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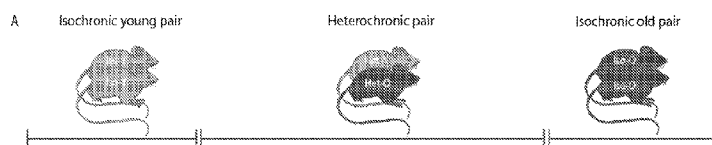
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(54) Title: METHODS AND COMPOSITIONS FOR INCREASING NEUROGENESIS AND ANGIOGENESIS



(57) Abstract: Methods and compositions for rejuvenating neural stem cells and/or progenitor cells, increasing neurogenesis, increasing angiogenesis, and treating or preventing neurodegenerative and neurovascular disorders are disclosed.



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METHODS AND COMPOSITIONS FOR INCREASING NEUROGENESIS AND ANGIOGENESIS

RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Application No. 61/833,813, filed June 11, 2013, and U.S. Provisional Application No. 61/988,862, filed May 5, 2014, the entire teachings of which are incorporated herein by reference.

GOVERNMENT SUPPORT

[0002] This invention was made with government support under R01 AG032977; 1R01 AG040019; 5U01 HL100402; 1DP2 OD004345 and 1R01AG033053 awarded by National Institutes of Health. The government has certain rights in the invention.

BACKGROUND OF THE INVENTION

[0003] Age-dependent dysfunction in adult stem cells is attributable to both cell-intrinsic and -extrinsic inputs. Critical mechanisms underlying the functional decline of aged stem cells remain elusive. Accordingly, there exists a need to identify factors that are able to promote or reverse age-associated changes in tissues as diverse as the skeletal muscle, liver and CNS (Wagers and Conboy, *Cell* 122: 659 (2005); Ruckh *et al.*, *Cell Stem Cell* 10:96 (2005).

SUMMARY OF THE INVENTION

[0004] The methods and compositions described herein are useful for example, for rejuvenating neural stem cells and/or progenitor cells, increasing neurogenesis and angiogenesis, treating or preventing neurodegenerative disorders and neurovascular disorders, and treating or preventing disorders, conditions, or symptoms associated with decreased neurogenesis and angiogenesis, for example, decreased neurogenesis or angiogenesis due to aging.

[0005] In some aspects, the disclosure provides a method for increasing neurogenesis in a subject in need thereof, comprising administering to the subject an agent or composition which increases the level of GDF11 polypeptide in the subject, thereby increasing neurogenesis in the subject.

[0006] In some aspects, the disclosure provides a method for treating or preventing a neurodegenerative disorder by increasing neurogenesis in a subject, comprising administering to the subject an effective amount of an agent or composition which increases the level of GDF11 polypeptide in the subject, thereby increasing neurogenesis in the subject, wherein increasing neurogenesis in the subject treats or prevents the neurodegenerative disorder in the subject.

[0007] In some aspects, the disclosure provides a pharmaceutical composition comprising a GDF11 polypeptide or a functional fragment or variant thereof, and a pharmaceutically acceptable carrier.

[0008] In some aspects, the disclosure relates to the use of a composition comprising a GDF11 polypeptide or functional fragment or variant thereof for increasing neurogenesis in a subject in need thereof, wherein increased levels of the GDF11 polypeptide or functional fragment or variant thereof in the subject increase neurogenesis in the subject.

[0009] In some aspects, the disclosure relates to the use of a composition comprising a GDF11 polypeptide or functional fragment or variant thereof for treating or preventing a neurodegenerative disorder by increasing neurogenesis in a subject in need thereof, wherein increased levels of the GDF11 polypeptide or functional fragment or variant thereof in the subject increase neurogenesis in the subject, thereby treating or preventing the neurodegenerative disorder in the subject.

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[0010] In some embodiments of any aspect described herein, a GDF11 polypeptide or functional fragment or variant thereof binds to, activates or signals through the ALK4/5 receptor. In some embodiments of any aspect described herein, activation or modulation of the ALK4/5 receptor causes the phosphorylation of SMAD2/3 proteins as the first component of the signaling cascade and increases SMAD phosphorylation. In some embodiments of any aspect described herein, GDF11 promotes proliferation of brain capillary endothelial cells through the SMAD signaling cascade.

[0011] In some aspects, the disclosure relates to the use of a composition comprising a GDF11 polypeptide or functional fragment or variant thereof for increasing angiogenesis in a subject in need thereof, wherein increased levels of the GDF11 polypeptide or functional fragment or variant thereof in the subject increase angiogenesis in the subject.

[0012] In some aspects, the disclosure relates to the use of a composition comprising a GDF11 polypeptide or functional fragment or variant thereof for treating or preventing a neurovascular disorder by increasing angiogenesis in a subject, wherein increased levels of the GDF11 polypeptide or functional fragment or variant thereof in the subject increase angiogenesis in the subject, thereby treating or preventing the neurovascular disorder in the subject.

[0013] In some embodiments of any aspect described herein, increasing neurogenesis is associated with increased neural cell proliferation, increased neural cell differentiation, increased number or frequency of neural stem cells, increased number of neural progenitor cells, increased number of neural precursor cells, and increased expression of at least one synaptic plasticity gene, at least one neuroprotective gene, or at least one neuronal specification gene selected from the group consisting of neuronal PAS domain protein 4 (Npas4), dual specificity phosphatase 1 (Dusp1), heat shock 70kDa protein 8 (Hspa8), BTG family, member 2 (Btg2), activity-regulated cytoskeleton-associated protein (Arc), nuclear receptor subfamily 4, group A, member 1 (Nr4a1), cysteine-rich, angiogenic inducer, 61 (Cyr61), FBJ murine osteosarcoma viral oncogene homolog (Fos), jun B proto-oncogene (Junb), Kruppel-like factor 10 (Klf10), nuclear receptor subfamily 4, group A, member 2 (Nr4a2), claudin 1 (Cldn1), early growth response 2 (Egr2), crystalline,

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mu (Crym), early growth response 1 (Egr1), and polio-like kinase 2 (Plk2), and increasing the number of new neurons. In some embodiments, neurogenesis is increased in the subventricular zone of the lateral ventricles. In some embodiments, neurogenesis is increased in the olfactory bulb. In some embodiments, increased neurogenesis results in improved olfactory behavior.

[0014] In some embodiments of any aspect described herein, a GDF11 polypeptide or functional fragment or variant thereof increases neurogenesis by activating or modulating the ALK4/5 receptor. In some embodiments of any aspect described herein, a GDF11 polypeptide or functional fragment or variant thereof increases neurogenesis through the SMAD signaling cascade.

[0015] In some embodiments of any aspect described herein, a GDF11 polypeptide or functional fragment or variant thereof increases angiogenesis by activating or modulating the ALK4/5 receptor. In some embodiments of any aspect described herein, a GDF11 polypeptide or functional fragment or variant thereof increases angiogenesis through the SMAD signaling cascade.

[0016] In some embodiments of any aspect described herein, the neurodegenerative disorder is selected from the group consisting of Huntington's disease, dentatorubropallidoluysian atrophy, Kennedy's disease, spinocerebellar ataxia, fragile X syndrome, fragile XE mental retardation, Friedreich's ataxia, myotonic dystrophy, spinocerebellar ataxia type 8, and spinocerebellar ataxia type 12, Alexander disease, Alper's disease, Alzheimer disease, amyotrophic lateral sclerosis (ALS), ataxia telangiectasia, Batten disease, Canavan disease, Cockayne syndrome, corticobasal degeneration, Creutzfeldt-Jakob disease, Guillain-Barré syndrome, ischemia stroke, Krabbe disease, kuru, Lewy body dementia, multiple sclerosis, multiple system atrophy, non-Huntingtonian type of Chorea, Parkinson's disease, Pelizaeus-Merzbacher disease, Pick's disease, primary lateral sclerosis, progressive supranuclear palsy, Refsum's disease, Sandhoff disease, Schilder's disease, spinal cord injury, spinal muscular atrophy (SMA), SteeleRichardson-Olszewski disease, and Tabes dorsalis.

[0017] In some embodiments of any aspect described herein, the subject has been diagnosed with a neurodegenerative disorder due to aging.

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[0018] In some aspects, the disclosure provides a method for increasing angiogenesis in a subject in need thereof, comprising administering to the subject an agent or composition which increases the level of GDF11 polypeptide in the subject, thereby increasing angiogenesis in the subject.

[0019] In some aspects, the disclosure provides a method for treating or preventing a neurovascular disorder by increasing angiogenesis in a subject, comprising administering to the subject an agent or composition which increases the level of GDF11 polypeptide in the subject, thereby increasing angiogenesis in the subject, wherein increasing angiogenesis in the subject treats or prevents the neurovascular disorder in the subject.

[0020] In some embodiments of any aspect described herein, increasing angiogenesis comprises increasing cerebrovascular architecture, increasing capillary density, increasing cerebral blood flow, and increasing cerebral vessel sprouting branch points.

[0021] In some embodiments of any aspect described herein, the neurovascular disorder is selected from the group consisting of brain atherothrombosis, brain aneurysms, brain arteriovenous malformations, brain embolism, brain ischemia, for example caused by atherothrombosis, embolism, or hemodynamic abnormalities, cardiac arrest, carotid stenosis, cerebrovascular spasm, headache, intracranial hemorrhage, ischemic stroke, seizure, spinal vascular malformations, reflex neurovascular dystrophy (RND), neurovascular compression disorders such as hemifacial spasms, tinnitus, trigeminal neuralgia, glossopharyngeal neuralgia, stroke, transient ischemic attacks, and vasculitis.

[0022] In some embodiments of any aspect described herein, the subject has been diagnosed with a neurovascular disorder due to aging.

[0023] In some embodiments of any aspect described herein, the level of GDF11 polypeptide is increased in at least one of the subject's systemic circulation, cerebral vasculature, cerebral tissue, cerebrospinal fluid, lateral ventricles, neurovasculature, and subventricular zone neurovascular niche.

[0024] In some embodiments of any aspect described herein, the agent or composition comprises an agonist antibody that increases expression of GDF11. In some embodiments of any aspect described herein, the agent or composition binds to,

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activates or signals through the ALK4/5 receptor. In some embodiments of any aspect described herein, the agent or composition comprises a GDF11 polypeptide. In some embodiments of any aspect described herein, the agent or composition comprises a GDF11 polypeptide comprising the amino acid sequence of SEQ ID NO: 3. In some embodiments of any aspect described herein, the agent or composition comprises a GDF11 polypeptide comprising the amino acid sequence of SEQ ID NO: 2. In some embodiments of any aspect described herein, the agent or composition comprises a GDF11 polypeptide comprising the amino acid sequence of SEQ ID NO: 1. In some embodiments of any aspect described herein, the agent or composition comprises a GDF11 polypeptide comprising the amino acid sequence of SEQ ID NO: 4. In some embodiments of any aspect described herein, the agent or composition comprises homodimers of GDF11 polypeptides comprising the amino acid sequence of any of SEQ ID NOs: 1, 2, 3 or 4. In some embodiments of any aspect described herein, the agent or composition comprises complexes of GDF11 polypeptides comprising the amino acid sequence of any of SEQ ID NOs: 1, 2, 3 or 4. In some embodiments of any aspect described herein, the agent or composition comprises a nucleic acid encoding a GDF11 polypeptide or a functional fragment or variant thereof.

[0025] In some embodiments of any aspect described herein, the GDF11 polypeptide comprises a modified GDF11 polypeptide. In some embodiments of any aspect described herein, the modified GDF11 polypeptide comprises a modification selected from the group consisting of fusion to an Fc fragment, pegylation, conjugation to albumin, an amino acid mutation that prevents or reduces proteolytic degradation, an amino acid mutation that prolongs half-life, and any combination thereof.

[0026] In some embodiments of any aspect described herein, the composition is administered via a route selected from the group consisting of intravenously, subcutaneously, intra-arterially, and intra-muscularly.

[0027] In some embodiments of any aspect described herein, the level of GDF11 polypeptide is increased by at least 100%. In some embodiments of any aspect described herein, the level of GDF11 polypeptide is increased to at least 75% of a healthy reference level.

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[0028] In some embodiments of any aspect described herein, the composition increases the level of GDF11 polypeptide in at least one of the subject's systemic circulation, cerebral vasculature, cerebrospinal fluid, cerebral tissue, lateral ventricles, neurovasculature, and subventricular zone neurovascular niche.

[0029] In some embodiments of any aspect described herein, the composition comprises an isolated or recombinant GDF11 polypeptide. In some embodiments of any aspect described herein, the isolated or recombinant GDF11 polypeptide comprises a modification selected from the group consisting of fusion to an Fc fragment, pegylation, conjugation to albumin, an amino acid mutation that prevents or reduces proteolytic degradation, an amino acid mutation that prolongs half-life, and any combination thereof.

[0030] The practice of the present invention will typically employ, unless otherwise indicated, conventional techniques of cell biology, cell culture, molecular biology, transgenic biology, microbiology, recombinant nucleic acid (e.g., DNA) technology, immunology, and RNA interference (RNAi) which are within the skill of the art. Non-limiting descriptions of certain of these techniques are found in the following publications: Ausubel, F., et al., (eds.), *Current Protocols in Molecular Biology*, *Current Protocols in Immunology*, *Current Protocols in Protein Science*, and *Current Protocols in Cell Biology*, all John Wiley & Sons, N.Y., edition as of December 2008; Sambrook, Russell, and Sambrook, *Molecular Cloning: A Laboratory Manual*, 3rd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, 2001; Harlow, E. and Lane, D., *Antibodies – A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, 1988; Freshney, R.I., "Culture of Animal Cells, A Manual of Basic Technique", 5th ed., John Wiley & Sons, Hoboken, NJ, 2005. Non-limiting information regarding therapeutic agents and human diseases is found in Goodman and Gilman's *The Pharmacological Basis of Therapeutics*, 11th Ed., McGraw Hill, 2005, Katzung, B. (ed.) *Basic and Clinical Pharmacology*, McGraw-Hill/Appleton & Lange; 10th ed. (2006) or 11th edition (July 2009). Non-limiting information regarding genes and genetic disorders is found in McKusick, V.A.: *Mendelian Inheritance in Man. A Catalog of Human Genes and Genetic Disorders*. Baltimore: Johns Hopkins University Press, 1998 (12th edition) or the more recent online database: Online Mendelian Inheritance in Man, OMIM™.

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McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University (Baltimore, MD) and National Center for Biotechnology Information, National Library of Medicine (Bethesda, MD), as of May 1, 2010, World Wide Web URL: <http://www.ncbi.nlm.nih.gov/omim/> and in Online Mendelian Inheritance in Animals (OMIA), a database of genes, inherited disorders and traits in animal species (other than human and mouse), at <http://omia.angis.org.au/contact.shtml>.

[0031] All patents, patent applications, and other publications (e.g., scientific articles, books, websites, and databases) mentioned herein are incorporated by reference in their entirety. In case of a conflict between the specification and any of the incorporated references, the specification (including any amendments thereof, which may be based on an incorporated reference), shall control. Standard art-accepted meanings of terms are used herein unless indicated otherwise. Standard abbreviations for various terms are used herein.

BRIEF DESCRIPTION OF THE DRAWINGS

[0032] The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawings will be provided by the Office upon request and payment of the necessary fee.

[0033] **Figs. 1A-1J** demonstrate that young blood stimulates neural stem and progenitor cell proliferation and expansion in the subventricular zone (SVZ). **Fig. 1A** shows a graphical scheme representing parabiotic pairs of young (2 months, CD45.1) and old (15-18 months, CD45.2) mice that were joined for 5 weeks. **Figs. 1B, 1C,** and **1D** show confocal images of coronal sections of the SVZ, double-labeled for Ki67 and GFAP (**Fig. 1B**), Sox2 and GFAP (**Fig. 1C**) and Olig2 and GFAP (**Fig. 1D**). Scale bar: 50µm. **Figs. 1E, 1F, 1G, 1H, 1I,** and **1J** show quantification of proliferative, neural stem and progenitor cell populations in the SVZ area, depicting the effect of parabiosis between isochronic and heterochronic parabionts. Quantification of Ki67⁺ (**Fig. 1E**), Sox2⁺ (**Fig. 1F**) and Olig2⁺ cells (**Fig. 1G**) in old parabionts. Quantification of Ki67⁺ (**Fig. 1H**), Sox2⁺ (**Fig. 1I**) and Olig2⁺ cells (**Fig. 1J**) in young parabionts (n Iso-O = 6-8, n Iso-Y = 6, n Het-O = 7-8, n Het-Y = 6, *p≤0.05, **p≤0.01). Data shown as mean ± S.E.M, statistical analysis by t-test.

[0034] **Figs. 2A-2F** demonstrate that heterochronic parabiosis enhances neurogenesis and cognitive functions in the old mouse. **Fig. 2A** shows the maximum projection of Z-stack images of olfactory bulb (OB) sections immunostained for BrdU and NeuN showing the increase in newborn neurons after heterochronic parabiosis in old mice whereas young mice are not affected. Scale bar: 100µm. **Figs. 2B and 2C** show quantification of newborn neurons in the OB of old (**Fig. 2B**) and young (**Fig. 2C**) parabionts (n=4, *p≤0.05). Numbers are shown per field. **Fig. 2D** shows measurement of the exploratory time during the olfaction assay (n=3, **p≤0.01). Data shown as mean ± S.E.M, statistical analysis by t-test. **Fig. 2E** shows the results of microarray analysis in tabular format showing the fold-increase up-regulation of genes in Het-O compared to Iso-O that were also up-regulated in the Iso-Y versus Iso-O comparison. **Fig. 2F** shows histograms showing the fold increase in gene expression after real-time RT-PCR.

[0035] **Figs. 3A-3I** demonstrate that young blood enhances vascularization and increases blood flow in the old mouse. **Fig. 3A** shows confocal images of coronal subventricular zone (SVZ) sections stained with CD31. Scale bar: 50µm. **Figs. 3B, 3C, and 3E** are bar graphs showing the results of volumetric analyses of 3D angiograms generated from z-stacks of 100µm thick sections (also see **Fig. 10**) of old-young (**Fig. 3B**), old (**Fig. 3C**) and young (**Fig. 3E**) parabionts (n old = 9, n young = 6, ***p≤0.001). **Figs. 3D and 3F** are bar graphs showing the quantification of blood vessel branchpoints per field in old (**Fig. 3D**) and young (**Fig. 3F**) parabionts (n old = 8, n young = 5, *p≤0.05). **Fig. 3G** shows perfusion MRI images of the SVZ. **Figs. 3H and 3I** are bar graphs showing measurements of cerebral blood flow in Iso-O and Iso-Y (**Fig. 3G**) or Het-O (**Fig. 3I**) mice from MRI perfusion experiments (n=4, *p≤0.05, **p≤0.01). Data shown as mean ± S.E.M, statistical analysis by t-test.

[0036] **Figs. 4A-4F** demonstrate that young circulating factor GDF11 recapitulates the effect of heterochronic parabiosis by enhancing angiogenesis and neurogenesis. **Figs. 4A and 4C** show confocal images of coronal subventricular zone (SVZ) sections showing that old mice (22 months) injected with GDF11 for 4 weeks have enhanced vascularization marked by CD31 (**Fig. 4A**) as well as increased Sox2⁺ neural stem cell populations (**Fig. 4C**) compared to control. **Fig. 4B** is a bar graph showing the results of volumetric analyses of 3D angiograms generated from z-stacks

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of 100µm thick sections showing that GDF11 treatment enhances blood vessel volume (n=9, **p≤0.01). **Fig. 4D** shows the quantification of Sox2⁺ cells in the SVZ area (n=6, *p≤0.05). **Fig. 4E** shows images of primary brain endothelial cell culture after treatment with or without GDF11 for 6 days and EdU for the last 4 hours, labeled with CD31, EdU and Hoechst. **Fig. 4F** is a bar graph showing quantification of the images depicted in **Fig. 4E** (n=3, *p≤0.05). Data shown as mean ± S.E.M, statistical analysis by pair-wise t-test. Scale bars: 50µm.

[0037] **Fig. 5** illustrates the confirmation of cross-circulation by blood chimerism by flow-cytometry analysis of the chimeric blood from Iso-Y, Het-Y, Het-O and Iso-O mice.

[0038] **Fig. 6** is a histogram showing the difference in neurosphere size of neurospheres derived by either Iso-O or Het-O mice and passaged at least once (n=15, **p≤0.01). Data shown as mean ± S.E.M, statistical analysis by t-test.

[0039] **Figs. 7A-7C** demonstrate that heterochronic parabiosis induces neuronal differentiation *in vitro*. **Fig. 7A** is a representative image showing the results of a neurosphere differentiation assay of Iso-O and Het-O derived neurospheres. **Fig. 7B** is a histogram showing the difference in TuJ1⁺ neurons. **Fig. 7C** is a histogram showing that no difference was observed in GFAP⁺ cell populations. (n=12, *p≤0.05). Data shown as mean ± S.E.M, statistical analysis by t-test.

[0040] **Fig. 8** shows an experimental setup for the olfaction assay. At the end of the 5-week parabiosis, mice are placed in a clean cage and exposed to the odorant.

[0041] **Figs. 9A and 9B** illustrate the results of microarray analyses on the forebrain of isochronic and heterochronic mice. **Fig. 9A** illustrates a microarray heat map and **Fig. 9B** illustrates Venn diagrams showing the numbers of genes identified in clusters that were either up-regulated or down-regulated. Note that the colored sections correspond to the list of genes on identified in **Fig. 2E**.

[0042] **Figs. 10A-10C** depict comparisons of the vasculature of Het-O and Iso-O paired mice. **Fig. 10A** depicts 3D reconstructions of the subventricular zone (SVZ) vasculature generated by confocal imaging of 100µm thick sections and processed with VOLOCITY software. **Fig. 10B** shows confocal images of coronal

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SVZ sections showing double-labeling with lectin and aquaporin-4 (AQP4). **Fig. 10C** shows confocal images of coronal cortical sections labeled with CD31.

[0043] **Fig. 11** shows a comparison of heterochronic parabiosis between young- GFP mice and old non-GFP mice. High magnification confocal image of the Het-O showing that there is no co-localization of circulating GFP progenitors with CD31⁺ (red) blood vessels.

[0044] **Figs. 12A and 12B** demonstrate the effects of either young or old mouse serum on primary brain capillary endothelial cells. **Fig. 12A** shows representative images of primary brain capillary endothelial cell cultures treated with serum-free media supplemented with either young or old mouse serum for 2 days and then pulsed for 4 hours with EdU. Scale bar: 50 μ m. **Fig. 12B** is a bar graph showing the quantification of **Fig. 12A**. (n=3, *p $\leq$ 0.05). Data shown as mean $\pm$ S.E.M, statistical analysis by pair-wise t-test.

[0045] **Fig. 13** depicts an example of an alignment between human GDF11 precursor peptide (query sequence; residues 62-407 of SEQ ID NO: 1) and human GDF8 precursor peptide created using the default settings of the alignment tool of the BLASTP program.

[0046] **Fig. 14** depicts an example of an alignment between human GDF11 precursor peptide (query sequence; residues 47-407 of SEQ ID NO: 1) and murine GDF11 precursor peptide created using the default settings of the alignment tool of the BLASTP program.

[0047] **Figs. 15A-15E** illustrate an age-dependent accumulation of factors in the blood of older mice that affect neurogenic zones in both the hippocampus and subventricular zone (SVZ) of the young mice. **Fig. 15A** graphically depicts parabiotic pairs of young (2 months, CD45.1) and old (21 months, CD45.2) mice that were joined for 5 weeks. **Figs. 15B and C** show confocal images of coronal sections of the SVZ, labeled for Ki67 (**Fig. 15B**) and Sox2 (**Fig. 15C**). Scale bar: 50 μ m. **Figs. 15D and E** are bar graphs respectively showing the quantification of Ki67⁺ cells and Sox2⁺ cells in the SVZ area of young parabionts (n = 4 animals per condition , *p<0.05, **p<0.01). Data shown as mean $\pm$ S.E.M; statistical analysis by Student's t-test.

[0048] **Figs. 16A and 16B** illustrate that increased vascularization in the Het-O mice extended to other neurogenic areas like the hippocampus. **Fig. 16A** shows confocal images of coronal sections of hippocampus dentate gyrus labeled with CD31. Scale bar: 50 μ m. **Fig. 16B** illustrates the results of a volumetric analysis of 3D angiograms generated from z-stacks of 100 μ m thick sections of the dentate gyrus (n = 7 animals per condition, *p<0.05). Data shown as mean $\pm$ S.E.M; statistical analysis by Student's t-test.

[0049] **Figs. 17A and 17B** show that the number of pericytes, which play a role in capillary vasoconstriction, were unaltered by heterochronic parabiosis. **Fig. 17A** illustrates confocal images of the subventricular zone (SVZ) coronal sections double-labeled with NG2 and CD31 to mark pericytes. Scale bar: 20 μ m. **Fig. 17B** shows the quantification of NG2⁺/CD31⁺ cells in the SVZ area of old parabionts (n = 4 animals per condition). Data shown as mean $\pm$ S.E.M; statistical analysis by Student's t-test.

[0050] **Figs. 18A and 18B** illustrate that GDF11 recapitulates the effect of heterochronic parabiosis by enhancing vascular remodeling and neurogenesis. **Fig. 18A** shows representative images of primary brain capillary endothelial cell cultures treated with either GDF11 (40ng/ml) or TFG β (10ng/ml) in the presence of the control sodium orthovanadate to preserve phosphorylation for 30 minutes. Scale bar: 100 μ m. **Fig. 18B** shows the quantification of the images presented in **Fig. 18A** (n=7, *p $\leq$ 0.05). Data shown as mean $\pm$ S.E.M; statistical analysis by t-test, compared to the control.

[0051] **Fig. 19** shows the amino acid sequence encoding a human GDF11 precursor polypeptide (SEQ ID NO: 1).

[0052] **Fig. 20** shows the amino acid sequence encoding a human GDF11 pro-peptide (SEQ ID NO: 2).

[0053] **Fig. 21** shows the amino acid sequence encoding a human mature GDF11 polypeptide (SEQ ID NO: 3).

[0054] **Fig. 22** shows the amino acid sequence encoding a human GDF11 N-terminal polypeptide (SEQ ID NO: 4),

[0055] **Fig. 23** shows the nucleic acid sequence encoding human GDF11 mRNA (SEQ ID NO: 5).

DETAILED DESCRIPTION OF THE INVENTION

[0056] Described herein are methods and compositions based on the discovery that as animals age, the level of GDF11 polypeptide in their blood decreases and deterioration of neural stem cells and/or progenitor cells increases, accompanied by a diminished capacity for neurogenesis and angiogenesis. The methods and compositions described herein are useful for rejuvenating the neurovascular niche (e.g., neural stem cells and/or progenitor cells), reversing decline (e.g., age-related decline) in neurogenesis and/or angiogenesis, increasing neurogenesis, increasing angiogenesis, treating or preventing neurodegenerative disorders, treating or preventing neurovascular disorders, for example, neurodegenerative disorders or neurovascular disorders associated with decreased neurogenesis and angiogenesis, respectively, due to aging. The methods and compositions described herein are also useful for increasing neuronal activity, inducing vascular remodeling, increasing neuroplasticity, increasing cognitive functioning, increasing olfactory sensitivity/behavior, or increasing neuronal stem cell and/or stem cell progenitor numbers and/or proliferation rate. The methods and compositions described herein generally relate to increasing the level of GDF11 (e.g., GDF11 polypeptide) in a subject to treat, prevent, or reverse the neurodegenerative and neurovascular disorders, conditions, and symptoms described herein. In particular embodiments the compositions and methods described herein have effect in the subventricular zone of the brain, while in other embodiments they have effect in one or more non-neurogenic regions of the brain.

[0057] For convenience, certain terms employed herein are collected here. Unless stated otherwise, or implicit from context, the following terms and phrases include the meanings provided below. Unless explicitly stated otherwise, or apparent from context, the terms and phrases below do not exclude the meaning that the term or phrase has acquired in the art to which it pertains. The definitions are provided to aid in describing particular embodiments, and are not intended to limit the claimed invention. Unless otherwise defined, all technical and scientific terms used herein have the meaning commonly understood by one of skill in the art.

[0058] As used herein, “neurodegenerative disorder” refers to a disease condition involving neuronal loss mediated or characterized at least partially by at least one of deterioration of neural stem cells and/or progenitor cells, a decreased capacity for neurogenesis, or a reduction in circulating GDF11 polypeptide in a subject. Non-limiting examples of neurodegenerative disorders include polyglutamine expansion disorders (e.g., HD, dentatorubropallidoluysian atrophy, Kennedy's disease (also referred to as spinobulbar muscular atrophy), and spinocerebellar ataxia (e.g., type 1, type 2, type 3 (also referred to as Machado-Joseph disease), type 6, type 7, and type 17)), other trinucleotide repeat expansion disorders (e.g., fragile X syndrome, fragile XE mental retardation, Friedreich's ataxia, myotonic dystrophy, spinocerebellar ataxia type 8, and spinocerebellar ataxia type 12), Alexander disease, Alper's disease, Alzheimer disease, amyotrophic lateral sclerosis (ALS), ataxia telangiectasia, Batten disease (also referred to as Spielmeyer-Vogt-Sjogren-Batten disease), Canavan disease, Cockayne syndrome, corticobasal degeneration, Creutzfeldt-Jakob disease, Guillain-Barré syndrome, ischemia stroke, Krabbe disease, kuru, Lewy body dementia, multiple sclerosis, multiple system atrophy, non-Huntingtonian type of Chorea, Parkinson's disease, Pelizaeus-Merzbacher disease, Pick's disease, primary lateral sclerosis, progressive supranuclear palsy, Refsum's disease, Sandhoff disease, Schilder's disease, spinal cord injury, spinal muscular atrophy (SMA), SteeleRichardson-Olszewski disease, and Tabes dorsalis.

[0059] In certain contexts, neurodegenerative disorders encompass neurological injuries or damages to the CNS or the PNS associated with physical injury (e.g., head trauma, mild to severe traumatic brain injury (TBI), spinal cord injury, diffuse axonal injury, craniocerebral trauma, cranial nerve injuries, cerebral contusion, intracerebral haemorrhage and acute brain swelling), ischemia (e.g., resulting from spinal cord infarction or ischemia, ischemic infarction, stroke, cardiac insufficiency or arrest, atherosclerotic thrombosis, ruptured aneurysm, embolism or haemorrhage), certain medical procedures or exposure to biological or chemic toxins or poisons (e.g., surgery, coronary artery bypass graft (CABG), electroconvulsive therapy, radiation therapy, chemotherapy, anti-neoplastic drugs, immunosuppressive agents, psychoactive, sedative or hypnotic drugs, alcohol, bacterial or industrial

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toxins, plant poisons, and venomous bites and stings), tumors (e.g., CNS metastasis, intraaxial tumors, primary CNS lymphomas, germ cell tumors, infiltrating and localized gliomas, fibrillary astrocytomas, oligodendrogliomas, ependymomas, pleomorphic xanthoastrocytomas, pilocytic astrocytomas, extraaxial brain tumors, meningiomas, schwannomas, neurofibromas, pituitary tumors, and mesenchymal tumors of the skull, spine and dura matter), infections (e.g., bacterial, viral, fungal, parasitic or other origin is selected from the group consisting of pyrogenic infections, meningitis, tuberculosis, syphilis, encephalomyelitis and leptomeningitis), metabolic or nutritional disorders (e.g., glycogen storage diseases, acid lipase diseases, Wemicke's or Marchiafava-Bignami's disease, Lesch-Nyhan syndrome, Farber's disease, gangliosidoses, vitamin B12 and folic acid deficiency), cognition or mood disorders (e.g., learning or memory disorder, bipolar disorders and depression), and various medical conditions associated with neural damage or destruction (e.g., asphyxia, prematurity in infants, perinatal distress, gaseous intoxication for instance from carbon monoxide or ammonia, coma, hypoglycaemia, dementia, epilepsy and hypertensive crises).

