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(54) Title: ENGINEERED REPROGRAMMING FACTORS AND USES THEREOF FOR TREATING EYE DISEASES

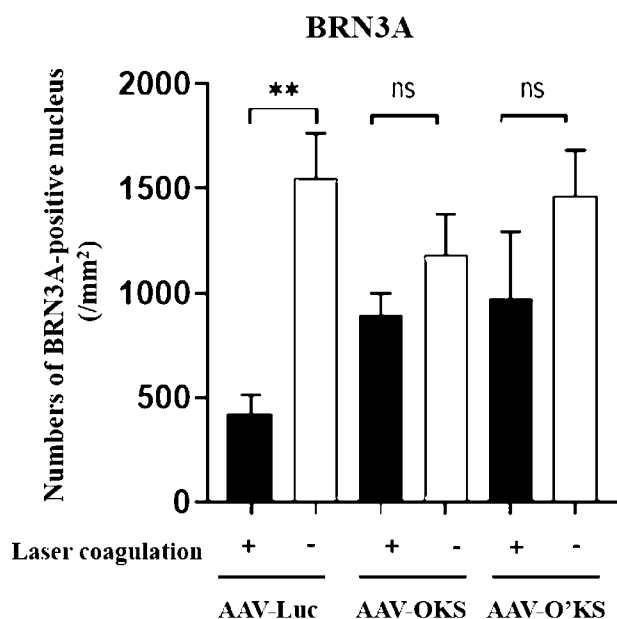


FIG. 39B

(57) Abstract: The present application provides methods of rejuvenating a population of cells (e.g., retinal cells) of an eye, comprising introducing into the population of cells (e.g., retinal cells), or to the eye, an effective amount of an engineered reprogramming factor that is deficient in liquid-liquid phase separation (LLPS). Also provided are methods of delaying or preventing the progression of aging of a retinal tissue, or vision impairment or loss, in an individual (e.g., human), methods of preserving or improving the viability and/or function of an retinal ganglion cell (RGC), methods of preserving or improving vision, methods of promoting retinal regeneration, and methods of treating and/or preventing aging, damage, or disease or condition in a retinal tissue, or vision impairment or loss, in an individual, using any of the rejuvenation methods and/or LLPS-deficient engineered reprogramming factors described herein.

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ENGINEERED REPROGRAMMING FACTORS AND USES THEREOF FOR TREATING EYE DISEASES

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This patent application claims priority benefits of Chinese Patent Application No. CN202310793813.X filed on June 30, 2023, the content of which is incorporated herein by reference in its entirety.

REFERENCE TO AN ELECTRONIC SEQUENCE LISTING

[0002] The contents of the electronic sequence listing (313682000240SEQLIST.xml; Size: 365,340 bytes; and Date of Creation: June 30, 2024) is herein incorporated by reference in its entirety.

FIELD OF THE INVENTION

[0003] The present application relates to methods of rejuvenating a population of cells of an eye, comprising introducing into the population of cells (e.g., retinal cells), or to the eye, an effective amount of an engineered reprogramming factor that is deficient in liquid-liquid phase separation (LLPS).

BACKGROUND OF THE INVENTION

[0004] An important approach for induced pluripotent stem cell (iPSC) reprogramming is using transcription factors such as OCT4, SOX2, KLF4, and MYC (referred to as “OSKM” or “4-Factor”) to reprogram somatic cells into pluripotent status, the process of which is called complete reprogramming, and can be divided into initiation phase, maturation phase, and stabilization phase (K. Tanabe *et al.*, *Proc Natl Acad Sci U S A.* 2013;110(30):12172-12179; N. Olova *et al.*, *Aging Cell.* 2019;18(1):e12877). During the reprogramming process, cells undergo drastic changes in transcription levels and epigenetic modifications, resulting in dramatic changes in cell identity and state. During the initiation phase, the expression levels of early-stage pluripotent genes, such as TRA-1-60, NANOG, SSEA4 and SALL4, increase rapidly, while the expression levels of somatic identity genes do not change much. Then, during the maturation phase, in addition to the above-mentioned early-stage pluripotent genes, the late-stage pluripotent genes, such as DPPA5 and LIN28, are highly expressed, while the the expression levels of somatic identity genes decrease rapidly (K. Tanabe *et al.*, 2013; N. Olova *et al.*, 2019). Finally, during the stabilization phase, the

cells have fully established the pluripotency gene expression network and become iPSCs, which express the above pluripotent genes and also other classical core pluripotency transcription factors, such as OCT4, SOX2 and KLF4, at high levels, while somatic identity marker genes are basically not expressed. This reprogramming process can lead to cell rejuvenation, which can be detected by changes in the transcription level of certain marker genes, cellular senescence markers, and especially epigenetic age (EpiAge) based on DNA methylation (N. Olova *et al.*, 2019; A. Daunay, *et al.*, *Aging (Albany NY)*. 2022;14(19):7718-7733; D. Gill *et al.*, *eLife*, 2022; 11: e71624).

[0005] Partial reprogramming refers to the cessation of complete reprogramming in the middle of the process mentioned above. Since cell rejuvenation occurs in the early-stage of the reprogramming process, and the loss of somatic identity occurs in the middle- and late-stages of the reprogramming process, there is a time window to rejuvenate the cells without altering the original identity of the cells, which is the technical principle of partial reprogramming to rejuvenate cells (A. Ocampo *et al.*, *Cell*. 2016;167(7):1719-1733.e12; N. Olova *et al.*, *Aging Cell*. 2019;18(1):e12877). The methods of using transcription factors to rejuvenate cells via partial reprogramming are mainly reported as follows: 1) *In vitro* induction of time-restricted partial reprogramming, also known as transient reprogramming. For example, transient induction of the 4-Factor expression by an inducible transgene expression system in human fibroblast was found to reverse the EpiAge of cells while maintaining the fibroblast identity (N. Olova *et al.*, 2019; D. Gill *et al.*, 2022). 2) *In vivo* induction of transient-pulse partial reprogramming. For example, transient-pulse induction of the 4-Factor expression in transgenic mice with an inducible expression system was found to make the organs (e.g., skin, spleen, liver, kidney, stomach and aorta) of Progeria mice younger, and also make the organs (e.g., pancreas and muscle) of wild-type old mice younger to a certain extent (A. Ocampo *et al.*, 2016; K. Browder *et al.*, *Nat Aging*. 2022;2(3):243-253). 3) Partial reprogramming by a reduced number of transcription factors (referred to 3-Factor reprogramming). For example, delivery of AAV vectors expressing 3 transcription factors (OSK) into the eyes of a mouse glaucoma model exhibited remarkable therapeutic efficacy (Lu *et al.*, *Nature*. 2020;588(7836):124-129).

[0006] Some protein and/or nucleic acid molecules in the cell can interact with each other to produce a phase with different physical and chemical properties in the surrounding homogeneous environment, usually showing droplet-like characteristics, and the result is the formation of membrane-free organelles or sub-cellular structures, namely, biomolecular condensates. This

process is referred to as phase separation or liquid-liquid phase separation (LLPS) (C. Brangwynne *et al.*, *Science*. 2009;324(5935):1729-1732; P. Li *et al.* *Nature*. 2012;483(7389):336-340). Biomolecular condensates are in size at the micron level, which create a relatively independent spatial region that selectively enriches molecules. Biomolecular condensates formed by phase separation typically have higher protein densities and reduced molecular micromotility compared to the surrounding environment, which can facilitate certain biochemical reactions more efficiently. The structural characteristics of proteins that can form condensates are still being studied.

[0007] In 2021, Wang *et al.* (*Cell Stem Cell*. 2021;28(10):1868-1883.e11) discovered that OCT4 undergoes phase separation to regulate topological-associated domain (TAD) reorganization, and TAD reorganization contributes to cell fate transition.

[0008] The disclosures of all publications, patents, patent applications and published patent applications referred to herein are hereby incorporated herein by reference in their entirety.

BRIEF SUMMARY OF THE INVENTION

[0009] One aspect of the present application provides a method of rejuvenating a population of cells (e.g., retinal cells) of an eye in an individual (e.g., human), comprising introducing into the population of cells (e.g., retinal cells) an effective amount of an engineered reprogramming factor that is deficient in LLPS. In some embodiments, the population of cells are retinal cells. In some embodiments, the population of retinal cells comprises retinal ganglion cells (RGCs), photoreceptor cells, bipolar cells, amacrine cells, horizontal cells, Müller glial cells, retinal pigment epithelium (RPE) cells, or any combination thereof.

[0010] In some embodiments according to any of the rejuvenation methods described above, the population of retinal cells are from a retinal tissue that is (i) an aged retinal tissue; (ii) a damaged retinal tissue; (iii) a retinal tissue with ocular disease or condition; and/or (iv) a retinal tissue after a surgical procedure. In some embodiments, the ocular disease or condition comprises traumatic optic neuropathy (TON), solar retinopathy, diabetic retinopathy, glaucoma, age-related macular degeneration (AMD), vision impairment or loss, or any combination thereof; and/or (ii) the damage is due to optic nerve crush or laser photocoagulation.

[0011] In some embodiments according to any of the rejuvenation methods described above, the method does not reprogram the population of retinal cells into a reprogrammed stem cell.

[0012] In some embodiments according to any of the rejuvenation methods described above, compared to a reference retinal cell not introduced with the engineered reprogramming factor, the rejuvenated population of retinal cells: (i) have reduced proportion of cell senescence biomarker-positive cells; (ii) have reduced expression and/or function of a biomarker of cell senescence; (iii) have reduced epigenetic age (EpiAge); (iv) have increased expression and/or function of a biomarker of retinal cell identity; and/or (v) have reduced expression and/or function of a biomarker of apoptosis. In some embodiments, (i) the biomarker of cell senescence is selected from the group consisting of senescence-associated- β -galactosidase (SA- β -gal), p16, and p21, or a combination thereof; (ii) the EpiAge is measured using Horvath's clock, Levine's clock, Hannum's clock, and/or AltumAge; (iii) the biomarker of retinal cell identity is selected from the group consisting of β III-tubulin, BRN3A, RBPMS, γ -synuclein, Thy 1, GAP43, MAP2, STMN2, NEFM, or a combination thereof; and/or (iv) the biomarker of apoptosis is selected from the group consisting of loss of membrane asymmetry, cleavage of a Bcl-2 family protein, caspase activation, caspase substrate cleavage, non-caspase protease activation, mitochondrial transmembrane potential ($\Delta \psi_m$) decrease, cytochrome C release, increase of sub G1 population, nuclear condensation, DNA fragmentation, and membrane blebbing, or a combination thereof.

[0013] In some embodiments according to any of the rejuvenation methods described above, compared to a reference retinal cell introduced with an effective amount of a reference reprogramming factor that is not deficient in LLPS, the rejuvenated population of retinal cells: (i) have increased expression and/or function of a biomarker of initiation phase reprogramming (IPR); (ii) have reduced expression and/or function of a biomarker of maturation phase reprogramming; and/or (iii) have reduced expression and/or function of a biomarker of stabilization phase reprogramming. In some embodiments, (i) the biomarker of IPR is NANOG and/or SALL4; (ii) the biomarker of maturation phase reprogramming is DPPA5 and/or LIN28A; and/or (iii) the biomarker of stabilization phase reprogramming is OCT4 and/or XACT.

[0014] In some embodiments according to any of the rejuvenation methods described above, compared to a reference retinal tissue not comprising retinal cells introduced with the effective amount of the engineered reprogramming factor, a retinal tissue comprising the population of retinal cells introduced with the effective amount of the engineered reprogramming factors has: (i) higher density of RGC axons; (ii) more retinal cells; (iii) improved electrophysiology, histology, and/or retinal function; and/or (iv) a connected optic nerve that has improved electrophysiology,

histology, and/or function. In some embodiments, compared to a reference retinal tissue comprising retinal ganglion cells introduced with an effective amount of a reference reprogramming factor that is not deficient in LLPS, a retinal tissue comprising the population of retinal cells introduced with the effective amount of the engineered reprogramming factors has: (i) higher density of RGC axons; (ii) more retinal cells; (iii) improved retinal electrophysiology, physiology, and/or function; and/or (iv) a connected optic nerve that has improved electrophysiology, histology, and/or function. In some embodiments, (i) the electrophysiology of the retina and/or the connected optic nerve is measured by electroretinogram (ERG), electro-oculography (EOG), and/or visual evoked response (VER); (ii) the physiology of the retina and/or the connected optic nerve is measured by optical coherence tomography (OCT), scanning laser ophthalmoscopy (SLO), and/or ocular ultrasound; (iii) the function of the retina and/or the connected optic nerve is measured by vision test; and/or (iv) the function of the connected optic nerve is measured by visual fields, pupillary light reflexes, the accommodation reflex, and/or funduscopy. In some embodiments, (i) the improved retinal physiology comprises reduced retinal lesion; and/or (ii) the improved electrophysiology of the retina and/or the connected optic nerve comprises improved photopic negative response (PhNR), improved pattern electroretinogram (pERG), and/or improved flash visual evoked potential (fVEP). In some embodiments, the reference retinal tissue and the retinal tissue are from a same individual. In some embodiments, the reference retinal tissue and the retinal tissue are from different individuals.

[0015] In some embodiments according to any of the rejuvenation methods described above, the engineered reprogramming factor comprises a mutation in an LLPS-related domain in reference to a corresponding wild-type reprogramming factor. In some embodiments, the LLPS-related domain is selected from the group consisting of an intrinsically disordered region (IDR), a region outside of DNA-binding domain (DBD), an ubiquitination site, or any combination thereof. In some embodiments, the mutation comprises: (i) substituting an acidic amino acid within the IDR with a neutral amino acid; (ii) substituting an amino acid that can be ubiquitinated with an amino acid that cannot be ubiquitinated; (iii) deleting an amino acid that can be ubiquitinated; (iv) deleting an amino acid within the IDR; and/or (v) deleting an amino acid within a region outside of DBD. In some embodiments, (i) the amino acid that can be ubiquitinated is K; and/or (ii) the amino acid to be deleted comprises an acidic amino acid.

[0016] In some embodiments according to any of the rejuvenation methods described above, the wild-type reprogramming factor is from a family selected from the group consisting of OCT family, SOX family, KLF family, MYC family, LIN28 family, NANOG family, and GLIS family. In some embodiments, the wild-type reprogramming factor is selected from the group consisting of OCT4, SOX2, KLF4, c-MYC, L-MYC, LIN28, NANOG, and GLIS1.

[0017] In some embodiments according to any of the rejuvenation methods described above, the engineered reprogramming factor is an engineered OCT4. In some embodiments, the engineered OCT4 comprises a mutation at amino acid positions 1-140 and/or 288-360, wherein the amino acid positions are in reference to SEQ ID NO: 2. In some embodiments, the engineered OCT4 comprises a substitution at an amino acid position selected from the group consisting of 8, 20, 26, 31, 56, 68, 91, 96, 98, 104, 108, 113, 125, 127, 130, 134, 135, 138, 291, 296, 297, 299, 341, and 343, and wherein the amino acid positions are in reference to SEQ ID NO: 2. In some embodiments, the engineered OCT4 comprises a substitution at an amino acid position selected from the group consisting of D8, D20, E26, D31, E56, E68, E91, E96, E98, E104, D108, E113, E125, E127, E130, E134, E135, D138, D291, E296, D297, E299, E341, and E343. In some embodiments, the substitution comprises substituting an acidic amino acid with a neutral amino acid. In some embodiments, the substitution comprises substituting a D or E with an A. In some embodiments, the engineered OCT4 comprises a substitution selected from the group consisting of D8A, D20A, D31A, D108A, E134A, D138A, D291A, or any combination thereof. In some embodiments, the engineered OCT4 comprises a substitution selected from the group consisting of: (i) D8A, D20A, E26A, D31A, E56A, E68A, E91A, E96A, E98A, E104A, D108A, E113A, E125A, E127A, E130A, E134A, E135A, D138A, D291A, E296A, D297A, E299A, E341A, and E343A; (ii) D8A, D20A, E26A, D31A, E56A, E68A, E91A, E96A, E98A, E104A, D108A, E113A, E125A, E127A, E130A, E134A, E135A, and D138A; (iii) D291A, E296A, D297A, E299A, E341A, and E343A; (iv) D8A, D20A, E26A, D31A, E56A, E68A, E91A, E96A, E98A, E104A, D108A, E113A, E125A, E127A, E130A, E134A, D138A, D291A, E296A, E299A, E341A, and E343A; (v) E134A, E135A, E296A, and D297A; (vi) D8A, D20A, D31A, D108A, D138A, D291A, and D297A; and (vii) E26A, E56A, E68A, E91A, E96A, E98A, E104A, E113A, E125A, E127A, E130A, E134A, E135A, E296A, E299A, E341A, and E343A. In some embodiments, the engineered OCT4 comprises an amino acid sequence of any of SEQ ID NOS: 4-10, or a variant thereof having at least about 85% sequence identity to any of SEQ ID NOS: 4-10. In some embodiments, the engineered OCT4

comprises a deletion at amino acid positions 2-140 and/or 288-360, wherein the amino acid positions are in reference to SEQ ID NO: 2. In some embodiments, the engineered OCT4 comprises a deletion selected from the group consisting of: (i) a deletion at amino acid positions 2-140 and 301-360; (ii) a deletion at amino acid positions 301-360; and (iii) a deletion at amino acid positions 2-140. In some embodiments, the engineered OCT4 comprises an amino acid sequence of any of SEQ ID NOs: 44-46, or a variant thereof having at least about 85% sequence identity to any of SEQ ID NOs: 44-46.

[0018] In some embodiments according to any of the rejuvenation methods described above, the engineered reprogramming factor is an engineered c-MYC. In some embodiments, the engineered c-MYC comprises a substitution of an amino acid that can be ubiquitinated with an amino acid that cannot be ubiquitinated, wherein the amino acid positions are in reference to SEQ ID NO: 12 or 85. In some embodiments, the substitution comprises substituting a K with an R. In some embodiments, the engineered c-MYC comprises a substitution at an amino acid position selected from the group consisting of 51, 52, 126, 143, 148, 157, 206, 269, 275, 289, 298, 317, 323, 326, 341, 355, 371, 389, 392, 397, 398, 412, 422, 428, and 430, and the amino acid positions are in reference to SEQ ID NO: 12 or 85. In some embodiments, the engineered c-MYC comprises a substitution selected from the group consisting of K51R, K52R, K148R, K275R, K289R, and K389R, or any combination thereof. In some embodiments, the engineered c-MYC comprises a substitution selected from the group consisting of: (i) K51R, K52R, K126R, K143R, K148R, K157R, K206R, K269R, K275R, K289R, K298R, K317R, K323R, K326R, K341R, K355R, K371R, K389R, K392R, K397R, K398R, K412R, K422R, K428R, and K430R; (ii) K148R; (iii) K389R; (iv) K148R and K389R; (v) K51R and K52R; (vi) K275R and K289R; and (vii) K51R, K52R, K275R, and K289R. In some embodiments, the engineered c-MYC comprises an amino acid sequence of any of SEQ ID NOs: 14-20, or a variant thereof having at least about 85% sequence identity to any of SEQ ID NOs: 14-20. In some embodiments, the engineered c-MYC comprises a deletion at amino acid positions 2-360, wherein the amino acid positions are in reference to SEQ ID NO: 12 or 85. In some embodiments, the engineered c-MYC comprises a deletion at amino acid positions 2-140. In some embodiments, the engineered c-MYC comprises the amino acid sequence of SEQ ID NO: 47 or 48, or a variant thereof having at least about 85% sequence identity to SEQ ID NO: 47 or 48.

[0019] In some embodiments according to any of the rejuvenation methods described above, the engineered reprogramming factor is an engineered KLF4. In some embodiments, the engineered KLF4 comprises a deletion at amino acid positions 2-374, wherein the amino acid positions are in reference to SEQ ID NO: 22. In some embodiments, the engineered KLF4 comprises the amino acid sequence of SEQ ID NO: 40, or a variant thereof having at least about 85% sequence identity to SEQ ID NO: 40.

[0020] In some embodiments according to any of the rejuvenation methods described above, the engineered reprogramming factor is an engineered SOX2. In some embodiments, the engineered SOX2 comprises a deletion at amino acid positions 2-40 and/or 201-317, wherein the amino acid positions are in reference to SEQ ID NO: 24. In some embodiments, the engineered SOX2 comprises an amino acid sequence of any of SEQ ID NOs: 41-43, or a variant thereof having at least about 85% sequence identity to any of SEQ ID NOs: 41-43.

[0021] In some embodiments according to any of the rejuvenation methods described above, the engineered reprogramming factor is encoded by a nucleic acid. In some embodiments, the nucleic acid comprises a nucleic acid sequence of SEQ ID NO: 3 or 13. In some embodiments, the nucleic acid is an RNA, such as a circular RNA (circRNA), a self-amplifying mRNA, or a linear mRNA. In some embodiments, the nucleic acid is a DNA. In some embodiments, the nucleic acid is comprised within a vector. In some embodiments, the vector is a viral vector, such as a lentiviral vector or an adeno-associated virus (AAV) vector. In some embodiments, the nucleic acid is delivered by an exosome, a liposome, a lipid nanoparticle (LNP), or a virus. In some embodiments, the method comprises administering a virus comprising a nucleic acid encoding the engineered reprogramming factor. In some embodiments, the virus is delivered via intravitreal injection or subretinal injection to the eye. In some embodiments, the method comprises administering an exosome, a liposome, or an LNP encapsulating an RNA (e.g., circRNA) encoding the engineered reprogramming factor. In some embodiments, the exosome, liposome, or LNP is delivered topically (e.g., as eye drops, ophthalmic ointment, or ophthalmic gel) to the eye.

[0022] In some embodiments according to any of the rejuvenation methods described above, the method comprises introducing a plurality of reprogramming factors into the population of retinal cells, wherein at least one of the plurality of reprogramming factors is an engineered reprogramming factor that is deficient in LLPS. In some embodiments, the plurality of

reprogramming factors comprises two or more reprogramming factors from two or more families selected from the group consisting of OCT family, SOX family, KLF family, MYC family, LIN28 family, NANOG family, and GLIS family. In some embodiments, the plurality of reprogramming factors comprises two or more reprogramming factors selected from the group consisting of OCT4, SOX2, KLF4, c-MYC, L-MYC, LIN28, NANOG, GLIS1, and variant thereof. In some embodiments, the plurality of reprogramming factors comprises three or all of OCT4, KLF4, SOX2, and c-MYC, wherein the at least one of the plurality of reprogramming factors is an engineered reprogramming factor that is deficient in LLPS. In some embodiments, the plurality of reprogramming factors comprises OCT4 and KLF4, wherein the at least one of OCT4 and KLF4 is an engineered reprogramming factor that is deficient in LLPS. In some embodiments, the OCT4 is an engineered OCT4 that is deficient in LLPS. In some embodiments, the KLF4 is an engineered KLF4 that is deficient in LLPS. In some embodiments, the plurality of reprogramming factors comprises a wild-type reprogramming factor. In some embodiments, the plurality of reprogramming factors comprise any of: (i) an engineered OCT4 that is deficient in LLPS, SOX2, and KLF4; (ii) an engineered OCT4 that is deficient in LLPS, KLF4, and an engineered c-MYC that is deficient in LLPS; or (iii) an engineered OCT4 that is deficient in LLPS, KLF4, an engineered c-MYC that is deficient in LLPS, and SOX2. In some embodiments, the plurality of reprogramming factors are encoded by a plurality of nucleic acids. In some embodiments, the plurality of nucleic acids are on separate nucleic acid constructs. In some embodiments, at least two of the plurality of nucleic acids are on a single nucleic acid construct. In some embodiments, the at least two of the plurality of nucleic acids are under the control of different promoters. In some embodiments, the at least two of the plurality of nucleic acids are under the control of the same promoter. In some embodiments, the at least two of the plurality of nucleic acids are connected by one or more linking nucleic acids. In some embodiments, the one or more linking nucleic acids comprise IRES or a linking sequence encoding a self-cleaving peptide. In some embodiments, the one or more linking nucleic acids comprise a linking sequence encoding a self-cleaving peptide, and wherein the self-cleaving peptide is P2A, T2A, or E2A. In some embodiments, the nucleic acid construct comprises from 5' to 3': (i) a first nucleic acid encoding an engineered OCT4 that is deficient in LLPS – a first linking sequence encoding P2A – a second nucleic acid encoding SOX2 – a second linking sequence encoding T2A – a third nucleic acid encoding KLF4; (ii) a first nucleic acid encoding an engineered OCT4 that is deficient in LLPS –

a first linking sequence encoding P2A – a second nucleic acid encoding KLF4 – a second linking sequence encoding T2A – a third nucleic acid encoding an engineered c-MYC that is deficient in LLPS; (iii) a first nucleic acid encoding an engineered OCT4 that is deficient in LLPS – a first linking sequence encoding P2A – a second nucleic acid encoding KLF4 – a second linking sequence encoding T2A – a third nucleic acid encoding an engineered c-MYC that is deficient in LLPS – a third linking sequence encoding E2A – a fourth nucleic acid encoding SOX2; or (iv) a first nucleic acid encoding an engineered OCT4 that is deficient in LLPS – a first linking sequence encoding P2A – a second nucleic acid encoding KLF4 – a second linking sequence encoding T2A – a third nucleic acid encoding SOX2. In some embodiments, (i) the engineered OCT4 that is deficient in LLPS comprises the amino acid sequence of SEQ ID NO: 4; and/or (ii) the engineered c-MYC that is deficient in LLPS comprises the amino acid sequence of SEQ ID NO: 14. In some embodiments, the nucleic acid construct comprises a nucleic acid sequence of any of SEQ ID NOs: 28, 30, 32, 35, 37, 39, 57, 59, 61, 94, 98, and 102.

[0023] In some embodiments according to any of the rejuvenation methods described above, the method further comprises introducing into the population of retinal cells an effective amount of a therapeutic agent. In some embodiments, the effective amount of the engineered reprogramming factor is administered once.

[0024] Another aspect of the present application provides a method of delaying or preventing the progression of aging of a retinal tissue of an eye in an individual (e.g., human, e.g., 22 years or older, such as 40 years or older), comprising introducing into a population of retinal cells of the retinal tissue, or introducing to the eye (e.g., topically, intravitreally, or subretinally), an effective amount of an engineered reprogramming factor that is deficient in LLPS (e.g., any of the engineered reprogramming factors described herein). In some embodiments, the method of delaying or preventing the progression of aging of a retinal tissue of an eye comprises rejuvenating a population of retinal cells of the retinal tissue using any of the rejuvenation methods described above.

[0025] Another aspect of the present application provides a method of preserving or improving the viability and/or function of an RGC of an eye in an individual (e.g., human), comprising introducing into the RGC, or introducing to the eye (e.g., topically, intravitreally, or subretinally), an effective amount of an engineered reprogramming factor that is deficient in LLPS (e.g., any of

the engineered reprogramming factors described herein). In some embodiments, the method of preserving or improving the viability and/or function of an RGC of an eye comprises rejuvenating the RGC using any of the rejuvenation methods described above.

[0026] Another aspect of the present application provides a method of delaying or preventing the progression of vision impairment or loss in an individual (e.g., human), comprising introducing into a population of retinal cells of an eye of the individual, or introducing to an eye of the individual (e.g., topically, intravitreally, or subretinally), an effective amount of an engineered reprogramming factor that is deficient in LLPS (e.g., any of the engineered reprogramming factors described herein). In some embodiments, the method of preventing the progression of vision impairment or loss in an individual comprises rejuvenating a population of retinal cells of an eye of the individual using any of the rejuvenation methods described above.

[0027] Another aspect of the present application provides a method of treating vision impairment or loss in an individual (e.g., human), comprising introducing into a population of retinal cells of an eye of the individual (e.g., topically, intravitreally, or subretinally), or introducing to an eye of the individual, an effective amount of an engineered reprogramming factor that is deficient in LLPS (e.g., any of the engineered reprogramming factors described herein). In some embodiments, the method of treating vision impairment or loss in an individual comprises rejuvenating a population of retinal cells of an eye of the individual using any of the rejuvenation methods described above.

[0028] Another aspect of the present application provides a method of preserving or improving vision in an individual (e.g., human), comprising introducing into a population of retinal cells of an eye of the individual (e.g., topically, intravitreally, or subretinally), or introducing to an eye of the individual, an effective amount of an engineered reprogramming factor that is deficient in LLPS (e.g., any of the engineered reprogramming factors described herein). In some embodiments, the method of preserving or improving vision in an individual comprises rejuvenating a population of retinal cells of the individual using any of the rejuvenation methods described above.

[0029] Another aspect of the present application provides a method of promoting retinal regeneration in an eye of an individual (e.g., human), comprising introducing into a population of retinal cells of the eye, or introducing to the eye (e.g., topically, intravitreally, or subretinally), an effective amount of an engineered reprogramming factor that is deficient in LLPS (e.g., any of the

engineered reprogramming factors described herein). In some embodiments, the method of promoting retinal regeneration in an eye comprises rejuvenating a population of retinal cells of the eye using any of the rejuvenation methods described above.

[0030] Another aspect of the present application provides a method of treating an individual (e.g., human) having aged or damaged retinal tissue or a retinal tissue with disease or condition in an eye, comprising introducing into a population of retinal cells of the eye, or introducing to the eye (e.g., topically, intravitreally, or subretinally), an effective amount of an engineered reprogramming factor that is deficient in LLPS (e.g., any of the engineered reprogramming factors described herein). In some embodiments, the method of treating aged or damaged retinal tissue or a retinal tissue with disease or condition in an eye comprises rejuvenating a population of retinal cells of the eye using any of the rejuvenation methods described above.

[0031] Another aspect of the present application provides a method of rejuvenating an optic nerve of an eye in an individual (e.g., human), comprising introducing to the eye (e.g., topically, intravitreally, or subretinally) an effective amount of an engineered reprogramming factor that is deficient in LLPS (e.g., any of the engineered reprogramming factors described herein). In some embodiments, the method of rejuvenating an optic nerve of an eye comprises rejuvenating an RGC of the eye using any of the rejuvenation methods described above.

[0032] Another aspect of the present application provides a method of delaying or preventing the progression of aging of an optic nerve of an eye in an individual (e.g., human, e.g., 22 years or older, such as 40 years or older), comprising introducing to the eye (e.g., topically, intravitreally, or subretinally) an effective amount of an engineered reprogramming factor that is deficient in LLPS (e.g., any of the engineered reprogramming factors described herein). In some embodiments, the method of delaying or preventing the progression of aging of an optic nerve of an eye comprises rejuvenating an RGC of the eye using any of the rejuvenation methods described above.

[0033] Another aspect of the present application provides a method of preserving or improving the viability and/or function of an optic nerve of an eye in an individual (e.g., human), comprising introducing to the eye (e.g., topically, intravitreally, or subretinally) an effective amount of an engineered reprogramming factor that is deficient in LLPS (e.g., any of the engineered reprogramming factors described herein). In some embodiments, the method of preserving or

improving the viability and/or function of an optic nerve of an eye comprises rejuvenating an RGC of the eye using any of the rejuvenation methods described above.

[0034] Another aspect of the present application provides a method of promoting optic nerve regeneration in an eye of an individual (e.g., human), comprising introducing to the eye (e.g., topically, intravitreally, or subretinally) an effective amount of an engineered reprogramming factor that is deficient in LLPS (e.g., any of the engineered reprogramming factors described herein). In some embodiments, the method of promoting optic nerve regeneration in an eye comprises rejuvenating an RGC of the eye using any of the rejuvenation methods described above.

[0035] Another aspect of the present application provides a method of treating an individual (e.g., human) having aged or damaged optic nerve or an optic nerve with disease or condition in an eye, comprising introducing to the eye (e.g., topically, intravitreally, or subretinally) an effective amount of an engineered reprogramming factor that is deficient in LLPS (e.g., any of the engineered reprogramming factors described herein). In some embodiments, the method of treating aged or damaged optic nerve or an optic nerve with disease or condition in an eye comprises rejuvenating an RGC of the eye using any of the rejuvenation methods described above.

[0036] Another aspect of the present application provides a method of preventing aging, damage, or disease or condition in a retinal tissue of an eye in an individual (e.g., human), comprising introducing into a population of retinal cells of the retinal tissue, or introducing to the eye (e.g., topically, intravitreally, or subretinally), an effective amount of an engineered reprogramming factor that is deficient in LLPS (e.g., any of the engineered reprogramming factors described herein). In some embodiments, the method of preventing aging, damage, or disease or condition in a retinal tissue of an eye comprises rejuvenating a population of retinal cells of the retinal tissue using any of the rejuvenation methods described above. In some embodiments, the retinal tissue and/or optic nerve is: (i) susceptible of aging or with predisposition toward aging; (ii) susceptible of being damaged or with predisposition toward damage; (iii) susceptible of or with predisposition toward developing a disease or condition; and/or (iv) to be administered a surgical procedure. In some embodiments, the damage is due to: a) optic nerve crush; and/or b) laser photocoagulation.

[0037] Another aspect of the present application provides a method of preventing aging, damage, or disease or condition in an optic nerve of an eye in an individual (e.g., human), comprising introducing to the eye (e.g., topically, intravitreally, or subretinally) an effective amount of an

engineered reprogramming factor that is deficient in LLPS (e.g., any of the engineered reprogramming factors described herein). In some embodiments, the method of preventing aging, damage, or disease or condition in an optic nerve of an eye comprises rejuvenating an RGC of the eye using any of the rejuvenation methods described above. In some embodiments, the retinal tissue and/or optic nerve is: (i) susceptible of aging or with predisposition toward aging; (ii) susceptible of being damaged or with predisposition toward damage; (iii) susceptible of or with predisposition toward developing a disease or condition; and/or (iv) to be administered a surgical procedure. In some embodiments, the damage is due to: a) optic nerve crush; and/or b) laser photocoagulation.

[0038] In some embodiments according to any of the methods described above, the aging, damage, or disease or condition of the retinal tissue or the eye is selected from the group consisting of: AMD, glaucoma, acute or chronic injury to the retina or optic nerve, Stargardt's disease, retinitis pigmentosa, retinal degeneration, optic atrophy, cataracts, optic neuropathy, diabetic retinopathy, TON, vision impairment or loss, and UV damage, or any combination thereof.

[0039] In some embodiments according to any of the methods described above, the individual is a human (e.g., or any age). In some embodiments, the human is 10 years or older (e.g., 15, 22, 30, 40, 50 years old, or older).

BRIEF DESCRIPTION OF THE DRAWINGS

[0040] FIGs. 1A-1B show verification of GFP-OCT4 and GFP-OCT4mutant (GFP-OCT4') expression and distribution pattern in DAPI-stained 293T cells. FIG. 1A shows distribution pattern of GFP-OCT4 and GFP-OCT4' in 293T cells, including after treatment with 1,6-hexanediol. FIG. 1B shows fluorescence recovery after photobleaching (FRAP) of GFP-Oct4 and GFP-Oct4' droplets.

[0041] FIGs. 2A-2D show schematic diagrams of AnaPIE circular RNA (circRNA) vector structures containing one or two nucleic acids encoding different reprogramming factors. FIG. 2A shows the vector structure of AnaPIE-OS (encoding OCT4 and SOX2). FIG. 2B shows the vector structure of AnaPIE-M (encoding c-MYC). FIG. 2C shows the vector structure of AnaPIE-K (encoding KLF4). FIG. 2D shows the vector structure of AnaPIE-LN (encoding LIN28 and NANOG).

[0042] FIG. 3 shows results of 1% agarose gel electrophoresis following digestion with RNase R enzyme to verify formation of AnaPIE-OS, AnaPIE-M, AnaPIE-K and AnaPIE-LN circRNAs.

[0043] FIG. 4 shows results of 2% agarose gel electrophoresis of test circRNAs AnaPIE-OS, AnaPIE-M, AnaPIE-K and AnaPIE-LN compared to control circRNA AnaPIE-GFP.

[0044] FIG. 5 shows Western blot analysis of protein expression from AnaPIE-OS, AnaPIE-M, AnaPIE-K and AnaPIE-LN circRNAs following transfection into HEK 293T cells.

[0045] FIGs. 6A-6F show schematic diagrams of AzoPIE circRNA plasmid structures containing three or four nucleic acids encoding different reprogramming factors. FIG. 6A shows the vector structure of AzoPIE-OSK (encoding OCT4, SOX2, and KLF4). FIG. 6B shows the vector structure of AzoPIE-O'SK (encoding OCT4', SOX2, and KLF4). FIG. 6C shows the vector structure of AzoPIE-OKM (encoding OCT4, KLF4, and c-MYC). FIG. 6D shows the vector structure of AzoPIE-O'KM' (encoding OCT4', KLF4, and c-MYC'). FIG. 6E shows the vector structure of AzoPIE-OKMS (encoding OCT4, KLF4, c-MYC, and SOX2). FIG. 6F shows the vector structure of AzoPIE-O'KM'S (encoding OCT4', KLF4, c-MYC', and SOX2).

[0046] FIG. 7 shows results of 1% agarose gel electrophoresis of AzoPIE-OSK, AzoPIE-O'SK, AzoPIE-O'KM', and AzoPIE-OKM circRNAs.

[0047] FIG. 8 shows results of 1% agarose gel electrophoresis of AzoPIE-OKMS and AzoPIE-O'KM'S circRNAs.

[0048] FIG. 9 shows Western blot analysis of protein expression from AzoPIE-OKM, AzoPIE-O'KM', and AzoPIE-OKMS circRNAs following transfection into HEK 293T cells.

[0049] FIGs. 10A-10C show schematic diagrams of AzoPIE circRNA vector structures containing nucleic acids encoding mouse OCT4 (wild-type (MsO) or mutant (MsOmut)), mouse KLF4 (MsKLF4) and mouse SOX2 (MsSOX2). FIG. 10A shows the vector structure of AzoPIE-MsOKS. FIGs. 10B-10C show the vector structure of AzoPIE-MsOmutKS encoding two different mouse OCT4 mutants. FIG. 10B shows the vector structure of AzoPIE-MsOmut1KS. FIG. 10C shows the vector structure of AzoPIE-MsOmut2KS.

[0050] FIG. 11 shows Western blot analysis of protein expression from the AzoPIE-MsOmut1KS circRNA following transfection into HEK 293T cells.

[0051] FIGs. 12A-12B show characterization of HEK cell exosomes. FIG. 12A shows a transmission electron microscope (TEM) image of exosome samples showing typical cup-shaped morphology. FIG. 12B shows nano flow cytometry results showing that the size of the exosomes is generally between 60-80 nm.

[0052] FIG. 13 shows nano flow cytometry results measuring concentration and size of HEK cell exosome (HEK-exo) and human MSC exosome (huMSC-exo) with and without the loading of circular RNA.

[0053] FIG. 14 shows RT-qPCR results indicating the copy number of circRNAs in HEK-exo, huMSC-exo, and control samples.

[0054] FIGs. 15A-15B show verification of circRNA delivery by exosomes using fluorescent reporters eGFP. FIG. 15A shows microscopy images showing eGFP expression 24 hours after incubating HEK-exo with circRNA-eGFP. FIG. 15B shows microscopy images showing eGFP expression 24 hours after incubating huMSC-exo with circRNA-eGFP.

[0055] FIG. 16 shows nano flow cytometry results showing no significant difference in loading efficiency of OSK-circRNA and O'SK circRNA in HEK-exo.

[0056] FIG. 17 shows immunofluorescence verification of expression of human KLF4, OCT4, and SOX4 proteins from OSK-circRNA-loaded HEK-exosome following transfection into human skin fibroblast cells. DAPI staining shows nuclei.

[0057] FIGs. 18A-18B show lentiviral vector construction. FIG. 18A shows a schematic diagram of four lentiviral vector structures: LV-TRE3G-OKM, LV-TRE3G-O'KM', LV-TRE3G-OKMS, and LV-TRE3G-O'KM'S. FIG. 18B shows results of enzyme digestion of the four lentiviral vector plasmids.

[0058] FIGs. 19A-19B show the lentiviral vector quality control assay. FIG. 19A shows lentiviral titer and sterility test results of LV-TRE3G-OKM, LV-TRE3G-O'KM', LV-TRE3G-OKMS, and LV-TRE3G-O'KM'S. FIG. 19B shows detection of KLF4 RNA expression levels of the four lentiviral vector plasmids following transduction into HEK 293T cells.

[0059] FIG. 20 shows a schematic diagram of the AAV-TRE3G-OKS vector structure.

[0060] FIG. 21 shows results of enzyme digestion of the AAV-TRE3G-OKS vector plasmid.

- [0061] FIG. 22 shows a schematic diagram of the AAV-TRE3G-O'KS vector structure.
- [0062] FIG. 23 shows results of enzyme digestion of the AAV-TRE3G-O'KS vector plasmid.
- [0063] FIGs. 24A-24C show RT-qPCR results for the AAV-TRE3G vectors. FIG. 24A shows the original amplification curve set. FIG. 24B shows the standard curve. FIG. 24C shows the titer measurement of AAV-TRE3G-OKS and AAV-TRE3G-O'KS using RT-qPCR.
- [0064] FIG. 25 shows immunostaining of KLF4 in mouse retinal ganglion cells two weeks after intravitreal injection with AAV-TRE3G-eGFP, AAV-TRE3G-OKS, or AAV-TRE3G-O'KS, together with co-injection of AAV-CMV-TET3G and followed by doxycyclin induction.
- [0065] FIG. 26 shows Western blot analysis of KLF4 expression from AAV-TRE3G-OKS and AAV-TRE3G-O'KS treated groups.
- [0066] FIG. 27 shows a schematic diagram of mouse AAV-CMV-OKS (AAV-CMV-MsOKS) vector structure.
- [0067] FIG. 28 shows results of enzyme digestion of the AAV-CMV-MsOKS vector plasmid.
- [0068] FIG. 29 shows a schematic diagram of AAV-CMV-MsOmut1KS vector encoding mouse Oct4 mutant 1.
- [0069] FIG. 30 show results of enzyme digestion of the AAV-CMV-MsOmut1KS vector plasmid.
- [0070] FIG. 31 shows a schematic diagram of AAV-CMV-MsOmut2KS vector encoding OCT4 mutant 2.
- [0071] FIG. 32 shows results of enzyme digestion of AAV-CMV-MsOmut2KS vector plasmid.
- [0072] FIGs. 33A-33C show RT-qPCR results for the AAV2-CMV vectors. FIG. 33A shows the original amplification curve set. FIG. 33B shows the standard curve. FIG. 33C shows viral particle titer measurement of AAV2-CMV-MsOKS, AAV2-CMV-MsOmut1KS, and AAV2-CMV-MsOmut2KS using RT-qPCR.
- [0073] FIGs. 34A-34C show RT-qPCR results for the AAV9-CMV vectors. FIG. 34A shows the original amplification curve set. FIG. 34B shows the standard curve. FIG. 34C shows viral particle titer measurement of AAV9-CMV-MsOKS and AAV9-CMV-MsOmut1KS using RT-qPCR.

[0074] FIGs. 35A-35B show a protocol for assessing the effects of AAV-TRE3G-OKS and AAV-TRE3G-O'KS on optic nerve regeneration and retinal ganglion cell (RGC) function following damage via laser coagulation. FIG. 35A shows the experimental timeline for assessing regenerative effects following retinal damage induced by laser coagulation. FIG. 35B shows a summary of the experimental groups. AAV-Luciferase served as negative control.

[0075] FIGs. 36A-36B show effects of AAV-TRE3G-OKS and AAV-TRE3G-O'KS on laser-induced retina lesion. FIG. 36A shows representative Optical Coherence Tomography (OCT) images. FIG. 36B shows a statistical analysis of laser-induced retina lesion area of each experimental group. AAV-Luciferase served as negative control.

[0076] FIGs. 37A-37B show effects of AAV-TRE3G-OKS and AAV-TRE3G-O'KS on ERG after laser coagulation. FIG. 37A shows representative full field ERG images. FIG. 37B shows a statistical analysis of photopic negative response (PhNR) amplitude by experimental group. PhNR is a measurement of the electrophysiological function of RGCs. AAV-Luciferase served as negative control.

[0077] FIGs. 38A-38B show effects of AAV-TRE3G-OKS and AAV-TRE3G-O'KS on optic nerve regeneration. FIG. 38A shows representative immunofluorescent images of nervous marker β III-tubulin in optic nerves. FIG. 38B shows a statistical analysis of β III-tubulin density. AAV-Luciferase served as negative control.

[0078] FIGs. 39A-39B show effects of AAV-TRE3G-OKS and AAV-TRE3G-O'KS on RGC survival. FIG. 39A shows representative immunofluorescent images of RGC marker BRN3A in retina. FIG. 39B shows quantification of RGC number per mm^2 area. AAV-Luciferase served as negative control.

[0079] FIGs. 40A-40B show a protocol for assessing the effects of AAV9-CMV-MsOKS and AAV9-CMV-MsO_{mut}KS on optic nerve regeneration and RGC function following damage via optic nerve crush. FIG. 40A shows the experimental timeline for assessing regenerative effects following optic nerve crush damage. FIG. 40B shows a summary of the experimental groups.

[0080] FIGs. 41A-41B show effects of AAV9-CMV-MsOKS and AAV9-CMV-MsO_{mut}KS on RGC electrophysiological function in response to optical nerve crush. FIG. 41A shows representative waveform graphs of pERG results. FIG. 41B shows a statistical analysis of P50-

N95 amplitude of the pERG. AAV-EGFP (no crush) and AAV-EGFR (crush) served as negative control.

[0081] FIG. 42 shows P1-N2 waveform graphs of fVEP for groups treated with AAV9-CMV-MsOKS and AAV9-CMV-MsO_{mut1}KS. AAV-EGFR (crush) served as negative control.

[0082] FIGs. 43A-43B show a protocol for assessing the effects of AAV2-CMV-MsOKS, AAV2-CMV-MsO_{mut1}KS, and AAV2-CMV-MsO_{mut2}KS on optic nerve regeneration and RGC survival following damage via optic nerve crush. FIG. 43A shows the experimental timeline for assessing regenerative effects following optic nerve crush damage. FIG. 43B shows a summary of the experimental groups.

[0083] FIGs. 44A-44B show effects of AAV2-CMV-MsOKS, AAV2-CMV-MsO_{mut1}KS, and AAV2-CMV-MsO_{mut2}KS on optic nerve regeneration. FIG. 44A shows representative images of CTB555-labeled axons regenerated in optic nerves following crush. FIG. 44B shows quantification of CTB intensity at different distances from the crush site.

[0084] FIGs. 45A-45B show effects of AAV2-CMV-MsOKS, AAV2-CMV-MsO_{mut1}KS, and AAV2-CMV-MsO_{mut2}KS on RGC survival. FIG. 45A shows representative wholemound retina images showing RGC marker RBPMS staining. FIG. 45B shows quantification of RGC number per 0.04 mm² area.

DETAILED DESCRIPTION OF THE INVENTION

[0085] Inventors of the present application discovered that engineered reprogramming factor(s) that is deficient in liquid-liquid phase separation (LLPS) (hereinafter also referred to as “LLPS-deficient reprogramming factor”) could mediate initiation phase reprogramming (IPR). Because of the deficiency in LLPS (or forming condensates), cellular reprogramming remains at the initiation phase. The benefits of the methods described herein include but are not limited to: i) cells and tissues (*e.g.*, retinal cells, retina or optic nerve) can be effectively rejuvenated, including effective reversion of EpiAge, reversal or amelioration of vision impairment or loss associated with aging, reducing biomarkers of cell senescence and/or apoptosis, inducing retinal cell regeneration, increasing retinal ganglion cell (RGC) axon density, improving electrophysiology, histology, and/or function of the retina/optic nerve, and effectively repairing injured or damaged cells and tissues (*e.g.*, eye damage due to optic nerve crush or laser photocoagulation), *etc.*; ii)

rejuvenated cells retain their cell identities (e.g., RGC) instead of becoming iPSCs; iii) the efficiency of cell rejuvenation is greatly improved while the risk of iPSC induction is greatly reduced, compared to that achieved by the traditional 4-Factor (non-engineered OSKM) or 3-Factor reprogramming methods; iv) various delivery methods are compatible with the LLPS-deficient reprogramming factors and rejuvenation methods described herein, such as DNA or RNA (e.g., circRNA or linear mRNA) delivery, viral vector delivery, or delivery by exosomes, liposome, or lipid nanoparticles (LNPs), hence suitable for feasible applications to various organ and tissue types (e.g., retina or optic nerve, such as using viral delivery). These findings demonstrate promising applications in various clinical settings, such as eye (e.g., retinal, optic nerve) rejuvenation, preventing or reducing the progression of aging, damage, disease or condition of retinal tissue or optic nerve, or vision impairment or loss associated with, preserving or improving the viability and/or function of RGC and/or optic nerve, treating aging, damage, disease or condition of retinal tissue or optic nerve, or associated vision impairment or loss, preserving or improving vision, and promoting retinal and/or optic nerve regeneration.

[0086] Hence in one aspect, the present application provides a method of rejuvenating a population of retinal cells of an eye in an individual (e.g., human) (herein after also referred to as “retinal rejuvenation method”), comprising introducing into the population of retinal cells, or to the eye (e.g., topically, intravitreally, or subretinally), an effective amount of an engineered reprogramming factor that is deficient in LLPS. In another aspect, the present application provides a method of rejuvenating an optic nerve of an eye in an individual (e.g., human) (herein after also referred to as “optic nerve rejuvenation method”), comprising introducing to the eye (e.g., topically, intravitreally, or subretinally) an effective amount of an engineered reprogramming factor that is deficient in LLPS. These methods are collectively referred herein as “eye rejuvenation methods” or “rejuvenation methods.” The rejuvenation methods provided herein can rejuvenate the eye, including but not limited to, the retinal tissue and the optic nerve. Also provided are methods of delaying or preventing the progression of aging of a retinal tissue, or vision impairment or loss, in an individual (e.g., human), methods of preserving or improving the viability and/or function of an retinal ganglion cell (RGC), methods of preserving or improving vision, methods of promoting retinal regeneration, and methods of treating and/or preventing aging, damage, or disease or condition in a retinal tissue, or vision impairment or loss, in an individual, using any of the rejuvenation methods and/or LLPS-deficient engineered reprogramming factors described herein.

Further provided are methods of delaying or preventing the progression of aging of an optic nerve of an eye, methods of preserving or improving the viability and/or function of an optic nerve of an eye, methods of promoting optic nerve regeneration in an eye, and methods of treating and/or preventing aging, damage, or disease or condition in an optic nerve of an eye in an individual (*e.g.*, human), using any of the rejuvenation methods and/or LLPS-deficient engineered reprogramming factors described herein.

[0087] In another aspect, the present application provides engineered reprogramming factors that are deficient in LLPS, such as for use in any of the eye rejuvenation methods described herein. Also provided are methods of preventing aging, damage, or disease or condition in a retinal tissue and/or an optic nerve of an eye in an individual (*e.g.*, human), or vision impairment or loss associated with, comprising administering to the eye (*e.g.*, to a population of retinal cells, or to the optic nerve) an effective amount of an engineered reprogramming factor that is deficient in LLPS (*e.g.*, any of the engineered reprogramming factors described herein), such as before the individual reaches certain age (*e.g.*, before 22 years or older), or before the retinal tissue/optic nerve is exposed to a damage factor (*e.g.*, optic nerve crush or laser photocoagulation).

I. Definitions

[0088] As used herein, “condensate” means a non-membrane-encapsulated compartment formed by phase separation of one or more proteins and/or other macromolecules such as nucleic acids (including all stages of phase separation).

[0089] The term “liquid-liquid phase separation” (LLPS) or “phase separation” refers to a process by which macromolecules (*e.g.*, protein, nucleic acid) condense into a liquid-like dense phase, demixing from a diluted surrounding environment. LLPS underlies the formation of biomolecular condensates. It allows for the enrichment of specific factors while excluding others from the condensates, thereby creating a unique environment that either favours or restricts certain biochemical reactions.

[0090] As used herein, “stem cell” refers to those cells which retain the ability to renew themselves through mitotic cell division and which can differentiate into a diverse range of specialized cell types. It includes both embryonic stem cells (ESCs) that are found in blastocysts, and adult stem cells that are found in adult tissues. “Totipotent cells” refers to cells that have the ability to develop into cells derived from all three embryonic germ layers (mesoderm, endoderm

and ectoderm) and an entire organism (e.g., human being if placed in a woman's uterus in the case of humans). Totipotent stem cells are produced by the fusion of an egg and a sperm. The cells produced by the first few divisions of a fertilized egg are also totipotent. Totipotent cells may give rise to an embryo, the extra embryonic membranes and all post-embryonic tissues and organs. ESCs are the offspring of totipotent cells. ESCs are pluripotent and can differentiate into cell types from any of the three germ layers.

[0091] As used herein, the term “pluripotent” refers to cells with the ability to give rise to progeny that can undergo differentiation, under appropriate conditions, into cell types that collectively exhibit characteristics associated with cell lineages from the three germ layers (endoderm, mesoderm, and ectoderm). Pluripotent stem cells (PSC) can contribute to tissues of a prenatal, postnatal or adult organism. A standard art-accepted test, such as the ability to form a teratoma in 8-12 week old SCID mice, can be used to establish the pluripotency of a cell population. However, identification of various pluripotent stem cell characteristics can also be used to identify pluripotent cells. Human pluripotent stem cells may express at least some, and optionally all, of the markers from the following non-limiting list: SSEA-3, SSEA-4, TRA-I-60, TRA-I-81, TRA-2-49/6E, ALP, Sox2, E-cadherin, UTF-I, Oct4, Lin28, REX-1, and Nanog.

[0092] As used herein, the term “induced pluripotent stem cell” (iPSC) refers to a pluripotent stem cell artificially derived from a non-pluripotent cell. A non-pluripotent cell can be a cell of lesser potency to self-renew and differentiate than a pluripotent stem cell. Cells of lesser potency can be, but are not limited to, somatic stem cells, tissue specific progenitor cells, primary or secondary cells. iPSCs can derive from adult cells that are induced into ESC-like pluripotency by cell reprogramming factors. iPSCs can be derived from adult somatic cells such as skin or blood cells. iPSCs have the potential to differentiate into any of the three germ layer cell types.

[0093] The term “cellular reprogramming” refers to the process of altering the epigenome of a cell using reprogramming factors (*e.g.*, reversing or preventing epigenetic changes in cells that are causes of dysfunction, deterioration, cell death, senescence or aging). Cellular reprogramming may be complete reprogramming, such that a differentiated cell (*e.g.*, somatic cell) is reprogrammed to a PSC. Cellular reprogramming may be incomplete, such that a differentiated cell (*e.g.*, somatic cell) retains its cellular identity (*e.g.*, lineage-specific stem cell). Cellular reprogramming may be incomplete, *e.g.*, a stem cell is not created, such that a cell is rejuvenated, or takes on more youthful

attributes (e.g., increased survival, reduced inflammation, or ability to divide). Cellular reprogramming may provide additional cellular functions, or prevent cellular aging (e.g., transdifferentiation, or transition into cellular senescence). Cellular reprogramming may induce temporary or permanent gene expression changes. In some embodiments, cellular reprogramming prevents senescence from occurring.

[0094] As used herein, the term “complete reprogramming” refers to the use of cell reprogramming factors to induce somatic cell conversion to iPSCs. The complete reprogramming process can be divided into three phases: the initiation phase, the maturation phase, and the stabilization phase. In the initiation phase, the cells still maintain their original somatic identity, but the expression of early pluripotency-related genes such as NANOG and SALL4 increases rapidly; in the maturation phase, the somatic identity characteristics are rapidly lost, and the expression of late pluripotency-related genes such as DPPA5 and LIN28 increases; and in the stabilization phase, the cells completely lose their cell identity and become iPSCs.

[0095] As used herein, the term “loss of cell identity” refers to the reprogramming of somatic cells to lose the morphology, function, epigenetic patterns, gene expression profiles, and other characteristics of the original somatic cells (*i.e.*, “cell identity”), and to obtain various characteristics of PSCs and become iPSCs. The cellular identity of somatic cells is gradually lost in the process of reprogramming. In the initiation phase, the cells still maintain their original cell identity; and in the maturation phase, the cells have lost most of their cell identity characteristics and obtained a higher degree of PSC characteristics.

[0096] The term “partial reprogramming” refers to the treatment or reprogramming of an aged or damaged cell using one or more cell reprogramming factors to restore some viability to the cells, but not enough to dedifferentiate into iPSCs and thus retain their original somatic identity. Transient reprogramming is an implementation of partial reprogramming, in which cells are exposed to cellular reprogramming factors for a sufficient period of time to rejuvenate the cells, but not enough to cause dedifferentiation into iPSCs. Due to the very short exposure time, the degree of rejuvenation of this instantaneous reprogramming is low.

[0097] “Initiation phase reprogramming” or “IPR” refers to reprogramming an aged or damaged cell in the initiation phase, so as to have a longer safety time window to rejuvenate the cells while greatly reduce the risk of becoming an iPSC.

[0098] A “reprogramming factor” is a factor, for example, it may be a transcription factor, which can be used to reprogram a target cell. The term “reprogramming factor” further includes any analogue molecule that mimics the function of the factor with respect to reprogramming capacity.

[0099] The term “rejuvenating” or “rejuvenation” as used herein is meant to include preventing or reversing the cellular causes of aging or damages without inducing a pluripotent state.

[0100] As used herein, the term “rejuvenated cell(s)” refers to aged or damaged cells that have been treated or reprogrammed with one or more cellular reprogramming factors such that the cells have a transcriptomic profile of a younger or healthier cell while still retaining one or more cell identity markers.

[0101] “Regeneration” refers to the capability of contributing to the repair or *de novo* construction of a cell, tissue or organ. In some embodiments, cell rejuvenation comprises cell regeneration.

[0102] The term “tissue regeneration” refers to restoration of existing tissue or cells (e.g., aged, damaged, or diseased) and/or production of new tissue or cells within a tissue that are the same type as the tissue of interest (e.g., same type as the damaged tissue or cell).

[0103] The term “tissue repair” in the context of damaged tissue refers to restoration of tissue architecture, function following tissue damage, or a combination thereof. Tissue repair includes tissue regeneration, cell growth, tissue replacement, and/or rewiring of existing tissue (reprogramming).

[0104] As used herein, the terms “treatment,” “treat,” and “treating” refer to reversing, alleviating, delaying the onset of, or inhibiting the progress of a disease or disorder, or one or more symptoms thereof, as described herein. In certain embodiments, treatment may be administered after one or more symptoms have developed. In other embodiments, treatment may be administered in the absence of symptoms. For example, treatment may be administered to a susceptible individual prior to the onset of symptoms, not yet been diagnosed with a disease or condition, or may be treated with another damaging agent (e.g., in light of a history of symptoms, in light of genetic or other susceptibility factors, a disease therapy, or any combination thereof). Treatment may also be continued after symptoms have resolved, for example, to prevent or delay their recurrence. Desirable effects of treatment include decreasing the rate of disease or condition

progression, ameliorating or palliating the disease state or symptoms, achieving desired pharmacological and/or physiological effect, and remission or improved prognosis. Said effects may be prophylactic in terms of complete or partial prevention of the disease or its symptoms, and/or therapeutic in terms of partial or complete cure of the disease and/or adverse effects resulting from the disease. For example, an individual is successfully “treated” if one or more symptoms associated with the disease or condition are mitigated or eliminated, including, but not limited to, delaying or preventing the progression of the disease or condition, decreasing symptoms resulting from the disease or condition, increasing the quality of life of those suffering from the disease or condition, decreasing the dose of other medications required to treat the disease or condition, and/or prolonging survival of individuals.

[0105] “Prevention” as used herein refers to all acts of avoiding symptoms or delaying the tension of a particular symptom by administering an agent before or after the onset of the development of the disease or condition.

[0106] As used herein, an “effective amount” refers to an amount of an agent effective to provide the desired treatment or prevention after being administered in single or multiple doses. As is understood in the clinical context, an effective amount of an agent or pharmaceutical composition may or may not be achieved in conjunction with another agent or pharmaceutical composition. Thus, an “effective amount” may be considered in the context of administering one or more agents, and a single agent may be considered to be given in an effective amount if, in conjunction with one or more other agents, a desirable result may be or is achieved.

[0107] As used herein, an “individual” or a “subject” refers to a mammal, including, but not limited to, human, bovine, horse, sheep, goat, pig, deer, donkey, feline, canine, rodent (e.g., rat, mouse, rabbit, hamster), or non-human primate (e.g., monkey, chimpanzee). In some embodiments, the individual is a human. In some embodiments, the individual is a pet, e.g., horse, dog, cat, rabbit, guinea pig, hamster, rat, mouse, ferret, chinchilla, etc. In some embodiments, the individual is a farm animal, e.g., horse, cattle, cow, goat, sheep, pig, donkey, etc.

[0108] “Tissue” refers to an ensemble of cells (identical or not) and an extracellular matrix (ECM) that together carry out a specific function or set of functions.

[0109] The term “variant” refers to a sequence that comprises a modification relative to a wild-type sequence. Non-limiting modifications in an amino acid sequence include insertions, deletions,

and point mutations. Non-limiting modifications to nucleic acid (e.g., engineered nucleic acid) sequences include frameshift mutations, nucleotide insertions, and nucleotide deletions.

[0110] As used herein, “percent (%) amino acid sequence identity” and “homology” with respect to a peptide or polypeptide sequence are defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the specific peptide or polypeptide sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN or MEGALIGN™ (DNASTAR) software. Those skilled in the art can determine appropriate parameters for measuring alignment, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared.

[0111] An amino acid substitution may include but is not limited to the replacement of one amino acid in a polypeptide with another amino acid. Exemplary substitutions are shown in **Table A**.

Table A. Exemplary amino acid substitutions.

Original Residue	Exemplary Substitutions
Ala (A)	Val; Leu; Ile
Arg (R)	Lys; Gln; Asn
Asn (N)	Gln; His; Asp, Lys; Arg
Asp (D)	Glu; Asn
Cys (C)	Ser; Ala
Gln (Q)	Asn; Glu
Glu (E)	Asp; Gln
Gly (G)	Ala
His (H)	Asn; Gln; Lys; Arg
Ile (I)	Leu; Val; Met; Ala; Phe; Norleucine
Leu (L)	Norleucine; Ile; Val; Met; Ala; Phe
Lys (K)	Arg; Gln; Asn
Met (M)	Leu; Phe; Ile
Phe (F)	Trp; Leu; Val; Ile; Ala; Tyr
Pro (P)	Ala
Ser (S)	Thr
Thr (T)	Val; Ser
Trp (W)	Tyr; Phe
Tyr (Y)	Trp; Phe; Thr; Ser
Val (V)	Ile; Leu; Met; Phe; Ala; Norleucine

[0112] Amino acids may be grouped according to common side-chain properties: (1) hydrophobic: Norleucine, Met, Ala, Val, Leu, Ile; (2) neutral hydrophilic: Cys, Ser, Thr, Asn, Gln; (3) acidic: Asp, Glu; (4) basic: His, Lys, Arg; (5) residues that influence chain orientation: Gly, Pro; (6) aromatic: Trp, Tyr, Phe. Non-conservative substitutions will entail exchanging a member of one of these classes for another class.

[0113] As used herein, the term “transfection” refers to the uptake of exogenous DNA or RNA by a cell. A cell has been “transfected” when exogenous DNA or RNA has been introduced inside the cell membrane. A number of transfection techniques are generally known in the art. See, e.g., Graham et al. (1973) *Virology*, 52:456, Sambrook et al. (2001) *Molecular Cloning*, a laboratory manual, 3rd edition, Cold Spring Harbor Laboratories, New York, Davis et al. (1995) *Basic Methods in Molecular Biology*, 2nd edition, McGraw-Hill, and Chu et al. (1981) *Gene* 13: 197. Such techniques can be used to introduce one or more exogenous DNA or RNA molecules into cells. The term refers to both stable and transient uptake of the DNA or RNA molecules. For example, transfection can be used for transient uptake of mRNAs encoding cellular reprogramming factors into cells in need of rejuvenation.

[0114] The terms “host cell,” “host cell line,” and “host cell culture” are used interchangeably and refer to cells into which exogenous nucleic acid has been introduced, including the progeny of such cells. Host cells include “transformants” and “transformed cells,” which include the primary transformed cell and progeny derived therefrom without regard to the number of passages. Progeny may not be completely identical in nucleic acid content to a parent cell, and may contain mutations. Mutant progenies that have the same function or biological activity as screened or selected for in the originally transformed cell are included herein.

[0115] The term “pharmaceutical formulation” of “pharmaceutical composition” refers to a preparation that is in such form as to permit the biological activity of the active ingredient to be effective, and that contains no additional components that are unacceptably toxic to a subject to which the formulation would be administered. Such formulations are sterile. A “sterile” formulation is aseptic or free from all living microorganisms and their spores.

[0116] It will be understood by one of ordinary skill in the art that uracil and thymine can both be represented by ‘t’, instead of ‘u’ for uracil and ‘t’ for thymine; in the context of a ribonucleic acid, it will be understood that ‘t’ is used to represent uracil unless otherwise indicated.

[0117] It is understood that embodiments of the invention described herein include “consisting” and/or “consisting essentially of” embodiments.

[0118] Reference to “about” a value or parameter herein includes (and describes) variations that are directed to that value or parameter *per se*. For example, description referring to “about X” includes description of “X”.

[0119] As used herein, reference to “not” a value or parameter generally means and describes “other than” a value or parameter. For example, the method is not used to treat cancer of type X means the method is used to treat cancer of types other than X.

[0120] The term “about X-Y” used herein has the same meaning as “about X to about Y.”

[0121] As used herein and in the appended claims, the singular forms “a,” “or,” and “the” include plural referents unless the context clearly dictates otherwise.

II. Methods of rejuvenating a population of retinal cells and/or an optic nerve

[0122] In some embodiments, there is provided a method of rejuvenating a population of retinal cells of an eye in an individual (e.g., human), comprising introducing into the population of retinal cells, or introducing to the eye (e.g., topically, intravitreally, or subretinally), an effective amount of an engineered reprogramming factor that is deficient in LLPS (e.g., any of the LLPS-deficient engineered reprogramming factors described herein), or a nucleic acid (DNA or RNA), a nucleic acid construct (e.g., circRNA or plasmid), or a virus encoding thereof, or a composition (e.g., pharmaceutical composition) comprising thereof. In some embodiments, there is provided a method of: (i) delaying or preventing the progression of aging of a retinal tissue of an eye in an individual (e.g., human, e.g., 22 years or older), (ii) preserving or improving the viability and/or function of an RGC of an eye in an individual (e.g., human), (iii) delaying or preventing the progression of vision impairment or loss in an individual (e.g., human), (iv) treating vision impairment or loss in an individual (e.g., human), (v) preserving or improving vision in an individual (e.g., human), (vi) promoting retinal regeneration in an eye of an individual (e.g., human), (vii) treating an individual (e.g., human) having aged or damaged retinal tissue or a retinal tissue with disease or condition in an eye, and/or (viii) preventing aging, damage, or disease or condition in a retinal tissue of an eye in an individual (e.g., human), comprising: (a) introducing into a population of retinal cells of the retinal tissue or the eye (e.g., introducing to the RGC), or

introducing to the eye (e.g., topically, intravitreally, or subretinally), of the individual, an effective amount of an engineered reprogramming factor that is deficient in LLPS (e.g., any of the LLPS-deficient engineered reprogramming factors described herein), or a nucleic acid (DNA or RNA), a nucleic acid construct (e.g., circRNA or plasmid), or a virus encoding thereof, or a composition (e.g., pharmaceutical composition) comprising thereof; or (b) rejuvenating a population of retinal cells of the retinal tissue or the eye (e.g., RGC) using any of the eye (e.g., retinal) rejuvenation methods described herein. In some embodiments, there is provided a method of: (i) rejuvenating an optic nerve of an eye in an individual (e.g., human), (ii) delaying or preventing the progression of aging of an optic nerve of an eye in an individual (e.g., human, e.g., 22 years or older), (iii) preserving or improving the viability and/or function of an optic nerve of an eye in an individual (e.g., human), (iv) promoting optic nerve regeneration in an eye of an individual (e.g., human), (v) treating an individual (e.g., human) having aged or damaged optic nerve or an optic nerve with disease or condition in an eye, and/or (vi) preventing aging, damage, or disease or condition in an optic nerve of an eye in an individual (e.g., human), comprising: (a) introducing to the eye (e.g., topically, intravitreally, or subretinally) an effective amount of an engineered reprogramming factor that is deficient in LLPS (e.g., any of the LLPS-deficient engineered reprogramming factors described herein), or a nucleic acid (DNA or RNA), a nucleic acid construct (e.g., circRNA or plasmid), or a virus encoding thereof, or a composition (e.g., pharmaceutical composition) comprising thereof; or (b) rejuvenating the optic nerve using any of the eye (e.g., optic nerve) rejuvenation methods described herein. In some embodiments, the population of retinal cells comprise RGCs, photoreceptor cells, bipolar cells, amacrine cells, horizontal cells, Müller glial cells, RPE cells, or any combination thereof. In some embodiments, the optic nerve is, or the population of retinal cells are from a retinal tissue that is: (i) aged; (ii) damaged; (iii) associated with (or has) ocular (e.g., retinal or optic nerve) disease or condition; and/or (iv) after a surgical procedure. In some embodiments, the retinal tissue or the optic nerve is: (i) susceptible of aging or with predisposition toward aging; (ii) susceptible of being damaged or with predisposition toward damage; (iii) susceptible of or with predisposition toward developing an ocular (e.g., retinal or optic nerve) disease or condition; and/or (iv) to be administered a surgical procedure. In some embodiments, the aging, damage, or disease or condition of the eye (e.g., retinal tissue or the optic nerve) is selected from the group consisting of: AMD, glaucoma, acute or chronic injury to the retina or optic nerve, Stargardt's disease, retinitis pigmentosa, retinal degeneration, optic atrophy,

cataracts, optic neuropathy, diabetic retinopathy, TON, vision impairment or loss, UV damage, or any combination thereof. In some embodiments, the damage is due to optic nerve crush and/or laser photocoagulation. In some embodiments, the method does not reprogram the population of retinal cells into a reprogrammed stem cell. In some embodiments, the engineered reprogramming factor comprises a mutation in an LLPS-related domain in reference to a corresponding wild-type reprogramming factor. In some embodiments, the LLPS-related domain is selected from the group consisting of an intrinsically disordered region (IDR), a region outside of DNA-binding domain (DBD), a ubiquitination site, or any combination thereof. In some embodiments, the mutation comprises: (i) substituting an acidic amino acid (e.g., D or E) within the IDR with a neutral amino acid (e.g., A); (ii) substituting an amino acid that can be ubiquitinated (e.g., K) with an amino acid that cannot be ubiquitinated (e.g., R); (iii) deleting an amino acid that can be ubiquitinated (e.g., K); (iv) deleting an amino acid (e.g., acidic amino acid) within the IDR; and/or (v) deleting an amino acid (e.g., acidic amino acid) within a region outside of DBD. In some embodiments, the wild-type reprogramming factor is from a family selected from the group consisting of OCT family, SOX family, KLF family, MYC family, LIN28 family, NANOG family, and GLIS family. In some embodiments, the wild-type reprogramming factor is selected from the group consisting of OCT4, SOX2, KLF4, c-MYC, L-MYC, LIN28, NANOG, and GLIS1. In some embodiments, the engineered reprogramming factor is an engineered OCT4, e.g., comprising an amino acid sequence of any of SEQ ID NOs: 4-10 and 44-46, such as SEQ ID NO: 4. In some embodiments, the engineered reprogramming factor is an engineered c-MYC, e.g., comprising an amino acid sequence of any of SEQ ID NOs: 14-20, 47, and 48, such as SEQ ID NO: 14. In some embodiments, the engineered reprogramming factor is an engineered SOX2, e.g., comprising an amino acid sequence of any of SEQ ID NOs: 41-43. In some embodiments, the engineered reprogramming factor is an engineered KLF4, e.g., comprising an amino acid sequence of SEQ ID NO: 40. In some embodiments, the engineered reprogramming factor is encoded by a nucleic acid, e.g., RNA such as circRNA, or DNA. In some embodiments, the nucleic acid is comprised within a vector, e.g., a viral vector, such as lentiviral vector or AAV vector. In some embodiments, the nucleic acid is delivered by an exosome, a liposome, an LNP, or a virus. In some embodiments, the method comprises administering a virus (e.g., LV or AAV) comprising a nucleic acid encoding the engineered reprogramming factor. In some embodiments, the virus is delivered via intravitreal injection or subretinal injection to the eye. In some embodiments, the method comprises

administering an exosome, a liposome, or an LNP encapsulating an RNA (e.g., circRNA) encoding the engineered reprogramming factor. In some embodiments, the exosome, liposome, or LNP is delivered topically to the eye (e.g., as eye drops, ophthalmic ointment, or ophthalmic gel). In some embodiments, the method further comprises introducing into the population of retinal cells, to the optic nerve, or to the eye, an effective amount of a therapeutic agent. In some embodiments, the effective amount of the engineered reprogramming factor is administered once. In some embodiments, the individual is a human (e.g., of any age). In some embodiments, the human is 10 years or older (e.g., at least about any of 15, 22, 30, 40, 50, 60, 70 years old, or older). In some embodiments, for preventing aging, damage, or disease or condition in a retinal cell/tissue, or in an optic nerve, the method is performed before the retinal cell/tissue or the optic nerve is aging, is exposed to a damage factor (e.g., UV irradiation, or surgical procedure), or has (e.g., diagnosed with) a disease or condition. In some embodiments, the method is performed at least about 12 hours (e.g., at least about any of 24 hours, 48 hours, 5 days, 1 week, 2 weeks, 4 weeks, or more) before the retinal cell/tissue or the optic nerve is exposed to a damage factor (e.g., laser photocoagulation).

[0123] The individual that can be treated here, either prophylactically or therapeutically, can be any mammal, including, but not limited to, human, bovine, horse, sheep, goat, pig, deer, donkey, feline, canine, rodent (e.g., rat, mouse, rabbit, hamster), or non-human primate (e.g., monkey, chimpanzee). In some embodiments, the individual is a human. In some embodiments, the individual is a non-human mammal (e.g., pet or farm animal). The human to be treated can be of any age, such as any of 10, 20, 22, 30, 40, 50, 60, 70, 80, 90 years old, or older. In some embodiments, the human is 22 years or older, such as 30 years or older, 40 years or older, 50 years or older, or 60 years or older. When the individual is a non-human mammal, the human ages described herein can be converted to equivalent non-human ages of the corresponding non-human mammal.

[0124] The engineered reprogramming factor(s) can be introduced into the population of retinal cells (e.g., RGC), to the optic nerve, or to the eye, as protein, nucleic acid (DNA or RNA, such as circRNA), nucleic acid construct (e.g., plasmid), virus (e.g., lentivirus or AAV), or a composition (e.g., pharmaceutical composition) comprising thereof. Hence, when referring to introducing into the population of retinal cells, to the optic nerve, or to the eye, an effective amount of an engineered reprogramming factor that is deficient in LLPS, it is not limited to introducing protein. Any

introduction or administration methods that can result in the engineered reprogramming factor(s) being introduced into the retinal cells, to the optic nerve, or to the eye, or can result in the engineered reprogramming factor(s) being expressed in the retinal cells, the optic nerve, or the eye, are contemplated by the methods described herein.

[0125] In some embodiments, the method comprises introducing a plurality of reprogramming factors into the population of retinal cells or to the eye (e.g., topically, intravitreally, or subretinally), wherein at least one of the plurality of reprogramming factors is an engineered reprogramming factor that is deficient in LLPS (e.g., any of the LLPS-deficient engineered reprogramming factors described herein). Hence in some embodiments, there is provided a method of rejuvenating a population of retinal cells of an eye in an individual (e.g., human), comprising introducing into the population of retinal cells, or introducing to the eye (e.g., topically, intravitreally, or subretinally), an effective amount of a plurality of reprogramming factors (or a pharmaceutical composition comprising thereof), wherein at least one of the plurality of reprogramming factors is an engineered reprogramming factor that is deficient in LLPS (e.g., any of the LLPS-deficient engineered reprogramming factors described herein). In some embodiments, there is provided a method of: (i) delaying or preventing the progression of aging of a retinal tissue of an eye in an individual (e.g., human, e.g., 22 years or older), (ii) preserving or improving the viability and/or function of an RGC of an eye in an individual (e.g., human), (iii) delaying or preventing the progression of vision impairment or loss in an individual (e.g., human), (iv) treating vision impairment or loss in an individual (e.g., human), (v) preserving or improving vision in an individual (e.g., human), (vi) promoting retinal regeneration in an eye of an individual (e.g., human), (vii) treating an individual (e.g., human) having aged or damaged retinal tissue or a retinal tissue with disease or condition in an eye, and/or (viii) preventing aging, damage, or disease or condition in a retinal tissue of an eye in an individual (e.g., human), comprising: (a) introducing into a population of retinal cells of the retinal tissue or the eye (e.g., introducing to the RGC), or introducing to the eye (e.g., topically, intravitreally, or subretinally), of the individual, an effective amount of a plurality of reprogramming factors (or a pharmaceutical composition comprising thereof), wherein at least one of the plurality of reprogramming factors is an engineered reprogramming factor that is deficient in LLPS (e.g., any of the LLPS-deficient engineered reprogramming factors described herein); or (b) rejuvenating a population of retinal cells of the retinal tissue or the eye (e.g., RGC) using any of the eye (e.g., retinal) rejuvenation methods

described herein. In some embodiments, there is provided a method of: (i) rejuvenating an optic nerve of an eye in an individual (e.g., human), (ii) delaying or preventing the progression of aging of an optic nerve of an eye in an individual (e.g., human, e.g., 22 years or older), (iii) preserving or improving the viability and/or function of an optic nerve of an eye in an individual (e.g., human), (iv) promoting optic nerve regeneration in an eye of an individual (e.g., human), (v) treating an individual (e.g., human) having aged or damaged optic nerve or an optic nerve with disease or condition in an eye, and/or (vi) preventing aging, damage, or disease or condition in an optic nerve of an eye in an individual (e.g., human), comprising: (a) introducing to the eye (e.g., topically, intravitreally, or subretinally) an effective amount of a plurality of reprogramming factors (or a pharmaceutical composition comprising thereof), wherein at least one of the plurality of reprogramming factors is an engineered reprogramming factor that is deficient in LLPS (e.g., any of the LLPS-deficient engineered reprogramming factors described herein); or (b) rejuvenating the optic nerve using any of the eye (e.g., optic nerve) rejuvenation methods described herein. In some embodiments, the plurality of reprogramming factors comprise two or more reprogramming factors from two or more families selected from the group consisting of OCT family, SOX family, KLF family, MYC family, LIN28 family, NANOG family, and GLIS family. In some embodiments, the plurality of reprogramming factors comprise two or more reprogramming factors selected from the group consisting of OCT4, SOX2, KLF4, c-MYC, L-MYC, LIN28, NANOG, GLIS1, and variant thereof. In some embodiments, the plurality of reprogramming factors comprise three or all reprogramming factors selected from the group consisting of OCT4, SOX2, KLF4, and c-MYC, wherein at least one of the plurality of reprogramming factors is an engineered reprogramming factor that is deficient in LLPS. In some embodiments, the plurality of reprogramming factors comprise OCT4 and KLF4, wherein at least one of OCT4 and KLF4 is an engineered reprogramming factor that is deficient in LLPS. In some embodiments, all of the plurality of reprogramming factors are engineered reprogramming factors that are deficient in LLPS. In some embodiments, the plurality of reprogramming factors comprise a wild-type reprogramming factor. In some embodiments, the OCT4 is an engineered OCT4 that is deficient in LLPS, e.g., comprising an amino acid sequence of any of SEQ ID NOs: 4-10 and 44-46, such as SEQ ID NO: 4. In some embodiments, c-MYC is an engineered c-MYC that is deficient in LLPS, e.g., comprising an amino acid sequence of any of SEQ ID NOs: 14-20, 47, and 48, such as SEQ ID NO: 14. In some embodiments, the population of retinal cells comprise RGCs,

photoreceptor cells, bipolar cells, amacrine cells, horizontal cells, Müller glial cells, RPE cells, or any combination thereof. In some embodiments, the optic nerve is, or the population of retinal cells are from a retinal tissue that is: (i) aged; (ii) damaged; (iii) associated with (or has) ocular (e.g., retinal or optic nerve) disease or condition; and/or (iv) after a surgical procedure. In some embodiments, the retinal tissue or the optic nerve is: (i) susceptible of aging or with predisposition toward aging; (ii) susceptible of being damaged or with predisposition toward damage; (iii) susceptible of or with predisposition toward developing an ocular (e.g., retinal or optic nerve) disease or condition; and/or (iv) to be administered a surgical procedure. In some embodiments, the aging, damage, or disease or condition of the eye (e.g., retinal tissue or the optic nerve) is selected from the group consisting of: AMD, glaucoma, acute or chronic injury to the retina or optic nerve, Stargardt's disease, retinitis pigmentosa, retinal degeneration, optic atrophy, cataracts, optic neuropathy, diabetic retinopathy, TON, vision impairment or loss, UV damage, or any combination thereof. In some embodiments, the damage is due to optic nerve crush and/or laser photocoagulation. In some embodiments, the method does not reprogram the population of retinal cells into a reprogrammed stem cell. In some embodiments, the plurality of reprogramming factors are encapsulated within an exosome, a liposome, or an LNP. In some embodiments, the exosome, liposome, or LNP is delivered topically to the eye (e.g., as eye drops, ophthalmic ointment, or ophthalmic gel). In some embodiments, the method further comprises introducing into the population of retinal cells, to the optic nerve, or to the eye, an effective amount of a therapeutic agent. In some embodiments, the effective amount of the plurality of reprogramming factors is administered once. In some embodiments, the individual is a human (e.g., of any age). In some embodiments, the human is 10 years or older (e.g., at least about any of 15, 22, 30, 40, 50, 60, 70 years old, or older). In some embodiments, for preventing aging, damage, or disease or condition in a retinal cell/tissue, or in an optic nerve, the method is performed before the retinal cell/tissue or the optic nerve is aging, is exposed to a damage factor (e.g., UV irradiation, or surgical procedure), or has (e.g., diagnosed with) a disease or condition. In some embodiments, the method is performed at least about 12 hours (e.g., at least about any of 24 hours, 48 hours, 5 days, 1 week, 2 weeks, 4 weeks, or more) before the retinal cell/tissue or the optic nerve is exposed to a damage factor (e.g., laser photocoagulation).

