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(54) Title: METHODS AND RELATED ASPECTS OF TREATING AND DIAGNOSING PROTEINOPATHIES

(57) Abstract: Provided herein are methods of treating a proteinopathy in a subject that include administering an effective amount of a fibrinogen-like protein 1 (FGL1), or a functional portion or variant thereof, to the subject having the proteinopathy. The effective amount elevates a level of the FGL1, or the functional portion or variant thereof, in a brain of the subject sufficient to inhibit a binding interaction between a pathogenic protein associated with the proteinopathy and a lymphocyte-activation protein 3 (LAGS) present in the brain of the subject. Related methods, uses, pharmaceutical compositions, and kits are also provided.

METHODS AND RELATED ASPECTS OF TREATING AND DIAGNOSING PROTEINOPATHIES

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to, and the benefit of, U.S. Provisional Patent Application Ser. No. 63/511,727, filed July 3, 2023, the disclosure of which is incorporated herein by reference.

STATEMENT OF GOVERNMENT INTEREST

[0002] This invention was made with government support under AG071820 and CA273333, awarded by the National Institutes of Health. The government has certain rights in the invention.

BACKGROUND OF THE INVENTION

[0003] Misfolded protein spread is a common theme in many aging-related neurodegenerative disorders, including Alzheimer's disease (AD), Lewy body dementia (LBD) and Parkinson's disease (PD). AD is the most common neurodegenerative disease and dementia, which is characterized with widely distributed neurofibrillary tangles. Misfolded microtubule-associated protein tau is the major component of these tangles. There are many tau-pathology-related diseases, such as Pick's disease (PiD), progressive supranuclear palsy (PSP), corticobasal degeneration (CBD), Argyrophilic gain disease (AGD) and chronic traumatic encephalopathy (CTE). LBD is the second most common dementia, including dementia with Lewy body (DLB) and PD with dementia (PDD), with substantial β-amyloid plaques, tau tangles, and α-synuclein (α-syn) inclusions. PD is the second most common neurodegenerative disease with motor and non-motor dysfunction, which is correlated to α-syn pathology distribution. Studies have shown that α-syn and tau are prion-like proteins from tissue-to-tissue, region-to-region, and cell-to-cell, that significantly drive disease progression. To date, however, therapies to treat

these and other proteinopathies and related diagnostic methods for such diseases have been limited.

[0004] Accordingly, there exists an unmet need for such therapies in addition to related diagnostic applications.

SUMMARY OF THE INVENTION

[0005] The present disclosure provides therapeutic proteins, namely, fibrinogen-like protein 1 (FGL1) for inhibiting prion-like proteinopathies, including Alzheimer's disease (AD), Lewy body dementia (LBD) and Parkinson's disease (PD), among other aging-related neurodegenerative disorders. More specifically, the therapeutic proteins elevate FGL1 protein levels in the brains of patients to inhibit the prion-like proteinopathies, an important step in the pathogenesis of PD, LBD, AD, and other neurological disorders via various routes of delivery, including AAV-mediated FGL1 gene delivery, exosome-mediated FGL1 protein delivery, and blood exchange. In some embodiments, FGL1 is also used as a blood biomarker to predict the risk of proteinopathy in, for example, PD, LBD and AD patients, as well as the normal aging population. These and other therapeutic and diagnostic applications of FGL1 will be apparent upon a complete review of the present disclosure, including the accompanying figures.

[0006] According to various embodiments, a method of treating a proteinopathy in a subject is presented. The method includes administering an effective amount of a fibrinogen-like protein 1 (FGL1), or a functional portion or variant thereof, to the subject having the proteinopathy, which effective amount elevates a level of the FGL1, or the functional portion or variant thereof, in a brain of the subject sufficient to inhibit a binding interaction between a pathogenic protein associated with the proteinopathy and a lymphocyte-activation protein 3 (LAG3) present in the brain of the subject, thereby treating the proteinopathy in the subject.

[0007] According to various embodiments, a method of inhibiting a binding interaction is presented. The method includes contacting an effective amount of a fibrinogen-like protein 1 (FGL1), or a functional portion or variant thereof, with a

composition that comprises a pathogenic protein and a lymphocyte-activation protein 3 (LAG3), wherein the effective amount of the FGL1, or the functional portion or variant thereof, reduces binding of the pathogenic protein to the LAG3 in the composition relative to the composition in the absence of the FGL1, or the functional portion or variant thereof, thereby inhibiting the binding interaction.

[0008] According to various embodiments, a method of inhibiting a binding interaction is presented. The method includes contacting an effective amount of an age-related rejuvenation factor, or a functional portion or variant thereof, with a composition that comprises a pathogenic protein and a lymphocyte-activation protein 3 (LAG3), wherein the effective amount of the age-related rejuvenation factor, or the functional portion or variant thereof, reduces binding of the pathogenic protein to the LAG3 in the composition relative to the composition in the absence of the age-related rejuvenation factor, or the functional portion or variant thereof, thereby inhibiting the binding interaction.

[0009] According to various embodiments, the present disclosure provides a use of a fibrinogen-like protein 1 (FGL1), or a functional portion or variant thereof, or a synthetic polynucleotide encoding the FGL1, or the functional portion or variant thereof, for the manufacture of a medicament for the therapeutic and/or prophylactic treatment of a proteinopathy.

[0010] According to various embodiments, a method of forming exosome vesicles (EVs) is presented. The method includes transfecting one or more induced pluripotent stem (iPS) cells with a synthetic polynucleotide encoding a fibrinogen-like protein 1 (FGL1), or a functional portion or variant thereof, such that the iPS cells express the FGL1, or the functional portion or variant thereof, to produce transfected iPS cells; and purifying one or more EVs that comprise the FGL1, or the functional portion or variant thereof, from the transfected iPS cells to produce purified EVs that comprise the FGL1, or the functional portion or variant thereof, thereby forming the EVs. In some embodiments, the iPS cells comprise human iPS cells.

[0011] According to various embodiments, a method of generating a recombinant adeno-associated virus (AAV) comprising an AAV capsid is presented. The method includes culturing a host cell containing: (a) a nucleic acid sequence encoding an

AAV capsid protein; (b) a functional rep gene; (c) a minigene comprising AAV inverted terminal repeats (ITRs) and a transgene encoding a fibrinogen-like protein 1 (FGL1), or a functional portion or variant thereof; and (d) sufficient helper functions to permit packaging of the minigene into the AAV capsid; and purifying the AAV capsid from the host cell, thereby generating the recombinant AAV comprising the AAV capsid.

[0012] According to various embodiments, a method of predicting a risk of a proteinopathy in a subject is presented. The method includes determining a fibrinogen-like protein 1 (FGL1) expression level in a sample obtained from a subject, which sample is indicative of the FGL1 expression level in a brain of the subject; and identifying the subject as likely to develop, or having, the proteinopathy when the FGL1 expression level in the brain of the subject is below a control expression level, or identifying the subject as unlikely to develop, or not having, the proteinopathy when the FGL1 expression level in the brain of the subject is above the control expression level, thereby the predicting the risk of the proteinopathy in the subject. In some embodiments, the method includes administering an effective amount of an exogenous FGL1, or a functional portion or variant thereof, to the subject when the FGL1 expression level in the brain of the subject is below the control expression level, which effective amount elevates a level of the exogenous FGL1, or the functional portion or variant thereof, in the brain of the subject sufficient to inhibit a binding interaction between a pathogenic protein associated with the proteinopathy and a lymphocyte-activation protein 3 (LAG3) present in the brain of the subject.

[0013] According to various embodiments, a pharmaceutical composition is presented. The pharmaceutical composition comprises a fibrinogen-like protein 1 (FGL1), or a functional portion or variant thereof, of use in the treatment of a proteinopathy in a subject. In some embodiments, the pharmaceutical composition comprises exosome vesicles (EVs) that comprise the FGL1, or the functional portion or variant thereof. In some embodiments, the pharmaceutical composition comprises adeno-associated virus (AAV) capsids that comprise a transgene encoding the FGL1, or the functional portion or variant thereof. In some embodiments, the

pharmaceutical composition comprises one or more blood components suitable for administering the pharmaceutical composition to the subject via blood exchange. In some embodiments, the proteinopathy comprises Alzheimer's disease (AD), Parkinson's disease (PD), and/or Lewy body dementia (LBD). In some embodiments, a kit comprises the pharmaceutical composition.

[0014] Various optional features of the above embodiments include the following. The composition is disposed *in vitro*. The composition is disposed *in vivo*. A brain of a subject comprises the composition. The subject is a human subject. The subject comprises a proteinopathy associated with the pathogenic protein. The proteinopathy comprises an α-synucleinopathy. The effective amount of the FGL1, or the functional portion or variant thereof, substantially treats the proteinopathy in the subject. The contacting step comprises administering the effective amount of the FGL1, or the functional portion or variant thereof, to the subject using at least one administration technique. The administration technique is selected from the group consisting of: a blood exchange, a stereotaxic injection, an intrastriatal injection, an intramuscular injection, an intradermal injection, a transmucosal injection, a subcutaneous injection, and an intravenous injection. A cell comprises the LAG3. The cell comprises a mammalian cell. The cell comprises a neuronal cell. The effective amount of the FGL1, or the functional portion or variant thereof, inhibits uptake of the pathogenic protein into the cell and/or inhibits spreading of the pathogenic protein to other cells in the composition. An aggregation comprises the pathogenic protein. The pathogenic protein comprises a prion-like protein. The prion-like protein comprises a monomeric α-synuclein protein (α-syn), an oligomeric α-syn, a preformed-fibrillar (PFF) α-syn, a functional portion or variant thereof, or any combination thereof. The prion-like protein comprises a monomeric a microtubule-associated protein tau (tau), an oligomeric tau, a preformed-fibrillar (PFF) tau, a functional portion or variant thereof, or any combination thereof.

[0015] Various additional optional features of the above embodiments include the following. The FGL1, or the functional portion or variant thereof, is human. The FGL1, or the functional portion or variant thereof, comprises a recombinant FGL1, or functional portion or variant thereof, or an exogenous FGL1, or functional portion or

variant thereof. The FGL1, or the functional portion or variant thereof, comprises a splice variant. A vector comprises the FGL1, or the functional portion or variant thereof. The vector is selected from the group consisting of: an exosome vesicle, a liposome, a lipid nanocrystal, and a lipid nanoparticle. A vector comprises a synthetic polynucleotide encoding the FGL1, or the functional portion or variant thereof, and wherein the method comprises contacting the vector with the composition such that the synthetic polynucleotide is expressed sufficiently to produce the effective amount of the FGL1, or the functional portion or variant thereof. An expression cassette comprises the synthetic polynucleotide. The vector is selected from the group consisting of: a virus, a cell, an exosome vesicle, a plasmid, a phagemid, a cosmid, a liposome, an expression cassette, a lipid nanocrystal, a lipid nanoparticle, and an artificial chromosome.

BRIEF DESCRIPTION OF THE DRAWINGS

[0016] FIGS. 1A-1E. The expression of FGL1 decreases with aging. (A) The liver FGL1 level by western blot in the young (3-month-old) and aged mice (20-month-old). **(B)** The quantification of liver FGL1. **(C)** The expression of FGL1 in the young and aged serum by ELISA. **(D)**The plasma FGL1 level by immunoblot in the aged mice (18-month-old), young mice (2, 3-month-old) and aged mice (18-month-old) by HBE with young mice (2, 3-month-old). **(E)** The quantification of plasma FGL1 in aged mice (18-month-old) were elevated 80-90% by HBE with young mice (2, 3-month-old). Data are the means ± SEM. Statistical significance was determined by using one-way ANOVA followed with Tukey's correction, *P < 0.05, **P < 0.01, n=4 in each group.

[0017] FIGS. 2A-2E. Effect of FGL1 on α-syn PFF-biotin uptake. (A) The cell binding assay in SH-SY5Y cells treated with Lag3, FGL1 and α-syn PFF biotin. **(B)** The quantitative analysis of α-syn PFF-biotin binding signal **(C)** Schematic showing the experimental design. The primary culture of cortical neurons were treated with FGL1-human Ig and mouse α-syn PFF-biotin (1μM) at DIV14. **(D)** The cells were used for neuron, biotin and Hoechst nuclear immunostaining in each group. **(E)** The

corresponding quantitative analysis of α-syn PFF-biotin signal. Scale bar, 20 μm. One-way ANOVA with Turkey's correction, data represent mean ± s.e.m. *P < 0.05, ***P < 0.001, nc, not significant, n=6 individual experiments.

[0018] FIGS. 3A and 3B. Ratio of FGL1 detected in CSF vs plasma. (A) Schematic showing the experimental design. A near infrared (IR) dye IRDye680RD was used to label FGL1. After tail vein injection (10 mg/kg) in WT mice, the CSF and plasma were collected at 3, 7 and 10 days for analysis. **(B)** The labeled FGL1 was increased at the 7 day by plate reader.

[0019] FIGS. 4A-4E. AAV-FGL1 reduces the pathological α-synuclein in the mice cerebral cortex. (A) Schematic showing the experimental design. Stereotaxic injection of α-syn PFF (5 μg per animal) was injected into the striatum and AAV-FGL1 (1.01×10^{13} vg/mouse) injection were performed into the lateral ventricle. At 30 days after injection, the mice were sacrificed for staining with FGL1 and synuclein antibodies in cortex (n = 3 mice in per group). **(B)** The cortex sections were used for FGL1 (red) and Hoechst nuclear staining (in blue) immunostaining. **(C)** The corresponding quantitative analysis of FGL1 signal. **(D)** The cortex were immunostained for pathologic α-synuclein and Hoechst nuclear staining. **(E)** The corresponding quantitative analysis of pathological α-synuclein signal. Scale bars, 100 μm. Student's t-test, data represent mean ± s.e.m. *P < 0.05, ***P < 0.001, n=3 mice, each group.

[0020] FIGS. 5A-5G. AAV-FGL1 reduces the pathological α-synuclein in the primary culture of cortical neurons. (A) Schematic showing the experimental design. The primary culture of cortical neurons were treated with AAV-Control, AAV-FGL1 (secreted) and AAV-FGL1 (none secreted) at DIV5. The cells were treated α-syn PFF at DIV7. The pathological of α-syn were tested at DIV14. **(B, C, D, E, F)** The cells were used for pathological of α-syn (red), neuron (green) and Hoechst nuclear staining (blue) immunostaining in each group. The corresponding quantitative analysis of FGL1 signal. The cortex was immunostained for pathologic α-synuclein (red) and Hoechst nuclear staining (blue). **(G)** The corresponding quantitative analysis of pathological α-synuclein signal. Scale bars, 50 μm. One-way ANOVA with

Turkey's correction, data represent mean ± s.e.m. **P < 0.01, ***P < 0.001, n=3 individual experiments.

[0021] FIGS. 6A-6E. The pathological of α-synuclein in the parabiosis groups. (A) Schematic showing the experimental design. The 16-month-old mice were paired with 16-month-old mice as isochronic parabiosis group, and the 16-month-old mice were paired with 2-month-old mice as heterochronic parabiosis group. After one month, the paired aged mice were injected α-syn PFF (5 μg/ml) in the striatum. After 2 months, the paired aged mice were sacrificed and stained for pS129 in the different brain regions. (B, C, D) The pS129 (red) staining for pathological α-synuclein in the cortex, amygdala, hippocampus. **(E)** The corresponding quantitative analysis of pathological α-synuclein signal. Scale bars, 10 μm. One-way ANOVA with Turkey's correction, data represent mean ± s.e.m. **P < 0.01, ***P < 0.001, nc, not significant, n=6 paired aged mice.

[0022] FIGS. 7A and 7B. Characterization of human iPSC-EVs. (A) Human iPSCs produce more EVs than MSCs. **(B)** particle size of human iPSC-EVs measured by nanoparticle tracking analysis (NTA). **(C)**Transmission electron microscopy (TEM) image of iPSC derived EVs. All data reflect mean ± SD from ≥3 independent experiments. Abbreviation: ns, not significant; ***,p< .001.

[0023] FIGS. 8A and 8B. iPS cells with enforced FGL1 expression show significantly higher level of FGL1 proteins in EVs. (A) Human iPS cells were transfected with synthetic FGL1 mRNA. After 24h, the level of FGL1 proteins was significantly higher than control cells transfected by GFP mRNA. **(B)** EVs were purified from culture medium from iPS cells as described in (A). FGL1 proteins were detected in EVs from iPS cells with enforced FGL1 expression, but not in control cells.

[0024] FIGS. 9A and 9B. iPS cells neon transfected with FGL1 showed secreted FGL1 proteins in EVs. (A) Human iPS cells were neon transfected (1100v) with plasmid FGL1. After 72h, the level of FGL1 protein was tested in the supernate and exosome by ELISA. **(B)** The ratio of the amount of exosome-FGL1 with secreted-FGL1.

DEFINITIONS

[0025] In order for the present disclosure to be more readily understood, certain terms are first defined below. Additional definitions for the following terms and other terms may be set forth throughout the specification. If a definition of a term set forth below is inconsistent with a definition in an application or patent that is incorporated by reference, the definition set forth in this application should be used to understand the meaning of the term.

[0026] As used in this specification and the appended claims, the singular forms "a," "an," and "the" include plural references unless the context clearly dictates otherwise. Thus, for example, a reference to "a method" includes one or more methods, and/or steps of the type described herein and/or which will become apparent to those persons skilled in the art upon reading this disclosure and so forth.

[0027] It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only and is not intended to be limiting. Further, unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure pertains. In describing and claiming the methods, systems, and computer readable media, the following terminology, and grammatical variants thereof, will be used in accordance with the definitions set forth below.

[0028] ***α-synuclein***: As used herein, the term "α-synuclein," "α-syn," "alpha-synuclein," or "SNCA" refers to a protein member of the synuclein family, which also includes beta- and gamma-synuclein. Synucleins are abundantly expressed in the brain and alpha- and beta-synuclein inhibit phospholipase D2 selectively. SNCA may serve to integrate presynaptic signaling and membrane trafficking. Defects in SNCA have been implicated in the pathogenesis of, for example, Parkinson disease. SNCA peptides are also a major component of amyloid plaques in the brains of patients with Alzheimer's disease. Alternatively spliced transcripts encoding different isoforms have been identified for this gene. A human α-synuclein NCBI Gene ID No. is 6622. α-synuclein can be present in various forms, such as monomeric α-syn, oligomeric α-syn, and preformed-fibrillar (PFF) α-syn.

[0029] ***Binding***: As used herein, the term "binding" or "binding interaction", typically refers to a non-covalent association between or among two or more entities. "Direct" binding involves physical contact between entities or moieties; "indirect" binding involves physical interaction by way of physical contact with one or more intermediate entities. Binding between two or more entities can be assessed in any of a variety of contexts—including where interacting entities or moieties are studied in isolation or in the context of more complex systems (e.g., while covalently or otherwise associated with a carrier entity and/or in a biological system or cell).

[0030] ***Determining FGL1 Expression***: As used herein, "determining FGL1 expression" refers to measuring FGL1 expression levels by any means or methods known to the person skilled in the art, in which FGL1 expression may be determined on a protein or mRNA level. Typically, the means or methods to measure FGL1 expression are able to quantitatively detect or determine FGL1 expression, e.g., human FGL1 expression. Quantitatively detecting or determining FGL1 expression includes detecting or determining relative or absolute amounts. Methods suitable for quantitatively detecting and determining FGL1 expression include contacting the sample with an anti-FGL1 antibody and detecting the binding between FGL1 and the antibody, such as by ELISA or by IHC, or detecting the amount of mRNA encoding FGL1 protein in the sample, such as by PCR.

[0031] ***Elevate***: As used herein, "elevate" or "increased" refers to an increase by at least about 10% as compared to a control, for example an increase by at least about 20%, or at least about 30%, or at least about 40%, or at least about 50%, or at least about 75%, or at least about 80%, or at least about 90%, or at least about 100%, or at least about 200%, or at least about 300%, or any integer decrease between 10-300% as compared to a control. As used herein, a "control" is a reference value or a reference sample for base line expression from at least one subject not having cancer or a non-cancerous tissue sample, wherein the non-cancerous tissue sample preferably originates from the same organ as the cancer and more preferably from the cancer patient. The methods and sample types used for establishing a cut-off value of a marker like FGL1 and for measuring the FGL1 level in a sample obtained from an individual or patient to be analyzed match each

other or are the same. Cut-off values, i.e., values above which elevated expression or overexpression (e.g., elevated expression of FGL1 compared to a control) is acknowledged can be obtained in a control group. The control group on which the cut-off value is based is chosen to match the group of individuals/patients under investigation.

[0032] ***Encode***: As used herein, "encode" refers broadly to any process whereby the information in a polymeric macromolecule is used to direct the production of a second molecule that is different from the first. In other aspects, a DNA molecule can encode an RNA molecule (e.g., by the process of transcription that uses a DNA-dependent RNA polymerase enzyme). Also, an RNA molecule can encode a polypeptide, as in the process of translation. When used to describe the process of translation, the term "encode" also extends to the triplet codon that encodes an amino acid. In some aspects, an RNA molecule can encode a DNA molecule, e.g., by the process of reverse transcription incorporating an RNA-dependent DNA polymerase. In another aspect, a DNA molecule can encode a polypeptide, where it is understood that "encode" as used in that case incorporates both the processes of transcription and translation.

