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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
16 March 2017 (16.03.2017)

WIPO | PCT

(10) International Publication Number
WO 2017/044801 A2

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

(21) International Application Number:
PCT/US2016/051035

(22) International Filing Date:
9 September 2016 (09.09.2016)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
62/217,476 11 September 2015 (11.09.2015) US
62/258,929 23 November 2015 (23.11.2015) US

(71) Applicants: **THE BROAD INSTITUTE, INC.** [US/US]; 415 Main Street, Cambridge, Massachusetts 02142 (US). **PRESIDENT AND FELLOWS OF HARVARD COLLEGE** [US/US]; 17 Quincy Street, Cambridge, Massachusetts 02115 (US). **THE BRIGHAM AND WOMEN'S HOSPITAL, INC.** [US/US]; 75 Francis Street, Boston, Massachusetts 02115 (US).

(72) Inventors: **MIKKELSEN, Tarjei**; c/o The Broad Institute, Inc., 415 Main Street, Cambridge, Massachusetts 02142 (US). **EBERT, Benjamin Levine**; c/o The Brigham and Women's Hospital, Inc., 75 Francis Street, Boston, Massachusetts 02115 (US). **SIEVERS, Quinlan**; c/o The Brigham and Women's Hospital, Inc., 75 Francis Street, Boston, Massachusetts 02115 (US).

(74) Agent: **HUNTER-ENSOR, Melissa**; c/o Greenberg Traurig, LLP, One International Place, Boston, Massachusetts 02110 (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, KW, 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, 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))*

WO 2017/044801 A2

(54) Title: METHODS OF IDENTIFYING DRUG-MODULATED POLYPEPTIDE TARGETS FOR DEGRADATION

(57) Abstract: In one aspect, the invention features a method for identifying a drug-modulated polypeptide substrate of cereblon (CRBN). In another aspect, the invention features a method of identifying a polypeptide target of a modulator of CRBN. In yet another aspect, the invention provides methods of monitoring or characterizing the sensitivity of a subject to a modulator of CRBN.

METHODS OF IDENTIFYING DRUG-MODULATED POLYPEPTIDE TARGETS FOR DEGRADATION

CROSS-REFERENCE TO RELATED APPLICATION

This application claims the benefit of the following U.S. Provisional Application No.: 62/258,929, filed November 23, 2015 and 62/217,476, filed September 11, 2015, the entire contents of which are incorporated herein by reference.

STATEMENT OF RIGHTS TO INVENTIONS MADE UNDER FEDERALLY SPONSORED RESEARCH

This invention was made with government support under Grant No. P01 CA066996 awarded by the National Institutes of Health. The government has certain rights in the invention.

BACKGROUND OF THE INVENTION

The drug thalidomide became infamous in the early 1960s when its use during the first trimester of pregnancy was linked to profound birth defects, most commonly a malformation of the upper limbs known as phocomelia. The discovery of thalidomide's teratogenic property was a major setback for the compound. However, thalidomide was later repurposed and is currently an FDA-approved therapy for a number of disorders, including erythema nodosum leparum, 5q- myelodysplastic syndrome (MDS), and the plasma cell malignancy multiple myeloma. Thalidomide's success as a treatment for these disorders motivated the synthesis of lenalidomide and pomalidomide, more potent derivatives which have largely replaced thalidomide in the treatment of 5q- MDS and multiple myeloma. It is therefore important to identify additional potentially therapeutically relevant targets of thalidomide, lenalidomide, and pomalidomide to improve clinical use of these drugs. Further, it is important to detect resistance to these drugs in patients, particularly at an early stage of a disease, so that alternate forms of therapy can be provided.

SUMMARY OF THE INVENTION

As described below, the present invention features methods of identifying drug-modulated polypeptide targets for cereblon (CRBN)-mediated degradation, particularly lenalidomide- or lenalidomide analog-modulated substrates of CRBN. The present invention

also features methods of characterizing and/or monitoring sensitivity of a subject to a modulator of CRBN.

In one aspect, the invention provides a method of identifying a cell resistant to a modulator of CRBN, the method comprising detecting the sequence of a region in a IKZF3 polynucleotide relative to a IKZF3 reference sequence, wherein the region encodes amino acids 146-168 of a IKZF3 polypeptide in the cell, and wherein detection of a mutation in the region indicates the cell is resistant to a modulator of CRBN.

In another aspect, the invention provides a method of characterizing the sensitivity of a subject to a modulator of CRBN, the method comprising detecting the sequence of a region in an IKZF3 polynucleotide in a biological sample obtained from the subject relative to a IKZF3 reference sequence, wherein the region encodes amino acids 146-168 of a IKZF3 polypeptide, and wherein detection of a mutation in the region is indicative of resistance to a modulator of CRBN and failure to detect a mutation is indicative of sensitivity to a modulator of CRBN.

In yet another aspect, the invention provides a method of monitoring sensitivity of a subject to a modulator of CRBN, the method comprising detecting the sequence of a region in an IKZF3 polynucleotide in a biological sample obtained from the subject relative to a IKZF3 reference sequence, wherein the region encodes amino acids 146-168 of a IKZF3 polypeptide, and wherein detection of a mutation in the region is indicative of resistance to a modulator of CRBN and failure to detect a mutation is indicative of sensitivity to a modulator of CRBN.

In still another aspect, the invention provides a method of monitoring sensitivity of a subject to a modulator of CRBN, the method comprising (a) administering to the subject an amount of lenalidomide or lenalidomide analog; and (b) detecting the sequence of a region in an IKZF3 polynucleotide in a biological sample obtained from the subject relative to a IKZF3 reference sequence, wherein the region encodes amino acids 146-168 of a IKZF3 polypeptide, and wherein detection of a mutation in the region is indicative of resistance to a modulator of CRBN and failure to detect a mutation is indicative of sensitivity to a modulator of CRBN.

In another aspect, the invention provides a method of selecting a subject for treatment with an alternative to a modulator of CRBN, the method comprising detecting the sequence of a region in an IKZF3 polynucleotide in a biological sample obtained from the subject relative to a IKZF3 reference sequence, wherein the region encodes amino acids 146-168 of a

IKZF3 polypeptide, wherein a subject having a mutation in the region is selected for treatment with an alternative to a modulator of CRBN.

In various embodiments of any of the aspects delineated herein, the mutation is at amino acid position 147, 148, 151, 152, 153, 155, 161, 164, or 168. In various embodiments, the sequence of the region in the IKZF3 polynucleotide is detected by sequencing or probe hybridization.

In various embodiments of any of the aspects delineated herein, the subject has a B cell neoplasia or related condition. In various embodiments, the B cell neoplasia or related condition is a plasma cell malignancy multiple myeloma or a myelodysplastic syndrome. In various embodiments, the biological sample is blood.

In yet another aspect, the invention provides a kit comprising a reagent detecting the sequence of a polynucleotide encoding amino acids 146-168 of an IKZF3 polypeptide. In various embodiments, the reagent is a sequencing primer or hybridization probe.

In still another aspect, the invention provides a method of identifying increased degradation of a polypeptide in a cell when the cell is contacted with a modulator of CRBN, the method comprising detecting in a polypeptide a sequence substantially identical to a IKZF3 zinc finger comprising amino acids 146-168 of IKZF3, wherein presence of the sequence indicates increased degradation of the polypeptide when the cell is contacted with a modulator of CRBN.

In another aspect, the invention provides a method of identifying a drug-modulated polypeptide substrate of CRBN, the method comprising detecting a sequence substantially identical to an IKZF3 zinc finger comprising amino acids 146-168 of IKZF3 in a candidate polypeptide, wherein presence of the sequence indicates the candidate polypeptide is a drug-modulated polypeptide substrate of CRBN.

In yet another aspect, the invention provides a method of identifying a polypeptide target of a modulator of CRBN, the method comprising detecting a sequence substantially identical to an IKZF3 zinc finger comprising amino acids 146-168 of IKZF3 in a candidate polypeptide, wherein presence of the sequence indicates the candidate polypeptide is a polypeptide target of a modulator of CRBN.

In still another aspect, the invention provides a method of depleting a polypeptide in a cell, the method comprising contacting the cell with a modulator of CRBN, wherein the polypeptide is identified as having a sequence substantially identical to an IKZF3 zinc finger

comprising amino acids 146-168 of IKZF3 in the polypeptide, thereby depleting the polypeptide in the cell.

In another aspect, the invention provides a method of depleting a polypeptide in a cell, the method comprising (a) fusing to the polypeptide a second polypeptide comprising a sequence substantially identical to a IKZF3 zinc finger comprising amino acids 146-168 of IKZF3; and (b) contacting the cell with a modulator of CRBN, thereby depleting the polypeptide in the cell.

In another aspect, the invention provides a method of identifying a drug-modulated polypeptide substrate of CRBN. The method contains the step of detecting a sequence substantially identical to a sequence of any one or more of the sequences of amino acids 146-168 of IKZF3, amino acids 149-172 of RNF166, amino acids 417-439 of ZNF692, and amino acids 400-422 of ZFP91, where presence of the sequence indicates the candidate polypeptide is a drug-modulated polypeptide substrate of CRBN.

In yet another aspect, the invention provides a method of identifying a drug-modulated polypeptide substrate of CRBN. The method contains the step of detecting a sequence substantially identical to any one or more of the sequences:

FQCNQCGASFTQKGNLLRHIKLH;

FACPYCGARNLDQQELVKHCVESH;

LQCEICGFTCRQKASLNWHQRKH; and

LQCEICGFTCRQKASLNWHMKKH;

where presence of the sequence indicates the candidate polypeptide is a drug-modulated polypeptide substrate of CRBN.

In various embodiments of any of the aspects delineated herein, the sequence comprises a C2H2 zinc finger sequence. In various embodiments, the C2H2 zinc finger sequence corresponding to amino acids 147, 152, and 153 in the IKZF3 zinc finger comprise Gln, Gly, or Ala. In various embodiments of any of the aspects delineated herein, the polypeptide is IKZF3, IKZF1, CSNK1a1, RNF166, ZNF692, or ZFP91. In various embodiments, the increased degradation is mediated by CRBN.

In various embodiments, the drug is lenalidomide, thalidomide, or pomalidomide. In various embodiments, the polypeptide substrate or polypeptide target is degraded by CRBN-mediated degradation in a cell when the cell is contacted with a modulator of CRBN. In various embodiments of any of the aspects delineated herein, the polypeptide is depleted by CRBN-mediated degradation of the polypeptide. In various embodiments of any of the

aspects delineated herein, the modulator of CRBN is lenalidomide, thalidomide, or pomalidomide.

Compositions and articles defined by the invention were isolated or otherwise manufactured in connection with the examples provided below. Other features and advantages of the invention will be apparent from the detailed description, and from the claims.

Definitions

Unless defined otherwise, all technical and scientific terms used herein have the meaning commonly understood by a person skilled in the art to which this invention belongs. The following references provide one of skill with a general definition of many of the terms used in this invention: Singleton et al., Dictionary of Microbiology and Molecular Biology (2nd ed. 1994); The Cambridge Dictionary of Science and Technology (Walker ed., 1988); The Glossary of Genetics, 5th Ed., R. Rieger et al. (eds.), Springer Verlag (1991); and Hale & Marham, The Harper Collins Dictionary of Biology (1991). As used herein, the following terms have the meanings ascribed to them below, unless specified otherwise.

By "agent" is meant any small molecule chemical compound, antibody, nucleic acid molecule, or polypeptide, or fragments thereof.

By “ameliorate” is meant decrease, suppress, attenuate, diminish, arrest, or stabilize the development or progression of a disease.

By "alteration" is meant a change (increase or decrease) in the expression levels or activity of a gene or polypeptide as detected by standard art known methods such as those described herein. As used herein, an alteration includes a 10% change in expression or activity levels, preferably a 25% change, more preferably a 40% change, and most preferably a 50% or greater change in expression or activity levels.

By "analog" is meant a molecule that is not identical, but has analogous functional or structural features. For example, a polypeptide analog retains the biological activity of a corresponding naturally-occurring polypeptide, while having certain biochemical modifications that enhance the analog's function relative to a naturally occurring polypeptide. Such biochemical modifications could increase the analog's protease resistance, membrane permeability, or half-life, without altering, for example, ligand binding. An analog may include an unnatural amino acid. Lenalidomide analogs include, but are not limited to, thalidomide or pomalidomide.

By “biological sample” is meant any liquid, cell, or tissue obtained from a subject.

By “biomarker” or “marker” is meant any protein or polynucleotide having an alteration in expression level or activity that is associated with a disease or disorder.

By “B cell neoplasia” is meant any neoplasia arising from a B-cell progenitor or other cell of B cell lineage. In particular embodiments, a B cell neoplasia arises from a cell type undergoing B cell differentiation. In other embodiments, a B cell neoplasia includes plasma cells.

In this disclosure, "comprises," "comprising," "containing" and "having" and the like can have the meaning ascribed to them in U.S. Patent law and can mean "includes," "including," and the like; "consisting essentially of" or "consists essentially" likewise has the meaning ascribed in U.S. Patent law and the term is open-ended, allowing for the presence of more than that which is recited so long as basic or novel characteristics of that which is recited is not changed by the presence of more than that which is recited, but excludes prior art embodiments.

By “CSNK1a1 polypeptide” or “casein kinase 1A1 polypeptide” is meant a polypeptide having at least about 85% or greater identity to Unit Pro Accession No. P48729-1 or P48729-2 (having a phosphor serine at position 156), or a fragment thereof, and having kinase activity. An exemplary CSNK1a1 polypeptide sequence is provided below.

```
        10         20         30         40         50         60
MASSSGSKAE FIVGGKYKLV RKIGSGSFGD IYLAINITNG EEVAVKLESQ KARHPQLLYE

        70         80         90        100        110        120
SKLYKILQGG VGIPHIRWYG QEKDYNVLVM DLLGPSLEDL FNFCSRRFTM KTVLMLADQM

       130        140        150        160        170        180
ISRIEYVHTK NFIHRDIKPD NFLMGIGRHC NKLFLIDFGL AKKYRDNRTR QHIPYREDKN

       190        200        210        220        230        240
LTGTARYASI NAHLGIEQSR RDDMESLGYV LMYFNRTSLP WQGLKAATKK QKYEKISEKK

       250        260        270        280        290        300
MSTPVEVLCK GFPAEFAMYL NYCRGLRFEE APDYMYLRQL FRILFRTLNH QYDYTFDWTM

       310        320        330
LKQKAAQQAA SSSGQGQQAQ TPTGKQTDKT KSNMKGF
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By “CSNK1a1 polynucleotide” or “casein kinase 1A1 polynucleotide” is meant a polynucleotide encoding a casein kinase 1A1 polypeptide. An exemplary CSNK1a1

polynucleotide sequence is provided at NCBI Accession No. NM_001025105. The sequence is provided below.

```
   1 atgcgcagct gggcggtgac agggtgacgc tcggagcgtg ggccgcgact ctcacggatc
  61 cggttccgcc ctctcgctgc cgatccttcg gagcgagcgc ccgagatccc tttcccagag
 121 tgctctgcgc cgtgaagaag cggctcccgg ggactggggg cattttgtgt tggctggagc
 181 tggagtaaca agatggcgtc gtccgcggag tgacaggggt ccctctgggc cggagccggc
 241 ggcagtggtg gcagcggtat cgccgcccta gctcaccgcg ccccttttcc agcccgcgac
 301 gtcgccgcgc aagcgaggca gcggcggccg ccgagaaaca agtggcccag cctggtaacc
 361 gccgagaagc ccttcacaaa ctgcggcctg gcaaaaagaa acctgactga gcggcggtga
 421 tcaggttccc ctctgctgat tctgggcccc gaaccccggt aaaggcctcc gtgttccgtt
 481 tcctgccgcc ctcctccgta gccttgccta gtgtaggagc cccgaggcct ccgtcctctt
 541 cccagaggtg tcggggcttg gccccagcct ccatcttcgt ctctcaggat ggcgagtagc
 601 agcggctcca aggctgaatt cattgtcgga gggaaatata aactggtacg gaagatcggg
 661 tctggctcct tcggggacat ctatttggcg atcaacatca ccaacggcga ggaagtggca
 721 gtgaagctag aatctcagaa ggccaggcat ccccagttgc tgtacgagag caagctctat
 781 aagattcttc aaggtggggt tggcatcccc cacatacggt ggtatggtca ggaaaaagac
 841 tacaatgtac tagtcatgga tcttctggga cctagcctcg aagacctctt caatttctgt
 901 tcaagaaggt tcacaatgaa aactgtactt atgttagctg accagatgat cagtagaatt
 961 gaatatgtgc atacaaagaa ttttatacac agagacatta aaccagataa cttcctaatg
1021 ggtattgggc gtcactgtaa taagtgttta gaatctccag tggggaagag gaaaagaagc
1081 atgactgtta gtacttctca ggacccatct ttctcaggat taaaccagtt attccttatt
1141 gattttggtt tggccaaaaa gtacagagac aacaggacaa ggcaacacat accatacaga
1201 gaagataaaa acctcactgg cactgcccga tatgctagca tcaatgcaca tcttggtatt
1261 gagcagagtc gccgagatga catggaatca ttaggatatg ttttgatgta ttttaataga
1321 accagcctgc catggcaagg gctaaaggct gcaacaaaga aacaaaaata tgaaaagatt
1381 agtgaaaaga agatgtccac gcctgttgaa gttttatgta aggggtttcc tgcagaattt
1441 gcgatgtact taaactattg tcgtgggcta cgctttgagg aagccccaga ttacatgtat
1501 ctgaggcagc tattccgcat tcttttcagg accctgaacc atcaatatga ctacacattt
1561 gattggacaa tgttaaagca gaaagcagca cagcaggcag cctcttccag tgggcagggt
1621 cagcaggccc aaacccccac aggcaagcaa actgacaaaa ccaagagtaa catgaaaggt
1681 ttctaagcat gaattgagga acagaagaag cagagcagat gatcggagca gcatttgttt
1741 ctccccaaat ctagaaattt tagttcatat gtacactagc cagtggttgt ggacaaccat
1801 ttacttggtg taaagaactt aatttcagta taaactgact ctgggcagca ttggtgatgc
1861 tgtatcctga gttgtagcct ctgtaattgt gaatattaac tgagatagtg aaacatggtg
1921 tccggttttc tattgcattt tttcaagtgg aaaagttaac taaatggttg acacacaaaa
1981 attggtggag aaattgtgca tatgccaatt ttttgttaaa acctttttgtt ttgaactata
2041 ctgctttgag atctcatttc agaagaacgg catgaacagt cttcagccac agttgtgatg
2101 gttgttaaat gctcacaatt gtgcattctt agggtttttc catccctggg gtttgcaagt
2161 tgttcactta aaacattctt aaaatggttg gcttcttgtc tgcaagccag ctgatatggt
2221 agcaaccaaa gattccagtg tttgagcata tgaaagactc tgcctgctta attgtgctag
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2281 aaataacagc atctaaagtg aagacttaag aaaaacttag tgactactag attatcctta
2341 ggactctgca ttaactctat aatgttcttg gtattaaaaa aaaagcatat ttgtcacaga
2401 aatttagtta acatcttaca actgaacatg tatgtatgtt gcttagataa atgtaatcac
2461 tgtaaacatc tatatgatct gggattttgt ttttattttg aaatgggagc ttttttgttt
2521 acaagttcat taaaaactaa aaactgtttc tgtaaggaaa tgagattttt tttaaacaac
2581 aaaaaatgcc ttgctgactc actattaaat aaaaatctcc ccaatttttt gatagactac
2641 ttcaaaaaaa aaaaaaaaaa a
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By "C2H2 zinc finger sequence" or "C2H2 zinc finger motif" is meant a sequence of amino acids which typically includes two conserved cysteines and two conserved histidine residues. The two conserved cysteines and two conserved histidines co-ordinate a zinc ion, although other combinations of cysteine/histidine as the zinc-chelating residues are possible. For example, in IKZF3, the cysteines at positions 148 and 151 and histidines at positions 164 and 168 are indicative of a C2H2 zinc finger motif.

By "CRBN polypeptide" or "Cereblon" is meant a polypeptide or fragment thereof having at least 85% amino acid sequence identity to NCBI Accession No. AAH67811.1 or NP_001166953.1 and having IKZF3 binding activity. Exemplary CRBN polypeptide sequences are provided below:

AAH67811.1

```
  1 magegdqqda ahnmgnhlpl lpeseeedem evedqdskea kkpniinfdt slptshtylg
 61 admeefhgrt lhdddscqvi pvlpqvmmil ipgqtlplql fhpqevsmvr nliqkdrtfa
121 vlaysnvqer eaqfgttaei yayreeqdfg ieivkvkaig rqrfkvlelr tqsdgiqqak
181 vqilpecvlp stmsavqles lnkcqifpsk pvsredqcsy kwwqkyqrrk fhcanltswp
241 rwlyslydae tlmdrikkql rewdenlkdd slpsnpidfs yrvaaclpid dvlriqllki
301 gsaiqrlrce ldimnkctsl cckqcqetei ttkneifsls lcgpmaayvn phgyvhetlt
361 vykacnlnli grpstehswf pgyawtvaqc kicashigwk ftatkkdmsp qkfwgltrsa
421 llptipdted eispdkvilc l
```

NP_001166953.1

```
  1 magegdqqda ahnmgnhlpl lpeseeedem evedqdskea kkpniinfdt slptshtylg
 61 admeefhgrt lhdddscqvi pvlpqvmmil ipgqtlplql fhpqevsmvr nliqkdrtfa
121 vlaysnvqer eaqfgttaei yayreeqdfg ieivkvkaig rqrfkvlelr tqsdgiqqak
181 vqilpecvlp stmsavqles lnkcqifpsk pvsredqcsy kwwqkyqkrk fhcanltswp
241 rwlyslydae tlmdrikkql rewdenlkdd slpsnpidfs yrvaaclpid dvlriqllki
301 gsaiqrlrce ldimnkctsl cckqcqetei ttkneifsls lcgpmaayvn phgyvhetlt
361 vykacnlnli grpstehswf pgyawtvaqc kicashigwk ftatkkdmsp qkfwgltrsa
421 llptipdted eispdkvilc l
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By "CRBN polynucleotide" is meant a nucleic acid molecule encoding a CRBN polypeptide. An exemplary CRBN polynucleotide sequence is provided at NCBI Accession No. BC067811, which is reproduced below:

```
   1 gcgtgtaaac agacatggcc ggcgaaggag atcagcagga cgctgcgcac aacatgggca
  61 accacctgcc gctcctgcct gagagtgagg aagaagatga aatggaagtt gaagaccagg
 121 atagtaaaga agccaaaaaa ccaaacatca taaattttga caccagtctg ccgacatcac
 181 atacatacct aggtgctgat atggaagaat ttcatggcag gactttgcac gatgacgaca
 241 gctgtcaggt gattccagtt cttccacaag tgatgatgat cctgattccc ggacagacat
 301 tacctcttca gctttttcac cctcaagaag tcagtatggt gcggaattta attcagaaag
 361 atagaacctt tgctgttctt gcatacagca atgtacagga aagggaagca cagtttggaa
 421 caacagcaga gatatatgcc tatcgagaag aacaggattt tggaattgag atagtgaaag
 481 tgaaagcaat tggaagacaa aggttcaaag tccttgagct aagaacacag tcagatggaa
 541 tccagcaagc taaagtgcaa attcttcccg aatgtgtgtt gccttcaacc atgtctgcag
 601 ttcaattaga atccctcaat aagtgccaga tatttccttc aaaacctgtc tcaagagaag
 661 accaatgttc atataaatgg tggcagaaat accagaggag aaagtttcat tgtgcaaatc
 721 taacttcatg gcctcgctgg ctgtattcct tatatgatgc tgagacctta atggacagaa
 781 tcaagaaaca gctacgtgaa tgggatgaaa atctaaaaga tgattctctt ccttcaaatc
 841 caatagattt ttcttacaga gtagctgctt gtcttcctat tgatgatgta ttgagaattc
 901 agctccttaa aattggcagt gctatccagc gacttcgctg tgaattagac attatgaata
 961 aatgtacttc cctttgctgt aaacaatgtc aagaaacaga aataacaacc aaaaatgaaa
1021 tattcagttt atccttatgt gggccgatgg cagcttatgt gaatcctcat ggatatgtgc
1081 atgagacact tactgtgtat aaggcttgca acttgaatct gataggccgg ccttctacag
1141 aacacagctg gtttcctggg tatgcctgga ctgttgccca gtgtaagatc tgtgcaagcc
1201 atattggatg gaagtttacg gccaccaaaa aagacatgtc acctcaaaaa ttttggggct
1261 taacgcgatc tgctctgttg cccacgatcc cagacactga agatgaaata agtccagaca
1321 aagtaatact ttgcttgtaa acagatgtga tagagataaa gttagttatc taacaaattg
1381 gttatattct aagatctgct ttggaaatta ttgcctctga tacataccta agtaaacata
1441 acattaatac ctaagtaaac ataacattac ttggagggtt gcagtttcta agtgaaactg
1501 tatttgaaac ttttaagtat actttaggaa acaagcatga acggcagtct agaataccag
1561 aaacatctac ttgggtagct tggtgccatt atcctgtgga atctgatatg tctggtagcg
1621 tgtcattgat gggacatgaa gacatctttg gaaatgatga gattatttcc tgtgttaaaa
1681 aaaaaaaaaa aatcttaaat tcctacaatg tgaaactgaa actaataatt tgatcctgat
1741 gtatgggaca gcgtatctgt accagtgctc taaataacaa aagctagggt gacaagtaca
1801 tgttcctttt ggaaagaagc aaggcaatgt atattaatta ttctaaaagg gctttgttcc
1861 tttccatttt ctttaacttc tctgagatac tgatttgtaa attttgaaaa ttagttaaaa
1921 tatgcagttt tttgagccca cgaatagttg tcatttcctt tatgtgcctg ttagtaaaaa
1981 gtagtattgt gtatttgctc agtatctgaa ctataagccc atttatactg ttccatacaa
2041 aagctatttt tcaaaaatta atttgaacca aaactactac tatagggaaa agatgccaaa
2101 acatgtcccc tcacccaggc taaacttgat actgtattat tttgttcaat gtaaattgaa
2161 gaaaatctgt aagtaagtaa accttaagtg tgaaactaaa aaaaaaaaaa aaa
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As used herein, a “degron” or “degron sequence” refers to an amino acid sequence in a polypeptide that is both necessary and sufficient for targeting by the polypeptide’s cognate ubiquitin ligase. In one embodiment, the degron of the IKZF3 polypeptide is amino acids 146-168 of IKZF3.

