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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
7 July 2016 (07.07.2016)

WIPO | PCT

(10) International Publication Number
WO 2016/109543 A1

(51) International Patent Classification:
C12N 5/0797 (2010.01) *C12N 5/0784* (2010.01)
C12N 5/078 (2010.01) *C12N 5/077* (2010.01)
C12N 5/0783 (2010.01) *A61K 35/12* (2006.01)

(21) International Application Number:
PCT/US2015/067873

(22) International Filing Date:
29 December 2015 (29.12.2015)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
62/098,048 30 December 2014 (30.12.2014) US
62/101,863 9 January 2015 (09.01.2015) 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, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*

(54) Title: METHODS TO IMPROVE CELL THERAPY

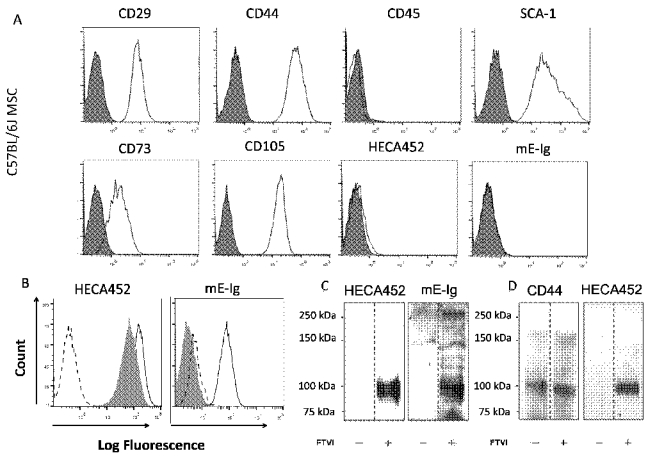

Figure 1

(57) Abstract: Methods of treatment are provided herein, including administration of a population cells modified to enforce expression of an E-selectin and/or an L-selectin ligand, the modified cell population having a cell viability of at least 70% after a treatment to enforce such expression.

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METHODS TO IMPROVE CELL THERAPY

[0001] This disclosure was made with government support under grants PO1 HL107146 (NHLBI Program of Excellence in Glycosciences), RO1 HL73714, and RO1 HL60528, awarded by National Institutes of Health. The government has certain rights in the disclosure.

[0002] This disclosure relates to compositions and methods for modifying the cell surface and the improved efficiency and applicability of such modified cells in cell-based treatment of inflammatory conditions, tissue injury/damage, and cancer.

[0003] The success of cell-based therapeutics (also known as "adoptive cellular therapeutics") depends on getting the relevant cells to the site(s) where they are needed in sufficient amount(s) to achieve intended biologic effect(s). Delivery of cells for clinical indications can be achieved by direct (local) injection into involved tissue(s), by intravascular administration (*e.g.*, systemically or by catheter-based delivery to a particular vascular bed), or by application/placement of cells directly onto the affected area (*e.g.*, for skin ulcers, burns, etc.). In all forms of cell administration, it would be advantageous for administered cells to possess membrane molecules that would promote lodgement of the cell within the administered site precisely within tissue microenvironments that are critical to achieve intended effect, *e.g.*, control of inflammation, tissue repair, elimination of rejection, eradication of cancer, etc. One such microenvironmental site are the "perivascular areas" present in and around microvessels within the injured tissue, as it is well known that integrity of the microvasculature, and production of new microvessels (*i.e.*, "angiogenesis"), is a critical prerequisite to tissue regeneration/repair. Indeed, at all sites of tissue injury, inflammation, and cancer, endothelial cells within the microvessels of affected tissue(s) display a characteristic set of adhesion molecules that serve a key role in recruitment of circulating (blood-borne) cells to the target site. These endothelial molecules are upregulated by inflammatory cytokines such as tumor necrosis factor (TNF) and interleukin 1 (IL-1), and, in humans include the molecule E-selectin, and, in mouse, the molecules E-selectin and P-selectin, which are lectins belonging to a family of adhesion molecules known as "selectins" (to be described in more detail below). In addition, leukocytes that have been recruited to any inflammatory site (including cancer) or to a site of tissue injury/damage display L-selectin, the "leukocyte" selectin, and, therefore,

expression of ligands for L-selectin on administered cells would promote lodgement of such cells to regions of leukocyte infiltrates within the affected tissue(s).

[0004] At first glance, direct delivery might seem to be the most efficient approach to cell administration. especially considering that a concentrated bolus of cells could be applied to an affected area. However, there are situations where local injection may actually be counterproductive to intended therapeutic effects, and, moreover, local injection is practical for only certain anatomic locations: (1) By introducing pertinent cells in media suspension under hydrostatic pressure, the injection procedure could harm the delivered cells and, furthermore, could further compromise tissue integrity and disrupt incipient tissue repair and/or host defense processes, thereby exacerbating the inflammatory condition or counteracting appropriate immune reactions *in situ*; (2) By virtue of being an invasive method, the injection needle/device (and the suspension solution) could induce target tissue damage and/or instigate collateral tissue damage; (3) Direct injection is most feasible for organs/tissues with well-defined anatomic boundaries (*e.g.*, the heart), and is impractical for tissues without extensive connective tissue support (*e.g.*, the lung); (4) The injection procedure could be technologically demanding and labor-intensive, requiring use of sophisticated delivery systems with substantial imaging support, especially for relatively inaccessible and/or fragile organs/tissues (*e.g.*, the central nervous system); (5) Most importantly, many degenerative and inflammatory conditions are widely distributed and multifocal in nature (*e.g.*, osteoporosis, inflammatory bowel disease, multiple sclerosis, etc.), and thus direct injection is neither practical nor effective. Thus, though there are clinical conditions/situations in which local injection is feasible, the vascular route of administration is mandated for all generalized “systemic” disorders, as well as for any tissue with problematic access and/or anatomy not amenable to local injection (*e.g.*, the pancreas in diabetes, the lung in chronic obstructive pulmonary disease). The capacity to administer cells repeatedly with minimal effort is another important practical advantage of systemic infusion. Therefore, creation of methodologies to optimize the expression/activity of molecular effectors directing both the adhesion/lodgement of directly injected cells within the inflammatory milieu and the physiologic migration of intravascularly administered cells to the affected site(s) is key to achieving the tremendous promise of all cell-based therapeutics.

[0005] The capacity to direct migration of blood-borne cells to a predetermined location ("homing") has profound implications for a variety of physiologic and pathologic processes. Recruitment of circulating cells to a specific anatomic site is initiated by discrete adhesive interactions between cells in flow and vascular endothelium at the target tissue(s). The molecules that mediate these contacts are called "homing receptors," and, as defined historically, these structures pilot tropism of cells in blood to the respective target tissue. Historically, three "tissue-specific homing receptors" were described: L-selectin for peripheral lymph nodes, $\alpha_4\beta_7$ (LPAM-1) for intestines and gut-associated lymphoid tissue, and a specialized glycoform of the molecule P-selectin Glycoprotein Ligand-1 (PSGL-1) known, specifically, as the "Cutaneous Lymphocyte Antigen" (CLA) that promotes cell migration to skin (R. Sackstein, Curr Opin Hematol 12, 444 (2005)). Notably, apart from these tissues, it had been recognized for several decades that circulating cells, especially hematopoietic stem cells (HSCs), navigate effectively to bone marrow (T. Lapidot, A. Dar, O. Kollet, Blood 106, 1901 (2005)), and several studies pointed to a role for selectins, predominantly E-selectin binding to HSC E-selectin ligands, in mediating recruitment of HSCs to marrow.

[0006] From a biophysical perspective, a homing receptor functions as a molecular brake, effecting initial tethering then sustained rolling contacts of cells in blood flow onto the vascular endothelium at velocities below that of the prevailing bloodstream (Step 1) (R. Sackstein, Curr Opin Hematol 12, 444 (2005)). Thereafter, a cascade of events ensue, typically potentiated by chemokines, resulting in activation of integrin adhesiveness (Step 2), firm adherence (Step 3) and endothelial transmigration (Step 4) (T. A. Springer, Cell 76, 301 (1994)). This "multi-step paradigm" holds that tissue-specific migration is regulated by a discrete combination of homing receptor and chemokine receptor expression on a given circulating cell, allowing for recognition of a pertinent "traffic signal" displayed by the relevant vascular adhesive ligands and chemokines expressed within target endothelium in an organ-specific manner. Following engagement of homing receptor(s) directing trafficking of cells to bone marrow, several lines of evidence indicate that one chemokine in particular, SDF-1 (CXCL12), plays an essential role in Step 2-mediated recruitment of cells to this site (T. Lapidot, A. Dar, O. Kollet, Blood 106, 1901 (2005); A. Peled et al., Science 283, 845 (1999); D. A. Sipkins et al., Nature 435, 969 (2005)). However, expression of SDF-1 is

not limited to the marrow, and this chemokine is typically expressed at all sites of tissue injury/inflammation (R. Sackstein, Immunol. Rev. 230: 140-163 (2009)).

[0007] The most efficient effectors of Step 1 rolling interactions are the selectins (E-, P- and L-selectin) and their ligands (R. Sackstein, Curr Opin Hematol 12, 444 (2005)). As the name implies, selectins are lectins that bind to specialized carbohydrate determinants, consisting of sialofucosylations containing an α(2,3)-linked sialic acid substitution(s) and an α(1,3)-linked fucose modification(s) prototypically displayed as the tetrasaccharide sialyl Lewis X (sLe^x; Neu5Acα2-3Galβ1-4[Fucα1-3]GlcNAcβ1-)) (R. Sackstein, Curr Opin Hematol 12, 444 (2005); M. J. Polley et al., Proc Natl Acad Sci USA 88, 6224 (1991)). The sLex glycan is recognized by a variety of monoclonal antibodies (mAbs), including the mAb known as "HECA452." E- and P-selectin are expressed on vascular endothelium (P-selectin also on platelets), and L-selectin is expressed on circulating leukocytes (R. Sackstein, Curr Opin Hematol 12, 444 (2005)). E- and P-selectin are typically inducible endothelial membrane molecules that are prominently expressed only at sites of tissue injury and inflammation, where their expression is generated in response to inflammatory cytokines. However, the microvasculature of bone marrow and skin constitutively expresses these selectins, and they play a key role in steady-state recruitment of blood-borne cells to these sites (R Sackstein J Invest. Dermatology, 122:1061-1069 (2004)). Importantly, within all inflammatory sites and sites of tissue injury/damage in primates (but not rodents), E-selectin is the principal vascular selectin mediating cell recruitment, as the promoter element responsive to the inflammatory cytokines TNF and IL-1 has been deleted from the P-selectin gene. Thus, at all inflammatory sites of humans, vascular E-selectin expression is more pronounced than that of P-selectin (R. Sackstein, Immunol. Rev. 230: 140-163 (2009)).

[0008] Two principal ligands for E-selectin have been identified on human hematopoietic stem/progenitor cells (HSPC), the highly sialofucosylated CLA glycoform of PSGL-1 (Z. Laszik et al., Blood 88, 3010 (1996), R. Sackstein, Immunol. Rev. 230: 140-163 (2009)) and a specialized sialofucosylated CD44 glycoform known as Hematopoietic Cell E-/L-selectin Ligand (HCELL) (C. J. Dimitroff, J. Y. Lee, S. Rafii, R. C. Fuhlbrigge, R. Sackstein, J Cell Biol 153, 1277 (2001); C. J. Dimitroff, J. Y. Lee, R. C. Fuhlbrigge, R. Sackstein, Proc Natl Acad Sci US A 97, 13841 (2000)). CD44 is a rather ubiquitous cell membrane protein, but the HCELL phenotype is found

predominantly on human HSPCs. In contrast to HCELL's restricted distribution, CLA/PSGL-1 is widely expressed among hematopoietic progenitors and more mature myeloid and lymphoid cells within the marrow (Z. Laszik et al., Blood 88, 3010 (1996), R. Sackstein, Immunol. Rev. 230: 140-163 (2009)). HCELL is operationally defined as CD44 that binds to E-selectin and L-selectin under shear conditions, and is identified by Western blot analysis of cell lysates as a CD44 glycoform reactive with E-selectin-Ig chimera (E-Ig) and with mAb HECA452, which recognizes sialyl Lewis X (and, in addition to sLex, HECA452 recognizes the tetrasaccharide isomer of sLex known as a "sialylated Lewis a" (sLea) in which fucose is attached in α(1,4)-linkage to N-acetylglucosamine within a type 1 lactosamine backbone). In addition to CLA and HCELL, human leukocytes and HSPCs can also express a CD43 glycoform known as "CD43-E" which can serve as an E-selectin ligand (Fuhlbrigge et al Blood 107:1421-1426 (2006), Merzaban et al Blood 118:1774-1783 (2011)), and, in mouse leukocytes, another E-selectin ligand known as E-selectin Ligand-1 (ESL-1) has been described (Merzaban et al Blood 118:1774-1783 (2011)) . In all glycoprotein selectin ligands (*e.g.*, CD43-E, CLA, and HCELL) binding to E-selectin (and, also, to L-selectin and P-selectin) is critically dependent on α(2,3)-sialic acid and α(1,3)-fucose modifications (C. J. Dimitroff, J. Y. Lee, S. Rafii, R. C. Fuhlbrigge, R. Sackstein, J Cell Biol 153, 1277 (2001); C. J. Dimitroff, J. Y. Lee, R. C. Fuhlbrigge, R. Sackstein, Proc Natl Acad Sci US A 97, 13841 (2000); R. Sackstein, C. J. Dimitroff, Blood 96, 2765 (2000); C. J. Dimitroff, J. Y. Lee, K. S. Schor, B. M. Sandmaier, R. Sackstein, J Biol Chem 276, 47623 (2001)). On human HSPCs, HCELL displays the pertinent sialofucosylated selectin binding determinants on N-glycans (C. J. Dimitroff, J. Y. Lee, S. Rafii, R. C. Fuhlbrigge, R. Sackstein, J Cell Biol 153, 1277 (2001); R. Sackstein, C. J. Dimitroff, Blood 96, 2765 (2000)). *In vitro* assays of E- and L-selectin binding under hemodynamic shear stress indicate that HCELL is the most potent ligand for these molecules expressed on any human cell (C. J. Dimitroff, J. Y. Lee, S. Rafii, R. C. Fuhlbrigge, R. Sackstein, J Cell Biol 153, 1277 (2001); C. J. Dimitroff, J. Y. Lee, K. S. Schor, B. M. Sandmaier, R. Sackstein, J Biol Chem 276, 47623 (2001)). Importantly, though E-selectin is constitutively expressed on microvascular endothelium of the marrow and skin, this molecule is prominently expressed on endothelial beds at all sites of inflammation - - both acute and chronic types - - regardless of whether it is induced by direct tissue injury (*e.g.*, burns, trauma, decubitus ulcers, etc.), ischemic/vascular events (*e.g.*, myocardial infarct,

stroke, shock, hemorrhage, coagulopathy, etc.), infections (*e.g.*, cellulitis, pneumonia, meningitis, SIRS, etc.), neoplasia (*e.g.*, breast cancer, lung cancer, lymphoma, etc.), immunologic/autoimmune conditions (*e.g.*, graft vs. host disease, multiple sclerosis, diabetes, inflammatory bowel disease, lupus erythematosus, rheumatoid arthritis, psoriasis, etc.), degenerative diseases (*e.g.*, osteoporosis, osteoarthritis, Alzheimer's disease, etc.), congenital/genetic diseases (*e.g.*, muscular dystrophies, lysosomal storage diseases, Huntington's disease, etc.), adverse drug effects (*e.g.*, drug-induced hepatitis, drug-induced cardiac injury, etc.), toxic injuries (*e.g.*, radiation exposure(s), chemical exposure(s), alcoholic hepatitis, alcoholic pancreatitis, alcoholic cardiomyopathy, cocaine cardiomyopathy, etc.), metabolic derangements (*e.g.*, uremic pericarditis, metabolic acidosis, etc.), iatrogenic conditions (*e.g.*, radiation-induced tissue injury, surgery-related complications, etc.), and/or idiopathic processes (*e.g.*, amyotrophic lateral sclerosis, Parsonnage-Turner Syndrome, etc.).

[0009] Among the various aspects of the present disclosure is the provision of methods for the treatment of a disease, disorder or medical condition having E-selectin expression on vascular endothelial cells and/or leukocyte infiltrates within affected tissue(s). E-selectin binds to sialylated, fucosylated carbohydrates (*e.g.*, members of the sialylated Lewis X and Lewis A families) present natively on the surface of certain leukocytes and hematopoietic stem/progenitor cells, *i.e.*, myeloid cells (*e.g.*, neutrophils, monocytes, eosinophils, macrophages, etc.), dendritic cells (both lymphoid- and myeloid-derived), lymphocytes (*e.g.*, naïve and memory T cells, naïve and memory B cells, effector T cells, regulatory T cells, natural killer cells (NK cells), etc.), hematopoietic progenitor cells, and hematopoietic stem cells. These cell types are thus found at acute and chronic inflammatory sites, recruited by vascular E-selectin to such inflammatory sites. Leukocytes and HSPCs characteristically display L-selectin. L-selectin, itself, binds to sialylated, fucosylated carbohydrates such as sLex. Thus, inflammatory sites have cells that express E-selectin (on endothelial cells) and L-selectin (on infiltrating leukocytes and HSPCs).

[0010] The ability to achieve intended outcome(s) of cell-based therapeutics is critically dependent on delivery of administered cells to sites where they are needed, and, also, to the localization of the administered cells within specific tissue microenvironments. Accordingly, there is a need in the art for methods to enhance vascular delivery of administered cells to sites of tissue injury/damage, to sites of

inflammation, to sites of cancer, and to sites of infection. There is also a need in the art to enhance lodgement/colonization of the administered cells within the affected tissue. Briefly, therefore, the present disclosure is directed to methods of treating a disease, disorder or medical condition manifesting as inflamed and/or damaged tissue and/or cancer in a subject, the methods comprising administering to the subject a cell population that expresses an E-selectin ligand and/or an L-selectin ligand at a level that exceeds that of a native population of the cells. The administration occurs coincident with expression of E-selectin on endothelial cells within the target tissue (*e.g.*, inflamed and/or damaged tissue, cancerous tissue) and/or coincident with accumulation of leukocytes (*e.g.*, leukocytic infiltrates) within the target tissue. The administration may be by direct injection within the target tissue and/or via the vasculature and/or via other means as described in further detail below.

[0011] The present disclosure is further directed to a method for the treatment of inflammation with a viable population of cells that express an E-selectin and/or L-selectin ligand. The viable cell population expresses an E-selectin ligand and/or an L-selectin ligand at a level that exceeds the level of expression of a native population of the cells. The administration occurs coincident with expression of E-selectin on endothelial cells within the target tissue (*e.g.*, inflamed and/or damaged tissue, cancerous tissue) and/or coincident with accumulation of leukocytes (*e.g.*, leukocytic infiltrates) within the target tissue. The administration may be by direct injection into the target tissue and/or via the vasculature (and/or via other means as described in further detail below) and treatment of the disease, disorder or medical condition need not be accompanied by long-term engraftment of the administered cells (*i.e.*, treatment could be achieved either with transient colonization or with long-term persistence/longevity of administered cells at the treatment site or with proliferation of administered cells at the treatment site or with differentiation and/or maturation of administered cells at the treatment site).

[0012] The present disclosure is further directed to a viable population of cells that express an E-selectin and/or L-selectin ligand for use in the manufacture of a medicament for treating inflammation in a subject. The viable cell population expresses an E-selectin ligand and/or an L-selectin ligand at a level that exceeds the level of expression of a native population of the cells. The administration occurs coincident with expression of E-selectin on endothelial cells within the target tissue (*e.g.*, inflamed

and/or damaged tissue, cancerous tissue) and/or coincident with accumulation of leukocytes (*e.g.*, leukocytic infiltrates) within the target tissue. The administration may be by direct injection into the target tissue and/or via the vasculature and/or via other means as described in further detail below.

[0013] The present disclosure is further directed to a viable population of cells that express an E-selectin ligand and/or an L-selectin ligand for use in the manufacture of a medicament for the treatment of a tumor/malignant disease, the treatment comprising administering to the subject the population of cells that express an E-selectin ligand and/or an L-selectin ligand, wherein the population expresses the E-selectin ligand and/or L-selectin ligand at a level that exceeds the level of expression of a native population of the cells, wherein said treatment comprises administering the population coincident with E-selectin expression on endothelial cells within the tumor/malignant tissue and/or coincident with accumulation of leukocytes within the tumor/malignant tissue.

[0014] The present disclosure is further directed to a viable population of cells that express an E-selectin ligand and/or an L-selectin ligand for use in the manufacture of a medicament for the treatment of a diseased state manifesting inflammation, the treatment comprising administering to the subject the population of cells that express an E-selectin ligand and/or an L-selectin ligand, wherein the population expresses the E-selectin ligand and/or L-selectin ligand at a level that exceeds the level of expression of a native population of the cells, wherein said treatment comprises administering the population coincident with the onset of E-selectin expression on endothelial cells within the tumor/malignant tissue and/or coincident with accumulation of leukocytes within the tumor/malignant tissue.

[0015] Other methods involving the administration (*e.g.*, via the vasculature and/or via direct injection into tissue and/or via other means) described herein include, for example, method of enhancing cell delivery and colonization in an inflamed and/or damaged tissue or site of cancer in a subject (collectively, a “target” tissue), methods of enhancing cell delivery into a target tissue of a subject and/or enhancing tissue colonization in the target tissue of the subject, methods of improving cellular delivery to a target tissue in a subject, methods of enhancing cell delivery and colonization into a target tissue of a subject, method of enhancing homing and engraftment of a cell

population within a target tissue in a subject, methods of treating an inflammatory condition in a subject, methods of enhancing tissue repair/regeneration in a subject, and methods of treating tumor/malignant disease in a subject.

[0016] In general, any of a variety of inflammatory conditions (*e.g.*, acute and/or chronic) and/or damaged tissue may be treated in accordance with the methods described herein, including, but not limited to those initiated by direct tissue injury (*e.g.*, burns, trauma, decubitus ulcers, etc.), ischemic/vascular events (*e.g.*, myocardial infarct, stroke, shock, hemorrhage, coagulopathy, etc.), infections (*e.g.*, cellulitis, pneumonia, meningitis, SIRS, etc.), neoplasia (*e.g.*, breast cancer, lung cancer, lymphoma, etc.), immunologic/autoimmune conditions (*e.g.*, graft vs. host disease, multiple sclerosis, diabetes, inflammatory bowel disease, lupus erythematosus, rheumatoid arthritis, psoriasis, etc.), degenerative diseases (*e.g.*, osteoporosis, osteoarthritis, Alzheimer's disease, etc.), congenital/genetic diseases (*e.g.*, epidermolysis bullosa, osteogenesis imperfecta, muscular dystrophies, lysosomal storage diseases, Huntington's disease, etc.), adverse drug effects (*e.g.*, drug-induced hepatitis, drug-induced cardiac injury, etc.), toxic injuries (*e.g.*, radiation exposure(s), chemical exposure(s), alcoholic hepatitis, alcoholic pancreatitis, alcoholic cardiomyopathy, cocaine cardiomyopathy, etc.), metabolic derangements (*e.g.*, uremic pericarditis, metabolic acidosis, etc.), iatrogenic conditions (*e.g.*, radiation-induced tissue injury, surgery-related complications, etc.), and/or idiopathic processes (*e.g.*, amyotrophic lateral sclerosis, Parsonnage-Turner Syndrome, etc.).

[0017] In certain embodiments, for example, the E-selectin ligand and/or L-selectin ligand expressed by the cell population is selected from one or more of Hematopoietic Cell E-/L-selectin Ligand (HCELL), Neural Cell Adhesion Molecule E-selectin Ligand (NCAM-E), CD43E, and CLA. In one embodiment, the E-selectin ligand is Hematopoietic Cell E-/L-selectin Ligand (HCELL) and/or Neural Cell Adhesion Molecule E-selectin Ligand (NCAM-E). In another embodiment, the E-selectin ligand is HCELL. In another embodiment, the E-selectin ligand is NCAM-E. In another embodiment, the L-selectin ligand is HCELL. Notably, since E-selectin is expressed within endothelial beds of the affected tissue and leukocytes characteristically express L-selectin, expression of HCELL, a potent E-selectin and L-selectin ligand, would serve to promote tissue lodgement expressly within the microenvironments of most intense immunoreactivity/tissue damage.

[0018] As discussed elsewhere herein in greater detail, a variety of methods may be utilized to prepare the population of cells for administration to the subject. In one embodiment, the population is prepared by contacting the cell or a population of cells with glycosyltransferase together with appropriate donor nucleotide sugar. For example, glycan engineering may be used to sialofucosylate CD44 to enforce the expression of Hematopoietic Cell E-selectin Ligand (HCELL), by glycosyltransferase-enforced expression of fucose residues (fucosylation), sialic acid residues (sialylation), or both (sialofucosylation). Alternatively, glycosyltransferases such as a fucosyltransferase may be used to transfer intact glycan structures such as sialyl-Lewis^X or sialyl-Lewis^a to cell surfaces. In addition, non-enzymatic methods may be used to covalently or non-covalently bind E-selectin and/or L-selectin ligands to cell surfaces. For example, aptamers, sLex (sialyl-Lewis^X), glycomimetics and/or peptidomimetics of sLex glycans, sLea (sialyl-Lewis^a), glycomimetics and/or peptidomimetics of sLea glycans, and other moieties that bind E-selectin and/or L-selectin may be non-covalently bound to cell surfaces using biotin-streptavidin pairs or covalently bound to the cell surfaces; whether covalent or non-covalent, the binding may be direct or via a linker. By way of further example, phage display particles or antibodies that bind E-selectin and/or L-selectin may be covalently or non-covalently bound to the cell surface, in each case mediating adherence of treated cells to E-selectin and/or L-selectin.

[0019] In general, and independent of the manner of preparing the cell population, the manner of preparation provides a modified cell population having a viability of at least 70% at 24 hours from the time of modification. In one such embodiment, the modified cell population has a viability of at least 80% at 24 hours from the time of the modification. In some embodiments, the modified cell population has a viability of at least 85% at 24 hours from the time of the modification. In some embodiments, the modified cell population has a viability of at least 90% at 24 hours from the time of the modification. Viability may be determined by methods known in the art such as trypan blue exclusion, or by dual color flow cytometry assessment for propidium iodide and Annexin V staining. Preferably, the phenotype of the cells (other than the cell surface modification) is preserved after treatment. By preserved phenotype it is meant the cell maintains its native function and/or activity. For example, if the cell is a stem cell it retains its regenerative potency, *e.g.*, its totipotency or its pluripotency or its multipotency or its unipotency.

[0020] The modified cell populations are viable (*i.e.*, have a viability of at least 70% at 24 hours from the time of modification as described above) and have enhanced binding to E-selectin and/or L-selectin relative a native population of the cells. The administration of cells may be via the vasculature and/or by direct injection into the tissue (and/or by other means as described in detail below). In one embodiment, the modified cell population is administered at a time coincident with the onset of inflammation or the infiltration of leukocytes into tissue. In another embodiment, the modified cell population is administered just prior to the onset of inflammation or the infiltration of leukocytes into the tissue.

[0021] In cases where cells undergo glycan engineering to enforce sLex expression or undergo decoration with sLex structures (*e.g.*, via avidin-spreptavidin techniques), measurement of increased sLex expression on treated cells can be performed by fluorescence staining with mAb HECA452 or any other mAb which recognizes sLex determinants, followed by flow cytometry to detect cell fluorescence intensity. The predetermined fluorescence threshold of the modified cell population is determined by first analyzing a sample of native (untreated) cells. Increases in sLex of treated cells is defined as increase percentage of marker-positive cells (*e.g.*, HECA452-reactive cells) of greater than 10% compared to native population of cells and/or by a 10% increase in mean channel fluorescence intensity over that of the baseline (untreated) cell population. For detecting whether the treated cell population has increased binding to E-selectin, binding to E-selectin can be assessed using either parallel plate flow chamber studies under shear stress conditions as described herein or via staining with E-selectin-Ig chimera and assessment of fluorescence intensity by flow cytometry. The control (baseline) sample of cells is assayed using the functional E-selectin binding assay described elsewhere herein, or by another generally accepted E-selectin fluorescence binding assay known in the art. E-selectin binding fluorescence levels are measured for the control (baseline) population sample. Enhanced binding to E-selectin is defined as treated cells having increased adherence to E-selectin in an E-selectin-specific binding assay. In one embodiment, enhanced binding to E-selectin can be defined by a fluorescence shift in an E-selectin binding assay using fluorochrome-conjugated reagents, *e.g.*, binding to E-selectin-Ig chimera as assessed by flow cytometry, in which the number of cells within the population that possess E-selectin binding increases by at least 10% more than that of the base-line binding (*i.e.*, increase

of 10% in marker-positive population) and/or is at least 10% greater in mean channel fluorescence than a predetermined fluorescence threshold (associated with the native cell population). In another embodiment, the percentage of cells that possess increased E-selectin reactivity is increased by 25% and/or the modified population exceeds the predetermined fluorescence threshold by 25%. In another embodiment, the modified population exceeds the baseline reactivity and/or predetermined fluorescence threshold by 50%. In another embodiment, the percentage of cells that possess increased E-selectin reactivity is increased by 75% over that of the baseline population of E-selectin-binding cells and/or the modified population exceeds the predetermined fluorescence threshold by 75%. In another embodiment, at least 90% of the cells in the modified population exceed the baseline E-selectin-binding population and/or the predetermined fluorescence threshold. In another embodiment, at least 95% of the cells in the modified population exceed the baseline E-selectin-binding population and/or the the predetermined fluorescence threshold.

[0022] Enhanced binding to L-selectin may be determined by an increase in binding to L-selectin using a functional L-selectin binding assay with high specificity such as a parallel plate flow chamber under dynamic shear stress conditions or a Stamper-Woodruff Assay, wherein the cell treatment increases the percentage of cells supporting L-selectin-mediated adherence. In the case of parallel plate assays, the treated cell population displays at least a 10% increase in tethering/rolling adhesive interactions to L-selectin (affixed and displayed on the chamber plastic or glass support surface) compared to that of baseline (untreated cells). In the case of the Stamper-Woodruff assay, increased L-selectin lymphocyte adherence is defined by at least a 10% increase in treated cell binding to L-selectin^{+} lymphocytes under a rotatory shear of 80 rpm; baseline cell binding is assessed on the untreated (control population), and directly compared to that in the treated cell population.

[0023] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present disclosure, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including

definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

[0024] Other objects and features will be in part apparent and in part pointed out hereinafter.

BRIEF DESCRIPTION OF THE DRAWINGS

[0025] **Figures 1(A)-1(D). Effects of Exofucosylation (FTVI treatment) on E-selectin ligand expression by mouse mesenchymal stem cells (MSCs).** *(A)* MSCs derived from C57BL/6 marrow lack expression of CD45 and express characteristic mouse MSC markers Sca-1, CD29, CD44, CD73 and CD105. MSCs natively lack reactivity with mAb HECA452 and with E-selectin-Ig chimera (mE-Ig) (istoype = red color and antibody = blue color). *(B)* Fucosyltransferase VI (FTVI)-modified MSC (solid line) stained positive for mAbs HECA452 and were reactive with mE-Ig. Digestion of FTVI-modified MSCs with bromelain and proteinase K (shaded histogram) significantly reduced murine E-selectin-Ig chimera (mE-Ig) reactivity, but not HECA452 staining, indicating that bromelain-sensitive glycoproteins serve as the principal E-selectin ligand(s) on glycan-modified (*i.e.*, FTVI-treated) MSCs. Dashed line represents staining controls (isotype control for HECA452 staining and calcium chelation with EDTA for mE-Ig staining). *(C)* Western blot analysis of HECA452 (left) and mE-Ig (right) reactivity of cell lysates of unmodified MSCs (-) and FTVI-modified MSCs (+). FTVI modification induced HECA452- and mE-Ig-reactive moieties predominantly on a doublet glycoprotein band of ~100 kDa. *(D)* CD44 was immunoprecipitated from equivalent amounts of cell lysate from FTVI-modified (+) or unmodified (-) MSCs. Immunoprecipitates were then electrophoresed and blotted with CD44 (mAbs KM114 and IM7; left) and HECA452 (right).

[0026] **Figure 2. Parallel plate flow chamber assay of FTVI-modified and unmodified wild type MSC adherence to TNF-α treated human umbilical vein endothelial cells (HUVEC).** FTVI modification markedly improved MSC adhesion to HUVEC at 0.5 dynes/cm^2, with binding evident at shear stress in excess of 20 dynes/cm^2 Treatment of HUVEC with anti-E-selectin (anti-CD62E) function-blocking mAb reduced rolling adhesive interactions of FTVI-modified MSCs to levels similar to that of unmodified MSCs. Similarly, removal of sLex determinants by sialidase treatment of FTVI-modified MSCs reduced adhesion to levels equivalent to unmodified MSCs.

[0027] **Figures 3(A)-3(C). Effects of intravenous administration of unmodified and FTVI-modified MSC on hyperglycemia in new onset diabetic NOD mice.** *(A)* Hyperglycemic NOD injected with PBS (untreated control) showed no reversal of hyperglycemia (glucose levels above 600 mg glucose/dL). As compared to infusion of unmodified MSCs *(B),* infusion of FTVI-modified MSCs *(C)* resulted in a marked increase in number of mice with reversion to normoglycemia and in the durability of diabetes reversal. Arrows in X-axis denote days of MSC infusion.

[0028] **Figures 4(A)-4(H). Immunofluorescence staining of islets to assess expression of E-selectin and localization of MSCs in the pancreas.** *Figures 4(A) and 4(B):* Pancreatic islets of *(A)* diabetic-resistant BALB/c mice and *(B)* NOD mice were stained for expression of insulin (green) and E-selectin (red). Islets are demarcated by dashed line. Compared to BALB/c (*A*), NOD mice *(B)* show diminished insulin production due to insulitis. In *Figures 4(C)-4(G)*, cryostat sections of pancreas from MSC-treated NOD mice stained with DAPI (blue). *Figures 4(C) and 4(D)*: Staining of sequential sections of NOD pancreas demonstrates co-localization of endothelial marker CD31 (*C*) and E-selectin (*D*), confirming the presence of E-selectin on peri-islet endothelial cells. *Figure 4(E)*: Co-staining of NOD islet with DAPI (blue), T-cell marker CD3 (green) and insulin (red) *(E)* reveals characteristic T-cell infiltration at the margins of the islet. *Figures 4(F) and 4(G)*: Immunofluorescence images of a cryostat section stained for infiltrating MSC (visualized with FITC-conjugated anti-sLex mAb HECA452; green), islet *(F)* (visualized by APC-conjugated anti-insulin mAb; red) and E-selectin-expressing microvessel *(G)* (visualized with PE-conjugated anti-E-selectin mAb; red). Staining identifies HECA452+ MSCs in zones of insulitis, in proximity to E-selectin-expressing microvessels in the peri-islet area and clustered within areas of lymphocytic infiltrates (*i.e.*, cells which characteristically express L-selectin). *Figure 4(H)*: Pancreatic infiltration of intravenously administered MSCs into NOD and BALB/c hosts. Accumulation of FTVI-modified MSCs into pancreata of NOD mice is 3-fold higher compared to that of unmodified MSCs ($p<0.01$), whereas no difference in pancreatic infiltrates is observed in BALB/c host (n=3 mice per group; minimum 30 fields counted per group at 60X magnification).

[0029] **Figures 5(A)-5(B). FTVI-modification of MSCs does not affect cell survival or immunosuppressive capacity.** *(A)* Similar levels of hGH was detected in the serum of NOD mice at different time points following injection with pHRST-hGH-

transduced FTVI-modified or unmodified MSCs. *(B)* FTVI-modified and unmodified MSCs equally suppress proliferation of NOD $CD4^+$ T cells stimulated with CD3/CD28, indicating that glycan-modification does not increase MSC capacity to suppress lymphocyte proliferation.

[0030] **Figures 6(A)-6(D). Lack of CD44 expression abrogates the anti-diabetic effect of systemically administered FTVI-modified MSC.** *(A)* As compared to unmodified wild type MSC (*Figure 3B*), administration of CD44-deficient MSCs shows modest anti-diabetic effect. Only 1 NOD mouse (out of 7) receiving unmodified CD44 KO MSCs showed reversal of hyperglycemia which was transient (diabetes recurrence at ~day 30), and 6 out of 7 diabetic NOD mice remained hyperglycemic. *(B)* As compared to results in mice receiving FTVI-modified wild-type MSCs *(Figure 3C),* administration of FTVI-modified CD44 KO MSCs conferred minimal anti-diabetic effects, with only 1 out of 6 NOD mice showing reversal of hyperglycemia. *(C)* Accumulation of MSCs in NOD pancreata was no different in mice receiving FTVI-modified CD44 KO MSCs (white bar) compared to mice receiving unmodified CD44 KO MSCs (black bar) ($p<0.01$), and in each case was similar to that receiving unmodified MSC (*Figure 4H*). MSC infiltrates were quantified at X60 magnification. *(D)* Both FTVI-modified and unmodified CD44 KO MSCs possess immunosuppressive capacity, similarly dampening T cell proliferation in the CD3/CD28 T cell stimulation assay.

[0031] **Figure 7. Characterization of mouse bone marrow derived MSCs.** MSCs were differentiated into osteocytes, adipocytes and chondrocytes (X200 magnification).

[0032] **Figure 8. FTVI modification of $CD44^{-/-}$ MSCs results in similar increase in HECA452 reactivity as wild-type (WT) MSCs.** Unmodified MSC (dotted line) show no reactivity with HECA452, while FTVI-modified MSC (solid line) and FTVI-modified CD44 KO MSC (filled grey) showed similar levels of staining with HECA452, indicating that exofucosylation creates sialofucosylated epitopes on alternative (*i.e.*, non-CD44) scaffolds in CD44 KO MSC.

[0033] **Figures 9(A)-9(B): Neural stem cells (NSCs) lack E-selectin ligands but express a number of other cell surface adhesion molecules.** *(A)* Flow cytometric analysis of HECA452, KM93, CSLEX-1, E-selectin ligand (binding to E-selectin-Ig chimera (E-Ig)), and P-selectin ligand (P-selectin-Ig chimera (P-Ig) binding)

expression on NSCs. The corresponding isotype controls showed overlapping signals for each antibody surveyed *i.e.* RatIgM (for HECA-452; MFI: 1.9), Mouse IgM (for KM93 and CSLEX-1; MFI: 2.7), and human IgG_1 (for E-Ig and P-Ig; MFI: 3.5). A histogram plot representing a typical E-Ig binding profile illustrates that over 99% of the cells consistently express E-Ig binding following glycosyltransferase-programmed stereosubstitution (GPS) via cell surface treatment with Fucosyltransferase VI. *(B)* Flow cytometric analysis of CD43 (S7 and 1B11), PSGL-1 (2PH1, 4RA10), CD44 (IM7, KM114), NCAM, PSA, CD49d, CD49e, CD29, LPAM-1, CD11a, CD18, and CXCR4. Dotted line is isotype control, black line is specific antibody. All results displayed are representative of n = 5 flow cytometry experiments performed on NSCs.

[0034] **Figures 10(A)-10(C): GPS treatment (*i.e.*, α(1,3)-exofucosylation via FTVI treatment) of NSCs generates sialofucosylations mainly on glycoproteins, some of which are glycophosphatidylinositol (GPI)-linked**. *(A)* Flow cytometric analysis of HECA452, CD15, KM93, CSLEX1, E-Ig and P-Ig reactivity on BT-NSCs (black bars) and GPS-NSCs (grey bars). The corresponding isotype controls for each antibody surveyed were: Rat IgM (for HECA-452; MFI: 1.8), Mouse IgM (for CD15, KM93, and CSLEX-1; MFI: 1.9), and human IgG_1 (for E-Ig and P-Ig; MFI: 3.2). Results displayed are representative of five separate experiments. *(B)* Flow cytometric analysis of HECA452 reactivity of GPS-NSCs undigested (black bars) or digested with bromelain (grey bars) prior to GPS treatment. Values are means ± SEM. (n = 3 for each group). *(C)* Flow cytometric analysis of HECA452 reactivity of GPS-NSCs undigested (black bars) or digested with phospholipase C (PI-PLC) to cleave GPI anchors (grey bars). Values are means ± SEM. (n = 3 for each group).

[0035] **Figures 11(A)-11(C): GPS treatment of NSCs creates transient E-selectin ligands at 100, 120 and 140-kDa which correspond to HCELL and N-CAM-E**. *(A)* CD44 was immunoprecipitated (with IM7 and KM114 mAb to CD44) from equivalent amounts of cell lysates from GPS-treated (GPS) or buffer-treated (BT) NSCs. Western blot analysis was performed on immunoprecipitates of NSCs and supernatants (SN) from the immunoprecipitates, which were electrophoresed and blotted with HECA452, E-Ig, and CD44. *(B)* N-CAM was immunoprecipitated (with N-CAM 13) from equivalent amounts of cell lysates from GPS-treated (+) or buffer-treated (-) NSCs lysates that had been either treated with PNGaseF (+) or not (-) Immunoprecipitates were then electrophoresed and blotted with E-Ig and N-CAM. Staining with E-Ig was

performed in the presence of Ca^{2+}. (C) NSCs were treated with GPS on Day 0 and cultured for another 3 days in normal growth media. Every 24 hours aliquots of cells were removed and assayed for E-selectin ligand activity by flow cytometry. See also **Figs. 11(A)-11(C), 12(A)-12(B), and 13(A)-13(F)**.

[0036] **Figure 12(A)-12(B): GPS-NSCs have markedly enhanced shear-resistant adhesive interactions with endothelial E-selectin under defined shear stress conditions**. *(A)* BT-NSCs or GPS-NSCs were perfused over IL-1β and TNF-α stimulated HUVECs at 1.0 dyn/cm^2. NSC accumulation was then determined at shear stresses of 1, 2, 4, 8, 16, 25 and 32 dyn/cm^2. GPS-NSCs show rolling adhesive interactions on HUVECs at a shear stress of up to 32 dyn/cm^2. To control for the specificity of binding of GPS-NSCs, EDTA was added to the assay buffer (EDTA group), or stimulated HUVECs were pretreated with a function blocking mAb to E-selectin (anti–E-Sel group) before use in adhesion assays. Values are means ± SEM. (n = 4 for each group). $P \leq 0.001$ for comparisons of GPS-NSCs to all other groups at all shear stress levels. *(B)* Adhesion bar graph for blot rolling assay (rolling cells/mm^2) for CHO-E cells perfused over SDS-PAGE immunoblots of HECA-452–reactive membrane glycoproteins of NSCs at 0.6 dyne/cm^2. Immunoprecipitates of CD44/HCELL and panNCAM from both BT-NSC (black bars) and GPS-NSCs (grey bars) were resolved by SDS-PAGE and blotted for HECA-452 prior to performing the assay. To control for the specificity of CHO-E binding to membrane glycoproteins, EDTA was added to the buffer containing the CHO-E cells before use in adhesion assays; no cells bound under this condition (data not shown). Results presented are representative of multiple runs (n = 4) on HECA-452 blots of multiple (n = 3) membrane preparations of NSCs.

[0037] **Figure 13(A)-13(F): GPS-NSCs exhibit improved homing in an EAE model in vivo**. *(A-D)* GPS-NSCs migrate to the CNS parenchyma more efficiently than BT-NSCs. 1 x 10^6 GPS-NSCs or BT-NSCs were labeled with PKH26 dye and were injected intravenously to MOG-induced EAE mice on day 9 and day 13 post-immunization (PI). *(A)* Analysis of the forebrain of EAE mice (on day 17 PI) that either received BT-NSCs or GPS-NSCs, revealed that lower numbers of PKH26-positive cells are seen in animals injected with BT-NSCs compared to GPS-NSCs. The yellow arrowheads indicate NSCs and the white arrows indicate infiltrates. The white dashed line indicates Meningeal borders. **Figs. 20(A)-20(B)** shows further analysis of these sections to confirm that the NSCs (PKH26; red) are located outside of Flk-1 vessels

(green) and that they are SOX-2 positive (green). *(B)* Lumbar-sacral spinal cords (insert) were harvested on day 17 PI. At day 17 PI, more GPS-NSCs migrated out of the blood vessels into the spinal cord parenchyma than BT-NSCs. Blood vessels were visualized by Flk-1 (VEGFR2; green) staining. The edge of the spinal cord parenchyma is highlighted with a white dotted line. NSCs labeled with PKH26 dye are shown in red and nuclei counterstained with TO-PRO-3 are blue. WM, white matter. V, blood vessels. *(C)* The insets show a 3-dimentional view of the migrated NSCs indicated by the arrows in A. *(D)* Quantification of numbers of BT-NSCs and GPS-NSCs per 200x migrating per spinal cord area at day 17 PI were determined. A significant increase in the numbers of migrating GPS-NSCs over BT-NSCs was evident, * $p < 0.05$. *(E)* Quantification of biodistribution of NSCs. GPS-NSCs (grey bars) or BT-NSCs (black bars) were labeled with CFDA-SE and injected intravenously into MOG-treated C57BL6 mice on day 9 and day 13 post-immunization. Brain, lymph nodes, spleen, liver and lung were analyzed 16 hours after the last injection to determine the percentage of CFSE positive cells present within a defined gate representing NSCs. Non-EAE mice that received GPS-NSCs or BT-NSCs were used to standardize the signals observed in each tissue tested. Mice that did not receive cells were used to determine the background signal. Error bars represent the standard error of the mean. Data are representative of 2 separate experiments where 10 mice per group were tested. *(F)* In vivo confocal demonstration that exofucosylated NSCs are found in close contact with CD4 T cells in the spleen in vivo (NSCs are labeled pink and CD4 T cells in green); this co-localization of NSCs and lymphocytes would be engendered by NSC HCELL binding to lymphocyte L-selectin.

[0038] **Figures 14(A)-14(I): GPS-NSCs contribute to significant amelioration of EAE symptoms through enhanced neuroprotection**. *(A)* The EAE clinical scores in C57BL/6 mice immunized with MOG 35-55 on Day 0 and subsequently injected with $1x10^6$ GFP-labeled buffer-treated NSCs (BT-NSC; filled red triangles; n = 30), GPS-treated NSCs (GPS-NSC; filled green circles; n = 30) or sham-treated mice (No NSC; filled black circles; n = 30) on day 9 and day 13 after immunization were determined. Mice that received GPS-NSCs displayed a pronounced clinical improvement compared with sham-treated mice (p=0.0001) and mice injected i.v. with BT-NSCs (p=0.006). *(B)* The cumulative burden of disease was assessed by performing a linear regression analysis comparing the slope of the curves in *(A)*. These

data highlight GPS-NSCs (green dotted line) significantly improve the clinical scores above that of BT-NSCs (red line). Mice receiving either GPS-NSCs or BT-NSCs displayed significantly improved clinical scores compared to mice that did not receive NSCs (No NSCs; black line). These data also suggest that BT-HSPCs (blue line) and GPS-HSPCs (purple line) worsen disease (see Fig. 21(C)). *(C)* Neuropathology at day 30 PI of the brain from EAE mice injected with NSCs was analyzed by staining with anti-CD11b mAb (green) and the nuclear label To-Pro3 (blue). GPS-NSC injection leads to significantly less injury per brain section as measured by numeration of CD11b macrophage/microglia from 20 different sections of 3 different spinal cords. Bar graphs depict the numeration of CD11b macrophage/microglia in spinal cord sections per high power field (HPF) from EAE mice that received No NSC, BT-NSC, or GPS-NSC and also the numeration of infiltrates per HPF were calculated based on To-Pro3 staining. White boxes correspond to higher magnification images. Yellow arrowheads correspond to activated microglia and white arrows correspond to infiltrates in the meninges (m). Note that the microglia in the No NSC samples are more activated than those found in the BT-NSC and GPS-NSC samples. Also the size of the infiltrates in the meninges is larger in the No NSC samples than in the BT-NSC samples. Scale bars, 100μm for top panels. Scale bars, 50 μm for bottom panels. *(D)* Neuropathology of the brain from EAE mice injected with NSCs was analyzed by staining 20 different sections of 3 separate brains from mice that either received no NSCs (EAE No NSCs), BT-NSC (EAE BT-NSCs), or GPS-NSC (EAE GPS-NSCs) with CD4 (to measure T cell infiltrations), CNPase (to quantify remyelinating cells), Olig-2 (to measure oligodendroglial differentiation) or SOX-9 (to measure multipotency of neural precursors) (green) and To-Pro3 (blue). GPS-NSC injection lead to significantly less T cell infiltrations, enhanced remyelination, higher numbers of oligodendroglia, and preservation of progenitor numbers. *(E)* Bar graphs depict the numeration of CD4 T, CNPase, Olig2, SOX-9 cells in brain sections per high power field (HPF) from EAE mice that received No NSC, BT-NSC, or GPS-NSC (as outlined in *(D)*), indicating that animals that received GPS-NSCs display enhanced neuroprotection of progenitor cells. *(F-I)* GPS-NSC and BT-NSC injection leads to increased axonal regeneration and axonal protection compared to No NSC control as measured by increased GAP-43 (green; $p<0.001$) staining *(F)* and by decreased staining with the monoclonal antibody SMI32 (red; $p<0.001$) *(H)* as assessed by quantitative confocal imaging of GAP43 pixel

intensity in more than 500 individual measurements. To-Pro3 staining dye was used to detect cell nuclei (blue). *(G,H)* Based on quantitative confocal imaging of more than 500 individual measurements of pixel intensity (Imitola, J., Cote, D., et al. 2011) graphical representation of GAP-43 *(G)* and SMI32 *(I)* was determined; note that SMI32 patterns *(I)* demonstrated axonal ovoids in animals with EAE but reduction in animals injected with NSCs *(I)* and SMI32 pixel intensity showed a gradual correction of axonal integrity in animals with GPS-NSCs. There is a reduction of axonal ovoid and axonal fragments compared to controls and BT-NSCs as depicted in the cartoon below *(I)* Scale bar, 100μm except for SMI32 staining where scale bar, 50μm.

[0039] **Figure 15: Inflammatory cytokine treatment does not induce selectin ligand expression on mouse NSCs**. NSCs were stimulated with 10 ng/ml of TNFα, 10 ng/ml IL-1β, 10 ng/ml IFNγ independently or in combination (all at 10 ng/ml). At 24 h, NSCs were harvested and analyzed by flow cytometry for E-selectin binding. Controls included untreated NSCs and Kg1a cells (positive control for E-selectin binding).

[0040] **Figure 16(A)-16(C): HCELL is the only E-selectin ligand created by GPS treatment in one (exemplary) human NSC line (CC-2599)**. *(A)* Flow cytometric analysis of CD15, CSLEX-1, HECA452, and E-selectin ligand (E-Ig binding), expression on human NSCs either treated with GPS (grey bars) or buffer-treated (BT; black bars). The mean fluorescence intensity is shown for each antibody measured. *(B)* Flow cytometric analysis of CD44, PSGL-1, NCAM, and CD43. *(C)* Western blot analysis of E-Ig reactivity of NSC lysates from mouse and human sources. CD44 and NCAM were immunoprecipitated from equivalent amounts of cell lysates from GPS-treated (+) or buffer-treated (-) mouse NSCs or human NSCs. Immunoprecipitates were then electrophoresed and blotted with E-Ig. Staining of GPS-treated NSCs with E-Ig was performed in the presence of Ca^{2+}.

[0041] **Figure 17: GPS treatment does not affect of the ability of NSCs to form neurospheres**. 200 viable GFP^{+} NSCs were plated per well in a 96 well plate immediately following GPS treatment for 7 days. The resulting neurospheres were counted and neurosphere frequency was calculated as the number of neurospheres divided by number of cells plated. There was no statistically significant difference in the density of neurospheres or the number of neurospheres formed between BT-NSCs and

GPS-NSCs. A representative image of the GFP^+ neurospheres used for these experiments is shown where the red fluorescence indicates Nestin expression and the green fluorescence indicates GFP. Note that BT- and GPS-GFP^+ NSCs were both able to form neurospheres equally. This figure is related to **Figs. 11(A)-11(C)**.

[0042] **Figure 18(A)-18(D): GPS treatment does not affect the differentiation capacity of NSCs into neurons *(A)*, astrocytes *(B, D)* or oligodendrocyte precursors *(C, D)***. $1x10^5$ BT- and GPS-NSC were cultured in the appropriate differentiation media and after 120 hours, numbers of $MAP\text{-}2^+$ neurons *(A)*, $GFAP^+$ astrocytes *(B,D)*, and $NG2^+$ oligodendrocytes *(C)* were counted per 20x vision field. There was no statistically significant difference between the ability of BT and GPS-NSCs to differentiate along these three lineages (p=0.1). *(D)* $GFAP^+$ astrocytes and $NG2^+$ oligodendrocytes are shown in red and To-Pro3 is used to stain nuclei of neural stem cells treated in vitro with control buffer (BT) or FTVI (GPS). Scale bar, 50μm. These figures are related to **Figs. 11(A)-11(C)**.

[0043] **Figures 19(A)-19(C): GPS treatment does not affect the immunomodulation function of NSCs in vitro**. Direct *in vitro* suppressive effects of NSCs on lymph node cells (LNCs) were measured by coculturing irradiated NSCs with LNCs isolated from naïve C57BL/6 mice. Both irradiated BT-NSC and GPS-NSC suppressed ^{3}H-thymidine incorporation *(A)* into LNCs in response to concanavalin A (ConA) in a dose-dependent manner and also suppress inflammatory cytokine production *(B)* as measured by ELISA to an equal degree; the ratios correspond to numbers of NSCs to numbers of LNCs (1:4, 1:2, 1:1, 2:1, and 4:1). Note that by increasing the ratio of NSC:LNC compared to LNC alone (first bar), the amount of ^{3}H-thymidine incorporation decreased significantly (p=0.0005). Also note that there was no statistically significant difference between the ability of BT-NSC and GPS-NSC to inhibit proliferation (p=0.2). *(C)* $1x10^6$ BT-NSCs or GPS-NSCs were treated for 24 h with or without inflammatory cytokines (10ng/mL IFN-γ and 15ng/mL TNF- α) and with or without E-Ig (5ng/mL) prior to RNA extraction and cDNA synthesis. Real-time RT-PCR revealed the fold change in gene expression (related to BT or GPS-NSCs alone) calculated using $2^{-\Delta\Delta CT}$ method and the relative expression of LIF mRNA was assayed relative to GAPDH housekeeping gene. Values are means ± SEM (n = 4 experiments). These figures are related to **Figs. 11(A)-11(C)**.

[0044] **Figures 20(A)-20(B): GPS-NSCs migrate to the CNS parenchyma more efficiently than BT-NSCs**. 1 x 10^6 GPS-NSCs or BT-NSCs were labeled with PKH26 dye and were injected intravenously to MOG-induced EAE mice on day 9 and day 13 post-immunization (PI). Brains were harvested on day 17 PI and snap-frozen before 20μm sections were prepared and stained with antibodies: anti-Flk-1 (VEGFR2; green) or anti-SOX-2 (red) to reveal blood vessels and the position of the NSCs respectively. *(A)* BT NSCs are primarily localized with FLK-1^+ endothelial cells whereas GPS-NSCs are found in the parenchyma as they have crossed the endothelium. These NSCs express sox-2, a marker for neural stem cells *(B)*. These figures are related to **Figs. 13(A)-13(F)**.

[0045] **Figures 21(A)-21(C): GPS treatment generates robust E-selectin ligand activity on mouse HSPCs but this does not translate into amelioration of EAE**. *(A)* Flow cytometric analysis of E-Ig reactivity on BT- and GPS- mouse hematopoietic stem/progenitor cells (HSPC; $Lineage^{neg}C\text{-}kit^{pos}$). The mean fluorescence intensity is shown above each histogram for the $hIgG_1$ isotype control, E-Ig reactivity on BT-HSPC and E-Ig reactivity on GPS-HSPC. These cells were used for in vivo EAE experiments as a control. *(B)* The EAE clinical scores in C57BL/6 mice immunized with MOG 35-55 on Day 0 and subsequently injected with 1x10^6 buffer-treated HSPC (BT-HSPC; open blue diamonds; n = 30), GPS-treated HSPC (GPS-HSPC; open purple diamonds; n = 30) or sham-treated mice (No NSC; filled black circles; n = 30) on day 9 and day 13 after immunization were determined. No significant improvement in the clinical scores was evident in mice receiving either BT- or GPS-HSPC. *(C)* The cumulative burden of disease was assessed by performing a linear regression analysis comparing the slope of the curves in *(B)*. These figures are related to **Figs. 14(A)-14(H)**.

[0046] **Figure 22: No evidence of GFP signal was observed at Day 30 PI from either BT- or GPS- NSCs injected into EAE mice.** C57BL/6 Mice were sacrificed 30 days after immunization with MOG 35-55 (on day 0) and subsequent injection with 1x10^6 buffer-treated NSCs (BT-NSC), GPS-treated NSCs (GPS-NSC) or sham-treated mice (No NSC) on day 9 and day 13 and immunohistochemistry for GFP^+ cells was performed. At 30 days PI GFP^+ cells were not identified in sections of relevant study groups, even when antibodies against GFP (red) were compared to endogenous GFP signal (EGFP; green). Cultured NSCs before injection were used as

a positive control (NSC-Pre Tx) and showed a robust GFP signal. Scale bar, 50μm. This figure is related to **Figs. 14(A)-14(H)**.

[0047] **Figure 23:** Model of neurorestoration afforded by GPS treatment of native-NSCs surface proteins, CD44 and NCAM, creating novel step 1 effectors, HCELL and NCAM-E, that efficiently bind endothelial E-selectin in EAE mice. As a result of injury, endothelial cells and glia may produce injury signals (*e.g.*, SDF-1α and E-selectin) that direct GPS-NSCs toward the injury-induced niches in higher number than in control mice. Despite the increased number of NSCs recruited, instead of cell replacement, NSCs offer local modulation of immunity and endogenous regeneration of CNS by increasing the number of SOX-9+/Olig-2+ oligodendroglia. This subsequently leads to more mature oligodendroglial cells (CNPase+), less axonal loss, and more axonal regeneration. Niche molecules secreted by primitive neural stem cells may promote some of the effects provided by their increased colonization.

[0048] **Figure 24. FTVII treatment of adipose-derived MSCs from lean subjects and from obese subjects results in creation of sLex structures on the cell surface.** Bar graphs display flow cytometry results (MFI, mean ± SEM) of sLex expression (HECA452-reactivity) following α(1,3)-exofucosylation of adipose-derived MSCs (n=4 samples of MSCs derived from lean and obese subjects).

[0049] **Figure 25. Western blot analysis of cell lysates of adipose-derived MSCs.** Lysates of MSCs from bone marrow (BM-MSCs) and from lean and obese adipose tissue (A-MSCs) were exofucosylated with FTVII (+) or treated with buffer alone (-), then were subjected to SDS-PAGE and blotted with HECA452 or with anti-human CD44 mAb. Lysates of the hematopoietic cell line KG1a (control) and the fibroblast cell line PIF were co-electrophoresed and blotted. KG1a cells express HCELL (HECA-452-reactive CD44 at mw of ~80 kDa). As shown in the figure, FTVII treatment results in enforced expression of HCELL (HECA452-reactive CD44) on PIF cells and on MSCs derived from adipose tissue of lean and obese subjects (upper panel); staining for expression of CD44 in each lysate is shown below the HECA452 blot. Expression of HCELL (~80 kDa HECA452-reactive band) is abrogated following sialidase treatment of cells (lower panel), consistent with sialidase sensitivity of HCELL expression (*i.e.*, elimination of sLex). Note that HCELL may appear as a doublet at ~80kDa (see profile of FTVII-treated A-MSC in lower panel), which reflects variable glycosylation of the core

CD44 glycoprotein that does not affect E-selectin or L-selectin ligand activity of the HCELL molecule.

[0050] **Figure 26. Results of parallel plate flow chamber studies of MSCs perfused over TNF-stimulated human umbilical vein endothelial cells (HUVEC).** As measured under defined hemodynamic shear conditions (dynes/cm^2, shown on x-axis), α(1,3)-exofucosylation (FTVII treatment) of A-MSCs derived from lean subjects (LA-MSCs) and from obese subjects (OA-MSCs) confers potent E-selectin binding on E-selectin displayed on TNF-stimulated HUVEC; the E-selectin ligand activity of FTVII-treated (HCELL+) adipose-derived MSCs is equivalent to that of FTVII-treated (HCELL+) bone marrow-derived MSCs (BM-MSCs; lower panel). Untreated MSCs have no significant binding interactions on HUVEC at any of the shear stress levels.

[0051] **Figures 27(A)-27(C). Analysis of effects of α(1,3)-exofucosylation on E-selectin binding of various leukocytes.** *(A)* Parallel plate flow chamber analysis of tethering and rolling interactions of native peripheral blood leukocytes with endothelial E-selectin (expressed on TNF-stimulated human umbilical vein endothelial cells (HUVEC)). Monocytes, CD4 T cells, CD8 T cells and B cells were freshly isolated and then perfused over TNF-simtulated HUVECs (*i.e.*, TNF induces HUVEC to express E-selectin) at a flow rate ranging from 0.5-16 dynes/cm^2 and cell rolling was observed and recorded for video analysis. Monocytes and CD4 T cells show rolling adhesive interactions on TNF-activated HUVEC at a shear stress up to 16 dynes/cm^2, whereas CD8 and B cells show minimal adhesive shear-resistant adhesive interactions. Note that monocytes cell rolling was 3-fold greater than CD4 T cells. Values are means ± SEM for a minimum of 3 independent experiments. *(B)* Representative flow cytometric histograms of staining with E-selectin-Ig chimera ("E-Ig", a probe for E-selectin ligands (from R&D Systems)) (left) and mAb HECA452 (right) of monocytes, CD4 and CD8 T cells, and B cells. Filled curves represent isotype control (or, for E-Ig staining, E-selectin binding in the absence of input Ca^{2+}) and open curves show specific reactivity (for E-Ig, binding in presence of input Ca^{2+}). *(C)* Untreated (solid black line) or protease (bromelain) treated (dotted line) cells were stained with HECA452 mAb and analyzed by flow cytometry. Protease (bromelain) treatment (dotted line) markedly decreases HECA452 staining of monocytes, CD4 T cells, CD8 T cells, and B cells, showing that glycoproteins are the principal carriers of sLex determinants on these cells.

[0052] **Figures 28(A)-28(C). Functional E-selectin ligand expression on monocytes and lymphocytes is increased by α(1,3)-exofucosylation treatment.** *(A)* Human monocytes were FTVII-treated or buffer treated (mock) and subjected to immunoprecipitation with E-selectin-Ig chimera ("E-Ig", a probe for E-selectin ligands (from R&D Systems)) followed by SDS-PAGE and blotted with HECA-452, anti-CD43, anti-CD44 and anti-PSGL-1 mAbs, respectively. Note increased immunoprecipitation with E-Ig following FTVII treatment of monocytes, prominently on CD44 (*i.e.*, creation of HCELL) and CD43 (creation of CD43-E-selectin ligand ("CD43-E"). *(B)* Western blot analysis of sequential immunoprecipitation of PSGL-1, CD43 and CD44 from lysates of CD4 T cells, CD8 T cells and B cells, followed by staining with E-Ig. *(C)* Parallel plate flow chamber analysis of tethering and rolling interactions of untreated ("unt") and FTVII-treated (*i.e.*, α(1,3)-exofucosylation; "FT7") of monocytes, CD4 and CD8T cells, and B cells on TNF-a activated HUVEC (which express E-selectin). FTVII treatment ("FT7") markedly augments E-selectin-mediated adherence of flowing cells to HUVEC under all shear stress levels tested.

[0053] **Figures 29(A)-29(C): CD44 expressed by monocyte-derived dendritic cells (*i.e.*, cultured following selection of monocytes by CD14 expression (CD14-S mo-DCs)) binds E-selectin.** ***(A)*** Representative flow cytometric histograms of HECA-452 mAb and E-Ig reactivity staining of mo-DCs. Grey lines represent isotype control or, for E-Ig, staining in the absence of Ca2+, whereas dotted black line represents PA-S mo-DCs and solid black lines are CD14-S mo-DCs. *(B)* Western blot analysis of E-selectin staining of CD14-S and PA-S mo-DC lysates. Whole cell lysates equivalent to 2×10^6 mo-DCs were resolved on a SDS-PAGE gel and immunoblotted. MW markers are along Y-axis (in kDa). Mo-DCs cultured on CD14 beads (CD14-S Mo-DCs) have higher HCELL expression (~80 kDa band) than those cultured on plastic (PA Mo-DCs). *(C)* Western blot analysis of CD44 immunoprecipitated from cell lysates of CD14-S and PA-S mo-DCs. CD44 immunoprecipitates (CD44 IP) were then blotted and stained with E-Ig chimera or anti-CD44 mAb. MW markers are as noted along Y-axis (in kDa). Note pronounced HCELL expression on CD14-S mo-DCs.

[0054] **Figures 30(A)-30(E). α(1,3)-Exofucosylation of human mo-DCs enforces higher E-selectin ligand activity and endothelial E-selectin expression promotes transendothelial migration (TEM) of exofucosylated DCs.** *(A)* Western

blot analysis comparing E-selectin binding (E-Ig) of lysates of 2 × 10^6 of KG1a cells and of mo-DCs. *(B)* Western blot analysis of E-Ig reactivity of mo-DC that were buffer treated (-) or FTVI-treated (+). FTVI treatment induces E-Ig binding at ~80 kDa, indicating enforced expression of HCELL. *(C)* Transendothelial migration (TEM) assay of buffer treated (BT) and FTVI-treated mo-DCs under shear flow conditions (2 dynes/cm^2) on TNF-α-stimulated HUVEC. The TEM value is the fold-increase in migration of cells on TNF-α-stimulated HUVEC compared with cells transmigrated on untreated HUVECs (which was minimal). TEM was analyzed under different conditions: HUVEC preincubated with function blocking anti-E-selectin mAb clone 68-5H11, mo-DC pre-incubated with function blocking anti-VLA-4 mAb HP2/1 or isotype mAb, and mo-DC treated with sialidase or pertussis toxin (PTX). As shown, exofucosylated DCs have higher TEM than untreated cells; TEM is abrogated by function-blocking antibodies to E-selectin and VLA-4, and by sialidase or PTX treatment of cells. *(D)* Analysis of the number of tethering and rolling mo-DCs (per cm^2 of endothelial surface area) and number of firmly adhering cells (per cm^2 of endothelial surface area) in the TEM assay. Note higher tethering/rolling interactions with exofucosylated DCs. *(E)* Average rolling velocity of buffer treated (BT) and FTVI-treated mo-DCs in the TEM assay. The cell number was counted at 2.0 dyne/cm2 shear stress (*i.e.*, perfused over TNF-α-stimulated HUVEC, at 2.0 dyne/cm2 shear stress). Values are means ± SD (n= 3). Statistical significant differences ($p<0.05$) are indicated by brackets and asterisks. The slower rolling of FTVI-treated DCs is indicative of higher efficiency of DC binding interactions to endothelial E-selectin under hemodynamic shear conditions.

[0055] **Figure 31. Expression of FoxP3 on regulatory T cells generated by ex vivo expansion of human CD4+ cells in presence of antibodies to CD3 and CD28, and IL-2 supplementation.** Dual color flow cytometry histograms showing expression of CD4 and FoxP3 on cultured CD4+/CD127-low T cells; high FoxP3 staining indicates that all culture-expanded cells are Tregs.

[0056] **Figure 32. Flow cytometry profiles of sLex expression (HECA452 staining) of buffer treated (light grey fill) and exofucosylated (FTVII-treated; black fill) Tregs.** Note that buffer-treated Tregs have no sLex expression (*i.e.*, profile is similar to isotype control staining (dashed line)), whereas FTVII-treated cells display high levels of sLex.

[0057] **Figure 33. Western blot analysis of E-Ig staining of FTVII-treated and buffer treated (BT) Tregs compared with the human myeloid leukemia cell line KG1a.** Buffer treated T regs have no E-selectin ligands. α(1,3)-Exofucosylation induces expression of several glycoprotein E-selectin ligands on Tregs, including expression of HCELL (~80 kDa band). KG1a cells natively express HCELL.

[0058] **Figure 34. Titration of regulatory T cell dosing in xenogenic graft versus host disease (GVHD).** Xenogeneic GVHD-associated weight loss (measured at day 50 post-injection of PBMCs) is abrogated by intravenous injection of 1.5×10^5 T regs but not by injection of similar dose of buffer treated Tregs (weight values are mean of N=3 mice). However, injection of 5×10^5 Tregs prevents GVHD weight loss regardless of α(1,3)-exofucosylation of administered Treg cells (mean of N=3 mice). Thus, α(1,3)-exofucosylation of Tregs increases potency in immunomodulation of GVHD by 3-fold.

EMBODIMENTS

[0059] The present disclosure is directed to methods of treating a disease, disorder, or medical condition manifesting as inflamed and/or damaged tissue or cancer in a subject. The methods involve the administration, to a subject in need of tissue repair/regeneration or cancer treatment, of a cell population that expresses an E-selectin ligand and/or an L-selectin ligand at levels that exceeds the level of E-selectin and/or L-selectin binding of a native population of the cells. The composition of cells is disposed in a pharmaceutically-acceptable solution, suspension, carrier or vehicle for administration to a subject or for storage (*e.g.*, cryopreserved) prior to administration to a subject.

[0060] Administration of cell populations described herein for therapeutic indications can be achieved in a variety of ways, in each case as clinically warranted/indicated, using a variety of anatomic access devices, a variety of administration devices, and a variety of anatomic approaches, with or without support of anatomic imaging modalities (*e.g.*, radiologic, MRI, ultrasound, etc.) or mapping technologies (*e.g.*, epiphysiologic mapping procedures, electromyographic procedures, electrodiagnostic procedures, etc.). Cells can be administered systemically, via either peripheral vascular access (*e.g.*, intravenous placement, peripheral venous access devices, etc.) or central vascular access (*e.g.*, central venous catheter/devices, arterial

access devices/approaches, *etc.*). Cells can be delivered intravascularly into anatomic feeder vessels of an intended tissue site using catheter-based approaches or other vascular access devices (*e.g.*, cardiac catheterization, *etc.*) that will deliver a vascular bolus of cells to the intended site. Cells can be introduced into the spinal canal and/or intraventricularly (*i.e.*, intrathecally, into the subarachnoid space to distribute within cerebrospinal fluid and/or within the ventricles). Cells can be administered directly into body cavities or anatomic compartments by either catheter-based approaches or direct injection (*e.g.*, intraperitoneal, intrapleural, intrapericardial, intravesicularly (*e.g.*, into bladder, into gall bladder, into bone marrow, into biliary system (including biliary duct and pancreatic duct network), intraurethrally, via renal pelvis/ intraureteral approaches, intravaginally, etc.)). Cells can be introduced by direct local tissue injection, using either intravascular approaches (*e.g.*, endomyocardial injection), or percutaneous approaches, or via surgical exposure/approaches to the tissue, or via laparoscopic/thoracoscopic/endoscopic/colonoscopic approaches, or directly into anatomically accessible tissue sites and/or guided by imaging techniques (*e.g.*, intra-articular, into spinal discs and other cartilage, into bones, into muscles, into skin, into connective tissues, and into relevant tissues/organs such as central nervous system, peripheral nervous system, heart, liver, kidneys, spleen, etc.). Cells can also be placed directly onto relevant tissue surfaces/sites (*e.g.*, placement onto tissue directly, onto ulcers, onto burn surfaces, onto serosal or mucosal surfaces, onto epicardium, *etc.*). Cells can also administered into tissue or structural support devices (*e.g.*, tissue scaffold devices and/or embedded within scaffolds placed into tissues, etc.), and/or administered in gels, and/or administered together with enhancing agents (*e.g.*, admixed with supportive cells, cytokines, growth factors, resolvins, anti-inflammatory agents, *etc.*).

[0061] The cell population is administered to the subject during a period of (*i.e.*, coincident with) induced expression (and, preferably, coincident with the onset of induced expression) of E-selectin on vascular endothelium within one or more tissues of the subject. The enforced expression of E-selectin ligands on the surface of administered cells will aid in revascularization, in host defense (*e.g.*, against infection or cancer) and/or in tissue repair/regeneration and/or mediate immunomodulatory processes that will dampen inflammation and/or prevent inflammation. The expression

of ligands for E-selectin guides delivery of intravascularly administered cells to sites of inflammation by mediating binding of blood-borne cells to vascular E-selectin expressed on endothelial cells at sites of inflammation. Moreover, whether cells are administered systemically, intravascularly, into the spinal canal and/or intraventricularly (*i.e.*, intrathecally, into the subarachnoid space to distribute within cerebrospinal fluid), directly into body cavities or compartments, by direct local tissue injection, or by placement onto relevant tissue surfaces/sites, the expression of ligands for E-selectin and/or L-selectin on administered cells promotes lodgement of cells within the affected tissue milieu, in apposition to cells bearing E-selectin (*i.e.*, endothelial cells) and/or L-selectin (*i.e.*, leukocytes), respectively, within the target site. Thus, the spatial distribution and localization of administered cells within the target tissue is modulated by the expression of E-selectin and/or L-selectin ligands on administered cells.

[0062] Particularly, the colonization of a desired cell type at a site of inflammation occurs as a result of the enforced expression of cell surface E-selectin ligands on the administered cells, such that the administered cells have augmented binding to E-selectin, thereby promoting the systemic delivery of the desired cells and/or the lodgement of cells when injected directly into the affected site. For example, the enforced expression of E-selectin ligands (*e.g.*, HCELL, CD43-E, CLA/PSLG-1, ESL-1, NCAM-E, etc.) is advantageously capable of anchoring directly injected cells within E-selectin-expressing vessels at sites of inflammation, tissue injury, or cancer. Thus, the present methods augment efficiency in the delivery of relevant cells at or to a site of inflammation, tissue injury, or cancer, including, for example, the capacity to deliver tissue-reparative stem cells, to deliver immunomodulatory cells (*e.g.*, mesenchymal stem cells, T-regulatory cells, NK-cells, dendritic cells, etc.), and the capacity to deliver immune effector cells to combat the inciting inflammatory process or cancer (*e.g.*, in the case of infection or malignancy, delivery of pathogen-specific immune effector T cells cells or cancer-specific cytotoxic T cells or NK cells, respectively); such immunologic cells (regulatory T-cells, NK cells, cytotoxic T-cells, dendritic cells, etc.) may be antigen-pulsed, tumor cell pulsed,virus pulsed, and other means to create antigen specificity. Similarly, the enforced expression of L-selectin ligands (*e.g.*, HCELL, PSGL-1, etc.) is advantageously capable of anchoring directly injected cells within L-selectin-expressing cells infiltrating sites of inflammation, tissue injury, or cancer.

[0063] The enhanced expression of an E-selectin ligand on the cell surface will drive vascular homing of cells to any site where E-selectin is expressed. In various embodiments, the cell population expresses Hematopoietic Cell E-/L-selectin Ligand (HCELL) and/or Neural Cell Adhesion Molecule E-selectin Ligand (NCAM-E). Other E-selectin ligands that may be expressed by cells include, for example, CD43-E, ESL-1, PSGL-1 and the CLA glycoform of PSGL-1. For example, since CD44 is a ubiquitously expressed cell membrane protein and is displayed on stem/progenitor cell populations of both "adult" and embryonic types, the capacity to modify glycosylation of this protein by *ex vivo* glycan engineering to create the HCELL (CD44 glycoform) phenotype will drive migration of injected (*e.g.*, intravascularly) (adoptively transferred) cells *in vivo* to marrow or to any tissue/organ site where E-selectin is expressed. Thus, the modified cells can be used in therapeutic settings to achieve targeted cell migration in a variety of physiologic and pathologic processes, including, for example, bone diseases, immune diseases, infectious diseases, and cancer therapeutics, to name just a few conditions.

[0064] It has also been discovered that the disease, disorder, or medical condition having associated inflammation can be treated using the instant methods even in the absence of differentiation of the cell population in the subject. That is, there are trophic effects of administered cells at the site of inflammation without persistent engraftment and/or repopulation of the administered cells, irrespective of the type of tissue involved. These trophic effects include release of cytokines/growth factors that promote revascularization (*e.g.*, VEGF), that promote tissue repair (*e.g.*, TGF-β), that are immunomodulatory (*e.g.*, IL-10), that stimulate growth/proliferation of tissue-resident progenitors (*e.g.*, SCF, LIF, etc) and many other tissue-reparative processes (*e.g.*, mitochondria delivery to cells). In addition, administered cells (*e.g.*, Tregs, MSCs, dendritic cells, etc.) may have potent immunomodulatory properties, including direct suppression of activated lymphocytes (*e.g.*, via expression of PDL-1).

[0065] As exemplified by **Figures 4(A) – 4(H)**, and as described in greater detail in the examples, cell populations modified to have enhanced E-selectin and/or L-selectin binding activity relative to native populations of the cell type have increased capacity to home, infiltrate and colonize tissue in the vicinity of E-selectin-expressing vasculature. Additionally, in one embodiment such cells lodge into sites of inflammation via binding to E-selectin on endothelial cells or lodge into sites of infiltrating leukocytes within the inflammatory site (*e.g.*, see **Figures 4(F) and 4(G)**). Further, beneficial

effects (*e.g.*, amelioration of the disease) may be realized by parenteral delivery or by direct injection into the site of inflammation or the site of infiltrating leukocytes even in the absence of differentiation of the cells (or long-term engraftment) after administered cell lodgement within the site (*i.e.*, transient engraftment may be sufficient to deliver intended biologic effect(s)).

[0066] In certain embodiments, the methods for glycan-engineering described in U.S. Pat. No. 8,084,236 for the enforcement of HCELL expression in viable cell populations, discussed in detail below, can induce higher amounts of expression of E-selectin ligands on such cells to promote their vascular delivery to sites of inflammation, and can induce higher amounts of E-selectin ligands and L-selectin ligands that can promote lodgement of cells within the affected tissue milieu.

[0067] Treatment of the subject refers to reducing or eliminating in an individual a clinical symptom of a disease, disorder, or medical condition having associated inflammation or delaying or preventing in an individual the onset of a clinical symptom of such disease, disorder, or medical condition. For example, treating can mean reducing a symptom of a condition characterized by an acute and/or chronic inflammation by, *e.g.*, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90% at least 95%, or at least 100%. The actual symptoms associated with acute and chronic inflammation are well known and can be determined by a person of ordinary skill in the art by taking into account factors, including, without limitation, the location of the acute and/or chronic inflammation, the cause of the acute and/or chronic inflammation, the severity of the acute and/or chronic inflammation, and/or the tissue or organ affected by the acute and/or chronic inflammation. Those of skill in the art will know the appropriate symptoms or indicators associated with a specific type of acute and/or chronic inflammation and will know how to determine if an individual is a candidate for treatment as disclosed herein.

[0068] Subjects that may be treated in accordance with the methods described herein includes any mammal (*e.g.*, a human), such as a mammal that can be susceptible to a disease. Examples include, for instance, a human, a non-human primate, a cow, a horse, a pig, a sheep, a goat, a dog, a cat, or a rodent such as a

mouse, a rat, a hamster, or a guinea pig. A subject can be a subject diagnosed with a disease, disorder, or medical condition having associated inflammation or otherwise known to have a disease, disorder, or medical condition the result or symptom of which is acute and/or chronic inflammation. In some embodiments, a subject can be diagnosed as, or known to be, at risk of developing a disease, disorder, or medical condition having associated inflammation. In certain embodiments, a subject can be selected for treatment on the basis of a known disease, disorder, or medical condition in the subject the result or symptom of which is an acute and/or chronic inflammation. In some embodiments, a subject can be selected for treatment on the basis of a suspected disease, disorder, or medical condition having associated inflammation in the subject. In some embodiments, the disease, disorder, or medical condition can be diagnosed by detecting a mutation or other abnormality in a biological sample (*e.g.*, urine, sputum, whole blood, serum, stool, etc., or any combination thereof. Accordingly, a modified cell population can be administered to a subject based, at least in part, on the fact that a mutation, symptom, or other abnormality is detected in at least one sample (*e.g.*, biopsy sample or any other biological sample) obtained from the subject. In some embodiments, diabetes, multiple sclerosis, or cancer (for example) cannot have been detected or located in the subject, but the presence of a mutation, symptom, or other abnormality associated with diabetes, multiple sclerosis, or cancer in at least one biological sample can be sufficient to administer modified cells to the subject in accordance with the methods described herein. In some embodiments, the composition can be administered to prevent the development of a disease, disorder, or medical condition such as diabetes, multiple sclerosis, or cancer. However, in some embodiments, the presence of an existing disease, disorder, or medical condition can be suspected, but not yet identified, and a composition of the disclosure can be administered to prevent further growth or development of the disease, disorder, or medical condition. The administered cells can be derived from autologous sources (*i.e.*, derived from the host itself), syngeneic sources (derived from an identical twin), or allogeneic sources (derived from non-genetically identical donor).

TIMING OF ADMINISTRATION

[0069] As noted above, the cell population is administered to the subject coincident with induced expression (and preferably coincident with the onset of induced

expression) of E-selectin by endothelial cells of the subject, or just prior to expected increased E-selectin expression within an affected tissue (*i.e.*, as prophylaxis prior to or coincident with delivery of E-selectin-inducing inflammatory stimulus (*e.g.*, radiation therapy)) or in anticipation of a reaction to a pathogen or disease process with subsequent upregulation of vascular E-selectin expression (*e.g.*, following exposure to or endogenous reactivation of an infectious agent, prior to overt flare of autoimmune disease, prior to elaboration of graft-versus-host disease (GVHD),etc.)). Accordingly, the treatment methods described herein may be implemented, for example, when the subject (*e.g.*, a human patient) presents to his/her physician or healthcare provider with one or more symptoms of inflammation or anticipated inflammatory flare. For a subject with diabetes, for example, such initial symptoms may include polyuria, nocturia, weight loss/gain, and/or lack of satiety, at which time (and prior to the onset of diabetic ketoacidosis) the subject may be administered the modified cells in accordance with the disclosure. For a subject with multiple sclerosis, for example, such initial symptoms may include transient sensory perturbations (*e.g.*, numbness, tingling) and/or fasciculations (twitching) and/or muscular weakness episodes.

[0070] In general, the temporal aspects of the administration of the modified cells may depend for example, on the particular modified cells, or the nature of the disease, disorder, or medical condition (and associated inflammation, tissue injury, or cancer) being treated. Other considerations may include the severity of the disease, disorder, or medical condition; activity of the specific cells or cell fragments and modifications employed; the specific composition employed; the age, body weight, general health, sex and diet of the subject; the time of administration, route of administration, and rate of excretion; the duration of the treatment; drugs used in combination or coincidental with the treatment regime; and like factors.

[0071] Accordingly, administration of the modified cells can occur as a single event or over a time course of treatment. For example, the modified cells can be administered hourly (*e.g.*, every hour, every two hours, every three hours, every four hours, every five hours, every six hours, and so on), daily, weekly, bi-weekly, bi-monthly or monthly. For treatment of acute conditions, for example, the time course of treatment may be at least several hours, days, or weeks. Certain conditions could extend treatment from several days to several weeks, months, or years. For example, treatment could extend over one week, two weeks, or three weeks, or longer (*e.g.*, one

to several months). For more chronic conditions, treatment could extend from several weeks to several months, a year or more, or the lifetime of the patient in need of such treatment. Alternatively, the compounds and agents can be administered hourly, daily, weekly, bi-weekly, bi-monthly, or monthly, for a period of several weeks, months, years, or over the lifetime of the patient as a prophylactic or as a therapeutic measure.

[0072] It will also be understood that the frequency of doses may be increased or decreased based upon the nature and severity of the disease, disorder, or medical condition (and associated inflammation) and/or the stage of the disease, disorder, or medical condition in the subject. For example, depending on these and other factors, less frequent administration may be desirable at the beginning of the treatment and more frequent administration may be desirable at or near end-stage, or *vice versa*. The various targets and symptoms may also be tracked (*e.g.*, insulin levels in a diabetic subject) and the administration and dose amount may be tailored accordingly.

[0073] If desired, the cells can be divided into multiple doses for purposes of administration; consequently, single dose compositions may contain such amounts or submultiples thereof to make up the dose.

INDICATIONS

[0074] The present disclosure is directed to the treatment of a disease, disorder, or medical condition wherein E-selectin is expressed in endothelial beds of the affected tissue(s) and/or L-selectin-expressing leukocytes have infiltrated/accumulated in the affected tissue(s). As discussed above, E-selectin and L-selectin each bind to sialylated, fucosylated carbohydrates, and enforced expression of these sialofucosylated glycan structures on the cell surface serves to program binding to these selectins. Accordingly, the disclosure describes methods to enhance homing to target tissue(s) by augmenting the expression of E-selectin ligands on administered cells; additionally, in describing methods to enhance expression of potent E-selectin and L-selectin ligands (such as HCELL) on administered cells to promote adherence to E-selectin on vascular endothelial cells and/or of L-selectin on tissue-infiltrating leukocytes within affected tissue(s), the disclosure provides a means to augment colonization/lodgement of the cells within relevant tissue microenvironments where

biologic effects are intended. In general, the methods described herein have utility in improving the outcome of any cell-based therapeutic approach, be it in immunotherapy applications (*e.g.*, administration of culture-expanded antigen-specific T cells and/or culture expanded NK cells for cancer or infectious disease applications, administration of culture-expanded chimeric antigen receptor (CAR) T cells, administration of antigen-pulsed dendritic cells, etc.), immunomodulatory/immunosuppressive therapeutic applications (*e.g.*, administration of culture-expanded regulatory T cells (Tregs), administration of antigen-pulsed dendritic cells, administration of mesenchymal stem cells, administration of culture-expanded NKT cells, etc.), or tissue repair/regenerative medicine applications (*e.g.*, use of stem and/or progenitor cells or other tissue-reparative cells for tissue regeneration/restoration; use of culture-expanded stem cells and/or culture-expanded progenitor cells for tissue regeneration/restoration). Within utility in regenerative medicine applications, it is understood that administered cells may themselves contribute to regenerate the target tissue by way of long-term engraftment (with attendant proliferation/differentiation) yielding tissue-specific cells (*e.g.*, such as in transplantation of hematopoietic stem cells for blood cell production) and/or may deliver a tissue restorative/reparative effect without long-term engraftment or differentiation into tissue-resident cells (*e.g.*, via delivery of trophic effects that stimulate resident stem/progenitors to repair the injured tissue(s) and/or by dampening inflammatory processes that promote injury and impede repair). All applications for all indications described herein can be used alone or in combination with enhancing agents (*e.g.*, growth factors, tissue scaffolds, etc.).

[0075] Any and all diseases, disorders, or medical conditions having associated inflammation (*e.g.*, acute and/or chronic), tissue injury/damage or neoplastic conditions may be treated in accordance with the methods described herein, including, but not limited to those initiated by direct tissue injury (*e.g.*, burns, trauma, bone fracture, bone deformities, decubitus ulcers, etc.), ischemic/vascular events (*e.g.*, myocardial infarct, stroke, shock, hemorrhage, coagulopathy, etc.), infections (*e.g.*, cellulitis, pneumonia, meningitis, cystitis, sepsis, SIRS, etc.), neoplasia (*e.g.*, breast cancer, lung cancer, prostate cancer, renal cell cancer, lymphoma, leukemia, etc.), immunologic/autoimmune conditions (*e.g.*, acute or chronic GVHD, multiple sclerosis, diabetes, inflammatory bowel disease (*e.g.*, Crohn's disease, ulcerative colitis), rheumatoid arthritis, psoriasis, etc.), degenerative diseases (*e.g.*, osteoporosis,

osteoarthritis, spinal disc degeneration, Alzheimer's disease, atherosclerosis, etc.), congenital/genetic diseases (*e.g.*, epidermolysis bullosa, osteogenesis imperfecta, muscular dystrophies, lysosomal storage diseases, Huntington's disease, etc.), adverse drug effects (*e.g.*, chemotherapy-induced tissue/organ toxicity, radiotherapy toxicity, drug-induced hepatitis, drug-induced cardiac injury, etc.), toxic injuries (*e.g.*, radiation exposure(s), chemical exposure(s), alcoholic hepatitis, alcoholic pancreatitis, alcoholic cardiomyopathy, cocaine cardiomyopathy, etc.), metabolic derangements (*e.g.*, uremic pericarditis, metabolic acidosis, etc.), iatrogenic conditions (*e.g.*, radiation-induced tissue injury, surgery-related complications, etc.), and/or idiopathic processes (*e.g.*, amyotrophic lateral sclerosis, Parsonnage-Turner Syndrome, etc.).

[0076] Other general and specific diseases, disorders, or medical condition that may be treated in accordance with the methods described herein include, but are not limited to:

[0077] Acute Leukemias, *e.g.*, Acute Biphenotypic Leukemia, Acute Lymphocytic Leukemia (ALL), Acute Myelogenous Leukemia (AML), and Acute Undifferentiated Leukemia;

[0078] Myelodysplastic Syndromes, *e.g.*, Amyloidosis Chronic Myelomonocytic Leukemia (CMML), Refractory Anemia (RA), Refractory Anemia with Excess Blasts (RAEB), Refractory Anemia with Excess Blasts in Transformation (RAEB-T), and Refractory Anemia with Ringed Sideroblasts (RARS);

[0079] Myeloproliferative Disorders, *e.g.*, Acute Myelofibrosis, Agnogenic Myeloid Metaplasia (Myelofibrosis), Essential Thrombocythemia, chronic myelogenous leukemia, and Polycythemia Vera;

[0080] Phagocyte Disorders, *e.g.*, Chediak-Higashi Syndrome, Chronic Granulomatous Disease, Leukocyte adhesion deficiencies, myeloperoxidase deficiency, Neutrophil Actin Deficiency, and Reticular Dysgenesis;

[0081] Lysosomal Storage Diseases, *e.g.*, Adrenoleukodystrophy, Alpha Mannosidosis, Gaucher's Disease, Hunter's Syndrome (MPS-II), Hurler's Syndrome (MPS-IH), Krabbe Disease, Maroteaux-Lamy Syndrome (MPS-VI), Metachromatic Leukodystrophy, Morquio Syndrome (MPS-IV), Mucolipidosis II (I-cell Disease), Mucopolysaccharidoses (MPS), Niemann-Pick Disease, Sanfilippo Syndrome (MPS-III),

Scheie Syndrome (MPS-IS), Sly Syndrome, Beta-Glucuronidase Deficiency (MPS-VII), and Wolman Disease;

[0082] Inherited Erythrocyte Abnormalities, *e.g.*, Beta Thalassemia, Blackfan-Diamond Anemia, Pure Red Cell Aplasia, and Sickle Cell Disease;

[0083] Inherited Platelet Abnormalities, *e.g.*, Amegakaryocytosis/Congenital Thrombocytopenia, Gray platelet syndrome;

[0084] Solid organ malignancies, *e.g.*, Brain Tumors, Ewing Sarcoma, Neuroblastoma, Ovarian Cancer, Renal Cell Carcinoma, Lung Cancers, Breast cancers, Gastric cancers, Esophageal cancers, Skin cancers, Oral cancers, Endocrine cancers, Liver cancers, Biliary system cancers, Pancreatic cancer, Prostate Cancer, and Testicular Cancer;

[0085] Other Applications, *e.g.*, Bone Marrow Transplants, Heart Disease (myocardial infarction), Liver Disease, Muscular Dystrophy, Alzheimer's Disease, Parkinson's Disease, Spinal Cord Injury, Spinal disc disease/degeneration, Bone disease, Bone fracture, Stroke, Peripheral Vascular Disease, Head trauma, Bullous diseases, Mitochondrial diseases, Ex vivo and In vivo expanded stem and progenitor cell populations, In vitro fertilization application and enhancement, Hematopoietic Rescue Situations (Intense Chemo/Radiation), Stem cells and progenitor cells derived from various tissues sources, Application in humans and animals, and Limb regeneration, reconstructive surgical procedures/indications, alone or in combination with enhancing agents;

[0086] Chronic Leukemias, *e.g.*, Chronic Lymphocytic Leukemia (CLL), Chronic Myelogenous Leukemia (CML), Juvenile Chronic Myelogenous Leukemia (JCML), and Juvenile Myelomonocytic Leukemia (JMML),

[0087] Stem Cell Disorders, *e.g.*, Aplastic Anemia (Severe), Congenital Cytopenia, Dyskeratosis Congenita, Fanconi Anemia, and Paroxysmal Nocturnal Hemoglobinuria (PNH);

[0088] Lymphoproliferative Disorders, *e.g.*, Hodgkin's Disease, Non-Hodgkin's Lymphomas, and Prolymphocytic Leukemia;

[0089] Histiocytic Disorders, *e.g.*, Familial Erythrophagocytic Lymphohistiocytosis, Hemophagocytosis, Hemophagocytic Lymphohistiocytosis, Histiocytosis-X, and Langerhans' Cell Histiocytosis;

[0090] Congenital (Inherited) Immune System Disorders, *e.g.*, Absence of T and B Cells, Absence of T Cells, Normal B Cell SCID, Ataxia-Telangiectasia, Bare Lymphocyte Syndrome, Common Variable Immunodeficiency, DiGeorge Syndrome, Kostmann Syndrome, Leukocyte Adhesion Deficiency, Omenn's Syndrome, Severe Combined Immunodeficiency (SCID), SCID with Adenosine Deaminase Deficiency, Wiskott-Aldrich Syndrome, and X-Linked Lymphoproliferative Disorder;

[0091] Other Inherited Disorders, *e.g.*, Cartilage-Hair Hypoplasia, Ceroid Lipofuscinosis, Congenital Erythropoietic Porphyria, Familial Mediterranean Fever, Glanzmann Thrombasthenia, Lesch-Nyhan Syndrome, Osteopetrosis, and Sandhoff Disease;

[0092] Plasma Cell Disorders, *e.g.*, Multiple Myeloma, Plasma Cell Leukemia, and Waldenstrom's Macroglobulinemia; and

[0093] Autoimmune Diseases, *e.g.*, Multiple Sclerosis, Rheumatoid Arthritis, Systemic Lupus Erythematosus, Scleroderma, Ankylosing spondylitis, Diabetes Mellitus, and Inflammatory Bowel Diseases.

[0094] Articular and skeletal diseases/conditions, *e.g.*, disc degeneration, synovial disease, cartilage degeneration, cartilage trauma, cartilage tears, arthritis, bone fractures, bone deformities, bone reconstruction, osteogenesis imperfecta, congenital bone diseases/conditions, genetic bone diseases/conditions, osteoporosis. Osteopetrosis, hypophosphatasia, metabolic bone disease, etc.

[0095] Skin/soft tissue diseases and conditions such as bullous diseases, psoriasis, eczema, epidermolysis bullosa, ulcerative skin conditions, soft tissue deformities (including post-surgical skin and soft tissue deformities), plastic surgery/reconstructive surgery indications, etc.

[0096] In general, associated inflammation symptoms that include, without limitation, fever, pain, edema, hyperemia, erythema, bruising, tenderness, stiffness, swollenness, chills, respiratory distress, hypotension, hypertension, stuffy nose, stuffy head, breathing problems, fluid retention, blood clots, loss of appetite, weight loss,

polyuria, nocturia, anuria, dyspnea, dyspnea on exertion, muscle weakness, sensory changes, increased heart rate, decreased heart rate, arrythmias, polydipsia, formation of granulomas, fibrinous, pus, non-viscous serous fluid, or ulcers. The actual symptoms associated with an acute and/or chronic inflammation are well known and can be determined by a person of ordinary skill in the art by taking into account factors, including, without limitation, the location of the inflammation, the cause of the inflammation, the severity of the inflammation, the tissue or organ affected, and the associated disorder.

[0097] Specific patterns of acute and/or chronic inflammation are seen during particular situations that arise in the body, such as when inflammation occurs on an epithelial surface, or pyogenic bacteria are involved. For example, granulomatous inflammation is an inflammation resulting from the formation of granulomas arising from a limited but diverse number of diseases, include, without limitation, tuberculosis, leprosy, sarcoidosis, and syphilis. Purulent inflammation is an inflammation resulting in large amount of pus, which consists of neutrophils, dead cells, and fluid. Infection by pyogenic bacteria such as staphylococci is characteristic of this kind of inflammation. Serous inflammation is an inflammation resulting from copious effusion of non-viscous serous fluid, commonly produced by mesothelial cells of serous membranes, but may be derived from blood plasma. Skin blisters exemplify this pattern of inflammation. Ulcerative inflammation is an inflammation resulting from the necrotic loss of tissue from the epithelial surface, exposing lower layers and forming an ulcer.

[0098] An acute and/or chronic inflammation symptom can be associated with a large, unrelated group of disorders which underlay a variety of diseases and disorders. The immune system is often involved with acute and/or chronic inflammatory disorders, demonstrated in both allergic reactions, arthritic conditions, and some myopathies, with many immune system disorders resulting in abnormal inflammation. Non-immune diseases with etiological origins in acute and/or chronic inflammatory processes include cancer, atherosclerosis, and ischaemic heart disease. Non-limiting examples of disorders exhibiting acute and/or chronic inflammation as a symptom include, without limitation, acne, acid reflux/heartburn, age related macular degeneration (AMD), allergy, allergic rhinitis, Alzheimer's disease, amyotrophic lateral sclerosis, anemia, appendicitis, arteritis, arthritis, asthma, atherosclerosis, autoimmune disorders, balanitis, blepharitis, bronchiolitis, bronchitis, a bullous pemphigoid, burn,

bursitis, cancer, cardiac arrest, carditis, celiac disease, cellulitis, cervicitis, cholangitis, cholecystitis, chorioamnionitis, chronic obstructive pulmonary disease (COPD) (and/or acute exacerbations thereof), cirrhosis, colitis, congestive heart failure, conjunctivitis, drug-induced tissue injury (*e.g.*, cyclophosphamide-induced cystitis), cystic fibrosis, cystitis, common cold, dacryoadenitis, decubitus ulcers, dementia, dermatitis, dermatomyositis, diabetes, diabetic neuropathy, diabetic retinopathy, diabetic nephropathy, diabetic ulcer, digestive system disease, eczema, emphysema, encephalitis, endocarditis, endocrinopathies, endometritis, enteritis, enterocolitis, epicondylitis, epididymitis, fasciitis, fibromyalgia, fibrosis, fibrositis, gastritis, gastroenteritis, gingivitis, glomerulonephritis, glossitis, heart disease, heart valve dysfunction, hepatitis, hidradenitis suppurativa, Huntington's disease, hyperlipidemic pancreatitis, hypertension, ileitis, infection, inflammatory bowel disease, inflammatory cardiomegaly, inflammatory neuropathy, insulin resistance, interstitial cystitis, interstitial nephritis, iritis, ischemia, ischemic heart disease, keratitis, keratoconjunctivitis, laryngitis, lupus nephritis, macular degeneration, mastitis, mastoiditis, meningitis, metabolic syndrome (syndrome X), a migraine, mucositis, multiple sclerosis, myelitis, myocarditis, myositis, nephritis, neuronitis, non-alcoholic steatohepatitis, obesity, omphalitis, oophoritis, orchitis, osteochondritis, osteopenia, osteomyelitis, osteoporosis, osteitis, otitis, pancreatitis, Parkinson's disease, parotitis, pelvic inflammatory disease, pemphigus vularis, pericarditis, peritonitis, pharyngitis, phlebitis, pleuritis, pneumonitis, polycystic nephritis, proctitis, prostatitis, psoriasis, pulpitis, pyelonephritis, pylephlebitis, radiation-induced injury, renal failure, reperfusion injury, retinitis, rheumatic fever, rhinitis, salpingitis, sarcoidosis, sialadenitis, sinusitis, spastic colon, stasis dermatitis, stenosis, stomatitis, stroke, surgical complication, synovitis, tendonitis, tendinosis, tenosynovitis, thrombophlebitis, thyroiditis, tonsillitis, trauma, traumatic brain injury, transplant rejection, trigonitis, tuberculosis, tumor, ulcers, urethritis, ursitis, uveitis, vaginitis, vasculitis, and vulvitis.

[0099] General categories of diseases, disorders, and trauma that can result in or otherwise cause acute and/or chronic inflammation include, but are not limited to genetic diseases, neoplasias, direct tissue injury, autoimmune diseases, infectious diseases, vascular diseases/complications (*e.g.*,ischemia/reperfusion injury), iatrogenic causes (*e.g.* drug adverse effects, radiation injury, etc.), and allergic manifestations.

[0100] In one embodiment, an acute and/or chronic inflammation comprises a tissue inflammation. In general, tissue inflammation is an acute and/or chronic inflammation that is confined to a particular tissue or organ. Thus, for example, a tissue inflammation may comprise a skin inflammation, a muscle inflammation, a tendon inflammation, a ligament inflammation, a bone inflammation, a cartilage/joint inflammation, a lung inflammation, a heart inflammation, a liver inflammation, a gall bladder inflammation, a pancreatic inflammation, a kidney inflammation, a bladder inflammation, an gum inflammation, an esophageal inflammation, a stomach inflammation, an intestinal inflammation, an anal inflammation, a rectal inflammation, a vessel inflammation, a vaginal inflammation, a uterine inflammation, a testicular inflammation, a penile inflammation, a vulvar inflammation, a neuron inflammation, an oral inflammation, an ocular inflammation, an aural inflammation, a brain inflammation, a ventricular/meningial inflammation and/or inflammation involving central or peripheral nervous system cells/elements.

[0101] In another embodiment, an acute and/or chronic inflammation comprises a systemic inflammation. Although the processes involved are similar if not identical to tissue inflammation, systemic inflammation is not confined to a particular tissue but rather involves multiple sites within the body, involving the epithelium, endothelium, nervous tissues, serosal surfaces and organ systems. When it is due to infection, the term sepsis can be used, with bacteremia being applied specifically for bacterial sepsis and viremia specifically to viral sepsis. Vasodilation and organ dysfunction are serious problems associated with widespread infection that may lead to septic shock and death.

[0102] In another embodiment, an acute and/or chronic inflammation is induced by an arthritis. Arthritis includes a group of conditions involving damage to the joints of the body due to the inflammation of the synovium including, for example, osteoarthritis, rheumatoid arthritis, juvenile idiopathic arthritis, spondyloarthropathies like ankylosing spondylitis, reactive arthritis (Reiter's syndrome), psoriatic arthritis, enteropathic arthritis associated with inflammatory bowel disease, Whipple disease and Behcet disease, septic arthritis, gout (also commonly referred to as gouty arthritis, crystal synovitis, metabolic arthritis), pseudogout (calcium pyrophosphate deposition disease), and Still's disease. Arthritis can affect a single joint (monoarthritis), two to four

joints (oligoarthritis) or five or more joints (polyarthritis) and can be either an auto-immune disease or a non-autoimmune disease.

[0103] In another embodiment, an acute and/or chronic inflammation is induced by an autoimmune disorder. Autoimmune diseases can be broadly divided into systemic and organ-specific autoimmune disorders, depending on the principal clinico-pathologic features of each disease. Systemic autoimmune diseases include, for example, systemic lupus erythematosus (SLE), Sjogren's syndrome, Scleroderma, rheumatoid arthritis and polymyositis. Local autoimmune diseases may be endocrinologic (Diabetes Mellitus Type 1, Hashimoto's thyroiditis, Addison's disease, etc.), dermatologic (pemphigus vulgaris), hematologic (autoimmune haemolytic anemia), neural (multiple sclerosis) or can involve virtually any circumscribed mass of body tissue. Types of autoimmune disorders include, without limitation, acute disseminated encephalomyelitis (ADEM), Addison's disease, an allergy or sensitivity, amyotrophic lateral sclerosis (ALS), anti-phospholipid antibody syndrome (APS), arthritis, autoimmune hemolytic anemia, autoimmune hepatitis, autoimmune inner ear disease, autoimmune pancreatitis, bullous pemphigoid, celiac disease, Chagas disease, chronic obstructive pulmonary disease (COPD) (including acute exacerbations thereof), diabetes mellitus type 1 (IDDM), endometriosis, fibromyalgia, Goodpasture's syndrome, Graves' disease, Guillain-Barre syndrome (GBS), Hashimoto's thyroiditis, hidradenitis suppurativa, idiopathic thrombocytopenic purpura, inflammatory bowel disease (IBD), interstitial cystitis, lupus (including discoid lupus erythematosus, drug-induced lupus erythematosus. lupus nephritis, neonatal lupus, subacute cutaneous lupus erythematosus and systemic lupus erythematosus), morphea, multiple sclerosis (MS), myasthenia gravis, myopathies, narcolepsy, neuromyotonia, pemphigus vulgaris, pernicious anaemia, primary biliary cirrhosis, recurrent disseminated encephalomyelitis (multiphasic disseminated encephalomyelitis), rheumatic fever, schizophrenia, scleroderma, Sjogren's syndrome, tenosynovitis, vasculitis, and vitiligo. In one particular embodiment, the acute and/or chronic inflammation results from or is otherwise caused by diabetes in the subject. In another particular embodiment, the acute and/or chronic inflammation results from or is otherwise caused by multiple sclerosis in the subject.

[0104] In another embodiment, an acute and/or chronic inflammation is induced by a myopathy. In general, myopathies are caused when the immune system

inappropriately attacks components of the muscle, leading to inflammation in the muscle. A myopathy includes, for example, an inflammatory myopathy and an auto-immune myopathy. Myopathies include, for example, dermatomyositis, inclusion body myositis, and polymyositis.

[0105] In another embodiment, an acute and/or chronic inflammation is induced by a vasculitis. Vasculitis is a varied group of disorders featuring inflammation of a vessel wall including lymphatic vessels and blood vessels like veins (phlebitis), arteries (arteritis) and capillaries due to leukocyte migration and resultant damage. The inflammation may affect any size blood vessel, anywhere in the body. It may affect either arteries and/or veins. The inflammation may be focal, meaning that it affects a single location within a vessel, or it may be widespread, with areas of inflammation scattered throughout a particular organ or tissue, or even affecting more than one organ system in the body. Vasculitis include, without limitation, Buerger's disease (thromboangiitis obliterans), cerebral vasculitis (central nervous system vasculitis), ANCA-associated vasculitis, Churg-Strauss arteritis, cryoglobulinemia, essential cryoglobulinemic vasculitis, giant cell (temporal) arteritis, Golfer's vasculitis, Henoch-Schonlein purpura, hypersensitivity vasculitis (allergic vasculitis), Kawasaki disease, microscopic polyarteritis/polyangiitis, polyarteritis nodosa, polymyalgia rheumatica (PMR), rheumatoid vasculitis, Takayasu arteritis, Wegener's granulomatosis, and vasculitis secondary to connective tissue disorders like systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), relapsing polychondritis, Behcet's disease, or other connective tissue disorders, vasculitis secondary to viral infection.

[0106] In another embodiment, an acute and/or chronic inflammation is induced by a skin disorder. Skin disorders include, for example, an acne, including acne vulgaris, a bullous phemigoid, a dermatitis, including atopic dermatitis and acute and/or chronic actinic dermatitis, an eczema-like atopic eczema, contact eczema, xerotic eczema, seborrhoeic dermatitis, dyshidrosis, discoid eczema, venous eczema, dermatitis, dermatitis herpetiformis, neurodermatitis, and autoeczematization, and stasis dermatitis, diabetic skin complications, hidradenitis suppurativa, lichen planus, psoriasis including plaqure psoriasis, nail psoriasis, guttate psoriasis, scalp psoriasis, inverse psoriasis, pustular psoriasis, erythrodermis psoriasis, and psoriatic arthritis, rosacea and scleroderma including morphea, ulcers.

[0107] In another embodiment, an acute and/or chronic inflammation is induced by a gastrointestinal disorder. A gastrointestinal disorder includes, for example, irritable bowel disease (IBD), an inflammatory bowel disease including Crohn's disease and an ulcerative colitis like ulcerative proctitis, left-sided colitis, pancolitis, and fulminant colitis.

[0108] In another embodiment, an acute and/or chronic inflammation is induced by a cardiovascular disease. When LDL cholesterol becomes embedded in arterial walls, it can invoke an immune response. Acute and/or chronic inflammation eventually can damage the arteries, which can cause them to burst. In general, cardiovascular disease is any of a number of specific diseases that affect the heart itself and/or the blood vessel system, especially the veins and arteries leading to and from the heart. There are over 60 types of cardiovascular disorders including, for example, a hypertension, endocarditis, myocarditis, heart valve dysfunction, congestive heart failure, myocardial infarction, a diabetic cardiac conditions, blood vessel inflammation like arteritis, phlebitis, vasculitis; arterial occlusive disease like arteriosclerosis and stenosis, inflammatory cardiomegaly, a peripheral arterial disease; an aneurysm; an embolism; a dissection; a pseudoaneurysm; a vascular malformation; a vascular nevus; a thrombosis; a thrombophlebitis; a varicose veins; a stroke. Symptoms of a cardiovascular disorder affecting the heart include, without limitation, chest pain or chest discomfort (angina), pain in one or both arms, the left shoulder, neck, jaw, or back, shortness of breath, dizziness, faster heartbeats, nausea, abnormal heartbeats, feeling fatigued. Symptoms of a cardiovascular disorder affecting the brain include, without limitation, sudden numbness or weakness of the face, arm, or leg, especially on one side of the body, sudden confusion or trouble speaking or understanding speech, sudden trouble seeing in one or both eyes, sudden dizziness, difficulty walking, or loss of balance or coordination, sudden severe headache with no known cause. Symptoms of a cardiovascular disorder affecting the legs, pelvis and/or arm include, without limitation, claudication, which is a pain, ache, or cramp in the muscles, and cold or numb feeling in the feet or toes, especially at night.

[0109] In another embodiment, an acute and/or chronic inflammation is induced by a cancer. In general, inflammation orchestrates the microenvironment around tumors, contributing to proliferation, survival and migration. For example, fibrinous inflammation results from a large increase in vascular permeability which

allows fibrin to pass through the blood vessels. If an appropriate procoagulative stimulus is present, such as cancer cells, a fibrinous exudate is deposited. This is commonly seen in serous cavities, where the conversion of fibrinous exudate into a scar can occur between serous membranes, limiting their function. In another example, a cancer is an inflammatory cancer like a NF-κB-driven inflammatory cancer.

[0110] In another embodiment, an acute and/or chronic inflammation is a pharmacologically-induced inflammation. Certain drugs or exogenic chemical compounds, including deficiencies in key vitamins and minerals, are known to effect inflammation. For example, Vitamin A deficiency causes an increase in an inflammatory response, Vitamin C deficiency causes connective tissue disease, and Vitamin D deficiency leads to osteoporosis. Certain pharmacologic agents can induce inflammatory complications, *e.g.*, drug-induced hepatitis. Certain illicit drugs such as cocaine and ecstasy may exert some of their detrimental effects by activating transcription factors intimately involved with inflammation (*e.g.*, NF-κB). Radiation therapy can induce pulmonary toxicity, burns, myocarditis, mucositis, and other tissue injuries depending on site of exposure and dose.

[0111] In another embodiment, an acute and/or chronic inflammation is induced by an infection. An infectious organism can escape the confines of the immediate tissue via the circulatory system or lymphatic system, where it may spread to other parts of the body. If an organism is not contained by the actions of acute inflammation it may gain access to the lymphatic system via nearby lymph vessels. An infection of the lymph vessels is known as lymphangitis, and infection of a lymph node is known as lymphadenitis. A pathogen can gain access to the bloodstream through lymphatic drainage into the circulatory system. Infections include, without limitation, bacterial cystitis, bacterial encephalitis, pandemic influenza, viral encephalitis, and viral hepatitis (A, B and C).

[0112] In another embodiment, an acute and/or chronic inflammation is induced by a tissue or organ injury. Tissue or organ injuries include, without limitation, a burn, a laceration, a wound, a puncture, or a trauma.

[0113] In another embodiment, an acute and/or chronic inflammation is induced by a transplant rejection. Transplant rejection occurs when a transplanted organ or tissue is not accepted by the body of the transplant recipient because the

immune system of the recipient attacks the transplanted organ or tissue. An adaptive immune response, transplant rejection is mediated through both T-cell-mediated and humoral immune (antibodies) mechanisms. A transplant rejection can be classified as a hyperacute rejection, an acute rejection, or a chronic rejection. Acute and/or chronic rejection of a transplanted organ or tissue is where the rejection is due to a poorly understood acute and/or chronic inflammatory and immune response against the transplanted tissue. Also included as transplant rejection is graft-versus-host disease (GVHD), either acute or chronic GVHD. GVHD is a common complication of allogeneic bone marrow transplantation in which functional immune cells in the transplanted marrow recognize the recipient as “foreign” and mount an immunologic attack. It can also take place in a blood transfusion under certain circumstances. GVHD is divided into acute and chronic forms. Acute and chronic GVHD appear to involve different immune cell subsets, different cytokine profiles, somewhat different host targets, and respond differently to treatment.In another embodiment, an acute and/or chronic inflammation is induced by a Th1-mediated inflammatory disease. In a well-functioning immune system, an immune response should result in a well-balanced pro-inflammatory Th1 response and anti-inflammatory Th2 response that is suited to address the immune challenge. Generally speaking, once a pro-inflammatory Th1 response is initiated, the body relies on the anti-inflammatory response invoked by a Th2 response to counteract this Th1 response. This counteractive response includes the release of Th2 type cytokines such as, *e.g.*, IL-4, IL-5, and IL-13 which are associated with the promotion of IgE and eosinophilic responses in atopy, and also IL-10, which has an anti-inflammatory response. A Th1-mediated inflammatory disease involves an excessive pro-inflammatory response produced by Th1 cells that leads to acute and/or chronic inflammation. The Th1-mediated disease may be virally, bacterially or chemically (*e.g.*, environmentally) induced. For example, a virus causing the Th1-mediated disease may cause a chronic or acute infection, which may cause a respiratory disorder or influenza.

[0114] In another embodiment, an acute and/or chronic inflammation comprises an acute and/or chronic neurogenic inflammation. Acute and/or chronic neurogenic inflammation refers to an inflammatory response initiated and/or maintained through the release of inflammatory molecules like SP or CGRP which released from peripheral sensory nerve terminals (*i.e.*, an efferent function, in contrast to the normal afferent signaling to the spinal cord in these nerves). Acute and/or chronic neurogenic

inflammation includes both primary inflammation and secondary neurogenic inflammation. Primary neurogenic inflammation refers to tissue inflammation (inflammatory symptoms) that is initiated by, or results from, the release of substances from primary sensory nerve terminals (such as C and A-delta fibers). Secondary neurogenic inflammation refers to tissue inflammation initiated by non-neuronal sources (*e.g.*, extravasation from vascular bed or tissue interstitium-derived, such as from mast cells or immune cells) of inflammatory mediators, such as peptides or cytokines, stimulating sensory nerve terminals and causing a release of inflammatory mediators from the nerves. The net effect of both forms (primary and secondary) of acute and/or chronic neurogenic inflammation is to have an inflammatory state that is maintained by the sensitization of the peripheral sensory nerve fibers. The physiological consequence of the resulting acute and/or chronic neurogenic inflammation depends on the tissue in question, producing, such as, *e.g.*, cutaneous pain (allodynia, hyperalgesia), joint pain and/or arthritis, visceral pain and dysfunction, pulmonary dysfunction (asthma, COPD), and bladder dysfunction (pain, overactive bladder).

