



(51) International Patent Classification:

A61K 35/32 (2015.01) C12N 5/07 (2010.01)

(21) International Application Number:

PCT/IB2017/054927

(22) International Filing Date:

11 August 2017 (11.08.2017)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

62/374,560 12 August 2016 (12.08.2016) US

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

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ,

(54) Title: METHODS FOR MAKING AND USING THERAPEUTIC EXOSOMES

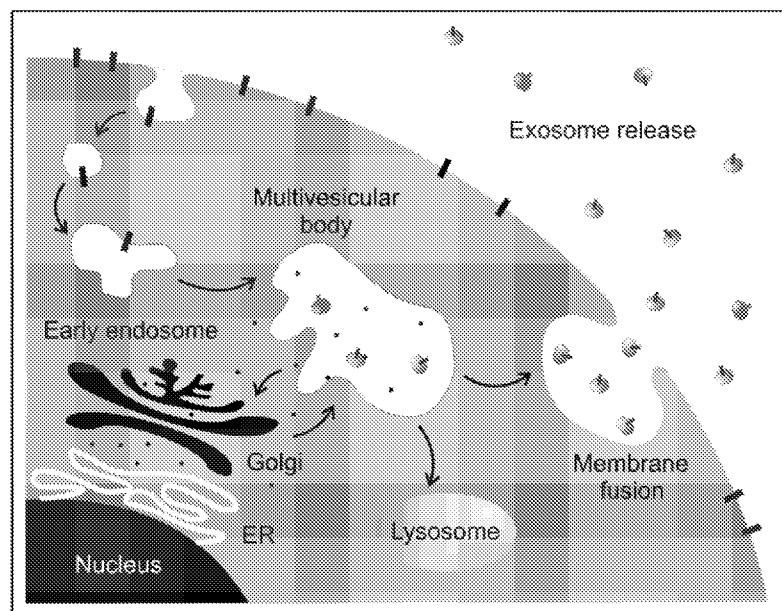


FIG. 1

(57) Abstract: Methods and compositions involving human exosomes or lipid nanovesicles are provided. For example, certain aspects relate to compositions comprising exosomes obtained from human cells that have been induced to undergo oxidative stress. Furthermore, some aspects of the invention provide methods of treating a subject at risk or having a demyelinating disorder using the compositions.





TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

- *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*

Published:

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

DESCRIPTION**METHODS FOR MAKING AND USING THERAPEUTIC EXOSOMES****CROSS-REFERENCE TO RELATED APPLICATIONS**

[0001] This application claims the benefit of priority of U.S. Provisional Patent
5 Application No. 62/374,560 filed August 12, 2016, which is hereby incorporated by reference
in its entirety.

STATEMENT OF GOVERNMENT SUPPORT

[0002] This invention was made with Government support under NS019108 and
TR000918, awarded by the National Institutes of Health. The Government has certain rights
10 in the invention.

1. Field of the Invention

[0003] The present invention relates generally to the field of medicine and neurology. In
particular, embodiments are directed to treatment of demyelinating disorders such as multiple
sclerosis (MS) and other neurological disorders associated with demyelination.

2. Description of Related Art

[0004] MS is a common neurological disease affecting more than 1 million people
worldwide. Its prevalence rate varies between races and geographical latitude, ranging from
more than 100 per 100,000 in Northern and Central Europe to 50 per 100,000 in Southern
Europe. MS is the most common cause of neurological disability in young and middle-aged
20 adults. Disease onset is before the age of 30 in about 50% of patients, between the ages of 30
to 40 in 25% of the patients, and between the ages of 40 to 50 in the remaining 25% of
patients. The female to male ratio is 2:1.

[0005] Neurological damage caused by MS can have a major physical, psychological,
social and financial impact on the patients and on their families. The most common clinical
25 symptoms of MS are paresis, paraesthesia, visual impairment, sexual, bowel, and urinary
dysfunction, spasticity, and incoordination. Cognitive dysfunction occurs in 40 to 50% of
patients. The extent of neurological deficit, rate of progression, and frequency of relapses are
highly variable among affected individuals.

[0006] Existing therapies for MS are designed to reduce inflammation and thus reduce
30 the degree of demyelination, and in some cases promoting remyelination. However, there

remains a need for a therapy that not only curtails demyelination, but also significantly promotes myelination and reduces oxidative stress (OS). Such a therapy would be instrumental in treating neurodegenerative disorders such as MS, as well as cognitive decline from normative aging and migraine.

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SUMMARY OF THE INVENTION

[0007] Aspects of the present invention overcome a major deficiency in the art by providing methods and compositions involving human-derived exosomes (which are referred to herein as exosomes, lipid nanovesicles, or nanovesicles) that promote myelination or repair demyelination, and can be modified or loaded to contain particular nucleic acid molecules (such as mRNAs and/or miRNAs) and/or proteins identified in these exosomes.

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[0008] Accordingly, aspects of the disclosure relate to a method of producing isolated exosomes from human cells comprising: a) inducing human dendritic cells to undergo external oxidative stress, wherein the cells produce exosomes; and b) isolating the produced exosomes.

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[0009] Further aspects relate to isolated exosomes produced by methods of the disclosure and pharmaceutical compositions comprising such exosomes. Further aspects relate to a method for treating a patient at risk for or having a demyelinating disorder comprising administering to the patient an effective amount of the pharmaceutical compositions or exosomes of the disclosure. Further method aspects relate to a method for treating a patient at risk for or having a demyelinating disorder comprising administering to the patient an effective amount of a pharmaceutical composition comprising isolated human-derived exosomes obtained from dendritic cells that have been induced to undergo oxidative stress, a method for increasing myelination in a patient at risk for or having a demyelinating disorder, the method comprising administering to the patient an effective amount of a pharmaceutical composition comprising isolated human-derived exosomes obtained from dendritic cells that have been induced to undergo oxidative stress, and/or a method for reducing or preventing spreading depression in a patient at risk for or having a demyelinating disorder, the method comprising administering to the patient an effective amount of a pharmaceutical composition comprising isolated human-derived exosomes obtained from dendritic cells that have been induced to undergo oxidative stress. The embodiments discussed below may be used in the method, composition, isolated exosomes, and isolated nanovesicle aspects described herein.

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[0010] In some embodiments, the dendritic cells are *in vitro* differentiated dendritic cells. In some embodiments, the dendritic cells are CD11b-. In some embodiments, the dendritic cells are CD11c+. In some embodiments, the dendritic cells are CD14-. The dendritic cells may be differentiated from a variety of sources such as wherein the dendritic cells are differentiated from human stem cells, human-derived stem cells, human progenitor cells, human induced pluripotent stem cells (iPSCs), human peripheral blood mononuclear cells (PBMCs), human bone marrow mononuclear cells (BMMCs), or human cord blood mononuclear cells (CBMCs). In some embodiments, the dendritic cells are differentiated from human stem cells, human derived stem cells, or human progenitor cells. In some embodiments, the human stem cells or human progenitor cells are cells isolated from bone marrow, cord blood, adipose tissue, or whole blood. In some embodiments, the dendritic cells are differentiated from BMMCs isolated from bone marrow or CBMCs isolated from cord blood. In some embodiments, the dendritic cells are differentiated from CD34+ cells. In some embodiments, the dendritic cells are differentiated from PBMCs isolated from whole blood. In some embodiments, the dendritic cells are differentiated from monocytes. In some embodiments, the dendritic cells are differentiated from iPSCs. In some embodiments, the iPSCs are derived from human fibroblasts. In some embodiments, the human fibroblasts are isolated from human dermal tissue or human adipose tissue. In some embodiments, the fibroblasts are from a skin biopsy. In some embodiments, the iPSCs are derived from human adipose stem cells, human adipose-derived stem cells, human keratinocytes, and human PBMCs.

[0011] In some embodiments, the method further comprises differentiating progenitor cells into dendritic cells.

[0012] In some embodiments, the progenitor cells are derived from human iPSCs. In some embodiments, the method further comprises contacting the cells with one or more of BMP4, VEGF, SCF, M-CSF, SCF, FL3, IL-3, TPO, GM-CSF, and IL-4. In some embodiments, the method comprises one or more sequential steps, wherein each step comprises or consists of a growth factor or cytokine described herein.

[0013] In some embodiments, differentiating the progenitor cells into dendritic cells comprises the sequential steps of: a. contacting the cells with GM-CSF, FL3, and M-CSF and b. contacting the cells with GM-CSF and IL-4. In some embodiments, differentiating the progenitor cells into dendritic cells comprises the sequential steps of: a. contacting the cells with M-CSF, SCF, FL3, IL-3, and TPO; b. contacting the cells with GM-CSF, FL3, and M-

CSF; and c. contacting the cells with GM-CSF and IL-4. In some embodiments, differentiating the progenitor cells into dendritic cells comprises the sequential steps of: a. contacting the cells with VEGF and SCF; b. contacting the cells with M-CSF, SCF, FL3, IL-3, and TPO; c. contacting the cells with GM-CSF, FL3, and M-CSF; and d. contacting the cells with GM-CSF and IL-4. In some embodiments, differentiating the progenitor cells into dendritic cells comprises the sequential steps of: a. contacting the cells with BMP4; b. contacting the cells with VEGF and SCF; c. contacting the cells with M-CSF, SCF, FL3, IL-3, and TPO; d. contacting the cells with GM-CSF, FL3, and M-CSF; and e. contacting the cells with GM-CSF and IL-4. In some embodiments, the method further comprises inducing pluripotent stem cells from human cells.

[0014] In some embodiments, the human cells comprise human fibroblasts, human adipose stem cells, human adipose-derived stem cells, human keratinocytes, or human PBMCs. In some embodiments, inducing pluripotent stem cells from human cells comprises contacting the cells with one or more of SOX2, KLF4, c-Myc, and LIN28.

[0015] In some embodiments, the progenitor cells are isolated from human mononuclear cells. In some embodiments, the human mononuclear cells are isolated from bone marrow or cord blood. In some embodiments, differentiating the progenitor cells into dendritic cells further comprises contacting the cells with one or more of GM-CSF, TNF α , and IL-4.

[0016] In some embodiments, differentiating the progenitor cells into dendritic cells comprises the sequential steps of: a. contacting the cells with GM-CSF and TNF α and b. contacting the cells with GM-CSF, TNF α , and IL4.

[0017] In some embodiments, differentiating the progenitor cells into dendritic cells comprises the sequential steps of: a. contacting the cells with a composition comprising BMP4, GM-CSF, SCF, and VEGF; b. contacting the cells with a composition comprising GM-CSF, SCF, and VEGF; wherein the composition excludes BMP4; c. contacting the cells with a composition comprising GM-CSF, and SCF; wherein the composition excludes BMP4 and VEGF; and d. contacting the cells with a composition comprising GM-CSF; wherein the composition excludes BMP4, VEGF, and SCF.

[0018] In some embodiments, differentiating the progenitor cells into dendritic cells comprises or further comprises the sequential steps of: a. contacting the cells with a composition comprising IL-4; and b. contacting the cells with a composition comprising IL-4 and GM-CSF.

[0019] In some embodiments, the progenitor cells are isolated from PBMCs. In some embodiments, the PBMCs are isolated from whole blood. In some embodiments, differentiating the progenitor cells into dendritic cells comprises contacting the cells with one or more of GM-CSF and IL4.

5 **[0020]** In some embodiments, inducing the cells to undergo oxidative stress comprises contacting the cells with IFN- γ . In some embodiments, inducing the cells to undergo oxidative stress comprises contacting the cells with a composition comprising IFN- γ , GM-CSF, and IL4. In some embodiments, the composition further comprises TNF α .

10 **[0021]** In some embodiments, the compositions described above and/or steps comprise contacting the cells with exosome-free serum.

[0022] In some embodiments differentiating progenitor cells into dendritic cells comprises the formation of embryoid bodies. In some embodiments, wherein the method further comprises or the formation of embryoid bodies comprises or further comprises detachment of the hiPSCs or progenitor cells from a substrate. In some embodiments, the
15 detachment of the hiPSCs or progenitor cells from the substrate comprises contacting the hiPSCs or progenitor cells with a protease. In some embodiments, the protease comprises dispase. In some embodiments, differentiating progenitor cells into dendritic cells comprises the maintenance of embryoid bodies in rotary orbital culture.. Rotary orbital culture is further described in Hookway et al., 2016, which is incorporated by reference.

20 **[0023]** In some embodiments, the method comprises one or more sequential steps, wherein each step comprises or consists of a growth factor or cytokine described herein. In some embodiments, the method comprises one or more sequential steps, wherein one or more growth factors and/or cytokines described herein are excluded in the one or more steps. In some embodiments, the compositions described herein exclude one or more components
25 described herein. In some embodiments, the compositions and/or steps described herein exclude any growth factors, cytokines, and/or proteins not specifically listed in the claim.

[0024] The growth factor or cytokine added in the method steps of the disclosure may be added to a final concentration of at least, at most, or exactly 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7,
30 1.8, 1.9, 2.0, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, 3.0, 3.1, 3.2, 3.3, 3.4, 3.5, 3.6, 3.7, 3.8, 3.9, 4.0, 4.1, 4.2, 4.3, 4.4, 4.5, 4.6, 4.7, 4.8, 4.9, 5.0, 5.1, 5.2, 5.3, 5.4, 5.5, 5.6, 5.7, 5.8, 5.9, 6.0, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, 7.0, 7.1, 7.2, 7.3, 7.4, 7.5, 7.6, 7.7, 7.8, 7.9, 8.0,

8.1, 8.2, 8.3, 8.4, 8.5, 8.6, 8.7, 8.8, 8.9, 9.0, 9.1, 9.2, 9.3, 9.4, 9.5, 9.6, 9.7, 9.8, 9.9, 10.0, 10.5, 11.0, 11.5, 12.0, 12.5, 13.0, 13.5, 14.0, 14.5, 15.0, 15.5, 16.0, 16.5, 17.0, 17.5, 18.0, 18.5, 19.0, 19.5, 20.0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 105, 110, 115, 120, 125, 130, 135, 140, 145, 150, 155, 160, 165, 170, 175, 180, 185, 190, 195, 200, 205, 210, 215, 220, 225, 230, 235, 240, 245, 250, 255, 260, 265, 270, 275, 280, 285, 290, 295, 300, 305, 310, 315, 320, 325, 330, 335, 340, 345, 350, 355, 360, 365, 370, 375, 380, 385, 390, 395, 400, 410, 420, 425, 430, 440, 445, 450, 460, 470, 475, 480, 490, 500, 510, 520, 525, 530, 540, 550, 560, 570, 575, 580, 590, 600, 610, 620, 625, 630, 640, 650, 660, 670, 675, 680, 690, 700, 710, 720, 725, 730, 740, 750, 760, 770, 775, 780, 790, 800, 810, 820, 825, 830, 840, 850, 860, 870, 875, 880, 890, 900, 910, 920, 925, 930, 940, 950, 960, 970, 975, 980, 990, 1000, 1100, 1200, 1300, 1400, 1500, 1600, 1700, 1800, 1900, 2000, 2100, 2200, 2300, 2400, 2500, 2600, 2700, 2800, 2900, 3000, 3100, 3200, 3300, 3400, 3500, 3600, 3700, 3800, 3900, 4000, 4100, 4200, 4300, 4400, 4500, 4600, 4700, 4800, 4900, 5000, 6000, 7000, 8000, 9000, 10000 pM, nM, μ M, or M (or any derivable range therein).

[0025] In some embodiments, the compositions of the disclosure are bovine-free or serum-free. In some embodiments, the methods exclude addition of bovine-containing products or include the addition of products/compositions that are serum-free.

[0026] In some embodiments, the compositions or sequential steps comprise contacting the cells for a defined period of time with the stated components. The defined period of time may be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, or 23 hours or 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50 days or any range derivable therein. During that time, the cells may be washed and/or re-plated with the same media.

[0027] In some embodiments, the method further comprises administering to a patient at risk for or having a demyelinating disorder an effective amount of a pharmaceutical composition comprising the isolated exosomes. In some embodiments, the dendritic cells are dendritic cells obtained from the patient or derived from cells obtained from the patient. In

some embodiments, the cells are obtained from the patient when the patient is less than 18 years old or when the patient is less than one year old or an age or age range described herein.

[0028] Further aspects of the disclosure relate to a method for treating a patient at risk for or having a demyelinating disorder comprising administering to the patient an effective amount of the pharmaceutical composition of the disclosure.

[0029] Yet further aspects relate to a method for treating a patient at risk for or having a demyelinating disorder comprising administering to the patient an effective amount of a pharmaceutical composition comprising isolated human-derived exosomes obtained from dendritic cells that have been induced to undergo oxidative stress.

[0030] Further aspects relate to a method for increasing myelination or for remyelination in a patient at risk for or having a demyelinating disorder, the method comprising administering to the patient an effective amount of a pharmaceutical composition comprising isolated human-derived exosomes obtained from dendritic cells that have been induced to undergo oxidative stress.

[0031] Further aspects relate to a method for reducing or preventing spreading depression in a patient at risk for or having a demyelinating disorder, the method comprising administering to the patient an effective amount of a pharmaceutical composition comprising isolated human-derived exosomes obtained from dendritic cells that have been induced to undergo oxidative stress.

[0032] In some embodiments, the dendritic cells are *in vitro* differentiated dendritic cells. In some embodiments, the dendritic cells are CD11b- and/or CD11c+ and/or CD1a+. The dendritic cells may be differentiated from a source described herein.

[0033] In some embodiments of the disclosure, the demyelinating disorder is cognitive decline, Alzheimer's disease, Parkinson's disease, stroke, epilepsy, migraine, traumatic brain injury, post-traumatic stress disorder, post-traumatic headache, multiple sclerosis, neuropathy, tauopathy, or ageing-induced cognitive decline. In some embodiments, the demyelinating disorder is multiple sclerosis or neuropathy. In some embodiments, the demyelinating disorder is migraine. In some embodiments, the migraine comprises migraine with aura. In some embodiments, the demyelinating disorder is traumatic brain injury.

[0034] A migraine can more specifically be a chronic migraine or episodic migraine, in some embodiments. In certain embodiments the migraine is with aura. Definitions of types of migraine are based on those set forth by the International Headache Society.

[0035] In some embodiments, a patient has been or will be treated with a drug to treat migraines, nausea, and/or pain. Drugs include, but are not limited to, triptans (for example, Almotriptan, Eletriptan, Frovatriptan, Naratriptan, Rizatriptan, Sumatriptan, Zolmitriptan), acetaminophen, dihydroergotamine, ergotamine tartrate, ibuprofen, and aspirin. Other drugs include those discussed in US Patent 9,399,053, which is hereby incorporated by reference for its disclosure of migraine treatment and migraines generally. In some embodiments, the patient is administered the composition nasally via inhalation or intravenously. In some embodiments, the patient is administered the composition by a route of administration described herein.

[0036] In some embodiments, the isolated exosomes have at least two different types of exosomes. In some embodiments, the cells have been induced to undergo oxidative stress by contact with IFN- γ . In some embodiments, the cells have been induced to undergo oxidative stress by a molecule or composition described herein.

[0037] In some embodiments, the isolated exosomes comprise at least an externally added therapeutic agent. In some embodiments, the externally added therapeutic agent is an siRNA. In some embodiments, the isolated exosomes comprise miR-219, miR-138, or miR-199a. In some embodiments, the isolated exosomes comprise mRNA encoding antioxidant system proteins.

[0038] Further embodiments comprise testing the exosomes for the presence or absence of a nucleic acid. In some embodiments, the nucleic acid comprises a miRNA or mRNA described herein. Further embodiments of the method or composition aspects comprise testing a population of cells for markers, such as cell-surface markers and/or dendritic markers. In some embodiments, the cell marker is one described herein. Further embodiments of the disclosure relate to isolating and/or enriching cells based on one or more cell markers.

[0039] Non-limiting examples of the demyelinating disorder include cognitive decline from aging, Alzheimer's disease, Parkinson's disease, stroke, epilepsy, migraine (acute, chronic or recurring), multiple sclerosis, post-traumatic stress disorder, post-traumatic headach, tauopathy, neuropathy, and ageing-induced cognitive decline. Also specifically contemplated are traumatic and ischemic brain injury, which can result in a significant loss of myelin. In particular examples, the demyelinating disorder is multiple sclerosis, neuropathy, traumatic brain injury, or neonatal brain injury.

[0040] Aspects of the disclosure relate to compositions comprising exosomes or nanovesicles, and the limitations described herein, as they relate to the exosome, nanovesicle, or composition, may be combined to form various embodiments. Furthermore, one or more embodiments or aspects described herein may be excluded from certain disclosed aspects of the disclosure.

[0041] In some embodiments, the compositions can be administered to a subject by any method known to those of ordinary skill in the art. Examples include intravenously, nasally, intradermally, intraarterially, intraperitoneally, intralesionally, intracranially, intraarticularly, intraprostatically, intrapleurally, intratracheally, intravitreally, intravaginally, intrarectally, topically, intratumorally, intramuscularly, subcutaneously, subconjunctival, intravesicularly, mucosally, intrapericardially, intraumbilically, intraocularly, orally, topically, locally, injection, infusion, continuous infusion, localized perfusion bathing target cells directly, via a catheter, via a lavage, directly into a heart chamber, directly injected into the organ or portion of organ or diseased site of interest, or by other method or any combination of the foregoing as would be known to one of ordinary skill in the art. In a particular aspect, the composition can be administered nasally or intravenously. In some embodiments, the composition is a liquid. In other embodiments, the composition is a gel or a powder. It is specifically contemplated that the composition may be a liquid that is provided to the patient as a mist.

[0042] Methods may involve administering a composition containing (or a composition comprising) about, at least about, or at most about 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2.0, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, 3.0, 3.1, 3.2, 3.3, 3.4, 3.5, 3.6, 3.7, 3.8, 3.9, 4.0, 4.1, 4.2, 4.3, 4.4, 4.5, 4.6, 4.7, 4.8, 4.9, 5.0, 5.1, 5.2, 5.3, 5.4, 5.5, 5.6, 5.7, 5.8, 5.9, 6.0, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, 7.0, 7.1, 7.2, 7.3, 7.4, 7.5, 7.6, 7.7, 7.8, 7.9, 8.0, 8.1, 8.2, 8.3, 8.4, 8.5, 8.6, 8.7, 8.8, 8.9, 9.0, 9.1, 9.2, 9.3, 9.4, 9.5, 9.6, 9.7, 9.8, 9.9, 10.0, 10.5, 11.0, 11.5, 12.0, 12.5, 13.0, 13.5, 14.0, 14.5, 15.0, 15.5, 16.0, 16.5, 17.0, 17.5, 18.0, 18.5, 19.0, 19.5, 20.0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 105, 110, 115, 120, 125, 130, 135, 140, 145, 150, 155, 160, 165, 170, 175, 180, 185, 190, 195, 200, 205, 210, 215, 220, 225, 230, 235, 240, 245, 250, 255, 260, 265, 270, 275, 280, 285, 290, 295, 300, 305, 310, 315, 320, 325, 330, 335, 340, 345, 350, 355, 360, 365,

370, 375, 380, 385, 390, 395, 400, 410, 420, 425, 430, 440, 445, 450, 460, 470, 475, 480, 490, 500, 510, 520, 525, 530, 540, 550, 560, 570, 575, 580, 590, 600, 610, 620, 625, 630, 640, 650, 660, 670, 675, 680, 690, 700, 710, 720, 725, 730, 740, 750, 760, 770, 775, 780, 790, 800, 810, 820, 825, 830, 840, 850, 860, 870, 875, 880, 890, 900, 910, 920, 925, 930, 940, 950, 960, 970, 975, 980, 990, 1000, 1100, 1200, 1300, 1400, 1500, 1600, 1700, 1800, 1900, 2000, 2100, 2200, 2300, 2400, 2500, 2600, 2700, 2800, 2900, 3000, 3100, 3200, 3300, 3400, 3500, 3600, 3700, 3800, 3900, 4000, 4100, 4200, 4300, 4400, 4500, 4600, 4700, 4800, 4900, 5000, 6000, 7000, 8000, 9000, 10000 nanograms (ng), micrograms (mcg), milligrams (mg), or grams of exosomes, or any range derivable therein. The above numerical values may also be the dosage that is administered to the patient based on the patient's weight, expressed as ng/kg, mg/kg, or g/kg, and any range derivable from those values.

[0043] Alternatively, the composition may have a concentration of exosomes that are 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2.0, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, 3.0, 3.1, 3.2, 3.3, 3.4, 3.5, 3.6, 3.7, 3.8, 3.9, 4.0, 4.1, 4.2, 4.3, 4.4, 4.5, 4.6, 4.7, 4.8, 4.9, 5.0, 5.1, 5.2, 5.3, 5.4, 5.5, 5.6, 5.7, 5.8, 5.9, 6.0, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, 7.0, 7.1, 7.2, 7.3, 7.4, 7.5, 7.6, 7.7, 7.8, 7.9, 8.0, 8.1, 8.2, 8.3, 8.4, 8.5, 8.6, 8.7, 8.8, 8.9, 9.0, 9.1, 9.2, 9.3, 9.4, 9.5, 9.6, 9.7, 9.8, 9.9, 10.0, 10.5, 11.0, 11.5, 12.0, 12.5, 13.0, 13.5, 14.0, 14.5, 15.0, 15.5, 16.0, 16.5, 17.0, 17.5, 18.0, 18.5, 19.0, 19.5, 20.0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 105, 110, 115, 120, 125, 130, 135, 140, 145, 150, 155, 160, 165, 170, 175, 180, 185, 190, 195, 200, 205, 210, 215, 220, 225, 230, 235, 240, 245, 250, 255, 260, 265, 270, 275, 280, 285, 290, 295, 300, 305, 310, 315, 320, 325, 330, 335, 340, 345, 350, 355, 360, 365, 370, 375, 380, 385, 390, 395, 400, 410, 420, 425, 430, 440, 441, 450, 460, 470, 475, 480, 490, 500, 510, 520, 525, 530, 540, 550, 560, 570, 575, 580, 590, 600, 610, 620, 625, 630, 640, 650, 660, 670, 675, 680, 690, 700, 710, 720, 725, 730, 740, 750, 760, 770, 775, 780, 790, 800, 810, 820, 825, 830, 840, 850, 860, 870, 875, 880, 890, 900, 910, 920, 925, 930, 940, 950, 960, 970, 975, 980, 990, 1000 ng/ml, µg/ml, mg/ml, or g/ml, or any range derivable therein.

[0044] In some embodiments, the composition may have at least, at most, or exactly, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, , 21, 22, 23, 24, 25, 26, 27, 28, 29,

30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 105, 110, 115, 120, 125, 130, 135, 140, 145, 150, 155, 160, 165, 170, 175, 180, 185, 190, 195, 200, 205, 210, 215, 220, 225, 230, 235, 240, 245, 250, 255, 260, 265, 270, 275, 280, 285, 290, 295, 300, 305, 310, 315, 320, 325, 330, 335, 340, 345, 350, 355, 360, 365, 370, 375, 380, 385, 390, 395, 400, 410, 420, 425, 430, 440, 441, 450, 460, 470, 475, 480, 490, 500, 510, 520, 525, 530, 540, 550, 560, 570, 575, 580, 590, 600, 610, 620, 625, 630, 640, 650, 660, 670, 675, 680, 690, 700, 710, 720, 725, 730, 740, 750, 760, 770, 775, 780, 790, 800, 810, 820, 825, 830, 840, 850, 860, 870, 875, 880, 890, 900, 910, 920, 925, 930, 940, 950, 960, 970, 975, 980, 990, 1000, 1200, 1400, 1600, 1800, 2000, 3000, 4000, 5000, 6000, 7000, 8000, 9000, 10000, 20000, 30000, 50000, or 100000 (or any derivable range therein) copies of a particular nucleic acid (eg. miRNA described herein).

[0045] In some embodiments, the composition may have at least, at most, or exactly, about 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 110, 120, 130, 140, 150, 200, 300, 400, 500, or 1000 fold more or less nucleic acid content than a naturally derived exosome or than an exosome isolated from a mammal.

[0046] The composition may be administered to (or taken by) the patient 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more times, or any range derivable therein, and they may be administered every 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 hours, or 1, 2, 3, 4, 5, 6, 7 days, or 1, 2, 3, 4, 5 weeks, or 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 months, or any range derivable therein. It is specifically contemplated that the composition may be administered once daily, twice daily, three times daily, four times daily, five times daily, or six times daily (or any range derivable therein) and/or as needed to the patient. Alternatively, the composition may be administered every 2, 4, 6, 8, 12 or 24 hours (or any range derivable therein) to or by the patient. In some embodiments, the patient is administered the composition for a certain period of time or with a certain number of doses after experiencing symptoms of a demyelinating disorder.

[0047] In certain embodiments, the isolated exosomes may include one type or at least two, three, four, five, six, seven, eight, nine, ten or more different types of exosomes (or any range derivable therein). The type of exosomes may be characterized by their compositions, for example, the types of nucleic acids and/or proteins of interest or effects.

[0048] The cells for producing exosomes can be any cells of one or more human subjects. For example, the cells may be immune cells, neural cells, or adipose cells. In particular aspects, the cells may be immune cells, such as dendritic cells, including dendritic cells that have been differentiated *in vitro* from human cell progenitor sources as described herein, lymphocytes (T cells or B cells), macrophages, or any cells of the immune system.

[0049] In a particular aspect, the cells for producing exosomes can be neural cells, such as microglia, astrocytes, neurons, oligodendrocytes, spindle neurons or any cells of the nervous systems. The cells can be in the form of a cell culture, a dissected tissue, or parts thereof. For example, the cells can be in the form of hippocampal slice cultures.

[0050] In a certain embodiment, the composition may be an autologous composition or the cells may be obtained from the same patient to be treated. Particularly, cells from a human subject may be harvested and cultured, and induced, stimulated or engineered to secrete an effective exosome-containing composition according to certain aspects of the invention. The exosome-containing composition may be then administered in a pharmaceutical composition to the same human donor.

[0051] In this particular embodiment, all the advantages of the autologous donation can apply. The skilled person will choose the nature and identity of the donor tissue or cells for exosome production depending on the use and as is expedient. Here, it may be necessary to consider the criteria and the advantages relevant for the decision to use autologous donation, and/or the choice of donor tissue/cells.

[0052] In another embodiment, the composition may be allogenic, that is to that the donor organism that provides exosome-producing cells and recipient organism to be treated are the same species but different individuals.

[0053] In an alternative embodiment, the composition may be xenogenic. This means that it is taken from an organism of a different species. For this purpose, cells are taken from a donor organism, for example an animal such as a, cow, pig, rat or yeast, and are induced, stimulated or engineered to produce an effective exosome-containing composition, which is administered in a pharmaceutical composition to the individual to be treated which belongs to a different species, for example a human.

[0054] In another embodiment, the composition may be obtained from autologous, allogenic, or xenogenic cells that have been preserved *ex vivo* and/or cultured *in vitro*.

[0055] The cells for producing exosomes may be obtained from a subject that is relatively young, for example, at an age that is at most one tenths, one fifths, one third, or half of the subject's expected life span. For example, the cells may be obtained from a human that is at most, less than or about one, two, three, four, five, six, seven, eight, nine, ten, 11, 12 months, or 1, 2, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 35, 40, 45, or 50 years old, or any age or range derivable therein. In a particular aspect, the exosomes may be obtained from a human that is less than one year old or less than 18 years old. In a particular aspect, the exosomes may be obtained from a human that is between 18 and 50 years old. The human may be the same patient that is to be treated.

[0056] Furthermore, in some aspects, the isolated exosomes or nanovesicles (e.g., the artificially engineered exosomes from *in vitro* reconstitution) may contain endogenous exosomes or may be loaded with externally added therapeutic agents, such as nucleic acids or protein molecules. In some embodiments, the nanovesicle is a liposome. The nucleic acids may be DNA or RNA, such as siRNA, miRNA, or mRNA. In certain aspects, the isolated exosomes may comprise miRNAs (mature or immature) such as 1, 2, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35 (or any range derivable therein) or more of miR-7a, miR-9, miR-9*, miR-17, miR-18a, miR-19a, miR-19b, miR-20a, miR-92a-1, miR-23a, miR-23a*, miR-23b, miR-32, miR-128, miR-138, miR-138*, miR-184, miR-199a-5p, miR-214, miR-219, miR-338, miR-338*, miR-27a, miR-27b, miR-106a, miR-124, miR-141, miR-144, miR-145, miR-146a, miR-181a, miR-200a, miR-451, miR-532-5p, and miR-665. In other embodiments, it is specifically contemplated that any one of these miRNAs or a combination of these miRNAs may be excluded as an embodiment. In certain aspects, the isolated exosomes may comprise proteins or mRNAs that encode antioxidant system proteins or miRNAs involved in oxidant/antioxidant homeostasis. In some embodiments, the externally added therapeutic agent is an engineered siRNA, mRNA, or miRNA (or combination of these agents) involved in oligodendrocyte differentiation and/or oxidant/antioxidant homeostasis that is added to naïve exosomes.

[0057] In certain aspects, the nanovesicles or loaded exosomes may comprise miRNAs such as one or more of miR-219, miR-138, miR-199a-5p, and/or miR-338. In certain aspects, the nanovesicles may comprise proteins or mRNAs that encode antioxidant system proteins.

[0058] The mRNA may encode antioxidant system proteins, such as enzymatic antioxidants (e.g., superoxide dismutase (SOD) or secreted antioxidants (e.g., glutathione). The skilled artisan will understand that methods for direct loading of agents, chimeric loading

of agents, or indirect loading (through modification of the producing cells) may be used. A particular example of direct loading may be via electroporation.

[0059] There may also be provided methods for obtaining isolated exosomes of cells. For example, the cells may be human cells. In some embodiments, the cells may be immune
5 cells. The methods may involve obtaining the cells that have been induced to undergo oxidative stress or stimulated via oxidative stress, where the cells produce exosomes. The methods may further involve isolating the produced exosomes.

[0060] In some embodiments, the cells are dendritic cells. In some embodiments, the dendritic cells are derived from human stem cells, human-derived stem cells, human
10 progenitor cells or human-derived progenitor cells. In some embodiments, the cells are bone marrow-derived, cord blood-derived or adipose-derived stem cells. In some embodiments the cells are bone marrow-derived, cord blood-derived or adipose-derived dendritic cells. In some embodiments, the cells are derived from *in vitro* cultured stem cells or dendritic cells. In some embodiments, the method further comprises differentiating the stem cells into
15 dendritic cells.

[0061] As described above, the isolated exosomes may be comprised in pharmaceutical compositions for the treating of a patient at risk for or having a demyelinating disorder, such as cognitive decline (e.g, from aging), Alzheimer's disease, Parkinson's disease, stroke, epilepsy, migraine, multiple sclerosis, neuropathy, traumatic brain injury, post traumatic
20 stress disorder, post-traumatic headache, and neonatal brain injury. In particular examples, the demyelinating disorder is multiple sclerosis, neuropathy, migraine, traumatic brain injury, or neonatal brain injury.

[0062] In certain aspects, the method may further comprise culturing the cells under conditions to induce oxidative stress before the isolation of exosomes. The oxidative stress
25 can be induced by an externally added cytokine, such as IFN- γ , by any other activating cytokines such as tumor necrosis factor alpha, or by an oxidant such as hydrogen peroxide. In other aspects, the compositions may comprise lipid nanovesicles that contain the same types or substantially similar types of nucleic acids such as mRNA, miRNAs, or proteins as those found in the isolated exosomes. The miRNAs may be 1, 2, 4, 5, 6, 7, 8, 9, 10, 11, 12,
30 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35 (or any range derivable therein) or more of miR-7a, miR-9, miR-9*, miR-17, miR-18a, miR-19a, miR-19b, miR-20a, miR-92a-1, miR-23a, miR-23a*, miR-23b, miR-32, miR-128, miR-138,

miR-138*, miR-184, miR-199a-5p, miR-214, miR-219, miR-338, miR-338*, miR-27a, miR-27b, miR-106a, miR-124, miR-141, miR-144, miR-145, miR-146a, miR-181a, miR-200a, miR-451, miR-532-5p, and miR-665. In some embodiments, the miRNAs may be one or more of miR-219, miR-138, miR-199a-5p, miR-338, miR-181a, miR-451, miR-532-5p, and miR-665. In some embodiments, the isolated lipid nanovesicles comprise at least two of miR-219, miR-138, and miR-199a. In some embodiments, the lipepid nanovesicles comprise miR-219 and miR-138. In certain aspects, the nanovesicles may be exosomes isolated from cells, like human cells, more particularly, a human that is at risk for or has a demyelinating disorder. In some embodiments, the human cells are dendritic cells or dendritic cells that have been differentiated *in vitro* from a human progenitor cell described herein. In some embodiments, the cells are human stem cells or human-derived stem cells. In some embodiments, the cells are bone marrow-derived, cord blood-derived, adipose-derived dendritic cells, PBMC-derived dendritic cells, or iPSC-derived dendritic cells. In some embodiments, the cells are derived from *in vitro* cultured cells.

[0063] In other aspects, the nanovesicles may be prepared from *in vitro* reconstitution of lipids. In other aspects, the nanovesicles may be loaded with one or more of the miRNAs listed above. The compositions may be comprised in pharmaceutical compositions and used for treating of subjects at risk for or having a demyelinating disorder. The nanovesicles may have a diameter of at least, about, or at most, 1, 2, 3, 4, 5, 10, 15, 20, 30, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000 nm or any range derivable therein. In a particular aspect, the exosome or vesicles may have a diameter of about 40 to about 100 nm. As used herein, “substantially similar” refers to at least 50, 55, 60, 65, 70, 75, 80, 90, 95, 99 or 100% identical or any range derivable therein. In other embodiments, it is specifically contemplated that any one of these miRNAs or a combination of these miRNAs may be excluded as an embodiment.

[0064] In some embodiments, the methods comprise altering the exosome surface to reduce potential inflammation caused by the exosomes. This can be done, for example, by stripping the surface of exosomes and adding back certain proteins. Stripping can be done by methods known in the art, and kits for performing such methods are commercially available (*e.g.* from System Biosciences, XPEP kits for Mass Spec, XPEP100A-1). In some embodiments, the exosomes and/or lipid nanovesicles have a modified exosome surface that reduces or eliminates an inflammation response when administered to a patient. In some

embodiments, the exosomes and/or nanovesicles are non-inflammatory or exhibit a low amount of inflammation that is easily tolerated by the patient.

[0065] Embodiments discussed in the context of methods and/or compositions of the invention may be employed with respect to any other method or composition described herein. Thus, an embodiment pertaining to one method or composition may be applied to other methods and compositions of the invention as well. For example, any of the disclosed methods of administration may be used to treat any of the disclosed demyelinating disorders. Embodiments of the invention include methods of treating patients having multiple sclerosis using a nasal administration route, an intravenous administration route, an inhalation administration route, or any other administration route. In other embodiments, the same routes of administration are used to treat patients with Alzheimer's disease, Parkinson's disease, stroke, or any other demyelinating disorder. As a further example, embodiments of the invention include treating multiple sclerosis with exosomes comprising miRNAs. Further embodiments of the invention include treating Alzheimer's disease, Parkinson's disease, stroke, or any other demyelinating disorder with exosomes comprising miRNAs. Further embodiments of the invention include treating multiple sclerosis with exosomes comprising mRNAs encoding antioxidant system proteins. Still further embodiments of the invention include treating Alzheimer's disease, Parkinson's disease, stroke, or any other demyelinating disorder with exosomes comprising mRNAs encoding antioxidant system proteins.

[0066] As used herein the specification, "a" or "an" may mean one or more. As used herein in the claim(s), when used in conjunction with the word "comprising", the words "a" or "an" may mean one or more than one.

[0067] The use of the term "or" in the claims is used to mean "and/or" unless explicitly indicated to refer to alternatives only or the alternatives are mutually exclusive, although the disclosure supports a definition that refers to only alternatives and "and/or." As used herein "another" may mean at least a second or more.

[0068] Throughout this application, the term "about" is used to indicate that a value includes the inherent variation of error for the device, the method being employed to determine the value, or the variation that exists among the study subjects.

[0069] Throughout this application, the term "effective" or "effective amount" is used to indicate that the compounds are administered at an amount sufficient to treat a condition in a subject in need thereof.

[0070] As used in this specification and claim(s), the words “comprising” (and any form of comprising, such as “comprise” and “comprises”), “having” (and any form of having, such as “have” and “has”), “including” (and any form of including, such as “includes” and “include”) or “containing” (and any form of containing, such as “contains” and “contain”) are inclusive or open-ended and do not exclude additional, unrecited elements or method steps. Although it is contemplated that any embodiment described using the terms comprising, having, including or containing (or variations thereof) may also be implemented with the term “consisting essentially of” or “consisting of”.

[0071] The term “human-derived” as used herein, refers to exosomes or cells derived from a cell of human origin.

[0072] As used herein the terms “encode” or “encoding” with reference to a nucleic acid are used to make the invention readily understandable by the skilled artisan; however, these terms may be used interchangeably with “comprise” or “comprising” respectively.

[0073] Other objects, features and advantages of the present invention will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples, while indicating preferred embodiments of the invention, are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

BRIEF DESCRIPTION OF THE DRAWINGS

[0074] The following drawings form part of the present specification and are included to further demonstrate certain aspects of the present invention. The invention may be better understood by reference to one or more of these drawings in combination with the detailed description of specific embodiments presented herein.

[0075] **FIG. 1:** Schematic of exosome formation. Exosomes form by invagination of the membrane of endocytic compartments, leading to formation of small interluminal vesicles that form as the endosome matures to become the multivesicular body (MVB). Molecular aggregation of surface receptors reroutes them to the MVB instead of the recycling compartment. Proteins and nucleic acids are specifically sorted to the MVB during biogenesis. The MVB can fuse with a lysosome, which leads to degradation of its contents, or with the plasma membrane to release them. When the MVB fuses with the cell’s plasma membrane, interluminal vesicles are released as exosomes into the interstitial space.

[0076] FIGS. 2A-C. Confirmation of exosome recovery from rat serum. Electron microscopy images of serum-exosomes at low (**A**) and high (**B**) power. Scale bars = 200 (**A**) and 100 (**B**) nm. Western blots (**C**) show presence two well-characterized exosomal protein markers: CD63 and Alix.

5 **[0077] FIGS. 3A-C.** Semi-quantitative analysis of OS in rat hippocampal slice cultures from exposure to interferon gamma (IFN- γ). (**A**) NeuN immunohistochemical labeling of a hippocampal slice cultures, for cytoarchitectural reference and to show the CA3 area of interest (dotted line box) used for quantification of OS via CellROXTM, a fluorogenic marker of OS (Grinberg, YY, et al. (2012), J Neurochem. 122:221-9). (**B-C**) Representative
10 CellROXTM-labeled images show IFN- γ exposure (500U/mL x 7 hours) significantly ($p < 0.02$; $n = 3$ /group) increased OS (**B**) compared to control (**C**). Scale bar = 200 μ m. Slice cultures were prepared and maintained as previously described (Grinberg, *et al.*, 2012; Mitchell, *et al.*, 2011; Pusic, *et al.*, 2011).

[0078] FIGS. 4A-B. Physiological and transient (i.e., phasic, to emulate conditions of
15 environmental enrichment (EE) consisting of exercise-rest-exercise cycles) stimulation with IFN- γ triggered nutritive effects. Transient (i.e., 500 U/mL x 12 hours; all groups $n \geq 5$) exposure of rat hippocampal slice cultures was nutritive when assessed seven days later. (**A**) myelin basic protein (MBP) was significantly ($p \leq 0.001$) increased above baseline and (**B**) OS was significantly ($p \leq 0.001$) reduced. OS was induced by via exposure to mitochondrial
20 inhibition (via menadione).

[0079] FIGS. 5A-B. IFN- γ , when pulsed onto rat slice cultures for 12 hours triggered the release of nutritive exosomes that mimic the nutritive effect of pulsed exposure to IFN- γ . Hippocampal slice cultures were exposed to IFN- γ (500 U/mL x 12 hours) and three days later exosomes were harvested from their surrounding incubation media. The latter were then
25 applied to naïve slice cultures and measurements made seven days later. All group sizes were ≥ 5 ; all significance measurements $p \leq 0.001$. (**A**) Exosomes from IFN- γ stimulated slice cultures triggered a significant rise in MBP above baseline levels and (**B**) a significant reduction in OS. OS was induced by via exposure to mitochondrial inhibition (via menadione).

30 **[0080] FIGS. 6A-B.** In addition, pulsed IFN- γ or exosomes significantly increased the anti-oxidant glutathione in microglia within rat hippocampal slice cultures, consistent with the increased resistance to OS seen in peripheral mast cells (Eldh M, *et al.* (2010). PLoS

ONE 5(12): e15353). The inventors detected an IFN- γ -induced rise in slice culture glutathione using ThiolTrackerTM, a fluorescent indicator of glutathione. (A) Confocal imaging for glutathione (long arrow) and a microglia marker (short arrow) confirmed that pulsed exposure to IFN- γ selectively increases microglial glutathione. Scale bar = 10 μ m.

(B) Furthermore, this increase was significant ($p \leq 0.001$; $n \geq 5$ /group) and could be mimicked by exposure to exosomes isolated from slice cultures activated by pulsed-exposure to IFN- γ .

[0081] FIGS. 7A-F. Marlau-style enrichment cage (Obiang, *et al.*, 2011; Sanchez, *et al.*, 2009) is shown where rats have free access to food and water, a maze, running wheel, and socialization area for 4 weeks to provide increased volitional opportunities for intellectual, physical, and social stimulation (i.e., EE). Non-enriched rats (NE) rats are housed in single standard cages. The EE cage consists (A) of a large two layer environment where a top layer maze (B) is changed three times a week (i.e., Monday, Wednesday, and Friday). Complexity is provided by the maze and novelty by changing the maze frequently as noted. (C) Activity is provided by a running wheel and (D) socialization by a red plastic resting area. (E) Rats climb a ladder to progress through the maze and (F) descend ramps to enter a feeding area. They move from the feeding area to the large socialization/exercise area via one-way doors.

[0082] FIGS. 8A-E. Young and environmentally enriched exosomes deliver functional miR-219 that impacts oligodendrocyte differentiation and myelination. Schematic illustration of the involvement of miR-219 in oligodendrocyte differentiation. (A) In multiple sclerosis oligodendrocyte precursor cells are actively prevented from differentiating into myelin producing cells in part due to deficiency of miR-219. (B) However, upon exposure to nutritive exosomes, neural stem cells preferentially enter the oligodendrocyte lineage due to inhibition of the proneurogenic factor NeuroD1. miR-219 also suppresses expression of a number of other factors that inhibit OPC differentiation, such as PDGFR α , a receptor for a mitogenic factor that promotes proliferation and prohibits differentiation. Finally, miR-219 decreases levels of ELOVL7, a regulator of lipid metabolism whose over-activity could lead to demyelination. (C-E) One day after application, nutritive exosome-treated naïve slice cultures ($n=3-10$ /group) expressed significantly (*) less NeuroD1, PDGFR α and ELOVL7 (p value ranges of 0.04-0.002, 0.03-0.003, and 0.02-0.001, for C, D, and E, respectively) relative to levels in untreated control slices. Significance determined by Student's t test.

[0083] FIGS. 9A-B. Young and environmentally enriched exosomes improved recovery from demyelinating injury in rat slice cultures. (A) Lysolecithin (0.5 mg/mL) exposure for 17

hours was used as a means to evoke demyelination followed by remyelination in slice cultures. Timecourse of recovery was determined via staining for myelin basic protein (MBP). Control slice shows typical MBP immunostaining in a healthy, mature slice culture. Lysolecithin induced demyelination that peaked at two days, showed first signs of recovery at five days, and progressively returned to normal by twelve days. Sham treatment (17 hour exposure to fresh media without lysolecithin) had no injurious effect on myelin content and/or distribution. Scale bar = 200 μ m. **(B)** Immediately after exposure to lysolecithin, cultures were treated with exosomes derived from young, young-EE, or aging-EE animals. Five days later, at the onset of remyelination, cultures were collected and MBP content analyzed via Western blot. While lysolecithin (Lyso) exposure triggered a significant ($*p < 0.001$; $n=6-16$) reduction of MBP in all groups compared to control, exosome exposure prompted a significant ($\#p<0.001$) increase in remyelination compared to that seen with lysolecithin alone. Similarly, slice cultures collected 5 days after lysolecithin treatment also revealed significantly ($*p < 0.001$; $n = 4-8/\text{group}$) increased presence of O4 positive cells. All exosome treatments significantly enhanced O4 staining ($\#p < 0.001$) above that seen with lysolecithin treatment alone. While O1 staining also significantly increased ($*p < 0.001$; $n = 3-4/\text{group}$) 5 days after lysolecithin treatment, no additional increase was seen with exosome treatment, suggesting that exosomes may directly increase remyelination by surviving mature oligodendrocytes. Significance was determined by ANOVA plus *post hoc* Holm-Sidak testing.

[0084] FIG. 10. IFN- γ -stimulated rat slice cultures released exosomes that mimicked the nutritive effects of pulsed-IFN- γ exposure and reduce susceptibility to spreading depression (SD), the most likely underlying cause of migraine. Immune cells exposed to oxidative stress can secrete exosomes that confer protection against oxidative stress to recipient cells. Likewise, slice cultures stimulated with a 12-hour pulse of IFN- γ released nutritive exosomes that mimicked the positive effects of exposure to pulsed IFN- γ . Slice cultures were exposed to IFN- γ (500 U/mL x 12 hours) and returned to normal incubation conditions. Three days later, exosomes were recovered from conditioned media. The latter were applied to naïve slice cultures and measurements made seven days later. IFN- γ -stimulated exosomes triggered a significant a significant ($p<0.01$), greater than 200-fold increase in SD threshold. Numerical data are mean $\pm$ SEM and significance ($*p<0.05$). Comparisons between groups made via paired Student's *t*-test.

