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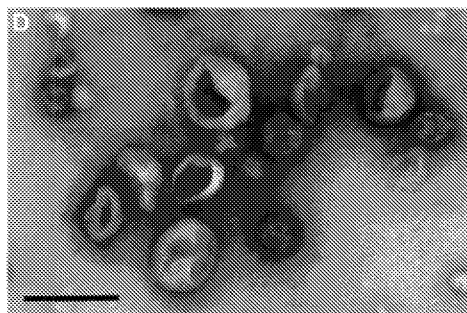


FIG. 2D

(57) Abstract: This disclosure relates to populations and compositions of purified cell-derived vesicles and uses thereof. One aspect of the disclosure relates to methods for purifying the cell-derived vesicles.



**METHODS FOR ENHANCED PRODUCTION AND ISOLATION OF
CELL-DERIVED VESICLES AND TREATMENT OF INFLAMMATION AND
NEUROLOGICAL DAMAGE**

STATEMENT OF GOVERNMENT SUPPORT

[0001] This invention was made with government support under the Grant No. RO1GM099688, awarded by the National Institute for Health (NIH). The government has certain rights in the invention.

CROSS-REFERENCE TO RELATED APPLICATION

[0002] This application claims the benefit under 35 U.S.C. § 119(e) of U.S. Serial No. 62/515406, filed June 5, 2017, the contents of which is incorporated by reference in its entirety herein.

TECHNICAL FIELD

[0003] The invention relates to populations and compositions of purified cell-derived vesicles and uses thereof. One aspect of the disclosure relates to methods for purifying the cell-derived vesicles.

BACKGROUND

[0004] Neurodegenerative and inflammatory diseases affect over 300 million people worldwide and often there are no satisfactory treatment options for these patients. Numerous studies have demonstrated that mesenchymal stem cells (MSCs) based therapeutics have robust neuroprotective and anti-inflammatory properties. However, there are several limiting factors that limit their potential clinical effectiveness.

[0005] MSCs mediate their neuroprotective and anti-inflammatory effects via the secretion of signaling factors, including exosomes and microvesicles. Exosomes and microvesicles are secreted cellular vesicles of endosomal origin and contain various proteins, lipids, and RNAs from the cytosol of the secreting cells. Upon release into the extracellular space, exosomes and microvesicles function as intercellular messengers, delivering their contents to a recipient target cell.

[0006] The characterization of the composition of stem cell derived exosome and/or microvesicles that are responsible for the observed tissue healing effects remains elusive. Identification of the exosome and/or microvesicle composition could have a great impact in the treatment of neurodegenerative and inflammation-related diseases. Thus, in order to develop promising vesicle-based therapeutics, there remains a need in the art to identify such components and to modify the exosomes to deliver the appropriate factors to a target cell to treat a specific disease.

SUMMARY

[0007] This disclosure relates to purified populations, compositions, and methods of treatment using secreted cell-derived vesicles (e.g., exosomes and/or microvesicles).

[0008] One aspect of the disclosure relates to a highly purified population of cell-derived vesicles prepared by culturing stem cells producing the cell-derived vesicles under conditions of hypoxia, low serum. In one aspect, the stem cells are cultured in the presence of one or more agents selected from an inflammatory agent, a neurotrophic factor, or an angiogenesis agent. In these methods, the the cell-derived vesicles can comprise exosomes and/or microvesicles. In some aspects, the inflammatory agent is selected from tumor necrosis factor alpha ("TNF α "), interleukin 6 ("IL-6"), interleukin 17 ("IL-17"), interleukin 1 β ("IL-1 β "), interferon gamma ("IFN γ "), lipopolysaccharide ("LPS"), or equivalents of each thereof. In some aspects, the neurotrophic factor is selected from brain derived neurotrophic factor ("BDNF"), nerve growth factor ("NGF"), Neurotrophin-3 ("NTF3"), ciliary neurotrophic factor ("CTNF"), glial cell derived neurotrophic factor ("GDNF"), fibroblast growth factors ("FGFs") 1-23 (e.g. FGF1, FGF2), insulin-like growth factors ("IGFs") (IGF1, IGF2), hepatocyte growth factor ("HGF"), Noggin ("NOG"), thyroid hormone triiodothyronine ("T3"), or equivalents of each thereof. In some aspects, the angiogenesis agent is selected from FGF2, vascular endothelial growth factor ("VEGF"), platelet derived growth factor ("PDGF"), HGF, FGF1, FGF2, epidermal growth factor ("EGF"), transforming growth factor beta 1-4 ("TGF β ," e.g. TGF β 1, TGF β 2, TGF β 3, or TGF β 4), proto-oncogene protein Wnt-1 ("WNT1"), or equivalents of each thereof. Preferably, the agent is selected from TNF α , Noggin, FGF2, or T3.

[0009] Another aspect of the disclosure relates to a highly purified population of modified cell-derived vesicles, optionally wherein the cell-derived vesicles comprise, consist essentially of, or yet further consist of, exosomes and/or microvesicles.

[0010] In a further aspect, the disclosure relates to a composition comprising, consisting essentially of, or yet further consisting of, the purified population of cell-derived vesicles according to any one of the embodiments described herein and one or more of a carrier, a preservative or a stabilizing agent. In a further aspect, the cell-derived vesicles are complexed to therapeutic agents, which include, without limitation, polynucleotides such as RNA and/or DNA and/or polypeptides or proteins such as neutropic factors.

[0011] In one aspect, the disclosure relates to a method for isolating and/or purifying a population of cell-derived vesicles, and in one aspect, exosomes, the method comprising, or consisting essentially of, or yet further consisting of: (a) isolating the cell-derived vesicles from conditioned media containing the cell-derived vesicles by an appropriate method, e.g., by applying a tangential flow filtration to conditioned media produced by a population of isolated stem cells to isolate a cell-derived vesicle containing fraction; and optionally (b) concentrating the cell-derived vesicle containing fraction to provide a purified population of cell-derived vesicles. Any appropriate method can be used to concentrate the cell-derived vesicles, e.g. exosomes. Non-limiting examples of such include centrifugation, ultrafiltration, filtration, differential centrifugation and column filtration with a 100 kDa to 750 kDa pore size, or either a 100 kDa to 750 kDa pore size. In some aspects, the pore size of the column is 100 kDa to 300 kDa. Further sub-populations can be isolated using antibodies or other agents that are specific for a specific marker expressed by the desired exosome population.

[0012] In another aspect, prior to isolation and/or purification of the cell-derived vesicles, the stem cells producing the vesicles are grown or cultured by any method known in the art, e.g. by a method comprising the use of a hollow fiber bioreactor prior to the isolation and/or purification of the cell-derived vesicles from the conditioned media. In one aspect, the cell-derived vesicles are exosomes. In one aspect, the stem cells (that produce the conditioned media containing the cell-derived vesicles and/or exosomes) are cultured under conditions of low serum and hypoxia or low oxygen conditions. In one aspect, the stem cells (that produce the conditioned media containing the cell-derived vesicles and/or exosomes) are cultured in

the presence of or contacted with one or more agents selected from a polynucleotide (RNA and/or DNA), an inflammatory agent, a neurotrophic factor, or an angiogenesis agent. In particular aspects, the inflammatory agent is selected from $\text{TNF}\alpha$, IL-6, IL-17, IL-1 β , IFN γ , lipopolysaccharide, or equivalents of each thereof; the neurotrophic factor is selected from BDNF, NGF, Neurotrophin-3, CTNF, GDNF, FGFs 1-23 (e.g. FGF1, FGF2), insulin-like growth factors (IGFs) (e.g IGF1, IGF2), HGF, Noggin, T3, or equivalents of each thereof; and/or the angiogenesis agent is selected from FGF2, VEGF, PDGF, HGF, FGF1, FGF2, EGF, TGF β 1-4, WNT1, or equivalents of each thereof. In some aspects, the agent is a recombinant protein. In some aspects, the culture conditions comprise about 1 to about 10 ng/mL, or alternatively about 5 to about 20 ng/mL, or alternatively about 5 to about 30 ng/mL, or alternatively about 5 to about 40 ng/mL, or alternatively about 5 to about 50 ng/mL, or alternatively about 5 to about 100 ng/mL, or alternatively about 5 to about 250 ng/mL, or alternatively about 5 to about 500 ng/mL, or alternatively about 25 to about 75 ng/mL, or alternatively about 50 to about 100 ng/mL, or alternatively about 100 to about 500 ng/mL, or or alternatively about 100 ng/mL to about 1 $\mu\text{g/mL}$, or alternatively about 1 $\mu\text{g/mL}$ to about 10 $\mu\text{g/mL}$, or alternatively about 10 $\mu\text{g/mL}$ to about 50 $\mu\text{g/mL}$, or alternatively about 50 $\mu\text{g/mL}$ to about 100 $\mu\text{g/mL}$, or alternatively about 100 $\mu\text{g/mL}$ to about 500 $\mu\text{g/mL}$, or alternatively about 100 $\mu\text{g/mL}$ to about 1000 $\mu\text{g/mL}$ of the agent. In particular aspects, the culture conditions comprise about 10 ng/mL, or alternatively about 15 ng/mL, or alternatively about 20 ng/mL, or alternatively about 25 ng/mL, or alternatively about 30 ng/mL, or alternatively about 40 ng/mL, or alternatively about 50 ng/ml, or alternatively about 100 ng/mL, or alternatively about 200 ng/mL, or alternatively about 250 ng/mL, or alternatively about 300 ng/mL, or alternatively about 400 ng/mL, or alternatively about 500 ng/mL, or alternatively about 1 $\mu\text{g/mL}$. Preferably, the agent is about 5 to about 100 ng/mL of the agent.

[0013] In some embodiments, the cell-derived vesicles of the population further comprise, or alternatively consist essentially of, or yet further consist of, at least one exogenous nucleic acid and/or at least one exogenous protein, i.e. a nucleic acid or protein that is not present in a naturally occurring cell-vesicle. Alternatively, the cell-derived vesicles can further comprise an endogenous nucleic acid and/or endogenous protein that is naturally present in the cell-derived vesicle but whose expression is to be enhanced or inhibited. Non-limiting examples

of nucleic acids include one or more or all of DNA and RNA, for example mRNA, RNAi, siRNA, pcRNA. In some embodiments, the exogenous or endogenous nucleic acid is or encodes one or more of a micro RNA (miRNA), for example, miR-181, miR-210, miR-214, miR-424, miR-150, miR-126, miR-132, miR-296, or let-7. In some embodiments, the exogenous or endogenous protein is one or more of platelet derived growth factor receptor (PDGFR), Collagen, Type 1, Alpha 2 (COL1A2), Collagen, Type VI, Alpha 3 (COL6A3), EGF-like repeats- and discoidin i-like domains-containing protein 3 (EDIL3), epidermal growth factor receptor (EGFR), fibroblast growth factor receptor (FGFR), fibronectin (FN1), Milk fat globule-EGF factor 8 (MFGE8), lectin, galactoside-binding, soluble, 3 binding protein (LGALS3BP), nuclear factor-kappaB (NFκB), transferrin (TF), vascular endothelial growth factor (VEGF), VEGF isoform 165A, or vascular endothelial growth factor receptor (VEGFR). In other embodiments, the population of cell-derived vesicles do not express or comprise VEGF, VEGFR or both. In some embodiments, the cell-derived vesicles of the present disclosure are modified to comprise one or more of an exogenous or endogenous protein, nucleic acid, metabolite, lipid, and/or membrane component, that can be detected in the exosomes and/or microvesicles of the present disclosure.

[0014] In some embodiments, the cell-derived vesicles of the population further comprise at least one exogenous nucleic acid and/or at least one exogenous protein, i.e. a nucleic acid or protein that is not present in a naturally occurring cell-vesicle. Alternatively, the cell-derived vesicles can further comprise an exogenous nucleic acid and/or exogenous protein that is naturally present in the cell-derived vesicle but whose expression is to be enhanced or inhibited. Non-limiting examples of nucleic acids include one or more or all of DNA and RNA, for example mRNA, RNAi, siRNA, pcRNA. In some embodiments, the exogenous nucleic acid is or encodes one or more of a micro RNA (miRNA), for example, miR-181, miR-210, miR-214, miR-424, miR-150, miR-126, miR-132, miR-296, or let-7. In some embodiments, the exogenous protein is one or more of platelet derived growth factor receptor (PDGFR), Collagen, Type 1, Alpha 2 (COL1A2), Collagen, Type VI, Alpha 3 (COL6A3), EGF-like repeats- and discoidin i-like domains-containing protein 3 (EDIL3), epidermal growth factor receptor (EGFR), fibroblast growth factor receptor (FGFR), fibronectin (FN1), Milk fat globule-EGF factor 8 (MFGE8), lectin, galactoside-binding, soluble, 3 binding protein (LGALS3BP), nuclear factor-kappaB (NFκB), transferrin (TF), vascular endothelial

growth factor (VEGF), VEGF isoform 165A, or vascular endothelial growth factor receptor (VEGFR). In other embodiments, the population of cell-derived vesicles do not express or comprise exogenous VEGF, VEGFR or both. In some embodiments, the cell-derived vesicles of the present disclosure are modified to comprise one or more of an exogenous protein, nucleic acid, metabolite, lipid, and/or membrane component, that can be detected in the exosomes and/or microvesicles of the present disclosure, (and listed in the molecular composition of exosomes section below).

[0015] A non-limiting example of a method and composition to provide a purified and/or isolated population of cell-derived vesicles comprising at least one exogenous nucleic acid is by transforming an isolated host cell, such as a stem cell with a vector comprising the coding polynucleotide. SEQ ID NO: 18 is an example of such a vector. Thus, in another aspect, provided herein is a lentiviral vector comprising the necessary regulatory elements. As is apparent to the skilled artisan, the marker sequence (nucleotides 5894 to 7321 of SEQ ID NO: 18) can be omitted as well as the enhancer element (nucleotides 7345 to 7941 of SEQ ID NO: 18) or be substituted with alternative markers or enhancers. In addition, nucleotides 5208 to 5363 correspond to the miR-132 element but other elements, as described herein or as known in the art, can be substituted therein. Alternative promoters (the PGK promoter provided as nucleotides 5364 to 5874) can be substituted as well. Alternative vectors are described in U.S. Patent Publication Nos. 2016/0046685 and WO 2014/035433, each incorporated by reference herein. One disclosed vector of WO 2014/035433 contains a gene encoding for the 165A isoform of VEGF and includes an MNLU3 promoter and an optional enhancer element.

[0016] Isolated host cells, such as stem cells, comprising such vectors are further provided as well as populations of such cells alone or in combination with the isolated or purified cell-derived vesicles as described herein. These compositions can be further combined with a carrier, preservative or stabilizer.

[0017] Also provided are methods for preparing the cell-derived vesicles by culturing the host cells to grow the cells, also as provided herein. As noted in more detail herein, in one aspect, mesenchymal stem cells were transfected with a plasmid expression vector

overexpressing miR-132 and tdTomato marker (SEQ ID NO: 18). Microvesicles were harvested from media that had been conditioned for 48 hours using ultracentrifugation.

[0018] In some embodiments, the population of cell-derived vesicles or isolated host cells is substantially homogeneous. In other embodiments, the population of cell-derived vesicles or isolated host cells is heterogeneous.

[0019] In some embodiments, the concentration of cell-derived vesicles in or isolated from the the population comprises between about 0.5 micrograms to about 200 micrograms of cell-derived vesicle protein collected per approximately 10^6 cells. In some embodiments, the concentration of cell-derived vesicles in or isolated from the population comprises between about 200 micrograms to about 5000 micrograms of cell-derived vesicle protein collected per approximately 10^6 cells. In other embodiments, the concentration of cell-derived vesicles in or isolated from the population comprises less than about 5000, or alternatively less than about 1000, or alternatively less than about 500, or alternatively less than about 200, or alternatively less than about 150, or alternatively less than about 125, or alternatively less than about 100, or alternatively less than about 75, or alternatively less than about 50, or alternatively less than about 30 micrograms, or alternatively less than about 25 micrograms, of cell-derived vesicle protein collected per approximately 10^6 cells. In yet other embodiments, the concentration of cell-derived vesicle protein in or isolated from the population is less than about 20 micrograms per 10^6 cells.

[0020] In some embodiments, the average diameter of the cell-derived vesicles in or isolated from the population is between about 0.1 nm and about 1000 nm, or alternatively between about 1.0 nm and about 1000 nm, or alternatively between about 1.5 nm and about 1000 nm. In other embodiments, the average diameter is between about 2 nm and about 800 nm, or alternatively about 2 nm to about 700 nm, or alternatively from about 2 nm to about 600 nm, or alternatively from about 2 nm to about 500 nm, or alternatively from about 2 nm to about 400 nm, or alternatively from about 2 nm to about 300 nm. In other embodiments, the average diameter is between about 10 nm and about 1000 nm, or alternatively 100 nm to about 1000 nm, or alternatively from about 300 nm to about 1000 nm, or alternatively from about 500 nm to about 1000 nm, or alternatively from about 750 nm to about 1000 nm, or alternatively from about 800 nm to about 1000 nm. In other embodiments, the average

diameter of the cell-derived vesicles in or isolated from the population is less than about 100 nm. In further embodiments, the average diameter of the cell-derived vesicles in or isolated from the population is less than about 50 nm. In still further embodiments, the average diameter of the cell-derived vesicles in the population is less than about 40 nm.

[0021] In some embodiments, the purified population of cell-derived vesicles described herein have been purified from by a methods known in the art, e.g. by a method comprising tangential flow filtration or other filtration method. Prior to isolation, the cells producing the cell-derived vesicles can be cultured by any appropriate method known in the art, e.g., in a hollow-fiber bioreactor. Prior to isolation, the cells producing the cell-derived vesicles can be cultured in the presence of or contacted with one or more agents that modify the vesicles to be anti-inflammatory, neuroprotective, and/or pro-angiogenesis. The one or more agents include but are not limited to a polynucleotide, an inflammatory agent, a neurotrophic factor, or an angiogenesis agent. In some embodiments, the inflammatory agent is selected from tumor necrosis factor alpha ("TNF α "), interleukin 6 ("IL-6"), interleukin 17 ("IL-17"), interleukin 1 β ("IL-1 β "), interferon gamma ("IFN γ "), lipopolysaccharide ("LPS"), or equivalents of each thereof. In some embodiments, the neurotrophic factor is selected from brain derived neurotrophic factor ("BDNF"), nerve growth factor ("NGF"), Neurotrophin-3 ("NTF3"), ciliary neurotrophic factor ("CTNF"), glial cell derived neurotrophic factor ("GDNF"), fibroblast growth factors ("FGFs") 1-23 (e.g. FGF1, FGF2), insulin-like growth factors ("IGFs") (IGF1, IGF2), hepatocyte growth factor ("HGF"), Noggin ("NOG"), thyroid hormone triiodothyronine ("T3"), or equivalents of each thereof. In some embodiments, the angiogenesis agent is selected from FGF2, vascular endothelial growth factor ("VEGF"), platelet derived growth factor ("PDGF"), HGF, FGF1, FGF2, epidermal growth factor ("EGF"), transforming growth factor beta 1-4 ("TGF β ," e.g. TGF β 1, TGF β 2, TGF β 3, or TGF β 4), proto-oncogene protein Wnt-1 ("WNT1"), or equivalents of each thereof. Preferably, the agent is selected from TNF α , Noggin, FGF2, or T3.

[0022] In some aspects, the agent is a recombinant protein. In some aspects, culture conditions comprise about 1 to about 10 ng/mL, or alternatively about 5 to about 20 ng/mL, or alternatively about 5 to about 30 ng/mL, or alternatively about 5 to about 40 ng/mL, or alternatively about 5 to about 50 ng/mL, or alternatively about 5 to about 100 ng/mL, or alternatively about 5 to about 250 ng/mL, or alternatively about 5 to about 500 ng/mL, or

alternatively about 25 to about 75 ng/mL, or alternatively about 50 to about 100 ng/mL, or alternatively about 100 to about 500 ng/mL, or alternatively about 100 ng/mL to about 1 µg/mL, or alternatively about 1 µg/mL to about 10 µg/mL, or alternatively about 10 µg/mL to about 50 µg/mL, or alternatively about 50 µg/mL to about 100 µg/mL, or alternatively about 100 µg/mL to about 500 µg/mL, or alternatively about 100 µg/mL to about 1000 µg/mL of agent. In particular aspects, the culture conditions comprise about 10 ng/mL, or alternatively about 15 ng/mL, or alternatively about 20 ng/mL, or alternatively about 25 ng/mL, or alternatively about 30 ng/mL, or alternatively about 40 ng/mL, or alternatively about 50 ng/mL, or alternatively about 100 ng/mL, or alternatively about 200 ng/mL, or alternatively about 250 ng/mL, or alternatively about 300 ng/mL, or alternatively about 400 ng/mL, or alternatively about 500 ng/mL, or alternatively about 1 µg/mL. Preferably, the stimulating agent is about 5 to about 100 ng/mL of agent.

[0023] In some embodiments, the population of cell-derived vesicles, e.g., exosomes is combined with a carrier, for example, a pharmaceutically acceptable carrier, that in one aspect, provides the composition with enhanced stability over an extended period of time. The compositions can be further combined with one or more other therapeutic agents, e.g. a neurotrophic factor (including but not limited to BDNF, NGF, Neurotrophin-3, CTNF, GDNF, FGF, IGF, HGF, Noggin, or T3), an angiogenesis promoter (including but not limited to FGF2, HGF, VEGF, PDGF, FGF1, EGF, TGFβ, or WNT1), an anti-inflammatory agent (including but not limited to TGFβ, IL-2, IL-10, IL-17, IL-35, IL-37), a phytochemical agent, a chemotherapeutic agent, and/or a Stat3 inhibitor. In one aspect, the therapeutic agent is added directly to the composition. In another aspect, the therapeutic agent is encapsulated by the exosome. Non-limiting examples of angiogenesis promoters include, angiotensin, prostaglandin E₁ (PGE₁), modified PGE₁ (see US Patent No. 6,288,113, incorporated by reference herein) and angiopoietin-1. Methods to encapsulate agents within exosomes are known in the art and described for example in U.S. Patent Publication No. 2014/0093557, published April 3, 2014, and incorporated by reference herein. In some embodiments, the compositions are formulated for therapeutic application and/or enhanced stability such as by drying, freeze drying, snap-freezing, or lyophilization.

[0024] In some embodiments, the compositions described herein further comprise one or more agents selected from an anti-inflammatory agent, a neurotrophic factor, or an

angiogenesis agent. In some embodiments, the anti-inflammatory agent is selected from TGF β 1-4, interleukin 2 (“IL-2”), interleukin 10 (“IL-10”), interleukin 17 (“IL-17”), interleukin 35 (“IL-35”), or interleukin – 1 family member 7 (“IL-37”). Preferably, the anti-inflammatory agent is TGF β and/or IL-2. In some embodiments, the neurotrophic factor is selected from BDNF, NGF, Neurotrophin-3, CTNF, GDNF, FGFs 1-23 (e.g. FGF1, FGF2), insulin-like growth factors (IGFs) (e.g IGF1, IGF2), HGF, Noggin, T3, or equivalents of each thereof. Preferably, the one or more neurotrophic factors is selected from FGF2, T3, NOG, BDNF, NGF, HGF, CTNF, GDNF, or IGF2. In some embodiments, the angiogenesis agent is selected from FGF2, VEGF, PDGF, HGF, FGF1, FGF2, EGF, TGF β 1-4, WNT1, or equivalents of each thereof. Preferably, the angiogenesis agent is FGF2 and/or HGF. In some aspects, the agent is recombinant. In some aspects, the compositions described herein comprise about 1 to about 10 ng/mL, or alternatively about 5 to about 20 ng/mL, or alternatively about 5 to about 30 ng/mL, or alternatively about 5 to about 40 ng/mL, or alternatively about 5 to about 50 ng/mL, or alternatively about 5 to about 100 ng/mL, or alternatively about 5 to about 250 ng/mL, or alternatively about 5 to about 500 ng/mL, or alternatively about 25 to about 75 ng/mL, or alternatively about 50 to about 100 ng/mL, or alternatively about 100 to about 500 ng/mL, or alternatively about 100 ng/mL to about 1 μ g/mL, or alternatively about 1 μ g/mL to about 10 μ g/mL, or alternatively about 10 μ g/mL to about 50 μ g/mL, or alternatively about 50 μ g/mL to about 100 μ g/mL, or alternatively about 100 μ g/mL to about 500 μ g/mL, or alternatively about 100 μ g/mL to about 1000 μ g/mL of agent. In particular aspects, the compositions comprise about 10 ng/mL, or alternatively about 15 ng/mL, or alternatively about 20 ng/mL, or alternatively about 25 ng/mL, or alternatively about 30 ng/mL, or alternatively about 40 ng/mL, or alternatively about 50 ng/mL, or alternatively about 100 ng/mL, or alternatively about 200 ng/mL, or alternatively about 250 ng/mL, or alternatively about 300 ng/mL, or alternatively about 400 ng/mL, or alternatively about 500 ng/mL, or alternatively about 1 μ g/mL of agent. Preferably, the agent is about 10 to about 1000 ng/mL.

[0025] In some embodiments, the compositions described herein further comprise an isolated stem cell, for example, one or more of an adult stem cell, an embryonic stem cell, an induced pluripotent stem cell, an embryonic-like stem cell, a mesenchymal stem cell, or a neural stem cell. In one aspect, the isolated stem cell further is modified, for example by the

introduction of a vector and/or gene for therapeutic use. A non-limiting example of such is a stem cell modified to express a pro-angiogenic factor, e.g., VEGF or an equivalent thereof as described in U.S. Patent Publication No. 2016/0046685 and WO 2014/035433. The compositions can be further combined with other therapeutic agents, e.g. an angiogenesis promoter, a phytochemical agent, a chemotherapeutic agent, neurotrophic factors, and/or a Stat3 inhibitor.

[0026] In a further aspect, the disclosure relates to a method for promoting angiogenesis in a subject in need thereof comprising administering to the subject an effective amount of a purified population and/or a composition according to any one of the embodiments described herein. The methods can further comprise administration of an effective amount of other agents, e.g. agents that facilitate or promote angiogenesis, e.g., angiotensin, prostaglandin E₁ (PGE₁), modified PGE₁ (see U.S. Patent No. 6,288,113) and angiopoietin-1. The administration can be concurrent or sequential as determined by the treating physician. The subject can be an animal, e.g., a mammal such as a human patient in need of such treatment, that in one aspect, has been pre-selected for the therapy by a treating physician or other health care professional.

[0027] In a further aspect, the disclosure relates to a method for treating peripheral arterial disease or stroke comprising administering to a subject an effective amount of a purified population and/or a composition according to any one of the embodiments described herein. The methods can further comprise administration of an effective amount of other agents, e.g., agents that facilitate or promote angiogenesis, e.g., angiotensin, prostaglandin E₁ (PGE₁), modified PGE₁ (see U.S. Patent No. 6,288,113) and angiopoietin-1. The administration can be concurrent or sequential as determined by the treating physician. The subject can be an animal, e.g., a mammal such as a human patient in need of such treatment, that in one aspect, has been pre-selected for the therapy by a treating physician or other health care professional.

[0028] In yet a further aspect, the disclosure relates to a method for treating a dermal wound in a subject comprising administering to the subject an effective amount of a purified population and/or a composition according to any one of the embodiments described herein. The methods can further comprise administration of an effective amount of other agents, e.g., agents that facilitate or promote angiogenesis, e.g., angiotensin, prostaglandin E₁ (PGE₁),

modified PGE₁ (see U.S. Patent No. 6,288,113) and angiopoietin-1. The administration can be concurrent or sequential as determined by the treating physician. The subject can be an animal, e.g., a mammal such as a human patient in need of such treatment, that in one aspect, has been pre-selected for the therapy by a treating physician or other health care professional.

[0029] In yet a further aspect, the disclosure relates to a method for treating a disease or condition involving an inflammatory response or related to inflammation in a subject in need thereof comprising administering to the subject an effective amount of a purified population and/or composition according to any one of the embodiments described herein. The diseases or conditions involving an inflammatory response or related to inflammation include but are not limited to multiple sclerosis (MS), primary and secondary progressive MS, relapsing remitting MS, brain inflammation, frailty, radiation induced soft tissue damage, neuroinflammatory disease, muscle injuries, radiation tissue damage, traumatic brain injury, myocardial infarction, graft versus host disease, Parkinson's disease, Alzheimer's, inflammatory bowel disease, Huntington's disease, amyotrophic lateral sclerosis, Bahcet's disease, sarcopenia, aging, spinal cord injury, wound repair, and dysphagia. In one aspect, the disease or condition is one or more of multiple sclerosis (MS), primary and secondary progressive MS, relapsing remitting MS. In one aspect, the inflammatory condition excludes stroke. In another aspect, the inflammatory condition excludes stroke when the cells are cultured in the absence of one or more agents selected from an inflammatory agent, a neurotrophic factor, or an angiogenesis agent. Additional diseases or conditions associated with or related to inflammation and/or inflammatory responses include auto-immune disease or disorders. The methods can further comprise administration of an effective amount of other agents, e.g., agents that suppress inflammatory responses. In some aspects, the other agents include anti-inflammatory agent, neurotrophic factors, or angiogenesis agents. In some embodiments, the anti-inflammatory agent is selected from TGF β 1-4, IL-2, IL-10, IL-17, IL-35, IL-37. Preferably, the anti-inflammatory agent is TGF β and/or IL-2. In some embodiments, the neurotrophic factor is selected from BDNF, NGF, Neurotrophin-3, CTNF, GDNF, FGFs 1-23 (e.g. FGF1, FGF2), insulin-like growth factors (IGFs) (e.g IGF1, IGF2), HGF, Noggin, T3, or equivalents of each thereof. Preferably, the one or more neurotrophic factors is selected from FGF2, T3, NOG, BDNF, NGF, HGF, CTNF, GDNF, or IGF2. In some embodiments, the angiogenesis agent is selected from FGF2, VEGF, PDGF, HGF,

FGF1, FGF2, EGF, TGF β 1-4, WNT1, or equivalents of each thereof. Preferably, the angiogenesis agent is FGF2 and/or HGF. The administration can be concurrent or sequential as determined by the treating physician. The subject can be an animal, e.g., a mammal such as a human patient in need of such treatment, that in one aspect, has been pre-selected for the therapy by a treating physician or other health care professional.

[0030] In some embodiments, the subject is administered at least one dose of between approximately 0.1 mg and 200 mg of cell-derived vesicle protein. In some embodiments, the dose is between approximately 0.1 and 1000 mg of cell-derived protein. In other embodiments, the subject is administered at least one dose of approximately 50 mg, or alternatively approximately 100 mg, or alternatively approximately 150 mg, or alternatively approximately 200 mg of cell-derived vesicle protein.

[0031] In some embodiments, the purified population and/or the composition according to any one of the embodiments as described herein is administered prior to or after administration of an isolated stem cell that may optionally be modified. In other embodiments, the purified population and/or the composition according to any one of the embodiments as described herein is administered simultaneously with an isolated stem cell. In one aspect, the stem cell has been transduced with VEGF or a VEGF isoform, as described above.

[0032] In some embodiments, the purified population and/or the composition according to any one of the embodiments as described herein, is administered by intravenous injection, intrathecal injection, direct injection, intramuscular injection, intracranial injection, or topically.

[0033] In some embodiments, the subject is a mammal, optionally a human patient. In a further aspect, the patient has been selected for the therapy by diagnostic criteria as known to those of skill in the art.

[0034] In some embodiments, according to the methods described herein, e.g., a method for purifying a population of cell-derived vesicles, comprising: (a) applying a tangential flow filtration to conditioned media produced by a population of isolated stem cells to isolate a cell-derived vesicles containing fraction; and (b) concentrating the cell-derived vesicle containing fraction to provide a purified population of cell-derived vesicles. After step (a) cell

debris and other contaminants are removed from the cell-derived vesicles containing fraction prior to step (b). In some embodiments, according to the methods described herein, the population of stem cells is cultured under hypoxic and low serum conditions for up to about 72 hours prior to performing step (a). In some embodiments, according to the methods described herein, step (a) is performed using an approximately 200 nanometer filter. In some embodiments, the methods described herein further comprise culturing the stem cells in the presence of, or contacting the stem cells with, one or more agents prior to isolating the cell-derived vesicles. In some embodiments, the one or more agents are selected from an inflammatory agent, a neurotrophic factor, or an angiogenesis agent. In some embodiments, the methods described herein further comprise the addition of one or agents selected from an anti-inflammatory agent, a neurotrophic factor, or an angiogenesis agent to the purified population of cell-derived vesicles. In some embodiments, the agents are recombinant. In particular embodiments, the inflammatory agent is selected from $\text{TNF}\alpha$, IL-6, IL-17, IL-1 β , IFN γ , lipopolysaccharide, or equivalents of each thereof; the anti-inflammatory agent is selected from $\text{TGF}\beta$ 1-4, IL-2, IL-10, IL-17, IL-35, IL-37, or equivalents of each thereof; the neurotrophic factor is selected from BDNF, NGF, Neurotrophin-3, CTNF, GDNF, FGFs 1-23 (e.g. FGF1, FGF2), insulin-like growth factors (IGFs) (e.g IGF1, IGF2), HGF, Noggin, T3, or equivalents of each thereof; and/or the angiogenesis agent is selected from FGF2, VEGF, PDGF, HGF, FGF1, FGF2, EGF, $\text{TGF}\beta$ 1-4, WNT1, or equivalents of each thereof.

[0035] In some embodiments, according to the methods described herein, the culture conditions for the stem cells comprise about 1 to about 10 ng/mL, or alternatively about 5 to about 20 ng/mL, or alternatively about 5 to about 30 ng/mL, or alternatively about 5 to about 40 ng/mL, or alternatively about 5 to about 50 ng/mL, or alternatively about 5 to about 100 ng/mL, or alternatively about 5 to about 250 ng/mL, or alternatively about 5 to about 500 ng/mL, or alternatively about 25 to about 75 ng/mL, or alternatively about 50 to about 100 ng/mL, or alternatively about 100 to about 500 ng/mL, or or alternatively about 100 ng/mL to about 1 $\mu\text{g/mL}$, or alternatively about 1 $\mu\text{g/mL}$ to about 10 $\mu\text{g/mL}$, or alternatively about 10 $\mu\text{g/mL}$ to about 50 $\mu\text{g/mL}$, or alternatively about 50 $\mu\text{g/mL}$ to about 100 $\mu\text{g/mL}$, or alternatively about 100 $\mu\text{g/mL}$ to about 500 $\mu\text{g/mL}$, or alternatively about 100 $\mu\text{g/mL}$ to about 1000 $\mu\text{g/mL}$ of agent (e.g. inflammatory agent, neurotrophic factor, or angiogenesis agent). In particular aspects, the culture conditions comprise about 10 ng/mL, or alternatively

about 15 ng/mL, or alternatively about 20 ng/mL, or alternatively about 25 ng/mL, or alternatively about 30 ng/mL, or alternatively about 40 ng/mL, or alternatively about 50 ng/mL, or alternatively about 100 ng/mL, or alternatively about 200 ng/mL, or alternatively about 250 ng/mL, or alternatively about 300 ng/mL, or alternatively about 400 ng/mL, or alternatively about 500 ng/mL, or alternatively about 1 µg/mL. Preferably, the agent is about 5 to about 100 ng/mL of agent.

[0036] In some embodiments, according to the methods described herein, about 1 to about 10 ng/mL, or alternatively about 5 to about 20 ng/mL, or alternatively about 5 to about 30 ng/mL, or alternatively about 5 to about 40 ng/mL, or alternatively about 5 to about 50 ng/mL, or alternatively about 5 to about 100 ng/mL, or alternatively about 5 to about 250 ng/mL, or alternatively about 5 to about 500 ng/mL, or alternatively about 25 to about 75 ng/mL, or alternatively about 50 to about 100 ng/mL, or alternatively about 100 to about 500 ng/mL, or alternatively about 100 ng/mL to about 1 µg/mL, or alternatively about 1 µg/mL to about 10 µg/mL, or alternatively about 10 µg/mL to about 50 µg/mL, or alternatively about 50 µg/mL to about 100 µg/mL, or alternatively about 100 µg/mL to about 500 µg/mL, or alternatively about 500 µg/mL to about 1000 µg/mL of agent (e.g. anti-inflammatory agent, neurotrophic factor, or angiogenesis agent) is added to the purified population of cell-derived vesicles. In particular aspects, about 10 ng/mL, or alternatively about 15 ng/mL, or alternatively about 20 ng/mL, or alternatively about 25 ng/mL, or alternatively about 30 ng/mL, or alternatively about 40 ng/mL, or alternatively about 50 ng/mL, or alternatively about 100 ng/mL, or alternatively about 200 ng/mL, or alternatively about 250 ng/mL, or alternatively about 300 ng/mL, or alternatively about 400 ng/mL, or alternatively about 500 ng/mL, or alternatively about 1 µg/mL of agent is added to the purified population of cell-derived vesicles. Preferably, the agent is about 10 to about 1000 ng/mL.

[0037] In some embodiments, according to the methods described herein, the isolated stem cells that produce the cell-derived vesicles are one or more of adult stem cells, embryonic stem cells, embryonic-like stem cells, neural stem cells, or induced pluripotent stem cells. In some embodiments, the stem cells are mesenchymal stem cells that in one aspect, are cultured under hypoxic and low serum conditions.

[0038] In some embodiments, according to the methods described herein, the hypoxic conditions are between approximately 1% to about 15% CO₂, for example about 5% CO₂, and between about 0.05% to about 20% oxygen tension. In some embodiments, the oxygen tension is less than 5%, or alternatively less than 10%. In some embodiments, the oxygen tension is about 1%, or alternatively about 5%. In some embodiments, the low serum conditions are serum free conditions.

[0039] In some embodiments, according to the methods described herein, the tangential flow filtration unit used for isolation and/or purification of the cell-derived vesicles is between about 50 kilodalton and about 750 kilodalton nominal molecular weight limit filtration unit, for example, about a 100 kilodalton nominal molecular weight limit filtration unit or about a 300 kilodalton nominal molecular weight limit filtration unit.

[0040] In some embodiments, the methods described herein further comprise formulating the purified population of cell-derived vesicles by mixing the population with a carrier and/or another therapeutic agent either by admixing the components or by encapsulation of the therapeutic agent using methods known in the art.

[0041] In some embodiments, the methods described herein further comprise freezing or freeze drying the purified population of cell-derived vesicles and/or compositions.

[0042] Also provided herein are populations of cell-derived vesicles obtainable from the methods according to any one of the embodiments as described herein.

[0043] Further provided herein are lyophilized or frozen populations of cell-derived vesicles of the purified population or the composition according to any one of the embodiments as described herein.

[0044] Still further provided herein are kits comprising populations of cell-derived vesicles of any one of the embodiments as described herein and instructions for use.

[0045] In a further aspect, the disclosure relates to a method for large-scale purification of a population of cell-derived vesicles, comprising applying a tangential flow filtration to conditioned media produced by a population of isolated stem cells cultured in a bioreactor to isolate a cell-derived vesicles containing fraction; and concentrating the cell-derived vesicle containing fraction to provide a purified population of cell-derived vesicles. In some aspects,

the isolated stem cells are cultured in the presence of or contacted by one or more agents selected from an inflammatory agent, a neurotrophic factor, or an angiogenesis agent. In other aspects, one or more anti-inflammatory agents, neurotrophic factors, or angiogenesis agents are added to the purified population of cell-derived vesicles. In some aspects, one or more agents are recombinant. In particular embodiments, the inflammatory agent is selected from $\text{TNF}\alpha$, IL-6, IL-17, IL-1 β , IFN γ , lipopolysaccharide, or equivalents of each thereof; the anti-inflammatory agent is selected from TGF β 1-4, IL-2, IL-10, IL-17, IL-35, IL-37, or equivalents of each thereof; the neurotrophic factor is selected from BDNF, NGF, Neurotrophin-3, CTNF, GDNF, FGFs 1-23 (e.g. FGF1, FGF2), insulin-like growth factors (IGFs) (e.g IGF1, IGF2), HGF, Noggin, T3, or equivalents of each thereof; and/or the angiogenesis agent is selected from FGF2, VEGF, PDGF, HGF, FGF1, FGF2, EGF, TGF β 1-4, WNT1, or equivalents of each thereof.

BRIEF DESCRIPTION OF DRAWINGS

[0046] FIGS. 1A and 1B show analysis of HiRIEF LC-MS/MS proteomics data from IC and PAD conditions compared to control condition EX. **(FIG. 1A)** Heatmap of MSC cluster analysis of differentially regulated proteins in IC and PAD conditions as compared to EX. **(FIG. 1B)** Panther pathway analysis of proteins upregulated in MSCs under PAD-like conditions show abundance of canonical angiogenesis related pathway proteins: EGF, FGF and PDGF. Analysis of 3 different donors for each condition. For differential expression T-tests with multiple testing correction with an FDR of 1% was used.

[0047] FIGS. 2A to 2D show mesenchymal stem cells increase secretion of exosomes upon exposure to PAD-like conditions. **(FIG. 2A)** Quantification of total protein content of vesicles derived from MSC under EX, IC and PAD culture conditions using DC assay. **(FIG. 2B)** Scanning electron micrograph of MSCs cultured in EX culture conditions indicating microvesicle release (arrows) from the cell surface (scale bar 5 μm , 5kX). **(FIG. 2C)** Scanning electron micrograph of MSCs cultured under PAD conditions (scale bar 2 μm , 10kX) indicating exosome adhesion to cell surface (arrows). **(FIG. 2D)** Transmission electron micrograph of MSC derived exosomes with 2% uranyl acetate negative staining (scale bar 200 nm, 25kX).

[0048] **FIG. 3** shows analysis of HiRIEF LC-MS/MS proteomics data of MSC exosomes comparing PAD to IC conditions. Panther pathway analysis of PAD exosomes shows abundance of angiogenesis related pathway proteins: EGFR, FGF and PDGF pathway associated proteins (red asterisk indicate angiogenesis associated pathways). Analysis of 3 different donors for each condition. For differential expression T-tests with multiple testing correction with an FDR of 1% was used.

[0049] **FIG. 4** shows MSC exosome-induced *in vitro* tubule formation of HUVECs. Basal media (Neg), 5 µg/ml, 10 µg/ml, 20 µg/ml of MSC exosomes in basal media, EndoGRO media positive control (Pos). Stained with Calcein AM and imaged at 14 hours post stimulation with 4X objective. Quantification of total segment length of tubule formation analyzed using ImageJ's Angiogenesis plugin. EndoGRO positive control media contains 2% FBS, EGF 5ng/ml and heparin sulfate 0.75 U/ml. (*) Indicates a p-value <0.05 using ANOVA, LSD post hoc analysis (n = 12).

[0050] **FIG. 5** shows NFkB inhibition abrogates MSC exosome-mediated tubule formation in HUVECs *in vitro*. Quantification of total segment length of tubule formation using ImageJ's Angiogenesis plugin. EndoGRO media contains 2% FBS, EGF 5ng/ml and heparin sulfate 0.75 U/ml. (*) Indicates a p-value <0.01 using ANOVA, LSD post hoc analysis (n = 6).

[0051] **FIGS. 6A and 6B** show representative concordance and variation between MSC donors. **(FIG. 6A)** Heatmap of cellular global proteome expression differentials between IC/EX and PAD/EX across all 3 donors reveals some donor to donor variation as well as intra-condition and intra-donor concordance. **(FIG. 6B)** Comparison of PAD/EX donor ratios from all 3 donors reveals some donor to donor variation as well as intra-condition and intra-donor concordance. Dots represent PAD/EX protein expression ratios of donor 3 vs donor 1 and PAD/EX protein expression ratios of donor 2 vs donor 1. Line represents regression analysis of PAD/EX protein expression ratios of donor 3 vs donor 1 and regression analysis of PAD/EX protein expression ratios of donor 2 vs donor 1.

[0052] **FIG. 7** shows upregulation of cholesterol biosynthesis pathway proteins in PAD/EX. Ingenuity Pathway Analysis of differentially expressed cellular proteins (FDR-1%) revealed upregulation of proteins associated with the cholesterol biosynthesis pathway in the

PAD condition as compared to the EX condition. Dark gray boxes indicate increased expression, light gray boxes indicate lack of detection. Analysis of 3 different donors per condition. For differential expression, T-tests with multiple testing correction with an FDR of 1% were used.

[0053] FIGS. 8A and 8B show upregulation of exosome biogenesis proteins in PAD/EX. **(FIG. 8A)** Relative expression of known exosome biogenesis proteins demonstrated a trend towards increased expression in PAD/EX. **(FIG. 8B)** Vesicle associated protein family members demonstrated a trend towards increased expression in PAD/EX.

[0054] FIG. 9 shows quantitative PCR (qPCR) detection of miR-132 in microvesicles isolated from MSCs modified with a miR-132 lentiviral vector.

[0055] FIGS. 10 shows qPCR analysis determined presence of angiogenic miRNAs demonstrating their presence at various concentrations, normalized to U6.

[0056] FIG. 11 shows that MSC-Stroke exosomes are packaged with lipid membrane components with signaling functions. Hydrophilic interaction chromatography mass spectrometry analysis (FDR 1%) demonstrates that MSC-Stroke exosomes are packaged lipid bilayer membrane components and their derivatives with important signaling functions include sphingomyelin (SM), phosphatidylcholines (PC), phosphatidylethanolamine (PE) and fatty acids (FA), many of which are also important for the biogenesis of exosomes.

[0057] FIG. 12 shows exosome yield based on total exosomal protein content of standard cell culture flasks, 50x T175's vs GMP grade bioreactor. This data demonstrates that GMP-grade manufacturing using a hollow fiber reactor system generates much higher yields of exosomes as compared to standard tissue culture flasks.

[0058] FIG. 13 shows that exosomes are readily taken up by many cell types within one hour. Exosomes will exert their function on cells that they are able to effectively interact with. Applicants investigated the ability of various cell types to take up exosomes. Applicants specifically labeled the lipid membrane of exosomes with a fluorescent dye and added them to the culture media of the cells. After one hour of co-incubation with the labeled exosomes, the media was removed, the cells were washed 3 times with PBS, and the cells then were quantitatively assessed for the presence of the exosome-conjugated fluorescent dye. The

negative control was the fluorescent “labeling” of just PBS to ensure there were no artifacts from dye aggregation due to the sample processing steps involved with labeling the exosomes. Analysis of the cells via flow cytometry clearly shows that the majority (>80%) of cells from each group were positive for the presence of exosomes. **FIG. 13** shows quantitative summary of flow cytometry data.

[0059] **FIG. 14** shows that MSC-stroke derived exosomes are up-taken by primary endothelial cells, induce migration and tubule formation. MSC-stroke exosomes labeled with PKH26 and exposed to human primary endothelial cells (HUVECs) for 1 hour, nuclear stain Hoechst. MSC-Stroke induced migration of HUVECs within 6 hours (Calcein AM) T-test $*=p<0.05$.

[0060] **FIG. 15** shows that exosomes induce stem cell proliferation. After tissues are damaged, the body tries to repair this damaged tissue. During this process, localized stem cells are activated to aid in this tissue repair process. The ability of the formulation of exosomes disclosed herein to induce the proliferation of a type of tissue resident stem cell, myoblasts, which are a common type of muscle stem cells were tested. Muscle stem cells were stimulated with increasing doses of exosomes for 24-hours and assessed their ability to induce the proliferation of myoblasts by determining the presence of a marker for cells in a proliferative state (Ki67) using flow cytometry. **FIG. 15** shows quantitative summary of the flow cytometry data. Without being bound by theory, this data demonstrates that this formulation of exosomes induces stem cell proliferation in a dose dependent manner, which is an indication of their intrinsic tissue healing properties.

[0061] **FIG. 16** illustrates the exosomes’ anti-inflammatory properties and mixed lymphocyte reaction. The exosome formulation disclosed herein was tested for its ability to diminish an inflammatory response in vitro, using a canonical inflammation assay called the mixed lymphocyte reaction. Primary white blood cells (lymphocytes) were isolated from fresh human blood and cultured in vitro. These immune cells were then stimulated with an antigen (PHA) derived from bacteria to stimulate a strong immune response. During this immune response to the bacterial based antigen (PHA), the cells responsible for the inflammation response proliferate, which is a canonical process of inflammation. Therefore the degree to which the cells proliferate is an indication of how strongly inflammation has

been induced. Without being bound by theory, this flow cytometry demonstrates that primary human lymphocytes' inflammatory response to a bacterial antigen (PHA) is diminished when co-stimulated with the exosome formulation disclosed herein in a dose dependent manner, indicating potent anti-inflammatory properties of the exosomes. Additionally, this experiment used lyophilized exosomes, instead of freshly thawed exosomes, demonstrating that lyophilized exosomes maintain their functionality as measured by their anti-inflammatory properties. **FIG. 16** shows a quantitative summary of results of this experiment.

[0062] **FIG. 17** shows the anti-inflammatory properties of freshly thawed exosomes were compared to lyophilized exosomes, on a dose for dose basis using the same mixed lymphocyte reaction assay as in **FIG. 16**. Without being bound by theory, this data established that the exosome formulation disclosed herein retains its anti-inflammatory properties post-lyophilization. This is a critical piece of data demonstrating the feasibility of commercialization. The lyophilized product will be substantially cheaper to store and ship and be readily available for clinicians and patients alike to administer therapy without the need for cumbersome use of liquid nitrogen tanks needed for most stem cell based therapeutics. **FIG. 17** is a quantitative summary of the data.

[0063] **FIG. 18** shows that exosomes induce T-regulatory cell proliferation. This assay was used to determine the mechanism of action for the exosomes' anti-inflammatory properties. T-regulatory cells (Tregs) are a type of immune cell with potent anti-inflammatory properties. The exosome formulation disclosed herein was tested for its ability to activate Tregs using flow cytometry. This study demonstrates that the exosomes induced Treg activation in a dose dependent manner and the figure is a quantitative summary of the flow cytometry data. Without being bound by theory, these results indicate that the exosome formulation disclosed herein mediates its anti-inflammatory properties through the activation of Tregs.

[0064] **FIG. 19** show that exosomes and lyophilized exosomes induce comparable levels of anti-inflammatory and neuroprotective cytokines. The exosome formulation disclosed herein was tested for its ability to modify the expression of various cytokines by primary human immune cells (lymphocytes). Fresh lymphocytes were isolated from human blood and cultured them in vitro. The lymphocytes were then stimulated with exosomes. A Quantibody

array was used to quantitatively assess cytokine expression of the cells after 24-hours. Without being bound by theory, this data indicates that the exosomes reduce the expression of key inflammatory cytokines and induces the expression of critical anti-inflammatory cytokines in primary human lymphocytes. Additionally, this data demonstrates that the modulation of cytokine expression is comparable between freshly thawed and lyophilized exosomes. The cytokines tested in this assay are: IL-11, G-CSF, Eotaxin, IL-4, IL-7, MCSF, IL-12p70, IL-1a, BLC, IL-8, GM-CSF, and MIP-1d.

[0065] FIG. 20 shows that exosomes and lyophilized exosomes induce comparable levels of anti-inflammatory and neuroprotective cytokines. The exosome formulation disclosed herein was tested for its ability to modify the expression of various cytokines by primary human immune cells (lymphocytes). Fresh lymphocytes were isolated from human blood and cultured them in vitro. The lymphocytes were then stimulated with exosomes. A Quantibody array was used to quantitatively assess cytokine expression of the cells after 24-hours. Without being bound by theory, this data indicates that the exosomes reduce the expression of key inflammatory cytokines and induces the expression of critical anti-inflammatory cytokines in primary human lymphocytes. Additionally, this data demonstrates that the modulation of cytokine expression is comparable between freshly thawed and lyophilized exosomes. The cytokines tested in this assay are: IL-2, IL-15, IL-13, IFN γ , IL-6sR, IL-16, IL-1b, IL-1ra, MIP-1b, TNFb, IL-17, IL-12p40, PDGF-BB, IL-5, IL-6, and Eotaxin-2.

[0066] FIG. 21 shows that exosomes and lyophilized exosomes induce comparable levels of anti-inflammatory and neuroprotective cytokines. The exosome formulation disclosed herein was tested for its ability to modify the expression of various cytokines by primary human immune cells (lymphocytes). Fresh lymphocytes were isolated from human blood and cultured them in vitro. The lymphocytes were then stimulated with exosomes. A Quantibody Array was used to quantitatively assess cytokine expression of the cells after 24-hours. Without being bound by theory, this data indicates that the exosomes reduce the expression of key inflammatory cytokines and induces the expression of critical anti-inflammatory cytokines in primary human lymphocytes. Additionally, this data demonstrates that the modulation of cytokine expression is comparable between freshly thawed and

lyophilized exosomes. The cytokines tested in this assay are: TNF RI, IL-10, MCP-1, I-309, TNFa, RANTES, MIP-1a, MIG, TNF RII, TIMP-1, ICAM-1, TIMP-2.

[0067] **FIG. 22** shows that MSC-exosomes contain miRNAs that modulate Tregs and oligodendrocytes. Data was generated by qPCR analysy of MSC-exosomes. The graph shows that there are numerous miRNAs associated with either R regulatory cell modulations (miR15, miR-1312 an dmiR-150), oligodendrocyte modulation (miR-210), or miRNAs involved in both (miR-92, miR-100, miR-126, miR-181 and miR-214).

[0068] **FIG. 23** shows that MSC-exosomes induce proliferation and differentiation of oligodencrocyte progenitor cells. Primary rat glial restricted precursor cells (GRPs) were treated either MSC-Exosomes or vehicle controls. MSC-Exosomes treatment for 48 hours resulted in significantly more GRPs cells as detected by both a CCK8 assay, with verification by nuclear staining with Hoescht imaging using fluorescent microscopy (**FIG. 23**). MSC-Exosome treatment for 72 hours increased expression of both early and late markers of oligodendrocyte maturation via flow cytometry evaluation following immunostaining for respective markers: Nestin, CNP, Olig2 and MBP.

[0069] **FIG. 24** shows that MSC-exosomes reduce disease in a model of MS. MSC-Exosome treatment reduces disease severity and increases survival in a model of relapse remitting multiple sclerosis. Right panel: Immunized mice were treated with either 250 µg MSC-exosomes (dark gray, n= 17) or vehicle controls (light gray, n= 21) via tail vein administration immediately prior to the onset of symptoms on Day 9. Repeated measures assessment demonstrated that MSCExosome treatment significantly reduced the clinical presentation of disease severity during both the peak of disease as well as during the chronic phase and initial relapse. Left Panel: MSC-exosome treatment also increased animal survival, 17 of 17 with MSC-Exosome treatment, with only 18 of 21 mice surviving in the vehicle control group.

[0070] **FIGS. 25** shows that MSC-exosomes potentiate Trg residency in brain. Applicants found that MSC-exosome treatment significantly increased the presence of Tregs by 40% in the brains of EAE mice as compared to vehicle controls. 35 days post-treatment, mice were euthanized, perfused and brain were processed to a single cell suspension (Miltenyi kit) prior

to staining for canonical Treg markers with monoclonal antibodies, CD25 and CD4 (Con = 21 brains, Exo = 18 brains), * $p < 0.005$.

[0071] FIG. 26 show that MSC-exosomes reduce loss of myelin. MSC-exosome treatment via IV administration attenuates loss of myelin up to 35 days post treatment in the spinal cords of EAE immunized mice compared to vehicle controls. Loss of myelin in the central nervous system is a key hallmark of multiple sclerosis. Control on left, treated mice on right.

[0072] FIG. 27 shows that MSC-exosomes attenuate immune activation in the central nervous system. MSC-exosome treatment attenuates immune cell activation (microglia, IBA1) up to 35 days post treatment in the spinal cords of EAE immunized mice compared to vehicle controls. IBA1 staining was quantified using ImageJ both for the number of IBA1+ cells per slide and total % area of IBA1+ staining (Con = 12 sections, Exo = 12 sections, 4 sections/mouse x 3 mice), * $p < 0.0005$.

[0073] FIG. 28 shows that MSC-exosomes attenuate activation of scar forming cells. MSC-Exosome treatment attenuates astrocyte activation (GFAP) up to 35 days post treatment in the spinal cords of EAE immunized mice, compared to vehicle controls. GFAP staining was quantified using ImageJ both for the number of GFAP+ cells per slide and total % area of GFAP+ staining (Con = 18 sections, Exo = 18 sections, 6 sections/mouse x 3 mice), * $p < 0.0005$.

DESCRIPTION OF EMBODIMENTS

[0074] It is to be understood that this invention is not limited to particular embodiments described, as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting, since the scope of this invention will be limited only by the appended claims.

[0075] The detailed description of the invention is divided into various sections only for the reader's convenience and disclosure found in any section may be combined with that in another section. Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can also be used in the practice or testing of the present invention, the

preferred methods and materials are now described. All publications mentioned herein are incorporated by reference to disclose and describe the methods and/or materials in connection with which the publications are cited.

[0076] All numerical designations, e.g., pH, temperature, time, concentration, and molecular weight, including ranges, are approximations which are varied (+) or (-) by increments of 0.1 or 1.0, where appropriate. It is to be understood, although not always explicitly stated, that all numerical designations are preceded by the term “about.” It also is to be understood, although not always explicitly stated, that the reagents described herein are merely exemplary and that equivalents of such are known in the art.

[0077] It must be noted that as used herein and in the appended claims, the singular forms “a”, “an”, and “the” include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to “a cell” includes a plurality of cells.

Definitions

[0078] The following definitions assist in defining the meets and bounds of the inventions as described herein. Unless specifically noted, the embodiments describing “cell-derived vesicles” shall include “exosomes,” “microvesicles” alone or in combination. In some aspects, only one or more of the vesicles are intended.

[0079] The term “about” when used before a numerical designation, *e.g.*, temperature, time, amount, concentration, and such other, including a range, indicates approximations which may vary by (+) or (-) 10 %, 5 % or 1 %.

[0080] The terms “administering” or “administration” in reference to delivering cell-derived vesicles to a subject include any route of introducing or delivering to a subject the cell-derived vesicles to perform the intended function. Administration can be carried out by any suitable route, including orally, intranasally, parenterally (intravenously, intramuscularly, intraperitoneally, or subcutaneously), intrathecally, intracranially, or topically. Additional routes of administration include intraorbital, infusion, intraarterial, intracapsular, intracardiac, intradermal, intrapulmonary, intraspinal, intrasternal, intrauterine, intravenous, subarachnoid, subcapsular, subcutaneous, transmucosal, or transtracheal. Administration includes self-administration and the administration by another.

