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[Continued on next page]

(54) Title: EXOSOMES AND USES THEREOF

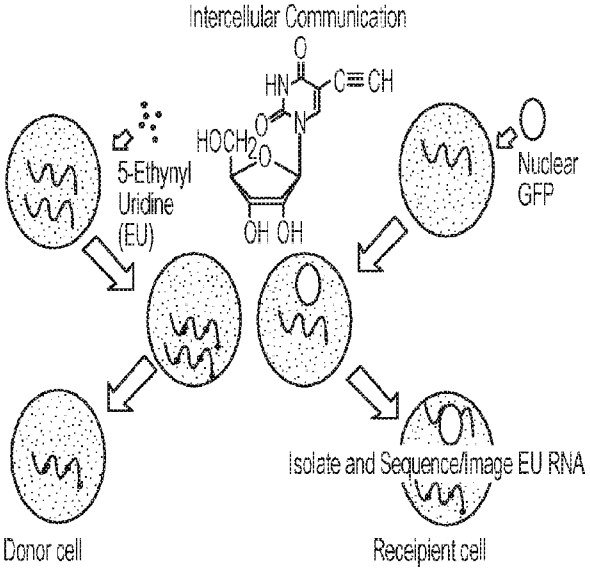

FIG. 5A

(57) Abstract: The present invention relates to the isolation and purification of exosomes from biological samples, and to methods for extracting RNA contained therein. The present invention provides methods and uses for the purification of exosomes, as well as compositions comprising same.

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EXOSOMES AND USES THEREOF

RELATED APPLICATIONS AND INCORPORATION BY REFERENCE

[0001] This application claims benefit of and priority to U.S. Provisional Application Nos. 62/151,142, 62/151,166 and 62/151,289 all filed April 22, 2015.

[0002] All documents cited or referenced herein ("herein cited documents"), and all documents cited or referenced in herein cited documents, together with any manufacturer's instructions, descriptions, product specifications, and product sheets for any products mentioned herein or in any document incorporated by reference herein, are hereby incorporated herein by reference, and may be employed in the practice of the invention. More specifically, all referenced documents are incorporated by reference to the same extent as if each individual document was specifically and individually indicated to be incorporated by reference.

FEDERAL FUNDING LEGEND

[0003] This invention was made with government support under grant numbers HG006193 and HG005550 awarded by the National Institutes of Health. The government has certain rights in the invention.

FIELD OF THE INVENTION

[0004] The present invention relates to the isolation and purification of exosomes from biological samples, and to methods for extracting RNA contained therein. The present invention provides methods and uses for the purification of exosomes, as well as compositions comprising same.

[0005] The present invention further relates to the use of exosomes for diagnosis and prognosis purposes. Provided are methods, uses and kits of parts useful in particular for RNA profiling, as well as for diagnostic and prognostic methods in a subject.

[0006] The present invention also relates for the use of exosomes in therapeutics. Provided are methods for treatment or prophylaxis of a disorder of interest.

BACKGROUND OF THE INVENTION

[0007] Exosomes are small extracellular vesicles that have been shown to contain RNA.

[0008] Exosomes can be isolated using ultracentrifugation steps. However, purified exosomes have proven to be difficult to isolate. In particular, the presence of cellular debris

amounts to 'contaminant' in a preparation, jeopardizing genetic and biochemical analysis of exosomes. While exosomes are isolated using ultracentrifugation as described herein, other methods such as filtration, chemical precipitation, size exclusion chromatography, microfluidics are known in the art.

[0009] Further, RNA content of exosomes was previously reported as uncorrelated to corresponding cellular RNA content (Skog J, Würdinger T, van Rijn S, Meijer DH, Gainche L, Sena-Esteves M, Curry WT Jr, Carter BS, Krichevsky AM, Breakefield XO. Nat Cell Biol. 2008 Dec;10(12):1470-6. doi: 10.1038/ncb1800. Epub 2008 Nov 16.).

[0010] Citation or identification of any document in this application is not an admission that such document is available as prior art to the present invention.

SUMMARY OF THE INVENTION

[0011] It would be of interest to provide methods that allow to establish a relationship between exosomal RNA content and corresponding cellular RNA content. This would have broad diagnostic and prognostic applications.

[0012] Further, exosomes could prove useful in the therapeutics field.

[0013] The present invention proves a method for the isolation of exosomes from a biological sample. In some aspects, said method comprises:

(a) providing a biological sample comprising exosomes from a cell population,

(b) preparing an exosome-enriched fraction from the biological sample of step (a),

(c) subjecting the exosome-enriched fraction of step (b) to a treatment with a proteinase.

[0014] The present invention also provides a method for the purification of exosomes from a biological sample. In some aspects, said method comprises:

(a) providing a biological sample comprising exosomes from a cell population,

(b) preparing an exosome-enriched fraction from the biological sample of step (a),

(c) subjecting the exosome-enriched fraction of step (b) to a treatment with a proteinase.

[0015] In some aspects, the proteinase of step (c) may be one or more independently selected from serine proteases, threonine proteases, cysteine proteases, aspartate proteases, glutamic acid proteases and metalloproteases. In some aspects, step (c) may comprise a treatment with a proteinase and subsequent inactivation thereof. According to some embodiments, proteinase

inactivation may be performed with one or more protease inhibitor(s). In some aspects, the proteinase of step (c) may be proteinase K.

[0016] In some aspects, step (b) may comprise one or more centrifugation steps, so as to remove live cells, dead cells and larger cellular debris from the biological sample of step (a). In some aspects, step (b) may comprise one or more filtration steps. In some embodiments, the filtration step may comprise filtration with a submicron filter, for example the submicron filter may be a 0.22 micron filter. In some aspects, wherein step (b) may comprise one or more centrifugation steps, so as to remove live cells, dead cells and larger cellular debris from the biological sample of step (a), followed by a filtration step with a submicron filter. In some aspects, step (b) may comprise one or more ultracentrifugation steps. In some aspects, step (b) may comprise:

(b-1) filtrating with a submicron filter,

(b-2) performing a first ultracentrifugation step, so as to provide a first exosome-enriched fraction,

(b-3) washing the exosome-enriched fraction of step (b-2), and

(b-4) performing a second ultracentrifugation step of the washed exosome-enriched fraction of step (b-3).

[0017] In some aspects, step (c) may be performed after the final ultracentrifugation step of step (b). In some aspects, step (c) may comprise a treatment with proteinase K and subsequent inactivation thereof. In some embodiments, the inhibitor may be diisopropyl fluorophosphate (DFP) or phenyl methane sulphonyl fluoride (PMSF).

[0018] In some aspects, the method may further comprise:

(d) subjecting the proteinase K-treated fraction of step (c) to a treatment with an RNase.

[0019] In some embodiments, the RNase may be one or more independently selected from RNase A, B, C, 1, and T1. In some embodiments, the RNase may be RNAse A/T1. In some aspects, step (d) may comprise a treatment with RNase and subsequent inactivation thereof. In some aspects, inactivation of RNase may comprise a treatment with one or more RNase inhibitor(s). In some embodiments, the RNase inhibitor may be selected from protein-based RNase inhibitors.

[0020] In some aspects, the method may provide exosomes which are essentially free of extra-exosomal material. In some aspects, the method may provide exosomes which are

essentially free of extra-exosomal nucleic acid-protein complexes. In some aspects, the method may provide exosomes which are essentially free of extra-exosomal RNA-protein complexes.

[0021] In still further aspects, the method may further comprise after step (c) or (d) one or more purification steps based on the affinity of a bait molecule for a prey exosome biomarker. In an embodiment, the method comprises a cell population comprising one or more cell types, 2 or more cell types, preferably 3 or more cell types, 4 or more cell types or 5 or more cell types. In an embodiment, the method comprises isolating or purifying cell type-specific exosomes, or cell-subtype-specific exosomes. In an embodiment, the method wherein the one or more cell type comprises cells derived from the endoderm, cells derived from the mesoderm, or cells derived from the ectoderm.

[0022] In another aspect, the method comprises cells, wherein the cells derived from the endoderm comprise cells of the respiratory system, the intestine, the liver, the gallbladder, the pancreas, the islets of Langerhans, the thyroid or the hindgut. In an embodiment, the method comprises cells, wherein the cells derived from the mesoderm comprise osteochondroprogenitor cells, muscle cells, cells from the digestive systems, renal stem cells, cells from the reproductive system, bloods cells or cells from the circulatory system (such as endothelial cells). In another embodiment, the method comprises cells, wherein the cells derived from the ectoderm comprise epithelial cells, cells of the anterior pituitary, cells of the peripheral nervous system, cells of the neuroendocrine system, cell of the teethes, cell of the eyes, cells of the central nervous system, cells of the ependymal or cells of the pineal gland. In an embodiment, the method comprises cells, wherein the cells from the central nervous system and the peripheral nervous system comprise neurons, Schwann cells, satellite glial cells, oligodendrocytes or astrocytes. In a further embodiment, the method comprises neurons wherein the neurons comprise interneurons, pyramidal neurons, gabaergic neurons, dopaminergic neurons, serotoninergic neurons, glutamatergic neurons, motor neurons from the spinal cord, or inhibitory spinal neurons.

[0023] In an aspect of the invention, the method provides cell types wherein the one or more cell-type is a cancer cell or a circulating tumor cell (CTC), such as cancer cell or CTC derived from any cell-types or cell subtypes. In an embodiment, the method provides a prey exosome biomarker, wherein the biomarker comprises a surface biomarker. In a further embodiment, the method wherein the prey exosome biomarker comprises a membrane protein. In another embodiment, the method comprises a prey exosome biomarker selected from the group

consisting of proteins as per Table D, column G; or proteins as per Table D, column H; or proteins as per Table D, column I; or proteins as per Table D, column J; or proteins as per Table D, column K; or proteins as per Table D, column L; or proteins as per Table D, column M. In one embodiment, the prey exosome biomarker is FLRT3 and/or L1CAM.

[0024] In an aspect, the method provides a bait molecule comprising a protein and more preferentially an antibody, such as a monoclonal antibody. In an embodiment, the bait molecule is recognized by an affinity ligand. In an embodiment, the bait molecule can also be an RNA aptamer. In a further embodiment, the affinity ligand comprises a protein, a peptide, a divalent metal-based complex or an antibody. In an embodiment, the bait molecule or the affinity ligand is immobilized on a solid substate. In another embodiment, the solid substrate is selced from a purification column, a microfluidic channel or beads, such as magnetic beads. In an embodiment, the method provides a purification, wherein the purification comprises a microfluidic affinity based purification, a magnetic based purification, a pull-down purification or a fluorescence activated sorting-based purification. In another embodiment, the method provides a biological sample, wherein the biological sample comprises a body fluid or is derived from a body fluid, wherein the body fluid was obtained from a mammal. In a further aspect, the body fluid is selected from the group consisting of amniotic fluid, aqueous humor, vitreous humor, bile, blood serum, breast milk, cerebrospinal fluid, cerumen (earwax), chyle, chyme, endolymph, perilymph, exudates, feces, female ejaculate, gastric acid, gastric juice, lymph, mucus (including nasal drainage and phlegm), pericardial fluid, peritoneal fluid, pleural fluid, pus, rheum, saliva, sebum (skin oil), semen, sputum, synovial fluid, sweat, tears, urine, vaginal secretion, vomit and mixtures of one or more thereof.

[0025] The present invention provides a method for the isolation of exosomes from a cell population, comprising steps of: (1) providing isolated exosomes from a biological sample comprising exosomes from said cell population, (2) performing on the isolated exosomes of step (1) one or more purification steps based on the affinity of a bait molecule for a prey exosome biomarker.

[0026] In another aspect, the present invention provides a method for the purification of exosomes from a cell population, comprising steps of: (1) providing purified exosomes from a biological sample comprising exosomes from said cell population; (2) performing on the purified

exosomes of step (1) one or more purification steps based on the affinity of a bait molecule for a prey exosome biomarker.

[0027] In an embodiment, the invention provides a method for either isolation or purification of exosomes from a cell population, wherein the cell population comprises one or more cell types, 2 or more cell types, 3 or more cell types, 4 or more cell types or 5 or more cell types. In an embodiment, the method isolates or purifies cell type-specific exosomes, or cell-subtype-specific exosomes. the method comprises a cell population comprising one or more cell types, 2 or more cell types, preferably 3 or more cell types, 4 or more cell types or 5 or more cell types. In an embodiment, the method comprises isolating or purifying cell type-specific exosomes, or cell-subtype-specific exosomes. In an embodiment, the method provides for isolation or purification of exosomes from a cell population, wherein the one or more cell type comprises cells derived from the endoderm, cells derived from the mesoderm, or cells derived from the ectoderm. In another aspect, the method comprises cells, wherein the cells derived from the endoderm comprise cells of the respiratory system, the intestine, the liver, the gallbladder, the pancreas, the islets of Langerhans, the thyroid or the hindgut. In an embodiment, the method comprises cells, wherein the cells derived from the mesoderm comprise osteochondroprogenitor cells, muscle cells, cells from the digestive systems, renal stem cells, cells from the reproductive system, bloods cells or cells from the circulatory system (such as endothelial cells). In another embodiment, the method comprises cells, wherein the cells derived from the ectoderm comprise epithelial cells, cells of the anterior pituitary, cells of the peripheral nervous system, cells of the neuroendocrine system, cell of the teethes, cell of the eyes, cells of the central nervous system, cells of the ependymal or cells of the pineal gland. In an embodiment, the method comprises cells, wherein the cells from the central nervous system and the peripheral nervous system comprise neurons, Schwann cells, satellite glial cells, oligodendrocytes or astrocytes. In a further embodiment, the method comprises neurons wherein the neurons comprise interneurons, pyramidal neurons, gabaergic neurons, dopaminergic neurons, serotoninergic neurons, glutamatergic neurons, motor neurons from the spinal cord, or inhibitory spinal neurons.

[0028] In an aspect of the invention, the method provides for isolation or purification of exosomes from a cell population, wherein the one or more cell-type is a cancer cell or a circulating tumor cell (CTC), such as cancer cell or CTC derived from any cell-types or cell subtypes. In an embodiment, the method provides a prey exosome biomarker, wherein the

biomarker comprises a surface biomarker. In a further embodiment, the method wherein the prey exosome biomarker comprises a membrane protein. In another embodiment, the method comprises a prey biomarker selected from the group consisting of proteins as per Table D, column G; or proteins as per Table D, column H; or proteins as per Table D, column I; or proteins as per Table D, column J; or proteins as per Table D, column K; or proteins as per Table D, column L; or proteins as per Table D, column M. In one embodiment, the prey exosome biomarker is FLRT3 and/or L1CAM.

[0029] In an aspect, the method provides for isolation or purification of exosomes from a cell population, wherein the bait molecule comprises a protein and more preferentially an antibody, such as a monoclonal antibody. In an embodiment, the bait molecule is recognized by an affinity ligand. In an embodiment, the bait molecule can also be an RNA aptamer. In a further embodiment, the affinity ligand comprises a protein, a peptide, a divalent metal-based complex or an antibody. In an embodiment, the bait molecule or the affinity ligand is immobilized on a solid substate. In another embodiment, the solid substrate is selced from a purification column, a microfluidic channel or beads, such as magnetic beads. In an embodiment, the method provides a purification, wherein the purification comprises a microfluidic affinity based purification, a magnetic based purification, a pull-down purification or a fluorescence activated sorting-based purification.

[0030] The present invention also provides a method for the preparation of exosomal RNA from a biological sample, said method comprising:

(i) providing a biological sample comprising exosomes from a cell population,

(ii) preparing purified exosomes from the biological sample of step (i),

(iii) extracting RNA from the purified exosomes of step (i).

[0031] In some aspects, step (ii) may comprise the method for the isolation/purification of exosomes as disclosed herein. In other aspects, the method for the preparation of exosomal RNA from a biological sample comprises a method wherein the purified exosomes prepared at step (ii) are exosomes from a single cell type or from a single cell subtype.

[0032] The present invention provides a method for the preparation of exosomal RNA of a cell population, comprising steps of: (1) providing purified exosomes from a biological sample comprising exosomes from said cell population; (2) performing on the purified exosomes of step

(1) one or more purification steps based on the affinity of a bait molecule for a prey exosome biomarker, and (3) extracting RNA from the purified exosomes of step (2).

[0033] In some aspects, the exosomal RNA may be total exosomal RNA. In some aspects, the exosomal RNA may comprise exosomal messenger RNA. In some aspects, the exosomal RNA may be total exosomal messenger RNA. In some aspects, the exosomal RNA is exosomal RNA from single cell type exosomes or single cell subtype exosomes.

[0034] The present invention also provides for a use of a proteinase in the purification of exosomes from a biological sample. The present invention also provides for a use of a proteinase and of an RNase in the purification of exosomes from a biological sample. The present invention also provides for a use of a proteinase in the purification of an ultracentrifugated exosome-containing sample. The present invention also provides for a use of a proteinase and of an RNase in the purification of an ultracentrifugated exosome-containing sample.

[0035] In the uses of the invention, in some aspects, the proteinase may be proteinase K. In the uses of the invention, in some aspects, the ultracentrifugated exosome-containing sample may be a washed ultracentrifugated exosome-containing sample.

[0036] In the uses of the invention, in some aspects, the ultracentrifugated exosome-containing sample may be a washed ultracentrifugated exosome-containing sample.

[0037] In the methods and uses of the invention as disclosed herein, in some aspects, the biological sample may be a bodily fluid or is derived from a bodily fluid, wherein the bodily fluid was obtained from a mammal. In some embodiments, the bodily fluid may be selected from amniotic fluid, aqueous humour, vitreous humour, bile, blood serum, breast milk, cerebrospinal fluid, cerumen (earwax), chyle, chyme, endolymph, perilymph, exudates, feces, female ejaculate, gastric acid, gastric juice, lymph, mucus (including nasal drainage and phlegm), pericardial fluid, peritoneal fluid, pleural fluid, pus, rheum, saliva, sebum (skin oil), semen, sputum, synovial fluid, sweat, tears, urine, vaginal secretion, vomit and mixtures of one or more thereof.

[0038] In the methods and uses of the invention as disclosed herein, in some aspects, the cell population may be a population of cells of the same cell type. In the methods and uses of the invention as disclosed herein, in some aspects, the cell population is a population of cells of different cell types. In another embodiment, the cell population comprises one or more cell types, 2 or more cell types, 3 or more cell types, 4 or more cell types, or 5 or more cell types.

[0039] In the methods and uses of the invention as disclosed herein, in some aspects, the biological sample comprises cultured cells. In some embodiments, the biological sample may comprise cells cultured *in vitro*. In some embodiments, the biological sample may comprise cells cultured *ex vivo*. In some embodiments, the biological sample may be a sample obtained by liquid biopsy. In some embodiments, the biological sample may comprise a cell type selected from cells types present in amniotic fluid, aqueous humour, vitreous humour, bile, blood serum, breast milk, cerebrospinal fluid, cerumen (earwax), chyle, chyme, endolymph, perilymph, exudates, feces, female ejaculate, gastric acid, gastric juice, lymph, mucus (including nasal drainage and phlegm), pericardial fluid, peritoneal fluid, pleural fluid, pus, rheum, saliva, sebum (skin oil), semen, sputum, synovial fluid, sweat, tears, urine, vaginal secretion, vomit.

[0040] The present invention also provides exosome preparations and compositions comprising exosomes. The present invention provides an exosome preparation obtainable with the method or the use as disclosed herein. The present invention also provides a composition comprising exosomes, wherein the composition is essentially free of extra-exosomal material. The present invention also provides a composition comprising exosomes, wherein the composition is essentially free of extra-exosomal nucleic acid-protein complexes. The present invention also provides a composition comprising exosomes, wherein the composition is essentially free of extra-exosomal RNA-protein complexes. In another aspect, the invention provides a composition comprising cell type specific exosomes or cell subtype specific exosomes. In an embodiment, the composition comprises exosomes, wherein the exosomes are specific for one or more cell types or cell subtypes. In another embodiment, the composition comprises purified exosomes, wherein said purified exosomes are exosomes from a single cell-type or of a single cell subtype.

[0041] The present invention provides a method for the determination of cellular RNA content in a cell population. In some aspects, said method comprises:

(a) providing a biological sample comprising exosomes from said cell population,

(b) preparing purified exosomes from the sample of step (a),

(c) extracting RNA from the purified exosomes of step (b), so as to provide exosomal RNA,

(d) analyzing the exosomal RNA extracted at step (c),

(e) estimating, as a function of the result from step (d), the cellular RNA content in the cell population.

[0042] In some aspects, step (b) may comprise the method for the purification of exosomes as disclosed herein.

[0043] In some aspects, the invention provides a method for the determination of cellular RNA content of a cell population, said method comprising (a) providing a biological sample comprising exosomes from said cell population; (b) preparing purified exosomes from the sample of step (a); (c) extracting RNA from the purified exosomes of step (b), so as to provide exosomal RNA; (d) analyzing the exosomal RNA extracted at step (c); (e) estimating, as a function of the result from step (d), the cellular RNA content in the cell population; wherein step (b) further comprises performing on the purified exosomes one or more purification steps based on the affinity of a bait molecule for a prey exosome biomarker.

[0044] In an embodiment, the method comprises the method of step (b) wherein the method comprises the isolation or the purification of exosomes from a biological sample. In an embodiment, the invention provides a method for either isolation or purification of exosomes from a cell population, wherein the cell population comprises one or more cell types, 2 or more cell types, 3 or more cell types, 4 or more cell types or 5 or more cell types. In an embodiment, the method isolates or purifies cell type-specific exosomes, or cell-subtype-specific exosomes. the method comprises a cell population comprising one or more cell types, 2 or more cell types, preferably 3 or more cell types, 4 or more cell types or 5 or more cell types. In an embodiment, the method comprises isolating or purifying cell type-specific exosomes, or cell-subtype-specific exosomes. In an embodiment, the method provides for isolation or purification of exosomes from a cell population, wherein the one or more cell type comprises cells derived from the endoderm, cells derived from the mesoderm, or cells derived from the ectoderm. In another aspect, the method comprises cells, wherein the cells derived from the endoderm comprise cells of the respiratory system, the intestine, the liver, the gallbladder, the pancreas, the islets of Langerhans, the thyroid or the hindgut. In an embodiment, the method comprises cells, wherein the cells derived from the mesoderm comprise osteochondroprogenitor cells, muscle cells, cells from the digestive systems, renal stem cells, cells from the reproductive system, bloods cells or cells from the circulatory system (such as endothelial cells). In another embodiment, the method comprises cells, wherein the cells derived from the ectoderm comprise epithelial cells, cells of the anterior pituitary, cells of the peripheral nervous system, cells of the neuroendocrine system, cell of the teethes, cell of the eyes, cells of the central nervous system, cells of the ependymal or cells of the

pineal gland. In an embodiment, the method comprises cells, wherein the cells from the central nervous system and the peripheral nervous system comprise neurons, Schwann cells, satellite glial cells, oligodendrocytes or astrocytes. In a further embodiment, the method comprises neurons wherein the neurons comprise interneurons, pyramidal neurons, gabaergic neurons, dopaminergic neurons, serotoninergic neurons, glutamatergic neurons, motor neurons from the spinal cord, or inhibitory spinal neurons.

[0045] In an aspect of the invention, the method provides for isolation or purification of exosomes from a cell population, wherein the one or more cell-type is a cancer cell or a circulating tumor cell (CTC), such as cancer cell or CTC derived from any cell-types or cell subtypes. In an embodiment, the method provides a prey exosome biomarker, wherein the biomarker comprises a surface biomarker. In a further embodiment, the method wherein the prey exosome biomarker comprises a membrane protein. In another embodiment, the method comprises a prey exosome biomarker selected from the group consisting of proteins as per Table D, column G; or proteins as per Table D, column H; or proteins as per Table D, column I; or proteins as per Table D, column J; or proteins as per Table D, column K; or proteins as per Table D, column L; or proteins as per Table D, column M. In one embodiment, the prey exosome biomarker is FLRT3 and/or L1CAM.

[0046] In an aspect, the method provides for isolation or purification of exosomes from a cell population, wherein the bait molecule comprises a protein and more preferentially an antibody, such as a monoclonal antibody. In an embodiment, the bait molecule is recognized by an affinity ligand. In an embodiment, the bait molecule can also be an RNA aptamer. In a further embodiment, the affinity ligand comprises a protein, a peptide, a divalent metal-based complex or an antibody. In an embodiment, the bait molecule or the affinity ligand is immobilized on a solid substate. In another embodiment, the solid substrate is selced from a purification column, a microfluidic channel or beads, such as magnetic beads. In an embodiment, the method provides a purification, wherein the purification comprises a microfluidic affinity based purification, a magnetic based purification, a pull-down purification or a fluorescence activated sorting-based purification.

[0047] In some aspects, step (e) may be performed based on a predicted correlation between exosomal RNA content and cellular RNA content.

[0048] In some aspects, said determination may comprise a qualitative determination. In some aspects, said determination may comprise a quantitative determination. In some embodiments, said quantitative determination may comprise determination of relative abundance of two RNAs. In some aspects, said determination may comprise determination of mRNA profiles.

[0049] In some aspects, said RNA may comprise messenger RNA (mRNA). In some aspects, said RNA may comprise micro RNA (miRNA). In some aspects, said RNA may comprise long non-coding RNA (lncRNA).

[0050] In some aspects, step (D) may comprise a qualitative determination. In some aspects, step (D) may comprise a quantitative determination. In some aspects, step (D) may comprise RNA sequencing (RNA seq). In some aspects, step (D) may comprise array analysis. In some aspects, step (D) may comprise reverse transcription polymerase chain reaction (RT-PCR). In some aspects, step (D) may comprise quantitative reverse transcription polymerase chain reaction (qRT-PCR). In some aspects, step (D) may comprise analyzing one or more sequence/s of interest.

[0051] In some aspects, the method of the invention comprises testing for the presence or absence of said sequence/s of interest. In some embodiments, step (D) may comprise analyzing for one or more allelic variants of a sequence of interest.

[0052] In some aspects, step (D) may comprise testing for presence or absence of said allelic variants. In some aspects, step (D) may comprise genome-wide analysis. In some aspects, step (D) may comprise transcriptome profiling.

[0053] In some aspects, the determination may be time-lapse.

[0054] In some aspects, the cell population may be a population of cells of the same cell type. In some aspects, the cell population may be a population of cells of different cell types.

[0055] In some aspects, the biological sample may comprise cultured cells. In some aspects, the biological sample may comprise cells cultured *in vitro*. In some aspects, the biological sample may comprise cells cultured *ex vivo*. In some aspects, the biological sample may be a sample obtained by liquid biopsy. In some aspects, the biological sample may comprise a cell type selected from blood, epithelia, muscle and neural cell types.

[0056] In some aspects, the biological samples is obtained from a body fluid, selected from amniotic fluid, aqueous humor, vitreous humor, bile, blood serum, breast milk, cerebrospinal

fluid, cerumen (earwax), chyle, chyme, endolymph, perilymph, exudates, feces, female ejaculate, gastric acid, gastric juice, lymph, mucus (including nasal drainage and phlegm), pericardial fluid, peritoneal fluid, pleural fluid, pus, rheum, saliva, sebum (skin oil), semen, sputum, synovial fluid, sweat, tears, urine, vaginal secretion, vomit and mixtures of one or more thereof.

[0057] In some aspects, the cell population of step (a) may be isolated as a subpart of a larger initial cell population. In some aspects, the cell population of step (a) may be obtained from a body fluid and isolated by immuno-magnetic separation.

[0058] In some aspects, the method of the invention may be for use in diagnosis. In some aspects, the method of the invention may be for use in prognosis. In some aspects, the method of the invention may be for use in identifying markers. In some aspects, the method of the invention may be for use in a screening process. In another aspect, the method determines the cellular RNA content of a single cell type or of a single cell subtype.

[0059] The present invention also provides a method for the diagnostic or prognostic of a disorder of interest in a subject. In some aspects, the method may comprise :

(I) selecting a marker, wherein said marker is associated with said disorder and wherein said marker may be determined in a cell type that is found in the subject to be in contact with a body fluid,

(II) providing a biological sample from said body fluid from said subject,

(III) estimating the cellular RNA content of said marker in the biological sample of step (II) by performing the method for the determination of cellular RNA content in a cell population as disclosed herein.

[0060] In an embodiment, the invention provides a method for the diagnostic or prognostic of a disorder of interest in a subject, wherein the cellular RNA content is the cellular content of a single cell type or of a single cell subtype.

[0061] In some aspects, the method further comprises:

(IV) determining, from the results of step (III), the status of the marker selected at step (I).

[0062] In some aspects, the marker may be selected from expression of a given open reading frame (ORF), overexpression of a given open reading frame (ORF), repression of a given open reading frame (ORF), over-repression of a given open reading frame (ORF), expression of a given allelic variant, relative level of expression of a given open reading frame (ORF), presence of a mutation in a given open reading frame (ORF).

[0063] In some aspects, said disorder may be a blood disorder and said marker is a marker that may be determined in one or more cell type/s that is/are found in the subject to be in contact with blood.

[0064] In some aspects, said disorder may be a brain or spine disorder and said marker may be a marker that may be determined in one or more cell type/s that is/are found in the subject to be in contact with cerebrospinal fluid.

[0065] In some aspects, said disorder may be a heart disorder and said marker may be a marker that may be determined in one or more cell type/s that is/are found in the subject to be in contact with blood or pericardial fluid.

[0066] In some aspects, said disorder may be said disorder is a prostate or bladder disorder and said marker may be a marker that may be determined in one or more cell type/s that is/are found in the subject to be in contact with urine.

[0067] In some aspects, said disorder may be an eye disorder and said marker may be a marker that may be determined in one or more cell type/s that is/are found in the subject to be in contact with tears.

[0068] In some aspects, said disorder may be a lung disorder and said marker may be a marker that may be determined in one or more cell type/s that is/are found in the subject to be in contact with pleural fluid.

[0069] The present invention also provides compositions comprising exosomes. In some aspects, the composition may be essentially free of extra-exosomal material, for use in diagnostics. In some aspects, the composition may be essentially free of extra-exosomal nucleic acid-protein complexes. In some aspects, the composition may be essentially free of extra-exosomal RNA-protein complexes.

[0070] The present invention provides a method for the treatment or prophylaxis of a disorder in a patient. In some aspects, said method may comprise exosome-mediated delivery of a therapeutic RNA to a cell.

[0071] In some aspects, said exosome-mediated delivery may occur from one donor cell to a recipient cell, and wherein the therapeutic RNA may result from transcription in the donor cell.

[0072] In some aspects, transcription in the donor cell may be inducible.

[0073] In some aspects, the delivery may be performed *ex vivo*. In some aspects, the delivery may be performed *in vivo*.

[0074] The present invention also an exosome for use in therapy. In some aspects, the present invention provides an exosome for use in delivering a therapeutic RNA to a cell. In some aspects, the exosome may be produced *in vitro*. In some aspects, the exosome may be produced *in vivo*.

[0075] The present invention also provides a therapeutic RNA for use in exosome-mediated delivery to a cell. In some aspects, the exosome may be produced *in vitro*. In some aspects, the exosome may be produced *in vivo*.

[0076] The present invention also provides a pharmaceutical composition comprising an exosome. In some aspects, said exosome may comprise a therapeutic RNA for delivery into a cell. In some aspects, the delivery may be performed *ex vivo*. In some aspects, the delivery may be performed *in vivo*. In some aspects, the cell may be capable of producing exosomes comprising a therapeutic RNA. In some aspects, the pharmaceutical composition is in a form suitable for injection.

[0077] The present invention also provides a use of a therapeutic RNA in the manufacture of a medicament for the treatment or prophylaxis of a disorder in a patient. In some aspects, the RNA may be delivered to a cell in an exosome-packaged form. In some aspects, the exosome may comprise a therapeutic RNA or delivery into a cell.

[0078] In the method, composition or use as disclosed herein, the therapeutic RNA may be translated in the recipient cell.

[0079] In the method, composition or use as disclosed herein, the therapeutic RNA may be a small interfering RNA (siRNA).

[0080] In the method, composition or use as disclosed herein, the therapeutic RNA may be a short hairpin RNA (shRNA).

[0081] Accordingly, it is an object of the invention not to encompass within the invention any previously known product, process of making the product, or method of using the product such that Applicants reserve the right and hereby disclose a disclaimer of any previously known product, process, or method. It is further noted that the invention does not intend to encompass within the scope of the invention any product, process, or making of the product or method of using the product, which does not meet the written description and enablement requirements of the USPTO (35 U.S.C. §112, first paragraph) or the EPO (Article 83 of the EPC), such that Applicants reserve the right and hereby disclose a disclaimer of any previously described

product, process of making the product, or method of using the product. It may be advantageous in the practice of the invention to be in compliance with Art. 53(c) EPC and Rule 28(b) and (c) EPC. Nothing herein is to be construed as a promise.

[0082] It is noted that in this disclosure and particularly in the claims and/or paragraphs, terms such as "comprises", "comprised", "comprising" and the like can have the meaning attributed to it in U.S. Patent law; e.g., they can mean "includes", "included", "including", and the like; and that terms such as "consisting essentially of" and "consists essentially of" have the meaning ascribed to them in U.S. Patent law, e.g., they allow for elements not explicitly recited, but exclude elements that are found in the prior art or that affect a basic or novel characteristic of the invention.

[0083] These and other embodiments are disclosed or are obvious from and encompassed by, the following Detailed Description.

BRIEF DESCRIPTION OF THE DRAWINGS

[0084] The following detailed description, given by way of example, but not intended to limit the invention solely to the specific embodiments described, may best be understood in conjunction with the accompanying drawings.

[0085] **Figure 1** shows graph of RNA fluorescence unit (FU) plotted against RNA size (nt) for various exosome purification methods.

[0086] **Figures 2A-2D** show electron microscopy (EM) photographs of exosome preparations for various exosome purification methods;(**2A**) Electron microscopy of exosomes with no treatment; (**2B**) Electron microscopy of exosomes with proteinase treated after spins; (**2C**) Side-by-side comparison of EM of untreated versus proteinase-treated; (**2D**) Electron microscopy of exosomes with proteinase treated between spins.

[0087] **Figure 3** shows results of a qRT-PCR experiment for various exosome purification methods.

[0088] **Figure 4A-4C** show RNA-Seq data, showing that the RNA profile of mRNAs in exosomes reflects that of the donor cells; (**4A**) illustrates mRNA profile in exosomes: PTMS; (**4B**) illustrates mRNA profile in exosomes: MT2A; (**4C**) illustrates mRNA profile in exosomes: Rab13.

[0089] **Figures 5A-5K** show principle and results for fluorescence imaging of cells using EU click chemistry, to assess possible exosome-mediated RNA transfer between cells; (**5A**) shows intercellular communication (**5B**) shows click-chemistry with 5-ethynyl uridine (**5C**) shows control HEK 293 cells grown in presence of 5-ethynyl uridine; (**5D**) shows negative control of HEK 293 cells with no 5-ethynyl uridine; (**5E**) illustrates RNA transfer experiment; (**5F**) shows negative control of HEK 293/ K562 cells with no 5-ethynyl uridine (**5G**) shows negative control of HEK 293/ K562 cells with no 5-ethynyl uridine with 640x magnification zoomed in; (**5H**) shows experimental #1 of HEK 293/ K562 cells with 5-ethynyl uridine (**5I**) shows experimental #1 of HEK 293/ K562 cells with 5-ethynyl uridine (**6J**) shows experimental #1 of HEK 293/ K562 cells with 5-ethynyl uridine (zoomed in); (**5K**) shows experimental #2 of HEK 293/ K562 cells with 5-ethynyl uridine.

[0090] **Figures 6A-6D** show principle and results of an experiment to assess possible exosome mediated RNA transfer between co-cultured cell lines; (**6A**) illustrates an alternative experiment of mouse-human co-culture; (**6B**) shows the experimental design; (**6C**) percentage of mouse genes with TMM > 2; (**6D**) shows mouse gene expression in human cells.

[0091] **Figures 7A-7D** illustrates Poly A selected from mRNA from two replicates of K562 cells and their exosomes was compared using RNA-Seq; (**7A**) compares cell 1 versus cell 2; (**7B**) compares exosome 1 versus exosome 2; (**7C**) compares cell 1 versus exosome 1 (**7D**) compares cell 2 versus exosome 2.

[0092] **Figure 8** illustrates that mRNA is inside the exosomes.

[0093] **Figure 9** illustrates Poly A enriched mRNA from untreated exosomes and proteinase/Rnase treated exosomes was compared using RNA-Seq.

[0094] **Figure 10** illustrates targeted pull down exosome subpopulations based on their protein marker using antibody conjugated magnetic beads.

[0095] **Figure 11** illustrates exosomes which were isolated from human CSF and mRNA for four genes (detected by qRT-PCR.) Cell RNA is used as a comparison.

DETAILED DESCRIPTION OF THE INVENTION

[0096] The terms “exosomes”, “micro-vesicles” and “extracellular vesicles” are herein used interchangeably. They refer to extracellular vesicles, which are generally of between 30 and 200

nm, for example in the range of 50-100 nm in size. In some aspects, the extracellular vesicles can be in the range of 20-300 nm in size, for example 30-250 nm in size, for example 50-200 nm in size. In some aspects, the extracellular vesicles are defined by a lipidic bilayer membrane.

[0097] As used herein, a "biological sample" may contain whole cells and/or live cells and/or cell debris. The biological sample may contain (or be derived from) a "bodily fluid". The present invention encompasses embodiments wherein the bodily fluid is selected from amniotic fluid, aqueous humour, vitreous humour, bile, blood serum, breast milk, cerebrospinal fluid, cerumen (earwax), chyle, chyme, endolymph, perilymph, exudates, feces, female ejaculate, gastric acid, gastric juice, lymph, mucus (including nasal drainage and phlegm), pericardial fluid, peritoneal fluid, pleural fluid, pus, rheum, saliva, sebum (skin oil), semen, sputum, synovial fluid, sweat, tears, urine, vaginal secretion, vomit and mixtures of one or more thereof. Biological samples include cell cultures, bodily fluids, cell cultures from bodily fluids. Bodily fluids may be obtained from a mammal organism, for example by puncture, or other collecting or sampling procedures.

[0098] The terms "subject," "individual," and "patient" are used interchangeably herein to refer to a vertebrate, preferably a mammal, more preferably a human. Mammals include, but are not limited to, murines, simians, humans, farm animals, sport animals, and pets. Tissues, cells and their progeny of a biological entity obtained in vivo or cultured in vitro are also encompassed.

[0099] The terms "therapeutic agent", "therapeutic capable agent" or "treatment agent" are used interchangeably and refer to a molecule or compound that confers some beneficial effect upon administration to a subject. The beneficial effect includes enablement of diagnostic determinations; amelioration of a disease, symptom, disorder, or pathological condition; reducing or preventing the onset of a disease, symptom, disorder or condition; and generally counteracting a disease, symptom, disorder or pathological condition.

[00100] As used herein, "treatment" or "treating," or "palliating" or "ameliorating" are used interchangeably. These terms refer to an approach for obtaining beneficial or desired results including but not limited to a therapeutic benefit and/or a prophylactic benefit. By therapeutic benefit is meant any therapeutically relevant improvement in or effect on one or more diseases, conditions, or symptoms under treatment. For prophylactic benefit, the compositions may be administered to a subject at risk of developing a particular disease, condition, or symptom, or to

a subject reporting one or more of the physiological symptoms of a disease, even though the disease, condition, or symptom may not have yet been manifested.

[00101] The term "effective amount" or "therapeutically effective amount" refers to the amount of an agent that is sufficient to effect beneficial or desired results. The therapeutically effective amount may vary depending upon one or more of: the subject and disease condition being treated, the weight and age of the subject, the severity of the disease condition, the manner of administration and the like, which can readily be determined by one of ordinary skill in the art. The term also applies to a dose that will provide an image for detection by any one of the imaging methods described herein. The specific dose may vary depending on one or more of: the particular agent chosen, the dosing regimen to be followed, whether it is administered in combination with other compounds, timing of administration, the tissue to be imaged, and the physical delivery system in which it is carried.

[00102] The practice of the present invention employs, unless otherwise indicated, conventional techniques of immunology, biochemistry, chemistry, molecular biology, microbiology, cell biology, genomics and recombinant DNA, which are within the skill of the art. See Sambrook, Fritsch and Maniatis, MOLECULAR CLONING: A LABORATORY MANUAL, 2nd edition (1989); CURRENT PROTOCOLS IN MOLECULAR BIOLOGY (F. M. Ausubel, et al. eds., (1987)); the series METHODS IN ENZYMOLOGY (Academic Press, Inc.): PCR 2: A PRACTICAL APPROACH (M.J. MacPherson, B.D. Hames and G.R. Taylor eds. (1995)), Harlow and Lane, eds. (1988) ANTIBODIES, A LABORATORY MANUAL, and ANIMAL CELL CULTURE (R.I. Freshney, ed. (1987)).

[00103] In aspects of the invention functional genomics screens allow for discovery of novel human and mammalian therapeutic applications, including the discovery of novel drugs, for, e.g., treatment of genetic diseases, cancer, fungal, protozoal, bacterial, and viral infection, ischemia, vascular disease, arthritis, immunological disorders, etc. As used herein assay systems may be used for a readout of cell state or changes in phenotype include, e.g., transformation assays, e.g., changes in proliferation, anchorage dependence, growth factor dependence, foci formation, growth in soft agar, tumor proliferation in nude mice, and tumor vascularization in nude mice; apoptosis assays, e.g., DNA laddering and cell death, expression of genes involved in apoptosis; signal transduction assays, e.g., changes in intracellular calcium, cAMP, cGMP, IP3, changes in hormone and neurotransmittor release; receptor assays, e.g., estrogen receptor and cell growth;

growth factor assays, e.g., EPO, hypoxia and erythrocyte colony forming units assays; enzyme product assays, e.g., FAD-2 induced oil desaturation; transcription assays, e.g., reporter gene assays; and protein production assays, e.g., VEGF ELISAs.

[00104] In the purification methods of the invention, it was found advantageous to perform a proteinase treatment, especially after the final ultracentrifugation step carried out for exosome preparation. Without being bound by theory, it is hypothesized that such treatment allows the removal of non exosomal nucleic acid-protein complexes, such as RNA-protein complexes. The proteinase treatment (and inactivation thereof), may then be followed by an RNAse treatment.

[00105] The exosome purification methods of the invention allows one to prepare compositions comprising exosomes, wherein the composition is essentially free of extra-exosomal material, and/or essentially free of extra-exosomal nucleic acid-protein complexes, and/or essentially free of extra-exosomal RNA-protein complexes. Such compositions may be used for exosomal RNA preparation.

[00106] The purification method of the invention may include the following: removal of live cells, dead cells and larger cell debris, which may be performed by centrifugation steps and collection of the corresponding supernatants; filtration using a submicron filter such as a 0.22 micron filter; collection of exosomes by ultracentrifugation (typically at 100*g*-130,000*g*, for example 120,000*g*); washing exosomes before an additional ultracentrifugation step; proteinase treatment and inactivation; RNase treatment and inactivation.

[00107] According to one aspect of the invention, a strong correlation can advantageously be established between the RNA profile, and notably the mRNA profile, of isolated or purified exosomes and the RNA profile of the corresponding donor cells. In particular, a correlation has been shown between the mRNA profile of exosomes from K562 cells which have been isolated or purified as per the purification method or the invention, notably after treatment with protease and then RNAse, and the RNA profile of donor K562 cells. Such a correlation has been shown for the first time and is advantageous for diagnostic applications, as the transcriptome profile from exosomes of a cell population very faithfully reflects the corresponding cellular transcriptome.

[00108] Furthermore, a correlation can also be established between the RNA content (notably the mRNA content) of purified or isolated exosomes treated with protease and RNase and the RNA content of protease/RNAse untreated exosomes. These results illustrate that the analyzed

RNA content of exosomes isolated or purified as per the purification method of the invention is actually inside said exosomes and not simply externally associated with exosomes. Analyses of the RNA exosomal content can be performed using any transcriptomics method (see notably Wang et.al, Nature Review Genetics (10) 57-63), such as RNA seq (for which a princeps protocol is notably described in Macosko E Z et al., 2015, Cell 161, 1202-1214) , RT-PCT (notably qRP-PCR), small RNA sequencing (Li et.al, NAR 41(6) 3619-3634) or microarray. RNA analysis can also be performed as described in "*Chip-based analysis of exosomal mRNA mediating drug resistance in glioblastoma*". Shao H, Chung J, Lee K, Balaj L, Min C, Carter BS, Hochberg FH, Breakefield XO, Lee H, Weissleder R. Nat Commun. 2015 May 11;6:6999. doi: 10.1038/ncomms7999. PMID: 25959588; "*Microfluidic isolation and transcriptome analysis of serum microvesicles*". Chen C, Skog J, Hsu CH, Lessard RT, Balaj L, Wurdinger T, Carter BS, Breakefield XO, Toner M, Irimia D. Lab Chip. 2010 Feb 21;10(4):505-11. doi: 10.1039/b916199f. Epub 2009 Dec 8. PMID: 20126692.

[00109] In some aspects, the purification method of the invention may further comprise a step of separating one or more sub-populations of exosomes from a purified pool of exosomes. Indeed in some aspects of the invention, a sub-population of exosomes from a mixed exosome population, found for example in a biological sample obtained from a body fluid, can be further purified or isolated, for example according to one or more specific donor cell types or donor cell subtypes. In some aspects, the purification method of the invention allows to isolate or purify subpopulations of exosomes from one or more cell types or cell subtypes, preferentially from a single cell type, or from a single cell subtype.

[00110] In some aspects, a cell population can comprise one or more cell types, notably 2 or more cell types, 3 or more cell types, 4 or more cell types, or 5 or more cell types. In some aspects, a cell population comprises at least 1 to 40 cell types, notably at least 1 to 30, at least 5 to 20, at least 5 to 10, at least 2 to 8 or at least 2 to 5 cell types. Therefore, cell type or cell subtype exosomes can be purified from a mixed exosome population obtained from a cell population.