ROUTES OF ADMINISTRATION

[0115] The cell population expressing an E-selectin and/or L-selectin ligand can be administered to subjects in accordance with a number of suitable routes of administration. Exemplary methods can include vascular injection, for example intravenous (i.v.) injection, or direct implantation of cells into a target site in a subject.

[0116] Other methods of delivery can include intratracheal delivery, intrathecal delivery, intraosseous delivery, pulmonary delivery, buccal delivery, aerosol delivery, inhalational delivery, and oral delivery. Still other methods can include intraarterial delivery, intracerebral delivery, intraintestinal delivery, intracardiac delivery, subcutaneous delivery, intramuscular delivery, intraorbital delivery, intracapsular delivery, intraspinal delivery, intraperitoneal delivery, intrasternal delivery, intravesical delivery, intralymphatic delivery, intracavital delivery, vaginal delivery, rectal delivery, transurethral delivery, intradermal delivery, intraocular delivery, aural delivery, intramammary delivery, orthotopic delivery, intratracheal delivery, intralesional delivery, percutaneous delivery, endoscopical delivery, transmucosal delivery, sublingual delivery, and direct application on body surfaces (*e,g.*, directly onto skin surface).

[0117] Cells can be inserted into a delivery device which facilitates introduction by injection or implantation into the subjects. Such delivery devices can include tubes, *e.g.*, catheters, for injecting cells and fluids into the body of a recipient subject. Such delivery devices may also include endoscopic delivery devices and methods. Preferably, the tubes additionally have a needle, *e.g.*, a syringe, through which the cells of the disclosure can be introduced into the subject at a desired location.

[0118] The cells can be delivered subsequent to manipulations/procedures to enhance E-selectin and/or L-selectin binding activity, or, in some embodiments, cryopreserved and then thawed prior to administration to a subject. The cells can be prepared for delivery in a variety of different forms. For example, the cells can be suspended in a solution or gel or embedded in a support matrix when contained in such a delivery device. Cells can be mixed with a pharmaceutically acceptable carrier or diluent in which the cells of the disclosure remain viable. Pharmaceutically acceptable carriers and diluents include saline, aqueous buffer solutions, solvents and/or dispersion media. The use of such carriers and diluents is well known in the art. The solution is preferably sterile and fluid. Preferably, the solution is stable under the conditions of manufacture and storage and preserved against the contaminating action of microorganisms such as bacteria and fungi through the use of, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. Solutions can be prepared by incorporating cells as described herein in a pharmaceutically acceptable carrier or diluent and, as required, other ingredients, followed by filtered sterilization.

[0119] Intravascular delivery via needles or vascular-assisted devices (*e.g.*, catheter-based) injection techniques for cell administration can also be used to achieve distribution through the entire vasculature, or to the vasculature of a particular organ, such as for example liver, or kidney or any other organ. This includes non-specific targeting of the vasculature. Alternatively, any organ can be targeted by selecting a specific injection site, such as *e.g.*, a liver portal vein. Alternatively, the injection can be performed systemically into any vein in the body. This method is useful for enhancing stem cell numbers in aging patients. In addition, the cells can function to populate vacant stem cell niches or create new stem cells to replenish the organ, thus improving organ function. For example, cells can take up pericyte locations within the vasculature.

[0120] In some embodiments, the cells are introduced into the subject as part of a cell aggregate (*e.g.*, a pancreatic islet), tissue, or organ, *e.g.*, as part of an organ transplant method.

[0121] Delivery of cells can also be used to target sites of active angiogenesis. For example, delivery of endothelial progenitor cells or mesenchymal stem or progenitor cells can enhance the angiogenic response at a wound site. Targeting of angiogenesis can also be useful for using cells as a vehicle to target drugs to tumors.

MODIFIED CELLS AND METHODS FOR THEIR PREPARATION

[0122] Due, at least in part, to their increased homing capabilities, certain E- and/or L-selectin expressing cells are useful in the methods described herein. By way of example, cells can improve engraftment of stem cells such as HSPCs in clinical transplantation, for use of MSC in cell-based therapy (*e.g.*, for bone diseases) or directing migration and infiltration of progenitor/stem cells at injured/damaged tissue(s) for regenerative therapeutics in accordance with the methods described herein.

[0123] Accordingly, the cells used in the methods described herein express an E-selectin ligand and/or an L-selectin ligand. Thus, for example, after modification, the cell binds E-selectin and/or L-selectin. In various embodiments, the modified cell can bind P-selectin. In one particular embodiment, after modification the cells express the sialofucosylated CD44 glycoform known as Hematopoietic Cell E-/L-selectin Ligand (HCELL). In another particular embodiment, after modification the cells express Neural Cell Adhesion Molecule E-selectin Ligand (NCAM-E). In certain embodiments, after modification the cells express both HCELL and NCAM-E. In certain embodiments the cells express HCELL and/or CD43-E and/or CLA. After modification, the cell is capable of homing *in vivo* to the bone marrow and or sites of inflammation.

[0124] In certain embodiments, the cells used in the methods described herein express an L-selectin ligand. In one particular embodiment, after modification the cells express the potent L-selectin ligand known as Hematopoietic Cell E-/L-selectin Ligand (HCELL), sialofucosylated CD44 glycoform. In another embodiment, the cells express increased amounts of the L-selectin ligands HCELL and/or PSGL-1, or the E-selectin ligands HCELL, CLA, CD43-E, NCAM-E, or ESL-1. Such cells may lodge into

sites of inflammation via binding to E-selectin on endothelial cells or via binding to L-selectin expressed on infiltrating leukocytes within the inflammatory site.

[0125] For *ex vivo* custom engineering of live cell surface glycans using glycosyltransferases, it is imperative that the treated cells remain viable and phenotypically conserved following treatment(s). Previously, divalent metal co-factors such as manganese had been deemed critical for enzymatic activity for α(1,3)-fucosyltransferases, with use of such co-factors at levels that were overtly cytotoxic (*e.g.*, 10 mM Mn^{++}). However, it is now known that these glycosyltransferases possess enzymatic activity in the absence of, or in presence of, relatively small amounts of, divalent metal co-factors (*e.g.*, divalent cations such as manganese, magnesium, calcium, zinc, cobalt or nickel). Moreover, these enzymes can be stored in absence of stabilizers such as glycerol which can themselves be toxic to cells. Certain glycosyltransferase compositions described in U.S. Pat. No. 7,875,585 are particularly useful in modification of glycans on live cells, which cells and/or particles or fragments thereof can then be used in the treatment methods described herein. In applications utilizing stem cells, it is also important to analyze whether differentiation along characteristic lineages is affected by enzymatic treatment.

[0126] Various methods can be utilized for preparing cells that bind E-selectin and/or L-selectin.

[0127] For example, U.S. Pat. Nos. 7,875,585 and 8,084,236, provide compositions and methods for *ex vivo* modification of cell surface glycans on a viable cell, the latter of which may in turn be utilized in methods of treatment of acute or chronic inflammation as described herein. The compositions include a purified glycosyltransferase polypeptide and a physiologically acceptable solution, for use together with appropriate donor nucleotide sugars in reaction buffers and reaction conditions specifically formulated to retain cell viability. In certain preferred embodiments, the physiologically acceptable solution is free or substantially free of divalent metal co-factors, to such extent that cell viability is not compromised. In these and other preferred embodiments, the composition is also free or substantially free of stabilizer compounds such as for example, glycerol. Glycosyltransferase include for example, fucosyltransferase, galactosyltransferase, sialytransferase and N-acetylglucosaminyltransferase. In one embodiment, the fucosyltransferase is an alpha

1,3 fucosyltransferase such as an alpha 1,3 fucosyltransferase III, alpha 1,3 fucosyltransferase IV, an alpha 1,3 fucosyltransferase V, an alpha 1,3 fucosyltransferase VI, an alpha 1,3 fucosyltransferase VII or an alpha 1,3 fucosyltransferase IX. The sialyltransferase can be ST3GalII, ST3GalIV, or ST3GalVI.

[0128] Glycans are modified on the surface of a cell by contacting a population of cells with one or more glycosyltransferase compositions described above. The cells are contacted with the glycosyltransferase composition together with appropriate nucleotide sugar donor (*e.g.*, GDP-fucose, CMP-sialic acid) under conditions in which the glycosyltransferase has enzymatic activity. Glycan modification according to this method results in cells that have at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or more viability at 24 hours or more after treatment. In one embodiment, for example, the cells have at least 70% viability at 48 hours after treatment. In one such embodiment, for example, the cells have at least 75% viability at 48 hours after treatment. In one embodiment, for example, the cells have at least 80% viability at 48 hours after treatment. In addition, the phenotype of the cells (other than the glycan modification) is preferably preserved after treatment. By preserved phenotype it is meant the cell maintains its native function and/or activity. For example, if the cell is a stem cell it retains its potency, *i.e.*, its relevant totipotency or pluripotency or multipotency or unipotency, as would be characteristic of that particular stem cell type.

[0129] Another method of modifying cells for use in the treatment methods described herein can be found in U.S. Patent Application Pub. No. 2013/0040837. In accordance with the methods described therein, nucleic acids (*e.g.*, an aptamer) that specifically bind to a non-nucleic acid target may be immobilized onto the cell membrane. The aptamers can be selected for a specific target, such as those capable of binding to particular proteins. Thus, for example, aptamers that bind to cell surface receptors/cell adhesion molecules (*e.g.*, E-selectin, L-selectin) may be immobilized onto the surface of the cell to improve cell targeting. The aptamers may be conjugated to the cell surface using a three step modification process that includes (i) treatment of cells (in a suspension after trypsinization) with sulfonated biotinyl-N-hydroxy-succinimide (NHS-biotin) to introduce biotin groups on the cell surface, (ii) complexing with streptavidin, and (iii) coupling with biotinylated aptamers. Using this approach, Karp et al. reported in US 2013/0040837 that typically ~21,000 molecules were attached per

cell using this procedure and that the site density of aptamers on the cell surface could be readily tuned by adjusting the aptamer concentration used in the conjugation.

[0130] Expression of selectin binding may also be induced by covalent addition of sLex or sLea moieties or other oligosaccharide complexes that bind selectins. For example, as described more fully and as exemplified by Karp et al. in US2011/0206740, biotin-streptavidin conjugation was utilized to chemically incorporate the sialyl-Lewis^X moiety onto the cell surface. More specifically, the free amine groups present on the surface of the cells were allowed to react with N-hydroxy-succinimide group of biotinyl-N-hydroxy-succinimide to biotinylate the cell surface. This step was subsequently followed by reacting the biotin moiety of the cell surface with a streptavidin molecule. The strong interaction between biotin and streptavidin allows the streptavidin molecule to be immobilized on the cell surface. The streptavidin is then reacted with biotinylated SLeX (Sialyl-Lex-PAA-Biotin) to introduce SLeX on the cell surface.

[0131] In an alternative approach to glycan engineering of cell surfaces, a fucosyltransferase is used incorporate an *en bloc* assembly of an sLex or sLea glycan onto a cell surface as described, for example, by Srivastava et al., *J. Biol. Chem.* 1992, 267:22356-22361. More specifically, Srivastava et al. reported that a partially purified Le-FucT from human milk, which normally used GDP-fucose as the donor for the transfer of a single fucose residue will also transfer a fucose residue substituted on C-6 by a very large sterically demanding structure. Accordingly, the fucosyltransferase could be used to transfer a glycan such as such as sialyl-Lewis^X to increase the level of expression of E-selectin ligand and/or an L-selectin ligand on the surface of of a population cells that is greater than the level of expression for a native population of such cells.

[0132] In another embodiment, a variety of bifunctional chemical linkers can be used to covalently attach sLex or sLea glycans, or glycomimetics of sLex or sLea, or peptidomimetics of sLex or sLea (*e.g.*, as could be generated by phage display technology). Similarly, mAb or mAb fragments thereof that bind to E-selectin and/or L-selectin could be conjugated to the cell surface to enhance binding to E-selectin and/or L-selectin, respectively.

[0133] In addition to the foregoing, any other methods of modifying a cell or cells to express an E-selectin ligand and/or an L-selectin ligand may be utilized. This

includes, for example, the promotion of covalent and/or non-covalent interactions, hydrogen bonding, and/or van der Wals forces between the cell surface and the ligand, phage display product, and the like.

[0134] In general, any type of cell can modified and used in the treatment methods described herein. For animal use it is preferable that the cell is of animal origin, while for human use it is preferably that the cell is a human cell; in each case, an autologous cell source could be used, a syngeniec source cell could be used, an allogeneic source cell could be used (including a combination of allogenic donor cells in a given cell product), or a xenogeneic cell source can be utilized. The cell can be a primary cell, *e.g.*, a primary hepatocyte, a primary neuronal cell, a primary myoblast, a primary mesenchymal stem cell, primary progenitor cell, or it can be a cell of an established cell line or of a culture expanded cell (*e.g.*, T cells, dendritic cells, etc). It is not necessary that the cell be capable of undergoing cell division; a terminally differentiated cell can be used in the methods described herein. In this context, the cell can be of any cell type including, but not limited to embryonic stem cells, adult stem cells, induced pluripotent stem cells, blood progenitor cells, tissue progenitor cells, epithelial, endothelial, neuronal, adipose, cardiac, skeletal muscle, fibroblast, immune cells (for example, dendritic cells, monocytes, macrophages, leukocytes (*e.g.*, a lymphocyte such as a B-lymphocyte, a T-lymphocyte, or a subset of T-lymphocytes, such as regulatory lymphocyte ($CD4^+/CD25^+/FOXP3^+$)), a naïve T cell, a central memory T cell, an effector memory T cell, an effector T cell, NK cells, etc.), hepatic, splenic, lung, circulating blood cells, platelets, reproductive cells, gastrointestinal cells, renal cells, bone marrow cells, cardiac cells, endothelial cells, endocrine cells, skin cells, muscle cells, neuronal cells, and pancreatic cells. The cell can be a cell line, a stem cell (*e.g.*, a mesenchymal stem cell, a hematopoietic stem cell, a tissue stem/progenitor cell (for example, a neural stem cell, gastrointestinal stem cell, myocyte stem cell, cardiomyocyte progenitor/stem cell, endothelial progenitor cell, or pulmonary stem cell), an umbilical cord stem cell, or an embryonic stem cell, or a primary cell isolated from any tissue including, but not limited to brain, liver, lung, gut, stomach, fat, muscle, testes, uterus, ovary, skin, spleen, endocrine organ and bone, and the like.

[0135] Where the cell is maintained under in vitro conditions, conventional tissue culture conditions and methods can be used, and are known to those of skill in

the art. Isolation and culture methods for various cells are well within the knowledge of one skilled in the art.

[0136] In addition, both heterogeneous and homogeneous cell populations are contemplated for use with the methods and compositions described herein. In addition, aggregates of cells, cells attached to or encapsulated within particles, cells within injectable delivery vehicles such as hydrogels, and cells attached to transplantable substrates (including scaffolds) or applied into tissue(s) that harbors scaffolds/transplantable substrates are contemplated for use with the methods and compositions described herein. Moreover, cells may be used in combination with tissue proliferative/enhancing agents and/or anti-inflammatory agents (*e.g.*, growth factors, cytokines, prostaglandins, trophic agents, Resolvins, NSAIDS, steroids, etc.)

[0137] The present disclosure further includes the following enumerated embodiments.

[0138] Embodiment 1. A method of enhancing cell delivery into a target tissue of a subject and/or enhancing tissue colonization in the target tissue of the subject, the method comprising: administering to the subject a population of cells that express an E selectin ligand and/or an L-selectin ligand, the population expressing the E selectin ligand and/or L-selectin ligand at a level that exceeds the level of expression of a native population of the cells, wherein said cell administration occurs coincident with E selectin expression on endothelial cells within the target tissue and/or coincident with accumulation of leukocytes within the target tissue.

[0139] Embodiment 2. A method of treating a disease, disorder or medical condition manifesting as inflamed and/or damaged tissue in a subject, the method comprising: administering to the subject a population of cells that express an E selectin ligand and/or an L-selectin ligand, the population expressing the E selectin ligand and/or L-selectin ligand at a level that exceeds the level of expression of a native population of the cells, wherein said cell administration occurs coincident with E selectin expression on endothelial cells within the target tissue and/or coincident with accumulation of leukocytes within the target tissue, and wherein said population exhibits enhanced localization and/or colonization within the inflamed and/or damaged tissue relative to a native population of the administered cells.

[0140] Embodiment 3. A method of improving cellular delivery to a target tissue in a subject, the method comprising: administering to the subject, via vascular delivery and/or via direct tissue injection (i.e., directly into affected tissue site(s)) and/or intrethecally and/or intracavitary and/or intravesically and/or within anatomic conduits (biliary system, urinary system, etc.) and/or onto tissue (i.e., placement onto affected tissue), a population of cells that express an E selectin ligand and/or an L-selectin ligand, the population expressing the E selectin ligand and/or L-selectin ligand at a level that exceeds the level of expression of a native population of the cells, wherein said cell administration occurs coincident with E selectin expression on endothelial cells within the target tissue and/or coincident with accumulation of leukocytes within the target tissue, and wherein said administered cell population achieves one or more of enhanced homing, colonization, and engraftment relative to a native population of cells.

[0141] Embodiment 4. A method of enhancing cell delivery and colonization in an inflamed and/or damaged tissue of a subject, the method comprising: directly injecting into or placing onto/within said inflamed and/or damaged tissue a population of cells that express an E selectin ligand and/or an L-selectin ligand, the population expressing the E selectin ligand and/or L-selectin ligand at a level that exceeds the level of expression of a native population of the cells, wherein said injection/placement occurs coincident with E selectin expression on endothelial cells within the target tissue and/or coincident with accumulation of leukocytes within the target tissue.

[0142] Embodiment 5. A method of enhancing cell delivery, colonization and/or engraftment into a target tissue of a subject, the method comprising: administering via the vasculature of the subject a population of cells that express an E selectin ligand and/or an L-selectin ligand, the population expressing the E selectin ligand and/or L-selectin ligand at a level that exceeds the level of expression of a native population of the cells, wherein said administration occurs coincident with E selectin expression on endothelial cells within the target tissue and/or coincident with accumulation of leukocytes within the target tissue.

[0143] Embodiment 6. A method of enhancing lodgement, colonization and/or engraftment of a cell population within a target tissue in a subject, the method comprising: directly injecting into the target tissue or placement onto the target tissue a population of cells that express an E selectin ligand and/or an L-selectin ligand, the

population expressing the E selectin ligand and/or L-selectin ligand at a level that exceeds the level of expression of a native population of the cells, wherein said injection occurs coincident with E selectin expression on endothelial cells within the target tissue and/or coincident with accumulation of leukocytes within the target tissue, and wherein said population achieves enhanced target tissue colonization relative to a native population of the cells.

[0144] Embodiment 7. A method of treating a disease, disorder or medical condition manifesting as inflamed and/or damaged tissue in a subject, the method comprising: administering to the subject a population of cells that express an E selectin ligand and/or an L-selectin ligand, the population expressing the E selectin ligand and/or L-selectin ligand at a level that exceeds the level of expression of a native population of the cells, and wherein said injection occurs coincident with E selectin expression on endothelial cells within the inflamed and/or damaged tissue and/or coincident with accumulation of leukocytes within the inflamed and/or damaged tissue.

[0145] Embodiment 8. A method of treating a tumor/malignant disease in a subject, the method comprising: administering to the subject a population of cells that express an E selectin ligand and/or an L-selectin ligand, the population expressing the E selectin ligand and/or L-selectin ligand at a level that exceeds the level of expression of a native population of the cells, wherein said administration occurs coincident with E selectin expression on endothelial cells within tissue(s) harboring tumor/malignant cells and/or coincident with accumulation of leukocytes within the tissue(s) harboring tumor/malignant cells.

[0146] Embodiment 9. A population of cells that express an E selectin ligand and/or an L-selectin ligand for use in the manufacture of a medicament for the treatment of a disease, disorder or medical condition manifesting as inflamed and/or damaged tissue in a subject, the treatment comprising administering to the subject the population of cells that express an E selectin ligand and/or an L-selectin ligand, wherein the population expresses the E selectin ligand and/or L-selectin ligand at a level that exceeds the level of expression of a native population of the cells, wherein said treatment comprises administering the population coincident with E selectin expression on endothelial cells within the inflamed and/or damaged tissue and/or coincident with accumulation of leukocytes within the inflamed and/or damaged tissue.

[0147] Embodiment 10. A population of cells that express an E selectin ligand and/or an L-selectin ligand for use in the manufacture of a medicament for the treatment of a tumor/malignant disease, the treatment comprising administering to the subject the population of cells that express an E selectin ligand and/or an L-selectin ligand, wherein the population expresses the E selectin ligand and/or L-selectin ligand at a level that exceeds the level of expression of a native population of the cells, wherein said treatment comprises administering the population coincident with E selectin expression on endothelial cells within the tumor/malignant tissue and/or coincident with accumulation of leukocytes within the tumor/malignant tissue.

[0148] Embodiment 11. The method or population of any one of Embodiments 1-10, wherein said cell administration occurs coincident with E selectin expression on endothelial cells and infiltrates of leukocytes bearing L-selectin within the target tissue.

[0149] Embodiment 12. The method or population of any one of Embodiments 1-11, wherein said administration/injection occurs during infiltration of leukocytes to an/the inflamed and/or damaged tissue.

[0150] Embodiment 13. The method or population of any one of Embodiments 1-12, further comprising engraftment of the administered population of cells in a milieu of an/the inflamed and/or damaged tissue.

[0151] Embodiment 14. The method or population of any one of Embodiments 1-13, wherein an immunomodulatory effect is achieved by colonization of cells within an/the inflamed and/or damaged tissue.

[0152] Embodiment 15. The method or population of any one of Embodiments 1-14, wherein a tissue reparative effect is achieved by colonization of administered cells within an/the inflamed and/or damaged tissue.

[0153] Embodiment 16. The method or population of any one of Embodiments 1-15, wherein an enhanced host defense/immune response effect is achieved by delivery and colonization of administered cells within an/the inflamed and/or damaged tissue.

[0154] Embodiment 17. The method or population of any one of Embodiments 1-16, wherein an anti-malignancy effect is achieved by colonization of administered cells within a/the tumor/malignant tissue site.

[0155] Embodiment 18. The method or population of any one of Embodiments 1-17, wherein said administration/injection comprises one or a series of injections of the cell population.

[0156] Embodiment 19. The method or population of cells of any one of Embodiments 1-18, wherein said administration/injection comprises daily, weekly, bi-weekly, monthly, or yearly injections of the cell population.

[0157] Embodiment 20. The method or population of cells of any one of Embodiments 1-9, further comprising one or more additional administrations/injection of the cell population upon a decrease in the subject of an immunomodulatory effect of a prior administration/injection.

[0158] Embodiment 21. The method or population of cells of any one of Embodiments 1-20, wherein the cell population is administered/injected intravenously.

[0159] Embodiment 22. The method or population of cells of any one of Embodiments 1-21, wherein the cell population is administered by direct injection to the inflamed and/or injured tissue.

[0160] Embodiment 23. The method or population of cells of any one of Embodiments 1-21, wherein the cell population is placed onto the inflamed tissue and/or injured tissue.

[0161] Embodiment 24. The method or population of cells of any one of Embodiments 1-21, wherein the cell population is placed onto an/the inflamed tissue and/or injured tissue.

[0162] Embodiment 25. The method or population of cells of any one of Embodiments 1-21, wherein the cell population is utilized in combination with other enhancing agents, anti-inflammatory agents, or with tissue scaffolds/transplantable devices/gels.

[0163] Embodiment 26. The method or population of any one of Embodiments 1-25, wherein the population of cells express an E selectin ligand and an L-selectin ligand.

[0164] Embodiment 27. The method or population of any one of Embodiments 1-26 wherein the E-selectin ligand and/or L-selectin ligand is selected from one or more

of Hematopoietic Cell E-/L-selectin Ligand (HCELL), Neural Cell Adhesion Molecule E-selectin Ligand (NCAM-E), CD43E, CLA, and ESL-1.

[0165] Embodiment 28. The method or population of any one of Embodiments 1-27, wherein the E-selectin ligand is Hematopoietic Cell E-/L-selectin Ligand (HCELL) and/or Neural Cell Adhesion Molecule E-selectin Ligand (NCAM-E).

[0166]

[0167] Embodiment 29. The method or population of any one of Embodiments 1-28, wherein the E-selectin ligand is HCELL.

[0168] Embodiment 30. The method or population of any one of Embodiments 1-29, wherein the E-selectin ligand is NCAM-E.

[0169] Embodiment 31. The method or population of any one of Embodiments 1-30, wherein the L-selectin ligand is HCELL.

[0170] Embodiment 32. The method or population of any one of Embodiments 1-31, wherein the cell population comprises one or more of epithelial, endothelial, neuronal, adipose, cardiac, skeletal muscle, fibroblast, and immune cells (for example, dendritic cells, monocytes, macrophages, leukocytes (e.g., a lymphocyte such as a NK cell, a B-lymphocyte, a T-lymphocyte, or a subset of T-lymphocytes, such as regulatory lymphocyte (CD4+/CD25+/FOXP3+)), a cytotoxic lymphocyte, etc.), hepatic, splenic, lung, circulating blood cells, platelets, reproductive cells, gastrointestinal, renal, bone marrow, pancreatic cells, a stem cell (e.g., a mesenchymal stem cell, a hematopoietic stem cell, a tissue stem/progenitor cell (for example, a neural stem cell, myocyte stem cell or pulmonary stem cell), an umbilical cord stem cell, or an embryonic stem cell, or an induced pluripotent stem cell, or a differentiated progenitor derived from an embryonic stem cell or from an induced pluripotent stem cells, or a differentiated progenitor derived from an adult stem cell, or a primary cell isolated from any tissue (e.g., brain, liver, lung, gut, stomach, fat, muscle, testes, uterus, ovary, skin, spleen, endocrine organ and bone), or a culture-expanded progenitor cell population, or a culture-expanded stem cell population, or a culture-expanded primary cell population.

[0171] Embodiment 33. The method or population of any one of Embodiments 1-32, wherein the cell population is a mesenchymal stem cell, a

hematopoietic stem cell, a tissue stem/progenitor cell, an umbilical cord stem cell, or an embryonic stem cell.

[0172] Embodiment 34. The method or population of any one of Embodiments 1-33, wherein the cell population is a leukocyte (e.g., a lymphocyte such as an NK cell, B-lymphocyte, a T-lymphocyte, or a subset of T-lymphocytes, such as regulatory lymphocyte (CD4+/CD25+/FOXP3+)), a cytotoxic T cell, etc.).

[0173] Embodiment 35. The method or population of any one of Embodiments 1-34, wherein the disease, disorder or medical condition is one or more of direct tissue injury (e.g., burns, trauma, decubitus ulcers, etc.), ischemic/vascular events (e.g., myocardial infarct, stroke, shock, hemorrhage, coagulopathy, etc.), infections (e.g., cellulitis, pneumonia, meningitis, sepsis, SIRS, etc.), neoplasia (e.g., breast cancer, lung cancer, prostate cancer, lymphoma, leukemia, etc.), immunologic/autoimmune conditions (e.g., graft vs. host disease, multiple sclerosis, diabetes, inflammatory bowel disease, lupus erythematosus, rheumatoid arthritis, psoriasis, etc.), degenerative diseases (e.g., osteoporosis, osteoarthritis, Alzheimer's disease, etc.), congenital/genetic diseases (e.g., epidermolysis bullosa, osteogenesis imperfecta, muscular dystrophies, lysosomal storage diseases, Huntington's disease, etc.), adverse drug effects (e.g., drug-induced hepatitis, drug-induced cardiac injury, etc.), toxic injuries (e.g., radiation exposure(s), chemical exposure(s), alcoholic hepatitis, alcoholic pancreatitis, alcoholic cardiomyopathy, cocaine cardiomyopathy, etc.), metabolic derangements (e.g., uremic pericarditis, metabolic acidosis, etc.), iatrogenic conditions (e.g., radiation-induced tissue injury, surgery-related complications, etc.), and/or idiopathic processes (e.g., amyotrophic lateral sclerosis, Parsonnage-Turner Syndrome, etc.).

[0174] Embodiment 36. The method or population of any one of Embodiments 1-35, wherein the disease, disorder or medical condition is diabetes.

[0175] Embodiment 37. The method or population of any one of Embodiments 1-36, wherein the disease, disorder or medical condition is multiple sclerosis.

[0176] Embodiment 38. The method or population of any one of Embodiments 1-37, wherein the subject is a human, non-human primate, mouse, rat, dog, cat, horse, or cow.

[0177] Embodiment 39. The method or population of any one of Embodiments 1-38, wherein the subject is a human patient.

[0178] Embodiment 40. The method or population of any one of Embodiments 1-39 wherein the cell population is treated to form a modified cell population having an enforced expression of an E-selectin and/or an L-selectin ligand, and the cell population has a viability of at least 70% at 24 hours after the treatment.

[0179] Embodiment 41. The method or population of any one of Embodiments 1-40 wherein the cell population has a viability of at least 80% at 24 hours after the treatment.

[0180] Embodiment 42. The method or population of any one of Embodiments 1-40 wherein the cell population has a viability of at least 70% at 48 hours after the treatment.

[0181] Embodiment 43. The method or population of any one of Embodiments 1-42 wherein, upon administration to the cell population to a subject, the cell population lodges within a tissue comprising infiltrating leukocytes.

[0182] Embodiment 44. The method or population of Embodiment 43 wherein the administration is a direct injection into the tissue or placement onto the tissue.

[0183] Embodiment 45. The method or population of Embodiment 43 wherein the administration is vascular.

[0184] Embodiment 46. The method or population of any of Embodiments 1-21 or 25-42 wherein the population is administered systemically, via either peripheral vascular access or central vascular access.

[0185] Embodiment 47. The method or population of any of Embodiments 1-21 or 25-42 wherein the population is administered intravascularly into anatomic feeder vessels of an intended tissue site using catheter-based approaches or other vascular access devices that will deliver a vascular bolus of cells to the intended site.

[0186] Embodiment 48. The method or population of any of Embodiments 1-21 or 25-42 wherein the population is administered by introduction into the spinal canal and/or intraventricularly.

[0187] Embodiment 49. The method or population of any of Embodiments 1-21 or 25-42 wherein the population is administered directly into body cavities by either catheter-based approaches or direct injection.

[0188] Embodiment 50. The method or population of any of Embodiments 1-21 or 25-42 wherein the population is administered by direct local tissue injection, using either intravascular approaches, or percutaneous approaches, or directly into anatomically accessible tissue sites and/or guided by imaging techniques.

[0189] Embodiment 51. The method or population of any of Embodiments 1-21 or 25-42 wherein the population is administered by placement directly onto relevant tissue surfaces/sites.

[0190] Embodiment 52. The method or population of any of Embodiments 1-21 or 25-42 wherein the population is administered into scaffolds or embedded within scaffolds placed into tissues, and/or administered in gels, and/or administered together with enhancing agents.

[0191] Embodiment 53. The method or population of any of Embodiments 1-21 or 25-42 wherein the population is administered into the cerebrospinal fluid.

[0192] Having described the disclosure in detail, it will be apparent that modifications and variations are possible without departing the scope of the disclosure defined in the appended claims. Furthermore, it should be appreciated that all examples in the present disclosure are provided as non-limiting examples.

EXAMPLES

[0193] The following non-limiting examples are provided to further illustrate the present disclosure. It should be appreciated by those of skill in the art that the techniques disclosed in the examples that follow represent approaches the inventors have found function well in the practice of the disclosure, and thus can be considered to constitute examples of 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 that are disclosed and still obtain a like or similar result without departing from the spirit and scope of the disclosure.

EXAMPLE 1: ALPHA(1,3)-FUCOSYLTRANSFERASE TREATMENT OF ADIPOSE-DERIVED MESENCHYMAL STEM CELLS ENFORCES EXPRESSION OF HCELL

[0194] Prior studies indicated that bone marrow-derived mesenchymal stem cells (MSCs) can be glycan engineered by α(1,3)-fucosyltransferases to create the potent E- and L-selectin ligand HCELL (R Sackstein et al, Nature Medicine 14:181-187 (2008)). To determine whether HCELL expression can be enforced on MSC derived from non-bone marrow sources, we analyzed the effects of α(1,3)-exofucosylation on adipose-derived mesenchymal stem cells. To this end, adipose tissue was obtained from liposuction material from both lean and obese subjects and mesenchymal stem cells were cultured as described (R Sackstein et al, Nature Medicine 14:181-187 (2008)). Cells were grown at low density (confluence <60%) under both normoxic (21% O_2) and hypoxic (<5% O_2) conditions in MSC medium supplemented with either FBS (DMEM with 20% FBS and 1% penicillin/streptomycin) or with human platelet lysate (DMEM with 5% human platelet lysate and 1% penicillin/streptomycin). MSCs were passaged when confluence approached 60%, and MSCs were harvested at passages 3-6. MSCs (20 x 10^6/ml) were then treated with Fucosyltransferase VII (FTVII; prepared by R&D Systems in suspension without divalent cations) at 20 ug/ml or with Fucosyltransferase VI (60 mU/ml (R Sackstein et al, Nature Medicine 14:181-187 (2008))) in each case in Hank's balanced salt solution (HBSS) without divalent cations containing 10 mM HEPES, 0.1% human serum albumin and 1 mM GDP-fucose (Sigma) for 60 min at 37ºC, whereas control cells (buffer-treated) were treated in the same buffer without FTVII or without FTVI. Cell viability was assessed routinely by dual laser flow cytometry using propidium iodide and annexin V staining. Cell viability was consistently greater than 80% at 24 hours following α(1,3)-exofucosylation with either FTVI or FTVII. See **Figures 24-26**.

EXAMPLE 2: ALPHA(1,3)-FUCOSYLTRANSFERASE TREATMENT OF HUMAN BLOOD LEUKOCYTES INDUCES HIGH LEVEL E-SELECTIN LIGAND ACTIVITY ON THE CELLS AND ENFORCES EXPRESSION OF HCELL

[0195] The enforced expression of E selectin ligands (*i.e.*, HCELL and other E-selectin ligands) on surfaces of desired cells (*e.g.* stem cells, progenitor cells, leukocytes, etc.) would confer heightened capacity of those cells to traffic intravascularly to sites of tissue inflammation/damage and to sites of tumor/cancer infiltrates on the basis of vascular E-selectin expression on endothelial cells within the

affected tissue(s). Additionally, once extravasated, the expression of HCELL (or other enforced E-selectin/ L-selectin ligands) on the relevant administered cell surface would serve to promote colonization of the administered cells within the target tissue, as administered cells would collect within perivascular areas via E-selectin ligand adherence to E-selectin expressed at endothelial cells, and, additionally, administered cells would also anchor via L-selectin ligand adherence to L-selectin displayed on the surfaces of infiltrating leukocytes within the inflamed/damaged or cancer tissue milieu. As such, enforcing the expression of HCELL and of other E-selectin ligands and/or L-selectin ligands on stem cells/progenitor cells and on specific leukocyte subsets (including, for example, NK cells, CD4 T cells, CD8 T cells, Tregs, monocytes, dendritic cells and granulocytes, as well as relevant leukocytes expanded in culture for therapeutic purposes (*e.g.*, antigen-specific T cells, expanded NK cells, chimeric antigen receptor T cells (CAR T cells), etc.)) could be harnessed in adoptive cell therapeutics (for tissue regeneration/repair and/or for immunotherapy to either augment immunity or to dampen immunity). Thus, critically, enforced expression of HCELL would enable efficient trafficking of intravascularly administered cells to target sites by E-selectin-dependent endothelial interactions yielding recruitment of blood-borne cells, thereafter followed by E-selectin-mediated and L-selectin-mediated lodgement of the extravasated cells with discrete tissue microenvironments. Moreover, similarly, if such cells were injected directly into the affected site(s) of tissue injury/damage or at sites of cancer, enforced expression of HCELL and other E-selectin/L-selectin ligands would promote E-selectin-mediated and L-selectin-mediated lodgement/colonization of the cells within the tissue milieu.

[0196] To analyze the molecular targets of cell surface α(1,3)-exofucosylation and the effects of exofucosylation on leukocyte E-selectin and L-selectin ligand activity, studies were performed on primary human peripheral blood leukocytes obtained from citrated whole blood. Leukocytes were separated by immunomagnetic bead sorting (Miltenyi) into monocytes (CD14+ cells), CD4+ lymphocytes, CD8+ lymphocytes, and B cells (CD19+ cells). Cells were treated with Fucosyltransferase VII (FTVII; prepared by R&D Systems in suspension without divalent cations) at 20 ug/ml or with Fucosyltransferase VI (at 60 mU/ml (R Sackstein et al, Nature Medicine 14:181-187 (2008))) in each case in Hank's balanced salt solution (HBSS) without divalent cations containing 10 mM HEPES, 0.1% human serum albumin and 1 mM GDP-fucose (Sigma)

for 60 min at 37°C, whereas control cells (buffer-treated) were treated in the same buffer without FTVII or without FTVI. Cell viability was assessed routinely by dual laser flow cytometry using propidium iodide and annexin V staining. Cell viability was consistently greater than 80% at 24 hours following exofucosylation with either FTVI or FTVII. See **Figures 27(A)-27(C)** and **Figures 28(A)-28(C)**.

EXAMPLE 3: ALPHA(1,3)-FUCOSYLTRANSFERASE TREATMENT OF PRIMARY CULTURES OF HUMAN DENDRITIC CELLS INDUCES HIGH LEVEL E-SELECTIN LIGAND ACTIVITY ON THE CELLS AND ENFORCES EXPRESSION OF HCELL.

[0197] To assess the capacity of α(1,3)-exofucosylation to modulate E-selectin and L-selectin ligand activity of human dendritic cells, monocytes were isolated from human peripheral blood mononuclear cells using anti-CD14 coated magnetic beads (Miltenyi Biotech) (CD14-S) or by plastic adherence (PA-S), and then cultured per standard protocols with cytokines IL-4 and GM-CSF for 6 days to induce differentiation into monocyte-derived dendritic cells (mo-DCs). Monocyte purity was evaluated by flow cytometry for expression of CD14, and differentiation and maturation of mo-DCs was evaluated by staining for expression of BDCA-1, HLA-DR, CD80, and CD86 (each from BD Biosciences). Mo-DCs were then treated with Fucosyltransferase VII (FTVII; prepared by R&D Systems in suspension without divalent cations) at 20 ug/ml or with Fucosyltransferase VI (60 mU/ml (R Sackstein et al, Nature Medicine 14:181-187 (2008))) in each case in Hank's balanced salt solution (HBSS) without divalent cations containing 10 mM HEPES, 0.1% human serum albumin and 1 mM GDP-fucose (Sigma) for 60 min at 37°C, whereas control cells (buffer-treated) were treated in the same buffer without FTVII or without FTVI. Cell viability was assessed routinely by dual laser flow cytometry using propidium iodide and annexin V staining. Cell viability was consistently greater than 80% at 24 hours following exofucosylation with either FTVI or FTVII. See **Figures 29(A)-29(C)** and **Figures 30(A)-30(E)**.

EXAMPLE 4: ALPHA(1,3)-FUCOSYLTRANSFERASE TREATMENT OF PRIMARY CULTURES OF HUMAN REGULATORY T CELLS (TREGS) INDUCES HIGH LEVEL E-SELECTIN LIGAND ACTIVITY ON THE CELLS AND ENFORCES EXPRESSION OF HCELL; ADMINISTRATION HCELL+ TREGS SUPPRESSES XENOGENEIC GVHD INDUCED BY AUTOLOGOUS CELLS.

[0198] The ability to enhance delivery of immunomodulatory cells (such as MSCs and Tregs) to sites of inflammation would serve to improve the potential of

adoptive cell therapeutics to dampen tissue damage in immunologic diseases (*e.g.*, GVHD, rheumatoid arthritis, etc.) and in exuberant inflammatory responses to infections (*e.g.*, sepsis, fulminant viral hepatitis, etc.). To this end, we investigated the effects of α(1,3)-exofucosylation on the capacity of intravascularly administered primary human Tregs to treat xenogeneic graft-versus-host disease in a human-mouse GVHD model. We sought to assess the capacity of Tregs derived from the autologous lymphocyte source that induced GVHD, as this strategy would be relevant to clinical hematopoietic stem cells transplantation (*i.e.*, Tregs would be expanded from donor hematopoietic/immune cell inoculum, and, therefore, donor Tregs would be used to treat GVHD induced by donor immune cells). To this end, peripheral blood mononuclear cells (PBMCs) were obtained by Ficoll gradient centrifugation of whole blood of a healthy human donor. CD4-high/CD127-low T cells were isolated using a negative selection magnetic immunobead system (Miltenyi) and expanded in culture in the presence of anti-CD3/anti-CD28 mAb supplemented with IL-2 to produce CD4+/FoxP3+/CD25+ cells (Tregs). Unfractionated PBMCs from the same donor subject were injected IP (10^7 cells/mouse) into NSG host mice intraperitoneally. Fourteen days after injection (accordingly, day 14 of Treg expansion) the expanded Treg cells were collected and then treated with Fucosyltransferase VII (FTVII; prepared by R&D Systems in suspension without divalent cations) at 20 ug/ml or with Fucosyltransferase VI (60 mU/ml (R Sackstein et al, Nature Medicine 14:181-187 (2008))) in each case in Hank's balanced salt solution (HBSS) without divalent cations containing 10 mM HEPES, 0.1% human serum albumin and 1 mM GDP-fucose (Sigma) for 60 min at 37ºC, whereas control cells (buffer-treated) were treated in the same buffer without FTVII or without FTVI (cell viability was assessed routinely by dual laser flow cytometry using propidium iodide and annexin V staining; cell viability was consistently greater than 80% at 24 hours following exofucosylation with either FTVI or FTVII). Mice were then injected intravascularly with either buffer-treated (BT) Tregs or with exofucosylated Tregs at a dose of 2.5×10^5 cells/mouse. Mice were then followed clinically and mouse weights were monitored for 50 days post-initial PBMC injection. Data as shown in Figures reveal that FTVII treatment of human Tregs induces expression of E-selectin ligands, including HCELL. Injection of exofucosylated autologously-derived Tregs into mice with xenogeneic GVHD (induced by prior administration of autologous PBMCs) results in dampening of GVHD, as evidenced by

reversal of weight loss; exofucosylated Tregs are 3-fold more potent in reversing GVHD than are unfucosylated Tregs. See **Figures 31-34**.

EXAMPLE 5: ALPHA(1,3)-FUCOSYLTRANSFERASE TREATMENT OF MSC AND OF HUMAN BLOOD LEUKOCYTES INDUCES L-SELECTIN LIGAND ACTIVITY ON THE CELLS AS ASSESSED BY STAMPER-WOODRUFF LYMPHOCYTE ADHERENCE ASSAY.

[0199] The Stamper Woodruff lymphocyte adherence assay is the conventional tool to assess L-selectin ligand activity (R. Sackstein, Immunol. Rev. 230: 140-163 (2009)) . This assay, which was devised in the mid-1970s to evaluate the molecular basis of lymphocyte binding to lymph node high endothelial venules, measures the capacity of L-selectin – as natively expressed on the surface of a live lymphocyte – to attach to its cognate ligand(s) expressed on the surface of a relevant cell. The assay is performed under rotatory shear conditions, thereby placing a discrete biophysical restriction on the binding interaction: only the most potent L-selectin ligands (presented on the surface of cells attached to glass) will support adherence of L-selectin displayed on the rotating suspension of lymphocytes. Indeed, the only L-selectin ligands that are robust enough to support L-selectin-mediated binding of lymphocytes in this assay are HCELL and the "endothelial" L-selectin ligands displayed on lymph node high endothelial venules (collectively, these L-selectin ligands are called "addressins") (R. Sackstein, Immunol. Rev. 230: 140-163 (2009)).

[0200] To assess the expression of L-selectin ligand activity induced by α(1,3)-exofucosylation, the Stamper-Woodruff was performed on cytospin preparations of native cells, buffer-treated cells, and α(1,3)-exofucosylated cells. Briefly, lymphocyte suspensions prepared fresh from human blood (10^7/ml lymphocytes in RPMI 1640 medium) were overlaid onto glass slides containing preparations of relevant cells placed on the slides by cytocentrifugation. Slides were placed on a rotating platform for incubation under shear (80 rpm) at 4°C for 30 minutes. Slides were then rinsed in PBS to remove nonadherent lymphocytes, fixed in 3% glutaraldehyde, and stained with methyl green-thionin. Slides were examined for lymphocyte adherence to cytocentrifuged cells by light microscopy. L-selectin ligand activity was measured by counting the number of lymphocytes adherent to a confluent area of cytocentrifuged cells, using an ocular grid under 250X magnification. The cytocentrifuged cells that

have adherent lymphcytes are scored, and the percentage of such cells within the total lawn of cytocentrifuged cells is quantified (as shown on Key in Table 1).

Table 1. Results of Stamper-Woodruff Assay
(L-selectin binding activity of cells)

	L-SELECTIN LIGAND ACTIVITY		
Type of Cell	*Native cells*	*Buffer-treated cells*	*α(1,3)-exofucosylated cells*
Human MSC	0	0	++++
Human CD4 T cell	+	+	++++
Human CD8 T cell	0	0	++
Human B cell	0	0	+
Human Treg cell	0	0	+++
Human Monocyte	+	+	++++
Human Dendritic Cell	+	+	++++

KEY:
0 <10% of cells bind L-selectin$^+$ lymphocytes
+ 10-30% of cells bind L-selectin$^+$ lymphocytes
++ 30-50% of cells bind L-selectin$^+$ lymphocytes
+++ 50-70% of cells bind L-selectin$^+$ lymphocytes
++++ >70% of cells bind L-selectin$^+$ lymphocytes

[0201] As shown in Table 1, native MSCs have no L-selectin binding activity, but α(1,3)-exofucosylation of MSCs induces profound L-selectin binding activity, with L-selectin-mediated lymphocyte binding observed on essentially all exofucosylated cells. Human CD4 T cells, human monocytes, and human dendritic cells each display modest L-selectin binding activity, but these cells support robust L-mediated lymphocyte adherence following α(1,3)-exofucosylation. Human Tregs, B cells and CD8 cells do not natively possess L-selectin ligand activity, but each cell type can be induced to bind L-selectin by α(1,3)-exofucosylation, with exofucosylated Tregs showing marked increases in L-selectin adherence. Thus, L-selectin binding is enhanced on essentially all human leukocytes following α(1,3)-exofucosylation, and, in particular, L-selectin ligand activity can be profoundly induced on human MSC, CD4 T cells, monocytes, dendritic cells and Tregs by α(1,3)-exofucosylation. In each case, this L-selectin binding activity is a reflection of enforced HCELL expression, and is commensurate with results of HCELL expression on these cells as exhibited by western blot data staining for sLex/E-Ig as displayed in **Figures 25, 28(A)-28(C), 30(A)-30(E) and 33**.

EXAMPLE 6 - HCELL EXPRESSION ON MURINE MSC LICENSES PANCREATOTROPISM AND CONFERS DURABLE REVERSAL OF AUTOIMMUNE DIABETES IN NOD MICE

Introduction

[0202] Despite significant advances in the pharmacotherapy of glycemia control, type 1 diabetes (T1D) is still associated with significant morbidity and mortality, and it continues to pose a major public health burden demanding innovative treatment strategies[1, 2]. Cell-based immunomodulatory therapy has emerged as a promising approach in the treatment of T1D[3]. Because of their immunomodulatory properties, safety profile, easy acquisition, and robust *ex vivo* expansion, mesenchymal stem cells (MSCs) have become the most rapidly growing cell therapy for the treatment of various refractory immune-mediated diseases including T1D[4-7]. In preclinical models using NOD mice, we and others have recently reported that systemically-administered MSCs have utility in dampening autoimmune diabetes[8-13]. However, the benefits of MSC therapy in reversal of hyperglycemia were temporary, highlighting a pressing need to develop strategies to improve the effectiveness of MSC-based therapy for T1D[6].

[0203] The efficacy of immunomodulatory cell therapy is closely related to the ability of the infused cells to traffic to the inflamed tissue[14, 15]. For some organs (*e.g.*, the heart), direct (local) injection of cells into the affected site can achieve requisite colonization for physiologic benefit[16]. However, for treatment of T1D, the vascular route of cell delivery is mandated, as direct injection of cells into the pancreatic parenchyma would trigger release of proteases and other enzymes that could induce profound, life-threatening pancreatic inflammation. The migration of blood-borne cells into tissues is initiated by tethering/rolling adhesive interactions on target tissue endothelium. The most potent mediators of these binding interactions are the selectins, a family of three Ca^{++}-dependent lectins (E-, P- and L-selectin, also known as CD62E, CD62P, and CD62L, respectively) that bind to sialofucosylated glycan determinants expressed on their respective ligands[17]. Importantly, within the microvasculature at all inflammatory sites, the endothelial selectin, E-selectin, is inducibly expressed in response to inflammatory cytokines such as TNF-α[17,18]. E-selectin binds to membrane glycoproteins and/or glycolipids on circulating cells that prototypically display the sialofucosylated tetrasaccharide known as “sialylated Lewis X” (sLex). However, MSCs do not natively express E-selectin ligands[19]. This deficit in trafficking limits the engraftment of MSCs in inflamed peripheral tissues following intravenous

administration[17,20], constraining the utility of MSC-based therapeutics. Accordingly, we sought to investigate whether MSC trafficking to inflamed pancreas could be licensed via cell surface glycan modification to enforce E-selectin ligand expression, and whether this would impact MSC therapeutic effect(s) in new onset autoimmune diabetes in NOD mice. Our findings provide new insights on the biology of MSC effects in diabetes, highlighting a unique and prominent role for enforced expression of the E-selectin ligand HCELL in enhancing the capacity of murine MSCs to reverse hyperglycemia in diabetic NOD mice.

MATERIALS AND METHODS

Mice

[0204] C57BL/6, B6.129(Cg)-Cd44^{tm1Hbg}/J (CD44$^{-/-}$ on CD57BL/6 genetic background; CD44-knock out ("CD44-KO")), BALB/c, and NOD mice were purchased from Jackson Laboratories and were housed and/or bred in a pathogen-free environment at the Harvard Medical School Facilities for Animal Care and Housing. Experiments requiring the use of mice were approved by the Institutional Animal Care and Use Committee.

MSC culture

[0205] Bone marrow MSCs were derived as described previously [9, 10]. In brief, isolated marrow cells from C57BL/6 (wild-type) or from CD44-KO mice were flask-seeded in culture medium consisting of Dulbecco's Modified Eagle Medium with 10% fetal bovine serum (Lonza), 10 ng/ml fibroblast growth factor (PeproTech), 100 U/ml penicillin and 100 ug/ml streptomycin (Gibco). MSCs in culture passage 4 to 6 were used for experiments.

MSC characterization and differentiation

[0206] MSCs were characterized by flow cytometry using anti-mouse antibodies (all from eBioscience) directed to cell surface markers Sca1, CD44, CD73, CD45, CD29, and CD105, together with relevant isotype controls. Recombinant mouse E-selectin (CD62E)-human Fc chimera was purchased from R&D Systems. MSCs were tested for their differentiation capacity into various mesodermal lineages as previously described[21, 22]. Briefly, chondrogenic differentiation of MSCs was induced with ascorbic acid (50 μg/ml) and TGFβ1 (1 ng/ml) in culture medium. After 3 weeks of culture, plates were washed with PBS, fixed with 4% paraformaldehyde, and stained

with 0.05% alcian blue for light microscopic visualization of cartilage. Osteogenic differentiation was induced with ascorbic acid (50 µg/ml), sodium β-glycerophosphate (10 mM), and dexamethasone (10 nM) in culture medium. Two weeks later, plates were washed, fixed with 4% paraformaldehyde, and stained with 2% alizarin red for visualization of characteristic calcium deposits. Adipogenic differentiation was induced by the addition of dexamethasone (100 nM) and insulin (6 ng/ml) in F12 medium supplemented with 1% fetal calf serum, 1% glutamine, 1% penicillin-streptomycin. Cells were fixed in 4% parafomaldehyde and stained with 0.3% Oil Red solution in 60% isopropanol to assess lipid-laden vacuoles.

MSC Exofucosylation

[0207] MSCs in suspension (10 x 10^6 per 200 ul reaction) were treated for 90 min at 37°C with 60 mU/mL fucosyltransferase VI (FTVI) in reaction buffer consisting of Ca^{2+}- and Mg^{2+}-free Hanks Balanced Salt Solution (HBSS) containing 20 mM HEPES, 0.1% human serum albumin and 1 mM GDP-fucose ("FTVI-modified MSC"), or with reaction buffer alone (unmodified MSC). Following treatment, MSCs were washed with HBSS containing 0.2% HSA and 20 mM HEPES. Cell viability was assessed by trypan blue exclusion and propidium iodide staining (consistently >85% viability 24 hours post-detachment and treatment). FTVI modification was assessed by flow cytometry. FTVI enzyme was provided by Dr. Roland Wohlgemuth (Sigma Chemical Corporation).

Western blot analysis

[0208] FTVI-modified and unmodified MSC lysates were prepared by incubation with 2% Nonidet P-40 (NP-40) in Buffer A (150 mM NaCl, 50 mM Tris-HCl, pH 7.4, 20 mg/mL phenylmethylsulfonyl fluoride, 0.02% sodium azide, and protease inhibitor cocktail tablet (Roche Molecular Biochemicals). All Western blots of whole cell lysates or of immunoprecipitated protein were performed under reducing conditions on 7.5% SDS-PAGE gels as described previously[19]. The amount of lysate in each lane was normalized to cell number for each Western blot performed. Blots were visualized with chemiluminescence using Lumi-Light Western Blotting Substrate (Roche).

Flow cytometry and Immunoprecipitation studies

[0209] Flow cytometry was performed as described previously[19]. For more details please refer to the Supplementary Methods section.

Parallel Plate Flow Chamber Adhesion Assay

[0210] A dynamic flow adhesion assay was performed using a parallel plate flow chamber (250 μm channel depth x 5.0 mm channel width), to assess E-selectin mediated MSC binding over stimulated human umbilical vein endothelial cells (HUVEC) as previously described [19]. For more details, please refer to the Supplementary Methods section.

Transduction of MSCs with hGH viral vector

[0211] MSCs were transduced with the lentivirus containing hGH plasmid construct as described previously[21]. Levels of hGH were measured by enzyme-linked immunosorbent assay (Roche Diagnostics) in MSC supernatants and in serum of injected animals.

Transduction of MSCs with GFP viral vector

[0212] MSCs were transduced with a retrovirus plasmid containing GFP (GFP-MSC) as described previously[21]. Transduced MSC were assessed for perinuclear expression of GFP by fluorescent microscopy. In a complementary approach to the use of GFP-MSCs to assess homing, MSCs (non-GFP-transduced) were labeled with the fluorescent dye CFSE.

Immunofluorescence

[0213] Pancreata were embedded in Tissue Tek OCT, frozen and sectioned in a cryomicrotome. Immunofluorescence images were acquired using a Nikon E-1000 epifluorescence microscope (X400 total magnification). For more details on specific staining, please refer to the Supplementary Methods section.

Mitogen-stimulation CD3/CD28 T cell proliferation assay

[0214] T cell proliferation assay using anti-CD3 and anti-CD28 stimulation was used to assess effects of FTVI modification on immunomodulatory capacity of MSCs. Briefly, $1x10^5$ NOD splenocytes were stimulated with purified mouse anti-CD3e and anti-CD28 (each at 1 μg per well; eBioscience) for 72 hours in RPMI media (Lonza), supplemented with 10% fetal bovine serum (Gemini Bio-Products), 1% penicillin streptomycin (Lonza) and 1% glutamine (Lonza), in the presence of a titrating concentration ($5x10^2$ – $5x10^4$) of irradiated (3000 rad) unmodified and FTVI-modified MSCs. In the last 12 hours of the 72 hour incubation period, cells were pulsed with 1 μCi of tritiated thymidine, and lymphocyte proliferation (^{3}H-thymidine incorporation) was

measured by scintillation counting. Results are reported as an average of triplicate samples (mean cpm ± SEM).

Reversal of new-onset hyperglycemia studies in NOD mice

[0215] Unmodified and FTVI-modified MSCs (0.5 x 10^6 cells in 200ul) were injected intravenously via tail vein without anesthesia into female NOD mice on the second day of hyperglycemia (>250 mg glucose/dL), and at 7 days and 30 days following onset of hyperglycemia. To assess effect of MSC administration on hyperglycemia, blood was obtained via tail vein and glucose measured as described previously[9, 10].

Statistics

[0216] Data from experimental assays and immunohistological experiments were analyzed using student t-test and Mann Whitney tests. Survival data were assessed using Kaplan-Meyer analysis. To perform the analysis and to generate graphs, Prism software was used (GraphPad Software, Inc., San Diego, CA). P value <0.05 was considered significant. Data represent mean ± SEM.

SUPPLEMENTARY METHODS

Flow cytometry and Immunoprecipitation studies

[0217] Flow cytometry was performed as described previously[19] using biotinylated mAb HECA452 (anti-human cutaneous lymphocyte antigen, which recognizes sLex; Rat IgM (BioLegend)), and anti-mouse CD44 mAb (KM114; Rat IgG1), purified anti-mouse CD44 mAb (IM7; Rat IgG2b), and phycoerythrin (PE)-labeled streptavidin (all obtained from BD Pharmingen). For western blotting, MSC lysates were incubated with anti-CD44 immunoprecipitating antibodies (KM114/IM7) or with appropriate isotype controls and then collected with Protein G-agarose (Invitrogen). Immunoprecipitates were washed extensively using Buffer A containing 2% NP-40, 1% SDS, and then subjected to SDS-PAGE, transferred to polyvinylidene difluoride membrane, and immunostained with HECA452 or anti-mouse CD44 antibodies (KM114/IM7), followed by incubation with appropriate HRP-conjugated secondary antibodies for visualization by chemiluminescence using Lumi-Light Western Blotting Substrate (Roche).

Parallel Plate Flow Chamber Adhesion Assay

[0218] Confluent HUVECs were stimulated for 6 h with TNF-α (40 ng/ml) to up-regulate E-selectin expression. Wild-type (WT) and CD44KO MSC were harvested with 0.005% Trypsin/EDTA, and were either FTVI modified or treated with buffer alone (control) for 90 minutes at 37°C. MSC were then resuspended at 10^6 cells/ml in Hanks' Balanced Salt Solution supplemented with 1 mM calcium chloride, 1 mM magnesium chloride, 5 mM HEPES, and 1 mg/ml BSA. MSC were perfused over the HUVEC monolayer at 0.5 dynes/cm^2, and then subjected to shear stress levels of 1.0, 2.0, 5.0, 10.0, 20.0, and 30.0 dynes/cm^2 at 3-minute intervals. MSC that interacted with HUVEC (cells that rolled in the field of view or rolled into it within a 10 second time frame) were viewed at the center of the chamber, minimum of 6 fields of view at each shear stress level. As controls to assess specificity of E-selectin binding to stimulated HUVEC, FTVI-modified MSC were treated with sialidase from *Vibrio cholerae* (0.1 U/ml;Roche) to cleave terminal sialic acid from sLex, and adherence was also assessed in presence of function-blocking anti-human E-selectin mAb (10 ug/ml) (BD Pharmingen).

Immunofluorescence

[0219] Tissues were cryostat-sectioned at 10um thickness and fixed on slides in cold acetone for 5 min. After drying, sections were blocked with 1% BSA and incubated in primary antibody overnight. Sections were washed 3X in Tris-buffered Saline (TBS) and incubated with secondary antibody for one hour. E-selectin (CD62E) staining was performed using primary rat anti-mouse CD62E (R&D Systems, 1:100), and secondary PE-conjugated goat anti-rat IgG (Southern Biotech, 1:500). Isotype-matched rat mAb was used as a staining control. CD31 staining was performed using rat anti-mouse CD31 (Biocare, 1:200) and secondary PE-conjugated goat anti-rat IgG (Southern Biotech, 1:500). Assessment of CD31 and E-selectin co-localization was performed on sequential sections. Insulin staining was undertaken using purified guinea pig anti-insulin (Dako, 1:5) and APC conjugated donkey anti-guinea Pig IgG (H+L) (Jackson Immunoresearch, 1:200). HECA452 mAb (Rat anti-CLA IgM mAb (Biolegend, 1:200)) and FITC conjugated mouse anti-rat IgM (Biolegend, 1:500) were used to detect FTVI-modified MSC within tissue sections. Sections were overlaid with mounting media alone or, when indicated, with mounting media containing DAPI (Vector Labs), cover-slipped, and then visualized by immunofluorescence microscopy.

RESULTS

Expression and functional analysis of FTVI-modified MSC

[0220] Bone marrow-derived MSCs from C57BL/6 mice, ("wild-type"), a diabetes-resistant strain, and from CD44-KO mice ($CD44^{-/-}$ on C57BL/6 background) showed characteristic small spindle fibroblast-like pattern and could be differentiated into mesodermal cell types (**Figure 7**). MSCs expressed cell surface markers characteristics of MSCs (**Figure 1(A)**). MSCs did not natively express E-selectin ligands, as shown by absence of reactivity with mAb HECA 452 (which recognizes canonical sialofucosylated E-selectin binding determinants (such as sialylated Lewis X (sLe^x)) and by absence of binding to E-selectin-Ig chimera (E-Ig) that serves as a probe for E-selectin ligand activity (**Figure 1(B)**).

[0221] A prior study of human MSCs showed that cell surface glycoengineering (*i.e.*, Glycosyltransferase-Programmed Stereosubstitution (GPS)) using the α-(1,3)-fucosyltransferase VI (FTVI) induces E-selectin adherence via conversion of native membrane CD44 into the molecule known as Hematopoietic Cell E-/L-selectin Ligand (HCELL), a fucosylated sialyllactosaminyl glycovariant of CD44 that potently binds E-selectin[19]. To determine whether cell surface exofucosylation could program E-selectin ligand activity on murine MSCs, cells were treated with FTVI using reaction conditions that were optimized to preserve cell viability. FTVI modification of MSCs markedly induced staining with mAb HECA452, as well as binding with a murine E-selectin-Ig chimera (mE-Ig) (**Figure 1(B)**). Notably, protease digestions of MSCs prior to FTVI modification markedly reduced mE-Ig reactivity, indicating that glycoproteins, not glycolipids, were predominant carriers of E-selectin binding determinants (**Figure 1(B)**). Following exofucosylation of mouse MSC, western blot of whole cell lysates (**Figure 1(C)**) and of immunoprecipitated CD44 (**Figure 1(D)**) revealed that the principal membrane glycoprotein decorated with the sialofucosylations recognized by E-Ig and HECA-452 was the "standard" (*i.e.*, containing no splice variant exons) form of CD44 (~100 kDa). These data indicate that FTVI modification converts murine MSC surface CD44 into HCELL.

FTVI-modified mouse MSCs have increased binding to E-selectin under physiologic shear stress conditions

[0222] To assess the capacity of FTVI-modified mouse MSCs to bind E-selectin under physiologic shear stress conditions, we performed parallel plate flow

chamber assays of both unmodified and FTVI-modified MSCs. To this end, HUVEC monolayers were first stimulated with TNF-α to upregulate E-selectin expression. As shown in **Figure 2**, FTVI-modification enabled MSC adhesion to HUVEC at shear stress levels of 0.5 dynes/cm^2 to as much as 20 dynes/cm^2. In flow chamber studies, MSC adherence to stimulated HUVEC was strictly dependent on E-selectin receptor/ligand interactions as incubation of HUVEC with function-blocking E-selectin mAb and sialidase treatment of MSCs (to eliminate sLe^x display) each profoundly reduced adhesion of FTVI-modified MSCs, to levels similar to that of unmodified MSCs (**Figure 2**). Altogether, these findings indicate that FTVI modification of murine MSC induces potent E-selectin binding activity, capable of sustaining E-selectin adherence at hemodynamic shear stress levels well beyond those typical of post-capillary venules[17].

Systemically-administered FTVI-modified MSCs potently reverse new onset hyperglycemia in NOD mice

[0223] To assess whether FTVI-modification affected the capacity of MSCs to modulate diabetes in NOD mice, new onset diabetic NOD mice either received no cells (untreated control), or received 5x10^5 FTVI-modified or 5x10^5 unmodified C57BL/6 MSCs intravenously (via tail vein) on day 2 of hyperglycemia (glucose >250 mg/dL), followed by intravenous injections at days 7 and 30 after onset of hyperglycemia. As shown in **Figure 3(A)**, untreated NOD mice showed a rapid increase in their blood glucose levels and, with exception of one animal, died within a few weeks of the onset of hyperglycemia. As compared to the administration of unmodified MSCs (**Figure 3(B)**), which resulted in a temporary reversal of autoimmune diabetes (*i.e.*, 3 of 9 mice were normoglycemic at 3 weeks, 2 of 9 animals were normoglycemic at 6 weeks, and all animals were hyperglycemic by 12 weeks), the infusion of FTVI-modified MSCs robustly and durably reversed hyperglycemia (**Figure 3(C)**). Strikingly, 7 out of 8 mice were normoglycemic for 3 weeks, 6 of 8 mice remained normoglycemic for 6 weeks, and 5 of 8 mice were free of diabetes for upwards of 12 weeks following initial treatment (**Figure 3(C)**). While the majority of the mice in the unmodified and FTVI-modified MSC group which had blood glucose levels above 600 mg/dl survived up to 90 days, only one (out of 7 mice) in the untreated group (*i.e.*, receiving no MSCs) survived 90 days.

E-selectin expression and MSC localization in the pancreas of NOD mice

[0224] To examine the effect of FTVI-modification on MSC trafficking to the pancreas, we first assessed the expression of E-selectin within microvasculature of the pancreas of 14-week-old NOD mice using immunofluorescence microscopy. Peri-islet microvessels are reported to be the primary site of inflammatory cell trafficking which results in insulitis [23, 24]. There was no E-selectin expression in pancreata of diabetic-resistant mice (**Figure 4(A)**), whereas peri-islet microvessels of the pancreas of NOD mice expressed E-selectin (**Figure 4(B)**), which co-localized with CD31 staining in sequential sections (**Figures 4(C) and 4(D)**). Infiltration of T-cells into the diabetic islet margins was confirmed by CD3 staining of NOD pancreata during diabetic onset (**Figure 4(E)**). To assess pancreatic infiltration of systemically administered FTVI-modified MSCs in NOD mice, we stained frozen sections of pancreata with antibody HECA452. As shown in **Figures 4(F) and 4(G)**, HECA452$^+$ MSCs were observed in the peri-islet area within the vicinity of E-selectin-expressing microvasculature 1 day after transplantation, colonizing (E-selectin-expressing) perivascular regions and (L-selectin-expressing) areas of T cell infiltrates. To assess the extent of homing of FTVI-modified and unmodified MSCs after systemic administration, GFP-transduced FTVI-modified and unmodified MSCs were intravenously injected into NOD mice, and their pancreata, pancreatic and mesenteric lymph nodes and spleen were harvested at day 4 post-injection, sectioned, and assessed for presence of MSCs by fluorescence microscopy. FTVI-modified MSCs showed 3-fold higher infiltration of pancreas as compared to unmodified MSCs, whereas no difference in pancreatic infiltrates of infused FTVI-modified and unmodified MSC were seen in diabetes-resistant BALB/c mice (**Figure 4(H)**). However, no difference was noted in the extent of homing of FTVI-modified and unmodified MSCs into lymphoid tissues of treated NOD mice.

Exofucosylation has no effect on MSC immunosuppressive capacity or on *in vivo* survival

[0225] We have previously reported that measuring human growth hormone (hGH) produced by hGH-transduced MSCs is a sensitive method to ascertain longevity of administered MSCs[21]. To assess if the enhanced reversal of hyperglycemia of administered FTVI-modified MSCs was consequent to a survival advantage, $5x10^5$ of hGH-transduced FTVI-modified and unmodified MSCs were injected into NOD mice at day 2 and 7 following onset of hyperglycemia, and serum hGH was measured at various

time-points post-injection. As shown in **Figure 5(A)**, there was no difference in the pattern of hGH production, indicating that FTVI modification did not affect MSC persistence *in vivo*. To evaluate whether exofucosylation modifies MSC immunomodulatory capabilities, we performed co-culture T cell suppression assays. T cell proliferation was equally dampened by FTVI-modified and unmodified MSCs (**Figure 5(B)**), indicating that exofucosylation does not impart additional immunomodulation properties on MSCs.

Absence of MSC CD44 expression abrogates the anti-diabetic effect of FTVI-modified MSC

[0226] To examine whether CD44 expression is requisite for the enhanced anti-diabetic potency of FTVI-modified MSCs, we generated MSCs from CD44 KO mice ($CD44^{-/-}$ on C57BL/6 genetic background). With exception of CD44, MSCs generated from marrow of CD44 KO mice showed identical levels of cell surface adhesion molecules. By flow cytometry, exofucosylated CD44 KO MSCs showed HECA452-reactivity equivalent to that of exofucosylated wild-type MSCs (**Figure 8**), indicating that sialofucosylated determinants were created on alternative (*i.e.*, non-CD44) scaffolds. To assess whether administration of CD44 KO MSCs conferred anti-diabetic activity, $5x10^5$ cells each of FTVI-modified and unmodified CD44 KO and of C57BL/6 wild-type MSCs were injected into new-onset diabetic NOD mice at days 2, 7 and 30 post-hyperglycemia onset. Compared to wild-type MSCs, CD44 deficiency of MSCs resulted in a significant impairment in their anti-diabetic effect, as demonstrated by failure in reversing autoimmune diabetes in 5 out 6 mice receiving unmodified CD44 KO MSCs, and in 6 out 7 mice receiving FTVI-modified CD44 KO MSCs (**Figures 6(A) and 6(B)**, respectively). Following injection in NOD mice, CFSE-labeled FTVI-modified and unmodified CD44 KO MSCs showed no difference in trafficking to pancreata (**Figure 6(C)**), with levels similar to that observed for wild-type MSCs (**Figure 4(H)**). MSC suppressive capacity *in vitro* was not abrogated by lack of MSC CD44 expression nor by exofucosylation of CD44 KO MSCs (**Figure 6(D)**), suggesting that the absence of euglycemic effect(s) of administered FTVI-treated $CD44^{-/-}$ MSCs is not attributable to inherent deficits in immunoregulation. Notably, the finding that exofucosylation renders expression of cell surface sialofucosylations recognizable by HECA452 on CD44 KO MSCs yet such cells do not realize intended biologic outcomes in diabetic NOD mice clearly highlights the importance of biochemical studies (*e.g.*, western blot analysis) –

beyond just simple flow cytometry studies to evaluate induced E-Ig and HECA452 immunoreactivity - in defining/predicting the biologic impact of enforced selectin binding engendered by cell surface glycan engineering.

DISCUSSION

[0227] Because of their immunomodulatory properties, safety profile, and robust expansion *ex vivo*, MSCs have become the focus of several human trials to treat various refractory immune-mediated diseases including T1D[6]. MSCs have been found to suppress insulitis and autoimmune diabetes via multifaceted immunomodulatory effects on pathogenic components critical to elaboration of T1D[25, 26]. These effects include the capacity to modulate expression of inflammatory vs. regulatory cytokines thereby promoting expansion of regulatory DC and T cells, and MSC expression of negative costimulatory molecules (*e.g.*, PDL-1) which can directly inhibit autoreactive T cells[8-10,13]. Hence, MSC therapy has great potential to be an exciting and unique strategy for T1D treatment. Nevertheless, there is a pressing need for studies to improve the efficacy MSC-based therapy in T1D[6].

[0228] In prior studies, we observed that intravenous administration of fully allogenic MSCs was associated with a transient reversal of hyperglycemia in diabetic NOD mice[9]. We hypothesized that a proximate hurdle in realizing a more robust anti-diabetic effect might be the relative paucity of MSC homing to inflamed pancreas. Here, we sought to assess whether systemic administration of MSCs with enhanced homing capacity via enforced HCELL expression would influence reversal of diabetes in the NOD model. To this end, we utilized the C57BL/6 strain as the allogenic source of MSCs since the CD44 KO phenotype was available on this genetic background. We focused on metabolic control as the primary end-point for assessing the efficacy of MSC therapy, as we had previously examined the immune mechanisms mediating anti-diabetic effects of MSC[9, 10].

[0229] In contrast to other studies which characteristically assess the effects of anti-diabetic interventions for relatively short time periods (typically <30 days), in this study the effects of MSC on reversal of autoimmune diabetes were assessed over a prolonged observation period (90 days). Our data here indicate that conversion of native membrane CD44 into HCELL by cell surface α-(1,3)-exofucosylation ("FTVI-modification") confers high efficiency mouse MSC binding to E-selectin. We observed E-

selectin expression in the peri-islet area of pancreata of NOD mice with insulitis, but not in pancreata of diabetic-resistant mice. Consistent with these findings, we did not observe differences in the level of homing of injected allogenic C57BL/6 FTVI-modified and unmodified MSCs into pancreata of diabetic-resistant mice (BALB/c hosts), but, compared with unmodified MSCs, there was heightened pancreatic infiltration of systemically administered C57BL/6 (allogenic) FTVI-modified MSCs in diabetic NOD mice, with concomitant durable reversal of diabetes. Notably, we did not observe differences in infiltration of lymphoid tissues between animals receiving FTVI-modified and unmodified MSCs, but there was marked increase in lodgement/engraftment of cells within the perivascular regions of E-selectin-expressing microvessels and within clusters of leukocytes. Collectively, these data draw attention to a primary role of MSC pancreatic colonization in suppressing autoimmune diabetes, but do not exclude the potential contribution of extrapancreatic effects of MSCs.

[0230] Compared to the anti-diabetic effects observed using intravenously administered fully allogenic unmodified MSCs in this study and in prior studies [9], the marked improvement in T1D reversal using fully allogeneic FTVI-modified (HCELL) MSCs observed here does not appear to be attributable to an enhanced immunosuppressive capacity of the glycoengineered MSC itself or to *in vivo* survival advantage endowed by glycoengineering of MSCs. In a prior study, we observed improved anti-diabetic effects in NOD mice following intravenous administration of semi-allogeneic MSCs obtained from NOR mice [10], likely a reflection of decreased rejection of the semi-allogeneic MSCs yielding improved *in vivo* tissue colonization/persistence of cells. However, in clinical applications, it would be advantageous to employ strategies to augment potency of fully allogeneic-source MSCs, especially as manufacturing and regulatory issues support the use of “off-the-shelf” (*i.e.*, culture-expanded, pooled) allogeneic MSC products. The finding that exofucosylated CD44 KO MSCs lack the capacity to induce durable reversal of hyperglycemia, despite evident creation of FTVI-dependent surface sialofucosylated determinants detectable by HEC452 reactivity, indicates that E-selectin binding determinants displayed expressly on the CD44 scaffold (*i.e.*, elaboration of HCELL) are required to achieve potent anti-diabetic effects. This may be related to a key role for HCELL expression to attain requisite tissue homing and extravasation[27] and/or could reflect a requirement for HCELL/CD44 expression to achieve appropriate lodgment in relevant microenvironmental sites[28]. A role for *in situ*

tissue lodgment to achieve MSC effect(s) has been highlighted by studies of MSC co-transplantation with islet allografts engendering immunoprivileged zones[29-31]. In this regard, the observed localization of HCELL$^+$ MSCs within islet perivascular areas and within islet leukocyte infiltrates highlights an operative role for HCELL in licensing immunomodulation: since E-selectin is expressed within endothelial beds of the affected tissue and leukocytes characteristically express L-selectin, expression of HCELL, a potent E-selectin and L-selectin ligand, serves to promote tissue lodgement expressly within the microenvironments of most intense immunoreactivity. While further studies of the immunomodulatory effects of MSC *in vivo* are warranted, the data here highlight the potential of enhanced pancreatotropism via systemic administration of HCELL$^+$ MSCs in the therapy of T1D. More broadly, the fact that E-selectin is upregulated by cytokines such as TNF and IL-1 within endothelial beds at all inflammatory sites[17,18] offers the opportunity to exploit enforced MSC HCELL expression to improve the efficacy of MSC-based therapy for a wide variety of inflammatory conditions.

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EXAMPLE 7 - CELL SURFACE GLYCAN ENGINEERING OF NEURAL STEM CELLS AUGMENTS NEUROTROPISM AND IMPROVES RECOVERY IN A MURINE MODEL OF MULTIPLE SCLEROSIS

INTRODUCTION

[0262] Nature has developed an extremely efficient mechanism to deliver circulating cells to sites of inflammation and injury. This process is controlled by a highly ordered cascade of molecular interactions (Butcher, E.C. 1991; Sackstein, R. 2005; Springer, T.A. 1994). The first essential event in cell recruitment involves shear-resistant adhesion of flowing cells on the endothelial surface, a process most efficiently mediated by selectins, a family of three Ca^{2+} dependent lectins (comprised of E-, P-, and L-selectin), binding to their respective counter-receptors. These interactions initially tether the cell to the vessel wall and, in the context of vascular shear flow, cause the cell to roll along the endothelial surface at velocities below that of the prevailing hemodynamic stream (Step 1). This process facilitates engagement of specific cell-borne chemokine receptors to pertinent chemokines present in the perivascular areas, thereby triggering inside-out signal transduction events leading to increased adhesiveness of integrin family members (Step 2). Adhesive interactions between the

activated cell integrins and their cognate endothelial cell counter-receptors then leads to arrest of rolling and firm adhesion of the cell to the vessel wall (Step 3), and, ultimately, transendothelial migration (extravasation, Step 4).

[0263] In multiple sclerosis (MS), and in its animal model, experimental autoimmune encephalomyelitis (EAE), recruitment of immunologic effectors is mediated by the upregulation of the vascular selectins, E- and P-selectin. E- and P-selectin are expressed on brain endothelium after *in vivo* activation with LPS or TNF-α, however, in murine EAE, P-selectin is expressed only transiently (Piccio, L., Rossi, B., et al. 2002). Notably, E-selectin is expressed throughout the inflammatory period with a patchy distribution at sites where vessels branch, suggesting the existence of preferential recruitment areas (Piccio, L., Rossi, B., et al. 2002). E-selectin is also characteristically found in vessels from acute plaques in MS patients (Lee, S.J. and Benveniste, E.N. 1999; Washington, R., Burton, J., et al. 1994). These findings suggest that E-selectin plays a dominant role in the recruitment of circulating cells to the brain in inflammatory diseases.

[0264] All three selectins bind to specialized carbohydrate determinants, comprised of sialofucosylations containing an α(2,3)-linked sialic acid substitution on galactose, and an α(1,3)-linked fucose modification on N-acetylglucosamine, prototypically displayed as the terminal tetrasaccharide sialyl Lewis X (sLe^x) (Polley, M.J., Phillips, M.L., et al. 1991; Sackstein, R. 2005). This structure, also known as "CD15s", may be displayed on either a protein scaffold (*i.e.*, a glycoprotein) or a lipid scaffold (*i.e.*, a glycolipid), and is recognized by mAb such as CSLEX-1 and HECA-452 (Alon, R., Feizi, T., et al. 1995; Dimitroff, C.J., Lee, J.Y., et al. 2001; Fuhlbrigge, R.C., Kieffer, J.D., et al. 1997; Gadhoum, S.Z. and Sackstein, R. 2008; Merzaban, J.S., Burdick, M.M., et al. 2011). Although additional structural modifications principally involving sulfation increase binding affinity of P- and L-selectin to sLe^x, no such modifications are needed for optimal binding of E-selectin (Leppanen, A., White, S.P., et al. 2000; Rosen, S.D. 2004).

[0265] Neural stem cell (NSC)-based therapy has generated great hope for halting and/or reversing disease progression in CNS inflammatory and/or degenerative diseases (Ben-Hur, T., Einstein, O., et al. 2003; Einstein, O., Fainstein, N., et al. 2007; Einstein, O., Grigoriadis, N., et al. 2006; Imitola, J., Raddassi, K., et al. 2004; Lee, S.T.,

Chu, K., et al. 2008; Pluchino, S., Quattrini, A., et al. 2003; Pluchino, S., Zanotti, L., et al. 2005; Ziv, Y., Avidan, H., et al. 2006). It is well known that NSCs express Step 2 and Step 3/4 effectors, such as CXCR4 (Bezzi, P., Domercq, M., et al. 2001; Flax, J.D., Aurora, S., et al. 1998; Imitola, J., Raddassi, K., et al. 2004) and VLA-4 (Pluchino, S., Zanotti, L., et al. 2005; Rampon, C., Weiss, N., et al. 2008), respectively, but there are no data on whether NSCs natively express Step 1 effectors. Accordingly, we examined expression of Step 1 effectors on mouse NSCs, and found that these cells are conspicuously devoid of Step 1 effectors, in particular, E-selectin ligands. Using the EAE model, we analyzed whether enforced E-selectin ligand activity via cell surface glycan engineering would impact migration of administered NSCs and, more importantly, the therapeutic effect(s) of administered cells. To this end, we utilized a technology called glycosyltransferase-programmed stereosubstitution (GPS) to create relevant selectin-binding glycan determinants on the cell surface (Sackstein, R., Merzaban, J.S., et al. 2008). We report here that GPS enforces expression of E-selectin ligands on NSCs by modifying glycans of two neural stem cell membrane glycoproteins, CD44 and of NCAM, creating the E-selectin ligands HCELL (Hematopoietic Cell E-/L-selectin Ligand) and NCAM-E, respectively. Glycoengineering of NSC E-selectin ligand activity resulted in increased neurotropism and yielded improved clinical outcome in EAE in absence of detectable long-term engraftment, indicating that these tissue-specific stem cells engendered a restorative effect, not a direct regenerative effect. Importantly, this neurorestorative effect is not a universal property of adult stem cells, as administration of GPS-engineered mouse hematopoietic stem/progenitor cells (HSPC) did not improve EAE clinical course. These findings have profound clinical implications in providing the first direct evidence that cell surface glycoengineering to create effectors of cell migration can improve the efficacy of stem cell-based therapeutics, and also provide new perspectives on the use of NSCs in the treatment of neuroinflammatory diseases.

RESULTS

Expression of molecular effectors of cell migration on neural stem cells

[0266] To analyze expression of molecular effectors of Step 1, Step 2 and Step 3/4 of cell migration, flow cytometry was performed on primary cultures of mouse NSCs. NSCs were devoid of reactivity with E-selectin-Ig chimera (E-Ig) and P-selectin-

Ig chimera (P-Ig), indicating absence of E- and P-selectin ligands (**Figure 9(A)**) even in the presence of inflammatory mediators (**Figure 15**); they also did not stain with mAb CSLEX1, KM93, or HECA452 (each of which identify sLe^x). NSCs expressed CD44 and the integrins VLA-4 (CD49d/CD29) and VLA-5 (CD49e/CD29), as well as the chemokine receptor CXCR4; this pattern of NSC marker expression has been observed by others (Back, S.A., Tuohy, T.M., et al. 2005; Campos, L.S., Decker, L., et al. 2006; Campos, L.S., Leone, D.P., et al. 2004; Imitola, J., Raddassi, K., et al. 2004; Ji, J.F., He, B.P., et al. 2004; Leone, D.P., Relvas, J.B., et al. 2005; Pluchino, S., Quattrini, A., et al. 2003; Pluchino, S., Zanotti, L., et al. 2005) (**Figure 9(B)**). The NSCs also characteristically expressed Neural Cell Adhesion Molecule (NCAM) in addition to the well-described polysialic acid (PSA) (Vitry, S., Avellana-Adalid, V., et al. 2001) (**Figure 9(B)**). NSCs did not express PSGL-1, CD43, LFA-1 (lacking both CD11a (α_1) and CD18 (β_2) chains), and LPAM-1 ($\alpha_4\beta_7$) (**Figure 9(B)**). These results indicate that NSCs are deficient in expression of Step 1 effectors of the multistep cascade of cell transmigration, yet express chemokine receptors and relevant integrin effectors that mediate Steps 2-4 in extravasation and that are also involved in their mobilization in the developing brain via radial migration (Imitola, J., Comabella, M., et al. 2004).

GPS enforces E-selectin ligand activity on neural stem cells

[0267] CD44, a molecule involved in migration of NSCs (Deboux, C., Ladraa, S., et al. 2013) and brain cancer stem cells (Fu, J., Yang, Q.Y., et al. 2013), is strongly expressed among NSCs in culture (**Figure 9(B)**). However, the finding that NSC lack E-selectin binding (**Figure 9(A)**) indicates that these cells do not natively express the E-selectin binding glycoform of CD44 known as HCELL (Dimitroff, C.J., Lee, J.Y., et al. 2000; Dimitroff, C.J., Lee, J.Y., et al. 2001; Sackstein, R. 2004). We thus sought to determine whether a non-genetic manipulation using glycosyltransferase-programmed stereosubstitution (GPS) of CD44 glycans would enforce HCELL expression (Sackstein, R., Merzaban, J.S., et al. 2008). To this end, we treated NSCs with the α(1,3)-linkage-specific fucosyltransferase, fucosyltransferase VI (FTVI). This enzyme specifically places a fucose onto a terminal type 2-lactosamine unit; if that lactosamine is capped with an α(2,3)-linked sialic acid, sLe^x is created. Following FTVI treatment of NSCs (GPS-NSC), reactivity with mAbs CSLEX1, KM93 and HECA452 was induced, consistent with strong expression of sLe^x epitopes (**Figure 10(A)**), with associated E-Ig binding (**Figure 10(A)**) but without induction of P-Ig binding (**Figure 10(A)**). Notably,

expression of CD15 (also known as SSEA-1 or Le^x) is high in NSCs (**Figure 10(A)**), and although FTVI can fucosylate unsialylated terminal lactosamines thereby yielding CD15 (SSEA-1), the expression of CD15 was unchanged following enforced fucosylation (**Figure 10(A)**). Altogether, these data indicate that α(1,3)-exofucosylation only occurred on sialylated lactosaminyl glycans. Bromelain digestion of NSCs prior to GPS treatment markedly reduced HECA452 reactivity (**Figure 10(B)**), demonstrating that glycoproteins, not glycolipids, were the predominant carriers of sialofucosylated determinants. Treatment of GPS-NSCs with phosphatidylinositol phospholipase C (PI-PLC) resulted in a modest but significant decrease in the HECA452 signal by FACS (**Figure 10(C)**), indicating that a minor population of sLe^x –decorated glycoproteins are glycophosphatidyl (GPI)-linked.

[0268] Western blot of cell lysates and of immunoprecipitated CD44 from GPS-NSCs revealed that one of the glycoproteins decorated with the essential sialofucosylations recognized by HECA452 was the standard, unspliced, form of CD44 (~100 kDa; **Figure 11(A)**), and CD44 also reacted with E-Ig (**Figure 11(A)**). However, following exhaustive immunoprecipitation of CD44, other candidate glycoprotein E-selectin ligand(s) were identified by evidence of reactivity with E-Ig and HECA452 in the residual supernatant fraction. Two bands were apparent at ~120 and ~140kDa. Based on the molecular weight profile of these bands (**Figure 11(A)**) and the partial PI-PLC sensitivity of E-selectin binding (**Figure 10(C)**), a characteristic of the 120 kDa form of NCAM (Gascon, E., Vutskits, L., et al. 2007; Maness, P.F. and Schachner, M. 2007; Rutishauser, U. 2008), we speculated that NCAM could be serving as an additional E-selectin ligand. We thus performed immunoprecipitation with a pan-NCAM mAb, and observed that the residual bands persisting after exhaustive immunoprecipitation of CD44 were indeed those of NCAM (**Figure 11(B)**). To determine if the relevant sialofucosylations on NCAM were displayed on N-glycans, we tested E-Ig reactivity on western blot of lysates of GPS-NSCs following digestion with *N*-glycosidase F (**Figure 11(A)**); no evident staining with E-Ig following digestion was observed, indicBing that the relevant E-selectin binding determinant(s) are displayed on N-glycans. The contribution of the GPI-anchored form of NCAM-E to overall sLe^x expression after enforced fucosylation of NSC is modest, as shown by a small decrease in HECA452 signal (and E-Ig signal – data not shown) following PI-PLC treatment (**Figure 10(C)**). Therefore, enforced α(1,3)-fucosylation of murine NSCs created HCELL as well as a

unique E-selectin ligand reactive form of the neural precursor molecule NCAM, which we named "NCAM-E". Interestingly, human NSCs (CC-2599) only express HCELL and not NCAM-E following GPS treatment (**Figures 16(A)-16(C)**).

[0269] To assess the stability of GPS-engineered E-selectin ligand activity on NSCs, we measured E-Ig reactivity by flow cytometry at 24 h intervals following enforced exofucosylation. E-selectin ligand activity was stable for up to 24 h, subsequently declining to undetectable levels by 72 h, presumably due to turnover of surface protein (**Figure 11(C)**). NSC viability (**Figure 17**) was unaffected by enforced exofucosylation and there were no differences in the number or proliferation of neurospheres in clonogenic assays or in differentiation of NSCs (**Figures 18(A)-18(D)**). Thus, there were no evident phenotypic differences induced by GPS treatment of NSCs, except for creation of E-selectin ligands.

[0270] NSCs have been shown to inhibit the proliferation T cells *in vitro* (Einstein, O., Fainstein, N., et al. 2007; Einstein, O., Grigoriadis, N., et al. 2006; Martino, G. and Pluchino, S. 2006), and, in particular, to dampen mitogenic responses of T cells *in vitro*. To assess whether this immunoregulatory function of NSCs is affected by enforced E-selectin ligand activity, we examined the ability of GPS-NSCs and control buffer-treated NSCs (BT-NSCs) to suppress the proliferation of lymph node cells in response to Con A activation. As displayed in **Figures 19(A)-19(C)**, both GPS- and BT-NSCs were able to decrease T cell proliferation as well as suppress the level of inflammatory cytokine production equally, suggesting that there is no immunomodulatory advantage or disadvantage provided by the GPS treatment itself on NSCs. Interestingly, we also observed equal release of leukemia inhibitory factor (LIF) from NSCs upon inflammatory cytokine treatment for both BT- and GPS-NSCs that was enhanced upon binding of GPS-treated cells to E-Ig (**Figure 19(C)**). Thus, enforced exofucosylation of NSCs induced transient E-selectin ligand activity without undesirable effects on NSC phenotype or function as determined by *in vitro* studies.

GPS-NSCs display robust physiological rolling interactions with E-selectin

[0271] To analyze the potency of E-selectin ligand activity of GPS-NSCs under physiologic blood flow conditions, parallel plate flow chamber studies were performed using human umbilical vein endothelial cells (HUVEC) stimulated by cytokines (IL-1β and TNF-α) to express E-selectin. As shown in **Figure 12(A)**, GPS-

NSCs exhibited prominent E-selectin ligand activity that was completely abrogated in the presence of EDTA or by the use of a blocking anti-E-selectin mAb. Significant shear-resistant rolling interactions were observed within usual post-capillary venular shear levels (1-4 dynes/cm^2), and persisted over 20 dynes/cm^2 (**Figure 12(A)**). To analyze which of the glycan-engineered E-selectin ligands, HCELL or NCAM-E, is the more potent E-selectin ligand on NSCs, we used the blot-rolling assay.(Fuhlbrigge, R.C., King, S.L., et al. 2002) This technique permits the detection of shear-dependent selectin ligand interactions on membrane proteins resolved by SDS-PAGE, thus allowing the evaluation of the individual contribution of HCELL and NCAM-E to the E-selectin ligand activity of GPS-NSC. Accordingly, to evaluate the respective E-selectin binding properties, E-selectin transfected CHO cells (CHO-E) were perfused over HECA-452 immunostained blots of GPS-NSC lysates. As illustrated in **Figure 12(B)**, more CHO-E cells interacted and adhered to HCELL compared to either the 120kD or the 140kD forms of NCAM-E. CHO-E cells suspended in flow medium containing 5 mM EDTA had negligible adhesion to any regions of the blot, confirming Ca^{2+}-dependent binding consistent with selectin ligand activity.

GPS-NSCs exhibit increased homing and tissue infiltration *in vivo* in EAE

[0272] To assess whether injected NSCs infiltrated the CNS parenchyma, we performed confocal microscopy studies. To this end, GPS-NSCs and BT-NSCs were labeled with PKH26 dye and were injected intravenously into MOG-induced chronic EAE mice in two separate i.v. injections: before disease onset (day 9 post-immunization (PI)) and at the onset of the disease (day 13 PI). These days were chosen based on prior studies of E-selectin expression on brain endothelium in EAE (Piccio, L., Rossi, B., et al. 2002). Lumbar-sacral spinal cords and brains were harvested 4 days (day 17 PI) after the second NSC injection. Confocal analysis of the brain demonstrated both higher amounts of GPS-NSCs (**Figure 13(A)**) and localization outside of Flk-1$^+$ vessels in the brain (**Figures 20(A)-20(B)**). Further, parallel analysis of the spinal cord (**Figures 13(B)-13(D)**), where the majority of pathology occurs in EAE, demonstrated two-fold greater numbers of extravascular GPS-NSCs infiltrates over BT-NSCs infiltrates by day 17 PI (**Figure 13(D)**). These data indicate that GPS-NSCs infiltrate the CNS parenchyma significantly more effectively than BT-NSCs in both the brain and the spinal cord. To further confirm our confocal data, we studied the effect of GPS-engineered E-

selectin ligand activity on short-term homing of NSCs *in vivo*. NSCs were stained using a fluorochrome tracking dye, CFSA-SE (carboxyfluorescein diacetate succinimidyl ester) and adoptively transferred into C57BL/6 mice on day 9 and day 13 PI with MOG as described in *Materials and Methods*. Within 16 h after the second cell injection, we observed that GPS-NSCs accumulated in the brain, spleen and liver three- to five-fold more efficiently than BT-NSCs (**Figure 13(E)**). The relative advantage of GPS-NSCs in infiltrating the brain, spleen and liver reflected a true difference in trafficking, and not simply the preferential expansion of these cells in situ, as their average CFDA-SE fluorescence was not reduced relative to BT-NSCs (data not shown). Indeed of those cells that migrated to the spleen, it is evident that there are close interactions of NSCs with $CD4^+$ T cells (**Figure 13(F)**). Injected cells also accumulated in lungs, but without difference between GPS- and BT-NSC, likely a reflection of steric trapping in pulmonary microvessels (**Figure 13(E)**). Thus, the sLe^x structure formed on NSCs following GPS-treatment licenses migration of these cells into these organs, and highlights the critical role of the E-selectin ligand activity in driving tissue-specific migration of NSCs in vivo.

GPS-NSCs increased homing translates into amelioration of neuropathology by enhanced endogenous indirect neuroregeneration in EAE

[0273] To address whether improved tissue homing of NSC had an enhanced therapeutic effect, we monitored the neurologic status of C57BL/6 mice receiving NSC injections following MOG-induced chronic EAE and analyzed clinical parameters as well as tissue restoration and pathology. Neural precursors were administered in two separate i.v. injections on day 9 PI and on day 13 PI. Five groups of EAE mice were tested: (1) GPS-NSCs; (2) BT-NSCs; (3) HBSS (sham; *i.e.* no cells); (4) BT-HSPC; and (5) GPS-HSPC. The clinical score was evaluated daily in individual mice in a blind fashion (**Figure 14(A)**), and the linear regression of cumulative burden of disease was calculated (**Figure 14(B)** and Table 2).

Treatment	Route of cell administration	No. of mice	Disease onset (days p.i)	Maximum clinical score	Cumulative disease score (0-20 p.i)	Cumulative disease score (21-30 p.i)
No NSC	-	30	13.22±0.6	2.2±0.4	15.75±0.6	14.9±2.4
NSCs BT	i.v	30	12±0.6	2.1±0.3	12.3±0.9	10.9±2
NSCs-GPS	i.v	30	12.2±0.5	1.8±0.1 *	10.8±1.4 *	7.1±1.5 *

* p<0.05 ANOVA multiple comparisons.

Table 2. EAE features in C57BL/6 mice treated i.v. GPS treated NSCs.

The injection of control NSCs (BT-NSC) attenuated the clinical severity of EAE compared to HSPC and sham treated animals ($p = 0.006$). However, i.v. injection of GPS-NSCs (n = 30) showed a more significantly improved clinical score compared to BT-NSCs (n = 30; $p = 0.006$), GPS-HSPC (n = 30; $p < 0.0001$), BT-HSPC (n = 30; $p < 0.0001$), and sham (n = 30; $p < 0.0001$) treated animals. It is important to note that this amelioration of disease severity is specific to NSCs, since neither the injection of GPS-HSPC (which display markedly increased E-selectin ligand activity, see **Figure 21(A)** (Merzaban, J.S., Burdick, M.M., et al. 2011)) or the injection of BT-HSPC showed an improvement in the clinical score of MOG-induced animals compared to control animals (**Figure 21(B)-21(C)** and Table 3).

Treatment	Route of cell administration	No. of mice	Disease onset (days p.i)	Maximum clinical score	Cumulative disease score (0-20 p.i)	Cumulative disease score (21-30 p.i)
No Cells	-	30	13.22±0.6	2.2±0.4	15.75±0.6	14.9±2.4
BT-HSPC	i.v	30	10±0.7	2.1±0.2	17.2±1*	16.7±1*
GPS-HSPC	i.v	30	9.5±0.4	2.5±0.6	18.3±2.2**	16.8±1.7*

* p<0.05 ANOVA multiple comparisons.

Table 3: EAE clinical features in C57BL/6 mice injected with i.v. GPS treated HSPCs.

Indeed, injection of either GPS- or BT-HSPCs showed a trend towards worsened clinical outcomes indicating that the observed salutary effects of NSC infusion are not a general property of adult stem cells.

[0274] Notably, examination of the neuropathology at day 30 after EAE induction showed statistically significant differences in several parameters of inflammation and neuroregeneration in mice that received GPS-NSC compared to BT-NSC and sham treated animals (**Figure 14(C)**). To determine the impact of NSC injections on the neuropathology of EAE, we evaluated a number of different markers. First, to assess the extent of inflammation, we stained sections of spinal cord from each study group of mice for CD11b, a marker that identifies infiltrating macrophages and microglia. Animals with EAE that received HBSS buffer alone displayed high levels of staining for CD11b, with cells exhibiting increased numbers of membrane processes, morphologic evidence of an activated phenotype (**Figure 14(C)**). The numbers of CD11b-stained cells were significantly decreased in mice that received BT-NSCs ($p < 0.0001$), and notably, these levels were even further decreased in animals injected with

GPS-NSCs ($p < 0.005$ compared to BT-NSCs). Importantly, the macrophage/microglia displayed decreased CD11b staining and more discrete membrane processes indicative of decreased activation (**Figure 14(C)**). In addition, quantification of brain CD4+ T cells in the different treatment groups revealed a significant decrease of infiltrating T cells in animals given GPS-NSCs (**Figures 14(D) and 14(E)**). To assess neuroregeneration, we stained sections of spinal cord for the markers GAP-43 and SMI-32 (**Figures 14(F)-14(I)**). In animals injected with GPS-NSCs, there was increased expression of GAP-43, a molecule associated with axon integrity and regeneration, compared to that in mice that received either BT-NSCs or HBSS buffer alone (**Figures 14(F)-14(G)**). Conversely, a specific reduction in the expression of SMI-32, a marker of axonal degeneration, was observed in animals that received GPS-NSCs compared to mice receiving either BT-NSCs or HBSS buffer (**Figures 14(H)-14(I)**). These data support the notion that the improved clinical effects afforded by GPS-NSCs over BT-NSCs are secondary to increased lesional migration of tissue-specific NSC yielding enhanced neuroprotection.

[0275] To further assess whether observed effects of GPS-NSCs reflect increased neuroregeneration, we stained for markers associated with oligodendrogenesis and mature oligodendrocytes, including CNPase, Olig-2, and SOX9. We observed a statistically significant enhancement in the number of Olig-2, SOX9 and CNPase cells in animals that received GPS-NSCs compared to BT-NSCs or sham control (**Figures 14(D) and 14(E)**). Although there was evidence of injected NSCs at day 17 PI (SOX-2^+ cells; **Figures 20(A)-20(B)**), we did not observe persistent colonization by NSC (tagged with GFP) at 30 days PI (**Figure 22**) in any of the injection groups, suggesting that the mechanism of NSC neuroprotection associated with improved homing does not necessitate the continued presence, nor differentiation towards neural lineage cells, of administered NSCs. Altogether, these data indicate that the treatment of mice with GPS-NSCs enhanced the delivery of NSCs to the CNS and provided neurorestoration via an indirect neuroregenerative effect, not through direct neuroregeneration (*i.e.*, cell replacement).

DISCUSSION

[0276] MS is a chronic, demyelinating disease of the CNS characterized by multifocal inflammatory lesions yielding gradual destruction of the myelin sheath, leading to axonal injury and loss (Imitola, J., Chitnis, T., et al. 2006). Stem cell-based

therapeutics offers the promise of repair of damaged/inflamed tissue by replacing affected cells (direct regeneration) and/or by production of supportive/trophic factors in the milieu that evoke tissue restoration by endogenous cells (indirect regeneration). In the case of disseminated neurologic diseases like MS, use of tissue-specific NSCs could prove clinically useful in achieving CNS recovery, but a proximate hurdle to accomplishing this goal is to deliver adequate numbers of cells to sites of neural injury. Prior studies have shown a benefit of transplanted NSCs in experimental models of stroke, spinal cord trauma and MS (Ben-Hur, T., Einstein, O., et al. 2003; Einstein, O., Fainstein, N., et al. 2007; Einstein, O., Grigoriadis, N., et al. 2006; Imitola, J., Raddassi, K., et al. 2004; Lee, S.T., Chu, K., et al. 2008; Pluchino, S., Quattrini, A., et al. 2003; Pluchino, S., Zanotti, L., et al. 2005; Ziv, Y., Avidan, H., et al. 2006). Direct injection into the affected site was used as route of delivery in these studies, however, in order to attain appropriate colonization of NSC within affected tissue(s) in multifocal CNS diseases, the vascular route of delivery is required as local administration (*i.e.*, in situ injection) is impractical given the diffuse nature of disease and associated anatomic constraints. To date, there have been no studies to evaluate the expression of molecular effectors of cell migration on NSCs, and, more importantly, no studies to address how optimizing expression of such effectors could enhance NSC neurotropism.

[0277] Our studies reveal that NSCs express relevant Step 2-4 effectors, but are conspicuously deficient in Step 1 effectors: (1) they do not natively express ligands for E-selectin or P-selectin (even when grown in the presence of inflammatory cytokines (**Figure 15**)); (2) they lack expression of the glycan sLe^x, which is the canonical selectin binding determinant; and (3) they do not express the glycoprotein PSGL-1, a selectin ligand on myelin-specific Th1 cells that has been reported to mediate trafficking to the brain (Piccio, L., Rossi, B., et al. 2005; Piccio, L., Rossi, B., et al. 2002). Importantly, expression of endothelial selectins, especially E-selectin, has been implicated in recruitment of immunologic effectors in MS and EAE (Lee, S.J. and Benveniste, E.N. 1999; Piccio, L., Rossi, B., et al. 2002). Thus, we sought here to assess whether glycan engineering of NSC to enforce expression of E-selectin ligands would enhance systemic delivery of the cells and, in consequence, have biologic effects in an MS model.

[0278] The dataset here show that cultured NSC express two well-characterized neural cell surface molecules, CD44 and NCAM (Back, S.A., Tuohy, T.M., et al. 2005; Pluchino, S., Quattrini, A., et al. 2003). Strikingly, exofucosylation of mouse

NSCs yielded high expression of sLe^x determinants prominently on these glycoproteins, programming conversion of CD44 into the potent E-selectin ligand HCELL (Dimitroff, C.J., Lee, J.Y., et al. 2000; Dimitroff, C.J., Lee, J.Y., et al. 2001) and also inducing expression of two sialofucosylated glycoforms of NCAM of ~120 kDa and ~140 kDa, which we designate "NCAM-E". Blot rolling assays revealed that HCELL is the principal E-selectin ligand expressed on GPS-NSCs (**Figure 12(B)**). These findings are corroborated by results of flow cytometry following the removal of NCAM-E by PI-PLC digestion, showing considerable retention of NSC sLe^x expression and E-Ig reactivity (**Figure 10(C)**).

[0279] NCAM, a member of the immunoglobulin superfamily, is expressed on both neurons and glia, and is conventionally viewed as a mediator of cell-cell interactions establishing a physical anchorage of cells to their environment. Post-translational glycan modifications consisting of α-2,8-linked polysialic acid (PSA) on the NCAM protein core provides unique properties in neural migration (Gascon, E., Vutskits, L., et al. 2007; Maness, P.F. and Schachner, M. 2007; Rutishauser, U. 2008) and PSA-NCAM appears to play a crucial role in mediating precursor cell migration in the brain (Glaser, T., Brose, C., et al. 2007; Lavdas, A.A., Franceschini, I., et al. 2006; Zhang, H., Vutskits, L., et al. 2004). Our data now provide the first evidence that NCAM displays relevant terminal α-(2,3)-sialyllactosaminyl glycans that can serve as acceptors for exofucosylation to create sLe^x determinants. Following cell surface glycoengineering of NSCs, PSA expression levels did not change and the induced E-selectin ligand activity is not permanent, as there was complete loss of sLe^x expression within 72 hours of enforced fucosylation (**Figures 11(A)-11(C)**). Thus, subsequent to extravasation, temporal reversion to native NCAM should occur, and infiltrating NSCs would then be capable of undergoing endogenous NCAM-mediated intraparenchymal CNS migration. Notably, though murine NSC express NCAM bearing α-(2,3)-sialyllactosaminyl glycans, our studies of human NSCs (CC-2599) indicate that these cells only express HCELL and not NCAM-E following exofucosylation (**Figure 15**); thus, human NSC may express relevant sialolactosaminyl glycans that serve as acceptors for enforced fucosylation only on CD44. These species differences should be considered when interpreting xenograft studies where human NSCs are used in rodent systems (Goncharova, V., Das, S., et al. 2014).

[0280] The induction of E-selectin ligand activity has profound implications for cell trafficking, serving to direct cell migration to endothelial beds that express E-selectin. As shown by others, we observed that NSCs display the chemokine receptor CXCR4 and the integrin VLA-4 (see **Figures 9(A)-9(B)**). It has been shown that expression of CXCR4 on human NSCs (Bezzi, P., Domercq, M., et al. 2001; Flax, J.D., Aurora, S., et al. 1998; Imitola, J., Raddassi, K., et al. 2004) promotes migration *in vivo* toward CNS injury wherein local astrocytes and endothelium up-regulate the cognate ligand stromal cell-derived factor 1α (SDF-1α, also known as CXCL12) (Imitola, J., Raddassi, K., et al. 2004). These observations define CXCR4 as a prominent Step 2 effector in promoting NSC homing to CNS injury. The corresponding endothelial receptor for VLA-4, VCAM-1, is also upregulated in the inflammatory response of the brain to injury (Justicia, C., Martin, A., et al. 2006). The VLA-4/VCAM-1 axis has similarly been shown to play a critical role in migration of NSC, in that only NSCs that constitutively express VLA-4, in addition to CXCR4, were able to accumulate around inflamed CNS microvessels in affected lesions in EAE (Pluchino, S., Zanotti, L., et al. 2005). A recent xenograft study suggests that integrins may play a role as step 1 mediators of migration on human NSCs in a rat stroke model suggesting that selectin interactions are not necessary in this model system (Goncharova, V., Das, S., et al. 2014). Although further work is warranted, the varying role(s) of integrins as mediators of Step 1 interactions could reflect differences in the inflammatory model used, the host animal system, the permeability of vessels, the expression of endothelial adhesion molecules at that site of inflammation, the presence of soluble adhesion molecules at the site, and the physical properties of the vessel that dictate the flow rate (*i.e.* diameter of vessel) (Berlin, C., Bargatze, R.F., et al. 1995; Ding, Z.M., Babensee, J.E., et al. 1999; Zarbock, A., Kempf, T., et al. 2012; Zarbock, A., Lowell, C.A., et al. 2007). In any case, expression of E-selectin ligands as Step 1 effectors on NSCs would serve to complement the constitutive expression of CXCR4 and VLA-4, thereby optimizing the recruitment of NSC to inflammatory sites. Accordingly, though we observed that intravenously administered (non-modified) BT-NSCs can infiltrate the brain of EAE mice (**Figure 13(E)**), enforced E-selectin ligand expression by glycoengineering yielded markedly increased migration of NSCs to the brain. This increased neurotropism was associated with markedly diminished CNS inflammation (**Figures 14(A)-14(I)**). Moreover, as shown in **Figure 13(A)-13(F)**, NSC accumulation in the CNS parenchyma

was two-fold higher among GPS-NSCs than BT-NSCs by day 17 post-injection, indicating that enforced expression of Step 1 effectors promotes extravasation.

[0281] In addition to enhanced neurotropism, short-term homing data also revealed a significantly higher accumulation of GPS-NSCs in the spleen and liver than in BT-NSCs (**Figure 13(E)**). These findings are consistent with results of a study reporting that systemically administered NSCs tend to accumulate in the brains of mice with EAE, and also in the spleen and the liver (Politi, L.S., Bacigaluppi, M., et al. 2007). E-selectin expression in the spleen has been described in humans, non-human primates and rodents (Alam, H.B., Sun, L., et al. 2000; Drake, T.A., Cheng, J., et al. 1993; Redl, H., Dinges, H.P., et al. 1991; Schweitzer, K.M., Drager, A.M., et al. 1996), and is upregulated by pro-inflammatory cytokines that are characteristically expressed in CNS inflammatory conditions (Emamgholipour, S., Eshaghi, S.M., et al. 2013; Weishaupt, A., Jander, S., et al. 2000); indeed, conjugation of sLe^x to polymers has been shown to markedly enhance the accumulation of such polymers within the spleen (Horie, K., Sakagami, M., et al. 2000; Horie, K., Sakagami, M., et al. 2004), providing direct evidence that sLe^x expression promotes splenic delivery. It has been reported that infiltration of the spleen by NSCs dampens production of inflammatory cytokines by resident spleen cells (*e.g.*, macrophages) resulting in anti-inflammatory effects (Lee, S.T., Chu, K., et al. 2008). Other studies have reported that intravascularly administered NSCs provide peripheral immunosuppression (Einstein, O., Fainstein, N., et al. 2007; Einstein, O., Grigoriadis, N., et al. 2006) or local immunomodulation that restrains CNS injury, rather than by enhancing neuroregeneration (Martino, G. and Pluchino, S. 2006; Pluchino, S., Zanotti, L., et al. 2009; Wang, L., Shi, J., et al. 2009). Thus, the observed increased splenic homing by enforced expression of E-selectin ligand activity on NSCs could be contributory to neuroprotection via immunomodulation by virtue of increased tissue ratio of NSCs to immune cells. This notion is supported by our *in vitro* data showing that although GPS-NSCs did not confer an immunomodulatory advantage compared to that observed with control NSCs, as the ratio of input NSCs to splenocytes is increased, T cell proliferation decreases (**Figure 19(A)**).

[0282] Previous studies of cell surface exoglycosylation had utilized human mesenchymal stem cells in a xenotransplant model and did not address the therapeutic impact on tissue injury, *i.e.*, whether glycoengineered stem cells would home to a site of inflammation and whether such cells would have desired regenerative and/or tissue-

protective effect(s). Though enhanced recruitment of NSCs to the CNS was observed following infusion of GPS-NSCs, there was no commensurate increase in long-term engraftment of NSCs. Thus, though enhanced CNS infiltration was achieved by exploiting physiologic cell migration (*i.e.*, in a non-invasive fashion that would preserve CNS tissue microenvironmental architecture), we did not observe that administered NSCs differentiated into progeny that regenerate functional CNS tissue in situ (*i.e.*, through direct regeneration). In fact, we observed that administration of GPS-NSCs improved the ability of endogenous neuronal progenitors to become oligodendroglia (**Figure 14(D) and 14(E)**). Our findings thus do not support the paradigm of direct neuroregeneration, but instead suggest that the predominant role of NSCs in CNS tissue repair is via trophic effects that support repair by endogenous cells (Caplan, A.I. 2009; Hess, D.C. and Borlongan, C.V. 2008; Laterza, C., Merlini, A., et al. 2013; Phinney, D.G. and Prockop, D.J. 2007), thus yielding neurorestoration (*i.e.*, through indirect neuroregeneration). Consistent with this notion, undifferentiated NSCs have been reported to significantly reduce scar formation and increase survival and function of endogenous glial and neuronal progenitors through the secretion of neurotrophins such as LIF (Laterza, C., Merlini, A., et al. 2013). In addition, NSCs support the formation of injury-induced growth niches through the expression of molecules such as BMP-4 and Noggin (Imitola, J., Raddassi, K., et al. 2004; Pluchino, S., Zanotti, L., et al. 2005) that trigger indirect neuroregeneration by endogenous cells.

[0283] Collectively, our data support a mechanism in which glycan engineering of NSC to enforce expression of E-selectin ligands heightens tissue colonization, mediating immunomodulation and tissue repair without necessitating persistent/long-term engraftment of administered NSCs. Notably, despite enhanced E-selectin ligand activity, infusion of GPS-HSPC did not improve the course of EAE (indeed, injection of HSPC worsens disease, see **Figures 21(A)-21(B)** and Table 3), indicating that the observed neuroprotective effects of GPS-NSCs are not a general property of adult stem cells and are due to neurorestorative effects inherent to NSC. Consistent with this finding of adult stem cell-specific biologic effect(s), human HSPCs have been found to elicit strong host immune rejection in a mouse model of congenital corneal disease while other adult stem cells such as mesenchymal stem cells suppress the host immune response (Liu, H., Zhang, J., et al. 2010). Though further studies on the molecular mechanism(s) mediating the observed neuroprotection by NSCs are

warranted, our results indicate that cell surface glycan engineering did not change the self-renewal capacity, differentiation potential or alter the innate immunomodulatory capacity of neural stem cells. Collectively, the data presented here lead us to propose a model (**Figure 23**) whereby enforced E-selectin ligand expression via exofucosylation of the surface of NSCs yields increased tissue recruitment at CNS inflammatory sites, thereby enhancing payload delivery of NSC trophic factors where they are needed. Because E-selectin expression is markedly upregulated at endothelial beds at all sites of inflammation in humans, our findings have profound translational implications for future clinical trials exploiting cell surface glycan engineering to improve the efficacy of stem cell therapeutics for MS as well as other devastating multifocal inflammatory diseases.

MATERIALS AND METHODS

[0284] ***Ethics Statement:*** All mouse experiments were performed in accordance with the NIH guidelines for the care and use of animals and under approval of the Institutional Animal Care and Use Committees of Harvard Medical School. The following are the specifics related to using mice these experiments. Justification for use: EAE is a valuable model for the human disease multiple sclerosis. There are no mathematical models, computer simulations or in vitro systems that can substitute for the in vivo disease. Many of the treatments available for MS and other autoimmune diseases were first tested and their mechanisms investigated in EAE. MOG induced EAE in C57/BL6 mice is valuable because of the availability of many knockout and transgenic mice on that background. Veterinary care: Mice are handled and cared for according to federal, institutional, and AAALAC guidelines. Procedures to minimize adverse effects: After immunization, animals are examined daily. Those affected by EAE develop a floppy tail (grade1), partial hind limbs (grade2), complete hind limb paralysis (grade 3), quadriparesis (grade 4), moribund or animal death (grade 5). Most animals have a grade 0 to 3, and, rarely, animals reach grade 4 and in such cases euthanasia is performed. When animals are affected by EAE, they are provided with gel packs to ensure access to fluids and are provided with access to food within the cage. Euthanasia is performed by CO2 inhalation administered according to institutional guidelines.