[0126] In some embodiments, there is provided a method of rejuvenating a population of retinal cells of an eye in an individual (e.g., human), comprising introducing into the population of retinal

cells, or introducing to the eye (e.g., topically, intravitreally, or subretinally), an effective amount of a plurality of reprogramming factors (or a pharmaceutical composition comprising thereof), wherein the plurality of reprogramming factors comprise an engineered OCT4 that is deficient in LLPS (e.g., any of SEQ ID NOs: 4-10 and 44-46, such as SEQ ID NO: 4), SOX2 (e.g., SEQ ID NO: 24), and KLF4 (e.g., SEQ ID NO: 22). In some embodiments, there is provided a method of: (i) delaying or preventing the progression of aging of a retinal tissue of an eye in an individual (e.g., human, e.g., 22 years or older), (ii) preserving or improving the viability and/or function of an RGC of an eye in an individual (e.g., human), (iii) delaying or preventing the progression of vision impairment or loss in an individual (e.g., human), (iv) treating vision impairment or loss in an individual (e.g., human), (v) preserving or improving vision in an individual (e.g., human), (vi) promoting retinal regeneration in an eye of an individual (e.g., human), (vii) treating an individual (e.g., human) having aged or damaged retinal tissue or a retinal tissue with disease or condition in an eye, and/or (viii) preventing aging, damage, or disease or condition in a retinal tissue of an eye in an individual (e.g., human), comprising: introducing into a population of retinal cells of the retinal tissue or the eye (e.g., introducing to the RGC), or introducing to the eye (e.g., topically, intravitreally, or subretinally), of the individual, an effective amount of a plurality of reprogramming factors (or a pharmaceutical composition comprising thereof), wherein the plurality of reprogramming factors comprise an engineered OCT4 that is deficient in LLPS (e.g., any of SEQ ID NOs: 4-10 and 44-46, such as SEQ ID NO: 4), SOX2 (e.g., SEQ ID NO: 24), and KLF4 (e.g., SEQ ID NO: 22). In some embodiments, there is provided a method of: (i) rejuvenating an optic nerve of an eye in an individual (e.g., human), (ii) delaying or preventing the progression of aging of an optic nerve of an eye in an individual (e.g., human, e.g., 22 years or older), (iii) preserving or improving the viability and/or function of an optic nerve of an eye in an individual (e.g., human), (iv) promoting optic nerve regeneration in an eye of an individual (e.g., human), (v) treating an individual (e.g., human) having aged or damaged optic nerve or an optic nerve with disease or condition in an eye, and/or (vi) preventing aging, damage, or disease or condition in an optic nerve of an eye in an individual (e.g., human), comprising introducing to the eye (e.g., topically, intravitreally, or subretinally) an effective amount of a plurality of reprogramming factors (or a pharmaceutical composition comprising thereof), wherein the plurality of reprogramming factors comprise an engineered OCT4 that is deficient in LLPS (e.g., any of SEQ ID NOs: 4-10 and 44-46, such as SEQ ID NO: 4), SOX2 (e.g., SEQ ID NO: 24), and KLF4 (e.g.,

SEQ ID NO: 22). In some embodiments, the population of retinal cells comprise RGCs, photoreceptor cells, bipolar cells, amacrine cells, horizontal cells, Müller glial cells, RPE cells, or any combination thereof. In some embodiments, the optic nerve is, or the population of retinal cells are from a retinal tissue that is: (i) aged; (ii) damaged; (iii) associated with (or has) ocular (e.g., retinal or optic nerve) disease or condition; and/or (iv) after a surgical procedure. In some embodiments, the retinal tissue or the optic nerve is: (i) susceptible of aging or with predisposition toward aging; (ii) susceptible of being damaged or with predisposition toward damage; (iii) susceptible of or with predisposition toward developing an ocular (e.g., retinal or optic nerve) disease or condition; and/or (iv) to be administered a surgical procedure. In some embodiments, the aging, damage, or disease or condition of the eye (e.g., retinal tissue or the optic nerve) is selected from the group consisting of: AMD, glaucoma, acute or chronic injury to the retina or optic nerve, Stargardt's disease, retinitis pigmentosa, retinal degeneration, optic atrophy, cataracts, optic neuropathy, diabetic retinopathy, TON, vision impairment or loss, UV damage, or any combination thereof. In some embodiments, the damage is due to optic nerve crush and/or laser photocoagulation. In some embodiments, the method does not reprogram the population of retinal cells into a reprogrammed stem cell. In some embodiments, the plurality of reprogramming factors are encapsulated within an exosome, a liposome, or an LNP. In some embodiments, the exosome, liposome, or LNP is delivered topically to the eye (e.g., as eye drops, ophthalmic ointment, or ophthalmic gel). In some embodiments, the method further comprises introducing into the population of retinal cells, to the optic nerve, or to the eye, an effective amount of a therapeutic agent. In some embodiments, the effective amount of the plurality of reprogramming factors is administered once. In some embodiments, the individual is a human (e.g., of any age). In some embodiments, the human is 10 years or older (e.g., at least about any of 15, 22, 30, 40, 50, 60, 70 years old, or older). In some embodiments, for preventing aging, damage, or disease or condition in a retinal cell/tissue, or in an optic nerve, the method is performed before the retinal cell/tissue or the optic nerve is aging, is exposed to a damage factor (e.g., UV irradiation, or surgical procedure), or has (e.g., diagnosed with) a disease or condition. In some embodiments, the method is performed at least about 12 hours (e.g., at least about any of 24 hours, 48 hours, 5 days, 1 week, 2 weeks, 4 weeks, or more) before the retinal cell/tissue or the optic nerve is exposed to a damage factor (e.g., laser photocoagulation).

[0127] In some embodiments, there is provided a method of rejuvenating a population of retinal cells of an eye in an individual (e.g., human), comprising introducing into the population of retinal cells, or introducing to the eye (e.g., topically, intravitreally, or subretinally), an effective amount of a plurality of reprogramming factors (or a pharmaceutical composition comprising thereof), wherein the plurality of reprogramming factors comprise an engineered OCT4 that is deficient in LLPS (e.g., any of SEQ ID NOs: 4-10 and 44-46, such as SEQ ID NO: 4), KLF4 (e.g., SEQ ID NO: 22), and an engineered c-MYC that is deficient in LLPS (e.g., any of SEQ ID NOs: 14-20, 47, and 48, such as SEQ ID NO: 14). In some embodiments, there is provided a method of: (i) delaying or preventing the progression of aging of a retinal tissue of an eye in an individual (e.g., human, e.g., 22 years or older), (ii) preserving or improving the viability and/or function of an RGC of an eye in an individual (e.g., human), (iii) delaying or preventing the progression of vision impairment or loss in an individual (e.g., human), (iv) treating vision impairment or loss in an individual (e.g., human), (v) preserving or improving vision in an individual (e.g., human), (vi) promoting retinal regeneration in an eye of an individual (e.g., human), (vii) treating an individual (e.g., human) having aged or damaged retinal tissue or a retinal tissue with disease or condition in an eye, and/or (viii) preventing aging, damage, or disease or condition in a retinal tissue of an eye in an individual (e.g., human), comprising introducing into a population of retinal cells of the retinal tissue or the eye (e.g., introducing to the RGC), or introducing to the eye (e.g., topically, intravitreally, or subretinally), of the individual, an effective amount of a plurality of reprogramming factors (or a pharmaceutical composition comprising thereof), wherein the plurality of reprogramming factors comprise an engineered OCT4 that is deficient in LLPS (e.g., any of SEQ ID NOs: 4-10 and 44-46, such as SEQ ID NO: 4), KLF4 (e.g., SEQ ID NO: 22), and an engineered c-MYC that is deficient in LLPS (e.g., any of SEQ ID NOs: 14-20, 47, and 48, such as SEQ ID NO: 14). In some embodiments, there is provided a method of: (i) rejuvenating an optic nerve of an eye in an individual (e.g., human), (ii) delaying or preventing the progression of aging of an optic nerve of an eye in an individual (e.g., human, e.g., 22 years or older), (iii) preserving or improving the viability and/or function of an optic nerve of an eye in an individual (e.g., human), (iv) promoting optic nerve regeneration in an eye of an individual (e.g., human), (v) treating an individual (e.g., human) having aged or damaged optic nerve or an optic nerve with disease or condition in an eye, and/or (vi) preventing aging, damage, or disease or condition in an optic nerve of an eye in an individual (e.g., human), comprising introducing to the eye (e.g., topically,

intravitreally, or subretinally) an effective amount of a plurality of reprogramming factors (or a pharmaceutical composition comprising thereof), wherein the plurality of reprogramming factors comprise an engineered OCT4 that is deficient in LLPS (e.g., any of SEQ ID NOs: 4-10 and 44-46, such as SEQ ID NO: 4), KLF4 (e.g., SEQ ID NO: 22), and an engineered c-MYC that is deficient in LLPS (e.g., any of SEQ ID NOs: 14-20, 47, and 48, such as SEQ ID NO: 14). In some embodiments, the population of retinal cells comprise RGCs, photoreceptor cells, bipolar cells, amacrine cells, horizontal cells, Müller glial cells, RPE cells, or any combination thereof. In some embodiments, the optic nerve is, or the population of retinal cells are from a retinal tissue that is: (i) aged; (ii) damaged; (iii) associated with (or has) ocular (e.g., retinal or optic nerve) disease or condition; and/or (iv) after a surgical procedure. In some embodiments, the retinal tissue or the optic nerve is: (i) susceptible of aging or with predisposition toward aging; (ii) susceptible of being damaged or with predisposition toward damage; (iii) susceptible of or with predisposition toward developing an ocular (e.g., retinal or optic nerve) disease or condition; and/or (iv) to be administered a surgical procedure. In some embodiments, the aging, damage, or disease or condition of the eye (e.g., retinal tissue or the optic nerve) is selected from the group consisting of: AMD, glaucoma, acute or chronic injury to the retina or optic nerve, Stargardt's disease, retinitis pigmentosa, retinal degeneration, optic atrophy, cataracts, optic neuropathy, diabetic retinopathy, TON, vision impairment or loss, UV damage, or any combination thereof. In some embodiments, the damage is due to optic nerve crush and/or laser photocoagulation. In some embodiments, the method does not reprogram the population of retinal cells into a reprogrammed stem cell. In some embodiments, the plurality of reprogramming factors are encapsulated within an exosome, a liposome, or an LNP. In some embodiments, the exosome, liposome, or LNP is delivered topically to the eye (e.g., as eye drops, ophthalmic ointment, or ophthalmic gel). In some embodiments, the method further comprises introducing into the population of retinal cells, to the optic nerve, or to the eye, an effective amount of a therapeutic agent. In some embodiments, the effective amount of the plurality of reprogramming factors is administered once. In some embodiments, the individual is a human (e.g., of any age). In some embodiments, the human is 10 years or older (e.g., at least about any of 15, 22, 30, 40, 50, 60, 70 years old, or older). In some embodiments, for preventing aging, damage, or disease or condition in a retinal cell/tissue, or in an optic nerve, the method is performed before the retinal cell/tissue or the optic nerve is aging, is exposed to a damage factor (e.g., UV irradiation, or surgical procedure), or has (e.g., diagnosed with) a disease or condition.

In some embodiments, the method is performed at least about 12 hours (e.g., at least about any of 24 hours, 48 hours, 5 days, 1 week, 2 weeks, 4 weeks, or more) before the retinal cell/tissue or the optic nerve is exposed to a damage factor (e.g., laser photocoagulation).

[0128] In some embodiments, there is provided a method of rejuvenating a population of retinal cells of an eye in an individual (e.g., human), comprising introducing into the population of retinal cells, or introducing to the eye (e.g., topically, intravitreally, or subretinally), an effective amount of a plurality of reprogramming factors (or a pharmaceutical composition comprising thereof), wherein the plurality of reprogramming factors comprise an engineered OCT4 that is deficient in LLPS (e.g., any of SEQ ID NOs: 4-10 and 44-46, such as SEQ ID NO: 4), KLF4 (e.g., SEQ ID NO: 22), an engineered c-MYC that is deficient in LLPS (e.g., any of SEQ ID NOs: 14-20, 47, and 48, such as SEQ ID NO: 14), and SOX2 (e.g., SEQ ID NO: 24). In some embodiments, there is provided a method of: (i) delaying or preventing the progression of aging of a retinal tissue of an eye in an individual (e.g., human, e.g., 22 years or older), (ii) preserving or improving the viability and/or function of an RGC of an eye in an individual (e.g., human), (iii) delaying or preventing the progression of vision impairment or loss in an individual (e.g., human), (iv) treating vision impairment or loss in an individual (e.g., human), (v) preserving or improving vision in an individual (e.g., human), (vi) promoting retinal regeneration in an eye of an individual (e.g., human), (vii) treating an individual (e.g., human) having aged or damaged retinal tissue or a retinal tissue with disease or condition in an eye, and/or (viii) preventing aging, damage, or disease or condition in a retinal tissue of an eye in an individual (e.g., human), comprising introducing into a population of retinal cells of the retinal tissue or the eye (e.g., introducing to the RGC), or introducing to the eye (e.g., topically, intravitreally, or subretinally), of the individual, an effective amount of a plurality of reprogramming factors (or a pharmaceutical composition comprising thereof), wherein the plurality of reprogramming factors comprise an engineered OCT4 that is deficient in LLPS (e.g., any of SEQ ID NOs: 4-10 and 44-46, such as SEQ ID NO: 4), KLF4 (e.g., SEQ ID NO: 22), an engineered c-MYC that is deficient in LLPS (e.g., any of SEQ ID NOs: 14-20, 47, and 48, such as SEQ ID NO: 14), and SOX2 (e.g., SEQ ID NO: 24). In some embodiments, there is provided a method of: (i) rejuvenating an optic nerve of an eye in an individual (e.g., human), (ii) delaying or preventing the progression of aging of an optic nerve of an eye in an individual (e.g., human, e.g., 22 years or older), (iii) preserving or improving the viability and/or function of an optic nerve of an eye in an individual (e.g., human), (iv) promoting optic nerve

regeneration in an eye of an individual (e.g., human), (v) treating an individual (e.g., human) having aged or damaged optic nerve or an optic nerve with disease or condition in an eye, and/or (vi) preventing aging, damage, or disease or condition in an optic nerve of an eye in an individual (e.g., human), comprising introducing to the eye (e.g., topically, intravitreally, or subretinally) an effective amount of a plurality of reprogramming factors (or a pharmaceutical composition comprising thereof), wherein the plurality of reprogramming factors comprise an engineered OCT4 that is deficient in LLPS (e.g., any of SEQ ID NOs: 4-10 and 44-46, such as SEQ ID NO: 4), KLF4 (e.g., SEQ ID NO: 22), an engineered c-MYC that is deficient in LLPS (e.g., any of SEQ ID NOs: 14-20, 47, and 48, such as SEQ ID NO: 14), and SOX2 (e.g., SEQ ID NO: 24). In some embodiments, the population of retinal cells comprise RGCs, photoreceptor cells, bipolar cells, amacrine cells, horizontal cells, Müller glial cells, RPE cells, or any combination thereof. In some embodiments, the optic nerve is, or the population of retinal cells are from a retinal tissue that is: (i) aged; (ii) damaged; (iii) associated with (or has) ocular (e.g., retinal or optic nerve) disease or condition; and/or (iv) after a surgical procedure. In some embodiments, the retinal tissue or the optic nerve is: (i) susceptible of aging or with predisposition toward aging; (ii) susceptible of being damaged or with predisposition toward damage; (iii) susceptible of or with predisposition toward developing an ocular (e.g., retinal or optic nerve) disease or condition; and/or (iv) to be administered a surgical procedure. In some embodiments, the aging, damage, or disease or condition of the eye (e.g., retinal tissue or the optic nerve) is selected from the group consisting of: AMD, glaucoma, acute or chronic injury to the retina or optic nerve, Stargardt's disease, retinitis pigmentosa, retinal degeneration, optic atrophy, cataracts, optic neuropathy, diabetic retinopathy, TON, vision impairment or loss, UV damage, or any combination thereof. In some embodiments, the damage is due to optic nerve crush and/or laser photocoagulation. In some embodiments, the method does not reprogram the population of retinal cells into a reprogrammed stem cell. In some embodiments, the plurality of reprogramming factors are encapsulated within an exosome, a liposome, or an LNP. In some embodiments, the exosome, liposome, or LNP is delivered topically to the eye (e.g., as eye drops, ophthalmic ointment, or ophthalmic gel). In some embodiments, the method further comprises introducing into the population of retinal cells, to the optic nerve, or to the eye, an effective amount of a therapeutic agent. In some embodiments, the effective amount of the plurality of reprogramming factors is administered once. In some embodiments, the individual is a human (e.g., of any age). In some embodiments, the human is 10 years or older (e.g., at least

about any of 15, 22, 30, 40, 50, 60, 70 years old, or older). In some embodiments, for preventing aging, damage, or disease or condition in a retinal cell/tissue, or in an optic nerve, the method is performed before the retinal cell/tissue or the optic nerve is aging, is exposed to a damage factor (e.g., UV irradiation, or surgical procedure), or has (e.g., diagnosed with) a disease or condition. In some embodiments, the method is performed at least about 12 hours (e.g., at least about any of 24 hours, 48 hours, 5 days, 1 week, 2 weeks, 4 weeks, or more) before the retinal cell/tissue or the optic nerve is exposed to a damage factor (e.g., laser photocoagulation).

[0129] In some embodiments, the method comprises introducing a plurality of nucleic acids (e.g., RNA (e.g., circRNA) or DNA) encoding a plurality of reprogramming factors into the population of retinal cells or to the eye (e.g., topically, intravitreally, or subretinally), wherein at least one of the plurality of reprogramming factors is an engineered reprogramming factor that is deficient in LLPS (e.g., any of the LLPS-deficient engineered reprogramming factors described herein). Hence in some embodiments, there is provided a method of rejuvenating a population of retinal cells of an eye in an individual (e.g., human), comprising introducing into the population of retinal cells, or introducing to the eye (e.g., topically, intravitreally, or subretinally), an effective amount of a plurality of nucleic acids (RNA (e.g., circRNA) or DNA, such as any of the nucleic acids described herein) (or a pharmaceutical composition comprising thereof) encoding a plurality of reprogramming factors, wherein at least one of the plurality of reprogramming factors is an engineered reprogramming factor that is deficient in LLPS (e.g., any of the LLPS-deficient engineered reprogramming factors described herein). In some embodiments, there is provided a method of: (i) delaying or preventing the progression of aging of a retinal tissue of an eye in an individual (e.g., human, e.g., 22 years or older), (ii) preserving or improving the viability and/or function of an RGC of an eye in an individual (e.g., human), (iii) delaying or preventing the progression of vision impairment or loss in an individual (e.g., human), (iv) treating vision impairment or loss in an individual (e.g., human), (v) preserving or improving vision in an individual (e.g., human), (vi) promoting retinal regeneration in an eye of an individual (e.g., human), (vii) treating an individual (e.g., human) having aged or damaged retinal tissue or a retinal tissue with disease or condition in an eye, and/or (viii) preventing aging, damage, or disease or condition in a retinal tissue of an eye in an individual (e.g., human), comprising: (a) introducing into a population of retinal cells of the retinal tissue or the eye (e.g., introducing to the RGC), or introducing to the eye (e.g., topically, intravitreally, or subretinally), of the individual, an effective

amount of a plurality of nucleic acids (RNA (e.g., circRNA) or DNA, such as any of the nucleic acids described herein) (or a pharmaceutical composition comprising thereof) encoding a plurality of reprogramming factors, wherein at least one of the plurality of reprogramming factors is an engineered reprogramming factor that is deficient in LLPS (e.g., any of the LLPS-deficient engineered reprogramming factors described herein); or (b) rejuvenating a population of retinal cells of the retinal tissue or the eye (e.g., RGC) using any of the eye (e.g., retinal) rejuvenation methods described herein. In some embodiments, there is provided a method of: (i) rejuvenating an optic nerve of an eye in an individual (e.g., human), (ii) delaying or preventing the progression of aging of an optic nerve of an eye in an individual (e.g., human, e.g., 22 years or older), (iii) preserving or improving the viability and/or function of an optic nerve of an eye in an individual (e.g., human), (iv) promoting optic nerve regeneration in an eye of an individual (e.g., human), (v) treating an individual (e.g., human) having aged or damaged optic nerve or an optic nerve with disease or condition in an eye, and/or (vi) preventing aging, damage, or disease or condition in an optic nerve of an eye in an individual (e.g., human), comprising: (a) introducing to the eye (e.g., topically, intravitreally, or subretinally) an effective amount of a plurality of nucleic acids (RNA (e.g., circRNA) or DNA, such as any of the nucleic acids described herein) (or a pharmaceutical composition comprising thereof) encoding a plurality of reprogramming factors, wherein at least one of the plurality of reprogramming factors is an engineered reprogramming factor that is deficient in LLPS (e.g., any of the LLPS-deficient engineered reprogramming factors described herein); or (b) rejuvenating the optic nerve using any of the eye (e.g., optic nerve) rejuvenation methods described herein. In some embodiments, the plurality of reprogramming factors comprise two or more reprogramming factors from two or more families selected from the group consisting of OCT family, SOX family, KLF family, MYC family, LIN28 family, NANOG family, and GLIS family. In some embodiments, the plurality of reprogramming factors comprise two or more reprogramming factors selected from the group consisting of OCT4, SOX2, KLF4, c-MYC, L-MYC, LIN28, NANOG, GLIS1, and variant thereof. In some embodiments, the plurality of reprogramming factors comprise three or all reprogramming factors selected from the group consisting of OCT4, SOX2, KLF4, and c-MYC, wherein at least one of the plurality of reprogramming factors is an engineered reprogramming factor that is deficient in LLPS. In some embodiments, the plurality of reprogramming factors comprise OCT4 and KLF4, wherein at least one of OCT4 and KLF4 is an engineered reprogramming factor that is deficient in LLPS. In some

embodiments, all of the plurality of reprogramming factors are engineered reprogramming factors that are deficient in LLPS. In some embodiments, the plurality of reprogramming factors comprise a wild-type reprogramming factor. In some embodiments, the OCT4 is an engineered OCT4 that is deficient in LLPS, e.g., comprising an amino acid sequence of any of SEQ ID NOs: 4-10 and 44-46, such as SEQ ID NO: 4. In some embodiments, c-MYC is an engineered c-MYC that is deficient in LLPS, e.g., comprising an amino acid sequence of any of SEQ ID NOs: 14-20, 47, and 48, such as SEQ ID NO: 14. In some embodiments, the population of retinal cells comprise RGCs, photoreceptor cells, bipolar cells, amacrine cells, horizontal cells, Müller glial cells, RPE cells, or any combination thereof. In some embodiments, the optic nerve is, or the population of retinal cells are from a retinal tissue that is: (i) aged; (ii) damaged; (iii) associated with (or has) ocular (e.g., retinal or optic nerve) disease or condition; and/or (iv) after a surgical procedure. In some embodiments, the retinal tissue or the optic nerve is: (i) susceptible of aging or with predisposition toward aging; (ii) susceptible of being damaged or with predisposition toward damage; (iii) susceptible of or with predisposition toward developing an ocular (e.g., retinal or optic nerve) disease or condition; and/or (iv) to be administered a surgical procedure. In some embodiments, the aging, damage, or disease or condition of the eye (e.g., retinal tissue or the optic nerve) is selected from the group consisting of: AMD, glaucoma, acute or chronic injury to the retina or optic nerve, Stargardt's disease, retinitis pigmentosa, retinal degeneration, optic atrophy, cataracts, optic neuropathy, diabetic retinopathy, TON, vision impairment or loss, UV damage, or any combination thereof. In some embodiments, the damage is due to optic nerve crush and/or laser photocoagulation. In some embodiments, the method does not reprogram the population of retinal cells into a reprogrammed stem cell. The plurality of nucleic acids can be on separate nucleic acid constructs (e.g., circRNA, plasmid, or recombinant viral genome), or on a single nucleic acid construct. The plurality of nucleic acids can be delivered using an exosome, a liposome, an LNP, or a virus (e.g., LV or AAV). In some embodiments, at least two of (e.g., all of) the plurality of nucleic acids are on a single nucleic acid construct. In some embodiments, the at least two of (e.g., all of) the plurality of nucleic acids are under the control of different promoters. In some embodiments, the at least two of (e.g., all of) the plurality of nucleic acids are under the control of the same promoter. In some embodiments, the at least two of (e.g., all of) the plurality of nucleic acids are connected by one or more linking nucleic acids. In some embodiments, the one or more linking nucleic acids comprise IRES or a linking sequence encoding a self-cleaving peptide, e.g.,

P2A, T2A, or E2A. In some embodiments, the nucleic acid construct comprises a nucleic acid sequence of any of SEQ ID NOs: 28, 30, 32, 35, 37, 39, 57, 59, 61, 94, 98, and 102. In some embodiments, the plurality of nucleic acids are one or more RNAs (e.g., one circRNA). In some embodiments, the one or more RNAs (e.g., one circRNA) are encapsulated within an exosome, a liposome, or an LNP. In some embodiments, the exosome, liposome, or LNP is delivered topically to the eye (e.g., as eye drops, ophthalmic ointment, or ophthalmic gel). In some embodiments, the plurality of nucleic acids are one or more DNAs. In some embodiments, the one or more (e.g., all) of the plurality of nucleic acids (DNA or RNA) are comprised within a vector (e.g., viral genome), such as comprised within a virus. In some embodiments, the method comprises administering a virus (e.g., LV or AVV) comprising one or more (e.g., all) of the plurality of nucleic acids (RNA or DNA). In some embodiments, the virus is delivered via intravitreal injection or subretinal injection to the eye. In some embodiments, the method further comprises introducing into the population of retinal cells, to the optic nerve, or to the eye, an effective amount of a therapeutic agent. In some embodiments, the effective amount of the plurality of nucleic acids is administered once. In some embodiments, the individual is a human (e.g., of any age). In some embodiments, the human is 10 years or older (e.g., at least about any of 15, 22, 30, 40, 50, 60, 70 years old, or older). In some embodiments, for preventing aging, damage, or disease or condition in a retinal cell/tissue, or in an optic nerve, the method is performed before the retinal cell/tissue or the optic nerve is aging, is exposed to a damage factor (e.g., UV irradiation, or surgical procedure), or has (e.g., diagnosed with) a disease or condition. In some embodiments, the method is performed at least about 12 hours (e.g., at least about any of 24 hours, 48 hours, 5 days, 1 week, 2 weeks, 4 weeks, or more) before the retinal cell/tissue or the optic nerve is exposed to a damage factor (e.g., laser photocoagulation).

[0130] In some embodiments, the plurality of nucleic acids encoding the plurality of reprogramming factors are on a single nucleic acid construct (e.g., RNA (e.g., circRNA) or DNA). In some embodiments, the plurality of nucleic acids are under the control of the same promoter. In some embodiments, the plurality of nucleic acids are connected by one or more linking nucleic acids. In some embodiments, the one or more linking nucleic acids comprise IRES or a linking sequence encoding a self-cleaving peptide, e.g., P2A, T2A, or E2A.

[0131] In some embodiments, there is provided a method of rejuvenating a population of retinal cells of an eye in an individual (e.g., human), comprising introducing into the population of retinal

cells, or introducing to the eye (e.g., topically, intravitreally, or subretinally), an effective amount of a plurality of nucleic acids (RNA (e.g., circRNA) or DNA; or nucleic acid construct(s) or virus comprising thereof) (or a pharmaceutical composition comprising thereof) encoding a plurality of reprogramming factors, wherein the plurality of reprogramming factors comprise an engineered OCT4 that is deficient in LLPS (e.g., any of SEQ ID NOs: 4-10 and 44-46, such as SEQ ID NO: 4), SOX2 (e.g., SEQ ID NO: 24), and KLF4 (e.g., SEQ ID NO: 22). In some embodiments, there is provided a method of: (i) delaying or preventing the progression of aging of a retinal tissue of an eye in an individual (e.g., human, e.g., 22 years or older), (ii) preserving or improving the viability and/or function of an RGC of an eye in an individual (e.g., human), (iii) delaying or preventing the progression of vision impairment or loss in an individual (e.g., human), (iv) treating vision impairment or loss in an individual (e.g., human), (v) preserving or improving vision in an individual (e.g., human), (vi) promoting retinal regeneration in an eye of an individual (e.g., human), (vii) treating an individual (e.g., human) having aged or damaged retinal tissue or a retinal tissue with disease or condition in an eye, and/or (viii) preventing aging, damage, or disease or condition in a retinal tissue of an eye in an individual (e.g., human), comprising introducing into a population of retinal cells of the retinal tissue or the eye (e.g., introducing to the RGC), or introducing to the eye (e.g., topically, intravitreally, or subretinally), of the individual, an effective amount of a plurality of nucleic acids (RNA (e.g., circRNA) or DNA; or nucleic acid construct(s) or virus comprising thereof) (or a pharmaceutical composition comprising thereof) encoding a plurality of reprogramming factors, wherein the plurality of reprogramming factors comprise an engineered OCT4 that is deficient in LLPS (e.g., any of SEQ ID NOs: 4-10 and 44-46, such as SEQ ID NO: 4), SOX2 (e.g., SEQ ID NO: 24), and KLF4 (e.g., SEQ ID NO: 22). In some embodiments, there is provided a method of: (i) rejuvenating an optic nerve of an eye in an individual (e.g., human), (ii) delaying or preventing the progression of aging of an optic nerve of an eye in an individual (e.g., human, e.g., 22 years or older), (iii) preserving or improving the viability and/or function of an optic nerve of an eye in an individual (e.g., human), (iv) promoting optic nerve regeneration in an eye of an individual (e.g., human), (v) treating an individual (e.g., human) having aged or damaged optic nerve or an optic nerve with disease or condition in an eye, and/or (vi) preventing aging, damage, or disease or condition in an optic nerve of an eye in an individual (e.g., human), comprising introducing to the eye (e.g., topically, intravitreally, or subretinally) an effective amount of a plurality of nucleic acids (RNA (e.g., circRNA) or DNA; or

nucleic acid construct(s) or virus comprising thereof) (or a pharmaceutical composition comprising thereof) encoding a plurality of reprogramming factors, wherein the plurality of reprogramming factors comprise an engineered OCT4 that is deficient in LLPS (e.g., any of SEQ ID NOs: 4-10 and 44-46, such as SEQ ID NO: 4), SOX2 (e.g., SEQ ID NO: 24), and KLF4 (e.g., SEQ ID NO: 22). In some embodiments, the population of retinal cells comprise RGCs, photoreceptor cells, bipolar cells, amacrine cells, horizontal cells, Müller glial cells, RPE cells, or any combination thereof. In some embodiments, the optic nerve is, or the population of retinal cells are from a retinal tissue that is: (i) aged; (ii) damaged; (iii) associated with (or has) ocular (e.g., retinal or optic nerve) disease or condition; and/or (iv) after a surgical procedure. In some embodiments, the retinal tissue or the optic nerve is: (i) susceptible of aging or with predisposition toward aging; (ii) susceptible of being damaged or with predisposition toward damage; (iii) susceptible of or with predisposition toward developing an ocular (e.g., retinal or optic nerve) disease or condition; and/or (iv) to be administered a surgical procedure. In some embodiments, the aging, damage, or disease or condition of the eye (e.g., retinal tissue or the optic nerve) is selected from the group consisting of: AMD, glaucoma, acute or chronic injury to the retina or optic nerve, Stargardt's disease, retinitis pigmentosa, retinal degeneration, optic atrophy, cataracts, optic neuropathy, diabetic retinopathy, TON, vision impairment or loss, UV damage, or any combination thereof. In some embodiments, the damage is due to optic nerve crush and/or laser photocoagulation. In some embodiments, the method does not reprogram the population of retinal cells into a reprogrammed stem cell. The plurality of nucleic acids can be on separate nucleic acid constructs (e.g., circRNA, plasmid, or recombinant viral genome), or on a single nucleic acid construct. The plurality of nucleic acids can be delivered using an exosome, a liposome, an LNP, or a virus (e.g., LV or AAV). In some embodiments, at least two of (e.g., all of) the plurality of nucleic acids are on a single nucleic acid construct. In some embodiments, the at least two of (e.g., all of) the plurality of nucleic acids are under the control of different promoters. In some embodiments, the at least two of (e.g., all of) the plurality of nucleic acids are under the control of the same promoter. In some embodiments, the at least two of (e.g., all of) the plurality of nucleic acids are connected by one or more linking nucleic acids. In some embodiments, the one or more linking nucleic acids comprise IRES or a linking sequence encoding a self-cleaving peptide, e.g., P2A, T2A, or E2A. In some embodiments, the plurality of nucleic acids are one or more RNAs (e.g., one circRNA). In some embodiments, the one or more RNAs (e.g., one circRNA) are encapsulated within an exosome, a

liposome, or an LNP. In some embodiments, the exosome, liposome, or LNP is delivered topically to the eye (e.g., as eye drops, ophthalmic ointment, or ophthalmic gel). In some embodiments, the plurality of nucleic acids are one or more DNAs. In some embodiments, the one or more (e.g., all) of the plurality of nucleic acids (DNA or RNA) are comprised within a vector (e.g., viral genome), such as comprised within a virus. In some embodiments, the method comprises administering a virus (e.g., LV or AVV) comprising one or more (e.g., all) of the plurality of nucleic acids (RNA or DNA). In some embodiments, the virus is delivered via intravitreal injection or subretinal injection to the eye. In some embodiments, the method further comprises introducing into the population of retinal cells, to the optic nerve, or to the eye, an effective amount of a therapeutic agent. In some embodiments, the effective amount of the plurality of nucleic acids is administered once. In some embodiments, the individual is a human (e.g., of any age). In some embodiments, the human is 10 years or older (e.g., at least about any of 15, 22, 30, 40, 50, 60, 70 years old, or older). In some embodiments, for preventing aging, damage, or disease or condition in a retinal cell/tissue, or in an optic nerve, the method is performed before the retinal cell/tissue or the optic nerve is aging, is exposed to a damage factor (e.g., UV irradiation, or surgical procedure), or has (e.g., diagnosed with) a disease or condition. In some embodiments, the method is performed at least about 12 hours (e.g., at least about any of 24 hours, 48 hours, 5 days, 1 week, 2 weeks, 4 weeks, or more) before the retinal cell/tissue or the optic nerve is exposed to a damage factor (e.g., laser photocoagulation).

[0132] In some embodiments, there is provided a method of rejuvenating a population of retinal cells of an eye in an individual (e.g., human), comprising introducing into the population of retinal cells, or introducing to the eye (e.g., topically, intravitreally, or subretinally), an effective amount of a nucleic acid construct (RNA (e.g., circRNA) or DNA; or a virus comprising thereof) (or a pharmaceutical composition comprising thereof), wherein the nucleic acid construct comprises from 5' to 3': a first nucleic acid encoding an engineered OCT4 that is deficient in LLPS (e.g., any of SEQ ID NOs: 4-10 and 44-46, such as SEQ ID NO: 4) – a first linking sequence (e.g., encoding P2A) – a second nucleic acid encoding SOX2 (e.g., SEQ ID NO: 24) – a second linking sequence (e.g., encoding T2A) – a third nucleic acid encoding KLF4 (e.g., SEQ ID NO: 22). In some embodiments, there is provided a method of: (i) delaying or preventing the progression of aging of a retinal tissue of an eye in an individual (e.g., human, e.g., 22 years or older), (ii) preserving or improving the viability and/or function of an RGC of an eye in an individual (e.g., human), (iii)

delaying or preventing the progression of vision impairment or loss in an individual (e.g., human), (iv) treating vision impairment or loss in an individual (e.g., human), (v) preserving or improving vision in an individual (e.g., human), (vi) promoting retinal regeneration in an eye of an individual (e.g., human), (vii) treating an individual (e.g., human) having aged or damaged retinal tissue or a retinal tissue with disease or condition in an eye, and/or (viii) preventing aging, damage, or disease or condition in a retinal tissue of an eye in an individual (e.g., human), comprising introducing into a population of retinal cells of the retinal tissue or the eye (e.g., introducing to the RGC), or introducing to the eye (e.g., topically, intravitreally, or subretinally), of the individual, an effective amount of a nucleic acid construct (RNA (e.g., circRNA) or DNA; or a virus comprising thereof) (or a pharmaceutical composition comprising thereof), wherein the nucleic acid construct comprises from 5' to 3': a first nucleic acid encoding an engineered OCT4 that is deficient in LLPS (e.g., any of SEQ ID NOs: 4-10 and 44-46, such as SEQ ID NO: 4) – a first linking sequence (e.g., encoding P2A) – a second nucleic acid encoding SOX2 (e.g., SEQ ID NO: 24) – a second linking sequence (e.g., encoding T2A) – a third nucleic acid encoding KLF4 (e.g., SEQ ID NO: 22). In some embodiments, there is provided a method of: (i) rejuvenating an optic nerve of an eye in an individual (e.g., human), (ii) delaying or preventing the progression of aging of an optic nerve of an eye in an individual (e.g., human, e.g., 22 years or older), (iii) preserving or improving the viability and/or function of an optic nerve of an eye in an individual (e.g., human), (iv) promoting optic nerve regeneration in an eye of an individual (e.g., human), (v) treating an individual (e.g., human) having aged or damaged optic nerve or an optic nerve with disease or condition in an eye, and/or (vi) preventing aging, damage, or disease or condition in an optic nerve of an eye in an individual (e.g., human), comprising introducing to the eye (e.g., topically, intravitreally, or subretinally) an effective amount of a nucleic acid construct (RNA (e.g., circRNA) or DNA; or a virus comprising thereof) (or a pharmaceutical composition comprising thereof), wherein the nucleic acid construct comprises from 5' to 3': a first nucleic acid encoding an engineered OCT4 that is deficient in LLPS (e.g., any of SEQ ID NOs: 4-10 and 44-46, such as SEQ ID NO: 4) – a first linking sequence (e.g., encoding P2A) – a second nucleic acid encoding SOX2 (e.g., SEQ ID NO: 24) – a second linking sequence (e.g., encoding T2A) – a third nucleic acid encoding KLF4 (e.g., SEQ ID NO: 22). In some embodiments, the nucleic acid construct encodes a protein sequence of SEQ ID NO: 93. In some embodiments, the nucleic acid construct further comprises an IRES at 5' of the first nucleic acid. In some embodiments, the nucleic acid construct is a

circRNA, a linear mRNA, or a self-amplifying RNA (e.g., RNA viral genome). In some embodiments, the nucleic acid construct is a DNA vector (e.g., lentival vector, AAV vector, or plasmid). In some embodiments, the nucleic acid construct comprises a nucleic acid sequence of any of SEQ ID NOs: 39, 57, and 94. In some embodiments, the population of retinal cells comprise RGCs, photoreceptor cells, bipolar cells, amacrine cells, horizontal cells, Müller glial cells, RPE cells, or any combination thereof. In some embodiments, the optic nerve is, or the population of retinal cells are from a retinal tissue that is: (i) aged; (ii) damaged; (iii) associated with (or has) ocular (e.g., retinal or optic nerve) disease or condition; and/or (iv) after a surgical procedure. In some embodiments, the retinal tissue or the optic nerve is: (i) susceptible of aging or with predisposition toward aging; (ii) susceptible of being damaged or with predisposition toward damage; (iii) susceptible of or with predisposition toward developing an ocular (e.g., retinal or optic nerve) disease or condition; and/or (iv) to be administered a surgical procedure. In some embodiments, the aging, damage, or disease or condition of the eye (e.g., retinal tissue or the optic nerve) is selected from the group consisting of: AMD, glaucoma, acute or chronic injury to the retina or optic nerve, Stargardt's disease, retinitis pigmentosa, retinal degeneration, optic atrophy, cataracts, optic neuropathy, diabetic retinopathy, TON, vision impairment or loss, UV damage, or any combination thereof. In some embodiments, the damage is due to optic nerve crush and/or laser photocoagulation. In some embodiments, the method does not reprogram the population of retinal cells into a reprogrammed stem cell. The nucleic acid construct can be delivered using an exosome, a liposome, an LNP, or a virus (e.g., LV or AAV). In some embodiments, the nucleic acid construct (e.g., circRNA or linear mRNA) is encapsulated within an exosome, a liposome, or an LNP. In some embodiments, the exosome, liposome, or LNP is delivered topically to the eye (e.g., as eye drops, ophthalmic ointment, or ophthalmic gel). In some embodiments, the nucleic acid construct is a vector (e.g., viral vector or viral genome). In some embodiments, the nucleic acid construct is comprised within or delivered by a virus (e.g., LV or AVV). In some embodiments, the virus is delivered via intravitreal injection or subretinal injection to the eye. In some embodiments, the method further comprises introducing into the population of retinal cells, to the optic nerve, or to the eye, an effective amount of a therapeutic agent. In some embodiments, the nucleic acid construct (RNA or DNA; or a virus comprising thereof) (or a pharmaceutical composition comprising thereof) is administered once. In some embodiments, the individual is a human (e.g., of any age). In some embodiments, the human is 10 years or older (e.g., at least about

any of 15, 22, 30, 40, 50, 60, 70 years old, or older). In some embodiments, for preventing aging, damage, or disease or condition in a retinal cell/tissue, or in an optic nerve, the method is performed before the retinal cell/tissue or the optic nerve is aging, is exposed to a damage factor (e.g., UV irradiation, or surgical procedure), or has (e.g., diagnosed with) a disease or condition. In some embodiments, the method is performed at least about 12 hours (e.g., at least about any of 24 hours, 48 hours, 5 days, 1 week, 2 weeks, 4 weeks, or more) before the retinal cell/tissue or the optic nerve is exposed to a damage factor (e.g., laser photocoagulation).

[0133] In some embodiments, there is provided a method of rejuvenating a population of retinal cells of an eye in an individual (e.g., human), comprising introducing into the population of retinal cells, or introducing to the eye (e.g., topically, intravitreally, or subretinally), an effective amount of a nucleic acid construct (RNA (e.g., circRNA) or DNA; or a virus comprising thereof) (or a pharmaceutical composition comprising thereof), wherein the nucleic acid construct comprises from 5' to 3': a first nucleic acid encoding an engineered OCT4 that is deficient in LLPS (e.g., any of SEQ ID NOs: 4-10 and 44-46, such as SEQ ID NO: 4) – a first linking sequence (e.g., encoding P2A) – a second nucleic acid encoding KLF4 (e.g., SEQ ID NO: 22) – a second linking sequence (e.g., encoding T2A) – a third nucleic acid encoding SOX2 (e.g., SEQ ID NO: 24). In some embodiments, there is provided a method of: (i) delaying or preventing the progression of aging of a retinal tissue of an eye in an individual (e.g., human, e.g., 22 years or older), (ii) preserving or improving the viability and/or function of an RGC of an eye in an individual (e.g., human), (iii) delaying or preventing the progression of vision impairment or loss in an individual (e.g., human), (iv) treating vision impairment or loss in an individual (e.g., human), (v) preserving or improving vision in an individual (e.g., human), (vi) promoting retinal regeneration in an eye of an individual (e.g., human), (vii) treating an individual (e.g., human) having aged or damaged retinal tissue or a retinal tissue with disease or condition in an eye, and/or (viii) preventing aging, damage, or disease or condition in a retinal tissue of an eye in an individual (e.g., human), comprising introducing into a population of retinal cells of the retinal tissue or the eye (e.g., introducing to the RGC), or introducing to the eye (e.g., topically, intravitreally, or subretinally), of the individual, an effective amount of a nucleic acid construct (RNA (e.g., circRNA) or DNA; or a virus comprising thereof) (or a pharmaceutical composition comprising thereof), wherein the nucleic acid construct comprises from 5' to 3': a first nucleic acid encoding an engineered OCT4 that is deficient in LLPS (e.g., any of SEQ ID NOs: 4-10 and 44-46, such as SEQ ID NO: 4) – a first linking sequence (e.g.,

encoding P2A) – a second nucleic acid encoding KLF4 (e.g., SEQ ID NO: 22) – a second linking sequence (e.g., encoding T2A) – a third nucleic acid encoding SOX2 (e.g., SEQ ID NO: 24). In some embodiments, there is provided a method of: (i) rejuvenating an optic nerve of an eye in an individual (e.g., human), (ii) delaying or preventing the progression of aging of an optic nerve of an eye in an individual (e.g., human, e.g., 22 years or older), (iii) preserving or improving the viability and/or function of an optic nerve of an eye in an individual (e.g., human), (iv) promoting optic nerve regeneration in an eye of an individual (e.g., human), (v) treating an individual (e.g., human) having aged or damaged optic nerve or an optic nerve with disease or condition in an eye, and/or (vi) preventing aging, damage, or disease or condition in an optic nerve of an eye in an individual (e.g., human), comprising introducing to the eye (e.g., topically, intravitreally, or subretinally) an effective amount of a nucleic acid construct (RNA (e.g., circRNA) or DNA; or a virus comprising thereof) (or a pharmaceutical composition comprising thereof), wherein the nucleic acid construct comprises from 5' to 3': a first nucleic acid encoding an engineered OCT4 that is deficient in LLPS (e.g., any of SEQ ID NOs: 4-10 and 44-46, such as SEQ ID NO: 4) – a first linking sequence (e.g., encoding P2A) – a second nucleic acid encoding KLF4 (e.g., SEQ ID NO: 22) – a second linking sequence (e.g., encoding T2A) – a third nucleic acid encoding SOX2 (e.g., SEQ ID NO: 24). In some embodiments, the nucleic acid construct further comprises an IRES at 5' of the first nucleic acid. In some embodiments, the nucleic acid construct is a circRNA, a linear mRNA, or a self-amplifying RNA (e.g., RNA viral genome). In some embodiments, the nucleic acid construct is a DNA vector (e.g., lentival vector, AAV vector, or plasmid). In some embodiments, the nucleic acid construct comprises a nucleic acid sequence of SEQ ID NO: 32 or 39. In some embodiments, the population of retinal cells comprise RGCs, photoreceptor cells, bipolar cells, amacrine cells, horizontal cells, Müller glial cells, RPE cells, or any combination thereof. In some embodiments, the optic nerve is, or the population of retinal cells are from a retinal tissue that is: (i) aged; (ii) damaged; (iii) associated with (or has) ocular (e.g., retinal or optic nerve) disease or condition; and/or (iv) after a surgical procedure. In some embodiments, the retinal tissue or the optic nerve is: (i) susceptible of aging or with predisposition toward aging; (ii) susceptible of being damaged or with predisposition toward damage; (iii) susceptible of or with predisposition toward developing an ocular (e.g., retinal or optic nerve) disease or condition; and/or (iv) to be administered a surgical procedure. In some embodiments, the aging, damage, or disease or condition of the eye (e.g., retinal tissue or the optic nerve) is selected from the group

consisting of: AMD, glaucoma, acute or chronic injury to the retina or optic nerve, Stargardt's disease, retinitis pigmentosa, retinal degeneration, optic atrophy, cataracts, optic neuropathy, diabetic retinopathy, TON, vision impairment or loss, UV damage, or any combination thereof. In some embodiments, the damage is due to optic nerve crush and/or laser photocoagulation. In some embodiments, the method does not reprogram the population of retinal cells into a reprogrammed stem cell. The nucleic acid construct can be delivered using an exosome, a liposome, an LNP, or a virus (e.g., LV or AAV). In some embodiments, the nucleic acid construct (e.g., circRNA or linear mRNA) is encapsulated within an exosome, a liposome, or an LNP. In some embodiments, the exosome, liposome, or LNP is delivered topically to the eye (e.g., as eye drops, ophthalmic ointment, or ophthalmic gel). In some embodiments, the nucleic acid construct is a vector (e.g., viral vector or viral genome). In some embodiments, the nucleic acid construct is comprised within or delivered by a virus (e.g., LV or AAV). In some embodiments, the virus is delivered via intravitreal injection or subretinal injection to the eye. In some embodiments, the method further comprises introducing into the population of retinal cells, to the optic nerve, or to the eye, an effective amount of a therapeutic agent. In some embodiments, the nucleic acid construct (RNA or DNA; or a virus comprising thereof) (or a pharmaceutical composition comprising thereof) is administered once. In some embodiments, the individual is a human (e.g., of any age). In some embodiments, the human is 10 years or older (e.g., at least about any of 15, 22, 30, 40, 50, 60, 70 years old, or older). In some embodiments, for preventing aging, damage, or disease or condition in a retinal cell/tissue, or in an optic nerve, the method is performed before the retinal cell/tissue or the optic nerve is aging, is exposed to a damage factor (e.g., UV irradiation, or surgical procedure), or has (e.g., diagnosed with) a disease or condition. In some embodiments, the method is performed at least about 12 hours (e.g., at least about any of 24 hours, 48 hours, 5 days, 1 week, 2 weeks, 4 weeks, or more) before the retinal cell/tissue or the optic nerve is exposed to a damage factor (e.g., laser photocoagulation).

[0134] In some embodiments, there is provided a method of rejuvenating a population of retinal cells of an eye in an individual (e.g., human), comprising introducing into the population of retinal cells, or introducing to the eye (e.g., topically, intravitreally, or subretinally), an effective amount of a plurality of nucleic acids (RNA (e.g., circRNA) or DNA; or nucleic acid construct(s) or virus comprising thereof) (or a pharmaceutical composition comprising thereof) encoding a plurality of reprogramming factors, wherein the plurality of reprogramming factors comprise an engineered

OCT4 that is deficient in LLPS (e.g., any of SEQ ID NOs: 4-10 and 44-46, such as SEQ ID NO: 4), KLF4 (e.g., SEQ ID NO: 22), and an engineered c-MYC that is deficient in LLPS (e.g., any of SEQ ID NOs: 14-20, 47, and 48, such as SEQ ID NO: 14). In some embodiments, there is provided a method of: (i) delaying or preventing the progression of aging of a retinal tissue of an eye in an individual (e.g., human, e.g., 22 years or older), (ii) preserving or improving the viability and/or function of an RGC of an eye in an individual (e.g., human), (iii) delaying or preventing the progression of vision impairment or loss in an individual (e.g., human), (iv) treating vision impairment or loss in an individual (e.g., human), (v) preserving or improving vision in an individual (e.g., human), (vi) promoting retinal regeneration in an eye of an individual (e.g., human), (vii) treating an individual (e.g., human) having aged or damaged retinal tissue or a retinal tissue with disease or condition in an eye, and/or (viii) preventing aging, damage, or disease or condition in a retinal tissue of an eye in an individual (e.g., human), comprising introducing into a population of retinal cells of the retinal tissue or the eye (e.g., introducing to the RGC), or introducing to the eye (e.g., topically, intravitreally, or subretinally), of the individual, an effective amount of a plurality of nucleic acids (RNA (e.g., circRNA) or DNA; or nucleic acid construct(s) or virus comprising thereof) (or a pharmaceutical composition comprising thereof) encoding a plurality of reprogramming factors, wherein the plurality of reprogramming factors comprise an engineered OCT4 that is deficient in LLPS (e.g., any of SEQ ID NOs: 4-10 and 44-46, such as SEQ ID NO: 4), KLF4 (e.g., SEQ ID NO: 22), and an engineered c-MYC that is deficient in LLPS (e.g., any of SEQ ID NOs: 14-20, 47, and 48, such as SEQ ID NO: 14). In some embodiments, there is provided a method of: (i) rejuvenating an optic nerve of an eye in an individual (e.g., human), (ii) delaying or preventing the progression of aging of an optic nerve of an eye in an individual (e.g., human, e.g., 22 years or older), (iii) preserving or improving the viability and/or function of an optic nerve of an eye in an individual (e.g., human), (iv) promoting optic nerve regeneration in an eye of an individual (e.g., human), (v) treating an individual (e.g., human) having aged or damaged optic nerve or an optic nerve with disease or condition in an eye, and/or (vi) preventing aging, damage, or disease or condition in an optic nerve of an eye in an individual (e.g., human), comprising introducing to the eye (e.g., topically, intravitreally, or subretinally) an effective amount of a plurality of nucleic acids (RNA (e.g., circRNA) or DNA; or nucleic acid construct(s) or virus comprising thereof) (or a pharmaceutical composition comprising thereof) encoding a plurality of reprogramming factors, wherein the plurality of reprogramming factors

comprise an engineered OCT4 that is deficient in LLPS (e.g., any of SEQ ID NOs: 4-10 and 44-46, such as SEQ ID NO: 4), KLF4 (e.g., SEQ ID NO: 22), and an engineered c-MYC that is deficient in LLPS (e.g., any of SEQ ID NOs: 14-20, 47, and 48, such as SEQ ID NO: 14). In some embodiments, the population of retinal cells comprise RGCs, photoreceptor cells, bipolar cells, amacrine cells, horizontal cells, Müller glial cells, RPE cells, or any combination thereof. In some embodiments, the optic nerve is, or the population of retinal cells are from a retinal tissue that is: (i) aged; (ii) damaged; (iii) associated with (or has) ocular (e.g., retinal or optic nerve) disease or condition; and/or (iv) after a surgical procedure. In some embodiments, the retinal tissue or the optic nerve is: (i) susceptible of aging or with predisposition toward aging; (ii) susceptible of being damaged or with predisposition toward damage; (iii) susceptible of or with predisposition toward developing an ocular (e.g., retinal or optic nerve) disease or condition; and/or (iv) to be administered a surgical procedure. In some embodiments, the aging, damage, or disease or condition of the eye (e.g., retinal tissue or the optic nerve) is selected from the group consisting of: AMD, glaucoma, acute or chronic injury to the retina or optic nerve, Stargardt's disease, retinitis pigmentosa, retinal degeneration, optic atrophy, cataracts, optic neuropathy, diabetic retinopathy, TON, vision impairment or loss, UV damage, or any combination thereof. In some embodiments, the damage is due to optic nerve crush and/or laser photocoagulation. In some embodiments, the method does not reprogram the population of retinal cells into a reprogrammed stem cell. The plurality of nucleic acids can be on separate nucleic acid constructs (e.g., circRNA, plasmid, or recombinant viral genome), or on a single nucleic acid construct. The plurality of nucleic acids can be delivered using an exosome, a liposome, an LNP, or a virus (e.g., LV or AAV). In some embodiments, at least two of (e.g., all of) the plurality of nucleic acids are on a single nucleic acid construct. In some embodiments, the at least two of (e.g., all of) the plurality of nucleic acids are under the control of different promoters. In some embodiments, the at least two of (e.g., all of) the plurality of nucleic acids are under the control of the same promoter. In some embodiments, the at least two of (e.g., all of) the plurality of nucleic acids are connected by one or more linking nucleic acids. In some embodiments, the one or more linking nucleic acids comprise IRES or a linking sequence encoding a self-cleaving peptide, e.g., P2A, T2A, or E2A. In some embodiments, the plurality of nucleic acids are one or more RNAs (e.g., one circRNA). In some embodiments, the one or more RNAs (e.g., one circRNA) are encapsulated within an exosome, a liposome, or an LNP. In some embodiments, the exosome, liposome, or LNP is delivered topically to the eye (e.g.,

as eye drops, ophthalmic ointment, or ophthalmic gel). In some embodiments, the plurality of nucleic acids are one or more DNAs. In some embodiments, the one or more (e.g., all) of the plurality of nucleic acids (DNA or RNA) are comprised within a vector (e.g., viral genome), such as comprised within a virus. In some embodiments, the method comprises administering a virus (e.g., LV or AVV) comprising one or more (e.g., all) of the plurality of nucleic acids (RNA or DNA). In some embodiments, the virus is delivered via intravitreal injection or subretinal injection to the eye. In some embodiments, the method further comprises introducing into the population of retinal cells, to the optic nerve, or to the eye, an effective amount of a therapeutic agent. In some embodiments, the effective amount of the plurality of nucleic acids is administered once. In some embodiments, the individual is a human (e.g., of any age). In some embodiments, the human is 10 years or older (e.g., at least about any of 15, 22, 30, 40, 50, 60, 70 years old, or older). In some embodiments, for preventing aging, damage, or disease or condition in a retinal cell/tissue, or in an optic nerve, the method is performed before the retinal cell/tissue or the optic nerve is aging, is exposed to a damage factor (e.g., UV irradiation, or surgical procedure), or has (e.g., diagnosed with) a disease or condition. In some embodiments, the method is performed at least about 12 hours (e.g., at least about any of 24 hours, 48 hours, 5 days, 1 week, 2 weeks, 4 weeks, or more) before the retinal cell/tissue or the optic nerve is exposed to a damage factor (e.g., laser photocoagulation).

[0135] In some embodiments, there is provided a method of rejuvenating a population of retinal cells of an eye in an individual (e.g., human), comprising introducing into the population of retinal cells, or introducing to the eye (e.g., topically, intravitreally, or subretinally), an effective amount of a nucleic acid construct (RNA (e.g., circRNA) or DNA; or a virus comprising thereof) (or a pharmaceutical composition comprising thereof), wherein the nucleic acid construct comprises from 5' to 3': a first nucleic acid encoding an engineered OCT4 that is deficient in LLPS (e.g., any of SEQ ID NOs: 4-10 and 44-46, such as SEQ ID NO: 4) – a first linking sequence (e.g., encoding P2A) – a second nucleic acid encoding KLF4 (e.g., SEQ ID NO: 22) – a second linking sequence (e.g., encoding T2A) – a third nucleic acid encoding an engineered c-MYC that is deficient in LLPS (e.g., any of SEQ ID NOs: 14-20, 47, and 48, such as SEQ ID NO: 14). In some embodiments, there is provided a method of: (i) delaying or preventing the progression of aging of a retinal tissue of an eye in an individual (e.g., human, e.g., 22 years or older), (ii) preserving or improving the viability and/or function of an RGC of an eye in an individual (e.g., human), (iii)

delaying or preventing the progression of vision impairment or loss in an individual (e.g., human), (iv) treating vision impairment or loss in an individual (e.g., human), (v) preserving or improving vision in an individual (e.g., human), (vi) promoting retinal regeneration in an eye of an individual (e.g., human), (vii) treating an individual (e.g., human) having aged or damaged retinal tissue or a retinal tissue with disease or condition in an eye, and/or (viii) preventing aging, damage, or disease or condition in a retinal tissue of an eye in an individual (e.g., human), comprising introducing into a population of retinal cells of the retinal tissue or the eye (e.g., introducing to the RGC), or introducing to the eye (e.g., topically, intravitreally, or subretinally), of the individual, an effective amount of a nucleic acid construct (RNA (e.g., circRNA) or DNA; or a virus comprising thereof) (or a pharmaceutical composition comprising thereof), wherein the nucleic acid construct comprises from 5' to 3': a first nucleic acid encoding an engineered OCT4 that is deficient in LLPS (e.g., any of SEQ ID NOs: 4-10 and 44-46, such as SEQ ID NO: 4) – a first linking sequence (e.g., encoding P2A) – a second nucleic acid encoding KLF4 (e.g., SEQ ID NO: 22) – a second linking sequence (e.g., encoding T2A) – a third nucleic acid encoding an engineered c-MYC that is deficient in LLPS (e.g., any of SEQ ID NOs: 14-20, 47, and 48, such as SEQ ID NO: 14). In some embodiments, there is provided a method of: (i) rejuvenating an optic nerve of an eye in an individual (e.g., human), (ii) delaying or preventing the progression of aging of an optic nerve of an eye in an individual (e.g., human, e.g., 22 years or older), (iii) preserving or improving the viability and/or function of an optic nerve of an eye in an individual (e.g., human), (iv) promoting optic nerve regeneration in an eye of an individual (e.g., human), (v) treating an individual (e.g., human) having aged or damaged optic nerve or an optic nerve with disease or condition in an eye, and/or (vi) preventing aging, damage, or disease or condition in an optic nerve of an eye in an individual (e.g., human), comprising introducing to the eye (e.g., topically, intravitreally, or subretinally) an effective amount of a nucleic acid construct (RNA (e.g., circRNA) or DNA; or a virus comprising thereof) (or a pharmaceutical composition comprising thereof), wherein the nucleic acid construct comprises from 5' to 3': a first nucleic acid encoding an engineered OCT4 that is deficient in LLPS (e.g., any of SEQ ID NOs: 4-10 and 44-46, such as SEQ ID NO: 4) – a first linking sequence (e.g., encoding P2A) – a second nucleic acid encoding KLF4 (e.g., SEQ ID NO: 22) – a second linking sequence (e.g., encoding T2A) – a third nucleic acid encoding an engineered c-MYC that is deficient in LLPS (e.g., any of SEQ ID NOs: 14-20, 47, and 48, such as SEQ ID NO: 14). In some embodiments, the nucleic acid construct encodes a protein sequence of

SEQ ID NO: 97. In some embodiments, the nucleic acid construct further comprises an IRES at 5' of the first nucleic acid. In some embodiments, the nucleic acid construct is a circRNA, a linear mRNA, or a self-amplifying RNA (e.g., RNA viral genome). In some embodiments, the nucleic acid construct is a DNA vector (e.g., lentival vector, AAV vector, or plasmid). In some embodiments, the nucleic acid construct comprises a nucleic acid sequence of any of SEQ ID NOs: 30, 35, 59, and 98. In some embodiments, the population of retinal cells comprise RGCs, photoreceptor cells, bipolar cells, amacrine cells, horizontal cells, Müller glial cells, RPE cells, or any combination thereof. In some embodiments, the optic nerve is, or the population of retinal cells are from a retinal tissue that is: (i) aged; (ii) damaged; (iii) associated with (or has) ocular (e.g., retinal or optic nerve) disease or condition; and/or (iv) after a surgical procedure. In some embodiments, the retinal tissue or the optic nerve is: (i) susceptible of aging or with predisposition toward aging; (ii) susceptible of being damaged or with predisposition toward damage; (iii) susceptible of or with predisposition toward developing an ocular (e.g., retinal or optic nerve) disease or condition; and/or (iv) to be administered a surgical procedure. In some embodiments, the aging, damage, or disease or condition of the eye (e.g., retinal tissue or the optic nerve) is selected from the group consisting of: AMD, glaucoma, acute or chronic injury to the retina or optic nerve, Stargardt's disease, retinitis pigmentosa, retinal degeneration, optic atrophy, cataracts, optic neuropathy, diabetic retinopathy, TON, vision impairment or loss, UV damage, or any combination thereof. In some embodiments, the damage is due to optic nerve crush and/or laser photocoagulation. In some embodiments, the method does not reprogram the population of retinal cells into a reprogrammed stem cell. The nucleic acid construct can be delivered using an exosome, a liposome, an LNP, or a virus (e.g., LV or AAV). In some embodiments, the nucleic acid construct (e.g., circRNA or linear mRNA) is encapsulated within an exosome, a liposome, or an LNP. In some embodiments, the exosome, liposome, or LNP is delivered topically to the eye (e.g., as eye drops, ophthalmic ointment, or ophthalmic gel). In some embodiments, the nucleic acid construct is a vector (e.g., viral vector or viral genome). In some embodiments, the nucleic acid construct is comprised within or delivered by a virus (e.g., LV or AVV). In some embodiments, the virus is delivered via intravitreal injection or subretinal injection to the eye. In some embodiments, the method further comprises introducing into the population of retinal cells, to the optic nerve, or to the eye, an effective amount of a therapeutic agent. In some embodiments, the nucleic acid construct (RNA or DNA; or a virus comprising thereof) (or a pharmaceutical composition

comprising thereof) is administered once. In some embodiments, the individual is a human (e.g., of any age). In some embodiments, the human is 10 years or older (e.g., at least about any of 15, 22, 30, 40, 50, 60, 70 years old, or older). In some embodiments, for preventing aging, damage, or disease or condition in a retinal cell/tissue, or in an optic nerve, the method is performed before the retinal cell/tissue or the optic nerve is aging, is exposed to a damage factor (e.g., UV irradiation, or surgical procedure), or has (e.g., diagnosed with) a disease or condition. In some embodiments, the method is performed at least about 12 hours (e.g., at least about any of 24 hours, 48 hours, 5 days, 1 week, 2 weeks, 4 weeks, or more) before the retinal cell/tissue or the optic nerve is exposed to a damage factor (e.g., laser photocoagulation).

[0136] In some embodiments, there is provided a method of rejuvenating a population of retinal cells of an eye in an individual (e.g., human), comprising introducing into the population of retinal cells, or introducing to the eye (e.g., topically, intravitreally, or subretinally), an effective amount of a plurality of nucleic acids (RNA (e.g., circRNA) or DNA; or nucleic acid construct(s) or virus comprising thereof) (or a pharmaceutical composition comprising thereof) encoding a plurality of reprogramming factors, wherein the plurality of reprogramming factors comprise an engineered OCT4 that is deficient in LLPS (e.g., any of SEQ ID NOs: 4-10 and 44-46, such as SEQ ID NO: 4), KLF4 (e.g., SEQ ID NO: 22), an engineered c-MYC that is deficient in LLPS (e.g., any of SEQ ID NOs: 14-20, 47, and 48, such as SEQ ID NO: 14), and SOX2 (e.g., SEQ ID NO: 24). In some embodiments, there is provided a method of: (i) delaying or preventing the progression of aging of a retinal tissue of an eye in an individual (e.g., human, e.g., 22 years or older), (ii) preserving or improving the viability and/or function of an RGC of an eye in an individual (e.g., human), (iii) delaying or preventing the progression of vision impairment or loss in an individual (e.g., human), (iv) treating vision impairment or loss in an individual (e.g., human), (v) preserving or improving vision in an individual (e.g., human), (vi) promoting retinal regeneration in an eye of an individual (e.g., human), (vii) treating an individual (e.g., human) having aged or damaged retinal tissue or a retinal tissue with disease or condition in an eye, and/or (viii) preventing aging, damage, or disease or condition in a retinal tissue of an eye in an individual (e.g., human), comprising introducing into a population of retinal cells of the retinal tissue or the eye (e.g., introducing to the RGC), or introducing to the eye (e.g., topically, intravitreally, or subretinally), of the individual, an effective amount of a plurality of nucleic acids (RNA (e.g., circRNA) or DNA; or nucleic acid construct(s) or virus comprising thereof) (or a pharmaceutical composition comprising thereof) encoding a

plurality of reprogramming factors, wherein the plurality of reprogramming factors comprise an engineered OCT4 that is deficient in LLPS (e.g., any of SEQ ID NOs: 4-10 and 44-46, such as SEQ ID NO: 4), KLF4 (e.g., SEQ ID NO: 22), an engineered c-MYC that is deficient in LLPS (e.g., any of SEQ ID NOs: 14-20, 47, and 48, such as SEQ ID NO: 14), and SOX2 (e.g., SEQ ID NO: 24). In some embodiments, there is provided a method of: (i) rejuvenating an optic nerve of an eye in an individual (e.g., human), (ii) delaying or preventing the progression of aging of an optic nerve of an eye in an individual (e.g., human, e.g., 22 years or older), (iii) preserving or improving the viability and/or function of an optic nerve of an eye in an individual (e.g., human), (iv) promoting optic nerve regeneration in an eye of an individual (e.g., human), (v) treating an individual (e.g., human) having aged or damaged optic nerve or an optic nerve with disease or condition in an eye, and/or (vi) preventing aging, damage, or disease or condition in an optic nerve of an eye in an individual (e.g., human), comprising introducing to the eye (e.g., topically, intravitreally, or subretinally) an effective amount of a plurality of nucleic acids (RNA (e.g., circRNA) or DNA; or nucleic acid construct(s) or virus comprising thereof) (or a pharmaceutical composition comprising thereof) encoding a plurality of reprogramming factors, wherein the plurality of reprogramming factors comprise an engineered OCT4 that is deficient in LLPS (e.g., any of SEQ ID NOs: 4-10 and 44-46, such as SEQ ID NO: 4), KLF4 (e.g., SEQ ID NO: 22), an engineered c-MYC that is deficient in LLPS (e.g., any of SEQ ID NOs: 14-20, 47, and 48, such as SEQ ID NO: 14), and SOX2 (e.g., SEQ ID NO: 24). In some embodiments, the population of retinal cells comprise RGCs, photoreceptor cells, bipolar cells, amacrine cells, horizontal cells, Müller glial cells, RPE cells, or any combination thereof. In some embodiments, the optic nerve is, or the population of retinal cells are from a retinal tissue that is: (i) aged; (ii) damaged; (iii) associated with (or has) ocular (e.g., retinal or optic nerve) disease or condition; and/or (iv) after a surgical procedure. In some embodiments, the retinal tissue or the optic nerve is: (i) susceptible of aging or with predisposition toward aging; (ii) susceptible of being damaged or with predisposition toward damage; (iii) susceptible of or with predisposition toward developing an ocular (e.g., retinal or optic nerve) disease or condition; and/or (iv) to be administered a surgical procedure. In some embodiments, the aging, damage, or disease or condition of the eye (e.g., retinal tissue or the optic nerve) is selected from the group consisting of: AMD, glaucoma, acute or chronic injury to the retina or optic nerve, Stargardt's disease, retinitis pigmentosa, retinal degeneration, optic atrophy, cataracts, optic neuropathy, diabetic retinopathy, TON, vision impairment or loss, UV damage, or

any combination thereof. In some embodiments, the damage is due to optic nerve crush and/or laser photocoagulation. In some embodiments, the method does not reprogram the population of retinal cells into a reprogrammed stem cell. The plurality of nucleic acids can be on separate nucleic acid constructs (e.g., circRNA, plasmid, or recombinant viral genome), or on a single nucleic acid construct. The plurality of nucleic acids can be delivered using an exosome, a liposome, an LNP, or a virus (e.g., LV or AAV). In some embodiments, at least two of (e.g., all of) the plurality of nucleic acids are on a single nucleic acid construct. In some embodiments, the at least two of (e.g., all of) the plurality of nucleic acids are under the control of different promoters. In some embodiments, the at least two of (e.g., all of) the plurality of nucleic acids are under the control of the same promoter. In some embodiments, the at least two of (e.g., all of) the plurality of nucleic acids are connected by one or more linking nucleic acids. In some embodiments, the one or more linking nucleic acids comprise IRES or a linking sequence encoding a self-cleaving peptide, e.g., P2A, T2A, or E2A. In some embodiments, the plurality of nucleic acids are one or more RNAs (e.g., one circRNA). In some embodiments, the one or more RNAs (e.g., one circRNA) are encapsulated within an exosome, a liposome, or an LNP. In some embodiments, the exosome, liposome, or LNP is delivered topically to the eye (e.g., as eye drops, ophthalmic ointment, or ophthalmic gel). In some embodiments, the plurality of nucleic acids are one or more DNAs. In some embodiments, the one or more (e.g., all) of the plurality of nucleic acids (DNA or RNA) are comprised within a vector (e.g., viral genome), such as comprised within a virus. In some embodiments, the method comprises administering a virus (e.g., LV or AAV) comprising one or more (e.g., all) of the plurality of nucleic acids (RNA or DNA). In some embodiments, the virus is delivered via intravitreal injection or subretinal injection to the eye. In some embodiments, the method further comprises introducing into the population of retinal cells, to the optic nerve, or to the eye, an effective amount of a therapeutic agent. In some embodiments, the effective amount of the plurality of nucleic acids is administered once. In some embodiments, the individual is a human (e.g., of any age). In some embodiments, the human is 10 years or older (e.g., at least about any of 15, 22, 30, 40, 50, 60, 70 years old, or older). In some embodiments, for preventing aging, damage, or disease or condition in a retinal cell/tissue, or in an optic nerve, the method is performed before the retinal cell/tissue or the optic nerve is aging, is exposed to a damage factor (e.g., UV irradiation, or surgical procedure), or has (e.g., diagnosed with) a disease or condition. In some embodiments, the method is performed at least about 12 hours (e.g., at least about any of 24 hours, 48 hours, 5

days, 1 week, 2 weeks, 4 weeks, or more) before the retinal cell/tissue or the optic nerve is exposed to a damage factor (e.g., laser photocoagulation).

[0137] In some embodiments, there is provided a method of rejuvenating a population of retinal cells of an eye in an individual (e.g., human), comprising introducing into the population of retinal cells, or introducing to the eye (e.g., topically, intravitreally, or subretinally), an effective amount of a nucleic acid construct (RNA (e.g., circRNA) or DNA; or a virus comprising thereof) (or a pharmaceutical composition comprising thereof), wherein the nucleic acid construct comprises from 5' to 3': a first nucleic acid encoding an engineered OCT4 that is deficient in LLPS (e.g., any of SEQ ID NOs: 4-10 and 44-46, such as SEQ ID NO: 4) – a first linking sequence (e.g., encoding P2A) – a second nucleic acid encoding KLF4 (e.g., SEQ ID NO: 22) – a second linking sequence (e.g., encoding T2A) – a third nucleic acid encoding an engineered c-MYC that is deficient in LLPS (e.g., any of SEQ ID NOs: 14-20, 47, and 48, such as SEQ ID NO: 14) – a third linking sequence (e.g., encoding E2A) – a fourth nucleic acid encoding SOX2 (e.g., SEQ ID NO: 24). In some embodiments, there is provided a method of: (i) delaying or preventing the progression of aging of a retinal tissue of an eye in an individual (e.g., human, e.g., 22 years or older), (ii) preserving or improving the viability and/or function of an RGC of an eye in an individual (e.g., human), (iii) delaying or preventing the progression of vision impairment or loss in an individual (e.g., human), (iv) treating vision impairment or loss in an individual (e.g., human), (v) preserving or improving vision in an individual (e.g., human), (vi) promoting retinal regeneration in an eye of an individual (e.g., human), (vii) treating an individual (e.g., human) having aged or damaged retinal tissue or a retinal tissue with disease or condition in an eye, and/or (viii) preventing aging, damage, or disease or condition in a retinal tissue of an eye in an individual (e.g., human), comprising introducing into a population of retinal cells of the retinal tissue or the eye (e.g., introducing to the RGC), or introducing to the eye (e.g., topically, intravitreally, or subretinally), of the individual, an effective amount of a nucleic acid construct (RNA (e.g., circRNA) or DNA; or a virus comprising thereof) (or a pharmaceutical composition comprising thereof), wherein the nucleic acid construct comprises from 5' to 3': a first nucleic acid encoding an engineered OCT4 that is deficient in LLPS (e.g., any of SEQ ID NOs: 4-10 and 44-46, such as SEQ ID NO: 4) – a first linking sequence (e.g., encoding P2A) – a second nucleic acid encoding KLF4 (e.g., SEQ ID NO: 22) – a second linking sequence (e.g., encoding T2A) – a third nucleic acid encoding an engineered c-MYC that is deficient in LLPS (e.g., any of SEQ ID NOs: 14-20, 47, and 48, such as

SEQ ID NO: 14) – a third linking sequence (e.g., encoding E2A) – a fourth nucleic acid encoding SOX2 (e.g., SEQ ID NO: 24). In some embodiments, there is provided a method of: (i) rejuvenating an optic nerve of an eye in an individual (e.g., human), (ii) delaying or preventing the progression of aging of an optic nerve of an eye in an individual (e.g., human, e.g., 22 years or older), (iii) preserving or improving the viability and/or function of an optic nerve of an eye in an individual (e.g., human), (iv) promoting optic nerve regeneration in an eye of an individual (e.g., human), (v) treating an individual (e.g., human) having aged or damaged optic nerve or an optic nerve with disease or condition in an eye, and/or (vi) preventing aging, damage, or disease or condition in an optic nerve of an eye in an individual (e.g., human), comprising introducing to the eye (e.g., topically, intravitreally, or subretinally) an effective amount of a nucleic acid construct (RNA (e.g., circRNA) or DNA; or a virus comprising thereof) (or a pharmaceutical composition comprising thereof), wherein the nucleic acid construct comprises from 5' to 3': a first nucleic acid encoding an engineered OCT4 that is deficient in LLPS (e.g., any of SEQ ID NOs: 4-10 and 44-46, such as SEQ ID NO: 4) – a first linking sequence (e.g., encoding P2A) – a second nucleic acid encoding KLF4 (e.g., SEQ ID NO: 22) – a second linking sequence (e.g., encoding T2A) – a third nucleic acid encoding an engineered c-MYC that is deficient in LLPS (e.g., any of SEQ ID NOs: 14-20, 47, and 48, such as SEQ ID NO: 14) – a third linking sequence (e.g., encoding E2A) – a fourth nucleic acid encoding SOX2 (e.g., SEQ ID NO: 24). In some embodiments, the nucleic acid construct encodes a protein sequence of SEQ ID NO: 101. In some embodiments, the nucleic acid construct further comprises an IRES at 5' of the first nucleic acid. In some embodiments, the nucleic acid construct is a circRNA, a linear mRNA, or a self-amplifying RNA (e.g., RNA viral genome). In some embodiments, the nucleic acid construct is a DNA vector (e.g., lentival vector, AAV vector, or plasmid). In some embodiments, the nucleic acid construct comprises a nucleic acid sequence of any of SEQ ID NOs: 28, 37, 61, and 102. In some embodiments, the population of retinal cells comprise RGCs, photoreceptor cells, bipolar cells, amacrine cells, horizontal cells, Müller glial cells, RPE cells, or any combination thereof. In some embodiments, the optic nerve is, or the population of retinal cells are from a retinal tissue that is: (i) aged; (ii) damaged; (iii) associated with (or has) ocular (e.g., retinal or optic nerve) disease or condition; and/or (iv) after a surgical procedure. In some embodiments, the retinal tissue or the optic nerve is: (i) susceptible of aging or with predisposition toward aging; (ii) susceptible of being damaged or with predisposition toward damage; (iii) susceptible of or with predisposition toward developing an ocular (e.g., retinal

or optic nerve) disease or condition; and/or (iv) to be administered a surgical procedure. In some embodiments, the aging, damage, or disease or condition of the eye (e.g., retinal tissue or the optic nerve) is selected from the group consisting of: AMD, glaucoma, acute or chronic injury to the retina or optic nerve, Stargardt's disease, retinitis pigmentosa, retinal degeneration, optic atrophy, cataracts, optic neuropathy, diabetic retinopathy, TON, vision impairment or loss, UV damage, or any combination thereof. In some embodiments, the damage is due to optic nerve crush and/or laser photocoagulation. In some embodiments, the method does not reprogram the population of retinal cells into a reprogrammed stem cell. The nucleic acid construct can be delivered using an exosome, a liposome, an LNP, or a virus (e.g., LV or AAV). In some embodiments, the nucleic acid construct (e.g., circRNA or linear mRNA) is encapsulated within an exosome, a liposome, or an LNP. In some embodiments, the exosome, liposome, or LNP is delivered topically to the eye (e.g., as eye drops, ophthalmic ointment, or ophthalmic gel). In some embodiments, the nucleic acid construct is a vector (e.g., viral vector or viral genome). In some embodiments, the nucleic acid construct is comprised within or delivered by a virus (e.g., LV or AAV). In some embodiments, the virus is delivered via intravitreal injection or subretinal injection to the eye. In some embodiments, the method further comprises introducing into the population of retinal cells, to the optic nerve, or to the eye, an effective amount of a therapeutic agent. In some embodiments, the nucleic acid construct (RNA or DNA; or a virus comprising thereof) (or a pharmaceutical composition comprising thereof) is administered once. In some embodiments, the individual is a human (e.g., of any age). In some embodiments, the human is 10 years or older (e.g., at least about any of 15, 22, 30, 40, 50, 60, 70 years old, or older). In some embodiments, for preventing aging, damage, or disease or condition in a retinal cell/tissue, or in an optic nerve, the method is performed before the retinal cell/tissue or the optic nerve is aging, is exposed to a damage factor (e.g., UV irradiation, or surgical procedure), or has (e.g., diagnosed with) a disease or condition. In some embodiments, the method is performed at least about 12 hours (e.g., at least about any of 24 hours, 48 hours, 5 days, 1 week, 2 weeks, 4 weeks, or more) before the retinal cell/tissue or the optic nerve is exposed to a damage factor (e.g., laser photocoagulation).

Retinal cells and tissues, optic nerve, and associated conditions

[0138] The eye has three layers. The outermost layer, known as the fibrous tunic, is composed of the cornea and sclera, which provide shape to the eye and support the deeper structures. The middle layer, known as the vascular tunic or uvea, consists of the choroid, ciliary body, pigmented

epithelium and iris. The innermost is the retina, which gets its oxygenation from the blood vessels of the choroid (posteriorly) as well as the retinal vessels (anteriorly). The spaces of the eye are filled with the aqueous humour anteriorly, between the cornea and lens, and the vitreous body, a jelly-like substance, behind the lens, filling the entire posterior cavity.

[0139] The retina is the innermost, light-sensitive layer of the eye. It is a delicate neural tissue lining the back of the eye that converts light stimuli into electric signals for processing by the brain. The retina comprises layers of neurons that collectively sense and process light, and sends visual information to the visual cortex of the brain via the optic nerve. The optic nerve is a cable of retinal ganglion cells (RGCs) that carry the electric signals from the retina to the brain. Diseases affecting the retina and optic nerve, e.g., glaucoma, and optic nerve injury can lead to vision loss and blindness. Early detection and treatment are critical in correcting problems before vision is lost in preventing further deterioration of vision.

[0140] The neural retina consists of ten layers of neurons interconnected by synapses and is supported by an outer layer of pigmented epithelial cells. The primary light-sensing cells in the retina are the photoreceptor cells, which are of two types: rods and cones. Rods function mainly in dim light and provide monochromatic vision. Cones function in well-lit conditions and are responsible for the perception of colour through the use of a range of opsins, as well as high-acuity vision used for tasks such as reading. A third type of light-sensing cell, the photosensitive ganglion cell, is important for entrainment of circadian rhythms and reflexive responses such as the pupillary light reflex.

[0141] The vertebrate retina has 10 distinct layers. From closest to farthest from the vitreous body: (i) inner limiting membrane – basement membrane elaborated by Müller cells; (ii) nerve fibre layer – axons of the ganglion cell bodies (a thin layer of Müller cell footplates exists between this layer and the inner limiting membrane); (iii) ganglion cell (RGC) layer – contains nuclei of ganglion cells, the axons of which become the optic nerve fibres, and some displaced amacrine cells; (iv) inner plexiform layer – contains the synapse between the bipolar cell axons and the dendrites of the ganglion and amacrine cells; (v) inner nuclear layer – contains the nuclei and surrounding cell bodies (perikarya) of the amacrine cells, bipolar cells, and horizontal cells; (vi) outer plexiform layer – projections of rods and cones ending in the rod spherule and cone pedicle, respectively, these make synapses with dendrites of bipolar cells and horizontal cells (in the

macular region, this is known as the Fiber layer of Henle); (vii) outer nuclear layer – cell bodies of rods and cones; (viii) external limiting membrane – layer that separates the inner segment portions of the photoreceptors from their cell nuclei; (ix) inner segment / outer segment layer – inner segments and outer segments of rods and cones, the outer segments contain a highly specialized light-sensing apparatus; and (x) retinal pigment epithelium (RPE) – single layer of cuboidal epithelial cells (with extrusions not shown in diagram). This layer is closest to the choroid, and provides nourishment and supportive functions to the neural retina, The black pigment melanin in the pigment layer prevents light reflection throughout the globe of the eyeball; this is extremely important for clear vision. These layers can be grouped into four main processing stages— photoreception; transmission to bipolar cells; transmission to ganglion cells, which also contain photoreceptors, the photosensitive ganglion cells; and transmission along the optic nerve. At each synaptic stage, horizontal and amacrine cells also are laterally connected.

[0142] Neural cell types of the retina have been well-characterized in terms of function, connectivity, and gene expression, for example, in Cowan et al., *Cell Types of the Human Retina and its Organoids at Single-Cell Resolution*, *Cell* (2020), and in Yan et al., *Cell Atlas of the Human Fovea and Peripheral Retina*, *Scientific Reports* (2020), each of which is herein incorporated by reference in its entirety.

[0143] The macula is an oval-shaped pigmented area in the center of the retina of the human eye and in other animals. The macula in humans has a diameter of around 5.5 mm. It is responsible for the central, high-resolution, color vision that is possible in good light. This kind of vision is impaired if the macula is damaged, as in macular degeneration.

[0144] RGCs are the final output neurons of the retina that process visual information and transmit it to discrete brain visual areas to form vision. Loss of RGCs is a leading cause of blindness in a group of diseases broadly categorized as optic neuropathies, including glaucoma, hereditary optic neuropathies, and disorders caused by toxins, nutritional defects and trauma. Vision loss in these patients is irreversible since humans and all mammals lack the ability to generate RGCs in adulthood.

[0145] In section, the retina is no more than 0.5 mm thick. It has three layers of nerve cells and two of synapses, including the unique ribbon synapse. The optic nerve carries the RGC axons to the brain, and the blood vessels that supply the retina. The ganglion cells lie innermost in the eye

while the photoreceptive cells lie beyond. Because of this counter-intuitive arrangement, light must first pass through and around the ganglion cells and through the thickness of the retina (including its capillary vessels), before reaching the rods and cones..

[0146] Each human optic nerve contains between 770,000 and 1.7 million nerve fibers (RGC axons). The optic nerve is a central tract of many axons of ganglion cells connecting primarily to the lateral geniculate body, a visual relay station in the diencephalon (the rear of the forebrain). It also projects to the superior colliculus, the suprachiasmatic nucleus, and the nucleus of the optic tract. It passes through the other layers, creating the optic disc in primates. Besides RGC axons, the optic nerve also contains glia, a non-neuronal cell. Glial cells maintain homeostasis, form myelin in the peripheral nervous system, and provide support and protection for neurons.

[0147] Damage to the optic nerve (ON) typically causes permanent and potentially severe loss of vision. Like most pathways in the mature central nervous system, the optic nerve cannot regenerate if injured. Optic nerve injury can be the result of glaucoma, trauma, toxicity, inflammation, ischemia, congenital diseases, or compression from tumors or aneurysms. To date, few effective treatments have been discovered to restore visual function and/or axon regeneration following optic nerve injury.

[0148] A retinal tissue or an optic nerve described herein may be an abnormal, damaged, or unhealthy retinal tissue or optic nerve, which may need to be treated. A retinal tissue or an optic nerve may also be a normal or healthy retinal tissue or optic nerve that is under a higher than normal risk of becoming abnormal, degenerative, or unhealthy (e.g., to be administered a surgical procedure, to be exposed to trauma, or belonging to an individual with a genetic predisposition for retinal/optic nerve degeneration or vision loss), which may need to be prevented. In certain embodiments, the retinal tissue or optic nerve is considered healthy but suboptimal for performance or survival in current or future conditions.