“Detect” refers to identifying the presence, absence or amount of the analyte to be detected.

By "detectable label" is meant a composition that when linked to a molecule of interest renders the latter detectable, via spectroscopic, photochemical, biochemical, immunochemical, or chemical means. For example, useful labels include radioactive isotopes, magnetic beads, metallic beads, colloidal particles, fluorescent dyes, electron-dense reagents, enzymes (for example, as commonly used in an ELISA), biotin, digoxigenin, or haptens.

By “disease” is meant any condition or disorder that damages or interferes with the normal function of a cell, tissue, or organ. Examples of diseases, include B cell neoplasia or other malignancies, for example, plasma cell malignancy, multiple myeloma or a myelodysplastic syndrome, erythema nodosum leparum, 5q- myelodysplastic syndrome.

By "effective amount" is meant the amount of a required to ameliorate the symptoms of a disease relative to an untreated patient. The effective amount of active compound(s) used to practice the present invention for therapeutic treatment of a disease varies depending upon the manner of administration, the age, body weight, and general health of the subject. Ultimately, the attending physician or veterinarian will decide the appropriate amount and dosage regimen. Such amount is referred to as an "effective" amount.

By "fragment" is meant a portion of a polypeptide or nucleic acid molecule. This portion contains, preferably, at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% of the entire length of the reference nucleic acid molecule or polypeptide. A fragment may contain 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100, 200, 300, 400, 500, 600, 700, 800, 900, or 1000 nucleotides or amino acids.

"Hybridization" means hydrogen bonding, which may be Watson-Crick, Hoogsteen or reversed Hoogsteen hydrogen bonding, between complementary nucleobases. For example, adenine and thymine are complementary nucleobases that pair through the formation of hydrogen bonds.

By "IKZF1 polypeptide" or "Ikaros" is meant a polypeptide having at least about 85% amino acid sequence identity to a sequence provided at NCBI Accession No. AAH18349, NP_006051, NP_001207694, or a fragment thereof and having DNA binding or transcriptional regulatory activity.

For IKZF1 Isoform 1, the degron is from 130-270. For IKZF1 Isoform 2, the degron is from amino acid 136-180/236-249. Both isoforms are responsive to lenalidomide. Exemplary amino acid sequences for the two isoforms are provided below:

IKZF1 isoform 2 NCBI Reference No. NP_001207694

```
1  mdadegqdms qvsgkesppv sdtpdegdep mpipedlstt sggqqssksd rvvasnvkve
61 tqsdeengra cemngeecae dlrmldasge kmngshrdqg ssalsgvggi rlpngklkcd
121 icgiicigpn vlmvhkrsht gerpfqcnqc gasftqkgnl lrhiklhsge kpfkchlcny
181 acrrrdaltg hlrthsvike etnhsemaed lckigsersl vldrlasnva krkssmpqkf
241 lgdkglsdtp ydssasyeke nemmkshvmd qainnainyl gaeslrplvq tppggsevvp
301 vispmyqlhk plaegtprsn hsaqdsaven llllskaklv psereaspsn scqdstdtes
361 nneeqrsgli yltnhiapha rnglslkeeh raydllraas ensqdalrvv stsgeqmkvy
421 kcehcrvlfl dhvmytihmg chgfrdpfec nmcgyhsqdr yefsshitrg ehrfhms
```

IKZF1 isoform 1 NCBI Reference No. NP_006051

```
1 mdadegqdms qvsgkesppv sdtpdegdep mpipedlstt sggqqssksd rvvasnvkve
61 tqsdeengra cemngeecae dlrmldasge kmngshrdqg ssalsgvggi rlpngklkcd
121 icgiicigpn vlmvhkrsht gerpfqcnqc gasftqkgnl lrhiklhsge kpfkchlcny
181 acrrrdaltg hlrthsvgkp hkcgycgrsy kqrssleehk erchnylesm glpgtlypvi
241 keetnhsema edlckigser slvldrlasn vakrkssmpq kflgdkglsd tpydssasye
301 kenemmkshv mdqainnain ylgaeslrpl vqtppggsev vpvispmyql hkplaegtpr
361 snhsaqdsav enllllskak lvpsereasp snscqdstdt esnneeqrsg liyltnhiap
421 harnglslke ehraydllra asensqdalr vvstsgeqmk vykcehcrvl fldhvmytih
481 mgchgfrdpf ecnmcgyhsq dryefsshit rgehrfhms
```

By "IKZF1 polynucleotide" is meant a polynucleotide encoding an IKZF1 polypeptide. An exemplary IKZF1 polynucleotide is provided at NM_006060.4 and reproduced below:

```
1 ggcagcagag gaacctttg gaggaggaag aggacacaga ggccctgtag ccaggcacca
61 agatccctcc caggtggctg ggtctgaggg gaactccgag cagccctagg tcctcaaagt
121 ctggatttgt gtggaaaagg cagctctcac ttggccttgg cgaggcctcg gttggttgat
181 aacctgagga ccatggatgc tgatgagggt caagacatgt cccaagtttc agggaaggaa
241 agccccccctg taagcgatac tccagatgag ggcgatgagc ccatgccgat ccccgaggac
301 ctctccacca cctcgggagg acagcaaagc tccaagagtg acagagtcgt ggccagtaat
361 gttaaagtag agactcagag tgatgaagag aatgggcgtg cctgtgaaat gaatggggaa
```

```
 421 gaatgtgcgg aggatttacg aatgcttgat gcctcgggag agaaaatgaa tggctcccac
 481 agggaccaag gcagctcggc tttgtcggga gttggaggca ttcgacttcc taacggaaaa
 541 ctaaagtgtg atatctgtgg gatcatttgc atcgggccca atgtgctcat ggttcacaaa
 601 agaagccaca ctggagaacg gcccttccag tgcaatcagt gcggggcctc attcacccag
 661 aagggcaacc tgctccggca catcaagctg cattccgggg agaagccctt caaatgccac
 721 ctctgcaact acgcctgccg ccggagggac gccctcactg gccacctgag gacgcactcc
 781 gtcattaaag aagaaactaa tcacagtgaa atggcagaag acctgtgcaa gataggatca
 841 gagagatctc tcgtgctgga cagactagca agtaacgtcg ccaaacgtaa gagctctatg
 901 cctcagaaat ttcttgggga caagggcctg tccgacacgc cctacgacag cagcgccagc
 961 tacgagaagg agaacgaaat gatgaagtcc cacgtgatgg accaagccat caacaacgcc
1021 atcaactacc tgggggccga gtccctgcgc ccgctggtgc agacgccccc gggcggttcc
1081 gaggtggtcc cggtcatcag cccgatgtac cagctgcaca agccgctcgc ggagggcacc
1141 ccgcgctcca accactcggc ccaggacagc gccgtggaga acctgctgct gctctccaag
1201 gccaagttgg tgccctcgga gcgcgaggcg tccccgagca acagctgcca agactccacg
1261 gacaccgaga gcaacaacga ggagcagcgc agcggtctca tctacctgac caaccacatc
1321 gccccgcacg cgcgcaacgg gctgtcgctc aaggaggagc accgcgccta cgacctgctg
1381 cgcgccgcct ccgagaactc gcaggacgcg ctccgcgtgg tcagcaccag cggggagcag
1441 atgaaggtgt acaagtgcga acactgccgg gtgctcttcc tggatcacgt catgtacacc
1501 atccacatgg gctgccacgg cttccgtgat ccttttgagt gcaacatgtg cggctaccac
1561 agccaggacc ggtacgagtt ctcgtcgcac ataacgcgag gggagcaccg cttccacatg
1621 agctaaagcc ctcccgcgcc cccaccccag accccgagcc accccaggaa aagcacaagg
1681 actgccgcct tctcgctccc gccagcagca tagactggac tggaccagac aatgttgtgt
1741 ttggatttgt aactgttttt tgttttttgt ttgagttggt tgattggggt ttgatttgct
1801 tttgaaaaga tttttatttt tagaggcagg gctgcattgg gagcatccag aactgctacc
1861 ttcctagatg tttccccaga ccgctggctg agattccctc acctgtcgct tcctagaatc
1921 cccttctcca aacgattagt ctaaattttc agagagaaat agataaaaca cgccacagcc
1981 tgggaaggag cgtgctctac cctgtgctaa gcacggggtt cgcgcaccag gtgtcttttt
2041 ccagtcccca gaagcagaga gcacagcccc tgctgtgtgg gtctgcaggt gagcagacag
2101 gacaggtgtg ccgccaccca agtgccaaga cacagcaggg ccaacaacct gtgcccaggc
2161 cagcttcgag ctacatgcat ctagggcgga gaggctgcac ttgtgagaga aaatactatt
2221 tcaagtcata ttctgcgtag gaaaatgaat tggttgggga aagtcgtgtc tgtcagactg
2281 ccctgggtgg agggagacgc cgggctagag cctttgggat cgtcctggat tcactggctt
2341 tgcggaggct gctcagatgg cctgagcctc ccgaggcttg ctgccccgta ggaggagact
2401 gtcttcccgt gggcatatct ggggagccct gttccccgct ttttcactcc catacctta
2461 atggccccca aaatctgtca ctacaattta aacaccagtc ccgaaatttg gatcttcttt
2521 ctttttgaat ctctcaaacg gcaacattcc tcagaaacca aagctttatt tcaaatctct
2581 tccttccctg gctggttcca tctagtacca gaggcctctt ttcctgaaga aatccaatcc
2641 tagccctcat tttaattatg tacatctgtt tgtagccaca agcctgaatt tctcagtgtt
2701 ggtaagtttc tttacctacc ctcactatat attattctcg ttttaaaacc cataaaggag
2761 tgatttagaa cagtcattaa ttttcaactc aatgaaatat gtgaagccca gcatctctgt
```

```
2821 tgctaacaca cagagctcac ctgtttgaaa ccaagctttc aaacatgttg aagctcttta
2881 ctgtaaaggc aagccagcat gtgtgtccac acatacatag gatggctggc tctgcacctg
2941 taggatattg gaatgcacag ggcaattgag ggactgagcc agaccttcgg agagtaatgc
3001 caccagatcc cctaggaaag aggaggcaaa tggcactgca ggtgagaacc ccgcccatcc
3061 gtgctatgac atggaggcac tgaagcccga ggaaggtgtg tggagattct aatcccaaca
3121 agcaagggtc tccttcaaga ttaatgctat caatcattaa ggtcattact ctcaaccacc
3181 taggcaatga agaatatacc atttcaaata tttacagtac ttgtcttcac caacactgtc
3241 ccaaggtgaa atgaagcaac agagaggaaa ttgtacataa gtacctcagc atttaatcca
3301 aacaggggtt cttagtctca gcactatgac attttgggct gactacttat ttgttaggca
3361 ggagctctcc tgtgcattgt aggataatta gcagtatccc tggtggctac ccaatagacg
3421 ccagtagcac cccgaattga caacccaaac tctccagaca tcaccaactg tcccctgcga
3481 ggagaaatca ctcctggggg agaaccactg acccaaatga attctaaacc aatcaaatgt
3541 ctgggaagcc ctccaagaaa aaaaaaaaaa aa
```

By "IKZF3 polypeptide" or "Aiolos" is meant a polypeptide having at least about 85% amino acid sequence identity to NCBI Accession No. NP_036613.2 (UnitPro Identifier No. Q9UKT9-1) or a fragment thereof and having DNA binding or transcriptional regulatory activity. An exemplary amino acid sequence of IKZF3 is provided below.

```
      10         20         30         40         50         60
MEDIQTNAEL KSTQEQSVPA ESAAVLNDYS LTKSHEMENV DSGEGPANED EDIGDDSMKV
        70         80         90        100        110        120
KDEYSERDEN VLKSEPMGNA EEPEIPYSYS REYNEYENIK LERHVVSFDS SRPTSGKMNC
       130        140        150        160        170        180
DVCGLSCISF NVLMVHKRSH TGERPFQCNQ CGASFTQKGN LLRHIKLHTG EKPFKCHLCN
       190        200        210        220        230        240
YACQRRDALT GHLRTHSVEK PYKCEFCGRS YKQRSSLEEH KERCRTFLQS TDPGDTASAE
       250        260        270        280        290        300
ARHIKAEMGS ERALVLDRLA SNVAKRKSSM PQKFIGEKRH CFDVNYNSSY MYEKESELIQ
       310        320        330        340        350        360
TRMMDQAINN AISYLGAEAL RPLVQTPPAP TSEMVPVISS MYPIALTRAE MSNGAPQELE
       370        380        390        400        410        420
KKSIHLPEKS VPSERGLSPN NSGHDSTDTD SNHEERQNHI YQQNHMVLSR ARNGMPLLKE
       430        440        450        460        470        480
VPRSYELLKP PPICPRDSVK VINKEGEVMD VYRCDHCRVL FLDYVMFTIH MGCHGFRDPF
       490        500
ECNMCGYRSH DRYEFSSHIA RGEHRALLK
```