[0085] FIGS. 11A-F. IFN- γ -induced modulation of rat slice culture glutathione content.

FIGS. 11A-D. Representative images show that glutathione-related fluorescence was assessed via ThiolTrackerTM under **(A)** control conditions and **(B)** the drop in glutathione evident 30 minutes after incubation with IFN- γ (500 U/mL). **(C)** Seven days after exposure to a 12-hour pulse of IFN- γ , glutathione content had recovered from the initial drop, and increased above baseline. **(D)** Treatment with exosomes from IFN- γ -stimulated slice cultures produced an even more robust increase. **(E)** In each case, these exposures were significantly ($p < 0.01$, 0.001, and 0.001, respectively) different from control. **(F)** High power microscopy showed that glutathione-positive cells were about 10 μ m in diameter, suggesting they were microglia. Confocal imaging and labeling of microglia with isolectin-GS-IB4 confirmed this suggestion. An exemplary image is shown. Arrowheads point to microglial surfaces and arrows to glutathione containing cell bodies. Numerical data are mean $\pm$ SEM and significance ($*p < 0.05$). Scale bar = 10 μ m. Comparisons between groups made via ANOVA plus Holm-Sidak *post hoc* testing.

[0086] FIGS. 12A-C. Myelin distribution in rat hippocampal slice cultures. **(A)** NeuN staining of hippocampal slice culture illustrates neuronal cytoarchitecture consisting of structurally preserved tri-synaptic loop (dentate gyrus-CA3-CA1). **(B)** Immunostaining for myelin basic protein shows regional distribution of grey matter myelin in 21 days *in vitro* hippocampal slice culture that closely parallels that seen *in vivo*. Scale bar = 250 μ m. **(C)** Electron microscopy confirmation of compact myelin in hippocampal slice culture. Exemplary image demonstrates presence of structurally normal, tightly laminated myelin sheath. Scale bar = 200 nm.

[0087] FIGS. 13A-D. Exosomes derived from rat serum were non-toxic and increased pre-oligodendrocyte levels in rat slice cultures. **(A)** Exosome isolation from serum was confirmed by electron microscopy and Western blot for surface markers CD63 and Alix. Scale bar = 40 nm. **(B)** Exosome application to hippocampal slice cultures was non-toxic. Exosomes were resuspended in PBS and applied to 21 days *in vitro* slice cultures. Slices were then stained with Sytox, a fluorescent marker of cell death, at 3, 5, 7 and 12 days post-treatment. NeuN immunostaining image (left) is shown to illustrate neuronal architecture. Sytox positive image (center) shows control with neuronal injury induced by 24 hour exposure to 20 μ M N-methyl-d-aspartate. Quantification of Sytox intensity ($n = 9$ /group) confirmed that exosome application caused no significant injury (right). Scale bar = 250 μ m. **(C)** Exemplary confocal image (left) of O4 positive cells in nutritive exosome-treated slice

culture. Scale bar = 25 μ m. Low power images were used for quantification of staining intensity from a stereotypic area of interest in CA3. Treatment with young, young-EE and aging-EE exosomes significantly ($*p \leq 0.001$; $n = 6-19/\text{group}$) increased O4+ fluorescence intensity of baseline and slices treated with aging, NE and aging-NE exosomes. Exposure of exosomes to 254 nm ultraviolet (UV) light for one hour before application to slices eliminated this increase. **(D)** Exemplary confocal image (left) of O1 positive cells in nutritive exosome-treated slice culture. Scale bar = 25 μ m. As before, quantification of low power images revealed significantly ($*p \leq 0.001$; $n = 6-19/\text{group}$) increased O1+ fluorescence intensity in nutritive exosome-treated slices compared to control and non-nutritive exosome-treated slices. Once again, exosomes exposed to UV light lost their effect, indicating that the responsible factor is an RNA species. Significance was determined by ANOVA plus *post hoc* Holm-Sidak testing.

[0088] FIGS. 14A-D. Young and environmentally enriched exosomes from rat serum enhanced myelination. **(A)** Nutritive exosomes enhanced baseline slice culture myelin levels. Slice cultures were treated with exosomes and harvested three days later for Western blot analysis of myelin basic protein (MBP) content. Young, young environmentally enriched (young-EE), and aging environmentally enriched (aging-EE) rat serum-derived exosomes ($n = 3-18$) all significantly ($*p = 0.004, 0.008, \text{ and } 0.003$, respectively) increased MBP content of slice cultures above control, whereas their aging, non-enriched (NE) and aging non-enriched (aging-NE) counterparts did not. Exposure of exosomes to 254 nm UV light for one hour before application to slices ablated their effect. **(B)** Representative electron micrograph images show myelin thickness in control cultures (left) and three days after exposure to young serum-derived (middle) or UV-exposed exosomes (right). Scale bar = 200 nm. **(C)** g ratio (axon diameter/fiber diameter) calculation ($n = 3/\text{group}$, with 20-27 axons measured per group) revealed a significant ($*p < 0.001$) decrease in young exosome-treated versus control and UV-exosome treated samples, indicating improved myelin thickness. **(D)** Axon diameter was not significantly different in young exosome-treated versus control and UV-exosome treated samples ($n = 3/\text{group}$, with 20-27 axons measured per group). Significance was determined by ANOVA plus *post hoc* Holm-Sidak testing.

[0089] FIG 15. Young and environmentally enriched exosomes from rat serum were enriched in miRNAs necessary for oligodendrocyte differentiation. miRNA content of young, young-EE and aging-EE exosomes were compared to aging-NE exosomal miRNA utilizing two different methods: (A) SBI's Rat Genome-wide microRNA qPCR Array Panel,

and (B) TaqMan Array Rodent MicroRNA Cards. Mature species that were significantly (i.e., >2 fold, $n=2-3$) enriched in each class of exosomes are shown in dark grey. miRNAs that were readily detectable but not significantly enriched are shown in white. miRNAs that could not be detected are shown in grey. † TaqMan microarray cards contained mmu-miR-219, which likely cross-reacted with rno-miR-219 species due to high sequence homology.

[0090] FIGS. 16A-C. Nasal administration of young serum-derived exosomes increased myelin in aging rats. 50 μ L of exosomes (~ 100 μ g protein) were intranasally delivered to aging rats. Three days later, brains were harvested, frozen, and motor cortex sectioned (14 μ m) for staining. **(A)** Staining for myelin basic protein (MBP) demonstrates myelin distribution in parasagittal motor cortex (left). Exemplary images illustrate corresponding cytochemical staining with FluoroMyelinTM to measure levels of compact myelin after nasal administration of UV-exposed sham exosomes (center) or young serum-derived exosomes (right). Stronger staining intensity at bottom of all images delineates the underlying white matter. Scale bar = 250 μ m. **(B)** Quantification of staining intensity shows significantly (* $p=0.001$; $n=3$ animals/group, with 9 images quantified per animal) increased compact myelin in cortex of animals treated with young exosomes. **(C)** Western blot for MBP confirms staining results and shows significantly (* $p=0.01$; $n=3$ animals/group) increased MBP in cortex of animals treated with young exosomes. Significance was determined by ANOVA plus *post hoc* Holm-Sidak testing.

[0091] FIGS 17A-D. Myelin increased from nasal application of exosomes. Rats were anesthetized with isoflurane and 50 μ L of exosomes (~100 μ g protein) delivered nasally. Three days later, brains were harvested. Images show olfactory bulb FluoroMyelinTM fluorescence in control **(A)** exosome treated **(B)** or UV-exposed exosome treated **(C)** cultures. **(D)** Quantification shows the EE-exosomes triggered a significant increase (* $p<0.001$) in olfactory bulb compact myelin that was abrogated to with administration of UV exposed EE-exosomes (UV-Exo). Significance was determined by ANOVA plus *post hoc* Holm-Sidak testing.

[0092] FIGS. 18A-D. EE-exosome-induced reduction in oxidative stress in rat slice cultures. OS was induced by menadione and measured via a fixable fluorescent marker for reactive oxygen species. Representative images are shown under control **(A)** conditions and three days after exposure to EE-exosomes **(B)** and NE-exosomes **(C)**. Scale bar = 200 μ m. **(D)** Quantifications show EE-exosome application for three days triggered a significant (* $p <$

0.001) reduction in OS, compared to control, NE-exosomes, and UV exposed EE-exosomes. Significance was determined by ANOVA plus *post hoc* Holm-Sidak testing.

[0093] FIGS. 19A-C. Nasal administration of IFN γ -stimulated-DC-Exos increased production of myelin in rat cortex. **(A)** Three days after nasally administered PBS-sham (left) or IFN γ -stimulated-DC-Exos (right), brains were harvested, frozen, and cortex sectioned for staining with FluoroMyelinTM to measure levels of compact myelin. Cal bar, 100 μ m. **(B)** Quantification showed a significant (*, $p < 0.001$; $n = 3$ animals/group) increase in FluoroMyelinTM staining intensity following nasal administration of IFN γ -stimulated-DC-Exos. **(C)** Western Blot for MBP shows a significant (*, $p = 0.019$; $n = 3$ animals/group) increase in MBP levels in the parasagittal motor cortex area of animals treated with IFN γ -stimulated-DC-Exos. Motor cortex was chosen as an exemplary area of brain. Significance was determined by Student's *t*-test.

[0094] FIGS. 20A-D. IFN γ -stimulated rat dendritic cells produced non-toxic exosomes. Exosome isolation confirmed by **(A)** Western Blot for surface markers CD63 and Alix, and by **(B)** electron microscopy. Scale bar = 25 nm. **(C)** Exosome application to rat slice cultures showed no toxic effects. Slices were stained with Sytox at 3, 6, 9, and 12 days post-treatment. NeuN immunostaining image (left) shows normal neuronal architecture. Sytox positive image (center) shows a control with neuronal injury induced by 24 hour exposure to 20 μ M N-methyl-d-aspartate. Sytox negative image (right) of exosome treated culture showed no injury. Images were inverted to enhance visualization. Scale bar = 250 μ m. **(D)** Quantification of Sytox fluorescence intensity ($n = 9$ slices/group) confirmed no significant change (ANOVA plus *post hoc* Holm-Sidak testing).

[0095] FIGS. 21A-C. IFN γ -stimulated-DC-Exos from rats increased myelination in slice cultures. **(A)** Exemplary electron microscopy images illustrate increased compact myelin in IFN γ -DC-Exo treated slice cultures. Treatments from left to right: control (no treatment); IFN γ -DC-Exo; UV-IFN γ -DC-Exo; and unstim-DC-Exo. Scale bar = 200 nm. **(B)** Quantification of myelin g ratios from electron microscopy images ($n = 3$ slices/group; and 10 cells/slice) showed a significant (*, $p = 0.008$) increase in compact myelin thickness with IFN γ -DC-Exo treatment. **(C)** Western blot confirmation and quantification showed significant (*, $p = 0.02$) increase in myelin basic protein levels in slice cultures treated with IFN γ -DC-Exo and a significant (#, $p = <0.001$; $n = 9, 15, 11, 9$ slices/group, respectively) decrease in slice cultures treated with UV-IFN γ -DC-Exo. Significance was determined by ANOVA plus *post hoc* Holm-Sidak testing and ANOVA testing, respectively.

[0096] FIGS. 22A-D. Progenitor cell populations in rat slice cultures were not affected by IFN γ -stimulated-DC-Exo treatments (derived from rats). Confocal typical images are shown for **(A)** neural stem cells (musashi, top) **(B)** oligodendrocyte progenitor cells (NG2, bottom) in control untreated slice cultures (left panel), IFN γ -stimulated-DC-Exos treated slice cultures (middle panel), and unstimulated-DC-Exos treated slice cultures (right panel). Scale bar = 10 μ m. Quantification of the number of positive **(C)** neural stem cells and **(D)** oligodendrocyte progenitor cells in each treatment group (determined from 9 images/group and $n = 3$ /group). No significant differences were seen between groups via ANOVA plus *post hoc* Holm-Sidak testing.

[0097] FIGS. 23A-D. IFN γ -stimulated-DC-Exos (derived from rats) reduced oxidative stress in slice culture. **(A)** Exemplary images show oxidative stress induced by acute exposure to menadione measured via CellROXTM, a fluorescent marker of oxidative stress after exposure to menadione alone (left) and after treatment with IFN γ -stimulated-DC-Exos (middle) and unstimulated-DC-Exos (right). Scale bar = 200 μ m. Quantification of fluorescence intensity **(B)** was performed at the CA3 area and showed that IFN γ -stimulated-DC-Exo treatment significantly (*, $p < 0.001$; $n = 8$ /group) reduced oxidative stress. **(C)** Exemplary images show glutathione (ThiolTrackerTM) and microglia (IsolectinB4) costaining. Control (left), IFN γ -stimulated-DC-Exos (middle) and unstimulated-DC-Exos (right). Scale bar = 10 μ m. **(D)** Quantification of ThiolTrackerTM fluorescence intensity revealed a significant (*, $p < 0.001$; $n = 9$) increase in glutathione content of microglia after treatment with IFN γ -stimulated-DC-Exos and unstimulated-DC-Exos compared to control. Significance was determined by ANOVA plus *post hoc* Holm-Sidak testing.

[0098] FIGS. 24A-B. IFN γ -stimulated-DC-Exo (derived from rats) were enriched in miRNA species involved in myelin production and anti-inflammatory response. miRNA content of IFN γ -stimulated DC-Exos were compared to that of unstimulated-DC-Exos. Results show expression levels of specific miRNAs involved in **(A)** myelin production / oligodendrocyte differentiation and **(B)** anti-inflammatory response. Black panels indicate mature miRNA species that could not be detected; medium light grey panels indicate miRNAs that were readily detectable but not significantly enriched; light grey indicate significantly enriched (i.e., >2 fold) miRNAs; and dark grey indicates very highly enriched (i.e., >10 fold) miRNAs.

[0099] FIGS. 25A-D. miR-219 mimic and IFN γ -stimulated-DC-Exos (derived from rats) similarly promote OPC differentiation. **(A)** Representative images of O4 positive staining

with DAPI counterstain. **(B)** Representative images of O1 positive staining with DAPI counterstain. Scale bar = 20 μ m. **(C)** Exemplary high-powered image of O4 staining to illustrate morphology, and quantification of percent of O4 positive cells (O4⁺ cells/total DAPI⁺ cells) per field (3 images per coverslip, $n = 3$ coverslips/group). Treatment with miR-219 mimic and IFN γ -stimulated-DC-Exos stimulated differentiation of OPCs into O4 expressing cells similar to T3 supplementation, and all groups were significantly (*, $p < 0.001$) increased from control. **(D)** Exemplary high-powered image of O1 staining to illustrate morphology, and quantification of percent of O1 positive cells (O1⁺ cells/total DAPI⁺ cells) per field (3 images per coverslip, $n = 3$ coverslips/group). Treatment with miR-219 mimic and IFN γ -stimulated-DC-Exos stimulated differentiation of OPCs into mature O1 expressing cells similar to T3 supplementation, and all groups were significantly (*, $p = 0.002$) increased from control. Significance was determined by ANOVA plus *post hoc* Holm-Sidak testing.

[0100] FIG 26. IFN γ -stimulated-DC-Exos (derived from rats) increased remyelination after acute lysolecithin induced demyelination. Slice cultures were exposed to lysolecithin to model acute demyelination followed by remyelination, then given different exosome treatments. At five days post-treatment, at the onset of remyelination, cultures were harvested and MBP content quantified via Western blot. While lysolecithin (Lyso) exposure caused a significant (*, $p < 0.001$; $n = 9$ slices/group) reduction of MBP in all groups compared to control, treatment with IFN γ -stimulated-DC-Exo induced a significant (#, $p < 0.001$) increase in remyelination compared to all other lysolecithin exposed groups. Significance was determined by ANOVA plus *post hoc* Holm-Sidak testing and ANOVA testing, respectively.

[0101] FIGS. 27A-B. Confirmation of rat IFN γ -stimulated DC-exosome quantum dot (QD) tagging. **(A)** Agarose gel electrophoresis of unconjugated QD nanoparticles (Lane 1) and CD63-conjugated QD nanoparticles (Lane 2). **(B)** Electron microscopy image of QD nanoparticle (arrowhead) tagged to exosomes (arrow). Scale bar = 25 nm.

[0102] FIGS. 28A-C. IFN γ -stimulated-DC-Exos (derived from rat) preferentially enter oligodendrocytes. **(A)** Merged images (top row) of QD tagged IFN γ -stimulated-DC-Exos (middle row) and cell-specific immunofluorescence (bottom row). **(B)** Merged images (top row) of QD tagged unstimulated-DC-Exos (middle row) and cell-specific immunofluorescence (bottom row). Left to right: oligodendrocytes (anti-CNPase), microglia (anti-Iba1), astrocytes (anti-GFAP), and neurons (anti-NeuN). Scale bar = 10 μ m. **(C)** Percent uptake of QD tagged IFN γ -stimulated-DC-Exos and QD tagged unstimulated-DC-Exos for

each cell type. Percent uptake calculated per 60 cells from $n = 3$ slices/group. These results indicate that DC exosomes can track to specific brain cell types. IFN γ -stimulated-DC-Exos were significantly (*, $p < 0.001$) localized to oligodendrocytes, while unstimulated-DC-Exos were significantly (*, $p < 0.001$) localized to astrocytes. Significance was determined by Student's t -test.

[0103] FIG. 29. Human bone marrow-derived dendritic cells immunostained for cell surface markers. Images show bright field immunostaining using diaminobenzidine to darken positive cells that confirm culturing procedures produce high percentage of immature dendritic cells. Upper panels **(1-3)** show immature dendritic cells stained for the dendritic cell marker CD11c. Lower panels **(1-3)** show the absence of staining for the macrophage marker CD11b. Scale bar = 50 μ m. Images were derived from bone marrow-derived cultures. Briefly, myeloid cells were isolated from adult bone marrow using a lymphocyte separation medium and differential centrifugation plus repeated washings. A magnetic bead-based technique was used to isolate CD34 positive hematopoietic cells, which were differentiated into immature dendritic cells (i.e., CD11c positive cells) and not macrophages (CD11b) by serial exposure to selected cytokines. Immature dendritic cells were stimulated with interferon gamma to produce neuroprotective exosomes that promote myelination and reduce oxidative stress.

[0104] FIG. 30. Western blot for surface marker of human bone marrow-derived dendritic cell exosomes. Human dendritic cell exosome isolation was confirmed via electron microscopy (e.g., FIG. 34) and via Western blotting for the exosome surface protein CD63. Image shows immunostaining for human dendritic cell protein lysate loaded to Western blot lanes at 12 μ g and 15 μ g. Note that human dendritic cell exosome CD63 was found to be slightly heavier than those derived from rat bone marrow, however consistent with post-translational modification of CD63 molecules during maturation of human dendritic cells.

[0105] FIG. 31A-D. Increased myelin basic protein (MBP) from exposure to exosomes from human bone marrow-derived dendritic cells stimulated with interferon gamma. Rat hippocampal brain slice cultures were used to screen for effects of exosomes. Hippocampal brain slice cultures are long-lived replicates of their *in vivo* counterparts. Images show myelin distribution in naïve brain slice cultures. (A) NeuN staining of a hippocampal slice culture illustrates neuronal cytoarchitecture consisting of structurally preserved trisynaptic loop (dentate gyrus-CA3-CA1). (B) Immunostaining for myelin basic protein (MBP), a

marker for myelin, shows regional distribution of gray matter myelin in 21 days *in vitro* hippocampal slice culture that closely parallels that seen *in vivo*. Scale bar = 250 μ m. (C) Electron microscopy confirmation of compact myelin in hippocampal slice culture. Representative image demonstrates presence of structurally normal, tightly laminated myelin sheath. Scale bar = 200 nm. (D) Cultures were exposed to 100 μ g of exosomes derived from human bone marrow-derived dendritic cells grown in culture and stimulated with interferon gamma (500 U/mL) for three days versus naïve (sham controls). Exosome exposure produced a significant ($P = 0.01$; $1-\beta$: 0.98) increase in MBP to 1.72 ± 0.13 versus 1.00 ± 0.09 . Thus, human stimulated dendritic cell exosomes parallel the promyelinating effects previously defined for these cells from rat bone marrow and like that seen from serum-derived exosomes after environmental enrichment of rats.

[0106] FIG. 32. MicroRNA-219 increase in exosomes derived from human bone marrow-derived dendritic cells stimulated with interferon gamma. RT-PCR strategies were used to show that human bone marrow-derived dendritic cells grown in culture release exosomes that contain significant (i.e., 1,486 fold increase) levels of miR-219 (SDC-Exo) compared to their unstimulated (USDC-Exo) counterparts. This supports the finding of a promyelinating capacity of exosomes from human bone-marrow derived dendritic cells stimulated with interferon gamma since miR-219 is known to be necessary and sufficient for the maturation of oligodendrocyte precursor cells into their mature, myelinating form.

[0107] FIG. 33A-E. Reduced inflammation from exposure to exosomes from human bone-marrow-derived dendritic cells stimulated with interferon gamma. Human bone marrow-derived dendritic cells stimulated with interferon gamma release exosome that reduce inflammation. Rat hippocampal brain slice cultures were treated with exosomes (70 μ g/1.2 mL medium) derived from human dendritic cells grown in culture and stimulated with interferon gamma (500 U/mL) for three days versus naïve (sham) controls. Microglia surface stained with FITC-labelled isolectin-GS-B4 served as a marker of inflammation. Representative images used for semi-quantitative analysis are shown [(A) sham (naïve) control culture; (B) stimulated dendritic cell exosome (SDC-Exo) treated cultures]. Scale bar = 200 μ m. (C) After three days, fluorescence intensity of microglial labeling fell significantly ($P = 0.0002$) from 1.00 ± 0.02 to 0.85 ± 0.02 , consistent with a drop in microglial activation (i.e., inflammation) which was confirmed by staining microglia. Representative confocal images (70 nm thick) of sham control (D) and SDC-Exo (E) treated hippocampal slice cultures imaged at the CA3 area. Scale bar = 50 μ m. Notice that the cell

bodies (arrowheads) are reduced in the treated cultures to an average of about 8 μm from 14 μm in naïve cultures. Processes are also shorter. Both characteristics are classical criteria of reduced inflammation.

[0108] FIG. 34A-B. Peripheral blood dendritic cell-derived exosomes. Human

dendritic cells grown in culture release exosomes. Images show electron microscopy evidence of human dendritic cell exosomes (i.e., 20-120 nm microvesicles) stained either with **(A)** phosphotungstic acid or **(B)** uranyl acetate. Scale bars to image lower left = 100 nm. Bottom right image is 2x magnified with scale bar = 200 nm. Monocyte derived dendritic cells from adult peripheral blood were grown in culture and exosomes isolated from culture medium.

[0109] FIG. 35A-B. (A) Rat EE releases serum-based exosomes containing miR-219 that enhance myelination (1) and reduce oxidative stress spreading depression (migraine), which inventors have shown triggers transient demyelination. **(B)** These effects can be mimicked in brain slice cultures via application of rat SDC-Exos which (1) enhance myelination, (2) reduce demyelination from lyssolecithin, (3) show no toxic effect on neural cells, and (4) reduce oxidative stress by increasing microglial (darker processes) glutathione (brighter cell bodies).

[0110] FIG. 36A-E. Nasal application of SDC-Exos in rats increases brain myelin **(A)** shown using FluoroMyelin staining (left) and **(B)** myelin basic protein immunostaining (right) with the same orientation. **(C-E)** show quantifications of FluoroMyelin, myelin basic protein immunostaining and western blot, respectively.

[0111] FIG. 37A-C. Nutritive rat-derived exosomes deliver functional miR-219 that transiently impacts oligodendrocyte differentiation and myelination. **(A)** Schematic illustration of miR-219 targets. Oligodendrocyte precursor cells are present MS lesions, but fail to differentiate into mature oligodendrocytes capable of repairing myelin. However, upon exposure to nutritive exosomes, neural stem cells preferentially enter the oligodendrocyte lineage due to the inhibition of the proneurogenic factor NeuroD1. miR-219 suppresses expression of repressors of OPC differentiation, such as PDGFR α , a receptor for a mitogenic factor that promotes proliferation/prohibits differentiation. miR-219 also decreases levels of ELOVL7, a regulator of lipid metabolism whose over-activity could lead to demyelination. **(B)** Treatment with SDC-Exos significantly ($*p < 0.001$; $n = 3$ slices/group) inhibited protein levels both PDGFR α and ELOVL7. **(C)** SDC-Exos stimulate a significant ($*p = 0.018$; $n = 6$

slices/group) increase in myelin basic protein (MBP, a marker for myelin) that returns to baseline over 12 days. This has important regulatory implications, as excessive myelination can have negative consequences. Significance determined by ANOVA plus *post hoc* Holm-Sidak testing. [(A) and (B) from Pusic and Kraig, *Glia*, 2014; (C) AD Pusic unpublished observations).

[0112] **FIG. 38A-C.** Impact of rat SDC-Exos on brain slice cultures.

[0113] **FIG. 39.** Schematic for production and impact of human SCD-Exos from adult immune cell sources.

[0114] **FIG. 40.** Immunohistochemical characterization of cultured dendritic cells derived from adult human bone marrow used to prepare hSDC-Exos. Immunostaining images are shown on top and associated phase contrast images on bottom.

[0115] **FIG. 41.** Characterization of hSDC-Exos.

[0116] **FIG. 42A-D.** HSDC-Exos promote myelination and reduce inflammation (i.e., microglial activation).

[0117] **FIG. 43.** Comparison of rat IFN γ -stimulated immune cell miRNA profiles compared to hSDC-Exos. (LEFT) Exosomes from environmental enrichment (EE)-derived T cells, B cells and blood dendritic cells all increased slice culture myelination, and were enriched in miRNA species involved in myelin production and anti-inflammatory responses. Peripheral blood monocytes were harvested from animals exposed to EE as before, then sorted into T cell, B cell and dendritic cell populations. When applied to hippocampal slice cultures, exosomes from all three cell populations significantly ($*p \leq 0.001$) increased myelin basic protein (MBP) relative to control, untreated cultures. Significance determined by ANOVA plus post hoc Holm-Sidak testing. miRNA expression profiles of EE-derived T cells, B cells and blood dendritic cell exosomes. Expression levels in individual EE blood cell exosomes were calculated relative to that of non-enriched (NE)-serum-Exos (from right to left; EE-serum-Exos, EE-T cell-Exos, EE-B cell-Exos and EE-blood DC-Exos). Results show expression levels of specific miRNAs involved in (A) myelin production / oligodendrocyte differentiation and (B) anti-inflammatory response. Black panels indicate mature miRNA species that could not be detected; medium gray panels indicate miRNAs that were readily detectable but not significantly enriched; medium gray indicate significantly enriched (i.e., >2 fold) miRNAs; and darker gray indicates highly enriched (i.e., >10 fold) miRNAs. (RIGHT) IFN γ -stimulated-hBMDC-Exos were enriched in miRNA species involved in myelin

production and anti-inflammatory response. miRNA content of IFN γ -stimulated-hBMDC-Exos were compared to that of unstimulated-hBMDC-Exos. Results show expression levels of specific miRNAs involved in myelin production / oligodendrocyte differentiation and anti-inflammatory response. Black panels indicate mature miRNA species that could not be detected; medium gray panels indicate miRNAs that were readily detectable but not significantly enriched; light gray indicate significantly enriched (i.e., >2 fold) miRNAs; and darker gray indicates very highly enriched (i.e., >10 fold) miRNAs.

[0118] FIG. 44A-I. Impact of nasally delivered rat SDC-Exos. (A-E) Nasal administration of SDC-Exos reduced lyssolecithin-induced demyelination. (A) Schematic depiction of the lyssolecithin injection site. Dark gray solid dot indicates craniotomy site. (B) India ink confirms accurate injection into the corpus callosum. Representative images of FluoroMyelin staining in rats that were (C) lyssolecithin injected and (D) lyssolecithin injected and nasally administered SDC-Exos (100 μ g in 50 μ l). (E) Quantification of demyelinated area showed significantly ($*p = 0.019$) less demyelination in treated animals. (F-G) Nasal administration of SDC-Exos significantly reduced expression of the M1 polarized microglia product iNOS by 4.37 fold compared to expression levels in Sham animals. Significance was defined by greater than 2-fold change. Though there was no significant change in other M1 and M2a markers measured, this reduction in iNOS was reflected by a similarly significant ($p = 0.035$) reduction in protein carbonyl content in Sham versus SDC-Exos-treated animals (H-I) Spreading depression (SD) was used to model migraine (Pusic, 2015). SDC-Exos increased SD threshold (SDT) *in vitro* and *in vivo*. (H) When applied to naïve hippocampal slice cultures, SDC-Exos significantly ($*p \leq 0.001$) increased SDT one day later, compared to untreated control slices. (I) Nasal administration of SDC-Exos to rats likewise significantly ($*p \leq 0.001$) increased neocortical SDT compared to untreated sham animals, or animals nasally administered unstimulated dendritic cell exosomes (Unstim-DC-Exos). Significance determined by ANOVA plus *post hoc* Holm-Sidak testing or Student t-test (Pusic KM et al., unpublished results).

[0119] FIG. 45. Schematic outlines protocols for preration and testing of HiSDC-Exos noted as Aims. Aim 1: This work shows the protocol for use of hiPSC-derived DCs for the production of hiSDC-Exos. The protocol will follow methods (with modifications as necessary) put forward by Baghbaderani et al. (2016) for the reprogramming of fibroblasts to hiPSCs via introduction of defined transcription factors (SOX2, KLF4, c-Myc and LIN28) using Lonza's 4D Nucleofector system. The differentiation protocol consists of the formation

of embryoid bodies from hiPSC colonies which are then cultured under serum-free, defined medium conditions in the presence of various growth factors/cytokines directing the cells into a hematopoietic and then myeloid lineage to generate immature DCs (Leishman and Fairchild, 2014; Silk et al, 2012; Tseng et al, 2009). Immature DCs derived from hiPSCs are then stimulated and SDC-Exos harvested from conditioned media. Exosome isolation is confirmed via Western blot (CD63 and Alix), electron microscopy, and fluorescent staining/ground state depletion microscopy. Aim 2: Experiments to substantiate the utility of hiSDC-Exos follow paradigms described in the rat SDC-Exo studies and utilize rat hippocampal slice cultures for screening. Slice cultures are exposed to hiPSCs-derived SDC-Exos for 3 and 7 days. Unstimulated DC-Exos will serve as sham controls (Pusic, 2014a; 2016ab). Aim 3. Experiments here test the ability of nasally administered hiSDC-Exos to reduce the impact of MS, migraine and TBI modeled in rats. Experimental models are briefly described. (A) Experimental autoimmune encephalitis (MS model) is used to induce demyelination in rats following procedures outlined by Tambalo and coworkers (2015, J Neurosci 35:10088-100000). (B) SD (migraine model) threshold is determined via the volume of micro-injections of KCl required to evoke a first SD in rat neocortex (Pusic, 2015). (C) A “hit and run” model of TBI will be utilized as outlined by Ren and coworkers [(2013) J Neurosci 33:834-845]. In MS and TBI studies, animals are administered SDC-Exos or sham controls at weekly to biweekly intervals. For migraine studies, animals are administered exosomes one hour before SD.

[0120] FIG. 46. Representative image of human fibroblast cultures and their vitality. Left hand image shows typical fibroblast culture using phase contrast microscopy. Right hand image shows associated Sytox staining. Sytox is a fluorescent dead cell marker. The results show that fibroblast culturing procedures produce dense, highly viable cells.

[0121] FIG. 47. Fibroblast transfection efficiency with low related toxicity. Upper left image shows phase contrast representative human fibroblast culture. Upper right image shows result of GFP plasmid transfection leading to GFP expression of this culture with high evidence of widespread transfection efficiency. Lower right hand image shows modest evidence of cellular toxicity (Sytox staining) from the transfection procedure. Images were taken two days after electroporation.

[0122] FIG. 48. Immunohistochemical staining used to confirm pluripotency of human stem cell colonies. Image to the top left shows positive immunostaining for Tra-1-60 with associated phase image to upper right in the panel. Lower images show absence of

fluorescence in negative control to the left with related phase contrast image to lower right in the panel. Middle panel shows positive immunostaining for Oct-3-4 (top) and related phase contrast image (Bottom). Arrow points to spontaneously differentiated cells which do not express Oct 3/4. Right hand panel shows positive immunostaining for SSEA-4 (top) and related phase contrast image (bottom).

[0123] FIG. 49. Representative human embryoid body development over time. hiPSCs differentiated to stage of embryoid bodies are shown at day 0 and day 14 and at low (1.25x objective or 12.5x gain and 10x objective or 100x gain). Notice the evolution of complexity shown in the higher power images. This is consistent with hiPSCs embryoid body development. The larger embryoid bodies shown at higher gain are approximately 500 μ m in diameter.

[0124] FIG. 50. Representative images demonstrating the ability to produce immature DCs from HiPSCs. Immunostaining images are shown at the top and associated phase contract images below (all using 40x objective and 400x gain). Notice that cultured cells are positively stained for CD1a, a marker for immature dendritic cells but few cells are positive for F4/80 a macrophage marker. Control images are shown [i.e., negative control (no primary antibody) and autofluorescence images].

[0125] FIG. 51. Representative immunostaining of hiPSC-derived immature dendritic cells. Image to left shows positive CD11c immunostaining of immature dendritic cells differentiated via the previously defined protocol from hiPSCs. Image to right is associated phase contrast photograph.

DESCRIPTION OF ILLUSTRATIVE EMBODIMENTS

I. Introduction

[0126] In some aspects, the invention generally relates to methods and compositions involving lipid nanovesicles, such as vesicles reconstituted *in vitro*, or exosomes obtained from cells that have been induced to undergo or stimulated via oxidative stress. The methods and compositions are suitable for treating subjects at risk for or having a neurological disorder, particularly a demyelination disorder. The invention is partly based on the discovery that certain exosomes, such as exosomes from cells that have been induced under or stimulated via oxidative stress, can enhance myelination capacity.

[0127] These methods and compositions have important improvements over existing state-of-the-art. In certain embodiments, the inventors describe development of a naturally

occurring process (i.e., youth and environmental enrichment, which can be mimicked via an oxidation signal such as cytokine exposure or oxidant exposure to stimulate blood-borne exosomes and related oxidative stress induction) as a novel treatment strategy for demyelinating disorders, including degenerative neurological disorders.

5 **[0128]** In certain embodiments of the invention, naturally occurring exosomes, small vesicles secreted by cells, can reduce oxidative stress (OS) and promote myelination by facilitating intercellular communication – even across the blood brain barrier. To the inventors' knowledge, no other treatments provide this combination. Exosomes can potentially enter all cell types and importantly, can cross the blood brain barrier when
10 administered intravenously or nasally. Furthermore, they may be targeted to specific cell types.

[0129] Existing therapies for MS are designed to reduce inflammation, thus reducing the degree of demyelination, and in some cases promoting remyelination. Advantages of certain embodiment of the invention include improving recovery from demyelination (e.g., by
15 >44%), promoting myelination above control levels, and reducing OS – an underlying factor in the pathogenesis of MS, cognitive decline from aging, and migraine.

[0130] For example, the methods and compositions in certain embodiments show a 40-45% reduction in OS, a 300-800% increase in O4 positive oligodendrocyte precursor cells, a 25-600% increase in O1 positive oligodendrocyte precursor cells, and a 50% increase in
20 myelin basic protein (MBP) when compared to controls. Additionally, the threshold of spreading depression, a likely cause of migraine that was recently shown to trigger demyelination, is elevated by more than 200-fold upon stimulation by the methods and compositions. Collectively, these nutritive changes illustrate the robust effect of the methods and compositions in some embodiments.

25 **[0131]** In certain embodiments, the methods and compositions enhance naturally occurring signaling pathways, and thus are likely to have a considerably better benefit/risk profile. This is especially important considering the elevated risk of infection posed by use of existing immunomodulating therapies for MS. Finally, the methods and compositions in certain embodiments may be a novel therapy for MS, as well as for treatment of other CNS
30 degenerative disorders whose pathogenesis involves OS and oligodendrocyte injury/dysmyelination.

II. Definitions

[0132] “Exosomes” are nanovesicles released from a variety of different cells. These small vesicles may be derived from large multivesicular endosomes and secreted into the extracellular milieu. The precise mechanisms of exosome release/shedding remain unclear.

5 They appear to form by invagination and budding from the limiting membrane of late endosomes, resulting in vesicles that contain cytosol and that expose the extracellular domain of membrane-bound cellular proteins on their surface. Using electron microscopy, studies have shown fusion profiles of multivesicular endosomes with the plasma membrane, leading to the secretion of the internal vesicles into the extracellular environment.

10 [0133] The term “therapeutic agent” is art-recognized and refers to any chemical moiety that is a biologically, physiologically, or pharmacologically active substance that acts locally or systemically in a subject. Examples of therapeutic agents, also referred to as “drugs”, are described in well-known literature references such as the Merck Index, the Physicians Desk Reference, and The Pharmacological Basis of Therapeutics, and they include, without
15 limitation, medicaments; vitamins; mineral supplements; substances used for the treatment, prevention, diagnosis, cure or mitigation of a disease or illness; substances which affect the structure or function of the body; or pro-drugs, which become biologically active or more active after they have been placed in a physiological environment.

[0134] The term “therapeutic effect” is art-recognized and refers to a local or systemic
20 effect in animals, particularly mammals, and more particularly humans caused by a pharmacologically active substance. The term thus means any substance intended for use in the diagnosis, cure, mitigation, treatment or prevention of disease or in the enhancement of desirable physical or mental development and/or conditions in an animal or human. The phrase “therapeutically-effective amount” means that amount of such a substance that
25 produces some desired local or systemic effect at a reasonable benefit/risk ratio applicable to any treatment. The therapeutically effective amount of such substance will vary depending upon the subject and disease condition being treated, the weight and age of the subject, the severity of the disease condition, the manner of administration and the like, which can readily be determined by one of ordinary skill in the art. For example, certain compositions in some
30 aspects may be administered in a sufficient amount to produce a at a reasonable benefit/risk ratio applicable to such treatment.

[0135] “About” and “approximately” shall generally mean an acceptable degree of error for the quantity measured given the nature or precision of the measurements. Typically, exemplary degrees of error are within 20 percent (%), preferably within 10%, and more preferably within 5% of a given value or range of values.

5 [0136] Alternatively, and particularly in biological systems, the terms “about” and “approximately” may mean values that are within an order of magnitude, preferably within 5-fold and more preferably within 2-fold of a given value. Numerical quantities given herein are approximate unless stated otherwise, meaning that the term “about” or “approximately” can be inferred when not expressly stated.

10 III. Exosomes

[0137] In certain aspects of the invention, exosomes may be prepared and used as a novel therapeutic modality for improvement of brain health or other related diseases.

[0138] Exosomes were first described as a means for reticulocytes to selectively discard transferrin receptors as they matured into erythrocytes (Johnstone, *et al.*, 1987). For a long
15 time thereafter, they were seen as mere ‘garbage cans’ for the removal of unwanted cellular components. However, since the discovery that B cells shed exosomes containing antigen-specific MHC II capable of inducing T cell responses (Raposo, *et al.*, 1996), an abundance of exosome research has revealed that these small vesicles are involved in a multitude of functions, both physiological and pathological.

20 [0139] Exosomes are small membrane vesicles of endocytic origin that are secreted by many cell types. For example, exosomes may have a diameter of about 40 to about 100 nm. They may be formed by inward budding of the late endosome leading to the formation of vesicle-containing multivesicular bodies (MVB) which then fuse with the plasma membrane to release exosomes into the extracellular environment. Though their exact composition and
25 content depends on cell type and disease state, exosomes all share certain characteristics.

[0140] In certain aspects, the exosomes may be purified by ultracentrifugation in a sucrose gradient, then identified by the presence of marker proteins such as Alix and CD63 (Schorey & Bhatnagar, 2008) or enrichment of tetraspanins and heat shock protein 70 (Lee, *et al.*, 2011), all of which are specifically expressed on exosomes. Furthermore, exosomes can
30 be isolated *in vivo* from malignant effusions and normal body fluids such as urine, blood, and cerebrospinal fluid, making them a promising source of diagnostic biomarkers. In some other aspects, exosomes can be isolated using ExoQuick-TC™ isolation kits.

[0141] Exosomes also have the potential for directional homing to specific target cells, dependent on the physical properties of their membranes. Their effect can be local, regional or systemic. Exosomes do not contain a random sampling of their parent cell's cytoplasm, but are enriched in specific mRNA, miRNA and proteins (Bobbie, *et al.*, 2011). This cargo is protected from degradation by proteases and RNases while the vesicle is in the interstitial space, and retains bioactivity once taken up by a recipient cell. In this way, they facilitate the transfer of interactive signaling and enzymatic activities that would otherwise be restricted to individual cells based on gene expression (Lee, *et al.*, 2011). For example, Skog and coworkers show that mRNA for a reporter protein can be incorporated into exosomes, transferred to a recipient cell, and translated (Skog, *et al.*, 2008).

[0142] According to certain aspects, the exosomes and compositions can be produced using various preparations of cells. For example, the exosome-producing cells may be cultured with cytokines or other reagents to induce oxidative stress, for example, cultured in the presence of interferon gamma (IFN γ), IL1- α , β , IL-2, IL-7, IL-12, IL-15, IL-18, IL-4 and/or IL-13; and/or antibodies against T cells surface markers, such as CD2, CD3, CD28, TCR, and/or soluble MHC class I or II tetramers and/or soluble CD1 tetramers. In a particular aspect, the exosome-producing cells may be cultured in the presence of IFN γ . The culturing may comprise acute treatment or phasic treatment of cells with reagents.

[0143] In another particular embodiment, the cells may be immune cells such as T cells. The T cells may have been cultured in the presence of a TCR-activating agent, or any one or more T cell subsets, such as CD4⁺ T cells, CD8⁺ T cells, $\gamma\delta$ T cells, NKT cells, or for NK cells. Particularly preferred T cell subsets for delivering MHC Class I/II peptides are CD4⁺ T cells and CD8⁺ T cells. NK cells may also produce exosomes in certain aspects. In additional aspects, the cell may be cultured with pharmaceutical reagents or particular treatments to induce maturation and/or activation of the cells, for example, in the presence of antigens, autologous or allogeneic APCs loaded with specific antigens or superantigens, mitogens (i.e., PHA), agrin, antibodies (such as anti-CD3 and anti-CD28 antibodies) or fragments thereof, reagents that trigger the activation of PKC (i.e., phorbol esters), cytoplasmic Ca²⁺ release (i.e., calcium ionophores), inhibition of phosphatases (i.e., okadaic acid) etc. In a particular embodiment, the cells may have been expanded and/or activated in culture.

[0144] In a further embodiment, the cells may be neural cells, such as glial cells, astrocytes, neurons, oligodendrocytes, spindle neurons, microglia, or any cells of the nervous

systems. The cells can be in the form of a cell culture, a dissected tissue, or parts thereof. For example, the cells can be in the form of hippocampal slice cultures.

[0145] In a further embodiment, the cells may be stem cells, such as human stem cells or induced pluripotent stem cells (iPSCs). In some embodiments, the cells are derived from human bone marrow, cord blood, PBMCs, or from adipose tissue. In some embodiments, the cells are human cells which are isolated, cultured *in vitro*, and induced to become an antigen presenting cell, such as a dendritic cells.

[0146] Furthermore, in a particular embodiment, the cells are autologous with respect to the patient to be treated, although allogeneic or even xenogeneic cells may be used. In a further particular embodiment, the cells may produce a recombinant polynucleotide encoding a biologically active molecule. This embodiment will be disclosed in more details below.

[0147] The exosomes produced or released by cells may be isolated and/or purified using several techniques. These include filtration, centrifugation, ion-chromatography, or concentration, either alone or in combinations. An exemplary purification method comprises a step of density gradient centrifugation. Another exemplary method comprises a step of ultrafiltration, either alone or coupled to a centrifugation step. Suitable purification methods have been described in WO99/03499, WO00/44389 and WO01/82958, which are incorporated therein by reference.

[0148] Selective purification or enrichment of physiologically active subpopulations of exosomes may be achieved via several procedures. In certain embodiments, effective exosomes may be concentrated to an enriched sample via use of specific surface protein markers and related separation techniques. In other embodiments, effective exosomes may be harvested from enriched primary cells cultures identified as capable of producing the effective exosomes. In further embodiments, based on screening procedures used to identify candidate effective exosome species, other exosomes may be fabricated using molecular engineering strategies designed to selectively produce exosomes containing the target (i.e., postulated) therapeutic molecular species. The latter may be confirmed by application of exosomes containing fabricated species to naïve cultures, where the desired effect (e.g., increased myelination) may be verified.

[0149] In certain embodiments, the exosomes or vesicles may be loaded with therapeutic agents such as nucleic acid molecules. The methods may include, but are not limited to:

[0150] (a) Electroporation. By this method, a number of holes are made in cells/exosomes by briefly shocking them with an electric field of 100-200 V/cm. The DNA/RNA can enter the cells/exosomes through the holes made by the electric field.

[0151] (b) Lipofection. The method commonly called transfection and can be used to transform cells/exosomes with DNA/RNA via vesicles containing the desired genetic constructs. The vesicles fuse with the cell membrane (similar to how two oil spots at the top of a broth will fuse) and the contents of the vesicles and the cells are combined. There are a number of transfection kits in the market, ready for use, e.g. DeliverX siRNA Transfection Kit (cat. No. DX0002) from Panomics, FuGENE® HD Transfection Reagent (Cat. no. 04709691001) from Roche and LIPOFECTAMINE™ 2000 (Cat. No. 11668-027) from Invitrogen.

[0152] (c) Transformation using heat shock. Chilling cells/exosomes in the presence of divalent cations such as Ca^{2+} (in CaCl_2) makes their membranes become permeable to RNA or DNA plasmids or fragments. Cells or exosomes are incubated with the DNA and then briefly heat shocked (42°C . for 30-120 seconds), which causes the DNA to enter the cell. This method may work well for condensed circular plasmid DNAs and may work for exosomal or lipid nanovesicle constituents.

[0153] The above methods describe briefly how production and delivery of modified exosomes can be achieved to transfer RNA and DNA to recipient cells. Exosomes can be engineered to contain RNA/DNA or modified to contain the gene of interest and may be isolated and shifted to the recipient cells, to affect their biological function or survival. Consequently, the exosomes may dispose their content into the cytoplasm of the target cells, which in turn leads to translation of mRNA to specific proteins in the target cell. Further, exosomes are capable of carrying and transferring small coding and non-coding RNA such as microRNA and siRNA that may regulate translation of a specific gene.

[0154] Modified or loaded exosomes being vesicles as carriers of DNA or RNA as described herein can be used to treat inherited diseases in hematopoietic, non-hematopoietic, stem cells, and organs. Modified or loaded exosome vesicles can also be used as carriers of DNA or RNA constructs for treatments of microbiological infections or diseases or dysfunctions in humans or animals, or for transfer through any biological membrane.

[0155] Changing or modifying the genetic material of exosomes by altering the conditions for the exosome-producing cells is achieved by changing pH, temperature,

growing conditions, or using antibodies/chemicals toward exosome-producing cells. This results in alteration of the nucleic acid content. Also, over-expression or repression of cytokines, chemokines and other genes in the exosome-producing cells can be used to change or modify the content of exosomes.

5 [0156] Transferring sense or anti-sense RNA to specific cells using exosome vesicles to switch off genes instead of adding new ones results in down regulation (slow down) or prevention of translation of the particular gene. The method is called RNA interference (siRNA).

10 [0157] To administer nucleic acids to recipient cells or tissues, DNA or RNA-containing exosomes can be administered to cells by addition of the exosomes to cell cultures *in vitro*, or injection of these exosomes intravenously, or by any other route, *in vivo* as is known in the art, such as nasally or intravenously. Exosomes can be targeted to any cell in the body, including cells in the cardiovascular system, skeletal muscle cells, joint cells, neural cells, gut cells, lung cells, liver cells or kidney cells, or cells in the immune system, or to any type of
15 cell with any function or dysfunction in the body of humans or animals, including malignant cells.

[0158] As disclosed in the invention herein, exosomes can be used to deliver genetic material to recipient cells to produce any drug or precursor of any drug, or to affect the function or metabolism of any drug, in any cell in humans or animals. Exosomes and
20 therapeutic methods using exosomes are further described in Pusic, K.M., *et al.*, Spreading depression requires microglia and is decreased by their M2a polarization from environmental enrichment. *Glia*. 2014 Jul;62(7):1176-94; Pusic, A.D. *et al.*, What are exosomes and how can they be used in multiple sclerosis therapy? *Expert Rev Neurother*. 2014 Apr;14(4):353-5; Pusic, A.D. *et al.*, Youth and environmental enrichment generate serum exosomes containing
25 miR-219 that promote CNS myelination. *Glia* 2014 Feb;62(2):284-99; Pusic, A.D. *et al.*, IFN γ Stimulated dendritic cell exosomes as a potential therapeutic for remyelination. *J Neuroimmunol*. 2014 Jan 15;266(1-2):12-23; and Patent Publications: WO 2013/016223 and WO 2014/028763, all of which are incorporated herein by reference.