[0081] “Comprising” or “comprises” is intended to mean that the compositions, for example media, and methods include the recited elements, but not excluding others. “Consisting essentially of” when used to define compositions and methods, shall mean excluding other elements of any essential significance to the combination for the stated purpose. Thus, a composition consisting essentially of the elements as defined herein would not exclude other materials or steps that do not materially affect the basic and novel characteristic(s) of the claimed invention. “Consisting of” shall mean excluding more than trace elements of other ingredients and substantial method steps. Embodiments defined by each of these transition terms are within the scope of this invention.

[0082] As used herein, the term “modified,” relative to cell-derived vesicles, refers to cell-derived vesicles (e.g., exosomes and/or microvesicles) that have been altered such that they differ from a naturally occurring cell-derived vesicles. Non-limiting examples of a modified cell-derived vesicle include an exosome and/or microvesicle that contains a nucleic acid or protein of a type or in an amount different than that found in a naturally occurring exosome and/or microvesicle.

[0083] The terms “patient,” “subject,” or “mammalian subject” are used interchangeably herein and include any mammal in need of the treatment or prophylactic methods described herein (e.g., methods for the treatment of inflammation). Such mammals include, particularly humans (e.g., fetal humans, human infants, human teens, human adults, *etc.*). Other mammals in need of such treatment or prophylaxis can include non-human mammals such as dogs, cats, or other domesticated animals, horses, livestock, laboratory animals (e.g., lagomorphs, non-human primates, *etc.*), and the like. The subject may be male or female. In certain embodiments the subject is at risk, but asymptomatic for diseases or conditions related to inflammation or an inflammatory response. In certain embodiments the subject is at risk, but asymptomatic for PAD. McDermott et al. (2008) *Circulation* 117(19) 2484-2491. In certain embodiments, the subject expresses symptoms of peripheral arterial disease (PAD), e.g., intermittent claudication (muscle pain, cramping of arms or legs), leg numbness or weakness, change of color of legs, weak or no pulse, and erectile dysfunction in men.

[0084] The term “purified population” or “enriched population” relative to cell-derived vesicles, as used herein refers to plurality of cell-derived vesicles that have undergone one or

more processes of selection for the enrichment or isolation of the desired exosome population relative to some or all of some other component with which cell-derived vesicles are normally found in culture media. Alternatively, "purified" can refer to the removal or reduction of residual undesired components found in the conditioned media (e.g., cell debris, soluble proteins, etc.). A "highly purified population" as used herein, refers to a population of cell-derived vesicles in which at least 50%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99% or 100% of cell debris and soluble proteins (e.g., proteins derived from fetal bovine serum and the like) in the conditioned media along with the cell-derived vesicles are removed.

[0085] The terms "treatment," "treat," "treating," etc. as used herein, include but are not limited to, alleviating a symptom of a disease or condition (e.g., a disease or condition involving an inflammatory response or related to inflammation in a subject in need thereof) and/or reducing, suppressing, inhibiting, lessening, ameliorating or affecting the progression, severity, and/or scope of the disease or condition. Additional treatments include but are not limited to promoting angiogenesis, treating inflammatory disease, treating brain inflammatory disease, treating stroke, treating muscular sclerosis (MS), treating primary and secondary progressive MS, treating relapsing remitting MS, treating brain inflammation treating radiation-induced soft tissue damage, treating frailty, treating treating peripheral arterial disease (PAD), treating wounds, treating ischemia, acute and chronic limb ischemia, Buerger's disease, and critical limb ischemia in diabetes. "Treatments" refer to one or both of therapeutic treatment and prophylactic or preventative measures. Subjects in need of treatment include those already affected by a disease or disorder or undesired physiological condition as well as those in which the disease or disorder or undesired physiological condition is to be prevented. In one aspect, the term "treatment" excludes prophylaxis. In another aspect, treatment is only prophylaxis.

[0086] The term "stem cell" refers to a cell that is in an undifferentiated or partially differentiated state and has the capacity to self-renew and to generate differentiated progeny. Self-renewal is defined as the capability of a stem cell to proliferate and give rise to more such stem cells, while maintaining its developmental potential (i.e., totipotent, pluripotent, multipotent, etc.). The term "somatic stem cell" is used herein to refer to any stem cell derived from non-embryonic tissue, including fetal, juvenile, and adult tissue. Natural

somatic stem cells have been isolated from a wide variety of adult tissues including blood, bone marrow, brain, olfactory epithelium, skin, pancreas, skeletal muscle, and cardiac muscle. Exemplary naturally occurring somatic stem cells include, but are not limited to, mesenchymal stem cells (MSCs) and neural stem cells (NSCs). In some embodiments, the stem or progenitor cells can be embryonic stem cells. As used herein, "embryonic stem cells" refers to stem cells derived from tissue formed after fertilization but before the end of gestation, including pre-embryonic tissue (such as, for example, a blastocyst), embryonic tissue, or fetal tissue taken any time during gestation, typically but not necessarily before approximately 10-12 weeks gestation. Most frequently, embryonic stem cells are pluripotent cells derived from the early embryo or blastocyst. Embryonic stem cells can be obtained directly from suitable tissue, including, but not limited to human tissue, or from established embryonic cell lines. "Embryonic-like stem cells" refer to cells that share one or more, but not all characteristics, of an embryonic stem cell.

[0087] A "mesenchymal stem cell," or MSC, is a multipotent stem cell that can differentiate into a variety of cell types. Cell types that MSCs have been shown to differentiate into *in vitro* or *in vivo* include osteoblasts, chondrocytes, myocytes, and adipocytes. Mesenchyme is embryonic connective tissue that is derived from the mesoderm and that differentiates into hematopoietic and connective tissue, whereas MSCs do not differentiate into hematopoietic cells. Stromal cells are connective tissue cells that form the supportive structure in which the functional cells of the tissue reside. Methods to isolate such cells, propagate and differentiate such cells are known in the technical and patent literature, e.g., U.S. Patent Publication Nos. 2007/0224171, 2007/0054399, 2009/0010895, which are incorporated by reference in their entirety. In one embodiment, the MSCs are plastic-adherent when maintained in standard culture conditions. In one embodiment, the MSC has the phenotype CD34⁻/CD45⁻/CD105⁺/CD90⁺/CD73⁺. In another embodiment, the MSC has the phenotype CD45⁻/CD34⁻/CD14⁻ or CD11b⁻/CD79a⁻ or CD19⁻/HLA-DR⁻ or HLA-DR^{low}/CD105⁺/CD90⁺/CD73⁺.

[0088] The term "induced pluripotent stem cells" as used herein is given its ordinary meaning and also refers to differentiated mammalian somatic cells (e.g., adult somatic cells, such as skin) that have been reprogrammed to exhibit at least one characteristic of pluripotency. See, for example, Takahashi et al. (2007) *Cell* 131(5):861-872, Kim et al.

(2011) *Proc. Natl. Acad. Sci.* 108(19): 7838–7843, Sell, S. *Stem Cells Handbook*. New York: Springer, 2013. Print.

[0089] The term “exogenous” in reference to a nucleic acid or protein refers to a polynucleotide or polypeptide sequence that has been artificially introduced into a cell, cell-derived vesicles, exosomes, microvesicle, or combination thereof. There may be an endogenous nucleic acid or protein having the same or substantially similar sequence as that of the polynucleotide or polypeptide encoding the exogenous nucleic acid or protein in the cell-derived vesicles or they may be a non-naturally occurring nucleic acid or protein to the a cell, cell-derived vesicles, exosomes, microvesicle, or combination thereof. For example, a mesenchymal stem cell can be genetically modified to overexpress a PDGFR-encoding polynucleotide. It is contemplated that a purified population of cell-derived vesicles isolated from the culture media collected from MSCs genetically modified to overexpress a gene or protein e.g., PDGFR would contain higher levels of PDGFR as compared to cell-derived vesicles isolated from MSCs that have not been modified to overexpress a PDGFR-encoding polynucleotide.

[0090] As used herein, the term “agent” or “factor” refers to a molecule, complex of molecules, cell, organelle, cellular product, or cellular component or fragment that is chemically, physically, and/or biologically active. Nonlimiting examples of agents include but are not limited to peptides, polypeptides, proteins, nucleic acids, polynucleotides, DNA, RNA, miRNA, siRNA, mRNA, lipids, small molecules, sugars, pharmaceutical compounds, cells, stem cells, cell-derived vesicles, cytokines, chemokines, steroids, microbes, viruses, vaccines, blood, blood components, allergenics, somatic cells, and tissues. In some aspects, administration or use of an agent or factor results in a desired effect in a target cell, cell product, population, cell-derived vesicle, and/or subject. For example, a neurotrophic factor may produce a neuroprotective effect. An angiogenesis agent may produce a pro-angiogenesis effect. An inflammatory agent may produce a pro-inflammatory response and/or trigger stem cells to react to inflammation by producing anti-inflammatory agents. An anti-inflammatory agent may produce an anti-inflammatory effect.

[0091] As used herein, the term "microRNAs" or "miRNAs" refers to post-transcriptional regulators that typically bind to complementary sequences in the three prime untranslated

regions (3' UTRs) of target messenger RNA transcripts (mRNAs), usually resulting in gene silencing. Typically, miRNAs are short, non-coding ribonucleic acid (RNA) molecules, for example, 21 or 22 nucleotides long. The terms "microRNA" and "miRNA" are used interchangeably.

[0092] As used herein, the terms "overexpress," "overexpression," and the like are intended to encompass increasing the expression of a nucleic acid or a protein to a level greater than the exosome naturally contains. It is intended that the term encompass overexpression of endogenous, as well as heterologous nucleic acids and proteins.

[0093] As used herein, the term "homogeneous" in reference to a population of cell-derived vesicles refers to population of cell-derived vesicles that have a similar amount of an exogenous nucleic acid, a similar amount of an exogenous protein, are of a similar size, or combinations thereof. A homogenous population is one wherein about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, about 98%, or 100% of the cell-derived vesicles share at least one characteristic. For example, in some embodiments about 90% of the cell-derived vesicles in the homogenous purified population overexpress miR-132. For example, in some embodiments about 90% of the cell-derived vesicles in the homogenous purified population overexpress miR-132 wherein the miR-132 is expressed at an amount that is at least 2 times greater than that typically found in cell-derived vesicles. Another example of a homogenous population is one wherein about 90% of the exosomes are less than 50 nm in diameter.

[0094] As used herein, the term "heterogeneous" in reference to a population of cell-derived vesicles refers to population of cell-derived vesicles that have differing amounts of an exogenous nucleic acid, differing amounts of an exogenous protein, are of a different size, or combinations thereof.

[0095] The term "substantially" refers to the complete or nearly complete extent or degree of a characteristic and in some aspects, defines the purity of the isolated or purified population of exosomes or microvesicle. For example, a substantially homogenous cell-derived vesicle population may be a cell-derived vesicle population that contains more than 50%, more than 60%, more than 70%, more than 80%, more than 90%, more than 95%, more

than 98%, or 100% cell-derived vesicles that comprise at least one exogenous nucleic acid, protein, or both.

[0096] As used herein, the term "tangential-flow filtration" (TFF) refers to a process in which the fluid mixture containing the cell-derived vesicles to be separated by filtration is recirculated at high velocities tangential to the plane of the membrane to increase the mass-transfer coefficient for back diffusion. In such filtrations a pressure differential is applied along the length of the membrane to cause the fluid and filterable solutes to flow through the filter. This filtration is suitably conducted as a batch process as well as a continuous-flow process. For example, the solution may be passed repeatedly over the membrane while that fluid which passes through the filter is continually drawn off into a separate unit or the solution is passed once over the membrane and the fluid passing through the filter is continually processed downstream. Tangential flow may contain cassette filters or cartridge (also called hollow fiber) filters that the membrane forms a set of parallel hollow fibers. The feed stream passes through the lumen of the fibers and the permeate is collected from outside the fibers. Cartridges are characterized in terms of fiber length, lumen diameter and number of fibers, as well as filter pore size.

[0097] As used herein, the term "pharmaceutically acceptable carrier" refers to any of the standard pharmaceutical carriers such as sterile solutions, tablets, coated tablets, and capsules. Typically such carriers contain excipients such as starch, milk, sugar, certain types of clay, gelatin, stearic acids or salts thereof, magnesium or calcium stearate, talc, vegetable fats or oils, gums, glycols, or other known excipients. Such carriers may also include flavor and color additives or other ingredients. Examples of pharmaceutically acceptable carriers include, but are not limited to, the following: water, saline, buffers, inert, nontoxic solids (e.g., mannitol, talc). Compositions comprising such carriers are formulated by well-known conventional methods. Depending on the intended mode of administration and the intended use, the compositions may be in the form of solid, semi-solid, or liquid dosage forms, such, for example, as powders, granules, crystals, liquids, suspensions, liposomes, pastes, creams, salves, etc., and may be in unit-dosage forms suitable for administration of relatively precise dosages.

[0098] An “effective amount” intends an amount sufficient to effect beneficial or desired results. An effective amount can be administered in one or more administrations, applications or dosages. Such delivery is dependent on a number of variables including the time period for which the individual dosage unit is to be used, the bioavailability of the therapeutic agent, the route of administration, etc. It is understood, however, that specific dose levels of the therapeutic agents of the present invention for any particular subject depends upon a variety of factors including the activity of the specific compound employed, the age, body weight, general health, sex, and diet of the subject, the time of administration, the rate of excretion, the drug combination, and the severity of the particular disorder being treated and form of administration. Treatment dosages generally may be titrated to optimize safety and efficacy. Typically, dosage-effect relationships from *in vitro* and/or *in vivo* tests initially can provide useful guidance on the proper doses for patient administration. In general, one will desire to administer an amount of the compound that is effective to achieve a serum level commensurate with the concentrations found to be effective *in vitro*. Determination of these parameters is well within the skill of the art. These considerations, as well as effective formulations and administration procedures are well known in the art and are described in standard textbooks.

[0099] As used herein, the term “peripheral arterial disease” or “PAD” refers is a subset of peripheral vascular disease. Peripheral arterial disease or peripheral artery disease can occur in arteries other than those supplying blood to the heart, but most often occurs in the legs and feet. The disease is characterized by segmental lesions causing stenosis or occlusion, usually in large and medium-sized arteries. Atherosclerosis is the leading cause of PAD, which results in atherosclerotic plaques with calcium deposition, thinning of the media, patchy destruction of muscle and elastic fibers, fragmentation of the internal elastic lamina, and thrombi composed of platelets and fibrin. Common sites for PAD are the femoral and popliteal arteries, (80 to 90% of patients), the abdominal aorta and iliac arteries (30% of patients) and the distal vessels, including the tibial artery and peroneal artery (40-50% of patients). The incidence of distal lesions increases with diabetes and with age. Conditions associated with PAD may be occlusive or functional. Examples of occlusive PAD include peripheral arterial occlusion, which may be acute, and Buerger's disease (thromboangiitis obliterans), Raynaud's disease, Raynaud's phenomenon and acrocyanosis.

Additional non-limiting examples of diseases to be treated include acute and chronic critical limb ischemia, Buerger's disease and critical limb ischemia in diabetes.

[0100] As used herein, the term “dermal wound” refers to an injury to the skin in which the skin is cut or broken.

[0101] As used herein, the term “promoting angiogenesis” refers to the stimulation of new blood vessels, repairing damaged blood vessels, or increasing the number of blood vessels.

[0102] The terms "inflammatory response" and "inflammation" as used herein indicate the complex biological response of vascular and lymphoid tissues of an individual to harmful stimuli, such as pathogens, damaged cells, or irritants, and includes secretion of cytokines and, more particularly, of pro-inflammatory cytokines, i.e. cytokines which are produced predominantly by activated immune cells and are involved in the amplification of inflammatory reactions. Exemplary pro-inflammatory cytokines and chemokines include but are not limited to IL-1 β , TNF- α , IFN- γ , IL-8, IL-6, IL-12, IL-15, IL-16, IL-17 (including family members IL17A, IL17B, IL-17C, IL-17D, IL-17E, IL-17F), IL-18, GM-CSF, IL-21, IL-23, IL-27 and TGF- β . Exemplary anti-inflammatory cytokines include but are not limited to TGF- β , IL-1R α , IL-4, IL-6, IL-10, IL-11, IL-13, IL-35, INF- α . A cytokine may have either pro-inflammatory and anti-inflammatory properties depending on the particular biological context (Cavaillon, J.M (2001) Cell Mol Biol 47(4): 695-702). Exemplary inflammations include acute inflammation and chronic inflammation. Acute inflammation indicates a short-term process characterized by the classic signs of inflammation (swelling, redness, pain, heat, and loss of function) due to the infiltration of the tissues by plasma and leukocytes. An acute inflammation typically occurs as long as the injurious stimulus is present and ceases once the stimulus has been removed, broken down, or walled off by scarring (fibrosis). Chronic inflammation indicates a condition characterized by concurrent active inflammation, tissue destruction, and attempts at repair. Chronic inflammation is not characterized by the classic signs of acute inflammation listed above. Instead, chronically inflamed tissue is characterized by the infiltration of mononuclear immune cells (monocytes, macrophages, lymphocytes, and plasma cells), tissue destruction, and attempts at healing, which include angiogenesis and fibrosis. An inflammation can be inhibited in the sense of the

present disclosure by affecting and in particular inhibiting any one of the events that form the complex biological response associated with an inflammation in an individual.

[0103] As used herein, exemplary diseases or conditions associated with or related to inflammation and/or inflammatory responses include but are not limited to multiple sclerosis, primary and secondary progressive MS, relapsing remitting MS, brain inflammation, radiation-induced soft tissue damage, frailty, neuroinflammatory disease, brain inflammatory disease, muscle injuries, radiation tissue damage, stroke, traumatic brain injury, myocardial infarction, graft versus host disease, Parkinson's disease, Alzheimer's, inflammatory bowel disease, Huntington's disease, amyotrophic lateral sclerosis, Bahcet's disease, sarcopenia, aging, spinal cord injury, wound repair, and dysphagia. Additional diseases or conditions associated with or related to inflammation and/or inflammatory responses include autoimmune disease or disorders.

[0104] As used herein, "neuroinflammatory disease" or "neuroinflammation" is inflammation of the nervous tissue and related diseases or conditions. In one embodiment, neuroinflammation is an immune response that causes damage to the central nervous system. Neuroinflammation can be caused by infection, traumatic brain injury, toxic metabolites, neurodegeneration, and/or autoimmunity. Exemplary neuroinflammatory diseases include but are not limited to acute disseminated encephalomyelitis (ADEM), Optic Neuritis (ON), Transverse Myelitis, Neuromyelitis Optica (NMO), Alzheimer's disease, Parkinson's disease, multiple sclerosis, primary and secondary progressive MS, relapsing remitting MS, brain inflammation and traumatic brain injury.

[0105] "Autoimmune disease or disorder" includes diseases or disorders arising from and directed against an individual's own tissues or organs or manifestation thereof or a condition resulting there from. In one embodiment, it refers to a condition that results from, or is aggravated by, the production by T cells that are reactive with normal body tissues and antigens. Examples of autoimmune diseases or disorders include, but are not limited to arthritis (rheumatoid arthritis such as acute arthritis, chronic rheumatoid arthritis, gout or gouty arthritis, acute gouty arthritis, acute immunological arthritis, chronic inflammatory arthritis, degenerative arthritis, type II collagen-induced arthritis, infectious arthritis, Lyme arthritis, proliferative arthritis, psoriatic arthritis, Still's disease, vertebral arthritis, and

juvenile-onset rheumatoid arthritis, osteoarthritis, arthritis chronica progrediente, arthritis deformans, polyarthritis chronica primaria, reactive arthritis, and ankylosing spondylitis), inflammatory hyperproliferative skin diseases, psoriasis such as plaque psoriasis, guttate psoriasis, pustular psoriasis, and psoriasis of the nails, atopy including atopic diseases such as hay fever and Job's syndrome, dermatitis including contact dermatitis, chronic contact dermatitis, exfoliative dermatitis, allergic dermatitis, allergic contact dermatitis, dermatitis herpetiformis, nummular dermatitis, seborrheic dermatitis, non-specific dermatitis, primary irritant contact dermatitis, and atopic dermatitis, x-linked hyper IgM syndrome, allergic intraocular inflammatory diseases, urticaria such as chronic allergic urticaria and chronic idiopathic urticaria, including chronic autoimmune urticaria, myositis, polymyositis/dermatomyositis, juvenile dermatomyositis, toxic epidermal necrolysis, scleroderma (including systemic scleroderma), sclerosis such as systemic sclerosis, multiple sclerosis (MS) such as spino-optical MS, primary primary and secondary progressive MS (PPMS), and relapsing remitting MS (RRMS), progressive systemic sclerosis, atherosclerosis, arteriosclerosis, sclerosis disseminata, ataxic sclerosis, neuromyelitis optica spectrum disorder (NMO, also known as Devic's Disease or Devic's Syndrome), inflammatory bowel disease (IBD) (for example, Crohn's disease, autoimmune-mediated gastrointestinal diseases, colitis such as ulcerative colitis, colitis ulcerosa, microscopic colitis, collagenous colitis, colitis polyposa, necrotizing enterocolitis, and transmural colitis, and autoimmune inflammatory bowel disease), bowel inflammation, pyoderma gangrenosum, erythema nodosum, primary sclerosing cholangitis, respiratory distress syndrome, including adult or acute respiratory distress syndrome (ARDS), meningitis, inflammation of all or part of the uvea, iritis, choroiditis, an autoimmune hematological disorder, rheumatoid spondylitis, rheumatoid synovitis, hereditary angioedema, cranial nerve damage as in meningitis, herpes gestationis, pemphigoid gestationis, pruritis scroti, autoimmune premature ovarian failure, sudden hearing loss due to an autoimmune condition, IgE-mediated diseases such as anaphylaxis and allergic and atopic rhinitis, encephalitis such as Rasmussen's encephalitis and limbic and/or brainstem encephalitis, uveitis, such as anterior uveitis, acute anterior uveitis, granulomatous uveitis, nongranulomatous uveitis, phacoantigenic uveitis, posterior uveitis, or autoimmune uveitis, glomerulonephritis (GN) with and without nephrotic syndrome such as chronic or acute glomerulonephritis such as primary GN, immune-

mediated GN, membranous GN (membranous nephropathy), idiopathic membranous GN or idiopathic membranous nephropathy, membrano- or membranous proliferative GN (MPGN), including Type I and Type II, and rapidly progressive GN, proliferative nephritis, autoimmune polyglandular endocrine failure, balanitis including balanitis circumscripta plasmacellularis, balanoposthitis, erythema annulare centrifugum, erythema dyschromicum perstans, erythema multiform, granuloma annulare, lichen nitidus, lichen sclerosus et atrophicus, lichen simplex chronicus, lichen spinulosus, lichen planus, lamellar ichthyosis, epidermolytic hyperkeratosis, premalignant keratosis, pyoderma gangrenosum, allergic conditions and responses, allergic reaction, eczema including allergic or atopic eczema, asteatotic eczema, dyshidrotic eczema, and vesicular palmoplantar eczema, asthma such as asthma bronchiale, bronchial asthma, and auto-immune asthma, conditions involving infiltration of T cells and chronic inflammatory responses, immune reactions against foreign antigens such as fetal A-B-O blood groups during pregnancy, chronic pulmonary inflammatory disease, autoimmune myocarditis, leukocyte adhesion deficiency, lupus, including lupus nephritis, lupus cerebritis, pediatric lupus, non-renal lupus, extra-renal lupus, discoid lupus and discoid lupus erythematosus, alopecia lupus, systemic lupus erythematosus (SLE) such as cutaneous SLE or subacute cutaneous SLE, neonatal lupus syndrome (NLE), and lupus erythematosus disseminatus, Type I diabetes, Type II diabetes, latent autoimmune diabetes in adults (or Type 1.5 diabetes). Also contemplated are immune responses associated with acute and delayed hypersensitivity mediated by cytokines and T-lymphocytes, sarcoidosis, granulomatosis including lymphomatoid granulomatosis, Wegener's granulomatosis, agranulocytosis, vasculitides, including vasculitis, large-vessel vasculitis (including polymyalgia rheumatica and giant cell (Takayasu's) arteritis), medium-vessel vasculitis (including Kawasaki's disease and polyarteritis nodosa/periarteritis nodosa), microscopic polyarteritis, immunovascularitis, CNS vasculitis, cutaneous vasculitis, hypersensitivity vasculitis, necrotizing vasculitis such as systemic necrotizing vasculitis, and ANCA-associated vasculitis, such as Churg-Strauss vasculitis or syndrome (CSS) and ANCA-associated small-vessel vasculitis, temporal arteritis, aplastic anemia, autoimmune aplastic anemia, Coombs positive anemia, Diamond Blackfan anemia, hemolytic anemia or immune hemolytic anemia including autoimmune hemolytic anemia (AIHA), Addison's disease, autoimmune neutropenia, pancytopenia, leukopenia, diseases involving leukocyte

diapedesis, CNS inflammatory disorders, brain inflammation, Alzheimer's disease, Parkinson's disease, multiple organ injury syndrome such as those secondary to septicemia, trauma or hemorrhage, antigen-antibody complex-mediated diseases, anti-glomerular basement membrane disease, anti-phospholipid antibody syndrome, anti-phospholipid syndrome, allergic neuritis, Behcet's disease/syndrome, Castleman's syndrome, Goodpasture's syndrome, Reynaud's syndrome, Sjogren's syndrome, Stevens-Johnson syndrome, pemphigoid such as pemphigoid bullous and skin pemphigoid, pemphigus (including pemphigus vulgaris, pemphigus foliaceus, pemphigus mucus-membrane pemphigoid, and pemphigus erythematosus), autoimmune polyendocrinopathies, Reiter's disease or syndrome, thermal injury, preeclampsia, an immune complex disorder such as immune complex nephritis, antibody-mediated nephritis, polyneuropathies, MS, primary and secondary progressive MS, relapsing remitting MS, chronic neuropathy such as IgM polyneuropathies or IgM-mediated neuropathy, autoimmune or immune-mediated thrombocytopenia such as idiopathic thrombocytopenic purpura (ITP) including chronic or acute ITP, acquired thrombocytopenic purpura, scleritis such as idiopathic cerato-scleritis, episcleritis, autoimmune disease of the testis and ovary including autoimmune orchitis and oophoritis, primary hypothyroidism, hypoparathyroidism, autoimmune endocrine diseases including thyroiditis such as autoimmune thyroiditis, Hashimoto's disease, chronic thyroiditis (Hashimoto's thyroiditis), or subacute thyroiditis, autoimmune thyroid disease, idiopathic hypothyroidism, Graves disease, polyglandular syndromes such as autoimmune polyglandular syndromes (or polyglandular endocrinopathy syndromes), paraneoplastic syndromes, including neurologic paraneoplastic syndromes such as Lambert-Eaton myasthenic syndrome or Eaton-Lambert syndrome, stiff-man or stiff-person syndrome, encephalomyelitis such as allergic encephalomyelitis or encephalomyelitis allergica and experimental allergic encephalomyelitis (EAE), myasthenia gravis such as thymoma-associated myasthenia gravis, cerebellar degeneration, neuromyotonia, opsoclonus or opsoclonus myoclonus syndrome (OMS), and sensory neuropathy, multifocal motor neuropathy, Sheehan's syndrome, autoimmune hepatitis, chronic hepatitis, lupoid hepatitis, giant cell hepatitis, chronic active hepatitis or autoimmune chronic active hepatitis, lymphoid interstitial pneumonitis (LIP), bronchiolitis obliterans (non-transplant) vs NSIP, Guillain-Barre syndrome, Berger's disease (IgA nephropathy), idiopathic IgA nephropathy,

linear IgA dermatosis, acute febrile neutrophilic dermatosis, subcorneal pustular dermatosis, transient acantholytic dermatosis, cirrhosis such as primary biliary cirrhosis and pneumonocirrhosis, autoimmune enteropathy syndrome, Celiac or Coeliac disease, celiac sprue (gluten enteropathy), refractory sprue, idiopathic sprue, cryoglobulinemia, amyotrophic lateral sclerosis (ALS; Lou Gehrig's disease), coronary artery disease, autoimmune ear disease such as autoimmune inner ear disease (AIED), autoimmune hearing loss, polychondritis such as refractory or relapsed or relapsing polychondritis, pulmonary alveolar proteinosis, Cogan's syndrome/nonsyphilitic interstitial keratitis, Bell's palsy, Sweet's disease/syndrome, rosacea autoimmune, zoster-associated pain, amyloidosis, a non-cancerous lymphocytosis, a primary lymphocytosis, which includes monoclonal B cell lymphocytosis (e.g., benign monoclonal gammopathy and monoclonal gammopathy of undetermined significance, MGUS), peripheral neuropathy, paraneoplastic syndrome, channelopathies such as epilepsy, migraine, arrhythmia, muscular disorders, deafness, blindness, periodic paralysis, and channelopathies of the CNS, autism, inflammatory myopathy, focal or segmental or focal segmental glomerulosclerosis (FSGS), endocrine ophthalmopathy, uveoretinitis, chorioretinitis, autoimmune hepatological disorder, fibromyalgia, multiple endocrine failure, Schmidt's syndrome, adrenalitis, gastric atrophy, presenile dementia, demyelinating diseases such as autoimmune demyelinating diseases and chronic inflammatory demyelinating polyneuropathy, Dressler's syndrome, alopecia areata, alopecia totalis, CREST syndrome (calcinosis, Raynaud's phenomenon, esophageal dysmotility, sclerodactyly, and telangiectasia), male and female autoimmune infertility, e.g., due to anti-spermatozoan antibodies, mixed connective tissue disease, Chagas' disease, rheumatic fever, recurrent abortion, farmer's lung, erythema multiforme, post-cardiotomy syndrome, Cushing's syndrome, bird-fancier's lung, allergic granulomatous angiitis, benign lymphocytic angiitis, Alport's syndrome, alveolitis such as allergic alveolitis and fibrosing alveolitis, interstitial lung disease, transfusion reaction, leprosy, malaria, parasitic diseases such as leishmaniasis, kypanosomiasis, schistosomiasis, ascariasis, aspergillosis, Sampter's syndrome, Caplan's syndrome, dengue, endocarditis, endomyocardial fibrosis, diffuse interstitial pulmonary fibrosis, interstitial lung fibrosis, pulmonary fibrosis, idiopathic pulmonary fibrosis, cystic fibrosis, endophthalmitis, erythema elevatum et diutinum, erythroblastosis fetalis, eosinophilic fasciitis, Shulman's syndrome, Felty's syndrome, flariasis,

cyclitis such as chronic cyclitis, heterochronic cyclitis, iridocyclitis (acute or chronic), or Fuch's cyclitis, Henoch-Schonlein purpura, human immunodeficiency virus (HIV) infection, SCID, acquired immune deficiency syndrome (AIDS), echovirus infection, sepsis, endotoxemia, pancreatitis, thyroxicosis, parvovirus infection, rubella virus infection, post-vaccination syndromes, congenital rubella infection, Epstein-Barr virus infection, mumps, Evan's syndrome, autoimmune gonadal failure, Sydenham's chorea, post-streptococcal nephritis, thromboangitis obliterans, thyrotoxicosis, tabes dorsalis, chorioiditis, giant cell polymyalgia, chronic hypersensitivity pneumonitis, keratoconjunctivitis sicca, epidemic keratoconjunctivitis, idiopathic nephritic syndrome, minimal change nephropathy, benign familial and ischemia-reperfusion injury, transplant organ reperfusion, retinal autoimmunity, joint inflammation, bronchitis, chronic obstructive airway/pulmonary disease, silicosis, aphthae, aphthous stomatitis, arteriosclerotic disorders, asperniogenesis, autoimmune hemolysis, Boeck's disease, cryoglobulinemia, Dupuytren's contracture, endophthalmitis, phacoanaphylactica, enteritis allergica, erythema nodosum leprosum, idiopathic facial paralysis, chronic fatigue syndrome, febris rheumatica, Hamman-Rich's disease, sensorineural hearing loss, haemoglobinuria paroxysmatica, hypogonadism, ileitis regionalis, leucopenia, mononucleosis infectiosa, transverse myelitis, primary idiopathic myxedema, nephrosis, ophthalmia sympathica, orchitis granulomatosa, pancreatitis, polyradiculitis acuta, pyoderma gangrenosum, Quervain's thyroiditis, acquired splenic atrophy, non-malignant thymoma, vitiligo, toxic-shock syndrome, food poisoning, conditions involving infiltration of T cells, leukocyte-adhesion deficiency, immune responses associated with acute and delayed hypersensitivity mediated by cytokines and T-lymphocytes, diseases involving leukocyte diapedesis, multiple organ injury syndrome, antigen-antibody complex-mediated diseases, antiglomerular basement membrane disease, allergic neuritis, autoimmune polyendocrinopathies, oophoritis, primary myxedema, autoimmune atrophic gastritis, sympathetic ophthalmia, rheumatic diseases, mixed connective tissue disease, nephrotic syndrome, insulinitis, polyendocrine failure, autoimmune polyglandular syndrome type I, adult-onset idiopathic hypoparathyroidism (AOIH), cardiomyopathy such as dilated cardiomyopathy, epidermolysis bullosa acquisita (EBA), hemochromatosis, myocarditis, nephrotic syndrome, primary sclerosing cholangitis, purulent or nonpurulent sinusitis, acute or chronic sinusitis, ethmoid, frontal, maxillary, or sphenoid sinusitis, an eosinophil-related

disorder such as eosinophilia, pulmonary infiltration eosinophilia, eosinophilia-myalgia syndrome, Löffler's syndrome, chronic eosinophilic pneumonia, tropical pulmonary eosinophilia, bronchopneumonic aspergillosis, aspergilloma, or granulomas containing eosinophils, anaphylaxis, seronegative spondyloarthritides, polyendocrine autoimmune disease, sclerosing cholangitis, sclera, episclera, chronic mucocutaneous candidiasis, Bruton's syndrome, transient hypogammaglobulinemia of infancy, Wiskott-Aldrich syndrome, ataxia telangiectasia syndrome, angiectasis, autoimmune disorders associated with collagen disease, rheumatism, neurological disease, lymphadenitis, reduction in blood pressure response, vascular dysfunction, tissue injury, cardiovascular ischemia, hyperalgesia, renal ischemia, cerebral ischemia, and disease accompanying vascularization, allergic hypersensitivity disorders, glomerulonephritides, reperfusion injury, ischemic re-perfusion disorder, reperfusion injury of myocardial or other tissues, lymphomatous tracheobronchitis, inflammatory dermatoses, dermatoses with acute inflammatory components, multiple organ failure, bullous diseases, renal cortical necrosis, acute purulent meningitis or other central nervous system inflammatory disorders, ocular and orbital inflammatory disorders, granulocyte transfusion-associated syndromes, cytokine-induced toxicity, narcolepsy, acute serious inflammation, chronic intractable inflammation, pyelitis, endarterial hyperplasia, peptic ulcer, valvulitis, emphysema, alopecia areata, adipose tissue inflammation/diabetes type II, obesity associated adipose tissue inflammation/insulin resistance, and endometriosis.

[0106] As used herein, the term “cytokine” encompasses low molecular weight proteins secreted by various cells in the immune system that act as signaling molecules for regulating a broad range of biological processes within the body at the molecular and cellular levels. “Cytokines” include individual immunomodulating proteins that fall within the class of lymphokines, interleukins, or chemokines.

[0107] Non-limiting examples of cytokines are disclosed herein, for example, IL-1A and IL-1B are two distinct members of the human interleukin-1 (IL-1) family. Mature IL-1A is a 18 kDa protein, also known as fibroblast-activating factor (FAF), lymphocyte-activating factor (LAF), B-cell-activating factor (BAF), leukocyte endogenous mediator (LEM), etc. IL-4 is a cytokine that induces T helper-2 (Th2) cell differentiation, and is closely related to and has similar functions to IL-13. IL-5 is produced by Th2 cells and mast cells. It acts to stimulate B cell growth and increase immunoglobulin secretion. It is also involved in

eosinophil activation. IL-6 is an interleukin that can act as either a pro-inflammatory or anti-inflammatory cytokine. It is secreted by T cells and macrophages to stimulate immune response to trauma or other tissue damage leading to inflammation. IL-6 is also produced from muscle in response to muscle contraction. IL-8 is a chemokine produced by macrophages and other cell types such as epithelial cells and endothelial cells, and acts as an important mediator of the immune reaction in the innate immune system response. IL-12 is involved in the differentiation of naïve T cells to T helper (Th1 or Th2) cells. As a heterodimeric cytokine, IL-12 is formed after two subunits encoded by two separate genes, IL-12A (p35) and IL-12B (p40), dimerize following protein synthesis. IL-12p70 indicates this heterodimeric composition. IL-13, a cytokine secreted by many cell types, especially Th2 cells, is an important mediator of allergic inflammation and disease. IL-17 is a cytokine produced by T helper cells and is induced by IL-23, resulting in destructive tissue damage in delayed-type reactions. IL-17 functions as a pro-inflammatory cytokine that responds to the invasion of the immune system by extracellular pathogens and induces destruction of the pathogen's cellular matrix. IP-10, or Interferon gamma-induced protein 10, is also known as C-X-C motif chemokine 10 (CXCL10) or small-inducible cytokine B10. As a small cytokine belonging to the CXC chemokine family, IP-10 is secreted by several cell types (including monocytes, endothelial cells and fibroblasts) in response to IFN- γ . Macrophage Inflammatory Proteins (MIP) belong to the family of chemokines. There are two major forms of human MIP, MIP-1 α and MIP-1 β , which are also known as chemokine (C-C motif) ligand 3 (CCL3) and CCL4, respectively. Both are produced by macrophages following stimulation with bacterial endotoxins. Granulocyte colony-stimulating factor (G-CSF or GCSF), also known as colony-stimulating factor 3 (CSF 3), is a colony-stimulating factor hormone. G-CSF is a glycoprotein, growth factor, and cytokine produced by a number of different tissues to stimulate the bone marrow to produce granulocytes and stem cells. G-CSF also stimulates the survival, proliferation, differentiation, and function of neutrophil precursors and mature neutrophils. Epidermal growth factor or EGF is a growth factor that plays an important role in the regulation of cell growth, proliferation, and differentiation by binding with high affinity to its receptor EGFR. Vascular endothelial growth factor (VEGF) is a family of growth factors that are important signaling proteins involved in both vasculogenesis (the de novo

formation of the embryonic circulatory system) and angiogenesis (the growth of blood vessels from pre-existing vasculature).

[0108] As used herein, the term “inflammatory agent” is used to refer to an agent that promotes an inflammatory response. Nonlimiting examples include but are not limited to pro-inflammatory signaling molecules, cytokines and chemokines (e.g. IL-1 β , TNF- α , IFN- γ , IL-8, IL-6, IL-12, IL-15, IL-16, IL-17 (including family members IL17A, IL17B, IL-17C, IL-17D, IL-17E, IL-17F), IL-18, GM-CSF, IL-21, IL-23, IL-27 and TGF- β), prostaglandins, as well as antigens such as bacterial lipopolysaccharide, double stranded RNA (e.g. viral genomes), and endotoxins that induce inflammation.

[0109] As used herein, the term “anti-inflammatory agent” is used to refer to an agent that suppresses an inflammatory response. Nonlimiting examples include but are not limited to anti-inflammatory cytokines and chemokines (e.g. TGF- β , IL-2, IL-1R α , IL-4, IL-6, IL-10, IL-17, IL-11, IL-13, IL-35, IL-37, INF- α), non-steroidal anti-inflammatory drugs (NSAIDs), antileukotrienes, and immune selective anti-inflammatory derivatives (ImSAIDs),.

[0110] As used herein, the term “neurotrophic factor” is used to refer to an agent that supports the growth, proliferation, survival, and/or differentiation of developing and/or mature neural tissue such as neurons. In some aspects, administration of a neurotrophic factor has neuroprotective effects. Many neurotrophic factors function through tyrosine kinase signaling pathways. Neurotrophic factors include but are not limited to neurotrophins, glial cell-line derived neurotrophic factor family ligands, and neuropoietic cytokines. In some embodiments, the neurotrophic factors of the disclosed compositions and methods include but are not limited to brain derived neurotrophic factor (BDNF, e.g. NP_001137277), nerve growth factor (NGF, NP_002497) Neurotrophin-3 (NTF3, NP_001096124, NP_002518), ciliary neurotrophic factor (CTNF, NP_000605), glial cell derived neurotrophic factor (GDNF, e.g. NP_000505), fibroblast growth factors (FGFs) 1-23 (e.g. FGF1, NP_000791, FGF2 NP_001997), insulin-like growth factors (IGFs) (IGF1, NP_000609, IGF2 e.g. NP_000603), hepatocyte growth factor (HGF, e.g. NP_000592), Noggin (NOG, NP_005441), thyroid hormone triiodothyronine (T3, (2S)-2-amino-3- [4-(4-hydroxy-3-iodophenoxy)- 3,5-diiodo-phenyl]propanoic acid, molecular formula C₁₅H₁₁I₃NNaO₄), and equivalents of each thereof. Preferably, the FGF is FGF2 and the IGF is IGF2. In some

aspects, the neurotrophic factors are recombinant. Exemplary recombinant neurotrophic factors are available from, for example, Peprotech (Rocky Hill, NJ, USA) (e.g. rh/m/rBDNF cat# 450-02, rhCTNF cat# 450-13, rhGDNF cat# 450-10, β -NGF cat# 450-01, rh NT-3 cat# 450-03, rhFGF2 cat# 100-18B, rhIGF2 cat# 100-12, rhHGF cat# 100-39, rhNOG cat# 120-10C). T3 is available from, for example, Santa Cruz Biotechnology (Santa Cruz, CA, USA) (e.g. T3 CAS# 55-06-1).

[0111] As used herein, the term “neuroprotective” refers to an effect that protects neural tissue against damage, degeneration, and/or impairment of function. In some aspects, neuroprotective means that an agent or factor enhances the efficacy of certain neurological indications. Neuroprotective effects include but are not limited to proliferation of neural stem cells (assayed by flow cytometry), differentiation of glial restricted precursor cells toward oligodendrocytes (assayed by flow cytometry and/or immunohistochemistry optionally through use of organotypic brain slice cultures and/or multiple sclerosis animal studies), reduction of apoptosis of neural cells when exposed to oxidative stress (assayed by flow cytometry), remyelination of axons (assayed by flow cytometry and/or immunohistochemistry optionally through use of organotypic brain slice cultures and/or multiple sclerosis animal studies), functional recovery in models with neurodeficits (assayed by behavioral test, immunohistochemistry, and/or flow cytometry optionally in MS animal studies), enhanced neurotrophic secretion (assayed by antibody array and/or RNA-seq, optionally in MS animal studies), and neurite outgrowth (assayed by immunohistochemistry).

[0112] As used herein, the term “angiogenesis agent” is used to refer to an agent that promotes angiogenesis (i.e. the stimulation of new blood vessels, repairing damaged blood vessels, or increasing the number of blood vessels). Nonlimiting examples of angiogenesis agents include but are not limited to FGF2, HGF, VEGF, PDGF, FGF1, EGF, TGF β , WNT1, angiotensin, prostaglandin E1 (PGE1), modified PGE1 (see US Patent No. 6,288,113, incorporated by reference herein) and angiopoietin-1.

[0113] As used herein, the terms “culture media” and “culture medium” are used interchangeably and refer to a solid or a liquid substance used to support the growth of cells (e.g., stem cells). Preferably, the culture media as used herein refers to a liquid substance capable of maintaining stem cells in an undifferentiated state. The culture media can be a

water-based media which includes a combination of ingredients such as salts, nutrients, minerals, vitamins, amino acids, nucleic acids, proteins such as cytokines, growth factors and hormones, all of which are needed for cell proliferation and are capable of maintaining stem cells in an undifferentiated state. For example, a culture media can be a synthetic culture media such as, for example, minimum essential media α (MEM- α) (HyClone Thermo Scientific, Waltham, MA, USA), DMEM/F12, GlutaMAX (Life Technologies, Carlsbad, CA, USA), Neurobasal Medium (Life Technologies, Carlsbad, CA, USA), KO-DMEM (Life Technologies, Carlsbad, CA, USA), OptiMEM (Life Technologies, Carlsbad, CA, USA), DMEM/F12 (Life Technologies, Carlsbad, CA, USA), supplemented with the necessary additives as is further described herein. In some embodiments, the cell culture media can be a mixture of culture media. Preferably, all ingredients included in the culture media of the present disclosure are substantially pure and tissue culture grade. “Conditioned medium” and “conditioned culture medium” are used interchangeably and refer to culture medium that cells have been cultured in for a period of time and wherein the cells release/secrete components (e.g., proteins, cytokines, chemicals, etc.) into the medium.

[0114] As used herein, a “bioreactor” refers to a culture system appropriate for supporting growth of cells. In some embodiments, cells may be cultured in a bioreactor system for large-scale growth of surface adherent cells. A non-limiting example of a bioreactor appropriate for practice of the methods disclosed herein is a hollow fiber bioreactor. A hollow fiber bioreactor maximizes the surface area for cells to adhere while minimizing the amount of culture medium needed to support the cells through use of hollow fibers. The hollow fibers are semi-permeable capillary membranes that can be bundled together to create a bioreactor cartridge capable of supporting a high cell density. Methods for use of hollow fiber bioreactors for growth of cells are known in the technical and patent literature, e.g., Sheu et al. “Large-scale production of lentiviral vector in a closed system hollow fiber bioreactor,” *Mol. Ther Methods Clin Dev* (2015) 2:15020. Other bioreactors suitable for practice of the disclosed methods include but are not limited to rocking bioreactor systems, stirred tank bioreactor systems, single use bioreactor systems, flow culture bioreactor systems, bioreactors with chambers appropriate for porous cylindrical scaffolds subjected to perfusion culture conditions, and bioreactors with tubular chambers.

[0115] As used herein, the term “vector” refers to a non-chromosomal nucleic acid comprising an intact replicon such that the vector may be replicated when placed within a cell, for example by a process of transformation. Vectors may be viral or non-viral. Viral vectors include retroviruses, lentiviruses, adenoviruses, herpesvirus, baculoviruses, modified baculoviruses, papovirus, or otherwise modified naturally occurring viruses. Exemplary non-viral vectors for delivering nucleic acid include naked DNA; DNA complexed with cationic lipids, alone or in combination with cationic polymers; anionic and cationic liposomes; DNA-protein complexes and particles comprising DNA condensed with cationic polymers such as heterogeneous polylysine, defined-length oligopeptides, and polyethylene imine, in some cases contained in liposomes; and the use of ternary complexes comprising a virus and polylysine-DNA.

[0116] A “viral vector” is defined as a recombinantly produced virus or viral particle that comprises a polynucleotide to be delivered into a cell, either *in vivo*, *ex vivo* or *in vitro*. Examples of viral vectors include retroviral vectors, lentiviral vectors, adenovirus vectors, adeno-associated virus vectors, alphavirus vectors and the like. Alphavirus vectors, such as Semliki Forest virus-based vectors and Sindbis virus-based vectors, have also been developed for use in gene therapy and immunotherapy. See, Schlesinger and Dubensky (1999) *Curr. Opin. Biotechnol.* 5:434-439 and Ying, et al. (1999) *Nat. Med.* 5(7):823-827.

[0117] In aspects where modification of the cell is mediated by a lentiviral vector, a vector construct refers to the polynucleotide comprising the lentiviral genome or part thereof, and a therapeutic gene. As used herein, “transfection” or “transduction” in reference to delivery of exogenous nucleic acids carries the same meaning and refers to the process by which a gene or nucleic acid sequences are stably transferred into the host cell by virtue of the virus entering the cell and integrating its genome into the host cell genome. The virus can enter the host cell via its normal mechanism of infection or be modified such that it binds to a different host cell surface receptor or ligand to enter the cell. Retroviruses carry their genetic information in the form of RNA; however, once the virus infects a cell, the RNA is reverse-transcribed into the DNA form which integrates into the genomic DNA of the infected cell. The integrated DNA form is called a provirus. As used herein, lentiviral vector refers to a viral particle capable of introducing exogenous nucleic acid into a cell through a viral or viral-like entry mechanism. A “lentiviral vector” is a type of retroviral vector well-known in

the art that has certain advantages in transducing nondividing cells as compared to other retroviral vectors. See, Trono D. (2002) *Lentiviral vectors*, New York: Springer-Verlag Berlin Heidelberg.

[0118] Lentiviral vectors of this invention are based on or derived from oncoretroviruses (the sub-group of retroviruses containing MLV), and lentiviruses (the sub-group of retroviruses containing HIV). Examples include ASLV, SNV and RSV all of which have been split into packaging and vector components for lentiviral vector particle production systems. The lentiviral vector particle according to the invention may be based on a genetically or otherwise (e.g., by specific choice of packaging cell system) altered version of a particular retrovirus.

Cell-derived Vesicles

[0119] Cell-derived vesicles, also referred to as extracellular vesicles, are membrane surrounded structures that are released by cells *in vitro* and *in vivo*. Extracellular vesicles can contain proteins, lipids, and nucleic acids and can mediate intercellular communication between different cells, including different cell types, in the body. Two types of extracellular vesicles are exosomes and microvesicles. Exosomes are small lipid-bound, cellularly secreted vesicles that mediate intercellular communication via cell-to-cell transport of proteins and RNA (El Andaloussi, S. et al. (2013) *Nature Reviews: Drug Discovery* 12(5):347-357). Exosomes range in size from approximately 30 nm to about 200 nm. Exosomes are released from a cell by fusion of multivesicular endosomes (MVE) with the plasma membrane. Microvesicles, on the other hand, are released from a cell upon direct budding from the plasma membrane (PM). Microvesicles are typically larger than exosomes and range from approximately 100 nm to 1 μ m.

Cells

[0120] Cell-derived vesicles (e.g., exosomes and/or microvesicles) can be isolated from eukaryotic cells. Non-limiting examples of cells that cell-derived vesicles can be isolated from include stem cells. Non-limiting examples of such stem cells include adult stem cells, embryonic stem cells, embryonic-like stem cells, neural stem cells, or induced pluripotent stem cells. In some embodiments, the stem cell is an adult stem cell that is optionally a mesenchymal stem cell. In one aspect the stem cell, e.g., the mesenchymal stem cells, has

been cultured under conditions of hypoxia and low serum or serum-free conditions. In a further aspect, the stem cells are cultured in the presence of one or more agents selected from an inflammatory agent, a neurotrophic factor, or an angiogenesis agent.

[0121] The cells of the present disclosure may be modified, for example, by genetic modification. In some embodiments, the cells are modified to express at least one exogenous nucleic acid and/or at least one exogenous protein. In some embodiments, the cells are modified to express at least one endogenous nucleic acid and/or at least one endogenous protein. The modification may be a transient modification. In other embodiments, the modification may be a stable modification. It is contemplated that by modifying the cells prior to collection of the cell-derived vesicles released by the modified cells, one can collect exosomes containing different amounts and types of proteins, lipids, and nucleic acids as compared to unmodified cells. Any method for cellular modification known to one of skill in the art can be used to modify the cells.

[0122] In some embodiments, the cells of the present disclosure are modified to express at least one exogenous or endogenous nucleic acid and/or at least one exogenous or endogenous protein. Non-limiting examples of nucleic acids include one or more or all of DNA and RNA, for example, a gene or gene fragment (for example, a probe, primer, EST or SAGE tag), exons, introns, messenger RNA (mRNA), transfer RNA, ribosomal RNA, ribozymes, cDNA, dsRNA, siRNA, miRNA, recombinant polynucleotides, branched polynucleotides, plasmids, vectors, isolated DNA of any sequence, isolated RNA of any sequence, nucleic acid probes and primers.

[0123] In some embodiments the exogenous or endogenous nucleic acid encodes a micro RNA (miRNA), for example, miR-150 (GenBank Accession No: NR_029703.1 (SEQ ID NO: 1)), miR-126 (GenBank Accession No: NR_029695.1 (SEQ ID NO: 2)), miR-132 (GenBank Accession No: NR_029674.1 (SEQ ID NO: 17)) miR-296 (GenBank Accession No: NR_029844.1 (SEQ ID NO: 3)), let-7 (GenBank Accession No: NR_029695.1 (SEQ ID NO: 4)), and equivalents thereof. In some embodiments the exogenous or endogenous protein is platelet derived growth factor receptor (PDGFR), wherein the PDGF is expressed by a transgene encoding PDGF (e.g., PDGFR-A (GenBank Accession No: NM_006206.4 (SEQ ID NO: 5)), PDGFR-B (GenBank Accession No: NM_002609.3 (SEQ ID NO: 6), or

equivalents thereof). In some embodiments the exogenous protein is Collagen, Type 1, Alpha 2 (COL1A2), (GenBank Accession No: NM_000089.3 (SEQ ID NO: 7), or equivalents thereof). In some embodiments the exogenous or endogenous protein is Collagen, Type VI, Alpha 3 (COL6A3), (GenBank Accession No: NM_004369.3 (SEQ ID NO: 8), or equivalents thereof). In some embodiments the exogenous protein is EGF-like repeats- and discoidin i-like domains-containing protein 3 (EDIL3), (GenBank Accession No: NM_005711.4 (SEQ ID NO: 9), or equivalents thereof. In some embodiments the exogenous or endogenous protein is epidermal growth factor receptor (EGFR) (GenBank Accession No: NM_005228.3 (SEQ ID NO: 10), or equivalents thereof. In some embodiments the exogenous protein or endogenous is fibroblast growth factor receptor (FGF) (GenBank Accession No: M60485.1 (SEQ ID NO: 11), or equivalents thereof. In some embodiments the exogenous or endogenous protein is fibronectin (FN1) (GenBank Accession No: M10905.1 (SEQ ID NO: 12), or equivalents thereof. In some embodiments the exogenous or endogenous protein is Milk fat globule-EGF factor 8 (MFGE8) (GenBank Accession No: NM_005928 (SEQ ID NO: 13), or equivalents thereof. In some embodiments the exogenous or endogenous protein is lectin, galactoside-binding, soluble, 3 binding protein (LGALS3BP) (GenBank Accession No: NM_005567 (SEQ ID NO: 14), or equivalents thereof. In some embodiments the exogenous or endogenous protein is transferrin (TF) (GenBank Accession No: M12530.1 (SEQ ID NO: 15), or equivalents thereof. In some embodiments the exogenous or endogenous protein is vascular endothelial growth factor (VEGF) (e.g. GenBank X62568.1 and GenBank AY04758) or isoform 165A of VEGF (SEQ ID NO: 19) or equivalents thereof. In some embodiments the exogenous or endogenous protein is vascular endothelial growth factor receptor (VEGFR) (GenBank Accession No: AF063657 (SEQ ID NO: 16), or equivalents thereof. In some embodiments, the cells of the present disclosure do not express exogenous or endogenous VEGF, VEGFR or both. In some embodiments, the cells of the present disclosure are modified to express at least one exogenous or endogenous nucleic acid encoding a protein or an endogenous or exogenous nucleic acid detected in exosomes and/or microvesicles of the present disclosure (and listed in the molecular composition of exosomes section below).

[0124] An equivalent or biological equivalent nucleic acid, polynucleotide or oligonucleotide or peptide is one having at least 80 % sequence identity, or alternatively at

least 85 % sequence identity, or alternatively at least 90 % sequence identity, or alternatively at least 92 % sequence identity, or alternatively at least 95 % sequence identity, or alternatively at least 97 % sequence identity, or alternatively at least 98 % sequence identity to the reference nucleic acid, polynucleotide, oligonucleotide or peptide. In alternative embodiment, the equivalent or biological equivalent hybridizes to the reference polynucleotide or oligonucleotide or its complement under conditions of high stringency. In a further aspect, the equivalent or biological equivalent is a peptide encoded by a polynucleotide that hybridizes to the polynucleotide encoding the reference peptide or its complement under conditions of high stringency.

[0125] The cells of the present disclosure can be cultured in any culture media known to those of skill in the art. For example, the cell culture media can comprise between 2% - 40% fetal bovine serum (FBS), preferably approximately 20% FBS; between 0.5% - 5% L-glutamine, preferably approximately 1% L-glutamine; and between 0.5% - 1% penicillin and streptomycin (Penn-strep), preferably approximately 1% penn-strep, in a basal media. In some embodiments, the cells are cultured in low levels of serum, for example, less than about 1% FBS, or alternatively from about 1% to about 2% FBS, or alternatively about 2% to about 5% FBS, or alternatively about 5% to about 10% FBS. In some embodiments, low serum conditions comprise less than 20% FBS. In some embodiments, at least a portion of the FBS is substituted with a serum replacement, for example, a platelet lysate (e.g., human platelet lysate (hPL)) or serum albumin (e.g. bovine serum albumin). In some embodiments, the amount of serum replacement (e.g., hPL) in the culture media is between 1% - 20%. In some embodiments, the cells are cultured in the absence of FBS. In other embodiments, the cells are cultured in the presence of high levels of serum, for example, 30% serum, 40% serum, 50% serum, or 60% serum.

[0126] The cells of the present disclosure can be cultured under any conditions known to those in the field. In some embodiments, the cells of the disclosure are cultured in conditions of about 1-20% oxygen (O₂) and about 5% carbon dioxide (CO₂). In some embodiments, the cells of the present disclosure are cultured under hypoxic or low oxygen conditions (e.g., in the presence of less than 10% O₂). In some embodiments, the hypoxic conditions are between approximately 1% to about 15% CO₂ and between 0.05% - 20% oxygen tension. In some embodiments, the cells are cultured under low serum conditions. In some

embodiments, the low serum conditions are serum free conditions. In some embodiments, the cells of the present disclosure are cultured at about 37 °C. In some embodiments, the cells of the present disclosure can be cultured at about 37 °C, 5% CO₂ and 10-20% O₂. In preferred embodiments, the cells of the present disclosure are cultured at about 5% CO₂.

[0127] In some embodiments, the cells are cultured in hypoxic conditions for a period of time. For example, the cells may be cultured under hypoxic and low serum conditions for up to about 72 hours prior to vesicle isolation or for up to about 40 hours prior to vesicle isolation. In other embodiments, the cells may be cultured under normoxic conditions for a period of time and then switched to hypoxic conditions and culture for a period of time.

[0128] It is surprising that stem cells cultured in hypoxic and/or serum free conditions released more exosomes as compared to conventional culture conditions. See, for example **FIG. 2A**. It is further surprising that these stressed conditions would produce cell-derived vesicles containing desirable components for use as therapeutics.

Augmented Stem Cell Stimulation Methods

[0129] In some embodiments, the stem cells are stimulated with one or more agents selected from an inflammatory agent, a neurotrophic factor, or an angiogenesis agent in combination with manufacturing/isolation methods disclosed herein. In some embodiments, the stimulation is achieved with one or more inflammatory agents and one or more neurotrophic factors in combination. In some embodiments, stimulation is achieved with one or more inflammatory agents and one or more angiogenesis agents in combination. In some embodiments, stimulation is achieved with one or more neurotrophic factors and one or more angiogenesis agents in combination. In some embodiments, stimulation is achieved with one or more inflammatory agents, one or more neurotrophic factors, and one or more angiogenesis agents in combination. In some embodiments, stimulation is achieved with one or more inflammatory agents. In some embodiments, stimulation is achieved with one or more neurotrophic factors. In some embodiments, stimulation is achieved with one or more angiogenesis agents.

