(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
1 September 2016 (01.09.2016)

WIPO | PCT

(10) International Publication Number
WO 2016/138488 A2

(51) International Patent Classification:
C12Q 1/68 (2006.01)

(21) International Application Number:
PCT/US2016/019949

(22) International Filing Date:
26 February 2016 (26.02.2016)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
62/176,796 26 February 2015 (26.02.2015) US
62/181,697 18 June 2015 (18.06.2015) US
62/386,073 16 November 2015 (16.11.2015) US

(71) Applicants: **THE BROAD INSTITUTE INC.** [US/US]; 415 Main Street, Cambridge, MA 02142 (US). **MASSACHUSETTS INSTITUTE OF TECHNOLOGY** [US/US]; 77 Massachusetts Ave, Cambrideg, MA 02142 (US). **PRESIDENT AND FELLOWS OF HARVARD COLLEGE** [US/US]; 17 Quincy Street, Cambridge, MA 02138 (US). **THE BRIGHAM AND WOMEN'S HOSPITAL, INC.** [US/US]; 75 Francis Street, Boston, MA 02115 (US). **THE REGENTS OF THE UNIVERSITY OF CALIFORNIA** [US/US]; 1111 Franklin Street, 12th Floor, Oakland, CA 94607 (US).

(72) Inventors: **REGEV, Aviv**; 53 Standish Street #3, Cambridge, MA 02138 (US). **KUCHROO, Vijay, K.**; 30 Fairhaven Road, Newton, MA 02149 (US). **GAUBLOMME, Jellert**; Oude Baan 373, 3630 Maasmechelen (BE). **LEE, Youjin**; 555 Mission Rock Street, Unit 624, San Francisco, CA 94158 (US). **WANG, Chao**; c/o The Brigham And Women's Hospital, Inc., 75 Francis Street, Boston, MA 02115 (US). **YOSEF, Nir**; 1520 Laurel Ave, Richmond, CA 94805 (US). **PARK, Hongkun**; 17 Hathaway Road, Lexington, MA 02420 (US). **KAMINSKI, James**; 1919 Curtis Street, Apt 6, Berkeley, CA 94702 (US).

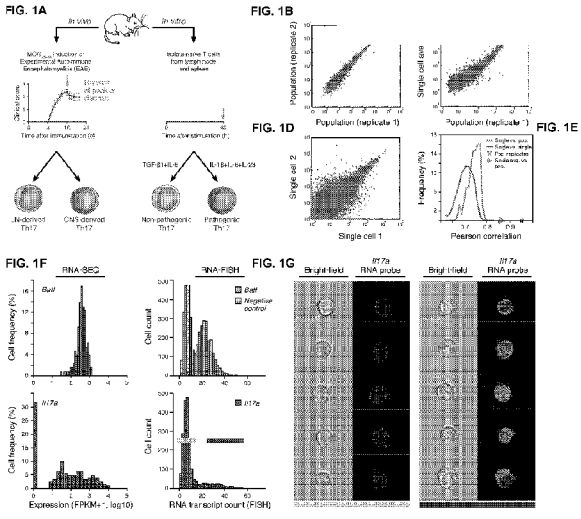
(74) Agents: **KOWALSKI, Thomas J.** et al.; Vedder Price P.C., 1633 Broadway, New York, NY 10019 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK,

[Continued on next page]

(54) Title: T CELL BALANCE GENE EXPRESSION, COMPOSITIONS OF MATTERS AND METHODS OF USE THEREOF



(57) Abstract: This invention relates generally to compositions and methods for identifying the regulatory network that modulates, controls or otherwise influences T cell balance, for example, Thl7 cell differentiation, maintenance and/or function, as well compositions and methods for exploiting the regulatory network that modulates, controls or otherwise influences T cell balance in a variety of therapeutic and/or diagnostic indications. This invention also relates generally to identifying and exploiting target genes and/or target gene products that modulate, control or otherwise influence T cell balance in a variety of therapeutic and/or diagnostic indications.

WO 2016/138488 A2

SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

— *without international search report and to be republished upon receipt of that report (Rule 48.2(g))*

T CELL BALANCE GENE EXPRESSION, COMPOSITIONS OF MATTERS AND METHODS OF USE THEREOF

RELATED APPLICATIONS AND INCORPORATION BY REFERENCE

[0001] This application claims priority to US provisional patent application 62/176,796, filed February 26, 2015; US provisional patent application 62/181,697, filed June 18, 2015 and US provisional patent application 62/386,073, filed November 16, 2015.

[0002] Reference is also made to PCT application PCT/US2015/017826, filed February 26, 2015 and published on September 3, 2015 as WO2015130968; WO/2012/048265; WO/2014/145631; WO/2014/134351; and US provisional patent application 61/945,641, filed February 27, 2014; and Wang et al., CD5L/AIM Regulates Lipid Biosynthesis and Restrains Th17 Cell Pathogenicity. Cell Volume 163, Issue 6, p1413–1427, 3 December 2015 and Gaublomme et al., Single-Cell Genomics Unveils Critical Regulators of Th17 Cell Pathogenicity. Cell Volume 163, Issue 6, p1400–1412, 3 December 2015, incorporated herein by reference.

[0003] The foregoing applications, and all documents cited therein or during prosecution ("appln cited documents") and all documents cited or referenced in the appln cited documents, and all documents cited or referenced herein ("herein cited documents"), and all documents cited or referenced in herein cited documents, together with any manufacturer's instructions, descriptions, product specifications, and product sheets for any products mentioned herein or in any document incorporated by reference herein, are hereby incorporated herein by reference, and may be employed in the practice of the invention. Appln cited documents, herein cited documents, all documents herein referenced or cited, and all documents indicated to be incorporated herein by reference, are incorporated by reference to the same extent as if each individual document was specifically and individually set forth herein in full and indicated to be incorporated by reference when or where cited or referenced.

FEDERAL FUNDING LEGEND

[0004] This invention was made with government support under grant numbers OD003958, HG006193, HG005062, OD003893, NS030843, NS045937, AI073748, AI045757 and AI056299 awarded by National Institutes of Health. The government may have certain rights in the invention.

FIELD OF THE INVENTION

[0005] This invention relates generally to compositions and methods for identifying the regulatory network that modulates, controls or otherwise influences T cell balance, for example, Th17 cell differentiation, maintenance and/or function, as well compositions and methods for exploiting the regulatory network that modulates, controls or otherwise influences T cell balance in a variety of therapeutic and/or diagnostic indications. This invention also relates generally to identifying and exploiting target genes and/or target gene products that modulate, control or otherwise influence T cell balance in a variety of therapeutic and/or diagnostic indications.

BACKGROUND OF THE INVENTION

[0006] Despite their importance, the molecular circuits that control the balance of T cells, including the differentiation of naïve T cells, remain largely unknown. Recent studies that reconstructed regulatory networks in mammalian cells have focused on short-term responses and relied on perturbation-based approaches that cannot be readily applied to primary T cells. Accordingly, there exists a need for a better understanding of the dynamic regulatory network that modulates, controls, or otherwise influences T cell balance, including Th17 cell differentiation, maintenance and function, and means for exploiting this network in a variety of therapeutic and diagnostic methods. Citations herein are not intended as an admission that anything cited is pertinent or prior art; nor does it constitute any admission as to the contents or date of anything cited.

SUMMARY OF THE INVENTION

[0007] The invention has many utilities. The invention pertains to and includes methods and compositions therefrom of Drug Discovery, as well as for detecting patients or subjects who may or may not respond or be responding to a particular treatment, therapy, compound, drug or combination of drugs or compounds; and accordingly ascertaining which drug or combination of drugs may provide a particular treatment or therapy as to a condition or disease or infection or infectious state, as well as methods and compositions for selecting patient populations (e.g., by detecting those who may or may not respond or be responding), or methods and compositions involving personalized treatment—a combination of Drug Discovery and detecting patients or subjects who may not respond or be responding to a particular treatment, therapy, compound, drug or combination of drugs or compounds (e.g., by as to individual(s), so detecting response, nor responding, potential to respond or not, and

adjusting particular treatment, therapy, compound, drug or combination of drugs or compounds to be administered or administering a treatment, therapy, compound, drug or combination of drugs or compounds indicated from the detecting).

[0008] The invention provides a method of diagnosing, prognosing and/or staging an immune response involving T cell balance, comprising detecting a first level of expression, activity and/or function of *Toso, advantageously Ctla2b*, *Gatm*, *Bdh2*, *Bcat1*, *Zfp36*, *Acsl4*, *Acat3*, *Adi1*, *Dot1l*, *Mett10d*, *Sirt6*, *Slc25a13*, *Chd2*, *Ino80c*, *Med21*, *Pdss1*, *Galk1*, *Gnpda2* or *Mtpap* or any one of the foregoing or any combination thereof with one or more of *Gpr65*, *Plzp* or *Cd5l* or any combination thereof *Gpr65*, *Plzp or Cd5l* in any combination of *Gpr65*, *Plzp*, *Toso* or *Cd5l* or one or more products of *Toso, advantageously Ctla2b*, *Gatm*, *Bdh2*, *Bcat1*, *Zfp36*, *Acsl4*, *Acat3*, *Adi1*, *Dot1l*, *Mett10d*, *Sirt6*, *Slc25a13*, *Chd2*, *Ino80c*, *Med21*, *Pdss1*, *Galk1*, *Gnpda2* or *Mtpap* or any one of the foregoing or any combination thereof with one or more of *Gpr65*, *Plzp* or *Cd5l* or any combination thereof *Gpr65*, *Plzp or Cd5l* in any combination of *Gpr65*, *Plzp*, *Toso* or *Cd5l*and comparing the detected level to a control of level of *Toso, advantageously Ctla2b*, *Gatm*, *Bdh2*, *Bcat1*, *Zfp36*, *Acsl4*, *Acat3*, *Adi1*, *Dot1l*, *Mett10d*, *Sirt6*, *Slc25a13*, *Chd2*, *Ino80c*, *Med21*, *Pdss1*, *Galk1*, *Gnpda2* or *Mtpap* or any one of the foregoing or any combination thereof with one or more of *Gpr65*, *Plzp* or *Cd5l* or any combination thereof *Gpr65*, *Plzp or Cd5l* in any combination of *Gpr65*, *Plzp*, *Toso* or *Cd5l* or gene product expression, activity and/or function, wherein a difference in the detected level and the control level indicates that the presence of an immune response in the subject.

[0009] The invention also provides a method of monitoring an immune response in a subject comprising detecting a level of expression, activity and/or function of *Toso, advantageously Ctla2b*, *Gatm*, *Bdh2*, *Bcat1*, *Zfp36*, *Acsl4*, *Acat3*, *Adi1*, *Dot1l*, *Mett10d*, *Sirt6*, *Slc25a13*, *Chd2*, *Ino80c*, *Med21*, *Pdss1*, *Galk1*, *Gnpda2* or *Mtpap* or any one of the foregoing or any combination thereof with one or more of *Gpr65*, *Plzp* or *Cd5l* or any combination thereof *Gpr65*, *Plzp or Cd5l* in any combination of *Gpr65*, *Plzp*, *Toso* or *Cd5l* at a first time point, detecting a level of expression, activity and/or function of one or more signature genes or one or more products of *Toso, advantageously Ctla2b*, *Gatm*, *Bdh2*, *Bcat1*, *Zfp36*, *Acsl4*, *Acat3*, *Adi1*, *Dot1l*, *Mett10d*, *Sirt6*, *Slc25a13*, *Chd2*, *Ino80c*, *Med21*, *Pdss1*, *Galk1*, *Gnpda2* or *Mtpap* or any one of the foregoing or any combination thereof with one or more of *Gpr65*, *Plzp* or *Cd5l* or any combination thereof *Gpr65*, *Plzp or Cd5l* in any combination of *Gpr65*, *Plzp*, *Toso* or *Cd5l* at a

second time point, and comparing the first detected level of expression, activity and/or function with the second detected level of expression, activity and/or function, wherein a change in the first and second detected levels indicates a change in the immune response in the subject.

[0010] The invention also provides a method of identifying a patient population at risk or suffering from an immune response comprising detecting a level of expression, activity and/or function of *Toso, advantageously Ctla2b*, *Gatm*, *Bdh2*, *Bcat1*, *Zfp36*, *Acsl4*, *Acat3*, *Adi1*, *Dot1l*, *Mett10d*, *Sirt6*, *Slc25a13*, *Chd2*, *Ino80c*, *Med21*, *Pdss1*, *Galk1*, *Gnpda2* or *Mtpap* or any one of the foregoing or any combination thereof with one or more of *Gpr65*, *Plzp* or *Cd5l* or any combination thereof *Gpr65*, *Plzp or Cd5l* in any combination of *Gpr65*, *Plzp*, *Toso* or *Cd5l* or one or more products of *Toso, advantageously Ctla2b*, *Gatm*, *Bdh2*, *Bcat1*, *Zfp36*, *Acsl4*, *Acat3*, *Adi1*, *Dot1l*, *Mett10d*, *Sirt6*, *Slc25a13*, *Chd2*, *Ino80c*, *Med21*, *Pdss1*, *Galk1*, *Gnpda2* or *Mtpap* or any one of the foregoing or any combination thereof with one or more of *Gpr65*, *Plzp* or *Cd5l* or any combination thereof *Gpr65*, *Plzp or Cd5l* in any combination of *Gpr65*, *Plzp*, *Toso* or *Cd5l* in the patient population and comparing the level of expression, activity and/or function of one or more signature genes or one or more products of *Toso, advantageously Ctla2b*, *Gatm*, *Bdh2*, *Bcat1*, *Zfp36*, *Acsl4*, *Acat3*, *Adi1*, *Dot1l*, *Mett10d*, *Sirt6*, *Slc25a13*, *Chd2*, *Ino80c*, *Med21*, *Pdss1*, *Galk1*, *Gnpda2* or *Mtpap* or any one of the foregoing or any combination thereof with one or more of *Gpr65*, *Plzp* or *Cd5l* or any combination thereof *Gpr65*, *Plzp or Cd5l* in any combination of *Gpr65*, *Plzp*, *Toso* or *Cd5l* in a patient population not at risk or suffering from an immune response, wherein a difference in the level of expression, activity and/or function of one or more of *Toso, advantageously Ctla2b*, *Gatm*, *Bdh2*, *Bcat1*, *Zfp36*, *Acsl4*, *Acat3*, *Adi1*, *Dot1l*, *Mett10d*, *Sirt6*, *Slc25a13*, *Chd2*, *Ino80c*, *Med21*, *Pdss1*, *Galk1*, *Gnpda2* or *Mtpap* or any one of the foregoing or any combination thereof with one or more of *Gpr65*, *Plzp* or *Cd5l* or any combination thereof *Gpr65*, *Plzp or Cd5l* in any combination of *Gpr65*, *Plzp*, *Toso* or *Cd5l* or one or more products of *Toso, advantageously Ctla2b*, *Gatm*, *Bdh2*, *Bcat1*, *Zfp36*, *Acsl4*, *Acat3*, *Adi1*, *Dot1l*, *Mett10d*, *Sirt6*, *Slc25a13*, *Chd2*, *Ino80c*, *Med21*, *Pdss1*, *Galk1*, *Gnpda2* or *Mtpap* or any one of the foregoing or any combination thereof with one or more of *Gpr65*, *Plzp* or *Cd5l* or any combination thereof *Gpr65*, *Plzp or Cd5l* in any combination of *Gpr65*, *Plzp*, *Toso* or *Cd5l* in the patient populations identifies the patient population as at risk or suffering from an immune response.

[0011] The invention also provides a method for monitoring subjects undergoing a treatment or therapy specific for a target gene selected from the group consisting of candidates *Toso, advantageously Ctla2b*, *Gatm*, *Bdh2*, *Bcat1*, *Zfp36*, *Acsl4*, *Acat3*, *Adi1*, *Dot1l*, *Mett10d*, *Sirt6*, *Slc25a13*, *Chd2*, *Ino80c*, *Med21*, *Pdss1*, *Galk1*, *Gnpda2* or *Mtpap* or any one of the foregoing or any combination thereof with one or more of *Gpr65*, *Plzp* or *Cd5l* or any combination thereof *Gpr65*, *Plzp or Cd5l* in any combination of *Gpr65*, *Plzp*, *Toso* or *Cd5l* for an aberrant immune response to determine whether the patient is responsive to the treatment or therapy comprising detecting a level of expression, activity and/or function of *Toso, advantageously Ctla2b*, *Gatm*, *Bdh2*, *Bcat1*, *Zfp36*, *Acsl4*, *Acat3*, *Adi1*, *Dot1l*, *Mett10d*, *Sirt6*, *Slc25a13*, *Chd2*, *Ino80c*, *Med21*, *Pdss1*, *Galk1*, *Gnpda2* or *Mtpap* or any one of the foregoing or any combination thereof with one or more of *Gpr65*, *Plzp* or *Cd5l* or any combination thereof *Gpr65*, *Plzp or Cd5l* in any combination of *Gpr65*, *Plzp*, *Toso* or *Cd5l* in the absence of the treatment or therapy and comparing the level of expression, activity and/or function of *Toso, advantageously Ctla2b*, *Gatm*, *Bdh2*, *Bcat1*, *Zfp36*, *Acsl4*, *Acat3*, *Adi1*, *Dot1l*, *Mett10d*, *Sirt6*, *Slc25a13*, *Chd2*, *Ino80c*, *Med21*, *Pdss1*, *Galk1*, *Gnpda2* or *Mtpap* or any one of the foregoing or any combination thereof with one or more of *Gpr65*, *Plzp* or *Cd5l* or any combination thereof *Gpr65*, *Plzp or Cd5l* in any combination of *Gpr65*, *Plzp*, *Toso* or *Cd5l* in the presence of the treatment or therapy, wherein a difference in the level of expression, activity and/or function of *Toso, advantageously Ctla2b*, *Gatm*, *Bdh2*, *Bcat1*, *Zfp36*, *Acsl4*, *Acat3*, *Adi1*, *Dot1l*, *Mett10d*, *Sirt6*, *Slc25a13*, *Chd2*, *Ino80c*, *Med21*, *Pdss1*, *Galk1*, *Gnpda2* or *Mtpap* or any one of the foregoing or any combination thereof with one or more of *Gpr65*, *Plzp* or *Cd5l* or any combination thereof *Gpr65*, *Plzp or Cd5l* in any combination of *Gpr65*, *Plzp*, *Toso* or *Cd5l* or products of *Toso, advantageously Ctla2b*, *Gatm*, *Bdh2*, *Bcat1*, *Zfp36*, *Acsl4*, *Acat3*, *Adi1*, *Dot1l*, *Mett10d*, *Sirt6*, *Slc25a13*, *Chd2*, *Ino80c*, *Med21*, *Pdss1*, *Galk1*, *Gnpda2* or *Mtpap* or any one of the foregoing or any combination thereof with one or more of *Gpr65*, *Plzp* or *Cd5l* or any combination thereof *Gpr65*, *Plzp or Cd5l* in any combination of *Gpr65*, *Plzp*, *Toso* or *Cd5l* in the presence of the treatment or therapy indicates whether the patient is responsive to the treatment or therapy.

[0012] In these methods the immune response is an autoimmune response or an inflammatory response; or the inflammatory response is associated with an autoimmune response, an infectious disease and/or a pathogen-based disorder; or the signature genes are Th17-associated genes; or the treatment or therapy is an antagonist as to expression of *Toso,*

advantageously Ctla2b, *Gatm*, *Bdh2*, *Bcat1*, *Zfp36*, *Acsl4*, *Acat3*, *Adi1*, *Dot1l*, *Mett10d*, *Sirt6*, *Slc25a13*, *Chd2*, *Ino80c*, *Med21*, *Pdss1*, *Galk1*, *Gnpda2* or *Mtpap* or any one of the foregoing or any combination thereof with one or more of *Gpr65*, *Plzp* or *Cd5l* or any combination thereof *Gpr65*, *Plzp or Cd5l* in any combination of *Gpr65*, *Plzp*, *Toso* or *Cd5l* in an amount sufficient to induce differentiation toward regulatory T cells (Tregs), Th1 cells, or a combination of Tregs and Th1 cells; or the treatment or therapy is an agonist that enhances or increases the expression of *Toso, advantageously Ctla2b*, *Gatm*, *Bdh2*, *Bcat1*, *Zfp36*, *Acsl4*, *Acat3*, *Adi1*, *Dot1l*, *Mett10d*, *Sirt6*, *Slc25a13*, *Chd2*, *Ino80c*, *Med21*, *Pdss1*, *Galk1*, *Gnpda2* or *Mtpap* or any one of the foregoing or any combination thereof with one or more of *Gpr65*, *Plzp* or *Cd5l* or any combination thereof *Gpr65*, *Plzp or Cd5l* in any combination of *Gpr65*, *Plzp*, *Toso* or *Cd5l* in an amount sufficient to induce T cell differentiation toward Th17 cells; or the treatment or therapy is an antagonist of a target gene selected from the group consisting of *Toso, advantageously Ctla2b*, *Gatm*, *Bdh2*, *Bcat1*, *Zfp36*, *Acsl4*, *Acat3*, *Adi1*, *Dot1l*, *Mett10d*, *Sirt6*, *Slc25a13*, *Chd2*, *Ino80c*, *Med21*, *Pdss1*, *Galk1*, *Gnpda2* or *Mtpap* or any one of the foregoing or any combination thereof with one or more of *Gpr65*, *Plzp* or *Cd5l* or any combination thereof *Gpr65*, *Plzp or Cd5l* in any combination of *Gpr65*, *Plzp*, *Toso* or *Cd5l* in an amount sufficient to switch Th17 cells from a pathogenic to non-pathogenic signature; or the treatment or therapy is an agonist that enhances or increases the expression of a target gene selected from the group consisting of *Toso, advantageously Ctla2b*, *Gatm*, *Bdh2*, *Bcat1*, *Zfp36*, *Acsl4*, *Acat3*, *Adi1*, *Dot1l*, *Mett10d*, *Sirt6*, *Slc25a13*, *Chd2*, *Ino80c*, *Med21*, *Pdss1*, *Galk1*, *Gnpda2* or *Mtpap* or any one of the foregoing or any combination thereof with one or more of *Gpr65*, *Plzp* or *Cd5l* or any combination thereof *Gpr65*, *Plzp or Cd5l* in any combination of *Gpr65*, *Plzp*, *Toso* or *Cd5l* in an amount sufficient to switch Th17 cells from a non-pathogenic to a pathogenic signature; or the T cell modulating agent is an antibody, a soluble polypeptide, a polypeptide agent, a peptide agent, a nucleic acid agent, a nucleic acid ligand, or a small molecule agent.

[0013] The invention also provides a method of modulating T cell balance, the method comprising contacting a T cell or a population of T cells with a T cell modulating agent in an amount sufficient to modify differentiation, maintenance and/or function of the T cell or population of T cells by altering balance between Th17 cells, regulatory T cells (Tregs) and other T cell subsets as compared to differentiation, maintenance and/or function of the T cell or population of T cells in the absence of the T cell modulating agent; wherein the T cell

modulating agent is an antagonist for or of *Toso, advantageously Ctla2b*, *Gatm*, *Bdh2*, *Bcat1*, *Zfp36*, *Acsl4*, *Acat3*, *Adi1*, *Dot1l*, *Mett10d*, *Sirt6*, *Slc25a13*, *Chd2*, *Ino80c*, *Med21*, *Pdss1*, *Galk1*, *Gnpda2* or *Mtpap* or any one of the foregoing or any combination thereof with one or more of *Gpr65*, *Plzp* or *Cd5l* or any combination thereof *Gpr65*, *Plzp or Cd5l* in any combination of *Gpr65*, *Plzp*, *Toso* or *Cd5l* in an amount sufficient to induce differentiation toward regulatory T cells (Tregs), Th1 cells, or a combination of Tregs and Th1 cells, or wherein the T cell modulating agent is an agonist that enhances or increases the expression of *Toso, advantageously Ctla2b*, *Gatm*, *Bdh2*, *Bcat1*, *Zfp36*, *Acsl4*, *Acat3*, *Adi1*, *Dot1l*, *Mett10d*, *Sirt6*, *Slc25a13*, *Chd2*, *Ino80c*, *Med21*, *Pdss1*, *Galk1*, *Gnpda2* or *Mtpap* or any one of the foregoing or any combination thereof with one or more of *Gpr65*, *Plzp* or *Cd5l* or any combination thereof *Gpr65*, *Plzp or Cd5l* in any combination of *Gpr65*, *Plzp*, *Toso* or *Cd5l* in an amount sufficient to induce T cell differentiation toward Th17 cells, or wherein the T cell modulating agent is specific for a target gene selected from the group consisting of *Toso, advantageously Ctla2b*, *Gatm*, *Bdh2*, *Bcat1*, *Zfp36*, *Acsl4*, *Acat3*, *Adi1*, *Dot1l*, *Mett10d*, *Sirt6*, *Slc25a13*, *Chd2*, *Ino80c*, *Med21*, *Pdss1*, *Galk1*, *Gnpda2* or *Mtpap* or any one of the foregoing or any combination thereof with one or more of *Gpr65*, *Plzp* or *Cd5l* or any combination thereof *Gpr65*, *Plzp or Cd5l* in any combination of *Gpr65*, *Plzp*, *Toso* or *Cd5l*, or wherein the T cell modulating agent is an antagonist of a target gene selected from the group consisting of *Toso, advantageously Ctla2b*, *Gatm*, *Bdh2*, *Bcat1*, *Zfp36*, *Acsl4*, *Acat3*, *Adi1*, *Dot1l*, *Mett10d*, *Sirt6*, *Slc25a13*, *Chd2*, *Ino80c*, *Med21*, *Pdss1*, *Galk1*, *Gnpda2* or *Mtpap* or any one of the foregoing or any combination thereof with one or more of *Gpr65*, *Plzp* or *Cd5l* or any combination thereof *Gpr65*, *Plzp or Cd5l* in any combination of *Gpr65*, *Plzp*, *Toso* or *Cd5l* in an amount sufficient to switch Th17 cells from a pathogenic to non-pathogenic signature, or wherein the T cell modulating agent is an agonist that enhances or increases the expression of a target gene selected from the group consisting of *Toso, advantageously Ctla2b*, *Gatm*, *Bdh2*, *Bcat1*, *Zfp36*, *Acsl4*, *Acat3*, *Adi1*, *Dot1l*, *Mett10d*, *Sirt6*, *Slc25a13*, *Chd2*, *Ino80c*, *Med21*, *Pdss1*, *Galk1*, *Gnpda2* or *Mtpap* or any one of the foregoing or any combination thereof with one or more of *Gpr65*, *Plzp* or *Cd5l* or any combination thereof *Gpr65*, *Plzp or Cd5l* in any combination of *Gpr65*, *Plzp*, *Toso* or *Cd5l* in an amount sufficient to switch Th17 cells from a non-pathogenic to a pathogenic signature. In these methods the T cell modulating agent is an antibody, a soluble polypeptide, a polypeptide agent, a peptide agent, a nucleic acid agent, a nucleic acid ligand, or a small molecule agent; or the T cells are naïve T

cells, partially differentiated T cells, differentiated T cells, a combination of naïve T cells and partially differentiated T cells, a combination of naïve T cells and differentiated T cells, a combination of partially differentiated T cells and differentiated T cells, or a combination of naïve T cells, partially differentiated T cells and differentiated T cells.

[0014] The invention also provides a method of enhancing Th17 differentiation in a cell population, increasing expression, activity and/or function of one or more Th17-associated cytokines or one or more Th17- associated transcription regulators selected from interleukin 17F (IL-17F), interleukin 17A (IL- 17A), STAT3, interleukin 21 (IL-21) and RAR-related orphan receptor C (RORC), and/or decreasing expression, activity and/or function of one or more non-Th17-associated cytokines or non-Th17-associated transcription regulators selected from FOXP3, interferon gamma (IFN-γ), GATA3, STAT4 and TBX21, comprising contacting a T cell with an agent that enhances expression, activity and/or function of *Toso, advantageously Ctla2b*, *Gatm*, *Bdh2*, *Bcat1*, *Zfp36*, *Acsl4*, *Acat3*, *Adi1*, *Dot1l*, *Mett10d*, *Sirt6*, *Slc25a13*, *Chd2*, *Ino80c*, *Med21*, *Pdss1*, *Galk1*, *Gnpda2* or *Mtpap* or any one of the foregoing or any combination thereof with one or more of *Gpr65*, *Plzp* or *Cd5l* or any combination thereof *Gpr65*, *Plzp or Cd5l* in any combination of *Gpr65*, *Plzp*, *Toso* or *Cd5l*.

[0015] In methods herein the agent enhances expression, activity and/or function of at least *Toso*. The agent can be an antibody, a soluble polypeptide, a polypeptide agonist, a peptide agonist, a nucleic acid agonist, a nucleic acid ligand, or a small molecule agonist; advantageously an antibody, such as a monoclonal antibody; or an antibody that is a chimeric, humanized or fully human monoclonal antibody.

[0016] The invention comprehends use of an antagonist for or of *Toso, advantageously Ctla2b*, *Gatm*, *Bdh2*, *Bcat1*, *Zfp36*, *Acsl4*, *Acat3*, *Adi1*, *Dot1l*, *Mett10d*, *Sirt6*, *Slc25a13*, *Chd2*, *Ino80c*, *Med21*, *Pdss1*, *Galk1*, *Gnpda2* or *Mtpap* or any one of the foregoing or any combination thereof with one or more of *Gpr65*, *Plzp* or *Cd5l* or any combination thereof *Gpr65*, *Plzp or Cd5l* in any combination of *Gpr65*, *Plzp*, *Toso* or *Cd5l* in an amount sufficient to induce differentiation toward regulatory T cells (Tregs), Th1 cells, or a combination of Tregs and Th1 cells for treating or Drug Discovery of or formulating or preparing a treatment for an aberrant immune response in a patient.

[0017] The invention comprehends use of an agonist that enhances or increases the expression of *Toso,* advantageously *Ctla2b*, *Gatm*, *Bdh2*, *Bcat1*, *Zfp36*, *Acsl4*, *Acat3*, *Adi1*,

Dot1l, *Mett10d*, *Sirt6*, *Slc25a13*, *Chd2*, *Ino80c*, *Med21*, *Pdss1*, *Galk1*, *Gnpda2* or *Mtpap* or any one of the foregoing or any combination thereof with one or more of *Gpr65*, *Plzp* or *Cd5l* or any combination thereof *Gpr65*, *Plzp or Cd5l* in any combination of *Gpr65*, *Plzp*, *Toso* or *Cd5l* in an amount sufficient to induce T cell differentiation toward Th17 cells for treating or Drug Discovery of or formulating or preparing a treatment for an aberrant immune response in a patient.

[0018] The invention comprehends use of an antagonist of a target gene selected from the group consisting of *Toso,* advantageously *Ctla2b*, *Gatm*, *Bdh2*, *Bcat1*, *Zfp36*, *Acsl4*, *Acat3*, *Adi1*, *Dot1l*, *Mett10d*, *Sirt6*, *Slc25a13*, *Chd2*, *Ino80c*, *Med21*, *Pdss1*, *Galk1*, *Gnpda2* or *Mtpap* or any one of the foregoing or any combination thereof with one or more of *Gpr65*, *Plzp* or *Cd5l* or any combination thereof *Gpr65*, *Plzp or Cd5l* in any combination of *Gpr65*, *Plzp*, *Toso* or *Cd5l* in an amount sufficient to switch Th17 cells from a pathogenic to non-pathogenic signature for treating or Drug Discovery of or formulating or preparing a treatment for an aberrant immune response in a patient.

[0019] The invention comprehends use of an agonist that enhances or increases the expression of a target gene selected from the group consisting of *Toso, advantageously Ctla2b*, *Gatm*, *Bdh2*, *Bcat1*, *Zfp36*, *Acsl4*, *Acat3*, *Adi1*, *Dot1l*, *Mett10d*, *Sirt6*, *Slc25a13*, *Chd2*, *Ino80c*, *Med21*, *Pdss1*, *Galk1*, *Gnpda2* or *Mtpap* or any one of the foregoing or any combination thereof with one or more of *Gpr65*, *Plzp* or *Cd5l* or any combination thereof *Gpr65*, *Plzp or Cd5l* in any combination of *Gpr65*, *Plzp*, *Toso* or *Cd5l* in an amount sufficient to switch Th17 cells from a non-pathogenic to a pathogenic signature for treating or Drug Discovery of or formulating or preparing a treatment for an aberrant immune response in a patient.

[0020] The invention comprehends a treatment method or Drug Discovery method or method of formulating or preparing a treatment comprising any one of the methods or uses herein discussed.

[0021] The invention comprehends a method of drug discovery for the treatment of a disease or condition involving an immune response involving T cell balance in a population of cells or tissue which express a target gene selected from the group consisting of *Toso,* advantageously *Ctla2b*, *Gatm*, *Bdh2*, *Bcat1*, *Zfp36*, *Acsl4*, *Acat3*, *Adi1*, *Dot1l*, *Mett10d*, *Sirt6*, *Slc25a13*, *Chd2*, *Ino80c*, *Med21*, *Pdss1*, *Galk1*, *Gnpda2* or *Mtpap* or any one of the foregoing or any combination thereof with one or more of *Gpr65*, *Plzp* or *Cd5l* or any combination thereof *Gpr65*, *Plzp or*

Cd5l in any combination of *Gpr65*, *Plzp*, *Toso* or *Cd5l* comprising the steps of (a) providing a compound or plurality of compounds to be screened for their efficacy in the treatment of said disease or condition; (b) contacting said compound or plurality of compounds with said population of cells or tissue; (c) detecting a first level of expression, activity and/or function of a target gene selected from the group consisting of *Toso, Ctla2b*, *Gatm*, *Bdh2*, *Bcat1*, *Zfp36*, *Acsl4*, *Acat3*, *Adi1*, *Dot1l*, *Mett10d*, *Sirt6*, *Slc25a13*, *Chd2*, *Ino80c*, *Med21*, *Pdss1*, *Galk1*, *Gnpda2* or *Mtpap* or any one of the foregoing or any combination thereof with one or more of *Gpr65*, *Plzp* or *Cd5l* or any combination of *Gpr65*, *Plzp* or *Cd5l* in any combination thereof *Gpr65*, *Plzp*, *Toso* or *Cd5l* or one or more products of a target gene selected from the group consisting of *Toso, Ctla2b*, *Gatm*, *Bdh2*, *Bcat1*, *Zfp36*, *Acsl4*, *Acat3*, *Adi1*, *Dot1l*, *Mett10d*, *Sirt6*, *Slc25a13*, *Chd2*, *Ino80c*, *Med21*, *Pdss1*, *Galk1*, *Gnpda2* or *Mtpap* or any one of the foregoing or any combination thereof with one or more of *Gpr65*, *Plzp* or *Cd5l* or any combination thereof *Gpr65*, *Plzp or Cd5l* in any combination of *Gpr65*, *Plzp*, *Toso* or *Cd5l*; (d) comparing the detected level to a control of level of a target gene selected from the group consisting of *Toso, Ctla2b*, *Gatm*, *Bdh2*, *Bcat1*, *Zfp36*, *Acsl4*, *Acat3*, *Adi1*, *Dot1l*, *Mett10d*, *Sirt6*, *Slc25a13*, *Chd2*, *Ino80c*, *Med21*, *Pdss1*, *Galk1*, *Gnpda2* or *Mtpap* or any one of the foregoing or any combination thereof with one or more of *Gpr65*, *Plzp* or *Cd5l* or any combination of *Gpr65*, *Plzp* or *Cd5l* in any combination thereof *Gpr65*, *Plzp*, *Toso* or *Cd5l* or gene product expression, activity and/or function; (e) evaluating the difference between the detected level and the control level to determine the immune response elicited by said compound or plurality of compounds.

[0022] The invention provides compositions and methods for modulating T cell balance. As used herein, the term "modulating" includes up-regulation of, or otherwise increasing, the expression of one or more genes, down-regulation of, or otherwise decreasing, the expression of one or more genes, inhibiting or otherwise decreasing the expression, activity and/or function of one or more gene products, and/or enhancing or otherwise increasing the expression, activity and/or function of one or more gene products.

[0023] As used herein, the term "modulating T cell balance" includes the modulation of any of a variety of T cell-related functions and/or activities, including by way of non-limiting example, controlling or otherwise influencing the networks that regulate T cell differentiation; controlling or otherwise influencing the networks that regulate T cell maintenance, for example, over the lifespan of a T cell; controlling or otherwise influencing the networks that regulate T

cell function; controlling or otherwise influencing the networks that regulate helper T cell (Th cell) differentiation; controlling or otherwise influencing the networks that regulate Th cell maintenance, for example, over the lifespan of a Th cell; controlling or otherwise influencing the networks that regulate Th cell function; controlling or otherwise influencing the networks that regulate Th17 cell differentiation; controlling or otherwise influencing the networks that regulate Th17 cell maintenance, for example, over the lifespan of a Th17 cell; controlling or otherwise influencing the networks that regulate Th17 cell function; controlling or otherwise influencing the networks that regulate regulatory T cell (Treg) differentiation; controlling or otherwise influencing the networks that regulate Treg cell maintenance, for example, over the lifespan of a Treg cell; controlling or otherwise influencing the networks that regulate Treg cell function; controlling or otherwise influencing the networks that regulate other CD4+ T cell differentiation; controlling or otherwise influencing the networks that regulate other CD4+ T cell maintenance; controlling or otherwise influencing the networks that regulate other CD4+ T cell function; manipulating or otherwise influencing the ratio of T cells such as, for example, manipulating or otherwise influencing the ratio of Th17 cells to other T cell types such as Tregs or other CD4+ T cells; manipulating or otherwise influencing the ratio of different types of Th17 cells such as, for example, pathogenic Th17 cells and non-pathogenic Th17 cells; manipulating or otherwise influencing at least one function or biological activity of a T cell; manipulating or otherwise influencing at least one function or biological activity of Th cell; manipulating or otherwise influencing at least one function or biological activity of a Treg cell; manipulating or otherwise influencing at least one function or biological activity of a Th17 cell; and/or manipulating or otherwise influencing at least one function or biological activity of another CD4+ T cell.

[0024] The invention provides T cell modulating agents that modulate T cell balance. For example, in some embodiments, the invention provides T cell modulating agents and methods of using these T cell modulating agents to regulate, influence or otherwise impact the level(s) of and/or balance between T cell types, *e.g.*, between Th17 and other T cell types, for example, regulatory T cells (Tregs), and/or Th17 activity and inflammatory potential. As used herein, terms such as "Th17 cell" and/or "Th17 phenotype" and all grammatical variations thereof refer to a differentiated T helper cell that expresses one or more cytokines selected from the group the consisting of interleukin 17A (IL-17A), interleukin 17F (IL-17F), and interleukin 17A/F heterodimer (IL17-AF). As used herein, terms such as "Th1 cell" and/or "Th1 phenotype" and

all grammatical variations thereof refer to a differentiated T helper cell that expresses interferon gamma (IFNγ). As used herein, terms such as "Th2 cell" and/or "Th2 phenotype" and all grammatical variations thereof refer to a differentiated T helper cell that expresses one or more cytokines selected from the group the consisting of interleukin 4 (IL-4), interleukin 5 (IL-5) and interleukin 13 (IL-13). As used herein, terms such as "Treg cell" and/or "Treg phenotype" and all grammatical variations thereof refer to a differentiated T cell that expresses Foxp3.

[0025] For example, in some embodiments, the invention provides T cell modulating agents and methods of using these T cell modulating agents to regulate, influence or otherwise impact the level of and/or balance between Th17 phenotypes, and/or Th17 activity and inflammatory potential. Suitable T cell modulating agents include an antibody, a soluble polypeptide, a polypeptide agent, a peptide agent, a nucleic acid agent, a nucleic acid ligand, or a small molecule agent.

[0026] For example, in some embodiments, the invention provides T cell modulating agents and methods of using these T cell modulating agents to regulate, influence or otherwise impact the level of and/or balance between Th17 cell types, *e.g.*, between pathogenic and nonpathogenic Th17 cells. For example, in some embodiments, the invention provides T cell modulating agents and methods of using these T cell modulating agents to regulate, influence or otherwise impact the level of and/or balance between pathogenic and non-pathogenic Th17 activity.

[0027] For example, in some embodiments, the invention provides T cell modulating agents and methods of using these T cell modulating agents to influence or otherwise impact the differentiation of a population of T cells, for example toward Th17 cells, with or without a specific pathogenic distinction, or away from Th17 cells, with or without a specific pathogenic distinction.

[0028] For example, in some embodiments, the invention provides T cell modulating agents and methods of using these T cell modulating agents to influence or otherwise impact the differentiation of a population of T cells, for example toward a non-Th17 T cell subset or away from a non-Th17 cell subset. For example, in some embodiments, the invention provides T cell modulating agents and methods of using these T cell modulating agents to induce T-cell plasticity, *i.e.*, converting Th17 cells into a different subtype, or into a new state.

[0029] For example, in some embodiments, the invention provides T cell modulating agents and methods of using these T cell modulating agents to induce T cell plasticity, *e.g.*, converting Th17 cells into a different subtype, or into a new state.

[0030] For example, in some embodiments, the invention provides T cell modulating agents and methods of using these T cell modulating agents to achieve any combination of the above.

[0031] In some embodiments, the T cells are naïve T cells. In some embodiments, the T cells are differentiated T cells. In some embodiments, the T cells are partially differentiated T cells. In some embodiments, the T cells are a mixture of naïve T cells and differentiated T cells. In some embodiments, the T cells are mixture of naïve T cells and partially differentiated T cells. In some embodiments, the T cells are mixture of partially differentiated T cells and differentiated T cells. In some embodiments, the T cells are mixture of naïve T cells, partially differentiated T cells, and differentiated T cells.

[0032] The T cell modulating agents are used to modulate the expression of one or more target genes or one or more products of one or more target genes that have been identified as genes responsive to Th17-related perturbations. These target genes are identified, for example, by contacting a T cell, *e.g.*, naïve T cells, partially differentiated T cells, differentiated T cells and/or combinations thereof, with a T cell modulating agent and monitoring the effect, if any, on the expression of one or more signature genes or one or more products of one or more signature genes. In some embodiments, the one or more signature genes are selected from those listed in Table 1 or Table 2 of WO/2014/134351, incorporated herein by reference; alone or with those of other herein disclosed methods.

[0033] In some embodiments, the target gene is one or more Th17-associated cytokine(s) or receptor molecule(s) selected from those listed in Table 3 of WO/2014/134351, incorporated herein by reference; alone or with those of other herein disclosed methods. In some embodiments, the target gene is one or more Th17-associated transcription regulator(s) selected from those shown in Table S3 (Gaublomme 2015) or listed in Table 4 of WO/2014/134351, incorporated herein by reference; alone or with those of other herein disclosed methods.

[0034] In some embodiments, the target gene is one or more Th17-associated transcription regulator(s) selected from those shown in Table S3 (Gaublomme 2015) or Table 5 of WO/2014/134351, incorporated herein by reference; alone or with those of other herein

disclosed methods. In some embodiments, the target gene is one or more Th17-associated receptor molecule(s) selected from those listed in Table 6 of WO/2014/134351, incorporated herein by reference; alone or with those of other herein disclosed methods. In some embodiments, the target gene is one or more Th17-associated kinase(s) selected from those listed in Table 7 of WO/2014/134351, incorporated herein by reference; alone or with those of other herein disclosed methods. In some embodiments, the target gene is one or more Th17-associated signaling molecule(s) selected from those listed in Table 8 of WO/2014/134351, incorporated herein by reference; alone or with those of other herein disclosed methods. In some embodiments, the target gene is one or more Th17-associated receptor molecule(s) selected from those listed in Table 9 of WO/2014/134351, incorporated herein by reference; alone or with those of other herein disclosed methods. In some embodiments, the target gene is one or more target genes involved in induction of Th17 differentiation such as, for example one or more of the target genes listed in Table 2 herein or Table 5 of WO/2014/134351, incorporated herein by reference (alone or with those of other herein disclosed methods), as being associated with the early stage of Th17 differentiation, maintenance and/or function. In some embodiments, the target gene is one or more target genes involved in onset of Th17 phenotype and amplification of Th17 T cells such as, for example, one or more of the target genes listed in Table 2 herein or Table 5 of WO/2014/134351, incorporated herein by reference (;alone or with those of other herein disclosed methods), as being associated with the intermediate stage of Th17 differentiation, maintenance and/or function. In some embodiments, the target gene is one or more target genes involved in stabilization of Th17 cells and/or modulating Th17-associated interleukin 23 (IL-23) signaling such as, for example, one or more of the target genes listed in Table 2 herein or Table 5 of WO/2014/134351, incorporated herein by reference (alone or with those of other herein disclosed methods), as being associated with the late stage of Th17 differentiation, maintenance and/or function. In some embodiments, the target gene is one or more of the target genes listed in Table 6 of WO/2014/134351, incorporated herein by reference (;alone or with those of other herein disclosed methods), as being associated with the early stage of Th17 differentiation. In some embodiments, the target gene is one or more of the target genes listed in Table 6 of WO/2014/134351, incorporated herein by reference (alone or with those of other herein disclosed methods), as being associated with the intermediate stage of Th17 differentiation, maintenance and/or function. In some

embodiments, the target gene is one or more of the target genes listed in Table 6 of WO/2014/134351, incorporated herein by reference (alone or with those of other herein disclosed methods), as being associated with the late stage of Th17 differentiation, maintenance and/or function. In some embodiments, the target gene is one or more of the target genes listed in Table 7 herein or Table 7 of WO/2014/134351, incorporated herein by reference (alone or with those of other herein disclosed methods), as being associated with the early stage of Th17 differentiation, maintenance and/or function. In some embodiments, the target gene is one or more of the target genes listed in Table 7 of WO/2014/134351, incorporated herein by reference (alone or with those of other herein disclosed methods), as being associated with the intermediate stage of Th17 differentiation, maintenance and/or function. In some embodiments, the target gene is one or more of the target genes listed in Table 7 of WO/2014/134351, incorporated herein by reference (alone or with those of other herein disclosed methods), as being associated with the late stage of Th17 differentiation, maintenance and/or function. In some embodiments, the target gene is one or more of the target genes listed in Table 8 of WO/2014/134351, incorporated herein by reference (alone or with those of other herein disclosed methods), as being associated with the early stage of Th17 differentiation, maintenance and/or function. In some embodiments, the target gene is one or more of the target genes listed in Table 8 of WO/2014/134351, incorporated herein by reference (alone or with those of other herein disclosed methods), as being associated with the intermediate stage of Th17 differentiation, maintenance and/or function. In some embodiments, the target gene is one or more of the target genes listed in Table 8 of WO/2014/134351, incorporated herein by reference (alone or with those of other herein disclosed methods), as being associated with the late stage of Th17 differentiation, maintenance and/or function. In some embodiments, the target gene is one or more of the target genes listed in Table 9 of WO/2014/134351, incorporated herein by reference (alone or with those of other herein disclosed methods), as being associated with the early stage of Th17 differentiation, maintenance and/or function. In some embodiments, the target gene is one or more of the target genes listed in Table S6 (Gaublomme 2015), Table 7 or in Table 9 of WO/2014/134351, incorporated herein by reference (alone or with those of other herein disclosed methods), as being associated with the intermediate stage of Th17 differentiation, maintenance and/or function. In some embodiments, the target gene is one or more of the target genes listed in Table S6 (Gaublomme 2015), Table

7 or Table 9 of WO/2014/134351, incorporated herein by reference (alone or with those of other herein disclosed methods), as being associated with the late stage of Th17 differentiation, maintenance and/or function.

[0035] In some embodiments, the target gene is one or more target genes that is a promoter of Th17 cell differentiation. In some embodiments, the target gene is GPR65. In some embodiments, the target gene is also a promoter of pathogenic Th17 cell differentiation and is selected from the group consisting of CD5L, DEC1, PLZP and TCF4.

[0036] In some embodiments, the target gene is one or more target genes that is a promoter of pathogenic Th17 cell differentiation. In some embodiments, the target gene is selected from the group consisting of CD5L, DEC1, PLZP and TCF4.

[0037] The desired gene or combination of target genes is selected, and after determining whether the selected target gene(s) is overexpressed or under-expressed during Th17 differentiation and/or Th17 maintenance, a suitable antagonist or agonist is used depending on the desired differentiation, maintenance and/or function outcome. For example, for target genes that are identified as positive regulators of Th17 differentiation, use of an antagonist that interacts with those target genes will shift differentiation away from the Th17 phenotype, while use of an agonist that interacts with those target genes will shift differentiation toward the Th17 phenotype. For target genes that are identified as negative regulators of Th17 differentiation, use of an antagonist that interacts with those target genes will shift differentiation toward from the Th17 phenotype, while use of an agonist that interacts with those target genes will shift differentiation away the Th17 phenotype. For example, for target genes that are identified as positive regulators of Th17 maintenance, use of an antagonist that interacts with those target genes will reduce the number of cells with the Th17 phenotype, while use of an agonist that interacts with those target genes will increase the number of cells with the Th17 phenotype. For target genes that are identified as negative regulators of Th17 differentiation, use of an antagonist that interacts with those target genes will increase the number of cells with the Th17 phenotype, while use of an agonist that interacts with those target genes will reduce the number of cells with the Th17 phenotype. Suitable T cell modulating agents include an antibody, a soluble polypeptide, a polypeptide agent, a peptide agent, a nucleic acid agent, a nucleic acid ligand, or a small molecule agent.

[0038] In some embodiments, the positive regulator of Th17 differentiation is a target gene selected from MINA, TRPS1, MYC, NKFB1, NOTCH, PML, POU2AF1, PROCR, RBPJ, SMARCA4, ZEB1, BATF, CCR5, CCR6, EGR1, EGR2, ETV6, FAS, IL12RB1, IL17RA, IL21R, IRF4, IRF8, ITGA3, and combinations thereof. In some embodiments, the positive regulator of Th17 differentiation is a target gene selected from MINA, PML, POU2AF1, PROCR, SMARCA4, ZEB1, EGR2, CCR6, FAS and combinations thereof.

[0039] In some embodiments, the negative regulator of Th17 differentiation is a target gene selected from SP4, ETS2, IKZF4, TSC22D3, IRF1 and combinations thereof. In some embodiments, the negative regulator of Th17 differentiation is a target gene selected from SP4, IKZF4, TSC22D3 and combinations thereof.

[0040] In some embodiments, the T cell modulating agent is a soluble Fas polypeptide or a polypeptide derived from FAS. In some embodiments, the T cell modulating agent is an agent that enhances or otherwise increases the expression, activity, and/or function of FAS in Th17 cells. As shown herein, expression of FAS in T cell populations induced or otherwise influenced differentiation toward Th17 cells. In some embodiments, these T cell modulating agents are useful in the treatment of an immune response, for example, an autoimmune response or an inflammatory response. In some embodiments, these T cell modulating agents are useful in the treatment of an infectious disease or other pathogen-based disorders. In some embodiments, the T cell modulating agent is an antibody, a soluble polypeptide, a polypeptide agonist, a peptide agonist, a nucleic acid agonist, a nucleic acid ligand, or a small molecule agonist. In some embodiments, the T cells are naïve T cells. In some embodiments, the T cells are differentiated T cells. In some embodiments, the T cells are partially differentiated T cells. In some embodiments, the T cells are a mixture of naïve T cells and differentiated T cells. In some embodiments, the T cells are mixture of naïve T cells and partially differentiated T cells. In some embodiments, the T cells are mixture of partially differentiated T cells and differentiated T cells. In some embodiments, the T cells are mixture of naïve T cells, partially differentiated T cells, and differentiated T cells. In some embodiments, the T cell modulating agent is an agent that inhibits the expression, activity and/or function of FAS. Inhibition of FAS expression, activity and/or function in T cell populations repressed or otherwise influenced differentiation away from Th17 cells and/or induced or otherwise influenced differentiation toward regulatory T cells (Tregs) and towards Th1 cells. In some embodiments, these T cell modulating agents

are useful in the treatment of an immune response, for example, an autoimmune response or an inflammatory response. In some embodiments, these T cell modulating agents are useful in the treatment of autoimmune diseases such as psoriasis, inflammatory bowel disease (IBD), ankylosing spondylitis, multiple sclerosis, Sjögren's syndrome, uveitis, and rheumatoid arthritis, asthma, systemic lupus erythematosus, transplant rejection including allograft rejection, and combinations thereof. In addition, enhancement of Th17 cells is also useful for clearing fungal infections and extracellular pathogens. In some embodiments, the T cell modulating agent is an antibody, a soluble polypeptide, a polypeptide antagonist, a peptide antagonist, a nucleic acid antagonist, a nucleic acid ligand, or a small molecule antagonist. In some embodiments, the T cells are naïve T cells. In some embodiments, the T cells are differentiated T cells. In some embodiments, the T cells are partially differentiated T cells that express additional cytokines. In some embodiments, the T cells are a mixture of naïve T cells and differentiated T cells. In some embodiments, the T cells are mixture of naïve T cells and partially differentiated T cells. In some embodiments, the T cells are mixture of partially differentiated T cells and differentiated T cells. In some embodiments, the T cells are mixture of naïve T cells, partially differentiated T cells, and differentiated T cells.

[0041] In some embodiments, the T cell modulating agent is an agent that inhibits the expression, activity and/or function of CCR5. Inhibition of CCR5 expression, activity and/or function in T cell populations repressed or otherwise influenced differentiation away from Th17 cells and/or induced or otherwise influenced differentiation toward regulatory T cells (Tregs) and towards Th1 cells. In some embodiments, these T cell modulating agents are useful in the treatment of an immune response, for example, an autoimmune response or an inflammatory response. In some embodiments, the T cell modulating agent is an inhibitor or neutralizing agent. In some embodiments, the T cell modulating agent is an antibody, a soluble polypeptide, a polypeptide antagonist, a peptide antagonist, a nucleic acid antagonist, a nucleic acid ligand, or a small molecule antagonist. In some embodiments, the T cells are naïve T cells. In some embodiments, the T cells are differentiated T cells. In some embodiments, the T cells are partially differentiated T cells. In some embodiments, the T cells are a mixture of naïve T cells and differentiated T cells. In some embodiments, the T cells are mixture of naïve T cells and partially differentiated T cells. In some embodiments, the T cells are mixture of partially

differentiated T cells and differentiated T cells. In some embodiments, the T cells are mixture of naïve T cells, partially differentiated T cells, and differentiated T cells.

[0042] In some embodiments, the T cell modulating agent is an agent that inhibits the expression, activity and/or function of CCR6. Inhibition of CCR6 expression, activity and/or function in T cell populations repressed or otherwise influenced differentiation away from Th17 cells and/or induced or otherwise influenced differentiation toward regulatory T cells (Tregs) and towards Th1 cells. In some embodiments, these T cell modulating agents are useful in the treatment of an immune response, for example, an autoimmune response or an inflammatory response. In some embodiments, the T cell modulating agent is an antibody, a soluble polypeptide, a polypeptide antagonist, a peptide antagonist, a nucleic acid antagonist, a nucleic acid ligand, or a small molecule antagonist. In some embodiments, the T cells are naïve T cells. In some embodiments, the T cells are differentiated T cells. In some embodiments, the T cells are partially differentiated T cells. In some embodiments, the T cells are a mixture of naïve T cells and differentiated T cells. In some embodiments, the T cells are mixture of naïve T cells and partially differentiated T cells. In some embodiments, the T cells are mixture of partially differentiated T cells and differentiated T cells. In some embodiments, the T cells are mixture of naïve T cells, partially differentiated T cells, and differentiated T cells.

[0043] In some embodiments, the T cell modulating agent is an agent that inhibits the expression, activity and/or function of EGR1. Inhibition of EGR1 expression, activity and/or function in T cell populations repressed or otherwise influenced differentiation away from Th17 cells and/or induced or otherwise influenced differentiation toward regulatory T cells (Tregs) and towards Th1 cells. In some embodiments, these T cell modulating agents are useful in the treatment of an immune response, for example, an autoimmune response or an inflammatory response. In some embodiments, the T cell modulating agent is an antibody, a soluble polypeptide, a polypeptide antagonist, a peptide antagonist, a nucleic acid antagonist, a nucleic acid ligand, or a small molecule antagonist. In some embodiments, the T cells are naïve T cells. In some embodiments, the T cells are differentiated T cells. In some embodiments, the T cells are partially differentiated T cells. In some embodiments, the T cells are a mixture of naïve T cells and differentiated T cells. In some embodiments, the T cells are mixture of naïve T cells and partially differentiated T cells. In some embodiments, the T cells are mixture of partially

differentiated T cells and differentiated T cells. In some embodiments, the T cells are mixture of naïve T cells, partially differentiated T cells, and differentiated T cells.

[0044] In some embodiments, the T cell modulating agent is an agent that inhibits the expression, activity and/or function of EGR2. Inhibition of EGR2 expression, activity and/or function in T cell populations repressed or otherwise influenced differentiation away from Th17 cells and/or induced or otherwise influenced differentiation toward regulatory T cells (Tregs) and towards Th1 cells. In some embodiments, these T cell modulating agents are useful in the treatment of an immune response, for example, an autoimmune response or an inflammatory response. In some embodiments, the T cell modulating agent is an antibody, a soluble polypeptide, a polypeptide antagonist, a peptide antagonist, a nucleic acid antagonist, a nucleic acid ligand, or a small molecule antagonist. In some embodiments, the T cells are naïve T cells. In some embodiments, the T cells are differentiated T cells. In some embodiments, the T cells are partially differentiated T cells. In some embodiments, the T cells are a mixture of naïve T cells and differentiated T cells. In some embodiments, the T cells are mixture of naïve T cells and partially differentiated T cells. In some embodiments, the T cells are mixture of partially differentiated T cells and differentiated T cells. In some embodiments, the T cells are mixture of naïve T cells, partially differentiated T cells, and differentiated T cells.

[0045] For example, in some embodiments, the invention provides T cell modulating agents and methods of using these T cell modulating agents to regulate, influence or otherwise impact the phenotype of a Th17 cell or population of cells, for example, by influencing a naïve T cell or population of cells to differentiate to a pathogenic or non-pathogenic Th17 cell or population of cells, by causing a pathogenic Th17 cell or population of cells to switch to a non- pathogenic Th17 cell or population of T cells (*e.g.*, populations of naïve T cells, partially differentiated T cells, differentiated T cells and combinations thereof), or by causing a non-pathogenic Th17 cell or population of T cells (*e.g.*, populations of naïve T cells, partially differentiated T cells, differentiated T cells and combinations thereof) to switch to a pathogenic Th17 cell or population of cells.

[0046] In some embodiments, the invention comprises a method of drug discovery for the treatment of a disease or condition involving an immune response involving T cell balance in a population of cells or tissue of a target gene comprising the steps of providing a compound or plurality of compounds to be screened for their efficacy in the treatment of said disease or

condition, contacting said compound or plurality of compounds with said population of cells or tissue, detecting a first level of expression, activity and/or function of a target gene, comparing the detected level to a control of level of a target gene, and evaluating the difference between the detected level and the control level to determine the immune response elicited by said compound or plurality of compounds. For example, the method contemplates comparing tissue samples which can be *inter alia* infected tissue, inflamed tissue, healthy tissue, or combinations of tissue samples thereof.

[0047] In one embodiment of the invention, the reductase null animals of the present invention may advantageously be used to modulate T cell balance in a tissue or cell specific manner. Such animals may be used for the applications hereinbefore described, where the role of T cell balance in product/drug metabolism, detoxification, normal homeostasis or in disease etiology is to be studied. It is envisaged that this embodiment will also allow other effects, such as drug transporter-mediated effects, to be studied in those tissues or cells in the absence of metabolism, e.g., carbon metabolism. Accordingly the animals of the present invention, in a further aspect of the invention may be used to modulate the functions and antibodies in any of the above cell types to generate a disease model or a model for product/drug discovery or a model to verify or assess functions of T cell balance.

[0048] In another embodiment, the method contemplates use of animal tissues and/or a population of cells derived therefrom of the present invention as an *in vitro* assay for the study of any one or more of the following events/parameters: (i) role of transporters in product uptake and efflux; (ii) identification of product metabolites produced by T cells; (iii) evaluate whether candidate products are T cells; or (iv) assess drug/drug interactions due to T cell balance.

[0049] The terms “pathogenic” or “non-pathogenic” as used herein are not to be construed as implying that one Th17 cell phenotype is more desirable than the other. As described herein, there are instances in which inhibiting the induction of pathogenic Th17 cells or modulating the Th17 phenotype towards the non-pathogenic Th17 phenotype is desirable. Likewise, there are instances where inhibiting the induction of non-pathogenic Th17 cells or modulating the Th17 phenotype towards the pathogenic Th17 phenotype is desirable.

[0050] As used herein, terms such as “pathogenic Th17 cell” and/or “pathogenic Th17 phenotype” and all grammatical variations thereof refer to Th17 cells that, when induced in the

presence of TGF-β3, express an elevated level of one or more genes selected from Cxcl3, IL22, IL3, Ccl4, Gzmb, Lrmp, Ccl5, Casp1, Csf2, Ccl3, Tbx21, Icos, IL17r, Stat4, Lgals3 and Lag, as compared to the level of expression in a TGF-β3-induced Th17 cells. As used herein, terms such as "non-pathogenic Th17 cell" and/or "non-pathogenic Th17 phenotype" and all grammatical variations thereof refer to Th17 cells that, when induced in the presence of TGF-β3, express a decreased level of one or more genes selected from IL6st, IL1rn, Ikzf3, Maf, Ahr, IL9 and IL10, as compared to the level of expression in a TGF-β3-induced Th17 cells.

[0051] In some embodiments, the T cell modulating agent is an agent that enhances or otherwise increases the expression, activity and/or function of Protein C Receptor (PROCR, also called EPCR or CD201) in Th17 cells. As shown herein, expression of PROCR in Th17 cells reduced the pathogenicity of the Th17 cells, for example, by switching Th17 cells from a pathogenic to non-pathogenic signature. Thus, PROCR and/or these agonists of PROCR are useful in the treatment of a variety of indications, particularly in the treatment of aberrant immune response, for example in autoimmune diseases and/or inflammatory disorders. In some embodiments, the T cell modulating agent is an antibody, a soluble polypeptide, a polypeptide agonist, a peptide agonist, a nucleic acid agonist, a nucleic acid ligand, or a small molecule agonist.

[0052] In some embodiments, the T cell modulating agent is an agent that inhibits the expression, activity and/or function of the Protein C Receptor (PROCR, also called EPCR or CD201). Inhibition of PROCR expression, activity and/or function in Th17 cells switches non-pathogenic Th17 cells to pathogenic Th17 cells. Thus, these PROCR antagonists are useful in the treatment of a variety of indications, for example, infectious disease and/or other pathogen-based disorders. In some embodiments, the T cell modulating agent is an antibody, a soluble polypeptide, a polypeptide antagonist, a peptide antagonist, a nucleic acid antagonist, a nucleic acid ligand, or a small molecule antagonist. In some embodiments, the T cell modulating agent is a soluble Protein C Receptor (PROCR, also called EPCR or CD201) polypeptide or a polypeptide derived from PROCR. In some embodiments, the invention provides a method of inhibiting Th17 differentiation, maintenance and/or function in a cell population and/or increasing expression, activity and/or function of one or more non-Th17-associated cytokines, one or more non-Th17 associated receptor molecules, or non-Th17-associated transcription regulators selected from FOXP3, interferon gamma (IFN-γ), GATA3, STAT4 and TBX21,

comprising contacting a T cell with an agent that inhibits expression, activity and/or function of MINA, MYC, NKFB1, NOTCH, PML, POU2AF1, PROCR, RBPJ, SMARCA4, ZEB1, BATF, CCR5, CCR6, EGR1, EGR2, ETV6, FAS, IL12RB1, IL17RA, IL21R, IRF4, IRF8, ITGA3 or combinations thereof. In some embodiments, the agent inhibits expression, activity and/or function of at least one of MINA, PML, POU2AF1, PROCR, SMARCA4, ZEB1, EGR2, CCR6, FAS or combinations thereof. In some embodiments, the agent is an antibody, a soluble polypeptide, a polypeptide antagonist, a peptide antagonist, a nucleic acid antagonist, a nucleic acid ligand, or a small molecule antagonist. In some embodiments, the antibody is a monoclonal antibody. In some embodiments, the antibody is a chimeric, humanized or fully human monoclonal antibody. In some embodiments, the T cell is a naïve T cell, and wherein the agent is administered in an amount that is sufficient to modulate the phenotype of the T cell to become and/or produce a desired non-Th17 T cell phenotype, for example, a regulatory T cell (Treg) phenotype or another CD4+ T cell phenotype. In some embodiments, the T cell is a partially differentiated T cell, and wherein the agent is administered in an amount that is sufficient to modulate the phenotype of the partially differentiated T cell to become and/or produce a desired non-Th17 T cell phenotype, for example, a regulatory T cell (Treg) phenotype or another CD4+ T cell phenotype. In some embodiments, the T cell is a Th17 T cell, and wherein the agent is administered in an amount that is sufficient to modulate the phenotype of the Th17 T cell to become and/or produce a CD4+ T cell phenotype other than a Th17 T cell phenotype. In some embodiments, the T cell is a Th17 T cell, and wherein the agent is administered in an amount that is sufficient to modulate the phenotype of the Th17 T cell to become and/or produce a shift in the Th17 T cell phenotype, *e.g.*, between pathogenic or non-pathogenic Th17 cell phenotype.

[0053] In some embodiments, the invention provides a method of inhibiting Th17 differentiation in a cell population and/or increasing expression, activity and/or function of one or more non-Th17-associated cytokines, one or more non-Th17-associated receptor molecules, or non-Th17-associated transcription factor selected from FOXP3, interferon gamma (IFN-γ), GATA3, STAT4 and TBX21, comprising contacting a T cell with an agent that enhances expression, activity and/or function of SP4, ETS2, IKZF4, TSC22D3, IRF1 or combinations thereof. In some embodiments, the agent enhances expression, activity and/or function of at least one of SP4, IKZF4, TSC22D3 or combinations thereof. In some embodiments, the agent is an antibody, a soluble polypeptide, a polypeptide agonist, a peptide agonist, a nucleic acid agonist, a

nucleic acid ligand, or a small molecule agonist. In some embodiments, the antibody is a monoclonal antibody. In some embodiments, the T cell is a naïve T cell, and wherein the agent is administered in an amount that is sufficient to modulate the phenotype of the T cell to become and/or produce a desired non-Th17 T cell phenotype, for example, a regulatory T cell (Treg) phenotype or another CD4+ T cell phenotype. In some embodiments, the T cell is a partially differentiated T cell, and wherein the agent is administered in an amount that is sufficient to modulate the phenotype of the partially differentiated T cell to become and/or produce a desired non-Th17 T cell phenotype, for example, a regulatory T cell (Treg) phenotype or another CD4+ T cell phenotype. In some embodiments, the T cell is a Th17 T cell, and wherein the agent is administered in an amount that is sufficient to modulate the phenotype of the Th17 T cell to become and/or produce a CD4+ T cell phenotype other than a Th17 T cell phenotype. In some embodiments, the T cell is a Th17 T cell, and wherein the agent is administered in an amount that is sufficient to modulate the phenotype of the Th17 T cell to become and/or produce a shift in the Th17 T cell phenotype, *e.g.*, between pathogenic or non-pathogenic Th17 cell phenotype.

[0054] In some embodiments, the invention provides a method of enhancing Th17 differentiation in a cell population increasing expression, activity and/or function of one or more Th17-associated cytokines, one or more Th17-associated receptor molecules, or one or more Th17-associated transcription regulators selected from interleukin 17F (IL-17F), interleukin 17A (IL-17A), STAT3, interleukin 21 (IL-21) and RAR-related orphan receptor C (RORC), and/or decreasing expression, activity and/or function of one or more non-Th17-associated cytokines, one or more Th17-associated receptor molecules, or one or more non-Th17-associated transcription regulators selected from FOXP3, interferon gamma (IFN-γ), GATA3, STAT4 and TBX21, comprising contacting a T cell with an agent that inhibits expression, activity and/or function of SP4, ETS2, IKZF4, TSC22D3, IRF1 or combinations thereof. In some embodiments, the agent inhibits expression, activity and/or function of at least one of SP4, IKZF4, TSC22D3 or combinations thereof. In some embodiments, the agent is an antibody, a soluble polypeptide, a polypeptide antagonist, a peptide antagonist, a nucleic acid antagonist, a nucleic acid ligand, or a small molecule antagonist. In some embodiments, the antibody is a monoclonal antibody. In some embodiments, the antibody is a chimeric, humanized or fully human monoclonal antibody. In some embodiments, the T cell is a naïve T cell, and wherein the agent is administered in an amount that is sufficient to modulate the phenotype of the T cell to become and/or produce a

desired Th17 T cell phenotype. In some embodiments, the T cell is a partially differentiated T cell, and wherein the agent is administered in an amount that is sufficient to modulate the phenotype of the partially differentiated T cell to become and/or produce a desired Th17 T cell phenotype. In some embodiments, the T cell is a CD4+ T cell other than a Th17 T cell, and wherein the agent is administered in an amount that is sufficient to modulate the phenotype of the non-Th17 T cell to become and/or produce a Th17 T cell phenotype. In some embodiments, the T cell is a Th17 T cell, and wherein the agent is administered in an amount that is sufficient to modulate the phenotype of the Th17 T cell to become and/or produce a shift in the Th17 T cell phenotype, *e.g.*, between pathogenic or non-pathogenic Th17 cell phenotype.

[0055] In some embodiments, the invention provides a method of enhancing Th17 differentiation in a cell population, increasing expression, activity and/or function of one or more Th17-associated cytokines, one or more Th17-associated receptor molecules, and/or one or more Th17-associated transcription regulators selected from interleukin 17F (IL-17F), interleukin 17A (IL-17A), STAT3, interleukin 21 (IL-21) and RAR-related orphan receptor C (RORC), and/or decreasing expression, activity and/or function of one or more non-Th17-associated cytokines, one or more Th17-associated receptor molecules, or one or more non-Th17-associated transcription regulators selected from FOXP3, interferon gamma (IFN-γ), GATA3, STAT4 and TBX21, comprising contacting a T cell with an agent that enhances expression, activity and/or function of MINA, MYC, NKFB1, NOTCH, PML, POU2AF1, PROCR, RBPJ, SMARCA4, ZEB1, BATF, CCR5, CCR6, EGR1, EGR2, ETV6, FAS, IL12RB1, IL17RA, IL21R, IRF4, IRF8, ITGA3 or combinations thereof. In some embodiments, the agent enhances expression, activity and/or function of at least one of MINA, PML, POU2AF1, PROCR, SMARCA4, ZEB1, EGR2, CCR6, FAS or combinations thereof. In some embodiments, the agent is an antibody, a soluble polypeptide, a polypeptide agonist, a peptide agonist, a nucleic acid agonist, a nucleic acid ligand, or a small molecule agonist. In some embodiments, the antibody is a monoclonal antibody. In some embodiments, the antibody is a chimeric, humanized or fully human monoclonal antibody. In some embodiments, the agent is administered in an amount sufficient to inhibit Foxp3, IFN-γ, GATA3, STAT4 and/or TBX21 expression, activity and/or function. In some embodiments, the T cell is a naïve T cell, and wherein the agent is administered in an amount that is sufficient to modulate the phenotype of the T cell to become and/or produce a desired Th17 T cell phenotype. In some embodiments, the T cell is a partially differentiated T

cell, and wherein the agent is administered in an amount that is sufficient to modulate the phenotype of the partially differentiated T cell to become and/or produce a desired Th17 T cell phenotype. In some embodiments, the T cell is a CD4+ T cell other than a Th17 T cell, and wherein the agent is administered in an amount that is sufficient to modulate the phenotype of the non-Th17 T cell to become and/or produce a Th17 T cell phenotype. In some embodiments, the T cell is a Th17 T cell, and wherein the agent is administered in an amount that is sufficient to modulate the phenotype of the Th17 T cell to become and/or produce a shift in the Th17 T cell phenotype, *e.g.*, between pathogenic or non-pathogenic Th17 cell phenotype.

[0056] In some embodiments, the invention provides a method of identifying genes or genetic elements associated with Th17 differentiation comprising: a) contacting a T cell with an inhibitor of Th17 differentiation or an agent that enhances Th17 differentiation; and b) identifying a gene or genetic element whose expression is modulated by step (a). In some embodiments, the method also comprises c) perturbing expression of the gene or genetic element identified in step b) in a T cell that has been in contact with an inhibitor of Th17 differentiation or an agent that enhances Th17 differentiation; and d) identifying a gene whose expression is modulated by step c). In some embodiments, the inhibitor of Th17 differentiation is an agent that inhibits the expression, activity and/or function of MINA, MYC, NKFB1, NOTCH, PML, POU2AF1, PROCR, RBPJ, SMARCA4, ZEB1, BATF, CCR5, CCR6, EGR1, EGR2, ETV6, FAS, IL12RB1, IL17RA, IL21R, IRF4, IRF8, ITGA3 or combinations thereof. In some embodiments, the agent inhibits expression, activity and/or function of at least one of MINA, PML, POU2AF1, PROCR, SMARCA4, ZEB1, EGR2, CCR6, FAS or combinations thereof. In some embodiments, the inhibitor of Th17 differentiation is an agent that enhances expression, activity and/or function of SP4, ETS2, IKZF4, TSC22D3, IRF1 or combinations thereof. In some embodiments, the agent enhances expression, activity and/or function of at least one of SP4, IKZF4 or TSC22D3. In some embodiments, the agent that enhances Th17 differentiation is an agent that inhibits expression, activity and/or function of SP4, ETS2, IKZF4, TSC22D3, IRF1 or combinations thereof. In some embodiments, wherein the agent that enhances Th17 differentiation is an agent that enhances expression, activity and/or function of MINA, MYC, NKFB1, NOTCH, PML, POU2AF1, PROCR, RBPJ, SMARCA4, ZEB1, BATF, CCR5, CCR6, EGR1, EGR2, ETV6, FAS, IL12RB1, IL17RA, IL21R, IRF4, IRF8, ITGA3 or combinations thereof. In some embodiments, the agent is an antibody, a soluble polypeptide, a polypeptide

antagonist, a peptide antagonist, a nucleic acid antagonist, a nucleic acid ligand, or a small molecule antagonist.

[0057] In some embodiments, the invention provides a method of modulating induction of Th17 differentiation comprising contacting a T cell with an agent that modulates expression, activity and/or function of one or more target genes or one or more products of one or more target genes selected from IRF1, IRF8, IRF9, STAT2, STAT3, IRF7, STAT1, ZFP281, IFI35, REL, TBX21, FLI1, BATF, IRF4, one or more of the target genes listed in Table 2 herein or Table 5 of WO/2014/134351, incorporated herein by reference (alone or with those of other herein disclosed methods), as being associated with the early stage of Th17 differentiation, maintenance and/or function, *e.g.*, AES, AHR, ARID5A, BATF, BCL11B, BCL3, CBFB, CBX4, CHD7, CITED2, CREB1, E2F4, EGR1, EGR2, ELL2, ETS1, ETS2, ETV6, EZH1, FLI1, FOXO1, GATA3, GATAD2B, HIF1A, ID2, IFI35, IKZF4, IRF1, IRF2, IRF3, IRF4, IRF7, IRF9, JMJD1C, JUN, LEF1, LRRFIP1, MAX, NCOA3, NFE2L2, NFIL3, NFKB1, NMI, NOTCH1, NR3C1, PHF21A, PML, PRDM1, REL, RELA, RUNX1, SAP18, SATB1, SMAD2, SMARCA4, SP100, SP4, STAT1, STAT2, STAT3, STAT4, STAT5B, STAT6, TFEB, TP53, TRIM24, and/or ZFP161, or any combination thereof.

[0058] In some embodiments, the invention provides a method of modulating onset of Th17 phenotype and amplification of Th17 T cells comprising contacting a T cell with an agent that modulates expression, activity and/or function of one or more target genes or one or more products of one or more target genes selected from one or more of the target genes listed in Table 2 herein or Table 5 of WO/2014/134351, incorporated herein by reference (alone or with those of other herein disclosed methods), as being associated with the intermediate stage of Th17 differentiation, maintenance and/or function. In some embodiments, the invention provides a method of modulating stabilization of Th17 cells and/or modulating Th17-associated interleukin 23 (IL-23) signaling comprising contacting a T cell with an agent that modulates expression, activity and/or function of one or more target genes or one or more products of one or more target genes selected from one or more of the target genes listed in Table 2 herein or Table 5 of WO/2014/134351 (alone or with those of other herein disclosed methods), incorporated herein by reference, as being associated with the late stage of Th17 differentiation, maintenance and/or function. In some embodiments, the invention provides a method of modulating one or more of the target genes listed in Table S6 (Gaublomme 2015), Table 7 or in Table 6 of

WO/2014/134351, incorporated herein by reference (alone or with those of other herein disclosed methods), as being associated with the early stage of Th17 differentiation, maintenance and/or function. In some embodiments, the invention provides a method of modulating one or more of the target genes listed in Table S6 (Gaublomme 2015), Table 7 herein or Table 6 of WO/2014/134351, incorporated herein by reference (alone or with those of other herein disclosed methods), as being associated with the intermediate stage of Th17 differentiation, maintenance and/or function. In some embodiments, the invention provides a method of modulating one or more of the target genes listed in Table S6 (Gaublomme 2015), Table 7 hereinor Table 6 of WO/2014/134351 (alone or with those of other herein disclosed methods), incorporated herein by reference, as being associated with the late stage of Th17 differentiation, maintenance and/or function. In some embodiments, the invention provides a method of modulating one or more of the target genes listed in Table 7 of WO/2014/134351 (alone or with those of other herein disclosed methods), incorporated herein by reference, as being associated with the early stage of Th17 differentiation, maintenance and/or function. In some embodiments, the invention provides a method of modulating one or more of the target genes listed in Table 7 of WO/2014/134351, incorporated herein by reference (alone or with those of other herein disclosed methods), asc being associated with the intermediate stage of Th17 differentiation, maintenance and/or function. In some embodiments, the invention provides a method of modulating one or more of the target genes listed in Table 7 of WO/2014/134351, incorporated herein by reference (alone or with those of other herein disclosed methods), as being associated with the late stage of Th17 differentiation, maintenance and/or function. In some embodiments, the invention provides a method of modulating is one or more of the target genes listed in Table 8 of WO/2014/134351, incorporated herein by reference (alone or with those of other herein disclosed methods), as being associated with the early stage of Th17 differentiation, maintenance and/or function. In some embodiments, the invention provides a method of modulating one or more of the target genes listed in Table 8 of WO/2014/134351, incorporated herein by reference (alone or with those of other herein disclosed methods), as being associated with the intermediate stage of Th17 differentiation, maintenance and/or function. In some embodiments, the invention provides a method of modulating one or more of the target genes listed in Table 8 of WO/2014/134351, incorporated herein by reference (alone or with those of other herein disclosed methods), as being associated

with the late stage of Th17 differentiation, maintenance and/or function. In some embodiments, the invention provides a method of modulating one or more of the target genes listed in Table 9 of WO/2014/134351, incorporated herein by reference (alone or with those of other herein disclosed methods), as being associated with the early stage of Th17 differentiation, maintenance and/or function. In some embodiments, the invention provides a method of modulating one or more of the target genes listed in Table 9 of WO/2014/134351, incorporated herein by reference (alone or with those of other herein disclosed methods), as being associated with the intermediate stage of Th17 differentiation, maintenance and/or function. In some embodiments, the invention provides a method of modulating one or more of the target genes listed in Table 9 of WO/2014/134351, incorporated herein by reference (alone or with those of other herein disclosed methods), as being associated with the late stage of Th17 differentiation, maintenance and/or function. In some embodiments, the invention provides a method of inhibiting tumor growth in a subject in need thereof by administering to the subject a therapeutically effective amount of an inhibitor of Protein C Receptor (PROCR). In some embodiments, the inhibitor of PROCR is an antibody, a soluble polypeptide, a polypeptide agent, a peptide agent, a nucleic acid agent, a nucleic acid ligand, or a small molecule agent. In some embodiments, the inhibitor of PROCR is one or more agents selected from the group consisting of lipopolysaccharide; cisplatin; fibrinogen; 1, 10-phenanthroline; 5-N-ethylcarboxamido adenosine; cystathionine; hirudin; phospholipid; Drotrecogin alfa; VEGF; Phosphatidylethanolamine; serine; gamma- carboxyglutamic acid; calcium; warfarin; endotoxin; curcumin; lipid; and nitric oxide.

[0059] In some embodiments, the invention provides a method of diagnosing an immune response in a subject, comprising detecting a level of expression, activity and/or function of one or more signature genes or one or more products of one or more signature genes selected from those listed in Table 1 or 2 of WO/2014/134351, incorporated herein by reference (alone or with those of other herein disclosed methods), and comparing the detected level to a control of level of signature gene or gene product expression, activity and/or function, wherein a difference between the detected level and the control level indicates that the presence of an immune response in the subject. In some embodiments, the immune response is an autoimmune response. In some embodiments, the immune response is an inflammatory response, including

inflammatory response(s) associated with an autoimmune response and/or inflammatory response(s) associated with an infectious disease or other pathogen-based disorder.

[0060] In some embodiments, the invention provides a method of monitoring an immune response in a subject, comprising detecting a level of expression, activity and/or function of one or more signature genes or one or more products of one or more signature genes, *e.g.*, one or more signature genes selected from those listed in Table 1 or 2 of WO/2014/134351, incorporated herein by reference (alone or with those of other herein disclosed methods), at a first time point, detecting a level of expression, activity and/or function of one or more signature genes or one or more products of one or more signature genes, *e.g.*, one or more signature genes selected from those listed in Table 1 or 2 of WO/2014/134351 (alone or with those of other herein disclosed methods), incorporated herein by reference, at a second time point, and comparing the first detected level of expression, activity and/or function with the second detected level of expression, activity and/or function, wherein a change between the first and second detected levels indicates a change in the immune response in the subject. In some embodiments, the immune response is an autoimmune response. In some embodiments, the immune response is an inflammatory response.

[0061] In some embodiments, the invention provides a method of monitoring an immune response in a subject, comprising isolating a population of T cells from the subject at a first time point, determining a first ratio of T cell subtypes within the T cell population at a first time point, isolating a population of T cells from the subject at a second time point, determining a second ratio of T cell subtypes within the T cell population at a second time point, and comparing the first and second ratio of T cell subtypes, wherein a change in the first and second detected ratios indicates a change in the immune response in the subject. In some embodiments, the immune response is an autoimmune response. In some embodiments, the immune response is an inflammatory response.

[0062] In some embodiments, the invention provides a method of activating therapeutic immunity by exploiting the blockade of immune checkpoints. The progression of a productive immune response requires that a number of immunological checkpoints be passed. Immunity response is regulated by the counterbalancing of stimulatory and inhibitory signal. The immunoglobulin superfamily occupies a central importance in this coordination of immune responses, and the CD28/cytotoxic T-lymphocyte antigen-4 (CTLA-4):B7.1/B7.2 receptor/ligand

grouping represents the archetypal example of these immune regulators (see e.g., Korman AJ, Peggs KS, Allison JP, "Checkpoint blockade in cancer immunotherapy." Adv Immunol. 2006; 90:297-339). In part the role of these checkpoints is to guard against the possibility of unwanted and harmful self-directed activities. While this is a necessary function, aiding in the prevention of autoimmunity, it may act as a barrier to successful immunotherapies aimed at targeting malignant self-cells that largely display the same array of surface molecules as the cells from which they derive. The expression of immune-checkpoint proteins can be dysregulated in a disease or disorder and can be an important immune resistance mechanism. Therapies aimed at overcoming these mechanisms of peripheral tolerance, in particular by blocking the inhibitory checkpoints, offer the potential to generate therapeutic activity, either as monotherapies or in synergism with other therapies.

[0063] Thus, the present invention relates to a method of engineering T-cells, especially for immunotherapy, comprising modulating T cell balance to inactivate or otherwise inhibit at least one gene or gene product involved in the immune check-point.

[0064] Suitable T cell modulating agent(s) for use in any of the compositions and methods provided herein include an antibody, a soluble polypeptide, a polypeptide agent, a peptide agent, a nucleic acid agent, a nucleic acid ligand, or a small molecule agent. By way of non-limiting example, suitable T cell modulating agents or agents for use in combination with one or more T cell modulating agents are shown in Table 10 of WO/2014/134351, incorporated herein by reference (alone or with those of other herein disclosed methods), of the specification.

[0065] One skilled in the art will appreciate that the T cell modulating agents have a variety of uses. For example, the T cell modulating agents are used as therapeutic agents as described herein. The T cell modulating agents can be used as reagents in screening assays, diagnostic kits or as diagnostic tools, or these T cell modulating agents can be used in competition assays to generate therapeutic reagents.

[0066] In some embodiments, the invention provides a method of diagnosing, prognosing and/or staging an immune response involving Th17 T cell balance, comprising detecting a first level of expression of one or more of saturated fatty acids (SFA) and/or polyunsaturated fatty acids (PUFA) in Th17 cells, and comparing the detected level to a control level of saturated fatty acids (SFA) and/or polyunsaturated fatty acids (PUFA), wherein a change in the first level of expression and the control level detected indicates a change in the immune response in the

subject. In one embodiment, a shift torwards polyunsaturated fatty acids (PUFA) and away from saturated fatty acids (SFA) indicates a non-pathogenic Th17 response.

[0067] In some embodiments, the invention provides a method for monitoring subjects undergoing a treatment or therapy involving T cell balance comprising, detecting a first level of expression of one or more of saturated fatty acids (SFA) and/or polyunsaturated fatty acids (PUFA) in Th17 cells in the absence of the treatment or therapy and comparing the detected level to a level of saturated fatty acids (SFA) and/or polyunsaturated fatty acids (PUFA) in the presence of the treatment or therapy, wherein a difference in the level of expression in the presence of the treatment or therapy indicates whether the subject is responsive to the treatment or therapy.

[0068] In another embodiment, the invention provides a method for monitoring subjects undergoing a treatment or therapy involving T cell balance comprising detecting a first level of expression of one or more of saturated fatty acids (SFA) and polyunsaturated fatty acids (PUFA) in Th17 cells in the absence of the treatment or therapy and comparing the ratio of detected level to a ratio of detected level of saturated fatty acids (SFA) and polyunsaturated fatty acids (PUFA) in the presence of the treatment or therapy, wherein a shift in the ratio in the presence of the treatment or therapy indicates whether the subject is responsive to the treatment or therapy. Not being bound by a theory, a shift in the ratio torwards polyunsaturated fatty acids (PUFA) and away from saturated fatty acids (SFA) indicates a non-pathogenic Th17 response.

[0069] In another embodiment, the therapy may be a lipid, preferably a mixture of lipids of the present invention. The lipids may be synthetic. Not being bound by a theory, a treatment comprising lipids may shift T cell balance.

[0070] In another embodiment, the treatment or therapy involving T cell balance is for a subject undergoing treatment or therapy for cancer. Not being bound by a theory, shifting Th17 balance torwards a pathogenic phenotype would allow a stronger immune response against a tumor.

[0071] In some embodiments, the invention provides a method of drug discovery for the treatment of a disease or condition involving an immune response involving Th17 T cell balance in a population of cells or tissue comprising: (a) providing a compound or plurality of compounds to be screened for their efficacy in the treatment of said disease or condition; (b) contacting said compound or plurality of compounds with said population of cells or tissue; (c)

detecting a first level of expression of one or more of saturated fatty acids (SFA) and/or polyunsaturated fatty acids (PUFA) in Th17 cells, optionally calculating a ratio; (d) comparing the detected level to a control level of saturated fatty acids (SFA) and/or polyunsaturated fatty acids (PUFA), optionally comparing the shift in ratio; and, (e) evaluating the difference between the detected level and the control level to determine the immune response elicited by said compound or plurality of compounds.

[0072] In some embodiments, a panel of lipids is detected. The panel may include saturated fatty acids (SFA) and/or polyunsaturated fatty acids (PUFA) whose expression is changed at least 1.5 fold when comparing wild type Th17 cells to $CD5L^{-/-}$ Th17 cells after treatment with non-pathogenic inducing cytokines. The non-pathogenic inducing cytokines may be TGF-β1+IL-6. The panel may include lipids whose expression is changed upon differentiation into a pathogenic or non-pathogenic Th17 cell. In another embodiment single saturated fatty acids (SFA) and/or polyunsaturated fatty acids (PUFA) representative of lipids whose expression is changed in response to CD5L loss or differentiation are detected. In a preferred embodiment, the SFA is a cholesterol ester or palmitic acid and the PUFA is a PUFA-containing triacylglyceride or arachidonic acid. In one embodiment only a single SFA or PUFA is detected.

[0073] In some embodiments, the treatment or therapy is a formulation comprising at least one lipid. The at least one lipid may be a synthetic lipid. Not being bound by a theory an autoimmune disease may be treated with polyunsaturated fatty acids (PUFA) and a disease requiring an enhanced immune response may be treated with saturated fatty acids (SFA).

[0074] Accordingly, it is an object of the invention to not encompass within the invention any previously known product, process of making the product, or method of using the product such that Applicants reserve the right and hereby disclose a disclaimer of any previously known product, process, or method. It is further noted that the invention does not intend to encompass within the scope of the invention any product, process, or making of the product or method of using the product, which does not meet the written description and enablement requirements of the USPTO (35 U.S.C. §112, first paragraph) or the EPO (Article 83 of the EPC), such that Applicants reserve the right and hereby disclose a disclaimer of any such subject matter.

[0075] It is noted that in this disclosure and particularly in the claims and/or paragraphs, terms such as “comprises”, “comprised”, “comprising” and the like can have the meaning

attributed to it in U.S. Patent law; e.g., they can mean "includes", "included", "including", and the like; and that terms such as "consisting essentially of" and "consists essentially of" have the meaning ascribed to them in U.S. Patent law, e.g., they allow for elements not explicitly recited, but exclude elements that are found in the prior art or that affect a basic or novel characteristic of the invention. Nothing herein is to be construed as a promise.

[0076] These and other embodiments are disclosed or are obvious from and encompassed by, the following Detailed Description.

BRIEF DESCRIPTION OF THE DRAWINGS

[0077] The novel features of the invention are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present invention will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the invention are utilized, and the accompanying drawings of which:

[0078] **Figure 1A-1G. Single-cell RNA-seq of Th17 cells *in vivo* and *in vitro*. (A)** Experimental setup; left: Procedure to isolate Th17 cells from *in vivo* tissues. EAE was induced by MOG immunization of IL-17A reporter mice, and $CD3^+CD4^+$IL-17A/GFP^+ cells were harvested at the peak of disease (inset cartoon graph: Y axis: disease score; X axis – days; Red arrow: the peak at clinical score 2.5-3) from the draining LNs and CNS and analyzed by single-cell RNA-Seq. Right: Procedure to differentiate Th17 cells *in vitro*. Naïve $CD4^+CD62L^+CD44^-$ T cells were isolated from the LN and the spleen of non-immunized mice and subsequently differentiated by CD3/CD28 activation and either TGF-β1+IL-6 to derive non-pathogenic Th17 cells, or IL-1β+IL-6+IL-23 to derive more pathogenic cells. Single-cell RNA-seq was performed at 48h into differentiation. **(B-E)** Quality of single-cell RNA-seq. Scatter plots (B-D) compare transcript expression (FPKM+1, $\log_{10}$) from the *in vitro* TGF-β1+IL-6 48hr condition, between two bulk population replicates (**B**), the 'average' of single-cell profile and a matched bulk population control (**C**), or two single cells (**D**). Histograms (**E**) depict the distributions of Pearson correlation coefficients (X axis) between single cells and their matched population control (red) and between pairs of single cells (blue). The Pearson correlation coefficient between the two replicates or between the single cell average and the matched population profile are

marked by a blue cross and red triangle, respectively. **(F,G)** Agreement between single-cell RNA-Seq and RNA Flow-FISH. (**F**) Comparison between expression distributions measured by RNA-seq (left) and transcript count distributions measured by RNA Flow-FISH (right) for the unimodally expressed gene *Batf* (top) and the bi-modally expressed *Il17a* (bottom). As a negative control, expression of the bacterial *DapB* gene was measured (light green). (**G**) Bright-field images of RNA Flow-FISH samples (n=5,000 cells) with the corresponding fluorescence channel for cells negative for *Il17a transcripts* (yellow) and positive for *Il17a* transcript (brown). Scale bar in the bright-field images is 7 μm. See also Figure 6, **Table S1,** related Figure 1.

[0079] Figure 2A-2F. Th17 cells span a progressive trajectory of states from the LN to the CNS. (A) Principal component analysis (PCA) separates CNS-derived cells (purple diamonds) from LN-derived cells (orange crosses). Shown are 302 cells in the space of the first two PCs. Numbered circles are selected features (signatures) that significantly correlate with PC1 or PC2 ($p < 10^{-6}$, **Table S2** (Gaublomme 2015) positioned based on the values of their Pearson correlation coefficient with each PC (axis values; to facilitate this view, the plotted PC values were normalized to be in the range between -1 and 1). Features were identified by the analysis depicted in (**B**) as either significantly diverse within a condition (with GSEA; FDR < 0.05); or between conditions (with a KS test comparing CNS and LN, FDR < 10^{-4}). **(B)** Functional annotation scheme. From top to bottom: Gene signatures are defined from literature (*e.g.*, by comparing $CD4^+$ memory and naïve T cells, top) distinguishing 'plus' and 'minus' genes (*e.g.*, genes that are, respectively, high and low in $CD4^+$ memory *vs.* naïve cells; bar plot). A signature score is calculated for each signature in each single cell, as the difference in weighted z scores between the 'plus' and 'minus' genes in the signature (**Experimental Procedures**). Finally (bottom), for each signature and PC Applicants compute the Pearson correlation coefficient between the signature score for each cell, and the loading on the PC for each cell. Applicants plot these Pearson correlation coefficients on the PCA plot (circled numbers in (**A**)). **(C)** Five progressive Th17-cell states from the LN to the CNS. Shown is the PCA plot as in A, but where Voronoi cells (defined by the signatures characterizing the cells populating the extremities of PCA space; **Experimental Procedures** (colored circles, **Table S2 (Gaublomme 2015)**) define five feature-specific subpopulations: Th17 self-renewing (green, defined by a LCMV-specific

CD4 signature comparing naïve cells to cells isolated 8 days post acute LCMV infection, GSE30431), Th17/pre-Th1 effector (pink, defined by a signature using TRP1 $CD4^+$ T cells comparing 5 day *ex vivo* Th17-polarized and stimulated cells to day 0 Th17 *in vitro* cells, GSE26030), Th17/Th1-like effector (yellow, LCMV-specific CD4 signature comparing cells isolated 8 days *vs.* 30 days post chronic LCMV infection, GSE30431), Th17/Th1-like memory (light blue, LCMV-specific CD4 signature comparing cells isolated 30 days post chronic infection to naïve cells, GSE30431), and Th17 dysfunctional/senescent (moss grey, inverse of a LCMV-specific CD4 signature comparing cells isolated 30 days post acute vs. chronic infection, GSE30431). The self-renewing state was observed in two technical replicates of one of the two *in vivo* biological replicates, potentially due to differences in disease induction or progression. **(D)** Example genes that distinguish each sub-population. For each of the five subpopulations in (C) (color coded rows) shown are cumulative distribution function (CDF) plots of expression for key selected genes. In each case, the gene's CDF is shown for cells from each sub-population. For the subpopulations that have a substantial mixture of LN and CNS cells, the dotted curve corresponds to cells from the CNS, and the solid line for cells from the LN of that subpopulation **(E,F)** Transcription factors (nodes) whose targets are significantly enriched in PC2 (**E**) or PC1 (**F**). Nodes are sized proportionally to fold enrichment (**Table S3** Gaublomme 2015) and colored according to the loading of the encoding gene in the respective PC (red and green: high and low PC loading, respectively; loadings were normalized to have zero mean and standard deviation of 1).

See also **Figure 7 and 13-14**, **Table S2-5** (Gaublomme 2015) , Table 2 and 6, related to **Figure 2**.

[0080] Figure 3A-3E. A spectrum of pathogenicity states *in vitro* (A) PCA plot of Th17 cells differentiated *in vitro*. PC1 separates cells from most (left) to least (right) pathogenic, as indicated both by the differentiation condition (color code), and by the correlated signatures (numbered circles). PC2 separates IL-17a+ sorted Th17 cells differentiated under pathogenic conditions (red triangles) from non-pathogenic cells (Light blue squares) and non-pathogenic cells not sorted to be IL-17A positive (Black circles) at 48h. Presented are features that correlate with PC1 or PC2 ($p<0.05$); and that were identified as significantly diverse within a condition (using GSEA; with an FDR cutoff of 0.05); or

between conditions (using KS-test to compare CNS and LN, with an FDR cutoff of 1e-4). **(B-D)** Key signatures related to pathogenicity. CDFs of the single-cell scores for key signatures for the three *in vitro* populations (colored as in A): (**B**) a signature distinguishing the *in vivo* Th17/Th1-like memory sub-population (blue in **Figure 2C**); (**C**) a signature distinguishing the *in vivo* Th17 self-renewing sub-population (green in **Figure 2C**); and (**D**) a signature of pathogenic Th17 cells (Lee et al., 2012). (**E**) CDFs of expression level (FPKM+1, $\log_{10}$) of *Il10* for the three *in vitro* populations.

See also **Table S2** (Gaublomme 2015) related to **Figure 3**.

[0081] Figure 4A-4E. Modules of genes that co-vary with pro-inflammatory and regulatory genes across single cells. (A) Single-cell expression distribution of genes. The heat map shows for each gene (row) its expression distribution across single cells differentiated under the TGF-β1+IL-6 condition for 48h (without further IL-17A-based sorting). Color scale: proportion of cells expressing in each of the 17 expression bins (columns). Genes are sorted from more unimodal (top) to bimodal (bottom). **(B)** Modules co-varying with pro-inflammatory and regulatory genes. Heat map of the Spearman correlation coefficients between the single-cell expression levels of signature genes of pathogenic T cells (Lee et al., 2012) or of other $CD4^+$ lineages (columns) and the single-cell expression of any other bimodally expressed gene (rows) in cells differentiated under the TGF-β1+IL-6 condition at 48h. Genes are clustered by similarity of these correlations, revealing two diametrically opposed modules of co-varying genes: a pro-inflammatory module (orange; *e.g.*, *Il17a, Il21, Ccl20*) and a regulatory module (green, *e.g.*, *Il10*, *Il24*, *Il27ra*). **(C)** The modules co-varying with pro-inflammatory and regulatory genes distinguish key variation. Each cell (TGF-β1+IL-6, 48h) is colored by a signature score comparing the two co-variation modules. Shown is a PCA plot (first two PCs) with the cells differentiated under the TGF-β1+IL-6 condition at 48h, where each cell is colored by a signature score (by the method of **Figure 2B**) comparing the two modules from **Figure 4B** (color code). Other signatures correlated to the PCs are marked by numbered circles. (**D**) Expression of key module genes. Each panel shows the PCA plot of (C) where cells are colored by an expression ranking score of a key gene, denoted on top. (from top left corner clockwise: *Il10*, *Toso*, *Il17a*, and *Plzp*. **(E)** A ranking of the top 100 candidate genes co-varying with pro-inflammatory or regulatory genes (out of 184; **Table 2 herein**), sorting

from high (left) to lower (right) ranking scores (bar chart). Bar chart (top) indicates ranking score deduced from single-cell data (**Experimental Procedures**). Genes are ordered from high (left) to low (right) scores. Purple-white heat map (middle) shows ranking scores for (top to bottom row): pathogenicity, pro-inflammatory *vs.* regulatory co-variation module and *in vitro* and *in vivo* PC's. Bottom matrix indicates 'known' (black, top row) genes previously associated with Th17 function; 'novel validated' (black, middle) genes that were tested and validated by follow-up experiments, and assignment to the 'pro-inflammatory/regulatory module' (orange & green, bottom) determined in this study.

See also **Figure 10 and 15**, **Table S2** (Gaublomme 2015) **&S8** related to **Figure 4**.

[0082] Figure 5A-5J. GPR65, TOSO and PLZP are validated as T-cell pathogenicity regulators. (A,B) Reduction in IL17A-producing cells in GPR65$^{-/-}$ T-cells differentiated *in vitro*. (**A**) Intracellular cytokine staining for IFN-γ (Y axis) and IL-17a (X axis) of CD4^{+} T cells from respective WT (top) or GPR65$^{-/-}$ (bottom) cells activated *in vitro* for 96h with anti-CD3 and anti-CD28, either without (Th0; left) or with Th17-polarizing cytokines (TGF-β1+IL-6, middle; or IL-1β+IL-6+IL-23, right). (**B**) Quantification of secreted IL-17A and Il-17F (Y axis) by cytometric bead assays (CBA) in corresponding samples (X axis). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. **(C)** Reduced IL-17A and IFN-γ production by GPR65$^{-/-}$ memory (CD62L^{-}CD44^{+}CD4^{+}) T cells in a recall assay. Rag1$^{-/-}$ mice were reconstituted with 2x10^{6} naïve CD4 T cells from WT or GPR65$^{-/-}$ mice, and, immunized with MOG_{35-55}/CFA one week post transfer. Draining LN and spleen cells were isolated 8 days after immunization and cultured *ex vivo* for 4 days with MOG_{35-55} for recall assay (**Experimental Procedures**). These cells were subsequently analyzed for production of IFN-γ (Y axis) and IL-17A (X axis). **(D)** Loss of GPR65 reduces tissue inflammation and autoimmune disease *in vivo*. Rag-1$^{-/-}$ mice (n = 10 per category) reconstituted with 2x10^{6} naïve CD4 T-cells from WT or GPR65$^{-/-}$ mice, then induced with EAE one week post transfer. Shown is the mean clinical score (Y axis) at days post immunization (X axis) for WT (black circles) or GPR65$^{-/-}$ (open circles) mice. Error bars indicate the standard deviation of the mean clinical score. **(E)** Transcriptional impact of a loss of GPR65, TOSO and PLZP. Shown is the significance of enrichment (-$\log_{10}$ (P-value); hypergeometric test, Y axis) of genes that are dysregulated compared to WT during the TGF-β1+IL-6 differentiation of GPR65$^{-/-}$ (96h), PLZP$^{-/-}$ (48h) and TOSO$^{-/-}$ (96h) cells. Red (blue) bars represent genes

characterizing PC1 of **Figure 4C** negatively (positively). Dashed red line: p = 0.01. **(F,G)** Reduction in IL17A-producing cells in $TOSO^{-/-}$ T cells differentiated *in vitro*. (**F**) Intracellular cytokine staining as in (A) but for WT or $TOSO^{-/-}$ $CD4^+$ T-cells, activated *in vitro* for 96h. (**G**) Quantification of secreted IL-17A and Il-17F for $CD4^+$ T cells from respective WT (dark green) or $TOSO^{-/-}$ (light green) mice as in (**B**) but at 48h. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. **(H)** Reduced IL-17A production by $TOSO^{-/-}$ LN memory T cells in a recall assay as in (C). **(I)** Hampered IL-17A production by $PLZP^{-/-}$ CD4+ T cells in an *in vitro* recall assay. $PLZP^{-/-}$ (bottom row) and littermate controls (top row) were immunized with 100 μg of MOG_{35-55}/CFA. Cells were harvested from the draining LNs and spleen 8 days post immunization and cultured *ex vivo* for 4 days with progressive concentrations of MOG_{35-55} (left column: 0 μg, middle: 5 μg and right: 20 μg) and 20ng/ml of IL-23. $CD4^+$ T cells were analyzed for IFN-γ (Y axis) and IL-17A (X axis) production by intracellular cytokine staining. **(J)** Quantification of secreted IL-17A and IL-17F of a MOG_{35-55} recall assay for littermate controls (dark green) and $PLZP^{-/-}$ mice (light green) at 96h post *ex vivo*. All experiments are a representative of at least three independent experiments with at least three experimental replicates per group.

[0083] Figure 6A-6I. related to Figure 1. Single-cell RNA-seq quality control. (A,B) Correlation between the first three PCs (X axis), and different RNA-seq quality measures (colored bars). (**A**) Before filtering and normalization, the main PCs highly correlate with various library quality scores (Legend below panel **A** & **B**), indicating that the dominant signal in the pre-normalization data may reflect experimental artifacts. (**B**) Normalization strongly reduces these correlations. Applicants find that before filtering and normalization (panel A) the main PCs highly correlate with the various library quality scores, as opposed to post-normalization (panel B). These results indicate that the dominant signal in the pre-normalization data might reflect experimental artifacts. **(C)** An example of a cell-specific false-negative curve (FNC). The false-negative rate (Y axis, percentage of genes in an expression bin that are detected in this cell (non zero estimated abundance)) is depicted as a function of transcript abundance in the bulk population (X axis, average expression level of genes within each bin). Each blue circle corresponds to a set of housekeeping genes (stratified according to their bulk-population expression levels). The false-negative curve (black solid line) is derived using a logistic function fit. **(D)** Correlations between single-cell

and bulk population profiles. Bar chart depicts the Spearman correlations coefficients (X axis) for each experimental batch (Y axis), where cells from each batch originate from a single mouse. A unique batch identifier is indicated in parentheses. Shown are Spearman correlations of gene expression profiles between pairs of single cells (blue bars, mean and standard deviation); between each single cell and a matched bulk population (orange bars, mean and standard deviation); between an average over all single cells and a matched bulk population (red bars); and between two bulk population replicates (green bars). **(E)** RNA-FlowFISH validation of expression distribution obtained by RNA-seq. Shown are the single-cell expression distributions for a set of select genes (rows) by RNA-seq (left column) and RNA-FlowFISH (right column). For RNA-seq distributions, the frequency of cells (Y axis) is shown as a function of expression (X axis, FPKM+1, $\log_{10}$), whereas RNA-FlowFISH is plotted as number of cells (Y axis) as a function of transcript (spot) count (X axis). Applicants find agreement for a variety of distributions, ranging from non-expressing (*Csf2, Itgax, Sdc1*) to unimodal distributions (*Irf4, Batf, Actb*) and bimodal distributions (*Il17a, Il2*).**(F)** Constitutively expressed genes are enriched for housekeeping functions. Shown is the fold enrichment of housekeeping genes among all the non-bimodally expressed genes (X axis) for each condition (Y axis) **(G)** As in (A), corresponding p-values (hypergeometric test). **(H, I)** Applicants find greater variation in expression levels for key immune genes. (H) Standard deviation (Yaxis) of all the detectably expressed genes in the non-pathogenic (TGF-β1+IL-6) condition is plotted *vs.* their single-cell average expression (X axis). Shown are housekeeping genes (green crosses), immune-response-related genes (red crosses, based on Gene Ontology) and other genes (blue dots). Selected outliers are highlighted by black squares. (I) As in (G), but where the standard deviation (Yaxis) and mean (X axis) of every gene are computed only for cells that express it (defined as those cells that are associated with the Gaussian distribution in our mixture model).

[0084] Figure 7A-7E. Population controls compared to single cell profiles. (A) Gene expression levels of selected genes for *in vivo* derived cells projected on PCs. Cells (CNS cells: diamonds, LN cells: crosses) are shown in a PCA plot as in **Figure 2C** and each cell is colored proportionally to the ranked expression of the denoted gene in this cell relative to the other cells (blue – low expression; red – high expression). Top: *Gpr65* is predominantly expressed in the CNS, and particularly high in the Th17/Th1-like memory subpopulation

(light blue). Bottom: *Ccr8*, previously associated with Th2 cells but not Th1/Th17 cells, is also highly expressed in most CNS derived cells. **(B)** Gene expression levels of selected genes for *in vitro* derived cells projected on PCs. Similar analysis as in (A) but for the different differentiation conditions *in vitro* and plotted on a PCA plot as in **Figure 3A**; (Left column) regulatory genes (IL-9, IL-16, Podoplanin and Foxp1) show high expression in the non-pathogenic condition (TGF-β1+IL-6), whereas inflammatory genes such as IL-22, IL-23r, Cxcr3 and Gm-csf are more highly expressed in the pathogenic differentiation condition (IL-1β+IL-6+IL-23). Figure 7 is sometimes also referred to as Supplementary Figure 2.**(C, D, E)** Shown are PCA plots based on single cell profiles (small circles, triangles, squares and crosses) along with projected matching population controls (large circles) and single cell averages (large squares) for **(C)** *In vitro* Th17 single cells only from the non-pathogenic conditions (TGF-β1+IL-6); **(D)** *In vivo* Th17 cells (CNS: purple, LN: orange); and **(E)** *In vitro* Th17 cells from all conditions: pathogenic (IL-1β+IL-6+IL-23; red icons); and non-pathogenic conditions (TGF-β1+IL-6. Black icons: cells not sorted for IL-17A/GFP+; light blue icons: IL-17A/GFP+ cells).

[0085] **Figure 8A-8D**. **(A)** $GPR65^{-/-}$ memory cells express less IL-17A upon IL-23 reactivation. Sorted memory ($CD62L^{-}CD44^{+}CD4^{+}$) T cells from wild type (WT, top row) and $GPR65^{-/-}$ (bottom row) mice were reactivated with IL-23 (20 ng/ml) for 96 h. Intracellular cytokine (ICC) analysis shows a reduction of ~45% IL-17A-positive cells (X axis) for $GPR65^{-/-}$ cells when compared to WT (**B**) IL-17A and IFN-γ production is hampered *in vivo* for $GPR65^{-/-}$ cells. A reduced frequency of IL-17A (X axis) and IFN- γ (Y axis) positive cells from the draining LNs and spleen of MOG_{35-55}/CFA-immunized $RAG\text{-}1^{-/-}$ mice reconstituted with WT (top row) or $GPR65^{-/-}$ (bottom row) naïve $CD4^{+}$ T-cells 30 days post EAE induction (**C**) $GPR65^{-/-}$ $CD4^{+}$ T-cells express less IL-17A and more IL-10. Quantification of secreted cytokines (Y axis) by cytometric bead assays (CBAs) for differentiation conditions (X axis) either without (Th0; left) or with Th17 polarizing cytokines (TGF-β1+IL-6, middle; or IL-1β+IL-6+IL-23, right) for $GPR65^{-/-}$ cells (light green) and littermate control cells (dark green). * $p<0.05$, ** $p<0.01$, *** $p<0.001$. All data presented here are a representative of three independent experiments, with at least 3 replicates per experiment. (**D**) Linear regression analysis of EAE disease progression for GPR65 KO *vs.* WT mice. Mean clinical score (Y axis) is shown as a function of days post

immunization (X axis) for WT (solid line) and GPR65$^{-/-}$ mice (dotted line). *** $p<0.001$. Data presented here is a representative of at least three independent experiments.

[0086] **Figure 9A-9C**. **(A)** TOSO$^{-/-}$ cells express less IL-17A but more IFN-γ upon IL-23 reactivation. Sorted memory (CD62L^{-}CD44^{+}CD4^{+}) T cells from WT and TOSO$^{-/-}$ mice were reactivated (anti-CD3/CD28) with IL-23 (20 ng/ml) for 96 h. The ICC analysis shows hardly any IL-17A (X axis) positive cells amongst TOSO$^{-/-}$ cells (bottom row) whereas WT does show a small IL-17A positive population (top row). On the other hand, IFN-γ (Y axis) gets induced to a larger extend in the TOSO$^{-/-}$ cells. (**B**) TOSO$^{-/-}$ cells exhibit lower FOXP3 levels during Treg differentiation. Naïve CD4^{+} T-cells from WT (top row) and TOSO$^{-/-}$ mice (bottom row) were differentiated *in vitro* with TGF-β1 (2 ng/ml) for 96h, and subsequently stained and analyzed by ICC for intracellular FOXP3 expression (Y axis) and CD4 expression (X axis). (**C**) TOSO$^{-/-}$ cells secrete less IL-17A, less IL-10, but more IFN- γ. Quantification of secreted cytokines (Y axis) by CBA for a 96h differentiation in conditions (X axis) without (Th0; left) or with Th17 polarizing cytokines (TGF-β1+IL-6, middle; or IL-1β+IL-6+IL-23, right) for TOSO$^{-/-}$ cells (light green) and WT cells (dark green). * $p<0.05$, ** $p<0.01$, *** $p<0.001$. All data presented here are a representative of three independent experiments, with at least three replicates per experiment.

[0087] **Figure 10A-10C**. **(A)** PLZP$^{-/-}$ T cells show comparable IL-17A and IFN-γ production to littermate controls (PLZP HET). ICC staining for IFN-γ (Y axis) and IL-17A (X axis) of CD4^{+} T cells from respective littermate controls (top) or PLZP$^{-/-}$ (bottom) cells activated *in vitro* for 48h with anti-CD3 and anti-CD28 either without (Th0; left) or with Th17 polarizing cytokines (TGF-β1+IL-6, middle; or IL-1β+IL-6+IL-23, right). **(B)** PLZP $^{-/-}$ cells produce less IL-17A cells upon IL-23 stimulation. PLZP$^{-/-}$ mice and littermate controls were immunized with 100 μg of MOG_{35-55}/CFA. Cells harvested 8 days after immunization from the draining LNs and spleen were cultured *ex vivo* for 4 days with (right column) or without (left) IL-23 (20 ng/ml). CD4^{+} T cells were analyzed for IFN-γ and IL-17A production by ICC staining. (**C**) PLZP$^{-/-}$ cells express significantly less pro-inflammatory cytokines in a MOG recall assay. Quantification of secreted cytokines (Y axis) by CBA in a MOG recall assay with different MOG_{35-55} concentrations (X axis) for PLZP$^{-/-}$ mice (light green) and littermate controls (dark green). * $p<0.05$, ** $p<0.01$, *** $p<0.001$, showing significant reduction of cytokine expression under MOG reactivation conditions. All data

presented here are a representative of three independent experiments, with at least 3 replicates per experiment.

[0088] Figure 11A-11M. CD5L shifts Th17 cell lipidome balance from saturated to unsaturated lipid, modulating Rorγt ligand availability and function. Figure 11A, B show Lipidome analysis of Th17 cells. **(A)** WT and CD5L$^{-/-}$ naïve T cells were differentiated. Cells and supernatant were harvested at 96 hours and subjected to MS/LC. Three independent mouse experiments were performed. Data shown are median expression of each metabolite identified that have at least 1.5 fold differences between WT and CD5L$^{-/-}$ under the TGFβ1+IL-6 condition. **(B,C)** Expression of representative metabolites including a cholesterol ester and a PUFA-containing TAG species**. (D)** Microscopy of wt and CD5L$^{-/-}$ cells stained for free cholesterol. (E,F) Rorγt ChIP from Th17 cells differentiated as described in A. under various conditions as indicated. **(G-J)** Dual luciferase reporter assays. **(G,H)** Dual luciferase reporter assays were performed in EL4 cells stably transfected with a control vector or Rorγt vector. CD5L retroviral vector was cotransfected in G. **(H)**.CD5L retroviral vector was cotransfected at 0, 25, 50 and 100ng / well. **(I-J)** 10μM of either arachidonic acid (PUFA) or 20μM of palmitic acid (SFA) were used whenever a single dose was indicated.. All ChIP and luciferase assay are representative of at least 3 independent experiments. Representative metabolites were used, including a cholesterol ester and a PUFA-containing TAG species. **(K)** Lipids from the two clusters in (A) are partitioned based on the length and saturation of their fatty acyl (FA) side chains. Those carrying more than one FA are further grouped by their FAs with the least saturation or longest carbon chain (in that order). Complete FA profile is shown in **(L)** Ratio of specific lipids in WT vs. CD5L$^{-/-}$ Th17 cells carrying various PUFA side chains. Phospholipids included in this analysis: phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine and their respective lyso-metabolites. Neutral lipid included in this analysis: Triacylglyceride, diacylglyceride and monoacylglyceride. Asterisk (*) denotes to $p < 0.05$ in Student's t-test. **(M)** Expression of *cyp51* and *sc4mol* mRNA in WT or CD5L$^{-/-}$ Th17 cells (TGF-β1+IL-6, left panels) or WT Th17 cells (TGF-β1+IL-6 with control or IL-23, right panels). SFA (palmitic acid, 25uM) or PUFA (arachidonic acid, 25uM) was added at 48h and cells analyzed at 96h.