[0285] ***Reagents:*** The following antibodies were purchased from BD Pharmingen: function blocking mouse anti-human E-selectin (68-5H11; IgG1), rat anti-human CLA (HECA-452; IgM), mouse anti-human sLe^x (CSLEX-1; IgM), mouse anti-human CD15 (IgM), rat anti-mouse PSGL-1 (2PH1 and 4RA10; IgG1), mouse anti-human PSGL-1 (KPL-1; IgG1), rat anti-mouse CD44 (KM114 and IM7; IgG1), mouse anti-human CD44 (515; IgG1), rat anti-mouse CD43 (S7; IgG2a), mouse anti-human CD43 (1G10; IgG1), mouse anti-(mouse anti-adult and embryonic pan N-Cam) CD56 (NCAM13; IgG2b), mouse anti-human CD56 (NCAM16.2; IgG2b), rat anti-human CXCR4 (2B11; IgG2b), mouse anti-human CXCR4 (12G5; IgG2a), rat anti-mouse CD18 (GAME-46; IgG1), rat anti-mouse CD29 (KM16; IgG2a), rat anti-mouse CD49d (9C10; IgG2b), rat anti-mouse CD11a (2D7; IgG2a), rat anti-mouse CD11b (M1/70; IgG2b), rat anti-mouse CD49e (MFR5; IgG2a), mouse IgG1,κ isotype, mouse IgG2a isotype, mouse IgM isotype, rat IgG isotype, rat IgM isotype, and human IgG_1 isotype. The following secondary antibodies were also purchased from BD Pharmingen: PE Streptavidin, biotin anti-rat IgM, and goat anti-mouse Ig-HRP. The following secondary antibodies were purchased from Southern Biotech.: rabbit anti-human IgG-biotin, goat anti-mouse IgM-PE, goat anti-rat IgG-PE, goat anti-mouse Ig-biotin, goat anti-rat Ig-HRP, and goat anti-human Ig-HRP. Recombinant mouse E-selectin/human Ig chimera (E-Ig) and recombinant mouse P-selectin/human Ig chimera (P-Ig) were from R&D. Mouse anti-human sLe^x (KM93; IgM) was purchased from Calbiochem. Rat anti-mouse CD43 (1B11; IgG2a) was purchased from Biolegend. Rat anti-mouse LPAM-1 (DATK32; IgG2a) and mouse anti-SMI32 (SMI-32; IgG1) were purchased from Abcam. Mouse anti-human CD15 (80H5; IgM) was purchased from Coulter-Immunotech. PNGase F was purchased from New England Biolabs. PI-PLC, Bromelain, Soybean Trypsin Inhibitor, DNase, mouse anti-Map2 (HM-2 ; IgG1) were purchased from Sigma. Mouse-anti GFP (B2, IgG_{2a}) was purchased from Santa Cruz Biotechnology, Inc (Santa Cruz, CA). To-pro3 was purchased from Invitrogen. Mouse anti-human polysialic acid (PSA; IgG2a) was a kind gift from Dr. Nicholas Stamatos. Fucosyltransferase VI (FTVI) enzyme was a gift of Dr. Roland Wohlgemuth (Sigma-Aldrich).

[0286] ***Cells:*** Mouse NSCs were isolated and cultured as described (Imitola, J., Comabella, M., et al. 2004; Imitola, J., Raddassi, K., et al. 2004) previously. Briefly, a suspension of dissociated NSCs ($5x10^5$ cells per ml), isolated from the telencephalic VZ of murine embryonic day 12, were cultured in 98% DMEM/F12 (GIBCO), 1% N2

supplement (GIBCO), 1% penicillin/streptomycin (GIBCO) 8 mg/ml heparin (Sigma), 10 ng/ml leukemia inhibiting factor (LIF, Chemicon), 20 ng/ml bFGF (Calbiochem) in uncoated 25-cm^2 flasks (Falcon) at 37°C in 5% CO_2. NSCs were used after second passage for most experiments. Single cell suspension of neural stem cells was achieved by mechanical dissociation of the neurospheres in Versene (Life Technologies).

[0287] For isolation of mouse HSPC, bone marrow was harvested from the femur and tibia of C57BL/6 mice and single cell suspensions were made. Red blood cells were lysed using red blood cell lysing buffer (Sigma). Cells were then washed and filtered through a 100μm cell strainer (BD Falcon) prior to lineage depletion using the Lineage Cell Depletion Kit from Miltenyi Biotec. Cell preparations were depleted using the autoMACS™ Separator (Miltenyi Biotec.). Following depletion, the cells were then positively selected for c-kit using CD117 MicroBeads (Miltenyi Biotec.). The resulting $Lineage^{neg}C\text{-}kit^{pos}$ population (referred to as HSPC) was used for in vivo EAE mouse studies.

[0288] Chinese hamster ovary cells transfected with full-length E-selectin (CHO-E) and were maintained as described previously (Dimitroff, C.J., Lee, J.Y., et al. 2001). The human βFGF-dependent NSC cell line, CC-2599, was cultured as previously described (Imitola, J., Raddassi, K., et al. 2004) and used for data presented in **Figures 16(A)-16(C)**).

[0289] ***GPS-treatment, PI-PLC and Bromelain reactions:*** The procedure for GPS treatment of murine NSCs was as previously described for MSCs (Sackstein, R., Merzaban, J.S., et al. 2008). Briefly, neurospheres were first harvested and dissociated into single cells by incubating with PBS/EDTA (0.02%) for 15 min at 37°C. Cells were then washed with HBSS, counted, and resuspended in 60 mU/ml FTVI in HBSS (without Ca^{2+} or Mg^{2+}) containing 20 mM HEPES, 0.1% human serum albumin and 1 mM GDP-fucose for 40 min at 37°C. PI-PLC reactions were performed at 37°C using 0.1U/ml for 1hr. Bromelain reactions were performed at 37°C in HBSS+2%BSA+0.1%bromelain for 1hr.

[0290] ***Flow cytometry***: Aliquots of cells (2 x 10^5 cells) were washed with PBS/2% FBS and incubated with primary mAbs or with isotype control mAbs (either unconjugated or fluorochrome conjugated). The cells were washed in PBS/2% FBS

and, for indirect immunofluorescence, incubated with appropriate secondary fluorochrome-conjugated anti-isotype antibodies. After washing cells, FITC or PE fluorescence intensity was determined using a Cytomics FC 500 MPL flow cytometer (Beckman Coulter Inc., Fullerton, CA).

[0291] ***Immunoprecipitation studies:*** Cell lysates of BT-NSC or GPS-NSC were incubated with immunoprecipitating antibodies or with appropriate isotype controls and then incubated with Protein G-agarose. Immunoprecipitates were washed extensively using Buffer A containing 2% NP-40, 1% SDS. In some experiments, immunoprecipitates were treated with N-glycosidase F (New England Biolabs) as previously described (Dimitroff, C.J., Lee, J.Y., et al. 2001). For Western blot analysis, all immunoprecipitates were diluted in reducing sample buffer, boiled, then subjected to SDS-PAGE, transferred to PVDF membrane, and immunostained with HECA-452 or E-Ig.

[0292] ***Western blot analysis:*** BT- and GPS-NSCs were lysed using 2% NP-40 in Buffer A (150mM NaCl, 50mM Tris-HCl, pH 7.4, 1 mM EDTA, 20 µg/ml PMSF, 0.02% sodium azide; and protease inhibitor cocktail tablet (Roche Molecular Biochemicals)). Western blots of quantified protein lysates or of immunoprecipitated protein were performed under reducing conditions as described previously (Dimitroff, C.J., Lee, J.Y., et al. 2001). Blots were visualized with chemiluminescence using Lumi-Light Western Blotting Substrate (Roche).

[0293] ***Parallel Plate Flow Chamber Adhesion Assays:*** E-selectin binding capacity of BT-NSCs and GPS-NSCs was compared using the parallel plate flow chamber assay (Glycotech; Gaithersburg, MD). NSCs (0.5×10^6 cells/ml, suspended in HBSS/10mM HEPES/2mM $CaCl_2$ solution) were drawn over confluent HUVEC monolayers. Initially, the NSCs were allowed to contact the HUVEC monolayer at a shear stress of 0.5 dyne/cm^2, subsequently the flow rate was adjusted to exert shear stress ranging from 0.5 to 30 dynes/cm^2. The number of BT- or GPS-NSCs adherent to the HUVEC monolayer were quantified in the final 15 sec interval at shear stress of 0.5, 1, 2, 5, 10, 20 and 30 dyne/cm^2. Each assay was performed at least 3 times and the values averaged. Control assays were performed by adding 5 mM EDTA to the assay buffer to chelate Ca^{2+} required for selectin binding or treating HUVEC with function-blocking anti-human E-selectin mAb at 37°C for 15 min. prior to use in adhesion assays.

[0294] ***Blot Rolling Assay:*** The blot rolling assay has been described previously (Dimitroff, C.J., Lee, J.Y., et al. 2000; Fuhlbrigge, R.C., King, S.L., et al. 2002; Sackstein, R. and Fuhlbrigge, R. 2006) and here was used to detect selectin-binding activity of NSC membrane proteins resolved by SDS-PAGE. Western blots of NSC membrane preparations were stained with anti-CLA (HECA-452) and rendered translucent by immersion in DMEM with 10% glycerol. CHO-E cells were resuspended (5×10^6/mL) in DMEM containing 2mM $CaCl_2$ and 10% glycerol. The blots were placed under a parallel plate flow chamber, and CHO-E cells were perfused at a physiologically relevant shear stress of 1.0 dyne/cm^2, an adjustment in the volumetric flow rate was made to account for the increase in viscosity due to the presence of 10% glycerol in the flow medium. Molecular weight markers were used as guides to aid placement of the flow chamber over stained bands of interest. The number of interacting cells per square millimeter was tabulated as a function of the molecular weight region and compiled into an adhesion histogram. Nonspecific adhesion was assessed by perfusing CHO-E cell suspensions containing 5mM EDTA in the flow medium.

[0295] ***Immunization for EAE-induction and neural stem cells Injection:*** NSCs or bone marrow HSPC (Lineage^{neg}C-kit^{pos}) were either treated with GPS or not and $1x10^6$ cells were injected into the tail vein of C57BL/6 mice on day 9 and day13 after immunization (PI) with MOG 35-55 (subcutaneously in the flanks; see details in Supplementary Material). EAE was scored in a blinded fashion; the investigator was not involved in the injections and was not aware of the composition of the groups. Details of the grading scale used are outlined in the Supplementary Material. BT-NSC, GPS-NSCs, BT-HSPCs or GPS-HSPCs were injected as a cell suspension into the tail-vein of EAE mice in a volume of 0.2 ml of HBSS. Sham treated mice (No NSCs) injected with HBSS alone were used as a negative control.

[0296] ***Short-term Homing Studies:*** BT-NSCs and GPS-NSCs were labelled with 5mM CFDA-SE (Invitrogen) for 5 min at room temperature in RPMI containing 10% FBS and injected intravenously into MOG-treated C57BL6 mice on day 9 and day 13 post-immunization (PI). Two million NSCs in a volume of 0.2mL of HBSS were injected into each mouse on each day. HBSS buffer alone was used to determine background signals. BT-NSCs and GPS-NSCs were also injected into animals that were not immunized with MOG and used to standardize the signals observed in each tissue assayed. Sixteen hours after the second NSC transfer, mice were sacrificed and

perfused with 1X PBS without Ca^{2+} and Mg^{2+}. The brain, spinal cord, lymph nodes, spleen, liver and lung were isolated. The brain and spinal cord were homogenized. Resulting pellets were resuspended in 0.25% Trypsin-EDTA (Invitrogen) and incubated at 37°C for 10 minutes. The digestion process was stopped using DMEM containing 0.01% SBTI, 0.001% DNase and 0.075% BSA. The lymph nodes, spleen and liver were mechanically dissociated and the resulting single-cell suspensions were assessed for frequencies of CFDA-SE positive cells by flow cytometry in the FL1 channel. Flow cytometric data was analyzed and expressed as percent of CFDA-SE-positive events detected in 200,000 cells scanned within a narrow gate that is set to include NSC. This gate was determined based on mixing cultured NSCs with suspensions of cells isolated from each tissue tested (brain, spinal cord, lymph node, spleen, liver and lung).

[0297] ***Analysis of NSC migration to CNS:*** BT-NSCs, GPS-NSCs were labelled with PKH26 dye (Invitrogen) and injected intravenously into MOG-treated C57BL6 mice on day 9 and day 13 post-immunization (PI). One million NSCs in a volume of 0.2mL of HBSS were injected into each mouse on each day. HBSS buffer alone was used to determine background signals. Four days after the second NSC transfer, mice were sacrificed and perfused with 1X PBS without Ca^{2+} and Mg^{2+} and lumbar-sacral spinal cords were harvested. The spinal cord was chosen because in the B6 model, the CNS lesions in the forebrain are very variable in size and location compared with the spinal cords, which possess a more predictable location (lumbosacral region) and also more exuberant pathology (Chitnis, T., Najafian, N., et al. 2001; Rasmussen, S., Wang, Y., et al. 2007; Wang, Y., Imitola, J., et al. 2008). For FACS analysis, the spinal cords were then homogenized and the resulting pellets were resuspended in 0.25% Trypsin-EDTA (Invitrogen) and incubated at 37°C for 10 minutes. The digestion process was stopped using DMEM containing 0.01% SBTI, 0.001% DNase and 0.075% BSA. For histology analysis, the brain and spinal cord were snap-frozen in liquid nitrogen and stored in -80°C until sectioning. The cryostat sections (20μm) of lumbar-sacral spinal cord or anterior, middle and posterior brain were fixed with 4% paraformaldehyde for 15 minutes and then stained with antibodies of interest, Blood vessels were visualized by anti-Flk-1 (VEGFR2, Sigma) and NSCs were stained with either anti-sox-2 (Millipore) or visualized by PKH26 dye in red. The spinal cords were then homogenized and the resulting pellets were resuspended in 0.25% Trypsin-EDTA (Invitrogen) and incubated at 37°C for 10 minutes. The digestion process was

stopped using DMEM containing 0.01% SBTI, 0.001% DNase and 0.075% BSA. Blood vessels were visualized by Flk-1 (VEGFR2) staining.

[0298] ***Immunization for EAE-induction and NSC/HSPC Injection:*** NSCs or HSPC (Lineage^{neg}C-kit^{pos}) were either treated with GPS or not and 1x10^6 cells were injected into the tail vein of C57BL/6 mice on day 9 and day 13 after immunized (PI) with MOG 35-55. MOG 35-55 (M-E-V-G-W-Y-R-S-P-F-S-R-O-V-H-L-Y-R-N-G-K) corresponding to mouse sequence is synthesized by QCB Inc. Division of BioSource International (Hopkinton, MA), and purified to >99% by HPLC. Mice are immunized subcutaneously in the flanks with 150-200 µg of MOG peptide in 0.1 ml PBS and 0.1 ml CFA containing 0.4 mg Mycobacterium Tuberculosis (H37Ra, Difco, Detroit, Michigan) and injected intraperitoneally with 200 ng Pertussis toxin (List Laboratories, Campbell, California) on the day of immunization and 2 days later. EAE was scored in a blinded fashion; the investigator was not involved in the injections and was not aware of the composition of the groups. The following grade was used: grade 1, limp tail or isolated weakness of gait without limp tail; grade 2, partial hind leg paralysis; grade 3, total hind leg or partial hind and front leg paralysis; grade 4, total hind leg and partial front leg paralysis; grade 5, moribund or dead animal. BT-NSC, GPS-NSCs, BT-HSPCs, GPS-HSPCs were injected as a cell suspension into the tail-vein of EAE mice in a volume of 0.2 ml of HBSS. Sham treated mice (No NSCs) injected with HBSS alone were used as a negative control.

[0299] ***Immunohistologic staining:*** Mice were perfused with 50 ml normal saline before sacrificing to remove any intravascular PBMCs. For confocal imaging animals were perfused intracardially with 10 ml of 4% paraformaldehyde in PBS. The brain and spinal cord were removed and embedded in O.C.T., quick frozen in liquid nitrogen and kept at -70°C until sectioning. Cryostat sections (10 µm) of spinal cords were fixed with acetone or 4% paraformaldehyde and then labelled with the antibody of interest. Isotype-matched Ig and omission of the primary antibody served as negative controls. Each specimen was evaluated at a minimum of three different levels of sectioning. The entire tissue section (a longitudinal spinal cord section) was evaluated for a given cellular marker at 40X magnification. The number of cells staining positive for the given markers were counted in ten 40X (high power fields) fields per section. The results for one section were totalled, and the results between sections were averaged.

[0300] ***Staining for confocal microscopy:*** Paraformaldehyde fixed sections (40µm) were washed in PBS, blocked in PBS containing 4% goat serum, 0.3% BSA, and 0.3% triton and subsequently incubated with primary antibodies overnight and secondary antibodies for 2h in blocking solution. We used highly cross-adsorbed secondary antibodies to avoid cross-reactivity (Alexa 488 and Alexa 594). Confocal microscopy was performed using a Zeiss Laser Scanning Microscope 3D analysis software (Zeiss, Thornwood, NY) with a multitrack acquisition protocol to avoid potential overlapping of the two fluorochromes.

[0301] ***Effects of GPS treatment on NSC differentiation, self-renewal capacity and immunosuppressive effects in vitro:*** The procedure for GPS treatment of murine NSCs was as previously described for MSCs (Sackstein, R., Merzaban, J.S., et al. 2008). BT- and GPS-NSCs were compared in vitro for their capacity to self-renew, form neurospheres, differentiate into $MAP2^+$ neurons, and inhibit the proliferation of ConA activated lymph node cells. *NSC differentiation and self-renewal capacity:* Neurospheres initially cultured in FGF/EGF containing media were plated on poly-D-lysine (PDL)-coated glass coverslips allowed to proliferate then harvested and treated for an hour with buffer (control) or enzymatic treatment with FTVI (60mU/mL), subsequently the resulting BT-NSCs and GPS-NSCs were plated at clonal density of 20 cells per µl and allowed to proliferate as neurospheres for 96 hours to 5 days. Neurosphere imaging was captured with an Axiovision microscope (NY). For neuronal, astrocyte, and oligodendrocyte differentiation, dissociated neurospheres were plated on PL-coated glass coverslips in a 24 well plate and cultured without FGF/EGF but in the presence of neurobasal medium containing 1% Glutamax, 1% Antibiotic/Antimycotic and 2% B27-Supplement. Fresh media was added every other day until day 5, and the cells were then subjected to immunofluorescence staining with MAP2 (neurons), GFAP (astrocytes), and NG2 (oligodendrocyte precursors). $MAP2^+$, $GFAP^+$, and $NG2^+$ cells were counted using standard stereological technique by an investigator blinded to the treatments. *Cocultures of Neural Stem Cells and Lymph Node Cells:* Lymph nodes were isolated from naïve C57BL/6 mice. Lymph node cells (LNC) were cultured as single-cell suspensions in a 96 well plate at 2×10^5 cells per well, as previously described (Einstein, O., Fainstein, N., et al. 2007). Culture medium consisted of RPMI-1640 supplemented with 10% fetal bovine serum, L-glutamine, sodium pyruvate, non-essential amino acid, 2-mercaptoethanol, HEPES and antibiotics (BioWhittaker) with 2.5

μg/ml concanavalin A (ConA, Sigma) or without. Neurospheres were dissociated and were first either treated with GPS or not (BT) and subsequently irradiated with 3,000 Rad. Following irradiation the dissociated NSCs were then added directly to the LNC culture medium at different ratios with ConA stimulated LNCs. The ratios tested of numbers of NSCs to numbers of LNCs were: 1:4, 1:2, 1:1, 2:1, and 4:1. The cells were then cultured for 48 hours before adding thymidine. Thymidine incorporation assays were performed 16 hours later.

[0302] ***Assessment of E-selectin ligands following treatment of NSCs with inflammatory cytokines:*** 1.5 x 10^6 cells/well were seeded in a 6 well plate containing 3 ml proliferation medium per well and stimulated with either 10 ng/ml of TNFα (R&D; 410-TRNC), 10 ng/ml IL-1β (R&D; 401-ML), 10 ng/ml IFNγ (R&D; 485-MI) independently or in combination (all three at 10 ng/ml). After 24 h and 48 h, the neurospheres were harvested by centrifugation and stained with E-Ig for flow cytometric analysis.

[0303] ***Measurement of LIF mRNA:*** 1x10^6 mouse embryonic NSCs were treated for 24 hours with or without inflammatory cytokines (IFN-Y at 10ng/ml and TNF-α at 15ng/ml). Total RNA was then extracted using Trizol reagent and the quality of the extracted RNA was measured using Agilent 2200 Tab station system. cDNA synthesis was done using high capacity cDNA reverse transcription kit (Applied Biosystems) and a Random Hexamer. mRNA level was measured using RT-PCR and the fold change in gene expression was calculated using $2^{-\Delta\Delta CT}$ method. The forward primer sequence for the LIF gene was CCTACCTGCGTCTTACTCCATCA and the reverse primer was CCCCAAAGGCTCAATGGTT (Sigma). The relative expression of LIF mRNA was assayed relative to GAPDH housekeeping gene in which TGCACCACCAACTGCTTAGC was used as a forward primer and GGCATGGACTGTGGTCATGAG as reverse primer.

[0304] ***Analysis of NSC migration to CNS:*** BT-NSCs, GPS-NSCs were labeled with PKH26 dye (Invitrogen) and injected intravenously into MOG-treated C57BL6 mice on day 9 and day 13 post-immunization (PI). One million NSCs were injected into each mouse on each day. HBSS buffer alone was used to determine background signals. Either 24 hours or 4 days after the second NSC transfer, mice were sacrificed, perfused, and lumbar-sacral spinal cords were harvested. The spinal

cord was chosen because in the B6 model, the CNS lesions in the forebrain are very variable in size and location compared with the spinal cords, which possess a more predictable location (lumbosacral region) and also more exuberant pathology (Chitnis, T., Najafian, N., et al. 2001; Rasmussen, S., Wang, Y., et al. 2007; Wang, Y., Imitola, J., et al. 2008). For flow cytometric analysis, the spinal cords were then homogenized and the resulting pellets were resuspended in 0.25% Trypsin-EDTA and incubated at 37°C for 10 minutes. The digestion process was stopped using DMEM containing 0.01% SBTI, 0.001% DNase and 0.075% BSA. For histology analysis, the brain and spinal cord were snap-frozen in liquid nitrogen and stored in -80°C until sectioning. The cryostat sections (20µm) of lumbar-sacral spinal cord or anterior, middle and posterior brain were fixed and then stained with antibodies of interest. Blood vessels were visualized by anti-Flk-1 (VEGFR2) and NSCs were stained with either anti-SOX-2 or visualized by PKH26 dye in red. For neuropathology analysis, cell quantification was performed by stereological analysis of animals in different groups. Spinal cords and brain were sectioned and every third section of the cervical and lumbosacral region was stained, cell quantification was performed in high power magnification of 3-5 sections. LSM 510 Confocal microscope with motorized stage was used to stereologically calculate the intensity of staining, and total cell numbers/per high power magnification.

[0305] ***Statistical Analysis-*** Data are expressed as the mean ±SEM. Statistical significance of differences between means was determined by two-way ANOVA. If means were shown to be significantly different, multiple comparisons were performed post-hoc by the Turkey t-test. Statistical significance was defined as $p<0.05$.

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WHAT IS CLAIMED IS:

1. A method of enhancing cell delivery into a target tissue of a subject and/or enhancing tissue colonization in the target tissue of the subject, the method comprising:

administering to the subject a population of cells that express an E-selectin ligand and/or an L-selectin ligand, the population expressing the E-selectin ligand and/or L-selectin ligand at a level that exceeds the level of expression of a native population of the cells,

wherein said cell administration occurs coincident with E-selectin expression on endothelial cells within the target tissue and/or coincident with accumulation of leukocytes within the target tissue.

2. A method of treating a disease, disorder or medical condition manifesting as inflamed and/or damaged tissue in a subject, the method comprising:

administering to the subject a population of cells that express an E-selectin ligand and/or an L-selectin ligand, the population expressing the E-selectin ligand and/or L-selectin ligand at a level that exceeds the level of expression of a native population of the cells,

wherein said cell administration occurs coincident with E-selectin expression on endothelial cells within the target tissue and/or coincident with accumulation of leukocytes within the target tissue, and

wherein said population exhibits enhanced localization and/or colonization within the inflamed and/or damaged tissue relative to a native population of the administered cells.

3. A method of improving cellular delivery to a target tissue in a subject, the method comprising:

administering to the subject, via vascular delivery and/or via direct tissue injection (*i.e.*, directly into affected tissue site(s)) and/or intrethecally and/or intracavitary and/or intravesically and/or within anatomic conduits (biliary system, urinary system, etc.) and/or onto tissue (*i.e.*, placement onto affected tissue), a population of cells that express an E-selectin ligand and/or an L-selectin ligand, the population expressing the E-selectin ligand and/or L-selectin ligand at a level that exceeds the level of expression of a native population of the cells,

wherein said cell administration occurs coincident with E-selectin expression on endothelial cells within the target tissue and/or coincident with accumulation of leukocytes within the target tissue, and

wherein said administered cell population achieves one or more of enhanced homing, colonization, and engraftment relative to a native population of cells.

4. A method of enhancing cell delivery and colonization in an inflamed and/or damaged tissue of a subject, the method comprising:

directly injecting into or placing onto/within said inflamed and/or damaged tissue a population of cells that express an E-selectin ligand and/or an L-selectin ligand, the population expressing the E-selectin ligand and/or L-selectin ligand at a level that exceeds the level of expression of a native population of the cells,

wherein said injection/placement occurs coincident with E-selectin expression on endothelial cells within the target tissue and/or coincident with accumulation of leukocytes within the target tissue.

5. A method of enhancing cell delivery, colonization and/or engraftment into a target tissue of a subject, the method comprising:

administering via the vasculature of the subject a population of cells that express an E-selectin ligand and/or an L-selectin ligand, the population expressing the E-selectin ligand and/or L-selectin ligand at a level that exceeds the level of expression of a native population of the cells,

wherein said administration occurs coincident with E-selectin expression on endothelial cells within the target tissue and/or coincident with accumulation of leukocytes within the target tissue.

6. A method of enhancing lodgement, colonization and/or engraftment of a cell population within a target tissue in a subject, the method comprising:

directly injecting into the target tissue or placement onto the target tissue a population of cells that express an E-selectin ligand and/or an L-selectin ligand, the population expressing the E-selectin ligand and/or L-selectin ligand at a level that exceeds the level of expression of a native population of the cells,

wherein said injection occurs coincident with E-selectin expression on endothelial cells within the target tissue and/or coincident with accumulation of leukocytes within the target tissue,

wherein said population achieves enhanced target tissue colonization relative to a native population of the cells.

7. A method of treating a disease, disorder or medical condition manifesting as inflamed and/or damaged tissue in a subject, the method comprising:

administering to the subject a population of cells that express an E-selectin ligand and/or an L-selectin ligand, the population expressing the E-selectin ligand and/or L-selectin ligand at a level that exceeds the level of expression of a native population of the cells,

wherein said injection occurs coincident with E-selectin expression on endothelial cells within the inflamed and/or damaged tissue and/or coincident with accumulation of leukocytes within the inflamed and/or damaged tissue.

8. A method of treating a tumor/malignant disease in a subject, the method comprising:

administering to the subject a population of cells that express an E-selectin ligand and/or an L-selectin ligand, the population expressing the E-selectin ligand and/or L-selectin ligand at a level that exceeds the level of expression of a native population of the cells,

wherein said administration occurs coincident with E-selectin expression on endothelial cells within tissue(s) harboring tumor/malignant cells and/or coincident with accumulation of leukocytes within the tissue(s) harboring tumor/malignant cells.

9. A population of cells that express an E-selectin ligand and/or an L-selectin ligand for use in the manufacture of a medicament for the treatment of a disease, disorder or medical condition manifesting as inflamed and/or damaged tissue in a subject, the treatment comprising administering to the subject the population of cells that express an E-selectin ligand and/or an L-selectin ligand, wherein the population expresses the E-selectin ligand and/or L-selectin ligand at a level that exceeds the level of expression of a native population of the cells,

wherein said treatment comprises administering the population coincident with E-selectin expression on endothelial cells within the inflamed and/or damaged tissue and/or coincident with accumulation of leukocytes within the inflamed and/or damaged tissue.

10. A population of cells that express an E-selectin ligand and/or an L-selectin ligand for use in the manufacture of a medicament for the treatment of a tumor/malignant disease, the treatment comprising administering to the subject the population of cells that express an E-selectin ligand and/or an L-selectin ligand, wherein the population expresses the E-selectin ligand and/or L-selectin ligand at a level that exceeds the level of expression of a native population of the cells,

wherein said treatment comprises administering the population coincident with E-selectin expression on endothelial cells within the tumor/malignant tissue and/or coincident with accumulation of leukocytes within the tumor/malignant tissue.

11. The method or population of any one of claims 1-10, wherein said cell administration occurs coincident with E-selectin expression on endothelial cells and infiltrates of leukocytes bearing L-selectin within the target tissue.

12. The method or population of any one of claims 1-11, wherein said administration/injection occurs during infiltration of leukocytes to an/the inflamed and/or damaged tissue.

13. The method or population of any one of claims 1-12, further comprising engraftment of the administered population of cells in a milieu of an/the inflamed and/or damaged tissue.

14. The method or population of any one of claims 1-13, wherein an immunomodulatory effect is achieved by colonization of cells within an/the inflamed and/or damaged tissue.

15. The method or population of any one of claims 1-14, wherein a tissue reparative effect is achieved by colonization of administered cells within an/the inflamed and/or damaged tissue.

16. The method or population of any one of claims 1-15, wherein an enhanced host defense/immune response effect is achieved by delivery and colonization of administered cells within an/the inflamed and/or damaged tissue.

17. The method or population of any one of claims 1-16, wherein an anti-malignancy effect is achieved by colonization of administered cells within a/the tumor/malignant tissue site.

18. The method or population of any one of claims 1-17, wherein said administration/injection comprises one or a series of injections of the cell population.

19. The method or population of cells of any one of claims 1-18, wherein said administration/injection comprises daily, weekly, bi-weekly, monthly, or yearly injections of the cell population.

20. The method or population of cells of any one of claims 1-9, further comprising one or more additional administrations/injection of the cell population upon a decrease in the subject of an immunomodulatory effect of a prior administration/injection.

21. The method or population of cells of any one of claims 1-20, wherein the cell population is administered/injected intravenously.

22. The method or population of cells of any one of claims 1-21, wherein the cell population is administered by direct injection to the inflamed and/or injured tissue.

23. The method or population of cells of any one of claims 1-21, wherein the cell population is placed onto the inflamed tissue and/or injured tissue.

24. The method or population of cells of any one of claims 1-21, wherein the cell population is placed onto an/the inflamed tissue and/or injured tissue.

25. The method or population of cells of any one of claims 1-21, wherein the cell population is utilized in combination with other enhancing agents, anti-inflammatory agents, or with tissue scaffolds/transplantable devices/gels.

26. The method or population of any one of claims 1-25, wherein the population of cells express an E-selectin ligand and an L-selectin ligand.

27. The method or population of any one of claims 1-26 wherein the E-selectin ligand and/or L-selectin ligand is selected from one or more of Hematopoietic Cell E-/L-selectin Ligand (HCELL), Neural Cell Adhesion Molecule E-selectin Ligand (NCAM-E), CD43E, CLA, and ESL-1.

28. The method or population of any one of claims 1-27, wherein the E-selectin ligand is Hematopoietic Cell E-/L-selectin Ligand (HCELL) and/or Neural Cell Adhesion Molecule E-selectin Ligand (NCAM-E).

29. The method or population of any one of claims 1-28, wherein the E-selectin ligand is HCELL.

30. The method or population of any one of claims 1-29, wherein the E-selectin ligand is NCAM-E.

31. The method or population of any one of claims 1-30, wherein the L-selectin ligand is HCELL.

32. The method or population of any one of claims 1-31, wherein the cell population comprises one or more of epithelial, endothelial, neuronal, adipose, cardiac, skeletal muscle, fibroblast, and immune cells (for example, dendritic cells, monocytes, macrophages, leukocytes (*e.g.*, a lymphocyte such as a NK cell, a B-lymphocyte, a T-lymphocyte, or a subset of T-lymphocytes, such as regulatory lymphocyte

($CD4^+/CD25^+/FOXP3^+$)), a cytotoxic lymphocyte, etc.), hepatic, splenic, lung, circulating blood cells, platelets, reproductive cells, gastrointestinal, renal, bone marrow, pancreatic cells, a stem cell (*e.g.*, a mesenchymal stem cell, a hematopoietic stem cell, a tissue stem/progenitor cell (for example, a neural stem cell, myocyte stem cell or pulmonary stem cell), an umbilical cord stem cell, or an embryonic stem cell, or an induced pluripotent stem cell, or a differentiated progenitor derived from an embryonic stem cell or from an induced pluripotent stem cells, or a differentiated progenitor derived from an adult stem cell, or a primary cell isolated from any tissue (*e.g.*, brain, liver, lung, gut, stomach, fat, muscle, testes, uterus, ovary, skin, spleen, endocrine organ and bone), or a culture-expanded progenitor cell population, or a culture-expanded stem cell population, or a culture-expanded primary cell population.

33. The method or population of any one of claims 1-32, wherein the cell population is a mesenchymal stem cell, a hematopoietic stem cell, a tissue stem/progenitor cell, an umbilical cord stem cell, or an embryonic stem cell.

34. The method or population of any one of claims 1-33, wherein the cell population is a leukocyte (*e.g.*, a lymphocyte such as an NK cell, B-lymphocyte, a T-lymphocyte, or a subset of T-lymphocytes, such as regulatory lymphocyte ($CD4^+/CD25^+/FOXP3^+$)), a cytotoxic T cell, etc.).

35. The method or population of any one of claims 1-34, wherein the disease, disorder or medical condition is one or more of direct tissue injury (*e.g.*, burns, trauma, decubitus ulcers, etc.), ischemic/vascular events (*e.g.*, myocardial infarct, stroke, shock, hemorrhage, coagulopathy, etc.), infections (*e.g.*, cellulitis, pneumonia, meningitis, sepsis, SIRS, etc.), neoplasia (*e.g.*, breast cancer, lung cancer, prostate cancer, lymphoma, leukemia, etc.), immunologic/autoimmune conditions (*e.g.*, graft vs. host disease, multiple sclerosis, diabetes, inflammatory bowel disease, lupus erythematosus, rheumatoid arthritis, psoriasis, etc.), degenerative diseases (*e.g.*, osteoporosis, osteoarthritis, Alzheimer's disease, etc.), congenital/genetic diseases (*e.g.*, epidermolysis bullosa, osteogenesis imperfecta, muscular dystrophies, lysosomal storage diseases, Huntington's disease, etc.), adverse drug effects (*e.g.*, drug-induced hepatitis, drug-induced cardiac injury, etc.), toxic injuries (*e.g.*, radiation exposure(s),

chemical exposure(s), alcoholic hepatitis, alcoholic pancreatitis, alcoholic cardiomyopathy, cocaine cardiomyopathy, etc.), metabolic derangements (*e.g.*, uremic pericarditis, metabolic acidosis, etc.), iatrogenic conditions (*e.g.*, radiation-induced tissue injury, surgery-related complications, etc.), and/or idiopathic processes (*e.g.*, amyotrophic lateral sclerosis, Parsonnage-Turner Syndrome, etc.).

36. The method or population of any one of claims 1-35, wherein the disease, disorder or medical condition is diabetes.

37. The method or population of any one of claims 1-36, wherein the disease, disorder or medical condition is multiple sclerosis.

38. The method or population of any one of claims 1-37, wherein the subject is a human, non-human primate, mouse, rat, dog, cat, horse, or cow.

39. The method or population of any one of claims 1-38, wherein the subject is a human patient.

40. The method or population of any one of claims 1-39 wherein the cell population is treated to form a modified cell population having an enforced expression of an E-selectin and/or an L-selectin ligand, and the cell population has a viability of at least 70% at 24 hours after the treatment.

41. The method or population of any one of claims 1-40 wherein the cell population has a viability of at least 80% at 24 hours after the treatment.

42. The method or population of any one of claims 1-40 wherein the cell population has a viability of at least 70% at 48 hours after the treatment.

43. The method or population of any one of claims 1-42 wherein, upon administration to the cell population to a subject, the cell population lodges within a tissue comprising infiltrating leukocytes.

44. The method or population of claim 43 wherein the administration is a direct injection into the tissue or placement onto the tissue.

45. The method or population of claim 43 wherein the administration is vascular.

46. The method or population of any of claims 1-21 or 25-42 wherein the population is administered systemically, via either peripheral vascular access or central vascular access.

47. The method or population of any of claims 1-21 or 25-42 wherein the population is administered intravascularly into anatomic feeder vessels of an intended tissue site using catheter-based approaches or other vascular access devices that will deliver a vascular bolus of cells to the intended site.

48. The method or population of any of claims 1-21 or 25-42 wherein the population is administered by introduction into the spinal canal and/or intraventricularly.

49. The method or population of any of claims 1-21 or 25-42 wherein the population is administered directly into body cavities by either catheter-based approaches or direct injection.

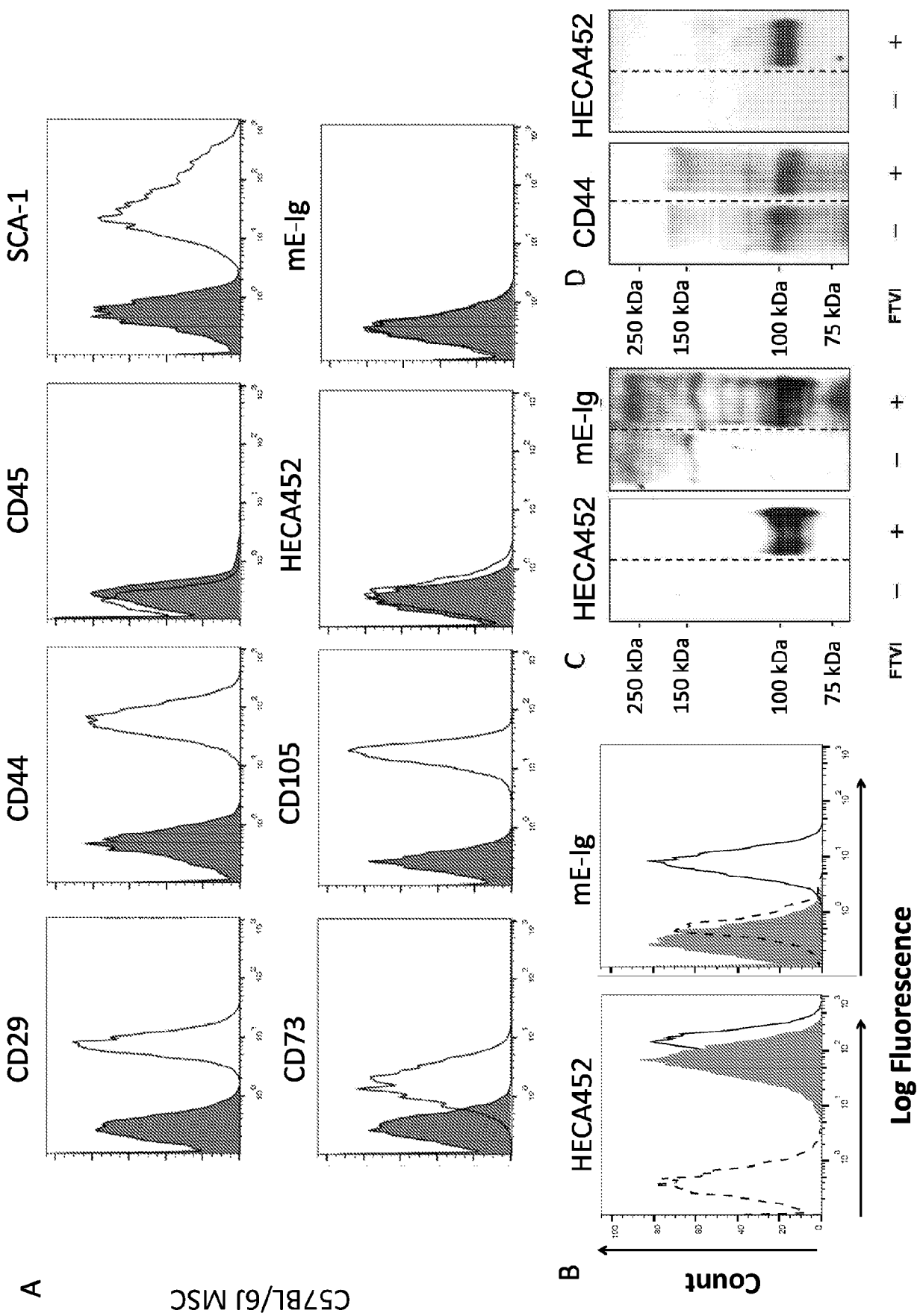
50. The method or population of any of claims 1-21 or 25-42 wherein the population is administered by direct local tissue injection, using either intravascular approaches, or percutaneous approaches, or directly into anatomically accessible tissue sites and/or guided by imaging techniques.

51. The method or population of any of claims 1-21 or 25-42 wherein the population is administered by placement directly onto relevant tissue surfaces/sites.

52. The method or population of any of claims 1-21 or 25-42 wherein the population is administered into scaffolds or embedded within scaffolds placed into tissues, and/or administered in gels, and/or administered together with enhancing agents.

53. The method or population of any of claims 1-21 or 25-42 wherein the population is administered into the cerebrospinal fluid.

5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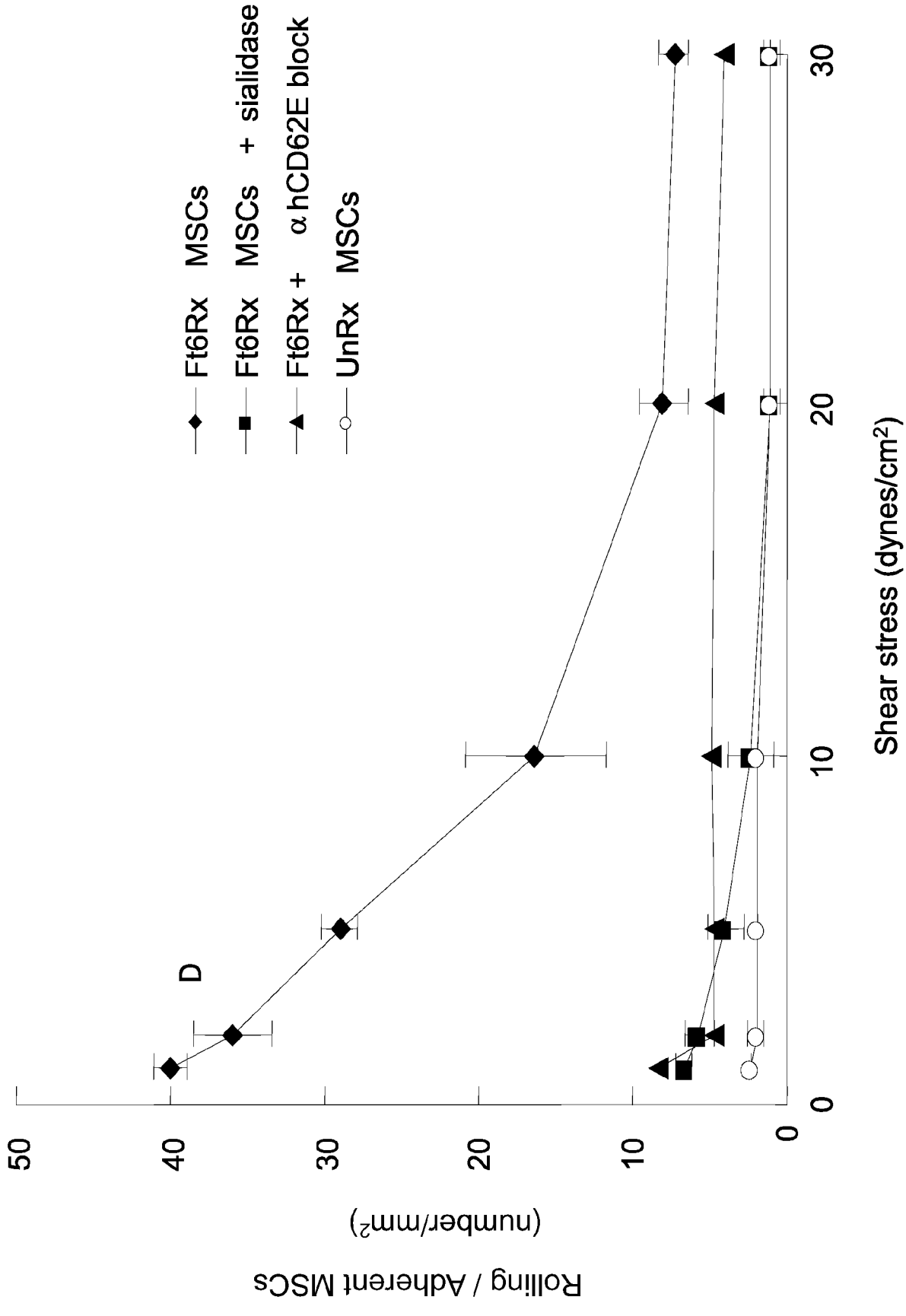
75 kDa
FTVI

Figure 1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UnRx MSCs
D
Rolling / Adherent MSCs
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0
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20
30
Shear stress (dynes/cm²)

Figure 2

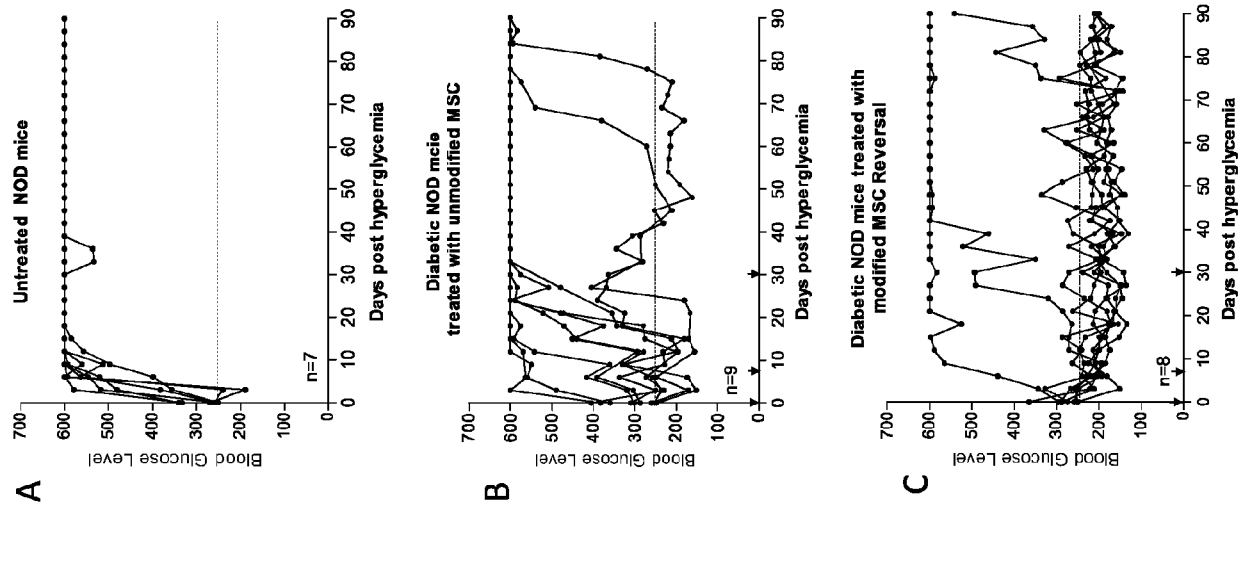

A
Untreated NOD mice
Blood Glucose Level
700
600
500
400
300
200
100
0
n=7
0 10 20 30 40 50 60 70 80 90
Days post hyperglycemia
B
Diabetic NOD mcie
treated with unmodified MSC
Blood Glucose Level
700
600
500
400
300
200
100
0
n=9
0 10 20 30 40 50 60 70 80 90
Days post hyperglycemia
C
Diabetic NOD mice treated with
modified MSC Reversal
Blood Glucose Level
700
600
500
400
300
200
100
0
n=8
0 10 20 30 40 50 60 70 80 90
Days post hyperglycemia

Figure 3

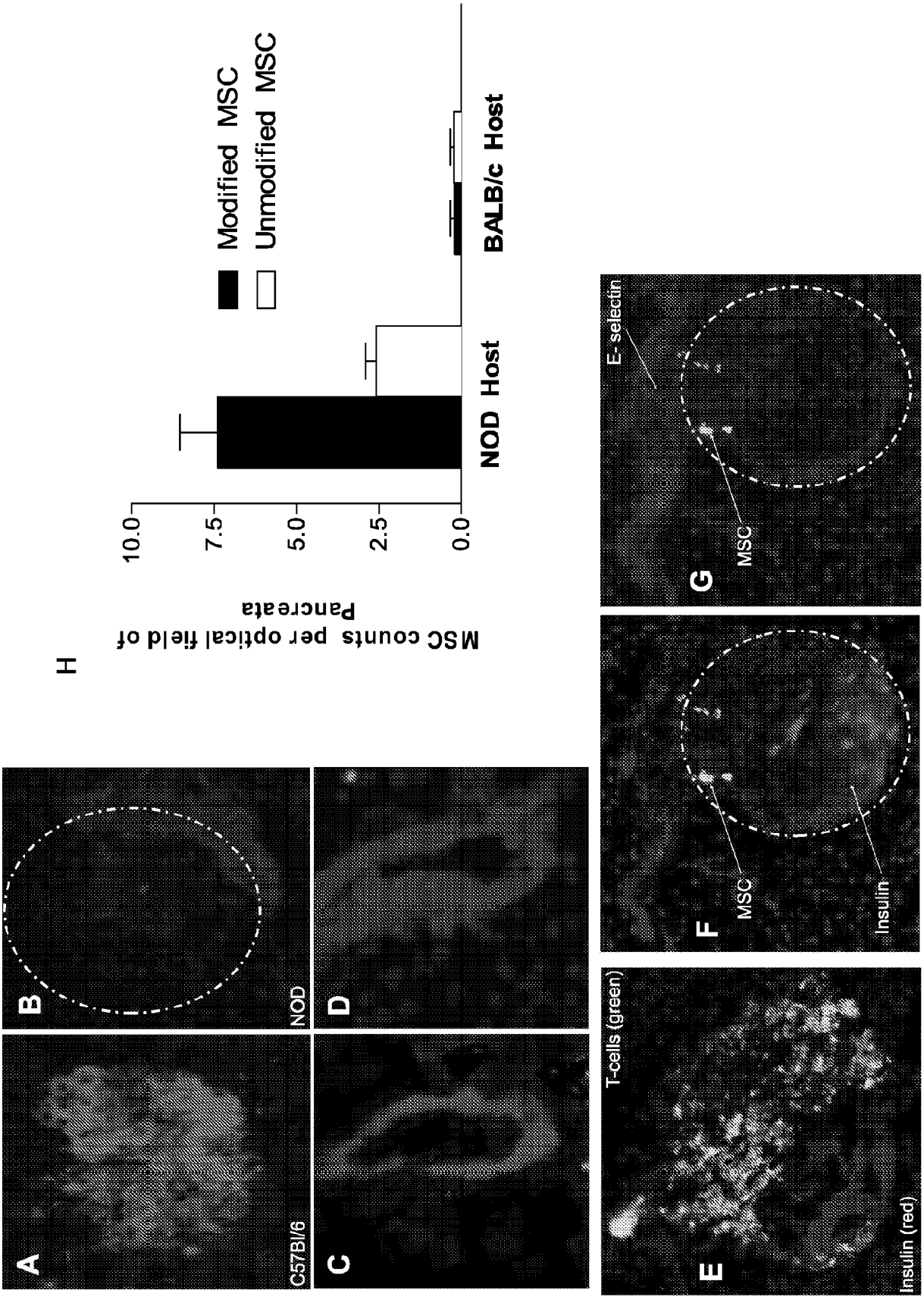
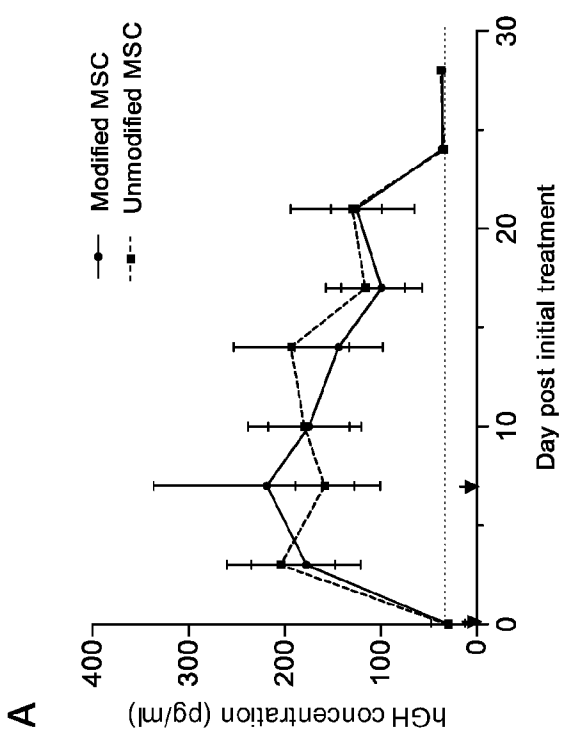

A
C57Bl/6
B
NOD
C
D
E
T-cells (green)
Insulin (red)
F
MSC
Insulin
G
E- selectin
MSC
H
MSC counts per optical field of Pancreata
10.0
7.5
5.0
2.5
0.0
Modified MSC
Unmodified MSC
NOD Host
BALB/c Host

Figure 4


A
Modified MSC
Unmodified MSC
hGH concentration (pg/ml)
400
300
200
100
0
0
10
20
30
Day post initial treatment

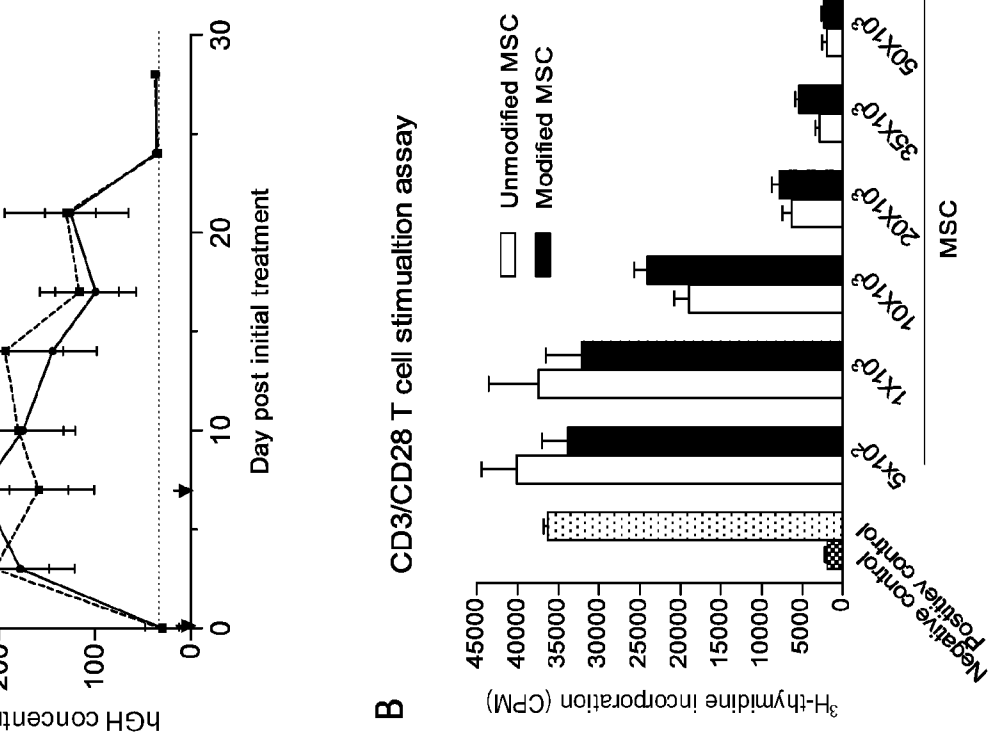

B
CD3/CD28 T cell stimualtion assay
Unmodified MSC
Modified MSC
3H-thymidine incorporation (CPM)
45000
40000
35000
30000
25000
20000
15000
10000
5000
0
Negative control
Positive control
5X10³
1X10⁴
10X10⁴
20X10⁴
35X10⁴
50X10⁴
MSC

Figure 5

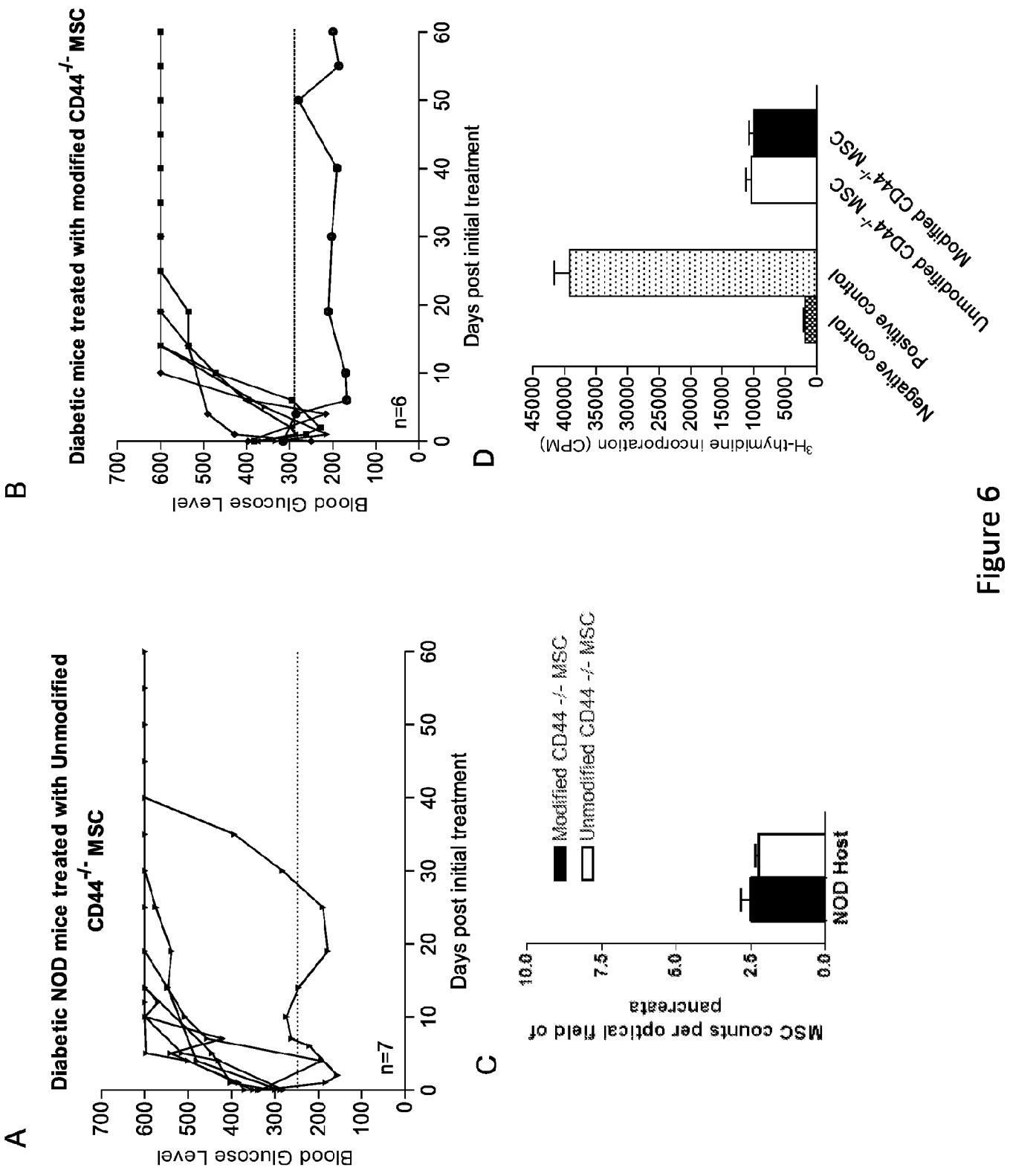

A
Diabetic NOD mice treated with Unmodified CD44-/- MSC
Blood Glucose Level
Days post initial treatment
n=7
B
Diabetic mice treated with modified CD44-/- MSC
Blood Glucose Level
Days post initial treatment
n=6
C
MSC counts per optical field of pancreata
Modified CD44 -/- MSC
Unmodified CD44 -/- MSC
NOD Host
D
3H-thymidine incorporation (CPM)
Negative control
Positive control
Unmodified CD44-/- MSC
Modified CD44-/- MSC

Figure 6

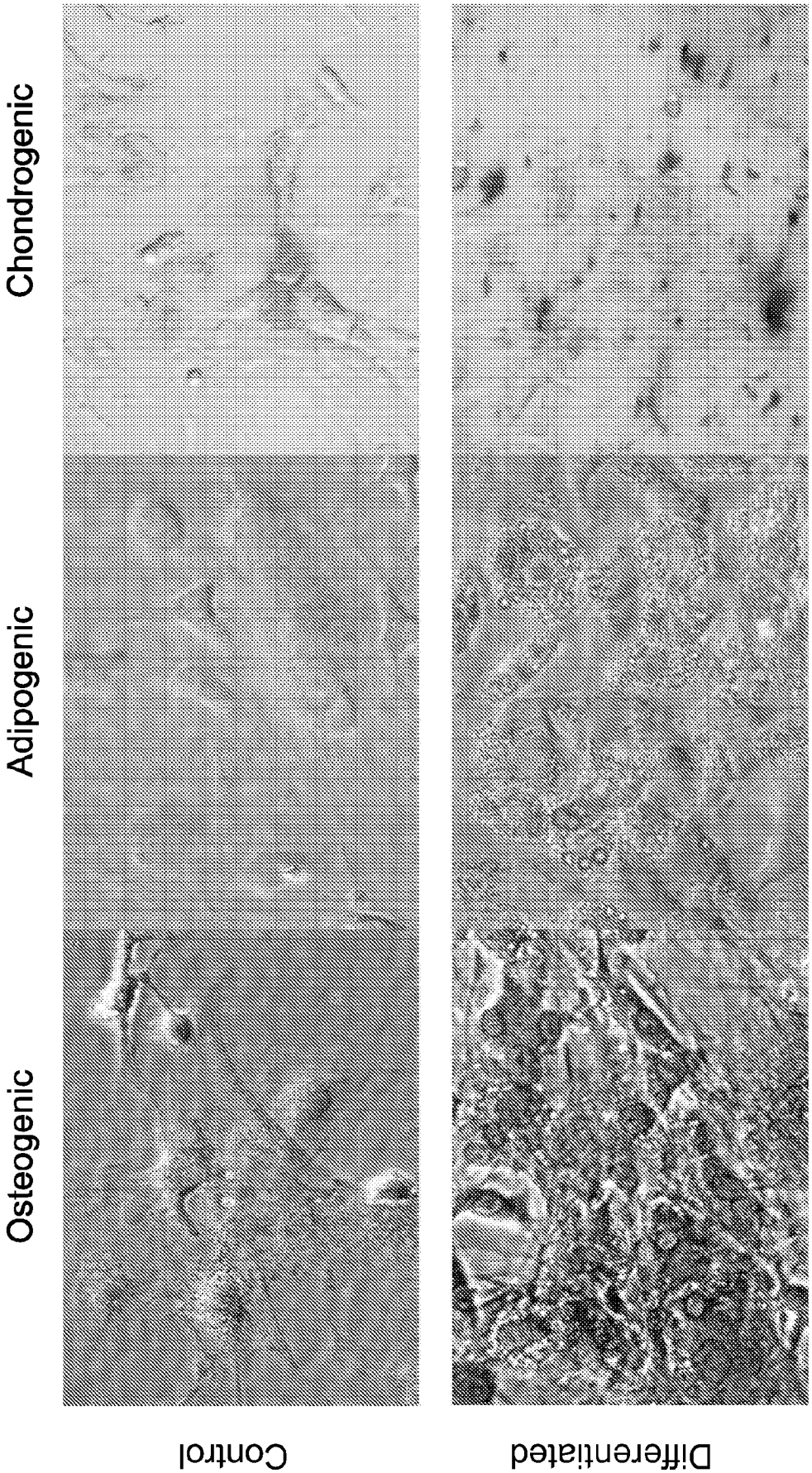

Osteogenic
Adipogenic
Chondrogenic
Control
Differentiated
Alizarin Red
Oil Red
Alcian Blue

Figure 7

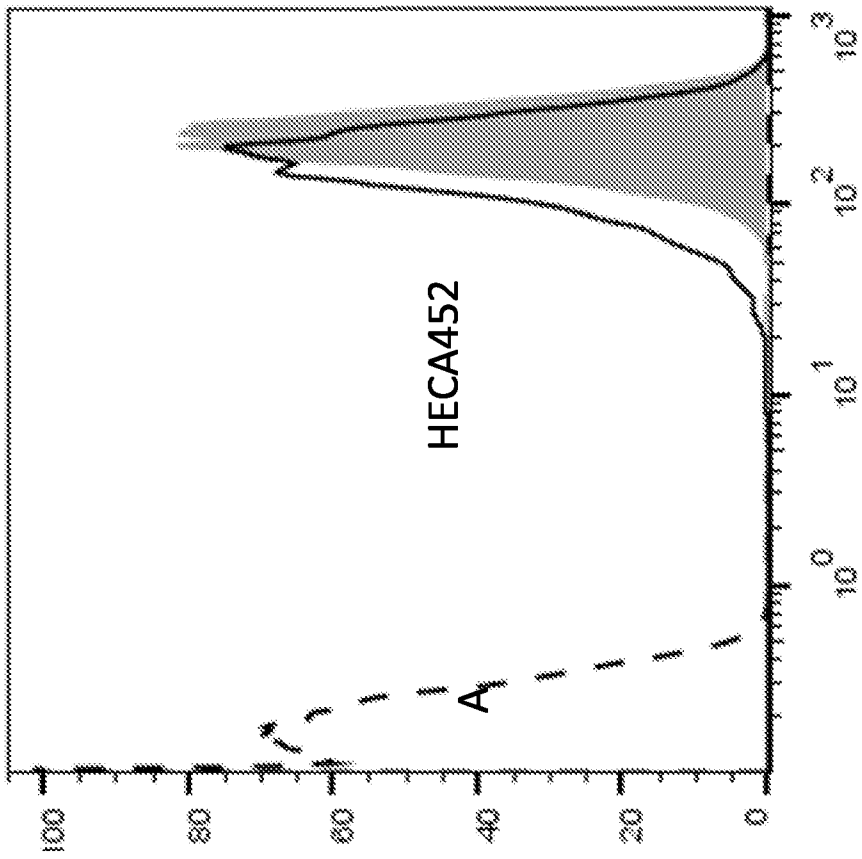


Figure 8

FIGURE 9

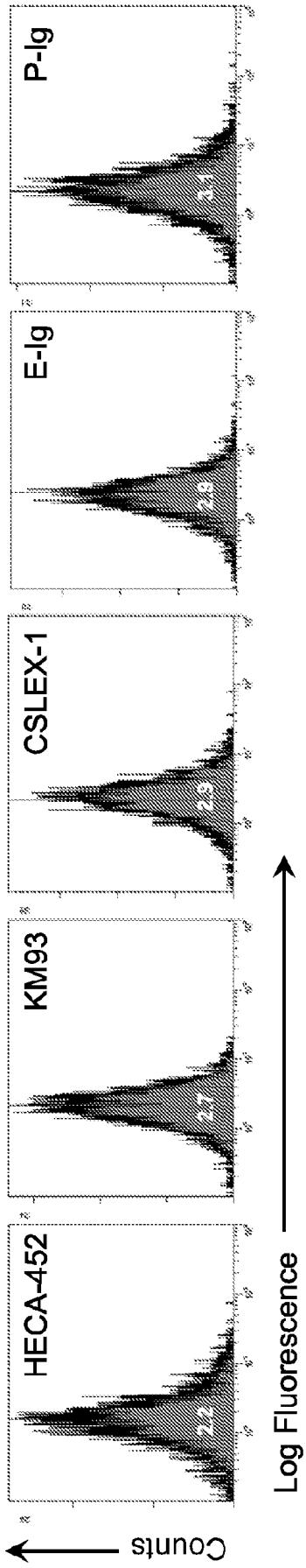


FIGURE 9

B

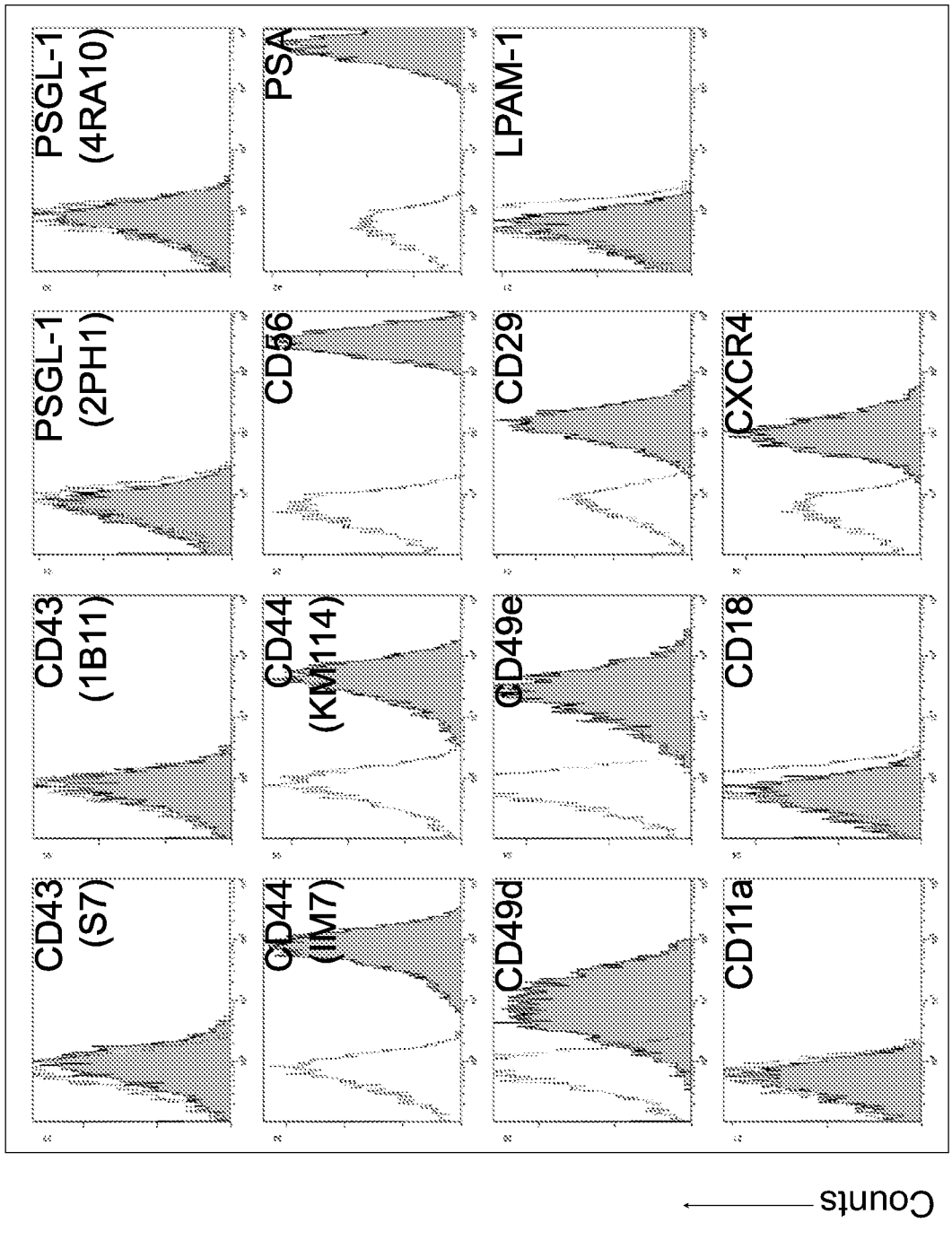

PSGL-1 (4RA10)
PSA
LPAM-1
PSGL-1 (2PH1)
CD56
CD29
CXCR4
CD43 (1B11)
CD44 (KM114)
CD49e
CD18
CD43 (S7)
CD44 (IM7)
CD49d
CD11a
Counts
Log Fluorescence

FIGURE 10

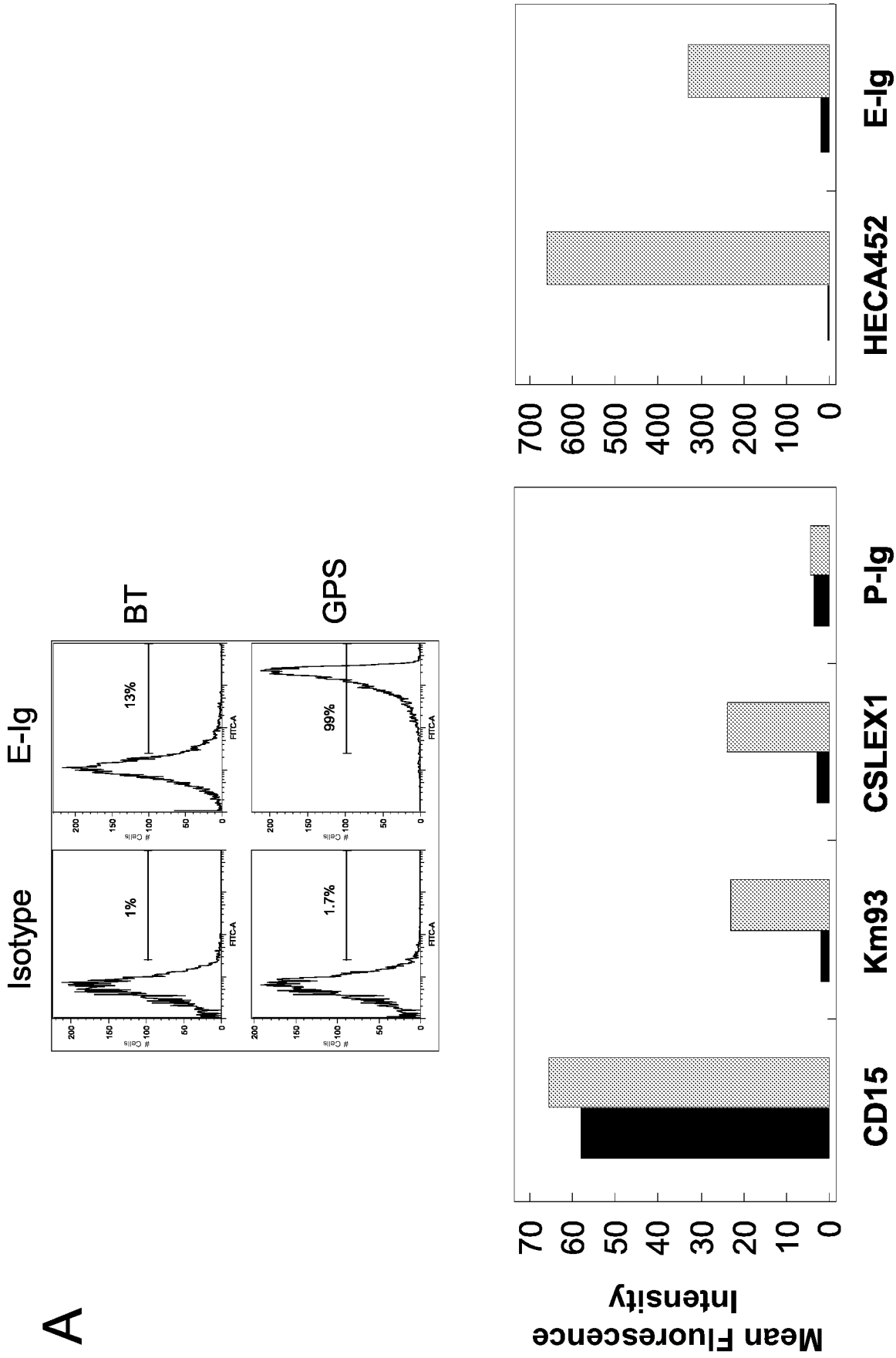

A
Isotype
E-Ig
BT
GPS
1%
13%
1.7%
99%
Cells
FITC-A
Mean Fluorescence Intensity
CD15
Km93
CSLEX1
P-Ig
HECA452
E-Ig

FIGURE 10

B

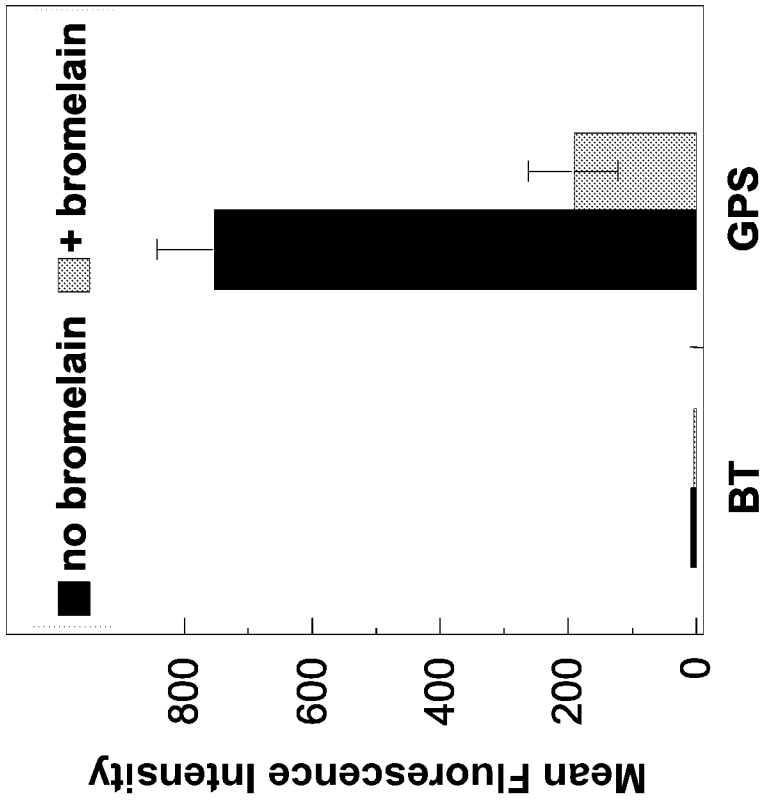
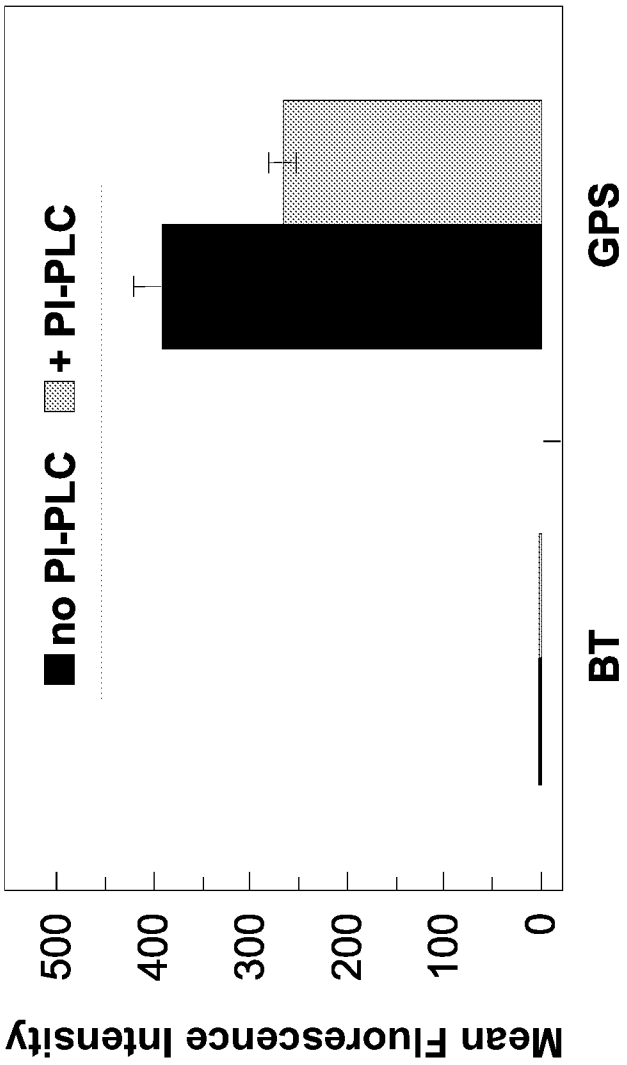

Mean Fluorescence Intensity
0
200
400
600
800
BT
GPS
no bromelain
+ bromelain

FIGURE 10


Mean Fluorescence Intensity
500
400
300
200
100
0
BT
GPS
no PI-PLC
+ PI-PLC

C

FIGURE 11

A

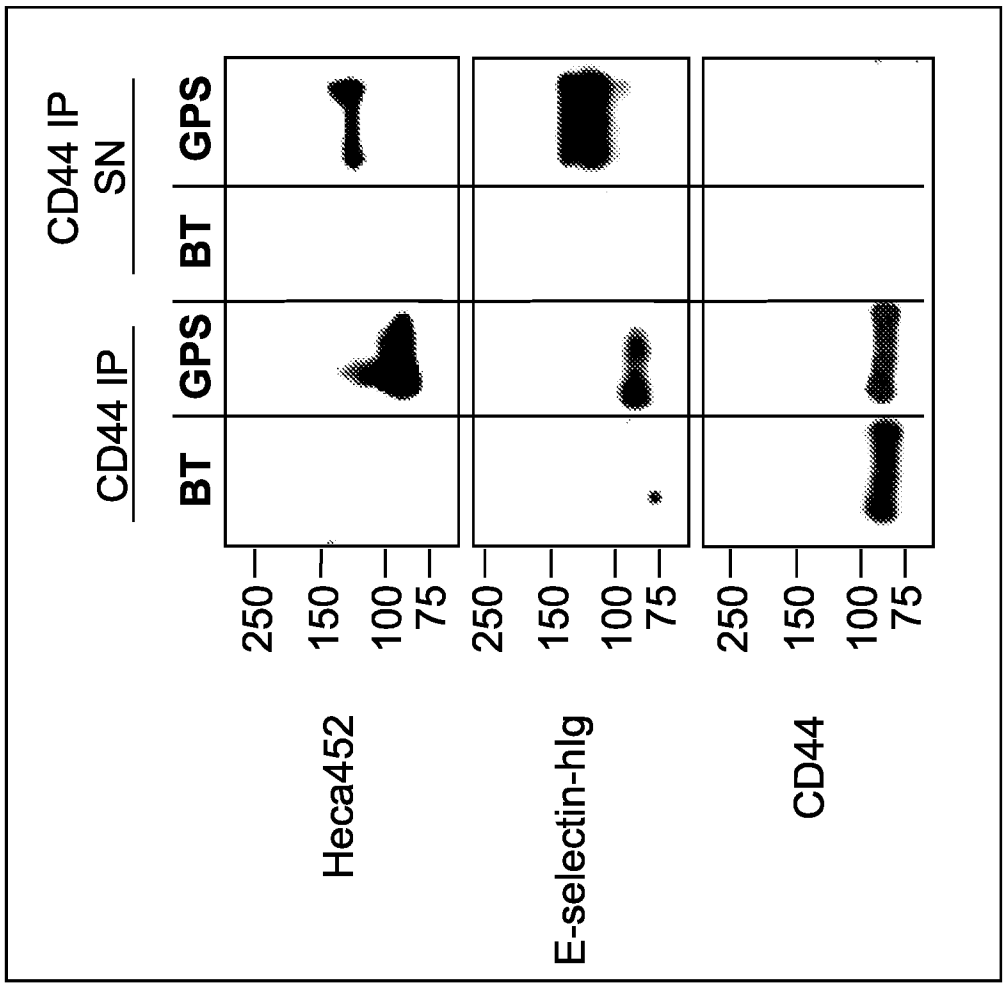

CD44 IP
BT
GPS
CD44 IP
SN
BT
GPS
Heca452
E-selectin-hlg
CD44
250
150
100
75

FIGURE 11

B

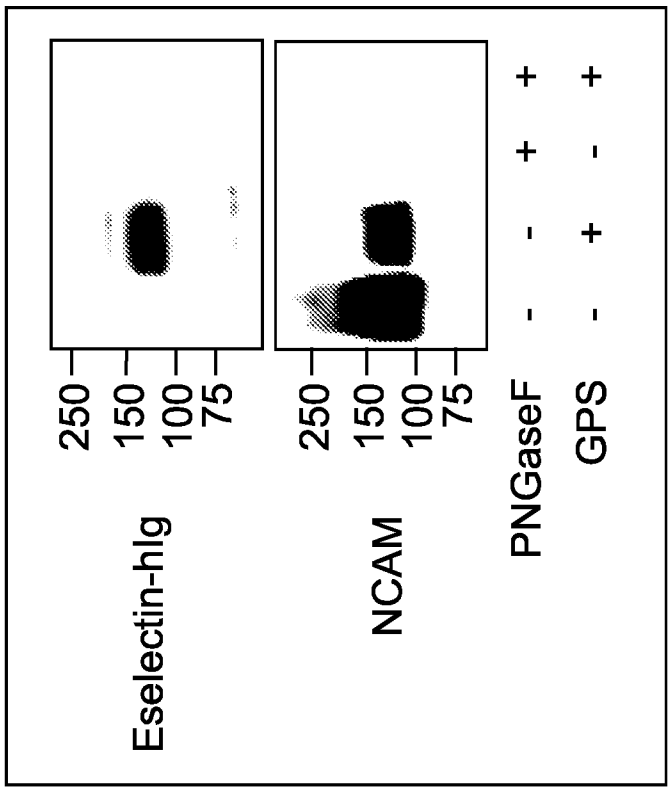


FIGURE 11

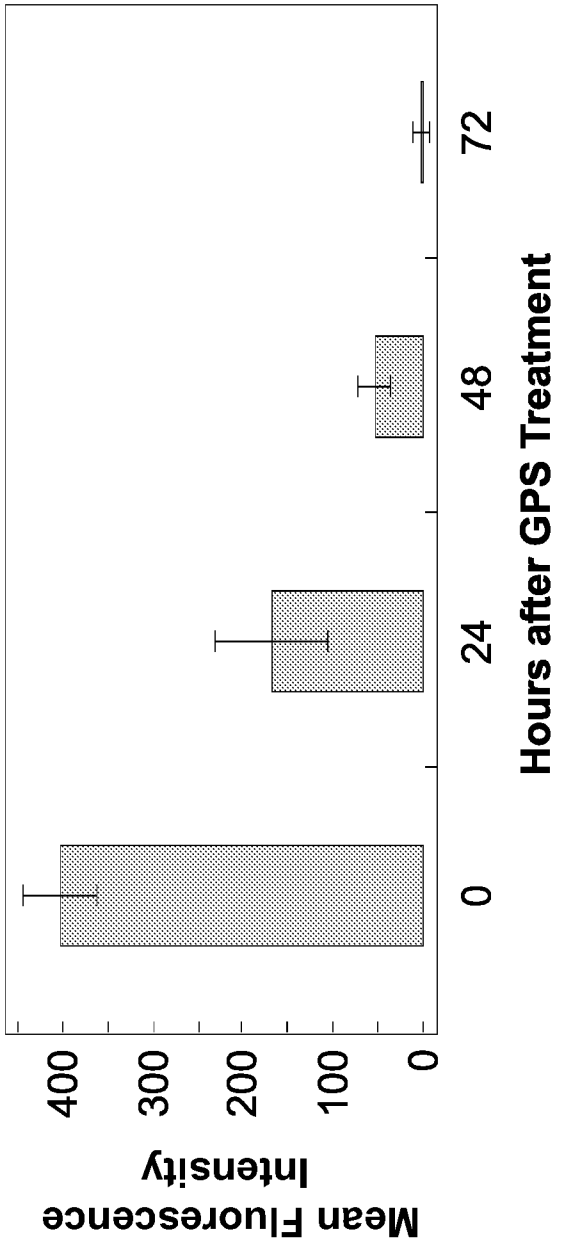

C
Mean Fluorescence Intensity
0
100
200
300
400
0
24
48
72
Hours after GPS Treatment

FIGURE 12

A

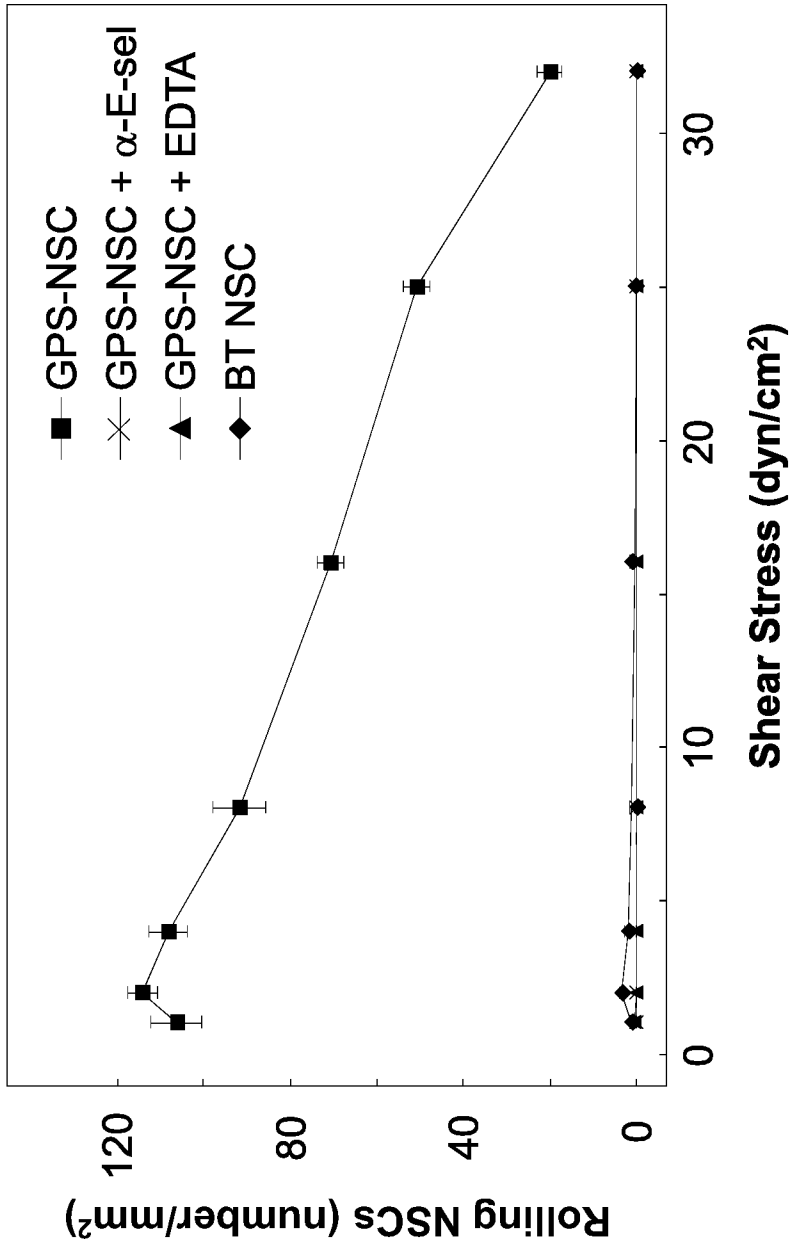

GPS-NSC
GPS-NSC + α-E-sel
GPS-NSC + EDTA
BT NSC
Rolling NSCs (number/mm²)
0
40
80
120
0
10
20
30
Shear Stress (dyn/cm²)

FIGURE 12

B

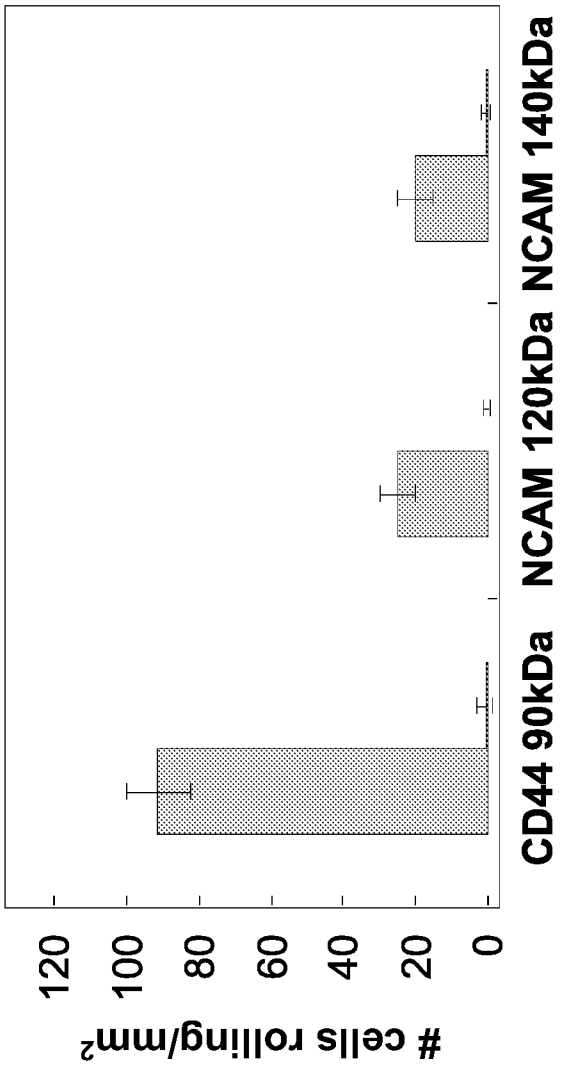

120
100
80
60
40
20
0
cells rolling/mm2
CD44 90kDa
NCAM 120kDa
NCAM 140kDa

FIGURE 13

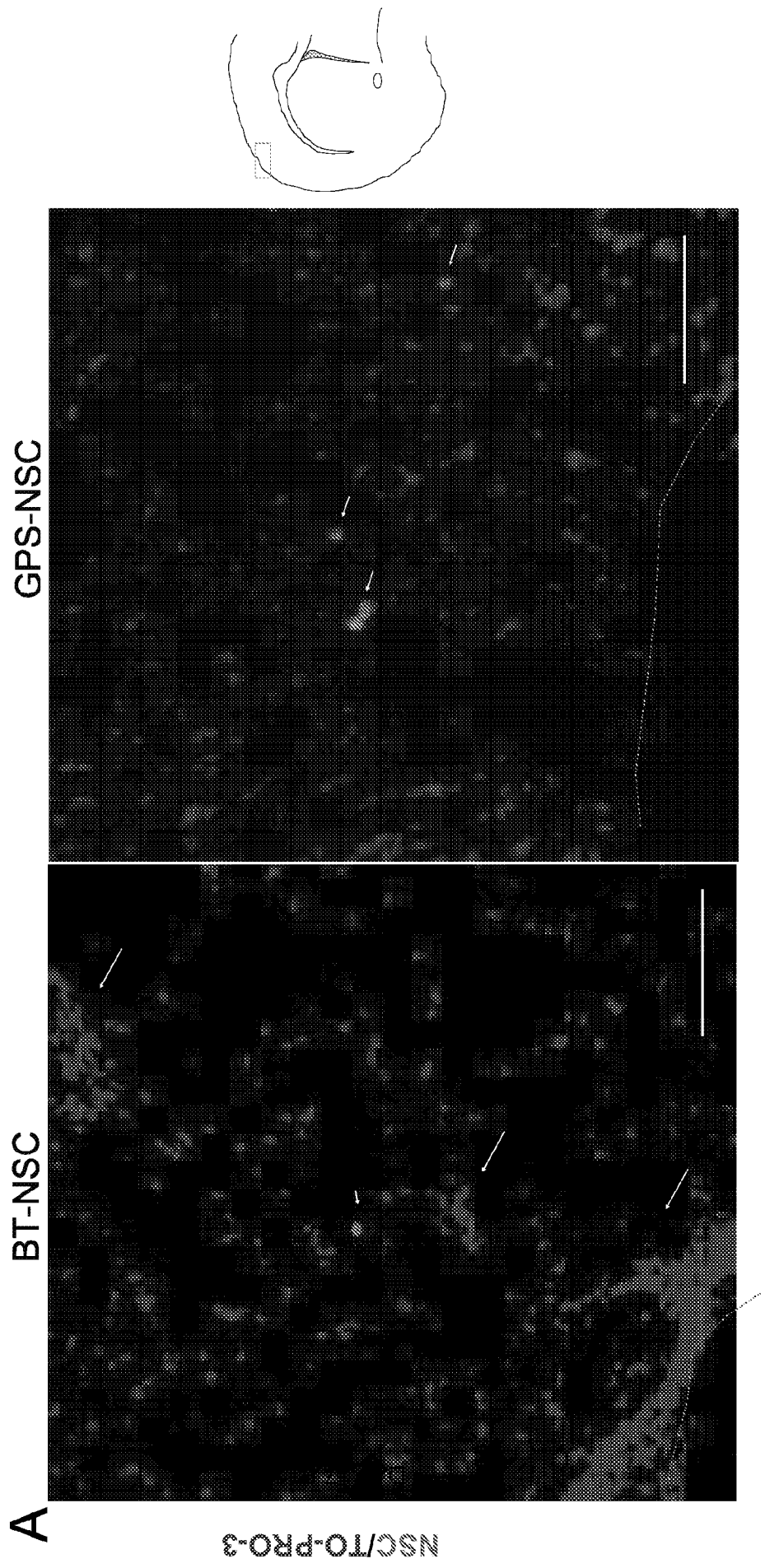


FIGURE 13

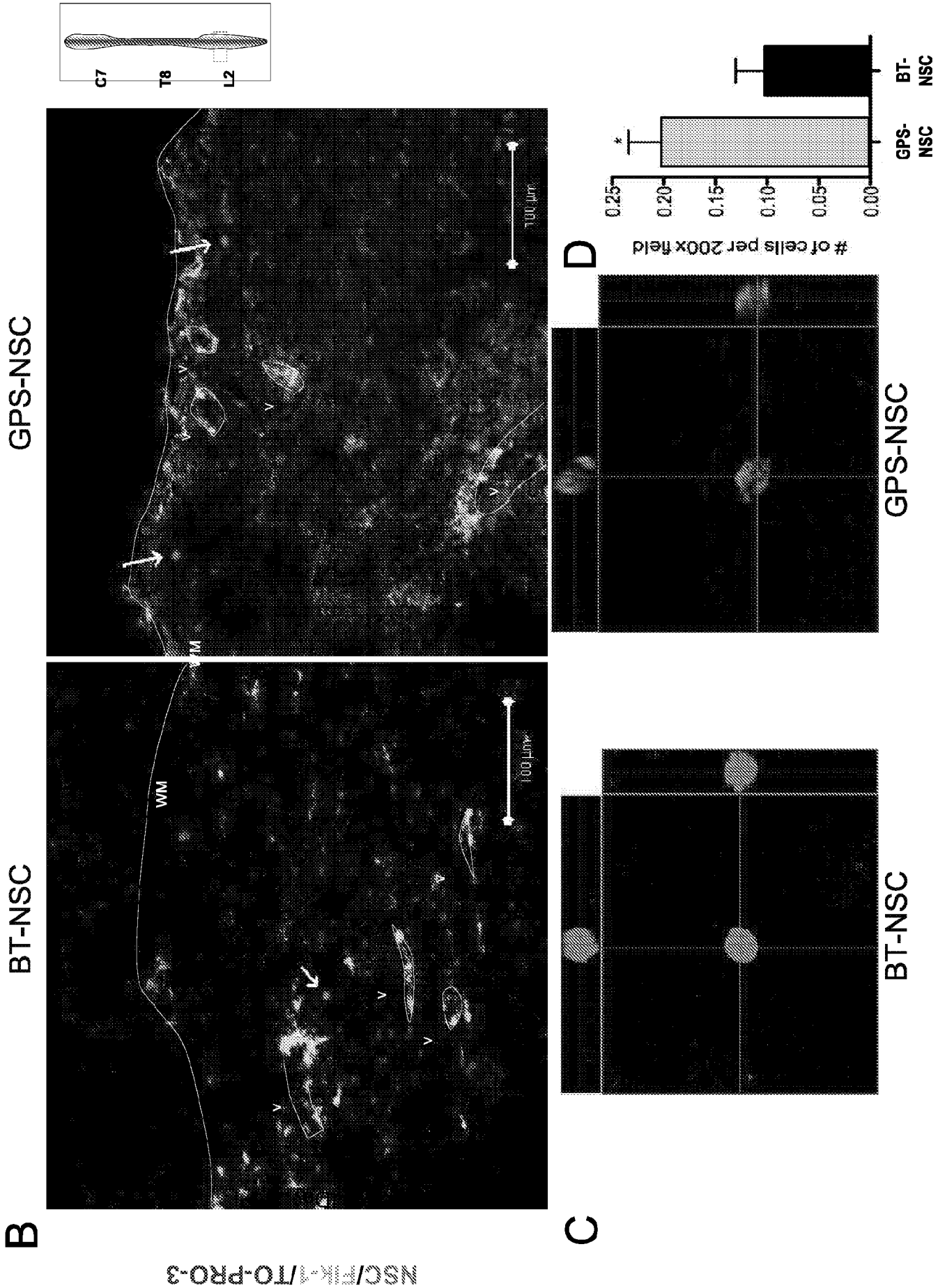
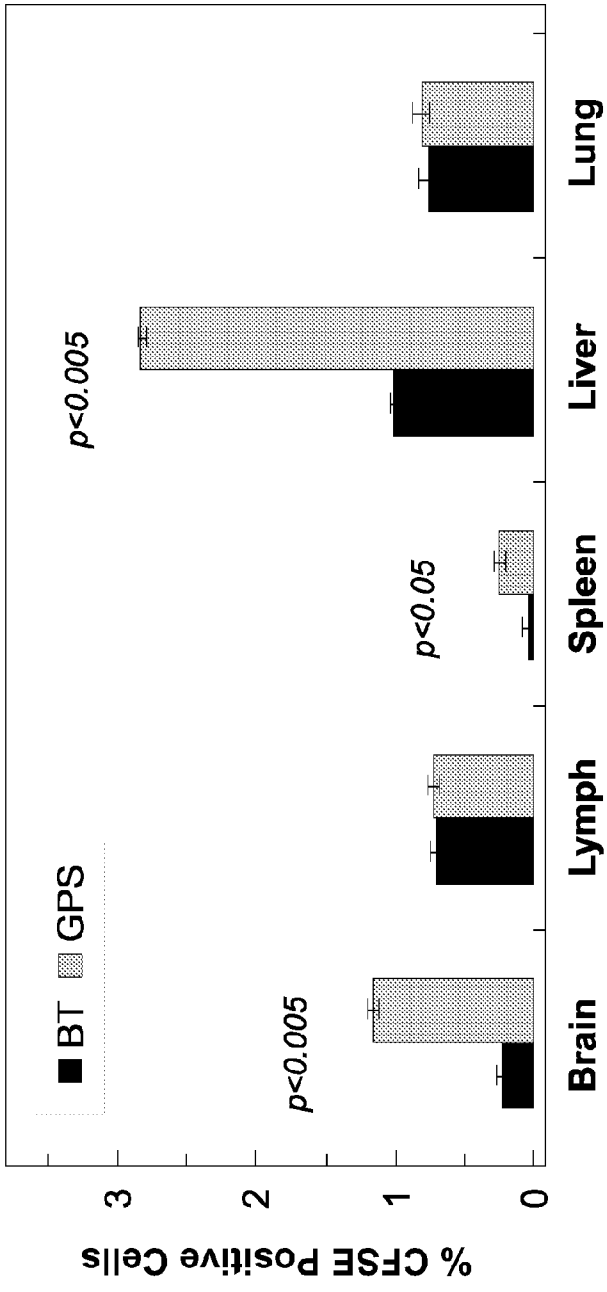

B
BT-NSC
GPS-NSC
WM
100 µm
C7
T8
L2
C
BT-NSC
D
GPS-NSC
# of cells per 200x field
0.25
0.20
0.15
0.10
0.05
0.00
*
GPS-NSC
BT-NSC
NSC/FLK-1/TO-PRO-3

FIGURE 13

E


% CFSE Positive Cells
0
1
2
3
BT
GPS
p<0.005
p<0.005
p<0.05
Brain
Lymph Node
Spleen
Liver
Lung

F

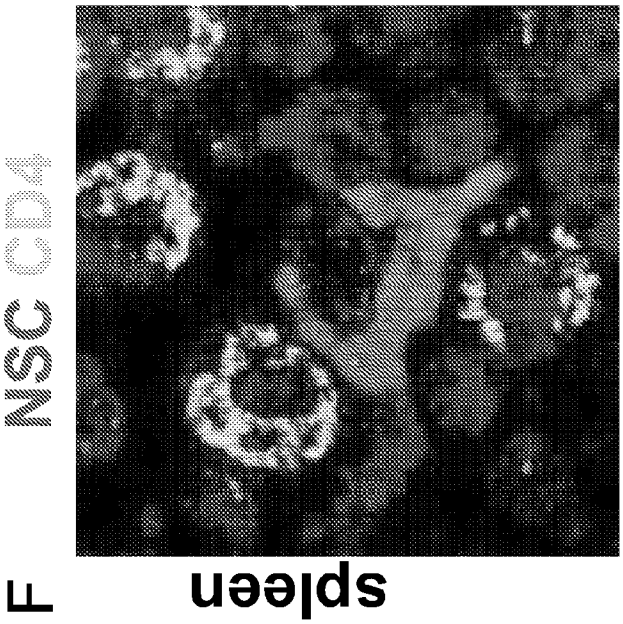

NSC CD4
spleen

FIGURE 14

A

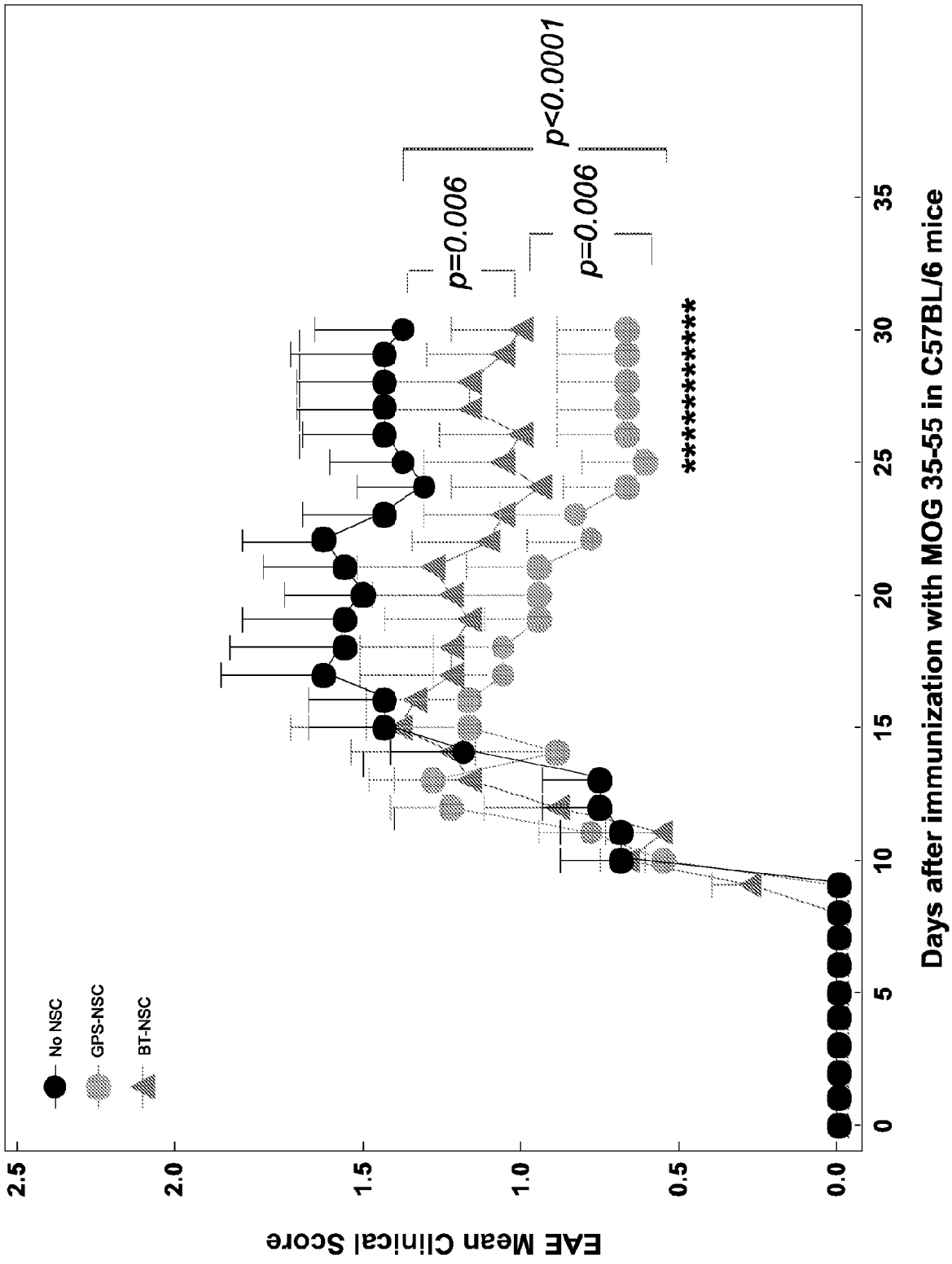

No NSC
GPS-NSC
BT-NSC
EAE Mean Clinical Score
Days after immunization with MOG 35-55 in C57BL/6 mice
p=0.006
p=0.006
p<0.0001
****

FIGURE 14

B

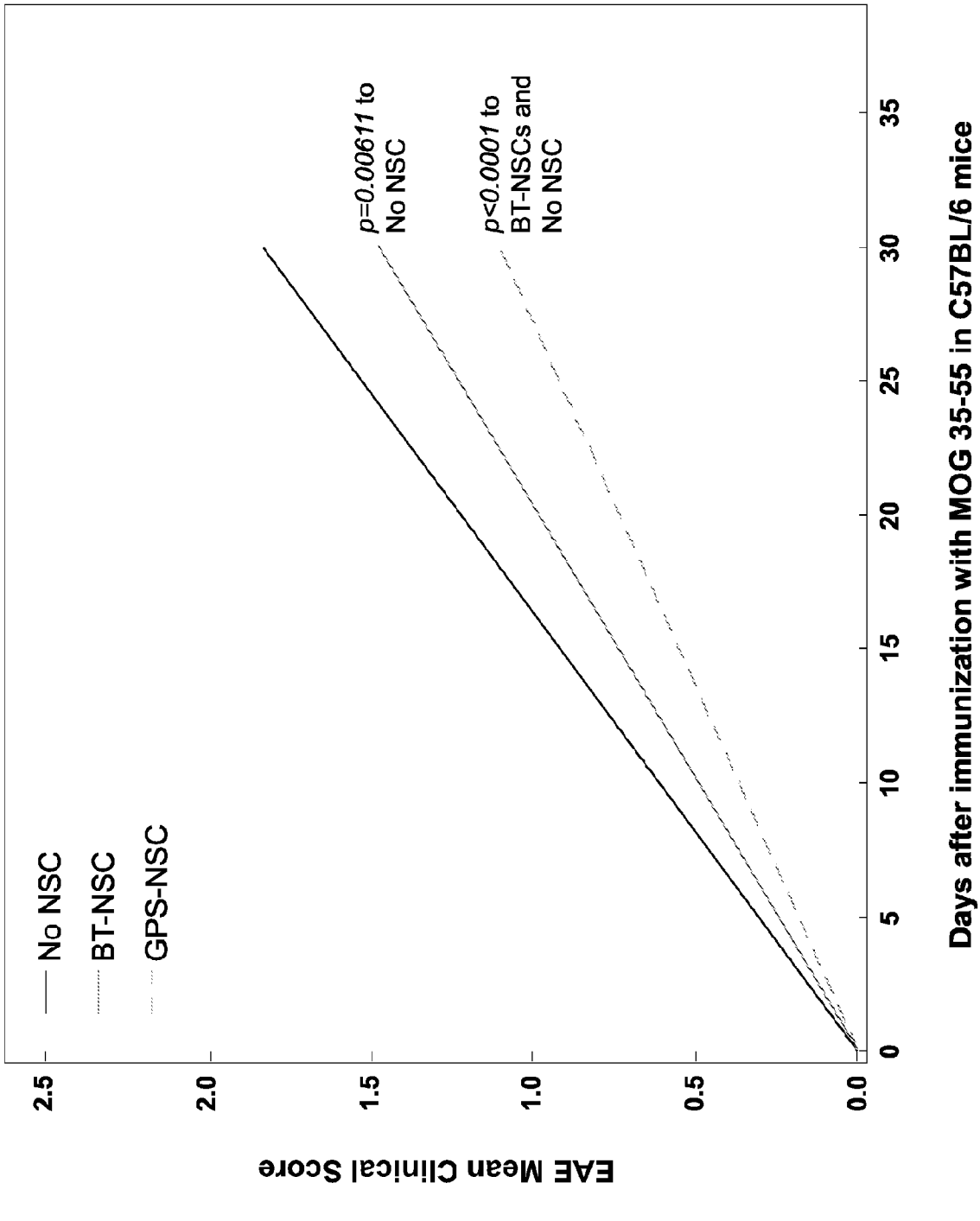

No NSC
BT-NSC
GPS-NSC
p=0.00611 to No NSC
p<0.0001 to BT-NSCs and No NSC
EAE Mean Clinical Score
0.0
0.5
1.0
1.5
2.0
2.5
0
5
10
15
20
25
30
35
Days after immunization with MOG 35-55 in C57BL/6 mice