[0033] ***Exosome***: As used herein, "exosome" or "exosome vesicle" or "EV' refers to a nanometer-size extracellular vesicle that transports proteins, RNAs, and/or lipids from a cellular origin.

[0034] ***Expression***: As used herein, the term "expression", when used in reference to a nucleic acid herein, refers to one or more of the following events: (1) production of an RNA transcript of a DNA template (e.g., by transcription); (2) processing of an RNA transcript (e.g., by splicing, editing, 5′ cap formation, and/or 3′ end formation); (3) translation of an RNA into a polypeptide; and/or (4) post-translational modification of a polypeptide.

[0035] ***Expression Cassette***: As used herein, the term "expression cassette" or "expression vector" as used herein refers to a nucleotide sequence which is capable of affecting expression of a protein coding sequence in a host compatible with such sequences. Expression cassettes typically include at least a promoter operably linked with the polypeptide coding sequence; and, optionally, with other sequences,

e.g., transcription termination signals. Additional factors necessary or helpful in effecting expression may also be included, e.g., enhancers. "Operably linked', refers to linkage of a promoter upstream from a DNA sequence such that the promoter mediates transcription of the DNA sequence. Thus, expression cassettes include plasmids, recombinant viruses, any form of a recombinant vector, and the like.

[0036] ***Fibrinogen-like Protein 1***: As used herein, the term "fibrinogen-like protein 1" or "FGL1" (synonyms LFIRE-1, HFREPI, Hepassocin, HP-041) refers to a protein of the fibrinogen family of proteins, which also includes fibrinogen-like protein 2 and clotting factors V, VIII, and XIII. However, the term "FGL1 expression" relates to both, protein and mRNA expression in humans unless otherwise stated. FGL1 is encoded by the *FGL1* gene. FGL-1 contains a fibrinogen related domain in its C-terminal portion, which contains the four conserved cysteines that are common to all members of the fibrinogen family. However, FGL-1 lacks the platelet-binding site, cross-linking region, and thrombin-sensitive site which allow the other members of the fibrinogen family to aid in fibrin clot formation. FGL1 is upregulated in regenerating liver and is abundantly associated with the fibrin matrix after clot formation. While the majority of FGL1 is found in plasma, approximately 20% of FGL1 remains in the serum after blood coagulation. Human FGL1 precursor has the amino acid sequence as provided by the NCBI Reference Sequence database under ID number NP_004458.3, wherein amino acids 1 to 22 correspond to the signal peptide and amino acids 23 to 312 correspond to the mature protein. The C-terminal globular domain of fibrinogen corresponds to amino acids 78 to 304. In some embodiments, the FGL1 is mammalian. In some embodiments, the FGL1 is murine, porcine, ovine, bovine, human, or combinations thereof.

[0037] ***Functional Portion Or Variant Thereof***: As used herein, the phrase "functional portion or variant thereof" in the context of a fibrinogen-like protein 1 (FGL1) refers to a modified or unmodified portion or fragment, or a sequence variant (e.g., amino acid sequence variant or encoding polynucleotide sequence variant) of FGL1 retains its ability to inhibit a binding interaction between a pathogenic protein associated with a proteinopathy (e.g., a prion-like protein, such as α-synuclein or tau) and a lymphocyte-activation protein 3 (LAG3) present in the brain of a subject. In

some embodiments, a "functional portion or variant thereof" of FGL1 has at least 80%, 85%, 90%, 95%, 99% identity with a wild-type FGL1 amino acid sequence. In some embodiments, a "functional portion or variant thereof" of a polynucleotide encoding FGL1 has at least 80%, 85%, 90%, 95%, 99% identity with a wild-type *FGL1* gene. In some embodiments, a "functional portion" of FGL1 or polynucleotide encoding FGL1 can comprise, for instance, about 10%, 25%, 30%, 50%, 68%, 80%, 90%, 95%, or more, of a corresponding full-length FGL1 or polynucleotide encoding FGL1 molecule.

[0038] ***Gene***: As used herein, the term "gene" refers to a DNA locus of heritable genomic sequence which affects an organism's trait by being expressed as a functional product or by regulation of gene expression. Genes and polynucleotides may include introns and exons as in genomic sequence, or just the coding sequences as in cDNAs, such as an open reading frame (ORF), comprising a start codon (methionine codon) and a translation stop codon. Genes and polynucleotides can also include regions that regulate their expression, such as transcription initiation, translation and transcription termination. Thus, also included are regulatory elements such as a promoter.

[0039] ***Host cell****:* As used herein, the phrase "host cell" refers to a cell into which exogenous DNA (recombinant or otherwise) has been introduced. For example, host cells may be used to produce the fibrinogen-like protein 1 (FGL1), or a functional portion or variant thereof, (e.g., wild-type, recombinant, or modified protein molecules) referenced herein by standard production techniques. Persons of skill upon reading this disclosure will understand that such terms refer not only to the particular subject cell, but, to the progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term "host cell" as used herein. In some embodiments, host cells include any prokaryotic and eukaryotic cells suitable for expressing an exogenous DNA (e.g., a recombinant nucleic acid sequence). Exemplary cells include those of prokaryotes and eukaryotes (single-cell or multiple-cell), bacterial cells (e.g., strains of *E. coli*, *Bacillus* spp., *Streptomyces* spp., etc.),

mycobacteria cells, fungal cells, yeast cells (e.g., *S. cerevisiae*, *S. pombe*, *P. pastoris*, *P. methanolica*, etc.), plant cells, insect cells (e.g., SF-9, SF-21, baculovirus-infected insect cells, *Trichoplusia ni*, etc.), non-human animal cells, human cells (e.g., induced pluripotent stem (iPS) cells, mesenchymal stem cells, etc.), or cell fusions such as, for example, hybridomas or quadromas. In some embodiments, the cell is a human, monkey, ape, hamster, rat, or mouse cell. In some embodiments, the cell is eukaryotic and is selected from the following cells: Chinese Hamster Ovary or CHO cells (e.g., CHO K1, DXB-11 CHO, Veggie-CHO), COS cells (e.g., COS-7), retinal cells, Vero cells, CV1 cells, kidney cells (e.g., HEK293, 293 EBNA, MSR 293, MDCK, HaK, BHK), HeLa cells, HepG2 cells, W138 cells, MRC 5 cells, Colo205 cells, HB 8065 cells, HL-60 cells, BHK21 cells, Jurkat cells, Daudi cells, A431 (epidermal) cells, CV-1 cells, U937 cells, 3T3 cells, L cells, C127 cells, SP2/0 cells, NS-0 cells, MMT 060562 cells, Sertoli cells, BRL 3A cells, HT1080 cells, myeloma cells, tumor cells, and a cell line derived from an aforementioned cell. In some embodiments, the cell comprises one or more viral genes, e.g., a retinal cell that expresses a viral gene (e.g., a PER.C6™ cell).

[0040] ***Isolated****:* As used herein, the term "isolated" refers to a substance and/or entity that has been (1) separated from at least some of the components with which it was associated when initially produced (whether in nature and/or in an experimental setting), and/or (2) designed, produced, prepared, and/or manufactured by the hand of man. Isolated substances and/or entities may be separated from about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or more than about 99% of the other components with which they were initially associated. In some embodiments, isolated agents are about 80%, about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or more than about 99% pure. As used herein, a substance is "pure" if it is substantially free of other components. In some embodiments, as will be understood by those skilled in the art, a substance may still be considered "isolated" or even "pure", after having been combined with certain other components such as, for

example, one or more carriers or excipients (e.g., buffer, solvent, water, etc.); in such embodiments, percent isolation or purity of the substance is calculated without including such carriers or excipients. To give but one example, in some embodiments, a biological polymer such as a polypeptide or polynucleotide that occurs in nature is considered to be "isolated" when, a) by virtue of its origin or source of derivation is not associated with some or all of the components that accompany it in its native state in nature; b) it is substantially free of other polypeptides or nucleic acids of the same species from the species that produces it in nature; c) is expressed by or is otherwise in association with components from a cell or other expression system that is not of the species that produces it in nature. Thus, for instance, in some embodiments, a polypeptide that is chemically synthesized or is synthesized in a cellular system different from that which produces it in nature is considered to be an "isolated" polypeptide. Alternatively or additionally, in some embodiments, a polypeptide that has been subjected to one or more purification techniques may be considered to be an "isolated" polypeptide to the extent that it has been separated from other components a) with which it is associated in nature; and/or b) with which it was associated when initially produced.

[0041] ***Lymphocyte-Activation Protein 3***: As used herein, "lymphocyte-activation protein 3" or "LAG3" refers to an inhibitory molecule involved in the regulation of T cell activation, proliferation and homeostasis. It is also referred to as CD223. LAG3 is a CD4-related activation-induced cell surface molecule that acts as an immune checkpoint receptor. LAG3 is expressed on activated T cells, natural killer cells (NK cells), B cells and plasmacytoid dendritic cells (pDCs). The protein negatively regulates proliferation, activation and homeostasis in a similar fashion to CTLA-4 and PD-1. It has been reported to play a role in Treg suppressive function and helps maintaining CD8+ T cell in a tolerogenic state. LAG3 is structurally related to CD4 and binds with high affinity to MHC class II molecules. A single lysine residue (K468) within a conserved "KIEELE" motif is essential for interaction with downstream signaling molecules. It has four extracellular Ig-like domains, with conserved structural motifs between the D1 and D3 domains as well as the D2 and D4 domains. The first domain is an Ig-like V-type domain and the following three

domains are Ig-like C2-type domains. The human LAG3 has approximately 70% homology with murine LAG3. A unique distinguishing feature of LAG3 is a proline-rich 30-aa loop between the C and C'β strands of the D1 domain. Although both, human and murine LAG-3, possess this loop, their homology is lowest in this region. Human LAG3 has the amino acid sequence as provided by the NCBI GenBank database under ID number AAH52589.

[0042] ***Nucleic Acid***: As used herein, "nucleic acid" refers to a naturally occurring or synthetic oligonucleotide or polynucleotide, whether DNA or RNA or DNA-RNA hybrid, single-stranded or double-stranded, sense or antisense, which is capable of hybridization to a complementary nucleic acid by Watson-Crick base-pairing. Nucleic acids can also include nucleotide analogs (e.g., bromodeoxyuridine (BrdU)), and non-phosphodiester internucleoside linkages (e.g., peptide nucleic acid (PNA) or thiodiester linkages). In particular, nucleic acids can include, without limitation, DNA, RNA, cDNA, gDNA, ssDNA, dsDNA, cfDNA, ctDNA, or any combination thereof.

[0043] ***Prion-like Protein***: As used herein, "prion like protein" refers to a neurodegenerative disease-related protein that shares similarities with prion replication and propagation processes, but which has noninfectious characteristics, unlike a prion. Examples of prion-like proteins, include amyloid-β (Aβ), α-synuclein, tau, and the transactive response DNA-binding protein of 43 kDa (TDP-43).

[0044] ***Protein***: As used herein, "protein" is used interchangeably with "polypeptide" and refers to polymers of amino acids of any length. These terms also include proteins that are post-translationally modified through reactions that include, but are not limited to, glycosylation, acetylation, phosphorylation, glycation or protein processing. Modifications and changes, for example fusions to other proteins, amino acid sequence substitutions, deletions or insertions, can be made in the structure of a polypeptide while the molecule maintains its biological functional activity. For example, certain amino acid sequence substitutions can be made in a polypeptide or its underlying nucleic acid coding sequence and a protein can be obtained with the same properties. The term "polypeptide" typically refers to a sequence with more than 10 amino acids and the term "peptide" means sequences with up to 10 amino acids in length. However, the terms may be used interchangeably.

[0045] ***Proteinopathy***: As used herein, "proteinopathy" or "protein conformational disorder," or "protein misfolding disease," is a class of diseases in which certain proteins become structurally abnormal, and thereby disrupt the function of cells, tissues and organs of the body. Frequently, the proteins fail to fold into their normal configuration; in this misfolded state, the proteins become toxic in some way (e.g., a toxic gain-of-function) or they lose their normal function. Examples of proteinopathies include diseases such as Creutzfeldt–Jakob disease and other prion diseases, Alzheimer's disease, Parkinson's disease, Lewy body dementia (LBD), amyloidosis, multiple system atrophy, and a wide range of other neurodegenerative disorders. In some embodiments, the proteinopathy is "α-synucleinopathy" which are a class of diseases involving misfolded prion-like neuronal protein α-synuclein. Other examples of prion-like proteins, include amyloid-β (Aβ), tau, and the transactive response DNA-binding protein of 43 kDa (TDP-43).

[0046] ***Pure****:* As used herein, an agent or entity is "pure" if it is substantially free of other components. For example, a preparation that contains more than about 90% of a particular agent or entity is typically considered to be a pure preparation. In some embodiments, an agent or entity is at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% pure.

[0047] ***Recombinant****:* As used herein, the term "recombinant" or "modified" is intended to refer to polypeptides (e.g., a fibrinogen-like protein 1 (FGL1), or a functional portion or variant thereof) that are designed, engineered, prepared, expressed, created or isolated by recombinant means, such as polypeptides expressed using a recombinant expression vector transfected into a host cell, polypeptides isolated from a recombinant, combinatorial polypeptide library or polypeptides prepared, expressed, created or isolated by any other means that involves splicing selected sequence elements to one another. In some embodiments, one or more of such selected sequence elements is found in nature. In some embodiments, one or more of such selected sequence elements is designed *in silico*. In some embodiments, one or more such selected sequence elements results from mutagenesis (e.g., *in vivo* or *in vitro*) of a known sequence element, e.g., from a

natural or synthetic source. In some embodiments, one or more such selected sequence elements results from the combination of multiple (e.g., two or more) known sequence elements that are not naturally present in the same polypeptide. In some embodiments, the term "recombinant" refers to (i) nucleic acid molecules that are constructed outside living cells by joining natural or synthetic nucleic acid segments to nucleic acid molecules that can replicate in a living cell, or (ii) nucleic acid molecules that result from the replication of those described in (i) above. For purposes herein, the replication can be *in vitro* replication or *in vivo* replication.

[0048] ***Sample***: As used herein, “sample” or “fluidic sample” refers to a tissue or organ from a subject; a cell (either within a subject, taken directly from a subject, or a cell maintained in culture or from a cultured cell line); a cell lysate (or lysate fraction) or cell extract; or a solution containing one or more molecules derived from a cell or cellular material (e.g., a polypeptide), which is assayed as described herein. A sample may also be any body fluid or excretion (for example, but not limited to, blood, urine, stool, saliva, tears, bile) that contains cells, cell components, or non-cellular fractions.

[0049] ***Subject***: As used herein, the term “subject” means any member of the animal kingdom. In some embodiments, “subject” refers to humans. In some embodiments, “subject” refers to non-human animals. In some embodiments, subjects include, but are not limited to, mammals, birds, reptiles, amphibians, fish, insects, and/or worms. In some embodiments, the non-human subject is a mammal (*e.g.*, a rodent, a mouse, a rat, a rabbit, a ferret, a monkey, a dog, a cat, a sheep, cattle, a primate, and/or a pig). In some embodiments, a subject may be a transgenic animal, genetically engineered animal, and/or a clone. In some embodiments, the subject is an adult, an adolescent or an infant. In some embodiments, terms “individual” or “patient” are used and are intended to be interchangeable with “subject.”

[0050] ***Tau***: As used herein, the term “tau,” “microtubule associated protein tau,” or “MAPT” refers to a protein encoded by a gene whose transcript undergoes complex, regulated alternative splicing, giving rise to several mRNA species. MAPT transcripts are differentially expressed in the nervous system, depending on stage of

neuronal maturation and neuron type. MAPT gene mutations have been associated with several neurodegenerative disorders such as Alzheimer's disease, Pick's disease, frontotemporal dementia, cortico-basal degeneration and progressive supranuclear palsy. A human tau NCBI Gene ID No. is 4137. Tau can be present in various forms, such as monomeric tau, oligomeric tau, and preformed-fibrillar (PFF) tau.

[0051] ***Vector***: As used herein, the term "vector" in the context of FGL1 therapeutic proteins and polynucleotides encoding FGL1 therapeutic proteins refers to a biological carrier or delivery entity used to introduce the proteins or polynucleotides into target organs or cells of a subject, such as the brain of the subject. In some embodiments, a "vector" is a nucleic acid that can be used to introduce a heterologous or synthetic polynucleotide into a cell. One type of vector is a "plasmid", which refers to a linear or circular double stranded DNA molecule into which additional nucleic acid segments can be ligated. Another type of vector is a viral vector (e.g., replication defective retroviruses, adenoviruses and adeno-associated viruses), in which additional DNA or RNA segments can be introduced into the viral genome. Other exemplary vectors in the context of polynucleotides encoding FGL1 therapeutic proteins, include cells, exosome vesicles, cosmids, liposomes, expression cassettes, lipid nanocrystals, lipid nanoparticles, and artificial chromosomes, among others. Exemplary vectors in the context of FGL1 therapeutic proteins, include exosome vesicles, liposomes, lipid nanocrystals, and lipid nanoparticles, among others.

DETAILED DESCRIPTION

[0052] The present disclosure provides therapeutic proteins, namely, fibrinogen-like protein 1 (FGL1), or functional portions or variants thereof, (collectively, sometimes referred to herein as the "FGL1 therapeutic proteins") for inhibiting prion-like proteinopathies, including Alzheimer's disease (AD), Lewy body dementia (LBD) and Parkinson's disease (PD), among other aging-related neurodegenerative disorders. Upon administration, FGL1 therapeutic proteins inhibit the prion-like

proteinopathies in the brains of patients, thereby interfering with the pathogenesis of these and other misfolded protein-related neurological disorders. Various routes of delivery to elevate FGL1 therapeutic protein levels in the brains of patients are disclosed herein, including, for example, adeno-associated virus (AAV)-mediated FGL1 gene delivery, exosome-mediated FGL1 therapeutic protein delivery, and blood exchange, among others. As further disclosed herein, FGL1 can also be used as a blood biomarker to predict the risk of proteinopathy in AD, LBD, PD, and other proteinopathy patients, as well as in the normal aging population. These and other treatment applications of FGL1 therapeutic proteins and diagnostic applications of FGL1 will be apparent upon a complete review of the present disclosure, including the accompanying figures.

[0053] Misfolded protein spread is commonly observed in various aging-related neurodegenerative disorders, including AD, LBD, and PD. AD is the most common neurodegenerative disease and dementia. It is characterized by having widely distributed neurofibrillary tangles. Misfolded microtubule-associated protein tau is the major component of these tangles. There are many tau-pathology-related diseases, such as Pick's disease (PiD), progressive supranuclear palsy (PSP), corticobasal degeneration (CBD), Argyrophilic gain disease (AGD), and chronic traumatic encephalopathy (CTE). LBD is another frequently observed dementia, including dementia with Lewy body (DLB) and PD with dementia (PDD). These diseases are associated with substantial β-amyloid plaques, tau tangles, and α-synuclein (α-syn) inclusions. PD is yet another commonly observed neurodegenerative disease with motor and non-motor dysfunction. It is correlated to α-syn pathology distribution. α-syn and tau are prion-like proteins that spread from tissue-to-tissue, region-to-region, and cell-to-cell, thereby significantly driving disease progression.

[0054] As described herein, the inoculation of, for example, a preformed fibrillar (PFF) of α-syn and tau can successfully recapitulate neurodegenerative disease progression, including pathology propagation, behavioral deficit phenotypes, neuroinflammation, and neurodegeneration. Lymphocyte-activation gene 3 (LAG3) is the receptor of, for example, α-syn PFF, and depletion and inhibition of LAG3 significantly reduces the pathogenic α-syn transmission *in vitro* and *in vivo*. FGL1 is

a major inhibitory ligand of LAG3. Accordingly, in some embodiments, the FGL1 therapeutic proteins of the present disclosure act as aging-related rejuvenation factors that block pathogenic proteins uptake and propagation via LAG3 and other pathways.

[0055] In some embodiments, the present disclosure provides methods of treating a proteinopathy (e.g., AD, LBD, PD, or the like) in a subject (e.g., a human subject). The methods include administering an effective amount of a fibrinogen-like protein 1 (FGL1), or a functional portion or variant thereof, to the subject having the proteinopathy. The effective amount elevates a level of the FGL1, or the functional portion or variant thereof, in a brain of the subject sufficient to inhibit a binding interaction between a pathogenic protein associated with the proteinopathy (e.g., α-syn, tau, etc.) and a lymphocyte-activation protein 3 (LAG3) present in the brain of the subject. Typically, a neuronal cell comprises the LAG3 on its surface.

[0056] In some embodiments, the present disclosure provides methods of inhibiting a binding interaction. The methods include contacting an effective amount of a fibrinogen-like protein 1 (FGL1), or a functional portion or variant thereof, with a composition that comprises a pathogenic protein (e.g., a prion-like protein, such as α-syn, tau, etc.) and a lymphocyte-activation protein 3 (LAG3). The effective amount of the FGL1, or the functional portion or variant thereof, reduces binding of the pathogenic protein to the LAG3 in the composition relative to the composition in the absence of the FGL1, or the functional portion or variant thereof. In some embodiments, the pathogenic protein is present as an aggregation of multiple pathogenic proteins. In some embodiments, the contacting step comprises administering the effective amount of the FGL1, or the functional portion or variant thereof, to the subject using an administration technique, such as a blood exchange, a stereotaxic injection, an intrastriatal injection, an intramuscular injection, an intradermal injection, a transmucosal injection, a subcutaneous injection, or an intravenous injection, among other techniques. The effective amount of the FGL1, or the functional portion or variant thereof, typically inhibits uptake of the pathogenic protein into the cell and/or inhibits spreading of the pathogenic protein to other cells in the composition.