[0149] In some embodiments, the population of retinal cells to be administered with the LLPS-deficient engineered reprogramming factor(s) described herein comprise retinal ganglion cells (RGCs), bipolar cells, photoreceptor cells (e.g., rod cells, cone cells), amacrine cells, horizontal cells, Muller glial cells, retinal pigment epithelium (RPE) cells, or any combination thereof. In some embodiments, the population of retinal cells comprise (or consist essentially of, or consist of) RGCs. In some embodiments, the population of retinal cells are from a retinal tissue that is (i)

an aged retinal tissue, or a retinal tissue susceptible of aging or with predisposition toward aging; (ii) a damaged retinal tissue, or a retinal tissue susceptible of being damaged or with predisposition toward retinal damage; (iii) a retinal tissue with or associated with ocular (e.g., retinal) disease or condition, or susceptible of or with predisposition toward developing ocular (e.g., retinal) disease, or condition; and/or (iv) a retinal tissue before or after a surgical procedure, such as laser eye surgery.

[0150] In some embodiments, the optic nerve to be treated (either therapeutically or prophylactically): (i) is aged, or is susceptible of aging or with predisposition toward aging; (ii) is damaged, or is susceptible of being damaged or with predisposition toward damage; (iii) has or is associated with an ocular (e.g., optic nerve) disease or condition, or is susceptible of or with predisposition toward developing ocular (e.g., optic nerve) disease or condition; and/or (iv) is before or after a surgical procedure (e.g., optic nerve sheath decompression).

[0151] In some embodiments, the population of retinal cells are from an aged retinal tissue, or a retinal tissue susceptible of aging or with predisposition toward aging. In some embodiments, the optic nerve is aged, or is susceptible of aging or with predisposition toward aging. Retinal or optic nerve aging can be intrinsic aging (i.e., chronological aging) or extrinsic aging (e.g., due to extrinsic damage factor such as UV irradiation, chemical exposure, or nutritional defects). In some embodiments, the aged retinal tissue or optic nerve is from a human who is 22 years or older (e.g., at least about any of 23, 24, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95 years old, or older), such as 24 years or older, 30 years or older, 40 years or older, 50 years or older, 55 years or older, or 60 years or older. In some embodiments, the retinal tissue or optic nerve is photoaged, or is susceptible of or with predisposition toward being photo-damaged (e.g., due to looking directly at the sun). The aged retinal tissue or optic nerve due to extrinsic damage factor(s), or retinal tissue or optic nerve susceptible to aging or with predisposition toward aging, can be from an individual (e.g., human) of any age, such as at least about any of 10, 12, 14, 16, 18, 20, 22, 30, 40, 50, 60, 70, 80, 90 years old, or older.

[0152] Extrinsic retinal aging can be due to retinal damage factors, which include but are not limited to, air pollution, sun radiation (e.g., UV), tobacco, alcohol, climate (e.g., high temperature, high altitudes, or desert environment), nutrition (e.g., fats, refined flour, highly processed foods), stress, lack of sleep, lack of exercise, toxicity (e.g., chemical toxin) exposure, or a combination

thereof. In some embodiments, the retinal damage factor can be a genetic disease a condition of the individual that speeds up eye aging.

[0153] UV light ages all structures of the eye, including but not limited to cornea and lens. Corneal damage, cataracts and macular degeneration are all possible chronic effects from UV exposure and can ultimately lead to decreased vision.

[0154] In some embodiments, the population of retinal cells are from a a damaged retinal tissue, or a retinal tissue susceptible of being damaged or with predisposition toward retinal damage. In some embodiments, the optic nerve is damaged, or is susceptible of being damaged or with predisposition toward damage. In some embodiments, the damage is ultraviolet (e.g., UVA or UVB)-induced. In some embodiments, the damage is due to optic nerve crush or compression, or to myelopathy of the optic tract or optic nerve. In some embodiments, the damage is due to invasive cut, abrasion, or wound. In some embodiments, the damage is due to laser photocoagulation. In some embodiments, the damage is due to fire or chemical burns, or chemical exposure. In some embodiments, the damage is due to surgical procedures, for example, damage from laser eye surgery. In some embodiments, the damaged optic nerve has optic neuropathy, such as traumatic optic neuropathy (TON). In some embodiments, the damaged retinal tissue has solar retinopathy. In some embodiments, the damage is due to ocular hypertension.

[0155] Optic neuropathy is damage to the optic nerve from any cause. Traumatic optic neuropathy (TON) refers to an acute injury of the optic nerve secondary to trauma. The optic nerve axons may be damaged either directly or indirectly, and the visual loss may be partial or complete. An indirect injury to the optic nerve typically occurs from the transmission of forces to the optic canal from blunt head trauma. This is in contrast to direct TON, which results from an anatomic disruption of the optic nerve fibers from penetrating orbital trauma, bone fragments within the optic canal, or nerve sheath hematomas. Direct TON can result from penetrating injury or from bony fragments in the optic canal or orbit piercing the optic nerve. Orbital hemorrhage (orbital compartment syndrome) and optic nerve sheath hematoma can also cause TON by direct compression. Neuroimaging studies (computed tomography [CT] scanning or magnetic resonance imaging [MRI]) are an important part of the assessment when TON is suspected. The main treatment options for TON include systemic corticosteroids and surgical optic nerve

decompression, either alone or in combination. The most common mechanisms of injury in TON include motor vehicle accidents, bike accidents, falls, and assaults.

[0156] Solar retinopathy (also known as, photic retinopathy, foveomacular retinitis, solar retinitis, and eclipse retinopathy) refers to a photochemical toxicity and resultant injury to retinal tissues, usually occurring at the fovea. This entity is commonly associated with sungazing or eclipse viewing, and often results in mild-to-moderate visual acuity deficit and/or central or paracentral scotomata. Recovery is spontaneous in nature and occurs over the course of 3-6 months after the inciting event, though visual recovery may be incomplete and patient may suffer from permanent visual acuity deficits and central or paracentral scotomata.

[0157] Laser photocoagulation is eye surgery using a laser to shrink or destroy abnormal structures in the retina, or to intentionally cause scarring that can help certain eye conditions. However, since each pulse of the laser causes a microscopic burn in the retina, there can be development of mild loss of vision, reduced night vision, blind spots, reduced side vision, difficulty focusing, blurred vision, and/or reduced color vision. Laser photocoagulation can induce retinal and optic nerve damage. It hurts RGCs and RGC axons more as they are more exposed in the retina, the other retina cell types can be less hurt as they are in the beneath layers of the retina.

[0158] Wounds to the retinal tissue or optic nerve can happen during and/or after laser eye surgery, or other procedures involving the eye, or due to concussions or other physical trauma. Acute and chronic wounds can comprise minor burns, minor wounds, scratches, accidental wounds, trauma to the retina or the surrounding tissues (e.g., optic nerve), or healing of same. Acute and chronic optic nerve wounds can comprise trauma to the optic nerve or optic tract, damage to axons of the optic nerve, damage to myelin sheaths of the optic nerve, compressed optic nerve, fully or partially severed optic nerve, optic nerve crush, and/or laser-induced coagulation.

[0159] In some embodiments, the population of retinal cells are from a retinal tissue with ocular (e.g., retinal) disease or condition, or susceptible of or with predisposition toward developing an ocular (e.g., retinal) disease or condition. In some embodiments, the optic nerve has ocular (e.g., optic nerve) disease or condition, or susceptible of or with predisposition toward developing an ocular (e.g., optic nerve) disease or condition. Any ocular diseases or conditions that are associated with retinal cell/tissue and/or optic nerve aging or damage, can be treated or prevented herein, include but are not limited to, macular degeneration, such as age-related macular degeneration

(AMD) (e.g., wet AMD, dry AMD), glaucoma, acute or chronic injury to the retina or optic nerve, Stargardt's disease, retinitis pigmentosa, retinal degeneration, optic atrophy, cataracts, optic neuropathy, diabetic retinopathy, TON, vision impairment or loss. In some embodiments, the ocular disease or condition is hereditary. In some embodiments, the ocular disease or condition is an aging-related disease or condition, such as glaucoma, dry eyes, cataracts, and AMD.

[0160] Optic atrophy or optic nerve degeneration is a pathological term referring to optic nerve shrinkage caused by the degeneration of RGC axons. It can lead to permanent vision loss. Optic atrophy can result from various causes, including increased intraocular pressure, compromised vascular supply, and physical trauma (e.g., from retro-orbital bleeding procedures). Since the optic nerve is composed of the axons of RGCs, primary lesions in the retina (especially those affecting the ganglion cell and nerve fiber layers) frequently result in concurrent optic nerve pathology, including degeneration. Since the optic nerve is an extracranial white matter tract of the brain, optic nerve degeneration can also be a “descending” change secondary to lesions in the brain. Optic nerve axonal degeneration and loss can also be an age-related incidental change.

[0161] In some embodiments, the retinal tissue is a degenerative retinal tissue, or susceptible to or with predisposition toward degeneration, which can be from an individual (e.g., human) of any age. Retinal degeneration refers to a group of diseases that result in gradual loss of light-sensitive cells in the retina and, therefore, progressive loss of vision. Retinal diseases can be genetic or can occur through injury to the eye, the natural aging process or by other conditions. In conditions such as age-related macular degeneration, environmental factors play a major role; whereas in inherited retinal degeneration, genetic variations are the major cause of the disease. Tools and biomarkers for the study of RGC degeneration are well known, e.g., see, Hwang et al., *Int J Mol Sci.* 2022; 23(8): 4287, the content of which is incorporated herein by reference in its entirety.

[0162] In some embodiments, the ocular (e.g., retinal or optic nerve) disease or condition is selected from the group consisting of: AMD (e.g., wet AMD, dry AMD), solar retinopathy, glaucoma, acute or chronic injury to the retina or optic nerve, Stargardt's disease, retinitis pigmentosa (an inherited degenerative disease), retinal or optic nerve degeneration, cataracts, optic neuropathy, diabetic retinopathy, TON, vision impairment or loss, UV damage, retinal tear, retinal detachment, epiretinal membrane, and macular hole. In some embodiments, a symptom of an ocular (e.g., retinal or optic nerve) disease, condition, or degeneration is selected from the group

consisting of: seeing floating specks or cobwebs, blurred or distorted vision, defects in peripheral vision, and vision loss. In some embodiments, the symptom of ocular disease or condition, such as associated with retinal and/or optic nerve aging or damage, comprises reduced night vision, blind spots, reduced side vision, difficulty focusing, blurred vision, and/or reduced color vision.

[0163] In some embodiments, the population of retinal cells are from a retinal tissue to be administered a surgical procedure or after a surgical procedure. In some embodiments, the optic nerve is to be administered a surgical procedure or after a surgical procedure. Ophthalmic surgery procedures include, but are not limited to, refractive surgery, laser eye surgery (e.g., Lasik™), procedures to remove a foreign object from the eye, photorefractive keratectomy (PRK), cataract surgery, corneal transplants (e.g., keratoplasty, corneal grafting), vitrectomy, glaucoma surgery, retinal surgery, plastic and reconstruction surgery, muscle surgery, and trauma repair.

[0164] The eye rejuvenation methods, treatment or prevention methods, or LLPS-deficient engineered reprogramming factors described herein can be used in combination with said surgical procedures; either before, after and/or during (in parallel to) the procedures.

Therapeutic and/or prophylactic effects of eye rejuvenation

[0165] The eye rejuvenation methods described herein can be therapeutic or prophylactic (*i.e.*, preventive), herein also referred to as “therapeutic eye rejuvenation method” or “treatment method”, and “prophylactic eye rejuvenation method” or “prevention method,” respectively. The methods or the LLPS-deficient engineered reprogramming factors (or compositions thereof) described herein can be used prophylactically or they can be used to reduce, treat, and/or prevent further retinal and/or optic nerve aging or damage, or reduce the effect on vision of retinal and/or optic nerve aging or damage in an individual who has predisposition toward eye damage, or has been exposed to an agent likely to cause eye damage, e.g., UV radiation or trauma, with the purpose to reduce, improve, alleviate, alter, remedy, ameliorate, or affect vision impairment or loss associated with aging or damage of the retina and/or optic nerve. Without being bound by theory, the treatment and prevention methods described herein comprising introducing an LLPS-deficient engineered reprogramming factor (or composition thereof) can function by any mechanisms, include but are not limited to eye rejuvenation.

[0166] For therapeutic eye rejuvenation methods, the retinal tissue to be treated can be (or the population of retinal cells to be treated are from): (i) an aged retinal tissue; (ii) a damaged retinal

tissue; (iii) a retinal tissue with retinal disease or condition (e.g., a degenerative retinal tissue); and/or (iv) a retinal tissue after surgical procedure. For therapeutic eye rejuvenation methods, the optic nerve to be treated can be: (i) an aged optic nerve; (ii) a damaged optic nerve (e.g., due to optic nerve crush or laser photocoagulation); (iii) an optic nerve tissue with retinal disease or condition; and/or (iv) an optic nerve after surgical procedure.

[0167] For prophylactic eye rejuvenation methods, the retinal tissue and/or the optic nerve to be treated can be (or the population of retinal cells to be treated are from): (i) susceptible of aging or with predisposition toward aging (e.g., before eye intrinsic aging starts); (ii) susceptible of being damaged or with predisposition toward damage (e.g., before exposure to eye damage factors such as UV irradiation, or has been exposed to an agent likely to cause eye damage); (iii) susceptible of or with predisposition toward developing an ocular disease or condition (e.g., before an ocular disease or condition is diagnosed); and/or (iv) to be administered a surgical procedure (e.g., before laser eye surgery).

[0168] The therapeutic eye rejuvenation methods described herein may achieve one or more of: i) improving the state (e.g., physiology, histology, electrophysiology, and/or function) of the retina and/or optic nerve (e.g., density of axons, width), and/or signs of ocular aging (e.g., losing the ability to see up close, having trouble distinguishing colors, needing more time to adjust to changing levels of light); ii) improving visual function (such as improving visual acuity (e.g., to 20/40 vision or to 20/20 vision), visual detection, visual sharpness, peripheral vision, night vision, color vision, or other visual functions; or decreasing, slowing or stopping a decline in visual function associated with aging, damage, disease or condition); iii) reducing scarring (e.g., macular pucker) of damaged retinal tissue; iv) enhancing restoration of the retina and/or optic nerve after surgical procedures, such as following laser eye surgery; v) treating and/or restoring damaged retina and/or optic nerve; vi) restoring retinal and/or optic nerve function; vii) reversing, halting, ameliorating, or slowing degeneration of the retina and/or optic nerve associated with aging, disease, or condition, viii) enhancing the restoration of retina after surgical procedures, e.g., enhancing scar or wound healing, or enhancing retinal and/or optic nerve regeneration, following procedures such as laser eye surgery; ix) preventing or delaying the progression of retinal and/or optic nerve aging; x) reducing the severity of an ocular (e.g., retinal or optic nerve) disease or condition; xi) preventing or delaying the progression of an ocular (e.g., retinal or optic nerve) disease or condition (e.g., optic neuropathy, or degeneration); xii) treating, alleviating, or

ameliorating a symptom associated with an ocular (e.g., retinal or optic nerve) disease or condition; xiii) improving the life quality (e.g., independence, or satisfaction) of an individual; xiv) reducing lesions (e.g., inflammation) of the retina and/or optic nerve, such as laser photocoagulation-induced lesions; xv) improving the viability and/or function of RGCs (e.g., cell body and/or axon) and/or optic nerve; xvi) delaying or preventing the progression of vision impairment or loss; xvii) treating, alleviating, or ameliorating vision impairment or loss; xviii) preserving or improving vision; xix) promoting retinal and/or optic nerve regeneration; xx) increasing (e.g., at least about any of 5%, 10%, 15%, 35%, 60%, 70%, 80%, or more) the thickness of the macula, such as measured by optical coherence tomography (OCT); xxi) improving visual field, e.g., by at least about 10% in the mean standard deviation on the Humphrey Visual Field Test; and/or xxii) improving ERG, a measurement of the electrical response of the retina to light stimulation (e.g., increasing ERG amplitude by at least about 10%). In some embodiments, compared to a reference, aging, damage, or disease or condition (or associated symptom, such as vision impairment or loss) of the retina and/or optic nerve treated by the eye rejuvenation methods described herein is reduced by at least about 5%, such as at least about any of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or 100%. In some embodiments, compared to a reference, the progression of aging, damage, or disease or condition (or associated symptom, such as vision impairment or loss) of the retina and/or optic nerve treated by the eye rejuvenation methods described herein is delayed by at least about 1 week, such as at least about any of 2 weeks, 1 month, 3 months, 6 months, 1 year, 2 years, 5 years, or longer. In some embodiments, compared to a reference, the viability and/or function of retinal cells (e.g., RGC) and/or optic nerve, thickness of the macula, the retinal and/or optic nerve regeneration, ERG, the visual function, and/or the life quality of the individual, is improved by at least about 5%, such as at least about any of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 100%, 2-fold, 5-fold, 10-fold, or more. In some embodiments, the visual function is restored to at least about 20% (e.g., at least about any of 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or 100%) of a healthy eye and/or a younger eye. The reference can be: i) a reference retinal cell or tissue, or a reference optic nerve, not introduced with the LLPS-deficient engineered reprogramming factor; and/or ii) a reference retinal cell or tissue, or a reference optic nerve, introduced with a reference reprogramming factor that is not deficient in LLPS. The reference retinal tissue/cell and the retinal tissue/cell can be from a same individual or from

different individuals. The reference optic nerve and the optic nerve can be from a same individual or from different individuals.

[0169] The prophylactic eye rejuvenation methods described herein may achieve one or more of: i) maintaining healthy retinal tissue and/or optic nerve, and/or healthy vision; ii) preventing or delaying the onset of retinal and/or optic nerve aging, damage (e.g., ocular hypertension or retinal scar), or disease or condition (or associated symptom, such as vision impairment or loss). In some embodiments, compared to a reference, the onset of retinal and/or optic nerve aging, damage, or disease or condition (or associated symptom) treated by the eye rejuvenation methods (or LLPS-deficient engineered reprogramming factors) described herein is delayed by at least about 1 week, such as at least about any of 2 weeks, 1 month, 3 months, 6 months, 1 year, 2 years, 5 years, or longer. In some embodiments, compared to a reference, the healthy retina and/or optic nerve, and/or the healthy vision, is maintained at least about 1 week longer, such as at least about any of 2 weeks, 1 month, 3 months, 6 months, 1 year, 2 years, 5 years, or more, longer. The reference can be: i) a reference retinal cell or tissue, or a reference optic nerve, not introduced with the LLPS-deficient engineered reprogramming factor; and/or ii) a reference retinal cell or tissue, or a reference optic nerve, introduced with a reference reprogramming factor that is not deficient in LLPS. The reference retinal tissue/cell and the retinal tissue/cell can be from a same individual or from different individuals. The reference optic nerve and the optic nerve can be from a same individual or from different individuals.

[0170] In some embodiments, the therapeutic and/or prophylactic treatment of an RGC can treat its cell body in the retina. In some embodiments, the therapeutic and/or prophylactic treatment of an RGC can treat its axon too, and/or the optic nerve formed by the axon.

[0171] In some embodiments, the therapeutic or prophylactic effects of the eye rejuvenation methods (or treatment or prevention methods) or LLPS-deficient engineered reprogramming factors described herein can be determined or quantified using one or more biomarkers, and/or one or more tests measuring the electrophysiology, histology, physiology, function, and/or health of the retina (e.g., RGCs), the optic nerve, and/or the eye.

[0172] In some embodiments, the treatment method improves the viability and/or function of a an RGC. The RGC may be a degenerated, damaged, aged, diseased, or even a normal/healthy RGC for which improved function is desired. In some embodiments, the method entails increasing the

biological activity, in the RGC, of one or more genes selected from the group consisting of Atoh7, Brn3B, Sox4, Sox11, and Ilsl.

[0173] In some embodiments, the treatment method improves the viability and/or function of a retinal neuron that is not an RGC. The non-RGC retinal neuron may be a photoreceptor cell, a bipolar cell, an amacrine cell, a Muller glial cell, an RPE cell, a horizontal cell, or any other subtype of retinal neuron.

[0174] In some embodiments, the treatment method improves the viability, histology, physiology, electrophysiology, function, and/or health of the optic nerve. In some embodiments, the methods provided herein promote regeneration of optic nerve structure, connectivity, and/or function. In some embodiments, the methods prevent, lessen, ameliorate, or reduce damage to the optic nerve of an individual, wherein the individual has damage to the optic nerve (e.g., due to optic nerve crush or laser photocoagulation), a disease or condition affecting the optic nerve, or is susceptible to a disease or condition affecting the optic nerve.

[0175] In some embodiments, the eye rejuvenation method does not reprogram the population of retinal cells into a reprogrammed stem cell (*i.e.*, iPSC). A reprogrammed stem cell or iPSC may express at least some, and optionally all, of the markers from the following non-limiting list: SSEA-3, SSEA-4, TRA-I-60, TRA-I-81, TRA-2-49/6E, ALP, Sox2, E-cadherin, UTF-I, Oct4, Lin28, Klf4, REX-1, and Nanog. OCT4, LIN28, and/or KLF4 can be expressed at high levels in iPSCs, while retinal identity marker genes are not expressed or barely expressed. In some embodiments, compared to a reference retinal cell introduced with an effective amount of a reference reprogramming factor that is not deficient in LLPS, the rejuvenated population of retinal cells: (i) have increased (e.g., increasing at least about 5%, such as at least about any of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 100%, 2-fold, 3-fold, 5-fold, 10-fold, or more) expression and/or function of a biomarker of initiation phase reprogramming (IPR); (ii) have reduced (e.g., reducing at least about 5%, such as at least about any of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or 100%) expression and/or function of a biomarker of maturation phase reprogramming; and/or (iii) have reduced (e.g., reducing at least about 5%, such as at least about any of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or 100%) expression and/or function of a biomarker of stabilization phase reprogramming. In some embodiments, compared to a reference retinal cell not introduced with the engineered reprogramming factor, the

rejuvenated population of retinal cells have increased (e.g., increasing at least about 5%, such as at least about any of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 100%, 2-fold, 3-fold, 5-fold, 10-fold, or more) expression and/or function of a biomarker of IPR. In some embodiments, (i) the biomarker of IPR is NANOG and/or SALL4; (ii) the biomarker of maturation phase reprogramming is DPPA5 and/or LIN28A; and/or (iii) the biomarker of stabilization phase reprogramming is OCT4 and/or XACT. The reference retinal tissue/cell and the retinal tissue/cell can be from a same individual or from different individuals.

[0176] In some embodiments, compared to a reference retinal cell not introduced with the engineered reprogramming factor, and/or compared to a reference retinal cell introduced with an effective amount of a reference reprogramming factor that is not deficient in LLPS, the rejuvenated population of retinal cells: (i) have reduced proportion of cell senescence biomarker-positive cells; (ii) have reduced expression and/or function of a biomarker of cell senescence; (iii) have reduced epigenetic age (EpiAge); (iv) have increased expression and/or function of a biomarker of retinal cell identity; and/or (v) have reduced expression and/or function of a biomarker of apoptosis. The reduction of the expression and/or function of the biomarker can be a reduction of at least about 5% (e.g., at least about any of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or 100%) of the expression and/or function of the biomarker in the reference retinal cell. The increase of the expression and/or function of the biomarker can be an increase of at least about 5% (e.g., at least about any of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 100%, 2-fold, 3-fold, 5-fold, 10-fold, or more) of the expression and/or function of the biomarker in the reference retinal cell. In some embodiments, (i) the biomarker of cell senescence is selected from the group consisting of senescence-associated- β -galactosidase (SA- β -gal), p16, and p21, or a combination thereof; (ii) the EpiAge is measured using Horvath's clock, Levine's clock, Hannum's clock, and/or AltumAge; (iii) the biomarker of retinal cell identity is selected from the group consisting of β III-tubulin, BRN3A, RBPMS, γ -synuclein, Thy 1, GAP43, MAP2, STMN2, NEFM, or a combination thereof; and/or (iv) the biomarker of apoptosis is selected from the group consisting of loss of membrane asymmetry, cleavage of a Bcl-2 family protein, caspase activation, caspase substrate cleavage, non-caspase protease activation, mitochondrial transmembrane potential ($\Delta\psi_m$) decrease, cytochrome C release, increase of sub G1 population, nuclear condensation, DNA fragmentation, and membrane blebbing, or a combination thereof. The reference retinal tissue/cell and the retinal tissue/cell can be from a same individual or from different individuals.

[0177] In some embodiments, compared to a reference retinal tissue not comprising retinal cells introduced with the effective amount of the engineered reprogramming factor, and/or compared to a reference retinal tissue comprising retinal ganglion cells introduced with an effective amount of a reference reprogramming factor that is not deficient in LLPS, a retinal tissue comprising the population of retinal cells introduced with the effective amount of the engineered reprogramming factors has: (i) higher (e.g., increasing at least about 5%) density of RGC axons; (ii) more (e.g., increasing at least about 5%) retinal cells (e.g., RGCs, or non-RGC cells); (iii) improved retinal electrophysiology, physiology, and/or function; and/or (iv) a connected optic nerve that has improved electrophysiology, histology, and/or function. In some embodiments, compared to a reference optic nerve and/or eye not introduced with the effective amount of the engineered reprogramming factor, and/or compared to a reference optic nerve and/or eye introduced with an effective amount of a reference reprogramming factor that is not deficient in LLPS, an optic nerve and/or eye introduced with the effective amount of the engineered reprogramming factors has: (i) higher (e.g., increasing at least about 5%) density of RGC axons; (ii) increased (e.g., increasing at least about 5%) density of regenerated axons in the optic nerve; and/or (iii) improved electrophysiology, histology, and/or function (e.g., vision). In some embodiments, (i) the electrophysiology of the retina, optic nerve (e.g., connected to the retina), and/or eye is measured by electroretinogram (ERG), electro-oculography (EOG), and/or visual evoked response (VER); (ii) the physiology of the retina, optic nerve (e.g., connected to the retina), and/or eye is measured by optical coherence tomography (OCT), scanning laser ophthalmoscopy (SLO), and/or ocular ultrasound; (iii) the function of the retina, optic nerve (e.g., connected to the retina), and/or eye is measured by vision test; and/or (iv) the function of the optic nerve (e.g., connected to the retina) and/or the eye is measured by visual fields, pupillary light reflexes, the accommodation reflex, and/or fundoscopy. In some embodiments, (i) the improved retinal physiology comprises reduced retinal lesion; and/or (ii) the improved electrophysiology of the retina, optic nerve (e.g., connected to the retina), and/or eye comprises improved photopic negative response (PhNR), improved pattern electroretinogram (pERG), and/or improved flash visual evoked potential (fVEP). The reference retinal tissue and the retinal tissue can be from the same individual or different individuals. The reference optic nerve and the optic nerve can be from the same individual or different individuals. The reference eye and the eye can be from the same individual or different individuals.

[0178] The biomarkers described herein can be detected and/or measured using any methods known in the art, such as RNA and/or protein expression tests, DNA methylation assays, and functional assays. Such methods include but are not limited to, northern blot, RNA sequencing (RNA-Seq), RT-qPCR, RNA-fluorescence *in situ* hybridization (RNA-FISH), western blot, mass spectrometry (MS), immunofluorescence (IF), reporter assay (*in vitro* or *in cell*), signal transduction assay, etc.

[0179] Any cellular senescence assay can be used herein, such as colorimetric assay (e.g., SA- β -gal assay), fluorescent assay (e.g., C12FDG or CellEvent Senescence Green Reagent, which function as fluorescent β -gal substrate), or flow cytometry (e.g., using a combination of antibody markers such as p16^{ARF} and p21).

[0180] Any apoptosis assay can be used herein, includes but is not limited to, Annexin V assays, DNA condensation and fragmentation (TUNEL) assays, Caspase activation and detection assays, mitochondrial membrane potential-dependent dyes, Cytochrome C release assays, glutathione assays, etc. *See, e.g., "Apoptosis assays and markers guide," abcam, at abcam.com/kits/apoptosis-assays, the content of which is incorporated herein by reference in its entirety.*

[0181] EpiAge is measured by age-associated changes of a collection of DNA methylation sites (e.g., clock CpGs, or age-associated CpG sites) whose aggregate methylation status (e.g., weighted methylation average) measures biological age. Different EpiAge models use different sets of methylation sites and different weighting algorithms. While Horvath's clock is the most popular model, any EpiAge model can be used herein, such as Levine's clock, Hannum's clock, or AltumAge (*see, e.g., Lucas Paulo de Lima Camillo et al., NPJ Aging. 2022; 8(1): 4; Horvath and Raj, Nat Rev Genet. 2018;19(6):371-384; the contents of each of which are incorporated herein by reference in their entirety*). Methods of detecting and/or measuring DNA methylation are well-known, include but are not limited to, methylation microarray, Reduced Representation Bisulfite Sequencing (RRBS), Tagmentation-based Indexing for Methylation Sequencing (TIME-Seq; Griffin *et al.*, *bioRxiv*. 2021), bisulfite barcoded amplicon sequencing (BBA-seq), pyrosequencing, droplet digital PCR (ddPCR), *etc.* Also *see* methods described in Han *et al.*, *BMC Biol.* 2020;18:71; Kurdyukov and Bullock, *Biology (Basel)*. 2016;5(1):3; the contents of each of which are incorporated herein by reference in their entirety.

[0182] In some embodiments, morphological characteristic(s), histology, and/or physiology of the retinal cell/tissue and/or optic nerve is used to assess the therapeutic or prophylactic effects of the eye rejuvenation methods (or treatment or prevention methods) or LLPS-deficient engineered reprogramming factors described herein. Such methods include but are not limited to, tissue biopsy, immunohistochemical staining, immunofluorescence, light microscopy, transmission electron microscopy, optical coherence tomography (OCT), scanning laser ophthalmoscopy (SLO), ocular ultrasound, etc. Retinal cell/tissue and/or optic nerve can also be visualized by fundus photography, color fundus photography, autofluorescence imaging, or fluorescein angiogram, etc.

[0183] OCT is an imaging method used to generate a picture of retina. The noninvasive method produces an image by measuring the amount of a dim red light that reflects off of the retina and optic nerve. OCT can measure the thickness of retina and optic nerve.

[0184] Clinical electrophysiological assessment of optic nerve and RGC function can be performed using the Pattern Electroretinogram (PERG), Visual Evoked Potential (VEP) or visual evoked response (VER) and the Photopic Negative Response (PhNR) amongst other more specialized techniques. The specific testing of the optic nerve involves visual acuity assessment, color perception, visual fields assessment, pupillary light reflexes assessment, accommodation assessment and fundusoscopic examination.

[0185] In some embodiments, the therapeutic or prophylactic effects of the eye rejuvenation methods or LLPS-deficient engineered reprogramming factors described herein can be determined or quantified using one or more assays to measure electrophysiology, physiology, health, and/or function of the retina, optic nerve, and/or the eye. Any electrophysiology, physiology, and function (e.g, vision test) tests of the eye are well-known in the art can be used herein, e.g., ERG, EOG, VEP or VER. See exemplary methods described in Marmoy and Viswanathan, *Eye (Lond)*. 2021;35(9):2386-2405, the content of which is incorporated herein by reference in its entirety.

[0186] In some embodiments, the treatment methods and engineered reprogramming factors of the invention can preserve or restore the visual function of an eye of a subject with an ocular condition (e.g., retinal and/or optic nerve condition, such as retinal degeneration). Assessment of axonal regeneration may be monitored through visual function tests as disclosed herein. Visual function can be measured using one or more of a variety of methods well-known in the art, include but are not limited to, visual acuity, automatic refraction, visual field, color vision test, etc. For

example, visual function can be assessed by measuring visual acuity. Visual acuity can be assessed, for example, by using conventional “eye charts” in which visual acuity is evaluated by the ability to discern letters of a certain size, with five letters of a given size present on each line (see, e.g., the “ETDRS” eye chart described in the Murphy, R. P., CURRENT TECHNIQUES IN OPHTHALMIC LASER SURGERY, 3rd Ed., edited by L. D. Singerman, and G. Cascas, Butterworth Heinemann, 2000). Evaluation of visual acuity may also be achieved by measuring reading speed and reading time. Visual acuity may be measured to evaluate whether administration of engineered rejuvenation factors to the affected eye preserves or permits improvement of visual acuity (e.g., to 20/40 vision or to 20/20 vision).

[0187] Visual function may also be measured by determining whether there is an increase in the thickness of the Nerve Fiber layer (NFL). NFL consists of ganglion cell axons. Their course runs parallel to the retinal surface; the fibers proceed to the optic disc, turn at a right angle, and exit the eye through the lamina cribrosa as the optic nerve. The fibers generally are unmyelinated within the retina. In some embodiments, the treatment methods can increase the NFL thickness by at least about any of 5%, 10%, 15%, 20%, 30%, 50%, 80%, 90%, 1-fold, 2-fold, or more, than a reference NFL without the treatment, such as measured by OCT. In some embodiments, the treatment methods can improve the ganglion cell layer (or other non-RGC layer of the retina), such as seen in the OCT. In some embodiments, the treatment methods can improve visual field (e.g., by at least about any of 5%, 10%, 20%, 50%, 80%, 1-fold, 2-fold, or more in the mean standard deviation on the Humphrey Visual Field Test).

[0188] The electroretinogram (ERG) is a diagnostic test that measures the electrical activity of the retina in response to a light stimulus. The ERG arises from currents generated directly by retinal neurons in combination with contributions from retinal glia. There are four types of ERGs. A full-field ERG (ff ERG) can check how well the entire retina is working. A focal ERG (fERG) is used primarily to measure the functional integrity of the central macula and is useful in providing information in diseases limited to the macula. A multifocal ERG (mfERG) evaluates the response of the retina to multifocal stimulation and tests part of the retina. It can check for diseases of the macula and for central vision loss. A pattern ERG (pERG) uses contrast reversing pattern stimuli (sinewave gratings or checkerboards) to assess macular RGC activity. See, e.g., eyewiki.aao.org/Electroretinogram. In some embodiments, the treatment methods described herein increase the ERG amplitude (e.g., P50-N95 (subtraction) amplitude), such as by at least about any

of 5%, 10%, 15%, 20%, 30%, 50%, 70%, 90%, 1-fold, 2-fold, or more, e.g., compared to a retina/optic nerve/eye not administered with the engineered reprogramming factors.

[0189] In ffERG, among the wave components, photopic negative response (PhNR) is a slow negative potential that follows the b-wave recorded under light adapted conditions. The PhNR is primarily driven by RGCs hence providing insight into RGC function. The PhNR is often measured from the pre-stimulus baseline to the trough of the response, or at a fixed time following the stimulus flash.

[0190] In pERG, the N95 component is likely generated by action potentials from RGCs. The P50 component may be generated by RGCs with additional contributions from more distal sites. The P50 and N95 components are dependent on macular cone function, as the photoreceptors provide input into the RGCs. Macular cone dysfunction can reduce the amplitude of the P50 and delay the response. Selective reduction of the N95 amplitude, with preservation of the P50 component, suggests RGC dysfunction. The pERG can be useful for assessing RGC function in conditions such as glaucoma and ischemic optic neuropathy. The pERG has also been shown to be abnormal in diabetic retinopathy and idiopathic intracranial hypertension.

[0191] Visual function or eye electrophysiology may also be measured by electrooculography (EOG), which is a technique for measuring the resting potential of the retina. EOG tests how well electrical currents are working in the whole eye. It can check certain eye and retina problems, such as Best's disease (congenital macular degeneration), retinitis pigmentos, and different types of macular dystrophy. Test results use a number measurement called the Arden ratio. This is the ratio of the eye's maximum electrical activity in light to the minimum electrical activity in the dark. EOG is particularly useful for the assessment of RPE function. In some embodiments, the treatment methods herein improve the Arden ratio of the eye, such as by at least about any of 5%, 10%, 15%, 20%, 30%, 50%, 70%, 90%, 1-fold, 2-fold, or more, e.g., compared to a retina/optic nerve/eye not administered with the engineered reprogramming factors.

[0192] Visual function may also be assessed through fundus autofluorescence (AF) imaging, which is a clinical tool that allows evaluation of the interaction between photoreceptor cells and the RPE. For example, increased fundus AF or decreased fundus AF has been shown to occur in AMD and other ocular disorders. Fundus AF imaging may be used to evaluate whether the treatment methods herein slow ocular disease progression.

[0193] Visual function may also be assessed by evaluation of contrast sensitivity, which a measurement of the ability to discern between luminances of different levels in a static image. An evaluation of contrast sensitivity may be used to assess whether the treatment methods herein preserve or permit improvement in the resolving power of the eye.

[0194] Visual function may also be assessed by microperimetry, which monitors retinal visual function against retinal thickness or structure and the condition of the subject's fixation over time. Microperimetry may be used to assess whether the treatment methods herein preserve or permit improvement in retinal sensitivity and fixation.

[0195] Visual function may also be assessed via electrophysiological testing, such as via electroretinogram (ERG) (e.g., pattern electrogram (pERG)) or flash visual evoked potential (fVEP), which monitors the electrophysiological function of RGCs. ERG (e.g., pERG) and/or fVEP testing may be used to assess whether the treatment methods herein preserve or permit improvement in retinal sensitivity and fixation.

[0196] The VEP is an important clinical test for assessing the functional integrity of the visual pathway from the retina to the striate cortex (primary visual cortex or V1). The VEP is produced from activation of cortical neurons in response to afferent pathway stimulation, which is recorded with electrodes placed over the occiput. Typically, VEPs are recorded to a high contrast pattern or diffuse flash stimuli or pattern VEPs, a checkerboard or grating stimulus is reversed in contrast over time whilst maintaining a constant mean luminance (PR-VEP), or alternatively the checkerboard appears and disappears on a background with the same mean luminance known as the pattern onset-offset VEP (PO-VEP), which provide information regarding the function of the macular pathways. Flash VEPs (F-VEP) are typically recorded to a strobe or LED flash stimulator and is useful in the examination of the generalised visual pathway function, particularly in eyes with poor optical quality where retinal image contrast is degraded. The interpretation of responses is performed by assessing the response amplitude (from the preceding negativity or baseline), peak-time/latency, morphology and transoccipital distribution. Generally, different conditions of the optic nerve or RGCs may affect response amplitude or latency preferentially, the VEP has a large macular contribution and can be affected by anything upstream in the visual pathway, for example a maculopathy may degrade responses as well as primary RGC disease.

[0197] In some embodiments, the treatment methods and/or engineered reprogramming factors described herein can achieve one or more of: (i) decreasing (e.g., decreasing at least about any of 5%, 10%, 20%, 50%, 80%, 90%, or more) laser-induced retina lesion area (e.g., assessed by OCT); (ii) increasing (e.g., increasing at least about any of 5%, 10%, 20%, 50%, 80%, 90%, 1-fold, 2-fold, or more) PhNR as measured by ERG; (iii) increasing P50-N95 (subtraction) amplitude (e.g., increasing at least about any of 5%, 10%, 20%, 50%, 80%, 90%, 1-fold, 2-fold, or more) as measured by pERG; and/or (iv) increasing (e.g., increasing at least about any of 5%, 10%, 20%, 50%, 80%, 90%, 1-fold, 2-fold, or more) fVEP waveforms.

[0198] Depending on therapeutic or prophylactic use, an effective amount of the LLPS-deficient engineered reprogramming factor described herein (or a composition thereof) is an amount which, upon administration to a population of retinal cells, optic nerve, eye, or an individual, achieves one or more of the therapeutic or prophylactic effects, biomarkers, and/or histology, physiology, electrophysiology, functional, and/or morphological characteristics described above. For example, the effective amount can prevent or reduce one or more signs of retinal/optic nerve aging or damage, e.g., vision loss, reduced visual sensitivity, loss of night vision, loss of peripheral vision, loss of retinal thickness, loss or degeneration of RGCs, or optic neuropathy. The effective amount to be administered to a retinal tissue, optic nerve, eye, or an individual is typically based on a variety of factors including age, sex, weight, and conditions of the retina or optic nerve. Effective doses will vary, as recognized by those skilled in the art, dependent on route for administration, excipient usage, and the possibility of co-usage with other treatments such as usage of agents for preventing or delaying vision loss, or surgical procedure. An experimental animal can be used in a method of determining an effective amount of the LLPS-deficient engineered reprogramming factor (or a composition thereof).

Engineered reprogramming factors that are deficient in LLPS

[0199] The present application in one aspect provides engineered reprogramming factors that are deficient in LLPS (also referred to herein as “engineered reprogramming factor” or “LLPS-deficient engineered reprogramming factor”). Any of the engineered reprogramming factors provided herein can be used in the eye rejuvenation methods (or treatment or prevention methods) described herein.

[0200] Any engineered reprogramming factors that are deficient in LLPS (e.g., reducing at least about 50% LLPS, such as reducing at least about any of 60%, 70%, 80%, 85%, 90%, 95%, 99%, or 100% LLPS) are encompassed and can be used herein. Deficiency in LLPS in some embodiments comprises deficiency in forming a condensate, such as deficiency in forming any condensate. Deficiency in LLPS in some embodiments comprises deficiency in forming a condensate comprising the same condensate components compared to that formed by a corresponding wild-type reprogramming factor. For example, a wild-type OCT4 may form a condensate with both wild-type SOX2 and wild-type KLF4; while an LLPS-deficient engineered OCT4 may form a condensate with only wild-type SOX2, but wild-type KLF4 is not within the condensate. In some embodiments, the LLPS-deficient engineered reprogramming factor does not reprogram a population of retinal cells into reprogrammed stem cells (or iPSCs), such as by retaining the reprogramming of the population of retinal cells at the initiation phase (IPR).

[0201] Any methods known in the art that can detect LLPS or condensate formation can be used herein to determine whether an engineered reprogramming factor is LLPS-deficient, or whether a mutation to a reprogramming factor causes LLPS-deficiency. See, e.g., Basturea, G.N. ("Biological Condensates," *MATER METHODS* 2019;9:2794) and Alberti et al. (*J Mol Biol.* 2018;430(23):4806–4820) for exemplary methods (the contents of each of which are incorporated herein by reference in their entirety), such as imaging technique, such as microscopy (e.g., fluorescent microscopy). LLPS or condensate formation of an engineered reprogramming factor can be detected *in vitro* or in cells. For example, the engineered reprogramming factor can comprise a detectable characteristic, such as fluorescent characteristic. In some embodiments, the engineered reprogramming factor comprises a label, such as a fluorescent label. In some embodiments, the engineered reprogramming factor is further labeled with a detection tag, such as a fluorescein, a fluorescent polypeptide (e.g., GFP), or a radioisotope label. In some embodiments, the label or tag does not affect the engineered reprogramming factor's action and/or activity in association/dissociation with one or more biomolecules (e.g., DNA, other condensate protein component, or other protein to form a condensate) or a condensate. In some embodiments, the engineered reprogramming factor or a condensate comprising thereof is detected using an antibody that specifically recognizes the engineered reprogramming factor, or a label/tag of the engineered reprogramming factor. The antibody can have a fluorescent label, or can be detected using a secondary antibody that has a fluorescent label. In some embodiments, the deficiency in LLPS of

the engineered reprogramming factor with another condensate component (e.g., DNA or another protein) is examined by detecting whether there is co-localization of the engineered reprogramming factor and the other condensate component, such as using fluorescent imaging technique. The dynamic nature of LLPS or condensates can be examined via FRAP.

[0202] In some embodiments, the imaging technique comprises any one or more of immunofluorescence (IF), in situ hybridization (ISH, such as FISH), gene fusion (e.g., GFP labeling), or a dye that is specific for the engineered reprogramming factor. In some embodiments, the imaging technique comprises use of an agent to visualize a cellular feature, such as a cell membrane or an organelle, such as nucleus (e.g., by DAPI staining). In some embodiments, the imaging technique further comprise measuring (such as quantifying) a signal of the detectable reagent. The microscopy technique can be one or more of: confocal microscopy, fluorescence microscopy, high-resolution microscopy, stimulated emission depletion (STED) microscopy, SoRa super-resolution spinning-disk microscopy, electron microscopy (such as cryo-EM or cryo-ET), and/or a total internal reflection fluorescence (TIRF) microscopy.

[0203] In some embodiments, there is provided an engineered reprogramming factor, which comprises a mutation (e.g., one or more mutations, such as insertion, deletion, and/or substitution) in an LLPS-related domain in reference to a corresponding wild-type reprogramming factor. The mutation can be in any LLPS-related domain, such as selected from the group consisting of an intrinsically disordered region (IDR), an activation domain (AD), a region outside of DNA-binding domain (DBD), an ubiquitination site, or any combination thereof. Mutations in other domains are also contemplated by the subject application, as long as such mutations can reduce (e.g., reducing at least about 50% LLPS, such as reducing at least about any of 60%, 70%, 80%, 85%, 90%, 95%, 99%, or more LLPS) or abolish LLPS of the reprogramming factor. The mutation can comprises one or more of: (i) substituting a charged amino acid (e.g., an acidic amino acid such as D or E, or a basic amino acid such as K, R, or H) within the IDR or AD with a neutral amino acid (e.g., Gly, Ala, Leu, Ile, Val, Phe, Pro, Met, Trp, Tyr, Ser, Thr, Cys, Gln, or Asn, such as A); (ii) substituting an amino acid that can be ubiquitinated (e.g., S, T, or K, such as K) with an amino acid that cannot be ubiquitinated (e.g., R); (iii) deleting an amino acid that can be ubiquitinated (e.g., S, T, or K, such as K); (iv) deleting an amino acid (e.g., a charged amino acid, such as an acidic amino acid (e.g., D or E) or a basic amino acid (e.g., K, R, or H)) within the IDR or AD; and/or (v) deleting an amino acid (e.g., a charged amino acid, such as an acidic amino acid (e.g., D or E) or a basic

amino acid (e.g., K, R, or H)) within a region outside of DBD. In some embodiments, the amino acid to be deleted comprises an acidic amino acid (e.g., D or E). In some embodiments, substituting a charged amino acid within the IDR or AD with a neutral amino acid comprises substituting an acidic amino acid (e.g., D or E) within the IDR or AD with a neutral amino acid (e.g., A). In some embodiments, the amino acid that can be ubiquitinated is K. In some embodiments, the engineered reprogramming factor does not comprise a mutation in IDR. In some embodiments, the engineered reprogramming factor does not comprise a mutation in DBD.

[0204] In some embodiments, the mutation that causes LLPS-deficiency of the engineered reprogramming factor comprises a partial or full deletion, such as partial or full deletion of an LLPS-related domain. In some embodiments, the mutation is a partial or full deletion of an IDR and/or AD, such as partial deletion of charged amino acids in the IDR. In some embodiments, the mutation is a terminal deletion, such as partial or full deletion of the N-terminal domain and/or C-terminal domain of a wild-type reprogramming factor. In some embodiments, the N-terminal domain and/or C-terminal domain of the wild-type reprogramming factor comprises an IDR and/or an AD. In some embodiments, the mutation is a partial or full deletion of the middle part of the wild-type reprogramming factor. In some embodiments, the middle part of the wild-type reprogramming factor comprises an IDR and/or an AD. In some embodiments, the mutation is a partial or full deletion of regulatory region(s) of a wild-type reprogramming factor, such as partial or full deletion of lysine(s), which can undergo ubiquitination modification, or partial or full deletion of other amino acid(s) that contribute to LLPS.

[0205] In some embodiments, the engineered reprogramming factor comprises (or further comprises) a mutation (e.g., insertion, deletion, and/or substitution) in the DBD. In some embodiments, the mutation in the DBD does not affect, or affects at most about 10% (e.g., at most about any of 9%, 8%, 7%, 6%, 5%, 2%, 1%, or less) of, the DNA-binding activity (e.g., affinity and/or specificity) of the engineered reprogramming factor.

[0206] In some embodiments, the DBD is in the middle of a wild-type reprogramming factor, hence the region outside of DBD can be at N-terminus and/or C-terminus of the wild-type reprogramming factor. In some embodiments, the DBD is at the N-terminus of a wild-type reprogramming factor, hence the region outside of DBD is C-terminal to the DBD or at the C-terminus of the wild-type reprogramming factor. In some embodiments, the DBD is at the C-

terminus of a wild-type reprogramming factor, hence the region outside of DBD is N-terminal to the DBD or at the N-terminus of the wild-type reprogramming factor. In some embodiments, the DBDs are at both the N-terminus and C-terminus of a wild-type reprogramming factor, hence the region outside of DBD(s) is C-terminal to the N-terminal DBD and N-terminal to the C-terminal DBD, such as in the middle of a wild-type reprogramming factor.

[0207] DBDs can be identified by many methods known in the art. Experimental approaches include but are not limited to, electrophoretic mobility-shift assay (EMSA), DNase I footprinting, exonuclease III footprinting, southwestern blotting, DNA pull-down assay, reporter assay, microplate capture and detection assay, X-ray crystallography, and nuclear magnetic resonance spectroscopy (NMR). Computational or database methods can also be used, such as DP-Bind, DeepDISE, DNAbinder, DNABIND, DRNAPred, GraphSite, DBS-pred, DBS-PSSM, BindN, DNABindR, DISIS, BindN-RF, DBindR, DBD-Threader, ProteDNA, BindN+, NAPS, MetaDBSite, DNABR, TargetS, SNBRFinder, DisoRDPbind, DQPred-DBR, TargetDNA, PRODNA, DRNAPred, PDRLGB, ENSEMBLE-CNN, NucBind, hybridNAP, DNAPred, ProNA2020, NCBRPred, MTDsite, DNAGenie, DeepDISOBind, HybridDBRpred, PDNAsite, RANSFAC, JASPAR, PROSITE, Pfam, and SMART.

[0208] Activation domain (AD) or transactivation domain (TAD) is a transcription factor scaffold domain which contains binding sites for other proteins such as transcription coregulators. There are usually 4 classes of TADs: acidic domains, glutamine-rich domains, proline-rich domains, and isoleucine-rich domains. AD can be identified by many methods known in the art. Experimental approaches include but are not limited to, reporter assay, co-IP, tAD-seq (Arnold et al., *EMBO J.* 2018;37(16):e98896), etc. Computational or database methods can also be used, such as ADpred, PADDLE, 9aaTAD Prediction Tool, etc.

[0209] Protein regions which lack stable three-dimensional structures are referred to as IDRs. IDRs can be inferred from sequence features (see, e.g., Lu et al., *PLoS Comput Biol.* 2022;18(6):e1010238; Trivedi and Nagarajaram, *Int J Mol Sci.* 2022;23(22):14050; R. van der Lee et al., *Chem Rev.* 2014;114(13):6589–6631; the contents of each of which are incorporated herein by reference in their entirety). IDRs are usually enriched with charged and polar amino acids and depleted in bulky hydrophobic residues, leading to a weakened hydrophobic effect, which is usually the main drive for the folding of polypeptides into their compact tertiary structure (natively

folded state). Methods to identify IDRs are well-known and can all be used herein. Computational methods for identifying IDRs can be divided into three categories: (1) Physicochemical-based methods, such as FoldIndex, GlobPlot, IUPred, FoldUnfold, and IsUnstruct, which rely on the amino acid physicochemical properties for identifying disorder. (2) Machine learning-based methods—for instance, DISvgg, RFPR-IDP, IDP-Seq2Seq, SPOT-Disorder, SPOT-Disorder2, DISOPRED3, SPINE-D, ESpritz, BVDEA, POODLE-S, RONN, and PONDRs—which treat the identification of IDRs as labeling each amino acid of a protein sequence or as a classification problem. (3) Meta methods, including MFDp, MetaPrDOS, and Meta-Disorder predictor, which fuse multiple predictors to yield the final prediction for IDRs. Other computing techniques can also be used, e.g., VSL2, VSL2B, IUPred2, ANCHOR2, DisEMBL, DISOPRED, and IUPred. Experimental methods used to detect IDRs include X-ray crystallography, NMR, small-angle X-ray scattering, circular dichroism and Förster resonance energy transfer.

[0210] Negatively charged IDRs of reprogramming factors may contribute to LLPS. IDR region within a reprogramming factor can be predicted by an algorithm that provides a Disorder Score. A region with a Disorder Score greater than a certain value has a high probability of being IDR. Using human OCT4 as an example, VSL2 was used as an algorithm to predict the IDR. A continuous region with a Disorder Score greater than a certain value has a high probability of being IDR - the value is not particularly limited, but can be in the range of 0.4-0.6, preferably 0.5. The exemplary prediction results of OCT4 showed that the IDR of OCT4 distributed in the activation domain (AD) at both ends of the protein, and more specifically, at amino acid residues 1-140 and 288-360; while the intermediate DBD did not belong to IDR. Alternative scoring criteria may also be used, such as IUPred2 and ANCHOR2. All scoring algorithm and criteria can be used herein, as long as they can be used for predictive scoring of the IDR. For example, as inventors predicted, the IDR prediction obtained by a variety of scoring criteria was the same (within an error range).

[0211] Ubiquitination site identification methods are well-known in the art. Biochemical approaches can be used, such as immunoblotting using anti-Ub antibodies. If the immunoblotting result suggests ubiquitination on the substrates, the putative ubiquitinated lysine is further mutated and analyzed by immunoblotting to evaluate whether the mutated lysine is ubiquitinated or not. Ubiquitination sites can also be identified by MS through the detection of peptide adducts derived from ubiquitin. Other methods of profiling ubiquitination sites include but are not limited to, Ub tagging-based approach, anti-diGly antibody-based approach, UbiSite antibody-based approach,

antibody-free approach (e.g., Ub COmbined FRActional DIagonal Chromatography (COFRADIC), StUbEx PLUS), etc. Computational methods can also be used, e.g., ESA-UbiSite, HUbipPred. Also *see* exemplary methods in, e.g., M. Pourmirzaei et al., *BMC Bioinformatics*. 2023 Nov;24(1):449; Sun and Zhang, *Cell Biosci.* 2022;12:126; the contents of each of which are incorporated herein by reference in their entirety.

[0212] When amino acids are modified by polyubiquitin, the polyubiquitin chains may mediate LLPS. Substituting an amino acid that can be ubiquitinated (e.g., K) with an amino acid that cannot be ubiquitinated (e.g., R) shall not produce polyubiquitin modification, hence avoiding LLPS. Any other mutations that can reduce or abolish ubiquitination modification of the reprogramming factors can also be used herein.

[0213] Any transcription factor that can reprogram (partial or complete) a cell can be used herein as the wild-type or reference reprogramming factor. The wild-type reprogramming factor can be from a family selected from the group consisting of OCT family, SOX family, KLF family, MYC family, LIN28 family, NANOG family, and GLIS family. In some embodiments, the wild-type reprogramming factor is selected from the group consisting of OCT4, SOX2, KLF4, c-MYC, L-MYC, LIN28, NANOG, and GLIS1. Other exemplary reprogramming factors that can be used herein or as reference reprogramming factors include DPPA2, DPPA4, ESRRB, GDF3, KLF2, KLF5, LMYC, NMYC, NR5A1, NR5A2, RCOR2, SALL1, SALL4, SOX1, SOX3, TDRD12, TET1, TH2A, TH2B, UTF1, ZFP42, MDM2, CyclinD1, SV40 large T antigen, SIRT6, TCL1A, and RAR γ .

[0214] The engineered reprogramming factor can be from any source, include but are not limited to, human, bovine, horse, sheep, feline, canine, rodent (e.g., rat, mouse, rabbit), avian (e.g., chicken), and non-human primate (e.g., monkey). In some embodiments, the engineered reprogramming factor is a human engineered reprogramming factor. In some embodiments, the engineered reprogramming factor is a mouse engineered reprogramming factor.

OCT

[0215] Oct family is a family of octamer (“Oct”) transcription factors which play a crucial role in maintaining pluripotency. Oct reprogramming factor herein refers to any naturally occurring member of the Oct family, or a functional variant thereof which maintains at least about 50% (e.g., at least about any of 60%, 70%, 80%, 90%, 95%, or 100%) reprogramming activity of the

corresponding wild-type Oct reprogramming factor. In some embodiments, an Oct reprogramming factor variant contains at least the DBD of the corresponding wild-type Oct reprogramming factor, and can further contain an AD. Exemplary Oct reprogramming factors include Oct-1, Oct-2, Oct-3/4, Oct-6, Oct-7, Oct-8, Oct-9, and Oct-11.

[0216] POU5F1 (POU domain, class 5, transcription factor 1) also known as Oct3/4 is one representative of Oct family. The absence of Oct3/4 in Oct-3/4+ cells, such as blastomeres and embryonic stem cells, leads to spontaneous trophoblast differentiation, and presence of Oct-3/4 thus gives rise to the pluripotency and differentiation potential of embryonic stem cells. Exemplary Oct3/4 proteins are the proteins encoded by the murine Oct3/4 gene (Genbank accession number NM_013633) and the human Oct3/4 gene (Genbank accession number NM_002701).

[0217] The terms “Oct3/4”, “Oct4,” “OCT4,” “Oct4 protein,” “OCT4 protein” and the like refer to any of the naturally-occurring forms of the Octomer 4 transcription factor, or variants thereof that maintain Oct4 transcription factor activity (e.g. at least about any of 50%, 80%, 90%, or 100% activity compared to wildtype Oct4 as measured by methods known in the art). In some embodiments, an OCT4 variant has at least about 85% (e.g., at least about any of 90%, 95%, 99%, or more) amino acid sequence identity across the whole sequence compared to the naturally occurring Oct4 polypeptide. In some embodiments, the Oct4 protein is the protein as identified by the Genbank reference ADW77327.1.

[0218] In some embodiments, the LLPS-deficient engineered reprogramming factor is an engineered OCT4, such as engineered human OCT4 or engineered mouse OCT4.

[0219] In some embodiments, the human wild-type OCT4 comprises the amino acid sequence of SEQ ID NO: 2, or is encoded by a nucleic acid sequence of SEQ ID NO: 1. In some embodiments, there is provided an engineered OCT4 that comprises a mutation (e.g., one or more mutations, such as deletion, insertion, and/or substitution) in the IDR(s) in reference to SEQ ID NO: 2. The IDR of OCT4 can be identified using any methods known in the art or described herein, such as VSL2. The IDR of wild-type OCT4 can account for about 38% to about 40% of the total sequence of SEQ ID NO: 2 at the N-terminus, and/or account for about 19% to about 24% of the total sequence of SEQ ID NO: 2 at the C-terminus. In some embodiments, the IDR of OCT4 is at amino acid positions 1-140 and 288-360 (can have a margin of error of about 10 to about 20 amino acids) in reference to SEQ ID NO: 2. In some embodiments, the DBD of OCT4 is at amino acid

positions 141-287 (can have a margin of error of about 10 to about 20 amino acids) in reference to SEQ ID NO: 2. In some embodiments, there is provided an engineered OCT4 that comprises a mutation (e.g., deletion, insertion, and/or substitution) at amino acid positions 1-140 and/or 288-360, wherein the amino acid positions are in reference to SEQ ID NO: 2. In some embodiments, the human wild-type OCT4 is GFP-tagged, e.g., encoded by a nucleic acid sequence of SEQ ID NO: 109.

[0220] In some embodiments, there is provided an engineered OCT4 that comprises a substitution in reference to SEQ ID NO: 2, wherein the substitution comprises substituting a polar amino acid (charged or uncharged, e.g., D, E, R, K, H, S, T, N, Q, C, Y) with a neutral amino acid (e.g., Gly, Ala, Leu, Ile, Val, Phe, Pro, Met, Trp, Tyr, Ser, Thr, Cys, Gln, or Asn, such as A). In some embodiments, there is provided an engineered OCT4 that comprises a substitution in reference to SEQ ID NO: 2, wherein the substitution comprises substituting a charged amino acid (e.g., an acidic amino acid, such as D or E, or a basic amino acid, such as R, K, or H) with a neutral amino acid (e.g., A). In some embodiments, the substitution comprises substituting an acidic amino acid (e.g., D or E) in reference to SEQ ID NO: 2 to a neutral amino acid (e.g., A). In some embodiments, the substitution comprises substituting an acidic amino acid (e.g., D or E) in the IDR(s) in reference to SEQ ID NO: 2 to a neutral amino acid (e.g., A). In some embodiments, there is provided an engineered OCT4 that comprises a substitution (e.g., substituting to a neutral amino acid, such as A) at an amino acid position selected from the group consisting of 8, 20, 26, 31, 56, 68, 91, 96, 98, 104, 108, 113, 125, 127, 130, 134, 135, 138, 145, 147, 166, 188, 209, 210, 215, 224, 238, 246, 270, 272, 291, 296, 297, 299, 341, and 343, wherein the amino acid positions are in reference to SEQ ID NO: 2. In some embodiments, the engineered OCT4 comprises a substitution (e.g., substituting to a neutral amino acid, such as A) at an amino acid position selected from the group consisting of 8, 20, 26, 31, 56, 68, 91, 96, 98, 104, 108, 113, 125, 127, 130, 134, 135, 138, 291, 296, 297, 299, 341, and 343, wherein the amino acid positions are in reference to SEQ ID NO: 2. In some embodiments, the engineered OCT4 comprises a substitution (e.g., substituting to a neutral amino acid, such as A) at an amino acid position selected from the group consisting of D8, D20, E26, D31, E56, E68, E91, E96, E98, E104, D108, E113, E125, E127, E130, E134, E135, D138, D291, E296, D297, E299, E341, and E343, wherein the amino acid positions are in reference to SEQ ID NO: 2. In some embodiments, the substitution comprises substituting a D or E with an A. In some embodiments, the engineered OCT4 comprises a substitution selected

from the group consisting of D8A, D20A, D31A, D108A, E134A, D138A, D291A, or any combination thereof, wherein the amino acid positions are in reference to SEQ ID NO: 2. In some embodiments, there is provided an engineered OCT4 that comprises a substitution in reference to SEQ ID NO: 2, wherein the substitution is selected from the group consisting of: (i) D8A, D20A, E26A, D31A, E56A, E68A, E91A, E96A, E98A, E104A, D108A, E113A, E125A, E127A, E130A, E134A, E135A, D138A, D291A, E296A, D297A, E299A, E341A, and E343A; (ii) D8A, D20A, E26A, D31A, E56A, E68A, E91A, E96A, E98A, E104A, D108A, E113A, E125A, E127A, E130A, E134A, E135A, and D138A; (iii) D291A, E296A, D297A, E299A, E341A, and E343A; (iv) D8A, D20A, E26A, D31A, E56A, E68A, E91A, E96A, E98A, E104A, D108A, E113A, E125A, E127A, E130A, E134A, D138A, D291A, E296A, E299A, E341A, and E343A; (v) E134A, E135A, E296A, and D297A; (vi) D8A, D20A, D31A, D108A, D138A, D291A, and D297A; and (vii) E26A, E56A, E68A, E91A, E96A, E98A, E104A, E113A, E125A, E127A, E130A, E134A, E135A, E296A, E299A, E341A, and E343A. In some embodiments, the engineered OCT4 comprises an amino acid sequence of any of SEQ ID NOS: 4-10, or a variant thereof having at least about 85% (e.g., at least about any of 90%, 95%, 99%, or more) sequence identity to any of SEQ ID NOS: 4-10. In some embodiments, the engineered OCT4 is encoded by a nucleic acid sequence of SEQ ID NO: 3.

[0221] In some embodiments, there is provided an engineered OCT4 that comprises a deletion (partial or complete) of the IDR(s) in reference to SEQ ID NO: 2. In some embodiments, the engineered OCT4 comprises a deletion at amino acid positions 2-140 and/or 288-360, wherein the amino acid positions are in reference to SEQ ID NO: 2. In some embodiments, the engineered OCT4 comprises a deletion selected from the group consisting of: (i) a deletion at amino acid positions 2-140 and 301-360; (ii) a deletion at amino acid positions 301-360; and (iii) a deletion at amino acid positions 2-140. In some embodiments, the engineered OCT4 comprises an amino acid sequence of any of SEQ ID NOS: 44-46, or a variant thereof having at least about 85% (e.g., at least about any of 90%, 95%, 99%, or more) sequence identity to any of SEQ ID NOS: 44-46. In some embodiments, the engineered OCT4 is GFP-tagged, e.g., encoded by a nucleic acid sequence of SEQ ID NO: 110.

[0222] In some embodiments, the mouse wild-type OCT4 (MsOCT4) comprises the amino acid sequence of SEQ ID NO: 66, or is encoded by a nucleic acid sequence of SEQ ID NO: 67. In some embodiments, there is provided an engineered mouse OCT4 (MsOCT4_{mut}) that comprises a

mutation (e.g., one or more mutations, such as deletion, insertion, and/or substitution) in the IDR(s) in reference to SEQ ID NO: 66. The IDR of MsOCT4 can be identified using any methods known in the art or described herein, such as VSL2. In some embodiments, the IDR of MsOCT4 is at amino acid positions 1-140 and 280-352 (can have a margin of error of about 10 to about 20 amino acids) in reference to SEQ ID NO: 66. In some embodiments, the DBD of MsOCT4 is at amino acid positions 141-279 (can have a margin of error of about 10 to about 20 amino acids) in reference to SEQ ID NO: 66. In some embodiments, there is provided an engineered MsOCT4 that comprises a mutation (e.g., deletion, insertion, and/or substitution) at amino acid positions 1-140 and/or 280-352, wherein the amino acid positions are in reference to SEQ ID NO: 66.

[0223] In some embodiments, there is provided an engineered MsOCT4 that comprises a substitution in reference to SEQ ID NO: 66, wherein the substitution comprises substituting a polar amino acid (charged or uncharged, e.g., D, E, R, K, H, S, T, N, Q, C, Y) with a neutral amino acid (e.g., Gly, Ala, Leu, Ile, Val, Phe, Pro, Met, Trp, Tyr, Ser, Thr, Cys, Gln, or Asn, such as A). In some embodiments, there is provided an engineered MsOCT4 that comprises a substitution in reference to SEQ ID NO: 66, wherein the substitution comprises substituting a charged amino acid (e.g., an acidic amino acid, such as D or E, or a basic amino acid, such as R, K, or H) with a neutral amino acid (e.g., A). In some embodiments, the substitution comprises substituting an acidic amino acid (e.g., D or E) in reference to SEQ ID NO: 66 to a neutral amino acid (e.g., A). In some embodiments, the substitution comprises substituting an acidic amino acid (e.g., D or E) in the IDR(s) in reference to SEQ ID NO: 66 to a neutral amino acid (e.g., A). In some embodiments, there is provided an engineered MsOCT4 that comprises a substitution (e.g., substituting to a neutral amino acid, such as A) at an amino acid position selected from the group consisting of D8, D19, E25, D30, E51, E63, E86, E91, E99, E103, E108, D112, E120, E123, E127, E128, D131, E284, E289, E290, E292, E333, and E335, wherein the amino acid positions are in reference to SEQ ID NO: 66. In some embodiments, the engineered MsOCT4 comprises the amino acid sequence of SEQ ID NO: 68, or a variant thereof having at least about 85% (e.g., at least about any of 90%, 95%, 99%, or more) sequence identity to SEQ ID NO: 68. In some embodiments, the engineered MsOCT4 is encoded by a nucleic acid sequence of SEQ ID NO: 69.

[0224] In some embodiments, there is provided an engineered MsOCT4 that comprises a deletion (partial or complete) of the IDR(s) in reference to SEQ ID NO: 66. In some embodiments, the engineered MsOCT4 comprises a deletion at amino acid positions 2-140 and/or 280-352,

wherein the amino acid positions are in reference to SEQ ID NO: 66. In some embodiments, the engineered MsOCT4 comprises a deletion at amino acid positions 324-326 in reference to SEQ ID NO: 66. In some embodiments, the engineered MsOCT4 comprises the amino acid sequence of SEQ ID NO: 70, or a variant thereof having at least about 85% (e.g., at least about any of 90%, 95%, 99%, or more) sequence identity to SEQ ID NO: 70. In some embodiments, the engineered MsOCT4 is encoded by a nucleic acid sequence of SEQ ID NO: 71.

MYC

[0225] Factors of the Myc family are factors encoded by myc proto-oncogenes implicated in cancer. c-Myc was shown to be a factor implicated in the generation of mouse iPSCs and of human iPSCs. Exemplary c-Myc proteins are the proteins encoded by the murine c-myc gene (Genbank accession number NM_010849) and the human c-myc gene (Genbank accession number NM_002467). N-Myc or L-myc was also used as possible reprogramming factor replacing c-Myc. N-Myc or L-myc can also be used as a possible reprogramming factor to replace c-Myc. Myc reprogramming factor herein refers to any naturally occurring member of the Myc family, or a functional variant thereof which maintains at least about 50% (e.g., at least about any of 60%, 70%, 80%, 90%, 95%, or 100%) reprogramming activity of the corresponding wild-type Myc reprogramming factor.

[0226] The terms “c-Myc,” “C-MYC,” “c-Myc protein”, “C-MYC protein” and the like as referred to herein includes any of the naturally-occurring forms of the cMyc transcription factor, or variants thereof that maintain cMyc transcription factor activity (e.g. within at least about any of 50%, 80%, 90% or 100% activity compared to wildtype cMyc as measured by methods known in the art). In some embodiments, a c-Myc variant has at least about 85% (e.g., at least about any of 90%, 95%, 99%, or more) amino acid sequence identity across the whole sequence compared to the naturally occurring c-Myc polypeptide. In some embodiments, the c-Myc protein is the protein as identified by the NCBI reference NP 002458.2.

[0227] In some embodiments, the LLPS-deficient engineered reprogramming factor is an engineered c-MYC, such as engineered human c-MYC or engineered mouse c-MYC.

[0228] In some embodiments, the human wild-type c-MYC comprises the amino acid sequence of SEQ ID NO: 12, or is encoded by a nucleic acid sequence of SEQ ID NO: 11, or 53. In some embodiments, the human wild-type c-MYC comprises the amino acid sequence of SEQ ID NO:

85, or is encoded by a nucleic acid sequence of SEQ ID NO: 86. In some embodiments, there is provided an engineered c-MYC that comprises a mutation (e.g., one or more mutations, such as deletion, insertion, and/or substitution) at a ubiquitination site (e.g., S, T, or K, such as K) in reference to SEQ ID NO: 12 or 85. The ubiquitination site(s) of c-MYC can be identified using any methods known in the art or described herein. In some embodiments, there is provided an engineered c-MYC that comprises a mutation (e.g., one or more mutations, such as deletion, insertion, and/or substitution) in the IDR in reference to SEQ ID NO: 12 or 85. The IDR of c-MYC can be identified using any methods known in the art or described herein, such as VSL2. In some embodiments, the IDR of c-MYC is at amino acid positions 1-360 (can have a margin of error of about 10 to about 20 amino acids) in reference to SEQ ID NO: 12 or 85. In some embodiments, the DBD of c-MYC (bHLH-LZ domain) is at amino acid positions 361-439 (can have a margin of error of about 10 to about 20 amino acids) in reference to SEQ ID NO: 12 or 85. In some embodiments, the engineered c-MYC comprises a deletion at amino acid positions 2-360, wherein the amino acid positions are in reference to SEQ ID NO: 12 or 85.

[0229] In some embodiments, there is provided an engineered c-MYC that comprises a substitution of an amino acid that can be ubiquitinated (e.g., S, T, or K, such as K) with an amino acid that cannot be ubiquitinated (e.g., R), wherein the amino acid positions are in reference to SEQ ID NO: 12 or 85. In some embodiments, the substitution comprises substituting a K with an R. In some embodiments, the engineered c-MYC comprises a (e.g., one or more) substitution at an amino acid position selected from the group consisting of 51, 52, 126, 143, 148, 157, 206, 269, 275, 289, 298, 317, 323, 326, 341, 355, 371, 389, 392, 397, 398, 412, 422, 428, and 430, wherein the amino acid positions are in reference to SEQ ID NO: 12 or 85. In some embodiments, 20 to 25 amino acid positions at any of 51, 52, 126, 143, 148, 157, 206, 269, 275, 289, 298, 317, 323, 326, 341, 355, 371, 389, 392, 397, 398, 412, 422, 428, and 430 in reference to SEQ ID NO: 12 or 85 are substituted. In some embodiments, the engineered c-MYC comprises a substitution selected from the group consisting of K51R, K52R, K148R, K275R, K289R, and K389R, or any combination thereof, wherein the amino acid positions are in reference to SEQ ID NO: 12 or 85. In some embodiments, the engineered c-MYC comprises a substitution selected from the group consisting of: (i) K51R, K52R, K126R, K143R, K148R, K157R, K206R, K269R, K275R, K289R, K298R, K317R, K323R, K326R, K341R, K355R, K371R, K389R, K392R, K397R, K398R, K412R, K422R, K428R, and K430R; (ii) K148R; (iii) K389R; (iv) K148R and K389R; (v) K51R

and K52R; (vi) K275R and K289R; and (vii) K51R, K52R, K275R, and K289R; wherein the amino acid positions are in reference to SEQ ID NO: 12 or 85. In some embodiments, the engineered c-MYC comprises an amino acid sequence of any of SEQ ID NOs: 14-20, or a variant thereof having at least about 85% (e.g., at least about any of 90%, 95%, 99%, or more) sequence identity to any of SEQ ID NOs: 14-20. In some embodiments, the engineered c-MYC is encoded by a nucleic acid sequence of SEQ ID NO: 13.

[0230] In some embodiments, there is provided an engineered c-MYC that comprises a deletion (partial or complete) of the IDR in reference to SEQ ID NO: 12 or 85. In some embodiments, the engineered c-MYC comprises a deletion at amino acid positions 2-360, wherein the amino acid positions are in reference to SEQ ID NO: 12 or 85. In some embodiments, the engineered c-MYC comprises a deletion at amino acid positions 2-140 in reference to SEQ ID NO: 12 or 85. In some embodiments, the engineered c-MYC comprises the amino acid sequence of SEQ ID NO: 47 or 48, or a variant thereof having at least about 85% (e.g., at least about any of 90%, 95%, 99%, or more) sequence identity to SEQ ID NO: 47 or 48.

[0231] In some embodiments, the engineered c-MYC comprises a substitution in the IDR in reference to SEQ ID NO: 12 or 85, wherein the substitution comprises substituting a polar amino acid (charged or uncharged, e.g., D, E, R, K, H, S, T, N, Q, C, Y) with a neutral amino acid (e.g., Gly, Ala, Leu, Ile, Val, Phe, Pro, Met, Trp, Tyr, Ser, Thr, Cys, Gln, or Asn, such as A). In some embodiments, the engineered c-MYC comprises a substitution in the IDR in reference to SEQ ID NO: 12 or 85, wherein the substitution comprises substituting a charged amino acid (e.g., an acidic amino acid, such as D or E, or a basic amino acid, such as R, K, or H) with a neutral amino acid (e.g., A). In some embodiments, the substitution comprises substituting an acidic amino acid (e.g., D or E) in the IDR in reference to SEQ ID NO: 12 or 85 to a neutral amino acid (e.g., A). In some embodiments, the IDR in reference to SEQ ID NO: 12 or 85 is at amino acid positions 2-360.

[0232] In some embodiments, the mouse wild-type c-MYC (Ms c-MYC) comprises the amino acid sequence of SEQ ID NO: 111. In some embodiments, the mouse wild-type c-MYC (Ms c-MYC) is encoded by a nucleic acid sequence of SEQ ID NO: 112. In some embodiments, there is provided an engineered mouse c-MYC (Ms c-MYC_{mut}) that comprises a mutation (e.g., one or more mutations, such as deletion, insertion, and/or substitution) in the IDR in reference to SEQ ID NO: 111. The IDR of Ms c-MYC can be identified using any methods known in the art or described

herein, such as VSL2. In some embodiments, the IDR of Ms c-MYC is at amino acid positions 1-360 (can have a margin of error of about 10 to about 20 amino acids) in reference to SEQ ID NO: 111. In some embodiments, there is provided an engineered Ms c-MYC that comprises a mutation (e.g., deletion, insertion, and/or substitution) at amino acid positions 2-360, wherein the amino acid positions are in reference to SEQ ID NO: 111. In some embodiments, there is provided an engineered Ms c-MYC that comprises a mutation (e.g., one or more mutations, such as deletion, insertion, and/or substitution) at a ubiquitination site (e.g., S, T, or K, such as K) in reference to SEQ ID NO: 111. The ubiquitination site(s) of Ms c-MYC can be identified using any methods known in the art or described herein. In some embodiments, the substitution comprises substituting a K with an R.

KLF

[0233] Klf family (or Krüppel-like family of transcription factors) refers to Klf genes initially identified as a factor for the generation of mouse iPSCs and also demonstrated to be a factor for generation of human iPSCs. Exemplary Klf4 proteins are the proteins encoded by the murine klf4 gene (Genbank accession number NM_010637) and the human klf4 gene (Genbank accession number NM_004235). Exemplary Klf family members include Klf1, Klf2, Klf3, Klf4, Klf5, Klf6, Klf7, Klf8, Klf9, Klf10, Klf11, Klf12, Klf13, Klf14, Klf15, Klf16, and Klf17. KLF reprogramming factor herein refers to any naturally occurring member of the Klf family, or a functional variant thereof which maintains at least about 50% (e.g., at least about any of 60%, 70%, 80%, 90%, 95%, or 100%) reprogramming activity of the corresponding wild-type Klf reprogramming factor.

[0234] The terms “KLF4,” “KLF4 protein” and the like as referred to herein includes any of the naturally-occurring forms of the KLF4 transcription factor, or variants thereof that maintain KLF4 transcription factor activity (e.g. within at least about any of 50%, 80%, 90% or 100% activity compared to wildtype KLF4 as measured by methods known in the art). In some embodiments, a KLF4 variant has at least about 85% (e.g., at least about any of 90%, 95%, 99%, or more) amino acid sequence identity across the whole sequence compared to the naturally occurring KLF4 polypeptide. In other embodiments, the KLF4 protein is the protein as identified by the NCBI reference NP 004226.3.

[0235] In some embodiments, the LLPS-deficient engineered reprogramming factor is an engineered KLF4, such as engineered human KLF4 or engineered mouse KLF4.

[0236] In some embodiments, the human wild-type KLF4 comprises the amino acid sequence of SEQ ID NO: 22 or 87, or is encoded by a nucleic acid sequence of SEQ ID NO: 21, 54, or 88. In some embodiments, there is provided an engineered KLF4 that comprises a mutation (e.g., one or more mutations, such as deletion, insertion, and/or substitution) in the IDR in reference to SEQ ID NO: 22. The IDR of KLF4 can be identified using any methods known in the art or described herein, such as VSL2. In some embodiments, the IDR of KLF4 is at amino acid positions 1-374 (can have a margin of error of about 10 to about 20 amino acids) in reference to SEQ ID NO: 22. In some embodiments, the DBD of KLF4 is at amino acid positions 375-470 (can have a margin of error of about 10 to about 20 amino acids) in reference to SEQ ID NO: 22. In some embodiments, there is provided an engineered KLF4 that comprises a mutation (e.g., deletion, insertion, and/or substitution) at amino acid positions 2-374, wherein the amino acid positions are in reference to SEQ ID NO: 22. In some embodiments, the engineered KLF4 comprises a deletion at amino acid positions 2-374, wherein the amino acid positions are in reference to SEQ ID NO: 22. In some embodiments, the engineered KLF4 comprises the amino acid sequence of SEQ ID NO: 40, or a variant thereof having at least about 85% (e.g., at least about any of 90%, 95%, 99%, or more) sequence identity to SEQ ID NO: 40.

[0237] In some embodiments, the engineered KLF4 comprises a substitution in the IDR in reference to SEQ ID NO: 22, wherein the substitution comprises substituting a polar amino acid (charged or uncharged, e.g., D, E, R, K, H, S, T, N, Q, C, Y) with a neutral amino acid (e.g., Gly, Ala, Leu, Ile, Val, Phe, Pro, Met, Trp, Tyr, Ser, Thr, Cys, Gln, or Asn, such as A). In some embodiments, the engineered KLF4 comprises a substitution in the IDR in reference to SEQ ID NO: 22, wherein the substitution comprises substituting a charged amino acid (e.g., an acidic amino acid, such as D or E, or a basic amino acid, such as R, K, or H) with a neutral amino acid (e.g., A). In some embodiments, the substitution comprises substituting an acidic amino acid (e.g., D or E) in the IDR in reference to SEQ ID NO: 22 to a neutral amino acid (e.g., A). In some embodiments, the IDR in reference to SEQ ID NO: 22 is at amino acid positions 2-374.

[0238] In some embodiments, the mouse wild-type KLF4 (MsKLF4) comprises the amino acid sequence of SEQ ID NO: 72, or is encoded by a nucleic acid sequence of SEQ ID NO: 73. In some embodiments, there is provided an engineered mouse KLF4 (MsKLF4_{mut}) that comprises a mutation (e.g., one or more mutations, such as deletion, insertion, and/or substitution) in the IDR in reference to SEQ ID NO: 73. The IDR of MsKLF4 can be identified using any methods known

in the art or described herein, such as VSL2. In some embodiments, the IDR of MsKLF4 is at amino acid positions 1-374 (can have a margin of error of about 10 to about 20 amino acids) in reference to SEQ ID NO: 73. In some embodiments, there is provided an engineered MsKLF4 that comprises a mutation (e.g., deletion, insertion, and/or substitution) at amino acid positions 2-374, such as partial or complete deletion of amino acids 2-374, wherein the amino acid positions are in reference to SEQ ID NO: 73.

SOX

[0239] Sox family refers to Sox genes associated with maintaining pluripotency similar to Oct-3/4, although it is associated with multipotent and unipotent stem cells in contrast with Oct-3/4, which is exclusively expressed in pluripotent stem cells. SOX genes (SRY-related HMG-box genes) encode a family of transcription factors that bind to the minor groove in DNA, and belong to a super-family of genes characterized by a homologous sequence called the HMG-box (for high mobility group). This HMG box is a DNA binding domain that is highly conserved throughout eukaryotic species. Homologues have been identified in insects, nematodes, amphibians, reptiles, birds and a range of mammals. Sox proteins (e.g., Sox1, Sox2, Sox3, Sox15, or Sox18) can be derived from human, mouse, rat, bovine, porcine, or other animals. SOX reprogramming factor herein refers to any naturally occurring member of the Sox family, or a functional variant thereof which maintains at least about 50% (e.g., at least about any of 60%, 70%, 80%, 90%, 95%, or 100%) reprogramming activity of the corresponding wild-type Sox reprogramming factor.

[0240] Exemplary Sox2 proteins are the proteins encoded by the murine Sox2 gene (Genbank accession number NM_011443) and the human Sox2 gene (Genbank accession number NM_003106).

[0241] The terms “Sox2,” “SOX2,” “Sox2 protein,” “SOX2 protein” and the like as referred to herein thus includes any of the naturally-occurring forms of the Sox2 transcription factor, or variants thereof that maintain Sox2 transcription factor activity (e.g. within at least about any of 50%, 80%, 90% or 100% activity compared to wildtype Sox2 as measured by methods known in the art). In some embodiments, a SOX2 variant has at least about 85% (e.g., at least about any of 90%, 95%, 99%, or more) amino acid sequence identity across the whole sequence compared to the naturally occurring Sox2 polypeptide. In some embodiments, the Sox2 protein is the protein as identified by the NCBI reference NP 003097.1.

[0242] In some embodiments, the LLPS-deficient engineered reprogramming factor is an engineered SOX2, such as engineered human SOX2 or engineered mouse SOX2.

[0243] In some embodiments, the human wild-type SOX2 comprises the amino acid sequence of SEQ ID NO: 24, or is encoded by a nucleic acid sequence of SEQ ID NO: 23. In some embodiments, there is provided an engineered SOX2 that comprises a mutation (e.g., one or more mutations, such as deletion, insertion, and/or substitution) in the IDR(s) in reference to SEQ ID NO: 24. The IDR(s) of SOX2 can be identified using any methods known in the art or described herein, such as VSL2. In some embodiments, the IDR of SOX2 is at amino acid positions 1-40 and 201-317 (can have a margin of error of about 10 to about 20 amino acids) in reference to SEQ ID NO: 24. In some embodiments, the DBD of SOX2 is at amino acid positions 41-200 (can have a margin of error of about 10 to about 20 amino acids) in reference to SEQ ID NO: 24. In some embodiments, there is provided an engineered SOX2 that comprises a mutation (e.g., deletion, insertion, and/or substitution) at amino acid positions 2-40 and/or 201-317, wherein the amino acid positions are in reference to SEQ ID NO: 24. In some embodiments, there is provided an engineered SOX2 that comprises a deletion at: (i) amino acid positions 2-40, (ii) amino acid positions 201-317; or (iii) 2-40 and 201-317; wherein the amino acid positions are in reference to SEQ ID NO: 24. In some embodiments, the engineered SOX2 comprises an amino acid sequence of any of SEQ ID NOs: 41-43, or a variant thereof having at least about 85% (e.g., at least about any of 90%, 95%, 99%, or more) sequence identity to any of SEQ ID NOs: 41-43.