Table 1: Factors to stimulate MSCs to modify cell-derived vesicle composition

Inflammatory agents	Neurotrophic factors	Angiogenesis agents
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TNF α	T3	FGF2
IL-6	FGF2	VEGF
IL-17	Noggin	PDGF
IL-1 β	BDNF	HGF
IFN γ	NGF	FGF1
LPS	HGF	EGF
	CTNF	TGF- β
	GDNF	WNT1
	IGF2	
	Neurotrophin-3	

[0130] In some embodiments, the inflammatory agent is selected from tumor necrosis factor alpha (“TNF α ,” NP_000585), interleukin 6 (“IL-6,” NP_000591, NP_001305024), interleukin 17 (“IL-17,” e.g. NP_002181), interleukin 1 β (“IL-1 β ”), interferon gamma (“IFN γ ,” NP_000610), lipopolysaccharides (“LPS,” available, for example, from Sigma Aldrich (St. Louis, MO, USA) e.g. cat# L3023, L9023, L3024), or equivalents of each thereof. Preferably, the inflammatory agent is TNF α .

[0131] In some embodiments, the neurotrophic factor is selected from brain derived neurotrophic factor (BDNF, e.g. NP_001137277), nerve growth factor (NGF, NP_002497) Neurotrophin-3 (NTF3, NP_001096124, NP_002518), ciliary neurotrophic factor (CTNF, NP_000605), glial cell derived neurotrophic factor (GDNF, e.g. NP_000505), fibroblast growth factors (FGFs) 1-23 (e.g. FGF1, NP_000791, FGF2 NP_001997), insulin-like growth factors (IGFs) (IGF1, NP_000609, IGF2 e.g. NP_000603), hepatocyte growth factor (HGF, e.g. NP_000592), Noggin (NOG, NP_005441), thyroid hormone triiodothyronine (T3, (2S)-2-amino-3-[4-(4-hydroxy-3-iodo-phenoxy)- 3,5-diiodo-phenyl]propanoic acid, molecular formula C₁₅H₁₁I₃NNaO₄, available from, for example, Santa Cruz Biotechnology (Santa

Cruz, CA, USA) (e.g. T3 CAS# 55-06-1)), or equivalents of each thereof. Preferably, the neurotrophic factor is FGF2 and/or T3.

[0132] In some embodiments, the angiogenesis agent is selected from FGF2, vascular endothelial growth factor (“VEGF”), platelet derived growth factor (“PDGF”), HGF, FGF1, FGF2, epidermal growth factor (“EGF,” NP_001171601, NP_001171602, NP_001954), transforming growth factor beta 1-4 (“TGF β ,” e.g. TGF β 1: NP_000651; TGF β 2: NP_001129071, NP_003229; TGF β 3: NP_001316867, NP_001316868, NP_003230; TGF β 4), proto-oncogene protein Wnt-1 (“WNT1,” NP_005421), or equivalents of each thereof. Preferably, the angiogenesis agent is FGF2.

[0133] In some embodiments, the agent or factor is a recombinant protein. Exemplary recombinant proteins are available from, for example, Peprotech (Rocky Hill, NJ, USA) (e.g. rhTNF α cat# 300-01A, rhIL-6 cat# 200-06, rhIL-17 cat# 200-17, rhIL-1 β cat# 200-01B, rhINF γ cat# 300-02, rh/m/rBDNF cat# 450-02, rhCTNF cat# 450-13, rhGDNF cat# 450-10, β -NGF cat# 450-01, rh NT-3 cat# 450-03, rhFGF2 cat# 100-18B, rhIGF2 cat# 100-12, rhHGF cat# 100-39, rhNOG cat# 120-10C, rhVEGF₁₆₅ cat# 100-20, rhPDGF-AA cat# 100-13A, rhPDGF-BB 1001-14B, rhPDGF-AB cat# 100-00AB, rhPDGF-CC cat# 100-00CC, rhFGF1 cat# 100-17A, rhTGF β 1 cat# 100-21, 100-21C, rhWNT-1 cat#120-17).

[0134] In some embodiments, the stimulation is achieved by culturing the stem cells in the presence of, or contacting the stem cells with an effective amount of the one or more inflammatory agents, neurotrophic factors, and/or angiogenesis agents. In some embodiments, the stem cells are cultured in the presence of the agent and/or factor. In some embodiments, the stem cells are expanded in the presence of the agent and/or factor. In some embodiments, the agent and/or factor is added to the stem cell expansion, maintenance, and/or growth medium(s) (i.e. the culture media used to culture stem cells prior to switching to cell-derived vesicle isolation medium). In some embodiments, the agent and/or factor is added to the cell-derived vesicle isolation medium (“isolation medium”). In some embodiments, the agent and/or factor is added to the stem cell expansion, maintenance, and/or growth media as well as the isolation medium. In some embodiments, the agent and/or factor is added only to the isolation medium. In some embodiments, the agent and/or factor is added to the culture medium immediately prior to switching the stem cells to the

isolation medium. In some embodiments, the agent and/or factor is added 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14 days prior to switching the stem cells to the isolation medium. In some embodiments, the agent and/or factor is added 1 hr, 2 hr, 3 hr, 4 hr, 6 hr, 8 hr, 10 hr, 12 hr, 16 hr, 18 hr, 20 hr, 24 hr, or 36 hr prior to switching the stem cells to the isolation medium. In some embodiments, the agent and/or factor is added to the stem cells 1 passage, 2 passages, 3 passages, 4 passages, or 5 passages prior to switching the cells to the isolation medium. In some embodiments, the stimulating agent is added more than once (e.g. twice, three times, four times, and/or daily). In some embodiments, the agents and/or factors are added sequentially.

[0135] In some embodiments, the concentration of the agent or factor is about 1 to about 10 ng/mL, or alternatively about 5 to about 20 ng/mL, or alternatively about 5 to about 30 ng/mL, or alternatively about 5 to about 40 ng/mL, or alternatively about 5 to about 50 ng/mL, or alternatively about 5 to about 100 ng/mL, or alternatively about 5 to about 250 ng/mL, or alternatively about 5 to about 500 ng/mL, or alternatively about 25 to about 75 ng/mL, or alternatively about 50 to about 100 ng/mL, or alternatively about 100 to about 500 ng/mL, or or alternatively about 100 ng/mL to about 1 µg/mL, or alternatively about 1 µg/mL to about 10 µg/mL, or alternatively about 10 µg/mL to about 50 µg/mL, or alternatively about 50 µg/mL to about 100 µg/mL, or alternatively about 100 µg/mL to about 500 µg/mL, or alternatively about 500 µg/mL to about 1000 µg/mL. In particular aspects, the agent or factor is about 10 ng/mL, or alternatively about 15 ng/mL, or alternatively about 20 ng/mL, or alternatively about 25 ng/mL, or alternatively about 30 ng/mL, or alternatively about 40 ng/mL, or alternatively about 50 ng/mL, or alternatively about 100 ng/mL, or alternatively about 200 ng/mL, or alternatively about 250 ng/mL, or alternatively about 300 ng/mL, or alternatively about 400 ng/mL, or alternatively about 500 ng/mL, or alternatively about 1 µg/mL. Preferably, the agent or factor is about 5 to about 50 ng/mL.

[0136] Without being bound by theory, the stem cells (e.g. MSCs) will respond to this stimulus and consequently modulate the contents of the resulting exosomes towards an increase in anti-inflammatory factors (e.g. with inflammatory agents such as TNF α stimulation), or alternatively towards neuroprotection (e.g. with neurotrophic factors such as Noggin and/or T3 stimulation), or alternatively towards angiogenesis (e.g. with angiogenesis agents such as FGF2 stimulation). Without being bound by theory, stimulation as described

herein results in significant modification of the composition of the exosomes with enhanced efficacy.

Augmented Cell-Derived Vesicle Composition Methods

In some embodiments, the population of highly purified cell-derived vesicles further comprise one or more of an anti-inflammatory agent, a neurotrophic factor, or an angiogenesis agent. In some embodiments, the population further comprises one or more anti-inflammatory agents and one or more neurotrophic factors in combination. In some embodiments, the further population comprises one or more anti-inflammatory agents and one or more angiogenesis agents in combination. In some embodiments, the population further comprises one or more neurotrophic factors and one or more angiogenesis agents in combination. In some embodiments, the population further comprises one or more anti-inflammatory agents, one or more neurotrophic factors, and one or more angiogenesis agents in combination. In some embodiments, the population further comprises one or more anti-inflammatory agents. In some embodiments, the population further comprises one or more neurotrophic factors. In some embodiments, the population further comprises one or more angiogenesis agents. In some aspects, the agent and/or factor is added directly to the already isolated cell-derived vesicles. In some aspects, the agent and/or factor is added after concentration of the isolated cell-derived vesicles. In some aspects, the agent and/or factor is formulated with the population of highly purified cell-derived vesicles.

Table 2: Agents or factors added directly to cell-derived vesicle product

Anti-Inflammatory	Neurotrophic	Angiogenesis
TGF- β	T3	FGF2
IL-2	FGF2	HGF
IL-10	Noggin	VEGF
IL-17	BDNF	PDGF
IL-35	NGF	FGF1
IL-37	HGF	EGF

	CTNF	TGF-beta
	GDNF	WNT1
	IGF2	
	Neurotrophin-3	

[0137] In some embodiments, the anti-inflammatory agent is selected from TGF β 1-4 (“TGF β ,” e.g. TGF β 1: NP_000651; TGF β 2: NP_001129071, NP_003229; TGF β 3: NP_001316867, NP_001316868, NP_003230; TGF β 4), interleukin 2 (“IL-2,” NP_000577), interleukin 10 (“IL-10,” NP_000563), interleukin 17 (“IL-17,” e.g. NP_002181), interleukin 35 (“IL-35”), or interleukin – 1 family member 7 (“IL-37,” NP_055254, NP_775294, NP_775295, NP_775296, NP_775297). Preferably, the anti-inflammatory agent is TGF β and/or IL-2.

[0138] In some embodiments, the neurotrophic factor is selected from brain derived neurotrophic factor (BDNF, e.g. NP_001137277), nerve growth factor (NGF, NP_002497) Neurotrophin-3 (NTF3, NP_001096124, NP_002518), ciliary neurotrophic factor (CTNF, NP_000605), glial cell derived neurotrophic factor (GDNF, e.g. NP_000505), fibroblast growth factors (FGFs) 1-23 (e.g. FGF1, NP_000791, FGF2 NP_001997), insulin-like growth factors (IGFs) (IGF1, NP_000609, IGF2 e.g. NP_000603), hepatocyte growth factor (HGF, e.g. NP_000592), Noggin (NOG, NP_005441), thyroid hormone triiodothyronine (T3, (2S)-2-amino-3- [4-(4-hydroxy-3-iodo-phenoxy)- 3,5-diiodo-phenyl]propanoic acid, molecular formula C₁₅H₁₁I₃NNaO₄, available from, for example, Santa Cruz Biotechnology (Santa Cruz, CA, USA) (e.g. T3 CAS# 55-06-1)), or equivalents of each thereof. Preferably, the one or more neurotrophic factors is selected from FGF2, T3, NOG, BDNF, NGF, HGF, CTNF, GDNF, or IGF2.

[0139] In some embodiments, the angiogenesis agent is selected from FGF2, vascular endothelial growth factor (“VEGF”), platelet derived growth factor (“PDGF”), HGF, FGF1, FGF2, epidermal growth factor (“EGF,” NP_001171601, NP_001171602, NP_001954), transforming growth factor beta 1-4 (“TGF β ,” e.g. TGF β 1: NP_000651; TGF β 2: NP_001129071, NP_003229; TGF β 3: NP_001316867, NP_001316868, NP_003230;

TGF β 4), proto-oncogene protein Wnt-1 ("WNT1," NP_005421), or equivalents of each thereof. Preferably, the angiogenesis agent is FGF2 and/or HGF.

[0140] In some embodiments, the agent or factor is a recombinant protein. Exemplary recombinant proteins are available from, for example, Peprotech (Rocky Hill, NJ, USA) (e.g. rhIL-2 cat# 200-02, rhIL-10 cat# 200-10, rhIL-35 cat# 200-37, rhIL-37 cat# 200-39, rhIL-17 cat# 200-17, rh/m/rBDNF cat# 450-02, rhCTNF cat# 450-13, rhGDNF cat# 450-10, β -NGF cat# 450-01, rh NT-3 cat# 450-03, rhFGF2 cat# 100-18B, rhIGF2 cat# 100-12, rhHGF cat# 100-39, rhNOG cat# 120-10C, rhVEGF₁₆₅ cat# 100-20, rhPDGF-AA cat# 100-13A, rhPDGF-BB 1001-14B, rhPDGF-AB cat# 100-00AB, rhPDGF-CC cat# 100-00CC, rhFGF1 cat# 100-17A, rhTGF β 1 cat# 100-21, 100-21C, rhWNT-1 cat#120-17).

[0141] In some aspects, the agent or factor is about 1 to 10 ng/mL, or alternatively 5 to 20 ng/mL, or alternatively 5 to 30 ng/mL, or alternatively 5 to 40 ng/mL, or alternatively 5 to 50 ng/mL, or alternatively 5 to 100 ng/mL, or alternatively 5 to 250 ng/mL, or alternatively 5 to 500 ng/mL, or alternatively 25 to 75 ng/mL, or alternatively 50 to 100 ng/mL, or alternatively 100 to 500 ng/mL, or or alternatively 100 ng/mL to 1 μ g/mL. In particular aspects, the protein is about 10 ng/mL, or alternatively about 15 ng/mL, or alternatively about 20 ng/mL, or alternatively about 25 ng/mL, or alternatively about 30 ng/mL, or alternatively about 40 ng/mL, or alternatively about 50 ng/mL, or alternatively about 100 ng/mL, or alternatively about 200 ng/mL, or alternatively about 250 ng/mL, or alternatively about 300 ng/mL, or alternatively about 400 ng/mL, or alternatively about 500 ng/mL, or alternatively about 1 μ g/mL. Preferably, the agent or factor is about 10 ng/mL to about 1 μ g/mL.

Isolation of Extracellular Vesicles

[0142] The purified populations of cell-derived vesicles (e.g., exosomes and/or microvesicles) of the present disclosure can be isolated using any method known by those in the art. Non-limiting examples include differential centrifugation by ultracentrifugation (Théry et al. (2006) Curr. Protoc. Cell Biol. 30:3.22.1-3.22.29; Witmer et al. (2013) J. Extracellular v.2), sucrose gradient purification (Escola et al. (1998) J. Biol. Chem. 273:20121-20127), and combination filtration/concentration (Lamparski et al. (2002) J. Immunol. Methods 270:211-226).

[0143] The purified populations of the cell-derived vesicles disclosed herein may be purified from by a method comprising tangential flow filtration (TFF) that may contain a hollow fiber filter or a cartridge filter. In some embodiments, the method for purifying a population of cell-derived vesicles comprises: (a) applying a tangential flow filtration to conditioned media produced by a population of isolated stem cells to isolate an cell-derived vesicle containing fraction; and (b) concentrating the cell-derived vesicle containing fraction to provide a purified population of cell-derived vesicles. In one aspect, the cells are grown under low serum and hypoxic or low oxygen conditions for a period of time prior to collecting the conditioned media from the cell population.

[0144] In some embodiments, after step (a) cell debris and other contaminants are removed from the cell-derived vesicle containing fraction prior to step (b).

[0145] In some embodiments, the population of stem cells were cultured under hypoxic and low serum conditions for up to about 72 hours prior to performing step (a). In some embodiments, the hypoxic conditions are between approximately 1% - 15% CO₂ and between 0.05% - 20% oxygen tension. In some embodiments, the low serum conditions are serum free conditions.

[0146] The isolated stem cells used for the methods described herein can be any stem cell known to those of skill in the art. Non-limiting examples of stem cells include adult stem cells, embryonic stem cells, embryonic-like stem cells, neural stem cells, or induced pluripotent stem cells. In some embodiments, the stem cells are mesenchymal stem cells.

[0147] The tangential flow filtration unit can be between about 50 kilodalton and about 750 kilodalton nominal molecular weight limit filtration unit. For example, the tangential flow filtration unit is about a 100 kilodalton nominal molecular weight limit filtration unit or about a 300 kilodalton nominal molecular weight limit filtration unit (e.g., Minimate™ Tangential Flow Filtration Capsules (Pall Corporation, Port Washington, NY, USA) and Pellicon Ultrafiltration Cassettes (EMD Millipore, Billerica, MA, USA)). In some embodiments, step (a) of the method disclosed herein is performed using an approximately 200 nanometer filter.

[0148] In some embodiments, step (b) of the method disclosed herein is performed using a filtration device. For example, the filtration device may be an approximately 100 kilodalton

nominal molecular weight limit filtration device or an approximately 300 kilodalton nominal molecular weight limit filtration device.

[0149] In some embodiments, the purified populations of cell-derived vesicles (e.g., exosomes and/or microvesicles) of the present disclosure can be isolated from conditioned media via direct isolation using membrane filtration devices (e.g. VivaSpin Centrifugal Concentrator, (Vivaproducts, Inc. Littleton, MA, USA)). For example, a 100 – 300 kDa membrane filtration device used with centrifugal force of 500 – 6000 x g may be used to perform the methods disclosed herein.

[0150] In some embodiments, the cells are grown in 20% FBS (or 4% hPL) at atmospheric oxygen percentages (~21% O₂) for approximately 24 – 72 hours in order to condition the media. The conditioned media is then precleared by centrifuging at 500 x g for 10 minutes. The media can then be cleared again by centrifuging at 2000 x g for 15 minutes. Then the sample is centrifuged at 17,000 x g for 45 minutes and the resulting pellet is resuspended in a solution (e.g., PBS).

[0151] In other embodiments, the cells are grown in 20% FBS (or 4% hPL) at atmospheric oxygen percentages (~21% O₂) for approximately 24 – 72 hours in order to condition the media. The conditioned media is then precleared by centrifuging at 500 x g for 10 minutes. The media can then be cleared again by centrifuging at 2000 x g for 15 minutes. The precleared media can then be placed in a TFF filter with 220 nm cutoff size (equivalent to approximately 2200 kDa) to allow at least a portion of the soluble proteins and smaller cell-derived vesicles to pass through the filter while keeping larger cell-derived vesicles. The cell-derived vesicles can then be washed in a sterile solution (e.g., PBS) to diafiltrate the sample. Then the sample can be further concentrated using a 200 nm filter (e.g., Vivaspin column (Viva Products, Littleton, MA, USA)).

[0152] In some embodiments, cell-derived vesicles (e.g. exosomes, microvesicles) are isolated from cells cultured in the presence of high levels of serum, for example, 30% serum, 40% serum, 50% serum, or 60% serum. In other embodiments, the cell-derived vesicles are isolated from cells cultured in the presence of from about 5% to about 25% serum (e.g., FBS). In some embodiments, at least a portion of the serum is substituted with a serum replacement, for example, a platelet lysate (e.g., human platelet lysate (hPL)). The cell-

derived vesicles can range in size from about 100 nm to about 1000 nm. The cell-derived vesicles can be isolated by any method known to those of skill in the art and, in particular, those described in the present disclosure. In some embodiments, the cell-derived vesicles are isolated using tangential flow filtration and filters (e.g., a hollow fiber filtration or a cartridge filter) with size cutoffs to select for a desired microvesicle population, for example, from about 100 nm to about 1000 nm, about 200 nm to about 900 nm, about 300 nm to about 800 nm, about 400 nm to about 700 nm, about 500 nm to about 600 nm. In some embodiments, the filters have a cutoff size of about 100 nm, about 200 nm, about 300 nm, about 400 nm, about 500 nm, about 600 nm, about 700 nm, about 800 nm, about 900 nm, or about 1000 nm.

[0153] After isolation, the cell-derived vesicles, e.g., exosomes can be concentrated to provide a purified population of cell-derived vesicles. Any appropriate method can be used to concentrate the cell-derived vesicles, e.g. exosomes. Non-limiting examples of such include centrifugation, ultrafiltration, filtration, differential centrifugation and column filtration with a 100 kDa to 750 kDa pore size, or either a 100 kDa to 750 kDa pore size. In some aspects, the pore size of the column is 100 kDa to 300 kDa. Further sub-populations can be isolated using antibodies or other agents that are specific for a specific marker expressed by the desired exosome population.

[0154] In some embodiments, the methods disclosed herein further comprise formulating the purified population of cell-derived vesicles by mixing the population with a carrier and/or a therapeutic agent such as a pro-angiogenic agent. Non-limiting examples are suitable carriers are described below. In addition or alternatively, the exosome composition can be combined with trehalose for enhanced stability, e.g., at a concentration of about 15 nM to about 50 nM of trehalose in carrier (e.g., PBS), or alternatively about 25 nM of trehalose in carrier (e.g., PBS). Methods to formulate exosomes with trehalose are described in Bosch et al. (2016) “*Trehalose prevents aggregation of exosomes and cryodamage*” Scientific Reports 6, Article number 36162, doi:10.1038/srep36162, incorporated herein by reference.

Molecular Composition of Cell-Derived Vesicles

[0155] In some embodiments, the purified populations of cell-derived vesicles (e.g., exosomes and/or microvesicles) of the present disclosure comprise proteins, lipids, metabolites, and/or nucleic acids (FIGS. 22-27). In some embodiments, the cell-derived

vesicles comprise therapeutic proteins and/or proteins associated with angiogenesis and immune modulation. In some embodiments, the protein content of the purified populations of cell-derived vesicles of the present disclosure is greater than the nucleic acid content of the cell-derived vesicles.

[0156] In some embodiments, the purified populations of cell-derived vesicles (e.g., exosomes and/or microvesicles) of the present disclosure may comprise one or more of, or alternatively two or more of, or alternatively three or more of, or alternatively four or more of, or alternatively five or more of, or alternatively six or more of, all of the following non-limiting examples of exogenous nucleic acids: miR-126, miR-132, miR-150, miR-210, miR-214, miR-296, and miR-424 (see FIG. 10). Several of the above-listed miRNAs are known in the art to mediate angiogenesis. The above-listed miRNAs were detected in exosomes and/or microvesicles of the present disclosure using a Bioanalyzer and qPCR analyses. Bioanalyzer analysis of exosomes demonstrated enrichment for small RNAs including rRNA2 and rRNA1 (see FIG. 10).

[0157] Surprisingly, the relative abundance of proteins in exosomes and/or microvesicles of the present disclosure was found to far exceed the relative abundance of RNA. This difference in relative abundance was statistically significant. In some embodiments, the relative abundance of protein exceeds the relative abundance of nucleic acids in exosomes and/or microvesicles of the present disclosure.

[0158] In some embodiments, the purified populations of cell-derived vesicles (e.g., exosomes and/or microvesicles) of the present disclosure may comprise one or more of, or alternatively two or more of, or alternatively three or more of, or alternatively four or more of, or alternatively five or more of, or alternatively six or more of, or alternatively seven or more of, or alternatively eight or more of, or alternatively nine or more of, or alternatively ten or more of, or alternatively all of (and integers therebetween) of the following non-limiting examples of metabolites: 3,6-anhydro-D-galactose, 4-aminobutyric acid, 5'-deoxy-5'-methylthioadenosine, 5-methoxytryptamine, s-adenosylmethionine, s-adenosylhomocysteine, adipic acid, aminomalonate, arabinose, aspartic acid, beta-alanine, cholesterol, citric acid, creatinine, cysteine, cytidine-5-monophosphate, erythritol, fructose, fumaric acid, galacturonic acid, glucose, glucose-1-phosphate, glucose-6-phosphate, glutamine, glyceric

acid, glycerol-alpha-phosphate, glycine, guanosine, hexitol, hexuronic acid, inosine, isohexonic acid, isomaltose, lactamide, lactic acid, lactose, leucine, levoglucosan, maleimide, malic acid, maltotriose, mannose, methanolphosphate, methionine, N-acetylaspartic acid, N-acetyl-D-galactosamine, nicotinamide, N-methylalanine, oxoproline, pantothenic acid, pentadecanoic acid, phenol, putrescine, pyruvic acid, ribitol, ribose, sorbitol, squalene, succinic acid, threitol, threonic acid, threonine, thymine, trans-4-hydroxyproline, trehalose, urea, uridine, valine, xylitol, and/or the any of the metabolites listed in Table 3. The above-listed metabolites were detected in exosomes and/or microvesicles of the present disclosure using an unbiased metabolomics approach. Several of the above-listed metabolites have been shown to modulate gene expression via epigenetic methylation marks on histone tails (e.g. S-adenosylmethionine (SAM) and S-Adenosyl-L-homocysteine (SAH)).

Table 3

Metabolites		
3,6-anhydro-D-galactose	glycerol-alpha-phosphate	N-methylalanine
4-aminobutyric acid	Glycine	oxoproline
5'-deoxy-5'-methylthioadenosine	Guanosine	pantothenic acid
5-methoxytryptamine	Hexitol	pentadecanoic acid
adipic acid	hexuronic acid	phenol
aminomalonate	Inosine	putrescine
arabinose	isohexonic acid	pyruvic acid
aspartic acid	Isomaltose	Ribitol
beta-alanine	Lactamide	Ribose
cholesterol	lactic acid	Sorbitol
citric acid	Lactose	Squalene
creatinine	Leucine	succinic acid
cysteine	Levoglucosan	Threitol
cytidine-5-monophosphate	Maleimide	threonic acid
erythritol	malic acid	Threonine
fructose	Maltotriose	Thymine
fumaric acid	Mannose	trans-4-hydroxyproline
galacturonic acid	methanolphosphate	Trehalose
glucose	Methionine	Urea
glucose-1-phosphate	N-acetylaspartic acid	Uridine
glucose-6-phosphate	N-acetyl-D-galactosamine	Valine

glutamine
glyceric acid

Nicotinamide

Xylitol

[0159] In some embodiments, the purified populations of cell-derived vesicles (e.g., exosomes and/or microvesicles) of the present disclosure may comprise one or more of, or alternatively two or more of, or alternatively three or more of, or alternatively four or more of, or alternatively, five or more of, or alternatively six or more of, or alternatively seven or more of, or alternatively eight or more of, or alternatively nine or more of, or alternatively ten or more of, or alternatively all of (and integers therebetween) of the following non-limiting examples of lipids and membrane components: Ceramide (d32:1), Ceramide (d33:1), Ceramide (d34:0), Ceramide (d34:1), Ceramide (d34:2), Ceramide (d34:2), Ceramide (d36:1), Ceramide (d38:1), Ceramide (d39:1), Ceramide (d40:0), Ceramide (d40:1), Ceramide (d40:2), Ceramide (d41:1), Ceramide (d42:1), Ceramide (d42:2) B, Ceramide (d44:1), Fatty Acid (20:4), Fatty Acid (22:0), Fatty Acid (22:6), Fatty Acid (24:0), Fatty Acid (24:1), glucosylceramides (d40:1), glucosylceramides (d41:1), glucosylceramides (d42:1), glucosylceramides (d42:2), Lysophosphatidylcholines (16:0), Lysophosphatidylcholines (18:0) A, Lysophosphatidylcholines (18:1), lysophosphatidylethanolamine (20:4), Phosphatidylcholines (32:1), Phosphatidylcholines (33:1), Phosphatidylcholines (34:0), Phosphatidylcholines (34:1), Phosphatidylcholines (34:2), Phosphatidylcholines (35:2), Phosphatidylcholines (36:1), Phosphatidylcholines (36:2), Phosphatidylcholines (36:3), Phosphatidylcholines (38:2), Phosphatidylcholines (38:3), Phosphatidylcholines (38:5), Phosphatidylcholines (38:6), Phosphatidylcholines (40:5), Phosphatidylcholines (40:6), Phosphatidylcholines (40:7), Phosphatidylcholines (p-34:0), Phosphatidylcholines (o-34:1), Phosphatidylethanolamines (34:1), Phosphatidylethanolamines (34:2), Phosphatidylethanolamines (36:3), Phosphatidylethanolamines (36:4), Phosphatidylethanolamines (38:4), B Phosphatidylethanolamines (38:6), Phosphatidylethanolamines (p-34:1), Phosphatidylethanolamines (o-34:2), Phosphatidylethanolamines (p-36:1), Phosphatidylethanolamines (o-36:2), Phosphatidylethanolamines (p-36:4), Phosphatidylethanolamines (o-36:5), Phosphatidylethanolamines (p-38:4), Phosphatidylethanolamines (o-38:5), Phosphatidylethanolamines (p-38:5), Phosphatidylethanolamines (o-38:6), Phosphatidylethanolamines (p-38:6), Phosphatidylethanolamines (o-38:7),

Phosphatidylethanolamines (p-40:4), Phosphatidylethanolamines (o-40:5), Phosphatidylethanolamines (p-40:5), Phosphatidylethanolamines (o-40:6), Phosphatidylethanolamines (p-40:6), Phosphatidylethanolamines (o-40:7), Phosphatidylethanolamines (p-40:7), Phosphatidylethanolamines (o-40:8), Sphingomyelin (d30:1), Sphingomyelin (d32:0), Sphingomyelin (d32:2), Sphingomyelin (d33:1), Sphingomyelin (d34:0), Sphingomyelin (d36:1), Sphingomyelin (d36:2), Sphingomyelin (d38:1), Sphingomyelin (d40:1), Sphingomyelin (d40:2), Sphingomyelin (d41:1), Sphingomyelin (d41:2), Sphingomyelin (d42:2), B Sphingomyelin (d42:3). The above-listed lipid and membrane components were detected in exosomes and/or microvesicles of the present disclosure using an unbiased lipidomics approach (see **FIG. 11** and Table 4). Several of the above-listed lipids have been shown to have therapeutic effects in multiple model systems (e.g. sphingomyelin and phosphatidylcholines).

Table 4**Lipid Membrane Components**

Ceramide (d32:1)	Phosphatidylcholines (32:1)
Ceramide (d33:1)	Phosphatidylcholines (34:0)
Ceramide (d34:0)	Phosphatidylcholines (34:1)
Ceramide (d34:1)	Phosphatidylcholines (34:2)
Ceramide (d34:2)	Phosphatidylcholines (35:2)
Ceramide (d34:2)	Phosphatidylcholines (36:1)
Ceramide (d36:1)	Phosphatidylcholines (36:2)
Ceramide (d38:1)	Phosphatidylcholines (36:3) B
Ceramide (d39:1)	Phosphatidylcholines (38:2)
Ceramide (d40:0)	Phosphatidylcholines (38:3)
Ceramide (d40:1)	Phosphatidylcholines (38:5) A
Ceramide (d40:2)	Phosphatidylcholines (38:6)
Ceramide (d41:1)	Phosphatidylcholines (40:5) A
Ceramide (d42:1)	Phosphatidylcholines (40:6) B
Ceramide (d42:2) B	Phosphatidylcholines (40:7)
Ceramide (d44:1)	Phosphatidylcholines (p-34:0)
	Phosphatidylethanolamines
Fatty Acid (20:4)	(34:1)
	Phosphatidylethanolamines
Fatty Acid (22:0)	(34:2)
	Phosphatidylethanolamines
Fatty Acid (22:6)	(36:3)
	Phosphatidylethanolamines
Fatty Acid (24:0)	(36:4)

Fatty Acid (24:1)	Phosphatidylethanolamines (38:4) B
glucosylceramides (d40:1)	Phosphatidylethanolamines (38:6)
glucosylceramides (d41:1)	Phosphatidylethanolamines (p-34:1)
glucosylceramides (d42:1)	Phosphatidylethanolamines (p-36:1)
glucosylceramides (d42:2)	Phosphatidylethanolamines (p-36:4)
Lysophosphatidylcholines (16:0)	Sphingomyelin (d30:1)
Lysophosphatidylcholines (18:0) A	Sphingomyelin (d32:0)
Lysophosphatidylcholines (18:1)	Sphingomyelin (d32:2)
lysophosphatidylethanolamine (20:4)	Sphingomyelin (d33:1)
Phosphatidylethanolamines (p-38:4)	Sphingomyelin (d34:0)
Phosphatidylethanolamines (p-38:5)	Sphingomyelin (d36:1)
Phosphatidylethanolamines (p-38:6)	Sphingomyelin (d36:2)
Phosphatidylethanolamines (p-40:4)	Sphingomyelin (d38:1)
Phosphatidylethanolamines (p-40:5)	Sphingomyelin (d40:1)
Phosphatidylethanolamines (p-40:6)	Sphingomyelin (d40:2) A
Phosphatidylethanolamines (p-40:7)	Sphingomyelin (d41:1)
Sphingomyelin (d42:2) B	Sphingomyelin (d41:2)
Sphingomyelin (d42:3)	

[0160] In some embodiments, the purified populations of cell-derived vesicles (e.g., exosomes and/or microvesicles) of the present disclosure may comprise one or more of, or alternatively two or more of, or alternatively three or more of, or alternatively four or more of, or alternatively, five or more of, or alternatively six or more of, or alternatively seven or more of, or alternatively eight or more of, or alternatively nine or more of, or alternatively ten or more of, or alternatively all of (and integers therebetween) of the following non-limiting examples of exosome-associated proteins: CD9, HSPA8, PDCD6IP, GAPDH, ACTB, ANXA2, CD63, SDCBP, ENO1, HSP90AA1, TSG101, PKM, LDHA, EEF1A1, YWHAZ, PGK1, EEF2, ALDOA, ANXA5, FASN, YWHAZ, CLTC, CD81, ALB, VCP, TPI1, PPIA,

MSN, CFL1, PRDX1, PFN1, RAP1B, ITGB1, HSPA5, SLC3A2, GNB2, ATP1A1, WHAQ, FLOT1, FLNA, CLIC1, CDC42, CCT2, A2M, YWHAG, RAC1, LGALS3BP, HSPA1A, GNAI2, ANXA1, RHOA, MFGE8, PRDX2, GDI2, EHD4, ACTN4, YWHAB, RAB7A, LDHB, GNAS, TFRC, RAB5C, ANXA6, ANXA11, KPNB1, EZR, ANXA4, ACLY, TUBA1C, RAB14, HIST2H4A, GNB1, UBA1, THBS1, RAN, RAB5A, PTGFRN, CCT5, CCT3, BSG, RAB5B, RAB1A, LAMP2, ITGA6, GSN, FN1, YWHAH, TKT, TCP1, STOM, SLC16A1, RAB8A, and/or the proteins listed in Table 5. The above-listed proteins were detected in exosomes and/or microvesicles of the present disclosure via gas chromatography and mass spectrometry analysis.

Table 5

Exosome Marker Proteins			
CD9	ALB	LGALS3BP	RAB14
HSPA8	VCP	HSPA1A	HIST2H4A
PDCD6IP	TPI1	GNAI2	GNB1
GAPDH	PPIA	ANXA1	UBA1
ACTB	MSN	RHOA	THBS1
ANXA2	CFL1	MFGE8	RAN
CD63	PRDX1	PRDX2	RAB5A
SDCBP	PFN1	GDI2	PTGFRN
ENO1	RAP1B	EHD4	CCT5
HSP90AA1	ITGB1	ACTN4	CCT3
TSG101	HSPA5	YWHAB	BSG
PKM	SLC3A2	RAB7A	RAB5B
LDHA	GNB2	LDHB	RAB1A
EEF1A1	ATP1A1	GNAS	LAMP2
YWHAZ	YWHAQ	TFRC	ITGA6
PGK1	FLOT1	RAB5C	GSN
EEF2	FLNA	ANXA6	FN1
ALDOA	CLIC1	ANXA11	YWHAH
ANXA5	CDC42	KPNB1	TKT
FASN	CCT2	EZR	TCP1
YWHAE	A2M	ANXA4	STOM
CLTC	YWHAG	ACLY	SLC16A1
CD81	RAC1	TUBA1C	RAB8A

[0161] In some embodiments, the purified populations of cell-derived vesicles (e.g., exosomes and/or microvesicles) of the present disclosure may comprise one or more of, or alternatively two or more of, or alternatively three or more of, or alternatively four or more of, or alternatively, five or more of, or alternatively six or more of, or alternatively seven or

more of, or alternatively eight or more of, or alternatively nine or more of, or alternatively ten or more of, or alternatively all of (and integers therebetween) of the following non-limiting examples of distinctive proteins which include proteins not previously associated with exosome identity: FN1, EDIL3, TF, ITGB1, VCAN, ANXA2, MFGE8, TGB1, TGFB2, TGFB1, TGFB2, TGFB1, TGFB2, TGFB1, TGFB2, TGFB1, TGFB2, BASP1, COL1, COL6, GAPDH, ITGA3, FBN1, ITGAV, ITGB5, NOTCH2, SDCBP, HSPA2, HSPA8, NT5E, MRGPRF, RTN4, NEFM, INA, NRP1, HSPA9, FBN1, BSG, PRPH, FBLN1, PARP4, FLNA, YBX1, EVA1B, ADAM10, HSPG2, MCAM, POSTN, GNB2, GNB1, ANPEP, ADAM9, ATP1A1, CSPG4, EHD2, PXDN, SERPINE2, CAV1, PKM, GNB4, NPTN, CCT2, LGALS3BP, and MVP. The above-listed proteins were detected in exosomes and/or microvesicles of the present disclosure via gas chromatography and mass spectrometry analysis.

[0162] In some embodiments, the purified populations of cell-derived vesicles (e.g., exosomes and/or microvesicles) of the present disclosure may comprise one or more of, or alternatively two or more of, or alternatively three or more of, or alternatively four or more of, or alternatively five or more of, or alternatively six or more of, or alternatively seven or more of, or alternatively eight or more of, or alternatively nine or more of, or alternatively ten or more of, or alternatively all of (and integers therebetween) of the following non-limiting examples of proteins associated with angiogenesis: FBLN2, TIMP1, NID1, IGFBP3, LTBP1, DUSP3, ITGAV, LAMA5, COL1A1, NOTCH2, NRG1, ERBB2, COL4A2, LDLR, TSB, MMP2, TIMP2, TPI1, ACVR1B, INHBA, EGFR, APOA1, NCSTN, TGFB2, SPARC, TGFB1, F2, SERPINE1, SDC4, SDC3, ACAN, IFI16, MMP14, PLAT, COL18A1, NOTCH3, DSP, PKP4, SERPINE2, SRGN, NRP2, EPHA2, ITGA5, NRP1, PLAUR, SERPINB6, CLEC3B, CD47, SDC1, PSMA7, ENG, S100A13, TIMP3, TMED10, TGFB1, CTGF, DCN, ITGB3, PDGFRA, JAG1, TGFB2, PLAUR, PDGFRB, FYN, THY1, HSPG2, TENC1, TGFB1, PLXNA1, LRP1, STAT1, CXCL12, VCAN, MET, FN1, CD36, STAT3, THBS1, FGFR1, GRB14, FGB, API5, HAPLN1, RECK, LAMC1, CYR61, GPC1, IGFBP4, ITGA4, MFAP2, SDC2, EFNB2, FGA, PLXND1, ADAM17, ADAM9, ANPEP, EPHB1, PPP2R5D, ANTXR2, IGFBP7, COL6A3, LAMB3, ADAMTS1, ADAM10, A2M, EFNB1, ITGA3, CLU, KHSRP, and EFEMP1 (Table 6). The above-listed proteins were detected in exosomes and/or microvesicles of the present disclosure via gas chromatography and mass spectrometry analysis.

Table 7**Immune Modulatory****Proteins**

TGFB1
 TGFB1
 TGFB2
 TGFB1
 TGFB2
 TGFBRAP1
 ADAM17
 ARG1
 CD274
 EIF2A
 EPHB2
 HLA-DRA
 ELAVL1
 IRAK1
 LGALS1
 PSME4
 STAT1
 STAT3

[0164] In some embodiments, the purified populations of cell-derived vesicles (e.g., exosomes and/or microvesicles) of the present disclosure may comprise one or more of, or alternatively two or more of, or alternatively three or more of, or alternatively four or more of, or alternatively, five or more of, or alternatively six or more of, or alternatively seven or more of, or alternatively eight or more of, or alternatively nine or more of, or alternatively ten or more of, or alternatively all of (and integers therebetween) of the following non-limiting examples of therapeutic proteins: EDIL3, TF, ITGB1, ANXA2, MFGE8, TGB1, TGBFR2, BASP1, COL1, COL6, GAPDH, FBN1, ITGB5, SDCBP, HSPA2, HSPA8, NT5E, MRGPRF, RTN4, NEFM, INA, HSPA9, FBN1, BSG, PRPH, FBLN1, PARP4, FLNA, YBX1, EVA1B, MCAM, POSTN, GNB2, GNB1, ATP1A1, CSPG4, EHD2, PXDN, CAV1, PKM, GNB4, NPTN, CCT2, LGALS3BP, and MVP(Table 8). The above-listed proteins were detected in exosomes and/or microvesicles of the present disclosure via gas chromatography and mass spectrometry analysis.

Table 8**Therapeutic
Proteins**

EDIL3	HSPA8	MCAM
TF	NT5E	POSTN
ITGB1	MRGPRF	GNB2
ANXA2	RTN4	GNB1
MFGE8	NEFM	ATP1A1
TGB1	INA	CSPG4
TGBFR2	HSPA9	EHD2
BASP1	FBN1	PXDN
COL1	BSG	CAV1
COL6	PRPH	PKM
GAPDH	FBLN1	GNB4
FBN1	PARP4	NPTN
ITGB5	FLNA	CCT2
SDCBP	YBX1	LGALS3BP
HSPA2	EVA1B	MVP

[0165] In some embodiments, the purified populations of cell-derived vesicles (e.g., exosomes and/or microvesicles) of the present disclosure may comprise one or more of, or alternatively two or more of, or alternatively three or more of, or alternatively four or more of, or alternatively, five or more of, or alternatively six or more of, or alternatively seven or more of, or alternatively eight or more of, or alternatively nine or more of, or alternatively ten or more of, or alternatively all of (and integers therebetween) of the following non-limiting examples of inflammation-related proteins: SERPINE1, ADAM17, ARG1, CD274, EIF2A, EPHB2, HLA-DRA, ELAVL1, IRAK1, LGALS1, PSME4, STAT1, STAT3, TGFB1, TGFB2, TGFB1, TGFB2, TGFB1, FBN1, HSP90AB1, SDCBP, LTBP1, JAK1, PIK3C2A, GRB2, HRAS, RAF1, MAP2K1, MAPK3, TYK2, CRIP2, IL6ST, JAK2, SQSTM1, DDX3X, PRMT5, SLC9A3R1, XPO1, TRAF3IP2, SPAG9, DIAPH1, CCDC22, PDCD6, PRPF40A, STAM2, TRIO, ERLIN2, AP2A2, MPZL1, AP2A1, EGFR, LMNA, EIF2S1, FYN, CDK1, NPM1, LYN, THBS1, ANXA5, RRAS, PCNA, SRC, XRCC6, HNRNPL, H2AFX, PRKCA, DDX5, PLCG1, FLNA, UBA1, S100A1, RPS3, SP100, AHCY, CFL1, F2R, RPA1, APEX1, MAPK1, EPHA2, PPP2R1A, PIF, PHB, NF2, LRPPRC, MSH2, CBX5, IQGAP1, TMED10, DNM2, VCP, EIF3B, EIF3E, ACTB, RPL26, SUMO2, PPP1CA, RAP1A, RAC1, AP2B1, PPP2CA, CSNK2A1, SIRPA, DAB2, CDK5, CLTC, CAV1, PRDX1, C1QBP, SREBF2, TRO, CHD3, TRIM28, SF3B2, ADAM9, ADAM15, PIN1, RIPK1, HDAC1, CUL2, EIF3A, FHL2, SMC1A, KPNB1, TMED2, SEC23B, CPSF6, WLS, DAB2IP, MICAL3, HUWE1, ABI1, RPTOR, CCAR2, COMMD1,

ARFGAP1, HSPH1, HDAC2, DDX17, RAD50, UPF1, COPS5, USP7, RHBDF1, AP2M1, EIF3C, PHB2, MAP1LC3B, SPNS1, PTPN23, CBX8, PDLIM7, DACT1, NXF1, MYO6, PA2G4, RUVBL1, THRAP3, ACOT9, CD2AP, and RBM8A. The above-listed proteins were detected in exosomes and/or microvesicles of the present disclosure via gas chromatography and mass spectrometry analysis.

[0166] In some embodiments, the purified populations of cell-derived vesicles (e.g., exosomes and/or microvesicles) of the present disclosure may comprise one or more of, or alternatively two or more of, or alternatively three or more of, or alternatively four or more of, or alternatively, five or more of, or alternatively six or more of, or alternatively seven or more of, or alternatively eight or more of, or alternatively nine or more of, or alternatively ten or more of, or alternatively all of (and integers therebetween) of the following non-limiting examples of canonical inflammation-related proteins: SERPINE1, ADAM17, ARG1, CD274, EIF2A, EPHB2, HLA-DRA, ELAVL1, IRAK1, LGALS1, PSME4, STAT1, STAT3, TGFB1, TGFB2, TGFBR1, TGFBR2, TGFBI, FBN1, HSP90AB1, SDCBP, LTBP1, JAK1, PIK3C2A, GRB2, HRAS, RAF1, MAP2K1, MAPK3, TYK2, STAT3, STAT1, STAT3, CRIP2, IL6ST, JAK2, CD274, and SQSTM1. The above-listed proteins were detected in exosomes and/or microvesicles of the present disclosure via gas chromatography and mass spectrometry analysis.

[0167] In further embodiments, the purified populations express one or more combinations of the above.

Formulations and Pharmaceutical Compositions

[0168] The present disclosure provides purified populations of cell-derived vesicles (e.g., exosomes and/or microvesicles). In some embodiments, the population of cell-derived vesicles is substantially homogeneous. In other embodiments, the population of cell-derived vesicles is heterogeneous.

[0169] In some embodiments, the substantially homogeneous population is a purified population where at least 90% of the cell-derived vesicles have a diameter of less than 100 nm as determined by a NanoSight LM10HS (available from Malvern Instruments Ltd, Amesbury, MA, USA).

[0170] In some embodiments, the concentration of cell-derived vesicles in the population comprises between about 0.5 micrograms and 100 micrograms of exosome and/or microvesicle protein collected per approximately 10^6 cells as determined by DC assay (Biorad, Hercules, CA, USA). In some embodiments, the concentration of cell-derived vesicles in the population comprises between about 100 micrograms and 5000 micrograms of exosome and/or microvesicle protein collected per approximately 10^6 cells. In other embodiments, the concentration of cell-derived vesicles in the population comprises between about 100 micrograms and 500 micrograms of exosome and/or microvesicle protein collected per approximately 10^6 cells. In other embodiments, the concentration of cell-derived vesicles in the population comprises between about 500 micrograms and 1000 micrograms of exosome and/or microvesicle protein collected per approximately 10^6 cells. In other embodiments, the concentration of cell-derived vesicles in the population comprises between about 1000 micrograms and 5000 micrograms of exosome and/or microvesicle protein collected per approximately 10^6 cells. In other embodiments, the concentration of cell-derived vesicles in the population comprises between about 40 micrograms and 100 micrograms of exosome and/or microvesicle protein collected per approximately 10^6 cells. In other embodiments, the concentration of cell-derived vesicles in the population comprises less than about 300 micrograms of cell-derived vesicles protein collected per approximately 10^6 cells. In other embodiments, the concentration of cell-derived vesicles in the population comprises less than about 200 micrograms of cell-derived vesicles protein collected per approximately 10^6 cells. In other embodiments, the concentration of cell-derived vesicles in the population comprises between about 10 micrograms and 40 micrograms of exosome and/or microvesicle protein collected per approximately 10^6 cells. In yet other embodiments, the concentration of cell-derived vesicles in the population comprises less than about 30 micrograms of cell-derived vesicles protein collected per approximately 10^6 cells. In yet other embodiments, the concentration of cell-derived vesicles in the population is less than about 20 micrograms per 10^6 cells.

[0171] The purified populations of cell-derived vesicles can be purified on the basis of average size of the cell-derived vesicles in the composition. Without being bound by theory, it is contemplated that the different sized cell-derived vesicles may contain different types and/or amounts of nucleic acids, protein, lipids, and other components. As such, it is

contemplated that compositions comprising cell-derived vesicles of an average size may have a different therapeutic efficacy as compared to a composition comprising cell-derived vesicles of a different average size. In some embodiments, the average diameter of the cell-derived vesicles in the population is between about 0.1 nm and about 1000 nm. In other embodiments, the average diameter of the cell-derived vesicles in the population is between about 2 nm and about 200 nm. In other embodiments, the average diameter of the cell-derived vesicles in the population is less than 100 nm. In yet other embodiments, the average diameter of the cell-derived vesicles in the population is less than 50 nm. In still other embodiments, the average diameter of the cell-derived vesicles in the population is less than about 40 nm.

[0172] The compositions disclosed herein may further comprise a carrier, for example, a pharmaceutically acceptable carrier. In some embodiments, more than one pharmaceutically acceptable carrier can be used. Any pharmaceutically acceptable carrier known to those of skill in the art can be used.

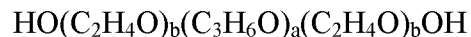
[0173] In some embodiments, the pharmaceutically acceptable carrier is a preservative, for example, a polymeric preservative or a stabilizing agent.

[0174] In some embodiments, the pharmaceutically acceptable carrier is selected from the group consisting of a polyethylene glycol (PEG) (e.g., PEG 150 Distearate), honey, a large molecular weight protein (e.g., bovine serum albumin or soy protein), polyvinyl alcohol, glyceryl monostearate, hyaluronic acid, glycerin, preferably vegetable-derived, proteins, preferably hydrolyzed proteins, (e.g., soy protein and silk protein), vasoline, citrosept, parabens, xanthan gum, i-carregaan, phytigel, Carbopol[®] polymers, and polyvinyl pyrrolidone.

[0175] In some embodiments, exosomes are preserved in serum albumin. Non-limiting examples of serum albumins appropriate for preservation of exosomes include bovine serum albumin (BSA), human serum albumin (HSA), ovalbumin (OVA), and lactalbumin.

[0176] Biocompatible gelation agents include thermosensitive sol-gel reversible hydrogels such as aqueous solutions of poloxamers. In one aspect, the poloxamer is a nonionic triblock copolymer composed of a central hydrophobic chain of polyoxypropylene (e.g.,

(poly(propylene oxide)) flanked by two hydrophilic chains of polyoxyethylene (*e.g.*, poly(ethylene oxide)). In one aspect, poloxamer has the formula



[0177] wherein a is from 10 to 100, 20 to 80, 25 to 70, or 25 to 70, or from 50 to 70; b is from 5 to 250, 10 to 225, 20 to 200, 50 to 200, 100 to 200, or 150 to 200. In another aspect, the poloxamer has a molecular weight from 2,000 to 15,000, 3,000 to 14,000, or 4,000 to 12,000. Poloxamers useful herein are sold under the tradename Pluronic[®] manufactured by BASF. Non-limiting examples of poloxamers useful herein include, but are not limited to, Pluronic[®] F68, P103, P105, P123, F127, and L121.

[0178] In one aspect, the biocompatible gelation agent is an agent that is liquid prior to application to a subject (*e.g.*, at room temperature or colder) and becomes a gel after application to the subject (*e.g.*, at body temperature). In one embodiment, the biocompatible gelation agent is a hydrogel.

[0179] In another aspect, disclosed herein is a composition comprising exosomes and/or microvesicles and a poloxamer wherein the composition is in a sol (liquid) phase at about 0 °C to about 20 °C and transitions a gel (solid) phase at or near the body temperature or higher, such as about 25 °C to about 40 °C, or about 30 °C to about 37 °C.

[0180] In some aspects, the pharmaceutically acceptable carrier is a pharmaceutically acceptable aqueous carrier such as water or an aqueous carrier. Examples of pharmaceutically acceptable aqueous carrier include sterile water, saline, phosphate buffered saline, aqueous hyaluronic acid, Ringer's solution, dextrose solution, Hank's solution, and other aqueous physiologically balanced salt solutions. In some embodiments, the pharmaceutically acceptable aqueous carrier is Normosol[™]-R.

[0181] Nonaqueous pharmaceutically acceptable carriers include, fixed oils, vegetable oils such as olive oil and sesame oil, triglycerides, propylene glycol, polyethylene glycol, and injectable organic esters such as ethyl oleate can also be used.

[0182] Pharmaceutically acceptable carrier can also contain minor amounts of additives, such as substances that enhance isotonicity, chemical stability, or cellular stability. Examples of buffers include phosphate buffer, bicarbonate buffer and Tris buffer, while examples of

preservatives include thimerosal, cresols, formalin and benzyl alcohol. In certain aspects, the pH can be modified depending upon the mode of administration. In some aspect, the composition has a pH in the physiological pH range, such as pH 7 to 9.

[0183] In one aspect, depending on the type of a pharmaceutically acceptable carrier used, the compositions described herein can comprise about 0.1-100%, 0.1-50%, or 0.1-30%, such as 0.1 %, 0.25 %, 0.5 %, 0.75%, 1 %, 2 %, 5 %, 7 %, 10 %, 15 %, 20 %, 25 %, 30 %, 40 %, 45 %, 50 %, 55 %, 60 %, 65 %, 70 %, 75 %, 80 %, 85 %, 90 % or 95 % of the pharmaceutically acceptable carrier used in the total weight of the composition, or any range between two of the numbers (end point inclusive).

[0184] In some embodiments, any one of the above listed pharmaceutically acceptable carriers is expressly excluded.

[0185] In some embodiments, the compositions disclosed herein may further comprise one or more neurotrophic factors. In some aspects, the neurotrophic factors are selected from BDNF, NGF, Neurotrophin-3, CTNF, GDNF, FGF, IGF, HGF, Noggin, and T3. In some aspects, the proteins are recombinant. In some aspects, the neurotrophic factor is about 1 to 10 ng/mL, or alternatively 5 to 20 ng/mL, or alternatively 5 to 30 ng/mL, or alternatively 5 to 40 ng/mL, or alternatively 5 to 50 ng/mL, or alternatively 5 to 100 ng/mL, or alternatively 5 to 250 ng/mL, or alternatively 5 to 500 ng/mL, or alternatively 25 to 75 ng/mL, or alternatively 50 to 100 ng/mL, or alternatively 100 to 500 ng/mL, or or alternatively 100 ng/mL to 1 µg/mL. In particular aspects, the factor is about 10 ng/mL, or alternatively about 15 ng/mL, or alternatively about 20 ng/mL, or alternatively about 25 ng/mL, or alternatively about 30 ng/mL, or alternatively about 40 ng/mL, or alternatively about 50 ng/mL, or alternatively about 100 ng/mL, or alternatively about 200 ng/mL, or alternatively about 250 ng/mL, or alternatively about 300 ng/mL, or alternatively about 400 ng/mL, or alternatively about 500 ng/mL, or alternatively about 1 µg/mL. Preferably, the neurotrophic factor is about 10 to about 1000 ng/mL.

[0186] In some embodiments, the cell-derived vesicles described herein are frozen (e.g., snap-frozen) or freeze-dried (e.g., lyophilized) to promote stability, preserve activity and increase shelf-life. One skilled in the art would understand how to reconstitute the lyophilized product before use.

[0187] In some embodiments, the populations of cell-derived vesicles described herein are used immediately after isolation. In other embodiments, the populations of cell-derived vesicles are cryopreserved (e.g. frozen), for example, using any cryopreservation techniques well-known to those skilled in the art. In some embodiments, all or substantially of the cells and/or cellular debris are removed from the culture medium prior to cryopreservation. In some embodiments, all or substantially of the cells and/or cellular debris are removed from the culture medium after cryopreservation.

Applications and Uses

[0188] The populations of cell-derived vesicles described herein can be used in numerous medial applications including for promoting angiogenesis, treating peripheral arterial disease or stroke, treating a disease or condition involving an inflammatory response or related to inflammation, and treating a dermal wound in a subject.

[0189] In one aspect, the disclosure is related to treating a disease or condition involving an inflammatory response or related to inflammation in a subject in need thereof, the method comprising administering to the subject a purified population of cell-derived vesicles, wherein the population is purified from a population of stem cells cultured under conditions of hypoxia and low serum, and optionally wherein the cell-derived vesicles comprise exosomes and/or microvesicles. In one aspect, the inflammatory disease or condition is selected from multiple sclerosis, primary and secondary progressive multiple sclerosis, relapsing remitting multiple sclerosis, radiation-induced soft tissue damage, fraility, a neuroinflammatory disease, muscle injuries, radiation tissue damage, stroke, brain inflammatory disease, traumatic brain injury, myocardial infarction, graft versus host disease, Parkinson's disease, Alzheimer's, inflammatory bowel disease, Huntington's disease, amyotrophic lateral sclerosis, Bahcet's disease, sarcopenia, aging, spinal cord injury, wound repair, or dysphagia, and optionally wherein the disease or condition excludes stroke. In a further aspect the treatment excludes prophylaxis. In a further aspect, the treatment is only prophylaxis. In a further aspect, the treatment is prophylaxis or treatment.

[0190] In a further aspect, the inflammatory disease or condition is selected from multiple sclerosis, progressive multiple sclerosis, or relapsing remitting multiple sclerosis. Methods for determining clinical efficacy are described herein and known in the art, e.g., see

ncbi.nlm.nih.gov/pmc/articles/PMC5250666/:

ncbi.nlm.nih.gov/pmc/articles/PMC3895761/.sciencedirect.com/science/article/pii/S0014488610002992?via%3Dihub:

sciencedirect.com/science/article/pii/S0165572808002257?via%3Dihub:

sciencedirect.com/science/article/pii/S0014488610002992#s0010, last accessed on June 5,

2018. The subject may be a mammal, for example, a human or non-human mammals such as a bovine, an ovine, or a porcine. In preferred embodiments, the subject is a human patient. In a further aspect, the subject has been selected for the therapy by diagnostic criteria as determined by the treating physician or health care professional.

[0191] In one aspect, provided herein are methods for promoting angiogenesis in a subject in need thereof comprising administering to the subject the purified population or an effective amount of the population and/or a composition described herein. In some embodiments, the subject is administered at least one dose of between approximately 0.1 mg and 200 mg of cell-derived vesicle protein. In some embodiments, the subject is administered at least one dose of between approximately 0.1 mg and 1000 mg of cell-derived vesicle protein. In other embodiments, the subject is administered at least one dose of approximately 50 mg of cell-derived vesicle protein. In some embodiments, the compositions of cell-derived vesicles are administered prior to or after administration of an isolated stem cell. In other embodiments, the compositions of cell-derived vesicles are administered simultaneously with an isolated stem cell. The compositions herein can be administered to the subject by any method known by those of skill in the art. In some embodiments, the compositions are administered by intravenous injection, intrathecal injection, direct injection, intramuscular injection, intracranial injection, or topically.