IV. miRNAs

30 [0159] In certain embodiments of the invention, isolated exosomes or lipid nanovesicles comprising microRNAs (abbreviated miRNAs) may be used in methods and compositions for treating patients at risk for or having demyelinating disorders. In particular embodiments, the

miRNAs may include one, two, or all of miR-7a, miR-9, miR-9*, miR-17, miR-18a, miR-19a, miR-19b, miR-20a, miR-92a-1, miR-23a, miR-23a*, miR-23b, miR-32, miR-128, miR-138, miR-138*, miR-184, miR-199a-5p, miR-214, miR-219, miR-338, miR-338*, miR-27a, miR-27b, miR-106a, miR-124, miR-141, miR-144, miR-145, miR-146a, miR-181a, miR-200a, miR-451, miR-532-5p, and miR-665. In particular embodiments, the miRNAs may include one, two, or all of miR-219, miR-138, miR-338, and miR-199a-5p. For example, the miRNAs may be miR-219 and miR-138; the miRNAs may be miR-219 and miR-338; the miRNAs may be miR-219 and miR-199a-5p.

[0160] As disclosed herein, specific miRNAs (miR-219, miR-138, miR-338, and miR-199a-5p) were selectively enriched in young exosomes, particularly miR-219, which showed the most significant enrichment, and is known to affect multiple steps of OPC differentiation into mature, myelinating oligodendrocytes.

[0161] miRNAs are naturally occurring, small non-coding RNAs that are about 17 to about 25 nucleotide bases (nt) in length in their biologically active form. miRNAs post-transcriptionally regulate gene expression by repressing target mRNA translation. It is thought that miRNAs function as negative regulators, i.e. greater amounts of a specific miRNA will correlate with lower levels of target gene expression.

[0162] There are three forms of miRNAs existing *in vivo*, primary miRNAs (pri-miRNAs), premature miRNAs (pre-miRNAs), and mature miRNAs. Primary miRNAs (pri-miRNAs) are expressed as stem-loop structured transcripts of about a few hundred bases to over 1 kb. The pri-miRNA transcripts are cleaved in the nucleus by an RNase II endonuclease called Drosha that cleaves both strands of the stem near the base of the stem loop. Drosha cleaves the RNA duplex with staggered cuts, leaving a 5' phosphate and 2 nt overhang at the 3' end.

[0163] The cleavage product, the premature miRNA (pre-miRNA) is about 60 to about 110 nt long with a hairpin structure formed in a fold-back manner. Pre-miRNA is transported from the nucleus to the cytoplasm by Ran-GTP and Exportin-5. Pre-miRNAs are processed further in the cytoplasm by another RNase II endonuclease called Dicer. Dicer recognizes the 5' phosphate and 3' overhang, and cleaves the loop off at the stem-loop junction to form miRNA duplexes. The miRNA duplex binds to the RNA-induced silencing complex (RISC), where the antisense strand is preferentially degraded and the sense strand mature miRNA

directs RISC to its target site. It is the mature miRNA that is the biologically active form of the miRNA and is about 17 to about 25 nt in length.

[0164] MicroRNAs function by engaging in base pairing (perfect or imperfect) with specific sequences in their target genes' messages (mRNA). The miRNA degrades or represses translation of the mRNA, causing the target genes' expression to be post-transcriptionally down-regulated, repressed, or silenced. In animals, miRNAs do not necessarily have perfect homologies to their target sites, and partial homologies lead to translational repression, whereas in plants, where miRNAs tend to show complete homologies to the target sites, degradation of the message (mRNA) prevails.

[0165] MicroRNAs are widely distributed in the genome, dominate gene regulation, and actively participate in many physiological and pathological processes. For example, the regulatory modality of certain miRNAs is found to control cell proliferation, differentiation, and apoptosis; and abnormal miRNA profiles are associated with oncogenesis. Additionally, it is suggested that viral infection causes an increase in miRNAs targeted to silence "pro-cell survival" genes, and a decrease in miRNAs repressing genes associated with apoptosis (programmed cell death), thus tilting the balance toward gaining apoptosis signaling.

V. Diseases

[0166] Diseases to be prevented, treated or diagnosed can be any disease that affects a subject that would be amenable to therapy or prevention through administration of a composition or a method as described herein. For example, the disease may be a disease amenable to the therapy for administering an exosome or lipid nanovesicle containing nucleic acids or other therapeutic agents that increase resistance to oxidative stress. In particular examples, there may be provided methods and compositions involving administering compositions involving isolated exosomes from cells that have been induced to undergo or stimulated via oxidative stress for treating demyelinating disorders.

[0167] A demyelinating disorder is any disorder or disease of the nervous system in which the myelin sheath of neurons is damaged. This impairs the conduction of signals in the affected nerves, causing impairment in sensation, movement, cognition, or other functions depending on which nerves are involved. The term describes the effect of the disease, rather than its cause; some demyelinating diseases are caused by genetics, some by infectious agents, some by autoimmune reactions, some by traumatic or ischemic injury, and some by unknown factors. Organophosphates, a class of chemicals which are the active ingredients in

commercial insecticides such as sheep dip, weed-killers, and flea treatment preparations for pets, etc., will also demyelinate nerves. Neuroleptics can cause demyelination.

[0168] Non-limiting examples of demyelinating disorders of the central nervous system include: multiple sclerosis (together with the similar diseases called idiopathic inflammatory demyelinating diseases), traumatic brain injury, post traumatic stress disorder, cognitive decline from aging, migraine, migraine without aura, migraine with aura, Vitamin B12 deficiency, Central pontine myelinolysis, Tabes Dorsalis, transverse myelitis, Devic's disease, progressive multifocal leukoencephalopathy, Optic neuritis, Leukodystrophies, traumatic brain injury and neonatal brain injury. Non-limiting examples of demyelinating disorders of the peripheral nervous system include: Guillain-Barré syndrome and its chronic counterpart, chronic inflammatory demyelinating polyneuropathy, Anti-MAG peripheral neuropathy, Charcot-Marie-Tooth Disease, and Copper deficiency.

[0169] Demyelination is the act of demyelinating, or the loss of the myelin sheath insulating the nerves. When myelin degrades, conduction of signals along the nerve can be impaired or lost, and the nerve eventually withers. This leads to certain neurodegenerative disorders like multiple sclerosis and chronic inflammatory demyelinating polyneuropathy.

[0170] Central nervous system (CNS) demyelination is a cause and consequence of a variety of neurological diseases and especially exemplified by MS and cognitive decline from aging, which follow a relapsing-remitting but then progressive course and a more protracted but progressive course, respectively. In both instances, these maladies involve increased oxidative stress (OS), which damages brain cells of oligodendrocyte lineage that are responsible for brain myelination, and production of myelination inhibitory factors including specific miRNAs.

[0171] According to an embodiment of the invention, the methods described herein are useful in inhibiting the development of and/or treating multiple sclerosis. Multiple sclerosis (MS), also known as “disseminated sclerosis” or “encephalomyelitis disseminata”, is an inflammatory disease in which the fatty myelin sheaths around the axons of the brain and spinal cord are damaged, leading to demyelination and scarring as well as a broad spectrum of signs and symptoms. Disease onset usually occurs in young adults, and it is more common in women. It has a prevalence that ranges between 2 and 150 per 100,000.

[0172] Demyelination may also play an important role in the pathophysiology of traumatic brain injury. In experimental studies, brain injuries have been shown to be accompanied by a loss of myelin (Johnson, *et al.*, 2013).

[0173] Neonatal brain disorders are also associated with demyelination and failure of remyelination. White matter injuries in the newborn brain, such as hypoxic ischemic encephalopathy and periventricular leukomalacia can result in cerebral palsy and cognitive disability. Failure of remyelination in such conditions contributes to permanent demyelinated lesions. (Fancy, *et al.*, 2011).

VI. Therapeutic agents or diagnostic agents for exosomes

[0174] In some embodiments, therapeutic agents or diagnostic agents may be loaded to the exosomes for delivery to a subject, such as by electroporation or other method known in the art. The therapeutic agents may be a therapeutic nucleic acid, a protein or antibody fragment, or a small molecule.

[0175] A “therapeutic nucleic acid” is defined herein to refer to a nucleic acid which can be administered to a subject for the purpose of treating or preventing a disease. The nucleic acid is one which is known or suspected to be of benefit in the treatment of a disease or health-related condition in a subject.

[0176] Therapeutic benefit may arise, for example, as a result of alteration of expression of a particular gene or genes by the nucleic acid. Alteration of expression of a particular gene or genes may be inhibition or augmentation of expression of a particular gene (e.g., via miRNA). In certain embodiments, the therapeutic nucleic acid encodes one or more proteins or polypeptides that can be applied in the treatment or prevention of a disease or health-related condition in a subject (i.e., via mRNA). The terms “protein” and “polypeptide” are used interchangeably herein. Both terms refer to an amino acid sequence comprising two or more amino acid residues.

[0177] Any nucleic acid known to those of ordinary skill in the art that is known or suspected to be of benefit in the treatment or prevention of a disease or health-related condition is contemplated in certain aspects as a therapeutic nucleic acid. The phrase “nucleic acid sequence encoding,” as set forth throughout this application, refers to a nucleic acid which directs the expression of a specific protein or peptide. The nucleic acid sequences include both the DNA strand sequence that is transcribed into RNA and the RNA sequence that is translated into protein. In some embodiments, the nucleic acid includes a therapeutic

gene. The term “gene” is used to refer to a nucleic acid sequence that encodes a functional protein, polypeptide, or peptide-encoding unit.

[0178] As will be understood by those in the art, the term “therapeutic nucleic acid” includes genomic sequences, cDNA sequences, and smaller engineered gene segments that express, or may be adapted to express, proteins, polypeptides, domains, peptides, fusion proteins, and mutants. The nucleic acid may comprise a contiguous nucleic acid sequence of about 5 to about 12000 or more nucleotides, nucleosides, or base pairs.

[0179] Encompassed within the definition of “therapeutic nucleic acid” is a “biologically functional equivalent” of a therapeutic nucleic acid that has proved to be of benefit in the treatment or prevention of a disease or health-related condition. Accordingly, sequences that have about 70% to about 99% homology to a known nucleic acid are contemplated in certain aspects.

A. Nucleic Acids Encoding Cytokines

[0180] In some embodiments of the pharmaceutical compositions set forth herein the nucleic acid encodes a cytokine. The term “cytokine” is a generic term for proteins released by one cell population which act on another cell as intercellular mediators. The nucleic acid sequences may encode the full length nucleic acid sequence of the cytokine, as well as non-full length sequences of any length derived from the full length sequences. It being further understood, as discussed above, that the sequence includes the degenerate codons of the native sequence or sequences which may be introduced to provide codon preference in a specific host cell.

[0181] Examples of such cytokines are lymphokines, monokines, growth factors and traditional polypeptide hormones. Included among the cytokines are growth hormones such as human growth hormone, N-methionyl human growth hormone, and bovine growth hormone; parathyroid hormone; thyroxine; insulin; proinsulin; relaxin; prorelaxin; glycoprotein hormones such as follicle stimulating hormone (FSH), thyroid stimulating hormone (TSH), and luteinizing hormone (LH); hepatic growth factor; prostaglandin, fibroblast growth factors (FGFs) such as FGF- α and FGF- β ; prolactin; placental lactogen, OB protein; tumor necrosis factor- α and - β ; mullerian-inhibiting substance; mouse gonadotropin-associated peptide; inhibin; activin; vascular endothelial growth factor; integrin; thrombopoietin (TPO); nerve growth factors such as NGF- β ; platelet-growth factor; transforming growth factors (TGFs) such as TGF- α and TGF- α ; insulin-like growth factor-I

and -II; erythropoietin (EPO); osteoinductive factors; interferons such as interferon- α , - β , and - γ ; colony stimulating factors (CSFs) such as macrophage-CSF (M-CSF); granulocyte-macrophage-CSF (GM-CSF); and granulocyte-CSF (G-CSF); interleukins (ILs) such as IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12; IL-13, IL-14, IL-15, IL-16, IL-17, IL-18, LIF, G-CSF, GM-CSF, M-CSF, EPO, kit-ligand, FLT-3 or MDA-7.

[0182] A non-limiting example of growth factor cytokines involved in wound healing include: epidermal growth factor, platelet-derived growth factor, keratinocyte growth factor, hepatocyte growth factor, transforming growth factors (TGFs) such as TGF- α and TGF- β , and vascular endothelial growth factor (VEGF). These growth factors trigger mitogenic, motogenic and survival pathways utilizing Ras, MAPK, PI-3K/Akt, PLC-gamma and Rho/Rac/actin signaling. Hypoxia activates pro-angiogenic genes (*e.g.*, VEGF, angiopoietins) via HIF, while serum response factor (SRF) is critical for VEGF-induced angiogenesis, re-epithelialization and muscle restoration. EGF, its receptor, HGF and Cox2 are important for epithelial cell proliferation, migration re-epithelialization and reconstruction of gastric glands. VEGF, angiopoietins, nitric oxide, endothelin and metalloproteinases are important for angiogenesis, vascular remodeling and mucosal regeneration within ulcers (Tarnawski, 2005).

B. Nucleic Acids Encoding Enzymes

[0183] Other examples of therapeutic nucleic acids include nucleic acids encoding enzymes. Examples include, but are not limited to, ACP desaturase, an ACP hydroxylase, an ADP-glucose pyrophosphorylase, an ATPase, an alcohol dehydrogenase, an amylase, an amyloglucosidase, a catalase, a cellulase, a cyclooxygenase, a decarboxylase, a dextrinase, an esterase, a DNA polymerase, an RNA polymerase, a hyaluron synthase, a galactosidase, a glucanase, a glucose oxidase, a GTPase, a helicase, a hemicellulase, a hyaluronidase, an integrase, an invertase, an isomerase, a kinase, a lactase, a lipase, a lipoxygenase, a lyase, a lysozyme, a pectinesterase, a peroxidase, a phosphatase, a phospholipase, a phosphorylase, a polygalacturonase, a proteinase, a peptidase, a pullanase, a recombinase, a reverse transcriptase, a topoisomerase, a xylanase, or a reporter gene.

[0184] Further examples of therapeutic genes include the gene encoding carbamoyl synthetase I, ornithine transcarbamylase, arginosuccinate synthetase, arginosuccinate lyase, arginase, fumarylacetoacetate hydrolase, phenylalanine hydroxylase, alpha-1 antitrypsin, glucose-6-phosphatase, low-density-lipoprotein receptor, porphobilinogen deaminase, factor

VIII, factor IX, cystathione beta.-synthase, branched chain ketoacid decarboxylase, albumin, isovaleryl-CoA dehydrogenase, propionyl CoA carboxylase, methyl malonyl CoA mutase, glutaryl CoA dehydrogenase, insulin, beta.-glucosidase, pyruvate carboxylase, hepatic phosphorylase, phosphorylase kinase, glycine decarboxylase, H-protein, T-protein, Menkes disease copper-transporting ATPase, Wilson's disease copper-transporting ATPase, cytosine deaminase, hypoxanthine-guanine phosphoribosyltransferase, galactose-1-phosphate uridylyltransferase, phenylalanine hydroxylase, glucocerebrosidase, sphingomyelinase, α -L-iduronidase, glucose-6-phosphate dehydrogenase, glucosyltransferase, HSV thymidine kinase, or human thymidine kinase.

[0185] A therapeutic nucleic acid may encode secreted antioxidants (e.g., ascorbic acid or glutathione) or enzymatic antioxidants (e.g., superoxide dismutase (SOD)). SOD, which exists in several isoforms, is a metalloenzyme which detoxifies superoxide radicals to hydrogen peroxide. Two isoforms are intracellular: Cu/Zn-SOD, which is expressed in the cytoplasm, and Mn-SOD, which is expressed in mitochondria (Linchey and Fridovich, 1997). Mn-SOD has been demonstrated to increase resistance to radiation in hematopoietic tumor cell lines transfected with MnSOD cDNA (Suresh *et al.*, 1993). Adenoviral delivery of Cu/Zn-SOD has been demonstrated to protect against ethanol induced liver injury (Wheeler *et al.*, 2001). Additionally adenoviral mediated gene delivery of both Mn-SOD and Cu/Zn-SOD are equally efficient in protection against oxidative stress in a model of warm ischemia-reperfusion (Wheeler *et al.*, 2001).

C. Nucleic Acids Encoding Hormones

[0186] Therapeutic nucleic acids also include nucleic acids encoding hormones. Examples include, but are not limited to, growth hormone, prolactin, placental lactogen, luteinizing hormone, follicle-stimulating hormone, chorionic gonadotropin, thyroid-stimulating hormone, leptin, adrenocorticotropin, angiotensin I, angiotensin II, β -endorphin, β -melanocyte stimulating hormone, cholecystokinin, endothelin I, galanin, gastric inhibitory peptide, glucagon, insulin, lipotropins, neurophysins, somatostatin, calcitonin, calcitonin gene related peptide, β -calcitonin gene related peptide, hypercalcemia of malignancy factor, parathyroid hormone-related protein, parathyroid hormone-related protein, glucagon-like peptide, pancreastatin, pancreatic peptide, peptide YY, PHM, secretin, vasoactive intestinal peptide, oxytocin, vasopressin, vasotocin, enkephalinamide, metorphinamide, alpha melanocyte stimulating hormone, atrial natriuretic factor, amylin, amyloid P component,

corticotropin releasing hormone, growth hormone releasing factor, luteinizing hormone-releasing hormone, neuropeptide Y, substance K, substance P, and thyrotropin releasing hormone.

D. Nucleic Acids Encoding Antibodies

- 5 [0187] The nucleic acids set forth herein may encode an antibody or fragment thereof. The term “antibody” is used to refer to any antibody-like molecule that has an antigen binding region, and includes antibody fragments such as Fab', Fab, F(ab')₂, single domain antibodies (DABs), Fv, scFv (single chain Fv), and the like. The techniques for preparing and using various antibody-based constructs and fragments are well known in the art. Means
10 for preparing and characterizing antibodies are also well known in the art. As used herein, the term “antibody” is intended to refer broadly to any immunologic binding agent such as IgG, IgM, IgA, IgD and IgE. Generally, IgG and/or IgM are preferred because they are the most common antibodies in the physiological situation and because they are most easily made in a laboratory setting.
- 15 [0188] In certain embodiments, the nucleic acid of the pharmaceutical compositions set forth herein encodes a single chain antibody. Single-chain antibodies are described in U.S. Patents 4,946,778 and 5,888,773, each of which are hereby incorporated by reference.

E. Diagnostic Nucleic Acids

- [0189] The exosomes or vesicles in some aspects may include a nucleic acid that is a
20 diagnostic nucleic acid. A “diagnostic nucleic acid” is a nucleic acid that can be applied in the diagnosis of a disease or health-related condition. Also included in the definition of “diagnostic nucleic acid” is a nucleic acid sequence that encodes one or more reporter proteins. A “reporter protein” refers to an amino acid sequence that, when present in a cell or tissue, is detectable and distinguishable from other genetic sequences or encoded
25 polypeptides present in cells. In some embodiments, a therapeutic gene may be fused to the reporter or be produced as a separate protein. For example, the gene of interest and reporter may be induced by separate promoters in separate delivery vehicles by co-transfection (co-infection) or by separate promoters in the same delivery vehicle. In addition, the two genes may be linked to the same promoter by, for example, an internal ribosome entry site, or a bi-
30 directional promoter. Using such techniques, expression of the gene of interest and reporter correlate. Thus, one may gauge the location, amount, and duration of expression of a gene of

interest. The gene of interest may, for example, be an anti-cancer gene, such as a tumor suppressor gene or pro-apoptotic gene.

[0190] Because cells can be transfected with reporter genes, the reporter may be used to follow cell trafficking. For example, *in vitro*, specific cells may be transfected with a reporter and then returned to an animal to assess homing. In an experimental autoimmune encephalomyelitis model for multiple sclerosis, Costa *et al.* (2001) transferred myelin basic protein-specific CD4⁺ T cells that were transduced to express IL-12 p40 and luciferase. *In vivo*, luciferase was used to demonstrate trafficking to the central nervous system. In addition, IL-12 p40 inhibited inflammation. In another system, using positron emission tomography (PET), Koehne *et al.* (2003) demonstrated *in vivo* that Epstein-Barr virus (EBV)-specific T cells expressing herpes simplex virus-1 thymidine kinase (HSV-TK) selectively traffic to EBV⁺ tumors expressing the T cells' restricting HLA allele. Furthermore, these T cells retain their capacity to eliminate targeted tumors. Capitalizing on sequential imaging, Dubey *et al.* (2003) demonstrated antigen specific localization of T cells expressing HSV-TK to tumors induced by murine sarcoma virus/Moloney murine leukemia virus (M-MSV/M-MuLV). Tissue specific promoters may also be used to assess differentiation, for example, a stem cell differentiating or fusing with a liver cell and taking up the characteristics of the differentiated cell such as activation of the surfactant promoter in type II pneumocytes.

[0191] Preferably, a reporter sequence encodes a protein that is readily detectable either by its presence, its association with a detectable moiety or by its activity that results in the generation of a detectable signal. In certain aspects, a detectable moiety may include a radionuclide, a fluorophore, a luminophore, a microparticle, a microsphere, an enzyme, an enzyme substrate, a polypeptide, a polynucleotide, a nanoparticle, and/or a nanosphere, all of which may be coupled to an antibody or a ligand that recognizes and/or interacts with a reporter.

[0192] In various embodiments, a nucleic acid sequence of the invention comprises a reporter nucleic acid sequence or encodes a product that gives rise to a detectable polypeptide. A reporter protein is capable of directly or indirectly generating a detectable signal. Generally, although not necessarily, the reporter gene includes a nucleic acid sequence and/or encodes a detectable polypeptide that are not otherwise produced by the cells. Many reporter genes have been described, and some are commercially available for the study of gene regulation (*e.g.*, Alam and Cook, 1990, the disclosure of which is incorporated

herein by reference). Signals that may be detected include, but are not limited to color, fluorescence, luminescence, isotopic or radioisotopic signals, cell surface tags, cell viability, relief of a cell nutritional requirement, cell growth and drug resistance. Reporter sequences include, but are not limited to, DNA sequences encoding β -lactamase, β -galactosidase (LacZ), alkaline phosphatase, thymidine kinase, green fluorescent protein (GFP), chloramphenicol acetyltransferase (CAT), luciferase, membrane bound proteins including, for example, G-protein coupled receptors (GPCRs), somatostatin receptors, CD2, CD4, CD8, the influenza hemagglutinin protein, symporters (such as NIS) and others well known in the art, to which high affinity antibodies or ligands directed thereto exist or can be produced by conventional means, and fusion proteins comprising a membrane bound protein appropriately fused to an antigen tag domain from, among others, hemagglutinin or Myc. Kundra *et al.*, 2002, demonstrated noninvasive monitoring of somatostatin receptor type 2 chimeric gene transfer *in vitro* and *in vivo* using biodistribution studies and gamma camera imaging.

[0193] In some embodiments, a reporter sequence encodes a fluorescent protein. Examples of fluorescent proteins which may be used in accord with the invention include green fluorescent protein (GFP), enhanced green fluorescent protein (EGFP), Renilla Reniformis green fluorescent protein, GFPmut2, GFPuv4, enhanced yellow fluorescent protein (EYFP), enhanced cyan fluorescent protein (ECFP), enhanced blue fluorescent protein (EBFP), citrine and red fluorescent protein from discosoma (dsRED). It is to be understood that these examples of fluorescent proteins is not exclusive and may encompass later developed fluorescent proteins, such as any fluorescent protein within the infrared, visible or ultraviolet spectra.

[0194] In various embodiments, the desired level of expression of at least one of the reporter sequence is an increase, a decrease, or no change in the level of expression of the reporter sequence as compared to the basal transcription level of the diagnostic nucleic acid. In a particular embodiment, the desired level of expression of one of the reporter sequences is an increase in the level of expression of the reporter sequence as compared to the basal transcription level of the reporter sequence.

[0195] In various embodiments, the reporter sequence encodes unique detectable proteins which can be analyzed independently, simultaneously, or independently and simultaneously. In other embodiments, the host cell may be a eukaryotic cell or a prokaryotic cell. Exemplary eukaryotic cells include yeast and mammalian cells. Mammalian cells include human cells and various cells displaying a pathologic phenotype, such as cancer cells.

[0196] For example, some reporter proteins induce color changes in cells that can be readily observed under visible and/or ultraviolet light. The reporter protein can be any reporter protein known to those of ordinary skill in the art. Examples include GFP, RFP, BFP and luciferase.

5 [0197] Nucleic acids encoding reporter proteins include DNAs, cRNAs, mRNAs, and subsequences thereof encoding active fragments of the respective reporter amino acid sequence, as well as vectors comprising these sequences.

[0198] Exemplary methods of imaging of reporter proteins include gamma camera imaging, CT, MRI, PET, SPECT, optical imaging, and ultrasound. In some embodiments,
10 the diagnostic nucleic acid is suitable for imaging using more than one modality, such as CT and MRI, PET and SPECT, and so forth.

[0199] Additional information pertaining to examples of reporters in imaging are set forth in Kumar, 2005; Kundra *et al.*, 2005; and Kundra *et al.*, 2002, each of which is herein specifically incorporated by reference in its entirety.

15 VII. Pharmaceutical compositions

[0200] In certain aspects, the compositions or agents for use in the methods are suitably contained in a pharmaceutically acceptable carrier. The carrier is non-toxic, biocompatible and is selected so as not to detrimentally affect the biological activity of the agent. The agents in some aspects of the invention may be formulated into preparations for local delivery
20 (i.e. to a specific location of the body, such as skeletal muscle or other tissue) or systemic delivery, in solid, semi-solid, gel, liquid or gaseous forms such as tablets, capsules, powders, granules, ointments, solutions, depositories, inhalants and injections allowing for oral, parenteral or surgical administration. Certain aspects of the invention also contemplate local administration of the compositions by coating medical devices and the like.

25 [0201] Suitable carriers for parenteral delivery via injectable, infusion or irrigation and topical delivery include distilled water, physiological phosphate-buffered saline, normal or lactated Ringer's solutions, dextrose solution, Hank's solution, or propanediol. In addition, sterile, fixed oils may be employed as a solvent or suspending medium. For this purpose any biocompatible oil may be employed including synthetic mono- or diglycerides. In addition,
30 fatty acids such as oleic acid find use in the preparation of injectables. The carrier and agent may be compounded as a liquid, suspension, polymerizable or non-polymerizable gel, paste or salve.

[0202] The carrier may also comprise a delivery vehicle to sustain (i.e., extend, delay or regulate) the delivery of the agent(s) or to enhance the delivery, uptake, stability or pharmacokinetics of the therapeutic agent(s). Such a delivery vehicle may include, by way of non-limiting examples, microparticles, microspheres, nanospheres or nanoparticles composed of proteins, liposomes, carbohydrates, synthetic organic compounds, inorganic compounds, polymeric or copolymeric hydrogels and polymeric micelles.

[0203] In certain aspects, the actual dosage amount of a composition administered to a patient or subject can be determined by physical and physiological factors such as body weight, severity of condition, the type of disease being treated, previous or concurrent therapeutic interventions, idiopathy of the patient and on the route of administration. The practitioner responsible for administration will, in any event, determine the concentration of active ingredient(s) in a composition and appropriate dose(s) for the individual subject.

[0204] In certain embodiments, pharmaceutical compositions may comprise, for example, at least about 0.1% of an active agent, such as an isolated exosome, a related lipid nanovesicle, or an exosome or nanovesicle loaded with therapeutic agents or diagnostic agents. In other embodiments, the active agent may comprise between about 2% to about 75% of the weight of the unit, or between about 25% to about 60%, for example, and any range derivable therein. In other non-limiting examples, a dose may also comprise from about 1 microgram/kg/body weight, about 5 microgram/kg/body weight, about 10 microgram/kg/body weight, about 50 microgram/kg/body weight, about 100 microgram/kg/body weight, about 200 microgram/kg/body weight, about 350 microgram/kg/body weight, about 500 microgram/kg/body weight, about 1 milligram/kg/body weight, about 5 milligram/kg/body weight, about 10 milligram/kg/body weight, about 50 milligram/kg/body weight, about 100 milligram/kg/body weight, about 200 milligram/kg/body weight, about 350 milligram/kg/body weight, about 500 milligram/kg/body weight, to about 1000 mg/kg/body weight or more per administration, and any range derivable therein. In non-limiting examples of a derivable range from the numbers listed herein, a range of about 5 microgram/kg/body weight to about 100 mg/kg/body weight, about 5 microgram/kg/body weight to about 500 milligram/kg/body weight, etc., can be administered.

[0205] Solutions of pharmaceutical compositions can be prepared in water suitably mixed with a surfactant, such as hydroxypropylcellulose. The compositions of the disclosure may comprise glycerol, liquid polyethylene glycols, and mixtures thereof. Under ordinary

conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms.

[0206] In certain aspects, the pharmaceutical compositions are advantageously administered in the form of injectable compositions either as liquid solutions or suspensions; solid forms suitable for solution in, or suspension in, liquid prior to injection may also be prepared. These preparations also may be emulsified. A typical composition for such purpose comprises a pharmaceutically acceptable carrier. For instance, the composition may contain less, than, equal to, or more than 10 mg, 25 mg, 50 mg or up to about 100 mg of human serum albumin per milliliter of phosphate buffered saline. Other pharmaceutically acceptable carriers include aqueous solutions, non-toxic excipients, including salts, preservatives, buffers and the like.

[0207] Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oil and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, saline solutions, parenteral vehicles such as sodium chloride, Ringer's dextrose, etc. Intravenous vehicles include fluid and nutrient replenishers. Preservatives include antimicrobial agents, anti-fungal agents, anti-oxidants, chelating agents and inert gases. The pH and exact concentration of the various components the pharmaceutical composition are adjusted according to well-known parameters.

[0208] Additional formulations are suitable for oral administration. Oral formulations include such typical excipients as, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate and the like. The compositions take the form of solutions, suspensions, tablets, pills, capsules, sustained release formulations or powders.

[0209] In further aspects, the pharmaceutical compositions may include classic pharmaceutical preparations. Administration of pharmaceutical compositions according to certain aspects may be via any common route so long as the target tissue is available via that route. This may include oral, nasal, buccal, rectal, vaginal or topical. Topical administration may be particularly advantageous for the treatment of skin cancers, to prevent chemotherapy-induced alopecia or other dermal hyperproliferative disorder. Alternatively, administration may be by orthotopic, intradermal, subcutaneous, intramuscular, intraperitoneal or intravenous injection. Such compositions would normally be administered as pharmaceutically acceptable compositions that include physiologically acceptable carriers,

buffers or other excipients. For treatment of conditions of the lungs, aerosol delivery can be used. Volume of the aerosol is between about 0.01 ml and 0.5 ml.

[0210] An effective amount of the pharmaceutical composition is determined based on the intended goal. The term “unit dose” or “dosage” refers to physically discrete units suitable for use in a subject, each unit containing a predetermined-quantity of the pharmaceutical composition calculated to produce the desired responses discussed above in association with its administration, *i.e.*, the appropriate route and treatment regimen. The quantity to be administered, both according to number of treatments and unit dose, depends on the protection or effect desired.

[0211] Precise amounts of the pharmaceutical composition also depend on the judgment of the practitioner and are peculiar to each individual. Factors affecting the dose include the physical and clinical state of the patient, the route of administration, the intended goal of treatment (*e.g.*, alleviation of symptoms versus cure) and the potency, stability and toxicity of the particular therapeutic substance.

VIII. Kits

[0212] Some embodiments concern kits, such as diagnostic and therapeutic kits, as well as kits for preparing and/or delivering exosomes. For example, a kit may comprise one or more pharmaceutical compositions as described herein and optionally instructions for their use. Kits may also comprise one or more devices for accomplishing administration of such compositions. For example, a subject kit may comprise a pharmaceutical composition and catheter for accomplishing direct administration of the composition to a patient having or at risk for a demyelination disorder. In other embodiments, a subject kit may comprise pre-filled ampoules of isolated exosomes, optionally formulated as a pharmaceutical, or lyophilized, for use with a delivery device.

[0213] Kits may comprise a container with a label. Suitable containers include, for example, bottles, vials, and test tubes. The containers may be formed from a variety of materials such as glass or plastic. The container may hold a composition which includes an antibody that is effective for therapeutic or non-therapeutic applications, such as described above. The label on the container may indicate that the composition is used for a specific therapy or non-therapeutic application, and may also indicate directions for either *in vivo* or *in vitro* use, such as those described above. In some embodiments, kits will comprise the container described above and one or more other containers comprising materials desirable

from a commercial and user standpoint, including buffers, diluents, filters, needles, syringes, and package inserts with instructions for use.

IX. Examples

[0214] The following examples are included to demonstrate preferred embodiments of the invention. It should be appreciated by those of skill in the art that the techniques disclosed in the examples which follow represent techniques discovered by the inventor to function well in the practice of the invention, and thus can be considered to constitute preferred modes for its practice. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments which are disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention.

EXAMPLE 1: Environmental Enrichment

[0215] The inventors established a Marlau-style EE cage for rats to test whether exosomes (FIG. 1) derived from peripheral blood (FIGS. 2A-2C) of EE-exposed (FIGS. 7A-7F) rats could reduce OS and promote myelination compared to non-enriched (NE) counterparts.

[0216] EE also generates low-level increased production of reactive oxygen species that trigger increased production of anti-oxidants. Accordingly, EE triggers a net reduction in brain OS. As a result, the inventors tested whether exosomes derived from the blood (FIGS. 2A-2C) of animals that received EE (FIGS. 7A-7F), when compared to NE counterparts, could recapitulate this effect when applied to hippocampal slice cultures. OS was induced by menadione exposure (FIGS. 3A-3B). These results confirm that exosomes from EE, but not NE animals, significantly reduced brain tissue OS when measured three days after exosome exposure. Furthermore, this decreased OS is not observed when exosomes are exposed to UV light prior to administration, suggesting that their effects were mediated by RNA species. Specific values were: Control: 1.00 ± 0.071 ; EE: 0.55 ± 0.03 ($p \leq 0.001$); NE: 0.95 ± 0.08 ; EE+UV: 0.99 ± 0.05 ($n = 6-18/\text{group}$). Further demonstration of reduction of brain OS by application of EE exosomes is in FIGS. 18A-18D.

[0217] Since EE mitigates cognitive decline from aging (Ahlskog, *et al.*, 2011), which occurs with increased OS (Sohal & Weindruch, 1996), and anti-oxidants promote myelination (Podratz, *et al.*, 2004), the inventors hypothesized that EE-derived exosomes could increase brain myelin content. Accordingly, the inventors measured MBP content in

hippocampal slice cultures exposed to exosomes and found that after three days, MBP was significantly increased after exposure to EE- but not NE-derived exosomes or controls. Furthermore, this effect of EE exosomes could be abrogated by exposure to UV light, again suggesting that RNA species mediate these effects. Specific values were: Control: 1.00 ± 0.08 ; EE: 1.47 ± 0.09 ($p \leq 0.001$); NE: 1.01 ± 0.19 ; EE+UV: 0.81 ± 0.02 ($n = 3-5/\text{group}$).

[0218] In the above EE experiments, adult animals were used. The inventors hypothesized that exosomes of EE exposed aging rats (>500 g), could also improve myelinating capacity of brain tissue. The inventors found that exosomes from aging EE (FIGS. 7A-7F) but not aging NE rats increased the number of differentiating OPCs (quantified via immunostaining for pre-oligodendrocyte marker O4) in hippocampal slice cultures. Specific values were: Control: 1.00 ± 0.23 ; Aging-EE: 2.95 ± 0.54 ; Aging-NE: 1.36 ± 0.13 ($n = 3-5/\text{group}$). In another experiment, treatment with young, young-EE and aging-EE exosomes significantly ($*p \leq 0.001$; $n=6-19/\text{group}$) increased O4+ fluorescence intensity compared to baseline and slices treated with aging, NE and aging-NE exosomes. (FIG. 13C) Exposure of exosomes to 254 nm ultraviolet light for one hour before application to slices eliminated this increase.

[0219] Similarly, OS was significantly ($p \leq 0.001$) reduced in naïve slice cultures by treatment for three hours with aging-EE-derived exosomes compared to their NE counterparts derived from aging animals. As above, this significant reduction in OS was abrogated by pretreatment of EE-exosomes with ultraviolet light. Specific values were: Control: 1.00 ± 0.06 ; aging-EE: 0.81 ± 0.04 ; aging-NE: 1.21 ± 0.02 ; aging-EE+UV: 1.22 ± 0.05 ($n = 6-9/\text{group}$). Interestingly, aging-NE exposed cultures showed a significant ($p = 0.001$) increase in OS compared to control, supporting the notion that aging is associated with pro-inflammatory change, which can promote demyelination.

[0220] To demonstrate that exosome treatment of hippocampal slice cultures was non-toxic, exosomes were resuspended in PBS and applied to 21 days in vitro slice cultures. Slices were then stained with Sytox, a fluorescent marker of cell death, at 3, 5, 7 and 12 days post-treatment. NeuN immunostaining (FIG. 13B left) is shown to illustrate neuronal architecture. Sytox positive image (FIG. 13B center) shows control with neuronal injury induced by 24 hour exposure to 20 μM N-methyl-d-aspartate. Quantification of Sytox intensity ($n = 9/\text{group}$) confirmed that exosome application caused no significant injury (FIG. 13B right).

EXAMPLE 2: Exosomes from Young/Aging Rats

[0221] Cognitive decline from aging occurs with increased OS (Sohal & Weindruch, 1996) and the systemic milieu of young animals can rejuvenate aspects of the aged CNS (Ruckh, *et al.*, 2012). Accordingly, the authors hypothesized that exosomes from young animals could mitigate OS (FIGS. 3A-3C), a critical aspect of cellular aging. Indeed, the authors found that exosomes from the blood of young but not aging rats significantly ($p \leq 0.001$) decreased OS in hippocampal slice cultures. Furthermore, this nutritive effect of young exosomes could be abrogated by exposing exosomes to UV light, suggesting that RNA species within young exosomes are responsible. Specific values were: Control: 1.00 ± 0.1 ; Young: 0.59 ± 0.04 ($p \leq 0.001$); Aging: 0.90 ± 0.09 ; Young+UV: 0.95 ± 0.05 ; ($n = 9/\text{group}$).

[0222] Since exosomes could stimulate increased myelin content (MBP), the inventors hypothesized that that exosomes could affect the OPC pool of brain tissue. Exosomes derived from the blood of young and old rats were applied to hippocampal slices as described above. OPC differentiation (quantified via immunostaining for oligodendrocyte precursor marker O4) was significantly ($p \leq 0.001$) increased in tissue exposed to exosomes from young but not aged animals. Again, this effect of ‘young’ exosomes could be abrogated by UV light, suggesting RNA species are responsible for the increased OPC pool. Specific values were: Control: 1.00 ± 0.22 ; Young: 2.36 ± 0.25 ; Aging: 0.69 ± 0.14 ; Young+UV: 1.21 ± 0.43 ($n = 7-9/\text{group}$).

[0223] As described above, the inventors described that exosomes from EE-stimulated animals could improve myelin content. The inventors hypothesized that EE may be rejuvenating animals. Accordingly, the inventors tested whether exosomes from the blood of young animals had a similar effect on brain myelin as exosomes from aging EE-stimulated animals. Indeed, MBP (quantified via Western blot) was significantly ($p < 0.05$) increased in hippocampal slices exposed to exosomes from young but not aging animal serum. Furthermore, while young exosomes already significantly increased MBP levels following 3-day exposure, this effect improved further following 7-day exposure to young exosomes. Specific values for 3-day exposure were: Control: 1.00 ± 0.10 ; Young: 1.56 ± 0.22 ; Aging: 0.86 ± 0.08 .

[0224] Specific values for 7-day significant ($p \leq 0.01$) increases in MBP were: Control: 1.00 ± 0.07 ; Young: 1.95 ± 0.15 ; Aging: 0.86 ± 0.08 ; $n = 4-6/\text{group}$. Collectively, these

data indicate that young exosomes promote myelination and may do so through mRNA and/or miRNA signaling.

[0225] As further demonstration that both young and environmentally enriched exosomes enhance myelination, slice cultures treated with young, young environmentally enriched (young-EE), and aging environmentally enriched (aging-EE) rat serum-derived exosomes ($n = 3-18$) all displayed significantly ($*p = 0.004, 0.008, \text{ and } 0.003$, respectively) increased MBP content above control, as shown by Western blot (FIG. 14A). In contrast, slice cultures treated with aging, non-enriched (NE) and aging non-enriched (aging-NE) exosomes did not display increased MBP content (FIG. 14A). Exposure of exosomes to 254 nm UV light for one hour before application to slices ablated their effect. The effect of young exosomes in increasing myelination is also demonstrated by electron micrographs showing enhanced thickness of the myelin sheath (FIGS. 14B-14D).

[0226] Given the increasing evidence that miRNAs are involved in the pathogenesis of demyelination from neurodegenerative disorders such as MS, the inventors next screened for differences in miRNA content of young and aging animal serum-derived exosomes. Levels of 21 microRNAs previously implicated in OPC maturation were assayed in exosomes derived from serum of young or aging animals. Both groups were positive for 10 microRNAs (miR-9, miR-19b, miR-23a, miR-23b, miR-128, miR-138, miR-145, miR-199a-5p, miR-219, miR-338, and miR-138). Specific microRNAs (miR-219, miR-138, miR-338, and miR-199a-5p) were enriched in young exosomes. The inventors plan to focus on miR-219, as it showed the most significant enrichment, and is known to affect multiple steps of OPC differentiation into mature, myelinating oligodendrocytes (FIG. 8A). Results of experiments showing enrichment of miRNAs necessary for oligodendrocyte differentiation in young and environmentally enriched exosomes are shown in FIG. 15. Furthermore, FIGS. 8C-8E show the changes in protein expression levels of mRNAs targeted by miR-219 (FIG. 8B) in slice cultures one day after application of nutritive (that is, myelin promoting and oxidative stress reducing) exosomes.

EXAMPLE 3: Remyelination using Young Exosomes

[0227] The inventors next hypothesized that young exosomes, which can improve baseline myelin content in healthy tissue, can also improve remyelination following an experimental model of demyelination. Indeed, the inventors demonstrated that young, young-EE and aging-EE exosomes significantly ($p \leq 0.001$) improved recovery following

acute demyelination induced by transient exposure to lysolecithin (FIGS. 9A-9B). Immediately after exposure to lysolecithin, cultures were treated with exosomes derived from young, young-EE, or aging-EE animals. Five days later, at the onset of remyelination, cultures were collected and MBP content analyzed via Western blot (FIG. 9B). While lysolecithin (Lyso) exposure triggered a significant ($*p < 0.001$; $n = 6-16$) reduction of MBP in all groups compared to control, exosome exposure prompted a significant ($\#p < 0.001$) increase in remyelination compared to that seen with lysolecithin alone. Similarly, slice cultures collected 5 days after lysolecithin treatment also revealed significantly ($*p < 0.001$; $n = 4-8/\text{group}$) increased presence of O4 positive cells. All exosome treatments significantly enhanced O4 staining ($\#p < 0.001$) above that seen with lysolecithin treatment alone. While O1 staining also significantly increased ($*p < 0.001$; $n = 3-4/\text{group}$) 5 days after lysolecithin treatment, no additional increase was seen with exosome treatment, suggesting that exosomes may directly increase remyelination by surviving mature oligodendrocytes. Significance was determined by ANOVA plus *post hoc* Holm-Sidak testing.

EXAMPLE 4: IFN γ -stimulated Dendritic Cell-derived Exosomes

[0228] The inventors studied dendritic cell-derived exosomes. Exosomes do not contain a random sampling of their parent cell's cytoplasm, but are enriched in specific mRNA, miRNA, and protein (Brobrie, et al., 2011). This cargo is protected from degradation by proteases and RNases while the vesicle is in the interstitial space, and retains bioactivity once taken up by a recipient cell. Thus, they facilitate transfer of signaling and enzymatic activities that would otherwise be restricted to individual cells based on gene expression (Lee, et al., 2011). Recent work shows that immune cells exposed to OS release exosomes that convey increased resistance against OS to neighboring cells (Eldh, *et al.*, 2010). Importantly, this effect is seen two hours after exposure, implying rapid translation of exosomal mRNA to protective proteins (Eldh, et al., 2010), most likely antioxidants or production of oxidant/antioxidant system related miRNAs.

[0229] Exosomes were isolated from rat hippocampal slice culture conditioned media and blood using ExoQuick-TCTM isolation kits. Interferon gamma (IFN γ) was used as an initial stimulus because of evidence suggesting that T cells (and their production of IFN γ) are involved in EE-based neuroprotection. Exposure to IFN γ was non-injurious, but triggered a significant ($p < 0.02$; $n = 3/\text{group}$) increase in OS from 1.00 ± 0.07 to 1.50 ± 0.27 in the CA3 region of the slices, as measured via CellROXTM, a fixable marker of reactive oxygen species.

[0230] Next, the inventors exposed rat hippocampal brain slices to a physiological dose of IFN γ and harvested exosomes from conditioned media three days later (conditioned media from slices exposed to media alone were collected for control). Then, new slices were treated with these exosomes for three hours, before exposure to OS via mitochondrial inhibition (menadione), and measured OS via CellROXTM. This treatment with exosomes from IFN γ stimulated cultures triggered a significant ($p < 0.002$; $n = 3/\text{group}$) protection from OS, from 1.00 ± 0.06 to 0.63 ± 0.05 . Thus, like immune cells (Eldh M et al. (2010) PLoS ONE), neural cells (microglia) exposed to OS can transfer increased resilience to OS via exosomes.

[0231] Next the inventors isolated exosomes from the blood of young and aging rats. When applied to slice cultures ($n = 9/\text{group}$) for a day, they found that exosomes from young rats triggered a significant ($p < 0.001$) reduction (0.59 ± 0.04) in OS compared to control, young-UV treated, and aging exosomes (1.00 ± 0.1 , 0.95 ± 0.05 , and 0.90 ± 0.10 , respectively).

[0232] Furthermore, exosomes from young rat blood triggered a significant ($p = 0.01$; $n = 3/\text{group}$) increase in OPC differentiation. Accordingly, serum-derived exosomes seem well-suited for development as a therapeutic to promote myelination.

[0233] The inventors stimulated brain slice cultures with 500 U/mL of IFN- γ , then harvested released exosomes. They chose IFN γ as the stress inducing signal since acute IFN γ exposure triggers a significant ($p < 0.02$; $n = 3/\text{group}$) rise of OS in the CA3 hippocampal region [Note: $p < 0.05$ (*); $p \leq 0.01$ (**); $p \leq 0.001$ (***)]: Control 1.00 ± 0.07 ; IFN γ $1.50 \pm 0.27^*$.

[0234] When IFN γ was applied phasically (i.e., every 12 hours for seven days), OS was significantly ($n = 5/\text{group}$) reduced: Control 1.00 ± 0.07 ; IFN γ $0.54 \pm 0.12^{***}$. Furthermore, these slice culture-IFN γ stimulated exosomes significantly reduced susceptibility to spreading depression (SD), the most likely cause of migraine. Thus, like peripheral immune cells, neural cells (microglia) exposed to OS transfer increased resilience to OS via exosomes. The inventors went on to show that nasal administration of IFN γ also reduces susceptibility to neocortical SD *in vivo*.

[0235] Notably, the above work also shows (data not described) that IFN γ stimulated rat slice culture exosomes increase tissue MBP when applied to separate slice cultures. Ruckh J and coworkers (2012) show that remyelination after experimentally induced demyelination in aged animals could be rejuvenated by exposure to the systemic milieu (i.e., blood) of younger

counterparts. The inventors showed this "systemic milieu" effect involves exosomes from serum that reduce OS, increase OPC differentiation and increase production of MBP. Specifically, comparisons of exosomes from young vs. aging rats (when applied to rat slice cultures) show that: OS was significantly reduced ($n = 9/\text{group}$) by exposure to "young vs. aging-derived" exosomes, an effect the inventors tested for involvement of mRNA or miRNA by destroying these signaling molecules via exposure to UV light: Control 1.00 ± 0.1 ; Young 0.59 ± 0.04 ***; Aging 0.90 ± 0.09 ; Young $\pm$ UV 0.95 ± 0.05 . These data suggests that an RNA species is responsible for the reduction in OS. Measurements were made using CelIROX™.

- 10 **[0236]** OPC differentiation was significantly increased ($n = 7-9/\text{group}$) by exposure to young vs. aging-derived exosomes from rat serum, an effect removed by UV exposure of young exosomes:

Control	1.00 ± 0.22
Young	2.36 ± 0.25 ***
Aging	0.69 ± 0.14
Young+UV	1.21 ± 0.43 .

- 15 **[0237]** These data also suggest that an RNA species is contained within young rat exosomes that promotes OPC differentiation. Measurements were made using semi-quantitative immunostaining.

- 20 **[0238]** Slice culture MBP content was significantly increased by exposure to young vs. aging-derived exosomes (derived from rat serum), effects removed by UV exposure of young exosomes:

3 hr:	Control	1.00 ± 0.03
	Young	0.85 ± 0.09
25	Aging	0.47 ± 0.07
	Young + UV	0.62 ± 0.16 ($n = 3-6/\text{group}$).
1 day:	Control	1.00 ± 0.03
	Young	1.05 ± 0.12
	Aging	0.53 ± 0.08 **
30	Young+UV	0.62 ± 0.16 ($n=3-6/\text{group}$).