[0244] In some embodiments, the engineered SOX2 comprises a substitution in the IDR(s) in reference to SEQ ID NO: 24, wherein the substitution comprises substituting a polar amino acid (charged or uncharged, e.g., D, E, R, K, H, S, T, N, Q, C, Y) with a neutral amino acid (e.g., Gly, Ala, Leu, Ile, Val, Phe, Pro, Met, Trp, Tyr, Ser, Thr, Cys, Gln, or Asn, such as A). In some embodiments, the engineered SOX2 comprises a substitution in the IDR(s) in reference to SEQ ID NO: 24, wherein the substitution comprises substituting a charged amino acid (e.g., an acidic amino acid, such as D or E, or a basic amino acid, such as R, K, or H) with a neutral amino acid (e.g., A). In some embodiments, the substitution comprises substituting an acidic amino acid (e.g., D or E) in the IDR(s) in reference to SEQ ID NO: 24 to a neutral amino acid (e.g., A). In some embodiments, the IDR(s) in reference to SEQ ID NO: 24 is at amino acid positions 1-40 and 201-317.

[0245] In some embodiments, the mouse wild-type SOX2 (MsSOX2) comprises the amino acid sequence of SEQ ID NO: 74, or is encoded by a nucleic acid sequence of SEQ ID NO: 75. In some embodiments, there is provided an engineered mouse SOX2 (MsSOX2_{mut}) that comprises a mutation (e.g., one or more mutations, such as deletion, insertion, and/or substitution) in the IDR(s) in reference to SEQ ID NO: 74. The IDR(s) of MsSOX2 can be identified using any methods known in the art or described herein, such as VSL2. In some embodiments, the IDR of MsSOX2 is at amino acid positions 1-40 and 203-319 (can have a margin of error of about 10 to about 20 amino acids) in reference to SEQ ID NO: 74. In some embodiments, the DBD of MsSOX2 is at amino acid positions 41-202 (can have a margin of error of about 10 to about 20 amino acids) in reference to SEQ ID NO: 74. In some embodiments, there is provided an engineered MsSOX2 that comprises a mutation (e.g., deletion, insertion, and/or substitution) at amino acid positions 2-40 and/or 203-319 such as partial or complete deletion of amino acids 2-40 and/or 203-319, wherein the amino acid positions are in reference to SEQ ID NO: 74.

NANOG

[0246] Nanog is a transcription factor critically involved with self-renewal of undifferentiated embryonic stem cells. It helps embryonic stem cells (ESCs) maintain pluripotency by suppressing cell determination factors. In humans, this protein is encoded by the NANOG gene. Exemplary nanog is the protein encoded by murine gene (Genbank accession number XM_132755) and human Nanog gene (Genbank accession number NM_024865). The human hNanog protein coded by the NANOG gene, consists of 305 amino acids and possesses 3 functional domains: the N-terminal domain, the C-terminal domain, and the conserved homeodomain motif (for DNA binding). The homeodomain in hNANOG ranges from residues 95 to 155. There are also additional NANOG genes (NANOG2, NANOG p8). Nanog reprogramming factor herein refers to any naturally occurring member of the Nanog family, or a functional variant thereof which maintains at least about 50% (e.g., at least about any of 60%, 70%, 80%, 90%, 95%, or 100%) reprogramming activity of the corresponding wild-type Nanog reprogramming factor.

[0247] The term “Nanog” or “nanog” and the like as referred to herein thus includes any of the naturally-occurring forms of the Nanog transcription factor, or variants thereof that maintain Nanog transcription factor activity (e.g. within at least about any of 50%, 80%, 90% or 100% activity compared to wildtype Nanog as measured by methods known in the art). In some

embodiments, a Nanog variant has at least about 85% (e.g., at least about any of 90%, 95%, 99%, or more) amino acid sequence identity across the whole sequence compared to the naturally occurring Nanog polypeptide. In some embodiments, the Nanog protein is the protein as identified by the NCBI reference NP_079141.

[0248] An LLPS-deficient engineered NANOG can comprise any of the mutations described herein that lead to LLPS deficiency. For example, partial or complete deletion of the N-terminal domain and/or the C-terminal domain outside of the DBD, substitution of a charged amino acid (e.g., an acidic amino acid, such as D or E, or a basic amino acid, such as R, K, or H) with a neutral amino acid (e.g., A), or a combination thereof.

LIN28

[0249] The LIN28 family of RNA-binding proteins promoting pluripotency has two members—LIN28A and LIN28B. Lin28 reprogramming factor herein refers to any naturally occurring member of the Lin28 family, or a functional variant thereof which maintains at least about 50% (e.g., at least about any of 60%, 70%, 80%, 90%, 95%, or 100%) reprogramming activity of the corresponding wild-type Lin28 reprogramming factor.

[0250] Lin28 or Lin-28 homolog A is a protein that is encoded by the LIN28 gene in humans. It is a marker of undifferentiated human embryonic stem cells and encodes a cytoplasmic mRNA-binding protein that binds to and enhances the translation of the IGF-2 (Insulin-like growth factor 2) mRNA. Lin28 has also been shown to bind to the let-7 pre-miRNA and block production of the mature let-7 microRNA in mouse embryonic stem cells. It is a factor in iPSCs generation, although it is not mandatory. Exemplary Lin28 is the protein encoded by murine gene (Genbank accession number NM_145833) and human Lin28 gene (Genbank accession number NM_024674).

[0251] The term “Lin28” or “Lin28 homolog A” and the like as referred to herein thus includes any of the naturally-occurring forms of the Lin28 transcription factor, or variants thereof that maintain Lin28 transcription factor activity (e.g. within at least about any of 50%, 80%, 90% or 100% activity compared to wildtype Lin28 as measured by methods known in the art). In some embodiments, a LIN28 variant has at least about 85% (e.g., at least about any of 90%, 95%, 99%, or more) amino acid sequence identity across the whole sequence compared to the naturally occurring Lin28 polypeptide. In other embodiments, the Lin28 protein is the protein as identified by the NCBI reference NP_078950.

[0252] Human LIN28 has an IDR at amino acid positions 1-31 and 178-209. An LLPS-deficient engineered LIN28 can comprise any of the mutations described herein that lead to LLPS deficiency. For example, partial or complete deletion of the IDR(s), substitution of a charged amino acid (e.g., an acidic amino acid, such as D or E, or a basic amino acid, such as R, K, or H) with a neutral amino acid (e.g., A), such as within the IDR(s), or a combination thereof.

Nucleic acids, constructs, and carriers

[0253] In some embodiments, the engineered reprogramming factor is encoded by a nucleic acid (e.g., RNA or DNA) or a nucleic acid construct (e.g., RNA construct, such as circRNA, or DNA construct, such as viral or bacterial vector). Hence also provided are nucleic acids (e.g., codon-optimized nucleic acids) and nucleic acid constructs that encode any of the LLPS-deficient engineered reprogramming factors described herein. In some embodiments, the nucleic acid comprises a nucleic acid sequence of SEQ ID NO: 3 or 13. In some embodiments, the nucleic acid comprises a nucleic acid sequence of SEQ ID NO: 69 or 71. The step of introducing into a population of retinal cells, or introducing to the eye, an effective amount of an engineered reprogramming factor that is deficient in LLPS in the eye rejuvenation methods (or treatment or prevention methods) described herein also encompass introducing into a population of retinal cells, or introducing to the eye, a nucleic acid that encodes an effective amount of an engineered reprogramming factor that is deficient in LLPS.

[0254] Any carriers or methods that can introduce the nucleic acids described herein into retinal cells or an eye can be used herein. In some embodiments, the delivery of the nucleic acid(s) is viral delivery (e.g., AAV or lentivirus). In some embodiments, the delivery of the nucleic acid(s) is non-viral delivery, such as lipofection, nucleofection, microinjection, biolistics, virosomes, liposomes, immunoliposomes, polycation or lipid:nucleic acid conjugates, electroporation, nanoparticles, exosomes, microvesicles, gene-gun, naked DNA, and artificial virions. Delivery using GalNAc conjugated linked delivery system, protamine, polymer, inorganic nanoparticle, or polymer matrix are also contemplated.

[0255] In some embodiments, the nucleic acid is an RNA (e.g., mRNA), such as a circular RNA (circRNA) or a linear RNA (e.g., linear mRNA). Circular mRNA has lower immunogenicity and higher stability than linear mRNA, and is suitable for long-term expression of encoded proteins *in vivo*. In some embodiments, the nucleic acid is comprised within a vector. In some embodiments,

the nucleic acid (e.g., circRNA) is delivered by an exosome, a liposome, or a lipid nanoparticle (LNP). In some embodiments, the nucleic acid is delivered by electroporation. In some embodiments, the mRNA comprises IRES. In some embodiments, the RNA may contain a sequence bound to L7Ae, TAT, etc., for enrichment of the RNA. In some embodiments, the RNA contains modification(s). In some embodiments, the RNA is a self-amplifying mRNA.

[0256] In some embodiments, the nucleic acid is a DNA. In some embodiments, the nucleic acid is comprised within a vector, such as a viral vector. In some embodiments, the viral vector is selected from the group consisting of adeno-associated virus (AAV) vector, adenovirus vector, retrovirus vector, herpes simplex virus (HSV) vector, murine stem cell virus (MSCV) vector, Moloney Murine Leukemia Virus (MoMuLV) vector, human immunodeficiency virus (HIV) vector, bovine papilloma virus vector, lentiviral vector, vaccinia virus vector, and polyoma virus vector. In some embodiments, the viral vector is a lentiviral vector or an AAV vector. In some embodiments, the nucleic acid is delivered by a virus, e.g., lentivirus or AAV. In some embodiments, the nucleic acid is delivered by electroporation.

[0257] The term “vector” or “nucleic acid construct” as used herein refers to DNA or RNA molecules that comprise a coding nucleotide sequence that can be transcribed into RNAs or expressed into proteins. The present application also provides vectors or nucleic acid constructs in which a nucleic acid of the present application is inserted. The nucleic acid can be cloned into a number of types of vectors. For example, the nucleic acid can be cloned into a vector including, but not limited to a plasmid, a phagemid, a phage derivative, an animal virus, and a cosmid. Vectors of particular interest include expression vectors, replication vectors, probe generation vectors, and sequencing vectors.

[0258] In some embodiments, the vector comprises a promoter that is operably linked to the coding nucleotide sequence, such that the promoter controls the transcription or expression of the coding nucleotide sequence. A promoter may be positioned 5' (upstream) of a coding nucleotide sequence under its control. The distance between the promoter and the coding sequence may be approximately the same as the distance between that promoter and the gene it controls in the gene from which the promoter is derived. As is known in the art, variation in this distance may be accommodated without loss of promoter function. In some embodiments, the vector comprises a 5' UTR and/or a 3'UTR that regulates the transcription or expression of the coding nucleotide

sequence. In some embodiments, a suitable vector contains an origin of replication functional in at least one organism, a promoter sequence, convenient restriction endonuclease sites, and one or more selectable markers. (See, e.g., WO 01/96584; WO 01/29058; and U.S. Pat. No. 6,326,193). Exemplary selectable markers can include, but are not limited to, peptide protein tags (e.g., FLAG-tag), fluorescent tags, and antibiotic resistance cassettes. Both selectable markers and reporter genes may be flanked by appropriate regulatory sequences to enable expression in the target cells. In some embodiments, the vector also comprises or encodes a Kozak sequence, a terminator sequence, a 5' cap, a poly(A) tail, or WPRE.

[0259] Suitable vectors include, but are not limited to, nucleic acid molecules that are single-stranded, double-stranded, or partially double-stranded; nucleic acid molecules that comprise one or more free ends, no free ends (e.g., circular); nucleic acid molecules that comprise DNA, RNA, or both; and other varieties of polynucleotides known in the art. One type of vector is a “plasmid,” which refers to a circular double stranded DNA loop into which additional DNA segments can be inserted, such as by standard molecular cloning techniques. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (e.g., bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (e.g., non-episomal mammalian vectors) are integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the transcription or expression of coding nucleotide sequences to which they are operatively linked. Such vectors are referred to herein as “expression vectors”.

[0260] Recombinant expression vectors can comprise a nucleic acid of the invention in a form suitable for transcription or expression of the nucleic acid in a host cell (e.g., an eye cell, such as retinal cell). In some embodiments, the recombinant expression vector includes one or more regulatory elements, which may be selected on the basis of the host cells to be used for transcription or expression, which is operatively linked to the nucleic acid sequence to be transcribed or expressed. Within a recombinant expression vector, “operably linked” is intended to mean that the nucleotide sequence of interest is linked to the regulatory element(s) in a manner that allows for expression of the nucleotide sequence (e.g., in an *in vitro* transcription/translation system or in a host cell when the vector is introduced into the host cell).

[0261] The expression vectors may contain a variety of elements for controlling expression, including without limitation, promoter sequences, transcription initiation sequences, enhancer sequences, selectable markers, and signal sequences. These elements may be selected as appropriate by a person of ordinary skill in the art. For example, the promoter sequences may be selected to promote the transcription of the polynucleotide in the vector. Suitable promoter sequences include, without limitation, T7 promoter, T3 promoter, SP6 promoter, beta-actin promoter, EF1a promoter, CMV promoter, CAG promoter, PGK promoter, and SV40 promoter. Enhancer sequences may be selected to enhance the transcription of the nucleic acids. Selectable markers may be selected to allow selection of the host cells inserted with the vector from those not, for example, the selectable markers may be genes that confer antibiotic resistance. Signal sequences may be selected to allow the expressed polypeptide to be transported outside of the host cell.

[0262] The expression vector may be in the form of a viral vector. Viral vector technology is well known in the art and is described, for example, in Sambrook *et al.* (2001, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, New York), and in other virology and molecular biology manuals. Viruses which are useful as vectors include, but are not limited to, retroviruses, adenoviruses, adeno-associated viruses, herpes viruses, and lentiviruses. In some embodiments, a murine stem cell virus (MSCV) vector is used to express a desired nucleic acid. MSCV vectors have been demonstrated to efficiently express desired nucleic acids in cells. Other examples of viral vectors are those based upon Moloney Murine Leukemia Virus (MoMuLV) and human immunodeficiency virus (HIV). The use of RNA or DNA viral based systems for the delivery of nucleic acids has high efficiency in targeting a virus to specific cells and trafficking the viral payload to the cellular nuclei.

[0263] In some embodiments, the viral vector is a recombinant AAV (rAAV) vector. In some embodiments, the rAAV vector is a vector derived from an AAV serotype, including without limitation, AAV ITRs are AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAVrh8, AAVrh8R, AAV9, AAV10, AAVrh10, AAV11, AAV12, AAV2R471A, AAV DJ, a goat AAV, bovine AAV, or mouse AAV capsid serotype or the like. In some embodiments, the nucleic acid in the AAV comprises an ITR of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAVrh8, AAVrh8R, AAV9, AAV10, AAVrh10, AAV11, AAV12, AAV2R471A, AAV DJ, a goat AAV, bovine AAV, or mouse AAV capsid serotype or the like. In some embodiments, the

nucleic acid in the AAV further encodes an engineered reprogramming factor as described herein. Use of any AAV serotype is considered within the scope of the present disclosure. In some embodiments, the vector is encapsidated in a rAAV particle. In some embodiments, the AAV viral particle comprises an AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAVrh8, AAVrh8R, AAV9, AAV10, AAVrh10, AAV11, AAV12, AAV2R471A, AAV2/2-7m8, AAV DJ, AAV2 N587A, AAV2 E548A, AAV2 N708A, AAV2 V708K, AAV2-HBKO, AAVDJ8, AAVPHP.B, AAVPHP.eB, AAVBR1, AAVHSC15, AAVHSC17, goat AAV, AAV1/AAV2 chimeric, bovine AAV, mouse AAV, or raAV2/HboV1 serotype capsid.

[0264] In some embodiments, the viral vector is a lentiviral vector. In some embodiments, the lentiviral vector is pseudotyped. In some embodiments, the lentiviral vector comprises a G glycoprotein. In some embodiments, the G glycoprotein is selected from the group consisting of vesicular stomatitis virus (VSV), Piry virus, Chandipura virus, Spring viremia of carp virus and Mokola virus G glycoproteins. In some embodiments, the lentiviral vector comprises long terminal repeats (LTRs) selected from the group consisting of HIV and equine infectious anemia long terminal repeats.

[0265] The viral vector (e.g., transfer vector) comprising the nucleic acid(s) encoding the reprogramming factor(s) can be introduced into a producer cell. In some embodiments, the nucleic acid(s) encoding the reprogramming factor(s) comprises long terminal repeat (LTR) sequences flanking the transgene to enable integration into the target cell genome. In some embodiments, the LTR (5' LTR and/or 3' LTR) is mutated (e.g., truncated, such as truncating U3) to generate self-inactivating viral vector. One or more packaging vectors can be introduced into the producer cell simultaneously or sequentially (in any order) to produce a recombinant virus. A recombinant virus can be produced by, for example, culturing the producer cell under conditions sufficient to make the recombinant virus. In some embodiments, the producer cell already comprises one or more nucleic acids that encode the components to produce the recombinant virus. These can be structural or non-structural viral components or proteins. Producer cell lines are known in the art and can be modified with the molecules of interest to produce the recombinant virus of interest. Any suitable producer cell line known in the art can be used, such as HEK293, 293T, HeLa, D-17, MDCK, BHK and Cf2Th cells. In some embodiments, the producer cell line is 293T.

[0266] In some embodiments, the viral vector promoter is selected from the group consisting of a Rous Sarcoma Virus (RSV) promoter, a Simian Virus 40 (SV40) promoter, a cytomegalovirus immediate early gene promoter (CMV IE), a murine stem cell virus promoter (MCSV), an elongation factor 1 alpha promoter (EF1- α), a phosphoglycerate kinase-1 (PGK) promoter, a ubiquitin-C (UBQ-C) promoter, a cytomegalovirus enhancer/chicken beta-actin (CAG) promoter, a polyoma enhancer/herpes simplex thymidine kinase (MC1) promoter, a beta actin (β -ACT) promoter, a “myeloproliferative sarcoma virus enhancer, negative control region deleted, d1587rev primer-binding site substituted (MND)” promoter, an NFAT promoter, a TETON[®] promoter, and an NF κ B promoter. The transfer vector (e.g., carrying the transgene) can employ a promoter same as or different from the envelope vector and/or packaging vector(s).

[0267] Genes necessary to produce a recombinant virus can be split across two or more vectors to increase recombinant virus safety. Recombinant virus is typically produced using an envelope vector encoding a viral glycoprotein, packaging vector(s) encoding viral structural proteins, and a transfer vector encoding the transgene of interest. The genes required produce a recombinant virus include *gag*, *pol*, *rev* and optionally *tat* (depending on the vectors used). *Gag* encodes for viral structural proteins, *pol* encodes for enzymes involved in reverse transcription and genome integration, while *rev* and *tat* encode for gene regulatory proteins. Wherein the transfer plasmid comprises a 5' LTR fused to a heterologous promoter, Tat is no longer required, as expression of the transgene is Tat-independent. Promoters may be chosen for each vector based on the desired application, such as depending on the cell type to be transduced (e.g., primary cells vs. cell lines), or the gene to be expressed (e.g., a dual promoter for co-expressing two transgenes).

[0268] In some embodiments, the rejuvenation method (or treatment or prevention method) described herein comprises introducing a plurality of reprogramming factors (e.g., a plurality of nucleic acids encoding the plurality of reprogramming factors) into a population of retinal cells, or an eye, wherein at least one of the plurality of reprogramming factors is an engineered reprogramming factor that is deficient in LLPS (e.g., any of the engineered reprogramming factors described herein). In some embodiments, the plurality of reprogramming factors comprise two or more reprogramming factors from two or more families selected from the group consisting of OCT family, SOX family, KLF family, MYC family, LIN28 family, NANOG family, and GLIS family. In some embodiments, the plurality of reprogramming factors comprise two or more reprogramming factors selected from the group consisting of OCT4, SOX2, KLF4, c-MYC, L-

MYC, LIN28, NANOG, GLIS1, and variant thereof, wherein at least one of the plurality of reprogramming factors is an engineered reprogramming factor that is deficient in LLPS. In some embodiments, the plurality of reprogramming factors comprise three or all of OCT4, KLF4, SOX2, and c-MYC, wherein at least one of the plurality of reprogramming factors is an engineered reprogramming factor that is deficient in LLPS. In some embodiments, the SOX2 is an engineered SOX2 that is deficient in LLPS, such as any of the engineered SOX2 described herein, e.g., any of SEQ ID NOs: 41-43. In some embodiments, the c-MYC is an engineered c-MYC that is deficient in LLPS, such as any of the engineered c-MYC described herein, e.g., any of SEQ ID NOs: 14-20, 47, and 48, such as SEQ ID NO: 14. In some embodiments, the plurality of reprogramming factors comprise OCT4 and KLF4, wherein at least one of OCT4 and KLF4 is an engineered reprogramming factor that is deficient in LLPS. In some embodiments, the OCT4 is an engineered OCT4 that is deficient in LLPS, such as any of the engineered OCT4 described herein, e.g., any of SEQ ID NOs: 4-10 and 44-46, such as SEQ ID NO: 4. In some embodiments, the KLF4 is an engineered KLF4 that is deficient in LLPS, such as any of the engineered KLF4 described herein, e.g., SEQ ID NO: 40. In some embodiments, all of the plurality of reprogramming factors are engineered reprogramming factors that are deficient in LLPS. In some embodiments, the plurality of reprogramming factors comprise a (e.g., one or more) wild-type reprogramming factor. In some embodiments, the plurality of reprogramming factors comprise any of: (i) an engineered OCT4 that is deficient in LLPS (e.g., any of SEQ ID NOs: 4-10 and 44-46, such as SEQ ID NO: 4), SOX2 (e.g., SEQ ID NO: 24), and KLF4 (e.g., SEQ ID NO: 22); (ii) an engineered OCT4 that is deficient in LLPS (e.g., any of SEQ ID NOs: 4-10 and 44-46, such as SEQ ID NO: 4), KLF4 (e.g., SEQ ID NO: 22), and an engineered c-MYC that is deficient in LLPS (e.g., any of SEQ ID NOs: 14-20, 47, and 48, such as SEQ ID NO: 14); (iii) an engineered OCT4 that is deficient in LLPS (e.g., any of SEQ ID NOs: 4-10 and 44-46, such as SEQ ID NO: 4), KLF4 (e.g., SEQ ID NO: 22), an engineered c-MYC that is deficient in LLPS (e.g., any of SEQ ID NOs: 14-20, 47, and 48, such as SEQ ID NO: 14), and SOX2 (e.g., SEQ ID NO: 24); or (iv) an engineered OCT4 that is deficient in LLPS (e.g., any of SEQ ID NOs: 4-10 and 44-46, such as SEQ ID NO: 4), KLF4 (e.g., SEQ ID NO: 22), and SOX2 (e.g., SEQ ID NO: 24).

[0269] In some embodiments, the plurality of reprogramming factors comprise three or all of mouse OCT4 (MsOCT4), mouse KLF4 (MsKLF4), mouse SOX2 (MsSOX2), and mouse c-MYC (Ms c-MYC), wherein at least one of the plurality of reprogramming factors is an engineered

reprogramming factor that is deficient in LLPS. In some embodiments, the MsSOX2 is an engineered MsSOX2 that is deficient in LLPS, such as any of the engineered MsSOX2 described herein. In some embodiments, the Ms c-MYC is an engineered Ms c-MYC that is deficient in LLPS, such as any of the engineered Ms c-MYC described herein. In some embodiments, the plurality of reprogramming factors comprise MsOCT4 and MsKLF4, wherein the at least one of MsOCT4 and MsKLF4 is an engineered reprogramming factor that is deficient in LLPS. In some embodiments, the MsOCT4 is an engineered MsOCT4 that is deficient in LLPS, such as any of the engineered MsOCT4 described herein, e.g., SEQ ID NO: 68 or 70. In some embodiments, the MsKLF4 is an engineered MsKLF4 that is deficient in LLPS, such as any of the engineered MsKLF4 described herein. In some embodiments, the plurality of reprogramming factors comprise a (e.g., one or more) wild-type reprogramming factor. In some embodiments, the plurality of reprogramming factors comprise: an engineered OCT4 that is deficient in LLPS (e.g., SEQ ID NO: 68 or 70), MsKLF4 (e.g., SEQ ID NO: 72), and MsSOX2 (e.g., SEQ ID NO: 74).

[0270] In some embodiments, the plurality of reprogramming factors are encoded by a plurality of nucleic acids, e.g., RNA (e.g., linear mRNA or circRNA) or DNA. The expression of the nucleic acid(s) can be constitutive or induced (e.g., using Tet-On system).

[0271] In some embodiments, the plurality of nucleic acids are on separate nucleic acid constructs. The nucleic acid construct can be an RNA construct (e.g., linear RNA or circRNA) or a DNA construct (e.g., lentival vector, AAV vector, or plasmid).

[0272] In some embodiments, at least two of the plurality of nucleic acids are on a single nucleic acid construct. The nucleic acid construct can be an RNA construct (e.g., linear RNA or circRNA) or a DNA construct (e.g., lentival vector, AAV vector, or plasmid). In some embodiments, at least two of the plurality of nucleic acids are on a single DNA construct (e.g., viral or bacterial vector). In some embodiments, the at least two of the plurality of nucleic acids are under the control of different promoters. In some embodiments, the at least two of the plurality of nucleic acids are under the control of the same promoter. The promoter can be a constitutive promoter (e.g., CMV) or an inducible promoter (e.g., Tet-On promoter, such as TRE3G (e.g., SEQ ID NO: 63), TRE, TRE3GS, TRE3GV, etc.). In some embodiments, the at least two of the plurality of nucleic acids are connected by one or more linking nucleic acids, such as IRES, or a linking sequence encoding a self-cleaving peptide (e.g., 2A peptide, such as P2A, T2A, E2A, F2A). In some embodiments,

the one or more linking nucleic acids comprise a linking sequence encoding a self-cleaving peptide, wherein the self-cleaving peptide is P2A, T2A, or E2A. In some embodiments, the nucleic acid encoding P2A comprises the nucleic acid sequence of SEQ ID NO: 25. In some embodiments, the nucleic acid encoding T2A comprises the nucleic acid sequence of SEQ ID NO: 26. In some embodiments, the nucleic acid encoding E2A comprises the nucleic acid sequence of SEQ ID NO: 27.

Circular RNA (circRNA)

[0273] In some embodiments, the nucleic acid or the nucleic acid construct is a circRNA. circRNA refers to a polyribonucleotide that forms a circular structure through covalent bonds. circRNA can be produced by any methods known in the art, include but are not limited to, back-splicing, *in vitro* synthesis of a linear RNA precursor followed by ligation of its ends to produce a covalently-closed loop (can be chemical or enzymatic approach), or generation in living cells by the overexpression of a plasmid harboring a minigene sequence that is naturally circularized following transcription, etc. During back-splicing, a 5' splice site attaches to an upstream 3' splice site on the same pre-mRNA, circularizing the intervening exon(s). *In vitro* or in cell circRNA synthesis can use the PIE (permuted intron exon) system, e.g., *Anabaena* permuted group I intron-exon system (Ana PIE), or *Azoarcus sp.* BH permuted group I intron circular system (AzoPIE), or the Twister-optimized RNA for durable overexpression system (Tornado system). For example, a nucleic acid sequence encoding one or more reprogramming factors can be recombined into the PIE system, and constructed within a plasmid (e.g., pBluescript II SK(+)), following *in vitro* transcription, the linear RNA is circularized to obtain the circRNA. See exemplary methods in Obi and Chen, *Methods*. 2021:196:85-103, WO2023115732, WO2023024500, US20230346921, US11679120, US11981909, US20240078661, and US20240042015, the contents of each of which are incorporated herein by reference in their entirety. For example, a nucleic acid sequence encoding one or more reprogramming factors can be recombined into the Tornado system, and constructed within a plasmid (e.g., pcDNA3.1(+), Piggybac), following the plasmid transfected into cells such as 293T, the circRNA can be produced and also exosome which containing circRNA can be collected. See exemplary methods in US20210340542, US011756183, the contents of each of which are incorporated herein by reference in their entirety.

[0274] In the PIE method, circularly permuted group I intron precursor RNAs (e.g., derived from *Tetrahymena* or *Anabena*) have end-to-end fused exons that interrupt half intron sequences. Circularly permuted group I intron precursor RNAs can self-splice to generate a circular RNA exon *in vitro*. Foreign sequences (e.g., encoding reprogramming factor(s)) can be placed in the exon of a permuted group I intron self-splicing system and made circular. This is because the exon sequence does not participate in the self-splicing reaction.

[0275] Methods of making any of the circRNAs described herein are provided. In some embodiments, the method comprises synthesizing a precursor RNA (linear RNA) by transcription (e.g., run-off transcription) using a DNA vector (e.g., a DNA vector comprising, in the following order, a 5' homology arm, a 3' group I intron fragment, a 5' spacer, an IRES, an expression sequence (e.g., encoding the plurality of reprogramming factors described herein), a 3' spacer, a 5' group I intron fragment, and a 3' homology arm) as a template, and circularizing the precursor RNA to form circRNA (e.g., incubating the precursor RNA under suitable conditions for circularization, such as in the presence of divalent cations (e.g., magnesium ions) and GTP). In some embodiments, the method further comprises constructing the DNA vector for transcription (e.g., *in vitro*) of the precursor RNA. DNA vector construction can be performed using any molecular cloning methods. Exemplary DNA vectors are shown in FIGs. 2A-2D, 6A-6F, 10A-10B. In some embodiments, the DNA vector is linearized (e.g., via enzyme digestion) to provide a transcription template. In some embodiments, the transcription template is digested (e.g., using DNaseI) after transcription of the precursor RNA is completed. In some embodiments, the method further comprises purifying the circRNA (e.g., via RNA precipitation and ethanol washing). In some embodiments, the method further comprises verification of the circRNA (e.g., Rnase R digestion analysis, or E-Gel electrophoresis). Successfully generated circRNA shall stay intact and not digested by exonuclease-like Rnase R, as circRNAs lack free ends. Also see exemplary circRNA making methods in Examples 2-4.

[0276] The present application hence also provides DNA vectors and linear precursor RNAs for making any of the circRNAs described herein.

[0277] In some embodiments, the DNA vector comprises, in the following order, an optional 5' homology arm, a 3' group I intron fragment, an optional 5' internal spacer, an IRES (e.g., CVB3 IRES), an expression sequence (e.g., encoding the plurality of reprogramming factors), an optional

3' internal spacer, a 5' group I intron fragment, and an optional 3' homology arm. In some embodiments, the 5' homology arm and the 3' homology arms are reverse complementary sequences which could help the two ends of linear precursor RNA get closer and improve circularization efficiency. In some embodiments, the 5' internal spacer and the 3' internal spacer are non-structural sequences (e.g., CAAA, or CAAAAA) for separating the ribozyme element (group I intron RNA) and the IRES element or the coding element. In some embodiments, the DNA vector comprises, in the following order, a 5' homology arm, a 3' group I intron fragment, a 5' internal spacer, an IRES (e.g., CVB3 IRES), an expression sequence (e.g., encoding the engineered reprogramming factor or the plurality of reprogramming factors described herein), a 3' internal spacer, a 5' group I intron fragment, and a 3' homology arm. In some embodiments, at least one of the 3' or 5' internal spacers has a length of about 3 to about 60 nucleotides. In some embodiments, the 3' and/or 5' homology arms has a length of about 10 to about 100 nucleotides, such as any of about 10 to about 80 nt, about 10 to about 60 nt, about 10 to about 50 nt, about 10 to about 30 nt, about 20 to about 80 nt, about 40 to about 60 nt, or about 10 to about 20 nt. In some embodiments, the 3' and 5' internal spacers each comprises a duplex forming region capable of forming a duplex. In some embodiments, the duplex forming regions of the 3' and 5' spacers each has a length of about 5 to about 100 nucleotides, such as any of about 10 to about 50 nt, about 20 to about 40 nt, or about about 8 to about 20 nt. The homology arms or the duplex forming regions may form perfect or imperfect duplexes. For example, at least about any of 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% of the homology arms or duplex forming regions may be base paired with one another. In some embodiments, the DNA vector further comprises a transcription regulatory sequence. In some embodiments, the transcription regulatory sequence comprises a promoter and/or an enhancer. In some embodiments, the promoter comprises a T7 promoter. The DNA vector can be a circular DNA or a linear DNA. In some embodiments, the T7 promoter is at the 5' end of 5' homology arm.

[0278] In some embodiments, the 5' elements of the DNA vector (contain 5' homology arm, 3' group I intron fragment, 5' internal spacer) upstream of IRES comprise the sequence of SEQ ID NO: 49. In some embodiments, the 3' elements of the DNA vector (contain 3' internal spacer, 5' group I intron fragment, 3' homology arm) downstream of the protein-coding sequence comprise the sequence of SEQ ID NO: 50.

[0279] In some embodiments, the 5' elements (contain 5' homology arm, 3' group I intron fragment, 5' internal spacer) upstream of IRES comprise the sequence of SEQ ID NO: 51. In some embodiments, the 3' elements (contain 3' internal spacer, 5' group I intron fragment, 3' homology arm) downstream of the protein-coding sequence comprise the sequence of SEQ ID NO: 52.

[0280] The term "3' group I intron fragment" refers to a sequence with 75% or higher similarity to the 3'-proximal end of a natural group I intron including the splice site dinucleotide and optionally a stretch of natural exon sequence. In some embodiments, the term "5' group I intron fragment" refers to a sequence with 75% or higher similarity to the 5'-proximal end of a natural group I intron including the splice site dinucleotide and optionally a stretch of natural exon sequence. The term "permutation site" refers to the site in a group I intron where a cut is made prior to permutation of the intron. This cut generates 3' and 5' group I intron fragments that are permuted to be on either side of a stretch of precursor RNA to be circularized. The term "splice site" refers to a dinucleotide that is partially or fully included in a group I intron and between which a phosphodiester bond is cleaved during RNA circularization. Two "duplex forming regions," "homology arms," or "homology regions," may be any two regions that are thermodynamically favored to cross-pair in a sequence specific interaction. In some embodiments, two duplex forming regions, homology arms, or homology regions, share a sufficient level of sequence identity to one another's reverse complement to act as substrates for a hybridization reaction. As used herein polynucleotide sequences have "homology" when they are either identical or share sequence identity to a reverse complement or "complementary" sequence. The percent sequence identity between a homology region and a counterpart homology region's reverse complement can be any percent of sequence identity that allows for hybridization to occur. In some embodiments, an internal duplex forming region of an inventive polynucleotide is capable of forming a duplex with another internal duplex forming region and does not form a duplex with an external duplex forming region.

[0281] Two types of spacers can improve precursor RNA circularization and/or gene expression from circRNA. The first type of spacer is external spacer, i.e., present in a precursor RNA but removed upon circularization. While not wishing to be bound by theory, it is contemplated that an external spacer may improve ribozyme-mediated circularization by maintaining the structure of the ribozyme itself and preventing other neighboring sequence elements from interfering with its folding and function. The second type of spacer is internal spacer, i.e., present in a precursor RNA

and retained in a resulting circular RNA. While not wishing to be bound by theory, it is contemplated that an internal spacer may improve ribozyme-mediated circularization by maintaining the structure of the ribozyme itself and preventing other neighboring sequence elements, particularly the neighboring IRES and coding region, from interfering with its folding and function. It is also contemplated that an internal spacer may improve protein expression from the IRES by preventing neighboring sequence elements, particularly the intron elements, from hybridizing with sequences within the IRES and inhibiting its ability to fold into its most preferred and active conformation.

[0282] Any IRES can be used herein. In some embodiments, the IRES is CVB3 IRES. Modifications of IRES and accessory sequences can increase or reduce IRES activities, for example, by truncating the 5' and/or 3' ends of the IRES, adding a spacer 5' to the IRES, modifying the 6 nucleotides 5' to the translation initiation site (Kozak sequence), modification of alternative translation initiation sites, and creating chimeric/hybrid IRES sequences.

[0283] In certain embodiments, the vectors, precursor RNA and circular RNA provided herein comprise a first (5') and/or a second (3') spacer. In some embodiments, including a spacer between the 3' group I intron fragment and the IRES may conserve secondary structures in those regions by preventing them from interacting, thus increasing splicing efficiency. In some embodiments, the first (between 3' group I intron fragment and IRES) and second (between the expression sequence and 5' group I intron fragment) spacers comprise additional base pairing regions that are predicted to base pair with each other and not to the first and second duplex forming regions. In some embodiments, such spacer base pairing brings the group I intron fragments in close proximity to each other, further increasing splicing efficiency. Additionally, in some embodiments, the combination of base pairing between the first and second duplex forming regions, and separately, base pairing between the first and second spacers, promotes the formation of a splicing bubble containing the group I intron fragments flanked by adjacent regions of base pairing. Typical spacers are contiguous sequences with one or more of the following qualities: 1) predicted to avoid interfering with proximal structures, for example, the IRES, expression sequence, or intron; 2) is at least 7 nt long and no longer than 100 nt; 3) is located after and adjacent to the 3' intron fragment and/or before and adjacent to the 5' intron fragment; and 4) contains one or more of the following: a) an unstructured region at least 5 nt long, b) a region of base pairing at least 5 nt long to a distal sequence, including another spacer, and c) a structured region at least 7 nt long limited in scope to

the sequence of the spacer. Spacers may have several regions, including an unstructured region, a base pairing region, a hairpin/structured region, and combinations thereof. In an embodiment, the spacer has a structured region with high GC content. In an embodiment, a region within a spacer base pairs with another region within the same spacer. In an embodiment, a region within a spacer base pairs with a region within another spacer. In an embodiment, a spacer comprises one or more hairpin structures. In an embodiment, a spacer comprises one or more hairpin structures with a stem of 4 to 12 nucleotides and a loop of 2 to 10 nucleotides. In an embodiment, there is an additional spacer (internal spacer) between the 3' group I intron fragment and the IRES. In an embodiment, this additional spacer prevents the structured regions of the IRES from interfering with the folding of the 3' group I intron fragment or reduces the extent to which this occurs. In some embodiments, the 5' spacer sequence is at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25 or 30 nucleotides in length. In some embodiments, the 5' spacer sequence is no more than 100, 90, 80, 70, 60, 50, 45, 40, 35, or 30 nucleotides in length. In some embodiments the 5' spacer sequence is between 3 and 50, 5 and 10, 10 and 50, 20 and 50, 20 and 40, and/or 25 and 35 nucleotides in length. In certain embodiments, the 5' spacer sequence is 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49 or 50 nucleotides in length. In one embodiment, the 5' spacer sequence is a polyA sequence. In another embodiment, the 5' spacer sequence is a polyAC sequence. In one embodiment, a spacer comprises about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% polyAC content. In one embodiment, a spacer comprises about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% polypyrimidine (C/T or C/U) content.

[0284] In some embodiments, the method comprises incubating the DNA vector under a suitable condition for transcription. In some embodiments, the DNA vector is transcribed *in vitro*. In some embodiments, the suitable condition comprises ATP, GTP, CTP, UTP, and an RNA polymerase (e.g., T7 RNA polymerase). In some embodiments, the suitable condition further comprise an RNase inhibitor.

[0285] It has been discovered that circRNA has reduced immunogenicity relative to a corresponding mRNA, at least partially because the mRNA contains an immunogenic 5' cap. When transcribing a DNA vector from certain promoters (e.g., a T7 promoter) to produce a precursor RNA, it is understood that the 5' end of the precursor RNA is G. To reduce the immunogenicity of a circular RNA composition that contains a low level of contaminant linear mRNA, an excess

of GMP relative to GTP can be provided during transcription such that most transcripts contain a 5' GMP, which cannot be capped.

[0286] In some embodiments, the linear precursor RNA can be circularized by autocatalysis of a Group I intron. Any Group I intron known in the art could be used to generate circRNA via self-splicing. Examples of Group I introns useful for the methods of this application are described in Puttaraju, M. & Been, M., *Nucleic Acids Res.* 20, 5357–5364 (1992) ; Ford, E. & Ares, M., *Proc. Natl Acad. Sci.* 91, 3117–3121 (1994) ; Vicens, Q., Paukstelis, P.J., Westhof, E., Lambowitz, A.M. & Cech, T.R., *RNA* 14, 2013–2029 (2008), which are incorporated herein by reference. The Group I intron can be derived from bacterial phage Group I intron or a bacterial Group I intron. In some embodiments, the group I intron is derived from a cyanobacteria Group I intron. In some embodiments, the group I intron is derived from an *Anabaena* Group I intron. In some embodiments, the linear RNA polynucleotide comprises a 3' *Anabaena* group I intron fragment and a 5' *Anabaena* group I intron fragment. In some embodiments, the reference 3' *Anabaena* group I intron fragment and reference 5' *Anabaena* group I intron fragment were generated using the L6-5 permutation site. In some embodiments, the linear RNA polynucleotide comprises a 3' *Azoarcus* sp. BH group I intron fragment and a 5' *Azoarcus* sp. BH group I intron fragment.

[0287] In some embodiments, the circRNA comprises an IRES followed by an expression sequence (e.g., encoding engineered reprogramming factor or the plurality of reprogramming factors). In some embodiments, the circRNA comprises, in the following order: a) a post-splicing intron fragment of a 3' group I intron fragment, b) an IRES, c) an expression sequence, and d) a post-splicing intron fragment of a 5' group I intron fragment. In some embodiments, the circRNA does not comprise the 5' homology arm and the 3' homology arm. In some embodiments, the circRNA does not comprise any group I intron fragment. In some embodiments, the circRNA retains the 5' internal spacer and the 3' internal spacer.

[0288] In some embodiments, the circular RNA polynucleotide is made via circularization of a RNA polynucleotide comprising, in the following order: a 3' group I intron fragment, an IRES, an expression sequence (e.g., encoding engineered reprogramming factor or the plurality of reprogramming factors described herein), and a 5' group I intron fragment.

[0289] In some embodiments, the circular RNA polynucleotide is made via circularization of a RNA polynucleotide comprising, in the following order: a 5' external duplex forming region (or

homology arm), a 3' group I intron fragment, a 5' internal spacer optionally comprising a 5' internal duplex forming region, an IRES, an expression sequence (e.g., encoding engineered reprogramming factor or the plurality of reprogramming factors described herein), a 3' internal spacer optionally comprising a 3' internal duplex forming region, a 5' group I intron fragment, and a 3' external duplex forming region (or homology arm).

[0290] In some embodiments, the circular RNA polynucleotide is made via circularization of a RNA polynucleotide comprising, in the following order: a 5' external duplex forming region (or homology arm), a 5' external spacer, a 3' group I intron fragment, a 5' internal spacer optionally comprising a 5' internal duplex forming region, an IRES, an expression sequence (e.g., encoding engineered reprogramming factor or the plurality of reprogramming factors described herein), a 3' internal spacer optionally comprising a 3' internal duplex forming region, a 5' group I intron fragment, a 3' external spacer, and a 3' external duplex forming region (or homology arm).

[0291] In some embodiments, the circular RNA polynucleotide is made via circularization of a RNA polynucleotide comprising, in the following order: a 3' group I intron fragment, a 5' internal spacer comprising a 5' internal duplex forming region, an IRES, an expression sequence (e.g., encoding engineered reprogramming factor or the plurality of reprogramming factors described herein), a 3' internal spacer comprising a 3' internal duplex forming region, and a 5' group I intron fragment.

[0292] In some embodiments, the circular RNA polynucleotide is made via circularization of a RNA polynucleotide comprising, in the following order: a 5' external duplex forming region (or homology arm), a 5' external spacer, a 3' group I intron fragment, a 5' internal spacer comprising a 5' internal duplex forming region, an IRES, an expression sequence (e.g., encoding engineered reprogramming factor or the plurality of reprogramming factors described herein), a 3' internal spacer comprising a 3' internal duplex forming region, a 5' group I intron fragment, a 3' external spacer, and a 3' external duplex forming region (or homology arm).

[0293] In some embodiments, the circular RNA polynucleotide is made via circularization of a RNA polynucleotide comprising, in the following order: a first polyA sequence, a 5' external duplex forming region (or homology arm), a 5' external spacer, a 3' group I intron fragment, a 5' internal spacer comprising a 5' internal duplex forming region, an IRES, an expression sequence (e.g., encoding engineered reprogramming factor or the plurality of reprogramming factors

described herein), a 3' internal spacer comprising a 3' internal duplex forming region, a 5' group I intron fragment, a 3' external spacer, a 3' external duplex forming region (or homology arm), and a second polyA sequence.

[0294] In some embodiments, the circular RNA polynucleotide is made via circularization of a RNA polynucleotide comprising, in the following order: a first polyA sequence, a 5' external spacer, a 3' group I intron fragment, a 5' internal spacer comprising a 5' internal duplex forming region, an IRES, an expression sequence (e.g., encoding engineered reprogramming factor or the plurality of reprogramming factors described herein), a 3' internal spacer comprising a 3' internal duplex forming region, a 5' group I intron fragment, a 3' external spacer, and a second polyA sequence.

[0295] In some embodiments, the circular RNA polynucleotide is made via circularization of a RNA polynucleotide comprising, in the following order: a first polyA sequence, a 5' external spacer, a 3' group I intron fragment, a 5' internal spacer comprising a 5' internal duplex forming region, an IRES, an expression sequence (e.g., encoding engineered reprogramming factor or the plurality of reprogramming factors described herein), a stop codon, a 3' internal spacer comprising a 3' internal duplex forming region, a 5' group I intron fragment, a 3' external spacer, and a second polyA sequence.

[0296] In some embodiments, at least one of the 3' or 5' internal or external spacers has a length of about 8 to about 60 nucleotides. In some embodiments, the 3' and 5' external duplex forming regions each has a length of about 10-50 nucleotides. In some embodiments, the 3' and 5' internal duplex forming regions each has a length of about 6-30 nucleotides.

[0297] circRNA can be administered as naked circRNA, or as a pharmaceutical composition comprising a transfection agent. In non-limiting examples, the transfection agent is polyethylenimine (PEI) or an LNP. Other examples of lipidosomes that can be used to administer the circRNA composition for administration include protamines, cationic nanoemulsions, modified dendrimer nanoparticles, protamine liposomes, cationic polymers, cationic polymer liposomes, polysaccharide particles, cationic lipid nanoparticles, cationic lipid-cholesterol nanoparticles, cationic lipid-cholesterol PEG nanoparticle, cationic lipid transfection reagents sold under the trademark LIPOFECTAMINE, nonliposomal transfection reagents sold under the trademark FUGENE, or any combination thereof can be used as the transfection agent. In some

embodiments, the liposome formulation may be influenced by, but not limited to, the selection of the cationic lipid component, the degree of cationic lipid saturation, the nature of the PEGylation, ratio of all components and biophysical parameters such as size. In some embodiments, the liposome formulation comprises a cationic lipid, a cholesterol and a PEGylated lipid. For example, a liposome formulation may comprise a cationic lipid, dipalmitoylphosphatidylcholine, cholesterol, and PEG-c-DMA. See, for example, Semple et al. *Nature Biotech.* 2010 28: 172-176, herein incorporated by reference in its entirety.

[0298] In some embodiments, the circRNA is not formulated with a transfection reagent. In some embodiments, the circRNA is delivered as naked RNA. In some embodiments, the circRNA is delivered by gene gun or by electroporation.

[0299] In some embodiments, the nucleic acid construct is a DNA construct (e.g., viral or bacteria vector, such as AAV vector or lentiviral vector). In some embodiments, the DNA construct comprises a promoter. In some embodiments, the promoter is TRE3G promoter, e.g., comprising the nucleic acid sequence of SEQ ID NO: 63. In some embodiments, the promoter is CMV promoter, e.g., comprising the nucleic acid sequence of SEQ ID NO: 76. In some embodiments, the DNA construct comprises a nucleic acid encoding a poly(A) tail. In some embodiments, the poly(A) tail is an SV40 poly(A) tail, e.g., encoded by a nucleic acid sequence of SEQ ID NO: 64. In some embodiments, the poly(A) tail is a human growth hormone (hGH) poly(A) tail, e.g., encoded by a nucleic acid sequence of SEQ ID NO: 65. In some embodiments, the DNA construct comprises a Kozak sequence, e.g., GCCGCCACC (SEQ ID NO: 113).

[0300] In some embodiments, there is provided a nucleic acid construct (e.g., RNA construct, such as circRNA, or DNA construct, such as viral or bacterial vector) encoding a plurality of reprogramming factors (e.g., OCT4, KLF4, SOX2, and/or c-MYC). In some embodiments, at least one of the plurality of reprogramming factors is an engineered reprogramming factor that is deficient in LLPS (e.g., any of the engineered reprogramming factors described herein). The nucleic acids encoding the plurality of reprogramming factors (wild-type or engineered) can be arranged in any sequences on the nucleic acid construct, either under the same promoter or different promoters.

[0301] In some embodiments, there is provided a nucleic acid construct (RNA (e.g., circRNA) or DNA construct) comprising from 5' to 3': a first nucleic acid encoding an engineered OCT4

that is deficient in LLPS (e.g., any of SEQ ID NOs: 4-10 and 44-46, such as SEQ ID NO: 4) – a first linking sequence (e.g., encoding P2A) – a second nucleic acid encoding SOX2 (e.g., SEQ ID NO: 24) – a second linking sequence (e.g., encoding T2A) – a third nucleic acid encoding KLF4 (e.g., SEQ ID NO: 22). In some embodiments, the nucleic acid construct encodes a protein sequence of SEQ ID NO: 93. In some embodiments, the nucleic acid construct further comprises an IRES (e.g., CVB3 IRES) at 5' of the first nucleic acid. In some embodiments, the nucleic acid construct is a circRNA. In some embodiments, the nucleic acid construct is a DNA vector (e.g., lentival vector, AAV vector, or plasmid). In some embodiments, the nucleic acid construct is a DNA vector or a linear RNA (e.g., transcribed by the DNA vector) (such as for generating a circRNA), comprising from 5' to 3': an optional 5' homology arm, a 3' group I intron fragment, an optional 5' spacer, an IRES, an expression sequence described herein (encoding O_{mut}SK interspaced by 2A peptide), an optional 3' spacer, a 5' group I intron fragment, and an optional 3' homology arm. In some embodiments, the nucleic acid construct comprises a nucleic acid sequence of any of SEQ ID NOs: 39, 57, and 94.

[0302] In some embodiments, there is provided a nucleic acid construct (RNA (e.g., circRNA) or DNA construct) comprising from 5' to 3': a first nucleic acid encoding an engineered OCT4 that is deficient in LLPS (e.g., any of SEQ ID NOs: 4-10 and 44-46, such as SEQ ID NO: 4) – a first linking sequence (e.g., encoding P2A) – a second nucleic acid encoding KLF4 (e.g., SEQ ID NO: 22) – a second linking sequence (e.g., encoding T2A) – a third nucleic acid encoding an engineered c-MYC that is deficient in LLPS (e.g., any of SEQ ID NOs: 14-20, 47, and 48, such as SEQ ID NO: 14). In some embodiments, the nucleic acid construct encodes a protein sequence of SEQ ID NO: 97. In some embodiments, the nucleic acid construct further comprises an IRES (e.g., CVB3 IRES) at 5' of the first nucleic acid. In some embodiments, the nucleic acid construct is a circRNA. In some embodiments, the nucleic acid construct is a DNA vector (e.g., lentival vector, AAV vector, or plasmid). In some embodiments, the nucleic acid construct is a DNA vector or a linear RNA (e.g., transcribed by the DNA vector) (such as for generating a circRNA), comprising from 5' to 3': an optional 5' homology arm, a 3' group I intron fragment, an optional 5' spacer, an IRES, an expression sequence described herein (encoding O_{mut}KM_{mut} interspaced by 2A peptide), an optional 3' spacer, a 5' group I intron fragment, and an optional 3' homology arm. In some embodiments, the nucleic acid construct comprises a nucleic acid sequence of any of SEQ ID NOs: 30, 35, 59, and 98.

[0303] In some embodiments, there is provided a nucleic acid construct (RNA (e.g., circRNA) or DNA construct) comprising from 5' to 3': a first nucleic acid encoding an engineered OCT4 that is deficient in LLPS (e.g., any of SEQ ID NOs: 4-10 and 44-46, such as SEQ ID NO: 4) – a first linking sequence (e.g., encoding P2A) – a second nucleic acid encoding KLF4 (e.g., SEQ ID NO: 22) – a second linking sequence (e.g., encoding T2A) – a third nucleic acid encoding an engineered c-MYC that is deficient in LLPS (e.g., any of SEQ ID NOs: 14-20, 47, and 48, such as SEQ ID NO: 14) – a third linking sequence (e.g., encoding E2A) – a fourth nucleic acid encoding SOX2 (e.g., SEQ ID NO: 24). In some embodiments, the nucleic acid construct encodes a protein sequence of SEQ ID NO: 101. In some embodiments, the nucleic acid construct further comprises an IRES (e.g., CVB3 IRES) at 5' of the first nucleic acid. In some embodiments, the nucleic acid construct is a circRNA. In some embodiments, the nucleic acid construct is a DNA vector (e.g., lentival vector, AAV vector, or plasmid). In some embodiments, the nucleic acid construct is a DNA vector or a linear RNA (e.g., transcribed by the DNA vector) (such as for generating a circRNA), comprising from 5' to 3': an optional 5' homology arm, a 3' group I intron fragment, an optional 5' spacer, an IRES, an expression sequence described herein (encoding O_{mut}KM_{mut}S interspaced by 2A peptide), an optional 3' spacer, a 5' group I intron fragment, and an optional 3' homology arm. In some embodiments, the nucleic acid construct comprises a nucleic acid sequence of any of SEQ ID NOs: 28, 37, 61, and 102.

[0304] In some embodiments, there is provided a nucleic acid construct (RNA (e.g., circRNA) or DNA construct) comprising from 5' to 3': a first nucleic acid encoding an engineered OCT4 that is deficient in LLPS (e.g., any of SEQ ID NOs: 4-10 and 44-46, such as SEQ ID NO: 4) – a first linking sequence (e.g., encoding P2A) – a second nucleic acid encoding KLF4 (e.g., SEQ ID NO: 22) – a second linking sequence (e.g., encoding T2A) – a third nucleic acid encoding SOX2 (e.g., SEQ ID NO: 24). In some embodiments, the nucleic acid construct further comprises an IRES (e.g., CVB3 IRES) at 5' of the first nucleic acid. In some embodiments, the nucleic acid construct is a circRNA. In some embodiments, the nucleic acid construct is a DNA vector (e.g., lentival vector, AAV vector, or plasmid). In some embodiments, the nucleic acid construct is a DNA vector or a linear RNA (e.g., transcribed by the DNA vector) (such as for generating a circRNA), comprising from 5' to 3': an optional 5' homology arm, a 3' group I intron fragment, an optional 5' spacer, an IRES, an expression sequence described herein (encoding O_{mut}KS interspaced by 2A peptide), an optional 3' spacer, a 5' group I intron fragment, and an optional 3'

homology arm. In some embodiments, the nucleic acid construct comprises a nucleic acid sequence of SEQ ID NO: 32 or 39.

[0305] In some embodiments, there is provided a nucleic acid construct (RNA (e.g., circRNA) or DNA construct) comprising from 5' to 3': a first nucleic acid encoding an engineered MsOCT4 that is deficient in LLPS (e.g., SEQ ID NO: 68 or 70) – a first linking sequence (e.g., encoding P2A) – a second nucleic acid encoding MsKLF4 (e.g., SEQ ID NO: 72) – a second linking sequence (e.g., encoding T2A) – a third nucleic acid encoding MsSOX2 (e.g., SEQ ID NO: 74). In some embodiments, the nucleic acid construct encodes a protein sequence of SEQ ID NO: 105 or 107. In some embodiments, the nucleic acid construct further comprises an IRES (e.g., CVB3 IRES) at 5' of the first nucleic acid. In some embodiments, the nucleic acid construct is a circRNA. In some embodiments, the nucleic acid construct is a DNA vector (e.g., lentival vector, AAV vector, or plasmid). In some embodiments, the nucleic acid construct is a DNA vector or a linear RNA (e.g., transcribed by the DNA vector) (such as for generating a circRNA), comprising from 5' to 3': an optional 5' homology arm, a 3' group I intron fragment, an optional 5' spacer, an IRES, an expression sequence described herein (encoding MsO_{mut}KS interspaced by 2A peptide), an optional 3' spacer, a 5' group I intron fragment, and an optional 3' homology arm. In some embodiments, the nucleic acid construct comprises a nucleic acid sequence of any of SEQ ID NOs: 78, 79, 81, 82, 106, and 108.

[0306] In some embodiments, there is provided a nucleic acid construct (RNA (e.g., circRNA) or DNA construct) comprising from 5' to 3': a first nucleic acid encoding OCT4 (e.g., SEQ ID NO: 2) – a first linking sequence (e.g., encoding P2A) – a second nucleic acid encoding SOX2 (e.g., SEQ ID NO: 24) – a second linking sequence (e.g., encoding T2A) – a third nucleic acid encoding KLF4 (e.g., SEQ ID NO: 22). In some embodiments, the nucleic acid construct further comprises an IRES (e.g., CVB3 IRES) at 5' of the first nucleic acid. In some embodiments, the nucleic acid construct encodes a protein sequence of SEQ ID NO: 91. In some embodiments, the nucleic acid construct is a circRNA. In some embodiments, the nucleic acid construct is a DNA vector (e.g., lentival vector, AAV vector, or plasmid). In some embodiments, the nucleic acid construct is a DNA vector or a linear RNA (e.g., transcribed by the DNA vector) (such as for generating a circRNA), comprising from 5' to 3': an optional 5' homology arm, a 3' group I intron fragment, an optional 5' spacer, an IRES, an expression sequence described herein (encoding OSK interspaced by 2A peptide), an optional 3' spacer, a 5' group I intron fragment, and an optional 3'

homology arm. In some embodiments, the nucleic acid construct comprises a nucleic acid sequence of SEQ ID NO: 58 or 92.

[0307] In some embodiments, there is provided a nucleic acid construct (RNA (e.g., circRNA) or DNA construct) comprising from 5' to 3': a first nucleic acid encoding OCT4 (e.g., SEQ ID NO: 2) – a first linking sequence (e.g., encoding P2A) – a second nucleic acid encoding KLF4 (e.g., SEQ ID NO: 22) – a second linking sequence (e.g., encoding T2A) – a third nucleic acid encoding c-MYC (e.g., SEQ ID NO: 12 or 85). In some embodiments, the nucleic acid construct encodes a protein sequence of SEQ ID NO: 95. In some embodiments, the nucleic acid construct further comprises an IRES (e.g., CVB3 IRES) at 5' of the first nucleic acid. In some embodiments, the nucleic acid construct is a circRNA. In some embodiments, the nucleic acid construct is a DNA vector (e.g., lentival vector, AAV vector, or plasmid). In some embodiments, the nucleic acid construct is a DNA vector or a linear RNA (e.g., transcribed by the DNA vector) (such as for generating a circRNA), comprising from 5' to 3': an optional 5' homology arm, a 3' group I intron fragment, an optional 5' spacer, an IRES, an expression sequence described herein (encoding OKM interspaced by 2A peptide), an optional 3' spacer, a 5' group I intron fragment, and an optional 3' homology arm. In some embodiments, the nucleic acid construct comprises a nucleic acid sequence of any of SEQ ID NOs: 31, 34, 60, and 96.

[0308] In some embodiments, there is provided a nucleic acid construct (RNA (e.g., circRNA) or DNA construct) comprising from 5' to 3': a first nucleic acid encoding OCT4 (e.g., SEQ ID NO: 2) – a first linking sequence (e.g., encoding P2A) – a second nucleic acid encoding KLF4 (e.g., SEQ ID NO: 22) – a second linking sequence (e.g., encoding T2A) – a third nucleic acid encoding c-MYC (e.g., SEQ ID NO: 12 or 85) – a third linking sequence (e.g., encoding E2A) – a fourth nucleic acid encoding SOX2 (e.g., SEQ ID NO: 24). In some embodiments, the nucleic acid construct encodes a protein sequence of SEQ ID NO: 99. In some embodiments, the nucleic acid construct further comprises an IRES (e.g., CVB3 IRES) at 5' of the first nucleic acid. In some embodiments, the nucleic acid construct is a circRNA. In some embodiments, the nucleic acid construct is a DNA vector (e.g., lentival vector, AAV vector, or plasmid). In some embodiments, the nucleic acid construct is a DNA vector or a linear RNA (e.g., transcribed by the DNA vector) (such as for generating a circRNA), comprising from 5' to 3': an optional 5' homology arm, a 3' group I intron fragment, an optional 5' spacer, an IRES, an expression sequence described herein (encoding OKMS interspaced by 2A peptide), an optional 3' spacer, a 5' group I intron fragment,

and an optional 3' homology arm. In some embodiments, the nucleic acid construct comprises a nucleic acid sequence of any of SEQ ID NOs: 29, 36, 62, and 100.

[0309] In some embodiments, there is provided a nucleic acid construct (RNA (e.g., circRNA) or DNA construct) comprising from 5' to 3': a first nucleic acid encoding OCT4 (e.g., SEQ ID NO: 2) – a first linking sequence (e.g., encoding P2A) – a second nucleic acid encoding KLF4 (e.g., SEQ ID NO: 22) – a second linking sequence (e.g., encoding T2A) – a third nucleic acid encoding SOX2 (e.g., SEQ ID NO: 24). In some embodiments, the nucleic acid construct further comprises an IRES (e.g., CVB3 IRES) at 5' of the first nucleic acid. In some embodiments, the nucleic acid construct is a circRNA. In some embodiments, the nucleic acid construct is a DNA vector (e.g., lentival vector, AAV vector, or plasmid). In some embodiments, the nucleic acid construct is a DNA vector or a linear RNA (e.g., transcribed by the DNA vector) (such as for generating a circRNA), comprising from 5' to 3': an optional 5' homology arm, a 3' group I intron fragment, an optional 5' spacer, an IRES, an expression sequence described herein (encoding OKS interspaced by 2A peptide), an optional 3' spacer, a 5' group I intron fragment, and an optional 3' homology arm. In some embodiments, the nucleic acid construct comprises a nucleic acid sequence of SEQ ID NO: 33 or 38.

[0310] In some embodiments, there is provided a nucleic acid construct (RNA (e.g., circRNA) or DNA construct) comprising from 5' to 3': a first nucleic acid encoding OCT4 (e.g., SEQ ID NO: 2) – a linking sequence (e.g., encoding P2A) – a second nucleic acid encoding SOX2 (e.g., SEQ ID NO: 24). In some embodiments, the nucleic acid construct encodes a protein sequence of SEQ ID NO: 83. In some embodiments, the nucleic acid construct further comprises an IRES (e.g., CVB3 IRES) at 5' of the first nucleic acid. In some embodiments, the nucleic acid construct is a circRNA. In some embodiments, the nucleic acid construct is a DNA vector (e.g., lentival vector, AAV vector, or plasmid). In some embodiments, the nucleic acid construct is a DNA vector or a linear RNA (e.g., transcribed by the DNA vector) (such as for generating a circRNA), comprising from 5' to 3': an optional 5' homology arm, a 3' group I intron fragment, an optional 5' spacer, an IRES, an expression sequence described herein (encoding OS interspaced by 2A peptide), an optional 3' spacer, a 5' group I intron fragment, and an optional 3' homology arm. In some embodiments, the nucleic acid construct comprises a nucleic acid sequence of SEQ ID NO: 56 or 84.

[0311] In some embodiments, there is provided a nucleic acid construct (RNA (e.g., circRNA) or DNA construct) comprising from 5' to 3': a first nucleic acid encoding LIN28 – a linking sequence (e.g., encoding P2A) – a second nucleic acid encoding NANOG. In some embodiments, the nucleic acid construct encodes a protein sequence of SEQ ID NO: 89. In some embodiments, the nucleic acid construct further comprises an IRES (e.g., CVB3 IRES) at 5' of the first nucleic acid. In some embodiments, the nucleic acid construct is a circRNA. In some embodiments, the nucleic acid construct is a DNA vector (e.g., lentival vector, AAV vector, or plasmid). In some embodiments, the nucleic acid construct is a DNA vector or a linear RNA (e.g., transcribed by the DNA vector) (such as for generating a circRNA), comprising from 5' to 3': an optional 5' homology arm, a 3' group I intron fragment, an optional 5' spacer, an IRES, an expression sequence described herein (encoding LN interspaced by 2A peptide), an optional 3' spacer, a 5' group I intron fragment, and an optional 3' homology arm. In some embodiments, the nucleic acid construct comprises a nucleic acid sequence of SEQ ID NO: 90.

[0312] In some embodiments, there is provided a nucleic acid construct (RNA (e.g., circRNA) or DNA construct) comprising from 5' to 3': a first nucleic acid encoding MsOCT4 (e.g., SEQ ID NO: 66) – a first linking sequence (e.g., encoding P2A) – a second nucleic acid encoding MsKLF4 (e.g., SEQ ID NO: 72) – a second linking sequence (e.g., encoding T2A) – a third nucleic acid encoding MsSOX2 (e.g., SEQ ID NO: 74). In some embodiments, the nucleic acid construct encodes a protein sequence of SEQ ID NO: 103. In some embodiments, the nucleic acid construct further comprises an IRES (e.g., CVB3 IRES) at 5' of the first nucleic acid. In some embodiments, the nucleic acid construct is a circRNA. In some embodiments, the nucleic acid construct is a DNA vector (e.g., lentival vector, AAV vector, or plasmid). In some embodiments, the nucleic acid construct is a DNA vector or a linear RNA (e.g., transcribed by the DNA vector) (such as for generating a circRNA), comprising from 5' to 3': an optional 5' homology arm, a 3' group I intron fragment, an optional 5' spacer, an IRES, an expression sequence described herein (encoding MsOKS interspaced by 2A peptide), an optional 3' spacer, a 5' group I intron fragment, and an optional 3' homology arm. In some embodiments, the nucleic acid construct comprises a nucleic acid sequence of any of SEQ ID NOs: 77, 80, and 104.

[0313] Exosomes can be derived from any applicable sources, as long as they can deliver the nucleic acid(s) to the eye, e.g., retinal cells. Exosomes can be released from many cell sources, such as lymphocytes, dendritic cells, epithelial cells, endothelial cells, mast cells or neurons. They

can also be found in most body fluids inclusive of blood, saliva, urine, breast milk, amniotic fluids, hydro thoracic fluids, ascitic fluid and in the culture medium of almost all cell types. In some embodiments, the exosome is derived from HEK293T cells or mesenchymal stem cells (MSCs).

[0314] LNPs can contain native or synthetic lipids, including conjugated lipids or polymers (e.g., pegylated lipids). LNPs can contain any one or more of neutral, zwitterionic lipids, ionizable lipids, cationic lipids, and anionic lipids. The LNPs can comprise one or more of: ionizable lipid (e.g., ALC-0315, SM-102, DLin-DMA, D-Lin-MC3-DMA), phospholipid (either saturated or unsaturated, e.g., DSPC, DPPC, DOPE, DOPC), cholesterol, and PEGylated lipid (e.g., ALC-0159, PEG-DMG). In some embodiments, LNPs contain natural or synthetic mono- or diacyl-form phosphatidylcholine (PC), phosphatidylglycerol (PG), phosphatidylserine (PS), phosphatidylethanolamine (PE), and/or phosphatidic acid (PA). LNPs can be micelles or reverse micelles (anti-micelles). LNPs can be monolamellar liposomes or multilayer liposomes.

[0315] The present application also includes variants to any of the nucleic acid sequences described herein. For example, the variants include nucleotide sequences that hybridize to the nucleic acid sequences encoding any of the one or more reprogramming factors described herein (e.g., wild-type and/or engineered) under at least moderately stringent hybridization conditions.

Methods of making LLPS-deficient engineered reprogramming factors

[0316] Also provided are methods of making any of the LLPS-deficient engineered reprogramming factors described herein. LLPS-deficient engineered reprogramming factors described herein can be made by introducing a nucleic acid into a host cell and allowing expression of the protein. In some embodiments, the preparation method comprises purifying the protein from the host cell extract or supernatant.

[0317] For example, DNA sequence encoding the LLPS-deficient engineered reprogramming factors can be synthesized. After obtaining such sequence, it is cloned into a suitable expression vector, then transfected into a suitable host cell. The transfected host cells are cultured (e.g., under a suitable condition to allow expression of the protein), and the supernatant is harvested and purified to obtain the protein. Purification can be followed.

[0318] Also contemplated here are isolated host cells (i) comprising any of the nucleic acids (DNA or RNA) or nucleic acid constructs (e.g., circRNA or plasmid) described herein encoding

any of the one or more reprogramming factors (e.g., wild-type and/or engineered), or (ii) expressing any of the one or more reprogramming factors described herein (e.g., wild-type and/or engineered). In some embodiments, the host cell is HEK 293T cell.

[0319] Suitable host cells can include, without limitation, prokaryotic cells, fungal cells, yeast cells, or higher eukaryotic cells such as mammalian cells. Higher eukaryotic cells, in particular, those derived from multicellular organisms can be used for expression of glycosylated polypeptides. Suitable higher eukaryotic cells include, without limitation, invertebrate cells and insect cells, and vertebrate cells. In some embodiments, the host cell is *E. coli*. In some embodiments, the host cell is a Chinese hamster ovary (CHO) cell or an HEK293 cell.

[0320] The vector can be introduced to the host cell using any suitable methods known in the art, including, but not limited to, DEAE-dextran mediated delivery, calcium phosphate precipitate method, cationic lipids mediated delivery, liposome mediated transfection, electroporation, microprojectile bombardment, receptor-mediated gene delivery, delivery mediated by polylysine, histone, chitosan, and peptides. In some embodiments, the host cells comprise two or more vectors each encoding a reprogramming factor described herein (e.g., wild-type and/or engineered). In some embodiments, the host cells comprise a single vector encoding two or more reprogramming factors described herein (e.g., wild-type and/or engineered).

[0321] In some embodiments, the present application provides methods of making an engineered reprogramming factor (e.g., any of the LLPS-deficient engineered reprogramming factors described herein), comprising i) culturing an isolated host cell comprising any of the isolated nucleic acids or vectors described herein, or any of the isolated host cells described herein, under a condition suitable for the expression of the engineered reprogramming factor, and ii) obtaining the expressed engineered reprogramming factor from said host cell (e.g., from the cell culture). Suitable conditions for expression of polynucleotides may include, without limitation, suitable medium, suitable density of host cells in the culture medium, presence of necessary nutrients, presence of supplemental factors, suitable temperatures and humidity, and absence of microorganism contaminants. In some embodiments, the method of making further comprises purifying the obtained engineered reprogramming factor described herein.

[0322] The expressed reprogramming factor (e.g., engineered reprogramming factor described herein) can be collected using any suitable methods. The polypeptide can be expressed

intracellularly, in the periplasmic space or be secreted outside of the cell into the medium. If the polypeptide is secreted into the medium, the supernatant of the cell culture may be collected and concentrated using a commercially available protein concentration filter, for example, an Amincon or Millipore Pellicon ultrafiltration unit. A protease inhibitor and/or an antibiotic may be included in the collection and concentration steps to inhibit protein degradation and/or growth of contaminated microorganisms.

[0323] The expressed polypeptide(s) can be further purified by a suitable method, such as without limitation, affinity chromatography, hydroxylapatite chromatography, size exclusion chromatography, gel electrophoresis, dialysis, ion exchange fractionation on an ion-exchange column, ethanol precipitation, reverse phase HPLC, chromatography on silica, chromatography on heparin sepharose, chromatography on an anion or cation exchange resin (such as a polyaspartic acid column), chromatofocusing, SDS-PAGE, and ammonium sulfate precipitation.

Pharmaceutical compositions, kits, and articles of manufacture

[0324] Also provided are compositions (e.g., pharmaceutical compositions) comprising i) any of the one or more reprogramming factors described herein (e.g., LLPS-deficient engineered reprogramming factor(s), or a plurality of reprogramming factors comprising at least one LLPS-deficient engineered reprogramming factor), and ii) an optional pharmaceutically acceptable carrier. In some embodiments, the one or more reprogramming factors described herein are encapsulated within an exosome, a liposome, or an LNP. In some embodiments, the pharmaceutical composition is for topical eye delivery, such as in the form of eye drops, ophthalmic ointment, or ophthalmic gel, or is coated onto or loaded into an ocular implant (e.g., contact lens).

[0325] Also provided are compositions (e.g., pharmaceutical compositions) comprising i) any of the nucleic acid(s) (DNA or RNA), nucleic acid construct(s) (e.g., circRNA, or DNA vector), or virus(es) (e.g., LV or AAV) described herein, and ii) an optional pharmaceutically acceptable carrier. Also provided are compositions (e.g., pharmaceutical compositions) comprising i) nucleic acid(s), nucleic acid construct(s), or virus(es) encoding any of the one or more reprogramming factors described herein (e.g., LLPS-deficient engineered reprogramming factor(s), or a plurality of reprogramming factors comprising at least one LLPS-deficient engineered reprogramming factor), and ii) an optional pharmaceutically acceptable carrier. In some embodiments, the

pharmaceutical composition comprising the nucleic acid, nucleic acid construct, or virus is for use as a gene therapy or as part of a gene therapy. In some embodiments, the nucleic acid or nucleic acid construct (e.g., circRNA, or linear mRNA) is encapsulated within an exosome, a liposome, or an LNP. In some embodiments, the pharmaceutical composition comprising an exosome, a liposome, or an LNP encapsulating a nucleic acid or nucleic acid construct (e.g., circRNA, or linear mRNA) is for topical eye delivery, such as in the form of eye drops, ophthalmic ointment, or ophthalmic gel, or is coated onto or loaded into an ocular implant (e.g., contact lens).

[0326] Suitable compositions (e.g., pharmaceutical composition) of the protein (or nucleic acid/vector/virus encoding the protein) can be obtained by mixing the protein (or nucleic acid/vector/virus encoding the protein) having the desired degree of purity with optional pharmaceutically acceptable carriers, excipients or stabilizers (*Remington's Pharmaceutical Sciences* 16th edition, Osol, A. Ed. (1980)). In some embodiments, the carrier comprises physiologic saline or other buffer system.

[0327] Pharmaceutically acceptable carriers, excipients, or stabilizers are usually nontoxic to the cell or mammal being exposed thereto at the dosages and concentrations employed. Often the physiologically acceptable carrier is an aqueous pH buffered solution. Acceptable carriers, excipients, or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride, benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrans; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose, or sorbitol; salt-forming counter-ions such as sodium; metal complexes (e.g. Zn-protein complexes); and/or non-ionic surfactants such as TWEEN™, PLURONICS™, or polyethylene glycol (PEG).

[0328] The compositions can contain auxiliary substances such as wetting, dispersing, or emulsifying agents (e.g., methylcellulose), pH buffering agents, gelling or viscosity enhancing additives, preservatives, and/or colors, depending upon the route of administration and the preparation desired. Buffers may be used to control the pH in a range which optimizes the therapeutic effectiveness, especially if stability is pH dependent. Suitable buffers comprise but are not limited to histidine-buffers, citrate-buffers, succinate-buffers, acetate-buffers and phosphate-buffers.

[0329] In some embodiments, the pharmaceutical composition is formulated to have a pH in the range of about 4.5 to about 9.0, including for example pH ranges of any one of about 5.0 to about 8.0, about 6.5 to about 7.5, about 6.5 to about 7.0, or about 7.0 to about 7.5. The buffer can include one or more buffering components such as a citrate, phosphate, borate, boric acid, succinate, acetate or a pharmaceutically acceptable salt thereof (e.g., sodium phosphate, sodium acetate, sodium citrate, sodium borate, sodium succinate, or sodium acetate), to changes in pH. The buffering component can be used especially under storage conditions, e.g., when the formulation will be subjected to prolonged storage.

[0330] The ophthalmic formulation can also include one or more of the following components as described herein: surfactants, tonicity agents, buffers, preservatives, co-solvents and viscosity building agents. Tonicity agents can be used to adjust the tonicity of the composition, e.g., to that of natural tears. Tonicity agents, particularly sugars, may also function as thermal stabilizers. In embodiments, potassium chloride, sodium chloride, magnesium chloride, calcium chloride, dextrose and/or mannitol may be added to achieve an appropriate tonicity, e.g., physiological tonicity. Tonicity agents can be added in an amount sufficient to provide an appropriate osmolality. In embodiments, a tonicity agent is added to provide an osmolality of about 150 mOsm per kg to 450 mOsm per kg or 250 mOsm per kg to 350 mOsm per kg. In embodiments, a tonicity agent is added to provide an osmolality that is isotonic in the eye.

[0331] The pharmaceutical compositions to be used for *in vivo* administration are generally formulated as sterile, substantially isotonic, and in full compliance with all Good Manufacturing Practice (GMP) regulations of the U.S. Food and Drug Administration. Sterility is readily accomplished by filtration through sterile filtration membranes. In some embodiments, the composition is free of any pathogens. For injection, the pharmaceutical composition can be in the

form of liquid solutions, for example in physiologically compatible buffers such as Hank's solution or Ringer's solution. In addition, the pharmaceutical composition can be in a solid form and re-dissolved or suspended immediately prior to use. Lyophilized compositions are also included.

[0332] In some embodiment, the pharmaceutical composition is formulated in accordance with routine procedures as a pharmaceutical composition adapted for ocular administration (e.g., topical, intravitreal, subretinal). Typically, compositions for injection are solutions in sterile isotonic aqueous buffer. Where necessary, the composition may also include a solubilizing agent and a local anesthetic such as lidocaine (e.g., eye gel) to ease pain at the site of the injection. Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampule or sachette indicating the quantity of active agent. Where the composition is administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

[0333] The carrier and/or excipient can be in a wide variety of forms. Non-limiting examples of suitable carriers and/or excipients include simple solutions (water or oil based), emulsions, dispersions, or multi-phase systems. For example, emulsion carriers and/or excipients can include, but are not limited to, oil-in-water, silicone-in-water, water-in-oil, water-in-silicone, water-in-oil-in-water, oil-in-water-in-oil, and oil-in-water-in-silicone emulsions.

[0334] Depending upon the desired product form, preferred carriers and/or excipients can contain an emulsion such as oil-in-water emulsions (e.g., silicone-in-water) and water-in-oil emulsions, (e.g., water-in-silicone emulsions). Emulsions can contain an aqueous phase and a lipid or oil. Lipids and oils may be derived from animals, plants, or petroleum and may be natural or synthetic (e.g., man-made). Emulsions can further contain an emulsifying agent (emulsifier), based on the weight of the composition. Emulsifier agents may be nonionic, anionic, or cationic. Suitable emulsifiers are disclosed for example in McCutcheon's Detergents and Emulsifiers, North American Edition, pages 317-324 (1986). Suitable emulsions may have a wide range of viscosities, depending on the desired product form. The compositions (e.g., solutions, eye ointments) may contain an acceptable emollient.

[0335] The compositions can be an emulsion, liquid, ointment, eye implant device or any other pharmaceutically acceptable formulation or device. Generally, an acceptable formulation for

pharmaceutical use would include any acceptable carrier, excipient, and/or substance suitable for ophthalmic use. The compositions may also contain one or more other biologically active agents including, but not limited to, growth factors, anti-inflammatory agents, and/or other peptides. Any of the compositions of the present invention may also be used in combination with other pharmaceutical products, and/or medical devices.

[0336] Ophthalmic formulations featured in the invention can be delivered for topical administration, e.g., for administration as a liquid drop, an ointment, or a gel, or for implantation (e.g., contact lens). Gels and ointments can also be administered using a dropper. Typically, the ophthalmic formulation is applied directly to the eye including onto the cornea, the eyelid or instillation into the space (cul-de-sac) between the eyeball and the eyelids. The ophthalmic formulation can be designed to mix readily with the lacrimal fluids and spread over the surfaces of the cornea and conjunctiva. With the usual technique of administration, the major portion of the active agent is typically deposited in the lower fornix. Capillarity, diffusional forces, and the blinking reflex drive incorporation of the active agent in the precorneal film from which it penetrates into and through the cornea.

[0337] Another strategy for sustained ocular delivery is the use of gelifying agents. These materials can be delivered in a liquid form, as an eye drop or intraocular injection. After instillation the polymer undergoes a phase change and forms a semi-solid or solid matrix that releases the active agent over prolonged period. The phase transition can be induced by changes in the temperature, ion concentration, or pH.

[0338] For topical ocular use, the gel forming solutions, such as Timoptic®-XE1 (Merck and Co. Inc., USA), which contains Gelrite® (purified anionic heteropolysaccharide from gellan gum); Pilogel®1 (Alcon, Inc., Switzerland) eye drops contain poly(acrylic acid); and Azasite®1 (Insite Vision, USA) have been tested clinically. These materials enhance the active agent retention relative to the conventional eye drops and lead to increased drug absorption into the eye and reduced dosing frequency. See Amo and Urtti, Drug Discovery Today, 13:135-143 (2008).

[0339] A formulation featured in the invention can be delivered by injection, e.g., intravitreal, subretinal, periocular, or subconjunctival injection. The formulation can be injected underneath the conjunctiva facilitating passage through the sclera and into the eye by simple diffusion. The formulation can also be injected underneath the conjunctiva and the underlying Tenon's capsule in

the more posterior portion of the eye to deliver the active agent to the ciliary body, choroid, and retina. The formulation may also be administered by retrobulbar injection.

[0340] One of the primary vehicles currently being developed for the delivery of ocular pharmacological agents is the poly(lactide-co-glycolide) (PLGA) microsphere for intraocular injection. The microspheres are composed of a polymer of lactic acid and glycolic acid, which are structured to form hollow spheres. These spheres can be approximately 15-30 μm in diameter and can be loaded with a variety of compounds varying in size from simple molecules to high molecular weight proteins such as antibodies. The biocompatibility of these microspheres is well established (see, Sintzel et al., (1996)), and microspheres have been used to deliver a wide variety of pharmacological agents in numerous biological systems. After injection, poly(lactide-co-glycolide) microspheres are hydrolyzed by the surrounding tissues, which cause the release of the contents of the microspheres (Zhu et al., (2000)). As will be appreciated, the in vivo half-life of a microsphere can be adjusted depending on the specific needs of the system.

[0341] In some embodiments, the pharmaceutical composition is suitable for administration to a mammal, such as a human. In some embodiments, the pharmaceutical composition is suitable for administration to a rodent (*e.g.*, mice, rats) or non-human primates (*e.g.*, Cynomolgus monkey). In some embodiments, the pharmaceutical composition is contained in a single-use vial, such as a single-use sealed vial. In some embodiments, the pharmaceutical composition is contained in a multi-use vial. In some embodiments, the pharmaceutical composition is contained in bulk in a container. In some embodiments, the pharmaceutical composition is cryopreserved.

[0342] In some embodiments, the composition (*e.g.*, pharmaceutical composition) comprises a virus described herein (*e.g.*, encoding LLPS-deficient engineered reprogramming factor(s), or a plurality of reprogramming factors comprising at least one LLPS-deficient engineered reprogramming factor) for gene therapy. In some embodiments, the composition further comprises an excipient. In some embodiments, the composition is delivered to the eye intraocularly (*e.g.*, intravitreally or subretinally).

[0343] Also provided are unit dosage forms of any of the reprogramming factors described herein (*e.g.*, LLPS-deficient engineered reprogramming factor(s), or a plurality of reprogramming factors comprising at least one LLPS-deficient engineered reprogramming factor). The term “unit dosage form” refers to a physically discrete unit suitable as unitary dosages for an individual (*e.g.*,

human), each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic or prophylactic effect, in association with a suitable pharmaceutical carrier, diluent, or excipient. These unit dosage forms can be stored in a suitable packaging in single or multiple unit dosages and may also be further sterilized and sealed. The formulation can be packaged for single or multi-dose use, e.g., in a bottle with an associated dropper or as a set of single-use droppers.

[0344] The present application further provides articles of manufacture comprising the compositions (such as pharmaceutical compositions) described herein in suitable packaging. Suitable packaging for compositions (such as pharmaceutical compositions) described herein are known in the art, and include, for example, vials (such as sealed vials), vessels, ampules, bottles, jars, flexible packaging (e.g., sealed Mylar or plastic bags), and the like. These articles of manufacture may further be sterilized and/or sealed.