[0060] As used herein, “neurovascular disorder” refers to any disease or condition that results in cerebrospinal ischemia, infarction, and hemorrhage mediated or characterized at least partially by a decreased capacity for angiogenesis or a reduction in circulating GDF11 polypeptide in a subject. Neurovascular disorders encompass any abnormality of the blood vessels within or supplying the brain and spine. This includes narrowing of arteries, which reduces blood flow to the brain and increases the risk of stroke (particularly “ischemic” stroke), and weakening of arteries, which may create brain aneurysms and increases the risk of intracranial bleeding (or “hemorrhagic” stroke.) Non-limiting examples of neurovascular disorders include brain atherothrombosis, brain aneurysms, brain arteriovenous malformations, brain embolism, brain ischemia, for example caused by atherothrombosis, embolism, or hemodynamic abnormalities, cardiac arrest, carotid stenosis, cerebrovascular spasm, headache, intracranial hemorrhage, ischemic stroke, seizure, spinal vascular malformations, reflex neurovascular dystrophy (RND), neurovascular compression disorders such as hemifacial spasms, tinnitus, trigeminal

neuralgia, glossopharyngeal neuralgia, stroke, transient ischemic attacks, and vasculitis.

[0061] The terms “decrease,” “reduce,” “reduced,” “reduction,” “decrease,” and “inhibit” are all used herein generally to mean a decrease by a statistically significant amount relative to a reference. However, for avoidance of doubt, “reduce,” “reduction” or “decrease” or “inhibit” typically means a decrease by at least 10% as compared to a reference level and can include, for example, a decrease by at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99% , up to and including, for example, the complete absence of the given entity or parameter as compared to the reference level, or any decrease between 10-99% as compared to the absence of a given treatment.

[0062] The terms “increased,” “increase” or “enhance” or “activate” are all used herein to generally mean an increase by a statically significant amount; for the avoidance of any doubt, the terms “increased”, “increase” or “enhance” or “activate” means an increase of at least 10% as compared to a reference level, for example an increase of at least about 20%, or at least about 30%, or at least about 40%, or at least about 50%, or at least about 60%, or at least about 70%, or at least about 80%, or at least about 90%, or up to and including a 100% increase or any increase between 10-100% as compared to a reference level, or at least about a 2-fold, or at least about a 3-fold, or at least about a 4-fold, or at least about a 5-fold or at least about a 10-fold increase, or any increase between 2-fold and 10-fold or more as compared to a reference level.

[0063] The term “isolated” or “partially purified” as used herein refers, in the case of a nucleic acid or polypeptide, to a nucleic acid or polypeptide separated from at least one other component (*e.g.*, nucleic acid or polypeptide) that is present with the nucleic acid or polypeptide as found in its natural source and/or that would be present with the nucleic acid or polypeptide when expressed by a cell, or secreted in the case of secreted polypeptides. A chemically synthesized nucleic acid or polypeptide or one synthesized using *in vitro* transcription/translation is considered “isolated.”

[0064] The term “biological sample” as used herein denotes a sample taken or isolated from a biological organism, e.g., cerebral tissue, cerebrospinal fluid, blood sample, cell lysate, a homogenate of a tissue sample from a subject, or a fluid sample from a subject. Exemplary biological samples include, but are not limited to, brain biopsies, cerebrospinal fluid, or blood and/or serum samples. In some embodiments, the sample is from a resection, biopsy, or core needle biopsy. In addition, fine needle aspirate samples can be used. Samples can include paraffin-embedded and frozen tissue. The term “biological sample” also includes untreated or pretreated (or pre-processed) biological samples. In some embodiments, the biological sample is an untreated biological sample. The sample can be obtained by removing a sample of cells from a subject, but can also be accomplished by using previously isolated cells (e.g. isolated at a prior time point and isolated by the same or another person).

[0065] As used herein, a “subject” means a human or animal. Usually the animal is a vertebrate such as a primate, rodent, domestic animal or game animal. Primates include chimpanzees, cynomolgous monkeys, spider monkeys, and macaques, e.g., Rhesus. Rodents include mice, rats, woodchucks, ferrets, rabbits and hamsters. Domestic and game animals include cows, horses, pigs, deer, bison, buffalo, feline species, e.g., domestic cat, canine species, e.g., dog, fox, wolf, avian species, e.g., chicken, emu, ostrich, and fish, e.g., trout, catfish and salmon. Patient or subject includes any subset of the foregoing, e.g., all of the above, but excluding one or more groups or species such as humans, primates or rodents. In certain embodiments, the subject is a mammal, e.g., a primate, e.g., a human. The terms, “patient”, “individual” and “subject” are used interchangeably herein. Preferably, the subject is a mammal. The mammal can be a human, non-human primate, mouse, rat, dog, cat, horse, or cow, but are not limited to these examples. Mammals other than humans can be advantageously used, for example, as subjects that represent animal models of, for example, neurodegenerative or neurovascular disorders (e.g., stroke). In addition, the methods described herein can be used to treat domesticated animals and/or pets. A subject can be male or female. A subject can be one who has been previously diagnosed with or identified as suffering from or having a condition in need of treatment (e.g., a neurodegenerative or neurovascular disorder, e.g., due to decreased capacity for neurogenesis or angiogenesis) or one or more complications related to

such a condition, and optionally, but need not have already undergone treatment for a condition or the one or more complications related to the condition. Alternatively, a subject can also be one who has not been previously diagnosed as having a condition in need of treatment or one or more complications related to such a condition. Rather, a subject can include one who exhibits one or more risk factors for a condition or one or more complications related to a condition. A “subject in need” of treatment for a particular condition can be a subject having that condition, diagnosed as having that condition, or at increased risk of developing that condition relative to a given reference population.

[0066] As used herein the term “comprising” or “comprises” is used in reference to compositions, methods, and respective component(s) thereof, that are essential to the method or composition, yet open to the inclusion of unspecified elements, whether essential or not.

[0067] The term “consisting of” refers to compositions, methods, and respective components thereof as described herein, which are exclusive of any element not recited in that description of the embodiment.

[0068] As used herein the term “consisting essentially of” refers to those elements required for a given embodiment. The term permits the presence of elements that do not materially affect the basic and novel or functional characteristic(s) of that embodiment.

[0069] The term “statistically significant” or “significantly” refers to statistical significance and generally means a “p” value greater than 0.05 (calculated by the relevant statistical test). Those skilled in the art will readily appreciate that the relevant statistical test for any particular experiment depends on the type of data being analyzed. Additional definitions are provided in the text of individual sections below.

[0070] Definitions of common terms in cell biology and molecular biology can be found in “The Merck Manual of Diagnosis and Therapy”, 19th Edition, published by Merck Research Laboratories, 2006 (ISBN 0-911910-19-0); Robert S. Porter et al. (eds.), The Encyclopedia of Molecular Biology, published by Blackwell Science Ltd., 1994 (ISBN 0-632-02182-9); The ELISA guidebook (Methods in molecular biology 149) by Crowther J. R. (2000); Immunology by Werner Luttmann, published by Elsevier, 2006. Definitions of common terms in molecular biology can

also be found in Benjamin Lewin, *Genes X*, published by Jones & Bartlett Publishing, 2009 (ISBN-10: 0763766321); Kendrew et al. (eds.), *Molecular Biology and Biotechnology: a Comprehensive Desk Reference*, published by VCH Publishers, Inc., 1995 (ISBN 1-56081-569-8) and *Cun-ent Protocols in Protein Sciences 2009*, Wiley Intersciences, Coligan et al., eds.

[0071] Unless otherwise stated, the present invention was performed using standard procedures, as described, for example in Sambrook et al., *Molecular Cloning: A Laboratory Manual* (3 ed.), Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., USA (2001) and Davis et al., *Basic Methods in Molecular Biology*, Elsevier Science Publishing, Inc., New York, USA (1995) which are both incorporated by reference herein in their entireties.

[0072] Described herein are methods comprising administering to a subject an agent or composition which increases the level of GDF11 polypeptide in the subject.

[0073] In some embodiments, the subject is one who is in need of rejuvenated neural stem cells and/or progenitor cells. As used herein “rejuvenated neural stem cells and/or progenitor cells” and “rejuvenating neural stem and/or progenitor cells” refer to a restoration of the neural stem cells and/or progenitor cells either partially or wholly to the quality and/or quantity of neural stem cells and/or progenitor cells typically found within a youthful healthy subject of the same species. In some embodiments, the number of neural stem cells and/or progenitor cells are restored completely to the number neural stem cells and/or progenitor cells of a youthful healthy subject of the same species. In some embodiments, the number of neural stem cells and/or progenitor cells in the subject are restored to within 95%, to within 90%, to within 85%, to within 80%, to within 75%, to within 70%, to within 65%, to within 60%, to within 55%, or to within 50% of the number of neural stem cells and/or progenitor cells of a youthful subject. In some contexts, “rejuvenated neural stem cells and/or progenitor cells” and “rejuvenating neural stem cells and/or progenitor cells” refers to an increase in the number or frequency of the neural stem cells and/or progenitor cells” in the subject compared to a number or frequency of the neural stem and/or progenitor cells before administration of an agent or composition which increased the level of GDF11 polypeptide in the subject. In some embodiments, the number or frequency of neural stem cells and/or progenitor cells are increased by at

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least 10% in the subject. In some embodiments, the number or frequency of neural stem cells and/or progenitor cells are increased by at least 15% in the subject. In some embodiments, the number or frequency of neural stem cells and/or progenitor cells are increased by at least 20% in the subject. In some embodiments, the number or frequency of neural stem cells and/or progenitor cells are increased by at least 25% in the subject. In some embodiments, the number or frequency of neural stem cells and/or progenitor cells are increased by at least 30% in the subject. In some embodiments, the number or frequency of neural stem cells and/or progenitor cells are increased by at least 35% in the subject. In some embodiments, the number or frequency of neural stem cells and/or progenitor cells are increased by at least 40% in the subject. In some embodiments, the number or frequency of neural stem cells and/or progenitor cells are increased by at least 45% in the subject. In some embodiments, the number or frequency of neural stem cells and/or progenitor cells are increased by at least 50% in the subject. In some embodiments, the number or frequency of neural stem cells and/or progenitor cells are increased by at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, or at least 90% in the subject. In some embodiments, the number or frequency of neural stem cells and/or progenitor cells are increased by at least 100% in the subject. In some embodiments, the number or frequency of neural stem cells and/or progenitor cells are increased by at least 1.1 fold, at least 1.2 fold, at least 1.3 fold, at least 1.4 fold, at least 1.5 fold, at least 1.6 fold, at least 1.7 fold, at least 1.8 fold, at least 1.9 fold, at least 2 fold, at least 3 fold, at least 4 fold, at least 5 fold, at least 10 fold or more in the subject.

[0074] In some embodiments, the subject is one who is in need of increased neurogenesis. In some embodiments, the subject is one who has, or has been diagnosed as having a neurodegenerative disorder. In some embodiments, the subject is one who has, or has been diagnosed as having a neurodegenerative disorder characterized by decreased neurogenesis. In some embodiments, the subject is one who has, or has been diagnosed as having a neurodegenerative disorder characterized at least partially by a decreased capacity for neurogenesis due to aging. In some embodiments, the subject is one who is at risk of developing a neurodegenerative disorder due to aging. In some embodiments, the subject is one who has, or has been diagnosed as having, or is at risk of developing, dementia. In some embodiments, the

subject is one who has, or has been diagnosed as having, or is at risk of developing, Alzheimer's disease. In some embodiments, the subject is one who has, or has been diagnosed as having, or is at risk of developing, mild cognitive impairment. In some embodiments, the subject is one who has, or has been diagnosed as having, or is at risk of developing, depression. In some embodiments, the subject is one who has, or has been diagnosed as having, or is at risk of developing, a learning disorder. In some embodiments, the subject is one who has, or has been diagnosed as having, or is at risk of developing, a memory disorder.

[0075] In some embodiments, the subject is one who is in need of increased angiogenesis. In some embodiments, the subject is one who has, or has been diagnosed as having a neurovascular disorder. In some embodiments, the subject is one who has, or has been diagnosed as having a neurovascular disorder characterized by decreased angiogenesis. In some embodiments, the subject is one who has, or has been diagnosed as having a neurovascular disorder characterized at least partially by a decreased capacity for angiogenesis due to aging. In some embodiments, the subject is one who is at risk of developing a neurovascular disorder due to aging. In some embodiments, the subject is one who has, or has been diagnosed as having, or is at risk of developing, stroke. In some embodiments, the subject is one who has, or has been diagnosed as having, or is at risk of developing, ischemia.

[0076] In some embodiments, the subject is an elderly subject. In some embodiments, an elderly subject is over the age of 50, 55, 60, 65, 70, 75, 80, 85, 90, or 100 years.

[0077] In some embodiments, the agent or composition which increases the level of GDF11 polypeptide is administered to a subject who has or has been diagnosed with a neurodegenerative disorder described herein. A health care professional may diagnose a subject as having a neurodegenerative disorder by the assessment of one or more symptoms of a neurodegenerative disorder in the subject. Non-limiting symptoms of a neurodegenerative disorder in a subject include difficulty lifting the front part of the foot and toes; weakness in arms, legs, feet, or ankles; hand weakness or clumsiness; slurring of speech; difficulty swallowing; muscle cramps; twitching in arms, shoulders, and tongue; difficulty chewing; difficulty breathing; muscle paralysis; partial or complete loss of vision; double vision; tingling or pain in

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parts of body; electric shock sensations that occur with head movements; tremor; unsteady gait; fatigue; dizziness; loss of memory; disorientation; misinterpretation of spatial relationships; difficulty reading or writing; difficulty concentrating and thinking; difficulty making judgments and decisions; difficulty planning and performing familiar tasks; depression; anxiety; social withdrawal; mood swings; irritability; aggressiveness; changes in sleeping habits; wandering; dementia; loss of automatic movements; impaired posture and balance; rigid muscles; bradykinesia; slow or abnormal eye movements; involuntary jerking or writhing movements (chorea); involuntary, sustained contracture of muscles (dystonia); lack of flexibility; lack of impulse control; and changes in appetite. A health care professional may also base a diagnosis, in part, on the subject's family history of a neurodegenerative disorder. A health care professional may diagnose a subject as having a neurodegenerative disorder upon presentation of a subject to a health care facility (e.g., a clinic or a hospital). In some instances, a health care professional may diagnose a subject as having a neurodegenerative disorder while the subject is admitted in an assisted care facility. Typically, a physician diagnoses a neurodegenerative disorder in a subject after the presentation of one or more symptoms.

[0078] In some embodiments, the agent or composition which increases the level of GDF11 polypeptide is administered to a subject who has or has been diagnosed with a neurological injury or damage to the CNS or the PNS associated with physical injury, ischemia, certain medical procedures or exposure to biological or chemic toxins or poisons, tumors, infections, metabolic or nutritional disorders, cognition or mood disorders, and various medical conditions associated with neural damage or destruction described herein.

[0079] In some embodiments, the agent or composition which increases the level of GDF11 polypeptide is administered to a subject who has or has been diagnosed with a neurovascular disorder described herein. A health care professional may diagnose a subject as having a neurovascular disorder by the assessment of one or more symptoms of a neurovascular disorder in the subject. Non-limiting symptoms of a neurovascular disorder in a subject include hypertension, platelet aggregation, altered cerebrovascular architecture (e.g., narrowing, stiffness, deformation), sudden

blood pressure shifts, facial weakness, visual impairment, loss of coordination, or balance, a sudden headache, and mental confusion with unintelligible speech.

[0080] In some embodiments, the agent or composition which increases the level of GDF11 polypeptide is administered to a subject who is at risk of developing a neurovascular disorder described herein. A subject who is at risk of developing a neurovascular disorder includes individuals who are elderly, have a history of diabetes, smoking, or ischemic heart disease, for example.

[0081] In some embodiments, the level of GDF11 polypeptide is the level of GDF11 in the systemic circulation of a subject. In some embodiments, the level of GDF11 polypeptide is the level of GDF11 in the cerebral vasculature of a subject. In some embodiments, the level of GDF11 polypeptide is the level of GDF11 in the neurovasculature of a subject. In some embodiments, the level of GDF11 polypeptide is the level of GDF11 in the cerebral tissue of a subject. In some embodiments, the level of GDF11 polypeptide is the level of GDF11 in the cerebrospinal fluid of a subject. In some embodiments, the level of GDF11 polypeptide is the level of GDF11 in the lateral ventricles of a subject. In some embodiments, the level of GDF11 polypeptide is the level of GDF11 in the subventricular zone neurovascular niche of a subject.

[0082] The level of GDF11 in a subject can be determined by obtaining a biological sample from the subject and determining the level of GDF11 in the biological sample. Methods for determining the level of a polypeptide in a subject or a sample obtained from a subject are well known in the art and include, but are not limited to, ELISA, radioimmunoassay, immunohistochemistry, methods involving a labeled antibody specific for GDF11, dot blot analysis, functional bioassays, Northern blot, in-situ hybridization, and RT-PCR, aptamer-based proteomic technology (e.g., SOMAscan™ commercially available from SomaLogic, Inc.) among others. Antibodies specific for GDF11 are commercially available, e.g. Cat. No. ab71347 from Abcam: Cambridge, MA. In some embodiments, the antibodies are antibodies which do not cross-react with GDF8. In some embodiments, the antibodies are selective GDF11 monoclonal antibodies. In some embodiments, the level of GDF11 polypeptide is determined by measuring the level of an mRNA encoding a GDF11 polypeptide. In some embodiments, the level of GDF11 can be measured as

described in Souza *et al.*, Molecular Endocrinology 2008 22:2689-2702; which is incorporated by reference herein in its entirety.

[0083] As animals age, neural stem cell and/or progenitor cells deteriorate (e.g., decrease in number or function) and animals exhibit diminished capacity for neurogenesis and decreased neuroplasticity, which is often accompanied with cognitive decline associated with one or more neurodegenerative disorders (e.g., dementia). Without wishing to be bound by theory, it is believed that deterioration of neural stem and/or progenitor cells and characteristic attendant reduced neurogenesis results in part from decreased levels of circulating GDF11 polypeptide. Surprisingly, the work described herein demonstrates that aged mice treated *in vivo* with daily IP injection of recombinant GDF11 (rGDF11) caused the subject's neural stem cells and/or progenitor cells to proliferate, which was accompanied by increased neurogenesis and angiogenesis.

[0084] In particular, the rGDF11 treatment increased the number of Sox2⁺ cells by 22.5% indicating increased neurogenesis, and increased cerebral blood flow by as much as 33.5%, as measured by volumetric assays of blood vessels of mice treated with GDF11 compared to PBS control.

[0085] Accordingly, in one aspect, the present invention provides a method of rejuvenating neural stem cells and/or progenitor cells in a subject in need thereof, comprising administering to the subject an agent or composition which increases the level of GDF11 polypeptide in the subject. In some embodiments, the agent or composition causes the subject's neural stem cells and/or progenitor cells to undergo at least one of an increase in frequency or number, proliferation, differentiation, and an increase in neurogenesis.

[0086] In another aspect, the present invention provides a method for increasing neurogenesis in a subject in need thereof, comprising administering to the subject an agent or composition which increases the level of GDF11 polypeptide in the subject, thereby increasing neurogenesis in the subject. In some embodiments, increasing neurogenesis is associated with increased neural cell proliferation, increased neural cell differentiation, increased number or frequency of neural stem cells, increased number of neural progenitor cells, increased number of neural precursor cells, and increased expression of at least one synaptic plasticity gene, at

least one neuroprotective gene, or at least one neuronal specification gene selected from the group consisting of neuronal PAS domain protein 4 (Npas4) (NCBI Gene ID: 266743), dual specificity phosphatase 1 (Dusp1) (NCBI Gene ID: 1843), heat shock 70kDa protein 8 (Hspa8) (NCBI Gene ID: 3312), BTG family, member 2 (Btg2) (NCBI Gene ID: 7832), activity-regulated cytoskeleton-associated protein (Arc) (NCBI Gene ID: 23237), nuclear receptor subfamily 4, group A, member 1 (Nr4a1) (NCBI Gene ID: 3164), cysteine-rich, angiogenic inducer, 61 (Cyr61) (NCBI Gene ID: 3491), FBJ murine osteosarcoma viral oncogene homolog (Fos) (NCBI Gene ID: 2353), jun B proto-oncogene (Junb) (NCBI Gene ID: 3726), Kruppel-like factor 10 (Klf10) (NCBI Gene ID: 7071), nuclear receptor subfamily 4, group A, member 2 (Nr4a2) (NCBI Gene ID: 4929), claudin 1 (Cldn1) (NCBI Gene ID: 9076), early growth response 2 (Egr2) (NCBI Gene ID: 1959), crystalline, mu (Crym) (NCBI Gene ID: 1428), early growth response 1 (Egr1) (NCBI Gene ID: 1958), and polio-like kinase 2 (Plk2) (NCBI Gene ID: 10769), and increasing the number of new neurons. In some embodiments, neurogenesis is increased in the subventricular zone of the lateral ventricles. In some embodiments, neurogenesis is increased in the olfactory bulb. In some embodiments, increased neurogenesis results in improved olfactory behavior.

[0087] In another aspect, the present invention provides a method for increasing angiogenesis in a subject in need thereof, comprising administering to the subject an agent or composition which increases the level of GDF11 polypeptide in the subject, thereby increasing angiogenesis in the subject. In some embodiments, increasing angiogenesis comprises increasing cerebrovascular architecture, increasing capillary density, increasing cerebral blood flow, and increasing cerebral vessel sprouting branch points.

[0088] The methods and compositions described herein relate to increasing the level of GDF11 polypeptide in a subject. As used herein, "GDF11" refers to "Growth and Differentiation Factor 11" (NCBI Gene ID No: 10220), a member of the Transforming Growth Factor-beta superfamily of growth factors. GDF11 is known to bind TGFβ3 superfamily type I receptors including ALK4, ALK5, and ALK7. For signaling in mammalian development, GDF11 predominantly uses ALK4 and ALK5. In some embodiments, GDF11 signaling can also occur via the ACVR2B receptor.

GDF11 is also closely related to GDF8 (also known as myostatin). GDF11 can also be referred to as bone morphogenic protein 11, i.e. BMP11. As used herein, "GDF11" can include the human precursor polypeptide (SEQ ID NO: 1, NCBI Ref Seq: NP_005802); the human pro-peptide (SEQ ID NO: 2); the human N-terminal polypeptide (SEQ ID NO: 4), and the human mature (SEQ ID NO: 3) forms of GDF11 as well as homologs from other species, including but not limited to bovine, dog, cat, chicken, murine, rat, porcine, bovine, turkey, horse, fish, baboon and other primates. The terms also refer to fragments or variants of GDF11 that maintain at least 50% of the neurogenesis and angiogenesis increasing effect of the full length GDF11 of SEQ ID NO: 2, SEQ ID NO: 1, or SEQ ID NO: 3, e.g. as measured in an appropriate animal model (e.g., heterochronic parabiosis of aged mice).

[0089] Conservative substitution variants that maintain any of the neural stem cell and/or progenitor cell rejuvenating effect, the neurogenesis effect, or the angiogenesis effect of wild type GDF11 will include a conservative substitution as defined herein. The identification of amino acids most likely to be tolerant of conservative substitution while maintaining at least 50% of the activity of the wild type GDF11 is guided by, for example, sequence alignment with GDF11 homologs or paralogs from other species. Amino acids that are identical between GDF11 homologs are less likely to tolerate change, while those showing conservative differences are obviously much more likely to tolerate conservative change in the context of an artificial variant. Similarly, positions with non-conservative differences are less likely to be critical to function and more likely to tolerate conservative substitution in an artificial variant. Variants can be tested for activity, for example, by administering the variant to an appropriate animal model (e.g., aged mice).

[0090] For human GDF11, the pro-peptide plus signal sequence (e.g. the precursor polypeptide, e.g., SEQ ID NO: 1) is 407 amino acids long. Cleavage of the 24 amino acid signal peptide generates a pro-peptide (e.g., SEQ ID NO: 2) of 383 amino acids and cleavage of the pro-peptide results in a mature GDF11 polypeptide (e.g., SEQ ID NO: 3) of 109 amino acids that corresponds to the C-terminal 109 amino acids of the pro-peptide. The mature polypeptide forms a disulfide-linked homodimer. Cleavage of the pro-peptide also generates the N-terminal polypeptide (e.g., SEQ ID NO: 4) comprising amino acids 25-298 of SEQ ID NO: 1. The N-

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terminal GDF11 polypeptide can antagonize the activity of, e.g., the polypeptides of SEQ ID NOs: 2 and 3, at least *in vitro* by forming a complex with other forms of GDF11 polypeptides and can thus be used to modulate the activity of GDF11 compositions as described herein. Thus, to the extent that GDF11 polypeptides as described herein rejuvenate neural stem cells and/or progenitor cells or increase neurogenesis or angiogenesis, and to the extent the N-terminal GDF11 polypeptide of, e.g., SEQ ID NO: 4, can antagonize such effects, the polypeptide of SEQ ID NO: 4 can be excluded from the meaning of “GDF11 polypeptide” as that term is used herein.

[0091] As used herein, the terms “proteins” and “polypeptides” are used interchangeably to designate a series of amino acid residues connected to the other by peptide bonds between the alpha-amino and carboxy groups of adjacent residues. The terms “protein”, and “polypeptide” refer to a polymer of protein amino acids, including modified amino acids (e.g., phosphorylated, glycosylated, etc.) and amino acid analogs, regardless of its size or function. “Protein” and “polypeptide” are often used in reference to relatively large polypeptides, whereas the term “peptide” is often used in reference to small polypeptides, but usage of these terms in the art overlaps. The terms “protein” and “polypeptide” are used interchangeably herein when refining to a gene product and fragments thereof.

[0092] Thus, exemplary polypeptides or proteins include gene products, naturally occurring proteins, homologs, orthologs, paralogs, fragments and other equivalents, variants, fragments, and analogs of the foregoing.

[0093] As used herein, “pro-peptide” used in reference to GDF11 refers to a GDF11 polypeptide in which the signal domain (e.g. amino acids 1-24 of SEQ ID NO: 1) has been cleaved off during formation of the mature and/or active forms of GDF11. As used herein, “precursor peptide” used in reference to a GDF11 polypeptide comprising the signal domain, e.g., a polypeptide comprising the amino acid sequence of SEQ ID NO: 1

[0094] In some embodiments, the level of GDF11 in a subject is increased by administering an agent or composition comprising a GDF11 agonist that increases the level or activity of GDF11 in the subject. Exemplary GDF11 agonists are described in U.S. Pat. No. 8,323,964.

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[0095] In some embodiments, the level of GDF11 in a subject is increased by administering an agent or composition comprising an agonist antibody that increases expression of GDF11 in the subject. Any antibody that is capable of increasing expression of GDF11 in the subject can be used. As used herein “antibody” includes, but is not limited to, full length antibodies, antibody fragments, single chain antibodies, bispecific antibodies, minibodies, domain antibodies, synthetic antibodies (sometimes referred to herein as “antibody mimetics”), chimeric antibodies, humanized antibodies, antibody fusions (sometimes referred to as “antibody conjugates”), and fragments of each, respectively. Exemplary antibody fragments include, but are not limited to, (i) the Fab fragment consisting of VL, VH, CL and CH I domains, (ii) the Fd fragment consisting of the VH and CH1 domains, (iii) the Fv fragment consisting of the VL and VH domains of a single antibody, (iv) the dAb fragment, which consists of a single variable domain, (v) isolated CDR regions, (vi) F(ab')₂ fragments, a bivalent fragment comprising two linked Fab fragments (vii) single chain Fv molecules (scFv), wherein a VH domain and a VL domain are linked by a peptide linker which allows the two domains to associate to form an antigen binding site, (viii) bispecific single chain Fv dimers, and (ix) “diabodies” or “triabodies”, multivalent or multispecific fragments constructed by gene fusion. The antibody fragments may be modified. For example, the molecules may be stabilized by the incorporation of disulfide bridges linking the VH and VL domains. Examples of antibody formats and architectures are described in Holliger & Hudson, 2006, Nature Biotechnology 23(9): 1126-1136, and Carter 2006, Nature Reviews Immunology 6:343-357 and references cited therein, all expressly incorporated by reference. Exemplary GDF11 agonist antibodies include the GDF11 agonist antibodies described in PCT International Application Publication WO/2002/010214.

[0096] In some embodiments, the level of GDF11 in a subject is increased by administering an agent or composition comprising a GDF11 polypeptide and/or a nucleic acid encoding a GDF11 polypeptide. A GDF11 polypeptide administered to a subject according to the methods described herein can comprise a GDF11 polypeptide as described herein above, e.g. a pro-peptide or mature form. In some embodiments, the GDF11 polypeptide comprises the amino acid sequence of SEQ ID NO: 1. In some embodiments, the GDF11 polypeptide comprises the amino acid sequence of

SEQ ID NO: 2. In some embodiments, the GDF11 polypeptide comprises the amino acid sequence of SEQ ID NO: 3. In some embodiments, the GDF11 polypeptide comprises the amino acid sequence of SEQ ID NO: 4.

[0097] In some embodiments, the agent or composition administered to the subject can comprise GDF11 polypeptide homodimers comprising polypeptides of the amino acid sequence of SEQ ID NO: 3. In some embodiments, the agent or composition administered to the subject can comprise GDF11 polypeptide homodimers comprising polypeptides of the amino acid sequence of SEQ ID NO: 4. In some embodiments, the agent or composition administered to the subject can comprise GDF11 polypeptide homodimers comprising polypeptides of the amino acid sequence of SEQ ID NO: 2. In some embodiments, the agent or composition administered to the subject can comprise GDF11 polypeptide homodimers comprising polypeptides of the amino acid sequence of SEQ ID NO: 1. In some embodiments, the agent or composition administered to the subject can comprise GDF11 polypeptide heterodimers comprising polypeptides of any of the amino acid sequence of SEQ ID NO: 4, SEQ ID NO: 3, SEQ ID NO: 2, and/or SEQ ID NO: 1.