By "IKZF3 polynucleotide" or "Aiolos polynucleotide" is meant a nucleic acid sequence encoding an IKZF3 polypeptide. An exemplary polynucleotide sequence is provided at NCBI Accession No. NM_012481, which is reproduced below:

```
   1 gcaggagcac gtggagaggc cgagtagcca cagcggcagc tccagcccgg cccggcagcg
  61 acatggaaga tatacaaaca aatgcggaac tgaaaagcac tcaggagcag tctgtgcccg
 121 cagaaagtgc agcggttttg aatgactaca gtttaaccaa atctcatgaa atggaaaatg
 181 tggacagtgg agaaggccca gccaatgaag atgaagacat aggagatgat tcaatgaaag
```

```
 241 tgaaagatga atacagtgaa agagatgaga atgttttaaa gtcagaaccc atgggaaatg
 301 cagaagagcc tgaaatccct tacagctatt caagagaata taatgaatat gaaaacatta
 361 agttggagag acatgttgtc tcattcgata gtagcaggcc aaccagtgga aagatgaact
 421 gcgatgtgtg tggattatcc tgcatcagct tcaatgtctt aatggttcat aagcgaagcc
 481 atactggtga acgcccattc cagtgtaatc agtgtggggc atcttttact cagaaaggta
 541 acctcctccg ccacattaaa ctgcacacag gggaaaaacc ttttaagtgt cacctctgca
 601 actatgcatg ccaaagaaga gatgcgctca cggggcatct taggacacat tctgtggaga
 661 aacccctacaa atgtgagttt tgtggaagga gttacaagca gagaagttcc cttgaggagc
 721 acaaggagcg ctgccgtaca tttcttcaga gcactgaccc aggggacact gcaagtgcgg
 781 aggcaagaca catcaaagca gagatgggaa gtgaaagagc tctcgtactg gacagattag
 841 caagcaatgt ggcaaaacga aaaagctcaa tgcctcagaa attcattggt gagaagcgcc
 901 actgctttga tgtcaactat aattcaagtt acatgtatga gaaagagagt gagctcatac
 961 agacccgcat gatggaccaa gccatcaata acgccatcag ctatcttggc gccgaagccc
1021 tgcgcccctt ggtccagaca ccgcctgctc ccacctcgga gatggttcca gttatcagca
1081 gcatgtatcc catagccctc acccgggctg agatgtcaaa cggtgcccct caagagctgg
1141 aaaagaaaag catccacctt ccagagaaga gcgtgccttc tgagagaggc ctctctccca
1201 acaatagtgg ccacgactcc acggacactg acagcaacca tgaagaacgc cagaatcaca
1261 tctatcagca aaatcacatg gtcctgtctc gggcccgcaa tgggatgcca cttctgaagg
1321 aggttccccg ctcttacgaa ctcctcaagc ccccgcccat ctgcccaaga gactccgtca
1381 aagtgatcaa caaggaaggg gaggtgatgg atgtgtatcg gtgtgaccac tgccgcgtcc
1441 tcttcctgga ctatgtgatg ttcacgattc acatgggctg ccacggcttc cgtgaccctt
1501 tcgagtgtaa catgtgtgga tatcgaagcc atgatcggta tgagttctcg tctcacatag
1561 ccagaggaga acacagagcc ctgctgaagt gaatatctgg tctcagggat tgctcctatg
1621 tattcagcat cgtttctaaa aaccaatgac ctcgcctaac agattgctct caaaacatac
1681 tcagttccaa acttcttttc ataccatttt tagctgtgtt cacaggggta gccagggaaa
1741 cactgtcttc cttcagaaat tattcgcagg tctagcatat tattactttt gtgaaacctt
1801 tgttttccca tcagggactt gaattttatg gaatttaaaa gccaaaaagg tatttggtca
1861 ttatcttcta cagcagtgga atgagtggtc ccggagatgt gctatatgaa acattctttc
1921 tgagatatat caaccacacg tggaaaagcc tttcagtcat acatgcaaat ccacaaagag
1981 gaagagctga ccagctgacc ttgctgggaa gcctcaccct tctgcccttc acaggctgaa
2041 gggttaagat ctaatctccc taatctaaat gacagtctaa gagtaagtaa aagaacagcc
2101 ataaaataag tatctgttac gagtaactga agaccccatt ctccaagcat cagatccatt
2161 tcctatcaca acatttttaa aaaatgtcat ctgatggcac ttctgcttct gtcctttacc
2221 ttcccatctc cagtgaaaag ctgagctgct ttgggctaaa ccagttgtct atagaagaaa
2281 atctatgcca gaagaactca tggttttaaa tatagaccat catcgaaact ccagaaattt
2341 atccactgtg gatgatgaca tcgctttcct ttggtcaagg ttggcagagc aagggtataa
2401 agggggaaat tgtttggcag caccaacaga aaacaaacaa acaaaaaaca gctacctaaa
2461 acttcttgaa agagttcatg gagaattggt gatacagacc caaagcaaat ttgccaatga
2521 tattttccac aaaaaaagtc caaaaagtat ggctcagcct ccccctcccc acaggagagg
2581 aattggagat agatggcatg tgtgtttaga tcggagttga gctccggaat ggggtgagga
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2641 gggacacctc tattgagagg ttctccttga tcaggcaggc ttcggccctt tttttcccat
2701 ttaaatggaa ctgctgtatt ccatgaaaat tcctgaaagt ctgatcacgg ttctgcagat
2761 gtataagtca tccttgtcac tcataatatg tacatactat caggaggagt gctgttatca
2821 tggtaaaatt agcactggaa taggaggtca caaaatgctg gctaattagc tatgtgactt
2881 tgagaaatcg tttaactttt ttttttttt tttttttgag acaggatctc actctgttgc
2941 ccaggctgga gtgcagtggt gcaatcatgg ctcagtgcag cctcgacctc cccaggctca
3001 ggtgatcctc ccacctcagc ctcttgagta ctgggacaac aagtgcacac caccatgtct
3061 ggctacattt tgttcttttt gtagagatag gggtctcact atgttgccca tgctggtctt
3121 gaactcctgg gctcaagcaa tcagcccgcc tcagcctcct aaagtgctgg gattacaggt
3181 gtgagccacc acacccagcc ttatttaact cttaaaactc agtttccggc caggctcggt
3241 ggctcacacc tgtaatccca acactttggg aagccgaggc aggcgcatca tttgaggtca
3301 ggagttcgag accagcctga cccacatggt gaaaccctgt ctctactaaa aatacaaaaa
3361 ttagctgggc agtagtggca catgcctgta atcccagcta ctccggaggc tgaggcagaa
3421 aaatcgctta agcctgggag gttgaggttg cggtgagtgg agatcacact actgcactcc
3481 agtctgggcg acagagtgag accctgtctc aaacaaaaca aaacaaaaac aaacaaacaa
3541 aaacaaaaaa aactcagttt cctcatccat aaaataggaa ttagatttca atgttctctt
3601 aggtcccttc tagctttaat tcatatgtga ttatgcagta accacaaggt atttttaaa
3661 cctcctaatg tatggatatt aagcagaaga gtatttatat gaatacatgt ttcacattcc
3721 tttggtatga aaatggtgtg ttaagttttt cctttaacca ctgagttgtg aatgtgaaga
3781 aggtggtgga gaggaacaaa aaacagaaag gtattttgat cttgccacaa agcatacaca
3841 caaattggca catgcagctg tttgccaaag ccttcttttt ttttttactt tttaagaaat
3901 tatgttaggg aaaataaatt ctgcttccag ggacaacttc atggagccta tttacaaatt
3961 aagagtcagc ttaatttgta acatttctac cagagccaag aatcccaaat tcctggtaga
4021 ttagtgtttt atttctaagg ggcttatgca ttcggctcca actcaactcg tctatgtgct
4081 gccagtaatt aaaatgttcc acctcagact gcacaaatgg cttatccttc tttgtggcat
4141 ggcgtctgtc tcaggaaaaa aggttttatg aaattccatg gcaacagtcc caacatgttt
4201 gagacttcag ctaaaggaat ggatgtattt tggtgtgtag tcttcagtat atcactgtat
4261 ttccgtaata ctagactcca agctatgcca gattgcttat tccctttgtg aaagaggagt
4321 tgctcattac gttcttgaaa tatcgcacat cctgttggtt cttcaaggga caagagaaag
4381 agaatttgga agcagggatt agtagaagag aaaacgaggg aaaggaagcc tttccaccag
4441 attagtgttc aagtctttgc agaggagacc aacttttttt gttttctttt gttttgagac
4501 agtctctcgc tctgttgccc aggctggagt gcagtggcgc gatctcggct cacggcaacc
4561 tccgcctccc gggttcaagc aattctcctg cctcagcctc ccaagtagct gggattacag
4621 gtgctcacca ccaagcccgg ctaatttttg tatttttagt agagacaagg tttcaccatg
4681 ttggccaggc cagtctcaaa ctcctgacct caggtgatct gcccgccttg gcctcccaca
4741 gtgctgggat tacaggcatg agctaccgca cccagcctga gaccaccttt tgcatctcaa
4801 gattgtgaaa ccaaggccca ttccaccagc ctggggactc tttttataga tatgatcctc
4861 ctttttcctg tgactaatga atttgctgca tgatttctat tcttctgagg ttagttttct
4921 gagtaaggtg accactcaca aaggcacttt ctttgtggca ttctgagcct agattggggc
4981 ccatcaattc cagaaaaaat ttatgtgtgg aaactctgca tccttaagtc ttgaagttga

5041 accagatatg cagtggttac catcacacag ataaacgctg ccttctgtac ataccccttа
5101 tgctgtacta attaacaaac cccttgccag ggctggggag gtgagggtga aggagaatct
5161 tagcagaagg gcagagtcag gacttgcatc tgccactgct gggcactgaa gccctggagc
5221 agcttcagat agtacctgta ctttctcatg cagactccct ctgaacaaga gccttgtagg
5281 cccctctcct tcatttccca ccagcctctt atcaggcggg ctttccacca tacacccagg
5341 aggccacggt ctgaggaaca accaaaccca tgcaaagggc cgggcgcgat agctcacgcc
5401 tgtaatgcca gcactttggg aggctggggc aggcagatca cctgaggttg ggagttcgag
5461 acctgcctga ccaacatgga gaaaccccca tctctactaa aaatacaaaa ttagccgggc
5521 gtgatggcac atgcctgtaa tcccagctac tcaggaggct gaggcaggag aatcgcttga
5581 acccgggagg cggaggttgc ggtgagccga gatggcacca ctgcactcca gcctcggcaa
5641 caagagcgaa actctgtcta aaacaaaaac aaacaaacaa acaaaaaaac ccaggcaaag
5701 tttccttgca gccaaggtga cagaactggg ctgagggtgg aaaagaaaca gaaccagtgc
5761 tccaggtgtt ttttaatttt ttaatttatt tttatttttt ttgtatatgt atatatatgt
5821 atgtatattt tagaggacca gggtctcact atgttgccta ggccagactc aaactcctgt
5881 gctcaagcaa tcctgcctca gcctcccaag tagctgggat tacaggcatg cacaaacaat
5941 gcccagctct ccaaatgttt tctgtcacta cctgaagtgt tgcatcggta cttcctacgg
6001 aaagaaaact aaatagaagt gtctctcccg tgagccccca ccactaccac cagaaaaaaa
6061 aaagagagaa aatgaactca tcagtcttta gtttcctcaa gttattctcc caaaaagaca
6121 ttcgccttgg cacagataag ccagctaatc ttatgcttta tgacccactg tgagctgttc
6181 ctgacacagc ttctgacttt gtcagtgaca aaatttctca ccttttaaat gcagtgctta
6241 acattttgtt aggcccatac tcaaaatcgg ccagatataa aatgacctca gattttgatc
6301 tcctaggctc aaacaatcct cctacctcag cctcccaagt agctgggact ataggcacac
6361 caccatgcac agctaatttt ttttgtattt ttctgcagag atggcgtttc gccatactgc
6421 ccaggctagt ctcaaaatcc tgggctcaag caatctgccc acctcagcct cccaaagtgc
6481 tggaactaca ggcaagagcc actgcgccca gccacaacct cagatttctt tggcaaacag
6541 aaatgtttaa aaacacaaaa ttttgctcag gtgaaacact gtgttactat caaatctcac
6601 atccacataa agtttttctt ttcggctttg tttcgtgagg aacagacaga acaaagtttt
6661 tccaggtagc atctgtatca ctattattct cctatttcct gtaccacccc cacctcccca
6721 agccctactg aatgtgaggt ttagaatgtt ttaaggaggg tcaggtgcgg tggctcacgc
6781 ctgtaatccc agcactttgg gaggccaagg cgggcggatc acctgagttt gggagttcga
6841 gaccagcctg accaacatgg agaaaccctg tctctactaa aaatacaaaa ttagccaggc
6901 gtggtggcac atgcctgtaa tcccagctac ttaggaggct gaggcaggag aatcgcttga
6961 acccaggagg aggaggttgt ggtgagccga gatcgtgcca ttgcactcca gcctgggtga
7021 cagagtgaga ctccatctcg aaaaaaaaaa tacaaaaatt agctgggtgt ggtggtgcac
7081 acctgtaatc ccagctactc gggaggctga cgcaggagaa ttgcttgaac ctgggaggtg
7141 gaggttgcag tgagccgaga tcgcgccatt gcaatccagc ctggacaaca gagtgagact
7201 ccatctcaaa aaaaaaaaaa aaaagaatgt tttaaggaaa aaaatagtac tgttacatat
7261 aatcccaggt gataagacca caatggaaat gtttaagtcc tcactttaaa gagtacccca
7321 ctgagaagag gtatgttgga ctctagcaga gatttggaaa ctctgggaca ctcaagatgt
7381 gaaagagcct ggctatctga ggactcaaag agtcagcatc gggacttgtg agctcaagaa

7441 gagaaaaggg agtggtgaaa ctttgtccta aaagttagca ccaggaacag aagaaaaaaa
7501 cccgatatat agtgatacct catcttttag agaatgggaa gctatttttg tgttcacaca
7561 gaaagtatag ttcaaaaaac ctctatatcc agagttcaga caaggagaat gatttgagat
7621 ataagtgccg atgaaggagg tcaattttga tctgaaacca gcagctggac ctgggccacc
7681 tcaggaaaag gactctgttc tccaaggcag cacgactgaa tggttctgag aataagccag
7741 ggttcaggac tcctgaccct ttaggaccat ggactcagaa gagcctgaag gacaattgtg
7801 ggctttaaac ttctgagagc ttgtaaagta acacaagact gtgcctctcc cttgccccag
7861 ctgtagatag tctttgcccc accattgtta tgaagataca cagggttttg cagtttgaat
7921 aaattggata caagtttcct ctttttttt ttctttttga gacaaagtct cgctctgttt
7981 ccccaggctg agtgcagtgg cacaatcaag gcttacttgc cgcctcaacc tcctgggctc
8041 aagcaacgag ccatcctccc gtcttagcct cccaactagc tgagactaca ggcgtgggtc
8101 accacaccca gctaattttt gtacttttg tagagacagg gtctcaccat gttgcccagg
8161 ctggtcctga actcctgggc tcaagtaatc tgcccacctc agcctcccaa agtgttgggg
8221 ttacaggcgt gaggcaccgc ggctggcctg agtttcttct taatactgta tcacaattgt
8281 gggctgtctt atgtgttgat atcgattgag ctatttgaaa taggaatgtt aatgggtgta
8341 ttaaattttt gtaaggatat aacaatatct accttccaag gatgttgtga ggttttccat
8401 gattttgtat atgagctaat gttacctttg aggggtggtg tgcattatgt tggatgattg
8461 taaattttca gtggaaaatg taccgtgtcc taaatttaaa gacatgaaaa atatcccaag
8521 atcatactag atcataatag caattccttt acaaatgaat tatggaggta actgatctct
8581 aacagtttcc ttcatgttgt tttaatgcac aagggcagag gatctgctga ccctttggaac
8641 cagcgtgagc taaccacgtg ctatagacac ttcatggtgt cgcacccagg gaagtcaaag
8701 cgctttgctc cctcactgtc tgtgagtcct cagccattag taccccaccc cccgctgctc
8761 caaaacttga gttatttcaa atgtttctca ctgttcatct ctccactgac cccactccag
8821 aaagcctgga gagagtccca agatgccacc caccttcccc aatccctcgc cacagatctg
8881 tgtctatctc acactctgta agtgccgctt tgcttcttcc tctcttgaaa agactgagaa
8941 cacacatttt aacatgttag gaaaatgggg cagcctaaaa aatgactgat cccaccgcca
9001 gtgactcatg tatactccag gctagcagac aaggcccttt ttggtgggcc tgcttctgtg
9061 ggttcacaga aaccaaatta ctgtgggttg caaagaatta gcaggtcatt tacaaagcag
9121 acatcccttc acccagactg tggttttgca tgctcaggtt ctcagtctat gagctttggt
9181 gcaggatcat tttggctact ggaaaaacca tagcttattt taaatttctg gttgccaaag
9241 ccaccacacg tgtggtctgt ggatgaccat tgtctgcaga atgacgagga aggaacagaa
9301 tgtggtttgg ggctcagggt ggccttccca ctgggaggga aggcgggagg gagcccttgc
9361 cctgggtttt gacacagcct gtgctcacag cctctcctct catctgcatt tctcagaaat
9421 gccctccctg cccagtggtg actttccctc gtcactccta tggagttcta cctggagccc
9481 agccatgtgt ggaactgtga agtttactcc tctgtaaaga tggtttaaag aaagtcagct
9541 tctgaaatgt aacaatgcta acccttgctg gaaccctgta agaaatagcc ctgctgatag
9601 ttttctaggt ttatcatgtt tgatttttac actgaaaaat aaaaaaatcc tggtatgttt
9661 gaaattaaaa aaaaaaaaaa aaaaaa

The terms "isolated," "purified," or "biologically pure" refer to material that is free to varying degrees from components which normally accompany it as found in its native state. "Isolate" denotes a degree of separation from original source or surroundings. "Purify" denotes a degree of separation that is higher than isolation. A "purified" or "biologically pure" protein is sufficiently free of other materials such that any impurities do not materially affect the biological properties of the protein or cause other adverse consequences. That is, a nucleic acid or peptide of this invention is purified if it is substantially free of cellular material, viral material, or culture medium when produced by recombinant DNA techniques, or chemical precursors or other chemicals when chemically synthesized. Purity and homogeneity are typically determined using analytical chemistry techniques, for example, polyacrylamide gel electrophoresis or high performance liquid chromatography. The term "purified" can denote that a nucleic acid or protein gives rise to essentially one band in an electrophoretic gel. For a protein that can be subjected to modifications, for example, phosphorylation or glycosylation, different modifications may give rise to different isolated proteins, which can be separately purified.

By "isolated polynucleotide" is meant a nucleic acid (e.g., a DNA) that is free of the genes which, in the naturally-occurring genome of the organism from which the nucleic acid molecule of the invention is derived, flank the gene. The term therefore includes, for example, a recombinant DNA that is incorporated into a vector; into an autonomously replicating plasmid or virus; or into the genomic DNA of a prokaryote or eukaryote; or that exists as a separate molecule (for example, a cDNA or a genomic or cDNA fragment produced by PCR or restriction endonuclease digestion) independent of other sequences. In addition, the term includes an RNA molecule that is transcribed from a DNA molecule, as well as a recombinant DNA that is part of a hybrid gene encoding additional polypeptide sequence.

By an "isolated polypeptide" is meant a polypeptide of the invention that has been separated from components that naturally accompany it. Typically, the polypeptide is isolated when it is at least 60%, by weight, free from the proteins and naturally-occurring organic molecules with which it is naturally associated. Preferably, the preparation is at least 75%, more preferably at least 90%, and most preferably at least 99%, by weight, a polypeptide of the invention. An isolated polypeptide of the invention may be obtained, for example, by extraction from a natural source, by expression of a recombinant nucleic acid encoding such a polypeptide; or by chemically synthesizing the protein. Purity can be

measured by any appropriate method, for example, column chromatography, polyacrylamide gel electrophoresis, or by HPLC analysis.

By "sensitivity to a modulator of CRBN" is meant that at least one symptom of a disease or condition is ameliorated by treatment with a modulator of CRBN.

By "resistant to a modulator of CRBN" is meant that a cell having a disease has acquired an alteration that allows it to escape an anti-disease effect of at least one modulator of CRBN. For example, a resistant cell may be a neoplastic cell that has acquired an alteration that allows it to escape an anti-neoplastic effect of the modulator of CRBN. Exemplary anti-neoplastic effects include, but are not limited to, any effect that reduces proliferation, reduces survival, and/or increases cell death (e.g., increases apoptosis).

By "lenalidomide sensitivity" is meant that at least one symptom of a disease or condition is ameliorated by treatment with lenalidomide. Likewise, by "lenalidomide analog sensitivity" is meant at least one symptom of a disease or condition is ameliorated by treatment with a lenalidomide analog.

By "lenalidomide resistant" is meant that a cell having a disease has acquired an alteration that allows it to escape an anti-disease effect of lenalidomide. Likewise, by "lenalidomide analog resistant" is meant that a cell having a disease has acquired an alteration that allows it to escape an anti-disease effect of a lenalidomide analog. For example, a lenalidomide resistant cell may be a neoplastic cell that has acquired an alteration that allows it to escape an anti-neoplastic effect of lenalidomide. Exemplary anti-neoplastic effects include, but are not limited to, any effect that reduces proliferation, reduces survival, and/or increases cell death (e.g., increases apoptosis).

By "modulator of CRBN" or "modulator of Cereblon" is meant any agent which binds Cereblon (CRBN) and alters an activity of CRBN. In some embodiments, an activity of CRBN includes binding with and/or mediating degradation of Ikaros (IKZF1), Aiolos (IKZF3), or Casein kinase 1 Alpha (CSNK1a1). Thus, a modulator of CRBN includes agents that alter binding of CRBN with IKZF1, IKZF3, or CSNK1a1 and agents that alter CRBN's mediation of IKZF1, IKZF3, or CSNK1a1 degradation. In particular embodiments, a modulator of CRBN is lenalidomide or an analog thereof (e.g., pomalidomide or thalidomide).

As used herein, "obtaining" as in "obtaining an agent" includes synthesizing, purchasing, or otherwise acquiring the agent.

As used herein, the terms “prevent,” “preventing,” “prevention,” “prophylactic treatment” and the like refer to reducing the probability of developing a disorder or condition in a subject, who does not have, but is at risk of or susceptible to developing a disorder or condition.

By “reduces” is meant a negative alteration of at least 10%, 25%, 50%, 75%, or 100%.

By “reference” is meant a standard or controlled condition.

A "reference sequence" is a defined sequence used as a basis for sequence comparison. A reference sequence may be a subset of or the entirety of a specified sequence; for example, a segment of a full-length cDNA or gene sequence, or the complete cDNA or gene sequence. For polypeptides, the length of the reference polypeptide sequence will generally be at least about 16 amino acids, preferably at least about 20 amino acids, more preferably at least about 25 amino acids, and even more preferably about 35 amino acids, about 50 amino acids, or about 100 amino acids. For nucleic acids, the length of the reference nucleic acid sequence will generally be at least about 50 nucleotides, preferably at least about 60 nucleotides, more preferably at least about 75 nucleotides, and even more preferably about 100 nucleotides or about 300 nucleotides or any integer thereabout or therebetween.

By “RNF166 polypeptide” is meant a polypeptide or fragment thereof having at least 85% amino acid sequence identity to NCBI Accession Nos. NP_849163, NP_001165286, or NP_001165287 (various isoforms) and having a C2H2 zinc finger targeted by lenalidomide or a lenalidomide analog. An exemplary RNF166 polypeptide sequence provided at NCBI Accession No. NP_849163is provided below:

```
  1 mamfrslvas aqqrqppagp aggdsgleaq ytcpiclevy hrpvaigscg htfcgeclqp
 61 clqvpsplcp lcrlpfdpkk vdkathvekq lssykapcrg cnkkvtlakm rvhissclkv
121 qeqmancpkf vpvvptsqpi psnipnrstf acpycgarnl dqqelvkhcv eshrsdpnrv
181 vcpicsampw gdpsyksanf lqhllhrhkf sydtfvdysi deeaafqaal alslsen
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By “RNF166 polynucleotide” is meant a nucleic acid sequence encoding an RNF166 polypeptide. An exemplary polynucleotide sequence is provided at NCBI Accession No. NM_178841, which is reproduced below:

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  1 ctacgatgac gtcagcgcgg cgcagtagcg gctgtgacta gcgggccggc ccgggccagg
 61 acagcgggcg gcgggcggcg cgggcctggc cccgggatgg ctatgttccg cagcctggtg
121 gcctcggctc agcagcggca gccgccggcc gggccggcgg gcggcgacag cggcctggag
181 gcgcagtaca cctgccccat ctgcctggag gtctatcacc ggcccgtggc catcggcagc
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 241 tgcggccaca cgttctgcgg ggagtgtctc cagccctgcc tgcaggtgcc atccccgctg
 301 tgcccactct gccgcctgcc cttcgacccc aagaaggtgg acaaggccac ccacgtggag
 361 aagcagctct catcctacaa agcgccctgt cgaggctgca acaaaaaggt gaccctggca
 421 aagatgagag tgcacatttc gtcctgcctg aaggtccagg agcagatggc caactgcccc
 481 aagttcgtcc ccgtggtgcc cacatcacag cctatcccca gcaacatccc caacaggtcc
 541 accttcgcct gcccgtactg tggtgcccgc aacctggacc agcaggagct ggtgaagcac
 601 tgtgtggaaa gccaccgcag cgaccccaac cgcgtggtgt gccccatctg ctcggcaatg
 661 ccctgggggg accccagcta caagagcgcc aacttcctgc agcacctgct tcaccgacac
 721 aagttctcct acgacacctt tgtggactac agtattgacg aggaggccgc cttccaggct
 781 gctctggccc tgtctctctc tgagaactga agggaagcgc agccacccgc ctgcgtctgg
 841 ggtcagggat gtccccgctc ctgtgtcgca cctggcacct gctcgggagc gcacctcacc
 901 ggactgagct cacaggagga gcctgcaccc gcgcagaagg ggagccgggg ccgagcctcc
 961 gggcctgaat acgggccagc cgccgaggcc gccagagcag ggccgcctgg tcccaccggc
1021 gtcgctgggt tcttcggtgc ttctggccga gcaggcggcc tacttgggca gggctggacg
1081 ctgggacctg gagctgccgc cgtctcttca aagccatgat acccccctcgt gggaagaagg
1141 gaccgacgcg cgagtcgcgc tccgcagtcg agccgggagg aacccaggct gctgccctgc
1201 ccagcccgac cctgccccgg ccccgcttcc accttgcgca tttggtactg gcttttgtga
1261 tacttaggaa ccctggcatc ttttctatat tatccagtgt gataatcttt tcacgtttta
1321 tagagcaaag acagagcagt tactcttcat attgcaatat ctgtgtttga ctaggaataa
1381 tagtattttt atggaacatt tacaaaatta tatttttaa gaaaacaatc aaaacaagca
1441 ttgggggatt ggggcaagga tggaaggagc agtggggcag ctgccagagc tcaggcgagc
1501 catggggtct gctgtggggt ctgccctggc cacccactgt gtgtctgggt ccttgaggtt
1561 tgtacgtttc tctttgatga ccaggaagaa atcccagcac cccagccaca ggctgtggct
1621 gctcccagca gaggcggggc cggcagagaa ggggcctcct ccacccagag tcctggcctt
1681 ggcccgctgt caccttcaaa gctgactgtg ccccgctgcg ggaggggacg gcaccccagt
1741 ggtggcagag cttgggggcc tgggcagggg cccgcttggc gggccgggca acacgtcaac
1801 attcttttct gttcttggca ttaattattg ctgtcttttt tttaaaaaaa aaagtttaaa
1861 taaaatgtct cagagcatct ctaaaaaa
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By "specifically binds" is meant a compound or antibody that recognizes and binds a polypeptide of the invention, but which does not substantially recognize and bind other molecules in a sample, for example, a biological sample, which naturally includes a polypeptide of the invention.

Nucleic acid molecules useful in the methods of the invention include any nucleic acid molecule that encodes a polypeptide of the invention or a fragment thereof. Such nucleic acid molecules need not be 100% identical with an endogenous nucleic acid sequence, but will typically exhibit substantial identity. Polynucleotides having "substantial identity" to an endogenous sequence are typically capable of hybridizing with at least one strand of a double-stranded nucleic acid molecule. Nucleic acid molecules useful in the methods of the invention include any nucleic acid molecule that encodes a polypeptide of the

invention or a fragment thereof. Such nucleic acid molecules need not be 100% identical with an endogenous nucleic acid sequence, but will typically exhibit substantial identity. Polynucleotides having "substantial identity" to an endogenous sequence are typically capable of hybridizing with at least one strand of a double-stranded nucleic acid molecule. By "hybridize" is meant pair to form a double-stranded molecule between complementary polynucleotide sequences (e.g., a gene described herein), or portions thereof, under various conditions of stringency. (See, e.g., Wahl, G. M. and S. L. Berger (1987) Methods Enzymol. 152:399; Kimmel, A. R. (1987) Methods Enzymol. 152:507).

For example, stringent salt concentration will ordinarily be less than about 750 mM NaCl and 75 mM trisodium citrate, preferably less than about 500 mM NaCl and 50 mM trisodium citrate, and more preferably less than about 250 mM NaCl and 25 mM trisodium citrate. Low stringency hybridization can be obtained in the absence of organic solvent, e.g., formamide, while high stringency hybridization can be obtained in the presence of at least about 35% formamide, and more preferably at least about 50% formamide. Stringent temperature conditions will ordinarily include temperatures of at least about 30° C, more preferably of at least about 37° C, and most preferably of at least about 42° C. Varying additional parameters, such as hybridization time, the concentration of detergent, e.g., sodium dodecyl sulfate (SDS), and the inclusion or exclusion of carrier DNA, are well known to those of ordinary skill in the art. Various levels of stringency are accomplished by combining these various conditions as needed. In a preferred embodiment, hybridization will occur at 30° C in 750 mM NaCl, 75 mM trisodium citrate, and 1% SDS. In a more preferred embodiment, hybridization will occur at 37° C in 500 mM NaCl, 50 mM trisodium citrate, 1% SDS, 35% formamide, and 100 μg/ml denatured salmon sperm DNA (ssDNA). In a most preferred embodiment, hybridization will occur at 42° C in 250 mM NaCl, 25 mM trisodium citrate, 1% SDS, 50% formamide, and 200 μg/ml ssDNA. Useful variations on these conditions will be readily apparent to those of ordinary skill in the art..

For most applications, washing steps that follow hybridization will also vary in stringency. Wash stringency conditions can be defined by salt concentration and by temperature. As above, wash stringency can be increased by decreasing salt concentration or by increasing temperature. For example, stringent salt concentration for the wash steps will preferably be less than about 30 mM NaCl and 3 mM trisodium citrate, and most preferably less than about 15 mM NaCl and 1.5 mM trisodium citrate. Stringent temperature conditions for the wash steps will ordinarily include a temperature of at least about 25° C, more

preferably of at least about 42° C, and even more preferably of at least about 68° C. In a preferred embodiment, wash steps will occur at 25° C in 30 mM NaCl, 3 mM trisodium citrate, and 0.1% SDS. In a more preferred embodiment, wash steps will occur at 42 C in 15 mM NaCl, 1.5 mM trisodium citrate, and 0.1% SDS. In a more preferred embodiment, wash steps will occur at 68° C in 15 mM NaCl, 1.5 mM trisodium citrate, and 0.1% SDS. Additional variations on these conditions will be readily apparent to those of ordinary skill in the art. Hybridization techniques are well known to those of ordinary skill in the art. and are described, for example, in Benton and Davis (Science 196:180, 1977); Grunstein and Hogness (Proc. Natl. Acad. Sci., USA 72:3961, 1975); Ausubel et al. (Current Protocols in Molecular Biology, Wiley Interscience, New York, 2001); Berger and Kimmel (Guide to Molecular Cloning Techniques, 1987, Academic Press, New York); and Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, New York.

By "substantially identical" is meant a polypeptide or nucleic acid molecule exhibiting at least 50% identity to a reference amino acid sequence (for example, any one of the amino acid sequences described herein) or nucleic acid sequence (for example, any one of the nucleic acid sequences described herein). Preferably, such a sequence is at least 60%, more preferably 80% or 85%, and more preferably 90%, 95% or even 99% identical at the amino acid level or nucleic acid to the sequence used for comparison.

Sequence identity is typically measured using sequence analysis software (for example, Sequence Analysis Software Package of the Genetics Computer Group, University of Wisconsin Biotechnology Center, 1710 University Avenue, Madison, Wis. 53705, BLAST, BESTFIT, GAP, or PILEUP/PRETTYBOX programs). Such software matches identical or similar sequences by assigning degrees of homology to various substitutions, deletions, and/or other modifications. Conservative substitutions typically include substitutions within the following groups: glycine, alanine; valine, isoleucine, leucine; aspartic acid, glutamic acid, asparagine, glutamine; serine, threonine; lysine, arginine; and phenylalanine, tyrosine. In an exemplary approach to determining the degree of identity, a BLAST program may be used, with a probability score between e^{-3} and e^{-100} indicating a closely related sequence.

By "subject" is meant a mammal, including, but not limited to, a human or non-human mammal, such as a bovine, equine, canine, ovine, or feline.

Ranges provided herein are understood to be shorthand for all of the values within the range. For example, a range of 1 to 50 is understood to include any number, combination of

numbers, or sub-range from the group consisting 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50.

As used herein, the terms "treat," "treating," "treatment," and the like refer to reducing or ameliorating a disorder and/or symptoms associated therewith. It will be appreciated that, although not precluded, treating a disorder or condition does not require that the disorder, condition or symptoms associated therewith be completely eliminated.