[0192] In one aspect, provided herein are methods for treating peripheral arterial disease or stroke in a subject in need thereof comprising administering to the subject the purified population or an effective amount of the population and/or a composition described herein. In some embodiments, the subject is administered at least one dose of between approximately 0.1 mg and 200 mg of cell-derived vesicle protein. In some embodiments, the subject is administered at least one dose of between approximately 0.1 mg and 1000 mg of cell-derived vesicle protein. In other embodiments, the subject is administered at least one dose of approximately 50 mg of cell-derived vesicle protein. In some embodiments, the

compositions of cell-derived vesicles are administered prior to or after administration of an isolated stem cell. In other embodiments, the compositions of cell-derived vesicles are administered simultaneously with an isolated stem cell. The compositions herein can be administered to the subject by any method known by those of skill in the art. In some embodiments, the compositions are administered by intravenous injection, intrathecal injection, direct injection, intrathecal injection, intramuscular injection, intracranial injection, or topically. In some embodiments, the compositions herein can be administered to a subject that has suffered a stroke within 24 hours following the stroke event. In other embodiments, the compositions herein can be administered to a subject that has suffered from a stroke about 24 – 48 hours following the stroke event. In other embodiments, the compositions herein can be administered to a subject that has suffered a stroke within about 48 – 72 hours following the stroke event. In other embodiments, compositions herein can be administered to a subject that has suffered a stroke within about 72 – 96 hours following the stroke event.

[0193] In one aspect, provided herein are methods for treating a dermal wound in a subject in need thereof comprising administering to the subject the purified population or an effective amount of the population and/or a composition described herein. In some embodiments, the subject is administered at least one dose of between approximately 0.1 mg and 200 mg of cell-derived vesicle protein. In some embodiments, the subject is administered at least one dose of between approximately 0.1 mg and 1000 mg of cell-derived vesicle protein. In other embodiments, the subject is administered at least one dose of approximately 50 mg of cell-derived vesicle protein. In some embodiments, the compositions of cell-derived vesicles are administered prior to or after administration of an isolated stem cell. In other embodiments, the compositions of cell-derived vesicles are administered simultaneously with an isolated stem cell. The compositions herein can be administered to the subject by any method known by those of skill in the art. In some embodiments, the compositions are administered by intravenous injection, intrathecal injection, direct injection, intramuscular injection, intracranial injection, or topically.

[0194] In one aspect, provided herein are methods for treating a disease or condition involving an inflammatory response or related to inflammation in a subject in need thereof comprising administering to the subject the purified population or an effective amount of the population and/or a composition described herein. In some embodiments, the subject is

administered at least one dose of between approximately 0.1 mg and 200 mg of cell-derived vesicle protein. In some embodiments, the subject is administered at least one dose of between approximately 0.1 mg and 1000 mg of cell-derived vesicle protein. In other embodiments, the subject is administered at least one dose of approximately 50 mg of cell-derived vesicle protein. In some embodiments, the compositions of cell-derived vesicles are administered prior to or after administration of an isolated stem cell. In other embodiments, the compositions of cell-derived vesicles are administered simultaneously with an isolated stem cell. The compositions herein can be administered to the subject by any method known by those of skill in the art. In some embodiments, the compositions are administered by intravenous injection, intrathecal injection, direct injection, intramuscular injection, intracranial injection, or topically. The methods can further comprise administration of an effective amount of other agents, e.g., agents that suppress inflammatory responses. In some aspects, the other agents include anti-inflammatory cytokines and neurotrophic factors. The neurotrophic factors include but are not limited to BDNF, NGF, Neurotrophin-3, CTNF, GDNF, FGF, IGF, HGF, Noggin, and T3. The administration can be concurrent or sequential as determined by the treating physician. The subject can be an animal, e.g., a mammal such as a human patient in need of such treatment, that in one aspect, has been pre-selected for the therapy by a treating physician or other health care professional.

[0195] In some embodiments, the purified populations of cell-derived vesicles disclosed herein reduce the expression of key inflammatory cytokines and induce the expression of critical anti-inflammatory cytokines in lymphocytes. Exemplary cytokines include but are not limited to IL-11, G-CSF, Eotaxin, IL-4, IL-7, MCSF, IL-12p70, IL-1a, BLC, IL-8, GM-CSF, MIP-1d, IL-2, IL-15, IL-13, IFNg, IL-6sR, IL-16, IL-1b, IL-1ra, MIP-1b, TNFb, IL-17, IL-12p40, PDGF-BB, IL-5, IL-6, Eotaxin-2, TNF RI, IL-10, MCP-1, I-309, TNFa, RANTES, MIP-1a, MIG, TNF RII, TIMP-1, ICAM-1, and TIMP-2 (**FIGS. 19-21**).

[0196] Methods to determine and monitor the therapy are known in the art and briefly described herein. When delivered in vitro, administration is by contacting the composition with the tissue or cell by any appropriate method, e.g., by administration to cell or tissue culture medium and is useful as a screen to determine if the therapy is appropriate for an individual or to screen for alternative therapies to be used as a substitute or in combination with the disclosed compositions. When administered in vivo, administration is by systemic

or local administration. In vivo, the methods can be practiced on a non-human animal to screen alternative therapies to be used as a substitute or in combination with the disclosed compositions prior to human administration. In a human or non-human mammal, they are also useful to treat the disease or disorder.

Kits

[0197] The agents described herein may, in some embodiments, be assembled into pharmaceutical or diagnostic or research kits to facilitate their use in therapeutic, diagnostic or research applications. A kit may include one or more containers housing the components of the invention and instructions for use. Specifically, such kits may include one or more agents described herein, along with instructions describing the intended application and the proper use of these agents. In certain embodiments agents in a kit may be in a pharmaceutical formulation and dosage suitable for a particular application and for a method of administration of the agents. Kits for research purposes may contain the components in appropriate concentrations or quantities for running various experiments.

[0198] The kit may be designed to facilitate use of the methods described herein and can take many forms. Each of the compositions of the kit, where applicable, may be provided in liquid form (e.g., in solution), or in solid form, (e.g., a dry powder). In certain cases, some of the compositions may be constitutable or otherwise processable (e.g., to an active form), for example, by the addition of a suitable solvent or other species (for example, water or a cell culture medium), which may or may not be provided with the kit. In some embodiments, the compositions may be provided in a preservation solution (e.g., cryopreservation solution). Non-limiting examples of preservation solutions include DMSO, paraformaldehyde, and CryoStor® (Stem Cell Technologies, Vancouver, Canada). In some embodiments, the preservation solution contains an amount of metalloprotease inhibitors.

[0199] As used herein, "instructions" can define a component of instruction and/or promotion, and typically involve written instructions on or associated with packaging of the invention. Instructions also can include any oral or electronic instructions provided in any manner such that a user will clearly recognize that the instructions are to be associated with the kit, for example, audiovisual (e.g., videotape, DVD, etc.), internet, and/or web-based communications, etc. The written instructions may be in a form prescribed by a

governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which instructions can also reflect approval by the agency of manufacture, use or sale for animal administration.

[0200] The kit may contain any one or more of the components described herein in one or more containers. As an example, in one embodiment, the kit may include instructions for mixing one or more components of the kit and/or isolating and mixing a sample and applying to a subject. The kit may include a container housing agents described herein. The agents may be in the form of a liquid, gel or solid (powder). The agents may be prepared sterilely, packaged in syringe and shipped refrigerated. Alternatively it may be housed in a vial or other container for storage. A second container may have other agents prepared sterilely. Alternatively the kit may include the active agents premixed and shipped in a syringe, vial, tube, or other container. The kit may have one or more or all of the components required to administer the agents to a subject, such as a syringe, topical application devices, or IV needle tubing and bag.

[0201] The therapies as describe herein can be combined with appropriate diagnostic techniques to identify and select patients for the therapy. For example, an ankle-brachial index (ABI) test may be performed to compare blood pressure in a patient's ankle from blood pressure in the patient's arm or Doppler ultrasound may look for blood flow in the major arteries and veins in the limbs. Thus, patients harboring the mutation can be identified prior to symptoms appearing or before advancement of the disease.

[0202] The following examples are provided to illustrate and not limit the disclosure.

EXAMPLES

[0203] Bone marrow derived mesenchymal stem cells (MSCs) exhibit tissue healing capabilities via signaling to endogenous cell populations including immune cells and endothelial cells (Meyerrose, T. et al. (2010) *Advanced Drug Delivery Reviews* 62(12):1167-1174). MSCs have also shown promise as a potential therapeutic for PAD through the secretion of a robust profile of angiogenic signaling proteins, however, it remains unclear which factors are the main drivers of MSC induced angiogenesis (Liew, A. et al. (2012) *Stem Cell Research & Therapy* 3(4):28). Exosomes are small lipid-bound, cellularly secreted vesicles that mediate intercellular communication via cell-to-cell transport of proteins and

RNA (El Andaloussi, S. et al. (2013) *Nature Reviews. Drug Discovery* 12(5):347-357). Interestingly, exosomes have been recently shown to also mediate some of the tissue healing properties of MSCs (Bian, S. et al. (2014) *Journal of Molecular Medicine* 92(4):387-397; Kordelas, L. et al. (2014) *Leukemia* 8(4):970-973; Zhang, B. et al. (2014) *Stem Cells* 33(7):2158-2168), however, the underlying mechanisms by which MSC derived exosomes exert their tissue healing properties remain unclear.

[0204] Additionally, the angiogenic potential of MSCs can vary due to differences in their microenvironment (Rosova, I. et al. (2008) *Stem Cells* 26(8):2173-2182). MSCs are generally expanded in high serum (10-20%) containing media under atmospheric oxygen (normoxic) conditions (21% O₂) prior to injection into animal models (Ikebe, C. et al. (2014) *BioMed Research International* 2014: 951512). However, MSCs experience a markedly different environmental niche upon injection into tissues affected by PAD, where they are exposed to significantly reduced oxygen tension and a reduced concentration of factors contained in serum due to a lack of proper blood flow (Banfi, A. et al. (2005) *Current Atherosclerosis Reports* 7(3):227-234). It has been recognized that the angiogenic potential of endothelial cells is enhanced when stimulated under hypoxic conditions (Humar, R. et al. (2002) *FASEB Journal: Official Publication of the Federation of American Societies for Experimental Biology* 16(8):771-780). Although there is evidence that hypoxic stimulation induces expression of angiogenic signaling proteins in endothelial cells, it is not clear to what extent such changes in the environmental niche affect the MSC proteome (Yamakawa, M. et al. (2003) *Circulation Research* 93(7):664-673; Beegle, J. et al. (2015) *Stem Cells* 33(6):1818-1828). Therefore, signaling pathways and gene networks that are differentially expressed at the protein level in MSCs exposed to PAD-like culture conditions as compared to normoxic, high serum expansion conditions were analyzed.

[0205] As proteins mediate most intracellular activity and communication between cells, mass spectrometry proteomics approaches have been invaluable in elucidating differential cell states and patterns of cellular communication (Johansson, H.J. et al. (2013) *Nature Communications* 4:2175). However, mass spectrometry based proteomics approaches have had limitations in depth of analysis, greatly limiting the characterization of signaling proteins within cells as they are often present at low levels as compared to other classes of proteins such as structural proteins, which are present at much higher levels (Hultin-Rosenberg, L. et

al. (2013) *Molecular & Cellular Proteomics: MCP* 12(7):2021-2031). A new mass spectrometry approach, termed high-resolution isoelectric focusing liquid coupled chromatography tandem mass spectrometry (HiRIEF LC-MS/MS), was recently developed and enables deep proteome coverage of cellular lysates (Branca, R.M. et al. (2014) *Nature Methods* 11(1):59-62). This approach has been demonstrated by Branca et al. to be capable of quantitatively characterizing >10,000 proteins per cell lysate, whereas other methods of mass spectrometry generate datasets with smaller depth of coverage (Branca, R.M. et al. (2014) *Nature Methods* 11(1):59-62).

[0206] The effects of a PAD-like microenvironment on angiogenic signaling protein expression within MSCs and their secreted exosomes were investigated. HiRIEF LC-MS/MS was used to investigate changes in MSC proteomic expression when cultured under normoxic, high serum expansion conditions as compared to conditions that mimic the microenvironment experienced by MSCs upon injection into tissues affected by PAD. It was found that exposure of MSCs to a PAD-like microenvironment increases expression of several pro-angiogenic signaling associated proteins including epithelial growth factor (EGF), fibroblast growth factor (FGF) and platelet derived growth factor (PDGF). In addition, it was observed that exposure of MSCs to a PAD-like microenvironment induces elevated exosome secretion and that these secreted exosomes contain a robust angiogenic signaling profile and are capable of inducing angiogenesis *in vitro* via the nuclear factor kappa-light-chain enhancer of activated B-cells (NFkB) pathway.

Example 1 – Culture Methods, Materials and Vesicle Isolation

Cell culture and reagents

[0207] Human bone marrow aspirates from young adult, non-smoking males were obtained from Lonza (Allendale, NJ, USA). For MSC isolation and expansion, bone marrow aspirates were passed through 90 µm pore strainers for isolation of bone spicules. Then, the strained bone marrow aspirates were diluted with equal volume of phosphate-buffered saline (PBS) and centrifuged over Ficoll (GE Healthcare, Waukesha, WI, USA) for 30 minutes at 700g. Next, mononuclear cells and bone spicules were plated in plastic culture flasks, using minimum essential media α (MEM-α) (HyClone Thermo Scientific, Waltham, MA, USA) supplemented with 10% fetal bovine serum (FBS; Atlanta Biologicals, Lawrenceville,

GA, USA) that had been screened for optimal MSC growth. After 2 days, nonadherent cells were removed by 2–3 washing steps with PBS. After passage 2 MSCs were expanded in 20% FBS and MSCs from passages 5–6 were used for experimentation. For serum starvation studies MSCs were washed 3 times with PBS and cultured in exosome isolation media consisting of OptiMEM without phenol red with 1% L-Glut (IC) (Life Technologies, Carlsbad, CA, USA) for 40 hours. For serum starvation plus low oxygen conditions (PAD) MSC were cultured in exosome isolation media under 1% oxygen tension for 40 hours. Pooled human HUVECS were purchased from Lonza (Allendale, NJ, USA) and cultured according to manufacturer's instructions using EndoGRO-LS Complete media from Millipore (Billerica, MA, USA).

Vesicle isolation and characterization

[0208] MSC were washed 3 times with PBS and switched to exosome isolation media; either 20% FBS media that was pre-cleared of exosomes via 18 hour 120,000 x g centrifugation, or OptiMEM (Life Technologies, Carlsbad, CA, USA) and were conditioned for 40 hours prior to vesicle isolation (Kordelas, L. et al. (2014) *Leukemia* 8(4):970-973). Microvesicles (MV) were isolated as in previous studies (Witwer, K.W. et al. (2013) *Journal of Extracellular Vesicles* 2:20360). Briefly conditioned media was cleared of cells and cell debris via centrifugation (500 x g and 1000 x g respectively), then spun at 17,000 x g pellet to isolate MVs. Exosomes were isolated as in previous studies (Witwer, K.W. et al. (2013) *Journal of Extracellular Vesicles* 2:20360). Briefly, for proteomics studies exosomes were isolated using 0.22 µm filtration to get rid of cells, cell debris and microvesicles prior to being spun at 120,000 x g for 2 hours, the pellet was then washed with 39 mLs of PBS and spun again at 120,000 x g for 2 hours. All ultracentrifuge steps were performed with a Ti70 rotor in polyallomer quick seal tubes (Beckman Coulter, Brea, CA, USA). Vesicle concentration was determined using DC (detergent compatible) assay (BioRad, Hercules, CA, USA) and size distribution assessed using NanoSight LM10HS (Malvern, Amesbury, MA, USA).

Electron microscopy

[0209] SEM images were taken with Philips XL30 TMP, (FEI Company, Hillsboro, OR, USA Sputter Coater: Pelco Auto Sputter Coater SC-7, (Ted Pella Inc., Redding, CA USA).

TEM images were taken on Philips CM120 Biotwin Lens, 9 (FEI Company, Hillsboro, OR, USA), with 2% uranyl acetate staining using facilities at Electron Microscopy Laboratory, School of Medicine, University of California at Davis.

Sample preparation for proteomics

[0210] Cell pellets were lysed with 4% SDS, 25 mM HEPES, 1mM DTT. EVs were lysed with 2% SDS, 25 mM HEPES, 1mM DTT. Lysates were heated to 95°C for 5 min followed by sonication for 1 min and centrifugation, 14,000g for 15 min. The supernatant was mixed with 1mM DTT, 8 M urea, 25 mM HEPES, pH 7.6 and transferred to a centrifugation filtering unit, 10 kDa cutoff (Nanosep®, Pall, Port Washington, NY, USA), and centrifuged for 15 min, 14,000g, followed by another addition of the 8 M urea buffer and centrifugation. Proteins were alkylated by 50 mM IAA, in 8 M urea, 25 mM HEPES for 10 min, centrifuged for 15 min, 14,000g, followed by 2 more additions and centrifugations with 8 M urea, 25 mM HEPES. Trypsin (Promega, Madison, WI, USA), 1:50, trypsin:protein was added to the cell lysate in 250 mM urea, 50 mM HEPES and incubated overnight at 37°C. The filter units were centrifuged for 15 min, 14,000g, followed by another centrifugation with MQ and the flow-through was collected (Branca, R.M. et al. (2014) *Nature Methods* 11(1):59-62). Peptides from EVs were TMT6 labelled and MSC cells with TMT10 labelled according to manufacturer's instructions (Thermo Fisher Scientific, San Jose, CA, USA). Peptides were cleaned by a strata-X-C-cartridge (Phenomenex, Torrance, CA, USA) (Branca, R.M. et al. (2014) *Nature Methods* 11(1):59-62; Wisniewski, J.R. et al. (2009) *Nature Methods* 6(5):359-362).

Proteomics on nLC-MS/MS on Thermo Scientific LTQ Orbitrap Velos

[0211] Before analysis of exosomes on LTQ-Orbitrap Velos (Thermo Fischer Scientific, San Jose, CA, USA), peptides were separated using an Agilent 1200 nano-LC system. Samples were trapped on a Zorbax 300SB-C18, and separated on a NTCC-360/100-5-153 (Nikkyo Technos., Ltd, Tokyo, Japan) column using a gradient of A (5% DMSO, 0.1% FA) and B (90% ACN, 5% DMSO, 0.1% FA), ranging from 3 % to 40% B in 45 min with a flow of 0.4 µl/min. The LTQ-Orbitrap Velos was operated in a data-dependent manner, selecting 5 precursors for sequential fragmentation by CID and HCD, and analyzed by the linear iontrap and orbitrap, respectively. The survey scan was performed in the Orbitrap at 30,000

resolution (profile mode) from 300-2000 m/z with a max injection time of 500 ms and AGC set to 1×10^6 ions. For generation of HCD fragmentation spectra, a max ion injection time of 500 ms and AGC of 5×10^4 were used before fragmentation at 37.5% normalized collision energy. For FTMS MS2 spectra, normal mass range was used, centroiding the data at 7500 resolution. Peptides for CID were accumulated for a max ion injection time of 200 ms and AGC of 3×10^4 , fragmented with 35% collision energy, wideband activation on, activation q 0.25, activation time 10 ms before analysis at normal scan rate and mass range in the linear iontrap. Precursors were isolated with a width of 2 m/z and put on the exclusion list for 60 seconds. Single and unassigned charge states were rejected from precursor selection.

Proteomic data analysis

[0212] GraphPAD Prism was used to calculate differential expression using multiple t-tests and a stringent false discovery cut off of 1% (GraphPAD Prism, La Jolla, CA, USA). Panther Pathway analysis was used to detect the number of pathways detected in each sample and the number of proteins of each pathway represented in each sample (see webpage: pantherdb.com). Ingenuity Pathway Analysis software was used to analyze enrichment for signaling pathway proteins and putative functionality of proteins present in and between each sample (Qiagen, Redwood City, CA, USA). ClueGO software was used for gene ontology analysis of each sample to detected broad classes of protein functionality (see webpage: ici.upmc.fr/cluego/cluegoDownload.shtml). CytoScape was used to generate network interactome maps for the angiogenesis interactome of MSCs and exosomes and the NFkB pathway interactome (see webpage: cytoscape.org). The constructed angiome dataset from Chu et al. (Chu, L.H. et al. (2012) *Physiol Genomics* 44:915-924) was used to search for the presence of canonical angiogenesis mediating proteins in data presented herein, with the addition of physically interacting proteins not found in the Chu et al. (Chu, L.H. et al. (2012) *Physiol Genomics* 44:915-924) was dataset. The Spike database was used to detect proteins for which there was experimental evidence for physical interactions (i.e., yeast-2-hybrid, co-immunoprecipitation) with the Chu et al. (Chu, L.H. et al. (2012) *Physiol Genomics* 44:915-924) was dataset and was accessed via CytoScape.

Tubule formation migration assay

[0213] Primary human umbilical cord vein endothelial cells were purchased from Lonza (Allendale, NJ, USA) and cultured in EndoGRO-LS Complete (Millipore, Billerica, MA, USA) media as per manufacturer's protocol and plated on growth factor reduced matrigel (Corning, Corning, NY, USA) and stained with Calcein AM (Life Technologies, Carlsbad, CA, USA) and imaged at 16 hours post stimulation at 4X on a Kenyence BZ-9000F (Keyence, Osaka, Japan). EndoGRO basal media was used for control and exosome stimulated wells and EndoGRO-LS Complete was used as a positive control (Millipore, Billerica, MA, USA). For NFkB inhibitor experiments pyrrolidine dithiocarbamate was used at a concentration of 50 μ M.

Results

MSCs exposed to PAD-like conditions show dynamic proteomic changes

[0214] To address what effect PAD-like microenvironment conditions have on the proteomic profile of MSCs, HiRIEF LC/MS-MS was used to quantify the proteome of MSCs. Human MSCs derived from the bone marrow of 3 young adult, non-smoking male donors were cultured under normoxic, high serum expansion conditions until passage 6. After three PBS washes, MSCs were cultured under one of three culture conditions for 40 hours: Normoxic, high serum expansion conditions (EX: 20% FBS, 21% O₂), PAD-like conditions (PAD: 0% FBS, 1% O₂) or an intermediate condition (IC: 0% FBS, 21% O₂).

[0215] A total of 6,342 proteins were identified and quantified in each of the 9 MSC samples, with 3 donors for each of the 3 conditions. A total of 580 membrane associated proteins were detected in each of the 9 MSC samples, including canonical MSC surface markers: CD73 (NT5E), CD90 (THY1) and CD105 (ENG). The data presented overlaps with and expands beyond the work by Mindaye *et al.* Statistical analysis of protein expression levels using a false discovery rate of 1% (FDR1%) revealed 315 and 843 differentially expressed proteins respectively between the EX vs IC and EX vs PAD conditions. Analysis of MSC differential expression ratios versus abundance (area) revealed differentially expressed proteins were distributed across the range of abundances of all cellular proteins (FIG. 1). This indicated that the effects of the culture conditions on protein expression were not limited to lowly expressed proteins. Analysis of MSC differential expression ratios versus P-value demonstrated that significantly differentially expressed

proteins (FDR1%) were distributed across the range of ratios for all cellular proteins. This indicated that the effects of the culture conditions on protein expression included many new and highly significant findings (FIG. 1).

[0216] Although global heatmap cluster analysis and linear regression analysis of PAD/EX ratios revealed donor to donor variation in MSCs, it also revealed robust intra-condition concordance between donors (**FIGS. 1, 6**), especially of significantly differentially expressed proteins. MSCs exposed to PAD-like conditions showed significant increases (FDR1%) in rate limiting proteins of glycolysis (ALDOB, ENO3 and PGK1) and the NRF2/glutathione pathway (ASK1, MKK3/6 and FTH1), which are metabolic and antioxidant associated pathways that have been shown to be modulated with exposure to lower oxygen tension (**FIG. 1**) (Lai, J.C. et al. (1993) *Dev Neurosci.* 15(3-5):181-193; Hayes, J.D. et al. (2014) *Trends Biochem Sci.* 39(4):199-218). Ingenuity Pathway Analysis of differentially expressed cellular proteins (FDR-1%) revealed increased expression of key regulators of the NRF2 pathway, which is the master regulator of glutathione synthesis, in the PAD condition as compared to the EX condition. Analysis was conducted on 3 different donors per condition. For differential expression T-tests with multiple testing correction with an FDR of 1% was used. IC-conditioned MSCs, in contrast, showed no such increases (FDR1%) in glycolysis and glutathione related pathway proteins as compared to the EX condition. Gene ontology analysis using Cytoscape's ClueGO plugin of significantly differentially expressed proteins (FDR1%), revealed numerous cell cycle checkpoint-related pathways (G1 phase, G2/M phase and cytokinesis) involved in the regulation of cellular proliferation were downregulated in both IC and PAD conditions as compared to the EX condition. Ingenuity Pathway Analysis of differentially expressed cellular proteins (FDR-1%) revealed downregulation of proteins involved in proliferation and cell cycle checkpoint-associated pathways, G1 phase progression, G2/M phase progression, cytokinesis, chromosomal segregation in the PAD condition as compared to the EX condition. Cholesterol and lipid biosynthesis pathways were upregulated in both IC and PAD conditions as compared to the EX condition (**FIGS. 1 and 7**) (Saito, R. et al. (2012) *Nature Methods* 9(11):1069-1076). Ingenuity Pathway Analysis of differentially expressed cellular proteins (FDR-1%) revealed down regulation of proteins associated with lipid biosynthesis in the PAD condition as compared to the EX condition.

[0217] Exposure of MSCs to a PAD-like environment induced significant changes in their proteome. Previous studies have indicated that MSCs are capable of inducing angiogenesis, therefore, Applicants analyzed how this PAD-like microenvironment modulated levels of their angiogenic signaling proteins (Duffy, G.P. et al. (2009) *Tissue Eng Part A* 15(9):2459-2470; Iwase, H. et al. (2005) *Radiat Prot Dosimetry* 116(1-4 Pt 2):640-646; Kwon, H.M. et al. (2014) *Vascular Pharmacology* 63(1):19-28). To investigate the interaction patterns of known angiogenic proteins in MSCs and to elucidate proteins that physically interact with these known angiogenic proteins, an angiogenesis interactome network map of the MSC proteome was developed. To generate the angiogenesis interactome network map a list of known angiogenic proteins from Chu et al. that were shown to be present in the MSC proteome (Chu, L.H. et al. (2012) *Physiol Genomics* 44(19):915-924) was derived. CytoScape was then used to include proteins that had experimental evidence of physical interaction with these MSC exosome angiogenic proteins and to show how they interacted with each other (Cline, M.S. et al. (2007) *Nat Protoc* 2(10):2366-2382). The advantage of this approach is that it not only elucidates the physical interactions of canonical angiogenesis proteins, but additionally reveals other non-canonical proteins that physically interact with the angiome, thereby shedding light on potentially novel mediators of angiogenesis. Analysis of the angiogenesis interactome of proteins present in MSCs across all 3 donors exposed to each of the 3 conditions revealed the most robust clustering of signaling protein interactions was with platelet derived growth factor receptor (PDGFR), epidermal growth factor receptor (EGFR) and NFkB nodes. This indicates that these pathways are likely drivers of MSCs' proangiogenic potential. Furthermore, using Panther Pathway analysis, Applicants found several angiogenic pathways to be significantly (FDR1%) upregulated in MSCs exposed to PAD-like conditions, including canonical angiogenic associated pathways of PDGF, EGF and FGF (FIG. 2) (Mi, H. et al. (2013) *Nat Protoc.* 8(8):1551-1566). These data collectively demonstrate significantly increased expression of several angiogenic signaling pathways and cholesterol/lipid biosynthesis pathways in MSCs exposed to the PAD condition as compared to the conventional EX condition.

MSC exosome secretion increases under PAD-like conditions

[0218] Newly synthesized membranes components such as lipids and cholesterol are transported from their site of genesis at the endoplasmic reticulum to the plasma membrane

via vesicular transport (Soccio, R.E. et al. (2004) *Arterioscler Thromb Vasc Biol.* 24(7):1150-1160; Lev, S. (2012) *Cold Spring Harb Perspect Biol.* 4(10)). However, as cells experience decreased rates of proliferation their need for newly synthesized plasma membrane components should also decrease (Baenke, F. et al. (2013) *Dis Model Mech.* 6(6):1353-1363). Applicants observed that a variety of cell cycle pathways decreased in expression in the IC and PAD conditions as expected, since the cells were exposed to a lower oxygen tension and deprived of growth factor stimulation. Interestingly however, Applicants observed that cholesterol/lipid biosynthesis proteins actually significantly (FDR1%) increased in expression and not decreased, in both IC and PAD conditions as compared to the expansion condition, EX (**FIG. 7**). This led the Applicants to speculate that an increase in exosome biogenesis could account for the increased expression of proteins involved in cholesterol/lipid biosynthesis. Indeed Applicants observed a trend towards increased expression of proteins involved in the biogenesis of exosomes, prompting us to analyze vesicle secretion of MSCs (**FIG. 8**).

[0219] Extracellular vesicles secreted from MSCs (microvesicles, exosomes) were isolated from media that had been conditioned for 40 hours under EX, IC and PAD culture conditions using ultracentrifugation. Analysis of vesicle yield via BCA protein concentration assays revealed that MSC microvesicle secretion decreased whereas exosome secretion substantially increased with MSCs exposed to IC and PAD conditions as compared to EX conditions (**FIG. 2**). However, exosomes isolated from the EX condition co-isolated with FBS protein from the media. Scanning electron microscopy (SEM) images of MSCs exposed to PAD conditions showed vesicle structures consistent with a decrease in microvesicle secretion and an increase of exosome secretion as compared to MSC exposed to EX conditions (**FIG. 2**). Furthermore, transmission electron microscopy of isolated PAD-derived MSC exosomes with negative staining is consistent with canonical exosome morphology; additionally, Nanosight analysis revealed that MSC exosomes were of expected size range and MSCs maintained low levels of apoptosis in all conditions.

MSC exosome proteome contains a robust profile of angiogenic signaling proteins

[0220] As two recent studies demonstrated that MSC exosomes are pro-angiogenic both *in vitro* and *in vivo* Applicants used MSC HiRIEF LC-MS/MS to characterize the proteome of

MSC derived exosomes from MSCs exposed to IC and PAD conditions (Bian, S. et al. (2014) *Journal of Molecular Medicine* 92(4):387-397; Zhang, H.C. et al. (2012) *Stem Cells and Development* 21(18):3289-3297). A total of 1927 proteins were quantified in each of the 6 samples generated from cells derived from 3 donors under both the PAD and IC conditions, 457 of which were not detected in MSCs, indicating exosomal enrichment. Applicants detected 92 of the top 100 most identified exosomal marker proteins from the ExoCarta database in each of Applicants' exosome samples from both conditions, IC and PAD (Simpson, R.J. et al. (2012) *Journal of Extracellular Vesicles* 1:18374; Mathivanan, S. et al. (2012) *Nucleic Acids Research* 40(Database issue):D1241-1244; Mathivanan, S. et al. (2009) *Proteomics* 9(21):4997-5000). Differential expression analysis of exosomes from IC and PAD conditions revealed few significant expression differences (FDR1%) in exosomes between IC and PAD conditions.

[0221] Gene ontology analysis using Cytoscape's ClueGO plugin of the 400 most abundant proteins in the MSC exosome proteome from all 3 donors from both conditions showed representation of vascular and endothelial associated proteins (Bindea, G. et al. (2009) *Bioinformatics* 25(8):1091-1093). GO analyses are generally broad based and helpful for a broad overview of the data, but are generally limited in their ability to identify specific signaling pathways. Applicants therefore performed Panther pathway analysis on the MSC exosome proteome and found high representation of several canonical angiogenic associated pathways: cadherin, EGFR, FGF and PDGF (**FIG. 3**).

[0222] Ingenuity Pathway Analysis (IPA) is a robust high throughput data analysis software that is able to predict the induction or inhibition of various cellular activities based on an expert, manually curated database of known protein associations and functions. IPA analysis showed that MSC exosomes contain numerous proteins with a variety of angiogenesis-related functionalities including induction of: angiogenesis, vasculogenesis, cell migration and endothelial cell proliferation.

[0223] Next Applicants performed network analysis of the angiogenesis interactome of MSC exosomes, as with the MSC proteome. Applicants showed the most robust representation of protein nodes clustered around the canonical angiogenic pathways of NFkB1/2, Avian Reticuloendotheliosis Viral Oncogene Homolog A (RELA), PDGFRB and

EGFR. Furthermore, network analysis of the NFkB pathway showed robust representation of MSC exosome proteins clustering around RELA, NFkB1/2 and TNF-receptor associated factor 6 (TRAF6). These data collectively showed that exosomes derived from MSCs exposed to PAD-like conditions contain a robust profile of angiogenic signaling proteins and putative functionalities closely mirroring those found in MSCs.

MSC exosomes induce angiogenesis via the NFkB pathway in endothelial cells

[0224] To test the angiogenic potential of MSC exosomes, human umbilical vein endothelial cells (HUVEC) were stimulated *in vitro* with PAD-derived MSC exosomes. To evaluate their ability to induce tubule formation, a canonical *in vitro* assay of angiogenesis, was applied. Traditionally, putative therapeutics are known to have a therapeutic index where they behave in a dose dependent manner with decreased effectiveness generally observed at higher doses (Jiang, W. et al. (2015) *AAPS J* 17(4):891-901). HUVECs were treated with increasing doses of PAD-derived MSC exosomes to test for their effective dose range. The low dose of PAD-derived MSC exosomes (1 µg/mL) induced significant tubule formation compared to the unstimulated control, as did the medium dose (10 µg/mL), measured by total segment length. However, the high dose of PAD-derived MSC exosomes (100 µg/mL) were less effective than the medium dose indicating the upper limits of the effective dose range (**FIG. 4**).

[0225] In Applicants' network analysis map of the MSC exosome angiogenesis interactome Applicants observed several hubs of clustering around nodes of the NFkB complex, which is known to mediate angiogenic signaling. Even though these particular nodes, which represent core components of the NFkB complex, were not detected in the MSC exosomes Applicants hypothesized that the presence of numerous NFkB interacting proteins may indicate a potential effector role of this pathway in HUVEC tubule formation. To test this hypothesis HUVECs were treated with pyrrolidine dithiocarbamate (PDTC), a specific inhibitor of NFkB signaling or vehicle control prior to stimulation with PAD-derived MSC exosomes in a tubule formation assay. PAD-derived MSC exosomes induced tubule formation in HUVECs treated with the vehicle control but not in HUVECs treated with PDTC, demonstrating that NFkB signaling is necessary for MSC exosome induction of tubule formation *in vitro* (**FIG.**

5). These results indicate that MSC exosomes mediate angiogenesis in a dose dependent manner via the NFkB pathway.

Discussion

[0226] This study presents, to Applicants' knowledge, the most robust proteomic characterization of MSCs and exosomes to date (MSC = 6,342 vs 1024, MSC exosome = 1927 vs 236) (Kim, H.S. et al. (2012) *Journal of Proteome Research* 11(2):839-849; Mindaye, S.T. et al. (2013) *Stem Cell Research* 11(2):793-805). Applicants detected 580 membrane associated proteins including those required to meet the minimal criteria for MSC classification (CD73, CD90, CD105) across all 9 MSC samples, and represents the most robust proteomic profiling of MSC membrane proteins to date (580 vs 172) (Mindaye, S.T. et al. (2013) *Journal of Proteomics* 78: 1-14). MSCs have been proposed as a therapeutic for PAD, however, the effect of the PAD microenvironment has on both the MSC physiology and MSC induced angiogenesis are poorly understood (Capoccia, B.J. et al. (2009) *Blood* 113(21):5340-5351). Even though several studies have demonstrated the efficacy of using MSCs for ischemic tissue related diseases, efforts towards identifying the underlying mechanisms of MSC induced angiogenesis have not been robustly investigated, as more focus has been placed on MSC secretion of VEGF and PDGF (Beckermann, B.M. et al. (2008) *British Journal of Cancer* 99(4):622-631; Deuse, T. et al. (2009) *Circulation* 120(11 Suppl):S247-S254; Fierro, F.A. et al. (2011) *Stem Cells* 29(11):1727-1737; Ding, W. et al. (2010) *Blood* 116(16):2984-2993). The quantitative proteomic methodology Applicants used underscores the need for an unbiased approach which in the present study, led to the finding that the MSC proteome is modulated upon exposure to a PAD-like microenvironment and multiple pathways are likely involved in MSC mediated angiogenesis.

[0227] Applicants show attenuation of various cell cycle initiation and glycolysis gene networks in MSCs exposed to PAD-like conditions. Network analysis of all 3 donors from all 3 culture conditions (9 samples total) demonstrated that the MSC angiogenesis interactome is enriched for nodes associated with PDGFR, EGFR, and NFkB. This indicated that these known angiogenesis mediating pathways are likely central hubs of intracellular angiogenic signaling within MSCs (Gianni-Barrera, R. et al. (2014) *Biochemical Society Transactions* 42(6):1637-1642; Tabernero, J. (2007) *Mol Cancer Res.* 5(3):203-220; Fujioka,

S. et al. (2003) *Clin Cancer Res.* (1):346-354; Hou, Y. et al. (2008) *Dev Dyn* 237(10):2926-2935). Furthermore, when MSCs were exposed to PAD-like conditions they significantly increased expression of proteins associated with a subset of angiogenic signaling pathways EGF, FGF, and PDGF.

[0228] MSCs are known to mediate much of their tissue healing effects through their secretome in various vascular disease models such as stroke and peripheral arterial disease (Meyerrose, T. et al. (2010) *Advanced Drug Delivery Reviews* 62(12):1167-1174; Bronckaers, A. et al. (2014) *Pharmacology & Therapeutics* 143(2):181-196). Recent studies have demonstrated that a new cell to cell communication system mediated by exosomes is capable of recapitulating much of the beneficial therapeutic effects of MSCs in these disease models (Bian, S. et al. (2014) *Journal of Molecular Medicine* 92(4):387-397; Kordelas, L. et al. (2014) *Leukemia* 8(4):970-973; Zhang, B. et al. (2014) *Stem Cells* 33(7):2158-2168; Lai, R.C. et al. (2010) *Stem Cell Research* 4(3):214-222). However, the underlying mechanisms by which MSC exosomes modulate these tissue-healing effects have yet to be elucidated.

[0229] Applicants characterized the proteome of exosomes derived from MSCs exposed to PAD-like conditions (PAD) and the intermediate condition (IC), but not from expansion conditions (EX) since Applicants' HiRIEF LC-MS/MS method requires large quantities of input material and the exosome yield from this condition was too small. Applicants quantitatively characterized 1,927 proteins in MSC exosomes from all three donors across both IC and PAD conditions, of which 457 were not detected in the MSC proteome. A potential explanation for this observed protein enrichment in MSC exosomes is that some proteins can be masked in more complex lysates when using mass spectrometry methodologies, but this does not preclude the possibility that some of these proteins are being directly shuttled into exosomes for secretion (Hultin-Rosenberg, L. et al. (2013) *Molecular & Cellular Proteomics: MCP* 12(7):2021-2031). Of note is the fact that the proteome of exosomes derived from MSCs appears to lack many canonical secretory signaling proteins such as cytokines and growth factors, but instead contain the downstream mediators of these pathways.

[0230] Applicants showed that exosomes from MSCs exposed to PAD-like conditions contain a robust profile of angiogenesis associated proteins that closely mirror the

upregulated angiogenic pathways found in MSCs exposed to PAD-like conditions including EGFR, FGF and PDGF pathways. These findings suggest that upon exposure to ischemic tissue conditions attempt to generate a more proangiogenic state via the secretion of exosomes, thereby facilitating localized tissue healing. Further, the main drivers of MSC exosome induced angiogenesis may act via direct signaling to endothelial cell populations or indirectly through inducing chemotaxis of immune cells such as monocytes.

[0231] Applicants also showed that proteins mediating cholesterol/lipid biosynthesis and metabolism are significantly upregulated in MSCs that are exposed to PAD-like conditions, while several known exosome biogenesis proteins trend towards increased expression under these same conditions. Numerous cell cycle pathways are significantly downregulated in MSCs exposed to PAD-like conditions and various cell types have substantially lower rates of proliferation when exposed to similar conditions (Rosova, I. et al. (2008) *Stem Cells* 26(8):2173-2182; Beegle, J. et al. (2015) *Stem Cells* 33(6):1818-1828). Since, ostensibly there should be much less demand for such high energy cost membrane components and exosomes are known to be enriched for lipid raft components such as cholesterol (Tan, S.S. et al. (2013) *Journal of Extracellular Vesicles* 2:22614), Applicants therefore speculated that the upregulation of these cholesterol/lipid biosynthesis proteins may be associated with exosome secretion. Applicants showed that MSCs increased secretion of exosomes upon exposure to PAD-like conditions which were of canonical size and morphology. Alternatively the observed increase in lipid biosynthesis may potentially be a cellular adaption to hypoxia in the PAD condition (Masson, N. et al. (2014) *Cancer Metab* 2(1):3).

[0232] Consistent with traditional broad range small molecule dose curves, Applicants show that exosomes derived from MSCs exposed to PAD-like conditions were able to induce angiogenesis *in vitro*, in a dose dependent manner. MSC exosomes at the highest concentration (100 µg/mL) induced less tubule formation as compared to lower doses, which may indicate an upper limit of the effective dosing range.

[0233] Applicants' network analysis indicated that MSC exosomes derived from PAD-like conditions are enriched for several nodes associated with NFkB signaling, which has previously been shown to be an important mediator of angiogenesis (Hou, Y. et al. (2008) *Dev Dyn* 237(10):2926-2935). Applicants demonstrated that MSC exosome induced

angiogenesis is dependent on NFkB signaling, since a specific chemical inhibitor of NFkB signaling completely abrogates the ability of MSC exosomes to induce tubule formation *in vitro*. It remains unclear, however, to what extent MSC induced angiogenesis can be attributed to exosome mediated effects. Overall, Applicants' data suggest that there are more signaling pathways involved which are worthy of further investigation.

Conclusion

[0234] A common trend that is becoming apparent across the MSC exosome literature is that exosomes derived from MSCs are able to mediate much of the functionality traditionally associated with canonical secretory proteins such as growth factors of the MSC secretome (Bian, S. et al. (2014) *Journal of Molecular Medicine* 92(4):387-397; Kordelas, L. et al. (2014) *Leukemia* 8(4):970-973; Zhang, B. et al. (2014) *Stem Cells* 33(7):2158-2168; Zhang, H.C. et al. (2012) *Stem Cells and Development* 21(18):3289-3297; Li, T. et al. (2013) *Stem Cells and Development* 22(6):845-854; Katsuda, T. et al. (2013) *Scientific Reports* 3: 1197; Lin, S.S. et al. (2014) *Neurochem Res.* 39(5):922-931; Bruno, S. et al. (2009) *Journal of the American Society of Nephrology: JASN* 2009; 20(5):1053-1067; Xin, H. et al. (2013) *Stem Cells* 31(12):2737-2746). Whether canonical secretory proteins or exosomally delivered proteins are the main drivers of the MSC secretome's functionality still needs further investigation; based on data presented herein it is likely microenvironment dependent.

Example 2 - Peripheral Artery Disease

[0235] Peripheral artery disease (PAD) of the lower extremities has become a major contributor to the cardiovascular public health burden. It is associated with high rates of morbidity and identifies a cohort of patients that is at increased risk of major cardiovascular ischemic events. PAD is estimated to affect 12% to 15% of people over the age of 65 years, approximately 8-10 million people in the United States. Prevalence is expected to increase significantly as the population ages, becomes more obese, and as diabetes mellitus becomes more common.

[0236] PAD is characterized by a lack of proper blood flow to the lower extremities due to narrowing or blockage of arterial vasculature from atherosclerotic plaques. Angioplasty and stent placement are commonly used to treat PAD, however, restenosis and re-occlusion from

subsequent blood clot formation and neo-intimal hyperplasia limit the effectiveness of these treatments in many patients.

[0237] A potential alternative therapeutic approach to treat PAD is localized induction of angiogenesis to restore blood flow to affected tissues. Studies in animal models of PAD have shown localized induction of angiogenesis via recombinant VEGF therapy. However, this straightforward approach has so far failed to show clear benefits in humans in late-stage clinical trials, perhaps due to the use of a monotherapeutic approach which only targeted a single signaling pathway responsible for one portion of the tissue healing process in PAD (Yla-Herttuala, S. et al. (2007) *Journal of the American College of Cardiology* 49(10):1015-1026).

[0238] Bone marrow derived mesenchymal stem cells (MSCs) promote enhanced tissue healing via signaling to endogenous cell populations including immune cells and endothelial cells. MSCs have shown promise as a therapeutic treatment for PAD through the secretion of a diverse profile of angiogenic signaling factors including exosomes. Exosomes are small lipid-bound, cellularly secreted vesicles that mediate intercellular communication via cell-to-cell transport of proteins, RNAs, lipids and metabolites. However, it remains unclear which of these secreted factors are of primary importance in MSC induced angiogenesis. Interestingly, exosomes have been recently shown to also mediate some of the tissue healing properties of MSCs, however, the underlying mechanisms by which MSC exosomes exert their tissue healing properties remain unclear.

[0239] The therapeutic application of MSCs in the clinic has advanced faster than the field's understanding of how the cells mediate tissue healing and currently it is not clear how MSC exosomes mediate angiogenesis in models of cardiovascular disease such as PAD. Exosomes are rapidly gaining interest as potential therapeutics for cardiovascular indications, perhaps serving as a safer and potentially more efficacious vehicle to deliver stem cell-derived therapeutics. In addition, the effective engineering of MSC exosomes holds the potential to allow for delivery of novel, therapeutically relevant biologics that have, heretofore, been impractical to deliver clinically, such as miRNA, mRNA, plasmids, membrane and cytosolic proteins.

[0240] Here, exosomes and microvesicles derived from MSCs were engineered with exogenous biologic components. MSCs were transduced with a lentivirus that overexpressed a fluorescent marker protein, tdTomato, and a miRNA, miR-132. After 16 hours the cells were washed 3X's and given fresh exosome isolation media (serum free) and placed in hypoxia (1% O₂) increases exosome secretion by MSCs. 48 hours later exosomes were isolated and purified from conditioned media using tangential flow filtration. Endothelial cells were then exposed to these isolated exosomes and imaged at 8 and 72 hour timepoints. Endothelial cells imaged at 8 hours post exosomes exposure show a small amount of fluorescence, indicating delivery of tdTomato on the protein level to cells. However, after 72 hours post exposure endothelial cells show a much higher fluorescent signal indicating additional tdTomato proteins translated from functional tdTomato mRNAs delivered via exosomes.

[0241] In a separate experiment, MSCs were transfected with a plasmid expression vector overexpressing miR-132 and tdTomato (SEQ ID NO: 10). After 16 hours the cells were washed 3X's and given fresh microvesicle isolation media. Microvesicles were harvested from media that had been conditioned for 48 hours using ultracentrifugation. DNA was isolated from purified microvesicles and PCR demonstrated the presence of the expression plasmid. The data herein demonstrate that these microvesicles delivered functional plasmids expressing tdTomato and miR-132 to endothelial cells as detected by fluorescence microscopy at 48 hours post exposure.

Example 3 - Large Scale Manufacturing Using a Hollow Fiber Reactor

[0242] A hollow fiber bioreactor may be used to scale up production of exosomes and/or microvesicles. This method reduces personnel labor and media usage, both of which can be costly expenditures. In this example, a hollow fiber cartridge was coated with an extracellular matrix (ECM) protein coating. Non-limiting examples of appropriate ECM and other coatings also appropriate for use with this method include fibronectin, gelatin, vitronectin, matrigel, and collagen. 10-100 million stem cells were seeded onto the coated hollow fiber cartridge. Cells were grown in expansion media: 5-20% FBS in basal media with 0-5% L-Glut, with a gas mixture of 20% oxygen, 5% CO₂, and 75% nitrogen. Alternatively, cells may be cultured at lower percentages of oxygen (between 1% and 20%),

with CO₂ at 5%. Following several days of cell expansion, the media is switched to isolation media, basal media with 0-5% L-Glut, with a gas mixture of 1-20% oxygen, 5% CO₂ with the balance being nitrogen. After 15-96 hours, exosomes and/or microvesicles are harvested from the resulting conditioned media. Exosomes and/or microvesicles may be isolated from the conditioned media either by TFF or by direct isolation using 100-300 kDa membrane filtration devices (e.g. VivaSpin) using centrifugal force of 500-6000 x g.

[0243] Cells cultured in a hollow fiber reactor system generate much higher yields of exosomes and/or microvesicles as compared to standard tissue culture flasks (**FIG. 12**). Further, use of the hollow fiber reactor system generates exosomes and/or microvesicles of canonical morphology and diameter. Exosomes may be quantified using a protein concentration kit (e.g. DC assay) and/or using a NanoSight machine. Size distribution of exosomes is obtained using a NanoSight machine or other particle analyzer such as Izon or flow cytometer. Electron microscopy is used to demonstrate that the exosomes are of canonical morphology and size. Further validation may be performed with in vitro assays including a migration assay, tubule formation, and immune modulation (e.g. mixed lymphocyte reaction) prior to in vivo studies.

Example 4 - Lyophilization of cell-derived vesicles

[0244] In some embodiments, lyophilization of cell-derived vesicles of the present disclosure is practiced with use of a condenser, a vacuum pump, and a freeze-dryer. In the above methods, the manifold is assembled to ensure that a good vacuum (100 μ bar or less) is achieved. The condenser should be set to -50°C or lower. Concentrated exosome and/or microvesicle solution is dispensed into microcentrifuge tubes or other suitable containers appropriate for the scale of the condense, vacuum pump, and/or freeze dryer used. The tubes should not be more than 33% full. The lid of the tubes is pierced with a hole or removed and replaced with Parafilm or other covering pierced with several holes. The microcentrifuge tubes are snap frozen by any method well known in the art, e.g. dipping until partially submersed in liquid nitrogen or dry/acetone or alternatively freezing in a suitable spark-proof deep freezer set to negative 40°C or lower. Once frozen, tubes are placed into a Quickfit style round-bottom flask or other suitable container for the size of tubes used. The outside of glass is cooled to -60°C or below and attached to the manifold. The vacuum is applied and

checked to ensure that it achieved returns to below 100 μ bar. Samples are then allowed to completely warm to room temperature overnight (approximately 16 hours) or less for volatile solvents. Following this warming, the vacuum is released by switching the manifold valve slowly to prevent material ablating from the tubes. In some embodiments, the system is left on and fractions are dried over several days before the condenser is thawed out. In some embodiments, multiple flasks on a manifold are used and different flasks are removed at different times depending on when they have completed drying.

Anti-Inflammatory Therapies

[0245] Without being bound by theory, there are 4 broad classes for MSC exosomes' mechanism of action: A) anti-inflammatory, B) regeneration, C) anti-fibrotic, D) neuroprotective. These categories can be interrelated and/or overlap. Cell-derived vesicles manufactured as described herein are used to treat the following diseases or conditions: (1) multiple sclerosis, (2) neuroinflammatory disease, (3) muscle injuries, (4) radiation tissue damage, (5) stroke, (6) traumatic brain injury, (7) myocardial infarction, (8) graft versus host disease, (9) Parkinson's disease, (10) Alzheimer's, (11) inflammatory bowel disease, (12) Huntington's disease, (13) amyotrophic lateral sclerosis, (14) Bahcet's disease, (15) sarcopenia, (16) aging, (17) spinal cord injury, (18) wound repair, and (19) dysphagia. Without being bound by theory, disease or conditions numbered (1-19) are treated through the cell-derived vesicles' anti-inflammatory mechanism. Without being bound by theory, disease or conditions numbered (1-7, 9-10, 12-13, 15, 17-19) are treated through the cell-derived vesicles' regenerative mechanism. Without being bound by theory, disease or conditions numbered (3-7, 17-19) are treated through the anti-fibrotic mechanism. Without being bound by theory, disease or conditions numbered (1-6, 9-10, 12-13 and 15-17) are treated through the neuroprotective mechanism.

Example 5 - Stroke

[0246] To establish a rat model of stroke with middle cerebral artery occlusion (MCAO), rats are first anesthetized using inhaled isofurane (3% for induction followed by 2% for maintenance). Fur on the incision site is removed using Nair and skin is cleaned and sterilized sequentially with sterile PBS, 75% ethanol and betadine. A midline neck incision is made and the soft tissues are pulled apart. The left common carotid artery (LCCA) is

carefully dissected free from the surrounding nerves (without harming the vagal nerve) and a ligature is made using 6.0/7.0 suture. 5.0 suture can also be used. The left external carotid artery (LECA) is then separated and a second knot is made. Next, the left internal carotid artery (LICA) is isolated and a knot is prepared with a 6.0 filament. After obtaining a good view of the left internal carotid artery (LICA) and the left pterygopalatine artery (LPA), both arteries are clipped, using a microvascular clip. A small hole is cut in the LCCA before it bifurcates to the LECA and the LICA. A monofilament made of 8.0 nylon coated with silicon hardener mixture is then introduced into the LICA, until it stops at the clip. Attention has to be paid not to enter the occipital artery. The clipped arteries are opened while the filament is inserted into the LICA to occlude the origin of the LMCA in the circle of Willis. The third knot on the LICA is closed to fix the filament in position.

[0247] Using the above MCAO model, Applicants demonstrated the therapeutic effects of exosomes in a rat model of stroke. To test whether exosomes are taken up by relevant target cell populations, MSC-Stroke exosomes are prepared by exposing MSCs to conditions that mimic the microenvironment experienced by MSC's upon injection into tissues affected by ischemia-related diseases (hypoxia, serum deprivation). Human bone marrow aspirates from young adult, non-smoking males were obtain from Lonza (Allendale, NJ). For MSC isolation and expansion, bone marrow aspirates were passed through 90 μ m pore strainers for isolation of bone spicules. Then, the strained bone marrow aspirates were diluted with equal volume of phosphate-buffered saline (PBS) and centrifuged over Ficoll (GE Healthcare, Waukesha, WI) for 30 minutes at 700g. Next, mononuclear cells and bone spicules were plated in plastic culture flasks, using minimum essential media α (MEM- α) (HyClone Thermo Scientific, Waltham, MA) supplemented with 10% fetal bovine serum (FBS; Atlanta Biologicals, Lawrenceville, GA) that had been screened for optimal MSC growth. After 2 days, non-adherent cells were removed by 2–3 washing steps with PBS. After passage 2 MSCs were expanded in 20% FBS and MSCs from passages 5-6 were used for experimentation. For serum starvation, MSCs were washed 3 times with PBS and cultured in exosome isolation media consisting of OptiMEM without phenol red with 1% L-Glut (IC) (Life Technologies, Carlsbad, CA) for 40 hours. For serum starvation plus low oxygen conditions (PAD) MSC were cultured in exosome isolation media under 1% oxygen tension for 40 hours. Pooled human HUVECS were purchased from Lonza (Allendale, NJ) and cultured according to

manufacturers instructions using EndoGRO-LS Complete media from Millipore (Billerica, MA).

[0248] MSCs were washed 3 times with PBS and switched to exosome isolation media; either 20% FBS media that was pre-cleared of exosomes via 18 hour 120,000 x g centrifugation, or OptiMEM (Life Technologies, Carlsbad, CA) and were conditioned for 40 hours prior to vesicle isolation. Microvesicles (MV) were isolated as described herein. Briefly conditioned media was cleared of cells and cell debris via centrifugation (500 x g and 1000 x g respectively), then spun at 17,000 x g pellet to isolate MVs. Exosomes were isolated as described herein. Briefly, for proteomics studies exosomes were isolated using 0.22 µm filtration to get rid of cells, cell debris and microvesicles prior to being spun at 120,000 x g for 2 hours, the pellet was then washed with 39 mLs of PBS and spun again at 120,000 x g for 2 hours. All ultracentrifuge steps were performed with a Ti70 rotor in polyallomer quick seal tubes (Beckman Coulter, Brea, CA). Vesicle concentration was determined using DC assay (BioRad, Hercules, CA) and size distribution assessed using NanoSight LM10HS (Malvern, Amesbury, MA).

[0249] To assess the ability of MSC exosomes to influence a target cell population, exosomes were labeled with a fluorescent label and exposed to human primary endothelial cells. Uptake of exosomes can be observed after 1 hour using fluorescence microscopy. This result demonstrates that exosomes are absorbed by cells that are therapeutic targets for human treatment of ischemic stroke. Further, exposure of target cell populations (e.g. endothelial cells) to MSC-Stroke exosomes induces migration within 6 hours and tubule formation within 15 hours, demonstrating that exosomes are capable of inducing an angiogenic effect, an important feature of a potential therapeutic for stroke.

[0250] Exosome treatment is capable of inducing therapeutic responses in the MCAO model. MSC-stroke derived exosomes (100 µg/mL) can be injected intracranially, intra-arterially, or intravenously into MCAO rats. Treatment with exosomes improved rat performance in a cylinder test of asymmetric paw usage and resulted in a reduction of the inflammatory cytokine IL-1β in area surrounding the stroke infarct. This data indicates the robustness and reproducibility of the exosomes' ability produce stroke-relevant therapeutic

effects (e.g. functional recovery via the motor skills assay and reduction in inflammation) by multiple routes of delivery.

Example 7 - Exosome uptake and cell proliferation

[0251] Exosomes will exert their function on cells that they are able to effectively interact with. Applicants investigated the ability of various cell types (muscle stem cells, kidney epithelial cells, and neural cells) to take up exosomes of the present disclosure. Applicants specifically labeled the lipid membrane of exosomes with a fluorescent dye and added them to the culture media of the cells. After one hour of co-incubation with the labeled exosomes, the media was removed, the cells were washed 3 times with PBS, the cells then were quantitatively assessed for the presence of the exosome-conjugated fluorescent dye. The negative control was the fluorescent “labeling” of just PBS to ensure there were no artifacts from dye aggregation due to the sample processing steps involved with labeling the exosomes. Analysis of the cells via flow cytometry clearly showed that the majority (>80%) of cells from each group were positive for the presence of exosomes (data not shown). This demonstrates that exosomes are readily taken up by many cell types within one hour. Applicants also demonstrated that MSC-stroke derived exosomes are taken up by primary endothelial cells and induce migration and tubule formation. MSC-stroke exosomes labeled with PKH26 were exposed to human primary endothelial cells (HUVECs) for 1 hour and stained with nuclear Hoechst. MSC-Stroke induced migration of HUVECs within 6 hours (Calcein AM) T-test $\ast=p<0.05$. (**FIG. 14**).