[0098] In some embodiments, a variant or fragment of a GDF11 polypeptide can be administered to a subject. In some embodiments, the variant of GDF11 is a conservatively modified variant. In some embodiments of any of the aspects described herein, the subject can be administered a variant or fragment (e.g. a conservatively modified variant or a functional fragment or a nucleic acid encoding such a polypeptide) of a polypeptide selected from Collectin kidney 1 (e.g. NCBI Gene ID No: 78989), Cathespin D (e.g. NCBI Gene ID No: 1509), Dickkopf-related protein 4 (e.g. NCBI Gene ID No: 27121), Erythrocyte membrane protein 4.1 (e.g. NCBI Gene ID No: 2035), esterase D (e.g. NCBI Gene ID No: 2098), hemoglobin (e.g. NCBI Gene ID No: 3043 or 3047), interleukin-1 receptor accessory protein (e.g. NCBI Gene ID No: 3556), natural killer group 2 member D (e.g. NCBI Gene ID No: 22914), Ras-related C3 botulinum toxin substrate 1 (e.g. NCBI Gene ID No: 5879), GTP-binding nuclear protein Ran (e.g. NCBI Gene ID No: 5901), tissue inhibitor of metalloproteases 3 (e.g. NCBI Gene ID No: 7078), and thymidylate synthase (e.g. NCBI Gene ID No: 7298).

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[0099] In some embodiments, the GDF11 polypeptide can be a variant of a sequence described herein, e.g. a variant of a GDF11 polypeptide comprising the amino acid sequence of SEQ ID NO: 4, SEQ ID NO: 3, SEQ ID NO: 1, or SEQ ID NO: 2. In some embodiments, the variant is a conservative substitution variant. Variants can be obtained by mutations of native nucleotide sequences, for example. A “variant,” as referred to herein, is a polypeptide substantially homologous to a native or reference polypeptide, but which has an amino acid sequence different from that of the native or reference polypeptide because of one or a plurality of deletions, insertions or substitutions. Polypeptide-encoding DNA sequences encompass sequences that comprise one or more additions, deletions, or substitutions of nucleotides when compared to a native or reference DNA sequence, but that encodes a variant protein or fragment thereof that retains the relevant biological activity relative to the reference protein, i.e., can rejuvenate neural stem cells and/or progenitor cells at least 50% as well as wild type GDF11. As to amino acid sequences, one of skill will recognize that individual substitutions, deletions or additions to a nucleic acid, peptide, polypeptide, or protein sequence which alters a single amino acid or a small percentage, (i.e. 5% or fewer, e.g. 4% or fewer, or 3% or fewer, or 1% or fewer) of amino acids in the encoded sequence is a “conservatively modified variant” where the alteration results in the substitution of an amino acid with a chemically similar amino acid. It is contemplated that some changes can potentially improve the relevant activity, such that a variant, whether conservative or not, has more than 100% of the activity of wild type GDF11, e.g. 110%, 125%, 150%, 175%, 200%, 500%, 1000% or more.

[0100] One method of identifying amino acid residues which can be substituted is to align, for example, human GDF11 to a GDF11 homolog from one or more non-human species. Alignment can provide guidance regarding not only residues likely to be necessary for function but also, conversely, those residues likely to tolerate change. Where, for example, an alignment shows two identical or similar amino acids at corresponding positions, it is more likely that that site is important functionally. Where, conversely, alignment shows residues in corresponding positions to differ significantly in size, charge, hydrophobicity, etc., it is more likely that that site can tolerate variation in a functional polypeptide. Similarly, alignment with a

related polypeptide from the same species, e.g. GDF8, which does not show the same activity, can also provide guidance with respect to regions or structures required for GDF11 activity. **Fig. 13** depicts an example of an alignment between human GDF11 precursor peptide (query sequence; residues 62-407 of SEQ ID NO: 1) and human GDF8 precursor peptide created using the default settings of the alignment tool of the BLASTP program, freely available on the world wide web at <http://blast.ncbi.nlm.nih.gov/>. **Fig. 14** depicts an example of an alignment between human GDF11 precursor peptide (query sequence; residues 47-407 of SEQ ID NO: 1) and murine GDF11 precursor peptide created using the default settings of the alignment tool of the BLASTP program, freely available on the world wide web at <http://blast.ncbi.nlm.nih.gov/>. The variant amino acid or DNA sequence can be at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or more, identical to a native or reference sequence, e.g. SEQ ID NO: 4, SEQ ID NO: 3, SEQ ID NO: 1, or SEQ ID NO: 2 or a nucleic acid encoding one of those amino acid sequences. The degree of homology (percent identity) between a native and a mutant sequence can be determined, for example, by comparing the two sequences using freely available computer programs commonly employed for this purpose on the World Wide Web. The variant amino acid or DNA sequence can be at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or more, similar to the sequence from which it is derived (referred to herein as an "original" sequence). The degree of similarity (percent similarity) between an original and a mutant sequence can be determined, for example, by using a similarity matrix. Similarity matrices are well known in the art and a number of tools for comparing two sequences using similarity matrices are freely available online, e.g. BLASTp (available on the world wide web at <http://blast.ncbi.nlm.nih.gov/>), with default parameters set.

[0101] It is noted that the mature GDF11 polypeptide includes likely intrachain disulfide bonds between, e.g. amino acid 313 and 372; 341 and 404; and 345 and 406 (numbered relative to the full length polypeptide, including the signal sequence) and that amino acid 371 likely participates in interchain disulfide bonding.

[0102] A given amino acid can be replaced by a residue having similar physiochemical characteristics, e.g., substituting one aliphatic residue for another

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(such as Ile, Val, Leu, or Ala for one another), or substitution of one polar residue for another (such as between Lys and Arg; Glu and Asp; or Gln and Asn). Other such conservative substitutions, e.g., substitutions of entire regions having similar hydrophobicity characteristics, are well known. Polypeptides comprising conservative amino acid substitutions can be tested in any one of the assays described herein to confirm that a desired apoptotic activity of a native or reference polypeptide is retained. Conservative substitution tables providing functionally similar amino acids are well known in the art. Such conservatively modified variants are in addition to and do not exclude polymorphic variants, interspecies homologs, and alleles consistent with the disclosure. Typically conservative substitutions for one another include: 1) Alanine (A), Glycine (G); 2) Aspartic acid (D), Glutamic acid (E); 3) Asparagine (N), Glutamine (Q); 4) Arginine (R), Lysine (K); 5) Isoleucine (I), Leucine (L), Methionine (M), Valine (V); 6) Phenylalanine (F), Tyrosine (Y), Tryptophan (W); 7) Serine (S), Threonine (T); and 8) Cysteine (C), Methionine (M) (see, e.g., Creighton, *Proteins* (1984)).

[0103] Any cysteine residue not involved in maintaining the proper conformation of the polypeptide also can be substituted, generally with serine, to improve the oxidative stability of the molecule and prevent aberrant crosslinking. Conversely, cysteine bond(s) can be added to the polypeptide to improve its stability or facilitate oligomerization.

[0104] In some embodiments, the GDF11 polypeptide administered to a subject can comprise one or more amino acid substitutions or modifications. In some embodiments, the substitutions and/or modifications can prevent or reduce proteolytic degradation and/or prolong half-life of the polypeptide in the subject. In some embodiments, a GDF11 polypeptide can be modified by conjugating or fusing it to other polypeptide or polypeptide domains such as, by way of non-limiting example, transferrin (W006096515A2), albumin (Yeh et al., 1992), growth hormone (US2003104578AA); cellulose (Levy and Shoseyov, 2002); and/or Fc fragments (Ashkenazi and Chamow, 1997). The references in the foregoing paragraph are incorporated by reference herein in their entireties.

[0105] In some embodiments, a GDF11 polypeptide as described herein can comprise at least one peptide bond replacement. A single peptide bond or multiple

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peptide bonds, e.g. 2 bonds, 3 bonds, 4 bonds, 5 bonds, or 6 or more bonds, or all the peptide bonds can be replaced. An isolated peptide as described herein can comprise one type of peptide bond replacement or multiple types of peptide bond replacements, e.g. 2 types, 3 types, 4 types, 5 types, or more types of peptide bond replacements. Nonlimiting examples of peptide bond replacements include urea, thiourea, carbamate, sulfonyl urea, trifluoroethylamine, ortho-(aminoalkyl)-phenylacetic acid, para-(aminoalkyl)-phenylacetic acid, meta (aminoalkyl)-phenylacetic acid, thioamide, tetrazole, boronic ester, olefinic group, and derivatives thereof.

[0106] In some embodiments, a GDF11 polypeptide as described herein can comprise naturally occurring amino acids commonly found in polypeptides and/or proteins produced by living organisms, e.g. Ala (A), Val (V), Leu (L), Ile (I), Pro (P), Phe (F), Trp (W), Met (M), Gly (G), Ser (S), Thr (T), Cys (C), Tyr (Y), Asn (N), Gln (Q), Asp (D), Glu (E), Lys (K), Arg (R), and His (H). In some embodiments, a GDF11 polypeptide as described herein can comprise alternative amino acids. Non-limiting examples of alternative amino acids include, D-amino acids; beta-amino acids; homocysteine, phosphoserine, phosphothreonine, phosphotyrosine, hydroxyproline, gamma-carboxyglutamate; hippuric acid, octahydroindole-2-carboxylic acid, statine, 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid, penicillamine (3-mercapto-D-valine), ornithine, citrulline, alpha-methyl-alanine, parabenzoylphenylalanine, para-amino phenylalanine, p-fluorophenylalanine, phenylglycine, propargylglycine, sarcosine, and tert-butylglycine), diaminobutyric acid, 7-hydroxytetrahydroisoquinoline carboxylic acid, naphthylalanine, biphenylalanine, cyclohexylalanine, aminoisobutyric acid, norvaline, norleucine, tert-leucine, tetrahydroisoquinoline carboxylic acid, pipecolic acid, phenylglycine, homophenylalanine, cyclohexylglycine, dehydroleucine, 2,2-diethylglycine, 1-aminocyclopentanecarboxylic acid, 1-amino-1-cyclohexanecarboxylic acid, amino-benzoic acid, aminonaphthoic acid, gamma-aminobutyric acid, difluorophenylalanine, nipecotic acid, alpha-amino butyric acid, thienyl-alanine, t-butylglycine, trifluorovaline; hexafluoroleucine; fluorinated analogs; azidemodified amino acids; alkyne-modified amino acids; cyano-modified amino acids; and derivatives thereof.

[0107] In some embodiments, a GDF11 polypeptide can be modified, e.g. by addition of a moiety to one or more of the amino acids comprising the peptide. In

some embodiments, a GDF11 polypeptide as described herein can comprise one or more moiety molecules, e.g. 1 or more moiety molecules per peptide, 2 or more moiety molecules per peptide, 5 or more moiety molecules per peptide, 10 or more moiety molecules per peptide or more moiety molecules per peptide. In some embodiments, a GDF11 polypeptide as described herein can comprise one more types of modifications and/or moieties, e.g. 1 type of modification, 2 types of modifications, 3 types of modifications or more types of modifications. Non-limiting examples of modifications and/or moieties include PEGylation; glycosylation; HESylation; ELPylation; lipidation; acetylation; amidation; end-capping modifications; cyano groups; phosphorylation; albumin, and cyclization. In some embodiments, an end-capping modification can comprise acetylation at the N-terminus, N-terminal acylation, and N-terminal formylation. In some embodiments, an end-capping modification can comprise amidation at the C terminus, introduction of C-terminal alcohol, aldehyde, ester, and thioester moieties. The half-life of a GDF11 polypeptide can be increased by the addition of moieties, e.g. PEG or albumin.

[0108] In some embodiments, the GDF11 polypeptide administered to the subject can be a functional fragment of one of the GDF11 amino acid sequences described herein. As used herein, a “functional fragment” is a fragment or segment of a peptide which can rejuvenate neural stem cells and/or progenitor cells or increase neurogenesis in a subject in accordance with the work described herein. A functional fragment can comprise conservative substitutions of the sequences disclosed herein. In some embodiments, a functional fragment can comprise the 12.5 kDa C-terminus of GDF11. In some embodiments, the 12.5 kDa C-terminus of GDF11 can function as a monomer. In some embodiments, the 12.5 kDa C-terminus of GDF11 can function as a homodimer. In some embodiments, the 12.5 kDa C-terminus of GDF11 can function as a heterodimer with the GDF11 pro-peptide.

[0109] Alterations of the original amino acid sequence can be accomplished by any of a number of techniques known to one of skill in the art. Mutations can be introduced, for example, at particular loci by synthesizing oligonucleotides containing a mutant sequence, flanked by restriction sites permitting ligation to fragments of the native sequence. Following ligation, the resulting reconstructed sequence encodes an analog having the desired amino acid insertion, substitution, or deletion.

Alternatively, oligonucleotide-directed site-specific mutagenesis procedures can be employed to provide an altered nucleotide sequence having particular codons altered according to the substitution, deletion, or insertion required. Techniques for making such alterations include those disclosed by Walder et al. (Gene 42:133, 1986); Bauer et al. (Gene 37:73, 1985); Craik (BioTechniques, January 1985, 12-19); Smith et al. (Genetic Engineering: Principles and Methods, Plenum Press, 1981); and U.S. Pat. Nos. 4,518,584 and 4,737,462, which are herein incorporated by reference in their entireties. In some embodiments, a GDF11 polypeptide as described herein can be chemically synthesized and mutations can be incorporated as part of the chemical synthesis process.

[0110] In some embodiments, a GDF11 polypeptide as described herein can be formulated as a pharmaceutically acceptable prodrug. As used herein, a “prodrug” refers to compounds that can be converted via some chemical or physiological process (e.g., enzymatic processes and metabolic hydrolysis) to a therapeutic agent. Thus, the term “prodrug” also refers to a precursor of a biologically active compound that is pharmaceutically acceptable. A prodrug may be inactive when administered to a subject, i.e. an ester, but is converted *in vivo* to an active compound, for example, by hydrolysis to the free carboxylic acid or free hydroxyl. The prodrug compound often offers advantages of solubility, tissue compatibility or delayed release in an organism. The term “prodrug” is also meant to include any covalently bonded carriers, which release the active compound *in vivo* when such prodrug is administered to a subject. Prodrugs of an active compound may be prepared by modifying functional groups present in the active compound in such a way that the modifications are cleaved, either in routine manipulation or *in vivo*, to the parent active compound. Prodrugs include compounds wherein a hydroxy, amino or mercapto group is bonded to any group that, when the prodrug of the active compound is administered to a subject, cleaves to form a free hydroxy, free amino or free mercapto group, respectively. Examples of prodrugs include, but are not limited to, acetate, formate and benzoate derivatives of an alcohol or acetamide, formamide and benzamide derivatives of an amine functional group in the active compound and the like. See Harper, “Drug Latentiation” in Jucker, ed. *Progress in Drug Research* 4:221-294 (1962); Morozowich et al, “Application of Physical Organic Principles to Prodrug Design” in

E. B. Roche ed. *Design of Biopharmaceutical Properties through Prodrugs and Analogs*, APHA Acad. Pharm. Sci. 40 (1977); *Bioreversible Carriers in Drug in Drug Design, Theory and Application*, E. B. Roche, ed., APHA Acad. Pharm. Sci. (1987); *Design of Prodrugs*, H. Bundgaard, Elsevier (1985); Wang et al. "Prodrug approaches to the improved delivery of peptide drug" in *Curr. Pharm. Design*. 5(4):265-287 (1999); Pauletti et al. (1997) Improvement in peptide bioavailability: Peptidomimetics and Prodrug Strategies, *Adv. Drug. Delivery Rev.* 27:235-256; Mizen et al. (1998) "The Use of Esters as Prodrugs for Oral Delivery of (3-Lactam antibiotics," *Pharm. Biotech.* 11,:345-365; Gagnault et al. (1996) "Designing Prodrugs and Bioprecursors I. Carrier Prodrugs," *Pract. Med. Chern.* 671-696; Asgharnejad, "Improving Oral Drug Transport", in *Transport Processes in Pharmaceutical Systems*, G. L. Amidon, P. I. Lee and E. M. Topp, Eds., Marcell Dekker, p. 185-218 (2000); Balant et al., "Prodrugs for the improvement of drug absorption via different routes of administration", *Eur. J. Drug Metab. Pharmacokinet.*, 15(2): 143-53 (1990); Balimane and Sinko, "Involvement of multiple transporters in the oral absorption of nucleoside analogues", *Adv. Drug Delivel)' Rev.*, 39(1-3): 183-209 (1999); Browne, "Fosphenytoin (Cerebyx)", *Clin. Neuropharmacol.* 20(1): 1-12 (1997); Bundgaard, "Bioreversible derivatization of drugs- principle and applicability to improve the therapeutic effects of drugs", *Arch. Pharm. Chemi* 86(1): 1-39 (1979); Bundgaard H. "Improved drug delivery by the prodrug approach", *Controlled Drug Delively* 17: 179-96 (1987); Bundgaard H. "Prodrugs as a means to improve the delivery of peptide drugs" ,*Arfv. Drug Delivel)' Rev.* 8(1): 1-38 (1992); Fleisher et al. "Improved oral drug delivery: solubility limitations overcome by the use of prodrugs", *Arfv. Drug Delivery Rev.* 19(2): 115-130 (1996); Fleisher et al. "Design of prodrugs for improved gastrointestinal absorption by intestinal enzyme targeting", *Methods Enzymol.* 112 (Drug Enzyme Targeting, Pt. A): 360-81, (1985); Farquhar D, et al., "Biologically Reversible Phosphate-Protective Groups", *Pharm. Sci.*, 72(3): 324-325 (1983); Freeman S, et al., "Bioreversible Protection for the Phospho Group: Chemical Stability and Bioactivation of Di(4-acetoxybenzyl) Methylphosphonate with Carboxyesterase," *Chern. Soc., Chern. Commun.*, 875-877 (1991); Friis and Bundgaard, "Prodrugs of phosphates and phosphonates: Novel lipophilic alphaacyloxyalkyl ester derivatives of phosphate- or phosphonate containing drugs

masking the negative charges of these groups”, *Eur. J. Pharm. Sci.* 4: 49-59 (1996); Gangwar et al., “Pro-drug, molecular structure and percutaneous delivery”, *Des. Biopharmz. Prop. Prodrugs Analogs, [Symp.] Meeting Date 1976*, 409-21. (1977); Nathwani and Wood, “Penicillins: a current review of their clinical pharmacology and therapeutic use”, *Drugs* 45(6): 866-94 (1993); Sinhababu and Thakker, “Prodrugs of anticancer agents”, *Adv. Drug Delivery Rev.* 19(2): 241-273 (1996); Stella et al., “Prodrugs. Do they have advantages in clinical practice?”, *Drugs* 29(5): 455-73 (1985); Tan et al. “Development and optimization of anti-HIV nucleoside analogs and prodrugs: A review of their cellular pharmacology, structure-activity relationships and pharmacokinetics”, *Adv. Drug Delivery Rev.* 39(1-3): 117-151 (1999); Taylor, “Improved passive oral drug delivery via prodrugs”, *Adv. Drug Delivery Rev.*, 19(2): 131-148 (1996); Valentino and Borchardt, “Prodrug strategies to enhance the intestinal absorption of peptides”, *Drug Discovery Today* 2(4): 148-155 (1997); Wiebe and Knaus, “Concepts for the design of anti-HIV nucleoside prodrugs for treating cephalic HIV infection”, *Adv. Drug Delivery Rev.*: 39(1-3):63-80 (1999); Waller et al., “Prodrugs”, *Br. J. Clin. Pharmac.* 28: 497-507 (1989), which are incorporated by reference herein in their entireties.

[0111] In some embodiments, a GDF11 polypeptide as described herein can be a pharmaceutically acceptable solvate. The term “solvate” refers to a peptide as described herein in the solid state, wherein molecules of a suitable solvent are incorporated in the crystal lattice. A suitable solvent for therapeutic administration is physiologically tolerable at the dosage administered. Examples of suitable solvents for therapeutic administration are ethanol and water. When water is the solvent, the solvate is referred to as a hydrate. In general, solvates are formed by dissolving the compound in the appropriate solvent and isolating the solvate by cooling or using an antisolvent. The solvate is typically dried or azeotroped under ambient conditions.

[0112] The peptides of the present invention can be synthesized by using well known methods including recombinant methods and chemical synthesis. Recombinant methods of producing a peptide through the introduction of a vector including nucleic acid encoding the peptide into a suitable host cell is well known in the art, such as is described in Sambrook et al., *Molecular Cloning: A Laboratory Manual*, 2d Ed, Vols 1 to 8, Cold Spring Harbor, NY (1989); M.W. Pennington and B.M. Dunn, *Methods*

in Molecular Biology: Peptide Synthesis Protocols, Vol 35, Humana Press, Totowa, NJ (1994), contents of both of which are herein incorporated by reference. Peptides can also be chemically synthesized using methods well known in the art. See for example, Merrifield et al., J. Am. Chem. Soc. 85:2149 (1964); Bodanszky, M., Principles of Peptide Synthesis, Springer-Verlag, New York, NY (1984); Kirnnerlin, T. and Seebach, D. J. Pept. Res. 65:229-260 (2005); Nilsson et al., Annu. Rev. Biophys. Biomol. Struct. (2005) 34:91-118; W.C. Chan and P.D. White (Eds.) Fmoc Solid Phase Peptide Synthesis: A Practical Approach, Oxford University Press, Cary, NC (2000); N.L. Benoiton, Chemistry of Peptide Synthesis, CRC Press, Boca Raton, FL (2005); J. Jones, Amino Acid and Peptide Synthesis, 2nd Ed, Oxford University Press, Cary, NC (2002); and P. Lloyd-Williams, F. Albericio, and E. Giralt, Chemical Approaches to the synthesis of peptides and proteins, CRC Press, Boca Raton, FL (1997), contents of all of which are herein incorporated by reference. Peptide derivatives can also be prepared as described in U.S. Pat. Nos. 4,612,302; 4,853,371; and 4,684,620, and U.S. Pat. App. Pub. No. 2009/0263843, contents of all which are herein incorporated by reference.

[0113] In some embodiments, the technology described herein relates to a nucleic acid encoding a GDF11 polypeptide as described herein. As used herein, the term “nucleic acid” or “nucleic acid sequence” refers to any molecule, preferably a polymeric molecule, incorporating units of ribonucleic acid, deoxyribonucleic acid or an analog thereof. The nucleic acid can be either single-stranded or double-stranded. A single-stranded nucleic acid can be one strand nucleic acid of a denatured double stranded DNA. Alternatively, it can be a single-stranded nucleic acid not derived from any double stranded DNA. In one aspect, the template nucleic acid is DNA. In another aspect, the template is RNA. Suitable nucleic acid molecules are DNA, including genomic DNA or cDNA. Other suitable nucleic acid molecules are RNA, including mRNA. The nucleic acid molecule can be naturally occurring, as in genomic DNA, or it may be synthetic, i.e., prepared based upon human action, or may be a combination of the two. The nucleic acid molecule can also have certain modification such as 2'-deoxy, 2'-deoxy-2'-fluoro, 2'-O-methyl, 2'-O-methoxyethyl (2'-O-MOE), 2'-O-aminopropyl (2'-O-AP), 2'-O-dimethylaminoethyl (2'-O-DMAOE), 2'-O-dimethylaminopropyl (2'-O-DMAP), 2'-O-dimethylaminoethoxyethyl (2'-O-

DMAEOE), or 2'-O--N-methylacetamido (2'-O-NMA), cholesterol addition, and phosphorothioate backbone as described in US Patent Application 20070213292; and certain ribonucleoside that are is linked between the 2' -oxygen and the 4' -carbon atoms with a methylene unit as described in US Pat No. 6,268,490, wherein both patent and patent application are incorporated hereby reference in their entirety.

[0114] In some embodiments, a nucleic acid encoding a GDF11 polypeptide can comprise the nucleotide sequence of SEQ ID NO: 5 (NCBI Ref Seq: NM_005811.3). In some embodiments, a nucleic acid encoding a GDF11 polypeptide as described herein is comprised by a vector. In some of the aspects described herein, a nucleic acid sequence encoding a GDF11 polypeptide as described herein, or any module thereof, is operably linked to a vector. The term “vector,” as used herein, refers to a nucleic acid construct designed for delivery to a host cell or for transfer between different host cells. As used herein, a vector can be viral or non-viral. The term “vector” encompasses any genetic element that is capable of replication when associated with the proper control elements and that can transfer gene sequences to cells. A vector can include, but is not limited to, a cloning vector, an expression vector, a plasmid, phage, transposon, cosmid, chromosome, virus, virion, etc.

[0115] As used herein, the term “expression vector” refers to a vector that directs expression of an RNA or polypeptide from sequences linked to transcriptional regulatory sequences on the vector. The sequences expressed will often, but not necessarily, be heterologous to the cell. An expression vector may comprise additional elements, for example, the expression vector may have two replication systems, thus allowing it to be maintained in two organisms, for example in human cells for expression and in a prokaryotic host for cloning and amplification. The term “expression” refers to the cellular processes involved in producing RNA and proteins and as appropriate, secreting proteins, including where applicable, but not limited to, for example, transcription, transcript processing, translation and protein folding, modification and processing. “Expression products” include RNA transcribed from a gene, and polypeptides obtained by translation of mRNA transcribed from a gene. The term “gene” means the nucleic acid sequence which is transcribed (DNA) to RNA *in vitro* or *in vivo* when operably linked to appropriate regulatory sequences. The gene may or may not include regions preceding and following the coding region, e.g. 5'

untranslated (5'UTR) or “leader” sequences and 3' UTR or “trailer” sequences, as well as intervening sequences (introns) between individual coding segments (exons).

[0116] As used herein, the term “viral vector” refers to a nucleic acid vector construct that includes at least one element of viral origin and has the capacity to be packaged into a viral vector particle. The viral vector can contain the nucleic acid encoding a GDF11 polypeptide as described herein in place of non-essential viral genes. The vector and/or particle may be utilized for the purpose of transferring any nucleic acids into cells either *in vitro* or *in vivo*. Numerous forms of viral vectors are known in the art.

[0117] By “recombinant vector” is meant a vector that includes a heterologous nucleic acid sequence, or “transgene” that is capable of expression *in vivo*. It should be understood that the vectors described herein can, in some embodiments, be combined with other suitable compositions and therapies. In some embodiments, the vector is episomal. The use of a suitable episomal vector provides a means of maintaining the nucleotide of interest in the subject in high copy number extra chromosomal DNA thereby eliminating potential effects of chromosomal integration.

[0118] In some embodiments the level of GDF11 in the subject is increased by at least 20% over the level of GDF11 in the subject prior to treatment, e.g. 20% or more, 30% or more, 40% or more, 50% or more, 100% or more, 150% or more, 200% or more, 250% or more, 300% or more, or 350% or more. In some embodiments the level of GDF11 in the subject is increased by at least 100% over the level of GDF11 in the subject prior to treatment. In some embodiments the level of GDF11 in the subject is increased by at least 200% over the level of GDF11 in the subject prior to treatment. In some embodiments the level of GDF11 in the subject is increased by about 250% over the level of GDF11 in the subject prior to treatment. In some embodiments, the level of GDF11 in the subject is increased to at least 50% of a healthy reference level, e.g. 50% or more, 60% or more, 70% or more, 80% or more, 90% or more, or 100% or more of a healthy reference level. In some embodiments, the level of GDF11 in the subject is increased to at least 60% of a healthy reference level. In some embodiments, the level of GDF11 in the subject is increased to at least 75% of a healthy reference level. In some embodiments, the level of GDF11 in the subject is increased to at least 90% of a healthy reference level. A healthy reference

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level can be the average level of GDF11 in a population of human subjects (e.g., young individuals) not exhibiting any signs or symptoms of neural stem cell and/or progenitor cell deterioration, diminished capacity for neurogenesis, or related conditions.

[0119] As used herein, “neural stem cell and/or progenitor cell deterioration” refers to a decrease in cell number or frequency of neural stem cells and/or progenitor cells.

[0120] In some embodiments, a healthy reference level can be the average level of GDF11 in a population of human subjects not exhibiting any signs or symptoms of neural stem cell and/or progenitor deterioration, diminished capacity for neurogenesis or related conditions and who are under the age of 70. In some embodiments, a healthy reference level can be the average level of GDF11 in a population of human subjects not exhibiting any signs or symptoms of neural stem cell and/or progenitor deterioration, diminished capacity for neurogenesis, or related conditions and who are under the age of 65. In some embodiments, a healthy reference level can be the average level of GDF11 in a population of human subjects not exhibiting any signs or symptoms of neural stem cell and/or progenitor deterioration, diminished capacity for neurogenesis, or related conditions and who are under the age of 60. In some embodiments, a healthy reference level can be the average level of GDF11 in a population of human subjects not exhibiting any signs or symptoms of neural stem cell and/or progenitor deterioration, diminished capacity for neurogenesis, or related conditions and who are under the age of 55. In some embodiments, a healthy reference level can be the average level of GDF11 in a population of human subjects not exhibiting any signs or symptoms of neural stem cell and/or progenitor deterioration, diminished capacity for neurogenesis, or related conditions and who are under the age of 50. In some embodiments, a healthy reference level can be the average level of GDF11 in a population of human subjects not exhibiting any signs or symptoms of neural stem cell and/or progenitor deterioration, diminished capacity for neurogenesis, or related conditions and who are under the age of 45. In some embodiments, a healthy reference level can be the average level of GDF11 in a population of human subjects not exhibiting any signs or symptoms of neural stem cell and/or progenitor deterioration, diminished capacity for

neurogenesis, or related conditions and who are under the age of 40. In some embodiments, a healthy reference level can be the average level of GDF11 in a population of human subjects not exhibiting any signs or symptoms of neural stem cell and/or progenitor deterioration, diminished capacity for neurogenesis, or related conditions and who are under the age of 35. In some embodiments, a healthy reference level can be the average level of GDF11 in a population of human subjects not exhibiting any signs or symptoms of neural stem cell and/or progenitor deterioration, diminished capacity for neurogenesis, or related conditions and who are under the age of 30. In some embodiments, a healthy reference level can be the average level of GDF11 in a population of human subjects not exhibiting any signs or symptoms of neural stem cell and/or progenitor deterioration, diminished capacity for neurogenesis, or related conditions and who are under the age of 25. In some embodiments, a healthy reference level can be the average level of GDF11 in a population of human subjects not exhibiting any signs or symptoms of neural stem cell and/or progenitor deterioration, diminished capacity for neurogenesis, or related conditions and who are under the age of 20.

[0121] In some embodiments, the methods described herein can comprise selecting a subject with a level of GDF11 which is lower than a healthy reference level and administering a treatment as described herein.

[0122] In some embodiments, the level of GDF11 in a subject is increased in order to treat a neurodegenerative disorder (e.g., Alzheimer's disease, dementia, mild cognitive decline, etc.) or neurological injury or damage to the CNS or the PNS associated with physical injury, ischemia, certain medical procedures or exposure to biological or chemic toxins or poisons, tumors, infections, metabolic or nutritional disorders, cognition or mood disorders, and various medical conditions associated with neural damage or destruction described herein. In some embodiments, the level of GDF11 in a subject is increased in order to treat a neurodegenerative disorder associated with decreased neurogenesis. In some embodiments, the level of GDF11 in a subject is increased in order to treat a neurodegenerative disorder by increasing neurogenesis in a subject. In some embodiments, the level of GDF11 in a subject is increased in order to prevent a neurodegenerative disorder or neurological injury or damage to the CNS or the PNS associated with physical injury, ischemia, certain

medical procedures or exposure to biological or chemic toxins or poisons, tumors, infections, metabolic or nutritional disorders, cognition or mood disorders, and various medical conditions associated with neural damage or destruction described herein.