[0057] In some embodiments, the present disclosure provides methods of inhibiting a binding interaction. The methods include contacting an effective amount of an age-related rejuvenation factor, or a functional portion or variant thereof, (e.g., FGL1 or another age-related rejuvenation factor) with a composition that comprises a pathogenic protein (e.g., α-syn, tau, etc.) and a lymphocyte-activation protein 3 (LAG3). The effective amount of the age-related rejuvenation factor, or the functional portion or variant thereof, reduces binding of the pathogenic protein to the LAG3 in the composition relative to the composition in the absence of the age-related rejuvenation factor, or the functional portion or variant thereof.

[0058] In some embodiments, the present disclosure provides a use of a fibrinogen-like protein 1 (FGL1), or a functional portion or variant thereof, or a synthetic polynucleotide encoding the FGL1, or the functional portion or variant thereof, for the manufacture of a medicament for the therapeutic and/or prophylactic treatment of a proteinopathy (e.g., AD, LBD, PD, or the like).

[0059] In some embodiments, the present disclosure provides methods of forming exosome vesicles (EVs). The methods include transfecting induced pluripotent stem (iPS) cells (e.g., human iPS cells) with a synthetic polynucleotide encoding a fibrinogen-like protein 1 (FGL1), or a functional portion or variant thereof, such that the iPS cells express the FGL1, or the functional portion or variant thereof, to produce transfected iPS cells; and purifying EVs that comprise the FGL1, or the functional portion or variant thereof, from the transfected iPS cells to produce purified EVs that comprise the FGL1, or the functional portion or variant thereof.

[0060] In some embodiments, the present disclosure provides methods of generating a recombinant adeno-associated virus (AAV) comprising an AAV capsid. The methods include culturing a host cell containing: (a) a nucleic acid sequence encoding an AAV capsid protein; (b) a functional rep gene; (c) a minigene comprising AAV inverted terminal repeats (ITRs) and a transgene encoding a fibrinogen-like protein 1 (FGL1), or a functional portion or variant thereof; and (d) sufficient helper functions to permit packaging of the minigene into the AAV capsid; and purifying the AAV capsid from the host cell.

[0061] In some embodiments, the present disclosure provides methods of predicting a risk of a proteinopathy (e.g., an aging-associated neurodegeneration, such as AD, LBD, PD, or the like) in a subject. The methods include determining a fibrinogen-like protein 1 (FGL1) expression level in a sample obtained from a subject. The sample is indicative of the FGL1 expression level in a brain of the subject. The methods also include identifying the subject as likely to develop, or having, the proteinopathy when the FGL1 expression level in the brain of the subject is below a control expression level, or identifying the subject as unlikely to develop, or not having, the proteinopathy when the FGL1 expression level in the brain of the subject is above the control expression level. In some embodiments, the methods include administering an effective amount of an exogenous FGL1, or a functional portion or variant thereof, to the subject when the FGL1 expression level in the brain of the subject is below the control expression level, which effective amount elevates a level of the exogenous FGL1, or the functional portion or variant thereof, in the brain of the subject sufficient to inhibit a binding interaction between a pathogenic protein associated with the proteinopathy (e.g., α-syn, tau, etc.) and a lymphocyte-activation protein 3 (LAG3) present in the brain of the subject.

[0062] In some embodiments, a pharmaceutical composition is presented. The pharmaceutical composition comprises a fibrinogen-like protein 1 (FGL1), or a functional portion or variant thereof, of use in the treatment of a proteinopathy in a subject. In some embodiments, the pharmaceutical composition comprises exosome vesicles (EVs) that comprise the FGL1, or the functional portion or variant thereof. In some embodiments, the pharmaceutical composition comprises adeno-associated virus (AAV) capsids that comprise a transgene or other synthetic polynucleotide encoding the FGL1, or the functional portion or variant thereof. In some embodiments, the pharmaceutical composition comprises one or more blood components suitable for administering the pharmaceutical composition to the subject via blood exchange. In some embodiments, the proteinopathy comprises Alzheimer's disease (AD), Parkinson's disease (PD), and/or Lewy body dementia (LBD). In some embodiments, a kit comprises the pharmaceutical composition.

[0063] In some embodiments, the compositions of the present disclosure are disposed *in vitro* (e.g., in a container or the like), whereas in other embodiments, the compositions are disposed *in vivo* (e.g., in the brain of a subject after being administered an FGL1 therapeutic protein as described herein).

[0064] In some embodiments, the FGL1, or the functional portion or variant thereof, comprises a recombinant FGL1, or the functional portion or variant thereof, or an exogenous FGL1, or the functional portion or variant thereof. In some embodiments the FGL1, or the functional portion or variant thereof, comprises a splice variant.

[0065] In some embodiments, a vector comprises the FGL1, or the functional portion or variant thereof. In some embodiments, the vector comprises an exosome vesicle, a liposome, a lipid nanocrystal, or a lipid nanoparticle. In some embodiments, a vector comprises a synthetic polynucleotide encoding the FGL1, or the functional portion or variant thereof. In these embodiments, the methods disclosed herein typically comprise administering the vector to a subject such that the synthetic polynucleotide is expressed sufficiently to produce the effective amount of the FGL1, or the functional portion or variant thereof, for example, in the brain of the subject. In these embodiments, an expression cassette comprises the synthetic polynucleotide. In these of these embodiments, the vector comprises a virus, a cell, an exosome vesicle, a plasmid, a phagemid, a cosmid, a liposome, an expression cassette, a lipid nanocrystal, a lipid nanoparticle, or an artificial chromosome.

[0066] In some embodiments, vectors are polynucleotides that can be single-stranded or double-stranded, synthesized or obtained (e.g., isolated and/or purified) from natural sources, which can contain natural, non-natural or altered nucleotides, and which can contain a natural, non-natural or altered internucleotide linkage, such as a phosphoroamidate linkage or a phosphorothioate linkage, instead of the phosphodiester found between the nucleotides of an unmodified oligonucleotide. It is generally preferred that the nucleic acid does not comprise any insertions, deletions, inversions, and/or substitutions. However, it may be suitable in some instances, as discussed herein, for the nucleic acid to comprise one or more insertions, deletions, inversions, and/or substitutions.

[0067] Typically, the nucleic acid vectors of the present disclosure are recombinant in which (i) nucleic acid molecules are constructed outside living cells by joining natural or synthetic nucleic acid segments to nucleic acid molecules that can replicate in a living cell, or (ii) nucleic acid molecules that result from the replication of those described in (i) above. For purposes herein, the replication can be *in vitro* replication or *in vivo* replication.

[0068] The nucleic acids can be constructed based on chemical synthesis and/or enzymatic ligation reactions using procedures known in the art. For example, a nucleic acid can be chemically synthesized using naturally occurring nucleotides or variously modified nucleotides designed to increase the biological stability of the molecules or to increase the physical stability of the duplex formed upon hybridization (e.g., phosphorothioate derivatives and acridine-substituted nucleotides). Examples of modified nucleotides that can be used to generate the nucleic acids include, but are not limited to, 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xanthine, 4-acetylcytosine, 5-(carboxyhydroxymethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-substituted adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, 3-(3-amino-3-N-2-carboxypropyl) uracil, and 2,6-diaminopurine. Alternatively, one or more of the nucleic acids of the invention can be purchased from companies, such as Macromolecular Resources (Fort Collins, CO) and Synthegen (Houston, TX).

[0069] In some embodiments, nucleic acid vectors may be optimized (e.g., codon optimized). Without being bound to a particular theory, it is believed that optimization of the nucleic acid sequence increases the translation efficiency of the mRNA transcripts. Optimization of the nucleic acid sequence may involve substituting a

native codon for another codon that encodes the same amino acid, but can be translated by tRNA that is more readily available within a cell, thus increasing translation efficiency. Optimization of the nucleic acid sequence may also reduce secondary mRNA structures that would interfere with translation, thus increasing translation efficiency.

[0070] The polynucleotides encoding an FGL1 therapeutic protein (e.g., an expression cassette) as described herein can be incorporated into a recombinant expression vector. A recombinant expression vector typically includes a genetically-modified oligonucleotide or polynucleotide construct that permits the expression of an mRNA, protein, polypeptide, or peptide by a host cell, when the construct comprises a nucleotide sequence encoding the mRNA, protein, polypeptide, or peptide, and the vector is contacted with the cell under conditions sufficient to have the mRNA, protein, polypeptide, or peptide expressed within the cell. The vectors of the present disclosure are not naturally occurring as a whole. However, parts of the vectors can be naturally occurring. The recombinant expression vectors can comprise any type of nucleotides, including, but not limited to DNA and RNA, which can be single-stranded or double-stranded, synthesized or obtained in part from natural sources, and which can contain natural, non-natural or altered nucleotides. The recombinant expression vectors can comprise naturally occurring, non-naturally occurring internucleotide linkages, or both types of linkages. Typically, the non-naturally occurring or altered nucleotides or internucleotide linkages do not hinder the transcription or replication of the vector.

[0071] The recombinant expression vector of the invention can be any suitable recombinant expression vector and can be used to transform or transfect any suitable host. Suitable vectors include those designed for propagation and expansion or for expression or both, such as plasmids and viruses. The vector can be selected from the group consisting of the pUC series (Fermentas Life Sciences), the pBluescript series (Stratagene, LaJolla, CA), the pET series (Novagen, Madison, WI), the pGEX series (Pharmacia Biotech, Uppsala, Sweden), and the pEX series (Clontech, Palo Alto, CA). Bacteriophage vectors, such as λGT10, λGT11, λZapII (Stratagene), λEMBL4, and λNM1149, also can be used. Examples of plant

expression vectors include pBI01, pBI101.2, pBI101.3, pBI121 and pBIN19 (Clontech). Examples of animal expression vectors include pEUK-Cl, pMAM and pMAMneo (Clontech). In some embodiments, the plasmid can be one of the pcDNA3 family of plasmids known in the art. In some embodiments, the plasmid can be the pNGVL4a expression vector.

[0072] The recombinant expression vectors of the present disclosure can be prepared using standard recombinant DNA techniques as known to those of ordinary skill in the art. Constructs of expression vectors, which are circular or linear, can be prepared to contain a replication system functional in a prokaryotic or eukaryotic host cell. Replication systems can be derived, e.g., from ColEl, 2 μ plasmid, λ, SV40, bovine papilloma virus, and the like.

[0073] Desirably, the recombinant expression vector comprises regulatory sequences, such as transcription and translation initiation and termination codons, which are specific to the type of host (e.g., bacterium, fungus, plant, or animal) into which the vector is to be introduced, as appropriate and taking into consideration whether the vector is DNA- or RNA-based.

[0074] A recombinant expression vector of the present disclosure can include one or more marker genes, which allow for selection of transformed or transfected hosts. Marker genes include biocide resistance, e.g., resistance to antibiotics, heavy metals, etc., complementation in an auxotrophic host to provide prototrophy, and the like. Suitable marker genes for the inventive expression vectors include, for instance, neomycin/G418 resistance genes, hygromycin resistance genes, histidinol resistance genes, tetracycline resistance genes, and ampicillin resistance genes.

[0075] In some embodiments, a recombinant expression vector can comprise a native or nonnative promoter operably linked to the nucleotide sequence encoding the FGL1, or the functional portion or variant thereof, or to the nucleotide sequence which is complementary to or which hybridizes to the nucleotide sequence encoding the FGL1, or the functional portion or variant thereof. The selection of promoters, e.g., strong, weak, inducible, tissue-specific and developmental-specific, is within the ordinary skill of the artisan. Similarly, the combining of a nucleotide sequence with a promoter is also within the skill of the artisan. The promoter can be a non-viral

promoter or a viral promoter, e.g., a cytomegalovirus (CMV) promoter, an SV40 promoter, an RSV promoter, and a promoter found in the long-terminal repeat of the murine stem cell virus.

[0076] In some embodiments, the present disclosure also provides a synthetic polypeptide molecule comprising at least one of the FGL1 polypeptides described herein along with at least one other polypeptide. The other polypeptide can exist as a separate polypeptide of the fusion protein, or can exist as a polypeptide, which is expressed in frame (in tandem) with one of the FGL1 polypeptides described herein. The other polypeptide can encode any peptidic or proteinaceous molecule, or a portion thereof. Suitable methods of making fusion proteins are known in the art, and include, for example, recombinant methods.

[0100] The FGL1 therapeutic proteins of the present disclosure (including functional portions and functional variants thereof) can be obtained by methods known in the art. Suitable methods of de novo synthesizing polypeptides and proteins are described in references, such as Chan et al., Fmoc Solid Phase Peptide Synthesis, Oxford University Press, Oxford, United Kingdom, 2005; Peptide and Protein Drug Analysis, ed. Reid, R., Marcel Dekker, Inc., 2000; Epitope Mapping, ed. Westwood et al., Oxford University Press, Oxford, United Kingdom, 2000; and U.S. Patent No. 5,449,752. Also, polypeptides and proteins can be recombinantly produced using the nucleic acids described herein using standard recombinant methods. See, for instance, Sambrook et al., Molecular Cloning: A Laboratory Manual, 3rd ed., Cold Spring Harbor Press, Cold Spring Harbor, NY 2001; and Ausubel et al., Current Protocols in Molecular Biology, Greene Publishing Associates and John Wiley & Sons, NY, 1994. Further, some of the FGL1 therapeutic proteins of the present disclosure can be isolated and/or purified from a source, such as a plant, a bacterium, an insect, a mammal, e.g., a rat, a human, etc. Methods of isolation and purification are well-known in the art. Alternatively, the FGL1 therapeutic proteins can be commercially synthesized by companies, such as Synpep (Dublin, CA), Peptide Technologies Corp. (Gaithersburg, MD), and Multiple Peptide Systems (San Diego, CA). In this respect, the FGL1 therapeutic proteins of the present disclosure can be synthetic, recombinant, isolated, and/or purified.

[0101] In some embodiments, the present disclosure provides pharmaceutical compositions that comprise or are capable of expressing (e.g., upon administration to a subject) an FGL1 therapeutic protein described herein. Typically, pharmaceutical compositions are administered or introduced into a subject, for example, a subject receiving treatment for a proteinopathy, and the FGL1 therapeutic proteins are allowed to come into contact with the one or more disease related cells or population of cells *in vivo* (e.g., cells comprising LAG3 on their surfaces).

[0102] In some embodiments, the pharmaceutical compositions of the present disclosure include a pharmaceutically acceptable carrier. With respect to pharmaceutical compositions, the carrier can be any of those conventionally used and is limited only by chemico-physical considerations, such as solubility and lack of reactivity with the active compound(s), and by the route of administration. The pharmaceutically acceptable carriers described herein, for example, vehicles, adjuvants, excipients, and diluents, are well known to those skilled in the art and are readily available to the public. Typically, the pharmaceutically acceptable carrier is one which is chemically inert to the active agent(s) and one which has no detrimental side effects or toxicity under the conditions of use.

[0103] The choice of carrier will be determined in part by the chemical properties of the particular pharmaceutical composition as well as by the particular method used to administer the pharmaceutical composition. Accordingly, there are a variety of suitable formulations of the pharmaceutical composition of the invention. In some embodiments, pharmaceutical compositions are formulated for blood exchange, stereotaxic, intrastriatal, parenteral, subcutaneous, intravenous, intramuscular, intraarterial, intrathecal, or intraperitoneal administration. More than one route can be used to administer the pharmaceutical compositions of the present disclosure, and in certain instances, a particular route can provide an immediate and more effective response than another route.

[0104] Injectable formulations are in accordance with the present disclosure. Effective pharmaceutical carriers for injectable compositions are well-known to those of ordinary skill in the art (see, e.g., *Pharmaceutics and Pharmacy Practice*, J.B.

Lippincott Company, Philadelphia, PA, Banker and Chalmers, eds., pages 238-250 (1982), and *ASHP Handbook on Injectable Drugs*, Trissel, 14th ed., (2007)). In some embodiments, the pharmaceutical compositions of the present disclosure use synthetic or naturally occurring compounds (e.g., cationic lipids, cationic polymers, lipid-polymer hybrid systems) as carriers to deliver the FGL1 therapeutic proteins or polynucleotides encoding those proteins into target cells or organs.

[0105] For purposes of the present disclosure, the amount or dose of the FGL1 therapeutic proteins or polynucleotides encoding FGL1 therapeutic proteins administered should be sufficient to effect, e.g., a therapeutic or prophylactic response, in the subject over a reasonable time frame. The dose will be determined by the efficacy of the given FGL1 therapeutic protein or the given polynucleotide encoding FGL1 therapeutic protein and the condition of the subject, as well as the body weight of the subject to be treated. Typically, the attending physician will decide the dosage of the given FGL1 therapeutic protein or the given polynucleotide encoding FGL1 therapeutic protein with which to treat each individual patient, taking into consideration a variety of factors, such as age, body weight, general health, diet, sex, to be administered, route of administration, and the severity of the condition being treated.

[0106] In accordance with some embodiments, the present disclosure provides a pharmaceutical composition comprising the FGL1 therapeutic protein or a polynucleotide encoding an FGL1 therapeutic protein disclosed herein in combination with at least one additional biologically active agent.

[0107] An “active agent” and a “biologically active agent” are used interchangeably herein to refer to a chemical or biological compound that induces a desired pharmacological and/or physiological effect, wherein the effect may be prophylactic or therapeutic. The terms also encompass pharmaceutically acceptable, pharmacologically active derivatives of those active agents specifically mentioned herein, including, but not limited to, salts, esters, amides, prodrugs, active metabolites, analogs and the like. When the terms “active agent,” “pharmacologically active agent” and “drug” are used, then, it is to be understood that the present disclosure includes the active agent per se as well as pharmaceutically acceptable,

pharmacologically active salts, esters, amides, prodrugs, metabolites, analogs etc. The active agent can be a biological entity, such as a virus or cell, whether naturally occurring or manipulated, such as transformed.

[0108] The biologically active agent may vary widely with the intended purpose for the composition. The term active is art-recognized and refers to any moiety that is a biologically, physiologically, or pharmacologically active substance that acts locally or systemically in a subject. Examples of biologically active agents, that may be referred to as “drugs”, are described in well-known literature references such as the Merck Index, the Physicians’ Desk Reference, and The Pharmacological Basis of Therapeutics, and they include, without limitation, medicaments; vitamins; mineral supplements; substances used for the treatment, prevention, diagnosis, cure or mitigation of a disease or illness; substances which affect the structure or function of the body; or pro-drugs, which become biologically active or more active after they have been placed in a physiological environment.

[0109] Non-limiting examples of biologically active agents include following: anti-inflammatory agents such as steroids, non-steroidal anti-inflammatory agents, anti-pyretic and analgesic agents, antigenic materials, and anti-viral drugs.

[0110] Various forms of the biologically active agents may be used. These include, without limitation, such forms as uncharged molecules, molecular complexes, salts, ethers, esters, amides, prodrug forms and the like, which are biologically activated when implanted, injected or otherwise placed into a subject.

[0111] Thus, in a further embodiment, the present disclosure provides a method for treating a proteinopathy in a subject in need thereof comprising administering to the subject an effective amount of an FGL1 therapeutic protein or a polynucleotide encoding an FGL1 therapeutic protein disclosed herein in combination with one or more additional biologically active agents (e.g., either simultaneously or serially with at least one other).

[0112] In accordance with some embodiments, the present disclosure also provides kits that contain the compositions or pharmaceutical compositions used with the methods, as described herein, to practice those methods. The kits can contain various combinations of pharmaceutical compositions and the like. The kit

can contain instructional material teaching methodologies, e.g., means to administer the compositions used to practice the methods disclosed herein, means to inject or infect cells, patients or animals with the pharmaceutical compositions of the present disclosure, means to monitor the resultant therapeutic response and assess the reaction of the individual to which the compositions have been administered, and the like.

[0113] Following long-standing patent law convention, the terms “a,” “an,” and “the” refer to “one or more” when used in this application, including the claims. Thus, for example, reference to “a subject” includes a plurality of subjects, unless the context clearly is to the contrary (e.g., a plurality of subjects), and so forth.

[0114] Throughout this specification and the claims, the terms “comprise,” “comprises,” and “comprising” are used in a non-exclusive sense, except where the context requires otherwise. Likewise, the term “include” and its grammatical variants are intended to be non-limiting, such that recitation of items in a list is not to the exclusion of other like items that can be substituted or added to the listed items.

[0115] For the purposes of this specification and appended claims, unless otherwise indicated, all numbers expressing amounts, sizes, dimensions, proportions, shapes, formulations, parameters, percentages, parameters, quantities, characteristics, and other numerical values used in the specification and claims, are to be understood as being modified in all instances by the term “about” even though the term “about” may not expressly appear with the value, amount or range. Accordingly, unless indicated to the contrary, the numerical parameters set forth in the following specification and attached claims are not and need not be exact, but may be approximate and/or larger or smaller as desired, reflecting tolerances, conversion factors, rounding off, measurement error and the like, and other factors known to those of skill in the art depending on the desired properties sought to be obtained by the presently disclosed subject matter. For example, the term “about,” when referring to a value can be meant to encompass variations of, in some embodiments, ± 100% in some embodiments ± 50%, in some embodiments ± 20%, in some embodiments ± 10%, in some embodiments ± 5%, in some embodiments ±1%, in some embodiments ± 0.5%, and in some embodiments ± 0.1% from the

specified amount, as such variations are appropriate to perform the disclosed methods or employ the disclosed compositions.