[0252] After tissues are damaged, the body tries to repair this damaged tissue. During this process, localized stem cells are activated to aid in this tissue repair process. The ability of the formulation of exosomes disclosed herein to induce the proliferation of a type of tissue resident stem cell, myoblasts, which are a common type of muscle stem cell was tested (**FIG. 15**). Muscle stem cells were stimulated with increasing doses of exosomes for 24-hours and assessed their ability to induce the proliferation of myoblasts by determining the presence of a marker for cells in a proliferative state (Ki67) using flow cytometry. Without being bound by theory, this data demonstrates that this formulation of exosomes induces stem cell proliferation in a dose dependent manner, which is an indication of their intrinsic tissue healing properties.

Example 8 - Inflammation

[0253] The exosome formulation disclosed herein was tested for its ability to diminish an inflammatory response in vitro, using a canonical inflammation assay called the mixed lymphocyte reaction. Primary white blood cells (lymphocytes) were isolated from fresh human blood and cultured in vitro. These immune cells were then stimulated with an antigen (PHA) derived from bacteria to stimulate a strong immune response. During this immune response to the bacterial based antigen (PHA), the cells responsible for the inflammation response proliferate, which is a canonical process of inflammation. Therefore, the degree to which the cells proliferate is an indication of how strongly inflammation has been induced. Without being bound by theory, this data demonstrates that primary human lymphocytes' inflammatory response to a bacterial antigen (PHA) is diminished when co-stimulated with the exosome formulation disclosed herein in a dose dependent manner, indicating potent anti-inflammatory properties of the exosomes (**FIG. 16**). Additionally, this experiment used lyophilized exosomes, instead of freshly thawed exosomes, demonstrating that lyophilized exosomes maintain their functionality as measured by their anti-inflammatory properties.

[0254] The anti-inflammatory properties of freshly thawed exosomes were compared to lyophilized exosomes, on a dose for dose basis using the same mixed lymphocyte reaction assay as described above (**FIG. 17**). Without being bound by theory, this data established that the exosome formulation disclosed herein retains its anti-inflammatory properties post-lyophilization. This is a critical piece of data demonstrating the feasibility of commercialization. The lyophilized product will be substantially cheaper to store and ship and be readily available for clinicians and patients alike to administer therapy without the need for cumbersome use of liquid nitrogen tanks needed for most stem cell based therapeutics.

Example 9 – T-Regulatory Cell Proliferation

[0255] Exosomes induce T-regulatory cell proliferation. A specific T-cell assay was used to determine the mechanism of action for the exosomes' anti-inflammatory properties. T-regulatory cells (Tregs) are a type of immune cell with potent anti-inflammatory properties. The exosome formulation disclosed herein was tested for its ability to activate Tregs using flow cytometry. As shown in **FIG. 18**, the exosomes induced Treg activation in a dose

dependent manner. Without being bound by theory, these results indicate that the exosome formulation disclosed herein mediates its anti-inflammatory properties through the activation of T-regs.

Example 10 – Exosomes Reduce Cell Death Due to Oxidative Stress

[0256] Exosomes reduce cell death due to oxidative stress. Oxidative stress is elevated in areas of tissue damage and induces cellular stress and cell death. The exosome formulation disclosed herein was tested for its ability to increase the survivorship of stem cells (myoblasts) exposed to high levels of oxidative stress (300 μ M H₂O₂). Stem cells were exposed to high levels of oxidative stress for 1 hour and subsequently probed for a marker of cell death (Annexin V) with or with co-stimulation with the exosome formulation (data not shown). Applicants determined that the high dose of exosomes was able to reduce stem cell death upon exposure to high levels of oxidative stress. The positive control was a media with high levels of antioxidants added to counteract the oxidative stress. Without being bound by theory, this data indicates that the exosome formulation disclosed herein contributes to tissue healing and cell survival by assisting cells cope with oxidative stress.

Example 11 - Lyophilized Exosomes Induce Comparable Levels of Anti-Inflammatory and Neuroprotective Cytokines

[0257] The exosome formulation disclosed herein was tested for its ability to modify the expression of various cytokines by primary human immune cells (lymphocytes). Fresh lymphocytes were isolated from human blood and cultured them in vitro. The lymphocytes were then stimulated with exosomes. A Quantibody array was used to quantitatively assess cytokine expression of the cells after 24-hours (**FIGS. 19-21**). Without being bound by theory, this data indicates that the exosomes reduce the expression of key inflammatory cytokines and induces the expression of critical anti-inflammatory cytokines in primary human lymphocytes. Additionally, this data demonstrates that the modulation of cytokine expression is comparable between freshly thawed and lyophilized exosomes. The cytokines tested in this assay are: IL-11, G-CSF, Eotaxin, IL-4, IL-7, MCSF, IL-12p70, IL-1a, BLC, IL-8, GM-CSF, MIP-1d, IL-2, IL-15, IL-13, IFN γ , IL-6sR, IL-16, IL-1b, IL-1ra, MIP-1b, TNFb, IL-17, IL-12p40, PDGF-BB, IL-5, IL-6, Eotaxin-2, TNF RI, IL-10, MCP-1, I-309, TNFa, RANTES, MIP-1a, MIG, TNF RII, TIMP-1, ICAM-1, and TIMP-2 (**FIGS. 19-21**).

[0258] Applicants also determined that MSC-exosomes are anti-inflammatory and induce Tregs. MSC-exosomes reduced inflammation and activated Tregs in vitro. Primary human lymphocytes activated with PHA, immediately followed by treatment with either MSC-exosomes or vehicle control. 48 hours post-treatment, cytokine expression of the conditioned media was assessed via Quantibody array analysis and proliferation status was evaluated via flow cytometry analysis of Ki67 expression. In a Treg activation assay, treatment with MSC-exosomes for 48 hours significantly increased the number of Tregs detected via flow cytometry by over 3 fold (data not shown).

Example 12 - Multiple Sclerosis

[0259] Multiple sclerosis affects about 2.5 million people worldwide and although there are currently several approved treatments, most of these treatments are limited in their application due to side effects and efficacy in only minor sub-populations of MS patients. In addition, none of the currently available treatments is regenerative in nature. Thus, there is a need in the art to address these underlying issues. There are several methods to establish pre-clinical and clinical efficacy. Example 12 describes several of these methods,

[0260] For humanization studies one can obtain human mobilized CD34+ cells from a commercial source, and transplant them into sublethally irradiated, naive newborn 2-5 day NRG mice intrahepatically. The mice are then transplanted with 250,000 total cells in a total volume no greater than 30 µl with an insulin syringe. Pups are placed on their backs and the syringe with the cells is inserted into the liver. The cells are then injected and the syringe is removed. The recipient mice are returned to their mothers and housed in autoclaved cages with sterile food and water to minimize the risk of infection. At 12 weeks posttransplantation, mice are bled via the tail vein (one capillary collected) following accepted guidelines, e.g., the UC Davis IACUC guidelines for blood collection. Engraftment of the human cells is evaluated.

[0261] For the EAE induction model, 5-18 week old mice are weighed and marked by ear punch or tail markings. Mice are injected subcutaneous (SC) with 300 µg of myelin oligodendrocyte glycoprotein (MOG) 35-55 in 200 µl of Complete Freund's Adjuvant (CFA) containing 4mg/ml killed Mycobacterium tuberculosis H37Ra over two sites at the back (100 µl in each site) only on day 0. Pertussis toxin (250 ng in 100 µl) is given i.p. on days 0 and 2

post-immunization (p.i.). Some mice will receive CFA without MOG for control purposes. Mice are weighed and clinically scored on day 0, 2 and 5, then daily starting on day 7 up to day 28. For studies that go beyond day 28, the mice will be weighed and scored 3x weekly until up to week 9. In certain experiments to examine the effect of various treatments mice are treated with IV or IP injections of various treatments as described below. Clinical scoring is as follows: (limp tail=1; waddle=2; paresis in one hind leg=2.5; paresis in both hind legs=3; paresis in 1 hindleg and paralysis in the other=3.5; paralysis in both hind legs=4; moribund=4.5; dead=5).

[0262] For perfusion studies, mice are perfused on day 7, 10, 14, 21, or 28 after immunization. For perfusion, mice will undergo anesthesia followed by tissue fixation. Mice are anesthetized with an injection of ketamine and xylazine i.p. When anesthesia is profound (determined by the lack of withdrawal response to foot compression), the skin from the upper abdomen and thorax is removed. The thoracic cage is then excised by cutting up from the abdomen and through the diaphragm and the rib cage removed to expose the heart. The perfusion needle is inserted into the left ventricle and the right atrium is snipped to allow blood returning to the heart to drain, prior to perfusion with saline followed by fixative. After perfusion, the central nervous system (brain and spinal cord) is removed for further immunohistochemical processing.

[0263] For mRNA studies, or flow cytometry studies, mice are euthanized by CO₂. When respiration has ceased, mice are perfused following the procedure for perfusion except that the mice are perfused with 20 ml of saline alone. After saline perfusion, the brain, spinal cord, spleen and lymph nodes are removed for mRNA isolation procedures or flow cytometric analyses.

[0264] Each of the following studies can be performed on both wild-type (WT) and humanized mice in separate experiments. Dosing is calculated based previous studies in and other published reports. Injection volumes for various treatments are 50 µl, however, timing and dosing of treatment injections may be re-evaluated and modified based on the findings from preliminary studies. Mice are weighed and clinically scored as stated above. Post-mortem tissues are evaluated via qPCR, RNA-seq, immunohistochemical analysis and/or flow cytometry analysis.

[0265] The purpose of dosing studies is to evaluate the efficacy of 3 different doses of MSC derived exosomes injected prior to the onset of symptoms in the EAE model. Mice are IV or IP injected with the following study design on day 4 following initial immunization injection: 1) CFA alone negative control, 2) CFA-MOG +vehicle control (PBS), 3) CFA-MOG +low dose exosomes (50 µg), 4) med dose exosomes (200 µg), 5) hi dose exosomes (800 µg). 12 mice/arm.

[0266] Standard of Care Comparison to evaluate the efficacy of MSC derived exosomes as compared to and in combination with a standard of care treatment for MS, copaxone, injected prior to the onset of symptoms in the EAE model. Mice are IV or IP injected with the following study design on day 4 following initial immunization injection: 1) CFA alone negative control, 2) CFA-MOG + vehicle control (PBS), 3) CFA-MOG + low dose exosomes (200 µg), 4) hi dose exosomes (800 µg), 5) CFA-MOG + copaxone (HED 1.6 mg), 6) CFA-MOG + medium dose copaxone (0.5mg) +medium dose exosomes (200 µg), 12 mice/arm.

[0267] Augmented exosome study can be done to assess the efficacy of exosomes that have been enhanced by augmenting their trophic factor contents. Mice are IV or IP injected with the following study design on day 4 following initial immunization injection: 1) CFA alone negative control, 2) CFA-MOG +vehicle control (PBS), 3) CFA-MOG +augmented exosomes I, 4) augmented exosomes II, 5) CFAMOG +augmented exosomes III, 6) CFA-MOG +augmented exosomes IV, 7) CFA-MOG augmented exosomes V, 12 mice/arm.

[0268] Immune cell invasion study, TP1 can be done to evaluate the effects of exosome treatment on immune cell invasion in the CNS at an early time point in the progression of disease (day 14). For flow studies animals are perfused but not fixed as stated above at the end of the study. Mice are IV or IP injected with the following study design on day 14 following initial immunization injection: 1) CFA alone negative control, 2) CFA-MOG + vehicle control (PBS), 3) CFA-MOG + low dose exosomes (50 µg), 4) CFA-MOG + high dose exosomes (800 µg), 12 animals/arm

[0269] Immune cell invasion study, TP2 can be done to evaluate the effects of exosome treatment on immune cell invasion in the CNS at a later time point in the progression of disease (day 21). For flow studies, animals will be perfused but not fixed as stated above at the end of the study. Mice are IV or IP injected with the following study design on day 21

following initial immunization injection: 1) CFA alone negative control, 2) CFA-MOG +vehicle control (PBS), 3) CFA-MOG +low dose exosomes (50 µg), 4) CFA-MOG +high dose exosomes (BOO µg), 12 animals/arm.

[0270] Exosome studies and the studies described below were performed with exosome preparations prepared as described above, without the addition of any exogenous agents to the culture conditions.

[0271] For exosome uptake assays, mExo were labeled with the lipophilic fluorochromatic dye, Cell Mask Green, according to manufacturer's instructions. Cells were exposed to 100 µg/ml of CellMask labelled exosomes for one hour then washed with PBS to eliminate excess exosomes. Cells were then analyzed using flow cytometry gating on FITC. T-tests were used to test for significance of mExo as compared to negative controls (PBS with CellMask Green dye).

[0272] For lymphocytes assays, lymphocytes were isolated from fresh human peripheral blood using Ficol separation and cultured in RPMI with 20% premium select FBS, 10ng IL-2, 1% L-glutamine and 1% Pen-Strep. For antigen-induced lymphocyte proliferation assay, lymphocytes were treated with 5 µg/ml phytohaemagglutinin (PHA) with 100 µg/ml mExo or vehicle control (PBS) treatment for 48 hours. Cells were then fixed, permeablized and stained with a primary monoclonal, fluorochrome conjugated (phycoerythrin) antibody against the canonical proliferation marker, PE-Ki67. Cells were subsequently analyzed using a flow cytometry.

[0273] For Treg activation assay, lymphocytes were isolated as above and cultured in medium containing 1µg/ml anti CD3, 1µg/ml anti CD28, 10% PS-FBS, 1% L-glutamine, 1% Pen-Strep, 1% sodium pyruvate, 1% HEPES, 50nM B-mercaptoethanol, 1X NEAA and 10 ng/ml IL-2. Lymphocytes were treated with exosomes or vehicle control (PBS) for 48 hours and analyzed via flow cytometry for canonical Treg surface markers CD4 and CD25 using primary monoclonal, fluorochrome conjugated antibodies. See **FIGS. 18**

[0274] For quantibody arrays, multiplexed ELISA arrays from Ray Biotech (Quantibody Arrays) were used to assess exosome modulation of lymphocyte secreted cytokines during the antigen induced lymphocyte proliferation assay. Lymphocyte activation assay was

performed as described above, except conditioned medium was obtained from treated cells just prior to the take down of the study. Conditioned medium was quantitatively assessed for the presence of 40 cytokines using Quantibody Arrays per manufacturer's instructions. See **FIG. 19-21**.

[0275] For glial restricted precursor cells and bioassays, primary rat glial restricted precursor cells (GRPs) were purchased from Thermo Fisher and cultured in Neurobasal medium with 10 ng/ml bFGF, 10 ng/ml PDGF-AA, GS22 Supplement and GlutaMax. Cells were treated with 100 µg exosomes and assessed for proliferation using both a colorimetric metabolic assay (CCK-8), with immunohistochemical verification via nuclear staining with DAPI and imaging on a fluorescent microscope. See **FIG. 23**. For differentiation assays GRPs are treated with 100 ug/ml exosomes or vehicle control (PBS) for 5 days prior to evaluation for both early (Nestin, CNP) and late markers (Olig2, MBP) of oligodendrocyte differentiation using flow cytometry. Primary monoclonal antibodies were used in conjunction with fluorochrome conjugated secondary antibodies prior to flow cytometry analysis. See **FIG 23**.

[0276] The exosome preparations were also used for the study of the relapse remitting mouse model of multiple sclerosis: Female SJL/J mice were purchased from Jackson Laboratory and immunized with 200 µg of proteolipid protein from Complete Freund's Adjuvant Emulsion and pertussis toxin on Day 0 according to manufacturer's instructions (Hooke Labs) to present RRMS phenotype (EAE model). See **FIG. 24**. Immediately prior to the onset of clinical symptoms (progressive paralysis starting from the tail and migrating anteriorly) on Day 9 mice were administered via tail vein injection 250 µg of exosomes (n= 18) or vehicle control (PBS) (n= 21) and evaluated daily for clinical scores by a blinded observer for the duration of the study. Clinical scores ranges from 0 to 5. 0= no phenotype, 0.5= tail end can lift for a few seconds before limping, 1= partial or complete limp tail, 2= waddle, 2.5= paresis in one hind limb, 3= paresis in both hind limbs, 3.5= paresis in one hind limb and paralysis in the other hind limb, 4= paralysis in both hindlimbs, 4.5= moribund, 5= death. Clinical scores evaluated using a repeated measures test to assess significance over time and across groups. See **FIG. 25**. At the end of the study on day 35, mice were euthanized, perfused with PBS and tissues were harvested for flow cytometry and immunohistochemical analysis. Brains were disassociated using the Brain Disassociation Kit

according to manufacturer's instruction (Miltenyi) and stained with primary monoclonal, fluorochrome conjugated antibodies against immune cell surface markers of T regulatory cells and evaluated using flow cytometry. Spinal cords were fixed in formalin for a day, and dehydrated in 30% sucrose for two days. Then the spinal cords were cryopreserved and sectioned at 30 micron for immunohistochemical analysis. Spinal cord sections were stained for myelin (Fluoromyelin), microglial (IBA1) and astrocyte (GFAP) activation markers using fluorochrome conjugated primary antibodies and imaged on a Keyence BZ-9000 fluorescent microscope. See **FIGS. 26, 27 and 28**.

[0277] To study therapeutic efficacy of the exosome preparations on progressive MS, CD1 (Charles River) and C57BL/6 (Jackson Labs) mice are used, aged from 6-15 weeks old. Intradermally inject the mice with MOG-CFA emulsion (Hooke Labs). About 25 μ l of emulsion is intradermally injected on the outside of each hind limb, for a total of 50 μ l per mouse, while mice are under anesthesia, 2-3% isoflurane (Halocarbon). On Day 12-14, a surgery scope is used to perform intracranial stereotaxic injection of 1 μ l of mycobacterium tuberculosis (H37Ra) suspended in sterile saline while mice are under anesthesia, (2-3% isoflurane (Halocarbon)) via heat pulled glass capillary needle (Sigma) while mice are kept at appropriate body temperature with a heating pad. Suture cranial incision with silk sutures. On days 21-28, mice are administered 300 μ g of exosome preparations or vehicle control (saline) via tail vein injection. On day 28-42 mice are euthanized via pentobarbital and perfused with fresh PFA prior to processing of brains for immunohistological analysis. Brains are placed in OCT (Tissue Tek) and frozen with a combination of isopentane and dry ice. Blocks are sectioned at 30 μ m increments with a cryostat (ThermoFisher) prior to staining with primary antibodies against: IBA1, GFAP, NeuN, Map2, CD19, CD4, CD25 or Olig2 incubated overnight and stained with fluorochrome conjugated secondary antibodies for one hour prior to imaging on a fluorescent microscope with stich function (Keyence). The resulting images of the brains lesions and associated pathology are quantified using ImageJ analysis. To determine and evaluate clinical efficacy, one can use a primary end point will the percentage of patients with disability progression confirmed at 12 weeks in a time-to-event analysis or evaluation via Expanded Disability Status Scale or evaluation via Multiple Sclerosis Functional Composite scores over a 12 week up to 28 week period.

Example 14 – Radiation Induced Soft Tissue Damage

[0278] C57BL/6 mice (000664), aged 8-12 weeks old, are purchased from Jackson Laboratory and are exposed to 50Gy of external beam x-ray radiation delivered (Elekta linear accelerator) which is focused on one hindlimb while under anesthesia (pentobarbital). Mice are given access to both nutrigenels and hydrogels (Clear H₂O). Animals are weighed and clinically observed for ulcerations twice weekly. Minor ulcerations were treated with topical antibiotic ointment. Animals were administered 300 µg MSC-derived exosomes or vehicle control (saline) via tail vein injection on week 1 post irradiation. Motor skills assessment was performed weekly via TreadScan (CleverSys), balance beam, fireman pole and open field (Columbus Instruments), and evaluated until the end of study up to week 10. At the end of the study, mice are euthanized, perfused prior to extraction of hindlimb skin and muscle tissue processing for cryosectioning, during which tissues are embedded in OCT (Tissue Tek) and frozen on a combination of isopentane and dry ice. Tissue blocks are sectioned on cryostat (ThermoFisher) at 30 µm increments and stained with Sirius Red (Sigma) and Fast Green (Sigma), or antibodies against collagen, vimentin or laminin (incubated overnight) followed by fluorochrome conjugated secondary antibodies (incubated one hour). Slides are dehydrated with sequential ethanol baths and then coverslip mounted with Permount (Fisher Chemical), prior to imaging on brightfield (Keyence) or fluorescent microscope (Keyence). Images can be quantified with ImageJ analysis.

[0279] For head and neck cancers, passive and stimulated saliva production, and clinical assessment of dysphagia via videofluoroscopy, CT scanning, endoscopic examination, MRI, chest radiography, transnasal esophagoscopy, cervical auscultation, blood tests including thyroid-stimulating hormone, vitamin B-12, and creatine kinase can be used to evaluate clinical efficacy.

[0280] For other forms of cancer such as breast cancer, reduction in fibrotic scarring may be a potential endpoint (eg skin elasticity). For sarcomas, one can evaluate clinical efficacy by assessing motor skills assessments.

[0281] As the efficacy of this therapy is clinical evaluated, one of skill in the art may be able to increase doses of irradiation which can be really helpful, as present radiation doses are determined based on a smaller subset of patients that are extra radiation sensitive. There are

no biomarkers for this population, so radiologists administer radiation in fractions suited toward this population, which in some cases limits the efficacy of the treatment of more aggressive tumors.

Equivalents

[0282] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs.

[0283] The inventions illustratively described herein may suitably be practiced in the absence of any element or elements, limitation or limitations, not specifically disclosed herein. Thus, for example, the terms “comprising,” “including,” “containing,” etc. shall be read expansively and without limitation. Additionally, the terms and expressions employed herein have been used as terms of description and not of limitation, and there is no intention in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the invention claimed.

[0284] Thus, it should be understood that although the present invention has been specifically disclosed by preferred embodiments and optional features, modification, improvement and variation of the inventions embodied therein herein disclosed may be resorted to by those skilled in the art, and that such modifications, improvements and variations are considered to be within the scope of this invention. The materials, methods, and examples provided here are representative of preferred embodiments, are exemplary, and are not intended as limitations on the scope of the invention.

[0285] The invention has been described broadly and generically herein. Each of the narrower species and subgeneric groupings falling within the generic disclosure also form part of the invention. This includes the generic description of the invention with a proviso or negative limitation removing any subject matter from the genus, regardless of whether or not the excised material is specifically recited herein.

[0286] All publications, patent applications, patents, and other references mentioned herein are expressly incorporated by reference in their entirety, including all formulas and figures, to

the same extent as if each were incorporated by reference individually. In case of conflict, the present specification, including definitions, will control.

[0287] Other embodiments are set forth within the following claims.

SEQUENCE LISTING

SEQ ID NO:1 - miR-150 - 1 ctcccatgg cctgtctcc caaccctgt accagtgtg ggctcagacc
ctggtacagg cctgggggac agggacctgg ggac

SEQ ID NO:2 - miR-126 - 1 cgctggcgac gggacattat tacttttggg acgcgctgtg acactcaaa
ctcgtaccgt gagtaataat gcgccgtcca cggca

SEQ ID NO:3 - miR-296

1 aggacccttc cagagggccc cccctcaatc ctgtgtgcc taattcagag ggttgggtgg
61 aggctctcct gaaggctct

SEQ ID NO:4 - let-7

1 tgggatgagg tagtaggtg tatagttta gggcacacc caccactggg agataactat
61 acaatctact gtctttccta

SEQ ID NO:5 - PDGFR-A

1 aagagcaaaa agcgaaggcg caatctggac actgggagat tcggagcgca gggagtttga
61 gagaaacttt tattttgaag agaccaaggt tgagggggggg cttatttcct gacagctatt
121 tacttagagc aaatgattag ttttagaagg atggactata acattgaatc aattacaaaa
181 cgcgggtttt gagcccatta ctgttgagc tacagggaga gaaacagagg aggagactgc
241 aagagatcat tggaggccgt gggcacgctc ttactccat gtgtgggaca ttcattgcgg
301 aataacatcg gaggagaagt ttccagagc tatggggact tccatccgg cgttctggt
361 cttaggctgt ctctcacag ggctgagcct aatcctctgc cagcttcat taccctctat
421 ccttccaaat gaaaatgaaa aggttggtgca gctgaattca tcctttctc tgagatgctt
481 tggggagagt gaagtgagct ggcagtacc catgtctgaa gaagagagct ccgatgtgga

541 aatcagaaat gaagaaaaca acagcggcct tttgtgacg gtcttggaag tgagcagtgc
601 ctggcgggcc cacacagggt tgtacacttg ctattacaac cacactcaga cagaagagaa
661 tgagcttgaa ggcaggcaca ttacatcta tgtgccagac ccagatgtag ccttgtacc
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781 tcgcacaact gatcccgaga ctctgtaac cttacacaac agtgaggggg tggtacctgc
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901 ggccaccgtc aaaggaaaga agttccagac catcccattt aatgtttatg ctttaaaagc
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1021 gattgtggtc acctgtgctg ttttaacaa tgagggtggt gaccttcaat ggacttacc
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1201 tgctgccgc caggctacca gggaggtaaa agaatgaag aaagtcacta ttctgtcca
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1501 ccattatact attgtagctc aaaatgaaga tgctgtgaag agctatactt ttgaactgtt
1561 aactcaagtt cttcatcca ttctggactt ggtcgatgat caccatggct caactggggg
1621 acagacgggt aggtgcacag ctgaaggcac gccgcttct gatattgagt ggatgatag
1681 caaagatatt aagaaatga ataataaaac ttctggact atttggcca acaatgtctc
1741 aaacatcatc acggagatcc actcccaga caggagtacc gtggagggcc gtgtgacttt
1801 cgccaaagtg gaggagacca tcgccgtcg atgcctggct aagaatctcc ttggagctga
1861 gaaccgagag ctgaagctgg tggctccac cctgcgttct gaactcacgg tggctgctgc
1921 agtctggtg ctgttggtga ttgtgatcat ctacttatt gtcctggtg tcatttgaa

1981 acagaaaccg aggtatgaaa ttcgctggag ggtcattgaa tcaatcagcc cagatggaca
2041 tgaatatatt tatgtggacc cgatgcagct gccttatgac tcaagatggg agtttccaag
2101 agatggacta gtgcttggc gggcttggg gtctggagcg ttgggaagg tggttgaagg
2161 aacagcctat ggattaagcc ggtccaacc tgtcatgaaa gttgcagtga agatgctaaa
2221 acccacggcc agatccagtg aaaaacaagc tctcatgtct gaactgaaga taatgactca
2281 cctggggcca catttgaaca ttgtaaactt gctgggagcc tgcaccaagt caggcccat
2341 ttacatcatc acagagtatt gcttctatgg agatttggc aactattgc ataagaatag
2401 ggatagcttc ctgagccacc acccagagaa gccaaagaaa gagctggata tcttggatt
2461 gaaccctgct gatgaaagca cacggagcta tgtatttta tctttgaaa acaatggta
2521 ctacatggac atgaagcagg ctgatactac acagtatgtc cccatgctag aaaggaaaga
2581 ggtttctaaa tattecgaca tccagagatc actctatgat cgtccagcct catataagaa
2641 gaaatctatg ttagactcag aagtcaaaaa cctccttca gatgataact cagaaggcct
2701 tactttattg gatttgtga gcttcaccta tcaagttgcc cgaggaatgg agttttggc
2761 ttcaaaaaat tgtgtccacc gtgatctggc tgctcgcaac gtcctcctgg cacaaggaaa
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2881 gtcgaaaggc agtaccttc tgcccgtaa gtggatggct cctgagagca tcttgacaa
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3181 ggagaatctg ctgcctggac aatataaaaa gagttatgaa aaaattcacc tggacttct
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3301 tgtcacctac aaaaacgagg aagacaagct gaaggactgg gaggggtggc tggatgagca
3361 gagactgagc gctgacagtg gctacatcat tcctctgcct gacattgacc ctgtccctga

3421 ggaggaggac ctgggcaaga ggaacagaca cagctcgag acctctgaag agagtccat
3481 tgagacgggt tccagcagtt ccacctcat caagagagag gacgagacca ttgaagacat
3541 cgacatgatg gatgacatcg gcatagactc ttcagacctg gtggaagaca gcttctgta
3601 actggcggat tcgaggggtt ccttccactt ctggggccac ctctggatcc cgttcagaaa
3661 accactttat tgcaatgcag aggttgagag gaggacttgg ttgatgttta aagagaagtt
3721 cccagccaag ggcctcgggg agcgttctaa atatgaatga atgggatatt ttgaaatgaa
3781 ctttgcagt gttgcctctt gcaatgcctc agtagcatct cagtgggtgtg tgaagtttg
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4621 gtgctgaaca taacttctca tgtatattac ccaatggaaa atataatgat cagcaaaaag
4681 actggatttg cagaagtttt tttttttt ttctcatgc ctgatgaaag ctttggcgac
4741 cccaatatat gtatttttg aatctatgaa cctgaaaagg gtcagaagga tgcccagaca
4801 tcagcctcct tcttccacc cttaccccaa agagaaagag ttgaaactc gagaccataa

4861 agatattctt tagtggaggc tggatgtgca ttagcctgga tcctcagttc tcaaatgtgt
4921 gtggcagcca ggatgactag atcctgggtt tccatccttg agattctgaa gtatgaagtc
4981 tgagggaac cagagtctgt attttctaa actccctggc tgttctgac gccagttt
5041 cggaacact gacttaggtt tcaggaagtt gccatgggaa acaataatt tgaactttg
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5161 gtgacattta atgcatcta gctagcaatt gcgacctaa ttaactttc cagtcttagc
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5281 cccactgtat gggggagatt gaactttcc cgtctccgt cttctgcctc ccactccata
5341 ccccgccaag gaaaggcatg tacaaaaatt atgcaattca gtgtccaag tctctgtga
5401 accagctcag tgttttggtg gaaaaacat ttaagttt actgataatt tgaggtaga
5461 tgggaggatg aattgtcaca tctatccaca ctgtcaaca ggttggtgtg ggttcattgg
5521 cattctttgc aactgtctt aattgctgat accatatgaa tgaaacatgg gctgtgatta
5581 ctgcaatcac tgtctatcg gcagatgatg ctttgaaga tgcagaagca ataataaagt
5641 acttgactac ctactggtgt aatctcaatg caagcccaa cttcttctc caacttttc
5701 atagtaagtg cgaagactga gccagattgg ccaattaaa acgaaacct gactaggttc
5761 ttagagcca attagactg aaatacgtt gtgtttctag aatcacagct caagcattct
5821 gttatcgct cactctcct tgtacagcct tttttgtg gtgcttgca tttgatatt
5881 gctgtgagcc ttgcatgaca tcatgaggcc ggatgaaact tctcagtcca gcagttcca
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SEQ ID NO: 10

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3901 a

SEQ ID NO: 12 - FN1

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SEQ ID NO: 13 - MFGE8

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241 ccctgccaca acggtgggtt atgcgaggag atttccaag aagtgcgagg agatgtcttc
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SEQ ID NO: 14 - LGALS3BP

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SEQ ID NO: 15 - TF

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2281 ttattgattt tatatttc

SEQ ID NO: 16 - VEGFR

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SEQ ID NO: 17 - miR-132

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Features	Nucleotide
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PGK promoter	5364 .. 5874
td-Tomato	5894 .. 7321
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Sequence ID No.: 19 -165A VEGF isoform

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What is claimed is:

1. A method for treating a disease or condition involving an inflammatory response or related to inflammation in a subject in need thereof, comprising administering to the subject a purified population of cell-derived vesicles, wherein the population is purified from a population of stem cells cultured under conditions of hypoxia and low serum, and optionally wherein the cell-derived vesicles comprise exosomes and/or microvesicles.
2. The method of claim 1, wherein the inflammatory disease or condition is selected from multiple sclerosis, primary and secondary progressive multiple sclerosis, relapsing remitting multiple sclerosis, radiation-induced soft tissue damage, fraility, a neuroinflammatory disease, muscle injuries, radiation tissue damage, stroke, brain inflammatory disease, traumatic brain injury, myocardial infarction, graft versus host disease, Parkinson's disease, Alzheimer's, inflammatory bowel disease, Huntington's disease, amyotrophic lateral sclerosis, Bahcet's disease, sarcopenia, aging, spinal cord injury, wound repair, or dysphagia, and optionally wherein the disease or condition excludes stroke.
3. The method of claim 1, wherein the inflammatory disease or condition is selected from multiple sclerosis, primary and secondary progressive multiple sclerosis, or relapsing remitting multiple sclerosis.
4. The method of claim 1, wherein the subject is administered at least one dose of cell-derived vesicles selected from the group of: from between approximately 0.1 mg to about 1000 mg of cell-derived vesicle protein from the purified population, or of of approximately 50 mg of cell-derived vesicle protein from the purified population.
5. The method of claim 1, wherein the cell-derived vesicle protein is administered prior to, simultaneously with, or after administration of an isolated stem cell.
6. The method of claim 1, wherein the purified population is administered by intravenous injection, intrathecal injection, direct injection, intramuscular injection, intracranial injection, or topically.
7. The method of claim 1, wherein the subject is a mammal, optionally a human patient.

8. The method of claim 1, wherein the population of stem cells comprise one or more populations selected from the group of adult stem cells, embryonic stem cells, embryonic-like stem cells, neural stem cells, mesenchymal stem cells, or induced pluripotent stem cells.
9. The method of claim 1, wherein the population of stem cells comprises an adult stem cells that are optionally a population of mesenchymal stem cells.
10. The method of claim 1, wherein the cell-derived vesicles further comprise at least one exogenous nucleic acid and/or at least one exogenous protein, and optionally wherein the exogenous nucleic acid encodes, or is, a micro RNA (miRNA).
11. The method of claim 10, wherein the miRNA is selected from the group of miR-150, miR-126, miR-132, miR-296, or let-7.
12. The method of claim 10, wherein the exogenous protein is one or more of platelet derived growth factor receptor (PDGFR), Collagen, Type 1, Alpha 2 (COL1A2), Collagen, Type VI, Alpha 3 (COL6A3), EGF-like repeats- and discoidin i-like domains-containing protein 3 (EDIL3), epidermal growth factor receptor (EGFR), fibroblast growth factor receptor (FGFR), fibronectin (FN1), Milk fat globule-EGF factor 8 (MFGE8), lectin, galactoside-binding, soluble, 3 binding protein (LGALS3BP), nuclear factor-kappaB (NFκB), or transferrin (TF), that optionally exclude VEGFR and/or VEGF.
13. The method of claim 1, wherein the cell-derived vesicles of the population comprise one or more of miR-126, miR-132, miR-150, miR-210, miR-214, miR-296, or miR-424.
14. The method of claim 1, wherein the cell-derived vesicles of the population comprise two or more of miR-126, miR-132, miR-150, miR-210, miR-214, miR-296, or miR-424.
15. The method of claim 1, wherein the cell-derived vesicles of the population comprise three or more of miR-126, miR-132, miR-150, miR-210, miR-214, miR-296, or miR-424.
16. The method of claim 1, wherein the cell-derived vesicles of the population comprise four or more of miR-126, miR-132, miR-150, miR-210, miR-214, miR-296, or miR-424.
17. The method of claim 1, wherein the cell-derived vesicles of the population comprise five or more of miR-126, miR-132, miR-150, miR-210, miR-214, miR-296, or miR-424.

18. The method of claim 1, wherein the cell-derived vesicles of the population comprise six or more of miR-126, miR-132, miR-150, miR-210, miR-214, miR-296, or miR-424.
19. The method of claim 1, wherein the cell-derived vesicles of the population comprise miR-126, miR-132, miR-150, miR-210, miR-214, miR-296, and miR-424.
20. The method of claim 1, wherein the cell-derived vesicles of the population comprise one or more of 3,6-anhydro-D-galactose, 4-aminobutyric acid, 5'-deoxy-5'-methylthioadenosine, 5-methoxytryptamine, s-adenosylmethionine, s-adenosylhomocysteine, adipic acid, aminomalonate, arabinose, aspartic acid, beta-alanine, cholesterol, citric acid, creatinine, cysteine, cytidine-5-monophosphate, erythritol, fructose, fumaric acid, galacturonic acid, glucose, glucose-1-phosphate, glucose-6-phosphate, glutamine, glyceric acid, glycerol-alpha-phosphate, glycine, guanosine, hexitol, hexuronic acid, inosine, isohexonic acid, isomaltose, lactamide, lactic acid, lactose, leucine, levoglucosan, maleimide, malic acid, maltotriose, mannose, methanolphosphate, methionine, N-acetylaspartic acid, N-acetyl-D-galactosamine, nicotinamide, N-methylalanine, oxoproline, pantothenic acid, pentadecanoic acid, phenol, putrescine, pyruvic acid, ribitol, ribose, sorbitol, squalene, succinic acid, threitol, threonic acid, threonine, thymine, trans-4-hydroxyproline, trehalose, urea, uridine, valine, or xylitol.
21. The method of claim 1, wherein the cell-derived vesicles of the population comprise two or more of 3,6-anhydro-D-galactose, 4-aminobutyric acid, 5'-deoxy-5'-methylthioadenosine, 5-methoxytryptamine, s-adenosylmethionine, s-adenosylhomocysteine, adipic acid, aminomalonate, arabinose, aspartic acid, beta-alanine, cholesterol, citric acid, creatinine, cysteine, cytidine-5-monophosphate, erythritol, fructose, fumaric acid, galacturonic acid, glucose, glucose-1-phosphate, glucose-6-phosphate, glutamine, glyceric acid, glycerol-alpha-phosphate, glycine, guanosine, hexitol, hexuronic acid, inosine, isohexonic acid, isomaltose, lactamide, lactic acid, lactose, leucine, levoglucosan, maleimide, malic acid, maltotriose, mannose, methanolphosphate, methionine, N-acetylaspartic acid, N-acetyl-D-galactosamine, nicotinamide, N-methylalanine, oxoproline, pantothenic acid, pentadecanoic acid, phenol, putrescine, pyruvic acid, ribitol, ribose, sorbitol, squalene, succinic acid, threitol, threonic acid, threonine, thymine, trans-4-hydroxyproline, trehalose, urea, uridine, valine, or xylitol.

22. The method of claim 1, wherein the cell-derived vesicles of the population comprise three or more of 3,6-anhydro-D-galactose, 4-aminobutyric acid, 5'-deoxy-5'-methylthioadenosine, 5-methoxytryptamine, s-adenosylmethionine, s-adenosylhomocysteine, adipic acid, aminomalonate, arabinose, aspartic acid, beta-alanine, cholesterol, citric acid, creatinine, cysteine, cytidine-5-monophosphate, erythritol, fructose, fumaric acid, galacturonic acid, glucose, glucose-1-phosphate, glucose-6-phosphate, glutamine, glyceric acid, glycerol-alpha-phosphate, glycine, guanosine, hexitol, hexuronic acid, inosine, isohexonic acid, isomaltose, lactamide, lactic acid, lactose, leucine, levoglucosan, maleimide, malic acid, maltotriose, mannose, methanolphosphate, methionine, N-acetylaspartic acid, N-acetyl-D-galactosamine, nicotinamide, N-methylalanine, oxoproline, pantothenic acid, pentadecanoic acid, phenol, putrescine, pyruvic acid, ribitol, ribose, sorbitol, squalene, succinic acid, threitol, threonic acid, threonine, thymine, trans-4-hydroxyproline, trehalose, urea, uridine, valine, or xylitol.

23. The method of claim 1, wherein the cell-derived vesicles of the population comprise four or more of 3,6-anhydro-D-galactose, 4-aminobutyric acid, 5'-deoxy-5'-methylthioadenosine, 5-methoxytryptamine, s-adenosylmethionine, s-adenosylhomocysteine, adipic acid, aminomalonate, arabinose, aspartic acid, beta-alanine, cholesterol, citric acid, creatinine, cysteine, cytidine-5-monophosphate, erythritol, fructose, fumaric acid, galacturonic acid, glucose, glucose-1-phosphate, glucose-6-phosphate, glutamine, glyceric acid, glycerol-alpha-phosphate, glycine, guanosine, hexitol, hexuronic acid, inosine, isohexonic acid, isomaltose, lactamide, lactic acid, lactose, leucine, levoglucosan, maleimide, malic acid, maltotriose, mannose, methanolphosphate, methionine, N-acetylaspartic acid, N-acetyl-D-galactosamine, nicotinamide, N-methylalanine, oxoproline, pantothenic acid, pentadecanoic acid, phenol, putrescine, pyruvic acid, ribitol, ribose, sorbitol, squalene, succinic acid, threitol, threonic acid, threonine, thymine, trans-4-hydroxyproline, trehalose, urea, uridine, valine, or xylitol.

24. The method of claim 1, wherein the cell-derived vesicles of the population comprise five or more of 3,6-anhydro-D-galactose, 4-aminobutyric acid, 5'-deoxy-5'-methylthioadenosine, 5-methoxytryptamine, s-adenosylmethionine, s-adenosylhomocysteine, adipic acid, aminomalonate, arabinose, aspartic acid, beta-alanine, cholesterol, citric acid, creatinine, cysteine, cytidine-5-monophosphate, erythritol, fructose, fumaric acid,

galacturonic acid, glucose, glucose-1-phosphate, glucose-6-phosphate, glutamine, glyceric acid, glycerol-alpha-phosphate, glycine, guanosine, hexitol, hexuronic acid, inosine, isohexonic acid, isomaltose, lactamide, lactic acid, lactose, leucine, levoglucosan, maleimide, malic acid, maltotriose, mannose, methanolphosphate, methionine, N-acetylaspartic acid, N-acetyl-D-galactosamine, nicotinamide, N-methylalanine, oxoproline, pantothenic acid, pentadecanoic acid, phenol, putrescine, pyruvic acid, ribitol, ribose, sorbitol, squalene, succinic acid, threitol, threonic acid, threonine, thymine, trans-4-hydroxyproline, trehalose, urea, uridine, valine, or xylitol.

25. The method of claim 1, wherein the cell-derived vesicles of the population comprise six or more of 3,6-anhydro-D-galactose, 4-aminobutyric acid, 5'-deoxy-5'-methylthioadenosine, 5-methoxytryptamine, s-adenosylmethionine, s-adenosylhomocysteine, adipic acid, aminomalonate, arabinose, aspartic acid, beta-alanine, cholesterol, citric acid, creatinine, cysteine, cytidine-5-monophosphate, erythritol, fructose, fumaric acid, galacturonic acid, glucose, glucose-1-phosphate, glucose-6-phosphate, glutamine, glyceric acid, glycerol-alpha-phosphate, glycine, guanosine, hexitol, hexuronic acid, inosine, isohexonic acid, isomaltose, lactamide, lactic acid, lactose, leucine, levoglucosan, maleimide, malic acid, maltotriose, mannose, methanolphosphate, methionine, N-acetylaspartic acid, N-acetyl-D-galactosamine, nicotinamide, N-methylalanine, oxoproline, pantothenic acid, pentadecanoic acid, phenol, putrescine, pyruvic acid, ribitol, ribose, sorbitol, squalene, succinic acid, threitol, threonic acid, threonine, thymine, trans-4-hydroxyproline, trehalose, urea, uridine, valine, or xylitol.

26. The method of claim 1, wherein the cell-derived vesicles of the population comprise seven or more of 3,6-anhydro-D-galactose, 4-aminobutyric acid, 5'-deoxy-5'-methylthioadenosine, 5-methoxytryptamine, s-adenosylmethionine, s-adenosylhomocysteine, adipic acid, aminomalonate, arabinose, aspartic acid, beta-alanine, cholesterol, citric acid, creatinine, cysteine, cytidine-5-monophosphate, erythritol, fructose, fumaric acid, galacturonic acid, glucose, glucose-1-phosphate, glucose-6-phosphate, glutamine, glyceric acid, glycerol-alpha-phosphate, glycine, guanosine, hexitol, hexuronic acid, inosine, isohexonic acid, isomaltose, lactamide, lactic acid, lactose, leucine, levoglucosan, maleimide, malic acid, maltotriose, mannose, methanolphosphate, methionine, N-acetylaspartic acid, N-acetyl-D-galactosamine, nicotinamide, N-methylalanine, oxoproline, pantothenic acid,

pentadecanoic acid, phenol, putrescine, pyruvic acid, ribitol, ribose, sorbitol, squalene, succinic acid, threitol, threonic acid, threonine, thymine, trans-4-hydroxyproline, trehalose, urea, uridine, valine, or xylitol.

27. The method of claim 1, wherein the cell-derived vesicles of the population comprise eight or more of 3,6-anhydro-D-galactose, 4-aminobutyric acid, 5'-deoxy-5'-methylthioadenosine, 5-methoxytryptamine, s-adenosylmethionine, s-adenosylhomocysteine, adipic acid, aminomalonate, arabinose, aspartic acid, beta-alanine, cholesterol, citric acid, creatinine, cysteine, cytidine-5-monophosphate, erythritol, fructose, fumaric acid, galacturonic acid, glucose, glucose-1-phosphate, glucose-6-phosphate, glutamine, glyceric acid, glycerol-alpha-phosphate, glycine, guanosine, hexitol, hexuronic acid, inosine, isohexonic acid, isomaltose, lactamide, lactic acid, lactose, leucine, levoglucosan, maleimide, malic acid, maltotriose, mannose, methanolphosphate, methionine, N-acetylaspartic acid, N-acetyl-D-galactosamine, nicotinamide, N-methylalanine, oxoproline, pantothenic acid, pentadecanoic acid, phenol, putrescine, pyruvic acid, ribitol, ribose, sorbitol, squalene, succinic acid, threitol, threonic acid, threonine, thymine, trans-4-hydroxyproline, trehalose, urea, uridine, valine, or xylitol.

28. The method of claim 1, wherein the cell-derived vesicles of the population comprise nine or more of 3,6-anhydro-D-galactose, 4-aminobutyric acid, 5'-deoxy-5'-methylthioadenosine, 5-methoxytryptamine, s-adenosylmethionine, s-adenosylhomocysteine, adipic acid, aminomalonate, arabinose, aspartic acid, beta-alanine, cholesterol, citric acid, creatinine, cysteine, cytidine-5-monophosphate, erythritol, fructose, fumaric acid, galacturonic acid, glucose, glucose-1-phosphate, glucose-6-phosphate, glutamine, glyceric acid, glycerol-alpha-phosphate, glycine, guanosine, hexitol, hexuronic acid, inosine, isohexonic acid, isomaltose, lactamide, lactic acid, lactose, leucine, levoglucosan, maleimide, malic acid, maltotriose, mannose, methanolphosphate, methionine, N-acetylaspartic acid, N-acetyl-D-galactosamine, nicotinamide, N-methylalanine, oxoproline, pantothenic acid, pentadecanoic acid, phenol, putrescine, pyruvic acid, ribitol, ribose, sorbitol, squalene, succinic acid, threitol, threonic acid, threonine, thymine, trans-4-hydroxyproline, trehalose, urea, uridine, valine, or xylitol.

29. The method of claim 1, wherein the cell-derived vesicles of the population comprise ten or more of 3,6-anhydro-D-galactose, 4-aminobutyric acid, 5'-deoxy-5'-methylthioadenosine, 5-methoxytryptamine, s-adenosylmethionine, s-adenosylhomocysteine, adipic acid, aminomalonate, arabinose, aspartic acid, beta-alanine, cholesterol, citric acid, creatinine, cysteine, cytidine-5-monophosphate, erythritol, fructose, fumaric acid, galacturonic acid, glucose, glucose-1-phosphate, glucose-6-phosphate, glutamine, glyceric acid, glycerol-alpha-phosphate, glycine, guanosine, hexitol, hexuronic acid, inosine, isohexonic acid, isomaltose, lactamide, lactic acid, lactose, leucine, levoglucosan, maleimide, malic acid, maltotriose, mannose, methanolphosphate, methionine, N-acetylaspartic acid, N-acetyl-D-galactosamine, nicotinamide, N-methylalanine, oxoproline, pantothenic acid, pentadecanoic acid, phenol, putrescine, pyruvic acid, ribitol, ribose, sorbitol, squalene, succinic acid, threitol, threonic acid, threonine, thymine, trans-4-hydroxyproline, trehalose, urea, uridine, valine, or xylitol.

30. The method of claim 1, wherein the cell-derived vesicles of the population comprise one or more of Ceramide (d32:1), Ceramide (d33:1), Ceramide (d34:0), Ceramide (d34:1), Ceramide (d34:2), Ceramide (d34:2), Ceramide (d36:1), Ceramide (d38:1), Ceramide (d39:1), Ceramide (d40:0), Ceramide (d40:1), Ceramide (d40:2), Ceramide (d41:1), Ceramide (d42:1), Ceramide (d42:2) B, Ceramide (d44:1), Fatty Acid (20:4), Fatty Acid (22:0), Fatty Acid (22:6), Fatty Acid (24:0), Fatty Acid (24:1), glucosylceramides (d40:1), glucosylceramides (d41:1), glucosylceramides (d42:1), glucosylceramides (d42:2), Lysophosphatidylcholines (16:0), Lysophosphatidylcholines (18:0) A, Lysophosphatidylcholines (18:1), lysophosphatidylethanolamine (20:4), Phosphatidylcholines (32:1), Phosphatidylcholines (33:1), Phosphatidylcholines (34:0), Phosphatidylcholines (34:1), Phosphatidylcholines (34:2), Phosphatidylcholines (35:2), Phosphatidylcholines (36:1), Phosphatidylcholines (36:2), Phosphatidylcholines (36:3), Phosphatidylcholines (38:2), Phosphatidylcholines (38:3), Phosphatidylcholines (38:5), Phosphatidylcholines (38:6), Phosphatidylcholines (40:5), Phosphatidylcholines (40:6), Phosphatidylcholines (40:7), Phosphatidylcholines (p-34:0), Phosphatidylcholines (o-34:1), Phosphatidylethanolamines (34:1), Phosphatidylethanolamines (34:2), Phosphatidylethanolamines (36:3), Phosphatidylethanolamines (36:4), Phosphatidylethanolamines (38:4), B Phosphatidylethanolamines (38:6),

Phosphatidylethanolamines (p-34:1), Phosphatidylethanolamines (o-34:2),
 Phosphatidylethanolamines (p-36:1), Phosphatidylethanolamines (o-36:2),
 Phosphatidylethanolamines (p-36:4), Phosphatidylethanolamines (o-36:5),
 Phosphatidylethanolamines (p-38:4), Phosphatidylethanolamines (o-38:5),
 Phosphatidylethanolamines (p-38:5), Phosphatidylethanolamines (o-38:6),
 Phosphatidylethanolamines (p-38:6), Phosphatidylethanolamines (o-38:7),
 Phosphatidylethanolamines (p-40:4), Phosphatidylethanolamines (o-40:5),
 Phosphatidylethanolamines (p-40:5), Phosphatidylethanolamines (o-40:6),
 Phosphatidylethanolamines (p-40:6), Phosphatidylethanolamines (o-40:7),
 Phosphatidylethanolamines (p-40:7), Phosphatidylethanolamines (o-40:8), Sphingomyelin
 (d30:1), Sphingomyelin (d32:0), Sphingomyelin (d32:2), Sphingomyelin (d33:1),
 Sphingomyelin (d34:0), Sphingomyelin (d36:1), Sphingomyelin (d36:2), Sphingomyelin
 (d38:1), Sphingomyelin (d40:1), Sphingomyelin (d40:2), Sphingomyelin (d41:1),
 Sphingomyelin (d41:2), Sphingomyelin (d42:2), or B Sphingomyelin (d42:3).

31. The method of claim 1, wherein the cell-derived vesicles of the population comprise two or more of Ceramide (d32:1), Ceramide (d33:1), Ceramide (d34:0), Ceramide (d34:1), Ceramide (d34:2), Ceramide (d34:2), Ceramide (d36:1), Ceramide (d38:1), Ceramide (d39:1), Ceramide (d40:0), Ceramide (d40:1), Ceramide (d40:2), Ceramide (d41:1), Ceramide (d42:1), Ceramide (d42:2) B, Ceramide (d44:1), Fatty Acid (20:4), Fatty Acid (22:0), Fatty Acid (22:6), Fatty Acid (24:0), Fatty Acid (24:1), glucosylceramides (d40:1), glucosylceramides (d41:1), glucosylceramides (d42:1), glucosylceramides (d42:2), Lysophosphatidylcholines (16:0), Lysophosphatidylcholines (18:0) A, Lysophosphatidylcholines (18:1), lysophosphatidylethanolamine (20:4), Phosphatidylcholines (32:1), Phosphatidylcholines (33:1), Phosphatidylcholines (34:0), Phosphatidylcholines (34:1), Phosphatidylcholines (34:2), Phosphatidylcholines (35:2), Phosphatidylcholines (36:1), Phosphatidylcholines (36:2), Phosphatidylcholines (36:3), Phosphatidylcholines (38:2), Phosphatidylcholines (38:3), Phosphatidylcholines (38:5), Phosphatidylcholines (38:6), Phosphatidylcholines (40:5), Phosphatidylcholines (40:6), Phosphatidylcholines (40:7), Phosphatidylcholines (p-34:0), Phosphatidylcholines (o-34:1), Phosphatidylethanolamines (34:1), Phosphatidylethanolamines (34:2), Phosphatidylethanolamines (36:3), Phosphatidylethanolamines (36:4),

Phosphatidylethanolamines (38:4), B Phosphatidylethanolamines (38:6),
 Phosphatidylethanolamines (p-34:1), Phosphatidylethanolamines (o-34:2),
 Phosphatidylethanolamines (p-36:1), Phosphatidylethanolamines (o-36:2),
 Phosphatidylethanolamines (p-36:4), Phosphatidylethanolamines (o-36:5),
 Phosphatidylethanolamines (p-38:4), Phosphatidylethanolamines (o-38:5),
 Phosphatidylethanolamines (p-38:5), Phosphatidylethanolamines (o-38:6),
 Phosphatidylethanolamines (p-38:6), Phosphatidylethanolamines (o-38:7),
 Phosphatidylethanolamines (p-40:4), Phosphatidylethanolamines (o-40:5),
 Phosphatidylethanolamines (p-40:5), Phosphatidylethanolamines (o-40:6),
 Phosphatidylethanolamines (p-40:6), Phosphatidylethanolamines (o-40:7),
 Phosphatidylethanolamines (p-40:7), Phosphatidylethanolamines (o-40:8), Sphingomyelin
 (d30:1), Sphingomyelin (d32:0), Sphingomyelin (d32:2), Sphingomyelin (d33:1),
 Sphingomyelin (d34:0), Sphingomyelin (d36:1), Sphingomyelin (d36:2), Sphingomyelin
 (d38:1), Sphingomyelin (d40:1), Sphingomyelin (d40:2), Sphingomyelin (d41:1),
 Sphingomyelin (d41:2), Sphingomyelin (d42:2), or B Sphingomyelin (d42:3).

32. The method of claim 1, wherein the cell-derived vesicles of the population comprise three or more of Ceramide (d32:1), Ceramide (d33:1), Ceramide (d34:0), Ceramide (d34:1), Ceramide (d34:2), Ceramide (d34:2), Ceramide (d36:1), Ceramide (d38:1), Ceramide (d39:1), Ceramide (d40:0), Ceramide (d40:1), Ceramide (d40:2), Ceramide (d41:1), Ceramide (d42:1), Ceramide (d42:2) B, Ceramide (d44:1), Fatty Acid (20:4), Fatty Acid (22:0), Fatty Acid (22:6), Fatty Acid (24:0), Fatty Acid (24:1), glucosylceramides (d40:1), glucosylceramides (d41:1), glucosylceramides (d42:1), glucosylceramides (d42:2), Lysophosphatidylcholines (16:0), Lysophosphatidylcholines (18:0) A, Lysophosphatidylcholines (18:1), lysophosphatidylethanolamine (20:4), Phosphatidylcholines (32:1), Phosphatidylcholines (33:1), Phosphatidylcholines (34:0), Phosphatidylcholines (34:1), Phosphatidylcholines (34:2), Phosphatidylcholines (35:2), Phosphatidylcholines (36:1), Phosphatidylcholines (36:2), Phosphatidylcholines (36:3), Phosphatidylcholines (38:2), Phosphatidylcholines (38:3), Phosphatidylcholines (38:5), Phosphatidylcholines (38:6), Phosphatidylcholines (40:5), Phosphatidylcholines (40:6), Phosphatidylcholines (40:7), Phosphatidylcholines (p-34:0), Phosphatidylcholines (o-34:1), Phosphatidylethanolamines (34:1), Phosphatidylethanolamines (34:2),

Phosphatidylethanolamines (36:3), Phosphatidylethanolamines (36:4),
 Phosphatidylethanolamines (38:4), B Phosphatidylethanolamines (38:6),
 Phosphatidylethanolamines (p-34:1), Phosphatidylethanolamines (o-34:2),
 Phosphatidylethanolamines (p-36:1), Phosphatidylethanolamines (o-36:2),
 Phosphatidylethanolamines (p-36:4), Phosphatidylethanolamines (o-36:5),
 Phosphatidylethanolamines (p-38:4), Phosphatidylethanolamines (o-38:5),
 Phosphatidylethanolamines (p-38:5), Phosphatidylethanolamines (o-38:6),
 Phosphatidylethanolamines (p-38:6), Phosphatidylethanolamines (o-38:7),
 Phosphatidylethanolamines (p-40:4), Phosphatidylethanolamines (o-40:5),
 Phosphatidylethanolamines (p-40:5), Phosphatidylethanolamines (o-40:6),
 Phosphatidylethanolamines (p-40:6), Phosphatidylethanolamines (o-40:7),
 Phosphatidylethanolamines (p-40:7), Phosphatidylethanolamines (o-40:8), Sphingomyelin
 (d30:1), Sphingomyelin (d32:0), Sphingomyelin (d32:2), Sphingomyelin (d33:1),
 Sphingomyelin (d34:0), Sphingomyelin (d36:1), Sphingomyelin (d36:2), Sphingomyelin
 (d38:1), Sphingomyelin (d40:1), Sphingomyelin (d40:2), Sphingomyelin (d41:1),
 Sphingomyelin (d41:2), Sphingomyelin (d42:2), or B Sphingomyelin (d42:3).