[00111] In some aspects, cell types according to the invention comprises cell types derived from the endoderm, cell types derived from the mesoderm, or cell types derived from the ectoderm. Cell types derived from the endoderm can comprise cell types of the respiratory system, the intestine, the liver, the gallbladder, the pancreas, the islets of Langerhans, the thyroid

or the hindgut. Cell types derived from the mesoderm can comprise osteochondroprogenitor cells, muscle cells, cell types from the digestive system, renal stem cells, cell types from the reproductive system, bloods cell types or cell types from the circulatory system (such as endothelial cells). Cell types derived from the ectoderm can comprise epithelial cells, cell types of the anterior pituitary, cell types of the peripheral nervous system, cell types of the neuroendocrine system, cell types of the teeth, cell types of the eyes, cell types of the central nervous system, cell types of the ependymal or cell types of the pineal gland. For example, a cell population from the central and peripheral nervous system can comprise cell types such as neurons, Schwann cells, satellite glial cells, oligodendrocytes or astrocytes. In some aspects of the invention, the one or more cell types comprise cancer cells or circulating tumor cells. Preferentially, said cancer cells or CTCs derive from the cell types as listed above. A cell type can also encompass one or more cell subtypes, notably 2 or more, 3 or more, 4 or more, 5 or more and up to 10 or more cell subtypes. For example neurons encompass various cell subtypes such as for example interneurons, pyramidal neurons, gabaergic neurons, dopaminergic neurons, serotoninergic neurons, glutamatergic neurons, motor neurons from the spinal cord, or inhibitory spinal neurons. Different cell types or cell subtypes can also be discriminated according to their respective transcriptome profile.

[00112] In some aspects, purification or isolation or exosomes according to a specific cell type or a cell subtype is achieved through one or more purification steps. In some aspects the one or more purification steps are based on the affinity of a bait molecule for a prey exosome biomarker.

[00113] In some aspects, bait molecules may be an antibody that binds exosome transmembrane protein. In some aspects, a bait molecule may be an RNA aptamer.

[00114] Prey exosome biomarkers according to the invention can be specific for one or more cell types or cell subtypes. Preferentially, prey exosomes biomarkers are membrane proteins. In this context, analysis of exosomal RNA content is highly relevant for diagnostic applications, as compared to the analysis of circulating DNA or RNA because it allows identification of the donor cell type or cell subtype though specific trans-membrane protein affinity purification, such as protein pull-up.

[00115] Exosome biomarkers can be typically identified through mass spectrometry analyses of exosomes obtained from specific cell types or cell subtypes, and if required confirmed through

western blotting or qRT-PCR analysis in said exosomes. For example exosomes from induced pluripotent stem cells (IPS cells) or IPS-derived- neurons can be used, but exosomes from any cell types or cell subtypes as defined above can be subjected to mass spectrometry analysis for identification of specific trans-membrane protein biomarkers. For example, mass spectrometry analysis can also be performed on total exosomes from a body fluid, such as CSF. Analysis of the transcriptome of CSF exosomes is of high interest because such exosome population is specific of the brain cell population.

[00116] Data obtained from such mass spectrometry analysis can be combined with genome or transcriptome analysis of corresponding donor cells in order to identify relevant biomarkers. This facilitates the identification of relevant exosome biomarkers useful for the present invention. For example, regarding CNS genetic information, lists of genes are available from e.g. "*Establishing the Proteome of Normal Human Cerebrospinal Fluid*" Schutzer S E et al., PLoS One, 2010; 5(6): e10980. "*An RNA-Sequencing Transcriptome and Splicing Database of Glia, Neurons, and Vascular Cells of the Cerebral Cortex*" Zhang Y et al., The Journal of Neuroscience, 2014, 34(36):11929 –11947. "*Purification and Characterization of Progenitor and Mature Human Astrocytes Reveals Transcriptional and Functional Differences with Mouse*" Zhang et al., 2016, Neuron 89, 37–53.

[00117] In some aspects of the invention, prey exosome biomarkers from neurons comprise one or more selected from proteins as per Table D, column G; or proteins as per Table D, column H; or proteins as per Table D, column I; or proteins as per Table D, column J; or proteins as per Table D, column K; or proteins as per Table D, column L; or proteins as per Table D, column M. In one embodiment, the prey exosome biomarker is FLRT3 and/or L1CAM. The presence of the at least one of these trans-membrane protein biomarkers in neuron exosomes can be confirmed through western blotting or RT-PCT analysis or neuron exosomes.

[00118] "*Dysfunctionally phosphorylated type 1 insulin receptor substrate in neural-derived blood exosomes of preclinical Alzheimer's disease*". Kapogiannis D, Boxer A, Schwartz JB, Abner EL, Biragyn A, Masharani U, Frassetto L, Petersen RC, Miller BL, Goetzl EJ. FASEB J. 2015 Feb;29(2):589-96. doi: 10.1096/fj.14-262048. Epub 2014 Oct 23. PMID: 25342129 and "*Plasma exosomal α-synuclein is likely CNS-derived and increased in Parkinson's disease*". Shi M, Liu C, Cook TJ, Bullock KM, Zhao Y, Ginghina C, Li Y, Aro P, Dator R, He C, Hipp MJ, Zabetian CP, Peskind ER, Hu SC, Quinn JF, Galasko DR, Banks WA, Zhang J. Acta

Neuropathol. 2014 Nov;128(5):639-50. doi: 10.1007/s00401-014-1314-y. Epub 2014 Jul 6. PMID: 24997849 describe analysis of exosomes obtained from plasma, but as such do not provide informative or conclusive evidence establishing a relationship with a specific organ of origin (such as brain) or specific tissue of origin or a fortiori specific cell types of origin such as neurons. This is because of the circulating nature of plasma that comes into contact with a number of various organs, tissues, etc., and thus may comprise exosomes stemming from a plurality of different cell types altogether. Further, it is unclear whether some exosomes are capable of corrsing the blood brain barrier. As a consequence, the data reported in these papers do not allow to identify the exact origin of the exosomes, and in particular cannot relate to exosomes from a specific cell type (such as neurons). Further, these papers do not disclose any RNA profiling, in particular, no RNA-seq analysis..

[00119] By contrast, the present invention provides methods for accessing information on tissue- or cell-type- specific exosomes, in particular tissue- or cell-type- specific transcription profiles. The present invention also provides very-high resolution diagnostic methods, wherein a subtle change in transcription profiles (e.g. a small up- or down-regulation in the transcription of a given gene in a given cell type or a given cell sub-type) can advantageously be efficiently detected, while it could not be in a total RNA or total exosome analysis.

[00120] In some aspects the one or more purification steps can comprise a microfluidic affinity based purification (see for example "*Chip-based analysis of exosomal mRNA mediating drug resistance in glioblastoma*". Shao H, Chung J, Lee K, Balaj L, Min C, Carter BS, Hochberg FH, Breakefield XO, Lee H, Weissleder R. Nat Commun. 2015 May 11;6:6999. doi: 10.1038/ncomms7999. PMID: 25959588; "*Microfluidic isolation and transcriptome analysis of serum microvesicles*". Chen C, Skog J, Hsu CH, Lessard RT, Balaj L, Wurdinger T, Carter BS, Breakefield XO, Toner M, Irimia D. Lab Chip. 2010 Feb 21;10(4):505-11. doi: 10.1039/b916199f. Epub 2009 Dec 8. PMID: 20126692.), a magnetic based purification, a pull-down purification or a fluorescence activated vesicle sorting-based purification (FAVS, see for example Van der Pol E et al., J Thromb Haemost., 2013 Jun;11 Suppl 1:36-45 "*Innovation in detection of microparticles and exosomes*" and Van des Pol E. et al., J Thromb Haemost. 2012 May;10(5):919-30), "*Single vs. swarm detection of microparticles and exosomes by flow cytometry*"; "*Glypican-1 identifies cancer exosomes and detects early pancreatic cancer*". Melo SA, Luecke LB, Kahlert C, Fernandez AF, Gammon ST, Kaye J, LeBleu VS, Mittendorf EA,

Weitz J, Rahbari N, Reissfelder C, Pilarsky C, Fraga MF, Piwnica-Worms D, Kalluri R. Nature. 2015 Jul 9;523(7559):177-82. doi: 10.1038/nature14581. Epub 2015 Jun 24. PMID: 26106858).. Commercial precipitation kits like ExoQuick™ and Total Exosome Isolation™ precipitation solutions are also available. Such kits are easy to use with only 1 or 2 steps and do not require any expensive equipment or advanced technical know-how..

[00121] In some aspects, the bait molecule can be a bait protein, such as an antibody and in some aspects is preferentially a monoclonal antibody directed against a prey exosome biomarker. In some aspects, the bait molecule can also be an RNA aptamer. If several prey exosomes are to be combined for purification, a mix of corresponding monoclonal antibodies directed against each of the said prey exosomes biomarkers to be pull-up can be used.

[00122] In some aspects, the bait molecule is recognized by an affinity ligand. Said affinity ligand can be a divalent metal-based complex, a protein, a peptide such as fusion protein tag or more preferentially an antibody.

[00123] In some aspects, the bait molecule or the affinity ligand is immobilized or "coupled" directly, or indirectly to a solid substrate material such as by formation of covalent chemical bonds between particular functional groups on the ligand (for example primary amines, thiols, carboxylic acids, aldehydes) and reactive groups on the substrate. A substrate, or a matrix, in the affinity purification steps of the method of the invention can be any material to which a biospecific ligand (i.e., the bait molecule or the affinity ligand) is coupled. Useful affinity supports may be those with a high surface-area to volume ratio, chemical groups that are easily modified for covalent attachment of ligands, minimal nonspecific binding properties, good flow characteristics and/or mechanical and chemical stability. Several substrates may be utilized as solid substrate, including for example agarose, cellulose, dextran, polyacrylamide, latex or controlled pore glass. Magnetic particles may also be used as a substrate instead of beaded agarose or other porous resins. Their small size provides the sufficient surface area-to-volume ratio needed for effective ligand immobilization and affinity purification. Magnetic beads may be produced as superparamagnetic iron oxide particles that may be covalently coated with silane derivatives. The coating makes the beads inert (i.e., to minimize nonspecific binding) and provides the particular chemical groups needed for attaching any affinity ligands of interest. Affinity purification with magnetic particles is generally not performed in-column. Instead, a few microliters of beads may be mixed with several hundred microliters of sample as a loose slurry.

During mixing, the beads remain suspended in the sample solution, allowing affinity interactions to occur with the immobilized ligand. After sufficient time for binding has been given, the beads are collected and separated from the sample using a powerful magnet. An exemplary bead purification method can be found in "*Proteomic comparison defines novel markers to characterize heterogeneous populations of extracellular vesicle subtypes*". Kowal J, Arras G, Colombo M, Jouve M, Morath JP, Primdal-Bengtson B, Dingli F, Loew D, Tkach M, Théry C. Proc Natl Acad Sci U S A. 2016 Feb 23;113(8):E968-77. doi: 10.1073/pnas.1521230113. Epub 2016 Feb 8. PMID: 26858453.

[00124] In some aspects of the invention, a pull down assay can be performed for the purification or isolation of a subpopulation of exosomes by pulling-down of one or more specific prey exosome biomarkers (preferentially a membrane protein as described below). Said prey exosome biomarkers may be specific of a at least one cell type or cell subtype and advantageously lead to enriching in exosomes from said selected cell type or cell subtype.

[00125] In some aspects the at least one or more purification steps for the purification of an exosome subpopulation comprise a pull down purification. In such pull-down purification, the prey exosome biomarker is generally a (trans)membrane protein, which has been found to be expressed in a cell type or a cell subtype. The bait protein is preferentially a monocolonal antibody directed against any of the prey exosome biomarker(s) which is to be pulled-up. Magnetic beads (for example Dynabeads® from Thermo Fisher Scientific) coated with an affinity ligand for the bait protein can be used to isolate said bait protein bound to said prey exosome biomarker(s). The affinity ligand is preferentially a class specific or a species specific antibody. As a matter of example, magnetic beads coated with anti-mouse antibodies can be used together with monoclonal mouse antibodies directed against a specific surface protein of a cell type or cell subtype subpopulation of exosomes (such as for example CD63 or CD81). Generally, a control antibody, such as a mouse mcherry monoclonal antibody, can be used.

[00126] A pull down assay can therefore be used to illustrate and validate the purification, or isolation of at least two exosome subpopulations expressing each at least one specific membrane protein, such as the canonical exosomes markers CD63 and CD81, which have previously been pooled. As shown in the results examples, said at least two exosomes subpopulations can be re-separated based on the selected protein biomarker. The purification or isolation of exosome

subpopulations by at least one specific prey exosome biomarker (preferentially a membrane protein) can be further confirmed using wertern blot or qRT-PCT.

[00127] Several control experiments can also be envisioned to compare the transcriptome of subpopulation of exosomes, purified or isolated by pull-up of at least one specific exosome biomarker, according to the method of the invention.

- It is advantageously possible to compare the transcriptome profile of at least two subpopulations of exosomes, purified from a mixed exosome population (*e.g.*: obtained from a cell population comprising one or more cell types, such as the K562 cells) using specific exosome biomarkers (such as CD63 or CD81) as described above (*e.g.*: using magnetic beads pull-down purification). The transcriptome profile of said exosomes subpopulations can also be further compared to the transcriptome profile of the total exosome population. Typically RNA seq analysis of exosomes is particulary well suited for such transcriptome comparisons.
- It is advantageously possible to compare the RNA seq analysis of total RNA, mRNA, micro RNA (miRNA), or long non coding RNA (lncRNA) of (i) at least one cell type and (ii) exosomes obtained from said at least one cell type. As a matter of example, it is possible to perform RNA seq analysis of mRNA from (i) IPS cells and IPS-derived neurons, and (ii) exosomes obtained respectively from said IPS cells and IPS-derived neurons and and then compare the obtained results.
- It is advantageously possible to compare (i) transcriptome profile analysis (notably the RNA seq analysis) of exosomes from the said different cell types or subtypes, isolated according through the purification method of the invention (notably using antibody-conjugated magnetic beads as described above) in order to enrich for exosomes expressing at least one cell type or cell subtype specific biomarker, with (ii) the transcriptome profile of total exosomes. For example the RNA seq results of exosomes from IPS cells and neuron exosomes isolated according to the pull down assay as described above can be compared to the RNA profile of total exosomes from both cell types.
- In vitro experiments for the control of the purification of exosome subpopulations can also comprise experiments, wherein exosomes subspopulations are purified or isolated from a complex biological sample obtained from at least two cell

populations, cell types, or cell subtypes. For example, from a mix of media obtained from cell culture of different cell types such as IPS cells and neurons. Exosomes of the specific cells types are then purified as described above and their transcriptome is analysed. Such an experiment allows reconstructing, ex post facto, the transcriptome of the original cell type.

[00128] Isolation or purification of total exosomes from biological samples derived from any body fluid such as CSF, urine, or blood etc. and transcriptome analysis of the obtained exosome population can also be envisioned. Using cell-type specific biomarkers, exosome subpopulations can be further purified through any of the purification steps as described above, and enrichment in expression of specific cell type biomarkers can be searched through transcriptome analysis of this subpopulation as compared to the total exosome population. Said analysis is of particular interest for CSF analysis and identification of exosomes from specific neuronal subtypes

[00129] According to the present invention, the RNA content of exosomes is found to correlate the RNA content of the corresponding cell. In other terms, in particular when exosomes are purified in accordance with purification method of the present invention, a correlation was found between said exosomal RNA content and corresponding cellular RNA content. Therefore, analyzing exosomal RNA provides both qualitative and quantitative information about the cellular RNA content of the corresponding cells. Advantageously, this makes it possible to provide non-invasive diagnostic methods. Indeed, the analysis (whether by RNA seq, transcriptome profiling, qRT-PCR or array) is performed on a biological sample derived from body fluids, such as derived from urine, blood or cerebrospinal fluid. Such fluids are more easily and readily available than corresponding organs (bladder, heart or brain). Correspondingly, the present invention provides diagnostic methods that are non-invasive and yet reliable. In some aspects, it is envisioned to use a subpopulation of exosomes as starting material to extract RNA. This may allow the analysis of exosome subpools/subpopulations.

[00130] If reasoning that exosomes contribute to RNA transport, then exosomes could provide a delivery system in therapeutics. This would allow the delivery of a therapeutic RNA to a cell, wherein said therapeutic RNA may silence or express a gene in a cell. The present invention contemplates delivery of the exosome itself, or of an exosome-shedding cell. The delivery may occur *in vivo* or *ex vivo*. Delivery may rely on a targeting ligang. Said targeting ligand may be one or more prey exosome biomarker as decribed herein. For example, the prey exosome

biomarker may be selected from proteins as per Table D, column G; or proteins as per Table D, column H; or proteins as per Table D, column I; or proteins as per Table D, column J; or proteins as per Table D, column K; or proteins as per Table D, column L; or proteins as per Table D, column M; the prey exosome biomarker may be FLRT3 and/or L1CAM; or efficient fragments thereof.

[00131] The term "library" as used herein generally means a multiplicity of member components constituting the library which member components individually differ with respect to at least one property, for example, a scFv library. Particularly, as will be apparent to the skilled artisan, "library" means a plurality of nucleic acids / polynucleotides, preferably in the form of vectors comprising functional elements (promoter, transcription factor binding sites, enhancer, etc.) necessary for expression of polypeptides or RNA molecules, either *in vitro* or *in vivo*, which are functionally linked to coding sequences for polypeptides or RNA molecules. The vector can be a plasmid or a viral-based vector suitable for expression in prokaryotes or eukaryotes or both, preferably for expression in mammalian cells. There should also be at least one, preferably multiple pairs of cloning sites for insertion of coding sequences into the library, and for subsequent recovery or cloning of those coding sequences. The cloning sites can be restriction endonuclease recognition sequences, or other recombination based recognition sequences such as loxP sequences for Cre recombinase, or the Gateway system (ThermoFisher, Inc.) as described in U.S. Pat. No. 5,888,732, the contents of which is incorporated by reference herein. Coding sequences for polypeptides can be cDNA, genomic DNA fragments, or random/semi-random polynucleotides. The methods for cDNA or genomic DNA library construction are well-known in the art, which can be found in a number of commonly used laboratory molecular biology manuals described herein.

[00132] In an aspect, the present invention provides for libraries of polynucleotide sequences encoding for interacting protein or RNA molecules. Methods of making libraries are well known in the art, in which the methods may use any of a variety of reverse transcriptases and optionally other DNA polymerases, vectors for cloning cDNAs, as well as adapters, linkers, restriction enzymes, and ligases or recombination enzymes for combining synthesized cDNA molecules with vectors. In some preferred embodiments of the invention, recombinational cloning is employed to insert cDNA molecules into expression vectors, and in these embodiments, adapters comprise recognition sites for recombination enzymes.

[00133] Members of a library may include any protein or RNA molecule chosen from any protein or RNA molecule of interest and includes protein or RNA molecules of unknown, known, or suspected diagnostic, therapeutic, or pharmacological importance. For example, the protein of interest can be a protein or RNA molecule suspected of being involved in a cellular process, for example, receptor signaling, apoptosis, cell proliferation, cell differentiation, immune responses or import or export of toxins and nutrients. The present invention can allow for genome wide interaction studies of key proteins expressed during these different immune cell states. As such, protein or RNA molecules of interest may be protein or RNA molecules expressed from an entire genome. Protein or RNA molecules expressed from a single cell type or from cells having a specific cell state may also be chosen.

[00134] The protein molecules of the present invention can be derived from all or a portion of a known protein or a mutant thereof, all or a portion of an unknown protein (e.g., encoded by a gene cloned from a cDNA library), or a random polypeptide sequence. Members of a DNA expression library, such as a cDNA or synthetic DNA library may be used. The full length of the protein or RNA molecule of interest, or a portion thereof, can be used. In the instance when the protein of interest is of a large size, e.g., has a molecular weight of over 20 kDa, it may be more convenient to use a portion of the protein.

[00135] Polynucleotide sequences which encode the protein or RNA molecule of interest may be inserted into a vector such that the desired protein or RNA molecule is produced in a host mammalian cell. The vectors may include a proximity detection molecule. The proximity detection molecules may be encoded in-frame with a polynucleotide sequence encoding for a protein library member. In the case of RNA molecules, the vector encoding an RNA molecule or a separate vector may encode for a fusion protein that recognizes a loop structure within the RNA molecule. In preferred embodiments, the fusion protein is encoded by a polynucleotide sequence on the same vector as the RNA molecule, such that if a cell expresses the RNA molecule it will also express the fusion protein. The fusion protein includes a proximity detection molecule, thereby allowing the RNA molecule to be bound by a proximity detection molecule after expression. Preferably, the recombinant expression vector includes one or more regulatory sequences operably linked to the polynucleotide sequence to be expressed. The term “regulatory sequence” includes promoters, enhancers and other expression control elements (e.g., polyadenylation signals).

[00136] In an exemplary embodiment, a cDNA library may be constructed from an mRNA population and inserted into an expression vector. Such a library of choice may be constructed de novo using commercially available kits or using well established preparative procedures (see, for example, Current Protocols in Molecular Biology, Eds. Ausubel et al. John Wiley & Sons: 1992). Alternatively, a number of cDNA libraries (from a number of different organisms) are publicly and commercially available. In the instance where it is preferable to replicate and store the polynucleotide sequences using a bacterial host cell, the DNA sequences are inserted into a vector which contains an appropriate origin of replication. It is also noted that protein or RNA molecules need not be naturally occurring full-length protein or RNA molecules. In certain embodiments, protein or RNA molecules can be encoded by synthetic DNA sequences.

[00137] The polynucleotide sequences encoding the desired protein or RNA molecule are typically operably linked to suitable transcriptional or translational regulatory elements. The regulatory elements typically include a transcriptional promoter, a sequence encoding suitable mRNA ribosomal binding sites, and sequences that control the termination of transcription and translation. The ability to replicate in a host, usually conferred by an origin of replication, and a selection gene to facilitate recognition of transformants may additionally be incorporated.

[00138] The nucleic acid sequences encoding the proteins or RNA molecules may be expressed in a variety of host cells, including E. coli and other bacterial hosts, and preferably eukaryotic host cells including but not limited to yeast, insect cells, and mammalian cells. The polynucleotide sequences will be operably linked to appropriate expression control sequences for each host. In a most preferred embodiment, the host cells comprise mammalian cells.

[00139] Many different mammalian cell types may be used in the practice of the invention. Cells suitable for use include primary cultures, cultures of immortalized cells or genetically manipulated strains of cells.

[00140] One of the main criteria for selection of a particular cell type may be the nature of post translational modification of target proteins expressed where the binding of such modified target proteins to a protein, RNA or small molecule may more accurately mimic the natural state. Cells that are associated with a particular disease state, or that originate from a particular tissue type may be chosen. Another criteria is the selection of a suitable cellular background to mimic the activity of a small molecule in its target tissue or cell type. If studying toxicity it may be appropriate to select a cell type associated with that toxicity, e.g. liver. Cell lines recognized in

the art as easy to transfect are particularly preferred. Different mammalian cell types may also be selected according to their permeability.

[00141] Cells may also be selected on the basis of their adherence to the chosen substrate, their rate of growth, and the ease with which they can be maintained in culture. Preferably the cells are human cells.

[00142] Any cultured mammalian cell can be used in the present invention, e.g., a primary, secondary, or immortalized cell. Exemplary mammalian cells are those of mouse, hamster, rat, rabbit, dog, cow, and primate including human. They may be of a wide variety of tissue types, including mast cells, endothelial cells, hepatic cells, kidney cells, or other cell types.

[00143] As used herein, the term primary cell means cells isolated from a mammal (e.g., from a tissue source), which are grown in culture for the first time before subdivision and transfer to a subculture. The term secondary cell means cells at all subsequent steps in culturing. That is, the first time a primary cell is removed from the culture substrate and passaged, it is referred to as a secondary cell, as are all cells in subsequent passages. Examples of mammalian primary and secondary cells which can be transfected include fibro-blasts, keratinocytes, epithelial cells (e.g., mammary epithelial cells, intestinal epithelial cells), endothelial cells, glial cells, neural cells, formed elements of the blood (e.g., lymphocytes, bone marrow cells), muscle cells and precursors of these somatic cell types.

[00144] Immortalized cells are cell lines that exhibit an apparently unlimited lifespan in culture. Examples of immortalized human cell lines useful for the present invention include, but are not limited to, HEK 293 cells and derivatives of HEK 293 cells (ATCC CRL 1573), HT1080 cells (ATCC CCL 121), HeLa cells and derivatives of HeLa cells (ATCC CCL 2, 2.1 and 2.2), MCF-7 breast cancer cells (ATCC HTB 22), K-562 leukemia cells (ATCC CCL 243), KB carcinoma cells (ATCC CCL 17), Raji cells (ATCC CCL 86), Jurkat cells (ATCC TIB 152), Namalwa cells (ATCC CRL 1432), HL-60 cells (ATCC CCL 240), Daudi cells (ATCC CCL 213), RPMI 8226 cells (ATCC CCL 155), U-937 cells (ATCC CRL 1593), Bowes Melanoma cells (ATCC CRL 9607), WI-38 cells (ATCC CLL 75), and MOLT-4 cells (ATCC CRL 1582).

[00145] The exosomes of the present invention may be loaded with exogenous cargoes, such as a therapeutic RNA, using electroporation protocols adapted for nanoscale applications (see, e.g., Alvarez-Erviti et al. 2011, Nat Biotechnol 29: 341). As electroporation for membrane particles at the nanometer scale is not well-characterized, nonspecific Cy5-labeled siRNA was

used for the empirical optimization of the electroporation protocol. The amount of encapsulated siRNA was assayed after ultracentrifugation and lysis of exosomes. Electroporation at 400 V and 125 μF resulted in the greatest retention of siRNA and was used for all subsequent experiments.

[00146] Alvarez-Erviti et al. administered 150 μg of each BACE1 siRNA encapsulated in 150 μg of RVG exosomes to normal C57BL/6 mice and compared the knockdown efficiency to four controls: untreated mice, mice injected with RVG exosomes only, mice injected with BACE1 siRNA complexed to an in vivo cationic liposome reagent and mice injected with BACE1 siRNA complexed to RVG-9R, the RVG pep tide conjugated to 9 D-arginines that electrostatically binds to the siRNA. Cortical tissue samples were analyzed 3 d after administration and a significant protein knockdown (45%, $P < 0.05$, versus 62%, $P < 0.01$) in both siRNA-RVG-9R-treated and siRNARVG exosome-treated mice was observed, resulting from a significant decrease in BACE1 mRNA levels (66% [+ or -] 15%, $P < 0.001$ and 61% [+ or -] 13% respectively, $P < 0.01$). Moreover, Applicants demonstrated a significant decrease (55%, $P < 0.05$) in the total [beta]-amyloid 1-42 levels, a main component of the amyloid plaques in Alzheimer's pathology, in the RVG-exosome-treated animals. The decrease observed was greater than the β-amyloid 1-40 decrease demonstrated in normal mice after intraventricular injection of BACE1 inhibitors. Alvarez-Erviti et al. carried out 5'-rapid amplification of cDNA ends (RACE) on BACE1 cleavage product, which provided evidence of RNAi-mediated knockdown by the siRNA.

[00147] Finally, Alvarez-Erviti et al. investigated whether siRNA-RVG exosomes induced immune responses in vivo by assessing IL-6, IP-10, TNFα and IFN-α serum concentrations. Following siRNA-RVG exosome treatment, nonsignificant changes in all cytokines were registered similar to siRNA-transfection reagent treatment in contrast to siRNA-RVG-9R, which potently stimulated IL-6 secretion, confirming the immunologically inert profile of the exosome treatment. Given that exosomes encapsulate only 20% of siRNA, delivery with RVG-exosome appears to be more efficient than RVG-9R delivery as comparable mRNA knockdown and greater protein knockdown was achieved with fivefold less siRNA without the corresponding level of immune stimulation. This experiment demonstrated the therapeutic potential of RVG-exosome technology, which is potentially suited for long-term silencing of genes related to neurodegenerative diseases. The exosome delivery system of Alvarez-Erviti et al. may be applied to deliver the exosome of the present invention to therapeutic targets, especially

neurodegenerative diseases. A dosage of about 100 to 1000 mg of a target RNA encapsulated in about 100 to 1000 mg of exosomes may be contemplated for the present invention.

[00148] El-Andaloussi et al. (Nature Protocols 7,2112–2126(2012)) discloses how exosomes derived from cultured cells can be harnessed for delivery of siRNA in vitro and in vivo. This protocol first describes the generation of targeted exosomes through transfection of an expression vector, comprising an exosomal protein fused with a peptide ligand. Next, El-Andaloussi et al. explain how to purify and characterize exosomes from transfected cell supernatant. Next, El-Andaloussi et al. detail crucial steps for loading siRNA into exosomes. Finally, El-Andaloussi et al. outline how to use exosomes to efficiently deliver siRNA in vitro and in vivo in mouse brain. Examples of anticipated results in which exosome-mediated siRNA delivery is evaluated by functional assays and imaging are also provided. The entire protocol takes ~3 weeks. Delivery or administration according to the invention may be performed using exosomes produced from self-derived dendritic cells.

[00149] In another embodiment, the plasma exosomes of Wahlgren et al. (Nucleic Acids Research, 2012, Vol. 40, No. 17 e130) are contemplated. Exosomes are nano-sized vesicles (30–90nm in size) produced by many cell types, including dendritic cells (DC), B cells, T cells, mast cells, epithelial cells and tumor cells. These vesicles are formed by inward budding of late endosomes and are then released to the extracellular environment upon fusion with the plasma membrane. Because exosomes naturally carry RNA between cells, this property might be useful in gene therapy.

[00150] The chemical transfection of a target RNA into exosomes may be conducted similarly to siRNA (see, e.g., Wahlgren et al. Nucleic Acids Research, 2012, Vol. 40, No. 17 e130). The exosomes may be co-cultured with monocytes and lymphocytes isolated from the peripheral blood of healthy donors. Therefore, it may be contemplated that exosomes containing a target RNA may be introduced to monocytes and lymphocytes of and autologously reintroduced into a human.

[00151] Markers are identified for a number of disorders. Such markers are useful in the diagnostic, prognostic and/or therapy of respective disorders. Such markers include disease-associated genes and polynucleotides.

[00152] Examples of disease-associated genes and polynucleotides are available from McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University (Baltimore, Md.)

and National Center for Biotechnology Information, National Library of Medicine (Bethesda, Md).

[00153] Examples of disease-associated genes and polynucleotides are listed in Tables A and B. Disease specific information is available from McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University (Baltimore, Md.) and National Center for Biotechnology Information, National Library of Medicine (Bethesda, Md.), available on the World Wide Web. Examples of signaling biochemical pathway-associated genes and polynucleotides are listed in Table C. Mutations in these genes and pathways can result in production of improper proteins or proteins in improper amounts which affect function.

Table A

DISEASE/DISORDERS	GENE(S)
Neoplasia	PTEN; ATM; ATR; EGFR; ERBB2; ERBB3; ERBB4;
	Notch1; Notch2; Notch3; Notch4; AKT; AKT2; AKT3; HIF;
	HIF1a; HIF3a; Met; HRG; Bcl2; PPAR alpha; PPAR
	gamma; WT1 (Wilms Tumor); FGF Receptor Family
	members (5 members: 1, 2, 3, 4, 5); CDKN2a; APC; RB
	(retinoblastoma); MEN1; VHL; BRCA1; BRCA2; AR
	(Androgen Receptor); TSG101; IGF; IGF Receptor; Igf1 (4
	variants); Igf2 (3 variants); Igf 1 Receptor; Igf 2 Receptor;
	Bax; Bcl2; caspases family (9 members:
	1, 2, 3, 4, 6, 7, 8, 9, 12); Kras; Apc
Age-related Macular	Abcr; Ccl2; Cc2; cp (ceruloplasmin); Timp3; cathepsinD;
Degeneration	Vldlr; Ccr2
Schizophrenia	Neuregulin1 (Nrg1); Erb4 (receptor for Neuregulin);
	Complexin1 (Cplx1); Tph1 Tryptophan hydroxylase; Tph2
	Tryptophan hydroxylase 2; Neurexin 1; GSK3; GSK3a;
	GSK3b
Disorders	5-HTT (Slc6a4); COMT; DRD (Drd1a); SLC6A3; DAOA;
	DTNBP1; Dao (Dao1)
Trinucleotide Repeat	HTT (Huntington's Dx); SBMA/SMAX1/AR (Kennedy's
Disorders	Dx); FXN/X25 (Friedrich's Ataxia); ATX3 (Machado-
	Joseph's Dx); ATXN1 and ATXN2 (spinocerebellar
	ataxias); DMPK (myotonic dystrophy); Atrophin-1 and Atn1
	(DRPLA Dx); CBP (Creb-BP - global instability); VLDLR
	(Alzheimer's); Atxn7; Atxn10
Fragile X Syndrome	FMR2; FXR1; FXR2; mGLUR5
Secretase Related	APH-1 (alpha and beta); Presenilin (Psen1); nicastrin
Disorders	(Ncstn); PEN-2
Others	Nos1; Parp1; Nat1; Nat2

Prion - related disorders	Prp
ALS	SOD1; ALS2; STEX; FUS; TARDBP; VEGF (VEGF-a;
	VEGF-b; VEGF-c)
Drug addiction	Prkce (alcohol); Drd2; Drd4; ABAT (alcohol); GRIA2;
	Grm5; Grin1; Htr1b; Grin2a; Drd3; Pdyn; Gria1 (alcohol)
Autism	Mecp2; BZRAP1; MDGA2; Sema5A; Neurexin 1; Fragile X
	(FMR2 (AFF2); FXR1; FXR2; Mglur5)
Alzheimer's Disease	E1; CHIP; UCH; UBB; Tau; LRP; PICALM; Clusterin; PS1;
	SORL1; CR1; Vldlr; Uba1; Uba3; CHIP28 (Aqp1,
	Aquaporin 1); Uchl1; Uchl3; APP
Inflammation	IL-10; IL-1 (IL-1a; IL-1b); IL-13; IL-17 (IL-17a (CTLA8); IL-
	17b; IL-17c; IL-17d; IL-17f); II-23; Cx3cr1; ptpn22; TNFa;
	NOD2/CARD15 for IBD; IL-6; IL-12 (IL-12a; IL-12b);
	CTLA4; Cx3cl1
Parkinson's Disease	x-Synuclein; DJ-1; LRRK2; Parkin; PINK1

Table B:

Blood and coagulation diseases and disorders	Anemia (CDAN1, CDA1, RPS19, DBA, PKLR, PK1, NT5C3, UMPH1, PSN1, RHAG, RH50A, NRAMP2, SPTB, ALAS2, ANH1, ASB, ABCB7, ABC7, ASAT); Bare lymphocyte syndrome (TAPBP, TPSN, TAP2, ABCB3, PSF2, RING11, MHC2TA, C2TA, RFX5, RFXAP, RFX5), Bleeding disorders (TBXA2R, P2RX1, P2X1); Factor H and factor H-like 1 (HF1, CFH, HUS); Factor V and factor VIII (MCFD2); Factor VII deficiency (F7); Factor X deficiency (F10); Factor XI deficiency (F11); Factor XII deficiency (F12, HAF); Factor XIIIA deficiency (F13A1, F13A); Factor XIIIB deficiency (F13B); Fanconi anemia (FANCA, FACA, FA1, FA, FAA, FAAP95, FAAP90, FLJ34064, FANCB, FANCC, FACC, BRCA2, FANCD1, FANCD2, FANCD, FACD, FAD, FANCE, FACE, FANCF, XRCC9, FANCG, BRIP1, BACH1, FANCJ, PHF9, FANCL, FANCM, KIAA1596); Hemophagocytic lymphohistiocytosis disorders (PRF1, HPLH2, UNC13D, MUNC13-4, HPLH3, HLH3, FHL3); Hemophilia A (F8, F8C, HEMA); Hemophilia B (F9, HEMB), Hemorrhagic disorders (PI, ATT, F5); Leukocyde deficiencies and disorders (ITGB2, CD18, LCAMB, LAD, EIF2B1, EIF2BA, EIF2B2, EIF2B3, EIF2B5, LVWM, CACH, CLE, EIF2B4); Sickle cell anemia (HBB); Thalassemia (HBA2, HBB, HBD, LCRB, HBA1).
Cell dysregulation and oncology diseases and disorders	B-cell non-Hodgkin lymphoma (BCL7A, BCL7); Leukemia (TAL1, TCL5, SCL, TAL2, FLT3, NBS1, NBS, ZNFN1A1, IK1, LYF1, HOXD4, HOX4B, BCR, CML, PHL, ALL, ARNT, KRAS2, RASK2, GMPS, AF10, ARHGEF12, LARG, KIAA0382, CALM, CLTH, CEBPA, CEBP, CHIC2, BTL, FLT3, KIT, PBT, LPP, NPM1, NUP214, D9S46E, CAN, CAIN, RUNX1, CBFA2, AML1, WHSC1L1, NSD3,

	FLT3, AF1Q, NPM1, NUMA1, ZNF145, PLZF, PML, MYL, STAT5B, AF10, CALM, CLTH, ARL11, ARLTS1, P2RX7, P2X7, BCR, CML, PHL, ALL, GRAF, NF1, VRNF, WSS, NFNS, PTPN11, PTP2C, SHP2, NS1, BCL2, CCND1, PRAD1, BCL1, TCRA, GATA1, GF1, ERYF1, NFE1, ABL1, NQO1, DIA4, NMOR1, NUP214, D9S46E, CAN, CAIN).
Inflammation and immune related diseases and disorders	AIDS (KIR3DL1, NKAT3, NKB1, AMB11, KIR3DS1, IFNG, CXCL12, SDF1); Autoimmune lymphoproliferative syndrome (TNFRSF6, APT1, FAS, CD95, ALPS1A); Combined immunodeficiency, (IL2RG, SCIDX1, SCIDX, IMD4); HIV-1 (CCL5, SCYA5, D17S136E, TCP228), HIV susceptibility or infection (IL10, CSIF, CMKBR2, CCR2, CMKBR5, CCCKR5 (CCR5)); Immunodeficiencies (CD3E, CD3G, AICDA, AID, HIGM2, TNFRSF5, CD40, UNG, DGU, HIGM4, TNFSF5, CD40LG, HIGM1, IGM, FOXP3, IPEX, AIID, XPID, PIDX, TNFRSF14B, TACI); Inflammation (IL-10, IL-1 (IL-1a, IL-1b), IL-13, IL-17 (IL-17a (CTLA8), IL-17b, IL-17c, IL-17d, IL-17f), II-23, Cx3cr1, ptpn22, TNFa, NOD2/CARD15 for IBD, IL-6, IL-12 (IL-12a, IL-12b), CTLA4, Cx3cl1); Severe combined immunodeficiencies (SCIDs)(JAK3, JAKL, DCLRE1C, ARTEMIS, SCIDA, RAG1, RAG2, ADA, PTPRC, CD45, LCA, IL7R, CD3D, T3D, IL2RG, SCIDX1, SCIDX, IMD4).
Metabolic, liver, kidney and protein diseases and disorders	Amyloid neuropathy (TTR, PALB); Amyloidosis (APOA1, APP, AAA, CVAP, AD1, GSN, FGA, LYZ, TTR, PALB); Cirrhosis (KRT18, KRT8, CIRH1A, NAIC, TEX292, KIAA1988); Cystic fibrosis (CFTR, ABCC7, CF, MRP7); Glycogen storage diseases (SLC2A2, GLUT2, G6PC, G6PT, G6PT1, GAA, LAMP2, LAMPB, AGL, GDE, GBE1, GYS2, PYGL, PFKM); Hepatic adenoma, 142330 (TCF1, HNF1A, MODY3), Hepatic failure, early onset, and neurologic disorder (SCOD1, SCO1), Hepatic lipase deficiency (LIPC), Hepatoblastoma, cancer and carcinomas (CTNNB1, PDGFRL, PDGRL, PRLTS, AXIN1, AXIN, CTNNB1, TP53, P53, LFS1, IGF2R, MPRI, MET, CASP8, MCH5; Medullary cystic kidney disease (UMOD, HNFJ, FJHN, MCKD2, ADMCKD2); Phenylketonuria (PAH, PKU1, QDPR, DHPR, PTS); Polycystic kidney and hepatic disease (FCYT, PKHD1, ARPKD, PKD1, PKD2, PKD4, PKDTS, PRKCSH, G19P1, PCLD, SEC63).
Muscular / Skeletal diseases and disorders	Becker muscular dystrophy (DMD, BMD, MYF6), Duchenne Muscular Dystrophy (DMD, BMD); Emery-Dreifuss muscular dystrophy (LMNA, LMN1, EMD2, FPLD, CMD1A, HGPS, LGMD1B, LMNA, LMN1, EMD2, FPLD, CMD1A); Facioscapulohumeral muscular dystrophy (FSHMD1A, FSHD1A); Muscular dystrophy (FKRP, MDC1C, LGMD2I, LAMA2, LAMM, LARGE, KIAA0609, MDC1D, FCMD, TTID, MYOT, CAPN3, CANP3, DYSF, LGMD2B, SGCG, LGMD2C, DMDA1, SCG3, SGCA, ADL, DAG2, LGMD2D, DMDA2, SGCB, LGMD2E, SGCD, SGD, LGMD2F, CMD1L, TCAP, LGMD2G, CMD1N, TRIM32, HT2A, LGMD2H, FKRP, MDC1C, LGMD2I, TTN,

	CMD1G, TMD, LGMD2J, POMT1, CAV3, LGMD1C, SEPN1, SELN, RSMD1, PLEC1, PLTN, EBS1); Osteopetrosis (LRP5, BMND1, LRP7, LR3, OPPG, VBCH2, CLCN7, CLC7, OPTA2, OSTM1, GL, TCIRG1, TIRC7, OC116, OPTB1); Muscular atrophy (VAPB, VAPC, ALS8, SMN1, SMA1, SMA2, SMA3, SMA4, BSCL2, SPG17, GARS, SMAD1, CMT2D, HEXB, IGHMBP2, SMUBP2, CATF1, SMARD1).
Neurological and neuronal diseases and disorders	ALS (SOD1, ALS2, STEX, FUS, TARDBP, VEGF (VEGF-a, VEGF-b, VEGF-c); Alzheimer disease (APP, AAA, CVAP, AD1, APOE, AD2, PSEN2, AD4, STM2, APBB2, FE65L1, NOS3, PLAU, URK, ACE, DCP1, ACE1, MPO, PACIP1, PAXIP1L, PTIP, A2M, BLMH, BMH, PSEN1, AD3); Autism (Mecp2, BZRAP1, MDGA2, Sema5A, Neurexin 1, GLO1, MECP2, RTT, PPMX, MRX16, MRX79, NLGN3, NLGN4, KIAA1260, AUTSX2); Fragile X Syndrome (FMR2, FXR1, FXR2, mGLUR5); Huntington's disease and disease like disorders (HD, IT15, PRNP, PRIP, JPH3, JP3, HDL2, TBP, SCA17); Parkinson disease (NR4A2, NURR1, NOT, TINUR, SNCAIP, TBP, SCA17, SNCA, NACP, PARK1, PARK4, DJ1, PARK7, LRRK2, PARK8, PINK1, PARK6, UCHL1, PARK5, SNCA, NACP, PARK1, PARK4, PRKN, PARK2, PDJ, DBH, NDUFV2); Rett syndrome (MECP2, RTT, PPMX, MRX16, MRX79, CDKL5, STK9, MECP2, RTT, PPMX, MRX16, MRX79, x-Synuclein, DJ-1); Schizophrenia (Neuregulin1 (Nrg1), Erb4 (receptor for Neuregulin), Complexin1 (Cplx1), Tph1 Tryptophan hydroxylase, Tph2, Tryptophan hydroxylase 2, Neurexin 1, GSK3, GSK3a, GSK3b, 5-HTT (Slc6a4), COMT, DRD (Drd1a), SLC6A3, DAOA, DTNBP1, Dao (Dao1)); Secretase Related Disorders (APH-1 (alpha and beta), Presenilin (Psen1), nicastrin, (Ncstn), PEN-2, Nos1, Parp1, Nat1, Nat2); Trinucleotide Repeat Disorders (HTT (Huntington's Dx), SBMA/SMAX1/AR (Kennedy's Dx), FXN/X25 (Friedrich's Ataxia), ATX3 (Machado- Joseph's Dx), ATXN1 and ATXN2 (spinocerebellar ataxias), DMPK (myotonic dystrophy), Atrophin-1 and Atn1 (DRPLA Dx), CBP (Creb-BP - global instability), VLDLR (Alzheimer's), Atxn7, Atxn10).
Occular diseases and disorders	Age-related macular degeneration (Abcr, Ccl2, Cc2, cp (ceruloplasmin), Timp3, cathepsinD, Vldlr, Ccr2); Cataract (CRYAA, CRYA1, CRYBB2, CRYB2, PITX3, BFSP2, CP49, CP47, CRYAA, CRYA1, PAX6, AN2, MGDA, CRYBA1, CRYB1, CRYGC, CRYG3, CCL, LIM2, MP19, CRYGD, CRYG4, BFSP2, CP49, CP47, HSF4, CTM, HSF4, CTM, MIP, AQP0, CRYAB, CRYA2, CTPP2, CRYBB1, CRYGD, CRYG4, CRYBB2, CRYB2, CRYGC, CRYG3, CCL, CRYAA, CRYA1, GJA8, CX50, CAE1, GJA3, CX46, CZP3, CAE3, CCM1, CAM, KRIT1); Corneal clouding and dystrophy (APOA1, TGFBI, CSD2, CDGG1, CSD, BIGH3, CDG2, TACSTD2, TROP2, M1S1, VSX1, RINX, PPCD, PPD, KTCN, COL8A2, FECD, PPCD2, PIP5K3, CFD); Cornea plana congenital (KERA, CNA2); Glaucoma (MYOC, TIGR, GLC1A, JOAG,

	GPOA, OPTN, GLC1E, FIP2, HYPL, NRP, CYP1B1, GLC3A, OPA1, NTG, NPG, CYP1B1, GLC3A); Leber congenital amaurosis (CRB1, RP12, CRX, CORD2, CRD, RPGRIP1, LCA6, CORD9, RPE65, RP20, AIPL1, LCA4, GUCY2D, GUC2D, LCA1, CORD6, RDH12, LCA3); Macular dystrophy (ELOVL4, ADMD, STGD2, STGD3, RDS, RP7, PRPH2, PRPH, AVMD, AOFMD, VMD2).