[0089] Figure 12A-12F. Characterization of WT and CD5L-/- mice with EAE. Mice were immunized **(A)** 15 days post immunization, lymphocytes from CNS were isolated and

directly stained and analyzed with flow cytometry for the expression of FoxP3. **(B)** Cells from CNS as in A were restimulated with PMA/ionomycin with Brefeldin A for 4 hours and profiled for cytokine production by flow cytometry . **(C)** Cells were isolated from Inguinal LN of mice 10 days after immunization. 3H Thymidine incorporation assays was used to determine T cell proliferation in response to MOG35-55 peptide; **(D)** Supernatant from C were harvested and the amount of IL-17 was determined by ELISA. **(E, F)** Summary data for Fig 17 G, H respectively.

[0090] Figure 13, related to Figure 2. Differential gene expression of Th17 cells derived from LPL, LN and CNS. Shown are the expression levels of immune response related genes (rows; Z normalized per row) that are differentially expressed between bulk population samples from CNS, LN and LPL derived Th17 cells (columns).

[0091] Figure 14A-14D, related to Figures 2 and 3. Temporal asynchrony between individual cells *in vivo* and *in vitro*. (A, B) Weighted Pearson correlation coefficient (red: positive; blue: negative) of each single cell's profile (row) with bulk profiles at each of 18 time points (columns) along a 72h time course of Th17 cell differentiation, previously collected with microarrays (Yosef et al., 2013). The weighted Pearson correlation weighs down the effect of false negatives, as done in the weighted PCA, and z-normalized per row. Cells collected *in vitro* (A) show more synchrony than those from in vivo samples (B). **(C, D)** Some of the cell-to-cell variation likely reflects time of differentiation. Shown are the PCA plots for *in vitro* cells (C, as in **Figure 3**; IL-1β+IL-6+IL-23, triangles, TGF-β1+IL-6, squares and circles) and *in vivo* cells (D, as in **Figure 2**; CNS cells: diamonds, LN cells: crosses). Each cell (point) is colored proportionally to the ranked associated time point of this cell's maximal correlation from the analysis in (A, B) (blue: early time points; red: late time points).

[0092] Figure 15A-15B, related to Figure 4. Population based studies do not prioritize genes that have top ranks for Th17 pathogenicity by single cell data Shown are the 184 genes from our co-variation matrix (rows, **Figure 4B**), ordered according to population based ranking (X-axis) along with their rank (log10 (#genes that are ranked equal to or better); Y-axis) based on either **(A)** a compendium of 41 studies of Th17 cells, or (**B**) a literature based ranking (Ciofani et al., 2012). Red crosses: our top ranking candidates that we followed up on. While the 184 genes from our covariation matrix are more highly ranked than the other 7,000 genes from the single cells *in vitro* ($p<10^{-10}$ and ~0.015 for A and B, respectively; Wilcoxon Ranksum test), they do not necessarily stand out.

[0093] Figure 16A-16I. CD5L is a candidate regulator of Th17 cell functional states. (A-C) Single-cell RNA-seq analysis. (A) Cd5l expression of single-cells from in-vitro generated and in-vivo sorted Th17 cells (IL-17.GFP+) from mice at the peak of EAE. **(B,C)** Correlation of Cd5l expression in non-pathogenic Th17 cells (TGF-β1+IL-6) with (B) the cell pathogenicity score (based on the pathogenic signature of (Lee et al., 2012)). p = 2.63 X 10-5 (Wilcoxon ranksum test, comparing signature scores of Cd5l expressing vs. non-expressing cells); **(C)** the founding signature genes of the single-cell based proinflammatory (red) and regulatory (green) modules (Solid bars, significant correlation ($p < 0.05$); striked bars, none significant correlation). **(D-F)** Validation of CD5L expression in vitro. Naïve T cells (CD4+CD62L+CD44-CD25-) were sorted and differentiated as indicated and analyzed by qPCR for CD5L expression at 48h (D) and 72h (E) and by flow cytometry at 48h (F); (E) IL-23 or control was added at 48h in fresh media. **(G-I)** Validation of Cd5l expression in vivo. (G,H) IL-17A.GFP reporter mice were immunized to induce EAE. Cells were sorted from spleen (G) and CNS (H) at the peak of disease. Cd5l and Il17a expression are measured by qPCR. Figure shown is representative data of three technical replicates from two independent experiments. (I) Cells were sorted from the gut of naïve mice and the number of RNA transcripts measured by nanostring nCounter platform.

[0094] Figure 17A-17H. CD5L represses effector functions without affecting Th17 cell differentiation. (A) EAE was induced by MOG/CFA (40μg) immunization. Left panel is pooled results from 3 independent experiments. Right panel: cytokine profile of CD4 T cells isolated from CNS at day15 post immunization. **(B-D)** Naïve splenic T cells were sorted and differentiated with TGF-β1+IL-6 for 48h. Th17 cell signature genes were measured by flow cytometry (**B**), ELISA (**C**) and qPCR **(D)**. **(E-F)** Effector Th17 cells were differentiated as in **B** and resuspended in fresh media with no cytokines for 72h followed by restimulation. Gene profile was measured by flow cytometry (**E**) and qPCR (**F**). **(G-H)** Effector memory T cells (CD4+CD62L-CD44+) (**G**) or Effector memory Th17 cells (CD4+CD62L-CD44+RorγtGFP+) (**H**) were sorted from spleen of naïve mice and activated with TCR stimulation.

[0095] Figure 18A-18F. CD5L and PUFA/SFA profile regulate Rorγt function in a ligand-dependent manner. (A, B) Rorγt ChIP-PCR analyses in WT and CD5L-/- Th17 cells. WT, CD5L-/- and Rorγt-/- Th17 cells were differentiated with TGF-β1+IL-6 for 96h. Enrichment of Rorγt binding to genomic regions of *Il17* (**A**) and *Il10* (**B**) is measured using qPCR. For fatty

acid experiments, 10μM of either SFA (palmitic acid) or PUFA (arachidonic acid or docosahexaenoic acid showed similar results) was added to WT Th17 cell culture at day 0. Three independent experiments were performed. (**C, D**) Rorγt transcriptional activity was measured by luciferase reporter of *Il17* promoter in EL4 cells transfected with CD5L-RV at 0, 25, 50, 100ng (**C**) or 100ng with 7, 27 dihydroxycholsterol (5, 0.5 or 0.05uM) (**D**). (**E**) Naïve WT T cells were activated without polarizing cytokines (Th0) and infected with retrovirus expressing Rorγt in the presence of control-RV or CD5L-RV with or without FF-MAS (5uM) as a source of Rorγt ligand. Each dot represents an independent infection. (**F**) WT or CD5L-/- naïve cells were differentiated with TGF-β1+IL-6. At 48h, cells were replated in fresh media with either control or FF-MAS (5uM) as a source of Rorγt ligand. Cells were harvested for FACS analysis 72h later.

[0096] Figure 19A-19E. Single cell RNA-seq identifies *Cd5l* as a gene in covariance with the pathogenic module within non-pathogenic Th17 cells. (**A**) Histogram of *Cd5l* expression in single cell from unsorted *in-vitro* derived Th17 cells differentiated under the TGF-β1+IL-6 condition. (**B**) The expression of *Cd5l* within single cell is shown in covariance with the first PC of *in-vitro* derived cells as in (**A**) where it correlates with the pro-inflammatory module. (**C**) Within the same PC space as in (**B**), score of pathogenic signature is shown to also correlate with PC1 as defined in the text. (**D, E**) Regulation of CD5L expression. (**D**) Naïve CD4 T cells were sorted from WT, Stat3$^{CD4Cre-/-}$, Rorgt$^{CD4Cre-/-}$ and CD5L-/- and differentiated under Th0 or Th17 (TGFb1+IL-6) condition as in Figure 17D. CD5L expression was measured intracellularly at 48 hour post differentiation. Upper panel: representative FACS plot; Lower panel: summary results from three independent experiments. (**E**) Naïve CD4 T cells were differentiated under Th0 condition and transfected with retrovirus carrying *Stat3* construct to overexpress STAT3. CD5L expression was measured as in **D**.

[0097] Figure 20A-20F. CD5L antagonizes pathogenicity of Th17 cells. (**A,B**) (A) Summary data for Cytokine profile of WT and CD5L-/- 2D2 cells isolated from CNS at day 27 post transfer. Cells were gated on Va3.2+CD4+. (B) Summary data for Cytokine profile of CD45.1 WT recipients that received 100,000 naïve WT or CD5L-/- 2D2 T cells and were immunized the following day with MOG/CFA without pertussis toxin. Cytokine profile of 2D2 T cells was examined on day 10 in draining LN (**C-F**) Passive EAE is induced. Briefly, naïve 2D2 cells were sorted from WT mice and differentiated under the pathogenic Th17 differentiation

conditions with IL-1β+IL-6+IL-23. At 24h, either CD5L-RV or control-RV retrovirus was used to infect the activated cells. The expression of CD5L was analyzed at day 3 post-infection. 50% of cells expressed GFP in both groups. (**C**) Representative flow cytometry analysis of cytokine profile prior to transfer; (**D**) Weight loss curve after transfer; (**E**) EAE score; Dotted green and red lines are linear regression analysis performed as in **Fig. 17A**. (**F**) Representative flow cytometry data of cytokine profile of $CD4_+$ T cells from CNS at day 30 post transfer.

[0098] Figure 21A-21E. CD5L regulates lipid metabolism in Th17 cells and modulate Rorγt ligand. (A) Lipidomics analysis. Entire set of 39 lipids (rows) resolved from cell lysates (columns) that have significantly different levels among any Th17 cell conditions and are with a fold difference of at least 1.5. (**B**) The ratio of specific lipids (from all those resolved) between WT and $CD5L_{-/-}$ Th17 cells (both in TGF-β1+IL-6 conditions) (Y-axis) partitioned by their PUFA content (X axis). (**C**) Left panel: The ratio of a particular lipid with specific SFA or MUFA content in WT vs $CD5L_{-/-}$ Th17 cells (TGF-β1+IL-6) is shown. Right panel, same data as left panel, segregating phospholipid from neutral lipids (**D**) MEVA analysis of all lipid species resolved (rows) comparing cell lysates or media in different Th17 cell conditions (1-6, legend). CE, cholesterolester; LPC, lysophosphatidylcholine; PC, phosphatidylcholine; SM, sphingomyelin; TAG, triacylglyceride. B623: IL-1β+IL-6+IL-23 condition; T16: TGF-β1+IL-6 condition. (**E**) Expression of free cholesterol in Th17 cells. WT and $CD5L_{-/-}$ Th17 cells were differentiated with TGF-β1+IL-6 for 48 hours and harvested for confocal microscopy. Cells were fixed using paraformaldehyde and stained with Filipin for 30 minutes, washed and sealed with DAPI-coatedcover slides and analyzed by confocal microscopy.

DETAILED DESCRIPTION

[0099] This invention relates generally to compositions and methods for identifying the regulatory networks that control T cell balance, T cell differentiation, T cell maintenance and/or T cell function, as well compositions and methods for exploiting the regulatory networks that control T cell balance, T cell differentiation, T cell maintenance and/or T cell function in a variety of therapeutic and/or diagnostic indications.

[00100] The invention provides compositions and methods for modulating T cell balance. The invention provides T cell modulating agents that modulate T cell balance. For example, in some embodiments, the invention provides T cell modulating agents and methods of using these

T cell modulating agents to regulate, influence or otherwise impact the level of and/or balance between T cell types, *e.g.*, between Th17 and other T cell types, for example, regulatory T cells (Tregs). For example, in some embodiments, the invention provides T cell modulating agents and methods of using these T cell modulating agents to regulate, influence or otherwise impact the level of and/or balance between Th17 activity and inflammatory potential. As used herein, terms such as "Th17 cell" and/or "Th17 phenotype" and all grammatical variations thereof refer to a differentiated T helper cell that expresses one or more cytokines selected from the group the consisting of interleukin 17A (IL-17A), interleukin 17F (IL-17F), and interleukin 17A/F heterodimer (IL17-AF). As used herein, terms such as "Th1 cell" and/or "Th1 phenotype" and all grammatical variations thereof refer to a differentiated T helper cell that expresses interferon gamma (IFNγ). As used herein, terms such as "Th2 cell" and/or "Th2 phenotype" and all grammatical variations thereof refer to a differentiated T helper cell that expresses one or more cytokines selected from the group the consisting of interleukin 4 (IL-4), interleukin 5 (IL-5) and interleukin 13 (IL-13). As used herein, terms such as "Treg cell" and/or "Treg phenotype" and all grammatical variations thereof refer to a differentiated T cell that expresses Foxp3.

[00101] These compositions and methods use T cell modulating agents to regulate, influence or otherwise impact the level and/or balance between T cell types, *e.g.*, between Th17 and other T cell types, for example, regulatory T cells (Tregs).

[00102] The invention provides methods and compositions for modulating T cell differentiation, for example, helper T cell (Th cell) differentiation. The invention provides methods and compositions for modulating T cell maintenance, for example, helper T cell (Th cell) maintenance. The invention provides methods and compositions for modulating T cell function, for example, helper T cell (Th cell) function. These compositions and methods use T cell modulating agents to regulate, influence or otherwise impact the level and/or balance between Th17 cell types, *e.g.*, between pathogenic and non-pathogenic Th17 cells. These compositions and methods use T cell modulating agents to influence or otherwise impact the differentiation of a population of T cells, for example toward the Th17 cell phenotype, with or without a specific pathogenic distinction, or away from the Th17 cell phenotype, with or without a specific pathogenic distinction. These compositions and methods use T cell modulating agents to influence or otherwise impact the maintenance of a population of T cells, for example toward the Th17 cell phenotype, with or without a specific pathogenic distinction, or away from the

Th17 cell phenotype, with or without a specific pathogenic distinction. These compositions and methods use T cell modulating agents to influence or otherwise impact the differentiation of a population of Th17 cells, for example toward the pathogenic Th17 cell phenotype or away from the pathogenic Th17 cell phenotype, or toward the non-pathogenic Th17 cell phenotype or away from the non-pathogenic Th17 cell phenotype. These compositions and methods use T cell modulating agents to influence or otherwise impact the maintenance of a population of Th17 cells, for example toward the pathogenic Th17 cell phenotype or away from the pathogenic Th17 cell phenotype, or toward the non-pathogenic Th17 cell phenotype or away from the non-pathogenic Th17 cell phenotype. These compositions and methods use T cell modulating agents to influence or otherwise impact the differentiation of a population of T cells, for example toward a non-Th17 T cell subset or away from a non-Th17 cell subset. These compositions and methods use T cell modulating agents to influence or otherwise impact the maintenance of a population of T cells, for example toward a non-Th17 T cell subset or away from a non-Th17 cell subset.

[00103] As used herein, terms such as "pathogenic Th17 cell" and/or "pathogenic Th17 phenotype" and all grammatical variations thereof refer to Th17 cells that, when induced in the presence of TGF-β3, express an elevated level of one or more genes selected from Cxcl3, IL22, IL3, Ccl4, Gzmb, Lrmp, Ccl5, Casp1, Csf2, Ccl3, Tbx21, Icos, IL17r, Stat4, Lgals3 and Lag, as compared to the level of expression in a TGF-β3-induced Th17 cells. As used herein, terms such as "non-pathogenic Th17 cell" and/or "non-pathogenic Th17 phenotype" and all grammatical variations thereof refer to Th17 cells that, when induced in the presence of TGF-β3, express a decreased level of one or more genes selected from IL6st, IL1rn, Ikzf3, Maf, Ahr, IL9 and IL10, as compared to the level of expression in a TGF-β3-induced Th17 cells.

[00104] These compositions and methods use T cell modulating agents to influence or otherwise impact the function and/or biological activity of a T cell or T cell population. These compositions and methods use T cell modulating agents to influence or otherwise impact the function and/or biological activity of a helper T cell or helper T cell population. These compositions and methods use T cell modulating agents to influence or otherwise impact the function and/or biological activity of a Th17 cell or Th17 cell population. These compositions and methods use T cell modulating agents to influence or otherwise impact the function and/or biological activity of a non-Th17 T cell or non-Th17 T cell population, such as, for

example, a Treg cell or Treg cell population, or another CD4+ T cell or CD4+ T cell population. These compositions and methods use T cell modulating agents to influence or otherwise impact the plasticity of a T cell or T cell population, *e.g.*, by converting Th17 cells into a different subtype, or into a new state.

[00105] The methods provided herein combine transcriptional profiling at high temporal resolution, novel computational algorithms, and innovative nanowire-based tools for performing perturbations in primary T cells to systematically derive and experimentally validate a model of the dynamic regulatory network that controls Th17 differentiation. *See e.g.*, Yosef et al., "Dynamic regulatory network controlling Th17 cell differentiation, Nature, vol. 496: 461-468 (2013)/doi: 10.1038/nature11981, the contents of which are hereby incorporated by reference in their entirety. The network consists of two self-reinforcing, but mutually antagonistic, modules, with novel regulators, whose coupled action may be essential for maintaining the level and/or balance between Th17 and other CD4+ T cell subsets. Overall, 9,159 interactions between 71 regulators and 1,266 genes were active in at least one network; 46 of the 71 are novel. The examples provided herein identify and validate 39 regulatory factors, embedding them within a comprehensive temporal network and reveals its organizational principles, and highlights novel drug targets for controlling Th17 differentiation.

[00106] A "Th17-negative" module includes regulators such as SP4, ETS2, IKZF4, TSC22D3 and/or, IRF1. It was found that the transcription factor Tsc22d3, which acts as a negative regulator of a defined subtype of Th17 cells, co-localizes on the genome with key Th17 regulators. The "Th17 positive" module includes regulators such as MINA, PML, POU2AF1, PROCR, SMARCA4, ZEB1, EGR2, CCR6, and/or FAS. Perturbation of the chromatin regulator Mina was found to up-regulate Foxp3 expression, perturbation of the co-activator Pou2af1 was found to up-regulate IFN-γ production in stimulated naïve cells, and perturbation of the TNF receptor Fas was found to up-regulate IL-2 production in stimulated naïve cells. All three factors also control IL-17 production in Th17 cells.

[00107] The immune system must strike a balance between mounting proper responses to pathogens and avoiding uncontrolled, autoimmune reaction. Pro-inflammatory IL-17-producing Th17 cells are a prime case in point: as a part of the adaptive immune system, Th17 cells mediate clearance of fungal infections, but they are also strongly implicated in the

pathogenesis of autoimmunity (Korn et al., 2009). In mice, although Th17 cells are present at sites of tissue inflammation and autoimmunity (Korn et al., 2009), they are also normally present at mucosal barrier sites, where they maintain barrier functions without inducing tissue inflammation (Blaschitz and Raffatellu, 2010). In humans, functionally distinct Th17 cells have been described; for instance, Th17 cells play a protective role in clearing different types of pathogens like *Candida albicans* (Hernandez-Santos and Gaffen, 2012) or *Staphylococcus aureus* (Lin et al., 2009), and promote barrier functions at the mucosal surfaces (Symons et al., 2012), despite their pro-inflammatory role in autoimmune diseases such as rheumatoid arthritis, multiple sclerosis, psoriasis systemic lupus erythematous and asthma (Waite and Skokos, 2012). Thus, there is considerable diversity in the biological function of Th17 cells and in their ability to induce tissue inflammation or provide tissue protection.

[00108] Mirroring this functional diversity, depending on the cytokines used for differentiation, *in vitro* polarized Th17 cells can either cause severe autoimmune responses upon adoptive transfer ('pathogenic Th17 cells') or have little or no effect in inducing autoimmune disease ('non-pathogenic cells') (Ghoreschi et al., 2010; Lee et al., 2012). *In vitro* differentiation of naïve CD4 T cells in the presence of TGF-β1+IL-6 induces an IL-17A and IL-10 producing population of Th17 cells, that are generally nonpathogenic, whereas activation of naïve T cells in the presence IL-1β+IL-6+IL-23 induces a T cell population that produces IL-17A and IFN-γ, and are potent inducers of autoimmune disease induction (Ghoreschi et al., 2010).

[00109] Charting this functional heterogeneity of Th17 cells to understand the molecular circuits that control it is thus of both fundamental and clinical importance. Previous transcriptional profiling studies have identified sets of genes, dubbed 'pathogenicity signatures', that consist of genes differentially expressed between 'pathogenic' *vs.* 'non-pathogenic' *in vitro* differentiated Th17 cells (Ghoreschi et al., 2010; Lee et al., 2012). However, such studies relied either on genomic profiling of cell populations, which are limited in their ability to detect distinct cellular states within a cell mixture, or on tracking a handful of pre-selected markers by fluorescence-based flow cytometry (Perfetto et al., 2004), which cannot discover novel molecular factors that regulate Th17 cell function. Emerging technological and computational approaches for single-cell RNA-seq (Shalek et al., 2013; Shalek et al., 2014; Trapnell et al., 2014) have opened up the exciting possibility of a more

unbiased and principled interrogation into the regulatory circuits underlying different cell states. Single-cell RNA-seq also facilitates the genomic study of samples with limited cell availability, such as *in vivo* derived Th17 cells from the sites of tissue inflammation during an autoimmune reaction.

[00110] Here, single-cell RNA-seq was performed of 806 mouse Th17 cells from *in vivo* and *in vitro* models and computationally analyzed the data to dissect the molecular basis of different functional Th17 cell states. It was found that Th17 cells isolated from the draining LNs and CNS at the peak of EAE span a spectrum of states ranging from self renewing cells in the LN to Th1-like effector/memory cells and a dysfunctional, senescent-like cell phenotype in the CNS. *In vitro* polarized Th17 cells also spanned a pathogenicity spectrum from potentially pathogenic to more regulatory cells. Genes associated with these opposing states include not only canonical regulators that were identified at a population level, but also novel candidates that have not been previously detected by population-level expression approaches (Ciofani et al., 2012; Yosef et al., 2013), which were prioritized for functional analysis. Testing four high-ranking candidates – *Gpr65*, *Plzp*, *Toso* and *Cd5l* – with knockout mice, substantial effects were found both on *in vitro* Th17-cell differentiation and on the development of EAE *in vivo*. This work provides novel insights into Th17 cellular and functional states *in vivo* leading to the discovery of novel regulators for targeted manipulation of pathogenic functions of Th17 cells in autoimmune disease.

[00111] The T cell modulating agents are used to modulate the expression of one or more target genes or one or more products of one or more target genes that have been identified as genes responsive to Th17-related perturbations. These target genes are identified, for example, by contacting a T cell, *e.g.*, naïve T cells, partially differentiated T cells, differentiated T cells and/or combinations thereof, with a T cell modulating agent and monitoring the effect, if any, on the expression of one or more signature genes or one or more products of one or more signature genes. In some embodiments, the one or more signature genes are selected from those listed in Table 1 or 2 of WO/2014/134351, incorporated herein by reference (alone or with those of other herein disclosed methods).

[00112] In some embodiments, the target gene is one or more Th17-associated cytokine(s) or receptor molecule(s) selected from those listed in Table 3 of WO/2014/134351, incorporated herein by reference (alone or with those of other herein disclosed methods). In some

embodiments, the target gene is one or more Th17-associated transcription regulator(s) selected from those shown in Table S3 (Gaublomme 2015) or Table 4 of WO/2014/134351, incorporated herein by reference (alone or with those of other herein disclosed methods).

[00113] In some embodiments, the target gene is one or more Th17-associated transcription regulator(s) selected from those shown in Table S3 (Gaublomme 2015) or Table 5 of WO/2014/134351, incorporated herein by reference (alone or with those of other herein disclosed methods). In some embodiments, the target gene is one or more Th17-associated receptor molecule(s) selected from those listed in Table 6 of WO/2014/134351, incorporated herein by reference (alone or with those of other herein disclosed methods). In some embodiments, the target gene is one or more Th17-associated kinase(s) selected from those listed in Table 7 of WO/2014/134351, incorporated herein by reference (alone or with those of other herein disclosed methods). In some embodiments, the target gene is one or more Th17-associated signaling molecule(s) selected from those listed in Table 8 of WO/2014/134351, incorporated herein by reference (alone or with those of other herein disclosed methods). In some embodiments, the target gene is one or more Th17-associated receptor molecule(s) selected from those listed in Table 9 of WO/2014/134351, incorporated herein by reference (alone or with those of other herein disclosed methods).

Automated Procedure for Selection of Signature Genes

[00114] The invention also provides methods of determining gene signatures that are useful in various therapeutic and/or diagnostic indications. The goal of these methods is to select a small signature of genes that will be informative with respect to a process of interest. The basic concept is that different types of information can entail different partitions of the "space" of the entire genome (>20k genes) into subsets of associated genes. This strategy is designed to have the best coverage of these partitions, given the constraint on the signature size. For instance, in some embodiments of this strategy, there are two types of information: (i) temporal expression profiles; and (ii) functional annotations. The first information source partitions the genes into sets of co-expressed genes. The information source partitions the genes into sets of co-functional genes. A small set of genes is then selected such that there are a desired number of representatives from each set, for example, at least 10 representatives from each co-expression set and at least 10 representatives from each co-functional set. The problem of working with multiple sources of information (and thus aiming to "cover" multiple partitions) is known in the

theory of computer science as Set-Cover. While this problem cannot be solved to optimality (due to its NP-hardness) it can be approximated to within a small factor. In some embodiments, the desired number of representatives from each set is one or more, at least 2, 5 or more, 10 or more, 15 or more, 20 or more, 25 or more, 30 or more, 35 or more, 40 or more, 50 or more, 60 or more, 70 or more, 80 or more, 90 or more, or 100 or more.

[00115] An important feature of this approach is that it can be given either the size of the signature (and then find the best coverage it can under this constraint); or the desired level of coverage (and then select the minimal signature size that can satisfy the coverage demand).

[00116] An exemplary embodiment of this procedure is the selection of the 275-gene signature (Table 1 of WO/2014/134351, incorporated herein by reference), which combined several criteria to reflect as many aspect of the differentiation program as was possible. The following requirements were defined: (1) the signature must include all of the TFs that belong to a Th17 microarray signature (comparing to other CD4+ T cells, *see e.g.*, Wei et al., in Immunity vol. 30 155-167 (2009)), see Methods in WO/2014/134351, incorporated herein by reference); that are included as regulators in the network and are at least slightly differentially expressed; or that are strongly differentially expressed; (2) it must include at least 10 representatives from each cluster of genes that have similar expression profiles; (3) it must contain at least 5 representatives from the predicted targets of each TF in the different networks; (4) it must include a minimal number of representatives from each enriched Gene Ontology (GO) category (computed over differentially expressed genes); and, (5) it must include a manually assembled list of ~100 genes that are related to the differentiation process, including the differentially expressed cytokines, receptor molecules and other cell surface molecules. Since these different criteria might generate substantial overlaps, a set-cover algorithm was used to find the smallest subset of genes that satisfies all of five conditions. 18 genes whose expression showed no change (in time or between treatments) in the microarray data were added to this list.

Use of Signature Genes

[00117] The invention provides T cell related gene signatures for use in a variety of diagnostic and/or therapeutic indications. For example, the invention provides Th17 related signatures that are useful in a variety of diagnostic and/or therapeutic indications. "Signatures" in the context of the present invention encompasses, without limitation nucleic acids, together

with their polymorphisms, mutations, variants, modifications, subunits, fragments, and other analytes or sample-derived measures.

[00118] Exemplary signatures are shown in Tables 1 and 2 of WO/2014/134351, incorporated herein by reference, and are collectively referred to herein as, *inter alia*, "Th17-associated genes," "Th17-associated nucleic acids," "signature genes," or "signature nucleic acids." These signatures are useful in methods of diagnosing, prognosing and/or staging an immune response in a subject by detecting a first level of expression, activity and/or function of one or more signature genes or one or more products of one or more signature genes selected from those listed in Table 1 or 2 of WO/2014/134351, incorporated herein by reference, and comparing the detected level to a control of level of signature gene or gene product expression, activity and/or function, wherein a difference in the detected level and the control level indicates that the presence of an immune response in the subject.

[00119] These signatures are useful in methods of monitoring an immune response in a subject by detecting a level of expression, activity and/or function of one or more signature genes or one or more products of one or more signature genes selected from those listed in Table 1 or 2 of WO/2014/134351, incorporated herein by reference, at a first time point, detecting a level of expression, activity and/or function of one or more signature genes or one or more products of one or more signature genes selected from those listed in Table 1 or 2 of WO/2014/134351, incorporated herein by reference, at a second time point, and comparing the first detected level of expression, activity and/or function with the second detected level of expression, activity and/or function, wherein a change in the first and second detected levels indicates a change in the immune response in the subject.

[00120] These signatures are useful in methods of identifying patient populations at risk or suffering from an immune response based on a detected level of expression, activity and/or function of one or more signature genes or one or more products of one or more signature genes selected from those listed in Table 1 or Table 2 of WO/2014/134351, incorporated herein by reference,. These signatures are also useful in monitoring subjects undergoing treatments and therapies for aberrant immune response(s) to determine efficaciousness of the treatment or therapy. These signatures are also useful in monitoring subjects undergoing treatments and therapies for aberrant immune response(s) to determine whether the patient is responsive to the treatment or therapy. These signatures are also useful for selecting or modifying therapies and

treatments that would be efficacious in treating, delaying the progression of or otherwise ameliorating a symptom of an aberrant immune response. The signatures provided herein are useful for selecting a group of patients at a specific state of a disease with accuracy that facilitates selection of treatments.

[00121] The present invention also comprises a kit with a detection reagent that binds to one or more signature nucleic acids. Also provided by the invention is an array of detection reagents, *e.g.*, oligonucleotides that can bind to one or more signature nucleic acids. Suitable detection reagents include nucleic acids that specifically identify one or more signature nucleic acids by having homologous nucleic acid sequences, such as oligonucleotide sequences, complementary to a portion of the signature nucleic acids packaged together in the form of a kit. The oligonucleotides can be fragments of the signature genes. For example the oligonucleotides can be 200, 150, 100, 50, 25, 10 or fewer nucleotides in length. The kit may contain in separate container or packaged separately with reagents for binding them to the matrix), control formulations (positive and/or negative), and/or a detectable label such as fluorescein, green fluorescent protein, rhodamine, cyanine dyes, Alexa dyes, luciferase, radiolabels, among others. Instructions (*e.g.*, written, tape, VCR, CD-ROM, etc.) for carrying out the assay may be included in the kit. The assay may for example be in the form of a Northern hybridization or DNA chips or a sandwich ELISA or any other method as known in the art. Alternatively, the kit contains a nucleic acid substrate array comprising one or more nucleic acid sequences.

Use of T Cell Modulating Agents

[00122] Suitable T cell modulating agent(s) for use in any of the compositions and methods provided herein include an antibody, a soluble polypeptide, a polypeptide agent, a peptide agent, a nucleic acid agent, a nucleic acid ligand, or a small molecule agent. By way of non-limiting example, suitable T cell modulating agents or agents for use in combination with one or more T cell modulating agents are shown in Table 10 of WO/2014/134351, incorporated herein by reference.

[00123] It will be appreciated that administration of therapeutic entities in accordance with the invention will be administered with suitable carriers, excipients, and other agents that are incorporated into formulations to provide improved transfer, delivery, tolerance, and the like. A multitude of appropriate formulations can be found in the formulary known to all pharmaceutical chemists: Remington's Pharmaceutical Sciences (15th ed, Mack Publishing

Company, Easton, PA (1975)), particularly Chapter 87 by Blaug, Seymour, therein. These formulations include, for example, powders, pastes, ointments, jellies, waxes, oils, lipids, lipid (cationic or anionic) containing vesicles (such as Lipofectin™), DNA conjugates, anhydrous absorption pastes, oil-in- water and water-in-oil emulsions, emulsions carbowax (polyethylene glycols of various molecular weights), semi-solid gels, and semi-solid mixtures containing carbowax. Any of the foregoing mixtures may be appropriate in treatments and therapies in accordance with the present invention, provided that the active ingredient in the formulation is not inactivated by the formulation and the formulation is physiologically compatible and tolerable with the route of administration. *See* also Baldrick P. "Pharmaceutical excipient development: the need for preclinical guidance." Regul. Toxicol Pharmacol. 32(2):210-8 (2000), Wang W. "Lyophilization and development of solid protein pharmaceuticals." Int. J. Pharm. 203(1-2):1-60 (2000), Charman WN "Lipids, lipophilic drugs, and oral drug delivery-some emerging concepts." J Pharm Sci. 89(8):967-78 (2000), Powell *et al.* "Compendium of excipients for parenteral formulations" PDA J Pharm Sci Technol. 52:238-311 (1998) and the citations therein for additional information related to formulations, excipients and carriers well known to pharmaceutical chemists.

[00124] Therapeutic formulations of the invention, which include a T cell modulating agent, are used to treat or alleviate a symptom associated with an immune-related disorder or an aberrant immune response. The present invention also provides methods of treating or alleviating a symptom associated with an immune-related disorder or an aberrant immune response. A therapeutic regimen is carried out by identifying a subject, *e.g.*, a human patient suffering from (or at risk of developing) an immune-related disorder or aberrant immune response, using standard methods. For example, T cell modulating agents are useful therapeutic tools in the treatment of autoimmune diseases and/or inflammatory disorders. In certain embodiments, the use of T cell modulating agents that modulate, *e.g.*, inhibit, neutralize, or interfere with, Th17 T cell differentiation is contemplated for treating autoimmune diseases and/or inflammatory disorders. In certain embodiments, the use of T cell modulating agents that modulate, *e.g.*, enhance or promote, Th17 T cell differentiation is contemplated for augmenting Th17 responses, for example, against certain pathogens and other infectious diseases. The T cell modulating agents are also useful therapeutic tools in various transplant indications, for example, to prevent, delay or otherwise mitigate transplant rejection and/or

prolong survival of a transplant, as it has also been shown that in some cases of transplant rejection, Th17 cells might also play an important role. (*See e.g.*, Abadja F, Sarraj B, Ansari MJ., "Significance of T helper 17 immunity in transplantation." Curr Opin Organ Transplant. 2012 Feb;17(1):8-14. doi: 10.1097/MOT.0b013e32834ef4e4). The T cell modulating agents are also useful therapeutic tools in cancers and/or anti-tumor immunity, as Th17/Treg balance has also been implicated in these indications. For example, some studies have suggested that IL-23 and Th17 cells play a role in some cancers, such as, by way of non-limiting example, colorectal cancers. (*See e.g.*, Ye J, Livergood RS, Peng G. "The role and regulation of human Th17 cells in tumor immunity." Am J Pathol. 2013 Jan;182(1):10-20. doi: 10.1016/j.ajpath.2012.08.041. Epub 2012 Nov 14). The T cell modulating agents are also useful in patients who have genetic defects that exhibit aberrant Th17 cell production, for example, patients that do not produce Th17 cells naturally.

[00125] The T cell modulating agents are also useful in vaccines and/or as vaccine adjuvants against autoimmune disorders, inflammatory diseases, etc. The combination of adjuvants for treatment of these types of disorders are suitable for use in combination with a wide variety of antigens from targeted self-antigens, *i.e.*, autoantigens, involved in autoimmunity, *e.g.*, myelin basic protein; inflammatory self-antigens, *e.g.*, amyloid peptide protein, or transplant antigens, *e.g.*, alloantigens. The antigen may comprise peptides or polypeptides derived from proteins, as well as fragments of any of the following: saccharides, proteins, polynucleotides or oligonucleotides, autoantigens, amyloid peptide protein, transplant antigens, allergens, or other macromolecular components. In some instances, more than one antigen is included in the antigenic composition.

[00126] Autoimmune diseases include, for example, Acquired Immunodeficiency Syndrome (AIDS, which is a viral disease with an autoimmune component), alopecia areata, ankylosing spondylitis, antiphospholipid syndrome, autoimmune Addison's disease, autoimmune hemolytic anemia, autoimmune hepatitis, autoimmune inner ear disease (AIED), autoimmune lymphoproliferative syndrome (ALPS), autoimmune thrombocytopenic purpura (ATP), Behcet's disease, cardiomyopathy, celiac sprue-dermatitis hepetiformis; chronic fatigue immune dysfunction syndrome (CFIDS), chronic inflammatory demyelinating polyneuropathy (CIPD), cicatricial pemphigold, cold agglutinin disease, crest syndrome, Crohn's disease, Degos' disease, dermatomyositis-juvenile, discoid lupus, essential mixed cryoglobulinemia, fibromyalgia-

fibromyositis, Graves' disease, Guillain-Barré syndrome, Hashimoto's thyroiditis, idiopathic pulmonary fibrosis, idiopathic thrombocytopenia purpura (ITP), IgA nephropathy, insulin-dependent diabetes mellitus, juvenile chronic arthritis (Still's disease), juvenile rheumatoid arthritis, Ménière's disease, mixed connective tissue disease, multiple sclerosis, myasthenia gravis, pernacious anemia, polyarteritis nodosa, polychondritis, polyglandular syndromes, polymyalgia rheumatica, polymyositis and dermatomyositis, primary agammaglobulinemia, primary biliary cirrhosis, psoriasis, psoriatic arthritis, Raynaud's phenomena, Reiter's syndrome, rheumatic fever, rheumatoid arthritis, sarcoidosis, scleroderma (progressive systemic sclerosis (PSS), also known as systemic sclerosis (SS)), Sjögren's syndrome, stiff-man syndrome, systemic lupus erythematosus, Takayasu arteritis, temporal arteritis/giant cell arteritis, ulcerative colitis, uveitis, vitiligo and Wegener's granulomatosis.

[00127] In some embodiments, T cell modulating agents are useful in treating, delaying the progression of, or otherwise ameliorating a symptom of an autoimmune disease having an inflammatory component such as an aberrant inflammatory response in a subject. In some embodiments, T cell modulating agents are useful in treating an autoimmune disease that is known to be associated with an aberrant Th17 response, *e.g.*, aberrant IL-17 production, such as, for example, multiple sclerosis (MS), psoriasis, inflammatory bowel disease, ulcerative colitis, Crohn's disease, uveitis, lupus, ankylosing spondylitis, and rheumatoid arthritis.

[00128] Inflammatory disorders include, for example, chronic and acute inflammatory disorders. Examples of inflammatory disorders include Alzheimer's disease, asthma, atopic allergy, allergy, atherosclerosis, bronchial asthma, eczema, glomerulonephritis, graft vs. host disease, hemolytic anemias, osteoarthritis, sepsis, stroke, transplantation of tissue and organs, vasculitis, diabetic retinopathy and ventilator induced lung injury.

[00129] Symptoms associated with these immune-related disorders include, for example, inflammation, fever, general malaise, fever, pain, often localized to the inflamed area, rapid pulse rate, joint pain or aches (arthralgia), rapid breathing or other abnormal breathing patterns, chills, confusion, disorientation, agitation, dizziness, cough, dyspnea, pulmonary infections, cardiac failure, respiratory failure, edema, weight gain, mucopurulent relapses, cachexia, wheezing, headache, and abdominal symptoms such as, for example, abdominal pain, diarrhea or constipation.

[00130] Efficaciousness of treatment is determined in association with any known method for diagnosing or treating the particular immune-related disorder. Alleviation of one or more symptoms of the immune-related disorder indicates that the T cell modulating agent confers a clinical benefit.

[00131] Administration of a T cell modulating agent to a patient suffering from an immune-related disorder or aberrant immune response is considered successful if any of a variety of laboratory or clinical objectives is achieved. For example, administration of a T cell modulating agent to a patient is considered successful if one or more of the symptoms associated with the immune-related disorder or aberrant immune response is alleviated, reduced, inhibited or does not progress to a further, *i.e.*, worse, state. Administration of T cell modulating agent to a patient is considered successful if the immune-related disorder or aberrant immune response enters remission or does not progress to a further, *i.e.*, worse, state.

[00132] A therapeutically effective amount of a T cell modulating agent relates generally to the amount needed to achieve a therapeutic objective. The amount required to be administered will furthermore depend on the specificity of the T cell modulating agent for its specific target, and will also depend on the rate at which an administered T cell modulating agent is depleted from the free volume other subject to which it is administered.

[00133] T cell modulating agents can be administered for the treatment of a variety of diseases and disorders in the form of pharmaceutical compositions. Principles and considerations involved in preparing such compositions, as well as guidance in the choice of components are provided, for example, in Remington: The Science And Practice Of Pharmacy 19th ed. (Alfonso R. Gennaro, et al., editors) Mack Pub. Co., Easton, Pa.: 1995; Drug Absorption Enhancement: Concepts, Possibilities, Limitations, And Trends, Harwood Academic Publishers, Langhorne, Pa., 1994; and Peptide And Protein Drug Delivery (Advances In Parenteral Sciences, Vol. 4), 1991, M. Dekker, New York.

[00134] Where polypeptide-based T cell modulating agents are used, the smallest fragment that specifically binds to the target and retains therapeutic function is preferred. Such fragments can be synthesized chemically and/or produced by recombinant DNA technology. (*See*, *e.g.*, Marasco et al., Proc. Natl. Acad. Sci. USA, 90: 7889-7893 (1993)). The formulation can also contain more than one active compound as necessary for the particular indication being treated, preferably those with complementary activities that do not adversely affect each other.

Alternatively, or in addition, the composition can comprise an agent that enhances its function, such as, for example, a cytotoxic agent, cytokine, chemotherapeutic agent, or growth-inhibitory agent. Such molecules are suitably present in combination in amounts that are effective for the purpose intended.

[00135] The invention comprehends a treatment method or Drug Discovery method or method of formulating or preparing a treatment comprising any one of the methods or uses herein discussed.

[00136] The present invention also relates to identifying molecules, advantageously small molecules or biologics, that may be involved in inhibiting one or more of the mutations in one or more genes selected from the group consisting of *Toso, advantageously Ctla2b*, *Gatm*, *Bdh2*, *Bcat1*, *Zfp36*, *Acsl4*, *Acat3*, *Adi1*, *Dot1l*, *Mett10d*, *Sirt6*, *Slc25a13*, *Chd2*, *Ino80c*, *Med21*, *Pdss1*, *Galk1*, *Gnpda2* or *Mtpap* or any one of the foregoing or any combination thereof with one or more of *Gpr65*, *Plzp* or *Cd5l* or any combination thereof *Gpr65*, *Plzp* or *Cd5l* in any combination of *Gpr65*, *Plzp*, *Toso* or *Cd5l*. The invention contemplates screening libraries of small molecules or biologics to identify compounds involved in suppressing or inhibiting expression of somatic mutations or alter the cells phenotypically so that the cells with mutations behave more normally in one or more of *Toso, advantageously Ctla2b*, *Gatm*, *Bdh2*, *Bcat1*, *Zfp36*, *Acsl4*, *Acat3*, *Adi1*, *Dot1l*, *Mett10d*, *Sirt6*, *Slc25a13*, *Chd2*, *Ino80c*, *Med21*, *Pdss1*, *Galk1*, *Gnpda2* or *Mtpap* or any one of the foregoing or any combination thereof with one or more of *Gpr65*, *Plzp* or *Cd5l* or any combination thereof *Gpr65*, *Plzp* or *Cd5l* in any combination of *Gpr65*, *Plzp*, *Toso* or *Cd5l*.

[00137] High-throughput screening (HTS) is contemplated for identifying small molecules or biologics involved in suppressing or inhibiting expression of somatic mutations in one or more of *Toso, advantageously Ctla2b*, *Gatm*, *Bdh2*, *Bcat1*, *Zfp36*, *Acsl4*, *Acat3*, *Adi1*, *Dot1l*, *Mett10d*, *Sirt6*, *Slc25a13*, *Chd2*, *Ino80c*, *Med21*, *Pdss1*, *Galk1*, *Gnpda2* or *Mtpap* or any one of the foregoing or any combination thereof with one or more of *Gpr65*, *Plzp* or *Cd5l* or any combination thereof *Gpr65*, *Plzp* or *Cd5l* in any combination of *Gpr65*, *Plzp*, *Toso* or *Cd5l*. The flexibility of the process has allowed numerous and disparate areas of biology to engage with an equally diverse palate of chemistry (see, e.g., Inglese et al., Nature Chemical Biology 3, 438 - 441 (2007)). Diverse sets of chemical libraries, containing more than 200,000 unique small molecules, as well as natural product libraries, can be screened. This includes, for example, the

Prestwick library (1,120 chemicals) of off-patent compounds selected for structural diversity, collective coverage of multiple therapeutic areas, and known safety and bioavailability in humans, as well as the NINDS Custom Collection 2 consisting of a 1,040 compound-library of mostly FDA-approved drugs (see, e.g., US Patent No. 8,557,746) are also contemplated.

[00138] The NIH's Molecular Libraries Probe Production Centers Network (MLPCN) offers access to thousands of small molecules – chemical compounds that can be used as tools to probe basic biology and advance our understanding of disease. Small molecules can help researchers understand the intricacies of a biological pathway or be starting points for novel therapeutics. The Broad Institute's Probe Development Center (BIPDeC) is part of the MLPCN and offers access to a growing library of over 330,000 compounds for large scale screening and medicinal chemistry. Any of these compounds may be utilized for screening compounds involved in suppressing or inhibiting expression of somatic mutations in one or more of *Toso, advantageously Ctla2b*, *Gatm*, *Bdh2*, *Bcat1*, *Zfp36*, *Acsl4*, *Acat3*, *Adi1*, *Dot1l*, *Mett10d*, *Sirt6*, *Slc25a13*, *Chd2*, *Ino80c*, *Med21*, *Pdss1*, *Galk1*, *Gnpda2* or *Mtpap* or any one of the foregoing or any combination thereof with one or more of *Gpr65*, *Plzp* or *Cd5l* or any combination thereof *Gpr65*, *Plzp* or *Cd5l* in any combination of *Gpr65*, *Plzp*, *Toso* or *Cd5l*.

[00139] The phrase "therapeutically effective amount" as used herein refers to a nontoxic but sufficient amount of a drug, agent, or compound to provide a desired therapeutic effect.

[00140] As used herein "patient" refers to any human being receiving or who may receive medical treatment.

[00141] A "polymorphic site" refers to a polynucleotide that differs from another polynucleotide by one or more single nucleotide changes.

[00142] A "somatic mutation" refers to a change in the genetic structure that is not inherited from a parent, and also not passed to offspring.

[00143] Therapy or treatment according to the invention may be performed alone or in conjunction with another therapy, and may be provided at home, the doctor's office, a clinic, a hospital's outpatient department, or a hospital. Treatment generally begins at a hospital so that the doctor can observe the therapy's effects closely and make any adjustments that are needed. The duration of the therapy depends on the age and condition of the patient, the stage of the a cardiovascular disease, and how the patient responds to the treatment. Additionally, a person

having a greater risk of developing a cardiovascular disease (e.g., a person who is genetically predisposed) may receive prophylactic treatment to inhibit or delay symptoms of the disease.

[00144] The medicaments of the invention are prepared in a manner known to those skilled in the art, for example, by means of conventional dissolving, lyophilizing, mixing, granulating or confectioning processes. Methods well known in the art for making formulations are found, for example, in Remington: The Science and Practice of Pharmacy, 20th ed., ed. A. R. Gennaro, 2000, Lippincott Williams & Wilkins, Philadelphia, and Encyclopedia of Pharmaceutical Technology, eds. J. Swarbrick and J. C. Boylan, 1988-1999, Marcel Dekker, New York.

[00145] Administration of medicaments of the invention may be by any suitable means that results in a compound concentration that is effective for treating or inhibiting (e.g., by delaying) the development of a cardiovascular disease. The compound is admixed with a suitable carrier substance, e.g., a pharmaceutically acceptable excipient that preserves the therapeutic properties of the compound with which it is administered. One exemplary pharmaceutically acceptable excipient is physiological saline. The suitable carrier substance is generally present in an amount of 1-95% by weight of the total weight of the medicament. The medicament may be provided in a dosage form that is suitable for oral, rectal, intravenous, intramuscular, subcutaneous, inhalation, nasal, topical or transdermal, vaginal, or ophthalmic administration. Thus, the medicament may be in form of, e.g., tablets, capsules, pills, powders, granulates, suspensions, emulsions, solutions, gels including hydrogels, pastes, ointments, creams, plasters, drenches, delivery devices, suppositories, enemas, injectables, implants, sprays, or aerosols.

[00146] In order to determine the genotype of a patient according to the methods of the present invention, it may be necessary to obtain a sample of genomic DNA from that patient. That sample of genomic DNA may be obtained from a sample of tissue or cells taken from that patient.

[00147] The tissue sample may comprise but is not limited to hair (including roots), skin, buccal swabs, blood, or saliva. The tissue sample may be marked with an identifying number or other indicia that relates the sample to the individual patient from which the sample was taken. The identity of the sample advantageously remains constant throughout the methods of the invention thereby guaranteeing the integrity and continuity of the sample during extraction and analysis. Alternatively, the indicia may be changed in a regular fashion that ensures that the data,

and any other associated data, can be related back to the patient from whom the data was obtained. The amount/size of sample required is known to those skilled in the art.

[00148] Generally, the tissue sample may be placed in a container that is labeled using a numbering system bearing a code corresponding to the patient. Accordingly, the genotype of a particular patient is easily traceable.

[00149] In one embodiment of the invention, a sampling device and/or container may be supplied to the physician. The sampling device advantageously takes a consistent and reproducible sample from individual patients while simultaneously avoiding any cross-contamination of tissue. Accordingly, the size and volume of sample tissues derived from individual patients would be consistent.

[00150] According to the present invention, a sample of DNA is obtained from the tissue sample of the patient of interest. Whatever source of cells or tissue is used, a sufficient amount of cells must be obtained to provide a sufficient amount of DNA for analysis. This amount will be known or readily determinable by those skilled in the art.

[00151] DNA is isolated from the tissue/cells by techniques known to those skilled in the art (see, e.g., U.S. Pat. Nos. 6,548,256 and 5,989,431, Hirota et al., Jinrui Idengaku Zasshi. September 1989; 34(3):217-23 and John et al., Nucleic Acids Res. Jan. 25. 1991 ;19(2):408; the disclosures of which are incorporated by reference in their entireties). For example, high molecular weight DNA may be purified from cells or tissue using proteinase K extraction and ethanol precipitation. DNA may be extracted from a patient specimen using any other suitable methods known in the art.

[00152] It is an object of the present invention to determine the genotype of a given patient of interest by analyzing the DNA from the patent, in order to identify a patient carrying specific somatic mutations of the invention that are associated with developing a cardiovascular disease. In particular, the kit may have primers or other DNA markers for identifying particular mutations such as, but not limited to, one or more genes selected from the group consisting of *Toso, advantageously Ctla2b*, *Gatm*, *Bdh2*, *Bcat1*, *Zfp36*, *Acsl4*, *Acat3*, *Adi1*, *Dot1l*, *Mett10d*, *Sirt6*, *Slc25a13*, *Chd2*, *Ino80c*, *Med21*, *Pdss1*, *Galk1*, *Gnpda2* or *Mtpap* or any one of the foregoing or any combination thereof with one or more of *Gpr65*, *Plzp* or *Cd5l* or any combination thereof *Gpr65*, *Plzp* or *Cd5l* in any combination of *Gpr65*, *Plzp*, *Toso* or *Cd5l*.

[00153] There are many methods known in the art for determining the genotype of a patient and for identifying or analyzing whether a given DNA sample contains a particular somatic mutation. Any method for determining genotype can be used for determining genotypes in the present invention. Such methods include, but are not limited to, amplimer sequencing, DNA sequencing, fluorescence spectroscopy, fluorescence resonance energy transfer (or "FRET")-based hybridization analysis, high throughput screening, mass spectroscopy, nucleic acid hybridization, polymerase chain reaction (PCR), RFLP analysis and size chromatography (e.g., capillary or gel chromatography), all of which are well known to one of skill in the art.

[00154] The methods of the present invention, such as whole exome sequencing and targeted amplicon sequencing, have commercial applications in diagnostic kits for the detection of the somatic mutations in patients. A test kit according to the invention may comprise any of the materials necessary for whole exome sequencing and targeted amplicon sequencing, for example, according to the invention. In a particular advantageous embodiment, a diagnostic for the present invention may comprise testing for any of the genes in disclosed herein. The kit further comprises additional means, such as reagents, for detecting or measuring the sequences of the present invention, and also ideally a positive and negative control.

[00155] The present invention further encompasses probes according to the present invention that are immobilized on a solid or flexible support, such as paper, nylon or other type of membrane, filter, chip, glass slide, microchips, microbeads, or any other such matrix, all of which are within the scope of this invention. The probe of this form is now called a "DNA chip". These DNA chips can be used for analyzing the somatic mutations of the present invention. The present invention further encompasses arrays or microarrays of nucleic acid molecules that are based on one or more of the sequences described herein. As used herein "arrays" or "microarrays" refers to an array of distinct polynucleotides or oligonucleotides synthesized on a solid or flexible support, such as paper, nylon or other type of membrane, filter, chip, glass slide, or any other suitable solid support. In one embodiment, the microarray is prepared and used according to the methods and devices described in U.S. Pat. Nos. 5,446,603; 5,545,531; 5,807,522; 5,837,832; 5,874,219; 6,114,122; 6,238,910; 6,365,418; 6,410,229; 6,420,114; 6,432,696; 6,475,808 and 6,489,159 and PCT Publication No. WO 01/45843 A2, the disclosures of which are incorporated by reference in their entireties.

[00156] The present invention further encompasses the analysis of lipids. Lipid profiling is a targeted metabolomics platform that provides a comprehensive analysis of lipid species within a cell or tissue. Profiling based on electrospray ionization tandem mass spectrometry (ESI-MS/MS) is capable of providing quantitative data and is adaptable to high throughput analyses. Additionally, Liquid chromatography–mass spectrometry (LC-MS, or alternatively HPLC-MS) may be used.

EXAMPLES & TECHNOLOGIES AS TO THE INSTANT INVENTION

[00157] The following examples are given for the purpose of illustrating various embodiments of the invention and are not meant to limit the present invention in any fashion. The present examples, along with the methods described herein are presently representative of preferred embodiments, are exemplary, and are not intended as limitations on the scope of the invention. Changes therein and other uses which are encompassed within the spirit of the invention as defined by the scope of the claims will occur to those skilled in the art.

[00158] In this regard, mention is made that mutations in cells and also mutated mice for use in or as to the invention can be by way of the CRISPR-Cas system or a Cas9-expressing eukaryotic cell or Cas-9 expressing eukaryote, such as a mouse. The Cas9-expressing eukaryotic cell or eukaryote, e.g., mouse, can have guide RNA delivered or administered thereto, whereby the RNA targets a loci and induces a desired mutation for use in or as to the invention. With respect to general information on CRISPR-Cas Systems, components thereof, and delivery of such components, including methods, materials, delivery vehicles, vectors, particles, and making and using thereof, including as to amounts and formulations, as well as Cas9-expressing eukaryotic cells, Cas-9 expressing eukaryotes, such as a mouse, all useful in or as to the instant invention, reference is made to: US Patents Nos. 8,697,359, 8,771,945, 8,795,965, 8,865,406, 8,871,445, 8,889,356, 8,889,418, 8,895,308, 8,932,814, 8,945,839, 8,906,616; US Patent Publications US 2014-0310830 (US APP. Ser. No. 14/105,031), US 2014-0287938 A1 (U.S. App. Ser. No. 14/213,991), US 2014-0273234 A1 (U.S. App. Ser. No. 14/293,674), US2014-0273232 A1 (U.S. App. Ser. No. 14/290,575), US 2014-0273231 (U.S. App. Ser. No. 14/259,420), US 2014-0256046 A1 (U.S. App. Ser. No. 14/226,274), US 2014-0248702 A1 (U.S. App. Ser. No. 14/258,458), US 2014-0242700 A1 (U.S. App. Ser. No. 14/222,930), US 2014-0242699 A1 (U.S. App. Ser. No. 14/183,512), US 2014-0242664 A1 (U.S. App. Ser. No. 14/104,990), US 2014-0234972 A1 (U.S. App. Ser. No. 14/183,471), US 2014-0227787 A1

(U.S. App. Ser. No. 14/256,912), US 2014-0189896 A1 (U.S. App. Ser. No. 14/105,035), US 2014-0186958 (U.S. App. Ser. No. 14/105,017), US 2014-0186919 A1 (U.S. App. Ser. No. 14/104,977), US 2014-0186843 A1 (U.S. App. Ser. No. 14/104,900), US 2014-0179770 A1 (U.S. App. Ser. No. 14/104,837) and US 2014-0179006 A1 (U.S. App. Ser. No. 14/183,486), US 2014-0170753 (US App Ser No 14/183,429); European Patents / Patent Applications: EP 2 771 468 (EP13818570.7), EP 2 764 103 (EP13824232.6), and EP 2 784 162 (EP14170383.5); and PCT Patent Publications WO 2014/093661 (PCT/US2013/074743), WO 2014/093694 (PCT/US2013/074790), WO 2014/093595 (PCT/US2013/074611), WO 2014/093718 (PCT/US2013/074825), WO 2014/093709 (PCT/US2013/074812), WO 2014/093622 (PCT/US2013/074667), WO 2014/093635 (PCT/US2013/074691), WO 2014/093655 (PCT/US2013/074736), WO 2014/093712 (PCT/US2013/074819), WO2014/093701 (PCT/US2013/074800), WO2014/018423 (PCT/US2013/051418), WO 2014/204723 (PCT/US2014/041790), WO 2014/204724 (PCT/US2014/041800), WO 2014/204725 (PCT/US2014/041803), WO 2014/204726 (PCT/US2014/041804), WO 2014/204727 (PCT/US2014/041806), WO 2014/204728 (PCT/US2014/041808), WO 2014/204729 (PCT/US2014/041809), and:

- *Multiplex genome engineering using CRISPR/Cas systems*. Cong, L., Ran, F.A., Cox, D., Lin, S., Barretto, R., Habib, N., Hsu, P.D., Wu, X., Jiang, W., Marraffini, L.A., & Zhang, F. *Science* Feb 15;339(6121):819-23 (2013);
- *RNA-guided editing of bacterial genomes using CRISPR-Cas systems*. Jiang W., Bikard D., Cox D., Zhang F, Marraffini LA. Nat Biotechnol Mar;31(3):233-9 (2013);
- *One-Step Generation of Mice Carrying Mutations in Multiple Genes by CRISPR/Cas-Mediated Genome Engineering*. Wang H., Yang H., Shivalila CS., Dawlaty MM., Cheng AW., Zhang F., Jaenisch R. Cell May 9;153(4):910-8 (2013);
- *Optical control of mammalian endogenous transcription and epigenetic states*. Konermann S, Brigham MD, Trevino AE, Hsu PD, Heidenreich M, Cong L, Platt RJ, Scott DA, Church GM, Zhang F. Nature. 2013 Aug 22;500(7463):472-6. doi: 10.1038/Nature12466. Epub 2013 Aug 23;
- *Double Nicking by RNA-Guided CRISPR Cas9 for Enhanced Genome Editing Specificity*. Ran, FA., Hsu, PD., Lin, CY., Gootenberg, JS., Konermann, S., Trevino, AE., Scott, DA., Inoue, A., Matoba, S., Zhang, Y., & Zhang, F. *Cell* Aug 28. pii: S0092-8674(13)01015-5. (2013*);*

- *DNA targeting specificity of RNA-guided Cas9 nucleases*. Hsu, P., Scott, D., Weinstein, J., Ran, FA., Konermann, S., Agarwala, V., Li, Y., Fine, E., Wu, X., Shalem, O., Cradick, TJ., Marraffini, LA., Bao, G., & Zhang, F. *Nat Biotechnol* doi:10.1038/nbt.2647 (2013);
- *Genome engineering using the CRISPR-Cas9 system*. Ran, FA., Hsu, PD., Wright, J., Agarwala, V., Scott, DA., Zhang, F. Nature Protocols Nov;8(11):2281-308. (2013);
- *Genome-Scale CRISPR-Cas9 Knockout Screening in Human Cells*. Shalem, O., Sanjana, NE., Hartenian, E., Shi, X., Scott, DA., Mikkelson, T., Heckl, D., Ebert, BL., Root, DE., Doench, JG., Zhang, F. *Science* Dec 12. (2013). [Epub ahead of print];
- *Crystal structure of cas9 in complex with guide RNA and target DNA*. Nishimasu, H., Ran, FA., Hsu, PD., Konermann, S., Shehata, SI., Dohmae, N., Ishitani, R., Zhang, F., Nureki, O. *Cell* Feb 27. (2014). 156(5):935-49;
- *Genome-wide binding of the CRISPR endonuclease Cas9 in mammalian cells*. Wu X., Scott DA., Kriz AJ., Chiu AC., Hsu PD., Dadon DB., Cheng AW., Trevino AE., Konermann S., Chen S., Jaenisch R., Zhang F., Sharp PA. *Nat Biotechnol*. (2014) Apr 20. doi: 10.1038/nbt.2889,
- *CRISPR-Cas9 Knockin Mice for Genome Editing and Cancer Modeling*, Platt et al., Cell 159(2): 440-455 (2014) DOI: 10.1016/j.cell.2014.09.014,
- *Development and Applications of CRISPR-Cas9 for Genome Engineering*, Hsu et al, Cell 157, 1262-1278 (June 5, 2014) (Hsu 2014),
- *Genetic screens in human cells using the CRISPR/Cas9 system*, Wang et al., Science. 2014 January 3; 343(6166): 80–84. doi:10.1126/science.1246981,
- *Rational design of highly active sgRNAs for CRISPR-Cas9-mediated gene inactivation*, Doench et al., Nature Biotechnology published online 3 September 2014; doi:10.1038/nbt.3026, and
- *In vivo interrogation of gene function in the mammalian brain using CRISPR-Cas9*, Swiech et al, Nature Biotechnology; published online 19 October 2014; doi:10.1038/nbt.3055,

each of which is incorporated herein by reference.

[00159] The invention involves a high-throughput single-cell RNA-Seq and/or targeted nucleic acid profiling (for example, sequencing, quantitative reverse transcription polymerase chain reaction, and the like) where the RNAs from different cells are tagged individually, allowing a single library to be created while retaining the cell identity of each read. In this regard, technology of US provisional patent application serial no. 62/048,227 filed September 9, 2014, the disclosure of which is incorporated by reference, may be used in or as to the invention.

A combination of molecular barcoding and emulsion-based microfluidics to isolate, lyse, barcode, and prepare nucleic acids from individual cells in high-throughput is used. Microfluidic devices (for example, fabricated in polydimethylsiloxane), sub-nanoliter reverse emulsion droplets. These droplets are used to co-encapsulate nucleic acids with a barcoded capture bead. Each bead, for example, is uniquely barcoded so that each drop and its contents are distinguishable. The nucleic acids may come from any source known in the art, such as for example, those which come from a single cell, a pair of cells, a cellular lysate, or a solution. The cell is lysed as it is encapsulated in the droplet. To load single cells and barcoded beads into these droplets with Poisson statistics, 100,000 to 10 million such beads are needed to barcode ~10,000-100,000 cells. In this regard there can be a single-cell sequencing library which may comprise: merging one uniquely barcoded mRNA capture microbead with a single-cell in an emulsion droplet having a diameter of 75-125 μm; lysing the cell to make its RNA accessible for capturing by hybridization onto RNA capture microbead; performing a reverse transcription either inside or outside the emulsion droplet to convert the cell's mRNA to a first strand cDNA that is covalently linked to the mRNA capture microbead; pooling the cDNA-attached microbeads from all cells; and preparing and sequencing a single composite RNA-Seq library. Accordingly, it is envisioned as to or in the practice of the invention provides that there can be a method for preparing uniquely barcoded mRNA capture microbeads, which has a unique barcode and diameter suitable for microfluidic devices which may comprise: 1) performing reverse phosphoramidite synthesis on the surface of the bead in a pool-and-split fashion, such that in each cycle of synthesis the beads are split into four reactions with one of the four canonical nucleotides (T, C, G, or A) or unique oligonucleotides of length two or more bases; 2) repeating this process a large number of times, at least six, and optimally more than twelve, such that, in the latter, there are more than 16 million unique barcodes on the surface of each bead in the pool. (See www.ncbi.nlm.nih.gov/pmc/articles/PMC206447). Likewise, in or as to the instant invention there can be an apparatus for creating a single-cell sequencing library via a microfluidic system, which may comprise: an oil-surfactant inlet which may comprise a filter and a carrier fluid channel, wherein said carrier fluid channel further may comprise a resistor; an inlet for an analyte which may comprise a filter and a carrier fluid channel, wherein said carrier fluid channel may further comprise a resistor; an inlet for mRNA capture microbeads and lysis reagent which may comprise a filter and a carrier fluid channel, wherein said carrier fluid

channel may further comprise a resistor; said carrier fluid channels have a carrier fluid flowing therein at an adjustable or predetermined flow rate; wherein each said carrier fluid channels merge at a junction; and said junction being connected to a mixer, which contains an outlet for drops. Similarly, as to or in the practice of the instant invention there can be a method for creating a single-cell sequencing library which may comprise: merging one uniquely barcoded RNA capture microbead with a single-cell in an emulsion droplet having a diameter of 125 μm lysing the cell thereby capturing the RNA on the RNA capture microbead; performing a reverse transcription either after breakage of the droplets and collection of the microbeads; or inside the emulsion droplet to convert the cell's RNA to a first strand cDNA that is covalently linked to the RNA capture microbead; pooling the cDNA-attached microbeads from all cells; and preparing and sequencing a single composite RNA-Seq library; and, the emulsion droplet can be between 50-210 μm. In a further embodiment, the method wherein the diameter of the mRNA capture microbeads is from 10 μm to 95 μm. Thus, the practice of the instant invention comprehends preparing uniquely barcoded mRNA capture microbeads, which has a unique barcode and diameter suitable for microfluidic devices which may comprise: 1) performing reverse phosphoramidite synthesis on the surface of the bead in a pool-and-split fashion, such that in each cycle of synthesis the beads are split into four reactions with one of the four canonical nucleotides (T,C,G,or A); 2) repeating this process a large number of times, at least six, and optimally more than twelve, such that, in the latter, there are more than 16 million unique barcodes on the surface of each bead in the pool. The covalent bond can be polyethylene glycol. The diameter of the mRNA capture microbeads can be from 10 μm to 95 μm. Accordingly, it is also envisioned as to or in the practice of the invention that there can be a method for preparing uniquely barcoded mRNA capture microbeads, which has a unique barcode and diameter suitable for microfluidic devices which may comprise: 1) performing reverse phosphoramidite synthesis on the surface of the bead in a pool-and-split fashion, such that in each cycle of synthesis the beads are split into four reactions with one of the four canonical nucleotides (T, C, G, or A); 2) repeating this process a large number of times, at least six, and optimally more than twelve, such that, in the latter, there are more than 16 million unique barcodes on the surface of each bead in the pool. And, the diameter of the mRNA capture microbeads can be from 10 μm to 95 μm. Further, as to in the practice of the invention there can be an apparatus for creating a composite single-cell sequencing library via a microfluidic system, which may comprise: an oil-

surfactant inlet which may comprise a filter and two carrier fluid channels, wherein said carrier fluid channel further may comprise a resistor; an inlet for an analyte which may comprise a filter and two carrier fluid channels, wherein said carrier fluid channel further may comprise a resistor; an inlet for mRNA capture microbeads and lysis reagent which may comprise a carrier fluid channel; said carrier fluid channels have a carrier fluid flowing therein at an adjustable and predetermined flow rate; wherein each said carrier fluid channels merge at a junction; and said junction being connected to a constriction for droplet pinch-off followed by a mixer, which connects to an outlet for drops. The analyte may comprise a chemical reagent, a genetically perturbed cell, a protein, a drug, an antibody, an enzyme, a nucleic acid, an organelle like the mitochondrion or nucleus, a cell or any combination thereof. In an embodiment of the apparatus the analyte is a cell. In a further embodiment the cell is a brain cell. In an embodiment of the apparatus the lysis reagent may comprise an anionic surfactant such as sodium lauroyl sarcosinate, or a chaotropic salt such as guanidinium thiocyanate. The filter can involve square PDMS posts; e.g., with the filter on the cell channel of such posts with sides ranging between 125-135 μm with a separation of 70-100 mm between the posts. The filter on the oil-surfactant inlet may comprise square posts of two sizes; one with sides ranging between 75-100 μm and a separation of 25-30 μm between them and the other with sides ranging between 40-50 μm and a separation of 10-15 μm. The apparatus can involve a resistor, e.g., a resistor that is serpentine having a length of 7000 - 9000 μm, width of 50 - 75 μm and depth of 100 - 150 mm. The apparatus can have channels having a length of 8000 - 12,000 μm for oil-surfactant inlet, 5000-7000 for analyte (cell) inlet, and 900 - 1200 μm for the inlet for microbead and lysis agent; and/or all channels having a width of 125 - 250 mm, and depth of 100 - 150 mm. The width of the cell channel can be 125-250 μm and the depth 100-150 μm. The apparatus can include a mixer having a length of 7000-9000 μm, and a width of 110-140 μm with 35-45o zig-zigs every 150 μm. The width of the mixer can be about 125 μm. The oil-surfactant can be a PEG Block Polymer, such as BIORAD™ QX200 Droplet Generation Oil. The carrier fluid can be a water-glycerol mixture. In the practice of the invention or as to the invention, a mixture may comprise a plurality of microbeads adorned with combinations of the following elements: bead-specific oligonucleotide barcodes; additional oligonucleotide barcode sequences which vary among the oligonucleotides on an indvidual bead and can therefore be used to differentiate or help identify those individual oligonucleotide molecules; additional oligonucleotide sequences that create

substrates for downstream molecular-biological reactions, such as oligo-dT (for reverse transcription of mature mRNAs), specific sequences (for capturing specific portions of the transcriptome, or priming for DNA polymerases and similar enzymes), or random sequences (for priming throughout the transcriptome or genome). The individual oligonucleotide molecules on the surface of any individual microbead may contain all three of these elements, and the third element may include both oligo-dT and a primer sequence. A mixture may comprise a plurality of microbeads, wherein said microbeads may comprise the following elements: at least one bead-specific oligonucleotide barcode; at least one additional identifier oligonucleotide barcode sequence, which varies among the oligonucleotides on an individual bead, and thereby assisting in the identification and of the bead specific oligonucleotide molecules; optionally at least one additional oligonucleotide sequences, which provide substrates for downstream molecular-biological reactions. A mixture may comprise at least one oligonucleotide sequence(s), which provide for substrates for downstream molecular-biological reactions. In a further embodiment the downstream molecular biological reactions are for reverse transcription of mature mRNAs; capturing specific portions of the transcriptome, priming for DNA polymerases and/or similar enzymes; or priming throughout the transcriptome or genome. The mixture may involve additional oligonucleotide sequence(s) which may comprise a oligio-dT sequence. The mixture further may comprise the additional oligonucleotide sequence which may comprise a primer sequence. The mixture may further comprise the additional oligonucleotide sequence which may comprise a oligo-dT sequence and a primer sequence. Examples of the labeling substance which may be employed include labeling substances known to those skilled in the art, such as fluorescent dyes, enzymes, coenzymes, chemiluminescent substances, and radioactive substances. Specific examples include radioisotopes (e.g., 32P, 14C, 125I, 3H, and 131I), fluorescein, rhodamine, dansyl chloride, umbelliferone, luciferase, peroxidase, alkaline phosphatase, β-galactosidase, β-glucosidase, horseradish peroxidase, glucoamylase, lysozyme, saccharide oxidase, microperoxidase, biotin, and ruthenium. In the case where biotin is employed as a labeling substance, preferably, after addition of a biotin-labeled antibody, streptavidin bound to an enzyme (e.g., peroxidase) is further added. Advantageously, the label is a fluorescent label. Examples of fluorescent labels include, but are not limited to, Atto dyes, 4-acetamido-4'-isothiocyanatostilbene-2,2'disulfonic acid; acridine and derivatives: acridine, acridine isothiocyanate; 5-(2'-aminoethyl)aminonaphthalene-1-sulfonic acid (EDANS); 4-amino-N-[3-

vinylsulfonyl)phenyl]naphthalimide-3,5 disulfonate; N-(4-anilino-1-naphthyl)maleimide; anthranilamide; BODIPY; Brilliant Yellow; coumarin and derivatives; coumarin, 7-amino-4-methylcoumarin (AMC, Coumarin 120), 7-amino-4-trifluoromethylcouluarin (Coumaran 151); cyanine dyes; cyanosine; 4',6-diaminidino-2-phenylindole (DAPI); 5'5"-dibromopyrogallol-sulfonaphthalein (Bromopyrogallol Red); 7-diethylamino-3-(4'-isothiocyanatophenyl)-4-methylcoumarin; diethylenetriamine pentaacetate; 4,4'-diisothiocyanatodihydro-stilbene-2,2'-disulfonic acid; 4,4'-diisothiocyanatostilbene-2,2'-disulfonic acid; 5-[dimethylamino]naphthalene-1-sulfonyl chloride (DNS, dansylchloride); 4-dimethylaminophenylazophenyl-4'-isothiocyanate (DABITC); eosin and derivatives; eosin, eosin isothiocyanate, erythrosin and derivatives; erythrosin B, erythrosin, isothiocyanate; ethidium; fluorescein and derivatives; 5-carboxyfluorescein (FAM), 5-(4,6-dichlorotriazin-2-yl)aminofluorescein (DTAF), 2',7'-dimethoxy-4'5'-dichloro-6-carboxyfluorescein, fluorescein, fluorescein isothiocyanate, QFITC, (XRITC); fluorescamine; IR144; IR1446; Malachite Green isothiocyanate; 4-methylumbelliferoneortho cresolphthalein; nitrotyrosine; pararosaniline; Phenol Red; B-phycoerythrin; o-phthaldialdehyde; pyrene and derivatives: pyrene, pyrene butyrate, succinimidyl 1-pyrene; butyrate quantum dots; Reactive Red 4 (Cibacron.TM. Brilliant Red 3B-A) rhodamine and derivatives: 6-carboxy-X-rhodamine (ROX), 6-carboxyrhodamine (R6G), lissamine rhodamine B sulfonyl chloride rhodamine (Rhod), rhodamine B, rhodamine 123, rhodamine X isothiocyanate, sulforhodamine B, sulforhodamine 101, sulfonyl chloride derivative of sulforhodamine 101 (Texas Red); N,N,N',N' tetramethyl-6-carboxyrhodamine (TAMRA); tetramethyl rhodamine; tetramethyl rhodamine isothiocyanate (TRITC); riboflavin; rosolic acid; terbium chelate derivatives; Cy3; Cy5; Cy5.5; Cy7; IRD 700; IRD 800; La Jolta Blue; phthalo cyanine; and naphthalo cyanine. A fluorescent label may be a fluorescent protein, such as blue fluorescent protein, cyan fluorescent protein, green fluorescent protein, red fluorescent protein, yellow fluorescent protein or any photoconvertible protein. Colormetric labeling, bioluminescent labeling and/or chemiluminescent labeling may further accomplish labeling. Labeling further may include energy transfer between molecules in the hybridization complex by perturbation analysis, quenching, or electron transport between donor and acceptor molecules, the latter of which may be facilitated by double stranded match hybridization complexes. The fluorescent label may be a perylene or a terrylen. In the alternative, the fluorescent label may be a fluorescent bar code. Advantageously, the label may be light

sensitive, wherein the label is light-activated and/or light cleaves the one or more linkers to release the molecular cargo. The light-activated molecular cargo may be a major light-harvesting complex (LHCII). In another embodiment, the fluorescent label may induce free radical formation. Advantageously, agents may be uniquely labeled in a dynamic manner (see, e.g., US provisional patent application serial no. 61/703,884 filed September 21, 2012). The unique labels are, at least in part, nucleic acid in nature, and may be generated by sequentially attaching two or more detectable oligonucleotide tags to each other and each unique label may be associated with a separate agent. A detectable oligonucleotide tag may be an oligonucleotide that may be detected by sequencing of its nucleotide sequence and/or by detecting non-nucleic acid detectable moieties to which it may be attached. Oligonucleotide tags may be detectable by virtue of their nucleotide sequence, or by virtue of a non-nucleic acid detectable moiety that is attached to the oligonucleotide such as but not limited to a fluorophore, or by virtue of a combination of their nucleotide sequence and the nonnucleic acid detectable moiety. A detectable oligonucleotide tag may comprise one or more nonoligonucleotide detectable moieties. Examples of detectable moieties may include, but are not limited to, fluorophores, microparticles including quantum dots (Empodocles, et al., Nature 399:126-130, 1999), gold nanoparticles (Reichert et al., Anal. Chem. 72:6025-6029, 2000), microbeads (Lacoste et al., Proc. Natl. Acad. Sci. USA 97(17):9461-9466, 2000), biotin, DNP (dinitrophenyl), fucose, digoxigenin, haptens, and other detectable moieties known to those skilled in the art. In some embodiments, the detectable moieties may be quantum dots. Methods for detecting such moieties are described herein and/or are known in the art. Thus, detectable oligonucleotide tags may be, but are not limited to, oligonucleotides which may comprise unique nucleotide sequences, oligonucleotides which may comprise detectable moieties, and oligonucleotides which may comprise both unique nucleotide sequences and detectable moieties. A unique label may be produced by sequentially attaching two or more detectable oligonucleotide tags to each other. The detectable tags may be present or provided in a plurality of detectable tags. The same or a different plurality of tags may be used as the source of each detectable tag may be part of a unique label. In other words, a plurality of tags may be subdivided into subsets and single subsets may be used as the source for each tag. One or more other species may be associated with the tags. In particular, nucleic acids released by a lysed cell may be ligated to one or more tags. These may include, for example, chromosomal DNA, RNA transcripts, tRNA, mRNA,

mitochondrial DNA, or the like. Such nucleic acids may be sequenced, in addition to sequencing the tags themselves, which may yield information about the nucleic acid profile of the cells, which can be associated with the tags, or the conditions that the corresponding droplet or cell was exposed to.

[00160] The invention accordingly may involve or be practiced as to high throughput and high resolution delivery of reagents to individual emulsion droplets that may contain cells, organelles, nucleic acids, proteins, etc. through the use of monodisperse aqueous droplets that are generated by a microfluidic device as a water-in-oil emulsion. The droplets are carried in a flowing oil phase and stabilized by a surfactant. In one aspect single cells or single organellesor single molecules (proteins, RNA, DNA) are encapsulated into uniform droplets from an aqueous solution/dispersion. In a related aspect, multiple cells or multiple molecules may take the place of single cells or single molecules. The aqueous droplets of volume ranging from 1 pL to 10 nL work as individual reactors. 104 to 105 single cells in droplets may be processed and analyzed in a single run. To utilize microdroplets for rapid large-scale chemical screening or complex biological library identification, different species of microdroplets, each containing the specific chemical compounds or biological probes cells or molecular barcodes of interest, have to be generated and combined at the preferred conditions, e.g., mixing ratio, concentration, and order of combination. Each species of droplet is introduced at a confluence point in a main microfluidic channel from separate inlet microfluidic channels. Preferably, droplet volumes are chosen by design such that one species is larger than others and moves at a different speed, usually slower than the other species, in the carrier fluid, as disclosed in U.S. Publication No. US 2007/0195127 and International Publication No. WO 2007/089541, each of which are incorporated herein by reference in their entirety. The channel width and length is selected such that faster species of droplets catch up to the slowest species. Size constraints of the channel prevent the faster moving droplets from passing the slower moving droplets resulting in a train of droplets entering a merge zone. Multi-step chemical reactions, biochemical reactions, or assay detection chemistries often require a fixed reaction time before species of different type are added to a reaction. Multi-step reactions are achieved by repeating the process multiple times with a second, third or more confluence points each with a separate merge point. Highly efficient and precise reactions and analysis of reactions are achieved when the frequencies of droplets from the inlet channels are matched to an optimized ratio and the volumes of the species are

matched to provide optimized reaction conditions in the combined droplets. Fluidic droplets may be screened or sorted within a fluidic system of the invention by altering the flow of the liquid containing the droplets. For instance, in one set of embodiments, a fluidic droplet may be steered or sorted by directing the liquid surrounding the fluidic droplet into a first channel, a second channel, etc. In another set of embodiments, pressure within a fluidic system, for example, within different channels or within different portions of a channel, can be controlled to direct the flow of fluidic droplets. For example, a droplet can be directed toward a channel junction including multiple options for further direction of flow (e.g., directed toward a branch, or fork, in a channel defining optional downstream flow channels). Pressure within one or more of the optional downstream flow channels can be controlled to direct the droplet selectively into one of the channels, and changes in pressure can be effected on the order of the time required for successive droplets to reach the junction, such that the downstream flow path of each successive droplet can be independently controlled. In one arrangement, the expansion and/or contraction of liquid reservoirs may be used to steer or sort a fluidic droplet into a channel, e.g., by causing directed movement of the liquid containing the fluidic droplet. In another, the expansion and/or contraction of the liquid reservoir may be combined with other flow-controlling devices and methods, e.g., as described herein. Non-limiting examples of devices able to cause the expansion and/or contraction of a liquid reservoir include pistons. Key elements for using microfluidic channels to process droplets include: (1) producing droplet of the correct volume, (2) producing droplets at the correct frequency and (3) bringing together a first stream of sample droplets with a second stream of sample droplets in such a way that the frequency of the first stream of sample droplets matches the frequency of the second stream of sample droplets. Preferably, bringing together a stream of sample droplets with a stream of premade library droplets in such a way that the frequency of the library droplets matches the frequency of the sample droplets. Methods for producing droplets of a uniform volume at a regular frequency are well known in the art. One method is to generate droplets using hydrodynamic focusing of a dispersed phase fluid and immiscible carrier fluid, such as disclosed in U.S. Publication No. US 2005/0172476 and International Publication No. WO 2004/002627. It is desirable for one of the species introduced at the confluence to be a pre-made library of droplets where the library contains a plurality of reaction conditions, e.g., a library may contain plurality of different compounds at a range of concentrations encapsulated as separate library elements for screening

their effect on cells or enzymes, alternatively a library could be composed of a plurality of different primer pairs encapsulated as different library elements for targeted amplification of a collection of loci, alternatively a library could contain a plurality of different antibody species encapsulated as different library elements to perform a plurality of binding assays. The introduction of a library of reaction conditions onto a substrate is achieved by pushing a premade collection of library droplets out of a vial with a drive fluid. The drive fluid is a continuous fluid. The drive fluid may comprise the same substance as the carrier fluid (e.g., a fluorocarbon oil). For example, if a library consists of ten pico-liter droplets is driven into an inlet channel on a microfluidic substrate with a drive fluid at a rate of 10,000 pico-liters per second, then nominally the frequency at which the droplets are expected to enter the confluence point is 1000 per second. However, in practice droplets pack with oil between them that slowly drains. Over time the carrier fluid drains from the library droplets and the number density of the droplets (number/mL) increases. Hence, a simple fixed rate of infusion for the drive fluid does not provide a uniform rate of introduction of the droplets into the microfluidic channel in the substrate. Moreover, library-to-library variations in the mean library droplet volume result in a shift in the frequency of droplet introduction at the confluence point. Thus, the lack of uniformity of droplets that results from sample variation and oil drainage provides another problem to be solved. For example if the nominal droplet volume is expected to be 10 pico-liters in the library, but varies from 9 to 11 pico-liters from library-to-library then a 10,000 pico-liter/second infusion rate will nominally produce a range in frequencies from 900 to 1,100 droplet per second. In short, sample to sample variation in the composition of dispersed phase for droplets made on chip, a tendency for the number density of library droplets to increase over time and library-to-library variations in mean droplet volume severely limit the extent to which frequencies of droplets may be reliably matched at a confluence by simply using fixed infusion rates. In addition, these limitations also have an impact on the extent to which volumes may be reproducibly combined. Combined with typical variations in pump flow rate precision and variations in channel dimensions, systems are severely limited without a means to compensate on a run-to-run basis. The foregoing facts not only illustrate a problem to be solved, but also demonstrate a need for a method of instantaneous regulation of microfluidic control over microdroplets within a microfluidic channel. Combinations of surfactant(s) and oils must be developed to facilitate generation, storage, and manipulation of droplets to maintain the unique

chemical/biochemical/biological environment within each droplet of a diverse library. Therefore, the surfactant and oil combination must (1) stabilize droplets against uncontrolled coalescence during the drop forming process and subsequent collection and storage, (2) minimize transport of any droplet contents to the oil phase and/or between droplets, and (3) maintain chemical and biological inertness with contents of each droplet (e.g., no adsorption or reaction of encapsulated contents at the oil-water interface, and no adverse effects on biological or chemical constituents in the droplets). In addition to the requirements on the droplet library function and stability, the surfactant-in-oil solution must be coupled with the fluid physics and materials associated with the platform. Specifically, the oil solution must not swell, dissolve, or degrade the materials used to construct the microfluidic chip, and the physical properties of the oil (e.g., viscosity, boiling point, etc.) must be suited for the flow and operating conditions of the platform. Droplets formed in oil without surfactant are not stable to permit coalescence, so surfactants must be dissolved in the oil that is used as the continuous phase for the emulsion library. Surfactant molecules are amphiphilic--part of the molecule is oil soluble, and part of the molecule is water soluble. When a water-oil interface is formed at the nozzle of a microfluidic chip for example in the inlet module described herein, surfactant molecules that are dissolved in the oil phase adsorb to the interface. The hydrophilic portion of the molecule resides inside the droplet and the fluorophilic portion of the molecule decorates the exterior of the droplet. The surface tension of a droplet is reduced when the interface is populated with surfactant, so the stability of an emulsion is improved. In addition to stabilizing the droplets against coalescence, the surfactant should be inert to the contents of each droplet and the surfactant should not promote transport of encapsulated components to the oil or other droplets. A droplet library may be made up of a number of library elements that are pooled together in a single collection (see, e.g., US Patent Publication No. 2010002241). Libraries may vary in complexity from a single library element to 1015 library elements or more. Each library element may be one or more given components at a fixed concentration. The element may be, but is not limited to, cells, organelles, virus, bacteria, yeast, beads, amino acids, proteins, polypeptides, nucleic acids, polynucleotides or small molecule chemical compounds. The element may contain an identifier such as a label. The terms "droplet library" or "droplet libraries" are also referred to herein as an "emulsion library" or "emulsion libraries." These terms are used interchangeably throughout the specification. A cell library element may include, but is not limited to, hybridomas, B-cells, primary cells, cultured

cell lines, cancer cells, stem cells, cells obtained from tissue, or any other cell type. Cellular library elements are prepared by encapsulating a number of cells from one to hundreds of thousands in individual droplets. The number of cells encapsulated is usually given by Poisson statistics from the number density of cells and volume of the droplet. However, in some cases the number deviates from Poisson statistics as described in Edd et al., "Controlled encapsulation of single-cells into monodisperse picolitre drops." Lab Chip, 8(8): 1262-1264, 2008. The discrete nature of cells allows for libraries to be prepared in mass with a plurality of cellular variants all present in a single starting media and then that media is broken up into individual droplet capsules that contain at most one cell. These individual droplets capsules are then combined or pooled to form a library consisting of unique library elements. Cell division subsequent to, or in some embodiments following, encapsulation produces a clonal library element. A bead based library element may contain one or more beads, of a given type and may also contain other reagents, such as antibodies, enzymes or other proteins. In the case where all library elements contain different types of beads, but the same surrounding media, the library elements may all be prepared from a single starting fluid or have a variety of starting fluids. In the case of cellular libraries prepared in mass from a collection of variants, such as genomically modified, yeast or bacteria cells, the library elements will be prepared from a variety of starting fluids. Often it is desirable to have exactly one cell per droplet with only a few droplets containing more than one cell when starting with a plurality of cells or yeast or bacteria, engineered to produce variants on a protein. In some cases, variations from Poisson statistics may be achieved to provide an enhanced loading of droplets such that there are more droplets with exactly one cell per droplet and few exceptions of empty droplets or droplets containing more than one cell. Examples of droplet libraries are collections of droplets that have different contents, ranging from beads, cells, small molecules, DNA, primers, antibodies. Smaller droplets may be in the order of femtoliter (fL) volume drops, which are especially contemplated with the droplet dispensors. The volume may range from about 5 to about 600 fL. The larger droplets range in size from roughly 0.5 micron to 500 micron in diameter, which corresponds to about 1 pico liter to 1 nano liter. However, droplets may be as small as 5 microns and as large as 500 microns. Preferably, the droplets are at less than 100 microns, about 1 micron to about 100 microns in diameter. The most preferred size is about 20 to 40 microns in diameter (10 to 100 picoliters). The preferred properties examined of droplet libraries include osmotic pressure balance, uniform size, and size

ranges. The droplets within the emulsion libraries of the present invention may be contained within an immiscible oil which may comprise at least one fluorosurfactant. In some embodiments, the fluorosurfactant within the immiscible fluorocarbon oil may be a block copolymer consisting of one or more perfluorinated polyether (PFPE) blocks and one or more polyethylene glycol (PEG) blocks. In other embodiments, the fluorosurfactant is a triblock copolymer consisting of a PEG center block covalently bound to two PFPE blocks by amide linking groups. The presence of the fluorosurfactant (similar to uniform size of the droplets in the library) is critical to maintain the stability and integrity of the droplets and is also essential for the subsequent use of the droplets within the library for the various biological and chemical assays described herein. Fluids (e.g., aqueous fluids, immiscible oils, etc.) and other surfactants that may be utilized in the droplet libraries of the present invention are described in greater detail herein. The present invention can accordingly involve an emulsion library which may comprise a plurality of aqueous droplets within an immiscible oil (e.g., fluorocarbon oil) which may comprise at least one fluorosurfactant, wherein each droplet is uniform in size and may comprise the same aqueous fluid and may comprise a different library element. The present invention also provides a method for forming the emulsion library which may comprise providing a single aqueous fluid which may comprise different library elements, encapsulating each library element into an aqueous droplet within an immiscible fluorocarbon oil which may comprise at least one fluorosurfactant, wherein each droplet is uniform in size and may comprise the same aqueous fluid and may comprise a different library element, and pooling the aqueous droplets within an immiscible fluorocarbon oil which may comprise at least one fluorosurfactant, thereby forming an emulsion library. For example, in one type of emulsion library, all different types of elements (e.g., cells or beads), may be pooled in a single source contained in the same medium. After the initial pooling, the cells or beads are then encapsulated in droplets to generate a library of droplets wherein each droplet with a different type of bead or cell is a different library element. The dilution of the initial solution enables the encapsulation process. In some embodiments, the droplets formed will either contain a single cell or bead or will not contain anything, i.e., be empty. In other embodiments, the droplets formed will contain multiple copies of a library element. The cells or beads being encapsulated are generally variants on the same type of cell or bead. In another example, the emulsion library may comprise a plurality of aqueous droplets within an immiscible fluorocarbon oil, wherein a single molecule may be encapsulated, such that

there is a single molecule contained within a droplet for every 20-60 droplets produced (e.g., 20, 25, 30, 35, 40, 45, 50, 55, 60 droplets, or any integer in between). Single molecules may be encapsulated by diluting the solution containing the molecules to such a low concentration that the encapsulation of single molecules is enabled. In one specific example, a LacZ plasmid DNA was encapsulated at a concentration of 20 fM after two hours of incubation such that there was about one gene in 40 droplets, where 10 μm droplets were made at 10 kHz per second. Formation of these libraries rely on limiting dilutions.

[00161] The present invention also provides an emulsion library which may comprise at least a first aqueous droplet and at least a second aqueous droplet within a fluorocarbon oil which may comprise at least one fluorosurfactant, wherein the at least first and the at least second droplets are uniform in size and comprise a different aqueous fluid and a different library element. The present invention also provides a method for forming the emulsion library which may comprise providing at least a first aqueous fluid which may comprise at least a first library of elements, providing at least a second aqueous fluid which may comprise at least a second library of elements, encapsulating each element of said at least first library into at least a first aqueous droplet within an immiscible fluorocarbon oil which may comprise at least one fluorosurfactant, encapsulating each element of said at least second library into at least a second aqueous droplet within an immiscible fluorocarbon oil which may comprise at least one fluorosurfactant, wherein the at least first and the at least second droplets are uniform in size and may comprise a different aqueous fluid and a different library element, and pooling the at least first aqueous droplet and the at least second aqueous droplet within an immiscible fluorocarbon oil which may comprise at least one fluorosurfactant thereby forming an emulsion library. One of skill in the art will recognize that methods and systems of the invention are not preferably practiced as to cells, mutations, etc as herein disclosed, but that the invention need not be limited to any particular type of sample, and methods and systems of the invention may be used with any type of organic, inorganic, or biological molecule (see, e.g, US Patent Publication No. 20120122714). In particular embodiments the sample may include nucleic acid target molecules. Nucleic acid molecules may be synthetic or derived from naturally occurring sources. In one embodiment, nucleic acid molecules may be isolated from a biological sample containing a variety of other components, such as proteins, lipids and non-template nucleic acids. Nucleic acid target molecules may be obtained from any cellular material, obtained from an animal, plant,

bacterium, fungus, or any other cellular organism. In certain embodiments, the nucleic acid target molecules may be obtained from a single cell. Biological samples for use in the present invention may include viral particles or preparations. Nucleic acid target molecules may be obtained directly from an organism or from a biological sample obtained from an organism, e.g., from blood, urine, cerebrospinal fluid, seminal fluid, saliva, sputum, stool and tissue. Any tissue or body fluid specimen may be used as a source for nucleic acid for use in the invention. Nucleic acid target molecules may also be isolated from cultured cells, such as a primary cell culture or a cell line. The cells or tissues from which target nucleic acids are obtained may be infected with a virus or other intracellular pathogen. A sample may also be total RNA extracted from a biological specimen, a cDNA library, viral, or genomic DNA. Generally, nucleic acid may be extracted from a biological sample by a variety of techniques such as those described by Maniatis, et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor, N.Y., pp. 280-281 (1982). Nucleic acid molecules may be single-stranded, double-stranded, or double-stranded with single-stranded regions (for example, stem- and loop-structures). Nucleic acid obtained from biological samples typically may be fragmented to produce suitable fragments for analysis. Target nucleic acids may be fragmented or sheared to desired length, using a variety of mechanical, chemical and/or enzymatic methods. DNA may be randomly sheared via sonication, e.g. Covaris method, brief exposure to a DNase, or using a mixture of one or more restriction enzymes, or a transposase or nicking enzyme. RNA may be fragmented by brief exposure to an RNase, heat plus magnesium, or by shearing. The RNA may be converted to cDNA. If fragmentation is employed, the RNA may be converted to cDNA before or after fragmentation. In one embodiment, nucleic acid from a biological sample is fragmented by sonication. In another embodiment, nucleic acid is fragmented by a hydroshear instrument. Generally, individual nucleic acid target molecules may be from about 40 bases to about 40 kb. Nucleic acid molecules may be single-stranded, double-stranded, or double-stranded with single-stranded regions (for example, stem- and loop-structures). A biological sample as described herein may be homogenized or fractionated in the presence of a detergent or surfactant. The concentration of the detergent in the buffer may be about 0.05% to about 10.0%. The concentration of the detergent may be up to an amount where the detergent remains soluble in the solution. In one embodiment, the concentration of the detergent is between 0.1% to about 2%. The detergent, particularly a mild one that is nondenaturing, may act to solubilize the sample. Detergents may

be ionic or nonionic. Examples of nonionic detergents include triton, such as the Triton™ X series (Triton™ X-100 t-Oct-C6H4--(OCH2--CH2)xOH, x=9-10, Triton™ X-100R, Triton™ X-114 x=7-8), octyl glucoside, polyoxyethylene(9)dodecyl ether, digitonin, IGEPAL™ CA630 octylphenyl polyethylene glycol, n-octyl-beta-D-glucopyranoside (betaOG), n-dodecyl-beta, Tween™. 20 polyethylene glycol sorbitan monolaurate, Tween™ 80 polyethylene glycol sorbitan monooleate, polidocanol, n-dodecyl beta-D-maltoside (DDM), NP-40 nonylphenyl polyethylene glycol, C12E8 (octaethylene glycol n-dodecyl monoether), hexaethyleneglycol mono-n-tetradecyl ether (C14E06), octyl-beta-thioglucopyranoside (octyl thioglucoside, OTG), Emulgen, and polyoxyethylene 10 lauryl ether (C12E10). Examples of ionic detergents (anionic or cationic) include deoxycholate, sodium dodecyl sulfate (SDS), N-lauroylsarcosine, and cetyltrimethylammoniumbromide (CTAB). A zwitterionic reagent may also be used in the purification schemes of the present invention, such as Chaps, zwitterion 3-14, and 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulf-onate. It is contemplated also that urea may be added with or without another detergent or surfactant. Lysis or homogenization solutions may further contain other agents, such as reducing agents. Examples of such reducing agents include dithiothreitol (DTT), β-mercaptoethanol, DTE, GSH, cysteine, cysteamine, tricarboxyethyl phosphine (TCEP), or salts of sulfurous acid. Size selection of the nucleic acids may be performed to remove very short fragments or very long fragments. The nucleic acid fragments may be partitioned into fractions which may comprise a desired number of fragments using any suitable method known in the art. Suitable methods to limit the fragment size in each fragment are known in the art. In various embodiments of the invention, the fragment size is limited to between about 10 and about 100 Kb or longer. A sample in or as to the instant invention may include individual target proteins, protein complexes, proteins with translational modifications, and protein/nucleic acid complexes. Protein targets include peptides, and also include enzymes, hormones, structural components such as viral capsid proteins, and antibodies. Protein targets may be synthetic or derived from naturally-occurring sources. The invention protein targets may be isolated from biological samples containing a variety of other components including lipids, non-template nucleic acids, and nucleic acids. Protein targets may be obtained from an animal, bacterium, fungus, cellular organism, and single cells. Protein targets may be obtained directly from an organism or from a biological sample obtained from the organism, including bodily fluids such as blood, urine, cerebrospinal fluid, seminal fluid, saliva, sputum, stool and tissue.

Protein targets may also be obtained from cell and tissue lysates and biochemical fractions. An individual protein is an isolated polypeptide chain. A protein complex includes two or polypeptide chains. Samples may include proteins with post translational modifications including but not limited to phosphorylation, methionine oxidation, deamidation, glycosylation, ubiquitination, carbamylation, s-carboxymethylation, acetylation, and methylation. Protein/nucleic acid complexes include cross-linked or stable protein-nucleic acid complexes. Extraction or isolation of individual proteins, protein complexes, proteins with translational modifications, and protein/nucleic acid complexes is performed using methods known in the art.

[00162] The invention can thus involve forming sample droplets. The droplets are aqueous droplets that are surrounded by an immiscible carrier fluid. Methods of forming such droplets are shown for example in Link et al. (U.S. patent application numbers 2008/0014589, 2008/0003142, and 2010/0137163), Stone et al. (U.S. Pat. No. 7,708,949 and U.S. patent application number 2010/0172803), Anderson et al. (U.S. Pat. No. 7,041,481 and which reissued as RE41,780) and European publication number EP2047910 to Raindance Technologies Inc. The content of each of which is incorporated by reference herein in its entirety. The present invention may relates to systems and methods for manipulating droplets within a high throughput microfluidic system. A microfluid droplet encapsulates a differentiated cell The cell is lysed and its mRNA is hybridized onto a capture bead containing barcoded oligo dT primers on the surface, all inside the droplet. The barcode is covalently attached to the capture bead via a flexible multi-atom linker like PEG. In a preferred embodiment, the droplets are broken by addition of a fluorosurfactant (like perfluorooctanol), washed, and collected. A reverse transcription (RT) reaction is then performed to convert each cell's mRNA into a first strand cDNA that is both uniquely barcoded and covalently linked to the mRNA capture bead. Subsequently, a universal primer via a template switching reaction is amended using conventional library preparation protocols to prepare an RNA-Seq library. Since all of the mRNA from any given cell is uniquely barcoded, a single library is sequenced and then computationally resolved to determine which mRNAs came from which cells. In this way, through a single sequencing run, tens of thousands (or more) of distinguishable transcriptomes can be simultaneously obtained. The oligonucleotide sequence may be generated on the bead surface. During these cycles, beads were removed from the synthesis column, pooled, and aliquoted into four equal portions by mass; these bead aliquots were then placed in a separate

synthesis column and reacted with either dG, dC, dT, or dA phosphoramidite. In other instances, dinucleotide, trinucleotides, or oligonucleotides that are greater in length are used, in other instances, the oligo-dT tail is replaced by gene specific oligonucleotides to prime specific targets (singular or plural), random sequences of any length for the capture of all or specific RNAs. This process was repeated 12 times for a total of 4^{12} = 16,777,216 unique barcode sequences. Upon completion of these cycles, 8 cycles of degenerate oligonucleotide synthesis were performed on all the beads, followed by 30 cycles of dT addition. In other embodiments, the degenerate synthesis is omitted, shortened (less than 8 cycles), or extended (more than 8 cycles); in others, the 30 cycles of dT addition are replaced with gene specific primers (single target or many targets) or a degenerate sequence. The aforementioned microfluidic system is regarded as the reagent delivery system microfluidic library printer or droplet library printing system of the present invention. Droplets are formed as sample fluid flows from droplet generator which contains lysis reagent and barcodes through microfluidic outlet channel which contains oil, towards junction. Defined volumes of loaded reagent emulsion, corresponding to defined numbers of droplets, are dispensed on-demand into the flow stream of carrier fluid. The sample fluid may typically comprise an aqueous buffer solution, such as ultrapure water (e.g., 18 mega-ohm resistivity, obtained, for example by column chromatography), 10 mM Tris HCl and 1 mM EDTA (TE) buffer, phosphate buffer saline (PBS) or acetate buffer. Any liquid or buffer that is physiologically compatible with nucleic acid molecules can be used. The carrier fluid may include one that is immiscible with the sample fluid. The carrier fluid can be a non-polar solvent, decane (e.g., tetradecane or hexadecane), fluorocarbon oil, silicone oil, an inert oil such as hydrocarbon, or another oil (for example, mineral oil). The carrier fluid may contain one or more additives, such as agents which reduce surface tensions (surfactants). Surfactants can include Tween, Span, fluorosurfactants, and other agents that are soluble in oil relative to water. In some applications, performance is improved by adding a second surfactant to the sample fluid. Surfactants can aid in controlling or optimizing droplet size, flow and uniformity, for example by reducing the shear force needed to extrude or inject droplets into an intersecting channel. This can affect droplet volume and periodicity, or the rate or frequency at which droplets break off into an intersecting channel. Furthermore, the surfactant can serve to stabilize aqueous emulsions in fluorinated oils from coalescing. Droplets may be surrounded by a surfactant which stabilizes the droplets by reducing the surface tension at the aqueous oil interface. Preferred surfactants

that may be added to the carrier fluid include, but are not limited to, surfactants such as sorbitan-based carboxylic acid esters (e.g., the "Span" surfactants, Fluka Chemika), including sorbitan monolaurate (Span 20), sorbitan monopalmitate (Span 40), sorbitan monostearate (Span 60) and sorbitan monooleate (Span 80), and perfluorinated polyethers (e.g., DuPont Krytox 157 FSL, FSM, and/or FSH). Other non-limiting examples of non-ionic surfactants which may be used include polyoxyethylenated alkylphenols (for example, nonyl-, p-dodecyl-, and dinonylphenols), polyoxyethylenated straight chain alcohols, polyoxyethylenated polyoxypropylene glycols, polyoxyethylenated mercaptans, long chain carboxylic acid esters (for example, glyceryl and polyglyceryl esters of natural fatty acids, propylene glycol, sorbitol, polyoxyethylenated sorbitol esters, polyoxyethylene glycol esters, etc.) and alkanolamines (e.g., diethanolamine-fatty acid condensates and isopropanolamine-fatty acid condensates). In some cases, an apparatus for creating a single-cell sequencing library via a microfluidic system provides for volume-driven flow, wherein constant volumes are injected over time. The pressure in fluidic cnannels is a function of injection rate and channel dimensions. In one embodiment, the device provides an oil/surfactant inlet; an inlet for an analyte; a filter, an inlet for for mRNA capture microbeads and lysis reagent; a carrier fluid channel which connects the inlets; a resistor; a constriction for droplet pinch-off; a mixer; and an outlet for drops. In an embodiment the invention provides apparatus for creating a single-cell sequencing library via a microfluidic system, which may comprise: an oil-surfactant inlet which may comprise a filter and a carrier fluid channel, wherein said carrier fluid channel may further comprise a resistor; an inlet for an analyte which may comprise a filter and a carrier fluid channel, wherein said carrier fluid channel may further comprise a resistor; an inlet for mRNA capture microbeads and lysis reagent which may comprise a filter and a carrier fluid channel, wherein said carrier fluid channel further may comprise a resistor; said carrier fluid channels have a carrier fluid flowing therein at an adjustable or predetermined flow rate; wherein each said carrier fluid channels merge at a junction; and said junction being connected to a mixer, which contains an outlet for drops. Accordingly, an apparatus for creating a single-cell sequencing library via a microfluidic system icrofluidic flow scheme for single-cell RNA-seq is envisioned. Two channels, one carrying cell suspensions, and the other carrying uniquely barcoded mRNA capture bead, lysis buffer and library preparation reagents meet at a junction and is immediately co-encapsulated in an inert carrier oil, at the rate of one cell and one bead per drop. In each drop, using the bead's barcode

tagged oligonucleotides as cDNA template, each mRNA is tagged with a unique, cell-specific identifier. The invention also encompasses use of a Drop-Seq library of a mixture of mouse and human cells. The carrier fluid may be caused to flow through the outlet channel so that the surfactant in the carrier fluid coats the channel walls. The fluorosurfactant can be prepared by reacting the perflourinated polyether DuPont Krytox 157 FSL, FSM, or FSH with aqueous ammonium hydroxide in a volatile fluorinated solvent. The solvent and residual water and ammonia can be removed with a rotary evaporator. The surfactant can then be dissolved (e.g., 2.5 wt %) in a fluorinated oil (e.g., Flourinert (3M)), which then serves as the carrier fluid. Activation of sample fluid reservoirs to produce regent droplets is based on the concept of dynamic reagent delivery (e.g., combinatorial barcoding) via an on demand capability. The on demand feature may be provided by one of a variety of technical capabilities for releasing delivery droplets to a primary droplet, as described herein. From this disclosure and herein cited documents and knowledge in the art, it is within the ambit of the skilled person to develop flow rates, channel lengths, and channel geometries; and establish droplets containing random or specified reagent combinations can be generated on demand and merged with the "reaction chamber" droplets containing the samples/cells/substrates of interest. By incorporating a plurality of unique tags into the additional droplets and joining the tags to a solid support designed to be specific to the primary droplet, the conditions that the primary droplet is exposed to may be encoded and recorded. For example, nucleic acid tags can be sequentially ligated to create a sequence reflecting conditions and order of same. Alternatively, the tags can be added independently appended to solid support. Non-limiting examples of a dynamic labeling system that may be used to bioninformatically record information can be found at US Provisional Patent Application entitled "Compositions and Methods for Unique Labeling of Agents" filed September 21, 2012 and November 29, 2012. In this way, two or more droplets may be exposed to a variety of different conditions, where each time a droplet is exposed to a condition, a nucleic acid encoding the condition is added to the droplet each ligated together or to a unique solid support associated with the droplet such that, even if the droplets with different histories are later combined, the conditions of each of the droplets are remain available through the different nucleic acids. Non-limiting examples of methods to evaluate response to exposure to a plurality of conditions can be found at US Provisional Patent Application entitled "Systems and Methods for Droplet Tagging" filed September 21, 2012. Accordingly, in or as to the invention it is

envisioned that there can be the dynamic generation of molecular barcodes (e.g., DNA oligonucleotides, flurophores, etc.) either independent from or in concert with the controlled delivery of various compounds of interest (drugs, small molecules, siRNA, CRISPR guide RNAs, reagents, etc.). For example, unique molecular barcodes can be created in one array of nozzles while individual compounds or combinations of compounds can be generated by another nozzle array. Barcodes/compounds of interest can then be merged with cell-containing droplets. An electronic record in the form of a computer log file is kept to associate the barcode delivered with the downstream reagent(s) delivered. This methodology makes it possible to efficiently screen a large population of cells for applications such as single-cell drug screening, controlled perturbation of regulatory pathways, etc. The device and techniques of the disclosed invention facilitate efforts to perform studies that require data resolution at the single cell (or single molecule) level and in a cost effective manner. The invention envisions a high throughput and high resolution delivery of reagents to individual emulsion droplets that may contain cells, nucleic acids, proteins, etc. through the use of monodisperse aqueous droplets that are generated one by one in a microfluidic chip as a water-in-oil emulsion. Being able to dynamically track individual cells and droplet treatments/combinations during life cycle experiments, and having an ability to create a library of emulsion droplets on demand with the further capability of manipulating the droplets through the disclosed process(es) are advantagous. In the practice of the invention there can be dynamic tracking of the droplets and create a history of droplet deployment and application in a single cell based environment. Droplet generation and deployment is produced via a dynamic indexing strategy and in a controlled fashion in accordance with disclosed embodiments of the present invention. Microdroplets can be processed, analyzed and sorted at a highly efficient rate of several thousand droplets per second, providing a powerful platform which allows rapid screening of millions of distinct compounds, biological probes, proteins or cells either in cellular models of biological mechanisms of disease, or in biochemical, or pharmacological assays. A plurality of biological assays as well as biological synthesis are contemplated. Polymerase chain reactions (PCR) are contemplated (see, e.g., US Patent Publication No. 20120219947). Methods of the invention may be used for merging sample fluids for conducting any type of chemical reaction or any type of biological assay. There may be merging sample fluids for conducting an amplification reaction in a droplet. Amplification refers to production of additional copies of a nucleic acid sequence and is

generally carried out using polymerase chain reaction or other technologies well known in the art (e.g., Dieffenbach and Dveksler, PCR Primer, a Laboratory Manual, Cold Spring Harbor Press, Plainview, N.Y. [1995]). The amplification reaction may be any amplification reaction known in the art that amplifies nucleic acid molecules, such as polymerase chain reaction, nested polymerase chain reaction, polymerase chain reaction-single strand conformation polymorphism, ligase chain reaction (Barany F. (1991) PNAS 88:189-193; Barany F. (1991) PCR Methods and Applications 1:5-16), ligase detection reaction (Barany F. (1991) PNAS 88:189-193), strand displacement amplification and restriction fragments length polymorphism, transcription based amplification system, nucleic acid sequence-based amplification, rolling circle amplification, and hyper-branched rolling circle amplification. In certain embodiments, the amplification reaction is the polymerase chain reaction. Polymerase chain reaction (PCR) refers to methods by K. B. Mullis (U.S. Pat. Nos. 4,683,195 and 4,683,202, hereby incorporated by reference) for increasing concentration of a segment of a target sequence in a mixture of genomic DNA without cloning or purification. The process for amplifying the target sequence includes introducing an excess of oligonucleotide primers to a DNA mixture containing a desired target sequence, followed by a precise sequence of thermal cycling in the presence of a DNA polymerase. The primers are complementary to their respective strands of the double stranded target sequence. To effect amplification, primers are annealed to their complementary sequence within the target molecule. Following annealing, the primers are extended with a polymerase so as to form a new pair of complementary strands. The steps of denaturation, primer annealing and polymerase extension may be repeated many times (i.e., denaturation, annealing and extension constitute one cycle; there may be numerous cycles) to obtain a high concentration of an amplified segment of a desired target sequence. The length of the amplified segment of the desired target sequence is determined by relative positions of the primers with respect to each other, and therefore, this length is a controllable parameter. Methods for performing PCR in droplets are shown for example in Link et al. (U.S. Patent application numbers 2008/0014589, 2008/0003142, and 2010/0137163), Anderson et al. (U.S. Pat. No. 7,041,481 and which reissued as RE41,780) and European publication number EP2047910 to Raindance Technologies Inc. The content of each of which is incorporated by reference herein in its entirety. The first sample fluid contains nucleic acid templates. Droplets of the first sample fluid are formed as described above. Those droplets will include the nucleic acid templates. In certain embodiments, the droplets will include only a

single nucleic acid template, and thus digital PCR may be conducted. The second sample fluid contains reagents for the PCR reaction. Such reagents generally include Taq polymerase, deoxynucleotides of type A, C, G and T, magnesium chloride, and forward and reverse primers, all suspended within an aqueous buffer. The second fluid also includes detectably labeled probes for detection of the amplified target nucleic acid, the details of which are discussed below. This type of partitioning of the reagents between the two sample fluids is not the only possibility. In some instances, the first sample fluid will include some or all of the reagents necessary for the PCR whereas the second sample fluid will contain the balance of the reagents necessary for the PCR together with the detection probes. Primers may be prepared by a variety of methods including but not limited to cloning of appropriate sequences and direct chemical synthesis using methods well known in the art (Narang et al., Methods Enzymol., 68:90 (1979); Brown et al., Methods Enzymol., 68:109 (1979)). Primers may also be obtained from commercial sources such as Operon Technologies, Amersham Pharmacia Biotech, Sigma, and Life Technologies. The primers may have an identical melting temperature. The lengths of the primers may be extended or shortened at the 5' end or the 3' end to produce primers with desired melting temperatures. Also, the annealing position of each primer pair may be designed such that the sequence and, length of the primer pairs yield the desired melting temperature. The simplest equation for determining the melting temperature of primers smaller than 25 base pairs is the Wallace Rule (Td=2(A+T)+4(G+C)). Computer programs may also be used to design primers, including but not limited to Array Designer Software (Arrayit Inc.), Oligonucleotide Probe Sequence Design Software for Genetic Analysis (Olympus Optical Co.), NetPrimer, and DNAsis from Hitachi Software Engineering. The TM (melting or annealing temperature) of each primer is calculated using software programs such as Oligo Design, available from Invitrogen Corp.

[00163] A droplet containing the nucleic acid is then caused to merge with the PCR reagents in the second fluid according to methods of the invention described above, producing a droplet that includes Taq polymerase, deoxynucleotides of type A, C, G and T, magnesium chloride, forward and reverse primers, detectably labeled probes, and the target nucleic acid. Once mixed droplets have been produced, the droplets are thermal cycled, resulting in amplification of the target nucleic acid in each droplet. Droplets may be flowed through a channel in a serpentine path between heating and cooling lines to amplify the nucleic acid in the droplet. The width and depth of the channel may be adjusted to set the residence time at each temperature, which may be

controlled to anywhere between less than a second and minutes. The three temperature zones may be used for the amplification reaction. The three temperature zones are controlled to result in denaturation of double stranded nucleic acid (high temperature zone), annealing of primers (low temperature zones), and amplification of single stranded nucleic acid to produce double stranded nucleic acids (intermediate temperature zones). The temperatures within these zones fall within ranges well known in the art for conducting PCR reactions. See for example, Sambrook et al. (Molecular Cloning, A Laboratory Manual, 3rd edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 2001). The three temperature zones can be controlled to have temperatures as follows: 95° C. (TH), 55° C. (TL), 72° C. (TM). The prepared sample droplets flow through the channel at a controlled rate. The sample droplets first pass the initial denaturation zone (TH) before thermal cycling. The initial preheat is an extended zone to ensure that nucleic acids within the sample droplet have denatured successfully before thermal cycling. The requirement for a preheat zone and the length of denaturation time required is dependent on the chemistry being used in the reaction. The samples pass into the high temperature zone, of approximately 95° C., where the sample is first separated into single stranded DNA in a process called denaturation. The sample then flows to the low temperature, of approximately 55° C., where the hybridization process takes place, during which the primers anneal to the complementary sequences of the sample. Finally, as the sample flows through the third medium temperature, of approximately 72° C., the polymerase process occurs when the primers are extended along the single strand of DNA with a thermostable enzyme. The nucleic acids undergo the same thermal cycling and chemical reaction as the droplets pass through each thermal cycle as they flow through the channel. The total number of cycles in the device is easily altered by an extension of thermal zones. The sample undergoes the same thermal cycling and chemical reaction as it passes through N amplification cycles of the complete thermal device. In other aspects, the temperature zones are controlled to achieve two individual temperature zones for a PCR reaction. In certain embodiments, the two temperature zones are controlled to have temperatures as follows: 95° C. (TH) and 60° C. (TL). The sample droplet optionally flows through an initial preheat zone before entering thermal cycling. The preheat zone may be important for some chemistry for activation and also to ensure that double stranded nucleic acid in the droplets is fully denatured before the thermal cycling reaction begins. In an exemplary embodiment, the preheat dwell length results in approximately 10 minutes preheat of the droplets

at the higher temperature. The sample droplet continues into the high temperature zone, of approximately 95° C., where the sample is first separated into single stranded DNA in a process called denaturation. The sample then flows through the device to the low temperature zone, of approximately 60° C., where the hybridization process takes place, during which the primers anneal to the complementary sequences of the sample. Finally the polymerase process occurs when the primers are extended along the single strand of DNA with a thermostable enzyme. The sample undergoes the same thermal cycling and chemical reaction as it passes through each thermal cycle of the complete device. The total number of cycles in the device is easily altered by an extension of block length and tubing. After amplification, droplets may be flowed to a detection module for detection of amplification products. The droplets may be individually analyzed and detected using any methods known in the art, such as detecting for the presence or amount of a reporter. Generally, a detection module is in communication with one or more detection apparatuses. Detection apparatuses may be optical or electrical detectors or combinations thereof. Examples of suitable detection apparatuses include optical waveguides, microscopes, diodes, light stimulating devices, (e.g., lasers), photo multiplier tubes, and processors (e.g., computers and software), and combinations thereof, which cooperate to detect a signal representative of a characteristic, marker, or reporter, and to determine and direct the measurement or the sorting action at a sorting module. Further description of detection modules and methods of detecting amplification products in droplets are shown in Link et al. (U.S. patent application numbers 2008/0014589, 2008/0003142, and 2010/0137163) and European publication number EP2047910 to Raindance Technologies Inc.