[0123] In some embodiments, the level of GDF11 in a subject is increased in order to treat a neurovascular disorder (e.g., stroke). In some embodiments, the level of GDF11 in a subject is increased in order to treat a neurovascular disorder associated with decreased angiogenesis. In some embodiments, the level of GDF11 in a subject is increased in order to treat a neurovascular disorder by increasing angiogenesis in a subject. In some embodiments, the level of GDF11 in a subject is increased in order to prevent a neurovascular disorder (e.g., stroke).

[0124] Neurodegenerative or neurovascular disorders related to low or decreased GDF11 polypeptide tend to develop with the decrease in GDF11 levels that occur with increasing age. Thus, it is expected that such conditions can be prevented or, at a minimum, delayed, by maintaining GDF11 polypeptide levels at or near the level found in normal, healthy young adults, e.g., by administering a GDF11 polypeptide or a nucleic acid encoding a GDF11 polypeptide with advancing age, but prior to the onset of a neurodegenerative or neurovascular disorder.

[0125] In another aspect, the present invention provides a method for treating or preventing a disorder associated with diminished capacity for neurogenesis in a subject, comprising administering to the subject an effective amount of an agent or composition which increases the level of GDF11 polypeptide in the subject, thereby increasing neurogenesis in the subject, wherein increasing neurogenesis in the subject treats or prevents the disorder associated with diminished capacity for neurogenesis in the subject.

[0126] In another aspect, the present invention provides a method for treating or preventing a neurodegenerative disorder in a subject, comprising administering to the subject an effective amount of an agent or composition which increases the level of GDF11 polypeptide in the subject, thereby treating the neurodegenerative disorder in the subject.

[0127] In another aspect, the present invention provides a method for treating or preventing a neurodegenerative disorder by increasing neurogenesis in a subject,

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comprising administering to the subject an effective amount of an agent or composition which increases the level of GDF11 polypeptide in the subject, thereby increasing neurogenesis in the subject, wherein increasing neurogenesis in the subject treats or prevents the neurodegenerative disorder in the subject. In some embodiments of this and other aspects disclosed herein, increasing neurogenesis is associated with increasing neural cell proliferation, increasing neural cell differentiation, increasing the number of neural stem cells, increasing the number of neural progenitor cells, increasing the number of neural precursor cells, increasing expression of at least one synaptic plasticity gene, at least one neuroprotective gene, or at least one neuronal specification gene selected from the group consisting of neuronal PAS domain protein 4 (Npas4) (NCBI Gene ID: 266743), dual specificity phosphatase 1 (Dusp1) (NCBI Gene ID: 1843), heat shock 70kDa protein 8 (Hspa8) (NCBI Gene ID: 3312), BTG family, member 2 (Btg2) (NCBI Gene ID: 7832), activity-regulated cytoskeleton-associated protein (Arc) (NCBI Gene ID: 23237), nuclear receptor subfamily 4, group A, member 1 (Nr4a1) (NCBI Gene ID: 3164), cysteine-rich, angiogenic inducer, 61 (Cyr61) (NCBI Gene ID: 3491), FBJ murine osteosarcoma viral oncogene homolog (Fos) (NCBI Gene ID: 2353), jun B proto-oncogene (Junb) (NCBI Gene ID: 3726), Kruppel-like factor 10 (Klf10) (NCBI Gene ID: 7071), nuclear receptor subfamily 4, group A, member 2 (Nr4a2) (NCBI Gene ID: 4929), claudin 1 (Cldn1) (NCBI Gene ID: 9076), early growth response 2 (Egr2) (NCBI Gene ID: 1959), crystalline, mu (Crym) (NCBI Gene ID: 1428), early growth response 1 (Egr1) (NCBI Gene ID: 1958), and polio-like kinase 2 (Plk2) (NCBI Gene ID: 10769), and increasing the number of new neurons

[0128] In some embodiments, neurogenesis is increased in the subventricular zone of the lateral ventricles. In some embodiments, neurogenesis is increased in the olfactory bulb. In some embodiments, increased neurogenesis results in improved olfactory behavior.

[0129] In some embodiments, the neurodegenerative disorder is one of polyglutamine expansion disorders (e.g., HD, dentatorubropallidoluysian atrophy, Kennedy's disease (also referred to as spinobulbar muscular atrophy), and spinocerebellar ataxia (e.g., type 1, type 2, type 3 (also referred to as Machado-Joseph disease), type 6, type 7, and type 17)), other trinucleotide repeat expansion disorders

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(e.g., fragile X syndrome, fragile XE mental retardation, Friedreich's ataxia, myotonic dystrophy, spinocerebellar ataxia type 8, and spinocerebellar ataxia type 12), Alexander disease, Alper's disease, Alzheimer disease, amyotrophic lateral sclerosis (ALS), ataxia telangiectasia, Batten disease (also referred to as Spielmeyer-Vogt-Sjogren-Batten disease), Canavan disease, Cockayne syndrome, corticobasal degeneration, Creutzfeldt-Jakob disease, Guillain-Barré syndrome, ischemia stroke, Krabbe disease, kuru, Lewy body dementia, multiple sclerosis, multiple system atrophy, non-Huntingtonian type of Chorea, Parkinson's disease, Pelizaeus-Merzbacher disease, Pick's disease, primary lateral sclerosis, progressive supranuclear palsy, Refsum's disease, Sandhoff disease, Schilder's disease, spinal cord injury, spinal muscular atrophy (SMA), SteeleRichardson-Olszewski disease, and Tabes dorsalis.

[0130] In some embodiments, the neurodegenerative disorder comprises a neurological injury or damage to the CNS or the PNS associated with physical injury, ischemia, certain medical procedures or exposure to biological or chemic toxins or poisons, tumors, infections, metabolic or nutritional disorders, cognition or mood disorders, and various medical conditions associated with neural damage or destruction described herein.

[0131] In another aspect, the present invention provides a method for treating or preventing a neurovascular disorder, comprising administering to the subject an agent or composition which increases the level of GDF11 polypeptide in the subject, thereby treating or preventing the neurovascular disorder in the subject.

[0132] In another aspect, the present invention provides a method for treating or preventing a neurovascular disorder by increasing angiogenesis in a subject, comprising administering to the subject an agent or composition which increases the level of GDF11 polypeptide in the subject, thereby increasing angiogenesis in the subject, wherein increasing angiogenesis in the subject treats or prevents the neurovascular disorder in the subject. In some embodiments of this and other aspects disclosed herein, increasing angiogenesis comprises increasing cerebrovascular architecture, increasing capillary density, increasing cerebral blood flow, and increasing cerebral vessel sprouting branch points.

[0133] In some embodiments, the neurovascular disorder is selected from the group consisting of brain atherothrombosis, brain aneurysms, brain arteriovenous malformations, brain embolism, brain ischemia, for example caused by atherothrombosis, embolism, or hemodynamic abnormalities, cardiac arrest, carotid stenosis, cerebrovascular spasm, headache, intracranial hemorrhage, ischemic stroke, seizure, spinal vascular malformations, reflex neurovascular dystrophy (RND), neurovascular compression disorders such as hemifacial spasms, tinnitus, trigeminal neuralgia, glossopharyngeal neuralgia, stroke, transient ischemic attacks, and vasculitis.

[0134] Aspects of the technology described herein relate to agents and which increase levels of GDF11 polypeptide (e.g., compositions comprising a GDF11 polypeptide as described herein or a nucleic acid encoding a GDF11 polypeptide as described herein). In some embodiments, the composition is a pharmaceutical composition. As used herein, the term “pharmaceutical composition” refers to the active agent in combination with a pharmaceutically acceptable carrier commonly used in the pharmaceutical industry. The phrase “pharmaceutically acceptable” is employed herein to refer to those compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

[0135] In some aspects, a composition described herein comprising a GDF11 polypeptide or functional fragment or variant thereof can be used for rejuvenating neural stem cells and/or progenitor cells in a subject in need thereof, wherein increased levels of the GDF11 polypeptide or functional fragment or variant thereof in the subject rejuvenate neural stem cells and/or progenitor cells in the subject.

[0136] In some embodiments, the composition causes the subject's neural stem cells to increase in frequency or number, thereby rejuvenating the neural stem cells and/or progenitor cells in the subject.

[0137] In some aspects, a composition described herein comprising a GDF11 polypeptide or a functional fragment or variant thereof can be used for increasing neurogenesis in a subject in need thereof, wherein increased levels of the GDF11

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polypeptide or functional fragment or variant thereof in the subject increases neurogenesis in the subject. In some embodiments of this and other aspects disclosed herein, increasing neurogenesis is associated with neural cell proliferation, increasing neural cell differentiation, increasing the number of neural stem cells, increasing the number of neural progenitor cells, increasing the number of neural precursor cells, increasing expression of at least one synaptic plasticity gene, at least one neuroprotective gene, or at least one neuronal specification gene selected from the group consisting of neuronal PAS domain protein 4 (Npas4) (NCBI Gene ID: 266743), dual specificity phosphatase 1 (Dusp1) (NCBI Gene ID: 1843), heat shock 70kDa protein 8 (Hspa8) (NCBI Gene ID: 3312), BTG family, member 2 (Btg2) (NCBI Gene ID: 7832), activity-regulated cytoskeleton-associated protein (Arc) (NCBI Gene ID: 23237), nuclear receptor subfamily 4, group A, member 1 (Nr4a1) (NCBI Gene ID: 3164), cysteine-rich, angiogenic inducer, 61 (Cyr61) (NCBI Gene ID: 3491), FBJ murine osteosarcoma viral oncogene homolog (Fos) (NCBI Gene ID: 2353), jun B proto-oncogene (Junb) (NCBI Gene ID: 3726), Kruppel-like factor 10 (Klf10) (NCBI Gene ID: 7071), nuclear receptor subfamily 4, group A, member 2 (Nr4a2) (NCBI Gene ID: 4929), claudin 1 (Cldn1) (NCBI Gene ID: 9076), early growth response 2 (Egr2) (NCBI Gene ID: 1959), crystalline, mu (Crym) (NCBI Gene ID: 1428), early growth response 1 (Egr1) (NCBI Gene ID: 1958), and polio-like kinase 2 (Plk2) (NCBI Gene ID: 10769), and increasing the number of new neurons.

[0138] In some aspects, a composition described herein comprising a GDF11 polypeptide or a functional fragment or variant thereof can be used for treating or preventing a neurodegenerative disorder in a subject in need thereof, wherein increased levels of the GDF11 polypeptide or functional fragment or variant thereof treat or prevent the neurodegenerative disorder.

[0139] In some aspects, a composition described herein comprising a GDF11 polypeptide or a functional fragment or variant thereof can be used for treating or preventing a neurodegenerative disorder by increasing neurogenesis in a subject, wherein increased levels of the GDF11 polypeptide or functional fragment or variant thereof increase neurogenesis in the subject, thereby treating or preventing the neurodegenerative disorder.

[0140] In some embodiments of this and other aspects described herein, the subject has been diagnosed with a neurodegenerative disorder due to aging.

[0141] In some aspects, a composition described herein comprising a GDF11 polypeptide or functional fragment or variant thereof can be used for increasing angiogenesis in a subject in need thereof, wherein increased levels of the GDF11 polypeptide or functional fragment or variant thereof in the subject increase angiogenesis in the subject. In some embodiments, increasing angiogenesis comprises increasing cerebrovascular architecture, increasing capillary density, increasing cerebral blood flow, and increasing cerebral vessel sprouting branch points.

[0142] In some aspects, a composition described herein comprising a GDF11 polypeptide or functional fragment or variant thereof can be used for treating or preventing a neurovascular disorder in a subject in need thereof, wherein increased levels of the GDF11 polypeptide or functional fragment or variant thereof in the subject treat or prevent the neurovascular disorder in the subject.

[0143] In some aspects, a composition described herein comprising a GDF11 polypeptide or functional fragment or variant thereof can be used for treating or preventing a neurovascular disorder by increasing angiogenesis in a subject, wherein increased levels of the GDF11 polypeptide or functional fragment or variant thereof in the subject increase angiogenesis in the subject, thereby treating or preventing the neurovascular disorder in the subject.

[0144] A still further aspect of the invention provides a pharmaceutical composition or kit of parts according to the invention for use in rejuvenating neural stem cells and/or progenitor cells, increasing neurogenesis, increasing angiogenesis, or in treating or preventing neurodegenerative or neurovascular disorders.

[0145] The preparation of a pharmacological composition that contains active ingredients dissolved or dispersed therein is well understood in the art and generally need not be limited based on formulation. Typically such compositions are prepared as injectable either as liquid solutions or suspensions, however, solid forms suitable for solution, or suspension, in liquid prior to use can also be prepared. The preparation can also be emulsified or presented as a liposome composition. The active ingredient can be mixed with excipients which are pharmaceutically acceptable and compatible with the active ingredient and in amounts suitable for use in the therapeutic methods

described herein. Suitable excipients are, for example, water, saline, dextrose, glycerol, ethanol or the like and combinations thereof. In addition, if desired, the composition can contain minor amounts of auxiliary substances such as wetting or emulsifying agents, pH buffering agents, and the like which enhance the effectiveness of the active ingredient. The therapeutic composition of the present invention can include pharmaceutically acceptable salts of the components therein.

Pharmaceutically acceptable salts include the acid addition salts (formed with the free amino groups of the polypeptide) that are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, tartaric, mandelic, and the like. Salts formed with the free carboxyl groups can also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, 2-ethylamino ethanol, histidine, procaine, and the like. Physiologically tolerable carriers are well known in the art. Exemplary liquid carriers are sterile aqueous solutions that contain no materials in addition to the active ingredients and water, or contain a buffer such as sodium phosphate at physiological pH value, physiological saline or both, such as phosphate-buffered saline.

[0146] Still further, aqueous carriers can contain more than one buffer salt, as well as salts such as sodium and potassium chlorides, dextrose, polyethylene glycol and other solutes. Liquid compositions can also contain liquid phases in addition to and to the exclusion of water. Exemplary of such additional liquid phases are glycerin, vegetable oils such as cottonseed oil, and water-oil emulsions. The amount of an active agent used in the invention that will be effective in the treatment of a particular disorder or condition will depend on the nature of the disorder or condition, and can be determined by standard clinical techniques.

[0147] In some embodiments, a GDF11 polypeptide or nucleic acid encoding a GDF11 polypeptide as described herein can be administered by controlled- or delayed-release means. Controlled release pharmaceutical products have a common goal of improving drug therapy over that achieved by their non-controlled release counterparts. Ideally, the use of an optimally designed controlled-release preparation in medical treatment is characterized by a minimum of drug substance being employed to cure or control the condition in a minimum amount of time. Advantages

of controlled-release formulations include: 1) extended activity of the drug; 2) reduced dosage frequency; 3) increased patient compliance; 4) usage of less total drug; 5) reduction in local or systemic side effects; 6) minimization of drug accumulation; 7) reduction in blood level fluctuations; 8) improvement in efficacy of treatment; 9) reduction of potentiation or loss of drug activity; and 10) improvement in speed of control of diseases or conditions. Kim, Cheng-ju, Controlled-release Dosage Form Design, 2 (Technomic Publishing, Lancaster, Pa.: 2000).

[0148] Conventional dosage forms generally provide rapid or immediate drug release from the formulation. Depending on the pharmacology and pharmacokinetics of the drug, use of conventional dosage forms can lead to wide fluctuations in the concentrations of the drug in a patient's blood and other tissues. These fluctuations can impact a number of parameters, such as dose frequency, onset of action, duration of efficacy, maintenance of therapeutic blood levels, toxicity, side effects, and the like.

[0149] Advantageously, controlled-release formulations can be used to control a drug's onset of action, duration of action, plasma levels within the therapeutic window, and peak blood levels. In particular, controlled or extended-release dosage forms or formulations can be used to ensure that the maximum effectiveness of a drug is achieved while minimizing potential adverse effects and safety concerns, which can occur both from under-dosing a drug (i.e., going below the minimum therapeutic levels) as well as exceeding the toxicity level for the drug.

[0150] Most controlled-release formulations are designed to initially release an amount of drug (active ingredient) that promptly produces the desired therapeutic effect, and gradually and continually release other amounts of drug to maintain this level of therapeutic or prophylactic effect over an extended period of time. In order to maintain this constant level of drug in the body, the drug must be released from the dosage form at a rate that will replace the amount of drug being metabolized and excreted from the body. Controlled-release of an active ingredient can be stimulated by various conditions including, but not limited to, pH, ionic strength, osmotic pressure, temperature, enzymes, water, and other physiological conditions or compounds.

[0151] A variety of known controlled- or extended-release dosage forms, formulations, and devices can be adapted for use with the salts and compositions of the disclosure. Examples include, but are not limited to, those described in U.S. Pat. Nos.: 3,845,770; 3,916,899; 3,536,809; 3,598,123; 4,008,719; 5,674,533; 5,059,595; 5,591,767; 5,120,548; 5,073,543; 5,639,476; 5,354,556; 5,733,566; and 6,365,185 B1; each of which is incorporated herein by reference. These dosage forms can be used to provide slow or controlled- release of one or more active ingredients using, for example, hydroxypropylmethyl cellulose, other polymer matrices, gels, permeable membranes, osmotic systems (such as OROS® (Alza Corporation, Mountain View, Calif. USA)), or a combination thereof to provide the desired release profile in varying proportions.

[0152] In some embodiments, the technology described herein relates to a syringe comprising a therapeutically effective amount of a composition e.g. a pharmaceutical preparation comprising a GDF11 polypeptide as described herein.

[0153] As used herein, the phrase “therapeutically effective amount”, “effective amount” or “effective dose” refers to an amount that provides a therapeutic or aesthetic benefit in the treatment, prevention, or management of, for example, neurodegenerative or neurovascular disorder, e.g., an amount that provides a statistically significant decrease in at least one symptom, sign, or marker of the neurodegenerative or neurovascular disorder.

[0154] Determination of a therapeutically effective amount is well within the capability of those skilled in the art. Generally, a therapeutically effective amount can vary with the subject's history, age, condition, sex, as well as the severity and type of the medical condition in the subject, and administration of other pharmaceutically active agents.

[0155] In one aspect, the technology described herein relates to a method comprising administering a GDF11 polypeptide or a nucleic acid encoding a GDF11 polypeptide to a subject. In some embodiments, the subject is in need of treatment for a neurodegenerative disorder, or a related condition as described herein. In some embodiments, the subject is in need of treatment for a neurovascular disorder, or a related condition as described herein. In some embodiments, the method is a method

of treating a subject. In some embodiments, the method is a method of treating or preventing sarcopenia in a subject. Such conditions are described herein.

[0156] As used herein, “treat,” “treatment,” “treating,” or “amelioration” when used in reference to a disease, disorder or medical condition, refer to therapeutic treatments for a condition, wherein the object is to reverse, alleviate, ameliorate, inhibit, slow down or stop the progression or severity of a symptom or condition. The term “treating” includes reducing or alleviating at least one adverse effect or symptom of a condition. Treatment is generally “effective” if one or more symptoms or clinical markers are reduced. Alternatively, treatment is “effective” if the progression of a condition is reduced or halted. That is, “treatment” includes not just the improvement of symptoms or markers, but also a cessation or at least slowing of progress or worsening of symptoms that would be expected in the absence of treatment. Beneficial or desired clinical results include, but are not limited to, alleviation of one or more symptom(s), diminishment of extent of the deficit, stabilized (i.e., not worsening) state of, for example, dementia, delay or slowing of dementia, and an increased lifespan as compared to that expected in the absence of treatment.

[0157] As used herein, the term “administering,” refers to the placement of the composition comprising a GDF11 polypeptide or a nucleic acid encoding a GDF11 polypeptide as disclosed herein into a subject by a method or route which results in delivery to a site of action. The pharmaceutical composition comprising a GDF11 polypeptide or a nucleic acid encoding a GDF11 polypeptide can be administered by any appropriate route which results in an effective treatment in the subject.

[0158] Data described herein indicate that systemic administration via the vascular system can be effective. Thus administration via the intravenous route is specifically contemplated. However, with appropriate formulation, other routes are contemplated, including, for example, intranasally, intraarterially; intra-coronary arterially; orally, by inhalation, intraperitoneally, intramuscularly, subcutaneously, intracavity, or by other means known by those skilled in the art. The compositions are administered in a manner compatible with the dosage formulation, and in a therapeutically effective amount. The quantity to be administered and timing depends on the subject to be treated, capacity of the subject's system to utilize the active ingredient, and degree of therapeutic effect desired.

[0159] Therapeutic compositions containing at least one agent can be conventionally administered in a unit dose, for example. The term “unit dose” when used in reference to a therapeutic composition refers to physically discrete units suitable as unitary dosage for the subject, each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect in association with the required physiologically acceptable diluent, i.e., carrier, or vehicle.

[0160] The dosage ranges for the agent depends upon the potency, and are amounts large enough to produce the desired effect e.g., rejuvenation of a subject's neural stem cells and/or progenitor cells, increased neurogenesis or angiogenesis, or a reversal of a neurodegenerative disorder or neurovascular disorder. The dosage should not be so large as to cause unacceptable adverse side effects.

[0161] Generally, the dosage will vary with the age, condition, and sex of the patient and can be determined by one of skill in the art. The dosage can also be adjusted by the individual physician in the event of any complication. Typically, the dosage can range from 0.001 mg/kg body weight to 0.5 mg/kg body weight. In one embodiment, the dose range is from 5 µg/kg body weight to 30 µg/kg body weight.

[0162] Administration of the doses recited above can be repeated. In some embodiments, the doses are given once a day, or multiple times a day, for example, but not limited to, three times a day. In some embodiments, the doses recited above are administered daily for weeks or months. The duration of treatment depends upon the subject's clinical progress and responsiveness to therapy. Without wishing to be bound by theory, where the GDF11 polypeptide apparently diminishes with age in affected individuals, it is expected that long-term therapy would be required to establish and maintain the benefit of GDF11-based treatment, e.g. a neurodegenerative or neurovascular disorder.

[0163] Precise amounts of active ingredient required to be administered depend on the judgment of the practitioner and are particular to each individual. However, suitable dosage ranges for systemic application are disclosed herein and depend on the route of administration. Suitable regimes for administration are also variable, but are typified by an initial administration followed by repeated doses at one or more intervals by a subsequent administration. Alternatively, continuous

intravenous infusion sufficient to maintain concentrations in the blood in the ranges specified for *in vivo* therapies are contemplated. In some embodiments, the dosage range is sufficient to maintain concentrations in the blood in the range found in the blood of a population of normal, healthy human subjects (e.g. those with no signs, symptoms, or markers of neural stem cell and/or progenitor cell deterioration) under the age of 50. In some embodiments, the dosage range is sufficient to maintain concentrations in the blood in the range found in normal, healthy human subjects under the age of 40. In some embodiments, the dosage range is sufficient to maintain concentrations in the blood in the range found in normal, healthy human subjects under the age of 30. In some embodiments, the dosage range is sufficient to maintain concentrations in the blood in the range found in normal, healthy human subjects under the age of 25. In some embodiments, the dosage range is sufficient to maintain concentrations in the blood in the range found in normal, healthy human subjects under the age of 21.

[0164] A therapeutically effective amount is an amount of an agent that is sufficient to produce a statistically significant, measurable change in, for example, a neurodegenerative disorder (e.g., dementia) or neurovascular disorder (e.g., stroke). Such effective amounts can be gauged in clinical trials as well as animal studies. Efficacy of an agent can be determined by assessing physical indicators of, for example neural stem cell and/or progenitor cell deterioration as described above herein. In experimental systems, assays for efficacy include assessing proliferation of neural cells using confocal imaging of SVZ stained by GFAP and Ki67⁺, Olig2⁺, and Sox2⁺, assessing differentiation of neural cells using neurosphere differentiation assays, assessing new neuron formation, for example, by dual staining for BrdU/NeuN⁺, assessing olfactory behavior using an olfaction assay, measuring expression of at least one of the synaptic plasticity, neuroprotective, and neuronal specification genes to assess up-regulation of these genes via microarray or RT-PCR, assessing cerebral blood flow by imaging the vasculature of the SVZ to produce an angiogram for volumetric analysis, and assessing vessel sprouting branch points. Such assays are well known in the art and described in detail in the Examples herein. Clinically acceptable methods for detecting or monitoring neural stem cell rejuvenation are described herein. In addition, efficacy of an agent can be measured

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by an increase in GDF11 polypeptides or fragments thereof in a subject being treated with an agent comprising a GDF11 polypeptide or a nucleic acid encoding GDF11 polypeptide.

[0165] The efficacy of a given treatment for a neurodegenerative or neurovascular disorder can be determined by the skilled clinician. However, a treatment is considered “effective treatment,” as the term is used herein, if any one or all of the signs or symptoms of e.g., a neurodegenerative or neurovascular disorder are altered in a beneficial manner, other clinically accepted symptoms are improved or ameliorated, e.g., by at least 10% following treatment with an agent or composition as described herein. Efficacy can also be measured by a failure of an individual to worsen as assessed by hospitalization or need for medical interventions (i.e., progression of the disease is halted). Methods of measuring these indicators are known to those of skill in the art and/or described herein.

[0166] In some embodiments, the methods further comprise administering the pharmaceutical composition described herein along with one or more additional agents, biologics, drugs, or treatments beneficial to a subject suffering from neural stem cell and/or progenitor cell deterioration or disorder associated with decreased neurogenesis or angiogenesis, or a neurodegenerative or neurovascular disorder as part of a combinatorial therapy. In some such embodiments, the agent, biologic, drug, or treatment can be selected from those found in *Harrison's Principles of Internal Medicine*, 13th Edition, Eds. T.R. Harrison *et al.* McGraw-Hill N.Y., NY; Physicians Desk Reference, 50th Edition, 1997, Oradell New Jersey, Medical Economics Co.; Pharmacological Basis of Therapeutics, 8th Edition, Goodman and Gilman, 1990; United States Pharmacopeia, The National Formulary, USP XII NF XVII, 1990, the complete contents of all of which are incorporated herein by reference. In some embodiments, the additional pharmaceutically active agent is selected from the group consisting of butyrates, valproic acid, hydroxyuirae and Riluzole.

[0167] The above other therapeutic agents, when employed in combination with the chemical entities described herein, may be used, for example, in those amounts indicated in the Physicians' Desk Reference (PDR) or as otherwise determined by one of ordinary skill in the art.

[0168] The description of embodiments of the disclosure is not intended to be exhaustive or to limit the disclosure to the precise form disclosed. While specific embodiments of, and examples for, the disclosure are described herein for illustrative purposes, various equivalent modifications are possible within the scope of the disclosure, as those skilled in the relevant art will recognize. For example, while method steps or functions are presented in a given order, alternative embodiments may perform functions in a different order, or functions may be performed substantially concurrently. The teachings of the disclosure provided herein can be applied to other procedures or methods as appropriate. The various embodiments described herein can be combined to provide further embodiments. Aspects of the disclosure can be modified, if necessary, to employ the compositions, functions and concepts of the above references and application to provide yet further embodiments of the disclosure. These and other changes can be made to the disclosure in light of the detailed description.

[0169] Specific elements of any of the foregoing embodiments can be combined or substituted for elements in other embodiments. Furthermore, while advantages associated with certain embodiments of the disclosure have been described in the context of these embodiments, other embodiments may also exhibit such advantages, and not all embodiments need necessarily exhibit such advantages to fall within the scope of the disclosure.

[0170] All patents and other publications identified are expressly incorporated herein by reference for the purpose of describing and disclosing, for example, the methodologies described in such publications that might be used in connection with the present invention. These publications are provided solely for their disclosure prior to the filing date of the present application. Nothing in this regard should be construed as an admission that the inventors are not entitled to antedate such disclosure by virtue of prior invention or prior publication, or for any other reason. All statements as to the date or representation as to the contents of these documents is based on the information available to the applicants and does not constitute any admission as to the correctness of the dates or contents of these documents.

[0171] One skilled in the art readily appreciates that the present invention is well adapted to carry out the objects and obtain the ends and advantages mentioned,

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as well as those inherent therein. The details of the description and the examples herein are representative of certain embodiments, are exemplary, and are not intended as limitations on the scope of the invention. Modifications therein and other uses will occur to those skilled in the art. These modifications are encompassed within the spirit of the invention. It will be readily apparent to a person skilled in the art that varying substitutions and modifications may be made to the invention disclosed herein without departing from the scope and spirit of the invention.

[0172] The articles “a” and “an” as used herein in the specification and in the claims, unless clearly indicated to the contrary, should be understood to include the plural referents. Claims or descriptions that include “or” between one or more members of a group are considered satisfied if one, more than one, or all of the group members are present in, employed in, or otherwise relevant to a given product or process unless indicated to the contrary or otherwise evident from the context. The invention includes embodiments in which exactly one member of the group is present in, employed in, or otherwise relevant to a given product or process. The invention also includes embodiments in which more than one, or all of the group members are present in, employed in, or otherwise relevant to a given product or process. Furthermore, it is to be understood that the invention provides all variations, combinations, and permutations in which one or more limitations, elements, clauses, descriptive terms, etc., from one or more of the listed claims is introduced into another claim dependent on the same base claim (or, as relevant, any other claim) unless otherwise indicated or unless it would be evident to one of ordinary skill in the art that a contradiction or inconsistency would arise. It is contemplated that all embodiments described herein are applicable to all different aspects of the invention where appropriate. It is also contemplated that any of the embodiments or aspects can be freely combined with one or more other such embodiments or aspects whenever appropriate. Where elements are presented as lists, e.g., in Markush group or similar format, it is to be understood that each subgroup of the elements is also disclosed, and any element(s) can be removed from the group. It should be understood that, in general, where the invention, or aspects of the invention, is/are referred to as comprising particular elements, features, etc., certain embodiments of the invention or aspects of the invention consist, or consist essentially of, such elements, features, etc.

For purposes of simplicity those embodiments have not in every case been specifically set forth in so many words herein. It should also be understood that any embodiment or aspect of the invention can be explicitly excluded from the claims, regardless of whether the specific exclusion is recited in the specification. For example, any one or more active agents, additives, ingredients, optional agents, types of organism, disorders, subjects, or combinations thereof, can be excluded.

[0173] Where the claims or description relate to a composition of matter, it is to be understood that methods of making or using the composition of matter according to any of the methods disclosed herein, and methods of using the composition of matter for any of the purposes disclosed herein are aspects of the invention, unless otherwise indicated or unless it would be evident to one of ordinary skill in the art that a contradiction or inconsistency would arise. Where the claims or description relate to a method, e.g., it is to be understood that methods of making compositions useful for performing the method, and products produced according to the method, are aspects of the invention, unless otherwise indicated or unless it would be evident to one of ordinary skill in the art that a contradiction or inconsistency would arise.