Table C:

CELLULAR FUNCTION	GENES
PI3K/AKT Signaling	PRKCE; ITGAM; ITGA5; IRAK1; PRKAA2; EIF2AK2;
	PTEN; EIF4E; PRKCZ; GRK6; MAPK1; TSC1; PLK1;
	AKT2; IKBKB; PIK3CA; CDK8; CDKN1B; NFKB2; BCL2;
	PIK3CB; PPP2R1A; MAPK8; BCL2L1; MAPK3; TSC2;
	ITGA1; KRAS; EIF4EBP1; RELA; PRKCD; NOS3;
	PRKAA1; MAPK9; CDK2; PPP2CA; PIM1; ITGB7;
	YWHAZ; ILK; TP53; RAF1; IKBKG; RELB; DYRK1A;
	CDKN1A; ITGB1; MAP2K2; JAK1; AKT1; JAK2; PIK3R1;
	CHUK; PDPK1; PPP2R5C; CTNNB1; MAP2K1; NFKB1;
	PAK3; ITGB3; CCND1; GSK3A; FRAP1; SFN; ITGA2;
	TTK; CSNK1A1; BRAF; GSK3B; AKT3; FOXO1; SGK;
	HSP90AA1; RPS6KB1
ERK/MAPK Signaling	PRKCE; ITGAM; ITGA5; HSPB1; IRAK1; PRKAA2;
	EIF2AK2; RAC1; RAP1A; TLN1; EIF4E; ELK1; GRK6;
	MAPK1; RAC2; PLK1; AKT2; PIK3CA; CDK8; CREB1;
	PRKCI; PTK2; FOS; RPS6KA4; PIK3CB; PPP2R1A;
	PIK3C3; MAPK8; MAPK3; ITGA1; ETS1; KRAS; MYCN;
	EIF4EBP1; PPARG; PRKCD; PRKAA1; MAPK9; SRC;
	CDK2; PPP2CA; PIM1; PIK3C2A; ITGB7; YWHAZ;
	PPP1CC; KSR1; PXN; RAF1; FYN; DYRK1A; ITGB1;
	MAP2K2; PAK4; PIK3R1; STAT3; PPP2R5C; MAP2K1;
	PAK3; ITGB3; ESR1; ITGA2; MYC; TTK; CSNK1A1;
	CRKL; BRAF; ATF4; PRKCA; SRF; STAT1; SGK
Glucocorticoid Receptor	RAC1; TAF4B; EP300; SMAD2; TRAF6; PCAF; ELK1;
Signaling	MAPK1; SMAD3; AKT2; IKBKB; NCOR2; UBE2I;
	PIK3CA; CREB1; FOS; HSPA5; NFKB2; BCL2;
	MAP3K14; STAT5B; PIK3CB; PIK3C3; MAPK8; BCL2L1;
	MAPK3; TSC22D3; MAPK10; NRIP1; KRAS; MAPK13;
	RELA; STAT5A; MAPK9; NOS2A; PBX1; NR3C1;
	PIK3C2A; CDKN1C; TRAF2; SERPINE1; NCOA3;
	MAPK14; TNF; RAF1; IKBKG; MAP3K7; CREBBP;
	CDKN1A; MAP2K2; JAK1; IL8; NCOA2; AKT1; JAK2;
	PIK3R1; CHUK; STAT3; MAP2K1; NFKB1; TGFBR1;
	ESR1; SMAD4; CEBPB; JUN; AR; AKT3; CCL2; MMP1;
	STAT1; IL6; HSP90AA1

Axonal Guidance Signaling	PRKCE; ITGAM; ROCK1; ITGA5; CXCR4; ADAM12;
	IGF1; RAC1; RAP1A; EIF4E; PRKCZ; NRP1; NTRK2;
	ARHGEF7; SMO; ROCK2; MAPK1; PGF; RAC2;
	PTPN11; GNAS; AKT2; PIK3CA; ERBB2; PRKCI; PTK2;
	CFL1; GNAQ; PIK3CB; CXCL12; PIK3C3; WNT11;
	PRKD1; GNB2L1; ABL1; MAPK3; ITGA1; KRAS; RHOA;
	PRKCD; PIK3C2A; ITGB7; GLI2; PXN; VASP; RAF1;
	FYN; ITGB1; MAP2K2; PAK4; ADAM17; AKT1; PIK3R1;
	GLI1; WNT5A; ADAM10; MAP2K1; PAK3; ITGB3;
	CDC42; VEGFA; ITGA2; EPHA8; CRKL; RND1; GSK3B;
	AKT3; PRKCA
Ephrin Receptor Signaling	PRKCE; ITGAM; ROCK1; ITGA5; CXCR4; IRAK1;
	PRKAA2; EIF2AK2; RAC1; RAP1A; GRK6; ROCK2;
	MAPK1; PGF; RAC2; PTPN11; GNAS; PLK1; AKT2;
	DOK1; CDK8; CREB1; PTK2; CFL1; GNAQ; MAP3K14;
	CXCL12; MAPK8; GNB2L1; ABL1; MAPK3; ITGA1;
	KRAS; RHOA; PRKCD; PRKAA1; MAPK9; SRC; CDK2;
	PIM1; ITGB7; PXN; RAF1; FYN; DYRK1A; ITGB1;
	MAP2K2; PAK4; AKT1; JAK2; STAT3; ADAM10;
	MAP2K1; PAK3; ITGB3; CDC42; VEGFA; ITGA2;
	EPHA8; TTK; CSNK1A1; CRKL; BRAF; PTPN13; ATF4;
	AKT3; SGK
Actin Cytoskeleton	ACTN4; PRKCE; ITGAM; ROCK1; ITGA5; IRAK1;
Signaling	PRKAA2; EIF2AK2; RAC1; INS; ARHGEF7; GRK6;
	ROCK2; MAPK1; RAC2; PLK1; AKT2; PIK3CA; CDK8;
	PTK2; CFL1; PIK3CB; MYH9; DIAPH1; PIK3C3; MAPK8;
	F2R; MAPK3; SLC9A1; ITGA1; KRAS; RHOA; PRKCD;
	PRKAA1; MAPK9; CDK2; PIM1; PIK3C2A; ITGB7;
	PPP1CC; PXN; VIL2; RAF1; GSN; DYRK1A; ITGB1;
	MAP2K2; PAK4; PIP5K1A; PIK3R1; MAP2K1; PAK3;
	ITGB3; CDC42; APC; ITGA2; TTK; CSNK1A1; CRKL;
	BRAF; VAV3; SGK
Huntington's Disease	PRKCE; IGF1; EP300; RCOR1; PRKCZ; HDAC4; TGM2;
Signaling	MAPK1; CAPNS1; AKT2; EGFR; NCOR2; SP1; CAPN2;
	PIK3CA; HDAC5; CREB1; PRKCI; HSPA5; REST;
	GNAQ; PIK3CB; PIK3C3; MAPK8; IGF1R; PRKD1;
	GNB2L1; BCL2L1; CAPN1; MAPK3; CASP8; HDAC2;
	HDAC7A; PRKCD; HDAC11; MAPK9; HDAC9; PIK3C2A;
	HDAC3; TP53; CASP9; CREBBP; AKT1; PIK3R1;
	PDPK1; CASP1; APAF1; FRAP1; CASP2; JUN; BAX;
	ATF4; AKT3; PRKCA; CLTC; SGK; HDAC6; CASP3
Apoptosis Signaling	PRKCE; ROCK1; BID; IRAK1; PRKAA2; EIF2AK2; BAK1;

	BIRC4; GRK6; MAPK1; CAPNS1; PLK1; AKT2; IKBKB;
	CAPN2; CDK8; FAS; NFKB2; BCL2; MAP3K14; MAPK8;
	BCL2L1; CAPN1; MAPK3; CASP8; KRAS; RELA;
	PRKCD; PRKAA1; MAPK9; CDK2; PIM1; TP53; TNF;
	RAF1; IKBKG; RELB; CASP9; DYRK1A; MAP2K2;
	CHUK; APAF1; MAP2K1; NFKB1; PAK3; LMNA; CASP2;
	BIRC2; TTK; CSNK1A1; BRAF; BAX; PRKCA; SGK;
	CASP3; BIRC3; PARP1
B Cell Receptor Signaling	RAC1; PTEN; LYN; ELK1; MAPK1; RAC2; PTPN11;
	AKT2; IKBKB; PIK3CA; CREB1; SYK; NFKB2; CAMK2A;
	MAP3K14; PIK3CB; PIK3C3; MAPK8; BCL2L1; ABL1;
	MAPK3; ETS1; KRAS; MAPK13; RELA; PTPN6; MAPK9;
	EGR1; PIK3C2A; BTK; MAPK14; RAF1; IKBKG; RELB;
	MAP3K7; MAP2K2; AKT1; PIK3R1; CHUK; MAP2K1;
	NFKB1; CDC42; GSK3A; FRAP1; BCL6; BCL10; JUN;
	GSK3B; ATF4; AKT3; VAV3; RPS6KB1
Leukocyte Extravasation Signaling	ACTN4; CD44; PRKCE; ITGAM; ROCK1; CXCR4; CYBA;
	RAC1; RAP1A; PRKCZ; ROCK2; RAC2; PTPN11;
	MMP14; PIK3CA; PRKCI; PTK2; PIK3CB; CXCL12;
	PIK3C3; MAPK8; PRKD1; ABL1; MAPK10; CYBB;
	MAPK13; RHOA; PRKCD; MAPK9; SRC; PIK3C2A; BTK;
	MAPK14; NOX1; PXN; VIL2; VASP; ITGB1; MAP2K2;
	CTNND1; PIK3R1; CTNNB1; CLDN1; CDC42; F11R; ITK;
	CRKL; VAV3; CTTN; PRKCA; MMP1; MMP9
Integrin Signaling	ACTN4; ITGAM; ROCK1; ITGA5; RAC1; PTEN; RAP1A;
	TLN1; ARHGEF7; MAPK1; RAC2; CAPNS1; AKT2;
	CAPN2; PIK3CA; PTK2; PIK3CB; PIK3C3; MAPK8;
	CAV1; CAPN1; ABL1; MAPK3; ITGA1; KRAS; RHOA;
	SRC; PIK3C2A; ITGB7; PPP1CC; ILK; PXN; VASP;
	RAF1; FYN; ITGB1; MAP2K2; PAK4; AKT1; PIK3R1;
	TNK2; MAP2K1; PAK3; ITGB3; CDC42; RND3; ITGA2;
	CRKL; BRAF; GSK3B; AKT3
Acute Phase Response Signaling	IRAK1; SOD2; MYD88; TRAF6; ELK1; MAPK1; PTPN11;
	AKT2; IKBKB; PIK3CA; FOS; NFKB2; MAP3K14;
	PIK3CB; MAPK8; RIPK1; MAPK3; IL6ST; KRAS;
	MAPK13; IL6R; RELA; SOCS1; MAPK9; FTL; NR3C1;
	TRAF2; SERPINE1; MAPK14; TNF; RAF1; PDK1;
	IKBKG; RELB; MAP3K7; MAP2K2; AKT1; JAK2; PIK3R1;
	CHUK; STAT3; MAP2K1; NFKB1; FRAP1; CEBPB; JUN;
	AKT3; IL1R1; IL6
PTEN Signaling	ITGAM; ITGA5; RAC1; PTEN; PRKCZ; BCL2L11;
	MAPK1; RAC2; AKT2; EGFR; IKBKB; CBL; PIK3CA;
	CDKN1B; PTK2; NFKB2; BCL2; PIK3CB; BCL2L1;

	MAPK3; ITGA1; KRAS; ITGB7; ILK; PDGFRB; INSR;
	RAF1; IKBKG; CASP9; CDKN1A; ITGB1; MAP2K2;
	AKT1; PIK3R1; CHUK; PDGFRA; PDPK1; MAP2K1;
	NFKB1; ITGB3; CDC42; CCND1; GSK3A; ITGA2;
	GSK3B; AKT3; FOXO1; CASP3; RPS6KB1
p53 Signaling	PTEN; EP300; BBC3; PCAF; FASN; BRCA1; GADD45A;
	BIRC5; AKT2; PIK3CA; CHEK1; TP53INP1; BCL2;
	PIK3CB; PIK3C3; MAPK8; THBS1; ATR; BCL2L1; E2F1;
	PMAIP1; CHEK2; TNFRSF10B; TP73; RB1; HDAC9;
	CDK2; PIK3C2A; MAPK14; TP53; LRDD; CDKN1A;
	HIPK2; AKT1; PIK3R1; RRM2B; APAF1; CTNNB1;
	SIRT1; CCND1; PRKDC; ATM; SFN; CDKN2A; JUN;
	SNAI2; GSK3B; BAX; AKT3
Aryl Hydrocarbon Receptor	HSPB1; EP300; FASN; TGM2; RXRA; MAPK1; NQO1;
Signaling	NCOR2; SP1; ARNT; CDKN1B; FOS; CHEK1;
	SMARCA4; NFKB2; MAPK8; ALDH1A1; ATR; E2F1;
	MAPK3; NRIP1; CHEK2; RELA; TP73; GSTP1; RB1;
	SRC; CDK2; AHR; NFE2L2; NCOA3; TP53; TNF;
	CDKN1A; NCOA2; APAF1; NFKB1; CCND1; ATM; ESR1;
	CDKN2A; MYC; JUN; ESR2; BAX; IL6; CYP1B1;
	HSP90AA1
Xenobiotic Metabolism	PRKCE; EP300; PRKCZ; RXRA; MAPK1; NQO1;
Signaling	NCOR2; PIK3CA; ARNT; PRKCI; NFKB2; CAMK2A;
	PIK3CB; PPP2R1A; PIK3C3; MAPK8; PRKD1;
	ALDH1A1; MAPK3; NRIP1; KRAS; MAPK13; PRKCD;
	GSTP1; MAPK9; NOS2A; ABCB1; AHR; PPP2CA; FTL;
	NFE2L2; PIK3C2A; PPARGC1A; MAPK14; TNF; RAF1;
	CREBBP; MAP2K2; PIK3R1; PPP2R5C; MAP2K1;
	NFKB1; KEAP1; PRKCA; EIF2AK3; IL6; CYP1B1;
	HSP90AA1
SAPK/JNK Signaling	PRKCE; IRAK1; PRKAA2; EIF2AK2; RAC1; ELK1;
	GRK6; MAPK1; GADD45A; RAC2; PLK1; AKT2; PIK3CA;
	FADD; CDK8; PIK3CB; PIK3C3; MAPK8; RIPK1;
	GNB2L1; IRS1; MAPK3; MAPK10; DAXX; KRAS;
	PRKCD; PRKAA1; MAPK9; CDK2; PIM1; PIK3C2A;
	TRAF2; TP53; LCK; MAP3K7; DYRK1A; MAP2K2;
	PIK3R1; MAP2K1; PAK3; CDC42; JUN; TTK; CSNK1A1;
	CRKL; BRAF; SGK
PPAr/RXR Signaling	PRKAA2; EP300; INS; SMAD2; TRAF6; PPARA; FASN;
	RXRA; MAPK1; SMAD3; GNAS; IKBKB; NCOR2;
	ABCA1; GNAQ; NFKB2; MAP3K14; STAT5B; MAPK8;
	IRS1; MAPK3; KRAS; RELA; PRKAA1; PPARGC1A;
	NCOA3; MAPK14; INSR; RAF1; IKBKG; RELB; MAP3K7;

	CREBBP; MAP2K2; JAK2; CHUK; MAP2K1; NFKB1;
	TGFBR1; SMAD4; JUN; IL1R1; PRKCA; IL6; HSP90AA1;
	ADIPOQ
NF-KB Signaling	IRAK1; EIF2AK2; EP300; INS; MYD88; PRKCZ; TRAF6;
	TBK1; AKT2; EGFR; IKBKB; PIK3CA; BTRC; NFKB2;
	MAP3K14; PIK3CB; PIK3C3; MAPK8; RIPK1; HDAC2;
	KRAS; RELA; PIK3C2A; TRAF2; TLR4; PDGFRB; TNF;
	INSR; LCK; IKBKG; RELB; MAP3K7; CREBBP; AKT1;
	PIK3R1; CHUK; PDGFRA; NFKB1; TLR2; BCL10;
	GSK3B; AKT3; TNFAIP3; IL1R1
Neuregulin Signaling	ERBB4; PRKCE; ITGAM; ITGA5; PTEN; PRKCZ; ELK1;
	MAPK1; PTPN11; AKT2; EGFR; ERBB2; PRKCI;
	CDKN1B; STAT5B; PRKD1; MAPK3; ITGA1; KRAS;
	PRKCD; STAT5A; SRC; ITGB7; RAF1; ITGB1; MAP2K2;
	ADAM17; AKT1; PIK3R1; PDPK1; MAP2K1; ITGB3;
	EREG; FRAP1; PSEN1; ITGA2; MYC; NRG1; CRKL;
	AKT3; PRKCA; HSP90AA1; RPS6KB1
Wnt & Beta catenin	CD44; EP300; LRP6; DVL3; CSNK1E; GJA1; SMO;
Signaling	AKT2; PIN1; CDH1; BTRC; GNAQ; MARK2; PPP2R1A;
	WNT11; SRC; DKK1; PPP2CA; SOX6; SFRP2; ILK;
	LEF1; SOX9; TP53; MAP3K7; CREBBP; TCF7L2; AKT1;
	PPP2R5C; WNT5A; LRP5; CTNNB1; TGFBR1; CCND1;
	GSK3A; DVL1; APC; CDKN2A; MYC; CSNK1A1; GSK3B;
	AKT3; SOX2
Insulin Receptor Signaling	PTEN; INS; EIF4E; PTPN1; PRKCZ; MAPK1; TSC1;
	PTPN11; AKT2; CBL; PIK3CA; PRKCI; PIK3CB; PIK3C3;
	MAPK8; IRS1; MAPK3; TSC2; KRAS; EIF4EBP1;
	SLC2A4; PIK3C2A; PPP1CC; INSR; RAF1; FYN;
	MAP2K2; JAK1; AKT1; JAK2; PIK3R1; PDPK1; MAP2K1;
	GSK3A; FRAP1; CRKL; GSK3B; AKT3; FOXO1; SGK;
	RPS6KB1
IL-6 Signaling	HSPB1; TRAF6; MAPKAPK2; ELK1; MAPK1; PTPN11;
	IKBKB; FOS; NFKB2; MAP3K14; MAPK8; MAPK3;
	MAPK10; IL6ST; KRAS; MAPK13; IL6R; RELA; SOCS1;
	MAPK9; ABCB1; TRAF2; MAPK14; TNF; RAF1; IKBKG;
	RELB; MAP3K7; MAP2K2; IL8; JAK2; CHUK; STAT3;
	MAP2K1; NFKB1; CEBPB; JUN; IL1R1; SRF; IL6
Hepatic Cholestasis	PRKCE; IRAK1; INS; MYD88; PRKCZ; TRAF6; PPARA;
	RXRA; IKBKB; PRKCI; NFKB2; MAP3K14; MAPK8;
	PRKD1; MAPK10; RELA; PRKCD; MAPK9; ABCB1;
	TRAF2; TLR4; TNF; INSR; IKBKG; RELB; MAP3K7; IL8;
	CHUK; NR1H2; TJP2; NFKB1; ESR1; SREBF1; FGFR4;
	JUN; IL1R1; PRKCA; IL6

IGF-1 Signaling	IGF1; PRKCZ; ELK1; MAPK1; PTPN11; NEDD4; AKT2;
	PIK3CA; PRKCI; PTK2; FOS; PIK3CB; PIK3C3; MAPK8;
	IGF1R; IRS1; MAPK3; IGFBP7; KRAS; PIK3C2A;
	YWHAZ; PXN; RAF1; CASP9; MAP2K2; AKT1; PIK3R1;
	PDPK1; MAP2K1; IGFBP2; SFN; JUN; CYR61; AKT3;
	FOXO1; SRF; CTGF; RPS6KB1
NRF2-mediated Oxidative	PRKCE; EP300; SOD2; PRKCZ; MAPK1; SQSTM1;
Stress Response	NQO1; PIK3CA; PRKCI; FOS; PIK3CB; PIK3C3; MAPK8;
	PRKD1; MAPK3; KRAS; PRKCD; GSTP1; MAPK9; FTL;
	NFE2L2; PIK3C2A; MAPK14; RAF1; MAP3K7; CREBBP;
	MAP2K2; AKT1; PIK3R1; MAP2K1; PPIB; JUN; KEAP1;
	GSK3B; ATF4; PRKCA; EIF2AK3; HSP90AA1
Hepatic Fibrosis/Hepatic	EDN1; IGF1; KDR; FLT1; SMAD2; FGFR1; MET; PGF;
Stellate Cell Activation	SMAD3; EGFR; FAS; CSF1; NFKB2; BCL2; MYH9;
	IGF1R; IL6R; RELA; TLR4; PDGFRB; TNF; RELB; IL8;
	PDGFRA; NFKB1; TGFBR1; SMAD4; VEGFA; BAX;
	IL1R1; CCL2; HGF; MMP1; STAT1; IL6; CTGF; MMP9
PPAR Signaling	EP300; INS; TRAF6; PPARA; RXRA; MAPK1; IKBKB;
	NCOR2; FOS; NFKB2; MAP3K14; STAT5B; MAPK3;
	NRIP1; KRAS; PPARG; RELA; STAT5A; TRAF2;
	PPARGC1A; PDGFRB; TNF; INSR; RAF1; IKBKG;
	RELB; MAP3K7; CREBBP; MAP2K2; CHUK; PDGFRA;
	MAP2K1; NFKB1; JUN; IL1R1; HSP90AA1
Fc Epsilon RI Signaling	PRKCE; RAC1; PRKCZ; LYN; MAPK1; RAC2; PTPN11;
	AKT2; PIK3CA; SYK; PRKCI; PIK3CB; PIK3C3; MAPK8;
	PRKD1; MAPK3; MAPK10; KRAS; MAPK13; PRKCD;
	MAPK9; PIK3C2A; BTK; MAPK14; TNF; RAF1; FYN;
	MAP2K2; AKT1; PIK3R1; PDPK1; MAP2K1; AKT3;
	VAV3; PRKCA
G-Protein Coupled	PRKCE; RAP1A; RGS16; MAPK1; GNAS; AKT2; IKBKB;
Receptor Signaling	PIK3CA; CREB1; GNAQ; NFKB2; CAMK2A; PIK3CB;
	PIK3C3; MAPK3; KRAS; RELA; SRC; PIK3C2A; RAF1;
	IKBKG; RELB; FYN; MAP2K2; AKT1; PIK3R1; CHUK;
	PDPK1; STAT3; MAP2K1; NFKB1; BRAF; ATF4; AKT3;
	PRKCA
Inositol Phosphate	PRKCE; IRAK1; PRKAA2; EIF2AK2; PTEN; GRK6;
Metabolism	MAPK1; PLK1; AKT2; PIK3CA; CDK8; PIK3CB; PIK3C3;
	MAPK8; MAPK3; PRKCD; PRKAA1; MAPK9; CDK2;
	PIM1; PIK3C2A; DYRK1A; MAP2K2; PIP5K1A; PIK3R1;
	MAP2K1; PAK3; ATM; TTK; CSNK1A1; BRAF; SGK
PDGF Signaling	EIF2AK2; ELK1; ABL2; MAPK1; PIK3CA; FOS; PIK3CB;
	PIK3C3; MAPK8; CAV1; ABL1; MAPK3; KRAS; SRC;
	PIK3C2A; PDGFRB; RAF1; MAP2K2; JAK1; JAK2;

	PIK3R1; PDGFRA; STAT3; SPHK1; MAP2K1; MYC;
	JUN; CRKL; PRKCA; SRF; STAT1; SPHK2
VEGF Signaling	ACTN4; ROCK1; KDR; FLT1; ROCK2; MAPK1; PGF;
	AKT2; PIK3CA; ARNT; PTK2; BCL2; PIK3CB; PIK3C3;
	BCL2L1; MAPK3; KRAS; HIF1A; NOS3; PIK3C2A; PXN;
	RAF1; MAP2K2; ELAVL1; AKT1; PIK3R1; MAP2K1; SFN;
	VEGFA; AKT3; FOXO1; PRKCA
Natural Killer Cell Signaling	PRKCE; RAC1; PRKCZ; MAPK1; RAC2; PTPN11;
	KIR2DL3; AKT2; PIK3CA; SYK; PRKCI; PIK3CB;
	PIK3C3; PRKD1; MAPK3; KRAS; PRKCD; PTPN6;
	PIK3C2A; LCK; RAF1; FYN; MAP2K2; PAK4; AKT1;
	PIK3R1; MAP2K1; PAK3; AKT3; VAV3; PRKCA
Cell Cycle: G1/S	HDAC4; SMAD3; SUV39H1; HDAC5; CDKN1B; BTRC;
Checkpoint Regulation	ATR; ABL1; E2F1; HDAC2; HDAC7A; RB1; HDAC11;
	HDAC9; CDK2; E2F2; HDAC3; TP53; CDKN1A; CCND1;
	E2F4; ATM; RBL2; SMAD4; CDKN2A; MYC; NRG1;
	GSK3B; RBL1; HDAC6
T Cell Receptor Signaling	RAC1; ELK1; MAPK1; IKBKB; CBL; PIK3CA; FOS;
	NFKB2; PIK3CB; PIK3C3; MAPK8; MAPK3; KRAS;
	RELA; PIK3C2A; BTK; LCK; RAF1; IKBKG; RELB; FYN;
	MAP2K2; PIK3R1; CHUK; MAP2K1; NFKB1; ITK; BCL10;
	JUN; VAV3
Death Receptor Signaling	CRADD; HSPB1; BID; BIRC4; TBK1; IKBKB; FADD;
	FAS; NFKB2; BCL2; MAP3K14; MAPK8; RIPK1; CASP8;
	DAXX; TNFRSF10B; RELA; TRAF2; TNF; IKBKG; RELB;
	CASP9; CHUK; APAF1; NFKB1; CASP2; BIRC2; CASP3;
	BIRC3
FGF Signaling	RAC1; FGFR1; MET; MAPKAPK2; MAPK1; PTPN11;
	AKT2; PIK3CA; CREB1; PIK3CB; PIK3C3; MAPK8;
	MAPK3; MAPK13; PTPN6; PIK3C2A; MAPK14; RAF1;
	AKT1; PIK3R1; STAT3; MAP2K1; FGFR4; CRKL; ATF4;
	AKT3; PRKCA; HGF
GM-CSF Signaling	LYN; ELK1; MAPK1; PTPN11; AKT2; PIK3CA; CAMK2A;
	STAT5B; PIK3CB; PIK3C3; GNB2L1; BCL2L1; MAPK3;
	ETS1; KRAS; RUNX1; PIM1; PIK3C2A; RAF1; MAP2K2;
	AKT1; JAK2; PIK3R1; STAT3; MAP2K1; CCND1; AKT3;
	STAT1
Amyotrophic Lateral	BID; IGF1; RAC1; BIRC4; PGF; CAPNS1; CAPN2;
Sclerosis Signaling	PIK3CA; BCL2; PIK3CB; PIK3C3; BCL2L1; CAPN1;
	PIK3C2A; TP53; CASP9; PIK3R1; RAB5A; CASP1;
	APAF1; VEGFA; BIRC2; BAX; AKT3; CASP3; BIRC3
JAK/Stat Signaling	PTPN1; MAPK1; PTPN11; AKT2; PIK3CA; STAT5B;

	PIK3CB; PIK3C3; MAPK3; KRAS; SOCS1; STAT5A;
	PTPN6; PIK3C2A; RAF1; CDKN1A; MAP2K2; JAK1;
	AKT1; JAK2; PIK3R1; STAT3; MAP2K1; FRAP1; AKT3;
	STAT1
Nicotinate and Nicotinamide	PRKCE; IRAK1; PRKAA2; EIF2AK2; GRK6; MAPK1;
Metabolism	PLK1; AKT2; CDK8; MAPK8; MAPK3; PRKCD; PRKAA1;
	PBEF1; MAPK9; CDK2; PIM1; DYRK1A; MAP2K2;
	MAP2K1; PAK3; NT5E; TTK; CSNK1A1; BRAF; SGK
Chemokine Signaling	CXCR4; ROCK2; MAPK1; PTK2; FOS; CFL1; GNAQ;
	CAMK2A; CXCL12; MAPK8; MAPK3; KRAS; MAPK13;
	RHOA; CCR3; SRC; PPP1CC; MAPK14; NOX1; RAF1;
	MAP2K2; MAP2K1; JUN; CCL2; PRKCA
IL-2 Signaling	ELK1; MAPK1; PTPN11; AKT2; PIK3CA; SYK; FOS;
	STAT5B; PIK3CB; PIK3C3; MAPK8; MAPK3; KRAS;
	SOCS1; STAT5A; PIK3C2A; LCK; RAF1; MAP2K2;
	JAK1; AKT1; PIK3R1; MAP2K1; JUN; AKT3
Synaptic Long Term	PRKCE; IGF1; PRKCZ; PRDX6; LYN; MAPK1; GNAS;
Depression	PRKCI; GNAQ; PPP2R1A; IGF1R; PRKD1; MAPK3;
	KRAS; GRN; PRKCD; NOS3; NOS2A; PPP2CA;
	YWHAZ; RAF1; MAP2K2; PPP2R5C; MAP2K1; PRKCA
Estrogen Receptor	TAF4B; EP300; CARM1; PCAF; MAPK1; NCOR2;
Signaling	SMARCA4; MAPK3; NRIP1; KRAS; SRC; NR3C1;
	HDAC3; PPARGC1A; RBM9; NCOA3; RAF1; CREBBP;
	MAP2K2; NCOA2; MAP2K1; PRKDC; ESR1; ESR2
Protein Ubiquitination	TRAF6; SMURF1; BIRC4; BRCA1; UCHL1; NEDD4;
Pathway	CBL; UBE2I; BTRC; HSPA5; USP7; USP10; FBXW7;
	USP9X; STUB1; USP22; B2M; BIRC2; PARK2; USP8;
	USP1; VHL; HSP90AA1; BIRC3
IL-10 Signaling	TRAF6; CCR1; ELK1; IKBKB; SP1; FOS; NFKB2;
	MAP3K14; MAPK8; MAPK13; RELA; MAPK14; TNF;
	IKBKG; RELB; MAP3K7; JAK1; CHUK; STAT3; NFKB1;
	JUN; IL1R1; IL6
VDR/RXR Activation	PRKCE; EP300; PRKCZ; RXRA; GADD45A; HES1;
	NCOR2; SP1; PRKCI; CDKN1B; PRKD1; PRKCD;
	RUNX2; KLF4; YY1; NCOA3; CDKN1A; NCOA2; SPP1;
	LRP5; CEBPB; FOXO1; PRKCA
TGF-beta Signaling	EP300; SMAD2; SMURF1; MAPK1; SMAD3; SMAD1;
	FOS; MAPK8; MAPK3; KRAS; MAPK9; RUNX2;
	SERPINE1; RAF1; MAP3K7; CREBBP; MAP2K2;
	MAP2K1; TGFBR1; SMAD4; JUN; SMAD5
Toll-like Receptor Signaling	IRAK1; EIF2AK2; MYD88; TRAF6; PPARA; ELK1;
	IKBKB; FOS; NFKB2; MAP3K14; MAPK8; MAPK13;

	RELA; TLR4; MAPK14; IKBKG; RELB; MAP3K7; CHUK;
	NFKB1; TLR2; JUN
p38 MAPK Signaling	HSPB1; IRAK1; TRAF6; MAPKAPK2; ELK1; FADD; FAS;
	CREB1; DDIT3; RPS6KA4; DAXX; MAPK13; TRAF2;
	MAPK14; TNF; MAP3K7; TGFBR1; MYC; ATF4; IL1R1;
	SRF; STAT1
Neurotrophin/TRK Signaling	NTRK2; MAPK1; PTPN11; PIK3CA; CREB1; FOS;
	PIK3CB; PIK3C3; MAPK8; MAPK3; KRAS; PIK3C2A;
	RAF1; MAP2K2; AKT1; PIK3R1; PDPK1; MAP2K1;
	CDC42; JUN; ATF4
FXR/RXR Activation	INS; PPARA; FASN; RXRA; AKT2; SDC1; MAPK8;
	APOB; MAPK10; PPARG; MTTP; MAPK9; PPARGC1A;
	TNF; CREBBP; AKT1; SREBF1; FGFR4; AKT3; FOXO1
Synaptic Long Term	PRKCE; RAP1A; EP300; PRKCZ; MAPK1; CREB1;
Potentiation	PRKCI; GNAQ; CAMK2A; PRKD1; MAPK3; KRAS;
	PRKCD; PPP1CC; RAF1; CREBBP; MAP2K2; MAP2K1;
	ATF4; PRKCA
Calcium Signaling	RAP1A; EP300; HDAC4; MAPK1; HDAC5; CREB1;
	CAMK2A; MYH9; MAPK3; HDAC2; HDAC7A; HDAC11;
	HDAC9; HDAC3; CREBBP; CALR; CAMKK2; ATF4;
	HDAC6
EGF Signaling	ELK1; MAPK1; EGFR; PIK3CA; FOS; PIK3CB; PIK3C3;
	MAPK8; MAPK3; PIK3C2A; RAF1; JAK1; PIK3R1;
	STAT3; MAP2K1; JUN; PRKCA; SRF; STAT1
Hypoxia Signaling in the	EDN1; PTEN; EP300; NQO1; UBE2I; CREB1; ARNT;
Cardiovascular System	HIF1A; SLC2A4; NOS3; TP53; LDHA; AKT1; ATM;
	VEGFA; JUN; ATF4; VHL; HSP90AA1
LPS/IL-1 Mediated Inhibition	IRAK1; MYD88; TRAF6; PPARA; RXRA; ABCA1;
of RXR Function	MAPK8; ALDH1A1; GSTP1; MAPK9; ABCB1; TRAF2;
	TLR4; TNF; MAP3K7; NR1H2; SREBF1; JUN; IL1R1
LXR/RXR Activation	FASN; RXRA; NCOR2; ABCA1; NFKB2; IRF3; RELA;
	NOS2A; TLR4; TNF; RELB; LDLR; NR1H2; NFKB1;
	SREBF1; IL1R1; CCL2; IL6; MMP9
Amyloid Processing	PRKCE; CSNK1E; MAPK1; CAPNS1; AKT2; CAPN2;
	CAPN1; MAPK3; MAPK13; MAPT; MAPK14; AKT1;
	PSEN1; CSNK1A1; GSK3B; AKT3; APP
IL-4 Signaling	AKT2; PIK3CA; PIK3CB; PIK3C3; IRS1; KRAS; SOCS1;
	PTPN6; NR3C1; PIK3C2A; JAK1; AKT1; JAK2; PIK3R1;
	FRAP1; AKT3; RPS6KB1
Cell Cycle: G2/M DNA	EP300; PCAF; BRCA1; GADD45A; PLK1; BTRC;
Damage Checkpoint	CHEK1; ATR; CHEK2; YWHAZ; TP53; CDKN1A;
Regulation	PRKDC; ATM; SFN; CDKN2A

Nitric Oxide Signaling in the	KDR; FLT1; PGF; AKT2; PIK3CA; PIK3CB; PIK3C3;
Cardiovascular System	CAV1; PRKCD; NOS3; PIK3C2A; AKT1; PIK3R1;
	VEGFA; AKT3; HSP90AA1
Purine Metabolism	NME2; SMARCA4; MYH9; RRM2; ADAR; EIF2AK4;
	PKM2; ENTPD1; RAD51; RRM2B; TJP2; RAD51C;
	NT5E; POLD1; NME1
cAMP-mediated Signaling	RAP1A; MAPK1; GNAS; CREB1; CAMK2A; MAPK3;
	SRC; RAF1; MAP2K2; STAT3; MAP2K1; BRAF; ATF4
Mitochondrial Dysfunction	SOD2; MAPK8; CASP8; MAPK10; MAPK9; CASP9;
	PARK7; PSEN1; PARK2; APP; CASP3
Notch Signaling	HES1; JAG1; NUMB; NOTCH4; ADAM17; NOTCH2;
	PSEN1; NOTCH3; NOTCH1; DLL4
Endoplasmic Reticulum	HSPA5; MAPK8; XBP1; TRAF2; ATF6; CASP9; ATF4;
Stress Pathway	EIF2AK3; CASP3
Pyrimidine Metabolism	NME2; AICDA; RRM2; EIF2AK4; ENTPD1; RRM2B;
	NT5E; POLD1; NME1
Parkinson's Signaling	UCHL1; MAPK8; MAPK13; MAPK14; CASP9; PARK7;
	PARK2; CASP3
Cardiac & Beta Adrenergic	GNAS; GNAQ; PPP2R1A; GNB2L1; PPP2CA; PPP1CC;
Signaling	PPP2R5C
Glycolysis/Gluconeogenesis	HK2; GCK; GPI; ALDH1A1; PKM2; LDHA; HK1
Interferon Signaling	IRF1; SOCS1; JAK1; JAK2; IFITM1; STAT1; IFIT3
Sonic Hedgehog Signaling	ARRB2; SMO; GLI2; DYRK1A; GLI1; GSK3B; DYRK1B
Glycerophospholipid	PLD1; GRN; GPAM; YWHAZ; SPHK1; SPHK2
Metabolism	
Phospholipid Degradation	PRDX6; PLD1; GRN; YWHAZ; SPHK1; SPHK2
Tryptophan Metabolism	SIAH2; PRMT5; NEDD4; ALDH1A1; CYP1B1; SIAH1
Lysine Degradation	SUV39H1; EHMT2; NSD1; SETD7; PPP2R5C
Nucleotide Excision Repair	ERCC5; ERCC4; XPA; XPC; ERCC1
Pathway	
Starch and Sucrose	UCHL1; HK2; GCK; GPI; HK1
Metabolism	
Aminosugars Metabolism	NQO1; HK2; GCK; HK1
Arachidonic Acid	PRDX6; GRN; YWHAZ; CYP1B1
Metabolism	
Circadian Rhythm Signaling	CSNK1E; CREB1; ATF4; NR1D1

Coagulation System	BDKRB1; F2R; SERPINE1; F3
Dopamine Receptor	PPP2R1A; PPP2CA; PPP1CC; PPP2R5C
Signaling	
Glutathione Metabolism	IDH2; GSTP1; ANPEP; IDH1
Glycerolipid Metabolism	ALDH1A1; GPAM; SPHK1; SPHK2
Linoleic Acid Metabolism	PRDX6; GRN; YWHAZ; CYP1B1
Methionine Metabolism	DNMT1; DNMT3B; AHCY; DNMT3A
Pyruvate Metabolism	GLO1; ALDH1A1; PKM2; LDHA
Arginine and Proline	ALDH1A1; NOS3; NOS2A
Metabolism	
Eicosanoid Signaling	PRDX6; GRN; YWHAZ
Fructose and Mannose	HK2; GCK; HK1
Metabolism	
Galactose Metabolism	HK2; GCK; HK1
Stilbene, Coumarine and	PRDX6; PRDX1; TYR
Lignin Biosynthesis	
Antigen Presentation	CALR; B2M
Pathway	
Biosynthesis of Steroids	NQO1; DHCR7
Butanoate Metabolism	ALDH1A1; NLGN1
Citrate Cycle	IDH2; IDH1
Fatty Acid Metabolism	ALDH1A1; CYP1B1
Glycerophospholipid	PRDX6; CHKA
Metabolism	
Histidine Metabolism	PRMT5; ALDH1A1
Inositol Metabolism	ERO1L; APEX1
Metabolism of Xenobiotics	GSTP1; CYP1B1
by Cytochrome p450	
Methane Metabolism	PRDX6; PRDX1
Phenylalanine Metabolism	PRDX6; PRDX1
Propanoate Metabolism	ALDH1A1; LDHA
Selenoamino Acid	PRMT5; AHCY
Metabolism	
Sphingolipid Metabolism	SPHK1; SPHK2
Aminophosphonate	PRMT5
Metabolism	
Androgen and Estrogen	PRMT5
Metabolism	
Ascorbate and Aldarate	ALDH1A1
Metabolism	
Bile Acid Biosynthesis	ALDH1A1
Cysteine Metabolism	LDHA

Fatty Acid Biosynthesis	FASN
Glutamate Receptor	GNB2L1
Signaling	
NRF2-mediated Oxidative	PRDX1
Stress Response	
Pentose Phosphate	GPI
Pathway	
Pentose and Glucuronate	UCHL1
Interconversions	
Retinol Metabolism	ALDH1A1
Riboflavin Metabolism	TYR
Tyrosine Metabolism	PRMT5, TYR
Ubiquinone Biosynthesis	PRMT5
Valine, Leucine and	ALDH1A1
Isoleucine Degradation	
Glycine, Serine and	CHKA
Threonine Metabolism	
Lysine Degradation	ALDH1A1
Pain/Taste	TRPM5; TRPA1
Pain	TRPM7; TRPC5; TRPC6; TRPC1; Cnr1; cnr2; Grk2;
	Trpa1; Pomc; Cgrp; Crf; Pka; Era; Nr2b; TRPM5; Prkaca;
	Prkacb; Prkar1a; Prkar2a
Mitochondrial Function	AIF; CytC; SMAC (Diablo); Aifm-1; Aifm-2
Developmental Neurology	BMP-4; Chordin (Chrd); Noggin (Nog); WNT (Wnt2;
	Wnt2b; Wnt3a; Wnt4; Wnt5a; Wnt6; Wnt7b; Wnt8b;
	Wnt9a; Wnt9b; Wnt10a; Wnt10b; Wnt16); beta-catenin;
	Dkk-1; Frizzled related proteins; Otx-2; Gbx2; FGF-8;
	Reelin; Dab1; unc-86 (Pou4f1 or Brn3a); Numb; Reln

Table D:

A	B	C	D	E	F	G	H	I	J	K	L	M
CSF mass spec transmembrane protein gene names	neuron specific genes in mouse	neuron specific genes in human	CSF transmembrane proteins that are neuron specific in mouse	CSF transmembrane proteins that are neuron specific in human	CSF transmembrane proteins that are neuron specific in mouse and human	Proteins found in neuron exosomes	CSF MS transmembrane proteins in neuron exosomes	neuron specific genes in mouse AND in neuron exosome	neuron specific genes in human AND in neuron exosome	CSF transmembrane proteins that are neuron specific in mouse AND in neuron exosome	CSF transmembrane proteins that are neuron specific in human AND in neuron exosome	CSF transmembrane proteins that are neuron specific in mouse and human AND in neuron exosome
APP	HTR7	GAD2	CACNA2D2	PCDHGC4	KIT	ABCA3	ACVR1B	ATP2B2	ATP2B1	CACNA2D1	SYT1	FLRT3
A4	NT5C1A	PCDHGC4	L1CAM	CNR1	LINGO2	ABCD2	APLP1	ATP2B3	ATP2B2	CDH4	ROBO2	L1CAM
AD1	YWHAG	SYNPR	NDST4	KIT	EPHA7	ACVR1B	APLP2	CACNA2D1	ATP2B3	CELSR2	RTN1	
SLC3A2	CHRNB2	CNR1	AJAP1	SYT1	GPR158	ACVR2A	APMAP	CADM3	ATP8A2	FLRT3	PLXNA4	
MDU1	ACBD7	DLX6-AS1	EPHA8	CDH9	RTN1	ANKAR	ATP2B1	CDH4	CACNA2D1	L1CAM	FLRT3	
ABCA2	HTR1B	RELN	WSCD2	LINGO2	CDH7	APLP1	ATP6AP1	CELSR2	CELSR3	PGRMC1	SCN3A	
ABC2	HTR1DB	GABRA1	KCNS2	ROBO2	WSCD2	APLP2	ATRN	CELSR3	DISP2	PLXNA4	L1CAM	
KIAA1062	HTR3A	KCNC2	CDH4	EPHA7	CDH18	APMAP	BMPR2	DISP2	EEF1E1	RTN1	LPHN2	
ACE	5HT3R	VIP	CACNA2D1	PCDH8	NDST4	ARL6IP5	CACNA2D1	EPHA3	EPHA3	STX1B	FXYD6	
DCP	HTR3	DLX1	CACNA2D3	PARM1	KCNS2	ASIC1	CADM1	FAM171A2	EPHA4		CACNA2D1	
DCP1	CHRM3	CCK	CDH7	GPR158	PLXNA4	ATP11C	CADM3	FLRT3	FLRT3		ATP2B1	
ADAM23	CHRM4	PTHLH	EPHA7	PTPRR	SCN3B	ATP2B1	CANX	IGSF3	FXYD6		SCAMP1	
MDC3	ABCC8	TAC3	D130043K22RIK	RTN1	FLRT3	ATP2B2	CDH2	ISLR2	GRIK2		EPHA4	
ABCA1	HRINS	TAC1	CNTNAP5A	ST8SIA3	L1CAM	ATP2B3	CDH4	L1CAM	GRIK3			
ABC1	SUR	ZMAT4	NPTXR	C11orf87	PLD5	ATP2B4	CELSR2	NGFR	IGSF3			
CERP	SUR1	CALB2	HCN3	HCN1	HS6ST2	ATP2C1	CPD	PCLO	ITFG1			
ADAM10	ABCG4	PENK	CNTNAP5C	PTPRT	CACNA2D3	ATP6AP1	EGFR	PGRMC1	ITPR1			
KUZ	WHITE2	GABRG2	SCN3B	CDH7	HCN3	ATP6V0A1	EPHA4	PLXNA4	L1CAM			
MADM	AFF3	NXPH2	AI593442	WBSCR17	CACNA2D1	ATP8A1	FAM171A1	ROBO2	LPHN2			
ADAM15	LAF4	GAD1	KIT	C9orf4	CLSTN3	ATP8A2	FLRT3	RTN1	PCLO			