[00164] Examples of assays are also ELISA assays (see, e.g., US Patent Publication No. 20100022414). The present invention provides another emulsion library which may comprise a plurality of aqueous droplets within an immiscible fluorocarbon oil which may comprise at least one fluorosurfactant, wherein each droplet is uniform in size and may comprise at least a first antibody, and a single element linked to at least a second antibody, wherein said first and second antibodies are different. In one example, each library element may comprise a different bead, wherein each bead is attached to a number of antibodies and the bead is encapsulated within a droplet that contains a different antibody in solution. These antibodies may then be allowed to form "ELISA sandwiches," which may be washed and prepared for a ELISA assay. Further, these contents of the droplets may be altered to be specific for the antibody contained therein to

maximize the results of the assay. Single-cell assays are also contemplated as part of the present invention (see, e.g., Ryan et al., Biomicrofluidics 5, 021501 (2011) for an overview of applications of microfluidics to assay individual cells). A single-cell assay may be contemplated as an experiment that quantifies a function or property of an individual cell when the interactions of that cell with its environment may be controlled precisely or may be isolated from the function or property under examination. The research and development of single-cell assays is largely predicated on the notion that genetic variation causes disease and that small subpopulations of cells represent the origin of the disease. Methods of assaying compounds secreted from cells, subcellular components, cell-cell or cell-drug interactions as well as methods of patterning individual cells are also contemplated within the present invention.

[00165] Another aspect of the invention is the combination of the technologies described herein. For example, the use of a high-throughput single-cell RNA-Seq and/or targeted nucleic acid profiling (for example, sequencing, quantitative reverse transcription polymerase chain reaction, and the like) where the RNAs from different cells are tagged individually, allowing a single library to be created while retaining the cell identity of each read, as explained above. RNA-Seq profiling of single cells (e.g. single Th17 cells) may be performed on cells isolated in vivo (e.g. isolated directly from a subject / patient, preferably without further culture steps). RNA-Seq profiling of single cells may be performed on any number of cells, including tumor cells, accociated infiltrating cells into a tumor, immune derived cells, microglia, astrocytes, CD4 cells, CD8 cells, most preferably Th17 cells. Computational analysis of the high-throughput single-cell RNA-Seq data. This allows, for example, to dissect the molecular basis of different functional cellular states. This also allows for selection of signature genes as described herein. Once selection of signature genes is performed, an optional further step is the validation of the signature genes using any number of technologies for knock-out or knock-in models. For example, as explained herein, mutations in cells and also mutated mice for use in or as to the invention can be by way of the CRISPR-Cas system or a Cas9-expressing eukaryotic cell or Cas-9 expressing eukaryote, such as a mouse.

[00166] Such a combination of technologies, e.g. in particular with direct isolation from the subject / patient, provides for more robust and more accurate data as compared to in vitro scenarious which cannot take into account the full in vivo system and networking. This combination, in several instances is thus more efficient, more specific, and faster. This

combinatinon provides for, for example, methods for identification of signature genes and validation methods of the same. Equally, screening platforms are provided for identification of effective therapeutics or diagnostics.

[00167] These and other technologies may be employed in or as to the practice of the instant invention.

EXAMPLE 1: Identification of novel regulators of Th17 cell pathogenicity by single cell genomics

[00168] Upon immunological challenge, diverse immune cells collectively orchestrate an appropriate response. Extensive cellular heterogeneity exists even within specific immune cell subtypes classified as a single lineage, but its function and molecular underpinnings are rarely characterized at a genomic scale. Here, single-cell RNA-seq was use to investigate the molecular mechanisms governing heterogeneity and pathogenicity of murine Th17 cells isolated from the central nervous system (CNS) and lymph nodes (LN) at the peak of autoimmune encephalomyelitis (EAE) or polarized *in vitro* under either pathogenic or non-pathogenic differentiation conditions. Computational analysis reveals that Th17 cells span a spectrum of cellular states *in vivo*, including a self-renewal state in the LN, and Th1-like effector/memory states and a dysfunctional/senescent state in the CNS. Relating these states to *in vitro* differentiated Th17 cells, novel genes governing pathogenicity and disease susceptibility were discovered. Using knockout mice, the crucial role in Th17 cell pathogenicity of four novel genes was tested: *Gpr65*, *Plzp*, *Toso* and *Cd5l*. Th17 cellular heterogeneity thus plays an important role in defining the function of Th17 cells in autoimmunity and can be leveraged to identify targets for seelctive suppression of pathogenic Th17 cells while sparing non-pathogenic tissue-protective ones.

[00169] *RNA-Seq profiling of single Th17 cells isolated in vivo and in vitro.* The transcriptome of 1,029 Th17 cells (subsequently retaining a final set of 806 cells, below), either harvested *in vivo* or differentiated *in vitro* (Figure 1A and Table S1) was profiled. For *in vivo* experiments, EAE was induced by myelin oligodendrocyte glycoprotein (MOG) immunization, $CD3^+CD4^+$IL-17A/GFP^+ cells were harvested from the draining LNs at the peak of disease and profiled immediately. For *in vitro* experiments, cells were collected during differentiation of $CD4^+$ naïve T cells under two polarizing conditions: TGF-β1+IL-6 and IL-1β+IL-6+IL-23; while both lead to IL-17A-producing cells, only the latter induces

EAE upon adoptive transfer of cell ensembles into wild type or RAG-1 -/- mice(Chung et al., 2009; Ghoreschi et al., 2010). At least two independent biological replicates were used for each *in vivo* and *in vitro* condition, and two technical replicates for two *in vivo* conditions. Single-cell mRNA SMART-Seq libraries were prepared using microfluidic chips (Fluidigm C_1) for single-cell capture, lysis, reverse transcription, and PCR amplification, followed by transposon-based library construction. Corresponding population controls (>50,000 cells for *in vitro* samples; ~2,000-20,000 cells for *in vivo* samples, as available) were also profiled, with at least two replicates for each condition.

[00170] The libraries were filtered by a set of quality metrics, removing 223 (~21%) of the 1,029 profiled cells, and controlled for quantitative confounding factors and batch effects (Figures S1A,B). ~7,000 appreciably expressed genes (fragments per kilobase of exon per million (FPKM) > 10) in at least 20% of each sample's cells) were retained for *in vitro* experiments and ~4,000 for *in vivo* ones. To account for expressed transcripts that are not detected (false negatives) due to the limitations of single-cell RNA-Seq (Deng et al., 2014; Shalek et al., 2014), subsequent analysis down-weighted the contribution of less reliably measured transcripts (Shalek et al., 2014) (Figure S1C. Following these filters, expression profiles were tightly correlated between population replicates (Figure 1C), and the average expression across all single cells correlated well with the matching bulk population profile (r ~ 0.76-0.89; Figure 1C, Figure S1D, red bars, and Table S1). While the average expression of single cells correlated well with the bulk population, substantial differences were found in expression between individual cells in the same condition (r ~ 0.3-0.8; Figure 1D and Figure S1D, blue bars) comparable to previous observations in other immune cells (Shalek et al., 2014). High-throughput, high-resolution, flow RNA-fluorescence was applied *in situ* hybridization (RNA-FlowFISH), an amplification-free imaging technique (Lalmansingh et al., 2013) to validate the observed patterns of gene expression heterogeneity for nine representative genes (Figure 1F, Figure 6E), chosen to span a wide range of expression and variation levels at 48h under the TGF-β1+IL-6 *in vitro* polarization condition. These experiments reveal that although canonical Th17 transcripts (*e.g.*, *Rorc, Irf4, Batf*) are expressed unimodally, other key immune transcripts (*e.g.*, *Il-17a, Il-2*) can vary in their expression across Th17 cells and exhibit a bimodal distribution. The analysis of this variation

can provide clues on the functional states of the Th17 cells that have been associated with different disease states or specificity to various pathogens.

[00171] *A functional annotation of single cell heterogeneity shows that Th17 cells span a spectrum of states in vivo.* To study the main sources of cellular variation *in vivo* and their functional ramifications, a principal component analysis (PCA, Figure 2A) was used followed by a novel analysis for functional annotation of the PC space based on the single cell expression of gene signatures of previously characterized T cell states (Figure 2B). Specifically, drawing from previous studies feature-specific gene signatures were assembled for various T-cell types and perturbation states, each consisting of a set of 'plus' and 'minus' genes that are highly and lowly expressed in each signature, respectively (Figure 2B). For every cell-signature pair, a score reflecting the difference in the average expression of 'plus' *vs.* 'minus' genes in that cell was computed, and then estimated whether each signature score significantly varied: either (1) across cells of the *same* source (either LN or CNS; using a one *vs.* all Gene Set Enrichment Analysis (GSEA); FDR < 0.05 in at least 10% of cells); or (2) between the LN and the CNS cells (KS-test, FDR<10^{-4}). For the signatures with significant variation in at least one test, the correlations of the respective single cell signature scores with the projection of cells to each of the first two principal components (PCs; Figure 2B and Table S2 (Gaublomme 2015)) were computed, and selected correlations were plotted on a normalized PCA map (Figure 2A, numbered open circles). To identify transcription factors that may orchestrate this heterogeneity, the single-cell RNA-seq data were combined with transcription factor target enrichment analysis (Yosef et al., 2013) to find factors whose targets are strongly enriched (Fisher exact test, p<10^{-5}) in genes that correlated with each PC (Pearson correlation, FDR <0.05; Figures 2E,F, Table S3 (Gaublomme 2015)).

[00172] Based on the functional annotation, the first PC (PC1) positively correlates with a recently defined effector *vs.* memory signature following viral infection (Crawford et al., 2014), and negatively correlates with an independent molecular signature characterizing memory T cells (Wherry et al., 2007) (Figure 2A, number 4 and 7, respectively; Table S2 (Gaublomme 2015)). This suggests that cells with high positive PC1 scores adopt an effector phenotype, and those with negative PC1 scores obtain a memory profile, and at the extreme – a dysfunctional/senescent profile. The second PC (PC2) separates cells by their source of origin (CNS and LN, Figure 2A) and correlates with a transition from a naïve-like self-

renewal state (negatively correlated with PC2; $p<10^{-33}$, Figure 2A, number 5; Table S2 (Gaublomme 2015)) with low cell cycle activity (negatively correlated with PC2, FDR<5%) to a Th1-like effector or memory effector state (positively correlated with PC2, Figure 2, number 2 and 3, $p<10^{-19}$ and $p<10^{-23}$, respectively). Consistently, an MsigDB analysis of genes that highly correlate with the PCs (Pearson correlation, FDR<5%) shows strong association with immune response (PC1; $p< 1.2X10^{-27}$ and PC2; $p< 1.2X10^{-28}$, hyoerhgeometric test) and cell cycle stage (PC1; $p< 10^{-30}$).

[00173] *A trajectory of progressing cell states from the LN to the CNS.* To further explore the diversity of LN and CNS cells, five of the key signatures discovered by functional annotation were used to divide the PCA space into distinct subsets of cells (Figure 2C, Table S2 (Gaublomme 2015)). To this end, a Voronoi diagram was computed that delineates regions that are most strongly associated with each of the five signatures. The resulting putative subpopulations exhibit a gradual progression from a self-renewing state to a pre-Th1 effector phenotype in the LN and CNS, to a Th1-like effector state and a Th1-like memory state in the CNS, and finally a dysfunctional/senescent state in the CNS, as detailed below.

[00174] First, self-renewing Th17 cells in the LN (Figure 2C, green) are characterized by: (1) a signature of Wnt signaling ($p<10^{-7}$, KS, Figure 2A, number 6, Table S4 (Gaublomme 2015)), Table 6, a known feature critical for self-renewal of hematopoietic stem cells and survival of thymocytes (Ioannidis et al., 2001; Reya et al., 2003), and supported by high expression of *Tcf7* ($p<10^{-12}$, Figure 2D, Table S4 (Gaublomme 2015)) Table 6, a key target of the Wnt pathway. *Tcf7* is a key transcription factor regulating the stem cell-like state of Th17 cells (Muranski et al., 2011), whose expression is lost when T-cells acquire an effector phenotype (Gattinoni et al., 2009; Willinger et al., 2006); (2) high expression ($p<10^{-10}$, KS-test, see Table S4 (Gaublomme 2015), Table 6) of the known naïve state marker *Cd62l* (De Rosa et al., 2001) (Figure 2D); and (3) up-regulation ($p<10^{-9}$) of *Cd27*, a pro-survival gene lacking in short-lived T cells (Dolfi et al., 2008; Hendriks et al., 2000; Hendriks et al., 2003; Snyder et al., 2008) (Figure 2D). Transcription factors analysis (negative PC2, Figure 2E, green) suggests that *Etv6*, *Med12* and *Zfx* specifically drive this self-renewing population. While neither of them has been linked to Th17 self-renewal, each is associated with such functions in other cells: Med12 is essential for *Wnt* signaling and early mouse development (Rocha et al., 2010); *Etv6*, a known positive regulator of Th17 cell differentiation (Ciofani et

al., 2012; Yosef et al., 2013), functions as an essential regulator of hematopoietic stem cell survival (Hock et al., 2004) and an initiator of self-renewal in pro-B cells (Tsuzuki and Seto, 2013); and *Zfx* is required for self renewal in embryonic and hematopoietic stem cells (Galan-Caridad et al., 2007; Harel et al., 2012), and of the tumorigenic, non-differentiated state in glioblastoma stem cells (Fang et al., 2014) and acute T-lymphoblastic and myeloid leukemia (Weisberg et al., 2014).

[00175] Second, cells from the LN and CNS adopt similar (overlapping) cell states only in the central state of PCA plot (Figure 2C, pink), reflecting effector Th17 cells with a pre-Th1 phenotype. Compared to the self-renewing subpopulöation, these effector Th17 cells (1) begin to express receptors for IFN (IFNAR-1, $p<10^{-3}$, KS, Table S4 (Gaublomme 2015), Table 6) and IL-18 (IL-18R1, $p<10^{-11}$, Figure 2D), both of which mediate differentiation of Th1 cells (Esfandiari et al., 2001; Shinohara et al., 2008); and (2) induce the Th1 associated chemokine receptor *Cxcr6* ($p<10^{-13}$, KS, Figure 2D) (Aust et al., 2005; Latta et al., 2007), and *Ccr2* ($p\leq10^{-6}$, KS, Figure 2D), associated with recruitment to the CNS in EAE/MS (Mahad and Ransohoff, 2003). Since these cells begin to express receptors that make them responsive to both IFN-γ and IL-18 and poised for recruitment to the CNS, they may therefore be the precursors that lead to the generation of Th17/Th1-like effector T cells observed in the CNS.

[00176] IL-17a/GFP+ sorted cells acquire a Th17/Th1-like effector phenotype in the CNS (Figure 2C, yellow), as indicated by up-regulation ($p<10^{-3}$, KS, Table S4 (Gaublomme 2015) , Table 6) of: (1) *Ifn-γ*, consistent with a Th1 phenotype (Figure 2D); (2) *Rankl* (Figure 2D), a marker of Th1 and IL-23 induced Th17 cells (Nakae et al., 2007), especially pathogenic Th17 cells in arthritis (Komatsu et al., 2014); and (3) cell cycle genes (*e.g.*, Geminin (Codarri et al., 2011), Figure 2D). Surprisingly, the Th1-like cells in the CNS (except dysfunctional/senescent state; Figure 2C,D grey) also induce Ccr8 (Figure 7A, bottom), previously described as a cell marker of Th2 cells (Zingoni et al., 1998), but not of Th17 / Th1 cells (Annunziato et al., 2007). Mice deficient for *Ccr8* exhibit later onset and milder signs of EAE (Ghosh et al., 2006; Hamann et al., 2008). Transcription factor analysis shows that these effector cells are associated with both canonical Th17 factors (Stat3, Irf4 and Hif1a) and Th1-associated factors, including *Rel* and *Stat4* (Kaplan et al., 1996; Nishikomori et al., 2002; Thierfelder et al., 1996) (Figure 2E, red), which are associated with EAE (Hilliard et al., 2002; Mo et al., 2008) or with autoimmune disease in humans (Gilmore and Gerondakis, 2011).

These sorted IL-17A/GFP+ cells could either be a stable population of double producers or reflect Th17 plasticity into the Th1 lineage, as Th17 cells transition into a Th1 state.

[00177] Next, Th1-like memory cells detected in the CNS (Figure 2C, light blue) correlate highly with both a memory phenotype (negative PC1) and a Th1-like phenotype (positive PC2). These cells are associated with an effector memory signature ($p<10^{-5}$, KS-test compared with all other sub-populations, see Table S4 (Gaublomme 2015) , Table 6), and up-regulate ($p<10^{-5}$, KS) memory signature genes (*e.g.*, *Nur77;* Figure 2D, *Samsn1*, *Il2ra*, *Il2rb*, *Tigit*, *Ifngr1* and *2*), and inflammatory genes (*Gm-csf* and *Gpr65;* Figure 2D). *Il-1r2* is a decoy receptor in the IL-1 pathway involved in Th17 pathogenicity (Sutton et al., 2006), the cytokine *Gm-csf* (Figure 2D) is essential for Th17 encephalitogenicity (El-Behi et al., 2011) and neuroinflammation (Codarri et al., 2011). *Nur77* (*Nr4a1*) (Figure 2D), a transcriptional repressor of IL-2 (Harant and Lindley, 2004), is strongly up-regulated, to maintain cells in a Th17 state despite acquiring a Th1 factor (Sester et al., 2008). Note that while IL-2 is a growth factor for Th1 cells, IL-2 affects Th17 differentiation and stability. Transcription factor analysis (Figure 2F) suggests that this cell state is in part driven by *Egr1*, a regulator of *Tbet* expression (Shin et al., 2009) that may help route Th1-like cells into the memory pool; *Bcl6*, a repressor of lymphocyte differentiation, inflammation, and cell cycle genes, essential for CD4 T-cell memory generation (Ichii et al., 2007); and *Hif1a*, crucial for controlling human Th17 cells to become long-lived effector memory cells (Kryczek et al., 2011) and particularly associated with cells that correlate highly with the memory and Th1 signatures (negative PC1, positive PC2).

[00178] Finally, Th17 cells acquire a dysfunctional, senescent-like state in the CNS (negative PC1 and PC2 scores; Figure 2C, moss grey), with (1) down-regulation ($p<10^{-3}$) of genes critical to T-cell activation, including Cd3 (Figure 2D) (Chai and Lechler, 1997; Lamb et al., 1987; Trimble et al., 2000), *Cd28* (Trimble et al., 2000; Wells et al., 2001), *Lat* (Figure 2D) (Hundt et al., 2006), *Lck* (Isakov and Biesinger, 2000; Nika et al., 2010), and *Cd2* (Bachmann et al., 1999; Lamb et al., 1987) (Table S4 (Gaublomme 2015), Table 6); (2) up-regulation of genes associated with senescence, such as *Ccrl2* (up regulated in exhausted CD8+ T-cells (Wherry et al., 2007)), *Marcks* (Figure 2D) (inducer of senescence (Jarboe et al., 2012)), and *Cd74* (a receptor to *Mif* in the *Hif-Mif* senescence pathway (Maity and Koumenis, 2006; Salminen and Kaarniranta, 2011; Welford et al., 2006)); and (3) association

with signatures for CD28 costimulation ($p<10^{-11}$, GSEA, Table S2 (Gaublomme 2015)) and PD-1 signaling ($p<10^{-10}$, GSEA, Table S2 (Gaublomme 2015)). Among the possible regulators of this cell state is *mir-144*, an inhibitor of TNF-α and IFN-γ production and of T-cell proliferation (Liu et al., 2011), whose targets are enriched ($p<10^{-4}$, hypergeometric test) in these cells.

[00179] *In vitro derived cells span a broad spectrum of pathogenicity states with key similarities and distinctions from in vivo isolated cells.* The analysis of *in vivo* Th17 cells harvested from mice undergoing EAE identified a progressive trajectory of at least five states, from self-renewing cells in the LN, through effector LN cells, effector Th1-like CNS cells, memory cells, and senescent ones. Given the limited number of cells available from *in vivo* samples, obtained as a mixed "snapshot" of an asynchronous process, it is difficult to determine their distinct pathogenic potential and underlying regulatory mechanisms. A complementary strategy is offered by profiling *in vitro* differentiated cells, where one can assess the heterogeneity of Th17 cells at the same condition (time point and cytokine stimulation). Furthermore, comparing *in vivo* and *in vitro* profiles can help uncover to what extent the *in vitro* differentiation conditions faithfully mirror *in vivo* states.

[00180] Single-cell RNA-seq profiles of 414 individual Th17 cells derived under non-pathogenic conditions (TGF-β1+IL-6, unsorted: 136 cells from 2 biological replicates, TGF-β1+IL-6, sorted for IL-17A/GFP+: 159 cells from 3 biological replicates) and pathogenic conditions (Il-1β+IL-6+IL-23, sorted for IL-17a/GFP+: 147 cells from 2 biological replicates) (Figure 3A) were then analyzed.

[00181] Using the functional annotation approach (Figure 2B) to annotate the cells with immune cell signatures, it was found that i*n vitro* differentiated Th17 cells vary strongly in a key signature of pathogenicity and tolerance (Lee et al., 2012), reflecting the conditions in which they were derived (Figure 3A, number 1, and 3D). High pathogenicity scores were associated with IL-17A/GFP+ sorted cells polarized under a pathogenic condition (Figure 3A,D red, number 1, PC1), whereas IL-17A/GFP+ sorted cells from non-pathogenic conditions correlate highly with the expression of regulatiory cytokines, such as IL-10, and their targets, which are barely detected in the pathogenic cells (Figure 3E). Finally, a signature obtained from the T-cells harvested from IL23R knockout mice and differentiated under the IL-1β+IL-6+IL23 condition correlates highly with the cells that adopt a more regulatory

profile, further confirming a crucial role of the IL-23 pathway in inducing a pathogenic phenotype in Th17 cells (Figure 3A, number 4, positive PC1).

[00182] Importantly, there is a clear zone of overlap in cell states between the pathogenic and non-pathogenic conditions, with pathogenic-like cells present (in a small proportion) in populations differentiated in non-pathogenic conditions (Figure 3A, red oval shading). In particular, cells polarized under the non-pathogenic (TGF-β1+IL-6) condition that were not specifically sorted to be IL-17A/GFP+ span the broadest pathogenicity spectrum: from cells resembling the least pathogenic cells in the IL-17A/GFP+ TGF-β1+IL-6 condition to those resembling more pathogenic cells in the IL-17A/GFP+IL-1β+IL-6+IL23 condition (Figure 3D, open black circles). At one end of this spectrum Th17 cells were observed with high expression of regulatory transcripts such as IL-9, IL-16, Foxp1 and Podoplanin Peters et al. 2014) (Figure 7B, left), and at the other end, Th17 cells were observed that express high levels of pro-inflammatory transcripts such as IL-22, IL23r, Cxcr3 and Gm-csf (Figure 7B, right).

[00183] To relate the *in vitro* differentiated cells to the *in vivo* observed behavior the *in vitro* cells (Figure 2B) were scored for immune related genes that characterize the *in vivo* identified subpopulations (Figure 2C) (Figure 3B,C). Cells derived in the non-pathogenic conditions scored more highly for the self-renewing signature ($p<1e-9$ KS test; Table S2 (Gaublomme 2015) and Figure 3A, number 6, and 3C), whereas those derived in pathogenic conditions resembled more the Th-17/Th-1 like memory phenotype identified in the CNS ($p<1e-7$ KS test; Table S2 (Gaublomme 2015) and Figure 3B).

[00184] *Co-variation with pro-inflammatory and regulatory modules in Th17 cells highlights novel candidate regulators.* The cellular heterogeneity within a single population of *in vitro* differentiated cells was then leveraged to identify regulators that might selectively influence pathogenic *vs.* nonpathogenic states of Th17 cells. Focusing on the (unsorted) cells from the TGF-β1+IL-6 *in vitro* differentiation condition, in which the broadest spectrum of cells spanning from pathogenic to nonpathogenic-like profiles was observed, first transcriptome-wide gene expression distributions across the population were analyzed. About 35% (2,252) of the detected genes are expressed in >90% of the cells (Figure 4A) with a unimodal distribution: these include housekeeping genes ($p<10^{-10}$, hypergeometric test, Figure 6F & 6G), the Th17 signature cytokine *IL-17f*, and transcription factors (*e.g.*, *Batf, Stat3 and*

Hif1a) that are essential for Th17 differentiation. On the other hand, bimodally expressed genes (Figure 4A, bottom) – with high expression in at least 20% of the cells and much lower (often undetectable) levels in the rest – include cytokines like *Il-17a* and *Il-10* and other pro-inflammatory (*e.g.*, *Il-21, Ccl20*) and regulatory cytokines or their receptors (*Il-24, Il-27ra,* Figure 4A). This suggests that variation in expression across Th17 cells may be related more to their (varying) pathogenicity state than to their (more uniform) differentiation state. Furthermore, while almost all cells express transcripts encoding the pioneer and master transcription factors for the Th17 lineage (*Rorc, Irf4, Batf*), a minority (<30%) also express transcripts encoding one or more of the transcription factors and cytokines that characterize other T-cell lineages (*e.g.*, *Stat4* for Th1 cells, and *Ccr4* for Th2 cells). This may suggest the presence of "hybrid" double-positive cells, consistent with reports on plasticity in T-cell differentiation (Antebi et al., 2013), and/or reflect the previous model of duality in the Th17 transcriptional network (Yosef et al., 2013). Finally, the expression of many key immune genes varies more than the rest of the genome, even with the same mean expression level (Figure 6H), or when only considering the expressing cells (Figure 6I), implying a greater degree of diversity in immune gene regulation. While such patterns may be biologically important, they must be interpreted with caution. First, some (*e.g.*, *Il-17a*, *Il-24* and *Ccl20*), but not all (*e.g.*, *Il-9*), of the transcripts with bi-modal patterns are also lowly expressed (on average) and thus may not be detected as reliably (Shalek et al., 2014). Second, transcription bursts coupled with instability of transcripts may lead to 'random' fluctuations in gene expression levels at any given cell.

[00185] To overcome these challenges and to identify candidate regulators of pathogenicity, co-variation between transcripts across cells (Figure 4B) was analyzed. It was reasoned that if transcript variation reflects distinct physiological cell states, entire gene modules should robustly co-vary across the cells. Furthermore, transcription factors and signaling molecules that are members of such modules may highlight new putative regulators of these modules and functional states. Focusing on significant co-variation (Spearman correlation; FDR < 0.05) between each bimodally expressed transcript (expressed by less than 90% of the cells; Figure 4B, rows) and a curated set of bimodally expressed immune response genes (cytokines, cytokine receptors, T helper cell specific signatures, Figure 4B, columns), two key transcript modules were found: a pro-inflammatory module (Figure 4B, orange) of

transcripts that co-vary with known Th17 cytokines, such as *Il-17a* and *Ccl-20*, and a regulatory module (Figure 4B, green) of transcripts that co-vary with known regulatory genes, such as *Il-10*, *Il-24*, and *Il-9*. Using these modules as signatures to annotate the original *in vitro* cell states (Figure 3A and 4C), the pro-inflammatory module (Figure 4C, number 1) and key inflammatory genes (Figure 4D, bottom) are correlated with the most pathogenic cells (PC1, negative correlation) and the regulatory module (Figure 4C), and key members (Figure 4ED, top), are correlated with the least pathogenic (PC1, positive correlation).

[00186] Co-variation of genes with each module highlights many novel putative regulators, many not detected by previous, population-level, approaches (Ciofani et al., 2012; Yosef et al., 2013). To select the most compelling candidate genes in the two modules (Figure 4b, rows) for follow-up functional studies, a computational ranking scheme was developed that considers each gene's correlation with the pro-inflammatory or regulatory modules, their loading on the first *in vitro* PC marking for pathogenic potential, and their role in the EAE context *in vivo* (Figure 4E, Table 2 herein). While the genes from our co-variation matrix (rows, **Figure 4B**) tend to be highly ranked compared to all genes also in bulk-population data ($p<10^{-10}$, Wilcoxon Ranksum test) or rankings (Ciofani et al., 2012), they do not necessarily stand out in bulk population rankings (**Figure 15**), highlighting the distinct signal from single-cell profiles. Based on this ranking and availability of knockout mice, three genes were chosen for functional follow up: *Plzp, Cd5l and Gpr65* that are co-expressed with the pro-inflammatory module, and *Toso*, co-expressed with the regulatory module. None of these genes was previously implicated in differentiation or effector function of Th17 cells.

[00187] *GPR65 promotes Th17 cell pathogenicity and is essential for EAE.* GPR65, a glycosphingolipid receptor, is co-expressed with the pro-inflammatory module (Figure 4B), suggesting that it might have a role in promoting pathogenicity. GPR65 is also highly expressed in the *in vivo* Th17 cells harvested from the CNS that attain a Th1-like effector/memory phenotype (Figure 2D). Importantly, genetic variants in the GPR65 locus are associated with multiple sclerosis (International Multiple Sclerosis Genetics et al., 2011), ankylosing spondylitis (International Genetics of Ankylosing Spondylitis et al., 2013), inflammatory bowel disease (Jostins et al., 2012), and Crohn's disease (Franke et al., 2010).

[00188] The role of GPR65 was tested in Th17 differentiation *in vitro* and in the development of autoimmunity *in vivo*. Naïve T-cells isolated from *Gpr65*$^{-/-}$ mice *in vitro*

were differentiated with TGF-β1+IL-6 (non-pathogenic condition) or with IL-1β+IL-6+IL-23 (pathogenic condition) for 96 hours. In both cases, there was a ~40% reduction of IL-17a positive cells in *Gpr65*$^{-/-}$ cells compared to their wild type (WT) controls as measured by intracellular cytokine staining (ICC) (Figure 5A). Memory cells from *Gpr65*$^{-/-}$ mice that were reactivated with IL-23 also showed a ~45% reduction in IL-17a-positive cells when compared to wild type controls (Figure S3A). Consistently, an enzyme-linked immunosorbent assay (ELISA) of the supernatant obrtained from the activated Th17 culture showed a reduced secretion of IL-17a ($p<0.01$) and IL-17f ($p<10^{-4}$) (Figure 5B) and increased IL-10 secretion ($p<0.01$, Figure S3A) under pathogenic (IL-1β+IL-6+ L-23) Th17 differentiation conditions in the knockout mice.

[00189] To further validate the effect of GPR65 on Th17 function, RNA-seq profiles were measured of a bulk population of *Gpr65*$^{-/-}$ Th17 cells, differentiated *in vitro* under both non-pathogenic (TGF-β1+IL-6) and pathogenic (IL-1β+IL-6+IL-23) conditions for 96 hours. Supporting a role for GPR65 as a driver of pathogenicity of Th17 cells, it was found that genes up-regulated in *Gpr65*$^{-/-}$ cells (compared to WT) are most strongly enriched ($P< 10^{-28}$, hypergeometric test, Figure 5E) for the genes characterizing the more regulatory cells under TGF-β1+IL-6 (positive PC1, Figure 4C, Table S6 (Gaublomme 2015), Table 7).

[00190] To determine the effect of loss of GPR65 on tissue inflammation and autoimmune disease *in vivo*, RAG-1$^{-/-}$ mice were reconstituted with naïve CD4+ T-cells from wild type or *Gpr65*$^{-/-}$, then induced EAE with myelin oligodendrocytes glycoprotein peptide emulsified with complete Freund's adjuvant (MOG35-55/CFA). It was found that in the absence of GPR65-expressing T cells, mice are protected from EAE (Figure 5D) and far fewer IL-17A and IFN-γ positive cells are recovered from the LN and spleen compared to wild-type controls transferred with wild-type cells (Figure S3B). Furthermore, *in vitro* restimulation with MOG_{35-55} of the spleen and LN cells from the immunized mice showed that loss of GPR65 resulted in dramatic reduction of MOG-specific IL-17A or IFN-γ positive cells compared to their wild-type controls (Figure 5C), suggesting that GPR65 regulates the generation of encephalitogenic T cells *in vivo*. Taken together, the data strongly validates that GPR65 is a positive regulator of the pathogenic Th17 phenotype, and its loss results in protection from EAE.

[00191] *TOSO is implicated in Th17-mediated induction of EAE.* TOSO (FAIM3) is an immune cell specific surface molecule, is known to negatively regulate *Fas*-mediated apoptosis (Hitoshi et al., 1998; Nguyen et al., 2011; Song and Jacob, 2005), and is co-expressed with the regulatory module in Th17 cells. Although its covariance with the regulatory module (Fogure 4B) may naively suggest that it positiviely regulates the regulatory module. *Toso* knockout mice were recently reported to be resistant to EAE (Lang et al., 2013). This may be consistent with a hypothesis that Toso is a negative regulatore of the non-pathogenic state, co-expressed with the regulatory module, as has been often obsreved for negative regulators and their targets in other systems (Amit et al., 2007; Segal et al., 2003) To test this hypothesis, *in vitro* differentiation and MOG recall assays on $TOSO^{-/-}$ cells were performed. Differentiation of $TOSO^{-/-}$ cells showed a defect in the production of pro-inflammatory cytokine IL-17A for both differentiation conditions (Figure 5F), which was confirmed by ELISA (Figure 5G). Moreover, memory cells stimulated with IL-23 show a lack of IL-17A production (Figure S4A). Consistently, in a MOG recall assay, $CD3^{+}CD4^{+}$ *Toso*$^{-/-}$ T cells showed no production of IL-17a across a range of MOG_{35-55} concentrations (Figure 5H). This supports a role for TOSO as a promoter of pathogenicity.

[00192] To further explore this, RNA-seq analysis of *Toso*$^{-/-}$ Th17 cell populations, differentiated *in vitro* under non-pathogenic conditions for 96 hours was performed. Loss of TOSO results in suppression of the key regulatory genes (e.g., IL-24 (FC=0.08), IL-9 (FC=0.33) and Procr (FC=0.41)(Table S6 (Gaublomme 2015), Table 7), consistent with the reduction of IL-10 production as measured by ELISA (Figure S4C), and a reduced number of FOXP3+ cells under Treg differentiation conditions (Figure S4B). On the other hand, in pathogenic conditions, IL-17a (FC=0.21) is down regulated in the absence of TOSO. Enrichment analysis with respect to PC1 of the non-pathogenic differentiation condition suggests that TOSO knockout cells, rather than up-regulating regulatory genes, down-regulate genes associated with a more pro-inflammatory cell phenotype (Figure 5E). Taken together, the data suggest that TOSO plays a critical role as a positive regulator of Th17-cell mediated pathogenticity.

[00193] *MOG-stimulated Plzp*$^{-/-}$ *cells have a defect in generating pathogenic Th17 cells.* PLZP (ROG), a transcription factor, is a known repressor of (the Th2 master regulator) GATA3 (Miaw et al., 2000), and regulates cytokine expression (Miaw et al., 2000) in T-

helper cells. Since *Plzp* is co-expressed with the pro-inflammatory module, it was hypothesized that it may regulate pathogenicity in Th17 cells.

[00194] While *in vitro* differentiated *Plzp*$^{-/-}$ cells produced IL-17A at comparable levels to wild-type (Figure S5A), a MOG-driven recall assay revealed that *Plzp*$^{-/-}$ cells do have a defect in IL-17A production that becomes apparent with increasing MOG concentration during restimulation (Figure 5I). Furthermore, *Plzp*$^{-/-}$ cells also produced less IL-17A than wild-type cells when reactivated in the presence of IL-23, which acts to expand previously *in vivo* generated Th17 cells (Figure S5B). Finally, *Plzp*$^{-/-}$ T cells secreted less IL-17A, IL-17F (Figure 5J), IFN-γ, IL-13 and GM-CSF (Figure S5C). These observations suggest that PLZP regulates the expression of a wider range of inflammatory cytokines. Based on RNA-Seq profiles, at 48 hours into the non-pathogenic differentiation of *Plzp*$^{-/-}$ cells, *Irf1* (FC=5.2), *Il-9* (FC= 1.8) and other transcripts of the regulatory module are up regulated compared to WT (Table S6 (Gaublomme 2015), Table 7), whereas transcripts from the pro-inflammatory module, such as *Ccl-20* (FC=0.38), *Tnf* (FC=0.10) and *Il-17a* (FC=0.42), are repressed. A similar pattern is observed with respect to PC1, where genes characterizing the more pro-inflammatory cells are strongly enriched among the down-regulated genes in *Plzp*$^{-/-}$ T cells (Figure 5E).

[00195] *DISCUSSION:* Genome-wide analysis of single-cell RNA expression profiles opens up a new vista for characterizing cellular heterogeneity in ensembles of cells, previously studied as a population. By profiling individual Th17 cells from the LN and CNS at the peak of EAE, it was found that Th17 cells adopt a spectrum of cellular states, ranging from cells with a self-renewing gene signature, to pro-inflammatory Th1-like effector or memory-like cells, to a dysfunctional/senescent phenotype. These findings shed light on the controversy in the field on whether Th17 cells are short-lived, terminally differentiated, effector cells (Pepper et al., 2010) or long-lived self-renewing T cells (Muranski et al., 2011). The analysis also shows that Th17 cells present in the lymph node and CNS generally appear to have different transcriptional profiles and that the only group of Th17 cells that transcriptionally overlap are those that attain a pre-Th1-like state with acquisition of cytokine receptors (like IL-18R) that push Th17 cells into a Th1 phenotype. This fits well with the data that most Th17 cells begin to co-express Th1 genes in the CNS and become highly pathogenic.

[00196] The Th1-like phenotype of Th17 cells observed in the CNS might facilitate memory cell formation, as the entry of Th1 cells into the memory pool is well established (Harrington et al., 2008; Sallusto et al., 1999). It is unclear if cells that adopt a Th1 phenotype are stable 'double producers' or if they show plasticity towards a Th1 fate. IL-23, which induces a pathogenic phenotype in Th17 cells has been shown to induce IFN-g in Th17 cells. Consistent with this data, *IL-23R*-deficient mice have lower frequencies of double producers (McGeachy et al., 2009) and chronic exposure of Th17 cells to IL-23 induces IFN-g production from Th17 cells. Additionally, a conversion from a Th17 to a Th1-like phenotype is also documented in other disease models and these are considered to be the most pathogenic T cells (Bending et al., 2009; Lee et al., 2009; Muranski et al., 2011; Palmer and Weaver, 2010; Wei et al., 2009b).

[00197] Despite being differentiated under the same culture conditions, *in vitro* differentiated Th17 cells also exhibit great cellular diversity, with a pathogenic, pro-inflammatory state on the one end of the spectrum and an immunosuppressive, regulatory state on the other end. A comparative analysis of *in vivo* and *in vitro* derived cells with respect to immune-related genes reveals that *in vitro* polarization towards a pathogenic Th17 phenotype (with IL-1β+IL-6+IL-23) produces cells that resemble more the Th17/Th1 memory cells in the CNS found during EAE (Figure 3A).

[00198] Single cell RNA-seq further showed that pro-inflammatory genes that render Th17 cells pathogenic and regulatory genes that render Th17 cell nonpathogenic are expressed as modules in groups of Th17 cells. This allowed for dissection of factors that relate to this specific facet of th17 cell functionality, rather than their general differentiation. Strong correlation (either positive or negative) between two genes suggests that their biological function may be linked. In this study, strong co-variation with key Th17 genes allowed us to recover many known regulators, but also to identify many promising novel candidates that were coexpressed with either a proinflammatory or a regulatory module in Th17 cells. For example, *Gpr65* positively correlated with the *in vitro* derived pro-inflammatory gene module. Consistently, *Gpr65*$^{-/-}$ CD4 T cells reconstituted to Rag1 mice were incapable of inducing EAE and had compromised IL-17A production. There are many genes similarly highlighted by this analysis, including *Gem*, *Cst7*, and *Rgs2*, all of which significantly correlate with the *in vitro* derived pro-inflammatory gene module and are highly expressed in

the *in vivo* Th17/Th1-like memory subpopulation the are present in the CNS during peak inflammaiton. *Foxp1*, on the other hand, one of the genes negatively correlated with the pro-inflammatory module, was lowly expressed in the inflammatory Th17/Th1-like subpopulations *in vivo*, but was highly expressed in the LN-derived Th17 self-renewing subpopulation ($p<10^{-7}$, KS test; Table S4 (Gaublomme 2015), Table 6). In line with this finding, in T follicular helper cells, Foxp1 has very recently been shown to directly and negatively regulate IL-21 (Wang et al., 2014), a driver of Th17 generation (Korn et al., 2007; Nurieva et al., 2007; Zhou et al., 2007), and to dampen the expression of the co-stimulatory molecule ICOS and its downstream signaling at the early stages of T-cell activation (Wang et al., 2014). Further functional studies with *Foxp1* knockout mice in the context of EAE could elucidate its potential role in regulating Th17 cell differentiation and development of autoimmune tissue inflammation.

[00199] Importantly, it should be noted that the co-variation of a gene with the pro-inflammatory or regulatory module does not necessarily indicate a pro-inflammatory or regulatory function to this gene. For example, one of the follow-up genes, *Toso*, co-varies with the regulatory module, but its absence protects mice from EAE (Brenner et al., 2014) and compromises IL-17A production, suggesting *Toso* does not serve as a regulatory factor. This is consistent with previous studies – from yeast (Segal et al 2003) to human (Amit et al 2007), showing how regulators with opposite, antagonistic functions, are co-regulated.

[00200] Examining the single-cell RNA-seq data together with ChIP data reveals transcription factors that regulate various cellular states observed in the study. For example, *Zfx* was identified as a strong candidate regulator of the self-renewing state of Th17 cells in the LN, because its targets are strongly enriched in this subpopulation, it is a known regulator of self-renewal in stem cells (Cellot and Sauvageau, 2007; Galan-Caridad et al., 2007; Harel et al., 2012), and it prevents differentiation in leukemias (Weisberg et al., 2014). In contrast, for the pathogenic effector and memory cells observed in the CNS during EAE, a prominent role is assigned to known Th17/Th1 transcription factors such as *Hif1a*, *Fosl2*, *Stat4* and *Rel*, and it is specified in which subpopulations their regulatory mechanisms contribute to disease. As such, this study elaborates on Th17 pathogenicity beyond differentiation and development. This data suggests that processes such as self-renewal, observed in the lymph node, may provide a pool of cells that are precursors for differentiating Th17 cells to effector/ memory formation in the CNS that may contribute to

Th17 pathogenicity in EAE. These cellular functional states enable us to map the contribution of novel and known genes to each of these processes during Th17 differentiation and function. Whereas population-based expression profiling has enabled identification of cytokines and transcription factors that set the differentiation states of Th17 cells, using single cell RNA-seq new granularity is provided in the transcriptome of a rather homogenous population of T cells. Many of the novel regulators that identified by single cell RNA-seq are regulating pathogenic *vs.* nonpathogenic functional states in Th17 cells. These novel regulators will allow the manipulation of pathogenic Th17 cells without affecting nonpathogenic Th17 cells that may be critical for tissue homeostasis and for maintaining barrier functions.

[00201] *Single-cell RNA-seq identifies CD5L as a candidate regulator of pathogenicity. Cd5l* is one of the high-ranking genes by single-cell analysis of potential regulators, exhibiting two surprising features: although *Cd5l* is expressed in Th17 cells derived under non-pathogenic conditions (**Figure 16A**), in these non-pathogenic cells, *Cd5l* positively correlates with the first PC of *in-vitro* derived cells and co-varies with other genes in the pro-inflammatory module (**Figure 19A, B, C**). In addition, *Cd5l* positively correlates with the cell pathogenicity score (**Figure 16B, C**). Comparing *Cd5l* expression at the single-cell level in Th17 cells (sorted IL-17.GFP+) derived *in vitro* showed ~80% of Th17 cells derived with IL-1β+IL-6+IL-23 lacked *Cd5l* expression, whereas Th17 cells differentiated with TGF-β1+IL-6 predominantly expressed *Cd5l* (**Figure 16A**). Neither Th17 cells differentiated under an alternative pathogenic condition (TGF-β3+IL-6) nor encephalitogenic Th17 cells sorted from the CNS of mice undergoing active EAE expressed *Cd5l* at the single-cell level (**Figure 16A**). However, *Cd5l* expressed in nonpathogenic Th17 cells (unsorted single-cell analysis, **Figure 19A**) correlates with the first PC and co-varies with the pro-inflammatory module (**Figure 19B**) that is indicative of the pathogenic signature (**Figure 19C**) as previously defined (Lee et al., 2012). Furthermore, *Cd5l* correlates with the defining signature of the pro-inflammatory module, and negatively correlates with that of the regulatory module (**Figure 16C**). Finally, it is among the top 8 genes in the single cell based pro-inflammatory module whose expression most strongly correlates with the previously defined pathogenic gene signature (**Figure 16B**, $p = 2.63 \times 10^{-5}$). CD5L is a member of the scavenger receptor cysteine rich superfamily (Sarrias et al., 2004). It is expressed in macrophages and can bind cytosolic fatty acid synthase in adipocytes following endocytosis (Miyazaki et al., 1999). CD5L is also a receptor for pathogen associated molecular patterns

(PAMPs), and may regulate innate immune responses (Martinez et al., 2014). However, its expression has not been reported in T cells, and its role in T-cell function has not been identified.

[00202] *CD5L expression is associated with non-pathogenic Th17 cells in vitro and in vivo.* Applicants determined that the preferential expression of CD5L in non-pathogenic Th17 cells, but in association with the pro-inflammatory module, may reflect a unique role for CD5L in regulating the transition between a non-pathogenic and pathogenic state. While co-expression with the proinflammatory module (**Figure 16C**) and correlation with a pathogenicity signature (**Figure 16B**) *per se* could suggest a function as a positive regulator of pathogenicity, the apparent absence of CD5L from Th17 cells differentiated *in vitro* under the pathogenic conditions or isolated from lesions in the CNS (**Figure 16A**) suggested a more nuanced role. Applicants hypothesized that CD5L is a negative regulator of pathogenicity, explaining its absence from truly pathogenic cells. In fact, mRNAs encoding negative regulators of cell states are often positively co-regulated with the modules they suppress in eukaryotes from yeast (Pe'er et al., 2002; Segal et al., 2003) to human (Amit et al., 2007).

[00203] Applicants first validated and extended the initial finding that CD5L is uniquely expressed in nonpathogenic Th17 cells by analyzing naïve CD4 T cells cultured under various differentiation conditions using qPCR and flow cytometry (**Figure 16D, E, F**). At the mRNA level, Applicants found little *Cd5l* expression in Th0, Th1 or Th2 helper T cells, high expression in Th17 cells differentiated with TGF-β1+IL-6, but low expression in Th17 cells differentiated with IL-1β+IL-6+IL-23 or in iTregs (**Figure 16D**). Protein measurements confirmed the presence of CD5L in a large proportion of non-pathogenic Th17 cells (**Figure 16F**).

[00204] Next, Applicants explored whether CD5L expression is associated with less pathogenic Th17 cells *in vivo*. Applicants analyzed Th17 cells isolated from mice induced with EAE. Th17 cells (CD3+CD4+IL-17.GFP+) sorted from the spleen expressed *Cd5l* but IL-17- T cells did not (**Figure 16G**). In contrast, *Cd5l* was not expressed in Th17 cells from the CNS despite significant expression of *Il17* (**Figure 16H**), consistent with the single-cell RNA-seq data (**Figure 16A**). Next, Applicants analyzed Th17 cells from mesenteric lymph nodes (mLN) and lamina propria (LP) of naïve mice, where Th17 cells contribute to tissue homeostasis and mucosal barrier function. IL-17+ but not IL-17- Tcells harvested from mLN and LP expressed high levels of *Cd5l* (**Figure 16I** and data not shown). Thus, CD5L is a gene expressed in non-pathogenic but not pathogenic Th17 cells *in vivo*. Applicants asked if IL-23, known to make

Th17 cells more pathogenic, can regulate *Cd5l* expression. Applicants hypothesized that if CD5L is a positive regulator of IL-23-dependent pathogenicity, its expression will be increased by IL-23, whereas if it is a negative regulator, its expression will be suppressed. As IL-23R is induced after T-cell activation, Applicants differentiated naïve T cells with TGF-β1+IL-6 for 48h and expanded them in IL-23 in fresh media. IL-23 suppressed *Cd5l* (**Figure 16E**), consistent with these cells acquiring a pro-inflammatory module and becoming pathogenic Th17 cells, and with our hypothetical assignment of CD5L as a negative regulator of pathogenicity. CD5L expression can be promoted by STAT3 but not RORγt (**Figure 19D, E**), as IL-23 can enhance STAT3 function further studies are required to elucidate the pathways involved in regulating CD5L expression.

[00205] *CD5L represses effector functions without affecting Th17 differentiation.* To analyze the functional role of CD5L *in vivo*, Applicants immunized mice with MOG_{35-55}/CFA to induce EAE. CD5L$^{-/-}$ mice exhibited more severe clinical EAE that persisted for at least 28 days, whereas wildtype (WT) mice began recovering 12 days post immunization (**Figure 17A**). Similar frequencies of FoxP3^{+} CD4^{+} Treg cells were found in WT and CD5L$^{-/-}$ mice, suggesting that the increased severity of the disease was not due to changes in the number of Tregs in CD5L$^{-/-}$ mice (**Figure 12A**). In contrast, more CD4 T cells produced IL-17 and fewer cells produced IFNγ in the CNS of CD5L$^{-/-}$ mice (**Figure 17A, 12B**). In response to MOG reactivation *in vitro*, cells from the draining lymph nodes of CD5L$^{-/-}$ mice showed higher proliferative responses and produced more IL-17 (**Figure 12C**, **12D**). These observations are consistent with either a direct or indirect role for CD5L in defining Th17 cell function. Applicants studied the impact of CD5L on Th17 cells differentiated from naïve WT and CD5L$^{-/-}$ T cells by analyzing signature gene expression. CD5L deficiency did not affect Th17 differentiation as measured by IL-17 expression (**Figure 17B**, **C**), nor did it affect other Th17 signature genes including *Il17f, Il21, Il23r, Rorc* or *Rorα* (**Figure 17D**). Of note, under the non-pathogenic differentiation condition, CD5L$^{-/-}$ Th17 cells made less IL-10 (**Figure 17C**, **D**). These observations suggest that changes in differentiation alone cannot explain the increased susceptibility to EAE in CD5L$^{-/-}$ mice, but that CD5L may indeed affect the internal state of differentiated Th17 cells. Applicants determined if CD5L regulates effector/memory Th17 cells by differentiation of nonpathogenic Th17 cells from naïve cells. Upon restimulation, more CD5L$^{-/-}$ Th17 cells produced IL-17 and expressed IL-23R without affecting viability (**Figure 17E** and data not shown), suggesting that

CD5L deficiency leads to more stable expansion of Th17 cells. Consistently, CD5L$^{-/-}$ Th17 cells expressed more *Il17* and *Il23r*, less *Il10* and similar levels of *Rorc* or *Rorα* (**Figure 17F**). Thus, CD5L does not regulate Th17 cell differentiation, but affects Th17 cell expansion and/or effector functions over time. Similarly, effector memory cells (CD4^{+}CD62LCD44^{+}) isolated *ex vivo* from CD5L$^{-/-}$ mice have higher frequencies of IL-17^{+} and lower frequencies of IL-10^{+} cells (**Figure 17G, 12E**), possibly reflecting the greater stability of Th17 cells that persist in the repertoire of CD5L$^{-/-}$ mice. To address if Th17 cells isolated *in vivo* also produced more IL-17 per-cell, Applicants sorted RORγt^{+} (GFP^{+}) effector/memory T cells from WT and CD5L$^{-/-}$ mice and found more IL-17^{+} and fewer IL-10^{+} cells in CD5L$^{-/-}$ cells, suggesting RORγt^{+} cells are better IL-17 producers in the absence of CD5L (**Figure 17H, 12F**).

[00206] *CD5L is a major switch that regulates Th17 cells pathogenicity.* To determine if loss of CD5L can convert non-pathogenic Th17 cells into disease-inducing Th17 cells, Applicants crossed CD5L$^{-/-}$ mice to 2D2 transgenic mice expressing a T-cell receptor specific for MOG$_{35-55}$/IA^{b} (Bettelli et al., 2003). Naïve CD5L$^{-/-}$ 2D2 T cells were differentiated with the nonpathogenic (TGF-β1+IL-6) Th17 condition and transferred into WT recipients. Applicants analyzed the phenotype of T cells from the CNS of mice undergoing EAE. The 2D2 CD5L$^{-/-}$ Th17 cells retained more IL-17^{+} and fewer IL-10^{+} cells (**Figure 20A**). A considerable proportion of endogenous T cells produced IL-10 compared to transferred 2D2 T cells (**Figure 20A**), suggesting that extracellular IL-10 is not sufficient to restrain the pathogenicity of CD5L$^{-/-}$ Th17 cells. WT 2D2 T cells also acquired IFNγ expression *in vivo*, whereas CD5L$^{-/-}$ 2D2 T cells produced little IFNγ, suggesting CD5L may also regulate Th17 cellstability. Consistently, naïve CD5L$^{-/-}$ 2D2 T cells transferred into WT hosts immunized with MOG$_{35-55}$/CFA without inducing EAE made more IL-17 and little IL-10 in contrast to WT 2D2 T cells (**Figure 20B**). As IL-23 suppresses CD5L (**Figure 16E**) and CD5L restrains Th17 cell pathogenicity, Applicants reasoned that sustained CD5L expression should antagonize IL-23-driven pathogenicity. To test this hypothesis, Applicants generated a retroviral vector for ectopic expression of CD5L. Naive 2D2 T cells were differentiated with IL-1β+IL-6+IL-23, transduced with CD5L, transferred into WT recipients, and followed for weight loss and the development of clinical EAE (**Experimental Procedures**). 2D2 T cells transduced with CD5L (CD5L-RV 2D2) had a small reduction in IL-17 and higher IL-10 levels (**Figure 20C**). Ectopic expression of CD5L in

pathogenic Th17 cells reduced their pathogenicity as CD5L-RV 2D2 recipients had reduced weight loss and a significant decrease in the incidence and peak severity of EAE (**Figure 20D, E**). Furthermore, CD5L-RV 2D2 Th17 cells transferred *in vivo* lost IL-17 production and began producing IFNγ (**Figure 20F**). Therefore, sustained expression of *Cd5l* in pathogenic Th17 cells converts them to a less pathogenic and less stable phenotype in that these cells lose the expression of IL-17 and acquire an IFNγ-producing phenotype *in vivo*. This observation, combined with the observation that the loss of CD5L converts non-pathogenic Th17 cells into pathogenic Th17 cells *in vivo,* unequivocally supports the role of CD5L as a negative regulator of the functional pathogenic state of Th17 cells.

[00207] *CD5L shifts the Th17 cell lipidome balance from saturated to unsaturated lipids, modulating Rorγt ligand availability and function*: Since CD5L is known to regulate lipid metabolism, by binding to fatty acid synthase in the cytoplasm of adipocytes (Kurokawa, Arai et al. 2010), it was speculated that CD5L may also regulate Th17-cell function by specifically regulating lipid metabolites in T cells. To test this hypothesis, it was analyzed whether lipid metabolism is regulated by CD5L and is associated with the increased pathogenicity observed in Th17 cells from $CD5L^{-/-}$ mice. The lipidome of WT and $CD5L^{-/-}$ Th17 cells differentiated under the non-pathogenic (TGFβ1+IL-6) and pathogenic (TGFβ1+IL-6+IL-23) conditions was profiled. It was possible to resolve and identify around 200 lipid metabolites intracellularly or in the supernatant of differentiating Th17 cells using mass spectrometry and liquid chromatography (Table 3 herein). Of those metabolites that were differentially expressed between WT and $CD5L^{-/-}$, a striking similarity between the lipidome of $CD5L^{-/-}$ Th17 cells differentiated under the non-pathogenic condition and WT Th17 cells differentiated under the pathogenic condition (Figure 11A) was observed. Among other metabolic changes, CD5L deficiency significantly increased the levels of saturated lipids (SFA), including metabolites that carry saturated fatty acyl and cholesterol ester (CE) as measured by liquid chromatography and mass spectrometry (Figure 11B), and free cholesterol as shown by microscopy (Figure 11D). Moreover, the absence of CD5L resulted in a significant reduction in metabolites carrying poly-unsaturated fatty acyls (PUFA) (Figure 11B). Similar increase in CE and reduction in PUFA is observed in the lipidome of Th17 cells differentiated under either of two pathogenic conditions (IL-1β+IL-6+IL-23 and TGFβ3+IL-6+IL-23) compared to non-pathogenic WT cells (Figure 11C). Thus, Th17 cell pathogenicity

is associated with a shift in the balance of lipidome saturation as reflected in the increase in saturated lipids and decrease in PUFA metabolites.

[00208] Cholesterol metabolites, such as oxysterols, have been previously reported to function as agonistic ligands of Rorγt (Jin, Martynowski et al. 2010, Soroosh, Wu et al. 2014). Previous ChIP-Seq analysis (Xiao, Yosef et al. 2014) suggests that Rorγt binds at several sites in the promoter and intronic regions of *Il23r* and *Il17* (Figure 11D) and near CNS-9 of *Il10*, where other transcription factors, such as cMaf, which regulates *Il10* expression, also binds. As showed above, CD5L restrains the expression of IL-23R and IL-17 and promotes IL-10 production in Rorγt^{+} Th17 cells, and because CD5L-deficient Th17 cells contain higher cholesterol metabolite and lower PUFA (Figure 11A,B). Putting these data together, it was hypothesized that CD5L regulates the expression of IL-23R, IL-17 and IL-10 by affecting the binding of Rorγt to these targets, through affecting the SFA-PUFA balance.

[00209] Applicants hypothesized that CD5L could regulate Th17-cell function by regulating fatty acid (FA) profiles in T cells. Applicants asked if lipid metabolites are regulated by CD5L and if any such changes are associated with the increased pathogenicity of CD5L$^{-/-}$ Th17 cells. Applicants profiled the lipidome of WT and CD5L$^{-/-}$ Th17 cells differentiated under the non-pathogenic (TGF-β1+IL-6) and pathogenic (TGF-β1+IL-6+IL-23) conditions using a non-targeted approach. Applicants detected 178 lipid metabolites from Th17 cells, 39 of which showed differences among various Th17 polarizing conditions (**Figure 11A,** $p < 0.05$, fold change > 1.5; **Table 4**). Strikingly, non-pathogenic WT Th17 cells had a unique lipidome profile that was distinct from those of CD5L$^{-/-}$ Th17 cells and WT Th17 cells differentiated with TGF-β1+IL-6+IL-23 (**Figure 11A**). Applicants analyzed the FA profile and lipid class in the Th17 cell lipidome. As Applicants did not detect free FA except myristic acid, Applicants analyzed the FA content (side-chain) of the lipids in Figure 11A. WT non-pathogenic Th17 cells (compared to CD5L$^{-/-}$ Th17 cells of the same conditions) have increased polyunsaturated fatty acid (PUFA), accompanied by a decrease in lipids containing saturated (SFA) and monounsaturated fatty acids (MUFA) (**Figure 11K**). Applicants then extended this analysis to the 178 lipids detected. Not all PUFA are different in WT vs. CD5L$^{-/-}$ Th17 cells: linoleic acid (C18:2) and linolenic acid (C18:3) are equally distributed in the lipidome, whereas downstream PUFA, in particular arachidonic acid (C20:4), are elevated in WT non-pathogenic Th17 cells (**Figure 21B**). In contrast, MUFA is equivalently distributed and the corresponding SFA is decreased in WT non-

pathogenic Th17 cells (**Figure 21C**). The PUFA increase in WT non-pathogenic Th17 is equivalently distributed among the phospholipid and neutral lipid compartments (**Figure 11L**), whereas the relative decrease of SFA is only significant in phospholipid (**Figure 11L**). Finally, comparing the difference in specific lipid species (**Figure 21D**), Applicants found a higher level of cholesterol ester (CE), lysophosphatidylcholine (LPC) and phosphatidylcholine (PC), as well as decreased triacylglyceride (TAG) in both the CD5L$^{-/-}$ and more pathogenic cells (**Figure 21D**). Taken together, these findings suggest CD5L predominantly regulates FA composition in Th17 cells, resulting in elevation of PUFA and changes in specific lipid species, including cholesterol metabolites. Similar changes are also observed in WT Th17 cells differentiated under the pathogenic condition. Cholesterol metabolites, such as oxysterols, can function as agonists of Rorγt (Jin et al., 2010; Soroosh et al., 2014), and the cholesterol synthesis pathway has been linked to the production of endogenous Rorγt ligand. While Applicants did not detect any oxysterols or intermediates of cholesterol synthesis, the higher level of cholesterol esters (**Figure 21D**) prompted us to further investigate the cholesterol pathway. Applicants confirmed the higher intensity of free cholesterol in CD5L$^{-/-}$ Th17 cells using microscopy (**Figure 21E**). Next, Applicants analyzed the expression of *cyp51* and *sc4mol*, two enzymes of the cholesterol synthesis pathway responsible for generating endogenous Rorγt ligands (Santori et al., 2015), and found both increased in CD5L$^{-/-}$ Th17 cells or in pathogenic WT Th17 cells (**Figure 11M**), suggesting this may be a common mechanism by which Th17 cells regulate their function. Applicants asked if the change in FA profile in CD5L$^{-/-}$ Th17 cells is responsible for the regulation of *cyp51* and *sc4mol*. Indeed, while SFA had a modest effect, PUFA abolished the increased expression of the enzymes in CD5L$^{-/-}$ Th17 cells (**Figure 11M**). Thus CD5L can regulate fatty acid composition in Th17 cells and alter the cholesterol synthesis pathway, a source of Rorγt ligand.

[00210] *CD5L and PUFA/SFA profile regulate Rorγt function in a ligand-dependent manner.* Applicants analyzed if CD5L and the PUFA/SFA profile can alter Rorγt binding and function. Our previous chromatin immunoprecipitation (ChIP)-Seq analysis (Xiao et al., 2014) suggested Rorγt binds at several sites in the promoter and intronic regions of *Il23r* and *Il17* and near CNS-9 of *Il10* (**Figure 54** WO2015130968) where other *Il10*-regulating transcription factors, such as cMaf, also bind (Xiao et al., 2014). As CD5L restrains IL-17 and promotes IL-10 in Rorγt^{+} Th17

cells (**Figure 46** WO2015130968) and CD5L-/- Th17 cells have more cholesterol metabolites and lower PUFA (**Figure 11A, 11K, 11M, 21E**), Applicants hypothesized that CD5L regulates the expression of IL-23R, IL-17, IL-10 and, in turn, pathogenicity by affecting the binding of Rorγt to these targets by changing the SFA/PUFA profile and cholesterol biosynthesis. Applicants assessed if CD5L regulates Rorγt binding and transcription using ChIP-PCR and luciferase reporter assays. ChIP of Rorγt showed higher binding in the *Il17* and *Il23r* region and reduced binding to the *Il10* region in CD5L-/- Th17 cells despite similar Rorγt expression compared to WT (**Figure 18A, B, Fig 54 WO2015130968**). Further, CD5L overexpression was sufficient to suppress Rorγt dependent transcription of *Il17* and *Il23r* luciferase reporters (**Figure 18C, Fig 54 WO2015130968**) and to enhance the transcription of the *Il10* reporter (**Figure Fig 54 WO2015130968**). This effect of CD5L is not observed with PPARγ, another regulator of *Il10*, further supporting the hypothesis that the effect of CD5L depends on Rorγt (**Fig 54 WO2015130968**). Applicants then examined whether changing the lipidome of WT Th17 cells with exogenous SFA or PUFA can regulate Rorγt binding to genomic regions (**Figure 18A, B** and **Fig 54 WO2015130968**). SFA enriched binding of Rorγt at *Il17* and *Il23r* loci and PUFA decreased such binding (**Figure 18A, Fig 54 WO2015130968**). Instead, PUFA increased Rorγt binding to the *Il10* CNS-9 locus (**Figure 18B**), suggesting that manipulation of the lipid content of Th17 cells can indeed modulate Rorγt binding to DNA. Applicants reasoned that if CD5L regulates Rorγt transcriptional activity by limiting Rorγt ligand, adding exogenous agonists of Rorγt would rescue CD5L-induced suppression. Indeed, 7β, 27-dihydroxycholesterol, previously shown as an endogenous ligand of Rorγt (Soroosh et al., 2014), rescued the CD5L-driven suppression of *Il17* reporter transcription, suggesting ligand availability partly contributes to the regulation of Rorγt function by CD5L (**Figure 18D**). Consistently, CD5L inhibited IL-17 expression in unpolarized Th0 cells with ectopic Rorγt expression and this inhibition could be partially rescued by the addition of a Rorγt ligand (**Figure 18E**). Addition of Rorγt ligand also increased IL-17 production from non-pathogenic Th17 cells (**Figure 18F**), suggesting that ligand restriction may be one of the mechanisms by which CD5L regulates Th17 cell pathogenicity. Applicants then determined if SFA/PUFA regulate Rorγt activity through Rorγt ligand. While Rorγt strongly transactivates the *Il23r* enhancer in the presence of an agonistic ligand, the addition of PUFA to the agonist ligand inhibited Rorγt-mediated *Il23r* transactivation and

enhanced *Il10* transactivation (**Fig 48 WO2015130968**). Similarly, adding SFA alone had little impact on Rorγt-dependent transcription, but it modified the transcriptional effect of oxysterol (**Fig 48 WO2015130968**). Thus, PUFA/SFA can modulate Rorγt transcriptional activity via a Rorγt-ligand dependent mechanism, although the precise mechanism of exogenous PUFA and SFA require further studies. Taken together, these observations suggest that CD5L shifts the FA composition in the lipidome, changes Rorγt ligand availability and Rorγt genomic binding, and regulates *Il23r* and *Il10*, members of the proinflammatory vs. regulatory modules.

[00211] *PUFA/SFA regulate Th17 cell and contribute to CD5L function.* As CD5L$^{-/-}$ Th17 cells have an altered balance in lipid saturation, and PUFA/SFA modulate Rorγt binding and function, Applicants analyzed the relevance of FA moieties to Th17 cell function and their contribution to CD5L-driven Th17 cell pathogenicity. Applicants first tested the effect of PUFA/SFA on the generation of Th17 cells. WT Th17 cells were differentiated with TGF-β1+IL-6 and expanded using IL-23 in fresh media with either PUFA or SFA. PUFA suppressed IL-17 and IL-23R expression consistent with reduced transactivation in WT but not in Rorγt$^{-/-}$ Th17 cells, suggesting PUFA can limit pathogenic Th17 cell function in a Rorγt dependent manner (**Fig 50 WO2015130968**). CD5L$^{-/-}$ Th17 cells differentiated with TGF-β1+IL-6 were also sensitive to PUFA treatment, resulting in reduced percentage of IL-17^{+} CD4^{+} T cells (**Fig 50 WO2015130968**). In contrast, addition of SFA only slightly increased the expression of both IL-17 and IL-23R expression, and this effect was not significant, possibly because pathogenic Th17 cells had already very high levels of SFA.Applicants studied the contribution of lipid saturation to Th17 cell pathogenicity. Applicants speculated that if the balance of lipid saturation distinguishes non-pathogenic WT Th17 cells and pathogenic CD5L$^{-/-}$ Th17 cells, the addition of SFA to WT and PUFA to CD5L$^{-/-}$ Th17 cells can result in reciprocal changes in the transcriptional signature relevant to Th17 cell pathogenicity. Applicants analyzed the expression of a 312 gene signature of Th17 cell differentiation and function (Yosef et al., 2013) in SFA- or control-treated WT Th17 cells and in PUFA- or control-treated CD5L$^{-/-}$ Th17 cells differentiated with TGF-β1+IL-6. Of those genes that are differentially expressed (**Table 5**, > 1.5 fold), PUFA-treated CD5L$^{-/-}$ Th17 cells resemble WT non-pathogenic Th17 cells, and SFA-treated WT non-pathogenic Th17 cells are more similar to CD5L$^{-/-}$ Th17 cells (**Fig 50 WO2015130968, Table 5**). qPCR analysis confirmed that PUFA and SFA reciprocally regulated effector molecule

expression of the pathogenicity signature (Lee et al., 2012), including *Il10*, *Il23r*, *Ccl5*, *Csf2* and *Lag3* (**Fig 50 WO2015130968**). Notably, in some cases PUFA and SFA have the same effects; for example, *Il22* expression is increased following either FA treatment. Taken together, these observations suggest that the balance of lipid saturation contributes to CD5L-dependent regulation of Th17 cells by regulating the Th17-cell transcriptome.

[00212] *DISCUSSION.* Th17 cells are a helper cell lineage capable of diverse functions ranging from maintaining gut homeostasis, mounting host defense against pathogens, to inducing autoimmune diseases. How Th17 cells can mediate such diverse and opposing functions remains a critical open question. Addressing this is especially important since anti-IL-17 and Th17-based therapies have been highly efficacious in some autoimmune diseases, but had no impact on others (Baeten and Kuchroo, 2013; Genovese et al., 2010; Hueber et al., 2012; Leonardi et al., 2012; Papp et al., 2012; Patel et al., 2013), even when Th17 cells have been genetically linked to the disease process (Cho, 2008; Lees et al., 2011). Using single-cell genomics Applicants have addressed this issue and have identified novel functional regulators of pathogenicity in Th17 cells. Here, Applicants highlight and investigate CD5L as one of the novel regulators that affect the pathogenicity of Th17 cells. Applicants show that: (**1**) Among CD4 T cells, CD5L is highly expressed only in non-pathogenic Th17 cells, but in them positively co-varies with a pro-inflammatory module, a pattern consistent with being a negative regulator of pathogenicity; (**2**) CD5L does not affect Th17 differentiation but affects their long-term expansion and function; (**3**) CD5Ldeficiency converts non-pathogenic Th17 cells into pathogenic Th17 cells; (**4**) CD5L regulates lipid metabolism in Th17 cells and alters their fatty acid composition; and (**5**) change in the lipidome in CD5L-/- Th17 cells affects the ligand availability and binding of Rorγt to its target genes.

[00213] In a seemingly paradoxical way, CD5L is expressed only in non-pathogenic Th17 cells, but in co-variance with the pro-inflammatory module. This observation led us to hypothesize that CD5L is a negative regulator of a non-pathogenic to pathogenic transition, since negative regulators are often known to co-vary in regulatory networks with the targets they repress in organisms from yeast (Segal et al., 2003) to mammals (Amit et al., 2007; Amit et al., 2009). Our functional analysis bears out this hypothesis, suggesting that CD5L might indeed be expressed to restrain the pro-inflammatory module in the non-pathogenic Th17 cells. Similarly, other genes with this specific pattern, *i.e.* exclusive expression in non-pathogenic cells but in co-

variance with the pro-inflammatory module, may also be repressors that quench pro-inflammatory effector functions and make Th17 cells non-pathogenic. Thus, depending on the environmental context or trigger, non-pathogenic Th17 cells can be readily converted into pathogenic Th17 cells by inhibiting a single gene like CD5L. This is supported by our data showing IL-23R signalling can suppress CD5L and persistent CD5L expression inhibits the pro-inflammatory function of Th17 cells. In addition to suppressing the pro-inflammatory module, CD5L also promotes the regulatory module, acting as a switch to allow rapid responses to environmental triggers such that Th17 cells can change their functional phenotype without intermediary pathways.

[00214] Both pathogenic and non-pathogenic Th17 cells are present in peripheral lymphoid organs, but pathogenic Th17 cells appear at sites of tissue inflammation (CNS) and non-pathogenic Th17 cells appear in the gut or other mucosal surfaces. This is mirrored in the expression of CD5L. IL-23, which is present in the CNS during EAE, can suppress CD5L and convert non-pathogenic Th17 cells into pathogenic Th17 cells. At steady state, it is unknown what promotes CD5L expression and non-pathogenicity in the gut. TGF-β could be a candidate given its abundance in the intestine and its role in both differentiation of IL-10-producing CD4 T cells *in vivo* (Konkel and Chen, 2011; Maynard et al., 2007) and Th17 cell differentiation (Bettelli et al., 2006; Veldhoen et al., 2006). Specific commensal bacteria (Ivanov et al., 2009; Yang et al., 2014) and metabolites from microbiota (Arpaia et al., 2013) can also regulate T cell differentiation. Notably, CD5L is reported as a secreted protein and can recognize PAMPs (Martinez et al., 2014). It is possible CD5L expressed by non-pathogenic Th17 cells in the gut can interact with the immune cells interacting with gut microbiota and maintain gut tolerance and a non-pathogenic Th17 phenotype. Other CD5L-expressing cells in the intestine may also contribute to such a function. Therefore, the two functional states of Th17 cells may be highly plastic, in that either pathogenic or non-pathogenic Th17 cells can be generated by sensing changes in the tissue microenvironment. CD5L is critical for maintaining the non-pathogenic functional state of Th17 cells, and IL-23 rapidly suppresses CD5L rendering the cells pathogenic. This hypothesis also predicts that non-pathogenic Th17 cells can be easily converted into pathogenic Th17 cells by production of IL-23 locally in the gut during inflammatory bowel disease. How does CD5L regulate Th17 cell pathogenicity? Applicants provide evidence CD5L can regulate Th17 cell function by regulating intracellular lipid metabolism and limiting Rorγt

ligand. CD5L inhibits the *de novo* synthesis of fatty acid through direct binding to fatty acid synthase. Applicants discovered that in Th17 cells CD5L is more than a general inhibitor, as it regulates the fatty acid composition of PUFA *vs.* SFA and MUFA. Applicants showed CD5L suppresses the cholesterol synthesis pathway by regulating critical enzymes sc4mol and cyp51 and the addition of PUFA could reverse this phenotype. Importantly, exogenous Rorγt ligand can rescue the suppressive effect of CD5L on IL-17 expression. PUFA metabolites can function as ligands of several transcription factors and the exact mode of function for PUFA requires further investigation. Applicants showed that PUFA limits ligand-dependent function for Rorγt, such that in the presence of CD5L or PUFA, Rorγt binding to the *Il17a* and *Il23r* loci is decreased, along with reduced transactivation of both genes, whereas binding at and expression from the *Il10* locus is enhanced. Notably, Rorγt's ability to regulate *Il10* expression was not reported previously. As CD5L does not impact overall Th17 cell differentiation, this suggests a nuanced effect of CD5L and lipid balance on Rorγt function, enhancing its binding to and transactivation at some loci, while reducing it in others. In Th17 cells, Stat3 and c-Maf can promote *Il10* (Stumhofer et al., 2007; Xu et al., 2009). As Stat3, C-Maf and Rorγt can all bind to the same *Il10* enhancer element, it is possible that, depending on the quality and quantity of the available ligands, Rorγt may interact with other transcription factors and regulate *Il10* transcription. This supports a hypothesis in which the spectrum of Rorγt ligands depends, at least in part, on the CD5L-regulated PUFA *vs.* SFA lipid balance in the cell, and these resulting ligands can impact the specificity of Rorγt, allowing it to assume a spectrum of functional states. Several metabolic pathways are associated with Th17 cell differentiation. HIF1α regulates Th17 cells through direct transactivation of Rorγt (Dang et al., 2011; Shi et al., 2011) and acetyl-coA carboxylase influences the Th17/Treg balance through the glycolytic and lipogenic pathways (Berod et al., 2014). Mice harbouring mutations in genes that regulate Th17 cell differentiation and function acquire an obese phenotype, associating Th17 cell development with obesity (Ahmed and Gaffen, 2010; Jhun et al., 2012; Mathews et al., 2014; Winer et al., 2009). A hallmark of obesity is the accumulation of saturated fat and cholesterol and mice fed with a diet rich in PUFA were reported to have reduced severity of chronic colitis and Th17 cell polarization (Monk et al., 2013; Monk et al., 2012). In this study, Applicants provided evidence that at the cellular level, lipidome saturation can promote Th17 cell function by regulating Rorγt function.

[00215] In conclusion, by using single-cell genomics and computational analysis, Applicants identified CD5L as a novel repressor of Th17 cell pathogenicity, highlighting the power of single-cell genomics to identify molecular switches that are otherwise obscured by population-level genomic profiles. CD5L appears to be a molecular switch that does not affect Th17 differentiation *per se* but one that impacts the function (pathogenic *vs.* non-pathogenic phenotype) of Th17 cells, potentially by regulating the quality and/or quantity of available Rorγt ligands, allowing a single master regulator to possibly assume multiple functional states. Our results connect the lipidome to essential functions of immune cells, opening new avenues for sensitive and specific therapeutic intervention.

[00216] *EXPERIMENTAL PROCEDURES. Mice:* C57BL/6 wild-type and $CD4^{-/-}$(2663) mice were obtained from Jackson Laboratory. IL-17A–GFP mice were from Biocytogen. All animals were housed and maintained in a conventional pathogen-free facility at the Harvard Institute of Medicine in Boston (IUCAC protocols: 0311-031-14 (V.K.K.) and 0609-058015 (A.R.)). All experiments were performed in accordance to the guidelines outlined by the Harvard Medical Area Standing Committee on Animals at the Harvard Medical School. In addition, spleens and lymph nodes from $GPR65^{-/-}$ mice were generously provided by Yang Li (IACUC protocol: 453). $PLZP^{-/-}$ mice and $TOSO^{-/-}$ mice were provided by Pier Paolo Pandolfi from Beth Israel Deaconess medical center and John Coligan from National instutite of Allergy and Infectious Diseases repectively.

[00217] *Cell sorting and in vitro T-cell differentiation:* CD4+ T cells were purified from spleen and lymph nodes using anti-CD4 microbeads (Miltenyi Biotech) then stained in PBS with 1% FCS for 20 min at room temperature with anti-CD4-$PerC^{P,}$ ^{a}nti-^{CD6}2l-APC and anti-CD44-PE antibodies (all Biolegend). Naive CD4+$CD62l^{high}CD44^{low}$ T cells were sorted using the BD FACSAria cell sorter. Sorted cells were activated with plate-bound anti-CD3 (2 μg ml−1) and anti-CD28 (2 μg ml−1) in the presence of cytokines. For Th17 differentiation, the following reagents were used: 2 ng/ml recombinant human TGF-β1 and recombinant human TGF-β3 (Miltenyi Biotec), 25 ng/ml recombinant mouse IL-6 (Miltenyi Biotec), 20 ng/ml recombinant mouse IL-23 (R&D Biosystems) and 20 ng/ml recombinant mouse IL-1β (Miltenyi Biotec). Cells were cultured for 48h and collected for RNA, intracellular cytokine staining, flow-fish, and flow cytometry.

[00218] *Active induction of EAE and disease analysis:* For active induction of EAE, mice were immunized by subcutaneous injection of 100 μg MOG(35–55) (MEVGWYRSPFSRVVHLYRNGK) in CFA, then received 200 ng pertussis toxin intraperitoneally (List Biological Laboratory) on days 0 and 2. Mice were monitored and were assigned scores daily for development of classical and atypical signs of EAE according to the following criteria (Jager et al., 2009): 0, no disease; 1, decreased tail tone or mild balance defects; 2, hind limb weakness, partial paralysis or severe balance defects that cause spontaneous falling over; 3, complete hind limb paralysis or very severe balance defects that prevent walking; 4, front and hind limb paralysis or inability to move body weight into a different position; 5, moribund state.

[00219] *Isolation of T-cells from EAE mice at the peak of disease:* At the peak of disease, T cells were collected from the draining lymph nodes and the CNS. For isolation from the CNS, mice were perfused through the left ventricle of the heart with cold PBS. The brain and the spinal cord were flushed out with PBS by hydrostatic pressure. CNS tissue was minced with a sharp razor blade and digested for 20 min at 37°C with collagenase D (2.5 mg/ml; Roche Diagnostics) and DNaseI (1 mg/ml; Sigma). Mononuclear cells were isolated by passage of the tissue through a cell strainer (70 μm), followed by centrifugation through a Percoll gradient (37% and 70%). After removal of mononuclear cells, the lymphocytes were washed, stained and sorted for CD3 (Biolegend), CD4 (Biolegend), 7AAD and IL-17a-GFP or FOXP3-GFP.

[00220] *Memory cell isolation/reactivation*: Spleen and lymph nodes were isolated from indicated mice and CD4+ T cells were purified using Automacs using the manufacturers protocol (Miltenyi Biotec, CA). Cells were stained with CD44-PE, CD62L-APC and CD4-Percp antibodies prior to being sorted on the Aria FACS sorter for CD4+CD44+CD62L- cells. Cells were plated on anti-CD3/anti-CD28 (2ug/ml each) coated flat- bottomed 96 well plate at 2x10^5 cells/well with or without IL-23 (20ng/ml) for reactivation. Cells were cultured in vitro for 96 hours and then live cells (7AAD-) were analyzed for intracellular cytokine staining or sorted for harvesting prior to RNA purification.

[00221] *Recall experiments*: Naïve CD4 T cells (CD4+CD62L+CD44-) were sorted from indicated KO and WT (or littermate) controls then adoptively transferred at 1x10^6 cells into Rag-1 KO mice for reconstitution. Two weeks post adoptive transfer; mice were immunized with

100ug of MOG_{35-55}/CFA. Cells were harvested from draining LNs and spleen 8 days post immunization and restimulated with MOG_{35-55} with or without IL-23 (20ng/ml) for 4 days. Cells were harvested for intracellular cytokine analysis.

[00222] *Isolation of T cells from lamina propria* : Cells were isolated from the lamina propria of the large intestine from 3-6 month old IL-17GFP KI mice using Miltenyi Biotec Lamina Propria Dissociation kit following the manufacturer's protocol (Militenyi Biotec, Calfornia). GFP+CD4+TCRb+7AAD- T cells were sorted using a MoFlow Astrios into RLT lysis buffer (Qiagen RNeasy micro kit) and subsequently taken through the 'RNA-seq of population controls' protocol described below.

[00223] *Whole transcriptome amplification:* Cell lysis and SMART-Seq (Ramskold et al., 2012) whole transcriptome amplification (WTA) was performed on the C_1 chip using the C_1 Single-Cell Auto Prep System (C_1 System) using the SMARTer Ultra Low RNA Kit for Illumina Sequencing (Clontech) with the following modifications:

Cell Lysis Mix:

Composition	**Stock Conc.**	**Volume**
C_1 Loading Reagent	20X	0.60 ul
SMARTer Kit RNase Inhibitor	40 x	0.30 ul
SMARTer Kit 3' SMART CDS Primer II A	12 μM	4.20 ul
SMARTer Kit Dilution Buffer	1X	6.90 ul

Cycling Conditions I:

a) 72°C, 3 min

b) 4°C, 10 min

c) 25°C, 1 min

Reverse Transcription (RT) Reaction Mix:

Composition	**Stock Conc.**	**Volume**

C_1 Loading Reagent	20.0 x	0.45 ul
SMARTer Kit 5X First-Strand Buffer (RNase-Free)	5.0 x	4.20 ul
SMARTer Kit Dithiothreitol	100 mM	0.53 ul
SMARTer Kit dNTP Mix (dATP, dCTP, dGTP, and dTTP, each at 10 mM)	10 mM	2.10 ul
SMARTer Kit SMARTer II A Oligonucleotide	12 uM	2.10 ul
SMARTer Kit RNase Inhibitor	40 x	0.53 ul
SMARTer Kit SMARTScribe™ Reverse Transcriptase	100.0 x	2.10 ul

Cycling Conditions II:

a) 42°C, 90 min

b) 70°C, 10 min

PCR Mix:

Composition	**Stock Conc.**	**Volume**
PCR Water	-	35.2 ul
10X Advantage 2 PCR Buffer	10.0 x	5.6 ul
50X dNTP Mix	10 mM	2.2 ul
IS PCR primer	12 uM	2.2 ul
50X Advantage 2 Polymerase Mix	50.0 x	2.2 ul
C1 Loading Reagent	20.0 x	2.5 ul

Cycling Conditions III:

a) 95°C, 1 min

b) 5 cycles of:

i) 95°C, 20s

ii) 58°C, 4 min

ii) 68°C, 6 min

c) 9 cycles of:

i) 95°C, 20s

ii) 64°C, 30s

ii) 68°C, 6 min

d) 7 cycles of:

i) 95°C, 30s

ii) 64°C, 30s

ii) 68°C, 7 min

e) 72°C, 10 min

[00224] *Single cell RNA-Seq.* WTA products were harvested from the C_1 chip and cDNA libraries were prepared using Nextera XT DNA Sample preparation reagents (Illumina) as per the manufacturer's recommendations, with minor modifications. Specifically, reactions were run at ¼ the recommended volume, the tagmentation step was extended to 10 minutes, and the extension time during the PCR step was increased from 30s to 60s. After the PCR step, all 96 samples were pooled without library normalization, cleaned twice with 0.9x AMPure XP SPRI beads (Beckman Coulter), and eluted in buffer TE. The pooled libraries were quantified using Quant-IT DNA High-Sensitivity Assay Kit (Invitrogen) and examined using a high sensitivity DNA chip (Agilent). Finally, samples were sequenced deeply using either a HiSeq 2000 or a HiSeq 2500 sequencer.

[00225] *Single-cell RNAseq data acquisition and analysis.* Applicants profiled the transcriptome of 806 Th17 cells, either harvested *in vivo* or differentiated *in vitro*. For *in vivo*

experiments, CD3+CD4+IL-17A.GFP+ cells were isolated from draining LNs and CNS of mice at peak of EAE. For *in vitro* experiments, cells were sorted at 48h post induction of differentiation of naïve CD4+ T cells under different conditions. Applicants had at least two independent biological replicates for each *in vivo* and *in vitro* condition (except for TGF-β3+IL-6 for which Applicants only had one replicate), as well as two technical replicates for two *in vivo* conditions.

[00226] Applicants prepared single-cell mRNA SMART-Seq libraries using microfluidic chips (Fluidigm C1) for single-cell capture, lysis, reverse transcription, and PCR amplification, followed by transposon-based library construction. For quality assurance, Applicants also profiled corresponding population controls (>50,000 cells for *in vitro* samples; ~2,000-20,000 cells for *in vivo* samples, as available), with at least two replicates for each condition. RNA-seq reads were aligned to the NCBI Build 37 (UCSC mm9) of the mouse genome using TopHat (Trapnell et al., 2009). The resulting alignments were processed by Cufflinks to evaluate the abundance (using FPKM) of transcripts from RefSeq (Pruitt et al., 2007). Applicants used log transform and quantile normalization to further normalize the expression values (FPKM) within each batch of samples (*i.e.*, all single-cells in a given run). To account for low (or zero) expression values Applicants added a value of 1 prior to log transform. Applicants filtered the set of analyzed cells by a set of quality metrics (such as sequencing depth), and added an additional normalization step specifically controlling for these quantitative confounding factors as well as batch effects. Our analysis is based on ~7,000 appreciably expressed genes (fragments per kilobase of exon per million (FPKM) > 10 in at least 20% of cells in each sample) for *in vitro* experiments and ~4,000 for *in vivo* ones. Applicants also developed a strategy to account for expressed transcripts that are not detected (false negatives) due to the limitations of single-cell RNA-seq (Deng et al., 2014; Shalek et al., 2014). Our analysis (e.g., computing signature scores, and principle components) down-weighted the contribution of less reliably measured transcripts. The ranking of regulators shown in **Figure 16** is based on having a strong correlation to at least one of the founding signature genes, and in addition, the significance of the overall pattern relative to the proinflammatory *vs.* regulatory signature by comparing the aggregates pattern across the individual correlations to shuffled data.

[00227] *Mice.* C57BL/6 wildtype (WT) was obtained from Jackson laboratory (Bar Harbor, ME). For EAE experiment, littermate control WT was used in comparison to CD5L$^{-/-}$ mice in one experiment which produced similar results compared to WT from Jackson. CD5L$^{-/-}$ mice were provided by Dr. Toru Miyazaki from the University of Tokyo (Miyazaki et al., 1999). CD5L$^{-/-}$ 2D2 mice were generated by crossing CD5L$^{-/-}$ mice with WT 2D2 transgenic mice. IL-23R GFP reporter mice were generated as previously published (Awasthi et al., 2009). Rorγt.GFP reporter mice were provided by Dr. Dan Littman and bred at the Harvard Institute of Medicine animal facility. All experiments were performed in accordance to the guidelines outlined by the Harvard Medical Area Standing Committee on Animals at the Harvard Medical School (Boston, MA).

[00228] *Experimental Autoimmune Encephalomyelitis (EAE).* For active EAE immunization, MOG_{35-55} peptide was emulsified in complete freund adjuvant (CFA). Equivalent of 40μg MOG peptide was injected per mouse subcutaneously followed by pertussis toxin injection intravenously on day 0 and day 2 of immunization. For adoptive transfer EAE, naïve 2D2 transgenic T cells were sorted as described in T cell culture and co-cultured with irradiated APC in the presence of soluble anti-CD3 and anti-CD28 antibodies (2.5μg/ml) and cytokines for five days. Cells were then harvested and restimulated with plate-bound anti-CD3 and anti-CD28 (2μg/ml) for 2 days prior to transfer. For overexpression of CD5L, retroviruses, MSCV, carrying either GFP empty vector control or GFP.CD5L vector was used to infect T cell culture as outlined above one day after T cell activation. Five million cells were transferred per mouse intravenously. EAE is scored as previously published (Jager et al., 2009).

[00229] *T cell differentiation culture.* Naïve CD4^{+}CD44^{-}CD62L^{+}CD25^{-} T cells or Effector memory CD4^{+}CD44^{+}CD62L^{-} were sorted using BD FACSAria sorter and activated with plate-bound anti-CD3 and anti- CD28 antibodies (both at 2μg/ml) in the presence of cytokines at a concentration of 2.5 X 10^5 cells/ml. For Th17 differentiation: 2ng/ml of rhTGFβ1, 2ng/ml of rhTGFβ3, 25ng/ml rmIL-6, 20ng/ml rmIL-1β (all from Miltenyi Biotec) and 20ng/ml rmIL-23 (R & D systems) were used at various combinations as specified in figures. For Th1 differentiation, 20ng/ml rmIL-12 (R & D systems); for Th2 differentiation 20ng/ml rmIL-4 (Miltenyi Biotec); for iTreg differentiation, 2.5ng/ml of rhTGFβ1 were used (Miltenyi Biotec). For differentiation experiments, cells were harvested at 48 hours. For restimulation experiments, cells were differentiated for 48 hours and resuspended in fresh media with no additional cytokines for 48-72 hours. Cells were re-stimulated with PMA/ionomycin for four hours before

analysis for cytokines by intracellular cytokine staining. For experiments with exogenous fatty acid, fatty acids were purchased and resuspended first with serum-free media containing BSA prior being added to culture.

[00230] *Lipidomics.* Th17 cells were differentiated from naïve WT and CD5L$^{-/-}$ T cells. Culture media were snap frozen. Cells were harvested at 96h. 10 X 10_6 cells per sample were snap frozen and extracted in either 80% methanol (for fatty acids and oxylipids) or isopropanol (for polar and nonpolar lipids). Two liquid chromatography tandem mass spectrometry (LC-MS) methods were used to measure fatty acids and lipids in cell extracts.

[00231] Fatty acid extracts (10 µL) were injected onto a 150 x 2 mm ACQUITY T3 column (Waters; Milford, MA). The column was eluted isocratically at a flow rate of 400 µL/min with 25% mobile phase A (0.1% formic acid in water) for 1 minute followed by a linear gradient to 100% mobile phase B (acetonitrile with 0.1% formic acid) over 11 minutes. MS analyses were carried out using electrospray ionization in the negative ion mode using full scan analysis over m/z 200-550 at 70,000 resolution and 3 Hz data acquisition rate. Additional MS settings were: ion spray voltage, -3.5 kV; capillary temperature, 320°C; probe heater temperature, 300 °C; sheath gas, 45; auxiliary gas, 10; and S-lens RF level 60. Lipids extracts (2 µL) were injected directly onto a 100 x 2.1 mm ACQUITY BEH C8 column (1.7 µm; Waters; Milford, MA). The column was eluted at a flow rate of 450 µL/min isocratically for 1 minute at 80% mobile phase A (95:5:0.1 vol/vol/vol 10 mM ammonium acetate/methanol/acetic acid), followed by a linear gradient to 80% mobile-phase B (99.9:0.1 vol/vol methanol/acetic acid) over 2 minutes, a linear gradient to 100% mobile phase B over 7 minutes, and then 3 minutes at 100% mobile-phase B. MS analyses were carried out using electrospray ionization in the positive ion mode using full scan analysis over *m/z* 200-1100 at 70,000 resolution and 3 Hz data acquisition rate. Additional MS settings were: ion spray voltage, 3.0 kV; capillary temperature, 300°C; probe heater temperature, 300 °C; sheath gas, 50; auxiliary gas, 15; and S-lens RF level 60. Raw data from methods 1-3 were processed using Progenesis CoMet and QI software (Nonlinear Dynamics Ltd.; Newcastle upon Tyne, UK) for feature alignment, nontargeted signal detection, and signal integration. Targeted processing of a subset of known metabolites was conducted using TraceFinder software (Thermo Fisher Scientific; Waltham, MA).

[00232] *ChIP-qPCR.* Chromatin ImmunoPrecipitation (ChIP) for Rorγt was performed as previously published (Xiao et al., 2014) using anti-Rorγt antibody (AFKJS-9) and RatIgG2a

isotype control antibody (eBioscience, CA). qPCR was performed using the following primers: *Il17a CNS2*: Fwd: 5'- TGG AAA GTT TTC TGA CCC ACT T; Rv: 5'- GGA AGC TGA GTA CGA GAA GGA A; *Il17a In1*: Fwd: 5'- ACC AAA GGA ACA AGT GGA AAG A; Rv:5'- TTT GAG AAC CAG TCA TGT CAC C; *Il17a p5*: Fwd: 5'- GGG GTA GGG TCA ATC TAA AAG C; Rv: 5'- GTG TGC TGA CTA ATT CCA TCC A; *Il10 CNS-9*: Fwd: 5' TTA CAG AAT GGC ACT TCC AGA G; Rv: 5' CGA TGT ATT AGT TCC GGT GTG T; *Il23r in3*: Fwd 5'- CTT GGC ATC ACA AAG CTT ACA G; Rv: 5'- ACT GCC AGG CAA GAA TTT ACT C; *Il23r in6*: Fwd 5'- TAC CTG AAA GCT GTG CAG AGA G; Rv: 5'- AAG TCC AAG CCT GTG AAA CAA T.

[00233] *Nanostring nCounter.* Nanostring nCounter platform (NanoString Technologies) is used to measure the number of RNA transcripts in RNA samples (**Fig 16I, Fig 18D**). A codeset containing 312 signature genes of Th17 cell differentiation and function as well as 4 additional house-keeping genes were custom-made (Yosef et al., 2013) and used in these experiments. Experimental procedures as detailed by the manufacturer is strictly followed.

[00234] *Antibodies.* Biotinylated anti-CD5L antibody used for flow cytometry analysis was purchased from R & D systems. All other flow cytometry antibodies were purchased from Biolegend. ELISA coating and capturing antibodies for IL-10 were from BD Biosciences and anti- IL-17 were purchased from Biolegend.

[00235] *Statistical Analysis.* Unless otherwise specified, all statistical analyses were performed using the two-tail student *t* test using GraphPad Prism software. P value less than 0.05 is considered significant ($P < 0.05$ = *; $P < 0.01$ = **; $P < 0.001$ = ***).

[00236] *RNA-Seq of population controls.* Population controls were generated by extracting total RNA using RNeasy plus Micro RNA kit (Qiagen) according to the manufacturer's recommendations. Subsequently, 1 μL of RNA in water was added to 2 μL of lysis reaction mix, thermocycled using cycling conditions I (as above). Next, 4 μL of the RT Reaction Mix were added and the mixture was thermocycled using cycling conditions II (as above). Finally, 1 μL of the total RT reaction was added to 9 μL of PCR mix and that mixture was thermocycled using cycling conditions III (as above). Products were quantified, diluted to 0.125 ng / μL and libraries were prepared, cleaned, and tested as above.

[00237] *RNA-Seq preprocessing.* RNA-Seq preprocessing was performed using the following. RNA-seq reads are aligned to the NCBI Build 37 (UCSC mm9) of the mouse

genome using TopHat (Trapnell et al., 2009). The resulting alignments are processed by Cufflinks to evaluate the abundance (using FPKM) of transcripts from RefSeq (Pruitt et al., 2007). Log transform and quantile normalization is used to further normalize the expression values (FPKM) within each batch of samples (*i.e.*, all single cells in a given run). To account for low (or zero) expression values a value of 1 prior to log transform was added.

[00238] *Sample filtering and normalization.* For each library quality scores were computated using Fastqc, Picard tools, and in-house scripts. Computed scores included: (1) Number of reads, (2) Number of aligned reads, (3) Percentage of aligned reads, (4) Percentage of transcripts identified (compared with the overall number of transcripts identified by at least one cell in the respective run), (5) Percentage of duplicate reads, (6) primer sequence contamination, (7) insert size (mean), (8) insert size (std), (9) Complexity, (10) Percentage of Ribosomal reads, (11) Percentage of Coding reads, (12) Percentage of UTR reads, (13) Percentage of Intronic reads, (14) Percentage of Intergenic reads, (15) Percentage of mRNA reads, (16) Coefficient of variation of coverage, (17) mean 5' Bias, (18) mean 3' Bias, (19) mean 5' to 3' Bias.

[00239] Libraries are excluded from further analysis with poor values in either the number of aligned reads, the percentage of aligned reads, or the percentage of identified transcripts. To this end, for a given performance measure x, a minimum cutoff value cx was set by taking the maximum over: $\{AVG(x) - 1.645* STD(x), MED(x) - 1.645* MAD(x)\}$ (*MED* stands for median and *MAD* is the median absolute deviation). For the latter two performance measures, a Gaussian mixture model is fit to x; if x fits a multi-modal distribution rather than a single Gaussian (using Bayesian Information Criteria to determine the best model), then an additional cutoff z determined as the boundary between the right-most distribution and the other distributions is used. Finally, hard lower bounds (*hlb*) are introduced for the cutoff values (#aligned reads >25k; percentage of aligned reads>20%; percentage of identified transcripts>20%). Then the cutoff is re-set to be $\max\{cx, z, hlb\}$. Only cells are retained that scored above the cutoff in all three cases.

[00240] As an additional pre-processing step a normalization technique (Risso et al., 2011) is employed to reduce the effects of the quality scores. To this end, a principal component analysis (PCA) is computed over the quality score matrix (a matrix with columns corresponding to cells and rows corresponding to quality scores). Then a global-scaling

normalization approach (previously used for GC content normalization in RNA-Seq data(Risso et al., 2011) is used to remove the effects of the top principal components (PCs), until >90% of the variance in the quality matrix is covered (Notably, the quality scores are correlated, and usually the top one or two principal components are sufficient). For a given PC, the cells are divided into 10 equally-sized bins based on their projected values. The normalized expression measures are defined as:

$$E'(i,j) = E(i,j) - Median(\{E(i,j'), \quad s.t.\ j' \in k(j)\}) + Median(\{E(i,:)\})$$

where *E(i,j)* is the original expression value of gene *i* in cell *j*; *k(j)* denotes the PC-value bin to which cell *j* belongs; and *E(i,:)* denotes the median value of gene *i* across all cells.

[00241] This approach was validated by computing PCA on the expression data (before filtering, after filtering, but before normalization, and after filtering and normalization) and calculating the correlation between the quality scores and the top PCs. It was found that before filtering and normalization the main PCs highly correlate with the various library quality scores; indicating that the dominant signal in the pre-normalization data might reflect experimental artifacts. These correlations are reduced after normalization, indicating that the remaining signal is less affected by artifacts (Figure 6).

[00242] *Batch correction.* Two or more replicates for the majority of the analyzed conditions were obtained. Since the replicates were divided into batches, a procedure to eliminate the pertaining batch effects was applied. Due to substantial differences in the number of detected genes between *in vivo* and *in vitro* samples, this analysis is performed separately for the *in vivo* and the *in vitro* samples. For a given sample, its *filtered gene set* is defined as the genes that have an expression level exceeding 10 FPKM in at least 20% of the cells. For a given set of samples (*in vivo* or *in vitro*), only the genes that appear in the filtered set of at least two of the samples are retained. This results in ~4,000 genes for the *in vivo* data and ~7,000 genes for the *in vitro*. Batch correction is then performed on the resulting matrices (generated by combining all the samples and filtering for the selected genes) using the COMBAT software (Johnson et al., 2007; Novershtern et al.). To eliminate the effects of quality scores on the resulting matrix (*i.e.*, systematic differences in the quality of different

samples, rather than cells within a sample), the correction procedure described in the previous section was re-applied.

[00243] *Taking into account false negatives using weighted analysis.* The estimation of transcript abundance as zero can be attributed to false-negatives in the RNA-Seq data. Different individual cells within a sample can have different rates of false-negatives, depending on the quality of the library, and cell integrity. To account for this, for every cell a false-negative curve (FNC) was constructed using the following. The cell-specific FNC represents the false-negative rate as a function of transcript abundance in the bulk population. The FNC is built by taking all the housekeeping genes that are detectable (non zero estimated abundance) in the bulk population and in at least one cell, and arranging them into 30 bins. Then for every bin, the ratio of housekeeping genes that are detectable is computed. Finally, a sigmoid function is fitted to the estimated values (See, e.g., Figure 6C). These values are used to weigh down possible false-negatives in the subsequent analysis: (1) For correlation-based analysis weighted correlations are used where a zero-value of a gene *i* in cell *j* is weighted by the value associated in the FNC of *j* with the expression of *i* in the bulk population. For lowly expressed genes the weight will be lower, indicating a higher chance for them to be false-negatives. Notably, the PCA analysis is done by computing the eigenvectors of the weighted covariance matrices. (2) For signature-based scores a weighted version of the gene set enrichment analysis algorithm is used, described next.

[00244] *RNA-FlowFish analysis of RNA-expression.* Cells prepared under the same conditions as the RNA-seq samples were prepared with the QuantiGene® ViewRNA ISH Cell Assay kit from Affymetrix following the manufacturers protocol. High throughput image acquisition at 60x magnification with an ImageStream X MkII allows for analysis of high-resolution images, including brightfield, of single cells. Genes of interest were targeted by type 1 probes, housekeeping genes by type 4 probes, and nuclei were stained with DAPI. Single cells were selected based on cell properties like area, aspect ratio (brightfield images) and nuclear staining. As a negative control, Bacterial DapB gene (Type 1 probe) were used. Spot counting was performed with the amnis IDEAS software to obtain the expression distributions.

[00245] *Weighted gene signature scores and gene set enrichment analysis.* To interpret the functional implications of the variation between cells, a set of gene signatures was assembled

that are indicative of various cell states, using the following. A typical signature is comprised of a "plus" subset and a "minus" subset. A strong match will have extreme, and opposite values for the expression of genes in the two sets (e.g., high values for the "plus" genes and low values for the "minus" genes). The signatures from the following sources are assembled: (1) The immunological signature (ImmSig) collection from MSigDB ((Liberzon et al., 2011); denoted as collection C7): ~2,000 gene sets (each divided into "plus" subset and a "minus" subset) found by comparing immune cells under different conditions (e.g., knockout vs. WT, different stimulations, time post infection etc.). (2) Cell cycle gene sets from MSigDB (Liberzon et al., 2011)and based on the gene ontology database (Huntley et al., 2009); (3) The NetPath database (Kandasamy et al., 2010): a collection of gene sets (each divided into "plus" subset and a "minus" subset) that are downstream of various immune signaling and are either positively or negatively regulated. (4) Signatures of T helper cell subsets, based on previous work (Wu et al., 2013)(Xiao et al., 2014). (5) Signatures of exhausted and memory T cells (Crawford et al., 2014); (6) Microarray data from Sarkar et al (Sarkar et al., 2008), comparing memory vs. effector CD8+ CT cells; (7) Microarray data from Muranski et al (Muranski et al., 2011),, tracking the development of Th17 and Th1 cell in an adoptive transfer model. (8) Microarray data from Kurachi et al (Kurachi et al., 2014), tracking the development of CD4+ and CD8+ T cells in acute and chronic infection models. **(9)** Microarray data comparing IL-23R knockout mice $CD4^+$ T cells differentiated in IL-1β+IL-6+IL-23 to WT (Y.L. and V.K.K, unpublished data).Notably, while sources 1-5 already provide processed gene sets, analysis of the remaining sources is based on the raw data (microarrays). This data was analyzed to infer differentially expressed genes. To this end, all genes with a fold change over 1.5 are reported; if there are at least two replicates, consistent (up or down) and >1.5 fold change in all pairwise comparisons is required (all replicates of condition "A" vs. all replicates of condition "B" must show fold change above the cutoff). To avoid spurious fold levels due to low expression values a small constant is added to the expression values (c=50) prior to the analysis. To search for signatures that are significantly expressed in a subset of cells the following test was performed: First, standardizing the rows of the expression matrix (i.e., every cell is normalized w.r.t. the other cells) and weighing down zero entries as above (multiplying the respective entries in the Z-normalized matrix by (1 – probability for false negative)). Given a signature $S=\{S^+,S^-\}$, a gene set enrichment analysis (GSEA) for every cell independently is

performed, using the values in the standardized, weighted matrix. To account for the direction, the values in the rows that correspond to the genes in S^- are negated. The standard GSEA formulation with 250 randomizations is used, where in each randomized run a random selection of S is considered, and 50 randomly selected cells. The reported p-values are computed empirically by comparing to the resulting 12,500 random scores. A 5% FDR cutoff is computed using the Benjamini-Hochberg scheme (Benjamini and Hochberg (1995) and only signatures that had a p-value below the cutoff in at least 10% of the cells is reported. To associate gene signatures with cell's location along the principle components, for every cell a signature score is computed. For every cell-signature pair, Applicants estimated whether the expression of genes in the signature significantly varied either: (**1**) across cells of the *same* source or (**2**) between conditions (*e.g.*, LN *vs.* CNS). A subset of the results for this analysis are presented in **Figures 2** and **4**. The complete result set is provided in **Table S2** (Gaublomme 2015). To identify signatures that significantly vary between conditions, Applicants then compute for every cell a **signature score**. Given a signature $S=\{S^+,S^-\}$, Applicants define the score as the weighted mean of the genes in S^+ minus the weighted mean of the genes in S^-. Applicants use the gene expression values under the same normalization and weighting scheme as in the weighted PCA analysis above. Signatures that significantly vary between two given conditions ("A", "B") were identified by comparing the distributions of signature scores of cells from condition "A" vs. cells of condition "B" (Kolmogorov-Smirnov (KS) test, FDR<10^{-4}). For the signatures with significant variation in at least one of the two tests above, Applicants next investigated whether they are significantly associated with the main PCs. To this end, Applicants computed a Pearson correlation coefficient between the signature score and each of the first two PCs (*i.e.*, comparing two vectors whose length equals the number of cells: one vector is the signature scores, the other vector is the projection value (i.e., x- or y- coordinate) of that cell in the PC space; **Figures 2-4** and **Table S2** (Gaublomme 2015)). Applicants plotted selected correlations on a normalized PCA map (for example: **Figure 2A**, numbered open circles).

[00246] *TF binding enrichment analysis.* TFs were looked for with a significant overlap between their previously annotated target genes and the genes that correlated with each principal component using the following. TF-target interaction data is obtained from public databases (Chen et al., 2011; Ciofani et al., 2012a; Lachmann et al., 2010; Liberzon et al.,

2011; Linhart et al., 2008). To select the set of genes for a given PC (PC1 or PC2), for every gene the Pearson correlation between its log expression value in every cell (adding a value of 1 to avoid effects of low expression levels) and the projection of this cell to that PC (i.e., the X [for PC1] or Y [for PC2] coordinate in the PC plot) is computed. Only genes with a p-value lower than a 5% FDR cutoff are retained. For every TF in the database, the statistical significance of the overlap between its putative targets and each of the groups defined above using a Fisher's exact test is computed. Cases where $p< 5*10^{-5}$ and the fold enrichment > 1.5 are included. Finally, in Figure 2, only cases in which the TF was expressed above a minimal level (5 FPKM) in at least one of the respective bulk population conditions are reported.

[00247] *Relating the in vitro differentiated cells to their in vivo counterparts.* To perform the analysis presented in Figures 3bB,C genes are identified that are significantly up- or down- regulated in each sub-population of in-vivo cells (FDR<0.05; one-vs-all KS test; Table S4 (Gaublomme 2015), Table 6). A signature is then defined by retaining only genes that are annotate with immune response function based on the gene ontology database (Huntley et al., 2009). Finally, the signature analysis above is repeated to score the in-vitro derived cells.

[00248] *Voronoi diagrams.* Voronoi diagrams were used in order to delineate areas (in the space of the first two principle components (PC)) that are most strongly associated with given signatures. Specifically, given a set of signature $S=\{s\_1,\ldots,s\_k\}$ is computed for every cell k signature scores (one for each signature). For each signature i the top 5 high-scoring cells are selected, and point c_i is computed as the centroid of these points in the PC map (be averaging over their x and y coordinates). Given a set of centroid points $\{c\_1,\ldots,c\_k\}$, the Voronoi diagram divides the space into respective regions $r\_1,\ldots,r\_k$ such that for every $1\leq i \leq k$, c_i is the closest centroid to all the points in r_i. Given a set of signatures that were significantly associated with the PC map in Figure 2a, the above procedure was followed to compute the Voronoi diagram in Figure 2b.

[00249] *Defining biomodal genes.* To quantify the shape of heterogeneity in the expression levels of genes across cells, the following scheme was devised: First, a number of statistical tests are applied in order to identify genes that exhibit a bimodal distribution: (1) Hartigans Dip Test (with a p-value cutoff of 5%); (2) Gaussian mixture model - comparing a 2- or 3-Gaussian model to a 1-Gaussian model using the Bayesian Information Criteria; (3) More than 10% of cells deviate from the mean by more than 2.32 times the standard deviation

(corresponding to a p-value of 1%); (4) More than 10% of cells deviate from the median by more than 2.32 times the median absolute deviation. For genes identified by at least one of the tests, two mixture models are fit using expectation maximization: (1) Exponential (for "non-expressing" cells) and normal (for "expressing" cells); and (2) Uniform (for "non-expressing" cells) and normal (for "expressing" cells). The model with the best fit us retained. Using this model a cutoff *x* is determined for each gene such that cells with expression higher than *x* are considered "expressing cells". *x* is determined as the maximum between {0, the boundary between the Gaussian distribution and the alternative distribution (for bi-modal genes)}. Finally, to define the set of bimodal genes, it is required (in addition to the aforementioned tests) that the percentage of "expressing cells" is smaller than 90%.

[00250] *Gene ranking.* An unbiased approach was used to select potential regulator of Th17 pathogenicity. The ranking is based on: (1) Correlation with the first principle component in the in-vitro derived Th17 cells (using Tgfb1+IL6; Figure 4c). To this end, the correlation between the expression of a given gene in each cell and the PC1 projection value of each cell (X coordinate in Figure 4b) is computed. A 5% FDR cutoff is computed using the Benjamini-Hochberg scheme and only correlations below that cutoff are reported. (2), (3) A similar analysis is performed for correlations with the first and second principle components in the in-vivo derived Th17 cells (Figure 2a). (4) Correlation with immune-related genes in the anti-correlated modules in Figure 4b (a "single cell pathogenicity signature" consisting of a pro-inflammatory module: Ccr6, Il18r1, Ccl4, Ccl20, Ctla4, Il17a, Il2, Cd40lg, Tnf, Il21, Cxcr3, Tnfsf9, Ebi3, and Stat4; and a regulatory module: Ccr4, Il10, Il24, Il9, Il16, Irf4, Sigirr, Il21r, and Il4ra). (5) A similar analysis using a curated pathogenicity signature (genes that are positively or negatively associated with pathogenic Th17). In the following the analysis done to evaluate selection criteria (4) and (5) is explained. For a given gene, and a signature (consisting of two opposing subsets; e.g., pro-inflammatory genes and regulatory genes) it is desirable to evaluate the statistical relationship between them. To this end, the values x1 and x2 are computed as its average correlation with the two opposing subsets respectively. Then for cases where sign(x1)!=sign(x2) its score is designated as sign(x1)*min{abs(x1), abs(x2)}. To estimate the significance of this score the original expression matrix is shuffled, and the test is repeated for 50 times. The shuffling is done independently for each row (gene), but it retains the original values of the gens in the

signature. This way it conserves the expression distribution of each gene, as well as correlations between the member genes of the signature. Only genes that "failed" at most twice are reported, when compared against the shuffled data (empirical p-value<=0.04). Finally, the genes are ranked based on their scores (correlation values for criteria (1)-(3) and an aggregate score for criteria (4)-(5)). Here genes are stratified into groups of 5 (first five genes are ranked 1st; next five genes are ranked 2nd, etc.). The final score is set as the second best rank among criteria (1-5), thus requiring a gene to preform well in at least two tests. This score is amended to prioritize (ranking 1st) genes that come up both in the in-vitro analysis (criteria 1, 4, 5; top 95%) and the in-vivo analysis (criteria 2, 3; top 75%). To break the ties between equally ranked genes, the following features are used, which are based on bulk-population studies: (a) whether the gene is significantly induced during Th17 differentiation (using previous analysis (Yosef et al., 2013), which considers only cases where the induction happened after 4 hours to exclude non-specific hits); (b) whether the gene was differentially expressed in response to Th17-related perturbations in previous studies, using the same collection of knockouts used for ranking in previous work (Yosef et al., 2013). (c) Whether the gene is bound by key Th17 transcription factors, and is affected by their perturbation during Th17 differentiation. To this end, the combined score computed by Ciofani et al. (Ciofani et al., 2012b) is used.

[00251] *Population based studies used to compare top ranking genes found by bulk population vs. single-cell analysis* : Population based data was based on either a compendium of 41 studies of Th17 cells from our labs, (**Table S7** (Gaublomme 2015)), or a literature based ranking (Ciofani et al., 2012). Each study from our labs is a comparison of two treatments (e.g., Th17 cells with or without sodium) for which Applicants identified differentially expressed genes (as described in the Methods section "Signature scores and gene set enrichment analysis"). Applicants then ranked each gene according to the number of studies (0-41) in which it was identified as differentially expressed. The literature based study (Ciofani et al., 2012) considers a combination of RNA-seq and ChIP-seq data, prioritizing genes that are differentially expressed, and bound by key Th17 transcription factors, such as Rorc.