[0116] Further, the term "about" when used in connection with one or more numbers or numerical ranges, should be understood to refer to all such numbers, including all numbers in a range and modifies that range by extending the boundaries above and below the numerical values set forth. The recitation of numerical ranges by endpoints includes all numbers, e.g., whole integers, including fractions thereof, subsumed within that range (for example, the recitation of 1 to 5 includes 1, 2, 3, 4, and 5, as well as fractions thereof, e.g., 1.5, 2.25, 3.75, 4.1, and the like) and any range within that range.

[0117] The following examples have been included to provide guidance to one of ordinary skill in the art for practicing representative embodiments of the presently disclosed subject matter. In light of the present disclosure and the general level of skill in the art, those of skill can appreciate that the following examples are intended to be exemplary only and that numerous changes, modifications, and alterations can be employed without departing from the scope of the presently disclosed subject matter. The descriptions and specific examples that follow are only intended for the purposes of illustration and are not to be construed as limiting in any manner to make compounds of the disclosure by other methods.

EXAMPLES

[0118] EXAMPLE 1: The protective role of aging-related FGL1 for prion-like proteinopathies

[0119] INTRODUCTION

[0120] By modeling with non-transgenic mice, the inoculation of preformed fibrillar (PFF) of α-syn and tau have successfully recapitulated neurodegenerative disease progression, including pathology propagation, behavioral deficit phenotypes, neuroinflammation, and neurodegeneration. Tremendous efforts have been made to study the cell-to-cell transmission mechanism of pathogenic α-syn and tau. Lymphocyte-activation gene 3 (Lag3) is the receptor of α-syn PFF, and depletion and

inhibition of Lag3 can significantly reduce the pathogenic α-syn transmission *in vitro* and *in vivo*. Recently, fibrinogen-like protein 1 (FGL1) was identified a major inhibitory ligand of Lag3. FGL1 is mainly secreted by liver and abundantly detected in human plasma. A significant reduction of FGL1 expression in various human cells has been shown and associated with the aging process. Accordingly, FGL1 is highly related to aging.

[0121] The propagation of pathogenic α-syn and tau have been extensively studied, and aging can significantly induce α-syn and tau spreading. However, the underlying aging-related mechanisms remain poorly understood. We hypothesize that FGL1 is an aging-related rejuvenation factor, which can block pathogenic proteins uptake and propagation via Lag3 and other pathways. The inhibitory efficacy of endogenous FGL1 decreases when FGL1 levels decline in aging. We used the heterochronic blood exchange (HBE), genetic depletion and viral-transduction to determine the efficacy of FGL1 in mediating prion-like proteins spreading and disease progression.

[0122] **RESULTS**

[0123] **The expression of FGL1 decreases with aging**

[0124] Previous studies have shown that FGL1 is aging-related. We tested the expression of FGL1 in livers and serum from young (3-month-old) and aged (20-month-old) mice by western blot (Fig. 1A) and ELISA (Fig. 1C), respectively. The results showed significant decreased protein level of FGL1 with immunoblot (Fig. 1B) and ELISA (Fig. 1C) from WT aged mice, compared to WT young mice. Furthermore, we assessed the FGL1 levels in the plasma and brain by HBE, and determined that FGL1 levels in the plasma and brain were significantly increased by HBE (Fig. 1D, E).

[0125] **FGL1 effectively inhibits neuronal uptake of α-syn PFF and consequential pathology propagation via Lag3**

[0126] Because FGL1 is the major ligand of Lag3, we then sought to determine if FGL1 can inhibit α-syn PFF binding to Lag3. We first purified recombinant FGL1 following an established protocol, and co-treated FGL1 (5, 10, 20, 40 nM) and biotin-labeled α-syn PFF (α-syn PFF-biotin, 63 nM) into the Lag3-expressing SH-SY5Y

cells for this well-established cell surface binding assay. The results show that FGL1 can significantly inhibit α-syn PFF-biotin binding to Lag3 (Fig. 2A, B). We further showed that recombinant FGL1 significantly decreased the neuronal uptake of α-syn PFF and the pS129 level in a dose-dependent manner (Fig. 2D, E). It is clearly that 30 and 150 nM FGL1 can effectively inhibit the neuronal uptake of α-syn PFF-biotin (Fig. 2E).

[0127] FGL1 is brain permeable and aging-dependent mediator of α-synucleinopathy

[0128] Because FGL1 is mainly secreted from the liver, we further tested whether plasma FGL1 can penetrate into the brain. We performed the intraperitoneal (i.p.) injection of recombinant FGL1 (10 mg/kg), and assessed the level of FGL1 in the cerebral spinal fluid (CSF) at 3, 7, 10 days afterwards (Fig. 3A). The CSF level of FGL1 reached high level at 7 days (Fig. 3B), exhibiting much higher brain penetration ratio to what has been reported with other proteins that cross the blood-brain barrier.

[0129] We injected α-syn PFF (5 μg per mouse) in striatum and Adeno Associated Virus (AAV)-FGL1 (1.01×10^{13} vg/mouse) in lateral ventricles (Fig. 4A). After 30 days, we tested the pathological α-synuclein with antibody pS129 by immunofluorescence in the cerebral cortex. Compared to the AAV-control group, there are more FGL1 (Fig. 4B, C) and less pS129 signal in the AAV-FGL1 injection group (Fig. 4D, E).

[0130] Furthermore, we cultured the primary cortical neurons and then treated AAV-FGL1 at DIV5 and α-syn PFF at DIV7. After another 7 days, we tested the pathological α-synuclein with antibody pS129 by immunofluorescence (Fig. 5A). The result showed that there was less positive signals in AAV-FGL1 treated group compared the AAV-control group (Fig. 5B-G). Taken *in vivo* and *in vitro* results together, they provide evidence for FGL1 inhibiting the pathological α-synuclein spreading as a treatment molecule.

[0131] Heterochronic blood exchange (HBE) can substantially inhibit α-syn pathology propagation in the brain

[0132] In the parabiosis design (Fig. 6A), we have heteroparabiosis between aged WT and young WT (aged-WT/young-WT) mice following with the intrastriatal-injection of α-syn PFF, compared to single aged WT mice, aged-WT/young-FGL1$^{-/-}$ mice pair. One month after the parabiosis surgery, we performed the stereotaxic injection of α-syn PFF into the dorsal striatum of aged mice with and without HBE. Two-month after PFF injection, substantial immunoreactivity of anti-pS129 can be observed in indicated brain regions of WT aged mice with α-syn PFF inoculation (Fig. 6B-D). Strikingly, HBE can significantly reduce pS129 signal in the indicated brain regions of aged-WT/young-WT, including the cortex, amygdala (Fig. 6E) compared to aged-WT mice.

[0133] **FGL1 can be packaged into exosome vesicles (EVs)**

[0134] EVs have been found as a potential carrier for the delivery of proteins, RNAs and chemical compounds to various organs. In particular, EVs have shown capability to cross the blood brain barrier and deliver therapeutic loads to the brain, thus providing a potential platform for the development of brain disease therapy.

[0135] EVs can be purified from various human cells (e.g. induced pluripotent stem (iPS) cells, mesenchymal stem cells, etc.) and other resources. Here, we provided an example to support the use of EVs derived from human iPS cells to package the FGL1 protein. We have established a pipeline to manufacture human iPS cell-derived EVs with a panel of quality control assays (Fig. 7). We established a strategy to achieve enforced FGL1 expression in human iPS cells using cell transfection by synthetic mRNAs coding the FGL1 gene (Fig. 8). Furthermore, we neon transfected plasmid FGL1 in iPS cells. After 72 hours, we tested the expression of FGL1 in the medium and exosome by ELISA (Fig. 9A). And then, we compared the ratio of FGL1 in exosome and in secreted (Fig. 9B). This novel strategy successfully generated EVs containing the FGL1 protein, which will allow the delivery of FGL1 proteins to cross the blood-brain barrier, and further block the uptake of α-syn PFF and following pathogenic processes.

[0136] **CONCLUSION**

[0137] A major finding of this example is that FGL1 elevation via parabiosis and viral-transduction can inhibit pathogenic α-syn spreading.

[0138] Parabiosis can alleviate the cognitive deficits, but the underlying mechanism is not clear.

[0139] FGL1 can reduce neuronal uptake and microglial activation via Lag3, which further validates the Lag3-related studies in α-synucleinopathy.

[0140] **METHODS**

[0141] ***Animals***

[0142] C57BL/6 mice were obtained from the Jackson Laboratory. All animal housing, breeding, and procedures were performed in accordance with the NIH Guide for the Care and Use of Experimental Animals and approved by Johns Hopkins University Animal Care and Use Committee.

[0143] ***Stereotaxic injection of AAV-FGL1 and α-syn PFF***

[0144] C57BL/6 mice (10–12 weeks old) were used for intrastriatal injections. α-syn PFF (5.0 mg/ml) were prepared and sonicated before injection. Mice were deeply anesthetized with ketamine (100 mg/kg) and xylazine (10 mg/kg). α-syn PFF (5 μg per animal) was injected into the striatum (coordinates: anteroposterior (AP) = +0.2 mm, mediolateral (ML) = +2.0 mm, dorsoventral (DV) = +2.6 mm relative to bregma). AAV-FGL1 (1.01×10^{13} vg/mouse) injection were performed using a 2 μl syringe at a rate of 0.4 μl/min into the lateral ventricle (coordinates: anteroposterior (AP) = -0.3 mm, mediolateral (ML) = +1.2 mm, dorsoventral (DV) = +4 mm relative to bregma), and the needle was left in place for an additional 5 min before slow withdrawal. Mice were monitored after surgery and 27 post-surgical care was provided. For histological studies, mouse brains were removed after transcardial perfusion with 4% PFA in 0.1 M sodium phosphate buffer (pH 7.2), followed by post-fixation in 4% PFA overnight and transfer to 30% sucrose, before being processed for serial sections.

[0145] ***Immunohistochemistry***

[0146] Serial brain sections (30 μm) were prepared using microtome (Micro HM450, Thermo Fisher Scientific). For the immunohistochemical detection of α-syn phosphorylation, sections were blocked with PBS containing 10% normal goat serum and 0.2% Triton X-100 for 1 hour, and then incubated with primary antibodies to p-α-syn (610787, BD), and FGL1 (215824, LifeSpan BioSciences) overnight at 4°C,

followed by incubations with appropriate fluorescent secondary antibodies conjugated to Alexa-fluor 488, 594 and DAPI. Detailed mapping of p-α-syn pathology was performed by tracing all visible immunoreactive inclusions/cells using microscope (Zeiss). Images were processed using Zen software 28 (Carl Zeiss). The signal intensity was measured using ImageJ.

[0147] ***Cell culture and neuronal treatment***

[0148] Primary culture of cortical neurons were prepared from 15.5-day-old embryo mouse brains. Neurons were plated on 48-well-plate coated with poly-L-orithine, at 37° C under 5% CO_2 in a humidified incubator. The Neurobasal, supplemented with 0.5 mM L-glutamine, B27 supplement, penicillin and streptomycin was used for culture. AAV-FGL1 ($1.01x10^{13}$ vg) was added to primary neuronal culture at DIV 5, and α-Syn PFF (5.0 μg/ml) was added at DIV 7. At DIV 14, the pathological α-synuclein (610787, BD Biosciences) and neuron (3854475, Millipore) was tested by immunofluorescence. Images were obtained by microscopy (Carl Zeiss). The images were analyzed and quantification by ImageJ.

[0149] ***PFF-endocytosis of primary Neuron***

[0150] For primary neuron culture, prepare at least 1x 10^6 cells (=1well of 6 well plate) and culture 14 days. FGL1-human Ig (0/3/30/150 nM), and mouse α-syn PFF-biotin (1μM) were treated cells. After 2 hours, the cells were fixed and stained for MAP2 (2896049, Invitrogen) and biotin (SA-647). Images were obtained by confocal scanning microscopy (Carl Zeiss). The images were analyzed and quantification by ImageJ.

[0151] ***Brain penetration of recombinant FGL1***

[0152] Recombinant FGL1 was labeled with a near infrared (IR) dye IRDye680RD. The FGL1 solution was mixed with IRDye680RD NHS Ester (molar ratio 1:2) for 16 hr shaking at 4C℃, followed with dialyzed with deionized water for 24 hr.

[0153] ***Plasmid FGL1 neon transfected in the iPS cells***

[0154] The plasmid of FGL1 (5 μg) was neon transfected (1100v, 30ms, 1 pulse) in the $1x\ 10^6$ iPS cells. After 72 hours, collected the medium and isolated exosome for ELISA test.

[0155] Some further aspects are also defined in the following clauses:

[0156] Clause 1: A method of treating a proteinopathy in a subject. The method comprising administering an effective amount of a fibrinogen-like protein 1 (FGL1), or a functional portion or variant thereof, to the subject having the proteinopathy, which effective amount elevates a level of the FGL1, or the functional portion or variant thereof, in a brain of the subject sufficient to inhibit a binding interaction between a pathogenic protein associated with the proteinopathy and a lymphocyte-activation protein 3 (LAG3) present in the brain of the subject, thereby treating the proteinopathy in the subject.

[0157] Clause 2: The method of Clause 1, wherein the proteinopathy comprises an α-synucleinopathy.

[0158] Clause 3: The method of Clause 1 or Clause 2, wherein the subject is a human subject.

[0159] Clause 4: The method of any of Clauses 1-3, wherein the administering step comprises using an administration technique is selected from the group consisting of: a blood exchange, a stereotaxic injection, an intrastriatal injection, an intramuscular injection, an intradermal injection, a transmucosal injection, a subcutaneous injection, and an intravenous injection.

[0160] Clause 5: The method of any of Clauses 1-4, wherein a cell comprises the LAG3.

[0161] Clause 6: The method of any of Clauses 1-5, wherein the cell comprises a mammalian cell.

[0162] Clause 7: The method of any of Clauses 1-6, wherein the cell comprises a neuronal cell.

[0163] Clause 8: The method of any of Clauses 1-7, wherein the effective amount of the FGL1, or the functional portion or variant thereof, inhibits uptake of the pathogenic protein into the cell and/or inhibits spreading of the pathogenic protein to other cells in the brain of the subject.

[0164] Clause 9: The method of any of Clauses 1-8, wherein an aggregation comprises the pathogenic protein.

[0165] Clause 10: The method of any of Clauses 1-9, wherein the pathogenic protein comprises a prion-like protein.

[0166] Clause 11: The method of any of Clauses 1-10, wherein the prion-like protein comprises a monomeric α-synuclein protein (α-syn), an oligomeric α-syn, a preformed-fibrillar (PFF) α-syn, a functional portion or variant thereof, or any combination thereof.

[0167] Clause 12: The method of any of Clauses 1-11, wherein the prion-like protein comprises a monomeric a microtubule-associated protein tau (tau), an oligomeric tau, a preformed-fibrillar (PFF) tau, a functional portion or variant thereof, or any combination thereof.

[0168] Clause 13: The method of any of Clauses 1-12, wherein the FGL1, or the functional portion or variant thereof, is human.

[0169] Clause 14: The method of any of Clauses 1-13, wherein the FGL1, or the functional portion or variant thereof, comprises a recombinant FGL1, or functional portion or variant thereof, or an exogenous FGL1, or functional portion or variant thereof.

[0170] Clause 15: The method of any of Clauses 1-14, wherein the FGL1, or the functional portion or variant thereof, comprises a splice variant.

[0171] Clause 16: The method of any of Clauses 1-15, wherein a vector comprises the FGL1, or the functional portion or variant thereof.

[0172] Clause 17: The method of any of Clauses 1-16, wherein the vector is selected from the group consisting of: an exosome vesicle, a liposome, a lipid nanocrystal, and a lipid nanoparticle.

[0173] Clause 18: The method of any of Clauses 1-17, wherein a vector comprises a synthetic polynucleotide encoding the FGL1, or the functional portion or variant thereof, and wherein the method comprises administering the vector to the subject such that the synthetic polynucleotide is expressed sufficiently to produce the effective amount of the FGL1, or the functional portion or variant thereof, in the brain of the subject.

[0174] Clause 19: The method of any of Clauses 1-18, wherein an expression cassette comprises the synthetic polynucleotide.

[0175] Clause 20: The method of any of Clauses 1-19, wherein the vector is selected from the group consisting of: a virus, a cell, an exosome vesicle, a plasmid, a phagemid, a cosmid, a liposome, an expression cassette, a lipid nanocrystal, a lipid nanoparticle, and an artificial chromosome.

[0176] Clause 21: A method of inhibiting a binding interaction. The method comprising contacting an effective amount of a fibrinogen-like protein 1 (FGL1), or a functional portion or variant thereof, with a composition that comprises a pathogenic protein and a lymphocyte-activation protein 3 (LAG3), wherein the effective amount of the FGL1, or the functional portion or variant thereof, reduces binding of the pathogenic protein to the LAG3 in the composition relative to the composition in the absence of the FGL1, or the functional portion or variant thereof, thereby inhibiting the binding interaction.

[0177] Clause 22: The method of Clause 21, wherein the composition is disposed *in vitro*.

[0178] Clause 23: The method of Clause 21 or Clause 22, wherein the composition is disposed *in vivo*.

[0179] Clause 24: The method of any of Clauses 21-23, wherein a brain of a subject comprises the composition.

[0180] Clause 25: The method of any of Clauses 21-24, wherein the subject is a human subject.

[0181] Clause 26: The method of any of Clauses 21-25, wherein the subject comprises a proteinopathy associated with the pathogenic protein.

[0182] Clause 27: The method of any of Clauses 21-26, wherein the proteinopathy comprises an α-synucleinopathy.

[0183] Clause 28: The method of any of Clauses 21-27, wherein the effective amount of the FGL1, or the functional portion or variant thereof, substantially treats the proteinopathy in the subject.

[0184] Clause 29: The method of any of Clauses 21-28, wherein the contacting step comprises administering the effective amount of the FGL1, or the functional portion or variant thereof, to the subject using at least one administration technique.

[0185] Clause 30: The method of any of Clauses 21-29, wherein the administration technique is selected from the group consisting of: a blood exchange, a stereotaxic injection, an intrastriatal injection, an intramuscular injection, an intradermal injection, a transmucosal injection, a subcutaneous injection, and an intravenous injection.

[0186] Clause 31: The method of any of Clauses 21-30, wherein a cell comprises the LAG3.

[0187] Clause 32: The method of any of Clauses 21-31, wherein the cell comprises a mammalian cell.

[0188] Clause 33: The method of any of Clauses 21-32, wherein the cell comprises a neuronal cell.

[0189] Clause 34: The method of any of Clauses 21-33, wherein the effective amount of the FGL1, or the functional portion or variant thereof, inhibits uptake of the pathogenic protein into the cell and/or inhibits spreading of the pathogenic protein to other cells in the composition.

[0190] Clause 35: The method of any of Clauses 21-34, wherein an aggregation comprises the pathogenic protein.

[0191] Clause 36: The method of any of Clauses 21-35, wherein the pathogenic protein comprises a prion-like protein.

[0192] Clause 37: The method of any of Clauses 21-36, wherein the prion-like protein comprises a monomeric α-synuclein protein (α-syn), an oligomeric α-syn, a preformed-fibrillar (PFF) α-syn, a functional portion or variant thereof, or any combination thereof.

[0193] Clause 38: The method of any of Clauses 21-37, wherein the prion-like protein comprises a monomeric a microtubule-associated protein tau (tau), an oligomeric tau, a preformed-fibrillar (PFF) tau, a functional portion or variant thereof, or any combination thereof.

[0194] Clause 39: The method of any of Clauses 21-38, wherein the FGL1, or the functional portion or variant thereof, is human.

[0195] Clause 40: The method of any of Clauses 21-39, wherein the FGL1, or the functional portion or variant thereof, comprises a recombinant FGL1, or functional

portion or variant thereof, or an exogenous FGL1, or functional portion or variant thereof.

[0196] Clause 41: The method of any of Clauses 21-40, wherein the FGL1, or the functional portion or variant thereof, comprises a splice variant.

[0197] Clause 42: The method of any of Clauses 21-41, wherein a vector comprises the FGL1, or the functional portion or variant thereof.

[0198] Clause 43: The method of any of Clauses 21-42, wherein the vector is selected from the group consisting of: an exosome vesicle, a liposome, a lipid nanocrystal, and a lipid nanoparticle.

[0199] Clause 44: The method of any of Clauses 21-43, wherein a vector comprises a synthetic polynucleotide encoding the FGL1, or the functional portion or variant thereof, and wherein the method comprises contacting the vector with the composition such that the synthetic polynucleotide is expressed sufficiently to produce the effective amount of the FGL1, or the functional portion or variant thereof.

[0200] Clause 45: The method of any of Clauses 21-44, wherein an expression cassette comprises the synthetic polynucleotide.

[0201] Clause 46: The method of any of Clauses 21-45, wherein the vector is selected from the group consisting of: a virus, a cell, an exosome vesicle, a plasmid, a phagemid, a cosmid, a liposome, an expression cassette, a lipid nanocrystal, a lipid nanoparticle, and an artificial chromosome.