- [0239]** These two sets (i.e., 3hr and 1day) of data suggest that "aging" exosomes (and young exosomes exposed to UV) contain signaling factors that can impede myelination.

3 day: Control	1.00 ± 0.10
Young	1.56 ± 0.22 *
Aging	0.86 ± 0.08 ($n=4-6$ /group).
7 day: Control	1.00 ± 0.07
Young	1.95 ± 0.15 **
Aging	0.86 ± 0.08 ($n=4$ /group).

[0240] Collectively, these data indicate that young exosomes promote myelination and may do so through mRNA and/or miRNA signaling. MBP was quantified via Western blot.

[0241] Given the increasing evidence that miRNAs are involved in the pathogenesis of demyelination from neurodegenerative disorders such as multiple sclerosis, the inventors next screened for miRNA expression differences between young and aging exosomes. Levels of 21 microRNAs previously implicated in OPC maturation were assayed in exosomes derived from serum of young or aging animals.

[0242] Both groups were positive for 10 microRNAs (miR-9, miR-19b, miR-23a, miR-23b, miR-128, miR-138, miR-145, miR-199a-5p, miR-219, miR-338, and miR-138). Specific microRNAs (miR-219, miR-138 and miR-199a-5p) were selectively enriched in young exosomes.

[0243] miR-219 will be emphasized, as it showed the most significant enrichment, and is known to affect multiple steps of OPC differentiation into mature, myelinating oligodendrocytes. Results of a further experiment showing enrichment of miRNAs necessary for oligodendrocyte differentiation in young and environmentally enriched exosomes are shown in FIG. 15. Furthermore, FIGS. 8C-8E show the changes in protein expression of mRNAs targeted by miR-219 (FIG. 8B) in slice cultures one day after application of nutritive exosomes.

[0244] The inventors next demonstrated that young exosomes significantly improved remyelination following acute demyelination produced by transient exposure to lysolecithin. For example, the inventors used 17 h exposure to lysolecithin to show that slice cultures transiently reduce their myelin content by about 80%.

[0245] As determined by Western blot quantification of MBP levels, exposure to young exosomes provided a significantly improved recovery from lysolecithin-induced demyelination at 5 days ($n = 5-6$).

Control	1.00 ± 0.11
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Lyso 0.34 ± 0.04
 Lyso+Young 0.73 ± 0.08 **

[0246] Environmental enrichment [(EE); i.e., volitionally increased social, intellectual, and physical activity] also generates low-level increases in reactive oxygen species that trigger increased production of anti-oxidants. Accordingly, EE triggers a net reduction in brain OS. As a result, the inventors tested whether exosomes derived from the serum of animals that received EE versus those that experienced normal animal housing [i.e., non-enriched (NE)] showed comparisons similar to those of young versus aging animals. The results confirm the following: OS was significantly reduced ($n = 6-18/\text{group}$) by exposure to EE versus NE exosomes, an effect that was abrogated by exposure of EE-exosomes to UV light.

Control 1.00 ± 0.071
 EE 0.55 ± 0.03 ***
 NE 0.95 ± 0.08
 EE+UV 0.99 ± 0.05 .

[0247] **General methods.** Experiments are performed in Wistar rats and hippocampal slice cultures. Slice cultures are prepared and used as previously described, using lysolecithin exposure as a means to evoke demyelination, and menadione to induce OS (Grinberg, *et al.*, 2012; Eldh, *et al.*, 2010). A newly developed rat enrichment cage (Lin, *et al.*, 2008) is used for EE. Non-enriched (NE) control rats are individually housed in standard cages. A visual recognition task is used to assess changes in hippocampus-based memory (Obiang, *et al.*, 2011)). The inventors screen for mRNAs using SABioscience PCR arrays and miRNAs using SeraMir exosome miRNA amplification kits followed by miRNA PCR arrays as previously described (Mitchell, *et al.*, 2010). OS is measured using CellROXTM in slice cultures and OxyBlotTM kits (to determine carbonyl levels) in whole animals. MBP [measured via Western blot (slice) or immunostaining (hippocampus of whole animals)] are used as a measure of myelin content.

[0248] Briefly, the inventors assess the ability of exosomes from young animals to reduce demyelination and OS (from lysolecithin) in slice cultures, and OS, myelin, and cognitive loss from aging in animals. Results are compared to those from exosomes of aging animal blood.

[0249] The stimulation paradigm prior to exosome harvest is EE versus NE. Groups are: EE-young, NE-young, EE-aging and NE-aging. Exosomes from these animals are applied to

slice cultures (A) or injected daily for seven days into naïve young and aging animals (B). Sham controls (for B) were injected daily with vehicle. mRNA and miRNA screening and subsequent confirmation of target proteins in brain are performed utilizing exosomes from groups defined above. Finally the inventors administer dendritic cell-derived exosomes engineered to contain specific RNA species (determined based on methods described above) to aging animals. Treatments are given intravenously or via nasal administration (Zhuang, *et al.*, 2011) daily for seven days. Endpoints are as described above and are to determine the impact of exosome treatment on OS, myelin, and cognition (i.e., hippocampus-based memory) in whole animals and OS and myelin content in slice cultures.

10 **EXAMPLE 5: Exosome-mediated Treatment**

[0250] Dendritic cells can be used as a source for exosomes for mitigation of OS and increased myelination/remyelination. Exosomes may also be used to treat traumatic brain injury and neonatal brain injury.

[0251] General methods. Slice cultures are prepared and maintained as previously described (Grinberg, *et al.*, 2012; Mitchell, *et al.*, 2011; Pusic, *et al.*, 2011). Oxidative stress is induced by brief exposure to the mitochondrial inhibitor, menadione (Grinberg, *et al.*, 2012) and OS quantitated using CellROX™ imaging (Grinberg, *et al.*, 2012). Exosomes are isolated using ExoQuick-TC™ isolation kits.

[0252] Dendritic cells are isolated from femurs and tibiae of CO₂-anesthetized and decapitated male rats (Wistar rats; 6-8 weeks old). After removing surrounding tissue, intact bones are disinfected with 70% ethanol for 2 min and rinsed with PBS before removing both ends. Then, a sterile syringe with a 21 gauge needle is used to flush marrow out with 10 mL of RPMI media through a cell strainer. Cells are then pelleted, treated with red blood cell lysis buffer, washed, and plated in culture media containing granulocyte-macrophage colony-stimulating factor at a density of 1 million cells/mL. After a week in culture, immature dendritic cells are collected. Finally, exosomes are harvested from media of cells treated with IFN-γ (Eldh, *et al.*, 2010). Exosomes from IFN-γ-stimulated dendritic cells (IFN-γ-DC-Exos) are non-toxic (FIGS. 20A-20D).

[0253] IFNγ-DC-Exos increase compact myelin levels in slice cultures. Different exosome treatments were applied to hippocampal slice cultures and EM imaging was performed three days later to determine changes in compact myelin. EM images demonstrated intact and tightly laminated myelin whose thickness was increased with the

application of IFN γ -DC-Exos (FIG 21A). Subsequent calculations of g ratios revealed significant ($p = 0.008$) improvement of laminated myelin with IFN γ -DC-Exo treatment compared to control (FIG. 21B).

[0254] When applied to naïve 24 DIV hippocampal slice cultures, IFN γ -DC-Exos significantly ($p = 0.02$) increased production of myelin basic protein (MBP) as measured via immunoblot (FIG. 21C). UV-treatment of IFN γ -DC-Exos (545 nm, 45 minutes 100 μ Watts/cm²) prior to application abrogated this effect, indicating involvement of RNA species in the observed increase in myelin production (Eldh *et al.*, 2010). Additionally, a significant ($p < 0.001$) decrease of MBP levels was seen with UV-treatment of IFN γ -DC-Exos compared to control (FIG. 21C). This is likely due to the delivery of contents damaged through UV treatment.

[0255] IFN γ -DC-Exo treatment does not cause progenitor depletion. To determine whether exosome-mediated increase of OPC differentiation has a deleterious effect on progenitor populations, the presence of neural progenitor cells and OPCs were assessed in hippocampal slice cultures treated with IFN γ -DC-Exos and Unstimulated-DC-Exos compared to untreated control. Staining with Musashi (Msi1/Msi2) (FIG. 22A) for neural stem cells revealed no significant difference in the number of positive cell counts between exosome treated slices and control (FIG. 22B). Similarly, staining with NG2 for cells in the oligodendrocyte progenitor cell stage showed no significant difference in the number of positive cells (FIGS. 22C-D), suggesting that the progenitor pool was not affected.

[0256] IFN γ -DC-Exo treatment also significantly increased oxidative tolerance of slice cultures. Administration of these exosomes three hours prior to menadione exposure significantly ($p < 0.001$) reduced oxidative stress, as seen by CellROXTM staining (FIGS. 23A-23B). Reduced glutathione levels, measured via ThiolTrackerTM staining, were significantly ($p = <0.001$) increased in cultures treated with both exosomes compared to untreated controls (FIGS. 23C-23D). However, treatment with IFN γ -DC-Exos triggered a significantly greater rise in reduced glutathione than that seen with unstimulated-DC-Exos alone. Glutathione was found localized to microglia, as seen by isolectin-GS-IB₄ double staining (Figure 4C).

[0257] Additionally, IFN γ -DC-Exos restore myelin levels post lysolecithin-induced demyelination. Lysolecithin was used as a means to induce demyelination, as a model of MS in hippocampal slice cultures (Birgbauer, *et al.*, 2004). Treatment with IFN γ -DC-Exos post

lysolecithin exposure significantly ($p < 0.001$) increased recovery of myelin, measured at five days post injury, compared to cultures treated with lysolecithin alone or given UV-IFN γ -DC-Exos (FIG. 26).

[0258] Specific miRNAs involved in oligodendrocyte differentiation and anti-inflammatory pathways are highly enriched in IFN γ -DC-Exos. Screening of exosomal miRNA revealed significant differences between the contents of IFN γ -DC-Exos and unstimulated-DC-Exos. IFN γ treatment of DC cells increased expression and packaging into exosomes of miRNAs involved in oligodendrocyte differentiation and myelin production pathways, listed in FIG. 24A. Notably, miR-219 was highly enriched in IFN γ -DC-Exos and undetectable in Unstimulated-DC-Exos. miRNA species involved in regulation of inflammatory pathways, such as miR-181a, miR-451, miR-532-5p, and miR-665 were especially highly enriched (>10 fold) in IFN γ -DC-Exos versus unstimulated-DC-Exos shown in FIG. 24B.. Increased presence of these miRNA species indicates the possibility that IFN γ -DC-Exos may reduce inflammation and oxidative stress.

[0259] To determine if IFN γ -DC-Exos increase OPC differentiation through miR-219, a miR-219 mimic was applied to primary OPC cultures. Primary OPC cultures were grown at low density on glass coverslips, and either treated with IFN γ -DC-Exos or transfected with a miR-219 mimic. Supplementation with T3, which induces OPC differentiation, was used as a positive control. Three days after treatment, IFN γ -DC-Exo treated OPCs showed increased differentiation compared to control cultures, as determined by increased staining for O4 (FIG. 25A) and O1 (FIG. 25B) positive cells. OPC cultures transfected with the miR-219 mimic likewise showed increased differentiation (FIGS. 25A-25B). Quantification of the percent O4 and O1 positive cells per treatment group revealed that both IFN γ -DC-Exos and the miR-219 mimic promoted OPC differentiation to the same extent as treatment with T3 (positive control), and were significantly ($p = 0.002$ and $p < 0.001$, respectively) increased from control (FIGS. 25C-25D).

[0260] Quantum dot (QD) tagged IFN γ -DC-Exos are preferentially taken up by oligodendrocytes. To determine whether QD nanoparticles was successfully conjugated to anti-CD63 antibody, unconjugated QD nanoparticles and conjugated QD-CD63 were analyzed on a 1.5% agarose gel. Conjugated QD-CD63 (FIG. 27A, lane 2) migrated at a higher molecular weight in comparison to unconjugated QD (FIG. 27A, lane 1) indicating the successful conjugation and a homologous species of conjugated QD-CD63. Further

confirmation of the coupling of QD-CD63, seen as a circular structure with an electron dense core (FIG. 27B, arrowhead), to exosomes (FIG. 27B, arrow) was visualized by EM imaging.

[0261] QD-IFN γ -DC-Exos (FIG. 28A) and QD-unstimulated-DC-Exos (FIG. 28B) were applied to hippocampal slice cultures and immunostained for specific cell types. Tracking of both types of QD-Exos resulted in co-localization with oligodendrocytes, microglia, and astrocytes at different rates; no uptake in neurons was observed.

[0262] QD positive cells are listed as a percentage of cells measured (FIG. 28C). The inventors counted 60 cells per cell-specific staining group and noted the number of QD-positive cells. QD-IFN γ -DC-Exos in slice showed that they were preferentially taken up by oligodendrocytes (72%) and to a lesser extent microglia (34%) and astrocytes (12%). In comparison, QD-unstimulated-DC-Exos were found to also co-localize with oligodendrocytes but to a lesser extent (7%), with uptake by microglia being similar (38%) to QD-IFN γ -DC-Exos, and astrocytes having the highest uptake at 63%. This suggests a difference in surface composition, where IFN γ -DC-Exos are significantly ($p < 0.001$) targeted to oligodendrocytes and unstimulated-DC-Exos are significantly ($p < 0.001$) targeted to astrocytes.

[0263] Exosomes can be used to treat whole animals. Nasal administration of young serum-derived exosomes increased myelin in aging rats. 50 μ L of exosomes (~ 100 μ g protein) were intranasally delivered to aging rats. Three days later, brains were harvested, frozen, and motor cortex sectioned (14 μ m) for staining. Cortices of animals treated with young exosomes had significantly increased compact myelin (FIGS. 16A-16C). Similar increases in myelin were observed in olfactory bulbs (FIGS. 17A-17D).

[0264] Similarly, nasal administration of exosomes from IFN γ -stimulated dendritic cells (IFN γ -DC-Exos) increases production of cortical myelin (FIGS. 19A-19C). To assess the ability of IFN γ -DC-Exos to increase myelin *in vivo*, IFN γ -DC-Exos were nasally administered to rats and their ability to increase myelin *in vivo* was determined. Three days post-nasal administration, brains were harvested and increased myelination was observed in the motor cortex by FluoroMyelinTM staining (FIG. 19A). FluoroMyelinTM staining intensity was significantly ($p < 0.001$) higher in IFN γ -DC-Exos treated animals than sodium succinate treated (sham) animals (FIG. 19B). Western blot analysis similarly showed significantly ($p = 0.019$) increased MBP levels in the cortex of animals treated with IFN γ -DC-Exos compared to sham (FIG. 19C).

[0265] Further effects of exosome treatment may be demonstrated with testing of hippocampus-based memory. The visual recognition task is used to assess changes in hippocampus-based memory. This task is non-stressful and robustly tests hippocampus-dependent memory (Gobbo & O'Mara, 2004). Recognition of a novel object versus a familiar one is used as a measure of hippocampus-dependent memory. The visual recognition task consists of four phases: habituation, training, retention, and test. Rats with normal object recognition memory will show an increase in exploration of a novel object versus a familiar one. Memory testing is quantified as the amount of time spent exploring the novel object as a percentage of the total time spent exploring both objects during the first 5 min of the testing phase.

[0266] Rats are an optimal species for aging/cognition research (Gallagher, *et al.*, 2011). The inventors used the Wistar strain because of its greater ambulatory behavior compared to other strains, which aids in EE-related aging research.

[0267] Exosome effects on OS after administration to whole animals may be determined via OxyBlotTM measurement of protein carbonyl levels. The inventors plan to deliver exosomes to briefly anesthetized rats daily for seven days before harvest and measurement of experimental variables. To determine if proteins contribute to exosome-induced reduction of OS, the inventors may (a) expose slice cultures to IFN γ for three days, harvest exosomes from media, and use an *in vitro* translation assay coupled to mass spec analysis (Valadi, *et al.*, 2007). The inventors will also (b) select proteins/peptides of interest from the aforementioned screen, and add them to slice cultures to determine if they can mimic application of IFN γ /OS-stimulated neural exosomes to reduce OS and increase myelin (i.e., MBP and thickened myelin measured by electron microscopy).

[0268] Groups are: (a) control slices; (b) slices exposed to stimulated exosomes; (c) slices exposed to stimulated exosomes depleted of RNA via UV light exposure; and (d) slices exposed to unstimulated exosomes.

[0269] The inventors use UV light to inactivate exosomal RNA as a sham control. This procedure effectively removes cell transfer of OS resistance in immune cells (Eldh, *et al.*, 2010), suggesting the effective OS protection from exosomes involves mRNA or miRNA, not protein.

EXAMPLE 6: Exosome-mediated Mitigation of Oxidative Stress and Demyelination

[0270] IFN γ has detrimental and beneficial brain effects, consistent with physiological conditioning hormesis (Kraig, *et al.*, 2010). IFN γ worsens demyelination from EAE, a model of multiple sclerosis. Yet, low-level IFN γ before the onset of disease protects against demyelination, an effect involving an oligodendrocyte oxidative stress response (OSR; Lin, *et al.*, 2008). Also, spreading depression (SD) triggers a transient (1 & 3 but not 7 day) drop in MBP in rat hippocampal slice cultures (Kunkler, *et al.*, 2006); and demyelination increases SD susceptibility *in vivo* (Merkler, *et al.*, 2009).

[0271] Since T cells are present in hippocampal slice cultures and SD increases their production of IFN- γ (Pusic, *et al.*, 2010), the inventors examined how T cells and IFN- γ affect SD susceptibility (Pusic, *et al.*, 2011). Results were based on $n \geq 3$ -6/group and comparisons made v. shams.

[0272] PCR arrays showed a 3.61 fold increase in osteopontin and a 2.22 fold decrease in IL-10, which indicate an enhanced Th1 effect from SD. Exposure to the Th1 cytokine IFN γ (500 U/mL) triggered significantly increased susceptibility to SD at 1 day but, importantly, triggered a significantly reduced susceptibility at 3 days. Removal of IFN γ by depletion of T cells by anti-CD4 or a neutralizing anti-IFN γ antibody prevented altered susceptibility to SD and prevented the SD-induced demyelination which otherwise triggered ruptured myelin sheaths shown via EM. Neocortical SD *in vivo* triggered a similar reduction in MBP a day later.

[0273] Finally, three-day treatment with IFN γ (500 U/mL) significantly reduced reactive oxygen species generated from chemical long-term potentiation (cLTP), a physiological means to increase brain excitability like that seen hours after SD (Grinberg, *et al.*, 2011). This beneficial effect of low-level IFN γ is supported by results from mice where enrichment, which occurs with hippocampal learning (Kraig, *et al.*, 2010), triggered a significant elevation in hippocampal T-cells, IFN- γ and MBP.

[0274] These results show that SD acutely activates T cells and may overwhelm the brain's oxidative tolerance, resulting in increased susceptibility to SD and demyelination. These effects may be prevented via enrichment, which modulates immune parameters that favor a Th1-skewed response (i.e., low-level IFN γ production) extended over time. The efforts are directed at deciphering neuroimmune signaling responsible for these dual effects

of T cells and how they relate to an activity-dependent stress response as a means to develop novel therapeutics that prevent recurrent migraine and its transition to chronic migraine.

EXAMPLE 7: Exosome-mediated Mitigation of Spreading Depression (Migraine)

[0275] The detrimental effects of T cell-secreted interferon gamma (IFN- γ) on oxidative stress (OS) and demyelination in multiple sclerosis are well recognized. However, it is also known that before disease onset low levels of IFN- γ , like that produced by EE, protect against demyelination and reduce OS. SD elevates IFN- γ , and SD threshold (SDT) is decreased in experimental models of demyelination, suggesting involvement of T cells in SD. Since the inventors found T cells within rat hippocampal slice cultures, the inventors utilized this preparation to probe for T cell-mediated effects of IFN- γ on SDT, OS, and MBP levels. SD triggered a significant loss of MBP that gradually recovered by seven days, a significant initial decrease in SDT and significantly increased OS. MBP loss was abrogated by T cell depletion, neutralization of IFN- γ , and blockade of neutral sphingomyelinase-2. Importantly, when IFN- γ was pulsed onto slices to emulate phasic changes of EE (e.g., activity-rest), significant yet opposite effects were seen: SDT was increased, OS reduced, and MBP elevated above control. The inventors next investigated the involvement of exosomes in these nutritive effects, as exosomes secreted by stressed immune cells can confer protection against OS. Results confirmed that exosomes from IFN- γ -stimulated slice cultures emulated the effects of treatment with phasic low-level IFN- γ . Finally, glutathione, an endogenous sphingomyelinase inhibitor, was significantly increased in microglia, suggesting their involvement in increasing myelin. These results support pulsed application of IFN- γ as a novel therapeutic target for prevention of SD, and by extension, migraine.

[0276] EE occurs with physiologically increased neural activity from phasically enhanced learning and memory, and lessened subsequent injury from neurodegenerative disorders including demyelinating diseases. EE promotes T-cell trafficking in the brain (Ziv, *et al.*, 2006), expression of IFN- γ (Pusic, *et al.*, 2010), increases production of myelin (Zhao, *et al.*, 2012), and reduces OS (Radak, *et al.*, 2008). Importantly, enhanced neuronal activity leads to elevated production of antioxidants (Papadia, *et al.*, 2008), including glutathione which inhibits demyelination by blocking sphingomyelinase (Liu *et al.*, 1998), and antioxidants stimulate myelin gene expression (Podratz, *et al.*, 2004).

[0277] The inventors probed for further evidence of potentially nutritive effects of IFN- γ /OS-antioxidant interactions on brain myelin using mature hippocampal slice cultures, as T-

cells are present and the tissue shows a rise in IFN- γ after SD (Kunkler, *et al.*, 2004). SD is a benign perturbation of brain that is thought to be the most likely cause of migraine aura, and perhaps migraine (Moskowitz, *et al.*, 1993; Lauritzen & Kraig, 2005). When recurrent, SD may also play a role in the conversion of episodic to high frequency and chronic migraine (Kraig *et al.*, 2010). Furthermore, SD increases OS (Grinberg *et al.*, 2012) which may contribute to demyelination, while experimental demyelination promotes SD (Merkler *et al.*, 2009).

[0278] The inventors' results show that SD disrupted the myelin sheath and caused significant but transient loss of MBP that resolved seven days later. Quantitative real-time PCR assay for gene expression analysis revealed mRNA changes consistent with the presence of T cells, a suggestion confirmed by immunostaining for the cells and their production of IFN γ . Continuous application of IFN γ to slices triggered a significant, acute reduction in SD threshold (SDT), increased OS, and reduced MBP. Removal of T cell/IFN- γ and pharmacological blockade of neutral sphingomyelinase-2 abrogated these changes. In contrast, IFN γ applied as a single 12 hour pulse or applied phasically to mimic EE produced opposite, significant effects – MBP increased, SDT increased, and OS was reduced. These effects were also obtained through application of exosomes recovered from IFN γ -stimulated slice culture media, and involved adaptive changes evoked by IFN- γ -induced production of glutathione localized to microglia (FIGS. 5 and 6) and increased SDT (FIG. 10).

[0279] SD was induced in a static, interface recording configuration as previously described (Pusic *et al.*, 2011; Grinberg *et al.*, 2011; Grinberg *et al.*, 2012). All recording were made at the genu of the CA3 interstitial pyramidal neuron area using 2-4 μ m tip diameter micropipettes filled with 150 mM sodium chloride. First, the normalcy of slice electrophysiological behavior was confirmed by monitoring the interstitial field potential responses to bipolar dentate gyrus electrical stimulation (100 μ s pulses at $\leq$ 0.2 Hz and 5-20 μ A). Slices with CA3 field post-synaptic responses $\geq$ 3 mV were used for experiments. Second, SD threshold was determined by progressively doubling the amount of applied current [10 pulses, 10 Hz (100 μ s/pulse)] beginning with that needed to trigger a half-maximal field potential response from a single stimulus. Applied currents for SD threshold were applied no faster than once every 1-3minutes and they ranged from 10-10,000 nC.

[0280] Since stimulated immune cells release exosomes that are capable of reducing OS in recipient cells (Eldh *et al.*, 2010), the inventors searched for involvement of exosomes in generating the nutritive effects of IFN- γ exposure. Slice cultures were exposed to IFN- γ for

12 hours and exosomes harvested from media three days later. They confirmed production and recovery of exosomes from media of IFN- γ stimulated cultures then applied these exosomes to naïve cultures and assessed spreading depression threshold (FIG. 10). Exosomes triggered a significant ($p < 0.01$) rise in spreading depression threshold [($n=8$ /group); Control: 1.00 ± 0.37 ; Exosomes: 279 ± 94], as well as a significant ($p < 0.001$) decrease in OS [measured using CellROXTM after menadione stimulation; Control: 1.00 ± 0.03 ($n=6$); Exosomes: 0.72 ± 0.02 ($n=11$)].

[0281] Exposure to pulsed IFN γ or to exosomes from IFN γ -stimulated cultures both reduced OS. Since the antioxidant glutathione is a naturally occurring inhibitor of neutral sphingomyelinase-2 (Liu *et al.*, 1998), implicated in demyelination, the inventors measured changes in glutathione using Thiol TrackerTM (FIG 11A-FIG 11E). Continuous acute IFN γ exposure triggered a significant decline in relative glutathione content, whereas pulsed exposure to IFN γ or exposure to IFN γ -stimulated slice culture exosomes triggered a significant rise in glutathione content at seven-days. Specific values were: Control: 1.00 ± 0.08 ($n = 21$); Acute IFN γ : 0.66 ± 0.07 ($n = 9$); IFN γ : 1.40 ± 0.06 ($n = 5$); Exosomes: 1.82 ± 0.09 ($n=6$). Cytochemical staining for microglia and confocal imaging confirmed microglia as predominant cell type containing glutathione (FIG. 11F). Thus, T cells/IFN γ stimulated exosomes are a novel therapeutic against high frequency or chronic migraine.

EXAMPLE 8: Exosome-mediated Treatment of Traumatic Brain Injury

[0282] Traumatic injury to brain is associated with loss of oligodendrocytes, the myelin producing cells of the brain, demyelination, and a failure of injured brain areas to adequately remyelinate (Flygt, *et al.*, 2013). However, new evidence indicates that injured brain may be able to remyelinate if adequately stimulated (Powers, *et al.*, 2013).

[0283] Experimental work from the inventors indicates that exosomes can be a novel therapeutic for recovery from traumatic brain injury. This conclusion follows from the following facts. Spreading depression (SD) worsens clinical outcome from traumatic brain injury (Hartings, *et al.*, 2011) and the inventors have shown that exosomes can significantly prevent SD.

[0284] For example, exosomes derived from the serum of rats exposed to environmental enrichment (EE) for four-eight weeks produce exosomes that reduce susceptibility to SD after application to hippocampal slice cultures for three days. EE-exosome application

significantly ($p = 0.006$) reduced SD susceptibility compared to control with specific values of: EE-Exo treated cultures: 304 ± 88 versus control: 1.00 ± 0.35 ($n = 6/\text{group}$).

[0285] In addition, application of IFN γ -stimulated dendritic cell exosomes to slice cultures for three days also produced a significant ($p < 0.001$) reduction in spreading depression susceptibility compared to control. Specific values were: IFN γ -dendritic cell exosome treated slices: 12.5 ± 1.52 versus control: 1.00 ± 0.45 ($n = 8/\text{group}$).

EXAMPLE 9: Exosome-mediated Treatment of Neonatal Brain Injury

[0286] Neonatal brain injury commonly results in injury to oligodendrocytes with associated hypomyelination (Kauer & Ling EA, 2009). Since exosomes derived from the serum of environmentally enriched animals as well as those derived from IFN γ -stimulated dendritic cells *in vitro*, promote oligodendrocyte differentiation and related myelin production, the inventors determined the impact of these potential therapeutic agents on neonatal ischemic brain injury.

[0287] Experiments were performed in developing hippocampal slice cultures. The slice cultures were prepared as previously described (Mitchell, *et al.*, 2010) using P9-P10 rat pups, except that cultures were transferred to serum-free media after four days *in vitro*. This was done to prevent any confounding effects of exosomes from horse-serum that is otherwise an early media constituent.

[0288] At the same time, cultures were exposed to oxygen-glucose deprivation (OGD; Markus, *et al.*, 2009) to model neonatal ischemic brain injury.

[0289] After OGD, cultures were treated with exosomes derived from IFN γ -stimulated dendritic cells grown *in vitro*, then allowed to mature until 21 days *in vitro* (i.e., consistent with P31 rats). Cultures were then harvested for measurement of myelin basic protein (MBP) via Western blot.

[0290] Exosome treatment resulted in a significant ($p = 0.002$) improvement of MBP levels post OGD exposure. Specific values were: control: 1.00 ± 0.20 ; OGD treated cultures: 0.40 ± 0.03 ; and OGD+IFN γ -stimulated dendritic cell exosomes: 1.37 ± 0.17 ($n = 6/\text{group}$).

EXAMPLE 10: PROTOCOL FOR HUMAN DENDRITIC CELL AND EXOSOME ISOLATION FROM BONE MARROW

Day 1

1. Bring centrifuge to room temperature.
2. Distribute unprocessed fresh human bone marrow sample over lymphocyte separation media (LSM) in 50mL conical tube (~20mL of bone marrow to 20mL of LSM).
3. Centrifuge tube at 400xg for 30 minutes with no brake at room temperature.
- 5 4. Collect bone marrow mononuclear cells (BM-MNCs) with a transfer pipette and transfer to a 50mL tube.
5. Fill tube to 45mL mark with RPMI 1640-10% heat inactivated FBS, mix, and centrifuge at 250xg for 10 min (with brake ON).
6. Discard supernatant and vortex pellet briefly.
- 10 7. Suspend cells in 45mL of RPMI-FBS medium and centrifuge at 250xg for 10 min
8. Discard supernatant and vortex pellet briefly.
9. Suspend cells in 1 mL RPMI-FBS medium and perform cell count.
10. Centrifuge cells at 250xg for 10 min to pellet cells.
11. Aspirate media, resuspend pellet in appropriate amount of cold MACs buffer for
- 15 further processing via magnetic beads.

Purification of CD34+ cells from BM-MNCs

12. Isolate CD34+ cells via magnetic column (MiniMACS CD34 Microbead kit) according to the Miltenyi manufacturer's guidelines.
13. Wash purified cells twice with RPMI-FBS, centrifuging at 300xg for 10 min to pellet
- 20 the cells.

DC generation from precursors

14. Resuspend cells in RPMI-FBS supplemented with GM-CSF (250ng/mL) and TNF α (50ng/mL and count cells.
15. Plate at a density of 4,000 cells/1 mL/well in 24-well plates containing RPMI-FBS
- 25 medium supplemented with GM-CSF (250ng/mL) and TNF α (50ng/mL).

Day 4

16. Bring media to 37°C. Remove supernatant from each well. Add 1 mL fresh RPMI-FBS media supplemented with GM-CSF (250ng/mL) and TNF α (50ng/mL) and IL4 (100ng/mL).

30 **Day 7**

17. Feed cells as mentioned above for Day 4.

Day 10

16. Feed cells as mentioned above with RPMI 1640 media supplemented 10% exosome depleted FBS, GM-CSF (250ng/mL), TNF α (50ng/mL), IL4 (100ng/mL) and 500 U/mL IFN γ and incubate for three days.

Day 13

- 5 Collect conditioned media for exosome isolation.

EXAMPLE 11: PROTOCOL FOR HUMAN DENDRITIC CELL AND EXOSOME ISOLATION FROM WHOLE BLOOD**Day 1**

- 10 1. Bring centrifuge to room temperature.
2. Collect/obtain ~500mL of whole blood into collection tubes (EDTA K2).
3. Distribute blood over lymphocyte separation media (LSM) in 50ml conical tube (~20ml of blood to 20mL of LSM). The interface must be clean.
4. Centrifuge tube(s) at 400xg for 30 minutes with no brake at room temperature.
- 15 5. Collect peripheral blood mononuclear cells (PBMCs, buffy coat) with a transfer pipette and transfer to a 50mL tube (pool 4 collected buffy coats into 1 x 50mL tube)
6. Fill tube to 45mL mark with X-VIVO 15 medium, mix, and centrifuge at 250xg for 10 min (with brake).
7. Discard supernatant and vortex pellet briefly.
- 20 8. Suspend cells in 45mL of X-VIVO 15 medium and centrifuge at 250xg for 10 min.
9. Discard supernatant, vortex pellet briefly.
10. Suspend cells in 45mL of X-VIVO 15 medium and centrifuge at 250xg for 10 min.
11. Discard supernatant, suspend cells in 5mL X-VIVO 15 and take sample to count
12. Plate PBMCs in a 24-well plate (10 million cells/mL in 0.5 mL of X-VIVO 15 per well) or in a 6-well plate (2.4 million cells/3 mL).
- 25 13. Incubate cells at 37°C, 5% CO₂ for 1 hour.
14. After 1-2 hours remove cells that did not adhere, and wash wells with DPBS three times.
15. Add 0.5 mL/well (24-well plate) or 3 mL/well (6-well plate) serum-free X-VIVO 15
- 30 (or RPMI 1640 -10% heat inactivated FBS) media supplemented with 100ng/mL GM-CSF and 25ng/mL IL4.

Day 3

17. Bring media to 37°C. Remove supernatant from each well. Wash each well by slowly adding 0.5 mL (24-well plate) or 3 mL (6-well-plate) of 1xDPBS without disturbing cells (do not add directly to the cells but slowly from the side of the well). Remove the 0.5 mL 1xDPBS from each well and discard. Add fresh media (0.5 mL/well/24-well plate or 3 mL/well/6-well plate) consisting of X-VIVO 15, or RPMI-FBS, supplemented with 100ng/mL GM-CSF and 25ng/mL IL4.

Day 5

18. Wash and feed cells as mentioned above.

Day 7

19. Cells are ready to be used for experiment (in our case ready to make exosomes for collection). Wash cells as mentioned above and feed with X-VIVO 15, or RPMI 1640 + 10% exosome-depleted FBS, containing GM-CSF (100ng/mL), IL4 (25ng/mL) and IFN γ (500 U/mL) and incubate for three days.

Day 10

- Collect conditioned media for exosome isolation.

EXAMPLE 12: PROTOCOL FOR HUMAN DENDRITIC CELL AND EXOSOME ISOLATION FROM CORD BLOOD

Day 1

- From frozen: Obtain 50-100mLs cord blood. Thaw and use immediately.

Isolation of cord blood mononuclear cells (CB-MNCs)*

1. Dilute CB sample with sterile filtered PBS in an appropriate sized sterile conical tube (1:1).
2. Prepare a 50mL conical tube with 15mLs of Ficoll-Paque.
3. Slowly and gently layer 35mLs of the diluted CB+PBS on top of the Ficoll-Paque (repeat as many tubes as needed until there is no more CB+PBS remaining)
4. Centrifuge Ficoll-Paque+CB tubes at 850xg for 30 minutes in a swinging-bucket rotor. Make sure to remove the brake (Brake OFF).
5. Collect the CB-MNCs [the cloudy white layer between the Ficoll-Paque and plasma (yellow top layer)] with a transfer pipette and transfer to a 50mL tube.
6. Add RPMI 1640-10% heat inactivated FBS (RPMI-FBS) to 30 mL mark on the tube to the wash cells.
7. Centrifuge at 480xg for 5 minutes at room temperature with the brake ON.

9. Remove and discard the supernatant. Pool the pellet from all the tubes together into one and fill again to the 30mL mark with RPMI-FBS media.
10. Mix gently with a 5mL serological pipet and centrifuge at 480xg for 5 minutes at room temperature with the brake ON.
- 5 11. Carefully remove supernatant and resuspend cells in 1-2 mLs of RPMI-FBS.
12. Count cells, centrifuge cell suspension at 300xg, aspirate media and resuspend pellet in appropriate amount of cold MACs buffer for further processing via magnetic beads.
*Alternatively, frozen cord blood mononuclear cells can be used in lieu of the density gradient isolation procedure.

10 Purification of CD34+ cells from CB-MNCs

13. Isolate CD34+ cells via magnetic column (MiniMACS CD34 Microbead kit) according to the Miltenyi manufacturer's guidelines.
14. Wash purified cells twice with RPMI-FBS, centrifuging at 300xg for 10 min to pellet the cells.

15 DC generation from precursors

15. Resuspend the cells in RPMI 1640 supplemented with 10% heat-inactivated FBS, GM-CSF (20ng/mL), TNF α (50U/mL), and 2.5% AB+ pooled human serum.
16. Count cells and plate in 24-well plates at a density of 10,000 cells/1 mL per well.

Day 3

- 20 17. Bring media to 37°C. Remove supernatant from each well. Add 1 mL fresh media containing RPMI -FBS, supplemented with GM-CSF (20ng/mL), TNF α (50U/mL), and 2.5% AB+ pooled human serum.

Days 5 and 7

18. Bring media to 37°C. Remove supernatant from each well. Add 1 mL fresh media containing RPMI-FBS supplemented with GM-CSF (20ng/mL), TNF α (50U/mL), and IL4 (50ng/mL).

Day 9

19. Bring media to 37°C. Remove supernatant from each well. Add 1 mL fresh media containing RPMI 1640 supplemented with 10% exosome-depleted FBS, GM-CSF (20ng/mL), TNF α (50U/mL), IL4 (50ng/mL) and IFN γ (500 U/mL).

Day 12

Collect conditioned media for exosome isolation.

EXAMPLE 13: PROTOCOL FOR HUMAN DENDRITIC CELL AND EXOSOME ISOLATION FROM iPSCs

[0291] Multiple sclerosis (MS) and migraine are interrelated healthcare burdens which cost the U.S. \$40B/year. Both disorders involve myelin damage and oxidative stress, processes that prevent brain cells from making new myelin. The inventors have developed a novel cell- based therapy – microRNA-containing exosomes from stimulated dendritic cells (SDC-Exos) – that for the first time remyelinates damaged brain and also prevents migraine in animal models. The data show that human SDC-Exos derived from bone marrow are equally effective. SDC-Exos are nanovesicles that easily enter brain and their natural occurrence creates a high benefit/risk for related therapeutics. Given the uncertainty in translating rodent studies to human therapeutics and difficulties in scaling exosome production to levels needed for human dosing, the goal of this project is to develop a strategy for production of unlimited SDC-

[0292] Exos from human pluripotent stem cells derived from fibroblasts obtained from skin biopsies. The aims are to: 1) Establish a protocol for the development of hiPSC derived DCs specifically for the production of SDC-Exos; 2) Determine the degree to which hiPSCs derived SDC-Exos are functionally similar to SDC-Exos derived from rat bone marrow-derived DCs.

[0293] The approach described herein to the development of novel neurotherapeutics is conceptually and technologically innovative. First, this work begins from the fact that EE reduces subsequent neurological disease by about half without negative sequelae. This means that naturally occurring factors could be harnessed to improve brain health. The inventors aim to further identify these factors and mechanisms in order to apply them as potential “EE-mimetics.” Second, the inventors focus on exosomes since these naturally occurring nanovesicles are released from stimulated cells as a novel means of intercellular communication made possible via their surface receptors or cargo. Third, the inventors focus especially on the microRNA cargo of exosomes since miRNAs modulate protein expression in an epigenetic fashion. This is most consistent with the basic tenets of how to improve brain function – through experiential learning. Taken together, these facts support the potential power of applying EE-mimetics involving exosomes and their miRNAs as a novel and potentially personalized new form of medicinals. Indeed, this work has led to the first neurotherapeutic that is capable of remyelinating brain and simultaneously reducing brain inflammation without evidence of negative sequelae.

[0294] The basis for the therapeutics development approach comes from the fact that environmental enrichment [i.e., increased intellectual, social, and physical activity (EE)] increases CNS myelination via serum-based exosomes that contain miR-219, which is necessary and sufficient for differentiation of oligodendrocyte precursors into myelinating cells (FIG. 35A). These EE- exosomes can be mimicked via SDC-Exos produced *in vitro* (FIG. 35B). Evidence suggests that miR-219 found in exosomes is well-conserved. Other work from the inventors' laboratory shows that circulating immune cells including microglia, which are derived from yolk sac blood islands, generate analogous exosomes containing miR-219 after stimulation with IFN γ .

[0295] Brain slice cultures, a long-lived *in vitro* replica of normal intact hippocampus, were used as a screening preparation to probe for CNS effects. However, the inventors have also shown that nasal administration of SDC-Exos significantly enhances CNS myelin (FIG. 36). In other work, the inventors showed that the SDC-Exos produce a significant mitigation of demyelination from lysolecithin exposure *in vivo*, a chemical model of MS. Additionally, the inventors have shown that nasal administration of SDC-Exos reduced OS and spreading depression, a model of migraine, which is otherwise enhanced by OS. The latter effect included enhanced anti-inflammatory marker expression.

[0296] The inventors are also working to detect the surface proteins on SDC-Exos that may help target the exosomes preferentially to oligodendrocytes using mass spectroscopy and a bioinformatics approach and are further tracking the entry of SDC-Exos into brain after nasal administration. The results thus far suggest that the exosomes move rapidly through brain, most likely via CSF pathways.

[0297] It was a goal to discover if human bone marrow-derived dendritic cells could be stimulated to produce exosomes with similar positive effects on myelination and OS (inflammation). Results show that human SDC-Exos when applied to rat hippocampal slice cultures significantly increased myelin basic protein, a marker for myelin, and reduced microglial activation, a marker of OS (FIG. 31).

[0298] Accordingly, this example describes methods and a standardized strategy for production of unlimited SDC-Exos from human pluripotent stem cells (hiPSCs) derived from fibroblasts obtained from skin biopsies. This will establish a robust and highly scalable means to produce therapeutic SDC-Exos.

[0299] Human pluripotent stem cells can provide several unique advantages for generation of exosomes. Donor cells can be obtained from adult patients, thus providing an autologous source of exosomes which should mitigate potential immune activation that otherwise might be seen with allogenic or xenogenic sources. Patient-specific human hiPSCs can be induced from adult fibroblasts obtained via skin biopsies. The plan is to use a non-integrative and non-viral reprogramming method using episomal vector-mediated transfection via Lonza's NucleofectorTM technology. This method would most likely retain FDA classification of derived SDC-Exo therapeutics as "biological medicinals" since resultant exosomes do not contain trans-gene products. Importantly, hiPSCs have unlimited growth capacity and can be used as planned for differentiation into immature DCs as well for subsequent ERCC and scientific community-wide generation of specific cell types and resultant exosomes from all three germ layers. Conceivably, exosome production via this strategy would come with little variability between batches since cell sources would be highly uniform.

[0300] This work will determine the degree to which hiPSCs derived from adult human fibroblasts can be driven to become functional DCs able to produce SDC-Exos. Ultimately, fibroblasts could be obtained from skin biopsies. For "proof of concept" purpose, adult human fibroblasts can be purchased from Lonza. Development of hiPSCs from cultured fibroblasts will be accomplished via introduction of defined transcription factors: SOX2, KLF4, c-Myc, LIN28 using the Lonza 4D NucleofectorTM system. Pluripotency will be assessed using immunomarkers. The differentiation protocol consists of the formation of embryoid-like bodies from hiPSC colonies which are then cultured under serum-free, defined medium conditions in the presence of various growth factors/cytokines (Leishman and Fairchild, 2014; Silk et al., 2012; Tseng et al., 2009). Embryoid bodies will be generated via dispase digestion and manual scraping and will be maintained in rotary orbital culture (or static) which has been shown to support the differentiation of hiPSCs. These cells will then be directed into the hematopoietic lineage (HSC) via exposure to BMP4, GM-CSF, SCF and VEGF. BMP4 is important to push hiPSCs into the initial stage of mesodermal commitment. Further differentiation into common myeloid progenitors is accomplished by the successive removal of BMP-4 on day 5, VEGF on day 14 and finally SCF on day 19 of culture. Common myeloid progenitors are differentiated into DC progenitors with the addition of a low concentration of IL-4 on days 14-18. Final differentiation of DC progenitors into immature DCs will be accomplished by adding GM-CSF and a higher concentration of IL-4

on days 19-30. Expression of specific markers will be checked at different stages of this process to confirm the differentiation of hiPSCs into functional immature DCs.

[0301] Immature DCs derived from hiPSCs will be stimulated with IFN γ in exosome free media. SDC- Exos will then be harvested and confirmed via Western blot (CD63, Alix),
5 electron microscopy, and fluorescent staining coupled to ground state depletion microscopy.

[0302] Endpoints will be to measure changes in myelin levels (myelin basic protein via western blot and immunostaining; oxidative stress in response to menadione via CellRox fluorescence). miR-219 content will be confirmed by real-time PCR. Additionally, the impact of miR-219 on the above variables will be assessed by inhibiting miR-219 and
10 evaluating the effects. A scrambled form of the inhibitor will be used as a sham control.

[0303] Currently, little commonality exists between methods used by various laboratories interested in generating therapeutic exosomes. This can lead to discrepancies in therapeutic efficacies and potentially variable side effects. The protocols described herein may lead to a common platform for the fabrication of large quantities of exosomes that can
15 be reproducible and standardized, consistent with good manufacturing practices.

[0304] It is contemplated that the ability of hiPSCs to differentiate into different cell types and produce specific exosomes can help aid in their reproducibility and mass production. This work here may further lead to subsequent development of “bioreactor-like” fabrication strategies that could extend the utility of the work to a wide array of users. This project
20 can begin to demonstrate the feasibility and utility of utilizing adult skin biopsies for the production of patient-specific therapeutic exosomes for a wide variety of diseases.

EXAMPLE 14: Development of Adult Human Dendritic Cell-Derived Exosomes.

[0305] Multiple sclerosis (MS) and migraine are interrelated healthcare burdens with considerable negative impact. Spreading depression (SD), the underlying cause of migraine with aura and a well-accepted animal model for migraine, occurs with transient demyelination (Pusic AD et al, Exp Neurol, 2015) and increased oxidative stress (OS) (Grinberg YY et al., J Neurochem, 2012). Likewise, myelin is damaged in MS and increased OS contributes to a progressive inability to remyelinate.
25

[0306] The inventors have developed a novel cell-based therapy – exosomes produced by IFN γ -stimulated dendritic cells (SDC-Exos) that contain microRNA species including miR-219. These exosomes can, for the first time, remyelinate damaged rat brain (Pusic AD et al., J Neuro Immunol, 2014) and prevent SD, perhaps by reducing OS (Pusic, unpublished
30

observation). Given the uncertainty in translating rodent studies to human therapeutics, the inventors propose human SDC-Exos, which recapitulate the effects seen from rodent SDC-Exos, as a novel therapeutic for brain demyelinating disorders.

[0307] Proof of concept studies follow for adult human dendritic cells (DCs) cultured from three sources: peripheral blood, cord blood and bone marrow. The methods involve selective derivation of adherent immature DCs, which are then stimulated by IFN γ for collection of conditioned media three days later. Identity of DCs was confirmed by morphology and immunostaining (i.e., CD11b⁺ for macrophages and CD11c⁺ for DCs). Exosome isolation was confirmed via electron microscopy (i.e., ~100 nm vesicles) and Western blot for exosomal surface protein CD63. Of the three tissue sources, the inventors found that human bone marrow-derived SDC-Exos triggered a significant increase (~170%) in myelin basic protein (MBP; as a measure of myelin), three days after application to rat hippocampal brain slice cultures. This is consistent with the ~122% increase seen in these cultures after application of rat SDC-Exos (Pusic AD, J Neuro Immunol, 2014) that reached a peak of 150% at five days before returning to baseline levels by seven days (Pusic AD, unpublished observations). IFN γ -stimulated Human SDC-Exos also contained significantly (i.e., > 2-fold) higher levels of miR-219 when compared to those derived from unstimulated human DCs. miR-219 is known to be necessary and sufficient for promoting oligodendrocyte precursor cell differentiation (Dugas JC et al., Neuron, 2010). Furthermore, SDC-Exos showed no evidence of causing microgliosis, and perhaps even reduce microglial activation.

[0308] MS and migraine are interrelated healthcare burdens with collective U.S. costs of \$40 billion annually, and represent the inventors' first disease targets. These disorders both involve myelin damage and increased OS.

[0309] Work from the inventors' laboratory demonstrates that exposure of animals to environmental enrichment (EE; increased physical, intellectual and social activity) promotes the release of myelination-promoting exosomes from peripheral immune cells. These exosomes also improve remyelination following acute demyelination of slice cultures. It is believed that they do so by delivering miRNA species that promote oligodendrocyte precursor cell (OPC) differentiation into mature, myelin producing cells (Pusic AD et al., J Neuro Immunol, 2014).

[0310] This work was extended to show that IFN γ -stimulated dendritic cells (SDC-Exos) can be used as a scalable *ex vivo* source of exosomes that are similarly therapeutic.

Additionally, SDC-Exos increase oxidative tolerance, reducing the impact of increased OS that occurs with neuroinflammation (in MS) and SD (in migraine). Thus, it is believed that that EE-mimetic exosomes derived from cultured DCs show great potential for development as a therapeutic for a wide array of neurological diseases that involve demyelination, including MS, migraine, cognitive decline from ageing, Alzheimer's disease and stroke.

[0311] The initial work was performed using exosomes produced by rat IFN γ -stimulated DCs delivered to rat cultured brain slices, and confirmed *in vivo* by nasally administering exosomes to rats (Schumer et al., Soc Neurosci abst, 2015). Here, the inventors translated the results of the rodent studies to humans by using human tissue to derive DCs for production of analogous exosomes.