[00252] *Flow cytometry and intracellular cytokine staining.* Sorted naive T cells were stimulated with phorbol 12-myristate 13-aceate (PMA) (50 ng/ml, Sigma-aldrich), ionomycin (1 μg /ml, Sigma-aldrich) and a protein transport inhibitor containing monensin (Golgistop)

(BD Biosciences) for 4 h before detection by staining with antibodies. Surface markers were stained in PBS with 1% FCS for 20 min at room temperature, then subsequently the cells were fixed in Cytoperm/Cytofix (BD Biosciences), permeabilized with Perm/Wash Buffer (BD Biosciences) and stained with Biolegend conjugated antibodies, that is, Brilliant violet 650 anti-mouse IFN- γ (XMG1.2) and allophycocyanin-anti-IL-17A (TC11-18H10.1), diluted in Perm/Wash buffer as described (Bettelli et al., 2006). Foxp3 staining was performed with the Foxp3 staining kit by eBioscience (00-5523-00) in accordance with their 'One-step protocol for intracellular (nuclear) proteins'. Data were collected using either a FACS Calibur or LSR II (Both BD Biosciences), then analysed using Flow Jo software (Treestar).

[00253] *Analysis of RNA-Seq data from knockout cells.* RNA-Seq was used to identify genes that are differentially expressed in knockout T cells, (compared with WT). To this end, replicate data was used to empirically infer a decision cutoff, above which the genes are reported. The decision cutoff is defined as a function of the magnitude of gene expression – genes that are lowly expressed are associated with a higher decision cutoff. To infer the cutoffs, first a set of replicate RNA-Seq experiments is collected. For each pair of replicates, the fold difference across all genes is calculated. The genes are then stratified into 10 bins (taking 10 quartiles), and then for each bin i the standard deviation d_i of fold changes between all pairs of replicates is computed. The fold change cutoff is then determined in each bin i to be $max\ \{1.5, d\_i\}$. As an additional stringent step, the obtained fold change cutoffs is smoothed, such that if the cutoff for a bin i is lower than bin $i+1$ (which includes genes with higher expression levels) then the cutoff of bin $i+1$ is set to that of bin i. For given knockout experiments with n "cases" and m "controls", differentially expressed only cases are expressed in which more than $(n \times m)/2$ comparisons are above the cutoff, and all comparisons are consistent (i.e., up- or down- regulation). As above, to avoid spurious fold levels due to low expression values a small constant to the expression values (5 FPKM) prior to the analysis is added. For the analysis in **Figure 5E** Applicants define the sets of all genes that either positively or negatively correlate with the first PC in cells differentiated with TGF-β1+IL-6 (**Figure 4C**; Pearson correlation, FDR<5%). Applicants then evaluate the significance of overlaps between these sets and the knockout-affected genes using a hypergeometric test. Applicants use the same approach to identify genes that are differentially expressed in the gut vs. the LN or CNS.

[00254] *RNA-FlowFISH.* RNA-fish using QuantiGene® FlowRNA Assay was performed in accordance with manufacturers guidelines for suspension cells, with minor modifications such as pipetting instead of vortexing, cells were stained with dapi and type 1 gene probes only. Cells were imaged using an ImageStream X MkII with a 60x objective. As a negative control, the expression of the bacterial *DapB* gene, in addition to *Csf2, Itgax and Scd1*, which are not expressed on Th17 cells in the TGF-β1/IL-6 condition at 48h was checked.

[00255] *Quantification of cytokine secretion using ELISA.* Naive T cells from knockout mice and their wild-type controls were cultured as described above, their supernatants were collected after 48h and 96h, and cytokine concentrations were determined by ELISA (antibodies for IL-17 and IL-10 from BD Bioscience) or by cytometric bead array for the indicated cytokines (BD Bioscience), according to the manufacturers' instructions.

[00256] ***TABLES***

[00257] The following Tables form a part of this disclosure:

[00258] **Table 1 Sample information: Columns Name;** indicates sample origin, **Batch;** samples with the same batch number originated from the same animal (in addition, batch 1&2 also come from the same animal and serve as technical replicates), **#Cells before filtering;** the number of captured, viable single cells on the Fluidigm C1 chip, **#Cells after filtering;** number of cells that survived filtering criteria (Experimental Procedures), **#Sequencing Reads;** Number of reads sequenced on Illumina HiSeq (average across all cells), **%Aligned reads:** percentage of reads that align to the NCBI Build 37 (UCSC mm9) of the mouse genome using TopHat (average across all cells).

Name	Batch	#Cells after filtering	#Cells before filtering	Average #Sequencing Reads	Average %Aligned reads
EAE-CNS-IL-17A/GFP+	1	48	86	2890292	41,134266
EAE-CNS-IL-17A/GFP+	2	61	75	2728575	45,292021
EAE-CNS-IL-17A/GFP+	4	57	68	2800285	48,0711565
EAE-LN-IL-17A/GFP+	1	39	33	2990609	35,9401461
EAE-LN-IL-17A/GFP+	2	40	38	2593529	45,8093984
EAE-LN-IL-17A/GFP+	3	57	70	3025209	74,5995014
TGFB1_IL6-48h	5	56	80	5468975	69,3823763
TGFB1_IL6-48h	6	74	93	2229856	67,1002098

TGFB1_IL6-48h-IL-17A/GFP+	7	67	86	1945212	63,6447485
TGFB1_IL6-48h-IL-17A/GFP+	8	67	94	3460935	61,2721476
TGFB1_IL6-48h-L-17A/GFP+	9	17	77	6316929	56,1096561
IL1B_IL6_IL23-48h-IL-17A/GFP+	8	69	90	3208148	61,2153719
IL1B_IL6_IL23-48h-IL-17A/GFP+	7	70	86	1936425	65,2173455

[00259] **Table 2.** Ranking of potential regulators of Th17 pathogenicity. Table 2: Potential regulators of Th17 pathogenicity (rows in Figure 4B) are ranked based on: (1) Correlation with the first principle component in the *in vitro* derived Th17 cells (using TGF-β1+IL-6; Figure 4C). (2, 3) Correlations with the first and second principle components in the *in vivo* derived Th17 cells (Figure 2A). (4) Correlation with immune-related genes in the columns of Figure 4B. (5) A Correlation with a curated pathogenicity signature (genes that are positively or negatively associated with pathogenic Th17 cells, (Lee et al., 2012)). The values in these respective columns indicate the rank (percentile) of the gene in the respective test, relative to all other candidate genes. Highly scoring genes are the ones that are bound by key Th17 transcription factors, and affected by perturbation of these factors during Th17 differentiation. The values in the respective column indicate the rank (percentile) of the gene, relative top all other candidate genes. Negative values indicate a negative correlation.

[00260]

					Sources for single-cell score				
Gene	Description	Rank	Attributes	Known	Profiled	Score	In-vivo PC1 rank		
In-vivo PC2 rank	In-vitro (Tgfb1+IL6)PC1 rank	Rank by correlation with single-cell pathogenicity signature (Figure 4c)	Rank by correlation with curate pathogenic signature (Lee et al. 2012)						
CTLA4	cytotoxic T-lymphocyte-associated protein 4	1	"ImmuneResponse,Known,CellSurface"						
1	0	1	0	0.994565217	-0.804347826	0.777173913	0.913043478		
GPR65	G-protein coupled receptor 65	1	0	1	1	0	0.967391304		
-0.369565217	0.586956522	0.777173913							
REL	reticuloendotheliosis oncogene	1	"TF,Known,PathogenicSignature(pos)"	1					
0	1	0	0.967391304	-0.804347826	0.722826087	0.451086957			
TMEM109	transmembrane protein 109	1	0	0	1	-0.967391304	0		
-0.614130435	0.668478261	0.804347826							
CD226	1	CellSurface	0	0	1	0	0.967391304	-0.423913043	
0.695652174	0.777173913								
BCL2A1B	1	0	0	1	0	0.994565217	-0.994565217	0.75	
0.913043478									
GBP2	guanylate binding protein 2	1	PathogenicSignature(pos)	0	0	1			
0.967391304	0.831521739	0.777173913	0	0					
ECE1	endothelin converting enzyme 1	1	0	0	1	0			
0.967391304	-0.47826087	0.668478261	0.885869565						

```
RAMP1   receptor (calcitonin) activity modifying protein 1   1       PathogenicSignature(pos)
        0       0       1       0       0.967391304     -0.695652174    0.559782609     0.695652174

BCL2A1D         1       ImmuneResponse 0        0       1       0       0.994565217     -0.994565217
        0.722826087     0.994565217

PLEK    pleckstrin      1       TF      0       0       1       0.994565217     0.885869565     -
0.858695652     0.722826087     0.940217391

BCL2A1A         1               0       0       1       0.858695652     0.994565217     -0.994565217
        0.777173913     0.967391304

ABCG1   "ATP-binding cassette, sub-family G (WHITE), member 1"      1               0       0
        1       0.967391304     -0.913043478    0.940217391     -0.858695652    -0.614130435

IL2     interleukin 2   2       "ImmuneResponse,Known,CytokineChemokine"        1       0
        0.994565217     0       0       -0.994565217    0.913043478     0.885869565

FAIM3           3               0       1       0.967391304     0       0       0.885869565     -
0.994565217     -0.994565217

1600014C10RIK   RIKEN cDNA 1600014C10 gene      3               0       0       0.967391304     0
        0       0.967391304     -0.940217391    -0.940217391

PDCD1   programmed cell death 1         3       "PathogenicSignature(neg),CellSurface"          0
        0       0.967391304     0       0.777173913     -0.967391304    0.858695652     0.722826087

ID3     inhibitor of DNA binding 3      3       "ImmuneResponse,TF,Known,PathogenicSignature(pos)"
        1       0       0.967391304     0       0       -0.967391304    0.858695652     0.994565217

SLFN2   schlafen 2      3               0       0       0.967391304     0       0       -0.967391304
        0.777173913     0.641304348

ZBTB32  zinc finger and BTB domain containing 32        3       TF      0       1       0.967391304
        0       0       -0.967391304    0.967391304     0.994565217

NFKBID          4               0       0       0.940217391     0       0.75    -0.994565217
        0.831521739     0.586956522

IL16    interleukin 16 4        "Known,CytokineChemokine"       1       0       0.940217391     0
        -0.940217391    0.913043478     0.451086957     0.505434783

SLA     src-like adaptor        4       PathogenicSignature(pos)        0       0       0.940217391
        0       0.804347826     -0.75   0.831521739     0.994565217

GM2792          4               0       0       0.940217391     0       0.831521739     -0.885869565
        0.967391304     0.967391304

MS4A4B  "membrane-spanning 4-domains, subfamily A, member 4B"   5
        PathogenicSignature(pos)        0       0       0.913043478     0       0       -0.586956522
        0.885869565     0.913043478

TGTP2           5       PathogenicSignature(pos)        0       0       0.913043478     0.913043478
        0.695652174     0.913043478     0.369565217     0.39673913

TGTP1           5               0       0       0.913043478     0.940217391     0.75    0.913043478
        -0.559782609    -0.586956522

IL17A   interleukin 17A         6
        "ImmuneResponse,PathogenicSignature(neg),Known,CytokineChemokine"       1       0
        0.885869565     0       0       -0.913043478    0.451086957     0.804347826

ACSL4   acyl-CoA synthetase long-chain family member 4        6       PathogenicSignature(neg)
        0       0       0.885869565     0.885869565     0.885869565     -0.641304348    0
        0.260869565

SOCS2   suppressor of cytokine signaling 2     6
        "PathogenicSignature(neg),Known,CytokineChemokine"      1       0       0.885869565     0
        0       0.885869565     -0.967391304    -0.885869565

FOXP1   forkhead box P1         6       "ImmuneResponse,TF"     0       0       0.885869565
        0.885869565     -0.994565217    0.206521739     0.288043478     0

SYTL3   synaptotagmin-like 3   6               0       0       0.885869565     -0.858695652
        0.940217391     -0.858695652    0.913043478     0.858695652

MAPKAPK3                6       "Kinase,PathogenicSignature(neg)"       0       0       0.885869565
        0       0.940217391     -0.885869565    0       0.179347826
```

```
PRKCSH  protein kinase C substrate 80K-H      6               0       0       0.885869565
        0.885869565     0       -0.315217391    0.994565217     0.940217391

GNG10   "guanine nucleotide binding protein (G protein), gamma 10"  6               0       0
        0.885869565     -0.940217391    0.885869565     0       0       0.423913043

GM2833          6               0       0       0.885869565     0       0.885869565     -0.804347826
        0.885869565     0.831521739

PPID            6               0       0       0.885869565     -0.885869565    0.75    -0.940217391
        0.668478261     0.233695652

CD5L    CD5 antigen-like        6       CellSurface     0       1       0.858695652     0       0
        -0.858695652    0.940217391     0.967391304

TNF     tumor necrosis factor  6       "ImmuneResponse,Known,CytokineChemokine"      1       0
        0.858695652     0.858695652     0.858695652     -0.559782609    0.39673913      0.423913043

IFI47   interferon gamma inducible protein 47 6               0       0       0.858695652     0
        0       0.940217391     -0.804347826    -0.668478261

CD44    CD44 antigen    6       CellSurface     0       0       0.858695652     0       0.858695652
        -0.804347826    0.858695652     0.858695652

GADD45B growth arrest and DNA-damage-inducible 45 beta        6               0       0
        0.858695652     0.858695652     0.913043478     -0.505434783    0.641304348     0.559782609

SH2D1A          6       "ImmuneResponse,PathogenicSignature(pos)"     0       0       0.858695652
        0       0       -0.369565217    0.994565217     0.858695652

GATM    glycine amidinotransferase (L-arginine:glycine amidinotransferase)  7               0
        0       0.831521739     0       0       0.831521739     -0.831521739    -0.858695652

N4BP1   NEDD4 binding protein 1         7               0       0       0.831521739     0       0
        0.722826087     -0.913043478    -0.913043478

NEK6    NIMA (never in mitosis gene a)-related expressed kinase 6   7
        "Kinase,PathogenicSignature(neg)"     0       0       0.831521739     0       0       -
0.831521739     0.641304348     0.75

SUSD3           7               0       0       0.831521739     -0.831521739    0.858695652     0.75
        0       0

MOV10   Moloney leukemia virus 10      7               0       0       0.831521739     0       0
        0.75    -0.967391304    -0.831521739

DUSP4           7               0       0       0.831521739     0       0       -0.831521739
        0.831521739     0.641304348

IER3    immediate early response 3      8       PathogenicSignature(neg)        0       0
        0.804347826     0.994565217     0.804347826     0       0       0.75

EEA1    early endosome antigen 1        8               0       0       0.804347826     0       0
        -0.940217391    0.804347826     0.315217391

BCAT1   "branched chain aminotransferase 1, cytosolic"        8               0       0
        0.804347826     0       0       -0.913043478    0.39673913      0.423913043

MAPKAPK2        MAP kinase-activated protein kinase 2 8       "Kinase,PathogenicSignature(neg)"
        0       0       0.804347826     0.913043478     0.804347826     0       -0.668478261    -
0.804347826

SASH3   SAM and SH3 domain containing 3        8       ImmuneResponse 0        0       0.804347826
        -0.913043478    -0.804347826    0.586956522     0.423913043     0.451086957

STAT4   signal transducer and activator of transcription 4   8
        "ImmuneResponse,TF,Known,PathogenicSignature(pos)"   1       0       0.804347826     0
        0.858695652     -0.315217391    0.885869565     0.804347826

CTLA2B  cytotoxic T lymphocyte-associated protein 2 beta      9               0       0
        0.777173913     0       0       0.831521739     -0.885869565    -0.777173913

CCL20   chemokine (C-C motif) ligand 20       9       "ImmuneResponse,Known,CytokineChemokine"
        1       0       0.777173913     0       0       -0.722826087    0.532608696     0.777173913

PDGFB   "platelet derived growth factor, B polypeptide"      9       PathogenicSignature(neg)
        0       0       0.777173913     0       0       -0.777173913    0.315217391     0.342391304
```

```
TNFSF9  "tumor necrosis factor (ligand) superfamily, member 9"      9
        "ImmuneResponse,Known,CellSurface,CytokineChemokine" 1      0       0.777173913     0
        0.777173913     -0.47826087     0.75    0.940217391

IFI35   interferon-induced protein 35 9     TF      0       0       0.777173913     0
        0.804347826     0.695652174     -0.695652174    -0.777173913

1810029B16RIK   RIKEN cDNA 1810029B16 gene    9             0       0       0.777173913     0
        0       -0.532608696    0.614130435     0.913043478

GEM     GTP binding protein (gene overexpressed in skeletal muscle) 10
        PathogenicSignature(pos)        0       0       0.75    0       0.831521739     -0.423913043
        0.369565217     0.75

IL4RA   "interleukin 4 receptor, alpha"       10
        "ImmuneResponse,SurfaceReceptor,Known,CellSurface,CytokineChemokine"       1       0
        0.75    0.885869565     0.722826087     0.451086957     -0.505434783    -0.641304348

INPP5B  inositol polyphosphate-5-phosphatase B       10             0       0       0.75    0
        0.722826087     -0.532608696    0.722826087     0.75

RHOF            10              0       0       0.75    0       0.75    -0.75   0.695652174
        0.559782609

PPAN    peter pan homolog (Drosophila)        10             0       0       0.75    0       0
        -0.940217391    0.75    0.179347826

MAGOHB  mago-nashi homolog B (Drosophila)     10             0       0       0.75    -0.913043478
        0       -0.75   0.532608696     0.532608696

TYW3            10              0       0       0.75    0       0       -0.777173913    0.777173913
        0.668478261

IRF1    interferon regulatory factor 1        11
        "ImmuneResponse,TF,Known,PathogenicSignature(pos)"  1       0       0.722826087     0
        0       0.885869565     0.369565217     0

CD40LG  CD40 ligand     11      "ImmuneResponse,Known,CellSurface,CytokineChemokine" 1       0
        0.722826087     0       0.75    -0.722826087    0.641304348     0.614130435

BCL2L1  BCL2-like 1     11      PathogenicSignature(neg)      0       0       0.722826087     0
        0.722826087     -0.804347826    0.342391304     0.369565217

SLC35A1         11              0       0       0.722826087     0       0       -0.777173913
        0.614130435     0.315217391

RPF2            11              0       0       0.722826087     -0.858695652    0       -0.722826087
        0.206521739     0

TM2D3   TM2 domain containing 3       11             0       0       0.722826087     0       0
        -0.722826087    0.777173913     0.722826087

IRF4    interferon regulatory factor 4        12
        "ImmuneResponse,TF,PathogenicSignature(neg),Known"  1       0       0.695652174     0
        0       0.559782609     -0.75   -0.722826087

IL18R1  interleukin 18 receptor 1     12
        "ImmuneResponse,SurfaceReceptor,Known,CellSurface,CytokineChemokine"       1       0
        0.695652174     0       0.940217391     0       0.722826087     0.695652174

ZFP36   zinc finger protein 36 12             0       0       0.695652174     0.994565217     0
        0.695652174     -0.586956522    0.423913043

ASRGL1  asparaginase like 1     12             0       0       0.695652174     0       0
        0.695652174     -0.532608696    0.47826087

CBWD1   COBW domain containing 1      12             0       0       0.695652174     0       0
        -0.777173913    0.505434783     0.668478261

GTPBP4  GTP binding protein 4  12             0       0       0.695652174     0       -0.885869565
        -0.695652174    0.206521739     0.206521739

IRF2    interferon regulatory factor 2        12      TF      0       0       0.695652174
        0.913043478     -0.695652174    0.233695652     0.179347826     0.668478261

HIVEP3  human immunodeficiency virus type I enhancer binding protein 3      13      TF      0
        0       0.668478261     0       0       -0.668478261    0.233695652     0.206521739
```

```
MS4A6B "membrane-spanning 4-domains, subfamily A, member 6B"       13
       "PathogenicSignature(pos),CellSurface"       0       0       0.668478261    0       -
0.668478261    0       0.967391304    0.722826087

OLFM2          13             0       0       0.668478261    0       0       -0.668478261   0
       0.152173913

CCR6   chemokine (C-C motif) receptor 6      13
       "SurfaceReceptor,PathogenicSignature(neg),Known,CellSurface,CytokineChemokine"     1
       0       0.641304348    0       -0.913043478   -0.641304348   0       0.369565217

COG6   component of oligomeric golgi complex 6      13      "ImmuneResponse,Known" 1       0
       0.641304348    0       0       0       -0.559782609   -0.641304348

PIK3R1 "phosphatidylinositol 3-kinase, regulatory subunit, polypeptide 1 (p85 alpha)"     13
       0       0       0.641304348    0.831521739    0       0.451086957    -0.614130435
       0.315217391

IL21R  interleukin 21 receptor       13
       "ImmuneResponse,SurfaceReceptor,Known,CellSurface,CytokineChemokine"       1       0
       0.641304348    0       0       0.668478261    -0.804347826   0.206521739

IMPA2  inositol (myo)-1(or 4)-monophosphatase 2     13             0       0       0.641304348
       0       0       0.641304348    0.179347826    -0.668478261

RSPH3A         13             0       0       0.641304348    0       0       0.532608696    -
0.940217391    -0.722826087

CDS2   CDP-diacylglycerol synthase (phosphatidate cytidylyltransferase) 2  13
       PathogenicSignature(neg)       0       0       0.641304348    0       0       0.641304348
       -0.695652174   0.39673913

CD24A  CD24a antigen  13      "ImmuneResponse,PathogenicSignature(pos),CellSurface"       0
       0       0.614130435    0       0       -0.614130435   0.559782609    0.233695652

IL24   interleukin 24 13      "PathogenicSignature(neg),Known,CytokineChemokine"   1       0
       0.614130435    0       0       0.614130435    -0.994565217   -0.940217391

SLC15A3 "solute carrier family 15, member 3"  13      PathogenicSignature(neg)       0       0
       0.614130435    0       0.913043478    -0.47826087    0.233695652    0.614130435

IKZF3          13      TF      0       0       0.614130435    0       0       0.559782609    -
0.940217391    -0.858695652

HIST1H4D               13             0       0       0.614130435    -0.994565217   0
       0.505434783    0.152173913    -0.614130435

ITGAV  integrin alpha V       13      CellSurface    0       0       0.614130435    0
       0.831521739    -0.586956522   0       0.342391304

PROCR  "protein C receptor, endothelial"     13      "ImmuneResponse,SurfaceReceptor,CellSurface"
       0       0       0.614130435    0       0       0.288043478    -0.913043478   -0.831521739

TPR    translocated promoter region  13             0       0       0.614130435    -0.967391304
       0       0       0.614130435    0.614130435

IL9    interleukin 9  14      "ImmuneResponse,PathogenicSignature(neg),Known,CytokineChemokine"
       1       0       0.586956522    0       0       0.559782609    -0.994565217   -0.967391304

CD84   CD84 antigen   14      CellSurface    0       0       0.586956522    0       0       -
0.586956522    0.315217391    0.342391304

TREML2         14             0       0       0.586956522    0       0       0.532608696    -
0.668478261    -0.804347826

POLB   "polymerase (DNA directed), beta"     14             0       0       0.586956522    0
       0       -0.668478261   0.858695652    0.233695652

SMAP1  stromal membrane-associated protein 1 14             0       0       0.559782609    0
       0       0.260869565    -0.641304348   -0.641304348

INSL6  insulin-like 6 14             0       0       0.559782609    0       0       -0.451086957
       0.451086957    0.559782609

CYLD           14             0       0       0.559782609    0       -0.858695652   0
       0.47826087     0.559782609

MAPRE2 "microtubule-associated protein, RP/EB family, member 2"    15             0       0
       0.532608696    0       0.940217391    -0.423913043   0.559782609    0.532608696
```

```
STK38L          15      Kinase  0       0       0.532608696     0       0       -0.858695652
        0.423913043     0.47826087

DOT1L           15              0       0       0.532608696     0       0.777173913     -0.532608696
        0.260869565     0.260869565

BDH2            15              0       0       0.532608696     0       0       -0.451086957
        0.315217391     0.831521739

ACAT3           15              0       0       0.532608696     0       0       0.233695652     -
0.586956522     -0.586956522

BTBD19          16              0       0       0.505434783     0       0       -0.505434783
        0.369565217     0.39673913

BC031181        cDNA sequence BC031181 16               0       0       0.505434783     0
        0.777173913     -0.39673913     0       0.505434783

SP3     trans-acting transcription factor 3     16      TF      0       0       0.505434783
        0.967391304     0       0       -0.614130435    0.505434783

IRAK1   interleukin-1 receptor-associated kinase 1      16
        "ImmuneResponse,Kinase,Known,CytokineChemokine"         1       0       0.505434783
        0.940217391     0       -0.206521739    0.505434783     0.505434783

EXOSC1  exosome component 1     16              0       0       0.505434783     0       0       -
0.505434783     0.315217391     0.586956522

EBI3    Epstein-Barr virus induced gene 3       17      "Known,CytokineChemokine"       1       0
        0.47826087      0       0       -0.315217391    0.831521739     0.967391304

ACIN1   apoptotic chromatin condensation inducer 1      17      TF      0       0       0.47826087
        0       0       0.315217391     -0.505434783    -0.75

FASTKD2 FAST kinase domains 2   17              0       0       0.47826087      0       0       -
0.858695652     0       0

PPP1R8  "protein phosphatase 1, regulatory (inhibitor) subunit 8"       17              0       0
        0.47826087      -0.940217391    0       0       -0.586956522    0.47826087

MAF1    MAF1 homolog (S. cerevisiae)    17      TF      0       0       0.47826087      0       0
        0.47826087      0.342391304     0.586956522

TRMU            17              0       0       0.47826087      0       0       -0.451086957
        0.804347826     0.695652174

STAT5B  signal transducer and activator of transcription 5B     18      "ImmuneResponse,TF,Known"
        1       0       0.451086957     0       0       0.288043478     0.423913043     0.451086957

LTA     lymphotoxin A   18      "ImmuneResponse,Known,CytokineChemokine"        1       0
        0.451086957     0       0.722826087     -0.206521739    0.423913043     0.451086957

EGR2    early growth response 2         18      "TF,PathogenicSignature(neg)"   0       0
        0.451086957     0       0.695652174     -0.369565217    0.369565217     0.39673913

SIRT6           18      TF      0       0       0.451086957     0       0       0.559782609     0
        0.233695652

EXT1    exostoses (multiple) 1  19              0       0       0.423913043     0       0       0
        0.39673913      0.423913043

NHEJ1   nonhomologous end-joining factor 1      19              0       0       0.423913043     0
        0       0.423913043     -0.47826087     -0.695652174

SERPINF1        "serine (or cysteine) peptidase inhibitor, clade F, member 1"           20
        0       0       0.39673913      0       0       -0.39673913     0.695652174     0.831521739

TGM2    "transglutaminase 2, C polypeptide"     20              0       0       0.39673913      0
        0       0.39673913      -0.885869565    -0.885869565

ADI1    acireductone dioxygenase 1      20              0       0       0.39673913      0       0
        0       -0.47826087     -0.532608696

RNF181  ring finger protein 181         20              0       0       0.39673913      0       0
        0.39673913      0.179347826     0.179347826

METT10D         20              0       0       0.39673913      0       0       -0.342391304
        0.586956522     0.532608696
```

```
NIP7    nuclear import 7 homolog (S. cerevisiae)     20            0       0       0.39673913
        0       0       -0.831521739    0.39673913      0

PSRC1   proline/serine-rich coiled-coil 1    20            0       0       0.369565217     0
        0       0.369565217     0.288043478     0.288043478

TBL2    transducin (beta)-like 2     20            0       0       0.369565217     0       0
        0.369565217     0.288043478     0.342391304

PQLC3   PQ loop repeat containing     20            0       0       0.369565217     0       0
        0.641304348     -0.47826087     0.233695652

NIF3L1  Ngg1 interacting factor 3-like 1 (S. pombe)  20            0       0       0.369565217
        0       0       -0.586956522    0.342391304     0.369565217

CYSLTR1 cysteinyl leukotriene receptor 1     21     PathogenicSignature(neg)      0       0
        0.342391304     0       0       0.342391304     -0.804347826    0.179347826

PDLIM5  PDZ and LIM domain 5   21     PathogenicSignature(neg)      0       0       0.342391304
        0       0       -0.614130435    0       0

LAG3    lymphocyte-activation gene 3  21
        "ImmuneResponse,PathogenicSignature(pos),CellSurface"       0       0       0.342391304
        0       0.777173913     -0.260869565    0.940217391     0.315217391

SLC25A13        "solute carrier family 25 (mitochondrial carrier, adenine nucleotide
translocator), member 13"       21            0       0       0.342391304     0       0       -
0.342391304     0       0.451086957

GTF2E1          21      TF      0       0       0.342391304     0       0       -0.614130435    0
        0.342391304

TSPAN6  tetraspanin 6  22     PathogenicSignature(neg)      0       0       0.315217391     0
        0       -0.505434783    0       0

CHD2            22      "TF,PathogenicSignature(pos)" 0       0       0.315217391     0       0
        0.668478261     0.179347826     0.152173913

ASB3    ankyrin repeat and SOCS box-containing 3     22            0       0       0.315217391
        0       0       0.315217391     0.233695652     0.260869565

DAPL1           23              0       0       0.288043478     0       0       0       0.913043478
        0.885869565

UBA3    ubiquitin-like modifier activating enzyme 3  23            0       0       0.288043478
        0       0       -0.288043478    0.233695652     0.559782609

ZUFSP   zinc finger with UFM1-specific peptidase domain      23      TF      0       0
        0.288043478     0       0       -0.288043478    0.641304348     0.315217391

MED21   mediator complex subunit 21   23            0       0       0.288043478     0       -
0.831521739     0       0.260869565     0.288043478

NGDN    "neuroguidin, EIF4E binding protein" 23            0       0       0.288043478     0
        -0.913043478    0       0       0.288043478

PIN4            23              0       0       0.288043478     0       0       0.288043478
        0.260869565     0

BCDIN3D BCDIN3 domain containing      23            0       0       0.288043478     0       0
        -0.342391304    0.532608696     0.152173913

RIPK3   receptor-interacting serine-threonine kinase 3       24      Kinase  0       0
        0.260869565     -0.940217391    0       0       0       0.260869565

CENPM   centromere protein M   24            0       0       0.260869565     0       0       0
        0       0.260869565

TACC3   "transforming, acidic coiled-coil containing protein 3"     24            0       0
        0.260869565     -0.994565217    0       0.260869565     0.233695652     0

STAG1           24      TF      0       0       0.260869565     0       0       0       0.451086957
        0.505434783

PDSS1   "prenyl (solanesyl) diphosphate synthase, subunit 1" 24            0       0
        0.260869565     0       0       -0.260869565    0       -0.532608696

CEP57           24              0       0       0.260869565     0       0       0.39673913
        0.315217391     0
```

```
MRPS22  mitochondrial ribosomal protein S22  24              0       0       0.260869565     0
        0       -0.260869565    0.47826087      0

KIF5B   kinesin family member 5B       25      PathogenicSignature(neg)        0       0
        0.233695652     0       -0.695652174    -0.233695652    0.423913043     0

BC055324                25              0       0       0.233695652     0       0       0       0.75
        0.695652174

CAMTA1          25      TF      0       0       0.233695652     0       0       0.233695652     -
0.532608696     0.47826087

C2CD3   C2 calcium-dependent domain containing 3      26              0       0       0.206521739
        0       0       0.342391304     0.206521739     0.206521739

NGLY1   N-glycanase 1   27              0       0       0.179347826     0       0       0.47826087
        0       0

DEGS1   degenerative spermatocyte homolog 1 (Drosophila)      27              0       0
        0.179347826     0       0       -0.423913043    0.39673913      0

GALK1   galactokinase 1         28      Kinase  0       0       0.152173913     0       0       0
        0       0.39673913

SPSB3   splA/ryanodine receptor domain and SOCS box containing 3     28              0       0
        0.152173913     0       0       0       0       0.152173913

CSNK1E  "casein kinase 1, epsilon"      29      Kinase  0       0       0.125   0       0       0
        0.342391304     0.369565217

TTC27   tetratricopeptide repeat domain 27     29              0       0       0.125   0       0
        -0.233695652    0.288043478     0

LINS            29              0       0       0.125   0       0       -0.206521739    0       0

INO80C  INO80 complex subunit C         30      PathogenicSignature(neg)        0       0
        0.097826087     0       0       0       0.288043478     0.288043478

FDX1    ferredoxin 1    30              0       0       0.097826087     0       0       0
        0.260869565     0.288043478

ITM2A   integral membrane protein 2A  31              0       0       0.070652174     0       0
        0       0.206521739     0.206521739

MTPAP           31              0       0       0.070652174     0       0.695652174     0
        0.505434783     0

DHX9    DEAH (Asp-Glu-Ala-His) box polypeptide 9      32              0       0       0.043478261
        0       0       0       0.152173913     0.152173913

CEP55   centrosomal protein 55 33               0       0       0       0       0       0       -
0.451086957     0.47826087

FAM118A "family with sequence similarity 118, member A"      33              0       0       0
        0       0       0       0       0

2500003M10RIK   RIKEN cDNA 2500003M10 gene      33              0       0       0       0       0
        0       0       0

ICAM1   intercellular adhesion molecule 1      33      "ImmuneResponse,CellSurface"  0       0
        0       0       0       0       0       0.369565217

GNPDA2  glucosamine-6-phosphate deaminase 2   33              0       0       0       0       0
        0       0.342391304     0

MTA3    metastasis associated 3         33      TF      0       0       0       0       0.722826087
        0       0.260869565     0

CCDC9   coiled-coil domain containing 9         33              0       0       0       0       0
        0       0.206521739     0.179347826

2210016L21RIK   RIKEN cDNA 2210016L21 gene      33              0       0       0       0       0
        0       0.179347826     0
```

[00261] **Table 3.** Normalized data of lipidome analysis. WT and CD5L$^{-/-}$ naïve T cells were differentiated. Cells and supernatant were harvested at 96 hours and subjected to MS/LC. Three independent mouse experiments were performed.

TGFb1+IL6_WT TGFb1+IL6_CD5LKO
TGFb1+IL6+IL23_WT TGFb1+IL6+IL23_CD5LKO
TGFb1+IL6_no cells TGFb1+IL6+IL23_no cells
TGFb1+IL6_WT TGFb1+IL6_CD5LKO TGFb1+IL6+IL23_WT
TGFb1+IL6+IL23_CD5LKO

Method	Compound	m/z	RT	HMDB ID	Metabolite	1_media	1a_media	1b_media
	2_media	2a_media	2b_media	3_media	3a_media	3b_media	4_media	
	4a_media	4b_media	5_media	5a_media	5b_media	6_media	6a_media	
	6b_media	1_cells	1a_cells	1b_cells	2_cells	2a_cells	2b_cells	
	3_cells	3a_cells	3b_cells	4_cells	4a_cells	4b_cells		
C18-NEG	TF1	355.2417	10.85	Internal Standard		PGE2-d4	26815668	26815668
	26815668	26815668	26815668	26815668		26815668	26815668	
	26815668	26815668	26815668	26815668		26815668	26815668	
	26815668	26815668	26815668	26815668		32190232	32190232	
	32190232	32190232	32190232	32190232		32190232	32190232	
	32190232	32190232	32190232	32190232				
C18-NEG	TF16	227.2006	16.5	HMDB00806	Myristic acid			
	3904	4592	5454	4734		6041	22362	4171
C18-NEG	TF18	255.2319	17.6	HMDB00220	Palmitic acid	5120		6669
	5595				6114			
		16628	3937	4288	4660	4573	5688	5506
C18-NEG	TF22	283.2632	18.45	HMDB00827	Stearic acid			
	5586							
		25493		4119	3574	5192	5674	
C18-NEG	TF6	303.2319	16.95	HMDB01043	Arachidonic acid			
						181344	214866	264314	172799
	212733	190235						
	4403							
C18-NEG	TF9	327.2319	16.7	HMDB02183	Docosahexaenoic acid			7338
		4140				388565	391793	411429	458935
	392193	415325						4137
C18-NEG	TF3	295.2279	14.3	HMDB04667	13-S-HODE		5135	
		9756	5592	4821	5142	24376	32547	28776	38269	36384
	17816	9894	27006	50636	57308	17034	17395	85814	41146	17985	63454
	20151									
C18-NEG	TF5	319.2268	15	HMDB11134	5-HETE			
					5686	13028	27430	17126	23621	
C18-NEG	TF2	319.2268	14.85	HMDB06111	12-HETE			
					605651	554051	571461	616076	556886	619527
C18-NEG	TF4	319.2268	14.6	HMDB03876	15-HETE			
					25361	7666	29546	49138	48717	
C18-NEG	TF20	351.2166	10.85	HMDB01220	PGE2	94502	165627	75971	144941	137472
	119832	128956	156919	133633	105390	116751	100092	113499	92244	105953	97876	96506
	111171	166567	145815	151644	150862	128839	143503	116128	138440	150043	153804	132273
	160265											
C18-NEG	TF21	378.2404	12.9	HMDB00277	Sphingosine 1-Phosphate			
	48562	4726		4961				28965	9479	

```
        17886   24158   12678   86777   199953  50675           66831   42635   87122   55959   58936
        32157   26314   41178

C18-NEG TF8     391.2843        13.7    HMDB00626       Deoxycholic acid/Chenodeoxycholic acid
        33842           129255  72931   24917   80429   57516   30651   56847   45052   71388   28804
        73469   66746   70604   67456   65810   87301   146975  5174    61510           28222   72116
        143146  11419   26116   130107  5832    79792

C18-NEG TF7     407.2792        12      HMDB00619       Cholic acid     841312  829989  1895854 1060513
        945565  959861  878522  914774  963595  874981  915457  937031  1044979 973879  1012834 1083731
        1055547 1066135 279570  14865   45923           22873   84326   291472  8592    74745   249696
        4172    158405

C18-NEG TF13    432.3109        13.65   HMDB00698       Glycolithocholic acid   358320  307449  804941
        446981  464141  452405  420928  417936  453376  399497  450928  417387  494639  474051  511277
        464895  434862  599613  306566  191381  221224          178104  218413  348429  112692  233360
        245261  143848  236992

C18-NEG TF10    448.3058        12.05   HMDB00637       Glycochenodeoxycholic acid      7723861 7584903
        16730744        9175994 8925907 8566471 8185757 8624925 8045296 7872327 8373366 8707438 9173126
        8790607 9130167 9715621 9130630 9973976 2371148 514553  301446          319299  342475  2726397
        287994  416282  2624579 298221  653840

C18-NEG TF12    448.3058        12.4    HMDB00631       Glycodeoxycholic acid   1294337 1218407 2895264
        1526275 1473921 1432269 1381864 1434129 1344172 1235504 1371185 1399989 1455338 1530555 1537812
        1682132 1596115 1620654 732878  147574  106136          96251   105425  870148  94741   119858
        813426  70296   214418

C18-NEG TF14    448.3058        11      HMDB00708       Glycoursodeoxycholic acid       27507   25021
        77979   26688   14758   6136    9701    21662   14785   11184   9364    25134   38008   22933
        28917           28088   30834


C18-NEG TF11    464.3007        11      HMDB00138/      Glycocholic acid        2936939 3041840 6885427
        3555056 3419546 3325521 3185794 3293510 3182289 3084720 3266052 3319168 3357090 3481738 3297525
        3631694 3399351 3897272 443415  113351  32011           66765   50376   627468  51569   58617
        545384  75607   127209

C18-NEG TF27    482.2935        12.85   HMDB00722       Taurolithocholic acid   2843408 2725802 6610292
        3637940 3436786 3187052 3098266 3137579 3268503 2985852 3226234 3153783 3478013 3330871 3486687
        3569226 3390069 3871627 678802  178585  5896            13015   36954   948858  55824   43382
        953183  55880   86020

C18-NEG TF23    498.2884        11.35   HMDB00951       Taurochenodesoxycholic acid     9711333 9187433
        21765418        10814147        11432901        10500138        10387928        10774996
        10569920        9857044 10085203        10135701        10588339        10195689
        10615260        10721686        10601011        11106218        3005206 1274941 983454  508378
        867034  921101  3540079 833310  946736  3469976 813296  1033422

C18-NEG TF25    498.2884        11.65   HMDB00896       Taurodeoxycholic acid   1293712 1299344 2209563
        1097889 1204800 1186702 1291165 1138396 1062944 1244038 1081470 1190853 1114176 1028242 1053480
        1520099 1145610 1164120 1108272 802664  673835  495822  718123  521982  1028133 564107  564084
        1012843 535227  539591

C18-NEG TF26    498.2884        10.3    HMDB00874       Taurohyodeoxycholic
acid/Tauroursodeoxycholic acid          607603  530169  749503  65618   564781  460525  409550  364218
        347874  292734  365255  317061  314646  277955  320850  281048  216878  298726  914503  837410
        858965  683257  700081  687038  778814  613338  617107  530759  531342  465380

C18-NEG TF24    514.2833        10.35   HMDB00036       Taurocholic acid        3683215 3788120 8155464
        4289030 4360551 4078206 3939955 3860197 3750381 3730889 3862014 4076709 4195791 4073666 3961542
        4543412 4209047 4584683 1022009 609445  530472  339078  487087  504578  1079071 478051  474946
        945912  412479  395216

C8-pos  2646    622.4444        6.88    Internal Standard       C24:0 PC        1012539 1158015 996417
        1161771 1043447 1087052 1030377 1037703 1023566 1019157 1013596 1032116 921771  1088590 1006026
        1059405 1018984 998964


C8-pos  1266    468.3088        4.58    HMDB10379       C14:0 LPC       3332    2679    5141    5977
        7970    7902    3713    6841    6850    5416    7519    6661    10608   9638    8547    9207
        8432    5929    8833    8161    6353    12478   9865    7275    7776    8962    5614    8336
        9238    6158
```

C8-pos	1392	494.3243		4.75	HMDB10383		C16:1 LPC		7014	5646	8282	13214
	14635	12487	7561	10624	11967	11642	13026	11105	42413	43253	46059	39641
	42162	25977	7062	9879	8556	13284	10300	8166	10118	12133	7968	9734
	10960	8711										
C8-pos	1685	496.3400		5.12	HMDB10382		C16:0 LPC		205378	185047	284161	287427
	351653	316290	164849	308496	316058	293955	316343	280511	615570	602681	657403	558708
	602325	451796	408232	274878	300060	310202	378407	255703	303547	344659	258328	314892
	371463	287901										
C8-pos	1536	520.3412		4.95	HMDB10386		C18:2 LPC		2120	1653	1606	2623
	3136	1558	161	895	1467	2115	1490	1991	29086	31806	28260	27216
	29300	20841	1254	1762	2827	1230	1764	692	1771	1427	975	2009
	1520	778										
C8-pos	1817	522.3559		5.31	HMDB02815		C18:1 LPC		37313	33235	47063	54536
	65990	54998	37484	53044	53484	58851	60620	51574	336070	323141	340550	305699
	331960	212335	74725	95810	121857	138419	130839	100262	116800	120798	109338	114039
	122626	109496										
C8-pos	2049	524.3716		5.73	HMDB10384		C18:0 LPC		89186	93212	150175	106945
	156320	139282	78631	143240	140446	115978	144991	131814	513627	484891	539919	452124
	541318	380513	515430	389585	382528	305556	425178	306964	290336	483268	341639	377311
	496284	398060										
C8-pos	1565	544.3408		4.98	HMDB10395		C20:4 LPC		315	546		2629
	1078	957	341	713	386	1538	1720	363	47160	48570	38948	43474
	45103	25078										
C8-pos	1686	518.3222		5.12	HMDB10393		C20:3 LPC		98536	79703	133563	127294
	161620	140310	78814	147798	143482	135002	147118	135523	283389	278500	302446	252437
	273364	205344	172119	123723	131367	139487	169528	118266	132188	154304	116932	147822
	175532	138138										
C8-pos	1543	568.3409		4.96	HMDB10404		C22:6 LPC		494			584
	207	363				399	194		16424	16085	13036	13927
	13541	8409										
C8-pos	1716	454.2916		5.15	HMDB11503		C16:0 LPE		476	111	111	799
	365	25	48	114	310	357	69	3	42	213	17	50
	22	290	6251	3178	2516	2983	3123	2174	2481	4162	1790	3408
	3299	1874										
C8-pos	1843	480.3093		5.33	HMDB11506		C18:1 LPE		2218	1466	1394	1398
	1209	2123	4672	1704	1918	1684	2263	1083	2374	1885	1870	1811
	2302	1519	10950	10311	12382	14075	12409	8842	12639	9670	11071	13057
	10742	9984										
C8-pos	2057	482.3243		5.75	HMDB11130		C18:0 LPE					
	35416	30237	24614	20595	26391	19130	19611	31352	21418	24054	30243	25746
C8-pos	1516	502.2929		4.89	HMDB11517		C20:4 LPE					
	13129	9742	8024	7203	7444	6125	8659	8705	6801	7602	9012	7862
C8-pos	3036	704.5219		8.17	HMDB07870		C30:1 PC		14998	25161	22315	25216
	19814	21383	57018	15177	13329	18092	17895	16663	1456	2479	2480	1441
	1314	2193	3179497	2637808	2115223	3934853	2521297	2762885	2991782	2105914	1788641	3155606
	2511736	2342464										
C8-pos	3174	706.5381		8.50	HMDB07869		C30:0 PC		95742	139255	118195	139529
	104080	121173	263005	100045	97722	103152	103096	99173	18413	22625	18592	15786
	19556	9024	23932912		13680597		9555005	11495872		11288552		
	11464986		8510608	11003330		8981976	12883235		12142872		11754712	
C8-pos	3094	730.5376		8.32	HMDB07874		C32:2 PC		294	3697	1418	1476
	619	26079	669			328						40
	41	1602014	1907726	1312863	2753385	1927112	1914619	1924029	1592890	1274248	2097057	1740467
	1617004											
C8-pos	3294	732.5539		8.66	HMDB07873		C32:1 PC		205285	286601	262106	282180
	231946	242071	498537	210272	231474	222549	207147	203388	97691	86987	90542	71470

	83548	51104	55194967		33848332		22246803		35107212		26193771	
	27784163		27670368		25706127		22602306		32424675		29776663	
	26605312											
C8-pos	3502	734.5693		8.96	HMDB07871		C32:0 PC		309174	390337	393097	385227
	321514	354180	391822	311399	324195	329510	336354	318227	287073	278414	320104	264113
	285151	190297	33287969		25702073		16256254		16464607		16426187	
	16731527		12179174		19370270		16431600		17890462		21505865	
	18037146											
C8-pos	3164	756.5530		8.49	HMDB08006		C34:3 PC					
		702								323		
	875171	988856	673483	1023525	867319	827735	701311	723025	644826	810044	783009	740564
C8-pos	3370	758.5690		8.80	HMDB07973		C34:2 PC		169769	212274	214180	212897
	212089	187074	402369	163864	177569	164380	162867	167024	188001	150595	175966	139347
	152171	104600	26166733		26958332		20067830		33686135		26641163	
	25560517		23405347		22095533		20436688		25485037		23869850	
	22262117											
C8-pos	3631	760.5849		9.11	HMDB07972		C34:1 PC		1437013	1781135	1933722	1716678
	1558530	1672468	2162247	1493713	1715862	1471713	1477760	1479214	1619969	1562252	1751912	1444734
	1523100	1105535	178901591		143335374		109157114		104342448		104833865	
	111146354		117279402		122152546		109711913		123307892		123029810	
	119131624											
C8-pos	3853	762.6004		9.40	HMDB07970		C34:0 PC		93512	121057	127626	122565
	102842	109523	108969	102870	117047	106010	107085	99058	126394	106850	122762	97715
	102705	67949	8968825	7919729	5888575	4726055	6062746	5415346	4727828	7213126	6142124	6076837
	7612206	5900373										
C8-pos	3481	784.5846		8.95	HMDB08105		C36:3 PC		147616	187415	202520	172746
	173027	186187	191142	161841	161995	153635	170015	158580	199672	189187	215520	175659
	194530	125204	7160245	9618295	7714713	8676343	7795045	8314128	6651223	7610487	7721078	6945637
	8164057	7771753										
C8-pos	3732	786.6005		9.24	HMDB08039		C36:2 PC		463073	626529	642678	540064
	501205	547609	1132092	467091	522166	430865	451021	454155	502200	468531	516696	427261
	454378	327946	80860255		94507728		89183697		104034202		90569180	
	92168585		91632373		86054248		86270047		80643093		88651540	
	86428668											
C8-pos	3995	788.6163		9.53	HMDB08038		C36:1 PC		888964	1120466	1153888	1063681
	962494	1020635	1279105	934110	1093301	915048	916238	903430	1180935	1052607	1154069	935346
	1039898	714352	100494858		77489842		61738737		47157821		55637534	
	60054259		55954680		71002509		60173420		59028380		68769288	
	63205618											
C8-pos	3355	806.5687		8.77	HMDB07991		C38:6 PC		102208	137870	139801	127593
	124626	127378	84574	121859	139186	107861	115065	114944	186101	151302	164681	144477
	153478	101856	731004	908514	411716	737076	484366	515978	497744	443482	419329	556145
	496703	475949										
C8-pos	3619	810.6002		9.11	HMDB08048		C38:4 PC		116733	150165	163751	136678
	137341	151632	95753	129561	153570	119951	128201	117325	162699	152936	176914	139421
	161637	109067	1119481	1728422	1303515	1786146	1480693	1516861	1485202	1370926	1331765	1448781
	1357728	1458638										
C8-pos	3856	812.6144		9.40	HMDB08047		C38:3 PC		338892	418953	471728	409861
	385841	425796	299347	376452	428895	383143	386432	367059	523006	455579	527243	426327
	475247	324421	3239362	3647621	2833978	2728332	2922200	3106003	2670371	2841476	2684190	2651238
	3038207	2840122										
C8-pos	4068	814.6319		9.62	HMDB08270		C38:2 PC		49919	73027	94939	71994
	55413	74436	154969	58418	76390	57351	52099	57746	75316	64649	72867	48600
	64364	36744	9293840	9520856	10010844		8082374	8421060	9506227	8312101	8578760	8643930
	7578026	8600347	8689160									
C8-pos	3148	826.5356		8.45	HMDB08511		C40:10 PC					
	17634	26748	5024	32216	9203	8120	8531	7348	1796	18414	3967	6035
C8-pos	3350	828.5511		8.77	HMDB08731		C40:9 PC		42395	52578	58279	50320
	52277	59335	35165	52676	56700	49452	47562	47608	80225	64631	74621	59199

66559 40590 194698 287249 130844 220042 150639 144369 158304 133617 123826 183219
152956 141857

C8-pos 3540 834.5999 9.01 HMDB08057 C40:6 PC 28677 40315 45841 42134
29354 35209 22163 29520 41487 25526 27385 27412 43491 39109 46984 35182
35893 28580 396980 487550 307629 580470 379342 413709 364802 296967 313224 412487
347813 331729

C8-pos 3518 740.5557 8.97 HMDB11212 C34:4 PC plasmalogen 4797 9848 5518
11398 2515 2578 59001 1064 1221 3052 2890 466
9752434 6913124 4965803 8048403 5528922 5895548 6481230 5421549 4687022 6946355
5858720 5478828

C8-pos 3632 744.5894 9.11 HMDB11210 C34:2 PC plasmalogen 12454 22450 18110
18086 6210 10566 70877 7970 8550 7945 6070 6583 7207 3521 6094
5643 6107 3172 11686985 11672448 8025822 11282617 8657446 9221373
10856382 8503632 7728070 9822941 8689498 8418221

C8-pos 3851 746.6058 9.40 HMDB11208 C34:1 PC plasmalogen-A 182689 244679 236142
233131 157412 181046 693064 142337 154211 145569 147135 140333 95137 84314 94238
70629 80503 54294 113475188 75081287 58906427 59320720
57590148 60744260 65669647 60648808 53946198 63244889
62086543 57585328

C8-pos 3653 768.5889 9.13 HMDB11310 C36:4 PC plasmalogen 5720 15177 13211
12744 7914 7883 30428 7626 6416 6726 4042 4683 2645 5287 3711
2480 3621 953 4637122 7535900 3963202 4920937 3908563 4539379 4602133 3752765 3707617
4063852 3774816 3949412

C8-pos 3756 770.6051 9.27 HMDB11244 C36:3 PC plasmalogen 3089 7963 6406
4909 6389 3488 8348 2732 1648 3257 2956 3199 2696 2295 2585
3912 2784 2092 3162551 3313974 2496483 3416792 2665852 2772151 3002241 2361016 2289667
2477037 2534783 2423949

C8-pos 3978 772.6202 9.52 HMDB11243 C36:2 PC plasmalogen 16843 30521 33682
28970 13453 22793 101233 13874 19007 13005 9935 9837 8362 8253 5283
6204 5842 4795 12949917 11791730 10168997 12408535 9858472
10216946 11686965 10265851 9667752 9810601 9890993 9199747

C8-pos 4219 774.6368 9.82 HMDB11241 C36:1 PC plasmalogen 17902 30859 33665
24992 9785 11869 121498 6439 8920 6577 7868 8346 2825 3981 5139
2422 5980 2867 29606412 15726047 14923394 10393625
11310871 11170466 13061965 13056046 11734350 11681531
12285980 11488631

C8-pos 3654 790.5715 9.13 HMDB11229 C38:7 PC plasmalogen 17836 25239 28831
24637 16221 21351 20049 20981 26327 19250 17832 21398 15472 19294 24064
15960 22703 16047 2077724 3170292 1773291 2377221 1907951 2113604 2111468 1714617 1634288
1900370 1741635 1798776

C8-pos 3752 792.5868 9.26 HMDB11319 C38:6 PC plasmalogen 8525 7806 7202
7860 6572 9847 3222 4317 9704 8082 7415 1953 11168 7556 14587
7786 6268 4227 902722 1142487 912151 1196680 981880 984157 1041252 822009 812605
898017 870846 884659

C8-pos 3909 796.6202 9.43 HMDB11252 C38:4 PC plasmalogen

987283 1078238 776557 928342 837424 905446 843780 727237 736031 760104 798661
768371

C8-pos 3912 818.6024 9.44 HMDB11294 C40:7 PC plasmalogen

295190 373860 270756 338174 294254 315094 290149 247372 244937 273112 266929
262422

C8-pos 3313 690.5064 8.67 HMDB08924 C32:1 PE

547852 447388 270517 356301 320563 333391 291585 317531 265621 360641 344883 322313

C8-pos 3061 692.5223 8.25 HMDB08923 C32:0 PE

198216 188000 108515 110176 101764 122280 91945 100596 84567 125078 116680 112959

C8-pos 3342 720.5538 8.74 HMDB08925 C34:0 PE 7869 11352 12481 11974
6597 9271 8556 7874 9767 7340 8328 8680 13062 8673 7546 7099

```
        5861    5549    955429  1117347 458844  457968  495784  535348  383557  494401  421738  501847
        558652  509220

C8-pos  3415    740.5222        8.83    HMDB08937       C36:4 PE

        146129  218649  118460  142636  124663  131621  118010  129102  104233  149847  143295  130559

C8-pos  3484    742.5376        8.95    HMDB09060       C36:3 PE

        340121  509273  450084  506655  416406  447064  416794  385030  424752  417774  438176  414984

C8-pos  3733    744.5532        9.24    HMDB08994       C36:2 PE        12784   14671   13163   10983
        5651    5681    52821   2602    5455    2245    4945    4081    866     541     873     1086
        181     319     3395962 4208916 4122531 5055936 3923968 4165180 4448509 3569714 3960404 3807167
        3680246 3786859

C8-pos  3999    746.5684        9.53    HMDB08993       C36:1 PE        24108   35440   31141   41722
        23969   34287   47783   20802   26522   19924   20270   19321   18675   13018   18511   11254
        19085   16742   2005824 2363433 1781099 1503895 1642338 1754345 1592292 1947173 1688759 1726200
        1855181 1744152

C8-pos  3357    764.5222        8.77    HMDB09102       C38:6 PE

        51149   48056   25196   34992   23821   28201   19572   30478   21866   27910   26143   21786

C8-pos  3506    766.5356        8.96    HMDB09069       C38:5 PE

        256395  514281  489954  539670  420889  472317  397375  396650  472420  403384  414368  435699

C8-pos  3765    768.5530        9.28    HMDB09003       C38:4 PE

        666081  903253  709029  539081  572674  633779  568470  699881  651459  591925  705578  672680

C8-pos  4080    772.5843        9.63    HMDB08942       C38:2 PE

        61436   71717   68990   65029   53722   65347   50958   54137   62557   56708   51835   56264

C8-pos  3700    792.5522        9.21    HMDB09012       C40:6 PE

        72994   96534   44341   26155   17398   47544   29885   41811   45483   47390   48158   42202

C8-pos  3637    700.5269        9.11    HMDB11343       C34:3 PE plasmalogen    3268    4529    3581
        3508    1001    3435    10436   948     927     994     2013    1205    315     529
        313             1512152 1676374 1416754 1564813 1473490 1601491 1663959 1502688 1403889 1554480
        1577519 1566274

C8-pos  3854    702.5428        9.40    HMDB08952       C34:2 PE plasmalogen    74128   87657   81951
        67685   44678   59755   129783  58357   55681   47280   53175   41392   14817   18415   15351
        14565   20190   16468   8486385 7744044 7398272 6531726 7174122 7703723 7111560 7030362 7049910
        7174980 7402199 7251904

C8-pos  3640    724.5267        9.11    HMDB11410       C36:5 PE plasmalogen    40752   54305   52853
        48563   37771   40184   73760   34993   34243   30355   34889   26915   10737   13546   13544
        13172   16303   15677   3922581 5501537 4908445 4965877 4841283 5262135 5122346 4843266 4855766
        4684771 5015778 5044780

C8-pos  3748    726.5419        9.26    HMDB11442       C36:4 PE plasmalogen

                1424357 1566291 1262383 1382156 1335926 1440304 1465597 1265627 1204243 1257516 1382164
        1317246

C8-pos  3985    728.5581        9.53    HMDB11441       C36:3 PE plasmalogen

                1925675 2110833 2005479 2351068 2067346 2197766 2208121 1976859 1969337 2019556 2059995
        2039071

C8-pos  4193    730.5743        9.81    HMDB09082       C36:2 PE plasmalogen

                4179185 3190190 3372145 2458572 2732591 2758344 2800156 2969895 2947390 2717266 2933454
        2973411

C8-pos  4416    732.5890        10.09   HMDB09016       C36:1 PE plasmalogen

                15518   11039   9973    3018    4934    6683    2890    10220   8834    1331    8348
        8091
```

C8-pos	3575	748.5270		9.05	HMDB11420		C38:7 PE plasmalogen		4996	7387	4712	
	5394	3730	5437	16837	4405	6788	3101	5421	3898	727	904	514
	375	211	1235	2187170	2299552	1919386	2068196	2006011	2193189	1979745	1993973	1841759
	1987230	2149232	2042178									
C8-pos	3673	750.5424		9.17	HMDB11387		C38:6 PE plasmalogen		2809	6062	4439	
	5818	5381	4312	15021	5771	3285	2400	3689	3794	2585	3186	2475
	3498	2401	3124	2584653	3264803	3002272	3250981	3055968	3338845	3050214	2818721	2867826
	2775695	3103546	3128792									
C8-pos	3895	752.5580		9.42	HMDB11386		C38:5 PE plasmalogen		1511	2365	1670	
	913	1473	1931	1042	1224	1391	1237	934	1487	564	798	1074
	868	1119	1878	728946	900779	808932	845912	841931	958588	821804	762124	779000
	802626	863949	855063									
C8-pos	4271	756.5900		9.89	HMDB11384		C38:3 PE plasmalogen					
		173311	150214	188897	121355	136541	163291	113677	134051	161849	109697	151354
	143403											
C8-pos	3669	796.5231		9.16	n/a		C42:11 PE plasmalogen					
	5289	29862	34910	47359	36147	36271	28431	25429	16635	21763	35286	40358
C8-pos	2944	772.5462		7.88	n/a		C36:3 PS plasmalogen					
	57141	39515	28147	24783	28719	20741	19626	33047	23444	28781	32426	30383
C8-pos	1809	300.2896		5.30	HMDB00252		sphingosine		6279	6262	5717	5830
	5610	5986	5683	5785	6437	5857	5782	6186	5987	6595	5958	6481
	8009	7013	3544	5081	7277	8406	7569	6961	7978	5487	5982	7425
	5822	6297										
C8-pos	3317	538.5193		8.68	HMDB04949		C16:0 Ceramide (d18:1)		2312	11317	4392	
	5381	2857	2784	6412	2148	2180	1640	1377	2053	172	202	
	386			935229	2245895	514680	649030	719654	667782	553474	704804	545020
	777682	826322	679639									
C8-pos	4349	622.6131		10.02	HMDB04952		C22:0 Ceramide (d18:1)					
		278913	453439	152636	145634	164607	139831	162430	182508	143835	167696	179750
	141571											
C8-pos	4556	650.6444		10.42	HMDB04956		C24:0 Ceramide (d18:1)		2098	9698	5789	
	7208	3892	2117	1429	604	1758	2579	3121	2785	866	1150	1290
	537	461	1163	1809880	2396651	915488	1066298	985035	867055	1032546	1083149	871600
	1097076	1074958	872705									
C8-pos	4396	648.6287		10.07	HMDB04953		C24:1 Ceramide (d18:1)					
		763334	1162889	576791	515576	551942	603086	578682	548454	518092	554878	595973
	567193											
C8-pos	3000	675.5434		8.04	HMDB12097		C14:0 SM		7959	9559	9222	10776
	9142	11591	12733	9087	9217	7689	7770	8883	12181	11585	9391	12347
	11087	4828	350938	253737	254918	195241	214284	227967	228444	238444	220909	230001
	253549	237241										
C8-pos	3051	701.5591		8.20	n/a		C16:1 SM		54686	68168	67177	64258	63072
	65943	57057	57853	62362	63926	57874	55303	70316	77401	78069	63207	69809
	39520	611116	522262	517495	583006	489869	541404	520994	481286	444595	509098	535642
	499032											
C8-pos	3204	703.5750		8.54	HMDB10169		C16:0 SM		620807	740512	748856	733877
	667158	695108	627640	662029	730328	664682	625295	632621	795200	868058	821109	673210
	729767	510351	16896485		8378801	6985095	7242786	7114819	7189596	6518633	7548428	6816746
	8195012	7790921	7269105									
C8-pos	3328	729.5909		8.70	HMDB12101		C18:1 SM		36412	45594	47549	44730
	41978	44233	26625	40095	46620	38147	40347	37361	61111	48186	52419	43633
	49478	31248	119011	78464	70080	54855	57517	63719	59973	62651	65769	61558
	65233	56986										
C8-pos	3555	731.6061		9.02	HMDB01348		C18:0 SM		117458	142563	158800	141233
	136149	146125	96927	119938	142660	132775	125791	121110	152121	157719	173907	142351

	153438	111808	855269	386046	297581	254760	241201	248992	256835	323721	305762	297472	
	310293	268467											
C8-pos	3948	759.6374		9.47	HMDB12102		C20:0 SM		22951	27503	34023	26421	
	24083	28583	13444	24970	30886	23770	22376	23359	39154	38233	38767	30489	
	35600	20834	201123	86391	61387	59219	48140	39850	68887	67020	63427	58245	
	58215	49057											
C8-pos	4071	785.6531		9.62	HMDB12104		C22:1 SM		73484	99878	106308	94758	
	92451	99558	60658	83489	107560	88959	85549	89952	127628	106901	117027	95753	
	114689	68821	185260	97669	93334	54181	51257	61902	72809	79917	86646	58221	
	63953	62029											
C8-pos	4287	787.6685		9.90	HMDB12103		C22:0 SM		164665	202116	213210	196619	
	182556	203372	120739	173962	224541	175775	165709	178380	254378	226522	242826	198254	
	248143	140499	702174	289858	252521	230953	174751	178482	279662	242316	214210	253557	
	226269	177955											
C8-pos	4305	813.6845		9.94	HMDB12107		C24:1 SM		319121	385323	410943	382997	
	358027	368273	255992	343329	404465	347245	320583	344153	474535	415063	462816	370610	
	439178	285569	4502687	1892962	2121997	1786867	1458200	1625235	2070210	1783827	1848698	1981652	
	1673538	1680676											
C8-pos	4505	815.7001		10.32	HMDB11697		C24:0 SM		97691	117738	128094	112782	
	109528	105809	73061	100043	108323	104885	99062	101794	147764	136788	140707	111109	
	143329	75575	2659819	1030387	915854	1131635	730826	742938	1153685	877032	731189	1077622	
	788961	723401											
C8-pos	5101	614.5885		11.99	HMDB06725		C14:0 CE		20792	28098	35585	32731	
	24454	26809	12157	25499	32126	21322	25565	20646	29756	26721	34784	23149	
	38303	13667	21564	51135	60333	19828	32618	52552	33034	35727	67047	16887	
	19236	38826											
C8-pos	5147	640.6030		12.07	HMDB00658		C16:1 CE		572917	702793	720891	675160	
	637519	705343	443695	623115	759708	633693	608399	599442	774345	826925	913589	674051	
	903438	516247	68186	152396	186523	106968	153775	172453	129404	131617	200886	91436	
	108872	140093											
C8-pos	5331	642.6188		12.41	HMDB00885		C16:0 CE		456623	544473	562196	456516	
	455614	583547	310548	488377	616083	443440	457486	429573	509366	582357	662568	423166	
	611127	361132	38921	100318	94829	51778	72865	88738	69459	83747	96204	54128	
	68309	66424											
C8-pos	5059	664.6030		11.88	HMDB10370		C18:3 CE		103755	106548	128366	118564	
	115511	127138	78785	106565	131011	109779	111078	111157	152048	148947	160984	122979	
	164341	91743	1748	5216	1989		2797	3445		1895	7354		
	2183	2988											
C8-pos	5207	666.6183		12.17	HMDB00610		C18:2 CE		1361063	1579498	1711370	1542494	
	1475380	1592270	1059467	1575118	1732351	1488233	1429105	1453388	1752063	1737348	2161771	1526199	
	2046971	1209840	35675	131075	86733	63029	150520	86322	55482	102986	87598	43174	
	87550	72046											
C8-pos	5396	668.6341		12.49	HMDB00918		C18:1 CE		2131567	2566278	2741344	2514407	
	2443677	2524289	1662052	2457691	2963070	2322218	2286733	2264956	2816063	2947078	3734819	2490701	
	3302551	1885560	273554	659720	1032233	577948	745875	1004224	698951	662546	946763	461988	
	563798	817379											
C8-pos	5559	670.6496		12.89	HMDB10368		C18:0 CE		38324	46335	51370	38640	
	43456	50426	22610	43185	59923	40826	40030	38444	62024	58531	81535	51476	
	73417	42521	46484	78197	106649	16117	55116	93063	61185	73524	90995	29667	
	45764	72789											
C8-pos	4997	688.6028		11.74	HMDB06731		C20:5 CE		81281	94461	100730	97839	
	98552	101133	65609	90393	106798	94865	87362	87704	123068	122341	129070	99677	
	132126	75271											
C8-pos	5107	690.6184		12.01	HMDB06726		C20:4 CE		1831098	2095091	2347139	2110729	
	2073841	2087516	1416513	1930944	2369481	2050243	1878830	1967259	2412243	2362367	2666967	2126271	
	2679860	1516435	12434	88553	16176	33519	125364	16232	19814	71971	22654	20978	
	68892	10559											
C8-pos	5256	692.6343		12.28	HMDB06736		C20:3 CE		189943	217622	244177	220165	
	207090	221011	134211	224788	244533	210119	190144	200855	263401	246441	316787	224163	

	291995	184042										
C8-pos	5050	714.6184		11.87	HMDB06733		C22:6 CE		200437	226618	254381	228037
	227977	230159	150814	217444	248072	224341	211038	220477	275331	284378	288663	235494
	301789	166812	447	5984	693		3830			2475	346	
	2070	1674										
C8-pos	5169	716.6337		12.11	HMDB10375		C22:5 CE		23888	31949	36043	36006
	30010	35279	14882	27978	41248	21732	25922	27832	42449	44796	52757	33758
	47488	16790										
C8-pos	1468	318.2641		4.80	HMDB11562		C14:1 MAG		4800	5000	4238	5011
	4748	5265	4819	4187	4631	4910	5004	4866	4967	4862	5080	4698
	5922	5192	2293	3382	4456	5693	5288	4732	5838	3899	4271	5093
	4284	4770										
C8-pos	1856	346.2952		5.35	HMDB11565		C16:1 MAG		61100	64093	67173	64524
	64680	64346	63207	64010	64584	64508	65003	63064	60145	67370	63131	61735
	74608	63049	35331	52234	69621	82482	76327	74667	85447	57468	60548	76804
	64817	67822										
C8-pos	2302	376.3422		6.19	HMDB11131		C18:0 MAG		1155974	887122	818196	894474
	846754	884764	863507	832565	875628	869287	841534	819586	872784	833236	906529	1174404
	843379	886238	400440	690212	889459	1003586	982395	989857	997477	767027	767494	975103
	803618	842776										
C8-pos	2744	430.3893		7.29	HMDB11582		C22:1 MAG		60148	64987	69591	60714
	59809	68593	61639	59519	60572	62221	58657	62191	57795	61219	64238	57780
	64660	58310	29805	52964	64071	74037	70243	71688	73082	58127	56793	69763
	59466	61118										
C8-pos	3571	558.5093		9.04	HMDB07011		C30:0 DAG		34891	39777	43685	42156
	35039	39350	36322	32217	36286	35543	36335	34588	31794	35529	34588	32658
	29191	33609	315638	239799	152405	243928	217691	165348	195972	205369	157756	264527
	234491	177298										
C8-pos	3436	582.5093		8.86	HMDB07128		C32:2 DAG				195	
	7226	63790	6454	25430	21102	10618	11192	14884	3917	15268	15458	7174
C8-pos	3683	584.5246		9.18	HMDB07099		C32:1 DAG		6253	8049	6538	9031
	5628	8382	7167	6024	6521	5339	5423	4936	4288	6340	4953	5884
	4425	5182	334952	475015	255061	569607	421645	299463	413401	352685	265995	438176
	372323	265994										
C8-pos	3958	591.4960		9.48	HMDB07098		C32:0 DAG		230834	309105	252115	244291
	240784	231703	224520	230244	233669	241661	244424	222337	215569	229119	230259	237316
	222946	234768	1026254	1182690	877121	1417285	1335693	897664	1173511	1389598	996304	1608300
	1487483	1030933										
C8-pos	3796	610.5404		9.31	HMDB07103		C34:2 DAG					
	144576	394267	156413	371997	294663	187924	241902	244659	172468	249012	254769	166382
C8-pos	4054	612.5560		9.61	HMDB07102		C34:1 DAG		7545	12588	10737	11297
	7436	7494	16464	6012	7927	4042	7814	6816	7559	5270	4753	2951
	3612	7475	1506955	1703845	1119435	1520909	1346234	1007241	1440289	1389832	1097630	1433900
	1334361	1023250										
C8-pos	4292	614.5720		9.91	HMDB07100		C34:0 DAG		292394	280602	287972	279073
	257524	265547	260852	242016	275783	263176	249314	255239	274650	250588	260634	305939
	272960	253428	1075007	971688	794560	887137	921584	702180	907993	1051245	826812	1019799
	1110093	775022										
C8-pos	3921	636.5556		9.44	HMDB07219		C36:3 DAG					
	18961	117295	29309	64187	50166	34538	35467	41778	36017	43252	43153	29550
C8-pos	4154	638.5716		9.73	HMDB07218		C36:2 DAG		1320	3629	2047	2802
	833	961	6083	1463	1348	3717	799	1588	273	798	336	258
	746	382395	937563	598305	986304	810333	545140	695589	656042	574819	708836	689206
	514261											

C8-pos	4369	640.5872	10.03	HMDB07216	C36:1 DAG	2121	6443	2613	3865	3661	3542	4325
	848	2312	3559	1843	936	2264	550	849	617	514	1164	816507
	968136	567275	608300	643713	482239	629550	701081	556750	682088	696032	533043	
C8-pos	4503	642.6030	10.31	HMDB07158	C36:0 DAG	508832	372153	387710	379161	343892	342740	375734
	323635	369944	368085	333929	328988	403680	355350	398580	566272	370959	338549	217481
	303614	366236	406492	408882	414941	426119	340417	332082	410756	366333	353108	
C8-pos	4826	740.6763	11.25	n/a	C42:0 TAG	37369	43923	48718	48554	40169	49632	39459
	34869	39625	35874	38684	35630	37042	43728	44105	39706	48263	36782	120722
	130972	98713	105263	109815	117394	97206	98394	112797	107826	98858	112682	
C8-pos	4806	764.6759	11.16	n/a	C44:2 TAG	5545	8885	9390	6005	5122	4567	5254
	4893	5909	5523	3516	5324	3659	5314	5215	3754	4250	5851	20116
	56884	19471	22108	16403	20781	13182	13510	37054	12137	16879	17821	
C8-pos	4859	766.6918	11.36	n/a	C44:1 TAG	55147	64327	70035	61652	54715	58227	55775
	55086	52502	59302	55884	57752	51945	57483	57147	53991	64503	56365	353201
	388320	265627	225393	240562	289308	227513	257786	322758	239002	254194	289403	
C8-pos	4936	768.7075	11.59	HMDB42063	C44:0 TAG	178738	194756	227900	226558	211935	209117	192809
	193037	199574	198534	189270	182887	186303	212158	186566	170578	217189	180753	825264
	744969	647869	588998	667889	677918	556458	690896	703949	610664	684995	673130	
C8-pos	4913	792.7073	11.51	HMDB10419	C46:2 TAG	83651	101588	95120	102028	86670	94319	91863
	81370	103115	94476	86713	89858	80248	99211	86847	88469	102817	90730	349382
	529890	351965	353238	332568	372316	318760	316671	450181	315919	315800	363068	
C8-pos	4976	794.7231	11.69	HMDB10412	C46:1 TAG	224904	260251	278319	284146	249655	275855	246132
	237058	270928	240293	241545	228513	227298	259268	248006	236908	277546	225823	3022706
	2609179	2048813	1552798	1745015	1964234	1670872	1976536	2335415	1717551	1949270	2164580	
C8-pos	5070	796.7388	11.94	HMDB10411	C46:0 TAG	461228	566849	578068	583191	488767	560364	477913
	483196	583093	489897	475003	467858	479370	518846	502407	458390	580670	456426	3143311
	2759877	2403404	1843221	2277657	2397312	1716677	2521591	2601075	1931286	2415192	2296616	
C8-pos	4923	818.7228	11.54	HMDB05432	C48:3 TAG	1494	1534	1958	489	1360	523	540
	1054	1419	1253	209	594	868	476	971	1147	142780	225768	160536
	136250	139403	160147	104767	130187	175511	110337	133041	153543			
C8-pos	5017	820.7387	11.79	HMDB05376	C48:2 TAG	226866	277617	291382	296356	246870	284240	243054
	240393	286231	241095	239610	235775	230134	250176	239586	220812	287917	227809	3092120
	2990127	2420528	1767866	2007367	2245860	1768998	2225242	2674619	1883797	2137050	2257235	
C8-pos	5127	822.7547	12.03	HMDB05359	C48:1 TAG	442318	567443	570644	561138	472862	532438	449152
	472466	565226	475726	459583	447573	476449	531643	540101	450695	588147	421709	14690946
	11586953	9809551	5813923	8000614	8568680	5922305	10202122	11195516	6761667	9539363	9053389	
C8-pos	5268	824.7697	12.30	HMDB05356	C48:0 TAG	515393	608742	634496	637841	518976	597758	524206
	571423	606066	544968	519445	511523	512039	543511	630404	498694	625044	537950	5396347
	5428185	4748081	3021429	4267458	4227163	3012102	5686474	5210582	3386390	5011027	4259963	
C8-pos	4954	844.7386	11.65	HMDB05435	C50:4 TAG							
	133168	218410	126176	81132	94366	100328	76652	102834	152575	69267	100758	104266
C8-pos	5054	846.7542	11.88	HMDB05433	C50:3 TAG	87259	110639	115424	108401	90772	111913	78272
	83616	117971	76652	84203	85939	75601	89680	92789	77011	119449	70843	1657497
	2186154	1786164	1262113	1480312	1667040	1167374	1568078	2045959	1172454	1430166	1592102	

C8-pos	5181	848.7699	12.13	HMDB05377	C50:2 TAG	274652	367013	389715	370911	289828	327021	291285
	332783	354176	287345	274108	275109	284678	308581	371907	275917	370283	291410	18085825
	16369602	13821058	7431549	10423726	10706423	7922560	13292884	14919640	8428689	11427553	11754062	
C8-pos	5302	850.7853	12.38	HMDB05360	C50:1 TAG	325014	448769	454143	415848	344924	408051	301505
	393750	445769	338992	317759	341316	352894	410302	461401	324123	430529	314618	24354437
	20387591	19590463	10135680	13695408	16210693	11248472	22401227	22104686	12548564	18665895	17287267	
C8-pos	5461	852.8023	12.66	HMDB05357	C50:0 TAG	163122	222828	233162	230123	195689	204302	188776
	191481	212823	192575	165080	156692	201973	208703	208102	160029	213253	170824	4613212
	4729959	4760887	2591195	4249729	3622921	2668501	6074739	5181511	3176897	5063839	4078311	
C8-pos	5038	870.7531	11.84	HMDB05380	C52:5 TAG	675	1873	1754	2272	408	2955	2501
	2131	688	1184	622	789	915	5	1401	429	122400	264436	117707
	43983	59933	80698	45008	90787	126888	38998	76295	82395			
C8-pos	5088	872.7693	11.98	HMDB05363	C52:4 TAG	4663	14837	15257	11073	5167	10729	3154
	4424	15324	5887	4181	3576	5854	9360	8703	4635	22401	4405	933225
	1264170	984175	610178	756166	822231	572291	868565	1129926	546278	749902	804516	
C8-pos	5239	874.7856	12.23	HMDB05384	C52:3 TAG	76035	111588	123729	98141	87433	99840	75256
	94645	101753	83664	62681	74607	86090	80429	103045	81090	109895	90060	6147894
	7074262	6815650	4092145	5369150	5624709	3934292	6107329	7482990	3764748	5293073	5677924	
C8-pos	5368	876.8006	12.48	HMDB05369	C52:2 TAG	216419	309244	331857	290766	251983	266518	217777
	261403	307597	227753	217432	220341	232763	259660	310781	214528	280596	186873	32020579
	26039568	29911870	14306828	19022015	22074541	15644212	28574897	31515704	16548978	23527421	24598791	
C8-pos	5500	878.8167	12.75	HMDB05367	C52:1 TAG	93308	143165	145841	121055	103946	108591	110988
	97290	122275	100814	106406	106518	101216	98972	105375	102183	120885	104117	15934169
	15497335	16709333	7202164	13365588	12426254	8874614	19194773	18549077	9938402	15084759	13784123	
C8-pos	5614	880.8327	13.06	HMDB05365	C52:0 TAG	77655	101915	100396	108753	84875	98707	82840
	92627	104488	86197	78099	75535	89258	94413	99162	82382	104111	90553	2169808
	2086724	2209777	1073673	1691964	1672756	1480353	3157899	2529786	1446319	2370374	1835281	
C8-pos	5013	894.7562	11.76	HMDB05447	C54:7 TAG	1712	1612	1835	1508	1988	1532	1964
	1943	1581	1614	1304	1258	1922	1279	1163	1361	565	566	61911
	107826	51498	28755	23372	41171	23504	37626	50395	20679	30312	30120	
C8-pos	5102	896.7682	11.99	HMDB05391	C54:6 TAG	24726	11704	19208	12277	29072	14490	30686
	26757	18124	17973	33584	25938	30347	14979	24544	24966	8941	27157	268344
	465398	226361	103193	126192	175891	77425	187077	272371	73058	150105	158620	
C8-pos	5168	898.7851	12.10	HMDB05385	C54:5 TAG	45839	47637	41436	50813	41779	46111	35255
	40502	47315	40648	52340	38500	57529	56038	52330	51168	46560	33745	852825
	1402450	902521	539037	696995	746485	543680	785829	1014925	496383	710805	753468	
C8-pos	5284	900.8078	12.32	HMDB05370	C54:4 TAG	3186895	2931103	2937012	2912884	3407822	2822252	3377080
	3209017	3024770	3348597	3329643	3340020	3163787	3168891	3207740	3323080	2975426	3077615	3452580
	4694005	5560806	4623356	5176966	5203833	5224874	5238530	5881843	4977277	5044262	5404865	
C8-pos	5437	902.8160	12.58	HMDB05405	C54:3 TAG	1015086	916038	957776	914912	1095589	887826	1091229
	1049941	1004249	1114526	1079602	1120857	1061604	1060923	1084945	1050798	994191	1072922	12686211
	12014459	15064281	7882052	11100007	11546223	8639554	14222512	16189069	8142347	11122552	12073765	
C8-pos	5539	904.8320	12.85	HMDB05403	C54:2 TAG	609741	581598	603193	539757	617872	607071	648497
	602083	570480	635317	655183	658020	623058	627414	588417	662444			

	576931	593008	14785027		12449009		15060181		6405154	11402769		
	11753355		8030046	16230612		16291399		8645345	12428285		12451251	
C8-pos	5645	906.8480		13.14	HMDB05395		C54:1 TAG		388587	328353	358229	356214
	399533	366077	421354	384306	360391	413574	431978	428456	384376	374731	406145	426703
	387199	420771	4527640	3581595	3941310	1984355	2770636	3109555	2668140	4892882	4351790	2645219
	3598771	3336165										
C8-pos	5045	920.7696		11.85	HMDB05392		C56:8 TAG					
	29238	81933	34039	17018	21029	25236	6251	19036	37027	13212	13406	24675
C8-pos	5165	922.7846		12.09	HMDB05462		C56:7 TAG					
	427333	639806	391359	159598	223479	246039	177444	304798	396523	161746	252993	287899
C8-pos	5292	924.8010		12.34	HMDB05456		C56:6 TAG					
	624616	973633	720460	385051	520191	582461	372169	607606	769519	366005	526222	555357
C8-pos	5362	926.8155		12.45	HMDB05406		C56:5 TAG					
	1207940	1599057	1528301	681656	922026	1162332	738401	1295519	1617719	692806	1041186	1160337
C8-pos	5478	928.8318		12.69	HMDB05398		C56:4 TAG					
	1472214	1396382	1611175	620597	1073538	1154684	706598	1513989	1684181	752946	1147682	1249745
C8-pos	5575	930.8478		12.94	HMDB05410		C56:3 TAG					
	2460671	1936380	2567613	989546	1570627	1843540	1292129	2484273	2674311	1211022	1728121	2008602
C8-pos	5674	932.8636		13.24	HMDB05404		C56:2 TAG					
	2439948	1739238	2119900	905634	1458441	1649493	1327623	2392210	2259069	1385794	1616184	1710332
C8-pos	5784	934.8792		13.60	HMDB05396		C56:1 TAG					
	1149546	862836	851145	473841	720330	688916	622476	1122804	954629	673128	921504	754286
C8-pos	5219	948.8011		12.18	HMDB05413		C58:8 TAG					
	166519	308720	263297	175703	208380	222760	138605	196049	287264	128092	164740	187556
C8-pos	5274	950.8160		12.30	HMDB05471		C58:7 TAG					
	87442	144455	115307	58099	69912	86387	42279	94168	131536	46198	59664	79383
C8-pos	5424	952.8316		12.56	HMDB05458		C58:6 TAG					
	305241	335779	295146	109192	196952	207320	124707	261118	315760	116415	209967	221993
C8-pos		887.5597		8.94	HMDB09815		C38:4 PI					
	478504	264239	130272	243273	236428	197145	274277	272264	213828	360193	283494	207122
C8-pos		706.4654		3.8	HMDB12333		C30:1 PS		39816	47219	42692	48479
	41451	54468	43314	39993	47442	48893	41921	40982	43167	38690	45510	42707
	38012	36694	17902	36204	44207	46292	50873	51551	59917	32869	41585	46242
	41000	45056										
C8-pos		764.5431		8.5	HMDB12356		C34:0 PS					
	182478	218389	103411	220445	162547	154758	151109	139892	111202	175856	167202	144916
C8-pos		808.5092		8.50	HMDB12362		C38:6 PS					
	94649	100121	67324	125913	79702	84159	102703	73505	60471	110177	82964	81884
C8-pos	3134	808.5071		8.41	HMDB10167		C40:6 PS		11597	17160	15134	12448
	9208	10603	14548	13971	11043	12764	8192	11421	19682	14296	16584	14854
	17050	8731	743019	646047	515868	572721	559644	557435	614265	577272	496869	663441
	630754	590984										

[00262] **Table 4.** Lipidomics data showing all lipids detected except those shown in Figure 21A. Data shown are normalized to WT (TGFb1+IL-6) condition showing average of 3 independent biological experiments.

Lipids that are not significantly different or have a fold change less than 1.5	Min P value	WT (TGFb1+IL-6)	CD5L-/- (TGFb1+IL-6)	WT (TGFb1+IL-6+IL-23)	CD5L-/- (TGFb1+IL-6+IL-23)
12-HETE	N/A	undetected	N/A	N/A	N/A
13-S-HODE	0.212	1	0.849	1.649	1.161
15-HETE	N/A	undetected	N/A	N/A	N/A
5 HETE	N/A	undetected	N/A	N/A	N/A
Arachidonic acid	N/A	undetected	N/A	N/A	N/A
C14:0 CE	0.096	1	0.783	1.021	0.563
C14:0 LPC	0.124	1	1.269	0.957	1.016
C14:0 SM	0.039	1	0.742	0.805	0.838
C16:0 CE	0.048	1	0.932	1.066	0.807
C16:0 LPC	0.277	1	0.960	0.921	0.991
C16:0 LPE	0.181	1	0.693	0.706	0.718
C16:0 SM	0.052	1	0.666	0.647	0.721
C16:1 CE	0.107	1	1.064	1.135	0.836
C16:1 LPC	0.140	1	1.245	1.188	1.158
C16:1 SM	0.072	1	0.878	0.876	0.935
C18:0 CE	0.083	1	0.719	0.976	0.641
C18:0 LPC	0.111	1	0.806	0.866	0.988
C18:0 LPE	0.052	1	0.732	0.802	0.887
C18:1 CE	0.163	1	1.164	1.174	0.918
C18:1 LPC	0.113	1	1.258	1.187	1.184
C18:1 LPE	0.366	1	1.050	0.982	1.004
C18:1 SM	0.059	1	0.658	0.704	0.687
C18:2 CE	0.165	1	1.183	0.971	0.800
C18:2 LPC	0.138	1	0.681	0.714	0.737
C18:2 CE	0.204	1	1.046	1.556	0.866
C20:3 CE	N/A	undetected	N/A	N/A	N/A
C20:3 LPC	0.141	1	1.000	0.944	1.080
C20:4 CE	0.276	1	1.495	0.977	0.857
C20:4 LPC	N/A	undetected	N/A	N/A	N/A
C20:4 LPE	0.048	1	0.672	0.782	0.792
C20:5 CE	N/A	undetected	N/A	N/A	N/A
C22:0 Ceramide (d18:1)	0.086	1	0.509	0.552	0.553
C22:0 SM	0.063	1	0.469	0.592	0.529
C22:5 CE	N/A	undetected	N/A	N/A	N/A
C22:6 CE	N/A	1	1.613	0.584	0.788
C22:6 LPC	N/A	undetected	N/A	N/A	N/A
C24:0 Ceramide (d18:1)	0.083	1	0.570	0.583	0.584
C24:0 SM	0.153	1	0.566	0.900	0.562
C24:1 Ceramide (d18:1)	0.088	1	0.567	0.657	0.686
C30:0 DAG	0.128	1	0.886	0.796	0.955
C30:0 PC	0.015	1	0.726	0.804	0.780
C30:1 PC	0.121	1	1.162	0.868	1.010
C32:0 DAG	0.078	1	1.188	1.158	1.337
C32:0 PE	0.008	1	0.676	0.580	0.717
C32:1 DAG	0.194	1	1.212	0.969	1.011
C32:1 PC	0.064	1	0.800	0.683	0.798
C32:1 PE	0.026	1	0.758	0.651	0.812
C32:2 DAG	0.086	1	0.738	0.387	0.489
C32:2 PC	0.072	1	1.368	0.953	1.133
C34:0 DAG	0.176	1	0.884	0.981	1.027
C34:0 PC	0.045	1	0.711	0.784	0.860
C34:0 PS	0.065	1	1.056	0.798	0.968
C34:1 DAG	0.222	1	0.895	0.907	0.876
C34:1 PC	0.002	1	0.743	0.809	0.847
C34:1 PC plasmalogen-A	0.112	1	0.716	0.726	0.739
C34:2 DAG	0.168	1	1.229	0.946	0.964
C34:2 PC plasmalogen	0.157	1	0.929	0.863	0.858
C34:2 PE plasmalogen	0.020	1	0.806	0.897	0.924
C34:3 PC	0.014	1	1.071	0.815	0.926
C34:3 PE plasmalogen	0.303	1	1.007	0.962	1.020
C34:4 PC plasmalogen	0.160	1	0.900	0.787	0.845
C36:1 DAG	0.090	1	0.737	0.852	0.813
C36:1 PC	0.049	1	0.679	0.781	0.797
C36:1 PE	0.043	1	0.797	0.850	0.866
C36:2 DAG	0.178	1	1.221	1.004	0.997
C36:2 PC	0.056	1	1.084	0.988	0.967
C36:2 PC plasmalogen	0.037	1	0.930	0.906	0.828
C36:2 PE	0.073	1	1.121	1.021	0.961
C36:2 PE plasmalogen	0.022	1	0.740	0.812	0.803
C36:3 DAG	0.124	1	0.880	0.684	0.700
C36:3 PC	0.046	1	1.012	0.898	0.934
C36:3 PC plasmalogen	0.036	1	0.987	0.853	0.829
C36:3 PE	0.088	1	1.054	0.944	0.976

C36:3 PE plasmalogen	0.058	1	1.095	1.019	1.013
C36:4 PC plasmalogen	0.081	1	0.829	0.748	0.791
C36:4 PE	0.029	1	0.826	0.727	0.877
C36:4 PE plasmalogen	0.113	1	0.978	0.925	0.930
C36:5 PE plasmalogen	0.280	1	1.051	1.034	1.029
C38:2 PC	0.005	1	0.902	0.886	0.863
C38:2 PE	0.011	1	0.911	0.825	0.815
C38:3 PC	0.051	1	0.901	0.843	0.877
C38:3 PE plasmalogen	0.051	1	0.822	0.795	0.788
C38:4 PC	0.068	1	1.152	1.009	1.027
C38:4 PC plasmalogen	0.009	1	0.960	0.812	0.819
C38:4 PE	0.043	1	0.786	0.843	0.885
C38:4 PI	0.140	1	0.775	0.871	0.975
C38:5 PE	0.084	1	1.237	1.005	0.954
C38:5 PE plasmalogen	0.044	1	1.085	0.969	1.034
C38:6 PC	0.084	1	0.847	0.663	0.745
C38:6 PC plasmalogen	0.038	1	1.059	0.905	0.897
C38:6 PE	0.059	1	0.699	0.578	0.610
C38:6 PE plasmalogen	0.025	1	1.050	0.987	1.018
C38:6 PS	0.206	1	1.106	0.905	1.049
C38:7 PC plasmalogen	0.045	1	0.911	0.778	0.775
C38:7 PE plasmalogen	0.055	1	0.978	0.908	0.964
C40:10 PC	0.093	1	1.003	0.958	0.575
C40:6 PC	0.056	1	1.152	0.818	0.916
C40:6 PE	0.019	1	0.887	0.886	0.990
C40:7 PC plasmalogen	0.010	1	1.008	0.833	0.854
C40:8 PC	0.116	1	0.840	0.675	0.780
C42:0 TAG	0.130	1	0.989	0.880	0.911
C44:0 TAG	0.091	1	0.872	0.880	0.888
C44:1 TAG	0.056	1	0.730	0.802	0.777
C44:2 TAG	0.084	1	0.615	0.661	0.486
C46:0 TAG	0.047	1	0.755	0.823	0.830
C46:1 TAG	0.029	1	0.685	0.779	0.759
C46:2 TAG	0.136	1	0.859	0.882	0.808
C48:0 TAG	0.072	1	0.740	0.883	0.813
C48:2 TAG	0.015	1	0.708	0.784	0.738
C48:3 TAG	0.096	1	0.824	0.776	0.750
C50:0 TAG	0.033	1	0.742	0.987	0.873
C50:3 TAG	0.038	1	0.783	0.849	0.745
C52:0 TAG	0.015	1	0.686	1.105	0.874
C52:1 TAG	0.030	1	0.685	0.966	0.806
C52:3 TAG	0.020	1	0.753	0.875	0.735
C52:4 TAG	0.025	1	0.688	0.808	0.660
C54:2 TAG	0.048	1	0.699	0.959	0.793
C54:3 TAG	0.053	1	0.768	0.982	0.788
C54:4 TAG	0.098	1	1.095	1.152	1.125
C54:5 TAG	0.052	1	0.638	0.742	0.623
C56:6 TAG	0.072	1	0.436	0.429	0.393
C58:8 TAG	0.065	1	0.822	0.842	0.650
Cholic acid	0.126	1	0.315	1.193	1.213
Deoxycholic acid/Chenodeoxycholic acid	0.204	1	0.470	0.848	1.010
Docosahexaenoic acid	N/A	undetected	N/A	N/A	N/A
Glycochenodeoxycholic acid	0.128	1	0.208	1.978	1.122
Glycocholic acid	0.117	1	0.193	1.253	1.271
Glycodeoxycholic acid	0.132	1	0.204	1.095	1.113
Glycolithocholic acid	0.114	1	0.551	0.966	0.871
Glycohyocholic acid	N/A	undetected	N/A	N/A	N/A
Palmitic acid	0.038	1	0.372	0.456	0.000
PGE2	0.083	1	0.912	0.872	0.962
Sphingosine	0.057	1	1.442	1.223	1.229
Stearic acid	0.208	1	0.453	0.204	0.223
Taurochenodeoxycholic acid	0.100	1	0.436	1.011	1.010
Taurocholic acid	0.080	1	0.618	0.940	0.811
Taurodeoxycholic acid	0.063	1	0.672	0.834	0.838
Taurohyodeoxycholic acid/Tauroursodeoxycholic acid	0.000	1	0.793	0.770	0.585
Taurolithocholic acid	0.125	1	0.058	1.214	1.269

[00263] Table 5. PUFA/SFA treatment recapitulates the transcriptome (restricted) of WT versus **CD5L-/- Th17 cells.** Data used to generate heatmap shown in **WO2015130968 Figure 50**. Nanostring data are shown using a Th17 cell codeset Applicants previously generated containing 312 genes. 3 independent experiments were performed and the median values are normalized to

WT. Only genes that show differential expression (1.5 fold) among any of the four groups are included.

	CD5LKO.PUFA	WT	CD5LKO	WT.SFA
Ccr4	1.69	1.00	0.33	0.61
Lgals3bp	1.34	1.00	0.34	0.58
Il12rb1	0.80	1.00	0.35	0.39
Vav3	1.20	1.00	0.41	0.56
Ifng	0.93	1.00	0.43	0.55
Il10	1.81	1.00	0.44	0.12
IL-33	0.66	1.00	0.44	1.33
Klrd1	0.63	1.00	0.46	0.92
Elk3	1.04	1.00	0.47	0.58
Itga3	0.76	1.00	0.47	0.50
nrp1	0.90	1.00	0.47	0.74
Sult2b1	0.61	1.00	0.48	0.38
Tmem229b	1.52	1.00	0.51	0.69
Cxcr3	1.44	1.00	0.52	0.48
Klf9	0.75	1.00	0.55	0.68
Peli2	0.83	1.00	0.55	0.88
Acvr2a	1.32	1.00	0.55	0.66
Ccl20	0.84	1.00	0.55	0.31
Gusb	0.94	1.00	0.56	1.02
Spp1	0.66	1.00	0.56	1.10
Maf	0.84	1.00	0.56	0.79
Tcf4	1.29	1.00	0.59	0.72
Rasgrp1	1.21	1.00	0.60	0.75
Cxcr5	1.42	1.00	0.60	1.17
Rela	0.86	1.00	0.60	0.70
Stat6	1.13	1.00	0.60	0.73
Hipir	0.89	1.00	0.60	0.78
Tgfb1	0.68	1.00	0.62	0.83
Grn	1.18	1.00	0.62	0.78
Ubiad1	1.16	1.00	0.62	0.94
Bcl11b	1.03	1.00	0.62	0.82
Irf4	0.65	1.00	0.62	0.68
Ccr8	0.71	1.00	0.63	0.74
Trat1	0.85	1.00	0.63	0.61
Ifih1	1.25	1.00	0.63	0.87
Map3k5	1.49	1.00	0.64	0.80
Foxo1	1.03	1.00	0.64	0.79
Bcl2l11	0.71	1.00	0.64	0.82
Il6st	0.89	1.00	0.64	0.87
Ski	0.86	1.00	0.64	0.88
Il7r	1.37	1.00	0.64	0.85
Il2ra	0.99	1.00	0.65	0.71
Serpinb1a	0.77	1.00	0.65	0.56
Il10ra	0.95	1.00	0.65	0.71
Litaf	0.61	1.00	0.65	1.48
Rfk	1.07	1.00	0.66	0.79
Slc6a6	1.03	1.00	0.66	0.79
Socs3	1.38	1.00	0.66	0.78

c

Smad3	1.03	1.00	0.66	0.81
Lad1	1.18	1.00	0.66	0.91
Tnip2	0.78	1.00	0.66	0.90
Tgfbr3	0.94	1.00	0.68	0.58
Ahr	1.08	1.00	0.68	0.83
Mina	1.08	1.00	0.68	0.72
Stat4	1.21	1.00	0.68	0.77
Il27ra	1.55	1.00	0.68	0.70
Mbnl3	1.30	1.00	0.69	0.71
Jak3	1.27	1.00	0.69	0.91
Tal2	1.52	1.00	0.69	1.15
Gmfg	0.76	1.00	0.70	0.62
Irf7	1.17	1.00	0.70	0.54
Abcg2	1.20	1.00	0.70	0.77
Il4ra	1.13	1.00	0.72	0.75
Notch2	1.20	1.00	0.72	0.78
Clcf1	1.25	1.00	0.72	0.74
Foxp1	1.25	1.00	0.72	0.77
Stat5b	1.19	1.00	0.73	0.82
Bcl3	1.13	1.00	0.73	0.85
Ikzf3	1.06	1.00	0.74	0.82
Il12rb2	1.60	1.00	0.74	0.88
Tgfb3	1.67	1.00	0.75	0.88
Irf8	1.29	1.00	0.75	0.99
Nfkbie	1.52	1.00	0.76	0.69
Trps1	1.44	1.00	0.77	0.84
Trim25	1.17	1.00	0.77	0.89
Tgm2	1.51	1.00	0.78	0.78
Ercc5	0.66	1.00	0.79	0.90
Etv6	1.70	1.00	0.79	0.94
Xrcc5	1.27	1.00	0.80	0.93
Il1r1	1.36	1.00	0.82	0.61
Csf2	1.20	1.00	0.83	0.97
Fli1	1.35	1.00	0.83	0.84
Klf10	1.30	1.00	0.83	0.91
Arl5a	1.33	1.00	0.84	0.93
Jun	0.64	1.00	0.84	1.11
Flna	1.10	1.00	0.84	0.65
Foxp3	1.22	1.00	0.85	0.71
Inhba	0.81	1.00	0.86	0.60
Cd247	1.32	1.00	0.88	0.81
Faim3	1.31	1.00	0.89	0.61
Pstpip1	1.24	1.00	0.90	1.16
Kat2b	1.22	1.00	0.90	0.69
Gja1	0.66	1.00	0.93	0.94
Cd86	1.73	1.00	0.94	0.99
Lpxn	1.39	1.00	0.94	0.85
Ccl1	0.67	1.00	0.95	0.58
Plagl1	1.07	1.00	0.95	2.19

Ctla4	1.63	1.00	0.96	0.81
Cd9	1.27	1.00	0.97	0.84
Pou2af1	0.86	1.00	1.00	1.30
Pmepa1	1.19	1.00	1.00	0.74
Prkd3	1.51	1.00	1.00	0.73
Il17f	0.71	1.00	1.04	0.90
EBF1	1.64	1.00	1.11	0.51
Gimap5	1.58	1.00	1.18	1.05
Tsc22d3	0.86	1.00	1.18	1.06
Gem	0.73	1.00	1.18	1.00
Gap43	0.68	1.00	1.21	1.30
Maff	0.77	1.00	1.22	0.99
pou2f1	0.86	1.00	1.23	1.34
Atf4	0.73	1.00	1.23	1.11
Rel	0.73	1.00	1.23	1.20
Frmd4b	1.28	1.00	1.26	1.05
Nkg7	1.40	1.00	1.31	0.62
Casp4	1.52	1.00	1.32	0.95
Mt2	0.84	1.00	1.33	1.33
BC021614	1.04	1.00	1.34	0.96
ATF2	0.89	1.00	1.38	1.18
Cxcr4	0.87	1.00	1.39	1.00
Bhlhe40	0.70	1.00	1.43	1.22
Il17a	1.11	1.00	1.44	0.96
Casp3	0.71	1.00	1.45	1.21
Sap30	0.81	1.00	1.47	1.23
Tnfrsf4	1.05	1.00	1.51	1.28
Plac8	0.85	1.00	1.51	1.04
Il23r	1.11	1.00	1.51	1.12
Rab33a	1.50	1.00	1.55	1.23
Sema7a	1.04	1.00	1.60	1.44
Il21	0.97	1.00	1.65	1.64
Oas2	0.82	1.00	1.66	1.28
Fzd7	0.72	1.00	1.71	1.07
Rorc	1.52	1.00	1.80	1.25
Mt1	0.79	1.00	1.85	1.56
Spry1	1.02	1.00	2.04	1.57
Egr2	1.64	1.00	2.21	1.53
Il3	1.45	1.00	2.24	2.11
Cd83	0.88	1.00	2.33	1.23
Cd70	0.77	1.00	2.51	0.89
Cxcl10	1.64	1.00	3.05	3.83

[00264] **Table 6.** Shown are genes that are significantly up or down regulated in different sections of the Voronoi diagram (subpopulations) (corresponding to Figure 2C).