MDC15	HTR1D	RAB3C	CALY	NETO1	AJAP1	ATP9A	FXYD6	SCN9A	PLXNA4			
ADAM17	HTR1DA	CRH	CLSTN3	SLITRK4	CALY	ATRN	ITGA7	SEZ6L2	PTPRO			
CSVP	HTRL	KIT	LINGO2	EPHA5	SCN2B	BMPR2	ITM2B	SLC38A1	RET			
TACE	HTR1F	PCP4L1	CDH18	NRXN3	MET	C5orf42	ITM2B	STX1B	ROBO2			
ADGRL2	HTR1EL	OPRK1	CXADR	WSCD2		CACNA2D1	ITM2C	SUSD2	RTN1			
KIAA0786	HTR2C	SERTM1	FLRT3	SLC12A5		CADM1	JAM3	SYP	RTN3			
LEC1	HTR1C	GABRB2	MET	CDH18		CADM3	L1CAM	SYT1	SCAMP1			
LPHH1	ACHE	CHRNA6	NPCD	D4S234E		CANX	LDLR	SYT2	SCN3A			
LPHN2	FAM132A	GRIN3A	HS6ST2	NDST4		CD151	LINGO1	SYT5	SCN9A			
ACVR1B	C1QDC2	SYT4	SCN2B	TRHDE		CD47	LPHN2	THSD7A	SERINC1			
ACVRLK4	CTRP12	ENTPD3	STX1B	KCNS2		CDH2	LPHN3		SLC38A1			
ALK4	CHRM5	ELAVL2	SLC24A2	SV2A		CDH4	LRP1		SLC4A10			
ADAM22	MLLT11	SYT1	RTN1	EFNB3		CELSR2	LRP1B		SLC4A8			
MDC2	AF1Q	C8orf34	GPR158	NOV		CELSR3	LRRN1		SYP			
ADGRB2	ACTN2	GRPR	RAMP3	CNTNAP2		CISD2	MMP15		SYT1			
BAI2	ADRA2C	ZNF385D	PODXL2	ATP1B1		CLCN3	NCAM1		SYT2			
ADGRL1	ADRA2L2	CBLN4	PRRT3	PLXNA4		CLCN6	NEO1		THSD7A			
KIAA0821	ADRA2RL2	NDNF	EFNB2	CLSTN2		CLDND1	NFASC		TMEFF2			
LEC2	NPPC	GRIK1-AS2	CHL1	CDH8		CPD	NPDC1					
LPHN1	CNP2	DLX6	D430041D05RIK	SCN3B		CXCR4	NRP1					
ADAM8	AFF2	PNOC	PLD5	ST8SIA5		CYB5A	NRXN1					
MS2	FMR2	SCG2	CELSR2	SEZ6		CYB5B	NRXN2					
ADAM11	OX19	SLC32A1	PGRMC1	RYR2		DCHS1	NSG1					
MDC	AJAP1	PLCXD3	PLXNA4	HS6ST3		DISP2	NTRK2					
ADAM28	MOT8	CKMT1B		FLRT3		EEF1E1	PGRMC1					
ADAM23	SHREW1	CALB1		SCN3A		EGFR	PLD3					
MDCL	AMY1A	CDH9		C11orf41		EPHA3	PLXNA1					
ADAM9	AMY1;	CCNA1		SORCS3		EPHA4	PLXNA3					
KIAA0021	AMY1B	RGS8		HMP19		EPHX1	PLXNA4					
MCMP	AMY1;	SV2C		L1CAM		ESYT1	PTPLAD1					
MDC9	AMY1C	RBM24		LPHN2		FAM171A1	PTPRD					

MLTNG	AMY1	PCDHA1		CD200		FAM171A2	PTPRF					
ADGRB3	PRMT8	VWC2L		PLD5		FDFT1	PTPRG					
BAI3	HRMT1L3	GLRA2		SORCS1		FER1L5	PTPRS					
KIAA0550	HRMT1L4	TACR1		GRIA4		FLRT3	ROBO1					
ADGRL3	ANKRD24	LINGO2		DPP6		FLT1	ROBO2					
KIAA0768	KIAA1981	NPY		HS6ST2		FOCAD	RTN1					
LEC3	AMIGO1	CKMT1A		ODZ1		FXYD6	RTN4					
LPHN3	ALI2	ZCCHC12		CNTNAP5		GRIK2	RTN4					
AJAP1	AMIGO	LNP1		SLITRK3		GRIK3	SCAMP1					
MOT8	KIAA1163	SLC10A4		LRFN5		GUCY2D	SCN3A					
SHREW1	ANKRD45	NPR3		LRRTM2		HECTD4	SDK1					
PAM	AP3B2	CAMK1G		EPHA10		HECTD4	SEMA4C					
ANO4	ARG2	ROBO2		FXYD6		HSD17B12	SEMA6D					
TMEM16D	ARMC2	KCNA3		PLXNC1		IGSF3	SEZ6L2					
ANTXR1	ATP2B3	LOC147670		CACNA2D3		ISLR2	SLC12A2					
ATR	ARX	SYNGR3		ADAM22		ITFG1	SORT1					
TEM8	ASGR1	GABRG3		PAM		ITGA7	STX12					
ANPEP	CLEC4H1	KCNJ3		SLITRK5		ITGA9	STX1B					
APN	ASPHD1	MAP7D2		C10orf35		ITGAM	STX7					
CD13	ATG9B	GRIN2B		ATRNL1		ITM2B	SYT1					
PEPN	APG9L2	SRRM4		ATP1A3		ITM2B	SYT11					
APLP2	NOS3AS	KCNQ5		ST8SIA2		ITM2C	TENM2					
APPL2	ATP2B2	OLFM3		HCN3		ITPR1	TENM4					
APMAP	PMCA2	KRT222		CCDC136		JAM3	TGOLN2					
C20orf3	DNAJC6	CAMKV		CACNA2D1		KIAA1109	TM9SF3					
UNQ1869/PRO4305	KIAA0473	EPHA7		SPINT2		KIAA1324L	TM9SF4					
AOC3	B3GALT1	GRIN2A		FNDC9		KIDINS220	TMEM132A					
VAP1	BAIAP3	STMN2		SLC8A2		KTN1	UNC5C					
APLP1	KIAA0734	SNAP25		NPTN		L1CAM	VAMP2					
ATF6B	BASP1	DLX5		FAR2		LDLR						

CREBL1	NAP22	INA		TPBG		LINGO1						
G13	ADAMTS15	SST		SEZ6L		LPHN2						
ATP11B	BARHL2	SCN2A		UNC5D		LPHN3						
ATPIF	ADAMTS14	CHRM2		TMEM132D		LPPR3						
ATPIR	B3GALT2	NPAS4		B4GALNT1		LRP1						
KIAA0956	BEX2	PCSK2		CLSTN3		LRP1B						
ATP2B1	BHLHE22	COCH		ATP2B1		LRRC59						
PMCA1	BHLHB5	PCDH8		AJAP1		LRRC8A						
ASTN2	TNRC20	BTBD11		B7H6		LRRN1						
KIAA0634	BFSP1	PARM1		ADAM23		MMP15						
ASTN1	BEX4	GPR158		LARGE		NALCN						
ASTN	BEXL1	PVALB		ERBB4		NCAM1						
KIAA0289	NADE3	MEG3		CALY		NCKAP1						
ATF6	BAIAP2L2	PDYN		PTPRN2		NEO1						
ASPH	UNQ9336/PRO34007	SVOP		LRP11		NFASC						
BAH	BMP5	HSPB3		TRPC3		NGFR						
ATP1A3	BMP6	PTPRR		SLC9A7		NPDC1						
ATP1A1	VGR	CHRNB3		DNER		NPR2						
ATP1A2	CACNA2D1	UCHL1		EPHA6		NRP1						
KIAA0778	CACNL2A	SIAH3		PVR		NRXN1						
ATP1B2	CCHL2A	SLC4A10		SCN2B		NRXN2						
B3GNT2	MHS3	NELL2		ATP1A1		NSDHL						
B3GALT7	CACNA2D3	RTN1		B3GALNT1		NSG1						
B3GNT1	CACNA1I	LGI2		GALNT13		NTRK2						
BACE1	KIAA1120	PGM2L1		CHST15		P2RX3						
BACE	CDH4	SCGB2A1		FLT3		PCDHB10						
KIAA1149	CDH7	ST8SIA3		DCC		PCDHB2						
ATP1B1	CDH7L1	C11orf87		SLITRK1		PCDHB5						
ATP1B	BEGAIN	GALNTL6		NAGPA		PCDHGA11						

ATRN	KIAA1446	LOC157627		SCAMP1		PCDHGB2						
KIAA0548	BEND6	GLS2		FAM20B		PCDHGB6						
MGCA	C6orf65	MYT1L		RNF150		PCLO						
B4GALT1	BSN	GRIP1		MET		PGRMC1						
GGTB2	KIAA0434	SYT10		IL1RAPL1		PIEZO2						
B3GNT4	ZNF231	SYTL5		EPHA4		PLD3						
UNQ1898/PRO4344	BTBD11	CCDC85A		GRIA2		PLXNA1						
B4GALNT4	C1QTNF1	SYT13		DCBLD2		PLXNA2						
B3GAT3	CTRP1	HCN1				PLXNA3						
B4GAT1	UNQ310/PRO353	PTPRT				PLXNA4						
B3GNT1	CDH12	CNTN5				PMEL						
B3GNT6	C2CD4C	SPHKAP				PRAF2						
BSG	FAM148C	MRAP2				PSEN1						
UNQ6505/PRO21383	KIAA1957	NRIP3				PTPLAD1						
BCAM	NLF3	CDH7				PTPN1						
LU	CABP7	BEX5				PTPRA						
MSK19	CALN2	CBLN2				PTPRD						
BET1L	CALY	NSF				PTPRF						
GS15	DRD1IP	DCLK1				PTPRG						
B3GNT8	BPIFA1	VSTM2A				PTPRO						
B3GALT7	LUNX	WBSCR17				PTPRS						
BGALT15	NASG	MTUS2				RET						
B4GALNT1	PLUNC	GABBR2				ROBO1						
GALGT	SPLUNC1	CRHBP				ROBO2						
SIAT2	SPURT	LOC285878				RTN1						
BAI1	UNQ787/PRO1606	STAT4				RTN3						
BAMBI	CACNA2D2	C9orf4				RTN4						

NMA	KIAA0558	CHGB				RTN4						
ATRNL1	CBLN4	CLVS1				SCAMP1						
KIAA0534	CBLNL1	ARL4C				SCAMP3						
B3GALNT 1	UNQ718/ PRO1382	NPY1R				SCARB2						
B3GALT3	CACNA1G	HTR2C				SCFD1						
UNQ531/ PRO1074	KIAA1123	DIRAS2				SCN3A						
BTN2A1	CDH18	RSPO3				SCN9A						
BT2.1	CDH14	NETO1				SDK1						
BTF1	CALCA	EGFL6				SEMA3D						
BST2	CALC1	HTR5A				SEMA4C						
CACNA2D 1	CCDC152	FSTL5				SEMA6D						
CACNL2A	Chr5_400	PCDH11Y				SERINC1						
CCHL2A	CACNG5	HOOK1				SEZ6L2						
MHS3	CCK	PRR16				SLC12A2						
CACNA2D 3	CAMKV	LOC10014 4602				SLC12A7						
CDH20	CASZ1	SLITRK4				SLC22A17						
CDH7L3	CST	VSNL1				SLC38A1						
CDH4	SRG	FRMPD4				SLC4A10						
CDH6	ZNF693	ATP8A2				SLC4A7						
CDH7	CCDC148	EPHA5				SLC4A8						
CDH7L1	CCDC6	CAPSL				SNRNP40						
CADM2	D10S170	RIMBP2				SORT1						
IGSF4D	TST1	FGF13				ST6GAL1						
NECL3	C4orf48	LAMP5				STARD3NL						
BOC	Chr4_55	SLC2A13				STX12						
UNQ604/ PRO1190	CELSR2	GRIK1				STX1B						
CACHD1	CDHF10	HBG2				STX7						
KIAA1573	EGFL2	RASD2				SUSD2						
VWCD1	KIAA0279	FGF14				SYNGR1						
BMPR2	MEGF3	RASGRF1				SYP						

PPH1	CACNG3	RBFOX1				SYT1						
CD93	C6orf222	MYO16				SYT11						
C1QR1	CGREF1	CPLX2				SYT2						
MXRA4	CGR11	SYN2				SYT5						
CDH11	CBLN1	GRIA1				TENM2						
CDH15	CCDC116	SCN1A				TENM3						
CDH14	CEACAM16	NRXN3				TENM4						
CDH3	CEAL2	KCNJ6				TEX10						
CDH2	CELF4	GRIK2				TGFBR1						
CDHN	BRUNOL4	PTCHD4				TGOLN2						
NCAD	CACNG2	FABP3				THSD7A						
CANX	CCL27	RAB3B				TM9SF2						
CALY	ILC	RGS4				TM9SF3						
DRD1IP	SCYA27	CDH13				TM9SF4						
CACNA2D2	CELF6	IL1RAPL2				TMEFF1						
KIAA0558	BRUNOL6	WSCD2				TMEFF2						
C3AR1	C8orf34	LINC00290				TMEM132A						
AZ3B	CDK5R2	ZNF804A				TMEM178B						
C3R1	NCK5AI	SLC7A14				TMEM55B						
HNFAG09	CELSR3	CYP4X1				TP53I11						
CADM3	CDHF11	NMNAT2				TSPAN15						
IGSF4B	EGFL1	SCGN				UBR4						
NECL1	FMI1	UBE2QL1				UNC5C						
SYNCAM3	KIAA0812	GPR83				VAMP2						
TSLL1	MEGF2	SCN7A				VANGL2						
UNQ225/PRO258	CDR1	GPR12				VAPB						
CD163	C11orf87	RXFP1				VSIG8						
M130	CEND1	FAM19A2				WDR11						
CDH10	BM88	CACNA1E				XPR1						
CADM1	CHAC1	REEP1				YIPF6						

IGSF4	BOTCH	SLC12A5																	
IGSF4A	C11orf42	TRPC4																	
NECL2	CLVS2	COL25A1																	
SYNCAM	C6orf212	WNT10B																	
TSLC1	C6orf213	SLC35F4																	
CADM4	RLBP1L2	GABRA5																	
IGSF4C	CNGA3	MAL2																	
NECL4	CNCG3	GREM2																	
TSLL2	CHSY3	RGS7BP																	
CA12	CHSY2	CDH18																	

Continuation of Data from Table D: *CSF mass spec transmembrane protein gene names No. 209 onwards*

209-243	244-278	279-314	315-349	350-384	385-419	420-454	455-489
CA14	CDHF10	MIC2X	COLEC12	NGC	DBH	EFNB2	ERAP1
UNQ690/PRO1335	EGFL2	MIC2Y	CLP1	CSPG4	DLK2	EPLG5	APPILS
CDH18	KIAA0279	CELSR1	NSR2	MCSP	EGFL9	HTKL	ARTS1
CDH14	MEGF3	CDHF9	SCARA4	GJA9	UNQ2903/PRO28633	LERK5	KIAA0525
CDH5	CASC4	FMI2	SRCL	GJA10	DPP10	EDNRB	UNQ584/PRO1154
CDH8	UNQ2573/PRO6308	CHST1	CNR1	CXADR	DPRP3	ETRB	ERAP2
CCR1	CD276	CHST8	CNR	CAR	KIAA1492	EMC10	LRAP
CMKBR1	B7H3	CLIC6	CRB2	CLSTN2	DSG3	C19orf63	ERBB3
CMKR1	PSEC0249	CLIC1L	CRELD1	CS2	CDHF6	HSM1	HER3
SCYAR1	UNQ309/PRO352	CLMP	CIRRIN	GJA1	DPP6	INM02	ESAM
CDH9	CD81	ACAM	UNQ188/PRO214	GJAL	DNER	UNQ764/PRO1556	UNQ220/PRO246
CANT1	TAPA1	ASAM	CSF1R	CXCL16	BET	EFNB3	EGFR
SHAPY	TSPAN28	UNQ318/PRO363	FMS	SCYB16	UNQ262/PRO299	EPLG8	ERBB
CD44	CD320	C11orf87	CSF2RA	SRPSOX	SLC1A2	LERK8	ERBB1
LHR	8D6A	CHST10	CSF2R	UNQ2759/PRO6714	EAAT2	EPHA5	HER1
MDU2	UNQ198/PRO224	CMTM1	CSF2RY	DAG1	GLT1	BSK	ENPP5
MDU3	CCDC136	CKLFSF1	COL23A1	DCC	DRD3	EHK1	UNQ550/PRO1107
MIC4	KIAA1793	CLCC1	CSF1	IGDCC1	DSG2	HEK7	PROCR
CDHR1	NAG6	KIAA0761	CCSMST1	DCBLD2	CDHF5	TYRO4	EPCR
KIAA1775	CD302	MCLC	C16orf91	CLCP1	DUOX2	EPHA8	EPHA7
PCDH21	CLEC13A	CHST14	CLSTN1	ESDN	LNOX2	EEK	EHK3
PRCAD	DCL1	D4ST1	CS1	DDR1	THOX2	HEK3	HEK11
CPD	KIAA0022	UNQ1925/PRO4400	KIAA0911	CAK	DSC2	KIAA1459	EPHB6
CCR10	ALCAM	CHST3	CYP26C1	EDDR1	CDHF2	EPHB1	ENPP4
GPR2	MEMD	CLIC1	CRIM1	NEP	DSC3	ELK	KIAA0879
CD248	CHST12	G6	S52	NTRK4	DSC3	EPHT2	NPP4
CD164L1	UNQ500/PRO1017	NCC27	UNQ1886/PRO4330	PTK3A	CDHF3	HEK6	ENTPD4
TEM1	CHST15	C9	CSMD2	RTK6	DSC4	NET	KIAA0392
CD6	BRAG	C14orf37	KIAA1884	TRKE	DSCAM	EPHB4	LALP70
CD9	GALNAC4S6ST	CNTNAP2	CLSTN3	DDR2	ECE1	HTK	LYSAL1
MIC3	KIAA0598	CASPR2	CS3	NTRKR3	EFNB1	MYK1	EPHA6
TSPAN29	C10orf35	KIAA0868	KIAA0726	TKT	EFL3	TYRO11	EHK2
GIG2	CD99	CNTNAP5	CSPG5	TYRO10	EPLG2	ENG	HEK12
CELSR2	MIC2	CASPR5	CALEB	DCBLD1	LERK2	END	EPHA10

490-524	525-559	560-594	595-629	630-664	665-699	700-734	735-769
EPHB2	C10orf38	FGFBR	GGT7	GALNTL3	GXYLT1	HLAF	IL3RB
DRT	FAM198B	FLG	GGTL3	GOLIM4	GLT8D3	ICAM2	IL5RB
EPHT3	C4orf18	FLT2	GGTL5	GIMPC	HS6ST2	HS2ST1	IGSF5
EPTH3	ENED	HBGFR	FZD3	GOLPH4	PSEC0092	HS2ST	JAM4
ERK	AD021	FKRP	GAL3ST3	GPP130	GRIA4	KIAA0448	IFNAR1
HEK5	UNQ2512/PRO6001	FLT3	GINM1	GOLM1	GLUR4	DGCR2	IFNAR
TYRO5	FAM174A	CD135	C6orf72	C9orf155	GRID2	IDD	IFNGR1
EPHB3	NS5ATP6	FLK2	UNQ710/PRO1361	GOLPH2	GLURD2	KIAA0163	ITGB8
ETK2	TMEM157	STK1	GALNT2	PSEC0242	HCN1	IL1RAP	IGSF8
HEK2	UNQ1912/PRO4371	FGFR3	GALNT11	UNQ686/PRO1326	BCNG1	C3orf13	CD81P3
TYRO6	FAR2	JTK4	GALNT16	GPNMB	HAVCR2	IL1R3	EWI2
GPR37L1	MLSTD1	FLRT2	GALNTL1	HGFIN	TIM3	ICAM1	KCT4
ETBRLP2	FCGR2A	KIAA0405	KIAA1130	NMB	TIMD3	ICOSLG	IL6ST
EXT1	CD32	UNQ232/PRO265	GLT8D1	UNQ1725/PRO9925	HCN2	B7H2	IMPAD1
FAM134A	FCG2	FLRT1	GALA4A	GPR37	BCNG2	B7RP1	IMPA3
C2orf17	FCGR2A1	UNQ752/PRO1483	AD-017	GPR56	HEPACAM	ICOSL	KIAA0319L
EPHA4	IGFR2	FNDC9	MSTP137	TM7LN4	HACD3	KIAA0653	KIAA1837
HEK8	FGFR2	C5orf40	UNQ572/PRO1134	TM7XN1	BIND1	IGSF11	PP791
SEK	BEK	FUT11	GLDN	UNQ540/PRO1083	PTPLAD1	BTIGSF	IFNLR1
TYRO1	KGFR	FXYD6	COLM	GLG1	HS6ST3	CXADRL1	IL28RA
FAM173A	KSAM	UNQ521/PRO1056	UNQ9339/PRO34011	CFR1	HEG1	VSIG3	LICR2
C16orf24	FLRT3	GALNT7	GYPC	ESL1	KIAA1237	IGSF1	ITM2B
RJD7	KIAA1469	FURIN	GLPC	MG160	HBEGF	IGDC1	BRI
FAM69C	UNQ856/PRO1865	FUR	GPC	SLC2A11	DTR	KIAA0364	BRI2
C18orf51	FAT1	PACE	GALNT18	GLUT10	DTS	PGSF2	JAM2
FCGR3A	CDHF7	PCSK3	GALNTL4	GLUT11	HEGFL	IMPG2	C21orf43
CD16A	FAT	GALNT6	GPR158	GPR179	HCN3	IPM200	VEJAM
FCG3	FAT2	FREM2	KIAA1136	GPR158L	KIAA1535	IFNAR2	UNQ219/PRO245
FCGR3	CDHF8	FRRS1L	GALNT10	GPR158L1	HLA-G	IFNABR	JAM3
IGFR3	KIAA0811	C9orf4	GPAA1	GPR180	HLA-6.0	IFNARB	UNQ859/PRO1868
EXT2	MEGF1	FZD7	GAA1	ITR	HLAG	ICAM5	KIAA1467
EXTL2	FGFR1	GALNT1	GALNT13	GP1BA	HMGCR	TLCN	ITGA2B
EXTR2	BFGFR	GGT5	KIAA1918	GRIA2	HLA-F	TLN	GP2B
FAM171A1	CEK	GGTLA1	WBSCR17	GLUR2	HLA-5.4	CSF2RB	ITGAB

770-804	805-839	840-874	875-909	910-944	945-979	980-1,014
A2MR	A2MR	LRFN5	UNQ671/PRO1305	CLEC13D	NFASC	KIAA0343
APR	APR	C14orf146	MERTK	CLEC13DL	KIAA0756	NOTCH3
LRP8	LRP8	SALM5	MER	MRC1L1	CHL1	NRG3
APOER2	APOER2	LRIG2	MET	MRC2	CALL	NRXN1
LRRC4	LRRC4	KIAA0806	MAG	CLEC13E	NEO1	NRXN2
BAG	BAG	LIG2	GMA	ENDO180	IGDCC2	KIAA0921
NAG14	NAG14	LRRN1	MEGF10	KIAA0709	NGN	NOTCH2
UNQ554/PRO1111	UNQ554/PRO1111	KIAA1497	KIAA1780	UPARAP	NCAM2	NPTXR
LSR	LSR	Nbla10449	MGAT1	MXRA7	NCAM21	NRXN3
LISCH	LISCH	UNQ693/PRO1338	GGNT1	TMAP1	NINJ1	C14orf60
LY75	LY75	MAN2A1	GLCT1	MUC16	NLGN4X	KIAA0743
CD205	CD205	MANA2	GLYT1	CA125	KIAA1260	NLGN3
CLEC13B	CLEC13B	MBOAT2	MGAT	SLC8A2	NLGN4	KIAA1480
MAN1C1	MAN1C1	OACT2	MEGF8	KIAA1087	UNQ365/PRO701	NL3
MAN1A3	MAN1A3	LRRTM2	C19orf49	NCX2	NLGN2	NOMO3
MAN1C	MAN1C	KIAA0416	EGFL4	MXRA8	KIAA1366	NPTN
LMAN1	LMAN1	LRRN2	KIAA0817	NAALADL1	NOMO1	SDFR1
ERGIC53	ERGIC53	LYVE1	MMP14	NAALADASEL	PM5	SDR1
F5F8D	F5F8D	CRSBP1	MGAT5	NAALADL	NOMO2	NSG1
LRRC4C	LRRC4C	HAR	GGNT5	NCKAP1L	NDST1	D4S234
KIAA1580	KIAA1580	XLKD1	MMP15	HEM1	HSST	NRXN2
NGL1	NGL1	UNQ230/PRO263	MGAT2	SLC8A1	HSST1	NRP2
UNQ292/PRO331	UNQ292/PRO331	MAN1A2	MOG	CNC	NDST2	VEGF165R2
SELL	SELL	MAN1B	MLEC	NCX1	HSST2	NRXN1
LNHR	LNHR	MAN1B1	KIAA0152	MYOF	NCSTN	KIAA0578
LYAM1	LYAM1	UNQ747/PRO1477	MYRF	FER1L3	KIAA0253	NRXN3
MAN2A2	MAN2A2	MANEA	C11orf9	KIAA1207	UNQ1874/PRO4317	KIAA0743
MANA2X	MANA2X	MANSC1	KIAA0954	SLC24A2	NPC1	NSG2
LRP1B	LRP1B	LOH12CR3	MRF	NCKX2	NCR3LG1	NTRK2
LRPDIT	LRPDIT	UNQ316/PRO361	MCAM	NAGPA	B7H6	TRKB
LRP5	LRP5	MAN1A1	MUC18	NCAM1	NDST4	OR2AK2
LR3	LR3	MEGF9	IGF2R	NCAM	HSST4	OR2AK1P
LRP7	LRP7	EGFL5	MPRI	NETO1	NPDC1	NTRK3
MANEAL	MANEAL	KIAA0818	MRC1	BTCL1	NRCAM	TRKC

1,015-1,049	1,050-1,084	1,085-1,119	1,120-1,154	1,155-1,189	1,190-1,224	1,225-1,259	1,260-1,294
NRP1	PCNXL1	PSEC0164	PIGA	DEP1	HVEC	RNF13	GREAT
NRP	PCDH10	UNQ289/PRO328	PKD1L3	PTPRR	PRR1	RZF	LGR8
VEGF165R	KIAA1400	PKD1	PLD3	ECPTP	PVRL3	RNF150	SLC39A6
OR52A1	PAPPA-AS1	PLXNA1	PLXDC1	PTPRQ	PRR3	KIAA1214	LIV1
CD200	DIPAS	NOV	TEM3	PTPRS	QSOX2	RPN2	ZIP6
MOX1	PAPPAS	PLXN1	TEM7	PTPRF	QSCN6L1	ROR1	RYR2
MOX2	PCDH17	PLXNA3	PLXNB3	LAR	SOXN	NTRKR1	SEZ6L2
My033	PCDH68	PLXN4	KIAA1206	PTPRZ1	EBAG9	ROBO2	PSK
OR13C3	PCH68	SEX	PLXN6	HTPZP2	RCAS1	KIAA1568	UNQ1903/PRO4349
OGFOD3	PCDHB14	PLXNA4	PLXNC1	PTPRZ	QSOX1	RTN1	SLC38A10
C17orf101	PCDHGC5	KIAA1550	VESPR	PTPRZ2	QSCN6	NSP	PP1744
OSMR	PCDH7	PLXNA4A	PLXND1	PTPZ	UNQ2520/PRO6013	SLC12A2	SLC39A12
OSMRB	BHPCDH	PLXNA4B	KIAA0620	PTCHD2	RAMP3	NKCC1	ZIP12
P2RY14	PDGFRB	UNQ2820/PRO34003	PODXL	DISP3	REEP2	S1PR3	SCN1B
GPR105	PDGFR	PLXNB2	PCLP	KIAA1337	C5orf19	EDG3	SCN3B
KIAA0001	PDGFR1	KIAA0315	PCLP1	PVRL2	SGC32445	SLC22A23	KIAA1158
PIK3IP1	PODXL2	POMGNT1	PRLR	HVEB	RHBDF1	C6orf85	SCN4A
HGFL	UNQ1861/ PRO3742	MGAT1.2	ACP2	PRR2	C16orf8	SLC12A5	SDC2
PARM1	PEAR1	UNQ746/PRO1475	PRRT2	PVR	DIST1	KCC2	HSPG1
UNQ1879/PRO4322	MEGF12	PKD1L2	PRRT3	PVS	IRHOM1	KIAA1176	SDC4
PCDHB15	PILRA	KIAA1879	UNQ5823/ PRO19642	PLXDC2	ATP6AP2	SLC4A4	SEZ6L
PCDHGA12	PGRMC1	PC1L2	PRSS8	TEM7R	ATP6IP2	NBC	KIAA0927
CDH21	HPR6.6	PLD4	PTPRG	UNQ2514/ PRO6003	CAPER	NBC1	UNQ2542/PRO6094
FIB3	PGRMC	C14orf175	PTPG	PTK7	ELDF10	NBCE1	SEMA6D
KIAA0588	PIGR	UNQ2488/PRO5775	PTPRK	CCK4	HT028	SCIMP	KIAA1479
UNQ371/PRO707	PROKR1	PLD5	PTPK	PTPRD	MSTP009	C17orf87	SLC39A10
PCDH9	GPR73	PNPLA6	PRRT1	PTPRM	PSEC0072	UNQ5783/ PRO16090	KIAA1265
PCDHGC4	PKR1	NTE	C6orf31	PTPRL1	RIC3	RTN4	ZIP10
PCDH8	PDGFRA	FXYD1	NG5	PTPRN	UNQ720/PRO1385	KIAA0886	SARAF
PCDHGC3	PDGFR2	PLM	PTPRN2	ICA3	RFNG	NOGO	TMEM66
PCDH2	RHEPDGFRA	PLXNB1	KIAA0387	ICA512	RHAG	My043	XTP3
PCNX	PI16	KIAA0407	PTPRB	PTPRT	RH50	SP1507	HSPC035
KIAA0805	CRISP9	PLXN5	PTPB	KIAA0283	ROBO1	RXFP2	NPD003
KIAA0995	PSPBP	SEP	PTPRJ	PVRL1	DUTT1	GPR106	PSEC0019

1,295 – 1,329	1,330 – 1,364	1,365 – 1,399	1,400 – 1,434	1,435 – 1,469	1,470 – 1,504	1,505 – 1,539	1,540 – 1,574
UNQ1967/PRO4499	UNQ783/PRO1317	SLAMF7	KIAA1854	C1orf9	ODZ4	TLR9	TRPC3
SDK1	SEMA6C	CS1	SLITL1	CH1	TNM4	UNQ5798/PRO19605	TRP3
SLC5A5	KIAA1869	UNQ576/PRO1138	UNQ9197/ PRO34756	OPT	SYT11	TMED4	TYRO3
NIS	SEMAY	SEMA4B	ST3GAL6	SLP1	KIAA0080	ERS25	BYK
SDC3	POMK	KIAA1745	SIAT10	STAB1	SYT9	TMED9	DTK
KIAA0468	SGK196	SEMAC	SLITRK3	FEEL1	TGOLN2	GP25L2	RSE
SEMA4D	ST3GAL4	UNQ749/PRO1480	KIAA0848	KIAA0246	TGN46	TMEM5	SKY
C9orf164	CGS23	SEMA4C	SGMS2	STX1B	TGN51	TNFRSF18	TIF
CD100	NANTA3	KIAA1739	SMS2	STX1B1	TMEM132E	AITR	UGT2B15
SEMAJ	SIAT4C	SEMAI	SLITRK6	STX1B2	TFRC	GITR	UGT2B8
SEMA6A	STZ	UNQ5855/PRO34487	SNPH	STX12	TIE1	UNQ319/PRO364	TYRP1
KIAA1368	ST8SIA3	SLITRK1	KIAA0374	SUSD6	TIE	TMEM25	CAS2
SEMAQ	SIAT8C	KIAA1910	SORCS1	DRAGO	TGFBR3	UNQ2531/PRO6030	TYRP
SCAMP1	SIRPB1	LRRC12	SORCS	KIAA0247	TENM1	TPBG	TYRRP
SCAMP	SLITRK4	UNQ233/PRO266	SORCS3	TMEM132A	ODZ1	5T4	AXL
SCN2B	SEL1L	SLITRK5	KIAA1059	HSPA5BP1	TNM1	TM9SF3	UFO
UNQ326/PRO386	TSA305	KIAA0918	SORL1	KIAA1583	PRRG1	SMBP	UNC5B
SCN3A	UNQ128/PRO1063	LRRC11	C11orf32	TMEM132B	PRGP1	UNQ245/PRO282	P53RDL1
KIAA1356	SELPLG	SEMA6B	SORT1	KIAA1786	TMG1	TM9SF4	UNC5H2
NAC3	SEZ6	SEMAN	SPINT2	KIAA1906	TGFBR2	KIAA0255	UNQ1883/PRO4326
ST8SIA2	ST6GALNAC1	SEMAZ	HAI2	TMEM132D	TLR1	TMED3	UNC5C
SIAT8B	SIAT7A	UNQ1907/PRO4353	KOP	HBE120	KIAA0012	C15orf22	UNC5H3
STX	UNQ543/PRO848	SGCE	SRPRB	KIAA1944	TMEM108	UNQ5357/PRO1078	VCAM1
ST8SIA5	ST8SIA4	ESG	PSEC0230	MOLT	KIAA1690	THBD	L1CAM
SIAT8E	PST	UNQ433/PRO840	SORCS2	SYT1	UNQ1875/PRO4318	THRM	UNC5D
ST6GAL2	PST1	SIGLEC8	KIAA1329	SVP65	TNFSF12	RELT	KIAA1777
KIAA1877	SIAT8D	SAF2	SV2A	SYT	APO3L	TNFRSF19L	UNC5H4
SIAT2	SIGLEC5	SLC9A7	KIAA0736	TMEM132C	DR3LG	TRPV5	UNQ6012/PRO34692
SCN4B	CD33L2	NHE7	PSEC0174	TENM2	UNQ181/PRO207	ECAC1	VAMP2
SECTM1	OBBP2	SHISA5	STX7	KIAA1127	TOR1AIP1	TRHDE	SYB2
K12	SIGLEC9	SCOTIN	SUSD5	ODZ2	LAP1	UNQ2507/PRO5995	VAPA
SEMA4A	UNQ668/PRO1302	PSEC0133	KIAA0527	TNM2	TNFRSF21	TXNDC15	VAP33
SEMAB	CD84	SLITRK2	SVOPL	TENM4	DR6	C5orf14	UST
SEMB	SLAMF5	CXorf2	SUCO	KIAA1302	UNQ437/PRO868	UNQ335/PRO534	DS2ST

1,575 – 1,609	1,610 – 1,644	1,645 – 1,679	1,680 – 1,714	1,715 – 1,749	1,750 – 1,784	1,785 – 1,819	1,820 – 1,854
SLC14A1	XXYLT1	hCG_37088	hCG_2045906	GALNT16	hCG_39124	SLITRK5	SEL1L
HUT11	C3orf21	CA14	B4GALT1	GALNTL1	NGL1	DKFZp686L0872	GRIA4
JK	PSEC0251	hCG_39384	SCN4B	hCG_21969	MST065	TM9SF3	DKFZp779G2333
RACH1	FAM20B	TNFSF13	hCG_1646677	XLKD1	hCG_2010808	DSC3	BDNF
UT1	KIAA0475	ENTPD4	FGFR3	hCG_23149	SLC2A11-a	GANAB	DKFZp686A04130
UTE	YIPF3	SEMA4A	TLR9	LST3	SLC2A11	DKFZp686D1354	UNC5C
VASN	C6orf109	C3AR1	GalNAc-T18	DIPLA1	hCG_41106	PTPsigma	SEMA4D
SLITL2	KLIP1	hCG_22220	GALNT18	EPHB4	SMBP	DKFZp686D04248	variant
UNQ314/PRO357/ PRO1282	ZP2	EPHB4	GALNTL4	hCG_20448	hCG_25781	HUT11	protein
ATP6AP1	ZPA	hCG_20448	hCG_1991780	tcag7.1248	ERBB4	HMP19	DKFZp686I11137
ATP6IP1	ZFPL1	SCN3A	HLA-G3	RHAG	EPHB2	DKFZp779D0769	ABC1
ATP6S1	PTPRN	CCR1	HLA-G	COX7A2	variant	NBC	ADAM17
VATPS1	hCG_15565	hCG_15324	CSF2RB	NEO1	protein	SORCS3	DST
XAP3	TGFBR2	ADAM22	ADAM17	UMOD	PCDH10	IL28RA	DKFZp761O2023
VLDLR	hCG_1997782	MERTK	ADAM18	MRDS1	ICAM5	CLSTN3	Tbeta
WSCD1	PTPRK	HLA-G	hCG_23065	OFCC1	NTRK2	ANTXR1/NNG1	RIIC
KIAA0523	CDH11	MOG	ADAM8	DKFZp761B182	EPHA4	fusion	FLJ00383
CX3CL1	hCG_26636	hCG_25629	EGFR	POMGNT1	DKFZp451E1911	ATP11B	SLC4A4
FKN	EXTL2	HLA-G	PGRMC1	SLC2A11-c	STK-1	AAT	KIAA0921
NTT	hCG_32848	hCG_1999524	hCG_23188	SLC2A11	JAG1	CLSTN1	DKFZp761D171
SCYD1	SLC12A2	HLA-G2.2	hEMMPRIN	hCG_41106	L1CAM	PTPRN	nma
A-152E5.2	hCG_27034	HLA-G	BSG	PIGA	Fc-gamma	SEMA4C	RH50
VSTM2B	ICOSLG	ENG	hCG_20562	hCG_1783055	receptor	HCN1	kit
WSCD2	hCG_401312	hCG_18549	SLITRK2	PTPRG	IIIB	TYRO3	ATP1B1
KIAA0789	NTRK2	PCDH9	hCG_1646164	SLC2A11	WUGSC:H_DJ1137M13.1	DLK2	KIAA0811
XYLT1	hCG_1985371	hCG_2026614	PRNP	hCG_41106	CD93	tmp_locus_20	DKFZp686I0613
XT1	RTN1	ICAM2	hCG_2045906	ATP1B1	LAR	IFNLR1	P3.58
XYLT2	NRP2	hCG_41817	RAMP3	hCG_37798	ITGA7	Nbla00445	DKFZp313P2036
XT2	hCG_15204	VLDLR	hCG_17148	SRPRB	variant	CRIM1	HCN3
UNQ3058/PRO9878	SCN2B	hCG_27927	tcag7.792	hCG_2023561	protein	CD58	ODZ1
VSIG4	hCG_41149	CX3CL1	RHAG	P2RY14	SCCA1	UT-B1	DKFZp686P14120
CRIg	PRNP	hCG_15105	hCG_20861	hCG_20914	DKFZp564A026	DKFZp761N1221	SEMA6B
Z39IG	hCG_1785425	ENG	NEU1	FAM3A	DKFZp781F1414	DKFZp686C2268	CSPG3
UNQ317/PRO362	SELL	TNFSF13	hCG_43692	D4S234E	LRIG1	MGAT5	variant

Continuation of Data from Table D: *neuron specific genes in mouse, gene numbers 209 onwards*

1,855-1,869
protein
DKFZp686P18250
BTCC-1
PVRL1
FLJ00329
DKFZp686J1169
DKFZp779F0871
FLJ00385
PVRL2
DKFZp434F011
Nbla00271
FLJ00095
DKFZp686B1310
DKFZp686F1789
KIAA1149

209-243	244-278	279-314	315-349	350-384	385-419
CHAT	COE2	KIAA0949	DUSP14	HEK3	FBXO41
CNIH3	CRH	STK21	MKP6	KIAA1459	FBX41
CILP	CPLX2	SDR39U1	DUSP4	EMX2	KIAA1940
UNQ602/PRO1188	COL11A1	C14orf124	MKP2	ELAVL2	FAM183A
CITED2	COLL6	HCDI	VH2	HUB	FAM131C
MRG1	CSRNP3	GJD2	ECEL1	ELAVL4	C1orf117
CAMK2N2	FAM130A2	GJA9	XCE	HUD	FAM184B
CLDN3	TAIP2	GAD1	UNQ2431/PRO4991	PNEM	KIAA1276
C7orf1	CPLX3	GAD	DEPTOR	EPHA7	FAM196A
CPETR2	Nbla11589	GAD67	DEPDC6	EHK3	C10orf141
CNKSR2	CPNE4	DAPK1	DRD5	HEK11	FNBP1L
CNK2	CRHR2	DAPK	DRD1B	ELMOD1	C1orf39
KIAA0902	CRF2R	DCX	DRD1L2	ERC2	TOCA1
KSR2	CRH2R	DBCN	DOC2A	KIAA0378	FAT3
CNTNAP3B	CPNE6	LISX	DPYSL5	FAM150B	CDHF15
CASPR3B	C18orf42	DCLK3	CRMP5	UNQ542/PRO1097	KIAA1989
CNNM1	CLSTN3	DCAMKL3	ULIP6	ENOX1	FIBCD1
ACDP1	CS3	DCDC3C	EEF1A2	PIG38	UNQ701/PRO1346
CNTN3	KIAA0726	KIAA1765	EEF1AL	EOMES	FLRT3
KIAA1496	CRTAC1	DSP	STN	TBR2	KIAA1469
PANG	ASPIC1	DIRAS2	EFHC2	ESRRG	UNQ856/PRO1865
CNTNAP5	CEP68	DLX1	EFNB2	ERR3	FBXL2
CASPR5	CTXN2	DLX5	EPLG5	ERRG2	FBL2
NR2F2	SS18L1	DGKK	HTKL	KIAA0832	FBL3
ARP1	CREST	DLG2	LERK5	NR3B3	FGF13
TFCOUP2	KIAA0693	DLX2	ENO2	FAM163B	FHF2
CYP4X1	TMEM63C	DISP2	EFNA3	C9orf166	FGF18
UNQ1929/PRO4404	C14orf171	DISPB	EFL2	FAM43B	UNQ420/PRO856
CORT	CSC1	KIAA1742	EPLG3	FAM78B	FGF14
UNQ307/PRO350	CXADR	DNAJC27	LERK3	FANK1	FHF4
CPNE5	CAR	RABJS	ENTPD3	HSD13	FGF9
KIAA1599	CYB561	RBJ	CD39L3	UNQ6504/PRO21382	FMN1
COL6A2	CIT	DMRTA2	EPHA8	FAM155A	FMN
EBF2	CRIK	DMRT5	EEK	FBLL1	LD

420-454	455-489	490-524	525-559	560-594	595-629	630-664	665-699
FIBIN	GHSR	GLUR1	HCN3	EWI3	IRX2	KCNG3	UNQ9234/PRO31993
PSEC0235	GABRA5	SLC2A14	KIAA1535	KIAA0466	IRXA2	KCNH4	PPFIA2
FSTL4	GDNF	GLUT14	HOOK1	ISOC1	MAPK8IP2	KCNK9	DLEU7
KIAA1061	GUCY1A2	GLUT3	HS3ST5	CGI-111	IB2	TASK3	LEU7
FBXL16	GUC1A2	GPR135	3OST5	IL34	JIP2	KLF5	LHX8
C16orf22	GUCSA2	GRIP1	HS3OST5	C16orf77	PRKM8IPL	BTEB2	L3MBTL1
FBL16	GFRA4	GRM1	HEXIM2	INHA	KIAA1549L	CKLF	KIAA0681
FRMPD3	GLRA2	GPRC1A	L3	INSL5	C11orf41	IKLF	L3MBT
KIAA1817	MSTN	MGLUR1	HAS3	UNQ156/PRO182	C11orf69	KIAA2022	L3MBTL
FSTL5	GDF8	GRPR	IGF2BP2	INSM2	KCTD16	CAMK1G	ALOXE3
KIAA1263	GIPC2	GDA	IMP2	IA6	KIAA1317	CLICK3	LSM11
FUT7	GLB1L2	KIAA1258	VICKZ2	Nbla106	KCNJ5	VWS1	LGI1
GALR2	MSTP014	GPR26	HPCA	IRS4	GIRK4	KLHL34	EPT
GALNR2	UNQ210/PRO236	GPR21	BDR2	ISLR	KCNF1	KCNIP2	UNQ775/PRO1569
GPRASP2	GPR149	GPR61	IGFBPL1	UNQ189/PRO215	KCNJ3	KCHIP2	PPFIA4
GNG3	PGR10	BALGR	IGFBPRP4	PKIA	GIRK1	KIFC2	KIAA0897
GNGT3	GNAZ	HS6ST2	ICA1L	PRKACN1	CAMK2B	KLHDC8A	LRRC3B
FOXO6	GNL3L	PSEC0092	ALS2CR14	IQSEC3	CAM2	LANCL3	LRP15
GNG2	GNRH1	GPR27	ALS2CR15	KIAA1110	CAMK2	LANCL2	UNQ195/PRO221
GABRB3	GNRH	SREB1	IGF1	JAKMIP1	CAMKB	GPR69B	L3MBTL4
FRMD3	GRH	GRID2IP	IBP1	GABABRBP	KCNA3	TASP	LMX1A
EPB41L4O	LHRH	GPR22	IL17B	JAMIP1	HGK5	LHFPL4	LONRF2
GARNL3	GPR151	GPRIN1	IL20	MARLIN1	KCNA4	LHX5	RNF192
GDF5	PGR7	KIAA1893	NIRF	PRKAR1B	KCNA4L	L1CAM	LRRC16B
BMP14	GLOD5	GPRIN3	ZCYTO7	INSM1	KCNB2	CAML1	C14orf121
CDMP1	GLRA3	KIAA2027	UNQ516/PRO1031	IA1	KCNC1	MIC5	LRRN4CL
GALR1	GPR158	GREM2	PKIB	KCNC2	KIT	LIN28B	UNQ728/PRO1410
GALNR	KIAA1136	CKTSF1B2	PRKACN2	KCNH1	SCFR	CSDD2	LRRC26
GALNR1	GPR45	DAND3	IKZF4	EAG	KIAA0319	LHFPL1	CAPC
GPRASP1	GPR88	PRDC	KIAA1782	EAG1	KIAA0895L	UNQ5824/PRO19643	MAGEE2
GASP	STRG	HAP1	ZNFN1A4	KCNK4	PRKAR2B	LHX9	HCA3
KIAA0443	GPR12	HAP2	INSRR	TRAAK	KCNIP4	LINGO2	MAGEL2
GATSL2	GRIA1	HLP1	IRR	KCNS2	CALP	LERN3	NDNL1
GATSL1	GLUH1	HCN4	IGSF3	KIAA1144	KCHIP4	LRRN6C	LY6H