FIGURE 14

C

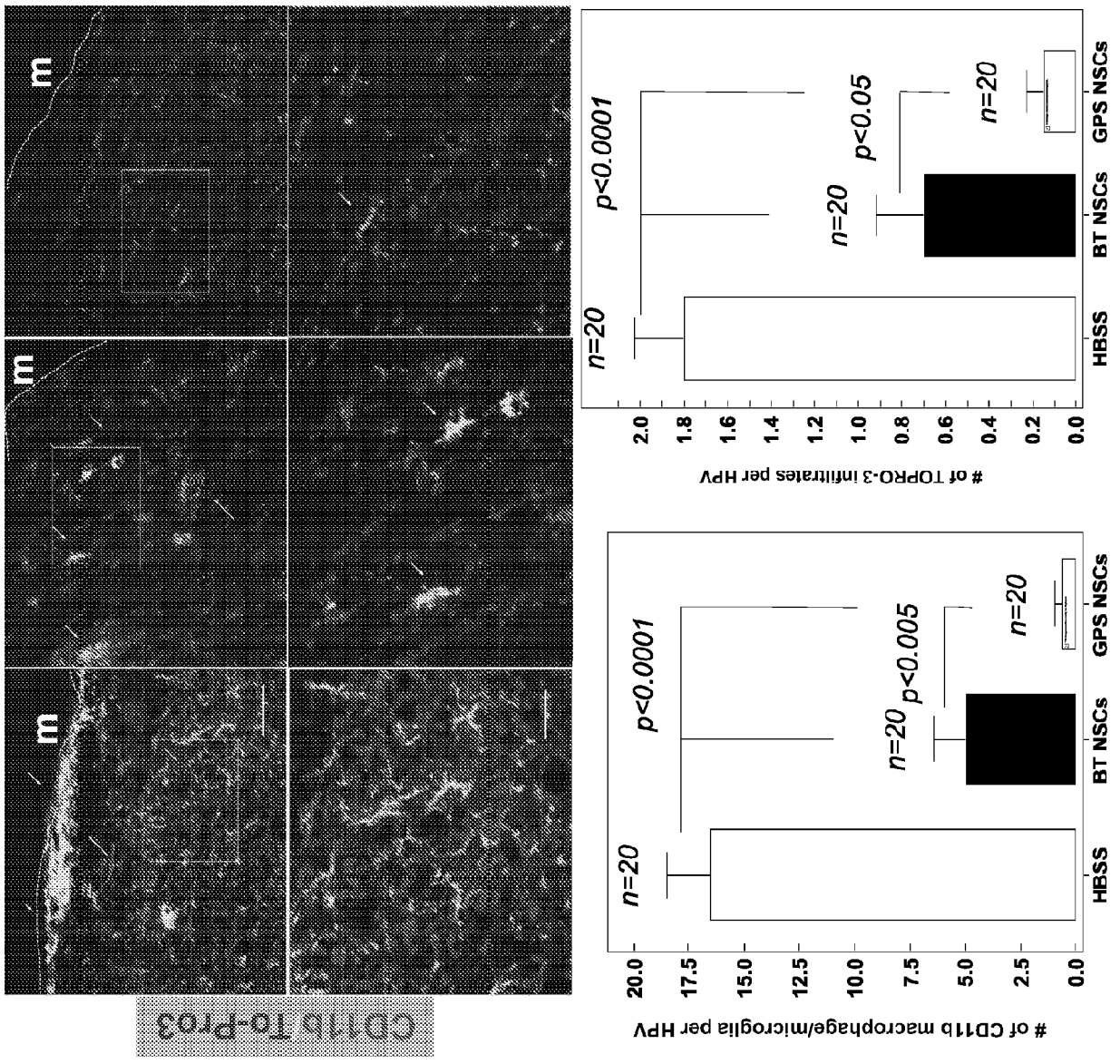

EAE No NSCs
EAE BT-NSCs
EAE GPS-NSCs
m
CD11b To-Pro3
# of CD11b macrophage/microglia per HPV
n=20
p<0.0001
n=20
p<0.005
n=20
HBSS
BT NSCs
GPS NSCs
# of TOPRO-3 infiltrates per HPV
n=20
p<0.0001
n=20
p<0.05
n=20

FIGURE 14

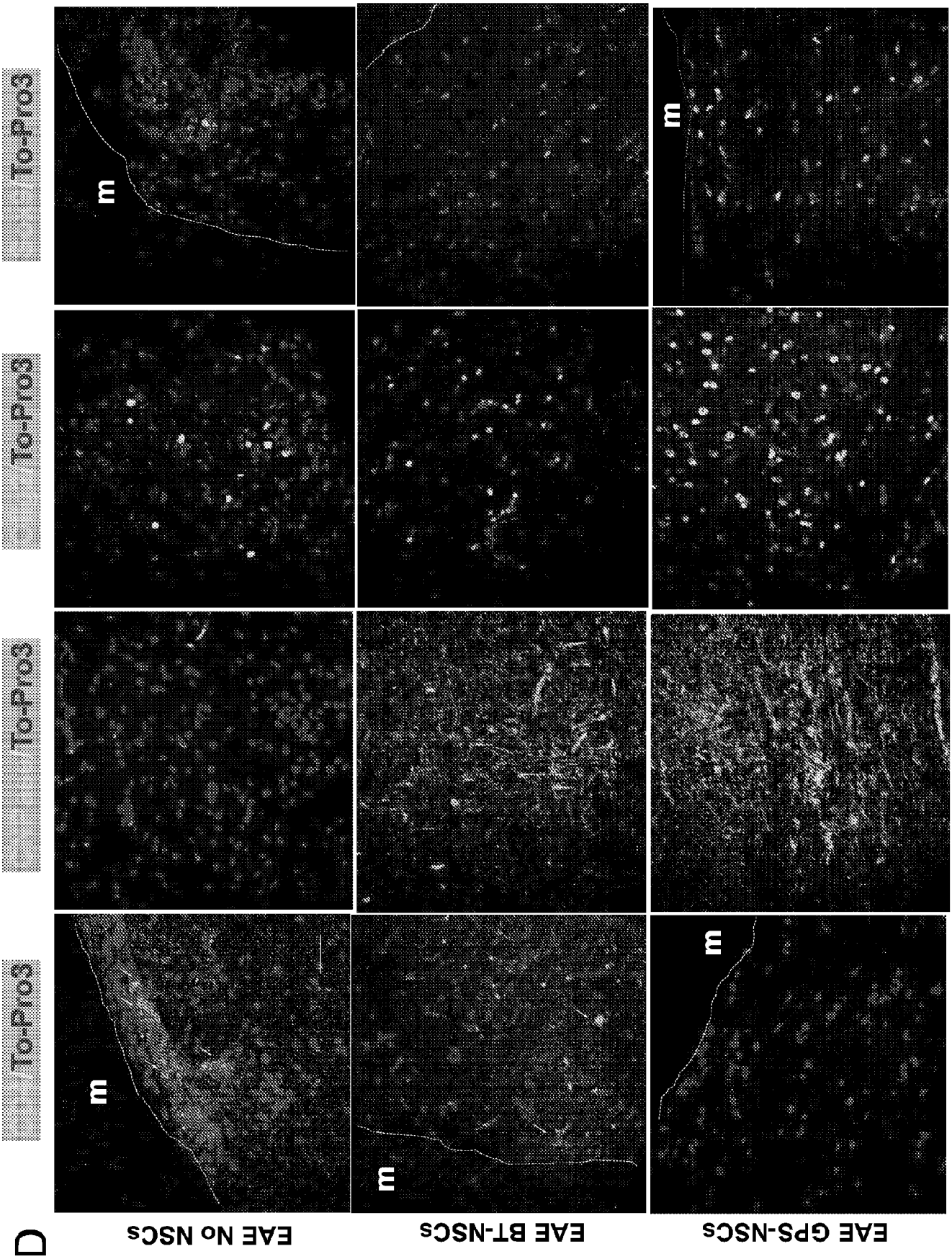

To-Pro3
To-Pro3
To-Pro3
To-Pro3
m
m
m
m
EAE No NSCs
EAE BT-NSCs
EAE GPS-NSCs
D

FIGURE 14

E

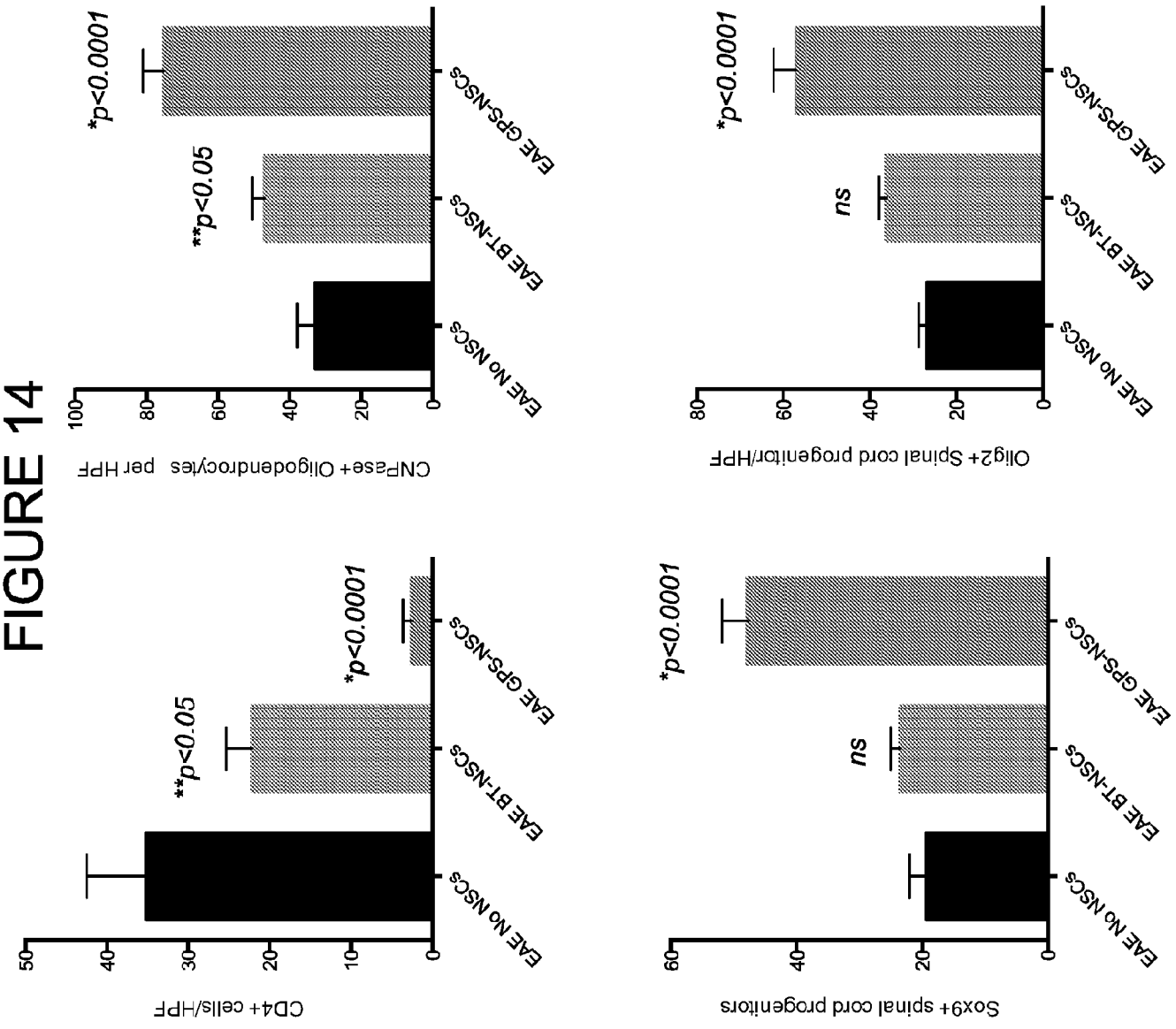

CD4+ cells/HPF
50
40
30
20
10
0
**p<0.05
*p<0.0001
EAE No NSCs
EAE BT-NSCs
EAE GPS-NSCs
CNPase+ Oligodendrocytes per HPF
100
80
60
40
20
0
**p<0.05
*p<0.0001
EAE No NSCs
EAE BT-NSCs
EAE GPS-NSCs
Sox9+ spinal cord progenitors
60
40
20
0
ns
*p<0.0001
EAE No NSCs
EAE BT-NSCs
EAE GPS-NSCs
Olig2+ Spinal cord progenitor/HPF
80
60
40
20
0
ns
*p<0.0001
EAE No NSCs
EAE BT-NSCs
EAE GPS-NSCs

FIGURE 14

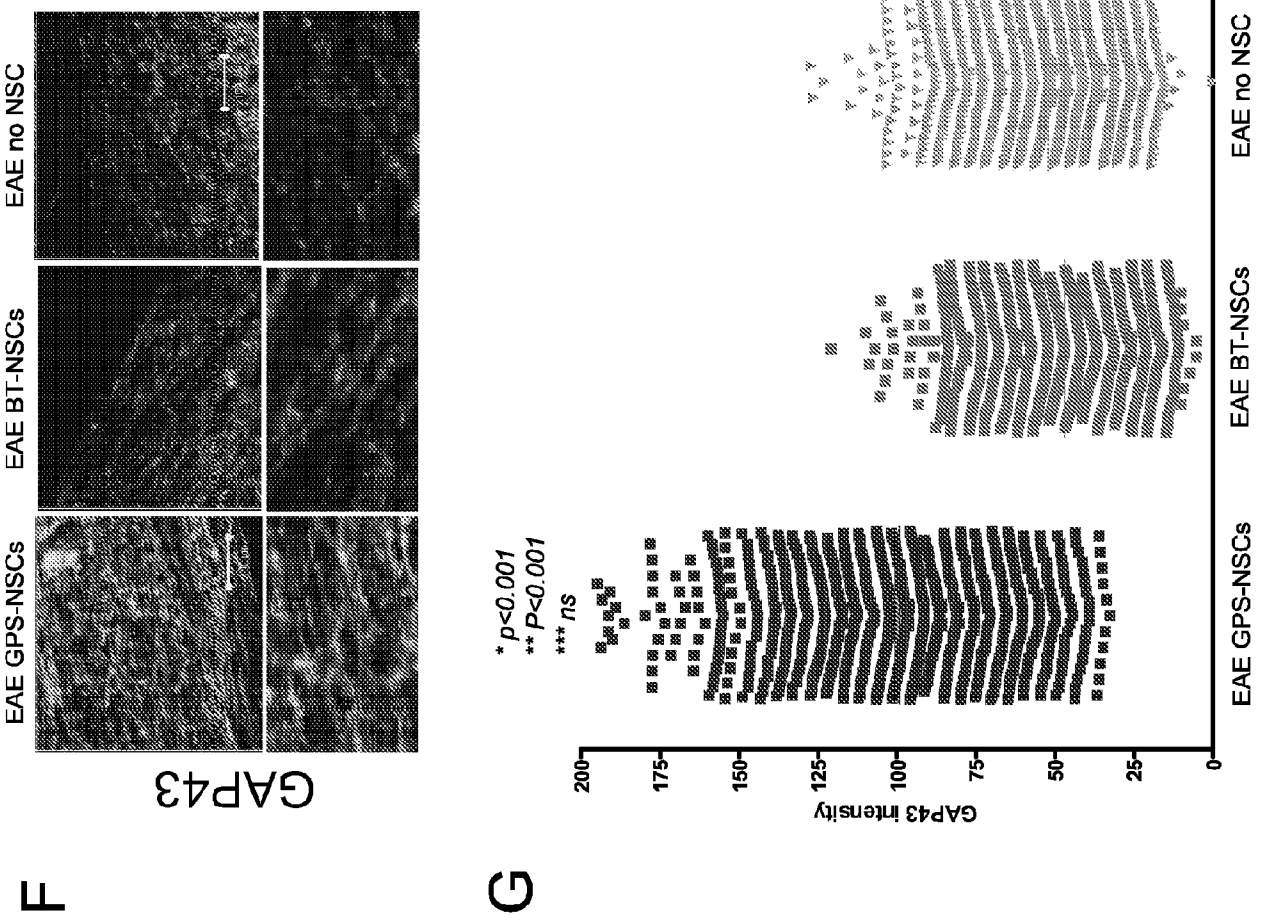

F
EAE GPS-NSCs
EAE BT-NSCs
EAE no NSC
GAP43
G
* p<0.001
** P<0.001
*** ns
EAE GPS-NSCs
EAE BT-NSCs
EAE no NSC
GAP43 intensity
200
175
150
125
100
75
50
25
0
EAE GPS-NSCs
EAE BT-NSCs
EAE no NSC

FIGURE 14

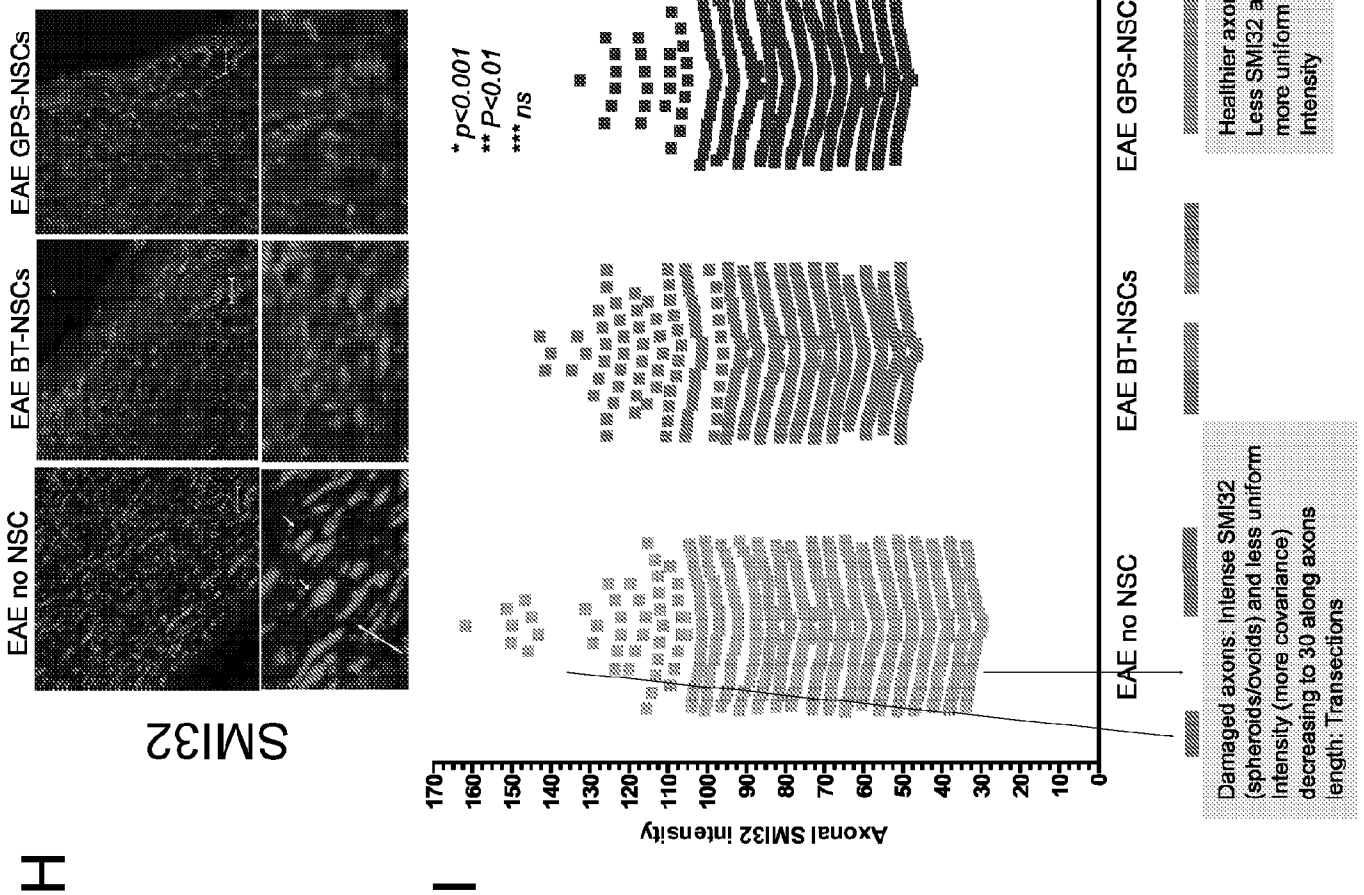

H
SMI32
EAE no NSC
EAE BT-NSCs
EAE GPS-NSCs
I
Axonal SMI32 intensity
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
EAE no NSC
EAE BT-NSCs
EAE GPS-NSCs
* p<0.001
** P<0.01
*** ns
Damaged axons: Intense SMI32 (spheroids/ovoids) and less uniform intensity (more covariance) decreasing to 30 along axons length: Transections
Healthier axons Less SMI32 and more uniform Intensity

Figure 15

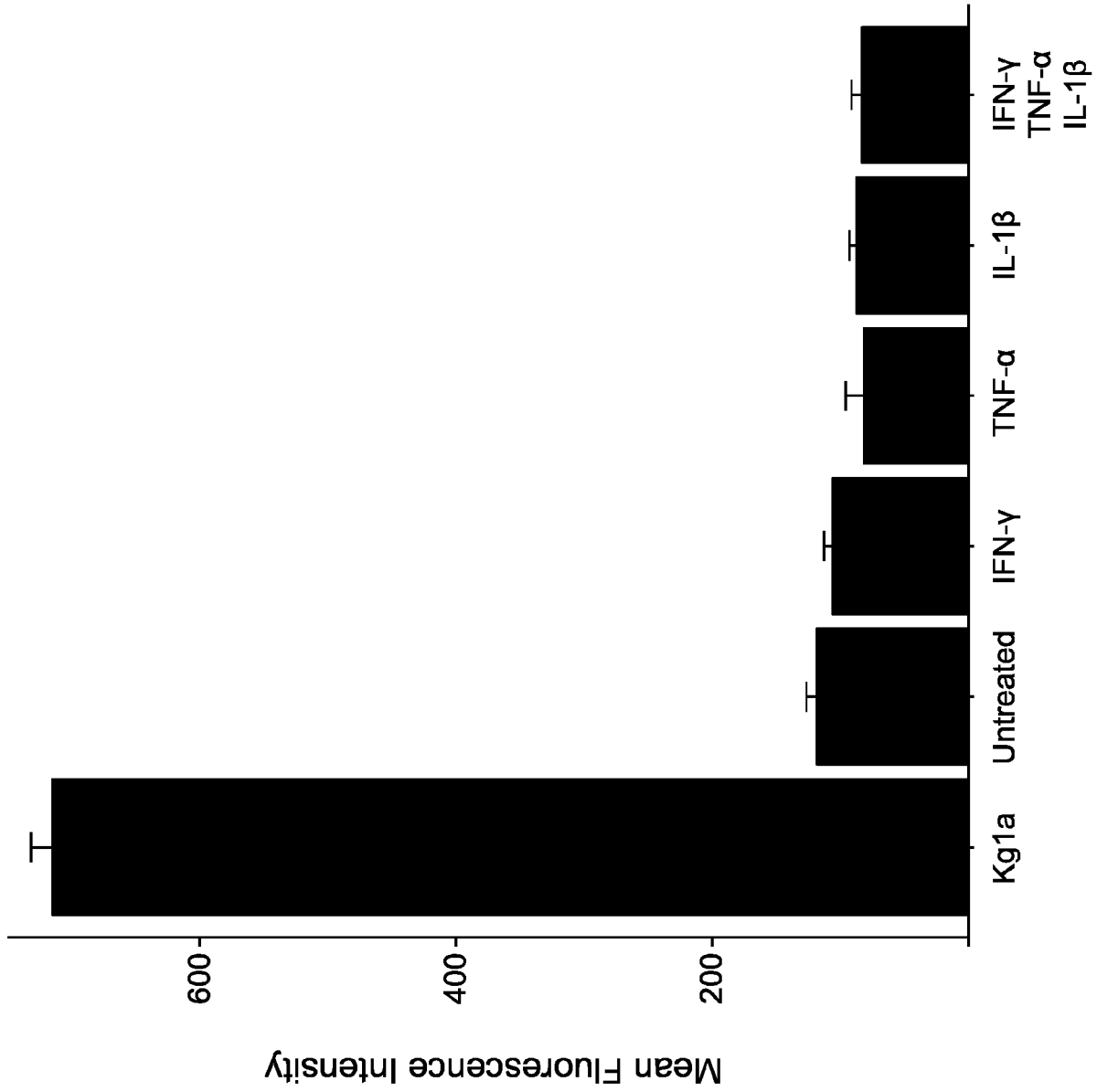

Mean Fluorescence Intensity
600
400
200
Kg1a
Untreated
IFN-γ
TNF-α
IL-1β
IFN-γ
TNF-α
IL-1β

Figure 16

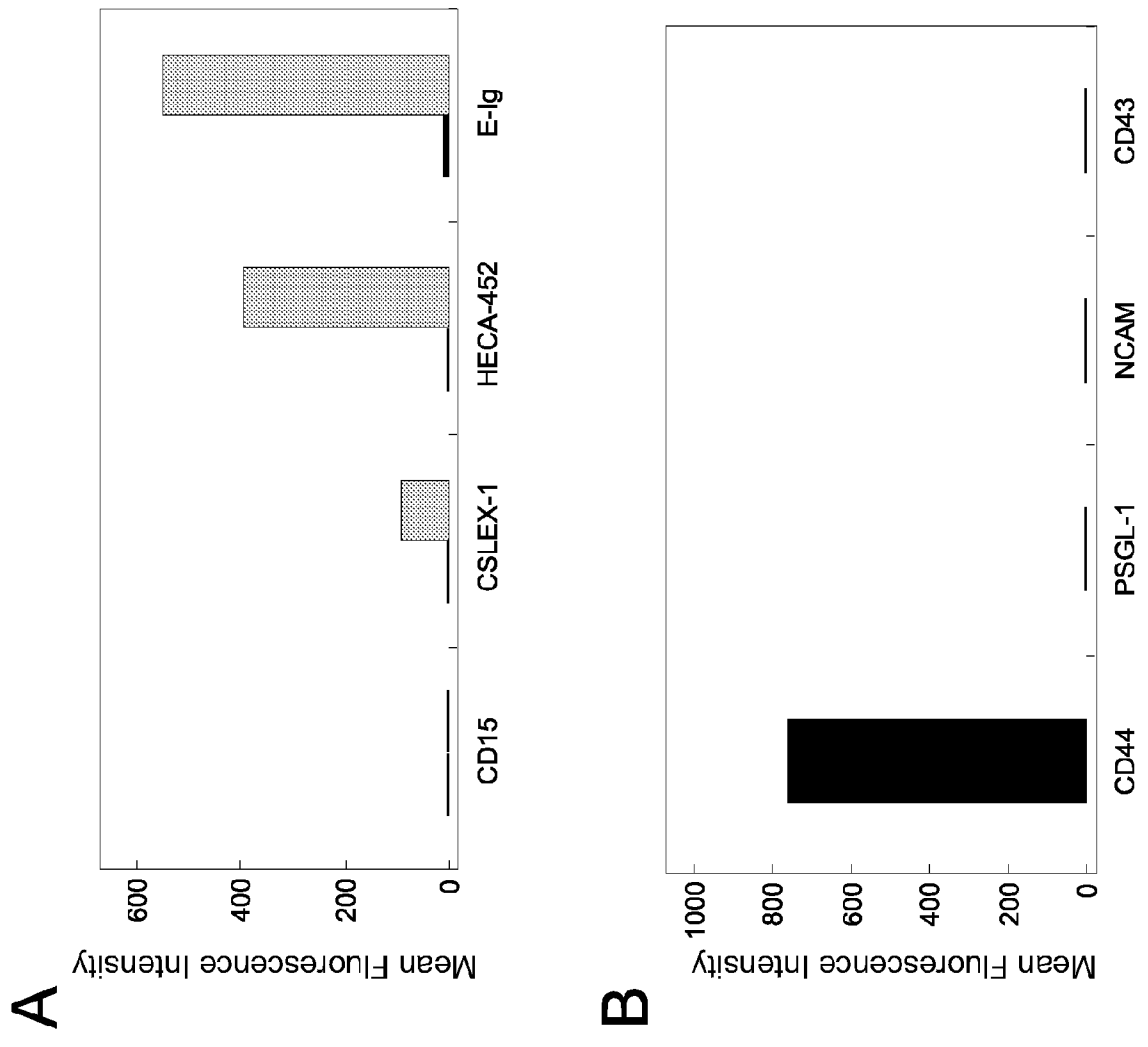

A
Mean Fluorescence Intensity
600
400
200
0
CD15
CSLEX-1
HECA-452
E-lg
B
Mean Fluorescence Intensity
1000
800
600
400
200
0
CD44
PSGL-1
NCAM
CD43
C
CD44IP NCAMIP
mouse
human
150
100
75
150
100
75
GPS - + - +

Figure 17

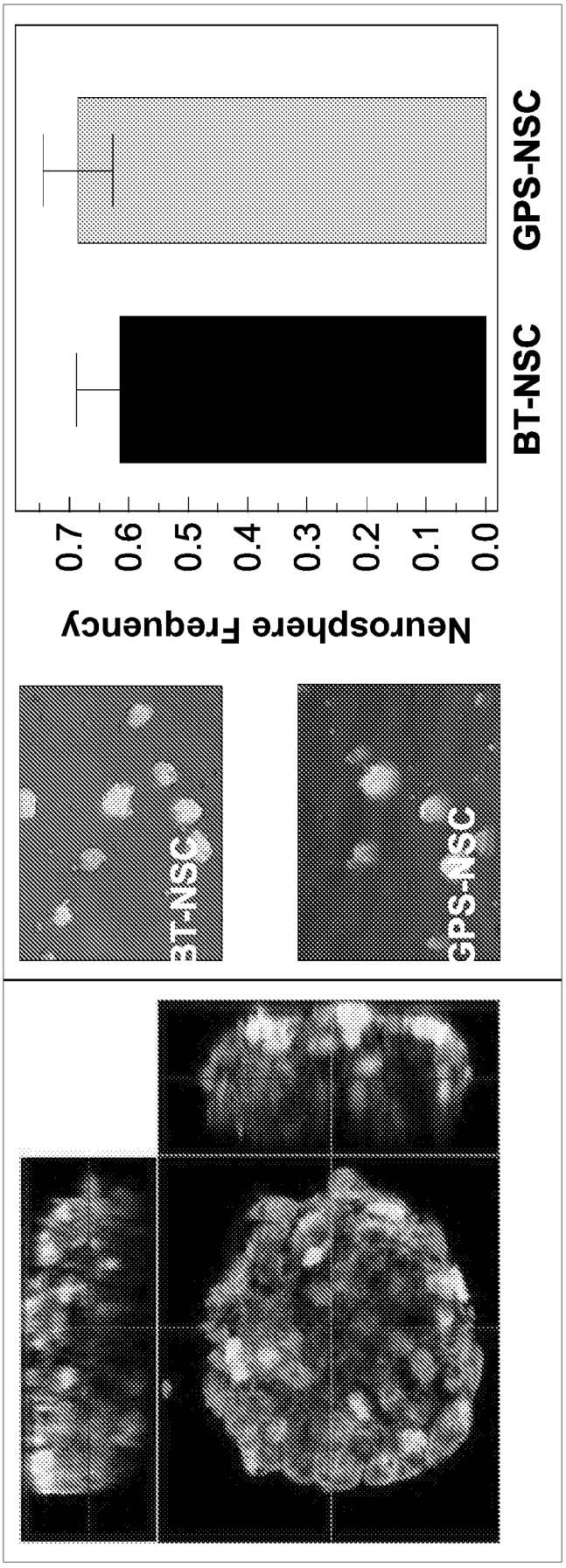
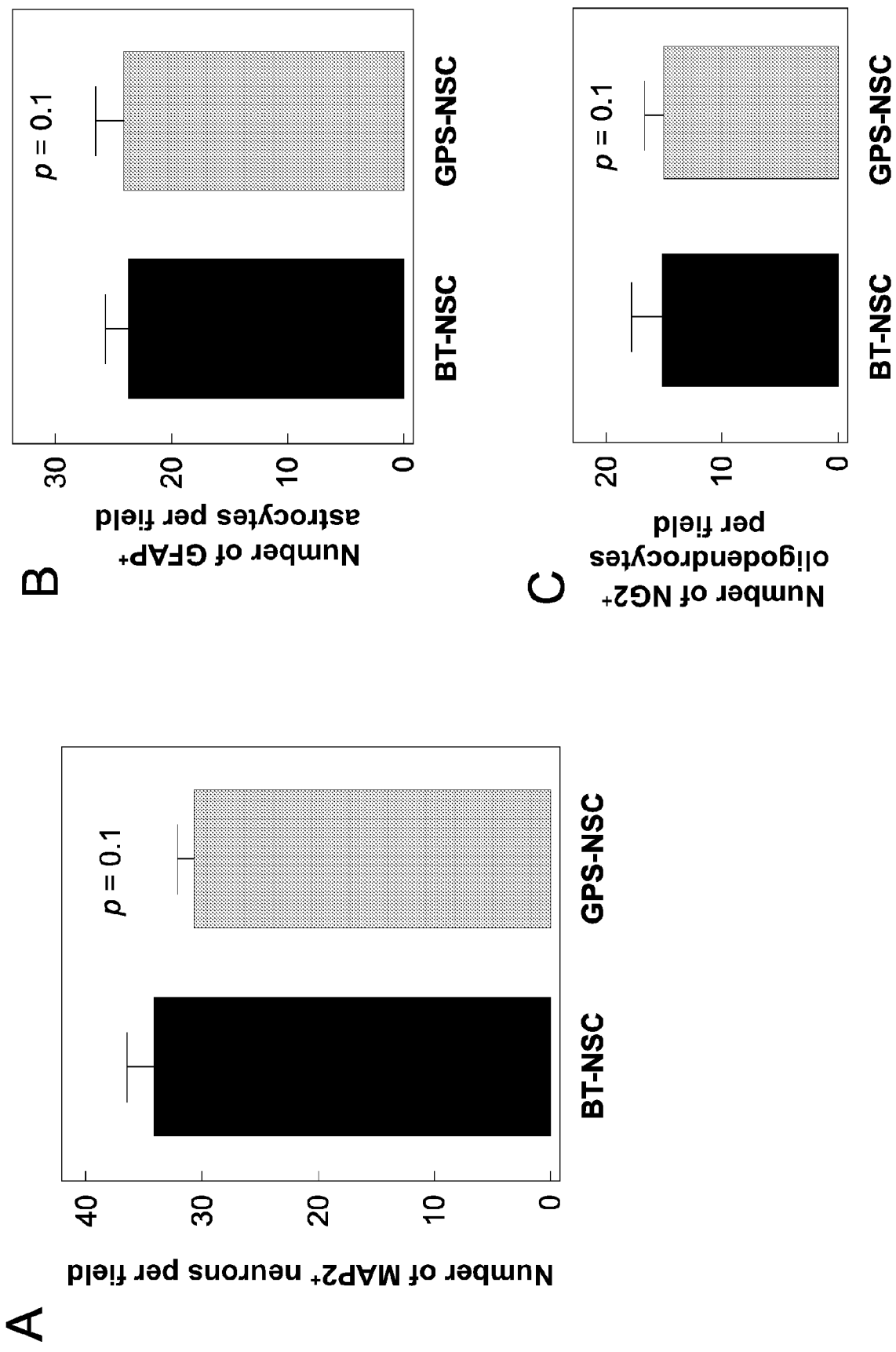

Neurosphere Frequency
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
BT-NSC
GPS-NSC
BT-NSC
GPS-NSC

Figure 18


A
Number of MAP2+ neurons per field
0
10
20
30
40
p = 0.1
BT-NSC
GPS-NSC
B
Number of GFAP+ astrocytes per field
0
10
20
30
p = 0.1
BT-NSC
GPS-NSC
C
Number of NG2+ oligodendrocytes per field
0
10
20
p = 0.1
BT-NSC
GPS-NSC

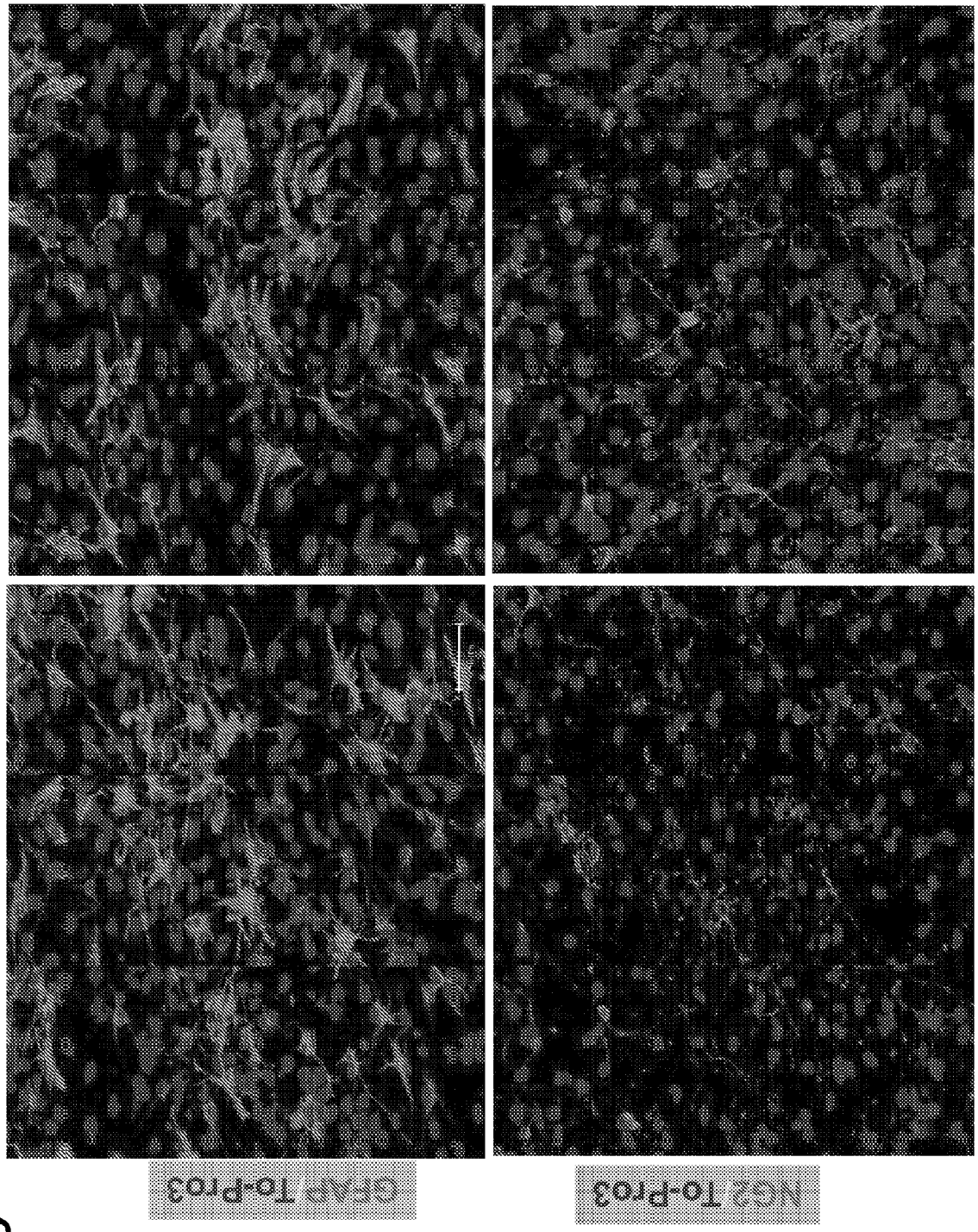


Figure 18 D

Figure 19

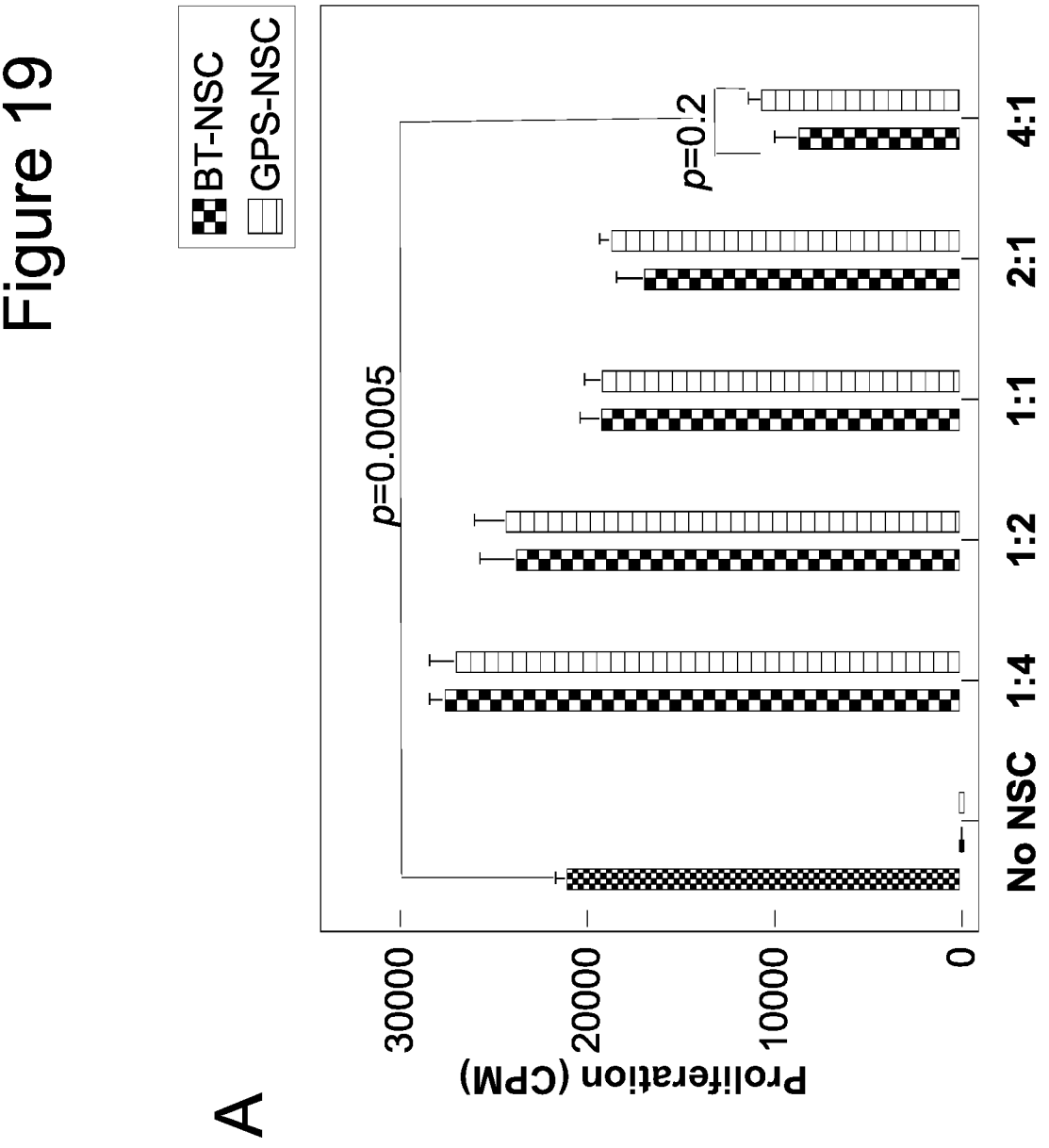

A
BT-NSC
GPS-NSC
Proliferation (CPM)
30000
20000
10000
0
p=0.0005
p=0.2
No NSC
1:4
1:2
1:1
2:1
4:1

Figure 19

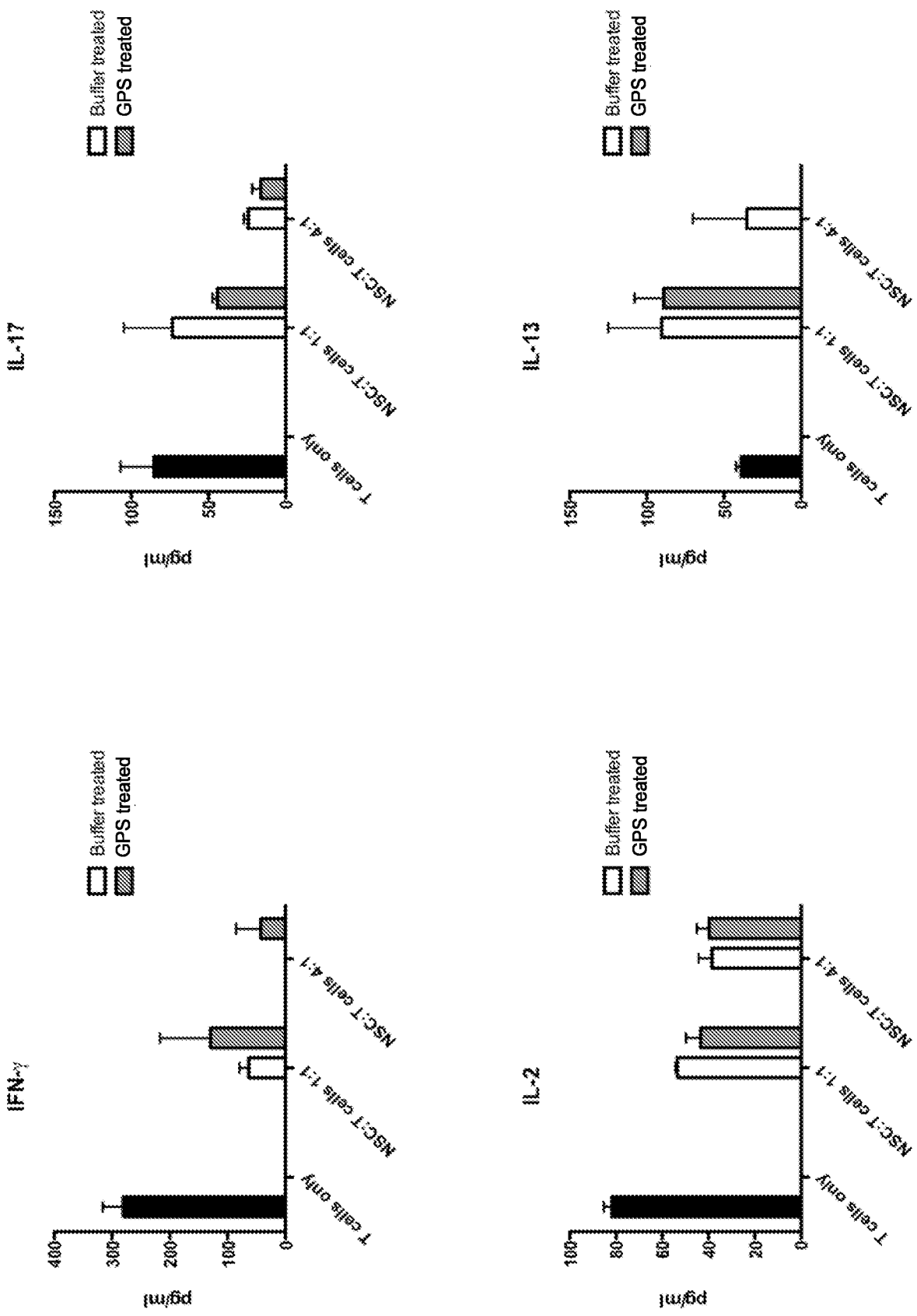
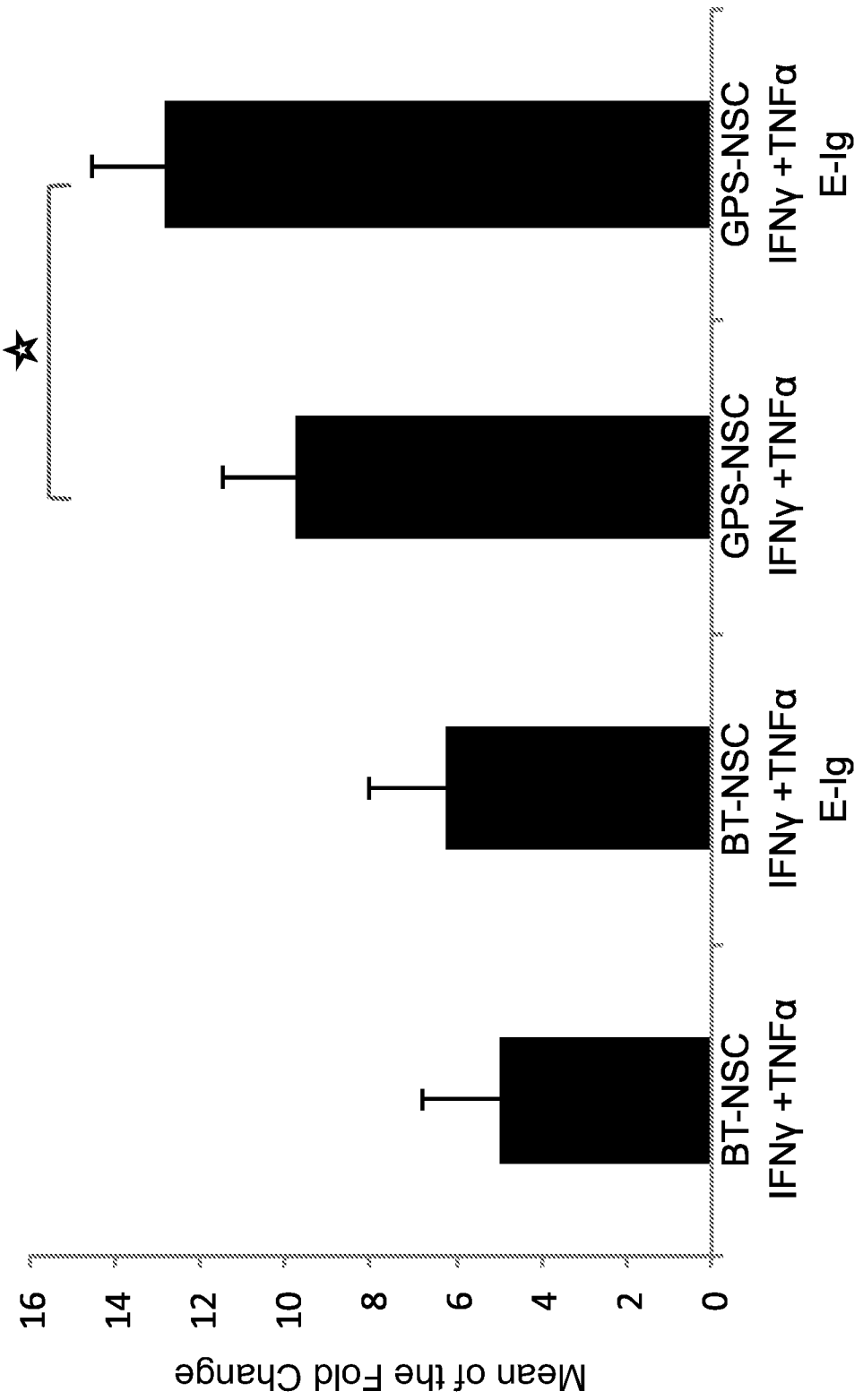

IL-17
IL-13
IFN-γ
IL-2
Buffer treated
GPS treated
pg/ml
T cells only
NSC:T cells 1:1
NSC:T cells 4:1

Figure 19


Mean of the Fold Change
0
2
4
6
8
10
12
14
16
BT-NSC
IFNγ +TNFα
BT-NSC
IFNγ +TNFα
E-Ig
GPS-NSC
IFNγ +TNFα
GPS-NSC
IFNγ +TNFα
E-Ig

C

Figure 20

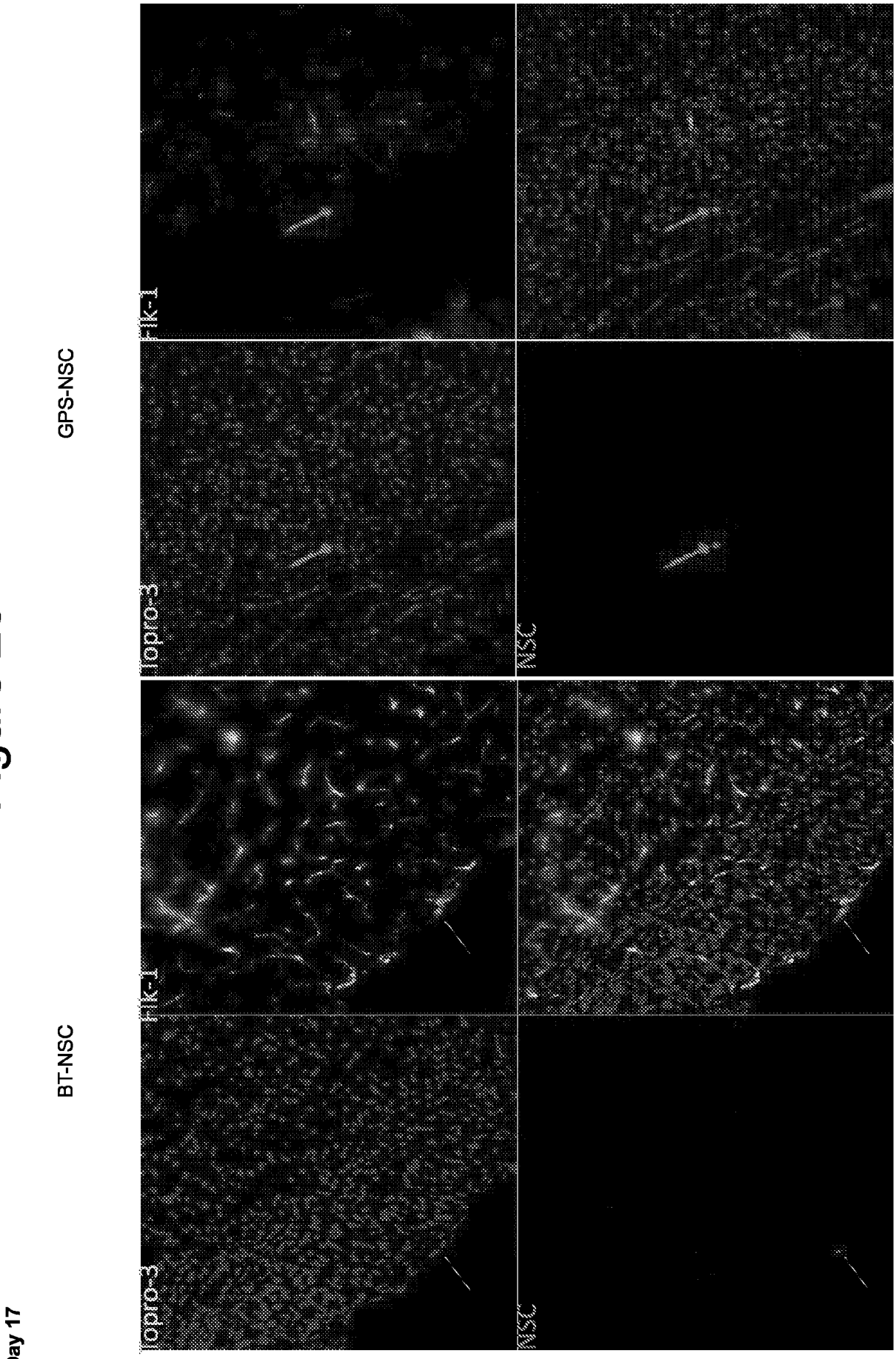


Figure 20

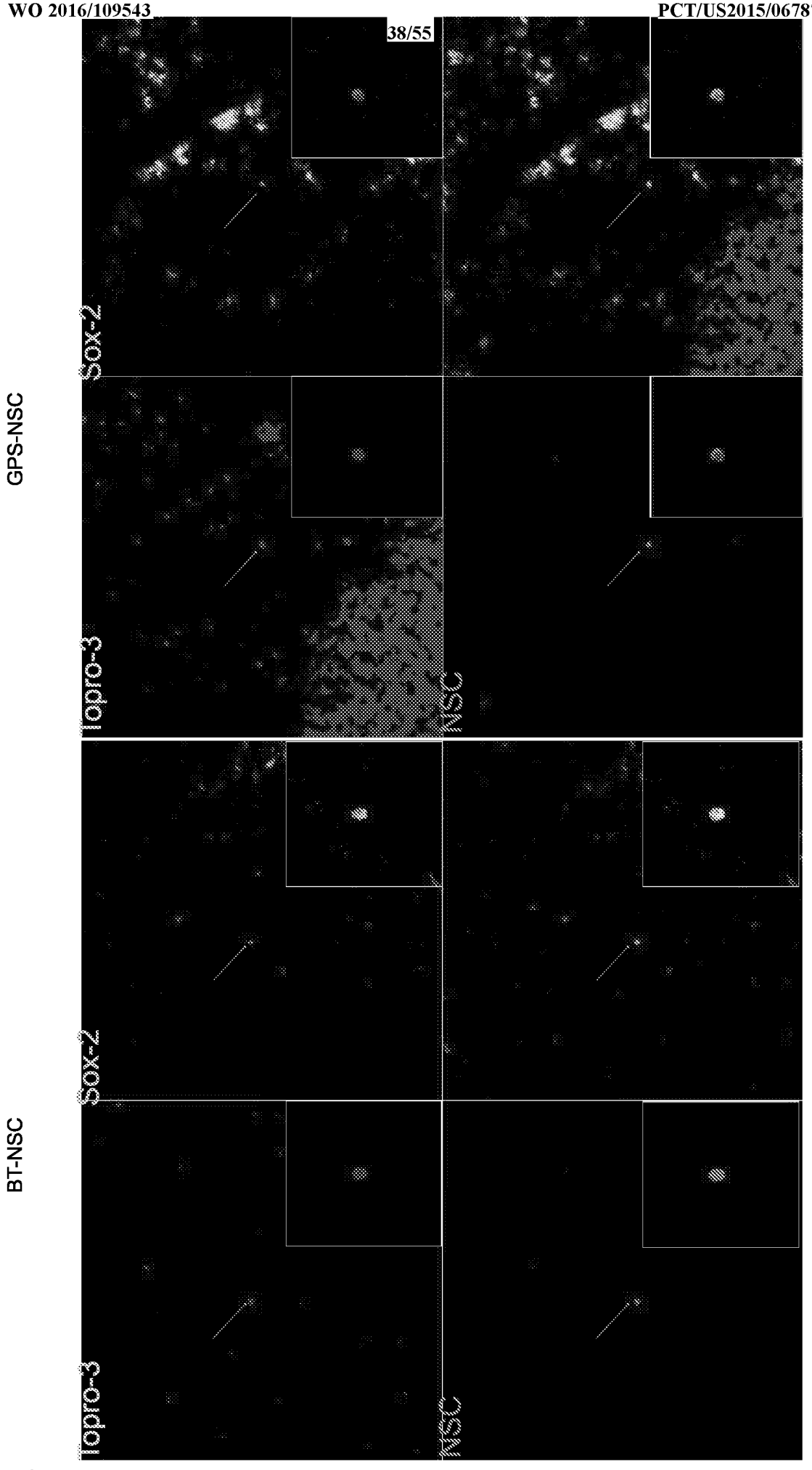


Figure 21

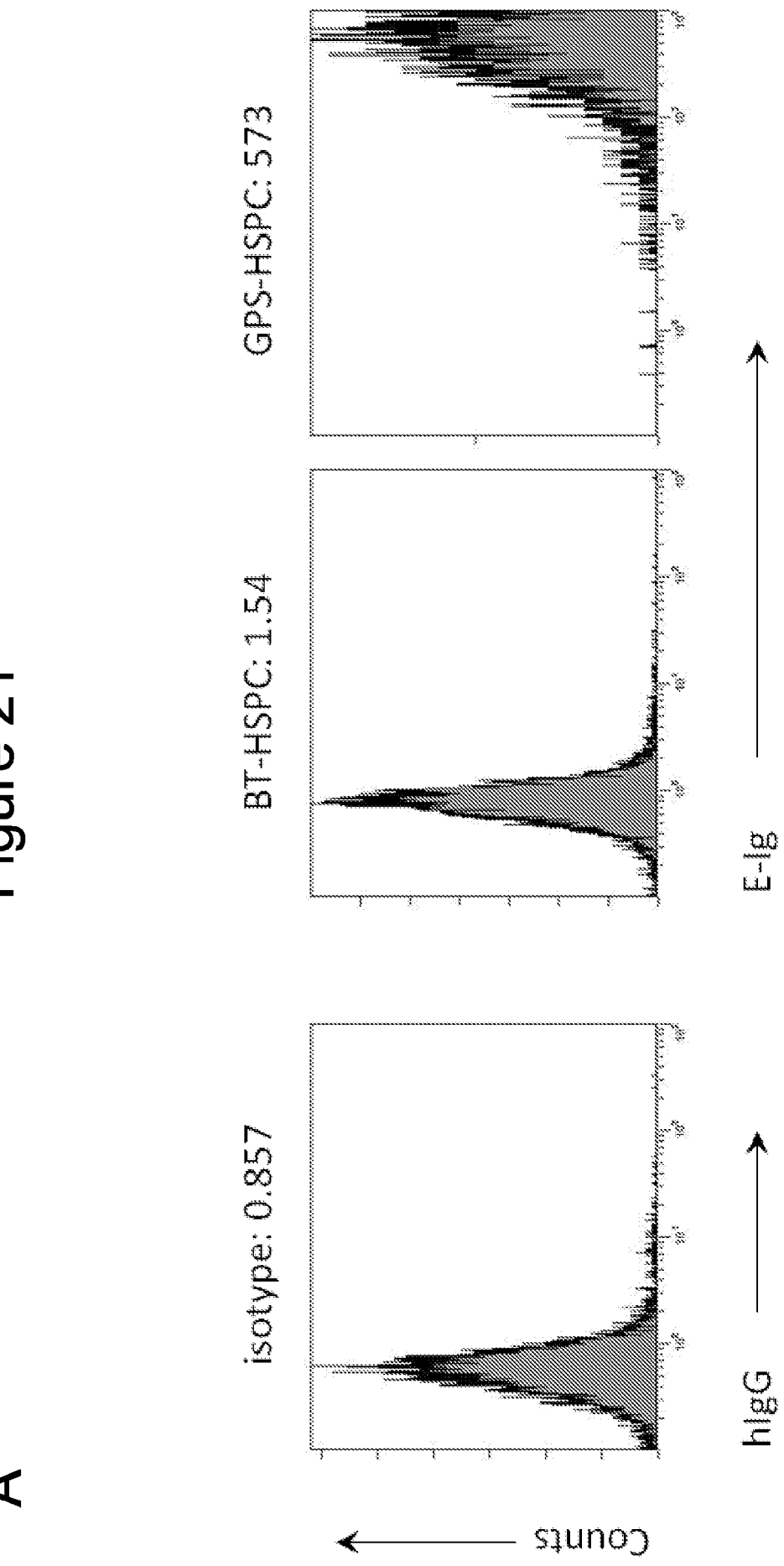


Figure 21

B

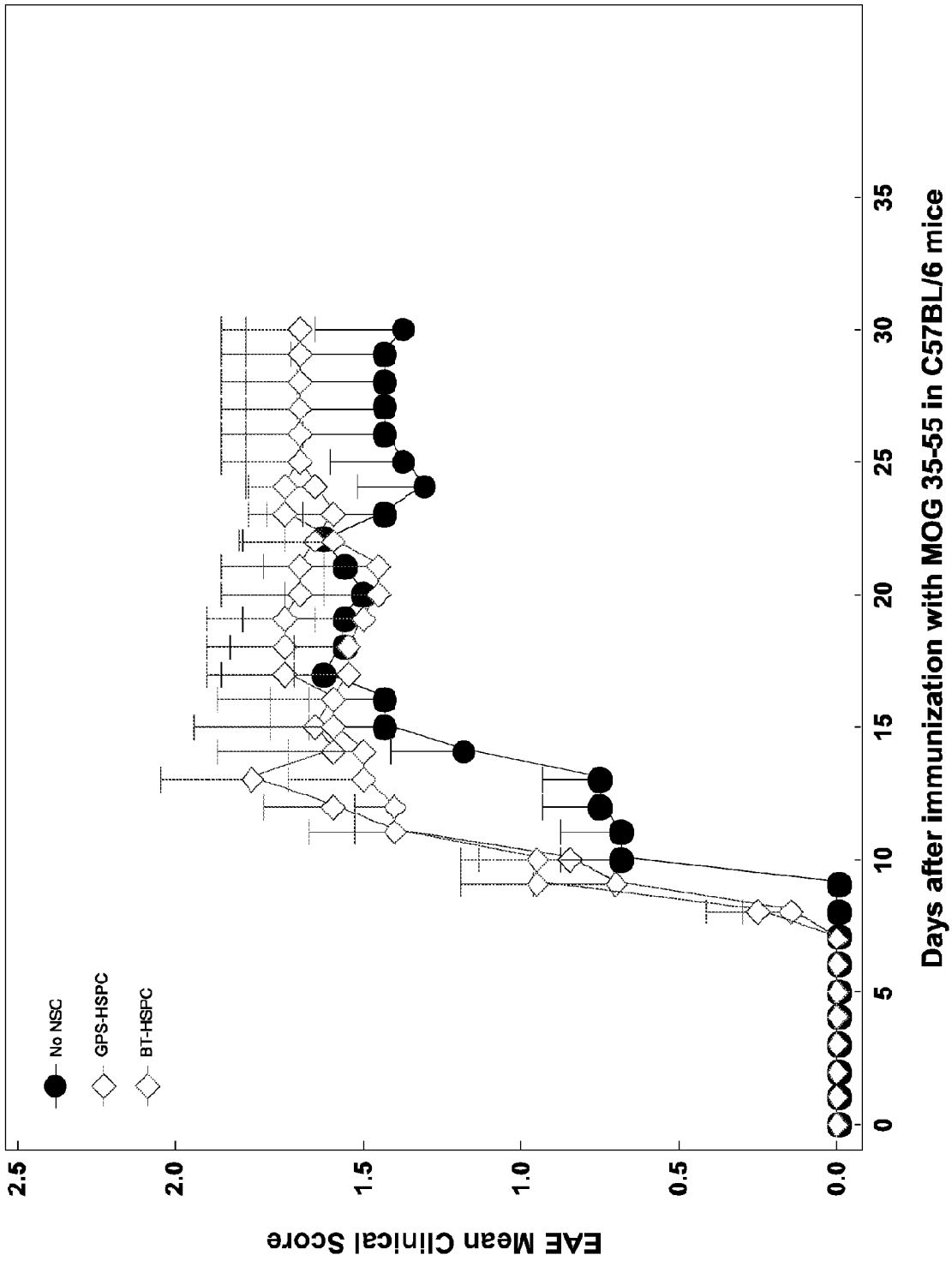

No NSC
GPS-HSPC
BT-HSPC
EAE Mean Clinical Score
Days after immunization with MOG 35-55 in C57BL/6 mice
0.0
0.5
1.0
1.5
2.0
2.5
0
5
10
15
20
25
30
35

Figure 21

C

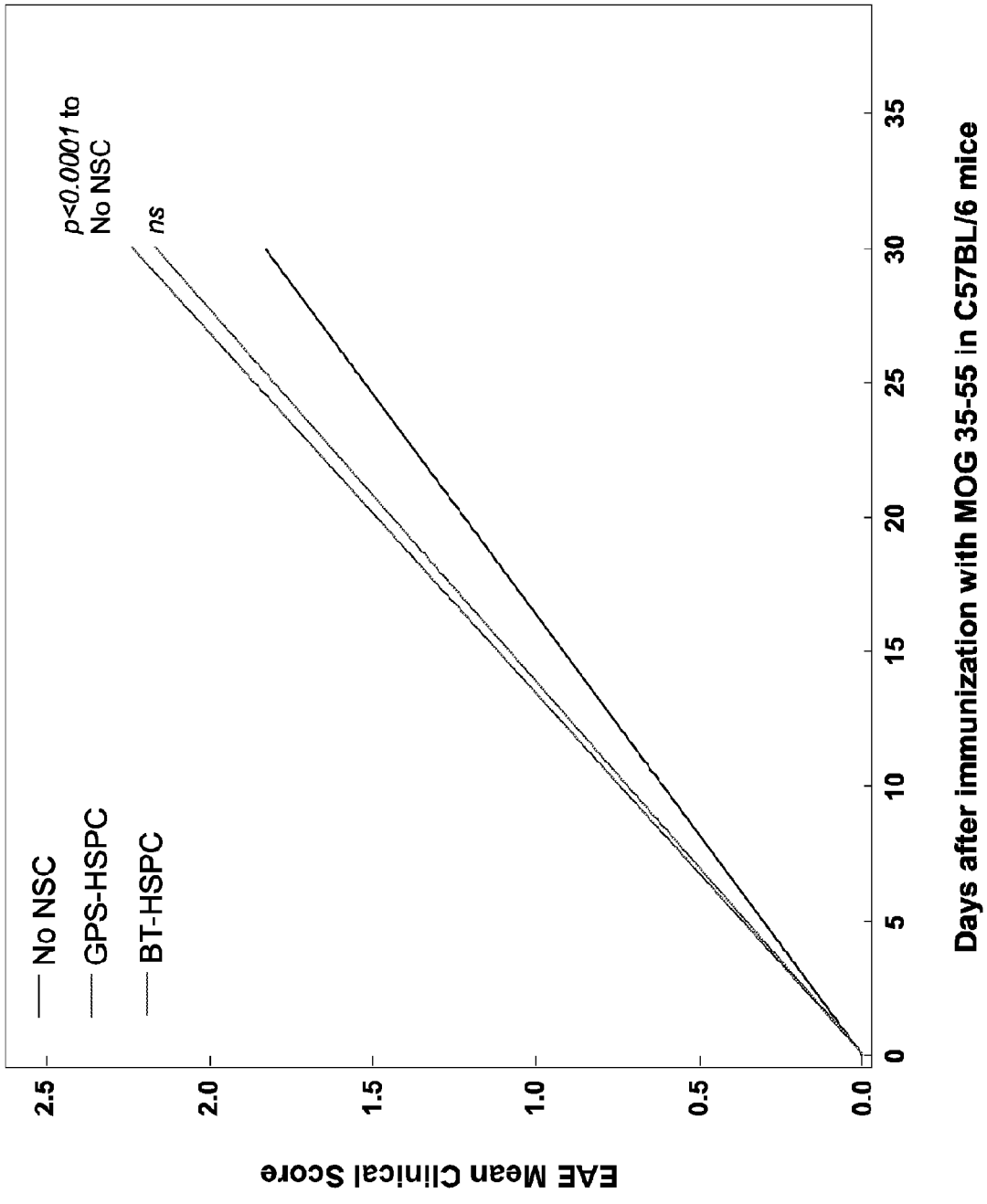

No NSC
GPS-HSPC
BT-HSPC
p<0.0001 to No NSC
ns
EAE Mean Clinical Score
0.0
0.5
1.0
1.5
2.0
2.5
0
5
10
15
20
25
30
35
Days after immunization with MOG 35-55 in C57BL/6 mice

Figure 22

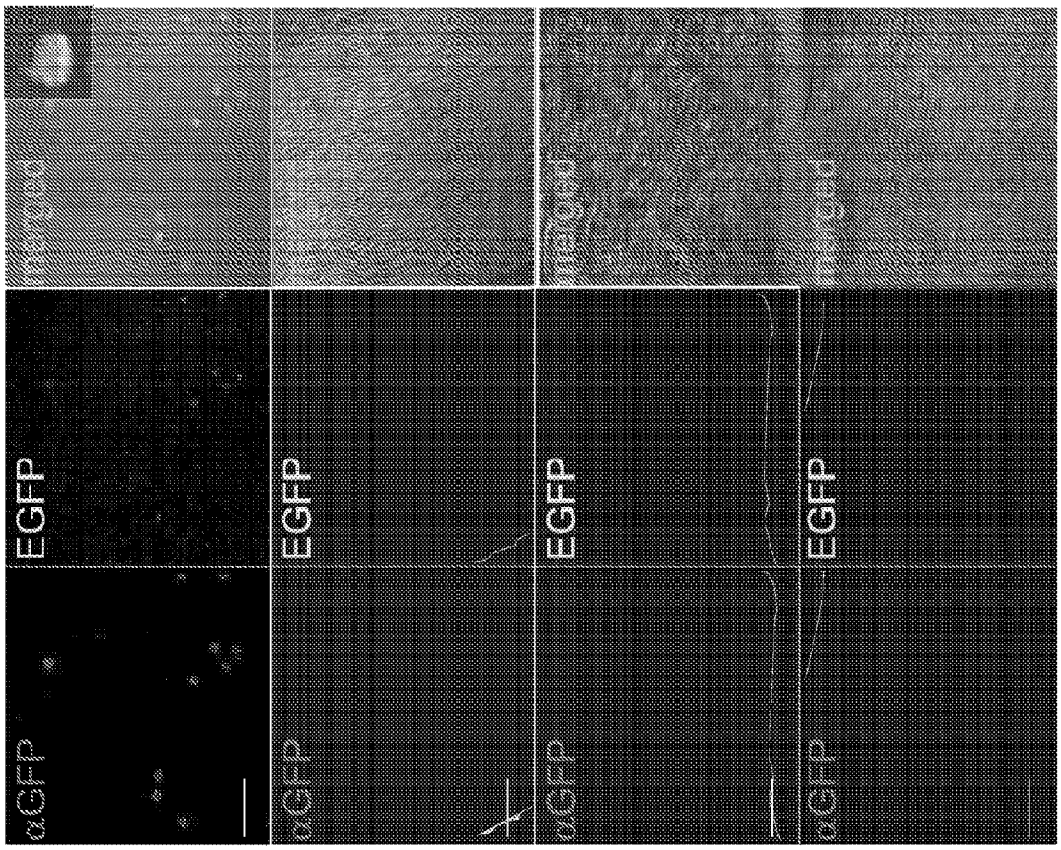


Figure 23

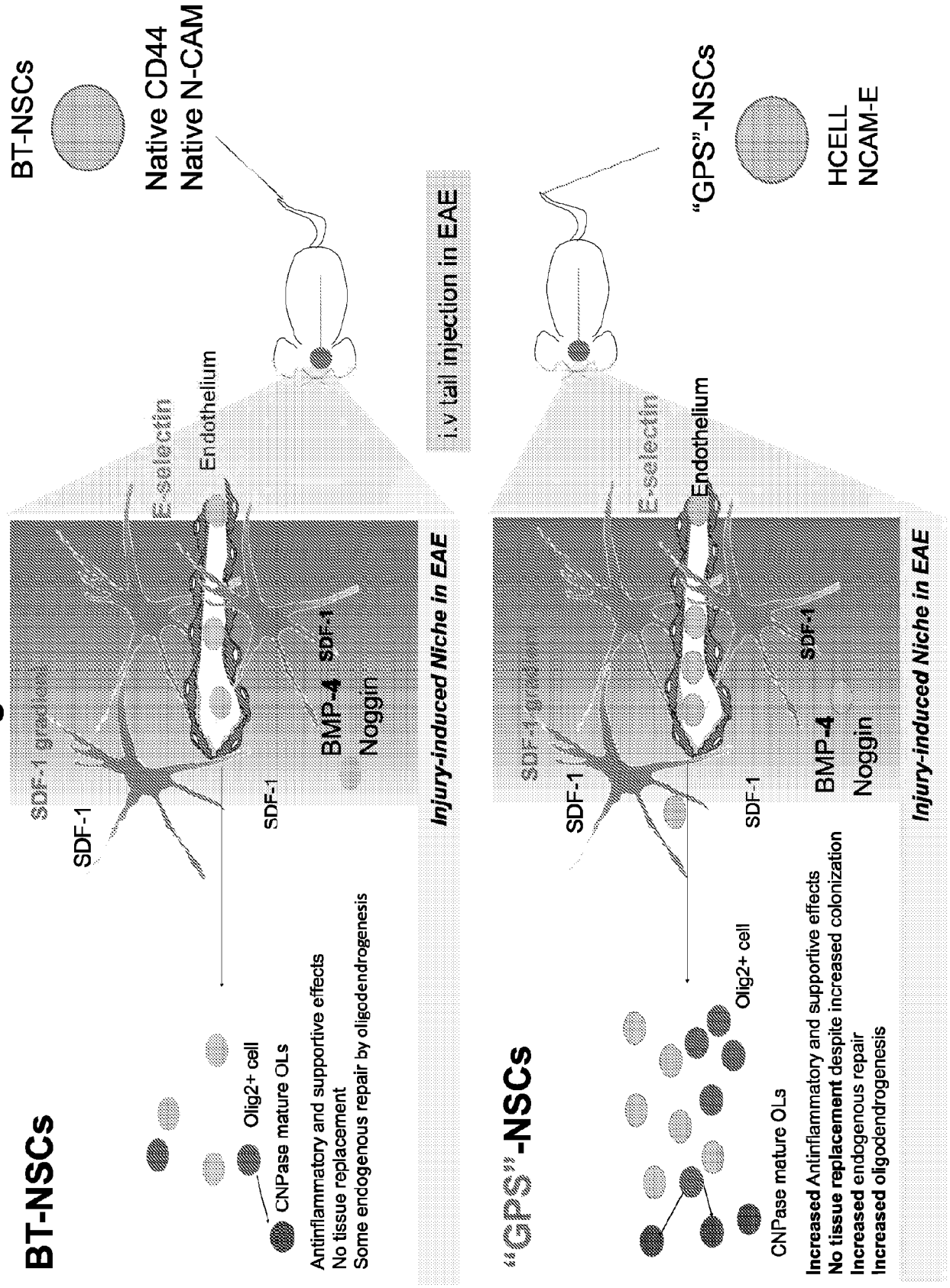

BT-NSCs
BT-NSCs
Native CD44
Native N-CAM
SDF-1 gradient
SDF-1
E-selectin
Endothelium
SDF-1
i.v tail injection in EAE
Olig2+ cell
CNPase mature OLs
Antinflammatory and supportive effects
No tissue replacement
Some endogenous repair by oligodendrogenesis
BMP-4
SDF-1
Noggin
Injury-induced Niche in EAE
"GPS"-NSCs
"GPS"-NSCs
HCELL
NCAM-E
SDF-1 gradient
SDF-1
E-selectin
Endothelium
SDF-1
Olig2+ cell
CNPase mature OLs
Increased Antinflammatory and supportive effects
No tissue replacement despite increased colonization
Increased endogenous repair
Increased oligodendrogenesis
BMP-4
SDF-1
Noggin
Injury-induced Niche in EAE

Figure 24. HECA452 reactivity (sLex expression) on Adipose-derived MSCs (A-MSCs) from Lean Subjects (LA-AMCs) and Obese subjects (OA-MSCs) after FTVII (FT7) treatment

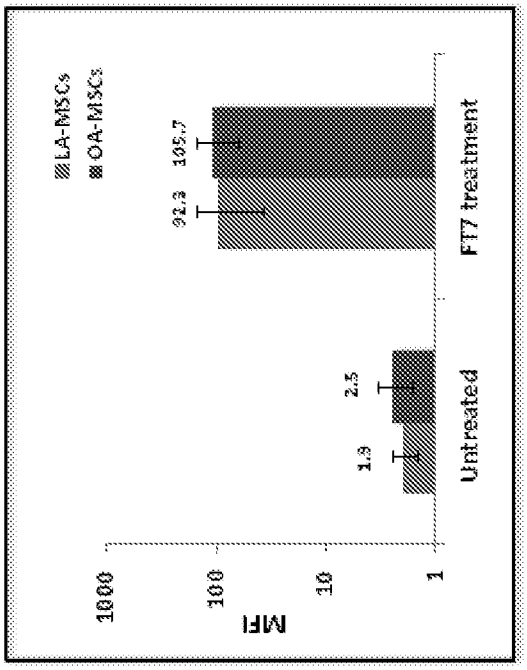
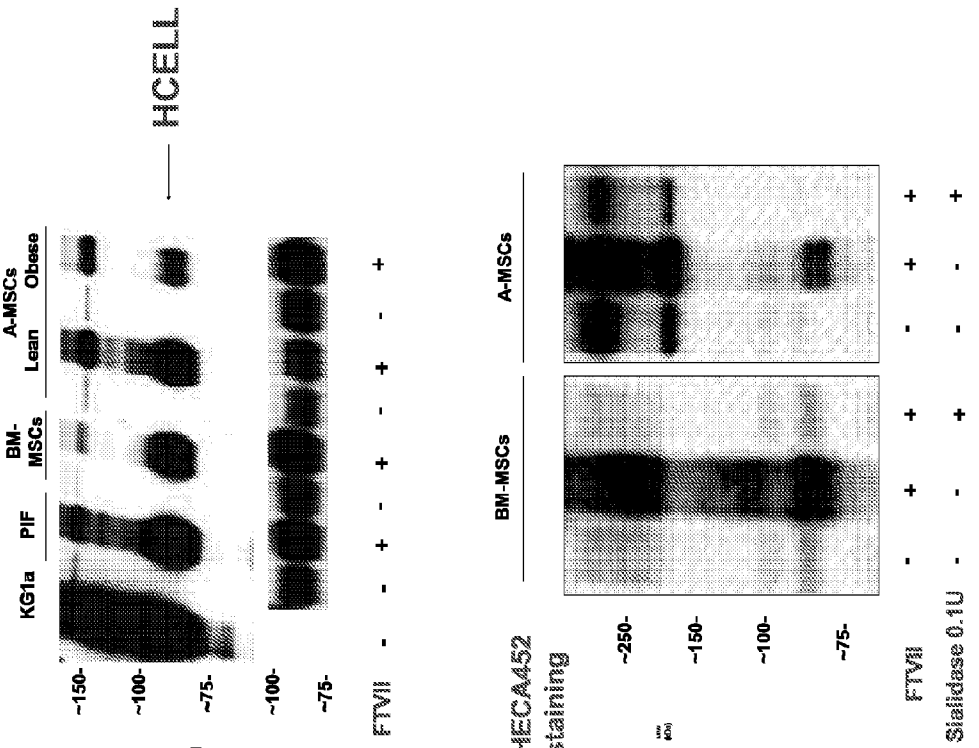


Figure 25. Biochemical analysis of human Bone Marrow-derived (BM-MSCs) and Adipose-derived (A-MSCs) MSCs after FTVII treatment



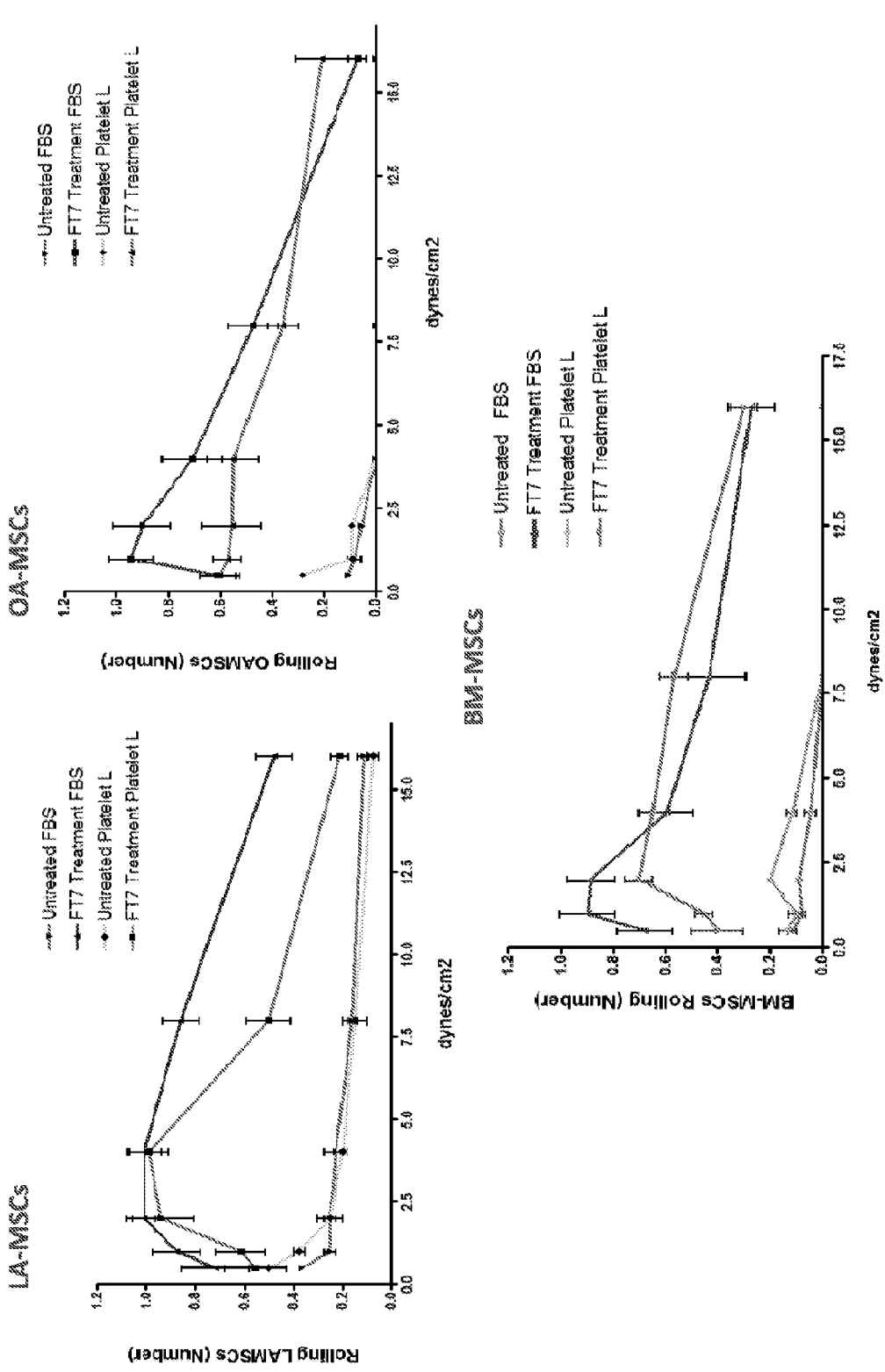
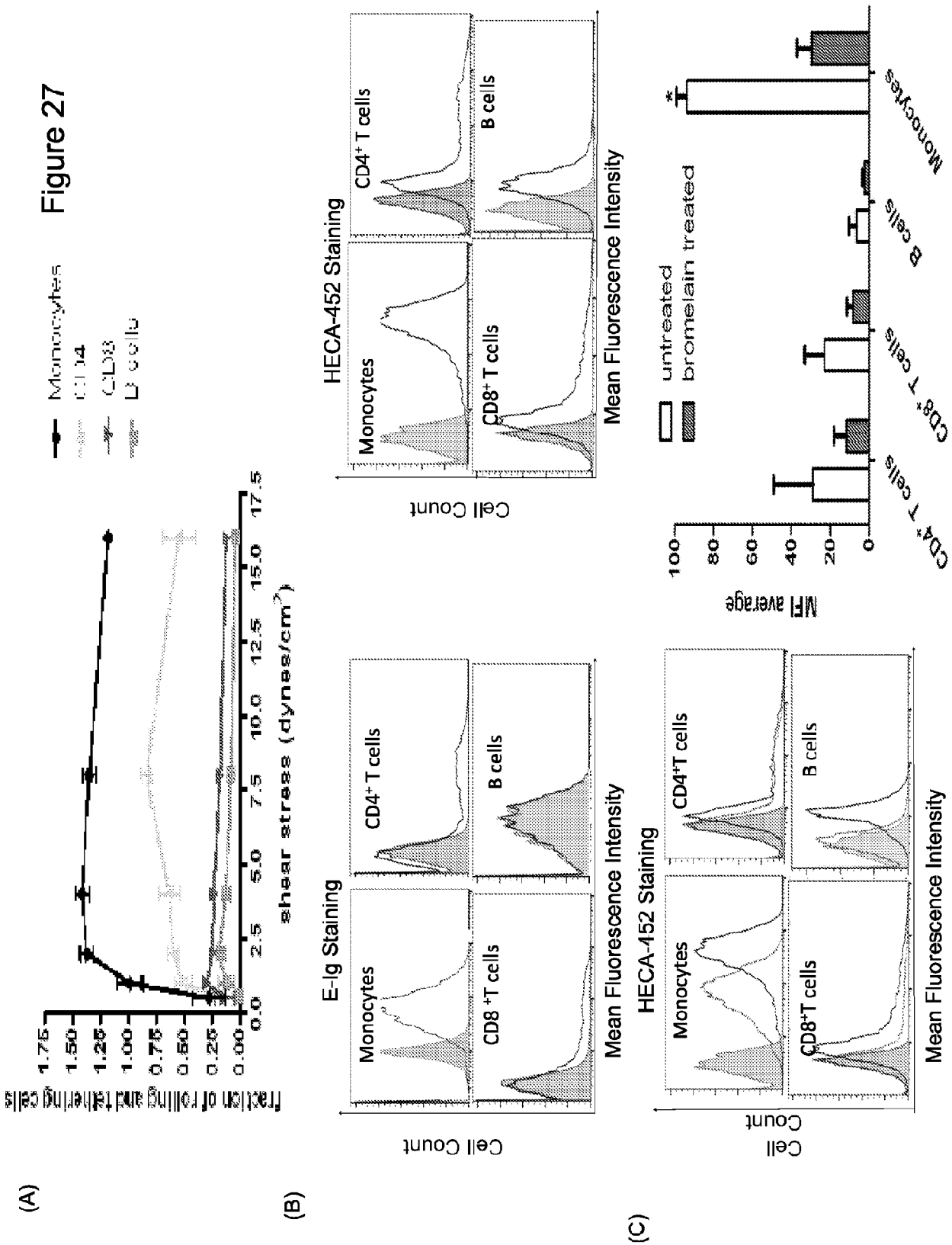


Figure 26. E-selectin binding (rolling capacity on TNF-stimulated HUVEC) of Lean (LA-MSCs) and Obese (OA-MSCs) adipose-derived MSCs compared to bone marrow-derived MSC s (BM-MSCs) after FTVII (FT7) treatment ; MSCs cultured with either fetal bovine serum (FBS) or Platelet lysate (Platelet L)

Figure 27


(A)
Monocytes
CD14
CD33
B cells
fraction of rolling and tethering cells
shear stress (dynes/cm²)
(B)
E-Ig Staining
Monocytes
CD4⁺T cells
CD8 ⁺T cells
B cells
Cell Count
Mean Fluorescence Intensity
HECA-452 Staining
(C)
untreated
bromelain treated
MFI average
CD4⁺ T cells
CD8⁺ T cells
B cells
Monocytes

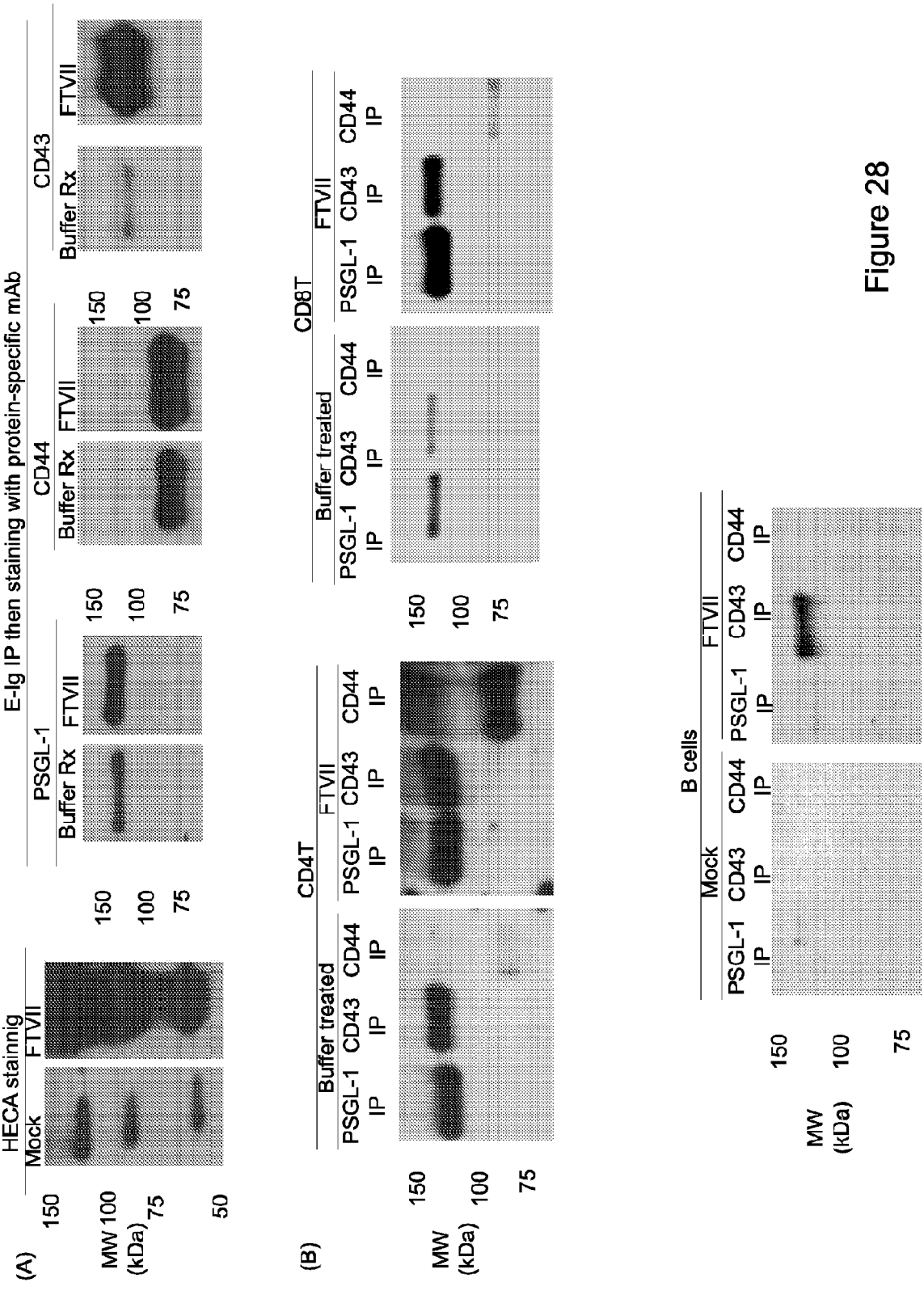

(A)
HECA stainnig
Mock
FTVII
E-Ig IP then staining with protein-specific mAb
PSGL-1
CD44
CD43
Buffer Rx
FTVII
MW (kDa)
150
100
75
50
(B)
CD4T
CD8T
Buffer treated
PSGL-1 IP
CD43 IP
CD44 IP
B cells

Figure 28

Figure 28

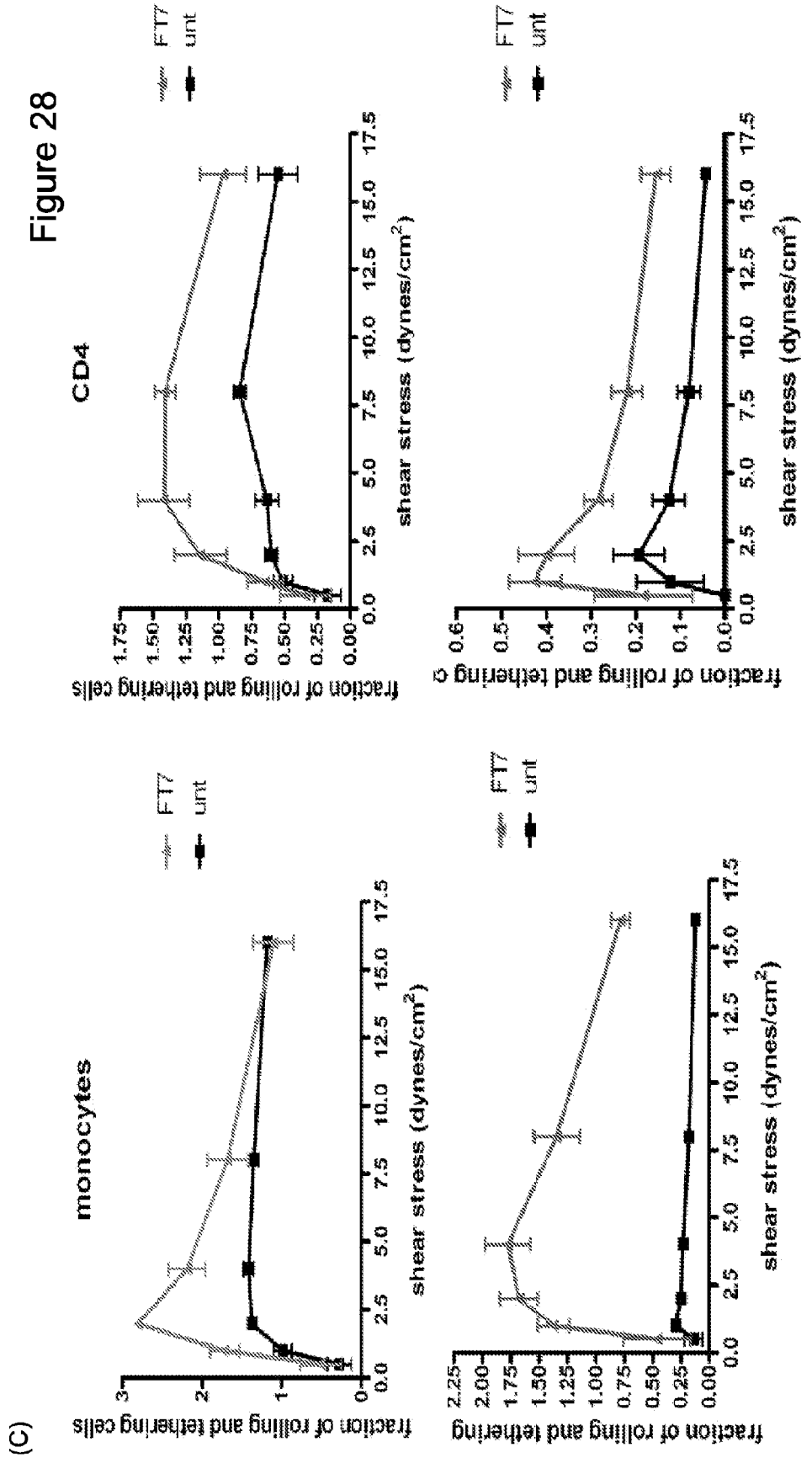

(C)
monocytes
CD4
FT7
unt
fraction of rolling and tethering cells
shear stress (dynes/cm2)
0.0
2.5
5.0
7.5
10.0
12.5
15.0
17.5

Figure 29

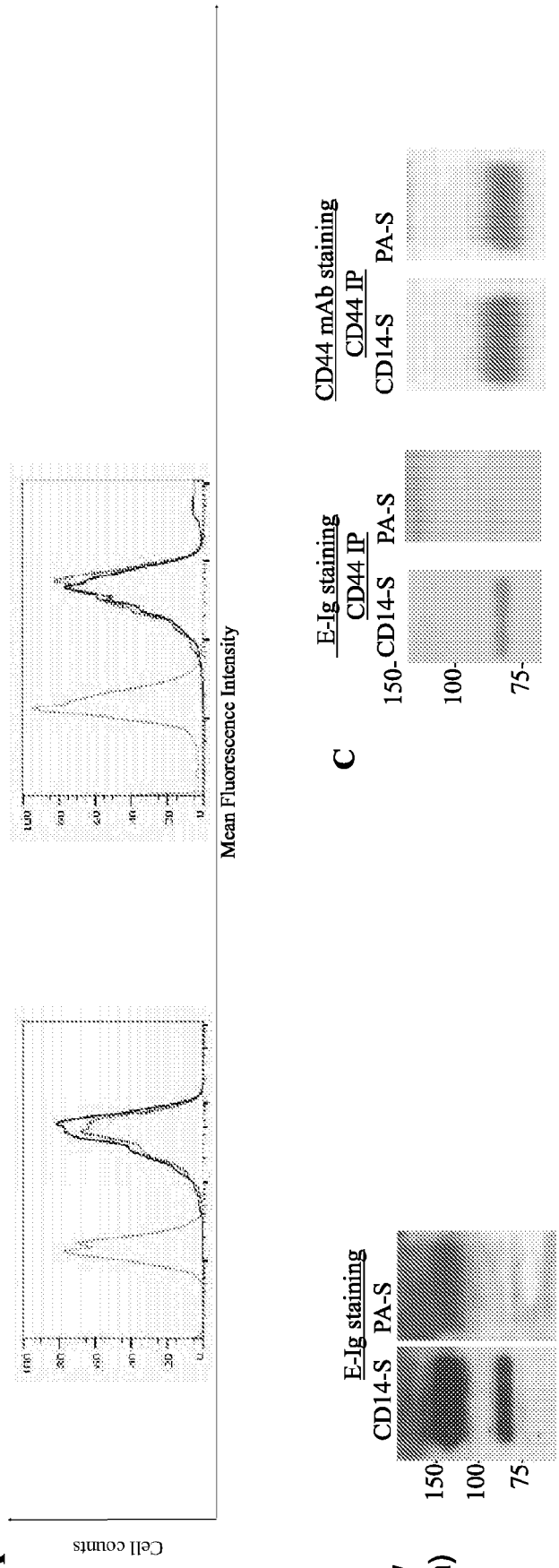

A
E-Ig
HECA-452
Cell counts
Mean Fluorescence Intensity
B
MW (kDa)
E-Ig staining
CD14-S PA-S
150
100
75
C
E-Ig staining
CD44 IP
CD14-S PA-S
150
100
75
CD44 mAb staining
CD44 IP
CD14-S PA-S

Figure 30

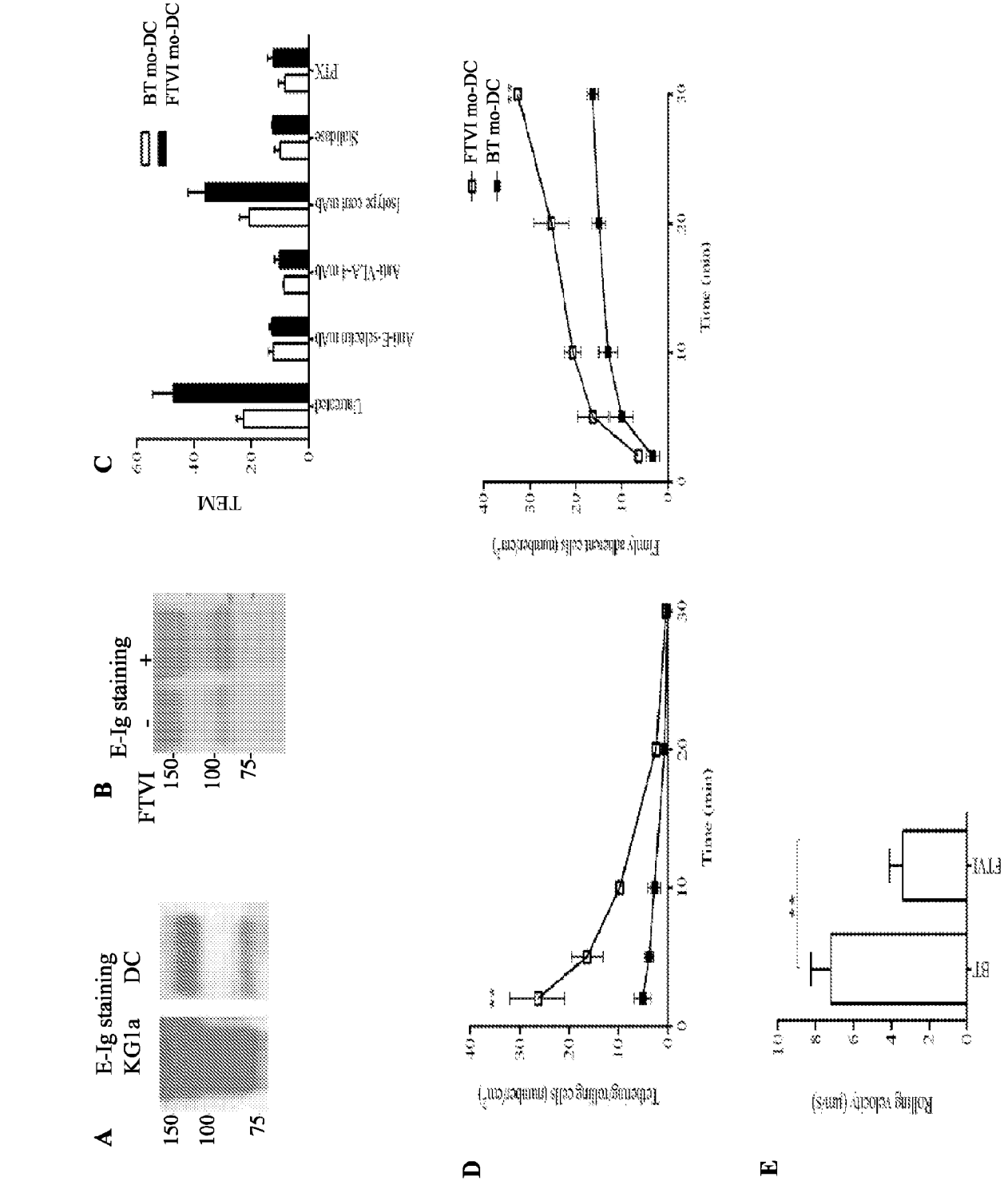

A
E-Ig staining
KG1a DC
MW (kDa)
150
100
75
B
E-Ig staining
FTVI - +
150
100
75
C
BT mo-DC
FTVI mo-DC
TEM
Lactosel
Anti-E-selectin mAb
Anti-VLA-4 mAb
Isotype cont mAb
Sialidase
PTX
D
FTVI mo-DC
BT mo-DC
Time (min)
Tethering/rolling cells (number/cm²)
Firmly adherent cells (number/cm²)
E
Rolling velocity (μm/s)
BT
FTVI

Figure 31

Flow cytometry staining of cultured Tregs for CD4 and FoxP3 expression

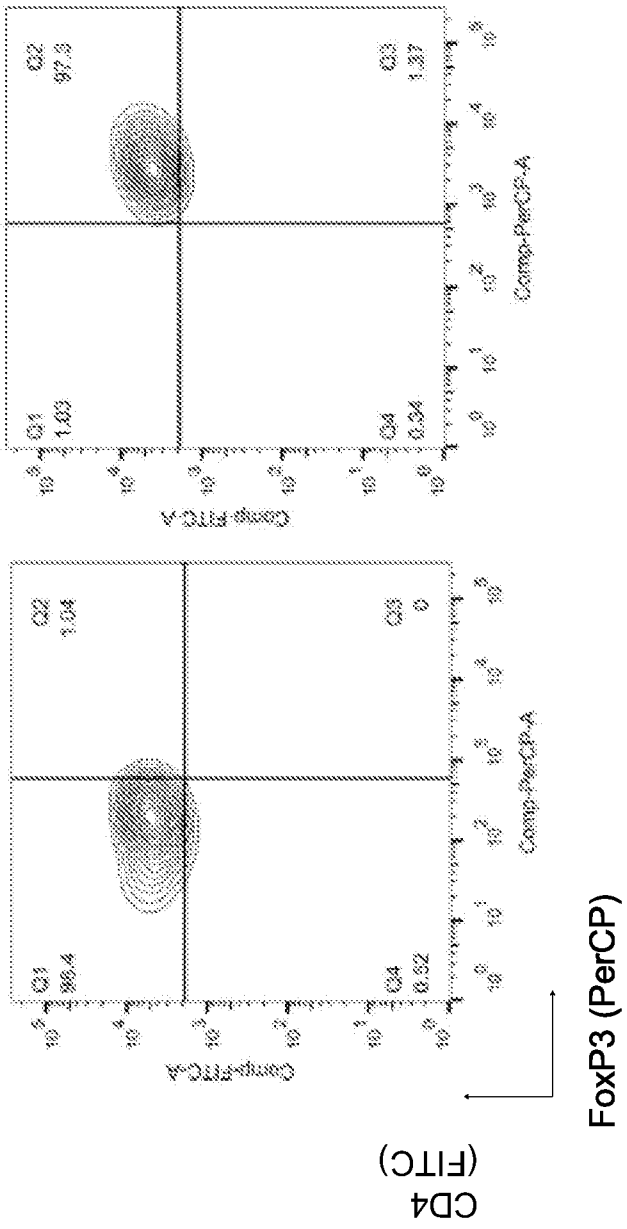
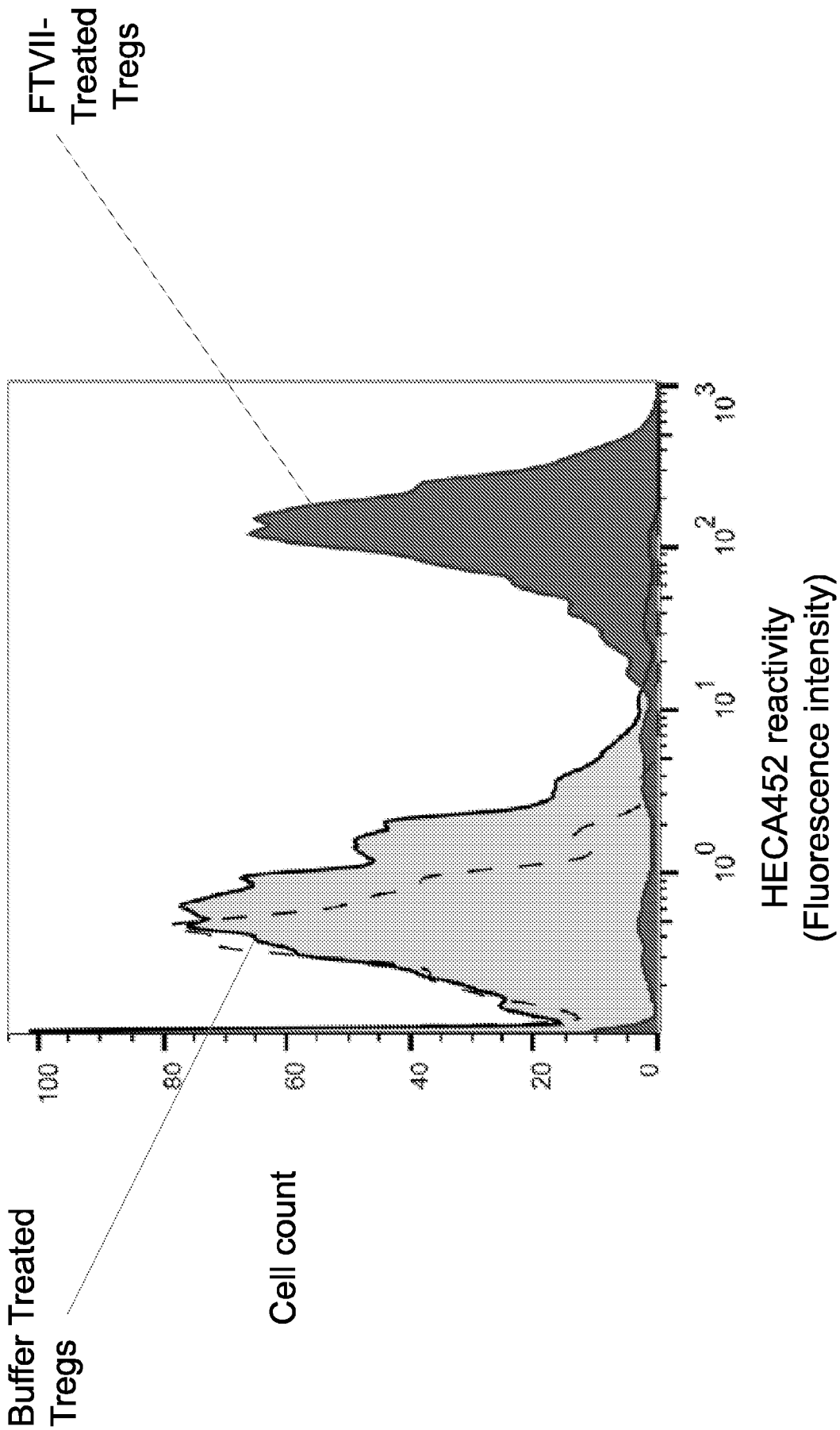


Figure 32
Flow cytometry staining of sLex (HECA452 mAb) of buffer treated and exofucosylated Tregs



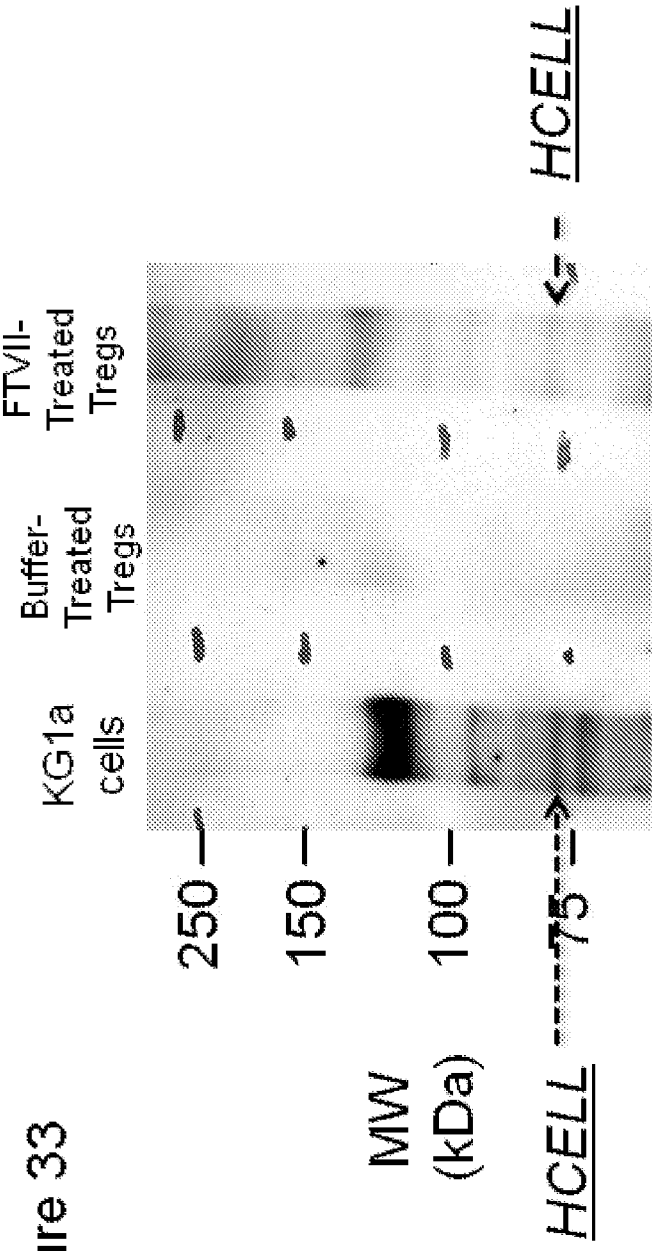
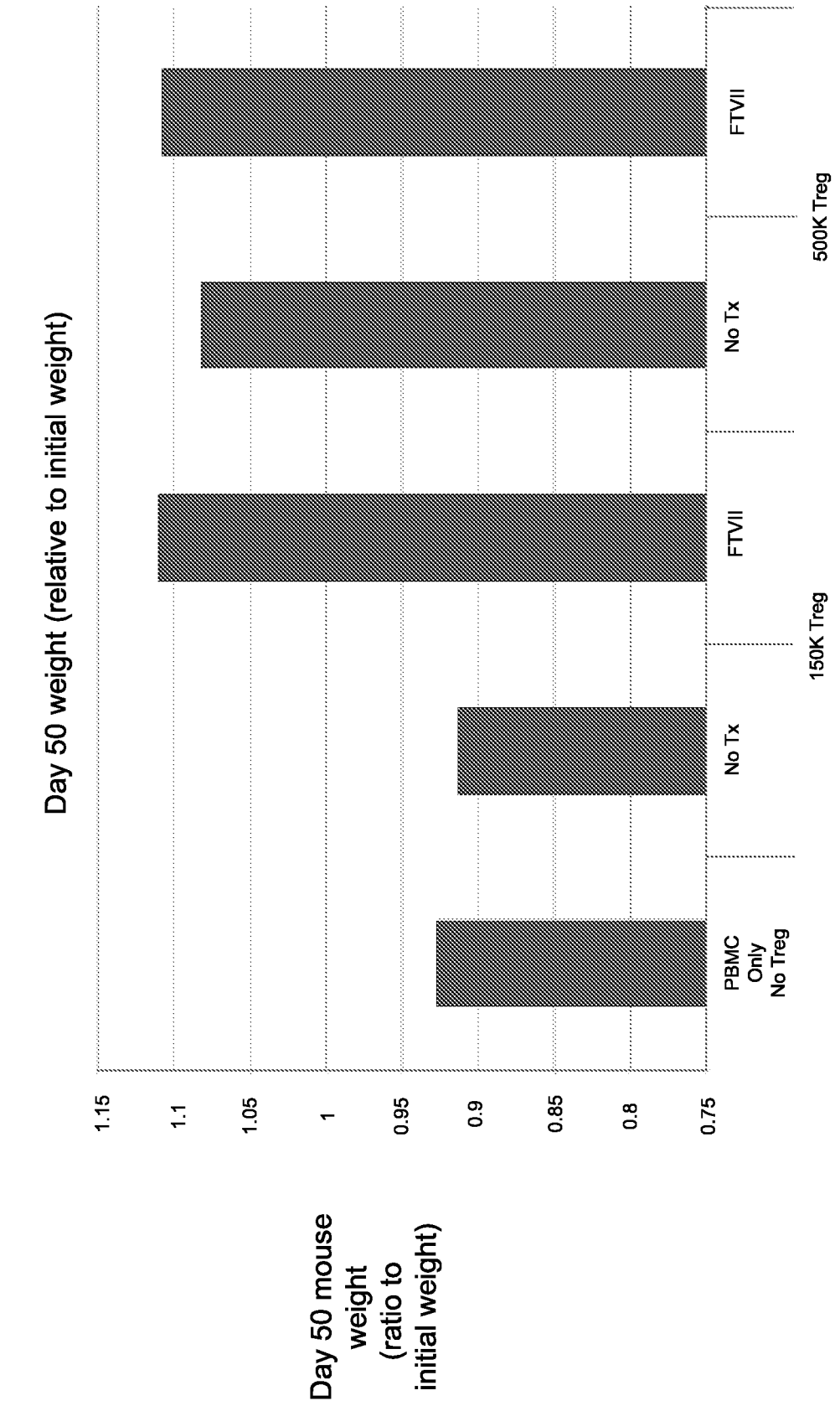