Differentially expressed genes in in-vivo sub-populations													
Th17/Th1-like memory		Th17/Th1-like effector-LN		Th17/Th1-like effector-CNS		Th17/Th1-like effector		Th17/pre-Th1-like effector		Th17 self-renewing		Th17 Dysfunctional /senescent	
STMN1	OSTF1	STMN1	PSPH	STMN1	RAB1	STMN1	ATOX1	CKS2	RPS8-PS1	TOP2A	EZR	STMN1	AC127419.1
RRM2	BCL2A1B	RRM2	CCDC21	RRM2	TNFSF11	RRM2	LYAR	FIGNL1	XAF1	UBE2C	GM10237	28104 17H13 RIK	48334 20G17 RIK

Differentially expressed genes in in-vivo sub-populations													
Th17/Th1-like memory		**Th17/Th1-like effector-LN**		**Th17/Th1-like effector-CNS**		**Th17/Th1-like effector**		**Th17/pre-Th1-like effector**		**Th17 self-renewing**		**Th17 Dysfunctional /senescent**	
2810417H13RIK	AA467197	2810417H13RIK	PRDX4	2810417H13RIK	PAPOLA	2810417H13RIK	GNG5	CIT	TXLNG	BIRC5	LEF1	HMGN2	SNRNP200
HMGN2	UBE2F	HMGN2	XPO1	HMGN2	CNOT6	HMGN2	RWDD1	MRPL27	NCK2	NDUFA5	FAM65B	TOP2A	FTH1
TOP2A	TMEM128	TOP2A	NOL12	TOP2A	HIST2H2AA2	TOP2A	D930014E17RIK	DOK2	CDK7	CCNB2	HK1	SMC2	SYT11
SMC2	GIT2	SMC2	SNRNP25	SMC2	DHRS3	SMC2	EIF4H	PPP1R8	MGA	NME1	EMB	GM7125	GM5148
GM7125	GM10247	GM7125	CAB39L	GM7125	HIST2H2AA1	GM7125	NUCKS1	HSPE1	ISCA1	TIPIN	2010011I01RIK	NUTF2-PS1	5830405N20RIK
SSNA1	IFITM3	SSNA1	MRPL15	NUTF2-PS1	AC131675.1	NUTF2-PS1	APIP	CDC26	POM121	SNRPB	MED21	SSNA1	MYSM1
BIRC5	RGS1	HIST1H4D	CLDND1	SSNA1	VAMP4	SSNA1	RIF1	IL22	LARP4B	NDUFA4	COMT1	HIST1H4D	WAS
PCNA	BHLHE40	SNRPA1	ILF2	HIST1H4D	NUBP1	HIST1H4D	EIF2S3X	YARS	POLR3C	NSMCE2	2310004N24RIK	SNRPA1	RNF5
H2AFV	GOT1	UBE2C	H2-KE2	SNRPA1	USP1	SNRPA1	LNP	IFNG	TNFRSF26	FAM36A	THADA	CKS1B	AGXT2L2
NDUFA5	RAB11A	BIRC5	UCHL5	UBE2C	STK39	UBE2C	DHX15	TBL3	TCF7	SNRPD2	SDF2	MRPL42	IRGM1
ASF1B	5430421N21RIK	CKS1B	PPP1R8	BIRC5	AP3S1	BIRC5	EXOSC10	ALDOA	MRPS7	DUT	FARSB	ANP32E	RP23-71J17.1
NME1	SELL	CDCA3	UCHL3	CKS1B	RAB4B	CKS1B	2610039C10RIK	CD7	RASSF1	SEC11C	H2-Q7	PCNA	AC120410.1
BCAP31	PTPRS	MRPS16	POLE4	MRPL42	GPS1	MRPL42	CD3E	CCR8	CPNE8	KIF23	THEMIS	MRPS16	HNRPDL
2700094K13RIK	GGH	H2AFV	HSP90B1	AC161456.1	RIOK1	AC161456.1	2400001E08RIK	MICAL1	TTC5	COMMD1	NUCB1	H2AFV	GM10155
TYMS	PGAM1	ASF1B	SNRPB2	ANP32E	CASP3	ANP32E	SYNCRIP	SDHC	GPR68	STRA13	S1PR1	NDUFA5	FXR1
TACC3	GM2574	CCNB2	NUP214	PCNA	PPME1	PCNA	HIST1H2BG	RPS15A	GRIPAP1	H2AFZ	BRP44L	ASF1B	GM10358
SNRPB	GPR171	TIPIN	PDLIM1	CDCA3	PDLIM2	CDCA3	POLR2B	TNFSF11	SFI1	TAGLN2	OSBPL3	RANBP1	SF3B3
GM11276	RAMP1	2700094K13RIK	MRPL53	MRPS16	IPO7	MRPS16	HSPA4	CCR6	LITAF	EMG1	B2M	NME1	USP50
HIST1H2AO	ITK	TIMM17A	RPAIN	H2AFV	ACTR1A	H2AFV	MRPS36-PS1	ASRGL1	AC151275.1	1810027O10RIK	TTC39C	BCAP31	GM5220
NUF2	H13	TYMS	BZW2	NDUFA5	HMOX2	NDUFA5	AKR1A4	DHRS3	BRAP	CISD3	2010002N04RIK	PSMD14	RPS19-PS2
HIST1H2AE	GM5138	TACC3	WDR12	ASF1B	NEDD1	ASF1B	WDYHV1	MDP1	GM5561	SRP19	NDFIP2	GM10349	GPSM3
HMGB2	P2RX7	GMNN	VRK1	RANBP1	NUDC	RANBP1	2810407C02RIK	GGPS1	ADO	LIG1	RPS12	TIMM17A	ATP2B4
MRPS14	RPL31	GM11276	PHPT1	CCNB2	CSDA	CCNB2	CENPL	POT1A	WBP11	MPHOSPH6	APOL7E	EXOSC8	CNOT3

Differentially expressed genes in in-vivo sub-populations													
Th17/Th1-like memory		Th17/Th1-like effector-LN		Th17/Th1-like effector-CNS		Th17/Th1-like effector		Th17/pre-Th1-like effector		Th17 self-renewing		Th17 Dysfunctional /senescent	
BANF1	HIF1A	HIST1H2AO	UFC1	NME1	LARP7	NME1	UFSP2	ORC3	EZH1	UHRF1	DDX18	GMNN	EXOC1
CDCA8	SMARCC1	NUF2	C330027C09RIK	CDK1	COPB2	CDK1	LGTN	ODF2	GM10054	ERGIC2	NSD1	SNRPB	SERBP1
MRPL18	PDHA1	HIST1H2AE	NFU1	BCAP31	GM9396	BCAP31	KPNB1	TMEM154	GM3940	TXN2	BCL2L1	NUF2	ZC3HC1
DDX39	HIGD2A	HMGB2	DPH3	PSMD14	TSPAN32	PSMD14	DCTN3	LSG1	GM7589	MRPS17	SATB1	TUBA1B	YTHDC1
NDUFA4	RPL30-PS8	CDCA8	MRPL11	TIPIN	SEPW1	TIPIN	DKKL1	UTP23	RPL12-PS1	TNFRSF4	SFPQ	MRPS14	RPL27A
MDH2	ARHGAP4	DDX39	ATP6V1H	2700094K13RIK	GM10036	2700094K13RIK	HIST1H2BC	PMPCA	EXOC4	HMMR	CAR5B	MRPL18	GM11273
SNRPD2	RGS16	NDUFA4	NUP93	CDC123	GM10071	CDC123	CCNC	SYPL	HSD3B2	MANF	OSTF1	DPY30	AC151275.1
SDHB	NDUFS1	RRM1	GABARAPL2	GM10349	PPP2R4	GM10349	FAIM	CDKAL1	SOCS2	LGALS1	BCL2A1B	PSMB6	GM5561
TK1	GM3272	MAD2L1	MKKS	TIMM17A	RPS23	TIMM17A	UBE2S	ERMN	2310016C08RIK	CISD1	UBAP2L	PSMC2	2810474O19RIK
SPC25	LGALS3	SPC25	GNG5	TYMS	ARMC1	TYMS	CTCF	TRAF2	KPNA1	TMEM49	AA467197	FAM36A	GM6139
CDK4	ANXA5	PSMB7	DHX15	TACC3	GM9000	TACC3	RPL12	CTSW	IL10RB	PLP2	AC134548.2	CCT5	FRMD4B
PMF1	STK38	DCTPP1	PRKAG1	EXOSC8	GM7808	EXOSC8	AP2S1	AGTPBP1	HK2	EMP3	UBE2F	CDK4	GM10054
KIF23	ITGB1BP1	FBXO5	TRAT1	GMNN	RSRC1	GMNN	FAM111A	DEGS1	REL	SRSF7	LY6G5B	DCTPP1	RPL13-PS3
AURKB	2510002D24RIK	PMF1	NGDN	DBI	NDFIP1	DBI	RAB1	SIKE1	GM6180	ACOT7	ACADL	MRPS18C	GM5805
HIST1H2AG	SERPINE2	KIF23	CCNC	SNRPB	RPS27A	SNRPB	ACP1	PFKL	RPL21-PS3	NOP56	GM10247	HPRT	SMG7
PSAT1	ECE1	HIST1H2AG	HMGN1	GM11276	UBAP2	GM11276	PAPOLA	PIGU	CDKN1A	TXN1	IFITM3	YWHAH	GM7589
ERH	GM2792	NDUFB7	PTCD2	HIST1H2AO	GM7536	HIST1H2AO	CNOT6	MUM1	IL4RA	CD48	RGS1	H2AFZ	QSOX1
TAGLN2	MED13	PSAT1	CCDC69	SNRPD1	HIST1H1C	SNRPD1	SNX4	TAF1	ZBTB20	TXNDC17	BHLHE40	NDC80	SAMHD1
BUB3	MAPKAPK3	CDKN3	FAM111A	NUF2	SMC6	NUF2	ANAPC1	MAP2K3	D14ABB1E	CKS2	HDLBP	NDUFB2	RPS2-PS6
NUSAP1	GIMAP3	ERH	CCR6	HIST1H2AE	CD2BP2	HIST1H2AE	ANAPC11	DNPEP	GM8815	RBBP4	PFDN2	EMG1	GM6180
NDC80	GPR65	MRPL54	4930453N24RIK	LSM6	RPL10A	LSM6	TRNT1	RINT1	GM10012	SEC61B	FAM129A	MED10	NSA2
EMG1	RPS13	H2AFZ	BAD	HMGB2	SF1	HMGB2	HIST2H2AA2	SLC3A2	HERC2	COX17	WDR43	SEC13	AL844854.1
SEC13	MAP3K8	BUB3	ELP2	TUBA1B	RPL19	TUBA1B	AGPAT3	NSF	GM10154	KRTCAP2	SELL	NDUFV2	4921517L17RIK
TPX2	EIF4EBP1	NUSAP1	PPP2R5A	MRPS14	MAP2K3	MRPS14	BAD	FAM65B	IFNGR2	HP1BP3	GGH	HMGB3	SRP54A
CCNB1	RCSD1	RFC3	PMPCB	BANF1	SETD8	BANF1	HIST2H2AA1	WIBG	4930412F15RIK	TMEM208	PGAM1	TAF9	GM6807

Differentially expressed genes in in-vivo sub-populations													
Th17/Th1-like memory		Th17/Th1-like effector-LN		Th17/Th1-like effector-CNS		Th17/Th1-like effector		Th17/pre-Th1-like effector		Th17 self-renewing		Th17 Dysfunctional /senescent	
HMGB3	RPL15-PS2	TPX2	RNASEK	RAN	UQCRFS1	RAN	AC131675.1	UBR1	GM10063	TFF1	RAMP1	0610010K14RIK	WTAP
HINT1	OSBPL9	CCNB1	MAPKSP1	CDCA8	ELK3	CDCA8	VAMP4	UPF3B	GABARAPL1	GM3090	ITK	EIF4A3	GM10695
RBBP7	BPTF	HMGB3	IL16	DPY30	RPL27	MRPL18	NUBP1	ARPC5L	KDM6A	CCT8	MTA3	THOC7	GCNT2
TUBB5	PBRM1	HINT1	DEDD	PSMB6	NOL7	2900010M23RIK	USP1	IL27RA	LRRC58	RPS17	BAX	TUBB5	GM8815
CLSPN	MGST2	TUBB5	TNFRSF25	DDX39	HAVCR2	DPY30	STK39	AHCYL2	RPL36-PS3	GNG10	EIF4G1	MRPL51	MEX3C
DTYMK	GM9858	MRPL51	CMAH	KIF22	GM9846	PSMB6	AP3S1	ZFP825	AP1B1	EEF1B2	PRKACB	PA2G4	GM10012
BAT1A	RARS	CLSPN	GPATCH8	NDUFA4	TUBB6	DDX39	RAB4B	GM5160	KRR1	SPC24	RPL31	NDUFB6	ZZEF1
ETFA	TRPC4AP	DTYMK	PSMG4	NSMCE2	NCBP1	KIF22	SRSF1	MIIP	1500012F01RIK	31-Aug	HIF1A	SSR2	GM10154
TUBB2C	FTH1	UHRF1	NAA15	MDH2	DGAT1	NDUFA4	GPS1	CLEC2D	NR4A1	NDUFA1	KHDRBS1	POLR2G	AC117259.1
CASC5	ARHGAP1	0610007P14RIK	NUDT3	LSMD1	AC119211.2	NSMCE2	ELP2	AA467197	CREBL2	IMMT	ALKBH4	UHRF1	GM8991
SNRPE	UBE2G1	CASC5	DLD	REXO2	GM10237	MDH2	THOC6	POGLUT1	H2-GS10	NKG7	DNAJB6	PCMT1	4930412F15RIK
PSMC1	COTL1	D2ERTD750E	PRPF4	FAM36A	FAM65B	LSMD1	RIOK1	HAUS8	CSRNP1	HSD17B10	PDS5A	CBX5	GM5453
CDCA2	UBE2J1	ERGIC2	DDRGK1	RRM1	ATAD2	REXO2	CASP3	IFITM3	GADL1	S100A4	RPS27	HAT1	GM10063
1700029F09RIK	GM4609	CDCA2	PIN1	MAD2L1	RPL10	PSMC2	ZCRB1	2410002O22RIK	ISCU	GM10120	RPL30-PS8	MRPS21	GM5908
RPP21	CMC1	LBR	E2F4	TK1	MED21	FAM36A	PPP2R5A	MTPN	UBXN11	CRIP1	MAGT1	1810006K21RIK	AC155816.1
WBP5	PDE4B	SLBP	TNFRSF9	CCT5	EIF4A1	RPS27L	PDLIM2	COX10	PLAC8	SRPK1	GOLM1	ORC6	RPL36-PS3
LBR	TNFRSF9	MCM7	CKB	SPC25	OSBPL3	RRM1	IPO7	SSBP2	RPL21-PS10	SET	GTPBP4	NDUFB11	MT1
TUBG1	TOX	POLD3	GM3150	CDK4	2010002N04RIK	SDHB	PMPCB	PHKG2	RPS6-PS1	S100A10	DHX9	LGALS1	ZMYND8
SLBP	FAM110A	MNS1	ARF6	DCTPP1	RPS12	MAD2L1	SMC3	TEX261	MS4A6C	CIT	RGS16	LAT	GM8054
TNFRSF4	HNRPLL	TUBA4A	PIM1	FBXO5	STX11	TK1	DDOST	BCAT2	TPD52	ZWINT	DDRGK1	ANXA6	MAPKAPK2
MCM7	D16ERTD472E	MCM3	ZFP488	RFC4	TSPO	CCT5	MRPL35	PLDN	TTF1	FKBP2	MRP63	POLR2F	AC142450.1
HMMR	CSF2	FH1	RGS10	MRPS18C	SMARCA4	SPC25	UBE2B	PDHA1	ATN1	NAP1L4	LGALS3	MRPL21	AC117184.1
ANP32A	RFC1	KPNA2	NR4A1	PMF1	SFPQ	PSMB7	ACTR1A	MAGT1	LY6I	CXCR6	LMAN2	TRAPPC1	GM3839
ORC6	TMEM87A	RPA1	GM3550	HPRT	AA467197	DCTPP1	SNRPC	RGS16	C330021F23RIK	GM6169	ANXA5	CWC15	GM10916

Differentially expressed genes in in-vivo sub-populations													
Th17/Th1-like memory		Th17/Th1-like effector-LN		Th17/Th1-like effector-CNS		Th17/Th1-like effector		Th17/pre-Th1-like effector		Th17 self-renewing		Th17 Dysfunctional /senescent	
LGALS1	BSCL2	KIF2C	PAN3	DUT	AC134548.2	FBXO5	DDB1	TAF13	NKIRAS1	MRPL41	WBP2	MCM6	A230046K03RIK
GTF2A2	AGXT2L2	AAAS	JUND	YWHAH	TMEM128	RFC4	CENPQ	2510002D24RIK	ABHD2	AIP	STK38	GTF2H5	GM9104
CD3G	H2-K1	MRPS33	TNFRSF1B	PSMA1	GM16477	MRPS18C	RECQL	GM4759	BAZ2B	UQCR11	RPL17	GLO1	LARS2
TMEM49	LARS	ANAPC5	IFI27L2B	LSM5	ACADL	PMF1	HMOX2	MAPKAPK3	RPL21-PS11	FABP5	RBM38	ANAPC5	HNRNPUL1
PLP2	REEP5	ACTL6A	ATN1	KIF23	GM8730	HPRT	RPL9-PS4	GPR65	RPL21-PS6	RPL22L1	ACTN2	HMGB1	KHSRP
MCM3	LZTR1	HMGB1	KIF24	AURKB	GM10247	DUT	RNASEK	EIF4EBP1	RPL29-PS2	10-Sep	ORC5	PSMD7	IRAK1
KPNA2	DHX40	PTMA	RABGAP1L	HIST1H2AG	IFITM3	SEC11C	DDX27	ARHGAP1	LILRB4	ZAP70	RPL5	PTMA	GM11167
ATP5G3	GM7665	GM6104	GM10313	NHP2	TMED9	YWHAH	STARD3NL	COTL1	KLHL24	POP4	FAM49B	VPS25	RAD9
NDUFV3	HNRNPA3	SPC24	BTG2	COMMD1	SCAND3	PSMA1	NEDD1	4732418C07RIK	FOSL2	EIF5A	AC127419.1	EIF3L	H2-GS10
RPA1	STK24	MRPL4	IGF2R	FKBP3	SELL	LSM5	SSR4	TOX	GM6316	PTPRCAP	4833420G17RIK	TMEM208	RC3H1
ACOT7	DDX42	AC087117.1	SKIL	PSAT1	PGAM1	KIF23	PDCL3	MYD88	GM6109	HNRNPA2B1	EIF4A2	GM6104	GADL1
WDR61	ZNHIT1	ATP5K	RAB10	CDKN3	CCDC59	AURKB	FTSJ3	DDHD2	LY6C2	ANXA2	ECE1	PPP1CA	GM10566
GM10108	PRKCH	IMMT	RPL21-PS7	STRA13	EIF2S2	HIST1H2AG	SMS	ARL5C	GM8909	TNFRSF18	GM2792	ARHGDIA	GM10293
CKS2	ELF2	RFC2	RPL21-PS11	ERH	GTPBP1	NDUFB7	NUDC		CTSH	PSMG2	ATP6V0B	SRPR	AC159008.1
RBBP4	OBFC2A	CIT	SRRM2	COMMD3	STAG1	NHP2	CSDA		GM11127	DKC1	MAPKAPK3	2700029M09RIK	GM3550
KIF2C	SS18	ZWINT	RPL29-PS2	MRPL54	RPL31	COMMD1	GOT2		EGR1	VIM	PIK3CD	MRPL4	ISCU
COX17	RBPSUH-RS3	CCDC34	GM10291	H2AFZ	BIRC2	FKBP3	RPL37		NR4A3	CCT7	GPR65	PHB	RPL7A-PS3
ANAPC5	EHD1	MKI67	GM10327	TAGLN2	RPS27	PSAT1	LARP7		GM7030	CNIH	TAP1	GM10120	UBXN11
HP1BP3	SAMSN1	NUDT1	GM5507	BUB3	RPL30-PS8	CDKN3	CCDC41		SDC4	HNRNPF	RPS13	PPIE	PICALM
HMGB1	XRN2	EXOSC9	GM6316	NUSAP1	PFDN5	STRA13	COPB2		H2-AB1	TPI1	MAP3K8	VDAC2	MYO1E
PTMA	HNRPDL	PHF5A	ALKBH5	NDC80	RGS16	ERH	SEPW1		C1QB	ENO1	STK4	NAP1L4	PPIP5K1
BC021614	GM10155	TIMM22	MLL2	RFC3	CNOT2	COMMD3	GM10071			DDX47	RPL15-PS2	SMC1A	HEXDC
SNRPG	ZFP148	NAA38	INSIG1	2310028O11RIK	MRP63	MRPL54	PPP2R4			1500032L24RIK	HBS1L	GM10123	CLINT1
GM6104	CYB5B	HELLS	GM8909	3200002M19RIK	FAU	H2AFZ	KCNAB2			PARK7	IL1R1	SUMO3	PAN3
NT5C	RUNX2	NGFRAP1	GM11127	PSMA4	RPL27-PS1	TAGLN2	API5			HSP90AA1	PRDM1	CCDC34	MFSD11

Differentially expressed genes in in-vivo sub-populations													
Th17/Th1-like memory		Th17/Th1-like effector-LN		Th17/Th1-like effector-CNS		Th17/Th1-like effector		Th17/pre-Th1-like effector		Th17 self-renewing		Th17 Dysfunctional /senescent	
RPS17	NFKBIA	RNASEH2B	H2-Q2	TPX2	RPL17	BUB3	SUGT1			TIGIT	GM9858	XLR4C	RPL21-PS10
MEAF6	ITM2B		CDKN1B	1810027O10RIK	ORC5	NUSAP1	PRPF18			GNG2	APPL1	MRPL34	RPS6-PS1
GNG10	BNIP3		NOTCH2	CCNB1	TSHZ1	NDC80	TARS			CAMK4	FTH1	PTTG1	GM5921
EEF1B2	GM5518		SGIP1	HMGB3	RPL5	NDUFB2	RPS23			CORO1A	RBM5	PPP6C	RNF149
BRD8	GM10358		NR4A3	HINT1	AC127419.1	MED10	ARMC1			IL18RAP	LIN7C	DCTN6	LRRC8D
SPC24	IFITM2		GVIN1	TAF9	VAMP3	NDUFV2	GM9000			DNAJC15	ARHGAP1	TIMM8B	RPL7A-PS10
DRG1	NEDD9			RBBP7	ING1	RFC3	RSRC1			TCEB2	CNP	PQBP1	JUND
ANAPC13	SF3B3			CDC45	SHISA5	2310028O11RIK	UBAP2			SUSD3	GM5148	HELLS	TMEM123
AC087117.1	CHSY1			0610010K14RIK	RAP2C	3200002M19RIK	GM7536			GM5506	UBE2J1	SARNP	MXD1
FIGNL1	CDK7			TUBB5	GPR65	PSMA4	HIST1H1C			ISY1	SERINC3	NUTF2	GAS2L3
NKG7	TCOF1			MRPL51	TAP1	TPX2	SIN3A			EEF1G	RNGTT	OLA1	TTF1
S100A4	FOXN2			CISD3	RPS13	1810027O10RIK	SUZ12			HSD17B12	LRRFIP1	PCIF1	TGFBR2
SRPK1	TAGAP			CLSPN	RPL15-PS2	CCNB1	SMC6			BCAS2	5830405N20RIK	NSMCE1	ANKFY1
CIT	CCPG1			NDUFC2	GM9858	HMGB3	MAP4K1			CTLA4	CMC1	EIF3C	DNAJB14
ZWINT	MGA			CENPA	GM5148	HINT1	SF1			PKP3	TULP4	PTPN2	CAMK2G
CXCR6	MAST4			NDUFB6	HSPH1	TAF9	RPL19			2900073G15RIK	PDE4B	UBE2V2	GM10362
GM6169	GM5220			RP23-378I13.5	FTL1	RBBP7	SETD8			ADSL	TNFRSF9	GIMAP4	TLCD1
MRPL41	RPS19-PS2			BAT1A	APOL7B	CDC45	UQCRFS1			PSMC4	MYSM1	ANAPC16	RAP2B
CCDC34	POLR2A			ETFA	TOX	EIF4A3	GM8394			1810037I17RIK	APOL7B	ADSL	DDX6
GM6984	GPSM3			LIG1	FAM110A	CHCHD1	IK			LPXN	TEX2	TRMT112	PYHIN1
MKI67	CREM			MPHOSPH6	RFC1	THOC7	RPL27			SDF4	YME1L1	CST7	TRAFD1
2610029G23RIK	POLR3C			UHRF1	RAPGEF6	TUBB5	ITGAV			CAPG	TOX	BRIX1	CAPN12
RPL22L1	TCF7			TUBB2C	GM7665	MRPL51	NOL7			SNX3	FAM110A	TPD52L2	SYNRG
BZW1	EPS15			NRM	RALBP1	TMEM14C	GPX1			ADK	1700123O20RIK	UFD1L	BAT2L

Differentially expressed genes in in-vivo sub-populations													
Th17/Th1-like memory		Th17/Th1-like effector-LN		Th17/Th1-like effector-CNS		Th17/Th1-like effector		Th17/pre-Th1-like effector		Th17 self-renewing		Th17 Dysfunctional /senescent	
FAM60A	CCDC50			CASC5	SLC24A5	PA2G4	GM9846			PRKAR1A	RBMS1	DCAF13	ATN1
EXOSC9	ATP2B4			SNRPE	EHD1	CLSPN	MSL3			RPLP0	D16ERTD472E	MRPS18B	TRPM2
CD2	P4HA1			D2ERTD750E	RPS8-PS1	NDUFC2	DNAJC2			PDLIM1	CSF2	ESF1	GM3222
ECH1	FBXO46			ATP5B	AC120410.1	DTYMK	NCBP1			CSNK2B	RFC1	EIF3D	MAP3K14
CBX3	IKBKB			ERGIC2	XRN2	CENPA	GPATCH8			28104 28I15RIK	TMEM87A	PSIP1	C330021F23RIK
HNRNPA2B1	CCR2			CBX5	GM10155	NDUFB6	EIF1AD			ACTG1	SNX2	BZW2	MLL3
CDCA7	PLIN2			SUMO2	SEC61G	RP23-378I13.5	ARGLU1			CALM1	DNAJB1	WDR12	ELMOD2
ANXA2	ISG20			CDCA2	CYB5B	BAT1A	CCDC107			YARS	H2-K1	KIF15	ABHD2
NAA38	ZYX			RBM3	RUNX2	ETFA	AC119211.2			EIF3K	FAM98B	UFC1	ADIPOR1
PRC1	UBASH3B			WBP5	GM5518	SRP19	GM10237			IFNG	TMEM149	C330027C09RIK	GM10313
DNAJC9	RORA			TCP1	GM12666	POLR2G	ATAD2			S100A13	REEP5	WDR33	DTX3L
TNFRSF18	GEM			LBR	GM10358	LIG1	TPT1			TMEM176B	GM7665	2410001C21RIK	PPM1K
DKC1	SLC15A3			TUBG1	NKAP	MPHOSPH6	OSBPL3			GSTP2	MPHOSPH10	MRPL48	GM8225
DNAJC8	PSD4			NAP1L1	CHSY1	UHRF1	UCP2			FAM162A	2610101N10RIK	ZCCHC17	MYCBP2
HNRNPF	1110007A13RIK			MRPS17	ZRANB2	TUBB2C	2010002N04RIK			GTF2E2	STK24	GABPB2	SMG1
TPI1	SFT2D1			TNFRSF4	GM5220	0610007P14RIK	A430093F15RIK			PSMD2	ZNHIT1	NOP58	RAB10
ENO1	ZC3HC1			MCM7	DYNLT1C	NRM	RPS12			CPSF3L	CNOT1	PNRC2	AL732476.1
CCDC21	YTHDC1			HMMR	DDX21	PCMT1	TSPO			CDC42	F2R	2610030H06RIK	RPL21-PS7
DDX47	IFNGR1			MRPL23-PS1	RPS19-PS2	NDUFS8	SMARCA4			RPS20	SS18	ACADVL	NPC2
NSMCE1	GOLGA7			POLD3	POM121	CASC5	SFPQ			BZW2	RBPSUH-RS3	TMED2	RPL21-PS11
TIGIT	IL18R1			PHGDH	GABARAP	SNRPE	GATAD1			SLAMF1	EHD1	CLP1	SIK1
TMEM50A	LITAF			NUDT21	HNRNPL	D2ERTD750E	AC134548.2			RPSA-PS10	GNL3	RIF1	AC163269.1
GNG2	ATF6			ORC6	TCF7	ATP5B	NAA15			ATP5L	RPS8-PS1	SDHC	TNRC6C
CORO1A	DOT1L			MNS1	CCND2	ERGIC2	GM16477			HAX1	NSG2	SCAMP4	4930470H14RIK

Differentially expressed genes in in-vivo sub-populations													
Th17/Th1-like memory		Th17/Th1-like effector-LN		Th17/Th1-like effector-CNS		Th17/Th1-like effector		Th17/pre-Th1-like effector		Th17 self-renewing		Th17 Dysfunctional /senescent	
CAB39L	TAB2			LGALS1	UAP1	CBX5	ACADL			CD226	SAMSN1	BIN2	GM10718
DNAJC15	USP4			HIST1H1E	A830010M20RIK	UQCR10	GM8730			HSP90AB1	AC120410.1	CPM	IFI203
GM5506	AC151275.1			LCK	RPL7A-PS5	AURKAIP1	SF3A1			PSMB8	XRN2	GM10250	RPL21-PS6
EZH2	INPP5F			SSB	SERBP1	NDUFB9	TMED9			NASP	GLUL	ARAF	MED13L
APOBEC3	CD44			LAT	LAMC1	VDAC3	SCAND3			SYTL3	GM10155	CCR6	RPL29-PS2
ISY1	KLF6			CISD1	GM10136	SUMO2	MTPN			OXCT1	FASL	GIMAP6	RASSF2
DLGAP5	PTP4A1			TMEM49	WDR92	HAT1	KIF2A			RPL36A	XAF1	4930453N24RIK	STAT1
CENPE	ZFP295			PLP2	U2AF1	FXC1	PUM2			RPL8	RASA3	RPL18	AHNAK
BCAS2	GM5561			MCM3	RPL27A	CDCA2	GTPBP1			GM8759	RUNX2	AC131675.1	ARID5B
H2-KE2	RASGRP1			FH1	LITAF	1700029F09RIK	STAG1			TBCB	NFKBIA	RRP1B	FMO1
SLC25A5	ATXN1			KPNA2	MDN1	RBM3	MED29			RPS8	HOPX	LONP2	GM10291
PSMD6	CD27			ATP5G3	YY1	WBP5	SMN1			RPSA	ITM2B	EEF1E1	RPL17-PS3
COX6C	SLC2A3			RPA1	TACC1	DEK	SREK1			RPL7	GM5518	ENY2	SLC39A1
PPP1R8	ZFML			ACOT7	AC151275.1	TCP1	RPL31			2410001C21RIK	PLEKHB2	GM10257	TAX1BP3
UCHL3	TNFAIP3			TXN1	GM5561	LBR	HMGA1			PRR13	GM10358	PRPF18	GM10327
UBL4	CORO2A			NDUFAB1	GM6139	TUBG1	KHDRBS1			RPLP1	PUM1	GM7808	BIRC6
CCL1	RPRD2			MCM6	CORO2A	NAP1L1	BIRC2			YWHAZ	NXF1	PPP1R7	IRF2BP2
XRCC6	NRIP1			GTF2H5	PRPF39	SLBP	RPS27			DDT	ELK4	YIF1A	GM5507
CTLA4	CCR1			ASNS	SLAMF6	MRPS17	FMR1			PPP1CC	ARHGEF3	INTS7	GM10800
2900073G15RIK	VPS54			GM10108	GM10054	MCM7	RPL30-PS8			ZCCHC17	IFITM2	TPT1	GM6316
NDE1	PRPF39			CKS2	SON	HMMR	PFDN5			MRPS36	NEDD9	PSME2B-PS	KDM6B
GLRX	SPIN1			GM10053	RPL13-PS3	POLD3	RGS16			CALM3	CHSY1	GM9234	GM6109
HNRNPR	SLAMF6			KIF2C	ZGPAT	CCT2	2810008M24RIK			SLC35D1	CDK7	ATP6V1F	PNRC1
LPXN	EIF2C2			HN1	GM5805	PSMA6	MRP63			SLA2	ZRANB2	TSPO	ZFP36L1
SDF4	UBL3			AAAS	GM3940	PHGDH	PIN1			PIH1D1	CHD4	RAB27A	ALKBH5

Differentially expressed genes in in-vivo sub-populations													
Th17/Th1-like memory		Th17/Th1-like effector-LN		Th17/Th1-like effector-CNS		Th17/Th1-like effector		Th17/pre-Th1-like effector		Th17 self-renewing		Th17 Dysfunctional /senescent	
CAPG	CD200R1			23100 61C15 RIK	GM7589	NUDT21	GNL3L			ALDOA	PPP1CB	GM2574	MLL2
NUP214	INPP4A			COX17	WDR70	ORC6	FAU			TSG101	TCOF1	GM5138	JUNB
PRKAR1A	SON			ANAPC5	RPL12-PS1	MNS1	RPL27-PS1			NDUFA13	NOL8	SREK1	PDCD4
CST7	RPL13-PS3			CKAP5	QSOX1	LGALS1	RPL17			L7RN6	MDM4	ING5	INSIG1
PDLIM1	ADAM19			MCM4	HSD3B2	HIST1H1E	ORC5			LXN	TRPS1	PFDN5	GM8909
SERPINB1A	GM5805			RANGAP1	AC156282.1	PSMC3	TSHZ1			CRMP1	AL732569.1	GTPBP4	C030046E11RIK
CDC26	GRINA			TUBA1C	GM10481	SSB	RPL5			SH3BGRL3	ZFP91	DHX9	PSAP
DERL2	ARAP2			HMGB1	TNRC6B	LAT	FAM49B			S100A6	AKNA	GM3272	ARID1B
YARS	CKB			PTMA	RPS2-PS6	CISD1	AC127419.1			GABARAPL2	MGA	RPL27-PS1	GM11127
GCLM	SQSTM1			SSBP1	23100 16C08 RIK	TUBA4A	VAMP3			RPLP2	GM5220	RPL5	H2-Q2
IFNG	GM7589			EIF3L	GM5619	PLP2	ING1			RAD21	RPS19-PS2		CDKN1B
SNRNP70	WDR70			BC021614	GM3150	MCM3	KRCC1			TBC1D10C	ZFP106		NOTCH2
PPIL2	BCL2A1A			SNRPG	REL	FH1	SHISA5			GM4294	BCL2A1C		SLFN5
FAM33A	VGLL4			TFF1	GM8910	KPNA2	GPR65			SEC22B	DYRK1A		SGIP1
FAM162A	RPL12-PS1			GM6104	GM6180	ATP5G3	TAP1			RPS15	CREM		NR4A3
PSMD2	ARIH1			PPP1CA	UTRN	SRSF7	RPS13			NCL	TNFSF10		GM4070
4933434E20RIK	ZFAND5			GM3090	CCRN4L	CWC15	RPL15-PS2			G3BP1	SEMA4A		BMP2K
CAPZA2	HSD3B2			ELOF1	BC005537	RPA1	GM9858			MRPS24	SRSF5		GM7030
SUPT16H	TEX10			MEAF6	TRIM12A	ACOT7	GM5148			ATP5G2	CASP8		GVIN1
OGDH	CTSD			MTHFD2	RPL21-PS3	TXN1	GM4609			NPTN	TSC22D4		ZFP36
RPS20	GM10481			ANP32B	NSA2	NDUFAB1	HSPH1			CISH	GLTSCR2		LYZ2
BZW2	23100 16C08 RIK			GNG10	ACSL4	CD48	FTL1			PRPF38A	TCF7		GRN
SFXN1	KPNA1			SPC24	AL844854.1	TXNDC17	WBP4			GM2000	16000 14C10 RIK		
RPSA-PS10	RUNX1			C79407	CDKN1A	MCM6	RFC1			RPL3	ATP2B4		
ATP5L	RNF13			27000 29M09 RIK	49215 17L17RIK	GTF2H5	GM6736			RPS29	CDK11B		

Differentially expressed genes in in-vivo sub-populations													
Th17/Th1-like memory		Th17/Th1-like effector-LN		Th17/Th1-like effector-CNS		Th17/Th1-like effector		Th17/pre-Th1-like effector		Th17 self-renewing		Th17 Dysfunctional /senescent	
VRK1	DENND4A			CHCHD3	GM6807	ASNS	GM10116			RPL28	PSMD9		
CD226	DCTN4			COPS6	FURIN	WDR61	REEP5			TMSB4X	CCND2		
SF3A3	HK2			RQCD1	COQ10B	GM10108	D19BWG1357E			RPL7A	LAG3		
NASP	REL			AC087117.1	KLF13	CKS2	GM7665			RPL38	PTPN18		
SYTL3	GM8910			FIGNL1	UPF1	TRP53	RALBP1			CCR8	CCR2		
HERPUD1	CD81			NKG7	RAB8B	GM10053	DDX42			TIMM17B	TMEM66		
TXNDC9	HSF2			CD6	ARF5	KIF2C	RP23-71J17.1			RPS3A	PLIN2		
RPL8	WDFY1			RFC2	PRKACA	GLO1	RPS8-PS1			SLA	ERO1L		
CSNK2A1	TRIM12A			PRDX1	CT033780.1	HN1	AC120410.1			ATOX1	UAP1		
MRPL10	TOB1			GOLT1B	WTAP	MRPL33	XRN2			RPS25	COX16		
PRR13	ZBP1			LSM2	CAPS2	AAAS	HNRPDL			RPS18	GPR68		
RPLP1	FAM102A			PFDN1	GM8815	MRPS33	GM10155			LLPH	NVL		
DDT	ACSL4			CUTA	TSC22D3	2310061C15RIK	PCBP1			RPS3	ARHGAP26		
LUC7L3	CDKN1A			TMPO	BAT2L2	COX17	BRD9			RWDD1	ZYX		
ZCCHC17	SRSF2IP			SMC4	ARF6	1810009A15RIK	SEC61G			EIF4H	GIMAP7		
BC031181	GM6807			6-Sep	GM10012	ANAPC5	CYB5B			1700012B07RIK	PMAIP1		
2310036O22RIK	FURIN			SSRP1	GM10154	ACTL6A	RUNX2			TMSB10	UBASH3B		
HJURP	IL4RA			ZC3H15	AC117259.1	CKAP5	ITM2B			RIF1	RORA		
MTIF2	COQ10B			SET	TGOLN1	MCM4	GM5518			IL1R2	APAF1		
ALDOA	TNF			MCM5	4930412F15RIK	RANGAP1	GM10358			RPL35	PIAS1		
TSG101	PDE4D			CLIC1	GM5453	TUBA1C	USP50			SNRNP27	RNF20		
PFKP	D14ABB1E			CIT	GM10063	HMGB1	NFATC2			BC016495	SLC15A3		
TAF6	GM8815			PDZD11	GM5908	PSMD7	GM5220			RPL22	PSD4		
LXN	ARF6			FKBP2	AC155816.1	DNAJC19	DYNLT1C			LASS2	1110007A13RIK		
CD40LG	PHC3			SMC1A	LRRC58	PTMA	RPS19-PS2			GM11353	WDR45L		

Differentially expressed genes in in-vivo sub-populations													
Th17/Th1-like memory		Th17/Th1-like effector-LN		Th17/Th1-like effector-CNS		Th17/Th1-like effector		Th17/pre-Th1-like effector		Th17 self-renewing		Th17 Dysfunctional /senescent	
CDK5RAP2	RALGPS2			GM10123	RPL36-PS3	SSBP1	GABARAP			RPL14	YTHDC1		
SKP1A	ANXA1			GM6169	AMD1	EIF3L	LARP4B			POLR2E	RHOH		
S100A6	JMJD1C			PSMA5	CFLAR	BC021614	HNRNPL			NACA	GM10136		
GABARAPL2	GABARAPL1			SUMO3	MACF1	SNRPG	TCF7			RPS19	IFNGR1		
RPLP2	SMPDL3A			AIP	FOXP1	YWHAQ	GRCC10			RPL39	DNAJC1		
TBC1D10C	RPL36-PS3			FDPS	PPP1R12A	UQCRC2	CCND2			GM10073	IL18R1		
HNRNPM	SEC62			CCDC34	MLL5	TFF1	TLN1			POLR2B	RPL27A		
PSMD5	FAM177A			GM6984	SP110	GM6104	A830010M20RIK			BCLAF1	USP7		
RPS15	FOXP1			FABP5	CDC42SE2	PPP1CA	GIMAP7			AC124742.1	C330019G07RIK		
NCL	CAMK2D			MKI67	ZMYND8	GM3090	RPL7A-PS5			MRPS36-PS1	DNAJB4		
CISH	ZMYND8			2610029G23RIK	KRR1	NAA10	SERBP1			COMMD6	LITAF		
GM2000	ANKRD12			MRPL34	ANKRD12	SRPR	WDR45L			SP1	GNGT2		
RPS29	NFKBIZ			SLC29A1	GM8054	ELOF1	GM10136			GM5559	MDN1		
RPL28	GM8054			POP4	AMD2	PPIG	WDR92			GM6472	DDX46		
TMSB4X	LARP4			TFDP1	GSK3B	MRPL28	RPL27A			RPS9	IFI35		
CCR8	MAPKAPK2			TTC1	AC108412.1	MEAF6	MDN1			RPS18-PS3	TAB2		
RPS25	SLFN1			ECH1	SPOPL	MTHFD2	DDX46			5-Sep	DMTF1		
LLPH	RBPJ			CBX3	GM7592	ANP32B	AC151275.1			RPL35A	AC151275.1		
HCST	RNF19A			MYG1	MNDAL	GNG10	GM5561			GM12033	CPD		
WBSCR22	1500012F01RIK			GM4737	ZFP488	COX7A2	2810474O19RIK			TRAT1	CD44		
NUCKS1	CTNNA1			UBE2A	AC142450.1	PPIB	GM6139			NGDN	KLF6		
RIF1	RGS10			CALM2	AC117184.1	SPC24	UBQLN1			IL2RA	GM5561		
IL1R2	CHD7			RDM1	JARID2	DRG1	WBP11			MRPL32	SPARC		
CD69	SEMA4B			HELLS	TMEM71	C79407	PRPF39			GNA15	EHMT1		
RPL35	NR4A1			PRC1	SEMA4B	2700029M09RIK	EZH1			SPAG7	NMNAT1		
BC016495	KLRD1			DNAJC9	GM2026	ICT1	GM10054			2810407C02RIK	PION		

Differentially expressed genes in in-vivo sub-populations													
Th17/Th1-like memory		Th17/Th1-like effector-LN		Th17/Th1-like effector-CNS		Th17/Th1-like effector		Th17/pre-Th1-like effector		Th17 self-renewing		Th17 Dysfunctional /senescent	
GM11353	GM3839			NUTF2	GM7609	CHCHD3	SON			TMEM179B	ATXN1		
CBX1	RBM47			PPIA	AMD-PS3	COPS6	RPL13-PS3			CENPL	CD27		
POLR2E	ZFP187			PCIF1	GM14305	CS	ZGPAT			RPS24	SLC2A3		
GM10073	GM9104			RPP30	GM14434	MRPL4	GM5805			GM10020	GM6139		
SSU72	RABGEF1			HSPA14	EMD	RQCD1	GM3940			DCTN3	CASP4		
PGK1	ASAH1			CNIH	LY6C1	AC087117.1	GM7589			ACOT9	TNFAIP3		
POLR2B	GPR132			MRPS11	GM3839	ATP5K	WDR70			RPS10	CORO2A		
BCLAF1	CTSB			HDGF	GM10916	DERA	RPL12-PS1			RPS7	MAF		
AC124742.1	ECM1			STIP1	A230046K03RIK	PDCD5	QSOX1			RPL21	SOAT1		
GM5559	CSRNP1			NSMCE1	GM9104	FIGNL1	HSD3B2			RPS21	BIRC3		
5-Sep	ZEB2			AHSA1	LARS2	CD6	AC110247.1			HIST1H2BC	NRIP1		
RPS15A	GM10293			GARS	B4GALT1	RFC2	AC156282.1			RPS13-PS1	CCR1		
GM12033	ANKRD17			TIGIT	HNRNPUL1	PRDX1	GM10481			TTC39B	VPS54		
TRAT1	AI848100			XPO1	KHSRP	GOLT1B	TNRC6B			HMGN1	PRPF39		
NGDN	SMAD7			CHMP2A	GM14391	LSM2	RPS2-PS6			CCDC55	RELL1		
ELOVL1	CCL4			CD160	GM11167	NDUFA9	GM5619			RPS16	SPIN1		
TPRKB	GP49A			PTGES3	RAD9	PFDN1	GM3150			GM10119	FRMD4B		
IL2RA	PELI1			SNRNP25	H2-GS10	PSMB3	RBM15			AC154908.2	RBM26		
PSMD4	XRN1			IL18RAP	06100 31J06RIK	CUTA	GM8910			RPL12	AIM1		
KPNB1	PLAC8			TMEM109	SPNA2	TMPO	GM6180			CTLA2A	SLAMF6		
RPL21	NRP1			MCM2	GADL1	SMC4	SLC38A6			TNFSF11	UBL3		
TTC39B	RPL21-PS10			NUP210	FAM113B	PPIE	UTRN			SPNB2	INPP4A		
HMGN1	RAB11FIP1			RPA3	GPBP1L1	SSRP1	CCRN4L			ERGIC3	GM10054		
CCDC55	GADD45B			EZH2	PTEN	BC056474	BC005537			RPL30	SON		
PPP1R16B	BTG1			D17WSU104E	GM10566	ZC3H15	TRIM12A			DENR	ANKRD44		
TNFSF11	CHD1			NXT1	GM10293	SET	RPL21-PS3			PECI	ADAM19		
PAPOLA	LGALS3BP			ISY1	ACOT2	MCM5	NSA2			RPL7L1	FRYL		

Differentially expressed genes in in-vivo sub-populations													
Th17/Th1-like memory		Th17/Th1-like effector-LN		Th17/Th1-like effector-CNS		Th17/Th1-like effector		Th17/pre-Th1-like effector		Th17 self-renewing		Th17 Dysfunctional /senescent	
GM10250	JUND			DLGAP5	AC159008.1	ICOS	ACSL4			GAPDH	ARAP2		
GAPDH	TNFRSF1B			PSMB1	GM3550	CLIC1	AL844854.1			CCR6	CKB		
CCR6	PLD3			CENPE	RPL7A-PS3	CIT	CDKN1A			HNRNPA0	SQSTM1		
ANAPC11	CTSC			SLC25A5	RALB	PDZD11	4921517L17RIK			P4HB	WDR70		
DHRS3	TTF1			CYC1	NIPBL	ZWINT	GM6807			MED11	BCL2A1A		
MIER1	ANKFY1			COX6C	UBXN11	FKBP2	FURIN			AGPAT3	QSOX1		
FXYD5	ANXA4			UCHL5	LNPEP	COPS3	KLF13			DHRS3	CTSD		
S100A11	EFHD2			ACTB	RRM2B	SMC1A	UPF1			GIMAP6	CLIC4		
CD4	HEXB			HMGN5	PSMB10	GM10123	ARF5			FXYD5	CCR7		
SRGN	ATN1			LSM4	PRPF4B	CCDC101	PRKACA			DGUOK	AP1S2		
GM10359	GM3222			ADH5	PPIP5K1	PSMD11	CT033780.1			RPS27A-PS2	RPS2-PS6		
ORC3	SLAMF7			DPYSL2	HEXDC	USMG5	WTAP			RPL18	SOCS2		
IL2RB	GBP7			2900073G15RIK	KDM5B	PSMA5	PDE4D			S100A11	2310016C08RIK		
SRSF1	H2-DMA			RNPS1	PAN3	CCDC56	GM10695			GM10159	RUNX1		
ABLIM1	C330021F23RIK			NFYB	RPL21-PS10	SUMO3	CAPS2			VAMP4	NFAT5		
KLRC1	SP3			MRPS25	RPS6-PS1	AIP	GM8815			SRGN	IGTP		
ID2	NFIL3			EFTUD2	GM5921	FDPS	EDEM1			GM10359	RNF13		
GM4963	DUSP5			GTL3	RPGRIP1	CCDC34	BAT2L2			PIGX	DENND4A		
PPP2R5A	GM10313			AI314976	BTG1	GM6984	ARF6			TRAF3IP3	KBTBD11		
SNX5	BTG2			SNX3	CLASP2	FABP5	GM10012			ACTR2	DCTN4		
BCL2A1D	PPM1K			PDHB	LRRC8D	NDUFB3	ZZEF1			AEBP2	HK2		
GM10263	IGF2R			SNRPB2	CAP1	PRPS1	GM10154			IL2RB	REL		
AC129078.1	TGTP1			NDUFAF2	RSBN1L	MKI67	AC117259.1			LAGE3	GM8910		
RPL15	SKIL			SNAPC5	RPL7A-PS10	2610029G23RIK	TGOLN1			GM10335	ARL15		
UNC13D	RAB10			HNRNPAB	JUND	IMPA1	GM8991			ABLIM1	HSF2		
CENPQ	GBP2			PRKAR1A	AP2A2	MRPL34	4930412F15RIK			KLRC1	WDFY1		
RECQL	RPL21-PS7			AHCY	DYNC1H1	ZFP207	GM5453			H2-Q8	CCRN4L		

Differentially expressed genes in in-vivo sub-populations													
Th17/Th1-like memory		Th17/Th1-like effector-LN		Th17/Th1-like effector-CNS		Th17/Th1-like effector		Th17/pre-Th1-like effector		Th17 self-renewing		Th17 Dysfunctional /senescent	
0910001L09RIK	C1QA			VDAC1	4632428N05RIK	SLC29A1	GM10063			GGNBP2	TRIM12A		
EIF3A	IER3			AP1S1	IFI27L2B	10-Sep	GM5908			CDC42SE1	ENTPD7		
GM2606	IFRD1			PPID	GAS2L3	MRPL40	AC155816.1			RPL9-PS6	ZBP1		
FOLR4	RPL21-PS11			BLMH	TTF1	CACYBP	LRRC58			ID2	FAM102A		
HSPA5	SIK1			TPD52L2	ANKFY1	POP4	ESCO1			ZCRB1	ACSL4		
GM6636	TET2			2810428I15RIK	DNAJB14	EXOSC9	RPL36-PS3			GM4963	CDKN1A		
LONP2	GBP6			FAM125A	GM10362	TFDP1	FNBP1			CD37	SRSF2IP		
FTSJ3	SPTY2D1			HAUS3	TLCD1	PSMB4	UBR4			PPP2R5A	GM6807		
ZFR	RPL21-PS6			ACTG1	RAP2B	TTC1	AMD1			SNX5	FURIN		
EXOSC2	NR4A2			CALM1	DDX6	CD5	SEC62			BCL2A1D	COQ10B		
GM9396	MED13L			YARS	PYHIN1	TIMM22	CFLAR			GM10263	VCPIP1		
TSPAN32	RRBP1			0610037P05RIK	CAPN12	ECH1	MACF1			DDOST	PRKACA		
GM10036	RASSF2			CRBN	SYNRG	CBX3	TXNIP			AC129078.1	ATPBD4		
SYPL	LILRB4			GM10076	ATN1	SMU1	PPP1R12A			RPL15	1110007C09RIK		
SUCLG2	AHNAK			IFNG	TRPM2	HNRNPA2B1	MLL5			RPL6	TNF		
LTA	RGS2			UBASH3A	GM3222	CDCA7	SP110			CYLD	PDE4D		
IL16	PLEK			TSN	MTMR2	MYG1	ZMYND8			EEF2	GCNT2		
TRA2B	FOSL2			FAM33A	KIF24	GM4737	KRR1			1810046J19RIK	BAT2L2		
VPS35	DUSP1			POLA1	C330021F23RIK	PPP6C	TNRC6A			CCM2	UPF2		
GM2833	PER1			PSIP1	IL7R	UBE2A	GM8054			SNRPC	RALGPS2		
GM10240	GM10327			OGDH	MS4A4C	BLVRA	AMD2			GM5879	GM10012		
ERMN	IRF2BP2			ARL1	MLL3	SRP9	GSK3B			RPL9-PS4	GATAD2A		
DENND2D	GM5507			ABCF2	2810422J05RIK	CNIH4	AC108412.1			0910001L09RIK	AP2B1		
FAM165B	TOB2			KIF15	PBXIP1	CALM2	SPOPL			EIF3A	GM10154		
RPS28	GM6316			TIPRL	GM2058	TIMM8B	UBE2H			RAC1	AC117259.1		

Differentially expressed genes in in-vivo sub-populations													
Th17/Th1-like memory		Th17/Th1-like effector-LN		Th17/Th1-like effector-CNS		Th17/Th1-like effector		Th17/pre-Th1-like effector		Th17 self-renewing		Th17 Dysfunctional /senescent	
CTSW	KDM6B			ACTN4	JHDM1D	NAA38	2310035C23RIK			GM2606	TGOLN1		
AQR	GM6109			CORO1C	KTN1	RDM1	GM7592			FKBP5	GM8991		
TMEM147	JUN			SYTL3	ELMOD2	HELLS	MNDAL			MYO1G	GM5453		
NDFIP1	ALKBH5			OXCT1	ABHD2	PRC1	SLFN1			FOLR4	GM10063		
RPS27A	JUNB			C330027C09RIK	GM10313	NGFRAP1	RBPJ			IFI27L2A	GM5908		
SAP30BP	CD63			WDR33	DTX3L	DNAJC9	ZFP488			RPS6	KDM6A		
UTP14A	MNDA			SNRPA	PPM1K	ITPRIPL1	AC142450.1			GM6636	FOXO1		
SIN3A	INSIG1			HIST1H4I	GM8225	NUTF2	AC117184.1			STARD3NL	ESCO1		
BSG	RNF213			ACSL5	RUNX3	ATP5J2	SEMA4B			CHMP5	AMD1		
SUZ12	FOSB			FAM96B	IGF2R	PPIA	GM2026			ZFR	AP1B1		
0610011F06RIK	PSAP			9130401M01RIK	MYCBP2	VIM	GM7609			RPL23A	MFSD4		
RPL10A	9930111J21RIK2			BAZ1B	SKIL	PCIF1	AMD-PS3			RPL37	MACF1		
MAP4K1	CCL5			ZCCHC17	SMG1	RNASEH2B	GM14434			GM9396	SAMD9L		
GATA3	GM11127			THOC4	ABCG1	RPP30	EMD			TSPAN32	FOXP1		
PTPN22	EGR1			2310036O22RIK	AL732476.1	PSPH	IRAK2			SEPW1	CAMK2D		
YIF1A	APOE			HJURP	RPL21-PS7	AK2	LY6C1			RPL9	PPP1R12A		
MUM1	NOTCH2			IFT27	NPC2	HSPA14	GM3839			RPL18A	SP110		
CMAH	CCRL2			SLC35D1	RPL21-PS11	NUDT5	GM10916			RPL37A	MT1		
YTHDF2	NR4A3			EXOSC5	SIK1	CNIH	NBR1			GM10036	PDCD11		
GBA	GM4070			SLC1A5	AC163269.1	HNRNPF	ZFP187			SYPL	H2-T10		
LIMD2	GM7030			NUP93	TNRC6C	MRPS11	A230046K03RIK			GM10071	ZMYND8		
MAP2K3	GVIN1			PPP1R11	4930470H14RIK	HDGF	GM9104			SIRT2	FOXN3		
SETD8	CD86			L7RN6	SRRM2	MRPS18A	LARS2			IL16	NFKBIZ		
DDX5	PRDX5			RNASEH2C	GM10718	STIP1	B4GALT1			TSPAN31	TNRC6A		
TAPBP	AIF1			TAF6	IFI203	CCDC21	HNRNPUL1			SUGT1	GM8054		

Differentially expressed genes in in-vivo sub-populations													
Th17/Th1-like memory		Th17/Th1-like effector-LN		Th17/Th1-like effector-CNS		Th17/Th1-like effector		Th17/pre-Th1-like effector		Th17 self-renewing		Th17 Dysfunctional /senescent	
LAPTM5	H2-AB1			CDK5RAP2	RPL21-PS6	H3F3A	H2-Q6			TRA2B	AMD2		
DGKA	H2-EB1			SH3BGRL3	HMHA1	15000 32L24 RIK	KHSRP			FKBP8	ACTN1		
HAUS2	LYZ2			CDKN2AIPNL	MED13L	NSMCE1	GM14391			RPS23	HELZ		
CST3	GRN			DNMT1	RPL29-PS2	AHSA1	GM11167			GM10268	CDK13		
ITGAV	C1QB			RAD21	RASSF2	MRPL46	RAD9			GM2833	23100 35C23 RIK		
HAVCR2	LY86			MEMO1	NCOA3	PRDX4	H2-GS10			AKAP13	RBPJ		
SLC3A2	FCER1G			MRPS24	STAT1	NUDCD2	06100 31J06R IK			GM10240	ZFP488		
MAPRE2	TYROBP			CISH	FMO1	GARS	TECPR1			AC124399.1	CTNNA1		
CLK3				PRPF38A	GM10291	XPO1	SPNA2			ERMN	TMEM71		
GPATCH8				CCDC124	RPL17-PS3	MRPL45	RC3H1			GM9000	SEMA4B		
ATP6V1G1				MRPS6	SLC39A1	CD160	GADL1			DENND2D	AMD-PS3		
SH2D2A				UBB	GM10327	PTGES3	FAM113B			RPS28	EMD		
DGAT1				GTF2A1	BIRC6	NOL12	GPBP1L1			RPL36	NR4A1		
EIF1AD				MKKS	IRF2BP2	GDI2	PTEN			CTSW	IRF2		
MRPS26				TRIM28	GM5507	SNRNP25	GM10566			ADRBK1	GM10916		
AW112010				CCR8	MAP3K1	TUBA1A	SETD2			MAT2A	RBM47		
FAM65B				PUF60	GM10800	59304 16I19R IK	UBN2			ODC1	ZFP187		
NUMA1				TMED2	TOB2	IL18RAP	GM10293			PPP1R7	ARHGAP31		
EMB				NEBL	GM6316	SIVA1	ACOT2			CSTB	A2300 46K03 RIK		
2010111I01RIK				HCFC1	KDM6B	TMEM109	AC159008.1			AC114007.1	CD9		
MED21				D9300 14E17 RIK	GM6109	MCM2	GM3550			TMEM147	GM9104		
2310004N24RIK				EIF4H	LY6C2	NUP210	BRWD1			NDFIP1	ASAH1		
ARPC5L				NUCKS1	ZFP36L1	RPA3	RPL7A-PS3			RPS27A	RBBP6		
AC114648.1				LNP	JUN	EZH2	AI848100			RBMX	KHSRP		
SDF2				SCAMP2	ALKBH5	TAF12	TRIM24			PFKL	CTSB		

Differentially expressed genes in in-vivo sub-populations													
Th17/Th1-like memory		Th17/Th1-like effector-LN		Th17/Th1-like effector-CNS		Th17/Th1-like effector		Th17/pre-Th1-like effector		Th17 self-renewing		Th17 Dysfunctional /senescent	
THEMIS				GALM	MLL2	CLDND1	NIPBL			GM7536	PTEN		
S1PR1				2810407C02RIK	PDCD4	GLTP	RNASET2A			BSG	ZEB2		
IL12RB2				CENPL	INSIG1	NT5C3L	UBXN11			RPL26	ACOT2		
GM9234				UFSP2	RNF213	NXT1	LNPEP			RPL10A	ISCU		
B2M				DCTN3	GM8909	ILF2	RRM2B			MRPL55	SMAD7		
ZFP825				DKKL1	C030046E11RIK	DLGAP5	PRPF4B			MAP4K1	UBXN11		
GM5160				RPS21	PSAP	PSMB1	RNASET2B			RPL19	GP49A		
MIIP				HIST1H2BC	9930011J21RIK2	CENPE	PPIP5K1			CMAH	KIF21B		
NSD1				UBE2S	ARID1B	HSD17B12	HEXDC			LIMD2	RRM2B		
SATB1				RPL12	GM11127	H2-KE2	KDM5B			SETD8	PRPF4B		
				AP2S1	H2-Q2	SLC25A5	PAN3			TMEM176A	PLAC8		
					CDKN1B	HAUS1	NRP1			BTLA	CLINT1		
					NOTCH2	FGFR1OP2	RPL21-PS10			GM5451	PAN3		
					SLFN5	CYC1	RPS6-PS1			DGKA	MFSD11		
					SGIP1	COX6C	GM5921			CST3	NRP1		
					GM4070	UCHL5	RPGRIP1			RPL27	RPL21-PS10		
					BMP2K	PPP1R8	BTG1			ITGAV	RAB11FIP1		
					GM7030	UCHL3	CLASP2			HAVCR2	GADD45B		
					GVIN1	UBL4	LRRC8D			GM9846	BTG1		
					ZFP36	XRCC6	CAP1			MAPRE2	RNF149		
					LYZ2	YWHAE	LGALS3BP			TUBB6	LGALS3BP		
					H2-AA	HMGN5	RSBN1L			U2AF1L4	TNFRSF1B		
					CTSS	CIAPIN1	RPL7A-PS10			VAMP8	SKI		
					CD74	LSM4	JUND			DGAT1	SSH2		
						PFDN4	DOCK8			RPS6KA1	MXD1		
						POLE4	AP2A2			AC119211.2	IFI27L2B		
						ADH5	DYNC1H1			AW112010	GAS2L3		

Differentially expressed genes in in-vivo sub-populations													
Th17/Th1-like memory		Th17/Th1-like effector-LN		Th17/Th1-like effector-CNS		Th17/Th1-like effector		Th17/pre-Th1-like effector		Th17 self-renewing		Th17 Dysfunctional /senescent	
						DPYSL2	4632428N05RIK				CTSC		
						2900073G15RIK	GBP10				ANKFY1		
						DCPS	IFI27L2B				ANXA4		
						M6PR	GAS2L3				GM10362		
						RNPS1	TTF1				TLCD1		
						NFYB	ANKFY1				SYNRG		
						MRPS25	DNAJB14				ATN1		
						EFTUD2	GM10362				LY6I		
						HSPE1	TLCD1				SOD2		
						ESD	RAP2B				GBP7		
						MFF	DDX6				C330021F23RIK		
						GTL3	SYNRG				IL7R		
						AI314976	CYTH4				KTN1		
						SNX3	ATN1				PPT1		
						ATP5C1	TRPM2				ASH1L		
						PDHB	GM3222				NFIL3		
						H47	MTMR2				ADIPOR1		
						SNRPB2	SOD2				BTG2		
						NDUFAF2	KIF24				PPM1K		
						NUP214	C330021F23RIK				GM8225		
						SNAPC5	IL7R				H2-OA		
						HNRNPAB	MS4A4C				RUNX3		
						AHCY	MLL3				MYCBP2		
						LSM10	2810422J05RIK				SKIL		
						PAK1IP1	GBP8				SMG1		
						GM10736	PBXIP1				ABCG1		
						MRPL53	GM2058				RAB10		
						VDAC1	JHDM1D				AL732476.1		
						AP1S1	KTN1				IFRD1		

Differentially expressed genes in in-vivo sub-populations													
Th17/Th1-like memory		Th17/Th1-like effector-LN		Th17/Th1-like effector-CNS		Th17/Th1-like effector		Th17/pre-Th1-like effector		Th17 self-renewing		Th17 Dysfunctional /senescent	
						MAD2L2	MLL1				RPL21-PS11		
						PPID	ELMOD2				SEPP1		
						UBA1	ASH1L				SIK1		
						BLMH	ABHD2				TET2		
						TPD52L2	GM10313				4930470H14RIK		
						MAGOHB	ZCCHC6				SRRM2		
						28104 28I15RIK	BTG2				CD38		
						RUVBL2	DTX3L				SPTY2D1		
						FAM125A	PPM1K				RPL21-PS6		
						HAUS3	GM8225				NR4A2		
						CALM1	RUNX3				HMHA1		
						YARS	IGF2R				RPL29-PS2		
						VBP1	MYCBP2				RRBP1		
						0610037P05RIK	SKIL				RASSF2		
						CRBN	TRP53INP1				LILRB4		
						GM10076	SMG1				AHNAK		
						UBASH3A	ABCG1				PLEK		
						TSN	RAB10				FMO1		
						FAM33A	AL732476.1				FOSL2		
						POLA1	RPL21-PS7				TAX1BP3		
						SBDS	NPC2				MS4A6D		
						PSIP1	RPL21-PS11				BIRC6		
						OGDH	SIK1				MAP3K1		
						ARL1	AC163269.1				GM10800		
						PPIH	PPP1R15A				GM6109		
						ABCF2	TNRC6C				JUN		
						KIF15	2610036A22RIK				MLL2		
						CNPY4	TET2				JUNB		
						TIPRL	GBP6				INSIG1		

Differentially expressed genes in in-vivo sub-populations													
Th17/Th1-like memory		Th17/Th1-like effector-LN		Th17/Th1-like effector-CNS		Th17/Th1-like effector		Th17/pre-Th1-like effector		Th17 self-renewing		Th17 Dysfunctional /senescent	
						ACTN4	E430029J22RIK				FOSB		
						POLR3K	4930470H14RIK				CCL5		
						CORO1C	SRRM2				LGMN		
						SSSCA1	GM10718				APOE		
						SF3A3	IFI203				NOTCH2		
						SYTL3	RPL21-PS6				SGIP1		
						OXCT1	HMHA1				NR4A3		
						C330027C09RIK	MED13L				GM4070		
						WDR33	RPL29-PS2				BMP2K		
						SNRPA	CD274				SDC4		
						ORC4	RASSF2				AIF1		
						HIST1H4I	NCOA3				LYZ2		
						ACSL5	KLHL24				H2-AA		
						NRF1	STAT1				GRN		
						9130401M01RIK	AHNAK				C1QB		
						MRPL11	ARID5B				FCER1G		
						CINP	FMO1						
						BAZ1B	GM10291						
						LUC7L3	RPL17-PS3						
						ZCCHC17	SLC39A1						
						PPIL1	GM10327						
						MRPS36	BIRC6						
						GABPB2	IRF2BP2						
						THOC4	GM5507						
						2310036O22RIK	MAP3K1						
						HJURP	GM10800						
						IFT27	TOB2						
						NOP58	GM6316						

Differentially expressed genes in in-vivo sub-populations													
Th17/Th1-like memory		Th17/Th1-like effector-LN		Th17/Th1-like effector-CNS		Th17/Th1-like effector		Th17/pre-Th1-like effector		Th17 self-renewing		Th17 Dysfunctional /senescent	
						SLC9A3R1	KDM6B						
						SLC35D1	GM6109						
						SLA2	LY6C2						
						EXOSC5	ZFP36L1						
						SLC1A5	JUN						
						NUP93	ALKBH5						
						PPP1R11	MLL2						
						IMPDH2	JUNB						
						L7RN6	PDCD4						
						PSMD13	MNDA						
						RNASEH2C	INSIG1						
						CRMP1	RNF213						
						UTP3	GM8909						
						UXT	C030046E11RIK						
						CDK5RAP2	PARP4						
						CDKN2AIPNL	PSAP						
						DNMT1	9930011J21RIK2						
						RAD21	ARID1B						
						ADPRH	GM11127						
						MEMO1	H2-Q2						
						ITPA	CDKN1B						
						RNF7	NOTCH2						
						EXOSC3	SLFN5						
						PRPF38A	SGIP1						
						CCDC124	GM4070						
						MRPS6	PCF11						
						MKKS	BMP2K						
						TRIM28	GM7030						
						CCR8	GVIN1						
						POLR2H	ZFP36						

Differentially expressed genes in in-vivo sub-populations													
Th17/Th1-like memory		Th17/Th1-like effector-LN		Th17/Th1-like effector-CNS		Th17/Th1-like effector		Th17/pre-Th1-like effector		Th17 self-renewing		Th17 Dysfunctional /senescent	
						PUF60	LYZ2						
						LTA4H	H2-AA						
						TMED2	CTSS						
						NEBL	FCER1G						
						HCFC1							

[00265] **Table 7.** Listed is the fold change (defined as the expression level of the knock out cells divided by the expression level of corresponding wild type or littermate controls) of all significantly differentially expressed genes (Experimental Procedures) for a given experimental condition. Experimental condition information includes; the knockout mouse ($GPR65^{-/-}$, $PLZP^{-/-}$ or $TOSO^{-/-}$), differentiation condition (TGF-β1+IL-6 or Il-1β+IL-6+IL-23), and the duration of differentiation before harvesting for RNA-seq analysis (48h or 96h). All differentiations were conducted as for the single cell *in vitro* data.

Differentially expressed genes for GPR65 -/-, PLZP-/- and TOSO-/- Th17 cells											
GPR65-KO-IL1B+IL6+ IL23-96h-1		GPR65-KO-TGFB1+IL6-96h-1		PLZP-KO-IL1B+IL6+IL23-48h-1		PLZP-KO-TGFB1+IL6-48h-1		TOSO-KO-IL1B+IL6+IL23-96h		TOSO-KO-IL1B+IL6+IL23-96h	
Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)
CT025533.1	638.963	LY6G	72.0601	CR478112.1	4828.97	AC112970.1	997.832	AC090432.1	19.4613	LY6G	20.5027
GM11042	219.403	CD3G	35.7993	AC163094.2	705.836	AC163330.1	0.00100217	GM10999	17.1617	GM10139	0.0744158
AC163330.1	57.6454	H2-Q8	20.2139	GM11035	469.257	AC118017.2	691.521	FAM132A	0.0731972	CCDC56	12.7227
GM10695	52.9557	ROMO1	18.4077	AC090563.1	181.836	GM10974	0.00177299	NDUFC1	12.6321	GM10192	12.3271
IL17F	20.8104	ATP5J	16.4856	GM10774	127.093	GM10774	0.00786822	IL24	0.0840608	IL24	0.0852024
GM11035	15.2049	MPP1	15.7176	GM11074	86.5719	GM11074	120.52	A2LD1	9.99009	PAM16	0.0870993
2210012G02RIK	14.137	UFM1	14.9395	GM11032	0.0235315	SND1	114.79	2010107H07RIK	9.64576	HMGA1-RS1	0.0887043
GM10222	12.7776	LY6I	14.4088	CISD3	0.0267441	DEDD	0.00957397	NHEJ1	9.40856	UCKL1	0.09253
S100A1	0.0863747	LY6C2	14.2462	IFI27L2A	29.7388	NUDT1	59.2046	RNF121	7.96782	PIH1D1	8.8086
SLC15A3	11.4418	GM10774	13.9351	TBC1D17	0.0363317	GM10222	0.017264	GM10495	7.43442	GNAQ	8.75423
MUTYH	10.8353	LY6C1	12.7774	AL732569.1	0.0430873	GM6293	0.0203867	NTAN1	0.152127	CCDC9	8.65022

Differentially expressed genes for GPR65-/-, PLZP-/- and TOSO-/- Th17 cells											
GPR65-KO-IL1B+IL6+ IL23-96h-1		GPR65-KO-TGFB1+IL6-96h-1		PLZP-KO-IL1B+IL6+IL23-48h-1		PLZP-KO-TGFB1+IL6-48h-1		TOSO-KO-IL1B+IL6+IL23-96h		TOSO-KO-IL1B+IL6+IL23-96h	
Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)
TEAD2	10.3068	IL17F	12.2224	EWSR1	21.7177	H2-Q8	48.6847	LSMD1	0.153423	MYCBP	0.12853
GM10490	9.71233	CCL5	0.0827434	AC121566.1	0.0476118	GM11032	47.0127	MED6	6.4984	FRG1	0.132368
IFFO2	8.85699	SGK1	11.4417	LIN37	0.052081	ATOX1	45.8514	MED7	6.43439	BCCIP	7.46098
TBCB	8.74941	2010107E04RIK	11.366	FAM36A	19.0056	AC121566.1	44.8555	CTSE	6.37075	0610037L13RIK	0.141592
AC102609.1	8.69175	BANF1	11.1666	GM10721	18.9873	PFN1	37.3129	TM2D3	0.160132	RABL3	6.66246
CATSPER4	8.30689	TIMM8B	11.0647	AC132391.1	0.0537352	AL845291.1	0.0348296	CCDC101	6.24144	COX6B2	6.63466
CCBL2	8.27697	VPS36	10.7432	AC163993.1	0.0554745	2310004I24RIK	0.0365274	SLC12A4	6.04047	MRPS30	0.150918
GM11074	8.19721	GAA	9.86035	2310030N02RIK	18.0003	SNX14	0.0369612	SAP30BP	5.90169	E130306D19RIK	6.53277
LINS	8.16618	COX7A1	9.68942	GM11167	17.8147	STRA13	26.8007	UBASH3B	5.82363	KLHDC1	6.48342
1700029F09RIK	8.12329	AC087540.1	9.67077	GM10106	17.065	1700054O19RIK	25.0357	8430419L09RIK	5.78915	FBXO9	6.32457
MCFD2	0.123505	NDUFC1	9.66605	CCDC34	16.9601	4930423O20RIK	24.9749	CT030170.2	5.45632	TMEM209	6.15855
TMEM33	7.73635	PPP2R5C	9.64143	AC131780.4	16.6801	1110051M20RIK	0.0422463	GOLGA1	5.358	FAM189B	0.164908
4930425F17RIK	7.56473	LY6A	9.61981	LYRM2	0.060334	GCDH	0.0422702	SRSF9	5.31367	SETD4	6.03598
CLEC12A	7.52948	IFI27L2A	9.53003	WBP11	16.5356	ARRDC1	22.4399	ZMPSTE24	5.2634	H2-Q8	5.89529
MCTS1	7.4317	LSMD1	9.38183	CES5A	16.1643	PAM	22.1904	TOMM5	0.190338	GM7367	5.88087
2010107G23RIK	7.38844	NGFRAP1	9.37045	MLLT10	16.0522	MED27	0.0482159	TSC22D1	5.2077	MRPS36	5.83917
UQCC	7.377	COX5B	9.302	AC125405.1	15.7217	NMNAT3	0.048537	PGLYRP1	5.16763	LEPREL1	5.77011
BCCIP	7.04936	GIMAP3	9.18091	GM10800	15.3943	NDUFS5	0.0489343	PACSIN3	5.13269	ATF7IP	0.177348
XPA	7.02998	SPAG7	9.17137	RWDD1	0.066117	PSENEN	20.1098	ZFP688	5.09008	WARS	0.180546
RAB34	7.02805	GMFG	9.11872	AC131780.2	15.0009	D8ERTD738E	0.0512351	PPAN	0.196514	ZCCHC17	5.50318
DFFA	6.96773	TFG	8.71842	GM10720	14.7899	MRPS23	18.1426	1700120B22RIK	5.073	A530032D15RIK	0.182743
GNG12	6.94052	XPA	0.117077	FANCE	14.53	POLR1D	17.8188	ZFP523	5.04854	HINT2	5.43973
ARL3	0.146797	MRPL2	8.47401	UBE2A	0.0707408	GMFG	17.5188	BSDC1	0.198872	GM1968	5.3797
TDP1	6.76641	CR974466.3	8.47128	CKLF	13.7256	MRPS5	17.1812	WDFY1	5.02185	GTPBP6	0.189111
SPG20	6.7321	AC118017.2	0.11863	PRNP	13.6676	GM10311	0.0585203	MUP11	4.90405	TMUB1	5.23194
CYTH1	0.150294	RPS6KA3	0.119074	LYRM7	13.446	RNASEK	16.9543	DIABLO	0.205542	BCL2L12	5.09994
GM10238	6.62969	PAICS	8.38958	GM10718	13.4418	2410015M20RIK	0.0598462	NUMB	4.81845	DOK2	0.196929

Differentially expressed genes for GPR65-/-, PLZP-/- and TOSO-/- Th17 cells											
GPR65-KO-IL1B+IL6+ IL23-96h-1		GPR65-KO-TGFB1+IL6-96h-1		PLZP-KO-IL1B+IL6+IL23-48h-1		PLZP-KO-TGFB1+IL6-48h-1		TOSO-KO-IL1B+IL6+IL23-96h		TOSO-KO-IL1B+IL6+IL23-96h	
Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)
HNRNPR	6.59627	TSPO	8.35239	A830010M20RIK	13.4368	42256	16.5496	HMGN3	4.79371	GM10416	5.05178
DRAM2	0.153594	GM10416	8.2616	GM10719	13.3625	MRPS18A	15.8322	FBXO6	0.210289	ACER2	5.04034
1810020D17RIK	0.154415	GM5215	8.11489	AEN	13.0909	RFC5	15.7095	HMGA1-RS1	0.213347	PYGO2	0.198435
CHCHD8	0.159584	NRBP1	7.90454	GM6396	12.781	LSM12	0.0640507	LACTB2	4.66939	CLEC16A	5.02465
LZIC	6.22633	GM7713	7.86697	GM10717	12.3342	1810035L17RIK	0.0643269	1110051M20RIK	4.65484	ACSL1	4.95276
PSMD13	6.14569	ATRX	7.85905	2010107H07RIK	11.8908	SPC25	15.407	SERTAD3	4.65467	MLEC	0.204293
PPDPF	6.0768	CCDC109B	7.85491	CCL3	11.8036	ORC5	15.3066	HRSP12	4.64663	ATPAF2	0.206001
TCF4	0.164742	PAPOLA	0.128481	ZFP668	0.0856954	GM11011	0.0660117	HIAT1	4.60585	FBXW20	4.84791
FASTK	6.03114	FUNDC2	7.75996	DPH3	11.5057	IPO9	0.0670124	IL17A	0.217419	9430002A10RIK	4.83616
SAFB2	5.93824	LEPREL1	7.71451	MRPL52	11.4306	ANAPC13	14.8538	UBAP1	4.57328	AKAP9	0.206832
WDR54	5.77242	DBI	7.66514	POLR2H	0.0878308	2810021J22RIK	0.0687575	CIC	4.56612	CBX5	0.208348
MED28	5.70363	PSMG4	7.54057	PIK3R1	0.0898843	PDCD2	0.06968	MMP16	4.56577	TULP4	4.79559
MOSPD3	5.68319	RGS19	7.53778	AC025786.1	0.0900128	PAF1	14.2702	PQBP1	4.55228	DOCK7	4.79084
RENBP	5.65082	AC112970.1	0.132869	MAPK3	0.0902236	CKLF	0.0702488	SEC61A2	4.54133	CRTC1	4.77533
ALDOB	5.63858	GM5830	7.43749	HMGXB4	11.0516	SLC39A14	14.0594	RRP8	4.49549	AC154631.1	4.75579
HELLS	5.48094	POLR2J	7.35784	MRPL54	0.0906724	AC132837.1	13.9914	IFT140	4.48953	2310003F16RIK	4.74871
GM11444	5.45078	TARBP2	7.2882	FBXL12	0.0941317	EXOSC3	0.0716036	CCDC109B	0.224915	DEB1	4.72541
TNFRSF22	5.41408	RSRC1	7.25371	4930431F12RIK	10.5765	ZCCHC7	13.8323	DSN1	4.43985	DFFA	4.66373
AC114625.1	5.37391	HSCB	7.21689	AC127590.1	10.5317	2310061C15RIK	13.8171	PHF20	4.43522	4930431F12RIK	4.66082
GM6003	5.33337	0610037L13RIK	7.19607	SEMA4F	0.0952178	BDH1	0.0726258	NPM3	4.38892	LHPP	4.60268
GALE	5.25036	NOP56	7.16844	MPND	0.0956693	HACL1	13.7256	RCAN3	4.37872	CSNK1G1	0.217973
GTDC1	5.21537	PIGK	7.16261	SLCO3A1	0.0961635	CCNE2	13.6676	MKNK1	4.3396	BC049349	4.57194
PHF21A	0.192406	RPL21-PS6	7.16	OPCML	10.354	GM9758	0.0731656	EXOC6B	4.31007	DRAM2	0.219853
ARHGAP4	5.17683	ANAPC13	7.05292	ZCCHC10	0.0984483	TMEM107	13.5403	ENPP2	0.233365	PCID2	4.5141
DLC1	5.16934	PDRG1	7.00334	AC155646.1	0.0995017	CCDC55	13.4463	ZC3H10	4.26288	GRAMD1B	4.50702
FMNL1	0.199325	ARRDC1	6.94928	PPP2R2B	9.89	GRCC10	13.3862	PIGF	0.236674	RUNX2	0.222122
PUSL1	4.98071	NAP1L4	6.94591	PDIK1L	9.83923	SCP2	13.1902	LY6C2	4.21301	GM5900	4.50071

Differentially expressed genes for GPR65-/-, PLZP-/- and TOSO-/- Th17 cells											
GPR65-KO-IL1B+IL6+ IL23-96h-1		GPR65-KO-TGFB1+IL6-96h-1		PLZP-KO-IL1B+IL6+IL23-48h-1		PLZP-KO-TGFB1+IL6-48h-1		TOSO-KO-IL1B+IL6+IL23-96h		TOSO-KO-IL1B+IL6+IL23-96h	
Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)
2610030H06RIK	4.94004	POP5	6.91452	EMP3	9.79589	GM16372	0.0762153	TRNAU1AP	0.237682	1200016B10RIK	4.49989
MECR	4.89871	0610037P05RIK	6.90089	MRPS12	9.73996	RDM1	0.0768956	TRMU	4.20472	GM16380	0.222513
TNFAIP8	0.204512	CKS1B	6.88764	GM10192	9.67502	MRPL23-PS1	12.9196	HIRIP3	4.18487	TRAFD1	4.49287
HRSP12	4.8833	A930005H10RIK	6.87052	GM10801	9.62219	ENTPD1	12.8249	2210016L21RIK	4.16073	FAM165B	4.45428
RHOQ	0.207277	GM10506	0.145568	GM10715	9.35018	INSL6	0.0791878	MDN1	4.15504	TMEM5	4.44872
GPATCH8	0.20735	CLK1	6.80099	1700026D08RIK	9.29877	AC125405.1	12.6184	SELK	0.242008	AIM1L	4.41539
IFNAR1	4.82106	PRDX4	6.78434	GM10842	0.107607	MRPL19	12.6066	FBXL12	4.06574	FAM129B	0.227632
TAF12	0.207562	WDR75	6.78281	XRCC4	0.10761	GNGT2	12.4445	LLGL2	4.0643	NUP85	4.36604
RASAL3	0.207598	SMEK2	6.76563	IL9	0.109536	AW112010	12.3828	MZT2	4.03573	HIST2H3B	4.33905
CCL4	4.78481	TMEM85	6.7621	A630001G21RIK	0.109616	AC102609.1	12.2887	MAD1L1	0.247878	FAM175A	0.231044
FAM69A	0.209402	DPM2	6.6864	ENTPD1	9.07642	ATP8B2	0.0822643	ZCRB1	0.247973	YAF2	0.232974
ME3	4.7724	UBL4	6.67639	IER3IP1	9.0603	GNG12	12.1127	DEB1	4.02094	GM10355	0.23349
MPND	4.77191	CLEC16A	0.151024	AC122006.1	8.8707	TMEM222	0.0832766	KLRC1	3.98576	NAB1	0.233585
NMB	4.67907	MPHOSPH8	6.58917	MED7	0.11446	EDF1	11.9815	HIBCH	3.97739	ADCK3	0.233826
SLC1A5	0.216752	PCMTD1	0.152472	MMADHC	0.115059	TIMM10	11.9333	A530032D15RIK	3.96486	PEX11B	0.234331
CIAPIN1	4.5998	PREB	6.55723	NSUN3	8.60642	BC057079	11.8981	MTX1	0.252901	BTBD10	0.235263
2810432D09RIK	4.56386	GM8394	6.54589	PHF10	0.116796	GM11110	0.0850407	UNC45A	3.93916	ACNAT1	4.24866
POLR2F	4.54368	FKBP3	6.5407	AC131780.1	8.51373	SLC35A1	0.0854511	KIT	3.9252	HIST1H4F	4.24366
LSM4	4.53477	FAM165B	6.54065	SNAP47	8.47662	POLR2I	0.0856321	NPAT	3.8405	CYSLTR1	0.235958
PNRC1	0.222174	BCCIP	6.50692	RAB11A	8.44774	UBE2B	11.6779	MLLT10	3.82477	PSMB9	4.21107
PUF60	4.47952	PPIG	6.50177	EXOC4	8.44629	ASAH1	0.0863792	1110005A03RIK	0.262623	NEBL	0.237823
1110001J03RIK	4.47334	NSMCE4A	6.47025	HIST1H2BH	0.119207	MRPL28	11.5026	DPY19L3	0.263324	HRSP12	4.20275
MLLT10	0.223694	CEPT1	6.39179	TRAFD1	8.36382	PCID2	0.0870048	CDKAL1	0.263647	PDIK1L	0.238132
0610010O12RIK	4.42709	SPCS1	6.36876	ATF7	0.119705	SRP9	11.4865	0610010O12RIK	3.79286	GM10482	0.238435
GM10482	0.226172	WDR61	6.36703	4930470H14RIK	8.2969	NISCH	11.4306	C1D	0.263877	POLR2I	4.19095
SLC25A11	4.39262	FANCC	6.33444	SOD1	8.20086	GM2178	11.3855	MANBA	3.78609	RPL21-PS4	0.239361
HDAC8	0.230041	RAD23A	6.20533	1700064H15RIK	0.121984	FUBP1	11.3077	SIN3A	3.78214	TMEM48	0.239496

Differentially expressed genes for GPR65-/-, PLZP-/- and TOSO-/- Th17 cells											
GPR65-KO-IL1B+IL6+ IL23-96h-1		GPR65-KO-TGFB1+IL6-96h-1		PLZP-KO-IL1B+IL6+IL23-48h-1		PLZP-KO-TGFB1+IL6-48h-1		TOSO-KO-IL1B+IL6+IL23-96h		TOSO-KO-IL1B+IL6+IL23-96h	
Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)
THAP7	4.34098	RNF5	6.20083	FASTK	0.122115	FANCE	11.298	NASP	3.76321	CLN3	0.240092
ECM1	4.33421	CKLF	6.13147	EVI2A	8.17236	RAB8A	11.2824	IQSEC1	3.7207	GABPA	0.240205
JUP	4.3205	CDK5RAP3	6.11741	GALK2	8.15188	RPL30-PS6	0.0889991	WIBG	0.270456	ITGAV	0.240239
1110004E09RIK	4.31718	DCAF17	0.163649	AC131780.3	8.14542	FAM64A	11.2121	RALGPS1	3.69553	AC114625.1	4.15154
TOR2A	4.30601	U2AF1L4	6.09837	DNAJC24	0.122813	TOR1AIP2	0.0899992	ARMC7	3.68615	MBTPS2	4.12905
ZFP54	4.29205	HMGB1	6.04354	4930534B04RIK	8.12667	PSMG3	11.0633	SNRPC	0.271861	1810074P20RIK	0.242547
GM6990	0.233534	PSMD6	5.99621	2310045N01RIK	0.123579	ALDH7A1	10.9234	CASP9	3.67579	CSNK1E	4.11864
AC155646.1	0.233606	AC132391.1	5.97323	SERGEF	8.03129	TSPAN32	10.724	KLHL15	3.67081	SELENBP2	4.11852
MTX1	4.27561	LY6K	5.94919	GGNBP1	0.124804	SSBP2	10.6665	MBTD1	3.67015	STYK1	4.09169
AL845291.1	4.2534	CD209C	0.16859	5730437N04RIK	7.93924	PPAPDC1B	0.093832	1110065P20RIK	3.66259	UFSP2	0.24606
GTF2H1	0.235342	DLG4	5.93042	DEB1	7.91466	UBL4	10.5765	NENF	0.273259	PHLDA3	0.24686
IER3	0.235664	VILL	5.92894	MTHFS	7.87981	MED28	10.5684	EIF4ENIF1	3.64441	KLC1	0.248587
AKTIP	0.235925	WDR13	5.90818	GM10576	7.8766	TRIM28	10.5317	ZFAND3	3.64348	PRL8A1	4.02006
WBSCR22	0.241006	HEMK1	5.89641	TTC39A	0.127576	GIMAP7	0.0953338	PDLIM1	0.276107	GM12166	4.01965
LY6K	0.241404	SLC35A1	5.83336	COX7A1	7.82902	IVD	10.4803	1110007A13RIK	3.61404	DUSP22	0.249121
BRIX1	4.14223	NDUFB6	5.81576	HSPA4	0.128114	HIST1H4C	10.4707	THNSL2	3.61171	CCDC23	4.00206
DNAJC24	4.12988	PRPS1	5.7819	RAB9	0.128579	AC025786.1	0.0955993	MYO1B	3.61009	NT5DC1	0.250004
ZMYND8	4.11359	IFI27L1	5.75922	DHODH	0.128681	UBR4	10.3681	ECE1	0.27729	RNF185	0.25078
RNF141	0.244327	ZMPSTE24	5.69972	PEX19	7.69973	PIGZ	0.0967107	SIVA1	0.277827	METTL1	3.97941
DDX49	4.07132	DEK	5.68906	DHPS	7.60305	MAGOHB	10.2556	TIPIN	0.277841	POLR3G	3.95579
SPAG5	4.06089	SERPINB1A	5.68805	CAP1	0.131667	KCTD9	10.2384	PSIP1	3.59527	H2-Q6	3.95138
2010107H07RIK	0.246574	KCNAB2	5.68376	AC102876.1	7.47393	CNDP2	10.2284	USP11	3.59442	GM10495	0.253919
TRIAP1	0.247873	NPRL2	5.65002	SSSCA1	7.4544	AC163993.1	10.1311	BATF3	0.278394	RAC3	0.256158
DHDPSL	4.03073	9030619P08RIK	5.64316	2010305A19RIK	7.43256	POP1	0.0992703	RALY	3.58739	1700054O19RIK	3.89993
CCDC130	4.01695	MAPRE1	5.63223	NDUFB2	7.39808	DHDDS	10.0429	MTHFS	3.5871	TMEM38B	3.89356
CPSF3L	4.00615	UFD1L	5.6314	GAA	7.38646	YIPF1	10.0231	3110001D03RIK	3.56035	BCAT2	0.257108
GUK1	4.0013	IL2	5.63133	HIST1H2BN	7.29384	RBM4B	9.97471	1500002O20RIK	3.55877	BATF3	3.85665

Differentially expressed genes for GPR65-/-, PLZP-/- and TOSO-/- Th17 cells											
GPR65-KO-IL1B+IL6+ IL23-96h-1		GPR65-KO-TGFB1+IL6-96h-1		PLZP-KO-IL1B+IL6+IL23-48h-1		PLZP-KO-TGFB1+IL6-48h-1		TOSO-KO-IL1B+IL6+IL23-96h		TOSO-KO-IL1B+IL6+IL23-96h	
Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)
TMEM85	3.98691	COPS7B	5.61304	DNAJC19	7.28404	MED24	9.95916	D6MM5E	3.55478	2310061I04RIK	3.85025
ACTRT2	3.96478	TEX14	0.178636	CLN3	0.1375	R3HDM2	9.89625	RIT1	3.55126	BMYC	0.260139
PPIL3	0.252903	42262	5.5932	PUS7L	7.24213	MTBP	9.84994	SYTL3	3.53773	CBX7	0.26126
RSRC1	0.253147	RNASEH2C	5.58174	FAM188A	7.23481	PUS7L	9.83923	GGT1	3.5364	EGFL7	0.261514
ZFP68	3.9485	PPOX	5.56957	IL3	0.139557	TNF	0.101767	ETFB	0.283229	AC125405.1	3.81229
SEL1L	3.94649	NUDT2	5.56538	GGNBP2	7.15053	SURF2	0.102144	GGPS1	0.283765	NDUFB2	3.79385
SLC12A6	3.93412	CD27	0.180344	ZFP353	7.12168	TFPT	0.10259	ZFP58	3.51272	ING3	0.264762
YBX1	0.254705	MOCS2	5.52865	VKORC1	7.08262	CERKL	0.10267	RTEL1	0.285742	THG1L	0.265805
ARID5A	3.92361	RGS1	5.52352	POP5	0.142477	GM10506	9.73403	BCL3	3.49539	TMEM147	3.74181
CCDC52	0.254901	LXN	5.51705	TXNL4A	0.142821	TAF12	0.102745	MFSD11	0.287788	ZCCHC10	3.73683
DULLARD	3.91929	PTPN6	5.47051	ZCRB1	0.142824	ALG5	9.71286	SPSB1	3.47315	POLR3GL	0.267958
CD209C	3.91841	GM10999	0.183075	GM14420	6.99722	GM7075	9.58803	PRL7B1	3.46403	CD72	3.72884
TTC33	3.90685	PES1	5.45618	AL732476.1	6.98499	FAM96B	9.58165	MAPRE2	0.288947	1110051M20RIK	3.72461
RBKS	0.256393	SNRPD2	5.44382	PARS2	0.143242	MYLPF	0.104864	CHAF1B	0.289552	MBD6	3.71439
PARP3	3.89675	ANKRD37	5.44157	2610044O15RIK	6.96583	KDM4C	0.10517	AP2A1	3.43984	RNF38	3.70742
FAM71F2	0.257088	CDCA2	0.183865	AC117259.1	6.9419	CTSW	9.48133	SCYL3	3.41864	GGT7	0.270077
2810405K02RIK	3.88632	E130306D19RIK	5.42898	GRSF1	6.93192	5730469M10RIK	9.47232	LRIG1	3.41702	C630004H02RIK	0.271104
GM10720	3.8603	WDR83	5.3926	BNIP2	0.144906	SH3KBP1	0.105824	RFK	3.40993	ORC5	3.67903
CSE1L	3.85295	EIF2B2	5.37028	PNKD	6.87634	FBXL17	9.42315	MFSD10	3.40474	PHTF2	3.67589
ANKRD40	3.85008	MAF1	5.35371	GM10203	6.8613	A330049M08RIK	9.37675	H2-Q8	3.40278	4930425F17RIK	3.67327
MCART6	3.80306	2310003L22RIK	5.35207	SLC7A4	0.146352	MAGED2	9.37124	MAFK	3.40214	TMEM199	0.272294
1700093K21RIK	0.263331	IFNAR2	5.34873	POLR2I	6.82307	NCF4	0.106786	HIATL1	0.29417	POLRMT	3.66929
MYC	3.79416	EIF2S3Y	0.187055	TMEM223	6.78242	SNRNP25	9.33426	GM8923	3.39724	CNOT6L	0.273167
AC131780.2	3.77596	ASNS	5.33487	TMSB15B1	0.148173	ABAT	9.31561	ETV6	0.294593	EXOC5	0.274182
GM10719	3.75154	PDLIM2	5.33001	R3HCC1	0.148619	CD3G	9.29877	2310079N02RIK	0.294964	ARL5C	3.63451
MGAT4C	3.74852	NAA16	5.32749	TMUB1	0.150806	PIGX	9.28618	CC2D1B	0.296056	PHYHD1	3.61167
MTA2	3.73852	GSTO1	5.31579	CWC27	0.151624	2410017P09RIK	0.107927	MTCH2	3.37335	BC055324	0.277808

Differentially expressed genes for GPR65-/-, PLZP-/- and TOSO-/- Th17 cells											
GPR65-KO-IL1B+IL6+ IL23-96h-1		GPR65-KO-TGFB1+IL6-96h-1		PLZP-KO-IL1B+IL6+IL23-48h-1		PLZP-KO-TGFB1+IL6-48h-1		TOSO-KO-IL1B+IL6+IL23-96h		TOSO-KO-IL1B+IL6+IL23-96h	
Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)
MAGOH	0.267975	GM10120	5.28972	B4GALT3	0.15171	1700128F08RIK	9.20197	POU2F2	0.296735	CHAF1B	3.59632
TSPAN4	3.73086	MKNK1	5.25695	AC142104.1	0.152305	PTPN2	9.18199	WWOX	3.36062	ARIH1	0.278113
GM11167	3.72546	IFRD1	5.24842	ACIN1	0.153182	POLR2A	0.108941	SUFU	3.35608	ROBLD3	0.280033
CLYBL	3.71736	SDCBP	5.24642	SYTL3	0.153535	CCDC9	9.15689	NMI	3.34913	TNFRSF14	3.57066
GM10717	3.71066	UBE2NL	5.24562	DNASE1	6.49588	PTPMT1	9.1473	WDR11	3.34188	FUCA2	0.280098
ACTR1B	3.70995	TMEM60	5.23158	GM16372	0.154587	TMEM85	9.07642	GM16416	3.33749	GM11275	3.54481
BOLA3	3.70413	GM8909	5.22564	MLLT3	6.46887	PLXND1	9.04785	MRPL27	3.3349	HERC3	3.53754
CEPT1	3.67288	GM9762	5.20942	RBM22	6.4636	GM8054	0.110711	COPZ2	3.33482	PSMD14	0.283245
LUC7L	3.67232	LZIC	5.1977	FKBP1A	0.155468	SEPSECS	0.110944	ZFP318	3.33439	TTC7	0.284516
LAIR1	3.67033	INSL6	5.1949	PLA2G16	6.4322	COX15	8.9846	APPL1	3.33289	AGPAT2	3.51269
MRPL17	3.67024	GM10925	5.17227	TMEM126A	0.156135	P4HA2	8.97898	TCF4	3.33168	AC152721.1	3.50828
ASAH1	0.273768	CDK11B	5.16631	METTL11A	0.156575	EED	8.97733	TMEM126A	3.3264	RAD23A	3.49641
SMARCD2	3.64906	ANXA5	5.1584	RPL21-PS7	6.35423	MAT2B	8.96162	VWA5A	3.32388	ADAR	3.48718
GM10718	3.64245	GPN3	5.13763	ZFP280C	0.158142	FUCA2	8.94968	MED10	0.300958	STX4A	0.286919
RUVBL2	3.63161	FXR2	5.13594	AC132837.1	6.32254	NDUFB4	0.111736	COX19	3.32126	LIAS	0.287101
TTC35	3.63095	TMBIM4	5.13572	MAP3K5	6.30122	ACAD8	0.112277	GM13147	3.31654	2210016L21RIK	3.47446
TPST2	3.62056	ELF2	5.09157	IL24	0.159647	WBSCR22	8.88424	IRF1	3.31264	IL15RA	3.47047
GM7713	3.60269	PDCD5	5.06988	SRCRB4D	0.159836	SMS	0.112965	BUB1	3.30274	9030617O03RIK	0.289013
CCDC107	3.59403	TTC4	5.05311	HCST	6.24208	NBR1	8.8219	LYAR	3.29979	TRPC2	3.45442
GOSR2	0.278504	MED6	5.05201	GM6096	6.23421	INSIG2	8.80943	KLHL22	3.29245	MPP6	3.44593
1110003E01RIK	3.58679	PTP4A2	5.02647	RRP8	0.160473	TMEM147	8.8022	TSR2	3.28397	TEAD2	0.290337
FAM175B	0.281168	FBXO6	5.02333	ALKBH3	0.1605	AC160471.1	0.1139	WFDC12	3.27084	DYNLT1B	3.43878
CCNDBP1	0.281381	KCTD13	0.199541	KLHL15	6.22658	POLE3	0.11417	METTL8	3.26973	HIST1H4C	3.43302
HCST	3.55104	AA467197	5.00576	SLC15A2	6.215	CDC25B	8.73177	ST6GAL1	0.306454	BX679668.1	3.42595
ZMPSTE24	3.51831	CREM	4.97069	GMPPA	6.20253	MMP16	8.69991	CLEC16A	0.307168	AQR	3.42194
SUCLA2	0.284944	MYCBP	0.201252	GM10695	6.2007	CAR9	8.6955	FOXJ3	0.308099	GLRX	3.42051
IRF5	3.50011	CUL1	4.96085	SPATA24	0.161365	4930425F17RIK	8.65193	MEN1	3.23788	AW112010	3.3997

Differentially expressed genes for GPR65-/-, PLZP-/- and TOSO-/- Th17 cells											
GPR65-KO-IL1B+IL6+ IL23-96h-1		GPR65-KO-TGFB1+IL6-96h-1		PLZP-KO-IL1B+IL6+IL23-48h-1		PLZP-KO-TGFB1+IL6-48h-1		TOSO-KO-IL1B+IL6+IL23-96h		TOSO-KO-IL1B+IL6+IL23-96h	
Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)
SNX11	3.49777	0610010K14RIK	4.95445	1700128F08RIK	6.18163	TEAD2	8.50191	CREB1	0.309475	CREB1	0.29428
CLN6	3.48154	RARS	4.95308	PRMT1	0.163087	TRPM7	0.117774	WDR91	3.22232	MKI67IP	3.38602
HEMK1	3.48009	MLX	4.94267	CENPT	6.11819	GM6396	0.118058	RPUSD3	3.19808	PACSIN3	3.3842
AC139042.1	0.287734	BC029214	4.92756	VAMP8	0.163897	IFI27L2B	8.45495	MAN1A2	3.19268	CDCA3	3.38042
MOSC2	3.46365	HCFC1R1	4.92552	FAM132A	6.1003	CLPP	8.44774	KDM4B	0.313314	SLCO3A1	0.295842
ADAMTSL4	3.44653	IDH3G	4.9087	ANKRD12	6.08043	4930431F12RIK	8.44682	RPS6KA1	3.18481	GABPB1	0.296055
ENTPD1	3.44534	GM11027	0.203723	MED12	6.05937	GM14326	0.118615	OPCML	0.314452	PPIL1	3.36445
HEATR7A	3.43505	CTSW	4.88659	USP48	0.165395	GM14399	0.118719	PSPC1	3.17489	GM13145	3.35155
RBMX2	0.291348	H2-T22	4.88453	ARF2	6.0275	TMEM41B	0.118866	GEMIN6	3.16916	HNRNPUL1	0.299266
H2-KE6	0.291727	UBAC1	4.88373	EP400	0.165979	FAM173A	0.118928	RSRC1	0.315601	HIST1H4D	3.33023
ACADM	0.292164	LRRC42	4.8802	SEC22A	6.02034	PARL	0.119111	PHRF1	0.316104	SRSF9	0.300436
ACAT1	0.29297	COMMD2	4.87675	POP4	0.167588	PFDN5	8.37525	MTMR2	3.16217	YIPF1	3.3284
TTC4	3.41016	UFC1	4.87361	CUL4A	5.96554	DUSP22	0.119563	CBX6	3.1587	1110034B05RIK	0.301017
MPP1	0.293362	EHMT1	4.85546	RHBDD2	5.95536	USP46	8.34247	NIPSNAP3B	0.316638	FXR2	0.301391
DNAJB6	3.40686	TSPAN32	4.85182	MED13	0.16848	RGS10	8.298	ZFP560	3.148	SEPP1	3.3074
PEX3	3.40459	NDUFA5	4.84453	GRIPAP1	0.168836	ZNHIT1	8.28155	PENK	3.14719	CTSE	3.30526
TM2D3	3.39927	SHBG	0.206932	POLB	0.168936	TMEM68	8.24996	DNAJC12	3.14601	GM16415	0.302846
ING3	0.294189	NDUFAF1	4.83055	AC117184.1	5.91584	MYD88	8.23977	ALAS1	3.146	LY6C1	3.29529
BC003331	3.3823	H2-Q2	4.82529	FAM45A	5.91331	ADRBK1	0.12153	ATHL1	3.1458	STT3B	0.304407
GM10721	3.37432	NAA38	4.82184	ATP8B2	5.90572	4933424B01RIK	0.121798	TRIM23	3.14356	ABHD10	3.28355
GM7204	3.36481	REXO4	4.81173	HIRIP3	5.88779	SQSTM1	8.20789	RPA3	0.31917	SKINT8	3.27906
GM11110	3.35826	ADRM1	4.75358	TPRKB	0.170218	GM11007	0.122161	MYSM1	0.319182	FANCE	0.304979
CLUAP1	0.298264	GEMIN7	4.74963	BRP44	0.170503	GM14430	0.122161	PI4K2B	3.12907	GSR	0.306238
CASP2	3.3411	GM16372	4.7397	GNAQ	0.170757	GM14432	0.122161	AKR1B10	0.320108	IL10RB	3.24925
PXT1	3.34066	INPP4B	4.73513	IMPA1	5.84994	GM2007	0.122161	AP1G2	3.11828	CCDC12	3.24378
IFT81	3.34042	MRPS33	4.73216	FXR2	0.170976	RNF214	8.18285	POLR2A	3.11809	GM9726	0.308711
INPP5B	0.299378	DRAM2	4.73138	ZCCHC11	0.172462	BBS5	8.18151	HOMEZ	3.10679	8430419L09RIK	0.308795

Differentially expressed genes for GPR65-/-, PLZP-/- and TOSO-/- Th17 cells											
GPR65-KO-IL1B+IL6+ IL23-96h-1		GPR65-KO-TGFB1+IL6-96h-1		PLZP-KO-IL1B+IL6+IL23-48h-1		PLZP-KO-TGFB1+IL6-48h-1		TOSO-KO-IL1B+IL6+IL23-96h		TOSO-KO-IL1B+IL6+IL23-96h	
Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)
KIN	0.30071	H2-Q7	4.72653	YY1	0.173566	PLA2G4C	8.17236	TCFE3	3.09882	0610011L14RIK	3.23236
GLUD1	3.30721	PHF5A	4.72302	ZFP687	0.173904	TIMM17B	8.16695	BLVRA	0.322872	RFC4	0.309401
ADCK5	0.30264	TANK	4.69966	ASAH1	5.73409	ITGB4	8.12447	PPP1R10	3.09185	CD69	3.21508
RANGRF	3.3018	STOML2	4.69151	1110018G07RIK	0.174577	STAM2	0.123152	FUCA1	3.0907	CCDC58	0.311259
OBFC1	0.303249	TBCA	4.6727	MT2	5.71646	RNMT	0.123306	WHSC1	0.323879	MCEE	0.311593
PREB	3.27993	GDI1	4.65362	COMTD1	5.68962	KIN	0.12338	CLYBL	3.0825	GM10576	3.20521
BRI3	0.304959	FAIM3	4.63947	SNAPC5	0.176233	GNG2	8.10003	SLC10A3	3.0807	RDH9	3.20507
GM5116	0.305402	ELMOD3	4.6303	EIF3G	5.66822	HSPB11	8.08051	PTPN5	3.08056	SDF2L1	3.20341
NINJ1	3.27329	ACBD5	4.62748	RASAL3	0.176644	TRMU	8.06635	MSL1	0.324943	FAM53B	3.19661
ANKRD5	3.27263	MCM7	4.61003	NBR1	0.176732	CCDC53	8.0588	PPP1CC	0.324984	ZFP687	3.19627
NAPA	3.27166	CDK1	0.217436	MKNK1	0.176948	ZFP120	0.124245	BLOC1S1	3.07662	TOR1AIP1	0.313011
PNKP	3.2648	LIMS1	4.5884	SFXN5	0.177569	2310045N01RIK	8.03129	RUNX2	0.325457	TSPAN5	0.313131
MRPL12	3.2625	CD53	4.58769	PRPSAP2	0.177826	WDR54	0.124942	ECHS1	3.06707	CASKIN2	0.313155
SMS	3.26183	PCNP	4.58745	CISH	5.61993	ZFP277	7.98906	BECN1	3.06641	TSEN34	0.313224
FTSJ3	0.306978	LTA	4.57147	WHSC1	0.178333	GM5830	7.96854	HINT3	3.06058	PLXNA3	0.313512
ALKBH7	3.25685	LST1	4.5675	PDCD6	5.60468	LST1	7.96261	RIN3	3.05864	DNTTIP2	0.314457
GTPBP2	0.307896	GM129	4.56142	A830080D01RIK	5.58829	GGNBP2	7.9446	STK38	3.03842	DEDD	0.314807
GIT2	3.24272	YWHAE	4.53645	GM9805	0.179155	STX4A	0.125957	CD74	0.329393	LSM7	3.17454
EDC4	3.23745	SNRPA1	4.52685	1700019E19RIK	0.179583	ITGA3	0.126311	RIPK2	3.03454	CD209C	3.17324
KIF18B	0.309756	CCDC55	0.221266	MTF1	0.179724	B9D1	0.126396	PLK4	0.330137	NPRL2	3.17313
MESDC2	0.311201	SIN3B	4.50388	NUDT1	5.54265	CASP8AP2	7.91164	NSUN5	0.330378	2010317E24RIK	3.16942
3200002M19RIK	3.21272	BUD31	4.49958	THAP3	0.180812	2310004N24RIK	7.8659	CCDC124	0.330746	COPS8	0.315522
TOP2B	0.311371	CAMTA1	0.222475	ABCB8	0.181246	GM10192	7.84084	UBE2L6	3.02117	1700128F08RIK	3.16253
DCTN3	3.20361	TSPAN31	4.49444	TOP2B	0.181282	EZH2	7.83613	D2WSU81E	3.02012	ALDH16A1	0.31636
2310061I04RIK	0.312456	GM7075	4.48701	AL844854.1	5.51339	MRPL47	0.127977	NUP85	0.331187	FBLIM1	3.15478
CTNNBL1	0.314975	NUP43	4.48542	SLMAP	0.181459	GM10576	7.80195	BC023829	3.01789	GM5356	3.15448
RASL2-9-PS	3.17011	TMEM223	4.47551	POLD3	0.182126	GIMAP5	7.78292	CLTC	3.00802	TRADD	0.317215

Differentially expressed genes for GPR65-/-, PLZP-/- and TOSO-/- Th17 cells											
GPR65-KO-IL1B+IL6+ IL23-96h-1		GPR65-KO-TGFB1+IL6-96h-1		PLZP-KO-IL1B+IL6+IL23-48h-1		PLZP-KO-TGFB1+IL6-48h-1		TOSO-KO-IL1B+IL6+IL23-96h		TOSO-KO-IL1B+IL6+IL23-96h	
Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)
IDH1	0.31565	0610007C21RIK	4.47073	SCLY	5.46754	MAPKSP1	7.76362	COG6	3.00697	EBNA1BP2	0.317965
KIF3A	3.16623	ZFP68	4.46896	JKAMP	0.183242	MFHAS1	0.129244	2310039H08RIK	0.332595	ABCC1	0.318111
LSM12	3.15031	CD2	4.46617	RFT1	0.183557	ARHGAP23	7.71607	MNT	3.00475	PDCL	3.13806
GM221	3.14642	NUSAP1	4.46516	C1D	0.184599	STARD4	0.129674	PCYOX1	0.333752	CCDC43	0.318846
AC131780.3	3.14591	AGPAT3	4.46119	CCDC59	0.184704	1600002K03RIK	0.1297	B230312A22RIK	2.99448	4930474N05RIK	3.1328
FAHD2A	3.14433	AC156550.1	4.45543	KDELC1	0.184761	SDR39U1	7.71011	CCNE1	2.98388	PDLIM2	3.12896
DOLPP1	3.13618	CDCA8	4.44608	ADCK3	5.41193	NFYB	0.129757	NDUFS5	2.98382	CCDC34	3.12826
STAM	3.13571	BTF3L4	4.44441	SEPSECS	0.184985	GRAP	7.6721	1110004E09RIK	2.98052	SRSF1	3.12446
TIMM10	0.319394	DDX52	4.44187	VEGFA	0.185212	LUZP1	7.63923	HMGB1	2.98022	DPP7	3.12375
GM10203	3.12599	NDUFS5	4.42774	SC5D	5.39603	HCST	7.61544	GTF2IRD2	2.97923	IL1F9	3.1218
NUBP1	0.320316	CDC42SE1	4.41984	FNBP1	5.39015	ZFP637	7.60926	TMED5	2.97866	SLC4A11	0.320514
NAT9	0.320397	UCHL5	4.41338	AIMP1	5.38091	SRP19	7.57363	FAF2	2.97736	ALG14	3.11635
RB1	0.320964	PIGYL	4.40893	1810020D17RIK	0.18622	OSGEPL1	7.56543	CCR4	0.336944	MFSD2A	0.321052
H2-GS10	3.11528	PDLIM7	4.4059	ECHDC1	5.36392	TMEM199	7.48781	NTNG2	2.9583	RB1CC1	0.321173
MYCBP	3.11017	VDAC3	4.40259	MTIF3	5.34337	SENP3	0.133839	RNF44	0.339022	FXR1	0.321184
AC132391.1	3.1075	4933424B01RIK	4.38958	RAPSN	5.33494	TSEN2	7.4544	NDUFAF3	0.339379	PINX1	3.11098
IPO13	0.322049	PPP6C	4.38589	MPHOSPH8	0.187887	GBP2	7.43783	IFT20	0.340482	D4ERTD22E	3.11053
PIP4K2B	3.10511	SUCLA2	4.37852	GM15401	0.188117	CIB1	7.4126	2310008H09RIK	0.340502	GNG2	3.10972
4930522L14RIK	3.10127	ENPP2	4.36614	TBCB	0.189179	IFRD1	7.40784	CAMK2B	2.93204	ERLEC1	3.10554
1810043H04RIK	3.09951	HCST	4.35327	5830405N20RIK	0.18951	3110003A17RIK	7.40034	COQ2	0.341951	ARMCX1	3.10231
PSPH	0.323327	GNPDA1	4.34901	OBFC1	0.189633	2010107H07RIK	7.38496	MRPL53	2.91647	GSTK1	3.09987
GM10106	3.0872	BAT1A	4.34702	AC161001.1	5.2627	ZFP51	0.135464	CD44	0.342897	UBE2D2	0.322793
ZFP451	3.08594	ZFP738	0.230576	AC156282.1	5.26252	ARHGAP15	7.37653	NFKBIL2	0.343165	HIST2H3C1	3.09785
R3HCC1	3.08076	MBD2	4.32965	GLUL	5.25996	POLD1	0.136672	RGS1	2.91313	8430426H19RIK	0.323762
CHCHD2	3.07856	PRR13	4.32946	LIME1	5.22859	TTLL12	0.13693	POLR2K	2.9129	SMARCD2	0.323887
PCCA	3.07753	DPY19L3	4.32468	CCDC43	0.191352	MAN1A2	0.136938	RNPC3	2.91133	BDP1	3.08415
WDR45	3.07381	GM6180	4.32311	DNAJC1	5.22394	HAUS1	7.29384	ARL5B	0.344485	MPV17	3.07741

Differentially expressed genes for GPR65-/-, PLZP-/- and TOSO-/- Th17 cells											
GPR65-KO-IL1B+IL6+ IL23-96h-1		GPR65-KO-TGFB1+IL6-96h-1		PLZP-KO-IL1B+IL6+IL23-48h-1		PLZP-KO-TGFB1+IL6-48h-1		TOSO-KO-IL1B+IL6+IL23-96h		TOSO-KO-IL1B+IL6+IL23-96h	
Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)
CFHR1	3.0702	SLC25A19	4.31144	MRPS24	5.22365	1700034H14RIK	7.27853	SLC2A6	0.344806	MPHOSPH6	0.325279
EMD	0.327152	TTF2	0.232143	IMMP2L	0.191798	PGAP2	7.26564	TBC1D9B	2.89908	CDCA5	3.07215
BCL2L11	0.3276	RPAIN	4.29849	COQ6	0.192374	RASA1	0.137688	B230208H17RIK	0.345136	1700106N22RIK	3.07201
AC131780.1	3.04934	CARS	4.27862	PIGK	5.17747	JMJD6	7.24938	ZBTB49	2.89711	ACADSB	0.325735
VTA1	0.328216	RCC2	4.26834	IL15RA	0.193169	AC132391.1	7.20559	WDR12	2.89619	AC125099.1	3.06864
ZMAT5	3.04487	DPF2	0.234501	VPS25	0.193271	COX16	0.139135	PTOV1	2.89529	SELENBP1	3.06799
PITPNA	0.328621	PHF10	4.24475	GM5116	5.16527	SDCCAG3	7.18597	SRR	2.8933	PLA2G16	3.06358
HNRNPH1	0.329638	BBS9	0.235876	PRM1	5.15044	2500003M10RIK	7.17577	2010111I01RIK	2.88992	HIST1H1B	3.06348
STX17	3.02886	RPP21	0.236253	CCL5	0.194269	SLC25A14	0.139358	BLCAP	0.347386	1810030N24RIK	0.326736
TFAM	3.01805	VMN2R7	0.236701	GRAMD1B	5.1398	TCTEX1D2	0.13985	PHF20L1	2.86916	ARID4B	0.327308
MXRA8	3.00578	EWSR1	4.20549	CAPS2	5.13789	4930447C04RIK	7.14502	FBXW4	2.86808	NOX4	3.05421
ACADSB	3.00199	BLOC1S1	4.2011	RPS6KB1	5.13778	MRPL53	7.13183	PHLDB1	0.348714	NEK8	3.04829
RPL21	2.99721	GOLT1B	4.18881	TBCA	0.194723	TMEM39A	7.07725	RAPH1	0.349641	BLCAP	0.328174
HAT1	2.99666	PFKL	4.184	CLUAP1	0.194896	MTIF2	7.06029	PIH1D1	2.85572	FAM49B	0.3283
AC151573.1	0.333857	FAM132A	4.17661	PUS1	0.195037	SOD1	7.0561	CREG1	2.85346	RHBDD3	0.328672
PHPT1	0.334679	GSN	4.17321	NAT9	5.11575	GM14391	0.142113	LMF1	2.85313	ECHDC1	0.328928
TMEM120A	2.98268	UGDH	4.16843	TMEM219	0.19559	GM16519	7.03028	BC003267	2.85291	UFD1L	0.329148
PEPD	0.335518	NR2C2	4.16575	ANAPC11	5.10652	ZWILCH	7.02727	NELF	2.83614	MGAT4C	3.03683
UXT	2.97617	MECR	0.240318	SEC63	0.196009	LENEP	0.142993	TTC9C	2.8299	SRD5A3	3.03372
AC131780.4	2.95956	ACAA2	4.14731	EIF2C4	5.09769	BC031181	6.98719	EPT1	2.82973	ZFP874A	0.329847
UCP2	2.94585	GM10028	4.14033	TRNAU1AP	5.09474	UNC5CL	6.96583	NEK8	2.82961	UGDH	0.329891
PML	2.92902	REXO2	4.13396	DIABLO	0.196302	CHUK	0.144161	MPND	0.353903	CUL1	0.330359
GM5531	2.92779	ATM	4.13183	CEP55	0.196627	SPIC	6.92498	MOBKL1A	2.82546	CMAH	3.02473
GM10715	2.91891	NUPR1	4.13177	GM7075	5.05552	HOOK3	0.144541	ZFP287	2.82431	LPL	3.02258
POLR3GL	2.91406	GM10203	0.242035	AIFM2	5.04453	DLG4	6.91142	TBCE	2.82358	SIDT2	3.01548
LSM1	2.91027	TAGLN2	4.11826	NUP210	0.198274	ARMCX6	0.145354	MARK2	2.81506	YBX1	0.333036
GM11152	0.343815	NBR1	4.11699	FAHD2A	0.198849	HIST1H1B	0.145354	GBA2	2.81383	IL9	0.333116

Differentially expressed genes for GPR65-/-, PLZP-/- and TOSO-/- Th17 cells											
GPR65-KO-IL1B+IL6+ IL23-96h-1		GPR65-KO-TGFB1+IL6-96h-1		PLZP-KO-IL1B+IL6+IL23-48h-1		PLZP-KO-TGFB1+IL6-48h-1		TOSO-KO-IL1B+IL6+IL23-96h		TOSO-KO-IL1B+IL6+IL23-96h	
Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)
STAT6	2.90777	CPT2	4.1068	ARHGAP29	0.19898	APIP	0.14542	FBF1	2.80213	PNPO	2.99467
DLG4	2.90687	GM1968	4.10351	MAN2C1	5.02447	DET1	0.145604	MRPS23	2.8007	CENPH	2.9921
MTCH1	0.344018	DERL2	4.09817	SPINK10	5.02384	CENPV	0.145745	MXRA8	2.80045	LTBP1	2.98882
MAGED2	0.344848	ERGIC2	4.09157	HSPB11	5.01869	GTF2F2	6.8613	GOSR1	2.80035	USP20	0.335411
SLC10A3	2.89944	PHOSPHO2	0.244986	2810428I15RIK	0.199368	COMMD6	6.83834	TAF11	0.357693	CD40LG	0.33575
CKLF	0.345303	ATP6V1D	4.08007	MFF	0.199484	B4GALT3	6.82285	CHCHD7	2.79479	CLP1	0.336059
RAE1	0.345495	LSM10	4.07981	TIMD2	5.0101	AARSD1	0.146637	TRIP11	2.79204	CBX8	0.33665
MED27	2.8884	ZNRD1	4.07458	UFD1L	0.19963	IFT46	6.8108	SQRDL	0.358218	TRNT1	0.336673
CTSE	2.88808	LGTN	4.07031	SDHC	0.199634	MAD2L1BP	6.80448	TIA1	2.79049	RG9MTD3	0.336739
IFT57	2.88579	WSB1	4.06913	1110001J03RIK	5.00685	NDUFB6	6.79376	EXOC5	2.78989	2610029I01RIK	2.95916
GM10217	0.346567	MTIF3	4.06886	GM11175	0.199973	0910001L09RIK	0.147261	1190007I07RIK	2.78892	BRE	0.338296
CIB1	0.347105	RNF7	4.05685	STYK1	4.98328	GLRX2	6.7192	LYSMD2	2.78888	CHCHD6	2.95512
HINT3	0.347157	AC116115.1	0.247894	RAD51L1	4.97228	CHD8	0.149339	ZFP317	0.358667	ZFP451	0.338711
TSC22D4	0.347171	2810474O19RIK	4.02276	MED6	0.201969	1110008F13RIK	0.149452	CDC34	0.35947	SEC61A2	0.338805
CDC23	0.34724	LUC7L3	4.00597	CNN2	0.202084	THAP7	0.149641	CCDC28A	2.77955	MAP3K14	0.338859
RPL21-PS14	2.87725	TNFRSF4	3.9988	PLAC8	4.93497	ATP5L-PS1	6.67932	BRD7	2.77765	SLC25A19	2.94723
UPP1	0.347912	OGT	3.99743	MRRF	0.203165	MED4	0.149821	SLC39A1	0.360242	RG9MTD2	2.94721
AI314180	0.348002	TNNC1	0.250444	DPM2	0.203226	SNX15	0.149921	CRTC2	2.77523	PQLC3	2.94393
KBTBD4	0.348425	GM12942	3.97863	3110001D03RIK	4.89187	AHSA2	0.15006	ATP6AP2	2.77394	PPT2	2.94232
2700094K13RIK	0.348921	JTB	3.97698	SMAD4	0.205077	PDCD1	6.62636	USP18	2.77391	TMTC2	2.94055
H2-Q6	2.85926	WBP5	3.97449	RGS10	0.205674	1110058L19RIK	6.61022	FANCL	2.7726	MRPL53	0.340076
ORC4	2.85828	4930522L14RIK	3.97073	4933427D14RIK	0.205823	WFDC12	0.151538	PAFAH1B1	2.77096	2610001J05RIK	0.3403
SLC4A8	2.8579	PHRF1	3.96914	TOR1A	0.205923	UFM1	6.59687	SVIL	2.76036	DYNLT3	0.340666
SDR39U1	2.85678	CCDC56	3.96355	DUT	4.85312	MRPS25	6.5844	MRPS25	2.75866	4931406P16RIK	0.340949
USP3	2.85498	LMAN2L	0.252321	SNX12	4.83276	CISH	0.151934	NUDT3	2.7582	DIP2A	2.93247
H2-D1	2.85043	ISCA2	3.9591	CAMTA1	4.82271	ACER3	6.58143	ATN1	2.75811	STAB1	2.92744
SLC1A2	0.350989	PARL	3.93771	EXOC7	4.81229	SLAMF1	6.5452	OLFR816	2.75542	BC017647	2.92608

Differentially expressed genes for GPR65-/-, PLZP-/- and TOSO-/- Th17 cells											
GPR65-KO-IL1B+IL6+ IL23-96h-1		GPR65-KO-TGFB1+IL6-96h-1		PLZP-KO-IL1B+IL6+IL23-48h-1		PLZP-KO-TGFB1+IL6-48h-1		TOSO-KO-IL1B+IL6+IL23-96h		TOSO-KO-IL1B+IL6+IL23-96h	
Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)
CARM1	2.8438	TMEM135	0.255381	COQ7	0.207907	ACTR1B	6.53341	2310044H10RIK	2.75278	ELP2	0.342265
TPK1	0.352289	IFT52	3.90514	RNF130	0.208222	GM6096	6.50942	CHD6	2.75194	ARHGAP4	0.342484
GM11678	2.83596	PSAT1	3.90383	FAM58B	0.208634	ELP4	6.50917	SNRPB2	0.363667	AP3S1	0.34362
ALPL	0.352687	STX18	0.256366	GPD1L	4.79143	TM4SF5	6.50305	NUCB1	0.364479	PTPN3	0.344161
H2AFY	2.83378	ANXA2	3.89173	SEC24B	0.208746	PXMP4	6.49205	DLGAP4	2.74225	KDM1A	0.344246
MRPL32	0.353268	AEN	0.257495	MBD2	4.76196	SPATA6	0.154734	DCXR	0.364747	PVR	2.90195
RASSF7	0.354535	TADA3	3.86131	ARMC6	0.210289	SNAPC4	0.154947	AHCY	2.74099	ERH	0.344634
GM14420	2.81884	MAT2B	3.86108	GM10125	4.74057	AA467197	0.155267	1110008P14RIK	0.365256	PPOX	0.344666
IGBP1	0.355248	42249	3.85924	9930111J21RIK2	4.73875	SUPV3L1	6.41432	TIAM1	0.365257	XLR4B	0.345325
NDFIP1	0.355598	NUDT1	0.259245	0610011F06RIK	4.73797	DHPS	0.156039	EIF2B1	2.72536	GM2938	2.88665
UHRF1	2.81099	RHOF	3.85418	CNPY2	4.72965	DDIT3	0.156589	5830405N20RIK	2.7248	PAIP2B	2.88615
TRIM50	2.81049	IMMP2L	3.84533	CHCHD8	4.7235	BOD1	0.156685	GM11276	0.367251	FBXW2	0.348007
CCDC43	0.355835	CELF2	3.83589	ACBD6	4.71636	9030625A04RIK	6.35456	HIST1H2AO	0.367251	MPST	2.8706
GTF2F2	0.355977	DENND2A	0.260843	ABT1	4.71447	COX5A	6.33911	RASSF2	2.72257	C2	2.85795
TNFRSF13B	2.80894	FAM114A1	0.261077	1810032O08RIK	0.212175	HERC3	6.3363	CRADD	2.72066	FAM78A	0.350322
TADA2A	2.79953	EXOSC9	3.82852	JUP	4.71286	PPIG	0.158134	SLCO4A1	0.367988	C330021F23RIK	2.85429
YIF1B	2.79885	RPL37-PS1	3.8276	C130022K22RIK	4.70846	APITD1	0.158176	ISCA2	0.368981	CDC25B	2.85421
NFKB1	0.357441	NCK1	0.262234	CDK14	4.70157	2310008H09RIK	6.31859	MRPL2	0.369111	ZFP369	2.85328
H2-K1	2.79752	SNX15	3.79972	HTRA2	4.69693	MBOAT1	0.158372	STOM	0.369478	BET1	0.350498
IFI27L2B	2.79246	RNASEH2A	3.79724	EIF2B2	0.213067	LMO4	0.158933	BAG1	0.370334	MRPS22	0.351299
IDH3B	0.359126	CNP	3.79312	ACBD5	4.688	STK19	0.159019	WSB2	2.70026	MTFR1	0.351334
MRPL55	0.359918	ACADVL	3.79189	GNGT2	4.68529	PHB	6.28423	BOP1	0.370403	INPP5D	2.83909
CDC40	0.359945	MRPL22	3.78887	0610031J06RIK	0.213485	UPP1	0.159191	FBXO18	2.69642	DNMT3B	2.83849
COMMD5	2.77285	SELK	3.7873	AI314180	0.21373	SLC15A2	6.28071	SERPINB6B	2.69283	D16H22S680E	0.352514
STXBP2	0.361123	MRPS24	0.264476	GM10417	4.66524	MOCS2	6.27195	5730494N06RIK	0.372059	UGGT2	2.83121
FAS	0.361673	ICOS	3.77963	CTPS2	0.214964	USE1	6.26846	BLOC1S2	0.372809	HRAS1	2.82848
CTR9	2.76177	CSDE1	3.77373	CLEC4A2	4.65076	DCTN5	6.26381	LAP3	2.68166	PDLIM5	2.82108