By "ZFP91 polypeptide" is meant a polypeptide or fragment thereof having at least 85% amino acid sequence identity to NCBI Accession No. NP_444251 or NP_001183980 (various isoforms) and and having a C2H2 zinc finger targeted by lenalidomide or a lenalidomide analog. An exemplary ZFP91 polypeptide sequence provided at NCBI Accession No. NP_444251 is reproduced below:

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  1 MPGETEEPRP PEQQDQEGGE AAKAAPEEPQ QRPPEAVAAA PAGTTSSRVL RGGRDRGRAA
 61 AAAAAAAVSR RRKAEYPRRR RSSPSARPPD VPGQQPQAAK SPSPVQGKKS PRLLCIEKVT
121 TDKDPKEEKE EEDDSALPQE VSIAASRPSR GWRSSRTSVS RHRDTENTRS SRSKTGSLQL
181 ICKSEPNTDQ LDYDVGEEHQ SPGGISSEEE EEEEEEMLIS EEEIPFKDDP RDETYKPHLE
241 RETPKPRRKS GKVKEEKEKK EIKVEVEVEV KEEENEIRED EEPPRKRGRR RKDDKSPRLP
301 KRRKKPPIQY VRCEMEGCGT VLAHPRYLQH HIKYQHLLKK KYVCPHPSCG RLFRLQKQLL
361 RHAKHHTDQR DYICEYCARA FKSSHNLAVH RMIHTGEKPL QCEICGFTCR QKASLNWHMK
421 KHDADSFYQF SCNICGKKFE KKDSVVAHKA KSHPEVLIAE ALAANAGALI TSTDILGTNP
481 ESLTQPSDGQ GLPLLPEPLG NSTSGECLLL EAEGMSKSYC SGTERVSLMA DGKIFVGSGS
541 SGGTEGLVMN SDILGATTEV LIEDSDSAGP
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By "ZFP91 polynucleotide" is meant a nucleic acid sequence encoding an ZFP91 polypeptide. An exemplary polynucleotide sequence is provided at NCBI Accession No. NM_053023, which is reproduced below:

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  1 gtgggggggg cgccctcgga gccgggcgga ggggaggggg gaaagaggag cgcagggtga
 61 gagtgagccg caggcttcgg gaggcgaggg ggcgggggga gcagcgccga ggccgccgcc
121 tccgcctccg ccgcctagga ctaggggtg ggggacggac aagccccgat gccgggggag
181 acggaagagc cgagaccccc ggagcagcag gaccaggaag ggggagaggc ggccaaggcg
241 gctccggagg agccccaaca acggcccct gaggcggtcg cggcggcgcc tgcagggacc
301 actagcagcc gcgtgctgag gggaggtcgg gaccgaggcc gggccgctgc ggccgccgcc
361 gccgcagctg tgtcccgccg gaggaaggcc gagtatcccc gccggcggag gagcagcccc
421 agcgccaggc ctcccgacgt ccccgggcag cagccccagg ccgcgaagtc cccgtctcca
481 gttcagggca agaagagtcc gcgactccta tgcatagaaa aagtaacaac tgataaagat
541 cccaaggaag aaaaagagga agaagacgat tctgccctcc ctcaggaagt ttccattgct
601 gcatctagac ctagccgggg ctggcgtagt agtaggacat ctgtttctcg ccatcgtgat
661 acagagaaca cccgaagctc tcggtccaag accggttcat tgcagctcat ttgcaagtca
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 721 gaaccaaata cagaccaact tgattatgat gttggagaag agcatcagtc tccaggtggc
 781 attagtagtg aagaggaaga ggaggaggaa gaagagatgt taatcagtga agaggagata
 841 ccattcaaag atgatccaag agatgagacc tacaaacccc acttagaaag ggaaacccca
 901 aagccacgga gaaaatcagg gaaggtaaaa gaagagaagg agaagaagga aattaaagtg
 961 gaagtagagg tggaggtgaa agaagaggag aatgaaatta gagaggatga ggaacctcca
1021 aggaagagag gaagaagacg aaaagatgac aaaagtccac gtttacccaa aaggagaaaa
1081 aagcctccaa tccagtatgt ccgttgtgag atggaaggat gtggaactgt ccttgcccat
1141 cctcgctatt tgcagcacca cattaaatac cagcatttgc tgaagaagaa atatgtatgt
1201 ccccatccct cctgtggacg actcttcagg cttcagaagc aacttctgcg acatgccaaa
1261 catcatacag atcaaaggga ttatatctgt gaatattgtg ctcgggcctt caagagttcc
1321 cacaatctgg cagtgcaccg gatgattcac actggcgaga agccattaca atgtgagatc
1381 tgtggattta cttgtcgaca aaaggcatct cttaattggc acatgaagaa acatgatgca
1441 gactccttct accagttttc ttgcaatatc tgtggcaaaa aatttgagaa gaaggacagc
1501 gtagtggcac acaaggcaaa aagccaccct gaggtgctga ttgcagaagc tctggctgcc
1561 aatgcaggcg ccctcatcac cagcacagat atcttgggca ctaacccaga gtccctgacg
1621 cagccttcag atggtcaggg tcttcctctt cttcctgagc ccttgggaaa ctcaacctct
1681 ggagagtgcc tactgttaga agctgaaggg atgtcaaagt catactgcag tgggacggaa
1741 cgggtgagcc tgatggctga tgggaagatc tttgtgggaa gcggcagcag tggaggcact
1801 gaagggctgg ttatgaactc agatatactc ggtgctacca cagaggttct gattgaagat
1861 tcagactctg ccggacctta gtggacagga agacttgggg catgggacag ctcagacttt
1921 gtatttaaaa gttaaaaagg acaaaaaaaa aatctaaagc atttaaaatc tagtgaaata
1981 actgaagggc ctgctctttc cattgtggat cacagcacac acatacatac accctccacc
2041 tccccatccc ctgttctccc tctgttgctc cccttataaa attgatgttg tctttaccag
2101 aaaggtagac aaaaaagaag cagcagcagc tcttaaagtg agggttattc tcatactcgg
2161 ttccagccat cagcagactt cctgctcatc ggcagatccc cctttccaac ctgtaactct
2221 gatgtgctct ggatcagctt ttaactttta atcatatatt actgtcttct aaatcccttc
2281 tcctcctcta ctgctgccct atggttctgg ctcctacccc ctgcggcaca cttatcttca
2341 aataccatag aattctaatc tctggaggct ggcagcttga cttggcactt tagggccct
2401 tagcagggtg agctgttaaa acagcacaca tctctcatcc cctcttcctt tattcccccc
2461 tgggtttcag aaaggaagga tatatgggga ccacctcccc cttctttgat cccagcatct
2521 cagtcccccc cccaaccctc catatggctc tcaatggtgc tcacttgctt ggaagcaggc
2581 tcccaatagg gaggggggctg ccctctacag tctctttgac tgtaagacag ggctctgtat
2641 cagtgagacg atgagaaaag tcccaggcta atggcagaaa tttgcacttt gaacatgtgt
2701 gtttttgtgt tgtggaacct gagattcctt atttattaac aggaagtctg attttttttt
2761 tttggagtct ttgttgctat attttgtggg gctgggagag agagattaga ttattttgac
2821 atgggatccc ttccataaca ggtactttga aggcaagaca tagggttgaa gaagcacagc
2881 cagcctctga aatcatagct ctccagtggc ttttaaagaa agctggtcct cagcactaac
2941 aaaatcacta caatagccta gtgctttttt ggaagccttt ttagggaaga atgttaggtt
3001 catggtaact agtatgctct ttgagatttt tacagtgttg aaacttaaga attttgagag
3061 ggtgaggagg gttgttcaga atctaaatta cagatagatg attgtttctt gtgaatttgt
3121 ttcttttcct tttttttgt ccctaccatt tccttacatt tcccttgggg cccatctctg
3181 gctccttgct ttttgtttct tgctttgctt tatcagttca ttccagctcc ctgttagtga
3241 aggacactgc tgttagtgaa ggaacaaagt ctatgagtcc taaaatttta agtcaaagaa
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3301 aactgctctg tttccccttt agtaacactt ctgaagagga aaaacttcaa tagccaaagt
3361 taataatcct atataataat tgctttggct ttcacctaaa attctgggca tcacaatttc
3421 cttgggatag aggttgtgtt ggggaataga ttgcttattg ctgttcactg gagagaaaag
3481 gtagtgtttt tgtacaaggt cataccgcca gaagccccaa atcctatttt ggctcatctt
3541 caggtaaaga gtaattccta tcctgtgtgc ctcagaagct agaatcgaag gcttacccta
3601 ttcattgttt attgtcagaa atgcatgatg gctcttggaa agaatgacgt tttgctggaa
3661 aaaaaaaaaa gaacagtttg tgtttcacaa acatggctta tcaatttttt caaagaattc
3721 ttttttccca aaaagaggag taacaaaatg tcatttctga aagaggctta ctttatacca
3781 actagtgtca gcatttggga tgccagggaa cagagagtga gacacctaca atcaccagtc
3841 tcaaatgcgc tattgtttct tttcagagtg ttgcagattt gccatttctc cataatatgg
3901 ggatagaaaa tggaataaag atagaaggga tgtagaatat gctttcctgc caacatggtt
3961 tggagtcgac tttggtatat tgactagatt tgaaaataca agattgatta gatgaatcta
4021 caaaaaagtt gtcctcctct caggtccctt ttacactttt tgactaacta gcatctatat
4081 tccacactta gctttttttgt cacacttatc ctttgtctcc gtaaatttca tttgcagtgg
4141 ttagtcatca gatattttag ccacctacac aaaagcaaac tgcattttta aaaatctttc
4201 tgagatggga gaaaatgtat tctcctttcc tataccgctc tcccaacaaa aaaacaacta
4261 gttagttcta ctaattagaa acttgctgta ctttttcttt tcttttaggg gtcaaggacc
4321 ctctttatag ctaccatttg cctacaataa attattgcag cagtttgcaa tactaaaata
4381 ttttttatag actttatatt tttccttttg ataaagggat gctgcatagt agagttggtg
4441 taattaaact atctcagccg tttccctgct ttcccttctg ctccatatgc ctcattgtcc
4501 ttccagggag ctcttttaat cttaaagttc tacatttcat gctcttagtc aaattctgtt
4561 acctttttaa taactcttcc cactgcatat ttccatcttg aattggtggt tctaaattct
4621 gaaactgtag ttgagataca gctatttaat atttctggga gatgtgcatc cctcttcttt
4681 gtggttgccc aaggttgttt tgcgtaactg agactccttg atatgcttca gagaatttag
4741 gcaaacactg gccatggccg tgggagtact gggagtaaaa taaaaatatc gaggtataga
4801 ctagcatcca catagagcac ttgaacctcc tttgtacctg tttggggaaa aagtataatg
4861 agtgtactac caatctaact aagattatta tagtctggtt gtttgaaata ccatttttt
4921 ctccttttgt gtttttccca ctttccaatg tactcaagaa aattgaacaa atgtaatgga
4981 tcaatttaaa atattttatt tcttaaaagc ctttttgcc tgttgtaatg tgcaggaccc
5041 ttctcctttc atgggagaga caggtagtta cctgaatata ggttgaaaag gttatgtaaa
5101 aagaaattat aataaaaggg atactttgct tttcaaatct ttgttttctc ttattctagg
5161 taaggcatat taaaaataaa tatgtaaaga agaaaaataa aagttgtctt catggaagca
5221 acttgtcttc cttggttgta ctgagttaca gttatcctag gggtgaaaca tgtgatgctg
5281 ctaagcaaac caaatgccct cagaacaggt gttatgtggg gcatactatt gtttgctttt
5341 gttgagaatc aggtggttaa tttttgactg ttcttgattt ctaatgctga aatgacatga
5401 ttctgttatt cagcaaactt ggaaatcttg atgttttgac aactgcctcc taggaaaact
5461 ggccatatgt taattaacct agtagatgga aaattaagga ttatgtgagg ttaattttac
5521 cctgataatg acaaaacctt gatagcattt aatattaata cttcttctca aaattgaatg
5581 tttatatcaa gtactgattt ttattttaaa aaagaaaaaa ctataatcct tctgccttcc
5641 aaaagccatg ctgtgatagc tgcccaggct gctctgttac atctcccatt tattgtttac
5701 ttttataaat ttgcttctaa gatggaaaaa aaaaa
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By "ZNF692 polypeptide" is meant a polypeptide or fragment thereof having at least 85% amino acid sequence identity to NCBI Accession No. NP_001129508,

NP_060335, or NP_001180257 (various isoforms) and and having a C2H2 zinc finger targeted by lenalidomide or a lenalidomide analog. An exemplary ZNF692 polypeptide sequence provided at NCBI Accession No. NP_001129508 is reproduced below:

```
  1 mplvhmassp avdvscrrre krrqldarrs kcrirlgghm eqwcllkerl gfslhsqlak
 61 flldrytssg cvlcagpepl ppkglqylvl lshahsrecs lvpglrgpgg qdgglvwecs
121 aghtfswgps lsptpseapk paslphttrr swcseatsgq eladlesehd ertqearlpr
181 rvgpppetfp ppgeeegeee ednedeeeem lsdaslwtys sspddsepda prllpspvtc
241 tpkegetppa paalssplav palsasslss rapppaevrv qpqlsrtpqa aqqtealast
301 gsqaqsaptp awdedtaqig pkrirkaakr elmpcdfpgc grifsnrqyl nhhkkyqhih
361 qksfscpepa cgksfnfkkh lkehmklhsd trdyicefca rsfrtssnlv ihrrihtgek
421 plqceicgft crqkaslnwh qrkhaetvaa lrfpcefcgk rfekpdsvaa hrskshpall
481 lapqespsgp lepcpsisap gplgssegsr psaspqaptl lpqq
```