KG1a cells
Buffer-Treated Tregs
FTVII-Treated Tregs
250
150
100
75
MW (kDa)
HCELL
HCELL

Figure 33

Figure 34


Day 50 weight (relative to initial weight)
Day 50 mouse weight (ratio to initial weight)
1.15
1.1
1.05
1
0.95
0.9
0.85
0.8
0.75
PBMC Only No Treg
No Tx
FTVII
No Tx
FTVII
150K Treg
500K Treg

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2015/067873

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12N5/0797 C12N5/078 C12N5/0783 C12N5/0784 C12N5/077
ADD. A61K35/12

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12N A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2006/068720 A2 (BRIGHAM & WOMENS HOSPITAL [US]; SACKSTEIN ROBERT [US]) 29 June 2006 (2006-06-29) claims 28, 31, 32, 47-49	1-53
X	WO 2008/011094 A2 (SACKSTEIN ROBERT [US]) 24 January 2008 (2008-01-24) claims 1-16	1-53
X	WO 2007/143204 A2 (SACKTEIN ROBERT [US]) 13 December 2007 (2007-12-13) claims 1, 24, 26, 31, 46, 47	1-53
X	WO 2007/005180 A2 (ADVANCED CARDIOVASCULAR SYSTEM [US]; CONSIGNY PAUL [US]; ASONGWE GABRI) 11 January 2007 (2007-01-11) claims 1, 4, 5, 6	1-53

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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

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"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
10 March 2016	22/03/2016

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Offermann, Stefanie

1

Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2014/161782 A1 (WOLPE STEPHEN D [US] ET AL) 12 June 2014 (2014-06-12) claims 1-17	1-53
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X	ROBERT SACKSTEIN: "Engineering cellular trafficking via glycosyltransferase-programmed stereosubstitution", ANNALS OF THE NEW YORK ACADEMY OF SCIENCES, vol. 1253, no. 1, 21 April 2012 (2012-04-21), pages 193-200, XP055255948, US ISSN: 0077-8923, DOI: 10.1111/j.1749-6632.2011.06421.x the whole document page 199	1-53

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
T	JASMEEN S MERZABAN ET AL: "Cell surface glycan engineering of neural stem cells augments neurotropism and improves recovery in a murine model of multiple sclerosis", GLYCOBIOLOGY, vol. 25, no. 12, 7 July 2015 (2015-07-07), pages 1392-1409, XP055255942, US ISSN: 0959-6658, DOI: 10.1093/glycob/cwv046 -----	
T	REZA ABDI ET AL: "HCELL Expression on Murine MSC Licenses Pancreatotropism and Confers Durable Reversal of Autoimmune Diabetes in NOD Mice", STEM CELLS., vol. 33, no. 5, 23 May 2015 (2015-05-23), pages 1523-1531, XP055255944, US ISSN: 1066-5099, DOI: 10.1002/stem.1948 -----	
T	C. I. SILVESCU ET AL: "G-CSF Induces Membrane Expression of a Myeloperoxidase Glycovariant that Operates as an E-selectin Ligand on Human Myeloid Cells", PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES, vol. 111, no. 29, 22 July 2014 (2014-07-22), pages 10696-10701, XP055255945, US ISSN: 0027-8424, DOI: 10.1073/pnas.1320833111 -----	

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2015/067873

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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		WO 2006068720 A2	29-06-2006
WO 2008011094 A2	24-01-2008	US 2009053198 A1	26-02-2009
		US 2011177040 A1	21-07-2011
		US 2014170120 A1	19-06-2014
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		US 2008044383 A1	21-02-2008
		US 2009239296 A1	24-09-2009
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		US 2012157751 A1	21-06-2012
		WO 2007005180 A2	11-01-2007
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(19)中华人民共和国国家知识产权局

(12)发明专利申请

(10)申请公布号 CN 107249601 A
(43)申请公布日 2017.10.13

(21)申请号 201580076144.8

(22)申请日 2015.12.29

(30)优先权数据
62/098,048 2014.12.30 US
62/101,863 2015.01.09 US

(85)PCT国际申请进入国家阶段日
2017.08.15

(86)PCT国际申请的申请数据
PCT/US2015/067873 2015.12.29

(87)PCT国际申请的公布数据
WO2016/109543 EN 2016.07.07

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(72)发明人 R·萨克斯滕

(74)专利代理机构 中国国际贸易促进委员会专利商标事务所 11038
代理人 袁泉

(51)Int.Cl.
A61K 35/14(2015.01)
A61K 35/28(2015.01)
A61K 35/30(2015.01)
A61K 35/32(2015.01)
A61K 35/33(2015.01)
A61K 35/34(2015.01)
A61K 35/17(2015.01)
A61K 35/35(2015.01)
A61K 35/37(2015.01)
A61K 35/407(2015.01)
A61K 35/42(2015.01)
A61P 35/00(2006.01)
A61P 35/02(2006.01)
A61P 29/00(2006.01)
A61P 17/02(2006.01)
A61P 9/10(2006.01)
A61P 7/04(2006.01)
A61P 7/02(2006.01)
A61P 31/04(2006.01)
A61P 3/10(2006.01)
A61P 1/00(2006.01)
A61P 19/02(2006.01)
A61P 17/06(2006.01)
A61P 19/10(2006.01)
A61P 25/28(2006.01)
A61P 21/00(2006.01)
A61P 25/14(2006.01)
A61P 1/16(2006.01)
A61P 1/18(2006.01)
A61P 9/00(2006.01)
A61P 13/12(2006.01)
C12N 5/00(2006.01)

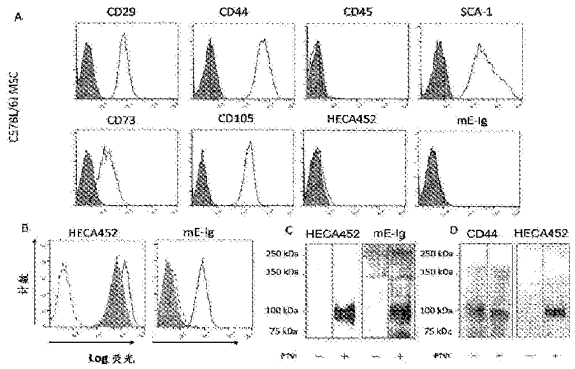
权利要求书5页 说明书65页 附图55页

(54)发明名称

改善细胞治疗的方法

(57)摘要

本文提供了治疗方法，包括施用经修饰以增强E-选择素和/或L-选择素配体表达的细胞群体，所述修饰的细胞群体在增强所述表达的处理后具有至少70%的细胞活力。



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1.一种增强细胞递送到受试者的靶组织中和/或增强在受试者的靶组织中的组织定殖的方法，所述方法包括：

向受试者施用表达E-选择素配体和/或L-选择素配体的细胞的群体，所述群体表达E-选择素配体和/或L-选择素配体的水平超过所述细胞的天然群体的表达水平，

其中所述细胞施用与靶组织内的内皮细胞上的E选择素表达同时进行，和/或与靶组织内白细胞的积累同时进行。

2.一种治疗在受试者中表现为发炎的和/或受损的组织的疾病、障碍或医学病况的方法，所述方法包括：

向受试者施用表达E-选择素配体和/或L-选择素配体的细胞的群体，所述群体表达E-选择素配体和/或L-选择素配体的水平超过所述细胞的天然群体的表达水平，

其中所述细胞施用与靶组织内的内皮细胞上的E-选择素表达同时进行，和/或与靶组织内白细胞的积累同时进行，并且

其中所述群体相对于所述施用的细胞的天然群体，在发炎的和/或受损的组织内表现出增强的定位和/或定殖。

3.一种改善向受试者中靶组织的细胞递送的方法，所述方法包括：

经由脉管递送和/或经由直接组织注射(即直接进入受影响的组织位点)和/或鞘内和/或腔内和/或膀胱内和/或在解剖管道内(胆道系统、尿道系统等)和/或在组织上(即，置于受影响的组织上)，向受试者施用表达E-选择素配体和/或L-选择素配体的细胞的群体，所述群体表达E-选择素配体和/或L-选择素配体的水平超过所述细胞的天然群体的水平，

其中所述细胞施用与靶组织内的内皮细胞上的E-选择素表达同时进行，和/或与靶组织内白细胞的积累同时进行，并且

其中所述施用的细胞群体相对于所述细胞的天然群体，实现增强的归巢、定殖及植入中的一项或多项。

4.一种增强在受试者的发炎的和/或受损的组织中的细胞递送和定殖的方法，所述方法包括：

将表达E-选择素配体和/或L-选择素配体的细胞的群体直接注射到所述发炎的和/或受损的组织内或放置在其上/内，所述群体表达E-选择素配体和/或L-选择素配体的水平超过所述细胞的天然群体的表达水平，

其中所述注射/放置与靶组织内的内皮细胞上的E-选择素表达同时进行，和/或与靶组织内白细胞的积累同时进行。

5.一种增强向受试者的靶组织中的细胞递送、定殖和/或植入的方法，所述方法包括：

通过所述受试者的脉管系统施用表达E-选择素配体和/或L-选择素配体的细胞的群体，所述群体表达E-选择素配体和/或L-选择素配体的水平超过所述细胞的天然群体的表达水平，

其中所述施用与靶组织内的内皮细胞上的E-选择素表达同时进行，和/或与靶组织内白细胞的积累同时进行。

6.一种增强细胞群体在受试者的靶组织内的沉积、定殖和/或植入的方法，所述方法包括：

将表达E-选择素配体和/或L-选择素配体的细胞的群体直接注射到靶组织内或放置在

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其上，所述群体表达E-选择素配体和/或L-选择素配体的水平超过所述细胞的天然群体的表达水平，

其中所述注射与靶组织内的内皮细胞上的E-选择素表达同时进行，和/或与靶组织内白细胞的积累同时进行，

其中所述群体相对于所述细胞的天然群体实现增强的靶组织定殖。

7.一种治疗在受试者中表现为发炎的和/或受损的组织的疾病、障碍或医学病况的方法，所述方法包括：

向受试者施用表达E-选择素配体和/或L-选择素配体的细胞的群体，所述群体表达E-选择素配体和/或L-选择素配体的水平超过所述细胞的天然群体的表达水平，

其中所述注射与发炎的和/或受损的组织内的内皮细胞上的E-选择素表达同时进行，和/或与发炎的和/或受损的组织内白细胞的积累同时进行。

8.一种治疗受试者的肿瘤/恶性疾病的方法，所述方法包括：

向受试者施用表达E-选择素配体和/或L-选择素配体的细胞的群体，所述群体表达E-选择素配体和/或L-选择素配体的水平超过所述细胞的天然群体的表达水平，

其中所述施用与具有肿瘤/恶性细胞的组织内的内皮细胞上的E-选择素表达同时进行，和/或与具有肿瘤/恶性细胞的组织内白细胞的积累同时进行。

9.表达E-选择素配体和/或L-选择素配体的细胞的群体，在制备用于治疗在受试者中表现为发炎的和/或受损的组织的疾病、障碍或医学病况的药物中的用途，所述治疗包括向所述受试者施用表达E-选择素配体和/或L-选择素配体的细胞的群体，其中所述群体表达E-选择素配体和/或L-选择素配体的水平超过所述细胞的天然群体的表达水平，

其中所述治疗包括与发炎的和/或受损的组织内的内皮细胞上E-选择素表达同时，和/或与发炎的和/或受损的组织内白细胞的积累同时施用所述群体。

10.表达E-选择素配体和/或L-选择素配体的细胞的群体，在制备用于治疗肿瘤/恶性疾病的药物中的用途，所述治疗包括向所述受试者施用表达E-选择素配体和/或L-选择素配体的细胞的群体，其中所述群体表达E-选择素配体和/或L-选择素配体的水平超过所述细胞的天然群体的表达水平，

其中所述治疗包括与肿瘤/恶性组织内的内皮细胞上的E-选择素表达同时，和/或与肿瘤/恶性受损组织内白细胞的积累同时施用所述群体。

11.权利要求1-10中任一项的方法，其中所述细胞施用与在靶组织内的内皮细胞及具有L-选择素的白细胞浸润物上的E-选择素表达同时进行。

12.权利要求1-11中任一项的方法或细胞群体，其中所述施用/注射在白细胞向某一/所述发炎的和/或受损的组织浸润期间进行。

13.权利要求1-12中任一项的方法或细胞群体，进一步包括所述施用的细胞的群体向某一/所述发炎的和/或受损的组织的微环境中的植入。

14.权利要求1-13中任一项的方法或细胞群体，其中通过细胞在某一/所述发炎的和/或受损的组织内的定殖来实现免疫调制作用。

15.权利要求1-14中任一项的方法或细胞群体，其中通过施用细胞在某一/所述发炎的和/或受损的组织内的定殖来实现组织修复作用。

16.权利要求1-15中任一项的方法或细胞群体，其中通过施用细胞在某一/所述发炎的

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和/或受损的组织内的递送/定殖来实现增强的宿主防御/免疫应答作用。

17.权利要求1-16中任一项的方法或群体，其中通过施用细胞在某一/所述肿瘤/恶性组织位点内的定殖来实现抗恶性肿瘤作用。

18.权利要求1-17中任一项的方法或群体，其中所述施用/注射包括所述细胞群体的一次或一系列注射。

19.权利要求1-18中任一项的方法或细胞的群体，其中所述施用/注射包括每日、每周、每两周、每月或每年注射所述细胞群体。

20.权利要求1-9中任一项的方法或细胞的群体，还包括在先前的施用/注射的免疫调制作用在受试者中降低时，进行一次或多次额外施用/注射所述细胞群体。

21.权利要求1-20中任一项的方法或细胞的群体，其中通过静脉内施用/注射所述细胞群体。

22.权利要求1-21中任一项的方法或细胞的群体，其中通过直接注射到发炎和/或损伤组织而施用所述细胞群体。

23.权利要求1-21中任一项的方法或细胞的群体，其中将所述细胞群体放置在发炎的组织和/或损伤的组织上。

24.权利要求1-21中任一项的方法或细胞的群体，其中将所述细胞群体放置在某一/所述发炎的组织和/或损伤的组织上。

25.权利要求1-21中任一项的方法或细胞的群体，其中所述细胞群体与其它增强剂、抗炎剂或与组织支架/可移植装置/凝胶组合使用。

26.权利要求1-25中任一项的方法或群体，其中所述细胞的群体表达E-选择素配体和L-选择素配体。

27.权利要求1-26中任一项的方法或群体，其中所述E-选择素配体和/或L-选择素配体选自造血细胞E-/L-选择素配体(HCELL)、神经细胞粘附分子E-选择素配体(NCAM-E)、CD43E、CLA和ESL-1中的一种或多种。

28.权利要求1-27中任一项的方法或群体，其中所述E-选择素配体是造血细胞E-/L-选择素配体(HCELL)和/或神经细胞粘附分子E-选择素配体(NCAM-E)。

29.权利要求1-28中任一项的方法或群体，其中所述E-选择素配体是HCELL。

30.权利要求1-29中任一项的方法或群体，其中所述E-选择素配体是NCAM-E。

31.权利要求1-30中任一项的方法或群体，其中所述L-选择素配体是HCELL。

32.权利要求1-31中任一项的方法或群体，其中所述细胞群体包含以下中的一种或多种：上皮、内皮、神经元、脂肪、心脏、骨骼肌、成纤维细胞、和免疫细胞(例如树突细胞、单核细胞、巨噬细胞、白细胞(例如淋巴细胞如NK细胞、B淋巴细胞、T淋巴细胞或T淋巴细胞亚群，如调节性淋巴细胞($CD4^+/CD25^+/FOXP3^+$))、细胞毒性淋巴细胞等)、肝、脾、肺、循环血细胞、血小板、生殖细胞、胃肠、肾、骨髓、胰细胞、干细胞(例如间充质干细胞、造血干细胞、组织干/祖细胞(例如神经干细胞、肌细胞干细胞或肺干细胞)、脐带干细胞、或胚胎干细胞、或诱导的多能干细胞、或衍生自胚胎干细胞或衍生自诱导的多能干细胞的分化的祖细胞、或衍生自成体干细胞的分化的祖细胞、或分离自任何组织(例如脑、肝、肺、肠、胃、脂肪、肌肉、睾丸、子宫、卵巢、皮肤、脾、内分泌器官和骨骼)的原代细胞、或经培养扩增的祖细胞群体、或经培养扩增的干细胞群体、或经培养扩增的原代细胞群体。

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33.权利要求1-32中任一项的方法或群体，其中所述细胞群体是间充质干细胞、造血干细胞、组织干/祖细胞、脐血干细胞或胚胎干细胞。

34.权利要求1-33中任一项的方法或群体，其中所述细胞群体是白细胞(例如淋巴细胞如NK细胞、B淋巴细胞、T淋巴细胞或T淋巴细胞亚群，例如调节性淋巴细胞($CD4^+/CD25^+/FOXP3^+$))、细胞毒性T细胞等)。

35.权利要求1-34中任一项的方法或群体，其中所述疾病、障碍或医学病况是以下中的一种或多种：直接组织损伤(例如烧伤、创伤、褥疮溃疡等)、缺血/脉管事件(例如心肌梗塞、中风、休克、出血、凝血病等)、感染(例如蜂窝组织炎、肺炎、脑膜炎、败血症、SIRS等)、瘤形成(例如乳腺癌、肺癌、前列腺癌、淋巴瘤、白血病等)、免疫/自身免疫病况(例如移植物抗宿主病、多发性硬化、糖尿病、炎性肠病、红斑狼疮、类风湿关节炎、银屑病等)、退行性疾病(例如骨质疏松症、骨关节炎、阿尔茨海默氏病等)、先天性/遗传性疾病(例如大疱性表皮松解、成骨不全、肌营养不良、溶酶体贮积病、亨廷顿氏病等)、药物不良反应(例如药物诱导的肝炎、药物-诱导的心脏损伤等)、中毒性损伤(例如辐射照射、化学品接触、酒精性肝炎，酒精性胰腺炎、酒精性心肌病、可卡因心肌病等)、代谢紊乱(例如尿毒症性心包炎、代谢性酸中毒等)、医源性病况(例如辐射诱导的组织损伤、外科手术相关的并发症等)，和/或特发性过程(例如肌萎缩性侧索硬化、Parsonnage-Turner综合征等)。

36.权利要求1-35中任一项的方法或群体，其中所述疾病、障碍或医学病况是糖尿病。

37.权利要求1-36中任一项的方法或群体，其中所述疾病、障碍或医学病况是多发性硬化。

38.权利要求1-37中任一项的方法或群体，其中所述受试者是人、非人灵长类动物、小鼠、大鼠、狗、猫、马或牛。

39.权利要求1-38中任一项的方法或群体，其中所述受试者是人类患者。

40.权利要求1-39中任一项的方法或群体，其中对细胞群体处理以形成具有增强表达E-选择素和/或L-选择素配体的修饰的细胞群体，并且所述细胞群体在处理后24小时具有至少70％的活力。

41.权利要求1-40中任一项方法或群体，其中所述细胞群体在处理后24小时具有至少80％的活力。

42.权利要求1-40中任一项方法或群体，其中所述细胞群体在处理后48小时具有至少70％的活力。

43.权利要求1-42中任一项的方法或群体，其中在向受试者施用所述细胞群体时，所述细胞群体沉积在包含浸润性白细胞的组织内。

44.权利要求43的方法或群体，其中所述施用是直接注射到组织内或放置在组织上。

45.权利要求43的方法或群体，其中所述施用是经脉管的。

46.权利要求1-21或25-42中任一项的方法或群体，其中经由外周脉管通路或中央脉管通路而系统地施用所述群体。

47.权利要求1-21或25-42中任一项的方法或群体，其中使用基于导管的方法或将细胞的脉管团块递送至预期位点的其它脉管通路装置，将所述群体经血管内施用到预期组织位点的解剖滋养层血管内。

48.权利要求1-21或25-42中任一项的方法或群体，其中通过引入到所述椎管和/或心

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室内地施用所述群体。

49.权利要求1-21或25-42中任一项的方法或群体，其中通过基于导管的方法或直接注射，将所述群体直接施用到体腔内。

50.权利要求1-21或25-42中任一项的方法或群体，其中通过直接局部组织注射，使用脉管内途径，或经皮方法，或直接进入解剖学上可接近的组织部位和/或由成像技术引导而施用所述群体。

51.权利要求1-21或25-42中任一项的方法或群体，其中通过直接放置在相关组织表面/位点上而施用所述群体。

52.权利要求1-21或25-42中任一项的方法或群体，其中将所述群体施用到支架内或包埋在放置于组织中的支架内，和/或施用于凝胶中，和/或与增强剂一起施用。

53.权利要求1-21或25-42中任一项的方法或群体，其中将所述群体施用到脑脊液内。

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改善细胞治疗的方法

[0001] 本公开依托国家卫生研究院给予的基金P01 HL107146(NHLBI糖科学卓越计划)、R01 HL73714和R01 HL60528,在政府支持下进行。政府在本公开中有特定权利。

[0002] 本公开涉及用于修饰细胞表面的组合物和方法,以及这些经修饰细胞在炎性病况、组织损伤/受损和癌症的基于细胞的治疗中的改善的功效和适用性。

[0003] 基于细胞的治疗剂(也称为"过继细胞治疗剂")的成功取决于将足够量的相关细胞提供到需要其的位点以实现预期的生物学作用。递送用于临床适应症的细胞可以通过直接(局部)注射到所涉及的组织中,通过血管内给药(例如系统地或通过基于导管的递送到达特定血管床),或通过直接将细胞应用/放置到受影响的区域(例如用于皮肤溃疡,烧伤等)来实现。在所有形式的细胞施用中,有利的是施用的细胞具有膜分子,其能够促进细胞在施用位点内精确地在组织微环境内沉积,这些组织微环境对于达到预期效果,例如控制炎症、组织修复、消除排异反应、癌症根除至关重要。所述微环境位点之一是存在于损伤组织内的微血管内和周围的"血管周围区域",因为众所周知,微脉管的完整性和新微血管的产生(即"血管生成")是组织再生/修复的关键先决条件。实际上,在组织损伤、炎症和癌症的所有位点,受影响组织的微血管内的内皮细胞显示出一组特征性的粘附分子,其在募集循环(血源性)细胞至靶位时起关键作用。这些内皮分子由炎性细胞因子如肿瘤坏死因子(TNF)和白细胞介素1(IL-1)上调,并且在人中其包括分子E-选择素,在小鼠包括分子E-选择素和P-选择素,这些是属于称为"选择素"的粘附分子家族的凝集素(在下文将更详细描述)。此外,已经被募集到任何炎症位点(包括癌症)或组织损伤/受损位点的白细胞显示有L-选择素,即"白细胞"选择素,因此,在被施用细胞上表达L-选择素的配体会促进这些细胞沉积到受影响组织内部的白细胞浸润物区域。

[0004] 乍看之下,直接递送似乎可能是细胞施用最有效的方法,特别是考虑到可以将浓缩的细胞团应用到受影响的区域。然而,也存在局部注射实际上对预期的治疗效果起反作用的情况,而且局部注射仅适用于某些解剖位置:(1)通过在静水压力下将相关细胞引入培养基悬浮液中,注射程序可能伤害到递送的细胞,并且进一步降低组织完整性并破坏早期组织修复和/或宿主防御过程,从而加剧炎症状况或原位抵消适当的免疫应答;(2)凭借侵入性方法,注射针/装置(和悬浮液)可引起靶组织受损和/或引起侧支组织受损;(3)直接注射对于具有明确解剖边界(例如心脏)的器官/组织最为可行,对于没有广泛结缔组织支持(例如肺)的组织是不易实施的。(4)注射程序可能在依赖技术且耗费劳动,需要使用具有显著成像支持的复杂递送系统,尤其是对于相对不易接近和/或脆弱的器官/组织(例如中枢神经系统)而言。(5)最重要的是,许多退行性和炎性疾病实际上是广泛分布和多灶性的(如骨质疏松症、炎性肠病、多发性硬化症等),因此直接注射既不实用也不实效。因此,尽管存在局部注射可行的临床状况/情况,但脉管途径的施用对于所有广泛的"系统"疾病,以及对于局部注射很难接近和/或解剖学上不适于局部注射的任何组织(例如糖尿病中的胰腺,慢性阻塞性肺疾病中的肺)是必须的。以最小的努力重复施用细胞的能力是系统输注的另一个重要的实际优点。因此,创建优化分子效应子的表达/活性的方法学是实现所有基于细胞的疗法巨大前景的关键,所述效应子既指导了炎症环境中直接注射的细胞的粘附/沉积,也

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指导脉管内施用的细胞向受影响部位的生理迁移。

[0005] 血源细胞直接迁移到预定位置的能力(“归巢”)对各种生理和病理过程有深刻的影响。循环细胞向特定解剖位点的募集是通过靶组织的流动的和脉管内皮中的细胞之间的离散的粘附相互作用引发的。介导这些接触的分子被称为“归巢受体”,并且如历史上所定义的,这些结构引导血液中的细胞对各自靶组织的向性。在历史中,描述了三种“组织特异性归巢受体”:L-选择素用于外周淋巴结,$\alpha_4\beta_7$(LPAM-1)用于肠和肠相关淋巴组织,以及P-选择素的一个专门的糖型P-选择素糖蛋白配体-1(PSGL-1),其特别地作为促进细胞向皮肤迁移的“皮肤淋巴细胞抗原”(CLA)而为人所知(R.Sackstein,Curr Opin Hematol 12,444(2005))。值得注意的是,除了这些组织,数十年来已经认识到循环细胞,特别是造血干细胞(HSC)能有效地导向骨髓(T.Lapidot,A.Dar,O.Kollet,Blood 106,1991(2005)),并且有几项研究指出了选择素的作用,主要是E-选择素与HSC E-选择素配体结合,介导HSC向骨髓的募集。

[0006] 从生物物理角度来看,归巢受体用作分子制动器,影响初始的聚集,然后使血流中细胞以低于主要血流速率的血液流动速率向脉管内皮上进行持续滚动接触(步骤1)(R.Sackstein,Curr Opin Hematol 12,444(2005))。此后,发生一系列事件,通常为通过趋化因子增强,导致整合素粘附性的激活(步骤2)、牢固粘附(步骤3)和内皮迁移(步骤4)(T.A.Springer,Cell 76,301(1994))。这种“多步骤范式”认为组织特异性迁移由给定循环细胞上的归巢受体和趋化因子受体表达的离散组合调节,允许识别由器官特异性形式表达于靶标内皮内的相关脉管粘附配体和趋化因子显示的相关“交通信号”。在指导细胞转运到骨髓的归巢受体参与之后,有几个证据表明一种趋化因子,具体而言是SDF-1(CXCL12)在步骤2介导的细胞向该位点的募集中起着重要作用(T.Lapidot,A.Dar,O.Kollet,Blood 106,1991(2005);A.Peled等人,Science 283,845(1999);D.A.Sipkins等人,Nature 435,969(2005))。然而,SDF-1的表达不限于骨髓,并且该趋化因子通常在组织损伤/炎症的所有位点表达(R.Sackstein,Immunol.Rev.230:140-163(2009))。

[0007] 步骤1滚动相互作用的最有效的效应子是选择素(E-,P-和L-选择素)及其配体(R.Sackstein,Curr Opin Hematol 12,444(2005))。顾名思义,选择素是结合特异化的碳水化合物决定簇的凝集素,其由含有α(2,3)连接的唾液酸取代及α(1,3)连接的岩藻糖修饰的唾液酸岩藻糖基化构成,四糖唾液酸Lewis X是其原型代表(sLe^x;Neu5Acα2-3Galβ1-4[Fucα1-3]GlcNAcβ1-))(R.Sackstein,Curr Opin Hematol 12,444(2005);M.J.Polley等人,Proc Natl Acad Sci USA 88,6224(1991))。sLex多糖被多种单克隆抗体(mAb)识别,包括称为“HECA452”的mAb。E-和P-选择素在脉管内皮(P-选择素还在血小板上)表达,L-选择素在循环白细胞上表达(R.Sackstein,Curr Opin Hematol 12,444(2005))。E-和P-选择素通常是可诱导的内皮细胞膜分子,其仅在组织损伤和炎症的位点显著表达,其中其表达是响应于炎性细胞因子产生的。然而,骨髓和皮肤的微血管组成型表达这些选择素,它们在这些位点的稳态募集血液细胞中起关键作用(R Sackstein J Invest.Dermatology,122:1061-1069(2004))。重要的是,在灵长类动物(但不是啮齿动物)中所有的炎症位点和组织损伤位点,E-选择素是介导细胞募集的主要脉管选择素,因为响应于炎性细胞因子TNF和IL-1的启动子元件已从P-选择素基因中删除。因此,在人的所有炎症位点,脉管E-选择素表达比P-选择素更显著(R.Sackstein,Immunol.Rev.230:140-163(2009))。

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[0008] 已经在人造血干细胞/祖细胞(HSPC)中鉴定了E-选择素的两种主要配体,即PSGL-1的高唾液酸岩藻糖基化的CLA糖型(Z.Laszik等人,Blood 88,3010(1996),R.Sackstein,Immunol.Rev.230:140-163(2009))和特异化的唾液酸岩藻糖基化的CD44糖型,称为造血细胞E-/L-选择素配体(HCELL)(C.J.Dimitroff,J.Y.Lee,S.Rafii,R.C.Fuhlbrigge,R.Sackstein,J Cell Biol 153,1277(2001);C.J.Dimitroff,J.Y.Lee,R.C.Fuhlbrigge,R.Sackstein,Proc Natl Acad Sci US A 97,13841(2000))。CD44是一种存在相当普遍的细胞膜蛋白,但HCELL表型主要发现于人类HSPC中。与HCELL的限制性分布相反,CLA/PSGL-1在骨髓中的造血祖细胞以及更成熟的骨髓和淋巴样细胞中广泛表达(Z.Laszik等人,Blood 88,3010(1996),R.Sackstein,Immunol.Rev.230:140-163(2009))。在操作上将HCELL定义为在剪切条件下与E-选择素和L-选择素结合的CD44,并且通过细胞裂解物的Western印迹分析鉴定为与E-选择素-Ig嵌合体(E-Ig)及mAb HECA452反应的CD44糖型,mAb HECA452识别唾液酸Lewis X(除了sLex之外,HECA452识别sLex的四糖异构体,被称为"唾液酸化的Lewis a"(sLea),其中岩藻糖以α(1,4)连接形式连接至1型乳糖胺骨架内的N-乙酰葡糖胺)。除了CLA和HCELL,人白细胞和HSPC还可以表达可作为E-选择素配体的称为"CD43-E"的CD43糖型(Fuhlbrigge等人Blood 107:1421-1426(2006),Merzaban等人Blood 118:1774-1783(2011)),并且在小鼠白细胞中,已经描述了称为E-选择素配体-1(ESL-1)的另一种E-选择素配体(Merzaban等人Blood 118:1774-1783(2011))。在所有糖蛋白选择素配体中(例如CD43-E、CLA和HCELL),与E-选择素(以及L-选择素和P-选择素)的结合都严重依赖于α(2,3)-唾液酸和α(1,3)-岩藻糖修饰(C.J.Dimitroff,J.Y.Lee,S.Rafii,R.C.Fuhlbrigge,R.Sackstein,J Cell Biol 153,1277(2001);C.J.Dimitroff,J.Y.Lee,R.C.Fuhlbrigge,R.Sackstein,Proc Natl Acad Sci US A 97,13841(2000);R.Sackstein,C.J.Dimitroff,Blood 96,2765(2000);C.J.Dimitroff,J.Y.Lee,K.S.Schor,B.M.Sandmaier,R.Sackstein,J Biol Chem 276,4762(2001))。在人HSPC上,HCELL显示在N-聚糖上的相关的唾液酸岩藻糖基化的选择素结合决定簇(C.J.Dimitroff,J.Y.Lee,S.Rafii,R.C.Fuhlbrigge,R.Sackstein,J Cell Biol 153,1277(2001);R.Sackstein,C.J.Dimitroff,Blood 96,2765(2000))。在血液动力学剪切应力下对E-和L-选择素结合的体外测定表明HCELL是在任何人类细胞上表达的这些分子的最有效配体(C.J.Dimitroff,J.Y.Lee,S.Rafii,R.C.Fuhlbrigge,R.Sackstein,J Cell Biol 153,1277(2001);C.J.Dimitroff,J.Y.Lee,K.S.Schor,B.M.Sandmaier,R.Sackstein,J Biol Chem 276,47623(2001))。重要的是,虽然E-选择素在骨髓和皮肤的微脉管内皮上组成型表达,但是在所有炎症位点——急性和慢性型的内皮层上都显著地表达这种分子——无论其由是否由以下原因引起:直接组织损伤(例如烧伤、创伤、褥疮溃疡等)、缺血/脉管事件(例如心肌梗塞、中风、休克、出血、凝血病等)、感染(例如蜂窝组织炎、肺炎、脑膜炎、SIRS等)、瘤形成(例如乳腺癌、肺癌、淋巴瘤等)、免疫/自身免疫病况(例如移植物抗宿主病、多发性硬化、糖尿病、炎性肠病、红斑狼疮、类风湿关节炎、银屑病等)、退行性疾病(例如骨质疏松症、骨关节炎、阿尔茨海默氏病等)、先天性/遗传性疾病(例如肌营养不良、溶酶体贮积病、亨廷顿氏病等)、药物不良反应(例如药物诱导的肝炎、药物-诱导的心脏损伤等)、中毒性损伤(例如辐射照射、化学品接触、酒精性肝炎,酒精性胰腺炎、酒精性心肌病、可卡因心肌病等)、代谢紊乱(例如尿毒症性心包炎、代谢性酸中毒等)、医源性病况(例如辐射诱导的组织损伤、外科

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手术相关的并发症等)，和/或特发性过程(例如肌萎缩性侧索硬化，Parsonnage-Turner综合征等)。

[0009] 在本公开的多个方面中，提供了用于治疗在受影响的组织内的脉管内皮细胞和/或白细胞浸润物上有E-选择素表达的疾病、障碍或医学病况的方法。E-选择素与天然存在于某些白细胞和造血干细胞/祖细胞表面上的唾液酸化的岩藻糖基化的碳水化合物(例如唾液酸化的Lewis X和Lewis A家族的成员)结合，所述细胞即骨髓细胞(例如嗜中性粒细胞、单核细胞、嗜酸性粒细胞、巨噬细胞等)、树突细胞(来自淋巴系和髓系)、淋巴细胞(例如初始和记忆T细胞、初始和记忆B细胞、效应T细胞、调节性T细胞、天然杀伤细胞(NK细胞)等)、造血祖细胞和造血干细胞。因此，发现这些细胞类型位于急性和慢性炎症位点，是由脉管E-选择素招募到这些炎症位点的。白细胞和HSPC特征性地显示有L-选择素。L-选择素本身结合唾液酸化的岩藻糖化的碳水化合物如sLex。因此，炎性位点具有表达E-选择素(内皮细胞上)和L-选择素(浸润性白细胞和HSPC上)的细胞。

[0010] 实现基于细胞的治疗剂的预期结果的能力在严重地依赖于向需要的位点递送施用的细胞，并且还涉及所述施用的细胞在特定组织微环境内的定位。因此，在本领域中需要增强施用的细胞向组织损伤/受损、向炎症位点、向癌症位点和向感染位点的脉管递送的方法。本领域还需要增强施用的细胞在受影响组织内的递送/定殖。因此，简而言之，本公开涉及治疗在受试者中表现为发炎的和/或受损的组织和/或癌症的疾病、障碍或医学病况的方法，所述方法包括向受试者施用以超过细胞的天然群体的水平表达E-选择素配体和/或L-选择素配体的细胞群体。在靶组织(例如发炎的和/或受损的组织、癌组织)内的内皮细胞上表达E-选择素的同时和/或在靶组织中白细胞积累(例如白细胞浸润物)的同时进行施用。可以通过在靶组织内的直接注射和/或通过脉管系统和/或通过如下文进一步详细描述的其它方式进行施用。

[0011] 本公开进一步针对用表达E-选择素和/或L-选择素配体的活细胞群体治疗炎症的方法。所述活细胞群体以超过所述细胞的天然群体表达水平的水平表达E-选择素配体和/或L-选择素配体。在靶组织(例如发炎的和/或受损的组织、癌组织)内的内皮细胞上表达E-选择素的同时和/或在靶组织中白细胞积累(例如白细胞浸润物)的同时进行施用。可以通过直接注射入靶组织内和/或通过脉管系统(和/或通过如下文进一步详细描述的其它方式)进行施用，并且对所述疾病、障碍或医学病况的治疗无需伴随长期植入所述施用细胞(即可通过所述施用细胞在治疗位点的瞬时定殖或长期持续/存活，或者通过所述施用细胞在治疗位点增殖，或者通过所述施用细胞在治疗位点分化和/或成熟来实现治疗)。

[0012] 本公开进一步针对表达E-选择素和/或L-选择素配体的活细胞群体，在制备用于治疗受试者的炎症的药物中的用途。所述活细胞群体以超过所述细胞的天然群体表达水平的水平表达E-选择素配体和/或L-选择素配体。在靶组织(例如发炎的和/或受损的组织、癌组织)内的内皮细胞上表达E-选择素的同时和/或在靶组织中白细胞积累(例如白细胞浸润物)的同时进行施用。可以通过直接注射入靶组织内和/或通过脉管系统和/或通过如下文进一步详细描述的其它方式进行施用。

[0013] 本公开进一步针对表达E-选择素配体和/或L-选择素配体的活细胞群体，在制备用于治疗肿瘤/恶性疾病的药物中的用途，所述治疗包括向受试者施用表达E-选择素配体和/或L-选择素配体的细胞群体，其中所述群体以超过所述细胞的天然群体的表达水平的

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水平表达所述E-选择素配体和/或L-选择素配体，其中所述治疗包括在肿瘤/恶性组织内的内皮细胞上的E-选择素表达同时和/或在肿瘤/恶性组织中白细胞的积累同时施用所述群体。

[0014] 本公开进一步针对表达E-选择素配体和/或L-选择素配体的活细胞群体，在制备用于治疗表现出炎症的患病状态的药物中的用途，所述治疗包括向受试者施用表达E-选择素配体和/或L-选择素配体的细胞的群体，其中所述群体以超过所述细胞的天然群体的表达水平的水平表达所述E-选择素配体和/或L-选择素配体，其中所述治疗包括在肿瘤/恶性组织内的内皮细胞上开始E-选择素表达同时和/或在肿瘤/恶性组织中白细胞的积累同时施用所述群体。

[0015] 涉及本文所述的施用(例如通过脉管系统和/或通过直接注射到组织内和/或经由其它方式)的其它方法包括例如增强受试者中发炎的和/或受损的组织或者癌症位点(统称为“靶”组织)中细胞递送和定殖的方法，增强细胞递送至受试者靶组织和/或增强在受试者靶组织中的组织定殖的方法，增强细胞群体在受试者靶组织内归巢和植入的方法，治疗受试者的炎性病况的方法，增强受试者中组织修复/再生的方法，以及治疗受试者的肿瘤/恶性疾病的方法。

[0016] 通常，根据本文所述的方法可以治疗多种炎性病症(例如急性和/或慢性)和/或受损的组织中的任何一种，包括但不限于由直接组织损伤(例如烧伤，创伤，褥疮溃疡等)、缺血/脉管事件(例如心肌梗塞、中风、休克、出血、凝血病等)、感染(例如蜂窝组织炎、肺炎、脑膜炎、SIRS等)、瘤形成(例如乳腺癌、肺癌、淋巴瘤等)、免疫/自身免疫病症(例如移植物抗宿主病、多发性硬化、糖尿病、炎性肠病、红斑狼疮、类风湿关节炎、银屑病等)、退行性疾病(例如骨质疏松症、骨关节炎、阿尔茨海默氏病等)、先天性/遗传性疾病(例如大疱性表皮松解、成骨不全、肌营养不良、溶酶体贮积病、亨廷顿氏病等)、药物不良反应(例如药物诱导的肝炎、药物-诱导的心脏损伤等)、中毒性损伤(例如辐射照射、化学品接触、酒精性肝炎，酒精性胰腺炎、酒精性心肌病、可卡因心肌病等)、代谢紊乱(例如尿毒症性心包炎、代谢性酸中毒等)、医源性病况(例如辐射诱导的组织损伤、外科手术相关的并发症等)，和/或特发性过程(例如肌萎缩性侧索硬化，Parsonnage-Turner综合征等)引发的那些。

[0017] 在某些实施方案中，例如由细胞群体表达的E-选择素配体和/或L-选择素配体选自以下中的一种或多种：造血细胞E-/L-选择素配体(HCELL)、神经细胞粘附分子E-选择素配体(NCAM-E)、CD43E和CLA。在一个实施方案中，E-选择素配体是造血细胞E-/L-选择素配体(HCELL)和/或神经细胞粘附分子E-选择素配体(NCAM-E)。在另一个实施方案中，E-选择素配体是HCELL。在另一个实施方案中，E-选择素配体是NCAM-E。在另一个实施方案中，L-选择素配体是HCELL。值得注意的是，由于E-选择素在受影响组织的内皮层内表达，而且白细胞特征性表达L-选择素，所以HCELL(一种有效的E-选择素和L-选择素配体)的表达将有助于明确地促进大多数强烈的免疫应答性/组织受损的微环境内的组织贮存。

[0018] 如本文更详细地讨论的，可以使用多种方法来制备用于施用于受试者的细胞群体。在一个实施方案中，通过使细胞或细胞的群体与糖转移酶及适当的供体核苷糖接触来制备群体。例如通过糖基转移酶增强表达岩藻糖残基(岩藻糖基化)、唾液酸残基(唾液酸化)或二者(唾液酸岩藻糖基化)，可以使用聚糖工程化来唾液酸岩藻糖基化CD44以增强造血细胞E-选择素配体(HCELL)的表达。备选地，可使用糖基转移酶如岩藻糖基转移酶将完整

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的聚糖结构如唾液酸Lewis^{X}或唾液酸Lewis^{a}转移至细胞表面。此外，非酶法可以用于共价或非共价地将E-选择素和/或L-选择素配体结合到细胞表面。例如可使用生物素-链霉亲和素对将适体、sLex(唾液酸Lewis^{X})、sLex聚糖的糖模拟物和/或肽模拟物、sLea聚糖的(唾液酸Lewis^{a})、sLea聚糖的糖模拟物和/或肽模拟物以及结合E-选择素和/或L-选择素的其它部分非共价地结合到细胞表面，或共价地结合到细胞表面；无论是共价还是非共价，所述结合可以是直接的或经由连接子。进一步的实例，结合E-选择素和/或L-选择素的噬菌体展示颗粒或抗体可以共价地或非共价地结合到细胞表面，在每种情况下介导经处理的细胞对E-选择素和/或L-选择素的粘附。

[0019] 通常，且与制备细胞群体的方式无关，制备方式提供了在修饰时间后24小时具有至少70％活力的修饰的细胞群体。在一个这样的实施方案中，修饰的细胞群体在修饰时间后24小时具有至少80％的活力。在一些实施方案中，修饰的细胞群体在修饰时间后24小时具有至少85％的活力。在一些实施方案中，修饰的细胞群体在修饰时间后24小时具有至少90％的活力。可以通过本领域已知的方法例如台盼蓝排除法或通过用于碘化丙啶和膜联蛋白V染色的双色流式细胞术评估来确定细胞活力。优选地，在处理后保留细胞的表型(除细胞表面修饰以外)。保留的表型是指细胞保持其天然功能和/或活性。例如如果细胞是干细胞，它保留其再生能力，例如其全能性或其多能性或其多潜能性或其单能性。

[0020] 经修饰的细胞群体是存活的(即如上所述在修饰时间后24小时具有至少70％的活力)，并且相对于所述细胞的天然群体具有增强的与E-选择素和/或L-选择素结合。可以通过脉管系统和/或通过直接注射到组织中(和/或通过下文详细描述的其它方式)进行细胞的施用。在一个实施方案中，在炎症或白细胞浸润到组织中发生的同时施用经修饰的细胞群体。在另一个实施方案中，恰好在炎症或白细胞浸润到组织中发生之前施用经修饰的细胞群体。

[0021] 在细胞经过聚糖工程化以增强sLex表达或经过用sLex结构(例如通过抗生物素蛋白-亲和链霉素技术)装饰的情况下，可以通过用mAb HECA452或识别sLex决定簇的任何其它mAb进行荧光染色然后用流式细胞术检测细胞荧光强度来进行对经处理的细胞上增加的sLex表达的测量。通过首先分析天然(未处理)细胞的样品来确定经修饰的细胞群体的预定荧光阈值。经处理细胞的sLex的增加被定义为相比细胞的天然群体，标记物阳性细胞(例如HECA452-反应细胞)比例增加超过10％，和/或平均通道荧光强度超过基线(未处理)细胞群体10％。为了检测经处理的细胞群体与E-选择素的结合是否增加，可以使用如本文所述的剪切应力条件下的平行板流动室研究或通过用E-选择素-Ig嵌合体进行染色以及通过流式细胞术进行荧光强度评估，而对与E-选择素的结合进行评估。使用本文别处描述的功能性E-选择素结合测定法或通过本领域已知的另一种普遍接受的E-选择素荧光结合测定法测定对照(基线)样品细胞。测量对照(基线)群体样品的E-选择素结合荧光水平。增强的与E-选择素的结合被定义为在E-选择素特异性结合测定中具有增加的对E-选择素粘附性的经处理细胞。在一个实施方案中，增强的与E-选择素的结合可以通过使用荧光染料缀合反应剂的E-选择素结合测定中的荧光偏移来定义，例如通过流式细胞术评估与E-选择素-Ig嵌合体的结合，其中具有E-选择素结合的群体内的细胞数目比基线结合的细胞数目增加至少10％(即，在标记阳性群体中增加10％)和/或平均通道荧光比(与天然细胞群体相关的)预定荧光阈值高至少10％。在另一个实施方案中，具有增加的E-选择素反应性的细胞的百分

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比增加25％和/或经修饰的群体超过预定的荧光阈值25％。在另一个实施方案中，经修饰的群体超过基线反应性和/或预定荧光阈值50％。在另一个实施方案中，具有增加的E-选择素反应性的细胞的百分比比E-选择素结合细胞的基线群体的百分比增加了75％，和/或经修饰的群体超过预定的荧光阈值75％。在另一个实施方案中，经修饰群体中至少90％的细胞超过基线E-选择素结合群体和/或预定荧光阈值。在另一个实施方案中，经修饰群体中至少95％的细胞超过基线E-选择素结合群体和/或预定荧光阈值。

[0022] 可以通过使用具有高特异性的功能性L-选择素结合测定法(例如在动态剪切应力条件下的平行板流动室)或Stamper-Woodruff测定法来确定增强的与L-选择素结合，其中细胞处理增加了支持L-选择素介导的粘附的细胞的百分比。在平行板测定的情况下，与基线(未处理的细胞)相比，经处理的细胞群体显示与L-选择素(固定并显示在小室塑料或玻璃支撑物表面上)的栓系/滚动(tethering/rolling)粘附相互作用有至少10％的增加。在Stamper-Woodruff测定的情况下，增加的L-选择素淋巴细胞粘附被定义为在80rpm的旋转剪切下，经处理的细胞与L-选择素$^{+}$淋巴细胞的结合有至少10％的增加；在未处理(对照)群体上评估基线细胞结合，并直接与经处理的细胞群体相比较。

[0023] 除非另有定义，本文使用的所有技术和科学术语具有与本公开所属领域的普通技术人员通常理解的相同的含义。尽管与本文所述类似或等同的方法和材料可以用于本公开的实践或测试中，但是在下文描述了合适的方法和材料。本文提到的所有出版物、专利申请、专利和其它参考文献通过引用整体并入本文。在发生冲突的情况下，以本说明书(包括定义)为准。此外，材料、方法和实施例是仅作说明而不是限制性的。

[0024] 将会在后文部分地展现和部分地指出其它主题和特征。

[0025] 附图简述

[0026] 图1(A)-1(D)：外岩藻糖基化(FTVI处理)对小鼠间充质干细胞(MSC)的E-选择素配体表达的影响。(A)衍生自C57BL/6骨髓的MSC缺乏CD45的表达并表达特征性小鼠MSC标记物Sca-1、CD29、CD44、CD73和CD105。MSC天然缺乏与mAb HECA452和E-选择素-Ig嵌合体(mE-Ig)的反应性(同种型＝红色；抗体＝蓝色)。(B)岩藻糖基转移酶VI(FTVI)修饰的MSC(实线)对于mAb HECA452染色呈阳性，并与mE-Ig可反应。用菠萝蛋白酶和蛋白酶K消化FTVI修饰的MSC(阴影直方图)显著减少与小鼠E-选择素-Ig嵌合体(mE-Ig的)反应性，但不显示HECA452染色，表明菠萝蛋白酶敏感性糖蛋白作为聚糖修饰的(即FTVI处理的)MSC上的主要E-选择素配体。虚线代表染色对照(用于HECA452染色的同种型对照和用于mE-Ig染色的与EDTA钙螯合)。(C)未修饰的MSC(-)和FTVI修饰的MSC(+)的细胞裂解物的HECA452(左)和mE-Ig(右)反应性的Western印迹分析。FTVI修饰诱导的HECA452-和mE-Ig反应性部分主要在～100kDa的双糖蛋白条带上。(D)从FTVI修饰(+)或未修饰(-)的MSC的等量细胞裂解物中免疫沉淀CD44。然后将免疫沉淀物电泳并用CD44(KM114和IM7mAb；左)和HECA452(右)进行印迹。

[0027] 图2：平行板流动室测定FTVI修饰的和未修饰的野生型MSC对TNF-α处理的人脐静脉内皮细胞(HUVEC)的粘附。FTVI修饰显著改善了在0.5达因/cm^{2}的MSC对HUVEC的粘附，在剪切应力超过20达因/cm^{2}结合明显。用抗E-选择素(抗CD62E)功能阻断性mAb处理HUVEC将FTVI修饰的MSC的滚动粘附相互作用降低到与未修饰的MSC相似的水平。类似地，通过唾液酸酶处理FTVI修饰的MSC去除sLex决定簇将粘附降低至与未修饰的MSC相当的水平。

[0028] 图3(A)-3(C)：静脉内施用未修饰的和FTVI修饰的MSC对新发糖尿病NOD小鼠的高

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血糖的影响。(A)用PBS注射的高血糖NOD(未处理对照)显示没有高血糖的逆转(葡萄糖水平高于600mg葡萄糖/dL)。与未修饰的MSC(B)的输注相比,输注FTVI修饰的MSC(C)导致逆转为正常血糖和持久保持糖尿病逆转的小鼠数量显著增加。X轴上的箭头表示MSC输注的天数。

[0029] 图4(A)-4(H):对胰岛进行免疫荧光染色以评估胰腺中E-选择素的表达和MSC的定位。图4(A)和4(B):就胰岛素(绿色)和E-选择素(红色)的表达,对(A)糖尿病抗性BALB/c小鼠和(B)NOD小鼠的胰岛染色。胰岛用虚线划分。与BALB/c(A)相比,NOD小鼠(B)显示由于胰岛炎导致的胰岛素产生减少。在图4(C)-4(G)中,用DAPI(蓝色)对来自MSC处理的NOD小鼠的胰腺的冷冻切片染色。图4(C)和4(D):NOD胰腺连续切片的染色显示内皮标志物CD31(C)和E-选择素(D)的共定位,证实E-选择素在胰岛周围内皮细胞上的存在。图4(E):用DAPI(蓝色)、T细胞标记物CD3(绿色)和胰岛素(红色)(E)对NOD胰岛的共染色显示在胰岛边缘的特征性T细胞浸润。图4(F)和4(G):就浸润性MSC(用FITC缀合的抗-SLex mAb HECA452显现;绿色)、胰岛(F)(通过APC-缀合的抗胰岛素mAb显现;红色)和E-选择素表达微血管(G)(用PE-缀合的抗E-选择素mAb显现;红色),对冷冻切片中的免疫荧光图像进行染色。染色鉴定出胰岛炎区域中的HECA452+MSC,其邻近于在胰岛周围区域中的E-选择素表达微血管,并聚集在淋巴细胞浸润物(即特征性表达L-选择素的细胞)的区域内。图4(H):向NOD和BALB/c宿主静脉内施用的MSC的胰腺浸润。FTVI修饰的MSC向NOD小鼠的胰腺中的积累是未修饰的MSC的3倍高($P<0.01$),而在BALB/c宿主(n=3只小鼠/组;以60X放大倍数计算每组至少30个视野)中未观察到胰腺浸润差异。

[0030] 图5(A)-5(B):MSC的FTVI修饰不影响细胞存活或免疫抑制能力。(A)在注射pHRST-hGH-转导的FTVI修饰的或未修饰的MSC后,在不同时间点的NOD小鼠的血清中检测到类似水平的hGH。(B)FTVI修饰的和未修饰的MSC同等程度地抑制用CD3/CD28刺激的NOD $CD4^+$T细胞的增殖,表明聚糖修饰不增加MSC抑制淋巴细胞增殖的能力。

[0031] 图6(A)-6(D):CD44表达的缺乏消除了系统性施用FTVI修饰的MSC的抗糖尿病作用。(A)与未修饰的野生型MSC(图3B)相比,施用CD44缺陷型MSC表现出微弱的抗糖尿病作用。只有1只(7只中)接受未修饰的CD44 KO MSC的NOD小鼠显示对高血糖的逆转,而且是短暂的(在~第30天糖尿病复发),7例糖尿病NOD小鼠中的6只保持高血糖。(B)与接受FTVI修饰的野生型MSC的小鼠的结果(图3C)相比,施用FTVI修饰的CD44 KO MSC赋予了最小的抗糖尿病作用,6只NOD小鼠中只有1只表现出对高血糖的逆转。(C)与接受未修饰的CD44 KO MSC的小鼠(黑色条)相比,在接受FTVI-修饰的CD44 KO MSC的小鼠(白色条)中,NOD胰腺中的MSC积累没有差异($p<0.01$),并且在每种情况下都类似于接受未修饰的MSC的小鼠(图4H)。在X60放大倍数下量化MSC浸润。(D)FTVI修饰的和未修饰的CD44 KO MSC均具有免疫抑制能力,类似地抑制CD3/CD28T细胞刺激测定中的T细胞增殖。

[0032] 图7:对小鼠骨髓来源的MSC的表征。MSC分化为骨细胞、脂肪细胞和软骨细胞(X200放大倍数)。

[0033] 图8:$CD44^{-/-}$MSC的FTVI修饰导致HECA452反应性产生与野生型(WT)MSC类似的增加。未修饰的MSC(虚线)显示与HECA452没有反应性,而FTVI修饰的MSC(实线)和FTVI修饰的CD44 KO MSC(填充灰色)显示出与HECA452相似的染色水平,表明外岩藻糖基化在CD44 KO MSC中的替代(即,非CD44)支架上产生唾液酸岩藻糖基化表位。

[0034] 图9(A)-9(B):神经干细胞(NSC)缺乏E-选择素配体,但表达许多其它细胞表面粘

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附分子。(A)HECA452、KM93、CSLEX-1、E-选择素配体(与E-选择素-Ig嵌合体(E-Ig)结合))和P-选择素配体(与P-选择素-Ig嵌合体(P-Ig)结合)在NSC上的表达。相应的同种型对照显示每个测定抗体的重叠信号,即大鼠IgM(对于HECA-452;MFI:1.9)、小鼠IgM(对于KM93和CSLEX-1;MFI:2.7)和人IgG_1(对于E-Ig和P-Ig;MFI:3.5)。代表典型E-Ig结合谱的直方图表明,超过99%的细胞在用岩藻糖基转移酶VI处理细胞表面进行糖基转移酶程序化立体置换(GPS)后持续表现出E-Ig结合。(B)CD43(S7和1B11)、PSGL-1(2PH1、4RA10)、CD44(IM7、KM114)、NCAM、PSA、CD49d、CD49e、CD29、LPAM-1、CD11a、CD18和CXCR4的流式细胞术分析。虚线是同种型对照,黑线是特异性抗体。所有显示的结果代表在NSC上进行的n=5次流式细胞术实验。

[0035] 图10(A)-10(C):NSC的GPS处理(即,通过FTVI处理的α(1,3)-外岩藻糖基化)生成唾液酸岩藻糖基化,主要在糖蛋白上,其中一些是糖磷脂酰肌醇(GPI)连接的。(A)对BT-NSC(黑色条)和GPS-NSC(灰色条)上的HECA452、CD15、KM93、CSLEX1、E-Ig和P-Ig反应性的流式细胞术分析。对于每个检测抗体的相应同种型对照是:大鼠IgM(对于HECA-452;MFI:1.8)、小鼠IgM(对于CD15,KM93和CSLEX-1;MFI:1.9)和人IgG_1(对于E-Ig和P-Ig;MFI:3.2)。显示的结果代表五次独立的实验。(B)在GPS处理前对用菠萝蛋白酶消化(灰色条)或未消化(黑条)的GPS-NSC的HECA452反应性进行流式细胞术分析。值为平均值±SEM。(每组n=3)。(C)用磷脂酶C(PI-PLC)消化以裂解GPI锚定物(灰色条)或未消化(黑条)的GPS-NSC的HECA452反应性的流式细胞术分析。值为平均值±SEM。(每组n=3)。

[0036] 图11(A)-11(C):NSC的GPS处理产生位于100、120和140kDa处的瞬时E-选择素配体,对应于HCELL和N-CAM-E。(A)从等量的来自GPS处理的(GPS)或缓冲液处理的(BT)NSC的细胞裂解物中免疫沉淀CD44(用针对CD44的IM7和KM114mAb)。对NSC的免疫沉淀物和与免疫沉淀物分离的上清液(SN)进行Western印迹分析,进行电泳并用HECA452、E-Ig和CD44进行印迹。(B)从已经用PNGaseF处理(+)或未处理(-)的来自GPS处理(+)或缓冲液处理的(-)NSC裂解物的等量细胞裂解物中免疫沉淀N-CAM(用N-CAM13),然后进行电泳并用E-Ig和N-CAM进行印迹。在Ca^{2+}存在下用E-Ig进行染色。(C)在第0天用GPS处理NSC并在正常生长培养基中再培养3天。每24小时移出细胞等分试样,并通过流式细胞术测定E-选择素配体活性。另见图11(A)-11(C)、12(A)-12(B)和13(A)-13(F)。

[0037] 图12(A)-12(B):GPS-NSC在限定的剪切应力条件下与内皮E-选择素有显著增强的抗剪切粘附相互作用。(A)在1.0达因/cm^2,将BT-NSC或GPS-NSC灌注于IL-1β和TNF-α刺激的HUVEC上。然后在剪切应力为1、2、4、8、16、25和32达因/cm^2,测定NSC积累情况。在高达32达因/cm^2的剪切应力,GPS-NSC显示在HUVEC上的滚动粘附相互作用。为了控制结合GPS-NSC的特异性,将EDTA加入到测定缓冲液(EDTA组)中,或者在粘附测定中使用之前用对E-选择素的功能阻断性mAb(抗E-Sel组)对经刺激的HUVEC进行预处理。值为平均值±SEM。(每组n=4)。在所有剪切应力水平,将GPS-NSC与所有其它组比较,$P\leq0.001$。(B)在0.6达因/cm^2,NSC的HECA-452反应性膜糖蛋白的SDS-PAGE免疫印迹上灌注的CHO-E细胞的印迹滚动测定(滚动细胞/mm^2)的粘附柱状图。在进行测定之前,通过SDS-PAGE解析来自BT-NSC(黑色条)和GPS-NSC(灰色条)的CD44/HCELL和panNCAM的免疫沉淀物,并对HECA-452进行印迹。为了控制CHO-E与膜糖蛋白结合的特异性,将EDTA加入到含有CHO-E细胞的缓冲液中,然后在粘附测定中使用;在这种条件下没有细胞结合(数据未显示)。所提供的结果代表了NSC的多个(n

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=3)膜制备物的多次(n=4)HECA-452印迹实验。

[0038] 图13(A)-13(F):GPS-NSC在体内EAE模型中表现出改善的归巢情况。(A-D)GPS-NSC比BT-NSC更有效地迁移到CNS实质组织中。用PKH26染料标记1×10^6个GPS-NSC或BT-NSC,并在免疫后(PI)第9天和第13天静脉内注射入MOG诱导的EAE小鼠。(A)对接受BT-NSC或GPS-NSC的EAE小鼠(PI第17天)的前脑进行分析,显示与GPS-NSC相比,在注射BT-NSC的动物中观察到PKH26阳性细胞数量更少。黄色箭头表示NSC,白色箭头表示渗透。白色虚线表示脑膜边界。图20(A)-20(B)显示了对这些切片的进一步分析,以证实NSC(PKH26;红色)位于Flk-1血管(绿色)之外,Flk-1血管是SOX-2阳性的(绿色)。(B)在PI第17天收获腰骶脊髓(插入物)。在PI第17天,比BT-NSC更多的GPS-NSC从血管迁移出到脊髓实质内。通过Flk-1(VEGFR2;绿色)染色显现出血管。脊髓实质的边缘用白色虚线突出显示。用PKH26染料标记的NSC显示为红色,用TO-PRO-3复染的核是蓝色的。WM,白质。V,血管。(C)小图显示了A中箭头所示的迁移的NSC的三维视图。(D)确定在PI第17天时每个脊髓区域每200x迁移的BT-NSC和GPS-NSC的量化数量。迁移GPS-NSC的数量比BT-NSC有显著增加,*$p<0.05$。(E)对NSC生物分布的定量。用CFDA-SE标记GPS-NSC(灰色条)或BT-NSC(黑色条),并在免疫后第9天和第13天静脉内注射到MOG处理的C57BL6小鼠中。在最后一次注射后16小时,分析脑、淋巴结、脾、肝和肺,以确定存在于表示NSC的限定门内的CFSE阳性细胞的百分比。使用接受GPS-NSC或BT-NSC的非EAE小鼠来标准化在每个测试组织中观察到的信号。使用未接受细胞的小鼠来确定背景信号。误差条代表平均值的标准误差。数据代表2个单独的实验,其中每个实验对每组10只小鼠进行了测试。(F)在体内共聚焦分析显示,发现外岩藻糖基化的NSC与体内脾脏中的CD4T细胞有密切接触(NSC标记为粉红色,CD4T细胞呈绿色);NSC和淋巴细胞的这种共定位由NSC HCELL结合至淋巴细胞L-选择素引起。

[0039] 图14(A)-14(I):GPS-NSC有助于通过增强的神经保护来显著缓解EAE症状。(A)确定第0天用MOG35-55免疫并随后在免疫后第9天和第13天注射1×10^6个GFP标记的缓冲液处理的NSC(BT-NSC;填充的红色三角形;n=30)、GPS-处理的NSC(GPS-NSC;填充的绿色圆圈;n=30)的C57BL/6小鼠或假处理的小鼠(无NSC;填充黑色圆圈;n=30)的EAE临床评分。与假处理的小鼠($p=0.0001$)以及i.v.注射BT-NSC的小鼠($p=0.006$)相比,接受GPS-NSC的小鼠显示出明显的临床改善。(B)通过对比(A)中的曲线的斜率进行线性回归分析来评估疾病的累积负担。这些数据凸显了GPS-NSC(绿色虚线)将临床评分改善到显著超过BT-NSC(红线)。与未接受NSC的小鼠相比(无NSC;黑线),接受GPS-NSC或BT-NSC的小鼠显示出显著改善的临床评分。这些数据还表明BT-HSPC(蓝线)和GPS-HSPC(紫线)使疾病恶化(见图21(C))。(C)通过用抗CD11b mAb(绿色)和核标签To-Pro3(蓝色)染色以分析来自注射NSC的EAE小鼠的脑的PI第30天的神经病理学。如通过对来自3个不同脊髓的20个不同切片的CD11b巨噬细胞/小胶质细胞的计数所测量的,GPS-NSC注射使得每个脑切片的损伤都显著减少。柱状图描绘了从不接受NSC,接受BT-NSC或GPS-NSC的EAE小鼠的每个高倍视野(HPF)下的脊髓切片中CD11b巨噬细胞/小胶质细胞的计数,并且也基于To-Pro3染色计算每HPF下的浸润物计数。白色方框对应于更高放大倍数的图像。黄色箭头对应于活化的小胶质细胞,白色箭头对应于脑膜(m)中的浸润物。要注意到的是无NSC样品中的小神经胶质细胞比BT-NSC和GPS-NSC样品中的小胶质细胞更活化。在无NSC样品中,脑膜内浸润物的大小也大于BT-NSC样品。顶图比例尺为100μm。底图比例尺为50μm。(D)通过对来自不接受NSC(EAE无NSC),接受BT-NSC

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(EAE BT-NSC)或GPS-NSC(EAE GPS-NSC)的小鼠的3个独立脑的20个不同切片进行CD4(用于测量T细胞浸润)、CNPase(用于定量髓鞘细胞)、Olig-2(用于测量少突胶质细胞分化)或SOX-9(用于测量神经前体的多潜能性)(绿色)和To-Pro3(蓝色)的染色以分析来自注射NSC的EAE小鼠的脑的神经病理学。GPS-NSC注射导致显著更少的T细胞浸润、增强的髓鞘再生、更多数量的少突神经胶质细胞以及保持祖细胞数量。(E)柱状图描绘了来自不接受NSC,接受BT-NSC或GPS-NSC的EAE小鼠的每个高倍视野(HPF)脑切片中CD4T、CNPase、Olig2、SOX-9细胞的计数(如(D)),表明接受GPS-NSC的动物显示祖细胞得到了增强的神经保护。(F-I)与无NSC对照相比,GPS-NSC和BT-NSC注射使得轴突再生和轴突保护增加,这是通过GAP-43(绿色;$p<0.001$)染色增加(F)和通过单克隆抗体SMI32(红色;$p<0.001$)染色减少(H)所测量的,如通过在超过500个独立测量中对GAP43像素强度的定量共焦成像评估的。To-Pro3染色染料用于检测细胞核(蓝色)。(G,H)基于像素强度的超过500个单个测量的定量共焦成像(Imitola,J.,Cote,D.等人2011)确定了GAP-43(G)和SMI32(I)的图示;要注意的是SMI32的模式(I)表明在EAE动物中有轴突卵球,而在注射NSC的动物中减少(I),且SMI32像素强度显示接受GPS-NSC的动物中轴突完整性的逐渐校正。与对照组和BT-NSC相比,轴突卵球和轴突片段的减少描绘在如下文卡通画(I)中。除了SMI32染色图中比例尺为50μm外,其余均为100μm。

[0040] 图15:炎症细胞因子处理未诱导小鼠NSC上的选择素配体表达。用10ng/ml的TNFα、10ng/ml的IL-1β、10ng/ml的IFNγ独立地或组合地(均为10ng/ml)刺激NSC。在24小时时,收集NSC并通过流式细胞术分析E-选择素结合。对照包括未处理的NSC和Kg1a细胞(E-选择素结合的阳性对照)。

[0041] 图16(A)-16(C):HCELL是对一个(示例性)人NSC系(CC-2599)进行GPS处理后产生的唯一E-选择素配体。(A)流式细胞术分析CD15、CSLEX-1、HECA452和E-选择素配体(E-Ig结合),在用GPS处理的(灰色条)或缓冲液处理(BT;黑色条)人NSC上的表达。显示的是每个抗体的平均荧光强度。(B)CD44、PSGL-1、NCAM和CD43的流式细胞术分析。(C)来自小鼠和人来源的NSC裂解物的E-Ig反应性的Western印迹分析。从来自GPS处理(+)或缓冲液处理的(-)小鼠NSC或人NSC的等量细胞裂解物中免疫沉淀CD44和NCAM。然后将免疫沉淀物进行电泳并用E-Ig进行印迹。在Ca^{2+}存在下用E-Ig对GPS处理的NSC进行染色。

[0042] 图17:GPS处理不影响NSC形成神经球的能力。在GPS处理7天后,立即以每孔200个活GFP^+NSC铺板在96孔板中。对得到的神经球计数,并将神经球的数量除以铺板的细胞数来计算神经球频率。BT-NSC与GPS-NSC之间在神经球的密度或形成的神经球的数量上没有统计学差异。显示了用于这些实验的GFP^+神经球的代表性图像,其中红色荧光表示巢蛋白(Nestin)表达,绿色荧光表示GFP。注意到BT-和GPS-GFP^+NSC都能够相等地形成神经球。该图与图11(A)-11(C)相关。

[0043] 图18(A)-18(D):GPS处理不影响NSC分化为神经元(A),星形胶质细胞(B,D)或少突胶质细胞前体(C,D)的能力。在适当的分化培养基中,培养1×10^5个BT-和GPS-NSC,120小时后,计数每个20x视野下$MAP-2^+$神经元(A)、$GFAP^+$星形胶质细胞(B,D)和$NG2^+$少突胶质细胞(C)的数量。BT和GPS-NSC沿这三个谱系分化的能力之间无统计学差异($p=0.1$)。(D)$GFAP^+$星形胶质细胞和$NG2^+$少突胶质细胞以红色显示,To-Pro3用于染色用对照缓冲液(BT)或FTVI(GPS)体外处理的神经干细胞的核。比例尺,50μm。这些图与11(A)-11(C)相关。

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[0044] 图19(A)-19(C):GPS处理不影响体外NSC的免疫调制功能。通过将经辐射的NSC与从初始C57BL/6小鼠分离的淋巴结细胞(LNC)共培养,测量NSC对淋巴结细胞(LNC)的直接体外抑制作用。经辐射的BT-NSC和GPS-NSC均以剂量依赖的方式响应伴刀豆球蛋白A(ConA)而抑制^{3}H-胸苷掺入(A)LNC中,并且同等程度地抑制炎症细胞因子产生(B),这是由ELISA所测量的;这些比率对应于NSC数量与LNC数量的比(1:4、1:2、1:1、2:1和4:1)。要注意的是,相比单独使用LNC(第一条线),通过增加NSC:LNC比率,^{3}H-胸苷掺入量显著降低($p=0.0005$)。还要注意,BT-NSC和GPS-NSC抑制增殖的能力之间无统计学差异($p=0.2$)。(C)将1×10^{6}个BT-NSC或GPS-NSC用或不用炎性细胞因子(10ng/mL的IFN-γ和15ng/mL的TNF-α)并在有或无E-Ig(5ng/mL)下处理24小时,之后进行RNA提取和cDNA合成。实时RT-PCR显示使用$2^{-\Delta\Delta CT}$方法计算的基因表达的倍数变化(相对单独用BT或GPS-NSC处理)和相对于GAPDH看家基因测定的LIF mRNA的相对表达。值为平均值±SEM($n=4$个实验)。这些图与图11(A)-11(C)相关。

[0045] 图20(A)-20(B):GPS-NSC比BT-NSC更有效地迁移到CNS实质组织。用PKH26染料标记1×10^{6}个GPS-NSC或BT-NSC,并在免疫后(PI)第9天和第13天静脉内注射入MOG诱导的EAE小鼠。在PI第17天收获脑,并快速冷冻制备20μm切片,用以下抗体染色:抗Flk-1(VEGFR2;绿色)或抗SOX-2(红色)分别显示血管和NSC位置。(A)BT NSC主要定位于FLK-1^{+}内皮细胞,而GPS-NSC由于其穿过内皮而在实质组织中发现。这些NSC表达sox-2——神经干细胞标记物(B)。这些图与图13(A)-13(F)相关。

[0046] 图21(A)-21(C):GPS处理在小鼠HSPC上产生稳健的E-选择素配体活性,但这不会转化为EAE的缓解。(A)对BT-和GPS-小鼠造血干/祖细胞(HSPC;Lineage^{neg}C-kit^{pos})上的E-Ig反应性进行流式细胞术分析。对于hIgG$_1$同种型对照、BT-HSPC上的E-Ig反应性和GPS-HSPC上的E-Ig反应性的平均荧光强度显示在每个分布图上方。这些细胞用于体内EAE实验作为对照。(B)在第0天用MOG35-55免疫并随后在免疫后第9天和第13天注射1×10^{6}个缓冲液处理的HSPC(BT-HSPC;蓝色空心菱形;$n=30$)、GPS处理的HSPC(GPS-HSPC;紫色空心菱形;$n=30$)的C57BL/6小鼠或假处理的小鼠(无NSC;填充黑色圆圈;$n=30$)中确定EAE临床评分。在接受BT-或GPS-HSPC的小鼠中,临床评分没有显着改善。(C)通过对(B)中的曲线的斜率比较进行线性回归分析来评估疾病的累积负担。这些图与图14(A)-14(H)相关。

[0047] 图22:在PI第30天没有观察到注射到EAE小鼠的BT-或GPS-NSC有GFP信号的证据。在用MOG35-55(第0天)免疫并在随后第9天和第13天注射1×10^{6}个缓冲液处理的NSC(BT-NSC)、GPS处理的NSC(GPS-NSC)后30天的C57BL/6小鼠或假处理的小鼠(无NSC)处死,随后进行GFP^{+}细胞的免疫组化。在PI第30天时,即使将针对GFP的抗体(红色)与内源GFP信号(EGFP;绿色)进行比较也未在相关研究组的切片中发现GFP^{+}细胞。将注射前培养的NSC用作阳性对照(NSC-Pre Tx),其显示出稳健的GFP信号。比例尺,50μm。该图与图14(A)-14(H)相关。

[0048] 图23:通过对天然NSC表面蛋白CD44和NCAM的GPS处理提供的神经恢复模型,产生有效结合EAE小鼠中的内皮E-选择素的新的步骤1效应子,HCELL和NCAM-E。作为损伤的结果,内皮细胞和神经胶质细胞可能产生比对照小鼠更多数目的损伤信号(例如SDF-1α和E-选择素),所述损伤信号引导GPS-NSC向损伤诱导的生态位(niche)。尽管募集的NSC数量增加,NSC通过增加SOX-9+/Olig-2+少突胶质细胞的数量来提供CNS的免疫力和内源性再生的局部调制而不是进行细胞置换。这随后导致产生更多成熟的少突神经胶质细胞(CNPase+),

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较少的轴突损失和更多的轴突再生。由原始神经干细胞分泌的生态位分子可以促进一些由其定殖增加而提供的作用。

[0049] 图24:对来自瘦的受试者和来自肥胖受试者的脂肪衍生MSC的FTVII处理导致在细胞表面产生sLex结构。柱状图显示脂肪衍生MSC的α(1,3)-外岩藻糖基化之后的sLex表达(HECA452-反应性)的流式细胞术结果(MFI,平均值±SEM)(n=4个来源于瘦和肥胖受试者的MSC的样品)。

[0050] 图25:脂肪衍生MSC的细胞裂解物的Western印迹分析。对来自骨髓的MSC(BM-MSC)及来自瘦的和肥胖的脂肪组织(A-MSC)的MSC的裂解物用FTVII(+)进行外岩藻糖基化,或单独用缓冲液(-)处理,然后进行SDS-PAGE,并用HECA452或与抗人CD44mAb进行印迹。将造血细胞系KG1a(对照)和成纤维细胞系PIF的裂解物进行共电泳并印迹。KG1a细胞表达HCELL(HECA-452-反应性CD44,mw为~80kDa)。如图所示,FTVII处理导致在来自瘦和肥胖受试者的脂肪组织的PIF细胞和MSC上增强表达HCELL(HECA452反应性CD44)(上图);对在每个裂解物中表达CD44的染色显示在下面的HECA452印迹中。在唾液酸酶处理细胞后,HCELL(~80kDa HECA452反应带)的表达下降(下图),与HCELL表达的唾液酸酶敏感性(即,消除sLex)一致。要注意的是,HCELL可能以~80kDa的双带出现(参见下图中FTVII处理的A-MSC的特征),其反映不影响HCELL分子的E-选择素或L-选择素配体活性的核心CD44糖蛋白可变糖基化的。

[0051] 图26:灌注于TNF刺激的人脐静脉内皮细胞(HUVEC)上的MSC的平行板流动室研究的结果。如在限定的血液动力学剪切条件(达因/cm^2,显示于x轴上)测量的,来自瘦受试者的A-MSC(LA-MSC)和来自肥胖受试者的A-MSC(OA-MSC)的α(1,3)-外岩藻糖基化(FTVII处理)赋予TNF-刺激的HUVEC上显示的在E-选择素上的有效E-选择素结合;FTVII处理(HCELL+)的脂肪衍生MSC的E-选择素配体活性等同于FTVII处理的(HCELL+)骨髓衍生的MSC(BM-MSC,底图)。未处理的MSC在任何剪切应力水平,在HUVEC上都没有显著的结合相互作用。

[0052] 图27(A)-27(C):α(1,3)-外岩藻糖基化对多种白细胞的E-选择素结合的作用分析。(A)平行板流动室分析天然外周血白细胞与(在TNF刺激的人脐静脉内皮细胞(HUVEC)上表达)的内皮E-选择素的栓系和滚动(tethering and rolling)相互作用)。新鲜分离单核细胞、CD4T细胞、CD8T细胞和B细胞,然后以0.5-16达因/cm^2的流速将其灌注在TNF-刺激的HUVEC(即,TNF诱导HUVEC表达E-选择素)上,观察并记录细胞滚动,用于视频分析。单核细胞和CD4T细胞在剪切应力高达16达因/cm^2时显示出在TNF激活的HUVEC上的滚动粘附相互作用,而CD8和B细胞显示出最小的粘附性抗剪切粘附相互作用。要注意的是,单核细胞的滚动是CD4T细胞的3倍高。值为最少3次独立实验的平均值±SEM。(B)使用E-选择素-Ig嵌合体("E-Ig",E-选择素配体的探针(来自R&D Systems))(左)和mAb HECA452(右)对单核细胞、CD4和CD8T细胞及B细胞染色的代表性流式细胞术分布图。填充曲线表示同种型对照(或者,对于E-Ig染色为没有输入Ca^{2+}的情况下的E-选择素结合),开放曲线显示特异反应性(对于E-Ig,为存在输入Ca^{2+}时的结合)。(C)用HECA452mAb对未处理(黑实线)或蛋白酶(菠萝蛋白酶)处理(虚线)的细胞染色并通过流式细胞术分析。蛋白酶(菠萝蛋白酶)处理(虚线)显著降低了单核细胞,CD4T细胞,CD8T细胞和B细胞的HECA452染色,表明糖蛋白是这些细胞上sLex决定簇的主要载体。

[0053] 图28(A)-28(C):通过α(1,3)-外岩藻糖基化处理增加单核细胞和淋巴细胞上的功

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能性E-选择素配体表达。(A)将人单核细胞进行FTVII处理或缓冲液处理(空白对照),并用E-选择素-Ig嵌合体("E-Ig",E-selectin配体的探针(来自R&D Systems))进行免疫沉淀,然后进行SDS-PAGE并分别使用HECA-452、抗CD43、抗CD44和抗PSGL-1mAb进行印迹。注意到FTVII处理单核细胞后用E-Ig的免疫沉淀增加,主要在CD44(即产生HCELL)和CD43(即产生CD43-E-选择素配体("CD43-E"))上。(B)对来自CD4T细胞,CD8T细胞和B细胞的裂解物的PSGL-1,CD43和CD44的顺序免疫沉淀进行Western印迹分析,随后用E-Ig进行染色。(C)平行板流动室分析未处理的("unt")和FTVII处理的(即,α(1,3)-外岩藻糖基化;"FT7")的单核细胞,CD4和CD8T细胞,以及B细胞对TNF-α激活的HUVEC(其表达E-选择素)的栓系和滚动相互作用。在所有测试的剪切应力水平下,FTVII处理("FT7")显著增强了流动细胞对HUVEC的E-选择素介导的粘附。

[0054] 图29(A)-29(C):由单核细胞衍生的树突细胞(即,通过CD14表达选择单核细胞后培养的树突细胞(CD14-S mo-DC))表达的CD44结合E-选择素。(A)mo-DC的HECA-452mAb及E-Ig反应性染色的代表性流式细胞分布图。灰线代表同种型对照,或者对于E-Ig是不存在Ca2+的情况下的染色,而虚线表示PA-S mo-DC,并且黑实线表示CD14-S mo-DC。(B)对CD14-S和PA-S mo-DC裂解物的E-选择素染色的Western印迹分析。在SDS-PAGE凝胶上解析相当于2×10^6mo-DC的全细胞裂解物并进行免疫印迹。MW标记沿Y轴(kDa)。在CD14珠子上培养的Mo-DC(CD14-S Mo-DC)比在塑料上培养的那些(PA Mo-DC)具有更高的HCELL表达(~80kDa条带)。(C)对来自CD14-S和PA-S mo-DC的细胞裂解物的CD44免疫沉淀物进行Western印迹分析。然后用E-Ig嵌合体或抗CD44 mAb对CD44免疫沉淀物(CD44IP)进行印迹并染色。MW标记沿着Y轴(以kDa表示)标注。注意CD14-S mo-DC上有显著的HCELL表达。

[0055] 图30(A)-30(E):人mo-DC的α(1,3)-外岩藻糖基化化促进更高的E-选择素配体活性,并且内皮E-选择素表达促进外岩藻糖基化的DC的穿内皮迁移(TEM)。(A)比较2×10^6个KG1a细胞和mo-DC的裂解物的E-选择素结合(E-Ig)的Western印迹分析。(B)经缓冲液处理的(-)或FTVI处理的(+)mo-DC的E-Ig反应性的Western印迹分析。FTVI处理在~80kDa诱导E-Ig结合,表明HCELL的增强表达。(C)在剪切流动条件(2达因/cm^2),缓冲液处理的(BT)和FTVI处理的mo-DC在TNF-α刺激的HUVEC上的穿内皮迁移(TEM)测定。TEM值是在TNF-α刺激的HUVEC上的细胞迁移与在未处理的HUVEC上的迁移细胞(这是最小迁移)相比的升高倍数。在不同条件下分析TEM:用功能阻断性抗E-选择素mAb克隆68-5H11预孵育的HUVEC,用功能阻断抗VLA-4mAb HP2/1或同种型mAb预孵育的mo-DC和用唾液酸酶或百日咳毒素(PTX)处理的mo-DC。如图所示,外岩藻糖基化的DC比未处理的细胞具有更高的TEM;通过E-选择素和VLA-4的功能阻断抗体以及通过唾液酸酶或PTX处理细胞消除了TEM。(D)在TEM测定中分析栓系和滚动mo-DC的数量(每cm^2内皮表面积)和牢固粘附细胞的数量(每cm^2内皮表面积)。要注意的是外岩藻糖基化的DC具有更高的栓系和滚动相互作用。(E)在TEM测定中缓冲液处理的(BT)和FTVI处理的mo-DC的平均滚动速率。在2.0达因/cm^2剪切应力,对细胞数量进行计数(即,在2.0达因/cm^2剪切应力,灌注在TNF-α刺激的HUVEC上)。值为平均±SD(n=3)。统计学显着性差异($p<0.05$)由括号和星号表示。FTVI处理的DC的较慢滚动表明在血液动力学剪切条件,DC与内皮E-选择素的结合相互作用的效率更高。

[0056] 图31:通过存在针对CD3和CD28的抗体以及IL-2补充物下离体扩增的人CD4+细胞而生成的调节性T细胞上FoxP3的表达。双色流式细胞术分布图显示在培养的CD4+/低

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CD127-low T细胞上CD4和FoxP3的表达；高FoxP3染色表明所有培养扩增的细胞都是Treg。

[0057] 图32：缓冲液处理的(浅灰色填充)和外岩藻糖基化的(FTVII处理；黑色填充)Treg的sLex表达(HECA452染色)的流式细胞谱。要注意的是，缓冲液处理的Tregs没有sLex表达(即，流式细胞谱类似于同种型对照染色(虚线))，而FTVII处理的细胞显示高水平的sLex。

[0058] 图33：与人骨髓性白血病细胞系KG1a相比，FTVII处理的和缓冲液处理的(BT)Treg的E-Ig染色的Western印迹分析。缓冲液处理的T reg没有E-选择素配体。α(1,3)-外岩藻糖基化诱导了几种糖蛋白E-选择素配体在Treg上的表达，包括HCELL的表达(~80kDa条带)。KG1a细胞天然表达HCELL。

[0059] 图34：异种移植物抗宿主病(GVHD)中调节性T细胞给药的滴度。通过静脉注射1.5×10^5个Treg而不是通过注射相似剂量的缓冲液处理的Treg消除了异源GVHD相关的体重减轻(在注射PBMC后第50天测量)(重量值是N=3只小鼠的平均值)。然而，注射5×10^5个Treg防止GVHD体重减轻(N=3只小鼠的平均值)，不论施用的Treg细胞是否为α(1,3)-外岩藻糖基化。因此，Treg的α(1,3)-外岩藻糖基化在GVHD的免疫调制中的效力增加3倍。

[0060] 实施方案

[0061] 本公开针对治疗在受试者中表现为发炎的和/或受损的组织的疾病、障碍或医学病况或者癌症的方法。所述方法涉及对需要组织修复/再生或者癌症治疗的受试者施用细胞群体，所述细胞群体以超过细胞的天然群体的E-选择素和/或L-选择素结合水平的水平表达E-选择素配体和/或L-选择素配体。将细胞的组合物置于药物可接受的溶液、悬浮液、载体或媒介物中，用于施用于受试者或在施用于受试者之前进行储存(例如冷冻保存)。

[0062] 本文所描述的用于治疗适应症的细胞群体的施用可以以多种方式实现，在每种情况下如临床保证/指示的，使用多种解剖使用设备、多种施用设备以及多种解剖手段，可以支持或不支持解剖成像模式(例如放射学，MRI，超声等)或作图技术(例如表生理(epiphysiologic)作图程序、肌电图程序、电诊断程序等)。可以通过外周脉管通路(例如静脉内置入、外周静脉通路装置等)或中央脉管通路(例如中心静脉导管/装置，动脉通路装置/方法等)系统地施用细胞。可以使用基于导管的方法或其它脉管通路装置(例如心脏导管插入术等)将细胞通过脉管内地递送到预期组织位点的解剖供体血管中，所述血管通路装置会将细胞的脉管团输送到预期位点。可以将细胞引入椎管内和/或脑室内(即鞘内地进入蛛网膜下腔以分布在脑脊液内和/或心室内)。可以通过基于导管的方式或直接注射(例如腹膜内、胸膜内、心包内、囊内(例如进入膀胱、进入胆囊、进入骨髓、进入胆道系统(包括胆管和胰管网))、尿道内、经肾盂/输尿管内手段、阴道内等))将细胞直接施用于体腔或解剖腔室内。可以通过直接局部组织注射引入细胞，使用脉管内手段(例如心内膜注射)，或经皮手段，或通过手术暴露/接近组织，或通过腹腔镜/胸腔镜/内窥镜/结肠镜检查方法，或直接进入解剖学上可接近的组织位点和/或通过成像技术指导(例如关节内、进入脊椎盘和其他软骨、进入骨骼、进入肌肉、进入皮肤、进入结缔组织以及相关组织/器官如中枢神经系统、周围神经系统、心、肝、肾、脾等)。还可以将细胞直接放置在相关的组织表面/位点(例如直接放置在组织上、溃疡上、烧伤表面上、浆膜或粘膜表面上、心外膜上等)。还可以将细胞施用入组织或结构支持装置中(例如组织支架装置和/或包埋在放置在组织中的支架内)，和/或在凝胶中施用，和/或与增强剂一起施用(例如与支持细胞、细胞因子、生长因子、溶解素、抗炎剂等)。

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[0063] 在受试者的一个或多个组织内的脉管内皮上的E-选择素的诱导表达期间(即,与此同时)内(并且优选地在诱导表达开始同时),将细胞群体施用于受试者。E-选择素配体在施用的细胞表面的增强表达将有助于血管再形成、宿主防御(例如针对感染或癌症)和/或组织修复/再生和/或介导抑制炎症和/或预防炎症的免疫调制过程。E-选择素配体的表达通过介导血源性细胞与在炎症位点的内皮细胞上表达的脉管E-选择素的结合来指导将血管内施用的细胞递送到炎症位点。此外,无论细胞是系统性施用还是血管内施用进入椎管和/或脑室内(即鞘内进入蛛网膜下腔内以分布在脑脊液内)、直接进入体腔或隔室、通过直接局部组织注射或是通过放置在相关组织表面/位点上,E-选择素和/或L-选择素的配体在施用的细胞上的表达都促进细胞在受影响组织微环境中的沉积,靠近携带E-选择素的细胞(即内皮细胞)和/或携带L-选择素的细胞(即,白细胞)。因此,靶组织中施用的细胞的空间分布和定位是通过施用的细胞上的E-选择素和/或L-选择素配体的表达而得到调制的。

[0064] 具体而言,由于在施用的细胞上增强表达细胞表面E-选择素配体而造成的所需细胞类型在发生炎症的位点的定殖使得施用的细胞与E-选择素的结合增加,从而当直接注射到受影响部位时促进所需细胞的系统性递送和/或细胞的沉积。例如E选择素配体(例如HCELL、CD43-E、CLA/PSLG-1、ESL-1、NCAM-E等)的增强表达有利地能够将直接注射的细胞锚定在炎症、组织损伤或癌症位点的表达E-选择素的血管内。因此,本发明的方法提高了相关细胞在或向炎症、组织损伤或癌症位点处的递送效率,包括例如递送组织修复干细胞的能力、递送免疫调制细胞(例如间充质干细胞、T调节细胞、NK细胞、树突细胞等)的能力,以及递送免疫效应细胞以抵抗激发性炎症过程的能力或癌症的能力(例如在感染或恶性肿瘤的情况下,分别递送病原体特异性免疫效应T细胞或癌特异性细胞毒性T细胞或NK细胞);这样的免疫细胞(调节性T细胞、NK细胞、细胞毒性T细胞、树突细胞等)可以是抗原致敏的、肿瘤细胞致敏的、病毒致敏的及用其他手段来产生抗原特异性。类似地,L-选择素配体(例如HCELL、PSGL-1等)的增强表达有利地能够将直接注射的细胞锚定在炎症、组织损伤或癌症的细胞浸润位点内表达L-选择素的细胞内。

[0065] E-选择素配体在细胞表面上的增强表达将驱动细胞的脉管归巢到任何表达E-选择素的位点。在多种实施方案中,细胞群体表达造血细胞E-/L-选择素配体(HCELL)和/或神经细胞粘附分子E-选择素配体(NCAM-E)。可以由细胞表达的其它E-选择素配体包括例如CD43-E、ESL-1、PSGL-1和PSGL-1的CLA糖型。例如由于CD44是普遍表达的细胞膜蛋白,并且显示在"成人"和胚胎类型的干细胞/祖细胞群体上,通过离体聚糖工程化来修饰该蛋白的糖基化以产生HCELL(CD44糖型)表型的能力将驱动体内注射(例如血管内)(过继性转移)的细胞迁移到骨髓或任何表达E-选择素的组织/器官位点。因此,修饰的细胞可以用于治疗的背景,以实现多种生理和病理过程(仅举几种病况,包括例如骨疾病,免疫疾病,感染性疾病和癌症治疗剂)中的靶细胞迁移。

[0066] 还已经发现,即使在受试者的细胞群体不存在分化的情况下,也可以使用即时方法来治疗具有相关炎症的疾病、障碍或医学病况。也就是,无论所涉及的组织类型如何,施用的细胞在所述的炎症位点处都存在营养作用,而不需要持续植入和/或再灌注施用的细胞。这些营养作用包括促进血管再形成(例如VEGF)、促进组织修复(例如TGF-β)、免疫调制(例如IL-10)、刺激组织驻留祖细胞的生长/增殖(例如SCF,LIF等)及许多其他组织修复过程(例如递送线粒体至细胞)的细胞因子/生长因子的释放。此外,施用的细胞(例如Treg、

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MSC、树突细胞等)可具有有效的免疫调制特性,包括直接抑制活化的淋巴细胞(例如通过PDL-1的表达)。

[0067] 如图4(A)-4(H)所示例,并且如实施例中更详细描述的那样,相对于所述细胞的天然群体,修饰为具有增强的E-选择素和/或L-选择素结合活性的细胞群体具有增加的归巢、浸润和定殖在表达E-选择素的脉管系统附近的组织的能力。另外,在一个实施方案中,这样的细胞通过与内皮细胞上的E-选择素结合而沉积在炎症位点,或者在炎症位点内沉积入浸润性白细胞的位点(例如参见图4(F)和4(G))。此外,有益效果(例如疾病缓解)可通过肠道外递送或者通过直接注射到炎症位点或浸润性白细胞位点实现,甚至在施用的细胞沉积进入位点内后缺乏这些细胞的分化(或长期植入)的情况下(即瞬时植入足以递送一种或多种预期的生物学作用)。

[0068] 在某些实施方案中,在美国专利第8,084,236号中描述的用于聚糖工程化方法用于在活细胞群体中增强HCELL表达,如下文详述的,可以在这些细胞上诱导更高量的E-选择素配体表达以促进它们脉管递送至炎症位点,并且可以诱导更高量的E-选择素配体和L-选择素配体以促进细胞在受影响组织环境中的沉积。

[0069] 受试者的治疗是指在个体中减少或消除具有相关炎症的疾病、障碍或医学病况的临床症状,或者在个体中延缓或预防这种疾病、障碍或医学病况的临床症状的发作。例如治疗可以意味着将特征为急性和/或慢性炎症的病况的症状减少例如至少20%、至少25%、至少30%、至少35%、至少40%、至少45%、至少50%、至少55%、至少60%、至少65%、至少70%、至少75%、至少80%、至少85%、至少90%至少95%、或至少100%。与急性和慢性炎症相关的实际症状是众所周知的,并且可以由本领域普通技术人员通过考虑以下因素来确定,包括但不限于急性和/或慢性炎症的位置、急性和/或慢性炎症的原因、急性和/或慢性炎症的严重性和/或受急性和/或慢性炎症影响的组织或器官。本领域技术人员将知道与特定类型的急性和/或慢性炎症相关的适当症状或指标,并且将知道如何确定个体是否成为本文所公开的治疗的候选者。

[0070] 可以根据本文所述的方法治疗的受试者包括任何哺乳动物(例如人),例如能对疾病易感的哺乳动物。实例包括例如人、非人灵长类动物、牛、马、猪、绵羊、山羊、狗、猫或啮齿动物(如小鼠、大鼠、仓鼠、或豚鼠)。受试者可以是被诊断患有具有相关炎症的疾病、障碍或医学病况的受试者或者其他已知患有结果或症状是急性和/或慢性炎症的疾病、障碍或医学病况的受试者。在一些实施方案中,受试者可以是被诊断为或已知存在发展具有相关炎症的疾病、障碍或医学病况的风险。在某些实施方案中,可以根据受试者中已知疾病、障碍或医学病况(其结果或症状是急性和/或慢性炎症)来选择用于治疗的受试者。在一些实施方案中,可以基于受试者中具有相关炎症的疑似疾病、障碍或医学病况来选择用于治疗的受试者。在一些实施方案中,可以通过检测生物样品(例如尿、痰、全血、血清、粪便等或其任何组合)中的突变或其他异常来诊断疾病、障碍或医学病况。因此,至少部分地基于从受试者获得的至少一个样品(例如活组织检查样品或任何其他生物样品)中检测到突变、症状或其他异常的事实将修饰的细胞群体施用于受试者。在某些实施方案中,在受试者中不能检测或定位有糖尿病,多发性硬化或癌症(例如),而存在于至少一种生物样品中的糖尿病,多发性硬化或癌症相关的突变、症状或其它异常足以根据本文所述的方法将修饰的细胞施用于受试者。在一些实施方案中,可以施用组合物以防止疾病、障碍或医学病况如糖尿病、多

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发性硬化症或癌症的发展。但是在某些实施方案中，可以怀疑但尚未鉴定现有疾病、障碍或医学病况的存在，可以施用本公开的组合物以防止疾病、障碍或医学病况的进一步滋生或发展。施用的细胞可以衍生自自体来源(即，源自宿主本身)、同基因来源(来自同卵双胞胎)或同种异源来源(源自非遗传相同的供体)。

[0071] 施用时间安排

[0072] 如上文提到的，在受试者的内皮细胞诱导表达E选择素同时(优选与诱导表达的发生同时)，或在受影响的组织中预期增加的E-选择素表达之前(即，在递送E-选择素诱导性炎症刺激(例如放射疗法)之前或同时进行预防)或预期对随后有脉管E-选择素表达上调的病原体或疾病过程发生反应(例如暴露于感染因子或在其内源性再激活之前、在自发性免疫疾病明显发作之前、先于移植物抗宿主病(GVHD)发挥等)而将细胞群体施用于受试者。因此，例如当受试者(例如人类患者)向他/她的医生或医疗保健提供者呈现炎症或预期的炎性发炎的一种或多种症状时，可执行本文所述的治疗方法。对于患有例如糖尿病的受试者，这种初始症状可包括多尿、夜尿、体重减轻/增加和/或饱腹感不足，在此时(并且糖尿病酮症酸中毒发作之前)，可以根据本公开对受试者施用经修饰的细胞。对于患有例如多发性硬化症的受试者，这种初始症状可以包括短暂的感觉扰动(例如麻木，刺痛)和/或肌束震颤(抽搐)和/或肌无力阵发。

[0073] 通常，施用修饰的细胞的时间方面可以取决于例如具体修饰的细胞，或待处理疾病、障碍或医学病况(以及相关的炎症、组织损伤或癌症)的性质。其他考虑可能包括疾病、障碍或医疗病况的严重性；特定细胞或细胞片段的活性和所用的修饰；所用的具体组合物；受试者的年龄、体重、一般健康状况、性别和饮食习惯；施用时间、施用途径、和排泄速率；治疗的持续时间；与治疗方案组合使用或同时使用的药物；以及诸如此类的因素。

[0074] 因此，施用修饰的细胞可以作为单次事件或在整个治疗的时间过中程发生。例如可以按小时(例如每小时、每两小时、每三小时、每四小时、每五小时、每六小时等)、每天、每周、每两周、每两月或每月施用修饰的细胞。为了治疗急性病况，治疗的时间过程可以是例如至少几个小时、几天或几周。某些病况可将治疗从几天延长至数周、数月或数年。例如治疗可以延长一周、两周或三周或更长时间(例如一至几个月)。对于更多的慢性病况，治疗可能会延续数周至数月、延续一年或更多、或延续至需要治疗的患者的生命期。备选地，可将所述化合物和试剂作为预防性或作为治疗性措施每小时、每天、每周、每两周、每两月或每月施用，施用长达数周、数月、数年或患者的整个生命期。

[0075] 还将理解，基于疾病、障碍或医学病况(和相关炎症)的性质和严重性和/或疾病、障碍或医疗病况在受试者中的阶段，可以增加或减少给药频率。例如根据这些和其它因素，在治疗开始时可能需要较少频率的施用，并且在末期或接近末期阶段可能需要更高频率的施用，反之亦然。还可以追踪多种靶标和症状(例如糖尿病受试者的胰岛素水平)，并且相应地调整施用和给药剂量。

[0076] 如果需要，为了施用目的，可将细胞分成多个剂量，所以，单次给药组合物可包含这些量或其约数以构成剂量。

[0077] 适应症

[0078] 本公开针对治疗疾病、障碍或医学病况，其中E-选择素表达于受影响组织的内皮层和/或已经在受影响的组织中浸润/积累的表达L-选择素的白细胞中。如上所述，E-选择

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素和L-选择素各自结合唾液酸化、岩藻糖基化的碳水化合物，且这些唾液酸岩藻糖基化的聚糖结构在细胞表面上的增强表达作用于调控对这些选择素的结合。因此，本公开描述了通过增加在施用细胞上E-选择素配体的表达来增强向靶组织归巢的方法；另外，描述了在施用的细胞上增强有效的E-选择素和L-选择素配体(例如HCELL)的表达以促进对脉管内皮细胞上的E-选择素和/或受影响的组织内组织浸润性白细胞上的L-选择素的粘附的方法，本公开提供了增加细胞在预期产生生物效应的相关组织微环境内的定殖/沉积的方法。通常，本文描述的方法可用于改善任何基于细胞的治疗方法的结果，无论是在免疫治疗应用中(例如施用经培养扩增的抗原特异性T细胞和/或经培养扩增的NK细胞用于癌症或感染性疾病应用，施用经培养扩增的嵌合抗原受体(CAR)T细胞的，施用抗原致敏的树突细胞的等)、免疫调制/免疫抑制治疗应用(例如施用经培养扩增的调节性T细胞(Treg)、施用抗原致敏的树突细胞、施用间充质干细胞，施用经培养扩增的NKT细胞等)、或组织修复/再生医学应用(例如使用干细胞和/或祖细胞或其他组织修复细胞进行组织再生/恢复；使用经培养扩增的干细胞和/或经培养扩增的祖细胞用于组织再生/恢复)。在再生医学应用中的用途中，应当理解施用的细胞本身可以通过长期植入(伴随的增殖/分化)产生组织特异性细胞(例如在移植造血干细胞用于血细胞生产中)的方式有助于再生靶组织，且/或可以传递组织恢复/修复作用而不需要长期植入或分化成组织驻留的细胞(例如通过传递营养作用，刺激驻留的干细胞/祖细胞以修复损伤的组织和/或通过抑制促进损伤和阻碍修复的炎症过程)。用于本文所述的所有适应症的所有应用可以单独使用或与增强剂(例如生长因子，组织支架等)组合使用。

[0079] 可以根据本文所述的方法来治疗具有相关(例如急性和/或慢性)炎症、组织损伤/受损或瘤形成病况的任何和所有疾病、障碍或医学病况，包括但不限于由直接组织损伤(例如烧伤、创伤、骨折、骨畸形、褥疮溃疡等)、缺血/脉管事件(例如心肌梗死、中风、休克、出血、凝血病等)、感染(例如蜂窝组织炎、肺炎、脑膜炎、膀胱炎、败血症、SIRS等)、瘤形成(例如乳腺癌、肺癌、前列腺癌、肾细胞癌、淋巴瘤、白血病等)、免疫/自身免疫病况(例如急性或慢性GVHD、多发性硬化、糖尿病、炎性肠病(例如克罗恩病、溃疡性结肠炎)、类风湿关节炎、银屑病等)、退行性疾病(例如骨质疏松症、骨关节炎、脊椎椎间盘退变、阿尔茨海默病、动脉粥样硬化等)、先天性/继承性疾病(例如大疱性表皮松解、成骨不全、肌营养不良、溶酶体贮积病、亨廷顿氏病等)、药物不良反应(例如化疗诱导的组织/器官毒性、放射治疗毒性、药物诱发型肝炎、药物性心脏损伤等)、毒性损伤(例如辐射暴露、化学暴露、酒精性肝炎、酒精性胰腺炎、酒精性心肌病、可卡因心肌病等)、代谢紊乱(例如尿毒症性心包炎、代谢性酸中毒等)、医源性病症(例如放射诱导的组织损伤、手术相关并发症等)、和/或特发性过程(例如肌萎缩性侧索硬化、Parsonnage-Turner综合征等)引起的那些。

[0080] 可以根据本文所述的方法治疗的其它一般的和特定的疾病、障碍或医学病况包括但不限于：

[0081] 急性白血病，例如急性双相型白血病、急性淋巴细胞白血病(ALL)、急性髓细胞白血病(AML)和急性未分化白血病；

[0082] 骨髓增生异常综合征，例如淀粉样变性慢性骨髓单核细胞白血病(CMML)、顽固性贫血(RA)、顽固性贫血伴原始细胞增多(RAEB)、顽固性贫血伴原始细胞增多转化型(RAEB-T)和顽固性贫血伴环状铁粒幼细胞增多(RARS)；

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[0083] 骨髓增生性障碍，例如急性骨髓纤维化、特发性骨髓外化生(骨髓纤维化)、基础性血小板增多症、慢性骨髓性白血病和真性红细胞增多症；

[0084] 吞噬细胞障碍，例如Chediak-Higashi综合征、慢性肉芽肿病、白细胞粘附缺陷、髓过氧化物酶缺乏、嗜中性粒细胞肌动蛋白缺乏和网状组织发育不全；

[0085] 溶酶体储存疾病，例如肾上腺脑白质营养不良、阿尔法糖尿病、戈谢氏病、猎人综合征(MPS-II)、赫勒综合征(MPS-IH)、克拉伯病、马库拉-拉米综合征(MPS-VI)、Metachromatic Leukodystrophy、Morquio综合征(MPS-IV)、粘液性脂肪病II(I-细胞病)、粘多糖贮积症(MPS)、尼曼-皮克病、Sanfilippo综合征(MPS-III)、Scheie综合征(MPS-IS)、Sly综合征、β-葡糖苷酸酶缺乏症(MPS-VII)和沃尔曼病；

[0086] 继承性红细胞异常，例如β地中海贫血、黑斑-钻石贫血、纯红细胞增生和镰状细胞病；

[0087] 继承性血小板异常，例如巨核细胞生成减少/先天性血小板减少症、灰色血小板综合征；

[0088] 实体器官恶性肿瘤，例如脑肿瘤、尤因肉瘤、神经母细胞瘤、卵巢癌、肾细胞癌、肺癌、乳腺癌、胃癌、食管癌、皮肤癌、口腔癌、内分泌癌、肝癌、胆道系统癌、胰腺癌、前列腺癌和睾丸癌；

[0089] 其他应用，例如骨髓移植物、心脏病(心肌梗塞)、肝病、肌营养不良症、阿尔茨海默氏病、帕金森病、脊髓损伤、椎间盘疾病/变性、骨病、骨折、中风、外周血管疾病、头部创伤、大疱性疾病、线粒体疾病、离体和体内扩增的干细胞和祖细胞群体、体外受精应用和增强、造血拯救情况(增强疗/放疗)、源自多种组织来源的干细胞和祖细胞、在人类和动物的应用、以及肢体再生、重建型外科手术/适应症，可单独或与增强剂组合使用；

[0090] 慢性白血病，例如慢性淋巴细胞白血病(CLL)、慢性骨髓性白血病(CML)、幼年性慢性骨髓性白血病(JCML)和幼年性骨髓单核细胞白血病(JMML)；

[0091] 干细胞疾病，例如再生障碍性贫血(严重)、先天性血细胞减少症、先天性角化不良、范可尼贫血和阵发性夜间血红蛋白尿(PNH)；

[0092] 淋巴增生性障碍，例如霍奇金病、非霍奇金淋巴瘤和淋巴细胞性白血病；

[0093] 组织细胞障碍，例如家族性噬红细胞淋巴组织细胞增多症、血细胞吞噬作用、噬血细胞淋巴组织细胞增多症、组织细胞增多症X、和朗格汉斯细胞组织细胞增多症；

[0094] 先天性(继承性)免疫系统疾病，例如T细胞和B细胞缺乏、T细胞缺乏、正常B细胞SCID、共济失调毛细血管扩张症、巴尔-淋巴细胞综合征、普通变异性免疫缺陷症、DiGeorge综合征、Kostmann综合征、白细胞粘附缺乏症、欧门综合征、严重的综合性免疫缺陷(SCID)、SCID伴腺苷脱氨酶缺乏、Wiskott-Aldrich综合征和X连锁淋巴细胞增生性障碍；

[0095] 其他继承性障碍，例如软骨毛发发育不良、蜡样脂褐质沉积症、先天性红血球卟啉症、家族性地中海热、血小板无力症(Glanzmann Thrombasthenia)、Lesch-Nyhan综合征、骨质疏松症和桑德霍夫病；

[0096] 血浆细胞障碍，例如多发性骨髓瘤、血浆细胞白血病和瓦尔登斯特伦的巨球蛋白血症；和

[0097] 自身免疫疾病，例如多发性硬化、类风湿关节炎、系统性红斑狼疮、硬皮病、强直性脊柱炎、糖尿病和炎性肠病。

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[0098] 关节和骨骼疾病/病况，例如椎间盘退化、滑膜疾病、软骨退化、软骨创伤、软骨撕裂、关节炎、骨折、骨畸形、骨重建、成骨不全、先天性骨病/病况、遗传性骨病/病况、骨质疏松症、骨石化症、低磷酸酯酶症、代谢性骨病等。

[0099] 皮肤/软组织疾病和病况如大疱性疾病、银屑病、湿疹、大疱性表皮松解症、溃疡性皮肤病、软组织畸形(包括手术后皮肤和软组织畸形)、整形手术/重建手术适应症等。

[0100] 通常，相关的炎症症状包括但不限于发烧、疼痛、水肿、充血、红斑、瘀伤、压痛、僵硬、肿胀、发冷、呼吸窘迫、低血压、高血压、鼻塞、头闷、呼吸问题、体液潴留、血块、食欲不振、体重减轻、多尿、夜尿、无尿、呼吸困难、运动性呼吸困难、肌肉无力、感觉改变、心率增加、心率降低、心律失常、烦渴、肉芽肿形成、纤维蛋白、脓液、非粘性浆液或溃疡。与急性和/或慢性炎症相关的实际症状是众所周知的，并且可以由本领域普通技术人员通过考虑以下因素来确定，包括但不限于炎症的位置、炎症的原因、炎症的严重性、受影响的组织或器官以及相关疾病。

[0101] 在体内发生的具体情况下，例如当上皮表面发生炎症或涉及脓性细菌时，可观察到急性和/或慢性炎症的具体模式。例如肉芽肿性炎症是由有限但不同数量的疾病产生的肉芽肿形成所导致的炎症，所述疾病包括但不限于结核病、麻风病、结节病和梅毒。脓性炎症是导致大量脓液的炎症，其由嗜中性粒细胞、死细胞和流体组成。化脓性细菌如葡萄球菌的感染是这种炎症的特征。浆液性炎症是通常由浆液膜的间皮细胞产生的非粘性浆液的大量积液引起的炎症，但也可以来自血浆。皮肤水泡是这种炎症模式的一个示例。溃疡性炎症是由上皮表面的组织的坏死损失、暴露下层并形成溃疡而引起的炎症。

[0102] 急性和/或慢性炎症症状可以与引起多种疾病和障碍的大量不相关的障碍相关联。免疫系统通常涉及急性和/或慢性炎性障碍，在两种过敏反应、关节炎病况和一些肌病中都有证实，同时许多免疫系统疾病导致异常炎症。具有急性和/或慢性炎症过程的病因来源的非免疫性疾病包括癌症、动脉粥样硬化和缺血性心脏病。表现出急性和/或慢性炎症作为症状的障碍的非限制性实例包括但不限于痤疮、酸反流/胃灼热、年龄相关性黄斑变性(AMD)、过敏、过敏性鼻炎、阿尔茨海默氏病、肌萎缩性侧索硬化症、贫血、阑尾炎、动脉炎、关节炎、哮喘、动脉粥样硬化、自身免疫性障碍、扁桃腺炎、眼睑炎、细支气管炎、支气管炎、大疱性类天疱疮、烧伤、滑囊炎、癌症、心脏骤停、心肌炎、乳糜泻、蜂窝组织炎、宫颈炎、胆管炎、胆囊炎、绒毛膜羊膜炎、慢性阻塞性肺病(COPD)(和/或其急性加重)、肝硬化、结肠炎、充血性心力衰竭、结膜炎、药物诱导的组织损伤(例如环磷酰胺诱导的膀胱炎)、囊性纤维化、膀胱炎、感冒、泪腺炎、褥疮性溃疡、痴呆、皮炎、皮肌炎、糖尿病、糖尿病性神经病、糖尿病性视网膜病变、糖尿病肾病、糖尿病性溃疡、消化系统疾病、湿疹、肺气肿、脑炎、心内膜炎、内分泌病、子宫内膜炎、肠炎、小肠结肠炎、上髁炎、附睾炎、筋膜炎、纤维肌痛、纤维化、纤维组织炎、胃炎、胃肠炎、牙龈炎、肾小球性肾炎、舌炎、心脏病、心脏瓣膜功能障碍、肝炎、化脓性红斑狼疮、亨廷顿舞蹈病、高脂血症胰腺炎、高血压、回肠炎、感染、炎性肠病、炎性心脏病、炎性神经病、胰岛素抵抗、间质性膀胱炎、间质性肾炎、虹膜炎、缺血、缺血性心脏病、角膜炎、角膜结膜炎、喉炎、狼疮性肾炎、黄斑变性、乳腺炎、乳突炎、脑膜炎、代谢综合征(X综合征)、偏头痛、粘膜炎、多发性硬化、脊髓炎、心肌炎、肌炎、肾炎、神经炎、非酒精性脂肪性肝炎、肥胖症、脑炎、卵巢炎、睾丸炎、骨软骨炎、骨质减少症、骨髓炎、骨质疏松症、骨炎、中耳炎、胰腺炎、帕金森病、腮腺炎、盆腔炎、天疱疮、心包炎、腹膜炎、咽炎、静脉炎、胸膜炎、肺