Differentially expressed genes for GPR65-/-, PLZP-/- and TOSO-/- Th17 cells											
GPR65-KO-IL1B+IL6+ IL23-96h-1		GPR65-KO-TGFB1+IL6-96h-1		PLZP-KO-IL1B+IL6+IL23-48h-1		PLZP-KO-TGFB1+IL6-48h-1		TOSO-KO-IL1B+IL6+IL23-96h		TOSO-KO-IL1B+IL6+IL23-96h	
Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)
STT3A	0.363716	SNX2	3.7713	GM7665	4.64326	TLE6	6.26098	CD48	0.373303	TTLL4	0.354725
H2-T23	2.74839	CNDP2	3.76083	SPINT2	0.215713	STX18	6.2564	CHCHD1	2.67635	ALKBH3	0.355442
GATAD1	0.364231	SLC25A5	3.75199	SPA17	0.215945	BCL2A1B	0.159862	MAPK1IP1	2.67331	SFI1	2.8129
RBM17	0.366844	SEMA4A	0.266764	TBC1D1	0.215945	CCNDBP1	6.23421	METTL4	0.374093	SYNJ1	0.355614
TIMM22	0.367561	FBXO4	3.74721	GM14399	0.216145	PHKG2	6.2254	ZFP605	2.67043	4930422I07RIK	0.356305
TMEM106A	2.71516	FXC1	3.74327	PRKRIP1	4.60596	GM10495	6.21918	SLC35A4	2.67036	PRKCZ	2.80052
AL732569.1	0.368325	DGAT1	3.73403	ZSCAN2	0.219106	RRP36	0.161141	PEA15A	2.66759	GGNBP2	0.35727
SDF2	0.368758	NSMCE1	3.73357	PRIM2	4.55993	POLR1E	0.161376	IQCE	0.375067	PRPS2	2.79576
AC132837.1	0.369262	GBP2	3.73272	QDPR	0.219302	ARFIP2	6.16931	MTG1	0.375758	NADSYN1	2.77753
5930416I19RIK	0.369793	EFTUD1	3.73038	CDCA5	0.219692	KRAS	0.16237	RALGAPA2	2.65799	NDUFAF1	0.360265
TUBA8	2.70237	GRHPR	3.73034	SCFD2	0.219781	MAD2L2	0.162373	NOL7	0.376463	LYSMD2	2.77269
H2-OB	0.370064	IL10RB	3.72675	HADHA	0.220075	EIF2B2	6.15565	FAIM3	0.37671	NUSAP1	2.77163
MED6	0.371113	DNAJC15	3.72299	GM4893	0.220436	8430423G03RIK	0.162475	RAB3D	0.377163	PXMP2	0.360913
RSU1	0.371744	IMMP1L	3.72249	RAB8A	0.22074	SGSM3	0.162516	2700094K13RIK	0.377586	SNX14	0.361047
TMEM179B	0.371842	ARMC7	0.269039	STAP1	0.220747	NSMCE4A	0.163059	LDB1	0.377679	BLOC1S2	2.76953
FLT3L	2.68853	CYP11A1	3.71276	FAM175A	4.51023	IPO13	0.163484	ADRBK1	2.64366	HIST1H4I	2.7683
TMC4	2.6825	EIF4G1	0.269833	TPST1	0.222301	GM561	6.09075	EPSTI1	2.64135	MUC2	2.76605
MORF4L2	0.373036	LGALS9	3.69407	FAM32A	4.4939	GALK2	6.08225	NRF1	2.6405	PPP1R13L	2.76466
DHPS	0.373179	ECHDC2	3.69165	MTUS2	0.222665	YIPF6	0.16479	PIGT	0.378962	PARVG	2.76232
BC030499	2.67153	S100A13	3.69113	CCNL1	4.47916	ASTE1	0.165034	TADA1	2.6381	CBARA1	2.75891
SYCE2	0.374728	RNASEH2B	3.67632	MLKL	0.223832	MRPL55	6.05937	CLTB	0.379157	EXOSC3	2.7582
ZRSR2	2.66741	ARPC5	3.66651	DNPEP	4.46494	HSD17B12	0.165137	TSEN34	2.6373	SRSF5	0.362666
RNMT	0.374968	CYTIP	3.66609	GM11276	4.46139	GM6843	0.165668	GM9774	0.380122	FBXL12	2.75597
GPCPD1	0.375771	RPA2	0.274174	HIST1H2AO	4.46139	ME3	6.03518	GM2833	2.62465	1500001M20RIK	0.363356
JAK1	2.66069	MRPL21	3.64363	CDK2AP1	0.224199	ABLIM2	6.01497	WBSCR22	2.62229	MANF	2.74877
MT2	0.375898	DYNLRB1	3.63555	ZFP35	4.45623	RPS12	0.1669	2410089E03RIK	2.61843	BECN1	0.363818
WAC	0.376308	MUS81	0.275812	SECISBP2	4.45206	VMN1R58	5.97815	UHRF1	0.382247	PSTK	2.74776

Differentially expressed genes for GPR65-/-, PLZP-/- and TOSO-/- Th17 cells											
GPR65-KO-IL1B+IL6+ IL23-96h-1		GPR65-KO-TGFB1+IL6-96h-1		PLZP-KO-IL1B+IL6+IL23-48h-1		PLZP-KO-TGFB1+IL6-48h-1		TOSO-KO-IL1B+IL6+IL23-96h		TOSO-KO-IL1B+IL6+IL23-96h	
Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)
THOC6	0.376726	GM10566	3.62112	NEK8	0.224899	AKIRIN1	5.97705	UBE2E1	2.61472	TMEM29	0.364208
USE1	2.65108	NINJ1	3.6157	42070	4.44642	TAF13	5.97344	GSTZ1	2.61459	PUS7	0.36489
THAP3	0.377346	BLZF1	3.59783	GM5474	4.43605	2400001E08RIK	5.96702	EIF3L	0.382819	CIT	0.365716
GM9574	0.377779	MRPL10	3.59223	TIMM44	4.43373	1110001J03RIK	5.94875	NPLOC4	2.61217	MLLT10	0.365901
MRPS6	0.377989	PRODH	3.58928	PPDPF	4.43157	ALKBH6	5.94376	BC026585	2.61077	PAQR3	2.73185
AC160471.1	0.378247	C1D	3.58896	IFI35	4.42972	DGKZ	5.9229	VPS29	0.383107	AHNAK	0.36641
FAM173A	2.64377	D10WSU52E	3.57659	CENPL	4.41587	MUS81	5.9194	GM5610	0.383109	NUDT3	0.366644
VEZT	2.64154	NUP54	3.5669	CDC7	4.41328	DCUN1D1	0.168965	PDLIM5	2.60311	LBR	0.367002
TMUB1	2.64146	OGFOD2	3.56196	AASDH	0.226953	CLNS1A	0.16917	SNTB1	2.60288	KCTD13	2.72178
LITAF	2.64108	RBM43	0.281272	ZMAT5	0.227216	SLC35C2	5.9112	GPR107	0.384602	CCDC109A	0.367499
CALD1	2.64051	GM5506	3.55487	PPAPDC1B	4.3942	CNN2	5.89954	1810035L17RIK	0.384739	SSRP1	2.71716
MAPRE1	0.378721	TXN1	3.5524	3110009E18RIK	0.228199	LRRC40	5.89635	AEN	0.385092	SPIC	2.71485
USP5	0.378759	ASL	3.55025	GPR19	4.38073	PRIM2	5.88398	USP25	2.59227	PDAP1	2.71339
PSMG2	2.63646	CD68	3.54467	SLC11A2	4.37804	ARFRP1	5.87869	FAM183B	0.38684	SNX32	2.71032
MRPL41	0.37975	GM11444	3.54297	ARL2	0.228445	MND1	0.170847	OAS1A	2.584	CTSC	0.369527
SERHL	0.379928	CCNL2	3.54173	DCTN3	0.228615	MOBKL3	0.170936	N4BP3	2.5831	NOL6	0.369607
GCC2	0.379996	DPH3	3.54104	DNAJC12	0.229118	THOC7	0.171761	NCKIPSD	2.57772	ZWILCH	0.370438
CRYGN	0.380065	C030039L03RIK	0.282425	BC057079	4.36401	UTY	0.171829	RIOK2	2.57744	OPRM1	2.69491
ABI1	0.380254	ENTPD1	0.282635	TMEM188	4.35828	GBA	5.81696	ASB7	2.57699	MRPS7	2.69191
XLR4C	2.62803	H2-Q6	3.52238	ARL3	4.3536	TMEM33	5.80804	ETOHI1	2.57488	MMP16	2.6919
MBOAT1	0.380531	WDR3	3.51936	FBXL17	4.3512	EIF4EBP1	5.79065	IL1R2	0.388562	PRDM11	2.69182
TMED3	2.62714	DHX8	3.51485	MUL1	4.34233	GTF2H1	5.78757	CYP4F13	2.57031	CCT6A	0.37176
GIMAP3	2.62097	NUBP2	3.51163	NUP188	4.33967	TUBGCP4	0.173065	NKG7	2.56692	VAV2	2.6868
NSUN5	2.61944	GM10324	3.50986	RBL2	4.33662	CD9	5.77675	GM14391	2.56275	DTL	2.68598
WIPF1	0.381938	NFIB	0.284944	MECR	4.32577	AFF1	0.173118	GRHPR	2.55795	ELOVL5	0.372757
CCT4	2.60936	IMPA2	3.50901	AI462493	4.32377	TANK	0.17324	PARP3	2.55783	PDPK1	0.372828
GPS2	0.383457	DCTN5	3.50543	ZFP26	4.31551	2310036O22RIK	0.173257	HDAC5	0.391103	TMEM69	2.68202

Differentially expressed genes for GPR65-/-, PLZP-/- and TOSO-/- Th17 cells											
GPR65-KO-IL1B+IL6+ IL23-96h-1		GPR65-KO-TGFB1+IL6-96h-1		PLZP-KO-IL1B+IL6+IL23-48h-1		PLZP-KO-TGFB1+IL6-48h-1		TOSO-KO-IL1B+IL6+IL23-96h		TOSO-KO-IL1B+IL6+IL23-96h	
Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)
NAA35	2.60468	DDX1	3.5049	GIMAP5	0.231722	4930473A06RIK	5.76841	SUPT3H	2.5565	RPA1	0.37316
RARS2	0.38393	TNFAIP8L2	3.50285	CYB5RL	0.231826	EPN2	5.75899	STRA6	0.391227	ITM2A	0.374049
NGFRAP1	0.384268	ARL6IP4	3.50197	RPS19BP1	4.31053	PNP2	0.173642	EHD1	2.55424	ERCC5	2.67242
IL1F9	2.59869	TMEM128	0.28566	CPM	0.232819	STIM2	0.173743	MRPL17	0.391555	BRD8	2.67235
TNFAIP8L2	0.384814	AC139042.1	3.49905	GM13147	4.29271	ZFP62	0.173835	TBC1D20	0.391907	CLDN7	0.374323
TMEM161A	0.38506	SLAMF8	0.286743	1110021L09RIK	4.2791	CAPZB	0.173926	TBCA	2.54932	SFT2D1	2.67093
GM10842	2.5922	CDC20	3.48721	PSMD10	4.27034	BCL2A1C	0.17394	ARHGEF18	2.54846	MRPS10	0.374692
CTTN	2.59036	FMNL1	3.4823	VIPAR	0.234174	TGS1	0.174071	ARL16	0.392431	ACAD11	2.66233
MLKL	0.386417	SEPP1	3.47958	CENPA	0.234317	PPIL3	0.174089	1500011H22RIK	0.392571	HDAC6	0.375756
GGTA1	0.386703	RPS17	3.47038	R3HDM2	4.26772	AC166169.1	0.174396	PTPN3	2.54622	RNF6	0.376193
METT11D1	2.58361	GPR18	3.46365	UBE2W	0.234509	FUS	0.174416	SETX	2.54107	CARKD	0.377398
TAF1D	0.387516	CTLA4	3.46255	TSC22D4	0.234692	PPIH	5.71612	ZDHHC13	0.393563	SBF2	2.64709
2310045N01RIK	2.57617	TMEM9	3.45673	OLA1	0.234852	MED11	0.174944	PHF7	2.53974	OSBPL7	2.64258
RNASEH2B	0.388366	EIF4E	3.45672	TATDN3	0.234926	MANBA	5.71117	MDFIC	2.53954	ZBTB7B	0.37856
THOC5	0.388575	PIH1D2	3.44643	CHKA	4.25282	TMEM223	5.7077	SUSD3	0.394206	RGS3	2.6388
RPL21-PS12	0.389232	GM8815	3.44583	RBM14	0.235463	BC017643	5.70458	RNMT	2.53629	DULLARD	0.379202
MRE11A	0.389622	HDAC7	3.4423	CHM	4.24299	ZZZ3	5.69466	GM12942	2.53483	NUDT14	0.380537
4632428N05RIK	0.390117	SLC25A39	3.44154	FAM3C	0.235902	L7RN6	5.69185	INO80C	2.53474	TYMS	2.62736
IMMP1L	0.390261	COMMD1	3.43923	MS4A6D	4.23668	POLK	0.175794	ZDHHC4	2.53286	SETD6	2.62561
8430419L09RIK	0.390402	CSTAD	0.290846	GM5900	4.23525	NUP43	5.68673	PBK	2.53258	INPP5F	2.6251
CCNC	2.56046	INSL3	3.43606	HDAC8	0.236114	IFT140	0.176033	NUDC	0.395065	CNPY2	2.62114
PSD4	0.390742	AKR1A4	3.43343	NRK	4.22303	DOHH	5.67803	ATPAF2	0.395464	NFIC	0.381718
IL15RA	0.390915	AP1S1	0.291393	VMN1R58	4.22215	SIRT2	5.63162	RACGAP1	2.52856	HPS5	0.382031
ALKBH1	0.390984	DUSP19	3.43144	ANAPC2	0.237303	MRPS34	0.177826	RFC4	0.395695	GM16181	2.60756
CINP	2.55745	PIH1D1	0.291703	BC055324	4.2104	ST6GAL1	0.178039	METTL10	2.52633	CHMP4B	2.60726
PFN1	2.55449	4930402H24RIK	0.291965	IFT20	0.237727	TGM4	5.61281	CDC45	2.52427	MNS1	2.60505
E030030I06RIK	0.391747	LSM5	3.41724	WDR85	4.19078	USP5	5.60719	CASP6	0.396329	DDA1	0.383999

Differentially expressed genes for GPR65-/-, PLZP-/- and TOSO-/- Th17 cells											
GPR65-KO-IL1B+IL6+ IL23-96h-1		GPR65-KO-TGFB1+IL6-96h-1		PLZP-KO-IL1B+IL6+IL23-48h-1		PLZP-KO-TGFB1+IL6-48h-1		TOSO-KO-IL1B+IL6+IL23-96h		TOSO-KO-IL1B+IL6+IL23-96h	
Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)
2700062C07RIK	0.392185	LAMA5	3.4158	1700047G07RIK	4.18925	LRRC31	0.178434	PDZD11	2.52099	BUB1B	0.384036
GTL3	2.54873	DBP	0.292969	GM11110	0.238794	SNAPC5	5.60413	FOXRED1	2.51945	MAP3K1	2.60194
AC087117.1	2.54855	BAD	0.293991	PFDN5	4.18382	AIP	5.59989	GM9762	0.397014	2900010M23RIK	2.60193
ATF7IP	0.392441	PFKFB3	0.294121	METTL5	4.17327	PHF20	5.59826	MGLL	0.397019	REXO1	0.384764
CEP250	0.392457	SNRPB2	3.39522	RHBDL2	4.16203	ACP6	0.178677	2310036O22RIK	2.51867	FKBP2	2.59551
HIST1H4I	2.54658	KCTD14	3.39439	AKAP13	4.15011	FAM3A	0.179502	NMT1	2.51497	CES5A	2.59475
TNFRSF25	2.545	TNFRSF18	3.39091	HIBADH	4.14954	2610528E23RIK	0.1799	2410002F23RIK	2.51263	RBMX	0.385616
GMFB	0.393779	EDF1	3.39068	ING2	0.241135	U2AF1L4	5.55635	USP21	2.51209	2500003M10RIK	0.385865
PARVG	2.53884	TM9SF4	3.38904	KIN	0.241463	MYSM1	0.180108	STAP1	2.50944	ABI3	0.386054
ACPL2	0.393929	MRPS36-PS1	3.38588	SNX14	4.13506	SPAG5	0.180124	TMEM120A	2.50846	PNPT1	2.59012
N6AMT2	0.395451	CCNE2	3.38245	BRD7	0.242433	CHAC2	5.55104	LY6I	2.50636	RPE	0.386085
B230208H17RIK	0.396264	C030048B08RIK	3.38235	LSM2	4.12415	FAM118B	0.180146	IKZF3	2.50618	GFM1	2.58967
3010026O09RIK	2.52186	TASP1	3.38082	GM71	0.242591	2310003H01RIK	5.54767	CLK2	0.399108	KPNA3	0.386292
MTIF3	2.51817	GMNN	3.37777	SUGP2	0.24264	SUSD3	5.54412	CSDA	0.39964	DEDD2	0.386389
BIN2	2.51792	SIT1	3.37462	WDR26	4.121	PJA2	0.180634	IFIT2	2.50035	BTBD9	0.387014
DCTPP1	0.397437	DAPP1	3.37257	CAB39L	4.12023	CHST12	5.52136	RFTN1	2.49718	ZZEF1	2.582
TM9SF4	0.397746	TUBA1A	3.37022	GM6132	4.1164	ZFP61	0.181115	U2AF1	0.400739	TWSG1	0.387342
PROP1	0.397841	PLIN2	3.36978	PSMC3IP	0.242976	A830010M20RIK	5.50564	LZTR1	2.49469	ASB6	2.58105
2310003C23RIK	0.397982	ACTB	3.36773	IFI27L1	4.09596	LRRC59	0.181713	9030025P20RIK	2.49125	TRAF3	2.58057
ATP1B3	2.51244	TMEM97	3.36432	GCC2	0.244318	PJA1	0.182048	RRAS	2.49102	SUFU	2.57907
PHAX	0.398858	TIMM22	0.298029	TRIP4	0.244704	RBM14	5.47441	NGFRAP1	0.401506	HAUS6	2.57873
KDM4C	0.399245	MRPS14	3.35244	6330416L07RIK	4.08248	SNX1	5.46754	TBRG4	2.49007	IRF3	0.387819
RRAS	2.50304	WDR54	3.35107	CASP2	4.08	MPDU1	5.46415	1110034B05RIK	0.402464	E330020D12RIK	2.57835
GM6483	0.39999	PHB2	3.34915	PPWD1	0.245456	GM4830	0.183093	H2-M2	2.48448	JMJD5	2.57693
TCTEX1D2	2.49548	CISD3	0.29859	ISL2	0.245584	PEX19	5.4617	CCDC76	2.48072	TRIAP1	0.388319
U2AF1L4	0.401416	FKBP1A	3.34809	AC117232.1	0.245801	H2-Q6	5.45725	ANKRD12	2.47797	LSM2	2.57473
HMOX1	0.401474	SLC25A11	3.34521	MMP16	4.06537	RBM22	5.45222	ZKSCAN14	2.4722	ZMAT5	2.57136

Differentially expressed genes for GPR65-/-, PLZP-/- and TOSO-/- Th17 cells											
GPR65-KO-IL1B+IL6+ IL23-96h-1		GPR65-KO-TGFB1+IL6-96h-1		PLZP-KO-IL1B+IL6+IL23-48h-1		PLZP-KO-TGFB1+IL6-48h-1		TOSO-KO-IL1B+IL6+IL23-96h		TOSO-KO-IL1B+IL6+IL23-96h	
Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)
AC166169.1	0.401762	AC090563.1	0.299305	APOBEC1	4.06336	MFN2	0.184369	CITED2	2.47108	AC132320.1	2.57098
SMEK2	0.40177	ATP6AP2	3.33938	CCDC40	0.246246	GM6710	0.184704	ING3	2.46922	UNC45A	2.56806
TGDS	0.402264	PFDN1	3.33829	TIAL1	4.05923	KIF2C	5.39762	ATP6V1D	2.46538	ZBTB20	0.389732
BBC3	0.402395	SNX1	3.33024	MRFAP1	0.246888	TBPL1	5.38661	KCNK7	0.406665	NXF1	0.391
CBARA1	2.48476	LUC7L	3.32473	GADL1	0.247037	CDC123	5.38034	HNRNPL	0.406792	GMEB1	0.391028
XRN2	0.403258	EIF4B	3.32463	SERPINF1	0.247061	RAG1AP1	5.37712	SPG11	2.45809	CIRH1A	0.391777
2810428I15RIK	0.403471	FYB	3.31789	KIF5B	4.04619	4933421E11RIK	5.37001	MIPOL1	2.45699	OAS1B	2.55216
LGALS3	0.403629	KNG1	0.302111	CORO1B	0.247187	AC127590.1	5.35724	COL4A3	2.45583	ARRB1	0.392411
S100A3	2.4669	LPCAT3	0.302157	LRRK1	4.04349	4930512M02RIK	5.3539	HSF2BP	2.45561	MRPL43	2.54414
GM6396	0.405591	GGA3	0.302824	TMEM104	4.04072	TREX1	5.34849	GM12789	2.45047	GM14443	2.54154
ITGA6	2.46387	ANKRD16	0.303006	ZFP488	4.0366	MRPL2	0.18728	AU022870	2.45027	SPAG5	2.53887
HMGN1	0.407101	ZSCAN21	0.303014	2310001H12RIK	0.247861	42248	5.33353	VAMP4	2.44973	ZFAND6	0.393917
EED	0.407365	VTA1	3.29714	GNB1L	0.248459	CUL4A	5.33054	CIAPIN1	0.408638	AC068006.1	2.53464
DNAJC21	0.407973	SATB1	3.29571	RPS2	0.248605	CENPL	0.187671	COQ5	2.44616	CD27	2.53458
NDUFS1	0.408024	NDUFS3	0.303751	ILK	0.248648	AU019823	0.187717	TATDN1	0.408903	PLBD2	2.52362
GM5617	2.44984	JKAMP	3.29212	COMMD2	4.01665	TRADD	5.32189	RNF7	0.408986	RAB2A	2.52042
WTIP	0.408332	SKAP2	3.28684	COQ9	0.248966	SNX12	0.188894	ATR	2.44494	ATRIP	2.52039
CD48	0.408816	H2-Q10	3.28673	D930014E17RIK	0.24934	LLPH	5.29397	H2-Q6	2.44355	SEC16A	0.396912
MFF	0.408963	COMMD3	3.28433	AC142450.1	4.0057	TNFRSF13B	5.28839	PTPN2	2.4435	MED31	2.5165
SRSF2	0.410079	MYSM1	3.28378	CLCF1	0.249709	MRE11A	0.189138	ATG4B	0.409998	PCCA	0.397718
SLC39A11	0.410655	1810020D17RIK	0.304718	AC102609.1	3.99958	IMPA1	5.27732	MED18	2.43574	SNAP23	0.398389
PPCS	2.42455	GM10800	3.28046	ORC4	0.250401	GALT	0.189633	1110049F12RIK	2.43525	IKBKE	2.5098
RPE	2.42392	TMEM50A	3.27816	YTHDC1	0.250938	LY6C2	5.27335	SPR	0.410802	NOP10	2.50758
BC049349	0.412584	CAPZA2	3.27751	MRPS23	3.98124	RP23-369M17.1	0.190228	TMEM121	2.43406	D19ERTD386E	0.399471
LRRC33	2.42129	MAP2K3	3.27284	TNFSF9	3.97682	LY6I	0.190318	BAK1	0.411164	CUL4A	2.50226
GM4953	2.42053	MDH2	3.27146	LSM12	3.97426	KIF1B	0.190499	WDR26	2.43141	MRPL12	2.50001

Differentially expressed genes for GPR65-/-, PLZP-/- and TOSO-/- Th17 cells											
GPR65-KO-IL1B+IL6+ IL23-96h-1		GPR65-KO-TGFB1+IL6-96h-1		PLZP-KO-IL1B+IL6+IL23-48h-1		PLZP-KO-TGFB1+IL6-48h-1		TOSO-KO-IL1B+IL6+IL23-96h		TOSO-KO-IL1B+IL6+IL23-96h	
Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)
SMAD2	0.413406	CD3D	3.27114	POLD4	3.94804	EIF3G	5.24109	MAPK3	0.411846	NCKAP5	2.4997
PTPRV	0.413851	PEX11B	3.27019	CEP57	0.253563	TMEM219	0.191107	CD226	2.42794	GM10125	2.49936
KLC1	2.4143	AHSA1	3.26954	SAMSN1	3.94248	4930529M08RIK	5.23014	TBC1D13	2.42405	GM5607	2.49751
CISH	2.41248	SPINK10	0.305927	2300009A05RIK	3.93763	H2AFV	5.22596	TIMM50	0.412533	FANCG	0.400578
1700007K09RIK	0.415149	BC017643	3.26754	AC156948.1	3.93241	DNAJC9	5.22093	1810020D17RIK	0.412683	FBXO44	0.400767
PIGZ	0.415217	A630001G21RIK	0.306225	S100A1	3.91733	TK1	5.21873	CUX2	2.42237	BIRC2	0.400962
PTTG1	0.415544	TBC1D10C	3.26349	GMDS	3.91583	RAD51AP1	0.191965	C130026I21RIK	2.42004	2310044H10RIK	0.401107
BC017643	2.40337	PARD6A	3.26276	CARS	0.255555	DHX33	5.20461	PRMT7	0.413402	BC048355	2.48897
YIF1A	0.416415	PAM16	3.2571	MRPL21	3.90612	IRF1	5.20163	LUC7L3	2.41893	5930416I19RIK	2.48462
FBXO5	0.41659	SCN9A	0.307586	PEA15A	0.256257	CAT	0.192378	TM2D1	0.413569	PTPN4	2.48407
PSEN2	2.39813	MOBKL2A	3.25054	ACP6	0.256389	CFLAR	0.19253	SUV420H2	2.41715	ANKRD13C	0.402613
LASS2	2.39778	SRSF7	3.24581	DHDDS	0.256606	BRP44	0.192542	MAPK7	2.41704	DNAJC16	0.40297
AC135633.1	2.39558	OTUB1	3.23621	RAB7L1	0.256733	ST7	5.19068	NDUFAF4	0.414396	SMARCB1	2.47527
LAMC1	2.39358	ATP5SL	0.309121	B9D1	0.256824	MS4A6B	5.18831	SLC35C2	2.41267	ZFP488	2.47522
PQBP1	0.418668	LDHA	3.2329	GPATCH8	3.89108	LXN	5.18433	FBXW17	2.40956	1110004E09RIK	2.47062
YIPF6	2.38573	CTPS2	3.23201	NT5C3	0.257359	NRD1	0.193068	GM6055	0.415127	DUSP10	2.46978
PPP1R15A	2.38156	GLMN	3.22448	2410017P09RIK	0.257389	1110002B05RIK	5.17424	COL11A2	2.40794	2610030H06RIK	0.406121
PHF20	0.420072	ZMAT5	3.22116	UTY	3.87871	AL844854.1	5.17249	1700034H14RIK	2.4071	SSSCA1	0.40619
GM9775	0.420155	MAP2K2	0.310823	1110012L19RIK	0.258256	MCCC2	5.16899	HNRNPM	2.40693	LGALS3BP	2.46169
H2-Q10	2.37859	COMMD4	3.2157	CCNH	3.86755	MRPL17	5.15904	BOLA1	0.415547	MTM1	2.45805
PHB2	2.37696	PIGP	3.21334	ANKRD32	0.258792	RPP38	0.194425	MNDAL	2.40581	ENTPD5	0.40683
BTF3L4	2.37421	CNPY2	3.21207	TBC1D7	0.259321	LGTN	5.13435	BIRC5	2.40443	TBCB	2.4579
HSCB	2.36574	CHCHD8	3.21086	XLR4A	3.85399	GM16181	5.13293	FOXP1	2.40424	GM2178	0.406996
A930005H10RIK	0.422718	HNRNPH1	3.20272	NINJ1	0.259964	ORC4	0.19501	ANKRD13D	2.4039	CCDC77	2.45456
PPP2R2C	0.422829	LCORL	3.20242	2610001J05RIK	0.260073	SCARB1	5.12455	AI452195	2.40212	ZFP259	0.407721
ATG13	2.36443	TMEM69	3.20107	4930579G24RIK	0.260613	DOT1L	5.11575	ARL8A	0.416667	ZDHHC12	0.407944
AATF	2.36391	S100A6	3.19886	GM14326	0.260714	MRPL23	5.11274	ARMCX6	0.4168	GSK3B	0.408054

Differentially expressed genes for GPR65-/-, PLZP-/- and TOSO-/- Th17 cells											
GPR65-KO-IL1B+IL6+ IL23-96h-1		GPR65-KO-TGFB1+IL6-96h-1		PLZP-KO-IL1B+IL6+IL23-48h-1		PLZP-KO-TGFB1+IL6-48h-1		TOSO-KO-IL1B+IL6+IL23-96h		TOSO-KO-IL1B+IL6+IL23-96h	
Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)
CDK1	0.423207	NFX1	3.19883	STARD3NL	3.83561	COMMD3	0.19575	TRIM56	2.39847	RMND1	0.408142
RABGGTB	0.423371	PDCD6IP	3.19865	VPS39	3.83015	FLAD1	0.195774	RAB43	2.39609	GM13147	0.40828
PNRC2	0.423764	ZRSR2	3.19603	ERCC1	3.82564	MRPS11	5.10387	FGD3	2.39565	AIM2	2.44856
HDAC1	2.35879	ABHD11	3.19565	APPL2	3.82255	IFNGR2	5.10147	TRIM37	2.39431	UHRF1	2.44582
GTF3C2	2.35778	CNBP	3.18437	MKRN1	0.26168	COX10	0.196107	NDUFB4	2.3924	DUSP19	0.409179
PPIL2	2.35711	GM10801	3.18078	PTP4A1	0.261791	ENDOG	0.196494	DUT	0.417992	BC023814	2.44176
TUBB2C	2.35326	H2-D1	3.17421	STK11	3.80655	GLS	5.08921	INSR	2.38695	TBX21	2.44142
UBE2W	2.35082	SPATA5	3.17178	GM10490	3.80099	UBE2E3	5.08578	MTF1	0.419149	LIFR	2.43915
NEIL1	2.34342	PSG23	0.315546	TMEM50B	0.263502	MRPL41	0.196729	FANCE	0.419595	COMMD5	0.410351
UBE2A	0.426773	BRD3	3.16708	GALT	0.264371	NUBP1	5.08253	0610037L13RIK	2.38209	RGP1	2.43434
SLC25A39	0.427532	CAPZA1	3.16705	NAA35	0.264406	DGUOK	5.0798	STK32C	2.3794	DCAF17	2.43375
SIL1	2.33407	ADAM33	0.315962	PCIF1	0.26458	TTC1	0.197229	MRM1	0.420347	TAZ	0.411156
LY6C1	2.33251	AC131780.2	3.16492	AI413582	0.264792	NUPL2	5.06885	FKBP5	2.37896	THAP7	0.411234
H2-KE2	2.32733	A930001N09RIK	3.15992	MRPL16	0.265274	FKBP1A	5.06112	RPL21-PS14	2.37893	TRAPPC3	0.411335
0910001L09RIK	2.32723	GPR19	0.31673	CETN4	0.266008	ABHD10	5.05799	TMEM209	0.420712	MINK1	2.42966
RGS1	2.32238	ARHGDIB	3.1569	RNMTL1	0.266008	GNPDA2	5.05258	PPP2R2D	2.37506	FBXO11	0.411741
MFAP3	0.4308	PSPH	3.14818	LPL	3.75591	4930470H14RIK	5.05027	SNAP23	2.37461	MCM3	0.411754
MTMR4	0.430925	GFPT1	3.14709	SPRYD4	0.266657	AMPD2	5.04453	CHD4	0.421301	UAP1	0.412061
ABHD11	0.431066	RC3H2	0.31779	IFT80	3.74813	ZFP386	0.198234	ZFP110	2.37356	6330577E15RIK	0.412524
THY1	2.3178	PJA2	3.146	GSN	3.74534	LMF1	5.03395	VPS26A	2.37266	SEPW1	2.42171
1500031L02RIK	2.3116	TIMM23	3.14596	PDCL	3.74384	IL2	0.198661	PNKP	0.421521	BAIAP2	2.41981
PEMT	0.432817	PSMA1	3.14408	AGTPBP1	3.7416	ANKHD1	5.02774	TMEM63B	0.422363	USF2	0.413563
CDK2AP1	0.432935	FANCE	0.318156	LUC7L	3.73929	TSPAN14	0.199012	DNAJB11	2.36543	FOLR4	2.41563
RAD54L	0.434173	CDCA7	0.319438	ECE2	0.267644	AC122006.1	5.01152	TYMP	2.36431	RAB14	0.414768
SMU1	0.434279	RPP30	3.12774	1810074P20RIK	3.73582	MAP3K5	5.01152	NDUFA10	0.423107	CRYZL1	0.415122
SMPD2	0.434936	ME2	3.12344	GTF2H4	3.72869	CASP2	0.199854	DARS2	2.36275	BRCC3	0.415153
IFI27L1	2.29594	NAA20	3.12119	1110058L19RIK	0.268254	TTC23	4.9978	CUL1	2.36253	WDR3	0.41543

Differentially expressed genes for GPR65-/-, PLZP-/- and TOSO-/- Th17 cells											
GPR65-KO-IL1B+IL6+ IL23-96h-1		GPR65-KO-TGFB1+IL6-96h-1		PLZP-KO-IL1B+IL6+IL23-48h-1		PLZP-KO-TGFB1+IL6-48h-1		TOSO-KO-IL1B+IL6+IL23-96h		TOSO-KO-IL1B+IL6+IL23-96h	
Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)
RSL24D1	2.29428	PKP4	0.320792	GM10212	3.72679	GM10695	4.99638	PAPD5	0.423809	FAM60A	0.415574
SFRS18	0.436007	FYTTD1	3.1168	TMEM93	3.72279	ETV4	4.9814	2700097O09RIK	2.35561	CAPN2	0.415688
PIK3CD	0.436753	PKN1	3.11594	GRCC10	3.7191	HSD3B2	4.97154	D4ERTD22E	2.35549	ADPGK	0.415859
HVCN1	2.28932	4933421E11RIK	3.11448	TFG	3.71634	ESF1	0.201313	GTF3A	0.424646	ADSSL1	0.41591
SEMA4D	0.436848	CDC23	3.09954	BRCC3	0.269708	SNX17	4.94502	ARNT	2.35173	MARCKSL1	0.415994
BC003266	2.28841	CLDND1	3.09523	PDK3	3.70624	CCND3	0.202376	PARD6A	2.3498	AGTPBP1	2.40301
FAM165B	0.43747	ACTR2	3.08416	GGCT	0.269918	NSMCE2	4.93656	INTS2	2.34851	RUFY1	0.417223
IL24	2.28483	ESF1	3.07944	TM9SF1	3.70468	TYMS	4.93404	ATP5K	2.34778	PROCR	0.41754
TBPL1	2.28311	STAT1	3.07825	EDC3	0.270247	KRT19	4.93225	DNAJA1	2.34478	HSD3B2	2.39353
CPNE8	0.438106	FPR2	0.325105	OSBPL9	3.69825	STXBP3A	4.91429	ADK	0.426564	L7RN6	0.418484
ANKRD37	2.28006	1700047G07RIK	0.32513	ACADM	0.270559	RHOQ	4.90855	ABHD11	2.34376	VWA5A	0.41858
MSL3	0.439112	PLEKHA2	3.07279	2900010J23RIK	0.270662	CRCP	4.90201	GM5830	2.34002	TESC	2.38873
PIGF	0.439876	EIF3E	3.07275	PMS1	0.270738	1700049G17RIK	4.89947	KPNB1	2.33972	LAP3	0.419111
EPHX4	2.27228	POR	3.07274	6530401N04RIK	3.68326	PSTK	0.204421	IFNAR1	2.33894	BIN3	2.38331
1500002O20RIK	2.26953	NSUN5	0.325629	DLGAP4	0.271558	FOXM1	0.204562	DNAJB4	0.427629	ZDHHC21	2.38218
BCAT1	0.440655	BHMT2	0.325884	PAFAH1B3	0.271558	TLCD2	4.88197	XPO6	2.33846	TRAF3IP3	2.37848
ZFP58	0.440674	ACAT1	3.06557	UTP3	0.271746	RDH9	4.8736	FAR1	2.33826	SLC25A10	0.420602
AHSA2	0.441142	SLC12A8	0.326475	GM7609	3.67655	OBFC2B	0.205823	RAB31	0.42773	WDR73	2.37632
AC154631.1	0.441629	MRPL23	3.05711	C630004H02RIK	0.272053	LSM2	4.85528	POLR2L	2.33638	PIF1	2.37403
FERMT3	2.2639	MAPK8IP3	0.327393	SIRT4	0.272685	WAC	4.8472	CYB561D2	0.429112	SUV420H2	2.36921
PDCD2L	0.441746	SUMO1	3.05309	2700007P21RIK	0.273377	1700021F05RIK	4.83563	KPNA2	0.429464	PHYHIPL	2.36779
LYRM4	0.442065	TESC	3.05301	TRIT1	0.273748	DSN1	4.82271	5730601F06RIK	0.429723	TMEM97	2.36561
PHOSPHO2	0.4425	TMEM9B	3.05154	TMC5	3.65153	GM9894	0.207352	PIGQ	0.429912	HOOK2	0.422724
OIP5	2.2582	ZFP637	3.04922	GM5244	3.63364	FBXW9	0.207469	AURKB	0.430777	GMFG	2.36501
PGAM5	2.25599	MRPL24	3.04409	GDE1	0.275214	PGAM5	4.81491	TH1L	2.32119	NOL11	0.422881
GM6293	2.25379	TBCB	3.04279	GTF2H3	3.62942	GM5576	0.207801	FAM184A	2.31742	CLTB	2.3623
PDSS1	0.444757	ETS1	3.04198	GNPAT	0.27554	SNAP23	4.81051	GSTT3	0.431811	FAM136A	2.35444

Differentially expressed genes for GPR65-/-, PLZP-/- and TOSO-/- Th17 cells											
GPR65-KO-IL1B+IL6+ IL23-96h-1		GPR65-KO-TGFB1+IL6-96h-1		PLZP-KO-IL1B+IL6+IL23-48h-1		PLZP-KO-TGFB1+IL6-48h-1		TOSO-KO-IL1B+IL6+IL23-96h		TOSO-KO-IL1B+IL6+IL23-96h	
Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)
VBP1	0.445036	SDR39U1	3.04125	HSD3B2	3.6278	C2CD3	4.81043	CHD8	0.432011	MOSPD3	0.425477
IFT46	0.445227	SERPINB1C	3.03727	DCP1B	0.275893	HMOX1	4.80258	ODF2	2.31431	PHF7	2.34785
GPR174	0.445264	FABP5	3.03405	DHX32	0.275893	HDDC2	0.208325	GM16380	0.432609	ITGB1	0.426033
TES	0.445357	1110003E01RIK	0.329743	GM5617	3.62459	HSPA12B	4.79693	DHX32	2.31124	TWF1	2.34335
H2-Q2	2.24237	UQCRC2	3.02946	ZCCHC7	0.275933	CCDC34	4.79632	CELF2	0.432691	CTSO	2.33801
GAPVD1	0.446533	MGAT4C	0.330276	CASP8AP2	0.276211	TMUB2	4.79307	TUT1	2.31019	ACP5	0.427721
PANX1	0.447449	TIPIN	3.02717	DPF2	0.276612	AC142104.1	4.79143	AFF1	2.30981	RBM43	2.33786
RBM38	2.23439	RPS6KB1	3.02517	MBD5	3.61261	CDK5RAP1	0.209144	POMP	0.432971	TMC5	0.427846
PUM1	0.448127	APOBEC3	3.02458	CERKL	3.60774	TMBIM1	4.77378	PITRM1	2.30782	GM3435	2.33728
PER1	2.22917	POLR2F	3.02026	THUMPD3	0.277258	IL11	0.209998	CYB5D1	2.3069	WASL	0.428335
MAEA	0.44936	TMEM218	3.01908	LMF1	3.60375	NIPSNAP3B	4.75276	MED25	0.434109	ANKRD16	0.429125
RBP7	0.449786	1700123O20RIK	3.01794	ARRDC1	3.60208	CDC40	0.210631	MTUS2	2.30321	GM5577	2.32926
6330439K17RIK	2.22281	OSBPL2	3.01609	GIMAP9	3.59942	ZC3H10	4.74138	AAGAB	0.434276	1810009A15RIK	2.3291
PPIL5	0.450513	RBMXRT	3.0158	CIZ1	0.278198	DEPDC5	4.73809	GTPBP5	2.30251	LRRC40	0.429404
TIMM8B	0.4519	PTMA	3.01477	ALG9	0.278323	CCDC6	4.73184	SLAIN1	2.3004	42068	0.429453
TKT	2.21139	RBM22	0.331773	ADRBK1	3.59128	ZDHHC12	0.211334	ZFP609	2.30028	ACSS2	0.429515
GM4877	0.452772	SAT1	3.01393	INSL6	0.278702	4930522L14RIK	4.72965	TRUB2	0.434877	GM4825	2.32433
TTC23	0.452878	2410004P03RIK	0.332288	KANK3	3.57822	METTL8	0.211586	VMAC	2.29809	H2-Q7	2.32323
DPYD	0.45312	ADAMTSL4	0.332405	VPS4B	0.279789	DCTN3	4.72301	S100A1	0.435407	STARD3	0.430584
FAM103A1	0.453256	D4WSU53E	3.00463	PTTG1IP	0.279925	BCCIP	0.211808	TOMM40L	2.29491	MPHOSPH9	0.431148
CYB5R3	2.20106	GM6104	0.333215	ZFP738	3.56755	CDKN2AIPNL	4.71636	INTS12	0.435833	METT5D1	0.432089
GPR89	0.454538	PRPSAP1	0.33323	NDRG1	3.5565	PIGN	4.71511	BC031181	2.29133	MYG1	0.432492
PICK1	2.19989	GM10979	0.333439	CENPH	3.55546	PIGQ	0.212219	ZFP60	2.29105	PPIH	2.31213
ARL6IP4	2.1967	ING3	2.99657	MLH1	0.281258	RBM28	0.212315	DBR1	0.436565	EIF5	0.433605
H2-K2	2.19661	SMARCA5	2.99274	GIT2	3.5547	TARBP2	4.70766	RBM34	2.29054	SNRNP35	0.433755
GDE1	0.455548	SRP19	2.98391	CDC20	0.281777	AC161001.1	4.70369	KIF21B	2.2878	0610011F06RIK	0.43426
AC079644.1	2.19361	INPP5F	0.335371	EXOSC4	3.54589	CDK14	4.70157	UBN2	2.28747	PPAN	0.434394

Differentially expressed genes for GPR65-/-, PLZP-/- and TOSO-/- Th17 cells											
GPR65-KO-IL1B+IL6+ IL23-96h-1		GPR65-KO-TGFB1+IL6-96h-1		PLZP-KO-IL1B+IL6+IL23-48h-1		PLZP-KO-TGFB1+IL6-48h-1		TOSO-KO-IL1B+IL6+IL23-96h		TOSO-KO-IL1B+IL6+IL23-96h	
Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)
GM16381	2.19361	STX6	0.335692	TRPM1	0.282101	RAD18	0.212948	BAT5	2.28739	ATP13A3	0.434517
GM2001	2.19361	GM10123	2.97672	CMAS	3.54427	DPY19L4	4.69293	RGS19	2.2834	ELOVL1	0.434824
GM5670	0.455882	CCT5	2.97616	HCFC2	3.53925	HIBADH	4.68299	TM9SF1	0.438314	GM7935	0.435003
LEPR	2.1916	NT5C3	2.97287	ADIG	3.53563	HNRNPL	0.213539	XBP1	0.43854	2310045N01RIK	2.29852
HOPX	2.18948	PIM2	2.97224	CATSPER4	3.53563	FAM175A	4.68088	H6PD	2.27792	4930555F03RIK	2.2952
CLSPN	2.18611	LY6E	2.96994	HERPUD1	0.282919	SYTL3	4.66871	SEMA4A	0.438999	MRPL16	0.435779
AKR1B8	0.457558	TTLL4	2.96786	IQCC	0.282927	GGA3	4.653	RABEP2	2.27703	CYBASC3	0.436256
GRCC10	2.18497	PTPRC	2.95901	EIF2B4	0.283034	IFFO2	4.65076	RG9MTD3	2.27613	HIST1H2BB	2.29128
POLE4	0.457719	PKM2	2.95759	S100A6	0.2837	POLB	4.64779	2610020H08RIK	0.439923	GBP5	2.29067
GPRASP2	0.457873	SYCP1	2.95358	TGS1	0.283813	GM2938	4.64434	MPDU1	2.27308	WDR77	0.436786
BC056474	0.458157	ACOT13	2.95291	PCCB	0.283949	GRAMD3	4.64089	HEMK1	2.27166	1700034H14RIK	0.436958
CIDEC	0.458486	ADAM19	0.339011	FOXK2	3.52152	9430023L20RIK	4.63579	NDOR1	0.440514	RBM7	0.437622
FNBP1	0.458581	1110065P20RIK	2.94794	LY6C2	3.51312	ATPBD4	4.63232	BZRAP1	0.440638	BRWD1	0.437664
SAE1	2.17964	AIMP2	2.94187	METT11D1	3.50825	CREBL2	4.62929	SRBD1	2.26829	WDR46	2.28303
TFIP11	0.458874	RDM1	2.9385	ARID4B	0.285507	HDHD3	0.216275	RDH14	2.26786	4930534B04RIK	2.28142
RPL30-PS6	2.17678	ZCRB1	0.340327	SGK1	3.4986	GSS	4.62133	DAZAP1	0.441077	4933427I04RIK	2.27929
ADAR	0.459489	DAPK2	0.340716	8430423G03RIK	3.49655	POLD4	4.61637	TRIB3	0.441663	BC023829	0.439785
PGS1	2.17398	LRRC41	0.341072	EXTL2	3.49509	DNAJB11	4.61387	2810422O20RIK	2.26358	SGSM3	2.27323
GPR107	0.460142	STARD3NL	2.93172	CENPK	0.286116	CDK2AP2	4.60874	STX2	2.26259	TOR1B	0.440344
TIMM17B	2.17137	GM11152	0.341478	PAM16	3.4935	VPS36	4.60218	GABPB2	2.26178	FLAD1	0.440699
STAM2	2.1672	MRPS18A	2.91805	RALB	3.49078	CD74	0.217596	FAM126A	2.26122	VEPH1	2.26833
GAA	0.461615	ORMDL3	0.343151	ZBED4	3.48917	TMEM106C	4.58509	TFB2M	2.25777	6030422M02RIK	2.26531
TRAPPC3	0.461743	GHITM	2.91234	STIM2	3.48912	ZFP353	4.58439	ECHDC1	2.25729	SCARB2	0.441666
PAFAH1B3	2.16551	STRN4	0.343765	4930547N16RIK	0.286625	PHRF1	4.57943	ANKRD32	2.25421	ST6GALNAC6	2.26353
PRAMEL6	0.461853	AZI2	2.90738	TRPC2	0.286652	PDDC1	0.218373	EPHA2	0.444115	NRF1	0.442264
LPHN3	0.462371	GM7030	2.90617	ING3	3.4874	CORO7	4.57843	NSUN3	0.444483	GJC3	2.26072
PCBP3	2.16243	RTP3	0.34424	DGCR6	3.48344	GTF2H4	4.57703	SHARPIN	2.24975	PPPDE2	0.442814

Differentially expressed genes for GPR65-/-, PLZP-/- and TOSO-/- Th17 cells											
GPR65-KO-IL1B+IL6+ IL23-96h-1		GPR65-KO-TGFB1+IL6-96h-1		PLZP-KO-IL1B+IL6+IL23-48h-1		PLZP-KO-TGFB1+IL6-48h-1		TOSO-KO-IL1B+IL6+IL23-96h		TOSO-KO-IL1B+IL6+IL23-96h	
Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)
SRSF3	0.46284	COPS2	2.90125	BOLA1	0.287478	TTC35	4.57584	LRRC8C	2.24954	L1CAM	0.442979
PET112L	0.465325	GM10451	0.344691	HIST1H4D	0.287859	6030408B16RIK	4.56696	ATP2B4	2.2494	RPAP2	2.25699
1500012F01RIK	0.465366	CALM2	2.90089	GM2938	3.46952	JAK1	4.55797	RASL11B	2.2486	DPY19L4	0.443354
SHISA5	2.14857	ICAM1	2.89977	PSAP	3.45928	PRAMEF8	4.55729	TTI1	2.24819	MFN2	0.443758
SH2D3C	0.466011	HSPA14	2.89926	AC161211.2	3.45693	GTPBP8	4.55576	RFXAP	2.24717	CCDC84	0.444341
MRPS28	0.466172	MED14	2.8974	SLC16A6	0.289278	FAM162A	0.219795	LRRC33	2.24323	NR4A2	0.444708
IL4	0.467198	EBP	2.89522	GNPDA2	0.289466	CNOT6L	0.219928	AC101875.1	2.23945	PARVA	2.24781
HNRNPC	0.467546	ACAT3	2.89508	COX17	3.44155	MTUS2	4.54291	CDK5RAP1	2.23785	CCPG1	0.445004
RTF1	2.13572	2310035K24RIK	2.89501	MPDU1	3.44092	ZMYND11	4.53646	SETDB1	0.447154	H2AFX	2.2465
IDH3G	2.13392	BC057079	0.345461	PNPLA7	3.4408	SFPQ	0.220524	TELO2	0.447155	MRPL1	2.24561
MFSD2A	0.469366	CRISP4	0.345759	COX10	0.291276	THUMPD3	0.22081	VTA1	2.2359	2900097C17RIK	2.2443
CLN3	0.470116	SNRNP25	0.346171	SETD5	3.43074	DNAJB6	4.52642	ZFP426	2.23532	ADI1	2.24225
CYP51	0.470341	ARRB1	0.346338	TNF	3.42383	CENPH	0.221034	MSL3	2.23499	GRAP2	0.446283
CARS	2.12414	GM10719	2.88708	TRAPPC6B	0.292286	STK38L	4.51851	SSNA1	2.23311	IKZF3	2.24007
ACAT3	0.471553	AL603711.1	0.346453	ERI3	3.4132	ZFP110	0.221331	SNRPG	0.448137	UTP6	0.44674
ETFB	2.11968	SLC25A1	2.88624	USP33	0.29313	ZDHHC6	4.51423	SLC28A2	0.448712	LCORL	0.447019
ATRIP	0.472654	CLK2	2.88431	DIAP1	0.293347	GM5623	0.221694	EXOSC7	2.22748	SEC23B	2.23703
NSMCE1	2.11554	GM11042	0.346709	PKP3	0.293441	HIST1H4K	4.51023	HELZ	0.44939	LEPREL2	2.23611
DHRS1	0.473178	LGALS4	0.347111	DCBLD2	3.40187	UBE2K	0.221837	MGAT4A	2.22469	GM9762	0.447916
GM10250	0.473386	CCDC97	2.87776	IKBKB	0.293957	AL732476.1	4.50643	C330027C09RIK	2.22406	SLC25A23	0.448019
SVOP	2.11244	ITGA3	2.87471	PRPF3	0.294636	RPF1	0.222192	FAM33A	2.22079	MRPS33	2.23185
GBP3	0.473443	BC026585	0.347865	FNBP4	3.39347	EFTUD1	0.222611	DIS3L2	2.22056	CORO2A	0.448298
TSPO	2.11212	NDUFB11	2.87217	PHOSPHO2	0.294693	METTL6	0.222665	PRPS2	0.450339	STK17B	0.448479
FAM45A	0.473528	SLC5A11	0.34834	NFYC	0.294786	AGA	0.222796	ELP4	2.21858	YKT6	0.448781
NEK2	2.1112	NDUFA8	2.86842	MCOLN2	3.3836	MGST2	4.486	GLRX2	2.21715	RCBTB2	0.449053
DGAT1	0.474097	BUB1B	2.86674	PDAP1	0.295633	PMPCB	4.47916	TCP11L1	2.21687	GIT1	2.2222
CENPH	0.474097	RHBDL2	0.349214	NFYB	3.37877	LZTFL1	0.223606	NFS1	2.21653	AC156948.1	2.22018

Differentially expressed genes for GPR65-/-, PLZP-/- and TOSO-/- Th17 cells											
GPR65-KO-IL1B+IL6+ IL23-96h-1		GPR65-KO-TGFB1+IL6-96h-1		PLZP-KO-IL1B+IL6+IL23-48h-1		PLZP-KO-TGFB1+IL6-48h-1		TOSO-KO-IL1B+IL6+IL23-96h		TOSO-KO-IL1B+IL6+IL23-96h	
Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)
SGSM3	0.474555	CYB5	2.86303	MRPL2	0.296363	DTWD1	4.47201	TMC6	0.451846	LEO1	0.450433
TRIM30B	0.474604	PDCD1	2.86295	DTWD1	0.29648	REPS1	4.46966	MYEOV2	2.21222	MVP	0.45048
FDXR	0.47544	CAPRIN2	0.349369	GM10033	3.37291	REXO4	4.46788	PFDN2	0.452543	RDM1	2.21862
TOMM20	2.10061	DHRS1	0.349492	STRN4	3.36855	MRPS15	4.46494	TMEM161A	2.20829	FAM192A	2.2176
PDAP1	0.477104	SH3GLB1	2.85718	SEC61A2	0.296884	RAC1	0.223967	CHRM4	2.2041	TBL3	2.21522
PTPMT1	2.09393	TCF4	0.350483	ACER2	3.3672	EIF4ENIF1	4.43929	E130309D02RIK	0.453787	1110008L16RIK	2.21368
SIGMAR1	0.478621	TRIAP1	2.85065	BUB1B	0.297187	NRF1	4.43836	NPEPPS	2.20295	UVRAG	0.452127
BBS7	0.47905	FUBP3	2.84969	GTDC1	3.36386	SPINT2	0.225426	DNAJB2	0.454667	GLRX5	2.20846
TNFSF13B	0.479792	CENPF	0.351001	GADD45G	3.36234	PLOD2	4.43373	GM2178	0.454756	2510003E04RIK	0.452882
PARP2	2.08299	LY6F	2.84688	TM2D2	0.297412	NDUFAF2	4.43157	MS4A6B	2.19789	NUFIP2	0.453053
NUDT3	2.08262	GM14181	0.351511	TOMM34	3.35824	ABHD6	0.225748	DOS	2.19472	TK1	0.453355
TTC5	2.08224	TPI1	2.84474	DYNLL2	0.297932	GTF3C5	4.42774	TBX21	2.19429	PPP1R12A	0.453602
LRRC24	0.480779	LMNA	2.83893	MTERFD1	3.35647	TXNIP	4.41587	FBXO44	0.456012	MAX	2.20405
NAA20	0.48164	TMEM55B	0.352678	TFAM	3.35624	SNX3	0.226596	CTLA2B	2.1924	PLIN2	0.453764
EIF1AX	0.481816	IFI47	2.82703	FLT3L	3.34759	TM9SF4	4.41067	4921517L17RIK	2.19238	DNAJA2	0.453795
MRPS36	0.481983	GM5145	2.82597	NOL7	0.298838	BBS9	4.40793	AC165266.1	0.456577	MTF2	0.453888
COX6B2	0.482287	ADK	2.82127	CTSE	3.34344	SEC23A	4.40537	PPRC1	2.18911	F2RL1	0.455044
GTPBP8	0.482307	AC149585.1	0.35473	2810422J05RIK	3.34306	UBLCP1	4.40451	BCAS3	0.457248	FBXO3	0.455736
CHI3L1	0.482918	NAT9	2.81543	MIA1	3.34135	NT5C	4.40436	PSMB6	0.457575	GM10417	2.19193
SIGIRR	2.07058	XRN2	2.81516	EIF4H	0.299847	POLR2H	4.40262	TMEM120B	0.457765	ZER1	0.456295
GM11273	2.06922	SCMH1	0.355375	THAP7	0.300809	CDC42SE1	4.40229	CDK16	2.1831	PREX1	0.456446
GM9830	0.483586	GM5160	2.81271	CREB1	3.32323	TNFAIP3	0.227358	2310011J03RIK	2.18273	RPL21-PS7	0.456737
DBR1	0.483831	HFM1	0.355716	GM2833	0.300988	PRR15	0.227365	GPR89	0.458367	IGSF8	0.456869
LEPREL1	0.483856	D18ERTD653E	2.80732	SRSF9	0.301296	TNFSF13B	4.3957	ARL5C	2.18109	MAPK3	0.457086
CRYZL1	0.484085	4933427I04RIK	0.356243	PFDN2	0.301424	NUDC	0.227573	GSTK1	0.45855	5730469M10RIK	2.1868
CCDC127	0.484708	ARHGAP4	2.80704	PIGYL	3.31608	ZFPL1	4.3942	DSTN	2.18006	SEMA4D	0.457713
RNF7	2.05833	PRAMEF8	2.80697	GM8055	3.31475	C2	0.227783	SEC23B	0.458803	MYCBP2	2.18452

Differentially expressed genes for GPR65-/-, PLZP-/- and TOSO-/- Th17 cells											
GPR65-KO-IL1B+IL6+ IL23-96h-1		GPR65-KO-TGFB1+IL6-96h-1		PLZP-KO-IL1B+IL6+IL23-48h-1		PLZP-KO-TGFB1+IL6-48h-1		TOSO-KO-IL1B+IL6+IL23-96h		TOSO-KO-IL1B+IL6+IL23-96h	
Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)
ACTC1	2.05784	CCR7	2.80169	REST	0.30191	NGRN	0.227815	FTSJ1	2.17933	STX8	2.17767
GM8815	2.05722	G3BP1	2.80063	SP100	3.31134	CRYZL1	4.38778	MEF2A	0.459317	NOL12	2.17683
TBC1D10C	2.05628	ADAMTSL5	0.357385	OAS1G	0.302131	PSMD5	4.38291	CDK2AP1	2.17715	TOP3B	0.460001
OSCAR	0.486345	HSDL2	2.79789	RASA1	3.30054	CBLL1	0.229251	TANK	2.1771	HECTD2	0.460161
GM8909	2.05336	SDHD	2.79732	MAPKAPK5	3.29877	FOLR4	4.36204	AC125221.1	0.459349	IKBKAP	0.460335
NCOA7	2.05066	LRRK1	0.35776	SLC4A1AP	0.303347	PRMT1	4.36011	MPHOSPH6	2.17579	DGUOK	0.460441
TRNT1	0.487822	PSMD5	2.79458	SQSTM1	3.29316	OPCML	4.35887	GM7367	2.1738	R3HDM2	0.460494
AIRE	2.04966	HSD17B12	2.79424	COX19	3.29302	CD200	0.229479	AC163101.1	0.460169	STIM2	2.17149
MRPS18B	0.48936	KIF18B	0.357954	GM12184	0.303672	HSD17B7	4.34864	CALD1	2.17236	IPO9	0.460607
AC113307.1	0.490348	GTF2E2	2.79364	MAPKAP1	0.304115	OTUD7B	4.34571	ZFP125	2.17183	TCP11L1	2.17006
PA2G4	0.490583	RP23-147O14.1	2.79357	TRMU	0.304377	ZCCHC9	4.3401	ALG5	0.460528	UQCRC1	0.46127
VPS8	0.490681	ACNAT1	2.79048	ITGB1	0.30453	ITGAM	0.230433	CNIH4	2.17113	DYNC1H1	2.16781
UBE2F	0.490797	GOSR2	2.78985	8430410A17RIK	3.28293	TIAL1	0.230539	GM10180	2.17074	TM7SF3	2.16685
DDX50	0.491492	SNRPE	2.78815	TMEM106B	3.27349	KATNAL2	4.33361	NAPG	0.460711	PAPOLG	2.16558
LCTL	0.491521	3110057O12RIK	2.78673	TUBD1	0.305922	FTO	0.231057	CCNK	0.460907	UBE1Y1	2.16452
PWP1	2.03349	TBPL1	2.78564	GET4	3.26735	SLC12A8	4.3262	1110014N23RIK	0.461185	COPG	0.462215
TMEM167	0.491829	5730437N04RIK	2.78518	ZFP560	0.306077	GM6624	4.32377	NDEL1	2.16644	CREB3	0.46359
TRABD	2.0272	FGGY	0.359534	RG9MTD3	0.307657	CEP63	0.231391	TOM1L2	2.16555	DHX32	2.15693
PCNA	2.02689	MAP4K2	2.77986	RPS6KB2	3.24669	TM9SF3	0.231488	VARS2	2.16514	PHRF1	2.15662
SFT2D1	0.493485	DIAP1	2.77962	1500011B03RIK	0.308119	ASCC1	4.31718	BBS9	0.461886	RNF220	2.15494
IFRD1	0.494308	TUBA1C	2.7781	MAP2K5	0.308611	TBCE	4.31053	ERH	0.461997	DNAJB6	0.464138
RPS6KA6	0.495289	AI462493	2.77233	GM5890	0.308934	ELMOD2	4.30615	EVL	2.16263	BCLAF1	0.464892
FBXO4	0.495816	N6AMT2	2.77103	LSM6	0.30901	SMARCD2	4.3011	FAM58B	2.1614	2210012G02RIK	0.464973
IRF6	2.01593	PPIA	2.76671	SESTD1	0.309995	BUB3	4.29962	1810014F10RIK	0.462829	TFPT	2.14718
TIMM13	2.0151	A430093F15RIK	2.76654	AIG1	3.22462	SLC20A1	4.29733	BPNT1	0.463089	H2-DMA	2.14258
HEATR3	0.497245	TSR1	2.76595	SLC25A14	0.310115	GPN2	0.233055	AKAP9	2.15875	UQCRQ	2.14201
CNN3	0.497368	AC120410.1	2.76426	TMEM39A	3.22291	SLU7	0.233292	SLC30A4	2.15757	RBBP6	2.14017

Differentially expressed genes for GPR65-/-, PLZP-/- and TOSO-/- Th17 cells											
GPR65-KO-IL1B+IL6+ IL23-96h-1		GPR65-KO-TGFB1+IL6-96h-1		PLZP-KO-IL1B+IL6+IL23-48h-1		PLZP-KO-TGFB1+IL6-48h-1		TOSO-KO-IL1B+IL6+IL23-96h		TOSO-KO-IL1B+IL6+IL23-96h	
Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)
GM6351	0.498605	TGOLN1	2.76399	0610010K14RIK	3.21932	MS4A6D	4.27783	UBTF	2.15703	WBSCR27	0.467399
RTN3	2.00547	1810012P15RIK	0.361885	AC132397.1	0.311155	VTI1B	4.27576	TSR1	0.463691	NLRC3	2.13944
OLFR345	0.499759	GM4979	0.362023	WWOX	3.21153	PI4KA	4.27384	INTS9	0.463911	NAAA	2.13651
CCDC55	0.500381	TMED7	0.362038	RP9	3.20928	GM10208	0.234478	AC132391.1	2.15509	SRR	0.468115
GAR1	0.502431	TRP53	2.758	CHCHD5	0.311661	MLX	4.25504	FKBP15	2.15391	BC016423	0.468265
CCR8	1.98996	CETN3	2.75738	RANGAP1	0.311673	HAUS7	0.235016	GM13308	2.15066	TMPRSS11BNL	2.13355
HSDL2	1.9894	CTNNBL1	2.75612	FYN	0.311934	ARGLU1	4.25041	TXNRD2	0.46555	MCM6	0.468971
RTCD1	0.502788	USMG5	2.75505	GPLD1	3.2021	TGIF1	0.235529	PWP1	0.465791	GABARAPL2	2.13081
2900092E17RIK	1.98882	ORF19	0.363004	DNAJA1	3.1971	GTF3C2	0.235537	TMEM220	2.14674	MYC	2.12935
ACLY	1.9886	RP23-389D15.1	0.363122	42253	0.312944	ADM	0.235992	PDE7A	2.14661	PSENEN	2.1288
1110059E24RIK	0.503225	CORO1C	0.363195	IL23A	0.313055	DSCR3	0.236114	CGRRF1	0.466117	ADCK4	2.12453
CAPRIN1	0.503311	AC131780.1	2.75298	PRL8A1	3.19363	RNF13	4.23063	IL17F	0.466476	2610020H08RIK	2.1236
FAM129B	1.98337	KBTBD4	2.75195	SEPP1	0.313428	PPAP2C	4.22014	HIST4H4	0.466639	COQ6	0.470918
MTHFS	0.504917	RPL7A-PS10	2.75035	NDUFB7	3.18801	GM129	0.237507	ALDH4A1	0.466655	TRRAP	2.12216
STAU1	1.97701	2610204G22RIK	0.364174	WDR35	0.313734	CRTC2	4.20833	MRPL20	2.14273	ERGIC2	0.471759
TLE6	0.505982	GM10750	0.364482	CSF2	0.313826	ANKRD46	4.20651	CLEC4A2	0.466949	HYOU1	0.471895
1190002H23RIK	1.97612	IKZF5	0.364538	RER1	0.314012	TOR1A	0.237885	UBXN2A	2.13985	PTPRCAP	2.1184
CD40LG	1.97553	NPEPPS	2.73802	RECQL	3.18209	ZNF512B	4.19972	FAM82B	0.467589	TOMM70A	0.472127
STAT5A	0.506535	4932425I24RIK	0.365333	STAG1	0.314267	SPRED1	0.238232	HIST1H1B	0.467605	TCIRG1	0.472379
FHDC1	0.506963	GNL2	2.73438	NKAP	3.18169	MRPL50	4.19615	MAP2K5	2.13721	MRPL35	2.11517
NRBP1	0.507055	UGT1A6A	0.366222	PTGR2	3.1815	ZC3H15	0.238561	STRN	2.13357	BRP16	2.1119
RHOC	0.507238	STAG1	0.366399	SIRT3	3.18125	GINS4	0.238992	GM10736	2.13349	CYB5R1	0.473998
SIDT2	0.507307	UBE2J2	0.366474	CCBL1	0.314523	1700020C11RIK	0.239037	CDKN2C	2.1312	PFKP	0.474076
LPCAT4	0.507401	NIPSNAP1	0.366488	KIF3A	3.17297	KDELR3	0.239351	EPS15	2.13044	TIMM22	0.474165
1700009P17RIK	0.50749	UBC	2.72581	2310061C15RIK	0.315197	DUSP23	0.239468	2510002D24RIK	0.469557	PRDX1	0.474435
GPN3	0.508025	PDIK1L	0.367074	PDHX	3.17102	ACAD11	4.17327	VTI1A	2.12789	TOP1MT	2.10729
POP7	1.96773	PFKFB2	0.367114	GALNT6	0.316141	CLCC1	4.17103	CCR8	0.469985	COX15	0.474648

Differentially expressed genes for GPR65-/-, PLZP-/- and TOSO-/- Th17 cells											
GPR65-KO-IL1B+IL6+ IL23-96h-1		GPR65-KO-TGFB1+IL6-96h-1		PLZP-KO-IL1B+IL6+IL23-48h-1		PLZP-KO-TGFB1+IL6-48h-1		TOSO-KO-IL1B+IL6+IL23-96h		TOSO-KO-IL1B+IL6+IL23-96h	
Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)
TMEM106C	0.508505	CCDC93	0.367484	ALG1	0.316257	NDUFA10	4.16873	IRGM1	2.12683	4933421E11RIK	0.475088
GBA2	0.509279	ZFP260	2.72025	ORAOV1	0.316266	SEPP1	4.16486	UBE2M	0.47037	AIF1L	2.10471
ING1	0.509737	RNF38	0.367695	PEX3	0.316448	ATG13	4.16056	RELT	0.470413	PATZ1	0.475465
ATP5G2	0.50999	ADD1	2.71941	TRIM12C	3.15835	ING2	4.15707	GBP8	2.12493	NDRG1	0.476038
ZMYND15	0.510139	EEF1G	2.71874	CR974466.3	3.1556	GM13540	0.24064	MFSD5	0.471448	GM6404	2.09989
RAMP1	1.95994	MARK2	0.368465	WIPI2	0.316989	H2-M3	0.240682	LCMT1	0.471778	SLC35C1	0.476217
TUBE1	1.95881	KLF7	2.71385	TRIB2	0.317126	ERP44	0.240825	KPNA6	2.1164	EPB4.1	0.476245
COMMD2	0.510898	5730403B10RIK	0.368507	HTT	0.317342	OVGP1	4.14954	TMX1	2.116	IL5RA	2.09889
FAM76A	0.511198	TMEM176B	2.713	GM10355	0.317373	TEX264	0.241296	BET1L	2.1144	DPH3	2.09784
OSGIN1	0.511961	IL1F9	0.36898	PABPC1	0.317586	GSPT1	4.14142	ADARB1	0.473036	MED30	0.476857
GM10479	0.512029	RNH1	2.709	METTL1	3.14705	MRPL24	4.14044	RPL30-PS6	0.473359	FGF13	0.477104
CCDC155	0.512097	TXNDC17	2.70692	BIN3	0.317891	NARFL	4.13729	FBXL8	2.11176	LRCH1	2.09545
AP2S1	0.513282	ARL3	2.70455	EIF1AD	0.318045	HMBOX1	0.241991	CTSL	0.47388	PHACTR4	0.477394
GM5356	1.94757	NAPG	2.70085	SLC7A3	0.318191	MRPL40	4.13221	0610007C21RIK	2.10994	ENTPD1	2.09064
GM2004	0.513559	COX5A	2.69935	ACSL6	3.14156	AP3M1	0.242416	AMDHD2	0.473971	ELF4	0.478486
ZMYM1	1.94678	ARFGAP3	2.69573	TIMP1	3.14129	RILPL2	4.12217	IFITM7	2.10784	5133401N09RIK	2.08776
YIPF3	1.94037	B230208H17RIK	0.371107	H2-M3	0.318527	BC056474	0.242985	PRKD3	2.10658	GM5244	2.08734
NDUFB4	1.93997	CCT2	2.69382	HNRNPD	0.318867	LAMC1	0.243258	DPP7	0.474707	TXNDC5	0.479354
SLC5A6	1.9379	EXTL1	0.371383	SMARCE1	0.318939	C1GALT1C1	0.243391	AHCYL1	0.475079	DBR1	0.479424
SLPI	0.516184	2210418O10RIK	0.371465	FYTTD1	0.318977	UTP6	4.10415	SNRPE	0.475442	PSME2	2.08388
STXBP3B	0.516692	PAK2	0.371564	ZFP68	0.319157	HELQ	0.243841	KDM1A	2.10326	GLB1	0.481116
ODF2	1.93096	MAN1B1	0.371606	GRK4	3.13139	CNPY2	4.0997	ASAH1	2.10298	PYGL	0.481326
MYO1B	1.92966	ABHD14A	2.68887	NCALD	3.12826	CTSE	4.09769	NBEAL2	2.1018	ZNRD1	2.07589
PABPN1	0.51825	AQP3	2.68602	VDAC2	0.320477	FUNDC2	4.09626	TMEM223	2.10067	DDB1	0.482269
FAM119A	0.519745	GM14443	0.372325	WDR5	3.11549	AATF	0.244143	BC016495	2.09905	RDH1	2.07068
HSP90B1	0.519761	PTS	2.68215	PIGN	3.11357	BAZ2B	4.09403	MTMR14	0.477007	1810006K21RIK	2.06959
FAAH	1.92212	COX7A2	2.67593	4933411K20RIK	3.10909	NPRL2	0.244258	TMEM194B	2.09601	SCAI	2.06911

Differentially expressed genes for GPR65-/-, PLZP-/- and TOSO-/- Th17 cells											
GPR65-KO-IL1B+IL6+ IL23-96h-1		GPR65-KO-TGFB1+IL6-96h-1		PLZP-KO-IL1B+IL6+IL23-48h-1		PLZP-KO-TGFB1+IL6-48h-1		TOSO-KO-IL1B+IL6+IL23-96h		TOSO-KO-IL1B+IL6+IL23-96h	
Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)
GNAQ	1.92071	TMX1	2.67553	UBFD1	0.321659	STRN3	0.244485	ANK	2.0958	GMPPA	2.06901
YWHAZ	0.521058	LIMD2	0.373978	USF1	0.321783	RBMX2	4.08875	PPP1R8	2.09564	OTUB1	2.06728
FAM98B	1.91469	SEC14L3	0.374268	EPB4.1	0.322107	TMEM161B	0.244574	GM11092	2.09303	MRPL54	2.06611
SYNGR1	0.523142	GM13247	0.37523	DNAJC9	3.10335	RHOT1	4.08433	ZHX2	0.477808	TNFSF9	2.06593
SHARPIN	0.523917	ABI1	2.6648	SPEN	0.322519	MOBKL2B	4.08057	IDE	0.478085	TPCN2	0.484625
PSMA4	1.90774	FAM53A	2.66272	MCEE	0.322623	ANKLE1	4.0788	HSBP1	0.478165	GPS2	2.0626
AMZ2	0.525351	SEC13	2.65611	CENPO	0.322861	HTATIP2	0.245456	BC029127	2.091	APPL2	2.06132
GM5590	0.525698	SUN1	0.376637	EBI3	3.09731	CORO1B	4.07192	PLSCR1	0.478294	GMIP	0.485289
PXMP4	0.525848	GTDC1	0.376912	NDUFS3	3.09465	D030074E01RIK	0.245584	MAVS	0.478734	EIF2AK4	0.485579
ESRRG	0.525993	4933427D14RIK	2.65234	ASH2L	0.323334	SERTAD2	4.06939	GM129	2.08859	TMEM123	0.485769
PFDN1	1.90048	UIMC1	2.6522	NAGK	3.08875	ITGA6	0.246102	TFPT	0.478798	UBE3B	0.486341
CCDC21	1.89879	PSMB2	2.64775	WDR37	0.323944	SPEN	4.05653	4931429L15RIK	2.08706	SEC11A	0.486873
MUS81	1.89522	SNX12	2.64757	MOBKL2A	3.08572	DAP	0.246516	BC056474	0.479157	4933439F18RIK	0.486967
RBM3	0.52776	GM5623	2.64667	PPP1R7	3.08366	DGCR6	4.0543	FAM96A	0.479384	OLFR613	2.0521
DLGAP4	0.52777	TEX13	0.377913	MOBKL3	0.324337	GRAMD1B	0.246709	TAF6	2.08572	KCTD10	2.05136
PSMG1	0.528081	GM10222	0.378032	2410017P07RIK	3.08185	ATP5S	0.246942	BRCC3	0.479527	CAST	0.487554
ABCF2	0.528096	HIST1H4D	2.64458	TMC6	3.08041	SEL1L	4.04563	0610007P08RIK	2.08457	RAPGEF2	2.05031
A430005L14RIK	1.89358	OLFR592	0.378312	RCC1	0.324706	LTBP1	4.04427	THYN1	0.480007	RPL23A-PS1	2.05018
PARS2	1.89236	DEFB36	0.378563	FAM98A	0.324901	BC002059	0.247311	PLRG1	0.480248	PKP4	2.05014
DDX23	0.529333	MAGEB18	0.378797	GSTO1	3.07349	FKBP2	4.04349	PEX19	0.480576	TTF2	0.487781
TRADD	0.529472	PRKRA	2.63855	ADI1	3.07244	PIH1D1	0.24734	MSRB2	0.48109	SNX11	0.488012
BRWD1	0.529774	ZCWPW1	2.63355	CAD	3.07003	CAMK4	0.247613	SGSM3	0.481319	AKIRIN1	0.489518
HOOK1	0.529863	TECR	2.63226	PRKAB1	0.326859	EPB4.1	4.03552	GOLPH3	0.482346	SHPRH	0.48972
BZW1	0.530277	ESCO2	0.380025	IDS	3.05883	TMEM120A	4.03342	TNFRSF1B	0.482373	MS4A6B	2.03976
CIZ1	0.531406	PPID	2.63042	PIGS	0.327095	ACY1	4.03144	NUDT1	0.48239	TAF6	2.03951
LPIN3	0.531659	SRP68	2.62526	UBE2K	3.05691	FBXO7	0.24829	PAG1	0.482728	STK25	0.490473
RHOG	1.87916	TXNRD2	2.62157	DHTKD1	0.327149	2700062C07RIK	4.0248	EAPP	2.06911	RGS14	2.03743

Differentially expressed genes for GPR65-/-, PLZP-/- and TOSO-/- Th17 cells											
GPR65-KO-IL1B+IL6+ IL23-96h-1		GPR65-KO-TGFB1+IL6-96h-1		PLZP-KO-IL1B+IL6+IL23-48h-1		PLZP-KO-TGFB1+IL6-48h-1		TOSO-KO-IL1B+IL6+IL23-96h		TOSO-KO-IL1B+IL6+IL23-96h	
Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)
TDRD7	0.534047	4930425F17RIK	0.381826	PNPO	0.327168	SLAMF7	0.248459	ADH5	0.483443	APEX1	0.491194
BRCC3	1.87235	ODF2	0.381901	ATOX1	3.0533	ECHDC1	0.248583	CHEK2	2.0683	WDR37	0.49142
NME2	0.534089	EEF1A1	2.61766	MTA1	3.05263	INPP5D	4.0219	ZDHHC5	0.483838	BC005624	2.03429
COMMD9	0.534538	GM4609	2.61683	MPP7	0.327679	OGFOD1	4.02036	SPATA2	0.483905	TAX1BP1	0.4917
CUL1	0.534786	EIF2S1	2.61263	ENO3	0.327796	PPIL5	0.248734	AKR1B8	0.484074	VAPA	0.491756
FGFR1OP2	1.86828	REPS1	2.61087	CTLA2B	0.328106	CD84	0.248964	TMEM160	0.484123	MFSD4	0.492919
GM5494	0.535808	HEXDC	0.383138	TRMT5	3.0478	AC142450.1	4.01427	TADA2A	2.06517	C130026I21RIK	2.02849
STARD4	0.536393	NUBPL	0.383279	L7RN6	0.328111	TUBB4	4.0125	BFAR	2.06511	GTF2H1	0.49316
SLC4A2	0.536914	H2-K1	2.60702	FBXO18	0.328343	HIGD2A	4.01059	CD55	2.06327	GUK1	2.02764
ACBD7	0.537082	3110003A17RIK	2.60608	OBFC2B	0.328937	ITPRIPL1	0.249459	CDYL2	2.0612	BAT4	0.493262
NUP188	0.537166	SLC12A9	0.384014	UBE2R2	0.329711	BOLA2	4.00643	5730460C07RIK	2.05794	PXN	0.494138
CCDC67	0.537188	CDADC1	2.60389	JAGN1	3.02432	TUBA3A	0.249604	5830418K08RIK	2.05734	BOLA3	0.494476
SCO2	0.537268	ATP6V1A	2.6038	DNASE2A	3.02216	UNC50	4.00634	LARP1B	2.05711	INSIG1	0.494544
RPL7A-PS8	0.537795	MLF2	0.384558	STX7	0.331134	PHF14	0.250137	NRD1	2.05564	CARM1	2.02201
SYNGR3	0.538227	MGST3	2.6002	PI4KA	3.01903	FAM114A2	0.250261	GPT2	0.486763	LGALS4	2.01707
6720456B07RIK	0.538249	CTSD	2.59728	WASF2	3.01724	AMT	3.99346	LGALS8	0.486918	STIM1	0.496023
SBDS	0.539336	FIGNL1	0.385147	RRBP1	0.331763	DHRS13	0.250712	G6PDX	2.05221	FAF1	0.496116
SRFBP1	0.539387	1110054O05RIK	0.385579	LRPPRC	0.332031	AC117259.1	3.98473	R3HDM2	2.05198	0610030E20RIK	2.0156
MANBA	0.539715	STXBP3A	2.58956	FAH	3.00849	FAM103A1	3.98124	ATP5H	2.05144	TUSC3	0.496643
MARK2	0.540156	RPS6	2.58683	SPC24	3.00563	ALKBH1	3.97894	TRAF3IP3	0.487575	BZW1	0.497008
CRNKL1	0.542027	GSTT2	2.58677	IPP	0.333073	CYSLTR1	3.97682	GNG12	0.487806	CYP4X1	2.00703
RAB8B	0.542064	TUBA1B	2.58618	SFMBT1	3.00142	DRAM2	0.251573	SLC25A10	0.488253	ERO1L	0.498334
CREBL2	0.542531	TEC	2.58595	CSTF2	3.00105	CKMT1	0.251619	B9D1	0.488281	LAMC1	0.498656
CRLF3	0.543038	OLFR57	0.386892	TCP11L1	3.00088	9930111J21RIK2	3.97353	MAPK1IP1L	0.488444	1110038D17RIK	2.00509
MBD6	0.543651	ZFP58	2.58133	BCAS3	3.00008	AIM2	3.97193	ETL4	2.04704	CDS2	0.498934
MPHOSPH8	0.544274	GM1840	2.57924	WBSCR22	0.333521	TASP1	3.96539	ABR	2.04692	ACSL4	0.499223
ORAOV1	0.545472	OPHN1	2.57923	XIAP	2.99495	TRIP13	3.95438	SMPDL3A	2.04613	LETMD1	2.003

Differentially expressed genes for GPR65-/-, PLZP-/- and TOSO-/- Th17 cells											
GPR65-KO-IL1B+IL6+ IL23-96h-1		GPR65-KO-TGFB1+IL6-96h-1		PLZP-KO-IL1B+IL6+IL23-48h-1		PLZP-KO-TGFB1+IL6-48h-1		TOSO-KO-IL1B+IL6+IL23-96h		TOSO-KO-IL1B+IL6+IL23-96h	
Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)
EFTUD1	0.546074	CENPH	0.388323	CTLA2A	2.9872	IDH3B	3.95381	PSMD6	0.488833	CIAO1	0.499491
SYNE2	0.546379	42253	0.388438	CCDC30	2.98622	PRAMEL6	3.94997	GATA3	0.488935	NARS	0.499662
GM16519	0.546936	GM8325	2.57395	ESF1	0.335338	H2-AB1	3.94804	MFN2	0.489162	GM10845	1.99974
GZMA	0.547503	CDKN2AIPNL	2.57245	RBBP9	2.98171	KPNA6	0.253314	RPP21	2.04171	ATP5SL	0.500147
SSBP3	0.547555	RASA1	2.57058	FRYL	0.335625	PSMB4	3.94607	PARK7	0.489843	TNK2	0.500251
AC154908.2	0.548873	MMAB	2.57045	WSB1	2.97883	FOXP1	0.253422	PTPN7	2.04112	TRPM7	0.500309
TEX10	0.549138	HNRNPA2B1	2.56681	GTF3C5	2.97865	PCCB	0.25368	VTI1B	2.04051	HEXDC	1.99764
ENTPD8	0.54997	DYNC1LI1	0.390009	MAN1A2	2.97706	CASZ1	0.253707	SYPL	2.03922	C79407	0.500689
CLU	0.550086	ACOT8	0.390187	CHURC1	2.9741	2310061I04RIK	3.94086	SLC35C1	0.490401	SMG5	0.500951
ATP6AP1	0.550153	GM6578	2.56122	APOO	2.97331	EDA	3.94086	GM10226	0.490733	ERCC1	0.501052
EXOC4	0.550339	RCAN3	2.56117	SPARC	2.9712	PDS5A	0.25396	FBXO22	2.036	ALKBH6	1.99384
AC121959.1	0.55083	PIGU	0.390626	RABL3	0.337272	CLEC16A	3.93383	BNIP3L	2.03506	GARS	0.501551
CLDND1	0.550984	A430078G23RIK	0.390774	AC163269.1	0.337421	URM1	3.92678	SUV420H1	2.03379	CINP	0.502082
PELP1	0.552241	CRIP2	2.55862	MDM2	0.337925	CDK2	3.92492	WDR77	2.03303	PHF20	0.502194
IAH1	0.552825	DPP6	0.391064	BC004004	2.95574	2900062L11RIK	0.255086	WDR47	2.03231	CBX6	0.502539
UFSP2	1.80831	ZFP772	0.391224	1810006K21RIK	0.338721	TMCO4	3.91832	SUGP1	0.492191	PI15	1.98937
PSAT1	0.553274	MRPS5	2.55298	SMARCA5	0.339057	YIPF3	3.91359	GFER	2.03044	HTATSF1	1.9889
RPL21-PS10	1.8074	TIMM13	2.55225	SMC4	0.33909	GM6531	3.90986	TNFAIP3	2.02887	MTHFD1L	0.502804
ATAD3A	0.553998	WDR70	0.39246	TLCD1	2.94781	TADA3	3.90802	SLC19A2	0.493046	CTPS2	0.502812
FANCC	0.554128	RPS8-PS1	2.54258	ZMYM4	2.94475	AC157595.1	3.907	GGA2	2.02625	RPL31	1.98629
RPL7A-PS3	0.556195	CIZ1	0.393323	CR1L	2.93625	RIN3	3.9052	MARK4	0.493923	IPO8	1.98393
DTWD1	0.556841	PDCD2L	2.54149	AC154908.2	2.929	NDUFV3	3.90298	ATP11A	0.494052	GM7964	0.504708
SOD1	0.558599	HAT1	0.39379	TRAT1	0.341417	SLC29A1	0.256339	KATNAL2	2.02335	SLC7A11	1.97586
SPEN	0.55987	UROS	0.393838	ARL1	2.92184	TOR1AIP1	0.25649	TPRKB	2.02206	DPF2	0.506625
FAM58B	0.561243	CENPM	2.53785	FH1	0.342486	DPF1	0.256606	RABGGTA	0.495179	GM9924	1.97165
KLHDC10	0.56306	KIF1B	0.394297	MSL1	2.91958	GEMIN4	3.89371	HEG1	2.01904	AC159008.1	1.96929
MMADHC	0.564054	TNNI3	0.394472	SLC4A11	0.342529	ARMC7	3.89219	CHD2	2.01572	UBE4B	0.508236

Differentially expressed genes for GPR65-/-, PLZP-/- and TOSO-/- Th17 cells											
GPR65-KO-IL1B+IL6+ IL23-96h-1		GPR65-KO-TGFB1+IL6-96h-1		PLZP-KO-IL1B+IL6+IL23-48h-1		PLZP-KO-TGFB1+IL6-48h-1		TOSO-KO-IL1B+IL6+IL23-96h		TOSO-KO-IL1B+IL6+IL23-96h	
Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)
GNA13	0.564115	DRAP1	0.394833	GEMIN6	0.342677	WARS	0.257273	ATF1	0.496124	STAM	0.508625
1110001A16RIK	1.77005	DCUN1D1	2.53234	PDXDC1	0.343355	2610001J05RIK	3.88639	GZMB	2.01541	SERPINF1	0.509771
AC112970.1	0.565013	RAD52	2.5261	TRAPPC2	0.344281	AC154908.2	0.257344	IKBIP	2.01523	CAPN7	0.51015
MRPL47	0.565422	TNIP2	0.395921	CTSA	2.90317	EBAG9	0.257504	HPVC-PS	2.01226	UAP1L1	0.510379
BCORL1	0.5655	GM4945	2.52316	CDK5RAP1	0.344497	MFNG	3.88175	MFAP1B	0.496986	SF3A3	1.95914
GM16514	0.566314	CHST12	2.52284	CIAO1	2.89879	HK1	3.88042	KAT2B	0.497207	DTX3	0.510586
DENR	0.567381	CSN3	0.396734	SMYD4	2.89716	MRPS10	0.257769	PIN4	2.01112	CTXN1	0.510613
ZBTB20	0.567625	DRG2	2.52055	GRHPR	0.345239	PIGYL	3.87436	SPRED2	2.0094	ATP13A2	0.510873
IPO4	0.567981	4930431F12RIK	0.396971	BATF	0.345388	RBM17	0.258107	CPM	2.0084	KPNA1	0.511448
CSTF1	0.568261	GM12216	0.397362	IFT46	2.8929	2310001H12RIK	0.258201	CRYZ	2.00822	NUP160	0.511725
DNALC1	0.569127	VEGFB	2.51584	HEXDC	2.89157	CDADC1	0.258521	PRDM9	2.00768	DOHH	0.511747
PPOX	0.570301	NDUFV1	2.51426	LIMS1	2.89128	EIF3K	0.258656	D17WSU104E	0.498499	CD84	1.95194
RP23-378I13.5	1.75336	WAC	2.50759	MTM1	2.88984	9330129D05RIK	3.86585	SLC25A23	0.498819	PPME1	0.513334
GSTT1	1.75265	PSMD7	2.50723	EMID2	0.346445	NADK	3.86219	SIT1	0.498906	GM8113	1.94732
UBAC1	0.570886	SET	2.50644	VPS36	0.346491	CISD3	3.85639	H2AFX	2.00404	RELT	0.513763
FAM114A2	1.75083	DAZAP2	2.50634	CSNK1G1	2.88601	2610021K21RIK	3.85623	MED29	0.499274	SIN3A	1.94621
ATP6V1D	0.571885	DPCD	2.50611	MRPS9	2.88388	TNNC1	3.84669	SPECC1L	2.00133	MAP2K2	0.514433
NUP210	0.572376	MYG1	2.50566	AC163101.1	2.87474	COPG2	3.84545	CFLAR	2.00132	GAD1	0.515378
FKBP4	0.573039	TRAF6	2.50285	CTSS	2.87188	GPS1	0.260265	POLK	0.499794	2010106G01RIK	0.516083
SF3B5	1.74422	2410002F23RIK	0.399637	ABCF3	0.348441	TWF2	0.260267	STX1A	0.500013	PIGX	1.93751
GNAS	0.57567	SLC1A5	2.50163	ATF2	0.348658	TRIAP1	3.83902	AAK1	1.99721	2510039O18RIK	0.516461
1600002K03RIK	0.57703	KATNAL1	0.399924	SND1	0.34901	GM12184	0.260714	OSBPL3	0.500811	TRAPPC4	0.516634
TRIM27	1.73294	SHISA5	2.49998	GM4978	2.86399	CNOT3	0.260816	TES	0.501326	PYCR2	0.517569
MTA3	0.577892	PLXNA2	0.400344	KBTBD4	2.86249	IER3	3.83412	FAM76A	0.501609	GM7334	1.93156
CDKN1A	1.7286	ENSA	2.49638	PDE7A	0.349459	PUM1	3.8327	THUMPD3	0.501862	VPS24	0.517816
LY6I	1.72847	PTPN2	2.49413	RPL30	0.349535	MRPS9	3.83118	ADORA2B	1.9918	ZBTB44	0.518369
MRPL4	1.72501	CCR8	2.49156	SRD5A3	0.349732	GLUL	0.261028	DLAT	0.502327	ZBTB25	1.92887

Differentially expressed genes for GPR65-/-, PLZP-/- and TOSO-/- Th17 cells											
GPR65-KO-IL1B+IL6+ IL23-96h-1		GPR65-KO-TGFB1+IL6-96h-1		PLZP-KO-IL1B+IL6+IL23-48h-1		PLZP-KO-TGFB1+IL6-48h-1		TOSO-KO-IL1B+IL6+IL23-96h		TOSO-KO-IL1B+IL6+IL23-96h	
Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)
STK16	0.582641	GPR171	2.49065	CCDC101	0.349885	TAF1D	0.261034	RCBTB2	1.98949	CDCA7L	1.92825
FAM19A1	0.582755	EAF2	0.401732	ZFP828	0.350727	2700060E02RIK	3.8289	BC003331	1.98914	DPP8	0.5189
1700022I11RIK	1.71165	LYZL6	0.401936	CNOT6L	2.84999	RAD52	0.261605	CYFIP1	1.98797	FOXRED1	0.519651
CCDC58	0.58524	SIGLEC5	2.48747	BET1	2.84451	CELF2	3.81935	2400001E08RIK	1.98759	PSG28	1.92329
CWC27	1.70688	NUMB	0.402097	ATP5J2	2.84365	2410002O22RIK	0.261916	RNPEP	0.503512	CDCA4	0.520308
NPC2	0.586183	SMOX	2.48543	MTA2	0.351893	PTTG1IP	0.262322	KIF2A	1.98526	NMT2	0.520384
CASC1	0.587311	PRKRIP1	2.48495	TSR2	2.84151	LRP1B	3.812	CNOT7	1.98499	SLC25A3	0.520812
FIGNL1	0.587706	1700040L02RIK	2.48404	APOO-PS	2.84093	1700084J12RIK	0.262448	ACER2	1.98398	TBCE	0.520816
GM10947	0.588948	HOMER3	0.402929	SRP9	0.352207	AMZ2	0.262804	CTNNB1	0.504068	FGFR1OP	0.521589
USP4	0.59223	AKT1S1	0.402934	CHD6	0.352869	TWF1	0.262902	2310001H12RIK	0.504076	UPB1	1.91671
IPO9	0.592784	CCDC52	2.48127	ST13	0.353181	9130011E15RIK	0.263089	1200016B10RIK	0.504231	ACO2	0.521775
GLUL	0.593623	MLXIPL	0.403803	GM10126	0.353969	PGM2	3.79693	COQ9	0.504426	ARID1A	1.91419
IK	0.594284	FAM96B	2.47507	YME1L1	0.35435	ZFP119B	0.263371	GM9920	1.98207	SCO1	0.522445
SMN1	0.598734	FAM192A	2.47499	LARS2	0.35468	MS4A4C	0.263921	LSR	0.504704	STK19	1.91279
RPF1	0.600117	D2WSU81E	2.47422	XRCC2	2.81685	BSCL2	3.78787	A230046K03RIK	1.98068	SLC9A7	1.9123
EIF3F	0.600944	KARS	2.4733	PPT1	0.355364	GPAA1	3.78783	PAIP2	0.505006	MEF2A	0.523401
STAMBPL1	0.601806	ZEB2	2.47291	ATP6V1G1	0.355986	SLC6A9	3.78783	NAB2	0.505161	4732465J04RIK	1.90773
NAP1L4	0.601861	EIF3I	2.47183	LRRC59	0.356549	RABEPK	0.264159	IBTK	1.97881	TRIM26	1.90526
SUMO3	1.66089	CCT7	2.47169	DAP	0.356992	POLR3G	0.264569	SCMH1	0.505617	PDLIM7	0.525343
ZFYVE20	1.65953	H2AFZ	2.46948	E130309D02RIK	0.357077	PHB2	3.77816	BC031353	1.9776	RAB8A	0.525506
SNX6	1.64709	CLIP1	0.405064	HMGB3	0.357559	VPS25	3.77655	UPF3A	1.97507	FAM172A	1.90216
TMEM208	0.608069	FLNA	2.46297	USP45	0.358041	APPL2	3.77447	FDXACB1	1.97505	HSP90B1	0.526021
CDYL2	1.64066	CMAH	0.406825	UBE2G2	0.358728	NAGA	0.264986	LY6C1	1.97364	TRAF2	1.90049
MRPS23	1.62324	PSMB3	2.45744	SLC13A4	0.35893	ZFP444	0.265217	RBBP6	1.97358	RTN3	0.526287
SDCCAG8	0.618133	NUP188	2.45588	DCTN6	0.359605	BTD	3.7705	DNAJC15	0.506779	HAT1	0.526603
GM10180	0.6231	TMEM50B	0.407658	BC005537	0.359731	ERCC8	0.265289	TBX6	1.97285	AI480653	0.52694
NFKBIL2	0.62363	PDIA6	2.45116	4930473A06RIK	0.360025	2310011J03RIK	0.265376	IRS2	1.97257	WDR13	0.527264

Differentially expressed genes for GPR65-/-, PLZP-/- and TOSO-/- Th17 cells											
GPR65-KO-IL1B+IL6+ IL23-96h-1		GPR65-KO-TGFB1+IL6-96h-1		PLZP-KO-IL1B+IL6+IL23-48h-1		PLZP-KO-TGFB1+IL6-48h-1		TOSO-KO-IL1B+IL6+IL23-96h		TOSO-KO-IL1B+IL6+IL23-96h	
Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)
TREX1	1.60079	SLC2A9	0.408452	NUP35	0.360156	SLC3A2	3.76824	ZFP260	0.506964	RPS12	1.89526
NMT1	0.629225	FBXO18	2.44665	DUS1L	0.360992	ADI1	0.265536	A630010A05RIK	1.97099	H2-GS10	1.89495
BOLA2	1.58747	IL2RG	2.44447	RNF25	2.76455	GSTZ1	0.265792	SYTL1	0.507613	RBPSUH-RS3	0.527976
RPS12-PS3	0.635083	SNRNP200	2.44421	ATP6V1D	0.362458	MTG1	0.265886	LYN	1.96963	CTNNA1	0.528139
EIF3K	0.640023	APLF	0.409141	AGK	0.362486	PPM1M	3.76093	ZMYND8	0.507888	POLD1	0.528232
RNF8	0.640552	TTC16	0.409214	EIF4E3	0.362549	MYBBP1A	0.265955	TGTP2	1.96875	FNDC3A	0.530053
GIMAP5	0.641094	FAM171A2	0.409287	PNO1	0.363285	TUSC2	0.266008	1600014C10RIK	0.507938	ECT2	0.530097
ICOS	1.55747	RDH11	2.44145	RPAP2	2.74578	CCDC40	0.266274	COG2	0.508092	ZBTB48	0.531218
AAAS	0.645299	GM9867	0.409753	CRYBG3	2.74507	RCCD1	3.75553	EIF1B	1.96748	AIMP2	0.531318
AACS	1.54713	SH3GL2	0.410064	YBX1	0.364351	UBE2G2	3.75208	AKR7A5	0.508268	GEM	0.532475
CLTB	0.646466	TGDS	2.43712	BBS9	2.74435	ZCCHC11	3.75014	A430033K04RIK	0.508386	SMOX	0.532485
TSTA3	0.64683	GM12355	2.43594	CCNC	0.364551	RFT1	3.74624	GNPTG	1.96637	GRK1	1.87782
GLTPD1	0.647385	SLC17A1	0.410597	ORC6	2.74063	BFAR	3.74384	CDC42SE2	1.96594	HSPH1	0.532596
USP33	0.652032	CHCHD2	2.43431	PSTK	2.74002	MLL5	0.267206	UBAC1	0.509005	EEF2	0.532776
HSF2BP	0.65726	2310004I24RIK	0.411066	PHF20	0.365273	AB041803	0.267228	STT3A	1.96405	SESN3	0.53345
EIF2B2	1.51353	RFC4	2.43237	GBP4	2.7348	EIF4E1B	3.74213	MEA1	1.96388	TMEM176B	0.534151
GM9846	0.661306	GM5449	2.43162	ATP2A2	0.365747	NUP54	0.267326	ALG6	1.96135	UBE2Q2	0.534491
AC068006.1	1.51037	RNMT	2.42932	CSDA	0.36588	TMEM111	3.74061	MAP2K4	0.510181	RASSF7	1.86971
BCL2A1D	0.663782	KIN	2.42826	CBR4	0.366082	GYG	0.267383	DAPK3	1.95949	MAVS	0.535261
EPHX1	0.664393	CRX	0.412069	CCDC111	2.73013	WAPAL	0.267453	GM6132	1.95886	FAM32A	1.86815
		NOSTRIN	2.42669	MBTPS2	0.366424	POLA1	3.73713	LRP1	0.511141	SMG7	0.535301
		TPMT	2.42108	GLA	0.36763	SCFD2	3.73631	VMN1R15	1.95617	CBLB	1.86343
		RIN2	0.414077	TUBGCP4	2.70223	ZBTB25	0.267644	2010005H15RIK	0.511384	VPS33B	0.537968
		MRPS21	2.41435	PTPN22	2.70201	CCDC137	3.73303	PUS1	1.955	RERE	0.538233
		LSS	0.41427	FAM82B	0.370392	GM16380	3.73135	HOXB1	1.95471	RAB7	0.539009
		ERCC6L	0.414813	OCIAD1	0.370722	ZFP870	0.268254	PTPN1	0.511705	TMEM222	1.85462
		CDH7	0.415362	PHF14	0.371514	BC021614	3.72679	TOP3A	1.9542	CDC26	0.539305

Differentially expressed genes for GPR65-/-, PLZP-/- and TOSO-/- Th17 cells											
GPR65-KO-IL1B+IL6+ IL23-96h-1		GPR65-KO-TGFB1+IL6-96h-1		PLZP-KO-IL1B+IL6+IL23-48h-1		PLZP-KO-TGFB1+IL6-48h-1		TOSO-KO-IL1B+IL6+IL23-96h		TOSO-KO-IL1B+IL6+IL23-96h	
Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)
		FLT1	0.415599	BC017643	0.371595	CENPO	3.72435	42066	1.95403	ARRB2	0.540067
		NHLRC3	2.40576	TAF12	2.69071	RGS11	0.268513	FAM65A	1.95385	REEP4	0.540384
		RAC2	2.4055	B230208H17RIK	2.68825	SIDT2	0.268642	TRA2A	1.95347	NFKBIL1	0.5408
		TTC35	0.416079	SMOX	2.68797	BHMT2	3.72138	CCDC34	1.95296	LUC7L	1.84785
		SERTAD2	2.40336	SNX1	0.372894	PRPSAP1	0.26896	SEC61B	0.512119	GM7263	1.84588
		BCL3	2.40316	GM10491	2.67982	ZNRD1	3.71802	UBTD1	0.512319	SGIP1	0.541977
		ORAOV1	0.416262	GLIPR1	2.67168	ZFP566	0.269243	BCAP29	0.512497	1810029B16RIK	1.83701
		GM10192	0.416457	CCDC55	2.66151	TFG	0.269249	F730047E07RIK	0.512933	GPR98	1.8369
		GM10576	0.416531	BCKDK	2.655	PIH1D2	3.7134	GTF2E2	0.512934	SYPL	1.83272
		1810062G17RIK	0.416542	OLFR613	2.65279	ATG4A	3.71131	BPTF	1.94905	TARDBP	0.546266
		ATF7	0.416542	MRPS28	0.378022	GRINA	0.269574	SURF6	0.513101	PAFAH1B3	1.82962
		PYGL	0.416898	GOSR2	2.64246	SIL1	0.269867	CDK4	1.94802	SNAPC1	0.547069
		B4GALT7	2.39738	SNX10	0.379113	FAM54B	3.70403	OAT	0.513348	PNRC1	0.547422
		SHKBP1	0.417187	PTPN7	2.63711	H2-Q7	3.70298	HSPBP1	0.513387	DHCR24	0.547732
		NEIL3	0.417589	RPL21-PS6	2.6325	LGALS4	3.70086	RP23-71J17.1	0.51361	EPT1	0.548464
		ARHGAP23	0.417865	CDK2AP2	0.380398	FZR1	0.27031	MINK1	0.513728	SERINC3	1.82304
		CCDC73	0.417948	LRRC33	0.38045	PAFAH1B3	3.69605	GPN2	0.513745	TRIM16	0.549268
		SERINC3	2.39241	PXMP4	2.62462	NFKB1	0.270657	LANCL1	0.514143	EIF4H	0.549483
		IRF3	2.39082	MAP3K1	2.62401	TAF8	3.69444	RNF214	0.514319	SERINC1	0.55107
		REEP3	0.418667	LCLAT1	0.381754	CD44	0.270938	NEURL3	1.94432	NFE2L2	0.551235
		NAPA	2.38692	TADA2A	2.61838	SLC12A6	3.68795	GJA1	1.94426	PSG16	1.80771
		RCCD1	2.38312	SBF2	2.61665	ADPRHL1	3.68326	CTPS	0.514334	PSD4	0.553898
		ZBTB48	0.419638	MED11	0.382379	GSTT2	0.271538	EPHB6	0.514382	BRP44L	1.80508
		ENO1	2.38291	SDR39U1	0.382638	NDUFS3	3.68165	SC4MOL	1.94234	NDUFS8	1.79949
		SRA1	2.38251	FLII	0.38277	WWOX	0.271617	GOLGB1	1.94176	PRKAG1	0.556035
		NRN1	2.3825	CCDC58	2.60645	GALNT1	3.67991	FAM53B	1.94035	VEGFA	0.557177
		RBMX2	2.38229	DCAF17	2.60515	AK157302	0.271878	AZIN1	0.515598	PML	0.557223

Differentially expressed genes for GPR65-/-, PLZP-/- and TOSO-/- Th17 cells											
GPR65-KO-IL1B+IL6+ IL23-96h-1		GPR65-KO-TGFB1+IL6-96h-1		PLZP-KO-IL1B+IL6+IL23-48h-1		PLZP-KO-TGFB1+IL6-48h-1		TOSO-KO-IL1B+IL6+IL23-96h		TOSO-KO-IL1B+IL6+IL23-96h	
Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)
		PLSCR2	0.419896	DPYSL5	2.60017	VPS52	0.271994	TBC1D7	1.93891	ZFP277	1.79357
		MRPL27	2.37937	D17WSU104E	2.59814	TPRGL	0.272112	GM5148	0.516057	EAPP	0.557704
		GM9920	0.420306	CRBN	0.384986	SDCCAG8	0.272155	GM15446	0.516669	UBR1	1.79211
		SNX11	0.420689	COMMD3	2.59712	JMY	0.272284	RPS19BP1	0.517113	NUP210	0.558386
		CCDC127	2.37554	GARS	0.385447	ZFP68	3.67263	BAT2L	1.93341	TRAF4	0.559497
		GM12166	2.37455	ADD1	0.385754	KDELR1	3.66723	THOC2	1.93184	NSMCE4A	1.78532
		CHCHD5	2.37221	IGF2BP1	2.5918	PRPSAP2	0.272685	GM7204	0.51812	TUBG1	0.560497
		PSMC1	2.37129	4930422I07RIK	0.385953	4921521F21RIK	3.66495	N4BP2L1	1.92934	HERC4	0.560835
		CDCA3	2.37031	SLC5A6	2.59072	AMDHD2	0.272858	DDB2	0.518393	TMEM128	0.561113
		HIGD1A	2.3696	STK38L	2.58657	SREK1	0.272866	UBR1	0.518747	ACP6	0.561274
		HK2	2.36845	PIGQ	2.58532	SPATA5	3.6635	CCDC64	1.92739	RAE1	1.77899
		HAX1	2.36709	CCNE2	0.387262	YWHAB	3.653	TIMM17A	0.519287	CRK	1.77824
		GM6616	0.422601	RNFT1	0.387617	AGPAT4	3.65153	NOL6	0.519525	PKM2	0.563075
		GOLGA2	2.36616	WDR83	2.5794	C130022K22RIK	3.64762	SNF8	0.519544	RAB3D	0.563213
		IDH3A	2.36555	TMEM208	2.57819	AAMP	0.274362	ZFAND2B	0.519692	ERI2	0.565008
		TUFT1	0.422771	LDHB	2.57679	BTNL7	0.274362	MAP2K6	1.92378	RAD9	1.76762
		IARS	2.36517	HIGD2A	0.388263	FARS2	0.274534	RPA2	1.92376	TRIM12C	0.566937
		SNRPC	0.423025	TRMT2A	2.57556	TMEM138	3.63884	FLCN	0.519922	BHLHE40	0.567591
		TAGAP1	2.36305	1810029B16RIK	0.388697	DHRS1	0.274822	CCDC109A	1.92333	GOLGA3	0.568098
		ESRRB	2.36259	WDR11	2.57205	FAM45A	0.274896	MICAL1	1.92329	DHODH	0.568201
		EGLN2	0.423727	PRPF4	0.389138	LRRC51	3.63652	YTHDF1	0.520077	CD2BP2	0.568371
		GNB3	0.423751	GM129	2.56113	HBP1	3.63527	6330512M04RIK	1.92214	NUP50	0.568502
		CETN2	2.35631	RNF8	0.390462	TM9SF1	0.275183	ARFGAP3	1.92052	RBBP4	0.570096
		SRSF2	2.35537	ENOPH1	2.55864	GM10125	3.63363	AHSA1	0.520838	SYCE2	0.57057
		GM6984	2.35439	CCDC21	2.55839	VPS4B	3.63172	KCTD20	1.91971	SDHB	0.57114
		ZDHHC2	0.424965	POLR3C	2.5568	SMYD4	0.275947	OLFR309	1.91784	IKZF1	0.571504
		ACTR5	2.35309	LZIC	0.391656	KDELC1	0.275947	1200011M11RIK	1.91752	TPM3	0.571603

Differentially expressed genes for GPR65-/-, PLZP-/- and TOSO-/- Th17 cells											
GPR65-KO-IL1B+IL6+ IL23-96h-1		GPR65-KO-TGFB1+IL6-96h-1		PLZP-KO-IL1B+IL6+IL23-48h-1		PLZP-KO-TGFB1+IL6-48h-1		TOSO-KO-IL1B+IL6+IL23-96h		TOSO-KO-IL1B+IL6+IL23-96h	
Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)
		BNIP1	0.425006	SMARCAL1	2.54702	RIOK2	3.62388	FUZ	0.52165	GNPDA2	0.572931
		FUNDC1	2.34964	CASP9	0.392795	ACTG2	0.276442	FBXL20	0.521882	BBS5	0.573259
		TMEM106C	2.3483	ATAD3A	2.54526	ACSL5	3.6173	CHCHD4	1.91611	WIPI1	0.575804
		RPL27A-PS2	2.34643	CREM	0.393101	IFT80	3.61578	CCDC99	1.91608	GM10126	1.72328
		2610020H08RIK	2.3461	NUSAP1	2.54381	C1QBP	3.61566	CNOT8	0.522017	EIF2B3	0.581782
		ALOXE3	0.426314	INTS4	2.54359	SAR1A	0.276578	RMND5A	0.522092	UBAP1	1.71853
		HELZ	2.34235	UBE3B	2.54343	EIF5	0.276583	VRK3	0.52243	SPSB1	0.582234
		FAM58B	2.34033	2210012G02RIK	2.54314	ZDHHC4	3.61425	TMEM70	0.52249	ALG1	0.583562
		TMEM29	2.33863	DCLRE1C	2.54308	RHEB	3.61261	WDR62	1.91337	EIF4G1	0.585375
		CCT8	2.33681	HUS1	0.393319	DEGS1	0.27711	PLA2G4C	0.52267	GM10154	1.69708
		BRD7	0.42797	UROS	0.39347	SETD3	0.277125	APLP2	1.91309	ARPC2	0.590214
		PSMD4	2.33652	CDCA2	2.54124	SNX5	3.60658	HEXB	0.522921	SIN3B	0.590385
		AC087117.1	2.33628	ATP5G2	0.393622	1700026D08RIK	3.60065	ITGA7	0.523022	NADK	0.591149
		CCNB1	2.33545	CHCHD2	2.53764	F730047E07RIK	3.59942	DDX3X	0.523083	SNX15	0.59177
		GLRX2	0.428297	RNF7	2.53293	AC102876.1	3.59924	LY75	1.90982	SUGP1	0.592918
		PRKAR1A	2.33459	ZFP871	0.394855	STRADA	3.59782	AMN1	0.523642	WBP11	0.593785
		FER1L4	0.428392	HDAC3	2.5311	MTCH1	3.59294	GM10126	1.9084	EIF4B	0.594521
		SERF1	2.33264	GM11444	0.395169	CLUAP1	0.278452	TBC1D14	1.90609	KPNA6	0.599164
		GLB1	2.33197	TRMT6	0.395306	TXNL4A	3.59025	TTC14	1.90396	EIF3G	0.603438
		RPL17-PS3	2.33185	PSMB4	0.395713	2010002N04RIK	3.5887	POLR2I	1.90363	CHCHD3	0.603518
		PCID2	2.32949	PSMB10	2.52609	WDR83	3.58864	CORO2A	0.525557	BLMH	0.604504
		SLC35A5	2.32855	FDPS	0.396001	ALG1	3.58642	CTSF	1.90247	NDUFV2	0.608703
		ACOT9	2.32667	PUS7	0.396696	SLC25A20	0.27883	CPEB4	1.90227	AC121959.1	0.60898
		XPO6	2.32366	H2-T10	0.396761	C1D	0.27899	ACAD11	1.90142	1700021K19RIK	0.612295
		DULLARD	0.430551	CDK6	2.52008	WDR5B	0.27904	ACADL	1.90024	JUNB	0.61636
		MBTPS2	0.430966	STXBP3A	0.397055	FBXW17	0.279342	USP34	1.89966	GM16372	0.620202
		GLRX3	2.31916	PRDX4	0.397077	MRPS2	3.57822	HECTD2	1.89902	TRIOBP	0.622737

Differentially expressed genes for GPR65-/-, PLZP-/- and TOSO-/- Th17 cells											
GPR65-KO-IL1B+IL6+ IL23-96h-1		GPR65-KO-TGFB1+IL6-96h-1		PLZP-KO-IL1B+IL6+IL23-48h-1		PLZP-KO-TGFB1+IL6-48h-1		TOSO-KO-IL1B+IL6+IL23-96h		TOSO-KO-IL1B+IL6+IL23-96h	
Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)
		FBXO7	0.431201	CAMK2D	0.397755	PARP2	0.279468	FAM18B	1.89846	SLC23A2	0.623297
		RFC1	2.31754	SURF6	0.397919	PVR	0.27983	SUB1	0.527024	TIMP1	0.62437
		BACH2	0.431518	NMT1	0.398118	SLC38A6	3.57343	GM4953	1.89734	NF2	0.627746
		EDIL3	0.431846	MRPL12	2.50676	CCDC117	0.280162	ORAOV1	0.527263	TM9SF2	0.630655
		MAPKAPK3	2.31328	UBA3	2.50203	SRSF1	3.56743	ARL13B	0.527358	BCL2L1	0.631318
		ACSL3	2.31253	CCDC53	0.400055	CCDC37	0.280558	VPS37A	0.527394	ZFP68	0.632526
		EIF2B1	2.31248	ARPC4	2.49874	NDUFA5	0.280768	RAD23B	1.89356	2410002F23RIK	0.637736
		RAB34	0.432762	SIP1	2.49633	LAPTM4A	0.280976	KLHDC1	1.89321	TIMD2	0.65466
		HIST1H1B	2.30999	DUSP11	0.40093	RHBDD3	3.55902	ACTN1	1.89312	GM11092	0.662806
		FAM86	2.30926	AQR	0.401972	CHURC1	3.55842	RNASEH1	0.528473		
		USP20	0.433214	PRPF6	2.48721	SRP72	0.281468	MFSD4	0.528688		
		ARRDC4	2.30811	SMCHD1	2.4853	VAPA	3.54427	PHKB	1.89121		
		GNB1L	2.30491	TOE1	0.402448	VCAM1	0.282146	CR974466.3	0.529469		
		OXSM	0.433947	ETF1	0.402612	C130026I21RIK	0.282669	FEN1	0.529593		
		KDELR1	2.30246	CSNK2B	0.402876	TAX1BP1	3.535	HK2	0.529764		
		PPWD1	0.434447	MRPL23	0.403481	H2-KE2	3.53448	ZFP64	1.88746		
		MTCH1	0.43445	PIP4K2B	2.47823	LRRC57	0.282927	CBFA2T2	0.529815		
		UBE2K	2.30119	GEMIN5	0.403858	MCFD2	0.282948	NUDT2	1.88732		
		MCM3	2.30089	MPP6	2.47593	RPUSD4	0.283046	TRIM26	1.88656		
		RAB26	2.29972	CHCHD3	2.47563	AHCTF1	3.53299	CAPN1	1.88608		
		COQ5	2.29929	BCAP29	0.404156	2610015P09RIK	0.28318	GPRASP2	0.530205		
		PPP1R12B	0.43505	HAX1	0.404298	AC161211.2	3.53132	WDR83	0.53028		
		GM1673	0.435322	RAB1	0.404338	GM10845	3.52711	ATP6V0A1	0.530309		
		BAT4	2.29618	2310008H09RIK	2.47301	RRP9	3.52632	1810012P15RIK	0.5304		
		HHAT	0.436199	PLEKHA2	0.404372	JKAMP	3.52345	GNPDA2	1.88521		
		IL11	0.4364	MRPS11	2.47198	BEND5	0.283881	1200011I18RIK	1.88463		
		TERF2IP	0.436546	GM10247	0.40515	RBM18	3.52106	RNF220	1.88316		