700-734	735-769	770-804	805-839	840-874	875-909	910-944	945-979
CCDC109B	JNK1	NDRF	NPTX1	NPH2	PDCD1LG2	PGF	BRN3A
MCUB	PRKM8	AVP	NPTXR	OPCML	B7DC	PGFL	RDC1
MAP3K15	SAPK1	ARVP	NRSN2	IGLON1	CD273	PLGF	PNMAL2
ASK3	SAPK1C	VP	C20orf98	OBCAM	PDCD1L2	PLXNA4	KIAA1183
MAGEE1	MLF1	CHL1	NTSR1	OPN3	PDL2	KIAA1550	PRRC2B
HCA1	MPPED1	CALL	NTRR	ECPN	PDE1A	PLXNA4A	BAT2L
KIAA1587	C22orf1	GAP43	NMBR	OPRL1	PCDHA1	PLXNA4B	BAT2L1
MARCH9	FAM1A	NEUROD1	GRIN2D	OOR	PCDHA8	UNQ2820/PRO34003	KIAA0515
RNF179	MRAP2	BHLHA3	GluN2D	ORL1	PCGF2	PLD5	PRRT3
MARCH4	C6orf117	NEUROD	NMDAR2D	PAFAH1B3	MEL18	PIP5KL1	UNQ5823/PRO19642
KIAA1399	MTUS2	NTS	GRIN1	PAFAHG	RNF110	PNMA2	PRDM8
RNF174	CAZIP	NOV	NMDAR1	ADCYAP1	ZNF144	KIAA0883	PFM5
MESP2	KIAA0774	CCN3	NPAS4	PACSIN1	PELI3	MA2	PPP1R1B
BHLHC6	TIP150	IGFBP9	BHLHE79	KIAA1379	PGBD5	PNOC	DARPP32
SCDO2	RUNX1T1	NOVH	NXF	OVGP1	PGM2L1	OFQ	PSD
MET	AML1T1	NDST3	PASD10	MUC9	BM32A	PROKR2	EFA6
MAB21L1	CBFA2T1	HSST3	NPFFR1	OGP	PCDHA9	GPR73L1	KIAA2011
CAGR1	CDR	UNQ2544/PRO4998	GPR147	PCDHAC2	KIAA0345	PKR2	PSD1
Nbla00126	ETO	NDST4	NPFF1	PCLO	CD274	PLCH2	TYL
MAB21L3	MTG8	HSST4	NPY2R	ACZ	B7H1	KIAA0450	PRLHR
C1orf161	ZMYND2	NNMT	NRN1	KIAA0559	PDCD1L1	PLCL4	GPR10
MCTP2	MSI2	NECAB1	NRN	P2RY1	PDCD1LG1	PNMA3	GR3
MEX3A	MYH8	EFCBP1	NTNG2	PCDHA2	PDL1	MA3	PRRT4
RKHD4	NANOS2	NDN	KIAA1857	PCDHA10	PODXL2	PPP3CB	PTCHD1
MESP1	NOS2	NEFH	LMNT2	CNRS8	UNQ1861/PRO3742	CALNA2	RAB9B
BHLHC5	SLC24A2	KIAA0845	UNQ9381/PRO34206	TP73	PGRMC1	CALNB	RAB9L
MGAT4C	NCKX2	NFH	NUMBL	P73	HPR6.6	CNA2	QRFPR
MMP24	NDRG4	TACR1	NAP1L2	PLA2G4E	PGRMC	PPM1E	GPR103
MT5MMP	BDM1	NK1R	BPX	PAQR9	PITPNM2	CAMKN	RAB3D
MICAL2	KIAA1180	TAC1R	NUDT11	PCDHA13	KIAA1457	KIAA1072	GOV
KIAA0750	SLC24A3	NPY	APS1	CNRS5	NIR3	POPX1	RAB16
MICAL2PV1	NCKX3	OLFM3	DIPP3B	PCSK5	PIWIL2	PNMA1	RTN4RL2
MICAL2PV2	NEUROD2	NOE3	SLC10A4	PC5	HILI	MA1	NGRH1
MAPK8	BHLHA1	UNQ1924/PRO4399	NXPH2	PC6	PLCXD3	POU4F1	NGRL3

980-1,014	1,015-1,049	1,050-1,084	1,085-1,119	1,120-1,154	1,155-1,189	1,190-1,224	1,225-1,259
RGS7BP	KIAA1391	SLC27A3	FRCL1	SIX3	SUSD2	TM4SF5	THH
R7BP	RGS17	ACSVL3	SLC5A7	SLCO5A1	STX1B	TUBB2B	THL
RAB6B	RGMB	FATP3	CHT1	OATP5A1	STX1B1	TCEAL7	TRHY
RASSF6	RHBDL1	PSEC0067	SCML4	SLC21A15	STX1B2	THSD7B	TRIM46
RBMS3	RHBDL	UNQ367/PRO703	CXCL12	SPRN	STX19	KIAA1679	TRIFIC
RAB3B	ARHGAP36	SLC45A1	SDF1	SHO	SYTL1	SPOCK3	TRIM66
RAB3C	RIMS3	DNB5	SDF1A	SNTG2	SLP1	TICN3	C11orf29
CRABP2	KIAA0237	SLC6A17	SDF1B	SRRM4	SB146	UNQ409/PRO771	KIAA0298
RAI2	RIMBP2	NTT4	SCN2B	KIAA1853	SNCA	TUBG2	NGFR
RAMP3	KIAA0318	RUNDC3B	UNQ326/PRO386	SPHKAP	NACP	TBR1	TNFRSF16
RARB	RBP2	RPIB9	SCRT1	KIAA1678	PARK1	TCP11	TRIM67
HAP	RIMS4	RPIP9	SBSN	SKIP	TMEM200A	TDRD6	TNL
NR1B2	C20orf190	SLC22A15	UNQ698/PRO1343	SULT2B1	KIAA1913	TAC3	TRPC6
REM2	RIPK4	FLIPT1	SEPT6	HSST2	HBE61	NKNB	TRP6
RBM24	ANKRD3	UNQ9429/PRO34686	KIAA0128	STMN1	SYN1	UNQ585/PRO1155	TRPC5
RNPC6	DIK	SLC35F4	SEP2	C1orf215	MAPT	TMEM59L	TRP5
RGAG1	RNF39	C14orf36	SHANK2	LAP18	MAPTL	BSMAP	TSPAN11
KIAA1318	HZFW	SATB2	CORTBP1	OP18	MTBT1	C19orf4	TH
RHOV	RNF165	KIAA1034	KIAA1022	SERTAD4	TAU	TMTC4	TYH
ARHV	RNASEL	SCN2A	PROSAP1	STK32B	TUBB2A	TEX15	UBE2QL1
WRCH2	RNS4	NAC2	SH3GL2	YANK2	TUBB2	TIAM2	USP11
RESP18	RPS6KL1	SCN2A1	CNSA2	UNQ3003/PRO9744	TCERG1L	KIAA2016	UHX1
RALGPS2	RPP25	SCN2A2	SH3D2A	SULT4A1	SYT2	STEF	TTC9B
RGS11	RSPO3	SCUBE3	SEPT3	SULTX3	TCEAL5	TNRC6C	SLC25A27
ADARB1	PWTSR	CEGF3	SEP3	STMN3	TERT	KIAA1582	UCP4
ADAR2	THSD2	SCRT2	SH2D4B	SCLIP	EST2	TNFAIP8L3	UNQ772/PRO1566
DRADA2	RTBDN	FP7030	SST	SOX11	TCS1	TIPE3	UPK3A
RED1	RTL1	SLC7A14	SP9	SPDEF	TRT	TMEM158	UPK3
RELL2	MAR1	KIAA1613	SH3BGRL2	PDEF	SYT3	HBBP	TSPYL4
C5orf16	MART1	SCN3B	FASH3	PSE	SYT6	RIS1	KIAA0721
UNQ9423/PRO34565	PEG11	KIAA1158	SH3RF3	SSTR4	TMEM178A	TMEM169	TTC9
REPS2	RSPH4A	SCN9A	POSH2	SV2B	TMEM178	LHFPL5	KIAA0227
POB1	RSHL3	NENA	SH3MD4	KIAA0735	PSEC0131	TMHS	TTC9A
ARHGAP20	RSPO1	SLC35D3	SIDT1	SSTR2	UNQ5926/PRO19820	TCHH	USP27X

1,260-1,294	1,295 – 1,329	1,330 – 1,364	1,365 – 1,399	1,400 – 1,434	1,435 – 1,469	1,470 – 1,504	1,505 – 1,539
USP22L	VGLUT2	RBFOX2	SPRYD3	TUFT1	LMTK3	STMN2	VWA5B2
USP27	SLC17A8	SGIP1	ZNF483	UNQ865	BCL11A	NEGR1	NXPH3
USP29	VGLUT3	MYO5B	TRPC7	OPRD1	MAL2	TRPC3	WNT7B
UBQLN2	VWC2	CKMT1B	LRRC24	MAPK10	KLHL14	GALNTL6	KSR2
N4BP4	UNQ739/PRO1434	CKMT1A	hCG_1818221	PDZD4	SYT17	GCA	DBN1
PLIC2	WSCD2	SYNGR3	WDR6	hCG_2004980	OSCAR	PIK3C2G	VIP
HRIHFB2157	KIAA0789	NELL1	LMCD1	ZDBF2	SDSL	EPHA3	SGSM1
UFSP1	XKR7	ALLC	PLEKHA5	DACT1	KLHL1	SH2D5	SNX16
UPP2	C20orf159	hCG_15830	ZMAT4	NBEA	RLTPR	ANKRD55	DBNDD1
ATP6AP1L	XRG7	RTN1	INPP5J	NAPB	CX3CL1	PPARGC1B	CXXC4
VAX1	YDJC	CHRFAM7A	ADCY1	hCG_22369	IPCEF1	SYT4	FSIP1
SLC32A1	ZFP57	PRTN3	hCG_19245	SEMA4G	RASGRF2	SYT13	CACNA1C
VGAT	C6orf40	EPHB6	GRIN2B	AUTS2	KCNT1	FRMPD4	SYT10
VIAAT	ZNF698	SLC38A1	SMARCA1	NOS1	CD24	SLC2A13	COL25A1
VWA5A	ZNF558	ISLR2	hCG_1980650	PCDHA4	HOMER2	GLIS1	GNAL
BCSC1	ZNF575	P2RX2	KCNT2	RSPO2	PCSK1	KLHL29	DYNC1I1
LOH11CR2A	ZWINT	RASGEF1C	NRG3	FAM171A2	CACNA1A	SYT5	RAB11FIP4
VSNL1	ZCCHC18	EYA2	NFIX	CYGB	SYT7	SYNPR	COL24A1
VISL1	ZFR2	CADPS	MMP11	SV2C	KCNH7	RGS8	ZIC2
SLC18A2	KIAA1086	DOC2B	HDGFRP3	CCDC73	SLIT1	CASP3	KIAA1211
SVMT	ZIC1	hCG_1741343	ATP6V1G2-DDX39B	NTRK1	GPR83	IL12A	SHISA6
VMAT2	ZIC	OCC-1	CALB2	CELF5	PLEK2	RCAN2	TMEM145
VSTM2L	ZNF201	C12orf75	hCG_2011413	RBMS1	CACNB2	NHLH2	RTN4R
C20orf102	ZIC4	SERF1B	SP8	RIT2	PLCD3	ZPBP	COCH
VWC2L	ZNF711	SERF1A	hCG_38927	FABP3	RXFP1	B3GALNT1	TRPV1
SLC18A3	CMPX1	FAM159B	TRPC4	DUSP26	LGI2	ANK1	CPLX1
VACHT	ZNF6	DIABLO	SCN5A	CHODL	PLEKHA7	TRHDE	SERPINI1
VASH2	TUBB3	hCG_1782202	CYP4B1	CDK5R1	CCNF	ANKRD42	TTC39A
VASHL	hCG_1983504	EPHB2	hCG_22100	LRFN5	SYT1	SSBP2	TMEM91
XKR4	AHI1	SLC27A2	ARHE	RBFOX1	KCNIP1	SRRM3	SLC1A6
KIAA1889	PNCK	hCG_39815	RND3	RALGAPA2	CACNA1B	SHANK1	GPC1
XRG4	DLGAP2	SNAP91	SNCB	FKBP1B	BCL11B	PEG3	DGKG
SLC17A6	VGF	SYP	CHCHD10	CELF3	HS3ST6	MTMR7	MARCH11
DNPI	FOXP2	KCNH2	PCBP3	RGS4	GRM2	CADM3	RNF152

1,540 - 1574	1,575 – 1,609	1,610 – 1,644	1,645 – 1,679	1,680 – 1,714	1,715 – 1,749	1,750 – 1,784	1,785 – 1,819
ZNF385B	PLA2G4F	COL19A1	MTSS1	PAK3	CGN	DLX6	CNRIP1
OSBPL5	SPATA2L	SNAP25	PDZD2	PCP4	SLC4A3	HOOK2	NOL4
FOSL2	ATP1B1	CNTNAP2	WIF1	SLC6A15	AGBL4	HLA-B	CHRNA3
SLC6A7	CLCN1	PPARGC1A	MAML3	CNTNAP4	GDAP1L1	NXPH4	PAK6
LYPD6B	MCHR1	GSE1	UNC5D	CDKL2	GRM8	LMO3	HSPA12A
DKK3	SAMD14	INA	OLFM1	NPY1R	STXBP1	RALYL	ADCY8
FLYWCH2	KCNQ5	GCGR	TEKT2	SNTB1	PANX2	COX7A1	ST8SIA2
MYT1L	ADARB2	EFNA5	GRIN2A	NOVA2	TRO	DIRAS1	PRPH
CLSTN2	FAM65B	DMRT3	PALM2	KIF5C	LRP11	JPH4	CDH9
CYP2S1	nan	ACTL6B	SLC16A14	KCNAB1	GRM7	PLEKHG4	PKNOX2
RAB3A	CRABP1	POU6F2	VPS37D	ACTA1	PCNXL2	ROBO2	SPOCK1
ELN	CNTN5	GABRG3	FAM57B	OXTR	CRHBP	CLRN1	CAMTA1
FAM167A	PLCZ1	SEZ6L2	C8orf89	PRKAA2	KCTD8	SCG2	SARM1
PENK	MAP3K12	KCNQ2	PALM3	VSTM2A	TMEM130	VWCE	ZNF804B
ACR	YPEL4	SPAG6	NETO1	CALN1	KCNG1	NTN5	RUFY3
GABRG2	MME	CLEC2L	NANOS1	SLC25A22	LRRC7	ITGA3	SMS
ANGPT1	TMEM210	LAMA3	NKX2-1	GALNT9	ANKRD34B	PTH2R	SDK2
COL23A1	TRIM17	CBLN2	RAC3	CD200	CIDEA	FXYD7	HRH3
KCNJ11	OSBP2	CHGB	PLCXD2	ATP1A3	HS6ST3	GOLGA7B	TMEM198
NXNL2	UNC13A	EML5	SEZ6	CA10	CCBE1	ANKRD35	ARHGAP15
MN1	CA11	SLC38A4	SELV	KIAA0895	AK5	STAC	HFM1
RELN	SLC7A4	MCTP1	SEMA6C	NPAS1	CDKL1	MAST1	CCDC120
RFX2	WNT2	EBF3	ADD2	PTPRN	ZDHHC22	CBX7	INPP5F
TBC1D30	NRG1	RNPC3	WNT5A	NRXN3	CHD5	C10orf35	CDH8
FAM189A1	GALNT14	DSCAML1	NFIB	ARMCX1	SPIRE2	SAMD3	C1orf95
RUSC1	CPNE7	HECW1	DNM1	RAPGEFL1	C2orf80	PTPRN2	SOBP
GIPR	CRMP1	DKKL1	ANKRD34A	SEMA3A	ABLIM3	CSRNP2	GABRA2
HTR1A	SERP2	C3orf18	B4GALNT4	ATL1	JAZF1	PPP2R2C	DPYSL3
ARMCX4	TAS1R1	CDKL4	nan	TTC22	KRT73	CIB2	YPEL1
DCC	SGTB	PITPNM3	STXBP5L	THPO	SAMD10	ADPRHL1	GAD2
PRKCE	POPDC3	RADIL	MAP3K9	C3orf67	GRIP2	ZNF804A	CDON
LGALS7	NEUROD6	NR1H4	GBX2	TMEM30C	RAB26	STK32C	NPNT
SMPD3	KIAA1107	TRPV6	LHX1	KIAA1324	PPIF	AMN	MGAT5B
ARHGDIG	CNR1	C14orf39	ARHGAP44	ITGBL1	KRT79	RHOF	SPINT2

Continuation of Data from Table D: *neuron specific genes in mouse, gene numbers 209 onwards*

1820 - 1854	1855 – 1881
MYL6B	GDAP1
SLC12A5	TRANK1
TMEM150C	C5orf49
NAP1L3	C1QTNF4
UCHL1	SYN3
SVOP	COL6A1
UBE2O	DACH1
MDGA1	ZNF667
THSD7A	LHX6
SYN2	UNC119
FAM49A	IGSF9
FAM169A	TWSG1
ABCA5	ASS1
CLVS1	CAMK4
RASGRP2	CNIH2
ZBTB16	SRGAP3
PRUNE2	NRIP3
CCDC64	CSDC2
HMGCLL1	CCDC136
NIPSNAP1	BEND4
MST1R	B4GALT6
SRSF12	UNC5A
YBX2	MKRN3
PGR	RASGEF1A
SFRP4	CDHR1
IL1RAPL2	THY1
EPHA6	
ISL1	
FNDC3B	
PNMAL1	
HRK	
ADAMTS17	
ERO1B	
LEFTY1	

209-243	244-278	279-314	315-349	350-384
IGF1	CDH12	TDO2	SLC7A3	KCTD16
IGSF3	AP1S2	NYAP2	SH2D5	GPR22
LYPD6B	LOC100128264	LOC728739	NEK10	PLK2
KIAA1324	DACH2	CLUL1	TRNP1	FLRT3
KIAA1217	SHISA9	TUSC3	TSPAN13	SCN3A
KCNC1	AFF2	SCG5	DNM1	FBLN7
XK	BCL11A	RFPL1-AS1	ITGBL1	PTPN5
GNG3	HAPLN1	ATP1B1	NT5DC3	MAGED4
FAM190A	TAGLN3	MEPE	CREG2	MAGED4
SP8	NETO2	PLXNA4	ST8SIA5	RFPL2
D4S234E	TMEM130	BSCL2	NRG1	LOC284408
NAPB	NOS1AP	CLSTN2	CADPS	HBG1
NEUROD6	COL12A1	ACCN1	FAM183A	TRPC6
CACNG3	TTC9	RSPO2	PCDH11X	C11orf41
FAXC	PPM1E	CDH8	TOX2	ANKRD34A
PKP2	CNTNAP2	GRM5	KIAA1024	KIAA1456
PPP1R1C	PRMT8	ATP2B3	SEZ6	OSBPL10
CNNM1	DYNC1I1	STXBP5L	LHX6	AMPH
NDST4	CNTN4	CHRM3	LINC00314	B4GALT6
TRHDE	BEND4	PAR1	PCLO	GPR149
EYA4	ELAVL4	FGF9	DLX2	LINC00473
SCN8A	SYCE1	LPPR4	PABPC1L2A	SYN1
KCNS2	CNTN3	GNG2	SULT4A1	RCAN2
TRPC5	PRKCE	SH3GL2	THSD7B	RNF207
PTH2R	MIMT1	SCN3B	RYR2	LPPR5
SNCB	RBM11	C2orf80	LOC440894	GNG4
SV2A	HTR2A	INPP5F	NAP1L2	SORCS3
TMEM196	TCEAL6	ELMOD1	ADD2	NAP1L3
EFNB3	FGF12	CAP2	HTR1E	ZNF385B
SSTR2	NSFP1	KITLG	RPS6KA6	HMP19
KCNT2	LOC440894	C1orf173	KCNH1	PNMA2
NOV	GDA	MLLT11	HS6ST3	SLC26A8
LOC653653	SYT16	NUDT11	KIAA1211	IL13RA2
CPNE4	LOC286002	CSRNP3	LOC730811	L1CAM

385-419	420-454	455-489	490-524	525-559	560-594	595-629	630-664
PAK6	SLC6A15	RNF165	SNTG1	KCNH5	SULF1	MAGEL2	ACYP1
PCDH18	SYP	LINC00246A	NRSN1	OPCML	FBXL2	ADAM22	MIF
BCL11B	PRKAR2B	ARL9	RGMB	NEFL	DIRAS3	MEX3B	GDAP1L1
RAB27B	DRP2	GLRA3	SLC6A17	PROK2	CABP1	THSD7A	GLS
ATP6V1G2	LOC100507043	VAT1L	LRRC7	KLHL13	FXYD6	SLC26A4	AP3B2
PAK7	GALNT14	SV2B	GYPE	RGS17	CCDC152	PSMG4	NR2F2
CDK5R1	PEG3	SNAP91	CLVS2	PLCB4	FAM123C	THRB	GULP1
PABPC1L2B	KLF5	PAR5	SGSM1	HPCAL4	HMGCLL1	GABRE	KLHL29
ADAMTS6	AKR1C2	CACNG2	CNTNAP5	LRFN5	FREM1	MOB4	MCF2
NPTX2	CIT	PIP5K1B	FRY	PTPN3	CELF5	ANKRD7	KIAA1644
LPHN2	GRM7	GPR137C	LOC158696	LOC100130155	CHRNA2	CSMD1	KIAA1524
NSFP1	PDE1B	STAR	ANKRD30BL	DLGAP2	BEX2	PAM	WASF1
VWC2	NELL1	GABRB3	FAM81A	ADRBK2	PLXNC1	WIPF3	SNCA
IGFBP5	CHRNA7	DOK6	KCNA4	KCNA6	THY1	MICAL2	CERS6
ENC1	KCNIP4	NCALD	PCP4	DLGAP1	CACNA2D3	ANKRD34C	DAB1
ZNF204P	GRIA4	CACNA1B	SETD6	ACRV1	RGS6	KIAA0408	GPRASP2
CD200	LMO7	C11orf63	CTXN2	CORT	LOC338651	FGF14-IT1	GUCY1B3
TOX3	XKR4	KMO	NMU	GPRASP1	LOC440040	FBXL21	SLC10A5
CPLX3	SNX10	EPB41L4B	DCX	LRRTM2	SGK494	HSFY2	GPR151
SLC27A2	LOC728730	C12orf68	ENO2	RNF175	LOC100506123	C3orf80	OCIAD2
ANK1	LOC389023	LOC285954	PDXP	IL12RB2	EXO1	SLITRK5	GLYATL1
CELF4	RPP25	ELOVL4	LANCL3	PAK3	HPRT1	CDK14	GPRIN1
PLD5	PTGFR	RAB3A	SUSD4	RASGRF2	TMEM178	HSFY2	FAM65B
UBE2T	CDKL2	SLC38A1	NDST3	FABP4	SHANK2	ROPN1L	TUBA8
NXPH1	C8orf85	CSMD3	DGKE	NUDT10	C17orf102	LRRC8B	C10orf35
BEX1	DPP6	RIMS3	DIRAS1	LOC100505483	ARHGEF3	TRPC7	SAMD12
ANKRD55	B3GALT2	GRIA3	TSPAN2	DNAH14	ZNF519	FAAH2	LOC100507341
KCNB1	LHFPL5	ARHGAP20	IFNA21	EPHA10	CACNG8	GLT1D1	SPATA17
KHDRBS2	ADRA2A	PLS1	UNC80	NTF3	JPH1	ABCA5	KIAA1239
GJB7	BASP1	HS3ST2	ZNF540	GRM1	AKAP5	KIAA1107	ANKRD30B
FAM216A	KCNMB2	HS6ST2	PNMAL1	IBSP	MAGEE1	GOT1	CES4A
SORCS1	SLC47A1	C8orf4	MAGEE2	SLC17A8	SYN3	RBFOX2	PPFIA4
TBR1	NAP1L5	ODZ1	NEGR1	YWHAH	PDP1	SLIT2	JAKMIP1
CCKBR	ZFR2	OSTN	SLITRK3	RET	DRD1	TCEAL5	ATRNL1

665-699	700-734	735-769	770-804	805-839	840-874	875-909	910-944
GPR63	TRIM36	ABI3BP	LOC283683	FRMPD2P1	LRRC55	TCEAL7	PART1
SLC35F3	PREPL	DACT1	TBC1D30	PLEKHA8P1	SNAI2	MME	MDH1
RGS7	CDCA7	FNDC9	ASNS	GDAP1	HAPLN4	CDKN3	CACNA1C
TRIM7	CNTNAP1	SLC8A2	PROX1	SLC8A3	RND3	KCNIP4-IT1	GUCY1A3
LOC100131208	C1orf53	CRABP1	SCARNA13	UNC5D	LEFTY1	LOC100506071	C9orf125
TRIM55	LDB2	CNTN6	VAMP1	LOC441666	SPRED3	REEP5	ATP6V1G2
ATP1A3	GRM8	C9orf11	CXXC4	FBXW12	SYNDIG1L	CLSTN3	CCNB1
OR7E2P	RPH3A	GABRA3	TCEAL2	LDOC1	CACNB4	SLC1A1	GAP43
NPTX1	RIMKLA	QPCT	PRKCB	RIIAD1	ACTC1	LOC285484	CHRNB2
NBEA	APITD1-CORT	GFOD1	C11orf80	PPEF1	BEST4	KCTD8	C6orf57
ST8SIA2	ZNF483	NPTN	CAMK2N2	ATP6V1G2	SCAI	ATP6V1G2	NREP
SLC16A7	SPINT2	AKAP14	KIAA0895	ATP6V1G2	ATP6V1G2	LAMB3	TRANK1
LURAP1L	KSR2	CELF3	CHGA	CNIH3	RAD51D	MAP3K9	TMEM233
LHFPL4	ANKRD19P	CALN1	DUSP26	FRAS1	GPR26	MFSD4	EREG
VAX1	ST6GALNAC5	WTH3DI	LOC100506124	SLC9B2	ATOH7	RASGRP1	CYP4Z1
HCN3	FAM216B	DMRTC1B	FAM106CP	ANKRD6	ITPR1	MPP3	CABP7
SLC17A6	WDR69	DMRTC1B	CHRFAM7A	GLT8D2	SYNJ1	ASXL3	PCDHAC2
TAS2R50	TCTEX1D1	RALYL	TPBG	MAGI1	TMEM132D	C15orf38-AP3S2	AJAP1
KCNV1	GUCY1A2	HIST1H4H	ARX	PATE4	ALK	FAM24B	B7H6
GRB14	ADCY1	DNAJC12	PKD2L1	TRAPPC2L	ODZ3	TC2N	CORO2A
NOL4	YWHAG	CLGN	ARHGAP28	TSPYL1	ATP6V1G2	NPY5R	NECAB2
TMEM155	GNB5	ZNF804B	EIF4E1B	DOC2A	C1orf216	INTS4L1	HTR4
SLC44A5	GCOM1	ARNTL2	SCAMP5	MTMR7	SLC17A7	GPR6	SPANXA1
MAP1LC3C	DLG3	GFRA2	SEZ6L	SUSD1	LENG9	NAALAD2	SPANXA1
CCDC136	FAM174B	DKK2	FAM135B	BEAN1	AP3M2	ADARB1	PPARGC1B
ARPP21	ARHGAP36	RFK	PITPNM3	HDAC9	SLC25A12	ATP2B1	RAB15
CACNA2D1	CORO6	PCSK1	SLC35F2	SLC16A14	LOC100128239	NECAP1	LRRTM1
TAS2R10	SERPINI1	RGS12	REPS2	GABRA4	B4GALNT1	LOC100506123	SUGT1P3
FBXO16	COL22A1	CELSR3	MC4R	PRICKLE1	ATP6V1A	LGALSL	CCDC65
TSHZ2	PLCXD2	MCHR2	GUCA1A	MCTP1	LONRF2	SLC24A4	ADAM23
STXBP1	DPT	FAR2	MOAP1	KLC1	MANSC4	SLC24A3	CHML
LNX1	FAM156B	GLRB	RASGEF1A	KCNB2	LOC285593	CGREF1	IRS1
LRRC2	HAR1B	ZNF815	HSPA12A	PCDH19	OXCT1	GPLD1	YPEL4
LOC284215	FAM84A	ADARB2	NTN4	CAMKK1	ADCYAP1	DPY19L2P4	CAMK4

945-979	980-1,014	1,015-1,049	1,050-1,084	1,085-1,119	1,120-1,154	1,155-1,189	1,190-1,224
MAP1B	PAK1	TAS2R16	SYT2	VDAC3	TRPC3	VSTM5	KCNAB1
BMP3	LRRTM3	EPHA3	CASQ1	TMEM35	LOC144481	DISP2	C18orf42
CBLN1	HTR7	PAR-SN	OR2D3	RPAIN	LOC201477	PPM1H	PXDNL
CNKSR2	CHST2	ECM1	SCAND3	KLHDC8A	KBTBD6	DNER	NTNG1
STS	SOX1	CCDC165	MZT1	PTER	KIF3A	TAF5	LOC729177
OXR1	C1orf115	FAM43A	MORN4	EIF4A2	TDRD9	LOC389493	TMTC1
CRYM	PKIB	PTPRN2	KCND2	AK5	MACROD2	HSPC072	PJA1
RUSC1	PTPRO	RUNDC3B	FAM126B	FAM169A	BLCAP	LOC729080	NLGN1
BHLHE22	SLC6A2	ZBBX	EPDR1	NEFH	SRSF12	PTS	MAPK9
BSN	C6orf115	SIDT1	AGBL4	DGKI	TRIB3	AFAP1-AS1	PDC
TLE2	LINC00277	RBP4	PLCE1	OLA1	SLC9A7	C1orf114	EEF1A2
DYDC2	LOC100505576	USP9Y	C1QL3	BAG4	MAP2	LOC100507254	LOC100379224
EGFEM1P	OR1F1	CRTAC1	C1QTNF9B	ADRA1B	MGC57346	MEF2C	UPP1
ITGA8	GNAL	DNAJC6	MARK1	RANBP17	RAB6B	MIAT	KLHL14
FAM133A	GJD2	PRPS2	KCNN2	C1orf96	FAM156A	HLA-F-AS1	LRRTM4
TMEM145	PRLHR	IPW	DOCK3	SPANXA2-OT1	LOC100126784	RHEBL1	PHTF1
MDGA2	CLEC4G	MYO5A	REM2	ABCA9	H2BFXP	EPHA6	ZNF215
ZNF273	UNC13A	HYLS1	TRH	MYOZ3	KIAA1804	KCNK2	ANKRD32
GRIK3	KIAA2022	TMEM63C	MYPOP	MAP1LC3A	ECE2	KCNH2	MAPT-AS1
ALDH1A2	CCL13	DPY19L2P1	HCN4	ZDHHC23	PLCB1	NEDD4L	SCN2B
TSPYL2	RP1-177G6.2	HPCAL1	RTN3	AFAP1	KIAA1383	FLJ43390	SUB1
GPR123	GRIP2	ARL15	TCTEX1D2	SGTB	PDE10A	SERP2	C14orf23
LARGE	PPP3CB	HUNK	LOC100130264	LBH	MBLAC2	AFF3	SAMD5
PLEKHA5	SLC4A8	BHLHB9	LRRC39	ZDHHC22	FABP6	CMAS	GPR52
CACNA1D	CD8A	JPH4	PPP1R17	FAM3C	LY86-AS1	KALRN	NECAB1
RASL11B	CALY	FKBP11	STBD1	MYO5B	THBS1	DCAF12L2	SCN9A
STMN1	TSPYL4	NKRF	CNRIP1	LOC81691	PPP1R2	ANKRD50	PIRT
CNIH2	CD3D	AASDHPPT	FREM3	HN1	PIK3R3	EEF1E1	LOC730101
PRKAA2	ARHGAP44	LOC441455	CNTNAP4	TFAMP1	FAM71D	PVR	COLQ
TNNT2	EFHA2	BBS7	JAZF1	FAM189A1	FSTL4	PDF	PCDH20
ERBB4	PDE8B	SCAND3	ATCAY	KIAA1586	P2RX5	CYP26B1	AAK1
PRR4	FGF10	MGC45800	PEG10	FKBP1B	TRIM67	LOC338817	FAM71C
NEU3	HTR3A	LOC100506757	C16orf45	SMPD3	SETBP1	ACOT7	C7
C17orf69	SYT14	LRP11	FBLN5	TIAM2	ATP2B2	FAM196A	OSCP1

1,225-1,259	1,260-1,294	1,295 – 1,329	1,330 – 1,364	1,365 – 1,399	1,400 – 1,434	1,435 – 1,469	1,470 – 1,504
SOX11	TEX26-AS1	ME3	CHST15	RAB9B	C3orf14	ATG16L1	MET
DPRX	LRRC66	FLJ41278	GLRX2	TACC2	RDH12	RNF150	TRAPPC6B
JPH3	ABCC5	UPP2	TNFAIP8L1	DCAF4	APOBEC4	MKL2	G3BP2
APOA2	TMEM182	UQCRHL	ELK1	DCC	PATE2	GPR88	NME5
MCOLN3	ASPHD2	BEND6	LOC400940	LMOD3	LOC644242	MORF4L2	PCDHB3
ZNF428	C5orf34	ATPIF1	PID1	DNAH6	PRKACB	MEST	ODF2L
UBE3D	DYNLT3	PKI55	ZNF667	KHDC1L	FCRLB	CACNA1G	OPN3
UBE2N	TBC1D3F	GGH	CPLX1	SRD5A1	ZDBF2	PCMT1	AKAP2
C1orf220	SORBS2	GALNT13	ANO1	SLITRK1	FARSB	AIM2	PACRG
DSCR9	ZFP37	R3HDM1	RS1	ALKBH8	LINC00282	C1orf94	PFDN6
LOC729911	C3orf26	SLC25A26	C9orf47	NAGPA	ANKRD20A2	PPP1R14C	IER3IP1
PANK1	RASAL2	ASAH2B	LOC728392	CLCN4	TAF7L	FAM83D	PEX10
GABRA6	GSTA4	FAN1	GPR153	ZNF697	IL5RA	FBXO34	NDN
C5orf25	VWDE	SLC22A3	LOC100129961	TMEM151B	FAM20B	SEMA4F	ABLIM2
WNT16	PLCH1	ZNF599	HIST1H2AB	CCL1	PKIA	DGKB	GFPT1
KCNH7	NYAP1	LOC400456	MYO1B	CUZD1	FAM49A	DUSP28	IL1RAPL1
HS3ST5	GPR162	DKK1	YEATS4	OCRL	LOC642852	UCHL5	HSF2
MAP2K4	POSTN	LSM11	KCNA1	INTS4L2	TTC9B	COX7A2L	ANO3
METTL21C	HSPA4L	HLF	ITFG1	HENMT1	PHYHIP	FGF17	NIPAL2
AHSG	TMEM183A	CDR2	DLG2	DLEU2L	CYP2C8	KIAA1377	NDFIP1
MYB	USP32P1	EYS	AHI1	CENPV	KCNK3	TRIM37	ANKRD20A4
XKR6	LOC100505738	LOC646214	HECW2	SYCE2	SLC9A2	CBWD3	SH3BP5
ZNF25	KAZN	NCRNA00185	LRRD1	CDKN2AIPNL	C14orf2	MKX	CHAF1B
SCOC	TASP1	FAM110C	KIAA1549	SCAMP1	FANCL	KIAA1199	CDK5
KCND3	STAC2	TAF9	KCNIP1	C9orf68	FAM18B2-CDRT4	LOC728758	SMYD2
LMTK2	SNRPN	TBC1D9	TAAR6	FAM20A	KIF3C	EIF4E3	LOC283177
CDKL5	SLC1A6	TRMT6	COL19A1	AP1S1	STRBP	FLJ41649	LOC100131289
ATP1A1	B3GALNT1	LOC728084	ABCA10	LOC139201	MAS1	CHIC1	KIAA0825
OPN5	KGFLP2	OCM	CTPS	PTTG1	IMMP2L	FBN1	RNF41
FAM110B	ZNF829	ZNF33B	KIRREL3	PFDN6	ANKRD20A2	SAPCD1	ADAMTSL3
ADAMTS9-AS2	NGFRAP1	LYPD6	FLT3	PFDN6	YWHAB	SEMA4G	ADAMTSL1
CPNE5	ERLEC1	ICA1	RABL2B	PFDN6	PKNOX2	BEX4	CRHR2
SOX11	TEX26-AS1	ME3	CHST15	RAB9B	C3orf14	ATG16L1	MET
DPRX	LRRC66	FLJ41278	GLRX2	TACC2	RDH12	RNF150	TRAPPC6B

1,505 – 1,539	1,540 – 1,573						
APOBEC3H	C6orf165						
EID2B	C6orf228						
ZNF280B	LYG1						
GAS7	GPC3						
PPME1	USMG5						
C6orf147	PPP1R2P3						
CDC42	BCAT1						
SEMA3E	GRIA2						
TMEM106C	STOX2						
SERINC1	SUCLA2						
NDUFAF4	MAPK13						
HSDL1	SUZ12P						
XCL1	FAM162B						
C14orf162	DCBLD2						
EPHA4	SEC61G						
CAMK2D	SNX25						
TPTE2P1	HDX						
MGC16142	CDON						
CABYR	FAM78B						
VN1R1	ZNF248						
GOT2	OR7E12P						
LOC284578	DNAJB14						
NUDT21	P4HA2						
KIAA0528	ATL1						
DCP2	GYPA						
TMEM17	AUH						
DNAJC5G	TTC33						
LIN7A	SPIN3						
LOC286367	C6orf165						
APOBEC3H	C6orf228						
EID2B	LYG1						
ZNF280B	GPC3						
GAS7	USMG5						
PPME1							

Table E: Key to Table D

Col. A-C in Table D are data derived from the published literature		
Col. A	Transmembrane proteins detected in cell-free CSF through mass spectrometry analysis.	"*Establishing the Proteome of Normal Human Cerebrospinal Fluid*" Schutzer S E et al., PLoS One, 2010; 5(6): e10980. This paper provides a list of proteins detected through mass spectrometry analysis in cell-free CSF.
Col. B	Neuron specific genes expressed in mouse	"*An RNA-Sequencing Transcriptome and Splicing Database of Glia, Neurons, and Vascular Cells of the Cerebral Cortex*" Zhang Y et al., The Journal of Neuroscience, 2014, 34(36):11929 –11947. This paper compares gene expression in different cells of the brain in mouse
Col. C	Neuron specific genes expressed in human	"*Purification and Characterization of Progenitor and Mature Human Astrocytes Reveals Transcriptional and Functional Differences with Mouse"* Zhang et al., 2016, Neuron 89, 37–53 This paper compares gene expression in different cells of the brain in human.
Col. D-F in Table D are data obtained from intersection of the data from col. A-C		
Col. D	CSF transmembrane proteins that are neuron specific in mouse	Intersect of (A,B)
Col. E	CSF transmembrane proteins that are neuron specific in human	Intersect of (A, C)
Col. F	CSF transmembrane proteins that are neuron specific in mouse AND human	Intersect of (A, B, C)
Col. G	Proteins found in neuron exosomes.	**Data from the applicants** obtained from mass spectrometry analysis of IPSC-derived neurons.
Col. H-M in Table D are data obtained from intersection between data from the applicant (col. G) and data derived from the published literature (Col. A-F).		
Col. H	CSF transmembrane proteins (detected through mass spectrometry analysis) in neuron exosomes	Intersect of (G, F) = intersect of (A, B, C, G)
Col. I	neuron specific genes in mouse AND in neuron exosomes	Intersect of (G, B)
Col. J	neuron specific genes expressed in human AND in neuron exosomes	Intersect of (G, C)

Col. K	CSF transmembrane proteins that are neuron specific in mouse AND in neuron exosomes	Intersect of (G, D) = intersect of (G, A, B)
Col. L	CSF transmembrane proteins that are neuron specific in human AND in neuron exosomes	Intersect of (G, E) = intersect of (G A, C)
Col. M	CSF transmembrane proteins that are neuron specific in mouse AND human AND in neuron exosomes	Intersect of (K, L) = intersect of (G, A, B, C)

[00154] The present invention may be applied to genetic mutations further described in Genetic Diseases of the Eye, Second Edition, edited by Elias I. Traboulsi, Oxford University Press, 2012. Several further aspects of the invention relate to diagnosing, prognosing and/or treating defects associated with a wide range of genetic diseases which are further described on the website of the National Institutes of Health under the topic subsection Genetic Disorders (website at health.nih.gov/topic/Genetic Disorders). The genetic brain diseases may include but are not limited to Adrenoleukodystrophy, Agenesis of the Corpus Callosum, Aicardi Syndrome, Alpers' Disease, Alzheimer's Disease, Barth Syndrome, Batten Disease, CADASIL, Cerebellar Degeneration, Fabry's Disease, Gerstmann-Straussler-Scheinker Disease, Huntington's Disease and other Triplet Repeat Disorders, Leigh's Disease, Lesch-Nyhan Syndrome, Menkes Disease, Mitochondrial Myopathies and NINDS Colpocephaly. These diseases are further described on the website of the National Institutes of Health under the subsection Genetic Brain Disorders.

[00155] In some embodiments, the condition (disorder) may be neoplasia. In some embodiments, where the condition is neoplasia, the genes to be diagnosed, prognosed and/or targeted are any of those listed in Table A (in this case PTEN and so forth). In some embodiments, the condition may be Age-related Macular Degeneration. In some embodiments, the condition may be a Schizophrenic Disorder. In some embodiments, the condition may be a Trinucleotide Repeat Disorder. In some embodiments, the condition may be Fragile X Syndrome. In some embodiments, the condition may be a Secretase Related Disorder. In some embodiments, the condition may be a Prion - related disorder. In some embodiments, the condition may be ALS. In some embodiments, the condition may be a drug addiction. In some embodiments, the condition may be Autism. In some embodiments, the condition may be

Alzheimer's Disease. In some embodiments, the condition may be inflammation. In some embodiments, the condition may be Parkinson's Disease.

[00156] Examples of proteins associated with Parkinson's disease include but are not limited to α-synuclein, DJ-1, LRRK2, PINK1, Parkin, UCHL1, Synphilin-1, and NURR1.

[00157] Examples of addiction-related proteins may include ABAT for example.

[00158] Examples of inflammation-related proteins may include the monocyte chemoattractant protein-1 (MCP1) encoded by the Ccr2 gene, the C-C chemokine receptor type 5 (CCR5) encoded by the Ccr5 gene, the IgG receptor IIB (FCGR2b, also termed CD32) encoded by the Fcgr2b gene, or the Fc epsilon R1g (FCER1g) protein encoded by the Fcer1g gene, for example.

[00159] Examples of cardiovascular diseases associated proteins may include IL1B (interleukin 1, beta), XDH (xanthine dehydrogenase), TP53 (tumor protein p53), PTGIS (prostaglandin I2 (prostacyclin) synthase), MB (myoglobin), IL4 (interleukin 4), ANGPT1 (angiopoietin 1), ABCG8 (ATP-binding cassette, sub-family G (WHITE), member 8), or CTSK (cathepsin K), for example.

[00160] Examples of Alzheimer's disease associated proteins may include the very low density lipoprotein receptor protein (VLDLR) encoded by the VLDLR gene, the ubiquitin-like modifier activating enzyme 1 (UBA1) encoded by the UBA1 gene, or the NEDD8-activating enzyme E1 catalytic subunit protein (UBE1C) encoded by the UBA3 gene, for example.

[00161] Examples of proteins associated Autism Spectrum Disorder may include the benzodiazapine receptor (peripheral) associated protein 1 (BZRAP1) encoded by the BZRAP1 gene, the AF4/FMR2 family member 2 protein (AFF2) encoded by the AFF2 gene (also termed MFR2), the fragile X mental retardation autosomal homolog 1 protein (FXR1) encoded by the FXR1 gene, or the fragile X mental retardation autosomal homolog 2 protein (FXR2) encoded by the FXR2 gene, for example.

[00162] Examples of proteins associated Macular Degeneration may include the ATP-binding cassette, sub-family A (ABC1) member 4 protein (ABCA4) encoded by the ABCR gene, the apolipoprotein E protein (APOE) encoded by the APOE gene, or the chemokine (C-C motif) Ligand 2 protein (CCL2) encoded by the CCL2 gene, for example.

[00163] Examples of proteins associated Schizophrenia may include NRG1, ErbB4, CPLX1, TPH1, TPH2, NRXN1, GSK3A, BDNF, DISC1, GSK3B, and combinations thereof.

[00164] Examples of proteins involved in tumor suppression may include ATM (ataxia telangiectasia mutated), ATR (ataxia telangiectasia and Rad3 related), EGFR (epidermal growth factor receptor), ERBB2 (v-erb-b2 erythroblastic leukemia viral oncogene homolog 2), ERBB3 (v-erb-b2 erythroblastic leukemia viral oncogene homolog 3), ERBB4 (v-erb-b2 erythroblastic leukemia viral oncogene homolog 4), Notch 1, Notch2, Notch 3, or Notch 4, for example.

[00165] Examples of proteins associated with a secretase disorder may include PSENEN (presenilin enhancer 2 homolog (C. elegans)), CTSB (cathepsin B), PSEN1 (presenilin 1), APP (amyloid beta (A4) precursor protein), APH1B (anterior pharynx defective 1 homolog B (C. elegans)), PSEN2 (presenilin 2 (Alzheimer disease 4)), or BACE1 (beta-site APP-cleaving enzyme 1), for example.

[00166] Examples of proteins associated with Amyotrophic Lateral Sclerosis may include SOD1 (superoxide dismutase 1), ALS2 (amyotrophic lateral sclerosis 2), FUS (fused in sarcoma), TARDBP (TAR DNA binding protein), VAGFA (vascular endothelial growth factor A), VAGFB (vascular endothelial growth factor B), and VAGFC (vascular endothelial growth factor C), and any combination thereof.

[00167] Examples of proteins associated with prion diseases may include SOD1 (superoxide dismutase 1), ALS2 (amyotrophic lateral sclerosis 2), FUS (fused in sarcoma), TARDBP (TAR DNA binding protein), VAGFA (vascular endothelial growth factor A), VAGFB (vascular endothelial growth factor B), and VAGFC (vascular endothelial growth factor C), and any combination thereof.

[00168] Examples of proteins related to neurodegenerative conditions in prior disorders may include A2M (Alpha-2-Macroglobulin), AATF (Apoptosis antagonizing transcription factor), ACPP (Acid phosphatase prostate), ACTA2 (Actin alpha 2 smooth muscle aorta), ADAM22 (ADAM metallopeptidase domain), ADORA3 (Adenosine A3 receptor), or ADRA1D (Alpha-1D adrenergic receptor for Alpha-1D adrenoreceptor), for example.

[00169] Examples of proteins associated with Immunodeficiency may include A2M [alpha-2-macroglobulin]; AANAT [arylalkylamine N-acetyltransferase]; ABCA1 [ATP-binding cassette, sub-family A (ABC1), member 1]; ABCA2 [ATP-binding cassette, sub-family A (ABC1), member 2]; or ABCA3 [ATP-binding cassette, sub-family A (ABC1), member 3]; for example.

Examples of proteins associated with Trinucleotide Repeat Disorders include AR (androgen receptor), FMR1 (fragile X mental retardation 1), HTT (huntingtin), or DMPK (dystrophia myotonica-protein kinase), FXN (frataxin), ATXN2 (ataxin 2), for example.