33. The method of claim 1, wherein the cell-derived vesicles of the population comprise four or more of Ceramide (d32:1), Ceramide (d33:1), Ceramide (d34:0), Ceramide (d34:1), Ceramide (d34:2), Ceramide (d34:2), Ceramide (d36:1), Ceramide (d38:1), Ceramide (d39:1), Ceramide (d40:0), Ceramide (d40:1), Ceramide (d40:2), Ceramide (d41:1), Ceramide (d42:1), Ceramide (d42:2) B, Ceramide (d44:1), Fatty Acid (20:4), Fatty Acid (22:0), Fatty Acid (22:6), Fatty Acid (24:0), Fatty Acid (24:1), glucosylceramides (d40:1), glucosylceramides (d41:1), glucosylceramides (d42:1), glucosylceramides (d42:2), Lysophosphatidylcholines (16:0), Lysophosphatidylcholines (18:0) A, Lysophosphatidylcholines (18:1), lysophosphatidylethanolamine (20:4), Phosphatidylcholines (32:1), Phosphatidylcholines (33:1), Phosphatidylcholines (34:0), Phosphatidylcholines (34:1), Phosphatidylcholines (34:2), Phosphatidylcholines (35:2), Phosphatidylcholines (36:1), Phosphatidylcholines (36:2), Phosphatidylcholines (36:3), Phosphatidylcholines (38:2), Phosphatidylcholines (38:3), Phosphatidylcholines (38:5), Phosphatidylcholines (38:6), Phosphatidylcholines (40:5), Phosphatidylcholines (40:6), Phosphatidylcholines (40:7), Phosphatidylcholines (p-34:0), Phosphatidylcholines (o-34:1),

Phosphatidylethanolamines (34:1), Phosphatidylethanolamines (34:2),
 Phosphatidylethanolamines (36:3), Phosphatidylethanolamines (36:4),
 Phosphatidylethanolamines (38:4), B Phosphatidylethanolamines (38:6),
 Phosphatidylethanolamines (p-34:1), Phosphatidylethanolamines (o-34:2),
 Phosphatidylethanolamines (p-36:1), Phosphatidylethanolamines (o-36:2),
 Phosphatidylethanolamines (p-36:4), Phosphatidylethanolamines (o-36:5),
 Phosphatidylethanolamines (p-38:4), Phosphatidylethanolamines (o-38:5),
 Phosphatidylethanolamines (p-38:5), Phosphatidylethanolamines (o-38:6),
 Phosphatidylethanolamines (p-38:6), Phosphatidylethanolamines (o-38:7),
 Phosphatidylethanolamines (p-40:4), Phosphatidylethanolamines (o-40:5),
 Phosphatidylethanolamines (p-40:5), Phosphatidylethanolamines (o-40:6),
 Phosphatidylethanolamines (p-40:6), Phosphatidylethanolamines (o-40:7),
 Phosphatidylethanolamines (p-40:7), Phosphatidylethanolamines (o-40:8), Sphingomyelin
 (d30:1), Sphingomyelin (d32:0), Sphingomyelin (d32:2), Sphingomyelin (d33:1),
 Sphingomyelin (d34:0), Sphingomyelin (d36:1), Sphingomyelin (d36:2), Sphingomyelin
 (d38:1), Sphingomyelin (d40:1), Sphingomyelin (d40:2), Sphingomyelin (d41:1),
 Sphingomyelin (d41:2), Sphingomyelin (d42:2), or B Sphingomyelin (d42:3).

34. The method of claim 1, wherein the cell-derived vesicles of the population comprise five or more of Ceramide (d32:1), Ceramide (d33:1), Ceramide (d34:0), Ceramide (d34:1), Ceramide (d34:2), Ceramide (d34:2), Ceramide (d36:1), Ceramide (d38:1), Ceramide (d39:1), Ceramide (d40:0), Ceramide (d40:1), Ceramide (d40:2), Ceramide (d41:1), Ceramide (d42:1), Ceramide (d42:2) B, Ceramide (d44:1), Fatty Acid (20:4), Fatty Acid (22:0), Fatty Acid (22:6), Fatty Acid (24:0), Fatty Acid (24:1), glucosylceramides (d40:1), glucosylceramides (d41:1), glucosylceramides (d42:1), glucosylceramides (d42:2), Lysophosphatidylcholines (16:0), Lysophosphatidylcholines (18:0) A, Lysophosphatidylcholines (18:1), lysophosphatidylethanolamine (20:4), Phosphatidylcholines (32:1), Phosphatidylcholines (33:1), Phosphatidylcholines (34:0), Phosphatidylcholines (34:1), Phosphatidylcholines (34:2), Phosphatidylcholines (35:2), Phosphatidylcholines (36:1), Phosphatidylcholines (36:2), Phosphatidylcholines (36:3), Phosphatidylcholines (38:2), Phosphatidylcholines (38:3), Phosphatidylcholines (38:5), Phosphatidylcholines (38:6), Phosphatidylcholines (40:5), Phosphatidylcholines (40:6),

Phosphatidylcholines (40:7), Phosphatidylcholines (p-34:0), Phosphatidylcholines (o-34:1), Phosphatidylethanolamines (34:1), Phosphatidylethanolamines (34:2), Phosphatidylethanolamines (36:3), Phosphatidylethanolamines (36:4), Phosphatidylethanolamines (38:4), B Phosphatidylethanolamines (38:6), Phosphatidylethanolamines (p-34:1), Phosphatidylethanolamines (o-34:2), Phosphatidylethanolamines (p-36:1), Phosphatidylethanolamines (o-36:2), Phosphatidylethanolamines (p-36:4), Phosphatidylethanolamines (o-36:5), Phosphatidylethanolamines (p-38:4), Phosphatidylethanolamines (o-38:5), Phosphatidylethanolamines (p-38:5), Phosphatidylethanolamines (o-38:6), Phosphatidylethanolamines (p-38:6), Phosphatidylethanolamines (o-38:7), Phosphatidylethanolamines (p-40:4), Phosphatidylethanolamines (o-40:5), Phosphatidylethanolamines (p-40:5), Phosphatidylethanolamines (o-40:6), Phosphatidylethanolamines (p-40:6), Phosphatidylethanolamines (o-40:7), Phosphatidylethanolamines (p-40:7), Phosphatidylethanolamines (o-40:8), Sphingomyelin (d30:1), Sphingomyelin (d32:0), Sphingomyelin (d32:2), Sphingomyelin (d33:1), Sphingomyelin (d34:0), Sphingomyelin (d36:1), Sphingomyelin (d36:2), Sphingomyelin (d38:1), Sphingomyelin (d40:1), Sphingomyelin (d40:2), Sphingomyelin (d41:1), Sphingomyelin (d41:2), Sphingomyelin (d42:2), or B Sphingomyelin (d42:3).

35. The method of claim 1, wherein the cell-derived vesicles of the population comprise six or more of Ceramide (d32:1), Ceramide (d33:1), Ceramide (d34:0), Ceramide (d34:1), Ceramide (d34:2), Ceramide (d34:2), Ceramide (d36:1), Ceramide (d38:1), Ceramide (d39:1), Ceramide (d40:0), Ceramide (d40:1), Ceramide (d40:2), Ceramide (d41:1), Ceramide (d42:1), Ceramide (d42:2) B, Ceramide (d44:1), Fatty Acid (20:4), Fatty Acid (22:0), Fatty Acid (22:6), Fatty Acid (24:0), Fatty Acid (24:1), glucosylceramides (d40:1), glucosylceramides (d41:1), glucosylceramides (d42:1), glucosylceramides (d42:2), Lysophosphatidylcholines (16:0), Lysophosphatidylcholines (18:0) A, Lysophosphatidylcholines (18:1), lysophosphatidylethanolamine (20:4), Phosphatidylcholines (32:1), Phosphatidylcholines (33:1), Phosphatidylcholines (34:0), Phosphatidylcholines (34:1), Phosphatidylcholines (34:2), Phosphatidylcholines (35:2), Phosphatidylcholines (36:1), Phosphatidylcholines (36:2), Phosphatidylcholines (36:3), Phosphatidylcholines (38:2), Phosphatidylcholines (38:3), Phosphatidylcholines (38:5),

Phosphatidylcholines (38:6), Phosphatidylcholines (40:5), Phosphatidylcholines (40:6), Phosphatidylcholines (40:7), Phosphatidylcholines (p-34:0), Phosphatidylcholines (o-34:1), Phosphatidylethanolamines (34:1), Phosphatidylethanolamines (34:2), Phosphatidylethanolamines (36:3), Phosphatidylethanolamines (36:4), Phosphatidylethanolamines (38:4), B Phosphatidylethanolamines (38:6), Phosphatidylethanolamines (p-34:1), Phosphatidylethanolamines (o-34:2), Phosphatidylethanolamines (p-36:1), Phosphatidylethanolamines (o-36:2), Phosphatidylethanolamines (p-36:4), Phosphatidylethanolamines (o-36:5), Phosphatidylethanolamines (p-38:4), Phosphatidylethanolamines (o-38:5), Phosphatidylethanolamines (p-38:5), Phosphatidylethanolamines (o-38:6), Phosphatidylethanolamines (p-38:6), Phosphatidylethanolamines (o-38:7), Phosphatidylethanolamines (p-40:4), Phosphatidylethanolamines (o-40:5), Phosphatidylethanolamines (p-40:5), Phosphatidylethanolamines (o-40:6), Phosphatidylethanolamines (p-40:6), Phosphatidylethanolamines (o-40:7), Phosphatidylethanolamines (p-40:7), Phosphatidylethanolamines (o-40:8), Sphingomyelin (d30:1), Sphingomyelin (d32:0), Sphingomyelin (d32:2), Sphingomyelin (d33:1), Sphingomyelin (d34:0), Sphingomyelin (d36:1), Sphingomyelin (d36:2), Sphingomyelin (d38:1), Sphingomyelin (d40:1), Sphingomyelin (d40:2), Sphingomyelin (d41:1), Sphingomyelin (d41:2), Sphingomyelin (d42:2), or B Sphingomyelin (d42:3).

36. The method of claim 1, wherein the cell-derived vesicles of the population comprise seven or more of Ceramide (d32:1), Ceramide (d33:1), Ceramide (d34:0), Ceramide (d34:1), Ceramide (d34:2), Ceramide (d34:2), Ceramide (d36:1), Ceramide (d38:1), Ceramide (d39:1), Ceramide (d40:0), Ceramide (d40:1), Ceramide (d40:2), Ceramide (d41:1), Ceramide (d42:1), Ceramide (d42:2) B, Ceramide (d44:1), Fatty Acid (20:4), Fatty Acid (22:0), Fatty Acid (22:6), Fatty Acid (24:0), Fatty Acid (24:1), glucosylceramides (d40:1), glucosylceramides (d41:1), glucosylceramides (d42:1), glucosylceramides (d42:2), Lysophosphatidylcholines (16:0), Lysophosphatidylcholines (18:0) A, Lysophosphatidylcholines (18:1), lysophosphatidylethanolamine (20:4), Phosphatidylcholines (32:1), Phosphatidylcholines (33:1), Phosphatidylcholines (34:0), Phosphatidylcholines (34:1), Phosphatidylcholines (34:2), Phosphatidylcholines (35:2), Phosphatidylcholines (36:1), Phosphatidylcholines (36:2), Phosphatidylcholines (36:3),

Phosphatidylcholines (38:2), Phosphatidylcholines (38:3), Phosphatidylcholines (38:5), Phosphatidylcholines (38:6), Phosphatidylcholines (40:5), Phosphatidylcholines (40:6), Phosphatidylcholines (40:7), Phosphatidylcholines (p-34:0), Phosphatidylcholines (o-34:1), Phosphatidylethanolamines (34:1), Phosphatidylethanolamines (34:2), Phosphatidylethanolamines (36:3), Phosphatidylethanolamines (36:4), Phosphatidylethanolamines (38:4), B Phosphatidylethanolamines (38:6), Phosphatidylethanolamines (p-34:1), Phosphatidylethanolamines (o-34:2), Phosphatidylethanolamines (p-36:1), Phosphatidylethanolamines (o-36:2), Phosphatidylethanolamines (p-36:4), Phosphatidylethanolamines (o-36:5), Phosphatidylethanolamines (p-38:4), Phosphatidylethanolamines (o-38:5), Phosphatidylethanolamines (p-38:5), Phosphatidylethanolamines (o-38:6), Phosphatidylethanolamines (p-38:6), Phosphatidylethanolamines (o-38:7), Phosphatidylethanolamines (p-40:4), Phosphatidylethanolamines (o-40:5), Phosphatidylethanolamines (p-40:5), Phosphatidylethanolamines (o-40:6), Phosphatidylethanolamines (p-40:6), Phosphatidylethanolamines (o-40:7), Phosphatidylethanolamines (p-40:7), Phosphatidylethanolamines (o-40:8), Sphingomyelin (d30:1), Sphingomyelin (d32:0), Sphingomyelin (d32:2), Sphingomyelin (d33:1), Sphingomyelin (d34:0), Sphingomyelin (d36:1), Sphingomyelin (d36:2), Sphingomyelin (d38:1), Sphingomyelin (d40:1), Sphingomyelin (d40:2), Sphingomyelin (d41:1), Sphingomyelin (d41:2), Sphingomyelin (d42:2), or B Sphingomyelin (d42:3).

37. The method of claim 1, wherein the cell-derived vesicles of the population comprise eight or more of Ceramide (d32:1), Ceramide (d33:1), Ceramide (d34:0), Ceramide (d34:1), Ceramide (d34:2), Ceramide (d34:2), Ceramide (d36:1), Ceramide (d38:1), Ceramide (d39:1), Ceramide (d40:0), Ceramide (d40:1), Ceramide (d40:2), Ceramide (d41:1), Ceramide (d42:1), Ceramide (d42:2) B, Ceramide (d44:1), Fatty Acid (20:4), Fatty Acid (22:0), Fatty Acid (22:6), Fatty Acid (24:0), Fatty Acid (24:1), glucosylceramides (d40:1), glucosylceramides (d41:1), glucosylceramides (d42:1), glucosylceramides (d42:2), Lysophosphatidylcholines (16:0), Lysophosphatidylcholines (18:0) A, Lysophosphatidylcholines (18:1), lysophosphatidylethanolamine (20:4), Phosphatidylcholines (32:1), Phosphatidylcholines (33:1), Phosphatidylcholines (34:0), Phosphatidylcholines (34:1), Phosphatidylcholines (34:2), Phosphatidylcholines (35:2),

Phosphatidylcholines (36:1), Phosphatidylcholines (36:2), Phosphatidylcholines (36:3), Phosphatidylcholines (38:2), Phosphatidylcholines (38:3), Phosphatidylcholines (38:5), Phosphatidylcholines (38:6), Phosphatidylcholines (40:5), Phosphatidylcholines (40:6), Phosphatidylcholines (40:7), Phosphatidylcholines (p-34:0), Phosphatidylcholines (o-34:1), Phosphatidylethanolamines (34:1), Phosphatidylethanolamines (34:2), Phosphatidylethanolamines (36:3), Phosphatidylethanolamines (36:4), Phosphatidylethanolamines (38:4), B Phosphatidylethanolamines (38:6), Phosphatidylethanolamines (p-34:1), Phosphatidylethanolamines (o-34:2), Phosphatidylethanolamines (p-36:1), Phosphatidylethanolamines (o-36:2), Phosphatidylethanolamines (p-36:4), Phosphatidylethanolamines (o-36:5), Phosphatidylethanolamines (p-38:4), Phosphatidylethanolamines (o-38:5), Phosphatidylethanolamines (p-38:5), Phosphatidylethanolamines (o-38:6), Phosphatidylethanolamines (p-38:6), Phosphatidylethanolamines (o-38:7), Phosphatidylethanolamines (p-40:4), Phosphatidylethanolamines (o-40:5), Phosphatidylethanolamines (p-40:5), Phosphatidylethanolamines (o-40:6), Phosphatidylethanolamines (p-40:6), Phosphatidylethanolamines (o-40:7), Phosphatidylethanolamines (p-40:7), Phosphatidylethanolamines (o-40:8), Sphingomyelin (d30:1), Sphingomyelin (d32:0), Sphingomyelin (d32:2), Sphingomyelin (d33:1), Sphingomyelin (d34:0), Sphingomyelin (d36:1), Sphingomyelin (d36:2), Sphingomyelin (d38:1), Sphingomyelin (d40:1), Sphingomyelin (d40:2), Sphingomyelin (d41:1), Sphingomyelin (d41:2), Sphingomyelin (d42:2), or B Sphingomyelin (d42:3).

38. The method of claim 1, wherein the cell-derived vesicles of the population comprise nine or more of Ceramide (d32:1), Ceramide (d33:1), Ceramide (d34:0), Ceramide (d34:1), Ceramide (d34:2), Ceramide (d34:2), Ceramide (d36:1), Ceramide (d38:1), Ceramide (d39:1), Ceramide (d40:0), Ceramide (d40:1), Ceramide (d40:2), Ceramide (d41:1), Ceramide (d42:1), Ceramide (d42:2) B, Ceramide (d44:1), Fatty Acid (20:4), Fatty Acid (22:0), Fatty Acid (22:6), Fatty Acid (24:0), Fatty Acid (24:1), glucosylceramides (d40:1), glucosylceramides (d41:1), glucosylceramides (d42:1), glucosylceramides (d42:2), Lysophosphatidylcholines (16:0), Lysophosphatidylcholines (18:0) A, Lysophosphatidylcholines (18:1), lysophosphatidylethanolamine (20:4), Phosphatidylcholines (32:1), Phosphatidylcholines (33:1), Phosphatidylcholines (34:0),

Phosphatidylcholines (34:1), Phosphatidylcholines (34:2), Phosphatidylcholines (35:2), Phosphatidylcholines (36:1), Phosphatidylcholines (36:2), Phosphatidylcholines (36:3), Phosphatidylcholines (38:2), Phosphatidylcholines (38:3), Phosphatidylcholines (38:5), Phosphatidylcholines (38:6), Phosphatidylcholines (40:5), Phosphatidylcholines (40:6), Phosphatidylcholines (40:7), Phosphatidylcholines (p-34:0), Phosphatidylcholines (o-34:1), Phosphatidylethanolamines (34:1), Phosphatidylethanolamines (34:2), Phosphatidylethanolamines (36:3), Phosphatidylethanolamines (36:4), Phosphatidylethanolamines (38:4), B Phosphatidylethanolamines (38:6), Phosphatidylethanolamines (p-34:1), Phosphatidylethanolamines (o-34:2), Phosphatidylethanolamines (p-36:1), Phosphatidylethanolamines (o-36:2), Phosphatidylethanolamines (p-36:4), Phosphatidylethanolamines (o-36:5), Phosphatidylethanolamines (p-38:4), Phosphatidylethanolamines (o-38:5), Phosphatidylethanolamines (p-38:5), Phosphatidylethanolamines (o-38:6), Phosphatidylethanolamines (p-38:6), Phosphatidylethanolamines (o-38:7), Phosphatidylethanolamines (p-40:4), Phosphatidylethanolamines (o-40:5), Phosphatidylethanolamines (p-40:5), Phosphatidylethanolamines (o-40:6), Phosphatidylethanolamines (p-40:6), Phosphatidylethanolamines (o-40:7), Phosphatidylethanolamines (p-40:7), Phosphatidylethanolamines (o-40:8), Sphingomyelin (d30:1), Sphingomyelin (d32:0), Sphingomyelin (d32:2), Sphingomyelin (d33:1), Sphingomyelin (d34:0), Sphingomyelin (d36:1), Sphingomyelin (d36:2), Sphingomyelin (d38:1), Sphingomyelin (d40:1), Sphingomyelin (d40:2), Sphingomyelin (d41:1), Sphingomyelin (d41:2), Sphingomyelin (d42:2), or B Sphingomyelin (d42:3).

39. The method of claim 1, wherein the cell-derived vesicles of the population comprise ten or more of Ceramide (d32:1), Ceramide (d33:1), Ceramide (d34:0), Ceramide (d34:1), Ceramide (d34:2), Ceramide (d34:2), Ceramide (d36:1), Ceramide (d38:1), Ceramide (d39:1), Ceramide (d40:0), Ceramide (d40:1), Ceramide (d40:2), Ceramide (d41:1), Ceramide (d42:1), Ceramide (d42:2) B, Ceramide (d44:1), Fatty Acid (20:4), Fatty Acid (22:0), Fatty Acid (22:6), Fatty Acid (24:0), Fatty Acid (24:1), glucosylceramides (d40:1), glucosylceramides (d41:1), glucosylceramides (d42:1), glucosylceramides (d42:2), Lysophosphatidylcholines (16:0), Lysophosphatidylcholines (18:0) A, Lysophosphatidylcholines (18:1), lysophosphatidylethanolamine (20:4),

Phosphatidylcholines (32:1), Phosphatidylcholines (33:1), Phosphatidylcholines (34:0), Phosphatidylcholines (34:1), Phosphatidylcholines (34:2), Phosphatidylcholines (35:2), Phosphatidylcholines (36:1), Phosphatidylcholines (36:2), Phosphatidylcholines (36:3), Phosphatidylcholines (38:2), Phosphatidylcholines (38:3), Phosphatidylcholines (38:5), Phosphatidylcholines (38:6), Phosphatidylcholines (40:5), Phosphatidylcholines (40:6), Phosphatidylcholines (40:7), Phosphatidylcholines (p-34:0), Phosphatidylcholines (o-34:1), Phosphatidylethanolamines (34:1), Phosphatidylethanolamines (34:2), Phosphatidylethanolamines (36:3), Phosphatidylethanolamines (36:4), Phosphatidylethanolamines (38:4), B Phosphatidylethanolamines (38:6), Phosphatidylethanolamines (p-34:1), Phosphatidylethanolamines (o-34:2), Phosphatidylethanolamines (p-36:1), Phosphatidylethanolamines (o-36:2), Phosphatidylethanolamines (p-36:4), Phosphatidylethanolamines (o-36:5), Phosphatidylethanolamines (p-38:4), Phosphatidylethanolamines (o-38:5), Phosphatidylethanolamines (p-38:5), Phosphatidylethanolamines (o-38:6), Phosphatidylethanolamines (p-38:6), Phosphatidylethanolamines (o-38:7), Phosphatidylethanolamines (p-40:4), Phosphatidylethanolamines (o-40:5), Phosphatidylethanolamines (p-40:5), Phosphatidylethanolamines (o-40:6), Phosphatidylethanolamines (p-40:6), Phosphatidylethanolamines (o-40:7), Phosphatidylethanolamines (p-40:7), Phosphatidylethanolamines (o-40:8), Sphingomyelin (d30:1), Sphingomyelin (d32:0), Sphingomyelin (d32:2), Sphingomyelin (d33:1), Sphingomyelin (d34:0), Sphingomyelin (d36:1), Sphingomyelin (d36:2), Sphingomyelin (d38:1), Sphingomyelin (d40:1), Sphingomyelin (d40:2), Sphingomyelin (d41:1), Sphingomyelin (d41:2), Sphingomyelin (d42:2), or B Sphingomyelin (d42:3).

40. The method of claim 1, wherein the cell-derived vesicles of the population comprise one or more of CD9, HSPA8, PDCD6IP, GAPDH, ACTB, ANXA2, CD63, SDCBP, ENO1, HSP90AA1, TSG101, PKM, LDHA, EEF1A1, YWHAZ, PGK1, EEF2, ALDOA, ANXA5, FASN, YWHAZ, CLTC, CD81, ALB, VCP, TPI1, PPIA, MSN, CFL1, PRDX1, PFN1, RAP1B, ITGB1, HSPA5, SLC3A2, GNB2, ATP1A1, WHAQ, FLOT1, FLNA, CLIC1, CDC42, CCT2, A2M, YWHAG, RAC1, LGALS3BP, HSPA1A, GNAI2, ANXA1, RHOA, MFGE8, PRDX2, GDI2, EHD4, ACTN4, YWHAB, RAB7A, LDHB, GNAS, TFRC, RAB5C, ANXA6, ANXA11, KPNB1, EZR, ANXA4, ACLY, TUBA1C, RAB14,

HIST2H4A, GNB1, UBA1, THBS1, RAN, RAB5A, PTGFRN, CCT5, CCT3, BSG, RAB5B, RAB1A, LAMP2, ITGA6, GSN, FN1, YWHAH, TKT, TCP1, STOM, SLC16A1, or RAB8A proteins.

41. The method of claim 1, wherein the cell-derived vesicles of the population comprise two or more of CD9, HSPA8, PDCD6IP, GAPDH, ACTB, ANXA2, CD63, SDCBP, ENO1, HSP90AA1, TSG101, PKM, LDHA, EEF1A1, YWHAZ, PGK1, EEF2, ALDOA, ANXA5, FASN, YWHAH, CLTC, CD81, ALB, VCP, TPI1, PPIA, MSN, CFL1, PRDX1, PFN1, RAP1B, ITGB1, HSPA5, SLC3A2, GNB2, ATP1A1, WHAQ, FLOT1, FLNA, CLIC1, CDC42, CCT2, A2M, YWHAG, RAC1, LGALS3BP, HSPA1A, GNAI2, ANXA1, RHOA, MFGE8, PRDX2, GDI2, EHD4, ACTN4, YWHAB, RAB7A, LDHB, GNAS, TFRC, RAB5C, ANXA6, ANXA11, KPNB1, EZR, ANXA4, ACLY, TUBA1C, RAB14, HIST2H4A, GNB1, UBA1, THBS1, RAN, RAB5A, PTGFRN, CCT5, CCT3, BSG, RAB5B, RAB1A, LAMP2, ITGA6, GSN, FN1, YWHAH, TKT, TCP1, STOM, SLC16A1, or RAB8A proteins.

42. The method of claim 1, wherein the cell-derived vesicles of the population comprise three or more of CD9, HSPA8, PDCD6IP, GAPDH, ACTB, ANXA2, CD63, SDCBP, ENO1, HSP90AA1, TSG101, PKM, LDHA, EEF1A1, YWHAZ, PGK1, EEF2, ALDOA, ANXA5, FASN, YWHAH, CLTC, CD81, ALB, VCP, TPI1, PPIA, MSN, CFL1, PRDX1, PFN1, RAP1B, ITGB1, HSPA5, SLC3A2, GNB2, ATP1A1, WHAQ, FLOT1, FLNA, CLIC1, CDC42, CCT2, A2M, YWHAG, RAC1, LGALS3BP, HSPA1A, GNAI2, ANXA1, RHOA, MFGE8, PRDX2, GDI2, EHD4, ACTN4, YWHAB, RAB7A, LDHB, GNAS, TFRC, RAB5C, ANXA6, ANXA11, KPNB1, EZR, ANXA4, ACLY, TUBA1C, RAB14, HIST2H4A, GNB1, UBA1, THBS1, RAN, RAB5A, PTGFRN, CCT5, CCT3, BSG, RAB5B, RAB1A, LAMP2, ITGA6, GSN, FN1, YWHAH, TKT, TCP1, STOM, SLC16A1, or RAB8A proteins.

43. The method of claim 1, wherein the cell-derived vesicles of the population comprise four or more of CD9, HSPA8, PDCD6IP, GAPDH, ACTB, ANXA2, CD63, SDCBP, ENO1, HSP90AA1, TSG101, PKM, LDHA, EEF1A1, YWHAZ, PGK1, EEF2, ALDOA, ANXA5, FASN, YWHAH, CLTC, CD81, ALB, VCP, TPI1, PPIA, MSN, CFL1, PRDX1, PFN1, RAP1B, ITGB1, HSPA5, SLC3A2, GNB2, ATP1A1, WHAQ, FLOT1, FLNA, CLIC1,

CDC42, CCT2, A2M, YWHAG, RAC1, LGALS3BP, HSPA1A, GNAI2, ANXA1, RHOA, MFGE8, PRDX2, GDI2, EHD4, ACTN4, YWHAB, RAB7A, LDHB, GNAS, TFRC, RAB5C, ANXA6, ANXA11, KPNB1, EZR, ANXA4, ACLY, TUBA1C, RAB14, HIST2H4A, GNB1, UBA1, THBS1, RAN, RAB5A, PTGFRN, CCT5, CCT3, BSG, RAB5B, RAB1A, LAMP2, ITGA6, GSN, FN1, YWHAH, TKT, TCP1, STOM, SLC16A1, or RAB8A proteins.

44. The method of claim 1, wherein the cell-derived vesicles of the population comprise five or more of CD9, HSPA8, PDCD6IP, GAPDH, ACTB, ANXA2, CD63, SDCBP, ENO1, HSP90AA1, TSG101, PKM, LDHA, EEF1A1, YWHAZ, PGK1, EEF2, ALDOA, ANXA5, FASN, YWHAH, CLTC, CD81, ALB, VCP, TPI1, PPIA, MSN, CFL1, PRDX1, PFN1, RAP1B, ITGB1, HSPA5, SLC3A2, GNB2, ATP1A1, WHAQ, FLOT1, FLNA, CLIC1, CDC42, CCT2, A2M, YWHAG, RAC1, LGALS3BP, HSPA1A, GNAI2, ANXA1, RHOA, MFGE8, PRDX2, GDI2, EHD4, ACTN4, YWHAB, RAB7A, LDHB, GNAS, TFRC, RAB5C, ANXA6, ANXA11, KPNB1, EZR, ANXA4, ACLY, TUBA1C, RAB14, HIST2H4A, GNB1, UBA1, THBS1, RAN, RAB5A, PTGFRN, CCT5, CCT3, BSG, RAB5B, RAB1A, LAMP2, ITGA6, GSN, FN1, YWHAH, TKT, TCP1, STOM, SLC16A1, or RAB8A proteins.

45. The method of claim 1, wherein the cell-derived vesicles of the population comprise six or more of CD9, HSPA8, PDCD6IP, GAPDH, ACTB, ANXA2, CD63, SDCBP, ENO1, HSP90AA1, TSG101, PKM, LDHA, EEF1A1, YWHAZ, PGK1, EEF2, ALDOA, ANXA5, FASN, YWHAH, CLTC, CD81, ALB, VCP, TPI1, PPIA, MSN, CFL1, PRDX1, PFN1, RAP1B, ITGB1, HSPA5, SLC3A2, GNB2, ATP1A1, WHAQ, FLOT1, FLNA, CLIC1, CDC42, CCT2, A2M, YWHAG, RAC1, LGALS3BP, HSPA1A, GNAI2, ANXA1, RHOA, MFGE8, PRDX2, GDI2, EHD4, ACTN4, YWHAB, RAB7A, LDHB, GNAS, TFRC, RAB5C, ANXA6, ANXA11, KPNB1, EZR, ANXA4, ACLY, TUBA1C, RAB14, HIST2H4A, GNB1, UBA1, THBS1, RAN, RAB5A, PTGFRN, CCT5, CCT3, BSG, RAB5B, RAB1A, LAMP2, ITGA6, GSN, FN1, YWHAH, TKT, TCP1, STOM, SLC16A1, or RAB8A proteins.

46. The method of claim 1, wherein the cell-derived vesicles of the population comprise seven or more of CD9, HSPA8, PDCD6IP, GAPDH, ACTB, ANXA2, CD63, SDCBP,

ENO1, HSP90AA1, TSG101, PKM, LDHA, EEF1A1, YWHAZ, PGK1, EEF2, ALDOA, ANXA5, FASN, YWHAЕ, CLTC, CD81, ALB, VCP, TPI1, PPIA, MSN, CFL1, PRDX1, PFN1, RAP1B, ITGB1, HSPA5, SLC3A2, GNB2, ATP1A1, WHAQ, FLOT1, FLNA, CLIC1, CDC42, CCT2, A2M, YWHAG, RAC1, LGALS3BP, HSPA1A, GNAI2, ANXA1, RHOA, MFGE8, PRDX2, GDI2, EHD4, ACTN4, YWHAB, RAB7A, LDHB, GNAS, TFRC, RAB5C, ANXA6, ANXA11, KPNB1, EZR, ANXA4, ACLY, TUBA1C, RAB14, HIST2H4A, GNB1, UBA1, THBS1, RAN, RAB5A, PTGFRN, CCT5, CCT3, BSG, RAB5B, RAB1A, LAMP2, ITGA6, GSN, FN1, YWHAH, TKT, TCP1, STOM, SLC16A1, or RAB8A proteins.

47. The method of claim 1, wherein the cell-derived vesicles of the population comprise eight or more of CD9, HSPA8, PDCD6IP, GAPDH, ACTB, ANXA2, CD63, SDCBP, ENO1, HSP90AA1, TSG101, PKM, LDHA, EEF1A1, YWHAZ, PGK1, EEF2, ALDOA, ANXA5, FASN, YWHAЕ, CLTC, CD81, ALB, VCP, TPI1, PPIA, MSN, CFL1, PRDX1, PFN1, RAP1B, ITGB1, HSPA5, SLC3A2, GNB2, ATP1A1, WHAQ, FLOT1, FLNA, CLIC1, CDC42, CCT2, A2M, YWHAG, RAC1, LGALS3BP, HSPA1A, GNAI2, ANXA1, RHOA, MFGE8, PRDX2, GDI2, EHD4, ACTN4, YWHAB, RAB7A, LDHB, GNAS, TFRC, RAB5C, ANXA6, ANXA11, KPNB1, EZR, ANXA4, ACLY, TUBA1C, RAB14, HIST2H4A, GNB1, UBA1, THBS1, RAN, RAB5A, PTGFRN, CCT5, CCT3, BSG, RAB5B, RAB1A, LAMP2, ITGA6, GSN, FN1, YWHAH, TKT, TCP1, STOM, SLC16A1, or RAB8A proteins.

48. The method of claim 1, wherein the cell-derived vesicles of the population comprise nine or more of CD9, HSPA8, PDCD6IP, GAPDH, ACTB, ANXA2, CD63, SDCBP, ENO1, HSP90AA1, TSG101, PKM, LDHA, EEF1A1, YWHAZ, PGK1, EEF2, ALDOA, ANXA5, FASN, YWHAЕ, CLTC, CD81, ALB, VCP, TPI1, PPIA, MSN, CFL1, PRDX1, PFN1, RAP1B, ITGB1, HSPA5, SLC3A2, GNB2, ATP1A1, WHAQ, FLOT1, FLNA, CLIC1, CDC42, CCT2, A2M, YWHAG, RAC1, LGALS3BP, HSPA1A, GNAI2, ANXA1, RHOA, MFGE8, PRDX2, GDI2, EHD4, ACTN4, YWHAB, RAB7A, LDHB, GNAS, TFRC, RAB5C, ANXA6, ANXA11, KPNB1, EZR, ANXA4, ACLY, TUBA1C, RAB14, HIST2H4A, GNB1, UBA1, THBS1, RAN, RAB5A, PTGFRN, CCT5, CCT3, BSG, RAB5B, RAB1A, LAMP2, ITGA6, GSN, FN1, YWHAH, TKT, TCP1, STOM, SLC16A1, or RAB8A proteins.

49. The method of claim 1, wherein the cell-derived vesicles of the population comprise ten or more of CD9, HSPA8, PDCD6IP, GAPDH, ACTB, ANXA2, CD63, SDCBP, ENO1, HSP90AA1, TSG101, PKM, LDHA, EEF1A1, YWHAZ, PGK1, EEF2, ALDOA, ANXA5, FASN, YWHAE, CLTC, CD81, ALB, VCP, TPI1, PPIA, MSN, CFL1, PRDX1, PFN1, RAP1B, ITGB1, HSPA5, SLC3A2, GNB2, ATP1A1, WHAQ, FLOT1, FLNA, CLIC1, CDC42, CCT2, A2M, YWHAG, RAC1, LGALS3BP, HSPA1A, GNAI2, ANXA1, RHOA, MFGE8, PRDX2, GDI2, EHD4, ACTN4, YWHAB, RAB7A, LDHB, GNAS, TFRC, RAB5C, ANXA6, ANXA11, KPNB1, EZR, ANXA4, ACLY, TUBA1C, RAB14, HIST2H4A, GNB1, UBA1, THBS1, RAN, RAB5A, PTGFRN, CCT5, CCT3, BSG, RAB5B, RAB1A, LAMP2, ITGA6, GSN, FN1, YWHAH, TKT, TCP1, STOM, SLC16A1, or RAB8A proteins.

50. The method of claim 1, wherein the cell-derived vesicles of the population comprise one or more of FN1, EDIL3, TF, ITGB1, VCAN, ANXA2, MFGE8, TGB1, TGFB2, TGFR1, TGBFR2, TGFBI, TGFBRAP1, BASP1, COL1, COL6, GAPDH, ITGA3, FBN1, ITGAV, ITGB5, NOTCH2, SDCBP, HSPA2, HSPA8, NT5E, MRGPRF, RTN4, NEFM, INA, NRP1, HSPA9, FBN1, BSG, PRPH, FBLN1, PARP4, FLNA, YBX1, EVA1B, ADAM10, HSPG2, MCAM, POSTN, GNB2, GNB1, ANPEP, ADAM9, ATP1A1, CSPG4, EHD2, PXDN, SERPINE2, CAV1, PKM, GNB4, NPTN, CCT2, LGALS3BP, or MVP proteins.

51. The method of claim 1, wherein the cell-derived vesicles of the population comprise two or more of FN1, EDIL3, TF, ITGB1, VCAN, ANXA2, MFGE8, TGB1, TGFB2, TGFR1, TGBFR2, TGFBI, TGFBRAP1, BASP1, COL1, COL6, GAPDH, ITGA3, FBN1, ITGAV, ITGB5, NOTCH2, SDCBP, HSPA2, HSPA8, NT5E, MRGPRF, RTN4, NEFM, INA, NRP1, HSPA9, FBN1, BSG, PRPH, FBLN1, PARP4, FLNA, YBX1, EVA1B, ADAM10, HSPG2, MCAM, POSTN, GNB2, GNB1, ANPEP, ADAM9, ATP1A1, CSPG4, EHD2, PXDN, SERPINE2, CAV1, PKM, GNB4, NPTN, CCT2, LGALS3BP, or MVP proteins.

52. The method of claim 1, wherein the cell-derived vesicles of the population comprise three or more of FN1, EDIL3, TF, ITGB1, VCAN, ANXA2, MFGE8, TGB1, TGFB2, TGFR1, TGBFR2, TGFBI, TGFBRAP1, BASP1, COL1, COL6, GAPDH, ITGA3, FBN1,

ITGAV, ITGB5, NOTCH2, SDCBP, HSPA2, HSPA8, NT5E, MRGPRF, RTN4, NEFM, INA, NRP1, HSPA9, FBN1, BSG, PRPH, FBLN1, PARP4, FLNA, YBX1, EVA1B, ADAM10, HSPG2, MCAM, POSTN, GNB2, GNB1, ANPEP, ADAM9, ATP1A1, CSPG4, EHD2, PXDN, SERPINE2, CAV1, PKM, GNB4, NPTN, CCT2, LGALS3BP, or MVP proteins.

53. The method of claim 1, wherein the cell-derived vesicles of the population comprise four or more of FN1, EDIL3, TF, ITGB1, VCAN, ANXA2, MFGE8, TGB1, TGFB2, TGFR1, TGBR2, TGFBI, TGFBRAP1, BASP1, COL1, COL6, GAPDH, ITGA3, FBN1, ITGAV, ITGB5, NOTCH2, SDCBP, HSPA2, HSPA8, NT5E, MRGPRF, RTN4, NEFM, INA, NRP1, HSPA9, FBN1, BSG, PRPH, FBLN1, PARP4, FLNA, YBX1, EVA1B, ADAM10, HSPG2, MCAM, POSTN, GNB2, GNB1, ANPEP, ADAM9, ATP1A1, CSPG4, EHD2, PXDN, SERPINE2, CAV1, PKM, GNB4, NPTN, CCT2, LGALS3BP, or MVP proteins.

54. The method of claim 1, wherein the cell-derived vesicles of the population comprise five or more of FN1, EDIL3, TF, ITGB1, VCAN, ANXA2, MFGE8, TGB1, TGFB2, TGFR1, TGBR2, TGFBI, TGFBRAP1, BASP1, COL1, COL6, GAPDH, ITGA3, FBN1, ITGAV, ITGB5, NOTCH2, SDCBP, HSPA2, HSPA8, NT5E, MRGPRF, RTN4, NEFM, INA, NRP1, HSPA9, FBN1, BSG, PRPH, FBLN1, PARP4, FLNA, YBX1, EVA1B, ADAM10, HSPG2, MCAM, POSTN, GNB2, GNB1, ANPEP, ADAM9, ATP1A1, CSPG4, EHD2, PXDN, SERPINE2, CAV1, PKM, GNB4, NPTN, CCT2, LGALS3BP, or MVP proteins.

55. The method of claim 1, wherein the cell-derived vesicles of the population comprise six or more of FN1, EDIL3, TF, ITGB1, VCAN, ANXA2, MFGE8, TGB1, TGFB2, TGFR1, TGBR2, TGFBI, TGFBRAP1, BASP1, COL1, COL6, GAPDH, ITGA3, FBN1, ITGAV, ITGB5, NOTCH2, SDCBP, HSPA2, HSPA8, NT5E, MRGPRF, RTN4, NEFM, INA, NRP1, HSPA9, FBN1, BSG, PRPH, FBLN1, PARP4, FLNA, YBX1, EVA1B, ADAM10, HSPG2, MCAM, POSTN, GNB2, GNB1, ANPEP, ADAM9, ATP1A1, CSPG4, EHD2, PXDN, SERPINE2, CAV1, PKM, GNB4, NPTN, CCT2, LGALS3BP, or MVP proteins.

56. The method of claim 1, wherein the cell-derived vesicles of the population comprise seven or more of FN1, EDIL3, TF, ITGB1, VCAN, ANXA2, MFGE8, TGB1, TGFB2, TGFB1, TGBFR2, TGFBI, TGFBRAP1, BASP1, COL1, COL6, GAPDH, ITGA3, FBN1, ITGAV, ITGB5, NOTCH2, SDCBP, HSPA2, HSPA8, NT5E, MRGPRF, RTN4, NEFM, INA, NRP1, HSPA9, FBN1, BSG, PRPH, FBLN1, PARP4, FLNA, YBX1, EVA1B, ADAM10, HSPG2, MCAM, POSTN, GNB2, GNB1, ANPEP, ADAM9, ATP1A1, CSPG4, EHD2, PXDN, SERPINE2, CAV1, PKM, GNB4, NPTN, CCT2, LGALS3BP, or MVP proteins.

57. The method of claim 1, wherein the cell-derived vesicles of the population comprise eight or more of FN1, EDIL3, TF, ITGB1, VCAN, ANXA2, MFGE8, TGB1, TGFB2, TGFB1, TGBFR2, TGFBI, TGFBRAP1, BASP1, COL1, COL6, GAPDH, ITGA3, FBN1, ITGAV, ITGB5, NOTCH2, SDCBP, HSPA2, HSPA8, NT5E, MRGPRF, RTN4, NEFM, INA, NRP1, HSPA9, FBN1, BSG, PRPH, FBLN1, PARP4, FLNA, YBX1, EVA1B, ADAM10, HSPG2, MCAM, POSTN, GNB2, GNB1, ANPEP, ADAM9, ATP1A1, CSPG4, EHD2, PXDN, SERPINE2, CAV1, PKM, GNB4, NPTN, CCT2, LGALS3BP, or MVP proteins.

58. The method of claim 1, wherein the cell-derived vesicles of the population comprise nine or more of FN1, EDIL3, TF, ITGB1, VCAN, ANXA2, MFGE8, TGB1, TGFB2, TGFB1, TGBFR2, TGFBI, TGFBRAP1, BASP1, COL1, COL6, GAPDH, ITGA3, FBN1, ITGAV, ITGB5, NOTCH2, SDCBP, HSPA2, HSPA8, NT5E, MRGPRF, RTN4, NEFM, INA, NRP1, HSPA9, FBN1, BSG, PRPH, FBLN1, PARP4, FLNA, YBX1, EVA1B, ADAM10, HSPG2, MCAM, POSTN, GNB2, GNB1, ANPEP, ADAM9, ATP1A1, CSPG4, EHD2, PXDN, SERPINE2, CAV1, PKM, GNB4, NPTN, CCT2, LGALS3BP, or MVP proteins.

59. The method of claim 1, wherein the cell-derived vesicles of the population comprise ten or more of FN1, EDIL3, TF, ITGB1, VCAN, ANXA2, MFGE8, TGB1, TGFB2, TGFB1, TGBFR2, TGFBI, TGFBRAP1, BASP1, COL1, COL6, GAPDH, ITGA3, FBN1, ITGAV, ITGB5, NOTCH2, SDCBP, HSPA2, HSPA8, NT5E, MRGPRF, RTN4, NEFM, INA, NRP1, HSPA9, FBN1, BSG, PRPH, FBLN1, PARP4, FLNA, YBX1, EVA1B, ADAM10, HSPG2, MCAM, POSTN, GNB2, GNB1, ANPEP, ADAM9, ATP1A1, CSPG4,

EHD2, PXDN, SERPINE2, CAV1, PKM, GNB4, NPTN, CCT2, LGALS3BP, or MVP proteins.

60. The method of claim 1, wherein the cell-derived vesicles of the population comprise one or more of FBLN2, TIMP1, NID1, IGFBP3, LTBP1, DUSP3, ITGAV, LAMA5, COL1A1, NOTCH2, NRG1, ERBB2, COL4A2, LDLR, TSB, MMP2, TIMP2, TPI1, ACVR1B, INHBA, EGFR, APH1A, NCSTN, TGFB2, SPARC, TGFB1, F2, SERPINE1, SDC4, SDC3, ACAN, IFI16, MMP14, PLAT, COL18A1, NOTCH3, DSP, PKP4, SERPINE2, SRGN, NRP2, EPHA2, ITGA5, NRP1, PLAUR, SERPINB6, CLEC3B, CD47, SDC1, PSMA7, ENG, S100A13, TIMP3, TMED10, TGFBI, CTGF, DCN, ITGB3, PDGFRA, JAG1, TGFBR2, PLAUR, PDGFRB, FYN, THY1, HSPG2, TENC1, TGFBR1, PLXNA1, LRP1, STAT1, CXCL12, VCAN, MET, FN1, CD36, STAT3, THBS1, FGFR1, GRB14, FGB, API5, HAPLN1, RECK, LAMC1, CYR61, GPC1, IGFBP4, ITGA4, MFAP2, SDC2, EFNB2, FGA, PLXND1, ADAM17, ADAM9, ANPEP, EPHB1, PPP2R5D, ANTXR2, IGFBP7, COL6A3, LAMB3, ADAMTS1, ADAM10, A2M, EFNB1, ITGA3, CLU, KHSRP, or EFEMP1 proteins associated with angiogenesis.

61. The method of claim 1, wherein the cell-derived vesicles of the population comprise two or more of FBLN2, TIMP1, NID1, IGFBP3, LTBP1, DUSP3, ITGAV, LAMA5, COL1A1, NOTCH2, NRG1, ERBB2, COL4A2, LDLR, TSB, MMP2, TIMP2, TPI1, ACVR1B, INHBA, EGFR, APH1A, NCSTN, TGFB2, SPARC, TGFB1, F2, SERPINE1, SDC4, SDC3, ACAN, IFI16, MMP14, PLAT, COL18A1, NOTCH3, DSP, PKP4, SERPINE2, SRGN, NRP2, EPHA2, ITGA5, NRP1, PLAUR, SERPINB6, CLEC3B, CD47, SDC1, PSMA7, ENG, S100A13, TIMP3, TMED10, TGFBI, CTGF, DCN, ITGB3, PDGFRA, JAG1, TGFBR2, PLAUR, PDGFRB, FYN, THY1, HSPG2, TENC1, TGFBR1, PLXNA1, LRP1, STAT1, CXCL12, VCAN, MET, FN1, CD36, STAT3, THBS1, FGFR1, GRB14, FGB, API5, HAPLN1, RECK, LAMC1, CYR61, GPC1, IGFBP4, ITGA4, MFAP2, SDC2, EFNB2, FGA, PLXND1, ADAM17, ADAM9, ANPEP, EPHB1, PPP2R5D, ANTXR2, IGFBP7, COL6A3, LAMB3, ADAMTS1, ADAM10, A2M, EFNB1, ITGA3, CLU, KHSRP, or EFEMP1 proteins associated with angiogenesis.

62. The method of claim 1, wherein the cell-derived vesicles of the population comprise three or more of FBLN2, TIMP1, NID1, IGFBP3, LTBP1, DUSP3, ITGAV, LAMA5,

COL1A1, NOTCH2, NRG1, ERBB2, COL4A2, LDLR, TSB, MMP2, TIMP2, TPI1, ACVR1B, INHBA, EGFR, APH1A, NCSTN, TGFB2, SPARC, TGFB1, F2, SERPINE1, SDC4, SDC3, ACAN, IFI16, MMP14, PLAT, COL18A1, NOTCH3, DSP, PKP4, SERPINE2, SRGN, NRP2, EPHA2, ITGA5, NRP1, PLAUI, SERPINB6, CLEC3B, CD47, SDC1, PSMA7, ENG, S100A13, TIMP3, TMED10, TGFBI, CTGF, DCN, ITGB3, PDGFRA, JAG1, TGFB2, PLAUR, PDGFRB, FYN, THY1, HSPG2, TENC1, TGFB1, PLXNA1, LRP1, STAT1, CXCL12, VCAN, MET, FN1, CD36, STAT3, THBS1, FGFR1, GRB14, FGB, API5, HAPLN1, RECK, LAMC1, CYR61, GPC1, IGFBP4, ITGA4, MFAP2, SDC2, EFNB2, FGA, PLXND1, ADAM17, ADAM9, ANPEP, EPHB1, PPP2R5D, ANTXR2, IGFBP7, COL6A3, LAMB3, ADAMTS1, ADAM10, A2M, EFNB1, ITGA3, CLU, KHSRP, or EFEMP1 proteins associated with angiogenesis.

63. The method of claim 1, wherein the cell-derived vesicles of the population comprise four or more of FBLN2, TIMP1, NID1, IGFBP3, LTBP1, DUSP3, ITGAV, LAMA5, COL1A1, NOTCH2, NRG1, ERBB2, COL4A2, LDLR, TSB, MMP2, TIMP2, TPI1, ACVR1B, INHBA, EGFR, APH1A, NCSTN, TGFB2, SPARC, TGFB1, F2, SERPINE1, SDC4, SDC3, ACAN, IFI16, MMP14, PLAT, COL18A1, NOTCH3, DSP, PKP4, SERPINE2, SRGN, NRP2, EPHA2, ITGA5, NRP1, PLAUI, SERPINB6, CLEC3B, CD47, SDC1, PSMA7, ENG, S100A13, TIMP3, TMED10, TGFBI, CTGF, DCN, ITGB3, PDGFRA, JAG1, TGFB2, PLAUR, PDGFRB, FYN, THY1, HSPG2, TENC1, TGFB1, PLXNA1, LRP1, STAT1, CXCL12, VCAN, MET, FN1, CD36, STAT3, THBS1, FGFR1, GRB14, FGB, API5, HAPLN1, RECK, LAMC1, CYR61, GPC1, IGFBP4, ITGA4, MFAP2, SDC2, EFNB2, FGA, PLXND1, ADAM17, ADAM9, ANPEP, EPHB1, PPP2R5D, ANTXR2, IGFBP7, COL6A3, LAMB3, ADAMTS1, ADAM10, A2M, EFNB1, ITGA3, CLU, KHSRP, or EFEMP1 proteins associated with angiogenesis.

64. The method of claim 1, wherein the cell-derived vesicles of the population comprise five or more of FBLN2, TIMP1, NID1, IGFBP3, LTBP1, DUSP3, ITGAV, LAMA5, COL1A1, NOTCH2, NRG1, ERBB2, COL4A2, LDLR, TSB, MMP2, TIMP2, TPI1, ACVR1B, INHBA, EGFR, APH1A, NCSTN, TGFB2, SPARC, TGFB1, F2, SERPINE1, SDC4, SDC3, ACAN, IFI16, MMP14, PLAT, COL18A1, NOTCH3, DSP, PKP4, SERPINE2, SRGN, NRP2, EPHA2, ITGA5, NRP1, PLAUI, SERPINB6, CLEC3B, CD47, SDC1, PSMA7, ENG, S100A13, TIMP3, TMED10, TGFBI, CTGF, DCN, ITGB3,

PDGFRA, JAG1, TGFBR2, PLAUR, PDGFRB, FYN, THY1, HSPG2, TENC1, TGFBR1, PLXNA1, LRP1, STAT1, CXCL12, VCAN, MET, FN1, CD36, STAT3, THBS1, FGFR1, GRB14, FGB, API5, HAPLN1, RECK, LAMC1, CYR61, GPC1, IGFBP4, ITGA4, MFAP2, SDC2, EFNB2, FGA, PLXND1, ADAM17, ADAM9, ANPEP, EPHB1, PPP2R5D, ANT XR2, IGFBP7, COL6A3, LAMB3, ADAMTS1, ADAM10, A2M, EFNB1, ITGA3, CLU, KHSRP, or EFEMP1 proteins associated with angiogenesis.

65. The method of claim 1, wherein the cell-derived vesicles of the population comprise six or more of FBLN2, TIMP1, NID1, IGFBP3, LTBP1, DUSP3, ITGAV, LAMA5, COL1A1, NOTCH2, NRG1, ERBB2, COL4A2, LDLR, TSB, MMP2, TIMP2, TPI1, ACVR1B, INHBA, EGFR, APH1A, NCSTN, TGFB2, SPARC, TGFB1, F2, SERPINE1, SDC4, SDC3, ACAN, IFI16, MMP14, PLAT, COL18A1, NOTCH3, DSP, PKP4, SERPINE2, SRGN, NRP2, EPHA2, ITGA5, NRP1, PLAUI, SERPINB6, CLEC3B, CD47, SDC1, PSMA7, ENG, S100A13, TIMP3, TMED10, TGFB1, CTGF, DCN, ITGB3, PDGFRA, JAG1, TGFBR2, PLAUR, PDGFRB, FYN, THY1, HSPG2, TENC1, TGFBR1, PLXNA1, LRP1, STAT1, CXCL12, VCAN, MET, FN1, CD36, STAT3, THBS1, FGFR1, GRB14, FGB, API5, HAPLN1, RECK, LAMC1, CYR61, GPC1, IGFBP4, ITGA4, MFAP2, SDC2, EFNB2, FGA, PLXND1, ADAM17, ADAM9, ANPEP, EPHB1, PPP2R5D, ANT XR2, IGFBP7, COL6A3, LAMB3, ADAMTS1, ADAM10, A2M, EFNB1, ITGA3, CLU, KHSRP, or EFEMP1 proteins associated with angiogenesis.

66. The method of claim 1, wherein the cell-derived vesicles of the population comprise seven or more of FBLN2, TIMP1, NID1, IGFBP3, LTBP1, DUSP3, ITGAV, LAMA5, COL1A1, NOTCH2, NRG1, ERBB2, COL4A2, LDLR, TSB, MMP2, TIMP2, TPI1, ACVR1B, INHBA, EGFR, APH1A, NCSTN, TGFB2, SPARC, TGFB1, F2, SERPINE1, SDC4, SDC3, ACAN, IFI16, MMP14, PLAT, COL18A1, NOTCH3, DSP, PKP4, SERPINE2, SRGN, NRP2, EPHA2, ITGA5, NRP1, PLAUI, SERPINB6, CLEC3B, CD47, SDC1, PSMA7, ENG, S100A13, TIMP3, TMED10, TGFB1, CTGF, DCN, ITGB3, PDGFRA, JAG1, TGFBR2, PLAUR, PDGFRB, FYN, THY1, HSPG2, TENC1, TGFBR1, PLXNA1, LRP1, STAT1, CXCL12, VCAN, MET, FN1, CD36, STAT3, THBS1, FGFR1, GRB14, FGB, API5, HAPLN1, RECK, LAMC1, CYR61, GPC1, IGFBP4, ITGA4, MFAP2, SDC2, EFNB2, FGA, PLXND1, ADAM17, ADAM9, ANPEP, EPHB1, PPP2R5D,

ANTXR2, IGFBP7, COL6A3, LAMB3, ADAMTS1, ADAM10, A2M, EFNB1, ITGA3, CLU, KHSRP, or EFEMP1 proteins associated with angiogenesis.

67. The method of claim 1, wherein the cell-derived vesicles of the population comprise eight or more of FBLN2, TIMP1, NID1, IGFBP3, LTBP1, DUSP3, ITGAV, LAMA5, COL1A1, NOTCH2, NRG1, ERBB2, COL4A2, LDLR, TSB, MMP2, TIMP2, TPI1, ACVR1B, INHBA, EGFR, APH1A, NCSTN, TGFB2, SPARC, TGFB1, F2, SERPINE1, SDC4, SDC3, ACAN, IFI16, MMP14, PLAT, COL18A1, NOTCH3, DSP, PKP4, SERPINE2, SRGN, NRP2, EPHA2, ITGA5, NRP1, PLAUR, SERPINB6, CLEC3B, CD47, SDC1, PSMA7, ENG, S100A13, TIMP3, TMED10, TGFBI, CTGF, DCN, ITGB3, PDGFRA, JAG1, TGFBR2, PLAUR, PDGFRB, FYN, THY1, HSPG2, TENC1, TGFBR1, PLXNA1, LRP1, STAT1, CXCL12, VCAN, MET, FN1, CD36, STAT3, THBS1, FGFR1, GRB14, FGB, API5, HAPLN1, RECK, LAMC1, CYR61, GPC1, IGFBP4, ITGA4, MFAP2, SDC2, EFNB2, FGA, PLXND1, ADAM17, ADAM9, ANPEP, EPHB1, PPP2R5D, ANTXR2, IGFBP7, COL6A3, LAMB3, ADAMTS1, ADAM10, A2M, EFNB1, ITGA3, CLU, KHSRP, or EFEMP1 proteins associated with angiogenesis.

68. The method of claim 1, wherein the cell-derived vesicles of the population comprise nine or more of FBLN2, TIMP1, NID1, IGFBP3, LTBP1, DUSP3, ITGAV, LAMA5, COL1A1, NOTCH2, NRG1, ERBB2, COL4A2, LDLR, TSB, MMP2, TIMP2, TPI1, ACVR1B, INHBA, EGFR, APH1A, NCSTN, TGFB2, SPARC, TGFB1, F2, SERPINE1, SDC4, SDC3, ACAN, IFI16, MMP14, PLAT, COL18A1, NOTCH3, DSP, PKP4, SERPINE2, SRGN, NRP2, EPHA2, ITGA5, NRP1, PLAUR, SERPINB6, CLEC3B, CD47, SDC1, PSMA7, ENG, S100A13, TIMP3, TMED10, TGFBI, CTGF, DCN, ITGB3, PDGFRA, JAG1, TGFBR2, PLAUR, PDGFRB, FYN, THY1, HSPG2, TENC1, TGFBR1, PLXNA1, LRP1, STAT1, CXCL12, VCAN, MET, FN1, CD36, STAT3, THBS1, FGFR1, GRB14, FGB, API5, HAPLN1, RECK, LAMC1, CYR61, GPC1, IGFBP4, ITGA4, MFAP2, SDC2, EFNB2, FGA, PLXND1, ADAM17, ADAM9, ANPEP, EPHB1, PPP2R5D, ANTXR2, IGFBP7, COL6A3, LAMB3, ADAMTS1, ADAM10, A2M, EFNB1, ITGA3, CLU, KHSRP, or EFEMP1 proteins associated with angiogenesis.