Differentially expressed genes for GPR65-/-, PLZP-/- and TOSO-/- Th17 cells											
GPR65-KO-IL1B+IL6+ IL23-96h-1		GPR65-KO-TGFB1+IL6-96h-1		PLZP-KO-IL1B+IL6+IL23-48h-1		PLZP-KO-TGFB1+IL6-48h-1		TOSO-KO-IL1B+IL6+IL23-96h		TOSO-KO-IL1B+IL6+IL23-96h	
Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)
		TOP1	0.43657	RFC3	2.46815	PFN2	0.284257	D11WSU47E	0.531055		
		PIGO	0.43692	C130026I21RIK	2.46687	COQ3	0.284301	UBXN11	0.531091		
		GAK	2.28802	ETFDH	0.40565	CYC1	3.51628	GSPT1	1.88263		
		SLC35B4	2.28551	2810474O19RIK	2.46509	GRINL1A	3.51311	FUS	1.88159		
		RWDD1	2.28476	ZMYM1	0.405929	CMTM5	3.51179	MAPK6	0.531495		
		ARFGAP1	2.28443	MYH9	2.46303	UVRAG	0.284938	2810006K23RIK	1.88005		
		RNF207	0.437931	RNF135	2.46229	SLC2A1	3.50806	TUBB6	0.532162		
		4933434E20RIK	2.28268	GBP2	2.46228	DCUN1D5	3.50632	BC003266	1.87822		
		1700049G17RIK	0.438122	RABGGTB	0.406451	RMND1	3.50373	ZKSCAN5	1.87779		
		MYST2	0.438131	BCL2L11	2.4598	PRKCQ	0.285425	PPARD	0.532756		
		HNRNPU	0.438283	CORO7	0.407067	BOLA1	3.50029	TOMM70A	0.532804		
		A2LD1	0.43857	CAB39	0.407257	2900010J23RIK	0.285879	ZCCHC10	1.87629		
		WDR67	0.438638	PFKFB4	2.45371	CLSPN	3.49183	LPIN2	0.533743		
		MAPKAPK5	0.438737	THOC4	2.45298	NUDT19	3.48855	MOCOS	1.87267		
		MRPL23-PS1	2.27896	R3HDM1	0.407704	TRP53BP1	0.286692	CCDC17	0.534254		
		BC046331	0.43885	LAMC1	0.407777	USP8	0.286742	PIK3AP1	0.534386		
		GM4666	0.43888	RBM4B	0.407937	2310008H04RIK	0.286929	SPNS1	0.534407		
		ADSL	2.27559	SERF2	0.408298	NR2C2	0.286973	EIF2B3	0.534832		
		VSIG10	0.439492	CINP	2.44822	CD97	3.48148	ACD	0.534847		
		ATP2B1	2.27493	KPNB1	0.408474	PGM3	0.287426	PTPMT1	1.8695		
		UCP2	2.27177	TAP1	0.409023	CLEC4A2	3.47531	UBA5	0.535227		
		GM8279	0.440209	EIF3F	2.44425	FCRL1	0.287744	SOCS1	1.86833		
		SSR4	2.27155	2700094K13RIK	2.43966	RAD51L1	0.287889	TRIM12A	1.86742		
		IGSF8	0.440973	CPNE8	2.43946	HIBCH	3.47339	KANK3	1.866		
		DAGLB	0.44098	AP1G2	2.43878	METTL7A1	0.288049	CSTF3	0.535954		
		UBB	2.26697	CNN3	0.410269	FAM58B	3.4704	RREB1	0.535999		
		KLHL18	0.441715	NUP93	2.43383	GM5507	0.288649	GM10749	1.86475		

Differentially expressed genes for GPR65-/-, PLZP-/- and TOSO-/- Th17 cells											
GPR65-KO-IL1B+IL6+ IL23-96h-1		GPR65-KO-TGFB1+IL6-96h-1		PLZP-KO-IL1B+IL6+IL23-48h-1		PLZP-KO-TGFB1+IL6-48h-1		TOSO-KO-IL1B+IL6+IL23-96h		TOSO-KO-IL1B+IL6+IL23-96h	
Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)
		SNRNP35	0.441746	SNAP23	0.410876	KCNQ5	3.46359	NCOA2	1.86434		
		DPM3	2.26299	IL17A	0.411163	D630004N19RIK	0.288787	ABCD1	1.86396		
		BC049349	0.442184	SCD3	2.43123	SLC25A39	3.46276	FOXO3	0.536658		
		HECTD3	2.26147	RRP36	0.41212	3110001D03RIK	3.46125	ASH2L	0.536841		
		MUM1	0.442346	WDR77	0.412209	ACRBP	3.45928	CD1D1	1.862		
		GM5879	2.26	1110059E24RIK	2.42555	2610301B20RIK	0.289335	EXOSC3	1.86032		
		ANAPC5	2.25775	RAB4B	2.42419	TIMM22	3.4562	2700073G19RIK	1.85974		
		MSN	2.25766	FAS	0.412615	ERCC3	0.289451	CLP1	0.537757		
		PRKAB1	0.44338	CDV3	0.41273	TTLL1	0.289466	GFM1	0.53782		
		OBFC2B	0.443448	TARS	0.412962	CYP4X1	3.45405	ANGEL1	0.537921		
		2010002N04RIK	2.25414	MCTS1	2.42132	FANCC	0.289578	TMEM55B	0.537935		
		GBA	2.25281	ADK	2.41902	ACP2	0.289725	AI314976	0.537974		
		WFDC12	0.444838	LUM	0.413837	BC068281	0.2898	FRG1	1.85879		
		HSD17B10	2.24676	DDB2	0.414031	STARD7	3.45065	IFI47	1.85754		
		RSF1	0.44561	CHCHD1	0.414192	PLAC8	3.44922	SLC39A14	0.538616		
		DHRS3	0.445627	HAUS5	0.414267	RPL21-PS4	3.44832	RPL10A-PS2	0.539089		
		APEX1	2.2438	GRAMD3	0.414562	AA960436	0.290163	MRPL12	1.85427		
		TULP4	0.445693	STAG2	2.41193	CBR1	3.44506	RAPGEF6	1.8531		
		KLHL6	2.2427	KIF23	2.41134	UROD	0.290278	ETFA	1.85182		
		TSTD2	0.445908	FANCG	0.414815	1110018G07RIK	3.44241	IL4RA	0.540125		
		SFXN5	0.445941	42249	2.40807	GABARAP	0.290549	BCL2L11	1.85096		
		A530064D06RIK	0.445959	MRPS5	2.40689	RFTN1	0.290802	CKAP5	1.8501		
		RG9MTD2	0.446327	9330129D05RIK	0.415476	TOR1B	3.43761	PPP1R11	1.84947		
		POLR3K	2.24048	MYCBP	2.40489	SIRT6	0.290991	MGAT1	0.540891		
		ZFAND1	2.24028	SMYD5	2.3976	HYAL2	0.291252	ACTL6A	0.540991		
		CHN2	0.446849	ZFP605	2.39591	AA415398	0.291482	PRR3	0.541067		
		GNA13	2.23782	POLR2F	0.418289	LAPTM4B	0.291771	TSPAN3	0.541238		

Differentially expressed genes for GPR65-/-, PLZP-/- and TOSO-/- Th17 cells											
GPR65-KO-IL1B+IL6+ IL23-96h-1		GPR65-KO-TGFB1+IL6-96h-1		PLZP-KO-IL1B+IL6+IL23-48h-1		PLZP-KO-TGFB1+IL6-48h-1		TOSO-KO-IL1B+IL6+IL23-96h		TOSO-KO-IL1B+IL6+IL23-96h	
Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)
		LRRC57	2.23676	TCTN2	2.3902	ACOT9	3.42735	SDHA	0.541254		
		RORA	2.23615	ZC3HAV1	2.38878	RIT1	0.291982	IRAK1	0.541435		
		STK38	0.447501	2410002I01RIK	0.419639	GATAD2B	0.292071	GM16372	0.541465		
		SDF4	2.23205	TOMM7	2.38206	GM454	3.42131	PRR13	0.541563		
		EMG1	2.23131	TBC1D9B	2.38033	RUSC1	0.292286	PHF21A	1.84613		
		FAM69A	0.448401	AC166253.1	2.37846	2610029G23RIK	0.292435	CCNDBP1	1.84601		
		IKBKG	0.448482	ID2	0.420475	MAX	3.41678	WBSCR16	0.5418		
		AC170752.1	2.22847	NUP43	0.420972	CIR1	3.41407	SURF2	0.542203		
		IAH1	0.448838	CLINT1	0.421182	SLC45A4	3.41407	HEATR7A	0.542304		
		ARMC6	2.22769	BAZ2B	2.37372	LEPROT	3.41029	UPF2	1.84338		
		RIMKLB	0.449006	NFS1	0.421373	DTWD2	0.29323	ZDHHC2	1.84324		
		ZNF512B	0.449096	CD40LG	0.421523	TMEM126A	3.40481	ZSWIM7	0.542856		
		PSMB1	2.22377	SEPHS1	0.421566	TSPAN6	3.40187	SNAPIN	1.84112		
		SPIC	0.449745	MSH2	2.37021	1700052N19RIK	3.40139	H2-OA	0.543218		
		NDUFB5	2.22266	1110001A16RIK	2.36988	ZFP239	3.40051	HIST1H3G	0.543763		
		ACTL6A	0.45008	IL16	2.36962	2410002I01RIK	3.39923	SH3BP5	1.83895		
		PLA2G2C	0.450326	SEC11A	2.36893	CST3	3.39793	ECT2	1.83892		
		RPL3	2.21903	UXT	0.422554	SLC9A3R1	0.294297	4933421E11RIK	0.544024		
		CLUAP1	2.21787	DECR2	2.3663	ZFP82	3.39489	CYB5R1	0.544334		
		S100A7A	0.45141	DPCD	0.422754	CABLES2	3.39347	GM10349	0.544527		
		USP7	0.451836	POLE	0.422899	RNF38	3.39295	PDCD1	0.544637		
		2900092E17RIK	0.452171	INPP5K	0.422982	2410002F23RIK	3.38695	PPP2R5E	1.83563		
		TUBG1	0.452948	FOXRED1	2.36406	HMGN5	0.295251	WHRN	0.545202		
		PECI	2.20688	VPS4A	2.36323	GALNT7	0.295262	WDYHV1	0.545738		
		DHX40	2.20161	BIRC3	2.3623	CRK	0.295838	GNPNAT1	1.83227		
		PAQR3	0.45431	GM10395	0.423351	1810063B05RIK	3.37945	6330416G13RIK	0.545776		
		AL592187.1	0.454499	3110043O21RIK	0.423484	PPP2R2D	0.295906	QDPR	0.545929		

Differentially expressed genes for GPR65-/-, PLZP-/- and TOSO-/- Th17 cells											
GPR65-KO-IL1B+IL6+ IL23-96h-1		GPR65-KO-TGFB1+IL6-96h-1		PLZP-KO-IL1B+IL6+IL23-48h-1		PLZP-KO-TGFB1+IL6-48h-1		TOSO-KO-IL1B+IL6+IL23-96h		TOSO-KO-IL1B+IL6+IL23-96h	
Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)
		MIF4GD	2.20019	SATB1	0.424146	AHNAK	0.295931	ZRANB2	1.83121		
		ABAT	0.454636	AC132320.1	2.35533	PTK2B	0.296081	GM71	1.83056		
		PBX1	0.455434	QRICH1	0.424793	ATP6AP2	3.37424	FBXW11	0.546354		
		MLPH	0.455592	TRP53	0.425101	WEE1	0.29648	GIMAP4	1.83026		
		WDR77	0.455613	SLC25A36	2.35234	LRRC41	3.37149	AGFG1	1.82946		
		FBXO44	2.19411	PIGX	2.35214	CENPQ	0.296665	AMIGO1	0.546986		
		PFDN2	0.455822	ASB13	0.425366	ATP6V1B2	0.297223	NDUFB9	0.547145		
		SULF2	0.456066	POP1	0.425439	NGFRAP1	3.36353	TMEM43	0.5472		
		4632433K11RIK	0.456427	TTC19	0.425544	GM4922	0.297412	EPHX1	0.547367		
		AP1B1	0.456439	AC087117.1	2.34081	HAUS3	0.297412	RALB	0.547377		
		MRPL20	2.18844	ZDHHC21	2.34042	IL1RL2	0.297412	SPATA7	1.82669		
		HINT1	2.18827	TCHP	0.427295	2610018G03RIK	0.297753	KHK	0.547649		
		6330439K17RIK	0.457064	DPY30	2.33847	RPL13-PS3	3.35846	CYTH2	1.82538		
		ANP32A	0.457393	NTAN1	0.427809	3110009E18RIK	0.297797	FAAH	0.5479		
		H2-T23	2.18171	PFDN4	0.428353	LDHC	3.35647	R3HCC1	0.548164		
		SAG	0.458565	SH3KBP1	2.33449	NHEDC2	0.298441	1700057G04RIK	0.548299		
		CTSZ	2.1802	GM10063	0.428916	MKLN1	0.298486	AHR	1.82235		
		GM10699	0.458737	CAML	0.428973	FBXL4	3.34996	RTCD1	1.82138		
		DGUOK	2.17906	LRRFIP1	0.429017	GM6816	3.34996	PLOD3	1.82037		
		PARP1	2.17543	SEMA4D	2.33063	CORO1C	3.34844	PSMD10	0.549959		
		UNC45B	0.459794	TRAPPC1	2.32803	SETX	0.298789	USMG5	0.551139		
		H2-K2	2.17147	ZFP655	0.429566	USP21	0.298838	CCDC127	0.551167		
		POLD4	2.17121	CD209C	2.32591	APEX2	3.3449	BRIX1	1.81416		
		ZFP53	2.16957	PPPDE1	2.32456	TMEM69	3.34327	BGLAP-RS1	1.81269		
		VPS54	2.16948	DEPDC5	0.431528	LRPPRC	0.299236	POLD1	0.551766		
		H2-OA	0.460969	EIF2B5	2.3157	CMTM6	3.33853	YWHAZ	0.551889		
		CCDC124	0.46117	AP1B1	2.31379	RER1	3.33853	PRPF3	0.551942		

Differentially expressed genes for GPR65-/-, PLZP-/- and TOSO-/- Th17 cells											
GPR65-KO-IL1B+IL6+ IL23-96h-1		GPR65-KO-TGFB1+IL6-96h-1		PLZP-KO-IL1B+IL6+IL23-48h-1		PLZP-KO-TGFB1+IL6-48h-1		TOSO-KO-IL1B+IL6+IL23-96h		TOSO-KO-IL1B+IL6+IL23-96h	
Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)
		TLCD2	0.461632	RNASEH2B	0.432267	CDK8	0.29975	HIST1H2BG	1.81161		
		LIMCH1	0.461684	RNF20	0.432732	KPNB1	0.299805	4930455C21RIK	0.55247		
		CLYBL	2.1649	GNPDA1	2.30957	GSN	3.33504	2810417H13RIK	1.80977		
		2610028H24RIK	2.16231	VTA1	0.433242	2010107G23RIK	3.33144	CHURC1	1.80936		
		POT1B	0.462653	NISCH	2.30736	CCNL1	0.300171	DNAJA3	0.552777		
		GM10359	2.161	INPP5B	0.434037	EIF2C4	3.32825	TMEM33	0.552949		
		FAM48A	2.16008	VEZT	2.30135	2310039H08RIK	3.32527	CRTC1	0.552987		
		SLC35A3	0.463086	DDX56	0.434647	GM4769	0.300727	MAN2C1	0.553075		
		VPS28	2.15942	MRPS18C	2.29971	CDKN3	3.32437	TBC1D2B	1.80746		
		GM16223	0.463771	TSSC1	2.29637	ALG8	0.300988	TMEM109	0.553279		
		PPIL1	0.463844	NIF3L1	0.435506	NDUFAF1	3.30854	DPH1	0.553309		
		SEC23A	2.15533	ACOT9	0.435567	BC026590	3.30791	ZFP617	1.8071		
		KCTD20	0.465161	RTN4	2.29576	VPS45	0.302474	GABPB1	0.553583		
		GM10491	0.465733	ICAM1	0.43576	RPL21-PS6	0.302832	PBX4	1.80478		
		GAPDH	2.14683	IFT52	0.436111	4932425I24RIK	3.30203	RPS6KB2	0.554403		
		EML1	0.466	GM10941	0.437625	ZUFSP	0.303095	WASF2	1.80256		
		GM11127	0.466028	CUL2	0.43766	FBXO25	0.303323	1810063B05RIK	0.5548		
		CTSL	2.14528	RABEPK	0.437762	CHCHD3	3.29527	GTF3C5	0.555342		
		FCGBP	0.466372	CCNA2	0.4381	AC156282.1	3.29316	NCOR1	1.80056		
		PMPCB	2.14362	NFU1	0.4384	APOO	3.29302	UFD1L	0.555419		
		GM11696	0.467028	4930512M02RIK	2.28097	TUBB2A	3.29053	INCENP	0.555529		
		EXPI	0.467245	MEMO1	0.43848	AC154727.1	0.304576	BC004004	1.79969		
		CD70	0.467704	AP2A1	2.28027	METT11D1	3.28058	ELOF1	0.555867		
		MRPL13	0.467833	9130011J15RIK	0.438852	TMEM55B	0.305017	ANKRD39	1.79863		
		FAM188A	2.13708	NAE1	0.43911	GM7792	0.305222	PEX5	1.79857		
		HELLS	2.1366	SET	0.439536	TBC1D14	3.2763	PML	1.79766		
		ZDHHC12	0.468237	PYCARD	0.43988	PRKACA	0.305346	PDGFA	0.556287		

Differentially expressed genes for GPR65-/-, PLZP-/- and TOSO-/- Th17 cells											
GPR65-KO-IL1B+IL6+ IL23-96h-1		GPR65-KO-TGFB1+IL6-96h-1		PLZP-KO-IL1B+IL6+IL23-48h-1		PLZP-KO-TGFB1+IL6-48h-1		TOSO-KO-IL1B+IL6+IL23-96h		TOSO-KO-IL1B+IL6+IL23-96h	
Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)
		GM6843	0.468988	FADS6	2.27168	CHCHD5	3.27182	ZMYM1	0.556484		
		H2-KE2	2.13071	NDUFB3	0.440946	PUF60	3.26996	RC3H2	0.556597		
		TTC39B	2.12776	CTNNB1	0.441276	B230315N10RIK	0.305922	IFI30	0.556767		
		GM9104	2.12771	PGAP2	0.441622	CASP9	3.26881	WDR74	0.556829		
		IFFO2	0.470055	SLC1A7	0.441665	CSMD3	3.26735	MAPKAPK5	0.557062		
		UNC119	0.470111	1110007A13RIK	2.26398	GIT2	3.26251	PLCXD1	0.557075		
		FARSA	2.12375	PARVG	2.26374	PARVG	3.26132	ABI1	0.55722		
		CDK5RAP1	0.471003	CCNG1	0.441766	JMJD1C	0.306647	PISD	0.557802		
		GM10481	2.12193	1810043G02RIK	2.2607	EXT1	0.306879	PRDX1	0.55786		
		HCN3	0.471413	CENPN	2.26031	STRBP	3.25784	CEPT1	0.558251		
		WDR26	2.11983	AHSA2	2.25986	HSD11B1	3.25586	GPSM3	1.79072		
		ZSCAN2	0.471782	NF1	2.25905	TSC22D3	0.307254	EGFR	0.558788		
		RABL2	0.472485	TIMM9	2.25653	FXR1	0.307377	CASZ1	1.78932		
		LRRC8D	0.47293	FAM192A	2.2561	M6PR	0.307753	SAR1B	1.78855		
		CPNE5	0.47295	CPA6	2.25489	CDIPT	3.24906	COMMD1	0.559244		
		WFDC5	0.473411	ACO1	2.25226	GM10120	0.30795	SMC4	0.559449		
		1110008J03RIK	0.473831	SUCLA2	2.24747	ME2	3.24437	NEDD9	1.78684		
		TPM1	0.474032	CCS	2.24671	UBE2R2	3.24431	GALT	0.559741		
		TAF11	0.474295	HDAC6	2.24613	NUP35	3.24336	FZR1	0.560072		
		1110049F12RIK	0.474416	BCL2A1D	0.44523	PRPS2	3.24211	LRSAM1	0.560257		
		CCDC101	2.10597	SS18	2.24356	S730403B10RIK	3.24185	FAM126B	0.560396		
		8430410A17RIK	0.475727	RPUSD4	0.446656	GJC3	3.24033	ZFP783	1.78415		
		GM16409	2.10033	STOML2	2.23691	RRP15	3.2377	RHEBL1	0.560621		
		4930423O20RIK	0.476418	TRUB2	2.23626	HERC4	0.309176	DLD	0.560748		
		IPO4	0.47698	LIAS	0.447179	PPIE	0.309526	AGPAT6	1.78293		
		OGFOD1	0.477246	EME1	2.23473	BRMS1	3.2304	CCDC88C	0.560936		
		GM16253	0.477298	PAPD5	2.23455	TOP2B	3.22874	TFG	0.561088		

Differentially expressed genes for GPR65-/-, PLZP-/- and TOSO-/- Th17 cells											
GPR65-KO-IL1B+IL6+ IL23-96h-1		GPR65-KO-TGFB1+IL6-96h-1		PLZP-KO-IL1B+IL6+IL23-48h-1		PLZP-KO-TGFB1+IL6-48h-1		TOSO-KO-IL1B+IL6+IL23-96h		TOSO-KO-IL1B+IL6+IL23-96h	
Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)
		AC087229.1	0.477627	MKKS	0.448136	FBXO22	3.22729	GM6404	1.78103		
		FTL1	2.09329	HDAC7	2.23062	USP7	3.22585	NUFIP2	1.77858		
		GM6177	2.09261	2610002J02RIK	0.448358	ST13	0.31005	PAM16	0.562297		
		EIF1AX	0.478225	GM10222	0.448984	CCDC47	3.22504	ACBD4	0.562414		
		OXSR1	0.478424	COX7B	0.449612	AC158559.1	0.310331	PICK1	0.562446		
		GM11011	0.478426	ARPP19	0.449695	CDK16	0.310356	LRP1B	0.562624		
		ZWILCH	2.08967	PYGB	2.22223	2410022L05RIK	3.21695	TMEM141	0.562888		
		APH1B	2.08549	1110004E09RIK	0.451634	IGFBP4	0.310854	GADD45A	0.562989		
		FNDC7	0.479946	MRPS36	0.452048	SRSF2	3.21548	PCGF1	0.563208		
		NUDT16L1	0.479973	P4HB	0.452445	D1BWG0212E	0.311108	MGAT4C	1.77522		
		AL589878.1	0.480025	FAM103A1	2.20774	FEN1	0.311256	NVL	1.77516		
		2010106G01RIK	0.480718	MCFD2	2.20704	YBX1	0.311269	PRPF40A	1.77313		
		AC153594.1	0.480831	SLC35A2	0.453334	CCNG1	3.21088	TMEM101	0.564739		
		RPL21-PS11	2.07755	HAUS7	0.453406	FAM40A	3.20928	CDCA3	0.564975		
		ATF4	2.07678	TMEM49	2.20502	5830433M19RIK	0.311726	SLFN8	1.76958		
		EMD	2.07543	SMAD3	0.454636	CTSS	3.20795	FUNDC2	0.565211		
		ABHD4	2.06755	MADD	2.19501	CLIP1	3.20692	1810013D10RIK	0.565296		
		PATZ1	0.483944	ZFP277	2.19468	GM10482	0.311882	RNASEH2A	0.565395		
		1700061G19RIK	0.48396	5930416I19RIK	2.19421	PRAMEL5	3.20611	GSN	0.565571		
		ERH	2.06622	HDGF	0.455778	GM10088	3.20581	PLCG1	1.768		
		SNX17	0.4842	CHRAC1	2.18984	ATP5L	0.312179	1600002H07RIK	0.56563		
		RHBDD2	0.484413	NUP214	2.18975	ZC3HAV1	3.20328	SYNGR1	0.565632		
		ILF2	2.06414	AGPAT6	2.18953	ING3	0.312252	MRPL13	1.76635		
		GM5045	2.06252	CUL1	0.456742	UPF3B	3.2021	CLPTM1L	0.566179		
		TRPM1	0.485193	FAM48A	2.18865	GM4885	3.19919	ATP5G1	0.566213		
		FURIN	0.486329	ADAMTSL4	0.45754	D2WSU81E	3.19707	ERN1	0.566827		
		GM7964	2.05608	PRDM11	2.1834	SMC4	0.312889	SMYD3	1.76394		

Differentially expressed genes for GPR65-/-, PLZP-/- and TOSO-/- Th17 cells											
GPR65-KO-IL1B+IL6+ IL23-96h-1		GPR65-KO-TGFB1+IL6-96h-1		PLZP-KO-IL1B+IL6+IL23-48h-1		PLZP-KO-TGFB1+IL6-48h-1		TOSO-KO-IL1B+IL6+IL23-96h		TOSO-KO-IL1B+IL6+IL23-96h	
Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)
		GPKOW	2.05294	BC026585	2.18302	POT1A	0.313051	PLAGL1	0.567379		
		IRGM1	2.05135	AKAP9	0.458226	PRKAB1	3.1937	MARCKSL1	1.762		
		METTL5	2.05114	DSTN	0.458411	BANF1	3.19157	ALDOART2	0.567735		
		PGAM1	2.0511	GOT2	0.459847	CDC20	3.18913	SELP	0.567803		
		MYBBP1A	0.487549	POLR2J	2.1702	TRMT61A	0.313689	DCTN4	0.568542		
		NUDT7	2.0507	GM15887	0.461531	MKKS	3.18457	CDK2	0.568548		
		2410017P09RIK	0.487772	CREB3	0.463233	1110002N22RIK	3.1836	PLA2G16	0.568624		
		NXT1	0.487864	NUP54	0.46331	4930555F03RIK	3.18179	6720489N17RIK	0.569815		
		HNRNPAB	0.487941	GM10495	2.15552	CGN	3.17972	1110007C09RIK	0.570179		
		PPP1R3F	2.04769	INPP5F	2.14687	KRT222	3.17941	USE1	0.570337		
		LEO1	2.04707	LGALS1	2.14651	SARNP	3.17917	VPS39	0.570866		
		CMTM6	2.04648	TIMM17A	0.466884	ARL6	3.17793	ATG9A	0.570933		
		MFF	0.48865	SURF4	0.467147	P2RX4	3.17721	ILF2	1.75104		
		PCBP3	0.488893	PSMC6	0.467773	COX18	3.17615	42068	0.571351		
		KLC1	0.489024	NDUFB11	2.1347	TADA2A	0.314846	YIF1A	0.571751		
		GM9808	2.04453	PSMD13	0.468583	TUFT1	0.314984	CIB1	1.74844		
		CBX1	2.0445	SSB	0.475177	RIOK1	3.17343	TNFRSF14	1.74806		
		IL4RA	2.04176	SDF4	0.479001	NUDT16L1	0.31515	AC090123.1	1.74715		
		2310045N01RIK	0.489897	RPL22L1	0.484688	0610009B22RIK	3.17238	AMFR	0.572472		
		CCT4	2.03971	NME1	2.05839	NAPA	3.17114	MAPKAP1	0.572544		
		BDH1	2.03947	RPS15A	0.489007	FNBP1	3.16923	GM13154	1.74582		
		GM10845	0.49045	ANP32A	0.495753	CTPS	3.16817	PAFAH2	0.573079		
		NUDC	2.03722	GM10036	0.501126	FAM195A	3.16733	EVI2A	0.57309		
		TSFM	2.03532	KDM5A	1.98696	ATP6V1E1	0.316043	CD69	0.573096		
		UHRF1	0.491655	MRPL20	1.98423	C330021F23RIK	3.16359	4930453N24RIK	0.573176		
		CHD3	0.491889	UBA1	0.508506	2810004N23RIK	3.15863	PLEK	0.573959		
		PI4KA	0.491991	CRIP1	1.94192	FUT8	0.316765	PIK3CD	0.573988		

Differentially expressed genes for GPR65-/-, PLZP-/- and TOSO-/- Th17 cells											
GPR65-KO-IL1B+IL6+ IL23-96h-1		GPR65-KO-TGFB1+IL6-96h-1		PLZP-KO-IL1B+IL6+IL23-48h-1		PLZP-KO-TGFB1+IL6-48h-1		TOSO-KO-IL1B+IL6+IL23-96h		TOSO-KO-IL1B+IL6+IL23-96h	
Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)
		CD247	0.492074	ATP5B	0.516133	PSMB5	0.316776	AKTIP	0.574124		
		PSG29	0.492256	TOMM5	1.92215	RNF14	0.316879	NUP50	0.574382		
		DDX56	0.492881	VPS29	1.89828	GM6498	3.15505	GM10108	0.574638		
		MGST2	0.493199	LY6A	1.89024	PPOX	3.15469	SF3B4	0.57473		
		PIP5K1A	0.493439	GPI1	0.529736	LIAS	0.317121	BC052040	1.73993		
		SCD2	2.02514	APEX1	0.536689	LIN37	0.317155	MFSD2A	1.73981		
		TNNI1	0.494042	1810009A15RIK	0.537057	CACNA1F	3.15288	PHLDA3	0.574792		
		SAA1	0.494437			FBXO18	3.15288	GFPT1	0.574973		
		GM11092	0.494518			ARHGDIA	3.15234	CDC26	1.73847		
		OLFR316	0.49502			BCL3	3.15086	CYP11A1	0.575584		
		MARCKSL1	0.495066			NUBPL	3.1491	MKKS	0.576672		
		CCDC61	0.496047			NARS2	3.14837	TMEM123	1.73129		
		HIST1H1E	0.496819			POP4	0.317653	SF3A2	0.577604		
		SIGMAR1	0.496855			RNF34	3.14724	RNF125	0.57771		
		EIF4G3	0.49691			EIF2B5	3.14567	A630033E08RIK	0.577835		
		NFKBID	0.496946			MYG1	0.31794	CIR1	0.577934		
		UNC50	0.496963			MS4A15	0.317994	RCSD1	0.577976		
		AI314976	2.01113			DDX41	0.318146	MANEA	1.72905		
		TRIM43A	0.4973			ARL3	3.14104	GIMAP9	0.578676		
		RAB7L1	0.497891			AEN	3.13723	TMEM138	0.578809		
		PI16	0.498177			BPGM	0.318753	JMJD6	0.579051		
		1110007A13RIK	0.498318			ARMC10	0.318867	ALDH7A1	0.57939		
		BTBD11	0.498889			SNUPN	3.1361	LZIC	0.579408		
		WDR69	0.499266			6330416G13RIK	3.13603	NAT9	1.72561		
		CDK2	0.499306			GORASP2	0.319013	UNC13D	0.5797		
		SEPW1	0.499344			WDR53	3.13378	MSI2	1.72493		
		ZBTB43	0.499355			CCDC58	0.319208	UBE2B	0.579806		

Differentially expressed genes for GPR65-/-, PLZP-/- and TOSO-/- Th17 cells											
GPR65-KO-IL1B+IL6+ IL23-96h-1		GPR65-KO-TGFB1+IL6-96h-1		PLZP-KO-IL1B+IL6+IL23-48h-1		PLZP-KO-TGFB1+IL6-48h-1		TOSO-KO-IL1B+IL6+IL23-96h		TOSO-KO-IL1B+IL6+IL23-96h	
Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)
		RELB	2.00243			KDM1A	3.13242	STK16	0.580011		
		RPL10	2.00217			BC011426	0.319263	RAB14	0.58029		
		AL8452911	0.499614			TMEM164	0.31954	AA467197	0.581573		
		GM4883	0.499929			MBTPS2	3.12778	EPN2	0.581642		
		FAM160A2	0.500259			QDPR	3.12635	MTMR1	1.71705		
		SLC22A23	0.501166			TFIP11	3.12476	FLII	0.582556		
		ECHDC1	0.501544			BC003267	3.12306	A630007B06RIK	1.71539		
		EFCAB1	0.501729			2210404J11RIK	0.320322	GPR98	1.71429		
		CIAPIN1	0.502094			NSG2	0.320617	ISYNA1	0.583808		
		PGAM5	0.502382			SGIP1	3.11884	SNRNP200	1.71092		
		ZDHHC19	0.502393			GIMAP6	3.11626	HIST1H3C	1.7103		
		PRDM10	1.99024			ATG16L2	3.11474	TFPI	0.585092		
		RPL39L	0.502504			NUPR1	3.11474	COX6A1	0.586233		
		RDH9	0.50263			GM10343	0.321806	GFM2	0.586276		
		ITPA	1.98861			TSPAN18	3.10729	PPIL3	0.586625		
		PTGES3	1.98596			KIF5B	0.32193	1810032O08RIK	1.70368		
		PTMS	0.503584			RPL27A-PS1	3.10625	KHDRBS1	0.587185		
		RNF135	0.50392			VPS72	3.10624	TMEM159	1.70133		
		MRPL50	1.98425			GM4978	0.322117	ALDOC	1.70114		
		BRAP	0.504061			FASTKD2	0.322465	SMAP1	0.588077		
		TMEM45B	0.504185			LUC7L3	3.10094	TM9SF4	1.7001		
		COMMD9	0.504361			STX11	0.322483	SUPT5H	0.58832		
		CNTN1	0.504447			NME7	3.09872	TMEM149	1.69819		
		ANO3	0.504602			TGFBR1	3.09731	ATP6V1H	0.589251		
		DCTN4	0.504703			SHQ1	3.09603	KCTD11	0.589528		
		MAPRE2	0.504727			LMAN1	3.09465	SOCS4	0.589616		
		HIST4H4	0.505159			HIP1R	3.09349	WASL	1.69599		

Differentially expressed genes for GPR65-/-, PLZP-/- and TOSO-/- Th17 cells											
GPR65-KO-IL1B+IL6+ IL23-96h-1		GPR65-KO-TGFB1+IL6-96h-1		PLZP-KO-IL1B+IL6+IL23-48h-1		PLZP-KO-TGFB1+IL6-48h-1		TOSO-KO-IL1B+IL6+IL23-96h		TOSO-KO-IL1B+IL6+IL23-96h	
Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)
		1500032L24RIK	0.505228			CSTB	3.09201	SMPD4	0.58987		
		DOK2	0.505314			GM5145	3.08822	FAM125A	0.590039		
		LIN37	1.97879			PDIA3	3.08642	SIGMAR1	1.69479		
		DCXR	1.97873			KYNU	3.0849	UHRF1BP1L	0.590182		
		RPS6-PS1	1.9786			CHD4	0.324318	EZH1	0.590285		
		PMS1	0.505608			AC117184.1	3.08207	SDCCAG8	1.69368		
		GPI1	1.97771			SERINC1	0.324744	PSMB9	1.69186		
		INSIG2	1.97708			UBE2E1	3.07896	MRPL19	0.591074		
		CEP250	0.505932			YWHAH	3.07799	A130022J15RIK	0.591458		
		TRMU	0.50683			OXNAD1	3.07753	DNAJC11	0.591491		
		AU017455	0.50733			TTC5	0.325023	SRSF4	0.591655		
		8430426H19RIK	0.50749			RWDD4A	3.07464	GM8973	0.591773		
		9030625A04RIK	0.507881			RPL26-PS2	3.07285	ARHGAP4	0.592326		
		ELMOD2	0.508684			PDHX	3.07277	SEPHS1	1.68819		
		MFN1	0.508852			GALE	3.07244	IL2	0.592404		
		GNGT1	1.96518			RHOH	3.07071	PRNP	1.68801		
		LRRTM4	0.509206			TAF1B	3.06934	LSP1	0.592415		
		HBXIP	1.96377			GM10916	0.325985	QPRT	0.592438		
		OBSL1	0.509404			CCDC132	0.326324	C80913	0.592481		
		RRP9	0.509527			SMCHD1	3.06356	LRRC24	0.59293		
		SRI	1.96225			CRIP2	3.06351	YTHDF2	0.592945		
		4930579K19RIK	0.509665			GRPEL2	0.326535	PYGB	0.593102		
		1700016D06RIK	0.509699			PARP4	3.06245	SEMA4F	0.593194		
		SEPHS1	0.509782			MSL3	0.326656	RILPL2	0.593397		
		OXNAD1	0.509827			AARS	0.326762	ATIC	0.593821		
		RPE	0.510997			TMEM179B	3.06001	CPNE3	1.68383		
		RPL7A-PS8	1.954			PYCRL	0.327028	IKBKG	0.594093		

Differentially expressed genes for GPR65-/-, PLZP-/- and TOSO-/- Th17 cells											
GPR65-KO-IL1B+IL6+ IL23-96h-1		GPR65-KO-TGFB1+IL6-96h-1		PLZP-KO-IL1B+IL6+IL23-48h-1		PLZP-KO-TGFB1+IL6-48h-1		TOSO-KO-IL1B+IL6+IL23-96h		TOSO-KO-IL1B+IL6+IL23-96h	
Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)
		SLC15A3	0.511777			LPL	3.05767	VHL	0.594121		
		GM561	0.511922			C030046E11RIK	3.05746	MRPL35	1.68226		
		FBXO3	1.95304			ZC3H12D	0.327301	H47	0.594489		
		OSGIN2	0.51206			2700007P21RIK	0.327512	ZNHIT1	0.594596		
		PXMP4	0.512182			4930583H14RIK	3.05263	ITPR2	1.68152		
		FXYD3	0.512375			ACAP2	0.327587	GP49A	1.67993		
		PLEKHG2	0.512695			CPNE8	0.327879	XLR4C	0.595291		
		MDH1	1.9488			LCMT1	0.327899	KPNA4	1.67867		
		LMO3	0.513707			CES2B	3.04897	DPF1	0.595754		
		THAP7	1.94632			MARK2	3.0478	ZFYVE20	0.595924		
		SLC1A7	0.513853			CDK2AP1	0.328236	FAF1	0.596011		
		PHPT1	0.514348			PLEK	0.328688	POLB	0.596191		
		TOMM5	1.94408			THOC1	0.328704	RPL37	1.67707		
		HNRPDL	1.94367			GTPBP2	3.04092	MOCS1	0.596294		
		WDR31	0.514637			CBWD1	0.329216	GNAI2	0.596532		
		TOR1AIP2	0.514874			BBS12	0.329239	YME1L1	1.67359		
		MYO1B	0.515039			TMEM167	0.32943	GPAA1	0.597772		
		RNF125	1.93985			CSDA	0.329624	INSL3	0.597842		
		2310016C08RIK	1.93825			CCDC22	0.329876	DNLZ	1.67102		
		NARFL	0.516157			VAMP4	3.02859	CLK4	1.66998		
		APEX2	0.516321			VPS16	3.02752	APBB1IP	1.66987		
		RANBP1	1.93556			SH3GLB1	3.02432	MRPS11	0.599032		
		HMCN1	0.517013			ZC3H14	0.330652	MAGED2	0.599116		
		AAGAB	0.517197			TRMT11	0.330748	ESCO1	0.599233		
		PSG16	0.517263			ABI3	0.331024	AC151578.1	1.66877		
		2610044O15RIK	0.517356			HBA-A2	3.02035	GPN1	0.60056		
		TMEM49	1.93192			NOP14	3.02006	UTRN	0.602289		

Differentially expressed genes for GPR65-/-, PLZP-/- and TOSO-/- Th17 cells											
GPR65-KO-IL1B+IL6+ IL23-96h-1		GPR65-KO-TGFB1+IL6-96h-1		PLZP-KO-IL1B+IL6+IL23-48h-1		PLZP-KO-TGFB1+IL6-48h-1		TOSO-KO-IL1B+IL6+IL23-96h		TOSO-KO-IL1B+IL6+IL23-96h	
Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)
		FCER1G	0.517759			ENOPH1	3.01903	BDP1	1.65868		
		KIF24	0.518046			SLC44A1	0.331232	AC148768.1	0.603		
		MEA1	0.51844			GM5614	3.01688	RPL35	1.65822		
		DHODH	0.518678			GM8225	0.332032	ENO2	1.65807		
		GM9574	0.519645			CD47	3.00969	DRAM2	1.65765		
		HNRNPK	1.92367			FTSJ1	0.332414	ATXN2	0.603542		
		NOC4L	0.520174			1700030K09RIK	0.33275	ABHD10	0.603967		
		AW146154	0.520334			PPP1CC	3.00449	TPRGL	0.605694		
		INTU	0.520955			NOL8	0.333129	OSGIN2	0.605746		
		YPEL5	1.91937			WSB1	3.00142	APOO-PS	0.605872		
		PTOV1	0.521626			WBP11	0.333203	RPL34	0.60602		
		GM11057	0.521738			MTERFD1	0.333307	GM16514	0.607024		
		4930429B21RIK	0.521955			VPS26A	0.333475	GNL3L	0.607071		
		LAPTM5	1.91483			ADAM17	2.99801	FXYD7	0.607867		
		NTNG2	0.522288			NUP188	0.333567	LIMK2	0.608276		
		CCM2	0.522776			ZFAND6	0.333577	ELAC2	0.608326		
		RPL9	1.9115			HPS5	0.334144	AW112010	1.64378		
		MS4A6D	0.523227			NUP85	0.334404	KIF2C	1.64323		
		USH2A	0.523684			GM5528	2.99039	GM14085	1.6432		
		PANX1	0.523705			PEX11B	0.334418	MTOR	0.608838		
		5430437P03RIK	1.90784			AL593857.1	0.334998	IMPA2	0.60909		
		DDX28	0.524218			CYFIP1	0.33539	RIC8	0.609158		
		PDXDC1	0.524505			4930451C15RIK	2.98157	GPR108	0.609424		
		1700025C18RIK	0.52592			SERBP1	0.335462	CD63	1.64047		
		PIN4	1.90123			PRL8A1	2.96933	EIF2S2	1.63999		
		9130011J15RIK	1.9008			GIMAP3	0.336894	TBCB	1.63952		
		NEK11	0.526292			SCFD1	0.337001	USP6NL	0.610349		

Differentially expressed genes for GPR65-/-, PLZP-/- and TOSO-/- Th17 cells											
GPR65-KO-IL1B+IL6+ IL23-96h-1		GPR65-KO-TGFB1+IL6-96h-1		PLZP-KO-IL1B+IL6+IL23-48h-1		PLZP-KO-TGFB1+IL6-48h-1		TOSO-KO-IL1B+IL6+IL23-96h		TOSO-KO-IL1B+IL6+IL23-96h	
Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)
		1700057G04RIK	0.526551			KDM5C	0.337333	PIK3R5	0.610793		
		CSF2RA	0.527			THYN1	0.337668	RABIF	0.610904		
		CDC14B	0.527155			RARS2	0.337682	YBX1	1.63684		
		ARID1A	0.527197			MLH3	0.337695	IFT52	0.61108		
		ABTB2	0.527331			RUVBL2	2.95935	CCS	0.611143		
		GLIPR1	0.527729			GADL1	2.95707	ADRM1	0.611145		
		ABL1	0.52888			SMARCB1	0.338306	FAM69A	1.63587		
		LRRC31	0.528966			HYOU1	0.339143	LRRC61	1.63562		
		PTN	0.529347			6030422M02RIK	2.94463	GM10257	1.63531		
		CTSH	1.88706			SPC24	0.3399	SDCBP	0.611715		
		STXBP2	1.88643			PAPD5	2.9409	DGKZ	0.612086		
		CHMP4B	0.530156			EIF2S2	0.340636	ZFP113	0.61223		
		ZBTB7B	0.530163			EPHA2	2.93381	YWHAE	1.63332		
		THNSL1	1.88547			RPL21-PS13	0.341839	GM2382	1.6316		
		BCHE	0.530597			RALGPS1	0.343168	H13	0.612962		
		NPNT	0.530949			WDR34	2.91354	TPST2	0.613058		
		SLC25A12	1.8827			TCOF1	0.343472	UTP18	1.63081		
		GM11744	0.531659			RAMP1	0.3436	DPF2	0.613245		
		MEN1	0.531763			AC132320.1	2.91022	SRSF10	1.62946		
		TDG	1.88037			1810046J19RIK	2.90959	GM6723	1.62727		
		SLCO1A4	0.532108			GM10071	2.90557	RPL21-PS4	0.614851		
		GM3150	1.87932			GTF2A2	2.90346	MRPL23	0.61524		
		DHTKD1	0.532265			RSRC1	2.90081	CKLF	1.62516		
		WFDC3	0.532408			ZFP738	0.345153	BCL2L12	0.61548		
		LY6G6C	0.532747			SEPW1	2.89617	SLC25A35	0.615825		
		SARS	1.87699			ICOS	0.345799	FABP5	0.615904		
		SMYD5	1.87569			CHSY1	0.346137	PRPF19	0.616463		

Differentially expressed genes for GPR65-/-, PLZP-/- and TOSO-/- Th17 cells											
GPR65-KO-IL1B+IL6+ IL23-96h-1		GPR65-KO-TGFB1+IL6-96h-1		PLZP-KO-IL1B+IL6+IL23-48h-1		PLZP-KO-TGFB1+IL6-48h-1		TOSO-KO-IL1B+IL6+IL23-96h		TOSO-KO-IL1B+IL6+IL23-96h	
Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)
		CC2D1B	0.533576			LSM6	0.346562	ACAD9	1.6219		
		DLEC1	0.533793			AU022252	2.88303	HSF2	0.617283		
		INVS	0.534027			MYO19	0.346902	SDC1	0.617848		
		COPA	0.534307			TULP4	2.88204	GM7551	1.61851		
		HHEX	0.534463			SCD1	0.347362	CRELD1	0.618095		
		TMEM43	0.534548			CD83	0.347481	IL21	0.618323		
		TMSB4X	1.8707			SIN3A	0.348585	LSG1	0.618479		
		NDUFAF2	0.534784			TMEM128	0.348728	BNIP1	0.618645		
		NUDT19	0.534909			ARF2	0.349221	SLC25A14	0.618958		
		GM10125	0.534953			YME1L1	0.349654	PSMG2	1.61508		
		SLC12A6	0.535677			PLEKHA1	0.350085	RWDD1	1.61433		
		0610011F06RIK	1.86601			CDC23	2.85513	4930431F12RIK	0.619471		
		TMEM149	1.86387			CWC22	0.350444	FAM53A	1.6121		
		GPR143	0.536753			RHOF	0.350505	9130011J15RIK	0.620392		
		LRPAP1	0.537166			HMGN2	2.85272	AMD-PS3	0.621165		
		AIP	1.86093			PFDN1	0.350644	XKRX	1.60901		
		CCDC142	0.537379			DMTF1	0.350683	ZFP382	0.622107		
		ITSN1	0.537442			CCDC56	2.84927	COMMD10	0.622673		
		PRAMEL6	0.537628			ANAPC11	2.84924	COPA	0.623015		
		COPE	1.8586			PPP2R3C	0.351018	IMMP1L	0.623121		
		SYNE1	0.538565			KBTBD4	0.351941	AC114007.1	1.6038		
		HBP1	1.85527			ATP11A	0.352003	2210012G02RIK	0.624415		
		YPEL1	0.539064			CD226	0.352127	HIPK3	0.624904		
		TMX2	1.85357			CEP97	2.83567	ZEB1	0.62508		
		5730403M16RIK	0.540161			FDPS	0.352866	C230096C10RIK	0.62563		
		TECTB	0.540828			BRCA1	0.353625	CCDC45	0.62605		
		AC132837.1	1.84883			ZFP71-RS1	2.82321	CCPG1	0.626144		

Differentially expressed genes for GPR65-/-, PLZP-/- and TOSO-/- Th17 cells											
GPR65-KO-IL1B+IL6+ IL23-96h-1		GPR65-KO-TGFB1+IL6-96h-1		PLZP-KO-IL1B+IL6+IL23-48h-1		PLZP-KO-TGFB1+IL6-48h-1		TOSO-KO-IL1B+IL6+IL23-96h		TOSO-KO-IL1B+IL6+IL23-96h	
Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)
		NDUFAF4	0.541102			DNAJA3	0.354683	HRAS1	0.626281		
		GCDH	0.541261			BAZ1B	2.81913	EIF2B5	0.626283		
		SCARB1	0.541408			SMC3	0.355663	RELB	1.59645		
		UBASH3A	1.8468			DHODH	2.80861	CCDC84	0.626489		
		ZZZ3	0.541756			INO80E	0.356211	ARF2	0.626727		
		MEGF6	0.543478			SELPLG	0.356485	AP1S1	0.628649		
		RPL9-PS6	1.83817			BBS4	0.356769	ZFP640	0.628656		
		AWAT2	0.544553			2700050L05RIK	0.356786	PRMT10	1.58977		
		BTBD16	0.544948			WDR43	0.356853	GTF3C2	0.62908		
		GCNT2	1.83502			NUDCD3	0.356972	DMTF1	1.58942		
		ARSK	0.545347			RARS	2.79838	GOSR2	0.629196		
		AASDH	0.545482			CYBASC3	0.357406	SAAL1	0.62955		
		TRMT2B	0.545657			BCKDK	0.357639	PTMS	0.629922		
		HIST1H4A	1.8326			PAIP2	0.357925	PSMD1	0.630357		
		EFTUD2	0.546041			RNMTL1	0.358145	CD72	1.58599		
		DTWD2	0.546128			LSG1	2.7903	EIF2S3Y	1.58457		
		GM10417	0.546155			1700008F21RIK	2.7852	NCBP2	0.631746		
		NGDN	0.546662			CPA6	2.78487	COG8	0.632996		
		HOXB1	0.54707			2700029M09RIK	0.359429	GM6396	0.633102		
		D11WSU47E	0.547455			WDR12	2.77938	ERP29	0.633491		
		GM10691	0.547725			NAT6	0.360026	NUBPL	0.634143		
		DHRS2	0.548037			GM7627	0.360486	ATP5L-PS1	0.634633		
		SRGN	1.82428			AP1B1	0.360615	ASNS	0.6348		
		GM14420	0.548174			DYNC1H1	0.360627	DTNB	1.57523		
		NUP210	0.548315			ANAPC1	2.77169	GM6843	1.5748		
		TMEM66	1.82364			ARAF	2.76739	TPT1	1.57464		
		4931408A02RIK	0.54868			GDI1	2.76562	LYRM2	0.635092		

Differentially expressed genes for GPR65-/-, PLZP-/- and TOSO-/- Th17 cells											
GPR65-KO-IL1B+IL6+ IL23-96h-1		GPR65-KO-TGFB1+IL6-96h-1		PLZP-KO-IL1B+IL6+IL23-48h-1		PLZP-KO-TGFB1+IL6-48h-1		TOSO-KO-IL1B+IL6+IL23-96h		TOSO-KO-IL1B+IL6+IL23-96h	
Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)
		CCDC60	0.54869			RPL21	0.361663	WAC	0.635559		
		VTI1B	0.549696			ADK	0.36299	TRIOBP	0.63562		
		PCYT2	1.81917			AIFM1	0.363256	GSDMD	0.636216		
		RPL13A-PS1	0.549725			PSD4	2.73921	NFKBIA	0.636642		
		GM6320	1.81274			H2-K1	2.7367	PLEKHF2	0.636936		
		UBE2A	0.551724			CEP57	0.365624	ZMIZ1	0.637048		
		TOP3B	0.551816			USP48	0.365786	DFFA	0.637145		
		TRAPPC6A	0.552441			NDUFA13	0.365819	THOP1	0.637365		
		RPL7	1.81007			PPP2R5D	0.366459	GSS	0.63771		
		DAZAP1	0.552963			COMT1	2.72784	BANF1	0.63781		
		CHD6	0.552991			EYA3	2.72297	MAP2K2	0.637887		
		SPRR1A	0.553582			PECR	0.367285	WSB1	0.638066		
		PHF20	1.80464			CFDP1	0.368433	CUL5	1.56587		
		VPS72	0.554352			IL4RA	0.368673	SHKBP1	0.638955		
		1700057K13RIK	0.55457			SDF2	0.369416	TECR	0.639414		
		TRIM24	0.555522			4732418C07RIK	2.69688	TMEM29	0.639476		
		GM14296	0.55609			ZFP446	0.370858	TWF1	0.640301		
		TXNDC11	0.556915			VGLL4	0.37087	HYOU1	0.641183		
		1700093K21RIK	0.557875			COG6	0.371414	1810049H13RIK	0.641337		
		SPP1	0.55804			COMMD1	0.372521	NFIA	0.641587		
		IVD	0.558086			CDC27	0.372858	DERL2	0.641603		
		YY1	0.560429			RPL26-PS4	0.373203	AKR1B3	1.55771		
		AC125405.1	0.560436			SLC11A2	0.373532	TSEN15	0.642102		
		CCDC18	0.561413			TUBA8	0.374051	ZFP593	1.55675		
		CTSC	0.562011			SRPR	0.374362	IL10RB	1.55656		
		GM4953	1.77831			STXBP2	0.37487	BID	0.642473		
		AL672068.1	0.563077			IKZF5	0.375049	SLC4A2	0.642706		

Differentially expressed genes for GPR65-/-, PLZP-/- and TOSO-/- Th17 cells											
GPR65-KO-IL1B+IL6+ IL23-96h-1		GPR65-KO-TGFB1+IL6-96h-1		PLZP-KO-IL1B+IL6+IL23-48h-1		PLZP-KO-TGFB1+IL6-48h-1		TOSO-KO-IL1B+IL6+IL23-96h		TOSO-KO-IL1B+IL6+IL23-96h	
Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)
		PSRC1	1.77182			RNF20	0.37522	HSD17B12	1.55536		
		KAT2B	0.565			RPS12-PS2	0.375391	SNRPB	0.643816		
		TMED4	1.76822			EIF1AX	0.37563	PRDM2	0.644808		
		OLFR1055	0.565775			NAT10	0.375687	PSMF1	0.645237		
		ME3	0.56733			GPATCH4	0.375755	TMEM106B	0.645351		
		ETL4	0.567722			PFAS	0.375796	BCAS2	0.645699		
		LRRC33	0.56973			SLC35B1	0.375953	EBNA1BP2	0.645891		
		FBXL21	0.569879			BLVRA	0.376773	RORC	0.646421		
		2810417H13RIK	1.75076			KPNA3	2.65139	SMYD2	1.54653		
		DCBLD2	0.571483			STAG2	0.377564	GGA1	0.647033		
		RALGDS	0.57227			CRNKL1	2.64761	PSME3	0.647243		
		SYF2	1.747			SVOP	0.378	SEL1L	1.54484		
		ALG13	0.572541			I0C0044D17RIK	0.378094	BCCIP	0.647653		
		FDXR	1.74544			TMEM80	2.63584	SRA1	0.648219		
		TCTEX1D2	0.573273			UQCC	2.63526	SERPINB1A	0.648369		
		SLC25A42	0.573647			CCL20	0.379952	PRKAB1	0.648723		
		ID2	1.73951			ISY1	0.380229	SYT11	1.54006		
		1110008P14RIK	0.57645			IFI47	2.62916	ENTPD4	0.649691		
		METTL14	0.577951			ASNS	0.3804	NDUFB5	0.650471		
		TXNDC16	0.580239			NAA40	0.380451	TRP53BP1	0.651022		
		RPS7	1.72318			CCNE1	0.380776	PIP5K1C	0.651315		
		FAM184A	0.580454			D330012F22RIK	0.381723	CMC1	0.652247		
		SNAP47	1.7222			CDK5RAP2	0.382085	RPS6KC1	0.652501		
		RAD18	1.72044			1700123O20RIK	0.383244	PAPOLA	1.53209		
		MAP3K7	1.72015			TSG101	2.60705	1110031I02RIK	0.653034		
		H2-AB1	1.71789			MTHFS	2.60336	IL15RA	0.653215		
		COLEC12	0.583908			RTN4IP1	0.38439	DDT	0.653474		

Differentially expressed genes for GPR65-/-, PLZP-/- and TOSO-/- Th17 cells											
GPR65-KO-IL1B+IL6+ IL23-96h-1		GPR65-KO-TGFB1+IL6-96h-1		PLZP-KO-IL1B+IL6+IL23-48h-1		PLZP-KO-TGFB1+IL6-48h-1		TOSO-KO-IL1B+IL6+IL23-96h		TOSO-KO-IL1B+IL6+IL23-96h	
Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)
		LIAS	0.584048			ADAMTSL4	0.384451	LXN	0.653775		
		VGLL4	1.71021			POLE	0.384793	CAML	0.654098		
		STAM2	0.584817			BCAP29	0.384893	NME6	0.654537		
		E230001N04RIK	0.585026			CD5	0.385786	GHDC	0.655175		
		SEL1L	0.585038			GLE1	0.385815	NT5C3L	1.52508		
		H2AFY	1.70815			SMC5	0.386445	DDX18	0.655943		
		R3HDM2	0.585866			MPI	2.5868	2900010J23RIK	0.656538		
		SPEN	0.586272			ARIH1	2.58613	RB1	0.656729		
		NCSTN	0.58805			OXCT1	0.387007	HIST1H3H	0.656763		
		PRL8A1	0.588641			PDPK1	2.57756	DPP3	0.657155		
		MRPS15	1.69495			PRODH	0.388288	DLG2	0.657191		
		GIGYF2	0.591335			DDX47	0.388346	ZFP703	1.52155		
		DERA	1.6895			2610507B11RIK	2.57237	AC090563.1	1.52069		
		GM12033	1.68909			DPM1	0.389039	HCFC1	0.657862		
		TM9SF1	0.594558			ANXA7	0.389075	MRPS30	0.657906		
		UCHL4	0.594589			KEAP1	0.389438	NBR1	0.658347		
		XRCC4	0.59578			4930453N24RIK	2.56778	BC029214	0.65973		
		AC068006.1	0.597766			CREM	0.38953	CARS2	0.659736		
		AUTS2	0.598334			RPP14	0.389539	SNAP47	0.659889		
		NPDC1	0.599026			IFT20	2.56423	D8ERTD738E	0.660079		
		CT033780.1	0.599051			1810022K09RIK	0.390263	RBM33	0.66011		
		1110001J03RIK	1.66896			GALM	0.390284	DYNLT3	1.51481		
		AUH	0.599389			GFM1	0.390367	HNRNPAB	0.66032		
		GBP5	0.601504			PDAP1	0.391077	MRPS36-PS1	0.661543		
		OAS3	0.602776			CUX1	0.391626	PPP5C	0.661659		
		MTG1	0.606137			SP100	2.54971	CLN6	0.661741		
		PNPLA8	0.606206			PPP4R2	0.392231	MEMO1	0.661783		

Differentially expressed genes for GPR65-/-, PLZP-/- and TOSO-/- Th17 cells											
GPR65-KO-IL1B+IL6+ IL23-96h-1		GPR65-KO-TGFB1+IL6-96h-1		PLZP-KO-IL1B+IL6+IL23-48h-1		PLZP-KO-TGFB1+IL6-48h-1		TOSO-KO-IL1B+IL6+IL23-96h		TOSO-KO-IL1B+IL6+IL23-96h	
Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)
		1500011B03RIK	0.606396			CAND1	2.54938	LSM7	0.661841		
		ZFP575	0.606493			CBX3	2.54808	ELK3	1.51042		
		RPL30-PS6	0.608551			BUD13	0.393549	CUTA	0.662154		
		ZFP560	0.610421			SACM1L	0.393831	RPRD1B	1.50924		
		INADL	0.611092			PRKCH	0.393957	LAMP1	1.50915		
		CAMK2D	1.63545			SUMO3	2.53558	INPP5D	0.662713		
		1700054O19RIK	0.612794			UBA6	0.394606	UTY	1.50869		
		ATG4A	0.612875			TIMELESS	2.53153	SMAP2	0.662836		
		TMEM219	1.63077			2410091C18RIK	0.395525	DNAJC19	0.662901		
		SNF8	0.613773			GTF2E2	0.396167	GM6666	0.663179		
		DDX58	0.615491			DLGAP5	0.396602	MRPS10	0.663256		
		GPAA1	0.617882			SGOL1	2.52105	PDE6D	0.663286		
		MID1	1.61553			SRPK1	0.396828	PCBP1	0.663767		
		PSMD2	0.61948			HCLS1	0.39702	ZAP70	0.663792		
		EEF1E1	1.61071			BRD7	0.397033	AI480653	1.50628		
		SRPK1	0.623737			MTA3	0.397085	NEK2	0.663933		
		ZFP259	0.623906			WDR26	0.397789	PGLYRP2	1.50557		
		KCNH6	0.625341			NFRKB	0.398091	ANAPC4	0.664398		
		RPL18A	0.625821			TMED9	0.398144	UBE4A	0.664403		
		AC110247.1	0.626334			AC125221.1	2.51118	PRDX5	0.665043		
		CKS2	1.59547			MYBL2	0.398438	GTPBP3	0.666361		
		SEC63	0.626857			BAX	0.398521				
		MDM2	0.630367			USPL1	0.398688				
		BLOC1S2	0.630729			SLC31A1	0.399003				
		TMEM154	0.631156			ELAVL1	0.400468				
		SBNO1	1.5767			GM14443	2.49621				
		RPRD1B	0.634263			LY6C1	2.49202				

Differentially expressed genes for GPR65-/-, PLZP-/- and TOSO-/- Th17 cells											
GPR65-KO-IL1B+IL6+ IL23-96h-1		GPR65-KO-TGFB1+IL6-96h-1		PLZP-KO-IL1B+IL6+IL23-48h-1		PLZP-KO-TGFB1+IL6-48h-1		TOSO-KO-IL1B+IL6+IL23-96h		TOSO-KO-IL1B+IL6+IL23-96h	
Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)
		CFLAR	0.638629			DCPS	0.401308				
		MAP3K5	0.638837			INPP5F	2.48702				
		ATP6V1B2	0.639371			THOC5	0.402952				
		ALDH7A1	0.640159			GM6736	2.4789				
		WBP7	1.55732			HIPK3	2.4774				
		EXOC4	0.642436			HSPA4	0.403747				
		AIFM1	0.643759			NDUFV1	0.403761				
		PUM1	0.645538			SYT11	2.47641				
		SLC38A6	0.646337			COX8A	0.40383				
		NMT1	0.648082			E230001N04RIK	0.404113				
		ING1	0.64957			ELOF1	0.404182				
		STX1A	0.650026			VBP1	0.404242				
		AA960436	1.53269			TDP1	0.404604				
		HMGCR	0.652542			COPS7A	2.47145				
		TUFM	0.653859			TBRG1	0.404881				
		RBMS2	0.654071			RAD54L	0.405062				
		ABHD5	0.654691			GM15887	0.40524				
		ITGB1BP3	0.656226			GPR171	0.405533				
		H2-DMA	1.52224			RFWD3	0.405537				
		DSN1	0.657136			SMEK2	0.406438				
		FAM18B	0.657681			D19ERTD386E	0.406513				
		FXYD5	1.51958			PMF1	0.407169				
		PEX1	0.659458			COMMD8	2.45588				
		PAFAH2	0.663406			ACOX2	2.45558				
		AP2S1	0.663464			FAM54A	0.407283				
		CT025683.2	0.664094			WDR89	0.407385				
		TIGIT	0.665111			SAMSN1	2.45027				

Differentially expressed genes for GPR65-/-, PLZP-/- and TOSO-/- Th17 cells											
GPR65-KO-IL1B+IL6+ IL23-96h-1		GPR65-KO-TGFB1+IL6-96h-1		PLZP-KO-IL1B+IL6+IL23-48h-1		PLZP-KO-TGFB1+IL6-48h-1		TOSO-KO-IL1B+IL6+IL23-96h		TOSO-KO-IL1B+IL6+IL23-96h	
Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)
		GM5436	0.666166			ATP6V1D	2.44873				
		AC121959.1	0.666529			FYTTD1	2.44867				
						NDUFB2	2.44813				
						RAD1	0.408477				
						OTUD6B	0.408922				
						RBM39	2.44457				
						EBNA1BP2	0.409552				
						1810013L24RIK	0.410077				
						STAT3	0.410132				
						RNASEH2A	2.43243				
						MLL1	0.411438				
						PIGA	0.411634				
						KIF24	2.42673				
						AP3B1	0.412271				
						RAD21	0.412759				
						ZFP330	0.412968				
						ACER2	2.41598				
						DHX9	0.4141				
						INTS9	0.41427				
						BC031781	0.415611				
						RCBTB1	0.416923				
						SUPT7L	2.39515				
						NARF	0.417643				
						MCM10	0.417936				
						TGTP2	2.39105				
						FADS6	2.39042				
						2310035K24RIK	2.38318				

Differentially expressed genes for GPR65-/-, PLZP-/- and TOSO-/- Th17 cells											
GPR65-KO-IL1B+IL6+ IL23-96h-1		GPR65-KO-TGFB1+IL6-96h-1		PLZP-KO-IL1B+IL6+IL23-48h-1		PLZP-KO-TGFB1+IL6-48h-1		TOSO-KO-IL1B+IL6+IL23-96h		TOSO-KO-IL1B+IL6+IL23-96h	
Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)
						FAM60A	2.38162				
						PSMC3IP	0.41999				
						RNF25	2.379				
						LPXN	2.37549				
						IL17A	0.421247				
						TMEM176B	2.37084				
						GNL2	0.422463				
						MYCBP2	2.36498				
						ALKBH5	2.36021				
						CALU	2.36001				
						RBPJ	0.423864				
						RINT1	0.424041				
						GM9396	2.35258				
						GCSH	0.425617				
						SPARC	2.34917				
						GLO1	2.3482				
						2410089E03RIK	2.34731				
						DPY19L3	2.34681				
						MCM6	2.34553				
						B020018G12RIK	0.426889				
						SNRPF	2.34229				
						TRP53	2.34163				
						C79407	2.34024				
						PAM16	0.427458				
						SNRNP27	2.33875				
						TMEM11	0.429165				
						CRIP1	2.32956				
						RPL18	2.32709				

Differentially expressed genes for GPR65-/-, PLZP-/- and TOSO-/- Th17 cells											
GPR65-KO-IL1B+IL6+ IL23-96h-1		GPR65-KO-TGFB1+IL6-96h-1		PLZP-KO-IL1B+IL6+IL23-48h-1		PLZP-KO-TGFB1+IL6-48h-1		TOSO-KO-IL1B+IL6+IL23-96h		TOSO-KO-IL1B+IL6+IL23-96h	
Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)
						MT2	2.32653				
						ITK	2.32166				
						CTSA	2.32073				
						MPP1	0.431271				
						DERL2	0.431818				
						CUL1	0.432041				
						UHRF1	2.31278				
						ALDOART1	2.31234				
						USP14	0.43273				
						FAM172A	2.30775				
						GM4825	0.433418				
						PDCD5	0.433425				
						MED12	0.433605				
						PPIL2	2.30371				
						INTS10	2.30127				
						CCNL2	0.434666				
						LY6A	2.2994				
						1110057K04RIK	2.29924				
						2310028O11RIK	2.29643				
						SCAI	2.29301				
						GRK4	2.29277				
						BIRC5	2.29248				
						RAD23A	2.29189				
						G3BP1	2.29103				
						SDCCAG1	0.437				
						SMC6	0.437145				
						NSUN5	2.28523				
						FAM48A	0.438003				

Differentially expressed genes for GPR65-/-, PLZP-/- and TOSO-/- Th17 cells											
GPR65-KO-IL1B+IL6+ IL23-96h-1		GPR65-KO-TGFB1+IL6-96h-1		PLZP-KO-IL1B+IL6+IL23-48h-1		PLZP-KO-TGFB1+IL6-48h-1		TOSO-KO-IL1B+IL6+IL23-96h		TOSO-KO-IL1B+IL6+IL23-96h	
Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)
						NSF	0.438358				
						HARS	2.2809				
						2510006D16RIK	2.27804				
						TRABD	2.27559				
						SYNCRIP	0.439523				
						SNX10	2.27209				
						SEC11A	0.440221				
						SEC61A1	2.2693				
						CSTF3	2.26916				
						HELLS	2.26881				
						LIG3	0.441041				
						ARL1	2.26717				
						ZFP488	2.26453				
						HCFC2	0.442179				
						CDC7	0.442591				
						HEATR6	2.25775				
						ETFDH	2.25742				
						GM9034	2.2546				
						TAPBPL	2.25428				
						IER3IP1	2.25291				
						BTRC	0.4439				
						AFF4	0.444174				
						WDR11	0.444467				
						CDC26	0.445353				
						HAGH	2.24485				
						NUP205	0.445805				
						BRIX1	2.24139				
						2310016M24RIK	2.23958				

Differentially expressed genes for GPR65-/-, PLZP-/- and TOSO-/- Th17 cells											
GPR65-KO-IL1B+IL6+ IL23-96h-1		GPR65-KO-TGFB1+IL6-96h-1		PLZP-KO-IL1B+IL6+IL23-48h-1		PLZP-KO-TGFB1+IL6-48h-1		TOSO-KO-IL1B+IL6+IL23-96h		TOSO-KO-IL1B+IL6+IL23-96h	
Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)
						PRDX6	2.23838				
						CHMP2A	0.44701				
						MRPS21	0.447083				
						TTPAL	2.23388				
						MYO1B	2.23376				
						EMB	2.23361				
						ANAPC16	2.23213				
						LSP1	2.22932				
						BRP44L	2.22887				
						ASL	2.22792				
						XPNPEP2	2.22638				
						SOAT2	0.449889				
						GM5745	2.22194				
						LPCAT3	0.450215				
						TOMM5	0.450963				
						PSMA3	2.21527				
						DENR	0.452926				
						NEK6	2.20784				
						POGLUT1	0.453388				
						BCL2A1A	0.453814				
						1110007A13RIK	2.20188				
						GGTA1	0.454425				
						HK2	0.454714				
						BSG	2.1981				
						WDR76	0.455091				
						BAT2L2	0.455459				
						IARS	0.455491				

Differentially expressed genes for GPR65-/-, PLZP-/- and TOSO-/- Th17 cells											
GPR65-KO-IL1B+IL6+ IL23-96h-1		GPR65-KO-TGFB1+IL6-96h-1		PLZP-KO-IL1B+IL6+IL23-48h-1		PLZP-KO-TGFB1+IL6-48h-1		TOSO-KO-IL1B+IL6+IL23-96h		TOSO-KO-IL1B+IL6+IL23-96h	
Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)
						GM6483	2.19173				
						PNP	0.456278				
						TMX1	2.1891				
						TBRG4	2.18888				
						SDHD	0.45686				
						RPP21	2.18707				
						PLCG1	0.457433				
						TRAP1	2.18582				
						ACO1	0.458043				
						GTF2H5	2.17756				
						LCP2	0.459844				
						GM10719	2.17187				
						METTL2	2.16859				
						GM7263	0.461366				
						TMEM109	2.16707				
						TSTA3	2.16432				
						2310003F16RIK	2.16375				
						MRPL12	2.16216				
						RPS7	0.463462				
						0610010K14RIK	0.465093				
						ASF1B	2.13197				
						EBP	0.470037				
						ACOT7	2.12369				
						AC101875.1	2.12289				
						ARL5C	0.472555				
						TCEB2	0.472686				
						LARS2	0.473056				

Differentially expressed genes for GPR65-/-, PLZP-/- and TOSO-/- Th17 cells											
GPR65-KO-IL1B+IL6+ IL23-96h-1		GPR65-KO-TGFB1+IL6-96h-1		PLZP-KO-IL1B+IL6+IL23-48h-1		PLZP-KO-TGFB1+IL6-48h-1		TOSO-KO-IL1B+IL6+IL23-96h		TOSO-KO-IL1B+IL6+IL23-96h	
Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)
						EIF3D	0.478682				
						PA2G4	0.481972				
						CAPZA2	0.482067				
						GM4838	0.486641				
						CD82	2.05396				
						NDUFA2	0.487634				
						SELK	0.489794				
						COX7C	2.04046				
						2610024G14RIK	2.0264				
						0610007P14RIK	0.497032				
						TIMP1	1.9987				
						GM4987	0.501073				
						AC131780.3	1.99065				
						NEDD8	0.503159				
						GCN1L1	1.98192				
						MRPL18	0.506898				
						UBR1	1.96833				
						ARF1	1.93639				
						PPP1CA	1.92795				
						RPS25	0.519359				
						SNRPD2	0.519897				
						COX7A2	1.9177				
						DAZAP2	1.91034				
						COX7B	1.90204				
						GM16382	1.90161				
						RPN2	1.89675				
						RPL30	1.89126				
						IL9	1.88491				

Differentially expressed genes for GPR65-/-, PLZP-/- and TOSO-/- Th17 cells											
GPR65-KO-IL1B+IL6+ IL23-96h-1		GPR65-KO-TGFB1+IL6-96h-1		PLZP-KO-IL1B+IL6+IL23-48h-1		PLZP-KO-TGFB1+IL6-48h-1		TOSO-KO-IL1B+IL6+IL23-96h		TOSO-KO-IL1B+IL6+IL23-96h	
Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)	Gene	Fold.Change (KO/WT)
						8430427H17RIK	1.88407				
						RPS12-PS3	0.531131				
						PSMD13	0.535643				
						TUBB2C	1.86526				
						GM6807	0.53642				
						UQCR11	1.85797				

[00266] REFERENCES

Ahmed, M., and Gaffen, S.L. (2010). IL-17 in obesity and adipogenesis. Cytokine & growth factor reviews *21*, 449-453.

Amit, I., Citri, A., Shay, T., Lu, Y., Katz, M., Zhang, F., Tarcic, G., Siwak, D., Lahad, J., Jacob-Hirsch, J., *et al.* (2007). A module of negative feedback regulators defines growth factor signaling. Nature genetics *39*, 503-512.

Amit, I., Garber, M., Chevrier, N., Leite, A.P., Donner, Y., Eisenhaure, T., Guttman, M., Grenier, J.K., Li, W., Zuk, O., *et al.* (2009). Unbiased reconstruction of a mammalian transcriptional network mediating pathogen responses. Science *326*, 257-263.

Annunziato, F., Cosmi, L., Santarlasci, V., Maggi, L., Liotta, F., Mazzinghi, B., Parente, E., Fili, L., Ferri, S., Frosali, F., *et al.* (2007). Phenotypic and functional features of human Th17 cells. The Journal of experimental medicine *204*, 1849-1861.

Antebi, Y.E., Reich-Zeliger, S., Hart, Y., Mayo, A., Eizenberg, I., Rimer, J., Putheti, P., Pe'er, D., and Friedman, N. (2013). Mapping differentiation under mixed culture conditions reveals a tunable continuum of T cell fates. PLoS biology *11*, e1001616.

Arpaia, N., Campbell, C., Fan, X., Dikiy, S., van der Veeken, J., deRoos, P., Liu, H., Cross, J.R., Pfeffer, K., Coffer, P.J., *et al.* (2013). Metabolites produced by commensal bacteria promote peripheral regulatory T-cell generation. Nature *504*, 451-455.

Aust, G., Kamprad, M., Lamesch, P., and Schmucking, E. (2005). CXCR6 within T-helper (Th) and T-cytotoxic (Tc) type 1 lymphocytes in Graves' disease (GD). European journal of endocrinology / European Federation of Endocrine Societies *152*, 635-643.

Awasthi, A., Riol-Blanco, L., Jager, A., Korn, T., Pot, C., Galileos, G., Bettelli, E., Kuchroo, V.K., and Oukka, M. (2009). Cutting edge: IL-23 receptor gfp reporter mice reveal distinct populations of IL-17-producing cells. Journal of immunology *182*, 5904-5908.

Bachmann, M.F., Barner, M., and Kopf, M. (1999). CD2 sets quantitative thresholds in T cell activation. The Journal of experimental medicine *190*, 1383-1392.

Baeten, D.L., and Kuchroo, V.K. (2013). How Cytokine networks fuel inflammation: Interleukin-17 and a tale of two autoimmune diseases. Nature medicine *19*, 824-825.

Bending, D., De la Pena, H., Veldhoen, M., Phillips, J.M., Uyttenhove, C., Stockinger, B., and Cooke, A. (2009). Highly purified Th17 cells from BDC2.5NOD mice convert into Th1-like cells in NOD/SCID recipient mice. The Journal of clinical investigation *119*, 565-572.

Benjamini, Y., and Hochberg, Y. (1995). Controlling the false discovery rate: a practical and powerful approach to multiple testing. Journal of the Royal Statistical Society Series B (Methodological), 289-300.

Berod, L., Friedrich, C., Nandan, A., Freitag, J., Hagemann, S., Harmrolfs, K., Sandouk, A., Hesse, C., Castro, C.N., Bahre, H., *et al.* (2014). De novo fatty acid synthesis controls the fate between regulatory T and T helper 17 cells. Nature medicine *20*, 1327-1333.

Bettelli, E., Carrier, Y., Gao, W., Korn, T., Strom, T.B., Oukka, M., Weiner, H.L., and Kuchroo, V.K. (2006). Reciprocal developmental pathways for the generation of pathogenic effector TH17 and regulatory T cells. Nature *441*, 235-238.

Bettelli, E., Pagany, M., Weiner, H.L., Linington, C., Sobel, R.A., and Kuchroo, V.K. (2003). Myelin oligodendrocyte glycoprotein-specific T cell receptor transgenic mice develop spontaneous autoimmune optic neuritis. The Journal of experimental medicine *197*, 1073-1081.

Blaschitz, C., and Raffatellu, M. (2010). Th17 cytokines and the gut mucosal barrier. Journal of clinical immunology *30*, 196-203.

Brenner, D., Brustle, A., Lin, G.H., Lang, P.A., Duncan, G.S., Knobbe-Thomsen, C.B., St Paul, M., Reardon, C., Tusche, M.W., Snow, B., *et al.* (2014). Toso controls encephalitogenic immune responses by dendritic cells and regulatory T cells. Proceedings of the National Academy of Sciences of the United States of America *111*, 1060-1065.

Cellot, S., and Sauvageau, G. (2007). Zfx: at the crossroads of survival and self-renewal. Cell *129*, 239-241.

Chai, J.G., and Lechler, R.I. (1997). Immobilized anti-CD3 mAb induces anergy in murine naive and memory CD4+ T cells in vitro. International immunology *9*, 935-944.

Chen, L., Wu, G., and Ji, H. (2011). hmChIP: a database and web server for exploring publicly available human and mouse ChIP-seq and ChIP-chip data. Bioinformatics *27*, 1447-1448.

Cho, J.H. (2008). The genetics and immunopathogenesis of inflammatory bowel disease. Nature reviews Immunology *8*, 458-466.

Chung, Y., Chang, S.H., Martinez, G.J., Yang, X.O., Nurieva, R., Kang, H.S., Ma, L., Watowich, S.S., Jetten, A.M., Tian, Q., *et al.* (2009). Critical regulation of early Th17 cell differentiation by interleukin-1 signaling. Immunity *30*, 576-587.

Ciofani, M., Madar, A., Galan, C., Sellars, M., Mace, K., Pauli, F., Agarwal, A., Huang, W., Parkurst, C.N., Muratet, M., *et al.* (2012a). A validated regulatory network for Th17 cell specification. Cell *151*, 289-303.

Ciofani, M., Madar, A., Galan, C., Sellars, M., Mace, K., Pauli, F., Agarwal, A., Huang, W., Parkurst, Christopher N., Muratet, M., *et al.* (2012b). A Validated Regulatory Network for Th17 Cell Specification. Cell.

Codarri, L., Gyulveszi, G., Tosevski, V., Hesske, L., Fontana, A., Magnenat, L., Suter, T., and Becher, B. (2011). RORgammat drives production of the cytokine GM-CSF in helper T cells, which is essential for the effector phase of autoimmune neuroinflammation. Nature immunology *12*, 560-567.

Crawford, A., Angelosanto, J.M., Kao, C., Doering, T.A., Odorizzi, P.M., Barnett, B.E., and Wherry, E.J. (2014). Molecular and transcriptional basis of CD4(+) T cell dysfunction during chronic infection. Immunity *40*, 289-302.

Dang, E.V., Barbi, J., Yang, H.Y., Jinasena, D., Yu, H., Zheng, Y., Bordman, Z., Fu, J., Kim, Y., Yen, H.R., *et al.* (2011). Control of T(H)17/T(reg) balance by hypoxia-inducible factor 1. Cell *146*, 772-784.

De Rosa, S.C., Herzenberg, L.A., Herzenberg, L.A., and Roederer, M. (2001). 11-color, 13-parameter flow cytometry: identification of human naive T cells by phenotype, function, and T-cell receptor diversity. Nature medicine *7*, 245-248.

Deng, Q., Ramskold, D., Reinius, B., and Sandberg, R. (2014). Single-cell RNA-seq reveals dynamic, random monoallelic gene expression in mammalian cells. Science *343*, 193-196.

Dolfi, D.V., Boesteanu, A.C., Petrovas, C., Xia, D., Butz, E.A., and Katsikis, P.D. (2008). Late signals from CD27 prevent Fas-dependent apoptosis of primary CD8+ T cells. Journal of immunology *180*, 2912-2921.

El-Behi, M., Ciric, B., Dai, H., Yan, Y., Cullimore, M., Safavi, F., Zhang, G.X., Dittel, B.N., and Rostami, A. (2011). The encephalitogenicity of T(H)17 cells is dependent on IL-1- and IL-23-induced production of the cytokine GM-CSF. Nature immunology *12*, 568-575.

Esfandiari, E., McInnes, I.B., Lindop, G., Huang, F.P., Field, M., Komai-Koma, M., Wei, X., and Liew, F.Y. (2001). A proinflammatory role of IL-18 in the development of spontaneous autoimmune disease. Journal of immunology *167*, 5338-5347.

Fang, X., Huang, Z., Zhou, W., Wu, Q., Sloan, A.E., Ouyang, G., McLendon, R.E., Yu, J.S., Rich, J.N., and Bao, S. (2014). The zinc finger transcription factor ZFX Is required for maintaining the tumorigenic potential of glioblastoma stem cells. Stem cells.

Franke, A., McGovern, D.P., Barrett, J.C., Wang, K., Radford-Smith, G.L., Ahmad, T., Lees, C.W., Balschun, T., Lee, J., Roberts, R., *et al.* (2010). Genome-wide meta-analysis increases to 71 the number of confirmed Crohn's disease susceptibility loci. Nature genetics *42*, 1118-1125.

Galan-Caridad, J.M., Harel, S., Arenzana, T.L., Hou, Z.E., Doetsch, F.K., Mirny, L.A., and Reizis, B. (2007). Zfx controls the self-renewal of embryonic and hematopoietic stem cells. Cell *129*, 345-357.

Gaffen, S.L., Hernandez-Santos, N., and Peterson, A.C. (2011). IL-17 signaling in host defense against Candida albicans. Immunologic research *50*, 181-187.

Gattinoni, L., Zhong, X.S., Palmer, D.C., Ji, Y., Hinrichs, C.S., Yu, Z., Wrzesinski, C., Boni, A., Cassard, L., Garvin, L.M., *et al.* (2009). Wnt signaling arrests effector T cell differentiation and generates CD8+ memory stem cells. Nature medicine *15*, 808-813.

Genovese, M.C., Van den Bosch, F., Roberson, S.A., Bojin, S., Biagini, I.M., Ryan, P., and Sloan-Lancaster, J. (2010). LY2439821, a humanized anti-interleukin-17 monoclonal antibody, in the treatment of patients with rheumatoid arthritis: A phase I randomized, double-blind, placebo-controlled, proof-ofconcept study. Arthritis and rheumatism *62*, 929-939.

Ghoreschi, K., Laurence, A., Yang, X.P., Tato, C.M., McGeachy, M.J., Konkel, J.E., Ramos, H.L., Wei, L., Davidson, T.S., Bouladoux, N., *et al.* (2010). Generation of pathogenic T(H)17 cells in the absence of TGF-beta signalling. Nature *467*, 967-971.

Ghosh, S., Elder, A., Guo, J., Mani, U., Patane, M., Carson, K., Ye, Q., Bennett, R., Chi, S., Jenkins, T., *et al.* (2006). Design, synthesis, and progress toward optimization of potent small molecule antagonists of CC chemokine receptor 8 (CCR8). Journal of medicinal chemistry *49*, 2669-2672.

Gilmore, T.D., and Gerondakis, S. (2011). The c-Rel Transcription Factor in Development and Disease. Genes & cancer *2*, 695-711.

Hamann, I., Zipp, F., and Infante-Duarte, C. (2008). Therapeutic targeting of chemokine signaling in Multiple Sclerosis. Journal of the neurological sciences *274*, 31-38.

Harant, H., and Lindley, I.J. (2004). Negative cross-talk between the human orphan nuclear receptor Nur77/NAK-1/TR3 and nuclear factor-kappaB. Nucleic acids research *32*, 5280-5290.

Harel, S., Tu, E.Y., Weisberg, S., Esquilin, M., Chambers, S.M., Liu, B., Carson, C.T., Studer, L., Reizis, B., and Tomishima, M.J. (2012). ZFX controls the self-renewal of human embryonic stem cells. PloS one *7*, e42302.

Harrington, L.E., Janowski, K.M., Oliver, J.R., Zajac, A.J., and Weaver, C.T. (2008). Memory CD4 T cells emerge from effector T-cell progenitors. Nature *452*, 356-360.

Hendriks, J., Gravestein, L.A., Tesselaar, K., van Lier, R.A., Schumacher, T.N., and Borst, J. (2000). CD27 is required for generation and long-term maintenance of T cell immunity. Nature immunology *1*, 433-440.

Hendriks, J., Xiao, Y., and Borst, J. (2003). CD27 promotes survival of activated T cells and complements CD28 in generation and establishment of the effector T cell pool. The Journal of experimental medicine *198*, 1369-1380.

Hernandez-Santos, N., and Gaffen, S.L. (2012). Th17 cells in immunity to Candida albicans. Cell host & microbe *11*, 425-435.

Hilliard, B.A., Mason, N., Xu, L., Sun, J., Lamhamedi-Cherradi, S.E., Liou, H.C., Hunter, C., and Chen, Y.H. (2002). Critical roles of c-Rel in autoimmune inflammation and helper T cell differentiation. The Journal of clinical investigation *110*, 843-850.

Hitoshi, Y., Lorens, J., Kitada, S.I., Fisher, J., LaBarge, M., Ring, H.Z., Francke, U., Reed, J.C., Kinoshita, S., and Nolan, G.P. (1998). Toso, a cell surface, specific regulator of Fas-induced apoptosis in T cells. Immunity *8*, 461-471.

Hock, H., Meade, E., Medeiros, S., Schindler, J.W., Valk, P.J., Fujiwara, Y., and Orkin, S.H. (2004). Tel/Etv6 is an essential and selective regulator of adult hematopoietic stem cell survival. Genes & development *18*, 2336-2341.

Hueber, W., Sands, B.E., Lewitzky, S., Vandemeulebroecke, M., Reinisch, W., Higgins, P.D., Wehkamp, J., Feagan, B.G., Yao, M.D., Karczewski, M., *et al.* (2012). Secukinumab, a human anti-IL-17A monoclonal antibody, for moderate to severe Crohn's disease: unexpected results of a randomised, double-blind placebo-controlled trial. Gut *61*, 1693-1700.

Hundt, M., Tabata, H., Jeon, M.S., Hayashi, K., Tanaka, Y., Krishna, R., De Giorgio, L., Liu, Y.C., Fukata, M., and Altman, A. (2006). Impaired activation and localization of LAT in anergic T cells as a consequence of a selective palmitoylation defect. Immunity *24*, 513-522.

Huntley, R.P., Binns, D., Dimmer, E., Barrell, D., O'Donovan, C., and Apweiler, R. (2009). QuickGO: a user tutorial for the web-based Gene Ontology browser. Database : the journal of biological databases and curation *2009*, bap010.

Ichii, H., Sakamoto, A., Arima, M., Hatano, M., Kuroda, Y., and Tokuhisa, T. (2007). Bcl6 is essential for the generation of long-term memory CD4+ T cells. International immunology *19*, 427-433.

International Genetics of Ankylosing Spondylitis, C., Cortes, A., Hadler, J., Pointon, J.P., Robinson, P.C., Karaderi, T., Leo, P., Cremin, K., Pryce, K., Harris, J., *et al.* (2013). Identification of multiple risk variants for ankylosing spondylitis through high-density genotyping of immune-related loci. Nature genetics *45*, 730-738.

International Multiple Sclerosis Genetics, C., Wellcome Trust Case Control, C., Sawcer, S., Hellenthal, G., Pirinen, M., Spencer, C.C., Patsopoulos, N.A., Moutsianas, L., Dilthey, A., Su, Z., *et al.* (2011). Genetic risk and a primary role for cell-mediated immune mechanisms in multiple sclerosis. Nature *476*, 214-219.

Ioannidis, V., Beermann, F., Clevers, H., and Held, W. (2001). The beta-catenin--TCF-1 pathway ensures CD4(+)CD8(+) thymocyte survival. Nature immunology *2*, 691-697.

Ivanov, II, Atarashi, K., Manel, N., Brodie, E.L., Shima, T., Karaoz, U., Wei, D., Goldfarb, K.C., Santee, C.A., Lynch, S.V., *et al.* (2009). Induction of intestinal Th17 cells by segmented filamentous bacteria. Cell *139*, 485-498.

Jager, A., Dardalhon, V., Sobel, R.A., Bettelli, E., and Kuchroo, V.K. (2009). Th1, Th17, and Th9 effector cells induce experimental autoimmune encephalomyelitis with different pathological phenotypes. Journal of immunology *183*, 7169-7177.

Jarboe, J.S., Anderson, J.C., Duarte, C.W., Mehta, T., Nowsheen, S., Hicks, P.H., Whitley, A.C., Rohrbach, T.D., McCubrey, R.O., Chiu, S., *et al.* (2012). MARCKS regulates growth and radiation sensitivity and is a novel prognostic factor for glioma. Clinical cancer research : an official journal of the American Association for Cancer Research *18*, 3030-3041.

Jhun, J.Y., Yoon, B.Y., Park, M.K., Oh, H.J., Byun, J.K., Lee, S.Y., Min, J.K., Park, S.H., Kim, H.Y., and Cho, M.L. (2012). Obesity aggravates the joint inflammation in a collagen-induced

arthritis model through deviation to Th17 differentiation. Experimental & molecular medicine *44*, 424-431.

Jin, L., Martynowski, D., Zheng, S., Wada, T., Xie, W., and Li, Y. (2010). Structural basis for hydroxycholesterols as natural ligands of orphan nuclear receptor RORgamma. Molecular endocrinology *24*, 923-929.

Johnson, W.E., Li, C., and Rabinovic, A. (2007). Adjusting batch effects in microarray expression data using empirical Bayes methods. Biostatistics *8*, 118-127.

Jostins, L., Ripke, S., Weersma, R.K., Duerr, R.H., McGovern, D.P., Hui, K.Y., Lee, J.C., Schumm, L.P., Sharma, Y., Anderson, C.A., *et al.* (2012). Host-microbe interactions have shaped the genetic architecture of inflammatory bowel disease. Nature *491*, 119-124.

Kandasamy, K., Mohan, S.S., Raju, R., Keerthikumar, S., Kumar, G.S., Venugopal, A.K., Telikicherla, D., Navarro, J.D., Mathivanan, S., Pecquet, C., *et al.* (2010). NetPath: a public resource of curated signal transduction pathways. Genome biology *11*, R3.

Kaplan, M.H., Sun, Y.L., Hoey, T., and Grusby, M.J. (1996). Impaired IL-12 responses and enhanced development of Th2 cells in Stat4-deficient mice. Nature *382*, 174-177.

Komatsu, N., Okamoto, K., Sawa, S., Nakashima, T., Oh-hora, M., Kodama, T., Tanaka, S., Bluestone, J.A., and Takayanagi, H. (2014). Pathogenic conversion of Foxp3+ T cells into TH17 cells in autoimmune arthritis. Nature medicine *20*, 62-68.

Konkel, J.E., and Chen, W. (2011). Balancing acts: the role of TGF-beta in the mucosal immune system. Trends in molecular medicine *17*, 668-676.

Korn, T., Bettelli, E., Gao, W., Awasthi, A., Jager, A., Strom, T.B., Oukka, M., and Kuchroo, V.K. (2007). IL-21 initiates an alternative pathway to induce proinflammatory T(H)17 cells. Nature *448*, 484-487.

Korn, T., Bettelli, E., Oukka, M., and Kuchroo, V.K. (2009). IL-17 and Th17 Cells. Annu Rev Immunol *27*, 485-517.

Kryczek, I., Zhao, E., Liu, Y., Wang, Y., Vatan, L., Szeliga, W., Moyer, J., Klimczak, A., Lange, A., and Zou, W. (2011). Human TH17 cells are long-lived effector memory cells. Science translational medicine *3*, 104ra100.

Kurachi, M., Barnitz, R.A., Yosef, N., Odorizzi, P.M., DiIorio, M.A., Lemieux, M.E., Yates, K., Godec, J., Klatt, M.G., Regev, A., *et al.* (2014). The transcription factor BATF operates as an essential differentiation checkpoint in early effector CD8+ T cells. Nature immunology *15*, 373-383.

Lachmann, A., Xu, H., Krishnan, J., Berger, S.I., Mazloom, A.R., and Ma'ayan, A. (2010). ChEA: transcription factor regulation inferred from integrating genome-wide ChIP-X experiments. Bioinformatics *26*, 2438-2444.

Lalmansingh, A.S., Arora, K., Demarco, R.A., Hager, G.L., and Nagaich, A.K. (2013). High-throughput RNA FISH analysis by imaging flow cytometry reveals that pioneer factor Foxa1 reduces transcriptional stochasticity. PloS one *8*, e76043.

Lamb, J.R., Zanders, E.D., Sewell, W., Crumpton, M.J., Feldmann, M., and Owen, M.J. (1987). Antigen-specific T cell unresponsiveness in cloned helper T cells mediated via the CD2 or CD3/Ti receptor pathways. European journal of immunology *17*, 1641-1644.

Lang, K.S., Lang, P.A., Meryk, A., Pandyra, A.A., Boucher, L.M., Pozdeev, V.I., Tusche, M.W., Gothert, J.R., Haight, J., Wakeham, A., *et al.* (2013). Involvement of Toso in activation of monocytes, macrophages, and granulocytes. Proceedings of the National Academy of Sciences of the United States of America *110*, 2593-2598.

Latta, M., Mohan, K., and Issekutz, T.B. (2007). CXCR6 is expressed on T cells in both T helper type 1 (Th1) inflammation and allergen-induced Th2 lung inflammation but is only a weak mediator of chemotaxis. Immunology *121*, 555-564.

Laurence, A., Tato, C.M., Davidson, T.S., Kanno, Y., Chen, Z., Yao, Z., Blank, R.B., Meylan, F., Siegel, R., Hennighausen, L., *et al.* (2007). Interleukin-2 signaling via STAT5 constrains T helper 17 cell generation. Immunity *26*, 371-381.

Lee, Y., Awasthi, A., Yosef, N., Quintana, F.J., Xiao, S., Peters, A., Wu, C., Kleinewietfeld, M., Kunder, S., Hafler, D.A., *et al.* (2012). Induction and molecular signature of pathogenic TH17 cells. Nature immunology *13*, 991-999.

Lee, Y.K., Turner, H., Maynard, C.L., Oliver, J.R., Chen, D., Elson, C.O., and Weaver, C.T. (2009). Late developmental plasticity in the T helper 17 lineage. Immunity *30*, 92-107.

Lees, C.W., Barrett, J.C., Parkes, M., and Satsangi, J. (2011). New IBD genetics: common pathways with other diseases. Gut *60*, 1739-1753.

Liberzon, A., Subramanian, A., Pinchback, R., Thorvaldsdottir, H., Tamayo, P., and Mesirov, J.P. (2011). Molecular signatures database (MSigDB) 3.0. Bioinformatics *27*, 1739-1740.

Leonardi, C., Matheson, R., Zachariae, C., Cameron, G., Li, L., Edson-Heredia, E., Braun, D., and Banerjee, S. (2012). Anti-interleukin-17 monoclonal antibody ixekizumab in chronic plaque psoriasis. The New England journal of medicine *366*, 1190-1199.

Lin, L., Ibrahim, A.S., Xu, X., Farber, J.M., Avanesian, V., Baquir, B., Fu, Y., French, S.W., Edwards, J.E., Jr., and Spellberg, B. (2009). Th1-Th17 cells mediate protective adaptive immunity against Staphylococcus aureus and Candida albicans infection in mice. PLoS pathogens *5*, e1000703.

Linhart, C., Halperin, Y., and Shamir, R. (2008). Transcription factor and microRNA motif discovery: the Amadeus platform and a compendium of metazoan target sets. Genome research *18*, 1180-1189.

Liu, Y., Wang, X., Jiang, J., Cao, Z., Yang, B., and Cheng, X. (2011). Modulation of T cell cytokine production by miR-144* with elevated expression in patients with pulmonary tuberculosis. Molecular immunology *48*, 1084-1090.

Mahad, D.J., and Ransohoff, R.M. (2003). The role of MCP-1 (CCL2) and CCR2 in multiple sclerosis and experimental autoimmune encephalomyelitis (EAE). Seminars in immunology *15*, 23-32.

Maity, A., and Koumenis, C. (2006). HIF and MIF--a nifty way to delay senescence? Genes & development *20*, 3337-3341.

Martinez, V.G., Escoda-Ferran, C., Tadeu Simoes, I., Arai, S., Orta Mascaro, M., Carreras, E., Martinez-Florensa, M., Yelamos, J., Miyazaki, T., and Lozano, F. (2014). The macrophage soluble receptor AIM/Api6/CD5L displays a broad pathogen recognition spectrum and is involved in early response to microbial aggression. Cellular & molecular immunology *11*, 343-354.

Mathews, J.A., Wurmbrand, A.P., Ribeiro, L., Neto, F.L., and Shore, S.A. (2014). Induction of IL-17A Precedes Development of Airway Hyperresponsiveness during Diet-Induced Obesity and Correlates with Complement Factor D. Frontiers in immunology *5*, 440.

Maynard, C.L., Harrington, L.E., Janowski, K.M., Oliver, J.R., Zindl, C.L., Rudensky, A.Y., and Weaver, C.T. (2007). Regulatory T cells expressing interleukin 10 develop from Foxp3+ and Foxp3- precursor cells in the absence of interleukin 10. Nature immunology *8*, 931-941.

McGeachy, M.J., Chen, Y., Tato, C.M., Laurence, A., Joyce-Shaikh, B., Blumenschein, W.M., McClanahan, T.K., O'Shea, J.J., and Cua, D.J. (2009). The interleukin 23 receptor is essential for the terminal differentiation of interleukin 17-producing effector T helper cells in vivo. Nature immunology *10*, 314-324.

Miaw, S.C., Choi, A., Yu, E., Kishikawa, H., and Ho, I.C. (2000). ROG, repressor of GATA, regulates the expression of cytokine genes. Immunity *12*, 323-333.

Miyazaki, T., Hirokami, Y., Matsuhashi, N., Takatsuka, H., and Naito, M. (1999). Increased susceptibility of thymocytes to apoptosis in mice lacking AIM, a novel murine macrophage-derived soluble factor belonging to the scavenger receptor cysteine-rich domain superfamily. The Journal of experimental medicine *189*, 413-422.

Mo, C., Chearwae, W., O'Malley, J.T., Adams, S.M., Kanakasabai, S., Walline, C.C., Stritesky, G.L., Good, S.R., Perumal, N.B., Kaplan, M.H., *et al.* (2008). Stat4 isoforms differentially regulate inflammation and demyelination in experimental allergic encephalomyelitis. Journal of immunology *181*, 5681-5690.

Monk, J.M., Hou, T.Y., Turk, H.F., McMurray, D.N., and Chapkin, R.S. (2013). n3 PUFAs reduce mouse CD4+ T-cell ex vivo polarization into Th17 cells. J Nutr *143*, 1501-1508.

Monk, J.M., Jia, Q., Callaway, E., Weeks, B., Alaniz, R.C., McMurray, D.N., and Chapkin, R.S. (2012). Th17 cell accumulation is decreased during chronic experimental colitis by (n-3) PUFA in Fat-1 mice. J Nutr *142*, 117-124.

Muranski, P., Borman, Z.A., Kerkar, S.P., Klebanoff, C.A., Ji, Y., Sanchez-Perez, L., Sukumar, M., Reger, R.N., Yu, Z., Kern, S.J., *et al.* (2011). Th17 cells are long lived and retain a stem cell-like molecular signature. Immunity *35*, 972-985.

Nakae, S., Iwakura, Y., Suto, H., and Galli, S.J. (2007). Phenotypic differences between Th1 and Th17 cells and negative regulation of Th1 cell differentiation by IL-17. Journal of leukocyte biology *81*, 1258-1268.

Nguyen, X.H., Lang, P.A., Lang, K.S., Adam, D., Fattakhova, G., Foger, N., Kamal, M.A., Prilla, P., Mathieu, S., Wagner, C., *et al.* (2011). Toso regulates the balance between apoptotic and nonapoptotic death receptor signaling by facilitating RIP1 ubiquitination. Blood *118*, 598-608.

Nishikomori, R., Usui, T., Wu, C.Y., Morinobu, A., O'Shea, J.J., and Strober, W. (2002). Activated STAT4 has an essential role in Th1 differentiation and proliferation that is independent of its role in the maintenance of IL-12R beta 2 chain expression and signaling. Journal of immunology *169*, 4388-4398.

Novershtern, N., Subramanian, A., Lawton, L.N., Mak, R.H., Haining, W.N., McConkey, M.E., Habib, N., Yosef, N., Chang, C.Y., Shay, T., *et al.* (2011). Densely interconnected transcriptional circuits control cell states in human hematopoiesis. Cell *144*, 296-309.

Nurieva, R., Yang, X.O., Martinez, G., Zhang, Y., Panopoulos, A.D., Ma, L., Schluns, K., Tian, Q., Watowich, S.S., Jetten, A.M., *et al.* (2007). Essential autocrine regulation by IL-21 in the generation of inflammatory T cells. Nature *448*, 480-483.

Palmer, M.T., and Weaver, C.T. (2010). Autoimmunity: increasing suspects in the CD4+ T cell lineup. Nature immunology *11*, 36-40.

Papp, K.A., Leonardi, C., Menter, A., Ortonne, J.P., Krueger, J.G., Kricorian, G., Aras, G., Li, J., Russell, C.B., Thompson, E.H., *et al.* (2012). Brodalumab, an anti-interleukin-17-receptor antibody for psoriasis. The New England journal of medicine *366*, 1181-1189.

Patel, D.D., Lee, D.M., Kolbinger, F., and Antoni, C. (2013). Effect of IL-17A blockade with secukinumab in autoimmune diseases. Annals of the rheumatic diseases *72 Suppl 2*, ii116-123.

Pe'er, D., Regev, A., and Tanay, A. (2002). Minreg: inferring an active regulator set. Bioinformatics *18 Suppl 1*, S258-267.

Pepper, M., Linehan, J.L., Pagan, A.J., Zell, T., Dileepan, T., Cleary, P.P., and Jenkins, M.K. (2010). Different routes of bacterial infection induce long-lived TH1 memory cells and short-lived TH17 cells. Nature immunology *11*, 83-89.

Perfetto, S.P., Chattopadhyay, P.K., and Roederer, M. (2004). Seventeen-colour flow cytometry: unravelling the immune system. Nature reviews Immunology *4*, 648-655.

Peters, A., Burkett, P.R., Sobel, R.A., Buckley, C.D., Watson, S.P., Bettelli, E., and Kuchroo, V.K. (2014). Podoplanin negatively regulates CD4+ effector T cell responses. The Journal of clinical investigation.

Pruitt, K.D., Tatusova, T., and Maglott, D.R. (2007). NCBI reference sequences (RefSeq): a curated non-redundant sequence database of genomes, transcripts and proteins. Nucleic Acids Res *35*, D61-65.

Quintana, F.J., Jin, H., Burns, E.J., Nadeau, M., Yeste, A., Kumar, D., Rangachari, M., Zhu, C., Xiao, S., Seavitt, J., *et al.* (2012). Aiolos promotes TH17 differentiation by directly silencing Il2 expression. Nature immunology *13*, 770-777.

Ramskold, D., Luo, S., Wang, Y.-C., Li, R., Deng, Q., Faridani, O.R., Daniels, G.A., Khrebtukova, I., Loring, J.F., Laurent, L.C., *et al.* (2012). Full-length mRNA-Seq from single-cell levels of RNA and individual circulating tumor cells. Nature biotechnology *30*, 777-782.

Reya, T., Duncan, A.W., Ailles, L., Domen, J., Scherer, D.C., Willert, K., Hintz, L., Nusse, R., and Weissman, I.L. (2003). A role for Wnt signalling in self-renewal of haematopoietic stem cells. Nature *423*, 409-414.

Risso, D., Schwartz, K., Sherlock, G., and Dudoit, S. (2011). GC-content normalization for RNA-Seq data. BMC bioinformatics *12*, 480.

Rocha, P.P., Scholze, M., Bleiss, W., and Schrewe, H. (2010). Med12 is essential for early mouse development and for canonical Wnt and Wnt/PCP signaling. Development *137*, 2723-2731.

Sallusto, F., Lenig, D., Forster, R., Lipp, M., and Lanzavecchia, A. (1999). Two subsets of memory T lymphocytes with distinct homing potentials and effector functions. Nature *401*, 708-712.

Salminen, A., and Kaarniranta, K. (2011). Control of p53 and NF-kappaB signaling by WIP1 and MIF: role in cellular senescence and organismal aging. Cellular signalling *23*, 747-752.

Santori, F.R., Huang, P., van de Pavert, S.A., Douglass, E.F., Jr., Leaver, D.J., Haubrich, B.A., Keber, R., Lorbek, G., Konijn, T., Rosales, B.N., *et al.* (2015). Identification of natural RORgamma ligands that regulate the development of lymphoid cells. Cell metabolism *21*, 286-297.

Sarkar, S., Kalia, V., Haining, W.N., Konieczny, B.T., Subramaniam, S., and Ahmed, R. (2008). Functional and genomic profiling of effector CD8 T cell subsets with distinct memory fates. The Journal of experimental medicine *205*, 625-640.

Sarrias, M.R., Gronlund, J., Padilla, O., Madsen, J., Holmskov, U., and Lozano, F. (2004). The Scavenger Receptor Cysteine-Rich (SRCR) domain: an ancient and highly conserved protein module of the innate immune system. Critical reviews in immunology *24*, 1-37.

Segal, E., Shapira, M., Regev, A., Pe'er, D., Botstein, D., Koller, D., and Friedman, N. (2003). Module networks: identifying regulatory modules and their condition-specific regulators from gene expression data. Nature genetics *34*, 166-176.

Sester, U., Presser, D., Dirks, J., Gartner, B.C., Kohler, H., and Sester, M. (2008). PD-1 expression and IL-2 loss of cytomegalovirus- specific T cells correlates with viremia and reversible functional anergy. American journal of transplantation : official journal of the American Society of Transplantation and the American Society of Transplant Surgeons *8*, 1486-1497.

Shalek, A.K., Satija, R., Adiconis, X., Gertner, R.S., Gaublomme, J.T., Raychowdhury, R., Schwartz, S., Yosef, N., Malboeuf, C., Gnirke, A., *et al.* (2013). Single-cell transcriptomics reveals bimodality in expression and splicing in immune cells. Nature *in press*.

Shalek, A.K., Satija, R., Shuga, J., Trombetta, J.J., Gennert, D., Lu, D., Chen, P., Gertner, R.S., Gaublomme, J.T., Yosef, N., *et al.* (2014). Single-cell RNA-seq reveals dynamic paracrine control of cellular variation. Nature *509*, 363-369.

Shi, L.Z., Wang, R., Huang, G., Vogel, P., Neale, G., Green, D.R., and Chi, H. (2011). HIF1alphadependent glycolytic pathway orchestrates a metabolic checkpoint for the differentiation of TH17 and Treg cells. The Journal of experimental medicine *208*, 1367-1376.

Shin, H.J., Lee, J.B., Park, S.H., Chang, J., and Lee, C.W. (2009). T-bet expression is regulated by EGR1-mediated signaling in activated T cells. Clinical immunology *131*, 385-394.

Shinohara, M.L., Kim, J.H., Garcia, V.A., and Cantor, H. (2008). Engagement of the type I interferon receptor on dendritic cells inhibits T helper 17 cell development: role of intracellular osteopontin. Immunity *29*, 68-78.

Snyder, C.M., Cho, K.S., Bonnett, E.L., van Dommelen, S., Shellam, G.R., and Hill, A.B. (2008). Memory inflation during chronic viral infection is maintained by continuous production of short-lived, functional T cells. Immunity *29*, 650-659.

Song, Y., and Jacob, C.O. (2005). The mouse cell surface protein TOSO regulates Fas/Fas ligand-induced apoptosis through its binding to Fas-associated death domain. The Journal of biological chemistry *280*, 9618-9626.

Soroosh, P., Wu, J., Xue, X., Song, J., Sutton, S.W., Sablad, M., Yu, J., Nelen, M.I., Liu, X., Castro, G., *et al.* (2014). Oxysterols are agonist ligands of RORgammat and drive Th17 cell differentiation. Proceedings of the National Academy of Sciences of the United States of America *111*, 12163-12168.

Stumhofer, J.S., Silver, J.S., Laurence, A., Porrett, P.M., Harris, T.H., Turka, L.A., Ernst, M., Saris, C.J., O'Shea, J.J., and Hunter, C.A. (2007). Interleukins 27 and 6 induce STAT3-mediated T cell production of interleukin 10. Nature immunology *8*, 1363-1371.

Sutton, C., Brereton, C., Keogh, B., Mills, K.H., and Lavelle, E.C. (2006). A crucial role for interleukin (IL)-1 in the induction of IL-17-producing T cells that mediate autoimmune encephalomyelitis. The Journal of experimental medicine *203*, 1685-1691.

Symons, A., Budelsky, A.L., and Towne, J.E. (2012). Are Th17 cells in the gut pathogenic or protective? Mucosal immunology *5*, 4-6.

Thierfelder, W.E., van Deursen, J.M., Yamamoto, K., Tripp, R.A., Sarawar, S.R., Carson, R.T., Sangster, M.Y., Vignali, D.A., Doherty, P.C., Grosveld, G.C., *et al.* (1996). Requirement for Stat4 in interleukin-12-mediated responses of natural killer and T cells. Nature *382*, 171-174.

Trapnell, C., Cacchiarelli, D., Grimsby, J., Pokharel, P., Li, S., Morse, M., Lennon, N.J., Livak, K.J., Mikkelsen, T.S., and Rinn, J.L. (2014). The dynamics and regulators of cell fate decisions are revealed by pseudotemporal ordering of single cells. Nature biotechnology *32*, 381-386.

Trapnell, C., Pachter, L., and Salzberg, S.L. (2009). TopHat: discovering splice junctions with RNA-Seq. In Bioinformatics, pp. 1105-1111.

Trimble, L.A., Kam, L.W., Friedman, R.S., Xu, Z., and Lieberman, J. (2000). CD3zeta and CD28 down-modulation on CD8 T cells during viral infection. Blood *96*, 1021-1029.

Tsuzuki, S., and Seto, M. (2013). TEL (ETV6)-AML1 (RUNX1) initiates self-renewing fetal pro-B cells in association with a transcriptional program shared with embryonic stem cells in mice. Stem cells *31*, 236-247.

Veldhoen, M., Hocking, R.J., Atkins, C.J., Locksley, R.M., and Stockinger, B. (2006). TGFbeta in the context of an inflammatory cytokine milieu supports de novo differentiation of IL-17-producing T cells. Immunity *24*, 179-189.

Waite, J.C., and Skokos, D. (2012). Th17 response and inflammatory autoimmune diseases. International journal of inflammation *2012*, 819467.

Wang, H., Geng, J., Wen, X., Bi, E., Kossenkov, A.V., Wolf, A.I., Tas, J., Choi, Y.S., Takata, H., Day, T.J., *et al.* (2014). The transcription factor Foxp1 is a critical negative regulator of the differentiation of follicular helper T cells. Nature immunology *15*, 667-675.

Wei, G., Wei, L., Zhu, J., Zang, C., Hu-Li, J., Yao, Z., Cui, K., Kanno, Y., Roh, T.Y., Watford, W.T., *et al.* (2009). Global mapping of H3K4me3 and H3K27me3 reveals specificity and plasticity in lineage fate determination of differentiating CD4+ T cells. Immunity *30*, 155-167.

Weisberg, S.P., Smith-Raska, M.R., Esquilin, J.M., Zhang, J., Arenzana, T.L., Lau, C.M., Churchill, M., Pan, H., Klinakis, A., Dixon, J.E., *et al.* (2014). ZFX controls propagation and prevents differentiation of acute T-lymphoblastic and myeloid leukemia. Cell reports *6*, 528-540.

Welford, S.M., Bedogni, B., Gradin, K., Poellinger, L., Broome Powell, M., and Giaccia, A.J. (2006). HIF1alpha delays premature senescence through the activation of MIF. Genes & development *20*, 3366-3371.

Wells, A.D., Walsh, M.C., Bluestone, J.A., and Turka, L.A. (2001). Signaling through CD28 and CTLA-4 controls two distinct forms of T cell anergy. The Journal of clinical investigation *108*, 895-903.

Wherry, E.J., Ha, S.J., Kaech, S.M., Haining, W.N., Sarkar, S., Kalia, V., Subramaniam, S., Blattman, J.N., Barber, D.L., and Ahmed, R. (2007). Molecular signature of CD8+ T cell exhaustion during chronic viral infection. Immunity *27*, 670-684.

Willinger, T., Freeman, T., Herbert, M., Hasegawa, H., McMichael, A.J., and Callan, M.F. (2006). Human naive CD8 T cells down-regulate expression of the WNT pathway transcription factors lymphoid enhancer binding factor 1 and transcription factor 7 (T cell factor-1) following antigen encounter in vitro and in vivo. Journal of immunology *176*, 1439-1446.

Winer, S., Paltser, G., Chan, Y., Tsui, H., Engleman, E., Winer, D., and Dosch, H.M. (2009). Obesity predisposes to Th17 bias. European journal of immunology *39*, 2629-2635.

Wu, C., Yosef, N., Thalhamer, T., Zhu, C., Xiao, S., Kishi, Y., Regev, A., and Kuchroo, V.K. (2013). Induction of pathogenic TH17 cells by inducible salt-sensing kinase SGK1. Nature *496*, 513-517.

Xiao, S., Yosef, N., Yang, J., Wang, Y., Zhou, L., Zhu, C., Wu, C., Baloglu, E., Schmidt, D., Ramesh, R., *et al.* (2014). Small-molecule RORgammat antagonists inhibit T helper 17 cell transcriptional network by divergent mechanisms. Immunity *40*, 477-489.

Xu, J., Yang, Y., Qiu, G., Lal, G., Wu, Z., Levy, D.E., Ochando, J.C., Bromberg, J.S., and Ding, Y. (2009). c-Maf regulates IL-10 expression during Th17 polarization. Journal of immunology *182*, 6226-6236.

Yosef, N., Shalek, A.K., Gaublomme, J.T., Jin, H., Lee, Y., Awasthi, A., Wu, C., Karwacz, K., Xiao, S., Jorgolli, M., *et al.* (2013). Dynamic regulatory network controlling TH17 cell differentiation. Nature *496*, 461-468.

Zhou, L., Ivanov, II, Spolski, R., Min, R., Shenderov, K., Egawa, T., Levy, D.E., Leonard, W.J., and Littman, D.R. (2007). IL-6 programs T(H)-17 cell differentiation by promoting sequential engagement of the IL-21 and IL-23 pathways. Nature immunology *8*, 967-974.

Zingoni, A., Soto, H., Hedrick, J.A., Stoppacciaro, A., Storlazzi, C.T., Sinigaglia, F., D'Ambrosio, D., O'Garra, A., Robinson, D., Rocchi, M., *et al.* (1998). The chemokine receptor CCR8 is preferentially expressed in Th2 but not Th1 cells. Journal of immunology *161*, 547-551.

* * *

[00267] While preferred embodiments of the present invention have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the invention. It should be understood that various alternatives to the embodiments of the invention described herein may be employed in practicing the invention.