[0202] Clause 47: A method of inhibiting a binding interaction. The method comprising contacting an effective amount of an age-related rejuvenation factor, or a functional portion or variant thereof, with a composition that comprises a pathogenic protein and a lymphocyte-activation protein 3 (LAG3), wherein the effective amount of the age-related rejuvenation factor, or the functional portion or variant thereof, reduces binding of the pathogenic protein to the LAG3 in the composition relative to the composition in the absence of the age-related rejuvenation factor, or the functional portion or variant thereof, thereby inhibiting the binding interaction.

[0203] Clause 48: The use of a fibrinogen-like protein 1 (FGL1), or a functional portion or variant thereof, or a synthetic polynucleotide encoding the FGL1, or the

functional portion or variant thereof, for the manufacture of a medicament for the therapeutic and/or prophylactic treatment of a proteinopathy.

[0204] Clause 49: A method of forming exosome vesicles (EVs). The method comprising: transfecting one or more induced pluripotent stem (iPS) cells with a synthetic polynucleotide encoding a fibrinogen-like protein 1 (FGL1), or a functional portion or variant thereof, such that the iPS cells express the FGL1, or the functional portion or variant thereof, to produce transfected iPS cells; and purifying one or more EVs that comprise the FGL1, or the functional portion or variant thereof, from the transfected iPS cells to produce purified EVs that comprise the FGL1, or the functional portion or variant thereof, thereby forming the EVs.

[0205] Clause 50: The method of Clause 49, wherein the iPS cells comprise human iPS cells.

[0206] Clause 51: The transfected iPS cells produced by the method of Clause 49 or Clause 50.

[0207] Clause 52: The purified EVs that comprise the FGL1, or the functional portion or variant thereof, produced by the method of Clause 49 or Clause 50.

[0208] Clause 53: A pharmaceutical composition comprising the purified EVs that comprise the FGL1, or the functional portion or variant thereof, produced by the method of Clause 49 or Clause 50.

[0209] Clause 54: A method of generating a recombinant adeno-associated virus (AAV) comprising an AAV capsid. The method comprising: culturing a host cell containing: (a) a nucleic acid sequence encoding an AAV capsid protein; (b) a functional rep gene; (c) a minigene comprising AAV inverted terminal repeats (ITRs) and a transgene encoding a fibrinogen-like protein 1 (FGL1), or a functional portion or variant thereof; and (d) sufficient helper functions to permit packaging of the minigene into the AAV capsid; and purifying the AAV capsid from the host cell, thereby generating the recombinant AAV comprising the AAV capsid.

[0210] Clause 55: The AAV capsid produced by the method of Clause 54.

[0211] Clause 56: A pharmaceutical composition comprising the AAV capsid produced by the method of Clause 54.

[0212] Clause 57: A method of predicting a risk of a proteinopathy in a subject. The method comprising: determining a fibrinogen-like protein 1 (FGL1) expression level in a sample obtained from a subject, which sample is indicative of the FGL1 expression level in a brain of the subject; and identifying the subject as likely to develop, or having, the proteinopathy when the FGL1 expression level in the brain of the subject is below a control expression level, or identifying the subject as unlikely to develop, or not having, the proteinopathy when the FGL1 expression level in the brain of the subject is above the control expression level, thereby the predicting the risk of the proteinopathy in the subject.

[0213] Clause 58: The method of Clause 57, comprising administering an effective amount of an exogenous FGL1, or a functional portion or variant thereof, to the subject when the FGL1 expression level in the brain of the subject is below the control expression level, which effective amount elevates a level of the exogenous FGL1, or the functional portion or variant thereof, in the brain of the subject sufficient to inhibit a binding interaction between a pathogenic protein associated with the proteinopathy and a lymphocyte-activation protein 3 (LAG3) present in the brain of the subject.

[0214] Clause 59: A pharmaceutical composition comprising a fibrinogen-like protein 1 (FGL1), or a functional portion or variant thereof, of use in the treatment of a proteinopathy in a subject.

[0215] Clause 60: The pharmaceutical composition of Clause 59, comprising exosome vesicles (EVs) that comprise the FGL1, or the functional portion or variant thereof.

[0216] Clause 61: The pharmaceutical composition of Clause 59, comprising adeno-associated virus (AAV) capsids that comprise a transgene encoding the FGL1, or the functional portion or variant thereof.

[0217] Clause 62: The pharmaceutical composition of Clause 59, comprising one or more blood components suitable for administering the pharmaceutical composition to the subject via blood exchange.

[0218] Clause 63: The pharmaceutical composition of any of Clauses 59-62, wherein the proteinopathy comprises Alzheimer's disease (AD), Parkinson's disease (PD), and/or Lewy body dementia (LBD).

[0219] Clause 64: A kit comprising the pharmaceutical composition of any of Clauses 59-63.

[0220] All references, including publications, patent applications, and patents, cited herein are hereby incorporated by reference to the same extent as if each reference were individually and specifically indicated to be incorporated by reference and were set forth in its entirety herein.

[0221] The use of the terms “a” and “an” and “the” and similar referents in the context of describing the invention (especially in the context of the following claims) are to be construed to cover both the singular and the plural, unless otherwise indicated herein or clearly contradicted by context. The terms “comprising,” “having,” “including,” and “containing” are to be construed as open-ended terms (i.e., meaning “including, but not limited to,”) unless otherwise noted. Recitation of ranges of values herein are merely intended to serve as a shorthand method of referring individually to each separate value falling within the range, unless otherwise indicated herein, and each separate value is incorporated into the specification as if it were individually recited herein. All methods described herein can be performed in any suitable order unless otherwise indicated herein or otherwise clearly contradicted by context. The use of any and all examples, or exemplary language (e.g., “such as”) provided herein, is intended merely to better illuminate the invention and does not pose a limitation on the scope of the invention unless otherwise claimed. No language in the specification should be construed as indicating any non-claimed element as essential to the practice of the invention.

[0222] Preferred embodiments of this invention are described herein, including the best mode known to the inventors for carrying out the invention. Variations of those preferred embodiments may become apparent to those of ordinary skill in the art upon reading the foregoing description. The inventors expect skilled artisans to employ such variations as appropriate, and the inventors intend for the invention to be practiced otherwise than as specifically described herein. Accordingly, this

invention includes all modifications and equivalents of the subject matter recited in the claims appended hereto as permitted by applicable law. Moreover, any combination of the above-described elements in all possible variations thereof is encompassed by the invention unless otherwise indicated herein or otherwise clearly contradicted by context.

Claims:

1. A method of treating a proteinopathy in a subject, the method comprising administering an effective amount of a fibrinogen-like protein 1 (FGL1), or a functional portion or variant thereof, to the subject having the proteinopathy, which effective amount elevates a level of the FGL1, or the functional portion or variant thereof, in a brain of the subject sufficient to inhibit a binding interaction between a pathogenic protein associated with the proteinopathy and a lymphocyte-activation protein 3 (LAG3) present in the brain of the subject, thereby treating the proteinopathy in the subject.

2. The method of claim 1, wherein the proteinopathy comprises an α-synucleinopathy.

3. The method of claim 1, wherein the subject is a human subject.

4. The method of claim 1, wherein the administering step comprises using an administration technique is selected from the group consisting of: a blood exchange, a stereotaxic injection, an intrastriatal injection, an intramuscular injection, an intradermal injection, a transmucosal injection, a subcutaneous injection, and an intravenous injection.

5. The method of claim 1, wherein a cell comprises the LAG3.

6. The method of claim 5, wherein the cell comprises a mammalian cell.

7. The method of claim 5, wherein the cell comprises a neuronal cell.

8. The method of claim 5, wherein the effective amount of the FGL1, or the functional portion or variant thereof, inhibits uptake of the pathogenic protein into

the cell and/or inhibits spreading of the pathogenic protein to other cells in the brain of the subject.

9. The method of claim 1, wherein an aggregation comprises the pathogenic protein.

10. The method of claim 1, wherein the pathogenic protein comprises a prion-like protein.

11. The method of claim 10, wherein the prion-like protein comprises a monomeric α-synuclein protein (α-syn), an oligomeric α-syn, a preformed-fibrillar (PFF) α-syn, a functional portion or variant thereof, or any combination thereof.

12. The method of claim 10, wherein the prion-like protein comprises a monomeric a microtubule-associated protein tau (tau), an oligomeric tau, a preformed-fibrillar (PFF) tau, a functional portion or variant thereof, or any combination thereof.

13. The method of claim 1, wherein the FGL1, or the functional portion or variant thereof, is human.

14. The method of claim 1, wherein the FGL1, or the functional portion or variant thereof, comprises a recombinant FGL1, or functional portion or variant thereof, or an exogenous FGL1, or functional portion or variant thereof.

15. The method of claim 1, wherein the FGL1, or the functional portion or variant thereof, comprises a splice variant.

16. The method of claim 1, wherein a vector comprises the FGL1, or the functional portion or variant thereof.

17. The method of claim 16, wherein the vector is selected from the group consisting of: an exosome vesicle, a liposome, a lipid nanocrystal, and a lipid nanoparticle.

18. The method of claim 1, wherein a vector comprises a synthetic polynucleotide encoding the FGL1, or the functional portion or variant thereof, and wherein the method comprises administering the vector to the subject such that the synthetic polynucleotide is expressed sufficiently to produce the effective amount of the FGL1, or the functional portion or variant thereof, in the brain of the subject.

19. The method of claim 18, wherein an expression cassette comprises the synthetic polynucleotide.

20. The method of claim 18, wherein the vector is selected from the group consisting of: a virus, a cell, an exosome vesicle, a plasmid, a phagemid, a cosmid, a liposome, an expression cassette, a lipid nanocrystal, a lipid nanoparticle, and an artificial chromosome.

21. A method of inhibiting a binding interaction, the method comprising contacting an effective amount of a fibrinogen-like protein 1 (FGL1), or a functional portion or variant thereof, with a composition that comprises a pathogenic protein and a lymphocyte-activation protein 3 (LAG3), wherein the effective amount of the FGL1, or the functional portion or variant thereof, reduces binding of the pathogenic protein to the LAG3 in the composition relative to the composition in the absence of the FGL1, or the functional portion or variant thereof, thereby inhibiting the binding interaction.

22. The method of claim 21, wherein the composition is disposed *in vitro*.

23. The method of claim 21, wherein the composition is disposed *in vivo*.

24. The method of claim 21, wherein a brain of a subject comprises the composition.

25. The method of claim 24, wherein the subject is a human subject.

26. The method of claim 24, wherein the subject comprises a proteinopathy associated with the pathogenic protein.

27. The method of claim 26, wherein the proteinopathy comprises an α-synucleinopathy.

28. The method of claim 26, wherein the effective amount of the FGL1, or the functional portion or variant thereof, substantially treats the proteinopathy in the subject.

29. The method of claim 24, wherein the contacting step comprises administering the effective amount of the FGL1, or the functional portion or variant thereof, to the subject using at least one administration technique.

30. The method of claim 29, wherein the administration technique is selected from the group consisting of: a blood exchange, a stereotaxic injection, an intrastriatal injection, an intramuscular injection, an intradermal injection, a transmucosal injection, a subcutaneous injection, and an intravenous injection.

31. The method of claim 21, wherein a cell comprises the LAG3.

32. The method of claim 31, wherein the cell comprises a mammalian cell.

33. The method of claim 31, wherein the cell comprises a neuronal cell.

34. The method of claim 31, wherein the effective amount of the FGL1, or the functional portion or variant thereof, inhibits uptake of the pathogenic protein into the cell and/or inhibits spreading of the pathogenic protein to other cells in the composition.

35. The method of claim 24, wherein an aggregation comprises the pathogenic protein.

36. The method of claim 21, wherein the pathogenic protein comprises a prion-like protein.

37. The method of claim 36, wherein the prion-like protein comprises a monomeric α-synuclein protein (α-syn), an oligomeric α-syn, a preformed-fibrillar (PFF) α-syn, a functional portion or variant thereof, or any combination thereof.

38. The method of claim 36, wherein the prion-like protein comprises a monomeric a microtubule-associated protein tau (tau), an oligomeric tau, a preformed-fibrillar (PFF) tau, a functional portion or variant thereof, or any combination thereof.

39. The method of claim 21, wherein the FGL1, or the functional portion or variant thereof, is human.

40. The method of claim 21, wherein the FGL1, or the functional portion or variant thereof, comprises a recombinant FGL1, or functional portion or variant thereof, or an exogenous FGL1, or functional portion or variant thereof.

41. The method of claim 21, wherein the FGL1, or the functional portion or variant thereof, comprises a splice variant.

42. The method of claim 21, wherein a vector comprises the FGL1, or the functional portion or variant thereof.

43. The method of claim 42, wherein the vector is selected from the group consisting of: an exosome vesicle, a liposome, a lipid nanocrystal, and a lipid nanoparticle.

44. The method of claim 21, wherein a vector comprises a synthetic polynucleotide encoding the FGL1, or the functional portion or variant thereof, and wherein the method comprises contacting the vector with the composition such that the synthetic polynucleotide is expressed sufficiently to produce the effective amount of the FGL1, or the functional portion or variant thereof.

45. The method of claim 44, wherein an expression cassette comprises the synthetic polynucleotide.

46. The method of claim 44, wherein the vector is selected from the group consisting of: a virus, a cell, an exosome vesicle, a plasmid, a phagemid, a cosmid, a liposome, an expression cassette, a lipid nanocrystal, a lipid nanoparticle, and an artificial chromosome.

47. A method of inhibiting a binding interaction, the method comprising contacting an effective amount of an age-related rejuvenation factor, or a functional portion or variant thereof, with a composition that comprises a pathogenic protein and a lymphocyte-activation protein 3 (LAG3), wherein the effective amount of the age-related rejuvenation factor, or the functional portion or variant thereof, reduces binding of the pathogenic protein to the LAG3 in the composition relative to the composition in the absence of the age-related rejuvenation factor, or the functional portion or variant thereof, thereby inhibiting the binding interaction.

48. The use of a fibrinogen-like protein 1 (FGL1), or a functional portion or variant thereof, or a synthetic polynucleotide encoding the FGL1, or the functional portion or variant thereof, for the manufacture of a medicament for the therapeutic and/or prophylactic treatment of a proteinopathy.

49. A method of forming exosome vesicles (EVs), the method comprising:

transfecting one or more induced pluripotent stem (iPS) cells with a synthetic polynucleotide encoding a fibrinogen-like protein 1 (FGL1), or a functional portion or variant thereof, such that the iPS cells express the FGL1, or the functional portion or variant thereof, to produce transfected iPS cells; and,

purifying one or more EVs that comprise the FGL1, or the functional portion or variant thereof, from the transfected iPS cells to produce purified EVs that comprise the FGL1, or the functional portion or variant thereof, thereby forming the EVs.

50. The method of claim 49, wherein the iPS cells comprise human iPS cells.

51. The transfected iPS cells produced by the method of claim 49.

52. The purified EVs that comprise the FGL1, or the functional portion or variant thereof, produced by the method of claim 49.

53. A pharmaceutical composition comprising the purified EVs that comprise the FGL1, or the functional portion or variant thereof, produced by the method of claim 49.

54. A method of generating a recombinant adeno-associated virus (AAV) comprising an AAV capsid, the method comprising:

culturing a host cell containing: (a) a nucleic acid sequence encoding an AAV capsid protein; (b) a functional rep gene; (c) a minigene comprising AAV inverted terminal repeats (ITRs) and a transgene encoding a fibrinogen-like protein 1 (FGL1),

or a functional portion or variant thereof; and (d) sufficient helper functions to permit packaging of the minigene into the AAV capsid; and,

purifying the AAV capsid from the host cell, thereby generating the recombinant AAV comprising the AAV capsid.

55. The AAV capsid produced by the method of claim 54.

56. A pharmaceutical composition comprising the AAV capsid produced by the method of claim 54.

57. A method of predicting a risk of a proteinopathy in a subject, the method comprising:

determining a fibrinogen-like protein 1 (FGL1) expression level in a sample obtained from a subject, which sample is indicative of the FGL1 expression level in a brain of the subject; and,

identifying the subject as likely to develop, or having, the proteinopathy when the FGL1 expression level in the brain of the subject is below a control expression level, or identifying the subject as unlikely to develop, or not having, the proteinopathy when the FGL1 expression level in the brain of the subject is above the control expression level, thereby the predicting the risk of the proteinopathy in the subject.

58. The method of claim 57, comprising administering an effective amount of an exogenous FGL1, or a functional portion or variant thereof, to the subject when the FGL1 expression level in the brain of the subject is below the control expression level, which effective amount elevates a level of the exogenous FGL1, or the functional portion or variant thereof, in the brain of the subject sufficient to inhibit a binding interaction between a pathogenic protein associated with the proteinopathy and a lymphocyte-activation protein 3 (LAG3) present in the brain of the subject.

59. A pharmaceutical composition comprising a fibrinogen-like protein 1 (FGL1), or a functional portion or variant thereof, of use in the treatment of a proteinopathy in a subject.

60. The pharmaceutical composition of claim 59, comprising exosome vesicles (EVs) that comprise the FGL1, or the functional portion or variant thereof.

61. The pharmaceutical composition of claim 59, comprising adeno-associated virus (AAV) capsids that comprise a transgene encoding the FGL1, or the functional portion or variant thereof.

62. The pharmaceutical composition of claim 59, comprising one or more blood components suitable for administering the pharmaceutical composition to the subject via blood exchange.

63. The pharmaceutical composition of claim 59, wherein the proteinopathy comprises Alzheimer's disease (AD), Parkinson's disease (PD), and/or Lewy body dementia (LBD).

64. A kit comprising the pharmaceutical composition of claim 59.

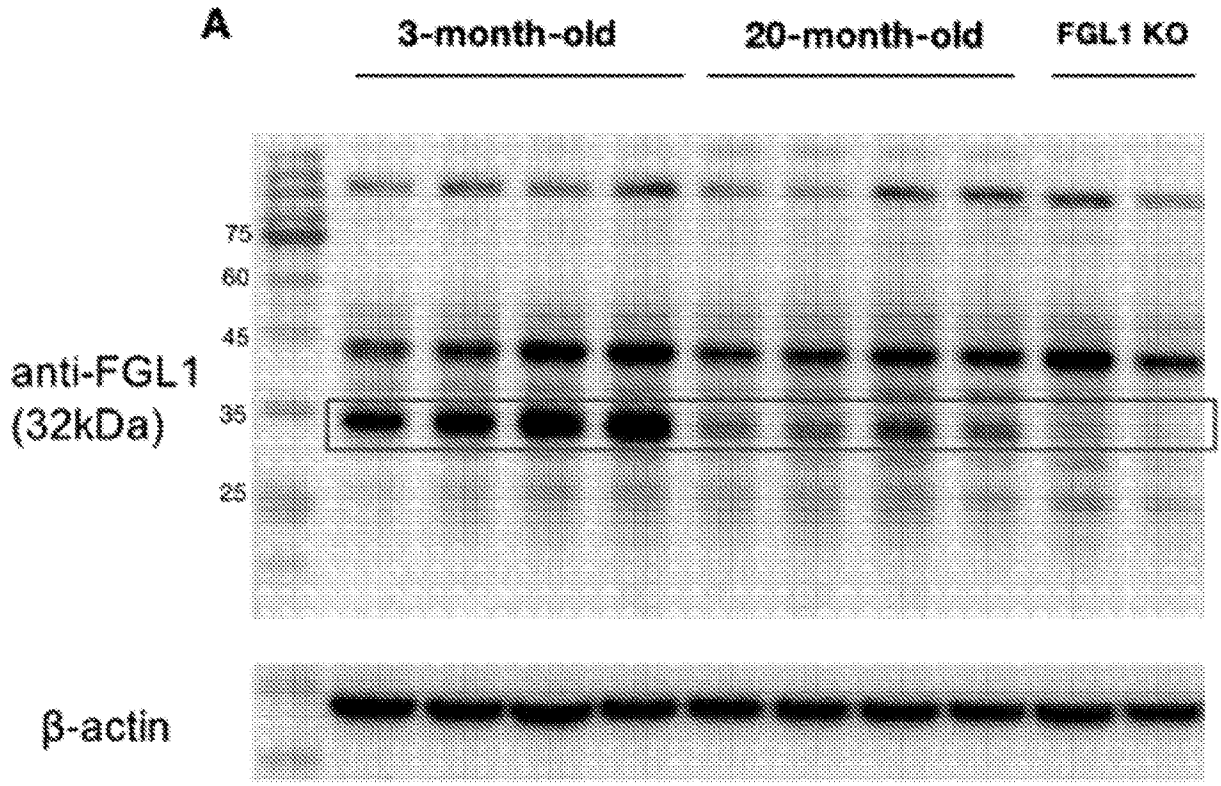
A
3-month-old
20-month-old
FGL1 KO
75
60
45
35
25
anti-FGL1
(32kDa)
β-actin