69. The method of claim 1, wherein the cell-derived vesicles of the population comprise ten or more of FBLN2, TIMP1, NID1, IGFBP3, LTBP1, DUSP3, ITGAV, LAMA5,

COL1A1, NOTCH2, NRG1, ERBB2, COL4A2, LDLR, TSB, MMP2, TIMP2, TPI1, ACVR1B, INHBA, EGFR, APH1A, NCSTN, TGFB2, SPARC, TGFB1, F2, SERPINE1, SDC4, SDC3, ACAN, IFI16, MMP14, PLAT, COL18A1, NOTCH3, DSP, PKP4, SERPINE2, SRGN, NRP2, EPHA2, ITGA5, NRP1, PLAUI, SERPINB6, CLEC3B, CD47, SDC1, PSMA7, ENG, S100A13, TIMP3, TMED10, TGFBI, CTGF, DCN, ITGB3, PDGFRA, JAG1, TGFB2, PLAUR, PDGFRB, FYN, THY1, HSPG2, TENC1, TGFB1, PLXNA1, LRP1, STAT1, CXCL12, VCAN, MET, FN1, CD36, STAT3, THBS1, FGFR1, GRB14, FGB, API5, HAPLN1, RECK, LAMC1, CYR61, GPC1, IGFBP4, ITGA4, MFAP2, SDC2, EFNB2, FGA, PLXND1, ADAM17, ADAM9, ANPEP, EPHB1, PPP2R5D, ANTXR2, IGFBP7, COL6A3, LAMB3, ADAMTS1, ADAM10, A2M, EFNB1, ITGA3, CLU, KHSRP, or EFEMP1 proteins associated with angiogenesis.

70. The method of claim 1, wherein the cell-derived vesicles of the population comprise one or more of TGFBI, TGFB1, TGFB2, TGFB1, TGFB2, TGFB1, TGFB2, TGFB1, ADAM17, ARG1, CD274, EIF2A, EPHB2, HLA-DRA, ELAVL1, IRAK1, LGALS1, PSME4, STAT1, or STAT3 proteins associated with immune modulation.

71. The method of claim 1, wherein the cell-derived vesicles of the population comprise two or more of TGFBI, TGFB1, TGFB2, TGFB1, TGFB2, TGFB1, ADAM17, ARG1, CD274, EIF2A, EPHB2, HLA-DRA, ELAVL1, IRAK1, LGALS1, PSME4, STAT1, or STAT3 proteins associated with immune modulation.

72. The method of claim 1, wherein the cell-derived vesicles of the population comprise three or more of TGFBI, TGFB1, TGFB2, TGFB1, TGFB2, TGFB1, ADAM17, ARG1, CD274, EIF2A, EPHB2, HLA-DRA, ELAVL1, IRAK1, LGALS1, PSME4, STAT1, or STAT3 proteins associated with immune modulation.

73. The method of claim 1, wherein the cell-derived vesicles of the population comprise four or more of TGFBI, TGFB1, TGFB2, TGFB1, TGFB2, TGFB1, ADAM17, ARG1, CD274, EIF2A, EPHB2, HLA-DRA, ELAVL1, IRAK1, LGALS1, PSME4, STAT1, or STAT3 proteins associated with immune modulation.

74. The method of claim 1, wherein the cell-derived vesicles of the population comprise five or more of TGFBI, TGFB1, TGFB2, TGFB1, TGFB2, TGFB1, ADAM17,

ARG1, CD274, EIF2A, EPHB2, HLA-DRA, ELAVL1, IRAK1, LGALS1, PSME4, STAT1, or STAT3 proteins associated with immune modulation.

75. The method of claim 1, wherein the cell-derived vesicles of the population comprise six or more of TGFBI, TGFB1, TGFBR2, TGFBR1, TGFB2, TGFBRAP1, ADAM17, ARG1, CD274, EIF2A, EPHB2, HLA-DRA, ELAVL1, IRAK1, LGALS1, PSME4, STAT1, or STAT3 proteins associated with immune modulation.

76. The method of claim 1, wherein the cell-derived vesicles of the population comprise seven or more of TGFBI, TGFB1, TGFB2, TGFB3, TGFB4, TGFB5, TGFB6, TGFB7, TGFB8, TGFB9, TGFB10, TGFB11, TGFB12, TGFB13, TGFB14, TGFB15, TGFB16, TGFB17, TGFB18, TGFB19, TGFB20, TGFB21, TGFB22, TGFB23, TGFB24, TGFB25, TGFB26, TGFB27, TGFB28, TGFB29, TGFB30, TGFB31, TGFB32, TGFB33, TGFB34, TGFB35, TGFB36, TGFB37, TGFB38, TGFB39, TGFB40, TGFB41, TGFB42, TGFB43, TGFB44, TGFB45, TGFB46, TGFB47, TGFB48, TGFB49, TGFB50, TGFB51, TGFB52, TGFB53, TGFB54, TGFB55, TGFB56, TGFB57, TGFB58, TGFB59, TGFB60, TGFB61, TGFB62, TGFB63, TGFB64, TGFB65, TGFB66, TGFB67, TGFB68, TGFB69, TGFB70, TGFB71, TGFB72, TGFB73, TGFB74, TGFB75, TGFB76, TGFB77, TGFB78, TGFB79, TGFB80, TGFB81, TGFB82, TGFB83, TGFB84, TGFB85, TGFB86, TGFB87, TGFB88, TGFB89, TGFB90, TGFB91, TGFB92, TGFB93, TGFB94, TGFB95, TGFB96, TGFB97, TGFB98, TGFB99, TGFB100, TGFB101, TGFB102, TGFB103, TGFB104, TGFB105, TGFB106, TGFB107, TGFB108, TGFB109, TGFB110, TGFB111, TGFB112, TGFB113, TGFB114, TGFB115, TGFB116, TGFB117, TGFB118, TGFB119, TGFB120, TGFB121, TGFB122, TGFB123, TGFB124, TGFB125, TGFB126, TGFB127, TGFB128, TGFB129, TGFB130, TGFB131, TGFB132, TGFB133, TGFB134, TGFB135, TGFB136, TGFB137, TGFB138, TGFB139, TGFB140, TGFB141, TGFB142, TGFB143, TGFB144, TGFB145, TGFB146, TGFB147, TGFB148, TGFB149, TGFB150, TGFB151, TGFB152, TGFB153, TGFB154, TGFB155, TGFB156, TGFB157, TGFB158, TGFB159, TGFB160, TGFB161, TGFB162, TGFB163, TGFB164, TGFB165, TGFB166, TGFB167, TGFB168, TGFB169, TGFB170, TGFB171, TGFB172, TGFB173, TGFB174, TGFB175, TGFB176, TGFB177, TGFB178, TGFB179, TGFB180, TGFB181, TGFB182, TGFB183, TGFB184, TGFB185, TGFB186, TGFB187, TGFB188, TGFB189, TGFB190, TGFB191, TGFB192, TGFB193, TGFB194, TGFB195, TGFB196, TGFB197, TGFB198, TGFB199, TGFB200, TGFB201, TGFB202, TGFB203, TGFB204, TGFB205, TGFB206, TGFB207, TGFB208, TGFB209, TGFB210, TGFB211, TGFB212, TGFB213, TGFB214, TGFB215, TGFB216, TGFB217, TGFB218, TGFB219, TGFB220, TGFB221, TGFB222, 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TGFB334, TGFB335, TGFB336, TGFB337, TGFB338, TGFB339, TGFB340, TGFB341, TGFB342, TGFB343, TGFB344, TGFB345, TGFB346, TGFB347, TGFB348, TGFB349, TGFB350, TGFB351, TGFB352, TGFB353, TGFB354, TGFB355, TGFB356, TGFB357, TGFB358, TGFB359, TGFB360, TGFB361, TGFB362, TGFB363, TGFB364, TGFB365, TGFB366, TGFB367, TGFB368, TGFB369, TGFB370, TGFB371, TGFB372, TGFB373, TGFB374, TGFB375, TGFB376, TGFB377, TGFB378, TGFB379, TGFB380, TGFB381, TGFB382, TGFB383, TGFB384, TGFB385, TGFB386, TGFB387, TGFB388, TGFB389, TGFB390, TGFB391, TGFB392, TGFB393, TGFB394, TGFB395, TGFB396, TGFB397, TGFB398, TGFB399, TGFB400, TGFB401, TGFB402, TGFB403, TGFB404, TGFB405, TGFB406, TGFB407, TGFB408, TGFB409, TGFB410, TGFB411, TGFB412, TGFB413, TGFB414, TGFB415, TGFB416, TGFB417, TGFB418, TGFB419, TGFB420, TGFB421, TGFB422, TGFB423, TGFB424, TGFB425, TGFB426, TGFB427, TGFB428, TGFB429, TGFB430, TGFB431, TGFB432, TGFB433, TGFB434, TGFB435, TGFB436, TGFB437, TGFB438, TGFB439, TGFB440, TGFB441, TGFB442, TGFB443, TGFB444, 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TGFB556, TGFB557, TGFB558, TGFB559, TGFB560, TGFB561, TGFB562, TGFB563, TGFB564, TGFB565, TGFB566, TGFB567, TGFB568, TGFB569, TGFB570, TGFB571, TGFB572, TGFB573, TGFB574, TGFB575, TGFB576, TGFB577, TGFB578, TGFB579, TGFB580, TGFB581, TGFB582, TGFB583, TGFB584, TGFB585, TGFB586, TGFB587, TGFB588, TGFB589, TGFB590, TGFB591, TGFB592, TGFB593, TGFB594, TGFB595, TGFB596, TGFB597, TGFB598, TGFB599, TGFB600, TGFB601, TGFB602, TGFB603, TGFB604, TGFB605, TGFB606, TGFB607, TGFB608, TGFB609, TGFB610, TGFB611, TGFB612, TGFB613, TGFB614, TGFB615, TGFB616, TGFB617, TGFB618, TGFB619, TGFB620, TGFB621, TGFB622, TGFB623, TGFB624, TGFB625, TGFB626, TGFB627, TGFB628, TGFB629, TGFB630, TGFB631, TGFB632, TGFB633, TGFB634, TGFB635, TGFB636, TGFB637, TGFB638, TGFB639, TGFB640, TGFB641, TGFB642, TGFB643, TGFB644, TGFB645, TGFB646, TGFB647, TGFB648, TGFB649, TGFB650, TGFB651, TGFB652, TGFB653, TGFB654, TGFB655, TGFB656, TGFB657, TGFB658, TGFB659, TGFB660, TGFB661, TGFB662, TGFB663, TGFB664, TGFB665, TGFB666, TGFB667, TGFB668, TGFB669, TGFB670, TGFB671, TGFB672, TGFB673, TGFB674, TGFB675, TGFB676, TGFB677, TGFB678, TGFB679, TGFB680, TGFB681, TGFB682, TGFB683, TGFB684, TGFB685, TGFB686, TGFB687, TGFB688, TGFB689, TGFB690, TGFB691, TGFB692, TGFB693, TGFB694, TGFB695, TGFB69

77. The method of claim 1, wherein the cell-derived vesicles of the population comprise eight or more of TGFBI, TGFB1, TGFBR2, TGFBR1, TGFB2, TGFBRAP1, ADAM17, ARG1, CD274, EIF2A, EPHB2, HLA-DRA, ELAVL1, IRAK1, LGALS1, PSME4, STAT1, or STAT3 proteins associated with immune modulation.

78. The method of claim 1, wherein the cell-derived vesicles of the population comprise nine or more of TGFBI, TGFBI, TGFBR2, TGFBR1, TGFBR2, TGFBRAP1, ADAM17, ARG1, CD274, EIF2A, EPHB2, HLA-DRA, ELAVL1, IRAK1, LGALS1, PSME4, STAT1, or STAT3 proteins associated with immune modulation.

79. The method of claim 1, wherein the cell-derived vesicles of the population comprise ten or more of TGFBI, TGFB1, TGFBR2, TGFB1, TGFB2, TGFBRAP1, ADAM17, ARG1, CD274, EIF2A, EPHB2, HLA-DRA, ELAVL1, IRAK1, LGALS1, PSME4, STAT1, or STAT3 proteins associated with immune modulation.

80. The method of claim 1, wherein the cell-derived vesicles of the population comprise one or more of EDIL3, TF, ITGB1, ANXA2, MFGE8, TGB1, TGBFR2, BASP1, COL1, COL6, GAPDH, FBN1, ITGB5, SDCBP, HSPA2, HSPA8, NT5E, MRGPRF, RTN4, NEFM, INA, HSPA9, FBN1, BSG, PRPH, FBLN1, PARP4, FLNA, YBX1, EVA1B, MCAM, POSTN, GNB2, GNB1, ATP1A1, CSPG4, EHD2, PXDN, CAV1, PKM, GNB4, NPTN, CCT2, LGALS3BP, or MVP therapeutic proteins.

81. The method of claim 1, wherein the cell-derived vesicles of the population comprise two or more of EDIL3, TF, ITGB1, ANXA2, MFGE8, TGB1, TGBFR2, BASP1, COL1, COL6, GAPDH, FBN1, ITGB5, SDCBP, HSPA2, HSPA8, NT5E, MRGPRF, RTN4, NEFM, INA, HSPA9, FBN1, BSG, PRPH, FBLN1, PARP4, FLNA, YBX1, EVA1B, MCAM, POSTN, GNB2, GNB1, ATP1A1, CSPG4, EHD2, PXDN, CAV1, PKM, GNB4, NPTN, CCT2, LGALS3BP, or MVP therapeutic proteins.

82. The method of claim 1, wherein the cell-derived vesicles of the population comprise three or more of EDIL3, TF, ITGB1, ANXA2, MFGE8, TGB1, TGBFR2, BASP1, COL1, COL6, GAPDH, FBN1, ITGB5, SDCBP, HSPA2, HSPA8, NT5E, MRGPRF, RTN4, NEFM, INA, HSPA9, FBN1, BSG, PRPH, FBLN1, PARP4, FLNA, YBX1, EVA1B, MCAM, POSTN, GNB2, GNB1, ATP1A1, CSPG4, EHD2, PXDN, CAV1, PKM, GNB4, NPTN, CCT2, LGALS3BP, or MVP therapeutic proteins.

83. The method of claim 1, wherein the cell-derived vesicles of the population comprise four or more of EDIL3, TF, ITGB1, ANXA2, MFGE8, TGB1, TGBFR2, BASP1, COL1, COL6, GAPDH, FBN1, ITGB5, SDCBP, HSPA2, HSPA8, NT5E, MRGPRF, RTN4, NEFM, INA, HSPA9, FBN1, BSG, PRPH, FBLN1, PARP4, FLNA, YBX1, EVA1B, MCAM, POSTN, GNB2, GNB1, ATP1A1, CSPG4, EHD2, PXDN, CAV1, PKM, GNB4, NPTN, CCT2, LGALS3BP, or MVP therapeutic proteins.

84. The method of claim 1, wherein the cell-derived vesicles of the population comprise five or more of EDIL3, TF, ITGB1, ANXA2, MFGE8, TGB1, TGBFR2, BASP1, COL1, COL6, GAPDH, FBN1, ITGB5, SDCBP, HSPA2, HSPA8, NT5E, MRGPRF, RTN4, NEFM, INA, HSPA9, FBN1, BSG, PRPH, FBLN1, PARP4, FLNA, YBX1, EVA1B, MCAM, POSTN, GNB2, GNB1, ATP1A1, CSPG4, EHD2, PXDN, CAV1, PKM, GNB4, NPTN, CCT2, LGALS3BP, or MVP therapeutic proteins.

85. The method of claim 1, wherein the cell-derived vesicles of the population comprise six or more of EDIL3, TF, ITGB1, ANXA2, MFGE8, TGB1, TGBFR2, BASP1, COL1, COL6, GAPDH, FBN1, ITGB5, SDCBP, HSPA2, HSPA8, NT5E, MRGPRF, RTN4, NEFM, INA, HSPA9, FBN1, BSG, PRPH, FBLN1, PARP4, FLNA, YBX1, EVA1B, MCAM, POSTN, GNB2, GNB1, ATP1A1, CSPG4, EHD2, PXDN, CAV1, PKM, GNB4, NPTN, CCT2, LGALS3BP, or MVP therapeutic proteins.

86. The method of claim 1, wherein the cell-derived vesicles of the population comprise seven or more of EDIL3, TF, ITGB1, ANXA2, MFGE8, TGB1, TGBFR2, BASP1, COL1, COL6, GAPDH, FBN1, ITGB5, SDCBP, HSPA2, HSPA8, NT5E, MRGPRF, RTN4, NEFM, INA, HSPA9, FBN1, BSG, PRPH, FBLN1, PARP4, FLNA, YBX1, EVA1B, MCAM, POSTN, GNB2, GNB1, ATP1A1, CSPG4, EHD2, PXDN, CAV1, PKM, GNB4, NPTN, CCT2, LGALS3BP, or MVP therapeutic proteins.

87. The method of claim 1, wherein the cell-derived vesicles of the population comprise eight or more of EDIL3, TF, ITGB1, ANXA2, MFGE8, TGB1, TGBFR2, BASP1, COL1, COL6, GAPDH, FBN1, ITGB5, SDCBP, HSPA2, HSPA8, NT5E, MRGPRF, RTN4, NEFM, INA, HSPA9, FBN1, BSG, PRPH, FBLN1, PARP4, FLNA, YBX1, EVA1B, MCAM, POSTN, GNB2, GNB1, ATP1A1, CSPG4, EHD2, PXDN, CAV1, PKM, GNB4, NPTN, CCT2, LGALS3BP, or MVP therapeutic proteins.

88. The method of claim 1, wherein the cell-derived vesicles of the population comprise nine or more of EDIL3, TF, ITGB1, ANXA2, MFGE8, TGB1, TGBFR2, BASP1, COL1, COL6, GAPDH, FBN1, ITGB5, SDCBP, HSPA2, HSPA8, NT5E, MRGPRF, RTN4, NEFM, INA, HSPA9, FBN1, BSG, PRPH, FBLN1, PARP4, FLNA, YBX1, EVA1B, MCAM, POSTN, GNB2, GNB1, ATP1A1, CSPG4, EHD2, PXDN, CAV1, PKM, GNB4, NPTN, CCT2, LGALS3BP, or MVP therapeutic proteins.

89. The method of claim 1, wherein the cell-derived vesicles of the population comprise ten or more of EDIL3, TF, ITGB1, ANXA2, MFGE8, TGB1, TGBFR2, BASP1, COL1, COL6, GAPDH, FBN1, ITGB5, SDCBP, HSPA2, HSPA8, NT5E, MRGPRF, RTN4, NEFM, INA, HSPA9, FBN1, BSG, PRPH, FBLN1, PARP4, FLNA, YBX1, EVA1B, MCAM, POSTN, GNB2, GNB1, ATP1A1, CSPG4, EHD2, PXDN, CAV1, PKM, GNB4, NPTN, CCT2, LGALS3BP, or MVP therapeutic proteins.

90. The method of claim 1, wherein the cell-derived vesicles comprise one or more of SERPINE1, ADAM17, ARG1, CD274, EIF2A, EPHB2, HLA-DRA, ELAVL1, IRAK1, LGALS1, PSME4, STAT1, STAT3, TGFB1, TGFB2, TGFB1, TGFB2, TGFB1, FBN1, HSP90AB1, SDCBP, LTBP1, JAK1, PIK3C2A, GRB2, HRAS, RAF1, MAP2K1, MAPK3, TYK2, STAT3, STAT1, STAT3, CRIP2, IL6ST, JAK2, CD274, or SQSTM1 proteins.

91. The method of claim 1, wherein the cell-derived vesicles comprise two or more of SERPINE1, ADAM17, ARG1, CD274, EIF2A, EPHB2, HLA-DRA, ELAVL1, IRAK1, LGALS1, PSME4, STAT1, STAT3, TGFB1, TGFB2, TGFBR1, TGFBR2, TGFBI, FBN1, HSP90AB1, SDCBP, LTBP1, JAK1, PIK3C2A, GRB2, HRAS, RAF1, MAP2K1, MAPK3, TYK2, STAT3, STAT1, STAT3, CRIP2, IL6ST, JAK2, CD274, or SQSTM1 proteins.
92. The method of claim 1, wherein the cell-derived vesicles comprise three or more of SERPINE1, ADAM17, ARG1, CD274, EIF2A, EPHB2, HLA-DRA, ELAVL1, IRAK1, LGALS1, PSME4, STAT1, STAT3, TGFB1, TGFB2, TGFBR1, TGFBR2, TGFBI, FBN1, HSP90AB1, SDCBP, LTBP1, JAK1, PIK3C2A, GRB2, HRAS, RAF1, MAP2K1, MAPK3, TYK2, STAT3, STAT1, STAT3, CRIP2, IL6ST, JAK2, CD274, or SQSTM1 proteins.
93. The method of claim 1, wherein the cell-derived vesicles comprise four or more of SERPINE1, ADAM17, ARG1, CD274, EIF2A, EPHB2, HLA-DRA, ELAVL1, IRAK1, LGALS1, PSME4, STAT1, STAT3, TGFB1, TGFB2, TGFBR1, TGFBR2, TGFBI, FBN1, HSP90AB1, SDCBP, LTBP1, JAK1, PIK3C2A, GRB2, HRAS, RAF1, MAP2K1, MAPK3, TYK2, STAT3, STAT1, STAT3, CRIP2, IL6ST, JAK2, CD274, or SQSTM1 proteins.
94. The method of claim 1, wherein the cell-derived vesicles comprise five or more of SERPINE1, ADAM17, ARG1, CD274, EIF2A, EPHB2, HLA-DRA, ELAVL1, IRAK1, LGALS1, PSME4, STAT1, STAT3, TGFB1, TGFB2, TGFBR1, TGFBR2, TGFBI, FBN1, HSP90AB1, SDCBP, LTBP1, JAK1, PIK3C2A, GRB2, HRAS, RAF1, MAP2K1, MAPK3, TYK2, STAT3, STAT1, STAT3, CRIP2, IL6ST, JAK2, CD274, or SQSTM1 proteins.
95. The method of claim 1, wherein the cell-derived vesicles comprise six or more of SERPINE1, ADAM17, ARG1, CD274, EIF2A, EPHB2, HLA-DRA, ELAVL1, IRAK1, LGALS1, PSME4, STAT1, STAT3, TGFB1, TGFB2, TGFBR1, TGFBR2, TGFBI, FBN1, HSP90AB1, SDCBP, LTBP1, JAK1, PIK3C2A, GRB2, HRAS, RAF1, MAP2K1, MAPK3, TYK2, STAT3, STAT1, STAT3, CRIP2, IL6ST, JAK2, CD274, or SQSTM1 proteins.
96. The method of claim 1, wherein the cell-derived vesicles comprise seven or more of SERPINE1, ADAM17, ARG1, CD274, EIF2A, EPHB2, HLA-DRA, ELAVL1, IRAK1, LGALS1, PSME4, STAT1, STAT3, TGFB1, TGFB2, TGFBR1, TGFBR2, TGFBI, FBN1, HSP90AB1, SDCBP, LTBP1, JAK1, PIK3C2A, GRB2, HRAS, RAF1, MAP2K1, MAPK3, TYK2, STAT3, STAT1, STAT3, CRIP2, IL6ST, JAK2, CD274, or SQSTM1 proteins.

97. The method of claim 1, wherein the cell-derived vesicles comprise eight or more of SERPINE1, ADAM17, ARG1, CD274, EIF2A, EPHB2, HLA-DRA, ELAVL1, IRAK1, LGALS1, PSME4, STAT1, STAT3, TGFB1, TGFB2, TGFBR1, TGFBR2, TGFBI, FBN1, HSP90AB1, SDCBP, LTBP1, JAK1, PIK3C2A, GRB2, HRAS, RAF1, MAP2K1, MAPK3, TYK2, STAT3, STAT1, STAT3, CRIP2, IL6ST, JAK2, CD274, or SQSTM1 proteins.

98. The method of claim 1, wherein the cell-derived vesicles comprise nine or more of SERPINE1, ADAM17, ARG1, CD274, EIF2A, EPHB2, HLA-DRA, ELAVL1, IRAK1, LGALS1, PSME4, STAT1, STAT3, TGFB1, TGFB2, TGFBR1, TGFBR2, TGFBI, FBN1, HSP90AB1, SDCBP, LTBP1, JAK1, PIK3C2A, GRB2, HRAS, RAF1, MAP2K1, MAPK3, TYK2, STAT3, STAT1, STAT3, CRIP2, IL6ST, JAK2, CD274, or SQSTM1 proteins.

99. The method of claim 1, wherein the cell-derived vesicles comprise ten or more of SERPINE1, ADAM17, ARG1, CD274, EIF2A, EPHB2, HLA-DRA, ELAVL1, IRAK1, LGALS1, PSME4, STAT1, STAT3, TGFB1, TGFB2, TGFBR1, TGFBR2, TGFBI, FBN1, HSP90AB1, SDCBP, LTBP1, JAK1, PIK3C2A, GRB2, HRAS, RAF1, MAP2K1, MAPK3, TYK2, STAT3, STAT1, STAT3, CRIP2, IL6ST, JAK2, CD274, or SQSTM1 proteins.

100. The method of claim 1, wherein the cell-derived vesicles comprise one or more of SERPINE1, ADAM17, ARG1, CD274, EIF2A, EPHB2, HLA-DRA, ELAVL1, IRAK1, LGALS1, PSME4, STAT1, STAT3, TGFB1, TGFB2, TGFBR1, TGFBR2, TGFBI, FBN1, HSP90AB1, SDCBP, LTBP1, JAK1, PIK3C2A, GRB2, HRAS, RAF1, MAP2K1, MAPK3, TYK2, CRIP2, IL6ST, JAK2, SQSTM1, DDX3X, PRMT5, SLC9A3R1, XPO1, TRAF3IP2, SPAG9, DIAPH1, CCDC22, PDCD6, PRPF40A, STAM2, TRIO, ERLIN2, AP2A2, MPZL1, AP2A1, EGFR, LMNA, EIF2S1, FYN, CDK1, NPM1, LYN, THBS1, ANXA5, RRAS, PCNA, SRC, XRCC6, HNRNPL, H2AFX, PRKCA, DDX5, PLCG1, FLNA, UBA1, S100A1, RPS3, SP100, AHCY, CFL1, F2R, RPA1, APEX1, MAPK1, EPHA2, PPP2R1A, PIF, PHB, NF2, LRPPRC, MSH2, CBX5, IQGAP1, TMED10, DNMT2, VCP, EIF3B, EIF3E, ACTB, RPL26, SUMO2, PPP1CA, RAP1A, RAC1, AP2B1, PPP2CA, CSNK2A1, SIRPA, DAB2, CDK5, CLTC, CAV1, PRDX1, C1QBP, SREBF2, TRO, CHD3, TRIM28, SF3B2, ADAM9, ADAM15, PIN1, RIPK1, HDAC1, CUL2, EIF3A, FHL2, SMC1A, KPNB1, TMED2, SEC23B, CPSF6, WLS, DAB2IP, MICAL3, HUWE1, ABI1, RPTOR, CCAR2, COMMD1, ARFGAP1, HSPH1, HDAC2, DDX17, RAD50, UPF1, COPS5, USP7,

RHBDF1, AP2M1, EIF3C, PHB2, MAP1LC3B, SPNS1, PTPN23, CBX8, PDLIM7, DACT1, NXF1, MYO6, PA2G4, RUVBL1, THRAP3, ACOT9, CD2AP, or RBM8A proteins.

101. The method of claim 1, wherein the cell-derived vesicles comprise two or more of SERPINE1, ADAM17, ARG1, CD274, EIF2A, EPHB2, HLA-DRA, ELAVL1, IRAK1, LGALS1, PSME4, STAT1, STAT3, TGFB1, TGFB2, TGFB1, TGFB2, TGFB1, FBN1, HSP90AB1, SDCBP, LTBP1, JAK1, PIK3C2A, GRB2, HRAS, RAF1, MAP2K1, MAPK3, TYK2, CRIP2, IL6ST, JAK2, SQSTM1, DDX3X, PRMT5, SLC9A3R1, XPO1, TRAF3IP2, SPAG9, DIAPH1, CCDC22, PDCD6, PRPF40A, STAM2, TRIO, ERLIN2, AP2A2, MPZL1, AP2A1, EGFR, LMNA, EIF2S1, FYN, CDK1, NPM1, LYN, THBS1, ANXA5, RRAS, PCNA, SRC, XRCC6, HNRNPL, H2AFX, PRKCA, DDX5, PLCG1, FLNA, UBA1, S100A1, RPS3, SP100, AHCY, CFL1, F2R, RPA1, APEX1, MAPK1, EPHA2, PPP2R1A, PIF, PHB, NF2, LRPPRC, MSH2, CBX5, IQGAP1, TMED10, DNM2, VCP, EIF3B, EIF3E, ACTB, RPL26, SUMO2, PPP1CA, RAP1A, RAC1, AP2B1, PPP2CA, CSNK2A1, SIRPA, DAB2, CDK5, CLTC, CAV1, PRDX1, C1QBP, SREBF2, TRO, CHD3, TRIM28, SF3B2, ADAM9, ADAM15, PIN1, RIPK1, HDAC1, CUL2, EIF3A, FHL2, SMC1A, KPNB1, TMED2, SEC23B, CPSF6, WLS, DAB2IP, MICAL3, HUWE1, ABI1, RPTOR, CCAR2, COMMD1, ARFGAP1, HSPH1, HDAC2, DDX17, RAD50, UPF1, COPS5, USP7, RHBDF1, AP2M1, EIF3C, PHB2, MAP1LC3B, SPNS1, PTPN23, CBX8, PDLIM7, DACT1, NXF1, MYO6, PA2G4, RUVBL1, THRAP3, ACOT9, CD2AP, or RBM8A proteins.

102. The method of claim 1, wherein the cell-derived vesicles comprise three or more of SERPINE1, ADAM17, ARG1, CD274, EIF2A, EPHB2, HLA-DRA, ELAVL1, IRAK1, LGALS1, PSME4, STAT1, STAT3, TGFB1, TGFB2, TGFB1, TGFB2, TGFB1, FBN1, HSP90AB1, SDCBP, LTBP1, JAK1, PIK3C2A, GRB2, HRAS, RAF1, MAP2K1, MAPK3, TYK2, CRIP2, IL6ST, JAK2, SQSTM1, DDX3X, PRMT5, SLC9A3R1, XPO1, TRAF3IP2, SPAG9, DIAPH1, CCDC22, PDCD6, PRPF40A, STAM2, TRIO, ERLIN2, AP2A2, MPZL1, AP2A1, EGFR, LMNA, EIF2S1, FYN, CDK1, NPM1, LYN, THBS1, ANXA5, RRAS, PCNA, SRC, XRCC6, HNRNPL, H2AFX, PRKCA, DDX5, PLCG1, FLNA, UBA1, S100A1, RPS3, SP100, AHCY, CFL1, F2R, RPA1, APEX1, MAPK1, EPHA2, PPP2R1A, PIF, PHB, NF2, LRPPRC, MSH2, CBX5, IQGAP1, TMED10, DNM2, VCP, EIF3B, EIF3E,

ACTB, RPL26, SUMO2, PPP1CA, RAP1A, RAC1, AP2B1, PPP2CA, CSNK2A1, SIRPA, DAB2, CDK5, CLTC, CAV1, PRDX1, C1QBP, SREBF2, TRO, CHD3, TRIM28, SF3B2, ADAM9, ADAM15, PIN1, RIPK1, HDAC1, CUL2, EIF3A, FHL2, SMC1A, KPNB1, TMED2, SEC23B, CPSF6, WLS, DAB2IP, MICAL3, HUWE1, ABI1, RPTOR, CCAR2, COMMD1, ARFGAP1, HSPH1, HDAC2, DDX17, RAD50, UPF1, COPS5, USP7, RHBDF1, AP2M1, EIF3C, PHB2, MAP1LC3B, SPNS1, PTPN23, CBX8, PDLIM7, DACT1, NXF1, MYO6, PA2G4, RUVBL1, THRAP3, ACOT9, CD2AP, or RBM8A proteins.

103. The method of claim 1, wherein the cell-derived vesicles comprise four or more of SERPINE1, ADAM17, ARG1, CD274, EIF2A, EPHB2, HLA-DRA, ELAVL1, IRAK1, LGALS1, PSME4, STAT1, STAT3, TGFB1, TGFB2, TGFBR1, TGFBR2, TGFBI, FBN1, HSP90AB1, SDCBP, LTBP1, JAK1, PIK3C2A, GRB2, HRAS, RAF1, MAP2K1, MAPK3, TYK2, CRIP2, IL6ST, JAK2, SQSTM1, DDX3X, PRMT5, SLC9A3R1, XPO1, TRAF3IP2, SPAG9, DIAPH1, CCDC22, PDCD6, PRPF40A, STAM2, TRIO, ERLIN2, AP2A2, MPZL1, AP2A1, EGFR, LMNA, EIF2S1, FYN, CDK1, NPM1, LYN, THBS1, ANXA5, RRAS, PCNA, SRC, XRCC6, HNRNPL, H2AFX, PRKCA, DDX5, PLCG1, FLNA, UBA1, S100A1, RPS3, SP100, AHCY, CFL1, F2R, RPA1, APEX1, MAPK1, EPHA2, PPP2R1A, PIF, PHB, NF2, LRPPRC, MSH2, CBX5, IQGAP1, TMED10, DNMT2, VCP, EIF3B, EIF3E, ACTB, RPL26, SUMO2, PPP1CA, RAP1A, RAC1, AP2B1, PPP2CA, CSNK2A1, SIRPA, DAB2, CDK5, CLTC, CAV1, PRDX1, C1QBP, SREBF2, TRO, CHD3, TRIM28, SF3B2, ADAM9, ADAM15, PIN1, RIPK1, HDAC1, CUL2, EIF3A, FHL2, SMC1A, KPNB1, TMED2, SEC23B, CPSF6, WLS, DAB2IP, MICAL3, HUWE1, ABI1, RPTOR, CCAR2, COMMD1, ARFGAP1, HSPH1, HDAC2, DDX17, RAD50, UPF1, COPS5, USP7, RHBDF1, AP2M1, EIF3C, PHB2, MAP1LC3B, SPNS1, PTPN23, CBX8, PDLIM7, DACT1, NXF1, MYO6, PA2G4, RUVBL1, THRAP3, ACOT9, CD2AP, or RBM8A proteins.

104. The method of claim 1, wherein the cell-derived vesicles comprise five or more of SERPINE1, ADAM17, ARG1, CD274, EIF2A, EPHB2, HLA-DRA, ELAVL1, IRAK1, LGALS1, PSME4, STAT1, STAT3, TGFB1, TGFB2, TGFBR1, TGFBR2, TGFBI, FBN1, HSP90AB1, SDCBP, LTBP1, JAK1, PIK3C2A, GRB2, HRAS, RAF1, MAP2K1, MAPK3, TYK2, CRIP2, IL6ST, JAK2, SQSTM1, DDX3X, PRMT5, SLC9A3R1, XPO1, TRAF3IP2,

SPAG9, DIAPH1, CCDC22, PDCD6, PRPF40A, STAM2, TRIO, ERLIN2, AP2A2, MPZL1, AP2A1, EGFR, LMNA, EIF2S1, FYN, CDK1, NPM1, LYN, THBS1, ANXA5, RRAS, PCNA, SRC, XRCC6, HNRNPL, H2AFX, PRKCA, DDX5, PLCG1, FLNA, UBA1, S100A1, RPS3, SP100, AHCY, CFL1, F2R, RPA1, APEX1, MAPK1, EPHA2, PPP2R1A, PIF, PHB, NF2, LRPPRC, MSH2, CBX5, IQGAP1, TMED10, DNMT2, VCP, EIF3B, EIF3E, ACTB, RPL26, SUMO2, PPP1CA, RAP1A, RAC1, AP2B1, PPP2CA, CSNK2A1, SIRPA, DAB2, CDK5, CLTC, CAV1, PRDX1, C1QBP, SREBF2, TRO, CHD3, TRIM28, SF3B2, ADAM9, ADAM15, PIN1, RIPK1, HDAC1, CUL2, EIF3A, FHL2, SMC1A, KPNB1, TMED2, SEC23B, CPSF6, WLS, DAB2IP, MICAL3, HUWE1, ABI1, RPTOR, CCAR2, COMMD1, ARFGAP1, HSPH1, HDAC2, DDX17, RAD50, UPF1, COPS5, USP7, RHBDF1, AP2M1, EIF3C, PHB2, MAP1LC3B, SPNS1, PTPN23, CBX8, PDLIM7, DACT1, NXF1, MYO6, PA2G4, RUVBL1, THRAP3, ACOT9, CD2AP, or RBM8A proteins.

105. The method of claim 1, wherein the cell-derived vesicles comprise six or more of SERPINE1, ADAM17, ARG1, CD274, EIF2A, EPHB2, HLA-DRA, ELAVL1, IRAK1, LGALS1, PSME4, STAT1, STAT3, TGFB1, TGFB2, TGFB1R1, TGFB1R2, TGFB1I, FBN1, HSP90AB1, SDCBP, LTBP1, JAK1, PIK3C2A, GRB2, HRAS, RAF1, MAP2K1, MAPK3, TYK2, CRIP2, IL6ST, JAK2, SQSTM1, DDX3X, PRMT5, SLC9A3R1, XPO1, TRAF3IP2, SPAG9, DIAPH1, CCDC22, PDCD6, PRPF40A, STAM2, TRIO, ERLIN2, AP2A2, MPZL1, AP2A1, EGFR, LMNA, EIF2S1, FYN, CDK1, NPM1, LYN, THBS1, ANXA5, RRAS, PCNA, SRC, XRCC6, HNRNPL, H2AFX, PRKCA, DDX5, PLCG1, FLNA, UBA1, S100A1, RPS3, SP100, AHCY, CFL1, F2R, RPA1, APEX1, MAPK1, EPHA2, PPP2R1A, PIF, PHB, NF2, LRPPRC, MSH2, CBX5, IQGAP1, TMED10, DNMT2, VCP, EIF3B, EIF3E, ACTB, RPL26, SUMO2, PPP1CA, RAP1A, RAC1, AP2B1, PPP2CA, CSNK2A1, SIRPA, DAB2, CDK5, CLTC, CAV1, PRDX1, C1QBP, SREBF2, TRO, CHD3, TRIM28, SF3B2, ADAM9, ADAM15, PIN1, RIPK1, HDAC1, CUL2, EIF3A, FHL2, SMC1A, KPNB1, TMED2, SEC23B, CPSF6, WLS, DAB2IP, MICAL3, HUWE1, ABI1, RPTOR, CCAR2, COMMD1, ARFGAP1, HSPH1, HDAC2, DDX17, RAD50, UPF1, COPS5, USP7, RHBDF1, AP2M1, EIF3C, PHB2, MAP1LC3B, SPNS1, PTPN23, CBX8, PDLIM7, DACT1, NXF1, MYO6, PA2G4, RUVBL1, THRAP3, ACOT9, CD2AP, or RBM8A proteins.

106. The method of claim 1, wherein the cell-derived vesicles comprise seven or more of SERPINE1, ADAM17, ARG1, CD274, EIF2A, EPHB2, HLA-DRA, ELAVL1, IRAK1, LGALS1, PSME4, STAT1, STAT3, TGFB1, TGFB2, TGFBR1, TGFBR2, TGFBI, FBN1, HSP90AB1, SDCBP, LTBP1, JAK1, PIK3C2A, GRB2, HRAS, RAF1, MAP2K1, MAPK3, TYK2, CRIP2, IL6ST, JAK2, SQSTM1, DDX3X, PRMT5, SLC9A3R1, XPO1, TRAF3IP2, SPAG9, DIAPH1, CCDC22, PDCD6, PRPF40A, STAM2, TRIO, ERLIN2, AP2A2, MPZL1, AP2A1, EGFR, LMNA, EIF2S1, FYN, CDK1, NPM1, LYN, THBS1, ANXA5, RRAS, PCNA, SRC, XRCC6, HNRNPL, H2AFX, PRKCA, DDX5, PLCG1, FLNA, UBA1, S100A1, RPS3, SP100, AHCY, CFL1, F2R, RPA1, APEX1, MAPK1, EPHA2, PPP2R1A, PIF, PHB, NF2, LRPPRC, MSH2, CBX5, IQGAP1, TMED10, DNMT2, VCP, EIF3B, EIF3E, ACTB, RPL26, SUMO2, PPP1CA, RAP1A, RAC1, AP2B1, PPP2CA, CSNK2A1, SIRPA, DAB2, CDK5, CLTC, CAV1, PRDX1, C1QBP, SREBF2, TRO, CHD3, TRIM28, SF3B2, ADAM9, ADAM15, PIN1, RIPK1, HDAC1, CUL2, EIF3A, FHL2, SMC1A, KPNB1, TMED2, SEC23B, CPSF6, WLS, DAB2IP, MICAL3, HUWE1, ABI1, RPTOR, CCAR2, COMMD1, ARFGAP1, HSPH1, HDAC2, DDX17, RAD50, UPF1, COPS5, USP7, RHBDF1, AP2M1, EIF3C, PHB2, MAP1LC3B, SPNS1, PTPN23, CBX8, PDLIM7, DACT1, NXF1, MYO6, PA2G4, RUVBL1, THRAP3, ACOT9, CD2AP, or RBM8A proteins.

107. The method of claim 1, wherein the cell-derived vesicles comprise eight or more of SERPINE1, ADAM17, ARG1, CD274, EIF2A, EPHB2, HLA-DRA, ELAVL1, IRAK1, LGALS1, PSME4, STAT1, STAT3, TGFB1, TGFB2, TGFBR1, TGFBR2, TGFBI, FBN1, HSP90AB1, SDCBP, LTBP1, JAK1, PIK3C2A, GRB2, HRAS, RAF1, MAP2K1, MAPK3, TYK2, CRIP2, IL6ST, JAK2, SQSTM1, DDX3X, PRMT5, SLC9A3R1, XPO1, TRAF3IP2, SPAG9, DIAPH1, CCDC22, PDCD6, PRPF40A, STAM2, TRIO, ERLIN2, AP2A2, MPZL1, AP2A1, EGFR, LMNA, EIF2S1, FYN, CDK1, NPM1, LYN, THBS1, ANXA5, RRAS, PCNA, SRC, XRCC6, HNRNPL, H2AFX, PRKCA, DDX5, PLCG1, FLNA, UBA1, S100A1, RPS3, SP100, AHCY, CFL1, F2R, RPA1, APEX1, MAPK1, EPHA2, PPP2R1A, PIF, PHB, NF2, LRPPRC, MSH2, CBX5, IQGAP1, TMED10, DNMT2, VCP, EIF3B, EIF3E, ACTB, RPL26, SUMO2, PPP1CA, RAP1A, RAC1, AP2B1, PPP2CA, CSNK2A1, SIRPA, DAB2, CDK5, CLTC, CAV1, PRDX1, C1QBP, SREBF2, TRO, CHD3, TRIM28, SF3B2, ADAM9, ADAM15, PIN1, RIPK1, HDAC1, CUL2, EIF3A, FHL2, SMC1A, KPNB1,

TMED2, SEC23B, CPSF6, WLS, DAB2IP, MICAL3, HUWE1, ABI1, RPTOR, CCAR2, COMMD1, ARFGAP1, HSPH1, HDAC2, DDX17, RAD50, UPF1, COPS5, USP7, RHBDF1, AP2M1, EIF3C, PHB2, MAP1LC3B, SPNS1, PTPN23, CBX8, PDLIM7, DACT1, NXF1, MYO6, PA2G4, RUVBL1, THRAP3, ACOT9, CD2AP, or RBM8A proteins.

108. The method of claim 1, wherein the cell-derived vesicles comprise nine or more of SERPINE1, ADAM17, ARG1, CD274, EIF2A, EPHB2, HLA-DRA, ELAVL1, IRAK1, LGALS1, PSME4, STAT1, STAT3, TGFB1, TGFB2, TGFBR1, TGFBR2, TGFBI, FBN1, HSP90AB1, SDCBP, LTBP1, JAK1, PIK3C2A, GRB2, HRAS, RAF1, MAP2K1, MAPK3, TYK2, CRIP2, IL6ST, JAK2, SQSTM1, DDX3X, PRMT5, SLC9A3R1, XPO1, TRAF3IP2, SPAG9, DIAPH1, CCDC22, PDCD6, PRPF40A, STAM2, TRIO, ERLIN2, AP2A2, MPZL1, AP2A1, EGFR, LMNA, EIF2S1, FYN, CDK1, NPM1, LYN, THBS1, ANXA5, RRAS, PCNA, SRC, XRCC6, HNRNPL, H2AFX, PRKCA, DDX5, PLCG1, FLNA, UBA1, S100A1, RPS3, SP100, AHCY, CFL1, F2R, RPA1, APEX1, MAPK1, EPHA2, PPP2R1A, PIF, PHB, NF2, LRPPRC, MSH2, CBX5, IQGAP1, TMED10, DNM2, VCP, EIF3B, EIF3E, ACTB, RPL26, SUMO2, PPP1CA, RAP1A, RAC1, AP2B1, PPP2CA, CSNK2A1, SIRPA, DAB2, CDK5, CLTC, CAV1, PRDX1, C1QBP, SREBF2, TRO, CHD3, TRIM28, SF3B2, ADAM9, ADAM15, PIN1, RIPK1, HDAC1, CUL2, EIF3A, FHL2, SMC1A, KPNB1, TMED2, SEC23B, CPSF6, WLS, DAB2IP, MICAL3, HUWE1, ABI1, RPTOR, CCAR2, COMMD1, ARFGAP1, HSPH1, HDAC2, DDX17, RAD50, UPF1, COPS5, USP7, RHBDF1, AP2M1, EIF3C, PHB2, MAP1LC3B, SPNS1, PTPN23, CBX8, PDLIM7, DACT1, NXF1, MYO6, PA2G4, RUVBL1, THRAP3, ACOT9, CD2AP, or RBM8A proteins.

109. The method of claim 1, wherein the cell-derived vesicles comprise ten or more of SERPINE1, ADAM17, ARG1, CD274, EIF2A, EPHB2, HLA-DRA, ELAVL1, IRAK1, LGALS1, PSME4, STAT1, STAT3, TGFB1, TGFB2, TGFBR1, TGFBR2, TGFBI, FBN1, HSP90AB1, SDCBP, LTBP1, JAK1, PIK3C2A, GRB2, HRAS, RAF1, MAP2K1, MAPK3, TYK2, CRIP2, IL6ST, JAK2, SQSTM1, DDX3X, PRMT5, SLC9A3R1, XPO1, TRAF3IP2, SPAG9, DIAPH1, CCDC22, PDCD6, PRPF40A, STAM2, TRIO, ERLIN2, AP2A2, MPZL1, AP2A1, EGFR, LMNA, EIF2S1, FYN, CDK1, NPM1, LYN, THBS1, ANXA5, RRAS, PCNA, SRC, XRCC6, HNRNPL, H2AFX, PRKCA, DDX5, PLCG1, FLNA, UBA1,

S100A1, RPS3, SP100, AHCY, CFL1, F2R, RPA1, APEX1, MAPK1, EPHA2, PPP2R1A, PIF, PHB, NF2, LRPPRC, MSH2, CBX5, IQGAP1, TMED10, DNMT2, VCP, EIF3B, EIF3E, ACTB, RPL26, SUMO2, PPP1CA, RAP1A, RAC1, AP2B1, PPP2CA, CSNK2A1, SIRPA, DAB2, CDK5, CLTC, CAV1, PRDX1, C1QBP, SREBF2, TRO, CHD3, TRIM28, SF3B2, ADAM9, ADAM15, PIN1, RIPK1, HDAC1, CUL2, EIF3A, FHL2, SMC1A, KPNB1, TMED2, SEC23B, CPSF6, WLS, DAB2IP, MICAL3, HUWE1, ABI1, RPTOR, CCAR2, COMMD1, ARFGAP1, HSPH1, HDAC2, DDX17, RAD50, UPF1, COPS5, USP7, RHBDF1, AP2M1, EIF3C, PHB2, MAP1LC3B, SPNS1, PTPN23, CBX8, PDLIM7, DACT1, NXF1, MYO6, PA2G4, RUVBL1, THRAP3, ACOT9, CD2AP, or RBM8A proteins.

110. The method of claim 1, wherein the purified population of cell-derived vesicles is substantially homogeneous.

111. The method of claim 1, wherein the purified population of cell-derived vesicles is heterogeneous.

112. The method of claim 1, wherein the population comprise a concentration of purified cell-derived vesicles from about 0.5 micrograms to about 5000 micrograms of exosome and/or microvesicle protein purified from about approximately 10^6 stem cells.

113. The method of claim 1, wherein the population comprise a concentration of purified cell-derived vesicles of less than about 300 micrograms of exosome and/or microvesicle protein collected per approximately 10^6 stem cells.

114. The method of claim 1, wherein the population comprise a concentration of purified cell-derived vesicles is less than about 200 micrograms per 10^6 stem cells.

115. The method of claim 1, wherein the average diameter of the cell-derived vesicles in the population is from about 0.1 nm to about about 1000 nm.

116. The method of claim 1, wherein the average diameter of the cell-derived vesicles in the population is less than 100 nm.

117. The method of claim 1, wherein the average diameter of the cell-derived vesicles in the population is less than 50 nm.

118. The method of claim 1, wherein the average diameter of the cell-derived vesicles in the population is less than about 40 nm.
119. The method of claim 1, wherein the cell-derived vesicles have been purified from by a method comprising filtration, optionally tangential flow filtration.
120. The method of claim 1, further comprising administering one or more agents selected from an anti-inflammatory agent, a neurotrophic factor, or an angiogenesis agent.
121. The c method of claim 120, wherein the anti-inflammatory agent is selected from TGF β , IL-2, IL-17, IL-35, or IL-37.
122. The method of claim 120, wherein the neurotrophic factors is selected from BDNF, NGF, Neurotrophin-3, FGF2, CTNF, GDNF, IGF2, HGF, Noggin, or T3.
123. The method of claim 120, wherein the angiogenesis agent is selected from FGF1, FGF2, HGF, VEGF, PDGF, EGF, TGF β , or WNT1.
124. The method of any one of claims 120-123, wherein the concentration of the agent is from about 5 ng/mL to about 1 μ g/mL.
125. The method of any one of claims 120-124, further comprising an isolated stem cell.
126. The method of claim 125, wherein the stem cell is selected from the group of an adult stem cell, an embryonic stem cell, an induced pluripotent stem cell, an embryonic-like stem cell, a mesenchymal stem cell, or a neural stem cell.
127. A composition comprising a highly purified population of cell-derived vesicles and one or more agents selected from an anti-inflammatory agent, a neurotrophic factor, or an angiogenesis agent, wherein the cell-derived vesicles are purified from a population of stem cells cultured under conditions of hypoxia and low serum conditions.
128. The composition of claim 127, wherein the anti-inflammatory agent is selected from TGF β , IL-2, IL-17, IL-35, or IL-37.
129. The composition of claim 127, wherein the neurotrophic factors is selected from BDNF, NGF, Neurotrophin-3, FGF2, CTNF, GDNF, IGF2, HGF, Noggin, or T3.
130. The composition of claim 127, wherein the angiogenesis agent is selected from FGF1, FGF2, HGF, VEGF, PDGF, EGF, TGF β , or WNT1.

131. The composition of any one of claims 127-130, wherein the concentration of agent is from about 5 to about 500 ng/mL.
132. The composition of any one of claims 127-131, further comprising a carrier.
133. The composition of claim 132, wherein the carrier is a pharmaceutically acceptable carrier.
134. The composition of any one of claims 127-133, further comprising an isolated stem cell.
135. The composition of claim 134, wherein the stem cell is selected from the group of an adult stem cell, an embryonic stem cell, an induced pluripotent stem cell, an embryonic-like stem cell, a mesenchymal stem cell, or a neural stem cell.
136. A method for promoting angiogenesis in a subject in need thereof comprising administering to the subject the composition of any one of claims 127-135.
137. The method of claim 136, wherein the subject is administered at least one dose of between approximately 0.1 mg and 1000 mg of cell-derived vesicle protein in the purified population.
138. The method of claim 136, wherein the subject is administered at least one dose of approximately 50 mg of cell-derived vesicle protein in the purified population.
139. The method of claim 136, wherein the composition of any one of claims 127-135 is administered prior to or after administration of an isolated stem cell.
140. The method of claim 136, wherein the composition of any one of claims 127-135 is administered simultaneously with an isolated stem cell.
141. The method of claim 136, wherein the composition of any one of claims 127-135 is administered by intravenous injection, intrathecal injection, direct injection, intramuscular injection, intracranial injection, or topically.
142. The method of claim 136, wherein the subject is a mammal, optionally a human patient.
143. A method for treating peripheral arterial disease or stroke comprising administering to a subject the composition of any one of claims 127-135.

144. The method of claim 143, wherein the subject is administered at least one dose of from about 0.1 mg to about 200 mg of cell-derived vesicle protein of the purified population.

145. The method of claim 143, wherein the subject is administered at least one dose of approximately 50 mg of cell-derived vesicle protein of the purified population.

146. The method of claim 143, wherein the composition of any one of claims 127-135 is administered prior to or after administration of a stem cell.

147. The method of claim 143, wherein the composition of any one of claims 127-135 is administered simultaneously with the stem cell.

148. The method of claim 143, wherein the composition of any one of claims 127-135 is administered by intravenous injection.

149. The method of claim 143, wherein the composition of any one of claims 127-135 is administered topically.

150. The method of claim 143, wherein the subject is a mammal, optionally a human patient.

151. A method for treating a dermal wound in a subject comprising administering to the subject the composition of any one of claims 127-135.

152. The method of claim 151, wherein the subject is administered at least one dose of from about 0.1 mg to about 200 mg of cell-derived vesicle protein of the purified population.

153. The method of claim 151, wherein the subject is administered at least one dose of approximately 50 mg of cell-derived vesicle protein.

154. The method of claim 151, wherein the composition of any one of claims 127-135 is administered prior to or after administration of an isolated stem cell.

155. The method of claim 151, wherein the composition of any one of claims 127-135 is administered simultaneously with an isolated stem cell.

156. The method of claim 151, wherein the composition of any one of claims 127-135 is administered topically.

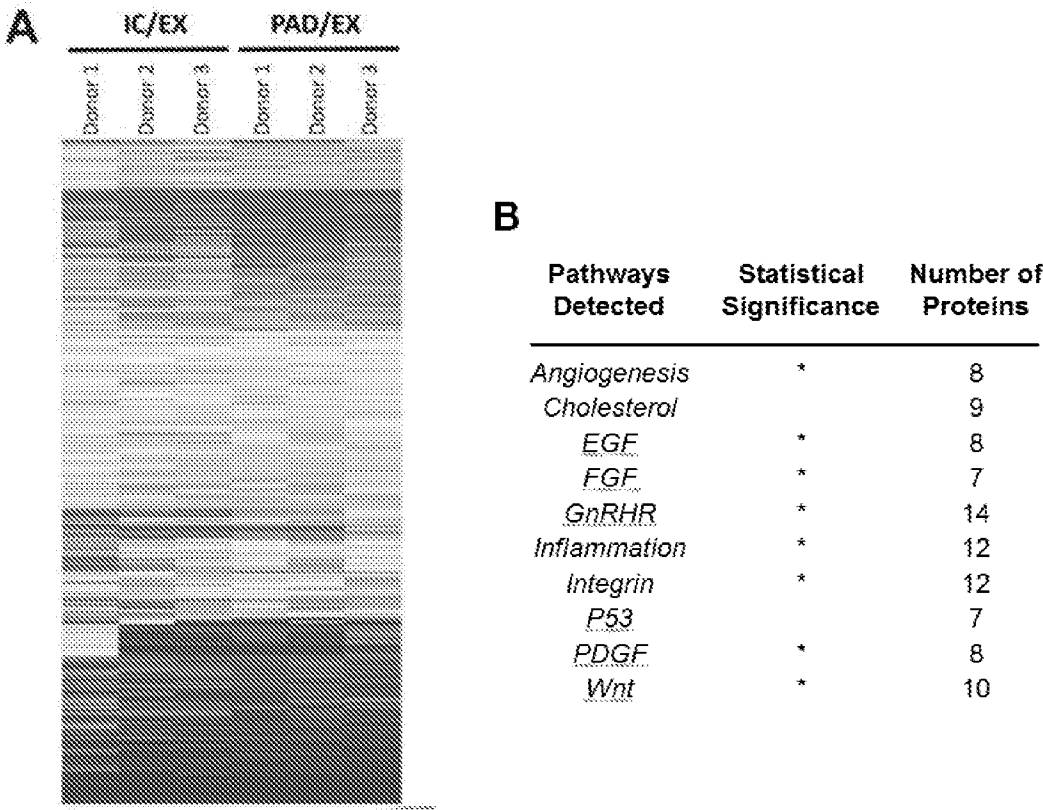
157. The method of claim 151, wherein the subject is a mammal, optionally a human patient.
158. A method for purifying a population of cell-derived vesicles, comprising: applying a tangential flow filtration to conditioned media produced by a population of isolated stem cells cultured in the presence of, or contacted with one or more agents selected from an inflammatory agent, a neurotrophic factor, or an angiogenesis agent; and optionally further concentrating the cell-derived vesicle containing fraction to provide a purified population of cell-derived vesicles.
159. The method of claim 158, wherein the inflammatory agent is selected from $\text{TNF}\alpha$, IL-6, IL-17, IL-1 β , IFN γ , or LPS.
160. The method of claim 158, wherein the neurotrophic factor is selected from T3, FGF2, Noggin, BDNF, NGF, HGF, CTNF, GDNF, IGF2, or Neurotrophin-3.
161. The method of claim 158, wherein the angiogenesis factor is selected from FGF2, VEGF, PDGF, HGF, FGF1, EGF, TGF β , or WNT1.
162. The method of claim 158, wherein the one or more agents is selected from $\text{TNF}\alpha$, Noggin, or T3.
163. A method for purifying a population of cell-derived vesicles, comprising:
- (a) applying a tangential flow filtration to conditioned media produced by a population of isolated stem cells to isolate a cell-derived vesicles containing fraction; and optionally
 - (b) concentrating the cell-derived vesicle containing fraction to provide a purified population of cell-derived vesicles; and further optionally
 - (c) adding one or more agents selected from an anti-inflammatory agent, a neurotrophic factor, or an angiogenesis agent to the purified population of cell-derived vesicles.
164. The method of claim 163, wherein the anti-inflammatory agent is selected from TGF β , IL-2, IL-17, IL-35, or IL-37.