[0174] Where ranges are given herein, the invention includes embodiments in which the endpoints are included, embodiments in which both endpoints are excluded, and embodiments in which one endpoint is included and the other is excluded. It should be assumed that both endpoints are included unless indicated otherwise. Furthermore, it is to be understood that unless otherwise indicated or otherwise evident from the context and understanding of one of ordinary skill in the art, values that are expressed as ranges can assume any specific value or subrange within the stated ranges in different embodiments of the invention, to the tenth of the unit of the lower limit of the range, unless the context clearly dictates otherwise. It is also understood that where a series of numerical values is stated herein, the invention includes embodiments that relate analogously to any intervening value or range defined by any two values in the series, and that the lowest value may be taken as a minimum and the greatest value may be taken as a maximum. Numerical values, as used herein, include values expressed as percentages. For any embodiment of the invention in which a numerical value is prefaced by “about” or “approximately”, the

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invention includes an embodiment in which the exact value is recited. For any embodiment of the invention in which a numerical value is not prefaced by “about” or “approximately”, the invention includes an embodiment in which the value is prefaced by “about” or “approximately”.

[0175] “Approximately” or “about” generally includes numbers that fall within a range of 1% or in some embodiments within a range of 5% of a number or in some embodiments within a range of 10% of a number in either direction (greater than or less than the number) unless otherwise stated or otherwise evident from the context (except where such number would impermissibly exceed 100% of a possible value). It should be understood that, unless clearly indicated to the contrary, in any methods claimed herein that include more than one act, the order of the acts of the method is not necessarily limited to the order in which the acts of the method are recited, but the invention includes embodiments in which the order is so limited. It should also be understood that unless otherwise indicated or evident from the context, any product or composition described herein may be considered “isolated”.

[0176] This invention is further illustrated by the following examples which should not be construed as limiting.

* * *

EXAMPLES

[0177] The examples that follow demonstrate that neurogenesis and angiogenesis in the aged brain can be rejuvenated by heterochronic parabiosis and by GDF11 administration.

[0178] Neurogenesis in the adult brain occurs primarily in two areas: the dentate gyrus of the hippocampus and the subventricular zone (SVZ) of the lateral ventricles (1, 2). The capacity of these areas to generate new neurons throughout life lies in the existence of neural stem cells (NSCs) and progenitors. In the SVZ, these cells reside in a three-dimensional heterogeneous niche connected to cerebrospinal fluid and the vasculature. The niche creates a special microenvironment for providing the necessary regulatory cues for proliferation and differentiation (3-6). The vasculature has emerged as an important regulator of neural stem cell proliferation and differentiation by providing both factors secreted locally from endothelial cells, as

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well as systemic regulatory signals from the blood. Systemic regulatory influences include the hormone prolactin (10), dietary restriction (11) and exercise/enriched environment (12), all of which positively modulate neurogenesis, and factors like increased the levels of glucocorticoids associated with stress that have the opposite effect (13). In aging, different components of the niche deteriorate. On one hand, the vasculature deteriorates with consequent reduction in blood flow and, on the other hand, the prevalence of NSCs and their capacity to produce new neurons greatly declines with age. This is associated with reduced neuroplasticity and cognitive functioning (14, 15). Since the neurovascular niche can respond to systemic signals, it follows that the age-related decline in neurogenesis may reflect alterations in the level of circulating factors, making it also possible to imagine reversing the decline by supplementing or inhibiting these factors. To investigate this concept, the inventors exploited the heterochronic parabiosis paradigm, whereby mice of different ages share a common blood circulation. The work described herein reports for the first time that heterochronic parabiosis resulted in a striking remodeling of the aged cerebral vasculature in response to young systemic factors, producing increased stem and progenitor cell numbers in the SVZ, notable increased angiogenesis and blood flow as well. Furthermore, the neural progenitors are capable of forming new neurons in the olfactory bulb (OB), which results in an overall improvement in olfactory behavior. Finally the inventors demonstrate that GDF11 is a systemic factor that recapitulates the beneficial effects of heterochronic parabiosis on angiogenesis and neurogenesis in the old mouse. These studies described herein expand our understanding of age-related decline of neurogenesis to include a direct impact on the neurogenic niche, and further demonstrate that age-dependent remodeling of this niche is reversible by systemic intervention and determinative of neurogenic function.

[0179] Example 1 - Young blood stimulates neural stem and progenitor cell proliferation and expansion in the subventricular zone (SVZ).

[0180] Heterochronic parabiotic pairs between old (Het-O) and young (Het-Y) male mice were generated, as well as control groups of age-matched pairs isochronic young (Iso-Y) and isochronic old (Iso-O) (Fig.1A). All parabiotic pairs remained surgically joined for 5 weeks prior to analysis. To allow identification of circulating

cells after parabiosis, young mice carrying the congenic marker CD45.1 and old mice carrying the congenic marker CD45.2 in the heterochronic pair were used, and young mice carrying either CD45.1 or CD45.2 in the Iso-Y pair were used (**Fig. 5**)(20). By taking advantage of these markers the degree of cross-circulation was assessed by analyzing spleen chimerism after parabiosis (**Fig. 5**) which reached around 60%. Coronal sections of the SVZ of heterochronic brains, and their isochronic controls were analyzed first. It was observed that the SVZ area marked by GFAP⁺ cells in the Het-O mice had significantly expanded compared to the same area in the Iso-O mice, suggesting an increase in the proliferative cell population. Indeed, confocal imaging of these sections stained with GFAP in combination with Ki67, Sox2, or Olig2 showed a marked increase in Ki67⁺, Sox2⁺ and Olig2⁺ cell populations (**Figs. 1B, 1C and 1D**, respectively). Quantification of these sections revealed that Ki67⁺ cells increased by 26.9% in the Het-O compared to the Iso-O SVZ (**Fig. 1E**). Further analysis of different SVZ stem/progenitor cell types revealed that the number of Sox2⁺ neural stem cells was increased by 112% in the Het-O compared to the Iso-O SVZ (**Fig. 1F**). Moreover, Olig2⁺ transit amplifying progenitors in the SVZ area were increased by 57% (**Fig. 1G**). On the contrary, these proliferative neural stem/progenitor cell populations remained unaffected by parabiosis in young Het-Y mice (**Figs. 1H, 1I and 1J**).

[0181] It has been previously reported that factors in old blood can have detrimental effects on hippocampal neurogenesis in young animals (31); however, as described above, the present investigators observed no decrease in neural stem/progenitor cell number in the SVZ in 2-month old young mice joined to 15-month old partners. In an effort to determine whether this discrepancy related to differences between the SVZ and the hippocampus or to the fact that the old mice were younger than the old mice used in the previous study (21 months of age), the present investigators joined 2-month old mice with 21-month old mice, as illustrated in **Fig. 15A**. In this system, the older blood did negatively affect young SVZ neurogenesis, as Het-Y₂₁ mice showed decreased proliferative Ki67⁺ cells (**Figs. 15B and 15D**) and Sox2⁺ neural stem cell populations (**Figs. 15C and 15E**) in the SVZ. These data are consistent with the previously reported negative effect of older blood on hippocampal neurogenesis (10) and indicate an age-dependent accumulation of

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factors in the blood of older mice that affect neurogenic zones in both the hippocampus and SVZ.

[0182] To further examine the effect of heterochronic parabiosis on NSC self-renewal and differentiation potential, neural stem cells from the SVZ area of parabiotic mouse brains were isolated and cultured *in vitro* (21). Neural stem cells derived from the SVZ area of parabiotic mouse brains were cultured *in vitro* in the form of neurospheres. After the first passage, neurospheres derived from the Het-O SVZ were 43% larger in diameter than those derived from the Iso-O SVZ (**Fig. 6**), suggesting that the proliferation rate was higher, and that NSC retain their ability to proliferate more, even *ex vivo*, once they have been exposed to young systemic factors, which is in accordance with the *in vivo* data. To assess if NSC differentiation was affected as well, a neurosphere differentiation assay was performed by removing the growth factors in culture (22). Indeed, Het-O differentiated neurospheres generated approximately 2.5-fold more TuJ1⁺ neurons than the Iso-O (**Figs. 7A, 7B, and 7C**), confirming that the rejuvenation effects of heterochronic parabiosis extend to promote neural progenitor cell differentiation *in vitro*. Collectively these data demonstrate that youthful circulating factors can restore the self-renewal and differentiation potential of aged stem cells and progenitors and that this effect can persist for some time after isolation from the mouse brain.

[0183] Example 2 - Heterochronic parabiosis enhances neurogenesis and cognitive functions in the old mouse.

[0184] Adult subventricular zone (SVZ) neural stem and progenitor cells differentiate into neuroblasts and migrate through the rostral migratory stream in order to finally reach the olfactory bulb where they mature locally into interneurons (23). The inventors next hypothesized that the increase in neural stem cells (NSCs) and progenitor cells reflects a concurrent change in olfactory neurogenesis. Parabiotic pairs were injected with BrdU (50mg/kg) one week after joining and pulsed every 12 hours for 3 days in order to label newborn neurons. After a total of 5 weeks, the animals were analyzed for double labeled BrdU⁺ and NeuN⁺ cells to quantify newborn neurons (**Fig. 2A**). Interestingly, increased self-renewal and differentiation potential of NSCs and progenitor cells resulted in increased neurogenesis in the

olfactory bulb. Het-O newborn neuron populations were enriched by 92% compared to the Iso-O (**Fig. 2B**). Neurons in the Het-Y condition seemed to be slightly negatively affected, although the decrease in neurogenesis was not statistically significant (**Fig. 2C**).

[0185] To further test the functional significance of these findings an olfaction assay was performed where naïve parabionts separated from the parabiotic partners after 5 weeks of joining were exposed to an odorant (**Fig. 8**) (24). After a short habituation period, each parabiont was presented with different concentrations of an odorant, and the total time that each parabiont spent exploring the odorant for each concentration was measured. In this assay, Het-O mice spent more time exploring the odorant at low concentration (10^{-5}), whereas high concentrations (10^{-1}) of the odorant seemed to produce a negative response (**Fig. 2D**). The same was true for young controls (**Fig. 2D**). On the other hand, Iso-O mice spent roughly the same amount of time exploring the odorant regardless of its concentration (**Fig. 2D**). To ensure that these results were not due to habituation to the odorant, the inventors performed the test starting from both lower and higher concentrations. In this paradigm Het-O mice showed an improved olfactory capability and higher olfactory sensitivity than the Iso-O mice, since they discriminate better between different concentrations of the odorant and needed far less time to discover the odorant compared to Iso-O pairs (**Fig. 2D**). Therefore, heterochronic parabiosis and exposure of the neurogenic niche to young systemic factors enhances functional neurogenesis culminating in an improvement in olfactory behavior.

[0186] This observation encouraged the inventors to explore the molecular changes that could be associated with the above functional modifications after parabiosis. A microarray analysis on the forebrain of isochronic and heterochronic mice was performed. Gene expression changes were compared between all different conditions for both up-regulation and down-regulation, resulting in multiple Venn diagrams (**Figs. 9A and 9B**). Strikingly, the cluster of genes that were up-regulated by up to 16-fold in both Iso-Y and Het-O conditions compared with Iso-O condition contains almost exclusively activity-dependent genes, largely known for their roles in synaptic plasticity, neuroprotection and neuron specification (**Fig. 2E**). Examples of highly up-regulated genes include Nasp4, Fos, Klf10, Dusp1, Btg2 and Arc. Npas4 is

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an immediate early gene that regulates the expression of activity-dependent genes (25). These results were further confirmed by real-time RT-PCR in which the inventors used different parabiotic animals than the ones used for the microarray analysis. Genes related to neuronal activity, like *Npas4*, *Dusp1*, *Btg2*, *Nra1* and *Cyr61* were up-regulated in the Het-O mice compared to the Iso-O mice (**Fig. 2F**). These data correlate with the above findings and provide additional evidence for the role of heterochronic parabiosis in the enhancement of neuroplasticity.

[0187] Example 3 - Young blood enhances vascularization and increases blood flow in the old mouse.

[0188] Since neural stem cells (NSCs) and the vasculature are functionally linked in the subventricular zone (SVZ) niche (4-6, 26), the inventors investigated whether parabiosis could affect the aged vasculature. Cerebrovascular architecture, capillary density and cerebral blood flow have been reported to decline during aging (17-19). The inventors reasoned that increases in neurogenesis in the aged brains might be due to a restoration of blood vessel number and function. For this purpose the vasculature of the SVZ was imaged and 3D reconstructions of the blood vessels or “angiograms” were created (**Fig. 10A**). Volumetric analysis of these angiograms showed that aging causes a decrease in blood vessel volume (comparison between a young and an old mouse) (**Figs. 3A and 3B**). However, heterochronic parabiosis reversed this decline, enhancing angiogenesis and increasing blood vessel volume by 87% in the Het-O compared to the Iso-O group (**Fig. 3A and 3C**). Further quantification of vessel sprouting branch points showed a 21% increase in Het-O versus Iso-O mice (**Fig. 3D**). Moreover, some of the blood vessels in the Het-O mice were not associated with AQP4⁺ astrocytic endfeet, suggesting that these vessels are newly formed and potentially leaky thus providing NSCs with enhanced nutritive support (**Fig. 10B**). This phenomenon of increased vascularization in the Het-O mice extended to other neurogenic areas like the hippocampus (**Figs. 16A and 16B**) and also to non-neurogenic areas in the brain, like the cortex (**Fig. 10C**). Young vasculature (Iso-Y and Het-Y), to the contrary, retained the same volumetric and branching characteristics despite heterochronic parabiosis (**Fig. 3A, 3E, and 3F**).

[0189] Because new vessels can form by either spontaneous sprouting from existing capillaries or *de novo* from circulating endothelial progenitors or both, the inventors asked which mechanism may be responsible for the increased volume of the vasculature that the inventors observed in heterochronic parabiosis. To determine the contribution of circulating progenitors, young GFP mice were joined with old non-GFP mice for 5 weeks. Analysis of these parabiotic brains demonstrated that endothelial progenitors from the young mice do not contribute substantially to the vascular remodeling in the Het-O mice (**Fig. 11**), reinforcing the idea that angiogenesis is stimulated by circulating factors. This conclusion was further supported when primary brain capillary endothelial cells were cultured *in vitro* and treated with serum isolated from either a young or an old mouse. As pericytes play a role in vasoconstriction in capillaries, the present inventors next sought to investigate whether their numbers were altered by heterochronic parabiosis. As illustrated in **Fig. 17**, quantification of the number of pericytes associated with blood vessels showed that they were unaffected by parabiosis. Young serum stimulated endothelial cell proliferation by 1.8-fold compared to old serum (**Fig. 12**), correlating with our *in vivo* data and indicating that the age of the mouse from which serum was isolated, rather than the age of the cells themselves, is critical to the stimulation of proliferation.

[0190] Next the inventors asked whether the increased blood vessel volume would translate into any type of functional improvement or modification. Since cerebral blood flow (CBF) is known to decrease with aging (27), CBF was measured by magnetic resonance imaging (MRI) in the SVZ of parabiotic mice (**Fig. 3G**). Indeed, CBF decreased with aging, as Iso-O mice had lower CBF than Iso-Y mice (**Fig. 3H**). However, when CBF was measured in Het-O mice it was noticeably higher than in Iso-O mice and close to the young levels (**Fig. 3G and 3I**) suggesting that the induction of angiogenesis observed in the Het-O mouse results in a change in the hemodynamics of the vascular system.

[0191] Example 4 - Young circulating factor GDF11 recapitulates the effect of heterochronic parabiosis by enhancing angiogenesis and neurogenesis.

[0192] The above data suggest that circulating factors in the young blood stimulate angiogenesis and neurogenesis in the older brain. This prompted the

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inventors to investigate whether GDF11, a circulating factor, could restore the age-related decline in angiogenesis and neurogenesis. For that reason, aged mice were treated with daily injections of either recombinant GDF11 (rGDF11, 0.1mg/kg mouse body weight, a dosing regimen that increases GDF11 levels in old mice toward youthful levels) or PBS (vehicle) for 4 weeks and their blood vessels were analyzed using the volumetric assay described above. Surprisingly, the volume of blood vessels in GDF11-treated old mice increased by 50% compared to the PBS-treated mice (**Figs. 4A and 4B**). Moreover, the population of Sox2⁺ cells in GDF11-treated old mice increased by 29% compared to the control (**Figs. 4C and 4D**). *In vitro* experiments confirmed that a 6-day treatment of primary brain capillary endothelial cells with rGDF11 (40ng/ml) *in vitro* increased their proliferation by 1.23-fold compared to controls, suggesting that GDF11 can act directly on endothelial cells (**Fig. 4E and 4F**). GDF11 is a member of the TGF β superfamily and its receptor is ALK4/5. Activation of the receptor should lead to phosphorylation of SMAD2/3 proteins as the first component of the signaling cascade. Indeed, treating endothelial cells with either rGDF11 (40ng/ml) or TGF β (positive control, 10ng/ml) increased SMAD phosphorylation significantly, although GDF11 was somewhat less active than TGF β under these conditions. Thus, GDF11 promotes proliferation of brain capillary endothelial cells through the SMAD signaling cascade (**Figs. 18A and 18B**).

[0193] Conclusions

[0194] The work described herein demonstrates that heterochronic parabiosis induces rejuvenation of the neurogenic niche at least in part by improving vascularity and blood flow, and that the age-related decline in neurogenesis can be reversed by exposure to young circulating factors. The work described herein further shows that the improved neurogenesis in the aged SVZ is associated with an increased number of capillaries in that region. The role of blood vessels in the brain is now believed to be more significant than that of simply providing oxygen and nutrient supply. Previous work has demonstrated a close association between neural stem cells and endothelial cells in the SVZ (4-6). Thus, it is possible that the primary effect of parabiosis is on blood vessels in that area, with an important consequence being the stimulation of NSC proliferation and differentiation. Importantly, the effect of parabiosis on blood

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vessel number was not restricted to the SVZ, and it may be that additional positive effects of increasing blood flow in the older brain can be observed.

[0195] Using one set of dosing conditions, GDF11, recently identified as an important parabiogenic factor, was able to increase both angiogenesis and neurogenesis, although not as much as parabiosis itself. It is possible that more optimized dosing will produce greater effects, but it is also possible that there are additional circulating or niche factors that remain to be identified. In any case, it is remarkable that one circulating factor can have such a significant effect and it lends credence to the idea that degenerative conditions of aging are amenable to relatively simple treatments.

MATERIALS AND METHODS

[0196] The materials and methods below were used to carry out the experiments described in the above Examples.

[0197] *Animals*

[0198] Aged C57Bl/6 mice (15-17 months) were obtained from the National Institute on Aging (NIA). Young C57Bl/6J, B6.SJL and GFP mice (2 months) were obtained from Jackson Laboratories (USA). All animal studies were approved by Harvard University Institutional Animal Care and Use Committee and performed in accordance with institutional and federal guidelines.

[0199] *Parabiosis and blood chimerism analysis*

[0200] Parabiosis was performed as described previously (28) on young (2 months) and old (15-16 months) male mice. Experiments where older (21 months) mice were used are coded as Iso-O₂₁ and Het-O₂₁. Het-Y₂₁ indicates a Het-Y animal in parabiosis with an Iso-O₂₁ mouse. For reversal of parabiosis mice were anesthetized with isoflurane. The skin was incised at the joining wound, separating the animals. The resulting incisions in the corresponding lateral aspects of separated mice were closed by staples or interrupting suture (5-0 coated vicryl) and animals were given buprenorphine every 12 hours for at least 48 hours after surgery.

[0201] For blood chimerism analysis spleens were dissected from the mice, the tissue was homogenized and erythrocytes lysed. Cells were stained with an antibody cocktail (CD45.2-FITC, CD45.1-PE, Ter119-APC, PI). The frequency of

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chimerism was measured by flow cytometry and analyzed using FLOWJO software (TreeStar, USA). Since old CD45.1⁺ are not commercially available we only used CD45.2⁺ in the Iso-O pair assuming that cross-circulation rates are similar to the Iso-Y and Het pairs.

[0202] *Immunohistochemistry*

[0203] Mice were perfused transcardially with 50ml PBS, followed by 50ml of 4% paraformaldehyde (PFA) and their brains were removed and post-fixed overnight in 4% PFA. Each brain was embedded in 4% agarose and 100µm-thick coronal were cut in a vibrating microtome (VT1000S, Leica) and pre-incubated in 10% normal goat or donkey serum, Triton 0.1% in PBS for 1 hour. For BrdU detection, sections were incubated for 40min with HCl 2N prior to blocking. Tissue sections or cell cultures were incubated overnight at 4°C with the following antibodies: rat monoclonal anti-BrdU (Abcam), mouse monoclonal anti-Olig2 (Millipore), polyclonal rabbit anti-Sox2 (Cell Signaling Technology), polyclonal rabbit anti-ki67 (Abcam), rat monoclonal CD31 (BD Biosciences), chicken polyclonal anti-GFAP (Abcam), mouse monoclonal anti-NeuN (Millipore), mouse monoclonal anti-TuJ1 (Millipore), rabbit polyclonal phospho-Smad2/3 (Cell Signaling), rabbit polyclonal anti-Aquaporin 4 and Fluorescein Lycopersicon Esculentum Lectin-FITC (Vector Laboratories). For endothelial proliferation assays the Click-iT® EdU Alexa Fluor® 488 Imaging Kit was used according to the manufacturer's instructions.

[0204] *Microarrays*

[0205] Brains from parabiotic old mice were dissected around the SVZ area and the tissue was processed for RNA extraction which was performed according to the manufacturer's instruction using the Stratagene Absolute RNA Microisolation kit (Stratagene, cat# 400805) and quality-checked on the Agilent 2100 Bioanalyzer. Complementary DNA was prepared with the Ambion WT Expression Kit (Ambion) and quality-checked on the Agilent 2100 Bioanalyzer. The Affymetric GeneChip WT Terminal Labeling Kit was used for cDNA fragmentation, labeling and hybridization to the GeneChip mouse Gene 1.0 ST Array (Affymetrix). The arrays were washed, stained, and scanned using the GeneChip Fluidics Station 450 and GeneChip Scanner 3000 7G (Affymetrix). The obtained raw intensity, .CEL files from the 8 chips, two replicates for each of the four, were then normalized by robust

multichip analysis (RMA) (Bioconductor release 2.11). Normalized expression values were analyzed with the Bioconductor limma package, an approach of implementation of the Empirical Bayes linear modeling (Smyth 2005). For all comparison tests, genes with P value <5% were selected for further analyses. The likelihood of overrepresentation of function categories in the up- or down-regulated gene list relative to a background of all array genes was calculated by Fisher's exact test for statistical analysis.

[0206] *Neural stem cell cultures, proliferation and differentiation*

[0207] Neural stem cell cultures (neurospheres) were performed as described previously (22). For proliferation neurospheres were maintained in serum-free medium containing EGF and bFGF (both 20ng/ml). For the differentiation assay neurospheres were trypsinized and plated on poly-lysine/laminin coated coverslips in serum-free, growth-factor-free medium as described previously (22).

[0208] *Endothelial cell cultures*

[0209] Primary mouse brain microvascular endothelial cells were purchased from Cell Biologics and maintained in Endothelial Cell Medium according to vendor's instructions. For proliferation assays, BVECs were trypsinized and seeded in a gelatin-coated 96 well plate at a density of 2,000 cells/well. Complete medium was replaced with the experimental growth medium 12 hours after plating. EdU was added at a concentration of 10 μ M four hours prior to fixation with 4% paraformaldehyde. Cells were stained with the EdU kit and the nuclear dye Hoechst 33342. For pSMAD assays, primary brain vessel endothelial cells were treated as indicated, in the presence of sodium orthovanadate, to preserve phosphorylation for 30 minutes, fixed with 4% paraformaldehyde, subjected to immunocytochemical analysis, and imaged using the Operetta high content imaging microscope (Perkin Elmer). Automated image analysis was performed using Columbus software (Perkin Elmer).

[0210] *Olfactory sensitivity assay*

[0211] This test is designed to detect a difference in olfactory sensitivity between two groups of mice. The olfactory sensitivity assay was performed as previously described (24). Before the assay control mice were exposed to different sets of odorants in order to measure the spontaneous response to the different odorants and concentrations. For the assay every naïve parabiotic mouse (3 days after pair

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separation) was placed in a new, clean cage and left for 15 min for habituation. Then different dilutions of the odorant (Peppermint, Sigma-Aldrich) that ranged from 10 to 10,000 were infused on a disk and the disk placed in the cage across from the mouse. Different doses of the odorant were presented to the animal. To avoid bias, the experiment was conducted both from high to low dilution and the reverse. The total time of exploration was measured and data were analyzed by Graph Pad Prism.

[0212] *Perfusion MRI*

[0213] Relative cerebral blood flow (CBF) was measured by using a flow-sensitive alternating inversion recovery (FAIR) sequence (29) on a Bruker Pharmascan 4.7T MRI using endogenous water protons as a tracer (TR = 16000 ms, TE = 23 ms, 64 x 64 x 1 matrix size, 0.625 mm x 0.625 mm x 2 mm voxel size) and with a Bruker 3.7 cm diameter transmit-receive coil to fit both mice in the scanner. This arterial spin labeling technique performs two echo planar images (EPI) with both flow sensitive and flow insensitive EPI scans. During the inversion delay time after slice-selective inversion, fully magnetized blood protons move into the imaging slice and exchange with tissue water protons. The difference between these two images is directly proportional to relative blood flow. In addition, a T2-weighted (T2w) scan (TR = 2000 ms, TE = 20 ms, effective TE = 60 ms, 8 averages, 192 x 192 x 22 matrix size, 0.133 mm x 0.130 mm x 0.7 mm voxel size) was performed to select a slice in the subventricular zone. The geometry was customized for each of the parabiosed mice to ensure axial slice selection for the images. The FAIR CBF map for each mouse was fused to the T2w anatomical images in OsiriX (<http://www.osirix-viewer.com>). Using the T2w images as anatomical reference, fixed-size oval regions-of-interest (ROI) were drawn in the SVZs on the FAIR CBF maps to measure the mean image intensities in the SVZs. The mean image intensities of the ventricles were used as the background. 3D images were generated using Amira (VSG, Burlington, MA) after segmentation for the brain on T2w images and fusing with the FAIR CBF maps.

[0214] *Volumetric 3D assays*

[0215] To measure the volume of blood vessels, 100µm-thick sections were imaged under a Zeiss LSM 510 inverted confocal microscope in order to create z-stacks. Those were analyzed using the Volocity 3D Image analysis software (Perkin

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Elmer) software to create three-dimensional angiograms and calculate the total volume.

[0216] *Imaging*

[0217] Imaging was performed using a Zeiss LSM 510 Inverted Confocal microscope, a Nikon Eclipse Ti microscope and the Operetta High Content Imaging System (Perkin Elmer).

[0218] *Quantification of immunostaining*

[0219] In all experiments, quantification was performed blindly: each experiment was assessed a code which was revealed after analysis. For the *in vivo* experiments, **n** represents the number of mice used for the analysis. For each mouse, 8-10 different sections comprising the same area of the SVZ were analyzed. Quantification of all figures was performed per field of view except for Olig2, which was measured only in the SVZ as marked by GFAP⁺ cells. Sox2 quantification in **Fig. 4C** was performed using a rectangular shape that was copied in all the images. Only cells inside the shape were counted. In endothelial cell proliferation assays, images of the whole well were acquired using the Operetta high content automated imaging microscope (Perkin Elmer) and were analyzed using Columbus Image Analysis Software (Perkin Elmer). Each *in vitro* experiment was repeated at least 3 times.

[0220] *Statistical analysis*

[0221] Statistical analyses were performed using Graph Pad Prism software, with Student's t-test assuming two-tailed distribution and equal or unequal variances depending on the experiment. Statistical significance was assigned for $p < 0.05$; results are shown as standard error of the mean.

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CLAIMS

What is claimed is:

1. A method for increasing neurogenesis in a subject in need thereof, comprising administering to the subject an agent or composition which increases the level of GDF11 polypeptide in the subject, thereby increasing neurogenesis in the subject.
2. A method for treating or preventing a neurodegenerative disorder by increasing neurogenesis in a subject, comprising administering to the subject an effective amount of an agent or composition which increases the level of GDF11 polypeptide in the subject, thereby increasing neurogenesis in the subject, wherein increasing neurogenesis in the subject treats or prevents the neurodegenerative disorder in the subject.
3. The method of claims 1 or 2, wherein increasing neurogenesis is associated with increased neural cell proliferation, increased neural cell differentiation, increased number or proliferation rate of neural stem cells, increased number or proliferation rate of neural progenitor cells, increased number or proliferation rate of neural precursor cells, increased expression of at least one synaptic plasticity gene, at least one neuroprotective gene, or at least one neuronal specification gene, or increased number of new neurons.
4. The method of any one of claims 1 to 3, wherein neurogenesis is increased in the subventricular zone of the lateral ventricles.
5. The method of any one of claims 1 to 3, wherein neurogenesis is increased in the olfactory bulb.
6. The method of claim 5, wherein increased neurogenesis results in improved olfactory behavior.

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7. The method of any one of claims 1 to 6, wherein the neurodegenerative disorder is selected from the group consisting of Huntington's disease, dentatorubropallidoluysian atrophy, Kennedy's disease, spinocerebellar ataxia, fragile X syndrome, fragile XE mental retardation, Friedreich's ataxia, myotonic dystrophy, spinocerebellar ataxia type 8, and spinocerebellar ataxia type 12, Alexander disease, Alper's disease, Alzheimer disease, amyotrophic lateral sclerosis (ALS), ataxia telangiectasia, Batten disease, Canavan disease, Cockayne syndrome, corticobasal degeneration, Creutzfeldt-Jakob disease, Guillain-Barré syndrome, ischemia stroke, Krabbe disease, kuru, Lewy body dementia, multiple sclerosis, multiple system atrophy, non-Huntingtonian type of Chorea, Parkinson's disease, Pelizaeus-Merzbacher disease, Pick's disease, primary lateral sclerosis, progressive supranuclear palsy, Refsum's disease, Sandhoff disease, Schilder's disease, spinal cord injury, spinal muscular atrophy (SMA), SteeleRichardson-Olszewski disease, and Tabes dorsalis.
8. The method of any one of claims 1 to 7, wherein the subject has been diagnosed with a neurodegenerative disorder due to aging.
9. A method for increasing angiogenesis in a subject in need thereof, comprising administering to the subject an agent or composition which increases the level of GDF11 polypeptide in the subject, thereby increasing angiogenesis in the subject.
10. A method for treating or preventing a neurovascular disorder by increasing angiogenesis in a subject, comprising administering to the subject an agent or composition which increases the level of GDF11 polypeptide in the subject, thereby increasing angiogenesis in the subject, wherein increasing angiogenesis in the subject treats or prevents the neurovascular disorder in the subject.
11. The method of claims 9 or 10, wherein increasing angiogenesis comprises increasing cerebrovascular architecture, increasing capillary density,

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increasing cerebral blood flow, and increasing cerebral vessel sprouting branch points.