FIG. 1A

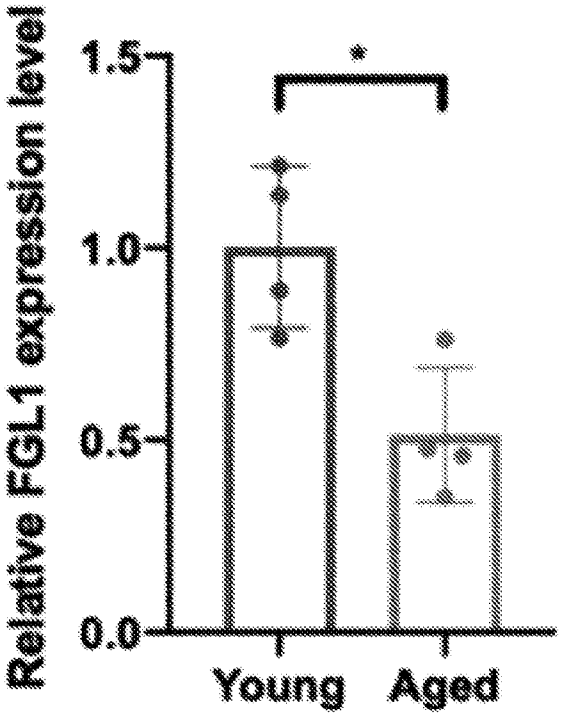
Relative FGL1 expression level
1.5
1.0
0.5
0.0
*
Young
Aged

FIG. 1B

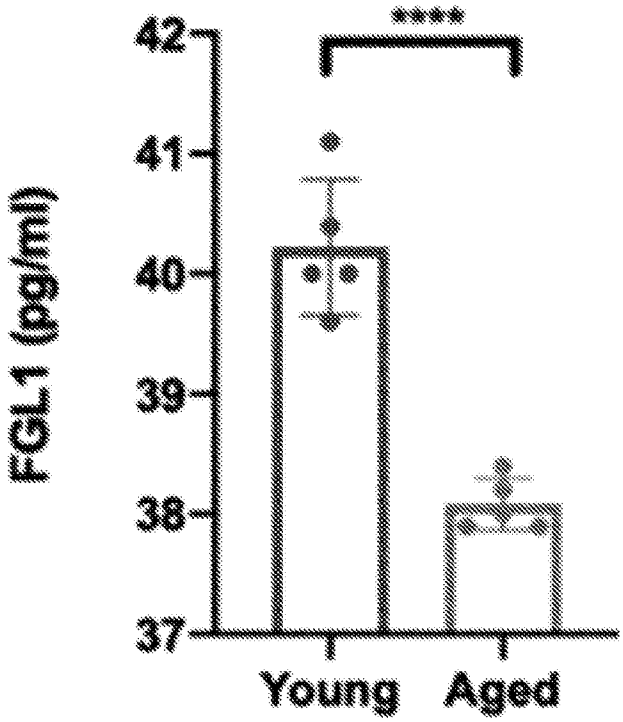
FGL1 (pg/ml)
42
41
40
39
38
37
****
Young
Aged

FIG. 1C

FIG. 1D

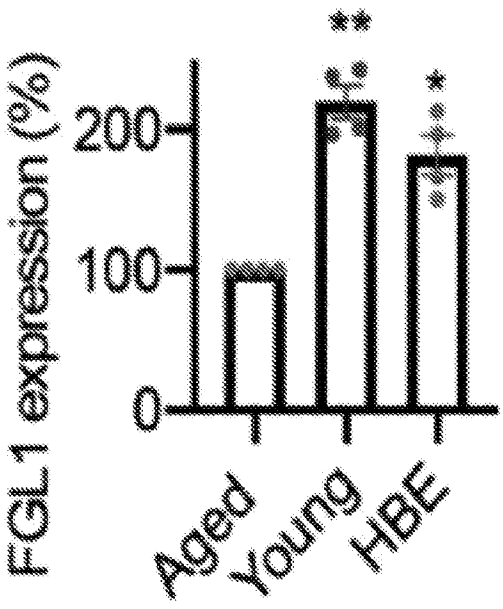

FIG. 1E

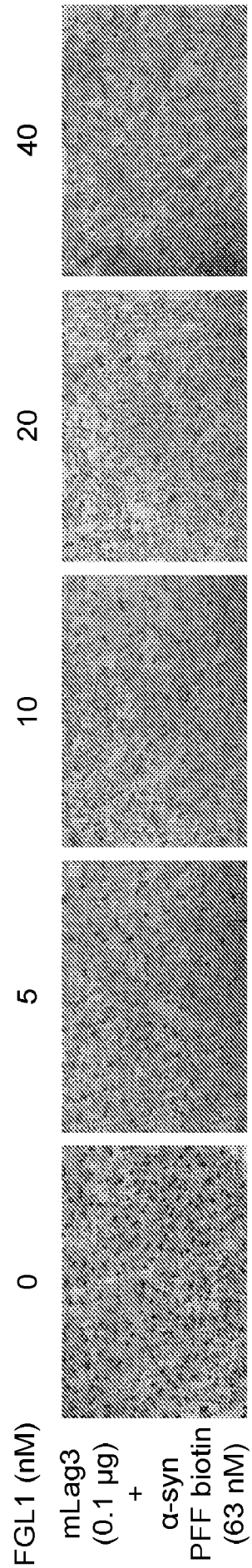

FIG. 2A

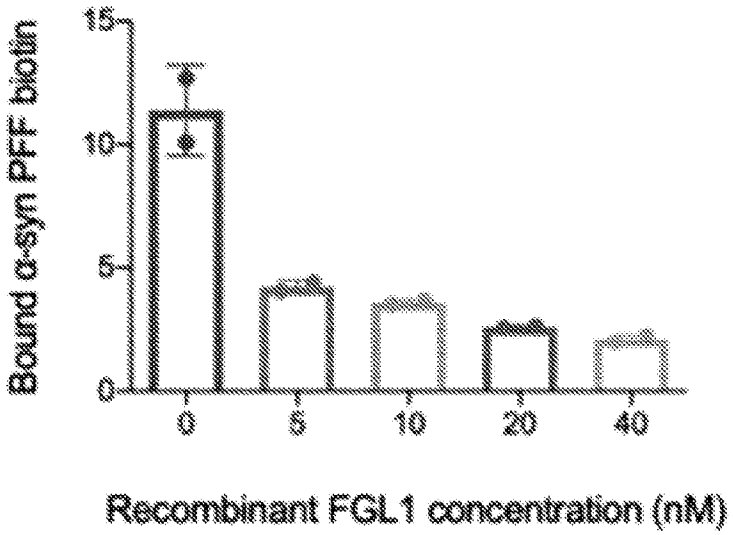
Bound α-syn PFF biotin
15
10
5
0
0
5
10
20
40
Recombinant FGL1 concentration (nM)

FIG. 2B


0 d
cell culture
14 d
FGL1-human Ig
α-syn PFF-biotin

FIG. 2C

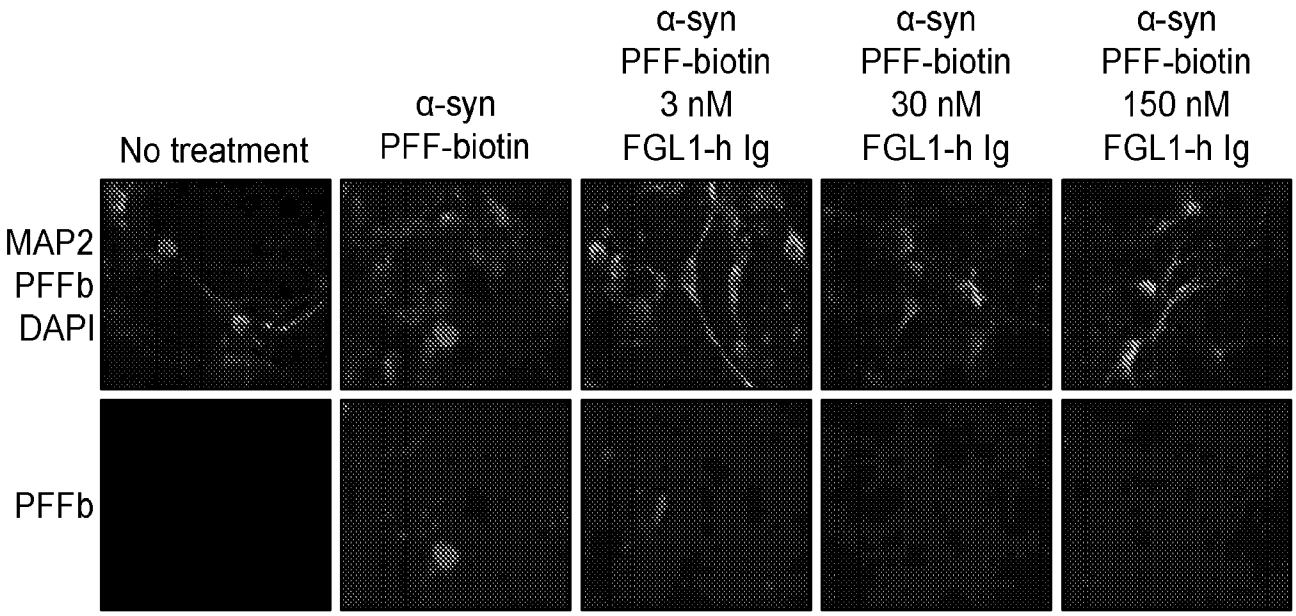
No treatment
α-syn PFF-biotin
α-syn PFF-biotin 3 nM FGL1-h Ig
α-syn PFF-biotin 30 nM FGL1-h Ig
α-syn PFF-biotin 150 nM FGL1-h Ig
MAP2
PFFb
DAPI
PFFb

FIG. 2D

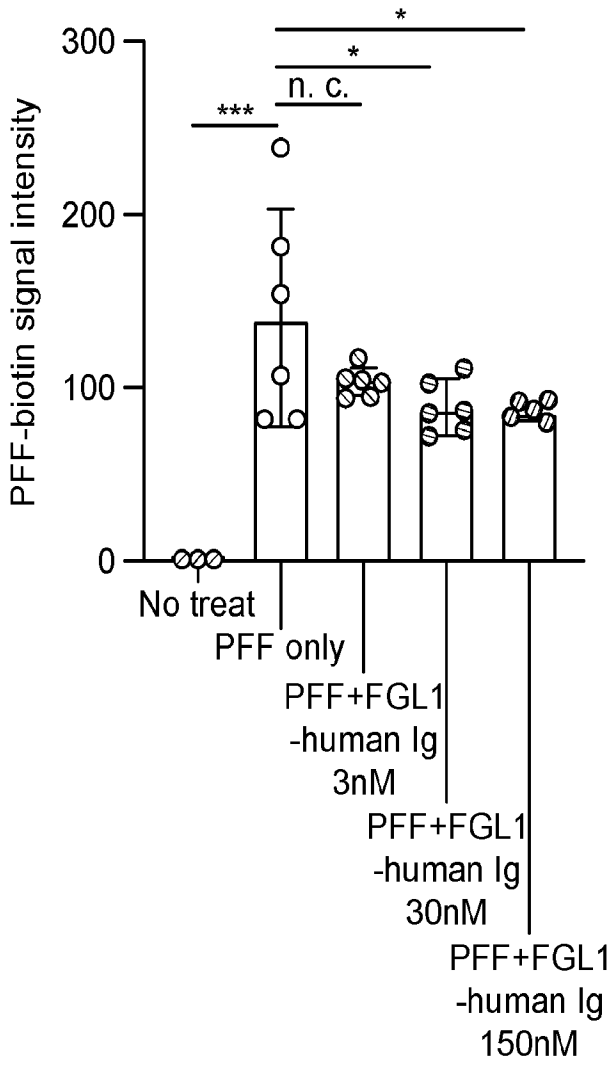
PFF-biotin signal intensity
300
200
100
0
***
n. c.
*
*
No treat
PFF only
PFF+FGL1 -human Ig 3nM
PFF+FGL1 -human Ig 30nM
PFF+FGL1 -human Ig 150nM

FIG. 2E

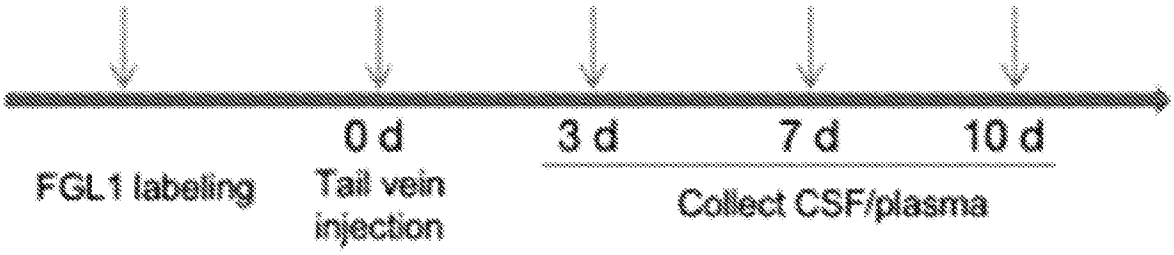
0 d
3 d
7 d
10 d
FGL1 labeling
Tail vein injection
Collect CSF/plasma

FIG. 3A

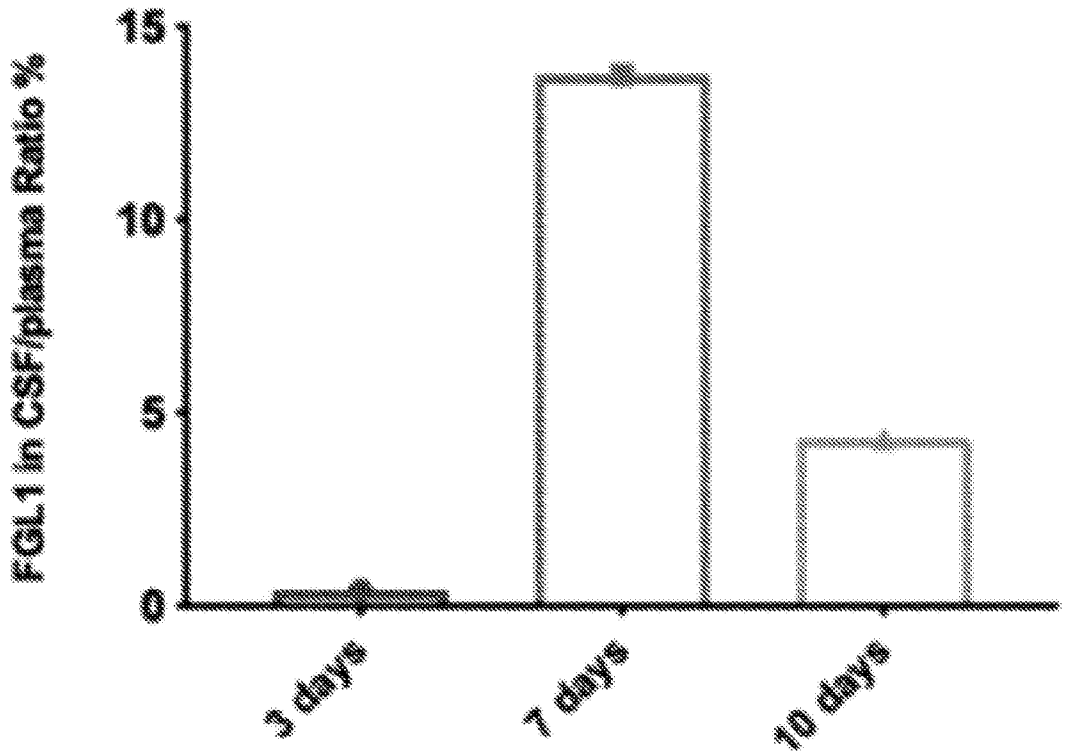
FGL1 in CSF/plasma Ratio %
15
10
5
0
3 days
7 days
10 days

FIG. 3B

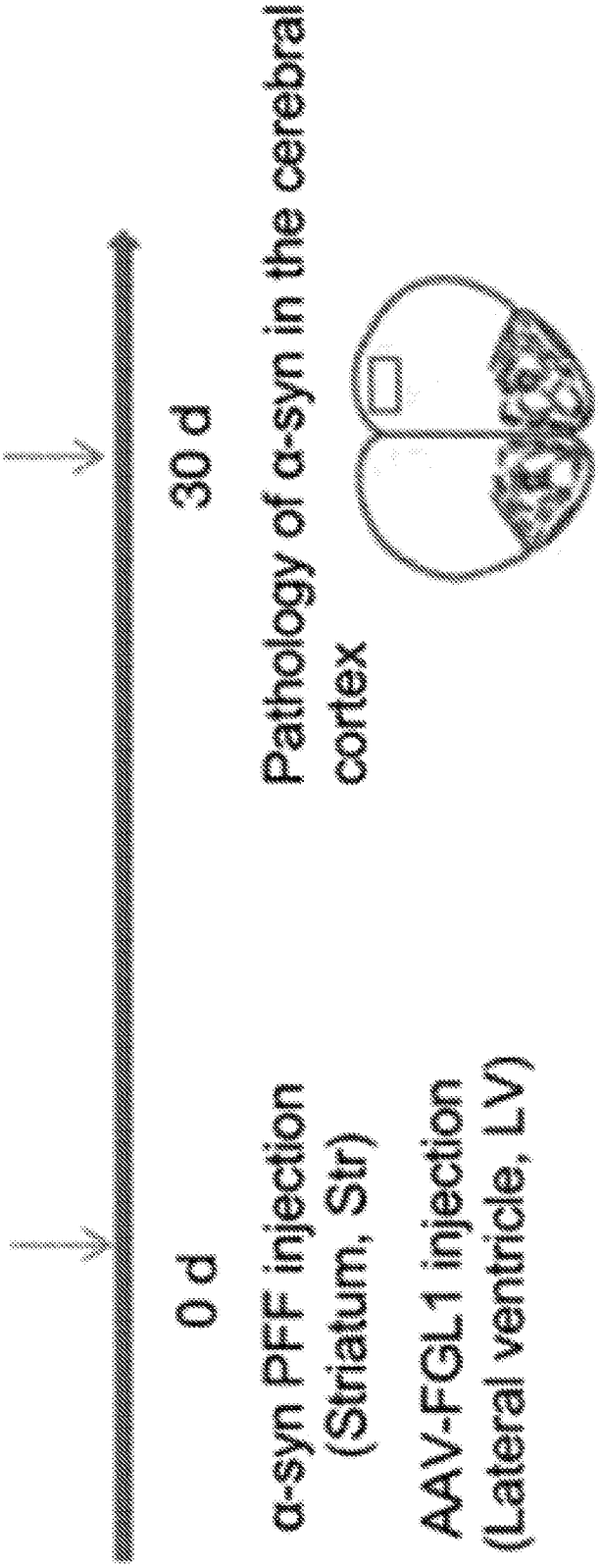
0 d
α-syn PFF injection
(Striatum, Str)
AAV-FGL1 injection
(Lateral ventricle, LV)
30 d
Pathology of α-syn in the cerebral cortex

FIG. 4A

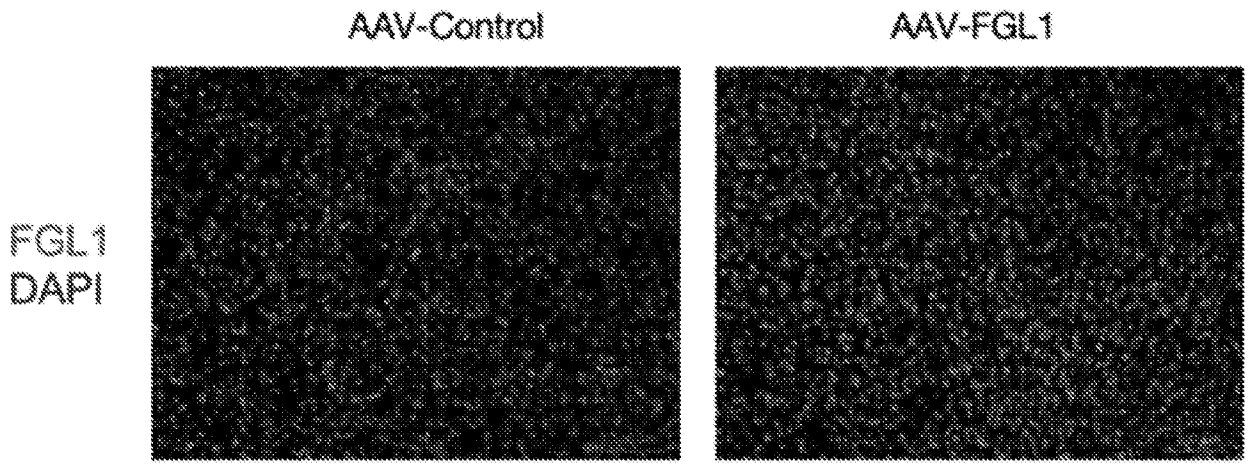
AAV-Control
AAV-FGL1
FGL1
DAPI

FIG. 4B

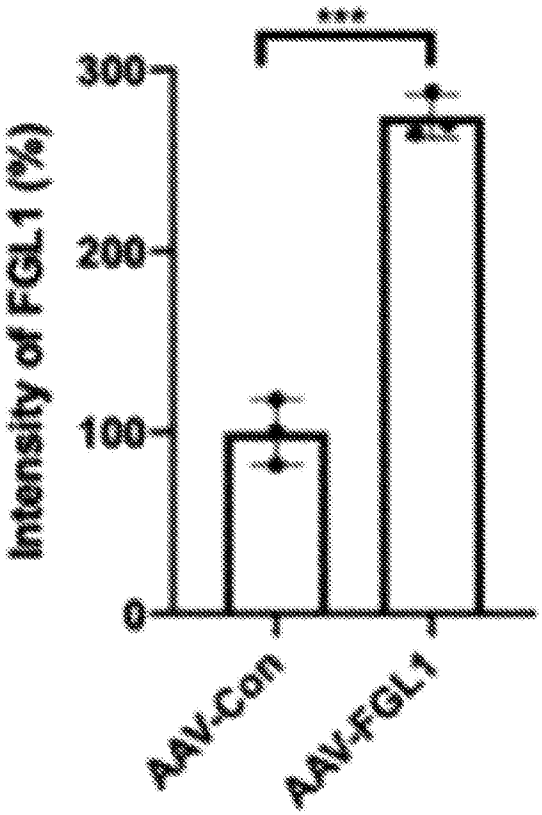
***
300
200
100
0
Intensity of FGL1 (%)
AAV-Con
AAV-FGL1