[00170] Examples of proteins associated with Neurotransmission Disorders include SST (somatostatin), NOS1 (nitric oxide synthase 1 (neuronal)), ADRA2A (adrenergic, alpha-2A-, receptor), ADRA2C (adrenergic, alpha-2C-, receptor), TACR1 (tachykinin receptor 1), or HTR2c (5-hydroxytryptamine (serotonin) receptor 2C), for example.

[00171] Examples of neurodevelopmental-associated sequences include A2BP1 [ataxin 2-binding protein 1], AADAT [aminoadipate aminotransferase], AANAT [arylalkylamine N-acetyltransferase], ABAT [4-aminobutyrate aminotransferase], ABCA1 [ATP-binding cassette, sub-family A (ABC1), member 1], or ABCA13 [ATP-binding cassette, sub-family A (ABC1), member 13], for example.

[00172] Further examples of preferred conditions treatable with the present system include may be selected from: Aicardi-Goutières Syndrome; Alexander Disease; Allan-Herndon-Dudley Syndrome; POLG-Related Disorders; Alpha-Mannosidosis (Type II and III); Alström Syndrome; Angelman; Syndrome; Ataxia-Telangiectasia; Neuronal Ceroid-Lipofuscinoses; Beta-Thalassemia; Bilateral Optic Atrophy and (Infantile) Optic Atrophy Type 1; Retinoblastoma (bilateral); Canavan Disease; Cerebrooculofacioskeletal Syndrome 1 [COFS1]; Cerebrotendinous Xanthomatosis; Cornelia de Lange Syndrome; MAPT-Related Disorders; Genetic Prion Diseases; Dravet Syndrome; Early-Onset Familial Alzheimer Disease; Friedreich Ataxia [FRDA]; Fryns Syndrome; Fucosidosis; Fukuyama Congenital Muscular Dystrophy; Galactosialidosis; Gaucher Disease; Organic Acidemias; Hemophagocytic Lymphohistiocytosis; Hutchinson-Gilford Progeria Syndrome; Mucolipidosis II; Infantile Free Sialic Acid Storage Disease; PLA2G6-Associated Neurodegeneration; Jervell and Lange-Nielsen Syndrome; Junctional Epidermolysis Bullosa; Huntington Disease; Krabbe Disease (Infantile); Mitochondrial DNA-Associated Leigh Syndrome and NARP; Lesch-Nyhan Syndrome; LIS1-Associated Lissencephaly; Lowe Syndrome; Maple Syrup Urine Disease; MECP2 Duplication Syndrome; ATP7A-Related Copper Transport Disorders; LAMA2-Related Muscular Dystrophy; Arylsulfatase A Deficiency; Mucopolysaccharidosis Types I, II or III; Peroxisome Biogenesis Disorders, Zellweger Syndrome Spectrum; Neurodegeneration with Brain Iron Accumulation Disorders; Acid Sphingomyelinase Deficiency; Niemann-Pick Disease Type C; Glycine

Encephalopathy; ARX-Related Disorders; Urea Cycle Disorders; COL1A1/2-Related Osteogenesis Imperfecta; Mitochondrial DNA Deletion Syndromes; PLP1-Related Disorders; Perry Syndrome; Phelan-McDermid Syndrome; Glycogen Storage Disease Type II (Pompe Disease) (Infantile); MAPT-Related Disorders; MECP2-Related Disorders; Rhizomelic Chondrodysplasia Punctata Type 1; Roberts Syndrome; Sandhoff Disease; Schindler Disease - Type 1; Adenosine Deaminase Deficiency; Smith-Lemli-Opitz Syndrome; Spinal Muscular Atrophy; Infantile-Onset Spinocerebellar Ataxia; Hexosaminidase A Deficiency; Thanatophoric Dysplasia Type 1; Collagen Type VI-Related Disorders; Usher Syndrome Type I; Congenital Muscular Dystrophy; Wolf-Hirschhorn Syndrome; Lysosomal Acid Lipase Deficiency; and Xeroderma Pigmentosum.

[00173] Some examples of disorders (conditions or diseases) that might be usefully treated, prognosed and/or diagnosed using the present invention are included in the Tables above and examples of genes or markers currently associated with those disorders are also provided there. However, the genes exemplified are not exhaustive.

[00174] In one aspect, the invention provides kits containing any one or more of the elements disclosed in the above methods and compositions. Elements may be provided individually or in combinations, and may be provided in any suitable container, such as a vial, a bottle, or a tube. In some embodiments, the kit includes instructions in one or more languages, for example in more than one language.

[00175] In some embodiments, a kit comprises one or more reagents for use in a process utilizing one or more of the elements described herein. Reagents may be provided in any suitable container. For example, a kit may provide one or more reaction or storage buffers. Reagents may be provided in a form that is usable in a particular assay, or in a form that requires addition of one or more other components before use (e.g. in concentrate or lyophilized form). A buffer can be any buffer, including but not limited to a sodium carbonate buffer, a sodium bicarbonate buffer, a borate buffer, a Tris buffer, a MOPS buffer, a HEPES buffer, and combinations thereof. In some embodiments, the buffer is alkaline. In some embodiments, the buffer has a pH from about 7 to about 10. In some embodiments, the kit comprises one or more oligonucleotides corresponding to a guide sequence for insertion into a vector so as to operably link the guide sequence and a regulatory element. In some embodiments, the kit comprises a homologous recombination template polynucleotide. In some embodiments, the kit comprises

one or more of the vectors and/or one or more of the polynucleotides described herein. The kit may advantageously allows to provide all elements of the systems of the invention.

[00176] Although the present invention and its advantages have been described in detail, it should be understood that various changes, substitutions and alterations can be made herein without departing from the spirit and scope of the invention as defined in the appended claims.

[00177] The present invention will be further illustrated in the following Examples which are given for illustration purposes only and are not intended to limit the invention in any way.

Examples

Example 1: isolation/purification of exosomes, and RNA extraction therefrom (no proteinase treatment): standard exosome isolation

[00178] The following protocol was used to isolate RNA from suspension cells such as K562 Cells. Buffers and some reagents refer to a mirVana RNA kit (Life technologies).

[00179] Day 1

- Spin down about 72 million cells total in 6 50 mL Falcon tubes (12 million cells per tube) at 300xg for 5 minutes.
- Aspirate media and resuspend each cell pellet in 43 mL exosome-free media. Transfer contents of each Falcon tube to T75 flask.

[00180] Day 2

- After 24 hours, take off all media and divide among 50 mL falcon tubes. Spin at 300xg for 10 minutes at 4 degrees.
- Transfer supernatant to new 50 mL tubes leaving cell pellet behind. Spin at 2000xg for 10 minutes at 4 degrees. Transfer supernatant to new 50 mL tubes leaving cell pellet behind.
- Spin supernatant at 16,500xg for 20 minutes at 4 degrees.
- Transfer supernatant to new 50 mL tubes, leaving pellet behind.
- Pass supernatant through Steriflip 0.22 micron filter.
- Transfer supernatant to pollyallomer ultracentrifuge tubes. Centrifuge at 120,000xg (26,500 RPM with SW32Ti rotor) for 70 minutes at 4 degrees.
- Remove supernatant, leaving ~2 cm of media above pellet. Add 5 mL PBS to each tube. Vortex on medium speed for a few seconds. Fill to top of each tube with PBS.
- Again, centrifuge at 120,000xg for 70 minutes at 4 degrees.

- Aspirate all of supernatant with Pasteur pipet without touching bottom of tube (where pellet is located).
- Add 2 µL Superasin to each tube (SUPERase• In™ RNase Inhibitor from Life technologies).
- Add 200 µL of Lysis/Binding Solution directly to the bottom of each ultracentrifuge tube. Pipet up and down. Transfer the contents of 3 ultracentrifuge tubes to one 1.5 mL Eppendorf tube.
- Vortex briefly and place on ice.
- Add 60 µL of miRNA Homogenate Additive to each tube (1/10 volume of lysate).
- Vortex each tube and place on ice for 10 minutes.
- Add a 600 µL of Acid-Phenol:Chloroform to each tube (volume that is equal to lysate volume before addition of miRNA Homogenate Additive).
- Vortex for 30 seconds to mix thoroughly.
- Centrifuge at maximum speed for 5 minutes (all spins at room temperature).
- While tubes are spinning, transfer some Elution Solution to new 1.5 mL tube and pre-heat Elution Solution in heating block to 95°C. Also, put filter cartridges into collection tubes.
- Carefully remove the upper (aqueous) phase and transfer to a new 1.5 mL tube.
- Add 1.25 volumes of 100% ethanol to the transferred aqueous phase.
- Pipet up and down and transfer up to 700 µL to a filter cartridge. Centrifuge at 10,000 RCF (10,000 RPM) for 15-30 seconds.
- Discard flow-through and load the rest of the lysate/ethanol mixture. Centrifuge at 10,000 RCF (10,000 RPM) for 15-30 seconds.
- Add 700 µL of miRNA Wash Solution 1 to filter and centrifuge at 10,000 RCF (10,000 RPM) for 15 seconds. Discard flow-through.
- Add 500 µL of miRNA Wash Solution 2/3 to filter and centrifuge at 10,000 RCF (10,000 RPM) for 15 seconds. Discard flow-through.
- Again, add 500 µL of miRNA Wash Solution 2/3 to filter and centrifuge at 10,000 RCF (10,000 RPM) for 15 seconds. Discard flow-through.
- Put filter back in collection tube and spin for 1 minute at 10,000 RCF (10,000 RPM) to remove any residual ethanol from the filter.
- Transfer filter cartridge with bound RNA to a new collection tube.

- Add 100 µL of pre-heated Elution Buffer to the center of each filter. Centrifuge for 30 seconds at maximum speed to recover the RNA.
- Store RNA at -80°C.

Example 2: isolation/purification of exosomes, and RNA extraction therefrom (with proteinase and RNase treatment): removal of Protein-RNA Complexes from the Exosome Pellet

[00181] The following protocol was used to isolate RNA from suspension cells such as K562 Cells. This protocol removes RNA-protein complexes from the exosomes. Buffers refer to a mirVana RNA kit (Life technologies).

- Execute exosome isolation protocol (see *example 1*) on 6 x 12 million cells up to the end of first ultracentrifugal spin.
- Take off complete supernatant of all six tubes. Resuspend each in 150µL PBS. Label two tubes **P1** and **P2** and to these, add 5 uL of proteinase K (active conc. 500µg/mL).
- Incubate all tubes at 37°C for 30 minutes.
- Fill tubes with PBS and ultracentrifuge again.
- After second spin, take off complete supernatant of all six tubes. Resuspend each in 150µL PBS. Label the four unlabeled tubes **NT1**, **NT2**, **PR1** and **PR2**.
- Add 5 µL of proteinase K (active conc. 500 µg/mL) to **PR1** and **PR2**.
- Incubate all tubes at 37°C for 30 minutes.
- Add 5µL PMSF (from 20 mM stock; active conc. 1mM) to **PR1** and **PR2**.
- Leave all tubes at RT for 10 minutes.
- Add 0.5µL RNase A/T1 (active conc. ~3 µg/mL) to **PR1** and **PR2**.
- Incubate all tubes at 37°C for 30 minutes.
- Add 2µL superasin to each tube. (SUPERase• In™ RNase Inhibitor from Life technologies).
- Move contents of each tube to 1 Eppendorf tube (total volume should be ~200µL per tube due to residual liquid in UC tube), labeled accordingly, and proceed with mirVana RNA isolation using 300µL lysis buffer.

- *Example 3: Chemical and enzymatic treatment of exosomes*

To achieve purified exosomes which are essentially free of extra-exosomal nucleic acid-protein complexes, the following procedure is provided. In sum, DNase is added during the preparation, then inactivated prior to lysing all of the vesicles which affords a composition which is

essentially free of extra-exosomal nucleic acid-protein complexes. Briefly, exosome pellet – either at the wash step between ultracentrifugations or after the final ultracentrifugation, as indicated – was resuspended in 50-500 µL PBS or 0.5% Triton X-100 as indicated. For proteinase treatment, Proteinase K (Life Technologies) was added to a final concentration of 500 µg/mL, and samples were incubated at 37°C for 30 minutes. Treatment was initially done in Proteinase K activity buffer (0.1 M NaCl, 10 mM Tris pH 8, 1 mM EDTA) rather than PBS, however reduced RNA yields from untreated exosomes resuspended in this buffer were observed; thus, all further treatments were performed in PBS. Proteinase was subsequently inactivated by the addition of phenylmethylsulfonyl fluoride (PMSF; Millipore) to 1 mM concentration. For RNase treatment, RNase Cocktail Enzyme Mix (Life Technologies) was added to a final concentration of 1.25 and 50 U/mL RNase A and T1, respectively, and samples incubated at 37°C for 30 minutes. RNase was inactivated by the addition of SUPERasein (Life Technologies) to 20 U/mL concentration and the addition of ≥ 2 volumes lysis buffer from mirVana miRNA isolation kit (Life Technologies). For DNase treatment, Turbo DNase (Life Technologies) was added to a concentration of 26 U/mL, with Turbo DNase buffer added to 1X concentration where indicated, and samples incubated at 37°C for 30 minutes. DNase was inactivated by the addition of EDTA to 15 mM followed by incubation at 75°C for 10 minutes.

- *Example 4: CD81 and CD63 exosome isolation with Mouse IgG beads (pull down purification)*

[00182] Day 0 (or earlier)

1. Mix 50 mL FBS with 500 mL IMDM and 5 mL P/S. Filter through 0.22 µM filter. Grow cells.

[00183] Day 1

2. Spin down 72 million cells total in 6 50 mL Falcon tubes at 300xg for 5 minutes.

3. Aspirate media and resuspend each cell pellet in 43 mL AIM-V. Transfer contents of each Falcon tube to T75 flask.

[00184] Day 2

4. After 24 hours, take off all media and divide among 50 mL falcon tubes. Spin at 300xg for 10 minutes at RT.

5. Transfer supernatant to new 50 mL tubes leaving cell pellet behind. Spin at 2000xg for 10 minutes at RT. Transfer supernatant to new 50 mL tubes leaving cell pellet behind.

6. Spin supernatant at 16,500xg for 20 minutes at 4 degrees.

7. Transfer supernatant to new 50 mL tubes, leaving pellet behind.

8. Pass supernatant through Steriflip 0.22 micron filter.

9. Transfer supernatant to pollyallomer ultracentrifuge tubes. Centrifuge at 120,000xg (26,500 RPM with SW32Ti rotor) for 70 minutes at 4 degrees.

10.During this spin, make fresh Isolation Buffer (PBS supplemented with 1 mg/mL BSA, filtered through 0.22μm filter) and prepare hot plate at 95°C

11.Also during first ultracentrifuge spin, prepare beads:

a. resuspend anti-mouse IgG Dynabeads by mixing for >10 min or vortexing gently for 30s.

b. transfer 100μL (4x107) beads each into 3 different Biotix 2mL tubes labelled **C, 81** and **63**.

c. wash the magnetic beads by adding 1 mL of Isolation Buffer. Mix well.

d. place tubes on the magnet for 2 minutes and remove supernatant carefully.

e. remove tubes from magnet and add 100μL isolation buffer.

f. To **81**, add 20μL (4μg) anti-human CD81 antibody, clone 1.3.3.22

g. To **63**, add 8μL (4μg) anti-human CD63 antibody, clone h5c6

h. To **C**, add 4μL (4μg) ctrl antibody (mouse mAb mCherry, 1C51)

i. Incubate on rotating rack in cold room until end of isolation (~3 hours)

12.Remove supernatant, leaving ~2 cm of media above pellet. Add 5 mL PBS to each tube. Vortex on medium speed for a few seconds. Fill to top of each tube with PBS.

13.Again, centrifuge at 120,000xg for 70 minutes at 4 degrees.

14.Aspirate all of supernatant with Pasteur pipet without touching bottom of tube (where pellet is located).

15.Add 80μL PBS to each tube and let sit for ~15 minutes.

16.Resuspend and pool all tubes into a biotix tube labelled P. Measure total volume, should be ~600μL due to 20μL residual liquid after aspiration.

17.Retrieve bead tubes from cold room, spin briefly and place on magnet.

18.Do 2X 900μL washes in isolation buffer to remove excess antibody.

19.Split pooled pellets 1/6 into each of the biotix tubes. Add isolation buffer to each bead tube to 200μL total volume. Put all on rotating rack in cold room overnight (16 hours)

20.Add 33µL **4X SB** (133µL total volume) to remaining 100µL of exosomes in **P** and boil at 95° for 5 min. Place immediately on ice and freeze at -80°

[00185] Day 3

21.After 16h, centrifuge all tubes from cold room briefly to collect samples.

22.Place **C, 81** and **63**. on magnet for two minutes. Collect supernatants and store in new tubes labelled **C-FT**, **81-FT, 63-FT** respectively.

23.Wash beads in each tube with 500µL Isolation Buffer. Leave 2 min on magnet before collecting wash supernatants. **Add each wash to respective FT tube.** Store at 4°C.

24. Add 133µL **1X** Sample Buffer to **C, 81** and **63**. and boil at 95° for 5 min. Place immediately on ice for 5 min, then place on magnet for 2 min, collect supernatants and freeze at -80° in new tubes.

25.Assemble all flow-through tubes and add each to its own ultracentrifuge tube. Fill tubes with PBS and spin 180 minutes at 120 000*g*.

26.After ultracentrifugation, remove supernatant entirely, add 80µL PBS and leave pellets for ~15 minutes.

27.Resuspend (should be about 100µL) move to labelled biotix tubes and add 33µL **4X** Sample Buffer to each.

28.Boil at 95°C for 5 min. Place immediately on ice and freeze at -80°C.

Example 5: analysis of RNA contents of exosomes as a function of exosome purification method – size distribution

[00186] Figure 1 shows graph of RNA fluorescence unit (FU) plotted against RNA size (nt), wherein "final spin" refers to the final centrifugation step.

[00187] The results allow comparison and validation of corresponding purification methods.

Example 6: analysis of RNA contents of exosomes as a function of exosome purification method – electron microscopy imaging

[00188] Figures 2A-D show electron microscopy (EM) photographs of exosome preparations, wherein "no treatment" refers to a protocol according to example 1; "after spins" refers to a protocol according to example 2; "between spins" denotes a protocol according to example 1, except that additional proteinase treatment occurred between the two ultracentrifugation steps.

[00189] The results show that the method used for exosome preparation affects exosome integrity. EM data allow comparison and validation of exosome purification methods.

[00190] Vesicles Electron Microscopy Prep

Stain prep

- Weigh 60 mg powdered Uranyl Formate into clean 10mL beaker with stir stick in radioactivity hood.
- Move this to the stir plate (make sure stirring is OFF) and cover with the big beaker with tin foil.
- Fill another clean 10 mL beaker with 3 mL water and heat this up (not on the same hot plate) until it's super boiling/as hot as possible. Ensure not to lose too much water to evaporation.
- Quickly pour this into other beaker with powder and start stirring. Stir vigorously for 2 minutes protected by tin foil.
- Using BD 5 mL syringe (with black lining inside, not the normject ones) suck up stain and then using corning 0.45 μm filter to filter it, deposit into 15 mL falcon tube. Label and wrap in tin foil.
- Wipe beaker with Kim wipe. throw this, gloves, syringe and filter into radioactive waste

Sample prep

- Good sample concentration is in the range of 1 nM
- Use special tweezers to put grids on parafilm-covered slide, dark shiny side up.
- Put slide in glow discharger, close lid carefully, hit start.
- Pick up grids with tweezers at the edge, don't pinch too hard. Put tweezers down (still holding grid) and pipet 3.5 μL of sample onto it. Leave 1 minute. This time changes depending on salt concentration etc.
- Wick away liquid with a piece of filter paper
- Add 3.5 μL stain, leave 30 seconds, then wick away this as well.
- Find a holder and carefully put grids down with dark side up, use this to carry to EM room

Example 7: analysis of RNA contents of exosomes as a function of exosome purification method – qRT-PCR analysis – validation of the purification method

[00191] Figure 3 shows qRT-PCR data of exosome RNA for 4 mRNAs that were previously found in exosome RNA-Seq data.

[00192] The qRT-PCR is performed for various conditions of exosome purification methods. All runs are normalized to RNA from the 'regular' exosome isolation (Example 1). The conditions for exosome purification are as follows :

(1) RNase treatment only

(which is expected not be sufficient if RNA is also protected by proteins as was shown for extracellular microRNAs in Arroyo et al, Proc Natl Acad Sci U S A. 2011 Mar 22;108(12):5003-8. doi: 10.1073/pnas.1019055108. Epub 2011 Mar 7.2011; Turchinovich et al Nucleic Acids Res. 2011 Sep 1;39(16):7223-33. doi: 10.1093/nar/gkr254. Epub 2011 May 24.)

(2) proteinase + RNase treatments after spins

(protocol as per Example 2; also see below)

(3) proteinase treatment (between spins)

This is the method described in previous publications such as Valadi et al, Nat Cell Biol. 2007 Jun;9(6):654-9. Epub 2007 May 7. As shown by EM (see above and Fig. 2d), this method compromises exosome integrity.

In accordance with the EM data, the qRT-PCR results show a decrease in mRNA levels.

(4) Triton + RNase treatments

This is a control run, wherein where Triton treatment is used to break open the vesicles, and samples are further treated with RNase. The results show drastic reduction in levels of mRNA.

[00193] R/T isolation for qPCR

1. Execute exosome isolation protocol on 6 x 12 million cells up to the end of second ultracentrifugal spin.
2. Take off complete supernatant of all six tubes.
3. Resuspend 4 pellets in 150µL PBS. Label them **NT1**, **NT2**, **R1** and **R2**.
4. Resuspend other 2 pellets in 3% Triton. Label them **TR1** and **TR2**,
5. Add 0.5µL RNase A/T1 (active conc. ~3µg/mL) to **R1**, **R2**, **TR1** and **TR2**,
6. Incubate all tubes at 37°C for 30 minutes.
7. Add 2µL superasin to each tube (SUPERase• In™ RNase Inhibitor from Life technologies).
8. Proceed with mirVana RNA isolation using 300µL lysis buffer (mirVana RNA kit from Life technologies).

[00194] P/R isolation for qPCR

1. Execute exosome isolation protocol on 6 x 12 million cells up to the end of first ultracentrifugal spin.
2. Take off complete supernatant of all six tubes. Resuspend each in 150μL PBS. Label two tubes **P1** and **P2** and to these, add 5 μL of proteinase K (active conc. 500μg/mL).
3. Incubate all tubes at 37°C for 30 minutes.
4. Fill tubes with PBS and ultracentrifuge again.
5. After second spin, take off complete supernatant of all six tubes. Resuspend each in 150 μL PBS. Label the four unlabeled tubes **NT1**, **NT2**, **PR1** and **PR2**.
6. Add 5 uL of proteinase K (active conc. 500 μg/mL) to **PR1** and **PR2**.
7. Incubate all tubes at 37°C for 30 minutes.
8. Add 5μL PMSF (from 20 mM stock; active conc. 1 mM) to **PR1** and **PR2**.
9. Leave all tubes at RT for 10 minutes.
10. Add 0.5μL RNase A/T1 (active conc. ~3μg/mL) to **PR1** and **PR2**.
11. Incubate all tubes at 37°C for 30 minutes.
12. Add 2 μL superasin to each tube (SUPERase• In™ RNase Inhibitor from Life technologies).
13. Proceed with mirVana RNA isolation using 300 μL lysis buffer (mirVana RNA kit from Life technologies).

[00195] qRT-PCR

1. After collecting cell and exosome RNA, dilute 100 ng cell RNA to 100 μL with H_2O. Add 2 μL Turbo DNase (Lifetech), 2 μL Superasin, and 10 μL DNase buffer to each sample.
2. Incubate at 37°C for 30 minutes.
3. Clean and concentrate using Zymo RNA Clean and Conncentrate kit according to instructions and elute in 16 μL H_2O.
4. Perform reverse transcription using Superscript VILO cDNA Synthesis kit with 14 μL of RNA in a 20 μL reaction.
5. Perform qPCR using KAPA Fast qPCR SYBR mix (KAPA Biosystems) with 2 μL of cDNA per reaction.

[00196] The following primers were used:

SRP14-F	GGGTACTGTGGAGGGCTTTG

SRP14-R	AGGAGGTTTGAATAAGCCATCTGA
B2M-F	GTATGCCTGCCGTGTGAAC
B2M-R	AAAGCAAGCAAGCAGAATTTGG
ACTB-F	CGGCATCGTCACCAACTG
ACTB-R	AACATGATCTGGGTCATCTTCTC
GAPDH-F	GGTGGTCTCCTCTGACTTCAACA
GAPDH-R	GTTGCTGTAGCCAAATTCGTTGT

Example 8: correlation of exosomal RNA content with cellular RNA content

[00197] Figures 4A-C show RNA-Seq data, showing that the RNA profile of mRNAs in exosomes reflects that of the donor cells. This indicates that the exosomes provide an accurate snapshot of the transcriptome of the cells they come from. Exosome preparation was according to the standard exosome isolation procedure (as in Example 1, without proteinase/RNase).

[00198] RNA-Seq

1. After collecting cell and exosome RNA, dilute 100 ng cell RNA to 100 μL with H_2O. Add 2 μL Turbo DNase (Lifetech), 2 μL Superasin, and 10 μL DNase buffer to each sample.
2. Incubate at 37°C for 30 minutes.
3. Clean and concentrate using Zymo RNA Clean and Conncentrate kit according to instructions and elute in 10 μL H_2O.
4. Perform a PolyA Selection using Dynabeads mRNA Purification kit (Lifetech).
5. Proceed with RNA-Seq library prep protocol as described in: *Perturbation of m6A writers reveals two distinct classes of mRNA methylation at internal and 5' sites.* Schwartz S, Mumbach MR, Jovanovic M, Wang T, Maciag K, Bushkin GG, Mertins P, Ter-Ovanesyan D, Habib N, Cacchiarelli D, Sanjana NE, Freinkman E, Pacold ME, Satija R, Mikkelsen TS, Hacohen N, Zhang F, Carr SA, Lander ES, Regev A. Cell Rep. 2014 Jul 10;8(1):284-96. doi: 10.1016/j.celrep.2014.05.048. Epub 2014 Jun 26.

Example 9: exosome-mediated RNA transfer experiment between HEK293 and K562 cells

[00199] Figures 5A-K show fluorescence imaging of cells using EUclick chemistry.

[00200] This example shows results from a system that allows detection of potential endogenous RNA transfer between cells in a co-culture system by feeding donor cells with a

modified nucleotide (5-ethynyl uridine, EU) that gets incorporated into its RNA and then co-culturing donor cells with unlabeled acceptor cells.

[00201] Click Chemistry is then used to detect RNA with the modified nucleotides by conjugate of a fluorophore to the EU. These results suggest the presence of RNA transfer. The white arrows point to spots of transferred RNA in the HEK293 acceptor cells. The green arrows just show the donor K562 cells.

[00202] EU-RNA Transfer Experiments

- K562 and HEK293 cells were both obtained from ATCC.
- K562 cells were incubated with 5-ethynyl uridine (Lifetech) diluted to 2 mM for 24 hours.
- K562 and HEK 293 cells were co-cultured for 24 hours.
- Cells were imaged using Click-IT RNA Alexa Fluor 594 Imaging kit (Lifetech)

Example 10: exosome-mediated RNA transfer experiment between co-cultured cell lines.

[00203] Figures 6A-D show principle and results of an experiment to assess possible exosome mediated RNA transfer between co-cultured cell lines.

[00204] This example illustrates a way to detect potential RNA transfer using unlabeled RNA. The principle is to co-culture mouse and human cells, separate them back out and use regular RNA-Seq to detect mouse transcripts in human cells. This technique relies on a principle similar to that of Example 7, but without using labeled nucleotides. Using this method, it was possible to detect some RNAs transferred but the strongest signal came from two mouse endogenous retrovirus RNAs (labeled as Gm3168 and Ctse).

[00205] Mouse Human RNA Transfer Experiments

- Human K562 and Mouse RAW Macrophage cells were both obtained from ATCC.
- K562 cells were infected with virus expressing GFP.
- K562 cells were FACS sorted to all be GFP positive.
- K562 GFP cells were co-cultured with Mouse RAW cells for 24 hours or 0 hours (as a control).
- K562 GFP cells were FACS sorted for GFP positive cells to separate from Mouse cells after 24 hour co-culture (2 biological replicates: Mix 1 and Mix 2). The 0 hour co-culture was also sorted, as well as a control of just K562 cells that never interacted with mouse cells.
- RNA was extracted using MirVana kit (Lifetech),

- 200 ng cell RNA to 100 µL with H2O. Add 2 µL Turbo DNase (Lifetech), 2 µL Superasin (Life technologies), and 10 µL DNase buffer to each sample.
- Incubation at 37°C for 30 minutes.
- Clean and concentrate using Zymo RNA Clean and Concentrate kit according to instructions and elute in 10 µL H_2O.
- PolyA Selection using Dynabeads mRNA Purification kit (Lifetech).
- Proceed with RNA-Seq library prep protocol as described in: *Perturbation of m6A writers reveals two distinct classes of mRNA methylation at internal and 5' sites*. Schwartz S, Mumbach MR, Jovanovic M, Wang T, Maciag K, Bushkin GG, Mertins P, Ter-Ovanesyan D, Habib N, Cacchiarelli D, Sanjana NE, Freinkman E, Pacold ME, Satija R, Mikkelsen TS, Hacohen N, Zhang F, Carr SA, Lander ES, Regev A. Cell Rep. 2014 Jul 10;8(1):284-96. doi: 10.1016/j.celrep.2014.05.048. Epub 2014 Jun 26.

Exosome Key Results.

[00206] Figures 7A-D show poly A selected mRNA from two replicates of K562 cells and their exosomes was compared using RNA-Seq. The bottom two panels show that cell and exosome mRNA is correlated in expression for protein-coding genes.

[00207] Applicants have sequenced the mRNA of exosomes from K562 cells and compared the RNA profile of the donor cells to that of the exosomes. Applicants have found that the mRNA profiles of exosomes reflects the trasnscriptome of the donor cells. Thus, using exosomes as a non-invasive read-out of the transcriptome of inaccessible cell types is possible.

[00208] Figure 8 illustrates mRNA in exosome pellet following enzymatic treatments. RNA from untreated exosomes and proteinase/RNase treated exosomes was compared using qRT-PCR for four mRNAs. There was very little or no change, indicating that the RNA is inside. As a control, vesicles with the detergent Triton were lysed and then treated with RNase.

[00209] Figure 9 illustrates Poly A enriched mRNA from untreated exosomes and proteinase/RNAse treated exosomes was compared using RNA-Seq. The mRNA is strongly correlated, indicating that the mRNA isolated *via* ultracentrifugation in the exosome pellet is inside the vesicles.

[00210] Applicants have confirmed that the mRNA in the exosome isolated product is really inside exosomes after developing a protocol to degrade all RNAs not in vesicles by enzymatic

treatment with proteinase and then RNAse. Applicants find a very high correlation between the mRNA profiles in the untreated exosome pellet and the proteinase/RNAse treated pellet, indicating the sequenced mRNA is really inside the vesicles. Applicants have confirmed these results through qRT-PCR as well.

[00211] Figure 10 illustrates targeted pull down exosome subpopulations based on their protein marker using antibody conjugated magnetic beads. CD63 is a glycosylated protein between 30 and 60 kDa. CD81 shows up as a distinct band between 20 and 30 kDa. mCherry is used as a non-specific control. This protocol/technique was developed to isolate specific exosome subpopulations by specific membrane proteins using antibody-conjugated magnetic beads. Further, the technique has been validated in K562 exosomes using the canonical exosome markers CD63 and CD81.

[00212] Figure 11 illustrates exosomes which were isolated from human CSF and mRNA for four genes (detected by qRT-PCR.) Cell RNA is used as a comparison. Two methods of isolating exosomes from CSF were demonstrated: one by running through 0.22 micron filter pelleting at 120,000g for 2 hours (CSF pellet) and one by extracting RNA directly from CSF after running through 0.22 micron filter without pellet. Similar results were observed by both methods.

Additional Examples

[00213] Mass spectrometry of exosomes from iPS cells and iPS-derived neurons is conducted to find neurons specific membrane proteins found on exosomes. These markers are verified by western blots in iPS and neurons exosomes.

[00214] RNA-Seq of exosomes from K562 cells are isolated using CD81 or CD63 antibody-conjugated magnetic beads. The RNA-Seq profiles of exosome subpopulation are compared to the RNA profiles of total exosomes.

[00215] RNA-Seq of mRNA from both cells and exosomes from iPS cells and iPS–derived neurons.

[00216] RNA-Seq of exosomes from iPS and neuron exosomes isolated using antibody-conjugated magnetic beads to enrich for exosomes expressing the cell type specific proteins. The RNA-Seq profiles of these exosome subpopulations are compared to the RNA profiles of total exosomes from each cell type.

[00217] In vitro proof of principle by mixing experiments where Applicants mix cell culture media from iPS cells and neurons and isolate exosomes from the mixed media. Applicants isolate exosomes from the original cell type using antibody-conjugated magnetic beads using the cell type specific markers. Applicants isolate RNA from these exosome subpopulations and perform RNA-Seq to confirm reconstruction of the transcriptome of the original cell type (iPS cells or neurons).

[00218] Applicants isolate exosomes from human cerebrospinal fluid (CSF) and perform RNA-Seq.

[00219] Applicants enrich for neuron specific exosomes in CSF using antibody-conjugated magnetic beads or a microfluidic device with immobilized antibodies. Applicants then sequence the RNA from these neuron-derived exosomes and to observe enriched expression of neuron-specific genes relative to total CSF exosomes.

* * *

[00220] Having thus described in detail preferred embodiments of the present invention, it is to be understood that the invention defined by the above paragraphs is not to be limited to particular details set forth in the above description as many apparent variations thereof are possible without departing from the spirit or scope of the present invention.

WHAT IS CLAIMED IS:

1. A method for the isolation of exosomes from a biological sample, said method comprising:
(a) providing a biological sample comprising exosomes from a cell population,
(b) preparing an exosome-enriched fraction from the biological sample of step (a),
(c) subjecting the exosome-enriched fraction of step (b) to a treatment with a proteinase.

2. A method for the purification of exosomes from a biological sample, said method comprising:
(a) providing a biological sample comprising exosomes from a cell population,
(b) preparing an exosome-enriched fraction from the biological sample of step (a),
(c) subjecting the exosome-enriched fraction of step (b) to a treatment with a proteinase.

3. The method of any one of the preceding claims, wherein the proteinase of step (c) is one or more independently selected from serine proteases, threonine proteases, cysteine proteases, aspartate proteases, glutamic acid proteases and metalloproteases.

4. The method of any one of the preceding claims, wherein step (c) comprises a treatment with a proteinase and subsequent inactivation thereof.

5. The method of the preceding claim, wherein proteinase inactivation is performed with one or more protease inhibitor(s).

6. The method of any one of the preceding claims, wherein the proteinase of step (c) is proteinase K.

7. The method of any one of the preceding claims, wherein step (b) comprises one or more centrifugation steps, so as to remove live cells, dead cells and larger cellular debris from the biological sample of step (a).

8. The method of any one of the preceding claims, wherein step (b) comprises one or more filtration steps.

9. The method of the preceding claim, wherein the filtration step comprises filtration with a submicron filter.

10. The method of the preceding claim, wherein the submicron filter is a 0.22 micron filter.

11. The method of any one of the preceding claims, wherein step (b) comprises one or more centrifugation steps, so as to remove live cells, dead cells and larger cellular debris from the biological sample of step (a), followed by a filtration step with a submicron filter.

12. The method of any one of the preceding claims, wherein step (b) comprises one or more ultracentrifugation steps.

13. The method of any one of the preceding claims,
wherein step (b) comprises:
(b-1) filtrating with a submicron filter,
(b-2) performing a first ultracentrifugation step, so as to provide a first exosome-enriched fraction,
(b-3) washing the exosome-enriched fraction of step (b-2), and
(b-4) performing a second ultracentrifugation step of the washed exosome-enriched fraction of step (b-3).

14. The method of any one of claims 12-13, wherein step (c) is performed after the final ultracentrifugation step of step (b).

15. The method of any one of the preceding claims, wherein step (c) comprises a treatment with proteinase K and subsequent inactivation thereof.

16. The method of the preceding claim, wherein the inhibitor is diisopropyl fluorophosphate (DFP) or phenyl methane sulphonyl fluoride (PMSF).

17. The method of any one of the preceding claims, further comprising:
(d) subjecting the proteinase K-treated fraction of step (c) to a treatment with an RNase.

18. The method of the preceding claim, wherein the RNase is one or more independently selected from RNase A, B, C, 1, and T1.

19. The method of the preceding claim, wherein the RNase is RNAse A/T1.

20. The method of any one of claims 17-19, wherein step (d) comprises a treatment with RNase and subsequent inactivation thereof.

21. The method of the preceding claim, wherein inactivation of RNase is comprises a treatment with one or more RNase inhibitor(s).

22. The method of the preceding claim, wherein the RNase inhibitor is selected from protein-based RNase inhibitors.

23. The method of any one of the preceding claims, wherein the method provides exosomes which are essentially free of extra-exosomal material.

24. The method of any one of the preceding claims, wherein the method provides exosomes which are essentially free of extra-exosomal nucleic acid-protein complexes.

25. The method of any one of the preceding claims, wherein the method provides exosomes which are essentially free of extra-exosomal RNA-protein complexes.

26. The method of any one of the preceding claims, wherein the method further comprises after step (c) or (d) one or more purification steps based on the affinity of a bait molecule for a prey exosome biomarker.

27. The method of any one of the preceding claims, wherein the cell population comprises one or more cell types, 2 or more cell types, preferably 3 or more cell types, 4 or more cell types or 5 or more cell types.

28. The method of any one of the preceding claims, wherein the method isolates or purifies cell type-specific exosomes, or cell-subtype-specific exosomes.

29. The method of any one of the preceding claims, wherein the one or more cell type comprises cells derived from the endoderm, cells derived from the mesoderm, or cells derived from the ectoderm.

30. The method of claim 29, wherein cells derived from the endoderm comprise cells of the respiratory system, the intestine, the liver, the gallbladder, the pancreas, the islets of Langerhans, the thyroid or the hindgut.

31. The method of claim 29, wherein cells derived from the mesoderm comprise osteochondroprogenitor cells, muscle cells, cells from the digestive systems, renal stem cells, cells from the reproductive system, bloods cells or cells from the circulatory system (such as endothelial cells).

32. The method of claim 29, wherein cells derived from the ectoderm, comprise epithelial cells, cells of the anterior pituitary, cells of the peripheral nervous system, cells of the neuroendocrine system, cell of the teethes, cell of the eyes, cells of the central nervous system, cells of the ependymal or cells of the pineal gland.

33. The method of claim 32, wherein cells from the central nervous system and the peripheral nervous system comprise neurons, Schwann cells, satellite glial cells, oligodendrocytes or astrocytes.

34. The method of claim 33, wherein neurons comprise interneurons, pyramidal neurons, gabaergic neurons, dopaminergic neurons, serotoninergic neurons, glutamatergic neurons, motor neurons from the spinal cord, or inhibitory spinal neurons.

35. The method of any one of claims 27 to 29, wherein the one or more cell-type is a cancer cell or a circulating tumor cell (CTC), such as cancer cell or CTC derived from any cell-types or cell subtypes as defined in claims 29 to 34.

36. The method of any one of claims 26 to 35, wherein the prey exosome biomarker comprises a surface biomarker.

37. The method of claim 36 wherein the prey exosome biomarker comprises a membrane protein.

38. The method of claim 36 or 37 wherein the prey biomarker is selected from the group comprising proteins as per Table D, column G; or proteins as per Table D, column H; or proteins as per Table D, column I; or proteins as per Table D, column J; or proteins as per Table D, column K; or proteins as per Table D, column L; or proteins as per Table D, column M; preferably the prey exosome biomarker is FLRT3 and/or L1CAM.

39. The method of any one of claims 26 to 38, wherein the bait molecule comprises a protein and preferentially an antibody, such as a monoclonal antibody or RNA aptamer.

40. The method of any one of claims 26 to 39, wherein the bait molecule is recognized by an affinity ligand.

41. The method of claim 40, wherein the affinity ligand comprises a protein, a peptide, a divalent metal-based complex or an antibody.

42. The method of claim of any one of claims 26 to 41, wherein the bait molecule or the affinity ligand is immobilized on a solid substrate.

43. The method of claim 42, wherein the solid substrate is selected from a purification column, a microfluidic channel or beads, such as magnetic beads.

44. The method of claim any one of claim 26 to 43, wherein the purification comprises a microfluidic affinity based purification, a magnetic based purification, a pull-down purification or a fluorescence activated sorting-based purification.

45. The method of any one of the preceding claims, wherein the biological sample comprises a body fluid or is derived from a body fluid, wherein the body fluid was obtained from a mammal.

46. The method of the preceding claim, wherein the body fluid is selected from amniotic fluid, aqueous humor, vitreous humor, bile, blood serum, breast milk, cerebrospinal fluid, cerumen (earwax), chyle, chyme, endolymph, perilymph, exudates, feces, female ejaculate, gastric acid, gastric juice, lymph, mucus (including nasal drainage and phlegm), pericardial fluid, peritoneal fluid, pleural fluid, pus, rheum, saliva, sebum (skin oil), semen, sputum, synovial fluid, sweat, tears, urine, vaginal secretion, vomit and mixtures of one or more thereof.

47. A method for the isolation of exosomes from a cell population, comprising steps of:
(1) providing isolated exosomes from a biological sample comprising exosomes from said cell population,
(2) performing on the isolated exosomes of step (1) one or more purification steps based on the affinity of a bait molecule for a prey exosome biomarker.

48. A method for the purification of exosomes from a cell population, comprising steps of:
(1) providing purified exosomes from a biological sample comprising exosomes from said cell population,
(2) performing on the purified exosomes of step (1) one or more purification steps based on the affinity of a bait molecule for a prey exosome biomarker.

49. The method of claim 47 or 48, wherein step (1) comprises the method for the isolation or the purification of exosomes from a biological sample as defined in claims 1 to 25.

50. The method of any one claims 47 to 49, wherein the cell population comprises one or more cell types, 2 or more cell types, 3 or more cell types, 4 or more cell types or 5 or more cell types.

51. The method of any one claims 47 to 50, wherein the method isolates or purifies cell type-specific exosomes, or cell-subtype-specific exosomes.

52. The method of any one of claims 47 to 51, wherein the one or more cell type comprises from cells derived from the endoderm, cells derived from the mesoderm, and cells derived from the ectoderm.

53. The method of claim 52, wherein cells derived from the endoderm comprise cells of the respiratory system, the intestine, the liver, the gallbladder, the pancreas, the islets of Langerhans, the thyroid or the hindgut.

54. The method of claim 52, wherein cells derived from the mesoderm comprise osteochondroprogenitor cells, muscle cells, cells from the digestive systems, renal stem cells, cells from the reproductive system, bloods cells or cells from the circulatory system (such as endothelial cells).

55. The method of claim 52, wherein cells derived from the ectoderm, comprise epithelial cells, cells of the anterior pituitary, cells of the peripheral nervous system, cells of the neuroendocrine system, cell of the teethes, cell of the eyes, cells of the central nervous system, cells of the ependymal or cells of the pineal gland.

56. The method of claim 55, wherein cells from the central nervous system and the peripheral nervous system comprises neurons, Schwann cells, satellite glial cells, oligodendrocytes or astrocytes.

57. The method of claim 56, wherein neurons comprise interneurons, pyramidal neurons, gabaergic neurons, dopaminergic neurons, serotoninergic neurons, glutamatergic neurons, motor neurons from the spinal cord, or inhibitory spinal neurons.

58. The method of any one of claims 50 to 52, wherein the cell-type is a cancer cell or a circulating tumor cell (CTC),such as a cancer cell or a CTC derived from any cell-types or cell subtypes as defined in claims 52 to 57.

59. The method of any one of claims 47 to 58, wherein the prey exosome biomarker comprises a surface biomarker.

60. The method of claim 59, wherein the prey exosome biomarker comprises a membrane protein.

61. The method of any of claim 59 or 60, wherein the prey biomarker is selected from the group comprising proteins as per Table D, column G; or proteins as per Table D, column H; or proteins as per Table D, column I; or proteins as per Table D, column J; or proteins as per Table D, column K; or proteins as per Table D, column L; or proteins as per Table D, column M; preferably the prey exosome biomarker is FLRT3 and/or L1CAM.

62. The method of any one of claims 47 to 61, wherein the bait molecule comprises a protein and more preferentially an antibody, such as a monoclonal antibody or RNA aptamer.

63. The method of any one of claims 47 to 62, wherein the bait molecule is recognized by an affinity ligand.

64. The method of claim 63, wherein the affinity ligand comprises a protein, a peptide, a divalent metal-based complex or an antibody.

65. The method of any one of claims 47 to 64, wherein the bait molecule or the affinity ligand is immobilized on a solid substrate.

66. The method of claim 65 wherein the solid substrate is selected from a purification column, a microfluidic channel or beads, such as magnetic beads.

67. The method of any one of claims 47 to 66, wherein the one or more purification steps comprises a microfluidic affinity based purification, a magnetic based purification, a pull-down purification or a fluorescence activated sorting-based purification.

68. A method for the preparation of exosomal RNA from a biological sample, said method comprising:
(i) providing a biological sample comprising exosomes from a cell population,
(ii) preparing purified exosomes from the biological sample of step (i),
(iii) extracting RNA from the purified exosomes of step (ii).

69. The method of claim 68, wherein step (ii) comprises the method of any one of claims 1-46.

70. The method of any one of claim 68 or 69, wherein the purified exosomes prepared at step (ii) are exosomes from a single cell type or from a single cell-subtype.

71. A method for the preparation of exosomal RNA of a cell population, comprising steps of
(1) providing purified exosomes from a biological sample comprising exosomes from said cell population,
(2) performing on the purified exosomes of step (1) one or more purification steps based on the affinity of a bait molecule for a prey exosome biomarker, and
(3) extracting RNA from the purified exosomes of step (2).

72. The method of claim 71 wherein step (1) comprises the method of any one of claims 1-25.

73. The method of any one of claim 71 or 72 wherein step (2) is performed as defined in any one of claims 26 to 46 or as defined in any one of claims 47 to 67..