12. The method of any one of claims 9 to 11, wherein the neurovascular disorder is selected from the group consisting of brain atherothrombosis, brain aneurysms, brain arteriovenous malformations, brain embolism, brain ischemia, for example caused by atherothrombosis, embolism, or hemodynamic abnormalities, cardiac arrest, carotid stenosis, cerebrovascular spasm, headache, intracranial hemorrhage, ischemic stroke, seizure, spinal vascular malformations, reflex neurovascular dystrophy (RND), neurovascular compression disorders such as hemifacial spasms, tinnitus, trigeminal neuralgia, glossopharyngeal neuralgia, stroke, transient ischemic attacks, and vasculitis.
13. The method of any one of claims 9 to 12, wherein the subject has been diagnosed with a neurovascular disorder due to aging.
14. The method of any one of claims 1 to 13, wherein the level of GDF11 polypeptide is increased in at least one of the subject's systemic circulation, cerebral vasculature, cerebral tissue, cerebrospinal fluid, lateral ventricles, neurovasculature, and subventricular zone neurovascular niche.
15. The method of any one of claims 1 to 14, wherein the agent or composition comprises an agonist antibody that increases expression of GDF11.
16. The method of any one of claims 1 to 14, wherein the agent or composition comprises a GDF11 polypeptide.
17. The method of any one of claims 1 to 14, wherein the agent or composition comprises a GDF11 polypeptide comprising the amino acid sequence of SEQ ID NO: 3.

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18. The method of any one of claims 1 to 14, wherein the agent or composition comprises a GDF11 polypeptide comprising the amino acid sequence of SEQ ID NO: 2.
19. The method of any one of claims 1 to 14, wherein the agent or composition comprises a GDF11 polypeptide comprising the amino acid sequence of SEQ ID NO: 1.
20. The method of any one of claims 1 to 14, wherein the agent or composition comprises a GDF11 polypeptide comprising the amino acid sequence of SEQ ID NO: 4.
21. The method of any one of claims 1 to 20, wherein the agent or composition comprises homodimers of GDF11 polypeptides comprising the amino acid sequence of any of SEQ ID NOs: 1, 2, 3 or 4.
22. The method of any one of claims 1 to 20, wherein the agent or composition comprises complexes of GDF11 polypeptides comprising the amino acid sequence of any of SEQ ID NOs: 1, 2, 3 or 4.
23. The method of any one of claims 1 to 22, wherein the agent or composition comprises a nucleic acid encoding a GDF11 polypeptide or a functional fragment or variant thereof.
24. The method of any one of claims 1 to 23, wherein the GDF11 polypeptide comprises a modified GDF11 polypeptide.
25. The method of claim 24, wherein the modified GDF11 polypeptide comprises a modification selected from the group consisting of fusion to an Fc fragment, pegylation, conjugation to albumin, an amino acid mutation that prevents or reduces proteolytic degradation, an amino acid mutation that prolongs half-life, and any combination thereof.

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26. The method of any one of claims 1 to 25, wherein the composition is administered via a route selected from the group consisting of intravenously, subcutaneously, intra-arterially, and intra-muscularly.
27. The method of any one of claims 1 to 26, wherein the level of GDF11 polypeptide is increased by at least 100%.
28. The method of any one of claims 1 to 27, wherein the level of GDF11 polypeptide is increased to at least 75% of a healthy reference level.
29. A pharmaceutical composition comprising a GDF11 polypeptide or a functional fragment or variant thereof, and a pharmaceutically acceptable carrier.
30. The use of a composition comprising a GDF11 polypeptide or functional fragment or variant thereof for increasing neurogenesis in a subject in need thereof, wherein increased levels of the GDF11 polypeptide or functional fragment or variant thereof in the subject increase neurogenesis in the subject.
31. The use of a composition comprising a GDF11 polypeptide or functional fragment or variant thereof for treating or preventing a neurodegenerative disorder by increasing neurogenesis in a subject in need thereof, wherein increased levels of the GDF11 polypeptide or functional fragment or variant thereof in the subject increase neurogenesis in the subject, thereby treating or preventing the neurodegenerative disorder in the subject.
32. The use of claims 30 or 31, wherein increasing neurogenesis is associated with increased neural cell proliferation, increased neural cell differentiation, increased number or proliferation rate of neural stem cells, increased number or proliferation rate of neural progenitor cells, increased number or proliferation rate of neural precursor cells, increased expression of at least one

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synaptic plasticity gene, at least one neuroprotective gene, or at least one neuronal specification gene, or increased number of new neurons.

33. The use of any one of claims 30 to 32, wherein neurogenesis is increased in the subventricular zone of the lateral ventricles.
34. The use of any one of claims 30 to 33, wherein neurogenesis is increased in the olfactory bulb.
35. The use of claim 34, wherein increased neurogenesis results in improved olfactory behavior.
36. The use of any one of claims 30 to 35, wherein the neurodegenerative disorder is selected from the group consisting of Huntington's disease, dentatorubropallidoluysian atrophy, Kennedy's disease, spinocerebellar ataxia, fragile X syndrome, fragile XE mental retardation, Friedreich's ataxia, myotonic dystrophy, spinocerebellar ataxia type 8, and spinocerebellar ataxia type 12, Alexander disease, Alper's disease, Alzheimer disease, amyotrophic lateral sclerosis (ALS), ataxia telangiectasia, Batten disease, Canavan disease, Cockayne syndrome, corticobasal degeneration, Creutzfeldt-Jakob disease, Guillain-Barré syndrome, ischemia stroke, Krabbe disease, kuru, Lewy body dementia, multiple sclerosis, multiple system atrophy, non-Huntingtonian type of Chorea, Parkinson's disease, Pelizaeus-Merzbacher disease, Pick's disease, primary lateral sclerosis, progressive supranuclear palsy, Refsum's disease, Sandhoff disease, Schilder's disease, spinal cord injury, spinal muscular atrophy (SMA), SteeleRichardson-Olszewski disease, and Tabes dorsalis.
37. The use of any one of claims 30 to 36, wherein the subject has been diagnosed with a neurodegenerative disorder due to aging.
38. The use of a composition comprising a GDF11 polypeptide or functional fragment or variant thereof for increasing angiogenesis in a subject in need

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thereof, wherein increased levels of the GDF11 polypeptide or functional fragment or variant thereof in the subject increase angiogenesis in the subject.

39. The use of a composition comprising a GDF11 polypeptide or functional fragment or variant thereof for treating or preventing a neurovascular disorder by increasing angiogenesis in a subject, wherein increased levels of the GDF11 polypeptide or functional fragment or variant thereof in the subject increase angiogenesis in the subject, thereby treating or preventing the neurovascular disorder in the subject.
40. The use of claims 38 or 39, wherein the neurovascular disorder is selected from the group consisting of brain atherothrombosis, brain aneurysms, brain arteriovenous malformations, brain embolism, brain ischemia, for example caused by atherothrombosis, embolism, or hemodynamic abnormalities, cardiac arrest, carotid stenosis, cerebrovascular spasm, headache, intracranial hemorrhage, ischemic stroke, seizure, spinal vascular malformations, reflex neurovascular dystrophy (RND), neurovascular compression disorders such as hemifacial spasms, tinnitus, trigeminal neuralgia, glossopharyngeal neuralgia, stroke, transient ischemic attacks, and vasculitis.
41. The use of any one of claims 38 to 40, wherein the subject has been diagnosed with a neurovascular disorder due to aging.
42. The use of any one of claims 30 to 41, wherein the composition increases the level of GDF11 polypeptide in at least one of the subject's systemic circulation, cerebral vasculature, cerebrospinal fluid, cerebral tissue, lateral ventricles, neurovasculature, and subventricular zone neurovascular niche.
43. The use of any one of claims 30 to 42, wherein the composition comprises an isolated or recombinant GDF11 polypeptide.
44. The use of any one of claims 30 to 43, wherein the isolated or recombinant GDF11 polypeptide comprises a modification selected from the group

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consisting of fusion to an Fc fragment, pegylation, conjugation to albumin, an amino acid mutation that prevents or reduces proteolytic degradation, an amino acid mutation that prolongs half-life, and any combination thereof.

45. The use of any one of claims 30 to 44, wherein the GDF11 polypeptide comprises the amino acid sequence of SEQ ID NO: 3.
46. The use of any one of claims 30 to 45, wherein the GDF11 polypeptide comprises the amino acid sequence of SEQ ID NO: 2.
47. The use of any one of claims 30 to 46, wherein the GDF11 polypeptide comprises the amino acid sequence of SEQ ID NO: 1.
48. The use of any one of claims 30 to 47, wherein the GDF11 polypeptide comprises the amino acid sequence of SEQ ID NO: 4.
49. The use of any one of claims 30 to 48, wherein the composition comprises homodimers of GDF11 polypeptides comprising the amino acid sequence of any of SEQ ID NOs: 1, 2, 3 or 4.
50. The use of any one of claims 30 to 49, wherein the composition comprises complexes of GDF11 polypeptides comprising the amino acid sequence of any of SEQ ID NOs: 1, 2, 3 or 4.
51. The use of any one of claims 30 to 50, wherein the composition comprises a nucleic acid encoding the GDF11 polypeptide or functional fragment or variant thereof.
52. The use of any one of claims 30 to 51 wherein the composition is administered via a route selected from the group consisting of intravenously, subcutaneously, intra-arterially, and intra-muscularly.

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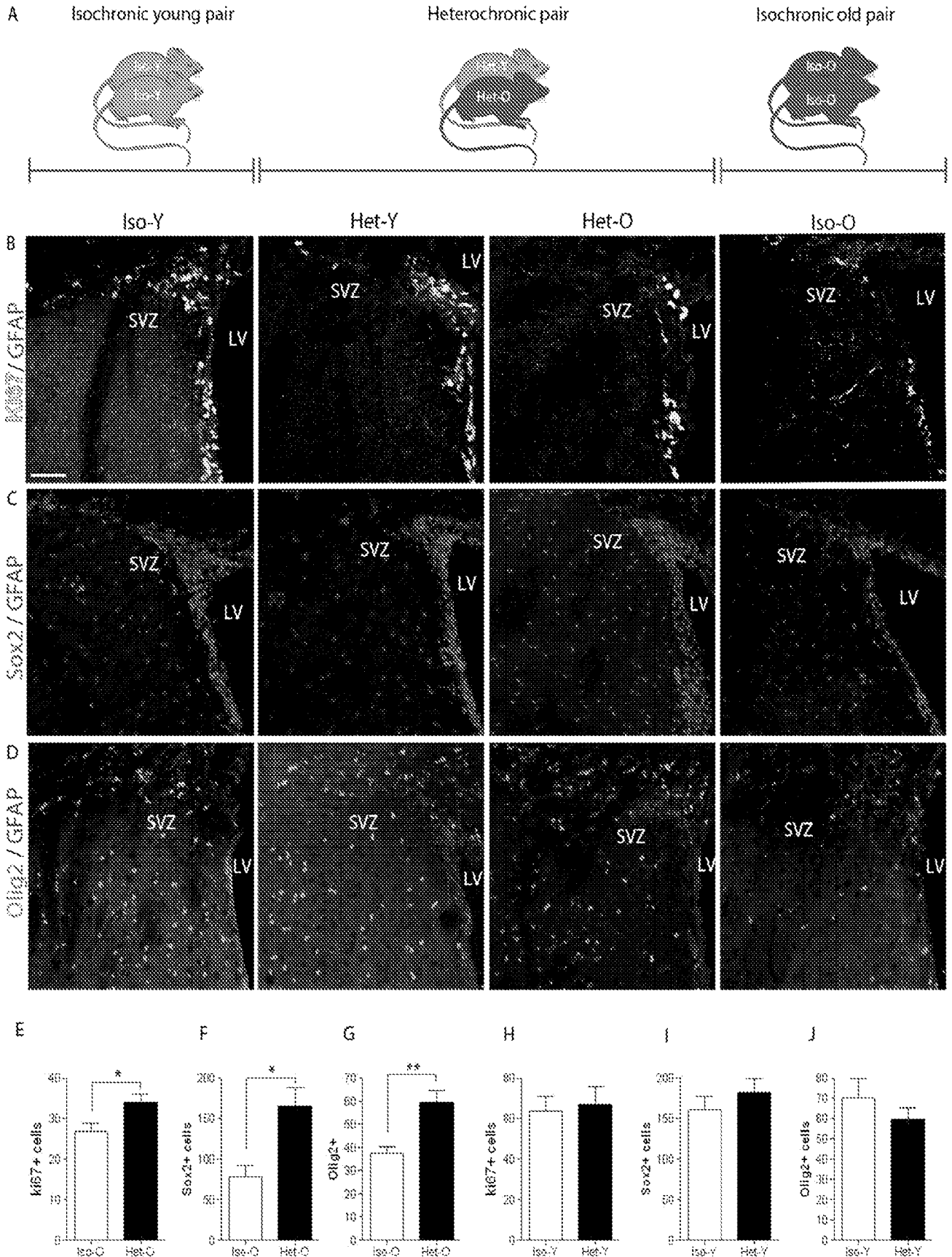
53. The use of any one of claims 30 to 52, wherein the composition increases the level of the GDF11 polypeptide by at least 100%.
54. The use of any one of claims 30-50, wherein the composition increases the level of GDF11 polypeptide to at least 75% of a healthy reference level.
55. The method of claim 3, wherein the at least one synaptic plasticity gene, the at least one neuroprotective gene, and/or the at least one neuronal specification gene is selected from the group consisting of neuronal PAS domain protein 4 (Npas4), dual specificity phosphatase 1 (Dusp1), heat shock 70kDa protein 8 (Hspa8), BTG family, member 2 (Btg2), activity-regulated cytoskeleton-associated protein (Arc), nuclear receptor subfamily 4, group A, member 1 (Nr4a1), cysteine-rich, angiogenic inducer, 61 (Cyr61), FBJ murine osteosarcoma viral oncogene homolog (Fos), jun B proto-oncogene (Junb), Kruppel-like factor 10 (Klf10), nuclear receptor subfamily 4, group A, member 2 (Nr4a2), claudin 1 (Cldn1), early growth response 2 (Egr2), crystalline, mu (Crym), early growth response 1 (Egr1), and polio-like kinase 2 (Plk2).
56. The use of claim 32, wherein the at least one synaptic plasticity gene, the at least one neuroprotective gene, and/or the at least one neuronal specification gene is selected from the group consisting of neuronal PAS domain protein 4 (Npas4), dual specificity phosphatase 1 (Dusp1), heat shock 70kDa protein 8 (Hspa8), BTG family, member 2 (Btg2), activity-regulated cytoskeleton-associated protein (Arc), nuclear receptor subfamily 4, group A, member 1 (Nr4a1), cysteine-rich, angiogenic inducer, 61 (Cyr61), FBJ murine osteosarcoma viral oncogene homolog (Fos), jun B proto-oncogene (Junb), Kruppel-like factor 10 (Klf10), nuclear receptor subfamily 4, group A, member 2 (Nr4a2), claudin 1 (Cldn1), early growth response 2 (Egr2), crystalline, mu (Crym), early growth response 1 (Egr1), and polio-like kinase 2 (Plk2).

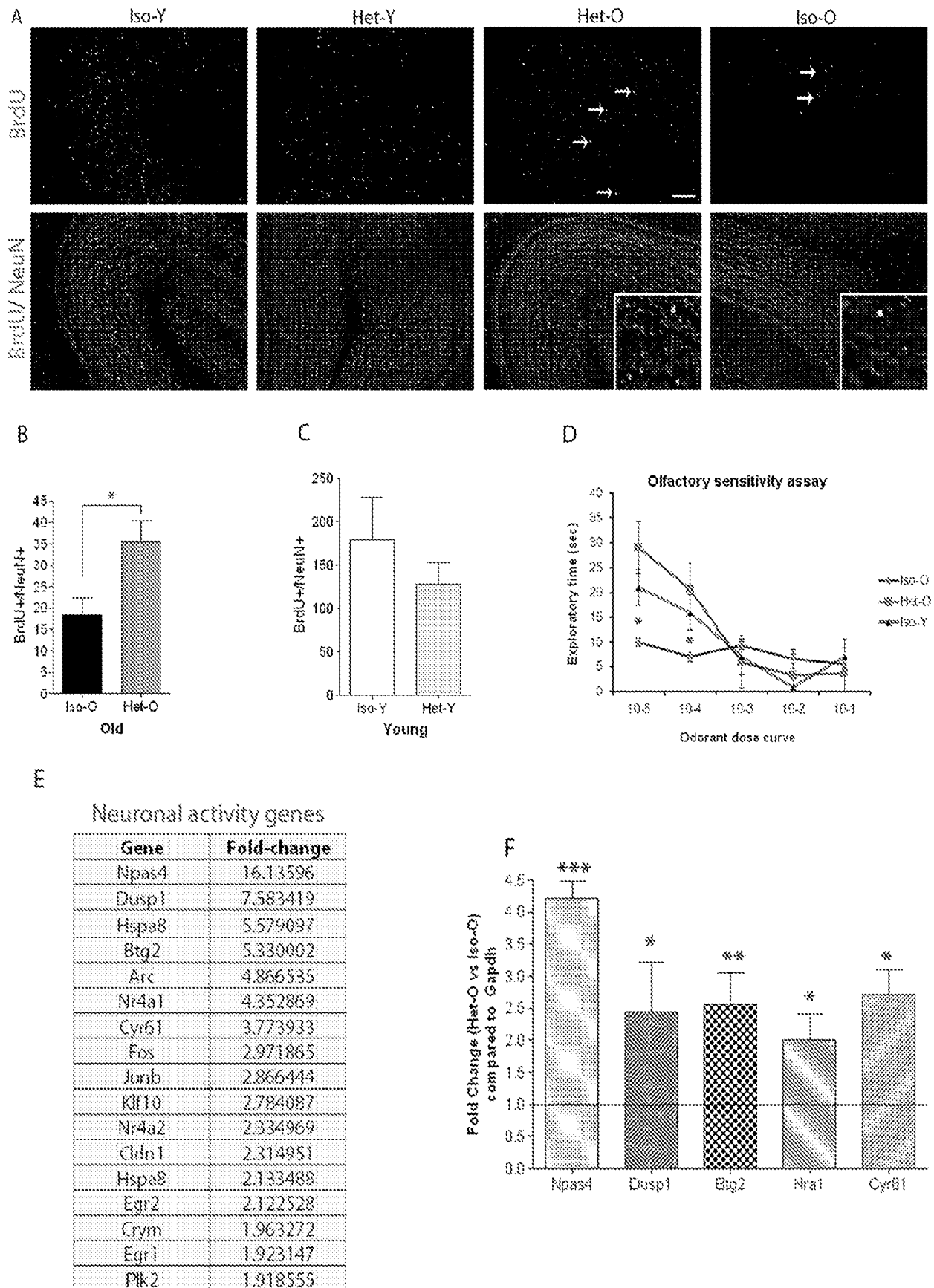
-83-

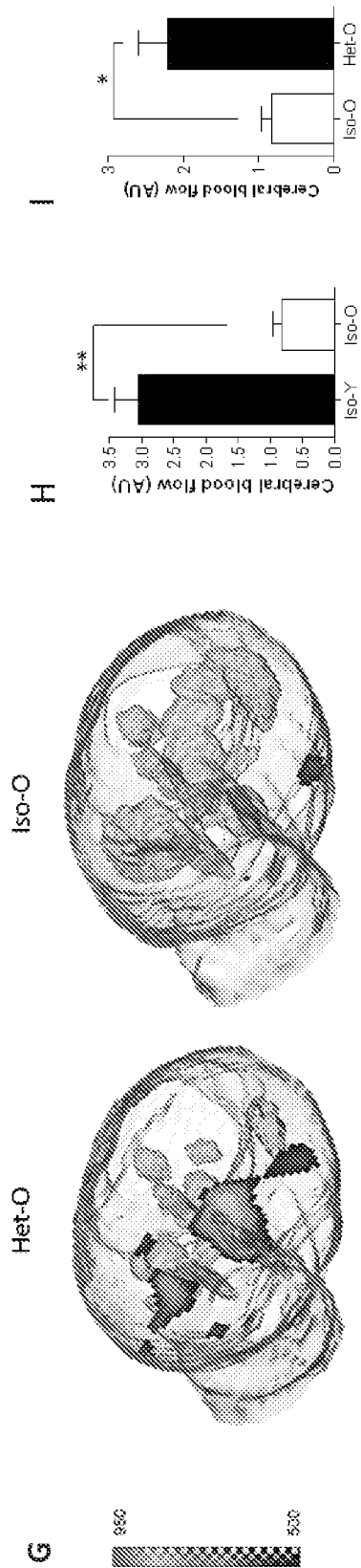
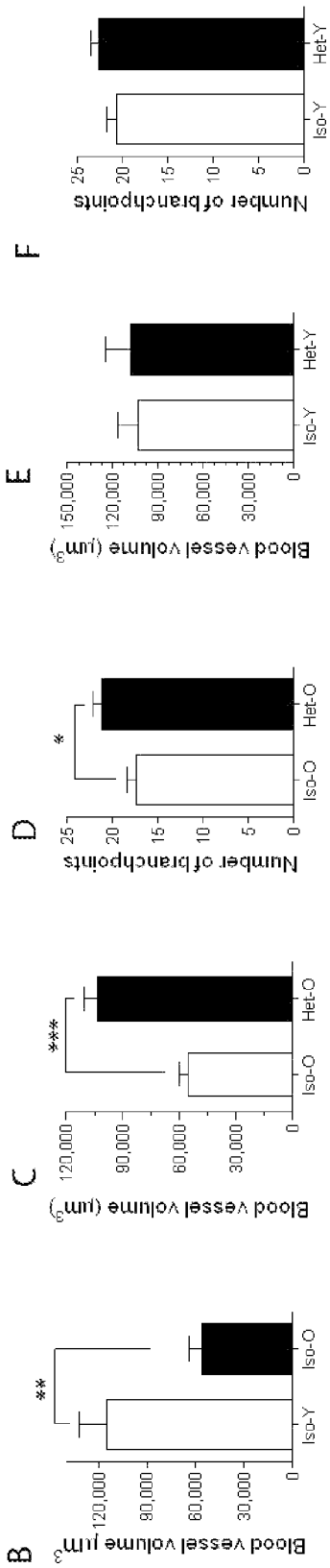
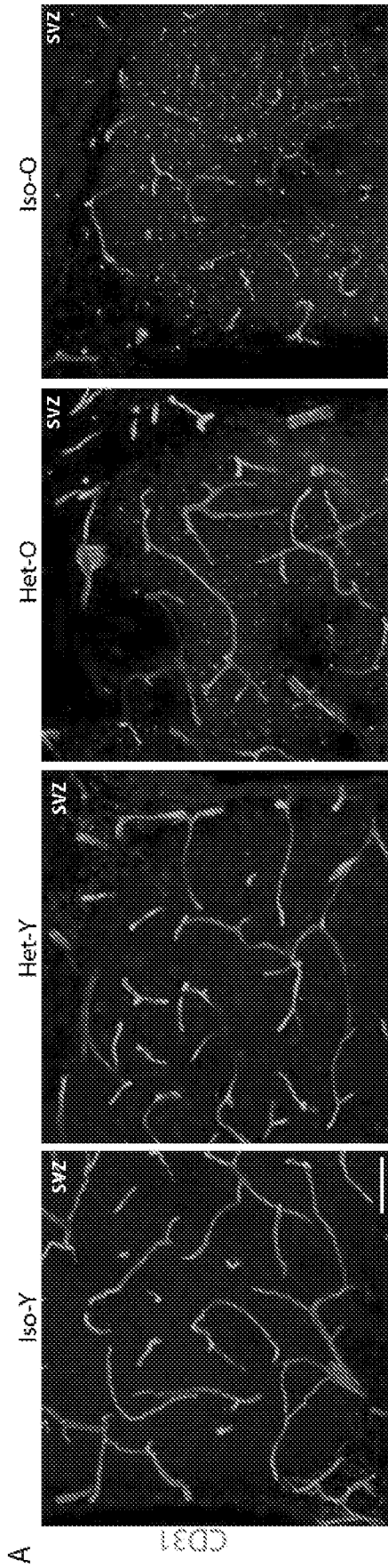
57. The method of any one of claims 1, 2, 9 and 10 wherein the GDF11 polypeptide binds to an ALK4/5 receptor.
58. The method of claim 57, wherein the modulation of the ALK4/5 receptor causes phosphorylation of SMAD2/3 proteins.
59. The method of claim 57, wherein the modulation of the ALK4/5 receptor increases SMAD phosphorylation.
60. The method of claims 1-2, wherein the GDF11 polypeptide increases neurogenesis through the SMAD signaling cascade.
61. The method of claims 9-10, wherein the GDF11 polypeptide increases angiogenesis through the SMAD signaling cascade.
62. The composition of claims 29, wherein the GDF11 polypeptide or functional fragment or variant thereof binds to an ALK4/5 receptor.
63. The composition of claim 62, wherein the modulation of the ALK4/5 receptor causes phosphorylation of SMAD2/3 proteins.
64. The composition of claim 62, wherein the modulation of the ALK4/5 receptor increases SMAD phosphorylation.
65. The use of any one of claims 38-39 wherein the GDF11 polypeptide or functional fragment or variant thereof binds to an ALK4/5 receptor.
66. The use of claim 65, wherein the modulation of the ALK4/5 receptor causes phosphorylation of SMAD2/3 proteins.
67. The use of claim 65, wherein the modulation of the ALK4/5 receptor increases SMAD phosphorylation.

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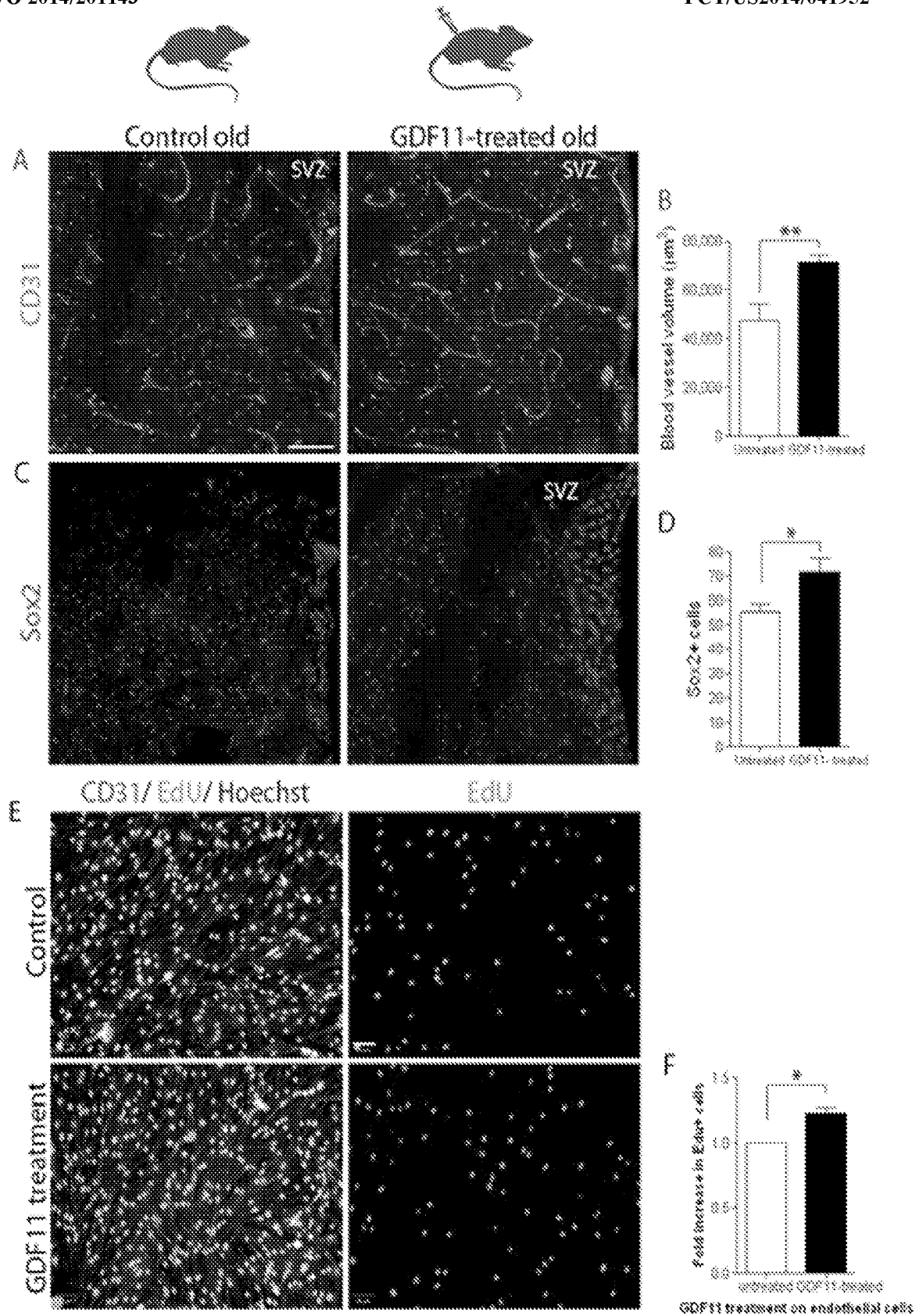
68. The use of claims 38-39, wherein the GDF11 polypeptide or functional fragment or variant thereof increases angiogenesis through the SMAD signaling cascade.