[0312] These results support the feasibility and utility of producing SDC-Exos from autologous and heterologous human DC sources as a novel therapeutic to mitigate the impact of neurodegenerative disorders involving myelin dysfunction and oxidative stress, without producing negative inflammatory sequelae.

[0313] In the following figures, the results of work with rat SDC-Exos is outlined. Screening of exosomal miRNA revealed significant differences in the content of stimulated versus unstimulated DC-Exos. IFN γ treatment of DCs increased expression and packaging of miRNAs involved in oligodendrocyte differentiation and myelin production pathways, with a notable increase in miR-219. miR-219 is important for oligodendrocyte maturation into myelinating cells (**FIG. 37A**) and is known to reduce the expression of growth factors that inhibit oligodendrocyte precursor (OPC) differentiation. Indeed, it was found that exposure of brain slice cultures to SDC-Exos reduced expression levels of two targets of miR-219 involved in the OPC differentiation pathway (**FIG. 37B**). Specific targets were: PDGFR α , the receptor for a mitogen that promotes OPC proliferation and inhibits their differentiation, and ELOVL7, which regulates lipid metabolism and redox homeostasis. Furthermore, transfecting SDC-Exos with a miR-219-specific inhibitor before applying them to brain slice cultures abrogated their promyelinating effect (Pusic and Kraig, 2014).

[0314] It is important to note that SDC-Exos do not permanently elevate myelin levels in normal (uninjured) brain (**FIG. 37C**), as doing so would produce detrimental effects. However, it is likely that based on need (e.g., demyelinating disease) or increased neural activity (e.g., learning), increased myelin levels from SDC-Exo exposure may be maintained.

[0315] SDC-Exo exposure increased myelin levels and improved recovery from lysolecithin-induced demyelination without producing a deleterious effect on progenitor populations (Pusic AD et al., J Neuro Immunol, 2014) (**FIG. 38A**). In addition, treatment with SDC-Exos also reduced menadione-induced OS [brighter shade shows increased OS (**FIG. 38B**)]. This effect is likely due to an increase in slice culture oxidative tolerance (**FIG. 38B**), as the inventors found increased levels of glutathione (brighter grey cell bodies) in treated cultures, specifically in microglia (darker grey processes). This reduction in OS was abrogated by transfection of SDC-Exos with a miR-219-specific inhibitor, but not by a scrambled microRNA inhibitor negative control (**FIG. 38C**) [Adapted from Pusic et al., J Neuro Immunol, 2014 (A and B); Schumer and Kraig, Soc Neurosci abst, 2015 (C)].

[0316] The plan for production of human SDC-Exos (hSDC-Exos) is depicted in **FIG. 39**. The inventors examined (1) the utility of deriving hSDC-Exos from three human sources: bone marrow, cord blood and peripheral blood. Isolated human dendritic cells were grown in culture and stimulated with IFN γ . Three days later, conditioned media was collected for harvest of exosomes (hSDC-Exos). (2) Next, the impact of hSDC-Exos on microglial activation (as an indicator of OS) was determined (Pusic AD et al., Soc Neurosci abst, 2016).

[0317] In order to determine the plausibility of producing functional, myelin-promoting human exosomes, three sources were tested: peripheral blood, cord blood and bone marrow. Out of these three sources, bone marrow was found to be the most effective. Using bone marrow as the primary source led to better DC yield and subsequent hSDC-Exos production. Furthermore, these exosomes increase myelin in rat brain slice culture, reduce inflammation, and contain increased levels of miR-219.

A. Cord Blood (n=1 trial).

1. Obtained one cryovial (100 million cord blood MNCs) from Lonza.
2. Good MNC viability upon thawing (>90%) and following CD34+ bead isolation (83-93%).
3. Plated 10,000 CD34+ isolated cells/well in 24-well plates (44 wells plated) with good attachment.
4. Observed fewer cells over time in culture (lost during feeding or did not survive?) despite being maintained in RPMI medium supplemented with serum, GM-CSF, TNF α and IL-4.

5. Conditioned medium was harvested from cells treated for 3 days with IFN γ , but exosomes were not isolated or assayed due to the low number of cells present at the end of the 12 day culture period.

B. Peripheral Blood Mononuclear Cells (PBMC)(n=6 trials).

- 5 1. PBMCs were isolated from 1 day old whole blood of adult male donors (from Zen-Bio).
2. Plated 5 million cells/well in 24-well plates (23-25 wells plated) or 2.4 million cells/well in 6-well plates (11-20 wells plated).
3. PBMCs maintained in serum-free XVIVO-15 medium supplemented with GM-CSF, and treated with IFN γ for 3 days yielded very few exosomes as measured by BCA protein assay
10 (low or negligible protein) and EM (very few vesicles).
4. Maintaining PBMCs in RPMI + 10% FBS +cytokines resulted in fewer healthy attached cells and many floating cells (phase-bright and shrunken morphology) which was not the case with XVIVO-15, where cells remained attached over the 10 day culture period.
5. PBMC exosome yield was too low to assay for effects on MBP in brain slice cultures.

15 C. Bone Marrow (n=14 trials).

1. CD34 $^{+}$ cells were isolated from fresh bone marrow obtained the previous day from adult male donors (Lonza).
2. Plated 4,000 CD34 $^{+}$ isolated cells/well in 24-well plates (72-528 wells plated) with good attachment.
- 20 3. Cells were maintained in RPMI+10% FBS supplemented with GM-CSF, TNF α and IL-4 and proliferated over the 13 day culture period.
4. Conditioned medium was harvested from human unstimulated bone marrow-derived DCs or bone marrow-derived DCs stimulated with IFN γ for 3 days. hSDC-Exos showed significant (i.e., > 2-fold) increase in miR-219 compared to those from unstimulated cells (see
25 below).
5. The average protein yield of exosomes isolated from 5 separate bone marrow-derived DC cultures was 867 μ g.
6. Treatment of OTCs for 3 days with 100 μ g of bone marrow SDC-Exos resulted in a significant increase (170%) in MBP (see below).

7. Treatment of OTCs for 3 days with 70 μ g of human bone marrow SDC exosomes did not result in microgliosis measured by Isolectin GS-IB4 staining (see below).

D. hSDC-Exo derived from human bone marrow characterization.

[0318] Human DC cultures were stained to determine purity. DC cultures expressed dendritic cell markers (CD11c⁺ and CD1a⁺), and did not express macrophage markers (CD11b⁻) (**FIG. 40**).

[0319] hSDC-Exos were also characterized (**FIG. 41**). Isolation was confirmed via Western blot for a well-characterized surface marker, CD63, which is larger in humans than in rats due to post translational modification. Electron microscopy image shows exosome size (scale bar, 100 nm), which was confirmed via NanoSight imaging (mode, 114 nm).

[0320] hSDC-Exos were applied to rat hippocampal slice cultures as a screen of function (**FIGS. 42 and 33**). FIG. 42 shows hippocampal slice culture architecture with NeuN staining of pyramidal and dentate gyrus neuronal layers (A), MBP immunostaining (B) and EM images of intact myelin sheaths (C). Quantification shows a significant rise in MBP, a marker for myelin, three days after exposure to SDC-Exos [compared to unstimulated DC-Exos (sham)]. FIG. 33 shows cytochemical staining (lectin isoB4) of microglia under control conditions (A and D) and three days after exposure to SDC-Exo (B and E). Quantification shows a significant reduction in microglial activation (C).

[0321] Details of the results shown in FIGS. 42 and 33 are as follows. (FIG. 42) Treatment with hSDC-Exos (versus sham treatment) produced a significant ($*p = 0.01$; $n = 5$ /group) increase in MBP. Thus, like EE-derived exosomes and rat SDC-Exos, hSDC-Exos likewise promote myelination. (FIG. 33).

[0322] Isolectin-GS-B4 staining served as a marker of microglial reactivity. Representative images taken at the CA3 area and used for semi-quantitative analysis are shown (A) sham (naïve) control culture; (B) hSDC-Exo-treated cultures. Scale bar = 200 μ m. (E) After three days, fluorescence intensity of microglial labeling fell significantly ($*p = 0.0002$) consistent with a drop in microglial activation (i.e., inflammation). Representative images of microglia from (D) sham control and (E) hSDC-Exo-treated hippocampal slice cultures are shown. Scale bar = 50 μ m.

[0323] RT-qPCR measurement of miR-219 in hSDC-Exos versus unstimulated human DC exosomes revealed a significant (>5.4 fold) (**FIG. 32**) increase in miR-219 content, consistent with the rat SDC-Exos. In addition, miRNA screening showed that the

promyelinating and anti-inflammatory miRNAs previously noted to be increased in exosomes from immune cells of animals exposed to EE and IFN γ -treated microglia are similarly elevated in hSDC-Exos (**FIG. 43**).

[0324] The above work shows for the first time that hSDC-Exos can significantly increase myelination and reduce inflammation. This strongly supports hSDC-Exo use as a novel neurotherapeutic for brain degenerative diseases including MS, migraine, Alzheimer' disease, epilepsy, stroke, and others as well as cognitive decline from ageing.

EXAMPLE 15: Development of Human SDC-Exos via Fibroblasts/Pluripotent Stem Cells Cultured to Become Immature Dendritic Cells

[0325] Multiple sclerosis (MS), migraine, and traumatic brain injury (TBI) are significant healthcare burdens which collectively cost the U.S. ~\$90 billion yearly. These disorders all involve myelin damage, which is exacerbated by generation of high levels of reactive oxygen species. The inventors have discovered that exosomes from stimulated dendritic cells (SDC-Exos) can remyelinate damaged rat brain and reduce oxidative stress. SDC-Exos are naturally occurring, deliver endogenous RNA species that promote myelination, are non-toxic and can be administered nasally to cross the blood brain barrier without use of an additive vehicle. **These traits make SDC-Exos well-suited for development as a novel exosome-based regenerative therapy.**

[0326] Example 14 shows, for the first time, that SDC-Exos derived from human bone marrow (hSDC-Exos) are likewise beneficial. However, given the uncertainty in translation of rodent studies to human therapeutics, the inventors have developed the ability to produce hSDC-Exos from a less invasive and scalable human source: fibroblast-derived induced human pluripotent stem cells (hiPSCs).

[0327] Environmental enrichment [(EE) i.e., increased intellectual, physical, and social activity] significantly reduces the severity of neurodegenerative disease. Understanding how this occurs may lead to novel neurotherapeutics. Work in the Inventor's laboratory directed to the development of EE-based therapeutics is conceptually and technologically innovative. The inventors studied the role of exosomes in EE and explored the use of dendritic cells (DCs) as a scalable *ex vivo* source of EE-mimetic exosomes.

[0328] SDC-Exos are also effective *in vivo* when nasally administered to rats (**FIG. 44**). Nasally administered rat SDC-Exos easily cross the blood brain barrier and distribute throughout the brain (Pusic, 2016a). The Thorne laboratory shows that agents administered

nasally enter brain along perineuronal (olfactory and trigeminal) routes to the CSF where they rapidly distribute via bulk flow of the CSF (Lockhead, 2015), and it is suspected that exosomes follow the same trajectory. Thus, the inventors have demonstrated for the first time that it is feasible to use cells from donor animals to generate exosomes that can be nasally administered to improve CNS health in recipient animals.

[0329] Described below are methods/results to document the feasibility/utility of culturing adult human fibroblasts and optimizing a transfection paradigm in preparation for making human pluripotent stem cells (iPSCs) that are then differentiated into dendritic cells as an alternate source of hSDC-Exos (i.e., hiSDC-Exos). An outline of the plans for hiPSC use is schematized in **FIG. 45** and stated below:

A. Culture and Passaging Fibroblasts

[0330] a) Method: Thaw frozen fibroblasts obtained from Lonza and initiate culture following vendor's instructions. Feed cultures every other day and passage once 70-80% confluent. b) Evaluation: Monitor cell confluency over time in culture and assess cell viability and cell number when subculturing. c) Milestone: Fibroblasts are ready for nucleofection after passaging twice when growth rate is stable and they have recovered from cryopreservation with little evidence of cell toxicity (Sytox staining) (**FIG. 46**).

B. Nucleofection Optimization

[0331] a) Method: Seven different nucleofection programs for transfection of human fibroblasts with a GFP reporter plasmid using the P2 Primary Cell 4D-Nucleofector X Kit in conjunction with the Amaxa 4D-Nucleofector (Lonza) were tested. The best was found to be the proprietary "DS-150" program. b) Evaluation: Assess fibroblast cell viability and GFP expression for each nucleofection program. c) Milestone: An optimal nucleofection program (DS-150) was achieved which demonstrated good transfection efficiency (>90%) and cell viability (>80%) and will be used for reprogramming fibroblasts. (**FIG. 47**).

C. Preparation of Plasmids Containing Reprogramming Factors (L-MYC, LIN28, SOX2, KLF4, sh-p53, OCT3/4, EBNA-1)

[0332] a) Method: Grow transformed bacteria expressing the 5 reprogramming episomal plasmids obtained from Addgene (pCE-hUL (L-MYC, LIN28), pCE-hSK (SOX2, KLF4), pCE-mp53DD, pCE-OCT3/4 and pCXB-EBNA-1) and purify plasmid DNA. b) Evaluation: Verify purified plasmid by analysis of DNA fragment size following restriction enzyme digest and by DNA sequencing c) Milestone: The plasmids are ready to use for fibroblast

nucleofection if the restriction enzyme digest pattern and DNA sequence match the predicted results (SEQ ID NOS:1-5).

[0333] pCE-hOCT-3/4:

CAGCTTGGGCTGCAGGTCGAGGGATCTCCATAAGAGAAGAGGGACAGCTATGAC
 5 TGGGAGTAGTCAGGAGAGGAGGAAAAATCTGGCTAGTAAAACATGTAAGGAAA
 ATTTTAGGGATGTTAAAGAAAAAAATAACACAAAACAAAATATAAAAAAAATCT
 AACCTCAAGTCAAGGCTTTTCTATGGAATAAGGAATGGACAGCAGGGGGGCTGTT
 TCATATACTGATGACCTCTTTATAGCCAACCTTTGTTCATGGCAGCCAGCATATG
 GGCATATGTTGCCAAACTCTAAACCAAATACTCATTCTGATGTTTTAAATGATTT
 10 GCCCTCCCATATGTCCTTCCGAGTGAGAGACACAAAAAATTCCAACACACTATTG
 CAATGAAAATAAATTTCTTTATTAGCCAGAAGTCAGATGCTCAAGGGGCTTCAT
 GATGTCCCCATAATTTTTTGGCAGAGGGAAAAAGATCTCAGTGGTATTTGTGAGCC
 AGGGCATTGGCCACACCAGCCACCACCTTCTGATAGGCAGCCTGCACCTGAGGA
 GTGAATTCGCCCTTACCTCAGTTTGAATGCATGGGAGAGCCCAGAGTGGTGACAG
 15 AGACAGGGGGAAAGGCTTCCCCCTCAGGGAAAGGGACCGAGGAGTACAGTGCA
 GTGAAGTGAGGGCTCCCATAGCCTGGGGGTACCAAATGGGGCCCTGGGGCCAG
 AGGAAAGGACACTGGTCCCCCTGAGAAAGGAGACCCAGCAGCCTCAAAATCCTC
 TCGTTGTGCATAGTCGCTGCTTGATCGCTTGCCCTTCTGGCGCCGGTTACAGAACC
 ACACTCGGACCACATCCTTCTCGAGCCCAAGCTGCTGGGCGATGTGGCTGATCTG
 20 CTGCAGTGTGGGTTTCGGGCACTGCAGGAACAAATTCTCCAGGTTGCCTCTCACT
 CGGTTCTCGATACTGGNTCGCTTTCTCTTTTCGGNNGCACGAGGGTTCTGCTTTGCA
 TATCTCTGAGATTTTCATGTGTCAGCTCTCACCNNTCTGCAGCAGGGNGCAGCTAC
 ACATGTCTGAGCTAGCTGCANAGCTCAAGCGCNATGTCNTGNTGATACTTCCAAA
 TAGANNCAGGTGANCCCATCGCNNGNNAATTCAGGNTCNTTCNNNNNNNNGGCAN
 25 GTCTNGAGNNNNNNNNNN (SEQ ID NO:1).

[0334] pCE-hSK:

CAGCTTGGGCTGCAGGTCGAGGGATCTCCATAAGAGAAGAGGGACAGCTATGAC
 TGGGAGTAGTCAGGAGAGGAGGAAAAATCTGGCTAGTAAAACATGTAAGGAAA
 ATTTTAGGGATGTTAAAGAAAAAAATAACACAAAACAAAATATAAAAAAAATCT
 30 AACCTCAAGTCAAGGCTTTTCTATGGAATAAGGAATGGACAGCAGGGGGGCTGTT
 TCATATACTGATGACCTCTTTATAGCCAACCTTTGTTCATGGCAGCCAGCATATG
 GGCATATGTTGCCAAACTCTAAACCAAATACTCATTCTGATGTTTTAAATGATTT
 GCCCTCCCATATGTCCTTCCGAGTGAGAGACACAAAAAATTCCAACACACTATTG

CAATGAAAATAAATTTTCCTTTATTAGCCAGAAGTCAGATGCTCAAGGGGCTTCAT
GATGTCCCCATAATTTTTGGCAGAGGGAAAAAGATCTCAGTGGTATTTGTGAGCC
AGGGCATTGGCCACACCAGCCACCACCTTCTGATAGGCAGCCTGCACCTGAGGA
GTGAATTCCTGCAGGCAATTCGCCCTTTCAATTCTGTGCCTCCGGGAGCAGGGTA
5 GGGCTGTGGATTCTTCTTCTTCCTCTCGAAAGTAGGTTGGCTTTCCCTGTGCACT
AGGGCCCTGCTGGGCCTTCAGCGGACATGAGGCTACCATATGGCTGATGCTCTGG
CAGAAGTGGCACTTCTTGGGCTGGGGTGGCAGCTTGCATTCCTTGGCATGATGAT
CTAGACCTCCACAGTTGTAGCACCTGTCTCCTTTTGATCTGCGCTTCTGCATGCTC
TTTCCTTTTGGCCGCCTCTCACTCCCAATACAGAATACTCCACCAGGTCCGGTGA
10 CACGGATGGATTCCAGACCCTTGGCTGACTTCTTAAAGGTGAACTCCACTGCCTC
ACCCNCCTTCAGCTCNGAACCTTTCATGTGCAGCTTACTCTGGTGCACAAAGAC
ATCCACTGGGGGGGTGAGCGCGACNCCNGCGCGGGCTGTCATGNCAGAAGNNG
NNCNCATGCGCACGTTGANNACTTACAGATTGNCCGCANCTGTTGCAGCAGCTG
AGNNNGTCGGCCGNCTGGNNNNNGTCTCAGNGCTCCTCGGGNGCCCTNCTGCNC
15 NNNNNGATACTNNCAACTGNNNNNGTTGANATCGGAGANCCAAATGGT (SEQ ID
NO:2).

[0335] pCE-hUL:

AGTCTTGGGCTGCAGGTCGAGGGATCTCCATAAGAGAAGAGGGACAGCTATGAC
TGGGAGTAGTCAGGAGAGGAGGAAAAATCTGGCTAGTAAAACATGTAAGGAAA
20 ATTTTAGGGATGTTAAAGAAAAAAATAACACAAAACAAAATATAAAAAAAATCT
AACCTCAAGTCAAGGCTTTTCTATGGAATAAGGAATGGACAGCAGGGGGCTGTT
TCATATACTGATGACCTCTTTATAGCCAACCTTTGTTCATGGCAGCCAGCATATG
GGCATATGTTGCCAAACTCTAAACCAAATACTCATTCTGATGTTTTAAATGATTT
GCCCTCCCATATGTCCTTCCGAGTGAGAGACACAAAAAATTCCAACACACTATTG
25 CAATGAAAATAAATTTTCCTTTATTAGCCAGAAGTCAGATGCTCAAGGGGCTTCAT
GATGTCCCCATAATTTTTGGCAGAGGGAAAAAGATCTCAGTGGTATTTGTGAGCC
AGGGCATTGGCCACACCAGCCACCACCTTCTGATAGGCAGCCTGCACCTGAGGA
GTGAATTCCTGCAGGCAATTCGCCCTTTCAATTCTGTGCCTCCGGGAGCAGGGTA
GGGCTGTGGATTCTTCTTCTTCCTCTCGAAAGTAGGTTGGCTTTCCCTGTGCACT
30 AGGGCCCTGCTGGGCCTTCAGCGGACATGAGGCTACCATATGGCTGATGCTCTGG
CAGAAGTGGCACTTCTTGGGCTGGGTGGCAGCTTGCATTCCTTGGCATGATGATC
TAGACCTCCACAGTTGTAGCACCTGTCTCCTTTTGATCTGCGCTTCTGCATGCTCT
TTCCTTTTGGCCGCCTCTCACTCCCAATACAGAATACTCCACCAGGTCCGGTGAC

ACGGATGGATTCCAGACCCTTGGCTGACTTCTTAAAGGTGAACTCCACTGCCTCA
 CCCTCCTTCAAGCTCCGGAACCCTTCCATGTGCAGCTTACTCTGGTGCACAAAGA
 CATCCACTGGGGGGGGTTCGAGCGCGACCCCGGCGCGGGGCGNCATGGNCAGGAGCC
 GACCCCATGCGNACGTTGANCACTACAGATGCCCCGCACCGTGCAGCAGCTGAGN
 5 TCGTCCGCGCCCNGNNGNGTCNNGNNTCCTCGGGGCCNTNCTGCGCNGNNNN
 NCNCCTGCANTGCTNNNNNANCGNNCATGTNNNAGGGGNATGNTNN (SEQ ID
 NO:3).

[0336] pCE-mp53DD:

CAGCTTGGGCTGCAGGTCGAGGGATCTCCATAAGAGAAGAGGGACAGCTATGAC
 10 TGGGAGTAGTCAGGAGAGGAGGAAAAATCTGGCTAGTAAAACATGTAAGGAAA
 ATTTTAGGGATGTTAAAGAAAAAAATAACACAAAACAAAATATAAAAAAATCT
 AACCTCAAGTCAAGGCTTTTCTATGGAATAAGGAATGGACAGCAGGGGGCTGTT
 TCATATACTGATGACCTCTTTATAGCCAACCTTTGTTTCATGGCAGCCAGCATATG
 GGCATATGTTGCCAAACTCTAAACCAAATACTCATTCTGATGTTTTAAATGATTT
 15 GCCCTCCCATATGTCCTTCCGAGTGAGAGACACAAAAAATTCCAACACACTATTG
 CAATGAAAATAAATTTCTTTATTAGCCAGAAGTCAGATGCTCAAGGGGCTTCAT
 GATGTCCCCATAATTTTTTGGCAGAGGGAAAAAGATCTCAGTGGTATTTGTGAGCC
 AGGGCATTGGCCACACCAGCCACCACCTTCTGATAGGCAGCCTGCACCTGAGGA
 GTGAATTCGCCCTTCAGTCTGAGTCAGGCCCCACTTTCTTGACCATTGTTTTTTTA
 20 TGGCGGGAAGTAGACTGGCCCTTCTTGGTCTTCAGGTAGCTGGAGTGAGCCCTGC
 TGTCTCCAGACTCCTCTGTAGCATGGGCATCCTTTAACTCTAAGGCCTCATTACAG
 TCCCGGAACATCTCGAAGCGTTTACGCCCGCGGATCTTGAGGGTGAAATACTCTC
 CATCAAGTGGTTTTTTTCTTTTGGCGGGGAGAGGGCGCTTGTGCAGGTGGGCAGCGC
 TCTCTTGAGGCTGATATCCGACTGTGACTCCTCCATGGCAGTCATCCTGGTGAAG
 25 GGCGAATTCTTTGCCAAATGATGAGACAGCACAATAACCAGCACGTTGCCCAGG
 AGCTGTAGAAAAAGAAGANCATGAACATGGTTAGCAGAGCTCTAGAGCCGCGTC
 ACACGCAGAGCGACCCCGCCTGCCCCGTCCNCCCGANCAGCGTCCCCCGCGACA
 GCNCGAGCTGANAGGANANGGNGCGCCGGCGACTNCGANNCTCCCCGTCCAT
 NCNTCTGCNGCGCGCACNNNTCGCTCGGNNGCTTAANNCNN (SEQ ID NO:4).

30 [0337] pCXB-EBNA1:

CAGCTTGGGCTGCAGGTCGAGGGATCTCCATAAGAGAAGAGGGACAGCTATGAC
 TGGGAGTAGTCAGGAGAGGAGGAAAAATCTGGCTAGTAAAACATGTAAGGAAA
 ATTTTAGGGATGTTAAAGAAAAAAATAACACAAAACAAAATATAAAAAAATCT

AACCTCAAGTCAAGGCTTTTCTATGGAATAAGGAATGGACAGCAGGGGGCTGTT
 TCATATACTGATGACCTCTTTATAGCCAACCTTTGTTTCATGGCAGCCAGCATATG
 GGCATATGTTGCCAAACTCTAAACCAAATACTCATTCTGATGTTTTAAATGATTT
 GCCCTCCCATATGTCCTTCCGAGTGAGAGACACAAAAAATTCCAACACACTATTG
 5 CAATGAAAATAAATTTTCCTTTATTAGCCAGAAGTCAGATGCTCAAGGGGCTTCAT
 GATGTCCCCATAATTTTTTGGCAGAGGGAAAAAGATCTCAGTGGTATTTGTGAGCC
 AGGGCATTGGCCACACCAGCCACCACCTTCTGATAGGCAGCCTGCACCTGAGGA
 GTGAATTCGCCCTTGGATCCTCACTCCTGCCCTTCCTCACCTCATCTCCATCACC
 TCCTTCATCTCCGTCATCTCCGTCATCACCTCCGCGGCAGCCCCCTTCCACCATAG
 10 GTGGAAACCAGGGAGGGCAAATCTACTCCATCGTCAAAGCTGCACACAGTCACCC
 TGATATTGCAGGTAGGAGCGGGCTTTGTCATAACAAGGTCCTTAATCGCATCCTT
 CAAAACCTCAGCAAATATATGAGTTTGTA AAAAGACCATGAAATAACAGACAAT
 GGA CTCCCTTAGCGGGCCAGGTTGTGGGCGGGTCCAGGGGGCCATTCCAAAGGG
 GAGACGACTCAATGGTGTAAGACGACATTGTGGAATAGCAAGGGCAGTTCCTCG
 15 CCTTAGGTTGTAAAGGGAGGTCTTACTACCTCCATATACGAACACACCGGCGACC
 CAAGTTCNTCGTCGGTAGTCCTTTCTACGTGACTCTAGCAGGAGAGCTCTAACT
 NCTGCATGTCTCAATTCGGTGACTNNGACACGATGCTTCAACCACNCCTTTTTGC
 GCTGCTCATCACCTGACCCGGGNCAGNGCTTGGGNCNTCTCCTN (SEQ ID NO:5).

[0338] SEQ ID NOS:1-5 show the plasmid sequence results. Episomal plasmids for
 20 reprogramming fibroblasts into iPSCs were obtained from Addgene as a stab culture.
 Plasmids were streaked onto LB Agar plates containing the appropriate selection antibiotic,
 and a single colony picked and inoculated into LB broth (also containing antibiotic). Plasmids
 were then purified using a commercially available kit. Plasmids were then sent for DNA
 sequencing and their identification verified by BLAST against their sequences provided by
 25 Addgene. The sequencing results are provided here in FASTA file format.

D. Integration-free Reprogramming of Dermal Fibroblasts with Episomal Plasmids

[0339] a) Method: The protocol is adapted from methods put forward by Baghbaderani
 et al., (2016) in which fibroblasts (passage 4-9) are transfected with 3-5 micrograms of an
 equimolar mixture of the 5 episomal plasmids using program DS-150 of Lonza's 4D
 30 Nucleofector system. After nucleofection, transfer the cells to a 6-well plate pre-coated with
 L7™ hPSC matrix (Lonza). Feed cells on days 1, 3 and 5 following transfection with
 DMEM + 10% FBS. Starting on Day 7 and every other day thereafter, feed with L7™ hPSC
 medium. b) Evaluation: Cell morphology is monitored over time in culture as

reprogrammed fibroblasts will lose their spindle shape and attain a rounder morphology forming compact colonies ~21-35 days post-transfection. Live cell staining is performed with Tra-1-60 or Tra-1-81 antibodies to distinguish reprogrammed colonies. c) Milestone: Colonies, approximately 0.5-1.0 mm in diameter, exhibiting embryonic stem cell (ESC) morphology (flat, cobblestone appearance with distinct borders; high nucleus-to-cytoplasm ratio; prominent nucleoli) , and/or positive for Tra-1-60/Tra-1-81, are large enough to be picked and expanded.

E. hiPSC Maintenance and Cryopreservation Banking

[0340] a) Method: The inventors manually pick colonies for expansion by cutting them into smaller fragments with a 25 gauge x 1-1/2 inch needle and transferring the pieces to a 12-well plate coated with L7™ hPSC matrix containing L7™ hPSC medium. The iPSC lines are cryopreserved at multiple passages (p4, p6, p9, p12) which can be subsequently expanded for characterization and banking. b) Evaluation: Monitor iPSC lines for changes in phenotype with each passage, i.e., showing signs of differentiation or irregular morphology. c) Milestone: hiPSC lines retaining ESC morphology at p10 are further characterized.

F. hiPSC Characterization of Pluripotency Markers

[0341] a) Method: hiPSC lines are evaluated for expression level, as well as stable expression, of undifferentiated stem cell markers (Tra-1-60, Tra-1-81, SSEA-4, Oct4, Sox2, Nanog) as compared to the differentiated iPSC marker (SSEA-1) or a fibroblast marker (CD13) via immunocytochemistry and/or flow cytometry. b) Evaluation: Expression of pluripotent stem cell markers will be monitored regularly through p10 and re-verified at ≥ p20. c) Milestone: By passage 10, completely reprogrammed hiPSC lines exhibit high expression of undifferentiated stem cell markers, no CD13 and little or no expression of SSEA-1 (**FIG. 48**).

G. Absence of Reprogramming Plasmids in hiPSC Lines

[0342] a) Method: PCR is performed on DNA from hiPSC lines for the EBNA-1 plasmid DNA sequence contained in all episomal vectors. b) Evaluation: Expression of EBNA-1 is compared between iPSC lines at early passage versus p10. c) Milestone: Absence of EBNA-1 expression indicates loss of episomal plasmids with hiPSC passaging (no plasmid integration).

H. Karyotyping hiPSC Lines

[0343] a) Method: G-banding chromosomal analysis is performed (WiCell, Madison, WI) on hiPSC lines. b) Evaluation: Karyotype analysis is performed comparing donor fibroblasts to hiPSC lines at p10 and $\geq$ p20 in order to assess genomic abnormalities and cell line stability during passaging. c) Milestone: Stable hiPSC lines without chromosomal abnormalities can be established.

I. *In Vitro* Assay of Embryoid Body Development

[0344] a) Method: Embryoid bodies are generated via dispase digestion and manual scraping and are maintained in rotary orbital culture which has been shown to support the differentiation of hiPSCs into the 3 germ layers (endoderm, mesoderm, ectoderm) after 14 days of culture. b) Evaluation: Assess the differentiation potential of hiPSC lines to form embryoid bodies and exhibit immunolabeling of germ layer markers for endoderm (alpha-fetoprotein), mesoderm (smooth muscle actin), and ectoderm (beta III tubulin). c) Milestone: hiPSC lines, exhibiting pluripotency as evidenced by the capacity to form embryoid bodies and differentiate into endoderm, mesoderm and ectoderm, will be used to generate dendritic cells. (FIG. 49).

J. Progressive Differentiation of hiPSCs to Dendritic Cells

[0345] a) Method: The differentiation protocol consists of the formation of embryoid-like bodies from hiPSC colonies which are maintained in rotary, orbital culture undercultured in serum-free, defined medium conditions in the presence of various growth factors/cytokines (Leishman and Fairchild, 2014; Silk et al., 2012; Tseng et al., 2009). The embryoid bodies are maintained in rotary, orbital culture during differentiation unlike the static, suspension culture method described previously for DCs derived from hiPSCs (Leishman and Fairchild, 2014; Silk et al., 2012). These cells are then directed into the hematopoietic lineage via exposure to BMP4, GM-CSF, SCF and VEGF. BMP4 is important to push hiPSCs into the initial stage of mesodermal commitment (Stage 1). Further differentiation into hematopoietic progenitors is accomplished by the successive removal of BMP-4 on day 5, VEGF on day 14 and finally SCF on day 19 of culture (Stage 2). Common myeloid progenitors are differentiated into DC progenitors with the addition of a low concentration of IL-4 on days 14-18. Final differentiation of DC progenitors into immature DCs will be accomplished by adding GM-CSF and a higher concentration of IL-4 on days 19-30 (Stage 3). Non-adherent, immature DCs derived from hiPSCs will be replated and then stimulated with IFN γ alone (in

contrast to treating cells with cocktail of cytokines/factors to generate mature DCs as described previously by others (Leishman and Fairchild, 2014; Silk et al., 2012). and SDC-Exos harvested from conditioned media. b) Evaluation: Expression of specific markers will be assessed using flow cytometry at different stages over the course of differentiation of hiPSCs into immature DCs: Stage 1 (Bry+, c-kit+), Stage 2 (CD34+, CD45+) and Stage 3 (CD1a, CD11c+). c) Milestone: hiPSC derived DCs expressing appropriate phenotypic surface markers can be generated and subsequently stimulated with IFN γ to produce exosomes for functional studies. (FIG 50).

K. Phenotypic Characterization of IFN γ -Stimulated hiPSC-derived DCs versus Primary Human and/or Rat Bone Marrow-derived DCs

[0346] a) Method: Immature hiPSC-derived DCs and primary human and/or rat bone marrow DC's are cultured in the presence or absence of IFN γ and characterized for expression of DC surface markers. b) Evaluation: DC surface marker expression (i.e., CD11c, CD1a, CD80, CD83, CD86, HLA-DR) is assessed using flow cytometry and comparisons made between tissue derived DC cell types with or without cytokine stimulation. c) Milestone: The findings provides information as to whether the pattern of cell surface marker expression of hiPSC-derived DCs in response to IFN γ stimulation is comparable to that observed with DCs derived from human or rat bone marrow. (FIG. 51).

L. Functional Assay of Exosomes (hiSDC-Exos) from IFN γ -Stimulated hiPSC-derived DCs as Compared to Primary Human and/or Rat Bone Marrow-derived DCs

[0347] a) Method: Harvest exosomes from IFN γ stimulated (SDC Exos) or non-stimulated DCs derived from hiPSCs, human bone marrow or rat bone marrow and apply to rat slice cultures for varying periods of time ranging from 3 hours to 7 days (see Figures above for bone marrow derived hSDC-Exos). b) Evaluation: Measure changes in myelin levels (myelin basic protein via Western blot and immunostaining) and oxidative stress (in response to menadione via CellRox fluorescence) in slice cultures. Quantify miR-219 content of exosomes by real-time PCR. Additionally, the impact of exosomal miR-219 on slice culture myelin and oxidative stress markers is assessed via transfection of exosomes with a miR-219 inhibitor. A scrambled form of the inhibitor will be used as a sham control. c) Milestone: The results will confirm whether SDC-Exos from hiPSC-derived DCs (originating from human fibroblasts) show functional equivalency to SDC-Exos derived from human or

rat bone marrow DCs and show increased levels of miR-219 and associated other miRNAs (see Figure 43). **miR-219 was detected in exosomes isolated from hiPSC DCs.**

* * *

[0348] All of the methods disclosed and claimed herein can be made and executed without undue experimentation in light of the present disclosure. While the compositions and methods of this invention have been described in terms of preferred embodiments, it will be apparent to those of skill in the art that variations may be applied to the methods and in the steps or in the sequence of steps of the method described herein without departing from the concept, spirit and scope of the invention. More specifically, it will be apparent that certain agents which are both chemically and physiologically related may be substituted for the agents described herein while the same or similar results would be achieved. All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit, scope and concept of the invention as defined by the appended claims.

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CLAIMS

1. A method of producing isolated exosomes from human cells comprising:
 - a. differentiating human bone marrow cells or human induced pluripotent stem cell (hiPSC)-derived progenitor cells into dendritic cells;
 - b. inducing human dendritic cells to undergo external oxidative stress, wherein the cells produce exosomes and wherein inducing the cells to undergo oxidative stress comprises contacting the cells with IFN- γ ; and
 - b. isolating the produced exosomes.
2. The method of claim 1, wherein differentiating the cells into dendritic cells comprises the formation of embryoid bodies and detachment of the human bone marrow cells or hiPSCs from a substrate.
3. The method of claim 2, wherein the detachment of the human bone marrow cells or hiPSCs from the substrate comprises contacting the human bone marrow cells or hiPSCs with a protease.
4. The method of claim 3, wherein the protease comprises dispase.
5. The method of claim 1 or 4, wherein differentiating progenitor cells into dendritic cells comprises the maintenance of embryoid bodies in rotary orbital culture.
6. A method of producing isolated exosomes from human cells comprising:
 - a. inducing human dendritic cells to undergo external oxidative stress, wherein the cells produce exosomes; and
 - b. isolating the produced exosomes.
7. The method of claim 6, wherein the dendritic cells are *in vitro* differentiated dendritic cells.
8. The method of claim 6 or 7, wherein the dendritic cells are CD11b-.
9. The method of any one of claims 6-8, wherein the dendritic cells are CD11c+or CD1a+.
10. The method of any one of claims 6-9, wherein the dendritic cells are CD14-.
11. The method of any one of claims 7-11, wherein the dendritic cells are differentiated from human stem cells, human-derived stem cells, human progenitor cells, human induced pluripotent stem cells (hiPSCs), human peripheral blood mononuclear cells (PBMCs), human bone marrow mononuclear cells (BMMCs), or human cord blood mononuclear cells (CBMCs).

12. The method of claim 11, wherein the dendritic cells are differentiated from human stem cells, human derived stem cells, or human progenitor cells.
13. The method of claim 12, wherein the human stem cells or human progenitor cells are cells isolated from bone marrow, cord blood, adipose tissue, or whole blood.
14. The method of claim 12, wherein the dendritic cells are differentiated from BMMCs isolated from bone marrow or CBMCs isolated from cord blood.
15. The method of claim 14, wherein the dendritic cells are differentiated from CD34+ cells.
16. The method of claim 11, wherein the dendritic cells are differentiated from PBMCs isolated from whole blood.
17. The method of claim 16, wherein the dendritic cells are differentiated from monocytes.
18. The method of claim 11, wherein the dendritic cells are differentiated from iPSCs.
19. The method of claim 18, wherein the iPSCs are derived from human fibroblasts.
20. The method of claim 19, wherein the human fibroblasts are isolated from human dermal tissue or human adipose tissue.
21. The method of claim 19 or 20, wherein the fibroblasts are from a skin biopsy.
22. The method of claim 18, wherein the iPSCs are derived from human adipose stem cells, human keratinocytes, and human PBMCs.
23. The method of any one of claims 6-22, wherein the method further comprises differentiating progenitor cells into dendritic cells.
24. The method of claim 23, wherein the progenitor cells are derived from human iPSCs.
25. The method of claim 24, wherein the method further comprises contacting the cells with BMP4.
26. The method of claim 24 or 25, wherein the method further comprises contacting the cells with VEGF and/or SCF.
27. The method of any one of claims 24-26, wherein the method further comprises contacting the cells with one or more of M-CSF, SCF, FL3, IL-3, TPO, GM-CSF, and IL-4.

28. The method of any one of claims 24-27, wherein differentiating the progenitor cells into dendritic cells comprises the sequential steps of:
- contacting the cells with GM-CSF, FL3, and M-CSF and
 - contacting the cells with GM-CSF and IL-4.
29. The method of any one of claims 24-28, wherein differentiating the progenitor cells into dendritic cells comprises the sequential steps of:
- contacting the cells with M-CSF, SCF, FL3, IL-3, and TPO;
 - contacting the cells with GM-CSF, FL3, and M-CSF; and
 - contacting the cells with GM-CSF and IL-4.
30. The method of any one of claims 24-29, wherein differentiating the progenitor cells into dendritic cells comprises the sequential steps of:
- contacting the cells with VEGF and SCF;
 - contacting the cells with M-CSF, SCF, FL3, IL-3, and TPO;
 - contacting the cells with GM-CSF, FL3, and M-CSF; and
 - contacting the cells with GM-CSF and IL-4.
31. The method of any one of claims 24-30, wherein differentiating the progenitor cells into dendritic cells comprises the sequential steps of:
- contacting the cells with BMP4;
 - contacting the cells with VEGF and SCF;
 - contacting the cells with M-CSF, SCF, FL3, IL-3, and TPO;
 - contacting the cells with GM-CSF, FL3, and M-CSF; and
 - contacting the cells with GM-CSF and IL-4.
32. The method of any one of claims 24-27, wherein differentiating the progenitor cells into dendritic cells comprises the sequential steps of:
- contacting the cells with a composition comprising BMP4, GM-CSF, SCF, and VEGF;
 - contacting the cells with a composition comprising GM-CSF, SCF, and VEGF; wherein the composition excludes BMP4;
 - contacting the cells with a composition comprising GM-CSF, and SCF; wherein the composition excludes BMP4 and VEGF; and
 - contacting the cells with a composition comprising GM-CSF; wherein the composition excludes BMP4, VEGF, and SCF.

33. The method of any one of claims 24-27 and 32, wherein differentiating the progenitor cells into dendritic cells comprises or further comprises the sequential steps of:
- contacting the cells with a composition comprising IL-4; and
 - contacting the cells with a composition comprising IL-4 and GM-CSF.
34. The method of any one of claims 24-33, wherein differentiating progenitor cells into dendritic cells comprises or further comprises the formation of embryoid bodies.
35. The method of claim 34, wherein the formation of embryoid bodies comprises or further comprises detachment of the hiPSCs from a substrate.
36. The method of claim 35, wherein the detachment of the hiPSCs from the substrate comprises contacting the hiPSCs with a protease.
37. The method of claim 36, wherein the protease comprises dispase.
38. The method of claim 34-37, wherein differentiating progenitor cells into dendritic cells comprises the maintenance of embryoid bodies in rotary orbital culture.
39. The method of any one of claim 24-38, wherein the method further comprises inducing pluripotent stem cells from human cells.
40. The method of claim 39, wherein the human cells comprise human fibroblasts, human adipose stem cells, human adipose-derived stem cells, human keratinocytes, or human PBMCs.
41. The method of claim 39 or 40, wherein inducing pluripotent stem cells from human cells comprises contacting the cells with one or more of SOX2, KLF4, c-Myc, and LIN28.
42. The method of claim 23, wherein the progenitor cells are isolated from human mononuclear cells.
43. The method of claim 42, wherein the human mononuclear cells are isolated from bone marrow or cord blood.
44. The method of claim 42 or 43, wherein differentiating the progenitor cells into dendritic cells further comprises contacting the cells with one or more of GM-CSF, TNF α , and IL-4.
45. The method of any one of claims 42-44, wherein differentiating the progenitor cells into dendritic cells comprises the sequential steps of:
- contacting the cells with GM-CSF and TNF α and
 - contacting the cells with GM-CSF, TNF α , and IL4.

46. The method of claim 23, The method of claim 28, wherein the progenitor cells are isolated from PBMCs.
47. The method of claim 46, wherein the PBMCs are isolated from whole blood.
48. The method of claim 46 or 47, wherein differentiating the progenitor cells into dendritic cells comprises contacting the cells with one or more of GM-CSF and IL4.
49. The method of any one of claims 6-48 wherein inducing the cells to undergo oxidative stress comprises contacting the cells with IFN- γ .
50. The method of any one of claims 6-49 wherein inducing the cells to undergo oxidative stress comprises contacting the cells with a composition comprising IFN- γ , GM-CSF, and IL4.
51. The method of claim 50, wherein the composition further comprises TNF α .
52. The method of claim 50 or 51, wherein the composition further comprises exosome-free serum.
53. The method of any one of claims 6-52, further comprising administering to a patient at risk for or having a demyelinating disorder an effective amount of a pharmaceutical composition comprising the isolated exosomes.
54. The method of claim 53, wherein the demyelinating disorder is cognitive decline, Alzheimer's disease, Parkinson's disease, stroke, epilepsy, migraine, traumatic brain injury, post-traumatic stress disorder, post-traumatic headache, multiple sclerosis, neuropathy, tauopathy, or ageing-induced cognitive decline.
55. The method of claim 54, wherein the demyelinating disorder is multiple sclerosis or traumatic brain injury.
56. The method of claim 54, wherein the demyelinating disorder is migraine.
57. The method of claim 56, wherein the migraine comprises migraine with aura.
58. The method of any one of claims 6-57, wherein the dendritic cells are dendritic cells obtained from the patient or derived from cells obtained from the patient.
59. The method of claim 58, wherein the cells are obtained from the patient when the patient is less than 18 years old.

60. The method of claim 59, wherein the cells are obtained from the patient when the patient is less than one year old.
61. Isolated exosomes produced according to the method of any of claims 6-52.
62. A pharmaceutical composition comprising the isolated exosomes of claim 61 and a pharmaceutically acceptable carrier.
63. A method for treating a patient at risk for or having a demyelinating disorder comprising administering to the patient an effective amount of the pharmaceutical composition of claim 62.
64. Isolated lipid nanovesicles comprising at least two of miR-219, miR-138, and miR-199a.
65. Isolated lipid nanovesicles of claim 64, wherein the isolated lipid nanovesicles comprise miR-219 and miR-138.
66. Isolated lipid nanovesicles of claim 64, wherein the isolated lipid nanovesicle are obtained from human cells.
67. Isolated lipid nanovesicles of claim 66, wherein the cells are dendritic cells.
68. Isolated lipid nanovesicles of claim 66-67, wherein the dendritic cells are derived from human stem cells, human-derived stem cells, human progenitor cells or human-derived progenitor cells.
69. Isolated lipid nanovesicles of any one of claims 66-68, wherein the cells are bone marrow-derived, cord blood-derived, adipose-derived, PBMC-derived, or iPSC-derived.
70. Isolated lipid nanovesicles of any one of claims 64-69, wherein the cells are derived from *in vitro* cultured cells.
71. Isolated lipid nanovesicles of any one of claims 64-70, wherein the isolated lipid nanovesicles are obtained from a patient at risk for or having a demyelinating disorder.
72. Isolated lipid nanovesicles of any one of claims 64-71, wherein the isolated lipid nanovesicles are *in vitro* reconstituted.
73. A pharmaceutical composition comprising the isolated lipid nanovesicles of any of claims 64-72 and a pharmaceutically acceptable carrier.

74. A method for treating a patient at risk for or having a demyelinating disorder comprising administering to the patient an effective amount of a pharmaceutical composition comprising isolated human-derived exosomes obtained from dendritic cells that have been induced to undergo oxidative stress.
75. A method for increasing myelination in a patient at risk for or having a demyelinating disorder, the method comprising administering to the patient an effective amount of a pharmaceutical composition comprising isolated human-derived exosomes obtained from dendritic cells that have been induced to undergo oxidative stress.
76. A method for reducing or preventing spreading depression in a patient at risk for or having a demyelinating disorder, the method comprising administering to the patient an effective amount of a pharmaceutical composition comprising isolated human-derived exosomes obtained from dendritic cells that have been induced to undergo oxidative stress.
77. The method of any one of claims 74-76, wherein the dendritic cells are *in vitro* differentiated dendritic cells.
78. The method of any one of claims 74-77, wherein the dendritic cells are CD11b-.
79. The method of any one of claims 74-78, wherein the dendritic cells are CD11c+ or CD1a+.
80. The method of any one of claims 74-79, wherein the dendritic cells are differentiated from human stem cells, human-derived stem cells, human progenitor cells, human induced pluripotent stem cells (iPSCs), human peripheral blood mononuclear cells (PBMCs), human bone marrow mononuclear cells (BMMCs), or human cord blood mononuclear cells (CBMCs).
81. The method of claim 80, wherein the dendritic cells are differentiated from human stem cells, human derived stem cells, or human progenitor cells.
82. The method of claim 81, wherein the human stem cells or human progenitor cells are stem cells isolated from bone marrow, cord blood, adipose tissue, or whole blood.
83. The method of claim 82, wherein the dendritic cells are differentiated from BMMCs isolated from bone marrow or CBMCs isolated from cord blood.
84. The method of claim 83, wherein the dendritic cells are differentiated from CD34+ cells.

85. The method of claim 80, wherein the dendritic cells are differentiated from PBMCs isolated from whole blood.
86. The method of claim 85, wherein the dendritic cells are differentiated from monocytes.
87. The method of claim 80, wherein the dendritic cells are differentiated from iPSCs.
88. The method of claim 87, wherein the iPSCs are derived from human fibroblasts.
89. The method of claim 88, wherein the human fibroblasts are isolated from human dermal tissue or human adipose tissue.
90. The method of claim 88, wherein the fibroblasts are from a skin biopsy.
91. The method of claim 87, wherein the iPSCs are derived from human adipose stem cells, human adipose-derived stem cells, human keratinocytes, and human PBMCs.
92. The method of any one of claims 74-91, wherein the demyelinating disorder is cognitive decline, Alzheimer's disease, Parkinson's disease, stroke, epilepsy, migraine, traumatic brain injury, post-traumatic stress disorder, post-traumatic headache, multiple sclerosis, neuropathy, tauopathy, or ageing-induced cognitive decline.
93. The method of claim 92, wherein the demyelinating disorder is multiple sclerosis or neuropathy.
94. The method of claim 92, wherein the demyelinating disorder is migraine.
95. The method of claim 94, wherein the migraine comprises migraine with aura.
96. The method of any one of claims 74-95, wherein the patient is administered the composition nasally via inhalation, or intravenously.
97. The method of any one of claims 74-96, wherein the cells have been induced to undergo oxidative stress by contact with IFN- γ .
98. The method of any one of claims 74-97, wherein the cells are cells obtained from the patient.
99. The method of claim 98, wherein the cells are cells obtained from the patient when the patient is less than 18 years old.
100. The method of claim 99, wherein the cells are cells obtained from the patient when the patient is less than one year old.