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炎、多囊性肾炎、直肠炎、前列腺炎、银屑病、牙髓炎、肾盂肾炎、脑膜炎、辐射诱发的损伤、肾衰竭、再灌注损伤、视网膜炎、风湿发热、鼻炎、输卵管炎、结节病、唾液腺炎、鼻窦炎、痉挛性结肠、淤滞性皮炎、狭窄、口腔炎、中风、手术并发症、滑膜炎、腱炎、肌腱变性、腱鞘炎、血栓性静脉炎、甲状腺炎、扁桃体炎、创伤、创伤性脑损伤、移植排斥反应、三角炎、结核病、肿瘤、溃疡、尿道炎、尿炎、葡萄膜炎、阴道炎、脉管炎和外阴炎。

[0103] 可导致或以其他方式引起急性和/或慢性炎症的疾病、障碍和创伤的一般类别包括但不限于遗传性疾病、肿瘤形成、直接组织损伤、自身免疫性疾病、感染性疾病、脉管疾病/并发症(例如:注射/再灌注损伤)、医源性诱因(如药物不良反应、放射线损伤等)和过敏表现。

[0104] 在一个实施方案中,急性和/或慢性炎症包括组织炎症。一般来说,组织炎症是限于特定组织或器官的急性和/或慢性炎症。因此,例如组织炎症可以包括皮肤炎症、肌肉炎症、肌腱炎症、韧带炎症、骨炎症、软骨/关节炎症、肺部炎症、心脏炎症、肝脏炎症、胆囊炎症、胰腺炎症、肾脏炎症、膀胱炎症、牙龈炎症、食道炎症、胃炎症、肠道炎症、肛门炎症、直肠炎症、血管炎症、阴道炎症、子宫炎症、睾丸炎症、阴茎炎症、外阴炎症、神经元炎症、口腔炎症、眼部炎症、耳炎症、脑炎症、脑室/脑膜炎症和/或涉及中枢或周围神经系统细胞/元件的炎症。

[0105] 在另一个实施方案中,急性和/或慢性炎症包括系统性炎症。尽管所涉及的过程与组织炎症即使不是相同也是相似的,但全身炎症并不局限于特定组织,而是涉及身体内的多个位点,涉及上皮、内皮、神经组织、浆膜表面和器官系统。当由于感染时,可以使用术语败血症,菌血症专门用于细菌性败血症,病毒血症专门用于病毒性败血症。血管舒张和器官功能障碍是与广泛的感染相关的严重问题,可能导致败血性休克和死亡。

[0106] 在另一个实施方案中,急性和/或慢性炎症由关节炎引起。关节炎包括由滑膜炎症引起的对身体关节损伤的一组病况,包括例如骨关节炎、类风湿性关节炎、幼年特发性关节炎、脊椎关节病如强直性脊柱炎、反应性关节炎(Reiter综合征)、银屑病关节炎、与炎性肠病相关的肠病性关节炎、惠普病和白塞病、败血性关节炎、痛风(通常也称为痛风性关节炎、晶状体滑膜炎、代谢性关节炎)、假痛风(焦磷酸钙沉积病)和斯蒂尔病(Still's disease)。关节炎可影响单个关节(单关节炎)、两个到四个关节(寡关节炎)或者五个或更多个关节(多关节炎),可以为自身免疫疾病或非自身免疫疾病。

[0107] 在另一个实施方案中,急性和/或慢性炎症是由自身免疫性障碍引起的。根据每种疾病的主要临床病理特征,自身免疫性疾病可大致分为系统性和器官特异性自身免疫性障碍。系统性自身免疫性疾病包括例如系统性红斑狼疮(SLE)、干燥综合征、硬皮病、类风湿性关节炎和多发性肌炎。局部自身免疫性疾病可能是内分泌性(1型糖尿病、桥本氏甲状腺炎、艾迪生病等)、皮肤性(寻常型天疱疮)、血液性(自身免疫性溶血性贫血)、神经性(多发性硬化症))的或几乎可以涉及任何外接的身体组织实质。自身免疫性疾病的类型包括但不限于急性弥漫性脑脊髓炎(ADEM)、艾迪生病、过敏或敏感、肌萎缩性侧索硬化(ALS)、抗磷脂抗体综合征(APS)、关节炎、自身免疫性溶血性贫血、自身免疫性肝炎、自身免疫性内耳疾病、自身免疫性胰腺炎、大疱性类天疱疮、乳糜泻、查加斯病、慢性阻塞性肺疾病(COPD)(包括其急性加重)、1型糖尿病(IDDM)、子宫内膜异位症、纤维肌痛、古德帕斯特综合征、格雷夫斯病、吉兰巴利综合征(GBS)、桥本氏甲状腺炎、化脓性紫癜、特发性血小板减少性紫癜、炎性肠病

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(IBD)、间质性膀胱炎、狼疮(包括盘状红斑狼疮、药物性红斑狼疮、狼疮性肾炎、新生儿狼疮、亚急性皮肤型红斑狼疮和系统性红斑狼疮)、恶性肿瘤、多发性硬化症(MS)、重症肌无力、肌病、发作性睡眠症、神经肌肉瘤、寻常型天疱疮、恶性贫血、原发性胆汁性肝硬化、复发性弥漫性脑脊髓炎(多发性散布性脑脊髓炎)、风湿热、精神分裂症、硬皮病、干燥综合征、腱鞘炎、脉管炎和白癜风。在一个具体实施方案中,急性和/或慢性炎症是受试者的糖尿病的结果,或者由糖尿病引起。在另一个具体实施方案中,急性和/或慢性炎症是受试者的多发性硬化的结果或者由其引起。

[0108] 在另一个实施方案中,由肌病引起急性和/或慢性炎症。一般来说,当免疫系统不适当地攻击肌肉的组分而导致肌肉炎症时,会引起肌病。肌病包括例如炎性肌病和自身免疫性肌病。肌病包括例如皮肌炎、包涵体肌炎和多发性肌炎。

[0109] 在另一个实施方案中,急性和/或慢性炎症由脉管炎引起。脉管炎是一组各种各样的障碍,其特征在于由于白细胞迁移和由此造成的损伤所致的管壁的炎症,所述管壁包括淋巴管和血管如静脉(静脉炎)、动脉(动脉炎)和毛细血管。炎症可以影响体内任何地方的任何大小的血管。它可以影响动脉和/或静脉。炎症可以是局灶性的,意味着它影响管内的单一位置,或者可以是广泛的,炎症区域散布在特定器官或组织内,或甚至影响身体中一个以上的器官系统。脉管炎包括但不限于Buerger氏病(血栓闭塞性脉管炎)、脑脉管炎(中枢神经系统脉管炎)、ANCA相关性脉管炎、Churg-Strauss动脉炎、冷球蛋白血症、原发性冷球蛋白血症性脉管炎、巨细胞(颞)动脉炎、Golfer's脉管炎、过敏性紫癜(Henoch-Schonlein purpura)、超敏脉管炎(过敏性脉管炎)、川崎病、微观多动脉炎/多脉管炎、多动脉炎结节、风湿性多发性肌痛(PMR)、类风湿性脉管炎、Takayasu动脉炎、韦格纳肉芽肿病、和继发于结缔组织障碍的脉管炎如系统性红斑狼疮(SLE)、类风湿性关节炎(RA)、复发的多发性软骨炎、白塞病或其他结缔组织疾病的脉管炎、继发于病毒感染的脉管炎。

[0110] 在另一个实施方案中,急性和/或慢性炎症由皮肤障碍引起。皮肤疾病包括例如痤疮包括寻常痤疮、大疱性类血液、皮炎包括特应性皮炎和急性和/或慢性光化性皮炎、湿疹样特应性湿疹、接触性湿疹、干性湿疹、脂溢性皮炎、汗疱症、盘状湿疹、静脉湿疹、皮炎、疱疹样皮炎、神经性皮炎、和自体湿疹化、和瘀血性皮炎、糖尿病性皮肤并发症、化脓性汗腺炎、扁平苔藓、银屑病(包括斑块状银屑病、指甲银屑病、滴状银屑病、头皮银屑病、皮褶银屑病、脓疱性银屑病、红皮病银屑病、和银屑病关节炎)、红斑痤疮和硬皮病包括硬斑病、溃疡。

[0111] 在另一个实施方案中,急性和/或慢性炎症由胃肠道障碍引起。胃肠障碍包括例如肠易激综合征(IBD),炎性肠病包括克罗恩病,和溃疡性结肠炎如溃疡性直肠炎、左侧结肠炎、全肠炎和暴发性结肠炎。

[0112] 在另一个实施方案中,急性和/或慢性炎症由心血管疾病引起。当LDL胆固醇嵌入动脉壁时,可以引起免疫应答。急性和/或慢性炎症最终会损伤动脉,这可能导致它们爆裂。一般来说,心血管疾病是影响心脏本身和/或血管系统的大量特定疾病中的任何一种,特别是影响通向和来自心脏的静脉和动脉。有超过60种类型的心血管疾病,包括例如高血压、心内膜炎、心肌炎、心脏瓣膜功能障碍、充血性心力衰竭、心肌梗塞、糖尿病心脏病、血管炎症,如动脉炎、静脉炎、脉管炎;动脉闭塞性疾病,如动脉硬化和狭窄、炎性心脏病、外周动脉疾病;动脉瘤;栓塞;剥离;假性动脉瘤;脉管畸形;脉管痣;血栓形成;血栓性静脉炎;静脉曲张;中风。影响心脏的心血管障碍的症状包括但不限于胸痛或胸部不适(心绞痛),单臂或双

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臂、左肩、颈部、下颌或背部的疼痛，呼吸急促，头晕，心跳加快，恶心，心跳异常，感觉疲惫。影响大脑的心血管障碍的症状包括但不限于，脸部、手臂或腿部(特别是在身体的一侧的)的突然麻木或虚弱，突然意识混乱或者说话或语言理解困难，单眼或双眼视觉困难，突然眩晕，步行困难或失去平衡或协调，突发不明原因的严重头痛。影响腿、骨盆和/或手臂的心血管障碍的症状包括但不限于，跛行，其是肌肉中的痛苦、疼痛或抽筋以及脚或脚趾感到寒冷或麻木，尤其是在晚上。

[0113] 在另一个实施方案中，急性和/或慢性炎症由癌症诱导。一般来说，炎症协调肿瘤周围的微环境，有助于增殖、存活和迁移。例如纤维蛋白性炎症导致血管通透性的大幅增加，其允许纤维蛋白通过血管。如果存在适当的促凝刺激物，例如癌细胞，则纤维蛋白渗出物被沉积下来。这通常在浆液腔中观察到，其中可以在浆膜之间发生纤维蛋白渗出物向瘢痕的转化，这限制了浆膜的功能。在另一个实例中，癌症是炎性癌症，像NF-κB驱动的那种炎性癌症。

[0114] 在另一个实施方案中，急性和/或慢性炎症是药理学诱导的炎症。已知某些药物或外源化学化合物，包括关键维生素和矿物质的缺乏，可以发挥炎症作用。例如维生素A缺乏导致炎症应答增加，维生素C缺乏导致结缔组织病，维生素D缺乏导致骨质疏松症。某些药物可诱导炎性并发症，例如药物诱导的肝炎。某些非法药物如可卡因和迷幻药可能通过激活与炎症密切相关的转录因子(如NF-κB)而发挥其部分有害影响。放射治疗可以根据暴露和剂量的部位而诱导肺部毒性，烧伤，心肌炎，粘膜炎和其他组织损伤。

[0115] 在另一个实施方案中，急性和/或慢性炎症由感染引起。传染性生物体可以通过循环系统或淋巴系统而逃离直接组织的限制，通过这些系统它可以扩散到身体的其他部位。如果生物体不包含在急性炎症的作用范围内，则可以通过附近的淋巴管进入淋巴系统。淋巴管的感染称为淋巴管炎，淋巴结感染称为淋巴结炎。病原体可以通过淋巴引流进入循环系统而得以进入血流。感染包括但不限于细菌性膀胱炎、细菌性脑炎、大流行性流感、病毒性脑炎和病毒性肝炎(A,B和C型肝炎)。

[0116] 在另一个实施方案中，急性和/或慢性炎症由组织或器官损伤所诱导。组织或器官损伤包括但不限于烧伤、裂伤、伤口、穿刺或创伤。

[0117] 在另一个实施方案中，由移植排斥诱导急性和/或慢性炎症。当移植受体的身体不接受移植的器官或组织时，因为受体的免疫系统攻击移植的器官或组织而发生移植排斥。适应性免疫应答，移植排斥是通过T细胞介导的和体液免疫(抗体)的机制所介导的。移植排斥可归类为超急性排斥反应、急性排斥反应或慢性排斥反应。移植的器官或组织的急性和/或慢性排斥是由针对移植组织急性和/或慢性炎症的免疫应答造成的排斥，对其理解甚微。移植排斥还包括移植物抗宿主病(GVHD)，无论是急性还是慢性GVHD。GVHD是同种异源骨髓移植的常见并发症，其中移植的骨髓中的功能性免疫细胞将受体识别为“外来的”并进行免疫攻击。在某些输血的情况下也发生类似情况。GVHD分为急性和慢性型。急性和慢性GVHD似乎涉及不同的免疫细胞亚群，不同的细胞因子谱，有似乎不同的宿主目标，并对治疗有不同的反应。在另一个实施方案中，急性和/或慢性炎症由Th1介导的炎性疾病所诱导。在功能良好的免疫系统中，免疫应答应产生平衡良好的促炎Th1应答和抗炎Th2应答，适合于解决免疫激发的问题。一般来说，一旦启动了促炎Th1应答，身体依赖于由Th2应答引起的抗炎应答，以抵消此Th1应答。这种对抗性应答包括Th2型细胞因子的释放，所述细胞因子例如IL-

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4、IL-5和IL-13，其与特应性中促进IgE和嗜酸性应答相关，还有IL-10，其具有抗炎性应答。Th1介导的炎性疾病涉及导致急性和/或慢性炎症的由Th1细胞产生的过度促炎症应答。Th1介导的疾病可以是病毒、细菌或化学(例如环境)诱导的。例如引起Th1介导的疾病的病毒可以引起慢性或急性感染，这可以引起呼吸系统疾病或流感。

[0118] 在另一个实施方案中，急性和/或慢性炎症包括急性和/或慢性神经源性炎症。急性和/或慢性神经源性炎症是指通过从周围感觉神经末梢(即，传出功能，与向这些神经中向脊髓的正常的传入信号相对)释放的诸如SP或CGRP的炎症分子的释放而引发和/或维持的炎症应答。急性和/或慢性神经源性炎症包括原发性炎症和继发性神经源性炎症。原发性神经源性炎症是指由主要感觉神经末梢(例如C和A-δ纤维)释放物质而引起或引发的组织炎症(炎性症状)。继发性神经源性炎症是指由非神经元来源(例如来自脉管床或组织间质来源，例如来自肥大细胞或免疫细胞的外渗物)的炎症介质如肽或细胞因子而引发的组织炎症，所述炎症介质刺激感觉神经末梢并导致炎症介质从神经释放。急性和/或慢性神经源性炎症的两种形式(原发和继发)的净效应是通过周围感觉神经纤维的敏化保持炎症状态。所产生的急性和/或慢性神经源性炎症的生理学后果取决于出现问题的组织，产生例如皮肤疼痛(异常性疼痛、痛觉过敏)、关节疼痛和/或关节炎、内脏痛和功能障碍、肺功能障碍(哮喘、COPD)和膀胱功能障碍(疼痛、膀胱过动症)。

[0119] 施用途径

[0120] 可以根据多种合适的施用途径将表达E-选择素和/或L-选择素配体的细胞群体施用于受试者。示例性方法可以包括脉管注射，例如静脉内(iv)注射，或将细胞直接植入受试者的靶位点。

[0121] 其他递送方法可以包括气管内递送、鞘内递送、骨内递送、肺部递送、颊部递送、气雾剂递送、吸入递送和口服递送。还有其他方法可以包括动脉内递送、脑内递送、肠内递送、心内递送、皮下递送、肌内递送、眶内递送、囊内递送、脊柱内递送、腹膜内递送、胸骨内递送、膀胱内递送、淋巴内递送、腔内递送、阴道递送、直肠递送、经尿道递送、皮内递送、眼内递送、经耳递送、乳房内递送、原位递送、气管内递送、病灶内递送、经皮递送、内窥镜递送、经粘膜递送、舌下递送、和直接应用于身体表面(例如直接应用于皮肤表面)。

[0122] 可以将细胞插入递送装置中，所述递送装置促进由注射或植入方式向受试者中的引入。这种递送装置可以包括用于将细胞和流体注入受体受试者体内的管，例如导管。这种递送装置还可以包括内窥镜递送装置和方法。优选地，所述管另外还具有针头，例如注射器，通过其可以将本公开的细胞引入到受试者中所期望的位置。

[0123] 可以在操作/步骤之后递送细胞以增强E-选凝素和/或L-选择素结合活性，或者在一些实施方案中，可以将细胞冻存并在施用于受试者之前解冻。可以将细胞制备成以多种不同形式进行递送。例如当包含在这种递送装置中时，细胞可以悬浮在溶液或凝胶中或者包埋在支持基质中。可以将细胞与本公开的细胞在其中能保持活力的药学上可接受的载体或稀释剂混合。药学上可接受的载体和稀释剂包括盐水、水性缓冲溶液、溶剂和/或分散介质。这些载体和稀释剂的用途是本领域公知的。所述溶液优选是无菌和液体。优选地，所述溶液在制造和储存条件下稳定，并可通过使用例如对羟基苯甲酸酯、氯丁醇、苯酚、抗坏血酸、硫柳汞等进行保存使其能对抗微生物如细菌和真菌的污染作用。可以通过将本文所述的细胞并入药学上可接受的载体或稀释剂中，并且根据需要，并入其它成分，然后进行过滤

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灭菌来制备溶液。

[0124] 经由针或用于细胞施用的脉管辅助装置(例如基于导管)注射装置的血管内递送也可以用于实现通过整个脉管系统的分布或使其分布于特定器官的脉管系统,所述器官例如肝、或肾或任何其他器官。这包括针对脉管系统的非特异性靶向。备选地,可以通过选择特定的注射位点(例如肝门静脉)来靶向任何器官。备选地,可以系统地注射进入体内的任何静脉。该方法对于增强老年患者的干细胞数量是有用的。此外,细胞可以起到填充空缺的干细胞生态位或产生新的干细胞来更新器官,从而改善器官功能的作用。例如细胞可以占据脉管系统内的细胞周边位置。

[0125] 在一些实施方案中,将细胞作为细胞聚集体(例如胰岛)、组织或器官的一部分引入受试者,例如作为器官移植方法的一部分引入。

[0126] 细胞的递送也可用于靶向活性血管生成的位点。例如内皮祖细胞或间充质干细胞或祖细胞的递送可增强伤口位点的血管生成应答。对血管生成的靶向也可用于使用细胞作为媒介物将药物靶向肿瘤。

[0127] 经修饰的细胞及其制备方法

[0128] 至少部分地由于其增加的归巢能力,某些E-和/或L-选择素表达细胞可用于本文所述的方法中。例如细胞可以改善临床移植中的干细胞(例如HSPC)的植入,用于在基于细胞(例如用于骨疾病)的治疗中使用MSC,或引导祖细胞/干细胞在损伤/受损组织的迁移和浸润以根据本文所述的方法进行再生治疗。

[0129] 因此,本文所述方法中使用的细胞表达E-选择素配体和/或L-选择素配体。因此,例如在修饰后,细胞结合E-选择素和/或L-选择素。在多个实施方案中,修饰的细胞可以结合P-选择素。在一个具体实施方案中,修饰后细胞表达称为造血细胞E-/L-选择素配体(HCELL)的唾液酸岩藻糖基化的CD44糖型。在另一个具体实施方案中,修饰后细胞表达神经细胞粘附分子E-选择素配体(NCAM-E)。在某些实施方案中,修饰后细胞表达HCELL和NCAM-E。在某些实施方案中,细胞表达HCELL和/或CD43-E和/或CLA。修饰后,细胞能够在体内归巢到骨髓和/或炎症位点。

[0130] 在某些实施方案中,用于本文所述方法的细胞表达L-选择素配体。在一个具体实施方案中,修饰后细胞表达有效的L-选择素配体,称为造血细胞E-/L-选择素配体(HCELL),唾液酸岩藻糖基化的CD44糖型。在另一个实施方案中,细胞表达增加量的L-选择素配体HCELL和/或PSGL-1或E-选择素配体HCELL、CLA、CD43-E、NCAM-E或ESL-1。这些细胞可以通过与内皮细胞上的E-选择素结合或通过与在炎性位点内的浸润性白细胞上表达的L-选择素结合而沉积入炎症位点。

[0131] 对于使用糖基转移酶进行活细胞表面聚糖的离体定制工程化,在处理后经处理的细胞必须保持活力并保留表型。先前,对于α(1,3)-岩藻糖基转移酶而言,二价金属辅因子如锰已被认为对于酶活性是至关重要的,而使用这些辅因子的水平具有明显的细胞毒性(例如10mM Mn^{++})。然而,现在已知这些糖基转移酶在不存在或存在相对少量的二价金属辅因子(例如二价阳离子如锰、镁、钙、锌、钴或镍)的情况下具有酶活性。此外,这些酶可以在不存在本身对细胞有毒性的稳定剂如甘油的情况下储存。描述于美国专利第7,875,585号的某些糖基转移酶组合物特别可用于在活细胞进行聚糖的修饰,则该细胞和/或其颗粒或片段可用于本文所述的治疗方法中。在使用干细胞的应用中,分析沿着特征谱系的分化是

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否受酶处理的影响也很重要。

[0132] 可以使用多种方法来制备结合E-选择素和/或L-选择素的细胞。

[0133] 例如美国专利第7,875,585和8,084,236号提供了用于在活细胞上离体修饰细胞表面聚糖的组合物和方法，后者进而可用于治疗如本文所述的急性或慢性炎症的方法。所述组合物包括纯化的糖基转移酶多肽和生理上可接受的溶液，与适当的供体核苷酸糖一起用于反应缓冲液并用于特异性配制以保留细胞活力的反应条件下。在某些优选实施方案中，生理上可接受的溶液是不含或基本上不含二价金属辅因子，以细胞活力不受影响为度。在这些和其它优选的实施方案中，组合物也是不含或基本上不含稳定剂化合物，例如甘油。糖基转移酶包括例如岩藻糖基转移酶、半乳糖基转移酶、唾液酸转移酶和N-乙酰葡糖胺基转移酶。在一个实施方案中，岩藻糖基转移酶是α1,3岩藻糖基转移酶，例如α1,3岩藻糖基转移酶III、α1,3岩藻糖基转移酶IV、α1,3岩藻糖基转移酶V、α1,3岩藻糖基转移酶VI、α1,3岩藻糖基转移酶VII或α1,3岩藻糖基转移酶IX。唾液酸转移酶可以是ST3GalII、ST3GalIV或ST3GalVI。

[0134] 通过将细胞群体与具有上述一种或多种糖转移酶组合物接触对细胞表面上的聚糖进行修饰。在糖基转移酶具有酶活性的条件下，将细胞与糖转移酶组合物连同适当的核苷酸糖供体(例如GDP-岩藻糖、CMP-唾液酸)接触。根据该方法进行的聚糖修饰，可以在修饰后24小时或更久时产生具有至少70％、75％、80％、85％、90％、95％、96％、97％、98％、99％或更高活力的细胞。在一个实施方案中，例如细胞在治疗后48小时具有至少70％的活力。在一个这样的实施方案中，例如细胞在处理后48小时具有至少75％的活力。在一个实施方案中，例如细胞在处理后48小时具有至少80％的活力。此外，优选地，在处理后保留细胞的表型(除细胞表面修饰以外)。通过“保留表型”是指细胞保持其天然功能和/或活性。例如如果细胞是干细胞，它保留其潜能，即其相关的全能性或多能性或其多潜能性或单能性，如特定干细胞类型的特征。

[0135] 对用于本文所述治疗方法的修饰细胞的另一种方法可见于在美国专利申请公开号2013/0040837。根据其中所述的方法，可以将特异性结合非核酸靶标的核酸(例如适体)固定化在细胞膜上。可以针对特定靶标选择适体，例如能够结合特定蛋白质的适体。因此，例如可以将结合细胞表面受体/细胞粘附分子(例如E-选择素、L-选择素)的适体可被固定化在细胞表面上以改善细胞靶向性。可以使用三步修饰方法将适体缀合至细胞表面，包括(i)用磺化的生物素-N-羟基琥珀酰亚胺(NHS-生物素)处理细胞(在胰蛋白酶消化后的悬浮液中的)以在细胞表面引入生物素基团，(ii)与链霉抗生物素蛋白复合，和(iii)与生物素化的适体偶联。使用这种方法，Karp等人在US 2013/0040837中报道，通常使用该方法为每个细胞连接～21,000个分子，并且可以通过调节缀合中使用的适体浓度容易地调节细胞表面上的适体的位点密度。

[0136] 也可以通过共价加入结合选择素的sLex或sLea部分或其它寡糖复合物来诱导选择素结合的表达。例如如Karp等人在US2011/0206740中更完整地描述并示例的，使用生物素-链霉亲和素缀合化学地将唾液酸-Lewis^{X}部分并入细胞表面上。更具体地，使细胞表面上存在的游离胺基与生物素-N-羟基-琥珀酰亚胺的N-羟基-琥珀酰亚胺基团反应以将细胞表面进行生物素化。在该步骤后将细胞表面的生物素部分与链霉亲和素分子反应。生物素与链霉抗生物素蛋白之间的强相互作用使链霉亲和素分子固定化在细胞表面上。然后将链

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霉亲和素与生物素化的SLeX反应(唾液酸-Lex-PAA-生物素)以将SLeX引入细胞表面上。

[0137] 在细胞表面的聚糖工程化的备选手段中,使用岩藻糖基转移酶将sLex或sLea聚糖的整体组合并入到细胞表面上,如由Srivastava等人,J.Biol.Chem.1992,267:22356-22361所描述的。更具体而言,Srivastava等人报道,来自人奶的部分纯化的Le-FucT通常使用GDP-岩藻糖作为转移单个岩藻糖残基的供体,其也将转移在C-6上置换有非常大的空间要求的结构的岩藻糖残基。因此,岩藻糖基转移酶可用于转移聚糖如唾液酸-Lewis^{X},以增加细胞群体表面上的E-选择素配体和/或L-选择素配体的表达水平,使其超过的这种细胞的天然群体的表达水平。

[0138] 在另一个实施方案中,可以使用多种双功能化学连接子以共价连接sLex或sLea聚糖,或者sLex或sLea的糖模拟物,或者sLex或sLea的拟肽(例如可以通过噬菌体展示技术产生的)。类似地,结合E-选择素和/或L-选择素的mAb或其mAb片段可以缀合到细胞表面以分别增强与E-选择素和/或L-选择素的结合。

[0139] 除了上述之外,可以使用修饰一种细胞或多种细胞来表达E-选择素配体和/或L-选择素配体的任何其它方法。这包括促进例如在细胞表面和配体、噬菌体展示产物等之间的共价和/或非共价相互作用、氢键合和/或范德华力等。

[0140] 通常,可以在本文所述的治疗方法中修饰和使用任何类型的细胞。用于动物用途时,优选细胞是动物来源的,而对于人类用途来说,优选细胞是人细胞;在每种情况下,可以使用自体细胞来源,可以使用同基因来源细胞,可以使用异基因来源细胞(包括给定细胞产物中的同种异源供体细胞的组合),或可以利用异种细胞来源。细胞可以是原代细胞,例如原代肝细胞、原代神经元细胞、原代成肌细胞、原代间充质干细胞、原代祖细胞,或者其可以是已建立的细胞系或经培养扩增细胞的细胞(例如T细胞、树突细胞等)。细胞不必具有进行细胞分裂的能力;终末分化的细胞可用于本文所述的方法中。在这种情况下,细胞可以是任何细胞类型,包括但不限于胚胎干细胞、成体干细胞、诱导的多能干细胞、血液祖细胞、组织祖细胞、上皮、内皮、神经元、脂肪、心脏、骨骼肌、成纤维细胞、免疫细胞(例如树突细胞、单核细胞、巨噬细胞、白细胞(例如淋巴细胞如B淋巴细胞、T淋巴细胞或T淋巴细胞亚群、如调节性淋巴细胞($CD4^{+}/CD25^{+}/FOXP3^{+}$))、初始T细胞、中央记忆T细胞、效应记忆T细胞、效应T细胞、NK细胞等)、肝、脾、肺、循环血细胞、血小板、生殖细胞、胃肠细胞、肾细胞、骨髓细胞、心脏细胞、内皮细胞、内分泌细胞、皮肤细胞、肌肉细胞、神经元细胞和胰腺细胞。细胞可以是细胞系、干细胞(例如间充质干细胞、造血干细胞、组织干/祖细胞(例如神经干细胞、胃肠干细胞、肌细胞干细胞、心肌细胞祖细胞/干细胞、内皮祖细胞或肺干细胞)、脐带干细胞或胚胎干细胞、或分离自包括但不限于脑、肝、肺、肠、胃、脂肪、肌肉、睾丸、子宫、卵巢、皮肤、脾脏、内分泌器官和骨骼等的任何组织的原代细胞。

[0141] 当细胞保持在体外条件下时,可以使用常规的组织培养条件和方法,这些是本领域技术人员已知的。各种细胞的分离和培养方法在本领域技术人员的知识范围之内。

[0142] 此外,考虑将异质和均质的细胞群体均与本文所述的方法和组合物一起使用。此外,细胞的聚集物,附着或包封在颗粒内的细胞,可注射递送媒介物(例如水凝胶)内的细胞和附着于可移植的基质(包括支架)或应用于锚定支架/可移植的基底的组织中的细胞,都考虑与本文所述的方法和组合物一起使用。此外,细胞可以与组织增生/增强剂和/或抗炎剂(例如生长因子、细胞因子、前列腺素、营养因子、消退素(Resolvin)、NSAIDS、类固醇等)

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组合使用。

[0143] 本公开还进一步包括以下列举的实施方案。

[0144] 实施方案1.一种增强细胞递送到受试者的靶组织中和/或增强在受试者的靶组织中的组织定殖的方法，所述方法包括：向受试者施用表达E选择素配体和/或L-选择素配体的细胞的群体，所述群体表达E选择素配体和/或L-选择素配体的水平超过所述细胞的天然群体的表达水平，其中所述细胞施用与靶组织内的内皮细胞上的E选择素表达同时进行，和/或与靶组织内白细胞的积累同时进行。

[0145] 实施方案2.一种治疗在受试者中表现为发炎的和/或受损的组织的疾病、障碍或医学病况的方法，所述方法包括：向受试者施用表达E选择素配体和/或L-选择素配体的细胞的群体，所述群体表达E选择素配体和/或L-选择素配体的水平超过所述细胞的天然群体的表达水平，其中所述细胞施用与靶组织内的内皮细胞上的E选择素表达同时进行，和/或与靶组织内白细胞的积累同时进行，并且其中所述群体相对于所述施用的细胞的天然群体，在发炎的和/或受损的组织内表现出增强的定位和/或定殖。

[0146] 实施方案3.一种改善向受试者靶组织的细胞递送的方法，所述方法包括：经由脉管递送和/或经由直接组织注射(即直接进入受影响的组织位点)和/或鞘内和/或腔内和/或膀胱内和/或在解剖管道内(胆道系统、尿道系统等)和/或在组织上(即，放置在受影响的组织上)，向受试者施用表达E选择素配体和/或L-选择素配体的细胞的群体，所述群体表达E选择素配体和/或L-选择素配体的水平超过所述细胞的天然群体的表达水平，其中所述细胞施用与靶组织内的内皮细胞上的E选择素表达同时进行，和/或与靶组织内白细胞的积累同时进行，并且其中所述施用的细胞群体相对于所述细胞的天然群体，实现增强的归巢、定殖及植入中的一项或多项。

[0147] 实施方案4.一种增强在受试者的发炎的和/或受损的组织中的细胞递送和定殖的方法，所述方法包括：将表达E选择素配体和/或L-选择素配体的细胞的群体直接注射到所述发炎的和/或受损的组织内或放置在其上/内，所述群体表达E选择素配体和/或L-选择素配体的水平超过所述细胞的天然群体的表达水平，其中所述注射/放置与靶组织内的内皮细胞上的E选择素表达同时进行，和/或与靶组织内白细胞的积累同时进行。

[0148] 实施方案5.一种增强向受试者的靶组织中的细胞递送、定殖和/或植入方法，所述方法包括：通过所述受试者的脉管系统施用表达E选择素配体和/或L-选择素配体的细胞的群体，所述群体表达E选择素配体和/或L-选择素配体的水平超过所述细胞的天然群体的表达水平，其中所述施用与靶组织内的内皮细胞上的E选择素表达同时进行，和/或与靶组织内白细胞的积累同时进行。

[0149] 实施方案6.一种增强细胞群体在受试者的靶组织内的沉积、定殖和/或植入的方法，所述方法包括：将表达E选择素配体和/或L-选择素配体的细胞的群体直接注射到靶组织内或放置在靶组织上，所述群体表达E选择素配体和/或L-选择素配体的水平超过所述细胞的天然群体的表达水平，其中所述注射与靶组织内的内皮细胞上的E选择素表达同时进行，和/或与靶组织内白细胞的积累同时进行，并且其中所述群体相对于所述细胞的天然群体实现了增强的靶组织定殖。

[0150] 实施方案7.一种治疗在受试者中表现为发炎的和/或受损的组织的疾病、障碍或医学病况的方法，所述方法包括：向受试者施用表达E选择素配体和/或L-选择素配体的细

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胞的群体，所述群体表达E选择素配体和/或L-选择素配体的水平超过所述细胞的天然群体的表达水平，并且其中所述注射与发炎的和/或受损的组织内的内皮细胞上的E选择素表达同时进行，和/或与发炎的和/或受损的组织内白细胞的积累同时进行。

[0151] 实施方案8.一种治疗受试者的肿瘤/恶性疾病的方法，所述方法包括：向受试者施用表达E选择素配体和/或L-选择素配体的细胞的群体，所述群体表达E选择素配体和/或L-选择素配体的水平超过所述细胞的天然群体的表达水平，其中所述施用与具有肿瘤/恶性细胞的组织内的内皮细胞上的E选择素表达同时进行，和/或与具有肿瘤/恶性细胞的组织内白细胞的积累同时进行。

[0152] 实施方案9.表达E选择素配体和/或L-选择素配体的细胞的群体，在制备用于治疗在受试者中表现为发炎的和/或受损的组织的疾病、障碍或医学病况的药物中用途，所述治疗包括向受试者施用表达E选择素配体和/或L-选择素配体的细胞的群体，其中所述群体表达E选择素配体和/或L-选择素配体的水平超过所述细胞的天然群体的表达水平，其中所述治疗包括与发炎的和/或受损的组织内的内皮细胞上的E选择素表达同时，和/或与发炎的和/或受损的组织内白细胞的积累同时施用所述群体。

[0153] 实施方案10.表达E选择素配体和/或L-选择素配体的细胞的群体，在制备用于治疗肿瘤/恶性疾病的药物中的用途，所述治疗包括向受试者施用表达E选择素配体和/或L-选择素配体的细胞的群体，其中所述群体表达E选择素配体和/或L-选择素配体的水平超过所述细胞的天然群体的表达水平，其中所述治疗包括与肿瘤/恶性组织内的内皮细胞的E选择素表达同时，和/或与肿瘤/恶性受损组织内白细胞的积累同时施用所述群体。

[0154] 实施方案11.实施方案1-10中任一项的方法或群体，其中所述细胞施用与靶组织内的内皮细胞及具有L-选择素的白细胞浸润物上的E选择素表达同时进行。

[0155] 实施方案12.实施方案1-11中任一项的方法或群体，其中所述施用/注射在白细胞向某一/所述发炎的和/或受损的组织浸润期间进行。

[0156] 实施方案13.实施方案1-12中任一项的方法或群体，进一步包括所述施用的细胞的群体在某一/所述发炎的和/或受损的组织的微环境中的植入。

[0157] 实施方案14.实施方案1-13中任一项的方法或群体，其中通过细胞在某一/所述发炎的和/或受损的组织内的定殖来实现免疫调制作用。

[0158] 实施方案15.实施方案1-14中任一项的方法或群体，其中通过施用的细胞在某一/所述发炎的和/或受损的组织内的定殖来实现组织修复作用。

[0159] 实施方案16.实施方案1-15中任一项的方法或群体，其中通过施用的细胞在某一/所述发炎的和/或受损的组织内的递送/定殖来实现增强的宿主防御/免疫应答作用。

[0160] 实施方案17.实施方案1-16中任一项的方法或群体，其中通过施用的细胞在某一/所述肿瘤/恶性组织位点内的定殖来实现抗恶性肿瘤作用。

[0161] 实施方案18.实施方案1-17中任一项的方法或群体，其中所述施用/注射包括所述细胞群体的一次或一系列注射。

[0162] 实施方案19.实施方案1-18中任一项的方法或细胞的群体，其中所述施用/注射包括每日、每周、每两周、每月或每年注射所述细胞群体。

[0163] 实施方案20.实施方案1-9中任一项的方法或细胞的群体，还包括先前的施用/注射的免疫调制作用在受试者中降低时，进行一次或多次额外施用/注射所述细胞群体。

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[0164] 实施方案21.实施方案1-20中任一项的方法或细胞的群体，其中通过静脉内施用/注射所述细胞群体。

[0165] 实施方案22.实施方案1-21中任一项的方法或细胞的群体，其中通过直接注射到发炎和/或损伤组织而施用所述细胞群体。

[0166] 实施方案23.实施方案1-21中任一项的方法或细胞的群体，其中将所述细胞群体放置在发炎的组织和/或损伤的组织上。

[0167] 实施方案24.实施方案1-21中任一项的方法或细胞的群体，其中将所述细胞群体放置在某一/所述发炎的组织和/或损伤的组织上。

[0168] 实施方案25.实施方案1-21中任一项的方法或细胞的群体，其中所述细胞群体与其它增强剂、抗炎剂或组织支架/可移植装置/凝胶组合使用。

[0169] 实施方案26.实施方案1-25中任一项的方法或群体，其中所述细胞的群体表达E选择素配体和L-选择素配体。

[0170] 实施方案27.实施方案1-26中任一项的方法或群体，其中所述E-选择素配体和/或L-选择素配体选自造血细胞E-/L-选择素配体(HCELL)、神经细胞粘附分子E-选择素配体(NCAM-E)、CD43E、CLA和ESL-1中的一种或多种。

[0171] 实施方案28.实施方案1-27中任一项的方法或群体，其中所述E-选择素配体是造血细胞E-/L-选择素配体(HCELL)和/或神经细胞粘附分子E-选择素配体(NCAM-E)。

[0172] 实施方案29.实施方案1-28中任一项的方法或群体，其中所述E-选择素配体是HCELL。

[0173] 实施方案30.实施方案1-29中任一项的方法或群体，其中所述E-选择素配体是NCAM-E。

[0174] 实施方案31.实施方案1-30中任一项的方法或群体，其中所述L-选择素配体是HCELL。

[0175] 实施方案32.实施方案1-31中任一项的方法或群体，其中所述细胞群体包含以下中的一种或多种：上皮、内皮、神经元、脂肪、心脏、骨骼肌、成纤维细胞、和免疫细胞(例如树突细胞、单核细胞、巨噬细胞、白细胞(例如淋巴细胞如NK细胞、B淋巴细胞、T淋巴细胞或T淋巴细胞亚群，如调节性淋巴细胞(CD4+/CD25+/FOXP3+))、细胞毒性淋巴细胞等)、肝、脾、肺、循环血细胞、血小板、生殖细胞、胃肠、肾、骨髓、胰细胞、干细胞(例如间充质干细胞、造血干细胞、组织干/祖细胞(例如神经干细胞、肌细胞干细胞或肺干细胞)、脐带干细胞、或胚胎干细胞、或诱导的多能干细胞、或者衍生自胚胎干细胞或来自诱导的多能干细胞的分化的祖细胞、或衍生自成体干细胞的分化的祖细胞、或分离自任何组织(例如脑、肝、肺、肠、胃、脂肪、肌肉、睾丸、子宫、卵巢、皮肤、脾脏、内分泌器官和骨骼)的原代细胞、或经培养扩增的祖细胞群体、或经培养扩增的干细胞群体、或经培养扩增的原代细胞群体。

[0176] 方案33.实施方案1-32中任一项的方法或群体，其中所述细胞群体为间充质干细胞、造血干细胞、组织干/祖细胞、脐血干细胞或胚胎干细胞。

[0177] 实施方案34.实施方案1-33中任一项的方法或群体，其中所述细胞群体是白细胞(例如淋巴细胞如NK细胞、B淋巴细胞、T淋巴细胞或T淋巴细胞亚群，例如调节性淋巴细胞(CD4+/CD25+/FOXP3+))、细胞毒性T细胞等)。

[0178] 实施方案35.实施方案1-34中任一项的方法或群体，其中所述疾病、障碍或医学病

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况是以下的一种或多种：直接组织损伤（例如烧伤、创伤、褥疮溃疡等）、缺血/脉管事件（例如心肌梗塞、中风、休克、出血、凝血病等）、感染（例如蜂窝组织炎、肺炎、脑膜炎、败血症、SIRS等）、瘤形成（例如乳腺癌、肺癌、前列腺癌、淋巴瘤、白血病等）、免疫/自身免疫病症（例如移植物抗宿主病、多发性硬化、糖尿病、炎性肠病、红斑狼疮、类风湿关节炎、银屑病等）、退行性疾病（例如骨质疏松症、骨关节炎、阿尔茨海默氏病等）、先天性/遗传性疾病（例如大疱性表皮松解、成骨不全、肌营养不良、溶酶体贮积病、亨廷顿氏病等）、药物不良反应（例如药物诱导的肝炎、药物-诱导的心脏损伤等）、中毒性损伤（例如辐射照射、化学品接触、酒精性肝炎，酒精性胰腺炎、酒精性心肌病、可卡因心肌病等）、代谢紊乱（例如尿毒症性心包炎、代谢性酸中毒等）、医源性病况（例如辐射诱导的组织损伤、外科手术相关的并发症等），和/或特发性过程（例如肌萎缩性侧索硬化、Parsonnage-Turner综合征等）。

[0179] 实施方案36.实施方案1-35中任一项的方法或群体，其中所述疾病、障碍或医学病况是糖尿病。

[0180] 实施方案37.实施方案1-36中任一项的方法或群体，其中所述疾病、障碍或医学病况是多发性硬化。

[0181] 实施方案38.实施方案1-37中任一项的方法或群体，其中所述受试者是人、非人灵长类动物、小鼠、大鼠、狗、猫、马或牛。

[0182] 实施方案39.实施方案1-38中任一项的方法或群体，其中所述受试者是人类患者。

[0183] 实施方案40.实施方案1-39中任一项的方法或群体，其中对细胞群体处理以形成具有增强表达E-选择素和/或L-选择素配体的修饰的细胞群体，并且所述细胞群体在处理后24小时具有至少70％的活力。

[0184] 实施例41.实施方案1-40中任一项方法或群体，其中所述细胞群体在处理后24小时具有至少80％的活力。

[0185] 实施方案42.实施方案1-40中任一项方法或群体，其中所述细胞群体在处理后48小时具有至少70％的活力。

[0186] 实施方案43.实施方案1-42中任一项的方法或群体，其中在向受试者施用所述细胞群体时，所述细胞群体沉积在包含浸润性白细胞的组织内。

[0187] 实施方案44.实施方案43的方法或群体，其中所述施用是直接注射到组织内或放置在组织上。

[0188] 实施方案45.实施方案43的方法或群体，其中所述施用是经血管的。

[0189] 实施方案46.实施方案1-21或25-42中任一项的方法或群体，其中经由外周脉管通路或中央脉管通路而系统地施用所述群体。

[0190] 实施方案47.实施方案1-21或25-42中任一项的方法或群体，其中使用基于导管的方法或将细胞的脉管团块递送至预期位点的其它脉管通路装置，将所述群体经血管内施用到预期组织位点的解剖滋养层血管内。

[0191] 实施方案48.实施方案1-21或25-42中任一项的方法或群体，其中通过引入到椎管中和/或心室内施用所述群体。

[0192] 实施方案49.实施方案1-21或25-42中任一项的方法或群体，其中通过基于导管的方法或直接注射，将所述群体直接施用到体腔内。

[0193] 实施方案50.实施方案1-21或25-42中任一项的方法或群体，其中通过直接局部组

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织注射，使用脉管内途径，或经皮方法，或直接进入解剖学上可接近的组织部位和/或由成像技术引导而施用所述群体。

[0194] 实施方案51.实施方案1-21或25-42中任一项的方法或群体，其中通过直接放置在相关组织表面/位点上而施用所述群体。

[0195] 实施方案52.实施方案1-21或25-42中任一项的方法或群体，其中将所述群体施用到支架内或包埋在放置于组织中的支架内，和/或施用于凝胶中，和/或与增强剂一起施用。

[0196] 实施方案53.实施方案1-21或25-42中任一项的方法或群体，其中将所述群体施用到脑脊液内。

[0197] 在已经对本公开进行详细描述后，显而易见的是，在不脱离所附权利要求限定的本发明的范围的情况下，可以进行修饰和变化。此外，应当理解，本公开中的所有实施例都作为非限制性实施例提供。

实施例

[0198] 提供以下非限制性实施例以进一步说明本公开内容。本领域技术人员应当理解，在下文实施例中公开的技术代表了发明人已经发现在本公开的实践中运行良好的手段，并因此可以被认为构成其实践的模式示例。然而，根据本公开内容，本领域技术人员应当理解，在不脱离本公开的精神和范围的情况下，可以在所公开的具体实施例中进行许多改变，并且仍然获得相像或相似的结果。

[0199] 实施例1:α(1,3)-岩藻糖基转移酶处理脂肪衍生的间充质干细胞增强HCELL的表达。

[0200] 先前的研究表明，骨髓来源的间充质干细胞(MSC)可以通过α(1,3)-岩藻糖转移酶进行聚糖工程化，以产生有效的E-和L-选择素配体HCELL(R Sackstein等，Nature Medicine 14:181-187(2008))。为了确定是否可以在源自非骨髓来源的MSC上增强HCELL表达，我们分析了α(1,3)-外岩藻糖基化对脂肪来源的间充质干细胞的作用。为此，从瘦和肥胖受试者的脂肪抽吸材料中获得脂肪组织，并且如所述方法培养间充质干细胞(R Sackstein等人，Nature Medicine 14:181-187(2008))。在补充有FBS的MSC培养基(含有20％FBS和1％青霉素/链霉素的DMEM)或人血小板裂解物的MSC培养基(含有5％人血小板裂解物和1％青霉素/链霉素的DMEM)中，在正常含氧(21％O_2)和缺氧(<5％O_2)条件下以低密度(汇合度<60％)培养细胞。在汇合度达到60％时，对MSC传代，在第3-6代时收获MSC。然后用20ug/ml的岩藻糖基转移酶VII(FTVII;通过R&D Systems在无二价阳离子的悬浮液中制备)或用60mU/ml的岩藻糖基转移酶VI(R Sackstein等人，Nature Medicine 14:181-187(2008))处理MSC(20×10^6/ml)，每种情况下都在无二价阳离子的Hank's平衡盐溶液(HBSS)中进行，其含有10mM HEPES、0.1％人血清白蛋白和1mM GDP-岩藻糖(Sigma)，在37℃作用60分钟，而对照细胞(缓冲液处理的)在没有FTVII或没有FTVI的相同缓冲液中进行处理。通过使用碘化丙啶和膜联蛋白V染色的双激光流式细胞术进行细胞活力的常规评估。在使用FTVI或FTVII进行α(1,3)-外岩藻糖基化后24小时，细胞活力持续超过80％。参见图24-26。

[0201] 实施例2:α(1,3)-岩藻糖基转移酶处理人血白细胞诱导在所述细胞上的高水平E-选择素配体的活性并增强HCELL的表达。

[0202] 所需细胞(例如干细胞、祖细胞、白细胞等)表面上的E选择素配体(即，HCELL及其

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它E-选择素配体)的增强表达将赋予这些细胞在血管内向组织炎症/受损位点以及肿瘤/癌症浸润位点的提高的运输能力,这是基于受影响组织内的内皮细胞上的脉管E-选择素表达。另外,一旦外渗,在相关施用的细胞表面上HCELL(或其他增强的E-选择素/L-选择素配体)的表达将有助于促进施用的细胞在靶组织内的定殖,因为施用的细胞将在血管周围区域内通过E选择素配体对内皮细胞上表达的E-选择素的粘附而收集,另外,施用的细胞还将通过L-选择素配体对在浸润性白细胞表面展示的L-选择素的粘附而锚定在发炎的/受损的或癌组织微环境中。因此,在干细胞/祖细胞上及在特定的白细胞亚群(包括例如NK细胞、CD4T细胞、CD8T细胞、Treg、单核细胞、树突细胞和粒细胞、以及用于治疗目的的在培养物中扩增的相关白细胞(例如抗原特异性T细胞、扩增的NK细胞、嵌合抗原受体T细胞(CAR T细胞)等))上增强表达HCELL和其他E-选择素和/或L-选择素配体可以在过继细胞治疗剂(用于组织再生/修复和/或用于免疫治疗以增强免疫力或抑制免疫力)中得到控制。因此,关键的是,HCELL的增强表达将能够通过E-选择素依赖性内皮相互作用而有效地将血管内施用的细胞运送到靶位点,从而产生血源性细胞的募集,之后是E-选择素介导的和L-选择素介导的外渗细胞在离散的组织微环境中的沉积。此外,类似地,如果这样的细胞直接被注射到组织损伤/受损的受影响位点或癌症位点、HCELL和其他E-选择素/L-选择素配体的增强表达会促进E-选择素介导的和L-选择素介导的细胞在组织微环境中的沉积/定殖。

[0203] 为了分析细胞表面α(1,3)-外岩藻糖基化的分子靶标以及外岩藻糖基化对白细胞E-选择素和L-选择素配体活性的作用,在从柠檬酸盐处理的全血中获得的原代人外周血白细胞上进行研究。通过免疫磁珠分选(Miltenyi)将白细胞分离成单核细胞(CD14+细胞)、CD4+淋巴细胞、CD8+淋巴细胞和B细胞(CD19+细胞)。用20ug/ml的岩藻糖基转移酶VII(FTVII;通过R&D Systems在无二价阳离子的悬浮液中制备)或用60mU/ml的岩藻糖基转移酶VI(R Sackstein等人,Nature Medicine 14:181-187(2008))处理细胞,每种情况下都在无二价阳离子的Hank's平衡盐溶液(HBSS)中进行,其含有10mM HEPES、0.1%人血清白蛋白和1mM GDP-岩藻糖(Sigma),在37℃进行60分钟,而对照细胞(缓冲液处理的)在没有FTVII或没有FTVI的相同缓冲液中处理。通过使用碘化丙啶和膜联蛋白V染色的双激光流式细胞术进行细胞活力的常规评估。使用FTVI或FTVII进行外岩藻糖基化后24小时,细胞活力持续高于80%。参见图27(A)-27(C)和图28(A)-28(C)。

[0204] 实施例3:α(1,3)-岩藻糖基转移酶对人树突细胞的原代培养物的处理诱导在所述细胞上的高水平E-选择素配体活性并增强HCELL的表达。

[0205] 为了评估α(1,3)-外岩藻糖基化调制人树突细胞的E-选择素和L-选择素配体活性的能力,使用抗CD14包被的磁珠(Miltenyi Biotech)(CD14-S)或通过塑料粘附(PA-S)从人外周血单核细胞中分离单核细胞,然后以标准操作流程用细胞因子IL-4和GM-CSF培养6天以诱导分化为单核细胞衍生的树突细胞(mo-DC)。通过流式细胞术以CD14表达评估单核细胞纯度,并通过对BDCA-1、HLA-DR、CD80和CD86(各来自BD Biosciences)的表达进行染色来评价mo-DC的分化和成熟。然后用20ug/ml的岩藻糖基转移酶VII(FTVII;通过R&D Systems在无二价阳离子的悬浮液中制备)或用60mU/ml的岩藻糖基转移酶VI(R Sackstein等人,Nature Medicine 14:181-187(2008))处理mo-DC,每种情况下都在无二价阳离子的Hank's平衡盐溶液(HBSS)中进行,其含有10mM HEPES、0.1%人血清白蛋白和1mM GDP-岩藻糖(Sigma),在37℃进行60分钟,而对照细胞(缓冲液处理的)在没有FTVII或没有FTVI的相同

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缓冲液中处理。通过使用碘化丙啶和膜联蛋白V染色的双激光流式细胞术进行细胞活力的常规评估。使用FTVI或FTVII进行外岩藻糖基化后24小时，细胞活力持续高于80％。参见图29(A)-29(C)和图30(A)-30(E)。

[0206] 实施例4：α(1,3)-岩藻糖基转移酶处理人调节T细胞(Treg)的原代培养物诱导在所述细胞上高水平的E-选择素配体活性并增强HCELL的表达；施用HCELL+TREG抑制由自体细胞诱导的异源GVHD。

[0207] 增强免疫调制细胞(例如MSC和Treg)至炎症位点递送的能力有助于改善过继细胞治疗剂在免疫疾病(例如GVHD、类风湿性关节炎等)中和在对感染的旺盛炎症应答(例如败血症、暴发性病毒肝炎等)中抑制组织受损的潜力。为此，我们调查了α(1,3)-外岩藻糖基化对血管内施用的原代人Treg在人-小鼠GVHD模型中治疗异种移植物抗宿主病的能力的作用。我们尝试评估衍生自自体淋巴细胞来源的Treg诱导GVHD的能力，因为该策略将与临床造血干细胞移植相关(即，从供体造血/免疫细胞接种物扩增Treg，由此，将使用供体Treg治疗由供体免疫细胞诱导的GVHD)。为此，通过Ficoll梯度离心获得健康人供体全血的外周血单核细胞(PBMC)。使用阴选的磁性免疫球蛋白系统(Miltenyi)分离CD4-高/CD127-低T细胞，并在补充有IL-2的抗CD3/抗-CD28mAb的存在下在培养物中扩增，以产生CD4+/FoxP3+/CD25+细胞(Treg)。将来自相同供体受试者的未分级PBMC经腹膜内IP注射入NSG宿主小鼠(10^7个细胞/小鼠)。注射后14天(也即Treg扩增的第14天)，收集扩增的Treg细胞，然后用20ug/ml的岩藻糖基转移酶VII(FTVII；通过R&D Systems在无二价阳离子的悬浮液中制备)或用60mU/ml的岩藻糖基转移酶VI(R Sackstein等人，Nature Medicine 14:181-187(2008))处理，每种情况下都在无二价阳离子的Hank's平衡盐溶液(HBSS)中进行，其含有10mM HEPES、0.1％人血清白蛋白和1mM GDP-岩藻糖(Sigma)，在37℃进行60分钟，而对照细胞(缓冲液处理的)在没有FTVII或没有FTVI的相同缓冲液中处理(通过使用碘化丙啶和膜联蛋白V染色的双激光流式细胞术进行细胞活力的常规评估；使用FTVI或FTVII进行外岩藻糖基化后24小时，细胞活力持续高于80％)。然后，以2.5×10^5个细胞/小鼠的剂量，用缓冲液处理的(BT)Treg或用外岩藻糖基化的Treg血管内注射小鼠。然后临床跟踪小鼠，并在最初PBMC注射后的50天监测小鼠体重。如图所示的数据显示，人Treg的FTVII处理诱导E-选择素配体(包括HCELL)的表达。将外岩藻糖基化的自体衍生的Treg注射到具有异源GVHD的小鼠中(由先前施用自体PBMC诱导)导致GVHD的抑制，可通过体重减轻的反转而证明；外岩藻糖基化的Treg在逆转GVHD方面是非糖基化Treg的效力的3倍。参见图31-34。

[0208] 实施例5：用STAMPER-WOODRUFF淋巴细胞粘附测定法评估α(1,3)-岩藻糖基转移酶对MSC和人血淋巴细胞的处理诱导在细胞上的L-选择素配体活性

[0209] Stamper Woodruff淋巴细胞粘附测定法是用于评估L-选择素配体活性的常规手段(R.Sackstein,Immunol.Rev.230:140-163(2009))。这项测定是在20世纪70年代中期设计的，用以评价淋巴细胞与淋巴结高内皮小静脉结合的分子基础，测量活淋巴细胞表面上天然表达的L-选择素附着于在相关细胞表面上表达的其同源配体的能力。该测定在旋转剪切条件下进行，从而对结合相互作用产生离散的生物物理学限制：只有最有效的L-选择素配体(呈现在附着于玻璃的细胞表面上)将支持粘附在淋巴细胞的旋转悬浮液上显示的L-选择素。确实，强到足以在该测定中支持L-选择素介导结合淋巴细胞的仅有的L-选择素配体是HCELL及显示于淋巴结高内皮小静脉上的“内皮”L-选择素配体(这些L-选择素配体统

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称为地址素(addressin))(R.Sackstein,Immunol.Rev.230:140-163(2009))。

[0210] 为了评估由α(1,3)-外岩藻糖基化诱导的L-选择素配体活性的表达,对天然细胞、缓冲液处理的细胞和α(1,3)-外岩藻糖基化的细胞进行Stamper-Woodruff。简言之,经过细胞离心使相关细胞制备物置于玻片上,将从人血中新鲜制备的淋巴细胞悬浮液(RPMI 1640培养基中的10^7/ml的淋巴细胞)重叠加至所述玻片上。将玻片置于旋转平台上,在4℃及剪切作用(80rpm)孵育30分钟。然后将玻片在PBS中漂洗以除去非粘附淋巴细胞,在3%戊二醛中固定,并用甲基绿-硫素染色。通过光学显微镜检查玻片,观察淋巴细胞对经细胞离心的细胞的粘附情况。在250X放大倍数下通过使用的目镜网格对粘附到经细胞离心的细胞的汇合区域的淋巴细胞的数量进行计数来测量L-选择素配体活性。对具有粘附淋巴细胞的经细胞离心的细胞进行评分,并定量经细胞离心的细胞总区域内的这些细胞的百分比(如表1中"Key"栏所示)。

[0211] 表1.Stamper-Woodruff测定的结果

[0212] (细胞的L-选择素结合活性)

[0213] L选择素配体活性

[0214]

细胞类型	天然细胞	缓冲液处理细胞	α(1,3)-外岩藻糖基化
人 MSC	0	0	++++
人 CD4 T 细胞	+	+	++++
人 CD8 T 细胞	0	0	++
人 B 细胞	0	0	+
人 Treg 细胞	0	0	+++
人单核细胞	+	+	++++
人树突细胞	+	+	++++

KEY 0 <10%的细胞结合 L-选择素$^+$淋巴细胞
+ 10-30%的细胞结合 L-选择素$^+$淋巴细胞
++ 30-50%的细胞结合 L-选蛋白$^+$淋巴细胞
+++ 50-70%的细胞结合 L-选蛋白$^+$淋巴细胞
++++ >70%的细胞结合 L-选择素$^+$淋巴细胞

[0215] 如表1所示,天然MSC没有L-选择素结合活性,但是对MSC进行α(1,3)-外岩藻糖基化诱导巨大的L-选择素结合活性,基本上在所有外岩藻糖基化的细胞上都观察到L-选择素介导的淋巴细胞结合。人CD4T细胞、人单核细胞和人树状细胞各自展示出微弱的L-选择素结合活性,但是这些细胞在α(1,3)-外岩藻糖基化后能支持强力的L介导的淋巴细胞粘附。人Treg、B细胞和CD8细胞天生不具有L-选择素配体活性,但是可以通过α(1,3)-外岩藻糖基化诱导每种细胞类型对L-选择素的结合,其中外岩藻糖基化的Treg显示在L-选择素粘附上有显著增加。因此,在α(1,3)-外岩藻糖基化之后,基本上所有人白细胞上的L-选择素结合都有增强,具体而言,可以通过α(1,3)-外岩藻糖基化在人MSC、CD4T细胞、单核细胞、树突细胞和Treg上深度诱导L-选择素配体活性。在每种情况下,所述L-选择素结合活性反映了增强的HCELL表达,并且与对sLex/E-Ig染色的western杂交数据展示的HCELL在这些细胞上的表达结果相当,如图25、28(A)-28(C)、30(A)-30(E)和33所示。

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[0216] 实施例6-小鼠MSC上的HCELL表达允许胰腺向性并赋予NOD小鼠中自发性糖尿病产生持久逆转

[0217] 引言

[0218] 尽管控制血糖)的药物疗法有显著进展,但是1型糖尿病(T1D)仍然与显著的发病率和死亡率相关,并且继续造成重大的公共卫生负担,需要创新的治疗策略[1,2]。基于细胞的免疫调制疗法已经成为有希望治疗T1D的手段[3]。由于其免疫调制特性,安全性,易于获取以及强有力的离体扩增,间充质干细胞(MSC)已经成为治疗各种难治性免疫介导疾病包括T1D的发展最快的细胞疗法[4-7]。在使用NOD小鼠的临床前模型中,我们和其他人近期报道,系统施用的MSC可用于抑制自身免疫性糖尿病[8-13]。然而,MSC疗法在逆转高血糖上的益处是暂时的,这强调了紧迫的需求,需要开发策略以改善基于MSC疗法对T1D的有效性的[6]。

[0219] 免疫调制细胞疗法的效力与所输注的细胞运输至发炎组织的能力密切相关[14,15]。对于一些器官(如心脏),直接(局部)将细胞注入受影响的部位可以实现产生生理益处的必要定殖[16]。然而,对于T1D的治疗,必须通过脉管途径进行细胞递送,因为直接注射细胞至胰腺实质中会引发蛋白酶和其他可以诱导严重威胁生命的胰腺炎症的酶的释放。血源性细胞迁移到组织中是通过在靶组织内皮上的栓系和滚动的粘附相互作用起始的。这些结合相互作用最有效的介质是选择素,一个具有三个Ca^{++}依赖性凝集素的家族(E-、P-和L-选择素,也分别称为CD62E、CD62P和CD62L),其结合在其各自配体上表达的唾液酸岩藻糖基化的聚糖决定簇[17]。重要的是,在所有炎症位点的微脉管系统内,内皮选择素E-选择素可以响应炎性细胞因子如TNF-α而被诱导表达[17,18]。E-选择素与循环细胞上的膜糖蛋白和/或糖脂结合,这些膜糖蛋白和/或糖脂典型地展示有被称为"唾液酸化的Lewis X"(sLex)的唾液酸岩藻糖基化的四糖。然而,MSC不天然表达E-选择素配体[19]。这种运输上的缺陷限制了在静脉内施用后MSC在发炎的外周组织中的植入[17,20],限制了基于MSC疗法的效用。因此,我们尝试调查是否可以经由细胞表面的聚糖修饰增强E-选择素配体的表达而允许MSC运输到发炎的胰腺,以及这是否会对NOD小鼠中新发的自身糖尿病的(一种或多种)MSC疗效产生影响。我们的发现提供了关于MSC在糖尿病中的作用的生物学新见解,强调了在糖尿病NOD小鼠中增强表达E-选择素配体HCELL在增强小鼠MSC逆转高血糖能力中的独特和突出作用。

[0220] 材料和方法

[0221] 小鼠

[0222] C57BL/6,B6.129(Cg)-$Cd44^{tm1\ Hbg}$/J(CD57BL/6遗传背景$CD44^{-/-}$;CD44敲除("CD44-KO"))、BALB/c及NOD小鼠购自Jackson Laboratories购买并被安置和/或饲养在哈佛医学院动物护理和饲养楼的无病原体环境中。需要使用小鼠的实验由机构动物护理和使用委员会批准。

[0223] MSC培养

[0224] 如前所述从得到骨髓MSC[9,10]。简言之,将来自C57BL/6(野生型)或CD44-KO小鼠的分离的骨髓细胞在培养瓶中接种于由含有10%胎牛血清(Lonza)、10ng/ml成纤维细胞生长因子(PeproTech)、100U/ml青霉素和100ug/ml链霉素(Gibco)的Dulbecco's Modified Eagle培养基构成的培养基质中。培养第4至6代的MSC用于实验。

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[0225] MSC表征和分化

[0226] 通过使用针对细胞表面标记物Sca1、CD44、CD73、CD45、CD29和CD105以及相关同种型对照的抗小鼠抗体(全部来自eBioscience)进行流式细胞术对MSC进行表征。重组小鼠E-选择素(CD62E)-人Fc嵌合体购自R&D Systems。如前所述,测试MSC分化为多种中胚层谱系的分化能力[21,22]。简言之,在培养基质中用抗坏血酸(50μg/ml)和TGFβ1(1ng/ml)诱导MSC的软骨形成分化。培养3周后,用PBS洗涤平板,用4%多聚甲醛进行固定,并用0.05%的阿辛蓝进行染色用于软骨的光学显微镜观察。在培养基质中用抗坏血酸(50μg/ml)、β-甘油磷酸钠(10mM)和地塞米松(10nM)诱导成骨分化。两周后,洗涤平板,用4%多聚甲醛进行固定,并用2%茜素红进行染色以观察特征性钙沉积物。通过在补充有1%胎牛血清、1%谷氨酰胺、1%青霉素-链霉素的F12培养基中加入地塞米松(100nM)和胰岛素(6ng/ml)诱导成脂分化。用4%多聚甲醛固定细胞,并用于60%异丙醇中的0.3%的油红溶液进行染色,以评估含脂质的空泡。

[0227] MSC外岩藻糖基化

[0228] 在37℃用添加60mU/mL岩藻糖转移VI(FTVI)的反应缓冲液("FTVI-修饰的MSC")或仅由反应缓冲液(未修饰的MSC)对MSC悬浮液(10×10^6个MSC每200ul反应体系)处理90分钟,所述反应缓冲液由不含Ca^{2+}-和Mg^{2+}的Hank's平衡盐溶液(HBSS)构成,其含有20mM HEPES、0.1%人血清白蛋白和1mM GDP-岩藻糖。处理后,用含有0.2%HSA和20mM HEPES的HBSS洗涤MSC。通过台盼蓝排除和碘化丙啶染色评估细胞活力(脱离和处理后24小时持续>85%的活力)。通过流式细胞术评估FTVI修饰。FTVI酶由Roland Wohlgemuth博士(Sigma Chemical Corporation)提供。

[0229] Western印迹分析

[0230] 通过与含2%Nonidet P-40(NP-40)的缓冲液A(150mM NaCl、50mM Tris-HCl,pH 7.4、20mg/ml苯基甲基磺酰氟化物、0.02%叠氮化钠和蛋白酶抑制剂混合片(Roche Molecular Biochemicals))一起孵育来制备FTVI-修饰的和未修饰的MSC裂解物。全细胞裂解物或免疫沉淀蛋白质的所有Western印迹都在还原性条件下在7.5%SDS-PAGE凝胶上进行,如前所述[19],对每次Western印迹进行在每个泳道中裂解物的量以细胞数进行标准化。使用Lumi-Light Western Blotting Substrate(Roche)用化学发光显现杂交结果。

[0231] 流式细胞术和免疫沉淀研究

[0232] 如前所述进行流式细胞术[19]。有关更多详情,请参阅补充方法部分。

[0233] 平行板流动室粘附试验

[0234] 使用平行板流动室(250μm通道深度×5.0mm通道宽度)进行动态流动粘附测定,以如前所述的方法评估经刺激的人脐静脉内皮细胞(HUVEC)上的E-选择素介导的MSC结合[19]。有关详细信息,请参阅补充方法部分。

[0235] 用hGH病毒载体转染MSC

[0236] 如前所述,用含有hGH质粒构建体的慢病毒转染MSC[21]。通过酶联免疫吸附测定(Roche Diagnostics)测量MSC上清液和注射动物血清中hGH水平。

[0237] 用GFP病毒载体转染MSC

[0238] 如前所述,用含有GFP的逆转录病毒质粒转染MSC(GFP-MSC)[21]。通过荧光显微镜评估经转染的MSC的GFP的核周表达。在使用GFP-MSC评估归巢的补充手段中,用荧光染料

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CFSE标记MSC(非GFP转染的)。

[0239] 免疫荧光

[0240] 将胰腺包埋于Tissue Tek OCT中,冷冻,并在冷冻切片机中切片。使用Nikon E-1000外荧光显微镜(总放大倍数X400)获得免疫荧光图像。有关特定染色的详细信息,请参阅补充方法部分。

[0241] 丝裂原刺激CD3/CD28T细胞增殖试验

[0242] 使用抗CD3和抗CD28刺激的T细胞增殖测定用于评估FTVI修饰对MSC的免疫调制能力的效应。简言之,在滴定浓度(5×10^{2}-5×10^{4})并经辐照(3000rad)的未修饰和FTVI修饰的MSC存在下,用纯化的小鼠抗CD3e和抗CD28(每种为1μg/孔;eBioscience)对RPMI培养基(Lonza)中的1×10^{5}个NOD脾细胞刺激72小时,所述RPMI培养基补充有10%胎牛血清(Gemini Bio-Products)、1%青霉素链霉素(Lonza)和1%谷氨酰胺(Lonza)。在72小时孵育期的最后12小时内,用1μCi的氚标记的胸腺嘧啶对细胞进行脉冲,并通过闪烁计数测量淋巴细胞增殖(^{3}H-胸苷掺入)。结果报告为一式三份样品的平均值(平均cpm±SEM)。

[0243] 逆转NOD小鼠的新发高血糖研究

[0244] 在高血糖(>250mg葡萄糖/dL)第二天,以及高血糖发作后的第7天和30天,无麻醉地通过尾静脉将未修饰的和FTVI修饰的MSC(0.5×10^{6}个细胞每200ul)静脉内注射入雌性NOD小鼠。如先前所述通过尾静脉获得血液并测量葡萄糖以评估MSC施用对高血糖的效应[9,10]。

[0245] 统计学分析

[0246] 使用学生t检验和Mann Whitney检验分析来自实验测定和免疫组织学实验的数据。使用Kaplan-Meyer分析来评估存活数据。使用Prism软件(GraphPad Software,Inc.,San Diego,CA)进行分析并生成图形。P值<0.05被认为是显著的。数据表示平均值±SEM。

[0247] 补充方法

[0248] 流式细胞术和免疫沉淀研究

[0249] 如先前所描述的方法进行流式细胞术[19],使用生物素化的mAb HECA452(抗人皮肤淋巴细胞抗原,其识别SLeX;大鼠IgM(BioLegend公司)),和抗小鼠CD44mAb(KM114;大鼠的IgG1),纯化的抗-小鼠CD44mAb(IM7;大鼠IgG2b)和藻红蛋白(PE)标记的链霉抗生物素蛋白(均得自BD Pharmingen)。对于western印迹,将MSC裂解物与抗CD44免疫沉淀抗体(KM114/IM7)或适当的同种型对照孵育,然后用蛋白质G-琼脂糖(Invitrogen)收集。使用含有2%NP-40、1%SDS的缓冲液A将免疫沉淀物充分洗涤,然后进行SDS-PAGE,转移至聚偏二氟乙烯膜,并用HECA452或抗小鼠CD44抗体(KM114/IM7)进行免疫染色,然后用合适的HRP缀合的二抗孵育用于使用Lumi-Light Western Blotting Substrate(Roche)通过化学发光显现条带。

[0250] 平行板流动室粘附试验

[0251] 使用TNF-α(40ng/ml)对汇合的HUVEC刺激6小时以上调E-选择素表达。用0.005%胰蛋白酶/EDTA收获野生型(WT)和CD44KO MSC,并在37℃用单独的缓冲液(对照)处理或进行FTVI修饰,持续90分钟。然后将MSC以10^{6}个细胞/ml重新悬浮于补充有1mM氯化钙、1mM氯化镁、5mM HEPES和1mg/ml BSA的Hank's平衡盐溶液中。以0.5达因/cm^{2}将MSC灌注于HUVEC单层上,然后每隔3分钟经受1.0、2.0、5.0、10.0、20.0和30.0达因/cm^{2}的剪切应力水平。在

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小室的中心观察到与HUVEC(在视野内滚动或在10秒钟内滚动至视野内的细胞)相互作用的MSC,在每个剪切应力水平下最少观察6个视野。作为评估E-选择素对经刺激HUVEC结合的特异性的对照,用来自霍乱弧菌(Vibrio cholerae)的唾液酸酶(0.1U/ml;Roche)处理FTVI修饰的MSC,从sLex裂解末端唾液酸,并在功能阻断性抗人E型选择素mAb(10ug/ml)(BD Pharmingen)存在下评估粘附性。

[0252] 免疫荧光

[0253] 将组织以10um的厚度进行低温冷冻切片,并在冷丙酮中固定在载玻片上5分钟。干燥后,用1%BSA封闭切片,并在一抗中孵育过夜。将切片在Tris缓冲盐水(TBS)中洗涤3次,并与二抗孵育1小时。使用一抗大鼠抗小鼠CD62E(R&D Systems,1:100)和二抗PE缀合的山羊抗大鼠IgG(Southern Biotech,1:500)进行E-选择素(CD62E)染色。使用同种型匹配的大鼠mAb作为染色对照。使用大鼠抗小鼠CD31(Biocare,1:200)和二抗PE缀合的山羊抗大鼠IgG(Southern Biotech,1:500)进行CD31染色。对系列切片进行CD31和E-选择素共定位的评估。使用纯化的豚鼠抗胰岛素(Dako,1:5)和APC缀合的驴抗豚鼠IgG(H+L)(Jackson Immunoresearch,1:200)进行胰岛素染色。使用HECA452mAb(大鼠抗CLA IgM mAb(Biolegend,1:200))和FITC缀合的小鼠抗大鼠IgM(Biolegend,1:500)检测组织切片内的FTVI修饰的MSC。在切片上滴加单独的封片剂,或在有指出时使用含DAPI的封片剂(Vector Labs),盖上盖玻片,然后通过免疫荧光显微镜显现。

[0254] 结果

[0255] FTVI修饰的MSC的表达和功能分析

[0256] 来自C57BL/6小鼠的("野生型")、抗糖尿病品系、及CD44-KO小鼠(C57BL/6背景的$CD44^{-/-}$)的骨髓衍生MSC显示特征性的小纺锤体成纤维细胞样的形式,并可被分化为中胚层细胞类型(图7)。MSC表达MSC的特征性细胞表面标记物(图1(A))。MSC天然不表达E-配体,如不与mAb HECA 452(其识别经典的唾液酸岩藻糖基化的E-选择素结合决定簇(例如唾液酸化的Lewis X(sLe^x))反应,并且不与用作E-选择素配体活性的探针的E选择素-Ig嵌合体(E-Ig)结合而显示的(图1(B))。

[0257] 先前对人MSC的研究表明使用α-(1,3)-岩藻糖基转移酶VI(FTVI)的细胞表面糖工程化(即糖基转移酶程序化的立体置换(GPS))通过将天然的膜CD44转化为成称为造血细胞E-/L-选择素配体(HCELL)的分子(其为CD44的岩藻糖基化唾液酸乳糖基化糖变体,可有效地结合E-选择素)来诱导E-选择素的粘附性[19]。为了确定细胞表面外岩藻糖基化是否可以对小鼠MSC的E-选择素配体活性进行程序化(program),使用经优化以保持细胞活力的反应条件用FTVI处理细胞。MSC的FTVI修饰显著诱导了使用mAb HECA452的染色,以及与鼠E-选择素-Ig嵌合体(mE-Ig)的结合(图1(B))。值得注意的是,在FTVI修饰之前,MSC的蛋白酶消化显著降低了mE-Ig反应性,表明了糖蛋白而非糖脂是E-选择素结合决定簇的主要载体(图1(B))。在小鼠MSC的外岩藻糖基化后,全细胞裂解物(图1(C))和免疫沉淀的CD44(图1(D))的western印迹显示用E-Ig和HECA-452所识别的唾液酸岩藻糖基化所装饰的主要膜糖蛋白是"标准"(即不含剪接变体外显子)形式的CD44(~100kDa)。这些数据表明,FTVI修饰将鼠MSC表面CD44转化为HCELL。

[0258] FTVI修饰的小鼠MSC在生理剪切应力条件下具有增加的E-选择素结合

[0259] 为了评估FTVI修饰的小鼠MSC在生理剪切应力条件下结合E-选择素的能力,我们

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进行了未修饰的和FTVI修饰的MSC的平行板流室测定。为此，首先用TNF-α刺激HUVEC单层以上调E-选择素表达。如图2所示，FTVI修饰使得MSC能够在0.5达因/cm^2至高达20达因/cm^2的剪切应力水平对HUVEC粘附。在流动室研究中，MSC对经刺激HUVEC的粘附性严格依赖于E-选择素受体/配体相互作用，因为HUVEC与功能阻断性E-选择素mAb的孵育及MSC的唾液酸酶处理(以消除sLe^x展示)每种都将FTVI修饰的MSC的粘附极度地降低至与未经修饰的MSC相似的水平(图2)。总之，这些发现表明，小鼠MSC的FTVI修饰诱导有效的E-选择素结合活性，能够维持在血液动力学剪切应力水平的E-选择素粘附，远远超过后毛细血管小静脉的典型水平[17]。

[0260] 系统施用的FTVI修饰的MSC有效地逆转NOD小鼠的新发高血糖

[0261] 为了评估FTVI修饰是否影响MSC调制NOD小鼠的糖尿病的能力，新发糖尿病NOD小鼠不接受细胞(未处理的对照)或在高血糖(葡萄糖>250mg/dL)的第2天静脉内(通过尾静脉)接受5×10^5个FTVI修饰的或5×10^5个未修饰的C57BL/6MSC，然后在高血糖发作后第7和30天静脉内注射。如图3(A)所示，未处理的NOD小鼠显示其血糖水平迅速升高，除一只动物外，其在高血糖发作的几周内死亡。与施用未修饰的MSC(图3(B))相比(其导致自身免疫性糖尿病的临时逆转，即，9只小鼠中的3只在3周时为正常血糖，9只动物中的2只在6周时为正常血糖，所有动物在12周时均为高血糖)，输注FTVI-修饰的MSC强力持久地逆转高血糖(图3(C))。引起注意的是，8只小鼠中有7只持续3周为正常血糖，8只小鼠中6只持续6周为正常血糖，8只小鼠中的5只在初始治疗后12周无糖尿病(图3(C))。虽然血糖水平高于600mg/dl的未修饰和FTVI修饰的MSC组中的大多数小鼠存活多至90天，但未处理组(即不接受MSC)中仅有一只(7只小鼠中)幸存了90天。

[0262] NOD小鼠胰腺中的E-选择素表达和MSC定位

[0263] 为了检查FTVI修饰对MSC向胰腺中运输的作用，我们首先使用免疫荧光显微镜评估在14周龄的NOD小鼠的胰腺的微脉管系统中E-选择素的表达。据报道，胰岛微血管是导致胰岛炎的炎症细胞运输的主要位点[23,24]。在糖尿病抗性小鼠的胰腺中没有E-选择素表达(图4(A))，而NOD小鼠胰腺的胰岛周围微血管表达E-选择素(图4(B))，其与系列切片的CD31染色共定位(图4(C)和4(D))。通过糖尿病发作期间NOD胰腺的CD3染色证实T细胞浸润到糖尿病胰岛边缘(图4(E))。为了评估在NOD小鼠中系统施用的FTVI修饰的MSC的胰腺浸润，我们用抗体HECA452对胰腺的冷冻切片进行了染色。如图4(F)和4(G)所示，在移植后1天，在表达E-选择素的微血管附近的胰岛周围区域观察到$HECA452^+$MSC，其定殖于血管周围区域(E-选择素表达)和T细胞浸润区域(L选择素表达)。为了评估系统施用后FTVI修饰和未修饰的MSC的归巢程度，将GFP转染的FTVI修饰的和未修饰的MSC静脉内注射到NOD小鼠中，并在注射后第4天收获其胰腺、胰腺淋巴结和肠系膜淋巴结和脾，进行切片，并通过荧光显微镜评估MSC的存在。与未修饰的MSC相比，FTVI修饰的MSC显示出3倍高的胰腺浸润，而在耐糖尿病的BALB/c小鼠中没有观察到输注的FTVI修饰的和未修饰的MSC在胰腺浸润上的差异(图4(H))。然而，在经治疗的NOD小鼠的淋巴组织中没有观察到FTVI修饰的和未修饰的MSC的归巢的程度差异。

[0264] 外岩藻糖基化不影响MSC免疫抑制能力或体内存活

[0265] 我们之前已经报道，测量由hGH转导的MSC产生的人生长激素(hGH)是确定施用的MSC寿命的敏感方法[21]。为了评估所施用的FTVI修饰的MSC对高血糖的增强逆转是否是带

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来生存优势，在高血糖发作后第2天和第7天将5×10^5个hGH转导的FTVI修饰的和未修饰的MSC注射到NOD小鼠中，在注射后的多个时间点测量血清hGH。如图5(A)所示，hGH生产模式没有差异，说明FTVI修饰不影响体内MSC持久性。为评估外岩藻糖基化是否修饰MSC的免疫调制能力，我们进行共培养T细胞抑制测定。T细胞增殖同样受到FTVI修饰的和未修饰的MSC的抑制(图5(B))，表明外岩藻糖基化不赋予MSC额外的免疫调制特性。

[0266] MSC CD44不表达消除FTVI修饰的MSC的抗糖尿病作用

[0267] 为了检测CD44表达是否为FTVI修饰的MSC的增强的抗糖尿病效力的前提，我们从CD44 KO小鼠(C57BL/6遗传背景$CD44^{-/-}$)产生MSC。除CD44外，由CD44 KO小鼠骨髓产生的MSC显示相同水平的细胞表面粘附分子。通过流式细胞术，外岩藻糖基化的CD44 KO MSC显示与外岩藻糖基化的野生型MSC相似的HECA452-反应性(图8)，表明唾液酸岩藻糖基化的决定簇是在备选的(即非CD44)支架上产生的。为了评估CD44 KO MSC的施用是否赋予抗糖尿病活性，在高血糖发生后第2、7和30天分别将5×10^5个FTVI修饰的和未修饰的CD44 KO和C57BL/6野生型MSC注射到新发糖尿病NOD小鼠中。与野生型MSC相比，MSC的CD44缺乏导致其抗糖尿病作用的受到显著损害，这通过在接受未修饰的CD44 KO MSC的6只小鼠中有5只以及接受FTVI修饰的CD44 KO MSC的7只小鼠中有6只无法逆转自身免疫性糖尿病所证实(分别见图6(A)和6(B))。在对NOD小鼠注射后，CFSE标记的FTVI修饰的和未修饰的CD44 KO MSC显示在向胰腺的运输上没有差异(图6(C))，其水平与对野生型MSC观察到的水平相似(图4(H))。MSC CD44表达的缺乏和CD44 KO MSC的外岩藻糖基化未消除MSC的体外抑制能力(图6(D))，这表明施用FTVI处理的$CD44^{-/-}$MSC的正常血糖作用的缺乏不归因于免疫调制的固有缺陷。值得注意的是，发现外岩藻糖基化在CD44 KO MSC上赋予细胞表面表达可被HECA452识别的唾液酸岩藻糖基化，但这些细胞在糖尿病NOD小鼠中未实现预期的生物学结果清楚地强调生物化学研究(例如Western印迹分析)的重要性—除了仅仅用简单流式细胞术研究评估诱导的E-Ig和HECA452免疫应答性—在定义/预测细胞表面聚糖工程化引起的增强选择素结合的生物学影响中。

[0268] 讨论

[0269] 由于其免疫调制特性、安全性和离体强力扩增，MSC已经成为几项人类试验的重点，用于治疗各种难治性免疫介导的疾病，包括T1D[6]。已经发现MSC通过对T1D发生至关重要的一些病理性组分的多方面的免疫调制作用而抑制胰岛炎和自身免疫性糖尿病[25,26]。这些作用包括调制炎性相对调节性细胞因子的表达的能力，从而促进调节性DC和T细胞的扩增，以及调制可以直接抑制自身反应性T细胞的负向共刺激分子(如PDL-1)的MSC表达[8-10,13]。因此，MSC疗法具有成为T1D治疗的一种令人激动并独特策略的巨大潜力。然而，迫切需要进行研究，以改善T1D中基于MSC的疗法的疗效[6]。

[0270] 在以前的研究中，我们观察到完全同基因MSC的静脉内施用与糖尿病NOD小鼠中高血糖的瞬时逆转相关[9]。我们假设实现更强大的抗糖尿病作用的最近障碍可以是MSC向发炎的胰腺中归巢的相对缺乏。本文中，我们尝试评估系统性施用通过增强HCELL表达而具有增强归巢能力的MSC将影响NOD模型中糖尿病的逆转。为此，我们利用C57BL/6品系作为MSC的同基因来源，因为CD44 KO表型可用于该遗传背景。因为我们以前检查了介导MSC的抗糖尿病作用的免疫机制，所以我们聚焦于代谢控制，作为评估MSC治疗功效的主要终点[9,10]。

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[0271] 与在相对较短的时间段(通常<30天)特征性地评估抗糖尿病干预的作用的其他研究相反,在该研究中,在长时间观察期间(90天)评估了MSC对逆转自身免疫性糖尿病的作用。我们的数据表明,通过细胞表面α-(1,3)-外岩藻糖基化("FTVI修饰")将初始膜CD44转化为HCELL赋予小鼠MSC与E-选择素高效的结合。我们观察到在具有胰岛炎的NOD小鼠的胰岛周围区域中有E-选择素表达,但在糖尿病抗性小鼠的胰腺中没有。与这些发现一致,我们没有观察到所注射的同基因C57BL/6的FTVI修饰的和未修饰的MSC向糖尿病抗性小鼠(BALB/c宿主)胰腺的归巢水平的差异,但是与未修饰的MSC相比,在糖尿病NOD小鼠中系统施用的C57BL/6(同种异源)FTVI修饰的MSC的系统性胰腺浸润升高,伴随着持久的糖尿病逆转。值得注意的是,我们没有观察到接受FTVI修饰的和未修饰的MSC的动物之间淋巴组织浸润的差异,但是在表达E-选择素的微血管周围区域内和白细胞簇内的细胞沉积/植入显着增加。总而言之,这些数据使人们注意到MSC的胰腺定殖在抑制自身免疫性糖尿病中的主要作用,但不排除MSC的胰腺外效应的贡献。

[0272] 与在本研究中和先前研究中使用静脉内施用的完全同基因的未修饰MSC观察到的抗糖尿病作用相比[9],在这里观察到的使用完全同种异源FTVI修饰的(HCELL)MSC的T1D逆转显著改善似乎不归因于糖工程化MSC本身的免疫抑制能力增强或通过对MSC糖工程化而赋予的体内生存优势。在先前的研究中,我们观察到静脉内施用获自NOD小鼠的半同种异源MSC后,NOR小鼠的抗糖尿病作用得到改善[10],这似乎反映了对半同种异源MSC的减少的排斥反应,从而改善了细胞在体内组织的定殖/持久性。然而,在临床应用中,使用策略来增加完全同种异源来源MSC的效力是有利的,尤其是制造和监管问题支持使用"现成的"(即培养扩增的,合并的)同种异源MSC。发现外岩藻糖基化的CD44 KO MSC缺乏诱导持续逆转高血糖的能力,尽管由HEC452反应性可检测到FTVI依赖性表面唾液酸岩藻糖基化的决定簇的明显产生,这表明在CD44支架上明确显示的E-选择素结合决定簇(即,HCELL的产生)是实现有效的抗糖尿病作用所必需的。这可以与HCELL表达以达到必要的组织归巢和外渗的关键作用相关[27]和/或可以反映对HCELL/CD44表达的需要以在相关的微环境中实现适当的沉积[28]。通过MSC与胰岛同种异体移植物共同移植产生免疫缺陷区的研究,已经强调了原位组织沉积对实现MSC效应的作用[29-31]。在这方面,观察到$HCELL^{+}$MSC在胰岛血管周围区域内和胰岛白细胞浸润物中的定位,强调了HCELL在允许免疫调制中的操作性作用:由于E-选择素在受影响组织的内皮层中表达并且白细胞特征性表达L-选择素,HCELL这种有效的E-选择素和L-选择素配体的表达作用于在最强烈免疫反应性的微环境中明确促进组织沉积。虽然有必要进一步研究MSC在体内的免疫调制作用,但是本文的数据强调了通过在T1D治疗中系统性施用$HCELL^{+}$MSC而增强胰腺向性的潜力。更广泛地说,在所有炎性位点内皮细胞层内的细胞因子如TNF和IL-1对E-选择素的上调的事实[17,18]提供了利用增强的MSC HCELL表达以改善基于MSC的治疗用于多种炎症病况的功效的机会。

[0273] 参考文献-实施例6

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[0305] 实施例7-神经干细胞的细胞表面糖蛋白工程化增加了神经向性并改善多发性硬化小鼠模型中的恢复情况

[0306] 引言

[0307] 大自然已经发展出一种非常有效的机制来将循环细胞递送到炎症和损伤的部位。该过程由分子相互作用的高度有序级联所控制(Butcher,E.C.1991;Sackstein,R.2005;Springer,T.A.1994)。细胞募集中的第一个至关重要的事件包括内皮表面上的流动细胞的抗剪切粘附,这过程被选择素最效介导,选择素是三种Ca^{2+}依赖性凝集素的家族(由E-、P-和L-选择素组成),与其各自的对应受体结合。这些相互作用最初将细胞束缚到血管壁,并且在脉管剪切流动的情况中,使细胞沿着内皮表面以低于主要血流动力学流的速率滚动(步骤1)。该过程有助于特定的细胞传导趋化因子受体与脉管周围区域中存在的相关趋化因子的会合(engagement),从而触发由内向外的信号转导事件,导致整合素家族成员的粘附性增加(步骤2)。激活的细胞整合素及其同源内皮细胞相对受体之间的粘附相互作用然后导致细胞滚动的停滞,并细胞牢固粘附在血管壁上(步骤3),最终导致穿内皮的迁移(外渗,步骤4)。

[0308] 在多发性硬化症(MS)及其动物模型(实验性自身免疫性脑脊髓炎(EAE))中,免疫效应子的募集由脉管选择素E-和P-选择素的上调所介导。E-和P-选择素在体内受到LPS或TNF-α激活后在脑内皮上表达,然而,在小鼠EAE中,P-选择素仅为瞬时表达(Piccio,L.,Rossi,B.等人,2002)。值得注意的是,E-选择素在整个炎症阶段表达,在血管分支位点发生斑块分布,表明存在优先募集区域(Piccio,L.,Rossi,B.等人,2002)。E-选择素还特征性地发现于来自MS患者急性斑块的血管中(Lee,S.J.和Benveniste,E.N.1999;Washington,R.,Burton,J.等人,1994)。这些发现表明在炎症疾病中,E-选择素在将循环细胞至脑的募集中有重要作用。

[0309] 所有三种选择素都结合特异化的碳水化合物决定簇,包括在半乳糖上含有α(2,3)连接的唾液酸置换的唾液酸岩藻糖基化,以及N-乙酰葡糖胺上的α(1,3)-连接的岩藻糖修饰,典型地显示为末端四糖唾液酸Lewis X(sLe^x)(Polley,M.J.,Phillips,M.L.等人,1991;Sackstein,R.2005)。这种结构也称为"CD15s",可以显示在蛋白质支架(即,糖蛋白)或脂质支架(即糖脂)上,并被mAb如CSLEX-1和HECA-452识别。(Alon,R.,Feizi,T.等人,1995;Dimitroff,C.J.,Lee,J.Y.等人,2001;Fuhlbrigge,R.C.,Kieffer,J.D.等人,1997;Gadhoum,S.Z.和Sackstein,R.2008;Merzaban,J.S.,Burdick,M.M.等人,2011)。虽然主要涉及磺酸化的其它结构修饰增加了P-和L-选择素对sLe^x的结合亲和力,但是对于E-选择素的最佳结合不需要这样的修饰(Leppanen,A.,White,S.P.等人2000;Rosen,S.D.2004)。

[0310] 基于神经干细胞(NSC)的治疗已经产生了在CNS炎症和/或退行性疾病中阻止和/或逆转病进展的巨大希望(Ben-Hur,T.,Einstein,O.等人,2003;Einstein,O.,Fainstein,N.等人,2007;Einstein,O.,Grigoriadis,N.等人,2006;Imitola,J.,Raddassi,K.等人,2004;Lee,S T.,Chu,K.等人2008;Pluchino,S.,Quattrini,A.等人,2003;Pluchino,S.,Zanotti,L等人,2005;Ziv,Y.,Avidan,H.等人,2006)。众所周知,NSC表达步骤2和步骤3/4的效应子,分别例如CXCR4(Bezzi,P.,Domercq,M.等人,2001;Flax,J.D.,Aurora,S.等人,1998;Imitola,J.,Raddassi,K.等人,2004)和VLA-4(Pluchino,S.,Zanotti,L等人,2005;

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Rampon,C,Weiss,N.等人,2008),但是没有关于NSC是否天然表达第1步效应子的数据。因此,我们检查了小鼠NSC上第1步效应子的表达,并且发现这些细胞明显缺乏第1步效应子,特别是E-选择素配体。使用EAE模型,我们分析了通过细胞表面聚糖工程化的增强E-选择素配体活性是否会影响所施用的NSC的迁移,重要的是,分析施用细胞的治疗效果。为此,我们使用了称为糖基转移酶程序化立体置换(GPS)的技术来在细胞表面上产生相关的选择素结合聚糖决定簇(Sackstein,R.,Merzaban,J.S.等人,2008)。我们在本文报道,GPS通过修饰两种神经干细胞膜糖蛋白(CD44和NCAM)的聚糖来增强NSC上E-选择素配体的表达,从而分别产生E-选择素配体HCELL(造血细胞E-/L-选择素配体)和NCAM-E。在没有检测到长期植入的情况下,NSC E-选择素配体活性的糖工程化导致增加的神经向性并且在缺乏可检测的长期移植的情况下在EAE中产生改善的临床结果,表明这些组织特异性干细胞产生恢复效应,而不是直接再生效应。重要的是,这种神经恢复作用不是成体干细胞的普遍性质,因为施用GPS工程化的小鼠造血干/祖细胞(HSPC)并没有改善EAE临床过程。这些发现具有深刻的临床意义,提供了第一个直接证据表明,细胞表面糖工程化产生细胞迁移的效应子可以提高基于干细胞的治疗剂的功效,也为NSC用于神经验证疾病的治疗的用途提供了新的视角。

[0311] 结果

[0312] 神经干细胞上细胞迁移的分子效应子的表达

[0313] 在小鼠NSC的原代培养物上进行流式细胞术,以分析细胞迁移中步骤1,步骤2及步骤3/4的分子效应子的表达。NSC与E-选择素-Ig嵌合体(E-Ig)和P-选择素-Ig嵌合体(P-Ig)没有反应性,表明不存在E-和P-选择素配体(图9(A)),即使在炎症介质的存在下(图15);它们也不会被mAb CSLEX1、KM93或HECA452(它们都识别sLe^x)染色。NSC表达CD44和整合素VLA-4(CD49d/CD29)和VLA-5(CD49e/CD29)以及趋化因子受体CXCR4;其他研究者已经观察到NSC标记物表达的这种模式(Back,S.A.,Tuohy,T.M.等人,2005;Campos,L.S.,Decker,L.等人,2006;Campos,L.S.,Leone,D.P.等人,2004;Imitola,J.,Raddassi,K.等人2004;Ji,J.F.,He,B.P.等人2004;Leone,D.P.,Relvas,J.B.等人2005;Pluchino,S.,Quattrini,A.等,2003;Pluchino,S.,Zanotti,L.等人2005)(图9(B))。除了明确描述的聚唾液酸(PSA)(外,NSC还特征性表达神经细胞粘附分子(NCAM)Vitry,S.,Avellana-Adalid,V.等人2001)(图9(B))。NSC不表达PSGL-1、CD43、LFA-1(缺乏CD11(α_1)和CD18(β_2)链两者),和LPAM-1($\alpha_4\beta_7$)(图9(B))。这些结果表明NSC在细胞迁移的多级级联阶段的步骤1效应子的表达不足,但表达趋化因子受体和相关的整合素效应子(介导外渗中的步骤2-4,并且也参与通过径向迁移在发育中的脑中的动员)(Imitola,J.,Comabella,M.等人,2004)。

[0314] GPS增强神经干细胞上的E-选择素配体活性

[0315] CD44是参与NSC(Deboux,C,Ladraa,S.等人,2013)和脑癌干细胞(Fu,J.,Yang,Q.Y.等人,2013)迁移的分子,在培养的NSC中强表达(图9(B))。然而,NSC缺乏E-选择素结合的发现(图9(A))表明这些细胞不天然表达称为HCELL的CD44的E-选择素结合糖型(Dimitroff,C.J.,Lee,J.Y.等人,2000;Dimitroff,C.J.,Lee,J.Y.等人,2001;Sackstein,R.2004)。因此,我们尝试确定对CD44的聚糖使用糖基转移酶程序化立体置换(GPS)的非遗传操作是否会增强HCELL表达(Sackstein,R.,Merzaban,J.S.等人,2008)。为此,我们用α(1,3)-连接的特异性岩藻糖基转移酶,岩藻糖基转移酶VI(FTVI)处理NSC。该酶特异性地将岩藻糖置于末端型2-乳糖胺单元上;如果该乳糖胺被α(2,3)连接的唾液酸封闭,则产生

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sLe^x。在对NSC进行FTVI处理后(GPS-NSC)，与mAb CSLEX1、KM93和HECA452的反应性得到诱导，这与sLe^x表位的强表达(图10(A))一致，具有相关的E-Ig结合(图10(A))，但不诱导P-Ig结合(图10(A))。值得注意的是，CD15(也称为SSEA-1或Le^x)在NSC中高表达(图10(A))，尽管FTVI可以将非唾液酸化的末端乳糖胺进行岩藻糖基化，从而产生CD15(SSEA-1)，但CD15的表达为在增强岩藻糖基化后不变(图10(A))。总之，这些数据表明，α(1,3)-外岩藻糖基化仅发生在唾液酸化的乳糖胺聚糖上。在GPS处理之前对NSC进行菠萝蛋白酶消化显著降低HECA452反应性(图10(B))，表明糖蛋白而不是糖脂是唾液酸岩藻糖基化的决定簇的主要载体。通过FACS显示用磷脂酰肌醇磷脂酶C(PI-PLC)处理GPS-NSC导致HECA452信号的中度但显著的降低(图10(C))，表明sLe^x修饰的糖蛋白的少量群体是糖磷脂酰基(GPI)-连接的。

[0316] 对来自GPS-NSC的细胞裂解物和免疫沉淀的CD44的western印迹显示用HECA452所识别的至关重要的唾液酸岩藻糖基化而修饰的糖蛋白之一是标准的未剪接形式的CD44(~100kDa;图11(A))，且CD44也与E-Ig反应(图11(A))。然而，在用CD44进行彻底免疫沉淀后，通过在残余上清液部分中与E-Ig和HECA452的反应性的证据可鉴定出其它候选糖蛋白E-选择素配体。在~120和~140kDa的两个条带是明显的。基于这些条带的分子量分布(图11(A))和E-选择素结合的部分PI-PLC敏感性(图10(C))，120kDa形式的NCAM的特征(Gascon,E.,Vutskits,L.等人,2007;Maness,P.F.和Schachner,M.2007;Rutishauser,U.2008)，我们推测NCAM可以作为额外的E-选择素配体。因此，我们用pan-NCAM mAb进行免疫沉淀，并观察到在CD44的全面免疫沉淀后保持的残留条带确实是NCAM的条带(图11(B))。为了确定NCAM上的相关唾液酸岩藻糖基化是否显示在N-聚糖上，我们用N-葡萄糖苷酶F消化后的GPS-NSC的裂解物进行western印迹来测试E-Ig反应性(图11(A))；观察到消化后没有E-Ig的明显染色，表明相关的E-选择素结合决定簇显示在N-聚糖上。GPI锚定形式的NCAM-E对NSC的增强岩藻糖基化后的总体sLe^x表达的贡献是中等的，如PI-PLC处理后HECA452信号(和E-Ig信号数据，未显示)的少量减少所示(图10(C))。因此，小鼠神经干细胞的增强α(1,3)-岩藻糖基化产生了HCELL以及我们命名为"NCAM-E"的神经前体分子NCAM的独特E-选择素配体反应形式。有趣的是，人NSC(CC-2599)在GPS处理后仅表达HCELL而非NCAM-E(图16(A)-16(C))。

[0317] 为了评估NSC上GPS-工程化的E-选择素配体活性的稳定性，我们在增强的外岩藻糖基化后每隔24小时通过流式细胞术测量E-lg反应性。E-选择素配体活性稳定多达24小时，随后可能由于表面蛋白的转换，到72小时下降到不可检测的水平(图11(C))。NSC活力(图17)不受增强的外岩藻糖基化的影响，且在克隆形成测定中神经球的数量或增殖或在神经干细胞分化中没有差异(图18(A)-18(D))。因此，除了产生E-选择素配体外，NSC的GPS处理没有诱导出明显的表型差异。

[0318] 已经显示NSC抑制体外的T细胞增殖(Einstein,O.,Fainstein,N.等人,2007;Einstein,O.,Grigoriadis,N.等人2006;Martino,G.和Pluchino,S.2006)，并且特别是抑制体外的T细胞有丝分裂原应答。为了评估NSC的这种免疫调制功能是否受到增强的E-选择素配体活性的影响，我们检测了GPS-NSC和对照缓冲液处理的NSC(BT-NSC)抑制淋巴结细胞响应Con A激活而增殖的能力。如图19(A)-19(C)所示，GPS和BT-NSC均能够降低T细胞增殖，还同等地抑制炎性细胞因子产生的水平，这表明在NSC上的GPS处理本身没有提供免疫调制优势或缺点。有趣的是，我们还观察到，在对BT-和GPS-NSC进行炎性细胞因子处理时，NSC等

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量释放白血病抑制因子(LIF),GPS处理的细胞与E-Ig结合时这种释放得到增强(图19(C))。因此,NSC的增强的外岩藻糖基化诱导瞬时E-选择素配体活性,而通过体外研究确定,这对NSC表型或功能没有不良影响。

[0319] GPS-NSC显示与E-选择素的强力的生理滚动相互作用

[0320] 为了分析在生理血流条件下GPS-NSC的E-选择素配体活性的效力,使用由细胞因子(IL-1β和TNF-α)刺激而表达E-选择素的人脐静脉内皮细胞(HUVEC)进行平行板流动室研究。如图12(A)所示,GPS-NSC表现出显著的E-选择素配体活性,其在EDTA存在下或通过使用阻断性抗E-选择素mAb而被完全消除。在通常的后毛细血管静脉剪切水平(1-4达因/cm^2)内观察到显著的抗剪切滚动相互作用,并且持续到超过20达因/cm^2(图12(A))。为了分析聚糖工程化的E-选择素配体——HCELL或NCAM-E中的哪个是NSC上更有效的E-选择素配体,我们使用印迹-滚动测定法。(Fuhlbrigge,R.C.,King,S.L.等人,2002)该技术允许对通过SDS-PAGE解析检测在膜蛋白上的剪切依赖性选择素配体相互作用,从而允许评价HCELL和NCAM-E对GPS-NSC的E-选择素配体活性的单独贡献。因此,为了评价各自的E-选择素结合特性,将E选择素转染的CHO细胞(CHO-E)灌注在GPS-NSC裂解物的HECA-452免疫染色的印迹上。如图12(B)所示,与NCAM-E的120kD或140kD形式相比,更多的CHO-E细胞与HCELL相互作用并粘附。悬浮在含有5mM EDTA的流动培养基中的CHO-E细胞对印迹的任何区域都是可忽略的粘附,确认了是Ca^{2+}依赖性结合,这与选择素配体活性一致。

[0321] GPS-NSC表现出在EAE中体内的增加的归巢和组织浸润

[0322] 为了评估注射的NSC是否浸润CNS实质,我们进行共聚焦显微镜研究。为此,用PKH26染料标记GPS-NSC和BT-NSC,并以两次独立的i.v.注射将其静脉内注射到MOG诱导的慢性EAE小鼠中:分别在发病前(免疫(PI)后第9天)和发病后(PI第13天)。所述天数是基于先前对EAE中脑内皮上的E-选择素表达的研究来选择的(Piccio,L.,Rossi,B.等人,2002)。在第二次NSC注射后4天(PI第17天)收获腰骶脊髓和脑。对脑的共聚焦分析显示脑中有更高量的GPS-NSC(图13(A))及在Flk-1^+血管外的定位(图20(A)-20(B))。进一步地,对脊髓进行平行分析(图13(B)-13(D)),其中绝大多数病理发生在EAE中,显示出在PI第17天血管外GPS-NSC浸润物的数量超过BT-NSC浸润物的两倍(图13(D))。这些数据表明在脑和脊髓两者中GPS-NSC浸润CNS实质比BT-NSC显著更有效。为了进一步确认我们的共聚焦数据,我们研究了GPS工程化的E-选择素配体活性对体内NSC短期归巢的影响。如在材料和方法中描述的,使用荧光染料跟踪染料CFSA-SE(羧基荧光素二乙酸酯琥珀酰亚胺酯)染色NSC,并在用MOG的PI第9天和第13天过继转移到C57BL/6小鼠中。在第二次细胞注射后的16h内,我们观察到GPS-NSC在脑、脾和肝中的累积是BT-NSC的三到五倍更有效(图13(E))。GPS-NSC在浸润脑、脾和肝的相对优势反映了在运输上的真正区别,而不仅仅是这些细胞在原位优先扩增,因为它们的平均CFDA-SE荧光相对于BT-NSC没有降低(数据未显示)。事实上,迁移到脾的那些细胞表明NSC与$CD4^+$T细胞显然有密切的相互作用(图13(F))。注射的细胞也积累在肺中,但在GPS和BT-NSC之间没有差异,似乎反映了在肺微血管中的空间捕获(图13(E))。因此,在GPS处理后,NSC上形成的sLe^x结构允许这些细胞迁移到这些器官中,并且强调了在体内E-选择素配体活性在驱动NSC的组织特异性迁移中的关键作用。

[0323] EAE中通过增强的内源性间接神经再生,GPS-NSC增加的归巢转化为神经病理学的缓解

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[0324] 为了解答NSC的改善的组织归巢是否具有增强的治疗效果，我们监测了接受NSC注射后MOG诱导的慢性EAE的C57BL/6小鼠的神经学状态，并分析了临床参数以及组织恢复和病理学。在PI第9天和PI第13天以两次分别i.v.注射来施用神经前体。测试了五组EAE小鼠：(1)GPS-NSC、(2)BT-NSC、(3)HBSS(假处理，即无细胞)、(4)BT-HSPC和(5)GPS-HSPC。以盲形式对小鼠每天评估临床评分(图14(A))，并计算累积疾病负担的线性回归(图14(B)和表2)。

[0325]

治疗	细胞施用途径	小鼠数量	疾病发作(p.i.天数)	最高临床评分	累积疾病评分(0-20 p.i.)	累积疾病评分(21-30 p.i.)
无NSC	-	30	13.22±0.6	2.2±0.4	15.75±0.6	14.9±2.4
NSC BT	i.v	30	12±0.6	2.1±0.3	12.3±0.9	10.9±2
NSC-GPS	i.v	30	12.2±0.5	1.8 ± 0.1^{no}	10.8 ± 1.4^{no}	7.1±1.5*

[0326] *p<0.05ANOVA多重比较。

[0327] 表2.经GPS处理的NSC进行i.v.处理C57BL/6小鼠的EAE临床特征。

[0328] 与HSPC和假处理的动物相比，注射对照NSC(BT-NSC)减轻了EAE的临床严重性(p=0.006)。然而，与BT-NSC(n=30;p=0.006)、GPS-HSPC(n=30;p<0.0001)、BT-HSPC(n=30;p<0.0001)和假处理(n=30;p<0.0001)的动物相比，i.v.注射GPS-NSC(n=30)显示出更显著改善的临床评分。重要的是要注意，疾病严重程度的缓解是特异于NSC的，因为注射GPS-HSPC(显示出显著增加的E-选择素配体活性，参见图21(A)(Merzaban,JS,Burdick,MM.等人，2011))或注射BT-HSPC都没有改善MOG诱导的动物相对于对照动物的临床评分(图21(B)-21(C)和表3)

[0329]

治疗	细胞施用途径	小鼠数量	疾病发作(p.i.天数)	最高临床评分	累积疾病评分(0-20 p.i.)	累积疾病评分(21-30 p.i.)
无细胞	-	30	13.22±0.6	2.2±0.4	15.75±0.6	14.9±2.4
BT-HSPC	i.v	30	10±0.7	2.1±0.2	17.2±1*	16.7±1*
GPS-HSPC	i.v	30	9.5±0.4	2.5±0.6	18.3±2.2**	16.8±1.7*

[0330] *p<0.05ANOVA多重比较

[0331] 表3:i.v.注射经GPS处理的HSPC的C57BL/6小鼠的EAE临床特征。

[0332] 实际上，注射GPS-或BT-HSPC都显示出临床结果恶化的趋势，表明观察到的NSC输注的有益作用不是成体干细胞的一般特性。

[0333] 值得注意的是，在EAE诱导后第30天检查神经病理学显示，与BT-NSC和假处理的动物相比，接受GPS-NSC的小鼠的炎症和神经再生的几个参数中有显著统计学差异(图14(C))。为了确定NSC注射对EAE的神经病理学的影响，我们评估了许多不同的标记物。首先，为了评估炎症的程度，我们对每个研究组的小鼠脊髓切片针对CD11b进行染色，CD11b是识别浸润性巨噬细胞和小胶质细胞的标记物。仅接受HBSS缓冲液的具有EAE的动物显示高水平的针对CD11b染色，细胞表现出增加的膜过程数量，这是活化表型的形态学证据(图14(C))。接受BT-NSC的小鼠中CD11b染色的细胞数显著减少(p<0.0001)，特别是注射GPS-NSC的动物中所述水平进一步降低(与BT-NSC相比，p<0.005))。重要的是，巨噬细胞/小胶质细

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胞显示减少的CD11b染色和更离散的膜过程，这表明活化减少(图14(C))。此外，对不同治疗组的脑CD4+T细胞的定量显示，在给予GPS-NSC的动物中，浸润性T细胞显著降低(图14(D)和14(E))。为了评估神经再生，我们对脊髓切片进行标记物GAP-43和SMI-32的染色(图14(F)-14(I))。在注射了GPS-NSC的动物中，与仅接受BT-NSC或HBSS缓冲液的小鼠相比，与轴突完整性和再生相关的分子GAP-43的表达有所增加(图14(F)-14(G))。相反，与接受BT-NSC或HBSS缓冲液的小鼠相比，在接受GPS-NSC的动物中观察到轴突变性标志物SMI-32的表达的特异性降低(图14(H)-14(I))。这些数据支持了GPS-NSC优于BT-NSC的改善临床效果是继发于组织特异性NSC的增加的损伤迁移而产生增强的神经保护。

[0334] 为了进一步评估所观察到的GPS-NSC的作用是否反映了增加的神经再生，我们针对与少突胶质细胞发生及成熟的少突胶质细胞相关的标记物进行染色，所述标记物包括CNP酶、Olig-2和SOX9。与BT-NSC或假处理对照相比，我们观察到在接受GPS-NSC的动物中Olig-2、SOX9和CNP酶的细胞数量有统计学显著的增强(图14(D)和14(E))。虽然在PI第17天有注射的NSC的证据($SOX\text{-}2^{+}$细胞；图20(A)-20(B))，我们在PI的30天在任何注射组中没有观察到NSC(用GFP标记)的持久定殖(图22)，提示与归巢改善相关的NSC神经保护机制不需要施用的NSC的持续存在，也不需要其向神经谱系细胞的分化。总而言之，这些数据表明，使用GPS-NSC处理小鼠增强NSC向CNS的递送，并通过间接神经再生作用提供神经恢复，而不是通过直接神经再生(即细胞置换)。

[0335] 讨论

[0336] MS是CNS的慢性脱髓鞘疾病，其特征在于多灶性炎性损害，产生髓鞘的逐渐破坏，导致轴突损伤和损失(Imitola,J.,Chitnis,T.等人2006)。基于干细胞的治疗剂提供了修复受损的/发炎的组织的希望，其是通过替代受影响的细胞(直接再生)和/或在微环境中产生能引发内源性细胞的组织再生的支持/营养因子(间接再生)。在散播性神经疾病如MS的情况下，已经证明组织特异性NSC的使用在临床上可用于实现CNS恢复，但是实现该目标的最近的障碍是将足够数量的细胞输送到神经损伤位点。先前的研究已经显示移植的NSC在中风、脊髓创伤和MS的实验模型中有益(Ben-Hur,T.,Einstein,O.等人,2003;Einstein,O.,Fainstein,N.等人,2007;Einstein,O.,Grigoriadis,N.等人,2006;Imitola,J.,Raddassi,K.等人,2004;Lee,S.T.,Chu,K.等人,2008;Pluchino,S.,Quattrini,A.等人,2003;Pluchino,S.,Zanotti,L.等人,2005;Ziv,Y.,Avidan,H.等人,2006)。在这些研究中，使用直接注射到受影响部位作为递送途径，然而，为了在多灶点CNS疾病的受影响组织内获得NSC的适当定殖，需要脉管递送途径，因为局部施用(即，原位注射)是不切实际的，这是由于疾病的扩散性质和相关的解剖学限制。迄今为止，还没有研究评估NSC上的细胞迁移的分子效应子的表达，更重要的是，没有研究如何优化这些效应子的表达可以增强NSC神经向性。

[0337] 我们的研究显示NSC表达相关的第2-4步效应子，但明显缺乏第1步效应子：(1)它们不天然表达E-选择素或P-选择素的配体(即使在存在炎性细胞因子下培养(图15))；(2)它们缺乏作为规范的选择素结合决定簇的聚糖sLe^{x}的表达；(3)它们不表达糖蛋白PSGL-1，一种已被报道介导向脑运输的在髓鞘特异性Th1细胞上的选择素配体(Piccio,L.,Rossi,B.等人,2005;Piccio,L.,Rossi,B.等人,2002)。重要的是，内皮选择素，特别是E-选择素的表达已经涉及MS和EAE中免疫效应子的募集(Lee,S.J.和Benveniste,E.N.1999;Piccio,

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L.,Rossi,B.等人,2002)。因此,我们尝试评估NSC的聚糖工程化以增强E-选择素配体的表达是否能增强细胞的系统性递送并由此在MS模型中有生物学作用。

[0338] 本文的数据集显示培养的NSC表达两个明确表征的神经细胞表面分子CD44和NCAM(Back,S.A.,Tuohy,T.M.等人,2005;Pluchino,S.,Quattrini,A.等人,2003)。引人注目的是,小鼠NSC的外岩藻糖基化导致sLe^x决定簇在这些糖蛋白上占优势地高表达,将CD44按程序转化为有效的E-选择素配体HCELL(Dimitroff,C.J.,Lee,J.Y.等人2000;Dimitroff,C.J.,Lee,J.Y.等人,2001),并且还诱导了我们称为"NCAM-E"的~120kDa和~140kDa的NCAM的两种唾液酸岩藻糖基化的糖型的表达。印迹滚动测定显示HCELL是在GPS-NSC上表达的主要E-选择素配体(图12(B))。通过PI-PLC消化去除NCAM-E后流式细胞术的结果证实了这些发现,显示出NSC sLe^x表达和E-Ig反应性的显著保留(图10(C))。