*Figs. 1A – 1J*

**Figs. 2A – 2F**



Figs. 3A – 3I



Figs. 4A-4F

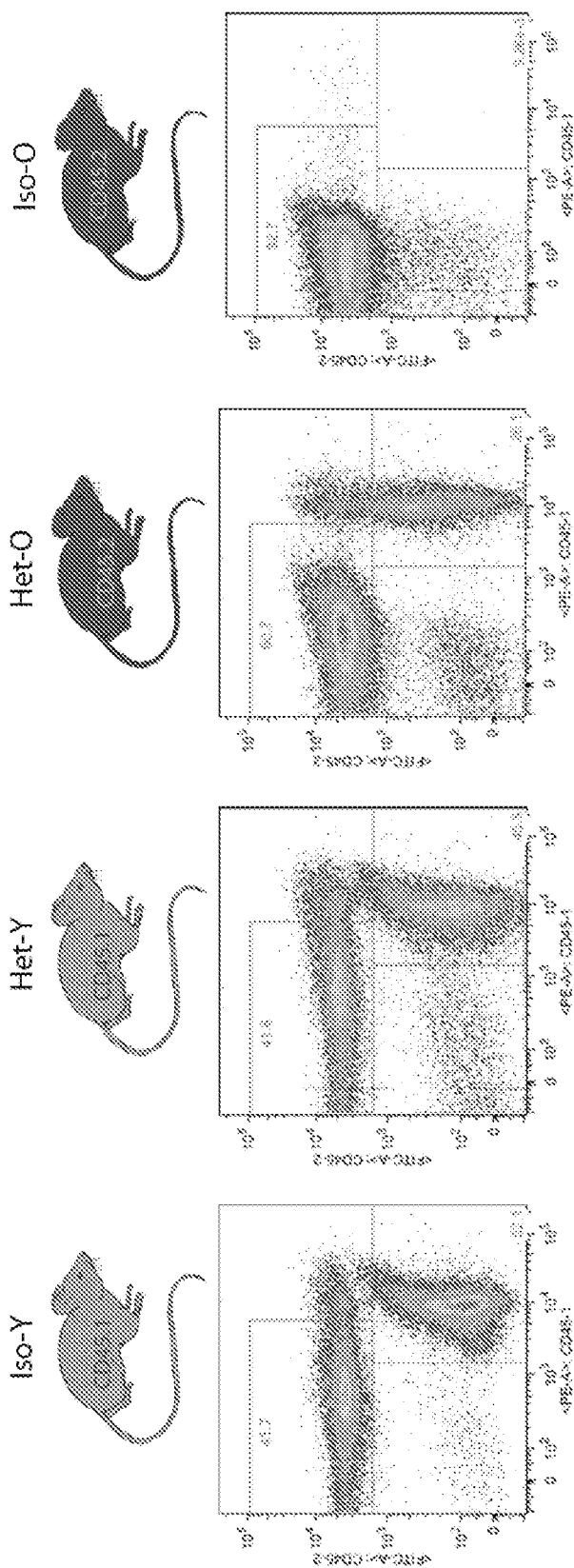


Fig. 5

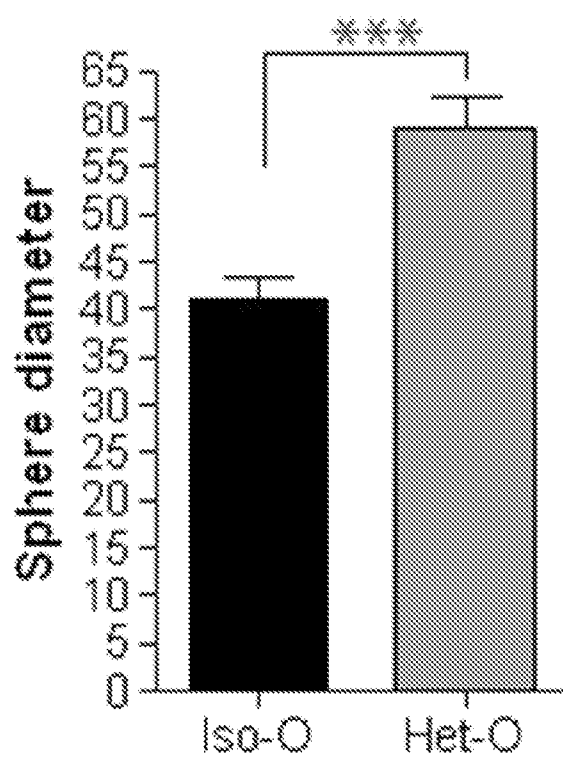
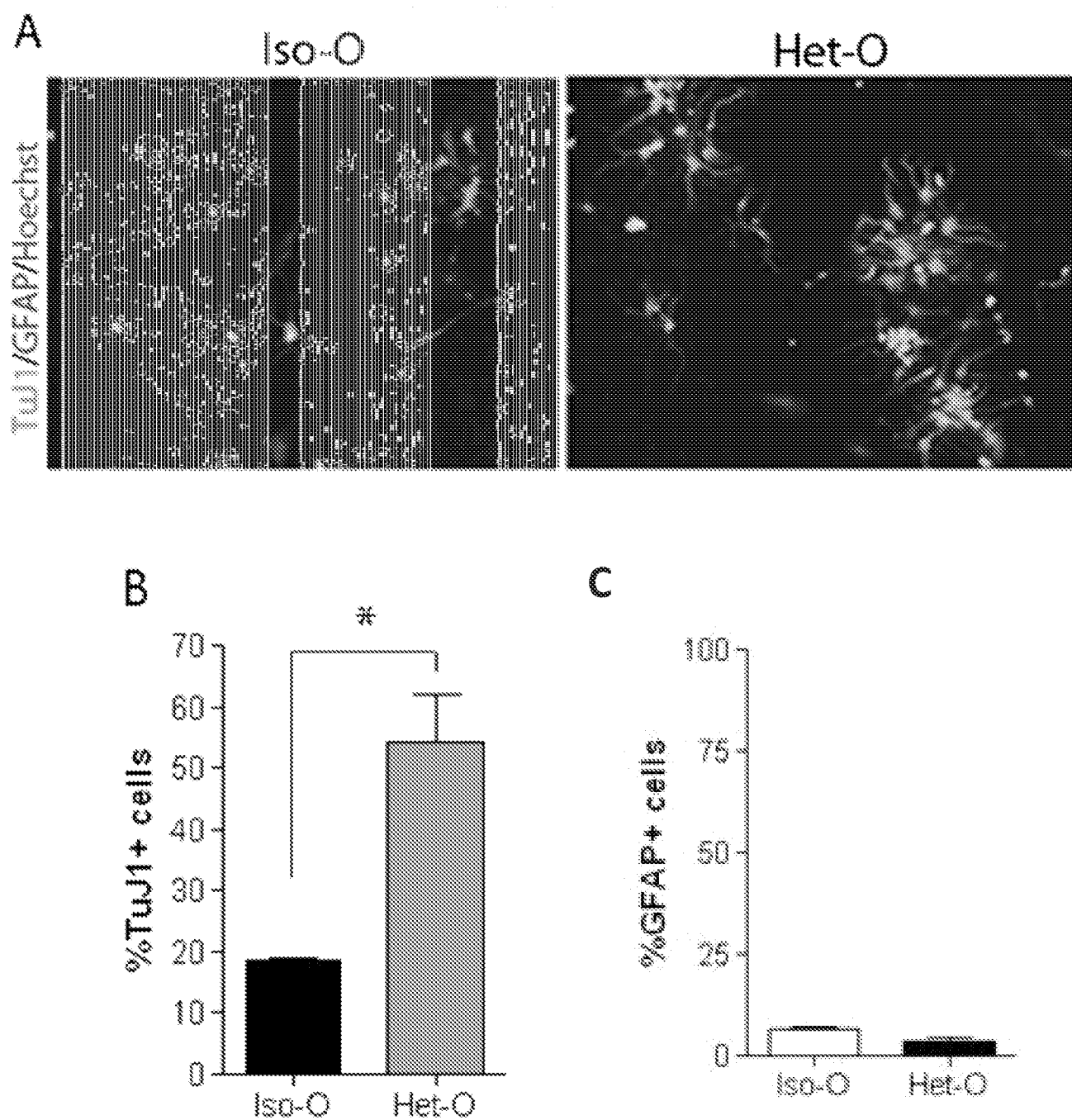


Fig. 6



Figs. 7A – 7C

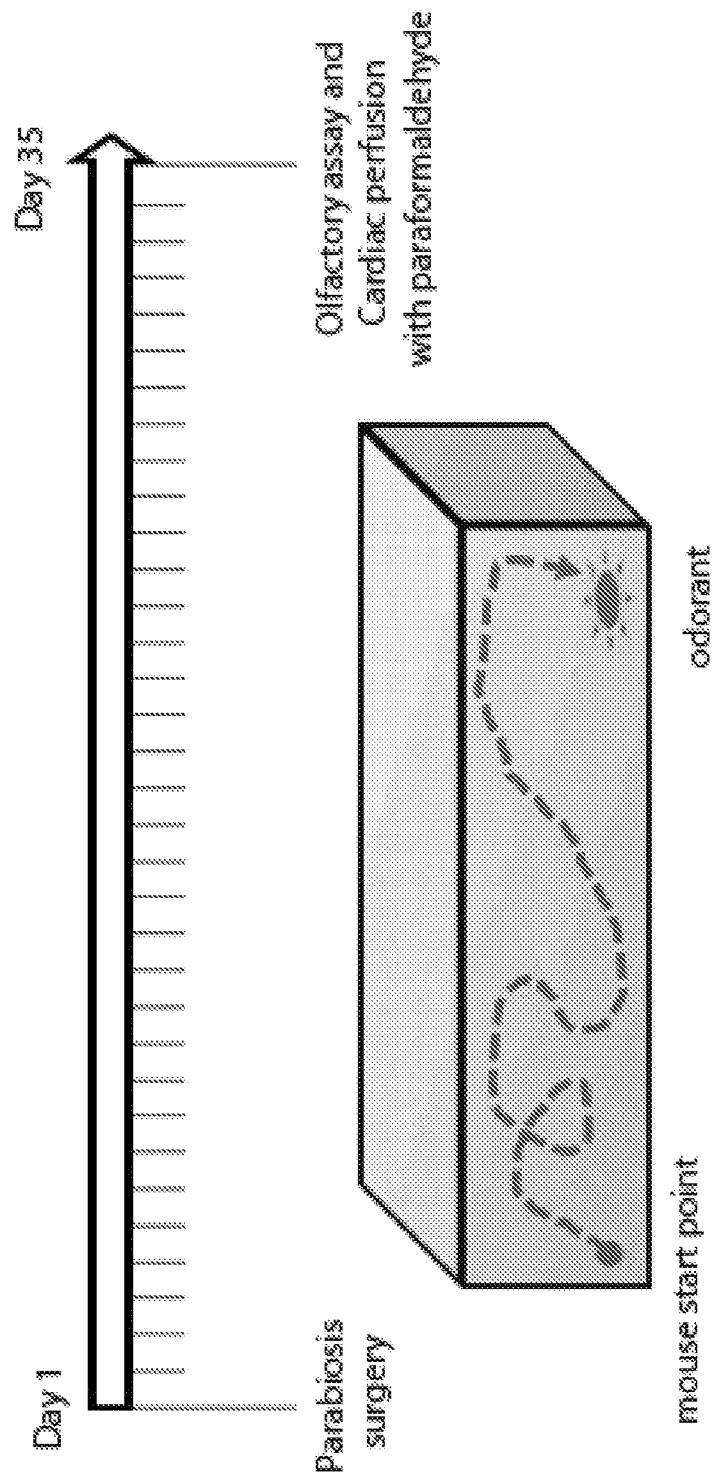
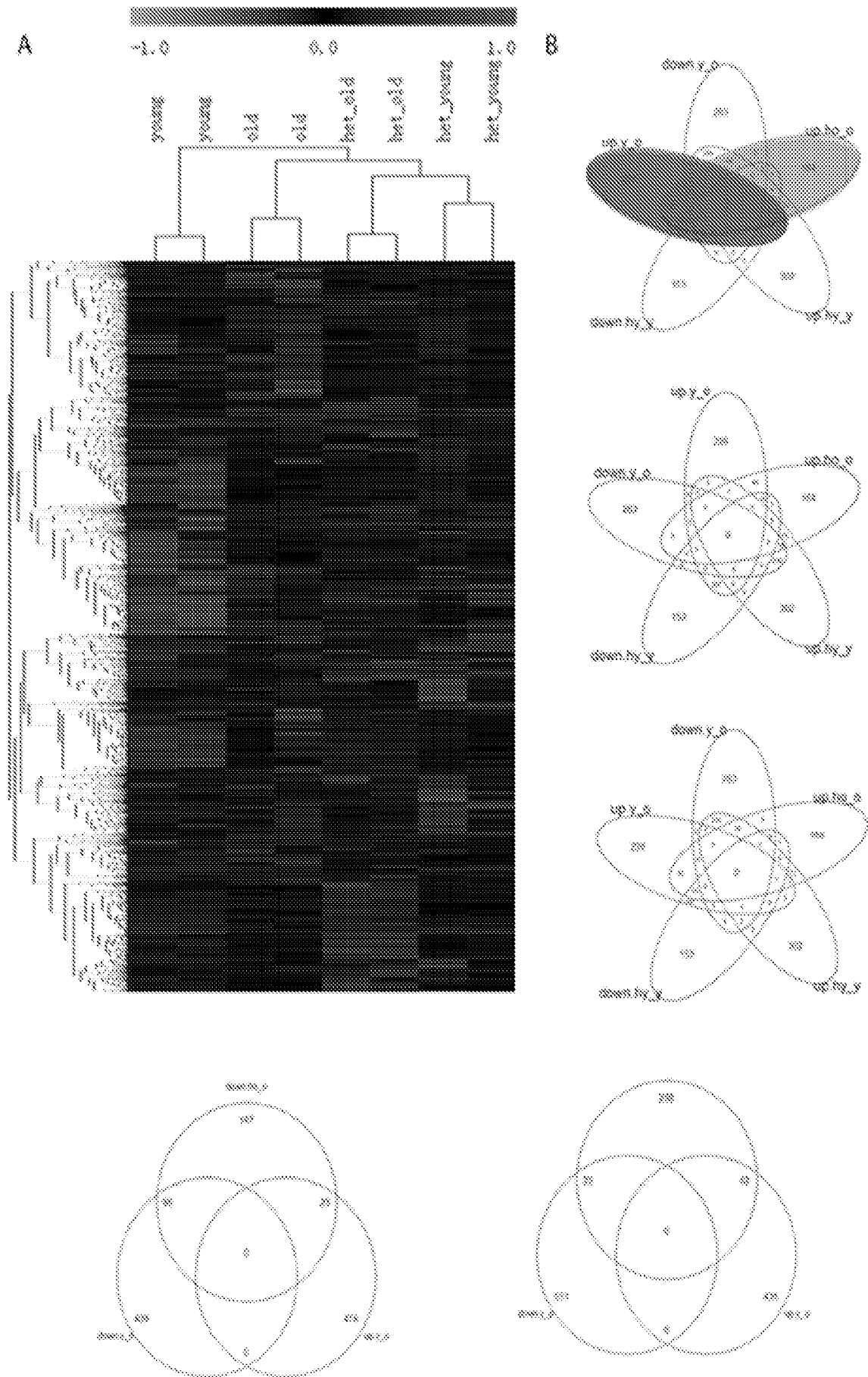
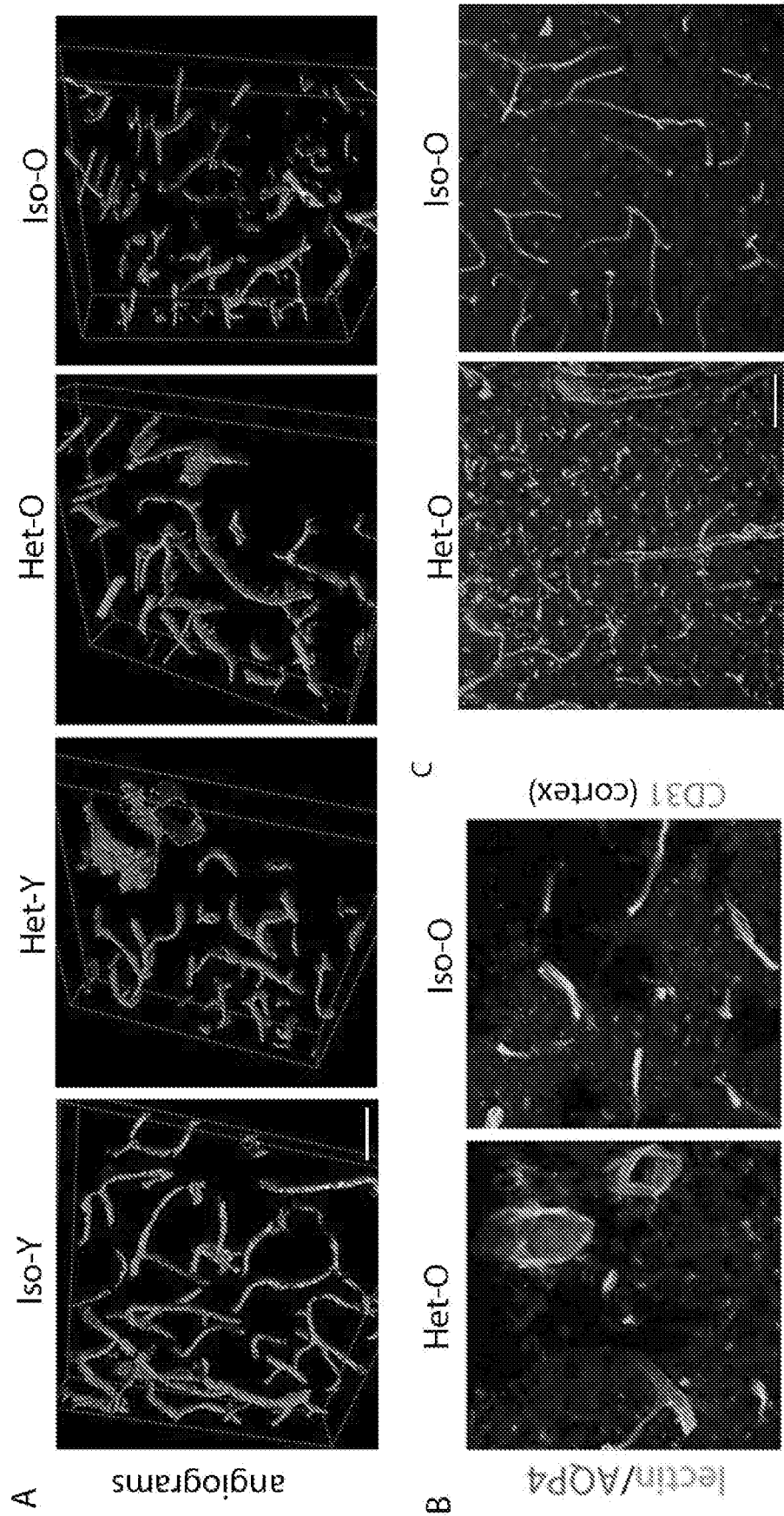


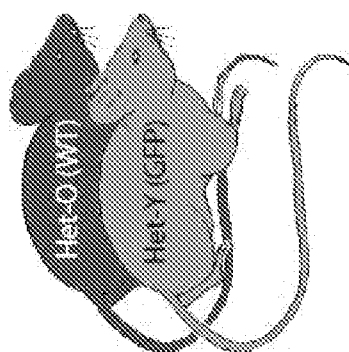
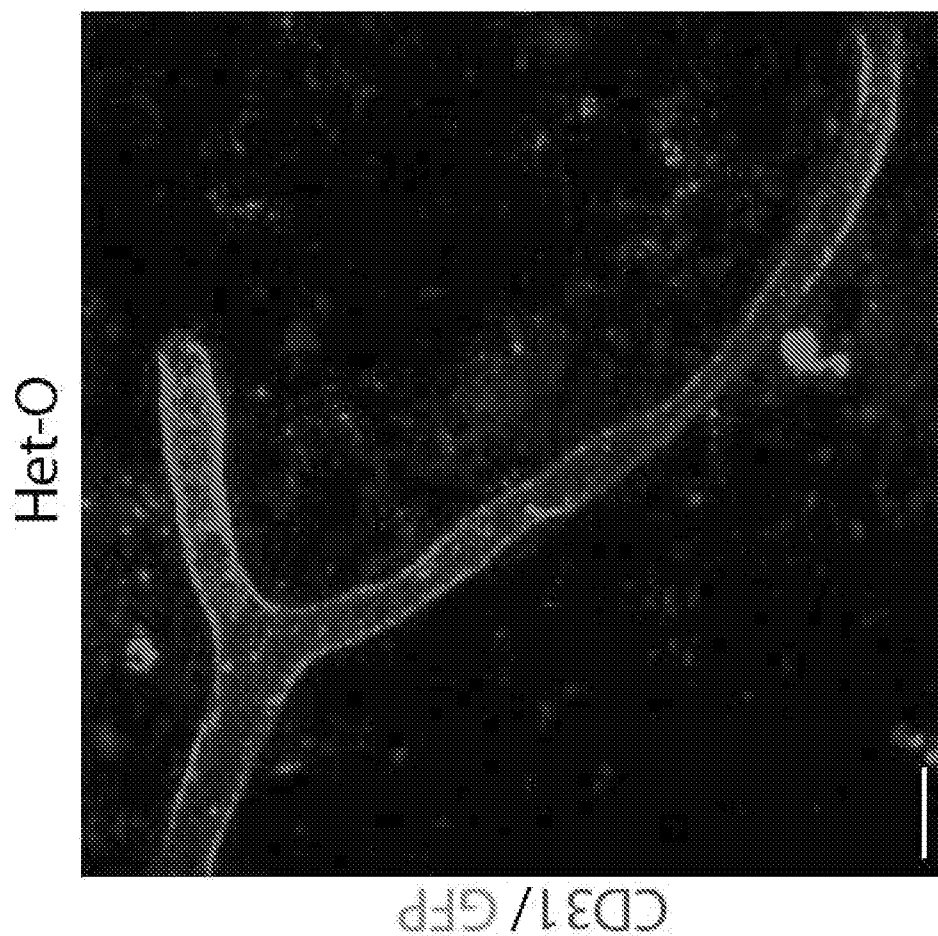
Fig. 8

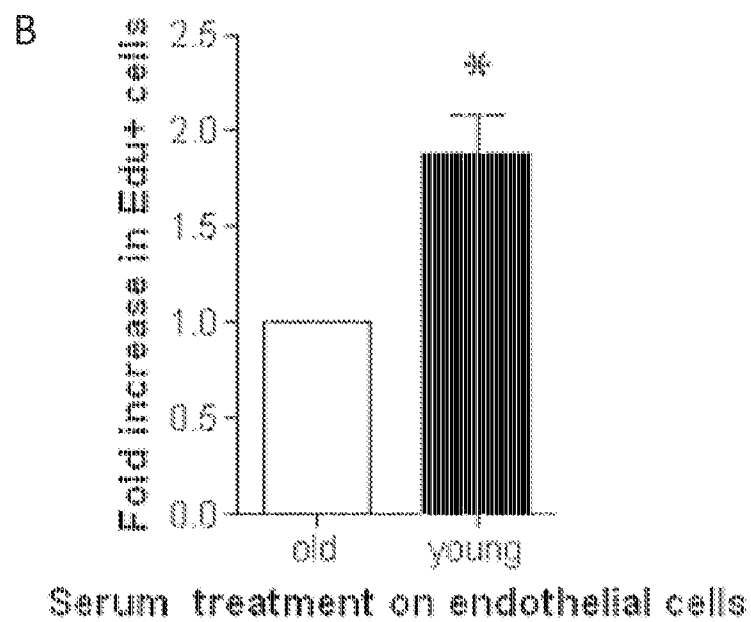
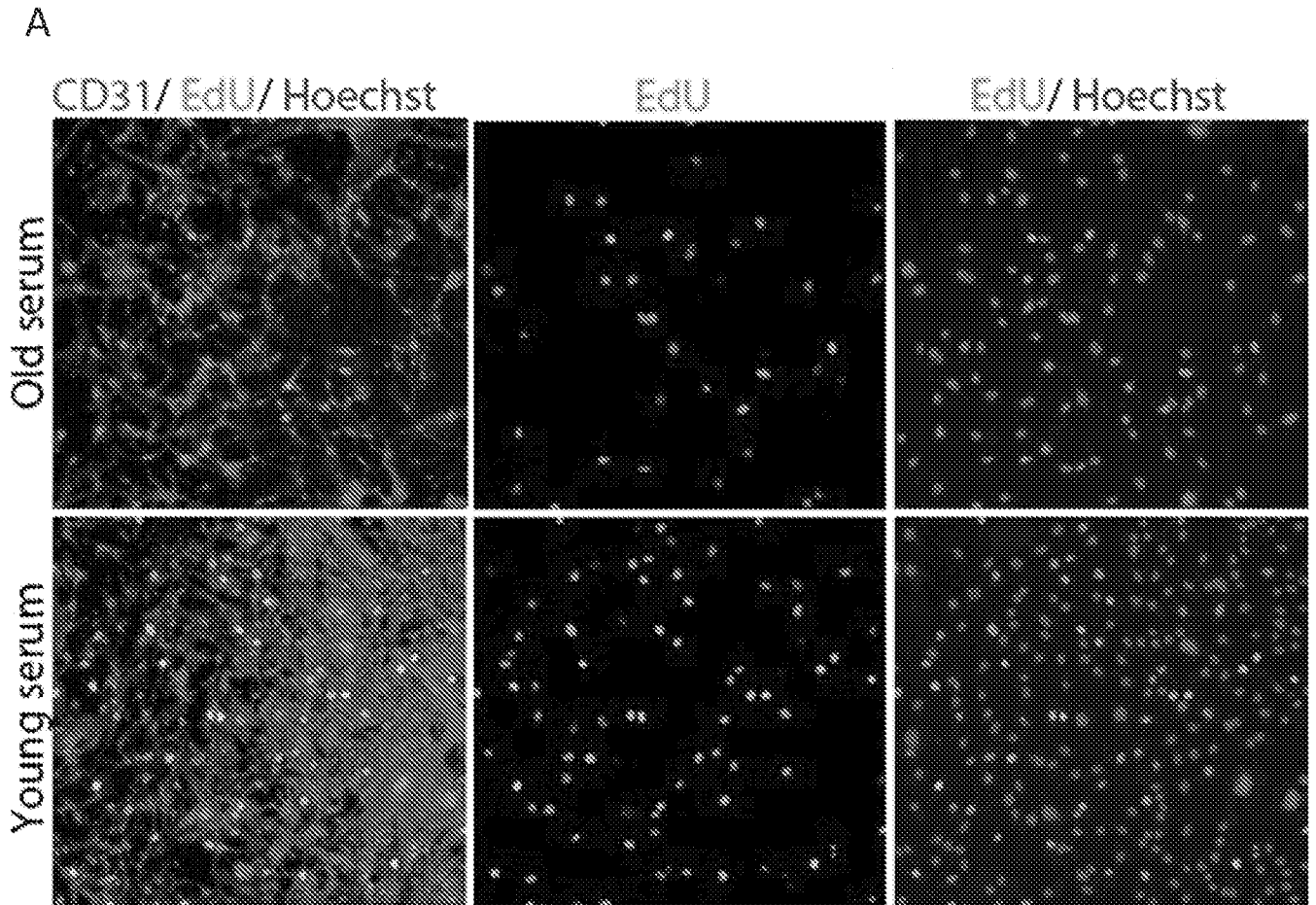


Figs. 9A-9B



Figs. 10A – 10C





Figs. 12A – 12B

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Sbjct 39 CNACTWRQNIKSSRIEAINIQILSKLRLLEIAPNISQDVIRQLLPKAPPLRELIDQYDVQR 98

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Fig. 13

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Query 47 RSRPAPSVAPEDGCPVCVMRCHSKELRLLESIKSQILSKLRLEAPNISREVVVKQLLPK 106
      RSRPAPSVAPEDGCPVCVMRCHSKELRLLESIKSQILSKLRLEAPNISREVVVKQLLPK
Sbjct 45 RSRPAPSVAPEDGCPVCVMRCHSKELRLLESIKSQILSKLRLEAPNISREVVVKQLLPK 104

Query 107 APPLQQILDLDLDFQSDALQPEDFLEEDETHATITETVISMAGETDPAVQIDCSFLCCHFF 166
      APPLQQILDLDLDFQSDALQPEDFLEEDETHATITETVISMAGETDPAVQIDCSFLCCHFF
Sbjct 105 APPLQQILDLDLDFQSDALQPEDFLEEDETHATITETVISMAGETDPAVQIDCSFLCCHFF 164

Query 167 SPKVMFTKVLKAQLWVYLRPVPEPATVYVQLRLRLPLTCEGTACGGGSGRRHIRIRSLKI 226
      SPKVMFTKVLKAQLWVYLRPVPEPATVYVQLRLRLPLTCEGTACGGGSGRRHIRIRSLKI
Sbjct 165 SPKVMFTKVLKAQLWVYLRPVPEPATVYVQLRLRLPLTCEGTACGGGSGRRHIRIRSLKI 224

Query 227 ELHSRSCHHQSIDFRQVVLHSDFERQPSNUGIEINAFDPSCGIDLAVTSLGPGAEGLHPPFME 286
      ELHSRSCHHQSIDFRQVVLHSDFERQPSNUGIEINAFDPSCGIDLAVTSLGPGAEGLHPPFME
Sbjct 225 ELHSRSCHHQSIDFRQVVLHSDFERQPSNUGIEINAFDPSCGIDLAVTSLGPGAEGLHPPFME 284

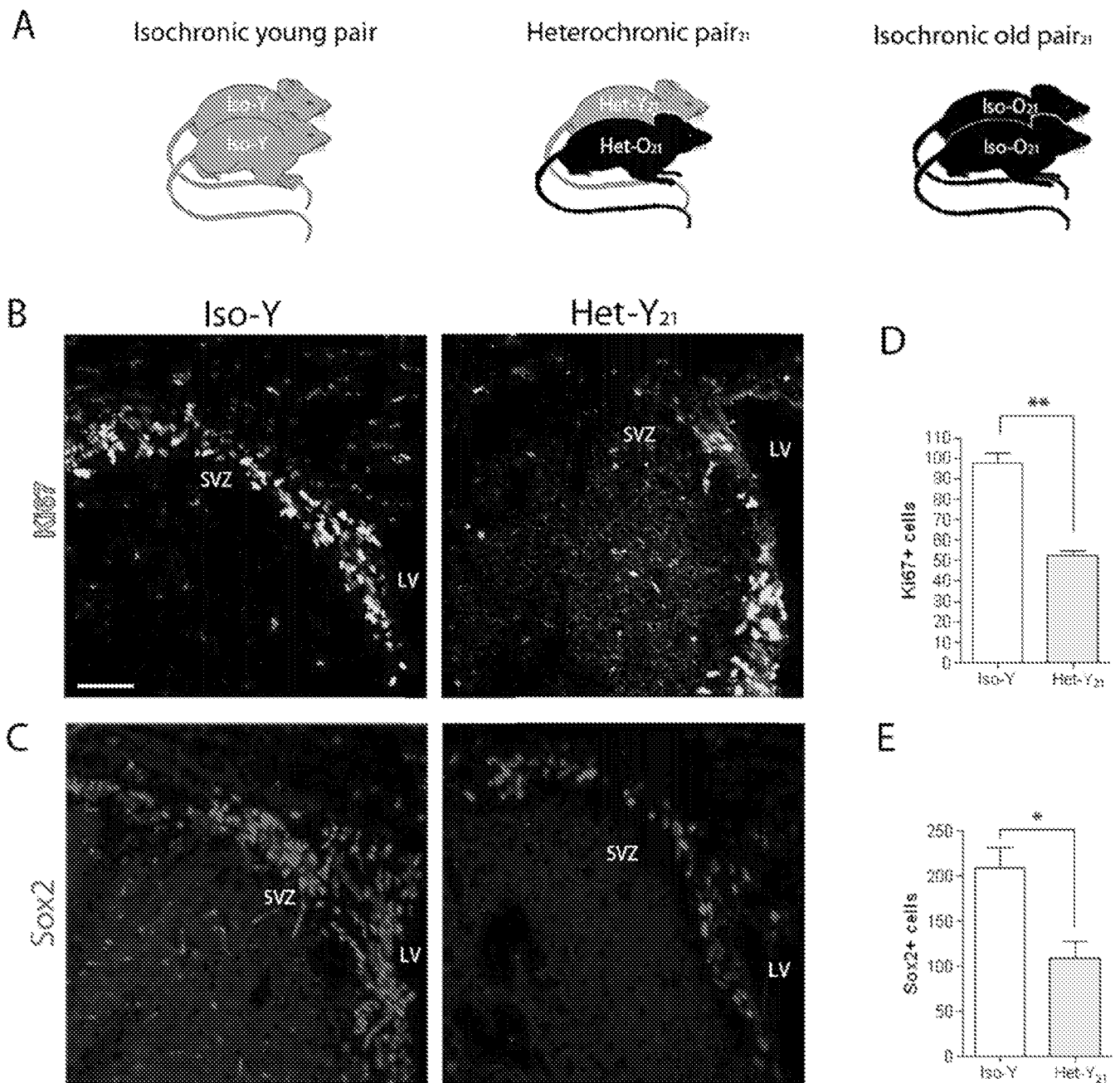
Query 287 LRVLENTKRSRRNLGLDCCDEHSSSESRCCKRYPLTVDFEAFGWDWIIAPKRYKANVYCSQCE 346
      LRVLENTKRSRRNLGLDCCDEHSSSESRCCKRYPLTVDFEAFGWDWIIAPKRYKANVYCSQCE
Sbjct 285 LRVLENTKRSRRNLGLDCCDEHSSSESRCCKRYPLTVDFEAFGWDWIIAPKRYKANVYCSQCE 344