101. The method of any one of claims 74-100, wherein the isolated exosomes comprise at least an externally added therapeutic agent.
102. The method of claim 101, wherein the externally added therapeutic agent is an siRNA.
103. The method of any one of claims 74-102, wherein the isolated exosomes comprise miR-219, miR-138, or miR-199a.
104. The method of any one of claims 74-103, wherein the isolated exosomes comprise mRNA encoding antioxidant system proteins.

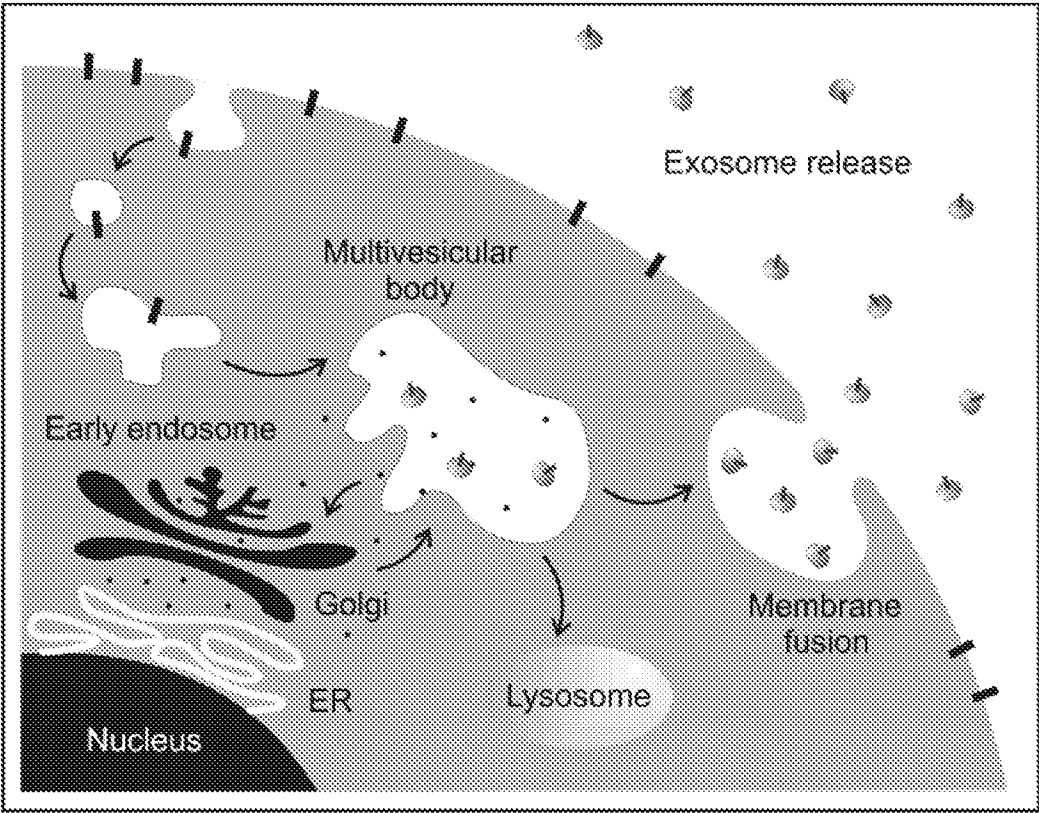


FIG. 1

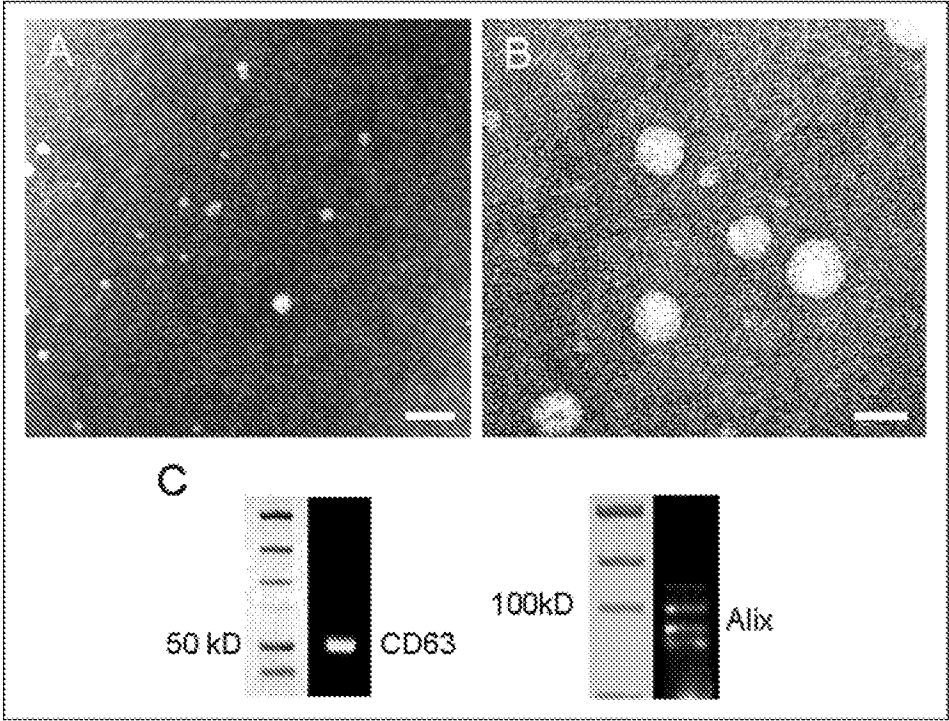


FIG. 2A-C

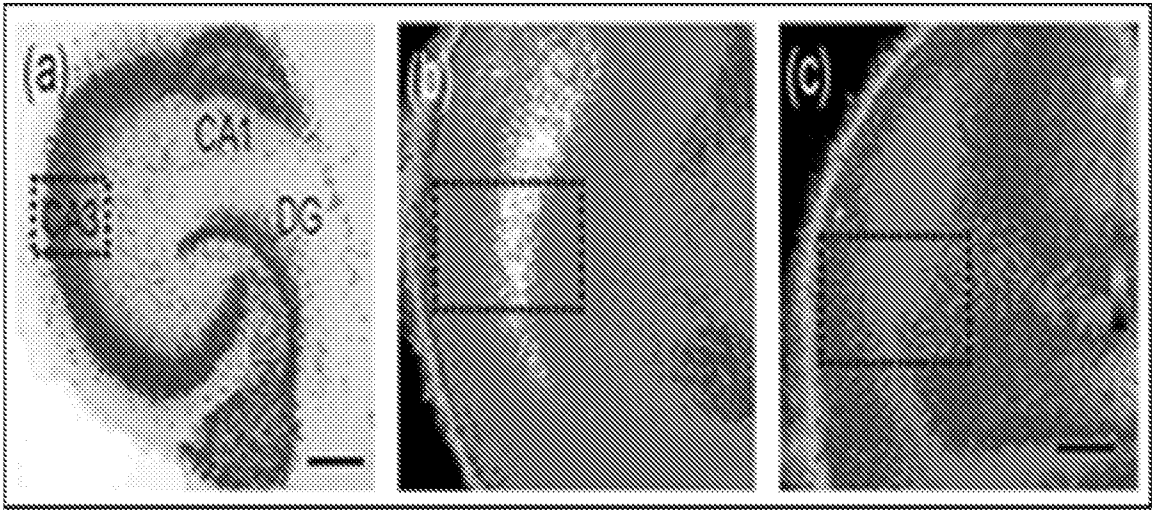


FIG. 3A-C

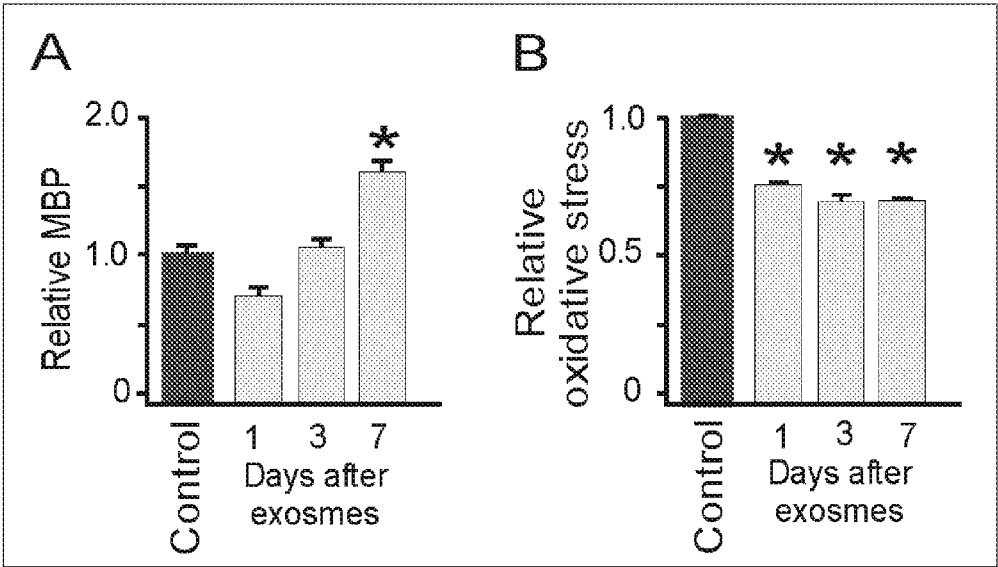


FIG. 4A-B

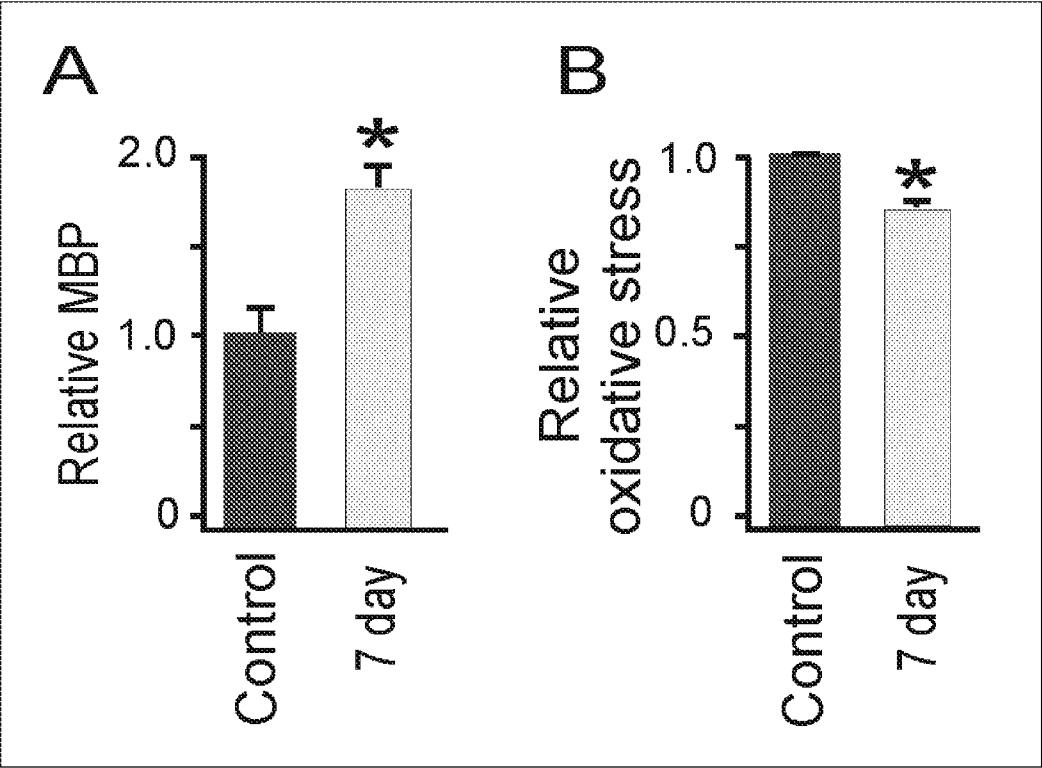


FIG. 5A-B

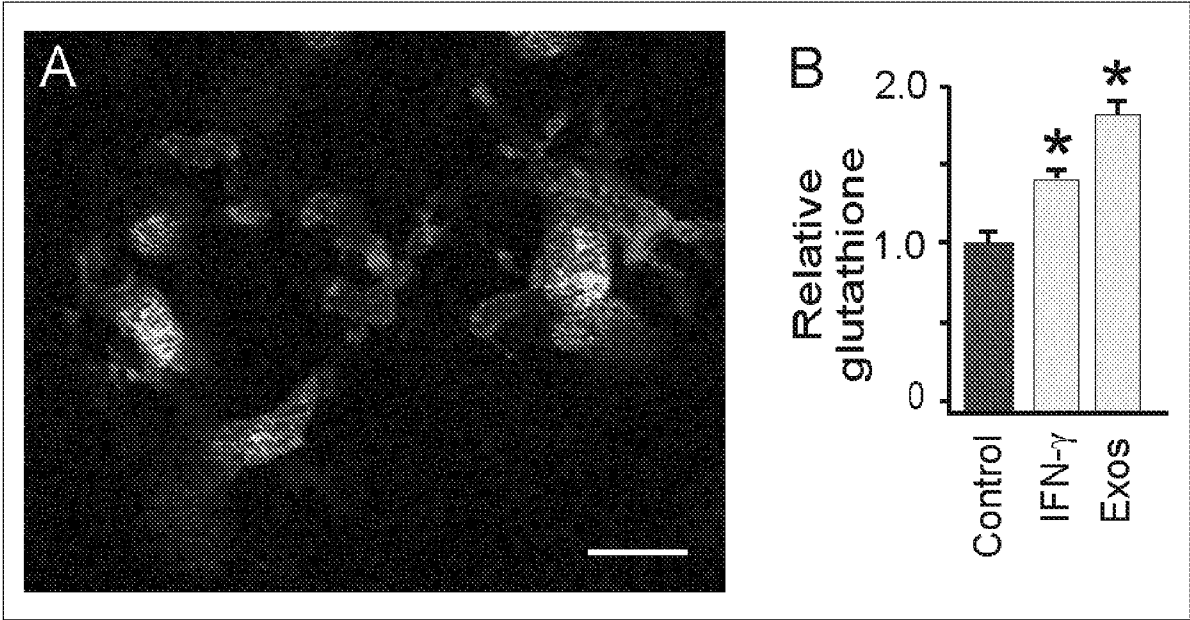


FIG. 6A-B

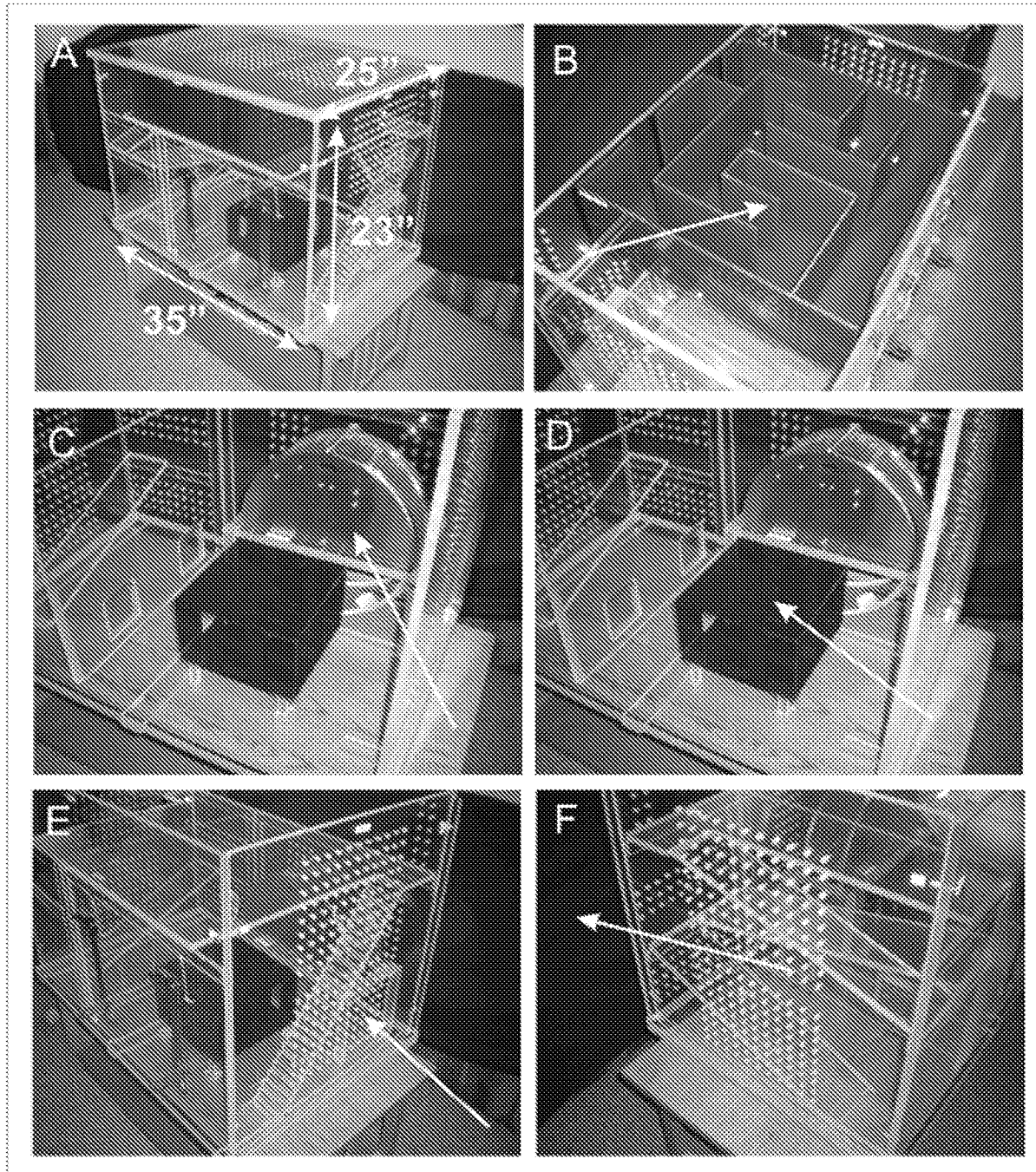


FIG. 7A-F

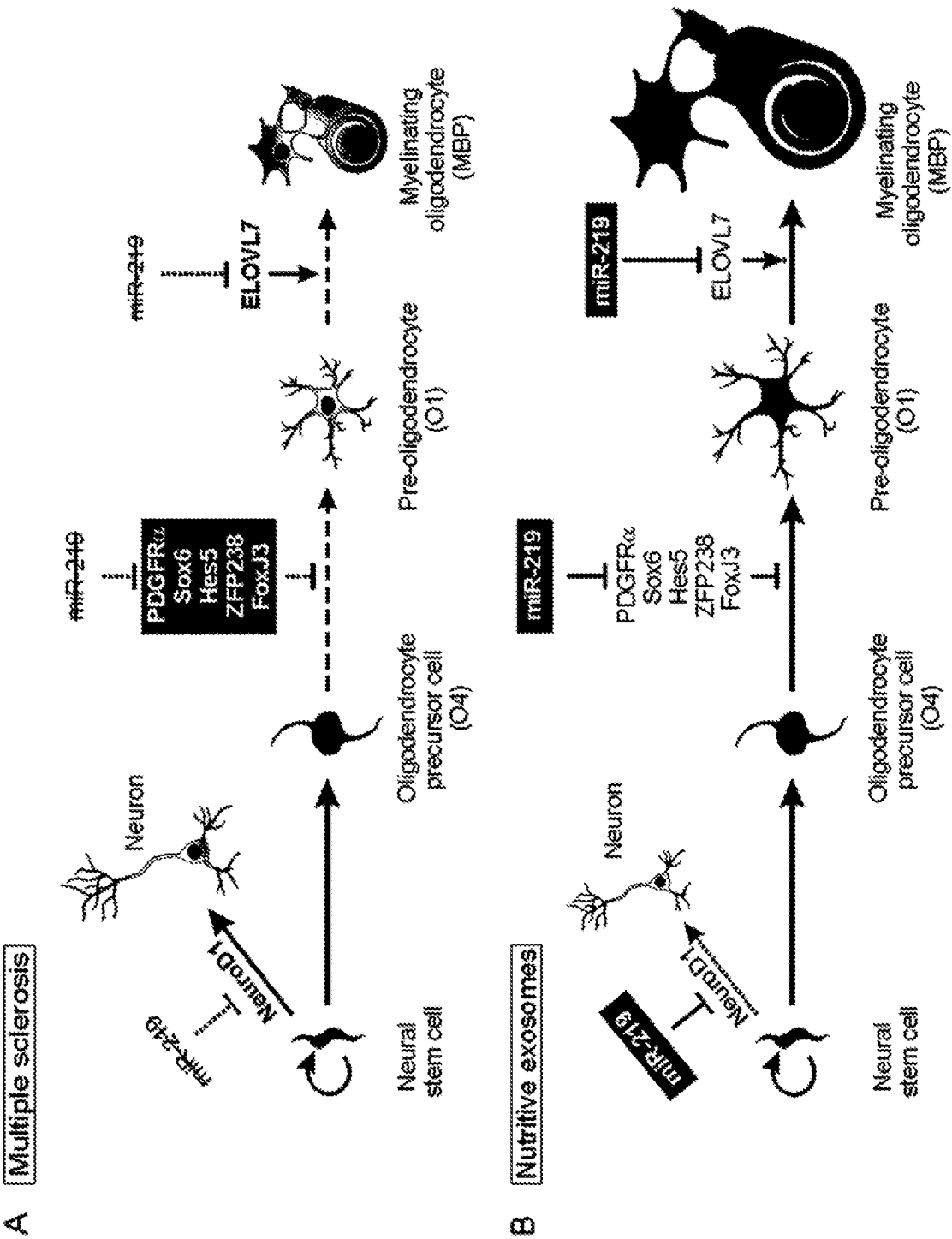




FIG. 8C-E

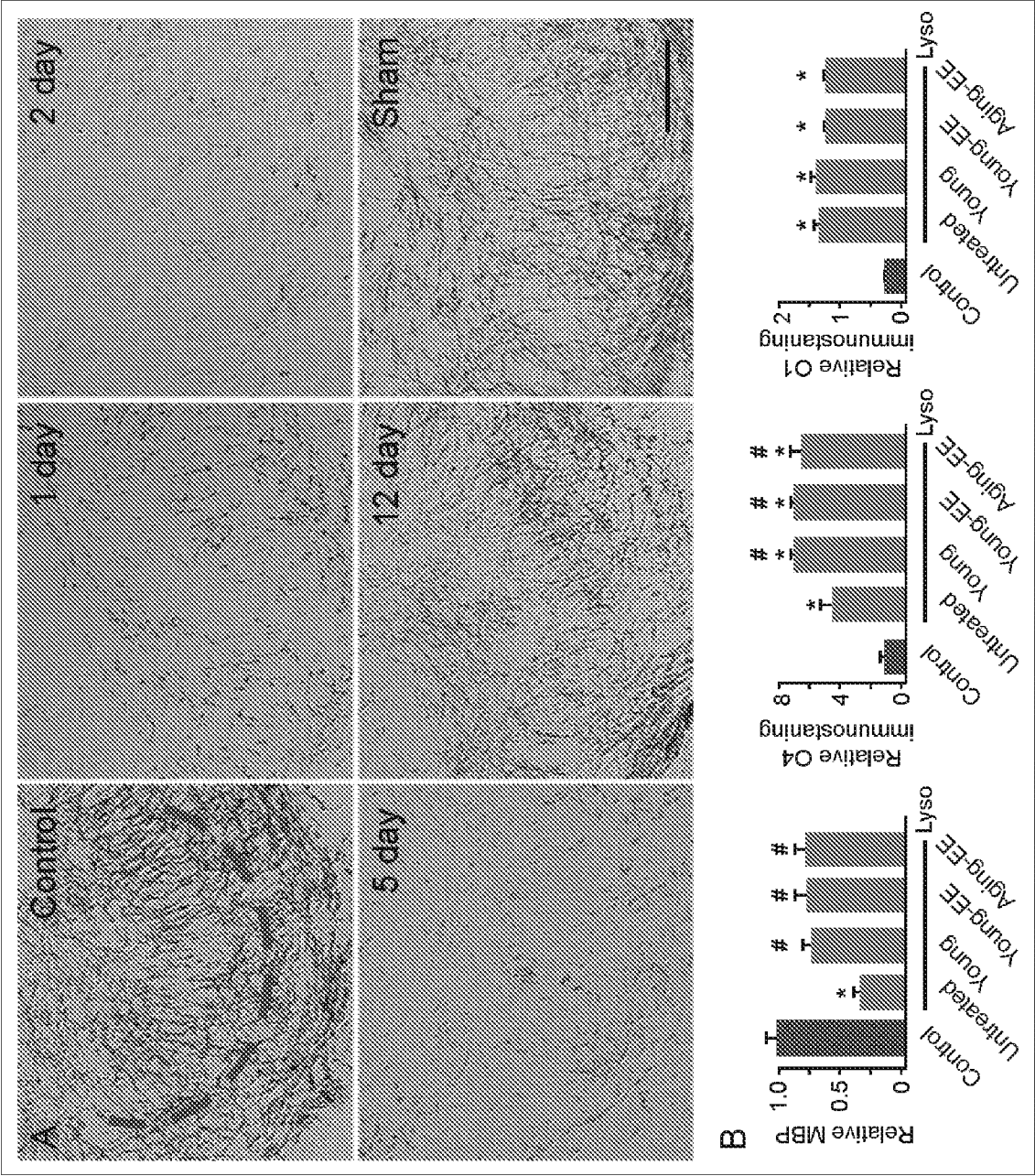


FIG. 9A-B

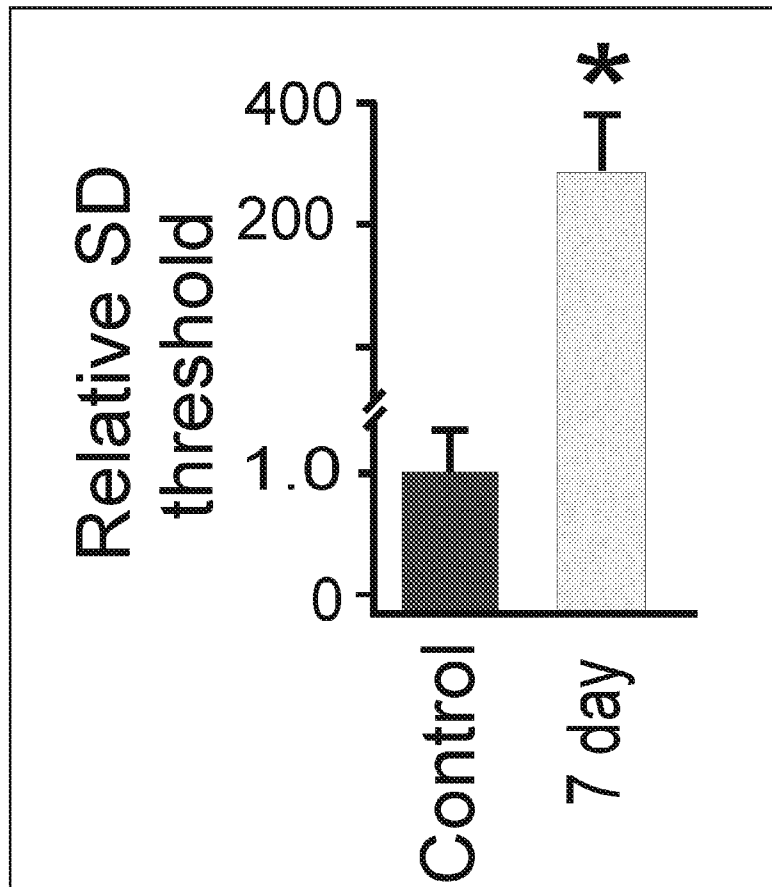


FIG. 10

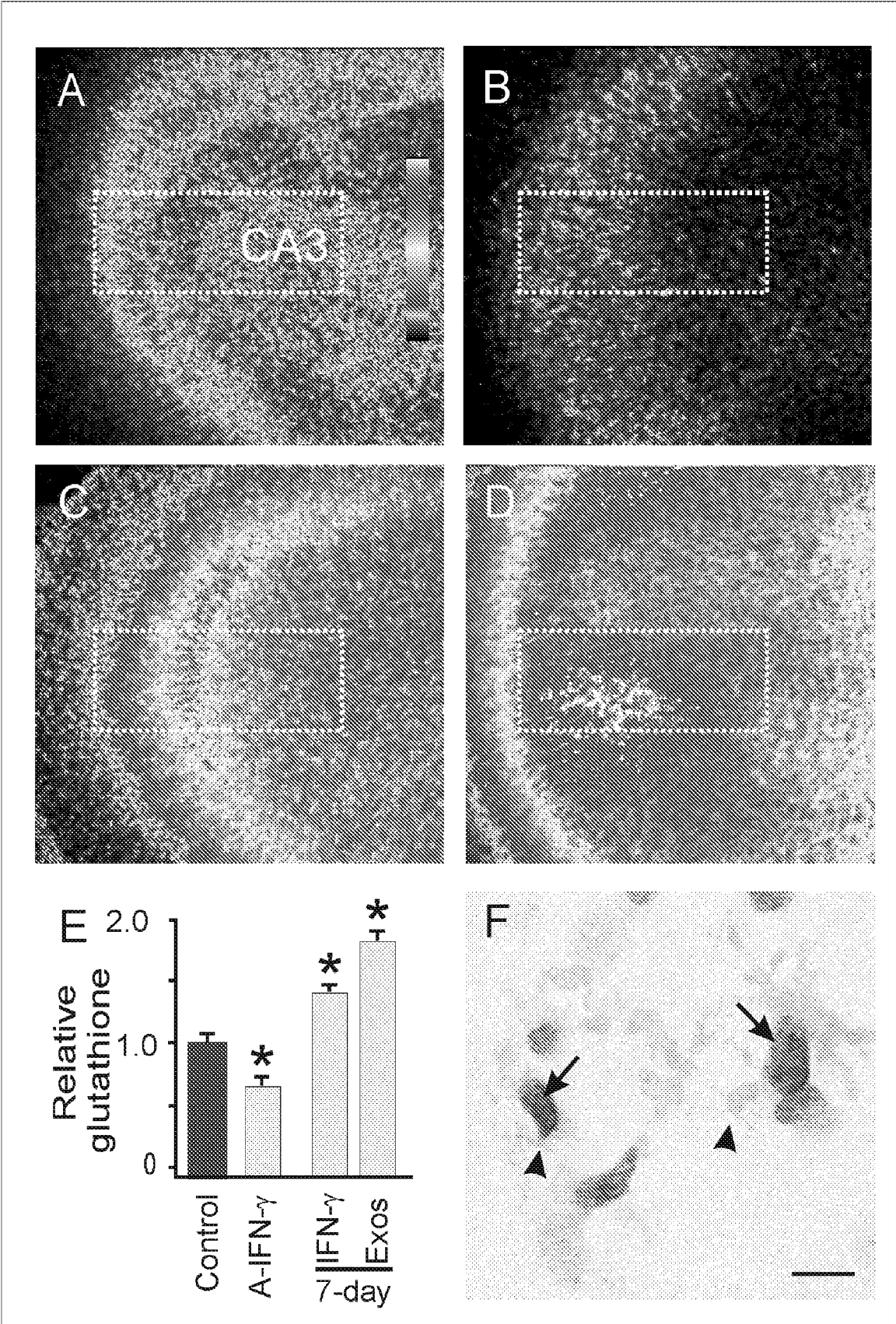
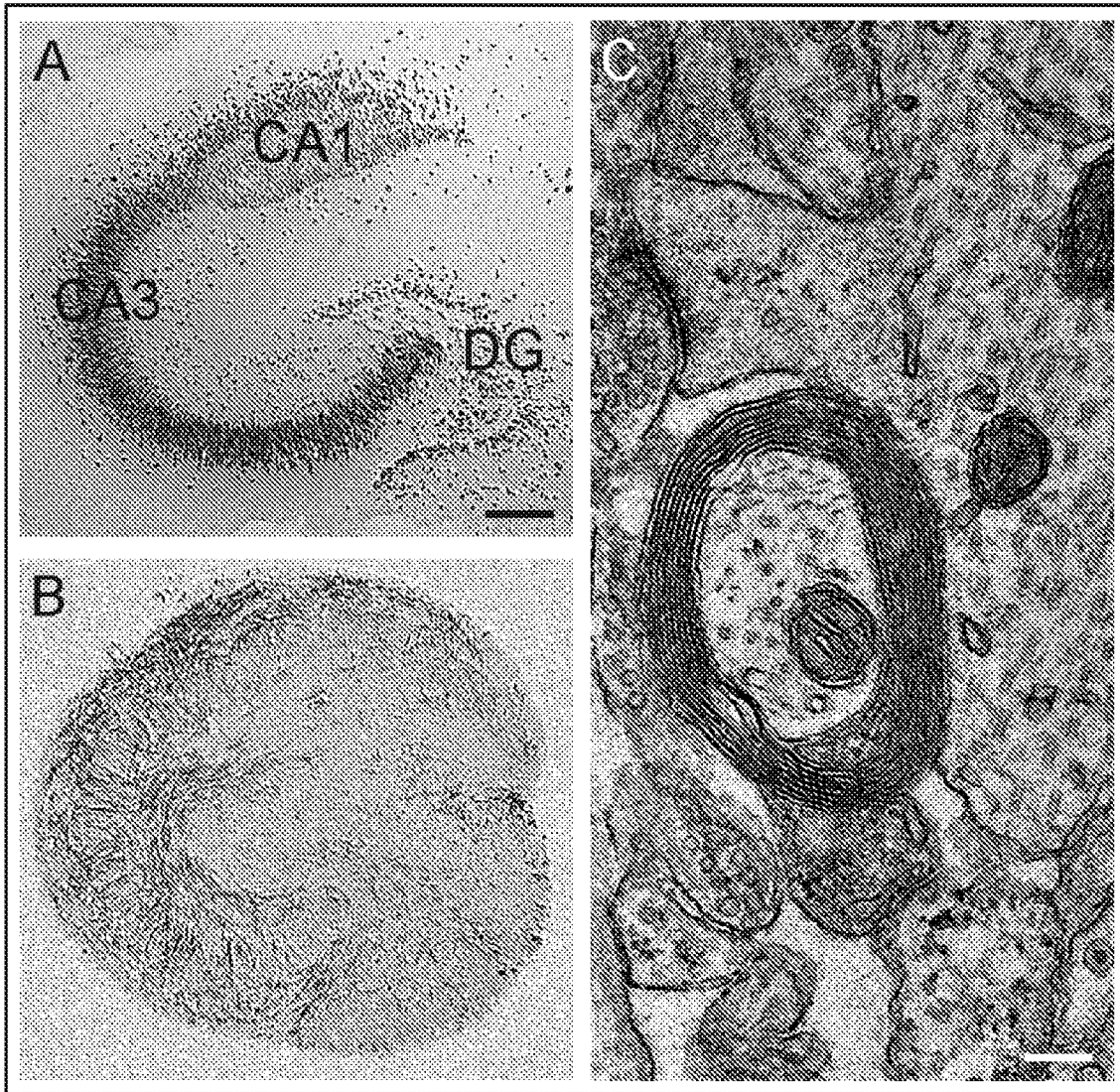