By "ZNF692 polynucleotide" is meant a nucleic acid sequence encoding an ZNF692 polypeptide. An exemplary polynucleotide sequence is provided at NCBI Accession No. NM_001136036, which is reproduced below:

```
   1 ggcgcacagg taaggccggg gtgggggtgg gtcgcgacgg gggctctggg cagcctggga
  61 actgccattg ggattagtcc gctccactca ctgtcagcat taagtggggg tgcccaagac
 121 ggggtggatg gggggcgccc tccagacctc tgaccacggc ctcaccgcca ctcgacccaa
 181 ctatgaagag cgccccccagc tgcacgccag gacacgacct ttccttcccc tagaaaccag
 241 taaaggccgc tgccctattc aagatgaaat gtgtggaccg cccccagccc agttgaaatt
 301 tcccgtgaaa gtctctcgcc ccttccccac agctccactt cagtggactg gagggcgcag
 361 gcctttgttc tgactgcttc tgtctgcctg cctcccaccc gacgacactc acatgcctct
 421 ggtgcacatg gcttcctccc cggcggtgga cgtgtcctgc aggcggcggg agaagcggcg
 481 gcagctggac gcgcgccgca gcaagtgccg catccgcctg ggcggccaca tggagcagtg
 541 gtgcctcctc aaggagcggc tgggcttctc cctgcactcg cagctcgcca agttcctgtt
 601 ggaccggtac acttcttcag gctgtgtcct ctgtgcaggt cctgagcctt tgcctccaaa
 661 aggtctgcag tatctggtgc tcttgtctca tgcccacagc cgagagtgca gcctggtgcc
 721 cgggcttcgg gggcctggcg gccaagatgg ggggcttgtg tgggagtgct cagcaggcca
 781 taccttctcc tggggaccct ctttgagccc tacaccttca gaggcaccca agccagcctc
 841 ccttccacat actactcgga gaagttggtg ttccgaggcc acgagtgggc aggagcttgc
 901 agatttggaa tctgagcatg atgagaggac tcaagaggcc aggttgccca ggagggtggg
 961 acccccacca gagaccttcc cacctccagg agaggaagag ggtgaggaag aagaggacaa
1021 tgatgaggat gaagaggaga tgctcagtga tgccagctta tggacctaca gctcctcccc
1081 agatgatagt gagcctgatg cccccagact actgccttcc cctgtcacct gcacacctaa
1141 agaggggggag acaccaccag cccctgcagc actctccagt cctcttgctg tgccggcctt
1201 gtcagcatcc tcattgagtt ccagagctcc tccacctgca gaagtcaggg tgcagccaca
1261 gctcagcagg acccctcaag cggcccagca gactgaggcc ctggccagca ctgggagtca
1321 ggcccagtct gctccaaccc cggcctggga tgaggacact gcacaaattg gccccaagag
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1381 aattaggaaa gctgccaaaa gagagctgat gccttgtgac ttccctggct gtggaaggat
1441 cttctccaac cggcagtatt tgaatcacca caaaaagtac cagcacatcc accagaagtc
1501 tttctcctgc ccagagccag cctgtgggaa gtctttcaac tttaagaaac acctgaagga
1561 gcacatgaag ctgcacagtg acacccggga ctacatctgt gagttctgcg cccggtcttt
1621 ccgcactagc agcaaccttg tcatccacag acgtatccac actggagaaa aacccctgca
1681 gtgtgagata tgcgggttta cctgccgcca gaaggcttcc ctgaactggc accagcgcaa
1741 gcatgcagag acggtggctg ccttgcgctt cccctgtgaa ttctgcggca agcgctttga
1801 gaagccagac agtgttgcag cccaccgtag caaaagtcac ccagccctgc ttctagcccc
1861 tcaagagtca cccagtggtc ccctagagcc ctgtcccagc atctctgccc ctgggcctct
1921 gggatccagc gaggggtcca ggccctctgc atctcctcag gctccaaccc tgcttcctca
1981 gcaatgagct ctcctccagc tttggctttg ggaagccaga ctccagggac tgaaaaggag
2041 caacaaggag agggtctgct tgagaaatgc cagatgcttg gtccccagga actaaggcga
2101 cagagtgcag ggtgggggca agactgggct gtaggggagc tggactactt tagtcttcct
2161 aaaggacaaa ataaacagta ttttatgcag gcaaaaaaaa aaaaaaaa
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Unless specifically stated or obvious from context, as used herein, the term "or" is understood to be inclusive. Unless specifically stated or obvious from context, as used herein, the terms "a", "an", and "the" are understood to be singular or plural.

Unless specifically stated or obvious from context, as used herein, the term “about” is understood as within a range of normal tolerance in the art, for example within 2 standard deviations of the mean. About can be understood as within 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, 0.5%, 0.1%, 0.05%, or 0.01% of the stated value. Unless otherwise clear from context, all numerical values provided herein are modified by the term about.

The recitation of a listing of chemical groups in any definition of a variable herein includes definitions of that variable as any single group or combination of listed groups. The recitation of an embodiment for a variable or aspect herein includes that embodiment as any single embodiment or in combination with any other embodiments or portions thereof.

Any compositions or methods provided herein can be combined with one or more of any of the other compositions and methods provided herein.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 depicts a schematic representation of the molecular structure of thalidomide and its derivatives.

FIG. 2depicts a schematic representation of the molecular mechanism of lenalidomide-mediated degradation. Lenalidomide binds to cereblon at its putative substrate recognition surface and in doing so, increases the affinity of cereblon for several key

substrates; the lymphocyte lineage transcription factors Ikaros (IKZF1) and Aiolos (IKZF3), and Casein Kinase 1 alpha (CSNK1a1). This increase in affinity subsequently results in the efficient CRL4-CRBN-dependent polyubiquitination of these target substrates, causing them to be rapidly degraded by the 26S proteasome. Degradation of Ikaros and Aiolos has been demonstrated to mediate the cell-autonomous effects in multiple myeloma as well as the increase in IL-2 secretion from T cells. Degradation of casein kinase 1 alpha appears to drive the therapeutic benefit observed in myelodysplastic syndrome.

FIG. 3 depicts a schematic diagram of the workflow described herein for identification of the degron sequence in Aiolos via screening of a comprehensive scanning mutagenesis library in a fluorescent reporter system.

FIG. 4 provides a set of plots depicting a representative analysis of flow cytometry data from the screen described herein. Upon 20 hours of lenalidomide treatment there is a clear reduction the level of GFP fluorescence in the wild-type (WT) control sample, however a single amino acid mutation (Q147H) in the mutant (MUT) control sample exhibits an attenuated response by comparison; this result highlights the ability of this fluorescence-based reporter system to distinguish functional, single amino acid changes that alter degradation. Of note, an increase in the GFP+ population upon treatment with lenalidomide is observed when comparing the degron library sample to the WT Control sample, likely indicating that constructs are present in the library in which the amino acid alterations have disrupted lenalidomide-mediated targeting by the CRL4-CRBN ubiquitin ligase.

FIGS. 5A-5B provide heat maps showing the results of the comprehensive scanning mutagenesis of Aiolos amino acids 130-189. FIGS. 5A-5B are gray scale versions of color figures. A copy of the original color heat map(s) are available upon request. The wild-type amino acid sequence of Aiolos is indicated on the x-axis, while each of the possible amino acid substitutions is indicated on the y-axis. Darker boxes indicate amino acids that depleted in the GFP negative fraction. Amino acids in the 146-168 region were generally depleted in the GFP negative fraction, particularly amino acids at positions 147, 148, 151, 152, 153, 155, 161, 164, and 168 (indicated by arrows). The screen clearly highlights in the case of all three compounds a series of residues that define the second zinc finger motif in Aiolos. The cysteines (C) at residues 148 and 151 and the histidines (H) at residues 164 and 168 are indicative of a C2H2 zinc finger motif, and their necessity here is likely driven by their role in maintaining the structure of the zinc finger via chelation of the zinc ion. The phenylalanine (F) at 155 and leucine (L) at 161 are also common within C2H2 zinc fingers

and their hydrophobic properties mediate proper folding of the tertiary structure. Most intriguing are the additional amino acids highlighted by the screen as being necessary for drug-induced targeting, including the glutamine (Q) at position 147, glycine (G) at position 152, and alanine (A) at position 153. The cysteines which are highlighted to the right of the zinc finger (i.e., the cysteines at positions 176 and 179) belong to the third zinc finger motif in Aiolos. The necessity of cysteines at positions 176 and 179 for targeting by lenalidomide or lenalidomide analogs is an artifact, as is the depletion of methionines (M) c-terminal to the second zinc finger highlighted by the screen.

FIG. 5B provides a set of schematics and a heat map showing results of a pooled saturation mutagenesis screen that established the second C2H2 zinc finger in Aiolos as the structural feature that is recognized by thalidomide, lenalidomide, and pomalidomide. At the top of FIG. 5B is a heat map depicting the lenalidomide/DMSO ratio of sequencing reads containing a given amino acid mutation (y-axis) at each position along the 60 amino acids included in the screen (x-axis). At the middle of FIG. 5B, an amino sequence of the second C2H2 zinc finger in Aiolos is provided. Single letter amino acid symbols depicted in medium gray (asterisk) and dark gray (black bar) indicate positions that were conserved in the saturation mutagenesis screen. Medium gray (asterisk) amino acids designate positions which are components of the C2H2 zinc finger motif and are found across the C2H2 family of zinc fingers. Dark gray (black bar amino acids are polymorphic sites in C2H2 zinc fingers. At the bottom of FIG. 5B, a PDB structure of a homologous zinc finger in Eos is provided (IZKF4, Q147H).

FIGS. 6A-6F provide a set of graphs and plots depicting that the second zinc finger within Aiolos is both necessary and sufficient for degradation by lenalidomide.

FIG. 6A provides a schematic depiction of a pooled saturation mutagenesis screen. At the top of FIG. 6A, a stick diagram of Aiolos (IKZF3) depicting the location of six C2H2 zinc finger domains as well as the region interrogated in the screen is shown.

FIG. 6B provides a schematic depiction of the linearized protein structure of Aiolos, which contains six C2H2 zinc fingers. The second zinc finger ("ZF2") comprising amino acids 146-168 was identified in the mutagenesis screen as the structural feature required for degradation by thalidomide, lenalidomide, and pomalidomide.

FIG. 6C provides a set of flow cytometry plots demonstrating that in Aiolos-GFP fusion constructs, zinc finger 2, specifically maintenance of its tertiary structure, is required for degradation.

FIG. 6D provides a flow cytometry plot depiciting lenalidomide-induced degradation of GFP which has been tagged with Aiolos zinc finger two (amino acids 146-168) via flexible linker.

FIG. 6E provides a plot showing normalized EGFP:mCherry ratios for Aiolos in the protein reporter vector (FIG. 6A, bottom) expressed via lentiviral transduction in HEK293T cells exposed to (A) DMSO control, (B) 1 μM thalidomide, (C) lenalidomide, and (D) pomalidomide.

FIG. 6F provides a flow cytometry histogram plot for Aiolos C2H2 zinc finger 2 (AA146-168) in the protein reporter vector (FIG. 6A, bottom) expressed via lentiviral transduction in HEK293T.

FIGS. 7A-7B provide a set of plots showing that three zinc finger proteins (RNF166, ZFP91, and ZNF692) exhibited significant decrease in abundance in the presence of lenalidomide or lenalidomide analogs, and that the zinc-finger containing regions of these proteins are targeted by lenalidomide and lenalidomide analogs.

FIG. 7A provides a replicate-by-replicate depiction of the log2 fold-changes in proteome abundance upon treatment with 1 uM lenalidomide in comparison to a DMSO-treated control. Arrows mark the zinc-finger containing proteins RNF166, ZFP91, and ZNF692.

FIG. 7B provides flow cytometry plots demonstrating that fusions of the zinc-finger-containing regions of RNF166, ZFP91, and ZNF692 to GFP are degraded with varying efficiencies by thalidomide, lenalidomide, and pomalidomide.

FIG. 8 provides a set of plots and diagrams showing that RNF166, ZNF692, and ZFP91 are C2H2 zinc finger-containing proteins which are degraded by thalidomide, lenalidomide, and pomalidomide in a cereblon and zinc finger-dependent fashion. FIG. 8 (top) provides plots showing normalized EGFP:mCherry ratios for RNF166, ZNF692, and ZFP91 in the protein reporter vector (FIG. 6A, bottom) which is over-expressed via lentiviral transduction in HEK293T cells exposed to (A) DMSO control, (B) 1 μM thalidomide, (C) lenalidomide, and (D) pomalidomide. . Bars corresponding to treatment groups A-D are consistent amongst all genetic background groups. Bar height is the average of three replicates, error bars represent 95% confidence intervals. At the bottom of FIG. 8, an alignment of the zinc finger degron sequences in Aiolos, Ikaros, RNF166, ZNF692, and ZFP91 is shown. Light gray (asterisk) and medium gray (black bar) bars indicate positions that were conserved in the saturation mutagenesis screen. Light gray (asterisk) amino acids

designate positions which are components of the C2H2 zinc finger motif and are found across the C2H2 family of zinc fingers. Medium gray (black bar) amino acids are polymorphic sites in C2H2 zinc fingers.

FIGS. 9A-9C provide plots and diagrams showing that a genome-scale CRISPR-Cas9 screen in lenalidomide-treated MM1S cells revealed genes whose loss conferred resistance to lenalidomide.

FIG. 9A provides a flow-chart of the screening method (top). "Len" refers to lenalidomide. The bottom of FIG. 9A provides a plot showing cell number throughout the duration of the 20 day assay (DMSO; 1 replicate, 1 uM Len; the average of three replicates).

FIG. 9B provides a plot showing the gRNA library ranked according to the Len/DMSO fold-change of the log2-transformed gRNA read count (average of 3 replicates). Light gray lines indicate 3 standard deviations above and below the mean.

FIG. 9C provides a diagram showing STARS algorithm output for the top 30 genes according to day 20 gRNA ranking.

FIGS. 10A-10B provide plots and diagrams showing that a Aiolos degradation reporter screen idenitified genes which are required for lenalidomide-induced degradation of Aiolos.

FIG. 10A provides a schematic of the reporter vector (top); features of the secondary library (middle); and a flow chart of the reporter screen (bottom).

FIG. 10B provides a diagram showing genes from the reporter screen ranked according to the average fold-change in the log-2 transformed gRNA sequencing read counts (Len-treated EGFP+/DMSO). "Len" refers to lenalidomide. Fold-change values are normalized to the average fold-change of 12 control gRNAs. Each point represents an individual gRNA, and each point is the average of three infection replicates. Light gray lines represent 2 standard deviations above and below the mean of the control gRNAs.

DETAILED DESCRIPTION OF THE INVENTION

The invention features methods that are useful for identifying proteins degraded in a CRL4-CRBN-dependent fashion by thalidomide, lenalidomide, and pomalidomide on the basis of their amino acid sequence.

The invention is based, at least in part, on the discovery of a degron sequence; an amino acid sequence within Aiolos (IKZF3) that mediates its association with thalidomide, lenalidomide, and pomalidomide in complex with cereblon, the substrate receptor for the

CRL4-CRBN E3 ubiquitin ligase. The discovery of the degron sequence in Aiolos (IKZF3) was achieved by means of a functional, comprehensive saturating mutagenesis screen of amino acids 130-189 in Aiolos (IKZF3). The amino acids identified fall within a zinc finger motif in Aiolos, suggesting that these compounds may target other transcription factors containing zinc finger motifs. Indeed, at least three other zinc-finger-containing proteins (RNF166, ZFP91, and ZNF692) have been preliminarily confirmed via multiple methods to be targets of these compounds. These findings indicate that the structural motif identified in the primary screen can be used to identify additional, potentially therapeutically relevant targets of these compounds.

It has recently been understood that this family of compounds derive their therapeutic properties from their unique ability to enforce degradation of several protein targets by the CRL4-CRBN E3 ubiquitin ligase. Specifically, these drugs are known to cause CRL4-CRBN-dependent ubiquitination and proteasomal degradation of the transcription factors Ikaros (IKZF1) and Aiolos (IKZF3), as well as Casein Kinase 1 Alpha (CSNK1a1). While the degradation of these targets explains the drugs' therapeutic efficacy in multiple myeloma and 5q- MDS, there are a number of cellular and clinical phenotypes elicited by thalidomide, lenalidomide, and pomalidomide which cannot yet be explained by the depletion of these proteins. Examples would include their sedative properties, teratogenicity, and anti-inflammatory effects.