[0345] The present application also provides kits comprising compositions (such as pharmaceutical compositions) described herein and may further comprise instruction(s) on methods of using the composition, such as uses described herein. The kits described herein may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, syringes, eye droppers, and/or applicators and package inserts with instructions for performing any methods described herein.

Administration methods

[0346] In some embodiments, the treatment or prevention methods described herein comprise introducing to the eye (e.g., to a population of retinal cells) any of the one or more reprogramming factors described herein (e.g., LLPS-deficient engineered reprogramming factor(s), or a plurality of reprogramming factors comprising at least one LLPS-deficient engineered reprogramming factor), or nucleic acid(s), nucleic acid construct(s), or virus(es) encoding thereof, or compositions (e.g., pharmaceutical compositions) comprising thereof.

[0347] In view of the fact that the volume of the eye in a given subject is known (for example, typical human eye contains 4 to 6 mL of fluid (humor), it is possible to calculate the dosage of the LLPS-deficient engineered reprogramming factors to be administered to give therapeutically effective concentrations.

[0348] The LLPS-deficient engineered reprogramming factors may be administered locally to the eye, for example, by topical, intravitreal, intraocular, intraorbital, subconjunctival, subretinal or transscleral routes. It is contemplated that local modes of administration may reduce or eliminate the incidence of potential side effects (e.g., systemic toxicity) that may occur during systemic administration.

[0349] Administration may be provided as a periodic bolus (for example, intravitreally or intravenously) or as continuous infusion from an internal reservoir (for example, from an implant disposed at an intra- or extra-ocular location (see, U.S. Pat. Nos. 5,443,505 and 5,766,242)) or from an external reservoir (for example, from a contact lens slow release formulation system). The LLPS-deficient engineered reprogramming factors may be administered locally, for example, by continuous release from a sustained release drug delivery device immobilized to an inner wall of the eye or via targeted transscleral controlled release into the choroid (see, for example, PCT/US00/00207, PCT/US02/14279, Ambati et al., (2000) INVEST. OPHTHALMOL. VIS. SCI. 41:1181-1185, and Ambati et al., (2000) INVEST. OPHTHALMOL. VIS. SCI. 41:1186-1191). A variety of devices suitable for administering active agents locally to the inside of the eye are known in the art. See, for example, U.S. Pat. Nos. 6,251,090, 6,299,895, 6,416,777, 6,413,540, and 6,375,972, and PCT/US00/28187, the contents of each of which are incorporated herein by reference in their entireties.

[0350] The treatment or prevention methods described herein, or LLPS-deficient engineered reprogramming factors described herein (or nucleic acid/vector/virus encoding thereof, or pharmaceutical composition comprising thereof), can be used in combination with any other therapeutic and/or prophylactic agents, and/or eye surgical procedure. The administration of other therapeutic agent can be simultaneously or sequentially with the LLPS-deficient engineered reprogramming factors described herein (or nucleic acid/vector/virus encoding thereof, or pharmaceutical composition comprising thereof).

[0351] The engineered reprogramming factor(s) described herein, or nucleic acid/vector/virus encoding thereof, or pharmaceutical composition comprising thereof, can be administered once or several times. In some embodiments, the engineered reprogramming factor(s) described herein, or nucleic acid/vector/virus encoding thereof, or pharmaceutical composition comprising thereof is administered once a day, once every three days, once every five days, once a week, once every 2

weeks, once every 3 weeks, once a month, once every 3 months, once every 6 months, or longer. The doses and dosing schedule can be determined by one skilled in the art, e.g., until one or more of the desired therapeutic and/or prophylactic effects, such as those described herein, has been achieved.

[0352] A typical protocol for use of the formulation as eye drops to treat or to alleviate the symptoms of an eye disorder is to place at least one drop of the formulation in the affected eye at least once daily until the severity of the symptoms is reduced to an acceptable level. In particularly severe eye disorders, more frequent applications may be necessary. For example, the formulation may be administered, for example 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 times daily.

[0353] In certain embodiments, the engineered reprogramming factor(s) described herein may be administered locally to the eye (e.g., topically, intravitreally, or subretinally) of an individual (e.g., a human), following other treatments of the retina and/or optic nerve. The engineered reprogramming factor(s) may be administered on the day of diagnosis and/or the same day that the retina and/or optic nerve has gone through other treatments and/or in the post-operative period.

EXAMPLES

[0354] The examples below are intended to be purely exemplary of the invention and should therefore not be considered to limit the invention in any way. The following examples and detailed description are offered by way of illustration and not by way of limitation.

Example 1: Verification of phase separation of wild-type and mutated Oct4 in HEK 293T cells

Construction of GFP-OCT4 and GFP-OCT4mutant (GFP-OCT4') in pCDNA3.4

[0355] Nucleic acids encoding GFP-OCT4 (SEQ ID NO: 109) and GFP-OCT4' (SEQ ID NO: 110) were synthesized by Genewiz and each cloned into a pCDNA3.4 plasmid. OCT4' (SEQ ID NO: 4) contains D/E to A mutations in the IDRs relative to the wild-type human OCT4 sequence (SEQ ID NO: 2). The inserted GFP-OCT4 and GFP-OCT4' nucleic acid sequences were verified via Sanger sequencing.

HEK 293T cell transfection and image acquisition

[0356] Plasmid constructs encoding GFP-OCT4 or GFP-OCT4' were transfected into HEK 293T cells and subsequently imaged, in order to verify successful expression of the constructs,

according to the following steps. HEK293 T cells were seeded on an imaging chamber prior to transfection. Plasmids encoding GFP-OCT4 or GFP-OCT4' were transfected by Lipofectamine 3000 Transfection Reagent according to the instructions of the manufacturer, using 0.75 μ L lipo3000 and 0.5 μ g plasmid per well. After 48 hours, the cells were dipped in a DAPI-containing mounting medium. A subset of cells were treated with 1.5% 1,6-Hexanediol (can dissolve protein condensates) for 5 minutes prior to mounting. Distribution of exogenous GFP-OCT4 or GFP-OCT4' in the cellular nucleus was imaged using Nikon A1 confocal microscopy (**FIG. 1A**). GFP-OCT4 was highly condensed as phase-separated droplets in the cellular nuclei of HEK 293T cells, suggesting that OCT4 exhibits a phase separation property. In contrast, GFP-OCT4' was evenly distributed in the cellular nuclei of HEK 293T cells. Following treatment with 1,6-Hexanediol, HEK 293T cells expressing GFP-OCT4 lost OCT4 condensates, showing similar phenotypes as GFP-OCT4'.

[0357] The liquid-like nature of OCT4 condensates was characterised through fluorescence recovery after photobleaching (FRAP) on GFP-OCT4 or GFP-OCT4' in transfected HEK 293T cells. As shown in **FIG. 1B**, GFP-OCT4 proteins showed rapid recovery of GFP fluorescence, demonstrating the dynamic nature of wild-type OCT4 condensates. On the contrary, GFP-OCT4' showed no FRAP even after 345 seconds post-photobleaching. This data confirmed that OCT4' could not phase separate or form condensates in cells like OCT4.

Example 2: Single or dual gene circRNA construction using AnaPIE method and protein expression verification

Construction of circular RNA (circRNA) vectors encoding reprogramming factors

[0358] Nucleic acids encoding six reprogramming factors (OCT4, SOX2, MYC, KLF4, LIN28A, NANOG) were constructed into four circRNA vectors (Table 1 and **FIGs. 2A-2D**). Dual-gene tandem combinations were constructed such that nucleic acids encoding two reprogramming factors are separated by a linking sequence encoding self-cleaving P2A peptide.

Table 1. Circular RNA constructs containing single and dual genes

Construct #	Construct Name	Genetic structure	Size (nt)	nt SEQ ID NO:	aa SEQ ID NO:
1	AnaPIE-OS	IRES-OCT4-P2A-SOX2	2100	84	83
2	AnaPIE-M	IRES-MYC	1320	86	85
3	AnaPIE-K	IRES-KLF4	1413	88	87

4	AnaPIE-LN	IRES-LIN28A-P2A-NANOG	1611	90	89
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[0359] *Anabaena* permuted group I intron-exon (Ana PIE) circRNA circularization system was selected in the following manner: the ring-forming framework PIE (permuted) was formed by rearrangement of *Anabaena* group I intron as AnaPIE. The nucleic acid sequences encoding different reprogramming factors were recombined into the AnaPIE framework and constructed into the pBlueScript II SK(+) plasmid as shown in **FIGs. 2A-2D**. The 5' elements (contain 5' homology arm, AnaPIE 3' intron, 5' spacer) upstream of IRES comprise the sequence of SEQ ID NO: 49. The 3' elements (contain 3' spacer, AnaPIE 5' intron, 3' homology arm) downstream of the protein-coding sequence comprise the sequence of SEQ ID NO: 50.

[0360] The recombinant circRNA plasmids were synthesized by GenScript. Synthesized plasmids were linearized via digestion with EcoR I to obtain the pre-transcriptional template.

Preparation and verification of circular RNAs encoding reprogramming factors

[0361] *In vitro* transcription was performed with an *in vitro* transcription system as described in Table 2. The reaction volume for the *in vitro* transcription system was 20 μ L per reaction.

Table 2. *In vitro* transcription system (20 μ L system)

Component	Volume
ATP/CTP/UTP/GTP (100 mM)	2 μ L of each rNTP
10 $\times$ T7 buffer	2 μ L
T7 RNA polymerase (50 units/ μ L)	2 μ L
RNase inhibitor (40 units/ μ L)	1 μ L
Inorganic pyrophosphatase (0.1 unit/ μ L)	2 μ L
Linear template DNA	1 μ g
Enzyme-free water	0-5 μ L (to yield total reaction volume of 10 μ L)

[0362] The prepared 20 μ L transcription system was incubated at 37°C in a PCR machine for 3 hours before the linear DNA template was digested with DNaseI (5 units/ μ L) at a concentration of 5 units/ μ g DNA template at 37°C for 20 minutes.

[0363] The circularization system was prepared according to the reaction components listed in Table 3, for a 50 μ L reaction volume.

Table 3. Circularization system for AnaPIE (50 μ L per reaction)

Component	Volume
GTP (100 mM)	1 μ L
10 $\times$ T7 enzyme buffer	3 μ L

<i>In vitro</i> transcripts	23 μ L
Enzyme-free water	0-23 μ L (to yield total reaction volume of 50 μ L)

[0364] The prepared circularization system was placed in a thermal cycler and incubated at 60°C for 20 minutes.

[0365] Purification of circRNAs was performed for each construct according to the following protocol. Following incubation, the circularization system was transferred to a 1.5 mL centrifuge tube. 0.5 volumes of lithium chloride at a concentration of 7.5 M and 50 mM EDTA RNA precipitation solution were added, mixed, and then placed in a -20°C freezer for 1 hour. Following the precipitation step, the circularization system was centrifuged at 18360 rcf at 4°C for 15 minutes before removal of the supernatant. 800 μ L 70% ethanol solution was added to the 1.5 mL tube, and the circularization system was centrifuged again at 18360 rcf at 4°C for 15 minutes. The supernatant, comprising the 70% ethanol, was removed again, and the pellet comprising the circularization system was resuspended with 100 μ L enzyme-free water.

[0366] Digestion analysis of circRNAs with RNase R was then performed. Because circRNAs lack free ends, circRNAs were not digested by exonuclease-like RNase R. RNase R digestion analysis was thus used to detect the formation of circRNAs. circRNA samples were treated with RNase R enzyme for 30 minutes at the following concentrations: 2 units/ μ g RNA, 4 units/ μ g RNA, and 8 units/ μ g RNA. Following digestion, 300 ng circRNA of each sample was loaded onto a 1% agarose gel. Gel electrophoresis was conducted at 120V for 30 minutes, and gels subsequently imaged using a gel documentation system. Results indicated that all four circRNAs generated stayed intact during RNase R digestion across different enzyme concentrations (**FIG. 3**), indicating successful circularization of the RNAs.

[0367] Further validation of circRNAs was then conducted using 2% E-Gel electrophoresis. About 500ng of each circRNA was diluted with nuclease-free to a volume of 20 μ L, mixed, heated at 70°C for 5 minutes, and then cooled on ice for 5 minutes. circRNA samples were then loaded onto a 2% E-Gel EX agarose gel (Thermo, catalog number: G401002) and electrophoresed for 15 minutes, using the preset settings for the gel type, and subsequently imaged on a gel documentation system. 2% agarose gel electrophoresis results showed that the migration rate of the test circRNAs AnaPIE-OS, AnaPIE-M, AnaPIE-K, and AnaPIE-LN were slower than a control circRNA AnaPIE-eGFP, indicating the correct molecular weight of the test circRNAs (**FIG. 4**).

Verification of eukaryotic cell transfection with reprogramming factor proteins

[0368] Cell preparation and mRNA transfection were conducted according to the following protocol. A T75 flask of HEK293T/17 cells at 80-90% confluency was prepared for plating. The culture medium was discarded, cells were rinsed with PBS and treated with 0.05% trypsin to detach. After removal of trypsin, cells were dispersed in 10mL of antibiotic-free culture medium with 10% FBS. Cell density was adjusted to 1×10^6 cells/mL $\pm 10\%$, and 0.2 mL of the cell suspension was seeded into each well of ten 48-well plates. Plates were incubated at 37°C in a 5% CO₂ incubator for 24 $\pm$ 4 hours.

[0369] CircRNA solutions (0.4 μ g each of MYC-circRNA, KLF4-circRNA, OCT4-P2A-SOX2-circRNA, Lin28A-P2A-Nanog-circRNA) were prepared in 25 μ L serum-free DMEM, mixed gently, and allowed to sit at room temperature for 5 minutes. A transfection reagent, LipoHigh (1 μ L) was diluted in 25 μ L of serum-free DMEM, mixed gently, and allowed to sit at room temperature for 5 minutes. The medium containing LipoHigh was then combined with the circRNA solutions, mixed gently, and incubated at room temperature for 20 minutes. The prepared cell culture plates were then taken out of the incubator, and the transfection mixtures were added dropwise to the seeded cells. After gently mixing, the plates were returned to the incubator set at 37°C with 5% CO₂. After 4-8 hours, the medium was replaced with 0.5mL of culture medium containing 2% FBS to continue cultivation.

[0370] Protein was collected at 48h post-transfection for Western Blot analysis. As shown in **FIG. 5**, Western blot results confirmed that reprogramming factors encoded by circRNAs (MYC-circRNA, KLF4-circRNA, OCT4-P2A-SOX2-circRNA, Lin28A-P2A-Nanog-circRNA) were successfully expressed in HEK293T cells, and that P2A peptide was successfully cleaved.

Example 3: Three or four gene circRNA construction using AzoPIE method, and protein expression verification*Construction of circRNA vectors with different tandem combinations of genes encoding three or four reprogramming factors*

[0371] Multigene vectors were designed according to the following tandem design strategy. Nucleic acids encoding reprogramming factors (wild-type or LLPS-deficient) were linked using linking sequences encoding P2A (SEQ ID NO: 25), T2A (SEQ ID NO: 26), and E2A (SEQ ID

NO: 27) peptides to form either a three-gene or four-gene tandem construct, as illustrated in Table 4. OCT4' (SEQ ID NO: 4) contains D/E to A mutations in the IDRs relative to the wild-type human OCT4 sequence (SEQ ID NO: 2). c-MYC mutant (c-MYC'; SEQ ID NO: 14) has K to R mutations to remove ubiquitination sites, relative to the wild-type human c-MYC sequence (SEQ ID NO: 85). Wild-type human KLF4 has the sequence of SEQ ID NO: 22. Wild-type human SOX2 has the sequence of SEQ ID NO: 24.

Table 4. Circular RNA constructs containing three and four genes

Construct #	Construct Name	Genetic Structure	Size (nt)	nt SEQ ID NO:	aa SEQ ID NO:
5	AzoPIE-OSK	IRES-OCT4-P2A-SOX2-T2A-KLF4	4457	92	91
6	AzoPIE-O'SK	IRES-OCT4mut-P2A-SOX2-T2A-KLF4	4457	94	93
7	AzoPIE-OKM	IRES-OCT4-P2A-KLF4-T2A-MYC	4825	96	95
8	AzoPIE-O'KM'	IRES-OCT4mut-P2A-KLF4-T2A-MYCmut	4825	98	97
9	AzoPIE-OKMS	IRES-OCT4-P2A-KLF4-T2A-MYC-E2A-SOX2	5843	100	99
10	AzoPIE-O'KM'S	IRES-OCT4mut-P2A-KLF4-T2A-MYCmut-E2A-SOX2	5843	102	101

[0372] After sequence optimization of the tandem-linked protein coding sequences, nucleic acid sequences were recombined into the *Azoarcus* sp. BH permuted group I intron circular system AzoPIE and constructed within the pBlueScript II SK (+) plasmid (**FIGs. 6A-6F**). The 5' elements (contain 5' homology arm, AzoPIE 3' intron, 5' spacer) upstream of IRES comprise the sequence of SEQ ID NO: 51. The 3' elements (contain 3' spacer, AzoPIE 5' intron, 3' homology arm) downstream of the protein-coding sequence comprise the sequence of SEQ ID NO: 52. Plasmid synthesis was conducted by GenScript as described above in Example 2.

[0373] *In vitro* transcription and circularization of the circRNAs was performed in the following manner: restriction enzyme EcoR I was used to linearize the circRNA plasmids, which passed DNA purity tests, to prepare the *in vitro* transcription (IVT) template. 20μL reaction systems were prepared for two sets of parallel experiments as described in Table 5. The IVT system was incubated in a PCR machine at 37°C for 3 hours to allow both *in vitro* transcription and circularization to occur. After the PCR reaction, 1 μL of DNase I and 2 μL of 10× DNase I buffer were added to each reaction system, followed by a 20-minute incubation at 37°C. Each IVT system was then transferred to a 1.5 mL centrifuge tube, with 23 μL of nuclease-free water and 23μL of a

precipitation solution containing 7.5M lithium chloride and 50mM EDTA added, mixed, and precipitated at -20°C for 1 hour. The solution was then centrifuged at 18360 rcf for 15 minutes at 4°C, the supernatant was discarded, and the pellet was washed with 500 μ L of 70% ethanol. After a second centrifugation (same conditions), the ethanol was removed, and the pellet was resuspended in 100 μ L of nuclease-free water. circRNA samples were then subjected to 1% agarose gel electrophoresis for 30 and 90 minutes, and band separation was analyzed at these time points. As shown in **FIG. 7** and **FIG. 8**, all six circRNAs produced distinct circRNA bands that separated effectively from precursor preRNA bands with extended electrophoresis time.

Table 5. *In vitro* transcription and circularization system for AzoPIE (20 μ L volume)

Reaction Component	Volume
ATP/CTP/UTP/GTP (100 mM)	2 μ L each rNTP
10 $\times$ T7 buffer	2 μ L
T7 RNA polymerase mix	2 μ L
Linear template DNA	1 μ g
Nuclease-free water	Adjust for total reaction volume of 20 μ L (0-8 μ L)

[0374] HEK293T cell preparation, mRNA transfection, and protein expression verified via Western blot analysis were conducted according to the steps outlined in Example 2. Briefly, three prepared circRNAs, OKM-circRNA, O'KM'-circRNA, and OKMS-circRNA, were selected for transfection into HEK293T/17 cells. After 48 hours of culturing post-transfection, cells were collected for analysis. Cell lysates were used for protein extraction, and extracted proteins were detected via Western blot analysis using corresponding antibodies. As shown in **FIG. 9**, cells transfected with any of the circRNAs successfully expressed corresponding reprogramming factors (wild-type or mutant), and that 2A peptides were successfully cleaved. Among the three circRNAs analyzed, AzoPIE-OKM showed a higher OCT4 expression level. The mutant OCT4 (OCT4') expressed by cells transfected with the AzoPIE-O'KM' circRNA exhibited a slightly lower molecular weight compared to the wild-type OCT4 protein. Without being bound by theory, the observed lower molecular weight of OCT4' was likely due to the substitutions of D/E to A (A is a smaller amino acid).

Example 4: Construction, synthesis, and protein expression of a three-gene tandem circRNA encoding mouse KLF4, mouse SOX2, and mouse OCT4

Construction of AzoPIE-MsOKS and AzoPIE-MsO_{mut}KS circRNA constructs

[0375] Three-gene tandem circRNA constructs encoding mouse wild-type OCT4 (MsOCT4; SEQ ID NO: 66) or mouse mutant OCT4 (MsOCT4_{mut}), mouse KLF4 (SEQ ID NO: 72), and mouse SOX2 (SEQ ID NO: 74) were designed, as shown in Table 6 and **FIGs. 10A-10C**. MsOCT4_{mut1} (SEQ ID NO: 68) contains D/E to A mutations in the IDRs. MsOCT4_{mut2} (SEQ ID NO: 70) has a deletion at amino acid positions 324-326 (within IDR) relative to wild-type MsOCT4 (SEQ ID NO: 66). Genes in the circRNA constructs were linked using linking sequences encoding P2A (SEQ ID NO: 25) and T2A (SEQ ID NO: 26) as shown in **FIGs. 10A-10C**. The tandem nucleic acid sequences were generated as described in Example 3, inserted into AzoPIE system and constructed within the pBlueScript II SK (+) plasmid.

Table 6. circRNA constructs containing three mouse genes

Construct #	Construct Name	Genetic Structure	Size (nt)	nt SEQ ID NO:	aa SEQ ID NO:
11	AzoPIE-MsOSK	IRES-MsOCT4-P2A-MsSOX2-T2A-MsKLF4	4457	104	103
12	AzoPIE-MsO _{mut1} SK	IRES-MsOCT4 _{mut1} -P2A-MsSOX2-T2A-MsKLF4	4457	106	105
13	AzoPIE-MsO _{mut2} SK	IRES-MsOCT4 _{mut2} -P2A-MsSOX2-T2A-MsKLF4	4457	108	107

[0376] *In vitro* transcription and circularization of the circRNAs were conducted as described in Example 3. After the circRNAs were generated, they were transfected into HEK 293T cells to verify protein expression via Western blot analysis, as described in Example 3. Western blot results, shown in **FIG. 11**, showed that expression of MsOCT4_{mut1} protein (SEQ ID NO: 68) was detected in cells following transfection with the AzoPIE-MsO_{mut1}SK circRNA. MsKLF4 protein (SEQ ID NO: 72) expression was also detected. β -actin served as a loading control.

Example 5: Delivery of circRNAs by exosomes

[0377] This Example presents a novel method for delivering circRNAs via exosomes, exemplified by AzoPIE11-eGFP, a model circRNA encoding the enhanced Green Fluorescent Protein (eGFP) reporter. This feature acts as a visual marker, such that circRNA delivery can be tracked and validated. The encapsulation of AzoPIE11-eGFP circRNA into exosomes was achieved through a chemical-assisted transfection method, demonstrating the efficiency of the technique. Successful integration of AzoPIE11-eGFP circRNA into exosomes and eGFP expression *in vitro* demonstrated the potential of exosomes as carriers for targeted circRNA delivery.

[0378] The method used exosomes from HEK293F cells (abbreviated “HEK-exo”) or from human umbilical cord mesenchymal stem cells (abbreviated as “huMSC-exo”), establishing that the method has applications across various cell sources. Briefly, exosomes were isolated from cell sources via tangential flow filtration (TFF) and differential ultracentrifugation. Isolated exosomes were characterized via transmission electron microscope (TEM) imaging and nano flow cytometry. **FIG. 12A** shows TEM images of exosome samples showing typical cup-shape morphology. **FIG. 12B** shows nano flow cytometry results showing that exosome size is distributed mainly between 60-80 nm. **FIG. 13** shows nano flow cytometry results measuring sizes of HEK-exo and huMSC-exo with and without circRNA. RT-qPCR results in **FIG. 14** show that circRNA constructs were included in HEK-exo and huMSC-exo but not in control (NC) sample (no exosome added).

[0379] A precise mixture of 2E11 exosomal particles, 60 µg AzoPIE11-eGFP circRNA, and 600 µg polyethylenimine (PEI) in PBS was prepared in a 1.5 mL Eppendorf tube and incubated at 37°C on a shaker for 30 minutes. Following incubation, HEK exosomes containing circRNAs were purified using a 100 kDA cutoff ultrafiltration technique, removing excess substances (including unincorporated circRNAs and unassociated PEI).

[0380] circRNA delivery to exosomes was verified via fluorescence microscopy of HEK-exo (**FIG. 15A**) and huMSC-exo (**FIG. 15B**) showing successful incorporation of the reporter circRNA-eGFP.

[0381] HEK-exo containing OSK-circRNA or O'SK-circRNA (produced from Example 3) were similarly prepared as above. Loading efficiency of OSK-circRNA and O'SK-circRNA in HEK-exo was measured and compared via nano flow cytometry, showing no significant difference (**FIG. 16**). OSK-circRNA-loaded HEK-exosome were transfected into human skin fibroblast cells and the protein expression of OCT4, SOX4, and KLF4 were verified by immunofluorescence (**FIG. 17**).

Example 6: Gene synthesis and lentivirus (LV) vector construction

Design of reprogramming factor unit sequences

[0382] Amino acid sequences for human mutant OCT4' (SEQ ID NO: 4) and its variants OCT4'1-6 (SEQ ID NOs: 5-10) were designed. All OCT4 mutants contain D/E to A mutations in the IDRs. Corresponding nucleotide sequences were designed, such as the nucleotide sequence for

Oct4', shown in SEQ ID NO: 3. Amino sequences for human mutant c-MYC' (SEQ ID NO: 14) and its variants c-MYC'1-6 (SEQ ID NOs: 15-20) were designed. All c-MYC mutants contain K to R mutations to remove ubiquitination sites. Corresponding nucleotide sequences were designed, such as the nucleotide sequence for c-Myc', shown in SEQ ID NO: 13. The amino acid sequence for human KLF4 (SEQ ID NO: 22) and corresponding nucleotide sequence (SEQ ID NO: 21) were designed. The amino acid sequence for human SOX2 (SEQ ID NO: 24) and corresponding nucleotide sequence (SEQ ID NO: 23) were designed. Linking sequences encoding P2A (SEQ ID NO: 25), T2A (SEQ ID NO: 26), and E2A (SEQ ID NO: 27) were designed. A Kozak sequence GCCGCCACC (SEQ ID NO: 113) was designed. A TRE3G promoter (Tet-On 3G tetracycline-inducible) sequence (SEQ ID NO: 63) was designed. An SV40 poly(A) sequence (SEQ ID NO: 64) was designed.

[0383] Tandem sequences were formed by linking the above components. Non-limiting examples of tandem sequences are shown in SEQ ID NOs: 28-33 and **FIG. 18A**. The O'KM'S encoding tandem sequence (SEQ ID NO: 28) was generated with the nucleic acid components OCT4'-P2A-KLF4-T2A-c-MYC'-E2A-SOX2. The OKMS encoding tandem sequence (SEQ ID NO: 29) was generated with the nucleic acid components OCT4-P2A-KLF4-T2A-c-MYC-E2A-SOX2. The O'KM' encoding tandem sequence (SEQ ID NO: 30) was generated with the nucleic acid components OCT4'-P2A-KLF4-T2A-c-MYC'. The OKM encoding tandem sequence (SEQ ID NO: 31) was generated with the nucleic acid components OCT4-P2A-KLF4-T2A-c-MYC. The O'KS encoding tandem sequence (SEQ ID NO: 32) was generated with the nucleic acid components OCT4'-P2A-KLF4-T2A-SOX2. The OKS encoding tandem sequence (SEQ ID NO: 33) was generated with the nucleic acid components OCT4-PO2A-KLF4-T2A-SOX2.

[0384] Lentiviral vector construction and packaging was carried out by Cyagen Biotechnology Co., Ltd. according to the following steps. First, the entire tandem sequences of O'KM'S, OKMS, O'KM', and OKM were synthesized. Appropriate restriction sites on the vector backbone were selected for digestion. Digestion was performed using the New England Biolabs (NEB) CutSmart system as detailed in Table 7. A schematic diagram of the four lentiviral vector structures is shown in **FIG. 18A**.

Table 7. CutSmart digestion system

Material	Quantity
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CutSmart buffer	17.5 μ L
Plasmid	600 ng-800 ng
Restriction enzymes	0.7 μ L or 2 $\times$ 0.5 μ L

[0385] Following digestion, bands were recovered using a gel recovery kit (NEB Monarch DNA, Catalog #: T1020S), and required backbone and target fragments were prepared for subsequent ligation.

[0386] Ligation and transformation of the lentiviral vector system was performed according to the following steps, with ligation system components as detailed in Table 8. Synthesized tandem sequences and other related vector components were each sequentially ligated with the linearized vector backbone. The ligation used the C115 Infusion ligation enzyme system, with ligation reaction parameters as described in Table 9.

Table 8. Ligation system

Reaction material	Volume/quantity
Processed backbone	100 ng
Connecting fragments	50 ng
2 $\times$ enzyme mixture	5 μ L
Sterile water	0-5 μ L (to yield total reaction volume of 10 μ L)

Table 9. C115 Infusion enzyme ligation reaction procedure

Reaction component	Parameter
Reaction temperature	50°C
Hot lid temperature	105°C
Reaction time	15 minutes
Reaction volume	10 μ L

[0387] Following ligation, the corresponding antibiotic-resistant plates (Amp⁺) were taken in advance to a 37°C incubator to dry in an inverted position, while 500 μ L of antibiotic-free culture medium was preheated. Competent cells were taken from storage at -80°C and thawed on ice for 15-20 minutes. Following thawing of competent cells, half the ligation mix was added to the competent cells, which was then mixed by flicking the tube gently five times prior to incubation in an ice bath for 30 minutes. Following the ice bath, the cells were quickly transferred to a preheated 42°C water bath for heat shock for 1 minute (strictly controlled between 45-90 seconds). Following heat shock, the cells were immediately placed on ice for 2 minutes, then transferred to the preheated 500 μ L culture medium and placed on a 37°C shaker for 1 hour. After 1 hour, the cell suspension was centrifuged at 4000 rpm for 2 minutes to remove the supernatant (discarding 400 μ L supernatant), the plate was inverted, and around 20 glass beads were added. The cell

suspension was pipetted, mixed thoroughly and spread onto the corresponding antibiotic-resistant plate, which was then placed in an incubator at 37°C overnight.

[0388] After culturing overnight, bacterial colonies were checked. The ultraviolet light in the laminar flow hood was turned on for 20 minutes for sterilization purposes, then turned off, and the airflow set to the third level. The hood was cleaned with 75% ethanol prior to beginning the bacterial check. On the clean bench, 25 μL of sterile water was added to the bottom of a 96-well PCR plate, and the bottom was checked against the light for the presence of air bubbles. Using sterile 10 μL pipette tips, single monoclonal colonies were gently picked and transferred into the corresponding wells of a 96-well reaction plate, mixed by pipetting 2-3 times with a 10 μL pipettor, and then 4 μL from each well was transferred to a new 96-well reaction plate to serve as a template for PCR reactions. 50 μL of antibiotic-free broth culture medium was added to the original 96-well plate template, which was then covered with a sterilized 96-well PCR plate cover, and placed in a 30°C incubator.

[0389] Bacterial colony screening was performed using the P222-AA Taq enzyme reaction system. Components for each reaction volume are shown in Table 10. Temperatures for each reaction stage are shown in Table 11.

Table 10. P222-AA Taq enzyme PCR reaction components

Reagent	Quantity
2 $\times$ Taq enzyme mixture	12.5 μL
Single colony suspension	4 μL
Primer pair	2 $\times$ 0.75 μL
Sterile water	0-7 μL (to yield total reaction volume of 25 μL)

Table 11. P222-AA Taq enzyme PCR reaction steps

PCR Reaction Stage	Temperature	Time	# of cycles
Pre-denaturation	95°C	3 min	23
Denaturation	95°C	15 s	23
Annealing	60°C	15 s	23
Extending	72°C	15 s/kb	23
Terminal Extension	72°C	5 min	n/a
Save	25°C	$+\infty$	n/a

[0390] Following the PCR reaction, samples were run on a TBE gel to confirm that the size of the bands matched the expected sizes. Then, 3.5 mL of Amp-resistant broth culture medium was

added to a 48-well plate containing the tested bacterial colonies and incubated overnight on a 37°C shaker.

[0391] Plasmid extraction was performed using Qiagen's QIAprep Spin Miniprep Kit (27106). The concentration of the extracted plasmids was measured and recorded. Concentrations greater than 50 ng/μL indicated a successful extraction. Successfully extracted plasmids were then verified via enzyme digestion.

[0392] NTI/SnapGene software was used to identify a suitable enzyme digestion scheme on the basis of existing restriction enzymes to verify the correctness of the plasmid. The enzyme digestion was set up as described in Table 12.

Table 12. Digestion System

Material	Quantity
Buffer	2 μL
Single/double enzymes	0.7 μL/0.5 μL
Plasmid	600-800 ng
Sterile water	Up to 20 μL

[0393] Digestion reactions were prepared according to Table 12, with materials as specified in Table 13, and were subsequently incubated at 37°C for 45 minutes. Following incubation, 5 μL of bromophenol blue/xylene cyanol was added, and samples were run on a TAE gel and imaged to verify the size of the bands (**FIG. 18B**). 30 μL of each plasmid was sequenced (Genewiz), confirming successful construction of the vectors.

Table 13. Materials

Material	Source
PCR enzymes	Vazyme P515
PCR gel recovery kit	QIA Gel Extraction Kit (28706)
Ligase	Vazyme C115
Bacteriometric Taq enzyme	Vazyme P222
Competent cells	Weidi Bio DH5α
Plasmid extraction kit	QIAprep Spin Miniprep Kit (27106)
PCR, bacterial screening, sequencing, primer synthesis, and gene synthesis services	GeneWiz

[0394] Successfully constructed lentiviral vectors include LV-TRE3G-OKM (SEQ ID NO: 34), LV-TRE3G-O'KM' (SEQ ID NO: 35), LV-TRE3G-OKMS (SEQ ID NO: 36), and LV-TRE3G-O'KM'S (SEQ ID NO: 37).

[0395] Lentiviral plasmids and helper plasmids (SL3, SL4, and SL5) were co-transfected into HEK 293T cells. Virus was harvested by centrifugation, collecting the cell supernatant, 48 hours post-transfection. Cell supernatant was treated with PEG8000 concentration and sucrose density gradient ultracentrifugation. Quality control measures including physical state examination, sterility testing, and titer testing were conducted (**FIG. 19A**). Titer testing was performed via RT-qPCR (after co-transfection cells with LV-EFS-Tet3G and doxycycline induction). In order to confirm transgene expression, the above-mentioned harvested lentiviruses were co-transduced into HEK 293T cells along with a TET3G (Tet-On 3G)-expressing lentiviral particle (LV-EFS-Tet3G, see **FIG. 35**). Doxycycline was added to induce expression. 48 hours post-induction, RNA samples were extracted, and the expression of transgenic KLF4 was detected using transgenic KLF4-specific primers (**FIG. 19B**). This data indicates that reprogramming factors used herein can be delivered by lentiviruses.

Example 7: Gene synthesis and adeno-associated virus (AAV) vector with inducible expression system construction

Design of reprogramming factor Unit Sequences

[0396] Amino acid and nucleic acid sequences of reprogramming factors were designed as described in Example 6. A Kozak sequence GCCGCCACC (SEQ ID NO: 113) was designed. A TRE3G promotor sequence (SEQ ID NO: 63) was designed. An hGH poly(A) sequence (SEQ ID NO: 65) was designed.

[0397] The O'KS encoding tandem sequence (SEQ ID NO: 32) was generated with the nucleic acid components OCT4'-P2A-KLF4-T2A-SOX2; a diagram of the AAV-TRE3G-O'KS vector components is shown in **FIG. 22**. The OKS encoding tandem sequence (SEQ ID NO: 33) was generated with the nucleic acid components OCT4-P2A-KLF4-T2A-SOX2; a diagram of the AAV-TRE3G-OKS vector components is shown in **FIG. 20**.

[0398] AAV vector construction was performed by Cyagen Biotechnology Co., Ltd. according to the following steps. First, appropriate restriction site combinations were selected on the AAV backbone plasmid. The AAV backbone plasmid and enzymes were incubated at 37°C for 1 hour, followed by agarose gel electrophoresis to recover the corresponding digested linear plasmid backbone.

[0399] The entire tandem sequences encoding OKS and O'KS were synthesized, and the tandem sequences and other related components were cloned into the linear backbone plasmid using a homologous recombination kit (Novagen, C115). The reaction system was transformed into competent cells, plated, and subsequently colonies were picked, cultured, and plasmids were extracted. Enzyme digestion verification and sequencing were performed as described in detail in Example 6.

[0400] After confirming that the enzyme digestion and sequencing results were correct (enzyme digestion verification results shown in **FIG. 21** for OKS vector and **FIG. 23** for O'KS vector), plasmids from the correct bacterial colonies were transformed again into competent bacteria, plated, colonies were picked, amplified, and extracted to provide the plasmids required for AAV packaging, including AAV-TRE3G-OKS (SEQ ID NO: 38) and AAV-TRE3G-O'KS (SEQ ID NO: 39). AAV2 serotype was used.

[0401] AAV viral plasmids, helper plasmids, and packaging plasmids were co-transfected into HEK 293T cells. 48 hours after transfection, cells were centrifuged, and the cell supernatant was collected. Cell supernatant was subjected to PEG8000 concentration and sucrose density gradient ultracentrifugation treatments. Quality control measures were conducted including physical status examination, sterility testing, and titer determination. Viral titer was measured using RT-qPCR (**FIGs. 24A-24C**).

AAV-mediated transgene expression in mouse retina

[0402] C57BL6/J mice were intravitreally injected with 0.75 μ L of AAV-TRE3G-GFP, AAV-TRE3G-OKS, or AAV-TRE3G-O'KS virus (AAV2) at day 0. 0.75 μ L of AAV-CMV-TET3G was co-injected and 2 mg/mL doxycyclin was added to the drinking water to induce transgene expression. Two weeks following injection of AAV virus, mice were sacrificed, and tissue was collected, frozen, and sectioned. Frozen tissue sections were analyzed via immunohistochemistry. KLF4 protein was expressed in the retinal ganglion cells (RGCs) of both the AAV-TRE3G-OKS group and the AAV-TRE3G-O'KS group (**FIG. 25**). Expression of KLF4 protein from both constructs was confirmed via Western blot analysis (**FIG. 26**).

[0403] These data indicate that reprogramming factors used herein can be delivered by AAVs.

Example 8: Gene synthesis and AAV2 vector construction with mouse-derived sequences*Design of mouse reprogramming factor unit sequences*

[0404] An amino acid sequence for MsOCT4 (SEQ ID NO: 66) and a corresponding MsOCT4 nucleotide sequence (SEQ ID NO: 67) were designed. An amino acid sequence for MsOCT4_{mut1} (SEQ ID NO: 68) and a corresponding nucleotide sequence for MsOCT4_{mut1} (SEQ ID NO: 69) were designed. An amino acid sequence for MsOCT4_{mut2} (SEQ ID NO: 70) and a corresponding nucleotide sequence for MsOCT4_{mut2} (SEQ ID NO: 71) were designed. An amino acid sequence for mouse KLF4 (MsKLF4) (SEQ ID NO: 72) and a corresponding nucleotide sequence (SEQ ID NO: 73) were designed. An amino acid sequence for mouse SOX2 (MsSOX2) (SEQ ID NO: 74) and a corresponding nucleotide sequence (SEQ ID NO: 75) were designed.

[0405] Linking sequences encoding P2A (SEQ ID NO: 25), T2A (SEQ ID NO: 26), and E2A (SEQ ID NO: 27), were designed. A Kozak sequence, GCCGCCACC (SEQ ID NO: 113), was designed. A CMV promotor sequence (SEQ ID NO: 76) was designed. An SV40 poly(A) sequence (SEQ ID NO: 64) was designed.

Construction of tandem sequences of mouse reprogramming factor units

[0406] Tandem sequences were formed by linking the above components. Non-limiting examples of tandem sequences include SEQ ID NOs: 77-79. The MsOKS-encoding tandem sequence (SEQ ID NO: 77) was designed with the nucleic acid components MsOCT4-P2A-MsKLF4-T2A-MsSOX2. Tandem sequences encoding MsOCT4_{mut1}KS and MsOCT4_{mut2}KS, were designed with the nucleic acid components MsOct4_{mut1}-P2A-MsKLF4-T2A-MsSOX2 (SEQ ID NO: 78) and MsOct4_{mut2}-P2A-MsKLF4-T2A-MsSOX2 (SEQ ID NO: 79), respectively.

AAV2 vector construction and packaging

[0407] AAV vector construction was performed by Cyagen Biotechnology Co., Ltd. according to the following steps. First, appropriate restriction site combinations were selected on the AAV backbone plasmid. The AAV backbone plasmid and enzymes were incubated at 37°C for 1 hour, followed by agarose gel electrophoresis to recover the corresponding digested linear plasmid backbone.

[0408] The entire tandem sequences encoding MsOKS, MSO_{mut1}KS, and MSO_{mut2}KS were synthesized, and the tandem sequences and other related components were cloned onto the linear

backbone plasmid using a homologous recombination kit (Novagen, C115). Diagrams of the vector components for AAV-CMV-MsOKS, AAV-CMV-MsO_{mut1}KS, and AAV-CMV-MsO_{mut2}KS are shown in **FIGs. 27, 29, and 31**, respectively. The reaction system was transformed into competent cells, plated, and subsequently colonies were picked, cultured, and plasmids were extracted. Enzyme digestion verification and sequencing were performed as described in detail in the preceding Examples.

[0409] After confirming the enzyme digestion and sequencing results were correct (enzyme digestion verification results shown in **FIG. 28** for AAV-CMV-MsOKS, **FIG. 30** for AAV-CMV-MsO_{mut1}KS, and **FIG. 32** for AAV-CMV-MsO_{mut2}KS), plasmids from the correct bacterial colonies were transformed again into competent bacteria, plated, colonies were picked, amplified, and extracted to provide the plasmids required for AAV2 packaging, including AAV-CMV-MsOKS (SEQ ID NO: 80), AAV-CMV-MsO_{mut1}KS (SEQ ID NO: 81), and AAV-CMV-MsO_{mut2}KS (SEQ ID NO: 82).

[0410] AAV viral plasmids, helper plasmids, and packaging plasmids were co-transfected into HEK 293T cells. 48 hours after transfection, cells were centrifuged and the cell supernatant was collected. Cell supernatant was subjected to PEG8000 concentration and sucrose density gradient ultracentrifugation treatments. Quality control measures were conducted including physical status examination, sterility testing, and titer determination. Titer for AAV2 particles was measured using RT-qPCR (results shown in **FIGs. 33A-33C**).

Example 9: Gene synthesis and AAV9 vector construction with mouse-derived sequences

Design of mouse reprogramming factor unit sequences

[0411] Nucleic acid sequences for encoding mouse reprogramming factors, linking sequences, Kozak sequence, CMV promotor sequence, and SV40 poly(A) sequence were similarly designed as described in Example 8.

Construction of tandem sequences of mouse reprogramming factor units

[0412] Tandem sequences for encoding MsOKS (SEQ ID NO: 77) and MsO_{mut1}KS (SEQ ID NO: 78) was designed as described in Example 8.

AAV9 vector construction and packaging

[0413] AAV vector construction for AAV-CMV-MsOKS (SEQ ID NO: 80) and AAV-CMV-MsO_{mutl}KS (SEQ ID NO: 81), verification, and AAV9 viral particle packaging were conducted similarly as in Example 8. Quality control measures were conducted including physical status examination, sterility testing, and titer determination. Titer for AAV9 particles was measured using RT-qPCR (results shown in **FIG. 34A-34C**).

[0414] These data collectively demonstrate that various AAV serotypes can be used to deliver the reprogramming factors, either of human or mouse origin.

Example 10: Initiation phase partial reprogramming promoted RGC survival and axon regeneration in a laser-induced traumatic optic neuropathy (TON) mouse model

[0415] C57BL6/J mice were intravitreally injected with 1.5 μ L of AAV2-CMV-GFP virus (Group 1; negative control), AAV2-TRE3G-OKS virus + AAV2-CMV-Tet3G virus (Group 2), or AAV2-TRE3G-O'KS virus + AAV2-CMV-Tet3G virus (Group 3) on day 0. AAV-TRE3G-OKS virus and AAV-TRE3G-O'KS virus (AAV2) were generated from Example 7, encoding human OKS and human O'KS, respectively. **FIG. 35A** shows the experimental timeline for assessing regenerative effects after laser photocoagulation. Laser photocoagulation is an eye surgery using a laser to shrink or destroy abnormal structures in the retina, or to intentionally cause scarring that can help certain eye conditions. **FIG. 35B** shows a summary table of each experimental group. Four weeks following injection of AAV constructs, a laser photocoagulation operation was conducted to induce traumatic optic neuropathy (TON). Laser photocoagulation can induce retina damage in general. It hurts RGCs and axons more as they are more exposed in the retina, the other retina cell types are less hurt as they are in the beneath layers of the retina. Mice were orally given drinking water containing 2 mg/mL doxycycline daily for the following three weeks.

[0416] Three weeks following laser coagulation, optical coherence tomography (OCT) and electroretinogram (ERG) tests were performed on mice from each experimental group. Treatment with O'KS showed retina regenerative effects relative to treatment with the control vector. Representative OCT images are shown in **FIG. 36A**, and statistical analysis of laser-induced retina lesion area among each group is shown in **FIG. 36B**. Damaged retina overgrew to heal the wound. As shown in **FIGs. 36A-36B**, the AAV-O'KS group outcompeted AAV-OKS group in reducing laser photocoagulation-induced retina lesion. Representative full-field ERG images are shown in **FIG. 37A**, and statistical analysis of Photopic Negative Response (PhNR) among each group is

shown in **FIG. 37B**. PhNR is a slow negative component of the full-field ERG that serves as an electrophysiological marker of RGC function. PhNR amplitude is greatly reduced in optic neuropathy. The ERG results indicate that AAV-OKS or AAV-O'KS treatment can greatly preserve RGC electrophysiological function and reduce RGC damage in response to laser photocoagulation, and that the AAV-O'KS group was slightly more effective. Two days after OCT and ERG testing, eyeballs were harvested and fixed with 4% paraformaldehyde (PFA). β -III-tubulin contributes to microtubule formation in neuronal cell bodies and axons, hence is a reliable marker for retinal ganglion cell (RGC) and axon. RGCs are the only neurons in retina that connect and relay visual signals to the central nervous system. β III-tubulin-labeled axons are shown in **FIG. 38A** and quantified in **FIG. 38B**. The data indicate that laser photocoagulation significantly reduced the density of axons; the group treated with AAV-OKS or AAV-O'KS showed no significant difference in axon density compared to that without laser damage, indicating effective axon regeneration. RGC survival was shown by BRN3A immunofluorescence staining and estimated via quantification of RGC cell numbers per mm² area, as shown in **FIGs. 39A-39B**. The data indicate that laser photocoagulation significantly reduced RGC number (suggesting RGC death); while the groups treated with AAV-OKS and AAV-O'KS greatly reduced RGC death following laser coagulation. There was no statistical difference for the RGC number per area with or without laser coagulation in these 2 experimental groups.

Example 11: Initiation phase partial reprogramming promoted restoration of vision following optic nerve crush in a mouse model

Optic nerve crush protocol

[0417] Initially, 2% methyl cellulose was administered to the right eye of each mouse to prevent dryness and cataract formation, followed by positioning the mouse on sterile gauze in a left lateral posture. This ensured that the right eye remained level for subsequent steps. Removal of excess methyl cellulose from the right eye was achieved with a moistened cotton swab, upon which 1-4 drops of a topical anesthetic were applied, necessitating a waiting period exceeding 15 seconds for effective anesthesia.

[0418] The surgical phase commenced with the elevation of the conjunctiva using toothed forceps, followed by a precise incision approximately 2mm from the lateral canthus utilizing corneal or conjunctiva scissors. Special care was taken to circumvent major blood vessels during

the blunt dissection of the external rectus muscle, with micro toothless bent forceps employed for this purpose. In instances of excessive bleeding, which could obscure the surgical field, the area was dabbed clean with a cotton swab.

[0419] Subsequently, a switch to micro toothless straight forceps facilitated the gentle advancement of the eyeball, revealing 3-4 mm of the optic nerve. This step necessitated adjusting the focus of the surgical microscope from the cornea to the optic nerve. The optic nerve was then clamped approximately 2mm posterior to the globe for around 10 seconds.

[0420] The procedure was concluded by retracting the surgical instruments, repositioning the eyeball, and administering tobramycin dexamethasone phosphate eye ointment to both eyes. Mice were then tagged for identification, placed on a heating pad for recovery, and closely monitored until full recuperation from anesthesia was observed, at which point they were returned to their respective cages. Detailed records of the surgical process, including the operation's fluidity, volume of bleeding, and any incidences of corneal or lens opacity, were meticulously maintained throughout.

Experimental procedure

[0421] As shown in **FIGs. 40A-40B**, C57BL6/J mice were intravitreally injected with 2 μ l of AAV9-CMV-EGFP virus (Groups 1 and 2), AAV9-CMV-MsOKS virus (titers at $1.65\text{E}+13\text{vg/mL}$; Group 3) or AAV9-CMV-MsO_{mut1}KS virus (titers at $1.23\text{E}+13\text{vg/mL}$; Group 4) at day 0. AAV9-CMV-MsOKS virus and AAV9-CMV-MsO_{mut1}KS virus were generated from Example 9, encoding mouse OKS and mouse O_{mut1}KS, respectively. Two weeks after injection of AAV virus, an optic nerve crush experiment was conducted as described above. Mice were then kept for an additional two weeks. Subsequently, electrophysiological tests including pattern electroretinogram (pERG) and flash visual evoked potentials (fVEP) were performed on both the untreated and optic nerve crush groups.

[0422] The standard, transient response separates the pERG into wave components, including a negative wave at about 35 msec (N35) followed by a positive wave at approximately 50 msec (P50) and a large, negative wave at around 95 msec (N95). The P50-wave is the initial positive deflection originating from RGCs as well as from outer retinal photoreceptor cells, namely the macular cones. This wave-component is largely a measure of outer retinal function. The N95-wave is the negative deflection following the P50-wave that originates from the inner retina. This wave-component

reflects the RGC function. The value of the P50-wave amplitude subtracted by the N95-wave amplitude (“P50-N95”) provides an index of outer to inner retinal function. As shown in **FIG. 41A-41B**, optic nerve crush significantly reduced ERG P50-N95 amplitude, and AAV-MsOKS and AAV-MsO_{mut1}KS treatment significant recovered the ERG P50-N95 amplitude.

[0423] A similar improvement in retinal function associated with AAV-MsOKS and AAV-MsO_{mut1}KS treatment was seen fVEP. fVEP is highly predictive of final visual outcome. P1-N2 amplitude of fVEP indicates vision function by flash stimulation. As shown in **FIG. 42**, after optic nerve crush, flash barely induced any change to the P1-N2 amplitude of fVEP, indicating vision loss; while AAV-MsOKS or AAV-MsO_{mut1}KS treatment greatly augmented the P1-N2 amplitude of fVEP, indicating that crushed optic nerve axons were rescued and impaired vision was restored.

[0424] Altogether, the MsOKS and MsO_{mut1}KS treatments significantly restored retinal function, optic nerve axon stimulation, and vision following optic nerve crush.

Example 12: Initiation phase partial reprogramming promoted RGC survival and optical nerve regeneration following optic nerve crush in a mouse model

[0425] C46BL6 mice were intravitreally injected with 2 μ L of AAV2-CMV-GFP, AAV2-CMV-MsOKS, AAV2-CMV-MsO_{mut1}KS, or AAV2-CMV-MsO_{mut2}KS at day 0, as shown in the experimental timeline (**FIG. 43A**) and cohort summary (**FIG. 43B**). AAV2-CMV-MsOKS virus, AAV2-CMV-MsO_{mut1}KS virus, and AAV2-CMV-MsO_{mut2}KS virus were generated from Example 8, encoding mouse OKS, mouse O_{mut1}KS, and mouse O_{mut2}KS respectively. Two weeks following injection of AAV, an optic nerve crush experiment was conducted as discussed above. Two weeks following optic nerve crush, 1 μ L of cholera toxin β subunit (CTB) (2 μ g/ μ L, Invitrogen) was injected into the vitreous bodies of the eye. Cholera toxin subunit B (CTB) is a sensitive neuroanatomical tracer that generally transports retrogradely in the nervous system. Two days later, eyeballs were harvested and fixed with 4% paraformaldehyde (PFA). CTB-labelled axons are shown in **FIG. 44A** and the fluorescent intensity is quantified in **FIG. 44B**, showing promotion of axon regeneration in the cohorts treated with all 3 test groups. AAV2-MsO_{mut1}KS outcompeted both AAV2-MsO_{mut2}KS and AAV2-MsOKS groups. AAV2-MsOKS showed the least axon regeneration effects. Further, the axon regeneration effects were most significant close to the crush site in the AAV2-MsO_{mut1}KS group, indicating its surprisingly high efficacy in damaged retina. RGC survival was assessed by RBPMS (selective marker of RGCs) staining and

quantification of RGC numbers per 0.04 mm² area. As shown in **FIGs. 45A-45B**, optical nerve crush significantly reduced RGC number, while a significant increase in survival of RGCs was observed for groups treated with AAV-MsOKS, AAV-MsO_{mut1}KS, and AAV-MsO_{mut2}KS. Altogether, these results demonstrate that administration of OSK, O_{mut1}SK, or O_{mut2}SK significantly increase survival of RGCs and optical nerve regeneration following optic nerve crush.

[0426] Collectively, all these data demonstrate that LLPS-deficient engineered reprogramming factors described herein and methods thereof are very effective in rejuvenating retina cells and optic nerve axon, treating and/or preventing retina or optic nerve axon damage, and retina/optic nerve axon disease (e.g., optic neuropathy). Further, the engineered reprogramming factors and methods described herein apply to various delivery methods, e.g., via AAV, and are not limited to serotypes of AAVs.

SEQUENCE LISTING

SEQ ID NO	Sequence Name	Sequence
2	OCT4-WT (aa)	MAGHLASDFAFSPPGGGGDGPGGPEPGWVDPRTWLSFQGGPPGGPGIGPGVGPSEVWGIPPCPPPYEFCGGMAYCGPQVGVGLVPQGGLETSQPEGEAGVGVESNSDASPEPCTVTPGAVKLEKEKLEQNPEESODIKALQKELEQFAKLLKQKRITLGYTQADVGLTLGVLFQKVFSSQTTICRFEALQLSFKNMCKLRPLLQKWVEEADNNENLQEICKAETLVQARKRKRTSIENRVRGNLENLFLQCPKPTLQQISHIAQQLGLEKD VVRVWFNRRQKGRSSSDYAQREDFEAAAGSPFSGGPVSFPLAPGPHFGTTPGYGSPHFTALYSSVFPFGEAAPPVSVTTLGSPMHNS
4	OCT4' (aa)	MAGHLASAFASPPGGGGAGPGGPAPGWVAPRTWLSFQGGPPGGPGIGPGVGPSEVWGIPPCPPPYAFCGGMAYCGPQVGVGLVPQGG LATSQAGAAAGVGASNSAGASAPACTVTPGAVKLAKAKLAQNPAASQAIALQKELEQFAKLLKQKRITLGYTQADVGLTLGVLFQKVFSSQTTICRFEALQLSFKNMCKLRPLLQKWVEEADNNENLQEICKAETLVQARKRKRTSIENRVRGNLENLFLQCPKPTLQQISHIAQQLGLE KD VVRVWFNRRQKGRSSSDYAQRAAFAAAGSPFSGGPVSFPLAPGPHFGTTPGYGSPHFTALYSSVFPFAGAAFPVSVTTLGSPMHNS
5	OCT4'-1 (aa)	MAGHLASAFASPPGGGGAGPGGPAPGWVAPRTWLSFQGGPPGGPGIGPGVGPSEVWGIPPCPPPYAFCGGMAYCGPQVGVGLVPQGG LATSQAGAAAGVGASNSAGASAPACTVTPGAVKLAKAKLAQNPAASQAIALQKELEQFAKLLKQKRITLGYTQADVGLTLGVLFQKVFSSQTTICRFEALQLSFKNMCKLRPLLQKWVEEADNNENLQEICKAETLVQARKRKRTSIENRVRGNLENLFLQCPKPTLQQISHIAQQLGLE KD VVRVWFNRRQKGRSSSDYAQREDFEAAAGSPFSGGPVSFPLAPGPHFGTTPGYGSPHFTALYSSVFPFGEAAPPVSVTTLGSPMHNS
6	OCT4'-2 (aa)	MAGHLASDFAFSPPGGGGDGPGGPEPGWVDPRTWLSFQGGPPGGPGIGPGVGPSEVWGIPPCPPPYEFCGGMAYCGPQVGVGLVPQGGLETSQPEGEAGVGVESNSDASPEPCTVTPGAVKLEKEKLEQNPEESODIKALQKELEQFAKLLKQKRITLGYTQADVGLTLGVLFQKVFSSQTTICRFEALQLSFKNMCKLRPLLQKWVEEADNNENLQEICKAETLVQARKRKRTSIENRVRGNLENLFLQCPKPTLQQISHIAQQLGLEKD VVRVWFNRRQKGRSSSDYAQRAAFAAAGSPFSGGPVSFPLAPGPHFGTTPGYGSPHFTALYSSVFPFAGAAFPVSVTTLGSPMHNS
7	OCT4'-3 (aa)	MAGHLASAFASPPGGGGAGPGGPAPGWVAPRTWLSFQGGPPGGPGIGPGVGPSEVWGIPPCPPPYAFCGGMAYCGPQVGVGLVPQGG LATSQAGAAAGVGASNSAGASAPACTVTPGAVKLAKAKLAQNPAESQAIALQKELEQFAKLLKQKRITLGYTQADVGLTLGVLFQKVFSSQTTICRFEALQLSFKNMCKLRPLLQKWVEEADNNENLQEICKAETLVQARKRKRTSIENRVRGNLENLFLQCPKPTLQQISHIAQQLGLE KD VVRVWFNRRQKGRSSSDYAQRAAFAAAGSPFSGGPVSFPLAPGPHFGTTPGYGSPHFTALYSSVFPFAGAAFPVSVTTLGSPMHNS
8	OCT4'-4 (aa)	MAGHLASDFAFSPPGGGGDGPGGPEPGWVDPRTWLSFQGGPPGGPGIGPGVGPSEVWGIPPCPPPYEFCGGMAYCGPQVGVGLVPQGGLETSQPEGEAGVGVESNSDASPEPCTVTPGAVKLEKEKLEQNPAASODIKALQKELEQFAKLLKQKRITLGYTQADVGLTLGVLFQKVFSSQTTICRFEALQLSFKNMCKLRPLLQKWVEEADNNENLQEICKAETLVQARKRKRTSIENRVRGNLENLFLQCPKPTLQQISHIAQQLGLEKD VVRVWFNRRQKGRSSSDYAQRAAFAAAGSPFSGGPVSFPLAPGPHFGTTPGYGSPHFTALYSSVFPFGEAAPPVSVTTLGSPMHNS
9	OCT4'-5 (aa)	MAGHLASAFASPPGGGGAGPGGPAPGWVAPRTWLSFQGGPPGGPGIGPGVGPSEVWGIPPCPPPYEFCGGMAYCGPQVGVGLVPQGGLETSQPEGEAGVGVESNSAGASPEPCTVTPGAVKLEKEKLEQNPEESQAIALQKELEQFAKLLKQKRITLGYTQADVGLTLGVLFQKVFSSQTTICRFEALQLSFKNMCKLRPLLQKWVEEADNNENLQEICKAETLVQARKRKRTSIENRVRGNLENLFLQCPKPTLQQISHIAQQLGLEKD VVRVWFNRRQKGRSSSDYAQRAAFAAAGSPFSGGPVSFPLAPGPHFGTTPGYGSPHFTALYSSVFPFGEAAPPVSVTTLGSPMHNS
10	OCT4'-6 (aa)	MAGHLASDFAFSPPGGGGDGPGGPEPGWVDPRTWLSFQGGPPGGPGIGPGVGPSEVWGIPPCPPPYAFCGGMAYCGPQVGVGLVPQGG LATSQAGAAAGVGASNSDASAPACTVTPGAVKLAKAKLAQNPAASQAIALQKELEQFAKLLKQKRITLGYTQADVGLTLGVLFQKVFSSQTTICRFEALQLSFKNMCKLRPLLQKWVEEADNNENLQEICKAETLVQARKRKRTSIENRVRGNLENLFLQCPKPTLQQISHIAQQLGLE KD VVRVWFNRRQKGRSSSDYAQRAAFAAAGSPFSGGPVSFPLAPGPHFGTTPGYGSPHFTALYSSVFPFAGAAFPVSVTTLGSPMHNS
12	MYC-WT (aa)	MPLNVSTNRYDLDYDSVQPYFYCDEENFYQQQQSELQPPAPSEDIWKKFELLTPPLSPSRSSGLCSPSYVAVTPFSLRGDNDGGGGSFSTADQLEMVTELLGGDMVNQSFCDPDDDEFIKNIIQDCMWSGFSAAAKLVSEKLASYQAARKDSGSPNPARGHSCVSTSSLYLQDLSAAASECIDPSVVPYPLNDSSSPKSCASQDSSAFSPSSDLSLSTESSPQGSPEPLVHEETPPTTSSDSEEEQDEEEIDVVSVEKRQAPGKRRESGSPSAGGHSKPPHSPVLVLRCHVSTHQHNYAAPSTRKDYPAARRVRLDSVRVLRQISNNRCKTSPRSSDTEENVKRTHNVLERQRRNELKRSFFALRDQIPELENNEKAPKVVLKATAYILSVQAEQKLISEEDLLRRRREQLRHRLEQLRNSCA
14	MYC' (aa)	MPLNVSTNRYDLDYDSVQPYFYCDEENFYQQQQSELQPPAPSEDIWRRFELLTPPLSPSRSSGLCSPSYVAVTPFSLRGDNDGGGGSFSTADQLEMVTELLGGDMVNQSFCDPDDDEFIRNIIQDCMWSGFSAAARLVSERLASQAARKDSGSPNPARGHSCVSTSSLYLQDLSAAASECIDPSVVPYPLNDSSSPKSCASQDSSAFSPSSDLSLSTESSPQGSPEPLVHEETPPTTSSDSEEEQDEEEIDVVSVERRQAPGRRRESGSPSAGGHSRPPHSPVLVLRCHVSTHQHNYAAPSTRRDYPAARRVRLDSVRVLRQISNNRCKTSPRSSDTEENVRRTHNVLERQRRNELR RSEFFALRDQIPELENNERAPRYVILRRATAYILSVQAEQRLISEEDLLRRRREQLRHRLEQLRNSCA
15	MYC'-1 (aa)	MPLNVSTNRYDLDYDSVQPYFYCDEENFYQQQQSELQPPAPSEDIWKKFELLTPPLSPSRSSGLCSPSYVAVTPFSLRGDNDGGGGSFSTADQLEMVTELLGGDMVNQSFCDPDDDEFIKNIIQDCMWSGFSAAAKLVSEKLASYQAARKDSGSPNPARGHSCVSTSSLYLQDLSA

		AASECIDPSVVFYPLNDSSSPKSCASQDSSAFSPSSDLSSTESSPQGSPEPLVLHEETPPTTSSDSEEFQDEEEEDVVSVEKRQAPGKRSES GSPSAGGHSKPPHSPVLVKRCHVSTHQHNYAAPSTRKDYPAAKRVKLDVSVRLRQISNNRKCTSPRSSDTEENVKRRTHNVLERQRRNE LKRSFFALRDQIPELENNEKAPKVILKKATAYILSVQAEFEQKLISEEDLLRKRREQLKHKLEQLRNSCA
16	MYC'-2 (aa)	MPLNVSFTRNRYDLDDYDSVQPYFYCDEEENFYQQQQSELOPPAPSEDIWKKFELLTPPLSPSRSSGLCSPSYVAVTPFSLRGDNDGGGG SFSTADQLEMVTELLGGDMVYNQSFICDPDDETFIKNIIQDCMWSGFSAAAKLVSEKLASYQAARKDSGSPNPARGHSVCSTSSLYLQDLS AAASECIDPSVVFYPLNDSSSPKSCASQDSSAFSPSSDLSSTESSPQGSPEPLVLHEETPPTTSSDSEEFQDEEEEDVVSVEKRQAPGKRSES ESGSPSAGGHSKPPHSPVLVKRCHVSTHQHNYAAPSTRKDYPAAKRVKLDVSVRLRQISNNRKCTSPRSSDTEENVKRRTHNVLERQRRNE NELKRSFFALRDQIPELENNERAPKVILKKATAYILSVQAEFEQKLISEEDLLRKRREQLKHKLEQLRNSCA
17	MYC'-3 (aa)	MPLNVSFTRNRYDLDDYDSVQPYFYCDEEENFYQQQQSELOPPAPSEDIWKKFELLTPPLSPSRSSGLCSPSYVAVTPFSLRGDNDGGGG SFSTADQLEMVTELLGGDMVYNQSFICDPDDETFIKNIIQDCMWSGFSAAAKLVSEKLASYQAARKDSGSPNPARGHSVCSTSSLYLQDLSA AAASECIDPSVVFYPLNDSSSPKSCASQDSSAFSPSSDLSSTESSPQGSPEPLVLHEETPPTTSSDSEEFQDEEEEDVVSVEKRQAPGKRSES GSPSAGGHSKPPHSPVLVKRCHVSTHQHNYAAPSTRKDYPAAKRVKLDVSVRLRQISNNRKCTSPRSSDTEENVKRRTHNVLERQRRNE LKRSFFALRDQIPELENNERAPKVILKKATAYILSVQAEFEQKLISEEDLLRKRREQLKHKLEQLRNSCA
18	MYC'-4 (aa)	MPLNVSFTRNRYDLDDYDSVQPYFYCDEEENFYQQQQSELOPPAPSEDIWRRFELLTPPLSPSRSSGLCSPSYVAVTPFSLRGDNDGGGGG SFSTADQLEMVTELLGGDMVYNQSFICDPDDETFIKNIIQDCMWSGFSAAAKLVSEKLASYQAARKDSGSPNPARGHSVCSTSSLYLQDLSA AAASECIDPSVVFYPLNDSSSPKSCASQDSSAFSPSSDLSSTESSPQGSPEPLVLHEETPPTTSSDSEEFQDEEEEDVVSVEKRQAPGKRSES GSPSAGGHSKPPHSPVLVKRCHVSTHQHNYAAPSTRKDYPAAKRVKLDVSVRLRQISNNRKCTSPRSSDTEENVKRRTHNVLERQRRNE LKRSFFALRDQIPELENNEKAPKVILKKATAYILSVQAEFEQKLISEEDLLRKRREQLKHKLEQLRNSCA
19	MYC'-5 (aa)	MPLNVSFTRNRYDLDDYDSVQPYFYCDEEENFYQQQQSELOPPAPSEDIWKKFELLTPPLSPSRSSGLCSPSYVAVTPFSLRGDNDGGGG SFSTADQLEMVTELLGGDMVYNQSFICDPDDETFIKNIIQDCMWSGFSAAAKLVSEKLASYQAARKDSGSPNPARGHSVCSTSSLYLQDLS AAASECIDPSVVFYPLNDSSSPKSCASQDSSAFSPSSDLSSTESSPQGSPEPLVLHEETPPTTSSDSEEFQDEEEEDVVSVEKRQAPGKRSES ESGSPSAGGHSKPPHSPVLVKRCHVSTHQHNYAAPSTRKDYPAAKRVKLDVSVRLRQISNNRKCTSPRSSDTEENVKRRTHNVLERQRRNE ELKRSFFALRDQIPELENNEKAPKVILKKATAYILSVQAEFEQKLISEEDLLRKRREQLKHKLEQLRNSCA
20	MYC'-6 (aa)	MPLNVSFTRNRYDLDDYDSVQPYFYCDEEENFYQQQQSELOPPAPSEDIWRRFELLTPPLSPSRSSGLCSPSYVAVTPFSLRGDNDGGGGG FSTADQLEMVTELLGGDMVYNQSFICDPDDETFIKNIIQDCMWSGFSAAAKLVSEKLASYQAARKDSGSPNPARGHSVCSTSSLYLQDLSA AAASECIDPSVVFYPLNDSSSPKSCASQDSSAFSPSSDLSSTESSPQGSPEPLVLHEETPPTTSSDSEEFQDEEEEDVVSVEKRQAPGKRSES GSPSAGGHSKPPHSPVLVKRCHVSTHQHNYAAPSTRKDYPAAKRVKLDVSVRLRQISNNRKCTSPRSSDTEENVKRRTHNVLERQRRNE LKRSFFALRDQIPELENNEKAPKVILKKATAYILSVQAEFEQKLISEEDLLRKRREQLKHKLEQLRNSCA
22	KLF4 (aa)	MAYSDALLPSFSTFASGPA GREKTLRQAGAPNNRWREELSHMKRLPPVLGPRPYDLAAATVADLES GGAGAGACGGSNLAPLRRETEEF NDLLDLDFILSNLTHPPESVAATVSSASASSSSSPSSGASAPSTCSFTYPIRAGNDPGVAPGGTGGGLLYGRESAPPPTAPFNADINDV SPSGGFAELLRPELDPVYIPQPPQPPGGGLMGKFVLKASLSAPGESYSPSVISVSKGSPDGSHPVVAPYNGGPPRTCPKIKQEA VSSCT HLGAGPPLSNHRPAADHDFLGRQLPSRTTPTLGLLEVLSSRDCHPALPLPPGFHPHPGPNYPSPFLPDQMOPQVPLPHYQELMPPGSCMPPEE PKPKRGRRSWPRKRTATHTCDYAGCGKTYTKSSHLKAHLRTHTEKPYHCDWDGCGWKFA RSDDELTRHYRKHTGHRFFQCQKCDRAFS RSDHLALHMKRHF
24	SOX2 (aa)	MYNNMETELKPPGPQQTSGGGGGNSTAAAAGGNQKNSPDRVKRPMNAFMVWSRGQRRKMAQENPKMHNSEISKRLGAEWKLLSETE KRPFIDEAKRIRALHMKHEHPDYKYRPRRKTKTLMKKDKYTLPGGLLAPGGNSMASGVGVGAGLGAGVNRQMDSYAHMNGWSNGSYS MMQDQLGYPQHPGLNAHGAQMOPMHRDYVLSALQYNSMTSSQTYMNGSPTYMSYSYQQGTGPMALGSMGVSVKSEASSPPVVTSSS HSRAPCQAGDLRDMISMYPGAEVPEPAAPSRLLHMSQHYGSGPVPTAINGTLPLSHM
28	O'KM'S (nt)	ATGGGCTGGCCACCTGGCCCTCTGCCTTCGCCTTCTCTCCCCACCTGGCGGGCGGGCTGCGCGGCCCGGGAGGACCCGCCCTGGATGG GTGGGCCCTAGAACCTGGCTGTCTTCCAGGGACCTCTGGCGGACCCGGCATCGGACCTGGCGGCGGCGGCGGCGGCGGCGGCGGCGG GGCATACTCTCCCTGCCCTCCCCCTACGCCCTTCTGCGGGGGCATGGCCTACTGTGGCCCCCAGGTTCGGGGTGGCGCTGGTGGCCCCAG GGCGGCTCGCCACAGCCAGCCCGCCGCGCGCGCTGGTGTCTGGAAGTGGCCAGCAACTCCGCGGGGCGAGCCAGCCCATGCAC CGTGACTCCCGGCGCAGTGAAGCTGGCCAGGCCAAGCTGGCCCAAGATCTCGCGGCTCTCAGGCCATCAAGGGCGCTCCAGAAGG AGCTGGAGCAGTTCGCGAAGCTGTGAAGCAGAAGCGGATCAGCTGGGGTACACCCAGGCCGACGTCGGCTGACCTGGGCGTC CTGTTCTGGCAAGGTGTTAGCCAGACCACTCTGCCGGTTCGAGGCCCTGCAAGTCTAGCTTCAAGAACATGTGCAAGCTGCGCCCA CTTCTGCAAGAGTGGGTGGAGGAGGCGGATAACACAGAACCTGCAGGAGATCTGCAAGGCGAGCGCTGGTGACGCTTCACTACCCCATC AGCGAAAGCGGACCTCCATCGAGAACCGGCTTCGCGGGAACCTGGAGAACCTGTTCTGCAAGTGTCCCAAGCCTACACTGCAGCAG ATCTCTCACATAGCCAGCAGCTGGGCTGGAGAAGGATGTGGTGCAGTGTGGTCTGCAATCGGAGCGCAAGCGGCAAGCGCTC TCTCCGCTACGCCAGAGGGCGGCTTCGCCGCGGCGGGGTCCCGCTCAGCGGCGGCGGCTGCTGCTTCCCTTCCCTTCCGCCCCGGG CCACACTCTCGGACTCCCGGCTACGGCAGCCACACTTACCGGCGCTGTACAGCAGCGTGCATTCCTCTGCCCGGGCGGCTTCCCC CCCGTGAGCGTGACGACGCTGGGACGCCCATGCACAGCAAT GGAAGCGGAGCCACGAACCTCTCTCTGTTAAAGCAAGCAGGAGATGTTGAAGAAAACCCCGGGCT ATGGCGCTGTCCGACGCCCTGCTGCCCTATTCAAGACCTTCGCTCAGGCCCCGCTGGCAGAGAAAAGACACTGAGACAGGCTGGA GCCCTTAACAACCGCTGGCGCGAGGAGCTGAGTCACATGAAACCTGCCCTGCCCTGTCTCTGGCGGCGCATACGACCTGGCGGCC GCCACGGTGGCCACCGACCTGGAGTCAGCGGGGGCGGAGCGGCTGTGGCGGCTCCAACCTGGCGGCCCTGCCCGCAGGGAGAGC TGAGGAGTCAACGATCTGCTGGATCTGGAATCTATCTCAGTAACCTCTGACCCACCCCGAGAGAGTGTGTGCTACCGTGAGC AGCAGCGCCAGCGCGAGCTTACGAGCAGCCCTAGCAGCTCGGAGCTGCAAGTGCACCTGAGCAGCTTCACTACCCCATC AGGGCCGGTAACGACCTGGTGTGGCCCCGGCGGACCGGGGGCGGACTCTGTACGGCCGGGAGTCCGCCCCCCCGCCACCGC CCCCCTCAACCTGGCGGACATCAACGATGTGAGCCCTCCGGGGGCTTCGTGCGCGAGCTGCTGCGGCCAGAGCTGACCCCTGTGTA CATCCCCCAGCAGCGCCGAGCCCCGGGCGCGGCTGATGGGGAAGTTCGTGCTCAAGGCCAGCTGAGCGCACCCGGCTCTG AGTACGGAAGCCCCCTCCGTGATGATGTGAGCAAGGGAAGCCCCGAGGGTCAACACCCCGTGGTGTGGGCCCTTACAACCGCGGG CGCGCACGAGCTGCCCAAGATCAAGCAGGAGGAGTGCAGCTGACCCATCTGGGTGCGGGGACCACCTGTCCAACCGGCCA CCGCCCGCTGCCCATGACTTCCCTCTGGCGAGGAGCTGCCACAGGAGACCCCTACGCTGGGCTGGAGGAGGTCTGTCTC CAGGGACTGCCACCGGCCCTGCCCTGCCCGGGGTTCACCCCAACCCCGGACCAACTACCCAGCTTCTGCTGACAGAGAT GCAGCCGAGGTTCCGCCCTGCATTACAGGAGCTGATGCCCGGCGAGCTGCATGCCCGGAGGCTAAGCCCTAAGCGGGGCC GGCGCAGCTGGCCCCGAAGCGGACCGCCACCCACACTTGCAGATTACGCCGGGTGGCGCAAGACCTACACAAGAGTTGCACTG AAGGCCACCTGCGAAGTACACGGGCGAGAAGCCCTACCACTGCGACTGGGACGGGTGCGGCTGGAAGTTGCCCGGTCCGATGA GCTGACCCGGCACTATCGGAAGCACACCGGCCACAGGCCCTTCCAGTGCCAGAAAGTGTGACGGGCCCTTACAGCGGTGACAGCATC TGGCACTGCACATGAAGCGCACTTC GGAAGCGGAGAGGGGACGGGGAAGTCTTCAACATGCGGGGAGCTGGAGGAGAAATCCCGGCCCC ATGCCCTGAATGTGCTCTTCAACAACGCAACTGATCTGAGACTGACTCTGTGCAAGCTTACTTCTACTGCGATGAGGAGGAG AACTTCTACAGCAGCAGCAGCAGGCAACTGACGCCACCCGCCCATCCGAGGATATCTGGAGGAGGTTGAGCTGTGCCGAC CCCTCTCTCAGCCCCCTCAGGAGGAGCGGCTGTGAGTCCAGCTACGTGGCTGTGACCCCTTCCCTGCGGCGGAGACAACGA CGCGGGGGGGGAGCTTACGACCGCTGACAGCTGGAGATGGTACCGAGCTGTGGGTGGCGACATGGTCAACAGAGCTTCA TCTGCGACCTGATGATGAGACATTCATCAGGAACATCATCATCCAGGACTGCATGTGGTCCGGCTTCTCGGCCGTGCCAGGCTGG TCAGCGAGAGCTGGCCAGCTACAGGCCCGCGGAGGAGTCCGGTGCCTAACCCCGCAGGCGCACAGGCTGCTGCTCCAGC AGCAGCTGTACCTGCAGGATCTGAGCGCGCGCGGAGGAGTGCATAGATCTTCACTGGTGTCCCATACCACTGAACGACAGC AGCTCTCCAGGAGCTGCCCTTCGAGGACAGCAGCGCTTACGCCCTCGTCCGACTCGCTGCTGTCAGCAGCAGGAGTCAAGCCCC CAGGGCTCTCTGAGCCCTGGTGTGCTGACGAGGAGAGCCCTCGTACCACTCTCGGACTCCGAGGAGGAGGAGGAGGATGAGGA