165. The method of claim 163, wherein the neurotrophic factors is selected from BDNF, NGF, Neurotrophin-3, FGF2, CTNF, GDNF, IGF2, HGF, Noggin, or T3.
166. The method of claim 163, wherein the angiogenesis agent is selected from FGF1, FGF2, HGF, VEGF, PDGF, EGF, TGF β , or WNT1.
167. The method of any one of claims 163-166, wherein the concentration of the one or more agents is from about 5 to about 100 ng/mL.
168. The method of any one of claims 163-166, wherein the concentration of the one or more agents is from about 10 ng/mL to about 1 μ g/mL.
169. The method of any one of claims 163-168, further comprising removing cell debris and other contaminants prior to concentrating the cell-derived vesicle containing fraction.
170. The method of any one of claims 163-169, wherein the population of stem cells are pre-cultured under hypoxic and low serum conditions prior for up to about 72 hours.
171. The method of any one of claims 163-170, wherein filtration is performed using an approximately 200 nanometer filter.
172. The method of any one of claims 163-171, wherein the isolated stem cells are one or more of adult stem cells, embryonic stem cells, embryonic-like stem cells, neural stem cells, or induced pluripotent stem cells.
173. The method of claim 172, wherein the stem cells are mesenchymal stem cells.
174. The method of claim 170, where the hypoxic conditions are independently from about 1% to about 15% CO₂ and from about 0.05% to about 20% oxygen tension.
175. The method of claim 170, wherein the low serum conditions are serum free conditions.
176. The method of any of claims 163-175, wherein the tangential flow filtration unit is between about 50 kilodalton and about 750 kilodalton nominal molecular weight limit filtration unit.
177. The method of any one of claims 163-175, wherein the tangential flow filtration unit is about a 100 kilodalton nominal molecular weight limit filtration unit.

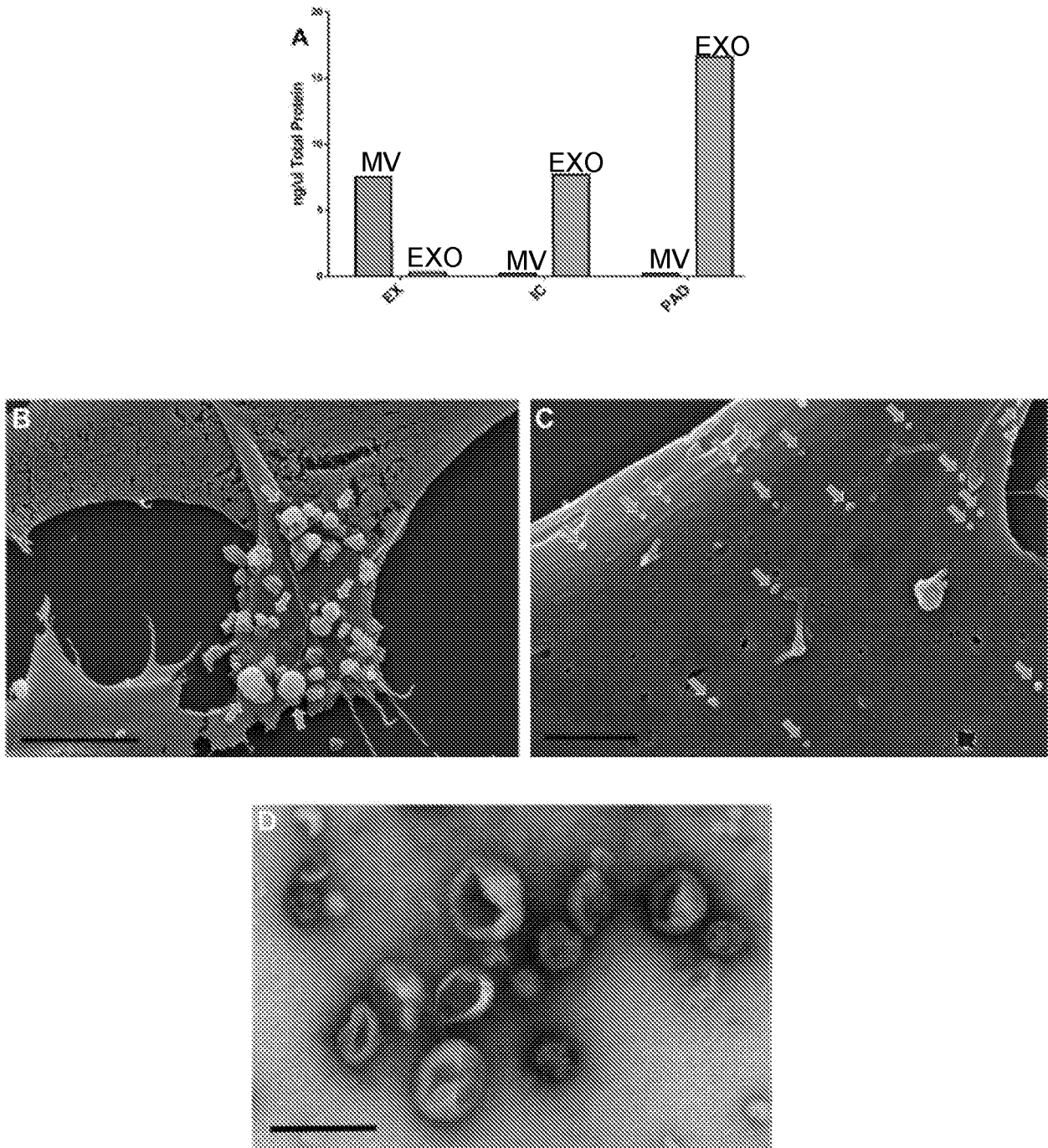
178. The method of any one of claims 163-175, wherein the tangential flow filtration unit is about a 300 kilodalton nominal molecular weight limit filtration unit.
179. The method of any one of claims 163-178, wherein the concentration step is performed using a filtration device.
180. The method of claim 179, wherein the filtration device is an approximately 100 kilodalton nominal molecular weight limit filtration device.
181. The method of claim 179, wherein the filtration device is an approximately 300 kilodalton nominal molecular weight limit filtration device.
182. The method of any one of claims 163-181, further comprising formulating the purified population of cell-derived vesicles by mixing the population with a carrier and/or a stabilizer.
183. The method of any one of claims 163-182, further comprising drying, freezing or freeze drying the purified population of cell-derived vesicles.
184. A population of cell-derived vesicles obtainable from the method of any one of claims 163-183.
185. A dried, lyophilized or frozen composition comprising the composition of any one of claims 127-135 or the population of claim 184.
186. A kit comprising the population of claim 184 and instructions for use.
187. A method for large-scale purification of a population of cell-derived vesicles, comprising:
- (a) contacting a population of isolated stem cells cultured in a bioreactor with one or more agents selected from an inflammatory agent, a neurotrophic factor, or an angiogenesis agent;
 - (b) applying a tangential flow filtration to conditioned media produced by the population of isolated stem cells to isolate a cell-derived vesicles containing fraction; and
 - (c) concentrating the cell-derived vesicle containing fraction to provide a purified population of cell-derived vesicles.

188. The method of claim 187, wherein the inflammatory agent is selected from $\text{TNF}\alpha$, IL-6, IL-17, IL-1 β , IFN γ , or LPS.
189. The method of claim 187, wherein the neurotrophic factor is selected from T3, FGF2, Noggin, BDNF, NGF, HGF, CTNF, GDNF, IGF2, or Neurotrophin-3.
190. The method of claim 187, wherein the angiogenesis factor is selected from FGF2, VEGF, PDGF, HGF, FGF1, EGF, TGF β , or WNT1.
191. The method of claim 187, wherein the one or more agents is selected from $\text{TNF}\alpha$, Noggin, or T3.
192. A method for large-scale purification of a population of cell-derived vesicles, comprising:
- (a) applying a tangential flow filtration to conditioned media produced by a population of isolated stem cells cultured in a bioreactor to isolate a cell-derived vesicles containing fraction;
 - (b) concentrating the cell-derived vesicle containing fraction to provide a purified population of cell-derived vesicles; and
 - (c) adding one or more agents selected from an anti-inflammatory agent, a neurotrophic factor, or an angiogenesis agent to the purified population of cell-derived vesicles.
193. The method of claim 192, wherein the anti-inflammatory agent is selected from TGF β , IL-2, IL-17, IL-35, or IL-37.
194. The method of claim 192, wherein the neurotrophic factors is selected from BDNF, NGF, Neurotrophin-3, FGF2, CTNF, GDNF, IGF2, HGF, Noggin, or T3.
195. The method of claim 192, wherein the angiogenesis agent is selected from FGF1, FGF2, HGF, VEGF, PDGF, EGF, TGF β , or WNT1.
196. The method of any one of claims 192-195, wherein the concentration of the one or more agents is from about 5 to about 100 ng/mL.
197. The method of any one of claims 192-195, wherein the concentration of the one or agents is from about 10 ng/mL to about 1 $\mu\text{g/mL}$.

198. The method of any one of claims 192-197, wherein the bioreactor is a hollow fiber bioreactor.
199. The method of any one of claims 192-198, wherein the population of stem cells is pre-cultured under hypoxic and low serum conditions for up to about 72 hours.
200. A method for treating a disease or condition related to an inflammatory response or inflammation in a subject in need thereof comprising administering to the subject a purified population of cell-derived vesicles prepared by culturing stem cells producing the cell-derived vesicles under conditions of hypoxia and low serum conditions, and optionally wherein the cell-derived vesicles comprise exosomes and/or microvesicles.
201. The method of claim 200, wherein the disease or condition is selected from the group of: multiple sclerosis, neuroinflammatory disease, muscle injuries, radiation tissue damage, stroke, traumatic brain injury, myocardial infarction, graft versus host disease, Parkinson's disease, Alzheimer's, inflammatory bowel disease, Huntington's disease, amyotrophic lateral sclerosis, Bahcet's disease, sarcopenia, aging, spinal cord injury, wound repair, or dysphagia.
202. The method of claim 200 or 201, wherein the subject is administered at least one dose of between approximately 0.1 mg and 1000 mg of cell-derived vesicle protein of the purified population.
203. The method of claim 200 or 201, wherein the subject is administered at least one dose of approximately 50 mg of cell-derived vesicle protein of the purified population.
204. The method of claim 200 or 201, wherein the purified population of cell derived vesicles is administered prior to or after administration of an isolated stem cell.
205. The method of claim 200 or 201, wherein the purified population of cell derived vesicles is administered simultaneously with an isolated stem cell.
206. The method of claim 200 or 201, wherein the purified population is administered by intravenous injection, intrathecal injection, direct injection, intramuscular injection, intracranial injection, or topically.
207. The method of claim 200 or 201, wherein the subject is a mammal, optionally a human patient.



FIGS. 1A, 1B



FIGS. 2A – 2D

Pathways Detected	Statistical Significance
<i>Gonadotropin</i>	*
<i>Angiogenesis</i>	*
<i>Proteasome</i>	
<i>Wnt</i>	*
<i>TGF-Beta</i>	*
<i>Parkinson's</i>	
<i>PDGF</i>	*
<i>Integrin</i>	*
<i>Inflammation</i>	*
<i>Huntington's</i>	
<i>Heterotrimeric Gq</i>	
<i>Heterotrimeric Gi</i>	
<i>FGF</i>	*
<i>EGF</i>	*
<i>Rho GTPase</i>	
<i>Cadherin</i>	*

FIG. 3

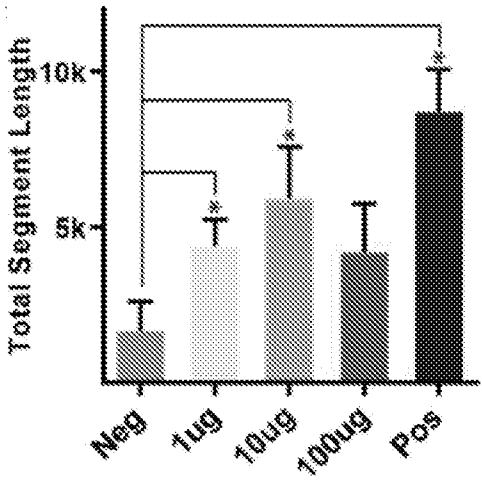


FIG. 4

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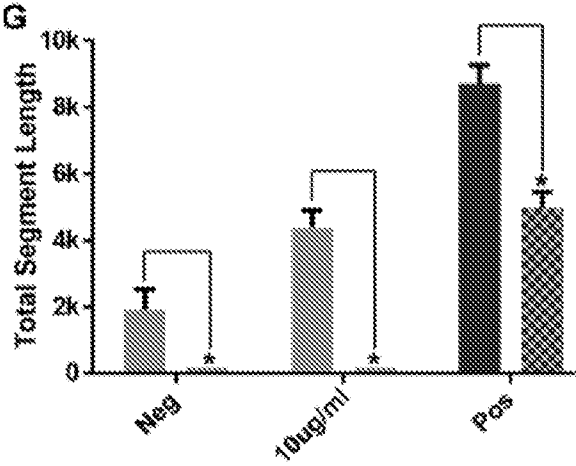


FIG. 5

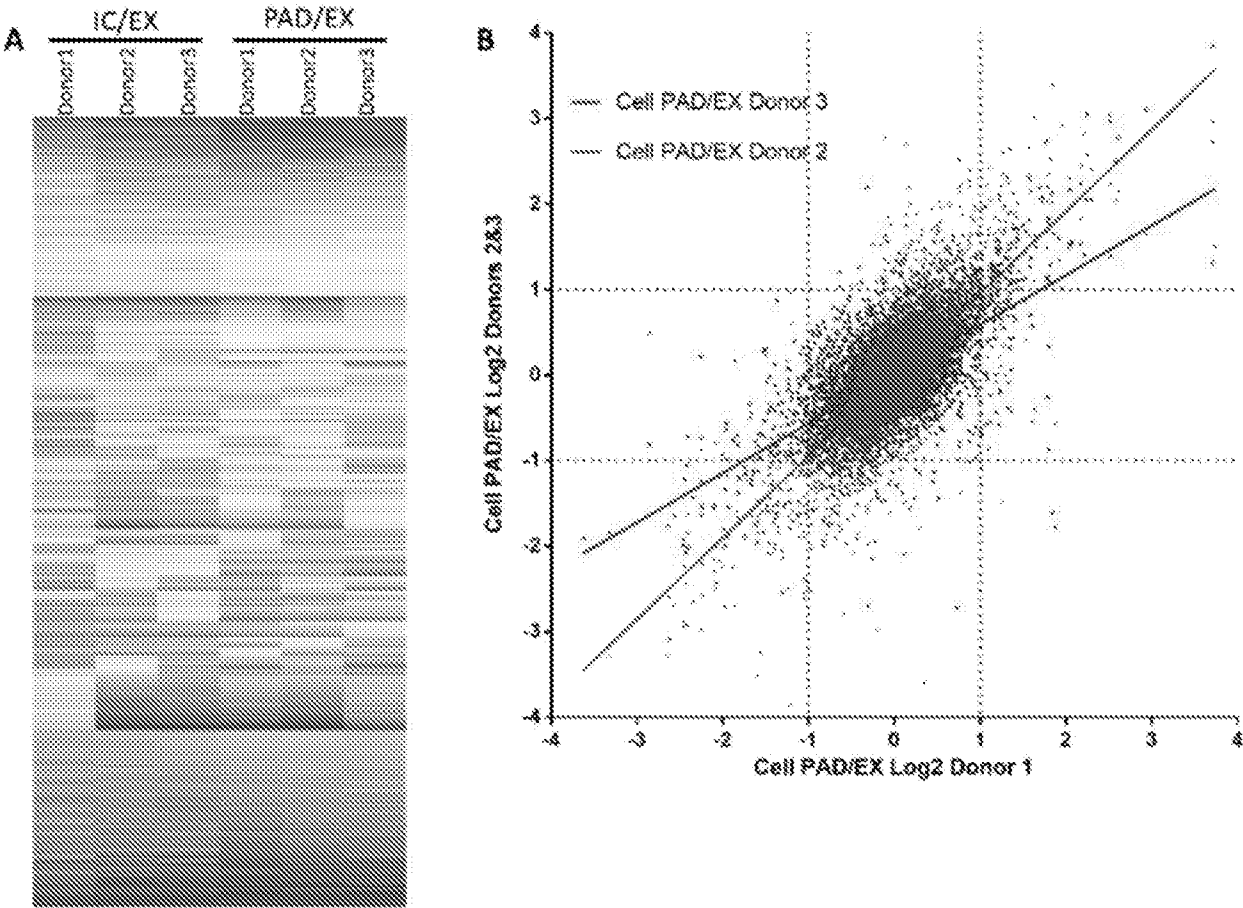


FIG. 6A, 6B

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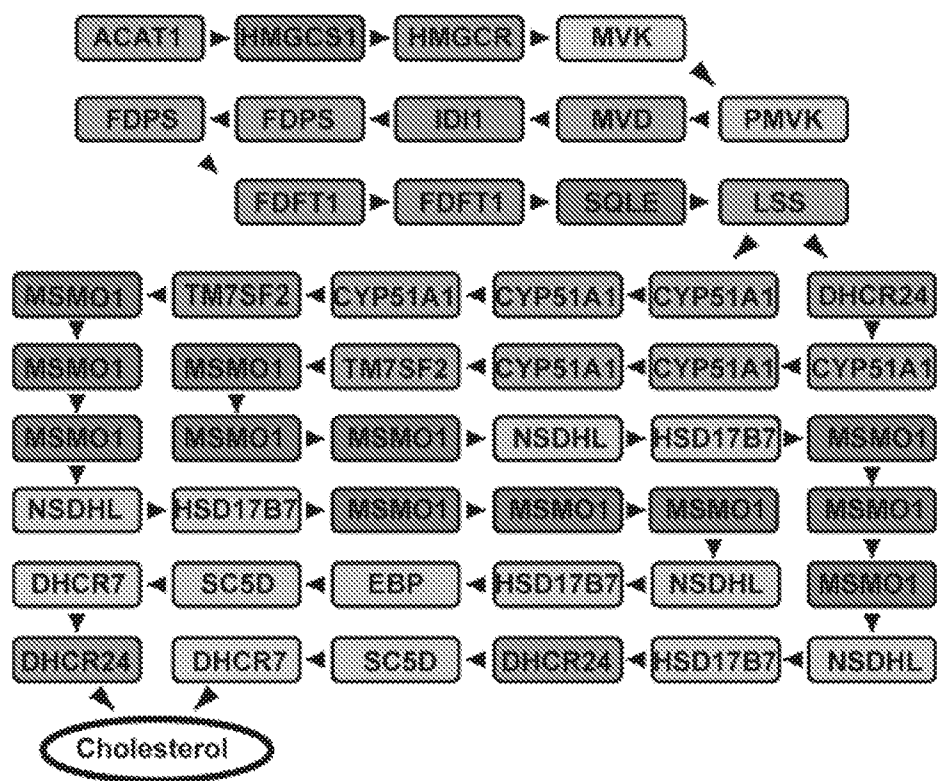


FIG. 7

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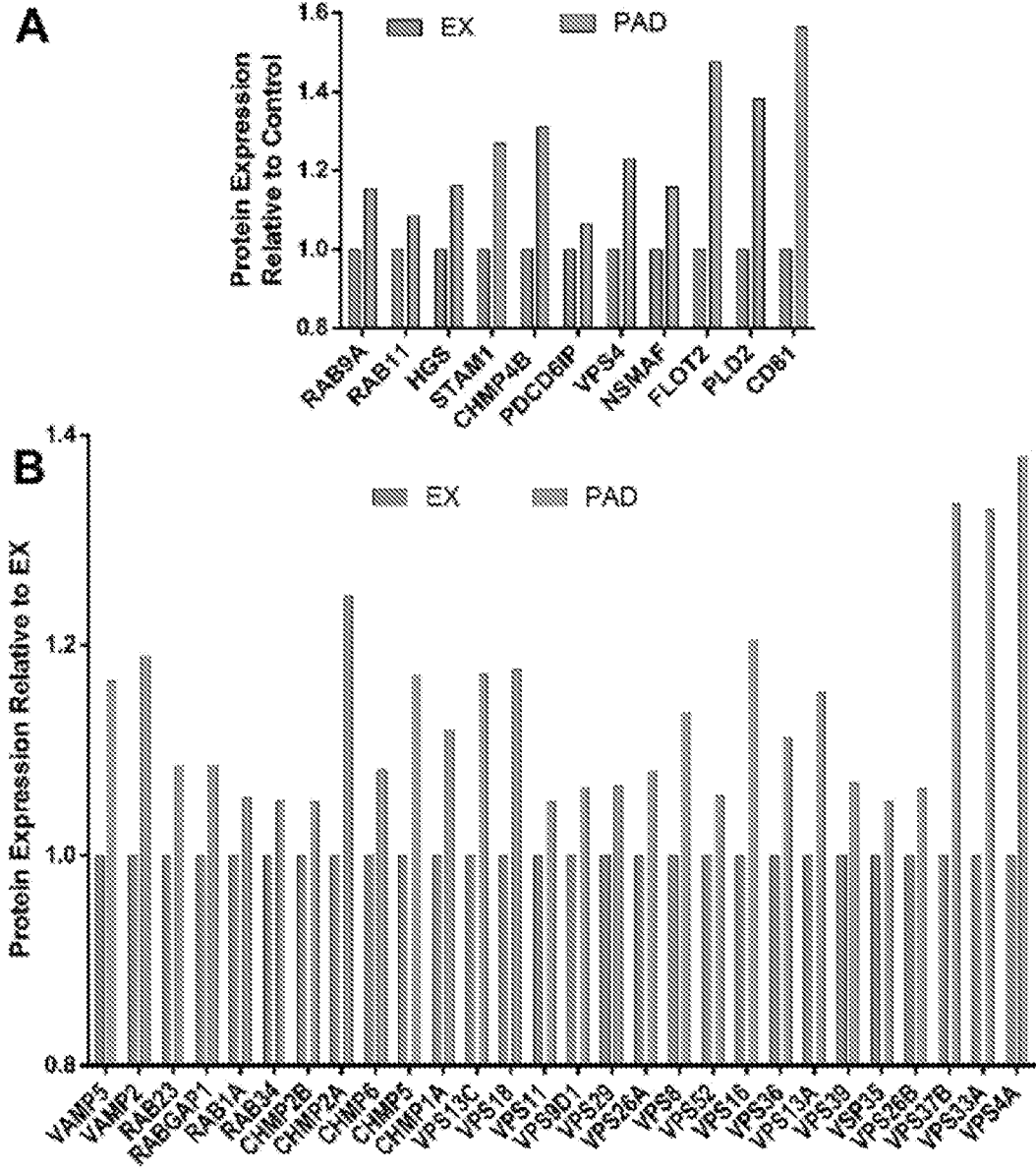


FIG. 8A, 8B

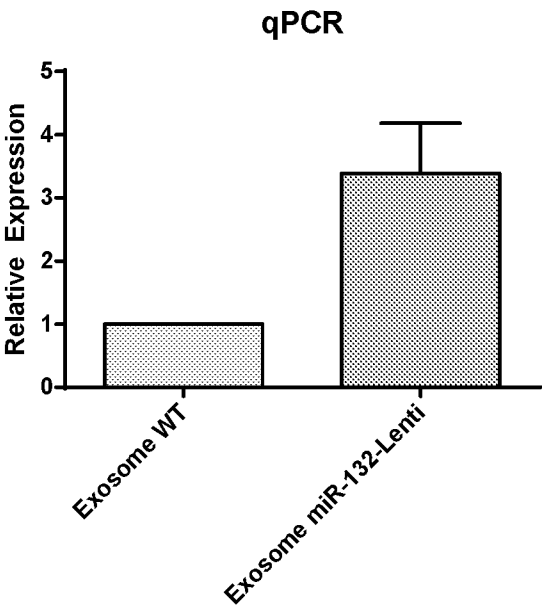


FIG. 9

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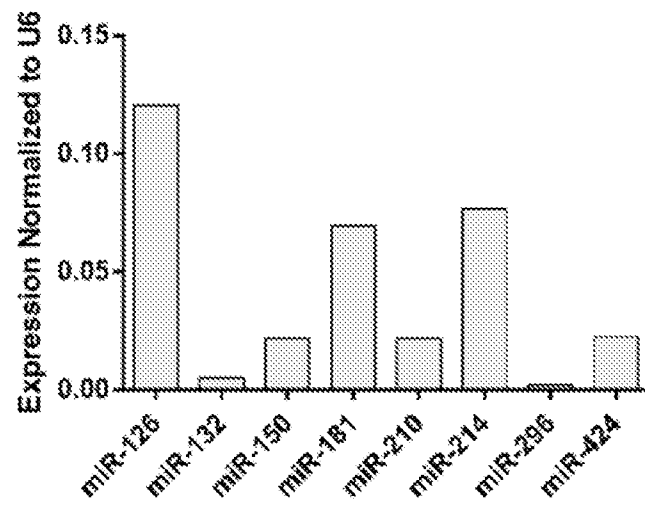


FIG. 10

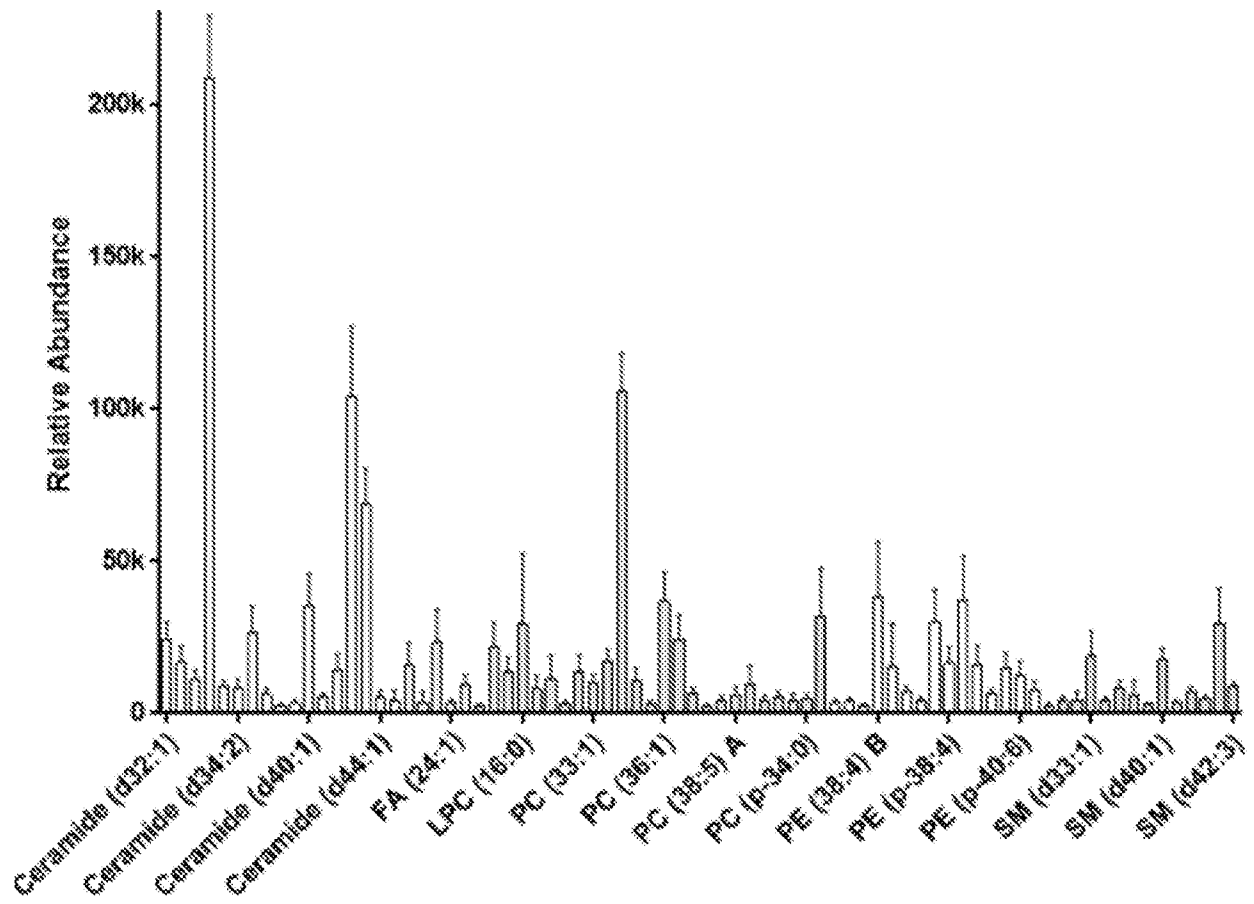


FIG. 11

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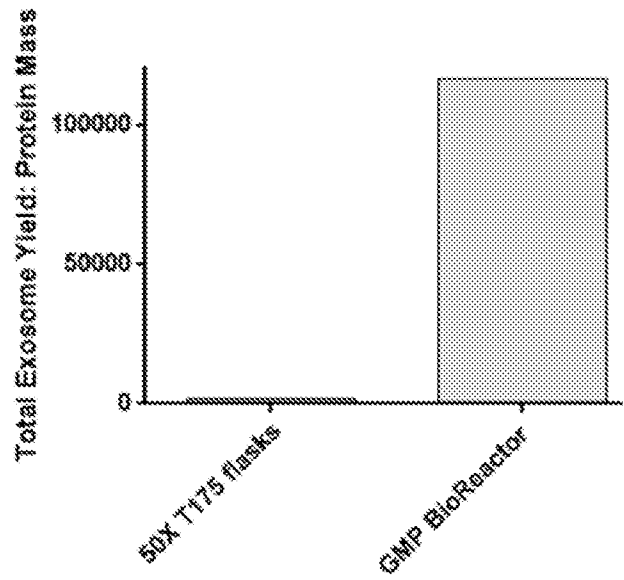


FIG. 12

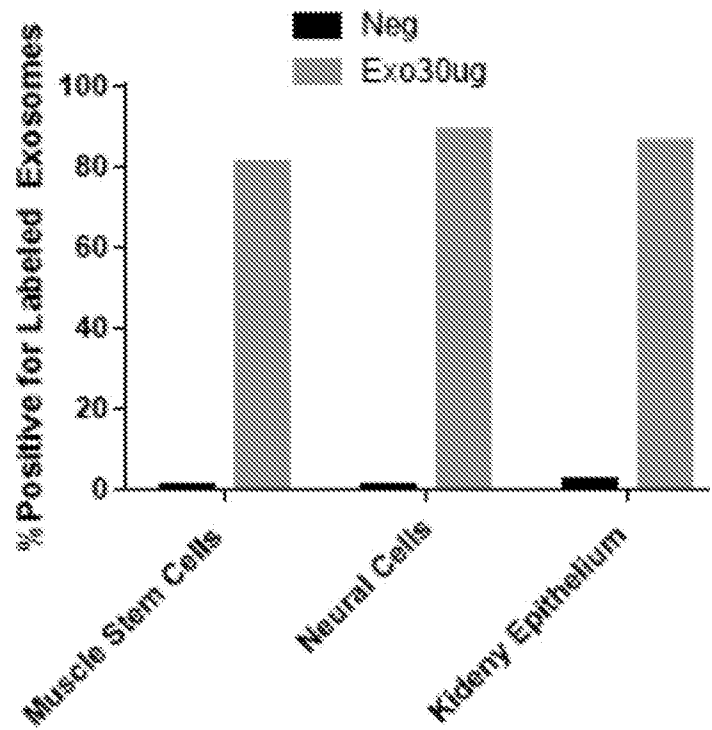


FIG. 13

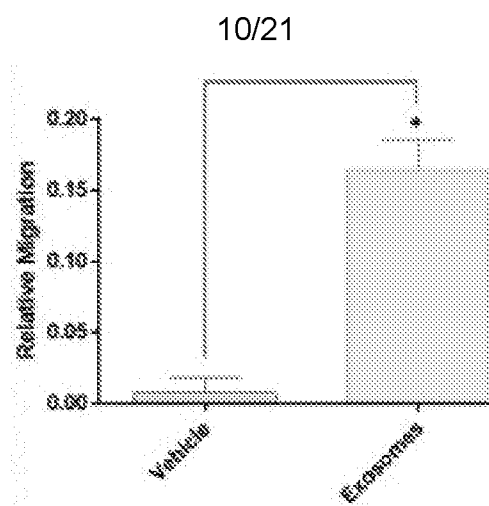


FIG. 14

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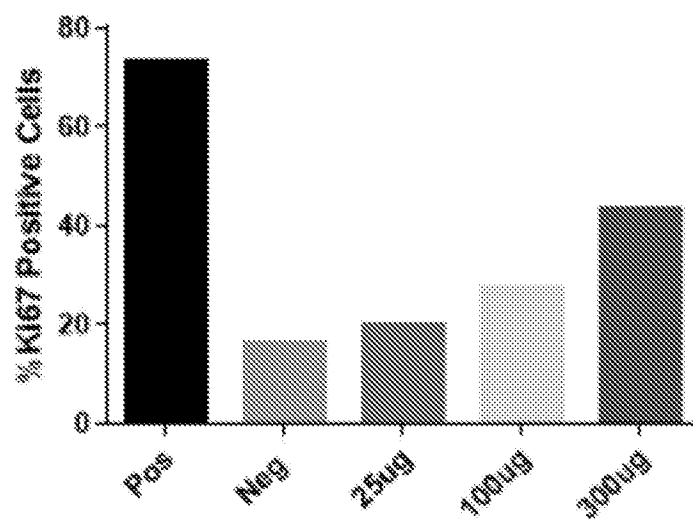


FIG. 15

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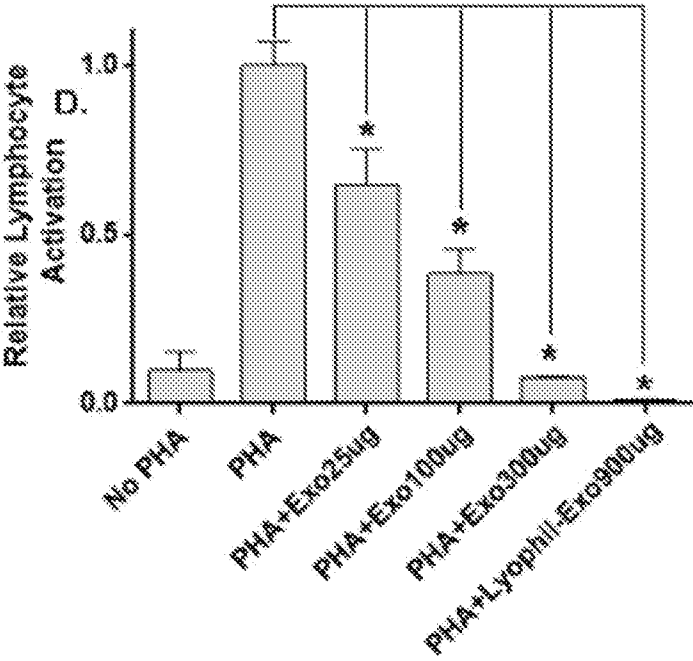


FIG. 16

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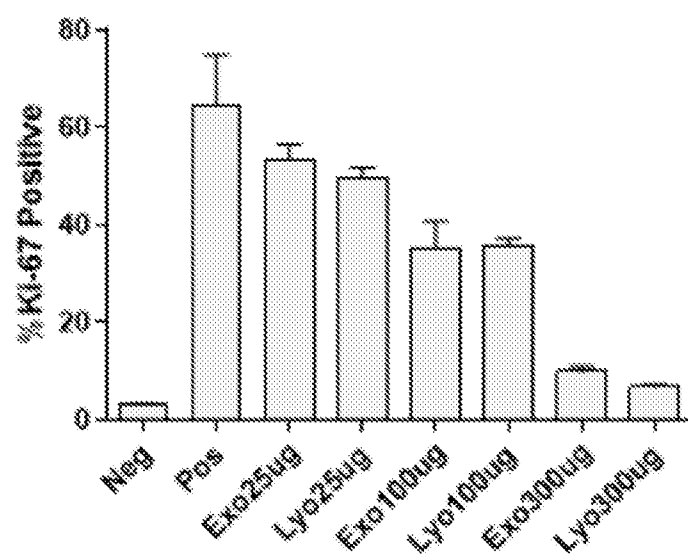


FIG. 17

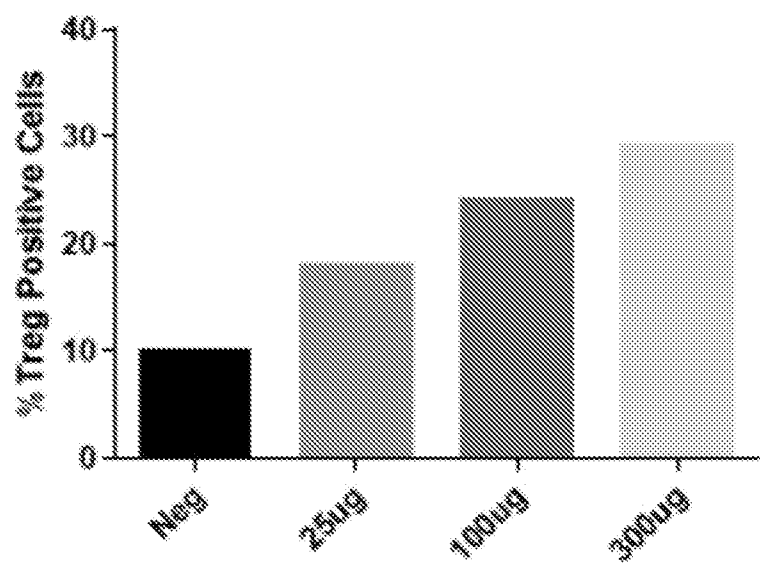


FIG. 18

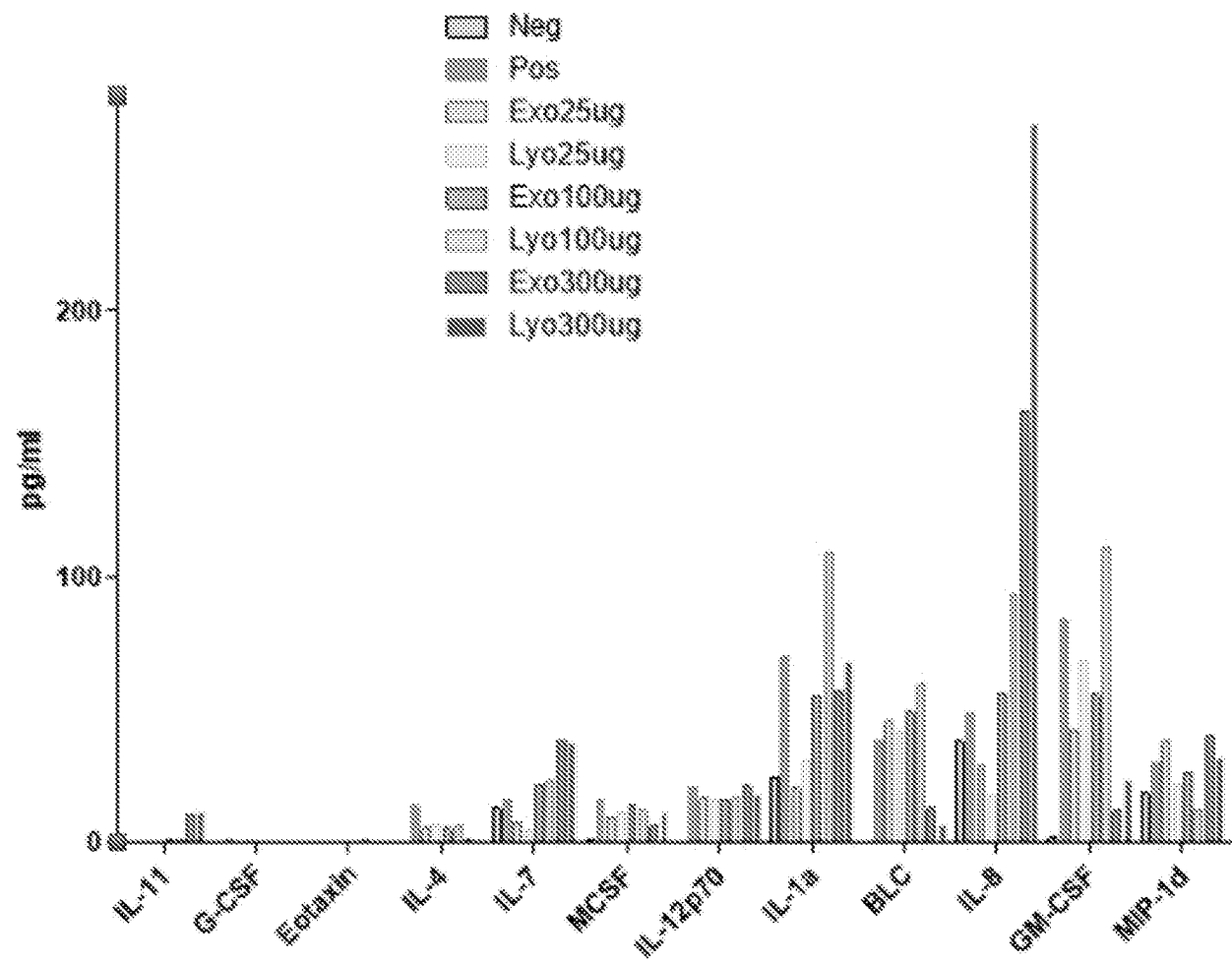


FIG. 19

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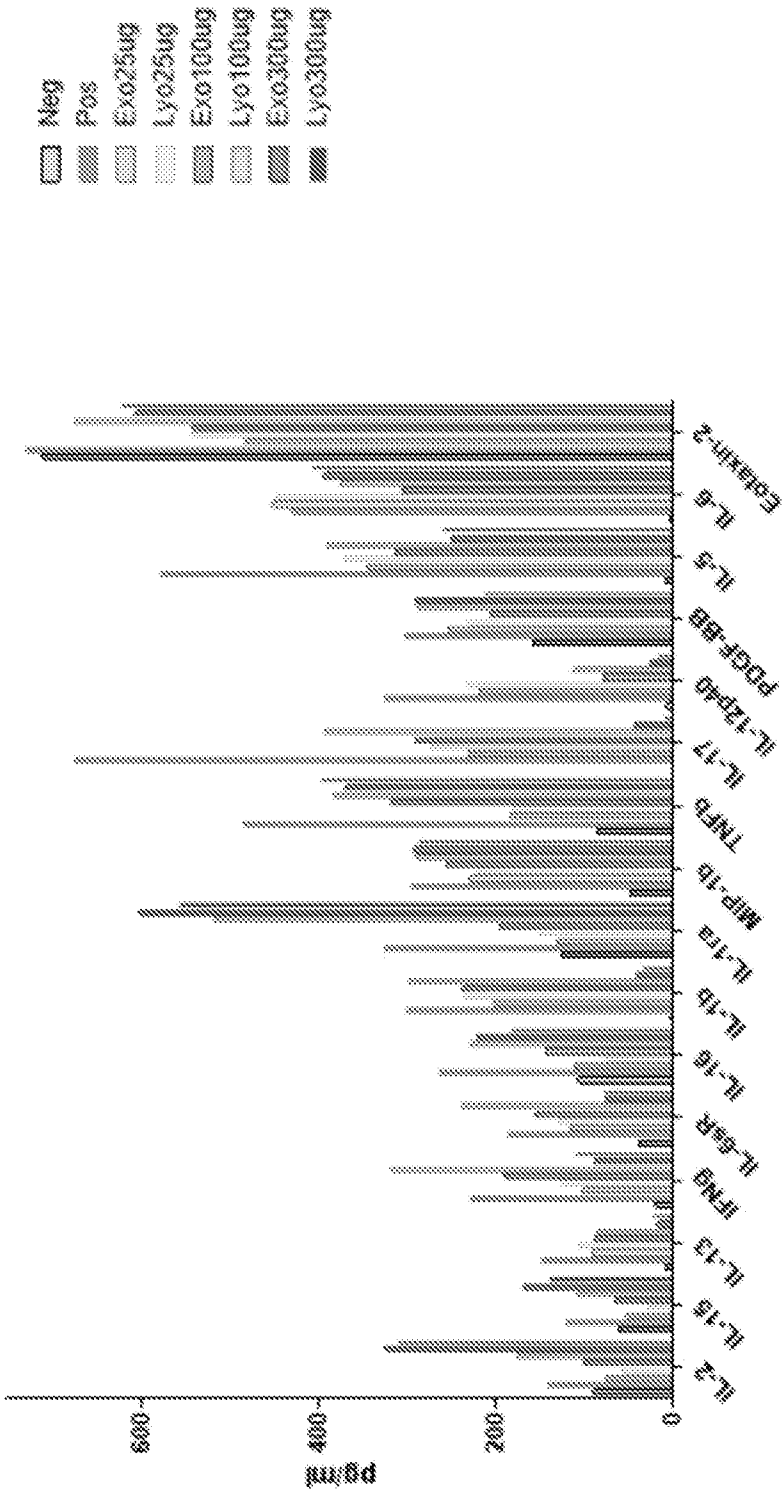


FIG. 20

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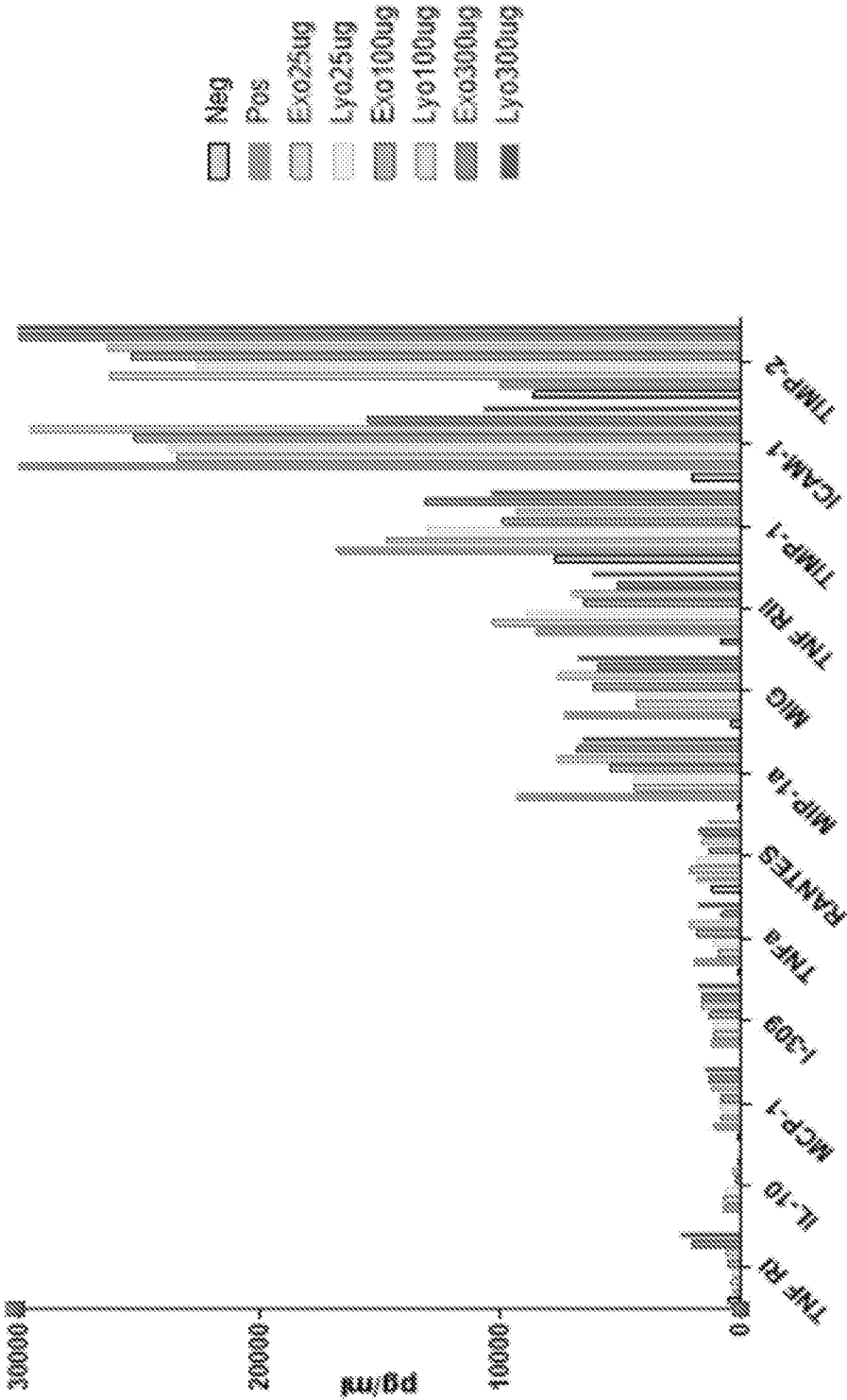


FIG. 21

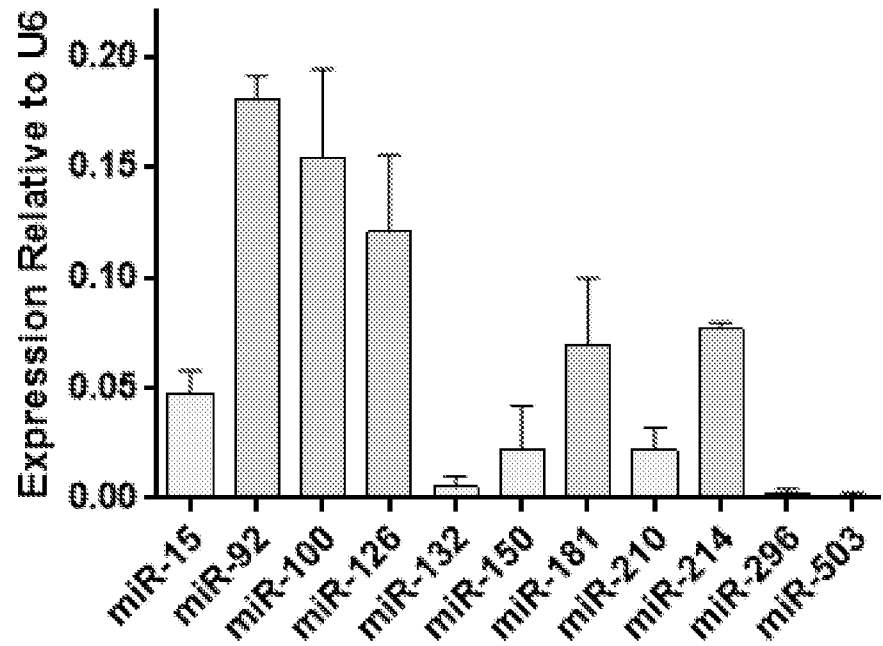


FIG. 22

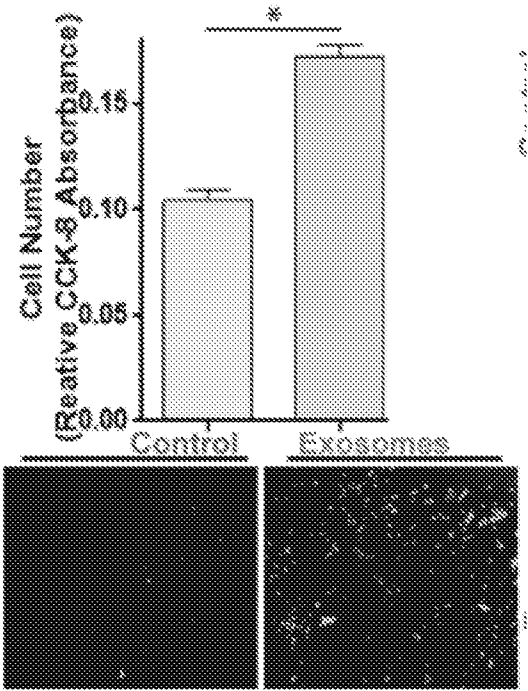


FIG. 23

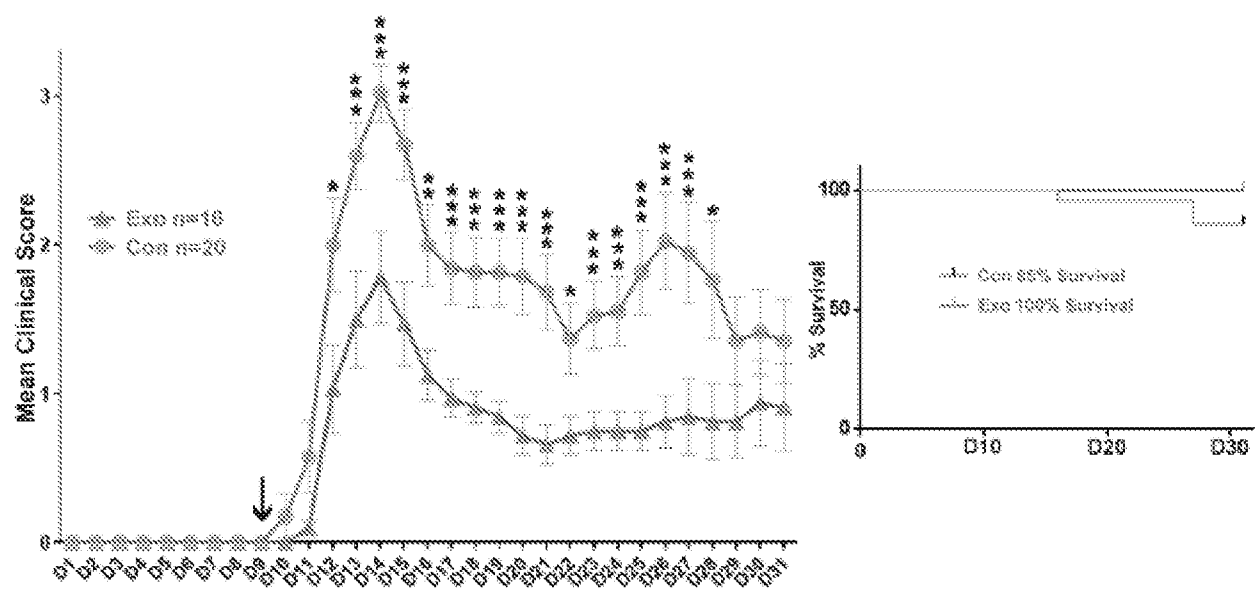


FIG. 24

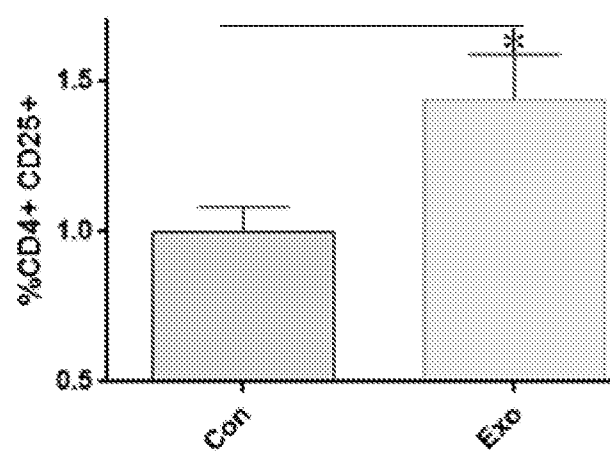


FIG. 25

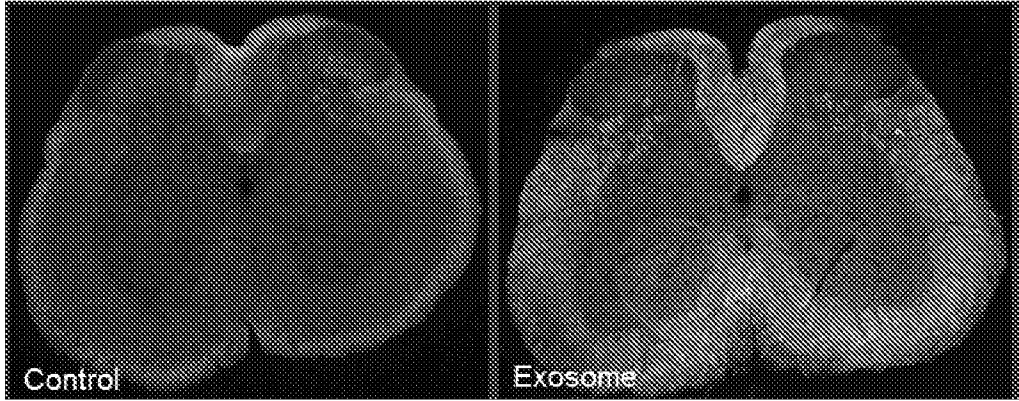


FIG. 26

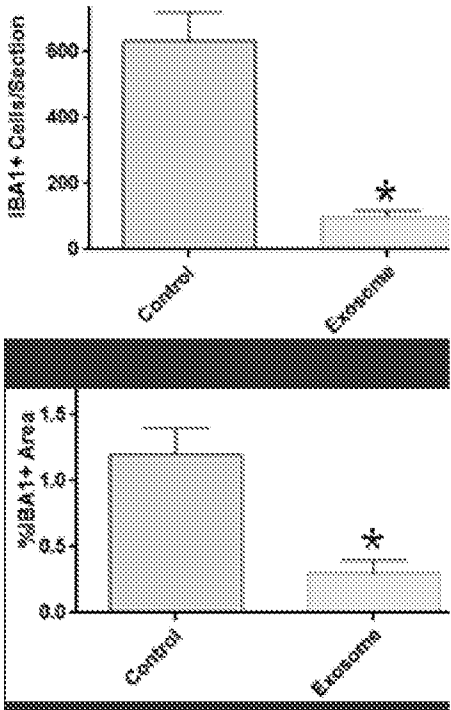


FIG. 27

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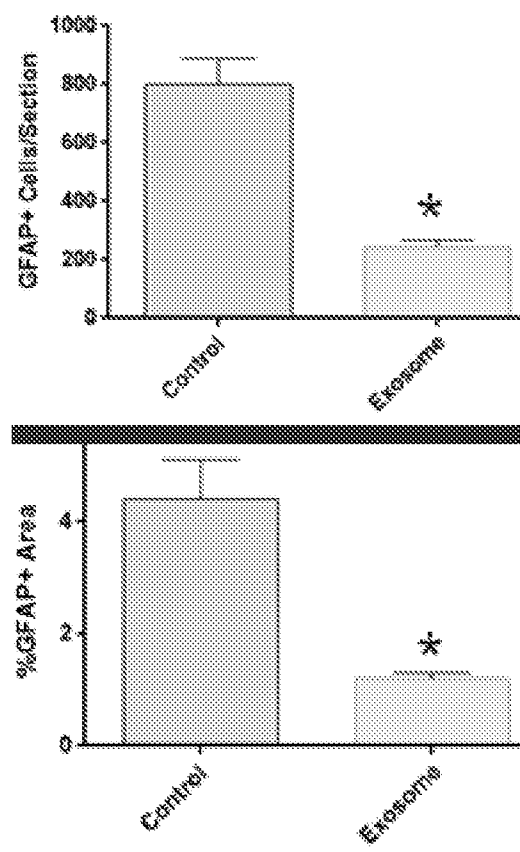


FIG. 28