Improved understanding of the mechanism through which these drugs function has provided knowledge necessary to design molecular technologies capable of identifying additional, potentially therapeutically relevant proteins which are degraded by thalidomide, lenalidomide, and pomalidomide. The identification of novel protein targets of these compounds could provide a molecular basis for the numerous cellular and clinical phenotypes which these drugs elicit, broaden the spectrum of disorders which may benefit from their use, and facilitate medicinal chemistry efforts to design more specific and potent compounds. The newly appreciated mechanism of action of thalidomide, lenalidomide, and pomalidomide has also provided a context allowing understanding and detection of resistance to these drugs in patients, particularly at an early stage of a disease, thereby facilitating expedient and rational choice of alternate therapies.

Lenalidomide- and Lenalidomide Analog-Dependent Mediation of Proteasomal Degradation

The drug thalidomide became infamous in the early 1960s when its use during the first trimester of pregnancy was linked to profound birth defects, most commonly a malformation of the upper limbs known as phocomelia. The discovery of thalidomide's teratogenic property was a major setback for the compound, however thalidomide was later repurposed and today is an FDA-approved therapy for a number of disorders, including erythema nodosum leparum, 5q- myelodysplastic syndrome (MDS), and the plasma cell malignancy multiple myeloma. Thalidomide's success as a treatment for these disorders motivated the synthesis of lenalidomide and pomalidomide, more potent derivatives which have largely replaced thalidomide in the treatment of 5q- MDS and multiple myeloma (FIG. 1).

Despite their clinical success, the mechanism behind the therapeutic benefit of thalidomide and its derivatives remained a mystery for over a decade. It is now understood that these drugs function by mediating efficient proteasomal degradation of several protein targets by the CRL4-CRBN E3 ubiquitin ligase. These targets include the lymphocyte lineage transcription factors Ikaros (IKZF1) and Aiolos (IKZF3), as well as the Wnt pathway regulator Casein Kinase 1 alpha (CSNK1a1). The CRL4-CRBN ubiquitin ligase belongs to the family of cullin-ring ligases and is a multi-subunit complex comprised of Ring Box Protein 1 (RBX1), DNA Damage Binding Protein 1 (DDB1), Cullin 4A (CUL4A), and Cereblon (CRBN). Thalidomide, lenalidomide, and pomalidomide bind specifically to cereblon, the substrate receptor for CRL4-CRBN. In doing so, these drugs increase Cereblon's affinity for Ikaros (IKZF1), Aiolos (IKZF3), and Casein Kinase 1 alpha (CSNK1a1). As a consequence of their increased association with the CRL4-CRBN ubiquitin ligase complex, these factors are efficiently ubiquitinated and degraded by the 26S proteasome (FIG. 2). Without wishing to be bound by theory, the degradation of Ikaros and Aiolos explains not only the tumoricidal effect on myeloma cells, but the increase in IL-2 secretion by T cells (Lu et al., 2014, Science 343, 305-309; Kronke et al., 2014, Science 343, 301-305; Ghandi et al., 2013, British Journal of Haematology, doi:10.1111/bjh. 12708). Similarly, the degradation of Casein Kinase 1 alpha mediates remission of the malignant stem cell clone in 5q- in myelodysplastic syndrome.

The present invention features methods that are useful for identifying proteins degraded in a CRL4-CRBN-dependent fashion by thalidomide, lenalidomide, and

pomalidomide on the basis of their amino acid sequence. In other aspects, the present invention features a method of depleting a polypeptide in a cell, the method comprising (a) detecting or fusing an IKFZ3 sequence to the polypeptide; and (b) contacting the cell with lenalidomide or a lenalidomide analog, degrading the target polypeptide in the cell. The methods of the present invention are based, at least in part, on the discovery of an amino acid sequence within Aiolos (IKZF3) that mediates its association with thalidomide, lenalidomide, and pomalidomide in complex with cereblon, the substrate receptor for the CRL4 CRBN E3 ubiquitin ligase. Thus, in some aspects, the present invention features methods capable of identifying or detecting a sequence substantially identical to this amino acid sequence in a polypeptide, wherein presence of the sequence indicates increased degradation of the polypeptide in a cell when the cell is contacted with lenalidomide or a lenalidomide analog.

Identification of Drug-induced Targets of Thalidomide, Lenalidomide, and Pomalidomide

The present invention features methods for identifying drug-modulated (in particular, lenalidomide- or lenalidomide analog-modulated) substrates of CRBN. The present invention also features methods for identifying polypeptide targets of thalidomide, lenalidomide, or pomalidomide. Proteomic methods, specifically mass spectrometry, have served as an effective approach to identify the drug-induced targets of thalidomide, lenalidomide, and pomalidomide. A caveat to this strategy, however, is that mass spectrometry can only detect changes in the levels of proteins which are expressed by the cell type being examined. Indeed, it is almost certain that all substrates whose protein levels are perturbed by this family of drugs have yet to be identified; the current list of targets fail to explain a number of these compounds' effects, most notably the sedative and anti-emetic properties for which thalidomide was originally marketed and the teratogenic effects which nearly eradicated these drugs from the armamentarium. An alternative strategy which has been used to discover ubiquitin ligase substrates in a cell-type independent manner is to take a structural approach and define the amino acid sequences responsible for targeting proteins to their cognate ubiquitin ligase (Nash et al., 2001, Nature 29,414(6863):514-21). In the study described herein, the consensus "degron" sequence which mediates binding of Aiolos (IKZF3) to the drug-cereblon complex was defined. It is planned that this consensus sequence will be used to examine the proteome for other possible drug-induced targets of the CRL4-CRBN ubiquitin ligase.

Described herein is a functional, comprehensive saturating mutagenesis screen which has revealed the amino acid sequence within Aiolos (IKZF3) that mediates its association with thalidomide, lenalidomide, and pomalidomide in complex with cereblon, the substrate receptor for the CRL4 CRBN E3 ubiquitin ligase. The amino acids identified fall within a zinc finger motif in Aiolos, suggesting the possibility that these compounds may target other transcription factors containing zinc finger motifs. Ikaros (IKZF1) contains a zinc finger motif identical to the motif identified in Aiolos (IKZF3). The implication of this work is therefore the potential to use the structural motif identified in the primary screen to identify additional, potentially therapeutically relevant targets of these compounds.

Lenalidomide and Lenalidomide Analog Therapies

Lenalidomide and lenalidomide analogs are effective therapies for a number of diseases or disorders, including 5q- myelodysplastic syndrome (MDS), erythema nodosum leparum, and several mature B-cell malignancies, most notably, the plasma cell malignancy multiple myeloma. Lenalidomide analogs approved for clinical use by the Food and Drug Administration (FDA) include thalidomide and pomalidomide. Lenalidomide is approved by the FDA for treatment of 5q- myelodysplastic syndrome (MDS), erythema nodosum leparum, and multiple myeloma. In some embodiments, lenalidomide and lenalidomide analogs are administered to a subject having 5q- myelodysplastic syndrome (MDS) or plasma cell malignancy multiple myeloma.

In some aspects, methods of the invention (which include prophylactic treatment) comprise administration of a therapeutically effective amount of lenalidomide or a lenalidomide analog, such as thalidomide or pomalidomide, to a subject (e.g., animal, human) in need thereof, including a mammal, particularly a human. Such treatment will be suitably administered to subjects, particularly humans, suffering from, having, susceptible to, or at risk for a disease, disorder, or symptom thereof. Determination of those subjects "at risk" can be made by any objective or subjective determination by a diagnostic test or opinion of a subject or health care provider (e.g., genetic test, enzyme or protein marker, family history, and the like). Lenalidomide or lenalidomide analogs may be also used in the treatment of any other disorders in which Ikaros (IKZF1), Aiolos (IKZF3), Casein Kinase 1 alpha (CSNK1a1), or other targets of lenalidomide may be implicated.

Characterizing and Monitoring Effectiveness of Lenalidomide and Lenalidomide Analog Therapies

Although thalidomide, lenalidomide, and pomalidomide are effective therapies for a number of disorders, most notably 5q- myelodysplastic syndrome and the plasma cell malignancy multiple myeloma, their effectiveness is hampered by development of resistance to these drugs. For example, lenalidomide is currently used in combination with dexamethasone as a front-line therapy for standard-risk multiple myeloma. While this combination offers distinct benefits with regard to disease-free and overall survival, the combination of dexamethasone and lenalidomide is not curative; on average disease progression develops 11 months after initiating treatment (Dimopoulos et al., 2007, N. Engl. J. Med., 357, 2123-2132; Weber et al., 2007, N. Engl. J. Med., 357, 2133-2142).

Without intending to be bound by theory, lenalidomide- or lenalidomide analog-induced association with cereblon (CRBN) and CRBN-mediated degradation of Ikaros (IKZF1) and Aiolos (IKZF3) are believed to confer the therapeutic effects of lenalidomide or lenalidomide analogs in disorders such as multiple myeloma. Thus, the identification of the amino acid sequence within Aiolos (IKZF3) that mediates its association with thalidomide, lenalidomide, and pomalidomide in complex with cereblon has potential clinical ramifications, as the mutation status of this region may serve as a biomarker capable of stratifying multiple myeloma patients with regard to their potential to respond to lenalidomide, and with regard to the choice of secondary therapies following relapse. Mutations in this region of Aiolos (IKZF3) may also be relevant biomarkers in the context of other malignancies treated with lenalidomide or lenalidomide analogs. In addition, the amino acid sequence identified in Aiolos (IKZF3) is within a zinc finger motif. Ikaros (IKZF1) contains a zinc finger motif identical to the motif identified in Aiolos (IKZF3). Without being bound by theory, it is believed that the amino acids in Ikaros' (IKZF1) zinc finger which correspond to those amino acids identified in Aiolos (IKZF3) are also responsible for mediating Ikaros' (IKZF1) association with cereblon (CRBN) and Cereblon-mediated degradation of Ikaros (IKZF1). Thus, mutations in the corresponding amino acids in IKZF1 may also serve as biomarkers of lenalidomide or lenalidomide analog resistance.

Accordingly, the present invention features methods of characterizing and/or monitoring the lenalidomide sensitivity of a subject comprising detecting the sequence of a region in an IKZF3 or IKZF1 polynucleotide relative to an IKZF3 or IKZF1 reference sequence. The methods include the step of detecting a sequence of a polypeptide or

polynucleotide of Aiolos (IKZF3) and/or Ikaros (IKZF1) in a biological sample from a subject suffering from or susceptible to a disorder or symptoms thereof associated with protein targets of lenalidomide, in which the subject has been administered a therapeutic amount of lenalidomide sufficient to treat the disease or symptoms thereof. The detection of a mutation in a polypeptide or polynucleotide of IKZF3 and/or IKZF1 is indicative of lenalidomide resistance and failure to detect a mutation is indicative of lenalidomide sensitivity.

The sequence of a polypeptide or polynucleotide of IKZF3 and/or IKZF1 detected in the method can be compared to a reference sequence. The reference sequence may be a known sequence of the gene in healthy normal controls. In some embodiments, a sequence of a polypeptide or polynucleotide of IKZF3 and/or IKZF1 in the subject is determined at a time point later than the initial determination of the sequence, and the sequences are compared to monitor the efficacy of the therapy. In other embodiments, a pre-treatment sequence of a polypeptide or polynucleotide of IKZF3 and/or IKZF1 in the subject is determined prior to beginning treatment according to this invention; this pre-treatment sequence of a polypeptide or polynucleotide of IKZF3 and/or IKZF1 can then be compared to the sequence of the polypeptide or polynucleotide of IKZF3 and/or IKZF1 in the subject after the treatment commences, to determine the efficacy of the treatment.

In some embodiments, thalidomide, lenalidomide, and pomalidomide are administered to a subject having a B cell neoplasia, such as multiple myeloma. Over time, many patients treated with lenalidomide acquire resistance to the therapeutic effects of lenalidomide. For example, lenalidomide is currently used in combination with dexamethasone as a front-line therapy for standard-risk multiple myeloma. While this combination offers distinct benefits with regards to disease-free and overall survival, the combination of dexamethasone and lenalidomide is not curative; on average disease progression develops 11 months after initiating treatment (Dimopoulos et al., 2007, N. Engl. J. Med., 357, 2123-2132; Weber et al., 2007, N. Engl. J. Med., 357, 2133-2142).

The early identification of lenalidomide resistance in a B cell neoplasia patient is important to patient survival because it allows for the selection of alternate therapies. Without wishing to be bound by theory, the anti-proliferative effect of lenalidomide in B cell neoplasias (in particular, multiple myeloma) is mediated by the combined depletion of Aiolos (IKZF3) and Ikaros (IKZF1). Accordingly, the invention provides methods for identifying the presence of lenalidomide resistant cells by detecting IKZF3 and/or IKZF1 polypeptides

that are resistant to lenalidomide-induced degradation. In one embodiment, a lenalidomide or lenalidomide analog resistant cell is identified by detection of a mutation in IKZF3 and/or IKZF1. Subjects identified as having a lenalidomide resistant B cell neoplasia are identified as in need of alternative treatment. Subjects identified as having a lenalidomide resistant myeloma, for example, are treated with Velcade, corticosteroids, or other anti-neoplastic therapy. For subjects identified as having lenalidomide resistant myelodysplastic syndrome are treated, for example, with azacitidine or decitabine.

In other embodiments, a lenalidomide or lenalidomide analog sensitivity in a subject is characterized by detecting a mutation in IKZF3 and/or IKZF1 polynucleotide or polypeptide sequence in a biological sample of the subject, such as a mutation in any one or more of amino acids 146-168. In particular embodiments, the invention provides for the detection of a mutation at amino acid 147, 148, 151, 152, 153, 155, 161, 164, or 168 in an IKZF3 polypeptide. These mutations are in a C2H2 zinc finger motif within Aiolos (IKZF3). Ikaros (IKZF1) contains an identical zinc finger. Thus, in other embodiments, the invention also provides for the detection of a mutation in Ikaros' (IKZF1) corresponding amino acids, which include amino acids at positions 146, 147, 150, 151,152, 163, or 167. Methods for detecting a mutation of the invention include immunoassay, direct sequencing, and probe hybridization to a polynucleotide encoding the mutant polypeptide. Exemplary methods of detecting a mutation of the invention are described in, for example, U.S. Patent Application Publication No. US2014/0127690, which is incorporated by reference herein in its entirety.