FIG. 11A-F

**FIG. 12A-C**

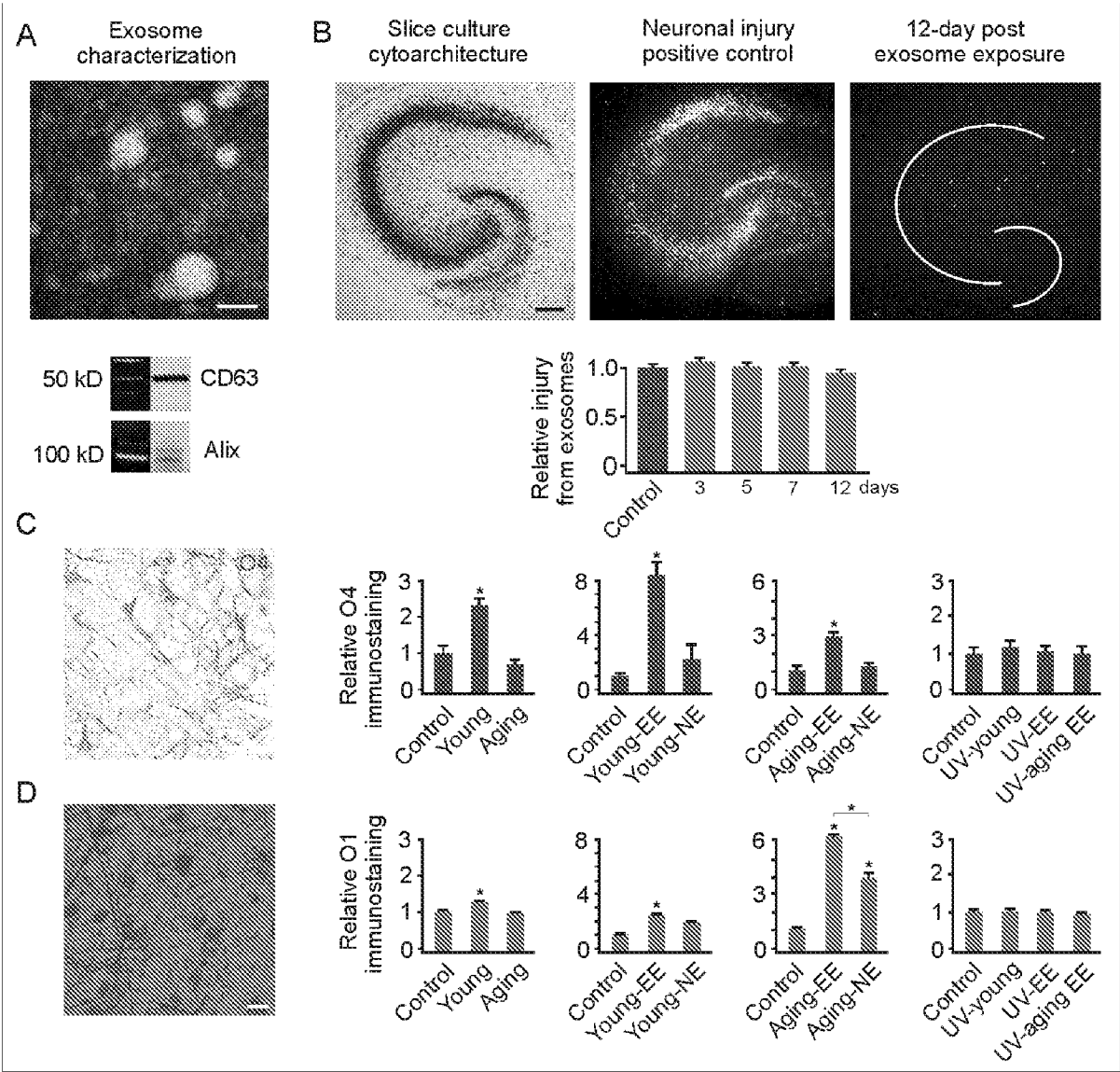


FIG. 13A-D

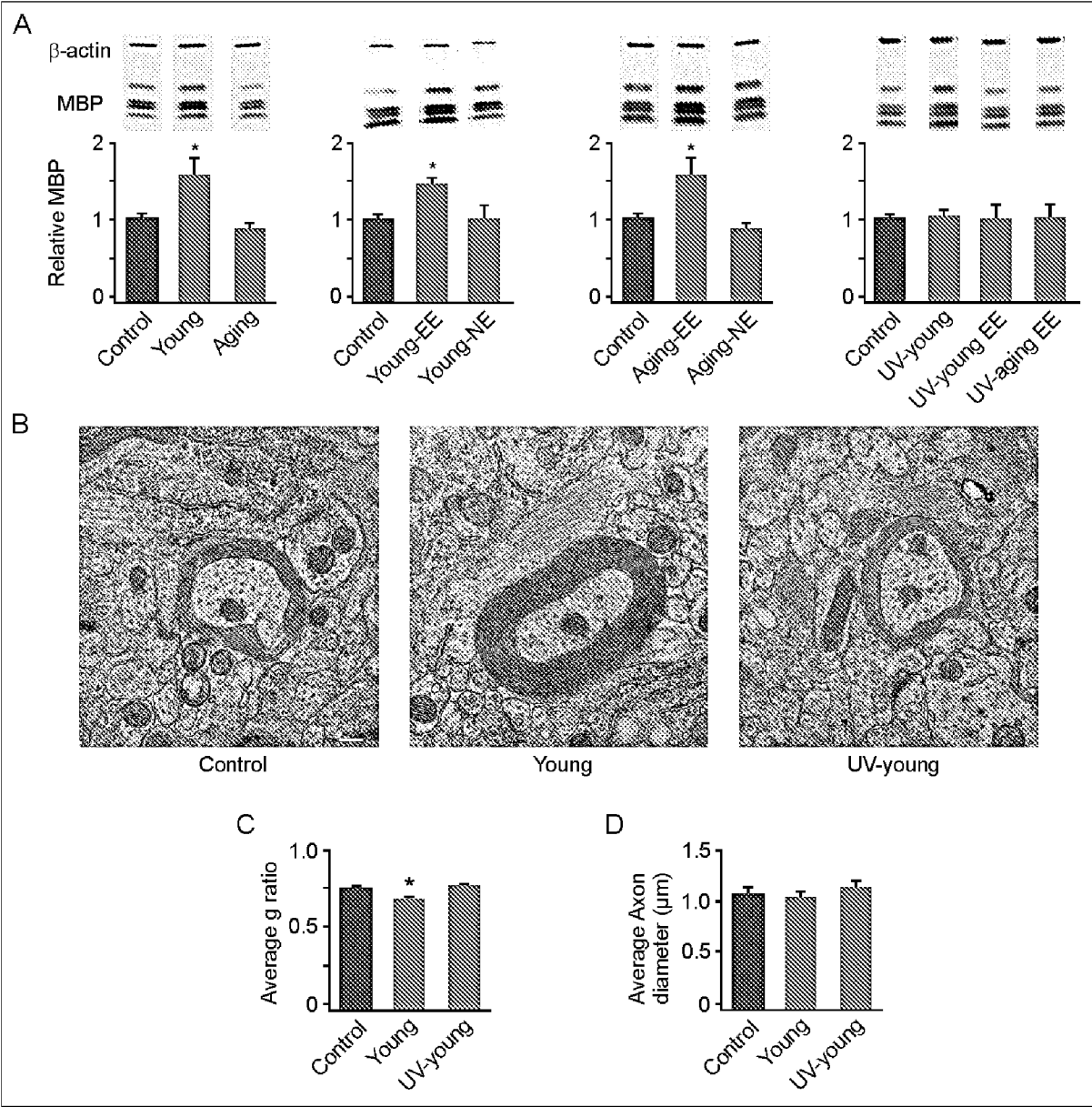


FIG. 14A-D

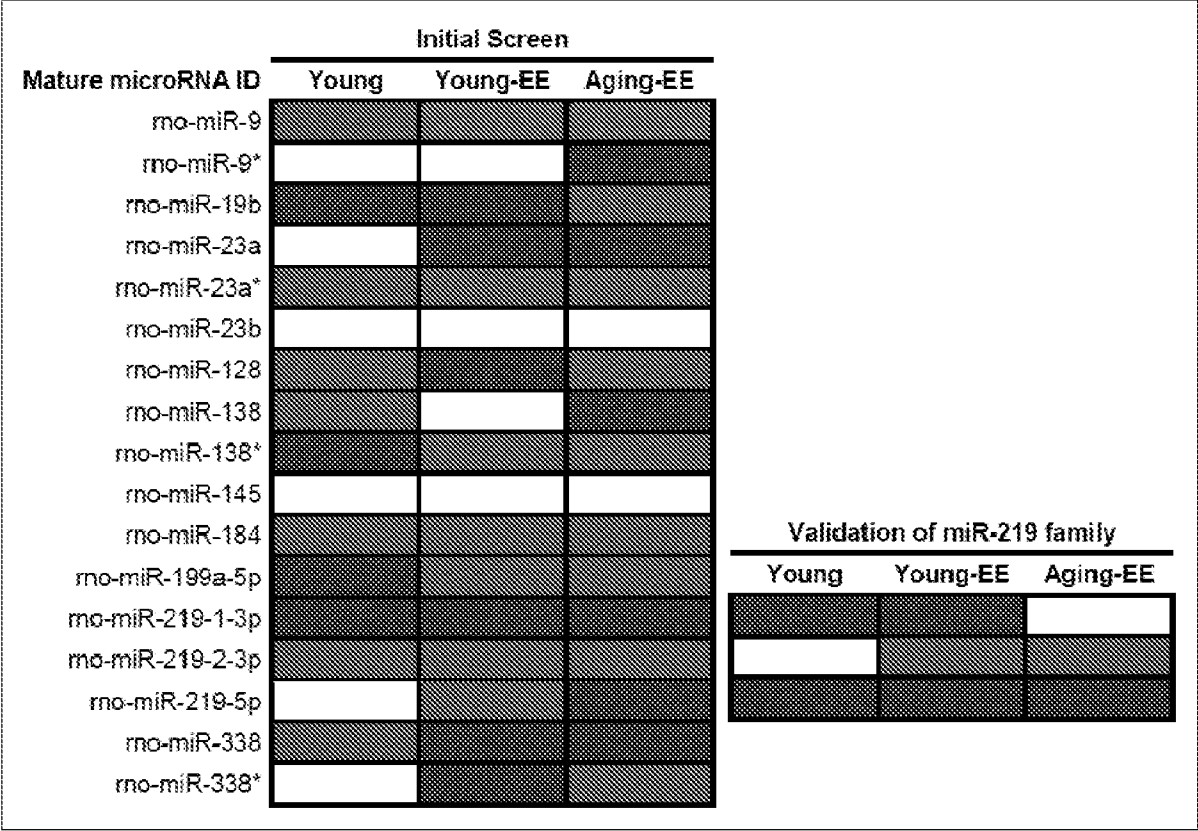


FIG. 15

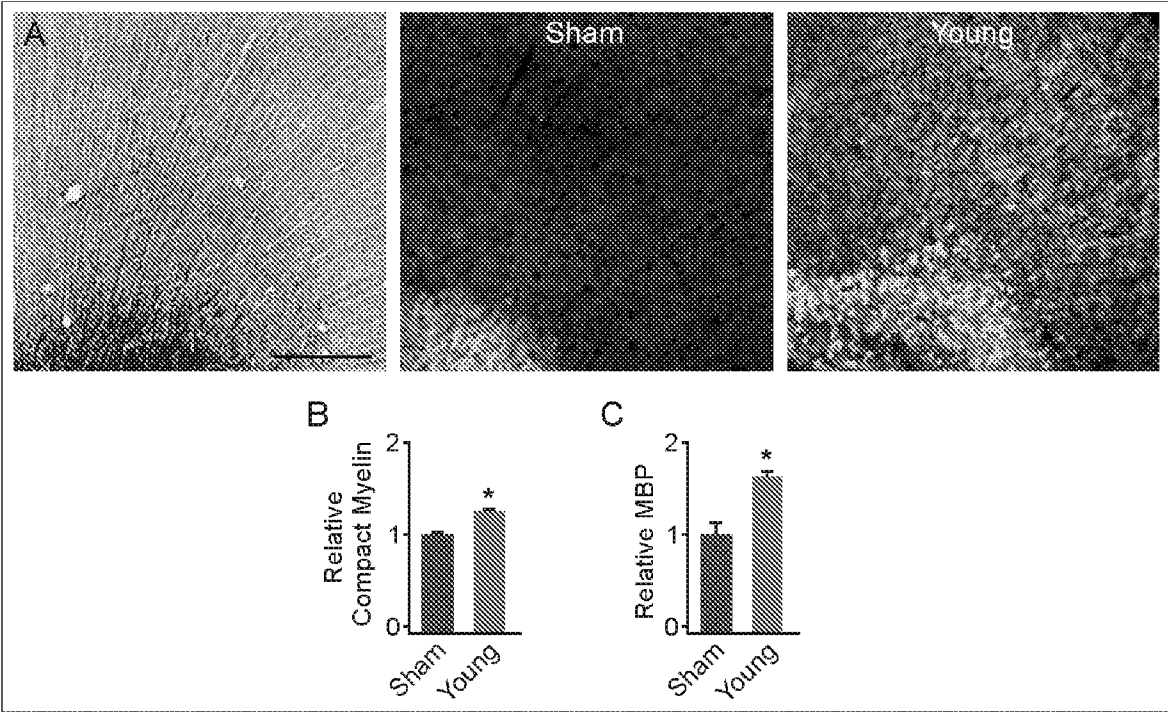


FIG. 16A-C

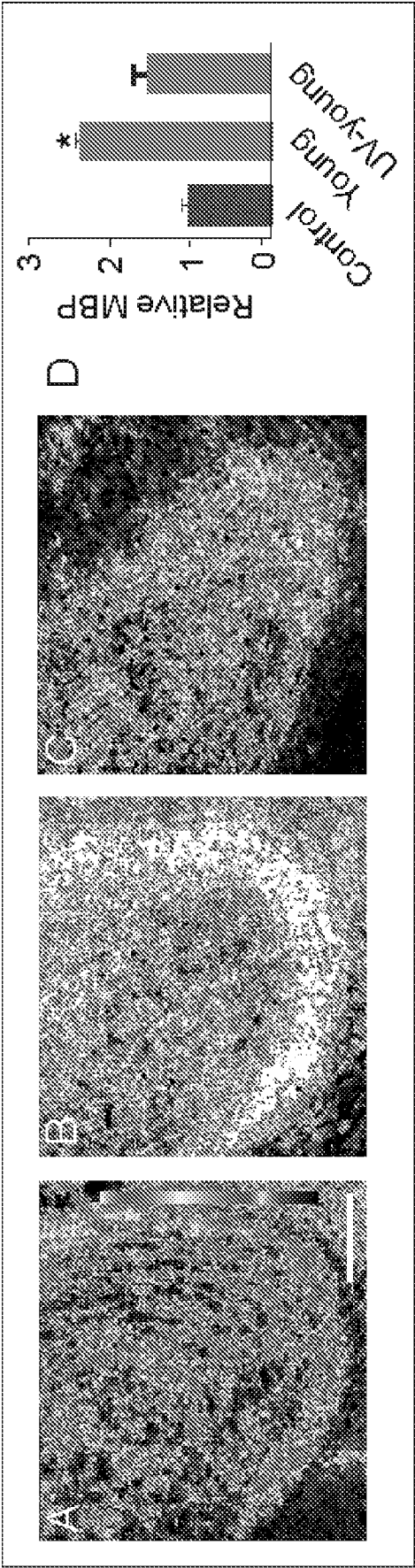


FIG. 17A-D

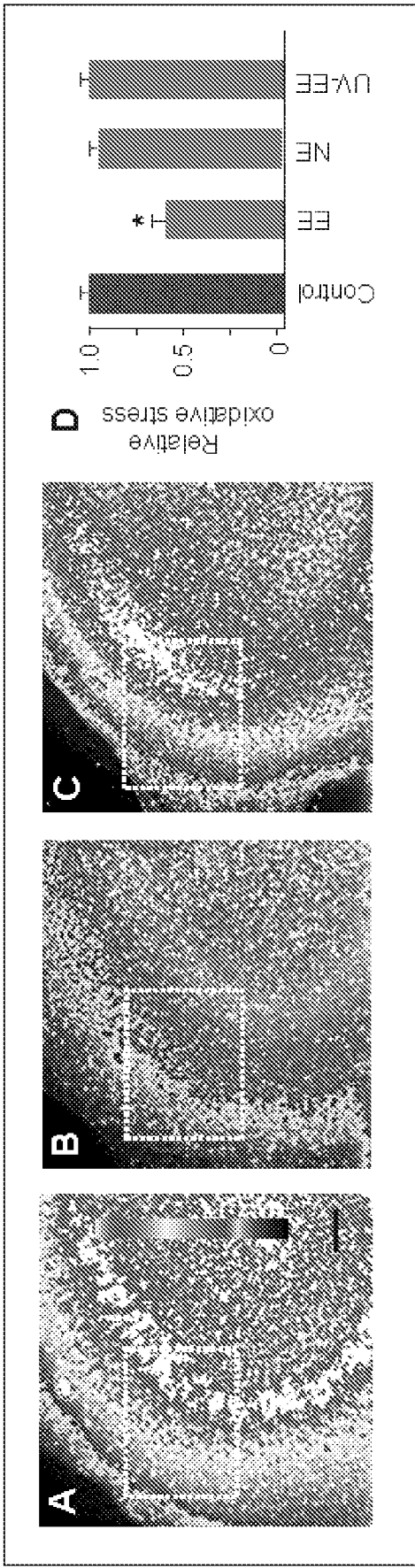
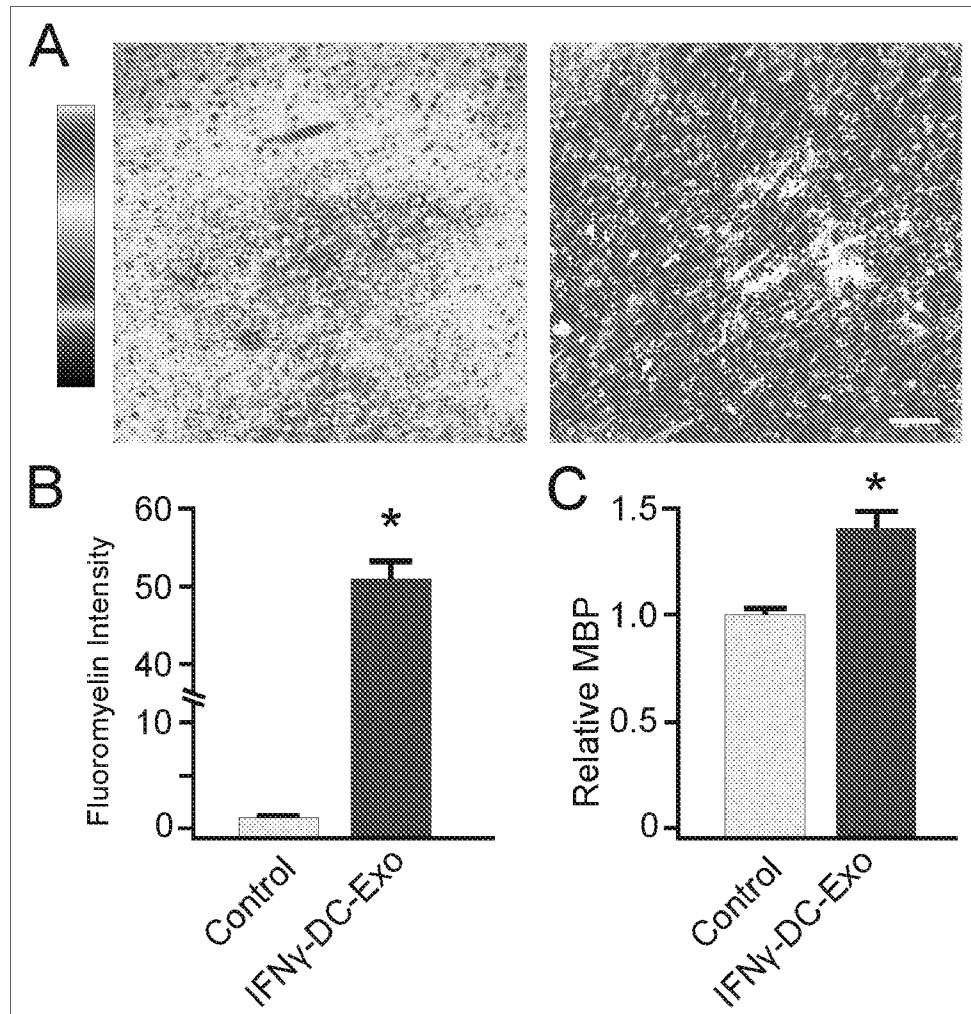
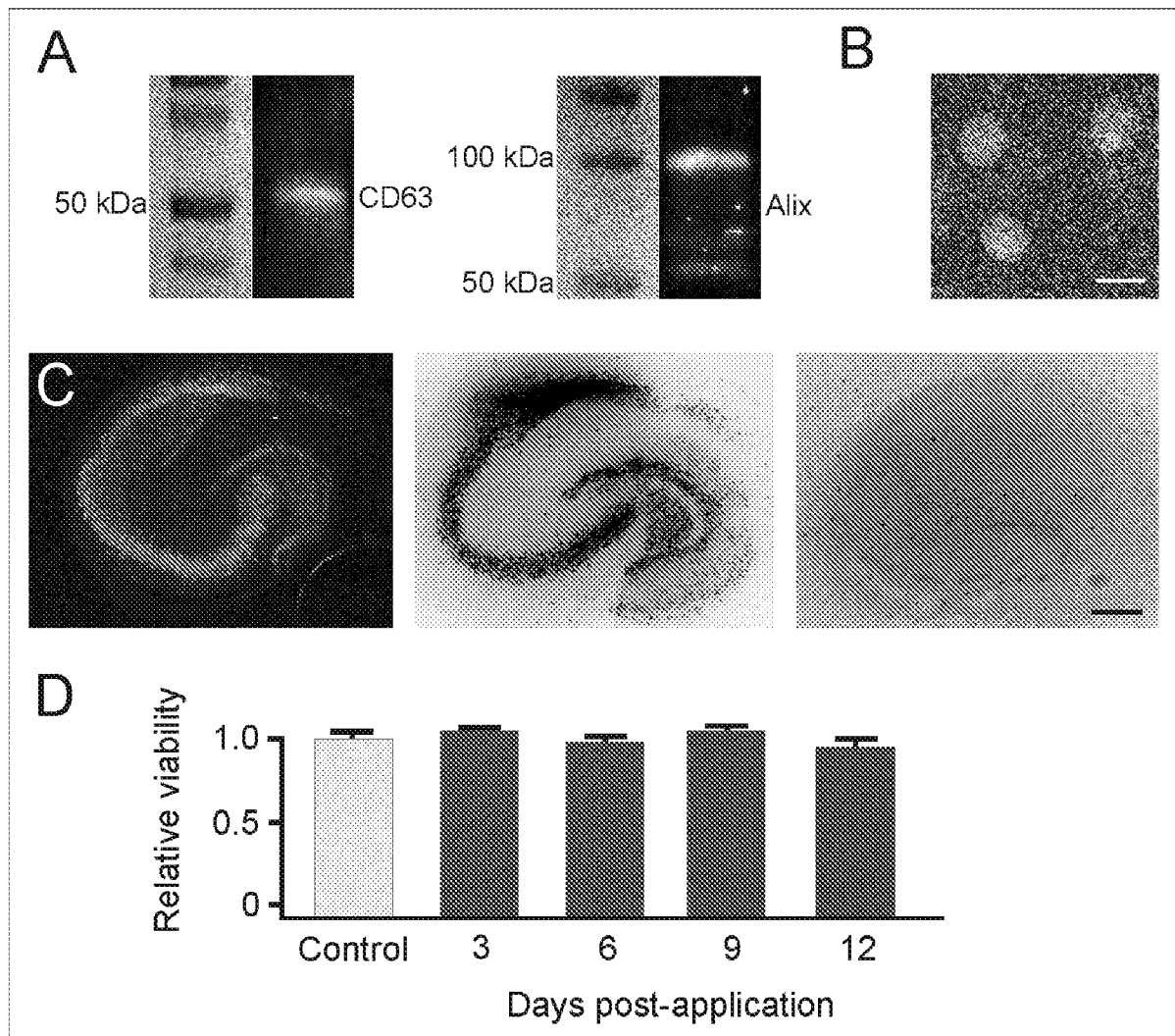


FIG. 18A-D

**FIG. 19A-C**

**FIG. 20A-D**

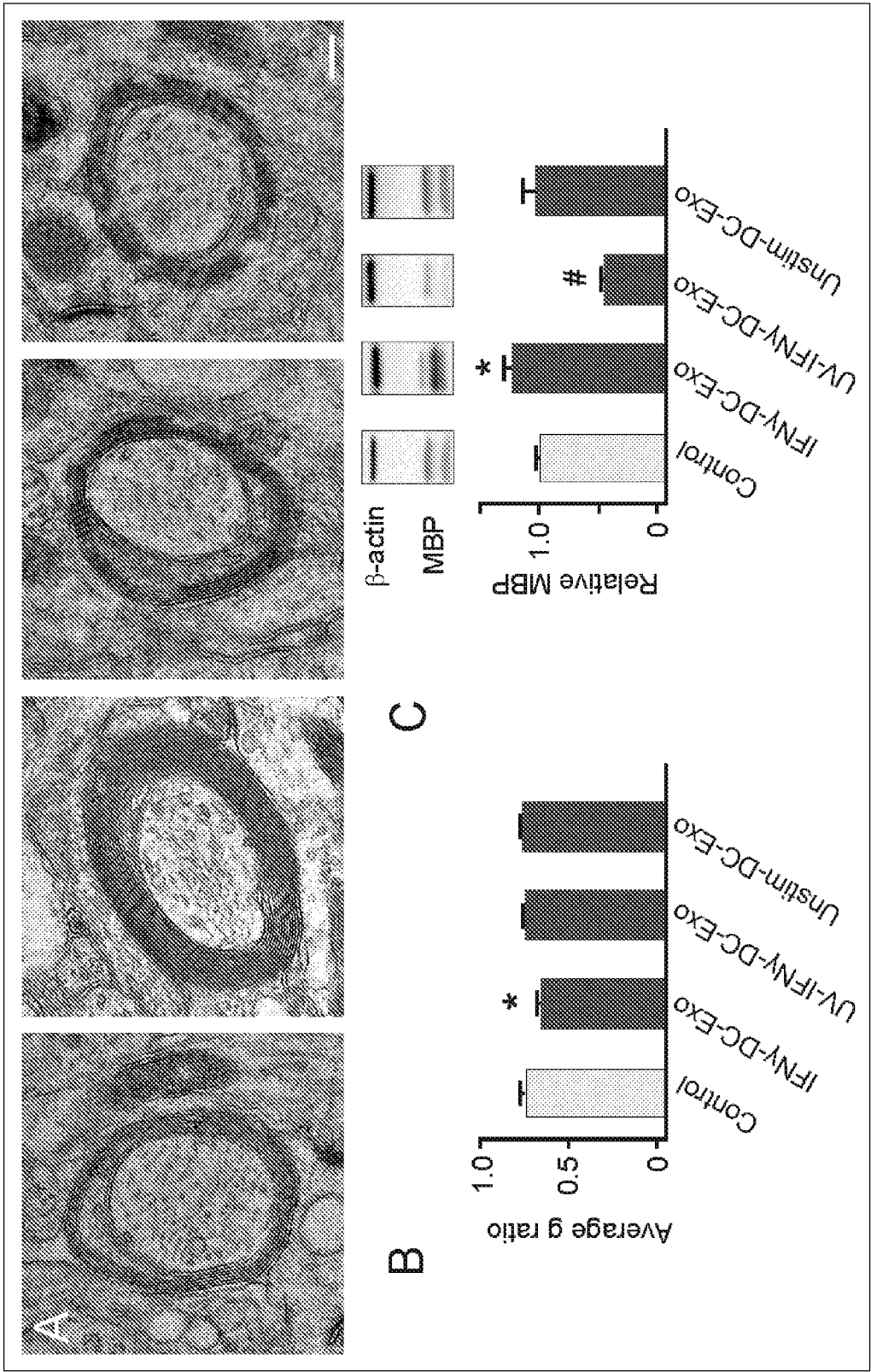


FIG. 21A-C

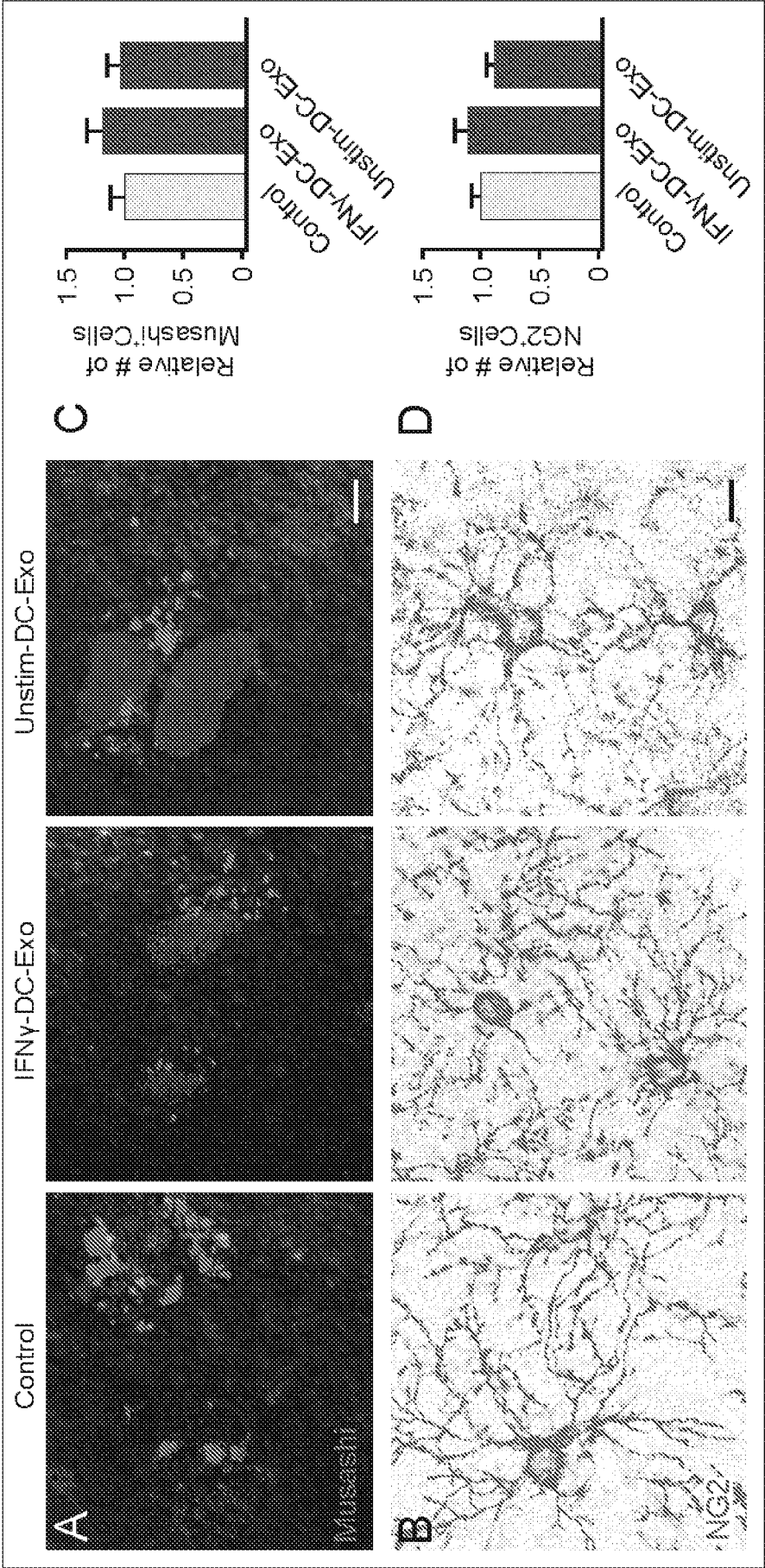


FIG. 22A-D

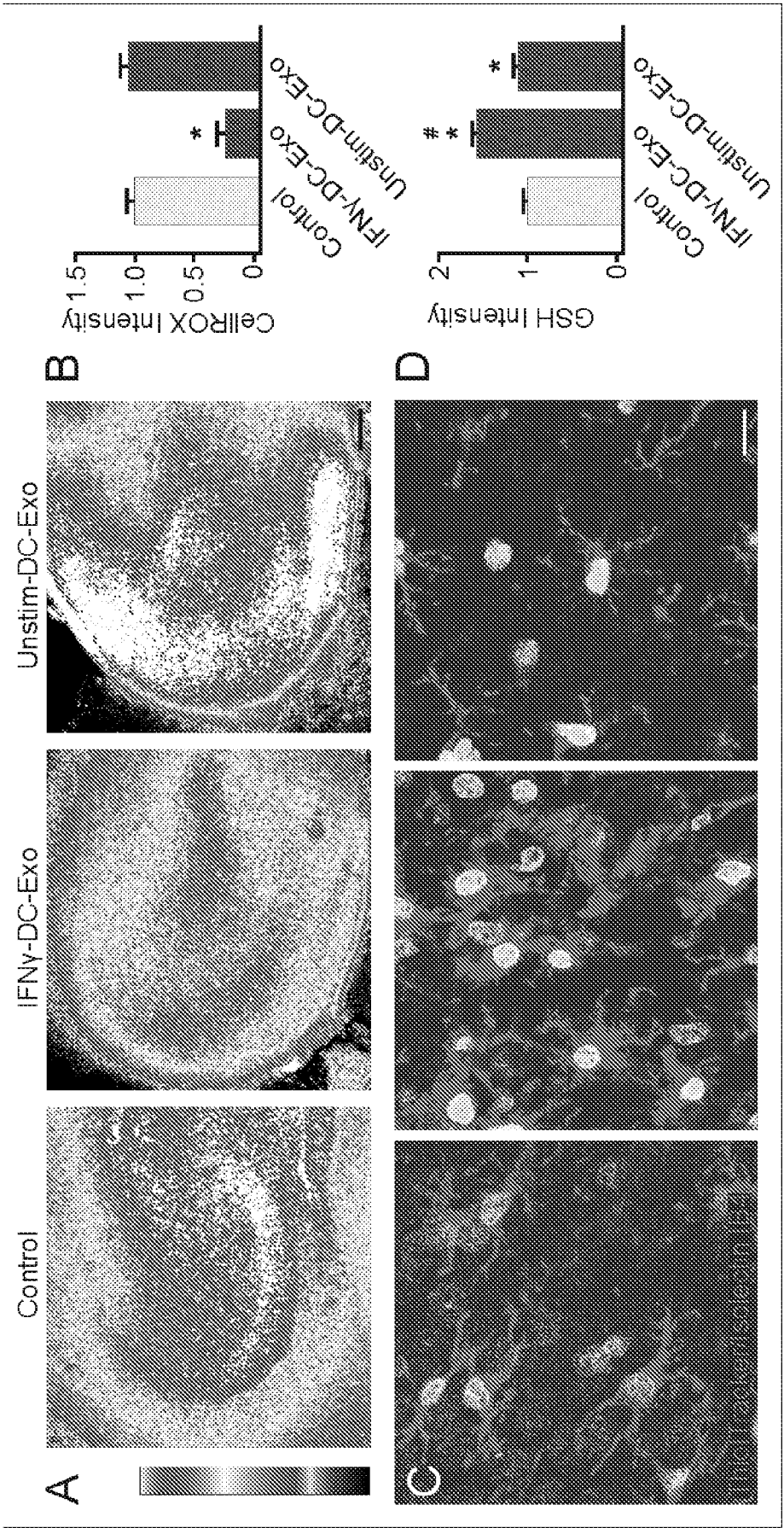


FIG. 23A-D

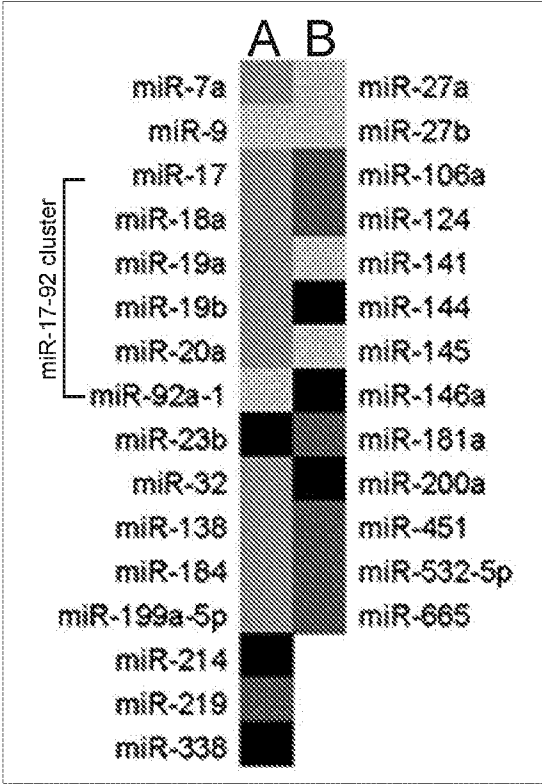


FIG. 24A-B

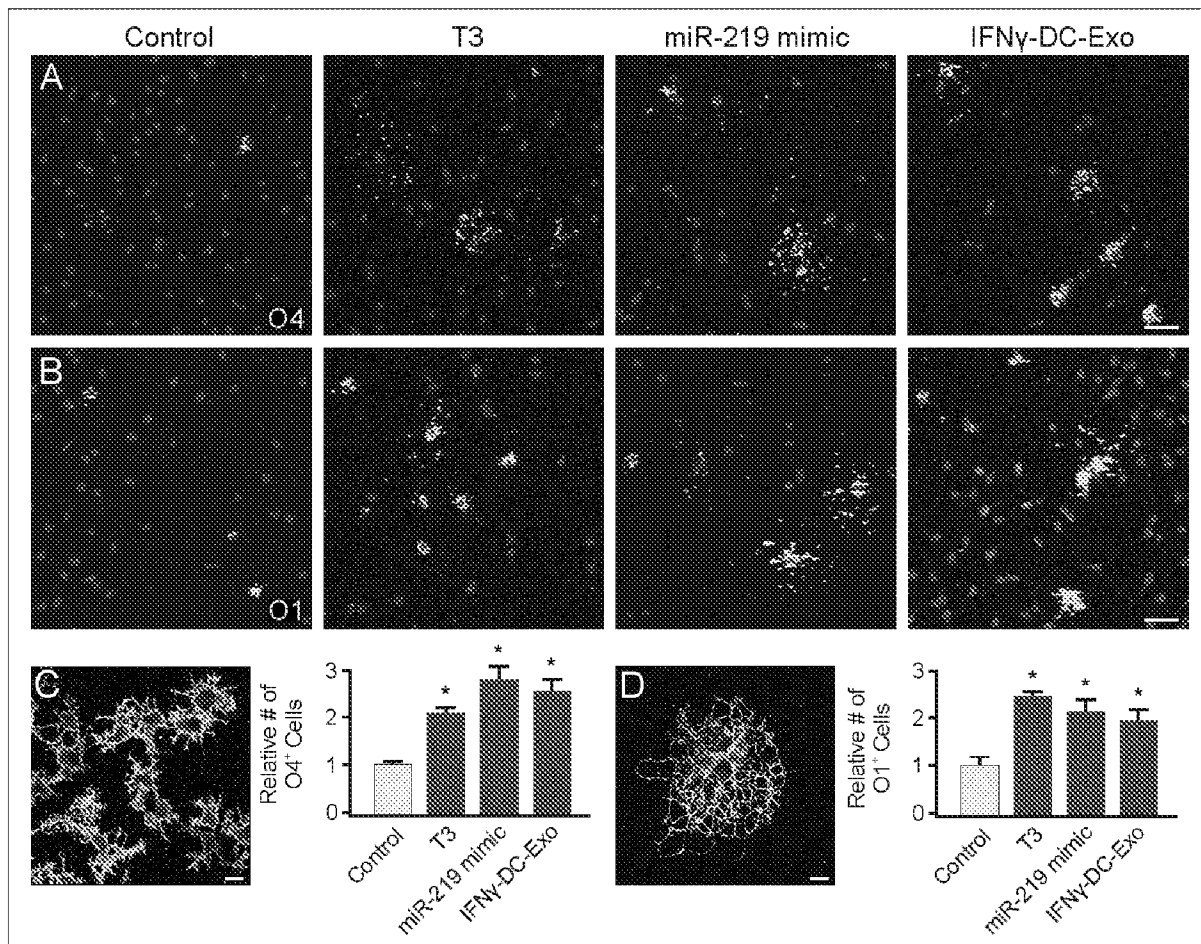


FIG. 25A-D

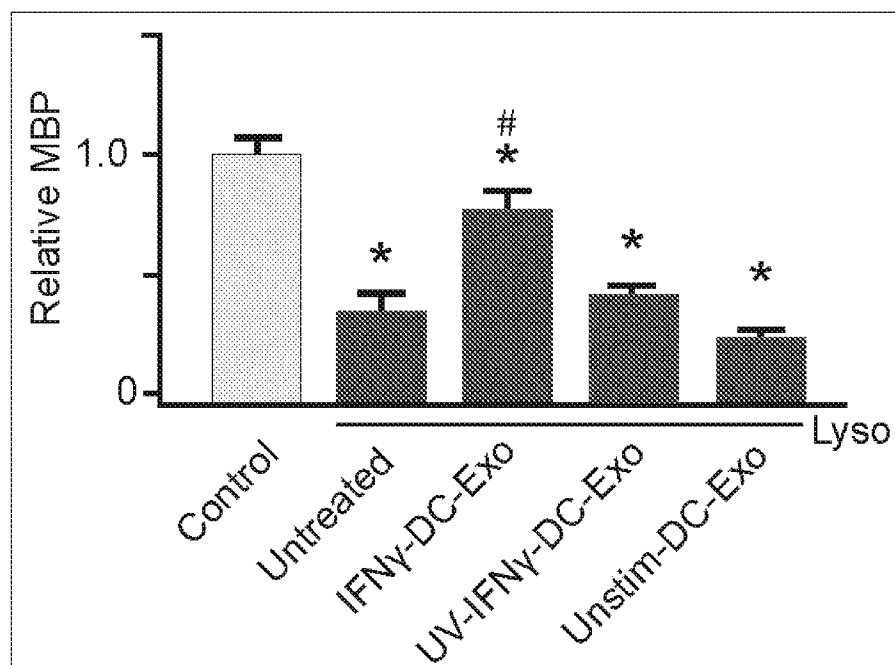


FIG. 26

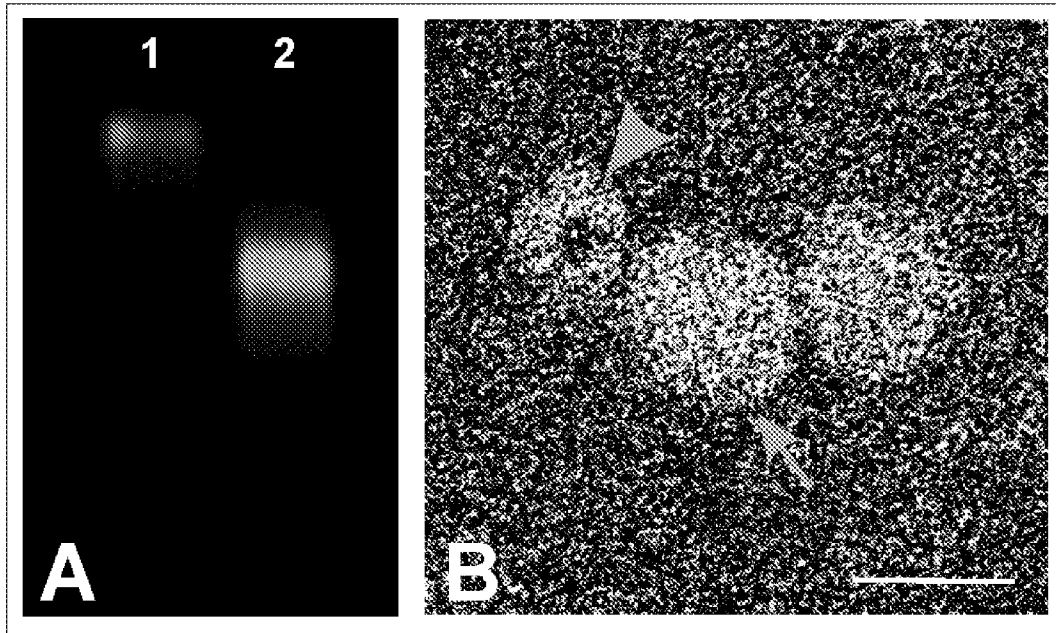


FIG. 27A-B

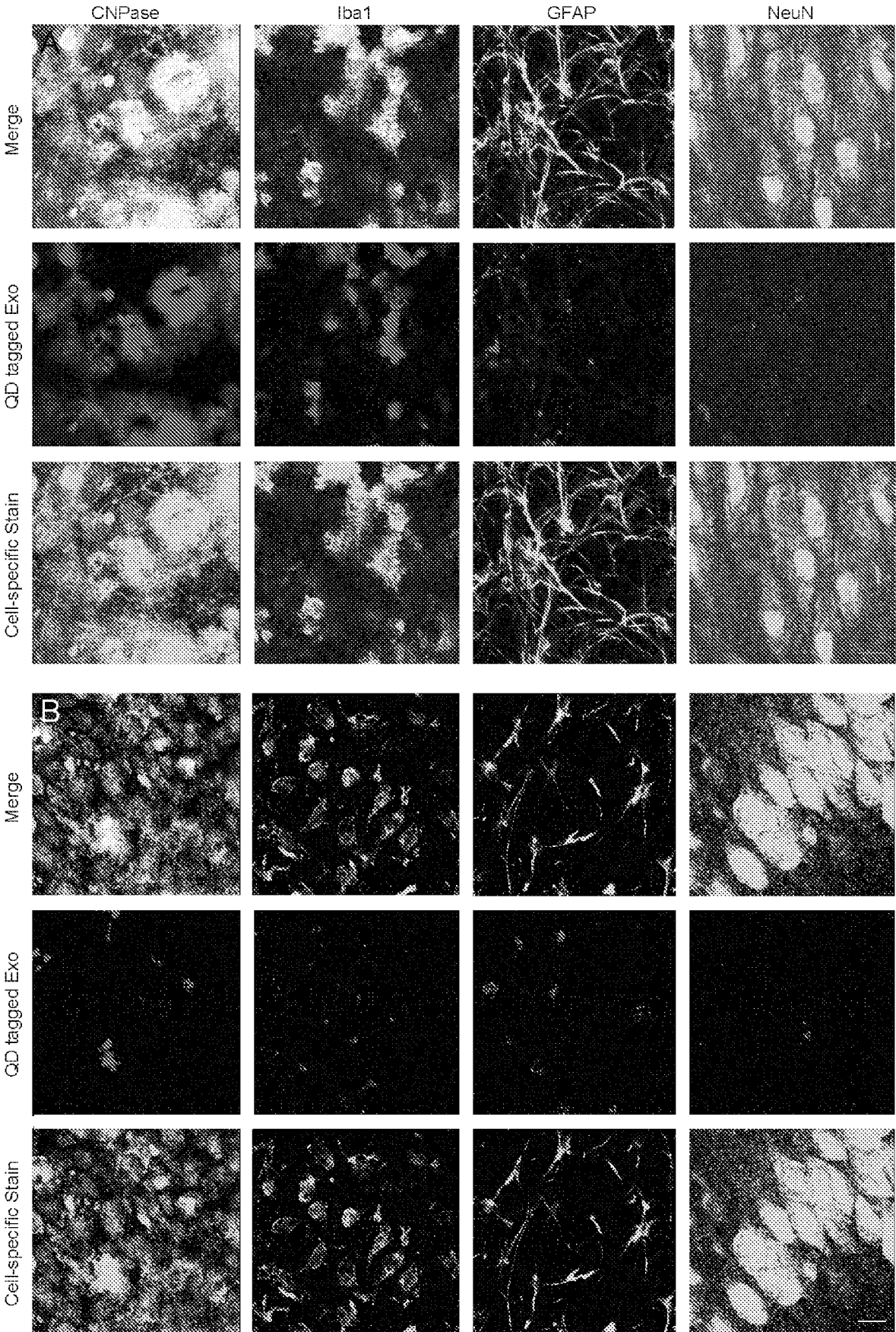


FIG. 28A-B

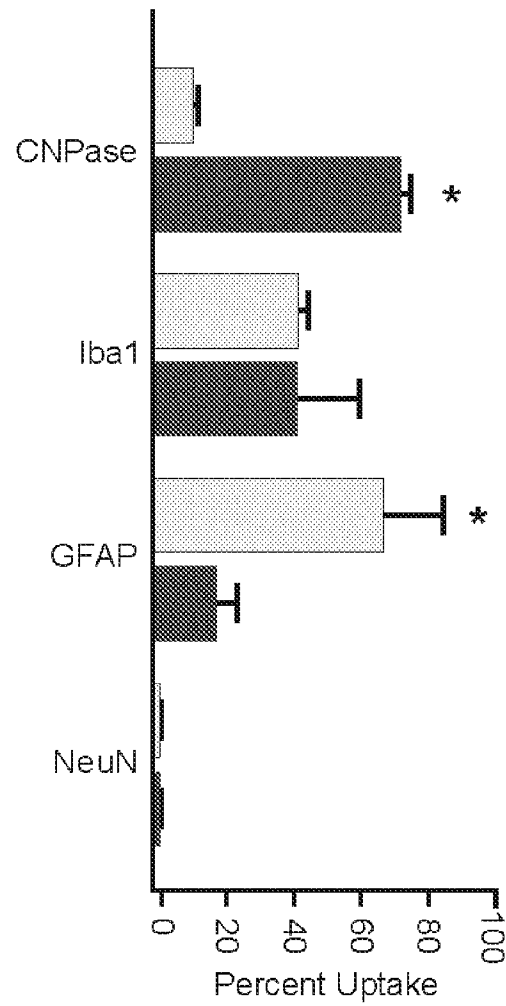


FIG. 28C

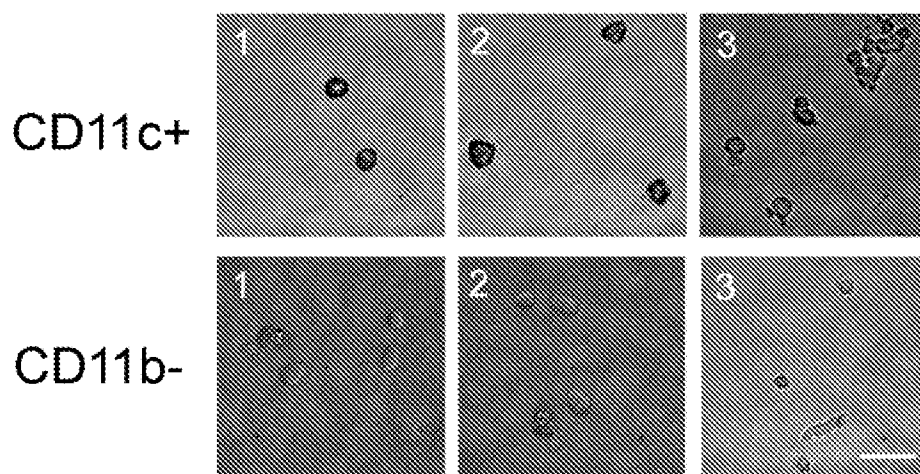
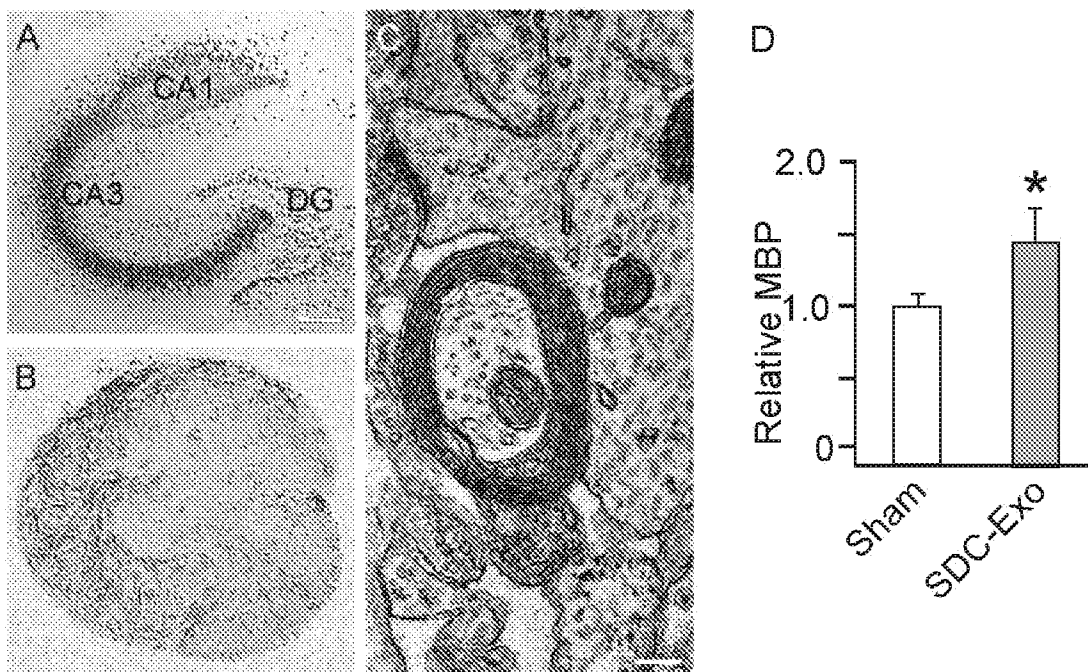


FIG. 29

**FIG. 30****FIG. 31A-D**

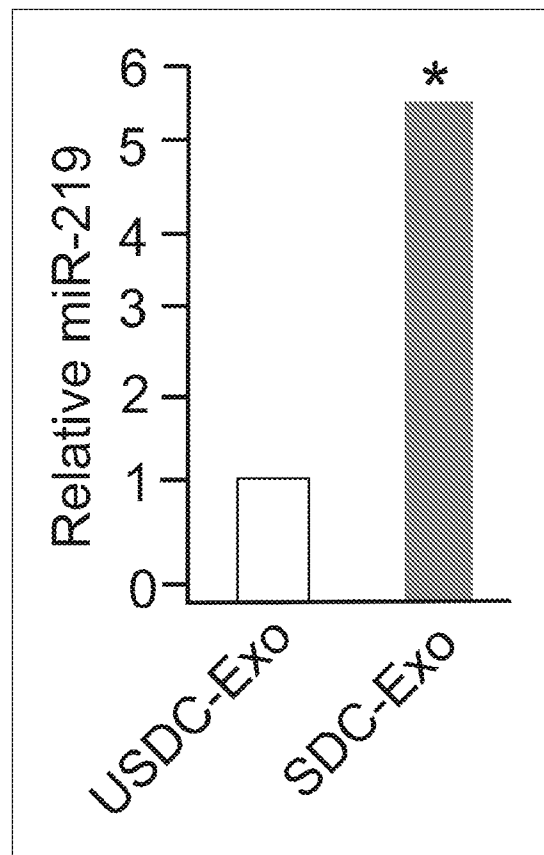


FIG. 32

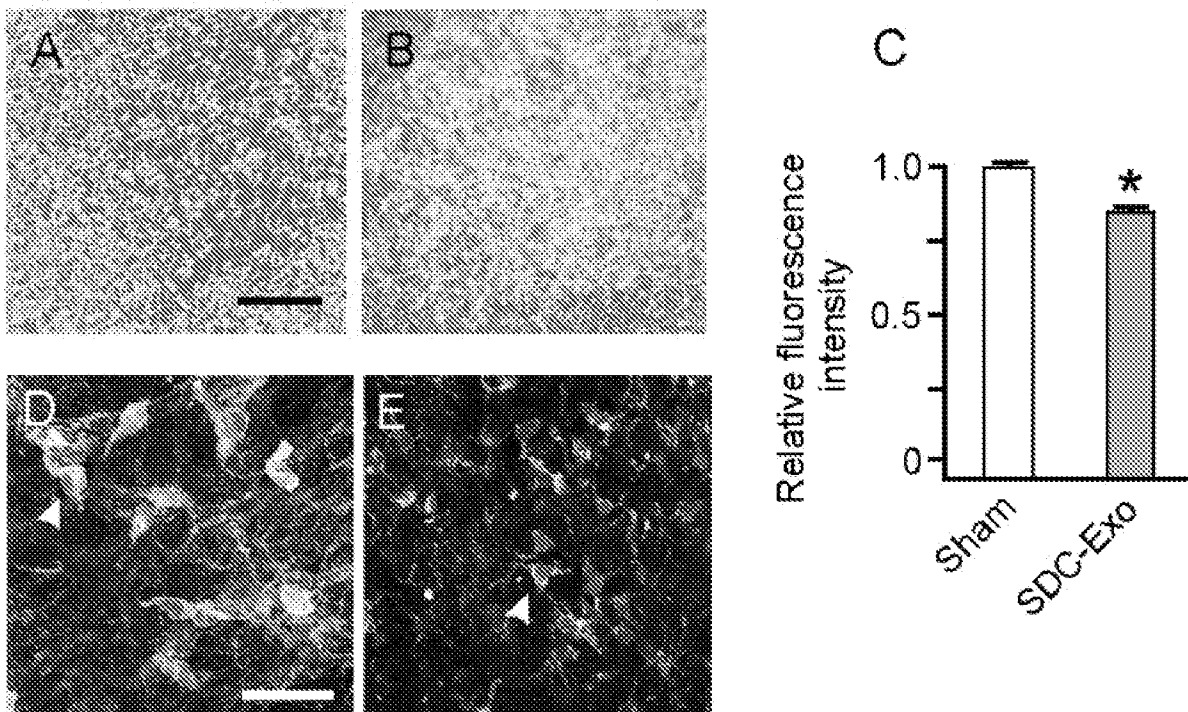
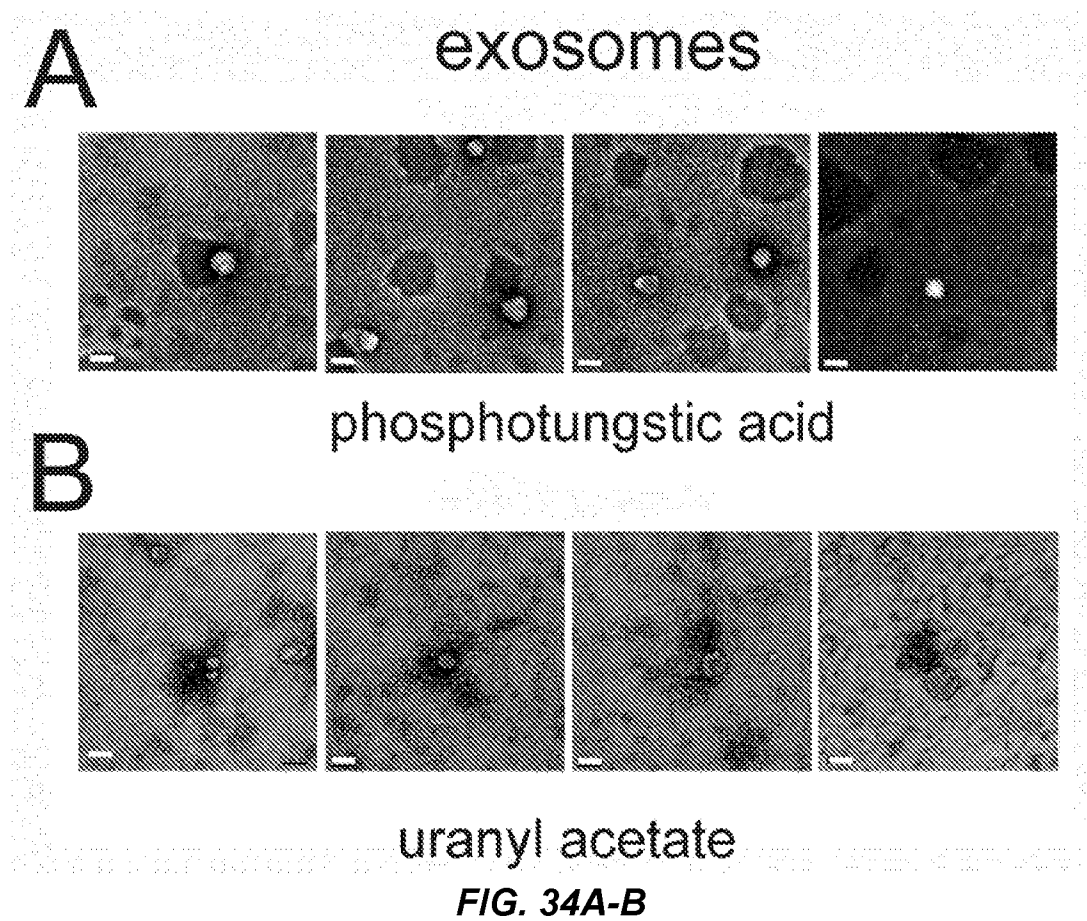