WHAT IS CLAIMED IS:

1. A method of diagnosing, prognosing and/or staging an immune response involving T cell balance, comprising detecting a first level of expression, activity and/or function of one or more of a gene in a herein Table or a combination of genes in herein Table(s) or *Toso, Ctla2b*, *Gatm*, *Bdh2*, *Bcat1*, *Zfp36*, *Acsl4*, *Acat3*, *Adi1*, *Dot1l*, *Mett10d*, *Sirt6*, *Slc25a13*, *Chd2*, *Ino80c*, *Med21*, *Pdss1*, *Galk1*, *Gnpda2* or *Mtpap* or any one of the foregoing or any combination thereof with one or more of *Gpr65*, *Plzp* or *Cd5l* or any combination of *Gpr65*, *Plzp* or *Cd5l* in any combination thereof *Gpr65*, *Plzp*, *Toso* or *Cd5l* or one or more products of one or more of a gene in a herein Table or a combination of genes in herein Table(s) or *Toso, Ctla2b*, *Gatm*, *Bdh2*, *Bcat1*, *Zfp36*, *Acsl4*, *Acat3*, *Adi1*, *Dot1l*, *Mett10d*, *Sirt6*, *Slc25a13*, *Chd2*, *Ino80c*, *Med21*, *Pdss1*, *Galk1*, *Gnpda2* or *Mtpap* or any one of the foregoing or any combination thereof with one or more of *Gpr65*, *Plzp* or *Cd5l* or any combination thereof *Gpr65*, *Plzp or Cd5l* in any combination of *Gpr65*, *Plzp*, *Toso* or *Cd5l* and comparing the detected level to a control of level of a gene in a herein Table or a combination of genes in herein Table(s) or *Toso, Ctla2b*, *Gatm*, *Bdh2*, *Bcat1*, *Zfp36*, *Acsl4*, *Acat3*, *Adi1*, *Dot1l*, *Mett10d*, *Sirt6*, *Slc25a13*, *Chd2*, *Ino80c*, *Med21*, *Pdss1*, *Galk1*, *Gnpda2* or *Mtpap* or any one of the foregoing or any combination thereof with one or more of *Gpr65*, *Plzp* or *Cd5l* or any combination of *Gpr65*, *Plzp* or *Cd5l* in any combination thereof *Gpr65*, *Plzp*, *Toso* or *Cd5l* or gene product expression, activity and/or function, wherein a difference in the detected level and the control level indicates that the presence of an immune response in the subject.

2. A method of monitoring an immune response in a subject comprising detecting a level of expression, activity and/or function of one or more of a gene in a herein Table or a combination of genes in herein Table(s) or *Toso, Ctla2b*, *Gatm*, *Bdh2*, *Bcat1*, *Zfp36*, *Acsl4*, *Acat3*, *Adi1*, *Dot1l*, *Mett10d*, *Sirt6*, *Slc25a13*, *Chd2*, *Ino80c*, *Med21*, *Pdss1*, *Galk1*, *Gnpda2* or *Mtpap* or any one of the foregoing or any combination thereof with one or more of *Gpr65*, *Plzp* or *Cd5l* or any combination of *Gpr65*, *Plzp* or *Cd5l* in any combination thereof *Gpr65*, *Plzp*, *Toso* or *Cd5l* or one or more products of one or more of a gene in a herein Table or a combination of genes in herein Table(s) or *Toso, Ctla2b*, *Gatm*, *Bdh2*, *Bcat1*, *Zfp36*, *Acsl4*, *Acat3*, *Adi1*, *Dot1l*, *Mett10d*, *Sirt6*, *Slc25a13*, *Chd2*, *Ino80c*, *Med21*, *Pdss1*, *Galk1*, *Gnpda2* or *Mtpap* or any one of the foregoing or any combination thereof with one or more of *Gpr65*, *Plzp* or *Cd5l* or any

combination thereof *Gpr65*, *Plzp or Cd5l* in any combination of *Gpr65*, *Plzp*, *Toso* or *Cd5l* at a first time point, detecting a level of expression, activity and/or function of one or more signature genes or one or more products of one or more of a gene in a herein Table or a combination of genes in herein Table(s) or *Toso, Ctla2b*, *Gatm*, *Bdh2*, *Bcat1*, *Zfp36*, *Acsl4*, *Acat3*, *Adi1*, *Dot1l*, *Mett10d*, *Sirt6*, *Slc25a13*, *Chd2*, *Ino80c*, *Med21*, *Pdss1*, *Galk1*, *Gnpda2* or *Mtpap* or any one of the foregoing or any combination thereof with one or more of *Gpr65*, *Plzp* or *Cd5l* or any combination thereof *Gpr65*, *Plzp or Cd5l* in any combination of *Gpr65*, *Plzp*, *Toso* or *Cd5l* at a second time point, and comparing the first detected level of expression, activity and/or function with the second detected level of expression, activity and/or function, wherein a change in the first and second detected levels indicates a change in the immune response in the subject.

3. A method of identifying a patient population at risk or suffering from an immune response comprising detecting a level of expression, activity and/or function of one or more of a gene in a herein Table or a combination of genes in herein Table(s) or *Toso, Ctla2b*, *Gatm*, *Bdh2*, *Bcat1*, *Zfp36*, *Acsl4*, *Acat3*, *Adi1*, *Dot1l*, *Mett10d*, *Sirt6*, *Slc25a13*, *Chd2*, *Ino80c*, *Med21*, *Pdss1*, *Galk1*, *Gnpda2* or *Mtpap* or any one of the foregoing or any combination thereof with one or more of *Gpr65*, *Plzp* or *Cd5l* or any combination of *Gpr65*, *Plzp* or *Cd5l* in any combination thereof *Gpr65*, *Plzp*, *Toso* or *Cd5l* or one or more products of one or more of a gene in a herein Table or a combination of genes in herein Table(s) or *Toso, Ctla2b*, *Gatm*, *Bdh2*, *Bcat1*, *Zfp36*, *Acsl4*, *Acat3*, *Adi1*, *Dot1l*, *Mett10d*, *Sirt6*, *Slc25a13*, *Chd2*, *Ino80c*, *Med21*, *Pdss1*, *Galk1*, *Gnpda2* or *Mtpap* or any one of the foregoing or any combination thereof with one or more of *Gpr65*, *Plzp* or *Cd5l* or any combination thereof *Gpr65*, *Plzp or Cd5l* in any combination of *Gpr65*, *Plzp*, *Toso* or *Cd5l* in the patient population and comparing the level of expression, activity and/or function of one or more signature genes or one or more products of a gene in a herein Table or a combination of genes in herein Table(s) or *Toso, Ctla2b*, *Gatm*, *Bdh2*, *Bcat1*, *Zfp36*, *Acsl4*, *Acat3*, *Adi1*, *Dot1l*, *Mett10d*, *Sirt6*, *Slc25a13*, *Chd2*, *Ino80c*, *Med21*, *Pdss1*, *Galk1*, *Gnpda2* or *Mtpap* or any one of the foregoing or any combination thereof with one or more of *Gpr65*, *Plzp* or *Cd5l* or any combination thereof *Gpr65*, *Plzp* or *Cd5l* in any combination of *Gpr65*, *Plzp*, *Toso* or *Cd5l*in a patient population not at risk or suffering from an immune response, wherein a difference in the level of expression, activity and/or function of one or more of a gene in a herein Table or a combination of genes in herein Table(s) or *Toso, Ctla2b*, *Gatm*,

Bdh2, *Bcat1*, *Zfp36*, *Acsl4*, *Acat3*, *Adi1*, *Dot1l*, *Mett10d*, *Sirt6*, *Slc25a13*, *Chd2*, *Ino80c*, *Med21*, *Pdss1*, *Galk1*, *Gnpda2* or *Mtpap* or any one of the foregoing or any combination thereof with one or more of *Gpr65*, *Plzp* or *Cd5l* or any combination of *Gpr65*, *Plzp* or *Cd5l* in any combination thereof *Gpr65*, *Plzp*, *Toso* or *Cd5l* or one or more products of one or more of a gene in a herein Table or a combination of genes in herein Table(s) or *Toso, Ctla2b*, *Gatm*, *Bdh2*, *Bcat1*, *Zfp36*, *Acsl4*, *Acat3*, *Adi1*, *Dot1l*, *Mett10d*, *Sirt6*, *Slc25a13*, *Chd2*, *Ino80c*, *Med21*, *Pdss1*, *Galk1*, *Gnpda2* or *Mtpap* or any one of the foregoing or any combination thereof with one or more thereof *Gpr65*, *Plzp* or *Cd5l* or any combination of *Gpr65*, *Plzp or Cd5l* in any combination of *Gpr65*, *Plzp*, *Toso* or *Cd5l* in the patient populations identifies the patient population as at risk or suffering from an immune response.

4. A method for monitoring subjects undergoing a treatment or therapy specific for a target gene selected from the group consisting of candidates comprising a gene in a herein Table or a combination of genes in herein Table(s) or *Toso, Ctla2b*, *Gatm*, *Bdh2*, *Bcat1*, *Zfp36*, *Acsl4*, *Acat3*, *Adi1*, *Dot1l*, *Mett10d*, *Sirt6*, *Slc25a13*, *Chd2*, *Ino80c*, *Med21*, *Pdss1*, *Galk1*, *Gnpda2* or *Mtpap* or any one of the foregoing or any combination thereof with one or more of *Gpr65*, *Plzp* or *Cd5l* or any combination thereof *Gpr65*, *Plzp* or *Cd5l* in any combination of *Gpr65*, *Plzp*, *Toso* or *Cd5l* for an aberrant immune response to determine whether the patient is responsive to the treatment or therapy comprising detecting a level of expression, activity and/or function of one or more of a gene in a herein Table or a combination of genes in herein Table(s) or *Toso, Ctla2b*, *Gatm*, *Bdh2*, *Bcat1*, *Zfp36*, *Acsl4*, *Acat3*, *Adi1*, *Dot1l*, *Mett10d*, *Sirt6*, *Slc25a13*, *Chd2*, *Ino80c*, *Med21*, *Pdss1*, *Galk1*, *Gnpda2* or *Mtpap* or any one of the foregoing or any combination thereof with one or more of *Gpr65*, *Plzp* or *Cd5l* or any combination thereof *Gpr65*, *Plzp* or *Cd5l* in any combination of *Gpr65*, *Plzp*, *Toso* or *Cd5l* in the absence of the treatment or therapy and comparing the level of expression, activity and/or function of a gene in a herein Table or a combination of genes in herein Table(s) or *Toso, Ctla2b*, *Gatm*, *Bdh2*, *Bcat1*, *Zfp36*, *Acsl4*, *Acat3*, *Adi1*, *Dot1l*, *Mett10d*, *Sirt6*, *Slc25a13*, *Chd2*, *Ino80c*, *Med21*, *Pdss1*, *Galk1*, *Gnpda2* or *Mtpap* or any one of the foregoing or any combination thereof with one or more of *Gpr65*, *Plzp* or *Cd5l* or any combination thereof *Gpr65*, *Plzp* or *Cd5l* in any combination of *Gpr65*, *Plzp*, *Toso* or *Cd5l* in the presence of the treatment or therapy, wherein a difference in the level of expression, activity and/or function of a gene in a herein Table or a combination of genes in

herein Table(s) or *Toso, Ctla2b*, *Gatm*, *Bdh2*, *Bcat1*, *Zfp36*, *Acsl4*, *Acat3*, *Adi1*, *Dot1l*, *Mett10d*, *Sirt6*, *Slc25a13*, *Chd2*, *Ino80c*, *Med21*, *Pdss1*, *Galk1*, *Gnpda2* or *Mtpap* or any one of the foregoing or any combination thereof with one or more of *Gpr65*, *Plzp* or *Cd5l* or any combination of *Gpr65*, *Plzp* or *Cd5l* in any combination thereof *Gpr65*, *Plzp*, *Toso* or *Cd5l* or one or more products of one or more of a gene in a herein Table or a combination of genes in herein Table(s) or *Toso, Ctla2b*, *Gatm*, *Bdh2*, *Bcat1*, *Zfp36*, *Acsl4*, *Acat3*, *Adi1*, *Dot1l*, *Mett10d*, *Sirt6*, *Slc25a13*, *Chd2*, *Ino80c*, *Med21*, *Pdss1*, *Galk1*, *Gnpda2* or *Mtpap* or any one of the foregoing or any combination thereof with one or more thereof *Gpr65*, *Plzp* or *Cd5l* or any combination of *Gpr65*, *Plzp or Cd5l* in any combination of *Gpr65*, *Plzp*, *Toso* or *Cd5l* in the presence of the treatment or therapy indicates whether the patient is responsive to the treatment or therapy.

5. The method of any one of claims 1 to 4 wherein the immune response is an autoimmune response or an inflammatory response.

6. The method of claim 5 wherein the inflammatory response is associated with an autoimmune response, an infectious disease and/or a pathogen-based disorder.

7. The method of any one of claims 1 to 6 wherein the signature genes are Th17-associated genes.

8. The method of any one of claims 4 to 7, wherein the treatment or therapy is an antagonist as to expression of a gene in a herein Table or a combination of genes in herein Table(s) or *Toso, Ctla2b*, *Gatm*, *Bdh2*, *Bcat1*, *Zfp36*, *Acsl4*, *Acat3*, *Adi1*, *Dot1l*, *Mett10d*, *Sirt6*, *Slc25a13*, *Chd2*, *Ino80c*, *Med21*, *Pdss1*, *Galk1*, *Gnpda2* or *Mtpap* or any one of the foregoing or any combination thereof with one or more of *Gpr65*, *Plzp* or *Cd5l* or any combination thereof *Gpr65*, *Plzp* or *Cd5l* in any combination of *Gpr65*, *Plzp*, *Toso* or *Cd5l*in an amount sufficient to induce differentiation toward regulatory T cells (Tregs), Th1 cells, or a combination of Tregs and Th1 cells.

9. The method of any one of claims 4 to 7, wherein the treatment or therapy is an agonist that enhances or increases the expression of a gene in a herein Table or a combination of genes in herein Table(s) or *Toso, Ctla2b*, *Gatm*, *Bdh2*, *Bcat1*, *Zfp36*, *Acsl4*, *Acat3*, *Adi1*, *Dot1l*, *Mett10d*, *Sirt6*, *Slc25a13*, *Chd2*, *Ino80c*, *Med21*, *Pdss1*, *Galk1*, *Gnpda2* or *Mtpap* or any one of the

foregoing or any combination thereof with one or more of *Gpr65*, *Plzp* or *Cd5l* or any combination thereof *Gpr65*, *Plzp* or *Cd5l* in any combination of *Gpr65*, *Plzp*, *Toso* or *Cd5l*in an amount sufficient to induce T cell differentiation toward Th17 cells.

10. The method of claim 4 to 7, wherein the treatment or therapy is an antagonist of a target gene selected from the group consisting of a gene in a herein Table or a combination of genes in herein Table(s) or *Toso, Ctla2b*, *Gatm*, *Bdh2*, *Bcat1*, *Zfp36*, *Acsl4*, *Acat3*, *Adi1*, *Dot1l*, *Mett10d*, *Sirt6*, *Slc25a13*, *Chd2*, *Ino80c*, *Med21*, *Pdss1*, *Galk1*, *Gnpda2* or *Mtpap* or any one of the foregoing or any combination thereof with one or more of *Gpr65*, *Plzp* or *Cd5l* or any combination thereof *Gpr65*, *Plzp* or *Cd5l* in any combination of *Gpr65*, *Plzp*, *Toso* or *Cd5l*in an amount sufficient to switch Th17 cells from a pathogenic to non-pathogenic signature.

11. The method of claim 4 to 7, wherein the treatment or therapy is an agonist that enhances or increases the expression of a target gene selected from the group consisting of a gene in a herein Table or a combination of genes in herein Table(s) or *Toso, Ctla2b*, *Gatm*, *Bdh2*, *Bcat1*, *Zfp36*, *Acsl4*, *Acat3*, *Adi1*, *Dot1l*, *Mett10d*, *Sirt6*, *Slc25a13*, *Chd2*, *Ino80c*, *Med21*, *Pdss1*, *Galk1*, *Gnpda2* or *Mtpap* or any one of the foregoing or any combination thereof with one or more of *Gpr65*, *Plzp* or *Cd5l* or any combination thereof *Gpr65*, *Plzp* or *Cd5l* in any combination of *Gpr65*, *Plzp*, *Toso* or *Cd5l* in an amount sufficient to switch Th17 cells from a non-pathogenic to a pathogenic signature.

12. The method according to any one of claims 8 to 11, wherein the T cell modulating agent is an antibody, a soluble polypeptide, a polypeptide agent, a peptide agent, a nucleic acid agent, a nucleic acid ligand, or a small molecule agent.

13. A method of modulating T cell balance, the method comprising contacting a T cell or a population of T cells with a T cell modulating agent in an amount sufficient to modify differentiation, maintenance and/or function of the T cell or population of T cells by altering balance between Th17 cells, regulatory T cells (Tregs) and other T cell subsets as compared to differentiation, maintenance and/or function of the T cell or population of T cells in the absence of the T cell modulating agent; wherein the T cell modulating agent is an antagonist for or of a gene in a herein Table or a combination of genes in herein Table(s) or *Toso, Ctla2b*, *Gatm*, *Bdh2*, *Bcat1*, *Zfp36*, *Acsl4*, *Acat3*, *Adi1*, *Dot1l*, *Mett10d*, *Sirt6*, *Slc25a13*, *Chd2*, *Ino80c*, *Med21*, *Pdss1*,

Galk1, *Gnpda2* or *Mtpap* or any one of the foregoing or any combination thereof with one or more of *Gpr65*, *Plzp* or *Cd5l* or any combination thereof *Gpr65*, *Plzp* or *Cd5l* in any combination of *Gpr65*, *Plzp*, *Toso* or *Cd5l*in an amount sufficient to induce differentiation toward regulatory T cells (Tregs), Th1 cells, or a combination of Tregs and Th1 cells, or wherein the T cell modulating agent is an agonist that enhances or increases the expression of a gene in a herein Table or a combination of genes in herein Table(s) or *Toso, Ctla2b*, *Gatm*, *Bdh2*, *Bcat1*, *Zfp36*, *Acsl4*, *Acat3*, *Adi1*, *Dot1l*, *Mett10d*, *Sirt6*, *Slc25a13*, *Chd2*, *Ino80c*, *Med21*, *Pdss1*, *Galk1*, *Gnpda2* or *Mtpap* or any one of the foregoing or any combination thereof with one or more of *Gpr65*, *Plzp* or *Cd5l* or any combination thereof *Gpr65*, *Plzp* or *Cd5l* in any combination of *Gpr65*, *Plzp*, *Toso* or *Cd5l* in an amount sufficient to induce T cell differentiation toward Th17 cells, or wherein the T cell modulating agent is specific for a target gene selected from the group consisting of a gene in a herein Table or a combination of genes in herein Table(s) or *Toso, Ctla2b*, *Gatm*, *Bdh2*, *Bcat1*, *Zfp36*, *Acsl4*, *Acat3*, *Adi1*, *Dot1l*, *Mett10d*, *Sirt6*, *Slc25a13*, *Chd2*, *Ino80c*, *Med21*, *Pdss1*, *Galk1*, *Gnpda2* or *Mtpap* or any one of the foregoing or any combination thereof with one or more of *Gpr65*, *Plzp* or *Cd5l* or any combination thereof *Gpr65*, *Plzp* or *Cd5l* in any combination of *Gpr65*, *Plzp*, *Toso* or *Cd5l,* or wherein the T cell modulating agent is an antagonist of a target gene selected from the group consisting of a gene in a herein Table or a combination of genes in herein Table(s) or *Toso, Ctla2b*, *Gatm*, *Bdh2*, *Bcat1*, *Zfp36*, *Acsl4*, *Acat3*, *Adi1*, *Dot1l*, *Mett10d*, *Sirt6*, *Slc25a13*, *Chd2*, *Ino80c*, *Med21*, *Pdss1*, *Galk1*, *Gnpda2* or *Mtpap* or any one of the foregoing or any combination thereof with one or more of *Gpr65*, *Plzp* or *Cd5l* or any combination thereof *Gpr65*, *Plzp* or *Cd5l* in any combination of *Gpr65*, *Plzp*, *Toso* or *Cd5l* in an amount sufficient to switch Th17 cells from a pathogenic to non-pathogenic signature, or wherein the T cell modulating agent is an agonist that enhances or increases the expression of a target gene selected from the group consisting of a gene in a herein Table or a combination of genes in herein Table(s) or *Toso, Ctla2b*, *Gatm*, *Bdh2*, *Bcat1*, *Zfp36*, *Acsl4*, *Acat3*, *Adi1*, *Dot1l*, *Mett10d*, *Sirt6*, *Slc25a13*, *Chd2*, *Ino80c*, *Med21*, *Pdss1*, *Galk1*, *Gnpda2* or *Mtpap* or any one of the foregoing or any combination thereof with one or more of *Gpr65*, *Plzp* or *Cd5l* or any combination thereof *Gpr65*, *Plzp* or *Cd5l* in any combination of *Gpr65*, *Plzp*, *Toso* or *Cd5l*in an amount sufficient to switch Th17 cells from a non-pathogenic to a pathogenic signature.

14. The method according to claim 13, wherein the T cell modulating agent is an antibody, a soluble polypeptide, a polypeptide agent, a peptide agent, a nucleic acid agent, a nucleic acid ligand, or a small molecule agent.

15. The method according to claim 13, wherein the T cells are naïve T cells, partially differentiated T cells, differentiated T cells, a combination of naïve T cells and partially differentiated T cells, a combination of naïve T cells and differentiated T cells, a combination of partially differentiated T cells and differentiated T cells, or a combination of naïve T cells, partially differentiated T cells and differentiated T cells.

16. A method of enhancing Th17 differentiation in a cell population, increasing expression, activity and/or function of one or more Th17-associated cytokines or one or more Th17-associated transcription regulators selected from interleukin 17F (IL-17F), interleukin 17A (IL-17A), STAT3, interleukin 21 (IL-21) and RAR-related orphan receptor C (RORC), and/or decreasing expression, activity and/or function of one or more non-Th17-associated cytokines or non-Th17-associated transcription regulators selected from FOXP3, interferon gamma (IFN-γ), GATA3, STAT4 and TBX21, comprising contacting a T cell with an agent that enhances expression, activity and/or function of a gene in a herein Table or a combination of genes in herein Table(s) or *Toso, Ctla2b*, *Gatm*, *Bdh2*, *Bcat1*, *Zfp36*, *Acsl4*, *Acat3*, *Adi1*, *Dot1l*, *Mett10d*, *Sirt6*, *Slc25a13*, *Chd2*, *Ino80c*, *Med21*, *Pdss1*, *Galk1*, *Gnpda2* or *Mtpap* or any one of the foregoing or any combination thereof with one or more of *Gpr65*, *Plzp* or *Cd5l* or any combination thereof *Gpr65*, *Plzp* or *Cd5l* in any combination of *Gpr65*, *Plzp*, *Toso* or *Cd5l*.

17. The method of claim 16, wherein the agent enhances expression, activity and/or function of at least *Toso*.

18. The method of claim 16 or 17, wherein the agent is an antibody, a soluble polypeptide, a polypeptide agonist, a peptide agonist, a nucleic acid agonist, a nucleic acid ligand, or a small molecule agonist.

19. The method of claim 18, wherein the agent is an antibody.

20. The method of claim 19 wherein the antibody is a monoclonal antibody.

21. The method of claim 20, wherein the antibody is a chimeric, humanized or fully human monoclonal antibody.

22. Use of an antagonist for or of a gene in a herein Table or a combination of genes in herein Table(s) or *Toso, Ctla2b*, *Gatm*, *Bdh2*, *Bcat1*, *Zfp36*, *Acsl4*, *Acat3*, *Adi1*, *Dot1l*, *Mett10d*, *Sirt6*, *Slc25a13*, *Chd2*, *Ino80c*, *Med21*, *Pdss1*, *Galk1*, *Gnpda2* or *Mtpap* or any one of the foregoing or any combination thereof with one or more of *Gpr65*, *Plzp* or *Cd5l* or any combination thereof *Gpr65*, *Plzp* or *Cd5l* in any combination of *Gpr65*, *Plzp*, *Toso* or *Cd5l* in an amount sufficient to induce differentiation toward regulatory T cells (Tregs), Th1 cells, or a combination of Tregs and Th1 cells for treating or Drug Discovery of or formulating or preparing a treatment for an aberrant immune response in a patient.

23. Use of an agonist that enhances or increases the expression of a gene in a herein Table or a combination of genes in herein Table(s) or *Toso, Ctla2b*, *Gatm*, *Bdh2*, *Bcat1*, *Zfp36*, *Acsl4*, *Acat3*, *Adi1*, *Dot1l*, *Mett10d*, *Sirt6*, *Slc25a13*, *Chd2*, *Ino80c*, *Med21*, *Pdss1*, *Galk1*, *Gnpda2* or *Mtpap* or any one of the foregoing or any combination thereof with one or more of *Gpr65*, *Plzp* or *Cd5l* or any combination thereof *Gpr65*, *Plzp* or *Cd5l* in any combination of *Gpr65*, *Plzp*, *Toso* or *Cd5l* in an amount sufficient to induce T cell differentiation toward Th17 cells for treating or Drug Discovery of or formulating or preparing a treatment for an aberrant immune response in a patient.

24. Use of an antagonist of a target gene selected from the group consisting of a gene in a herein Table or a combination of genes in herein Table(s) or *Toso, Ctla2b*, *Gatm*, *Bdh2*, *Bcat1*, *Zfp36*, *Acsl4*, *Acat3*, *Adi1*, *Dot1l*, *Mett10d*, *Sirt6*, *Slc25a13*, *Chd2*, *Ino80c*, *Med21*, *Pdss1*, *Galk1*, *Gnpda2* or *Mtpap* or any one of the foregoing or any combination thereof with one or more of *Gpr65*, *Plzp* or *Cd5l* or any combination thereof *Gpr65*, *Plzp* or *Cd5l* in any combination of *Gpr65*, *Plzp*, *Toso* or *Cd5l* in an amount sufficient to switch Th17 cells from a pathogenic to non-pathogenic signature for treating or Drug Discovery of or formulating or preparing a treatment for an aberrant immune response in a patient.

25. Use of an agonist that enhances or increases the expression of a target gene selected from the group consisting of a gene in a herein Table or a combination of genes in herein Table(s) or *Toso, Ctla2b*, *Gatm*, *Bdh2*, *Bcat1*, *Zfp36*, *Acsl4*, *Acat3*, *Adi1*, *Dot1l*, *Mett10d*, *Sirt6*, *Slc25a13*,

Chd2, *Ino80c*, *Med21*, *Pdss1*, *Galk1*, *Gnpda2* or *Mtpap* or any one of the foregoing or any combination thereof with one or more thereof *Gpr65*, *Plzp* or *Cd5l* or any combination of *Gpr65*, *Plzp* or *Cd5l* in any combination of *Gpr65*, *Plzp*, *Toso* or *Cd5l* in an amount sufficient to switch Th17 cells from a non-pathogenic to a pathogenic signature for treating or Drug Discovery of or formulating or preparing a treatment for an aberrant immune response in a patient.

26. A treatment method or Drug Discovery method or method of formulating or preparing a treatment comprising any one of the methods or uses of any of the preceding claims.

27. The method of claim 26 or the use of claim 27wherein an agent, agonist or antagonist of any of the preceding claims is a putative drug or treatment in Drug Discovery or formulating or preparing a treatment; and formulating or preparing a treatment comprises admixing the agent, agonist or antagonist with a pharmaceutically acceptable carrier or excipient.

28. A method of drug discovery for the treatment of a disease or condition involving an immune response involving T cell balance in a population of cells or tissue which express one or more of a gene in a herein Table or a combination of genes in herein Table(s) or *Toso, Ctla2b*, *Gatm*, *Bdh2*, *Bcat1*, *Zfp36*, *Acsl4*, *Acat3*, *Adi1*, *Dot1l*, *Mett10d*, *Sirt6*, *Slc25a13*, *Chd2*, *Ino80c*, *Med21*, *Pdss1*, *Galk1*, *Gnpda2* or *Mtpap* or any one of the foregoing or any combination thereof with one or more of *Gpr65*, *Plzp* or *Cd5l* or any combination of *Gpr65*, *Plzp* or *Cd5l* in any combination thereof *Gpr65*, *Plzp*, *Toso* or *Cd5l* comprising the steps of:

(a) providing a compound or plurality of compounds to be screened for their efficacy in the treatment of said disease or condition;

(b) contacting said compound or plurality of compounds with said population of cells or tissue;

(c) detecting a first level of expression, activity and/or function of one or more of a gene in a herein Table or a combination of genes in herein Table(s) or *Toso, Ctla2b*, *Gatm*, *Bdh2*, *Bcat1*, *Zfp36*, *Acsl4*, *Acat3*, *Adi1*, *Dot1l*, *Mett10d*, *Sirt6*, *Slc25a13*, *Chd2*, *Ino80c*, *Med21*, *Pdss1*, *Galk1*, *Gnpda2* or *Mtpap* or any one of the foregoing or any combination thereof with one or more of *Gpr65*, *Plzp* or *Cd5l* or any combination of *Gpr65*, *Plzp* or *Cd5l* in any combination thereof *Gpr65*, *Plzp*, *Toso* or *Cd5l* or one or more products of one or more of a gene in a herein Table or

a combination of genes in herein Table(s) or *Toso, Ctla2b*, *Gatm*, *Bdh2*, *Bcat1*, *Zfp36*, *Acsl4*, *Acat3*, *Adi1*, *Dot1l*, *Mett10d*, *Sirt6*, *Slc25a13*, *Chd2*, *Ino80c*, *Med21*, *Pdss1*, *Galk1*, *Gnpda2* or *Mtpap* or any one of the foregoing or any combination thereof with one or more of *Gpr65*, *Plzp* or *Cd5l* or any combination thereof *Gpr65*, *Plzp or Cd5l* in any combination of *Gpr65*, *Plzp*, *Toso* or *Cd5l*;

(d) comparing the detected level to a control of level of a gene in a herein Table or a combination of genes in herein Table(s) or *Toso, Ctla2b*, *Gatm*, *Bdh2*, *Bcat1*, *Zfp36*, *Acsl4*, *Acat3*, *Adi1*, *Dot1l*, *Mett10d*, *Sirt6*, *Slc25a13*, *Chd2*, *Ino80c*, *Med21*, *Pdss1*, *Galk1*, *Gnpda2* or *Mtpap* or any one of the foregoing or any combination thereof with one or more of *Gpr65*, *Plzp* or *Cd5l* or any combination of *Gpr65*, *Plzp* or *Cd5l* in any combination thereof *Gpr65*, *Plzp*, *Toso* or *Cd5l* or gene product expression, activity and/or function; and,

(e) evaluating the difference between the detected level and the control level to determine the immune response elicited by said compound or plurality of compounds.

29. A method of diagnosing, prognosing and/or staging an immune response involving Th17 T cell balance in a subject, comprising detecting a first level of expression of one or more of saturated fatty acids (SFA) and/or polyunsaturated fatty acids (PUFA) in Th17 cells, and comparing the detected level to a control level of saturated fatty acids (SFA) and/or polyunsaturated fatty acids (PUFA), wherein a change in the first level of expression and the control level detected indicates a change in the immune response in the subject.

30. The method of claim 29, further comprising determining the ratio of SFA to PUFA and comparing the ratio to a control level, wherein a shift in the ratio indicates a change in the immune response in the subject.

31. The method of claim 29 or 30, wherein a shift torwards polyunsaturated fatty acids (PUFA) and/or away from saturated fatty acids (SFA) indicates a non-pathogenic Th17 response.

32. A method for monitoring subjects undergoing a treatment or therapy involving T cell balance comprising, detecting a first level of expression of one or more of saturated fatty acids (SFA) and/or polyunsaturated fatty acids (PUFA) in Th17 cells in the absence of the treatment or

therapy and comparing the detected level to a level of saturated fatty acids (SFA) and/or polyunsaturated fatty acids (PUFA) in the presence of the treatment or therapy, wherein a difference in the level of expression in the presence of the treatment or therapy indicates whether the subject is responsive to the treatment or therapy.

33. The method of claim 32, wherein the treatment or therapy involving T cell balance is for a subject undergoing treatment or therapy for cancer or an autoimmune disease.

34. A method of drug discovery for the treatment of a disease or condition involving an immune response involving Th17 T cell balance in a population of cells or tissue comprising:

(a) providing a compound or plurality of compounds to be screened for their efficacy in the treatment of said disease or condition;

(b) contacting said compound or plurality of compounds with said population of cells or tissue;

(c) detecting a first level of expression of one or more of saturated fatty acids (SFA) and/or polyunsaturated fatty acids (PUFA) in Th17 cells;

(d) comparing the detected level to a control level of saturated fatty acids (SFA) and/or polyunsaturated fatty acids (PUFA); and,

(e) evaluating the difference between the detected level and the control level to determine the immune response elicited by said compound or plurality of compounds.

35. A method of treatment of a disease or condition involving an immune response involving Th17 T cell balance comprising administering at least one lipid to a patient in need thereof, wherein the at least one lipid is sufficient to cause a shift in the ratio of SFA to PUFA, whereby there is a change in T cell balance.

FIG. 1A

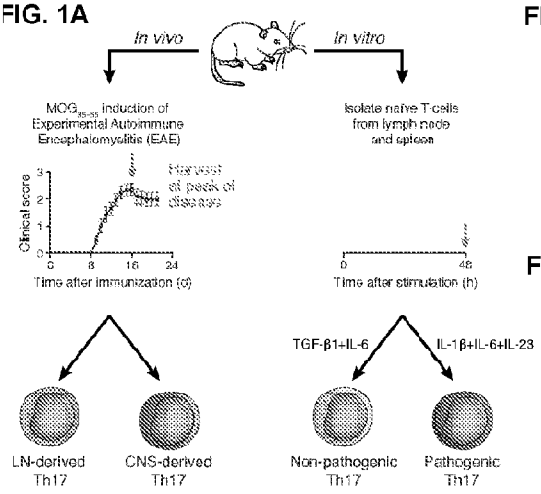


FIG. 1B

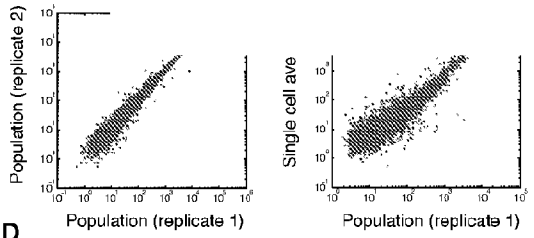


FIG. 1D

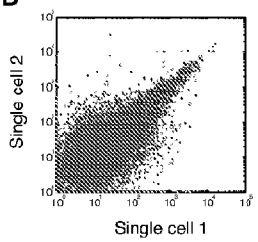


FIG. 1E

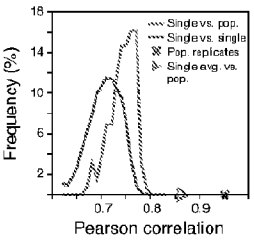


FIG. 1F

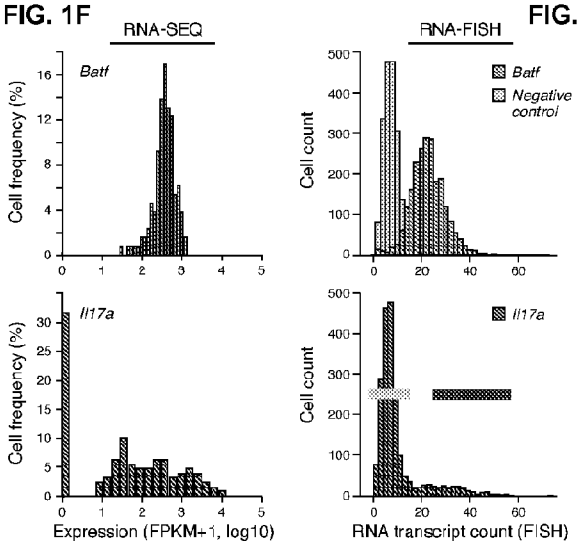


FIG. 1G

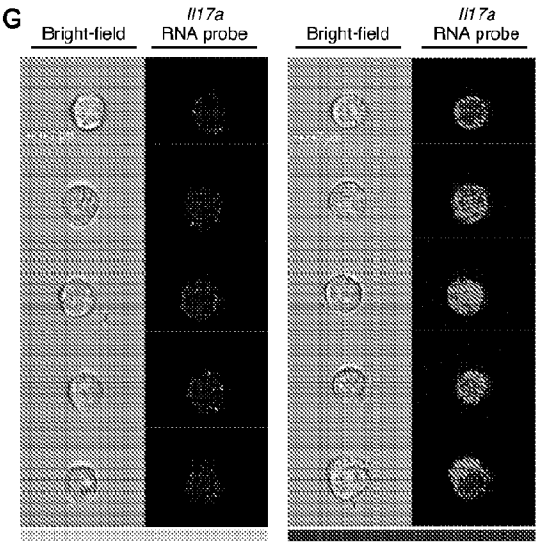
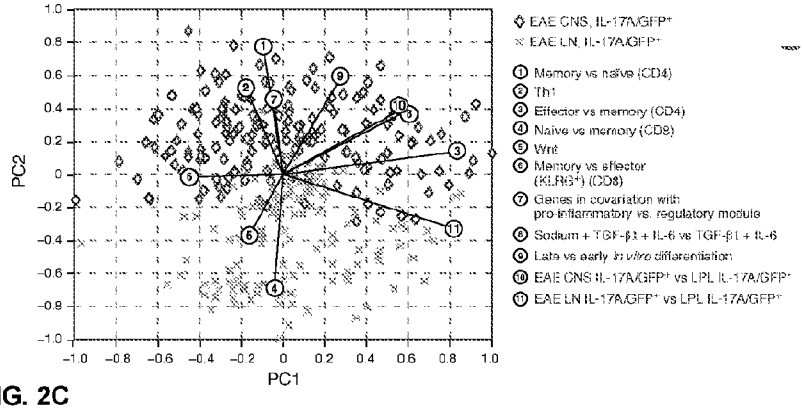


FIG. 2A



FI

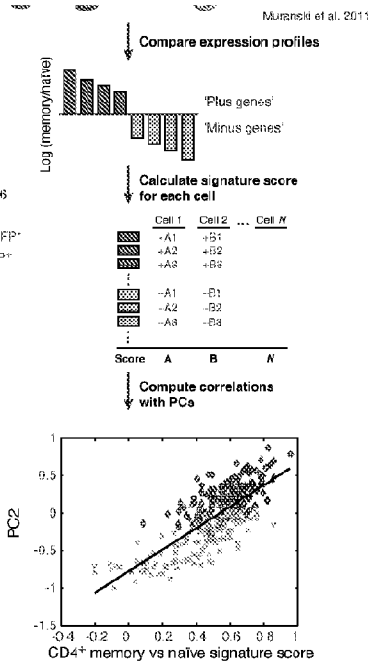


FIG. 2C

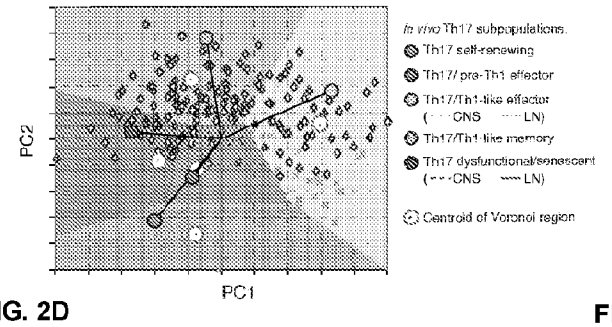


FIG. 2D

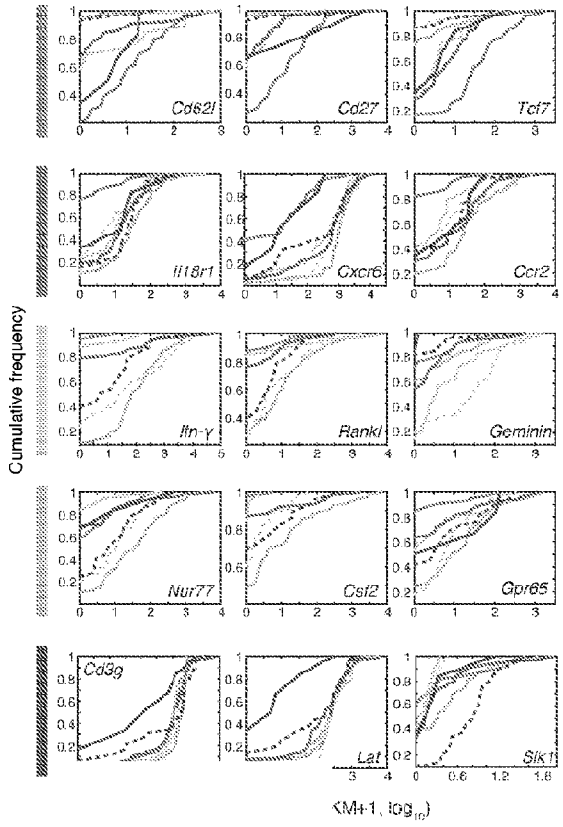


FIG. 2E

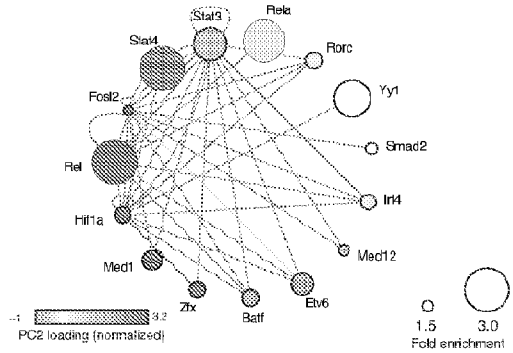


FIG. 2F

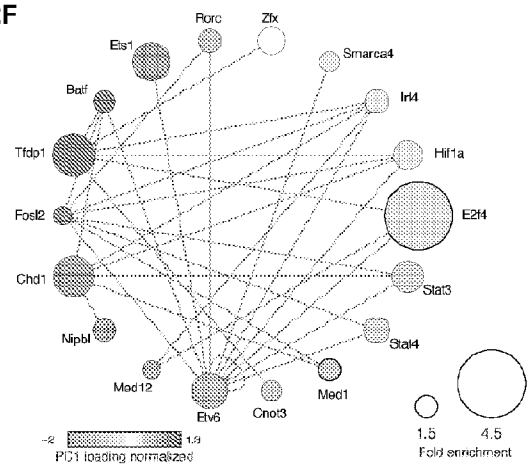


FIG. 3A

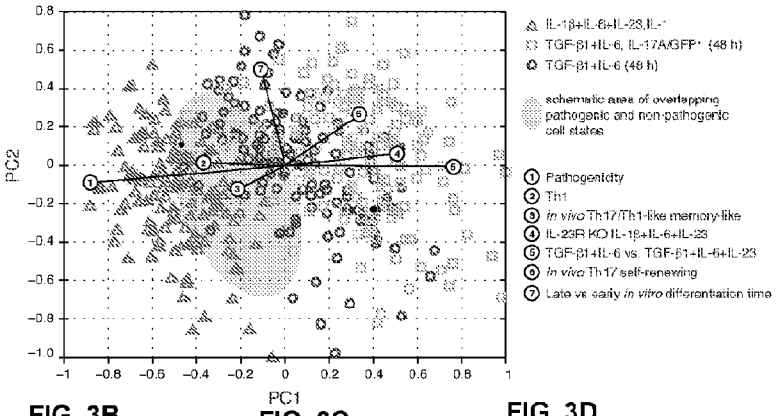


FIG. 3B

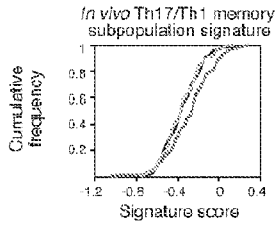


FIG. 3C

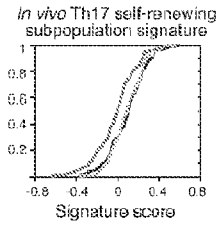


FIG. 3D

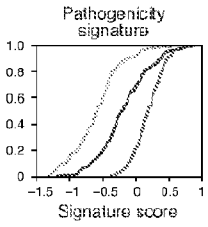


FIG. 3E

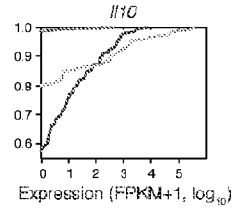


FIG. 4A

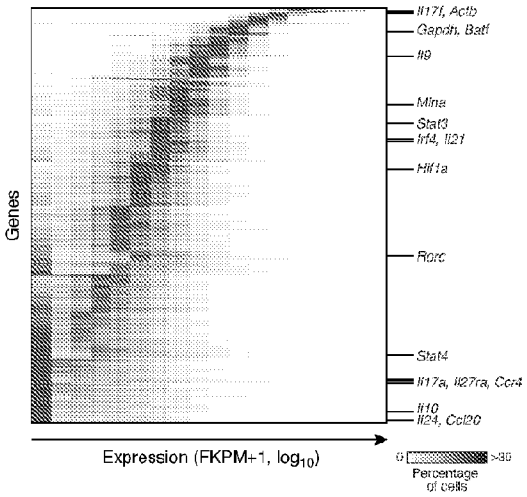


FIG. 4B

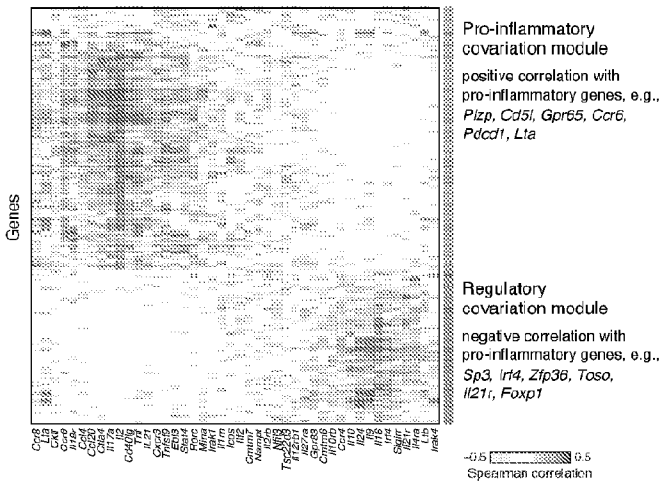


FIG. 4C

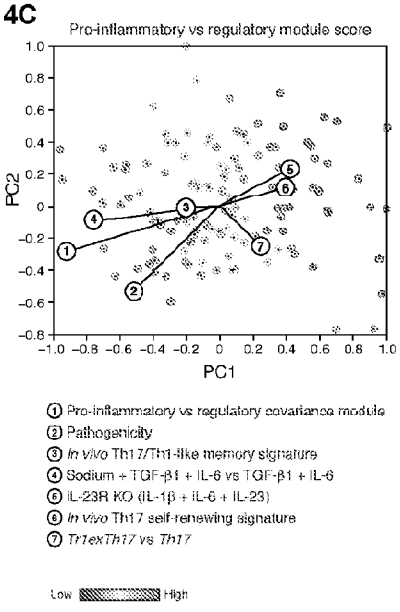


FIG. 4D

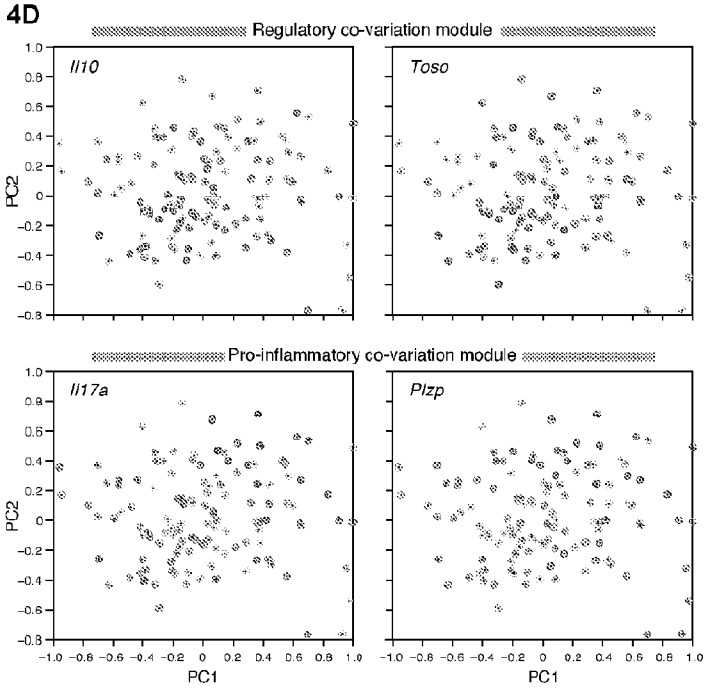


FIG. 4E

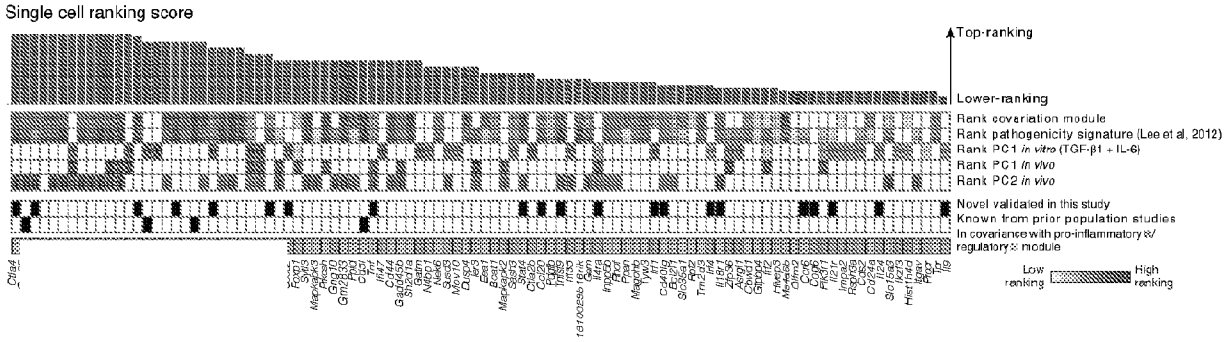


FIG. 5A

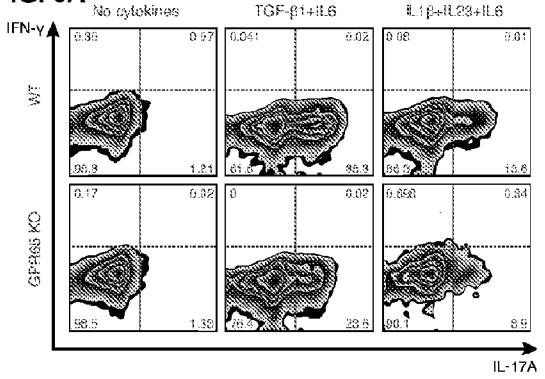


FIG. 5B

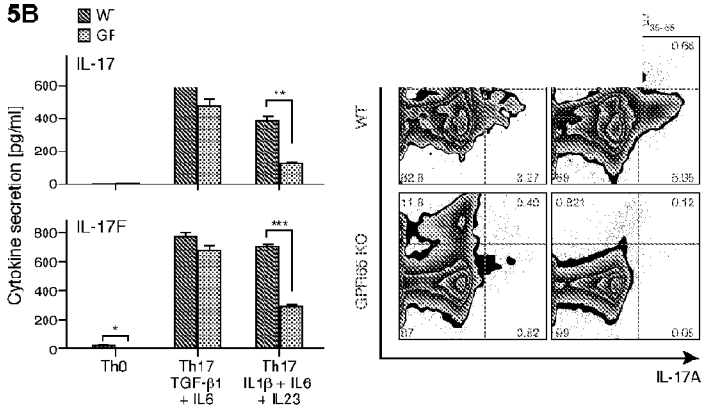


FIG. 5D

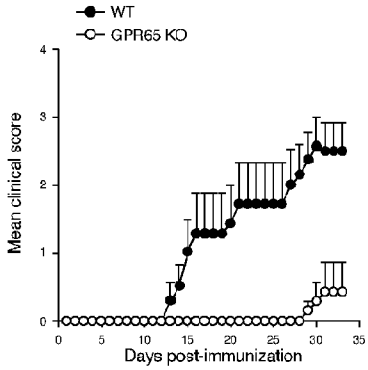


FIG. 5E

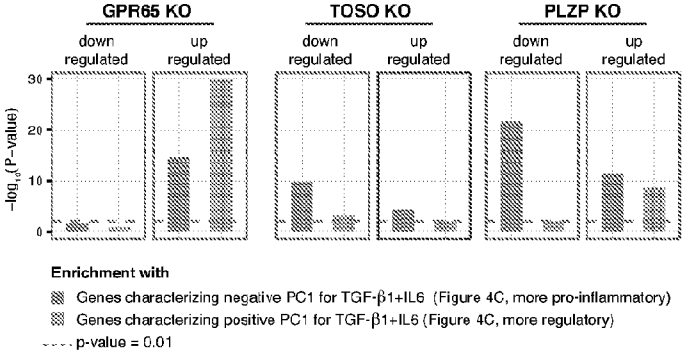


FIG. 5F

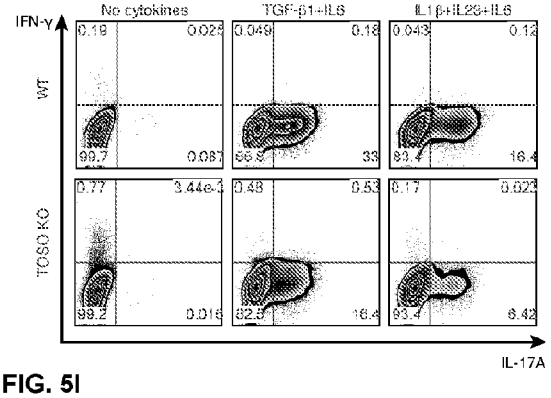


FIG. 5G

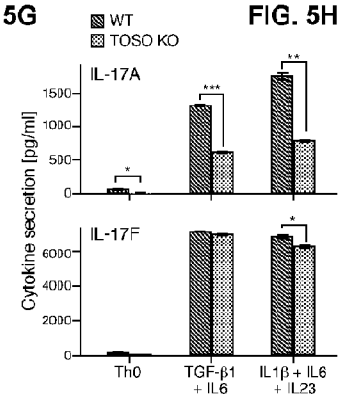


FIG. 5H

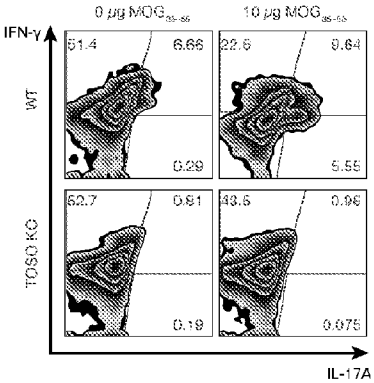


FIG. 5I

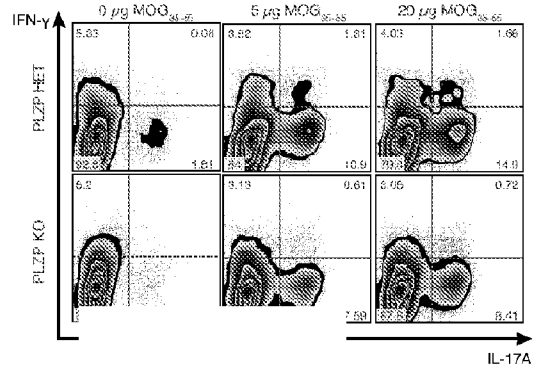


FIG. 5J

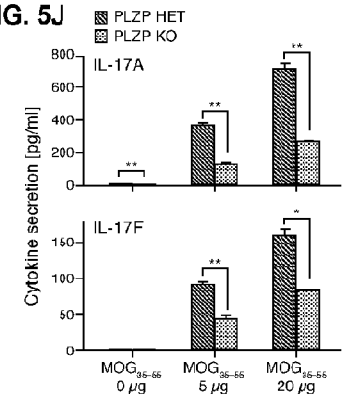


FIG. 6A

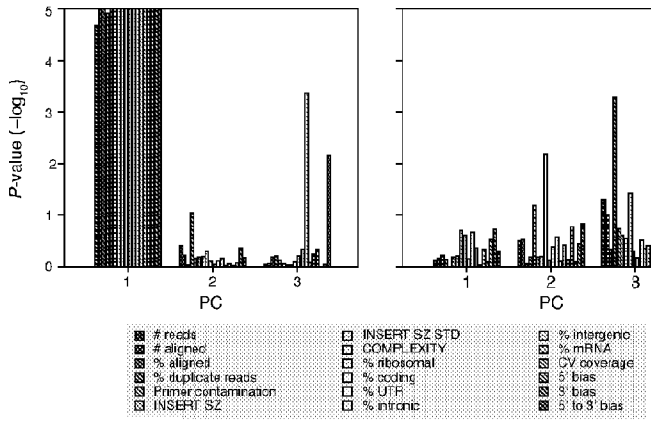


FIG. 6B

FIG. 6C

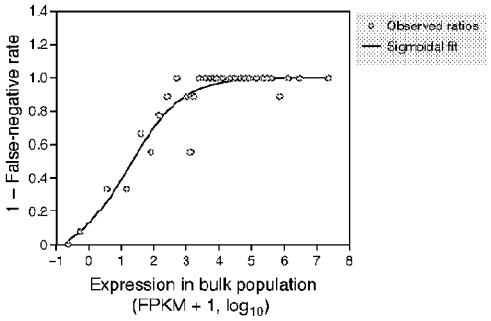


FIG. 6D

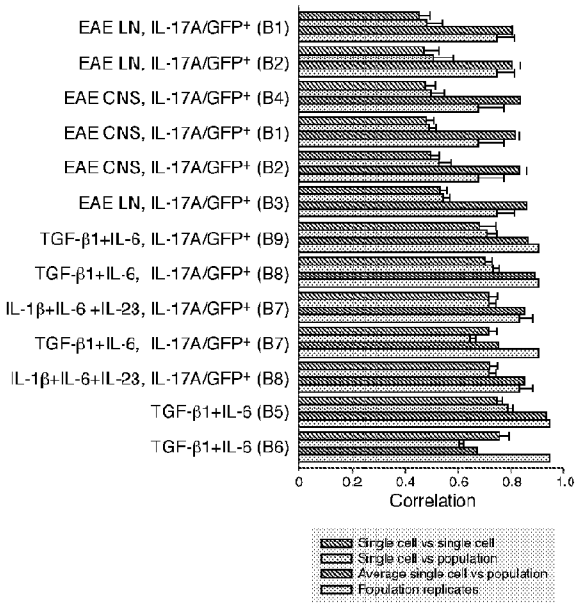


FIG. 6E

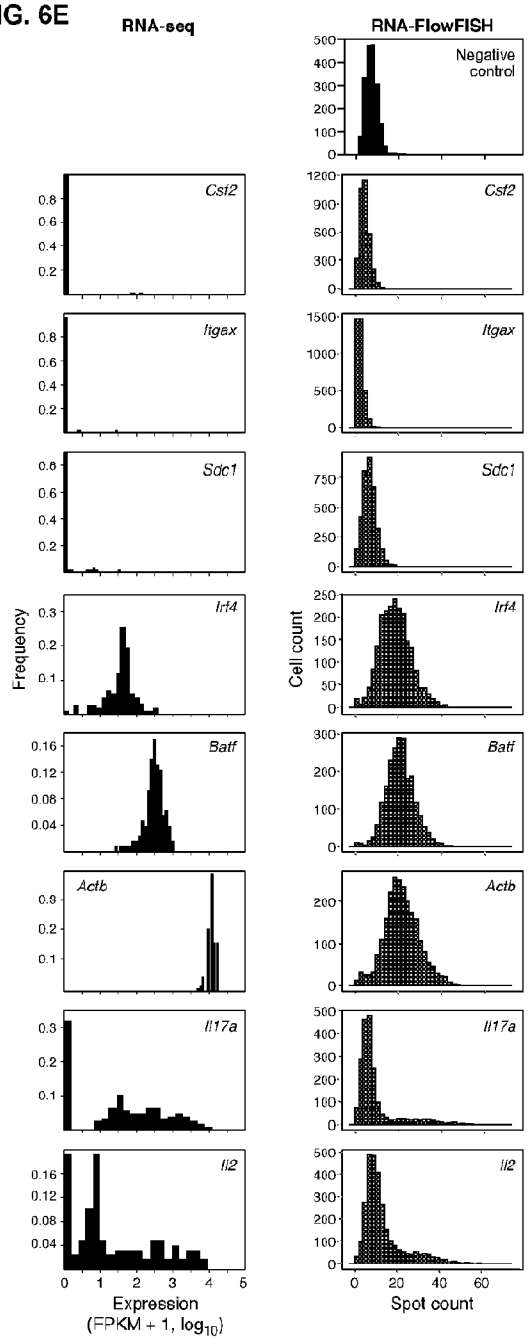


FIG. 6F

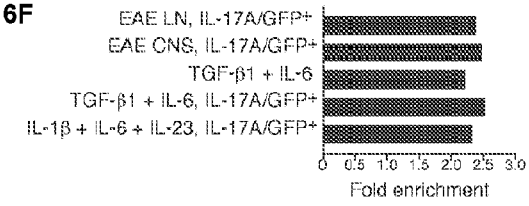


FIG. 6G

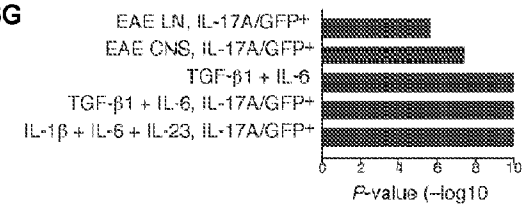
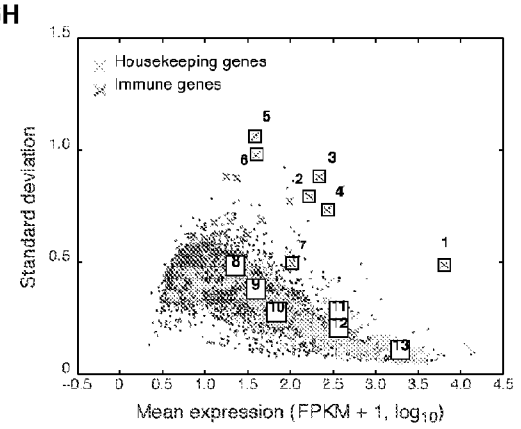
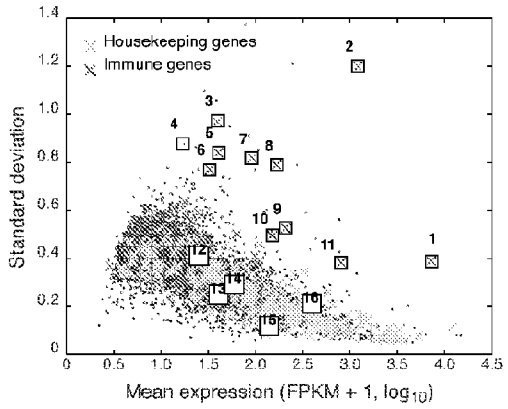


FIG. 6H



1 *Il17f* 6 *Il2* 9 *Irf4*
2 *Il17a* 6 *Ccl20* 10 *Stat3*
3 *Il9* 7 *Ccr7* 11 *Batf*
4 *Lta* 8 *Rorc* 12 *Il2ra*
13 *Mif*

FIG. 6I



1 *Il17i* 6 *Tg* 9 *Mt2* 13 *Irf4*
2 *Il22* 6 *Il23r* 10 *Ctla4* 14 *Stat3*
3 *Ccl20* 7 *Upp1* 11 *Mt1* 15 *Csf2*
4 *Ccl4* 8 *Il17a* 12 *Rorc* 16 *Batf*

FIG. 7A

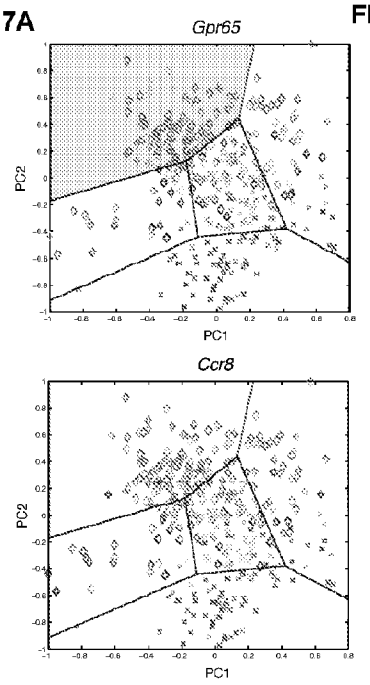
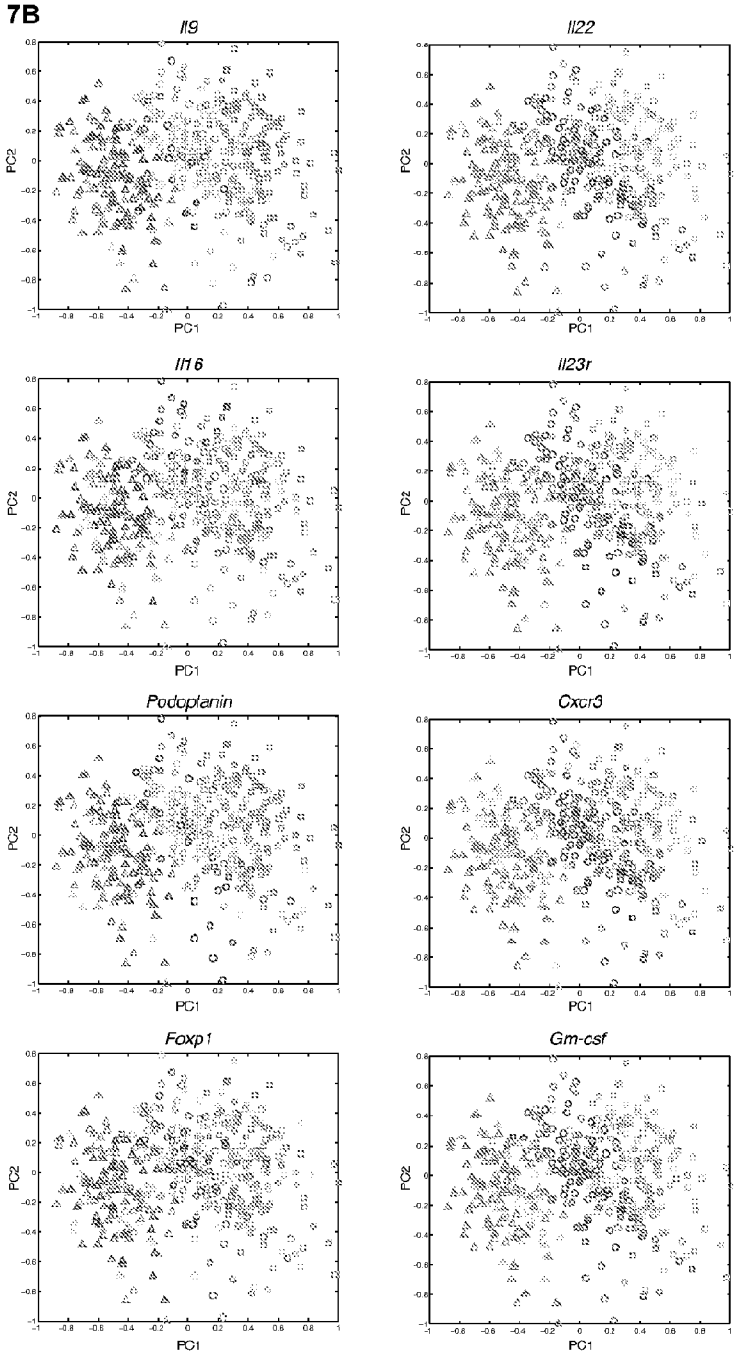


FIG. 7B



▲ *In vitro* IL-1β+IL-6+IL-23, IL-17A/GFP+
○ *In vitro* TGF-β1+IL-6
□ *In vitro* TGF-β1+IL-6, IL-17A/GFP+
◇ EAE CNS, IL-17A/GFP+
✖ EAE LN , IL-17A/GFP+

FIG. 7C

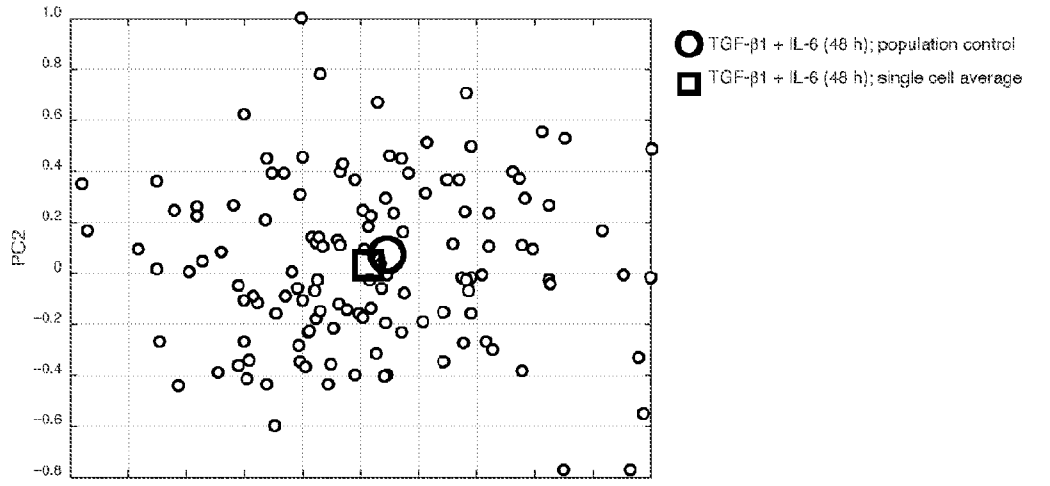


FIG. 7D

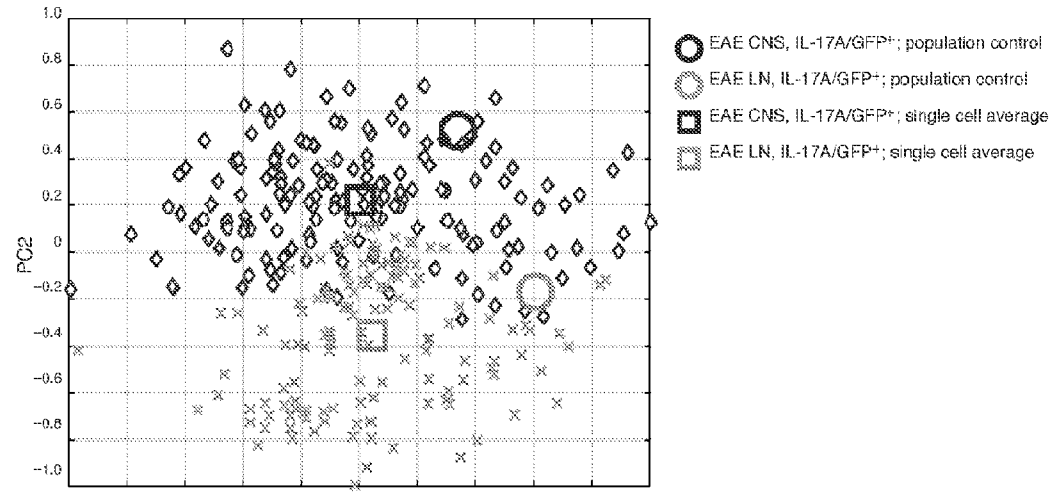


FIG. 7E

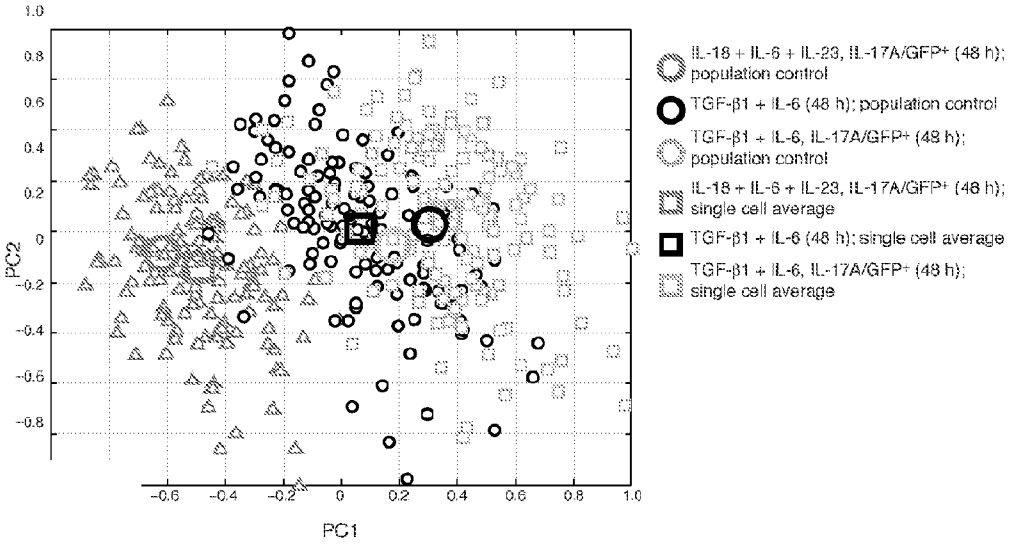


FIG. 8A

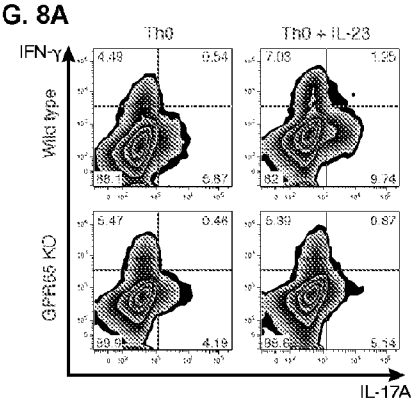


FIG. 8B

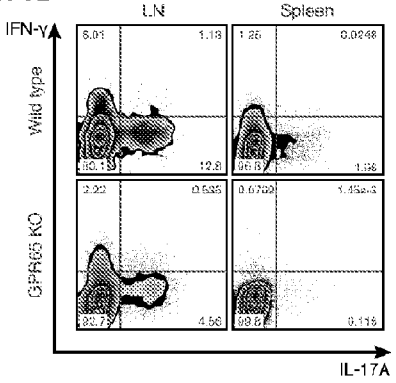


FIG. 8C

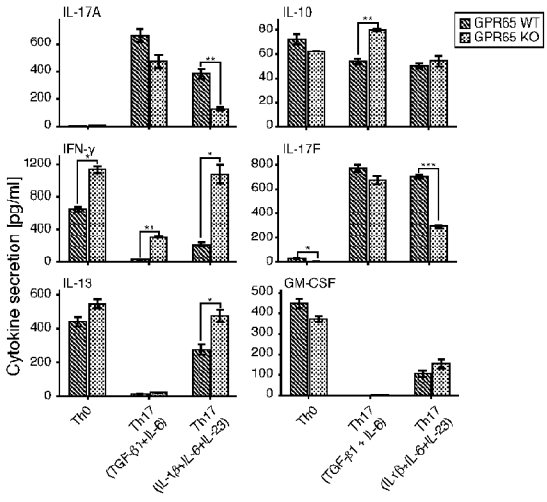


FIG. 8D

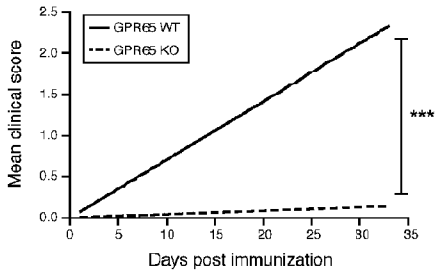


FIG. 9A

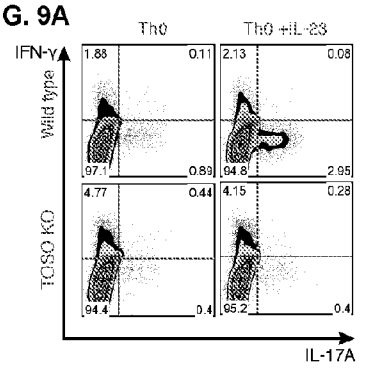


FIG. 9B

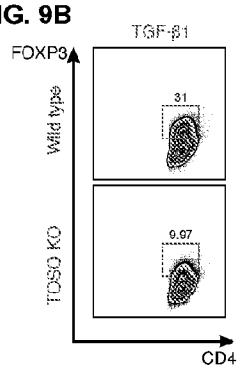


FIG. 9C

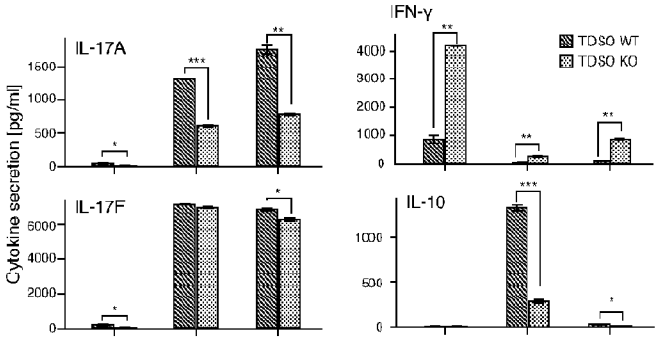


FIG. 10A

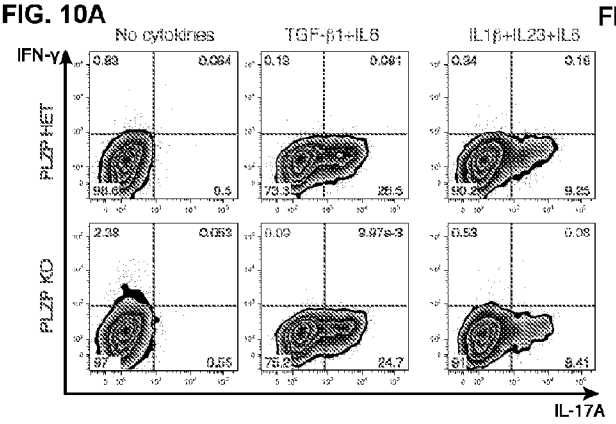


FIG. 10B

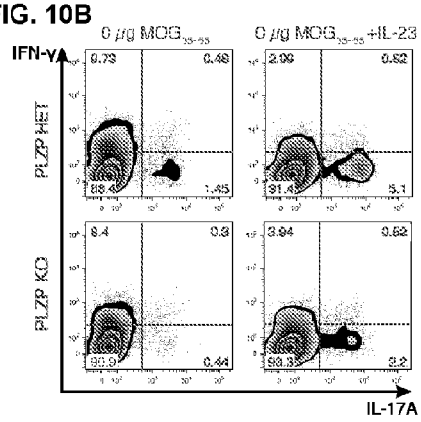


FIG. 10C

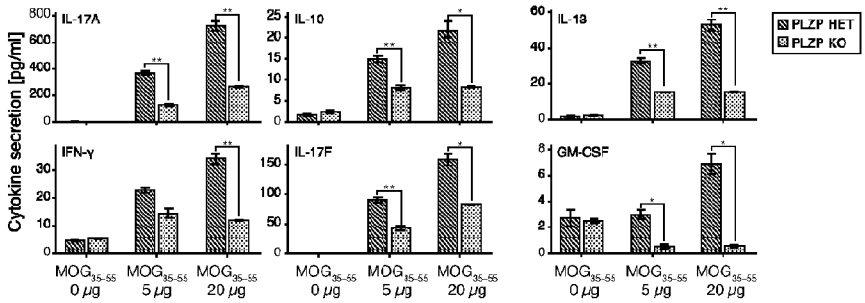


FIG. 11A

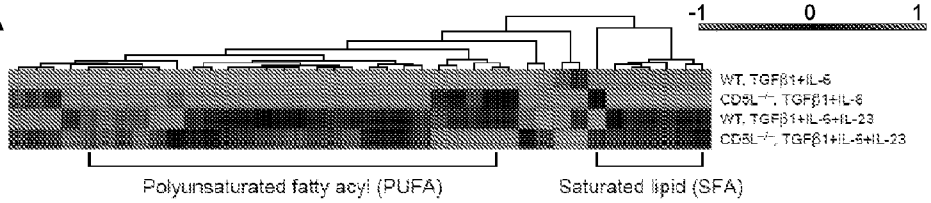


FIG. 11B

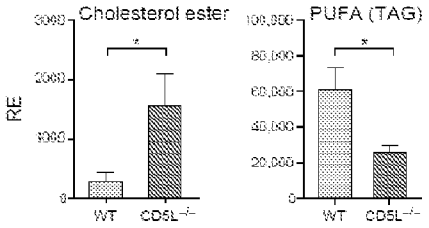


FIG. 11C

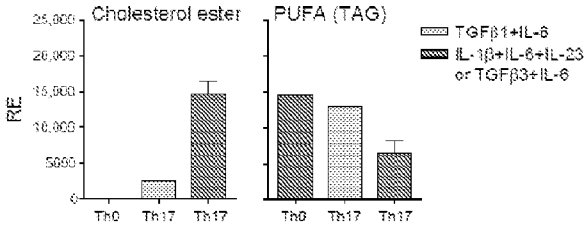


FIG. 11D

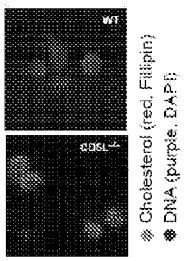


FIG. 11E

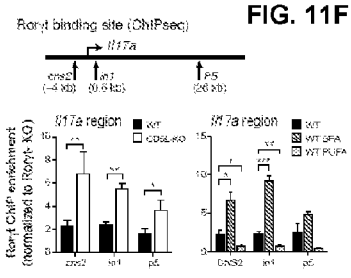


FIG. 11F

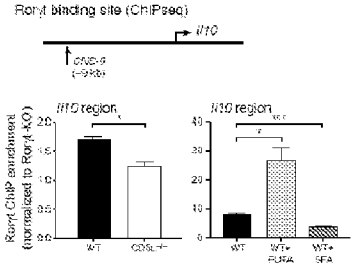


FIG. 11G

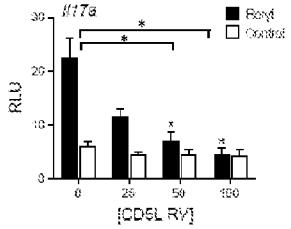


FIG. 11H

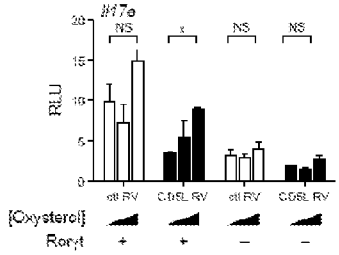


FIG. 11I

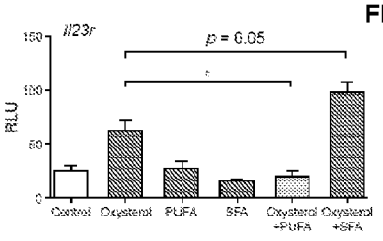


FIG. 11J

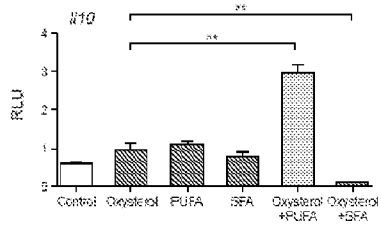


FIG. 11K

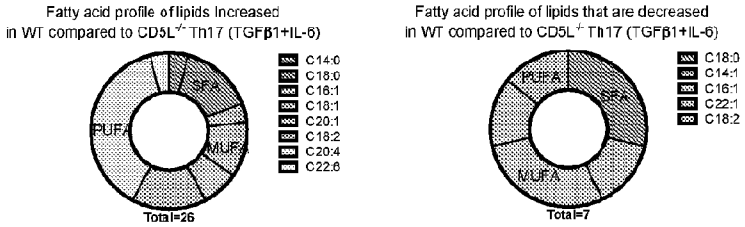


FIG. 11L

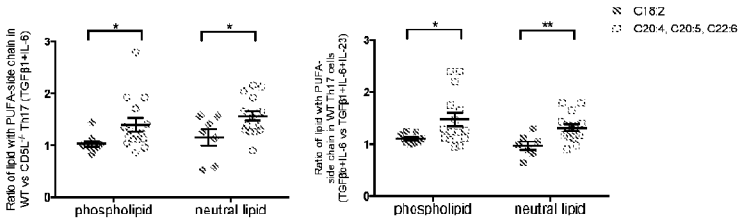


FIG. 11M

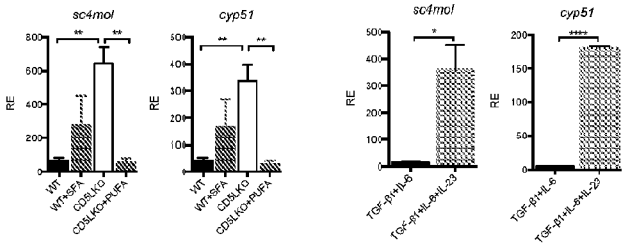


FIG. 12A

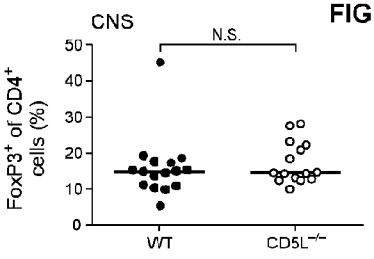


FIG. 12B

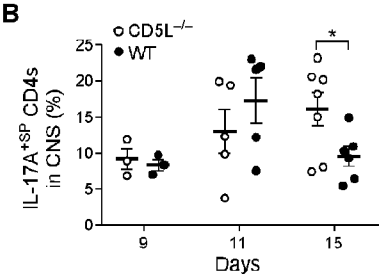


FIG. 12C

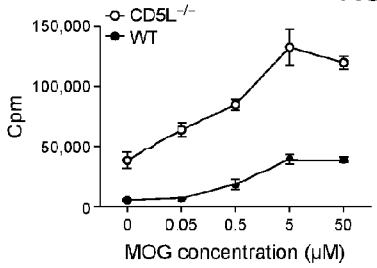


FIG. 12D

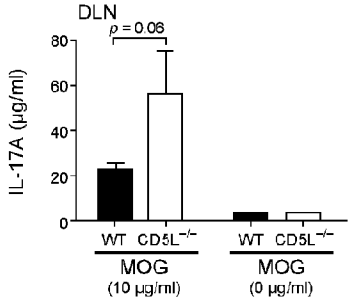


FIG. 12E

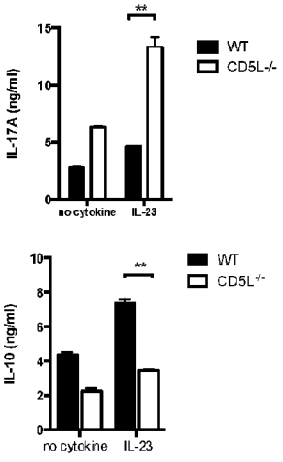
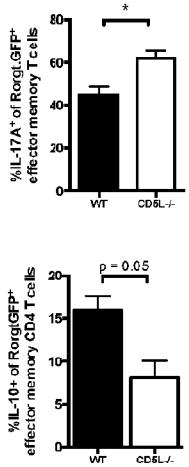


FIG. 12F



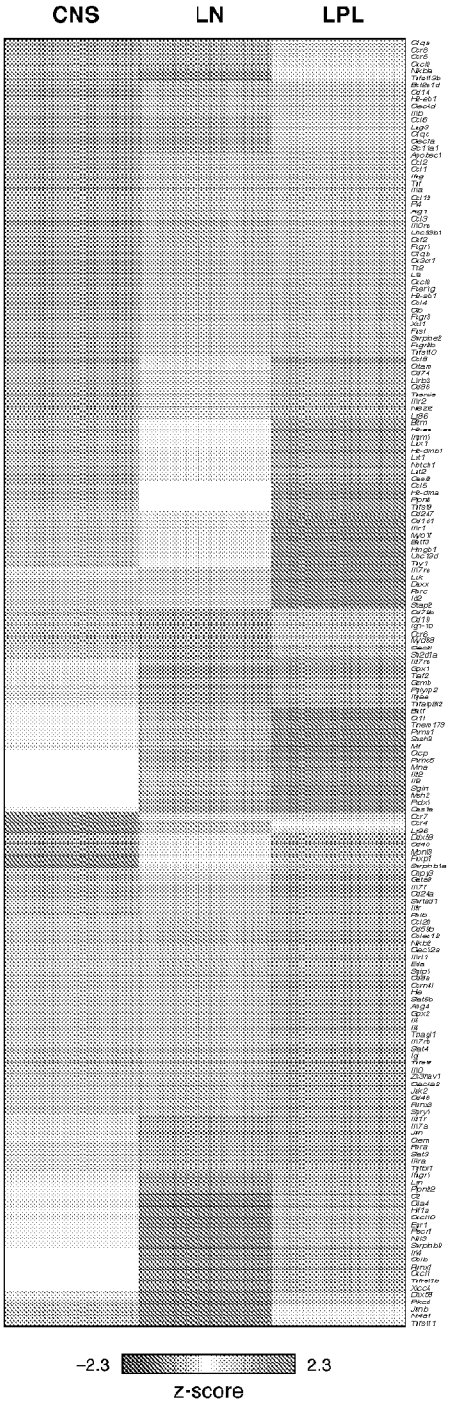

CNS
LN
LPL
−2.3
2.3
z-score

FIG. 14A

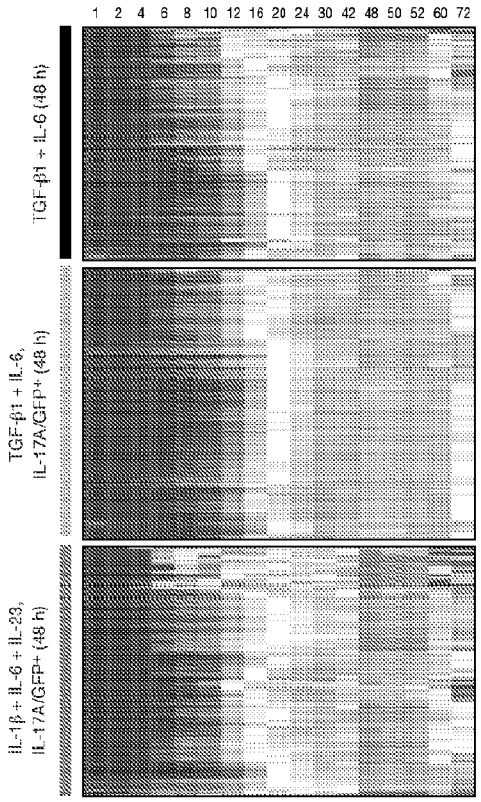


FIG. 14B

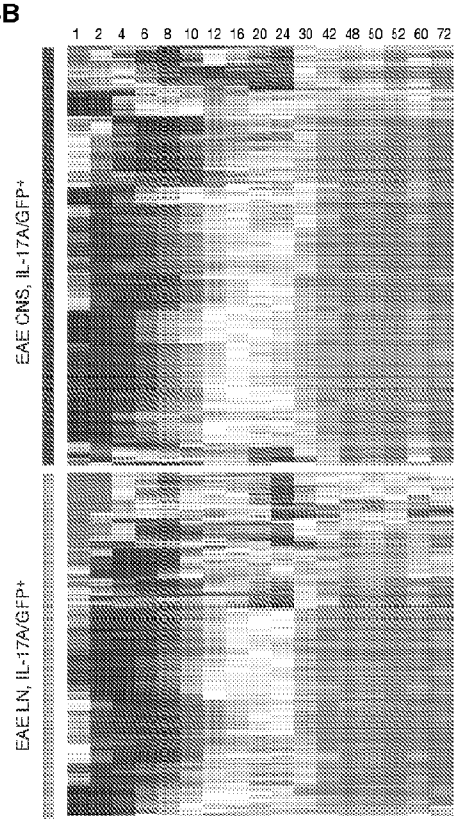


FIG. 14C

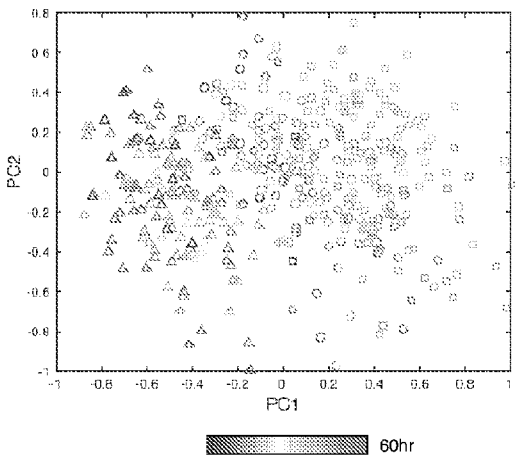


FIG. 14D

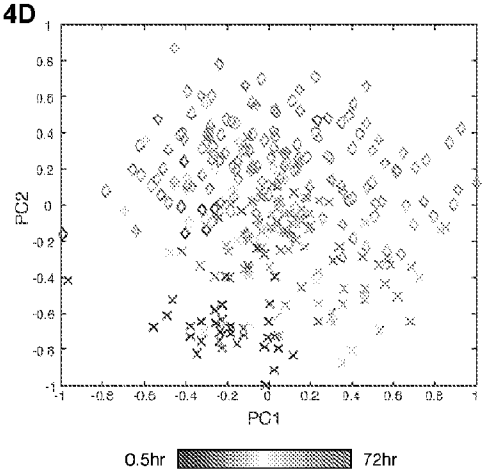


FIG. 15A

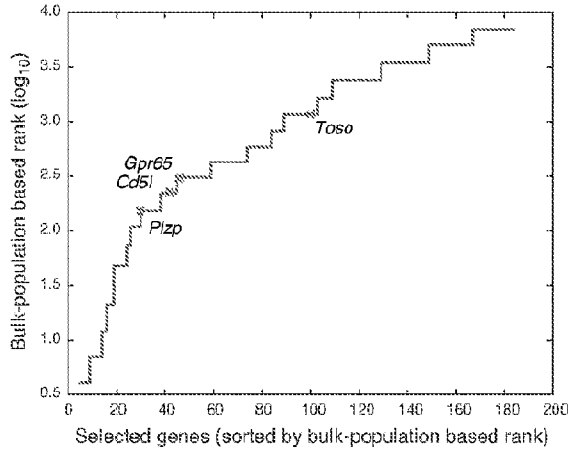


FIG. 15B

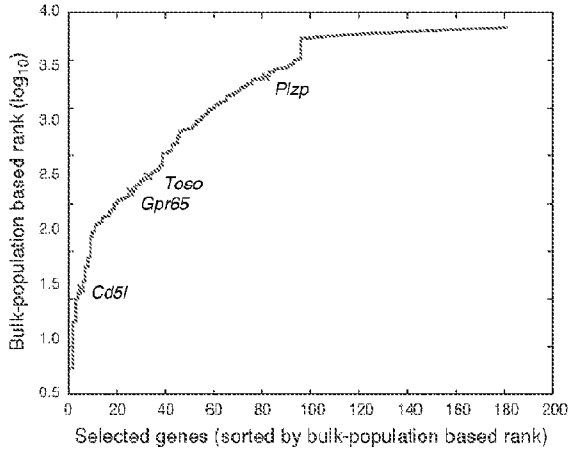


FIG. 16A

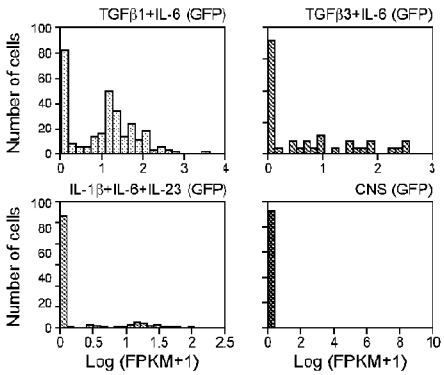


FIG. 16B

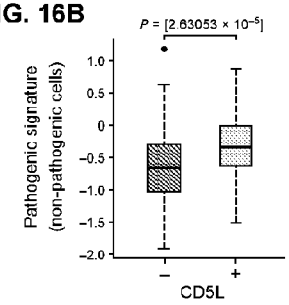


FIG. 16C

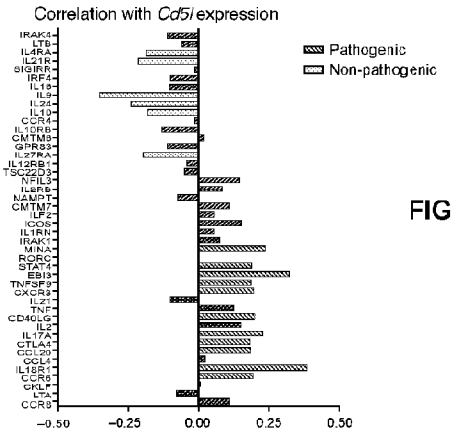


FIG. 16D

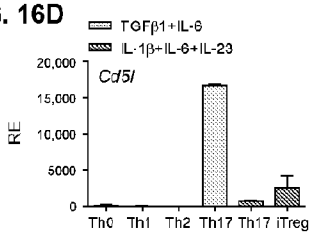


FIG. 16E

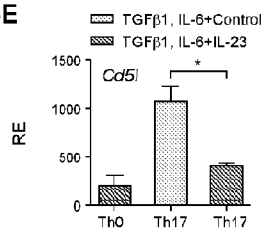


FIG. 16F

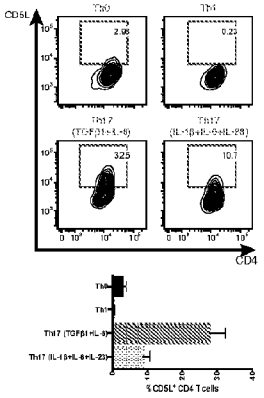


FIG. 16G

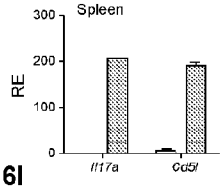


FIG. 16H

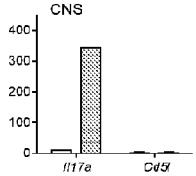


FIG. 16I

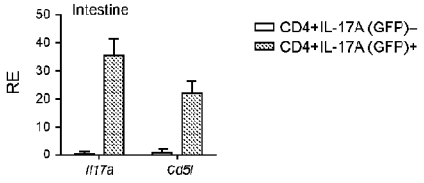
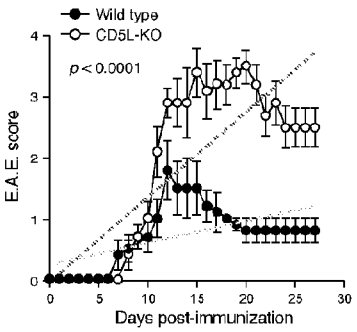


FIG. 17A



WT	13/13	8.6 ± 0.7	2.5 ± 0.4
CD5L$^{-/-}$	12/12	8.7 ± 0.9	4.0 ± 0.4

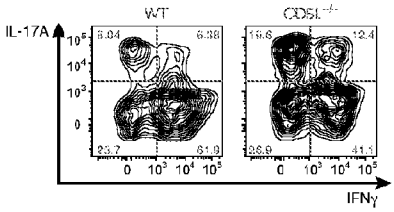


FIG. 17B

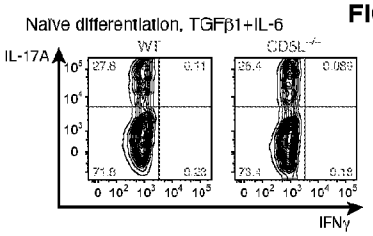


FIG. 17C

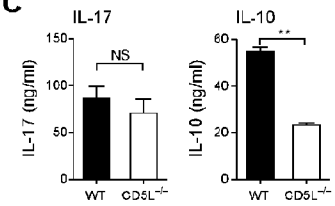


FIG. 17D

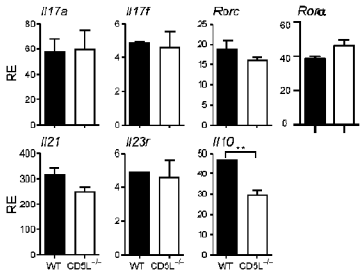


FIG. 17E

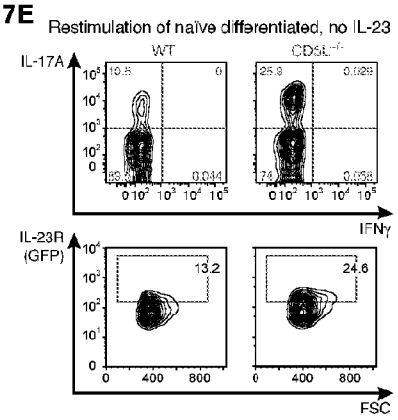


FIG. 17F

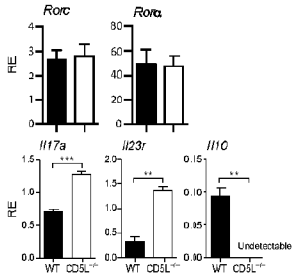


FIG. 17G

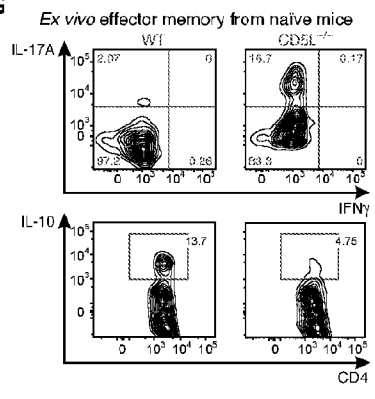


FIG. 17H

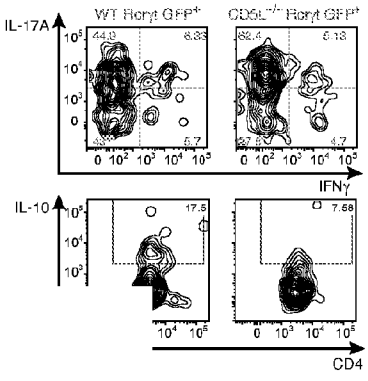


FIG. 18A

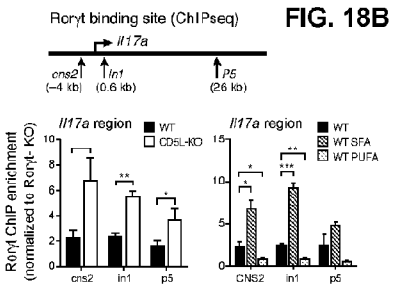


FIG. 18B

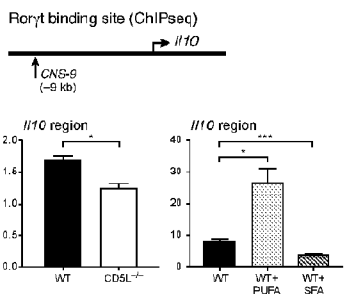


FIG. 18C

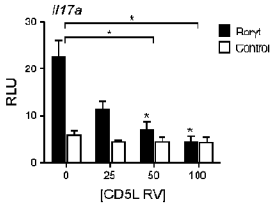


FIG. 18D

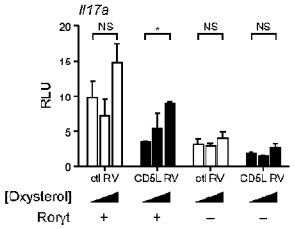


FIG. 18E

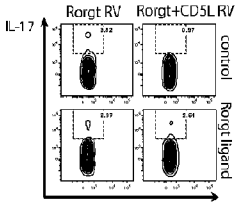


FIG. 18F

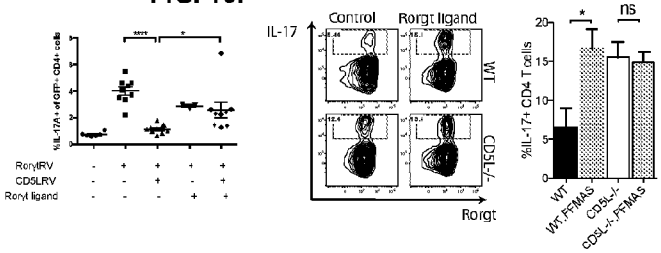


FIG. 19A

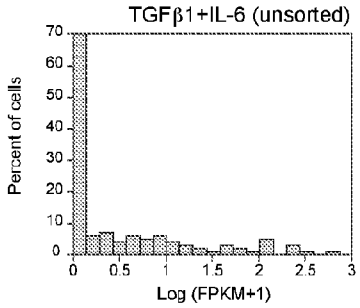


FIG. 19B

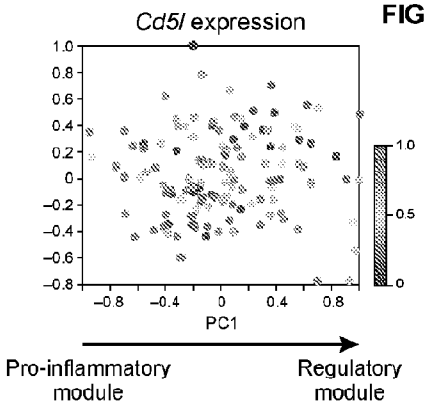


FIG. 19C

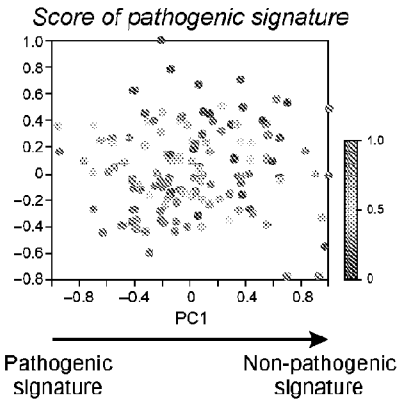


FIG. 19D

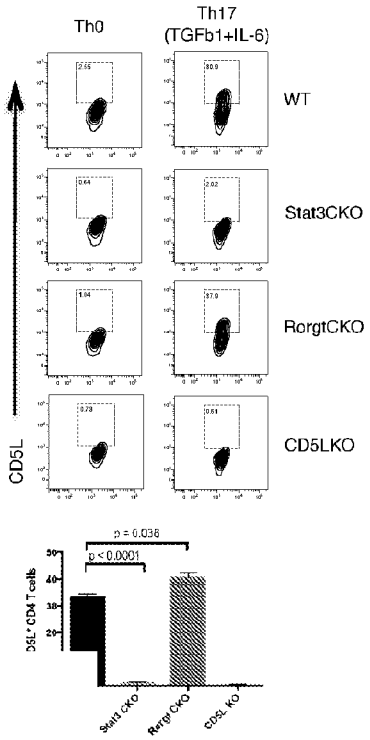


FIG. 19E

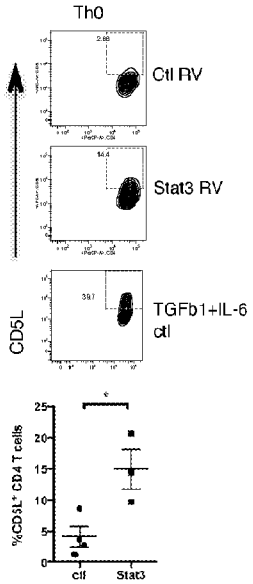


FIG. 20A

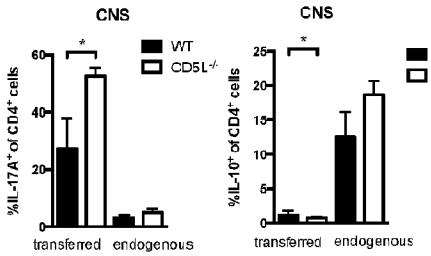


FIG. 20B

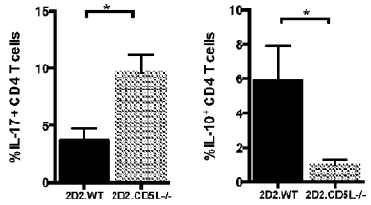


FIG. 20C

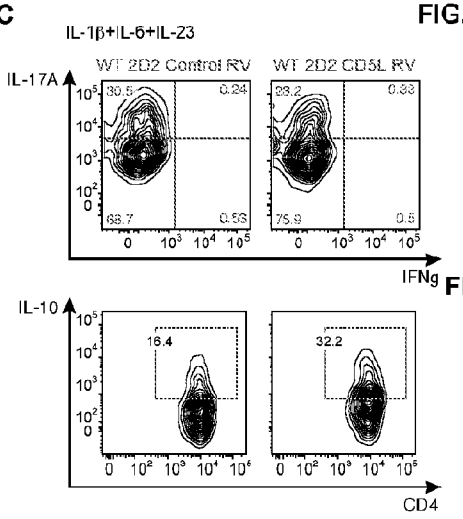


FIG. 20D

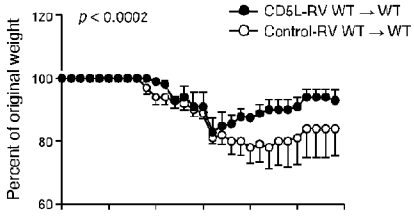


FIG. 20E

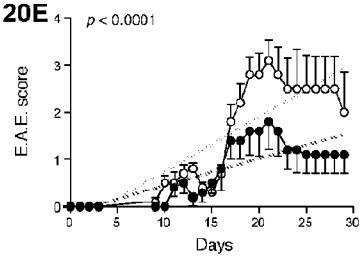


FIG. 20F

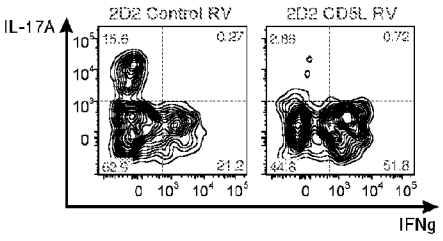
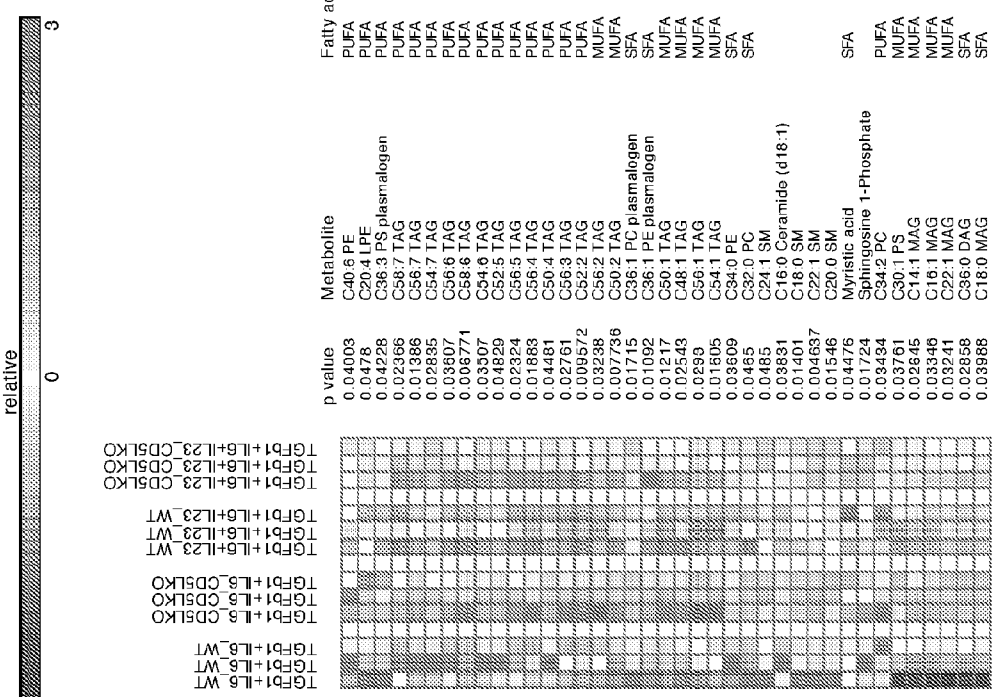


FIG. 21A



p value	Metabolite	Fatty acid content	Ratio: CD5LKO/WT (TGFb1+IL-6)
0.04003	C40:6 PE	PUFA	0.4259
0.0478	C20:4 LPE	PUFA	0.6723
0.04228	C36:3 PS plasmalogen	PUFA	0.5949
0.02366	C58:7 TAG	PUFA	0.6175
0.01386	C56:7 TAG	PUFA	0.4313
0.02835	C54:7 TAG	PUFA	0.4217
0.03607	C56:6 TAG	PUFA	0.6416
0.006771	C58:6 TAG	PUFA	0.5485
0.03507	C54:6 TAG	PUFA	0.4221
0.04829	C52:5 TAG	PUFA	0.3659
0.02324	C56:5 TAG	PUFA	0.638
0.01883	C56:4 TAG	PUFA	0.6359
0.04481	C50:4 TAG	PUFA	0.5773
0.02761	C56:3 TAG	PUFA	0.6323
0.009572	C52:2 TAG	PUFA	0.6298
0.03238	C56:2 TAG	MUFA	0.6372
0.007736	C50:2 TAG	MUFA	0.5916
0.01715	C36:1 PC plasmalogen	SFA	0.5456
0.01092	C36:1 PE plasmalogen	SFA	0.4006
0.01217	C50:1 TAG	MUFA	0.6224
0.02543	C48:1 TAG	MUFA	0.6202
0.0293	C56:1 TAG	MUFA	0.6576
0.01605	C54:1 TAG	MUFA	0.6526
0.03609	C34:0 PE	SFA	0.5882
0.0465	C32:0 PC	SFA	0.6595
0.0485	C24:1 SM		0.5718
0.03831	C16:0 Ceramide (d18:1)		0.551
0.01401	C18:0 SM		0.4841
0.004637	C22:1 SM		0.4447
0.01546	C20:0 SM		0.4219
0.04476	Myristic acid	SFA	0.3394
0.01724	Sphingosine 1-Phosphate		0.3244
0.03434	C34:2 PC	PUFA	1.5001
0.03761	C30:1 PS	MUFA	1.5127
0.02645	C14:1 MAG	MUFA	1.5509
0.03346	C16:1 MAG	MUFA	1.4554
0.03241	C22:1 MAG	MUFA	1.4708
0.02858	C36:0 DAG	SFA	1.5012
0.03988	C18:0 MAG	SFA	1.5029

FIG. 21B

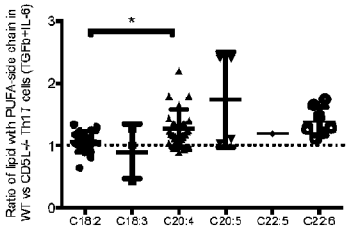
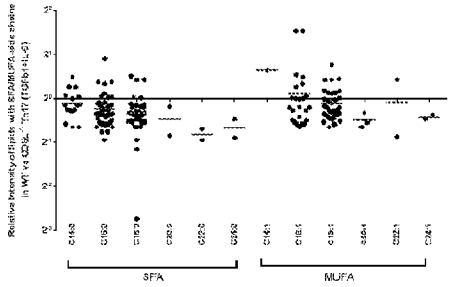


FIG. 21C



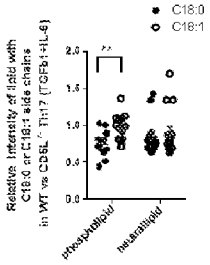


FIG. 21D

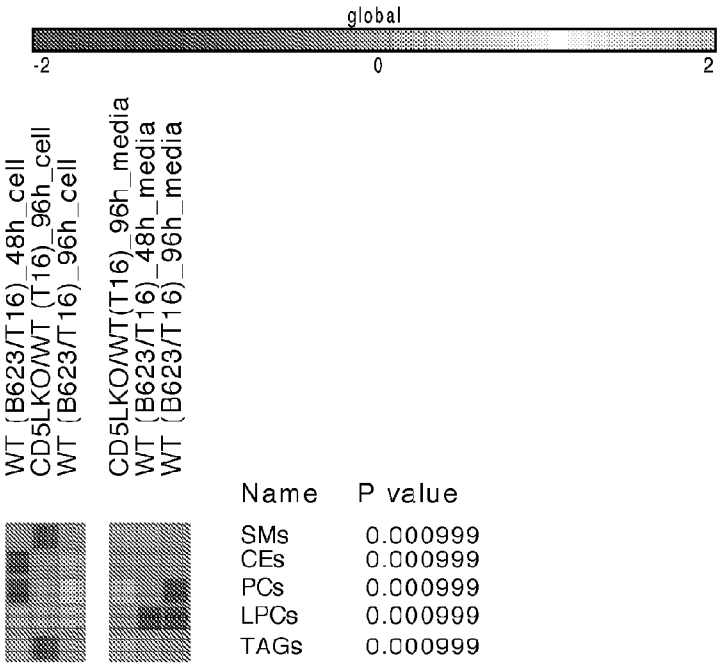


FIG. 21E