Methods of monitoring the sensitivity to lenalidomide or lenalidomide analog of a subject having a disease (e.g., a B cell neoplasia) are useful in managing subject treatment. Provided herein are methods where alterations in a polynucleotide or polypeptide of IKZF3 and/or IKZF1 (e.g., sequence, level, post-transcriptional modification, biological activity) are analyzed, such as before and again after subject management or treatment. In these cases, the methods are used to monitor the status of lenalidomide sensitivity (e.g., response to lenalidomide treatment, resistance to lenalidomide, amelioration of the disease, or progression of the disease).

For example, polypeptides or polynucleotides of IKZF3 and/or IKZF1 can be used to monitor a subject's response to certain treatments of a disease (e.g., B cell neoplasia). The level, biological activity, sequence, post-transcriptional modification, or sensitivity to lenalidomide induced degradation of a polypeptide or polynucleotide of IKZF3 and/or IKZF1 may be assayed before treatment, during treatment, or following the conclusion of a treatment

regimen. In some embodiments, multiple assays (e.g., 2, 3, 4, 5) are made at one or more of those times to assay resistance to lenalidomide.

Alterations in polynucleotides or polypeptides of IKZF3 and/or IKZF1 (e.g., sequence, level, post-transcriptional modification, biological activity) are detected in a biological sample obtained from a patient that has or has a propensity to develop a disease, such as B cell neoplasia. Such biological samples include, but are not limited to, peripheral blood, bone marrow, or lymphoid tissue obtained from the subject relative to the level of such biomarkers in a reference.

Combination Therapies

In some aspects, the present invention provides methods for detecting alterations in a polypeptide or polynucleotide of IKZF3 and/or IKZF1 in a biological sample (e.g., peripheral blood, bone marrow) derived from a subject having a B cell neoplasia to determine whether the B cell neoplasia is sensitive to treatment with lenalidomide or whether it has acquired lenalidomide resistance. Alterations in IKZF3 and/or IKZF1 are useful individually, or in combination with other markers typically used in characterizing a B cell neoplasia.

B-cell neoplasms typically recapitulate the normal stages of B-cell differentiation, and can be classified according to their putative cell of origin. Accordingly, alterations in IKZF1 and/or IKZF3 may be assayed alone or in combination with the neoplasm's cytogenetic profile, genotype, and immunophenotype. B cell markers useful in the methods of the invention include, but are not limited to, characterization of CD5, CD10, CD19, CD20, CD22, CD23, FMC7, CD79a, CD40, CD38, and CD138.

Kits

In one aspect, the invention provides kits for monitoring lenalidomide- or lenalidomide analog sensitivity, including the development of lenalidomide- or lenalidomide analog resistance. For example, the kits can be used to detect an alteration in a polypeptide or polynucleotide of IKZF3 and/or IKZF1 (e.g., sequence level, post-transcriptional modification, biological activity). If desired a kit includes any one or more of the following: capture molecules that bind a polynucleotide or polypeptide of IKZF3 and/or IKZF1. The capture molecules may be sequencing primers or hybridization probes for detecting the sequence of a polynucleotide of IKZF3 and/or IKZF1. The kits have many applications. For example, the kits can be used to determine if a subject has a lenalidomide sensitive disorder

(e.g., a lenalidomide sensitive multiple myeloma) or if the subject has developed resistance to lenalidomide.

The kits may include instructions for the assay, reagents, testing equipment (test tubes, reaction vessels, needles, syringes, etc.), standards for calibrating the assay, and/or equipment provided or used to conduct the assay. The instructions provided in a kit according to the invention may be directed to suitable operational parameters in the form of a label or a separate insert.

The practice of the present invention employs, unless otherwise indicated, conventional techniques of molecular biology (including recombinant techniques), microbiology, cell biology, biochemistry and immunology, which are well within the purview of the skilled artisan. Such techniques are explained fully in the literature, such as, "Molecular Cloning: A Laboratory Manual", second edition (Sambrook, 1989); "Oligonucleotide Synthesis" (Gait, 1984); "Animal Cell Culture" (Freshney, 1987); "Methods in Enzymology;" "Handbook of Experimental Immunology" (Weir, 1996); "Gene Transfer Vectors for Mammalian Cells" (Miller and Calos, 1987); "Current Protocols in Molecular Biology" (Ausubel, 1987); "PCR: The Polymerase Chain Reaction", (Mullis, 1994); "Current Protocols in Immunology" (Coligan, 1991). These techniques are applicable to the production of the polynucleotides and polypeptides of the invention, and, as such, may be considered in making and practicing the invention. Particularly useful techniques for particular embodiments will be discussed in the sections that follow.

The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the assay, screening, and therapeutic methods of the invention, and are not intended to limit the scope of what the inventors regard as their invention.

EXAMPLES

Example 1: Identification of amino acid sequence in Aiolos (IKZF3) that mediates targeting by thalidomide, lenalidomide, and pomalidomide.

Described herein is a study defining an amino acid sequence in Aiolos (IKZF3) which mediates binding of Aiolos (IKZF3) to the drug-cereblon complex. This sequence may be used to examine the proteome for other possible drug-induced targets of the CRL4-CRBN ubiquitin ligase.

In this study, a region within Aiolos (IKZF3) which mediates lenalidomide- or lenalidomide analog mediated binding of Aiolos to the CRL4-CRBN ubiquitin ligase was identified. The degron region within Aiolos had previously been narrowed down to amino acids 130-189, a stretch of 60 amino acids that is necessary and sufficient to confer lenalidomide-induced degradation by the CRL4-CRBN ubiquitin ligase (Kronke et al., 2014, Science, 343: 301-305). Traditional cloning methods, however, had failed to reduce this region further and specifically delineate which amino acids are functionally relevant for drug-induced binding to cereblon. As an alternative approach, an array-based synthesis of DNA oligos to generate a comprehensive scanning mutagenesis library of amino acids 130-189 in Aiolos was utilized (FIG. 3). In this mutagenesis library each construct contained approximately one amino acid mutation, and within the total library, each amino acid was mutated such that each of the other 19 amino acids were represented at that location (Melnikov et al., 2014, J. Vis. Exp.,doi: 10.3791/51719). The library, which contained approximately 1,200 constructs, was cloned in-frame with GFP in a lentiviral plasmid. This plasmid additionally contained an IRES.mCherry sequence as an internal control to distinguish fluctuations in GFP that were occurring at the transcriptional or post-translational level, as well as a puromycin resistance cassette to serve as a pharmacologic selection marker. Previous optimization had demonstrated that this fluorescence-based degron reporter system was capable of discriminating single amino acid alterations that disrupted the functionality of the degron, specifically a Q147H mutation.

When examining the flow cytometry data from the screen, it was apparent that approximately 25% of the constructs in the library contained amino acid substitutions that impaired degradation by each of the three compounds. Representative data and the gating strategies for sorting are shown in FIG. 4. A cancer expressing one of these degradation resistant forms of Aiolos would be resistant to treatment with thalidomide, pomalidomide or lenalidomide. Analysis of the sequencing data from all three compounds clearly highlighted a number of amino acid residues necessary for degradation in the second C2H2 zinc finger motif of Aiolos (FIGS. 5A-5B). Without intending to be bound by theory, strong conservation of the cysteines (C) at residues 148 and 151, and the histidines (H) at residues 164 and 168 likely reflects their role in maintaining the structure of the zinc finger fold via chelation of a zinc ion. The phenylalanine (F) at 155 and leucine (L) at 161 are also common within C2H2 zinc fingers and likely were conserved due to their hydrophobic properties, which are also required for proper folding of the tertiary structure. Perhaps most intriguing

then were the glutamine (Q) at position 147, as well as the glycine (G) and alanine (A) at positions 152 and 153, respectively; these residues are variable amongst C2H2 zinc fingers, and are candidates for being the amino acids which articulate with the drug-ubiquitin ligase complex. The cysteines at positions 176 and 179 belong to the adjacent C2H2 zinc finger motif which was truncated in the fragment which was screened, indicating that conservation of these residues is an artifact due to the fact that they cannot have formed a proper tertiary structure. Indeed, the cysteines at positions 176 and 179 were confirmed to be artifacts. The depletion of methionine C-terminal to the second C2H2 zinc finger motif is also suspected to represent an artifact, likely due to the fact that methionine may serve as an alternate start codon, facilitating "skipping" of the relevant sequence needed for degradation.

Following the screen described herein, several relevant avenues of questioning were pursued. First, the identification of the degron sequence within Aiolos (IKZF3) was validated by experimentally demonstrating that the second C2H2 zinc finger in Aiolos (IKZF3) (amino acids 146-169) was both necessary and sufficient to induce targeting by thalidomide, lenalidomide, and pomalidomide, as described further herein. Second, an active search of existing proteomic data for potential alternative protein targets of thalidomide, lenalidomide, and pomalidomide was performed. This examination preliminarily identified RNF166, ZNF692, and ZFP91 as candidates, as described further herein. If indeed these proteins are degraded in the presence of thalidomide, lenalidomide, or pomalidomide, the same comprehensive saturating mutagenesis screen will be performed to gain orthogonal information on what residues within the zinc fingers are relevant for drug induced targeting by the CRL4-CRBN ubiquitin ligase

An implication of this work is to use a greater understanding of the consensus degron sequence or structural motif targeted by thalidomide, lenalidomide, and pomalidomide to either computationally or functionally search the proteome for novel targets of these compounds. Without intending to be bound by theory, novel targets may explain side effects of these compounds, the neurologic phenotype elicited by thalidomide, the teratogenicity of the drugs, or perhaps most desirably, the discovery of novel targets may warrant the clinical use of thalidomide, lenalidomide, and/or pomalidomide in other disorders.

Example 2: Identification of amino acid sequence in Aiolos (IKZF3) that is necessary and sufficient to mediate degradation by lenalidomide

As described herein, a structural motif within the transcription factor Aiolos (IKZF3) that mediates its targeting by the CRL4-CRBN E3 ubiquitin ligase in complex with thalidomide, lenalidomide, and pomalidomide was identified in a screen. Specifically, the screen revealed that the drug-ubiquitin ligase complex recognizes the second, C2H2 zinc finger within Aiolos (IKZF3), with critical amino acids being those which mediate the tertiary structure of the zinc finger, as well as residues 146, 151, and 152, which are polymorphic between individual zinc fingers.

The results from the screen were confirmed by demonstrating that the second zinc finger within Aiolos is both necessary and sufficient for degradation by lenalidomide or lenalidomide analogs (FIGS. 6A-6F). Indeed, both deletion of the second zinc finger region or ablation of its zinc finger fold by mutating a key cysteine residue abrogated targeting of a GFP-tagged Aiolos by all three compounds (necessity) (FIG. 6B). Additionally, attaching zinc finger 2 (amino acids 146-168) to GFP via a flexible linker conferred lenalidomide-induced degradation of GFP (sufficiency) (FIG. 6C).

Example 3: Alternative targets of lenalidomide or lenalidomide analogs for CRL4-CRBN mediated ubiquitination and degradation

With the knowledge that these compounds are capable of directing CRL4-CRBN mediated ubiquitination and degradation of proteins containing zinc finger motifs, two proteomic datasets derived from treatment of the cell lines MM1S (multiple myeloma) and KG1 (Acute Myeloid Leukemia) with thalidomide and lenalidomide were more closely examined (Krönke et al., Science 343, 301–305 (2014); Krönke et al., Nature 523, 183–188 (2015)). Indeed, there were three zinc finger proteins which exhibited a significant decrease in abundance in the presence of drug: RNF166, ZFP91, and ZNF692(FIG. 7A). Preliminary data shown herein confirmed that the zinc-finger containing regions of these proteins are targeted by thalidomide, lenalidomide, and pomalidomide for degradation at the protein level (FIG. 7B; FIG. 8).

Signaling through the NFKB pathway has been noted to be impaired in the presence of thalidomide, lenalidomide, and pomalidomide. However, this effect has yet to be explained by a molecular target. ZFP91 is therefore of interest because it is a critical member of the non-canonical NFKB signaling pathway, with existing evidence that a reduction of its

protein levels is capable of impairing non-canonical NFKB signaling (Jin et al., Journal of Biological Chemistry 285, 30539–30547 (2010); Jin et al., Biochem. Biophys. Res. Commun. 400, 581–586 (2010)). The hypothesis that degradation of ZFP91 by these compounds explains the ability of these drugs to inhibit NFKB signaling will be pursued. Without intending to be bound by theory, this property may also mechanistically illuminate additional, unexplained cellular and clinical phenotypes such as the inhibition of TNFa secretion by monocytes, anti-angiogenesis, anti-inflammatory properties, and tumoricidal effects of these drugs in multiple myeloma and acute myeloid leukemia.

Example 4: Results of Screen for Genes that Mediate Resistance to Lenalidomide in Multiple Myeloma

In an effort to discover genes whose loss confers resistance to lenalidomide, a pooled, genome-wide CRISPR-Cas9 screen in the lenalidomide-sensitive myeloma cell line, MM1S, was performed. Loss of cereblon has been noted to promote resistance to lenalidomide in cell line models (Zhu et al., 2011, Blood 118, 4771-4779; Lopez-Girona et al., 2012, Leukemia 26, 2326-2335). Therefore, parameters for the screen, including dose and endpoints, were optimized using cereblon gRNAs as a positive control.

In this study, a set of genes whose loss conferred resistance to lenalidomide was identified from a genome-wide screen performed in a lenalidomide-sensitive myeloma cell line. The screen was carried out as follows: on day 8, Cas9-expressing MM1S cells were infected at an efficiency of 46% with the second-generation "GEKO" gRNA library designed by the Zhang lab and Genetic Perturbations Platform at the Broad Institute; this library contains approximately 120,000 gRNAs targeting 18,000 genes (~6 gRNA/gene) (Sanjana et al., 2014, Nature Methods 11, 783-784). On day 0, a baseline control sample of 120 million cells was taken and the remaining infected cells began treatment with either DMSO (1x 60 million cells) or 1 μM lenalidomide (2 sets of 3x 120 million cells). The number of cells per replicate in the DMSO and 1 μM lenalidomide treatment groups ensured an estimated representation of each gRNA in 500 and 1000 cells, respectively. Endpoint samples were collected on days 12 (D12) and 20 (D20) (FIG. 9A). Genomic DNA was isolated from each of the collected samples and relative gRNA abundance was determined via barcoded PCR amplification of the genomic gRNA insert and pooled sequencing of the resultant amplicons across four lanes of the Illumina HiSeq. Read counts were normalized and log2 transformed, and the D12 and D20 replicates were averaged. The fold-change in gRNA abundance upon