		AGAGATCGACGTGGTCAGCGTGGAGAGGCGCCAGGCTCCTGGCAGGCGCAGCGAGTCTGGCAGCCCTCCGCCGGGGGCCACAGCA GGCCCCCGCACAGCCCGCTGGTGCTGAGGCGGTGTACGTGAGCACCACCAGCATAACTATGCTGCGCCTCCTTCCACCAGGAGGG ACTACCCCGCTGCCAGGCGGGTGAGGCTGGATAGCGTCCGGGTGCTGAGGCGAGATCAGCAATAACCGTAGGTGACCTCCCCCAGG AGCAGTGACACAGAGGAGAACGTGAGGAGGCGGACTCAACACGTTCTGGAGCGCCAGCGCCGGAACGAGCTGAGGCGGTCTCTT CGCTCTGAGGGACCAGATCCAGAGCTGGAGAAACATGAGAGGGCACCGAGGGTGGTATCTCTGAGGAGGGCCACCAGCTATATCC TGAGCGTGCAAGGCTGAGGAGCAGAGGCTGATCAGCGAGGAGGACTTGCTGAGGAGGCGGCGGAGCAGCTGAGGACAGGCTGGA GCAGCTGCGAAACAGCTGCGCC GGAAGCGGACAGTGTACTAATTATGCTCTCTTGAATTGGCTGGAGATGTTGAGAGCAACCCAGGTCCC ATGTACAACATGATGGAGACAGAGCTGAAGCCCCCGGGCTCAGCAGACCAGCGGCGGGGGCACTCCACC GCCGCCG TGCTGGGGGCAACCAGAAGAACAGCCCCGATAGGGTGAAGCGCCCTATGAACGCTTCATGGTGTGGTCCCGGGCCAGCGCCGTA AGATGGCTCAGGAGAACCTAAGATGCACAACCTCCGAGATCAGCAAGCGGCTGGGCGCGGAGTGGAAGCTGTGTGCGAGACCGA GAAGCGGCCATTTCATCGACGAGGCCAAGAGGCTTCTGCGCCTGCACATGAAGGAGCATCTGACTACAAGTACCGGCCCGGCCGA AGACCAAGACACTGATGAAGAAGGACAAGTACACCTTGCTGGGGGCTGCTGGCCCCCGGGGCAACTCCATGGCTTCCGGCGTG GGCGTGGGAGCCGCTAGGCGCTGGCGTGAACAGCGCATGGACTCCTACGCTCAGATGAACGGCTGGAGCAACGGGTCTACAG CATGATGCAGGACCAAGTTGGGCTACCCCCAGCACCTGGGCTGAACGCCCATGGGGTGGCCAGATGAGGCGCTGCATCGGTAG ACGTGAGTGCCCTGCACTACAACCTCCATGACATCTAGCCAGACCTACATGAACGGGAGTCCACCTACAGCATGAGCTATAGCCAGC AGGGGACCCAGCATGGCCCTGGGGTCCATGGGCTCCGTGGTGAAGTCGGAGGCCAGCTCCTACCTCCCGTGGTGACAAGCTCCT GCCACAGCCGGGCCCTGCCAGGCTGGGATCTGAGGGACATGATCTCCATGTACCTCCGGGCGCGAGGTGCCGAGCCTGTG TCCACAGCAGGCTGCATATGAGCCAGCATTACAGTCCGGGCCCTGCCGGCACTGCCATCAATGGGACACTGCCACTGTCCCAT TGTGA
30	O'KM' (nt)	ATGGCTGGCCACCTGGCCTCTGCCCTTCTCTCCCCACCTGGCGGCGGGGTGCCGGCCCCGGAGGAGCCCGCCCTGGATGG GTGGCCCCAGAACCTGGCTGTACTTCCAGGGACCTCTTGGCGGACCCGGCATCGGACCTGGCGTGGGGCCCGGAGCGCCGTGTGG GGCATACCTCCCTGCCCTCCCCCTACGCCCTTCTGCGGGGCTATGGCTACTGTGGCCCCAGGTGCGGGTGGCCCTGTGCCACG GGCGGCTCGCCACCAGCCAGCCGCCCGCGCGCTGGTGTGCGAGTGCCAGCAACTCCGCCGGGCCAGCCAGCCCCATGCAC CGTGACTCCCGGCGCAGTGAAGCTGGCCCAAGGCCAAGCTGGCCCAAGAACTCTGCCGCTCTCAGGCCATCAAGGGCTCCAGAGG AGCTGGAGCAGTTTCGCGAAGCTGTGAAGCAGAAGCGGATCACGCTGGGGTACACCCAGGCCAGCTGGGCTGACCTGGGCGT CTGTTCGGCAAGGTGTTAGCCAGACCACTCTGCCGGTTCGAGGCCCTGCAAGCTTAGCTTCAAGAACATGTGCAAGCTGGCCCA CTTCTGCAGAAAGTGGTGGAGGAGGCGGATAACAACGAGAACCTGCAGGAGATCTGCAAGGCCAGAGCGCTGGTGCAGGCCAGGA AGCGAAAGCGGACCTCCATCGAGAACCGGTTTCGGGGAACCTGGAGAACCTGTTCTGAGTGTCCCAAGCTGTCCAGCAGAG ATCTCTACATAGCCACAGCTGGGCTGGAGAAAGATGTGGTGGAGTGTGGTTCGCAATCGGAGGAGCAAGGGCAAGCGCTC TTCTCCGCTACGCCAGAGGGCGGCTTCCGCCGCGCGGGGTCCCCCTTACGCGGCGGCCCTGTCTTCCCTTCCCTTCCCTCCCGG CCACACTTCCGGACTCCCGGCTACGGCAGCCACACTTACCAGCGCTGTACAGCAGCGTGCCATTCCCTGCCGGGGCGGCTTCCCC CCCGTGAGCGTGACGACGCTGGGCGAGCCCATGCACAGCAAT GGAAGCGGAGCCACGAACTTCTCTGTAAAGCAAGCAGGAGATGTTGAAGAAAACCCCGGGCT ATGGCCGTGTCCGACGCCCTGCTGCCCTCATTACGACCTTCCGCTCAGGCCCCGCTGGCAGAGAAAAGACACTGAGACAGGCTGGA GGCCCTTAACAAACCGCTGGCGCAGGAGCTGAGTACATGAACACGCTGCCCTGTCTGCTGGCGGCCATACGACCTGGCCGCC GCCACCTGGCCACCGACCTGGAGTCAAGCGGGGCGGAGCGCTGTGGCGGCTCAGCTGTGGCGGCTCAGCTGTGGCGGCTGAGG TGAGGAGTTCAACGATCTGCTGGATCTGGAATTCATCCTCAGTAACTCTGACCCACCCCGCAGAGAGTGTGTGCTACCGTGAGC AGCAGCGCCAGCGGAGCTCTAGCAGCAGCCCTAGCAGCTCCGGGCTGCCAGTGCCCCAGCACCTGCAGCTTACCTACCCCATC AGCGAGCGGTAAACGACCTGGTGTGGCCCCCGGGGCGGACCTGTATCGGCGGGGAGTCCGCCCGCCGCCACCGC CCCCCTCAACCTGGCGGACATCAACGATGTGAGCCCTCCGGGGGCTTCTGTCGCGGAGCTGCTCGCGGCAAGCTGCAACCTGTGTA CATCCCCCACAGCAGCGAGCCCGCGGCGGCGCTGATGGGGAAGTTCTGTGCTCAAGGCCAGCTGAGCGCACCCGGCTCTG AGTACGGGAAGCCCCCTCCGTGATCAGTGTGAGCAAGGGAAGCCCCGAGCGCTACACCCCGTGGTGTGTGGCCCCCTACAACGGCGGG CCGCCACGGACCTGCCCAAGATCAAGCAGGAGGCAAGTGAAGCTGACCCATCTGGGTGCGGGGCCACCACTGTCCAAAGCGGCC CCGCCCCGTGCCCATGACTTCCCTTGGGCGAGGAGCTGCCAGCAGGACCAACCTACGCTGGGGCTGGAGGAGTCTCTGCCCTC CAGGGACTGCCACCCGGCCCTGCCCTGCCCGGGGTTCCACCCCAACCCCGGACCAACTACCCAGCTTCTGCTGCTGACAGAT GCAGCCGAGGTTCCGCCCTGCAATACAGGAGCTGATGCCCGGGGAGCTGCATGCCCGAGGAGCTTAAGCCTAAGCGGGGCC GGCGAGCTGGCCCGCAAGCGGAGCCACACTTGGGATACGCCGGGTGCGGCAAGACTACACAAAGAGTTCCGACCTG AAGGCCACCTGCGAACTCACACGGGCGAGAAGCCCTACCACTGCCAGTGGGACGGGTGCGGGCTGGAAAGTTGCCCGGTCCGATGA GCTGACCCGGCACTATCGGAAGCACACCGGGCACAGGCCCTTCCAGTGCCAGAAGTGTGACCGGGGCTTACGCCGCTCAGACCATC TGGCACTGCATGAAGCGCACTT GGAAGCGGAGGAGGCGAGGGGAAGTCTTCAACATGCGGGGACGTGGAGGAAAATCCCGGCC ATGCCCTGAATGTGCTTCAACAAACGCACTATGATCTGGATGACTCTGTGACGCTTACTTCTACTGCGATGAGGAGGAG AACTTCTACAGCAGCAGCAGCAGAGGCAACTGCAGCCACCCGCCCATCCGAGGATATCTGGAGGAGGTTTCGAGCTGTGCCGAC CCCTCTCTCAGCCCCCTCAGGAGGAGCGGGCTGTGAGTCCAGCTACGTGGCTGTGACCCCTTCTCCCTGCGCGGGGACACGA CGCGCGGGGGGAGGCTTCAACAGCAGCTGACAGCTGGAGTGTGACAGGAGCTGCTGGGTGGGAGGAGTCAACCAAGAGCTTCA TCTGCGACCTGATGATGAGACATTATCAGGAACATCATCATCAGGAGTGCATGTGGTCCGGCTTCTCGGCCGCTGCCAGGCTGG TGAGCGAGAGGCTGGCCAGCTACAGGCCCGCCGAGGGATTCCGGGTGCGCTAACCCCGCAGGGGCCACAGCGTCTGTCTCAGC AGCAGCTGTACCTGCAGGATCTGAGCGCCGCCGCCAGCGAGTGCATAGATCCTTCAAGTGGTGTCCCATACCCACTGAACGACAGC AGCTCTCCAGGAGCTGCGCTCGCAGGACAGCAGCGCTTACGCCCCCTGTCGAGCTGCTGTGTCAGCAGCCAGGTGAGCCCC CAGGGCTCTCTGAGCCCTGGTGTGACGAGGAGACCCCTCCTACACCTCCTCGGACTCCGAGGAGGAGCAGGAGGATGAGGA AGAGATCGAGCTGGTCAAGCTGGAGAGGCGGAGGCTCTGGCAGGCGCAGCGAGTGTGGCAGCCCTCCCGCGGGGGCCACAGCA GGCCCCCGCACAGCCCGCTGGTGTGAGGCGGTGTACGTGAGCAGCCACAGCATAACTATGCTGCGCTCTTCCACAGGAGGG ACTACCCCGCTGCCAGGCGGTGAGGCTGGATAGCGTCCGGGTGCTGAGGCGAGTACAGCAATAACCGTAGGTGACCTCCCCAGG AGCAGCTGACAGAGGAGAGACGTGAGGAGGCGGACTACAACAGTCTTGGAGCGCCAGCGCGGAACGAGCTGAGGCGGTCTTCT CGCTCTGAGGGAGCAGATCCAGAGCTGGAGAAACATGAGAGGGCACCGAGGGTGGTATCTCTGAGGAGGGCCACCAGCTATATCC TGAGCGTGCAGGCTGAGGAGCAGAGGCTGATCAGCGAGGAGGACTTGCTGAGGAGGCGGCGGAGCAGCTGAGGCACAGGCTGGA GCAGCTGCGAAACAGCTGCGCCTGA
32	O'KS (nt)	ATGGCTGGCCACCTGGCCTCTGCCCTTCTCTCCCCACCTGGCGGCGGGGTGCCGGCCCCGGAGGAGCCCGCCCTGGATGG GTGGCCCCAGAACCTGGCTGTACTTCCAGGGACCTCTTGGCGGAGCCCGGCATCGGACCTGGCGTGGGGCCCGGAGCGCCGTGTGG GGCATACCTCCCTGCCCTCCCCCTACGCCCTTCTGCGGGGCTATGGCTACTGTGGCCCCAGGTGCGGGTGGCCCTGTGTGCCAG GGCGGCTCGCCACCAGCCAGCCGCCCGCGCGCTGGTGTGCGAGTGCCAGCAACTCCCGGGGGCCAGCCAGCCCCATGCAC CGTGACTCCCGGCGCAGTGAAGCTGGCCCAAGGCCAAGCTGGCCCAAGAACTCTGCCGCTCTCAGGCCATCAAGGCGCTCAGAAGG AGCTGGAGCAGTTTCGCGAAGCTGTGAAGCAGAAGCGGATCACGCTGGGGTACACCCAGGCCAGCTGGGCTGACCTGGGCGT CTGTTCCGCAAGGTGTTAGCCAGACCACTCTGCCGTTTCGAGGCCCTGCAAGCTTAGCTTCAAGAACATGTGCAAGCTGCGCCCA CTTCTGAGGAAGTGGTGGAGGAGGCGGATAACAACGAGAACTGCAAGGAGATCTGCAAGGCCAGAGCGCTGGTGCAGGCCAGGA AGCGAAAGCGGACCTCCATCGAGAACCGGCTTCCGGGGAACCTGGAGAACCTGTTCTGCAAGTGTCCCAAGCCTACACTGCAGCAG ATCTCTACATAGCCAGCAGCTGGGCTGGAGAAAGATGTGGTGGAGTGTGGTTCGCAATCGGAGGCAAGGGCAAGCGGCTC TTCTCCGCTACGCCAGAGGGCGGCTTCCGCCGCGGGGTCCCCCTTACGCGGCGGCCCGGTGTCTTCCCTTCCCTTGGCCCCGGG CCACACTTCCGGACTCCCGGCTACGGCAGCCACACTTACCCGCGCTGTACAGCAGCGTGCCATTCCCTGCCGGGGCGGCTTCCCC

		CCCGTGAGCGTGACGACGCTGGGCAGCCCCATGCACAGCAATGGAAGCGGAGCCACGAACCTTCTCTGTGTTAAAGCAAGCAGGAGA TGTGTAAGAAAACCCCGGGCCTATGGCCGTGTCCGACGCCTGCTGCCCTCATTACGACCTTCGCCTCAGGCCCCGCTGGCAGAGA AAAGACACTGAGACAGGCTGGAGCCCCAACAACCGCTGGCGCGAGGAGCTGAGTCACATGAAACGCCTGCCCCCTGTCCTGCCCTG GGCGGCCATACGACCTGGCCGCCGCCACGGTGGCCACCGACCTGGAGTCAGGCGGGGCCGGAGCCGCTGTGGCGGCTCCAACCTG GCGCCCTGCCCGCAGGGAGACTGAGGAGTTCAACGATCTGCTGGATCTGGACTTCATCTCAGTAACCTCTGACCCACCCCCCA GAGAGTGTGTCTACCGTGAGCAGCAGCGCCAGCGAGCTCTAGCAGCAGCCCTAGCAGCTCCGGGCTGCCAGTCCCCCAG CACCTGCAGCTTACCTACCCCATCAGGGCCGGTAACGACCTGGTGTGGCCCCCGCGGCCACCGGGGGCGACTCTGTACGGCCG GGAGTCCGCCCGCCCGCCACCGCCCTTCAACCTGGCGGACATCAACGATGTGAGCCCTCCGGGGGCTTCGTGCCGAGCTGCT GCGGCCAGAGCTCGACCTGTGTACATCCCCCACAAGCAGCCGCAAGCCCCGGGCGGGCCCTGATGGGGAAGTTCGTGCTCAAGG CCAGCCTGAGCGCACCCGGCTCTGAGTACGGAAGCCCTCCGTGATCAGTGTGAGCAAGGGAAGCCCCGACGGCTCACACCCCGTG GTTGTGGCCCCCTACAACGGCGGGCGGCCACGGACCTGCCCAAGATCAAGCAGGAGGCAAGTGAAGCTGCACCCATCTGGGTGC GGGGCCACCACTGTCCAACGGCCACCGCCCGCTGCCATGACTTCCCTCTGGGCAGGCAGCTGCCAGCAGGACACCCCTACGCT GGGCCTGGAGGAGTCTGTCTCCAGGACTGCCACCCGGCCCTGCCCTGCCCGGGGTTCACCCCAACCCCGGACCCAACTA CCCCAGCTTCTGCTGACAGATGACAGCGCAGGTTCCGCCCTGCATTACCAGGAGCTGATGCCCGGGGAGCTGCATGCCCGA GGAGCCTAAGCCTAAGCGGGGCCGGCGCAGCTGGCCCGCAAGCGAACCCACCACTTGCATTACGCGGGGTGCGCGGCAAGA CCTACACAAAGAGTTGCGACCTGAAGGCCCACTGCGAATCACACGGCGGAGAAAGCCCTACCACTGCGACTGGGACGGGTGCGGC TGGAACTTGCCCGGTCCGATGAGCTGACCCGGCACTATCGGAAGCACACCGGGCACAGCCCTTCCAGTGCCAGAAAGTGTGACCC GGCCCTCAGCGGTGACACCTGTCGGCACTGGAAGCGCACTTCGGAAGCGGAGAGGCGAGGGAAGTCTTCAACATCGG GGGACGTGGAGGAAAAATCCCGGCCCATGTACAACATGATGGAGACAGAGCTGAAGCCCCCGGGCCTCAGCAGACAGCGGGCG CGGCGGGGCAACTCCACCGCGCCGTGCTGGGGCAACAGAGAAGAACAGCCCGGATAGGCTGAAGCCCTATGAACGCCCTTCA TGGTGTGGTCCCGGGCCAGCGCCGTAAGATGGCTCAGGAGAAACCTAAGATGCACAACCTCCGAGATCAGCAAGCGGTGGGCGCG GAGTGGAAAGTGTCTGTCGAGAGCCGAGAAAGCGGCCATTCATCGACAGAGGCCAAGAGGCTTCGTGCCCTGCACATGAAGGAGCATCC TGACTACAAGTACCGGCCCGCCGGAAGCAAGACACTGATGAAGGAAGCAAGTACACCTTGCTGGGGGCTGCTGGCCCGG GGGGCAACTCCATGCTTCCGGCGTGGCGTGGGAGCCGCTTAGCGCTGGCGTGAACAGCGCATGGACTCTACGCTCACATG AACGGCTGGAGCAACGGGTCTACAGCATGATGCAGGACCAAGTTGGGCTACCCCAAGCACCCTGGGCTGAACGCCCATGGGGTGC CCAGATGACGCCATGTCATCGGTACGAGCTGAGTGGCTGACAGTGAACCTCCATGACATCTAGCCAGCTGATGAACGGGATCC CACCTACAGCATGAGCTATAGCCAGCAGGGGACCCCAAGGCATGGCCCTGGGCTCCATGGGCTCCGTGGTGAAGTCCGAGGCCAGCT CCTCACCTCCCGTGGTGACAAGCTCCTCCACAGCCGGGCCCTGCCAGGCTGGGATCTGAGGACATGATCTCCATCTCACTCC CGGGCCGAGGTGCCGAGCTGCTGCTCCAGCAGGCTGCATAGAGCCAGCAATACCACTCCGGGCGCTGCGGCGCATGCCA TCAATGGGCACTGCCACTGTCCCATATGTA
35	LV-TRE3G- OKM' (nt)	AATGTAGTCTTATGCAATACTCTGTAGTCTTGCAACATGGTAACGATGAGTTAGCAACATGCCTTACAAGGAGAGAAAAAGCACCG TGCATGCCGATTGGTGGAAAGTAAGGTGGTACGATCGTGCCTTATTAGGAAGGCAACAGACGGGTCTGACATGGATTGGACGAACCA CTGAATTGCCGCTTGCAGAGATATTGTATTTAAGTGCCTAGCTCGATACATAAACGGGTCTCTCTGGTTAGACAGATCTGAGCCCTG GGAGCTCTGGCTAACTAGGGAACCCACTGCTTAAGCCTCAATAAGCTTGCCTTGAAGTGTGCTTGAAGTGTGCTGCTGCTGCTG TGTGACTCTGGTAAGTACAGATCCCTCAGACCTTTTACTGCTGAGTGTGGAATACTCTAGCAGTGGCGCCCAACAGGCACTTGAAG CGAAAGGGAAACAGAGGAGCTCTCTGACGCGAGGACTCGGCTTGTCTGAAGCGCGCACGGCAAGAGGCGAGGGGCGGCGACTGGT GAGTACGCAAAAAATTTTACTAGCGGAGGCTAGAAGGAGAGAGATGGGTGCGAGAGCGCTCAGTATTAAGCGGGGAGAGAACTTGA TCGCATGGGAAAAAATTCGGTTAAGGCCAGGGGGAAGAAAAATATAAATTAACATATAGTATGGGCAAGCAGGGAGCTAG AACGATTGCGAGTTAATCTGGCCTGTTAGAAACATCAGAAAGGCTGTAGACAATACTGGGACAGCTACAACCATCCCTTCAGACAG TAGCAGAAAGAACTTAGATCATTATATAATACAGTAGCAACCTCTGATTGTGTGCATCAAGGATAGAGATAAAAGCAGACCAAGGAA GCTTTAGACAAGATAGAGGAAGAGCAAAACAAAGTAAGACCCAGCCAGCAGCAAGCGCCGCTGATCTTCAGACCTGGAGGAGGA GATATGAGGCAAAATGGAGAGTGAATTATATAAATAAAGTAGTAAAAATTAAGCACTTAGGAGGACACCCCAAGGCAAA GAGAAGAGTGTGTCAGAGAGAAAAAGAGCAGTGGGAATAGGAGCTTTGTTCTTGGGTTCTTGGGAGCAGCAGGAAGCACTATG GCGCAGCGCTCAATGACGCTGACGCTACAGGCCAGACAATTATTGTCTGGTATAGTGCAGCAGCAGACAATTTGCTGAGGGCTATT GAGGCGCAACAGCATCTGTTGCAACTACAGCTCTGGGGCATCAAGCAGCTCCAGGCAAGAATCTGGCTGTGGAAAGATGCTTAA GGATCAACAGCTCTGGGGATTGGGGTGTCTTGAAAACTCATTTGCACCACTGCTGTGCCCTTGGAAATGCTAGTTGGAGTAATAA ATCTCTGGAAACAGATTTGGAATCACACGACCTGGATGGAGTGGGACAGAGAAATTAACAAATTCACAAAGCTTAATACACTCTTAAT TGAAGAACTCGCAAAACAGCAAGAAAGAAATGGAACAAAGAAATTTGGAATTAGATAAATGGAAATTTGTTGGAATTTGTTTAA TAACAAATTTGCTGTGGTATATAAATTAATCATAATGATAGTAGGAGGCTTGGTGGTTAAGAAATAGTTTGTGCTGACTTTCTAT AGTGAATAGAGTTAGGCGGGATATTCACCATATCGTTTCAGACCCACCTCCCAACCCGAGGGGACCCGACAGGCCCGAAGGAA TAGAAGAAAGGTTGAGAGAGAGAGACAGAGACAGATCCATTCTGATTAGTGAACGGATCTCGACGTTTACCGTATCTGCTTTAAAGAA AAGGGGGGATTGGGGGTACAGTGCAGGGGAAAGAAATAGTAGACATAATAGCAACAGACATACAAAGTAAAGAAATCAAAAAACA AATTACAAAAATTCAAAAATTTTACTAGTGATTATCGGATTGATAATCGAATTCACCGTITTTACTCCCTATCAGTGTAGTATGAGCA TGAAGAGTTTACTCCCTATCAGTGTAGAGAACGTATGACAGCTTTACTCCCTATCAGTGTAGAGAACGTATAAGGAGTTTACTCC CTATCAGTGTAGAGAACGTATGACAGTTTACTCCCTATCAGTGTAGAGAACGTATCTACAGTTTACTCCCTATCAGTGTAGAGAG AACGTATCCAGTTTACTCCCTATCAGTGTAGAGAACGTATGAGCAAGCTATAAGCTTTAGGCGTGTACGCTGGCGCCTATAAAAGCAGT TTTAGTGAACCGTCAGATCGCCTGGAGCAATTCACAACACTTTTGTCTTATACCAACTTTCCGTACCACCTTCTACCCTCGTAACT CGAGGCCGCCACCATGGGTGGCCACCTGGCCCTGCTGCTTCCGCTTCTCTCCCCACCTGGCGGGCGGCTGCCGGCCCCGGAGGACC CGCCCCCTGGATGGGTGGCCCCTAGAACCTGGCTGTCATTCCAGGGACCTCCTGGCGGACCCGGCATCGGACCTGGCGTCCGGCCCGG CAGCGCGGTGTGGGCATACCTCCCTGCCCTCCCCCTACGCCCTTCTGCGGGGGCATGGCTACTGTGCCCCCAAGGTCGGGGTCCG CCTGGTGGCCAGGGCGGCTCGCCACCCAGCCAGCCCGCGGGCGCGCTGGTGTGGAGTGGCCAGCAACTCCCGCGGGGCCAGCC CAGCCCCATGACCGTGACTCCCGGCCAGTGAAGCTGGCCAGGCCAAGCTGGCCCAAGTCTGCCCTCTCAGGCAATCAAG GCGCTCGGAGAGGAGCTGGAGCAGTTGCGGAAGCTGCTGAAGCAGAAAGCGGATCAGCTGGGGTACACCCAGGCCAGGTCGGCCT GACCTTGGGGCTCTGTGGCAAGTGTTCAGCCAGACCACTCTGCCGTTTCGAGGGCTCGAGCTTAGCTTACGAACATGCTG CAAGCTGCGCCCACTTCTGCAAGAGTGGGTGGAGGCGGATAACAACGAGAACCTGCAAGGAGATCTGCAAGGCCAGACGCTG GTGACAGCCAGGAAGCGAAAGCGGACCTCCATCGAGAACCAGCTTCCGCGGAACCTGGAGAACCTGTCTGCAAGTGTCCCAAGCC TACACTGCGAGCATCTCTACATAGCCAGCAGCTGGGCTGGAGAGGATGTGGTGGAGTGTGGTCTGCAATCGGAGGCAGA AGGGCAAGCGCTCTCTCCGCTACGCCAGAGGGCGGCTTCCGCGCGGCGGGTCCCCCTTACGCGGCGGCCCCGTGTCTCTCC CTCTGGCCCCCGGGCCACACTTCGGGACTCCCGGCTACGGCAGGCCACACTTACCAGCGCTGTACAGCAGCGTGCATCTCCCTGGC GGGCGGCTTCTCCCCCGTGAAGGTGACGAGCTGGGCGGCCATGCAACAGCAATGGAAGCGGAGGCCACGAACTTCTCTGTGTA AGCAAGCAGGAGATGTTGAAGAAACCCCGGGCTATGGCGGTGTCCGACGCCCTGCTGCCCTCATTCAGCACCTTCGCTCAGGCC CCGCTGGCAGAGAAAAAGCACTGAGACAGGCTGGAGCCCTAACAACCGCTGGCGCGAGGAGCTGAGTCACATGAAACGCTGCCC CCGTCTGCTGCTGGCGGCCATACGACCTGGCCCGCCGACCGTGGCCACCGACTGGAAGTACGGCGGGCGGAGCCGCTGTGG CGGCTCCAACCTGGCGCCCTGCCCGCAGGGAGACTGAGGAGTTCAACGATCTGCTGGATCTGGAATCTCATCTCAGTAATCTCT GACCCACCCCCAGAGAGTGTGCTGCTACCGTGAGCAGCGCCGAGCTCTAGCAGCAGCTTACGAGCTTCCGCGGCTG CCAGTGGCCCGACCTGACGCTTACCTACCCATCAGGGCCGGTAACGACCTGGTGTGGCCCCCGGGGACCGGGGCGGA CTCCTGACGGCCGGAGTCCGCCCCCCCGCCACCGCCCTTCAACCTGGCGGACATCAACGATGTGAGCCCTCCGGGGGCTTC GTCGCGAGAGTGTGCGGCCAGAGCTCGACCTGTGTACATCCGCCACAGCAGCGCCGCGGCGGCTGATGGGGA GTTCTGTCTCAAGGCCAGCTGAGCGCACCCGGCTGTGATACGGAAGCCCTCCGTGATCAGTGTGAGCAAGGGAAGCCCCGACG GCTCACACCCCGTGGTGTGGCCCCCTACAACGGCGGGCGCCACGGACCTGCCCAAGATCAAGCAGGAGGCAAGTGTGAGCAGCTGC

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[illegible]

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[illegible]

		ACCCTGGTGTGCCCCCGGCGGCACCGGGGGGACTCCTGTACGGCCGGAGTCCGCCCCCGCCACCGCCCCCTTCAACTGG CGGACATCAACGATGTGAGCCCTCCGGGGGCTTCGTGCGCGAGCTGCTGCGGCCAGAGCTCGACCTGTGTACATCCCCACAGC AGCCGAGCCCCGGGGCGGGCTGATGGGGAAGTTCTGCTCAAGGCCAGCCTGAGCGCACCCGGCTCTGAGTACGGAAGCCCC TCCGTGATCAGTGTGAGCAAGGGAAGCCCCGACGGCTCACACCCCGTGGTTGTGGCCCCCTACAACGGCGGGCCCGCCACGGACCTG CCCCAAGATCAAGCAGGAGGCACTGAGCAGCTGCACCCATCTGGGTGCGGGGCCACCACTGTCCAACGGCCACCGCCCCGTGCC ATGACTTCCCTTGGGACGGCAGCTGCCAGCAGGACCACCCCTACGCTGGGCTGGAGGAGGCTCTGTCTCCAGGGACTGCCACC CGGCCCTGCCCTGCCCGGGGTTCCACCCCCACCCGGGACCCAACTACCCAGCTTCTGTCTGACCAAGATGCAGCCGAGGTTT CGCCCCGTGATTACAGGAGCTGATGCCCGGGGAGCTGCATGCCGAGGAGGCTAAGCCTAAGCGGGGCCGGCGACGTGGGCC CGCAAGCGGACCGCCACCCACACTTGGGATTACGCCGGGTGCGGCAAGACCTACACAAAGAGTTCCACCTGGAAGGCCACCTGGC AACTCACACGGGCGAGAAGCCCTACCCTGCGACTGGGACGGGTGCGGTGGAAGTTCCGCCGGTCCGATGAGCTGACCCGGCACT ATCGGAAGCACACCGGGCACAGGCCCTTCCAGTGCCAGAAGTGTGACCGGGCTTACGCCGGTCAGACCATCTGGCACTGCACATG AAGCGCCACTTCTGATGATAAAAAAACAACAAACAAACAGGCTATTATGCGTTACCGGGGATGCTCAATGTACCTATTTCTATGGG CCTTGGCGGGGAAACACGCAAGGGATGGTGTCAAATTCGGCGAAACCTAAGCGCCCCCGGGCGTATGGCAACGCCGAGCCAA GCTTCGGCGCCAATTCCATTATCAGATTCTAGAG
59	O'KM'- circRNA (nt)	GGGAGACCTCGAATGGAATTTGCGCCGATGAAGGTGTAGAGACTAGACGGCACCCACCTAAGGCAACACGCTATGGTGAAGGCATA GTCCAGGGAGTGGCGAAAGTACACAAACCGGAATATTGGACTCGCCGGAACGCAATAGCCGAAAAACAAAAACAAAAAAC AAAAAACAACAAAAACAAAAACACATTAACAGCCTGTGGGTTGATCCACCCACAGGCCCATTTGGGCGCTAGCACTCTGGT ATCACGGTACCTTTGTGCGCTGTTTATACCCCTCCCCCACTTAACCTTAGAAGTAACACACAGCACTAACCGGAGCTGCGGCA CACCAGCCACGTTTGTATCAAGCACTTGTATACCCCGGACTGAGTATCAATAGACTGCTACCGGGTTGAAGGAGAAAGCGTTCGT TATCCGGCAACTACTTCGAAAAACCTAGTAACACCGTGGAGTTGACAGAGTGTTCGCTCAGCACTACCCAGTGTAGATCAGGTC GATGAGTACCCGATTCCCCACGGGCGACCGTGGCGGTGGCTGCGTTGGCGGCTGCCATGGGGAACCCATGGGACGCTCTAAT ACAGACATGGTGCGAAGAGTCTATTGAGCTAGTTGGTAGTCTCCGGCCCCGTAATGCGGGTAATCCTAAGTGGGACACACACCC TCAAGCCAGAGGGCAGTGTGTCGTAACGGCAACTTGCAGCGGAACCGACTACTTTGGGTGTCGGTGTTCATTTTATCTATACT GGCTGCTTATGGTGACAATTGAGAGATCGTTACCATATAGCTATTGGATTGGCCATCCGGTGACTAATAGAGCTATTATATATCCCTT TGTGGGTTTATACCACTAGCTTGAAAGAGGTTAAAAACATTACCAATTCATTGTTAAGTTGAATACAGCAAAATGGCTGGCCACCTG GCTCTGCTTCCCTTCTCTCCCCCAGCTGGCGGGCGCGGTGGCCCGGGAGGACCGCCCTGATGGGTGGCCCTAGAACCC TGGCTGTCTTCCAGGGACCTCTGGCGGACCGGGCATCGGACCTGGCGTGGGCCCGGCGAGCGCGTGTGGGGCATACCTCCCTGC CCTCCCCCTACGCTTCTGGCGGGCATGGCTACTGTGGCCCCAGGTCGGGGTGGGCTGGTGGCCAGGGCGGCTCGCCAC AGCCAGCCCCCGCGCGCGCTGGTGTGCGAGTGGCCAGAACTCCGCGGGGGCAGCCAGCCCTATGCAACCGTACTCCCGGCC AGTGAAGCTGGCCAAAGGCCAAGCTGGCCGAGAATCTGCGCCCTCTCAGGCCATCAAGGCGCTCCAGGAAGGAGCTGGAGCAGTTCG CGAAGCTGCTGAAGCAGAAGCGGATCAGCTGGGGTACACCCAGCGACGTCGGCTGAGCTGGGCGCTGTCTGTTCAAGGTT TTCAGCCAGACCCATCTGCCGTTGAGGGCTGACGCTTAGCTTCAAGAACATGTGCAAGCTGCGCCCACTTCTGCAAGAGTGG GTGGAGAGGGCGGATAAACAAGGAAACCTGCAGGAGATCTGCAAGGCCGAGACGCTGGTGCAGGCCAGGAAGCGGAGCT CCATGAGGAAACCGCTTCCGGGAAACCTGGAGAACCTGTTCTGCGAGTGTCCCAAGCTACGCTCAGCAGGATCTCTACATAGCC AGCAGCTGGGCTGGAGAAGGATGTGGTGGAGTGTGGTCTGCAATCGGAGGCGAGAAGGGCAAGCGCTTCTCTCCGCTACGCC CAGAGGGCGGCTTCCGCGCGGGGGTCCCCCTTACGCGCGGGCGCCGTGTCTTCCCTTGGCCCCGGGGCACACTTCGGGACT CCCGGCTACGGCAGCCACACTTACCGCGCTGTACAGCGAGCTGATGCGCCATTCCCTGCGGGGGCGCTTCCCGCCGTCAGCG ACGCTGGGCGAGCCCATGCACAGCAATGGAAGCGGAGCCACGAATCTCTCTGTAAAGCAAGCAGGAGATGTTGAAGAAACCC CGGCTATGGCGTGTCCGACGCCCTGCTGCCCTATTACAGACCTTCGCTCAGGCCCGCTGGCAGAGAAAAGACACTGAGACA CGCTGGAGCCCTAACAAACCGCTGGCGCGAGGAGCTGAGTACATGAAACCGCTGCCCTGTCTGCTGGCGGGCGCATACGACCT GGCCGGCGCCACCGTGGCCACCGACCTGGAGTCAAGCGGGGGCGGAGCGGCTGTGGCGGCTCAACCTGGCGCCCTGCCCGCA GGGAGACTGAGGAGTTCAACGATCTGCTGGATCTGGAATCTCAGTAACTCTCTGACCCACCCCAAGAGTGTGCTGCTA CCGTGAGCGAGCGCCAGCGGAGCTTAGCAGAGCCCTAGCAGCTCCGGGCTGCCAGTGGCCAGGACCTGACGCTTACCT ACCCCATCAGGGCCCGTAACGACCTGGTGTGGCCCCGGCGGCAACCGGGGGCGGACTCTGTACGGCGGGGAGTCCGCCCCCG CCACCGGCCCTTCAACCTGGCGGACATCAACGATGTGAGTGTGAGCTCCGCGGGGCTTCGTCGCGGAGCTGCTGCTCAGCTGCAG CCTGTGTACATCCCCCACAGCAGCCGACGCCCGGGCGGCGGCTGATGGGGAAGTTCTGTCTCAAGGCCAGCTGAGCGCACC CGGCTCTGAGTACGGAAGCCCTCCGTGATCAGTGTGAGCAAGGGAAGCCCCGACGGCTACACCCCGTGGTTGTGGCCCCCTACAA CGGCGGGCGCCACGACCTGCCCAAGATCAAGCAGGAGGAGTGAAGCAGCTGCACCCATCTGGGTGCGGGCCACCACTGTCCA ACGGCCACCGCCCCGTGCCATGACTTCCCTCTGGGCAAGGAGCTGCCAGCAGGACCACCCCTACGCTGGGCTGGAGGAGGTCC TGTCTCCAGGGAATGCCACCCGGCCCTGCCCTGCCCGGGGTTCCACCCCCACCCCGGACCCAACTACCCAGCTTCTCTGCTGA CCAGATGCAAGCGCAGGTTCCGCCCCCTGATTACAGGAGCTGATGCCCCCGGGCAGCTGATGCCCGAGGAGCCATAAGCCTGA GGGCGCGGCGAGCTGCCCGCGCAAGCGGACCGCCACCCACACTTGGATTACCGCGGGTGGCGCAAGACCTACACAAAGATGCTG CACTGAAGGCCACCTGCGAATCAACAGGGCGAGAAGCCCTTACCACTGCGACTGGGAGTGGGAGGAGTTCCGCGGCTC CGATGAGCTGACCCGGCACTATCGGAAGCACACCGGGCACAGGCCCTTCCAGTGCCAGAAGTGTGACCGGGCTTCAGCCGGTCA ACCATCTGGCACTGCATGAAAGCGCCACTTCGGAAGCGGAGAGGCGAGGGGAAGTCTTCAATACGCGGGGACGCTGGAGGAAAAAT CCCGGCCCATGCCCTGAATGTGCTTCAACCAACCGCAACTATGATCTGGAATGACTATGACTCTACTTCTACTGCGATG AGGAGGAGAACTTCTACCAGCAGCAGCAGCAGAGCGAACTGCAGCCACCCGCCCCATCCGAGGATATCTGGAGGAGGTTGAGCTG CTGCCGACCCCTCTCTAGCCCCCTCAGGAGGAGCGGGCTGTGAGTCCAGCTACGTGGCTGTGACCCCTTCTCCCTGCGCGGC GACAACGACGCGCGGGGGGGGAGCTTACGACCCGCTGACCAAGCTGGAGATGGTACCAGAGCTGCTGGGTGGCGACATGGTCAACCA GAGCTTCACTGCGACCTGATGATGAGACATTATCAGGAACATCATCATCCAGGACTGCATGTGGTCCGGCTTCTCGGCGCTGC CAGGCTGGTCAGGAGAGGCTGGCCAGTACCAGGCCCGCGGAGGAGTCCGGGTGCGCTAACCCCGCCAGGGGCCACAGCGTCT GCTCCACGAGCAGCCTGTACCTGCAGGATCTGAGCGCCGCGCCAGCGAGTGCATAGATCCTTCACTGGTGTGCCATACCCACTGA ACGACAGCAGCTTCCAGGAGCTGCGCTCGCAGGACAGCAGCGCTTACGCCCTCGTCCGACTGCTGCTGCTCAGGAGT CGAGCCCCCAGGGCTCTCTGAGCCCTGGTGTGACAGGAGGAGCCCTTCTACCACTCTCGGACTCCGAGGAGGAGCAGGAG GATGAGGAAGAGATGACGCTGGTCAAGCTGGAGAGGGCGCAGGCTCTGGCAGGCGCAGCGAGTGTGGCAGCCCTCCGCGGGG GCCACAGCAGGCCCGGCACAGCCCGCTGGTGTGAGGCGGTGTACGTCAGGACCCACAGCATAACTATGCTGCGCTCTTCCA CCAGGAGGAGTACCCCGCTGCCAGCGGGTGAAGTGAAGTACGCTCCGGGTGCTGAGGCGAGTACGCAATAACCCGATGTTGCAAC TCCCCAGGAGCAGTGACACAGAGGAGAAGCTGAGGAGGCGGACTCACAACGTTCTGGAGCGCCAGCGCCGGAACGAGCTGAGGC GGTCTTCTTGGCTGTGAGGAGCAGATCCAGAGCTGGAGAACAAATGAGAGGGCAGCGAGGGTGGTCACTGAGGAGGGCCACC GCGTATATCTGAGCGTGCAGGCTGAGGAGCAGAGGCTGATCAGCGAGGAGGAGTGTGAGGAGGGCGCGAGCAGCTGAGGC ACAGGCTGGAGCAGCTGCCAAACAGCTGCGCTGATGATAAAAAAACAACAAACAAACAGGCTATTATGCGTTACCGCGATGCT CAATGTACCTATTTCTATGGCCCTTGGCGGGGAAACACGCAAGGGATGGTGTCAAATTCGGCGAAACCTAAGCGCCCCCGGGG GTATGACACCGCGAGCCAACTTCGGCGCAATTCCATTATCAGATTCTAGAG
61	O'KM'S- circRNA (nt)	GGGAGACCTCGAATGGAATTTGCGCCGATGAAGGTGTAGAGACTAGACGGCACCCACCTAAGGCAACACGCTATGGTGAAGGCATA GTCCAGGGAGTGGCGAAAGTACACAAACCGGAATATTGGACTCGCCGGAACGCAATAGCCGAAAAACAAAAACAAAAAAC AAAAAACAACAAAAACAAAAACACATTAACAGCCTGTGGGTTGATCCACCCACAGGCCCATTTGGGCGCTAGCACTCTGGT ATCACGGTACCTTTGTGCGCTGTTTATACCCCTCCCCCACTTAACCTTAGAAGTAACACACACCGATCAACAGTACGCTGGCA CACCAGCAGTTTTGTATCAAGCACTTGTATACCCCGGACTGAGTATCAATAGACTGCTCAGCGGTTGAAGGAGAAAGCGTTCGT TATCCGGCAACTACTTCGAAAAACCTAGTAACACCGTGGAGTTGACAGAGTGTTCGCTCAGCACTACCCAGTGTAGATCAGGTC GATGAGTACCCGATTCCCCACGGGCGACCGTGGCGGTGGCTGCGTTGGCGGCTGCCATGGGGAACCCATGGGACGCTCTAAT

		ACAGACATGGTGCAGAGTCTATTGAGCTAGTTGGTAGTCTCCGGCCCTGAATGCGGCTAATCCTAACTGCGGAGCACACACC TCAAGCCAGAGGGCAGTGTGCTGTAACGGGCACTCTGACGGGAAACCGACTACTTTGGGTGTCCGTGTTTATTATTCCTATACT GGCTGCTTATGGTGACAATTGAGAGATCGTTACCATAATAGCTATTGGATTGGCCATCCGGTGACTAATAGAGCTATTATATATCCCTT TGTTGGGTTTATACCACTTAGCTTGAAAGAGGTTAAACATTACAATTATTGTTAAGTTGAATACAGCAAAATGGCTGGCCACCTG GCCTCTGCCTTCGCTTCTCTCCCCCACTGGCGGGCGGCTGCCGGCCCCGGAGGACCCGCCCTGGATGGGTGGCCCCTAGAACCC TGGCTGTCTATCCAGGGACCTCCTGGCGGACCCGGCATCGACCTGGCGTGGGGCCGGACGCGCGTGTGGGGCATACCTCCCTGC CCTCCCCCTACGCCTTCTGCGGGGGCATGGCTACTGTGGCCCCAGGTCGGGGTGGGCTGTGCCCCAGGGCGGCTCGCCACC AGCCAGCCCGCCGGCGCCGCTGGTGTGCGAGTGGCCAGCACTCCGCCGGGGCCAGCCAGCCCATGACCGTGAATCCCGGCGC AGTGAAGCTGGCCAAGGCCAAGCTGGCCCAAGATCTGCGCGCTCTCAGGCCATCAAGGCGCTCCAGAAGGAGCTGGAGAGTTCCG CGAAGCTGCTGAAGCAGAAGCGGATCACGCTGGGGTACACCCAGGCCGACGTGGCTGACCTGGGCGTCTGTTCGGCAAGGTG TTCAGCCAGACCCATCTGCGGGTTCGAGGCCCTGCAGCTTAGCTTCAAGAACATGTGCAAGCTGCGCCCACTTTCGAGAAAGTGG GTGGAGGAGCGGATAACAACGAGAACTGCAGGAGATCTGCAAGGCCGAGACGCTGGTGCAGGCCAGGAAGCGAAAGCGGACCT CCATCGAGAACCCTGCTTCGCGGAACCTGGAGAACCTGTTCTCTGAGTGTCCCAAGCTTACACTGCAGCAGATCTCTCATATAGCCC AGCAGCTGGGCTGGAGAAGGATGTGGTGCAGTGTGGTCTGCAATCGGAGGCAAGAGGCAAGCGCTCTTCTCCGCTACGCC CAGAGGCCCGCTTCGCCCGGGCGGGTCCCTTACGGCGGGCGGGTGTCTTCTTCCCTGCGCCAGCCACTTCCGGGACT CCCGGCTACGGCAGCCACACTTACCGCGCTGTACAGCAGCGTGCCTTCTGCGGGGCGGCTTCCCCCGCTGAGCGTGACG ACGCTGGGAGCCCATGCACAGCAATGGAAGCGGAGGCCAGCACTTCTCTGTAAAGCAAGCAGGAGATGTTGAAGAAAACCC CGGGCTATGGCGTGTCCGACGCCCTGTGCTCCCTATTAGCACTTCCGCTCAGGCCCGCTGGGAGAGAAAGACTAGAGACA GGCTGGAGCCCTAACAACCGCTGGCGGAGGAGTGTGATCATGAAACGCTGCCCCCTGTCTGCTGGCGGGCCATACGACCT GGCGCGCGCACGGTGGCCACCGACTGGAGTCAAGCGGGCGGAGCGGCTGTGGCGGCTCAACCTGGCGGCTCTGCCCGCA GGGAGACTGAGGAGTTCAACGATCTGCTGGATCTGGACTTCATCTCAGTAACTCTGACCCACCCCCAGAGAGTGTGCTGCTA CCGTGAGCAGCAGCGCCAGCGGAGCTTAGCAGCAGCCCTAGCAGCTCCGGGCTGCCAGTGCCTCCAGCACCTGCAGCTTACCT ACCCCATCAGGGCCGTAACGACCTGGTGTGGCCCCGGCGGACCCGGGGCGGACTCTGTACGGCCGGGAGTCCGCCCCCGC CCCACCGCCCCCTTCAACTGGCGGACATCAACGATGTGAGCCCTCCGGGGGCTTCGTGCGGAGCTGCTGCGGCCAGAGCTGAC CCTGTGTACATCCCCCACAGCAGCCGAGCCCCGGGCGGGGCTGATGGGGAAGTTGCTGCTCAAGGCCAGCTGAGCGCACC CGGCTGTAGTACGGAAGCCCTCCGTGATGATGTGAGTGAAGAACGCCGAGCCGAGGCTACACGGTGTGTGGCGGCTTACAA CGGCGGGCGCCACCGACCTGCCCCAAGATCAAGCAGGAGGCAAGTGAAGCTGACCCATCTGGGTGCGGGGCGCCACTGTCCA ACGGCCACCGCCCCGCTGCCATGACTTCCCTCTGGGAGGAGCTGCCAGCAGGACCCCTACGCTGGGCTGGAGAGGTTCC TGTCCTCAGGGACTGCCACCCGGCTGCCCTGCCCGGGGTTCACACCCCAACCCCGGACCTACCCAGCTTCTGCTGTA CCAGATGAGCGCAGGTTCCGCCCCCTGCTATTACAGGAGCTGATGCCCGGGGAGCTGATGCCCGAGGAGCTTAAAGCTAAGC GGGCGGGCGCAGCTGCGCCCGCAAGCGGACCGCCACCTTACGCGATTACGCCGGGTGCGGATACACAAAGAGTTGCTG CACCTGAAGGCCCACTGCGAAGTACACCGGGCGAGAAGCCCTACCAGTGGGAGTGGGAGCGGCTGGAGTTCGCCGGTTC CGATGAGCTGACCCGGCACTATCGGAAGCACACCGGGGCAAGGCGCTTCCAGTGCAGAAAGTGTGACCGGGCTTACGGCGGTGAG ACCATCTGGCACTGCACATGAAGCGCCACTTGGAAAGCGGAGGAGGAGGAGGAGTCTTAACTACAGGACCTGAGGAGAAAT CCCGGCCCCATGCCCTGAATGTGCTTACCAACCGCAACTATGATCTGGAATGACTCTGTGAGCCTTACTTCTACTGCGATG AGGAGGAGAACTTCTACCAGCAGCAGCAGCAGAGCGAAGTGCAGCCACCCCGCCCATCCGAGGATATCTGGAGGAGGTTGAGCTG CTGCCACCCCTCTCTCAGCCCTCCAGGAGGAGCGGGTGTGAGTCCCAAGTACGTGCGCTTGCCTTCTCCCTGCGGGCG GACAACGACCGCGGGGGGGGAGCTTACGACCCGCTGACCAAGTGGAGATGTCACCGAGCTGCTGGGTGGCGACATGCTCAACCA GAGCTTACTCTGCGACCTGATGATGAGACATTCATCAGGAACATCATCTCAGGACTGCATGTGGTCCGGCTTCTCGGCCGCTGC CAGGCTGGTACGCGAGAGGCTGGCCAGTACCAAGCGCGCGGAGGAGTTCGGGTGCGCTAACCCCGCAGGGGCCACAGCGTCT GCTCCACGAGCGAGCTGTACTCTGAGGATCTGAGCGCCGCCGCGCAGCAGGAGTGCATAGATCTTCACTGAGTGTGCTCCATACCACTGA ACGACAGCAGCTTCTCCAGGAGCTGCGCTCGCAGGACAGCGGCTTACGCCCCCTGCTCGAGCTGCTGCTTCCAGCAGGAGT CGAGCCCCCAGGGCTCTCTGAGCCCCGTGGTGTGACAGGAGGAGCCCTCTACCACCTCTCGGACTCCGAGGAGGAGCAGGAG ATGAGGAGAGAGATGACGTGGTCAAGCTGGAGAGGCGGAGGCTCTGCGAGGCGCAGCGAGTGTGCGAGCCCTCGCGGGG GCCACGACGAGGCCCGCGCACAGCCGCTGGTGTGAGGCGGTGTACGTGAGCAGCCACCAAGATGATGCTGCGCTCTCTTCCA CCAGGAGGAGTACCCCGCTGCCAGGCGGCTGAGGCTGGATAGCTCCGGGTGCTGAGGAGATCAGCAATAACCGTAGGTGCACC TCCCCAGGAGCAGTGACACAGAGGAGAACGTGAGGAGGCGGACTCACAACGTTCTGGAGCGCCAGCGCCGGAACGAGCTGAGGC GGTCTTCTTCTGCTGAGGAGCAGATCCAGAGCTGGAGAACATGAGAGGCGACCGAGGTTGCTGAGGAGGCGGACCC GCGTATATCTGAGCGTGCAGGCTGAGGAGCAGAGGCTGATCAGCGAGGAGGAGTGTGCTGAGGAGGCGGCGGAGCAGCTGAGGC ACAGGCTGGAGCAGTGCAGAACAGCTGCGCCGGAAGCGGACAGTGTACTAATTATGCTCTCTGAAATTGGCTGGAGATGTTGAG AGCAACCCAGGTCCATGTGACAACTGATGAGACAGAGCTGAAAGCCCCCGGGCTCAGCAGACCGCGCGCGCGGGGCA ACTCCACCGCGCCGCTGCTGGGGGCAACAGAAAGACGCCCGATAGGGTGAAGCGCCCTATGAACCGCTTACGTGGTGTGGTCC CGCGGCCAGCGCCGTAAGATGGCTCAGGAGAACCTAAGTGCACAACTCCGAGATAGCAAGCGCTGGCGCGGAGTGGAAAG TGCTGTGCGGAGACCGAGAAGCGGCCATTATCGACGAGGCGCAAGAGGCTTCTGCTGCTGCACATGAAGGAGCATCTGACTACAAG TACCGCCCCCGCGGAGAGCAAGACACTGATGAAGAAAGGCAAGTACACCTTGCCTGGGGGCTGCTGGCCCCCGGGGCAATC CATGGCTTCCGGCTGGCGTGGGCGTGGGAGCGGCTAGGCGCTGGCGTGAACAGCGCATGGAATCTACATGACAGCGGCTGA GCAACGGGTCTACAGCATGATGACAGGACAGTGGGCTACCCCCAGCACCCTGGGCTGAACGCCCATGGGCTGCCAGATGACG CCATGACATCGGTACGAGTGTGCTGAGTACAACCTTCCATGACATCTAGCAGACCTACATGAACGGGAGTCCCACCTACAGC CGTGGTGACAAAGCTCTCCACAGCGGGGCCCTGCCAGGCTGGGGATCTGAGGGAGATGATCTCCATGATACCTCCCGGGCGCCGA GGTGCCCGAGCCTGCTGCTCCAGCAGGCTGCATATGAGCAGCAATTACCAGTCCGGGCGCGTCCCGGCACTGCCATCAATGGGAC ACTGCCACTGTCCCATATGTGATGATAAAAAAACAACCAACCGCTATTATGCTTACCGGCGATGCTCAATGACCTATT CTATGGGCTTCCGCGGGAACCGCAAGGATGGTGTCAAAATTCGGCGAAACCTAAGCGCCCCCGCGGGCGTATGGCAACGCC GAGCCAAAGCTTCCGCGCAATTCCATTATCAGATTCTAGAG
93	AzoPIE-O'SK (aa)	MAGHLASAFSPPPGGGGAGPGGAPGWVAPRTWLSFQPPGGPGIGPGVPGSAVWGIPPCPPPYAFCCGGMAYCGPQVGVGLVPQGG LATSQPAGAAGVGVASNSAGAPAPCTVTPGAVKLAKAKLAQNPAASQAIKALQKELEQFAKLLKQKRITLGYTQADVGLTLGVLFKGV FSQTTICRFEALQLSFKNMCKLRPLLQKWVEADNNENLQEIKAETLVQARKRKRTSIENRVRGNLENLFLQCPKPTLQOISHIAQQLGLE KDVVRVWFCNRRQKQKRSSSAYAQRAFAAAGSPFSGGPVSFPLAPGPHFGTTPGYGSPHFTALYSSVFPFAGAAFPVSVTLTGSMPHSN GSGATNFSLLKQAGDVEENPGPMYNNMETELKPPGPQQTSGGGGGNSTAAAGGNQKNSPDRVKRPMNAFWMVWSRGQRKMAQENP KMHNSEISKRLGAEWKLSETEKRFIDEAKRLRALHMKHEHPDYKYRPRRKTTLMKDKYTLPGGLLAPGNSMASGCVGVGAGLGAG VNORMDSYAHMNGWSNGSYMMQDQLGYPPHPLNAHGAQMOPMHRVYDVSALQYNSMTSSQTYMNGSPTYMSYSQQGTGPGMAL GSMGSVVKSEASSPPVVTSSSHRAPCQAGDLRDMISMYLPGAEPPEPAAPSRHMSQHYQSQGPVPGTAINGTLPLSHMGSGEGRGSLT CGDVEENPGPMAVSDALLPSFTFASGPAGREKTLRQAGAPNNRWREELSHMKRLPPVLPGRPYDLAAATVATDLESAGAGAACCGSNL APLPRRETEEFNDLLDLDLILSNLTHPPESVAATVSSSASASSSSSSSPSPASAPSTCSFTYPIRAGNDPVPAGGTGGGLLYGRESAPPTA PFNLADINDVSPSGFVAELLRPDLVPVIPPQQPQPPGGGLMKGFVLKASLAPGSEYGSPSVSKGSPDGSHPVVAPYNGGPPRTPCK IKQEA VSSCTHLGAGPPLSNGHRPAAHDFPLGRQLPSRTTPTLGLLEEVSSRDCHPALPLPPGFHPPHGPYPNPSFLPDQMOPQVPLHYQEL MPPGSCMPPEPKPKRGRRSWPKRTATHTCDYAGCGKTYTKSSHLKAHLRHTGEPYHCDWDGCGWKFAKRSDELTRHYRKHTGHRPF QCKQCDRAFSRSDHLALHMKRHF
94	AzoPIE-O'SK (nt)	taatagaactactataGGGAGACCTCGAATGGAATTTGCGCCGATGAAGGTGTAGAGACTAGACGGCACCCACCTAAGGCAAAACGCTATGG TGAAGGCATAGCTCAGGAGTGGCGAAAGTACACAAACCGGAATATTGGACTCGCCGGAACGCAATAGCCGAAAAACAAAAA

		CAAAAAAAAAAAAAAAAAAACAAAAAAAAACACATTAACACAGCCTGTGGGTTGATCCACCCACAGGCCATTGGGCGCT AGCACTCTGGTATCACGGTACCTTTGTGCGCTGTTTTATACCCCTCCCCAACTGTAACCTAGAAGTAACACACACCGATCAACAG TCAGCGTGGCACCACAGCCACGTTTTGATCAAGCACTTCTGTTACCCCGGACTGAGTATCAATAGACTGCTACGCGGTTGAAGGAG AAAGCGTTTCGTTATCCGGCCAACCTACTTCGAAAAACCTAGTAACACCGTGGAAGTTGCAGAGTGTTTCGCTCAGCACTACCCCAAGT TAGATCAGGTCGATGAGTACCACGATTCGCCACGGGCGACCGTGGCGGTGGCTGCGTGGCGGCCCTGCCCATGGGGAACCCATGG GACGCTCTAATACAGACATGGTGCGAAGAGTCTATTGAGCTAGTGGTAGTCTCCGGCCCTGAATGCGGCTAATCCTAATCTGCGG AGCACACACCTCAAGCCAGAGGGCAGTGTGCTGAACGGGCAACTTCGAGCGGAACCGACTCTTTGGGTGTCGCTGTTTCATTT TATTCTATCTGGCTGCTTATGGTGACATTTGAGAGATCGTACCATATAGCTATTGGATTGGCCATCCGGTGAATAAGAGCTAT TATATATCCCTTTGTTGGGTTTATACCCTTAGCTTGAAGAGGTTAAACATTACAATTCATTGTTAAGTTGAATACAGCAAAATGG CTGGCCACCTGGCCTCTgceTTCGCCTTCTCTCCCCACCTGGCGGCGGCGGTgceGGCCCCGGAGGACCCgceCCTGGATGGGTGgceCC TAGAACCTGGCTGTCTATCCAGGGACCTCTGGCGGACCCGGCATCGGACCTGGCGTGGGGCCCGGACGgceGTGTGGGGCATACCT CCCTGCCCTCCCCCTACgceTTCGCGGGGCGCATGGCTACTGTGGCCCCCAGGTCGGGGTGGGCTGGTGCCCGAGGGCGGCTCge eACCAGCCAGCCCgceGGCgceGCTGGTGTGGAGTgceAGCAATCCgceGGGGCCAGCCAGceCATGACCCGTGACTCCCGGCGCA GTGAAGCTGgceAAGgceAAGCTGgceCAGAATCTTgcegceTCTCAGgceATCAAGCGCTCCAGAAGGAGCTGGAGCAGTTCCGCGAAGCT GCTGAAGCAGAAGCGGATCACGCTGGGGTACACCCAGGCCGAGCTCGGCTGACCTGGGCGCTCTGTCGGCAAGGTGTTACGCC AGACCACCATCTGCCGGTTCGAGGCGCTGCAGCTTAGCTTCAAGAACATGTGCAAGCTGCGGCCACTTCTGCAAGAAGTGGGTGGAGG AGGCGGATAACACGAGAACCTGCAGGAGATCTGCAAGGCGGAGACGCTGGTGACGGCCAGGAAGCGAAAGCGGACCTCCATCGA GAACCCGGTTCGCGGAAACCTGGAGAACCTGTTCTGCAAGTGTCCAAAGCTACACTGCAGCTGCTCTACATAGCCAGCTGCTAGC GGGCGTGGAGAAGGATGTGTGCGAGTGTGGTTCGCAATCGGAGGCGAGAAGGGCAAGCGCTCTTCTCCgceTACGCCAGAGGgce gceTTgceCGGCGGGGTCCCCCTTACGCGGCGGCGGCTGTCTTCCCTCTGGCCCCGGGCGCACACTTCGGGACTCCCCGGCTACGG CAGCCACACTTACCGCGCTGTACAGCAGCGTGCCATTCCCTgceGGGgceGCTTCCCCCGCTGAGCGTGACGACGCTGGGCGGCC CCATGCACAGCAATGGAAGCGGAGCCACGAATTTCTCTCTGTTAAAGCAAGCAGGAGATGTTGAAGAAAACCCCGGCGCTATGTAC AACATGATGGAGACAGAGCTGAAGCCCCCGGGCTTCAGCAGCAGCAGCGGCGGCGGCGGAGCACTCCACCCCGGCGCTGCTGG GGGCAACCGAGAAGAACAGCCCCGATAGGGTGAAGCGCCCTATGAACGCTTCATGGTGTGGTCCCGCGGCCAGCGCGTAAGATGG CTCAGGAGAACCCTAAGATGCACAACCTCCGAGATCAGCAAGCGGCTGGGCGCGGAGTGAAGCTGCTGTGGAGACCGGAGAAGCG GCCATTCTGACGAGGCCAAGAGGCTTCTGCGCTTGCACATGAAGGAGCATCTGACTACAGTCCGCGCCCGCGGAGAACCA AGACACTGATGAAGAAAGGACAAGTACACCTTGCTGGGGGCTGCTGGCCCCCGGGGCAACTCCATGGCTTCCGCGCTGGGCGTG GGAGCGGCTAGGCGCTGGCGTGAACACGCGCATGGAATCTACGCTACATGAACGGCTGGAGCAACGGGTCTTACAGCATGAT GCAGGACAGTGGGCTACCCCGAGCACCCTGGGCTGAACGCCATGGGCTGCGCAGATGCAGCCATGCTGACAGCTGACAGCTGA GTGCCCTGCGATACAACCTCATGACATCTAGCCAGACCTACATGAACGGGAGTCCCACTACAGATGAGCTATGAGCAGCAAGGGG AGCCAGGATGGCTGGGCTGCGGTGCTGGTGAAGTGAAGGCGGAGTCTACCTACCTGGGTGAGCAAGGCTCTCTCCAC AGCCGGGCCCCCTGCCAGGCTGGGATCTGAGGGACATGATCTCCATGTACCTCCCGGGCGCGGAGGTGCCCGAGCCTGCTGCTCCC AGCAGGCTGCATATGAGCCAGCATTACAGTCCGGGCGCGTGGCGGCGACTGCCATCAATGGGACACTGCCACTGTCCCATATGGGA AGCGGAGGGCAGGGGAAGTCTTCAACATCGGGGAGCTGGAGGCAAAATCCCGGCCCATGGCGTGTCCGACCGCCCTGCTGCC CTCATTCAGCACCTTCGCTCAGGCCCGCTGGCAGAGAAAGACACTGAGACAGGCTGGAGCCCCTAACACCCGCTGGCGCGAGG AGCTGAGTGCATGAACCGCTGCCCTTGTCTGCTGGGCGGCCATACGACCTGGCGCGCCACCGGTGGCCACCGACCTGGAGT CAGGCGGGGCGGAGCGCGCTGTGCGGCTTCAACACTGGCGCCCTGCGCCCGCAGGGAGACTGAGGAGTCAACGATCTGGGAT CTGGACTTCATCTCAGTAACCTCTGACCCACCCCGCAGAGAGTGTGTGCTGCTACCGTGAGCAGCAGCGCCAGCGCGAGCTCTAGC AGCACCCCTAGCAGCTCCGGGCTGCCAGTGGCCCGACCGCTGCAAGCTTCACTACCCCATCAGGGCGGTTAACGACCTGGTGTG GCCCGGGCGGACCGGGGCGGACTCCTGTACGGCGGGAGTCCCGCCCCCGGCCACCGCCCTTCAACCTGGCGGACATCAAC GATGTGAGCCCTCCGGGGGCTTCTGTCGCGGAGCTGCTGCGGCGCAGAGCTGCAACCTGTGTACATCCCCCGCAGCAGCGCGGACGCC CCGGCGGGCGGCGCTGATGGGGAAGTTCGTGCTCAAGGCCAGCTGAGCGCACCCGGCTCTGAGTACCGAAGCCCTCCGTGATCAG TGTGAGCAAGGGAAGCCCCGACGGCTCACACCCCGTGGTGTGGCCCCCTACAACGGCGGGCGGCCACGGACCTGCCCCAAGATCA AGCAGGAGGCGAGTGAGCAGCTGCACCATCTGGGTGCGGGGCGCACCCTGTCAACCGGCCACCGCCCGCTGCCCATGACTTCCCTC TGGGACGGCAGCTGCCAGCAGGACCCCTACGCTGGGCTGAGGAGGTCTGTCTCAGGGACTGCCACCGCGGCTGCCCTC TGCCCCCGGGGTTCCACCCCCACCCCGGACCCAACTACCCAGCTTCTGCTGACAGATGCAGCGCAGGTTCCGCCCTGCATT ACCAGGAGCTGATGCCCGGGGCGAGCTGCATGCCCGAGGAGCTTAAGCCTAAGCGGGGCGGGCGAGCTGGCCCGCAAGCGGAGC GCCACCCACTTGCATTACGCCGGGTGCGGCAAGACTACACAAAGAGTTTCGCACTGAAGGGCCACTGCGCACTCACACGGG CGAGAAGCCCTACCACTGCGACTGGGACGGGTGCGGCTGGAAGTTTCGCCCCGTCCGATGAGCTGACCGGCGACTATCGGAAGCACA CCGGGCACAGGCCCTTCCAGTGCCAGAAGTGTGACCGGGCTTACGCCGCTGACAGCATCTGGCATGCATGAAGCGCCACTTCT GATGATTAATAAATAAATAAATAAATAAAGCGCTATTATGCGTTACCGGCGAGTCTCAATGTACTATTTCATGCGCTTGGCGGGA ACCACGAAGGATGTTGTCAAATTCGGCGAAACCTAAGCGCGCGCGCGGCGTATGGCAACGCCAGGCCAAGCTTCGGCGCCAAT TCCATTATCAGATTTCTAGAg
97	AzoPIE- O'KM' (aa)	MAGHLASAFAPSPPPGGGAGPGGPAPGWVAPRTWLSFQPPGGPGIGPGVGPBSAVWGIPPCCPPYAFCCGGMAYCGPQVGVGLVPQGG LATSPAGAAAGVGVASNSAGASPACTVTPGAVKLAKAKLAQNPAAQAIKALQKELEQFAKLLKQKRITLGYTQADVGLTLGVLFKGV FSQTFGRFEALQLSFKNMCKLRPLLQKWVEEADNNENLQKEALETLVQARKRKRTSIENRVRNLENLFLQCPKPLTQISHIAQQGLGLE KDVVRVWFENRRQKQGRSSSAYAQRAFAAAGSPFSGGPVSFPLAPGPHFGTGPYGSPHFTALYSVPFPAGAAFPVSVTTLGSPMHNS GSGATNFSLLKQAGDVEENPGPMVSDALLPSFSTFASGPAGREKTLRQAGAPNNRWRELSHMKRLPPVLPGRPYDLAAATVATDLES GAGAGCGGSLNAPLPRRETEEFNDLLDLDLILNSLTHPPESVAATVSSASASSSSSPSSGASAPSTCSFTYPIRAGNDPGVAPGGTGGGL LYGRESAPPTAPFNADINDVSPSGGFVAELRLPELDPVYIPQPPQPPGGGLMGKFVVKASLAPGSEYGPSVISVSKGSPDGSHPVVVA PYNGGPPRTCPKIQEAVSSCTHLGAGPPLSNHHRPAHDPLGRQLPSRTPTLGLLEVLSSRDCHPALPLPPGFHPHPNYPSPFLPDQM QPQVPLPHYQELMPPGSCMPEEPKPKRGRRSWPRKRTATHTCDYAGCGKTYTKSSHLKAHLRTHTGEPYHCDWDGCGWKFAKSDLT RHYRKHTGHRPFQCKQCDRAFSSDLHLHMKRHFSGSEGRVSLTCDGVEENPGMPLNVSFNTRNRYDLDYDSVQPYFYFDEEENFYQ QQQSELOPPAPSEDIWRRFELLPTPLSPSRSSGLCSPSYVAVTSPFLSRGDNDDGGGSFSTADQLEMVTELLGGDMVYNQSFICDPDDTEFIR NHIQDCMWSGFSAAARLVSERLASQAARRDSGSPNARGHSVCSTSSLYLQDLASAAASECIDPSVVPYPLNDSSSPRSCQDSSAFSPS SDSLSSSTESSPQGSPEPLVLHEETPTTSSDSEEEQDEEEDVVSVERRQAPGRRESGSPSAGGHSRPPHSPLVLRCHVSTHQHNYAAP STRRDYPAARRVRLDSVRVLQISNNRRCTSPRSSDTEENVRRRTHNVLQRRLNRRSFFALRDQIPELENNERAPRVVLRRATAYILS VQAEQRLISEEDLLRRRREQLRHLRLEQLRNSCA
98	AzoPIE- O'KM' (nt)	taatacgaactaataGGGAGACCTTCGAATGGAATTTGCGCGGATGAAGGTGTAGAGACTAGACGGCACCCACCTAAGGCAACACGCTATGG TGAAGGCATAGTCCAGGGAGTGCGGAAAGTGCACACAAACCGGAATATTGGACTCGCCGGAACCGCAATAGCGGAAAAACAAAAA CAAAAAAACAAAAAAACCAAAAAACAAAAACACATTAACACAGCCTGTGGGTTGATCCACCCACAGGCCCATTTGGGCGCT AGCACTCTGGTATCACGGTACCTTTGTGCGCTGTTTTATACCCCTCCCCAACTGTAACCTAGAAGTAACACACACCGCATCAACAG TCAGGCTGGCACACCGGACCGTTCGATCAAGCACTTGTTCACCCGGACTGAGTATCACTGCTCAGCGGCTTGAAGGAG AAAGCGTTTCGTTATCCGGCCAACCTACTTCGAAAAACCTAGTAACACCGTGGAAGTTGCAGAGTGTTTCGCTCAGCACTACCCCAAGT TAGATCAGGTCGATGAGTACCACATTCACCGGCGACCGTGGCGGTGGCTGCGTGGCGGCCCTGCCATGGGGAACCCATGG GACGCTCTAATACAGACATGGTGCGCAAGAGCTATTGAGCTAGTGGTAGTCTCCGGCCCTGAATGCGGCTAATCGCTACCGG AGCACACACCTCAAGCCAGAGGGCAGTGTGCTGAACGGGCAACTTCGAGCGGAACCGACTACTTGGGTGTCGCTGTTTCATTT TATTCTATCTGGCTGCTTATGGTGACAATTGAGAGATCGTACCATATAGCTATTGGATTGGCCATCCGGTGAATAAGAGCTAT TATATATCCCTTTGTTGGGTTTATACCCTTAGCTTGAAGAGGTTAAACATTACAATTCATTGTTAAGTTGAATACAGCAAAATGG CTGGCCACCTGGCCTCTgceTTCGCCTTCTCTCCCCACCTGGCGGCGGCGGTgceGGCCCCGGAGGACCCgceCCTGGATGGGTGgceCC

	eACCAGCCAGCCCgcccGGCgcccGCTGGTGTGCGGAGTGgcccAGCAACTCCgcccGGGGCCAGCCCAGcccCATATGCACCGTGACTCCCGGCCGCA GTGAAGCTGgcccAAGgcccAAGCTGgcccCAGAAATCCTgcccTCTCAGgcccATCAAGGCGCTCCAGAAGGAGCTGGAGCAGTTCCGCGAAGCT GCTGAAGCAGAAAGCGGATCAGCTGGGGTACACCCAGGCCGAGCTGGGCTGACCTGGGGCTCTGTTCGGCAAGGTGTTACAGCC AGACCACCATCTGCCGTTTCGAGGCCCTGCAGCTTAGCTTCAAGAACATGTGCAAGCTGCCCCACTTCTGCAGAAAGTGGGTGGAGG AGGCGGATAACAACGAGAACCTGCAGGAGATCTGCAAGGCCGAGACGCTGGTGCAGGCCAGGAAGCGAAAGCGGACCTCCATCGA GAACCGCTTCGCGGGAACCTGGAGAACCTGTCTCTGCAAGTGTCCCAAGCCTACACTGCAGCAGATCTCTACATAGCCAGCAGCT GGGCTTGGAGAAGGATGTGGTGCAGTGTGGTTCTGCAATCGGAGGCGAGAAGGGCAAGCGCTCTTCTTCgcccTACGCCCAAGAGGgccc gcccTTTgcccGCGGCGGGTCCCCCTTCAGCGGCGGCCCGGTGTCTTCCCTCTGGCCCCCGGGCCACACTTCGGGACTCCCGGCTACGG CAGCCCACTTCACCGCGCTGTACAGCAGCGTGCCATTCCCTTgcccGGGgcccGCTTCCCCCGCTGAGCGGTGACGACGCTGGGCAAGCC CCATGCACAGCAATGGAAGCGGAGCCACGAACCTCTCTCTGTTAAAGCAAGCAGGAGATGTTGAAGAAAACCCCGGCCATATGGCC GTGTCCGACGCCCTGTCTGCCCTCATTACAGCACCTTCGCTCAGGCCCGCTGGCAGAGAAAAGACACTGAGACAGGCTGGAGCCCCCT AACAACCGCTGGCGGAGGAGCTGAGTCACATGAAACGCTGCCCCCTGTCTGCTTGGCGGCCATACGACCTGGCGGCCGCCAC GGTGGCCACCGACCTGGAGTCAGCGGGGCCGGAGCCGCTGTGGCGGCTCCAACCTGGCGGCCCTGCCCGCAGGGAGACTGAGG AGTTCAACGATCTGCTGGATCTGGACTTCATCCTCAGTAACTCTTGACCCACCCCCAGAGAGTGTGTGCTACCGTGGAGCAGCA GGCCAGCGGAGCTCTAGCAGCAGCCCTAGCAGCTCCGGGCTGCCAGTGGCCCCAGCACCTGACGCTTCACTACCCATACCCATCAGGG CCGGTAACGACCTGGTGTGGCCCCCGGCGGCCACCGGGGCGGACTCTGTACGGCCGGGAGTCCGCCCCCCCGCCACCGCCCCCT TCAACCTGGCGGACATCAACGATGTGAGCCCCCTCCGGGGGCTTCGTGCGCGAGCTGCTGCGGCCAGAGCTCGACCTGTGTACATCC CCCCACAGCAGCGCAGCCCCCGGGCGCGGCTGATGGGGAAAGTTCGTGCTCAAGGCCAGCTGAGCGCACCCCGCTGAGTAC GGAAAGCCCTCCGTGATCAGTGTGAGCAAGGGAAGCCCCGAGCGCTCACACCCCTGTGTGTGGCCCCCTACACGGCGGGCGGCC ACGGACCTGCCCAAGATCAAGCAGGAGGAGTGAGCAGCTGCACCCATCTGGGTGCGGGCCACCACTGTCCAACGGGCCACCGCC CCGCTGCCCATGACTTCCCTCTGGGCAGGAGCTGCCAGCAGGACCACCCCTACGCTGGGCTGGAGGAGGTCTGTCTCCAGGG ACTGCCACCCGGCCCTGCCCTTGCCCCCGGGGTTCACCCCCACCCCGGACCCAACTACCCAGCTTCTGCTGACCATGAGCAGC CGCAGTTCCGCCCTGCATTACAGGAGCTGATGCCCGGGCAGCTGCATGCCCGAGGAGCTTAAGCCTAAGCGGGCGCGGCCG AGCTGGCCCCGCAAGCGGACCGCCACCCACACTTGCGATTACGCGGGGTGCGGCAAGACTACACAAAAGATTTCGACCTGAAGGC CCACCTGCGAACTCACACGGGCGAGAAGCCCTACACTGCGACTGGGACGGGTGCGGCTGGAAGTTTCGCCCGGTCCGATGAGTGCA CCCCGCACTATCGGAAGCACACCGGGCACAGGCCCTTCCAGTGGCCAGAAAGTGTGACCGGGCTTCAGCCGGTCAGACCATCTGGCA CTGACATGAAGCGCCACTTCGGAAGCGGAGAGGGCAGGGGAAGTCTTCTAACATGCGGGGACGCTGGAGGAAAATCCCGGCCCAT GCCCTGAATGTGTCTTACCAACCGCAACTATGATCTGGACTATGACTCTGTGCAGCCTTACTTCTACTGCGATGAGGAGGAGAA CTTCTACAGCAGCAGCAGCAGAGCGAACTGCAGCCACCCGCCCATCCGAGGATATCTGGAGGAgGTTTCGAGCTGCTGCCGgccc CCTCTCAGCCCCCTCAGGAGGAGCGGGCTGTGCAGTCCAGCTACGTGGCTGTGACCCCCCTTCTCCCTGCGCGGCGCAACAGCAGCG GGGGGGGGAGCTTCAGCACCGCTGAGCAGCTGAGATGGAGTGTCAACGAGCTGCTGGGTGCGGACATGTTCAACCAAGCTTCTATCTG CGACCTGATGATGAGACATTCATCAgGAACATCATATCCAGGACTGCATGTGGTCCGGCTTCTCGGCCGCTGCCAGCTGGTCAGC GAGAgGCTGGCCAGCTACAGGCCCGCCGGAGGGATTCCGGGTGCGCTAACCCCGCCAGGGGCCACAGCGTCTGCTCCACGAGCAGC CTGTAACTGCAGGATCTGAGCGCGCGGCCAGCGAGTGCATAGATCCTTCAGTGGTGTCCCATACCACTGAACGACAGCAGCTCT CCCAgGAGCTGCGCTTCGAGGACAGCAGCGCTTCAGGCCCTCGTCCGACTCGTGTCTCCAGCAGCGAGTTCAGCCCCAGGGC TCTCTGAGCCCCCTGGTGTGCACGAGGAGACCCCTCCTACCCTCTCCGACTCCGAGGAGGAGCAGGAGGATGAGGAAGAGAT CGACGTGGTCAGCGTGGAGAgGCGCCAGGCTCTGGCAGGCGCAAGCAGTCTGGCAGCCCCCTCCGCCGGGGGCCACAGCAGGCCCCC GCACAGCCCCCTGTGTGTGAgGCGGTGTACGTGAGCACCCACAGCATAACTATGCTGCGCTCTTCCACCAGGAgGGACTACCC CGCTGCCAgGCGGGTGAgGCTGGATAGCGTCCGGGTGCTGAGGCGATCAGCAATAACCGTAgGTGACCTCCCCAGGAGCAGTGA CACAGAGGAGAACGTGAgGAGGCGGACTCACAACTTCTGGAGCGCCAGCGCCGGAACGAGCTGAgGCGGTCTTCTTCGCTCTGAG GGACAGATCCAGAGCTGGAGAACAATGAGAgGGCAGCGAgGGTGTATCCTGAgGAgGGCCACCGCGTATATCTGAGCGTGA GGCTGAGGAGCAGAgGCTGATCAGCGAGGAGGACTTGTCTGAGGAgGCGGCGCAGCAGCTGAgGCACAgGCTGGAGCAGCTGCGAA ACAGCTGCGCCGGAAGCGGACAGTGTACTAATTATGTCTCTTGAATTGGCTGGAGATGTTGAGAGCAACCCAGGTCCCATGTACA ACATGATGGAGACAGAGCTGAAGCCCCCGGGCTCAGCAGACCAGCGCGCGCGCGGGGCAACTCCACCGCGCGCGCTGCTGGG GGCAACCAAGAAAGACAGCCCCGATAGGGTGAAGCGCCCTATGAACGCTTCTATGGTGTGGTCCCGCGGCCAGCGCGCTAAGATGGC TCAGGAGAACCCTAAGATGCACAACTCCGAGATCAGCAAGCGGCTGGGCGCGAGTGAAGCTGCTGTGCGAGACCGAGAAGCGG CCATTCTACGAGGAGGCAAGAGGCTTCGTGCCCTGCACATGAAGGAGCATCTGACTACAAGTACCGGCCCGCGGAAAGACCAA GACACTGATGAAGAAGGACAAGTACACCTTGCTGGGGGCTGCTGGCCCCCGGGGCAACTCCATGGCTTCGGCGTGGCGGTGG GAGCCGCTTATAGCGCTGGCGTGAACAGCGCATGGACTCCTACGCTCATATGAACGGCTGGAGCAACGGGTCTACAGCATGATG CAGGACCAGTTGGGCTACCCCCAGCACCTGGGCTGAACGCCATGGGGCTGCCAGATGCAGCCCATGCATGGTACGACGTGAG TGCCCTGCAGTACAACCTCATGACATCTAGCCAGACCTACATGAACGGGAGTCCCACTACAGCATGAGCTATAGCCAGCAGGGGA CCCCAGGATGGCCCTGGGGTCCATGGGCTCCGTGGTGAAGTCCGAGGCCAGCTCCTACCTCCCGTGGTGACAAGCTCCTCCACA GCGGGGCCCTGCCAGGCTGGGGATCTGAGGGACATGATCTCCATGTACCTCCCGGGCGCGGAGGTGCCGAGCTGCTGTCTCCA GCAGGCTGCATATGAGCCAGCATTACAGTCCGGGCGCTGCCGGCACTGCCATCAATGGGACACTGCCACTGTCCCATATGTGAT GATAAAAAAACAACAAACAGGCTATTATGCGTTACCGCGCATGCTCAATGTACATATTTCATGgCCTTGGCGCGGAAAC ACGCAAGGGATGGTGTCAAATTCGGCGAAACCTAAGCGCCCCCGGGCGTATGGCAACGCCAGCCAAGCTTCGGCGCCAATTCC ATTATCAGATTCTAGAg
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CLAIMS

What is claimed is:

1. A method of rejuvenating a population of retinal cells of an eye in an individual, comprising introducing into the population of retinal cells an effective amount of an engineered reprogramming factor that is deficient in liquid-liquid phase separation (LLPS).
2. The method of claim 1, wherein the population of retinal cells comprise retinal ganglion cells (RGCs), photoreceptor cells, bipolar cells, amacrine cells, horizontal cells, Müller glial cells, retinal pigment epithelium (RPE) cells, or any combination thereof.
3. The method of claim 1 or 2, wherein the population of retinal cells are from a retinal tissue that is:
 - (i) an aged retinal tissue;
 - (ii) a damaged retinal tissue;
 - (iii) a retinal tissue with ocular disease or condition; and/or
 - (iv) a retinal tissue after a surgical procedure.
4. The method of claim 3, wherein
 - (i) the ocular disease or condition comprises traumatic optic neuropathy (TON), solar retinopathy, diabetic retinopathy, glaucoma, age-related macular degeneration (AMD), vision impairment or loss, or any combination thereof; and/or
 - (ii) the damage is due to optic nerve crush or laser photocoagulation.
5. The method of any one of claims 1-4, wherein the method does not reprogram the population of retinal cells into a reprogrammed stem cell.
6. The method of any one of claims 1-5, wherein the engineered reprogramming factor comprises a mutation in an LLPS-related domain in reference to a corresponding wild-type reprogramming factor.

7. The method of claim 6, wherein the LLPS-related domain is selected from the group consisting of an intrinsically disordered region (IDR), a region outside of DNA-binding domain (DBD), an ubiquitination site, or any combination thereof.
8. The method of claim 7, wherein the mutation comprises:
 - (i) substituting an acidic amino acid within the IDR with a neutral amino acid;
 - (ii) substituting an amino acid that can be ubiquitinated with an amino acid that cannot be ubiquitinated;
 - (iii) deleting an amino acid that can be ubiquitinated;
 - (iv) deleting an amino acid within the IDR; and/or
 - (v) deleting an amino acid within a region outside of DBD.
9. The method of claim 8, wherein:
 - (i) the amino acid that can be ubiquitinated is K; and/or
 - (ii) the amino acid to be deleted comprises an acidic amino acid.
10. The method of any one of claims 1-9, wherein the engineered reprogramming factor is an engineered OCT4.
11. The method of claim 10, wherein the engineered OCT4 comprises a mutation at amino acid positions 1-140 and/or 288-360, wherein the amino acid positions are in reference to SEQ ID NO: 2.
12. The method of claim 11, wherein the engineered OCT4 comprises a substitution at an amino acid position selected from the group consisting of 8, 20, 26, 31, 56, 68, 91, 96, 98, 104, 108, 113, 125, 127, 130, 134, 135, 138, 291, 296, 297, 299, 341, and 343, and wherein the amino acid positions are in reference to SEQ ID NO: 2.
13. The method of claim 12, wherein the substitution comprises substituting an acidic amino acid with a neutral amino acid.

14. The method of claim 12 or 13, wherein the engineered OCT4 comprises a substitution selected from the group consisting of:

(i) D8A, D20A, E26A, D31A, E56A, E68A, E91A, E96A, E98A, E104A, D108A, E113A, E125A, E127A, E130A, E134A, E135A, D138A, D291A, E296A, D297A, E299A, E341A, and E343A;

(ii) D8A, D20A, E26A, D31A, E56A, E68A, E91A, E96A, E98A, E104A, D108A, E113A, E125A, E127A, E130A, E134A, E135A, and D138A;

(iii) D291A, E296A, D297A, E299A, E341A, and E343A;

(iv) D8A, D20A, E26A, D31A, E56A, E68A, E91A, E96A, E98A, E104A, D108A, E113A, E125A, E127A, E130A, E134A, D138A, D291A, E296A, E299A, E341A, and E343A;

(v) E134A, E135A, E296A, and D297A;

(vi) D8A, D20A, D31A, D108A, D138A, D291A, and D297A; and

(vii) E26A, E56A, E68A, E91A, E96A, E98A, E104A, E113A, E125A, E127A, E130A, E134A, E135A, E296A, E299A, E341A, and E343A.

15. The method of claim 14, wherein the engineered OCT4 comprises an amino acid sequence of any of SEQ ID NOs: 4-10, or a variant thereof having at least about 85% sequence identity to any of SEQ ID NOs: 4-10.

16. The method of claim 11, wherein the engineered OCT4 comprises a deletion at amino acid positions 2-140 and/or 288-360, wherein the amino acid positions are in reference to SEQ ID NO: 2.

17. The method of claim 16, wherein the engineered OCT4 comprises an amino acid sequence of any of SEQ ID NOs: 44-46, or a variant thereof having at least about 85% sequence identity to any of SEQ ID NOs: 44-46.

18. The method of any one of claims 1-9, wherein the engineered reprogramming factor is an engineered c-MYC.

19. The method of claim 18, wherein the engineered c-MYC comprises a substitution of an amino acid that can be ubiquitinated with an amino acid that cannot be ubiquitinated, wherein the amino acid positions are in reference to SEQ ID NO: 12 or 85.

20. The method of claim 19, wherein the substitution comprises substituting a K with an R.

21. The method of claim 19 or 20, wherein the engineered c-MYC comprises a substitution at an amino acid position selected from the group consisting of 51, 52, 126, 143, 148, 157, 206, 269, 275, 289, 298, 317, 323, 326, 341, 355, 371, 389, 392, 397, 398, 412, 422, 428, and 430, and wherein the amino acid positions are in reference to SEQ ID NO: 12 or 85.

22. The method of claim 21, wherein the engineered c-MYC comprises a substitution selected from the group consisting of:

(i) K51R, K52R, K126R, K143R, K148R, K157R, K206R, K269R, K275R, K289R, K298R, K317R, K323R, K326R, K341R, K355R, K371R, K389R, K392R, K397R, K398R, K412R, K422R, K428R, and K430R;

(ii) K148R;

(iii) K389R;

(iv) K148R and K389R;

(v) K51R and K52R;

(vi) K275R and K289R; and

(vii) K51R, K52R, K275R, and K289R.

23. The method of any one of claims 19-22, wherein the engineered c-MYC comprises an amino acid sequence of any of SEQ ID NOs: 14-20, or a variant thereof having at least about 85% sequence identity to any of SEQ ID NOs: 14-20.

24. The method of claim 18, wherein the engineered c-MYC comprises a deletion at amino acid positions 2-360, wherein the amino acid positions are in reference to SEQ ID NO: 12 or 85.

25. The method of claim 24, wherein the engineered c-MYC comprises the amino acid sequence of SEQ ID NO: 47 or 48, or a variant thereof having at least about 85% sequence identity to SEQ ID NO: 47 or 48.
26. The method of any one of claims 1-9, wherein the engineered reprogramming factor is an engineered KLF4.
27. The method of claim 26, wherein the engineered KLF4 comprises a deletion at amino acid positions 2-374, wherein the amino acid positions are in reference to SEQ ID NO: 22.
28. The method of claim 27, wherein the engineered KLF4 comprises the amino acid sequence of SEQ ID NO: 40, or a variant thereof having at least about 85% sequence identity to SEQ ID NO: 40.
29. The method of any one of claims 1-9, wherein the engineered reprogramming factor is an engineered SOX2.
30. The method of claim 29, wherein the engineered SOX2 comprises a deletion at amino acid positions 2-40 and/or 201-317, wherein the amino acid positions are in reference to SEQ ID NO: 24.
31. The method of claim 30, wherein the engineered SOX2 comprises an amino acid sequence of any of SEQ ID NOs: 41-43, or a variant thereof having at least about 85% sequence identity to any of SEQ ID NOs: 41-43.
32. The method of any one of claims 1-31, wherein the engineered reprogramming factor is encoded by a nucleic acid.
33. The method of claim 32, wherein the nucleic acid is an RNA or a DNA.
34. The method of claim 33, wherein the RNA is a circular RNA (circRNA).

35. The method of any one of claims 32-34, wherein the nucleic acid is delivered by an exosome, a liposome, a lipid nanoparticle (LNP), or a virus.