[0339] NCAM是免疫球蛋白超家族的成员,在神经元和神经胶质上均表达,并且通常被视为细胞-细胞相互作用建立细胞与其环境的物理锚定的调控子。由NCAM蛋白质核心上的α-2,8连接的聚唾液酸(PSA)组成的翻译后聚糖修饰提供了在神经迁移中的独特性质(Gascon,E.,Vutskits,L.等人,2007;Maness,P.F.和Schachner,M.2007;Rutishauser,U.2008),且PSA-NCAM似乎在脑中介导前体细胞迁移中起关键作用(Glaser,T.,Brose,C.等人2007;Lavdas,A.A.,Franceschini,I.等人,2006;Zhang,H.,Vutskits,L.等人,2004)。我们的数据现在提供了第一个证据,证明NCAM显示相关末端α-(2,3)-唾液酸乳糖胺聚糖可以作为外岩藻糖基化的受体来产生sLe^x决定簇。在对NSC进行细胞表面糖工程化之后,PSA表达水平没有变化,且诱导的E-选择素配体活性不是永久性的,因为在增强岩藻糖基化的72小时内sLe^x表达完全丧失(图11(A)-11(C))。因此,在外渗之后,应该发生暂时的向初始NCAM的反转,并且浸润性NSC之后能够进行经内源性NCAM介导的实质内CNS迁移。值得注意的是,尽管小鼠NSC表达携带α-(2,3)-唾液酸乳糖胺聚糖的NCAM,我们对人类NSC(CC-2599)的研究表明,这些细胞在外岩藻糖基化后只表达HCELL而不表达NCAM-E(图15);因此,人NSC可以表达相关的唾液酸乳糖聚糖,其作为只在CD44上增强岩藻糖基化的受体。在解释人NSC用于啮齿动物系统的异种移植研究时,应考虑这些物种的差异(Goncharova,V.,Das,S.等人,2014)。

[0340] E-选择素配体活性的诱导对细胞运输具有深远的影响,其可用于指导细胞迁移到表达E-选择素的内皮细胞层。如他人所示,我们观察到NSC显示趋化因子受体CXCR4和整合素VLA-4(参见图9(A)-9(B))。已经显示CXCR4在人NSC上的表达(Bezzi,P.,Domercq,M.等人,2001;Flax,J.D.,Aurora,S.等人1998;Imitola,J.,Raddassi,K.等人,2004)促进体内向CNS损伤的迁移,其中局部星形胶质细胞和内皮上调同源配体基质细胞衍生因子1α(SDF-1α,也称为CXCL12)(Imitola,J.,Raddassi,K.等人,2004)。这些观察将CXCR4定义为促进NSC归巢到CNS损伤中重要的第2步效应子。VLA-4、VCAM-1的相应内皮受体也在脑对损伤的炎症应答中被上调(Justicia,C,Martin,A.等人,2006)。类似地,也显示VLA-4/VCAM-1轴在NSC的迁移中发挥关键作用,因为只有除CXCR4外还组成型表达VLA-4的NSC能够在EAE的受影响的损伤中发炎的CNS微血管周围积累(Pluchino,S.,Zanotti,L.等人,2005)中。最近的异种移植研究表明,整合素可以在大鼠中风模型中作为第1步介质在人NSC的迁移中起作用,这表明在该模型系统中选择素相互作用是不必要的(Goncharova,V.,Das,S.等人,2014)。虽然进一步的工作是有必要的,但作为第1步相互作用介质的整合素的不断变化的

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作用可以反映在所用炎症模型、宿主动物系统、血管通透性、在炎症位点内皮粘附分子表达、在该位点可溶性粘附分子的存在、控制流速的血管物理特性(即,血管直径)中的差异(Berlin,C.,Bargatze,R.F.等,1995;Ding,Z.M.,Babensee,JE.等人,1999;Zarbock,A.,Kempf,T.等人2012;Zarbock,A.,Lowell,CA.等人,2007)。在任何情况下,作为步骤1效应子的E-选择素配体在NSC上的表达将有助于补充CXCR4和VLA-4的组成型表达,从而优化NSC向炎症位点的募集。因此,尽管我们观察到静脉内施用的(未修饰的)BT-NSC可以浸润EAE小鼠的脑(图13(E)),而通过糖工程化的增强E-选择素配体表达导致NSC向脑的迁移显著增加。这种增加的神经向性与明显减少的CNS炎症相关(图14(A)-14(I))。此外,如图13(A)-13(F)所示,注射后第17天,GPS-NSC比BT-NSC在CNS实质中的NSC积累高两倍,表明第1步效应子的增强表达促进外渗。

[0341] 除了增强的神经向性之外,短期归巢数据还显示了脾和肝中GPS-NSC的积累比BT-NSC显著更高(图13(E))。这些发现与一项研究结果一致,该研究报道了系统性施用的NSC倾向于积累在具有EAE的小鼠的脑中,以及在脾和肝中(Politi,L.S.,Bacigaluppi,M.等人,2007)。已经在人、非人灵长类动物和啮齿动物中描述了脾中的E-选择素表达(Alam,H.B.,Sun,L等人,2000;Drake,T.A.,Cheng,J.等人,1993;Redl,H.,Dinges,H.P.等人,1991;Schweitzer,K.M.,Drager,A.M.等人,1996)并且通过在CNS炎性病况中特征性表达的促炎细胞因子被上调(Emamgholipour,S.,Eshaghi,S.M.等人,2013;Weishaupt,A.,Jander,S.等人,2000年);实际上,已经显示sLe^x与聚合物的缀合显著增强了该聚合物在脾内的积累(Horie,K.,Sakagami,M.等人2000;Horie,K.,Sakagami,M.等人2004),这提供sLe^x表达促进脾脏递送的直接证据。据报道,NSC的脾脏浸润抑制驻留的脾细胞(如巨噬细胞)产生炎性细胞因子,从而产生抗炎作用(Lee,S.T.,Chu,K.等人,2008)。其他研究报道血管内施用的NSC提供周围免疫抑制(Einstein,O.,Fainstein,N.等人,2007;Einstein,O.,Grigoriadis,N.等人,2006)或局部免疫调制而阻止CNS损伤,而不是通过增强神经再生(Martino,G.和Pluchino,S.2006;Pluchino,S.,Zanotti,L.等人,2009;Wang,L,Shi,J.等人,2009)。因此,观察到NSC上增强表达E-选择素配体活性而增加的脾脏归巢有助于通过NSC对免疫细胞的组织比率增加经由免疫调制的神经保护作用。我们的体外数据支持这一观点,表明尽管与在对照NSC所观察到的相比,GPS-NSC没有赋予免疫调制优势,但随着输入的NSC与脾细胞的比率增加,T细胞增殖减少(图19(A))。

[0342] 对细胞表面外岩藻糖基化的先前研究已经在异种移植模型中使用了人间充质干细胞,并没有指出对组织损伤的治疗影响,即糖工程化的干细胞是否将归巢至炎症位点,以及这样的细胞是否具有期望的再生和/或组织保护作用。虽然在输注GPS-NSC后,观察到NSC向CNS的募集增强,但NSC的长期植入没有相应的增加。因此,虽然通过利用生理细胞迁移(即以非侵入性方式保留CNS组织微环境结构)实现了增强的CNS浸润,但是我们没有观察到施用的NSC分化为原位再生(即,通过直接再生)功能性CNS组织的后代。事实上,我们观察到GPS-NSC的施用提高了内源性神经元祖细胞变成少突神经胶质细胞的能力(图14(D)和14(E))。因此,我们的研究结果不支持直接神经再生的范式,而是表明NSC在CNS组织修复中的主要作用是通过支持通过内源性细胞进行修复的营养作用(Caplan,A.I.2009;Hess,D.C.和Borlongan,C.V.2008;Laterza,C,Merlini,A.等人,2013;Phinney,D.G.和Prockop,D.J.2007)从而产生神经恢复(即通过间接神经再生)。与此一致,据报道未分化的NSC通过

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分泌神经营养因子如LIF(Laterza,C,Merlini,A.等人,2013)显著减少瘢痕形成并增加内源性胶质细胞和神经元祖细胞的存活和功能。此外,NSC通过表达分子如BMP-4和Noggin来支持损伤诱导的生长生态位的形成(Imitola,J.,Raddassi,K.等人,2004;Pluchino,S.,Zanotti,L.等人,2005),其引发内源性细胞的间接神经再生。

[0343] 总的来说,我们的数据支持其中NSC的聚糖工程化增强E-选择素配体的表达的机制增加组织定殖,介导免疫调制和组织修复而不需要施用的NSC的持续/长期植入。值得注意的是,尽管E-选择素配体活性增强,但是输注GPS-HSPC并没有改善EAE的过程(实际上,注射HSPC会使疾病恶化,见图21(A)-21(B)和表3),表明观察到GPS-NSC的神经保护作用不是成体干细胞的一般特性,并且是由于NSC固有的神经恢复作用。与成体干细胞的特异性生物学作用的这种发现一致,已经发现人HSPC在先天性角膜疾病的小鼠模型中引起强的宿主免疫排斥,而其他成体干细胞例如间充质干细胞则抑制宿主免疫应答(Liu,H.,Zhang,J.等人,2010)。虽然有必要进一步研究通过NSC介导所观察到的神经保护作用的分子机制,但我们的研究结果表明细胞表面聚糖工程化并未改变神经干细胞的自我更新能力,分化潜能或改变其先天免疫调制能力。总而言之,本文提供的数据引导我们提出一个模型(图23),其中通过NSC表面的外岩藻糖基化的增强E-选择素配体表达在CNS炎症位点产生增加的组织募集,从而提高NSC营养因子在需要其的地方的载荷递送。因为在人类所有炎症位点的内皮层上E-表达都显著上调,所以我们的研究结果对未来的临床试验有深刻的转化意义,其中利用细胞表面聚糖工程化来提高干细胞治疗剂对MS以及其他破坏性多灶点验证疾病的效力。

[0344] 材料和方法

[0345] 伦理声明:所有小鼠实验均按照NIH的动物护理及使用指导方针进行,并经哈佛医学院的机构动物护理和使用委员会批准。以下是使用小鼠的这些实验的具体细节。使用理由:EAE是人疾病多发性硬化症的有价值模型。没有可以代替这种体内疾病的数学模型、计算机模拟或体外系统。首先测试了许多可用于MS和其他自身免疫性疾病的治疗方法,并在EAE中对其机制进行了研究。C57/BL6小鼠中MOG诱导的EAE是有价值的,因为在该背景下许多敲除和转基因小鼠都可用。兽医护理:根据联邦、委员会和AAALAC指南处置和护理小鼠。尽量减少不良反应的程序:免疫后,每天检查动物。受EAE影响的小鼠发展为软尾(1级)、后肢残缺(2级)、后肢完全麻痹(3级)、四肢麻痹(4级)、濒死或动物死亡(5级)。大多数动物具有0至3级,很少动物达到4级,在这种情况下,执行安乐死。当动物受到EAE的影响时,为其提供凝胶包,以确保摄入流体,使其能够摄取笼子内的食物。安乐死是根据委员会指导方针施用CO2吸入进行。

[0346] 试剂:以下抗体购自BD Pharmingen:功能阻断性小鼠抗人E-选择素(68-5H11;IgG1)、大鼠抗人CLA(HECA-452;IgM)、小鼠抗人sLe^x(CSLEX-1;IgM)、小鼠抗人CD15(IgM)、大鼠抗小鼠PSGL-1(2PH1和4RA10;IgG1)、小鼠抗人PSGL-1(KPL-1;IgG1)、大鼠抗小鼠CD44(KM114和IM7;IgG1)、小鼠抗人CD44(515;IgG1)、大鼠抗小鼠CD43(S7;IgG2a)、小鼠抗人CD43(1G10;IgG1)、小鼠抗(小鼠抗成人和胚胎泛N-Cam)CD56(NCAM13;IgG2b)、小鼠抗人CD56(NCAM16.2;IgG2b)、大鼠抗人CXCR4(2B11;IgG2b)、小鼠抗人CXCR4(12G5;IgG2a)、大鼠抗小鼠CD18(GAME-46;IgG1)、大鼠抗小鼠CD29(KM16;IgG2a)、大鼠抗小鼠CD49d(9C10;IgG2b)、大鼠抗小鼠CD11a(2D7;IgG2a)、大鼠抗小鼠CD11b(M1/70;IgG2b)、大鼠抗小鼠CD49e(MFR5;IgG2a)、小鼠IgG1,κ同种型、小鼠IgG2a同种型、小鼠IgM同种型、大鼠IgG同种

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型、大鼠IgM同种型和人IgG_1同种型。以下二抗也购自BD Pharmingen:PE链霉亲和素、生物素抗大鼠IgM和山羊抗小鼠Ig-HRP。以下二抗抗体购自Southern Biotech:兔抗人IgG生物素、山羊抗小鼠IgM-PE、山羊抗大鼠IgG-PE、山羊抗小鼠Ig-生物素、山羊抗大鼠Ig-HRP和山羊抗人Ig-HRP。重组小鼠E-选择素/人Ig嵌合体(E-Ig)和重组小鼠P-选择素/人Ig嵌合体(P-Ig)购自R&D。小鼠抗人sLe^x(KM93;IgM)购自Calbiochem。大鼠抗小鼠CD43(1B11;IgG2a)购自Biolegend。大鼠抗小鼠LPAM-1(DATK32;IgG2a)和小鼠抗SMI32(SMI-32;IgG1)购自Abcam。小鼠抗人CD15(80H5;IgM)购自Coulter-Immunotech。PNGase F购自New England Biolabs。PI-PLC、菠萝蛋白酶、大豆胰蛋白酶抑制剂、DNase、小鼠抗Map2(HM-2;IgG1)购自Sigma。小鼠抗GFP(B2,IgG_{2a})购自Santa Cruz Biotechnology,Inc(Santa Cruz,CA)。To-pro3购自Invitrogen。小鼠抗人唾液酸(PSA;IgG2a)由Nicholas Stamatos博士惠赠。岩藻糖基转移酶VI(FTVI)酶由Roland Wohlgemuth博士(Sigma-Aldrich)惠赠。

[0347] 细胞:如之前所述分离和培养小鼠NSC(Imitola,J.,Comabella,M.等人,2004;Imitola,J.,Raddassi,K.等人,2004)。简言之,将分离自第12天小鼠胚胎的端脑VZ的离解的NSC(5×10^5个细胞/ml)的悬浮液在未包被的25-cm^2培养瓶(Falcon)中于37℃在5%CO_2中培养在98%的DMEM/F12(GIBCO)、1%的N2补充剂(GIBCO)、1%青霉素/链霉素(GIBCO)、8mg/ml肝素(Sigma)、10ng/ml白血病抑制因子(LIF,Chemicon)、20ng/ml bFGF(Calbiochem)中。在大多数实验中使用传代二代后的NSC。通过在Versene(LifeTechnologies)中对神经球进行机械解离来获得神经干细胞的单细胞悬浮液。

[0348] 为了分离小鼠HSPC,从C57BL/6小鼠的股骨和胫骨收获骨髓,并制备单细胞悬浮液。使用红细胞裂解缓冲液(Sigma)裂解红血细胞。然后洗涤细胞并通过100μm细胞过滤器(BD Falcon)进行过滤,之后使用Miltenyi Biotec的Lineage Cell Depletion Kit进行谱系消减。使用autoMACS Separator(Miltenyi Biotec)消减细胞制备物。在消减之后,使用CD117MicroBeads(Miltenyi Biotec),对细胞进行c-kit阳选。所得的$Lineage^{neg}$-$C\text{-}kit^{pos}$群体(简称HSPC)用于体内EAE小鼠研究。

[0349] 用全长E-选择素(CHO-E)转染中国仓鼠卵巢细胞并如前所述进行维持(Dimitroff,CJ,Lee,JY.等人,2001)。如前所述(Imitola,J.,Raddassi,K.等人,2004)培养人βFGF依赖性NSC细胞系CC-2599,并用于图16(A)-16(C)中所示的数据。

[0350] GPS处理,PI-PLC和菠萝蛋白酶反应:小鼠NSC的GPS处理过程如先前针对MSC所述(Sackstein,R.,Merzaban,J.S.等人,2008)。简言之,首先收获神经球,并在37℃用PBS/EDTA(0.02%)孵育15分钟将其解离成单个细胞。然后用HBSS洗涤细胞,计数,并重新悬浮于含60mU/ml的FTVI的HBSS中(不含Ca^{2+}或Mg^{2+}),其中含有20mM HEPES、0.1%人血清白蛋白和1mM GDP-岩藻糖,在37℃处理40分钟。在37℃用0.1U/ml进行PI-PLC反应1小时。菠萝蛋白酶反应在HBSS+2%BSA+0.1%菠萝蛋白酶中,在37℃进行1小时。

[0351] 流式细胞术。用PBS/2%FBS对等份的细胞(2×10^5个细胞)进行洗涤,并与一抗mAb或同型对照mAb(无缀合的或荧光染料缀合的)孵育。在PBS/2%FBS中对细胞进行洗涤,用于间接免疫荧光,与合适的荧光染料缀合的抗同种型二抗孵育。洗涤细胞后,使用Cytomics FC 500MPL流式细胞仪(Beckman Coulter Inc.,Fullerton,CA)测定FITC或PE荧光强度。

[0352] 免疫沉淀研究:将BT-NSC或GPS-NSC的细胞裂解物与免疫沉淀抗体或与适当的同种型对照孵育,然后与蛋白G-琼脂糖一起孵育。使用含有2%NP-40、1%SDS的缓冲液A充分

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洗涤免疫沉淀物。在一些实验中，如先前所述，用N-糖苷酶F(New England Biolabs)处理免疫沉淀物(Dimitroff,C.J.,Lee,J.Y.等人，2001)。对于Western印迹分析，将所有免疫沉淀物在还原性样品缓冲液中稀释，煮沸，然后进行SDS-PAGE，转移至PVDF膜，并用HECA-452或E-Ig进行免疫染色。

[0353] Western印迹分析：使用含2％NP-40的缓冲液A(150mM NaCl、50mM Tris-HCl，pH7.4、1mM EDTA、20μg/ml PMSF、0.02％叠氮化钠；蛋白酶抑制剂混合片(Roche MolecularBiochemicals))裂解BT-和GPS-NSC。如前所述，在还原条件下对经过定量的蛋白质裂解物或免疫沉淀的蛋白质进行Western印迹(Dimitroff,C.J.,Lee,J.Y.等人，2001)。使用Lumi-Light Western Blotting Substrate(Roche)进行化学发光以显现印迹。

[0354] 平行板流动室粘附测定：使用平行板流动室测定(Glycotech;Gaithersburg,MD)比较BT-NSC和GPS-NSC的E-选择素结合能力。吸取NSC(0.5×10^6个细胞/ml，悬浮在HBSS/10mM HEPES/2mM $CaCl_2$溶液中)于汇合的HUVEC单层上。最初，允许NSC以0.5达因/cm^2的剪切应力接触HUVEC单层，随后调节流速以施加0.5至30达因/cm^2的剪切应力。在剪切应力为0.5、1、2、5、10、20和30达因/cm^2，对在最终15秒间隔内粘附于HUVEC单层的BT-或GPS-NSC的数量进行定量。每次测定进行至少3次，取平均值。在用于粘附测定前，通过向测定缓冲液中加入5mM EDTA以螯合选择素结合所需的Ca^{2+}或用功能阻断性抗人E-选择素mAb在37℃处理HUVEC 15分钟来进行对照测定。

[0355] 印迹滚动测定：先前已经有印迹滚动测定法的描述(Dimitroff,C.J.,Lee,JY.等人，2000;Fuhlbrigge,R.C.,King,S.L.等人，2002;Sackstein,R.和Fuhlbrigge,R.2006)，并在本文用于检测通过SDS-PAGE解析的NSC膜蛋白的选择素结合活性。用抗CLA(HECA-452)对NSC膜制备物的Western印迹进行染色，并浸入含有10％甘油的DMEM中使其呈半透明。将CHO-E细胞(5×10^6/mL)重新悬浮于含有2mM $CaCl_2$和10％甘油的DMEM中。将印迹置于平行板流动室下，并且在1.0达因/cm^2的生理相关剪切应力灌注CHO-E细胞，进行体积流速的调节以抵消由于在流动介质中存在10％甘油造成的粘度增加。使用分子量标记物作为引导物，以帮助流动室在目的染色带上的放置。将每平方毫米的相互作用细胞的数量作为分子量区域的函数进行列表并编制成粘附分布图。通过在流动介质中灌注含有5mM EDTA的CHO-E细胞悬浮液来评估非特异性粘附。

[0356] 免疫用于EAE诱导和神经干细胞注射：NSC或骨髓HSPC(Lineage^{neg}C-kit^{pos})用GPS处理或不处理，并且在用MOG 35-55免疫(在侧腹皮下；细节见补充材料)后(PI)第9天和第13天将1×10^6个细胞注射到C57BL/6小鼠的尾静脉。以盲实验方式为EAE评分；研究者没有参与注射，并不知道各组的组成。“补充材料”中列出了所使用的分级尺度的细节。将BT-NSC、GPS-NSC、BT-HSPC或GPS-HSPC于体积为0.2ml的HBSS中的细胞悬浮液注射入EAE小鼠的尾静脉中。使用注射单独HBSS的假处理小鼠(无NSC)作为阴性对照。

[0357] 短期归巢研究：将BT-NSC和GPS-NSC在室温下于含有10％FBS的RPMI中用5mM CFDA-SE(Invitrogen)标记5分钟，并在免疫后(PI)第9天和第13天静脉内注射到MOG-处理的C57BL6小鼠中。每天对每只小鼠注射于0.2mL HBSS中的200万个NSC。使用仅HBSS缓冲液来确定背景信号。将BT-NSC和GPS-NSC也注射到未用MOG免疫的动物中，并用于标准化每种所测定的组织中观察到的信号。在第二次NSC转移后16小时，处死小鼠，并用不含Ca^{2+}和Mg^{2+}的1×PBS灌注。分离脑、脊髓、淋巴结、脾、肝和肺。将脑和脊髓匀浆。将所得团块重新悬浮于

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0.25％胰蛋白酶-EDTA(Invitrogen)中，并在37℃孵育10分钟。使用含有0.01％SBTI、0.001％DNA酶和0.075％BSA的DMEM终止消化过程。机械解离淋巴结、脾和肝，并通过流式细胞术在FL1通道中评估所得单细胞悬浮液的CFDA-SE阳性细胞的频率。分析流式细胞术数据，并将数据表示为所扫描的200,000个细胞中检测到的在设置为包括NSC的窄门内的CFDA-SE阳性事件的百分比。该门是基于将培养的NSC与从每个测试组织(脑、脊髓、淋巴结、脾、肝和肺)分离的细胞悬浮液混合而确定的。

[0358] NSC向CNS迁移的分析：用PKH26染料(Invitrogen)标记BT-NSC、GPS-NSC，并在免疫后(PI)第9天和第13天静脉内注射到MOG处理的C57BL6小鼠中。每天在每只小鼠中注射于0.2mL的HBSS中的100万个NSC。使用仅HBSS缓冲液来确定背景信号。在第二次NSC转移后四天，处死小鼠并用不含Ca^{2+}和Mg^{2+}的1x PBS灌注，收获腰骶脊髓。选择脊髓是因为在B6模型中，前脑中的CNS损伤在尺寸和位置上与脊髓相比是非常可变的，脊髓具有更可预测的位置(腰骶部区域)并具有更丰富的病理(Chitnis,T.,Najafian,N.等人，2001;Rasmussen,S.,Wang,Y.等人，2007;Wang,Y.,Imitola等人，2008)。然后将脊髓匀浆，并将得到的团块重新悬浮于0.25％胰蛋白酶-EDTA(Invitrogen)中，并在37℃孵育10分钟，用于FACS分析。使用含有0.01％SBTI、0.001％DNA酶和0.075％BSA的DMEM终止消化过程。将脑和脊髓在液氮中快速冷冻并储存在-80℃直到进行切片，用于组织学分析。腰骶脊髓或前脑、中脑和后脑的冷冻切片(20μm)用4％多聚甲醛固定15分钟，然后用目的抗体染色，通过抗Flk-1(VEGFR2,Sigma)显现血管，而NSC用抗-sox-2(Millipore)进行染色或用PKH26染料以显现为红色。然后对脊髓匀浆，将所得的团块重新悬浮于0.25％胰蛋白酶-EDTA(Invitrogen)中，并在37℃孵育10分钟。使用含有0.01％SBTI、0.001％DNA酶和0.075％BSA的DMEM终止消化过程。通过Flk-1(VEGFR2)染色显现血管。

[0359] 免疫用于EAE诱导和NSC/HSPC注射：NSC或HSPC($Lineage^{neg}C\text{-}kit^{pos}$)用GPS处理或不处理，并且在用MOG 35-55免疫后(PI)第9天和第13天将1×10^{6}个细胞注射到C57BL/6小鼠的尾静脉中。对应于小鼠序列的MOG 35-55(M-E-V-G-W-Y-R-S-P-F-S-R-O-V-H-L-Y-R-N-G-K)由QCB公司Division of BioSource International(Hopkinton,MA)合成，并通过HPLC纯化至>99％。使用于0.1ml PBS和0.1ml含有0.4mg结核分枝杆菌(H37Ra,Difco,Detroit,Michigan)的CFA中的150-200μg MOG肽在两侧皮下免疫小鼠，并在免疫接种当天和2天后腹膜内注射200ng百日咳毒素(List Laboratories,Campbell,California)。以盲实验方式为EAE评分；研究者没有参与注射，并不知道各组的组成。使用以下分级：1级，尾无力或单纯步态虚弱而没有尾无力；2级，部分后肢麻痹；3级，全后肢麻痹或部分后肢和前肢麻痹；4级，全后肢和部分前肢麻痹；5级，濒死或死亡动物。将BT-NSC、GPS-NSC、BT-HSPC、GPS-HSPC于体积为0.2ml的HBSS中的细胞悬浮液注射入EAE小鼠的尾静脉中。使用注射单独HBSS的假处理小鼠(无NSC)作为阴性对照。

[0360] 免疫组织化学染色：在处死之前，用50ml生理盐水灌注小鼠以除去任何血管内的PBMC。用10ml于PBS中的4％多聚甲醛心脏内灌注动物用于共聚焦成像。去除脑和脊髓并将其包埋在O.C.T中，在液氮中快速冷冻，并保持在-70℃直至切片。用丙酮或4％多聚甲醛固定脊髓的冷冻切片(10μm)，然后用目的抗体标记。同种型匹配的Ig和不加一抗作为阴性对照。在至少三种不同水平的切片对每个标本进行评价。在40X放大倍数下评价整个组织切片(纵向脊髓切片)的给定细胞标记物情况。在每个切片中取十个40X(高倍视野)视野中计数

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对于给定标记物染色阳性的细胞数量。将一个切片的结果相加，并且取各切片结果的平均数。

[0361] 共焦显微镜的染色：在PBS中洗涤经多聚甲醛固定的切片(40μm)，用含有4％山羊血清、0.3％BSA和0.3％triton的PBS对其进行封闭，并随后将其与封闭液中的一抗孵育过夜，并与封闭液中的二抗中孵育2小时。我们使用经高度交叉吸附的二抗来避免交叉反应(Alexa 488和Alexa 594)。使用Zeiss Laser Scanning Microscope 3D分析软件(Zeiss，Thornwood，NY)进行共焦显微镜成像，具有多轨捕获操作流程，以避免两种荧光染料的潜在重叠。

[0362] 在体外GPS处理对NSC分化、自我更新能力和免疫抑制作用的作用：小鼠NSC的GPS处理方法如前文对MSC所述(Sackstein，R.，Merzaban，J.S.等人，2008)。对其自我更新、形成神经球、分化成$MAP2^+$神经元及抑制经ConA活化的淋巴结细胞的增殖的能力在体外比较BT-和GPS-NSC。NSC分化和自我更新能力：将最初培养在含FGF/EGF的培养基中的神经球铺板在允许增殖的聚D-赖氨酸(PDL)包被的玻璃盖玻片上，然后收获，并用缓冲液(对照)处理或用FTVI(60mU/mL)进行酶处理1小时，随后将所得到的BT-NSC和GPS-NSC以20个细胞每μl的集落密度铺板并允许增殖为神经球96小时至5天。用Axiovision显微镜(NY)捕获神经球成像。为进行神经元、星形胶质细胞和少突胶质细胞分化，将解离的神经球铺板在24孔板中PL包被的玻璃盖玻片上，并在不含FGF/EGF但存在含1％Glutamax、1％抗生素/抗真菌剂和2％B27补充剂的神经基础培养基中培养。每隔一天添加新鲜培养基至第5天，然后用MAP2(神经元)、GFAP(星形胶质细胞)和NG2(少突胶质细胞前体)对细胞进行免疫荧光染色。由对处理不知情的研究者使用标准体视学技术计数$MAP2^+$、$GFAP^+$和$NG2^+$细胞。神经干细胞和淋巴结细胞的共培养：从初始C57BL/6小鼠中分离淋巴结。淋巴结细胞(LNC)以单细胞悬浮液在96孔板中以每孔2×10^5个细胞进行培养，如前所述(Einstein，O.，Fainstein，N.等人，2007)。培养基由补充有10％胎牛血清、L-谷氨酰胺、丙酮酸钠、非必需氨基酸、2-巯基乙醇、HEPES和抗生素的RPMI-1640(BioWhittaker)组成，其含有或不含2.5μg/ml刀豆球蛋白A(ConA，Sigma)。解离神经球，并且首先用GPS或不用(BT)进行处理，然后用3000Rad照射。照射后，以与经ConA刺激的LNC的不同比率，将解离的NSC直接加入到LNC培养基中。NSC数量与LNC数量的测试比率为：1:4、1:2、1:1、2:1和4:1。然后将细胞培养48小时，之后加入胸苷。16小时后进行胸苷并入测定。

[0363] 在用炎性细胞因子处理NSC后评估E-选择素配体：将1.5×10^6个细胞/孔接种在每孔含有3ml增殖培养基的6孔板中，并用10ng/mlTNFα(R&D；410-TRNC)、10ng/ml IL-1β(R&D；401-ML)、10ng/ml IFNγ(R&D；485-MI)单独或组合(全部为10ng/ml)进行刺激。24h和48h后，通过离心收获神经球，并用E-Ig进行染色用于流式细胞术分析。

[0364] LIF mRNA的测量：用或不用炎性细胞因子(10ng/ml的IFN-γ和15ng/ml的TNF-α)对1×10^6个小鼠NSC处理24小时。然后使用Trizol试剂提取总RNA，并使用Agilent 2200TabStation系统测量提取的RNA的质量。使用高容量cDNA逆转录试剂盒(Applied Biosystems)和Random Hexamer进行cDNA合成。使用RT-PCR测量mRNA水平，并使用$2^{-\Delta\Delta CT}$法计算基因表达的倍数变化。LIF基因的正向引物序列为CCTACCTGCGTCTTACTCCATCA，反向引物为CCCCAAAGGCTCAATGGTT(Sigma)。LIF mRNA的相对表达是相对于GAPDH管家基因测定的，对于后者TGCACCACCAACTGCTTAGC用作正向引物，GGCATGGACTGTGGTCATGAG作为反向引物。

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[0365] NSC向CNS迁移的分析:用PKH26染料(Invitrogen)标记BT-NSC、GPS-NSC,并在免疫后(PI)第9天和第13天静脉内注射到MOG处理的C57BL6小鼠中。每天每只小鼠注射100万个NSC。使用仅HBSS缓冲液来确定背景信号。在第二次NSC转移后24小时或第4天,处死小鼠,灌注,收获腰骶脊髓。选择脊髓是因为在B6模型中,前脑中的CNS损伤在尺寸和位置上与脊髓相比是非常可变的,脊髓具有更可预测的位置(腰骶部区域)并具有更丰富的病理(Chitnis,T.,Najafian,N.等人,2001;Rasmussen,S.,Wang,Y.等人,2007;Wang,Y.,Imitola,J.等人,2008)。然后将脊髓匀浆,将得到的团块重新悬浮于0.25%胰蛋白酶-EDTA中,并在37℃孵育10分钟,用于流式细胞术分析。使用含有0.01%SBTI、0.001%DNA酶和0.075%BSA的DMEM终止消化过程。将脑和脊髓在液氮中快速冷冻并储存在-80℃直到进行切片,用于组织学分析。腰骶脊髓或前脑、中脑和后脑的冷冻切片(20μm)进行固定,然后用目的抗体进行染色。通过抗Flk-1(VEGFR2)显现血管,而NSC用抗-SOX-2进行染色或用PKH26染料以显现为红色。通过不同组动物的立体分析进行细胞定量,用于神经病理学分析。对脊髓和脑进行切片,并对宫颈和腰骶区域的每第三切片进行染色,在高放大倍数下对3-5个切片进行细胞定量。使用具有电动平台的LSM 510共聚焦显微镜用于立体地计算染色强度和总细胞数/每高放大倍数。

[0366] 统计分析-数据表示为平均值±SEM。由双尾ANOVA确定平均值之间的差异的统计学意义。如果平均值显著不同,则事后通过Turkey t检验进行多次比较。统计学显著性定义为$p<0.05$。

[0367] 参考文献-实施例7

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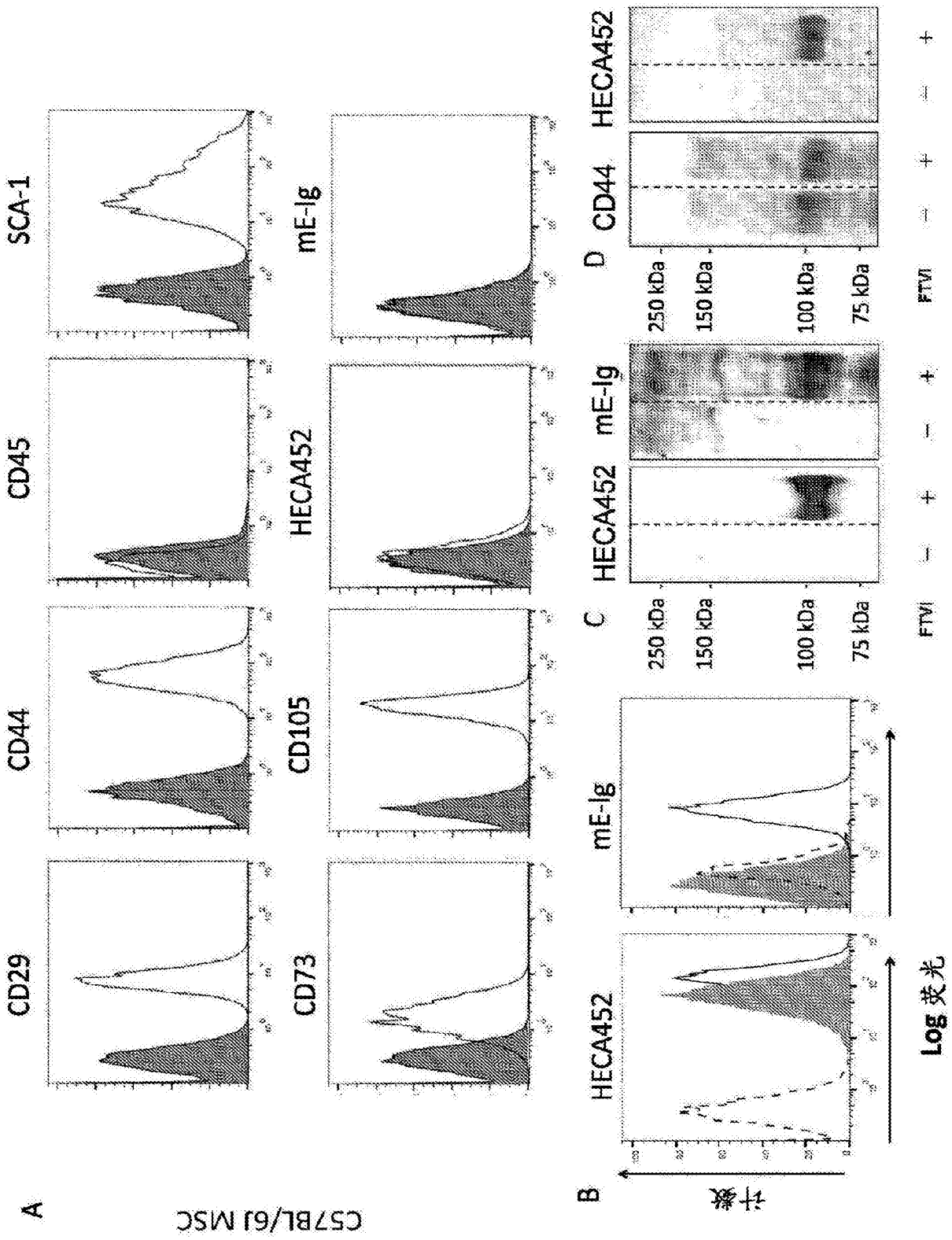

A
C57BL/6J MSC
SCA-1
CD45
CD44
CD29
mE-Ig
HECA452
CD105
CD73
B
HECA452
mE-Ig
计数
Log 荧光
C
HECA452
mE-Ig
250 kDa
150 kDa
100 kDa
75 kDa
FTVI
D
CD44
HECA452
250 kDa
150 kDa
100 kDa
75 kDa
FTVI

图1

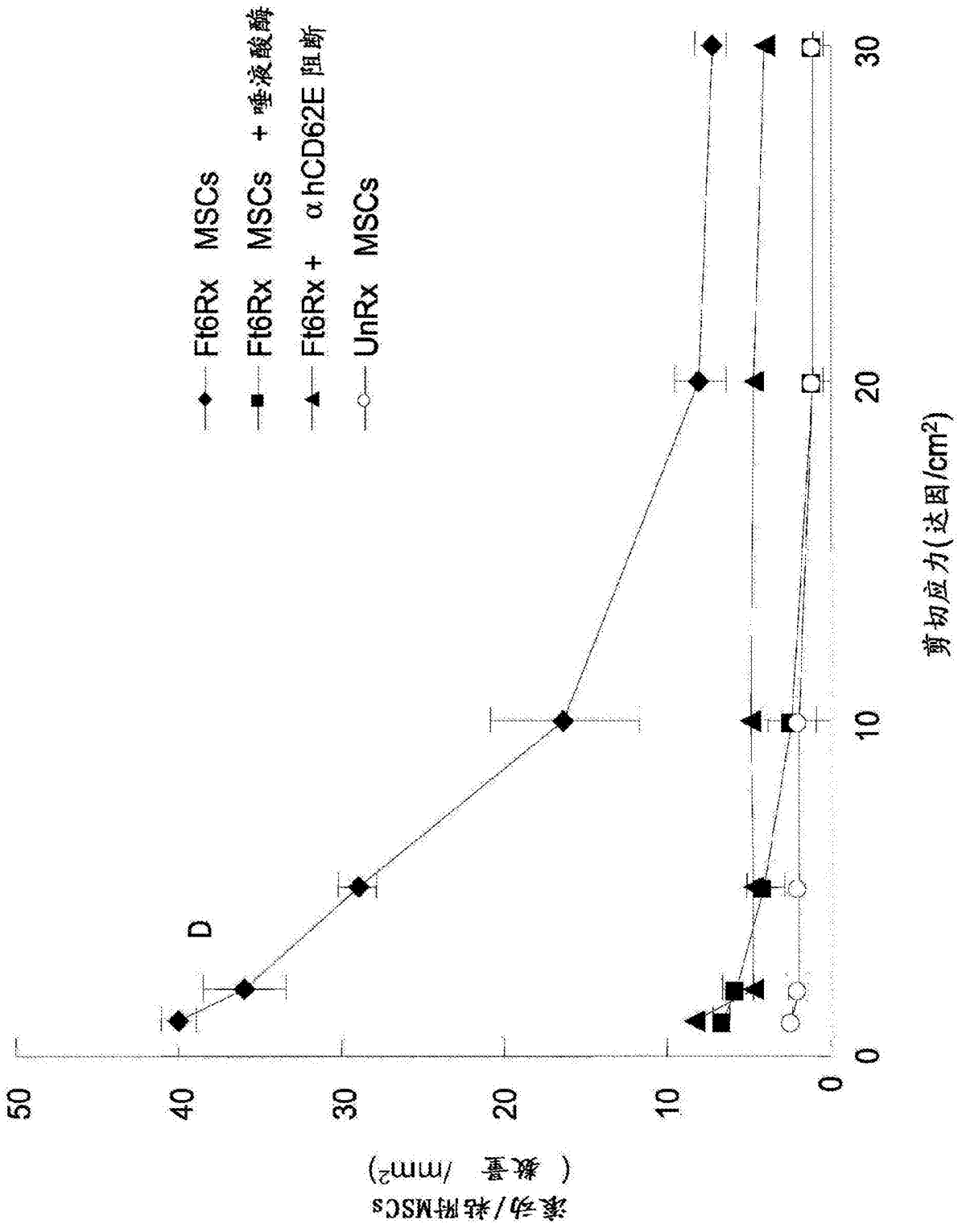

Ft6Rx MSCs
Ft6Rx MSCs + 唾液酸酶
Ft6Rx + αhCD62E 阻断
UnRx MSCs
D
滚动/粘附MSCs
(数量 /mm²)
剪切应力(达因/cm²)
0
10
20
30
40
50
0
10
20
30

图2

打印ID7891292 Pg 74

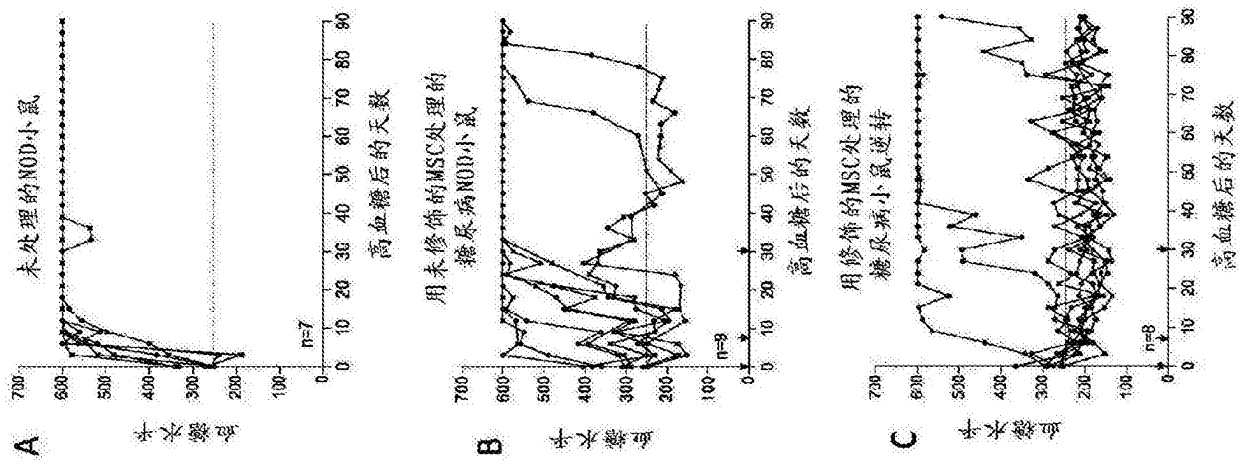


图3

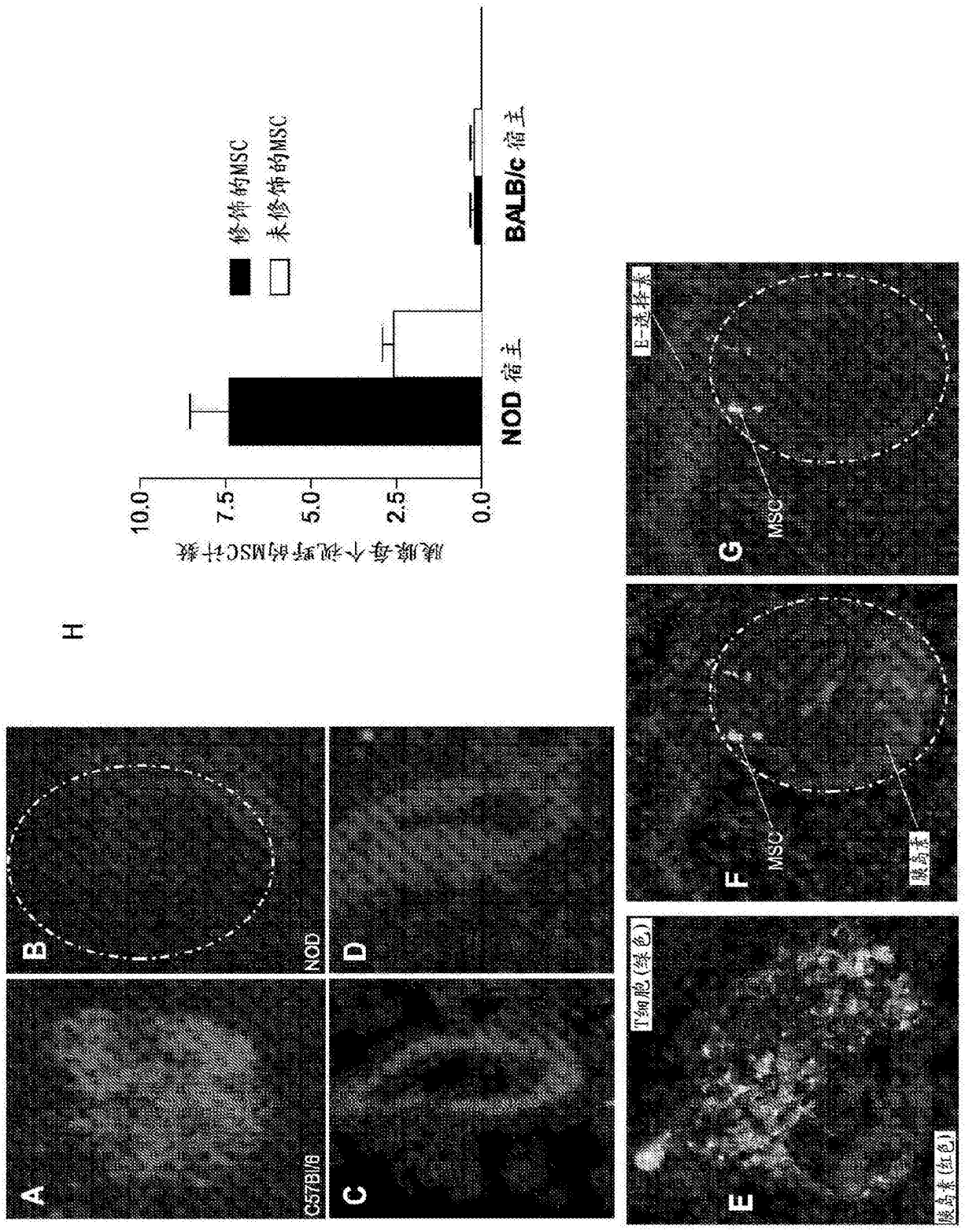

A
C57BL/6
B
NOD
C
D
E
T细胞(绿色)
胰岛素(红色)
F
MSC
胰岛素
G
MSC
E-选择素
H
修饰的MSC
未修饰的MSC
胰腺每个视野的MSC计数
10.0
7.5
5.0
2.5
0.0
NOD 宿主
BALB/c 宿主

图4

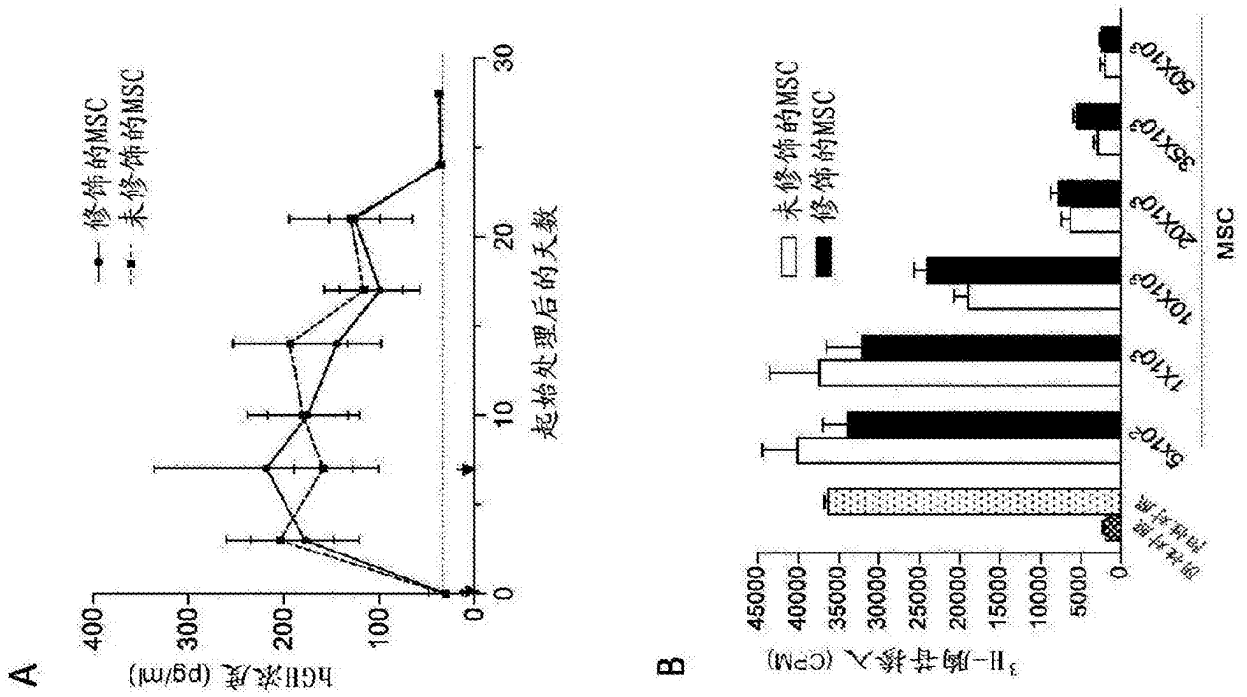

A
hGH浓度 (pg/ml)
400
300
200
100
0
0
10
20
30
起始处理后的天数
修饰的MSC
未修饰的MSC
B
^{3}H-胸苷掺入 (CPM)
45000
40000
35000
30000
25000
20000
15000
10000
5000
0
仅培养基
肾癌对照
5X10^5
1X10^6
10X10^6
20X10^6
35X10^6
50X10^6
MSC
未修饰的MSC
修饰的MSC

图5

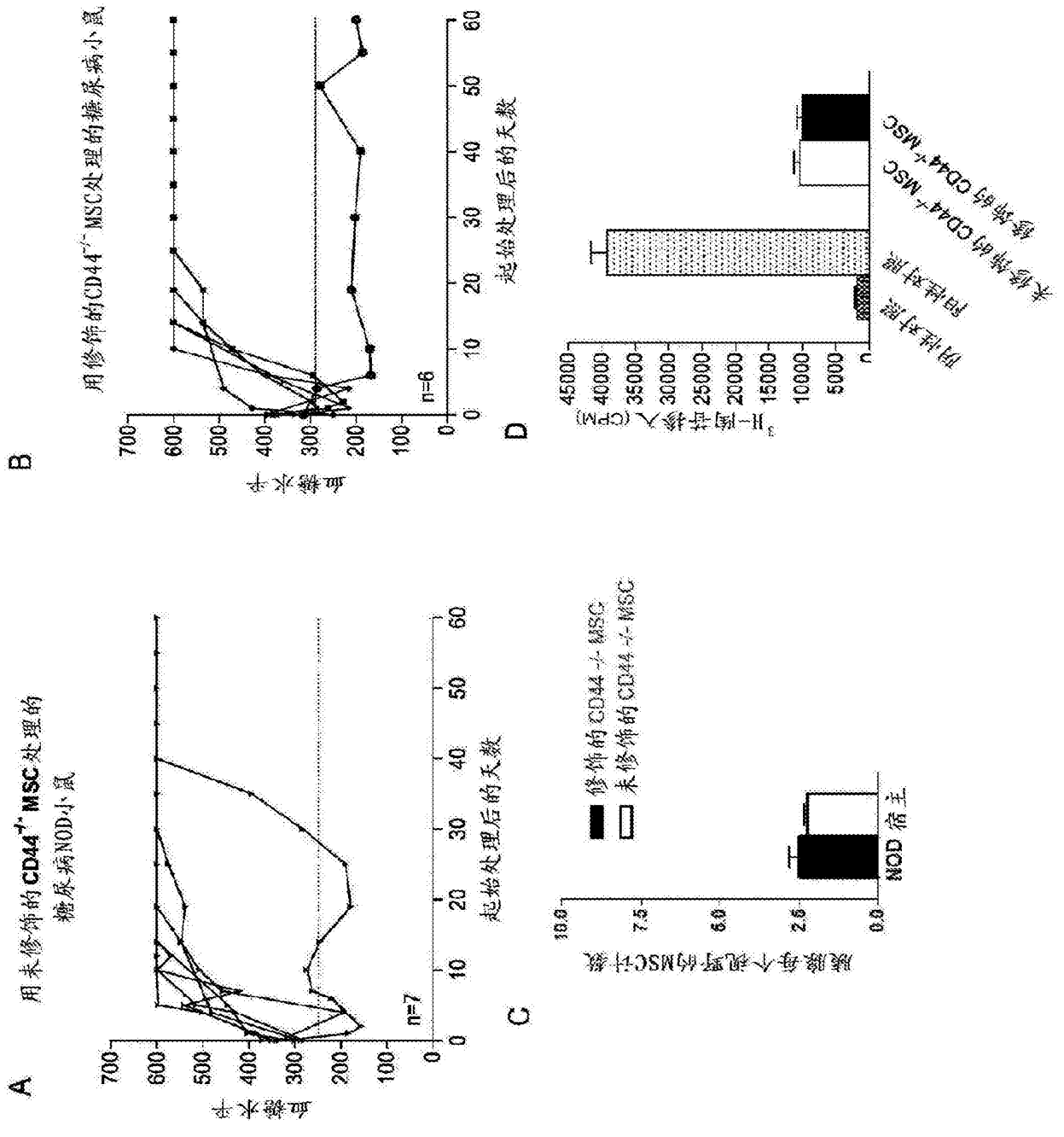

A
用未修饰的CD44-/- MSC处理的
糖尿病NOD小鼠
血糖水平
起始处理后的天数
n=7
B
用修饰的CD44-/- MSC处理的糖尿病小鼠
血糖水平
起始处理后的天数
n=6
C
修饰的CD44-/- MSC
未修饰的CD44-/- MSC
聚集每个视野的MSC计数
NOD 宿主
D
3H-胸苷掺入 (CPM)
n
同性对照
同性对照
未修饰的CD44-/- MSC
修饰的CD44-/- MSC

图6

打印ID7891292 Pg 78

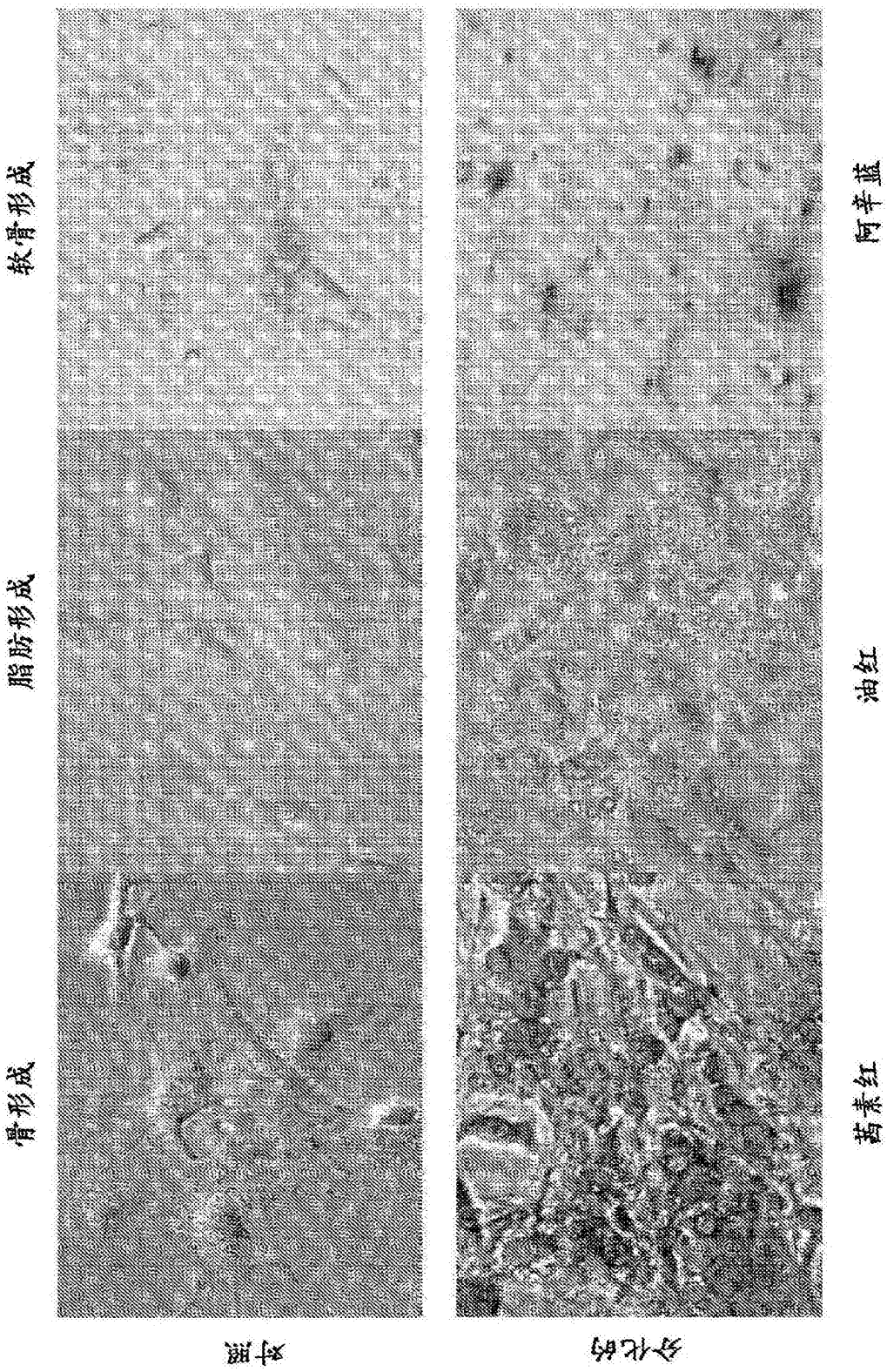


图7

打印ID7891292 Pg 79

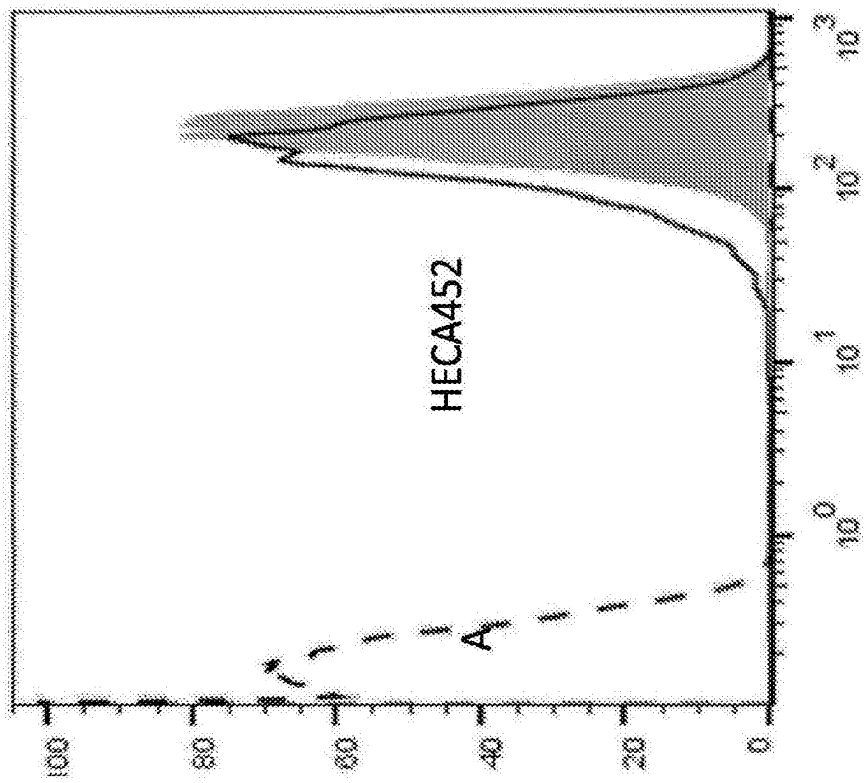
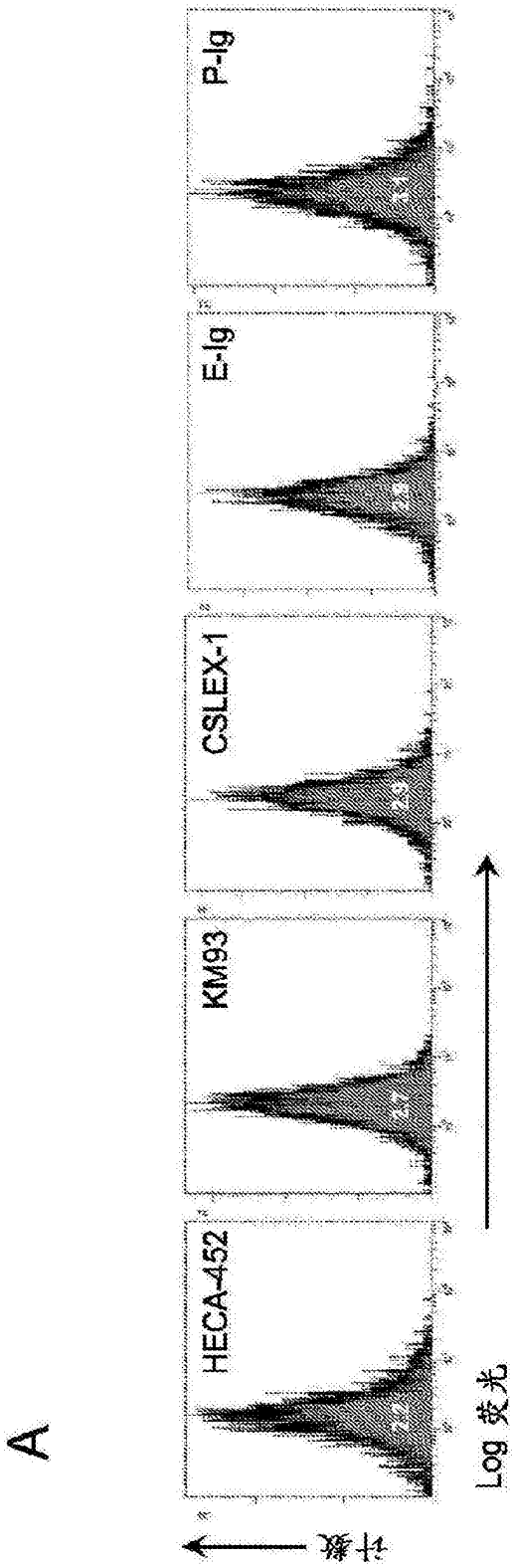
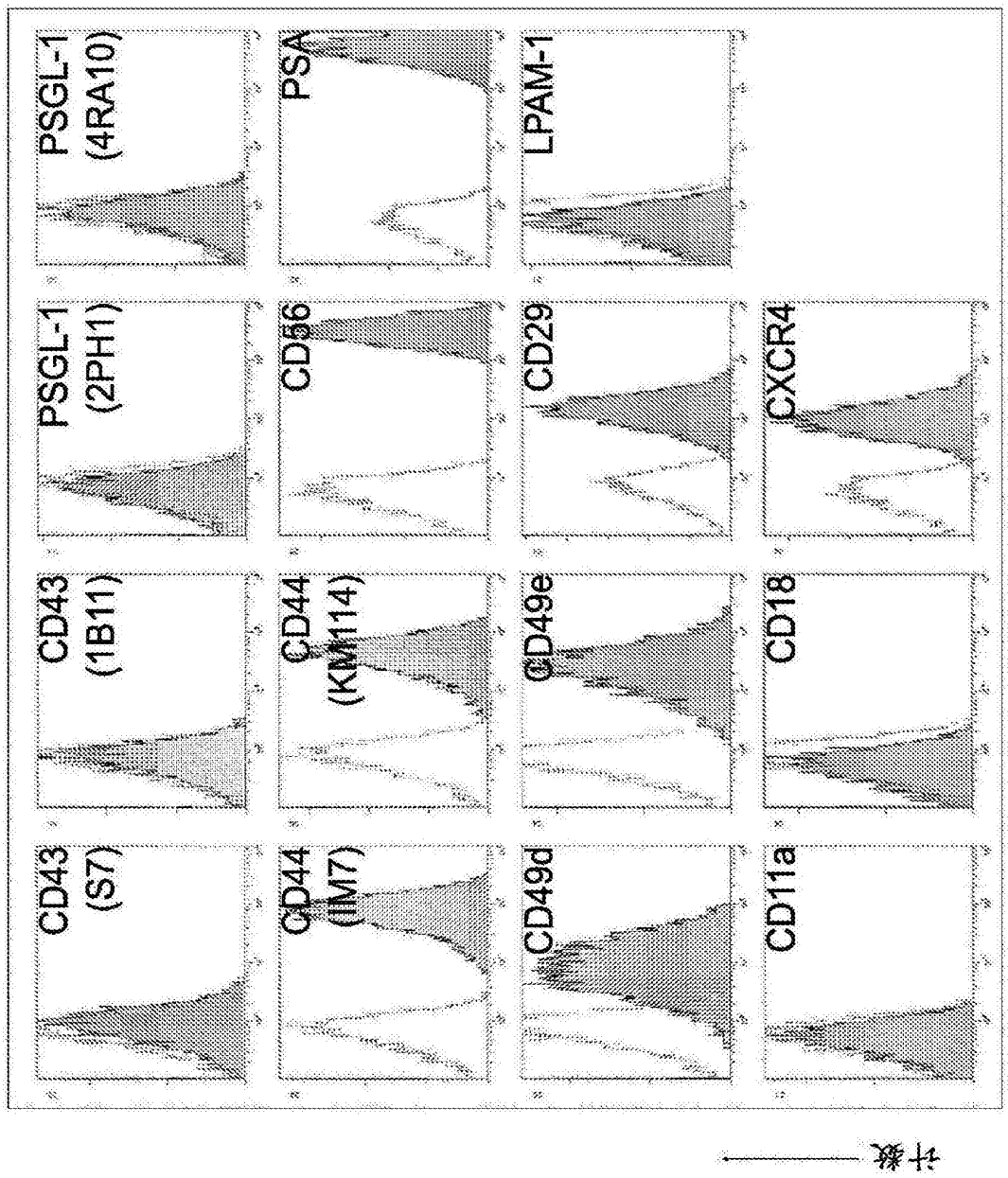


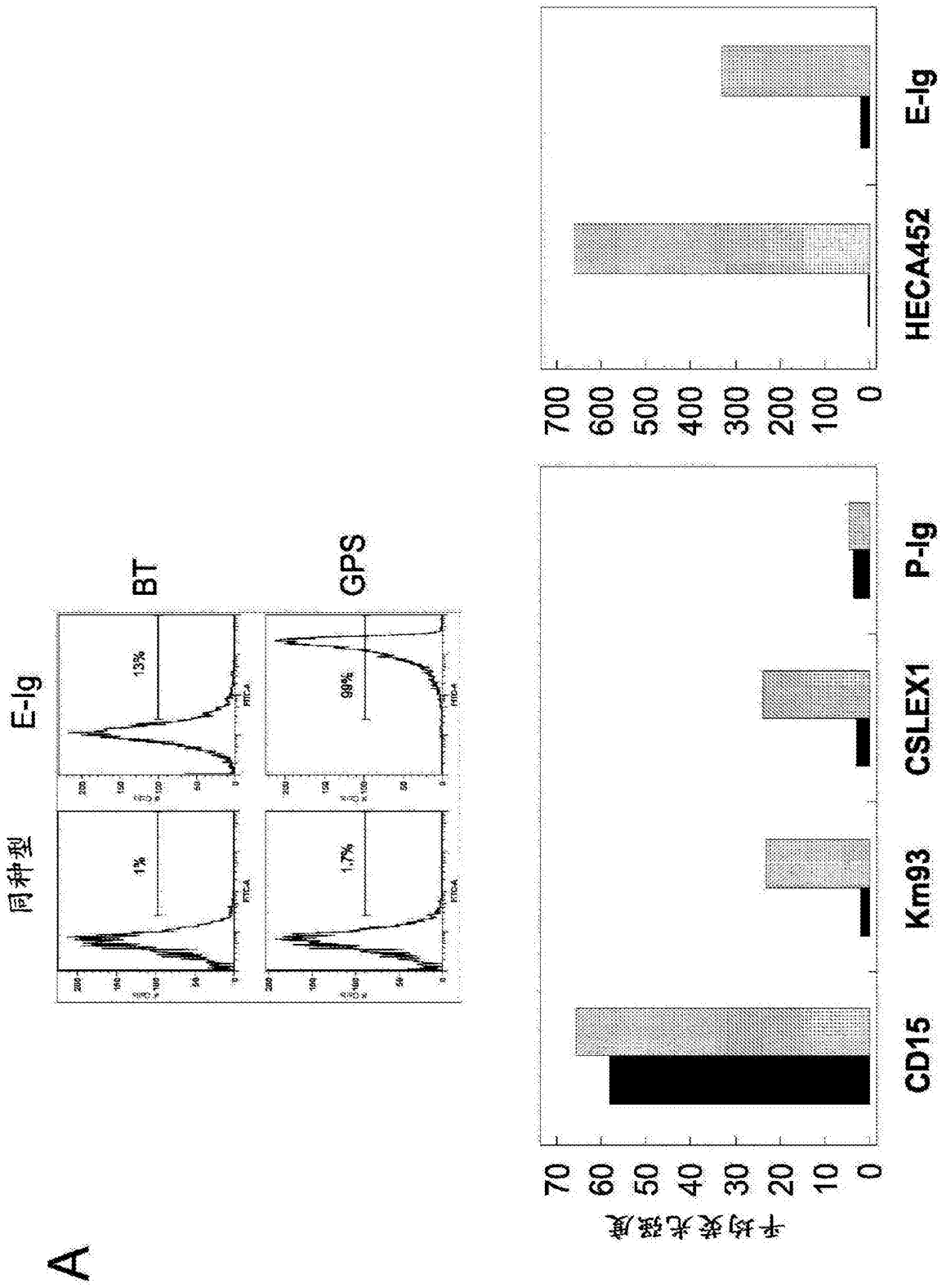
图8

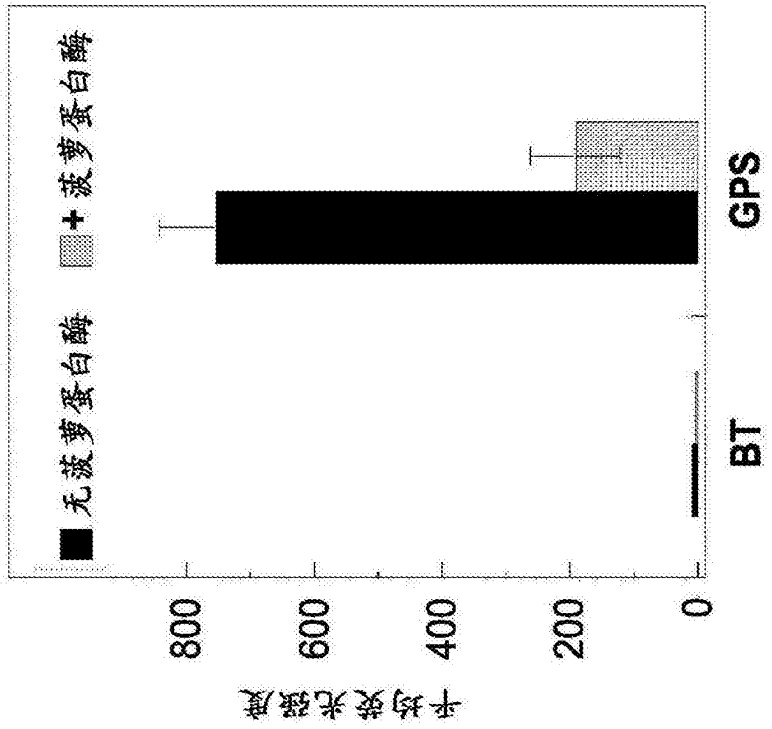

A
HECA-452
KM93
CSLEX-1
E-lg
P-lg
计数
Log 荧光


PSGL-1 (4RA10)
PSA
LPAM-1
PSGL-1 (2PH1)
CD29
CXCR4
CD43 (1B11)
CD18
CD43 (S7)
CD49d
CD11a
计数
Log 荧光

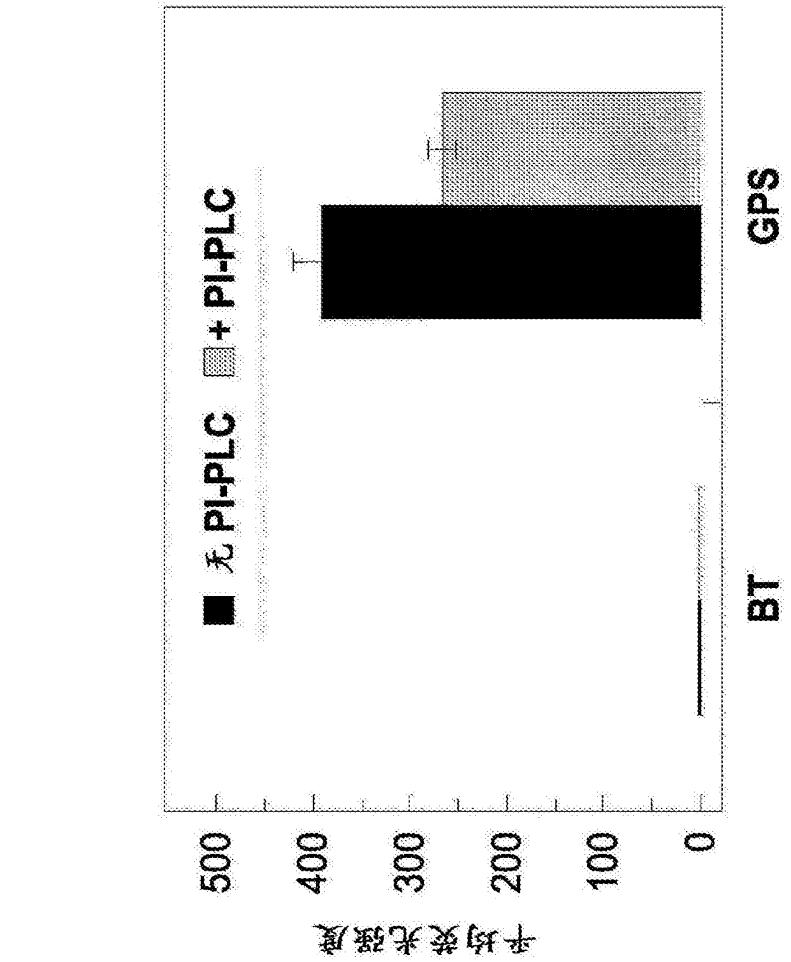
B

图9


A
同种型
E-Ig
BT
GPS
1%
13%
1.7%
99%
平均荧光强度
CD15
Km93
CSLEX1
P-Ig
HECA452
E-Ig

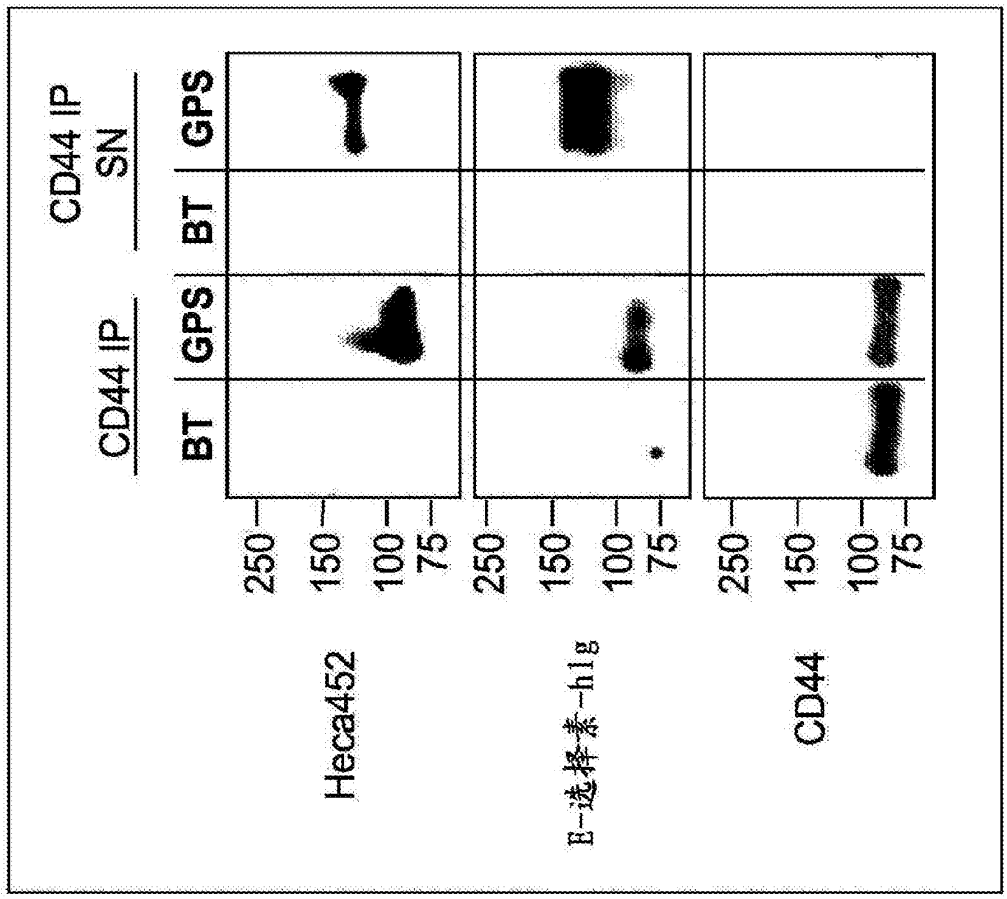


B



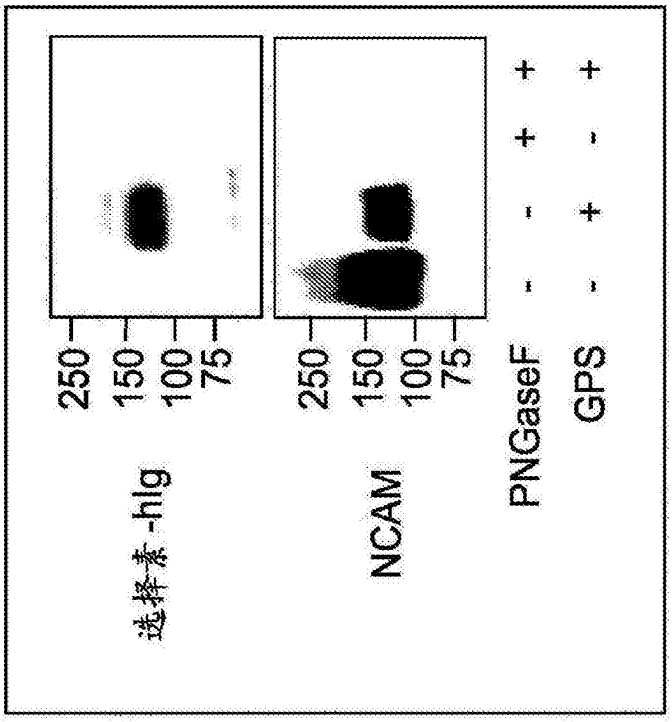
C

图10

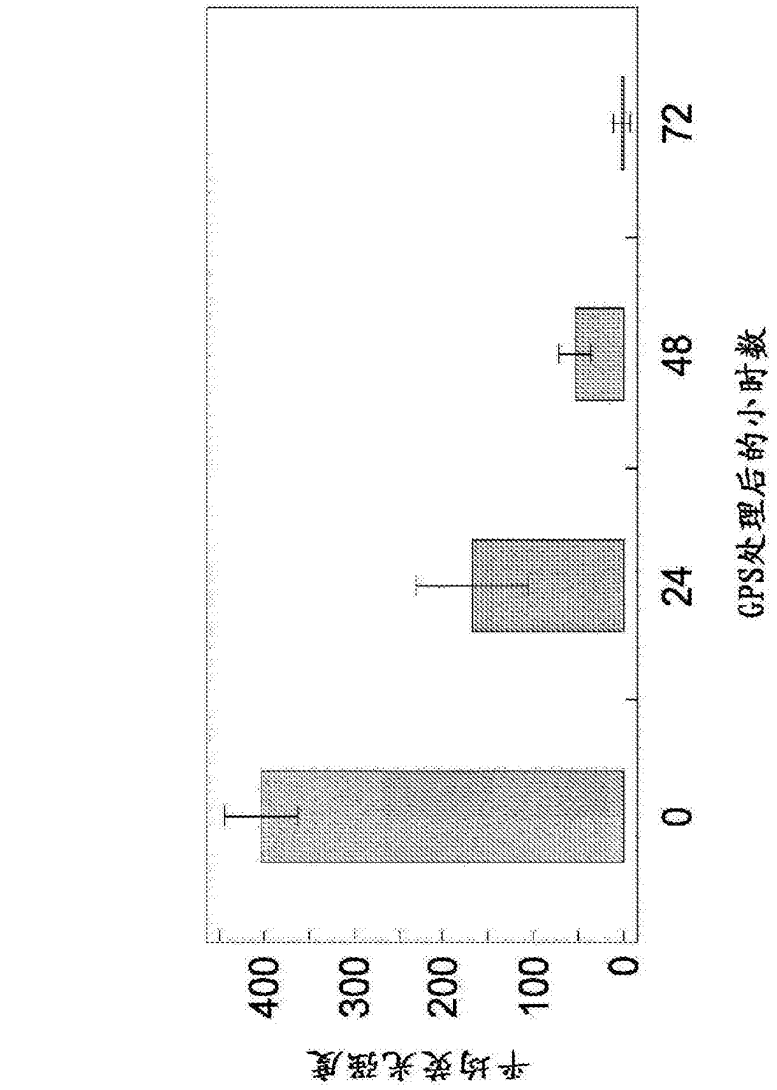

CD44 IP
SN
BT
GPS
CD44 IP
BT
GPS
250
150
100
75
Heca452
E-选择素-hIg
CD44

A

打印ID7891292 Pg 86



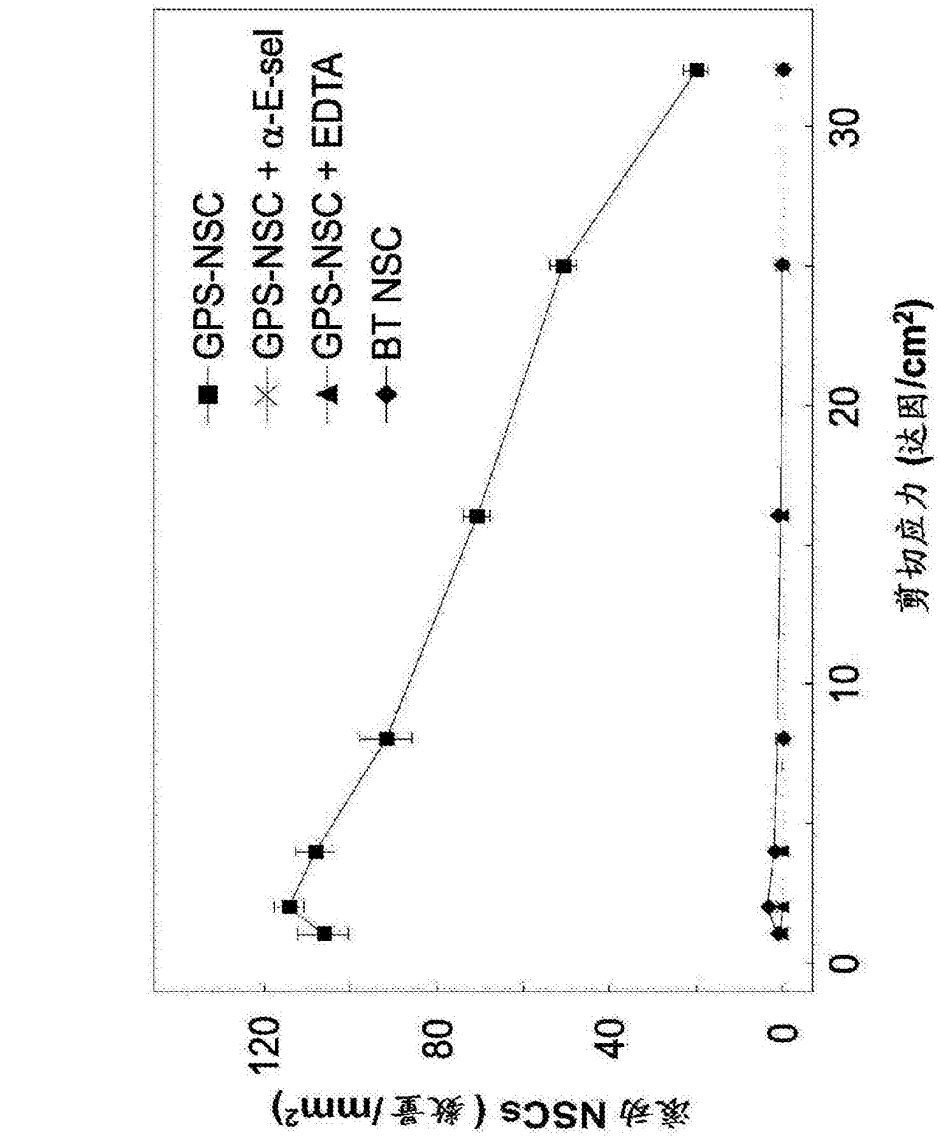
B



C

图11

打印ID7891292 Pg 88



A

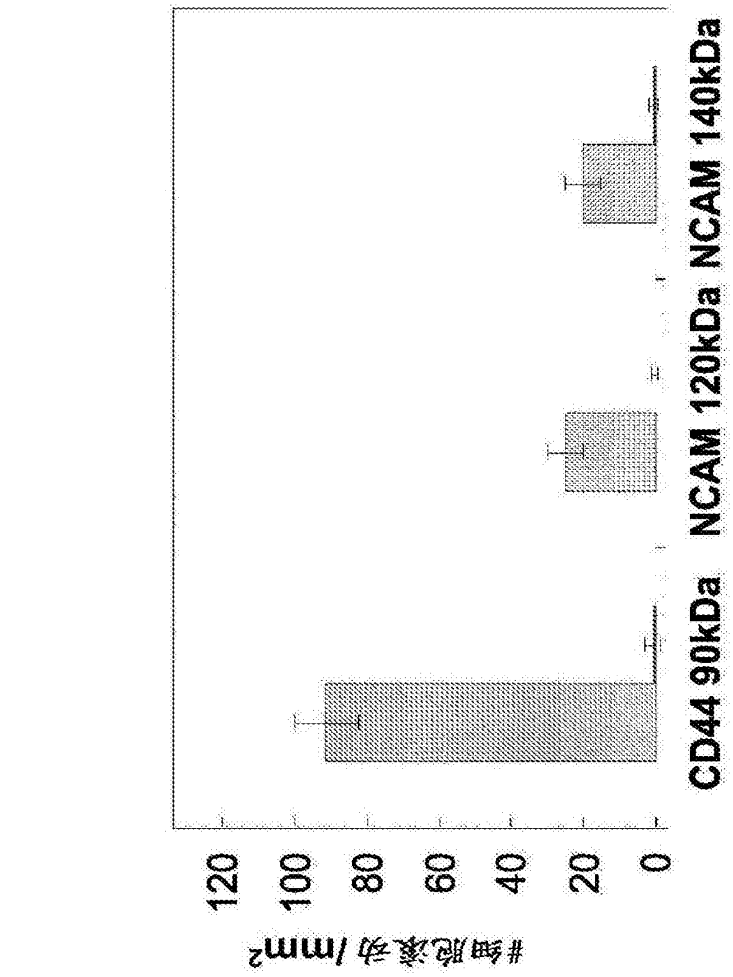
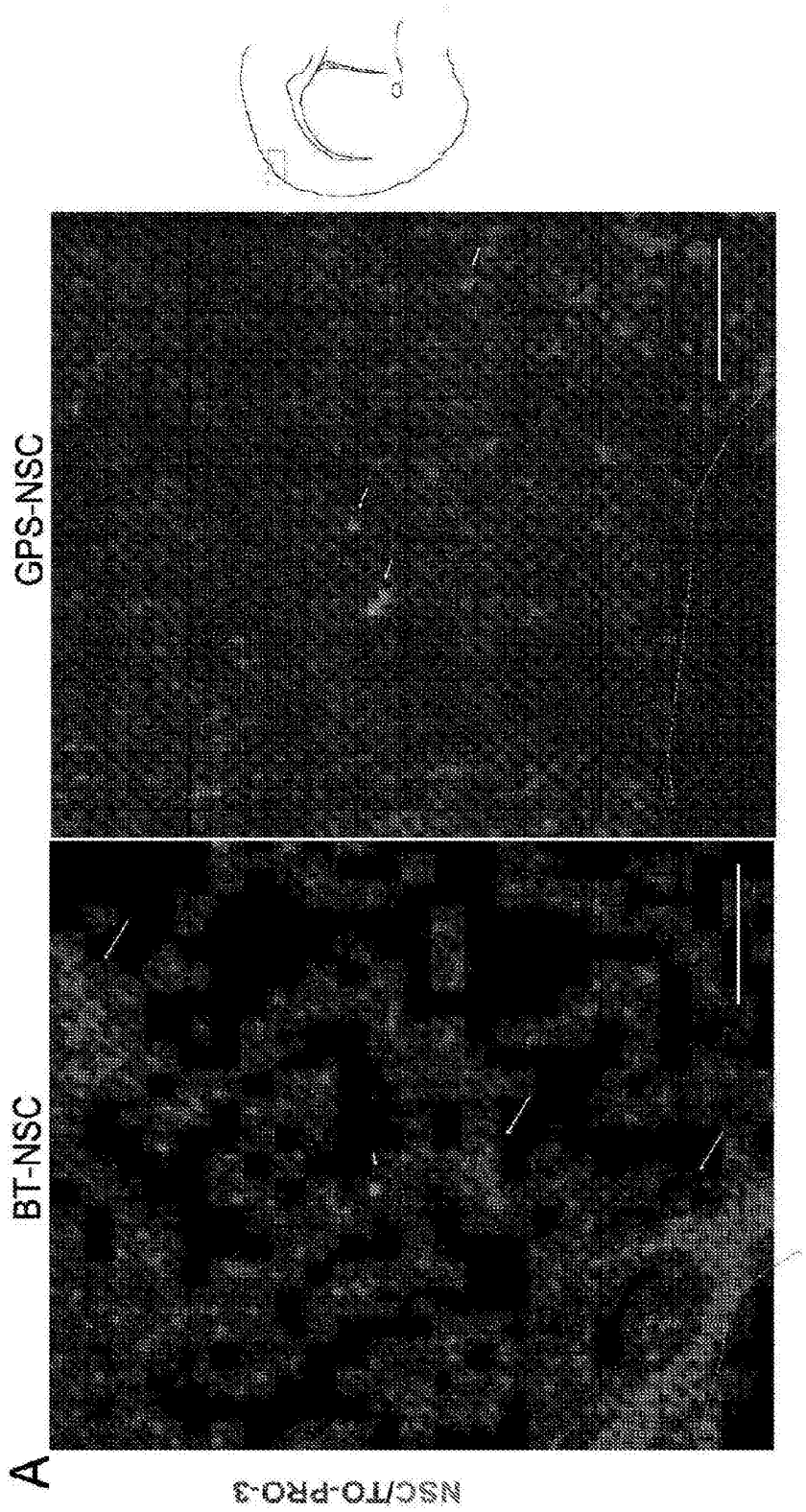
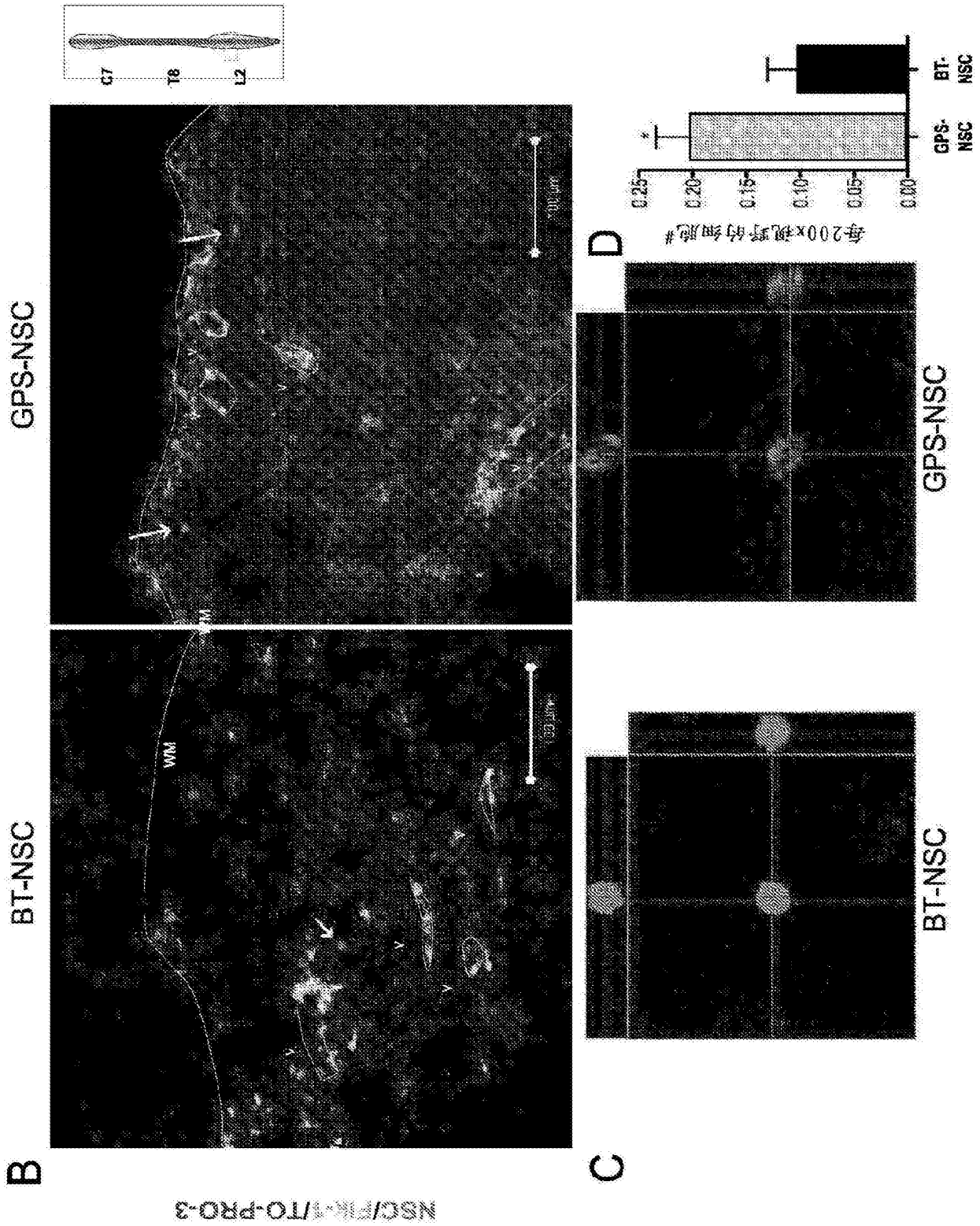

B
#细胞滚动/mm²
120
100
80
60
40
20
0
CD44 90kDa
NCAM 120kDa
NCAM 140kDa

图12

打印ID7891292 Pg 90

FIGURE 13





NSC/PDL-1/TO-PRO-3

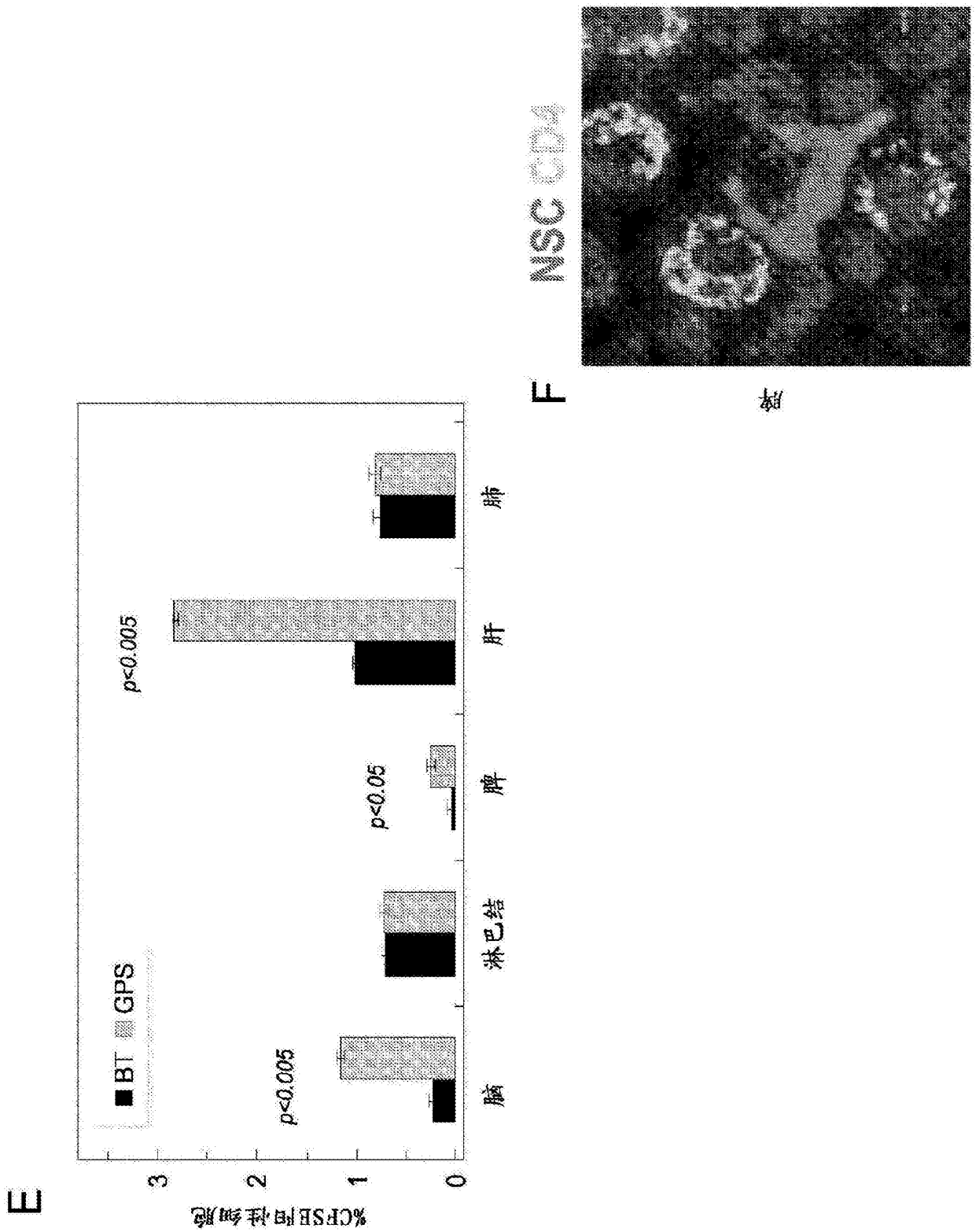
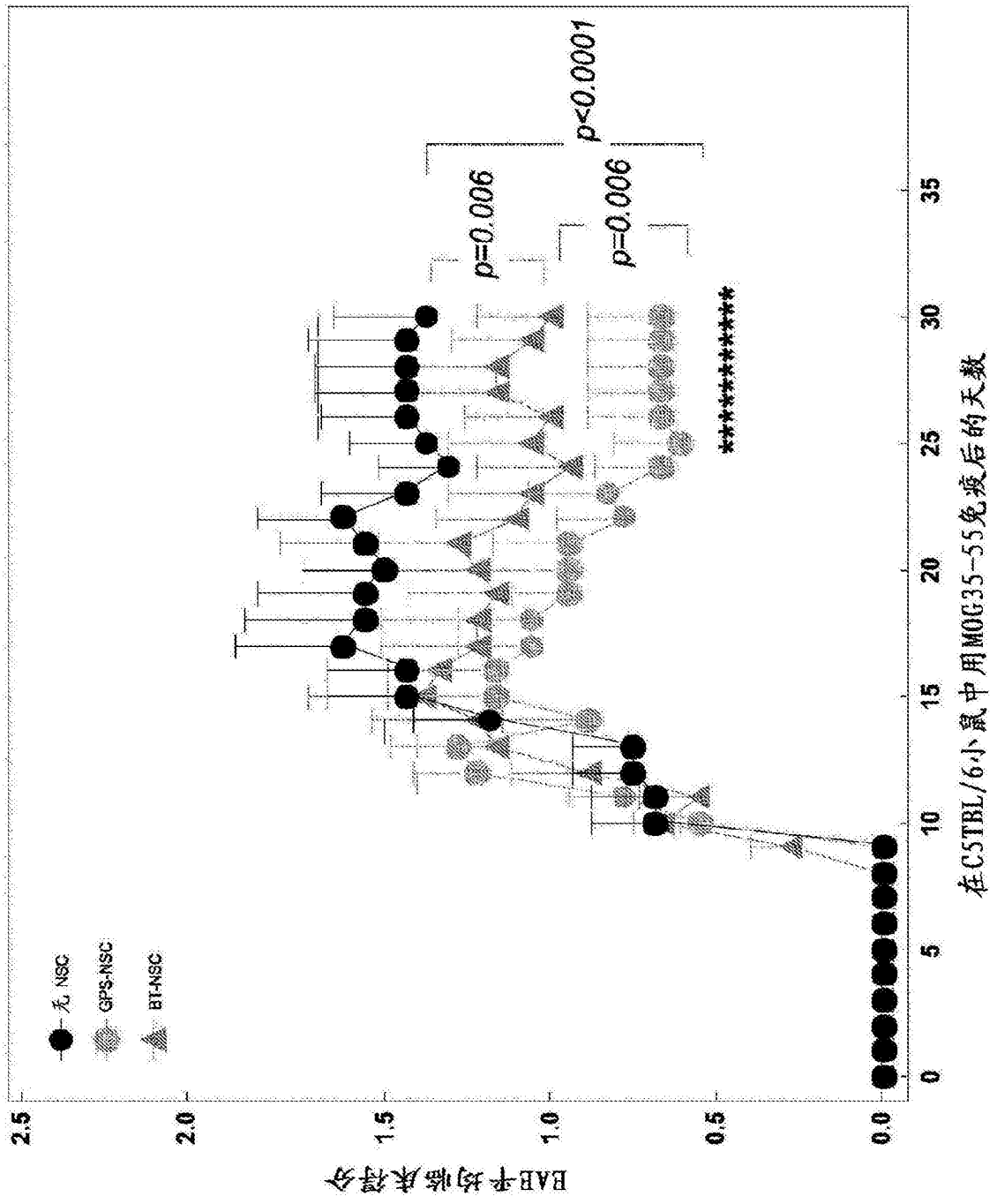
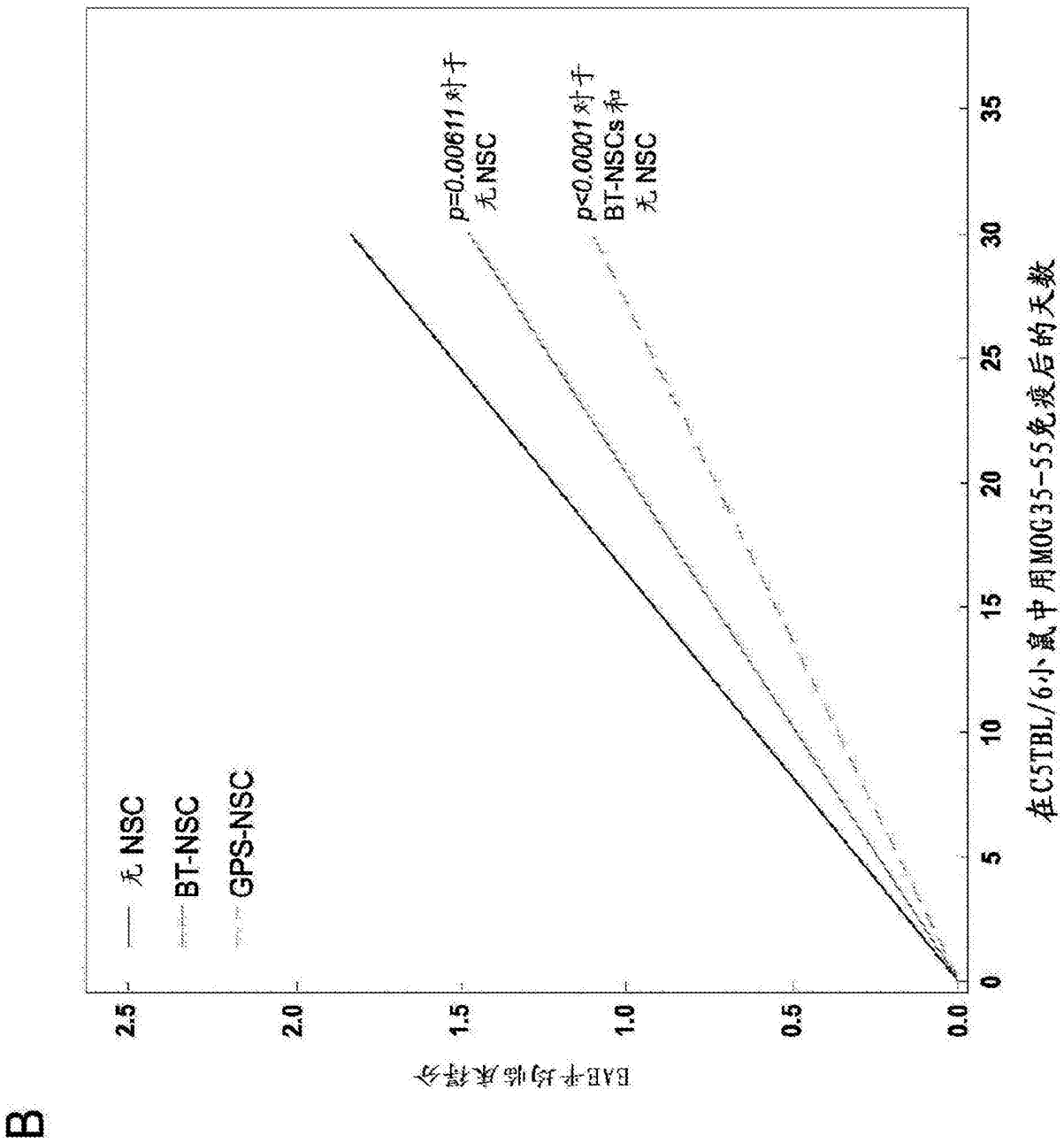
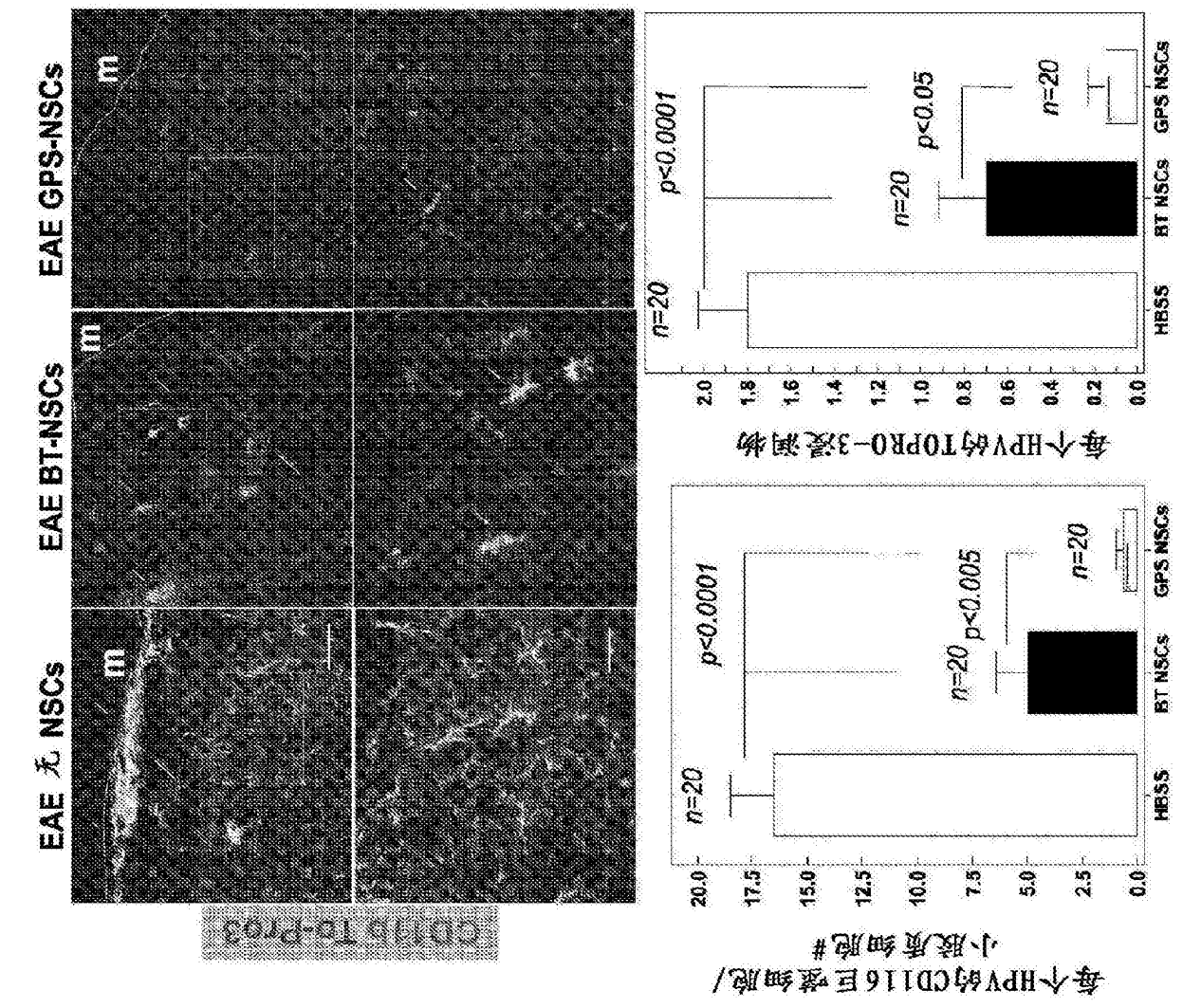


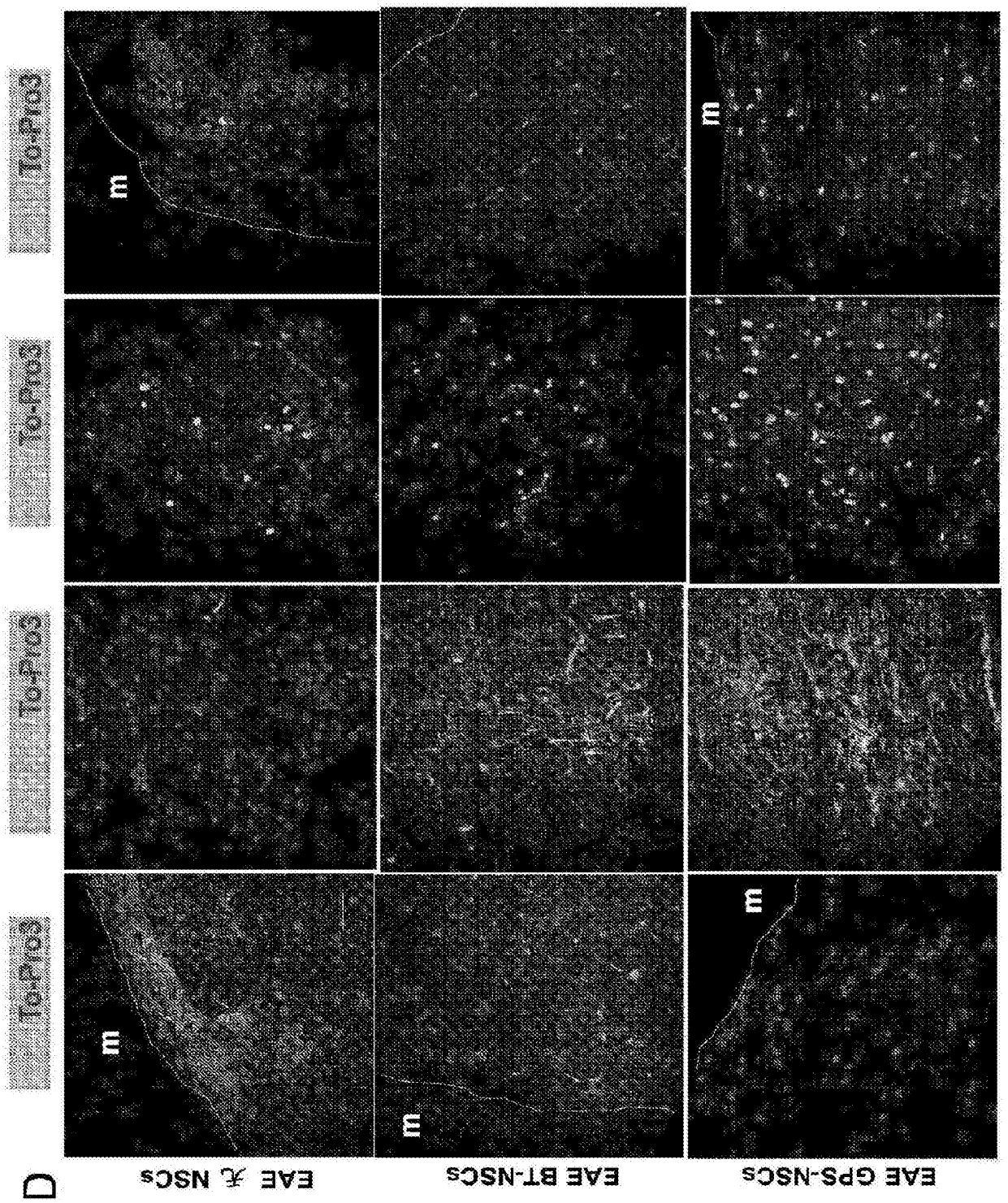
图13

A


EAE平均临床评分
在C5TBL/6小鼠中用MOG35-55免疫后的天数
无 NSC
GPS-NSC
BT-NSC
p=0.006
p<0.0001
p=0.006
****
0.0
0.5
1.0
1.5
2.0
2.5
0
5
10
15
20
25
30
35


B
EAE平均临床评分
在C5TBL/6小鼠中用MOG35-55免疫后的天数
无 NSC
BT-NSC
GPS-NSC
p=0.00611对于无NSC
p<0.0001对于BT-NSCs和无NSC
0.0
0.5
1.0
1.5
2.0
2.5
0
5
10
15
20
25
30
35