selection with lenalidomide was calculated by comparing the relative abundance of a given gRNA in the lenalidomide-treated experimental condition to its relative abundance in the corresponding DMSO control (FIGS. 9A-9B). A plot showing the gRNA library ranked according to the Len/DMSO fold-change of the log2-transformed gRNA read count (average of 3 replicates) is shown in FIG. 9B.

An examination of the gRNA rankings at D20 revealed that all six of the gRNAs targeting cereblon (CRBN) to be amongst the top 7 and top 6 gRNAs, respectively, confirming the screen optimization procedures (FIG. 9C; Table 1). To discover additional genes whose loss confers resistance to lenalidomide, the STARS algorithm (Genetic Perturbations Platform) was used to collapse gRNA rankings by gene and assign p, FDR (false discovery rate), and q values, as well as a composite STARS score. In comparison to D12, the D20 data yielded hits with much higher confidence, with the top 30 genes possessing FDR values below 0.05. In keeping with the mechanism of lenalidomide, cereblon was ranked first, and of the top 30 genes, 18 are regulators of cullin-ring ligases and/or participants in the ubiquitin-proteasome pathway. Most notably, all 9 members of the COP9 signalosome complex in scored with FDRs less than 0.05 (GPS1 [12], COPS2 [2], COPS3 [27], COPS4 [10], COPS5 [30], COPS6 [9], COPS7A [14], COPS7B [3], COPS8 [6]). Additional genetic modules that emerged as themes in the D20 STARS ranking of genes are CRL4-CRBN complex members (CRBN [1], DDB1 [17], CUL4B [52]), NFKB pathway (TRAF2 [5], NFKBIA [32]), members of the 5' mRNA decapping complex (EDC4 [7], XRN1 [19], DCP2 [36]), nuclear hormone receptor signaling (NCOR1 [15], RARA [25]), and tumor suppressors which have recently been noted to be relevant in melanoma (PPP6C [26], SPOP [28]). Novel components of the CRL4-CRBN E3 ubiquitin ligase pathway identified in the screen included two E2 enzymes, UBE2G1 and UBE2D3.

Table 1: Genes whose loss conferred resistance to lenalidomide

CRBN
COPS2
COPS7B
CAND1
TRAF2
COPS8
EDC4
PLAA

COPS6
UBE2G1
GPS1
UBE2D3
COPS7A
NCOR1
DEPDC5
DDB1
SRP14
XRN1
EIF4A1
SNRNP25
UBE2M
GLMN
OTUB1
RARA
PPP6C
COPS3
SPOP
SYCP2L
COPS5
RBX1
CUL4A
CUL4B

A focused, pooled viral gRNA library was made containing an orthogonal set of gRNAs targeting the top 30 hits from the screen as well as NFKBIA [32], DCP2 [36], CUL4B [52], and the CRL4-CRBN complex members which did not score in the screen, CUL4A and RBX1. The focused library was designed using an on-target prediction algorithm and specifically contains three gRNAs per gene, each targeting a different exon in the first 50% of the protein (Doench et al., 2014, Nat. Biotechnol. doi:10.1038/nbt.3026). In the same manner as the original screen, this library was used to validate the hits in Cas9-expressing MM1S cells as well as three other lenalidomide-sensitive myeloma cell lines: OPM2, U266, and NCIH929. To determine which of the hits prevent degradation of the Aiolos transcription factor the same focused viral library was screened in an MM1S, NCIH929, and HEK293 T reporter cell lines expressing Aiolos tagged to GFP; flow cytometry-based sorting of GFP high and low cells following a 20 hour incubation with lenalidomide was used to

isolate cells carrying gRNAs that did or did not impair Aiolos degradation. Subsequently, gDNA isolation, PCR amplification of the gRNA insert, and Illumina-based sequencing were used as a readout. Results of the screen of this library are shown in FIGS. 10A-10B.

Other Embodiments

From the foregoing description, it will be apparent that variations and modifications may be made to the invention described herein to adopt it to various usages and conditions. Such embodiments are also within the scope of the following claims.

The recitation of a listing of elements in any definition of a variable herein includes definitions of that variable as any single element or combination (or subcombination) of listed elements. The recitation of an embodiment herein includes that embodiment as any single embodiment or in combination with any other embodiments or portions thereof.

All patents and publications mentioned in this specification are herein incorporated by reference to the same extent as if each independent patent and publication was specifically and individually indicated to be incorporated by reference.

What is claimed is:

1. A method of identifying a cell resistant to a modulator of CRBN, the method comprising detecting the sequence of a region in a IKZF3 polynucleotide relative to a IKZF3 reference sequence, wherein said region encodes amino acids 146-168 of a IKZF3 polypeptide in said cell, and wherein detection of a mutation in said region indicates the cell is resistant to a modulator of CRBN.

2. A method of characterizing the sensitivity of a subject to a modulator of CRBN, the method comprising detecting the sequence of a region in an IKZF3 polynucleotide in a biological sample obtained from the subject relative to a IKZF3 reference sequence, wherein said region encodes amino acids 146-168 of a IKZF3 polypeptide, and wherein detection of a mutation in said region is indicative of resistance to a modulator of CRBN and failure to detect a mutation is indicative of sensitivity to a modulator of CRBN.

3. A method of monitoring sensitivity of a subject to a modulator of CRBN, the method comprising detecting the sequence of a region in an IKZF3 polynucleotide in a biological sample obtained from the subject relative to a IKZF3 reference sequence, wherein said region encodes amino acids 146-168 of a IKZF3 polypeptide, and wherein detection of a mutation in said region is indicative of resistance to a modulator of CRBN and failure to detect a mutation is indicative of sensitivity to a modulator of CRBN.

4. A method of monitoring sensitivity of a subject to a modulator of CRBN, the method comprising

(a) administering to the subject an amount of a modulator of CRBN; and

(b) detecting the sequence of a region in an IKZF3 polynucleotide in a biological sample obtained from the subject relative to a IKZF3 reference sequence, wherein said region encodes amino acids 146-168 of a IKZF3 polypeptide, and wherein detection of a mutation in said region is indicative of resistance to a modulator of CRBN and failure to detect a mutation is indicative of sensitivity to a modulator of CRBN.

5. A method of selecting a subject for treatment with an alternative to a modulator of CRBN, the method comprising detecting the sequence of a region in an IKZF3

polynucleotide in a biological sample obtained from the subject relative to a IKZF3 reference sequence, wherein said region encodes amino acids 146-168 of a IKZF3 polypeptide, wherein a subject having a mutation in said region is selected for treatment with an alternative to a modulator of CRBN.

6. The method of any one of claims 1-5, wherein the mutation is at amino acid position 147, 148, 151, 152, 153, 155, 161, 164, or 168.

7. The method of any one of claims 1-6, wherein the modulator of CRBN is lenalidomide, thalidomide, or pomalidomide.

8. The method of any one of claims 1-7, wherein the sequence of said region in the IKZF3 polynucleotide is detected by sequencing or probe hybridization.

9. The method of any one of claims 2-8, wherein the subject has a B cell neoplasia or related condition.

10. The method of claim 9, wherein the B cell neoplasia or related condition is a plasma cell malignancy multiple myeloma or a myelodysplastic syndrome.

11. The method of any one of claims 2-10, wherein the biological sample is blood.

12. A kit comprising a reagent detecting the sequence of a polynucleotide encoding amino acids 146-168 of an IKZF3 polypeptide.

13. The kit of claim 12, wherein the reagent is a sequencing primer or hybridization probe.

14. A method of identifying increased degradation of a polypeptide in a cell when the cell is contacted with a modulator of CRBN, the method comprising detecting in a polypeptide a sequence substantially identical to a IKZF3 zinc finger comprising amino acids 146-168 of IKZF3, wherein presence of said sequence indicates increased degradation of said polypeptide when the cell is contacted with a modulator of CRBN.

15. A method of identifying a drug-modulated polypeptide substrate of CRBN, the method comprising detecting a sequence substantially identical to a IKZF3 zinc finger comprising amino acids 146-168 of IKZF3 in a candidate polypeptide, wherein presence of said sequence indicates said candidate polypeptide is a drug-modulated polypeptide substrate of CRBN.

16. A method of identifying a polypeptide target of a modulator of CRBN, the method comprising detecting a sequence substantially identical to a IKZF3 zinc finger comprising amino acids 146-168 of IKZF3 in a candidate polypeptide, wherein presence of said sequence indicates the candidate polypeptide is a polypeptide target of a modulator of CRBN.

17. A method of depleting a polypeptide in a cell, the method comprising contacting the cell with a modulator of CRBN, wherein the polypeptide is identified as having a sequence substantially identical to a IKZF3 zinc finger comprising amino acids 146-168 of IKZF3 in said polypeptide, thereby depleting the polypeptide in the cell.

18. A method of depleting a polypeptide in a cell, the method comprising (a) fusing to the polypeptide a second polypeptide comprising a sequence substantially identical to a IKZF3 zinc finger comprising amino acids 146-168 of IKZF3; and (b) contacting the cell with a modulator of CRBN, thereby depleting the polypeptide in the cell.

19. A method of identifying a drug-modulated polypeptide substrate of CRBN, the method comprising detecting a sequence substantially identical to a sequence selected from the group consisting of: amino acids 146-168 of IKZF3, amino acids 149-172 of RNF166, amino acids 417-439 of ZNF692, and amino acids 400-422 of ZFP91, wherein presence of said sequence indicates said candidate polypeptide is a drug-modulated polypeptide substrate of CRBN.

20. A method of identifying a drug-modulated polypeptide substrate of CRBN, the method comprising detecting a sequence substantially identical to a sequence selected from the group consisting of the sequences:

FQCNQCGASFTQKGNLLRHIKLH;

FACPYCGARNLDQQELVKHCVESH;

LQCEICGFTCRQKASLNWHQRKH; and

LQCEICGFTCRQKASLNWHMKKH;

wherein presence of said sequence indicates said candidate polypeptide is a drug-modulated polypeptide substrate of CRBN.

21. The method of any one of claims 14-20, wherein said sequence comprises a C2H2 zinc finger sequence.

22. The method of claim 21, wherein positions in said C2H2 zinc finger sequence corresponding to amino acids 147, 152, and 153 in said IKZF3 zinc finger comprise Gln, Gly, or Ala.

23. The method of any one of claims 14-17 or 19-20, wherein the polypeptide is IKZF3, IKZF1, CSNK1a1, RNF166, ZNF692, or ZFP91.

24. The method of claim 14, wherein the increased degradation is mediated by CRBN.

25. The method of any one of claims 15 or 19-20, wherein the drug is lenalidomide, thalidomide, or pomalidomide.

26. The method of any one of claims 15-16, wherein the polypeptide substrate or polypeptide target is degraded by CRBN-mediated degradation in a cell when the cell is contacted with the modulator of CRBN.

27. The method of any one of claims 17-18, wherein the polypeptide is depleted by CRBN-mediated degradation of the polypeptide.

28. The method of any one of claims 14-27, wherein the modulator of CRBN is lenalidomide, thalidomide, or pomalidomide.

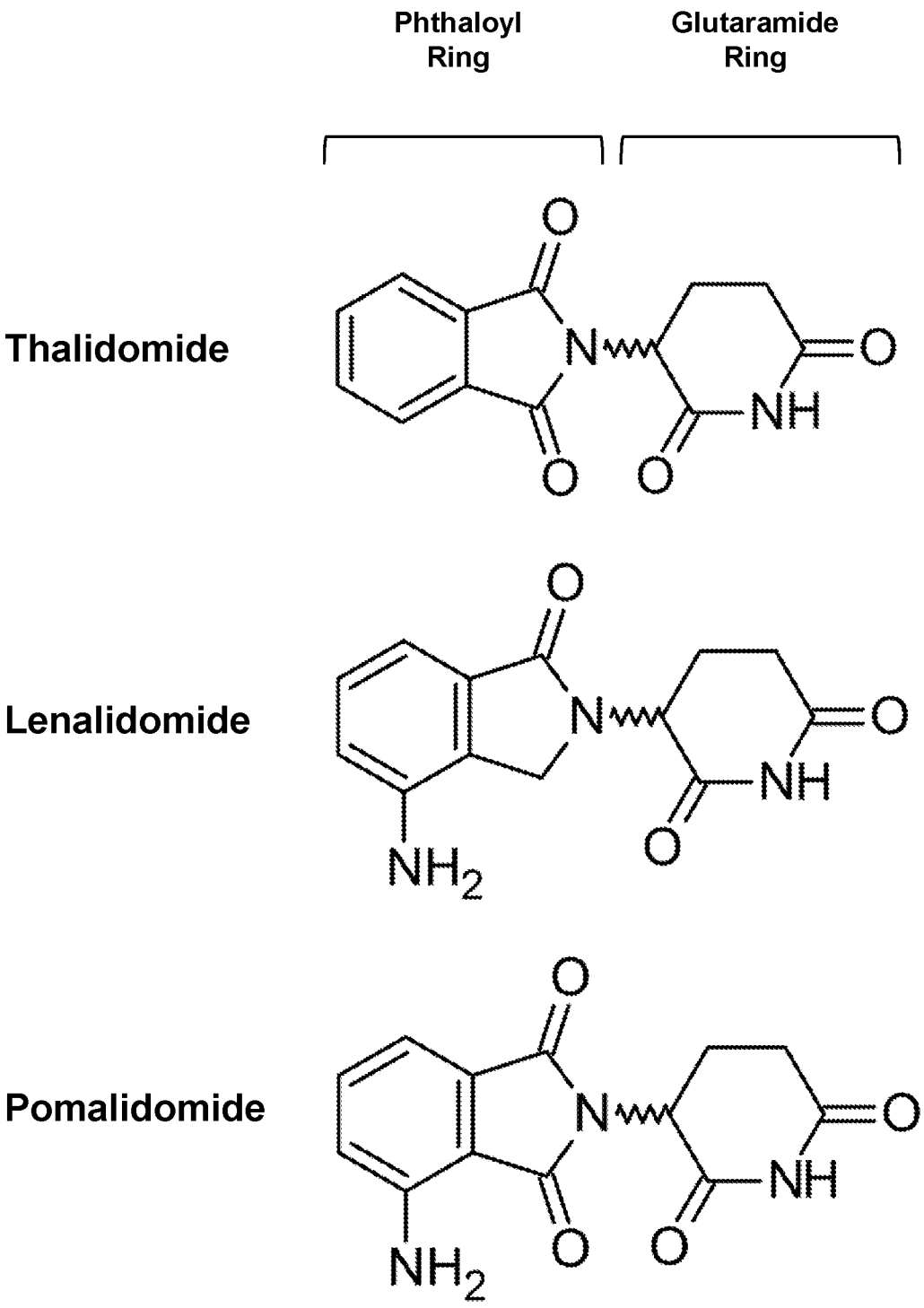

Phthaloyl Ring
Glutaramide Ring
Thalidomide
Lenalidomide
Pomalidomide
O
N
NH
NH2

FIG. 1

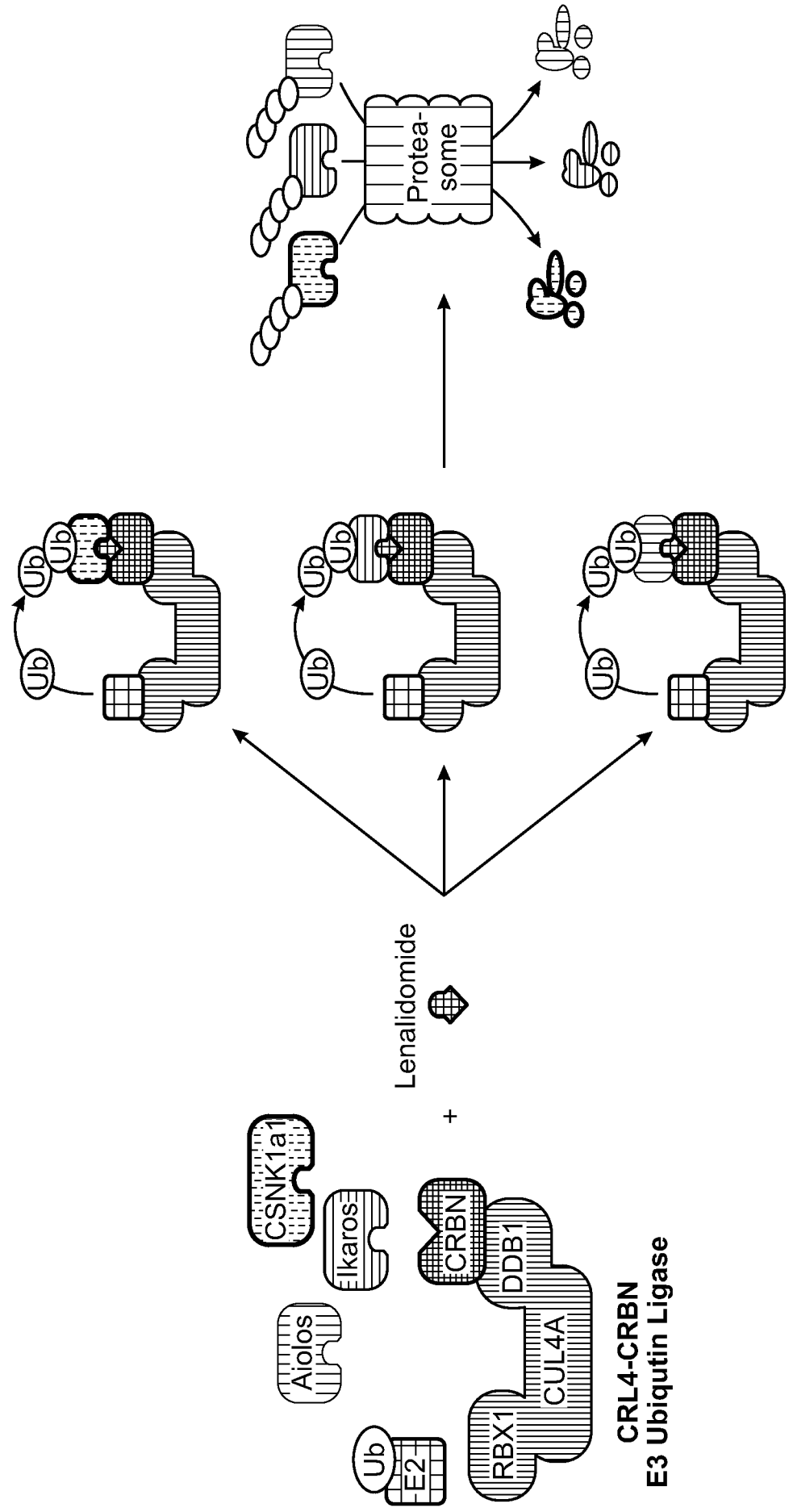

Protea-
some
Ub
Lenalidomide
+
CSNK1a1
Ikaros
Aiolos
CRBN
DDB1
CUL4A
RBX1
Ub
E2
CRL4-CRBN
E3 Ubiqutin Ligase

FIG. 2

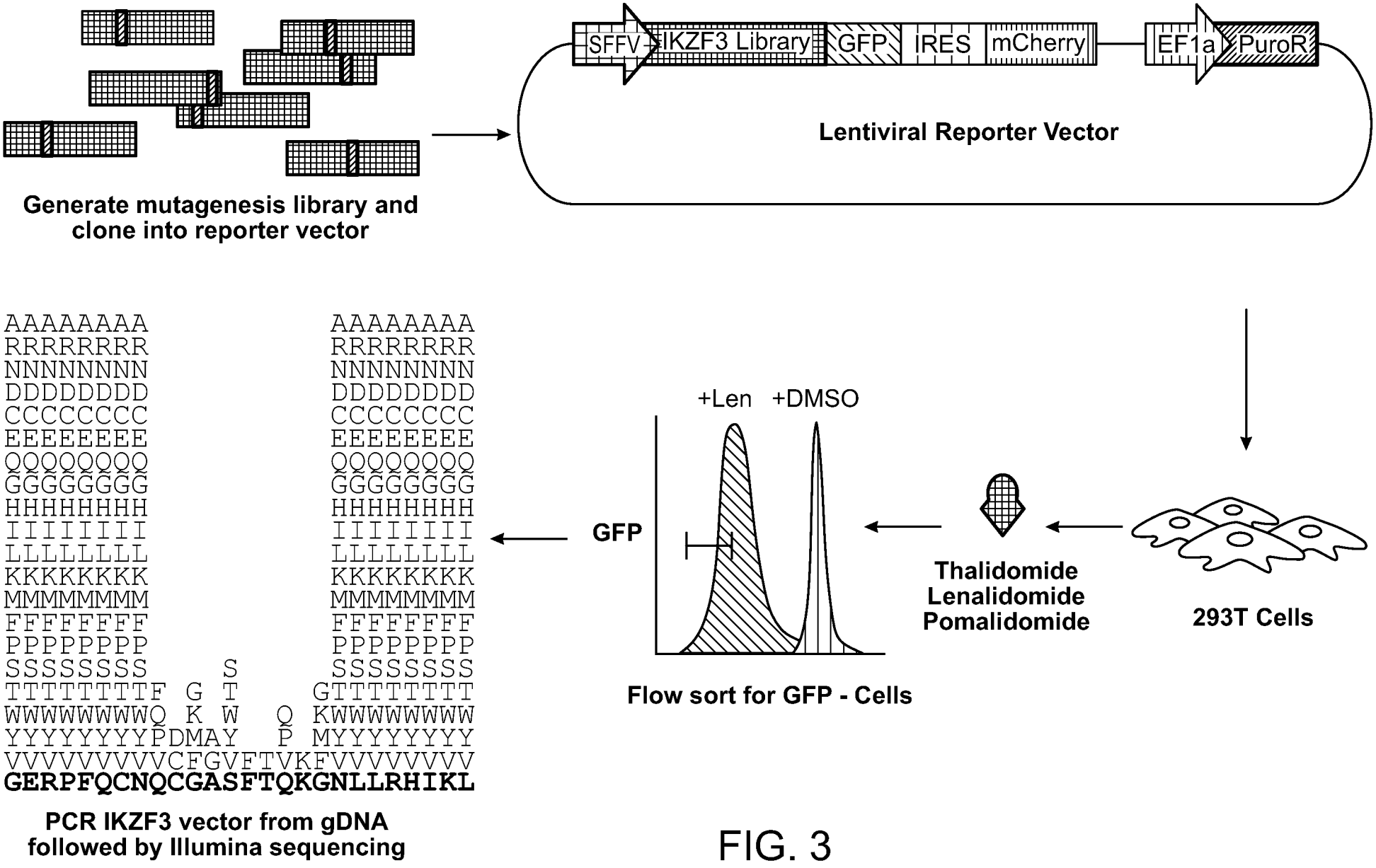


FIG. 3

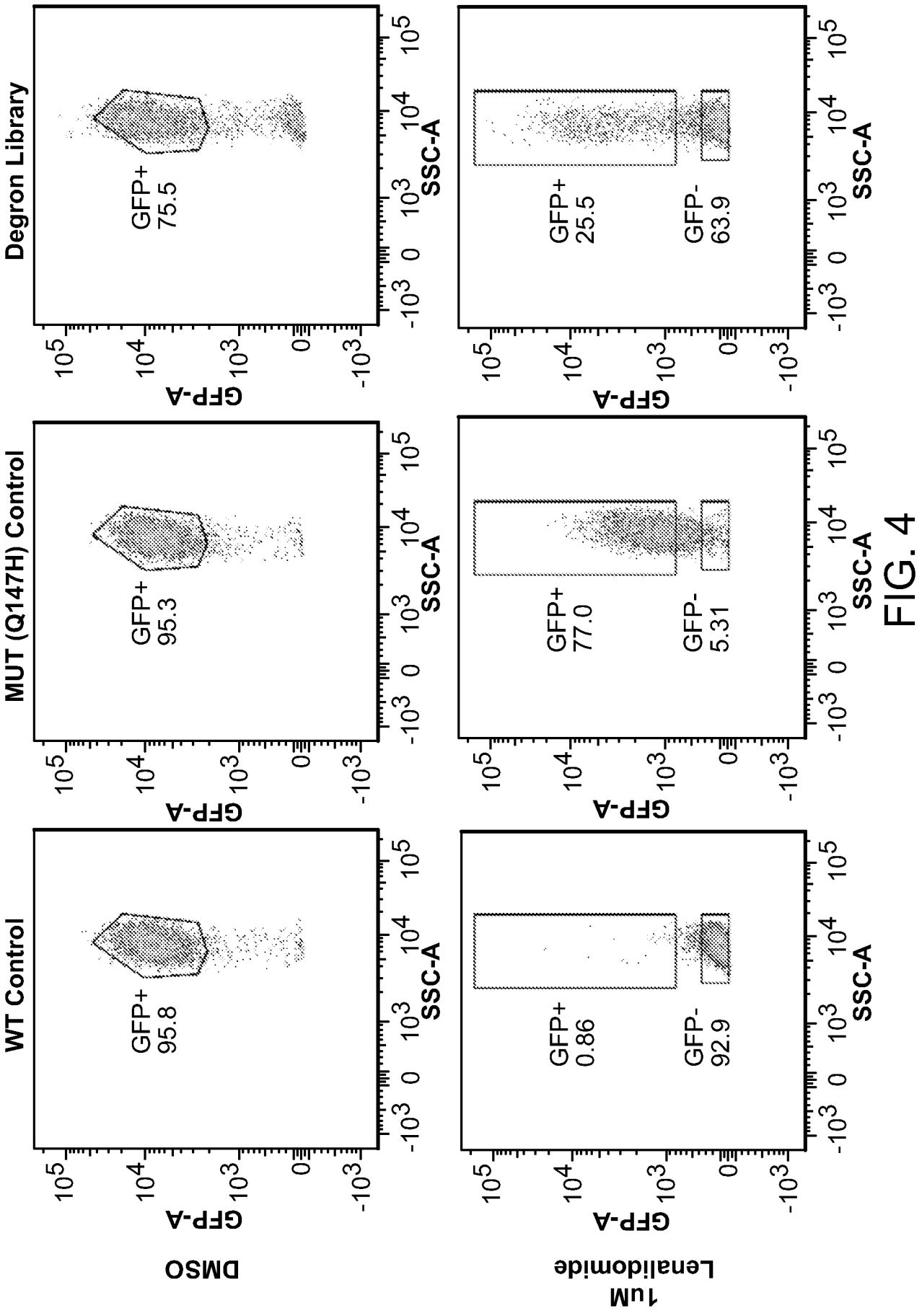

WT Control
MUT (Q147H) Control
Degron Library
DMSO
1uM Lenalidomide
GFP-A
SSC-A
GFP+ 95.8
GFP- 92.9
GFP+ 95.3
GFP+ 77.0
GFP- 5.31
GFP+ 75.5
GFP+ 0.86
GFP+ 25.5
GFP- 63.9

FIG. 4

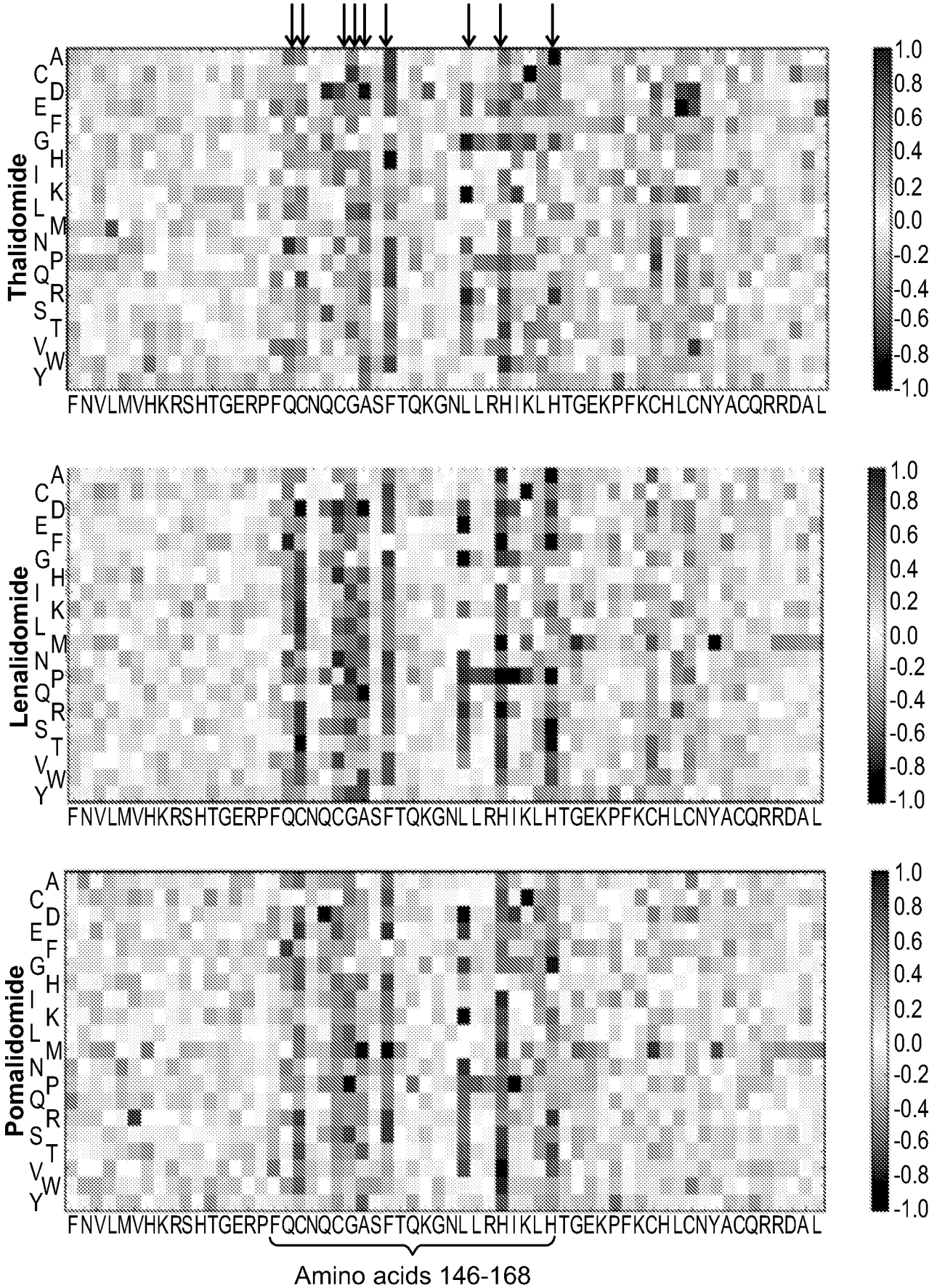

Thalidomide
Lenalidomide
Pomalidomide
A C D E F G H I K L M N P Q R S T V W Y
F N V L M V H K R S H T G E R P F Q C N Q C G A S F T Q K G N L L R H I K L H T G E K P F K C H L C N Y A C Q R R D A L
1.0 0.8 0.6 0.4 0.2 0.0 -0.2 -0.4 -0.6 -0.8 -1.0
Amino acids 146-168

FIG. 5A

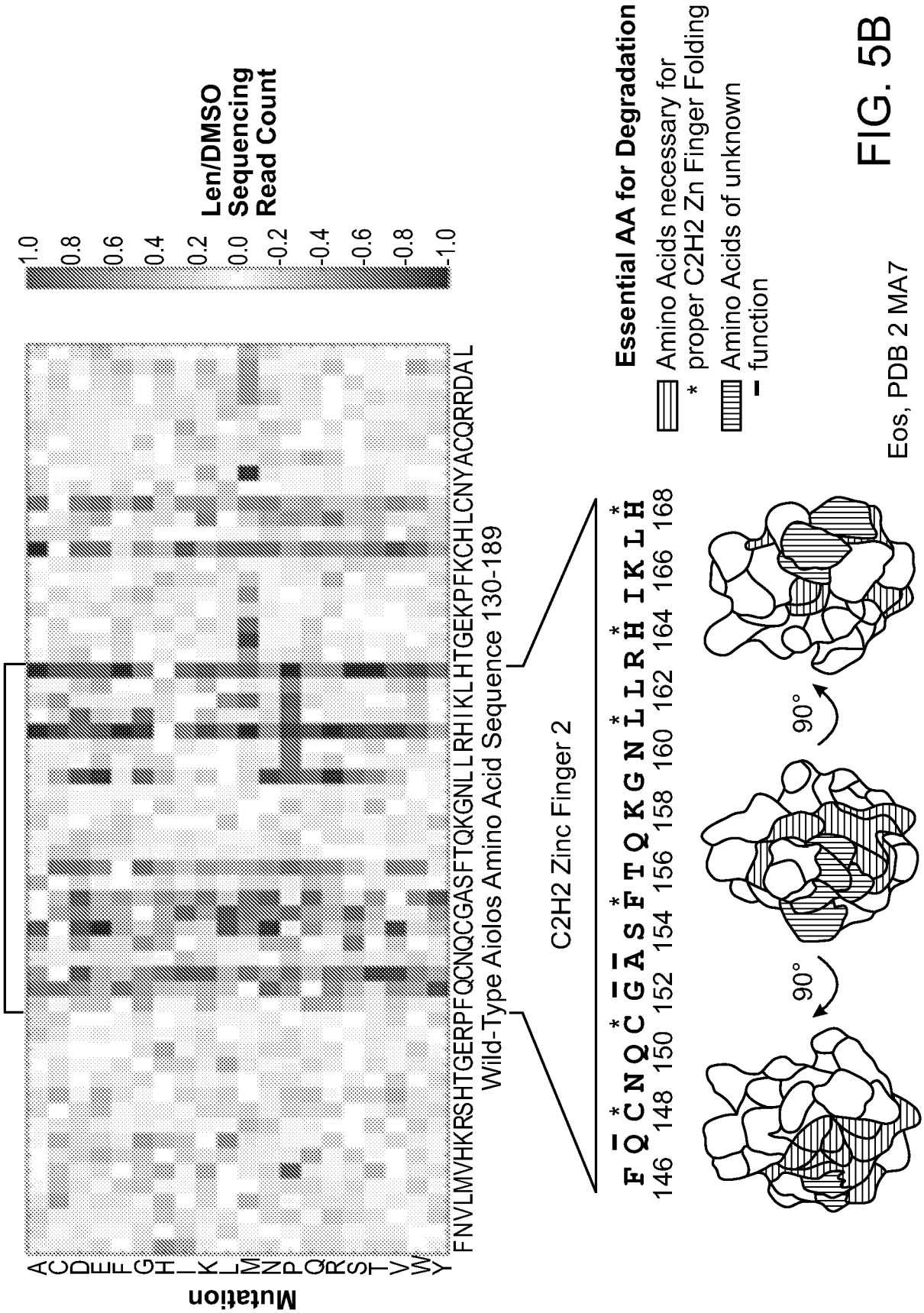

Len/DMSO Sequencing Read Count
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
-0.4
-0.6
-0.8
-1.0
Mutation
A C D E F G H I K L M N P Q R S T V W Y
FNVLMVHKRSHTGERPFQCNQCGASFTQKGNLLRHIKLHTGEKPFKCHLCNYACQRRDAL
Wild-Type Aiolos Amino Acid Sequence 130-189
C2H2 Zinc Finger 2
F Q C N Q C G A S F T Q K G N L L R H I K L H
146 148 150 152 154 156 158 160 162 164 166 168
Essential AA for Degradation
Amino Acids necessary for
* proper C2H2 Zn Finger Folding
Amino Acids of unknown
– function
90°
90°
Eos, PDB 2 MA7

FIG. 5B

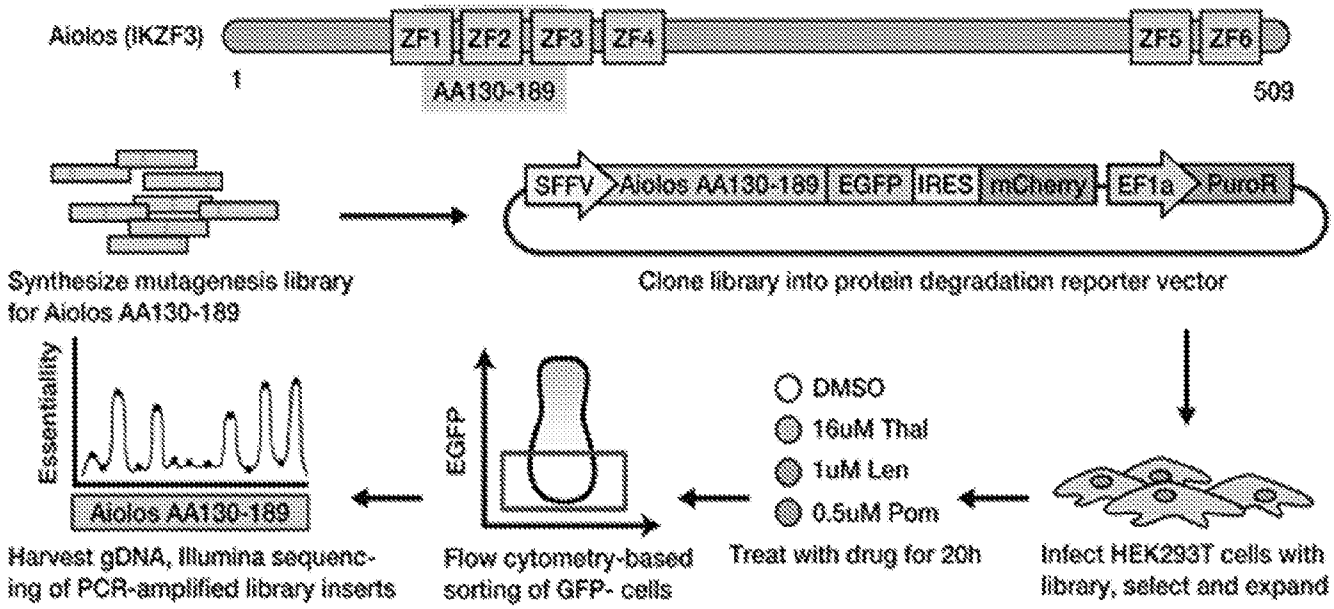

Aiolos (IKZF3)
ZF1
ZF2
ZF3
ZF4
ZF5
ZF6
1
AA130-189
509
SFFV
Aiolos AA130-189
EGFP
IRES
mCherry
EF1a
PuroR
Synthesize mutagenesis library for Aiolos AA130-189
Clone library into protein degradation reporter vector
Essentiality
Aiolos AA130-189
EGFP
DMSO
1uM Len
16uM Thal
0.5uM Pom
Harvest gDNA, Illumina sequencing of PCR-amplified library inserts
Flow cytometry-based sorting of GFP- cells
Treat with drug for 20h
Infect HEK293T cells with library, select and expand

FIG. 6A

FIG. 6B

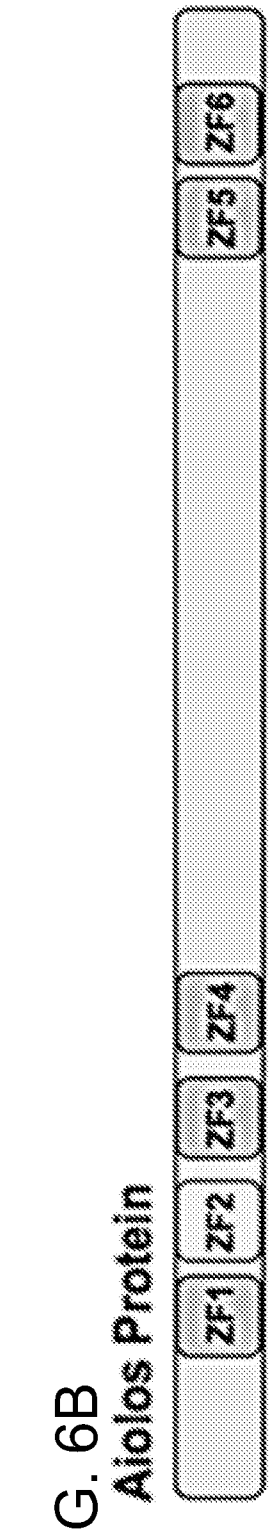

Aiolos Protein
ZF1
ZF2
ZF3
ZF4
ZF5
ZF6

FIG. 6C

WT Aiolos
Aiolos del146-168
Aiolos C148A
Normalized to Mode
GFP-A
DMSO
Thalidomide (16uM)
Lenalidomide (1uM)
Pomalidomide (0.5uM)
16 uM Thalidomide
DMSO
1uM Lenalidomide
0.5 uM Pomalidomide

FIG. 6D

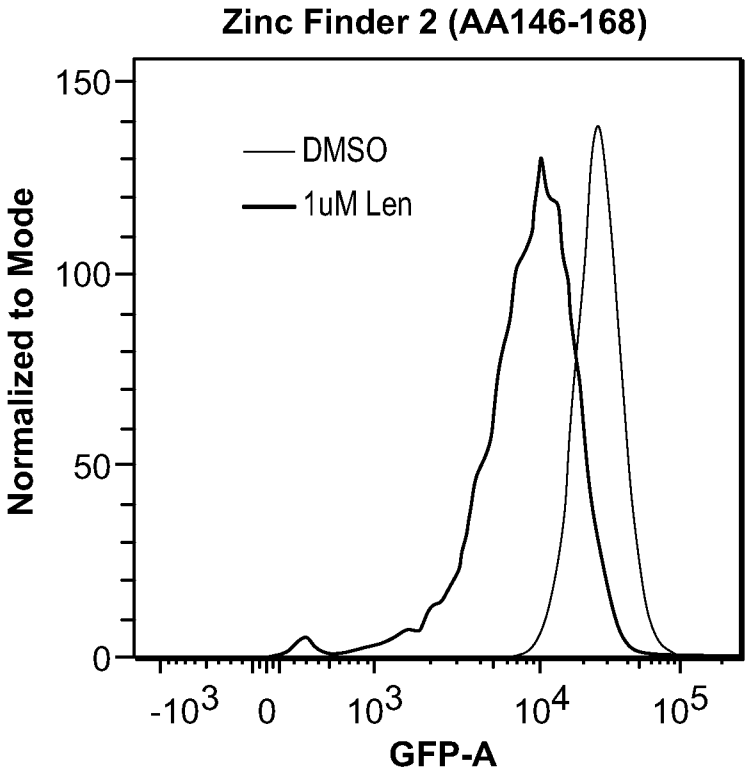

Zinc Finder 2 (AA146-168)
150
100
50
0
Normalized to Mode
DMSO
1uM Len
-10^3
0
10^3
10^4
10^5
GFP-A

FIG. 6E

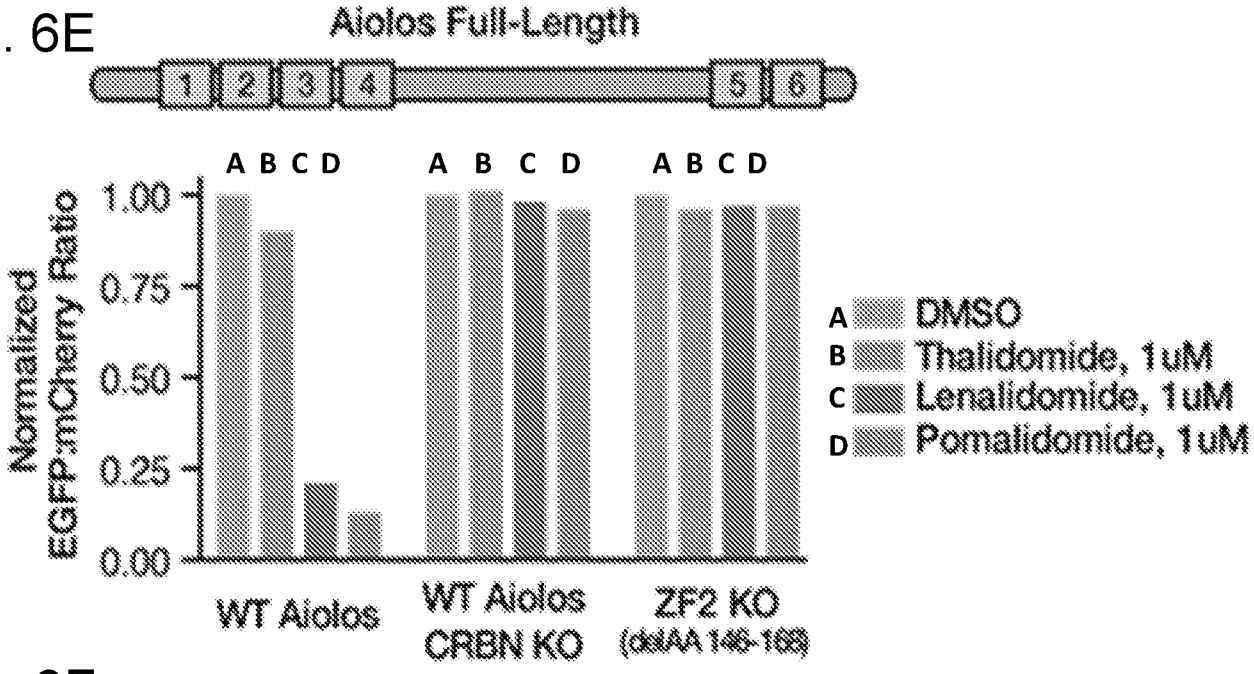
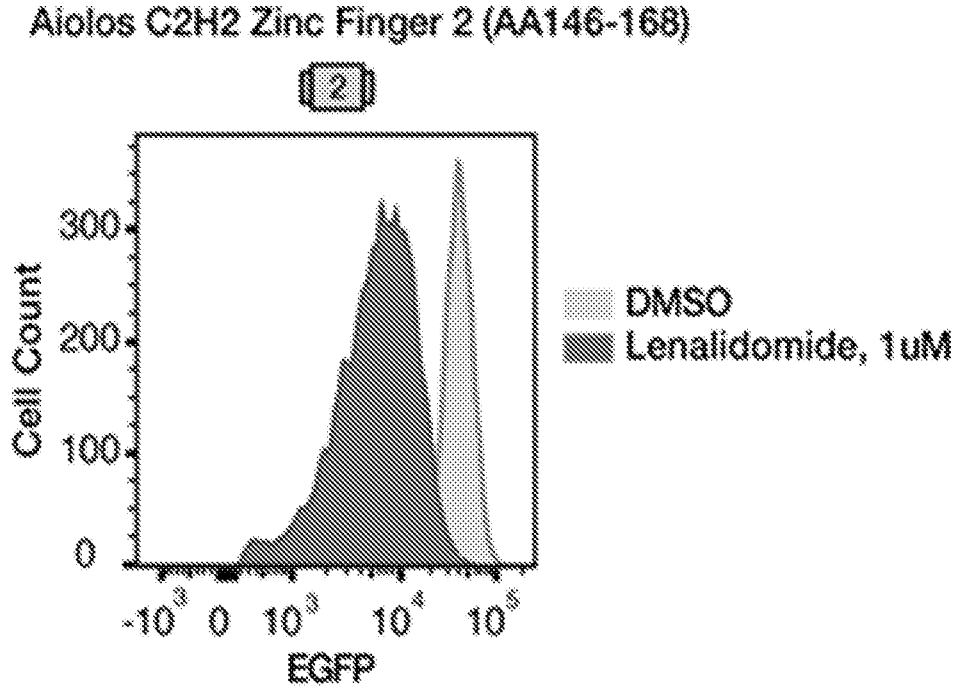

Aiolos Full-Length
1
2
3
4
5
6
A B C D
A B C D
A B C D
Normalized EGFP:mCherry Ratio
1.00
0.75
0.50
0.25
0.00
WT Aiolos
WT Aiolos CRBN KO
ZF2 KO (delAA 146-168)
A DMSO
B Thalidomide, 1uM
C Lenalidomide, 1uM
D Pomalidomide, 1uM

FIG. 6F


Aiolos C2H2 Zinc Finger 2 (AA146-168)
2
Cell Count
300
200
100
0
-10^3
0
10^3
10^4
10^5
EGFP
DMSO
Lenalidomide, 1uM

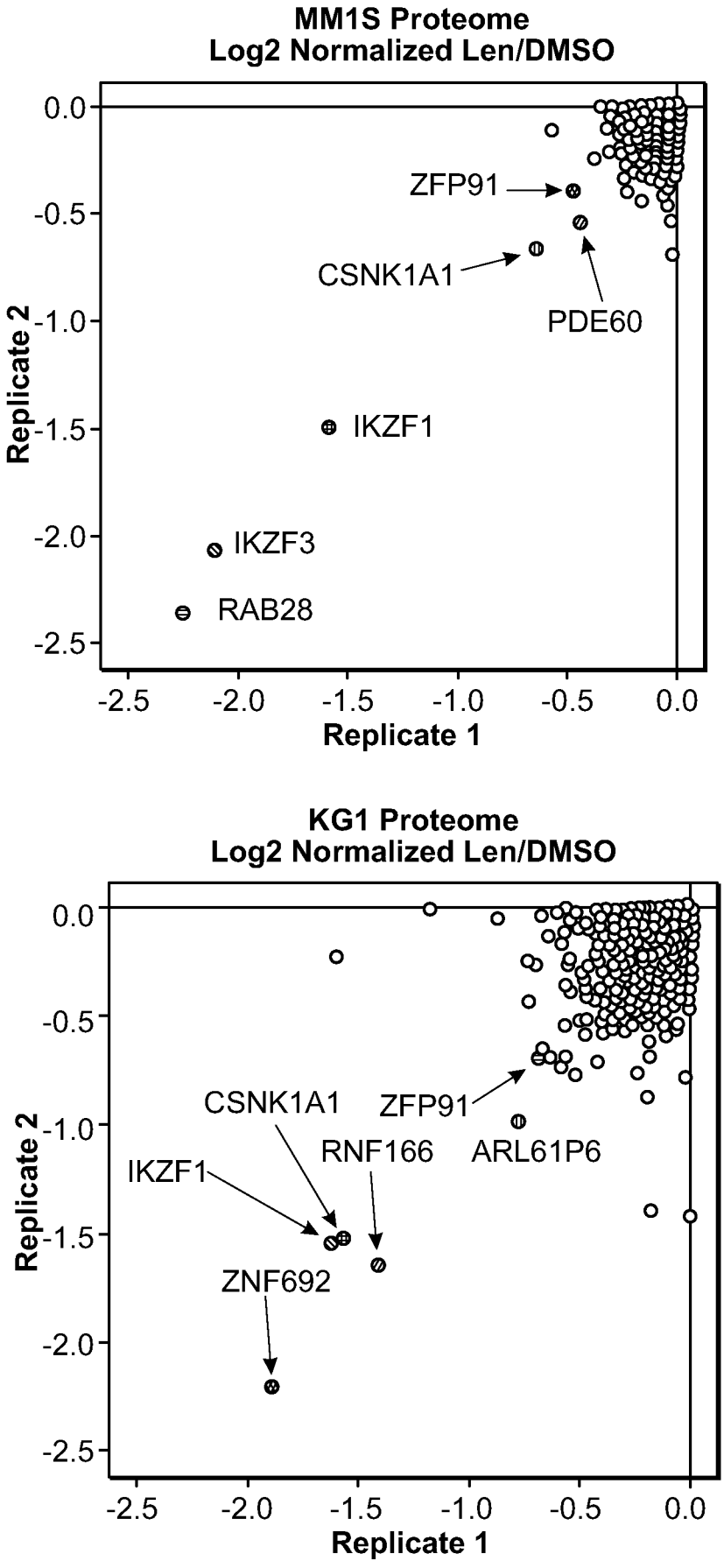

MM1S Proteome
Log2 Normalized Len/DMSO
Replicate 2
Replicate 1
0.0
-0.5
-1.0
-1.5
-2.0
-2.5
ZFP91
CSNK1A1
PDE60
IKZF1
IKZF3
RAB28
KG1 Proteome
Log2 Normalized Len/DMSO
CSNK1A1
ZFP91
RNF166
ARL61P6
IKZF1
ZNF692

FIG. 7A

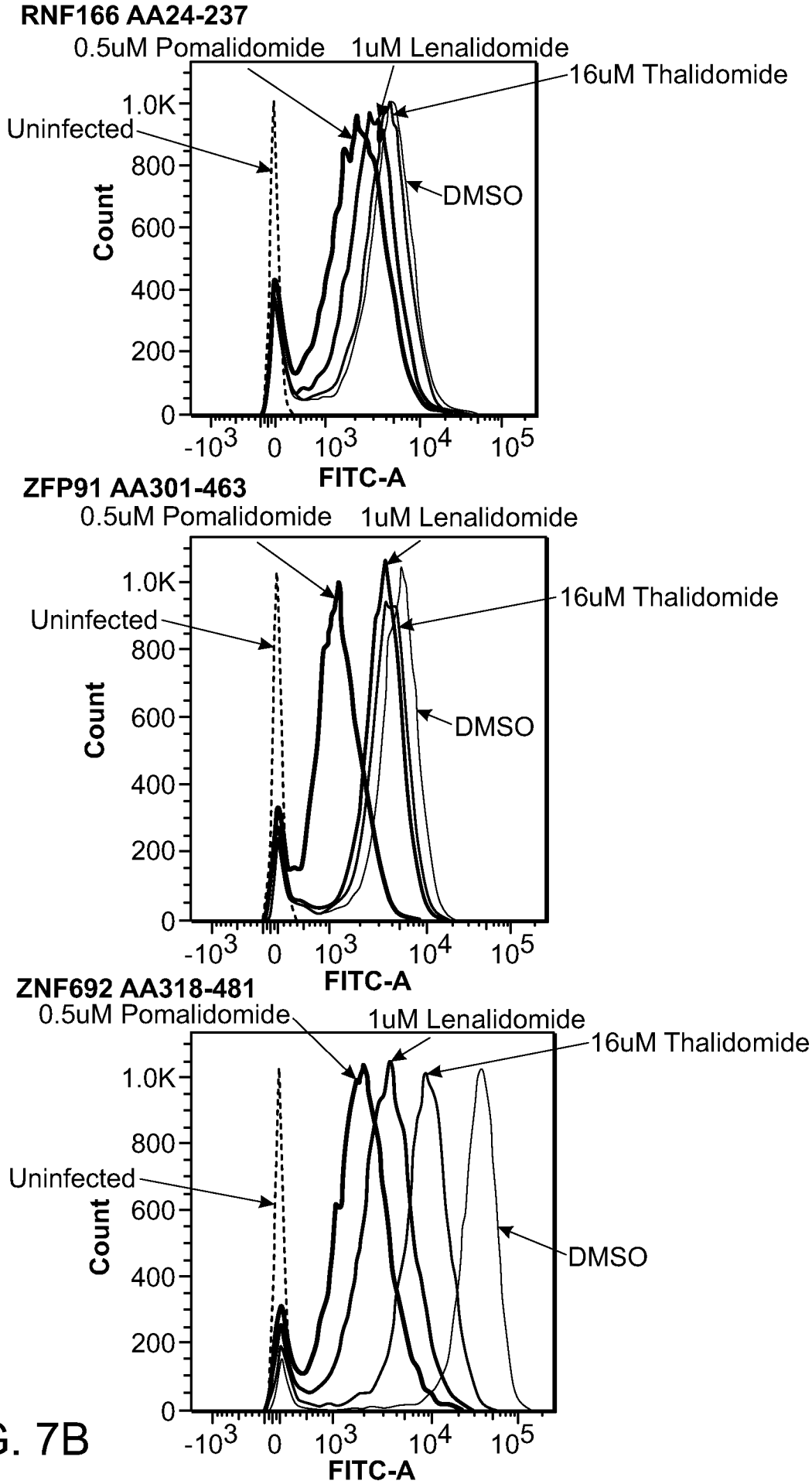

RNF166 AA24-237
0.5uM Pomalidomide
1uM Lenalidomide
16uM Thalidomide
Uninfected
DMSO
Count
1.0K
800
600
400
200
0
-10^3
0
10^3
10^4
10^5
FITC-A
ZFP91 AA301-463
0.5uM Pomalidomide
1uM Lenalidomide
16uM Thalidomide
Uninfected
DMSO
Count
FITC-A
ZNF692 AA318-481
0.5uM Pomalidomide
1uM Lenalidomide
16uM Thalidomide
Uninfected
DMSO
Count
FITC-A

FIG. 7B

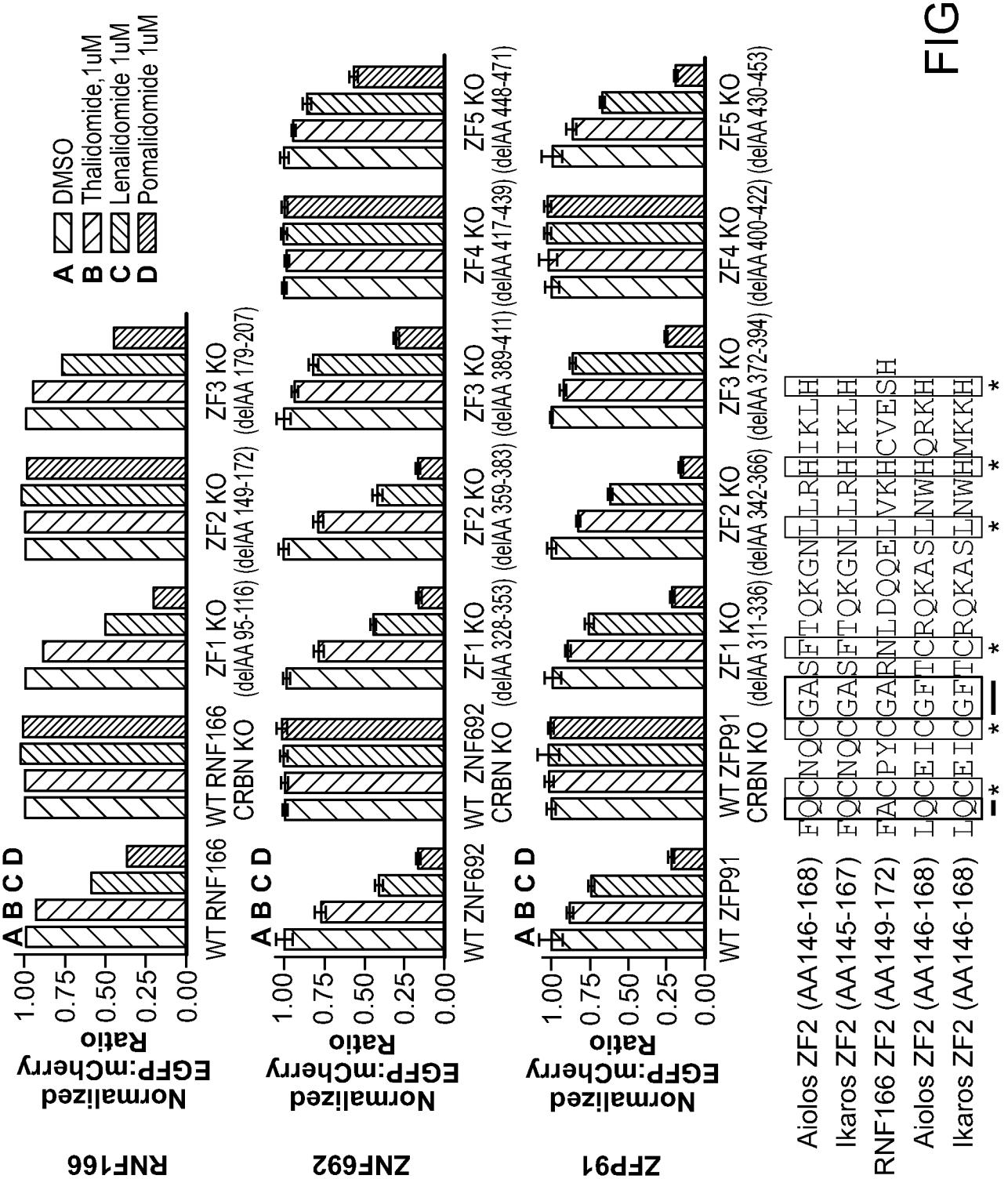

A DMSO
B Thalidomide,1uM
C Lenalidomide 1uM
D Pomalidomide 1uM
RNF166
ZNF692
ZFP91
Normalized EGFP:mCherry Ratio
1.00
0.75
0.50
0.25
0.00
A B C D
WT RNF166
CRBN KO
ZF1 KO (delAA 95-116)
ZF2 KO (delAA 149-172)
ZF3 KO (delAA 179-207)
WT ZNF692
CRBN KO
ZF1 KO (delAA 328-353)
ZF2 KO (delAA 359-383)
ZF3 KO (delAA 389-411)
ZF4 KO (delAA 417-439)
ZF5 KO (delAA 448-471)
WT ZFP91
CRBN KO
ZF1 KO (delAA 311-336)
ZF2 KO (delAA 342-366)
ZF3 KO (delAA 372-394)
ZF4 KO (delAA 400-422)
ZF5 KO (delAA 430-453)
Aiolos ZF2 (AA146-168) FQCNQCGASFTQKGNLLRHIKLH
Ikaros ZF2 (AA145-167) FQCNQCGASFTQKGNLLRHIKLH
RNF166 ZF2 (AA149-172) FACPYCGARNLDQQELVKHCVESH
Aiolos ZF2 (AA146-168) LQCEICGFTCRQKASLNWHQRKH
Ikaros ZF2 (AA146-168) LQCEICGFTCRQKASLNWHMKKH

FIG. 8

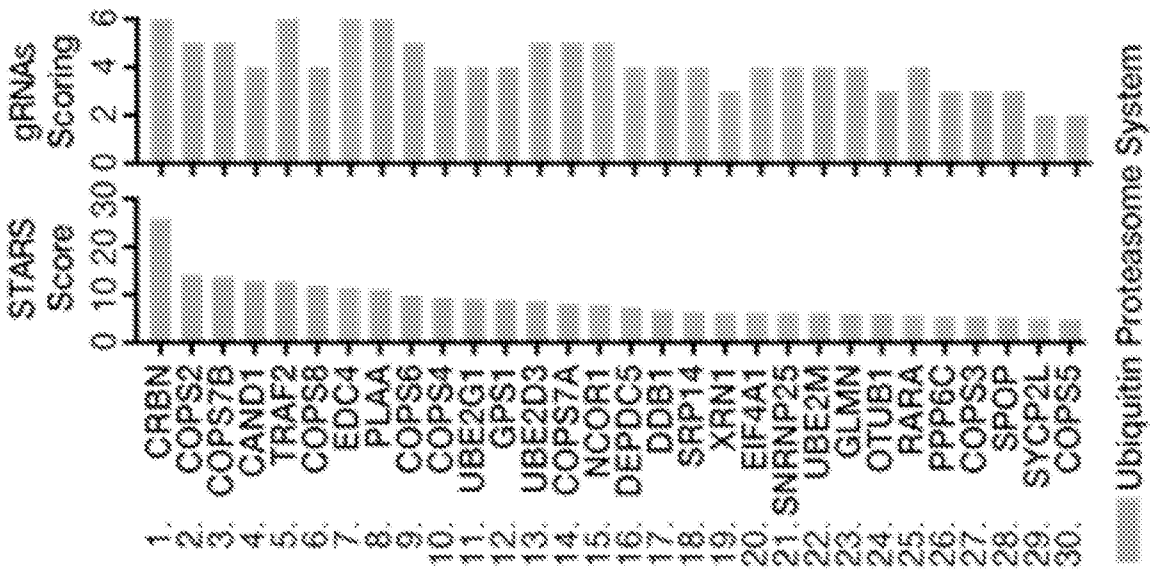


FIG. 9C

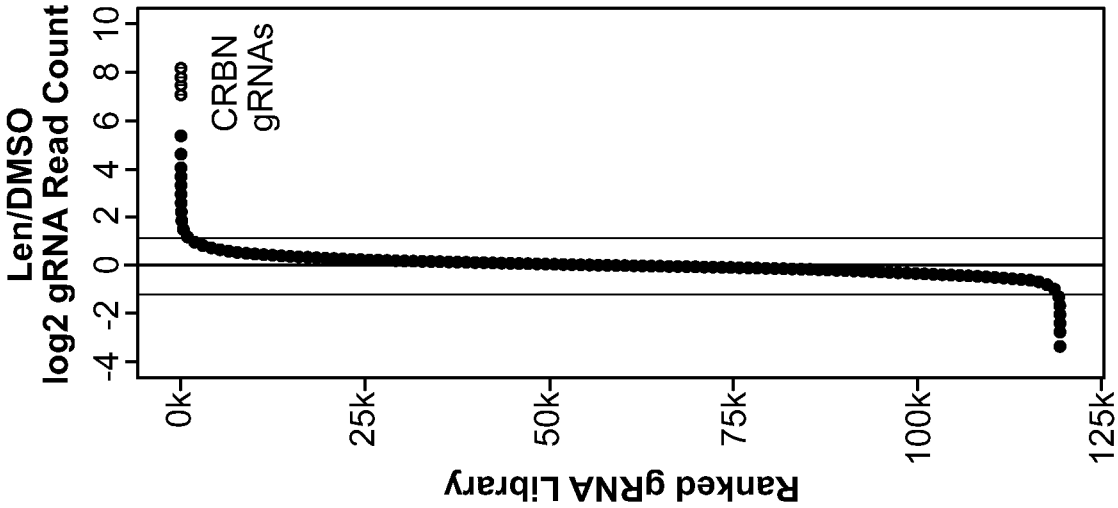


FIG. 9B

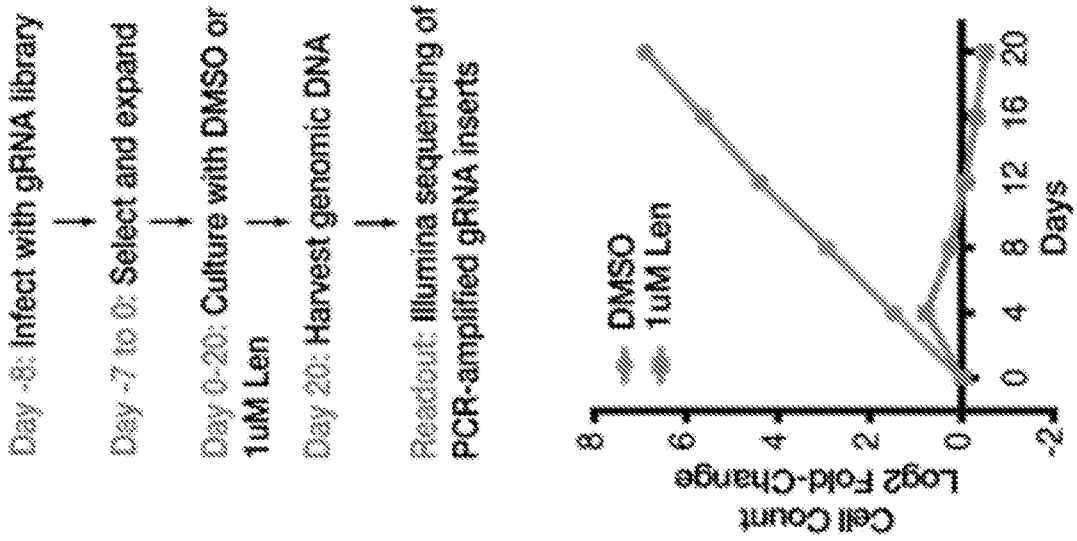


FIG. 9A

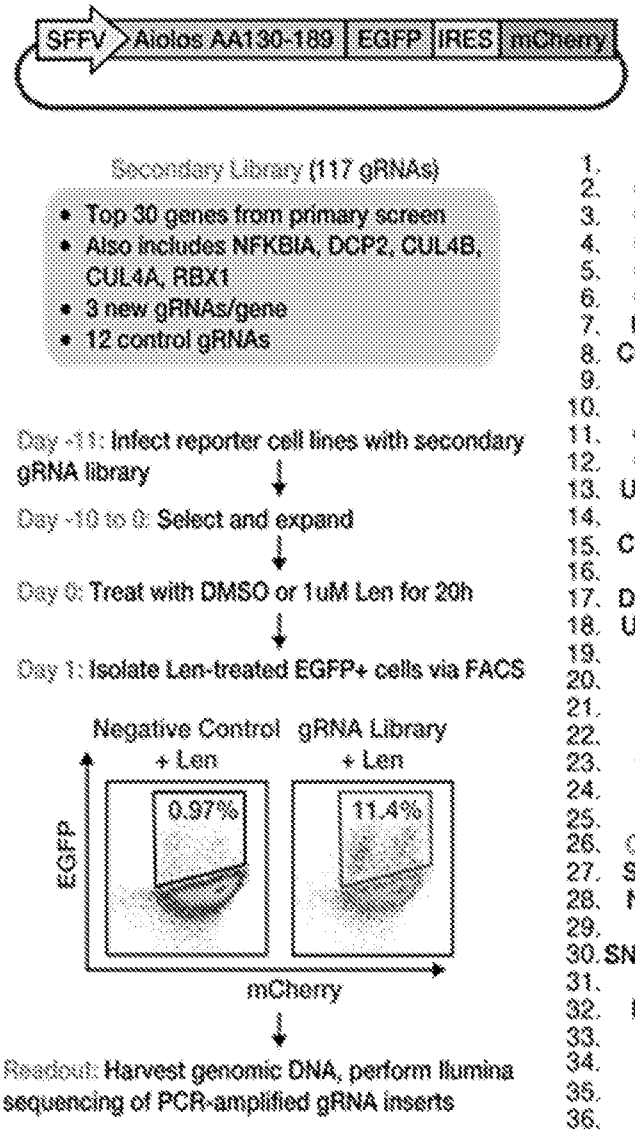


FIG. 10A

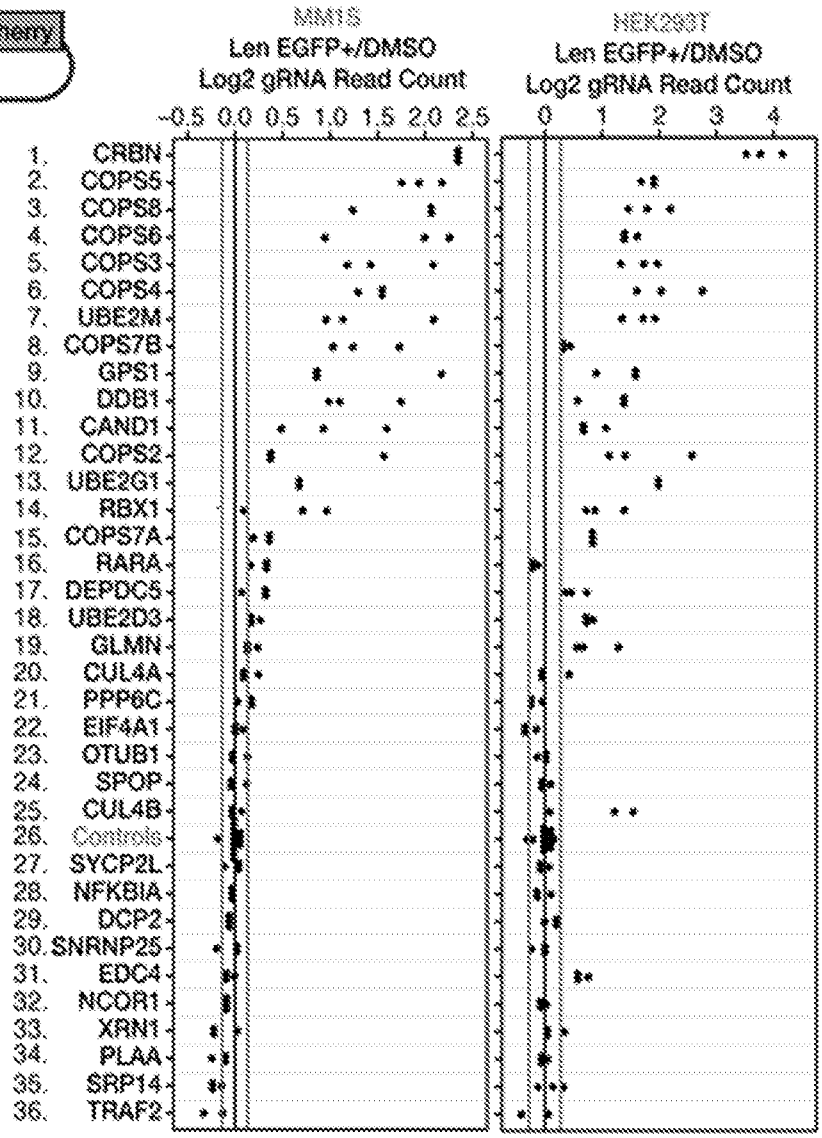


FIG. 10B