36. The method of claim 35, wherein the method comprises administering a virus comprising a nucleic acid encoding the engineered reprogramming factor.

37. The method of claim 36, wherein the virus is delivered via intravitreal injection or subretinal injection to the eye.

38. The method of any one of claims 1-37, wherein the method comprises introducing a plurality of reprogramming factors into the population of retinal cells, wherein at least one of the plurality of reprogramming factors is an engineered reprogramming factor that is deficient in LLPS.

39. The method of claim 38, wherein the plurality of reprogramming factors comprise two or more reprogramming factors selected from the group consisting of OCT4, SOX2, KLF4, c-MYC, L-MYC, LIN28, NANOG, GLIS1, and variant thereof.

40. The method of claim 39, wherein the plurality of reprogramming factors comprise any of:
(i) an engineered OCT4 that is deficient in LLPS, SOX2, and KLF4;
(ii) an engineered OCT4 that is deficient in LLPS, KLF4, and an engineered c-MYC that is deficient in LLPS; or
(iii) an engineered OCT4 that is deficient in LLPS, KLF4, an engineered c-MYC that is deficient in LLPS, and SOX2.

41. The method of any one of claims 38-40, wherein the plurality of reprogramming factors are encoded by a plurality of nucleic acids.

42. The method of claim 41, wherein at least two of the plurality of nucleic acids are on a single nucleic acid construct.

43. The method of claim 42, wherein the at least two of the plurality of nucleic acids are under the control of the same promoter.

44. The method of claim 43, wherein the nucleic acid construct comprises from 5' to 3':

(i) a first nucleic acid encoding an engineered OCT4 that is deficient in LLPS – a first linking sequence encoding P2A – a second nucleic acid encoding SOX2 – a second linking sequence encoding T2A – a third nucleic acid encoding KLF4;

(ii) a first nucleic acid encoding an engineered OCT4 that is deficient in LLPS – a first linking sequence encoding P2A – a second nucleic acid encoding KLF4 – a second linking sequence encoding T2A – a third nucleic acid encoding an engineered c-MYC that is deficient in LLPS;

(iii) a first nucleic acid encoding an engineered OCT4 that is deficient in LLPS – a first linking sequence encoding P2A – a second nucleic acid encoding KLF4 – a second linking sequence encoding T2A – a third nucleic acid encoding an engineered c-MYC that is deficient in LLPS – a third linking sequence encoding E2A – a fourth nucleic acid encoding SOX2; or

(iv) a first nucleic acid encoding an engineered OCT4 that is deficient in LLPS – a first linking sequence encoding P2A – a second nucleic acid encoding KLF4 – a second linking sequence encoding T2A – a third nucleic acid encoding SOX2.

45. A method of delaying or preventing the progression of aging of a retinal tissue of an eye in an individual, comprising introducing into a population of retinal cells of the retinal tissue, or introducing to the eye, an effective amount of an engineered reprogramming factor that is deficient in LLPS.

46. A method of treating an individual having aged or damaged retinal tissue or a retinal tissue with disease or condition in an eye, comprising introducing into a population of retinal cells of the eye, or introducing to the eye, an effective amount of an engineered reprogramming factor that is deficient in LLPS.

47. A method of treating an individual having aged or damaged optic nerve or an optic nerve with disease or condition in an eye, comprising introducing to the eye an effective amount of an engineered reprogramming factor that is deficient in LLPS.

48. A method of preventing aging, damage, or disease or condition in a retinal tissue of an eye in an individual, comprising introducing into a population of retinal cells of the retinal tissue, or introducing to the eye, an effective amount of an engineered reprogramming factor that is deficient in LLPS.

49. The method of any one of claims 45-48, wherein introducing to the eye is topically, intravitreally, or subretinally.

50. The method of any one of claims 1-49, wherein the individual is a human.

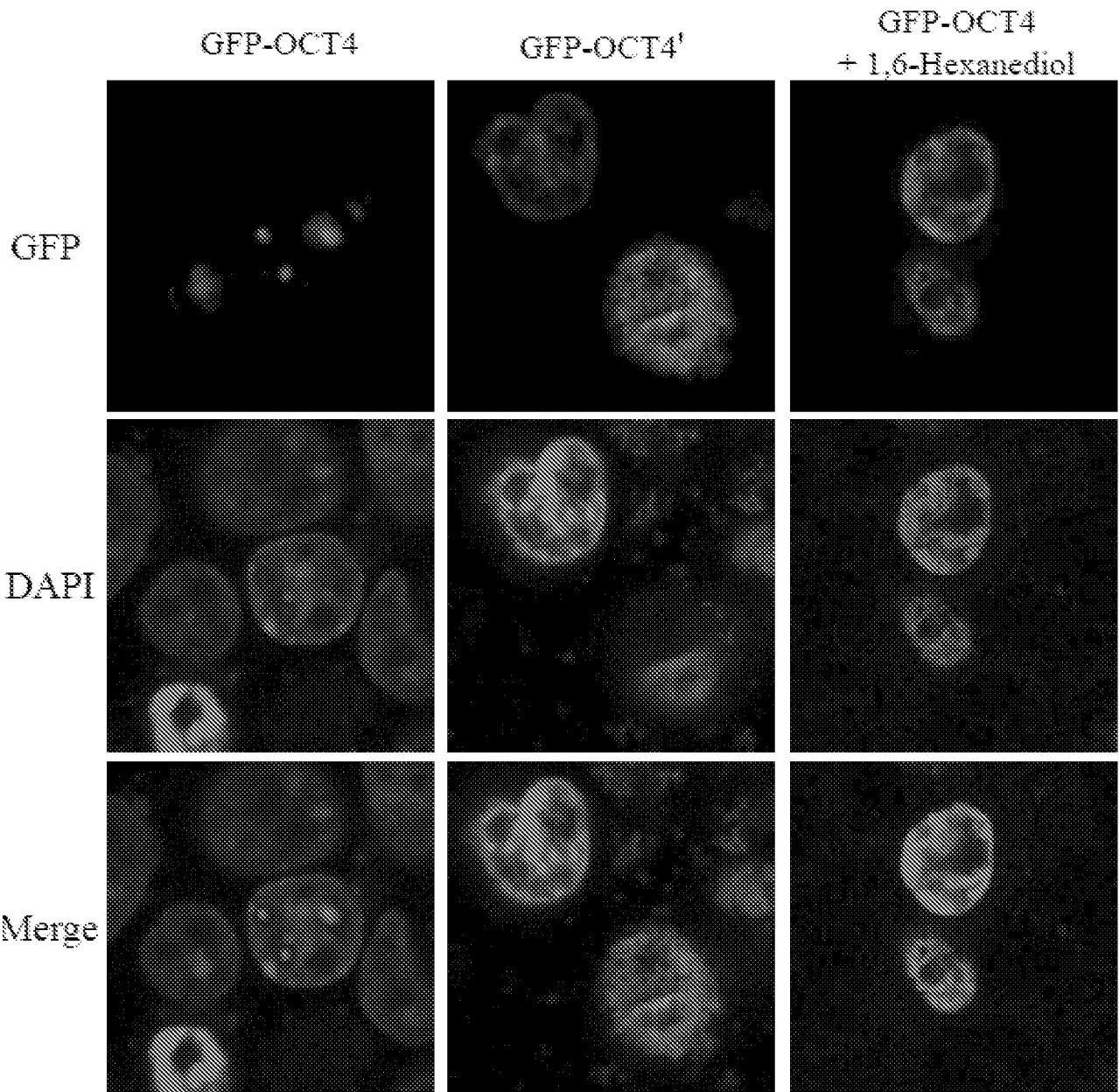


FIG. 1A

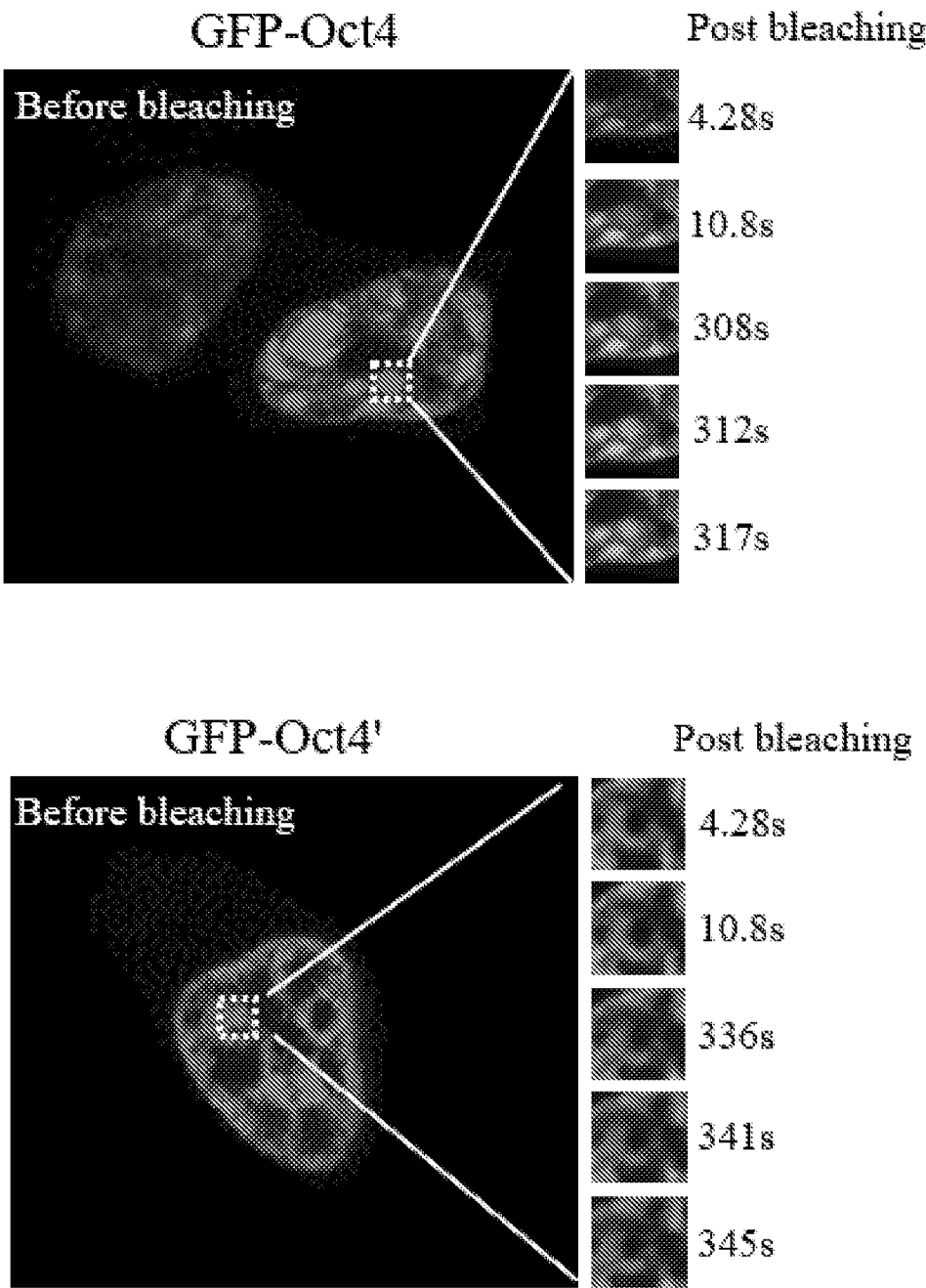


FIG. 1B

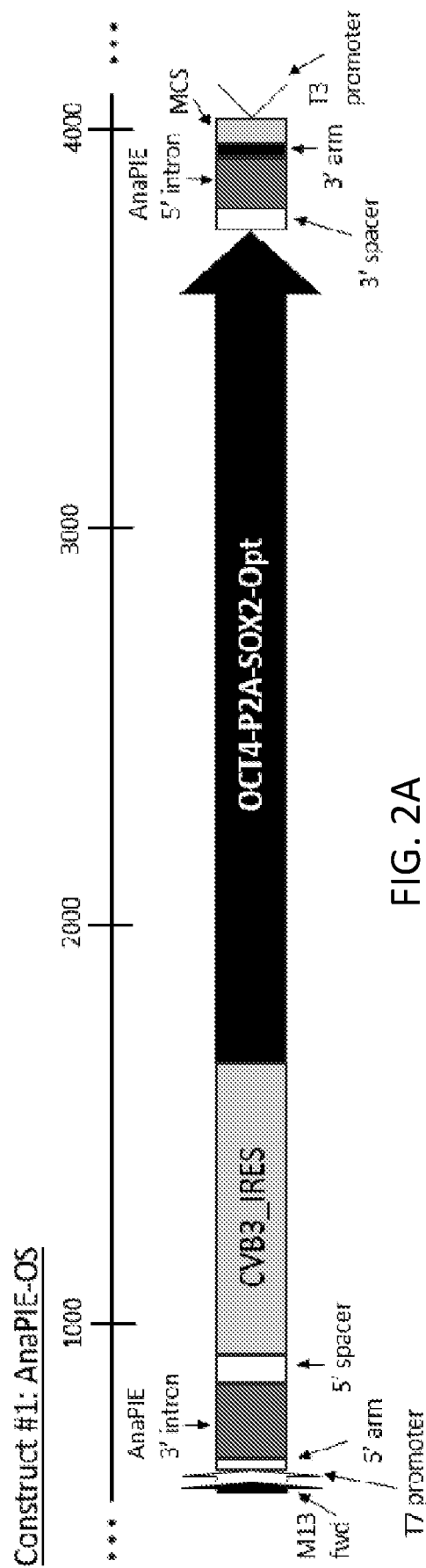


FIG. 2A

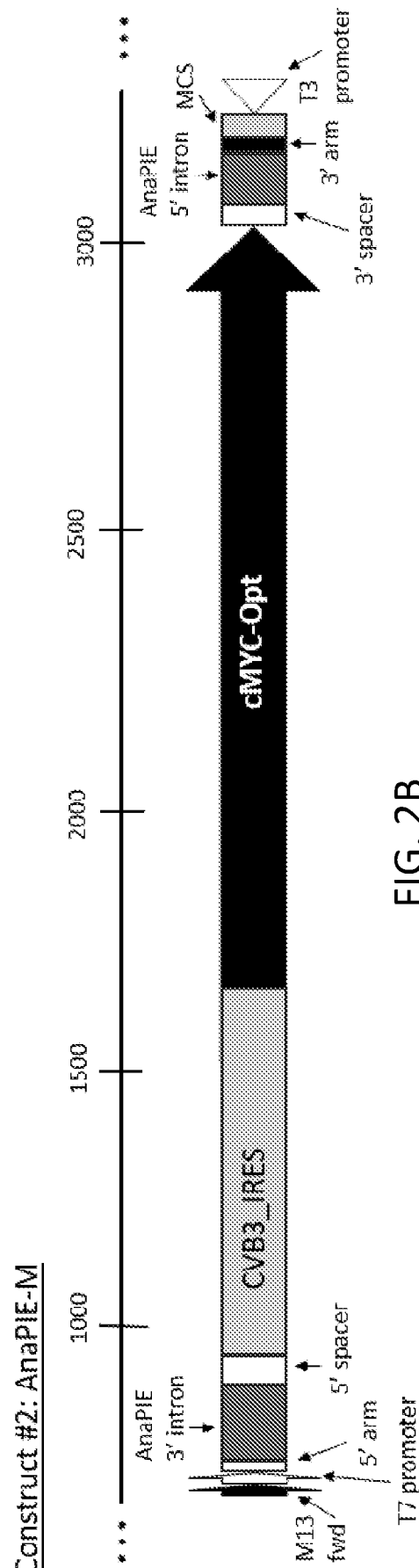


FIG. 2B

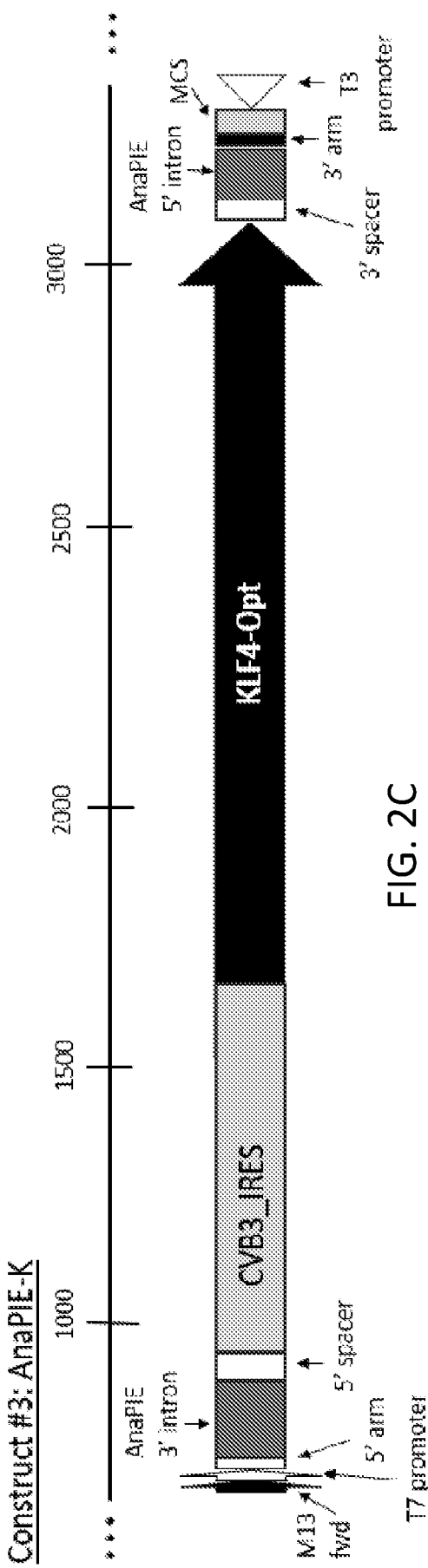


FIG. 2C

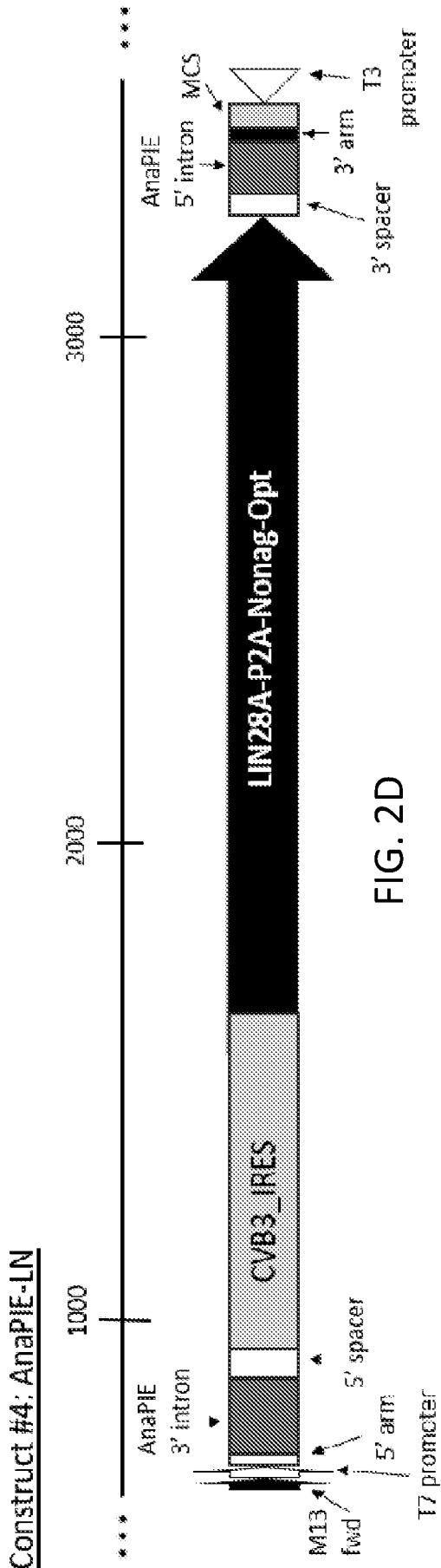


FIG. 2D

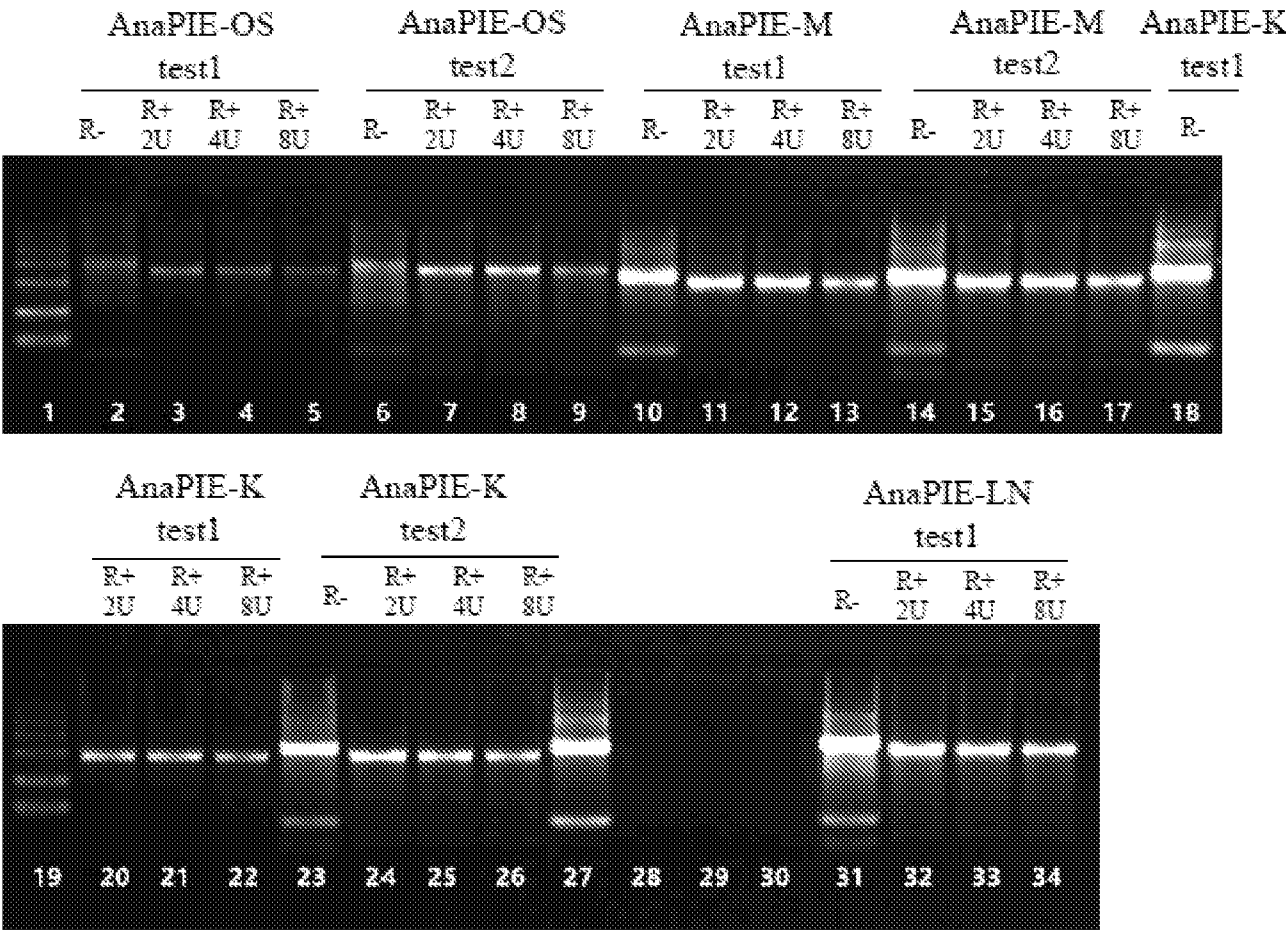


FIG. 3

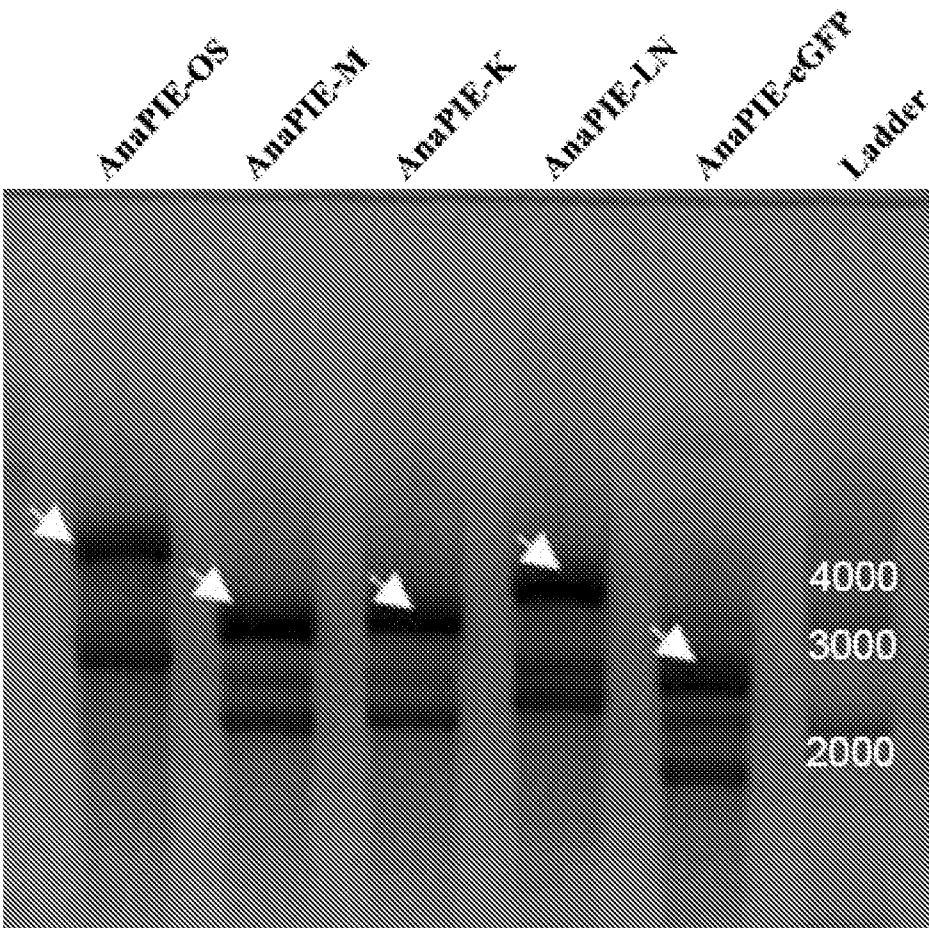


FIG. 4

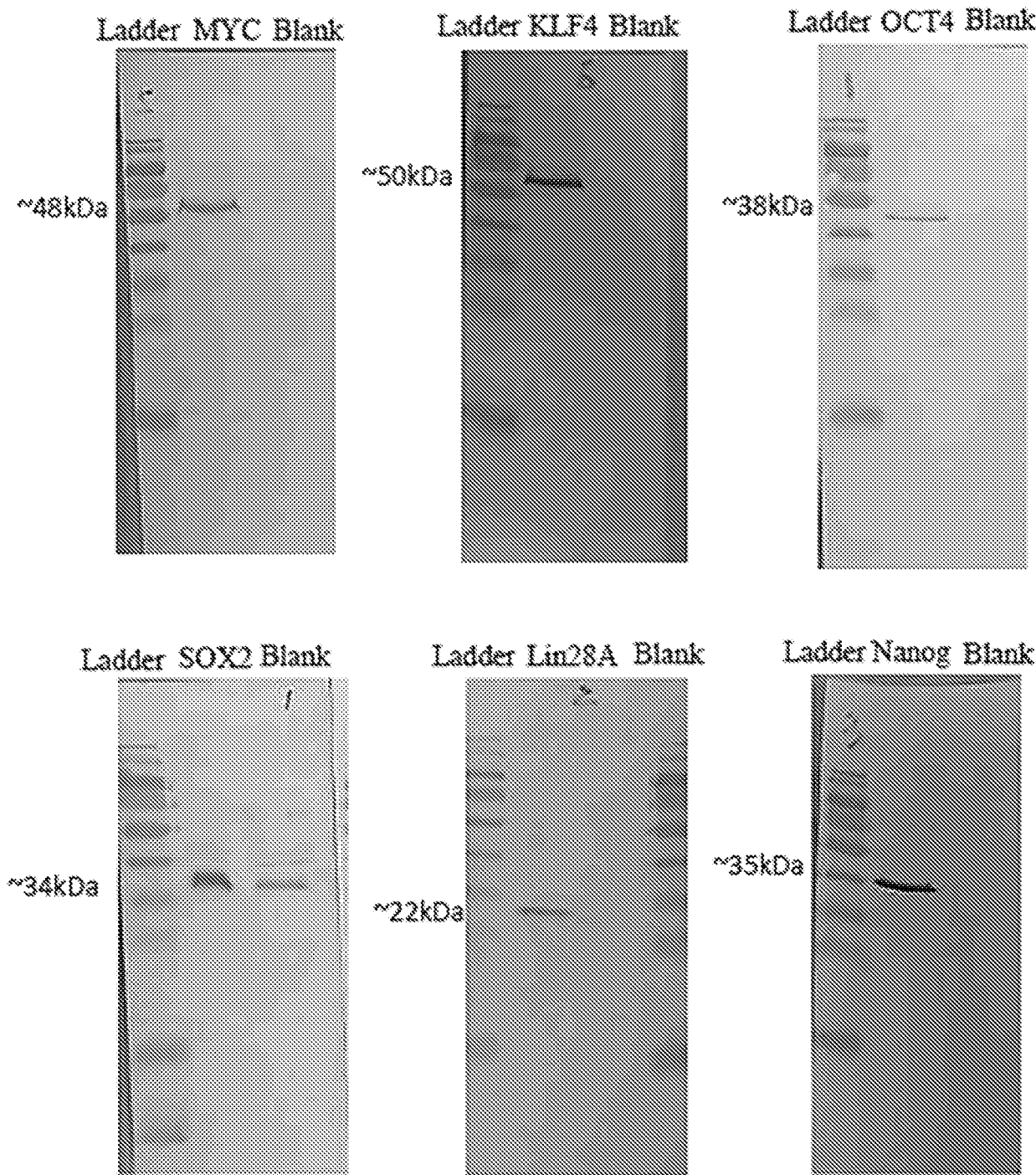


FIG. 5

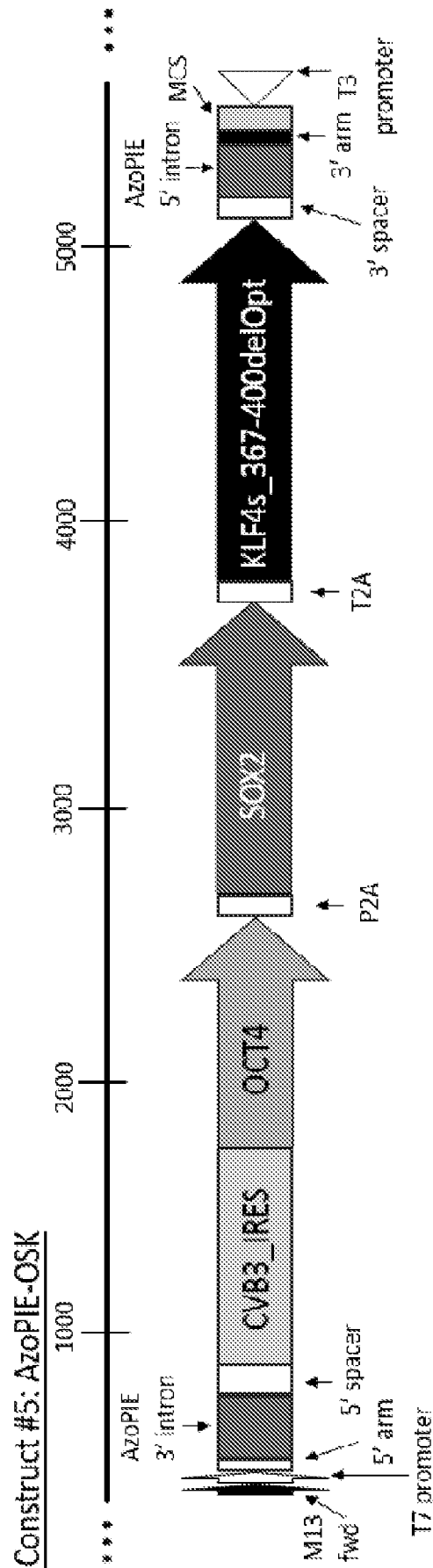


FIG. 6A

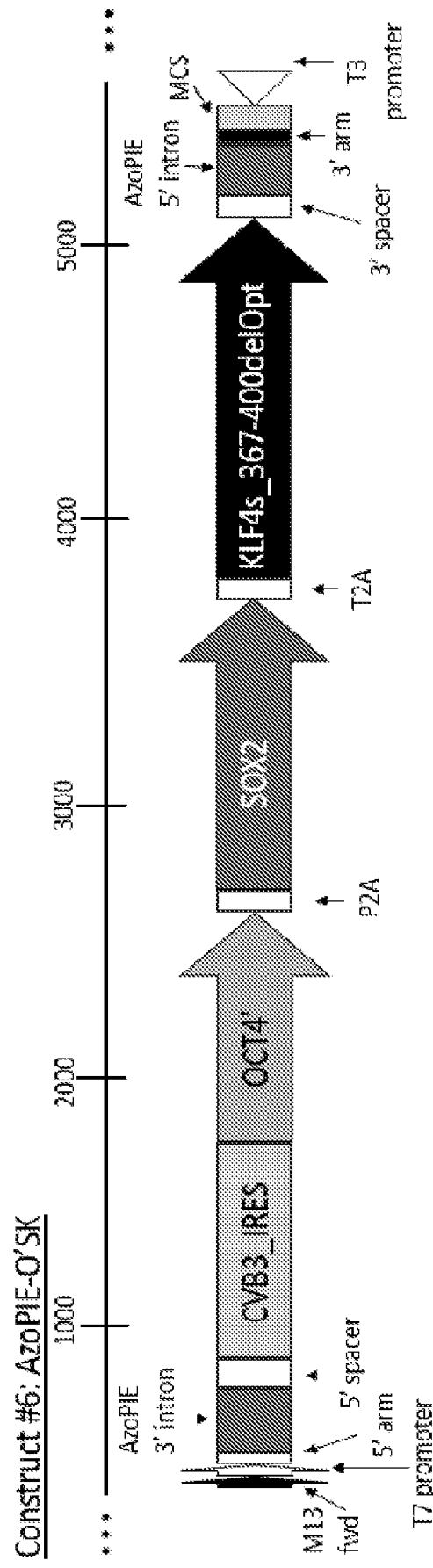
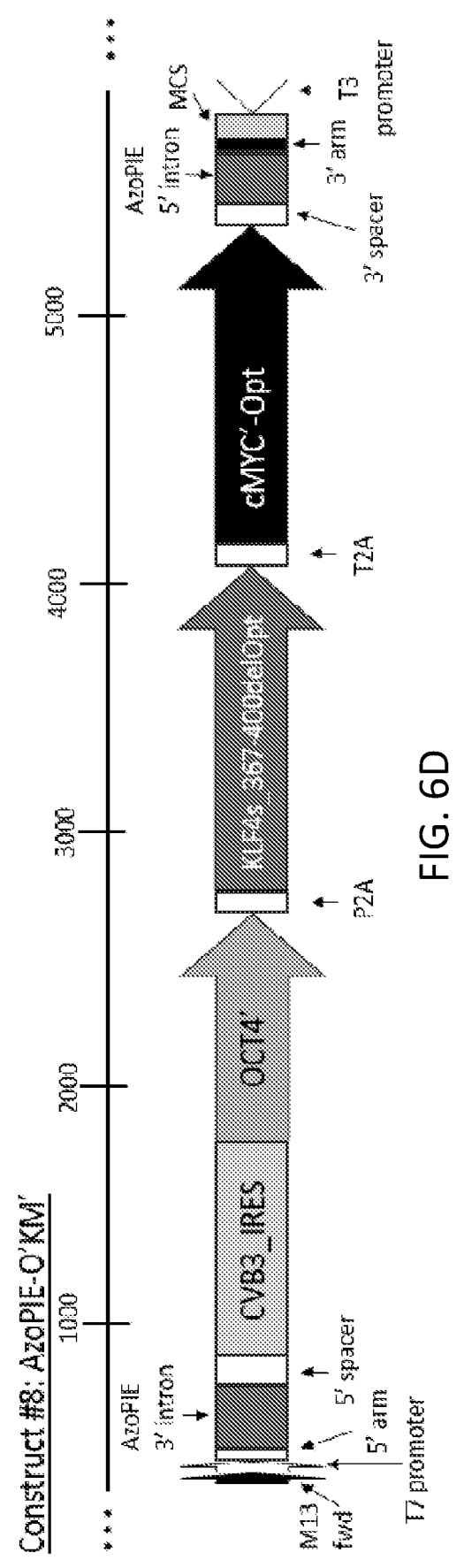
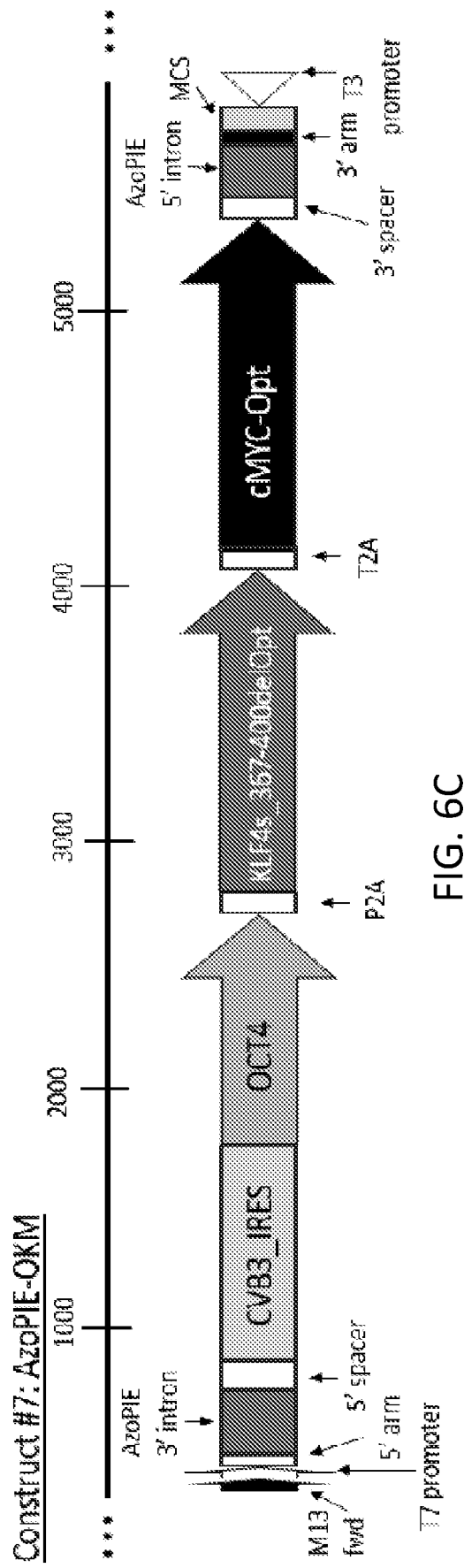


FIG. 6B



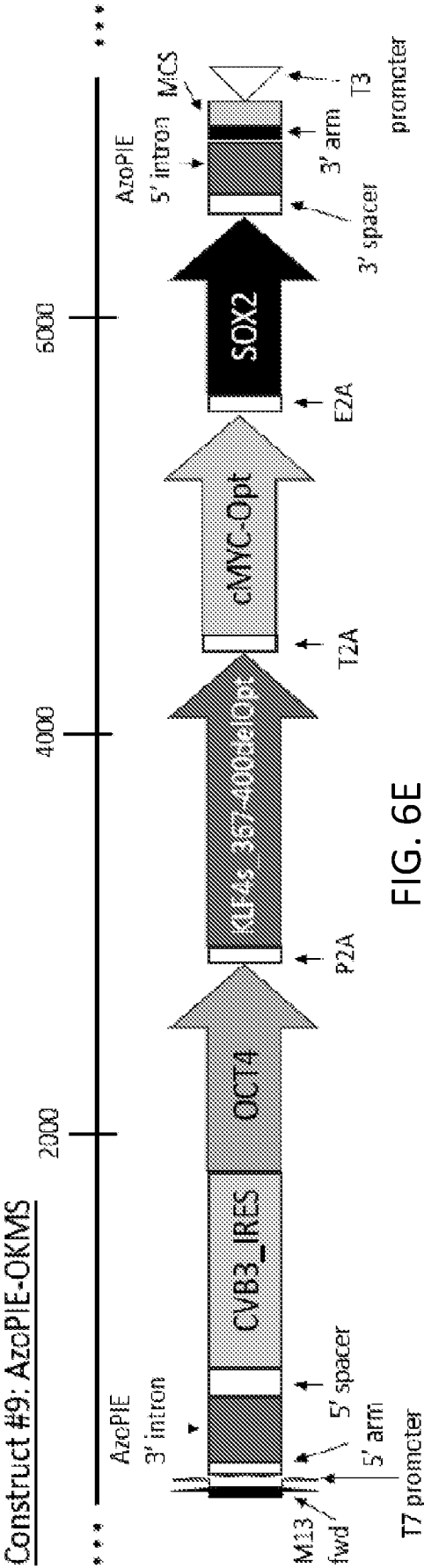


FIG. 6E

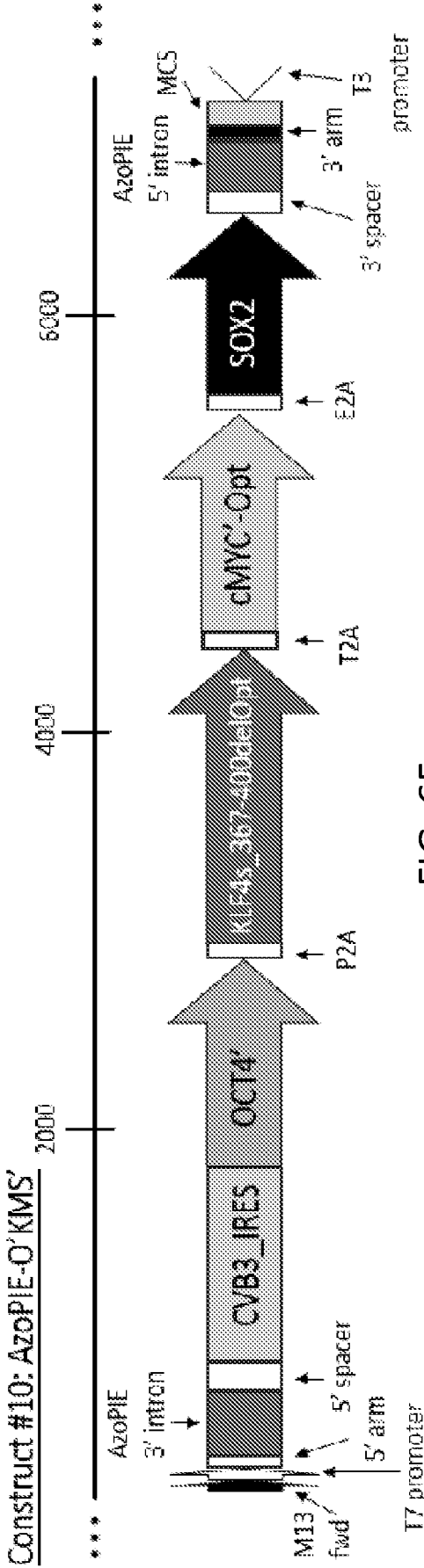


FIG. 6F

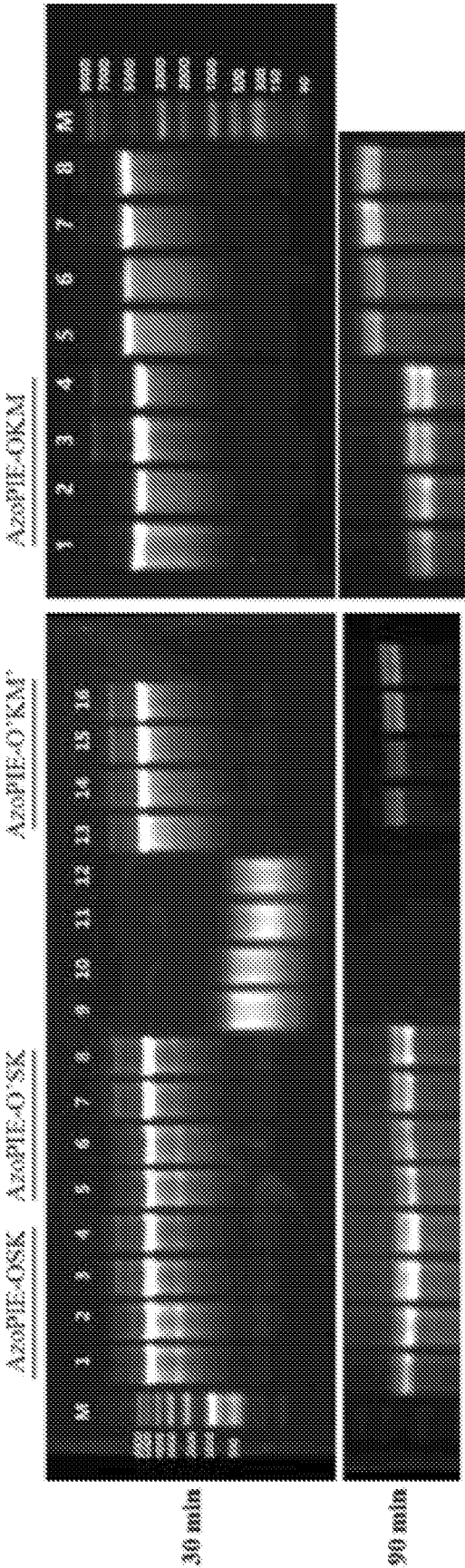


FIG. 7

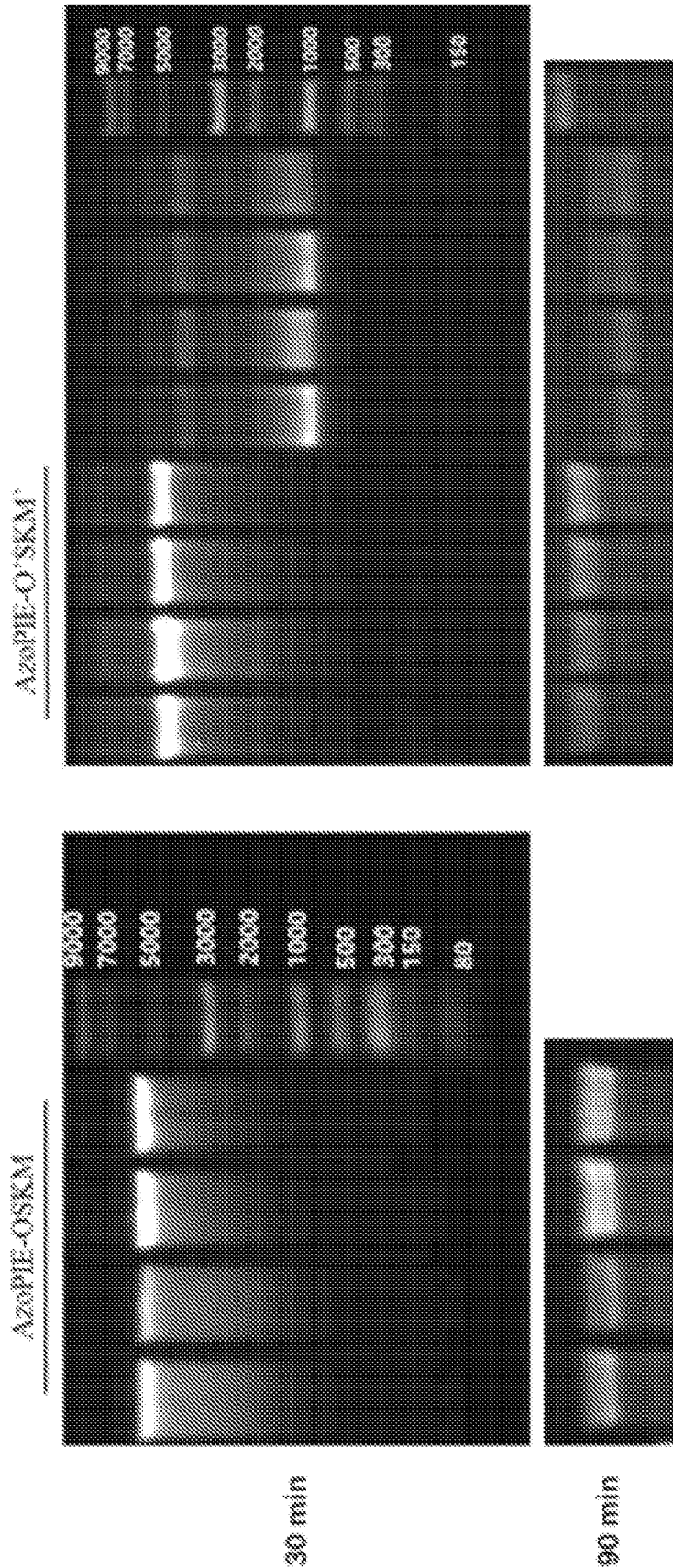


FIG. 8

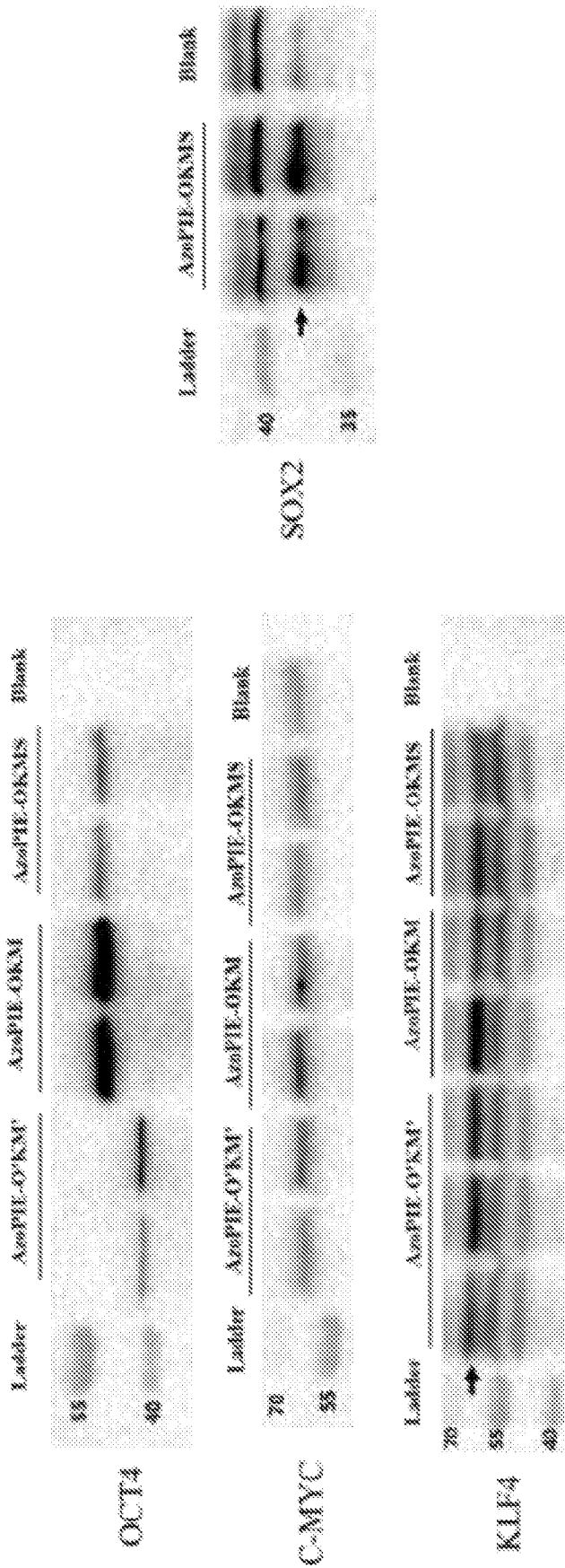


FIG. 9

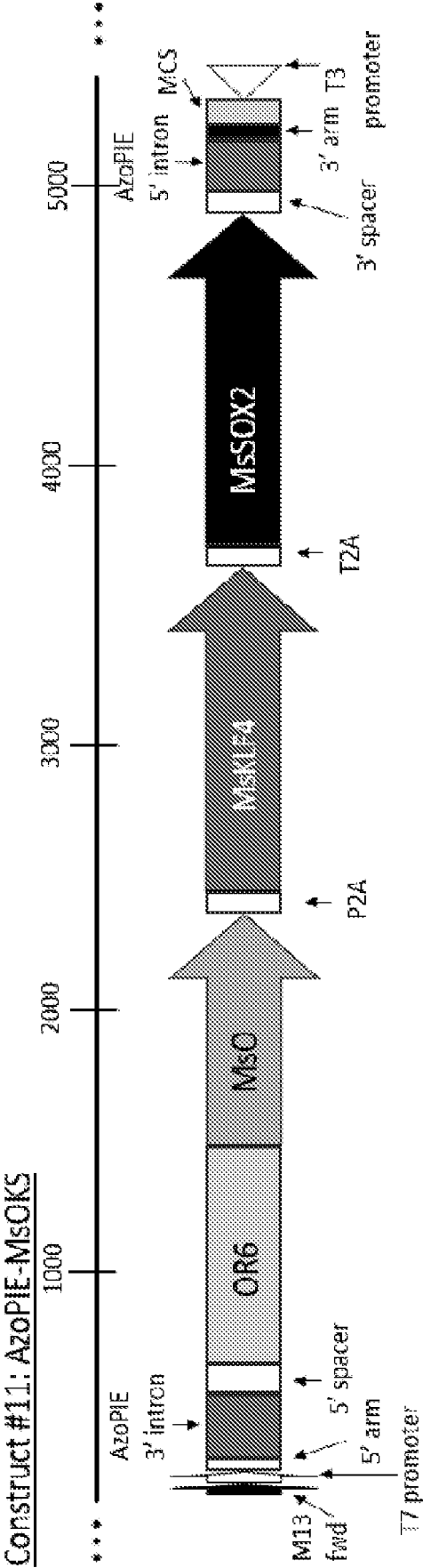


FIG. 10A

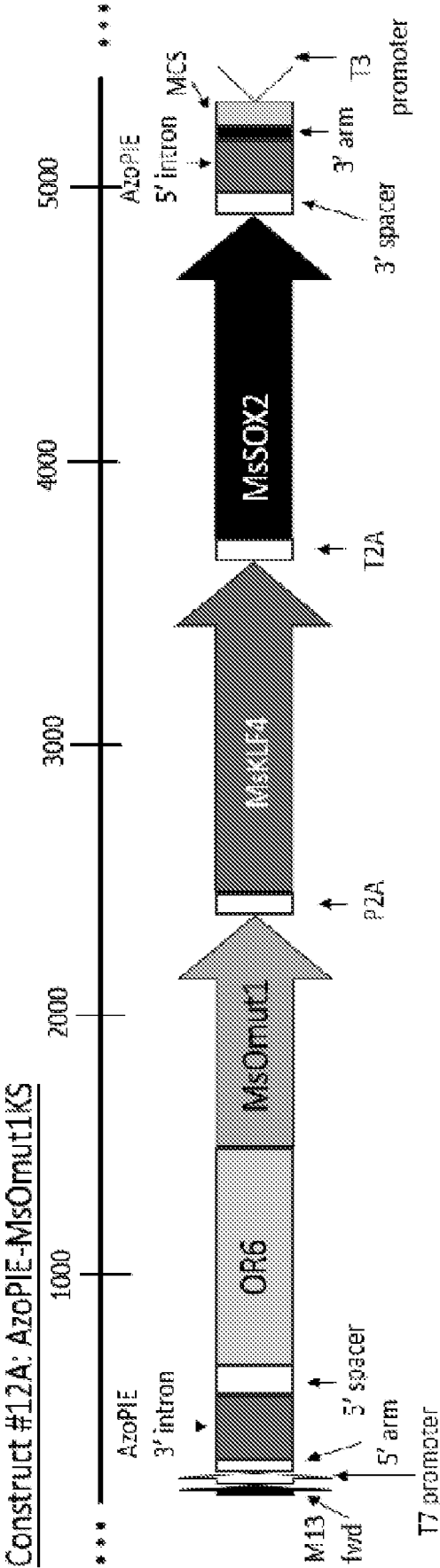


FIG. 10B

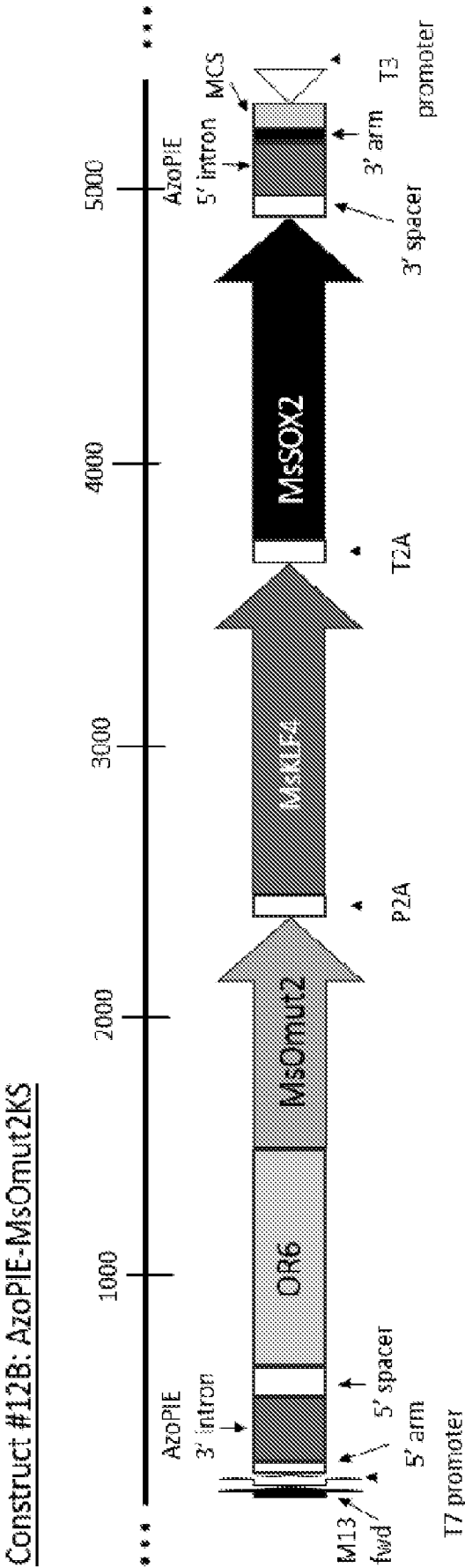


FIG. 10C

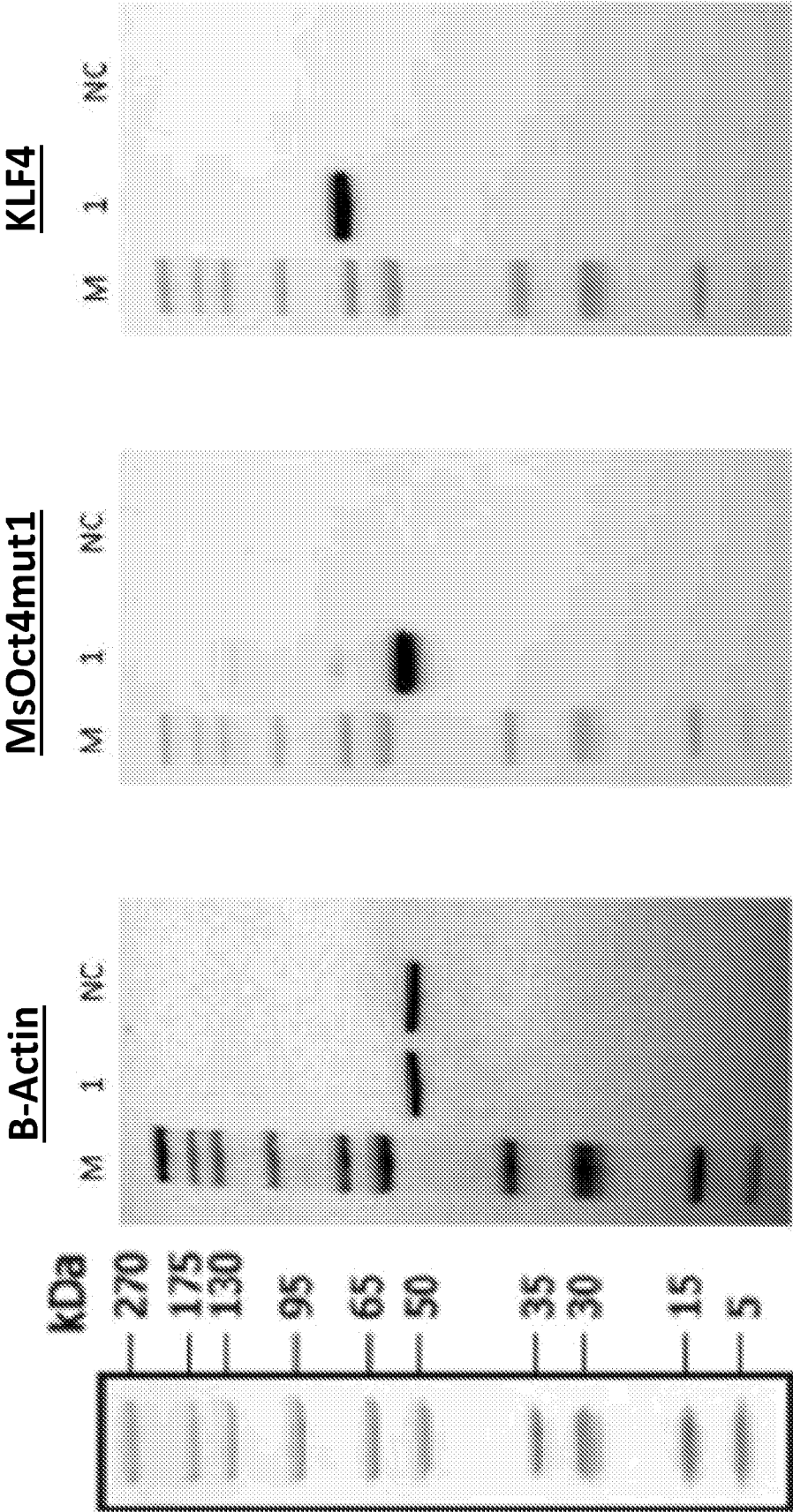


FIG. 11

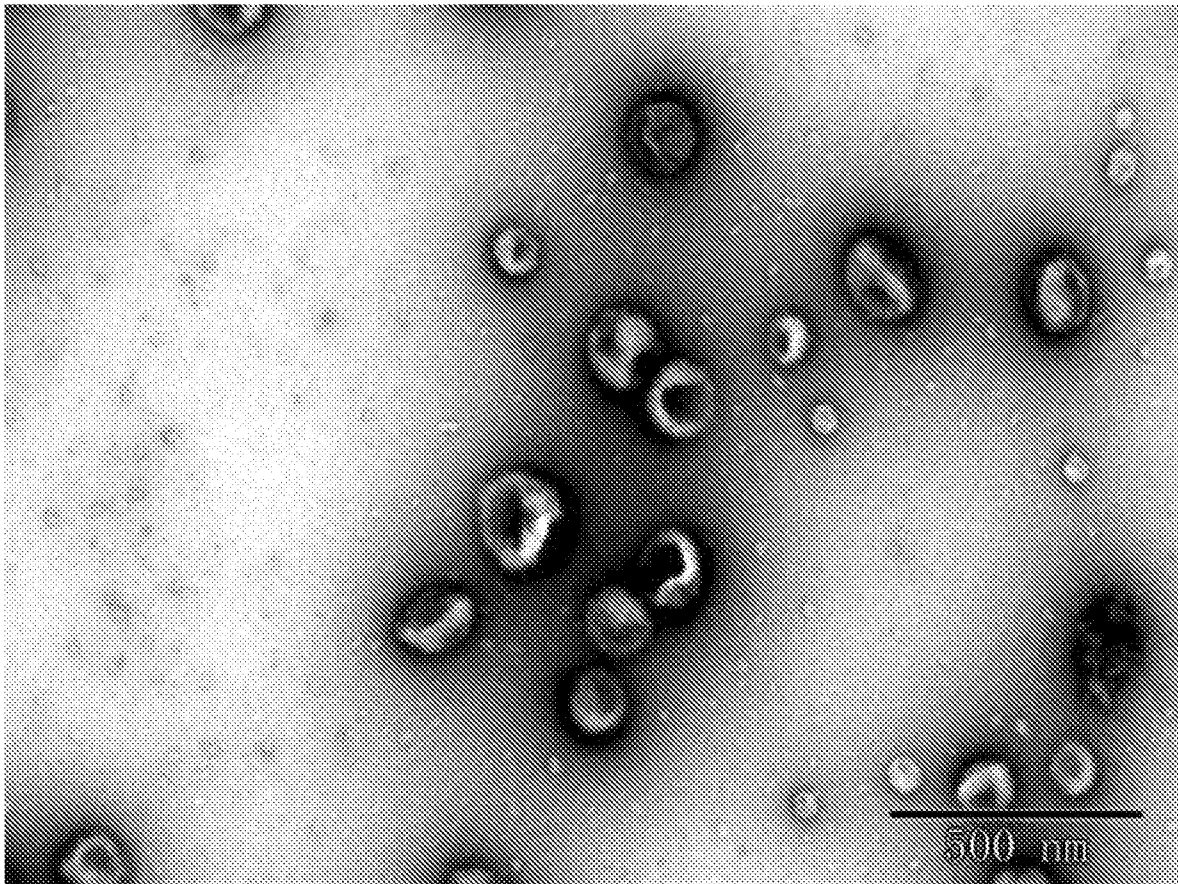


FIG. 12A

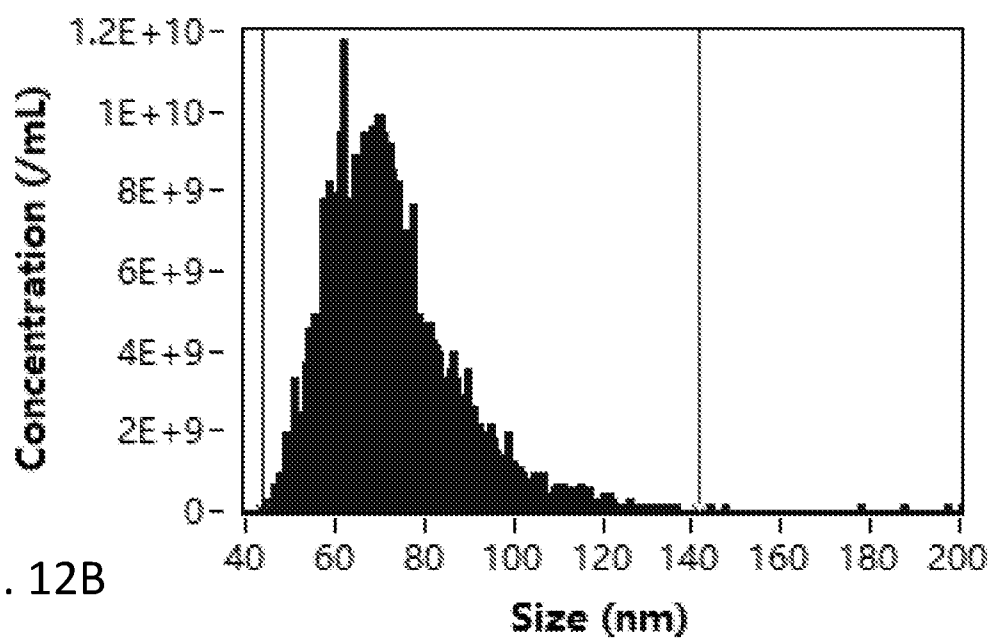


FIG. 12B

Sample	Particle concentration (particles/mL)	Particle size (nm)
HEK-exo	4.83E11	72.4
HEK-exo with circRNA	1.58E11	151.8
huMSC-exo	2.99E11	75.5
huMSC-exo with circRNA	1.76E11	137.9

FIG. 13

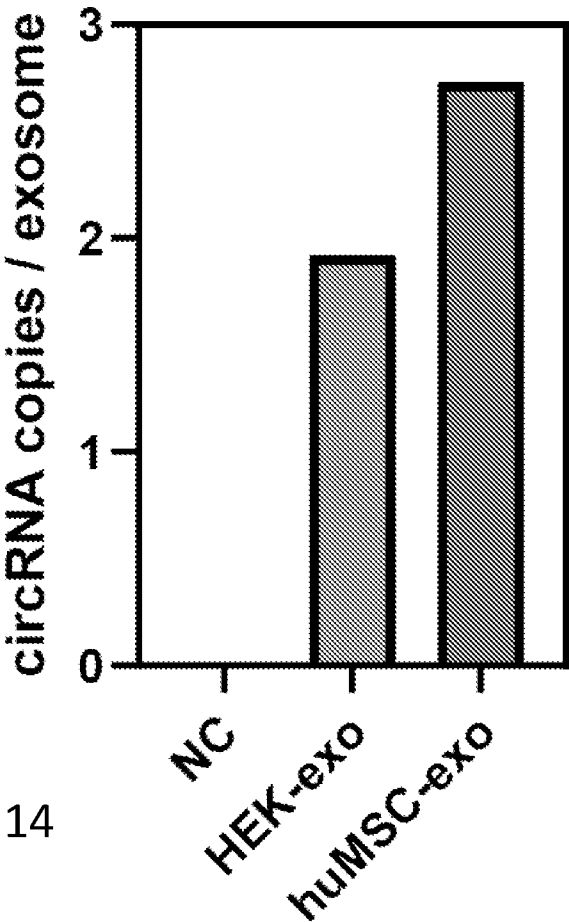


FIG. 14

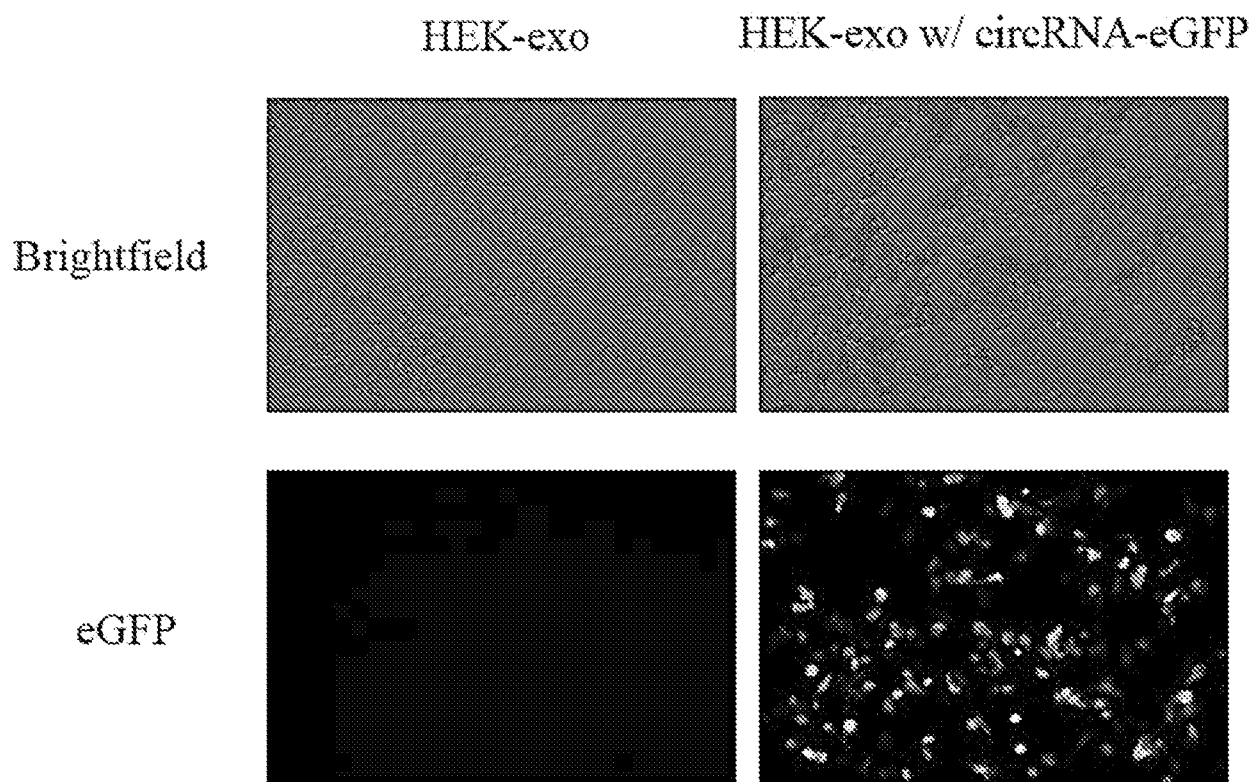


FIG. 15A

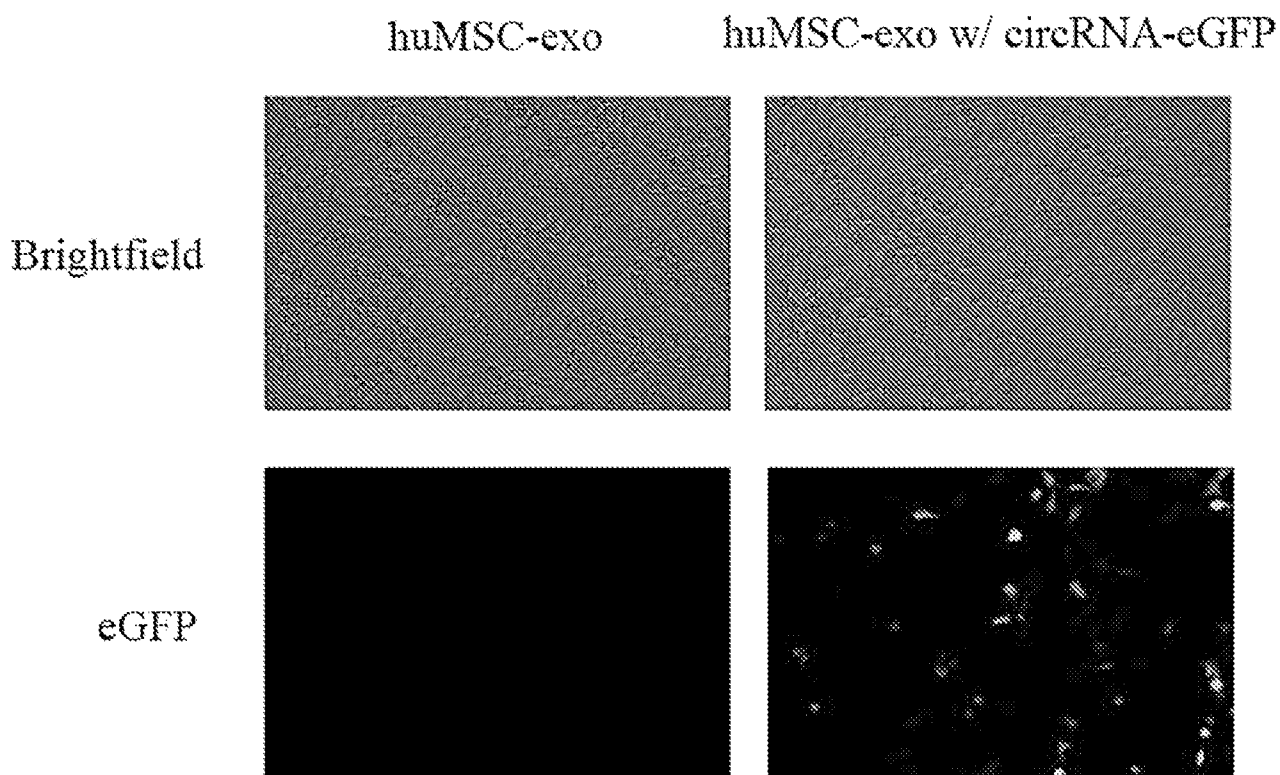


FIG. 15B

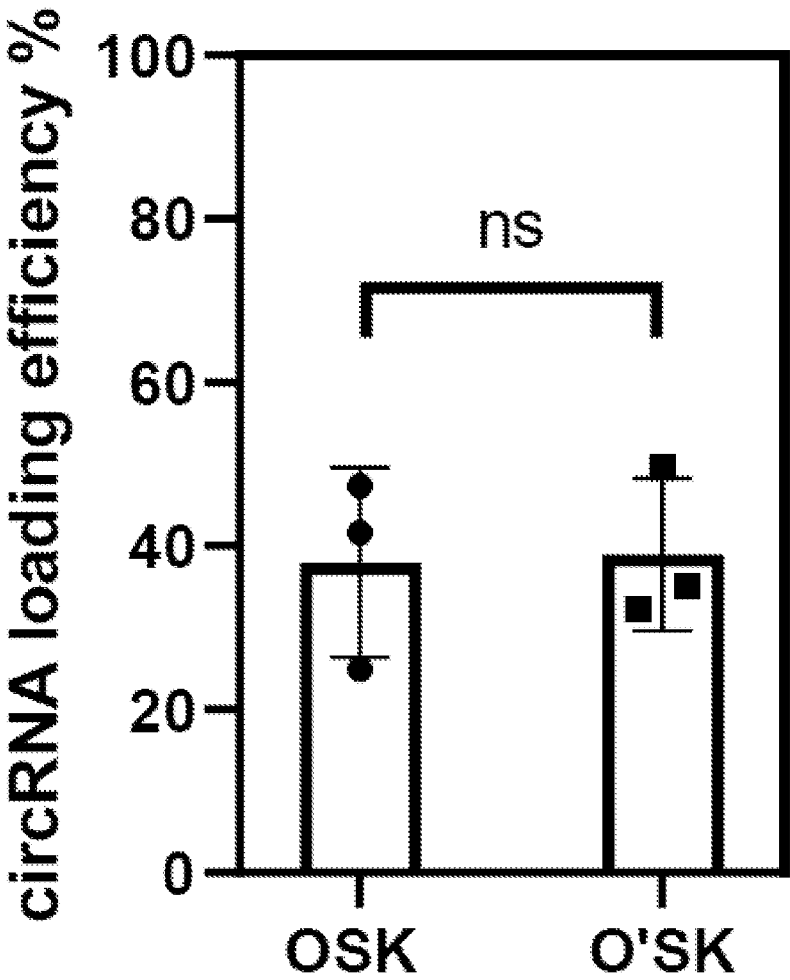


FIG. 16

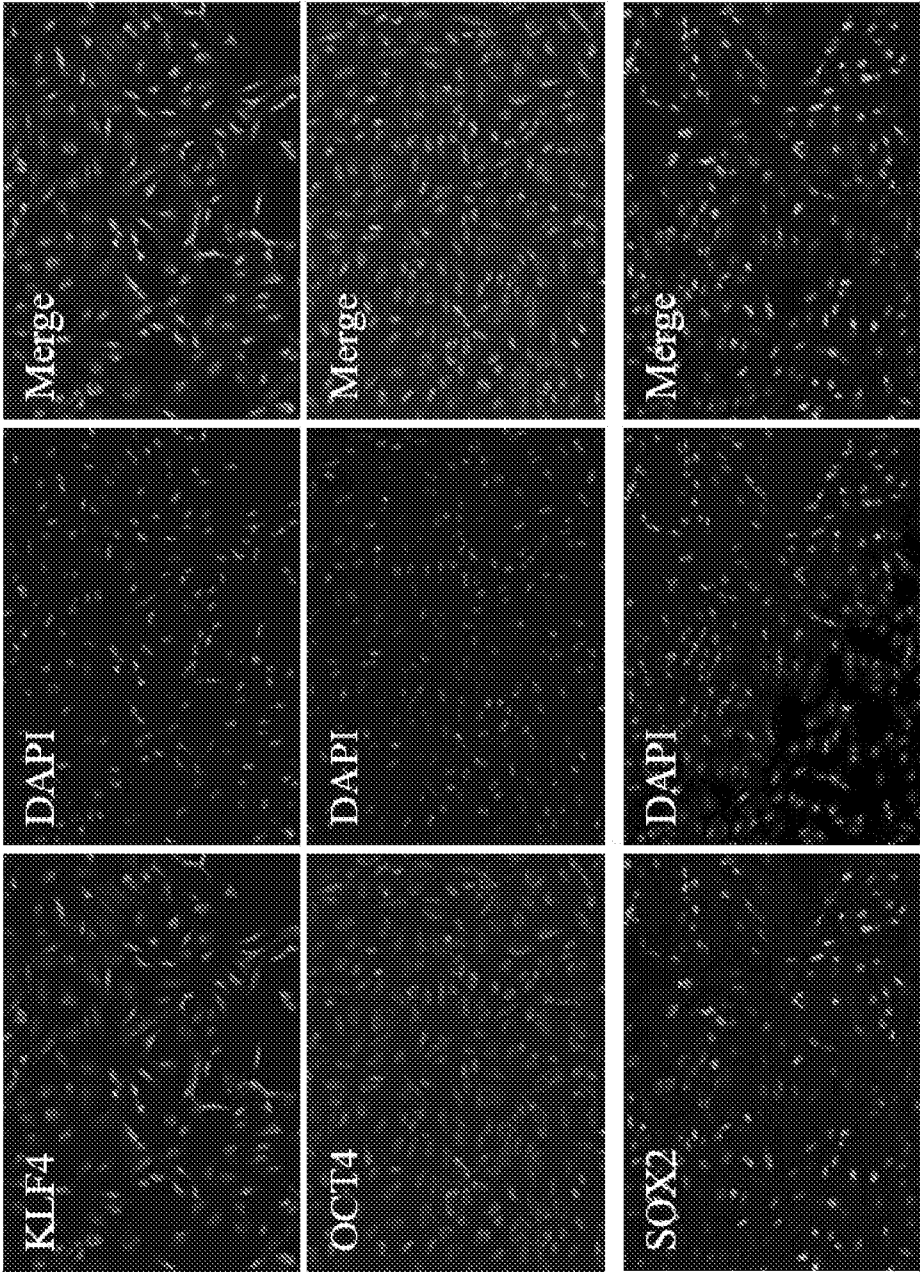


FIG. 17

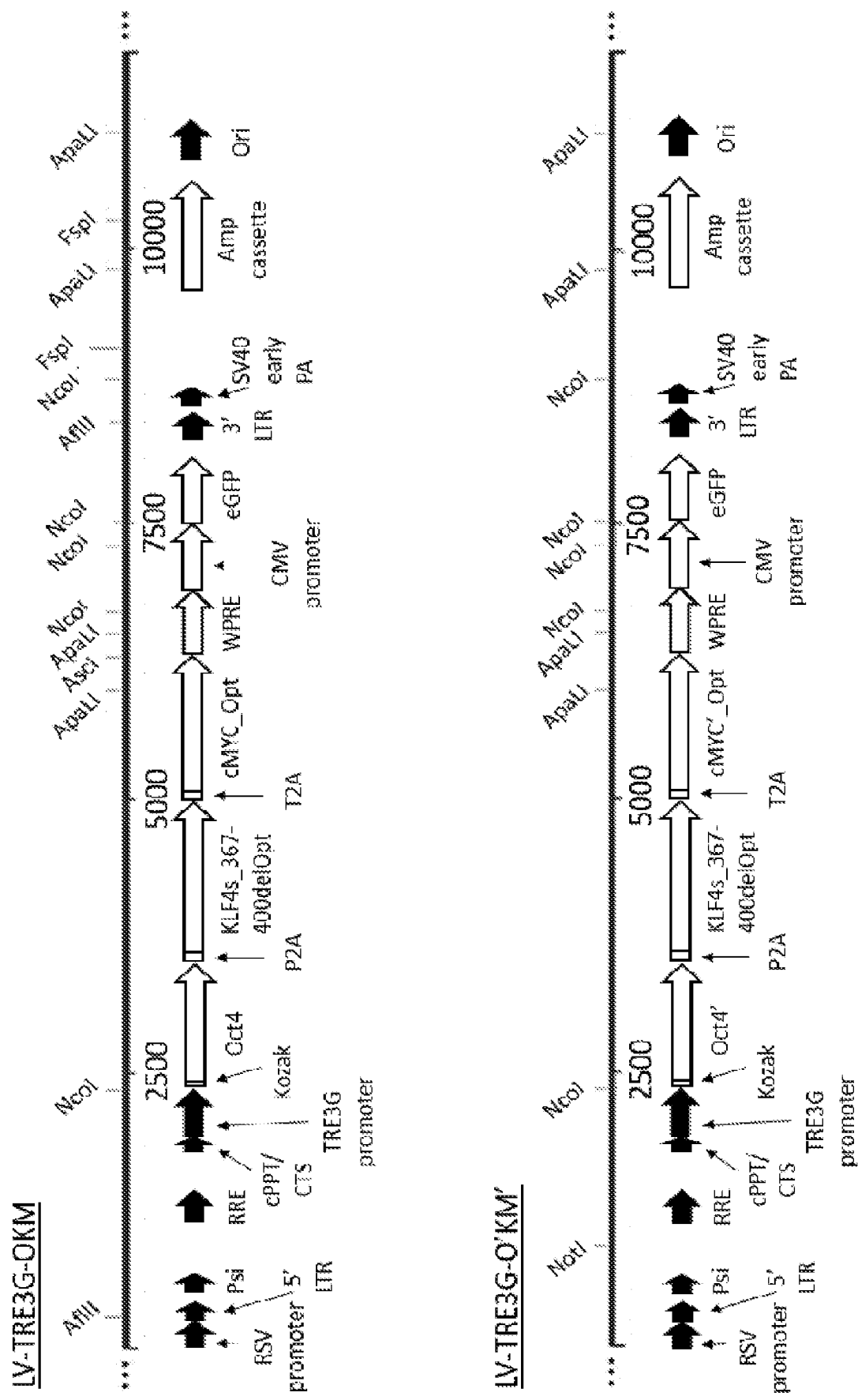


FIG. 18A

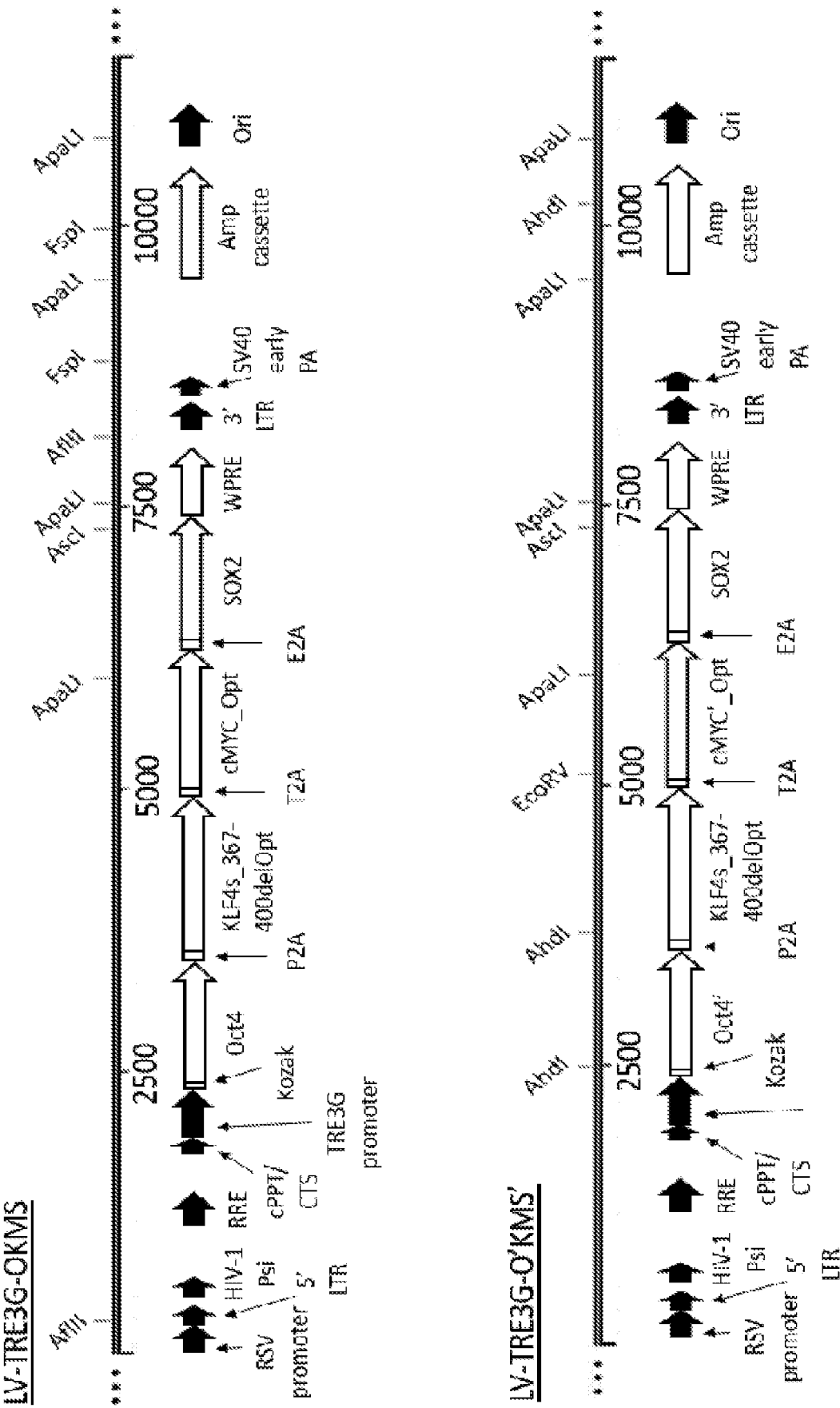
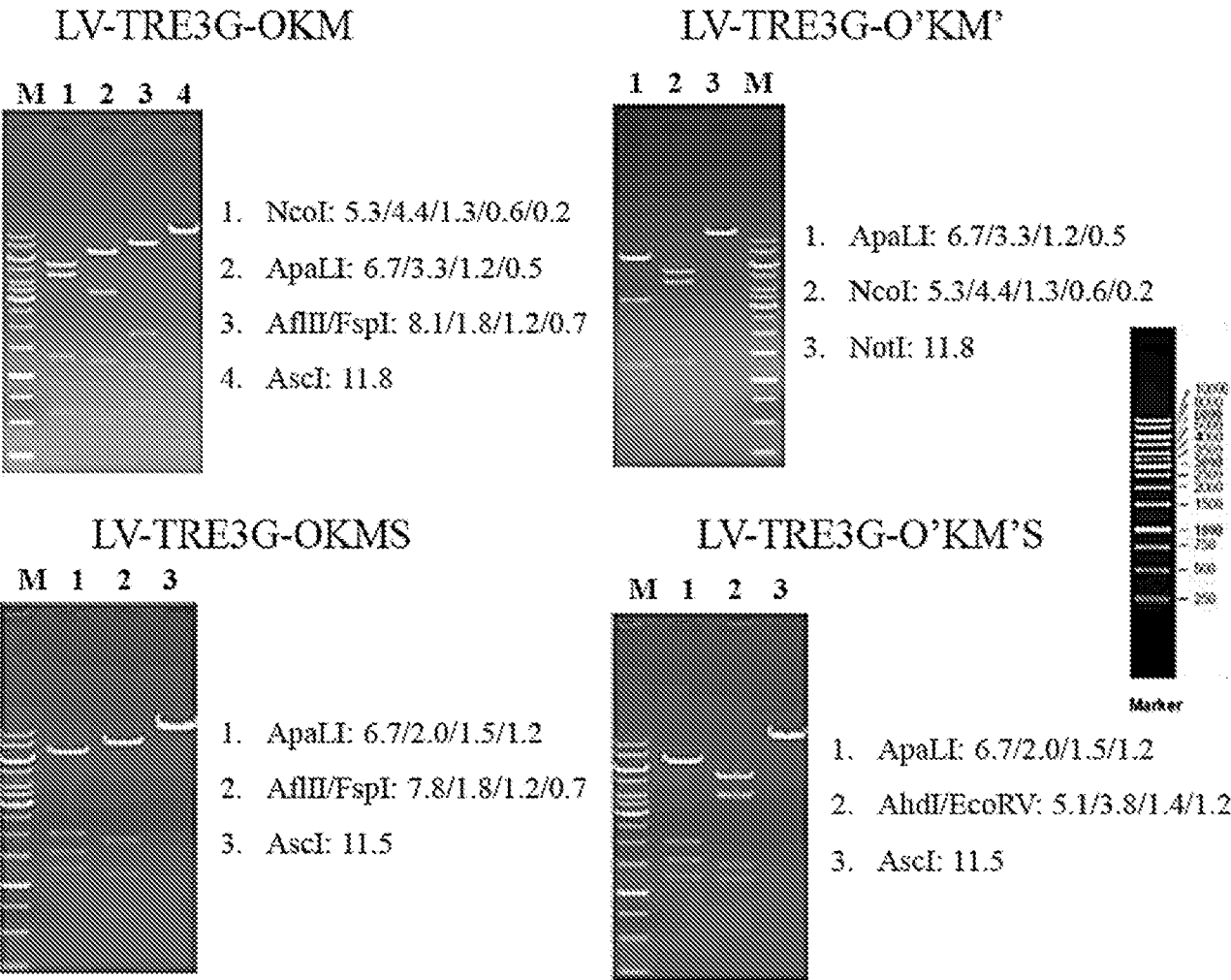


FIG. 18A (cont.)