C
EAE 无 NSCs
EAE BT-NSCs
EAE GPS-NSCs
m
每个HPV的CD116巨噬细胞/
小胶质细胞#
HBSS
BT NSCs
GPS NSCs
n=20
p<0.0001
p<0.005
每个HPV的TOPRO-3浸润物
p<0.05


To-Pro3
To-Pro3
To-Pro3
To-Pro3
m
m
m
m
EAE 大 NSCs
EAE BT-NSCs
EAE GPS-NSCs
D

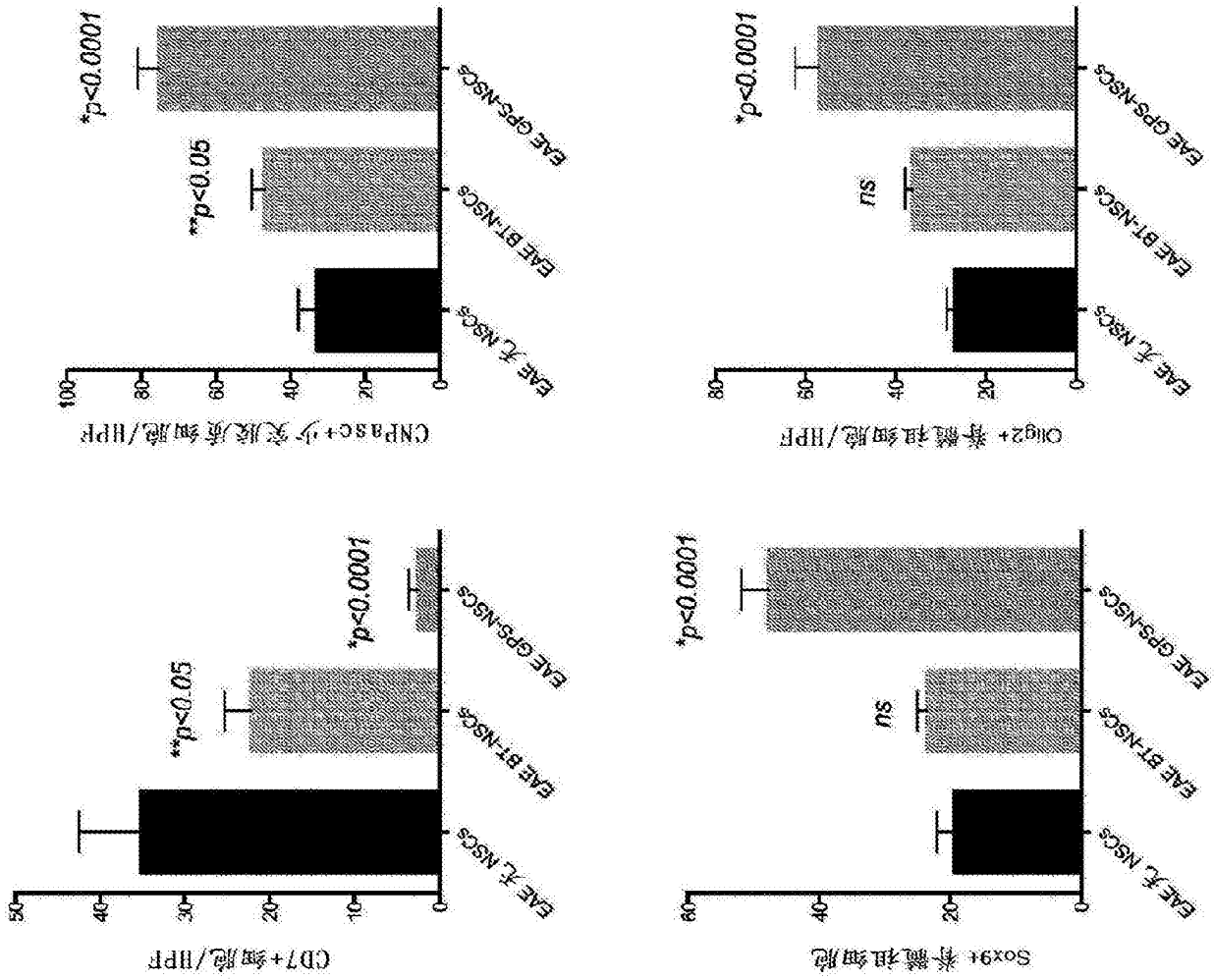
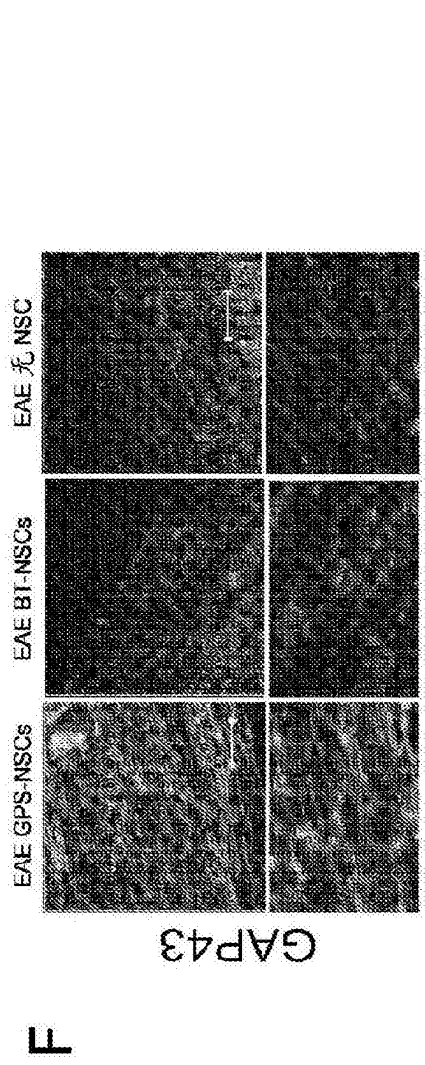
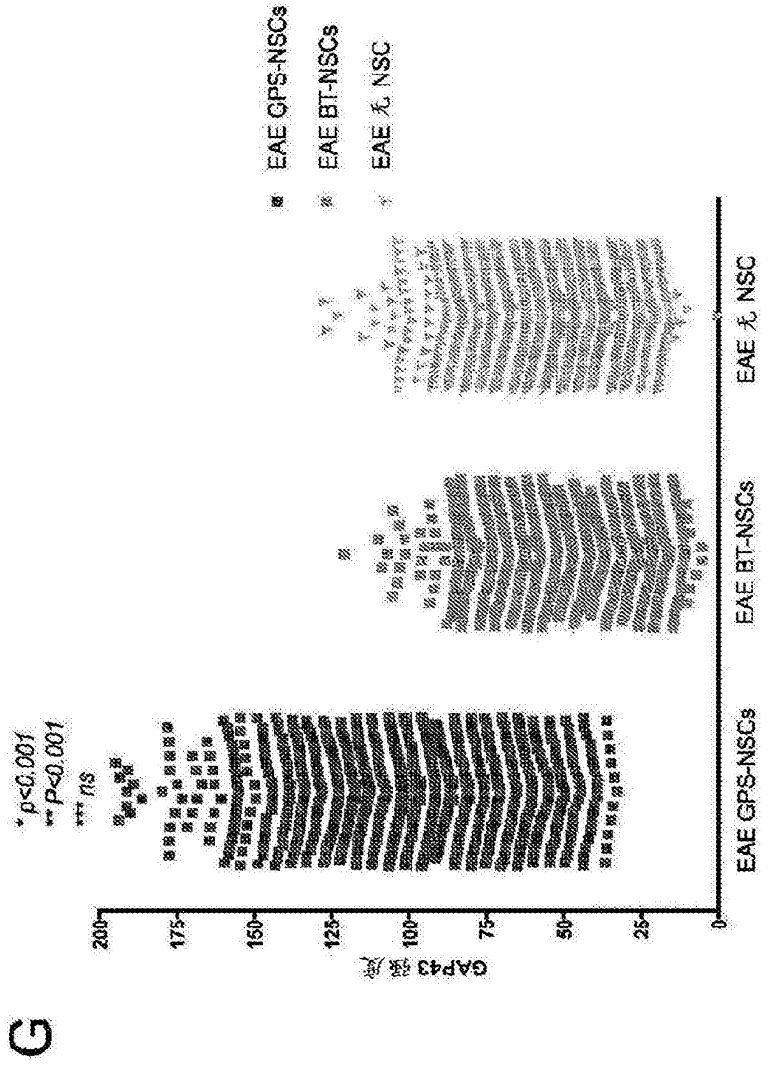

Sox9+ 神经祖细胞
Olig2+ 神经祖细胞/HPF
CD7+细胞/HPF
CNPase+少突胶质细胞/HPF
EAE无 NSCs
EAE BT-NSCs
EAE GPS-NSCs
*p<0.0001
ns
**p<0.05

图3


EAE GPS-NSCs
EAE BT-NSCs
EAE 无 NSC
GAP43

F


EAE GPS-NSCs
EAE BT-NSCs
EAE 无 NSC
* p<0.001
** P<0.001
*** ns
GAP43 荧光
200
175
150
125
100
75
50
25
0
EAE GPS-NSCs
EAE BT-NSCs
EAE 无 NSC

G

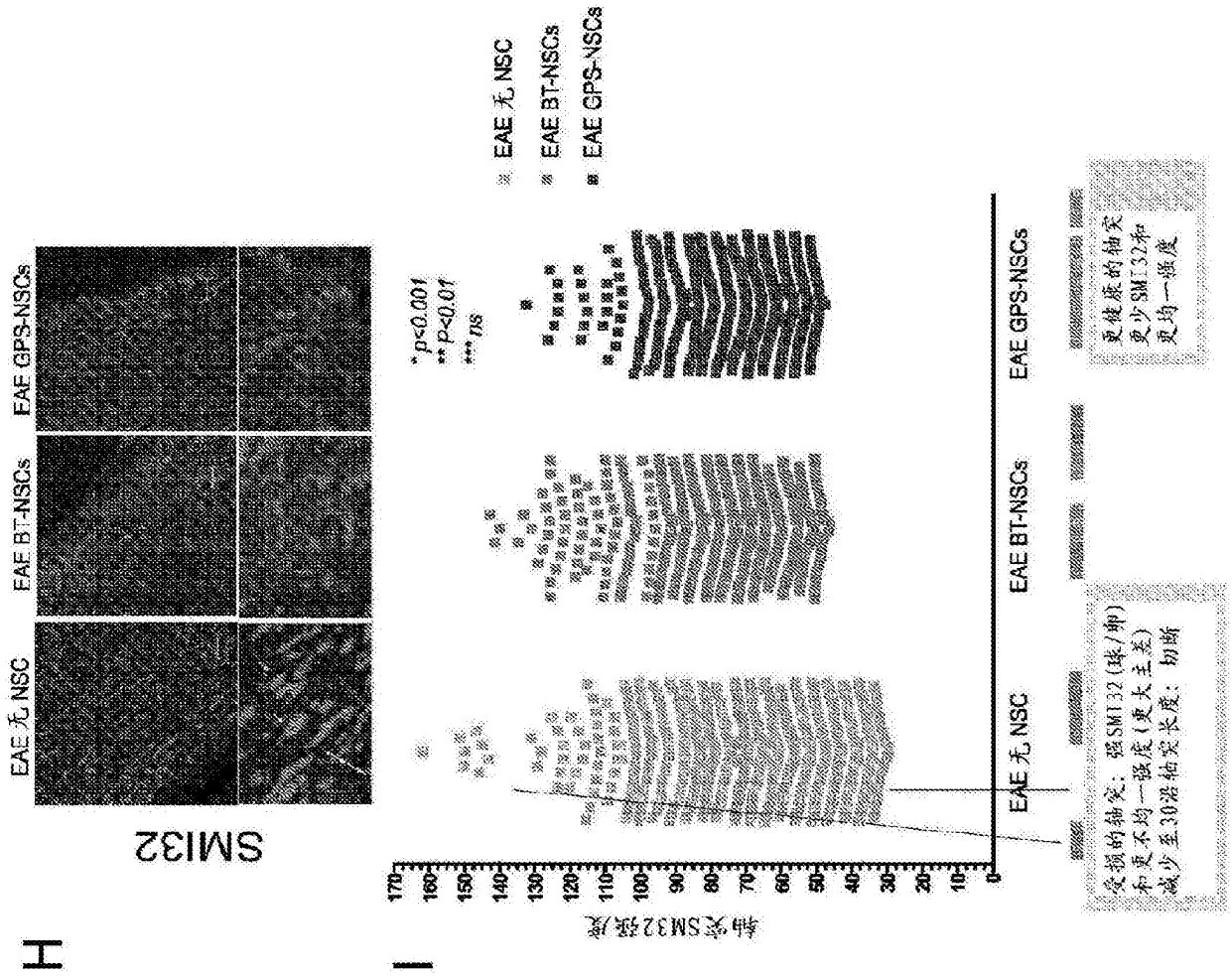

H
SMI32
EAE 无 NSC
EAE BT-NSCs
EAE GPS-NSCs
I
轴突SMI32强度
EAE 无 NSC
EAE BT-NSCs
EAE GPS-NSCs
*p<0.001
**p<0.01
***ns
受损的轴突：强SMI32(染/伸)
和更不均一强度(更大主差)
或少至30沿轴突长度：切断
更健康的轴突
更少SMI32和
更均一强度

图14

打印ID7891292 Pg 100

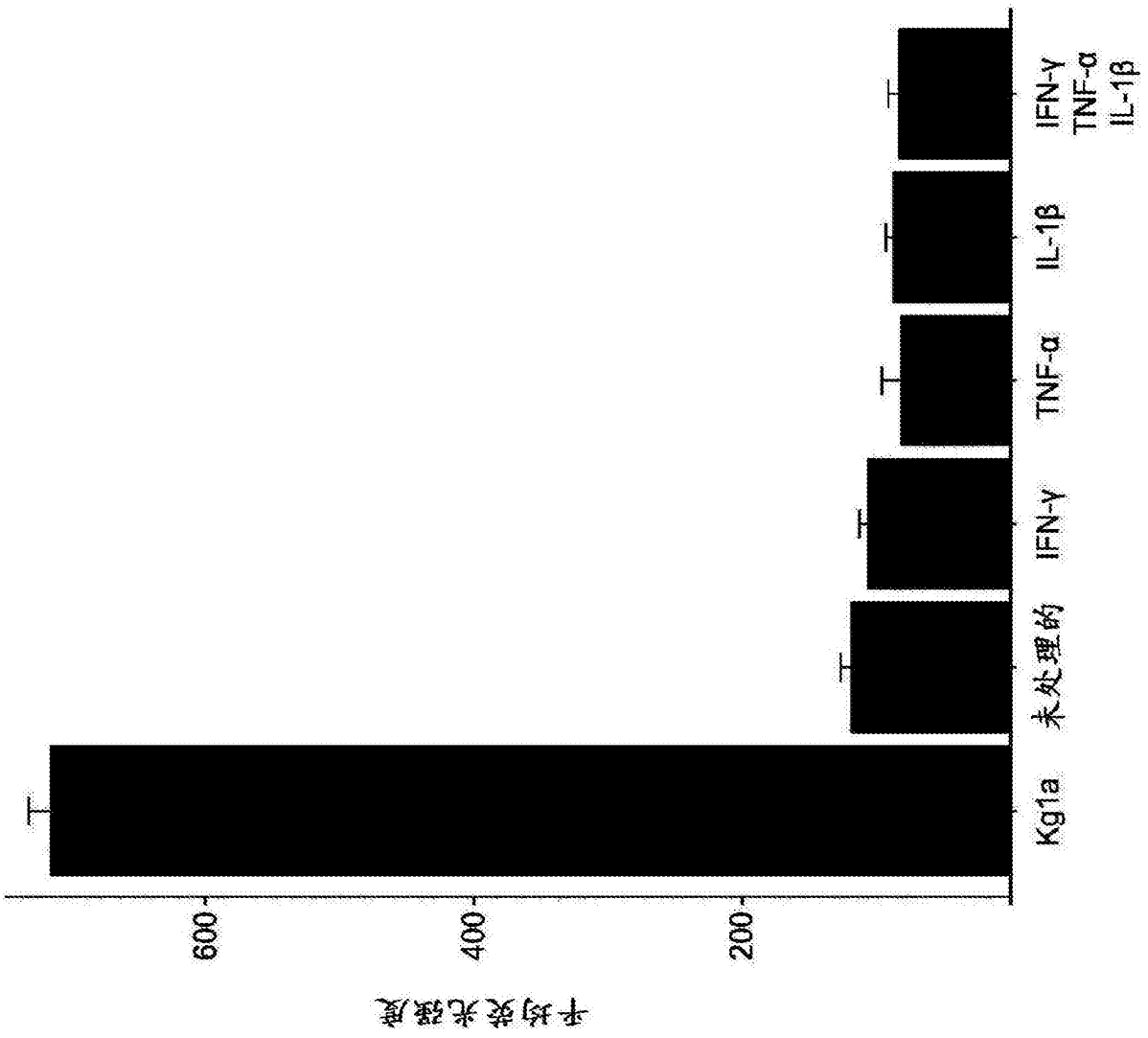


图15

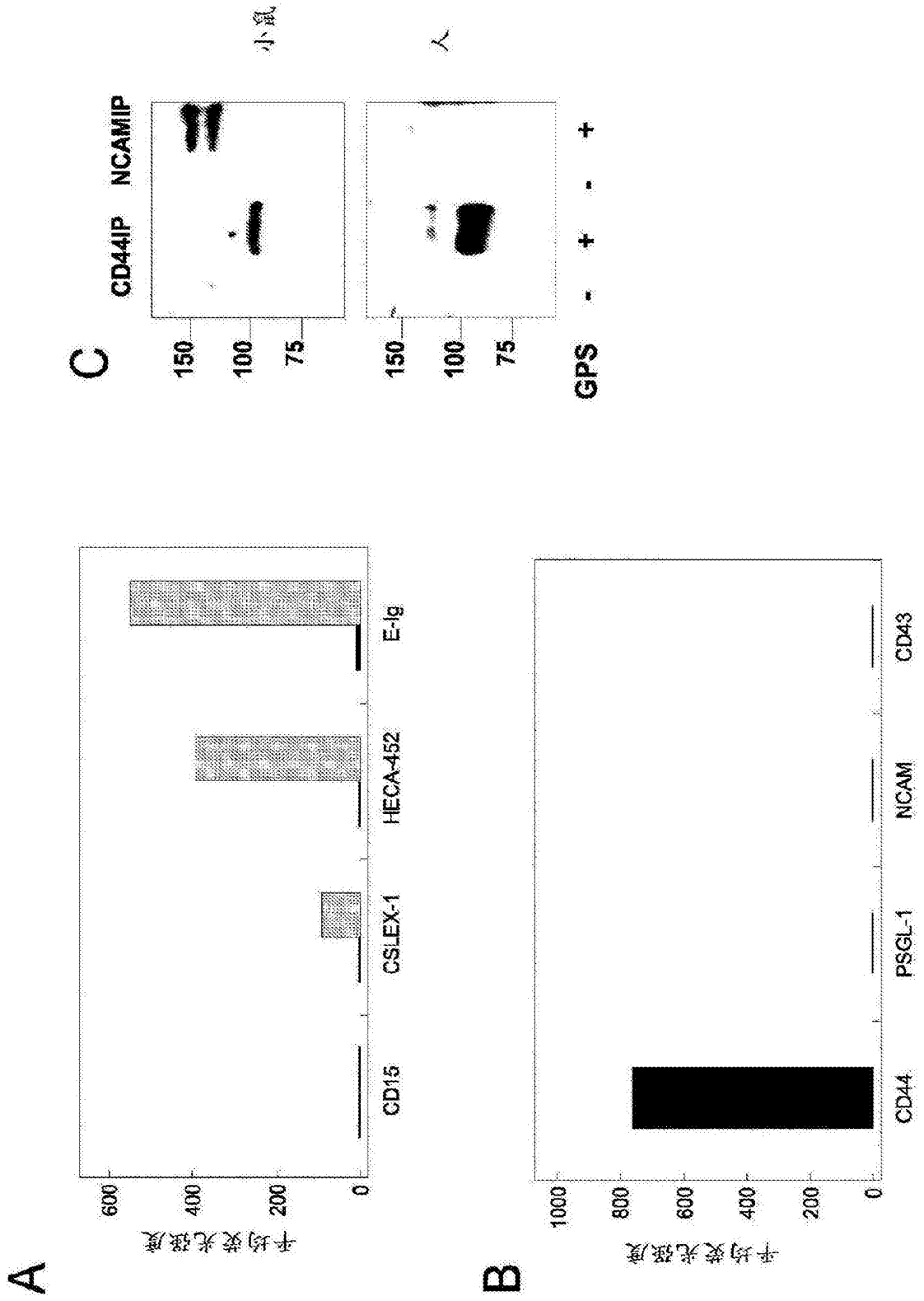

A
平均荧光强度
600
400
200
0
CD15
CSLEX-1
HECA-452
E-Ig
B
平均荧光强度
1000
800
600
400
200
0
CD44
PSGL-1
NCAM
CD43
C
小鼠
人
CD44IP
NCAMIP
150
100
75
GPS
-
+
-
+
+
-

图16

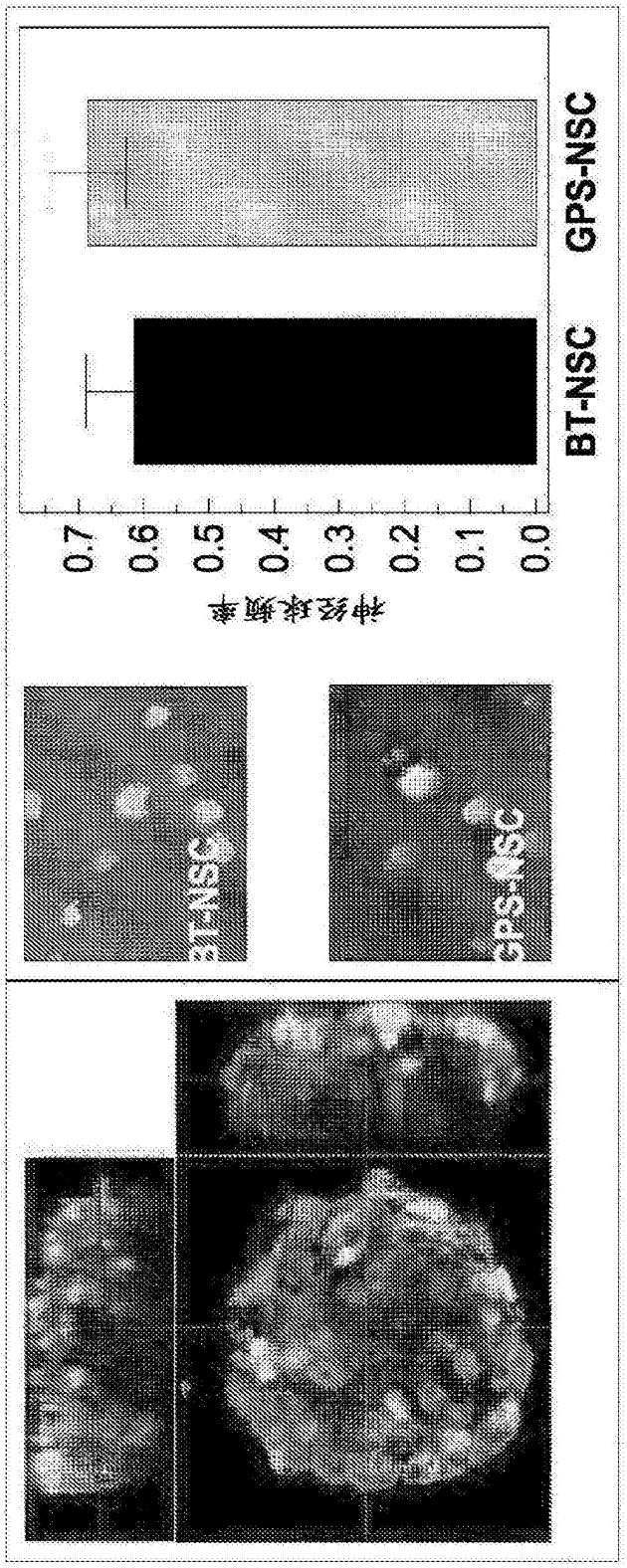
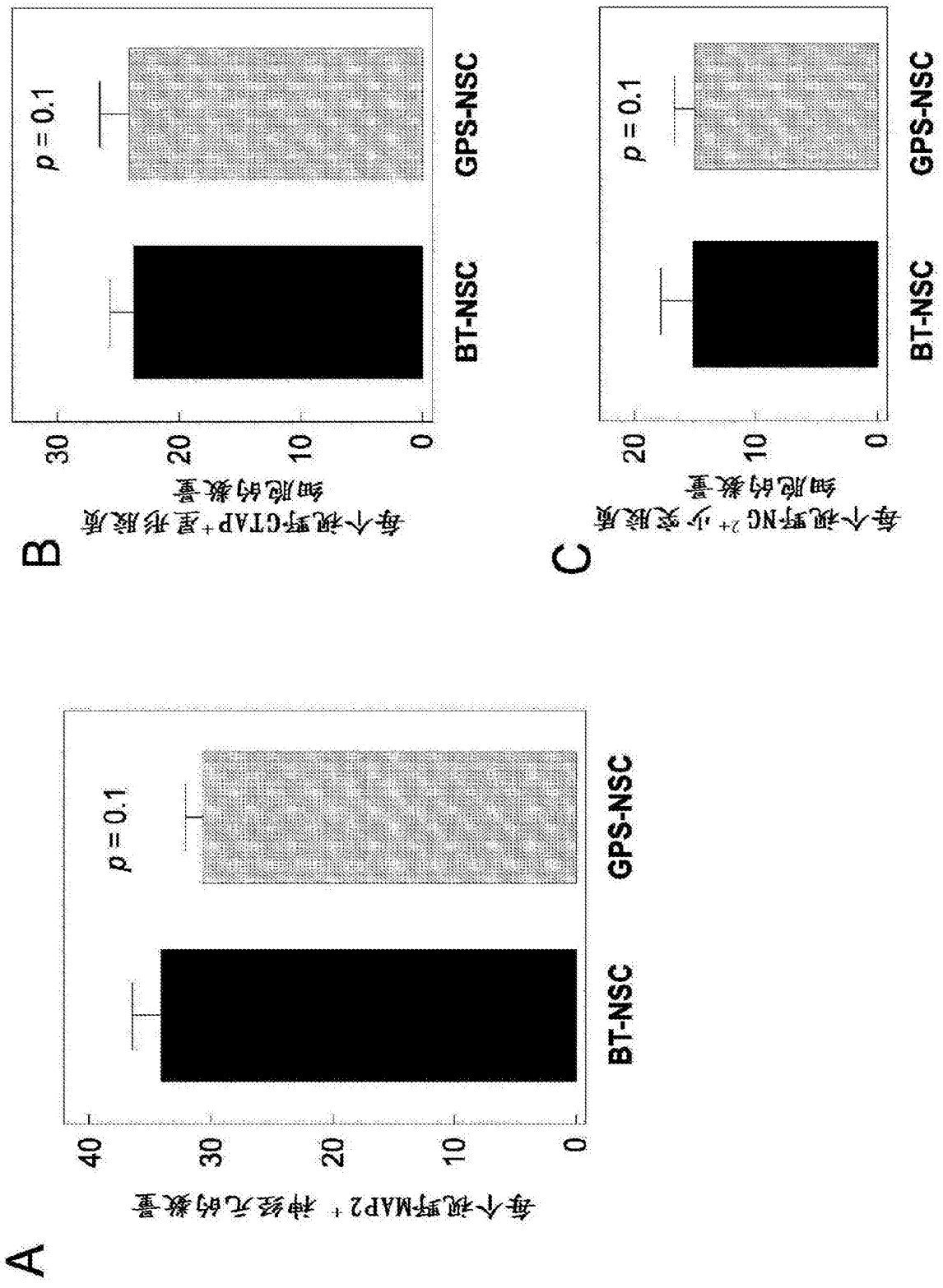


图17


A
每个视野MAP2+ 神经元的数量
0
10
20
30
40
p = 0.1
BT-NSC
GPS-NSC
B
每个视野GTAP+星形胶质
细胞的数量
0
10
20
30
p = 0.1
BT-NSC
GPS-NSC
C
每个视野NG2+少突胶质
细胞的数量
0
10
20
p = 0.1
BT-NSC
GPS-NSC

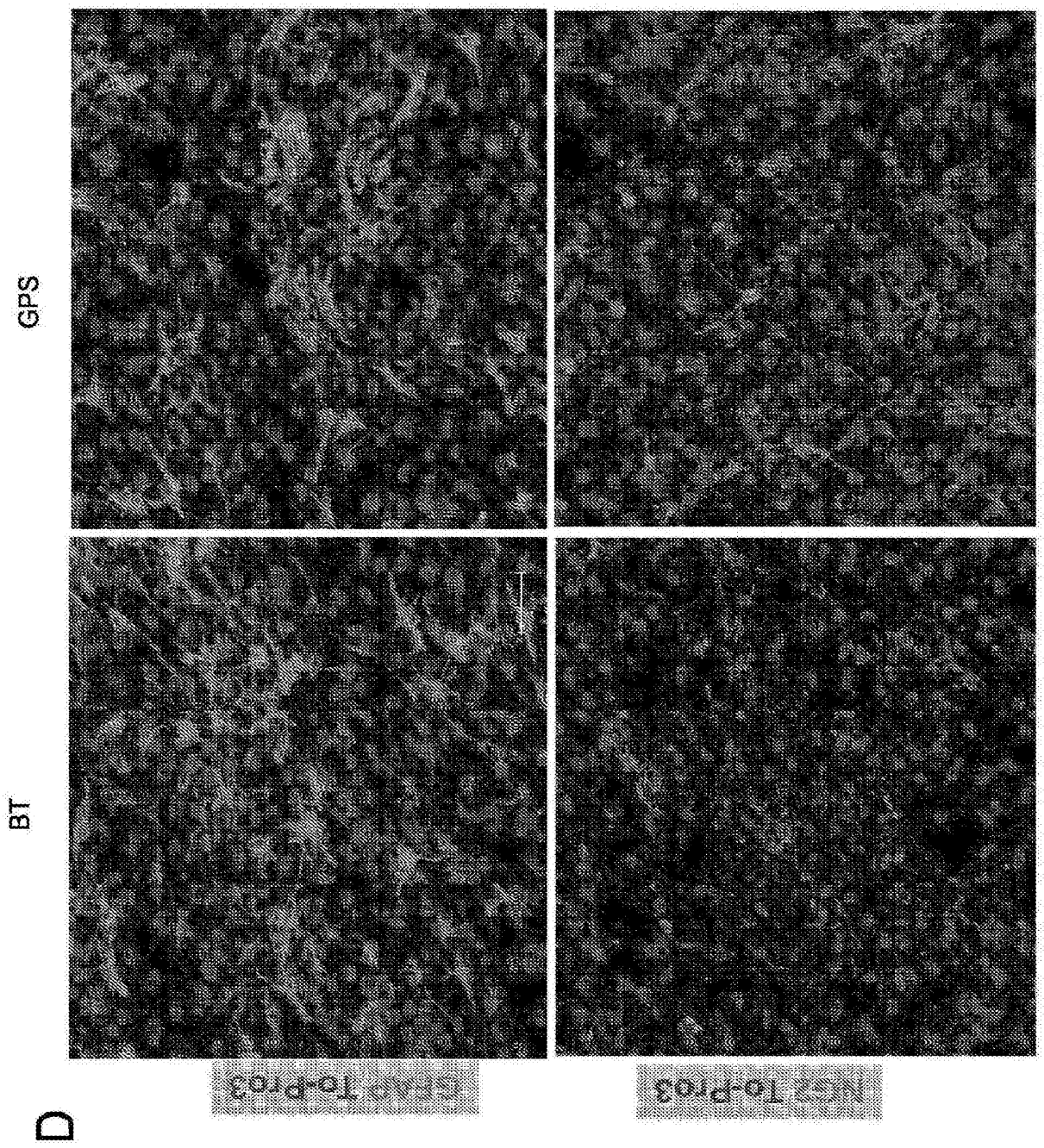
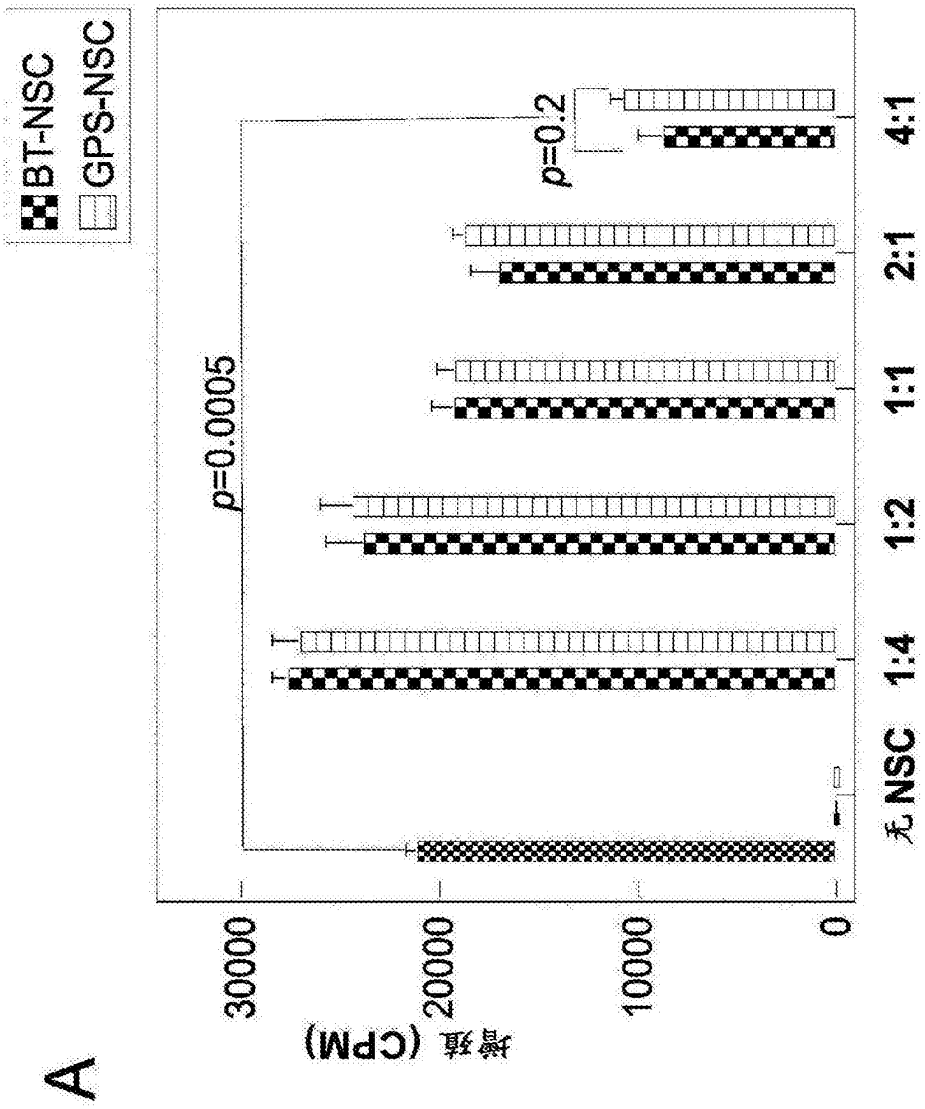
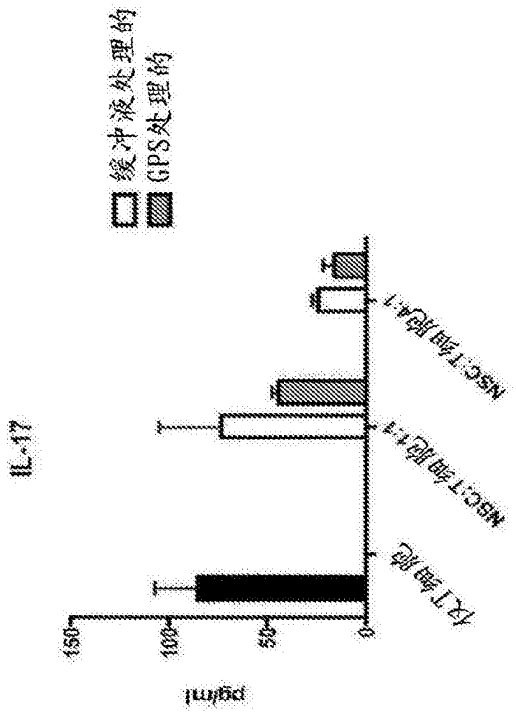
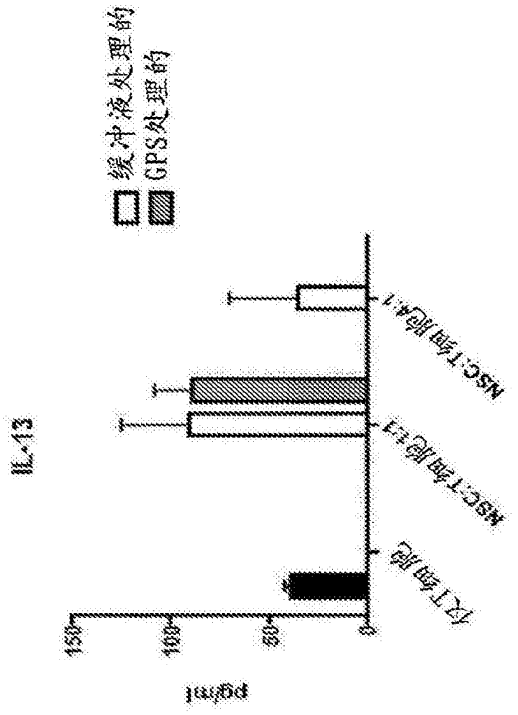
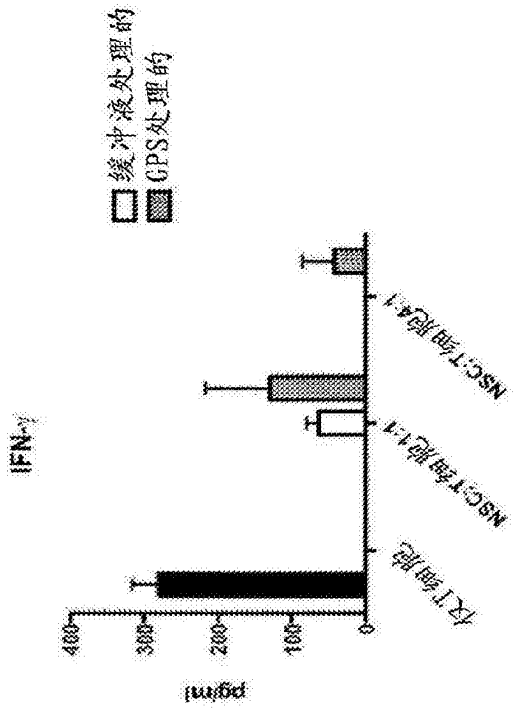


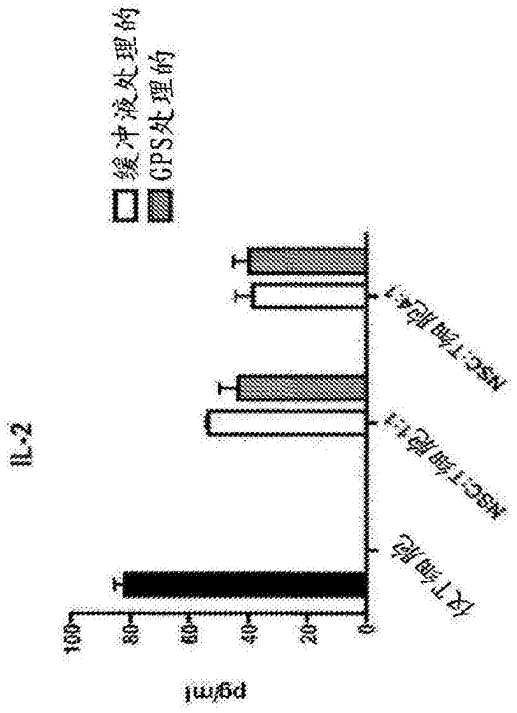
图18


A
增殖 (CPM)
30000
20000
10000
0
BT-NSC
GPS-NSC
p=0.0005
p=0.2
无 NSC
1:4
1:2
1:1
2:1
4:1


缓冲液处理的
GPS处理的
IL-17
pg/ml
150
100
50
0
抗T细胞
NSC-T细胞1:1
NSC-T细胞4:1


缓冲液处理的
GPS处理的
IL-13
pg/ml
150
100
50
0
抗T细胞
NSC-T细胞1:1
NSC-T细胞4:1


缓冲液处理的
GPS处理的
IFN-γ
pg/ml
400
300
200
100
0
抗T细胞
NSC-T细胞1:1
NSC-T细胞4:1


缓冲液处理的
GPS处理的
IL-2
pg/ml
100
80
60
40
20
0
抗T细胞
NSC-T细胞1:1
NSC-T细胞4:1

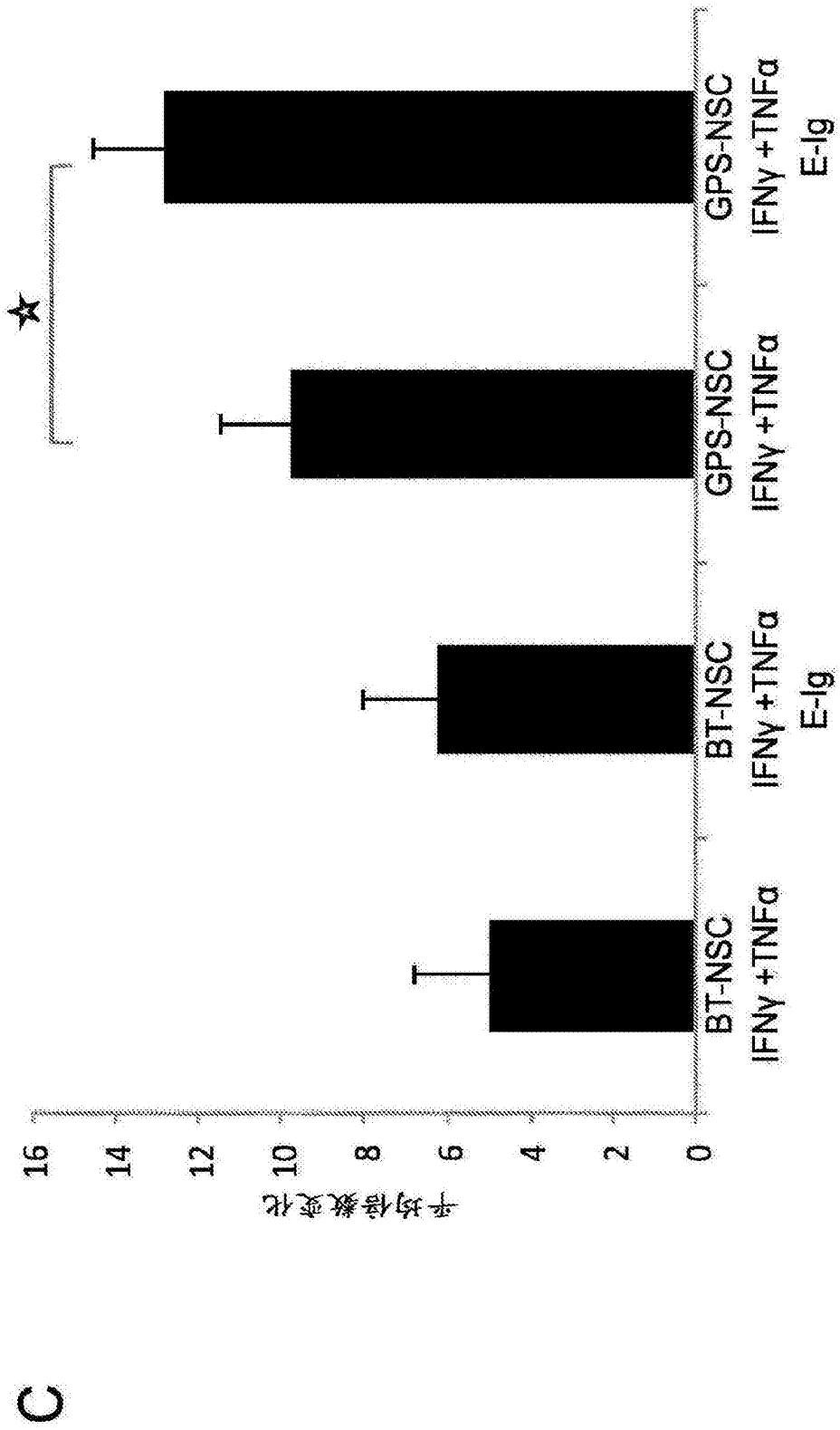
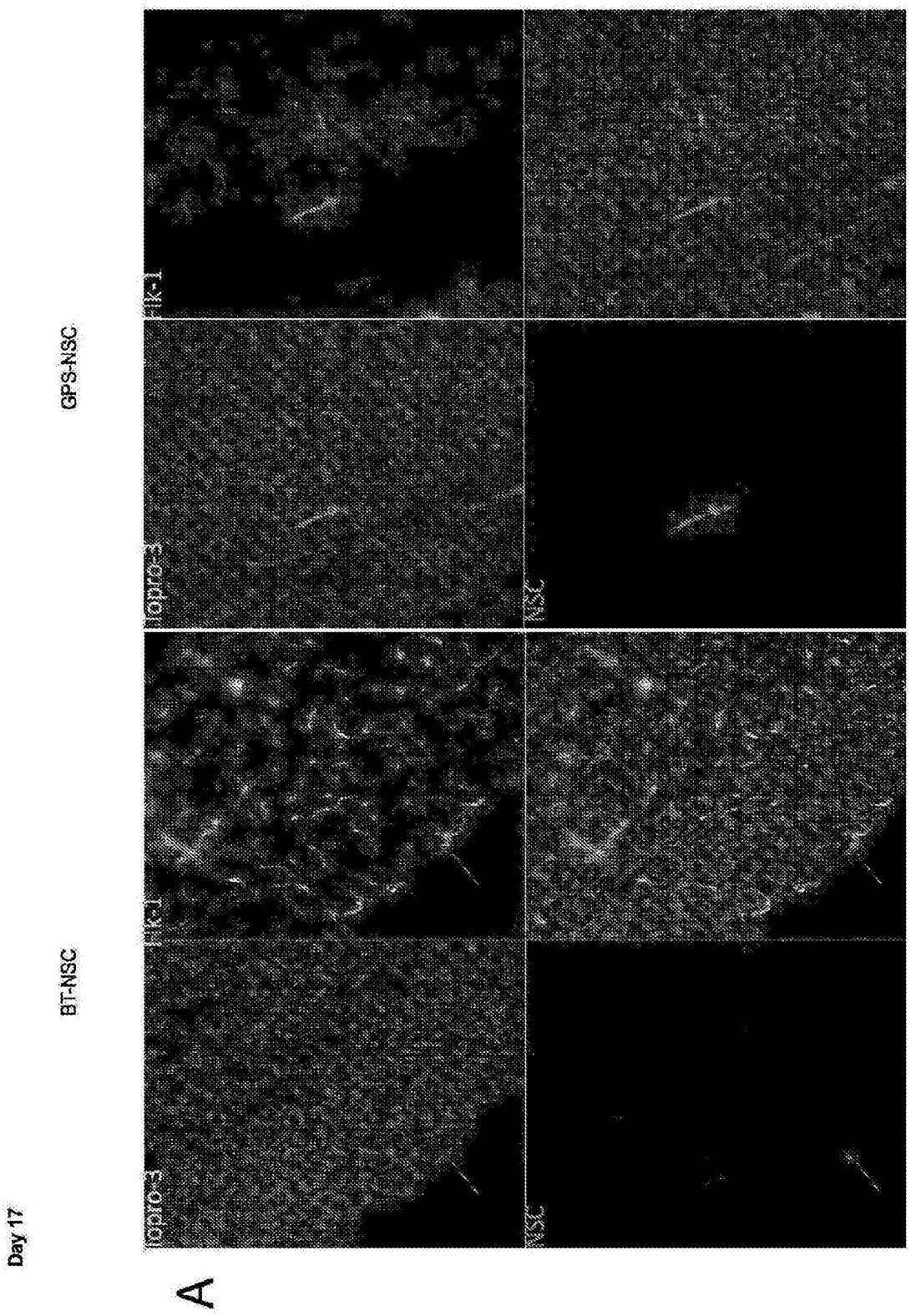

平均倍数变化
0
2
4
6
8
10
12
14
16
BT-NSC
IFNγ +TNFα
BT-NSC
IFNγ +TNFα
E-Ig
GPS-NSC
IFNγ +TNFα
GPS-NSC
IFNγ +TNFα
E-Ig
☆
C

图19


A
Day 17
BT-NSC
GPS-NSC
Iopro-3
Tik-1
NSC
Iopro-3
Tik-1
NSC

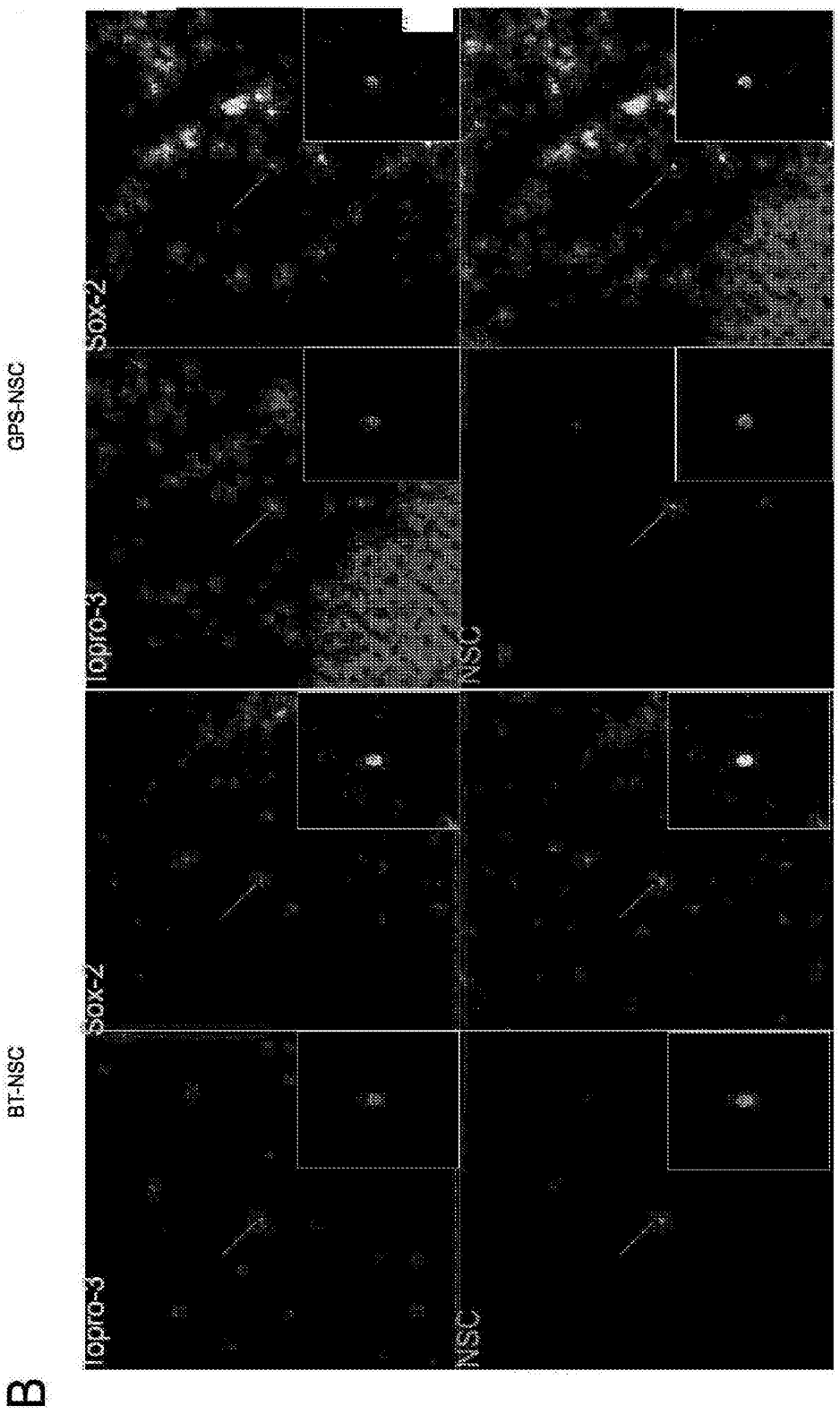
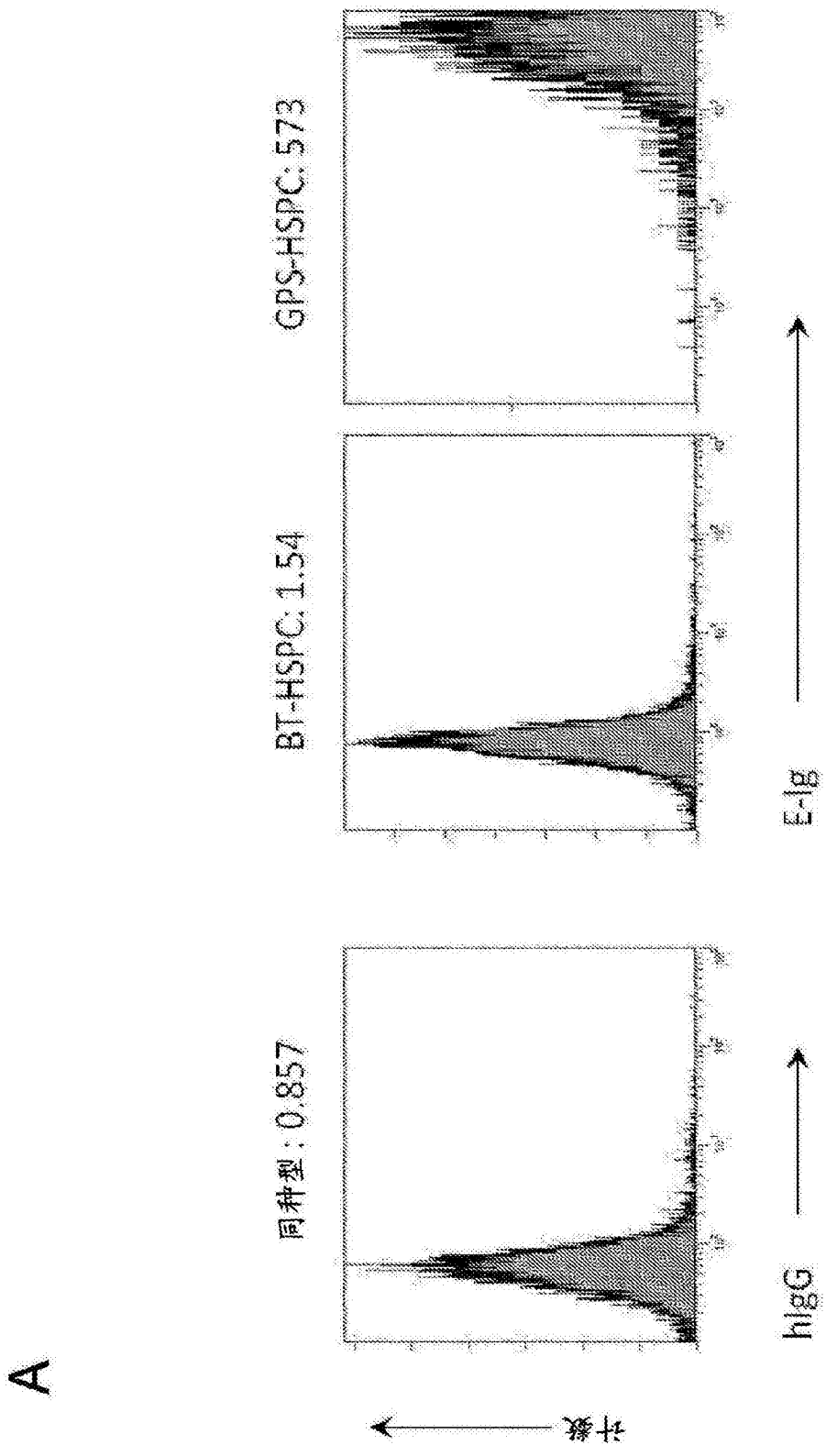
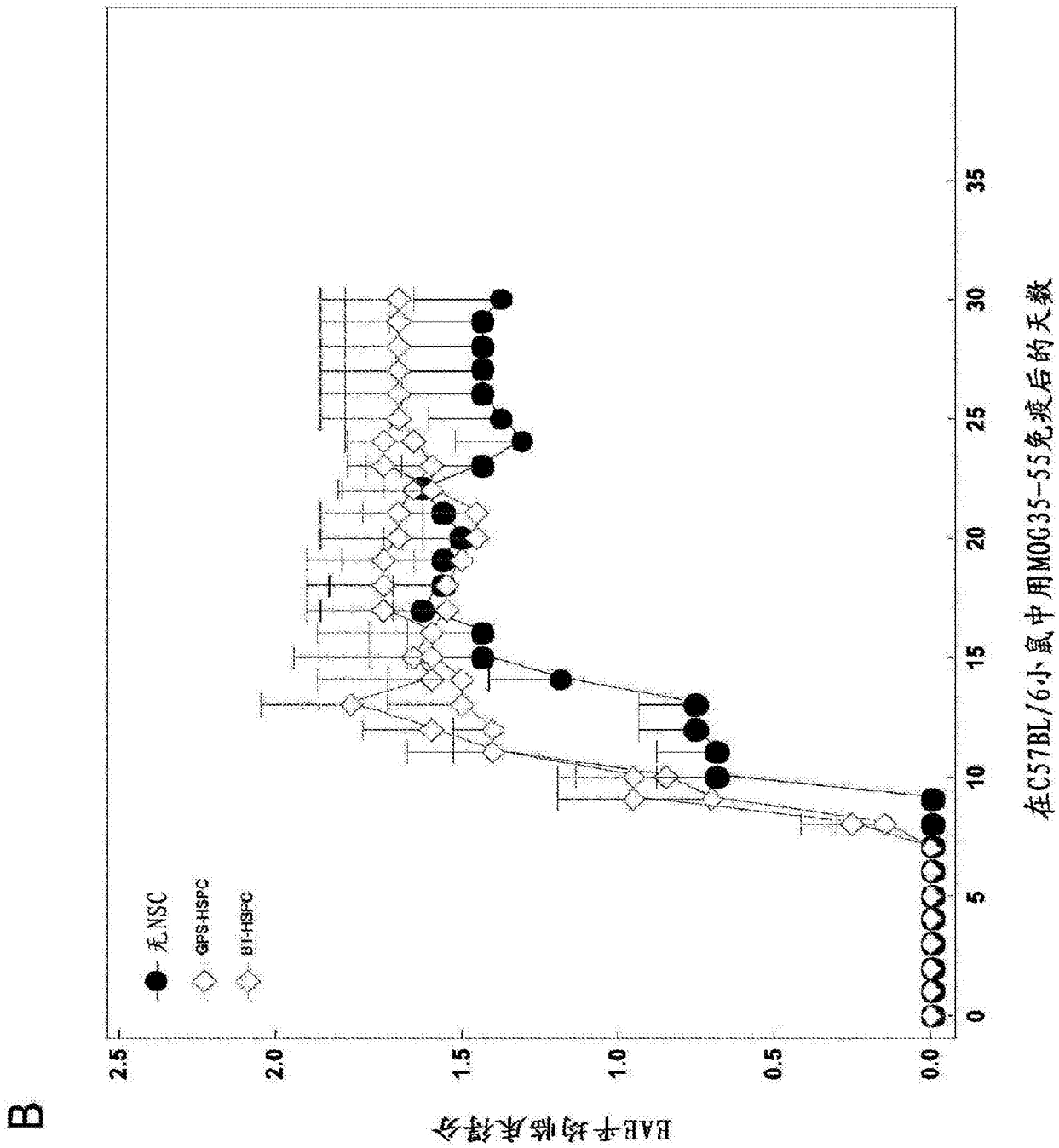


图20


A
同种型：0.857
BT-HSPC: 1.54
GPS-HSPC: 573
计数
hIgG
E-Ig


B
EAE平均临床评分
2.5
2.0
1.5
1.0
0.5
0.0
0
5
10
15
20
25
30
35
在C57BL/6小鼠中用MOG35-55免疫后的天数
无NSC
GFS-HSPC
BT-HSPC

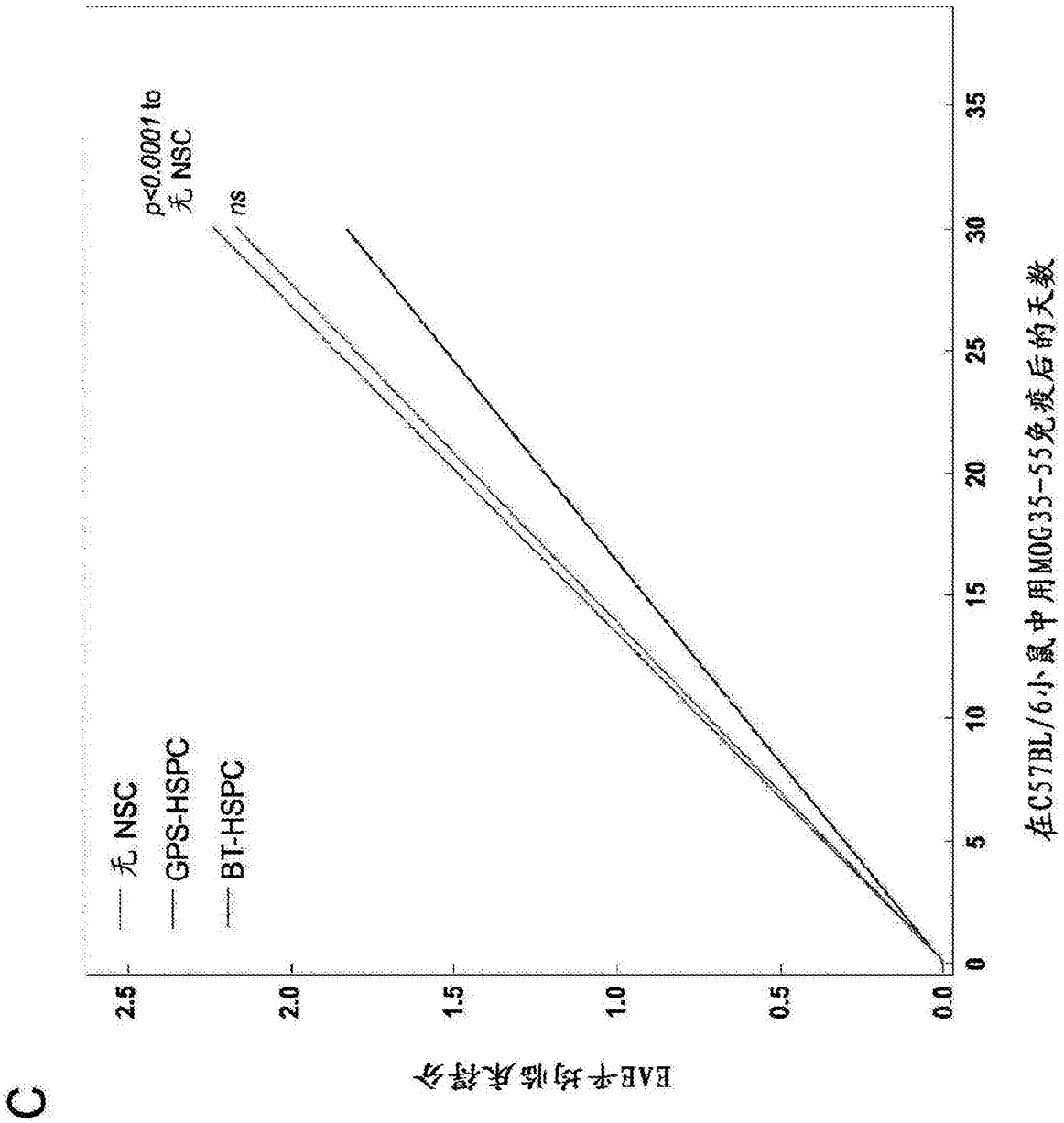

C
EAE平均临床得分
2.5
2.0
1.5
1.0
0.5
0.0
0
5
10
15
20
25
30
35
在C57BL/6小鼠中用MOG35-55免疫后的天数
无 NSC
GPS-HSPC
BT-HSPC
p<0.0001 to 无 NSC
ns

图21

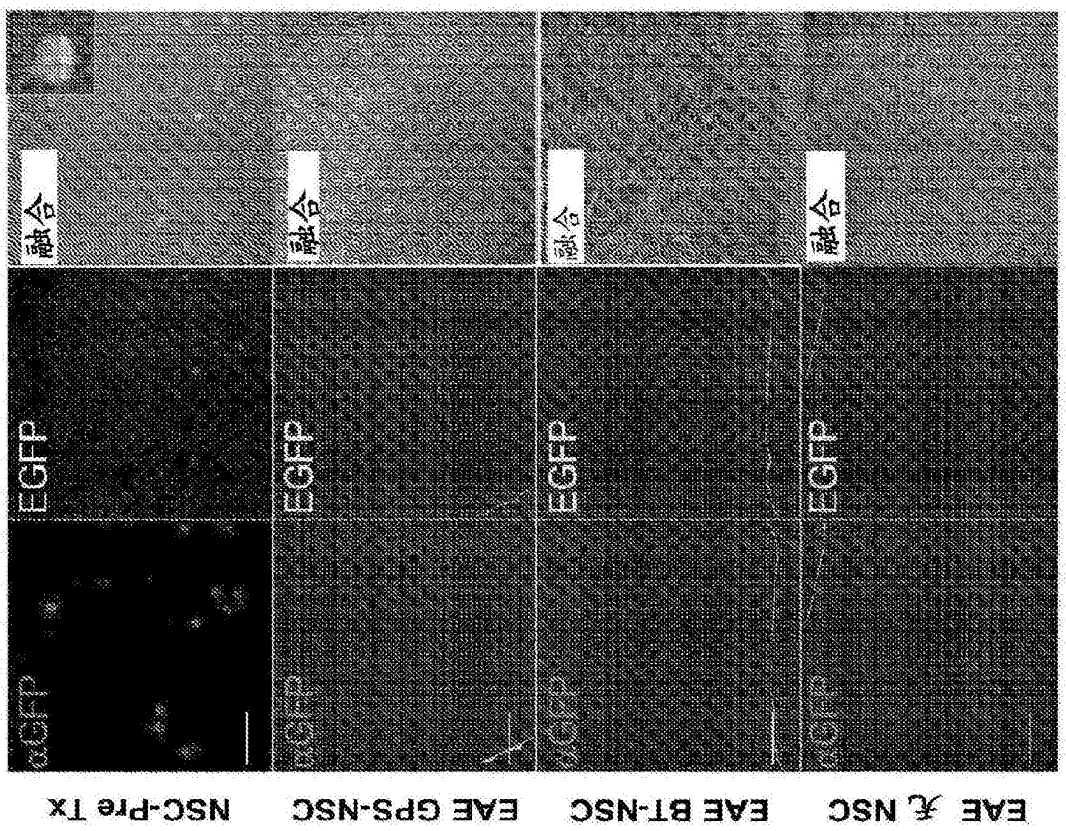


图22

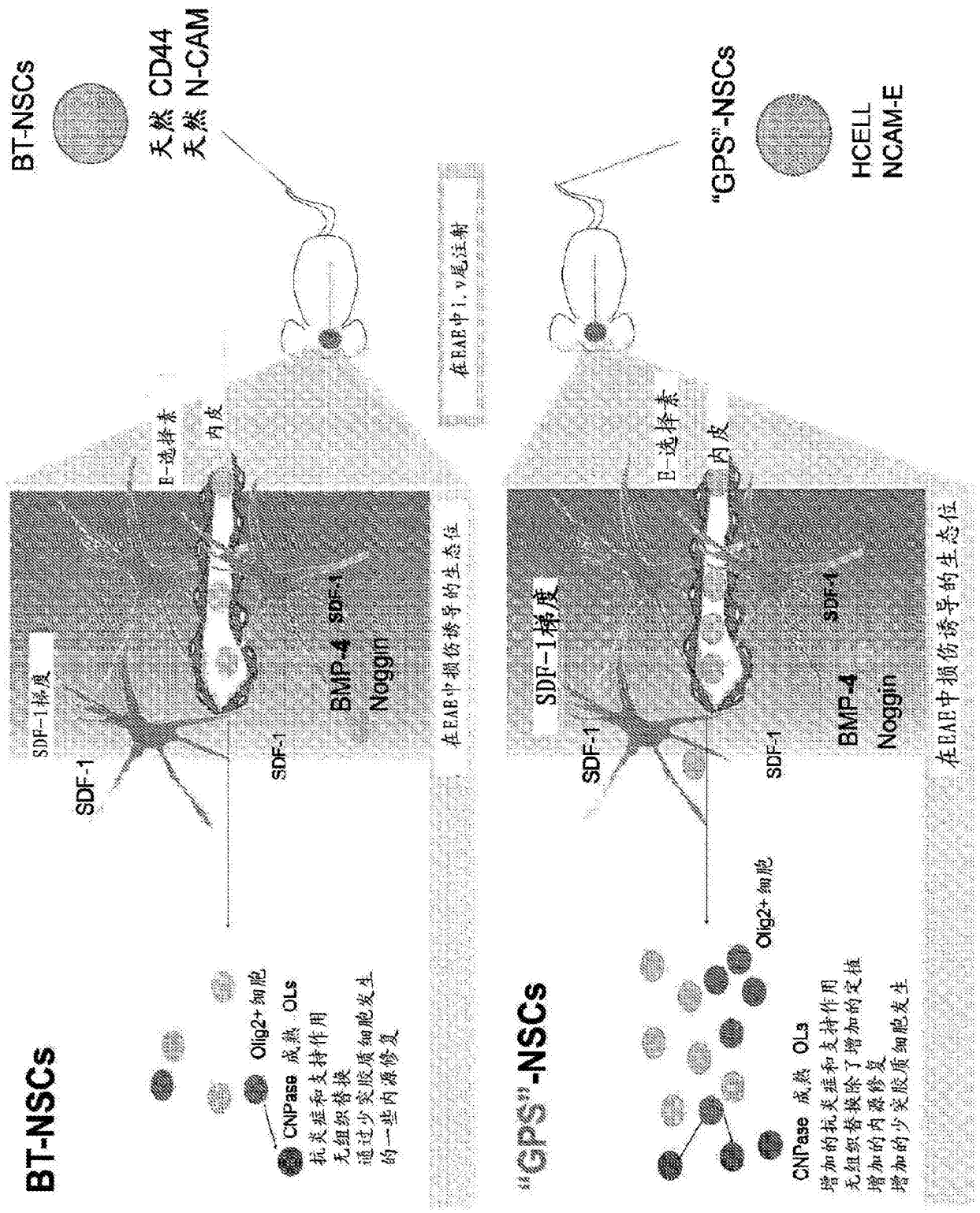

BT-NSCs
SDF-1梯度
SDF-1
天然 CD44
天然 N-CAM
E-选择素
内皮
SDF-1
BMP-4 SDF-1
Noggin
在EAE中损伤诱导的生态位
在EAE中i.v尾注射
Olig2+ 细胞
CNPase 成熟 OLs
抗炎症和支持作用
无组织替换
通过少突胶质细胞发生
的一些内源修复
"GPS"-NSCs
SDF-1梯度
SDF-1
"GPS"-NSCs
HCELL
NCAM-E
E-选择素
内皮
SDF-1
BMP-4
Noggin
在EAE中损伤诱导的生态位
Olig2+ 细胞
CNPase 成熟 OLs
增加的抗炎症和支持作用
无组织替换除了增加的定植
增加的内源修复
增加的少突胶质细胞发生

图23

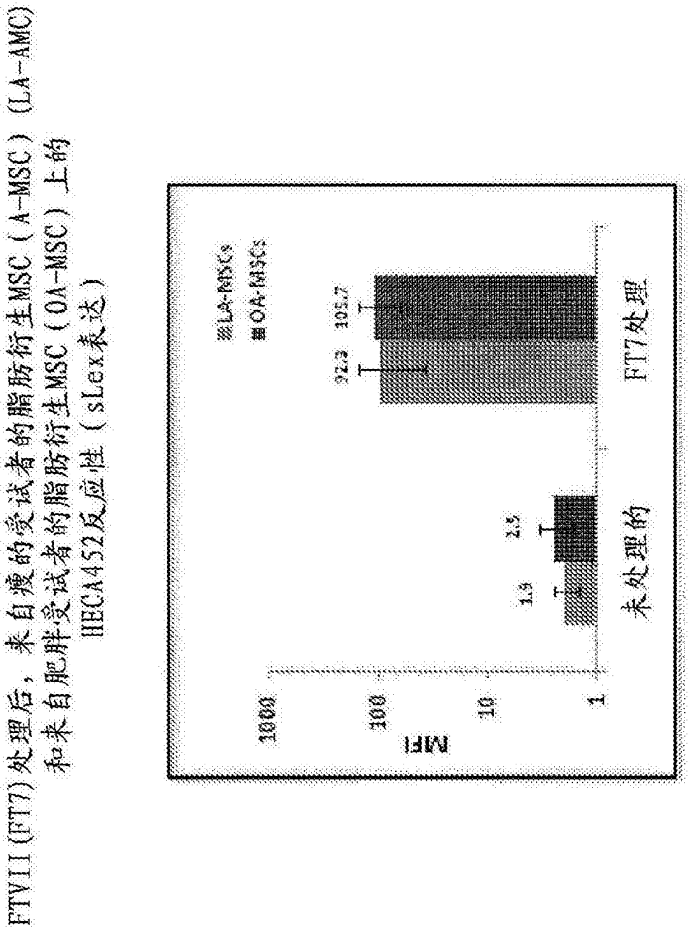

FTVII(FT7)处理后，来自瘦的受试者的脂肪衍生MSC（A-MSC）(LA-AMC)
和来自肥胖受试者的脂肪衍生MSC（OA-MSC）上的
HECA452反应性（sLex表达）
LA-MSCs
OA-MSCs
MFI
1000
100
10
1
1.9
2.5
92.3
102.7
未处理的
FT7处理

图24

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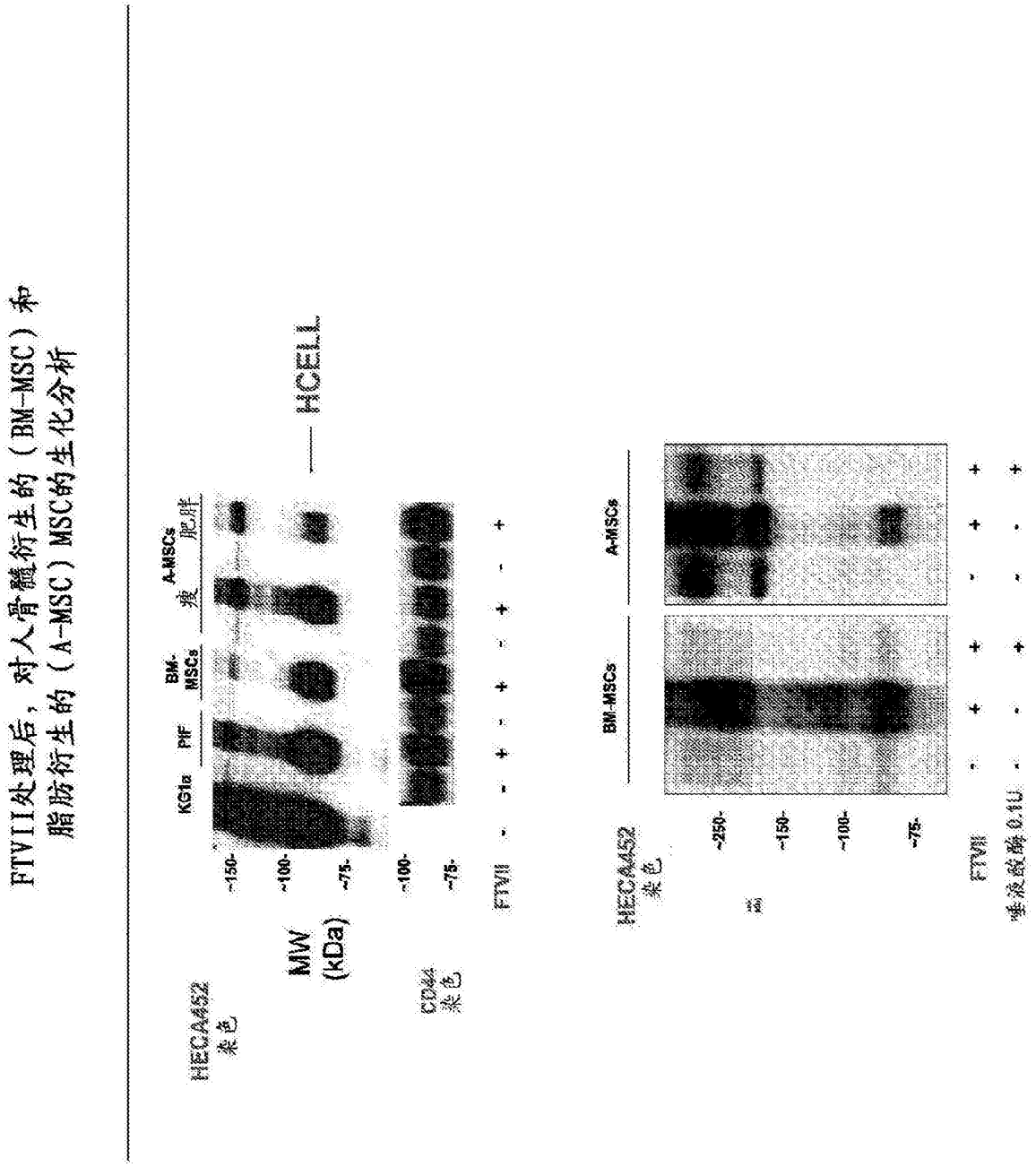


图25

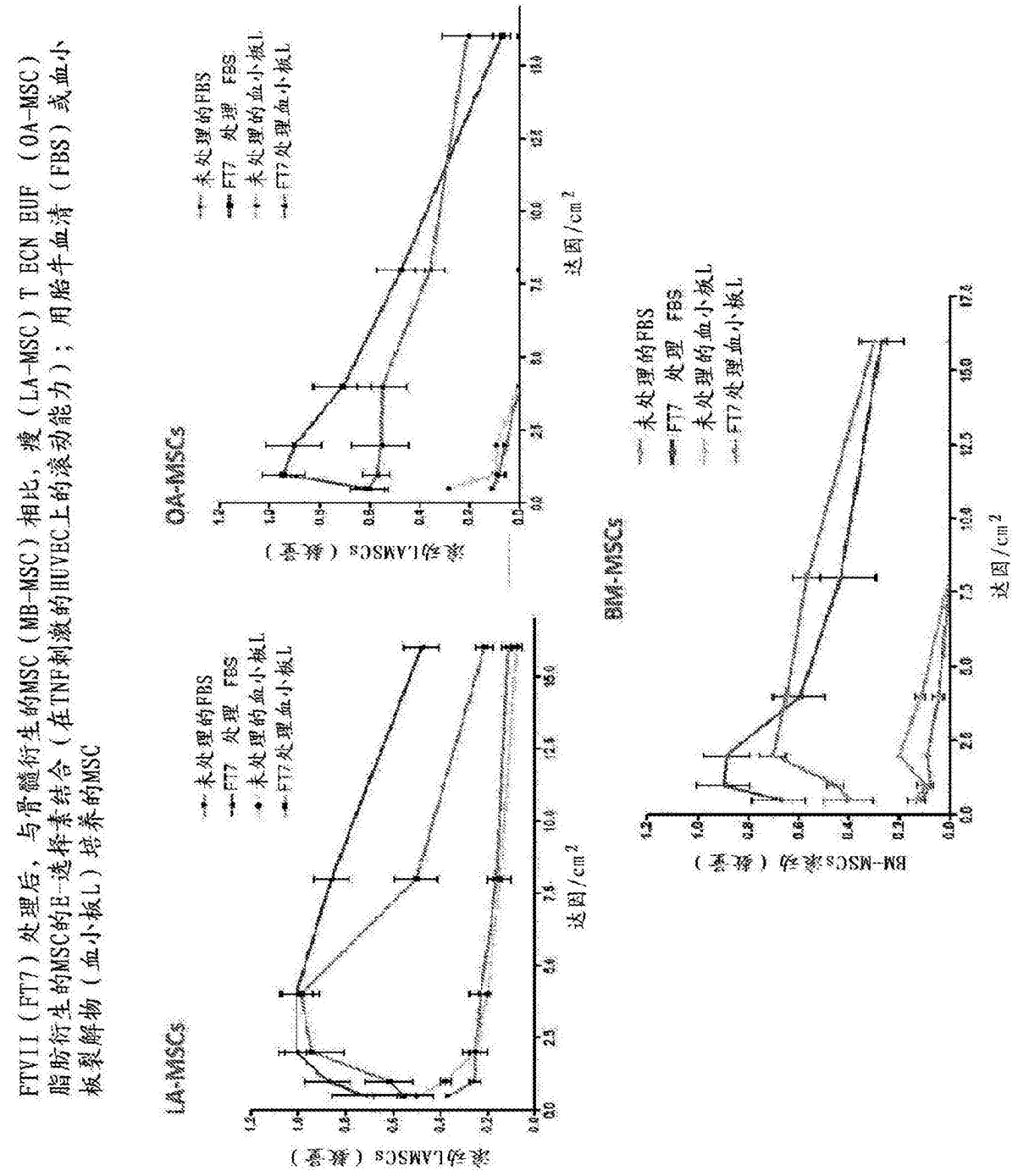

FTVII（FT7）处理后，与骨髓衍生的MSC（MB-MSC）相比，瘦（LA-MSC）T ECN EUF （OA-MSC）脂肪衍生的MSC的E-选择素结合（在TNF刺激的HUVEC上的滚动能力）；用胎牛血清（FBS）或血小板裂解物（血小板L）培养的MSC
LA-MSCs
OA-MSCs
BM-MSCs
未处理的FBS
FT7 处理 FBS
未处理的血小板L
FT7处理血小板L
滚动LAMSCs（数量）
达因/cm²

图26

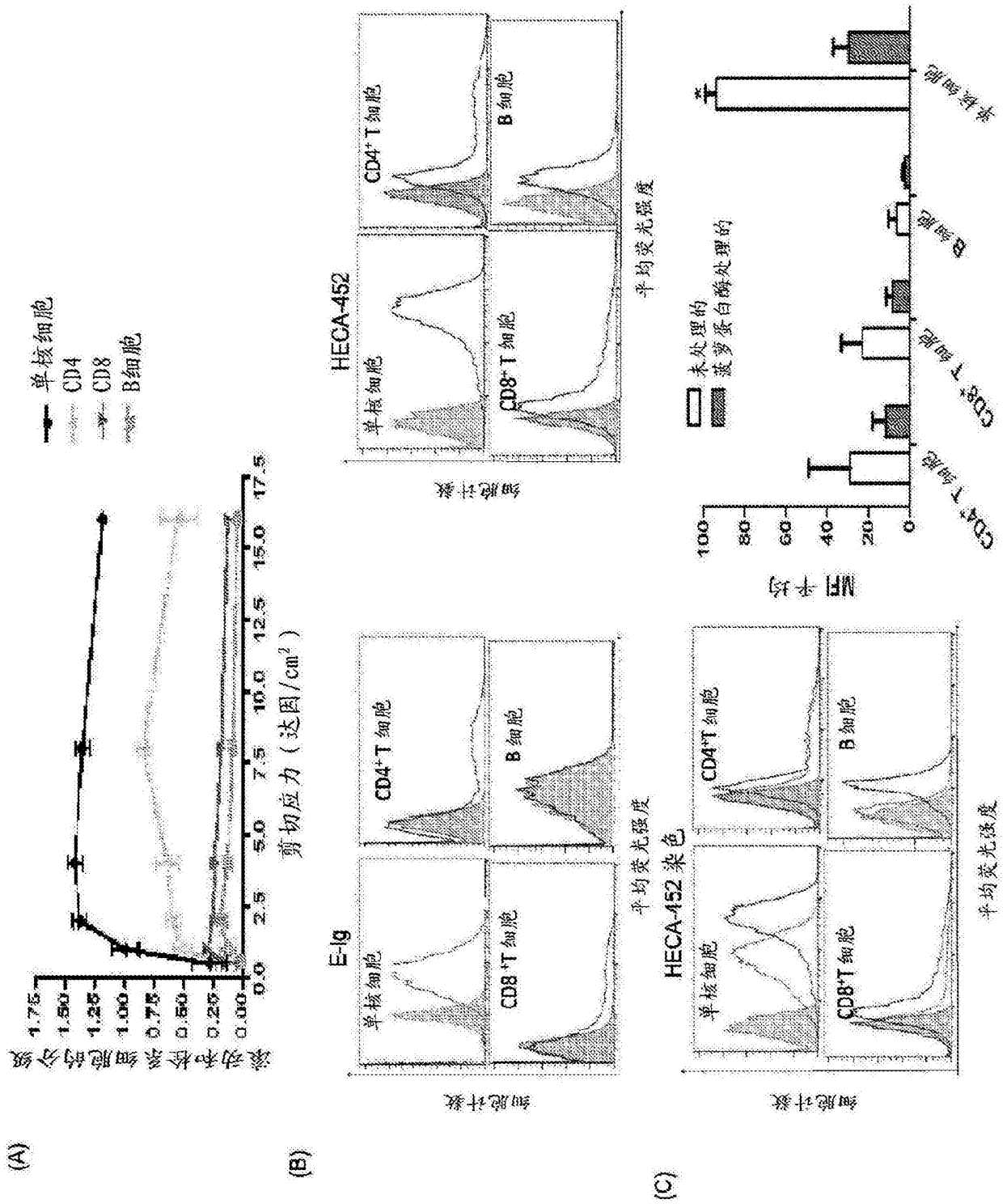
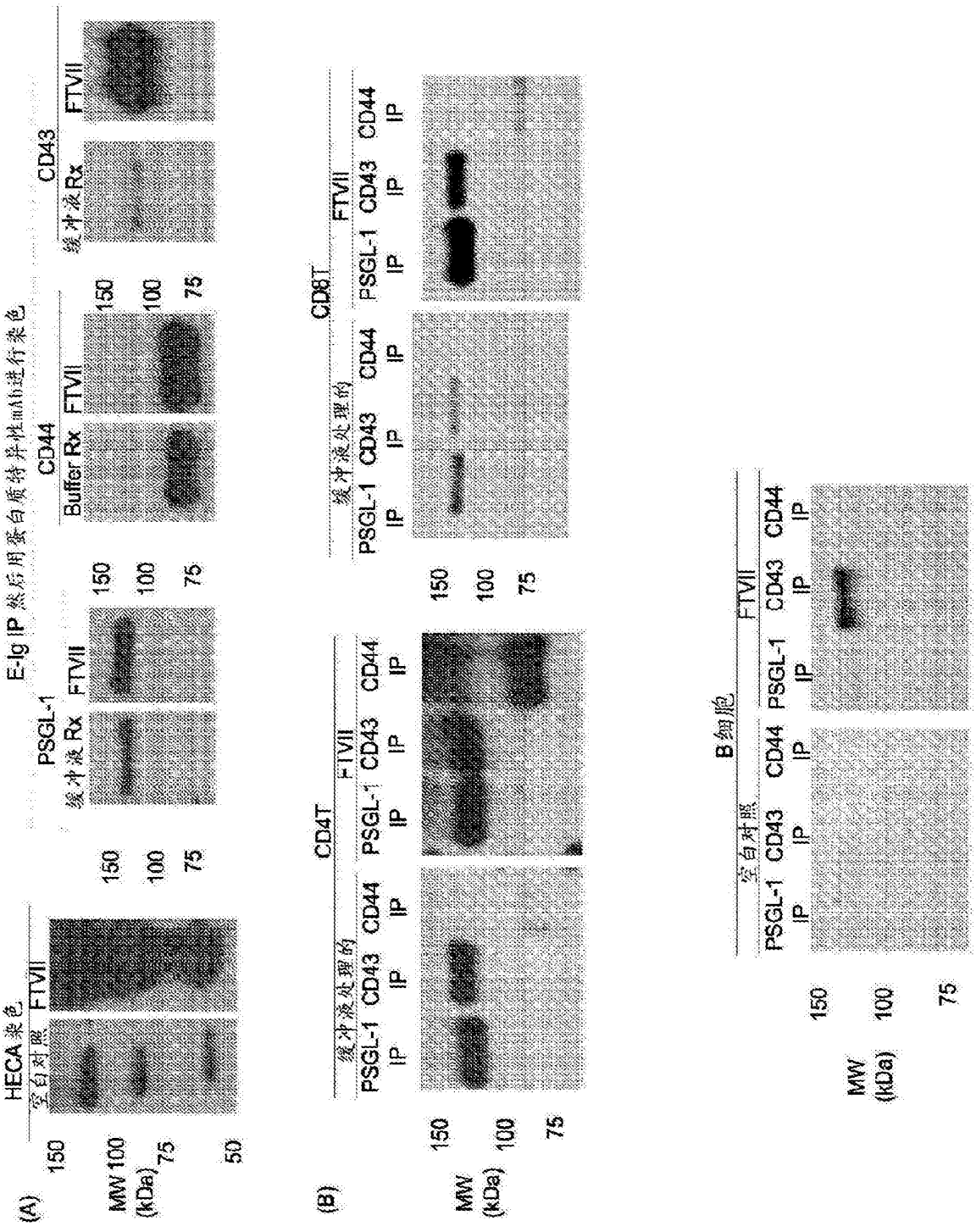

(A)
单核细胞
CD4
CD8
B细胞
粘附细胞的分级
剪切应力（达因/cm²）
(B)
E-lg
单核细胞
CD8⁺T细胞
CD4⁺T细胞
B细胞
细胞计数
平均荧光强度
HECA-452
(C)
HECA-452 染色
未处理的
唾液酸酶处理的
平均荧光强度

图27


(A)
HECA染色
空白对照
FTVII
150
MW 100
(kDa) 75
50
E-Ig IP 然后用蛋白质特异性mAb进行染色
PSGL-1
缓冲液Rx
FTVII
CD44
Buffer Rx
FTVII
CD43
缓冲液Rx
FTVII
150
100
75
(B)
CD4T
缓冲液处理的
FTVII
PSGL-1 CD43 CD44 PSGL-1 CD43 CD44
IP IP IP IP IP IP
CD8T
缓冲液处理的
FTVII
PSGL-1 CD43 CD44 PSGL-1 CD43 CD44
IP IP IP IP IP IP
B细胞
空白对照
FTVII
PSGL-1 CD43 CD44 PSGL-1 CD43 CD44
IP IP IP IP IP IP
MW
(kDa)

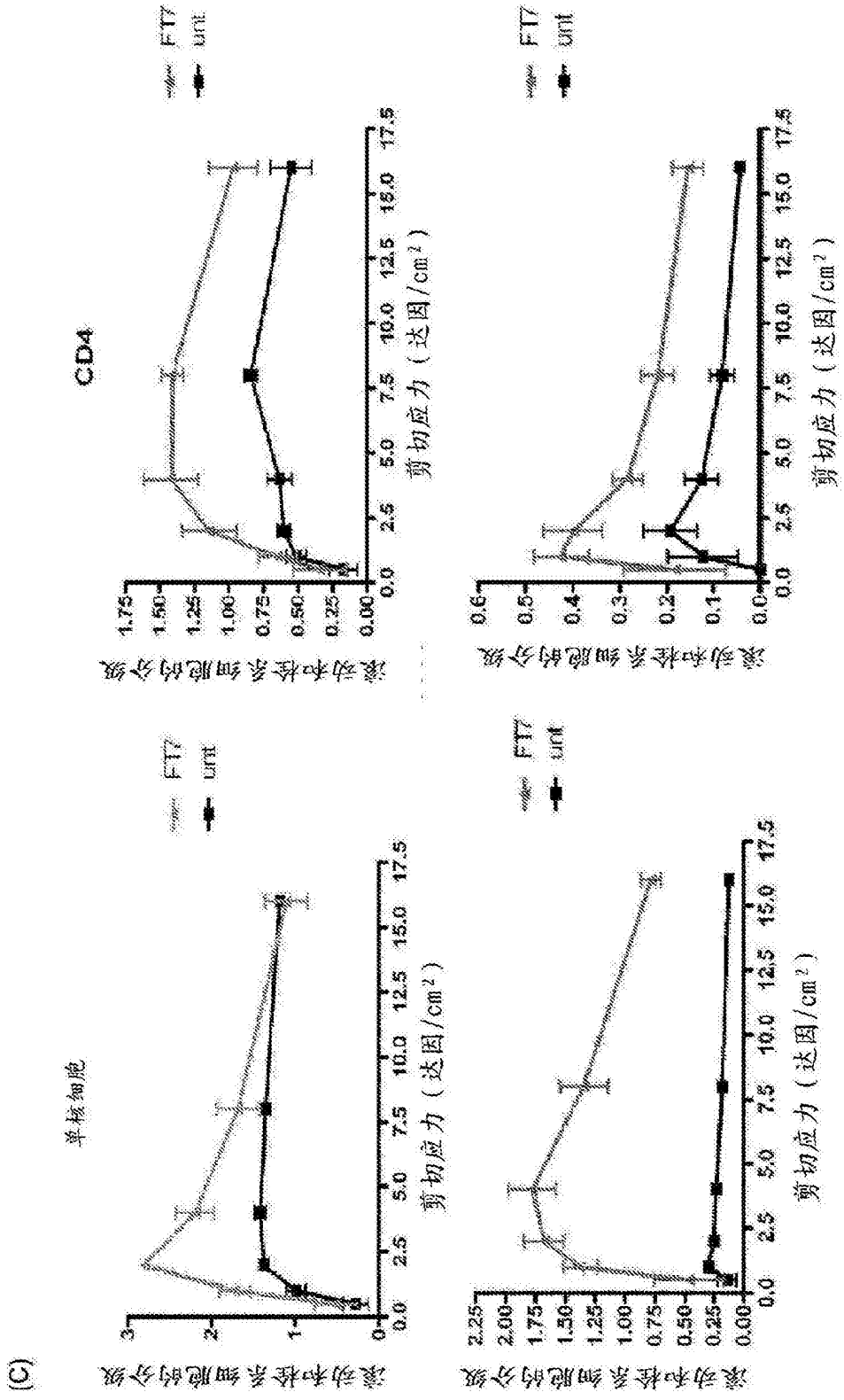


图28

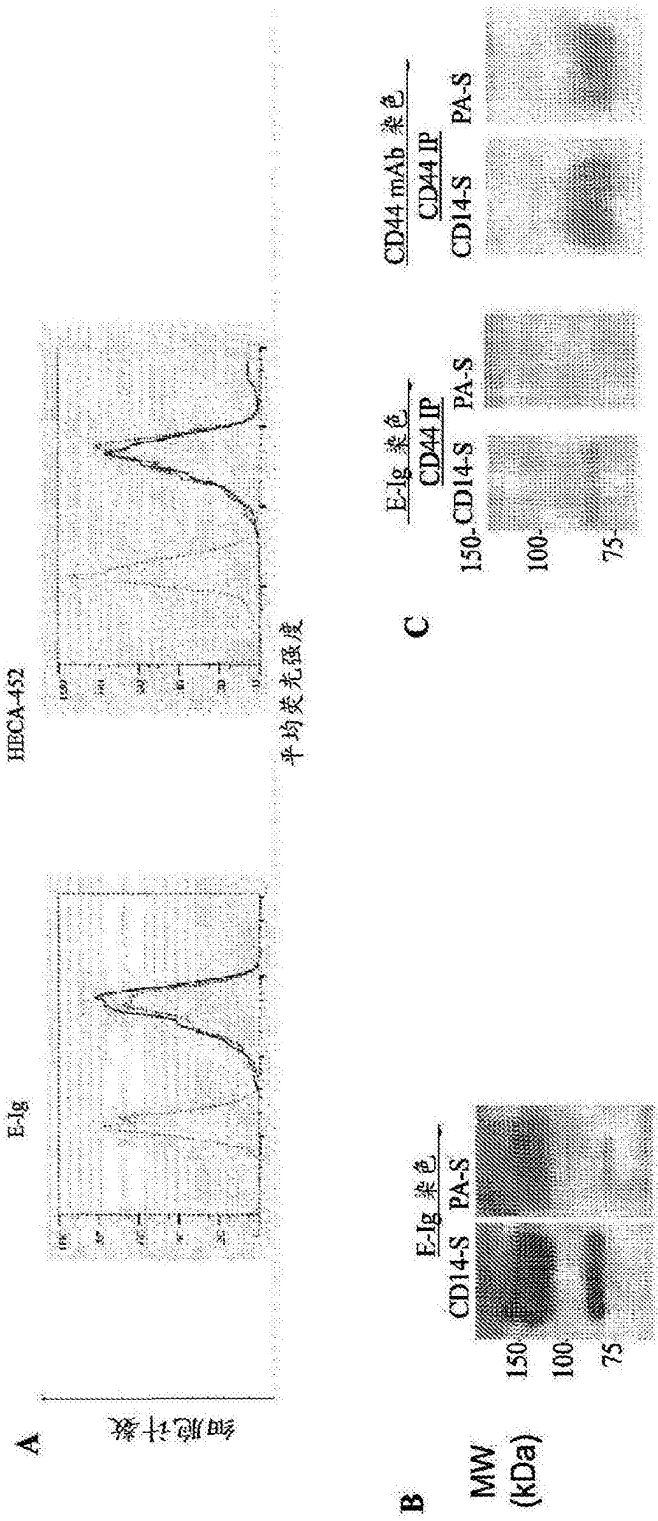

A
细胞计数
E-Ig
HECA-452
平均荧光强度
B
E-Ig 来色
CD14-S PA-S
MW (kDa)
150
100
75
C
E-Ig 来色
CD44 IP
CD14-S PA-S
150
100
75
CD44 mAb 来色
CD44 IP
CD14-S PA-S

图29

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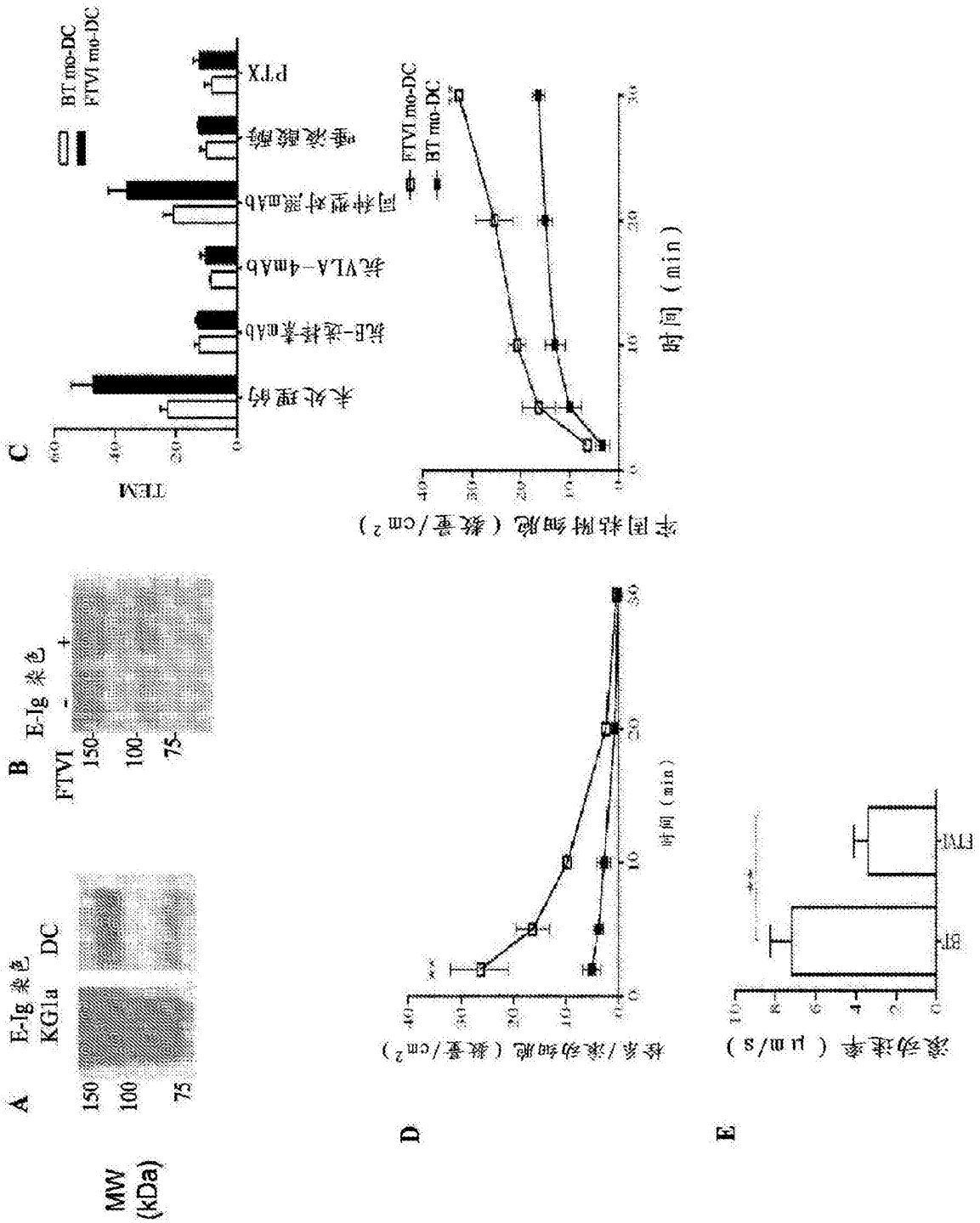

图30

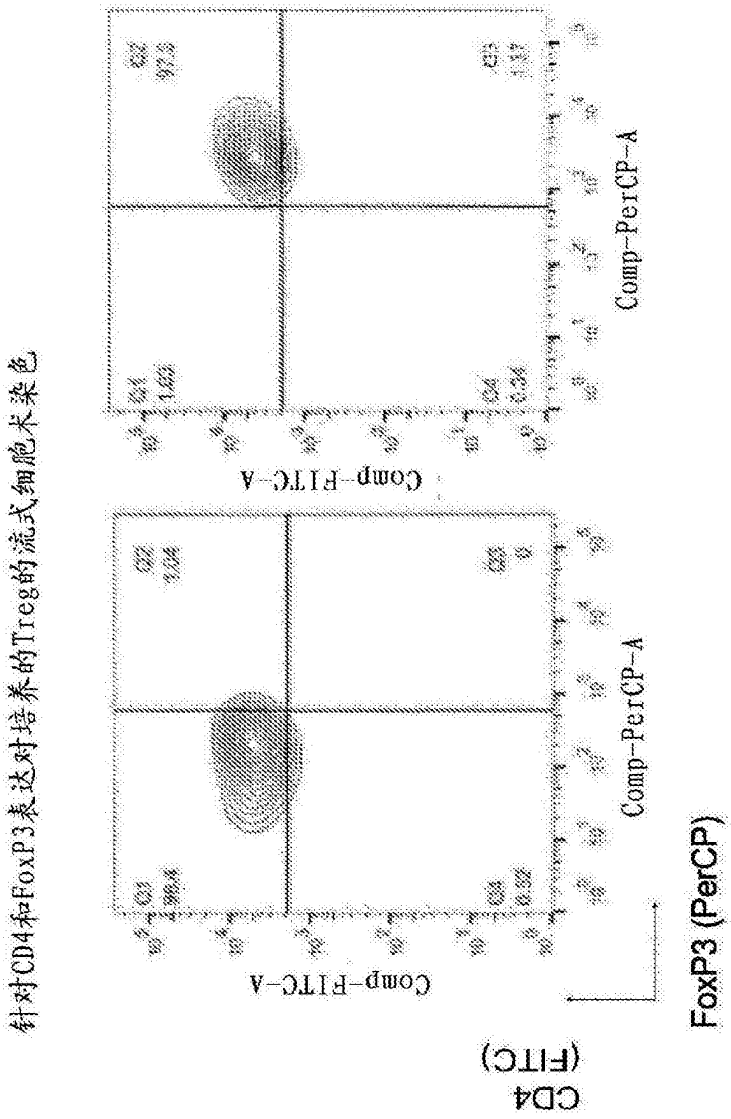
针对CD4和FoxP3表达对培养的Treg的流式细胞术染色
Comp-FITC-A
Comp-PerCP-A
FoxP3 (PerCP)
CD4
(FITC)

图31

打印ID7891292 Pg 124

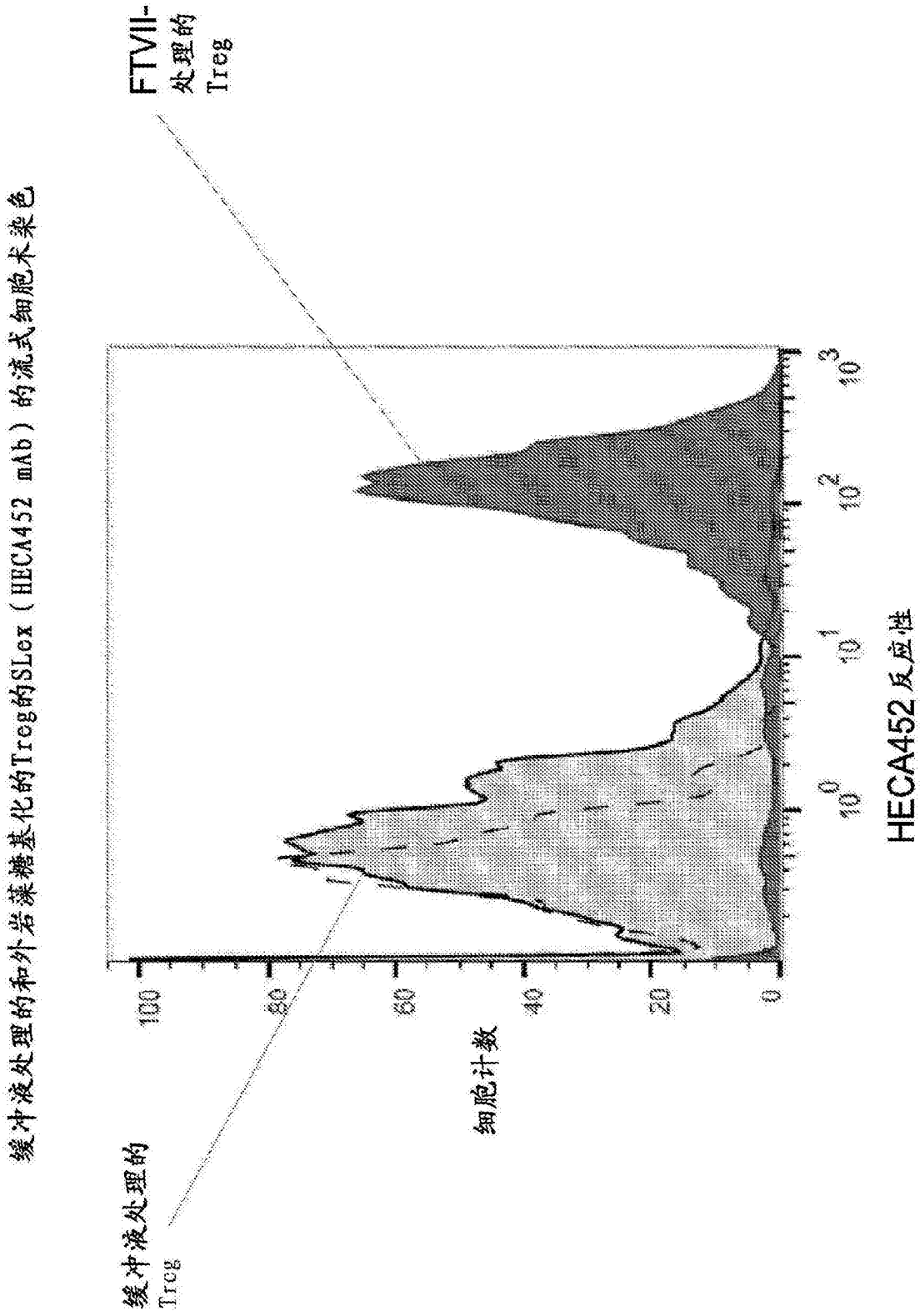

图32

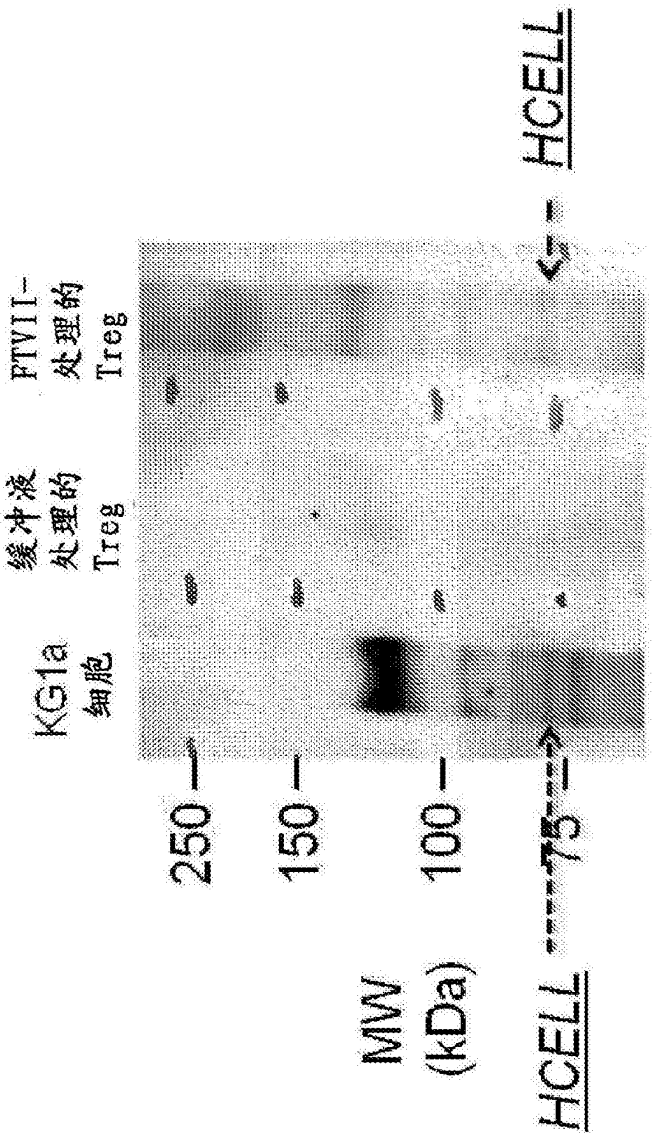
KG1a 细胞
缓冲液处理的 Treg
PTVII-处理的 Treg
250
150
100
75
MW (kDa)
HCELL
HCELL

图33

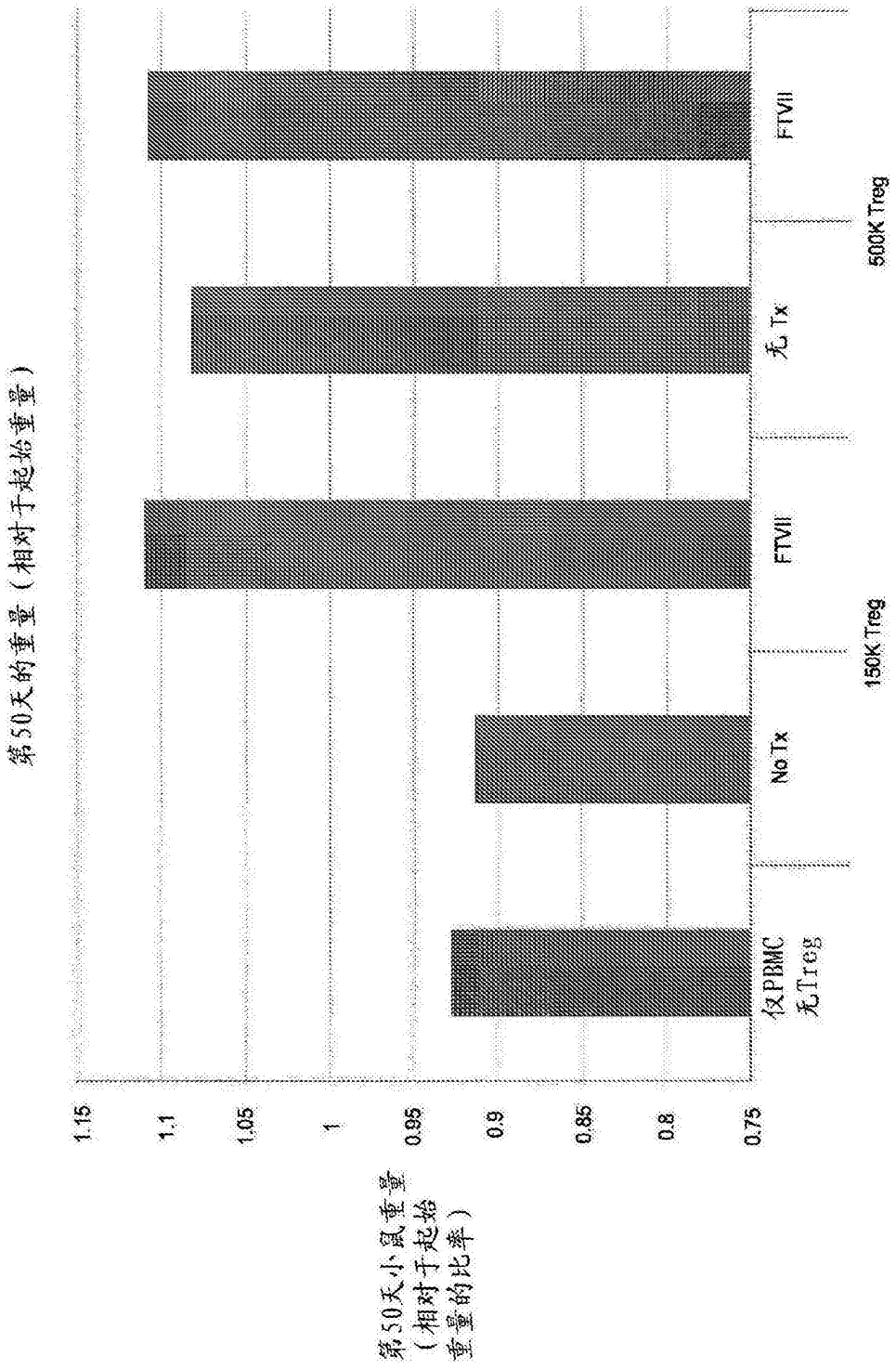
第50天的重量（相对于起始重量）
1.15
1.1
1.05
1
0.95
0.9
0.85
0.8
0.75
第50天小鼠重量（相对于起始重量的比率）
仅PBMC无Treg
No Tx
FTVII
150K Treg
无 Tx
FTVII
500K Treg

图34