74. The method of any one of claims 71to 73, wherein the exosomal RNA is total exosomal RNA.

75. The method of any one of claims 71 to 74, wherein the exosomal RNA comprises exosomal messenger RNA.

76. The method of any one of claims 71-75, wherein the exosomal RNA is total exosomal messenger RNA.

77. The method of any one of claims 71 to 76 wherein the exosomal RNA is exosomal RNA from single cell type exosomes or single cell subtype exosomes.

78. Use of a proteinase in the purification of exosomes from a biological sample.

79. Use of a proteinase and of an RNase in the purification of exosomes from a biological sample.

80. Use of a proteinase in the purification of an ultracentrifugated exosome-containing sample.

81. Use of a proteinase and of an RNase in the purification of an ultracentrifugated exosome-containing sample.

82. Use according to any one of claims 78-81, wherein the proteinase is proteinase K.

83. Use according to any one of claims 78-82, wherein the ultracentrifugated exosome-containing sample is a washed ultracentrifugated exosome-containing sample.

84. Use according to any one of claims 78-83, wherein the ultracentrifugated exosome-containing sample is a washed ultracentrifugated exosome-containing sample.

85. The method or use of any one of the preceding claims, wherein the biological sample is a bodily fluid or is derived from a bodily fluid, wherein the bodily fluid was obtained from a mammal.

86. The method or use of the preceding claim, wherein the bodily fluid is selected from amniotic fluid, aqueous humour, vitreous humour, bile, blood serum, breast milk, cerebrospinal fluid, cerumen (earwax), chyle, chyme, endolymph, perilymph, exudates, feces, female ejaculate, gastric acid, gastric juice, lymph, mucus (including nasal drainage and phlegm), pericardial fluid, peritoneal fluid, pleural fluid, pus, rheum, saliva, sebum (skin oil), semen, sputum, synovial fluid, sweat, tears, urine, vaginal secretion, vomit and mixtures of one or more thereof.

87. The method or use of any one of the preceding claims, wherein the cell population is a population of cells of the same cell type.

88. The method or use of any one of the preceding claims, wherein the cell population is a population of cells of different cell types.

89. The method or use of any one of the preceding claims, wherein the cell population comprises one or more cell types, 2 or more cell types, 3 or more cell types, 4 or more cell types, or 5 or more cell types.

90. The method or use of any one of the preceding claims, wherein the biological sample comprises cultured cells.

91. The method or use of any one of the preceding claims, wherein the biological sample comprises cells cultured *in vitro*.

92. The method or use of any one of the preceding claims, wherein the biological sample comprises cells cultured *ex vivo*.

93. The method or use of any one of the preceding claims, wherein the biological sample is a sample obtained by liquid biopsy.

94. The method or use of any one of the preceding claims, wherein the biological sample comprises a cell type selected from cells types present in amniotic fluid, aqueous humour, vitreous humour, bile, blood serum, breast milk, cerebrospinal fluid, cerumen (earwax), chyle, chyme, endolymph, perilymph, exudates, feces, female ejaculate, gastric acid, gastric juice, lymph, mucus (including nasal drainage and phlegm), pericardial fluid, peritoneal fluid, pleural fluid, pus, rheum, saliva, sebum (skin oil), semen, sputum, synovial fluid, sweat, tears, urine, vaginal secretion, vomit.

95. An exosome preparation obtainable with the method or the use of any one of the preceding claims.

96. A composition comprising exosomes, wherein the composition is essentially free of extra-exosomal material.

97. A composition comprising exosomes, wherein the composition is essentially free of extra-exosomal nucleic acid-protein complexes.

98. A composition comprising exosomes, wherein the composition is essentially free of extra-exosomal RNA-protein complexes.

99. A composition comprising cell type specific exosomes or cell subtype specific exosomes.

100. The composition of claim 99, wherein the exosomes are specific for one or more cell types or cell subtypes.

101. The composition of claim 100 comprising purified exosomes, wherein said purified exosomes are exosomes from a single cell-type or of a single cell subtype.

102. A method for the determination of cellular RNA content in a cell population, said method comprising:

(a) providing a biological sample comprising exosomes from said cell population,

(b) preparing purified exosomes from the sample of step (a),

(c) extracting RNA from the purified exosomes of step (b), so as to provide exosomal RNA,

(d) analyzing the exosomal RNA extracted at step (c),

(e) estimating, as a function of the result from step (d), the cellular RNA content in the cell population.

103. The method of the preceding claim, wherein step (b) comprises the method for the purification of exosomes as disclosed in any of claims 1 to 46.

104. The method of claim 102 or 103, wherein step (B) comprises the method for the purification of exosomes from a cell population as disclosed in any of claims 48 to 67.

105. A method for the determination of cellular RNA content of a cell population, said method comprising:

(a) providing a biological sample comprising exosomes from said cell population;

(b) preparing purified exosomes from the sample of step (a);

(d) extracting RNA from the purified exosomes of step (b), so as to provide exosomal RNA;

(d) analyzing the exosomal RNA extracted at step (c);

(e) estimating, as a function of the result from step (d), the cellular RNA content in the cell population

wherein step (b) further comprises performing on the purified exosomes one or more purification steps based on the affinity of a bait molecule for a prey exosome biomarker.

106. The method of claim 105, wherein step (b) comprises the method for the isolation or the purification of exosomes from a biological sample as defined in claims 1 to 25.

107. The method of claims 105 or 106, wherein the cell population comprises one or more cell types, 2 or more cell types, 3 or more cell types, 4 or more cell types, 5 or more cell types.

108. The method of any one claims 105 to 107, wherein the method isolates or purifies cell type-specific exosomes, or cell subtype-specific exosomes.

109. The method of any one of claims 105 to 108, wherein the cell type comprises cells derived from the endoderm, cells derived from the mesoderm or cells derived from the ectoderm.

110. The method of claim 109, wherein cells derived from the endoderm comprise cells of the respiratory system, the intestine, the liver, the gallbladder, the pancreas, the islets of Langerhans, the thyroid or the hindgut.

111. The method of claim 109, wherein cells derived from the mesoderm comprise osteochondroprogenitor cells, muscle cells, cells from the digestive systems, renal stem cells, cells from the reproductive system, bloods cells or cells from the circulatory system (such as endothelial cells).

112. The method of claim 109, wherein cells derived from the ectoderm, comprise epithelial cells, cells of the anterior pituitary, cells of the peripheral nervous system, cells of the neuroendocrine system, cell of the teethes, cell of the eyes, cells of the central nervous system, cells of the ependymal or cells of the pineal gland.

113. The method of claim 112, wherein cells from the central nervous system and the peripheral nervous system comprises neurons, Schwann cells, satellite glial cells, oligodendrocytes or astrocytes.

114. The method of claim 113, wherein neurons comprise interneurons, pyramidal neurons, gabaergic neurons, dopaminergic neurons, serotoninergic neurons, glutamatergic neurons, motor neurons from the spinal cord, or inhibitory spinal neurons.

115. The method of any one of claims 107 to 109, wherein the cell-type is a cancer cell or a circulating tumor cell (CTC), such as a cancer cell or a CTC derived from any cell-types or cell subtypes as defined in claims 107 to 114.

116. The method of any one of claims 105 to 115, wherein the prey exosome biomarker comprises a surface biomarker.

117. The method of claim 116, wherein the prey exosome biomarker comprises a membrane protein.

118. The method of claim 116 or 117, wherein the prey exosome biomarker is selected from the group comprising proteins as per Table D, column G; or proteins as per Table D, column H; or proteins as per Table D, column I; or proteins as per Table D, column J; or proteins as per Table D, column K; or proteins as per Table D, column L; or proteins as per Table D, column M; preferably the prey exosome biomarker is FLRT3 and/or L1CAM.

119. The method of any one of claims 105 to 118, wherein the bait molecule comprises a protein and more preferentially an antibody, such as a monoclonal antibody or RNA aptamer.

120. The method of any one of claims 105 to 119, wherein the bait molecule is recognized by an affinity ligand.

121. The method of claim 120, wherein the affinity ligand comprises a protein, a peptide, a divalent metal-based complex or an antibody.

122. The method of claim 120 or 121, wherein the bait molecule or the affinity ligand is immobilized on a solid substrate.

123. The method of claim 122, wherein the solid substrate is selected from a purification column, a microfluidic channel or beads such as magnetic beads.

124. The method of any one of claims 105 to 123, wherein the purification is comprises a microfluidic affinity based purification, a magnetic based purification, a pull-down purification or a fluorescence activated sorting-based purification.

125. The method of any one of claims 102-124, wherein step (e) is performed based on a predicted correlation between exosomal RNA content and cellular RNA content.

126. The method of any one of claims 102-125, wherein said determination comprises a qualitative determination.

127. The method of any one of claims 102-126, wherein said determination comprises a quantitative determination.

128. The method of any one of claims 102-127, wherein said quantitative determination comprises determination of relative abundance of two RNAs.

129. The method of any one of claims 102-128, wherein said determination comprises determination of mRNA profiles.

130. The method of any one of claims 102-129, wherein said RNA comprises messenger RNA (mRNA).

131. The method of any one of claims 102-160, wherein said RNA comprises micro RNA (miRNA).

132. The method of any one of claims 102-131, wherein said RNA comprises long non-coding RNA (lncRNA).

133. The method of any one of claims 102-132, wherein step (D) comprises a qualitative determination.

134. The method of any one of claims 102-133, wherein step (D) comprises a quantitative determination.

135. The method of any one of claims 102-134, wherein step (D) comprises RNA sequencing (RNA seq).

136. The method of any one of claims 102-135, wherein step (D) comprises array analysis.

137. The method of any one of claims 102-136, wherein step (D) comprises reverse transcription polymerase chain reaction (RT-PCR).

138. The method of claim 137, wherein step (d) comprises quantitative reverse transcription polymerase chain reaction (qRT-PCR).

139. The method of any one of claims 102-138, wherein step (d) comprises analyzing one or more sequence/s of interest.

140. The method of claim 139, comprising testing for the presence or absence of said sequence/s of interest.

141. The method of claim 140, wherein step (d) comprises analyzing for one or more allelic variants of a sequence of interest.

142. The method according to claims 102-141, wherein step (d) comprises testing for presence or absence of said allelic variants.

143. The method of any one of claims 102-142, wherein step (d) comprises genome-wide analysis.

144. The method of any one of claims 102-143, wherein step (d) comprises transcriptome profiling.

145. The method of any one of claims 102-144, wherein the determination is time-lapse.

146. The method of any one of claims 102-145, wherein the cell population is a population of cells of the same cell type.

147. The method of any one of claims 102-146, wherein the cell population is a population of cells of different cell types.

148. The method of any one of claims 102-147, wherein the biological sample comprises cultured cells.

149. The method of any one of claims 102-148, wherein the biological sample comprises cells cultured *in vitro*.

150. The method of any one of claims 102-149, wherein the biological sample comprises cells cultured *ex vivo*.

151. The method of any one of claims 102-150, wherein the biological sample is a sample obtained by liquid biopsy.

152. The method of any one of claims 102-151, wherein the biological sample comprises a cell type selected from blood, epithelia, muscle and neural cell types.

153. The method of any of claims 102-152, wherein the biological sample is obtained from a body fluid selected from amniotic fluid, aqueous humor, vitreous humor, bile, blood serum, breast milk, cerebrospinal fluid, cerumen (earwax), chyle, chyme, endolymph, perilymph, exudates, feces, female ejaculate, gastric acid, gastric juice, lymph, mucus (including nasal drainage and phlegm), pericardial fluid, peritoneal fluid, pleural fluid, pus, rheum, saliva, sebum (skin oil), semen, sputum, synovial fluid, sweat, tears, urine, vaginal secretion, vomit and mixtures of one or more thereof.

154. The method of any one of claims 102-153, wherein the cell population of step (a) is isolated as a subpart of a larger initial cell population.

155. The method of any one of claims 102-154, wherein the cell population of step (a) is obtained from a body fluid and isolated by immuno-magnetic separation.

156. The method of any one of any one of claims 102-155, for use in diagnosis.

157. The method of any one of claims 102-156, for use in prognosis.

158. The method of any one of claims 102-157, for use in identifying markers.

159. The method of any one of claims 102-158, for use in a screening process.

160. The method of any one of claims 102-159, wherein the method determines the cellular RNA content of a single cell type or of a single cell subtype.

161. A method for the diagnostic or prognostic of a disorder of interest in a subject, comprising:
(I) selecting a marker, wherein said marker is associated with said disorder and wherein said marker may be determined in a cell type that is found in the subject to be in contact with a body fluid,
(II) providing a biological sample from said body fluid from said subject,
(III) estimating the cellular RNA content of said marker in the biological sample of step (II) by performing the method of any one of claims 102-155.

162. The method of claim 161, wherein the cellular RNA content is the cellular content of a single cell type or of a single cell subtype.

163. The method of claim 161 or 162, further comprising (IV) determining, from the results of step (III), the status of the marker selected at step (I).

164. The method of any one of claims 160-163, wherein the marker is selected from expression of a given open reading frame (ORF), overexpression of a given open reading frame (ORF), repression of a given open reading frame (ORF), over-repression of a given open reading

frame (ORF), expression of a given allelic variant, relative level of expression of a given open reading frame (ORF), presence of a mutation in a given open reading frame (ORF),

165. The method of any one of claims 161-164, wherein said disorder is a blood disorder and said marker is a marker that may be determined in one or more cell type/s that is/are found in the subject to be in contact with blood.

166. The method of any one of claims 161-165, wherein said disorder is a brain or spine disorder and said marker is a marker that may be determined in one or more cell type/s that is/are found in the subject to be in contact with cerebrospinal fluid.

167. The method of any one of claims 161-166, wherein said disorder is a heart disorder and said marker is a marker that may be determined in one or more cell type/s that is/are found in the subject to be in contact with blood or pericardial fluid.

168. The method of any one of claims 161-167, wherein said disorder is a prostate or bladder disorder and said marker is a marker that may be determined in one or more cell type/s that is/are found in the subject to be in contact with urine.

169. The method of any one of claims 161-168, wherein said disorder is an eye disorder and said marker is a marker that may be determined in one or more cell type/s that is/are found in the subject to be in contact with tears.

170. The method of any one of claims 161-169, wherein said disorder is a lung disorder and said marker is a marker that may be determined in one or more cell type/s that is/are found in the subject to be in contact with pleural fluid.

171. Composition comprising exosomes, wherein the composition is essentially free of extra-exosomal material, for use in diagnostics.

172. Composition comprising exosomes, wherein the composition is essentially free of extra-exosomal nucleic acid-protein complexes.

173. Composition comprising exosomes, wherein the composition is essentially free of extra-exosomal RNA-protein complexes.

174. A method for the treatment or prophylaxis of a disorder in a patient, said method comprising exosome-mediated delivery of a therapeutic RNA to a cell.

175. The method of claim 174, wherein said exosome-mediated delivery occurs from one donor cell to a recipient cell, and wherein the therapeutic RNA results from transcription in the donor cell.

176. The method of claim 175, wherein transcription in the donor cell is inducible.

177. The method of any one of claims 174-176, wherein the delivery is performed *ex vivo*.

178. The method of any one of claims 174-176, wherein the delivery is performed *in vivo*.

179. Exosome for use in delivering a therapeutic RNA to a cell.

180. Exosome of claim 179, wherein the exosome is produced according to the method or the use as defined in any one of claims 1-70 and 78-94.

181. Exosome of claim 179, wherein the exosome is in a preparation obtainable according to claim 95.

182. Exosome of claim 179, wherein the exosome is produced *in vitro*

183. Exosome of claim 179, wherein the exosome is produced *in vivo*.

184. Therapeutic RNA for use in exosome-mediated delivery to a cell.

185. Therapeutic RNA of claim 184, wherein the exosome is produced *in vitro*

186. Therapeutic RNA of claim 184, wherein the exosome is produced *in vivo*.

187. Therapeutic RNA of claim 184, wherein the exosome is produced according to the method or the use as defined in any one of claims 1-70 and 78-94.

188. Therapeutic RNA of claim 184, wherein the exosome is in a preparation obtainable according to claim 95.

189. Pharmaceutical composition comprising an exosome, wherein said exosome comprises a therapeutic RNA for delivery into a cell.

190. The pharmaceutical composition of claim 189, wherein the delivery is performed *ex vivo*.

191. The pharmaceutical composition of claim 189, wherein the delivery is performed *in vivo*.

192. Pharmaceutical composition comprising a cell, wherein the cell is capable of producing exosomes comprising a therapeutic RNA.

193. Pharmaceutical composition of any one of claims any one of claims 189-192, in a form suitable for injection.

194. Use of a therapeutic RNA in the manufacture of a medicament for the treatment or prophylaxis of a disorder in a patient, wherein the RNA is delivered to a cell in an exosome-packaged form.

195. Use of an exosome in the manufacture of a medicament for the treatment or prophylaxis of a disorder in a patient, wherein the exosome comprises a therapeutic RNA or delivery into a cell.

196. The method, composition or use of any one of claims 174-195, wherein the therapeutic RNA is translated in the recipient cell.

197. The method, composition or use of any one of claims 174-195, wherein the therapeutic RNA is a small interfering RNA (siRNA).

198. The method, composition or use of any one of claims 174-195, wherein the therapeutic RNA is a short hairpin RNA (shRNA).

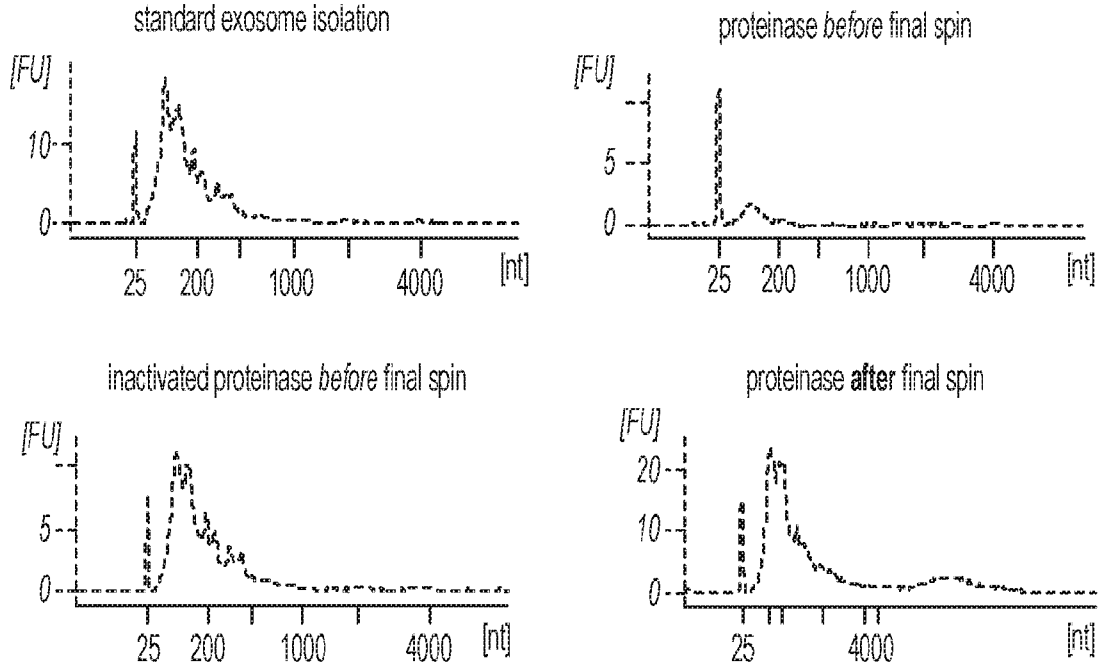

standard exosome isolation
[FU]
10
0
25
200
1000
4000
[nt]
proteinase *before* final spin
[FU]
5
0
25
200
1000
4000
[nt]
inactivated proteinase *before* final spin
[FU]
5
0
25
200
1000
4000
[nt]
proteinase **after** final spin
[FU]
20
10
0
25
4000
[nt]

FIG. 1

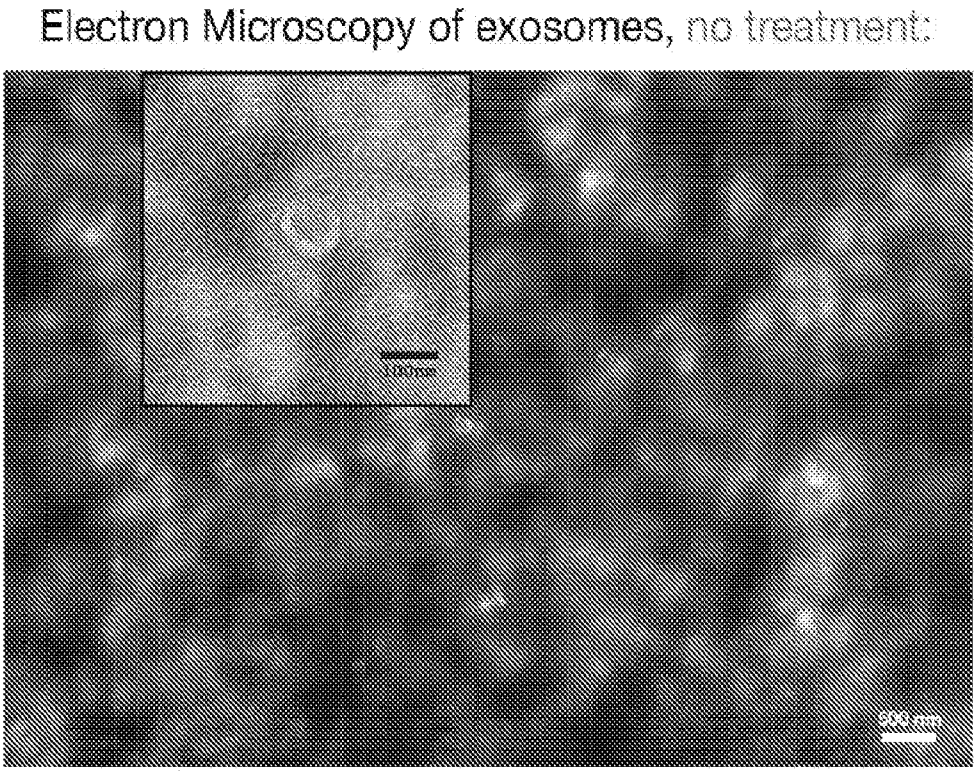

Electron Microscopy of exosomes, no treatment:

FIG. 2A

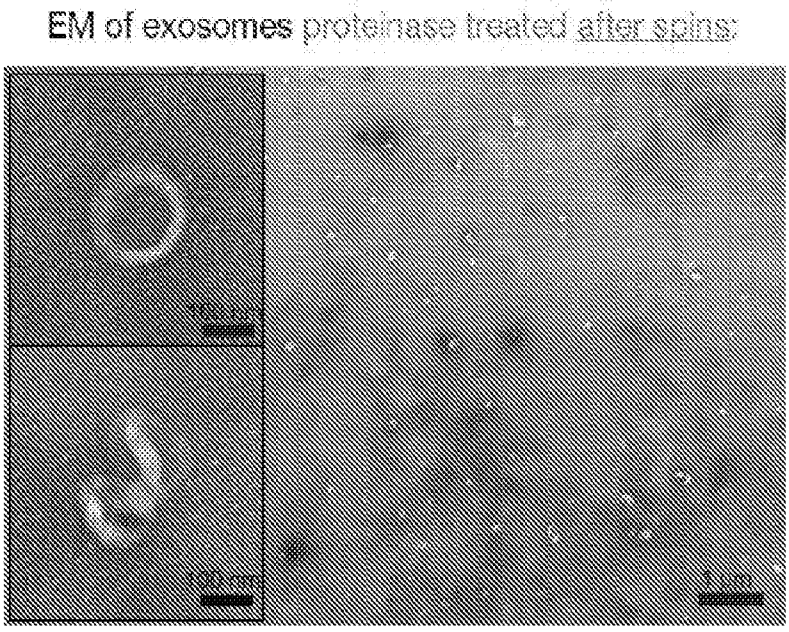

EM of exosomes proteinase treated after spins:

FIG. 2B

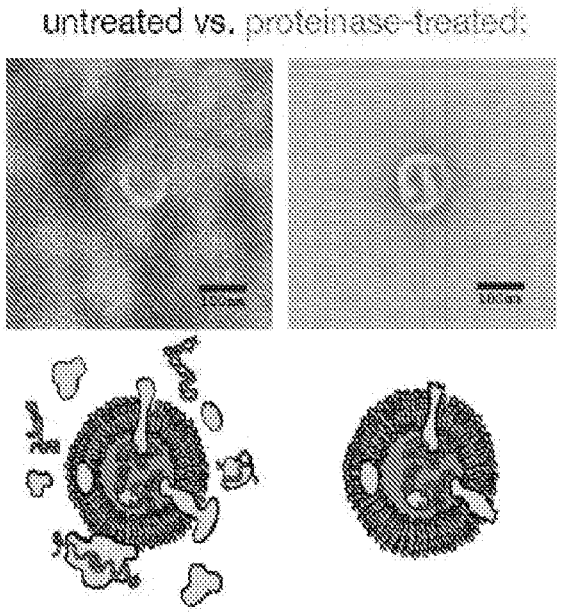

untreated vs. proteinase-treated:

FIG. 2C

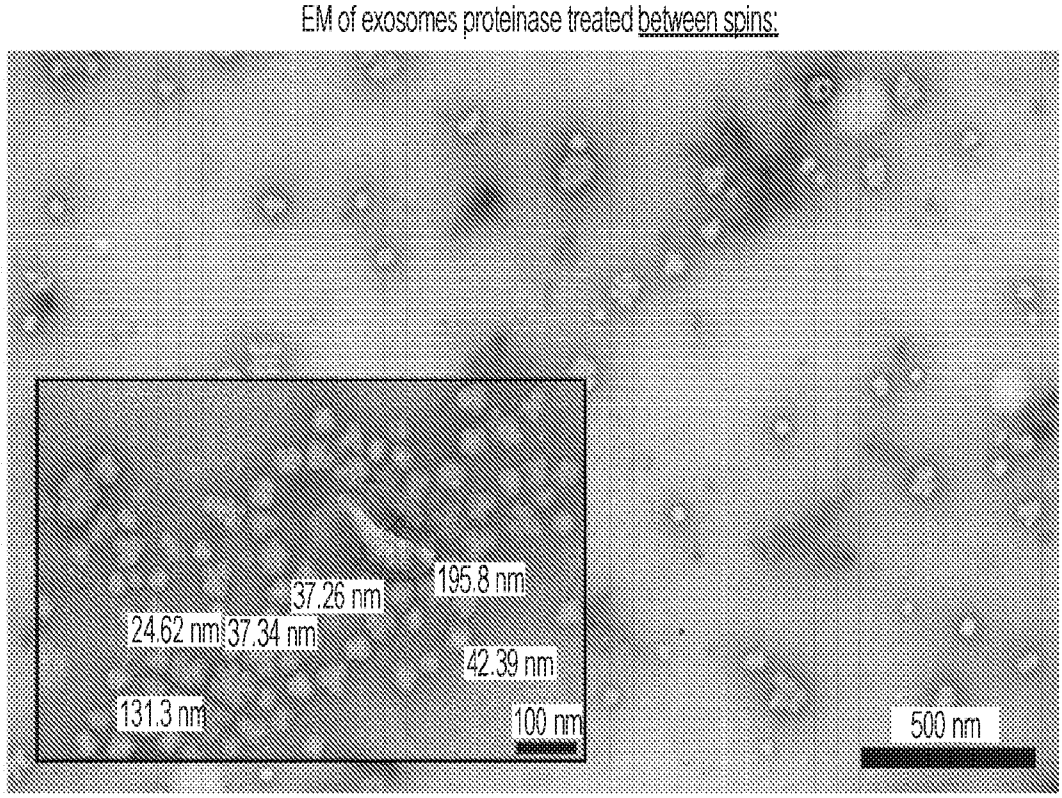

EM of exosomes proteinase treated between spins:
37.26 nm
195.8 nm
24.62 nm
37.34 nm
42.39 nm
131.3 nm
100 nm
500 nm

FIG. 2D

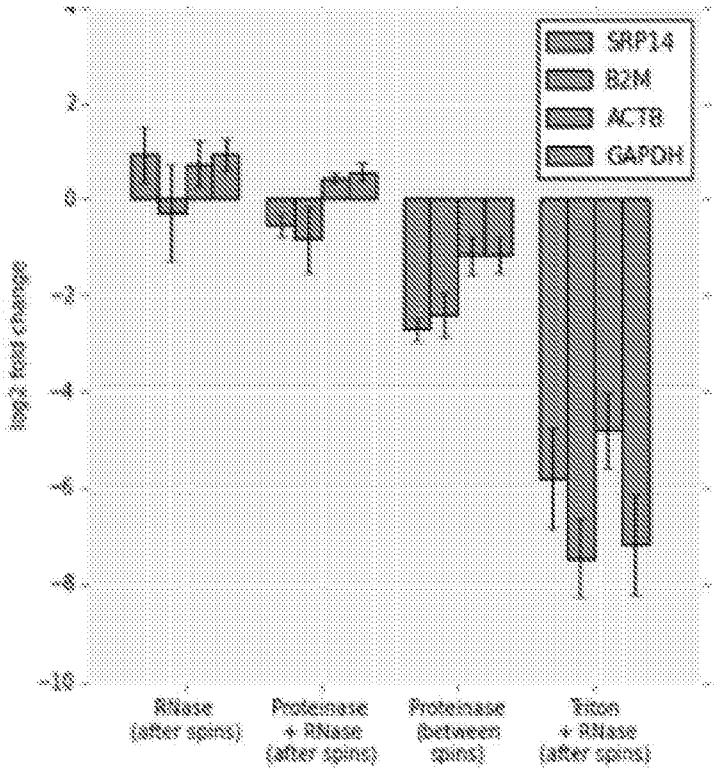

SRP14
B2M
ACTB
GAPDH
log2 fold change
RNase (after spins)
Proteinase + RNase (after spins)
Proteinase (between spins)
Triton + RNase (after spins)

FIG. 3

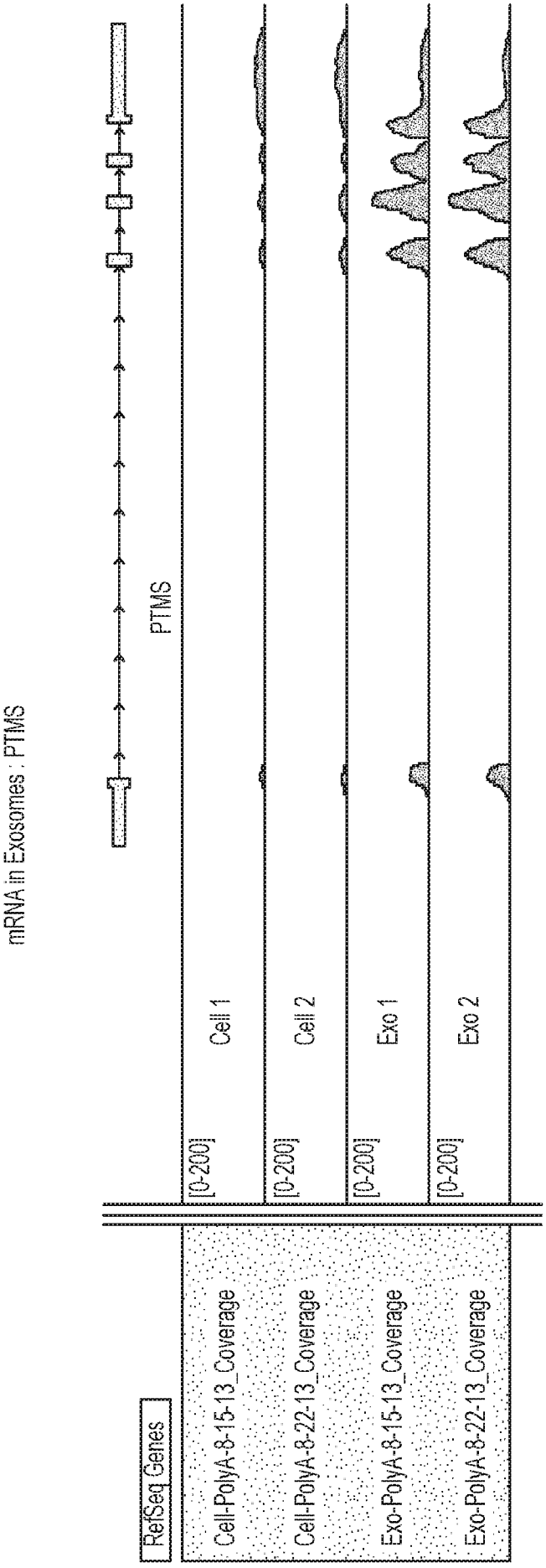

mRNA in Exosomes : PTMS
PTMS
RefSeq Genes
Cell-PolyA-8-15-13_Coverage
Cell-PolyA-8-22-13_Coverage
Exo-PolyA-8-15-13_Coverage
Exo-PolyA-8-22-13_Coverage
[0-200]
Cell 1
Cell 2
Exo 1
Exo 2

FIG. 4A

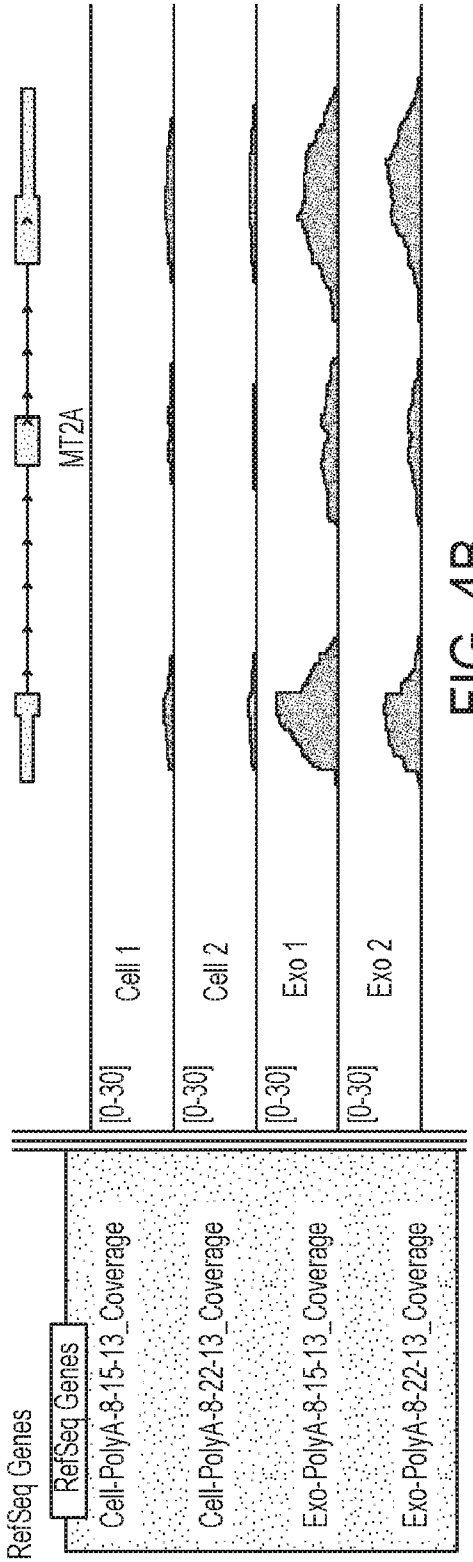

mRNA in Exosomes : MT2A
MT2A
RefSeq Genes
RefSeq Genes
Cell-PolyA-8-15-13_Coverage
Cell-PolyA-8-22-13_Coverage
Exo-PolyA-8-15-13_Coverage
Exo-PolyA-8-22-13_Coverage
[0-30]
Cell 1
Cell 2
Exo 1
Exo 2

FIG. 4B

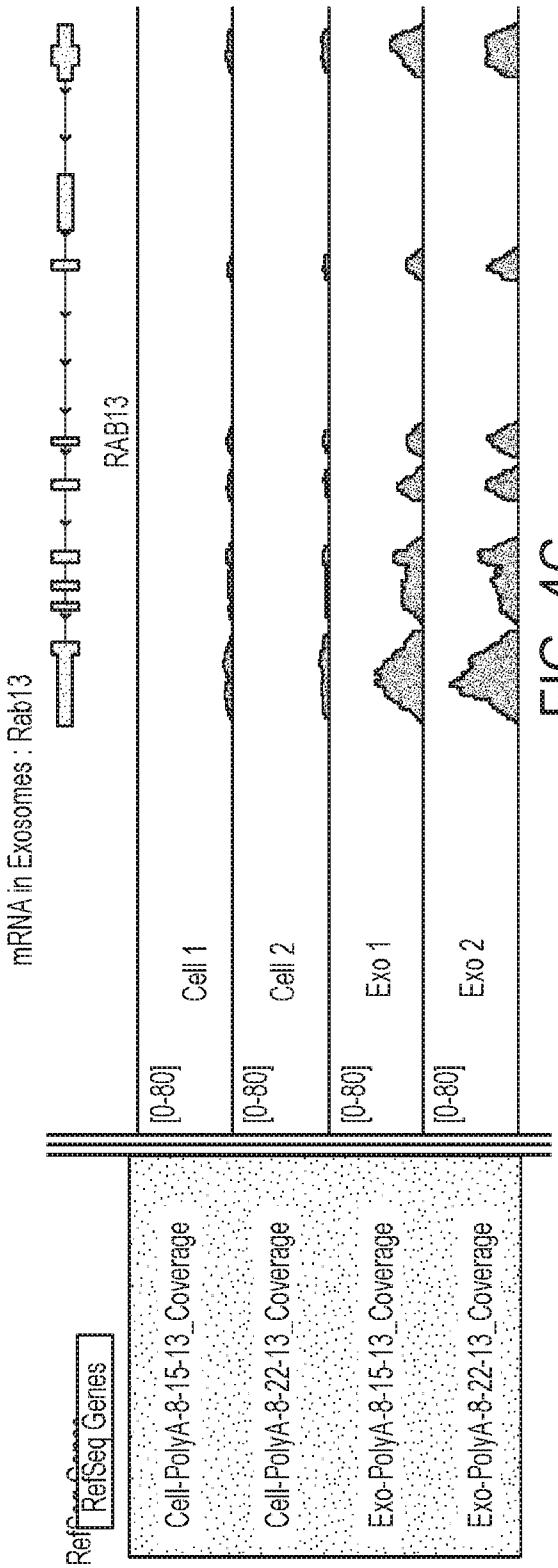

mRNA in Exosomes : Rab13
RAB13
RefSeq Genes
Cell-PolyA-8-15-13_Coverage
Cell-PolyA-8-22-13_Coverage
Exo-PolyA-8-15-13_Coverage
Exo-PolyA-8-22-13_Coverage
[0-80]
Cell 1
Cell 2
Exo 1
Exo 2

FIG. 4C

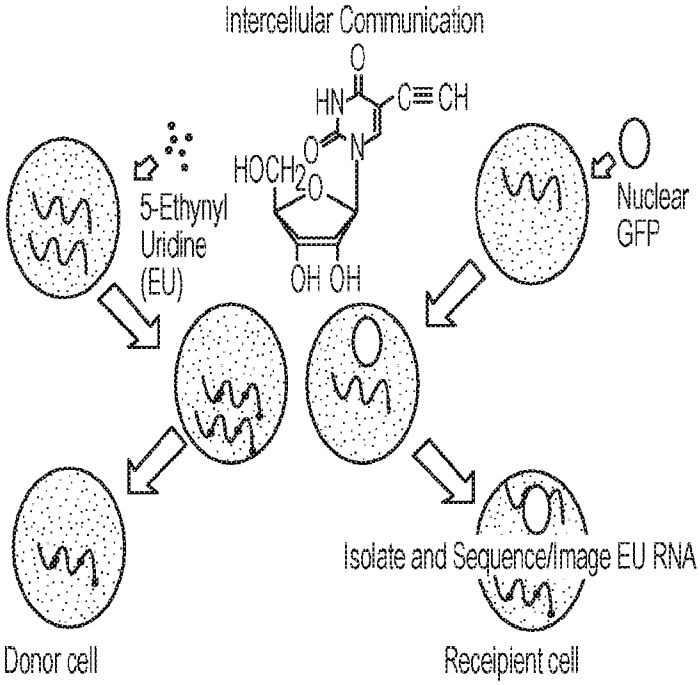

Intercellular Communication
5-Ethynyl Uridine (EU)
Nuclear GFP
Isolate and Sequence/Image EU RNA
Donor cell
Receipient cell

FIG. 5A

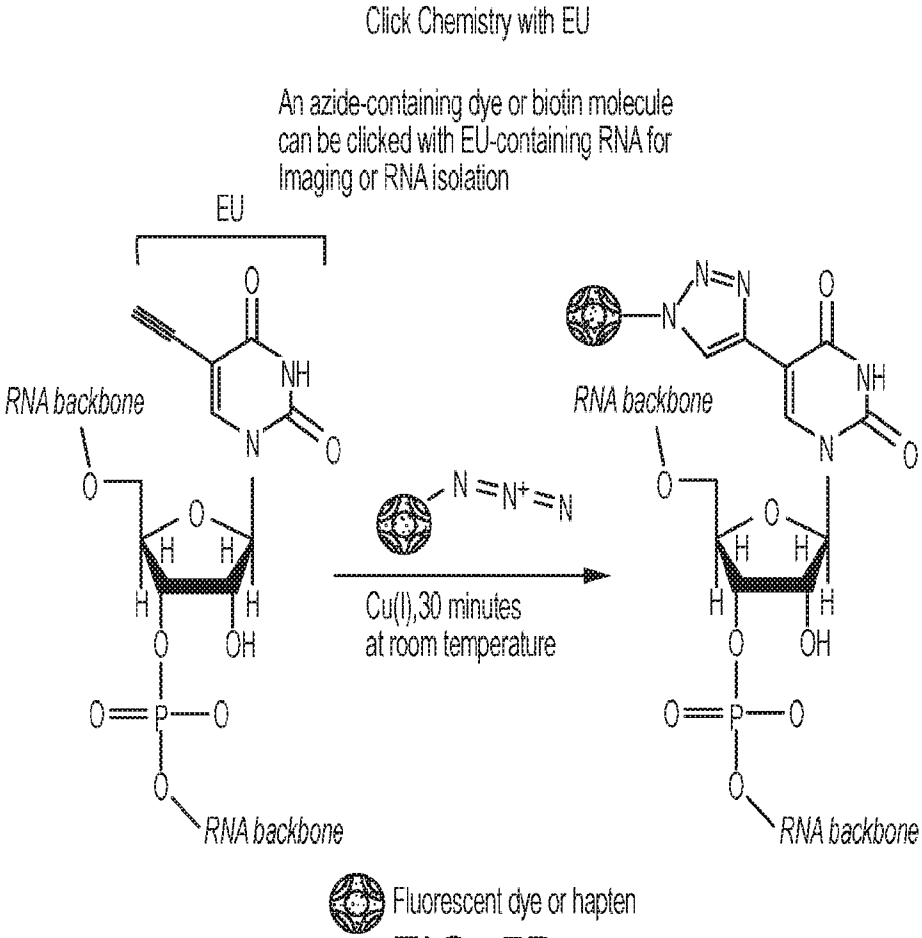

Click Chemistry with EU
An azide-containing dye or biotin molecule can be clicked with EU-containing RNA for Imaging or RNA isolation
EU
RNA backbone
Cu(I),30 minutes at room temperature
RNA backbone
RNA backbone
Fluorescent dye or hapten

FIG. 5B

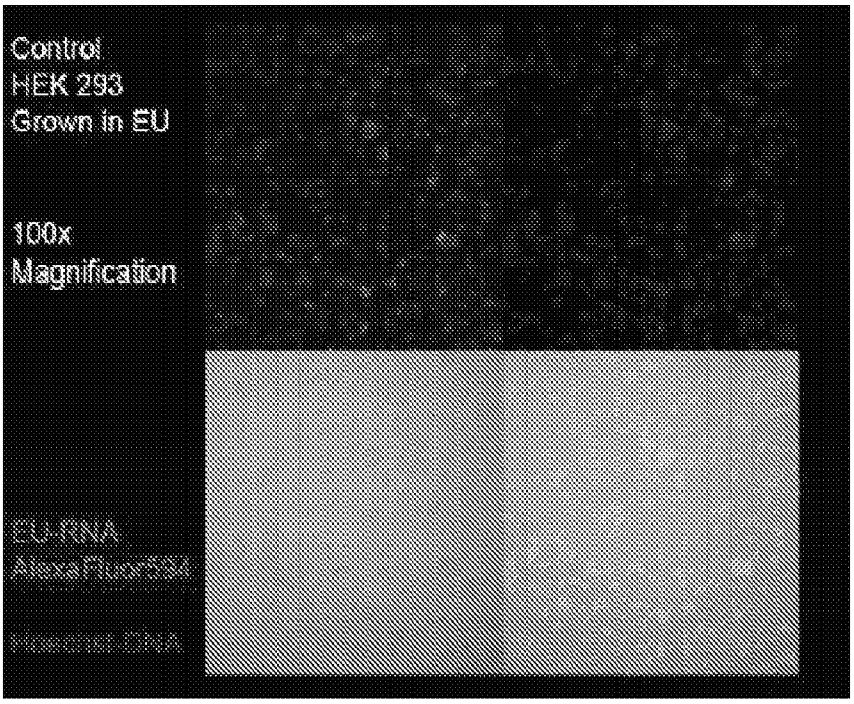

Control
HEK 293
Grown in EU
100x
Magnification

FIG. 5C


Negative
Control:
HEK 293
No EU
100x
magnification

FIG. 5D

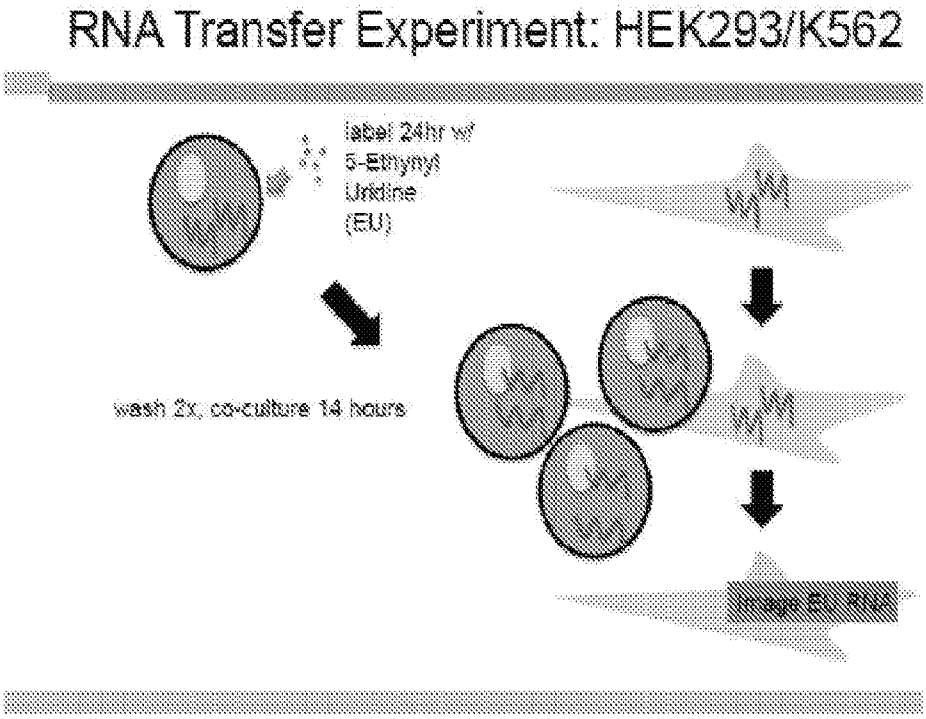

RNA Transfer Experiment: HEK293/K562
label 24hr w/
5-Ethynyl
Uridine
(EU)
wash 2x, co-culture 14 hours