FIG. 33A-E



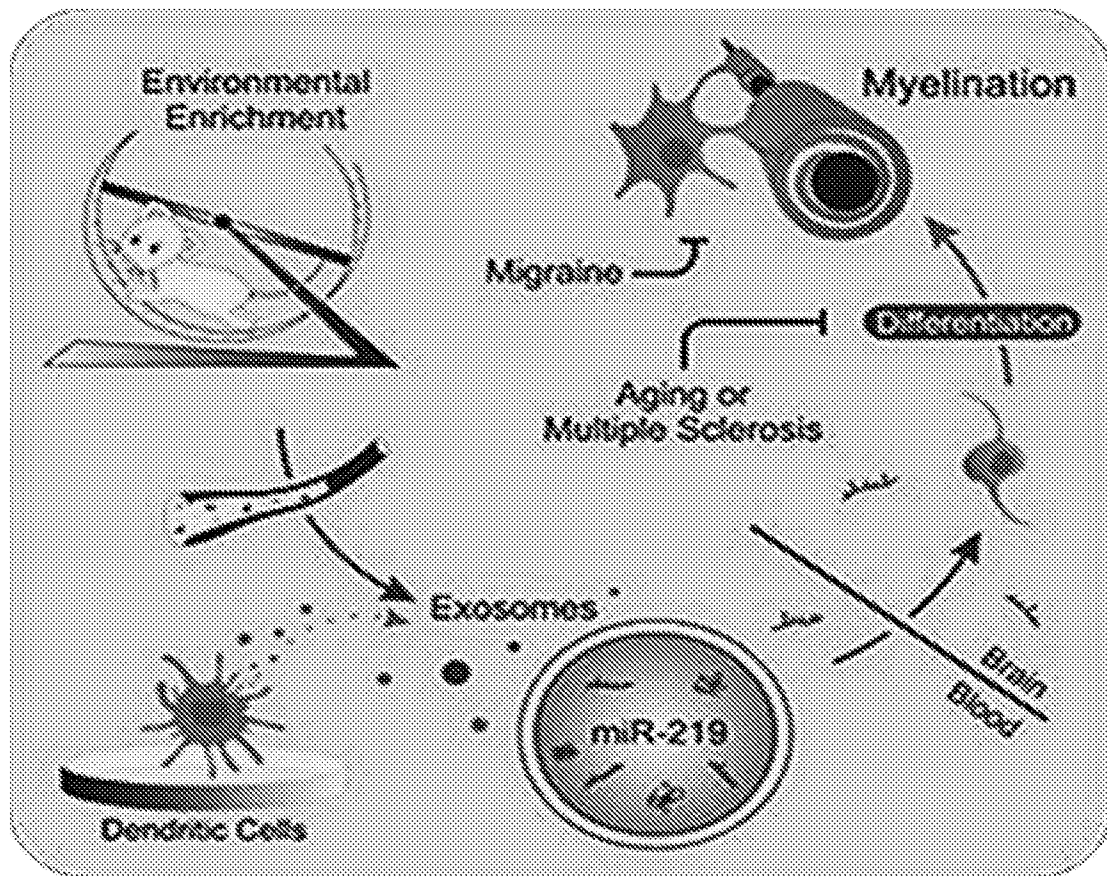


FIG. 35A

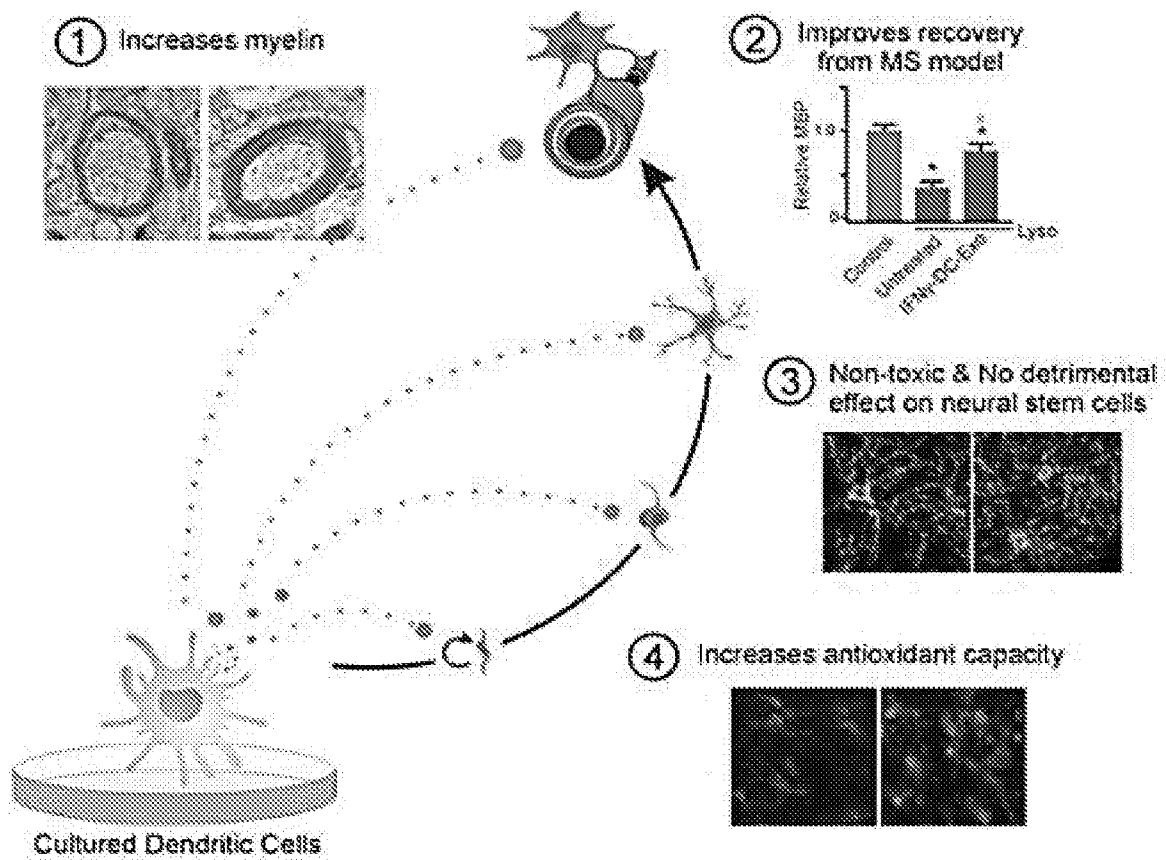


FIG. 35B

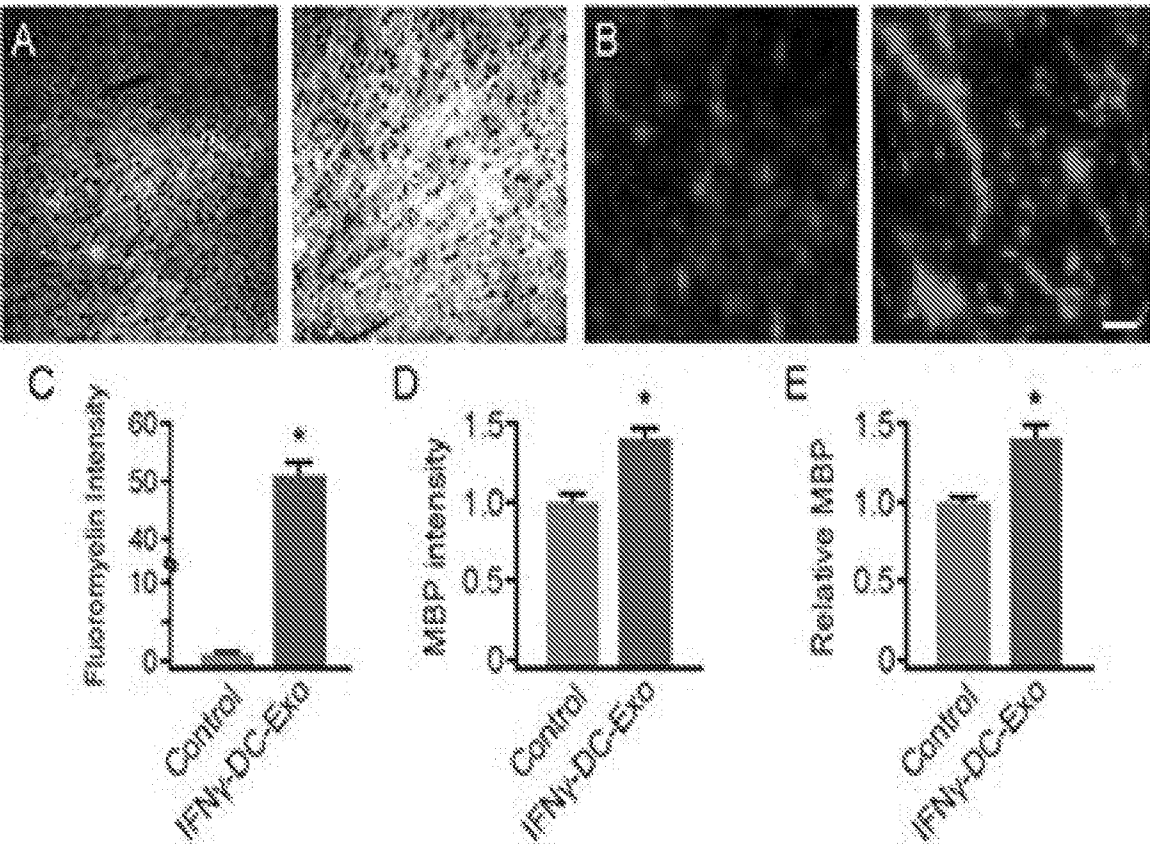


FIG. 36A-E

A Nutritive Exosomes

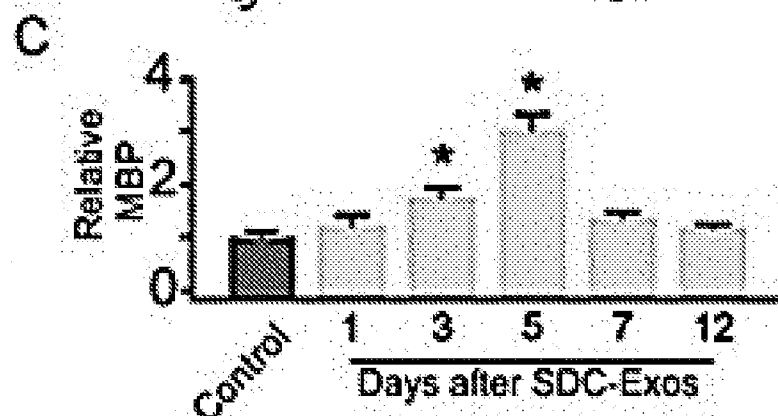
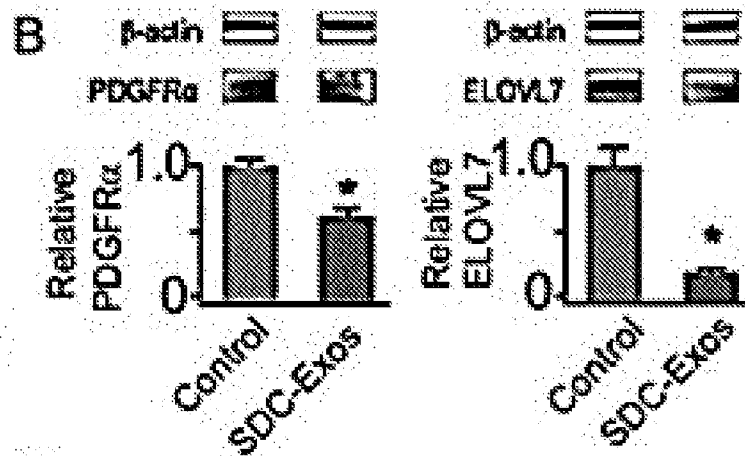
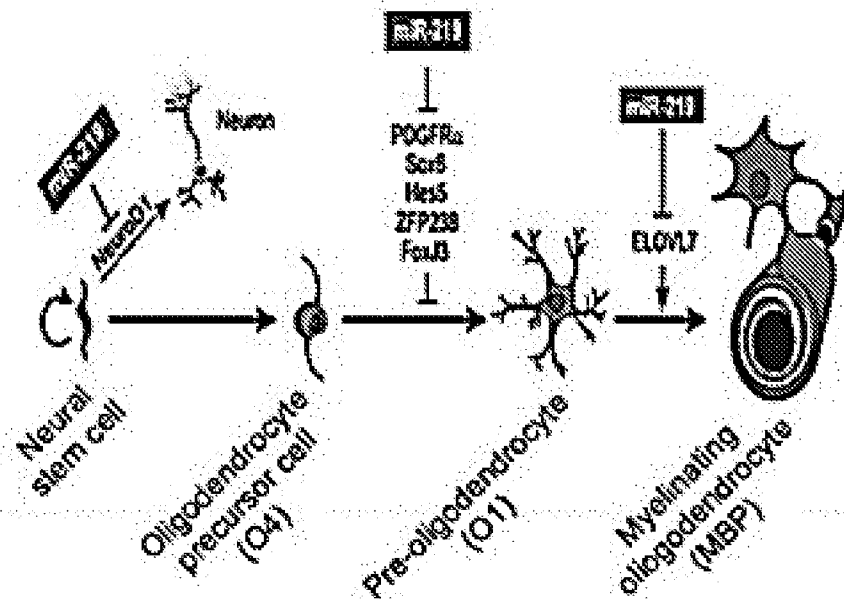


FIG. 37A-C

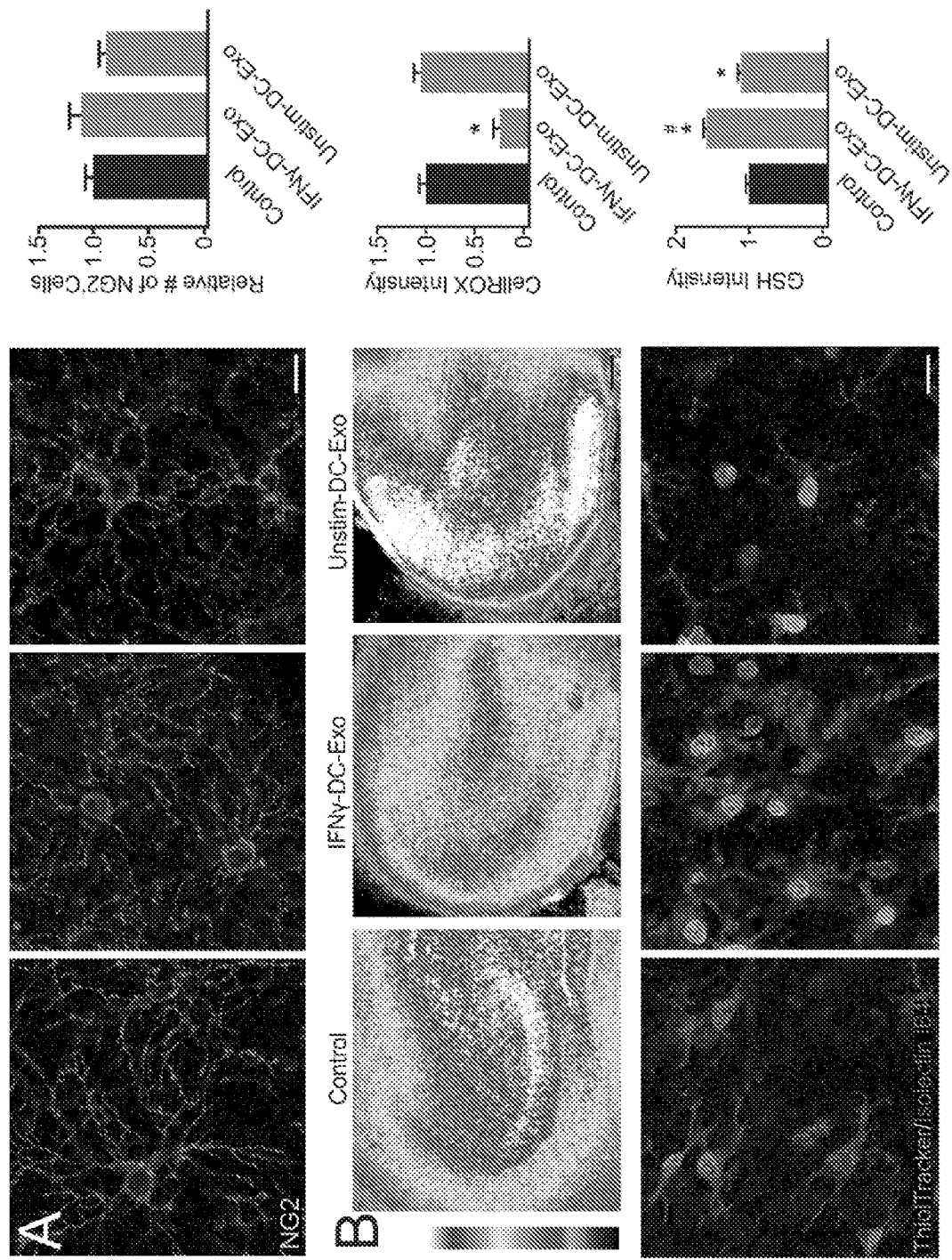


FIG. 38A-B

C

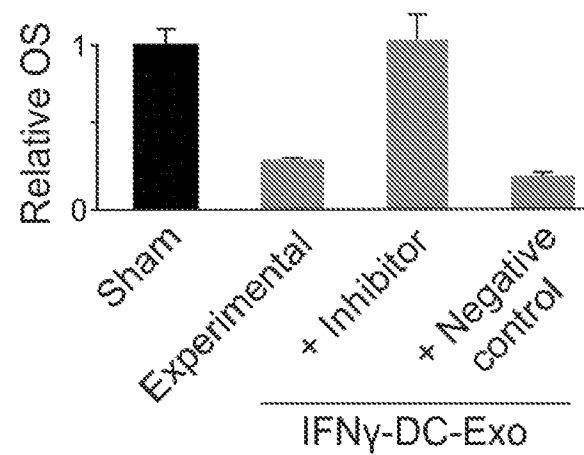


FIG. 38C

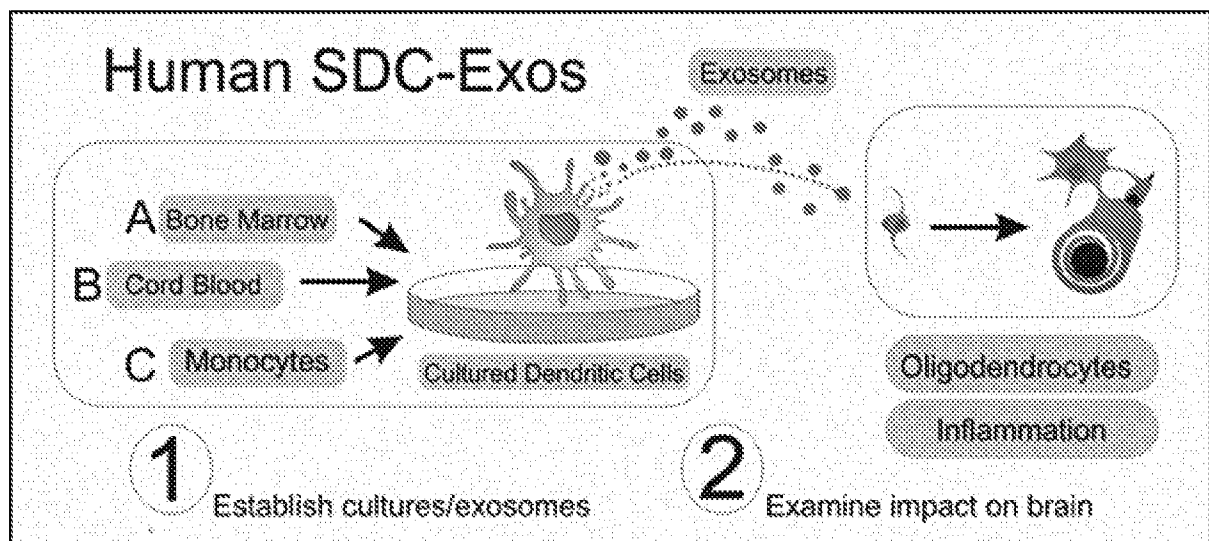
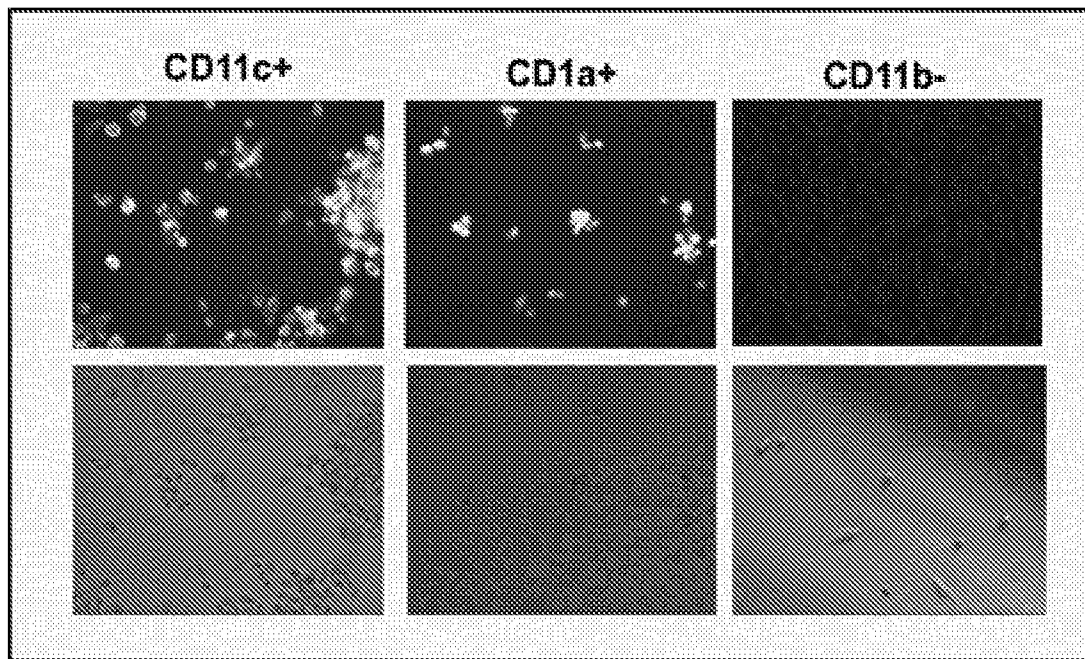
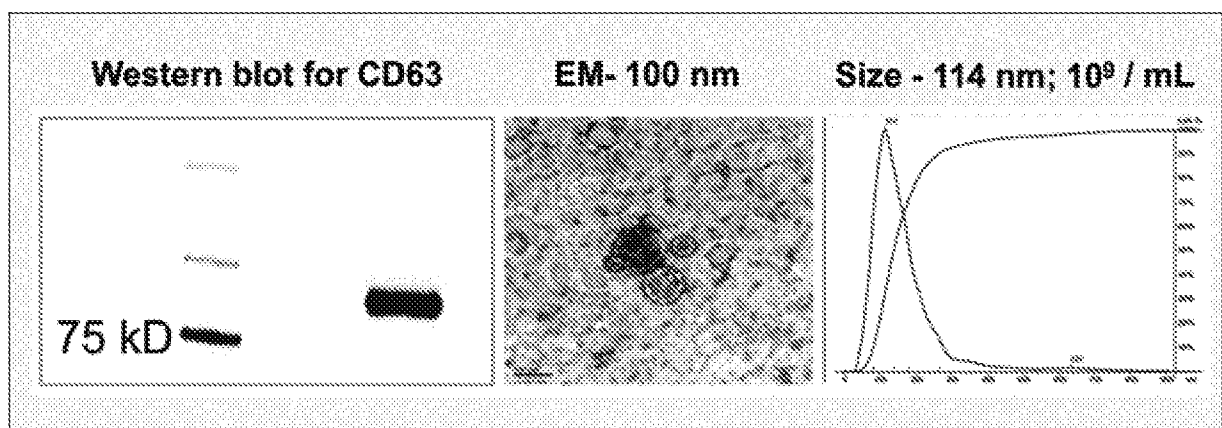


FIG. 39

**FIG. 40****FIG. 41**

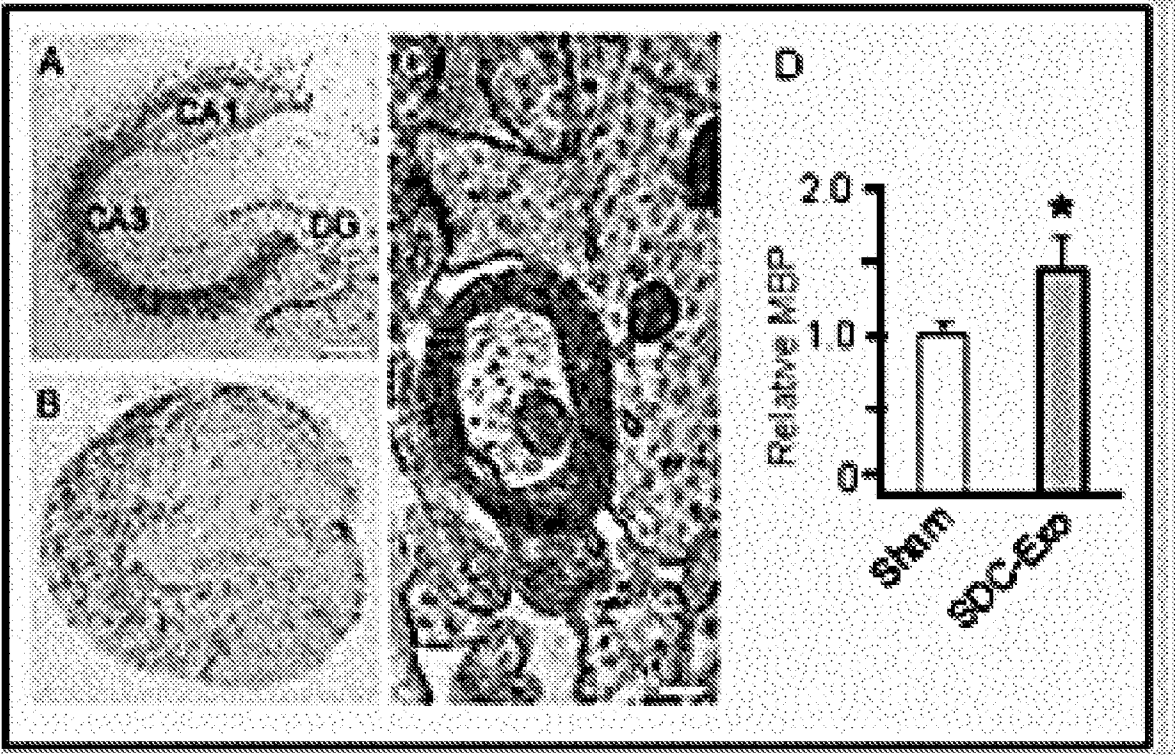


FIG. 42A-D

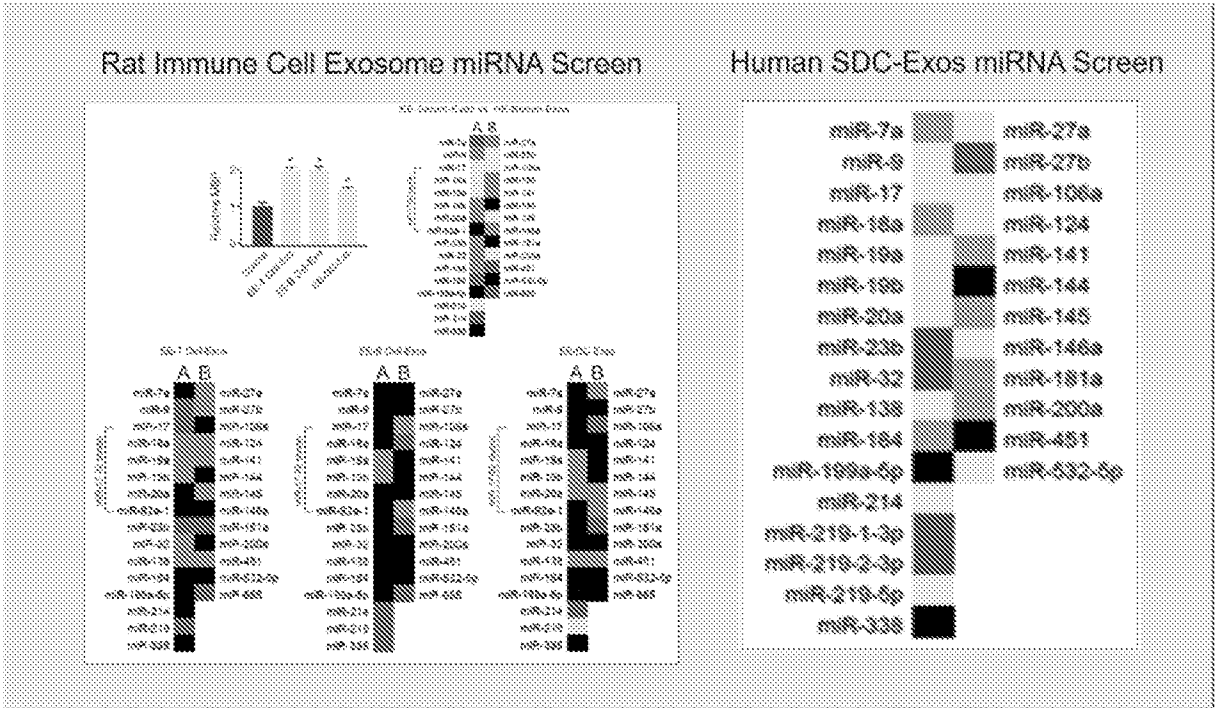


FIG. 43

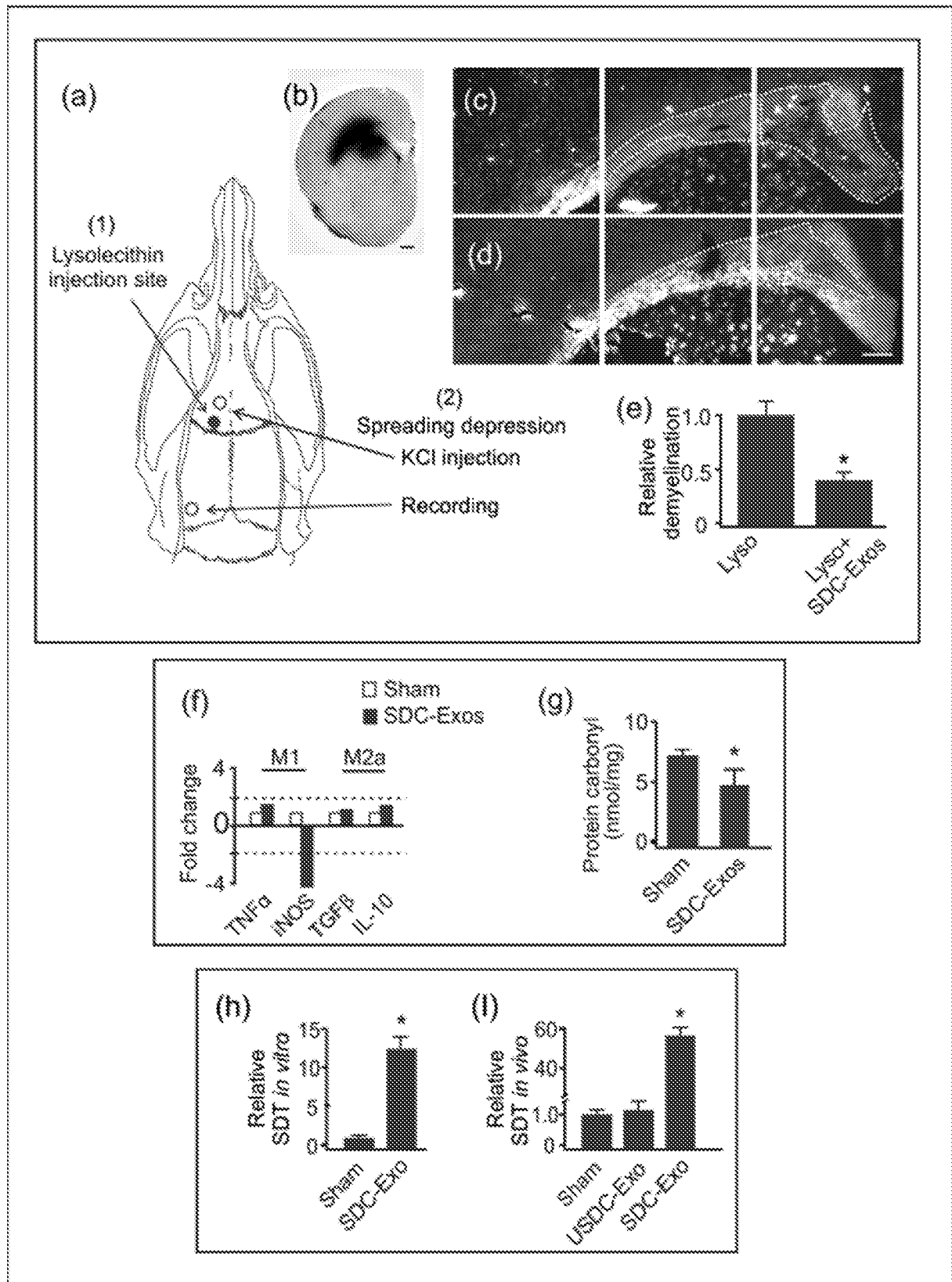


FIG. 44A-I

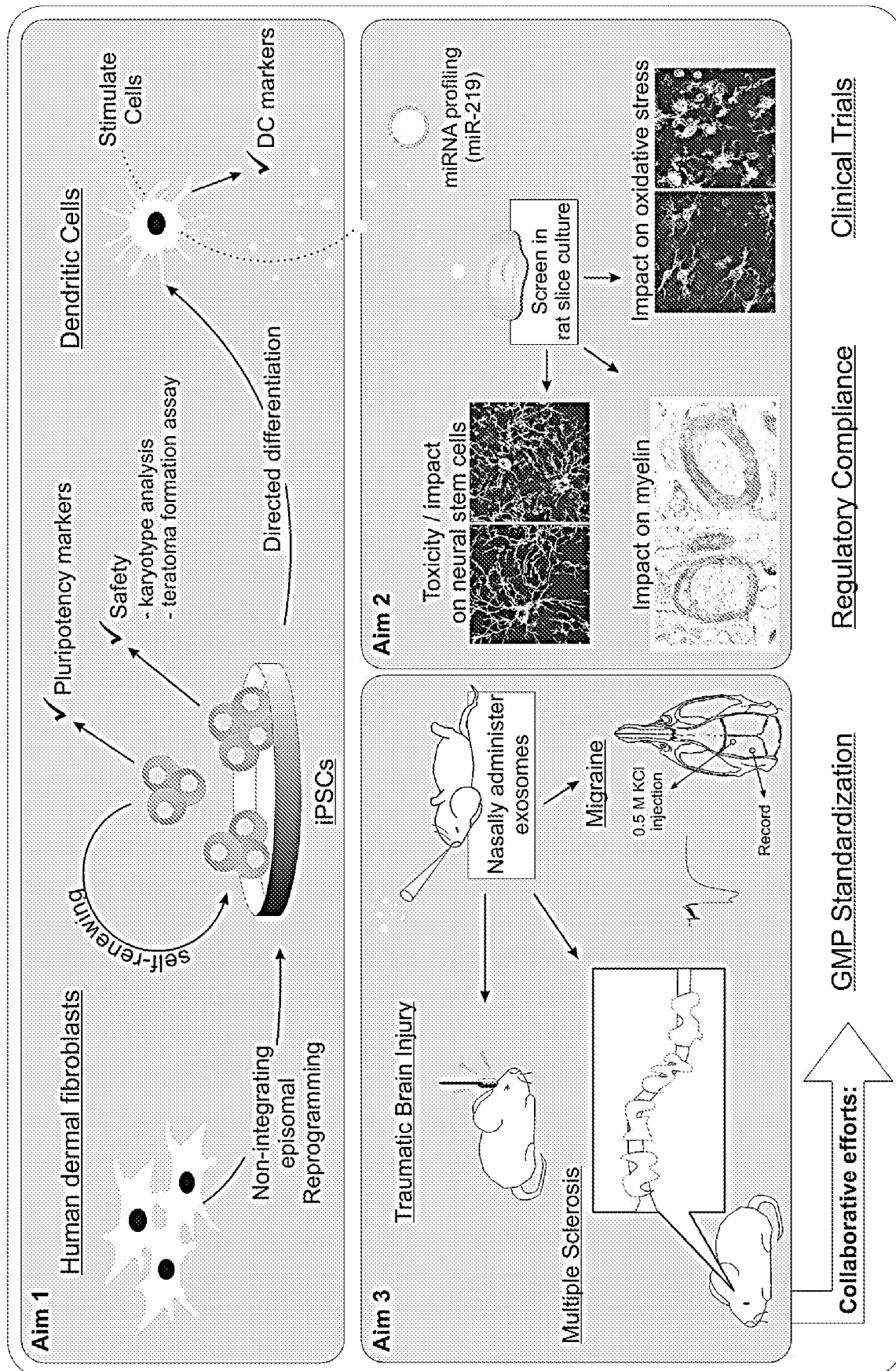


FIG. 45

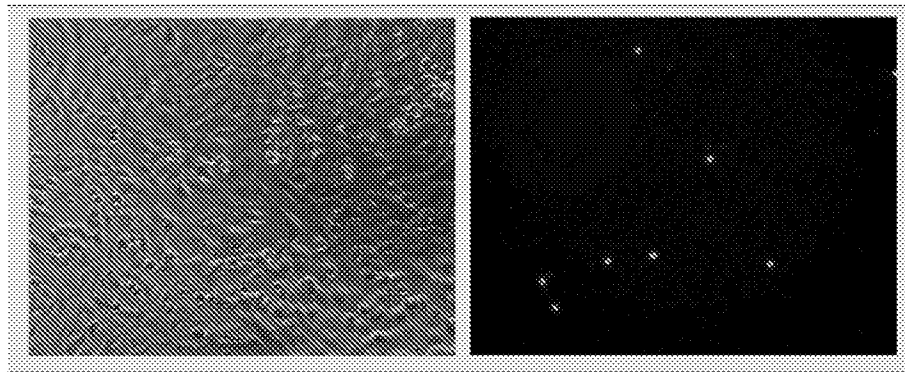


FIG. 46

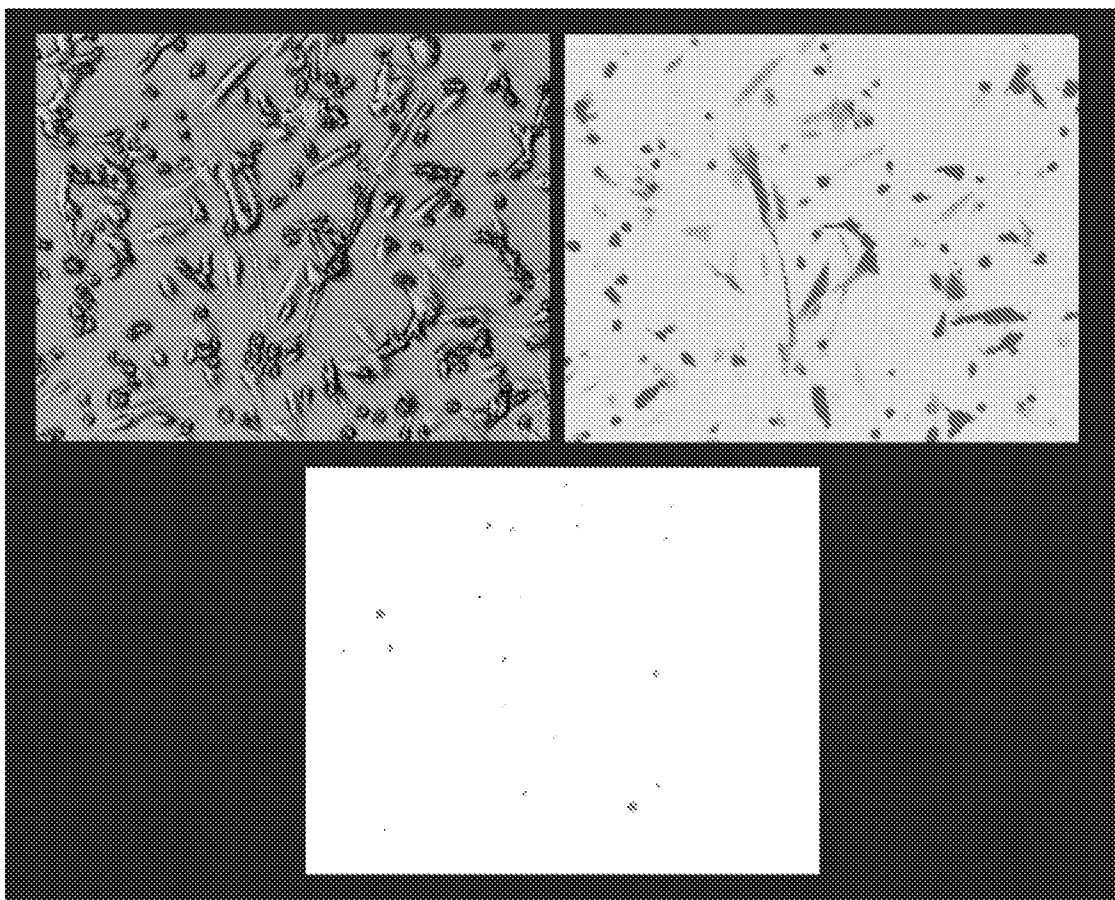
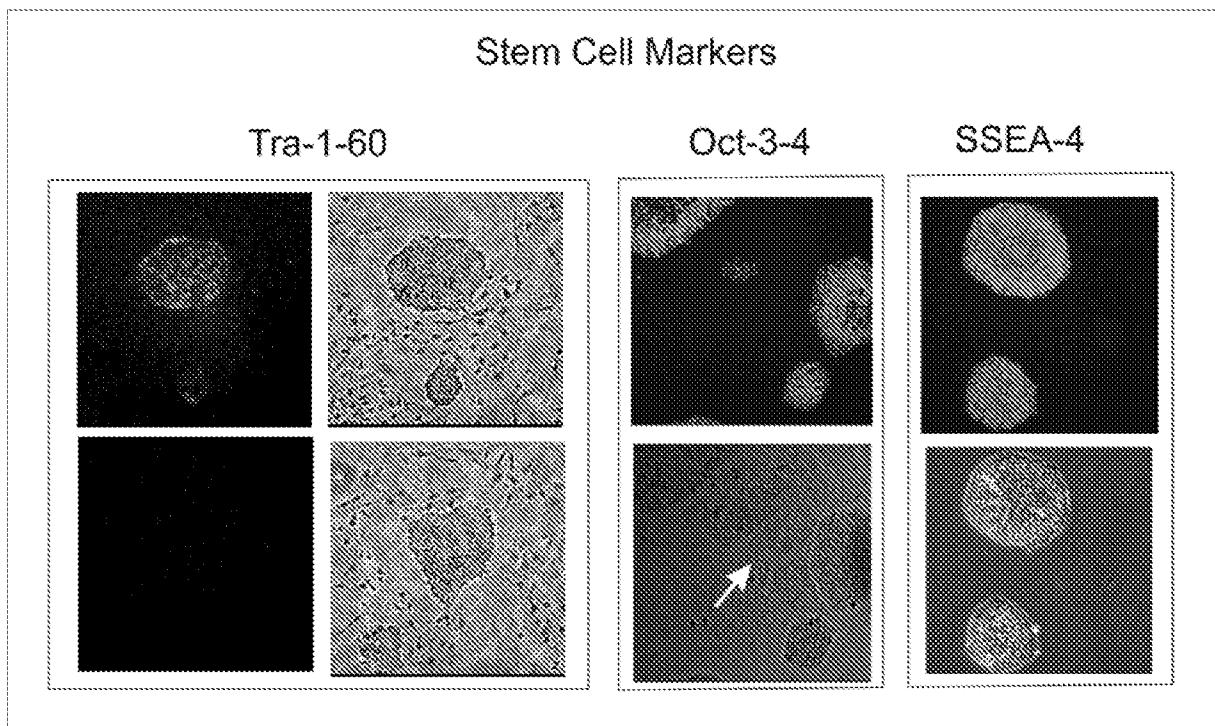
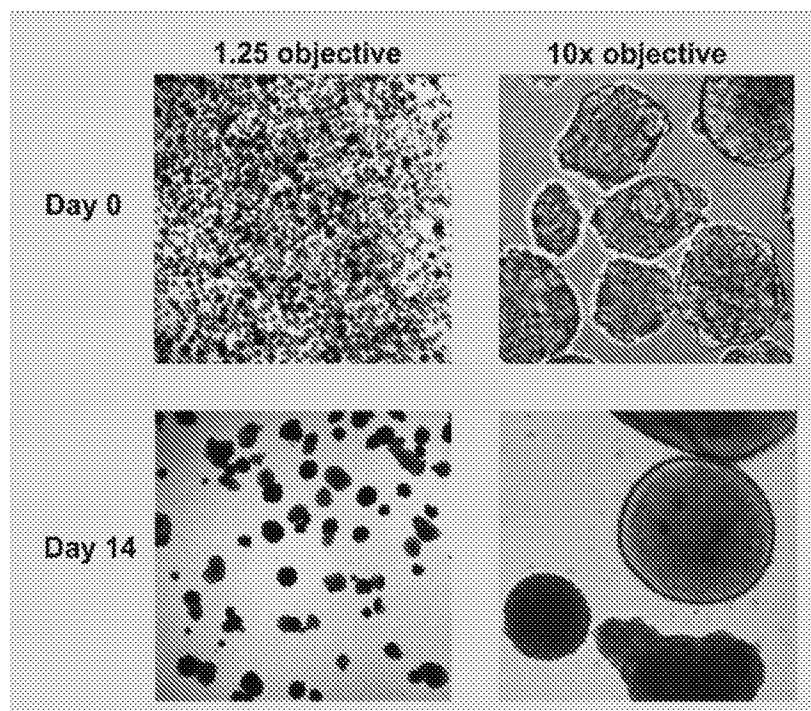
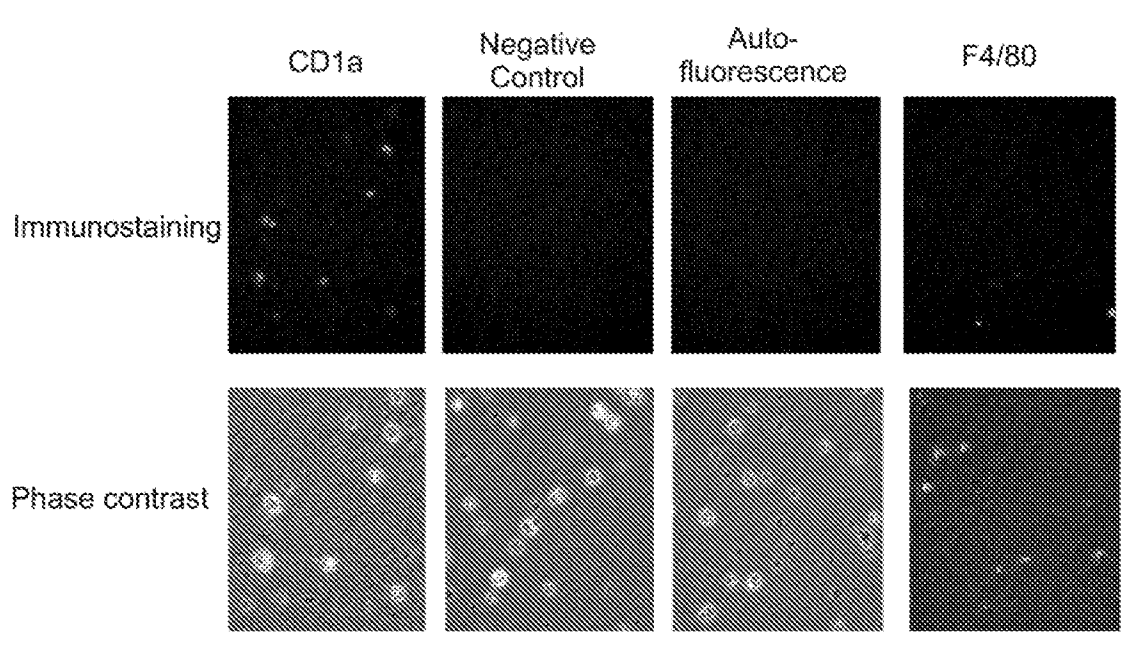
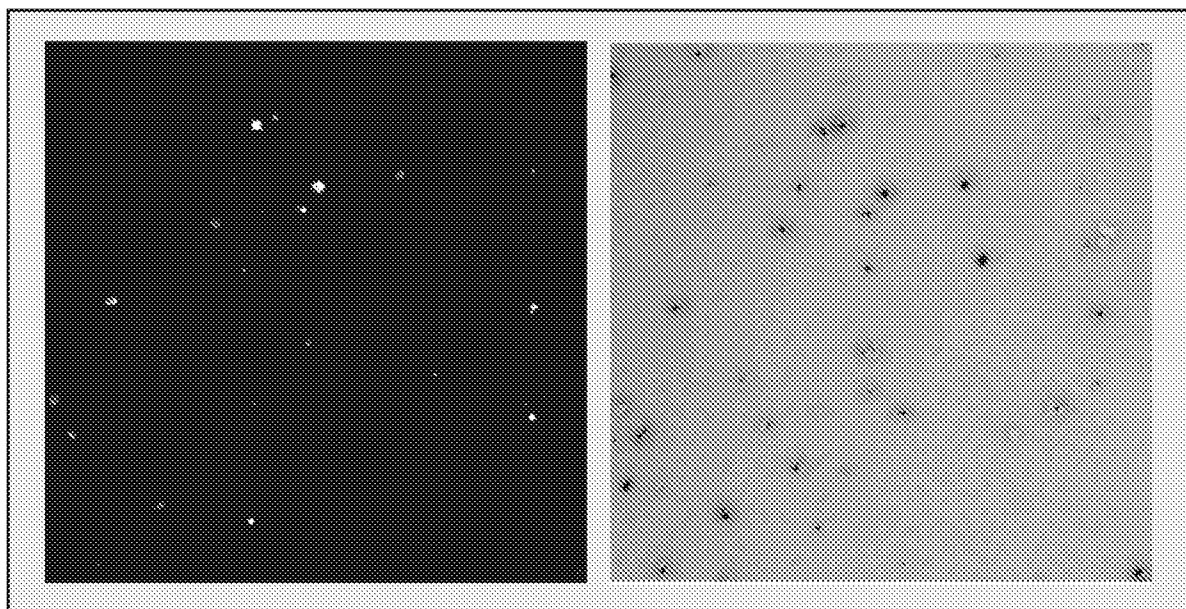


FIG. 47

**FIG. 48****FIG. 49**

**FIG. 50****FIG. 51**