Query 347 YNFMQKYPHITHLVQQANPRGSAGPCCPTTKNSPINMLYFNDKQQLIYCKIPGMVYDRCGC 405
      YNFMQKYPHITHLVQQANPRGSAGPCCPTTKNSPINMLYFNDKQQLIYCKIPGMVYDRCGC
Sbjct 345 YNFMQKYPHITHLVQQANPRGSAGPCCPTTKNSPINMLYFNDKQQLIYCKIPGMVYDRCGC 404

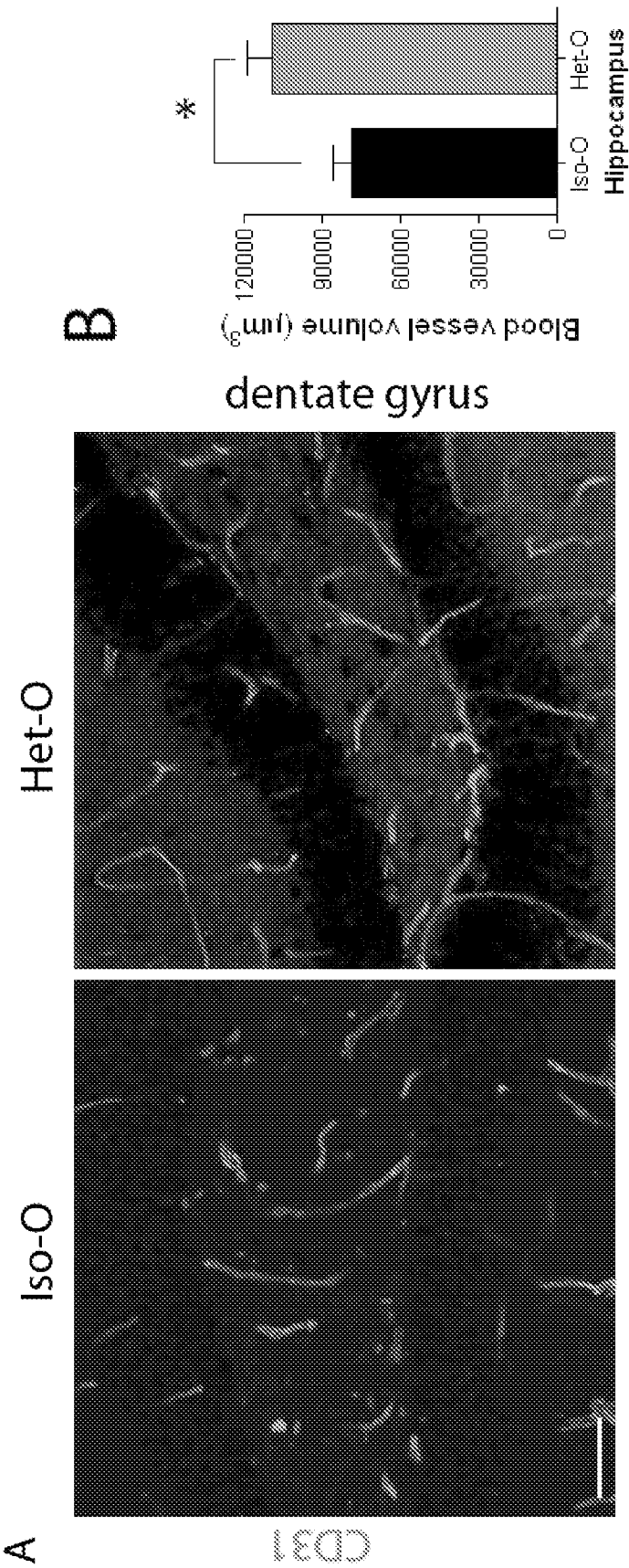
Query 407 S 407
Sbjct 405 S 405

```

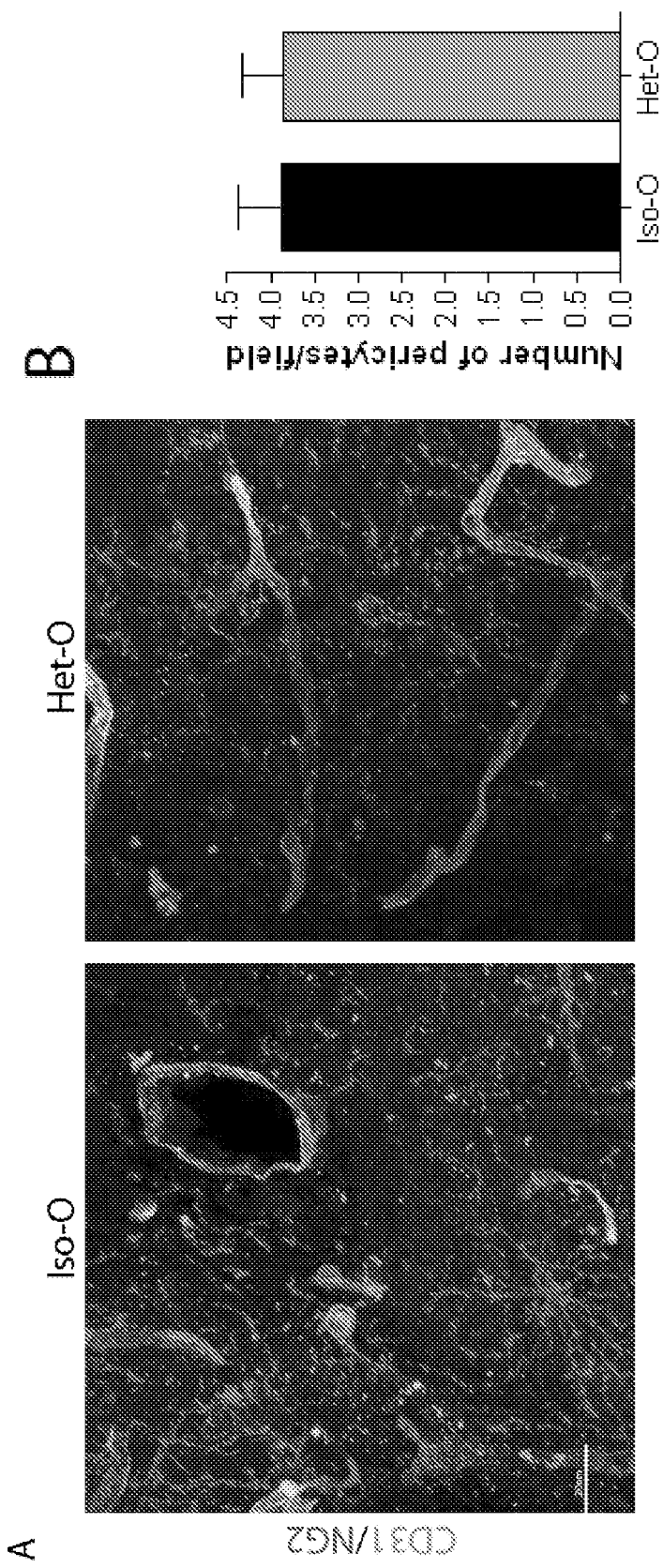
Fig. 14



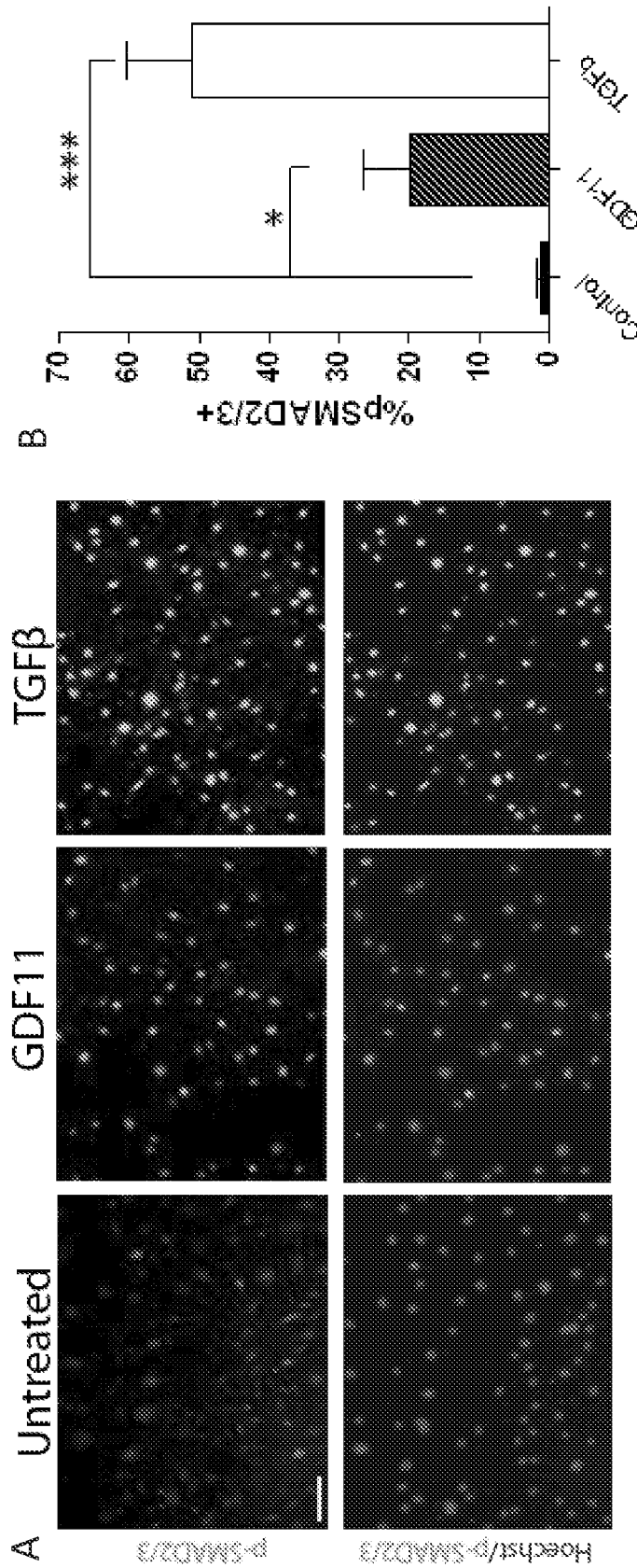
Figs. 15A – 15E



Figs. 16A – 16B



Figs. 17A – 17B



Figs. 18A – 18B

SEQ ID NO: 1 (Fig. 19)

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Met Val Leu Ala Ala Pro Leu Leu Leu Gly Phe Leu Leu Leu Ala Leu
1      5      10      15
Glu Leu Arg Pro Arg Gly Glu Ala Ala Glu Gly Pro Ala Ala Ala
20      25      30
Ala Ala Ala Ala Ala Ala Ala Ala Gly Val Gly Gly Glu Arg Ser
35      40      45
Ser Arg Pro Ala Pro Ser Val Ala Pro Glu Pro Asp Gly Cys Pro Val
50      55      60
Cys Val Trp Arg Gln His Ser Arg Glu Leu Arg Leu Glu Ser Ile Lys
65      70      75      80
Ser Gln Ile Leu Ser Lys Leu Arg Leu Lys Glu Ala Pro Asn Ile Ser
85      90      95
Arg Glu Val Val Lys Gln Leu Leu Pro Lys Ala Pro Pro Leu Gln Gln
100     105     110
Ile Leu Asp Leu His Asp Phe Gln Gly Asp Ala Leu Gln Pro Glu Asp
115     120     125
Phe Leu Glu Glu Asp Glu Tyr His Ala Thr Thr Glu Thr Val Ile Ser
130     135     140
Met Ala Gln Glu Thr Asp Pro Ala Val Gln Thr Asp Gly Ser Pro Leu
145     150     155     160
Cys Cys His Phe His Phe Ser Pro Lys Val Met Phe Thr Lys Val Leu
165     170     175
Lys Ala Gln Leu Trp Val Tyr Leu Arg Pro Val Pro Arg Pro Ala Thr
180     185     190
Val Tyr Leu Gln Ile Leu Arg Leu Lys Pro Leu Thr Gly Glu Gly Thr
195     200     205
Ala Gly Gly Gly Gly Gly Gly Arg Arg His Ile Arg Ile Arg Ser Leu
210     215     220
Lys Ile Glu Leu His Ser Arg Ser Gly His Trp Gln Ser Ile Asp Phe
225     230     235     240
Lys Gln Val Leu His Ser Trp Phe Arg Gln Pro Gln Ser Asn Trp Gly
245     250     255
Ile Glu Ile Asn Ala Phe Asp Pro Ser Gly Thr Asp Leu Ala Val Thr
260     265     270
Ser Leu Gly Pro Gly Ala Glu Gly Leu His Pro Phe Met Glu Leu Arg
275     280     285
Val Leu Glu Asn Thr Lys Arg Ser Arg Arg Asn Leu Gly Leu Asp Cys
290     295     300
Asp Glu His Ser Ser Glu Ser Arg Cys Cys Arg Tyr Pro Leu Thr Val
305     310     315     320
Asp Phe Glu Ala Phe Gly Trp Asp Trp Ile Ile Ala Pro Lys Arg Tyr
325     330     335
Lys Ala Asn Tyr Cys Ser Gly Gln Cys Glu Tyr Met Phe Met Gln Lys
340     345     350
Tyr Pro His Thr His Leu Val Gln Gln Ala Asn Pro Arg Gly Ser Ala
355     360     365
Gly Pro Cys Cys Thr Pro Thr Lys Met Ser Pro Ile Asn Met Leu Tyr
370     375     380
Phe Asn Asp Lys Gln Gln Ile Ile Tyr Gly Lys Ile Pro Gly Met Val
385     390     395     400
Val Asp Arg Cys Gly Cys Ser
405

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FIG. 19

SEQ ID NO: 2 (Fig. 20)

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Ala Glu Gly Pro Ala Ala Ala Ala Ala Ala Ala Ala Ala Ala Ala
1      5      10      15
Ala Gly Val Gly Gly Glu Arg Ser Ser Arg Pro Ala Pro Ser Val Ala
20      25      30
Pro Glu Pro Asp Gly Cys Pro Val Cys Val Trp Arg Gln His Ser Arg
35      40      45
Glu Leu Arg Leu Glu Ser Ile Lys Ser Gln Ile Leu Ser Lys Leu Arg
50      55      60
Leu Lys Glu Ala Pro Asn Ile Ser Arg Glu Val Val Lys Gln Leu Leu
65      70      75      80
Pro Lys Ala Pro Pro Leu Gln Gln Ile Leu Asp Leu His Asp Phe Gln
85      90      95
Gly Asp Ala Leu Gln Pro Glu Asp Phe Leu Glu Glu Asp Glu Tyr His
100     105     110
Ala Thr Thr Glu Thr Val Ile Ser Met Ala Gln Glu Thr Asp Pro Ala
115     120     125
Val Gln Thr Asp Gly Ser Pro Leu Cys Cys His Phe His Phe Ser Pro
130     135     140
Lys Val Met Phe Thr Lys Val Leu Lys Ala Gln Leu Trp Val Tyr Leu
145     150     155     160
Arg Pro Val Pro Arg Pro Ala Thr Val Tyr Leu Gln Ile Leu Arg Leu
165     170     175
Lys Pro Leu Thr Gly Glu Gly Thr Ala Gly Gly Gly Gly Gly Gly Arg
180     185     190
Arg His Ile Arg Ile Arg Ser Leu Lys Ile Glu Leu His Ser Arg Ser
195     200     205
Gly His Trp Gln Ser Ile Asp Phe Lys Gln Val Leu His Ser Trp Phe
210     215     220
Arg Gln Pro Gln Ser Asn Trp Gly Ile Glu Ile Asn Ala Phe Asp Pro
225     230     235     240
Ser Gly Thr Asp Leu Ala Val Thr Ser Leu Gly Pro Gly Ala Glu Gly
245     250     255
Leu His Pro Phe Met Glu Leu Arg Val Leu Glu Asn Thr Lys Arg Ser
260     265     270
Arg Arg Asn Leu Gly Leu Asp Cys Asp Glu His Ser Ser Glu Ser Arg
275     280     285
Cys Cys Arg Tyr Pro Leu Thr Val Asp Phe Glu Ala Phe Gly Trp Asp
290     295     300
Trp Ile Ile Ala Pro Lys Arg Tyr Lys Ala Asn Tyr Cys Ser Gly Gln
305     310     315     320
Cys Glu Tyr Met Phe Met Gln Lys Tyr Pro His Thr His Leu Val Gln
325     330     335
Gln Ala Asn Pro Arg Gly Ser Ala Gly Pro Cys Cys Thr Pro Thr Lys
340     345     350
Met Ser Pro Ile Asn Met Leu Tyr Phe Asn Asp Lys Gln Gln Ile Ile
355     360     365
Tyr Gly Lys Ile Pro Gly Met Val Val Asp Arg Cys Gly Cys Ser
370     375     380

```

FIG. 20

SEQ ID NO: 3 (Fig. 21)

Asn	Leu	Gly	Leu	Asp	Cys	Asp	Glu	His	Ser	Ser	Glu	Ser	Arg	Cys	Cys
1			5						10					15	
Arg	Tyr	Pro	Leu	Thr	Val	Asp	Phe	Glu	Ala	Phe	Gly	Trp	Asp	Trp	Ile
			20					25					30		
Ile	Ala	Pro	Lys	Arg	Tyr	Lys	Ala	Asn	Tyr	Cys	Ser	Gly	Gln	Cys	Glu
		35					40					45			
Tyr	Met	Phe	Met	Gln	Lys	Tyr	Pro	His	Thr	His	Leu	Val	Gln	Gln	Ala
	50					55					60				
Asn	Pro	Arg	Gly	Ser	Ala	Gly	Pro	Cys	Cys	Thr	Pro	Thr	Lys	Met	Ser
65					70					75				80	
Pro	Ile	Asn	Met	Leu	Tyr	Phe	Asn	Asp	Lys	Gln	Gln	Ile	Ile	Tyr	Gly
			85					90					95		
Lys	Ile	Pro	Gly	Met	Val	Val	Asp	Arg	Cys	Gly	Cys	Ser			
			100				105								

FIG. 21

SEQ ID NO: 4 (Fig. 22)

Ala Glu Gly Pro Ala Ala Ala Ala Ala Ala Ala Ala Ala Ala Ala
 1 5 10 15
 Ala Gly Val Gly Gly Glu Arg Ser Ser Arg Pro Ala Pro Ser Val Ala
 20 25 30
 Pro Glu Pro Asp Gly Cys Pro Val Cys Val Trp Arg Gln His Ser Arg
 35 40 45
 Glu Leu Arg Leu Glu Ser Ile Lys Ser Gln Ile Leu Ser Lys Leu Arg
 50 55 60
 Leu Lys Glu Ala Pro Asn Ile Ser Arg Glu Val Val Lys Gln Leu Leu
 65 70 75 80
 Pro Lys Ala Pro Pro Leu Gln Gln Ile Leu Asp Leu His Asp Phe Gln
 85 90 95
 Gly Asp Ala Leu Gln Pro Glu Asp Phe Leu Glu Glu Asp Glu Tyr His
 100 105 110
 Ala Thr Thr Glu Thr Val Ile Ser Met Ala Gln Glu Thr Asp Pro Ala
 115 120 125
 Val Gln Thr Asp Gly Ser Pro Leu Cys Cys His Phe His Phe Ser Pro
 130 135 140
 Lys Val Met Phe Thr Lys Val Leu Lys Ala Gln Leu Trp Val Tyr Leu
 145 150 155 160
 Arg Pro Val Pro Arg Pro Ala Thr Val Tyr Leu Gln Ile Leu Arg Leu
 165 170 175
 Lys Pro Leu Thr Gly Glu Gly Thr Ala Gly Gly Gly Gly Gly Gly Arg
 180 185 190
 Arg His Ile Arg Ile Arg Ser Leu Lys Ile Glu Leu His Ser Arg Ser
 195 200 205
 Gly His Trp Gln Ser Ile Asp Phe Lys Gln Val Leu His Ser Trp Phe
 210 215 220
 Arg Gln Pro Gln Ser Asn Trp Gly Ile Glu Ile Asn Ala Phe Asp Pro
 225 230 235 240
 Ser Gly Thr Asp Leu Ala Val Thr Ser Leu Gly Pro Gly Ala Glu Gly
 245 250 255
 Leu His Pro Phe Met Glu Leu Arg Val Leu Glu Asn Thr Lys Arg Ser
 260 265 270
 Arg Arg

FIG. 22

SEQ ID NO: 5 (Fig. 23)

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1      tccccgcccc ccagtcctcc ctccccctccc ctccagcatg gtgctcgccg ccccgctgct
61     gctgggcttc ctgctcctcg ccctggagct ggggccccgg ggggagggcg ccgagggccc
121    cgcgggcgcg gggcgggcgg cgggcgggcg ggcagcgggc ggggtcgggg gggagcgctc
181    cagccggcca gccccgtccg tggcgcccg gcccgaacggc tgccccgtgt gcgtttggcg
241    gcagcacagc cgcgagctgc gcctagagag catcaagtcg cagatcttga gcaaaactgcg
301    gctcaaggag ggcgccaaca tcagccgcga ggtgggtgaag cagctgctgc ccaaggcgcc
361    gccgctgcag cagatcctgg acctacacga ctccagggc gacgcgctgc agcccagga
421    cttcctggag gaggacgagt accacgccac caccgagacc gtcattagca tggcccagga
481    gacggacca gacgtacaga cagatggcag ccctctctgc tgccattttc acttcagccc
541    caaggtgatg ttcacaaagg tactgaaggc ccagctgtgg gtgtacctac ggctgtacc
601    cgcgccagcc acagtctacc tgcagatctt gcgactaaaa ccctaactg gggaagggac
661    cgcaggggga gggggcgag gccggcgta catccgtatc cgctcaactga agattgagct
721    gcactcacgc tcaggccatt ggcagagcat cgacttcaag caagtgcctac acagctgggt
781    cgcgcagcca cagagcaact ggggcatcga gatcaacgcc tttgatccca gtggcacaga
841    cctggctgtc acctccctgg ggcggggagc cgaggggctg catccattca tggagcttcg
901    agtcctagag aacacaaaac gttcccggcg gaacctgggt ctggactgcg acgagcactc
961    aagcgagtc cgtgctgccc gatatccct cacagtggac tttgaggctt tcggctggga
1021   ctggatcatc gcacctaagc gctacaaggc caactactgc tccggccagt gcgagtacat
1081   gttcatgcaa aaatatccgc ataccattt ggtgcagcag gccaatccaa gagctctgc
1141   tgggcccgtg tgtaccccca ccaagatgtc cccaatcaac atgctctact tcaatgacaa
1201   gcagcagatt atctacggca agatccctgg catgggtggtg gatcgctgtg gctgctctta
1261   aggtggggga tagaggatgc ctccccaca gacctacc caagacctc agccctgcc
1321   ccaccccccc aagccctaga gctccctcca ctcttccgc gaacatcaca ccgttccccg
1381   accaagccgt gtgcaataca acagagggag gcaggtggga attgaggggt aggggtttgg
1441   gggaaagggg aagcaggggc atagtcaggg tggggagtggt ttgaagtttg cagatgagaa
1501   ggtttgacaa aaagacagag agatgtagag acagtgatag agacagagga acaaaaagag
1561   cagcagtgag aaggcaaaag gagaggcaga agagacagac gaggcagaga caaaacactg
1621   agaaagagac tgaatggag taataaatga aagccccaca ccaagcctcc tttcttccac
1681   tggcaagggt aggggcttg tatagtttg ggagatcccc tgactattca gtaggagaag
1741   aaatcaaaaa tccattcttt tctcttctc tccctccaac agtggccagg ggaaggggaa
1801   gtgagggcag gggcaaaaag atttggaat ttttatttat ttatttattg tgaactttca
1861   tttttttggg atttggcttt actggaatag gagggccct gccactgtg cccggtttat
1921   ccttatttcc ccaaaccctg ctctcccaa cactactca cttaagcact tgtataaagc
1981   ctccaggggt ggggaatggg gtaaagggca agagggcgga cacatgaagt ttagtttcta
2041   accatcatc accctaactc aaccttttct gagccaaatg gottgaattg aagccagttg
2101   tcatggaaat agtaagaggt tagggtttaa gagctgggga tgcgggggtg ggagagagaa
2161   cctcaacat ccaggatcta tataatgaga gctactttaa accctcaggt ccacctcat
2221   gatgctgagt tatttagcca gaggggtcag cctgcttatg cccaaattcc ctacgccaag
2281   agagagacca aagagcctct ggaatggccc tgcctccagc ctctatcttc aggtcaatta
2341   gagagagtat agagacccca ggtccccctg ggtctggaaa gcgttaggag aggtcaagaa
2401   aggagcagta aggaggtga aggttacagg gcatttgaat ccaaatcact gctctgggct
2461   agggaataga gccagcagac caaggtggga aggattctgg aagggggaca ttttagtctc
2521   ctaaccccaa agctcagggg ggaagagggg agaacaagga agcagagtggt ataattattt

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FIG. 23

2581 tttccttttta tttttggaat ctaacagtac ctggcagcag ggaggggaaa gtacagtggg
2641 gaaaagcatc tgacaaggcc agttagaaca gaggatggga aggatggaga ctccggggct
2701 tggaaggcta ggaagcaggc agagactggg tggcatttca agtcaactagc taggcccatt
2761 cattcctccc acaaccctga cccattctcc totggactca ctgtgcctca gtttcttccc
2821 ctcaatggaa tgagaaatga cagcaccgc cacagccaag agatgaattc tgagcactta
2881 ccacgggcac tttatggaca taaaatacct ctgctgtgg gacagataac cagggcacca
2941 gagtagtggg gaagagatgt gaggcttaag aggagtcaca ggcttcagag tacaagttcc
3001 cctctgcctc ccagctggac agtgcttaga agccaaggag ttgagaatct cctgatccac
3061 accctatcct tacttcacca ccaggcctct tggctccagg caagagctta gaggatgtca
3121 ggagaggtgg gggtaagaat cttcagcaaa actgtcactc taagtagagc cagcagttac
3181 gggctgata aaaacagtac tgaactaaag taaagcccaa gctgggtgac aaaactggat
3241 ggctcattct tcccagagc atgactctcc cccttgcca gttggtggaa ggggcaagg
3301 tatgtgacca cccttgagaa ggtgatgttg gtgagcttta acatcttatt cctattctta
3361 tagtgagaaa gtgaaacaag atctttcagt agaggaatgg gcagggctgt taggctcttc
3421 agcttgctt caccatata gcagctatgc taacccaag cctctctggc cctgttcttc
3481 atccttcctt ctgccccaat cctgaaggac aagacacacc cgccatcaa caccactcac
3541 atttccttgg tggagggaaa ggaacagaga agtgaagaac agatacctcc ctccaaggtc
3601 aaatgcctcg tgatcttggc agagtaggga ttgggcaata agcatcaggt atcttccctc
3661 tacagattct agagagctgg ggcattaaat atgggggaca cttagaatac agctccttaa
3721 ataccaccaa ataaagacct ttgtgtgtgt gtggtgggtg gggggggggc aggggtcttt
3781 ctcttatgaa cataaatctg tgagctgaag tctcattccc ctgttccctc ctaccccaa
3841 agaggcacag agtgaaggga cttggggggc acagctcagc aaccagtggt gaggtagcac
3901 cccctccac cttatgatgt gtgtggacct ggccagtgc cctctgaaca tatcattatt
3961 agtgtaatta tcatttatct tgtgtatttg tcacattgtg tgcattgacag cctttgttaa
4021 ggggtgtctga ggagtatgga gctgacagg gcattggaat gccaggaaa aactcttca
4081 actgagatca aggttctctg gaggaacca ctgcaaaaag gccatcaggc agttttcaag
4141 ttatgtgaca gagggcaaag acggccatag ggtgctctga gttttgggt ggtcacatga
4201 cacaatccag cacttgaacc tgaaaaaaaa aataaaagcg gtcaaagagt ttagaattca

FIG. 23 cont.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US14/41952

A. CLASSIFICATION OF SUBJECT MATTER IPC(8) - A61K 38/18 (2014.01) CPC - A61K 38/18 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) IPC(8): A61K 38/18, 38/22, 38/1875, 48/00; A61P 27/02, 27/06 (2014.01) CPC: A61K 38/18, 38/22, 38/1875, 48/00; USPC: 435/37; 514/1.1, 8.1, 8.6, 8.8, 9.1 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) MicroPatent (US-Granted, US-Applications, EP-A, EP-B, WO, JP, DE-G, DE-A, DE-T, DE-U, GB-A, FR-A); ProQuest; IP.com; Google; Google Scholar; 'GDF11,' angiogenesis, neurogenesis, 'neurovascular disorder,' 'neurodegenerative disorder'		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 2009/0215671 A1 (CALOF, AL et al.) August 27, 2009; abstract; paragraphs [0007], [0028], [0030], [0033], [0034], [0038], [0041], [0043], [0050], [0055]	1-3, 29-32, 57-59, 62-64 ----- 55, 56, 65-67
X --- Y	US 2003/0104977 A1 (RIPAMONTI, U et al.) June 5, 2003; abstract; paragraphs [0034], [0040], [0097], [0106], [0120]	9-10, 38, 39 ----- 11, 40, 65-67
Y	WO 2012/135623 A1 (GIORDANO, A et al.) October 4, 2012; page 13; lines 30-32; page 15, lines 15-16; page 16, lines 3-5; page 16, lines 21-24	11, 40
Y	US 2011/0105395 A1 (FALLON, JH et al.) May 5, 2011; abstract; paragraph [0053]	55, 56
Y	US 2011/0027177 A1 (JACOBY, DB et al.) February 3, 2011; abstract; paragraphs [0023], [0231]	11
☐ Further documents are listed in the continuation of Box C. ☐		
* Special categories of cited documents: "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier application or patent but published on or after the international filing date "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) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "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 "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family		
Date of the actual completion of the international search 01 October 2014 (01.10.2014)		Date of mailing of the international search report 31 OCT 2014
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201		Authorized officer: Shane Thomas PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US14/41952

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☒ Claims Nos.: 4-8, 12-28, 33-37, 41-54, 60, 61, 68
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.