FIG. 5E

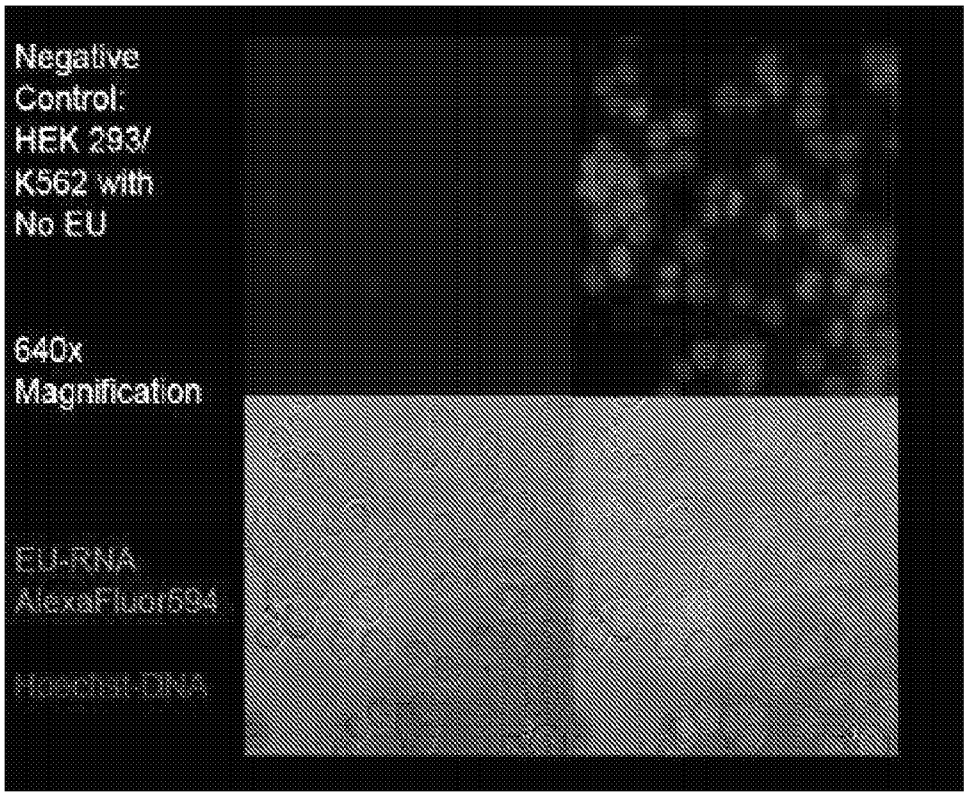

Negative
Control:
HEK 293/
K562 with
No EU
640x
Magnification

FIG. 5F

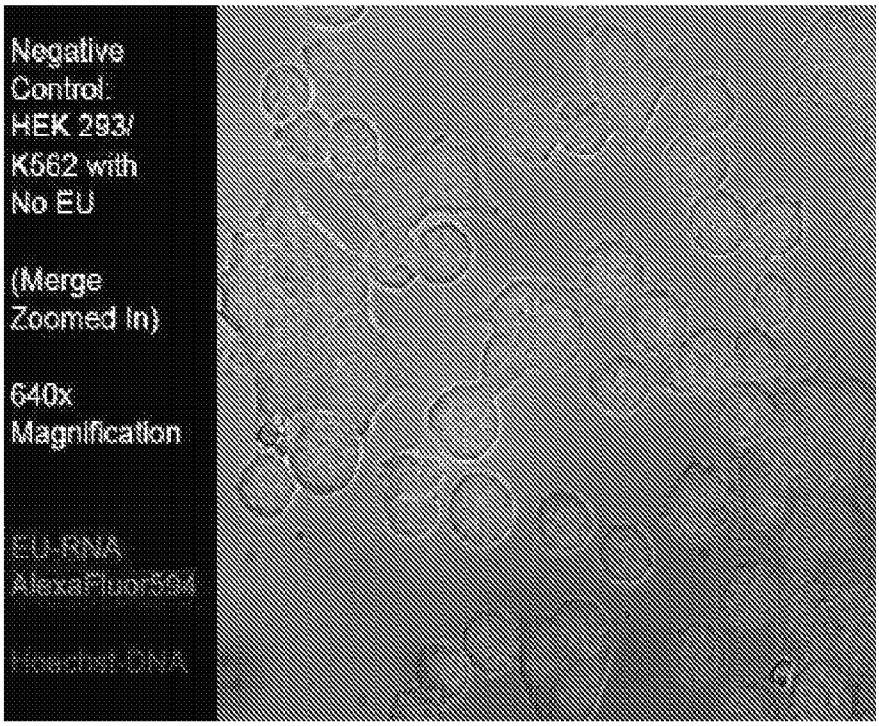

Negative Control: HEK 293/ K562 with No EU
(Merge Zoomed In)
640x Magnification
EU-RNA: AlexaFluor®488
Nuclear DNA

FIG. 5G

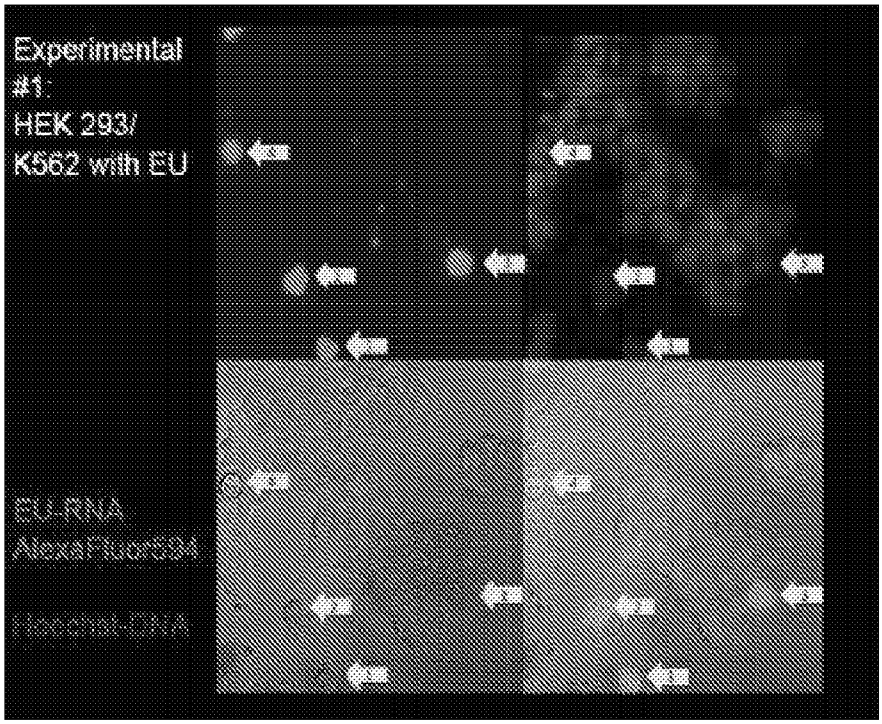

Experimental #1: HEK 293/ K562 with EU
EU-RNA: AlexaFluor®488
Nuclear DNA

FIG. 5H

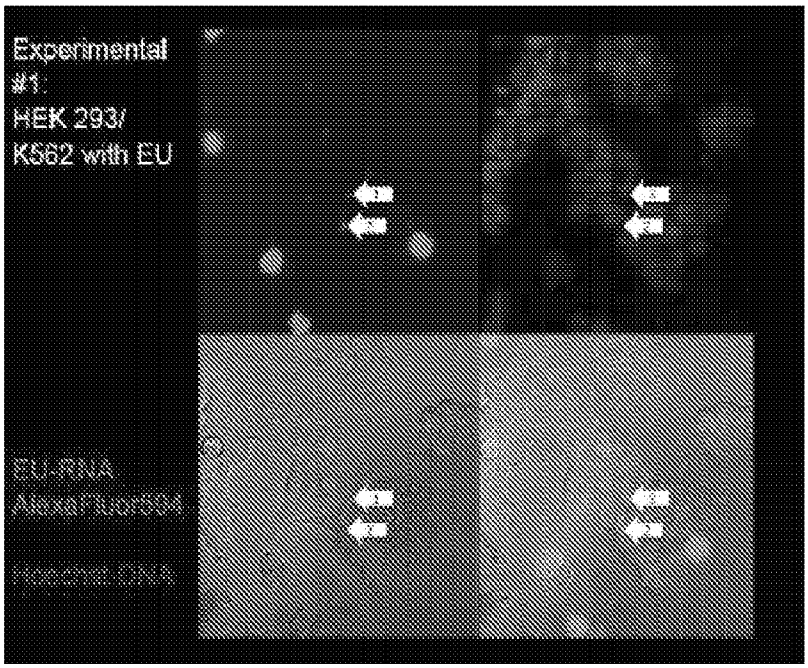

Experimental
#1:
HEK 293/
K562 with EU

FIG. 5I

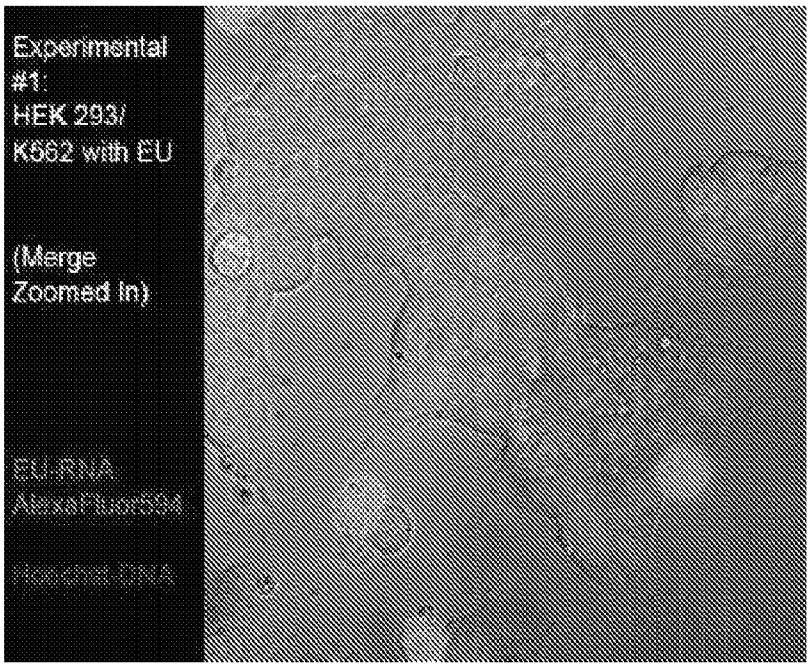

Experimental
#1:
HEK 293/
K562 with EU
(Merge
Zoomed In)

FIG. 5J

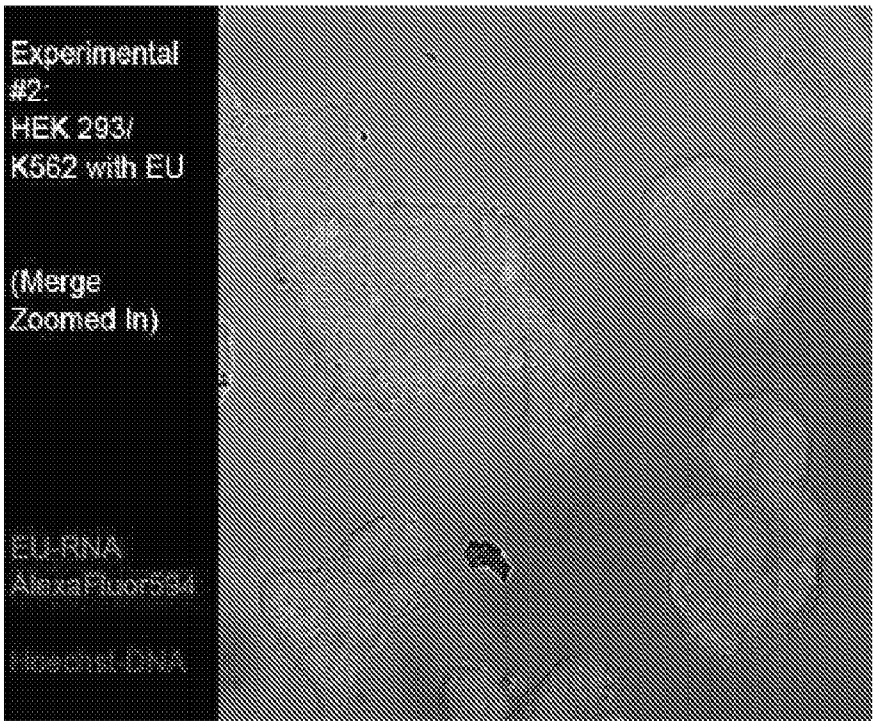

Experimental #2:
HEK 293/
K562 with EU
(Merge
Zoomed In)

FIG. 5K

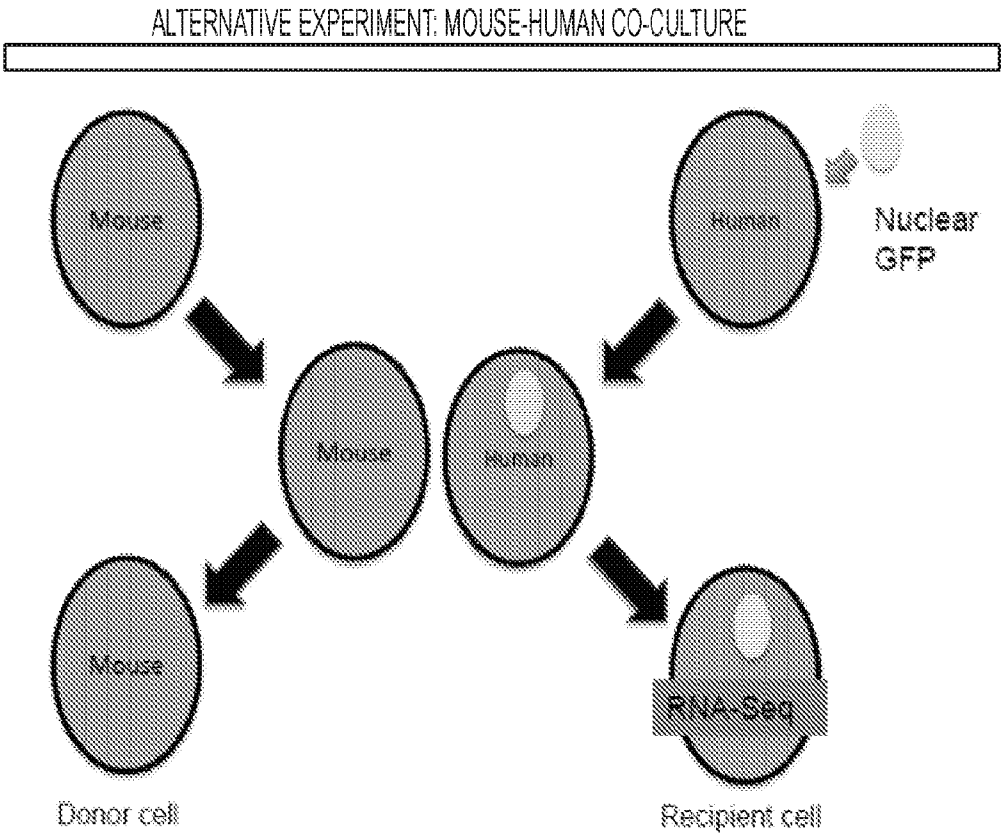

ALTERNATIVE EXPERIMENT: MOUSE-HUMAN CO-CULTURE
Mouse
Human
Nuclear GFP
Mouse
Human
Mouse
RNA-Seq
Donor cell
Recipient cell

FIG. 6A

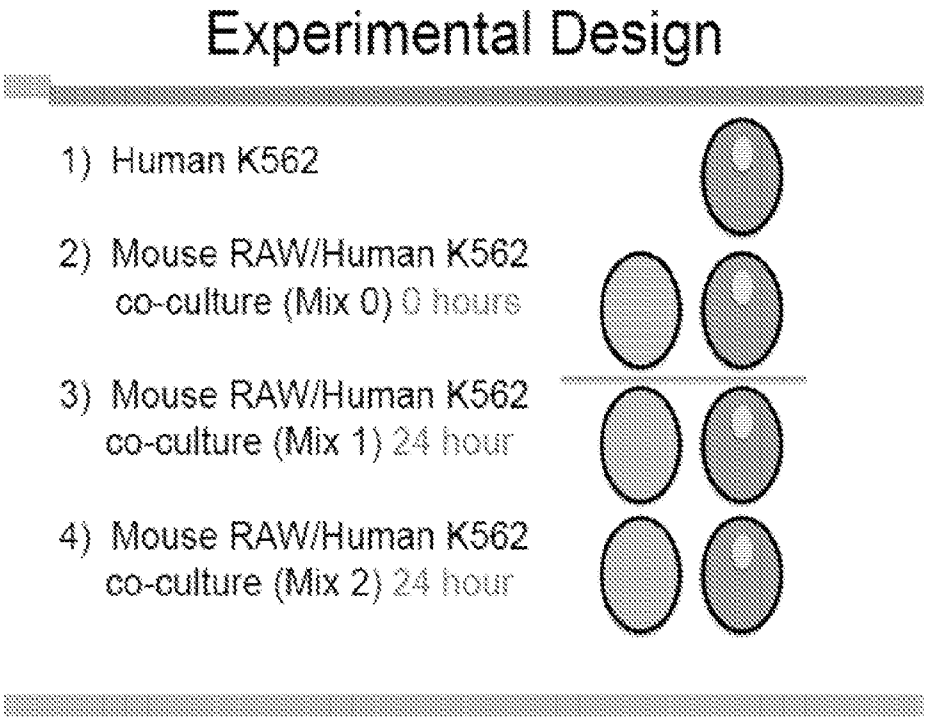

Experimental Design
1) Human K562
2) Mouse RAW/Human K562 co-culture (Mix 0) 0 houre
3) Mouse RAW/Human K562 co-culture (Mix 1) 24 hour
4) Mouse RAW/Human K562 co-culture (Mix 2) 24 hour

FIG. 6B

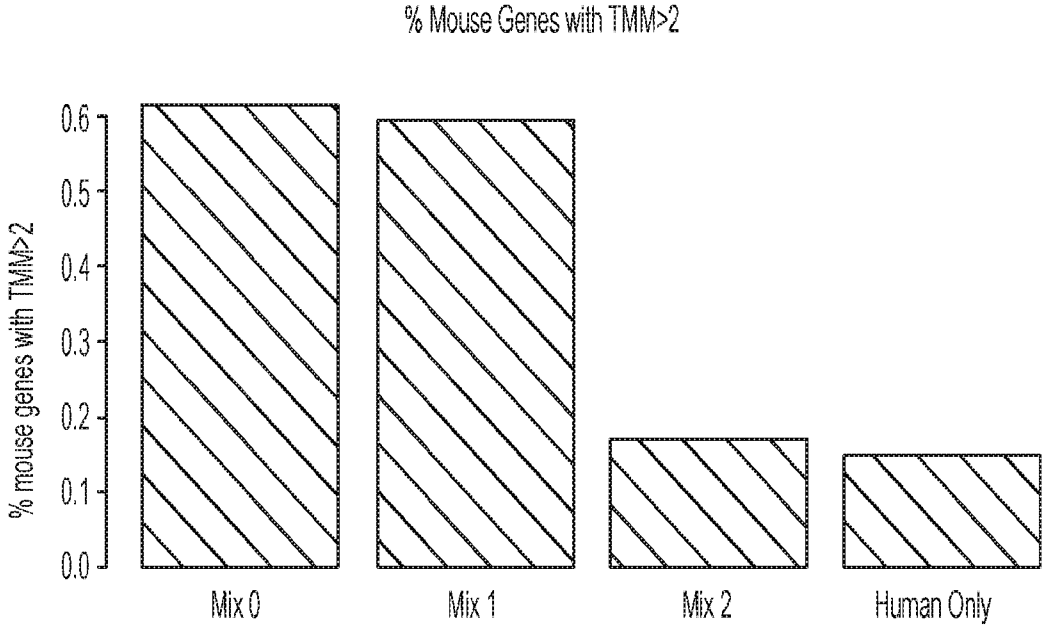

% Mouse Genes with TMM>2
% mouse genes with TMM>2
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Mix 0
Mix 1
Mix 2
Human Only

FIG. 6C

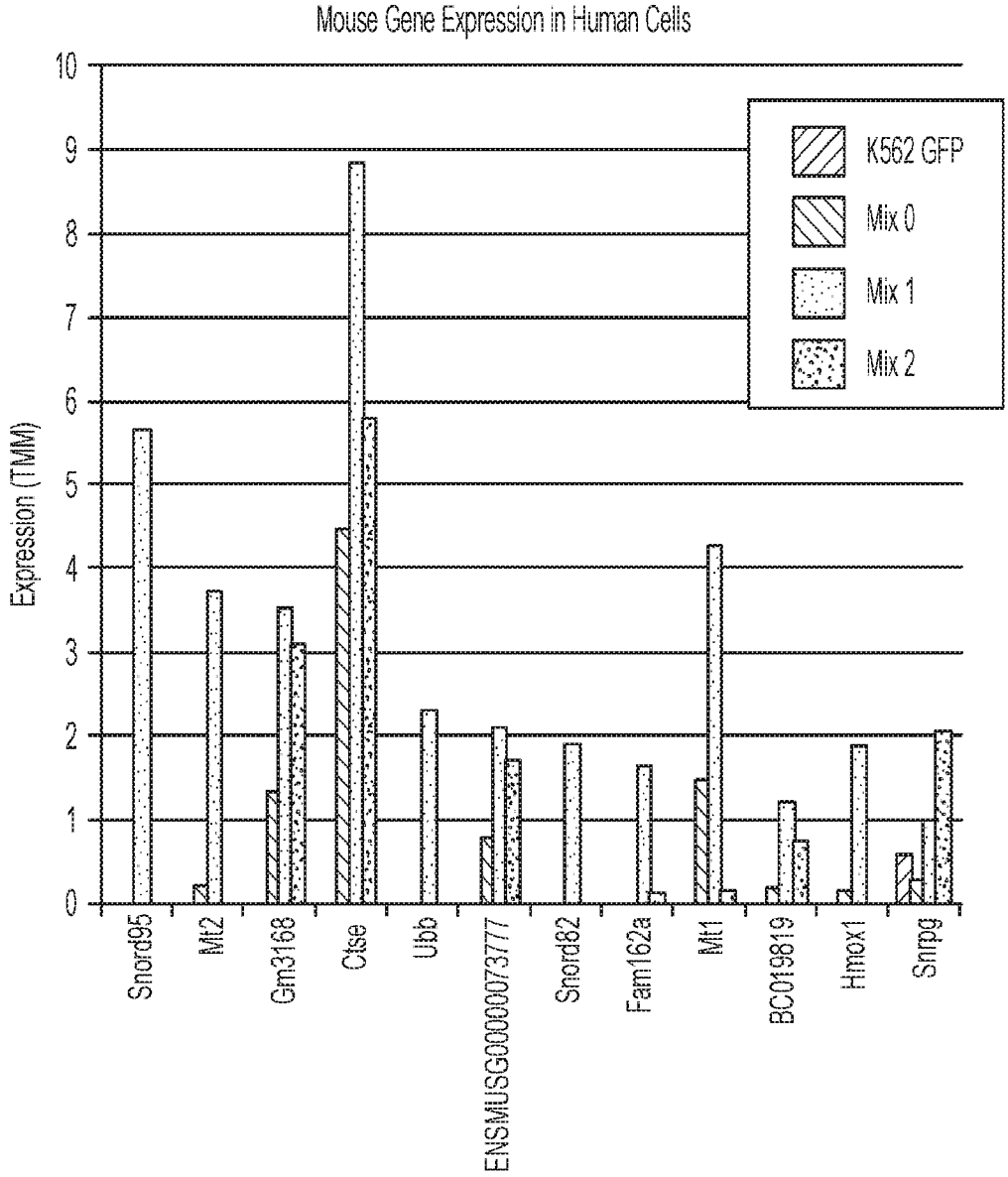

Mouse Gene Expression in Human Cells
Expression (TMM)
10
9
8
7
6
5
4
3
2
1
0
K562 GFP
Mix 0
Mix 1
Mix 2
Snord95
Mt2
Gm3168
Ctse
Ubb
ENSMUSG00000073777
Snord82
Fam162a
Mt1
BC019819
Hmox1
Snrpg

FIG. 6D

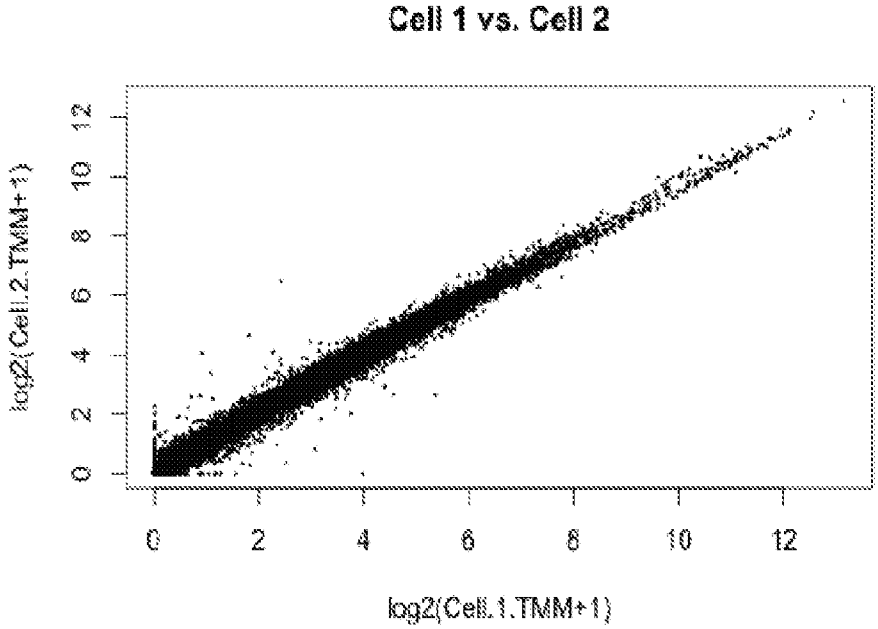

Cell 1 vs. Cell 2
log2(Cell.2.TMM+1)
log2(Cell.1.TMM+1)
0
2
4
6
8
10
12

FIG. 7A

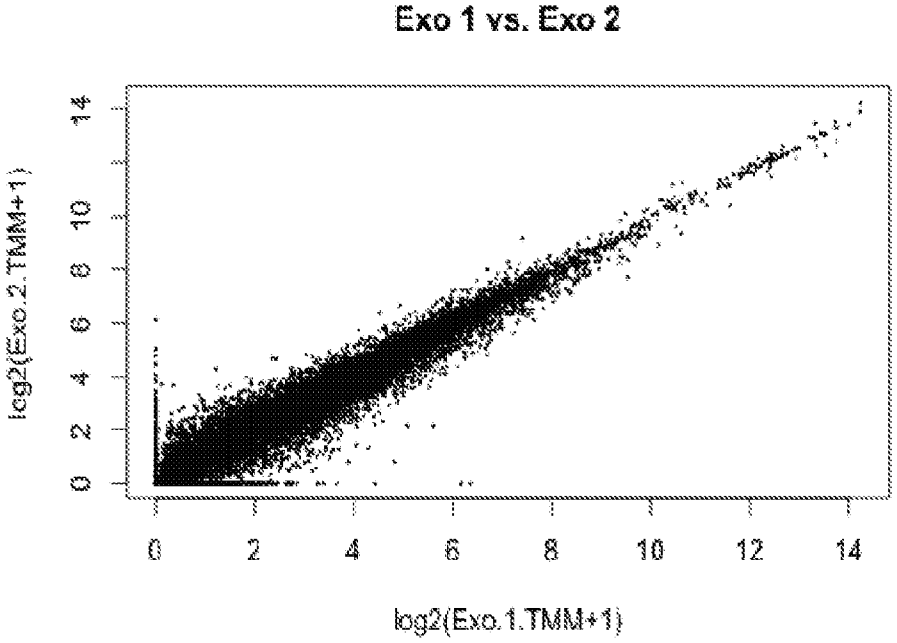

Exo 1 vs. Exo 2
log2(Exo.2.TMM+1)
log2(Exo.1.TMM+1)
0
2
4
6
8
10
12
14

FIG. 7B

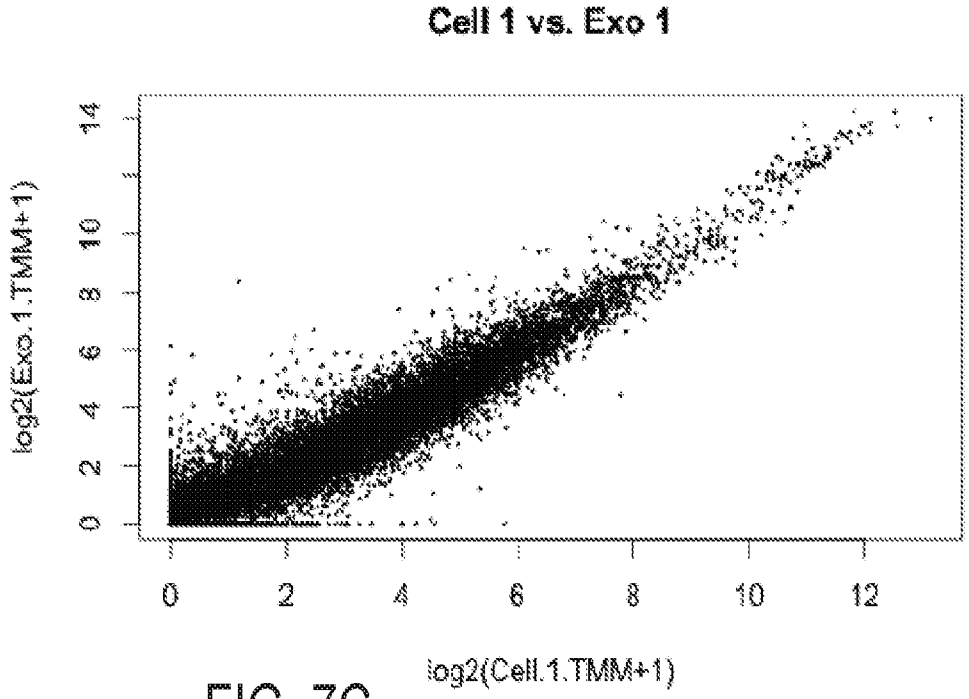

Cell 1 vs. Exo 1
log2(Exo.1.TMM+1)
log2(Cell.1.TMM+1)
0
2
4
6
8
10
12
14

FIG. 7C

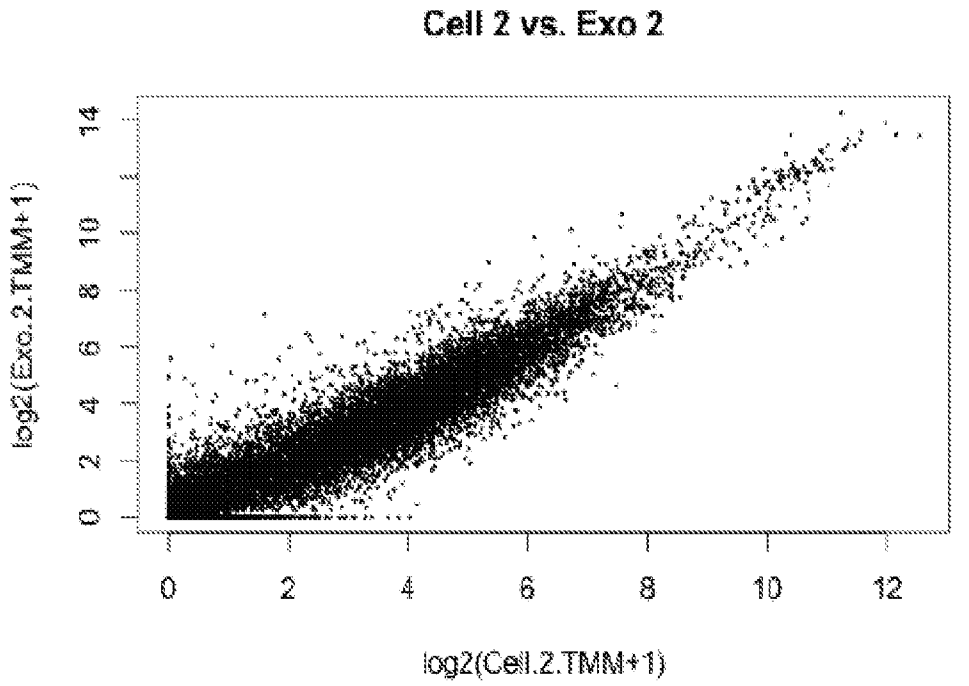
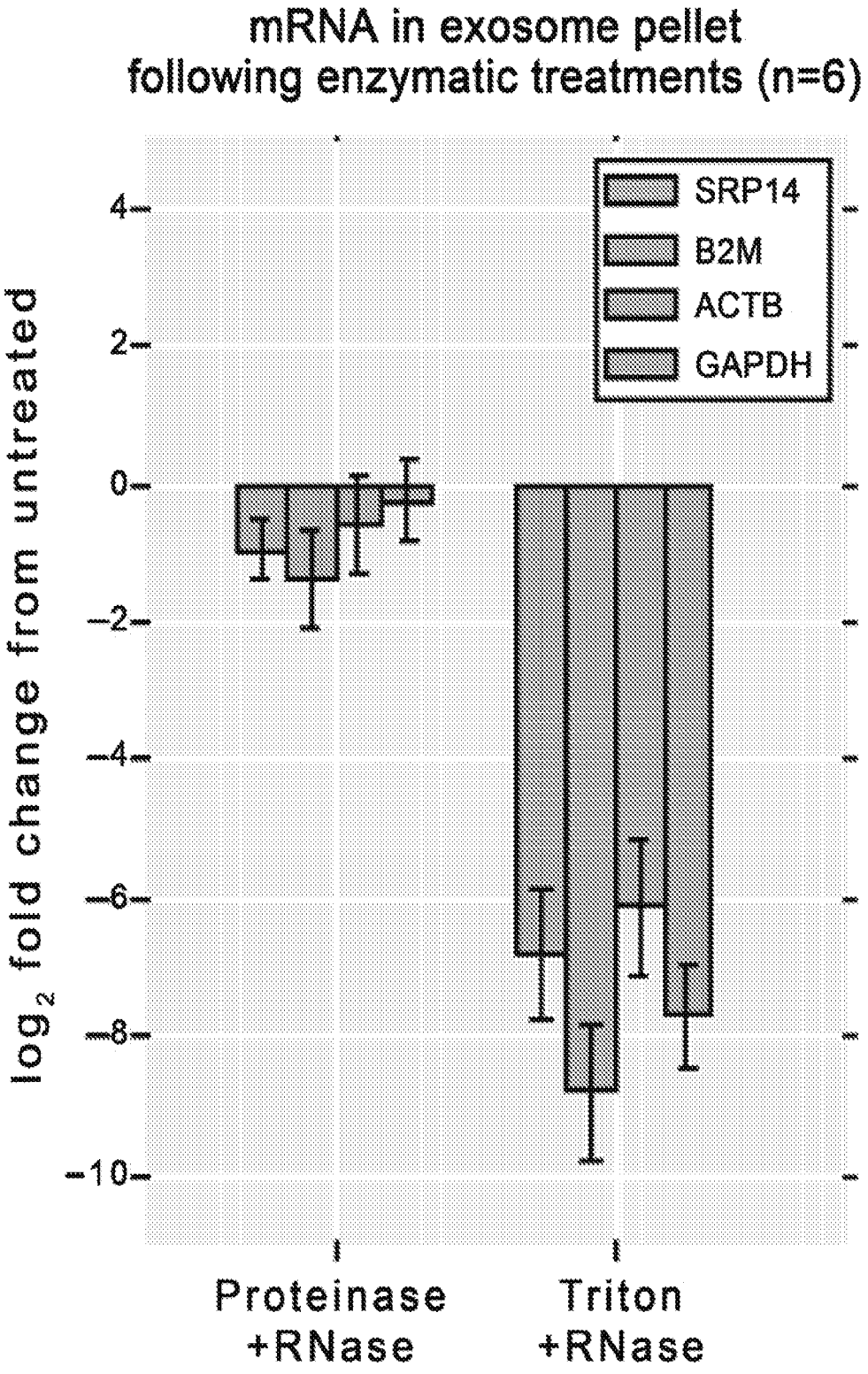

Cell 2 vs. Exo 2
log2(Exo.2.TMM+1)
log2(Cell.2.TMM+1)
0
2
4
6
8
10
12
14

FIG. 7D

FIG. 8


mRNA in exosome pellet
following enzymatic treatments (n=6)
SRP14
B2M
ACTB
GAPDH
log2 fold change from untreated
4
2
0
-2
-4
-6
-8
-10
Proteinase
+RNase
Triton
+RNase

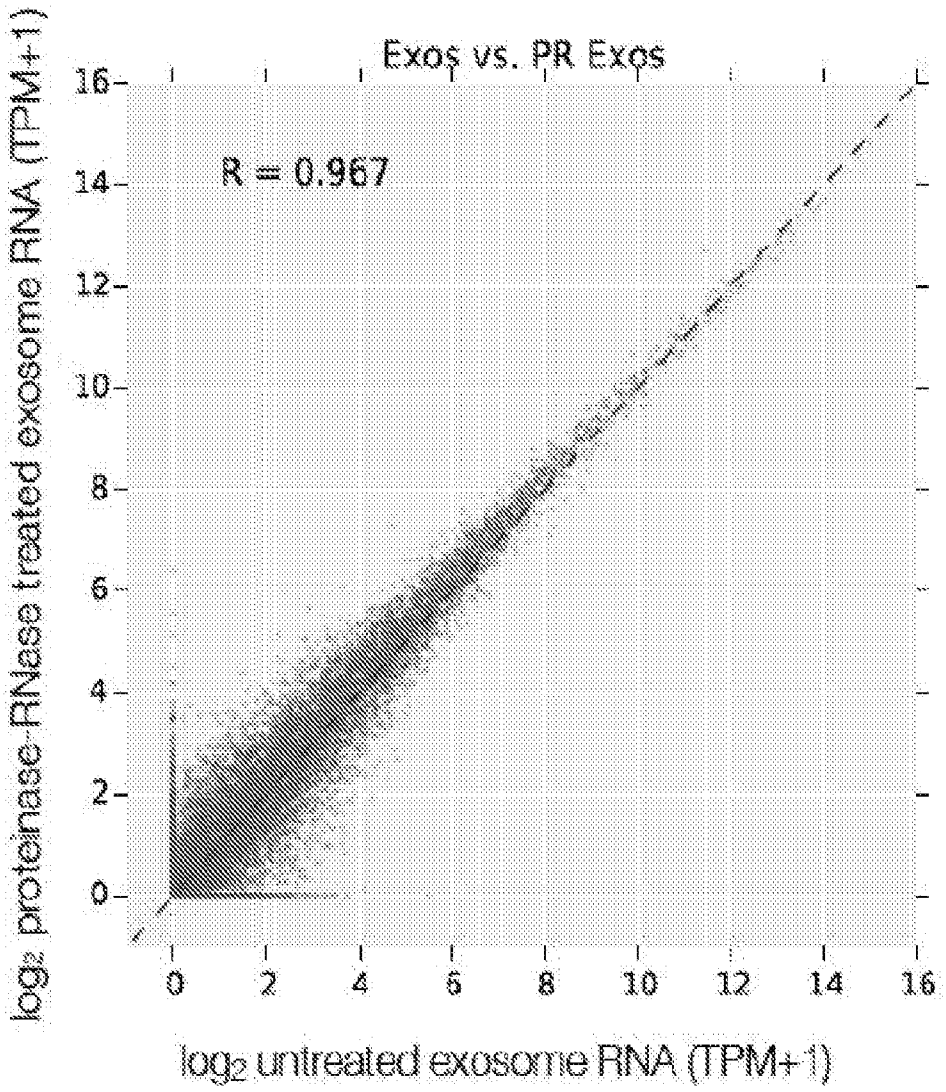

Exos vs. PR Exos
R = 0.967
log2 proteinase-RNase treated exosome RNA (TPM+1)
log2 untreated exosome RNA (TPM+1)
0
2
4
6
8
10
12
14
16

FIG. 9

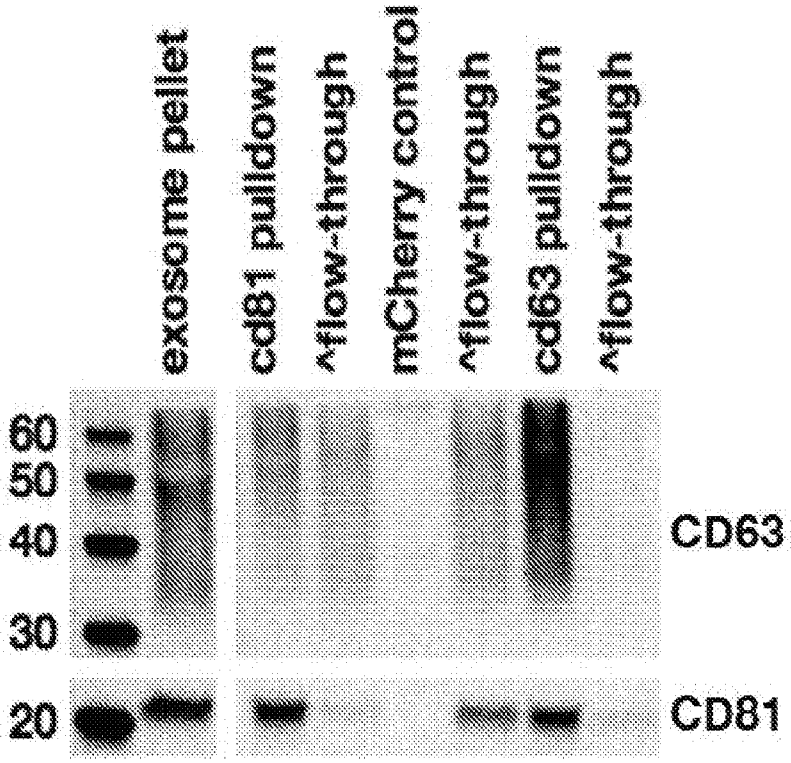

exosome pellet
cd81 pulldown
^flow-through
mCherry control
^flow-through
cd63 pulldown
^flow-through
60
50
40
30
20
CD63
CD81

FIG. 10

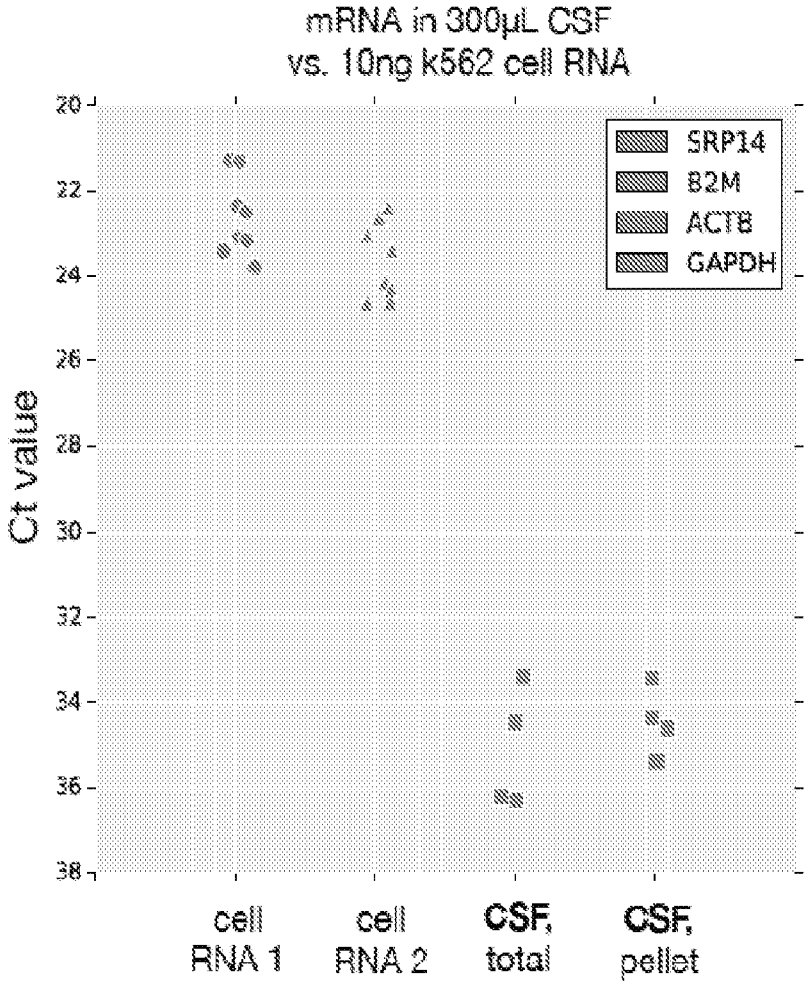

mRNA in 300µL CSF
vs. 10ng k562 cell RNA
Ct value
20
22
24
26
28
30
32
34
36
38
SRP14
B2M
ACTB
GAPDH
cell RNA 1
cell RNA 2
CSF, total
CSF, pellet

FIG. 11