FIG. 4C

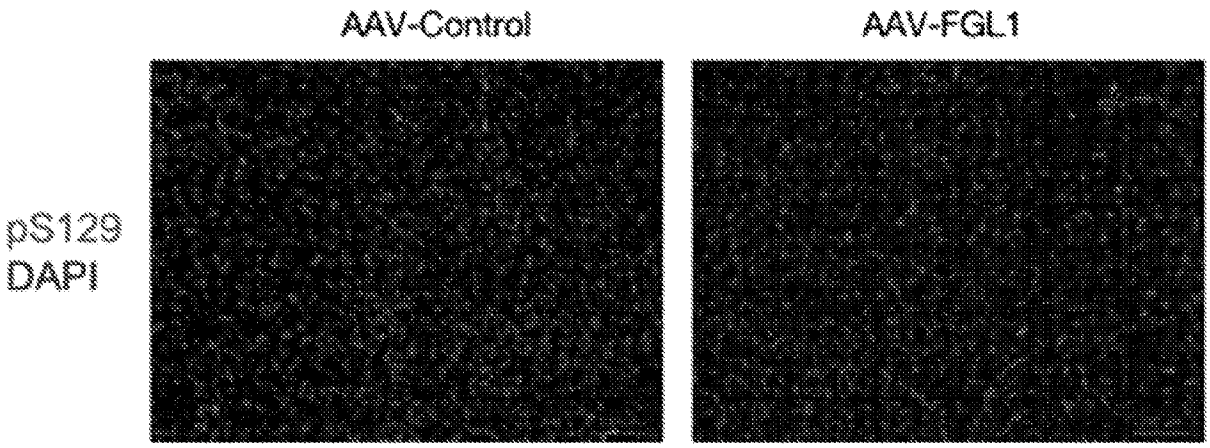
AAV-Control
AAV-FGL1
pS129
DAPI

FIG. 4D

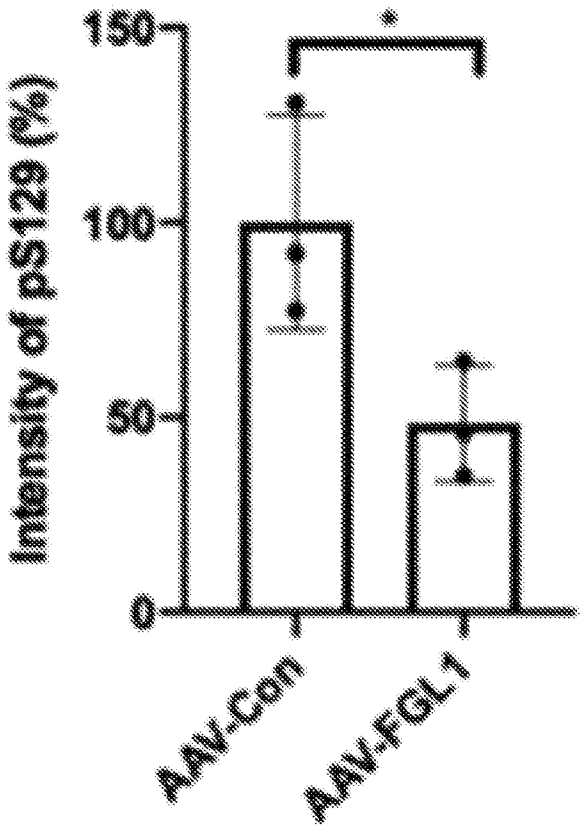
Intensity of pS129 (%)
150
100
50
0
*
AAV-Con
AAV-FGL1

FIG. 4E

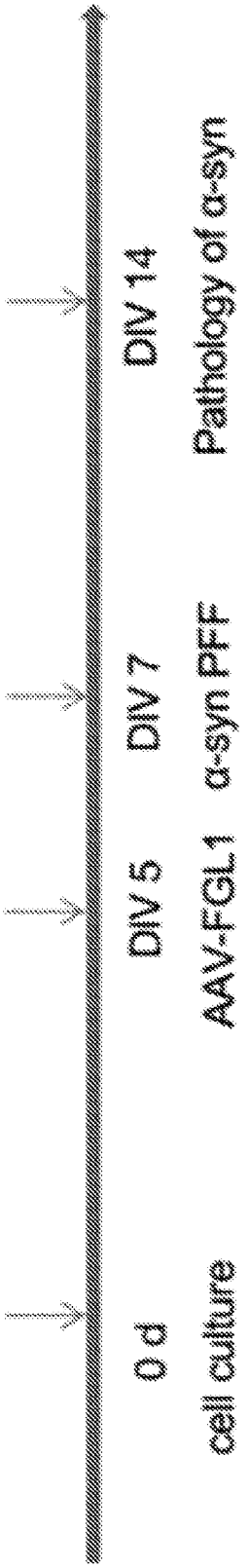
0 d
cell culture
DIV 5
AAV-FGL1
DIV 7
α-syn PFF
DIV 14
Pathology of α-syn

FIG. 5A

PBS

pS129
NeuN
DAPI

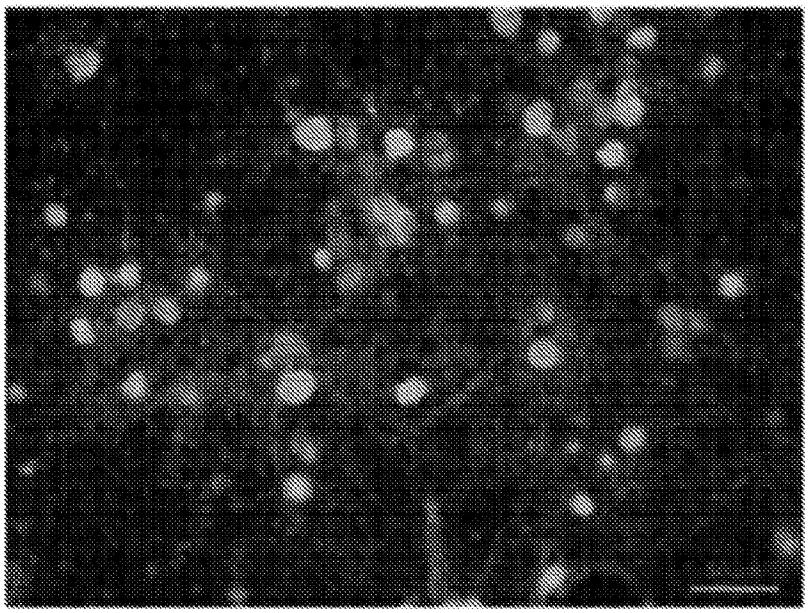

FIG. 5B

α-syn PFF

pS129
NeuN
DAPI

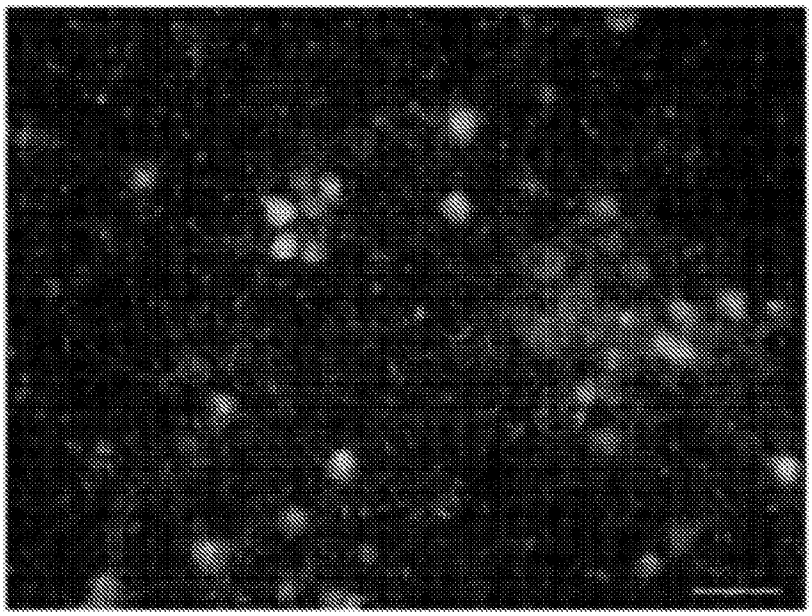

FIG. 5C

AAV-Control
+α-syn PFF

pS129
NeuN
DAPI

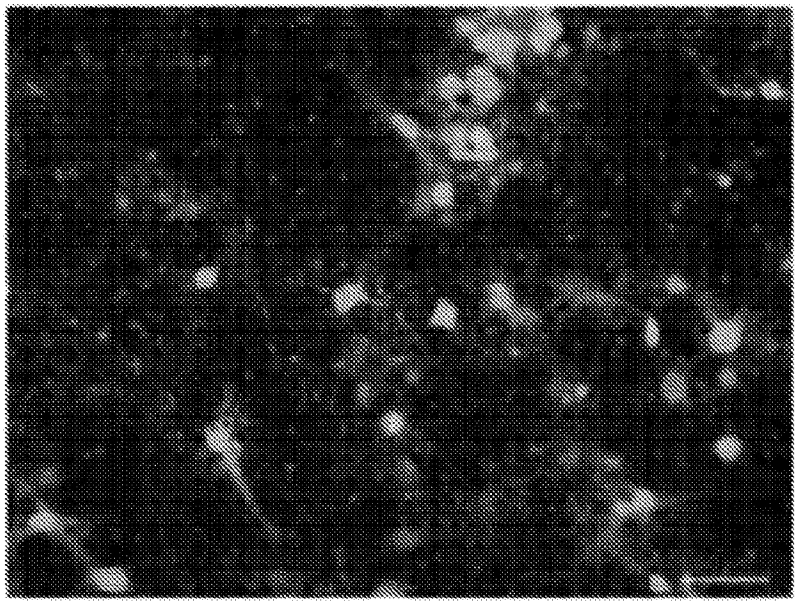

FIG. 5D

LF17A (secreted FGL1)
+α-syn PFF

pS129
NeuN
DAPI

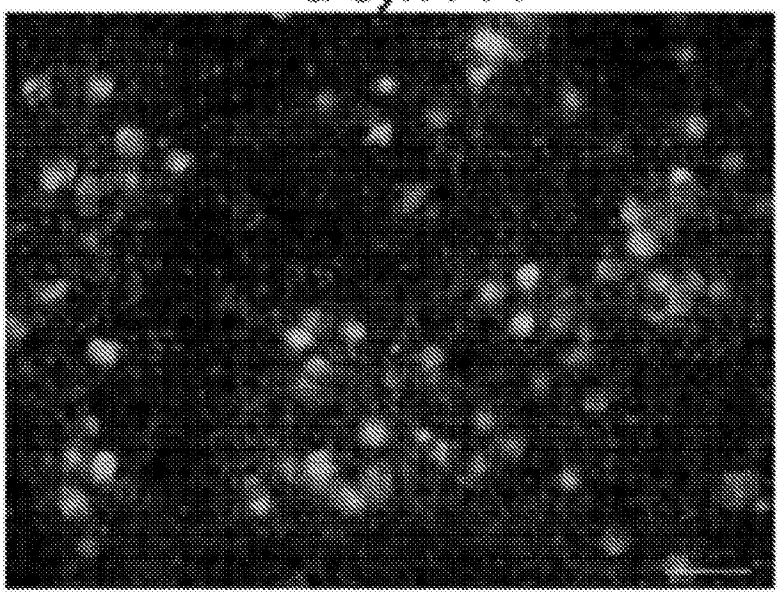

FIG. 5E

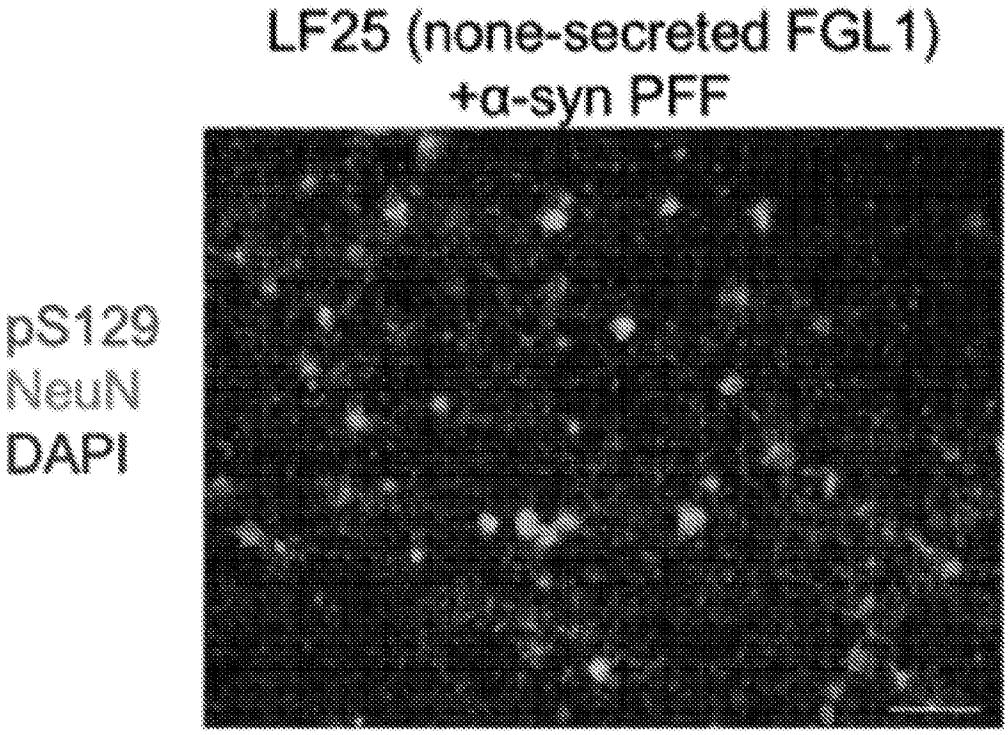
LF25 (none-secreted FGL1)
+α-syn PFF
pS129
NeuN
DAPI

FIG. 5F

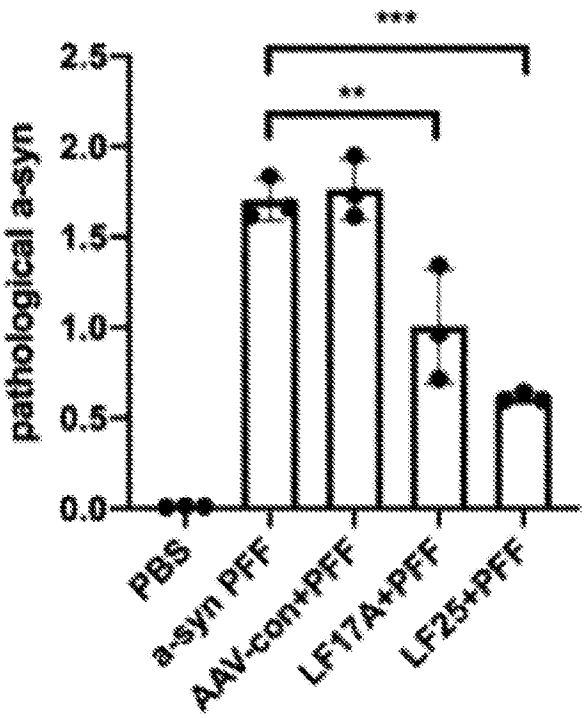
***
**
pathological a-syn
2.5
2.0
1.5
1.0
0.5
0.0
PBS
a-syn PFF
AAV-con+PFF
LF17A+PFF
LF25+PFF

FIG. 5G

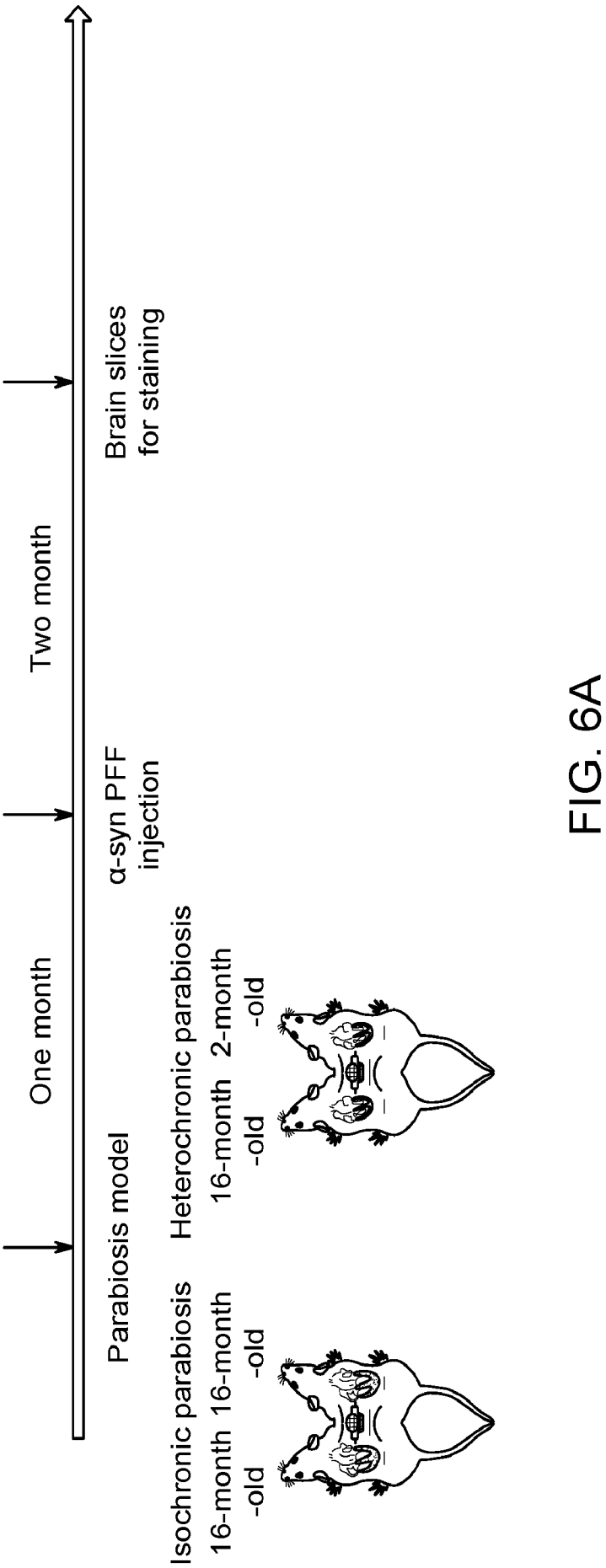
Parabiosis model
One month
α-syn PFF injection
Two month
Brain slices for staining
Isochronic parabiosis
16-month -old
16-month -old
Heterochronic parabiosis
16-month -old
2-month -old

FIG. 6A

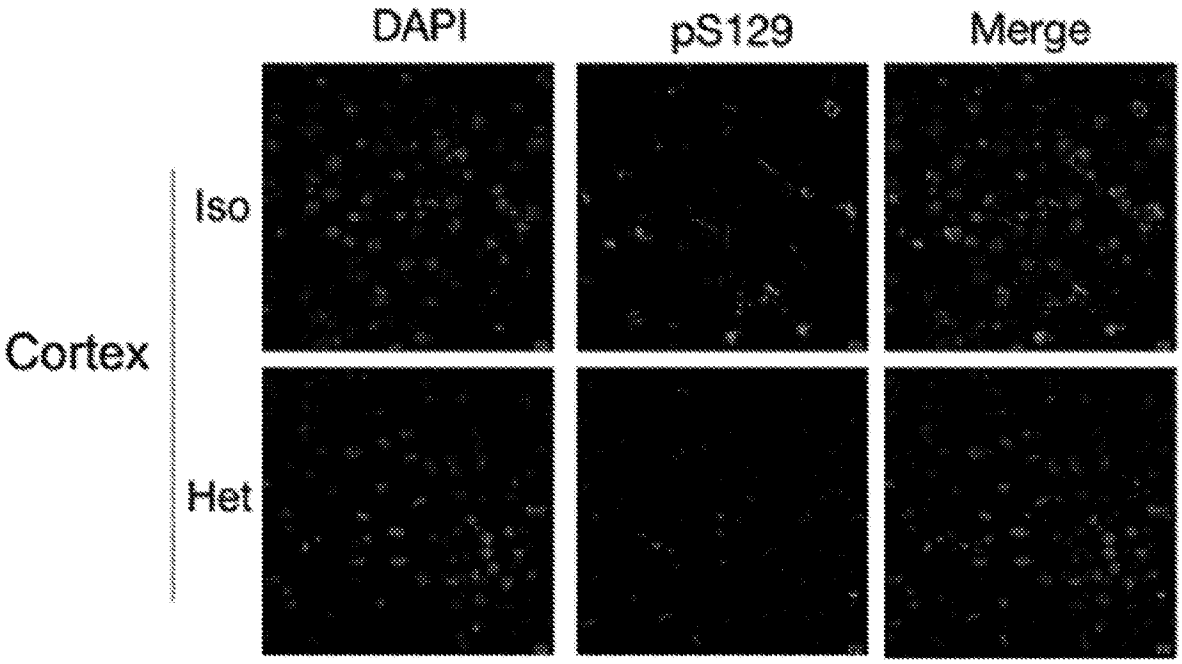
DAPI
pS129
Merge
Cortex
Iso
Het

FIG. 6B

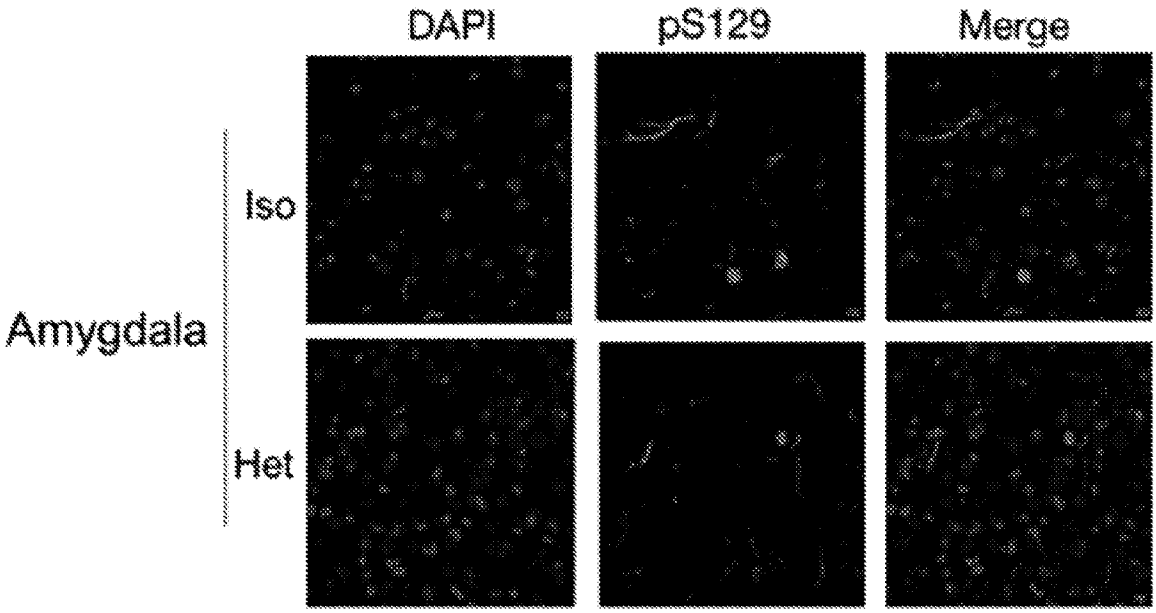
DAPI
pS129
Merge
Amygdala
Iso
Het

FIG. 6C

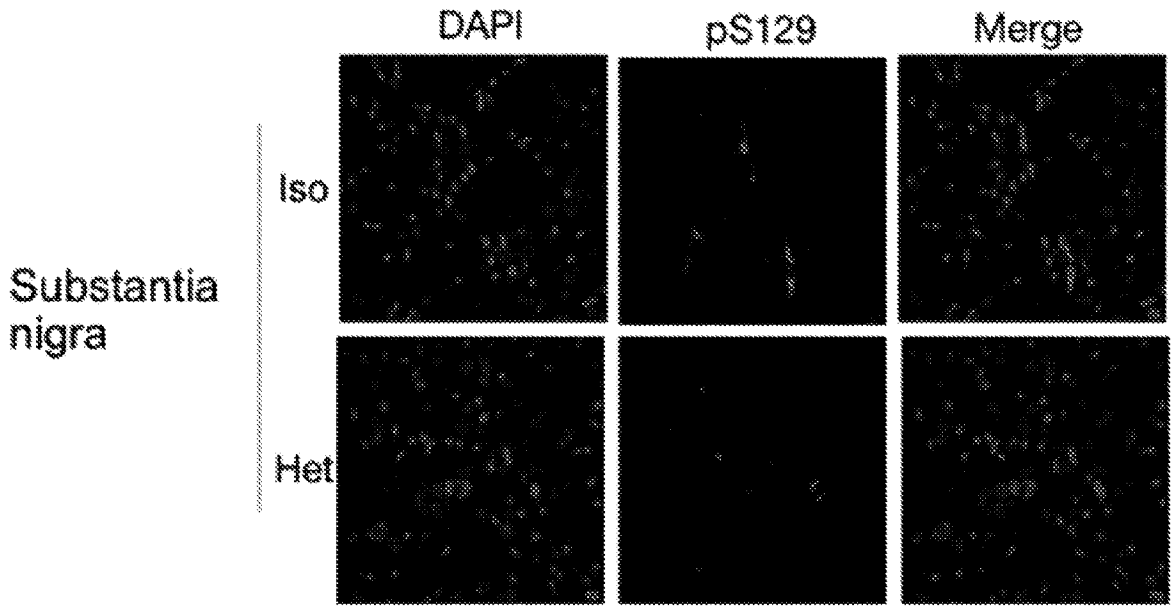
DAPI
pS129
Merge
Substantia nigra
Iso
Het

FIG. 6D

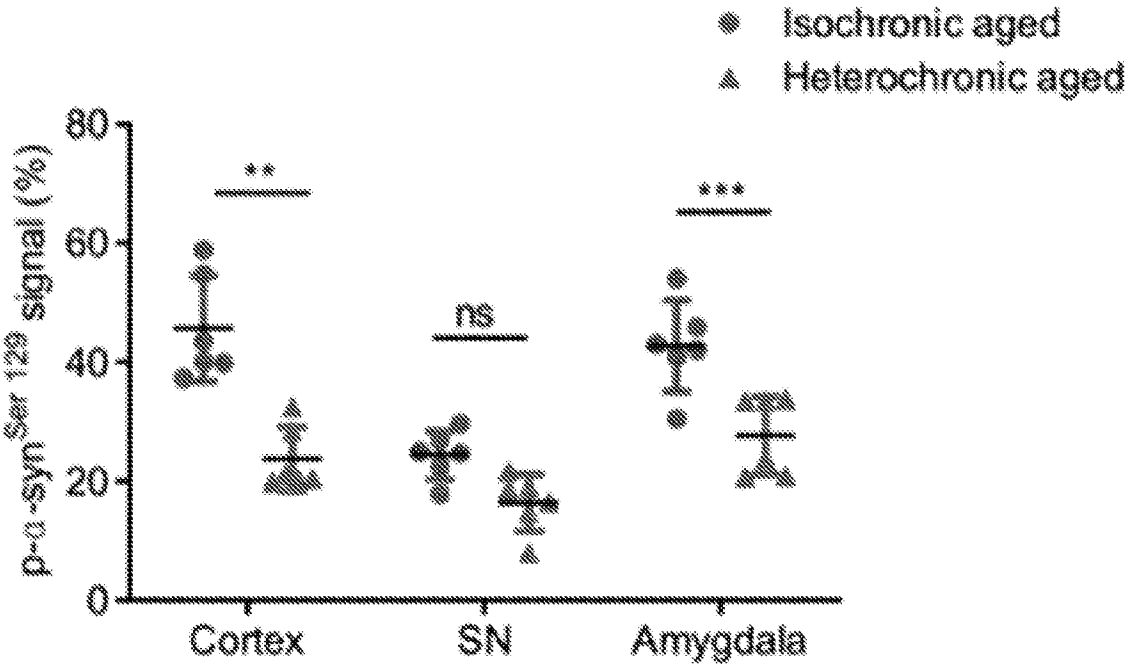
Isochronic aged
Heterochronic aged
p-α-syn Ser 129 signal (%)
80
60
40
20
0
**
ns
***
Cortex
SN
Amygdala

FIG. 6E

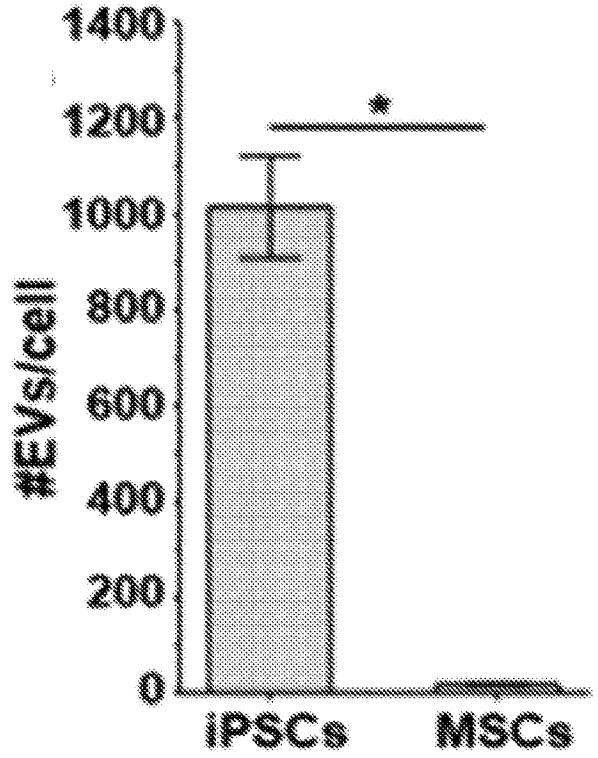
1400
1200
1000
800
600
400
200
0
#EVs/cell
*
iPSCs
MSCs

FIG. 7A

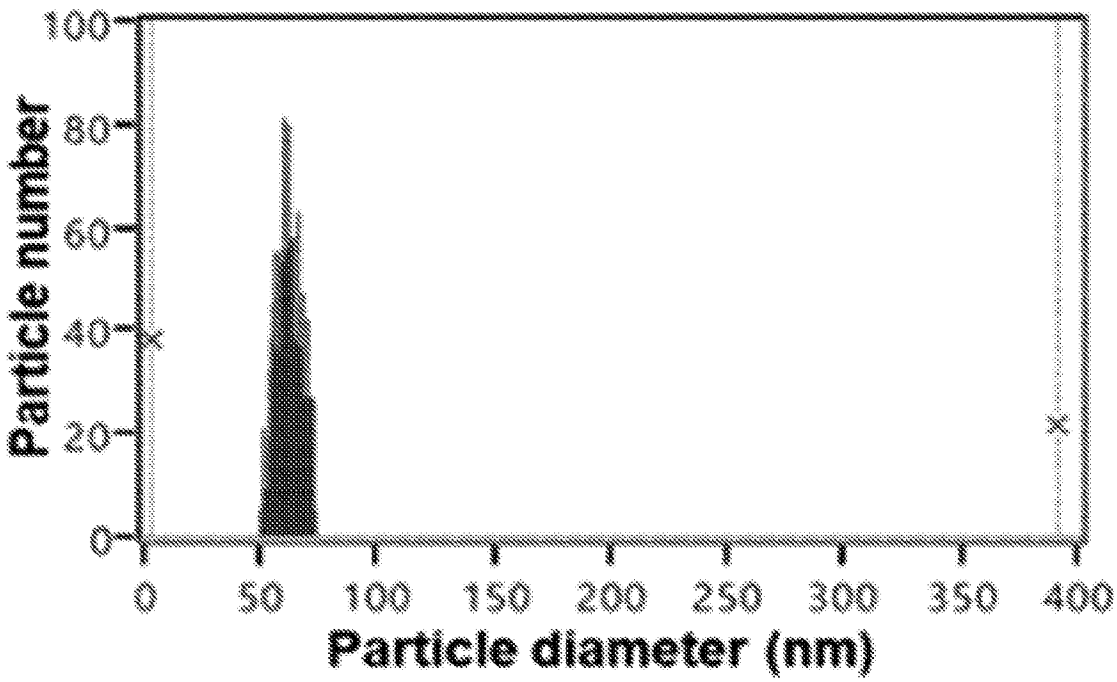
100
80
60
40
20
0
Particle number
0
50
100
150
200
250
300
350
400
Particle diameter (nm)

FIG. 7B

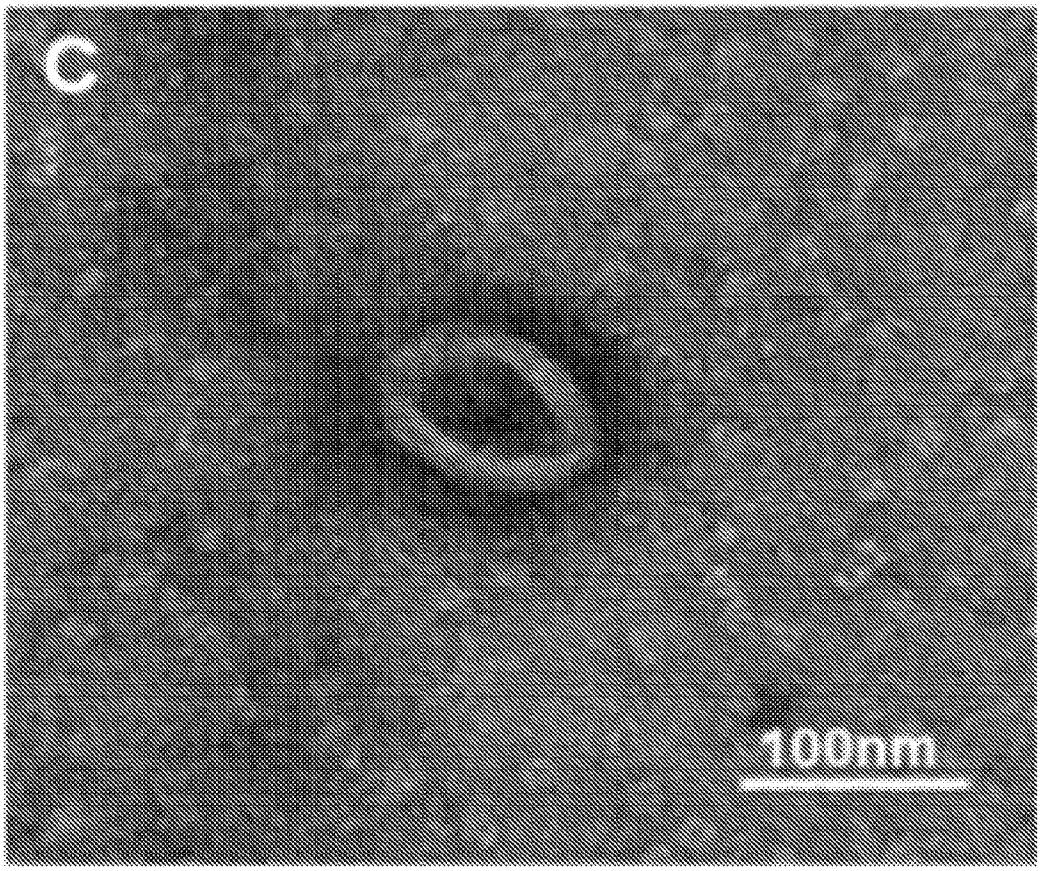

FIG. 7C

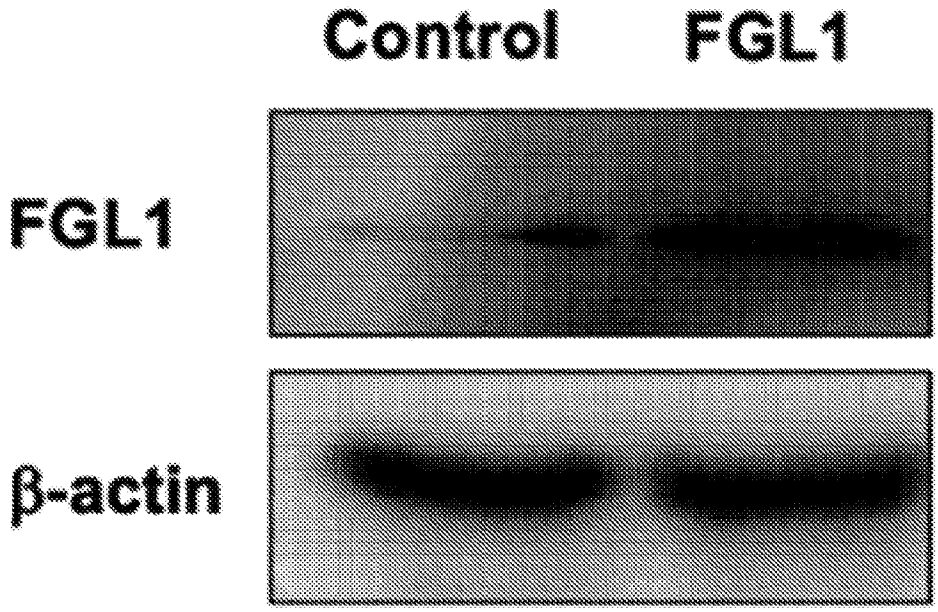

FIG. 8A

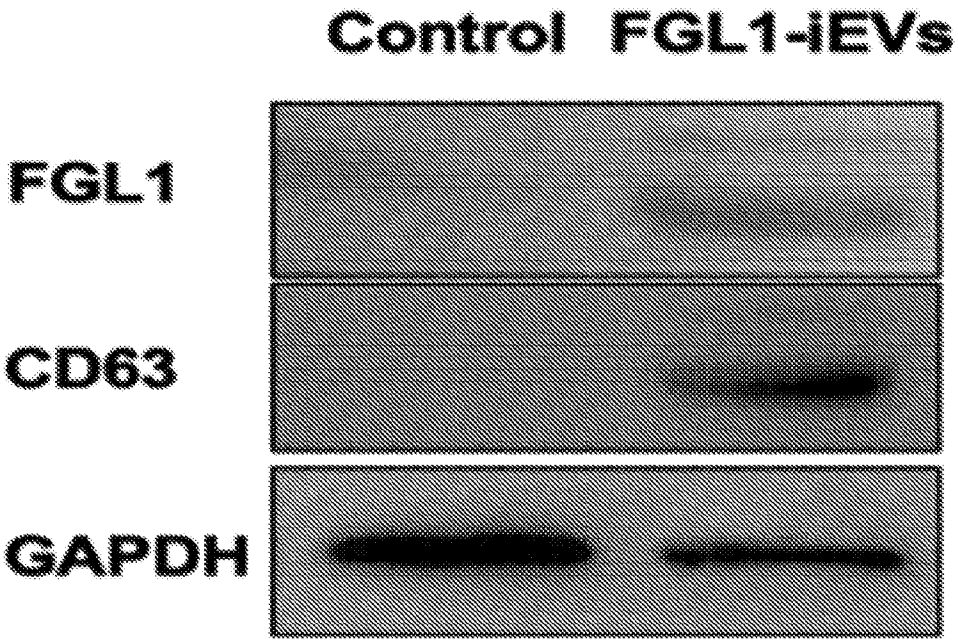

FIG. 8B

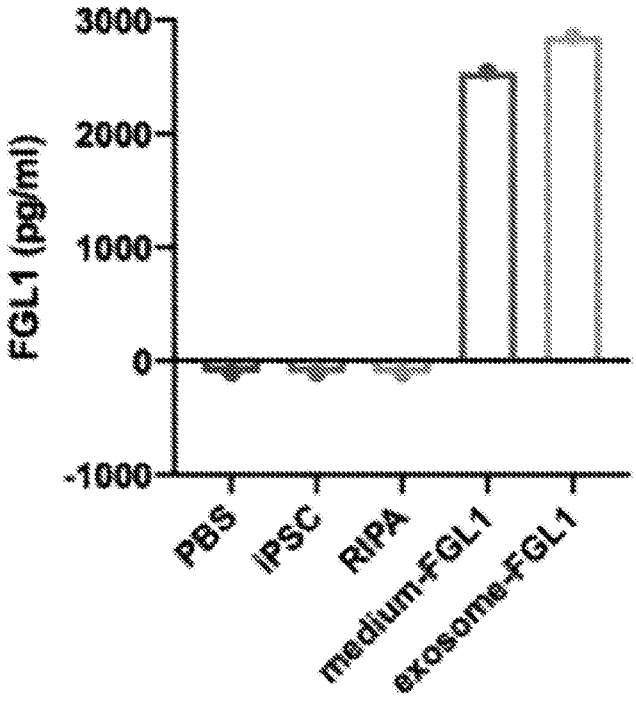
3000
2000
1000
0
-1000
FGL1 (pg/ml)
PBS
IPSC
RIPA
medium-FGL1
exosome-FGL1

FIG. 9A

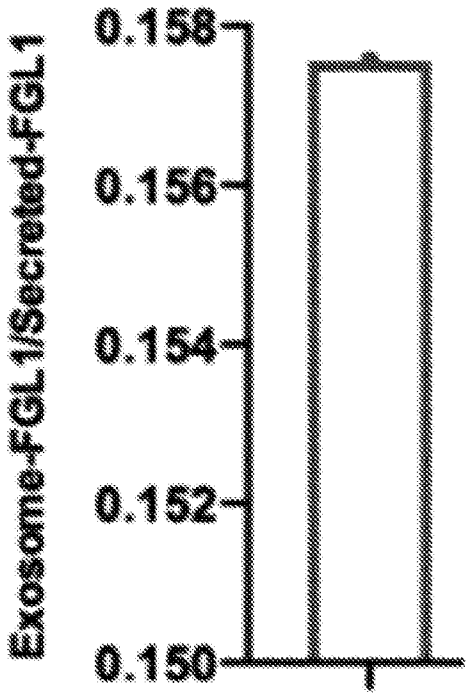
0.158
0.156
0.154
0.152
0.150
Exosome-FGL1/Secreted-FGL1

FIG. 9B