Enzyme digestion of final vector

FIG. 18B

Sample No.	Bacterial Test	Mycoplasma Test	Titer (TU/mL)
LV-TRE3G-OKM	Negative	Negative	1.04E+8
LV-TRE3G-O'KM'	Negative	Negative	2.09E+8
LV-TRE3G-OKMS	Negative	Negative	3.88E+8
LV-TRE3G-O'KM'S	Negative	Negative	7.08E+8

FIG. 19A

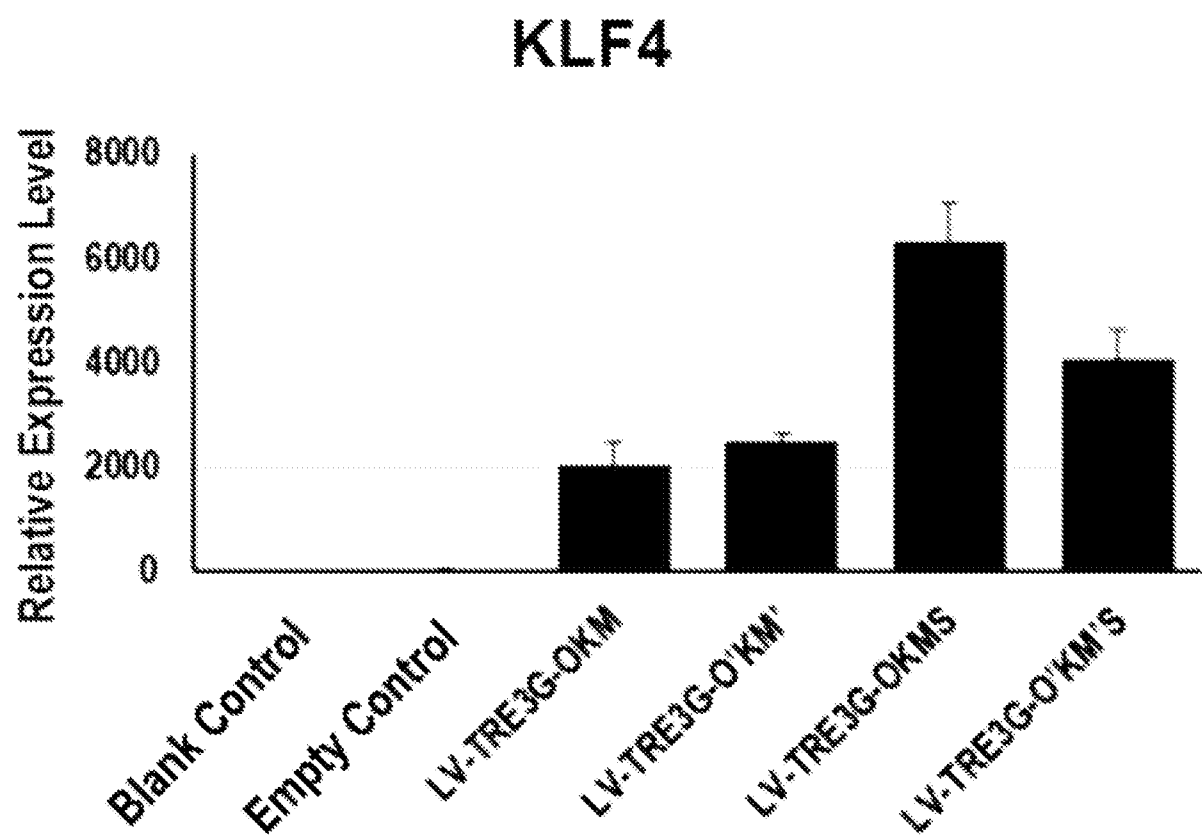


FIG. 19B

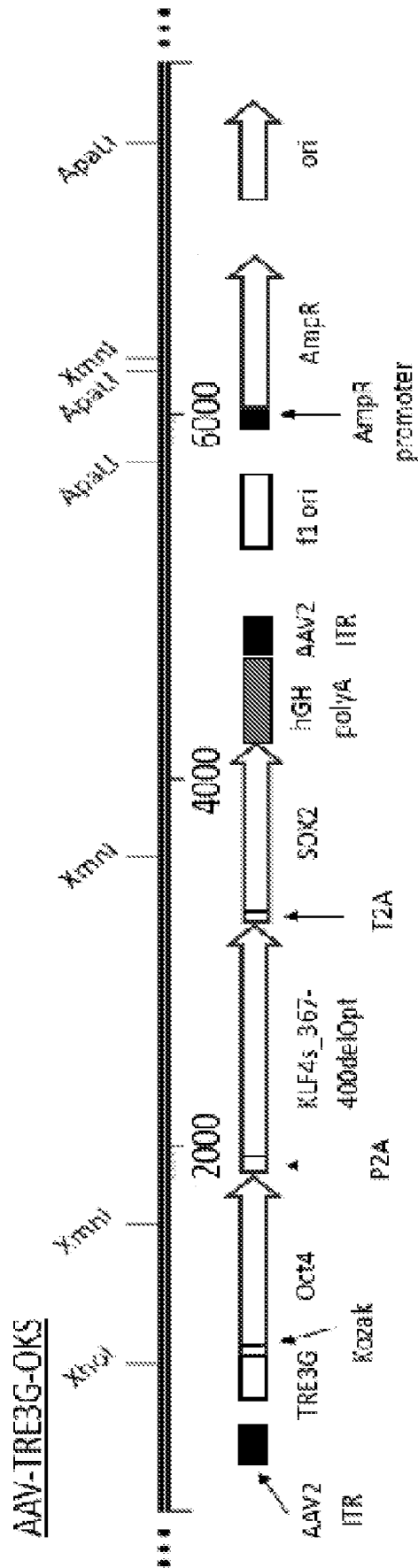


FIG. 20

AAV-TRE3G-OKS

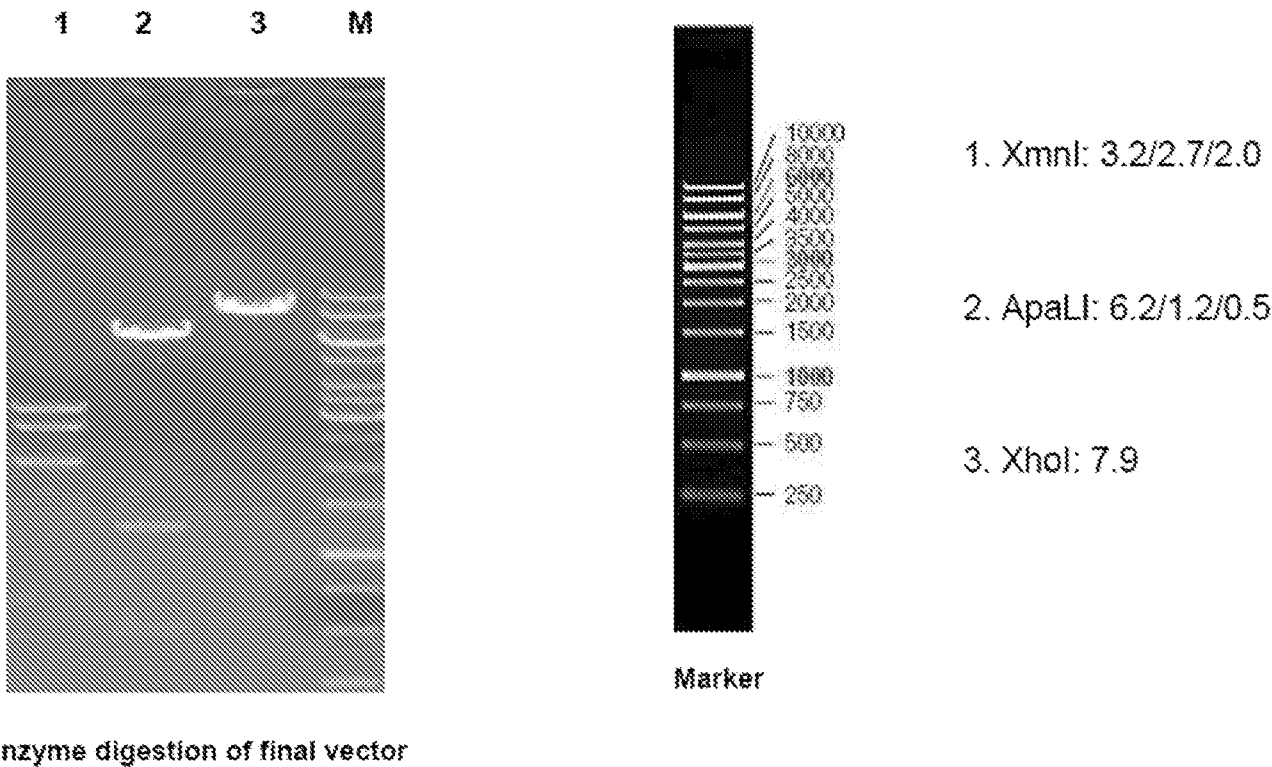


FIG. 21

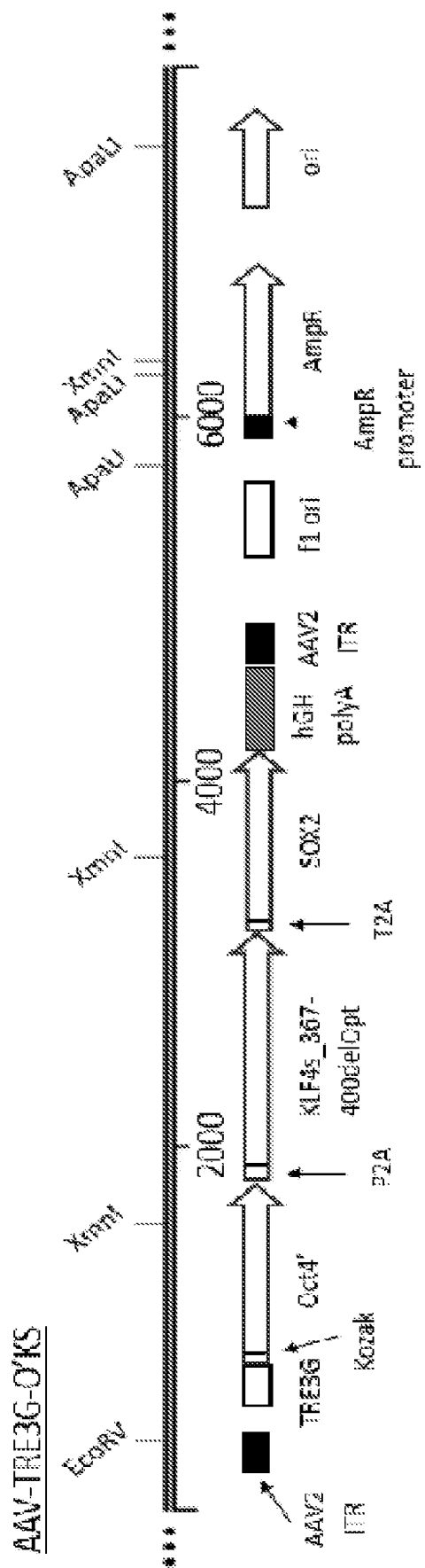


FIG. 22

AAV-TRE3G-O'KS

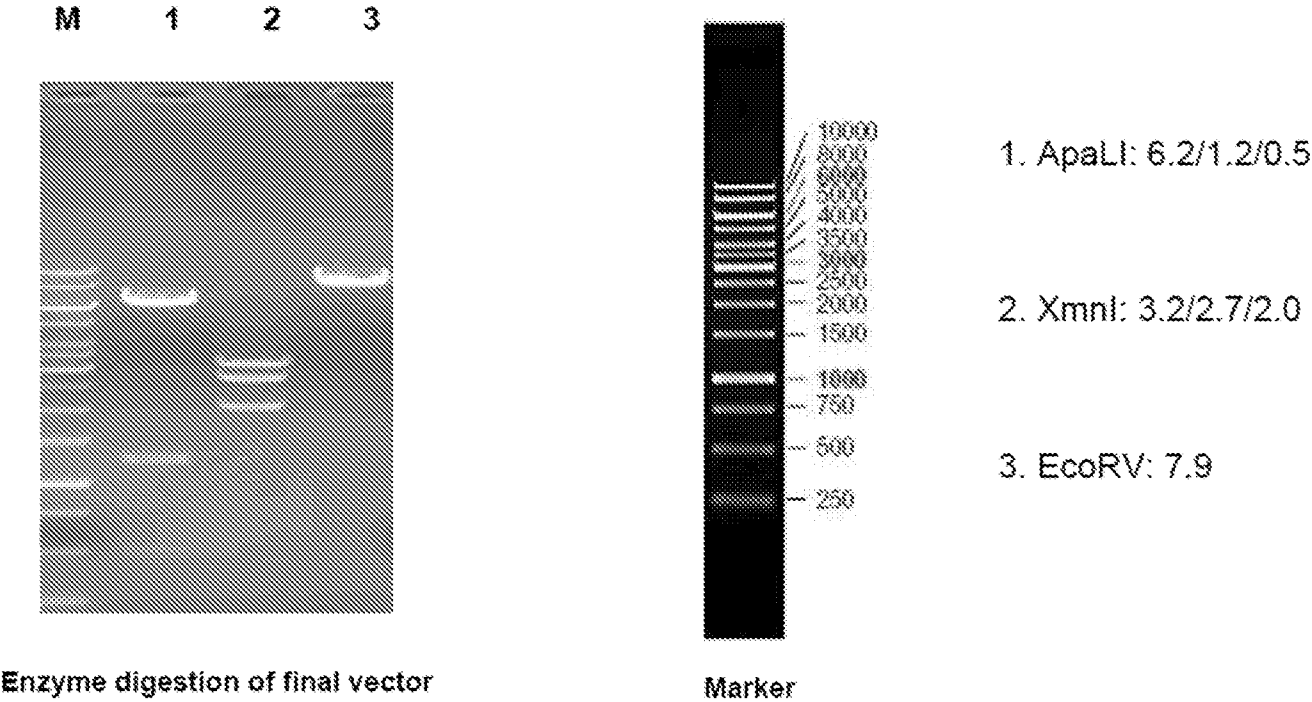


FIG. 23

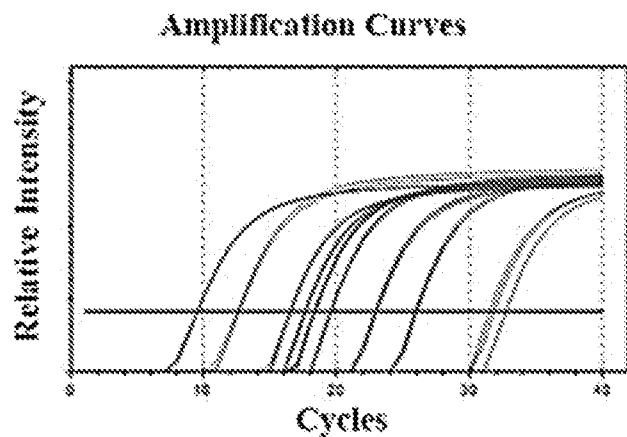


FIG. 24A

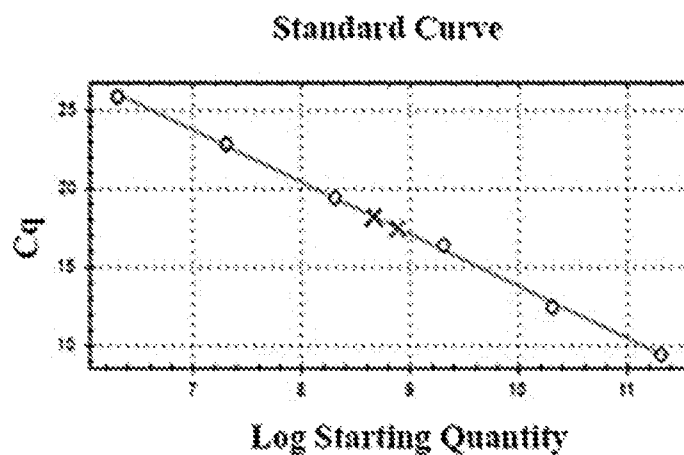


FIG. 24B

Sample No.	Target	CT	q-PCR Titer	Average	Dilution	Titer (GC/mL)
AAV2-TRE3G-OKS-Rep1	ITR	18.28	4.60E+08	4.63E+08	20000	9.26E+12
AAV2-TRE3G-OKS-Rep2	ITR	18.26	4.65E+08	4.63E+08	20000	9.26E+12
AAV2-TRE3G-OKS-Rep3	ITR	18.27	4.64E+08	4.63E+08	20000	9.26E+12
AAV2-TRE3G-O'KS-Rep1	ITR	18.32	4.47E+08	4.55E+08	20000	9.10E+12
AAV2-TRE3G-O'KS-Rep2	ITR	18.27	4.63E+08	4.55E+08	20000	9.10E+12
AAV2-TRE3G-O'KS-Rep3	ITR	18.30	4.54E+08	4.55E+08	20000	9.10E+12
Reference AAV-Rep1	ITR	17.53	7.73E+08	7.55E+08	1000	7.55E+11
Reference AAV-Rep2	ITR	17.62	7.26E+08	7.55E+08	1000	7.55E+11
Reference AAV-Rep3	ITR	17.54	7.66E+08	7.55E+08	1000	7.55E+11

FIG. 24C

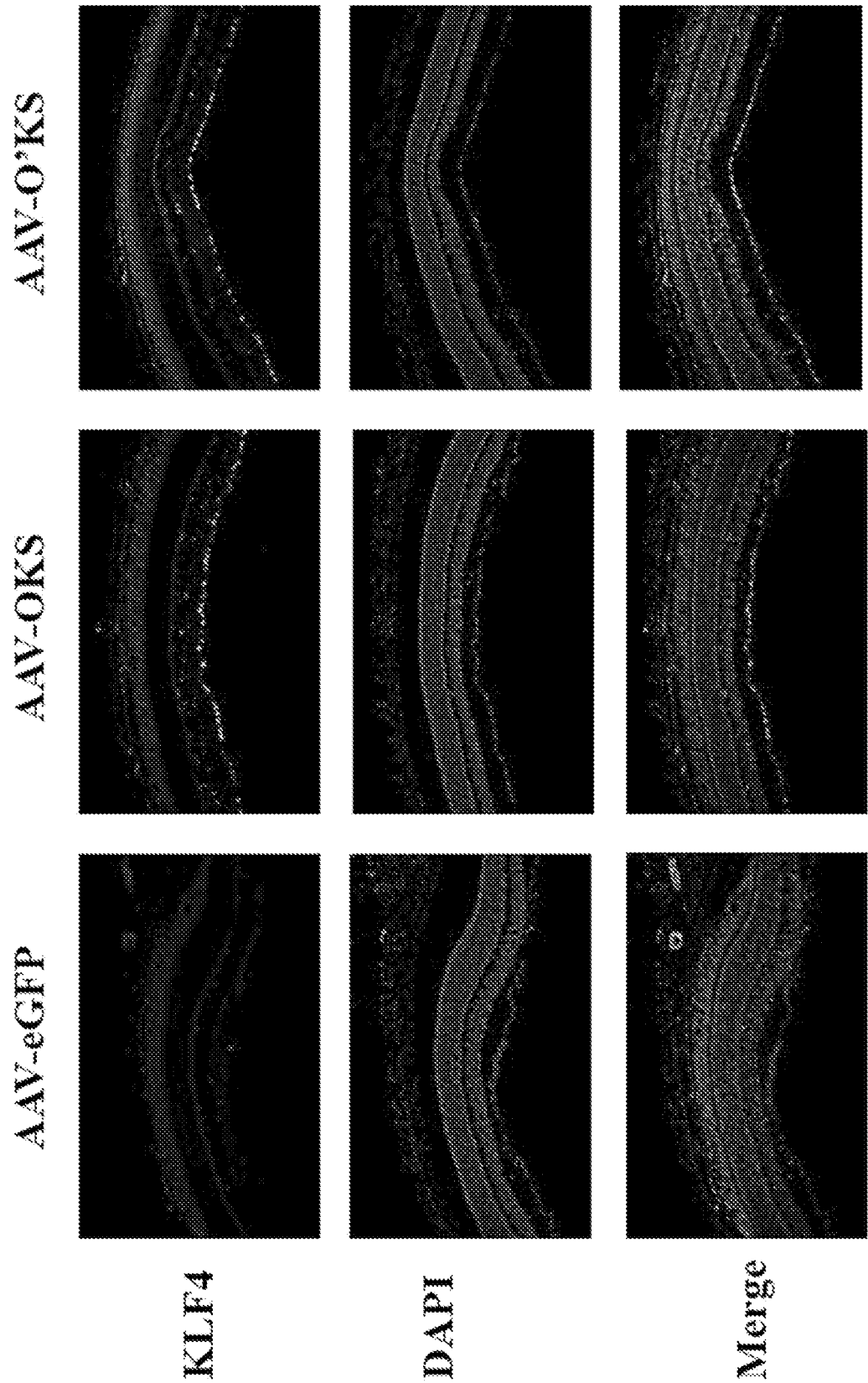


FIG. 25

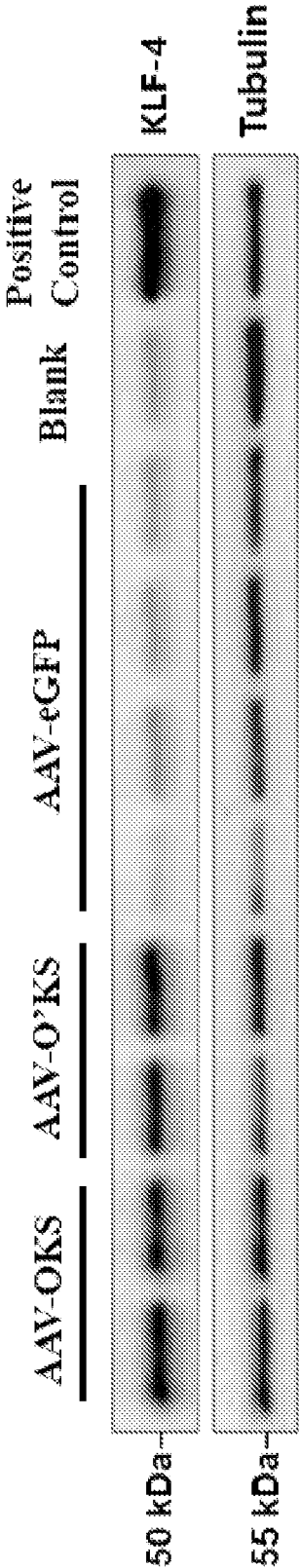


FIG. 26

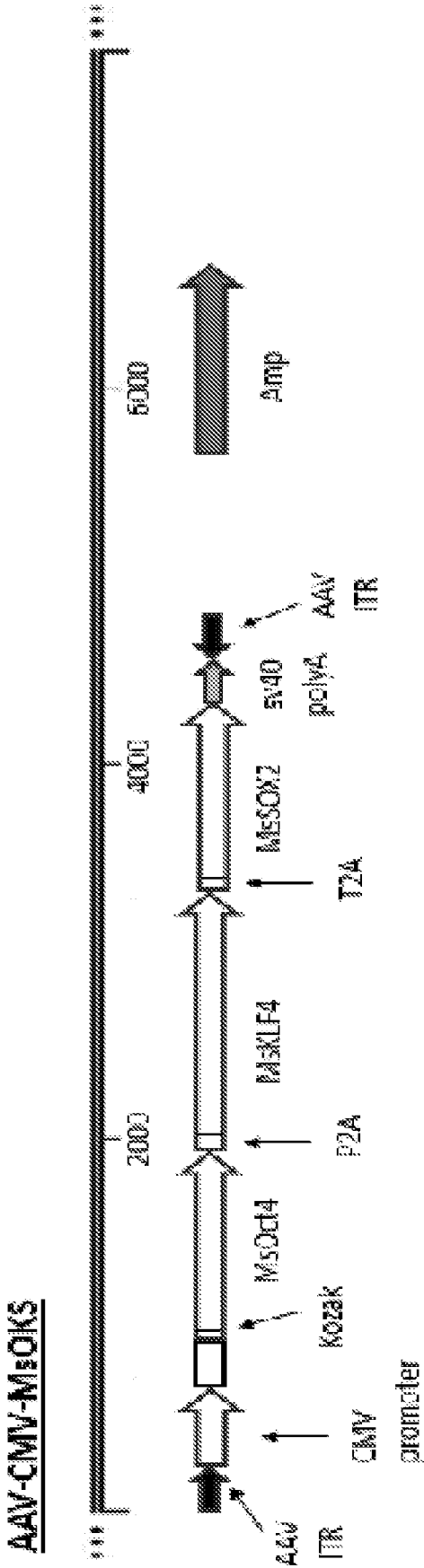


FIG. 27

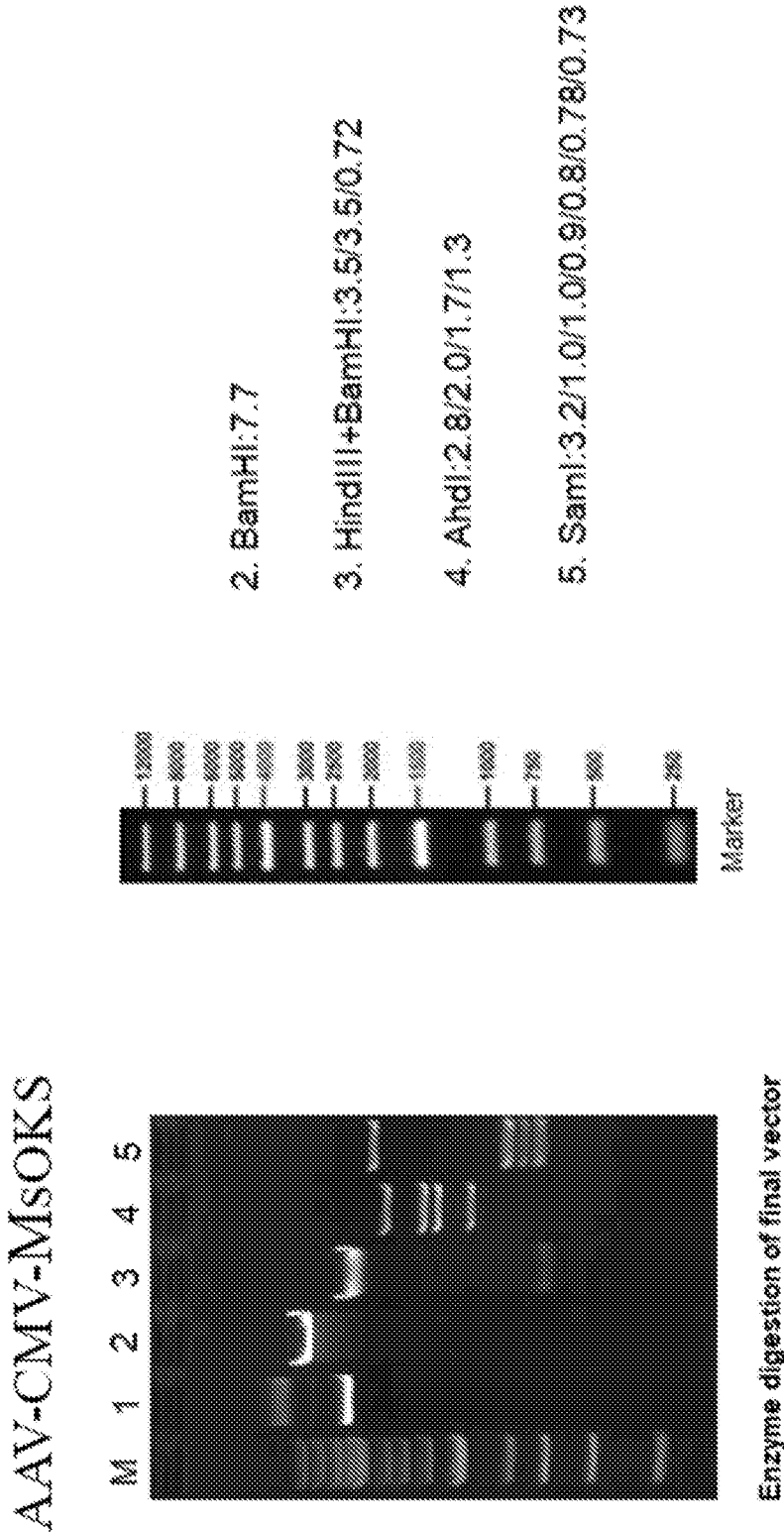


FIG. 28

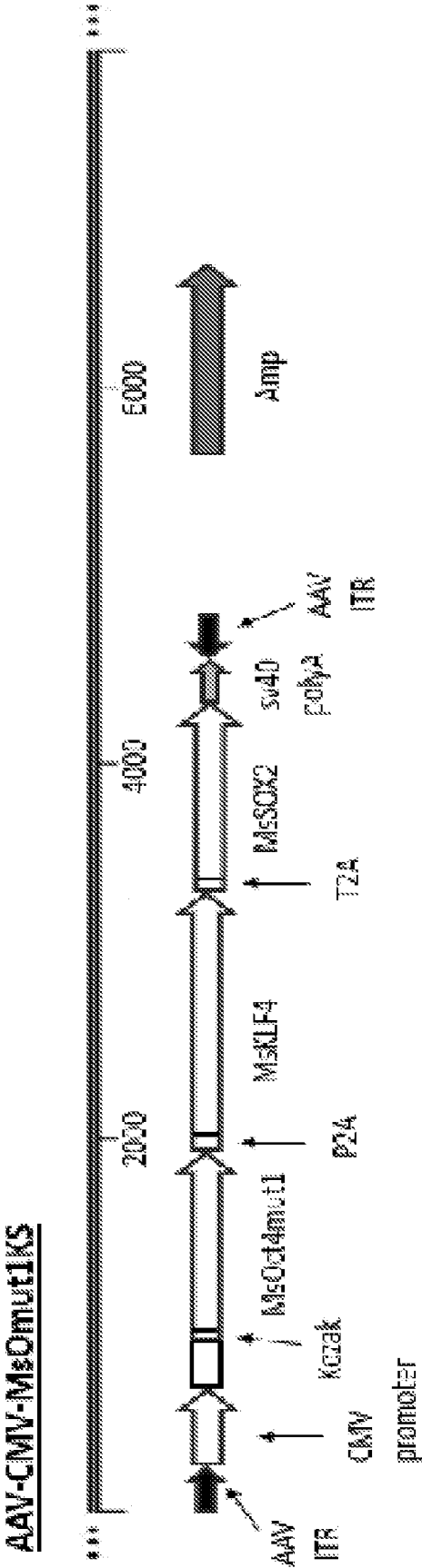


FIG. 29

AAV-CMV-MsOmut1KS

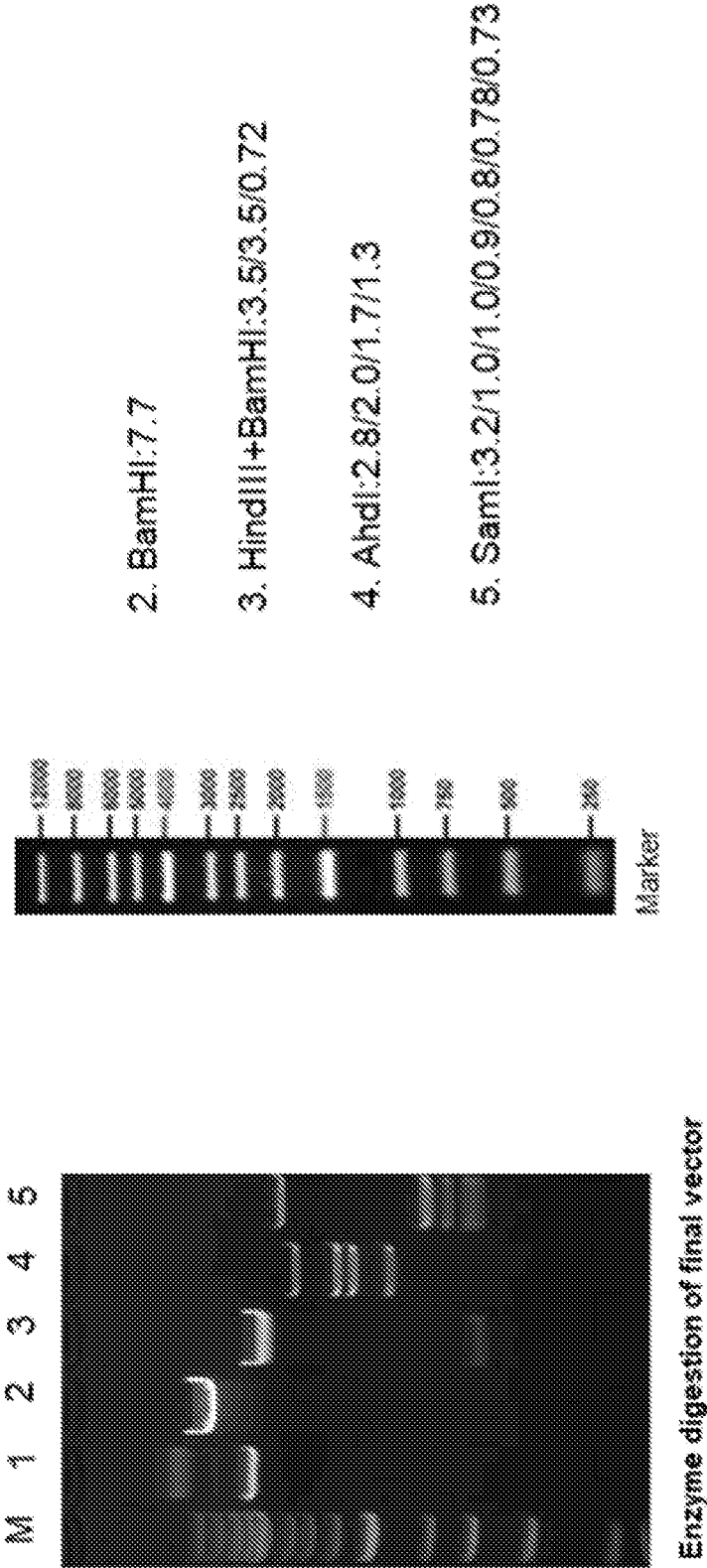


FIG. 30

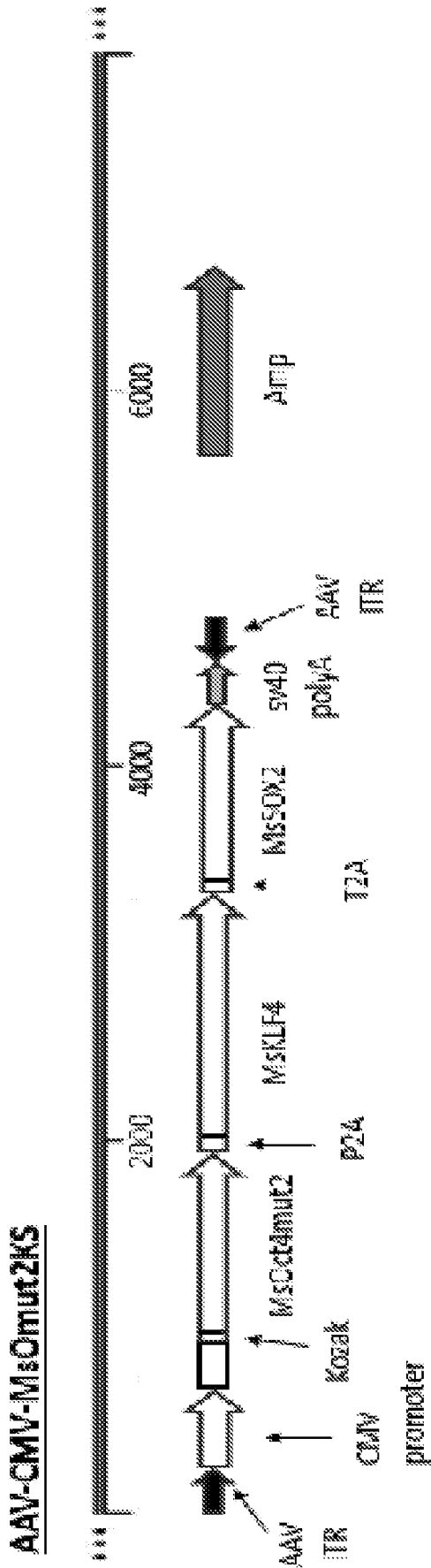
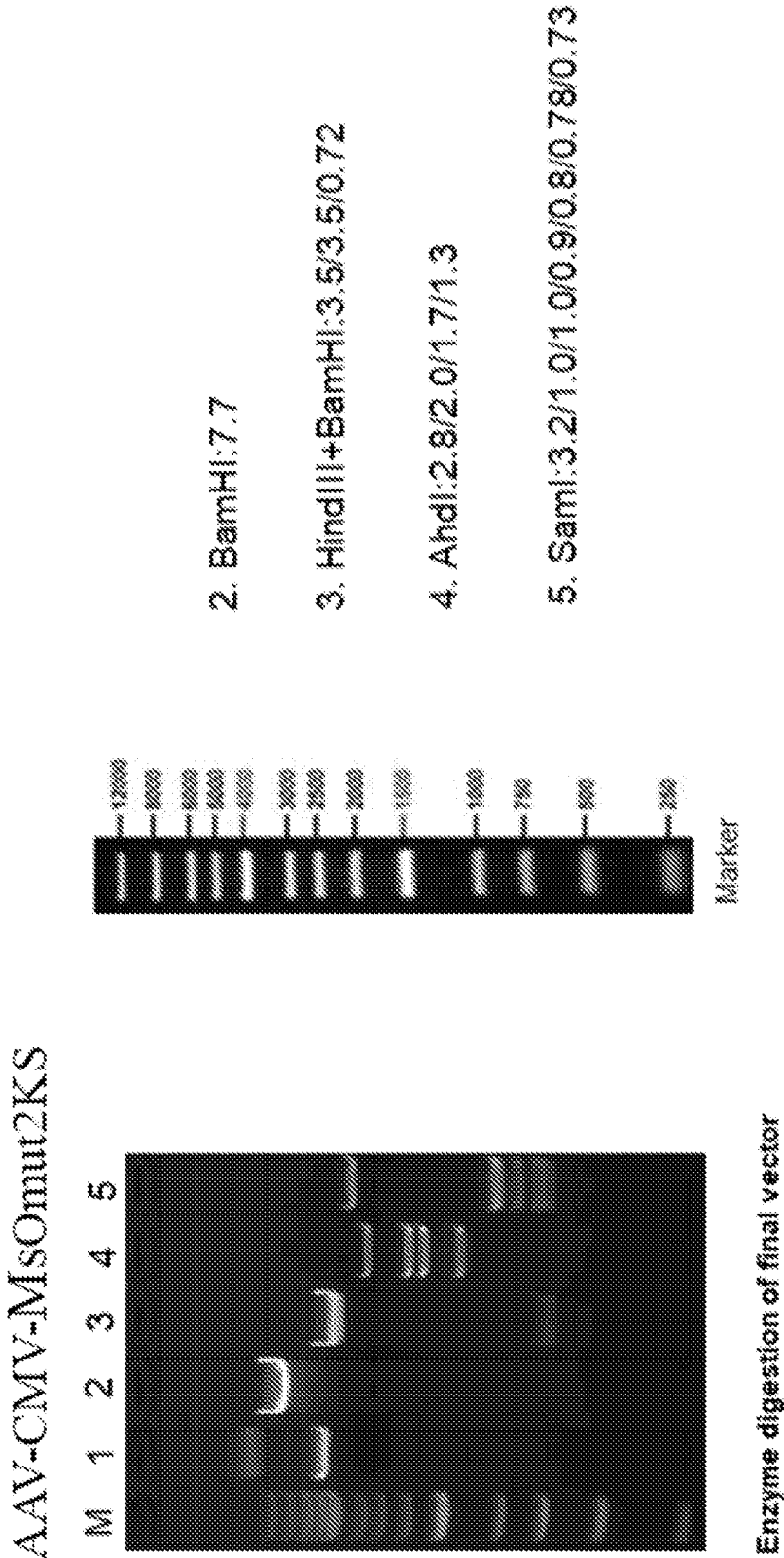


FIG. 31



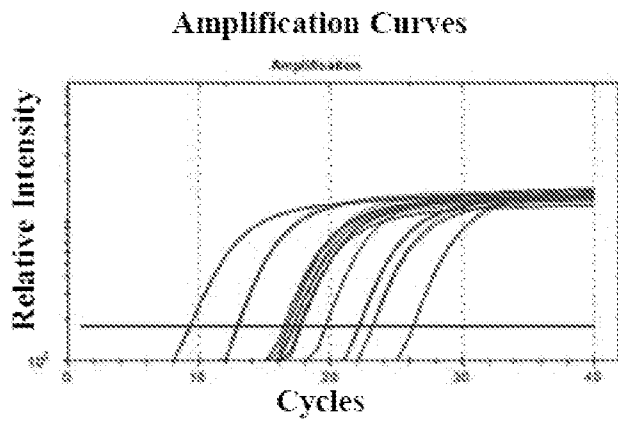


FIG. 33A

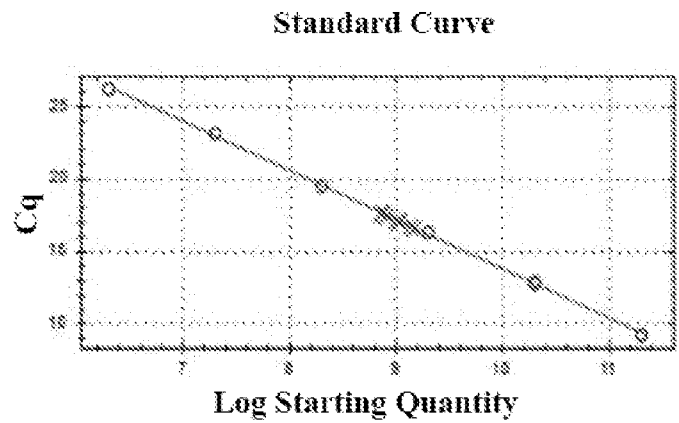


FIG. 33B

Sample No.	Target	CT	q-PCR Titer	Average	Dilution	Titer (GC/mL)
AAV2-CMV-MsOKS-Rep1	ITR	17.13	1.06E+09	1.09E+09	2000	1.83E+13
AAV2-CMV-MsOKS-Rep2	ITR	17.10	1.09E+09	1.09E+09	2000	1.83E+13
AAV2-CMV-MsOKS-Rep3	ITR	17.08	1.09E+09	1.09E+09	2000	1.83E+13
AAV2-CMV-MSOmut1KS-Rep1	ITR	17.51	8.19E+08	8.82E+08	2000	1.48E+13
AAV2-CMV-MSOmut1KS-Rep2	ITR	17.49	8.31E+08	8.82E+08	2000	1.48E+13
AAV2-CMV-MsOmut1KS-Rep3	ITR	17.22	9.97E+08	8.82E+08	2000	1.48E+13
AAV2-CMV-MsOmut2KS-Rep1	ITR	16.78	1.35E+09	1.44E+09	2000	2.42E+13
AAV2-CMV-MsOmut2KS-Rep2	ITR	16.68	1.44E+09	1.44E+09	2000	2.42E+13
AAV2-CMV-MsOmut2KS-Rep3	ITR	16.60	1.52E+09	1.44E+09	2000	2.42E+13
Ref AAV	ITR	17.79	6.75E+08	7.02E+08	1000	5.90E+11
Ref AAV	ITR	17.72	7.12E+08	7.02E+08	1000	5.90E+11
Ref AAV	ITR	17.70	7.19E+08	7.02E+08	1000	5.90E+11

FIG. 33C

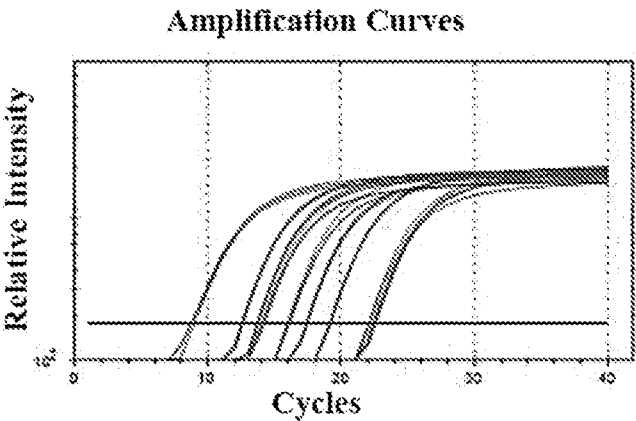


FIG. 34A

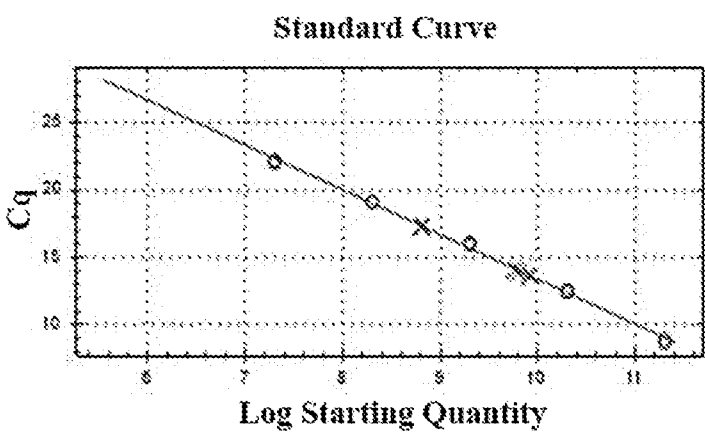


FIG. 34B

Sample No.	Target	CT	q-PCR Titer	Average	Dilution	Titer (GC/mL)
AAV9-CMV-McOKS-Rep1	ITR	13.70	8.06E+09	8.27E+09	2000	1.65E+13
AAV9-CMV-MsOKS-Rep2	ITR	13.69	8.13E+09	8.27E+09	2000	1.65E+13
AAV9-CMV-MsOKS-Rep3	ITR	13.61	8.61E+09	8.27E+09	2000	1.65E+13
AAV9-CMV-MsOmut1KS-Rep1	ITR	14.10	6.11E+09	6.17E+09	2000	1.23E+13
AAV9-CMV-MsOmut1KS-Rep2	ITR	13.98	6.65E+09	6.17E+09	2000	1.23E+13
AAV9-CMV-MsOmut1KS-Rep3	ITR	14.19	5.74E+09	6.17E+09	2000	1.23E+13
Ref AAV	ITR	17.79	6.75E+08	7.02E+08	1000	6.48E+11
Ref AAV	ITR	17.72	7.12E+08	7.02E+08	1000	6.48E+11
Ref AAV	ITR	17.70	7.19E+08	7.02E+08	1000	6.48E+11

FIG. 34C

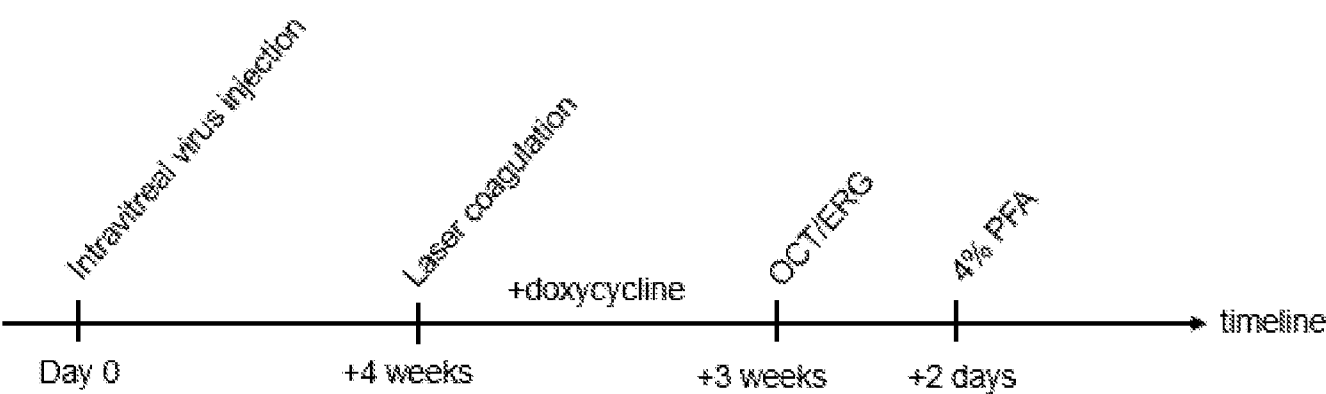


FIG. 35A

Group number	Group	Route of administration	Dosage volume (μL/Eye)	Number of successful eye injections
1	AAV2-CMV-Luciferase	IVT	1.5	6
2	AAV2-TRE3G-DKS + AAV2-CMV-Tet3G	IVT	0.75 + 0.75	6
3	AAV2-TRE3G-D'KS + AAV2-CMV-Tet3G	IVT	0.75 + 0.75	6

FIG. 35B

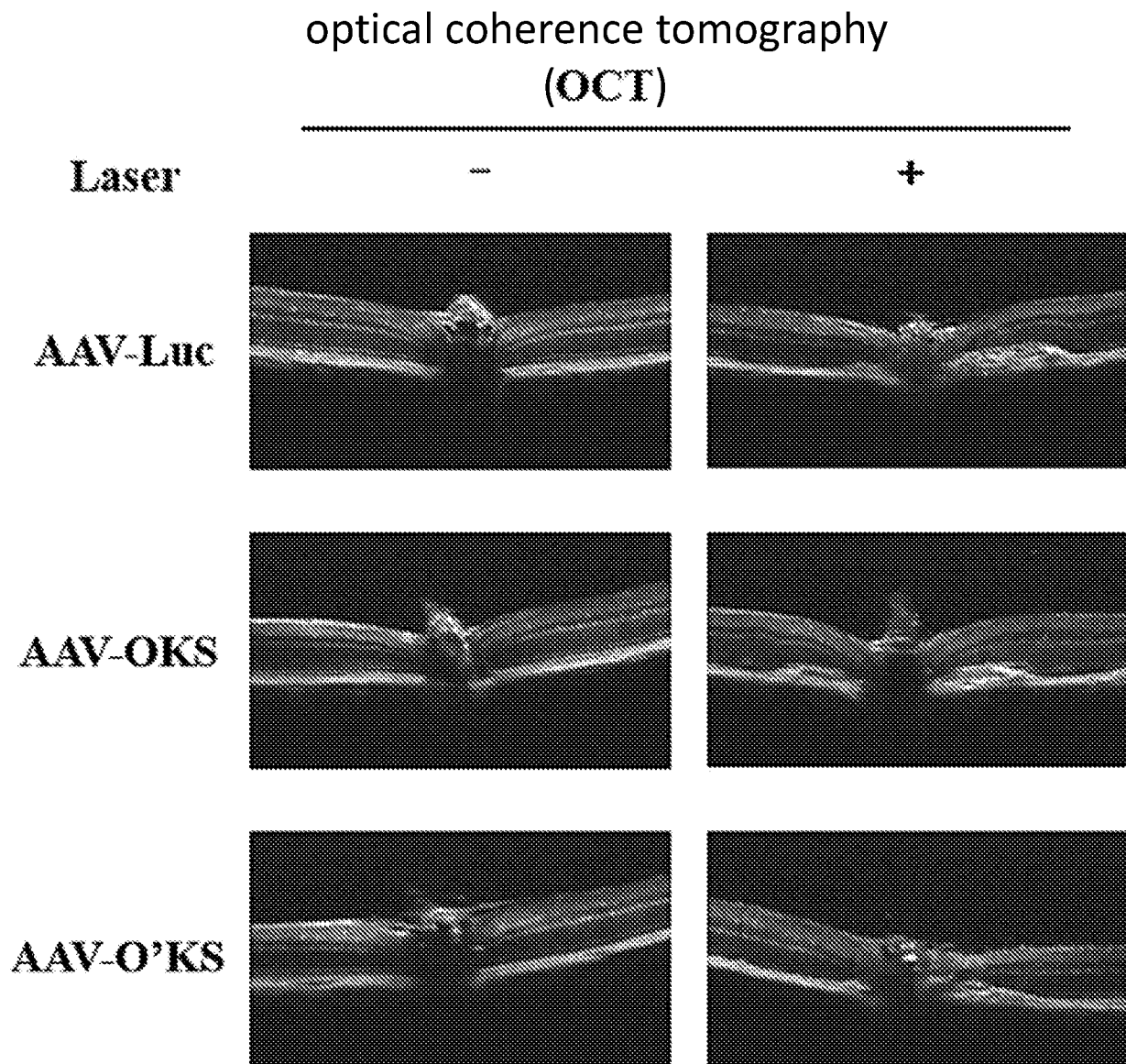


FIG. 36A

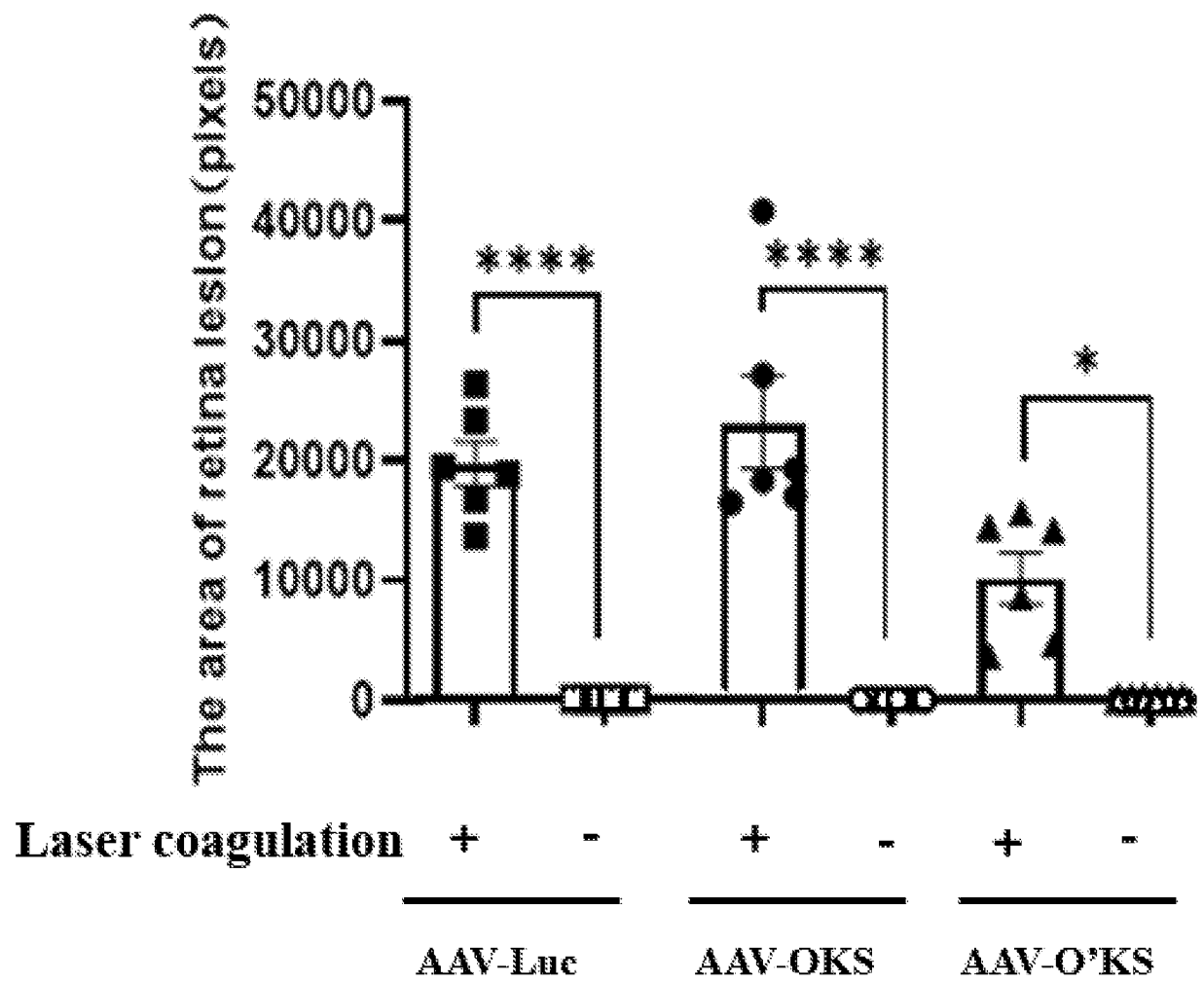


FIG. 36B

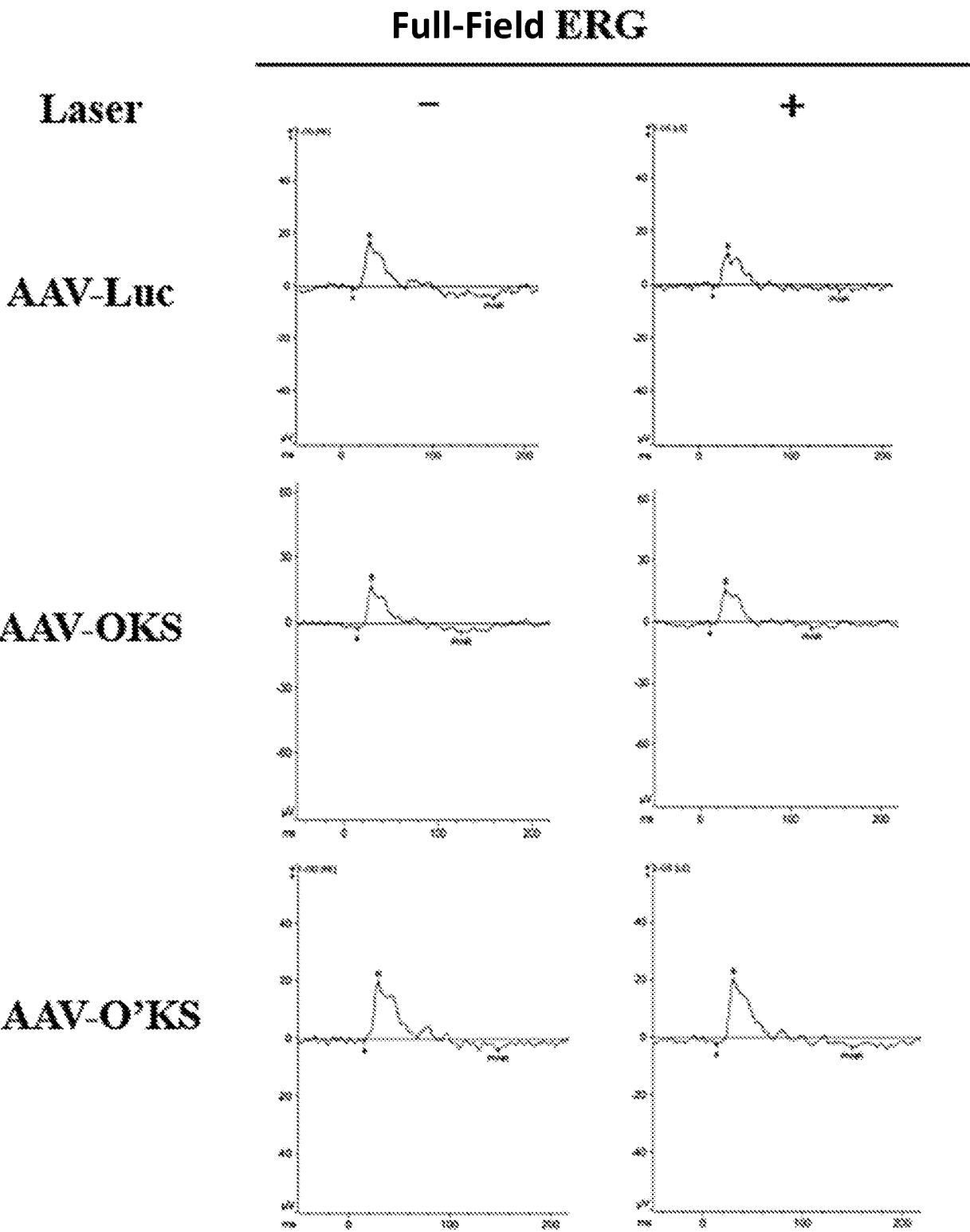


FIG. 37A

Photopic Negative Response (PhNR)

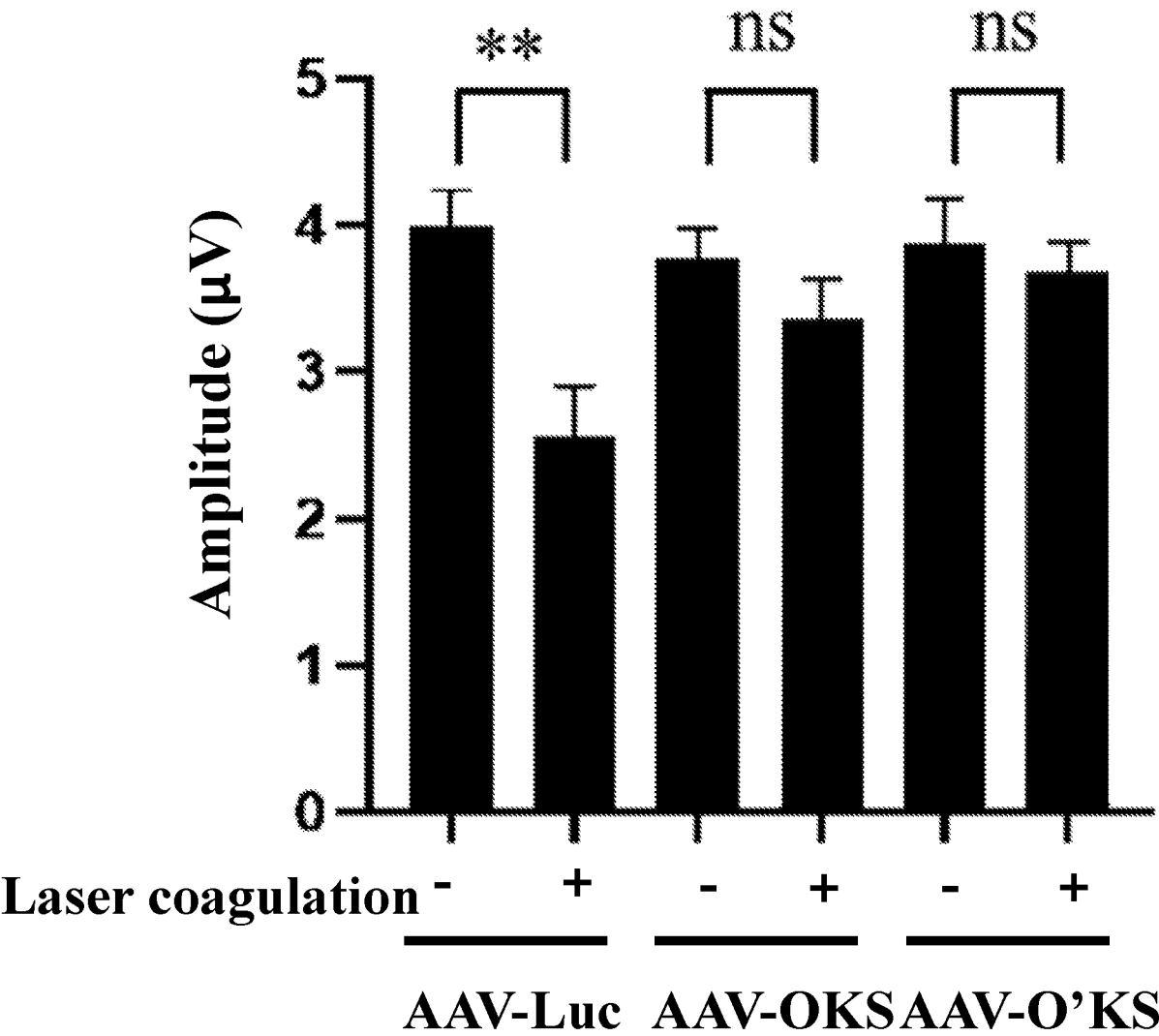


FIG. 37B

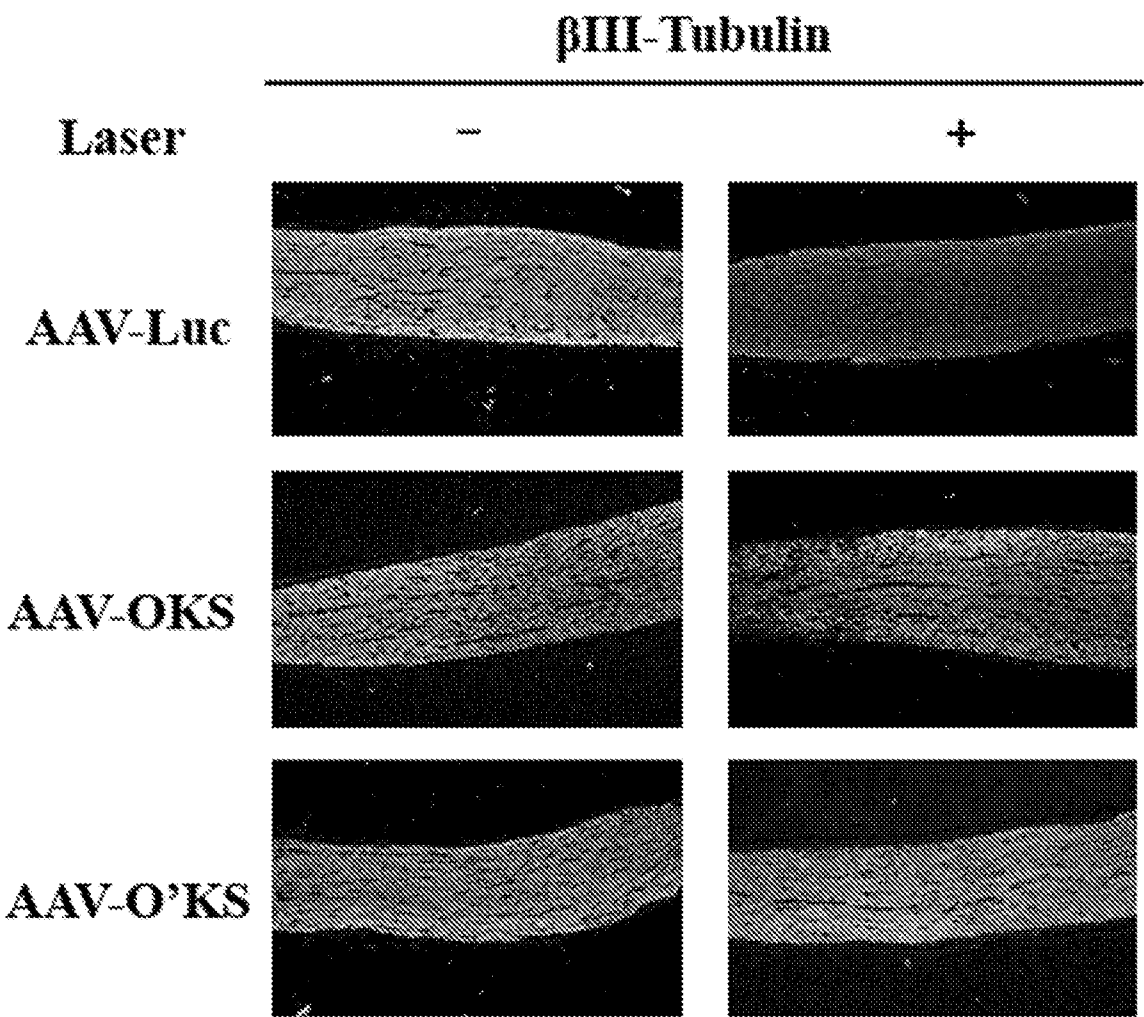


FIG. 38A

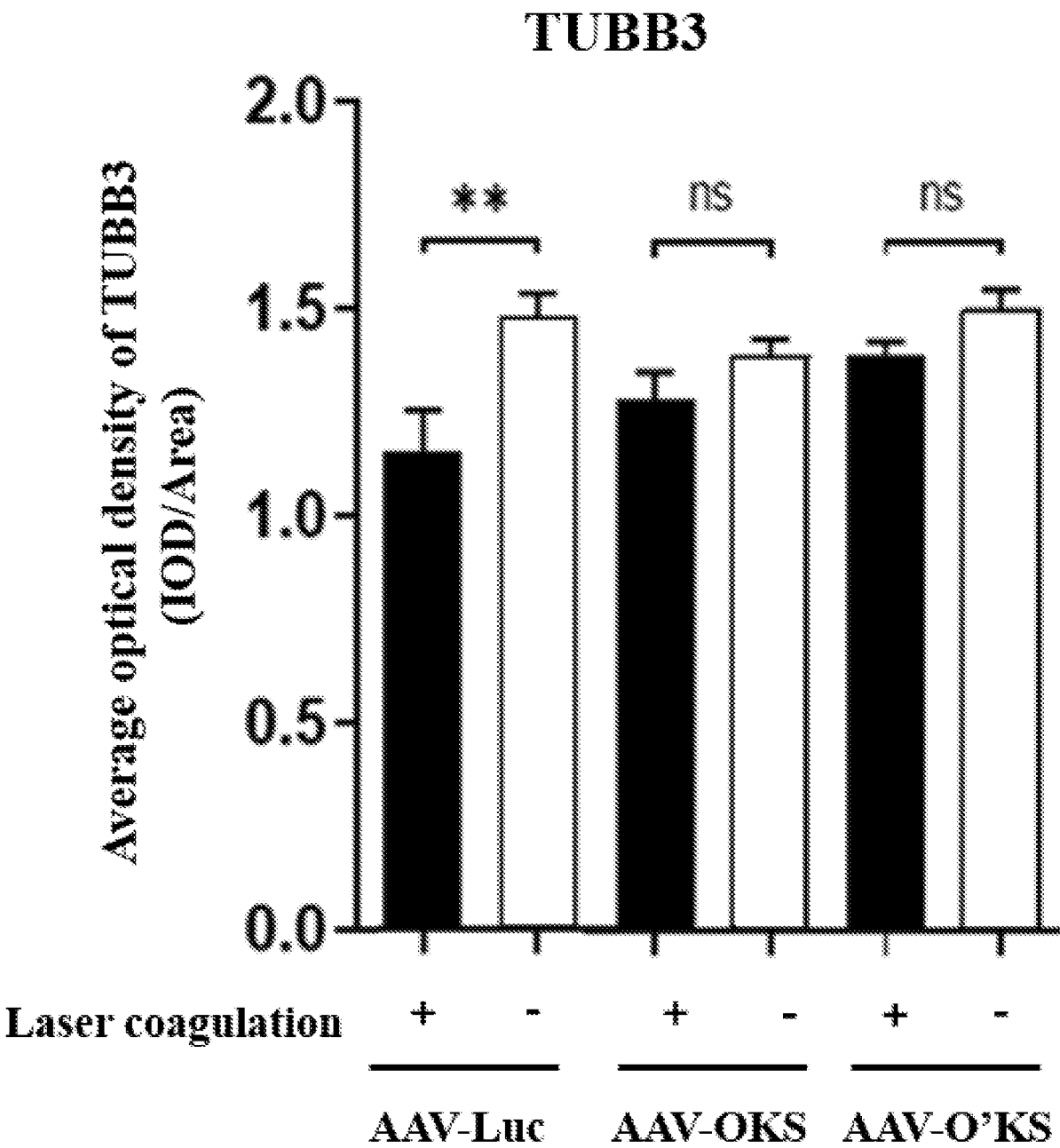


FIG. 38B

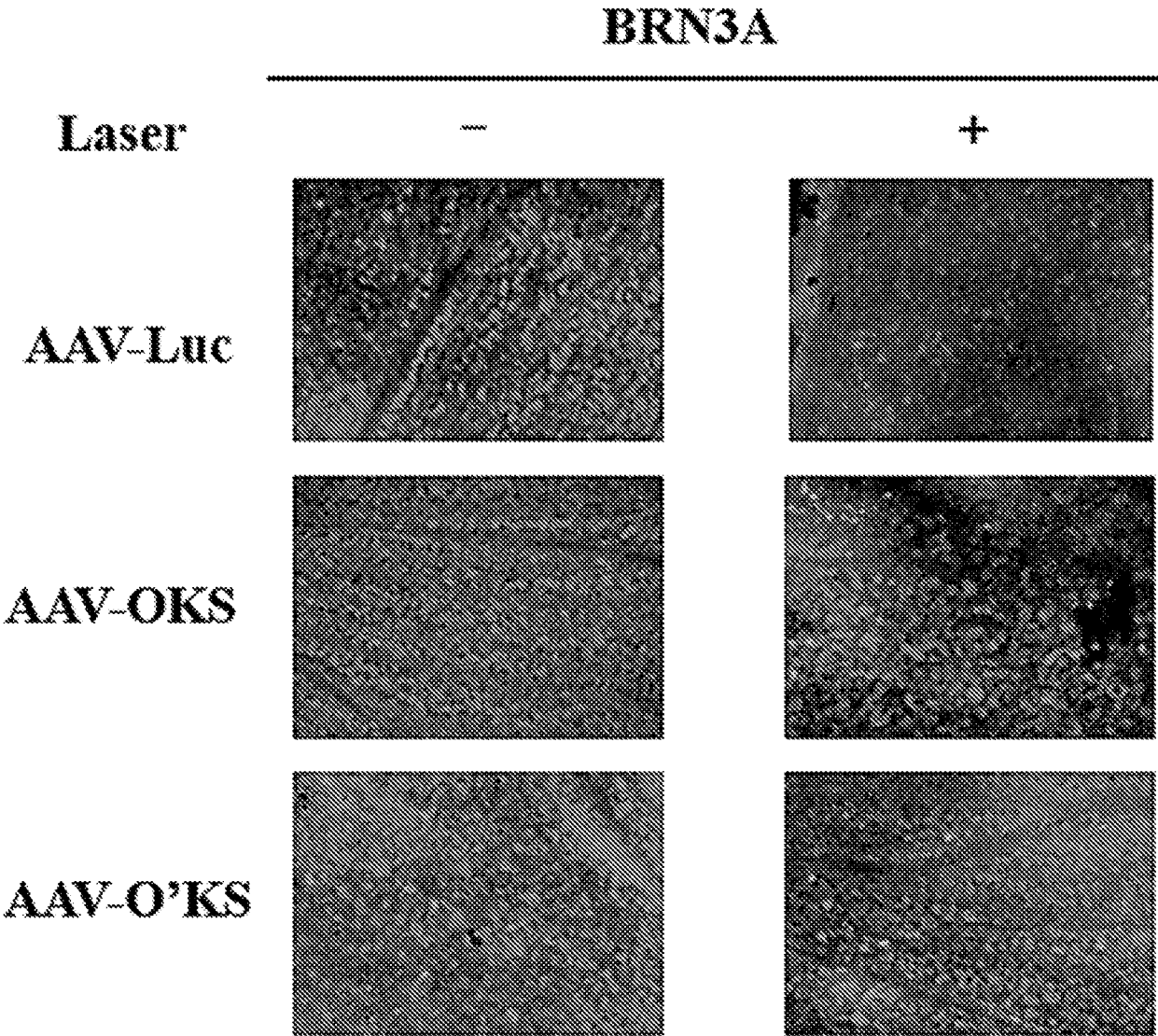


FIG. 39A

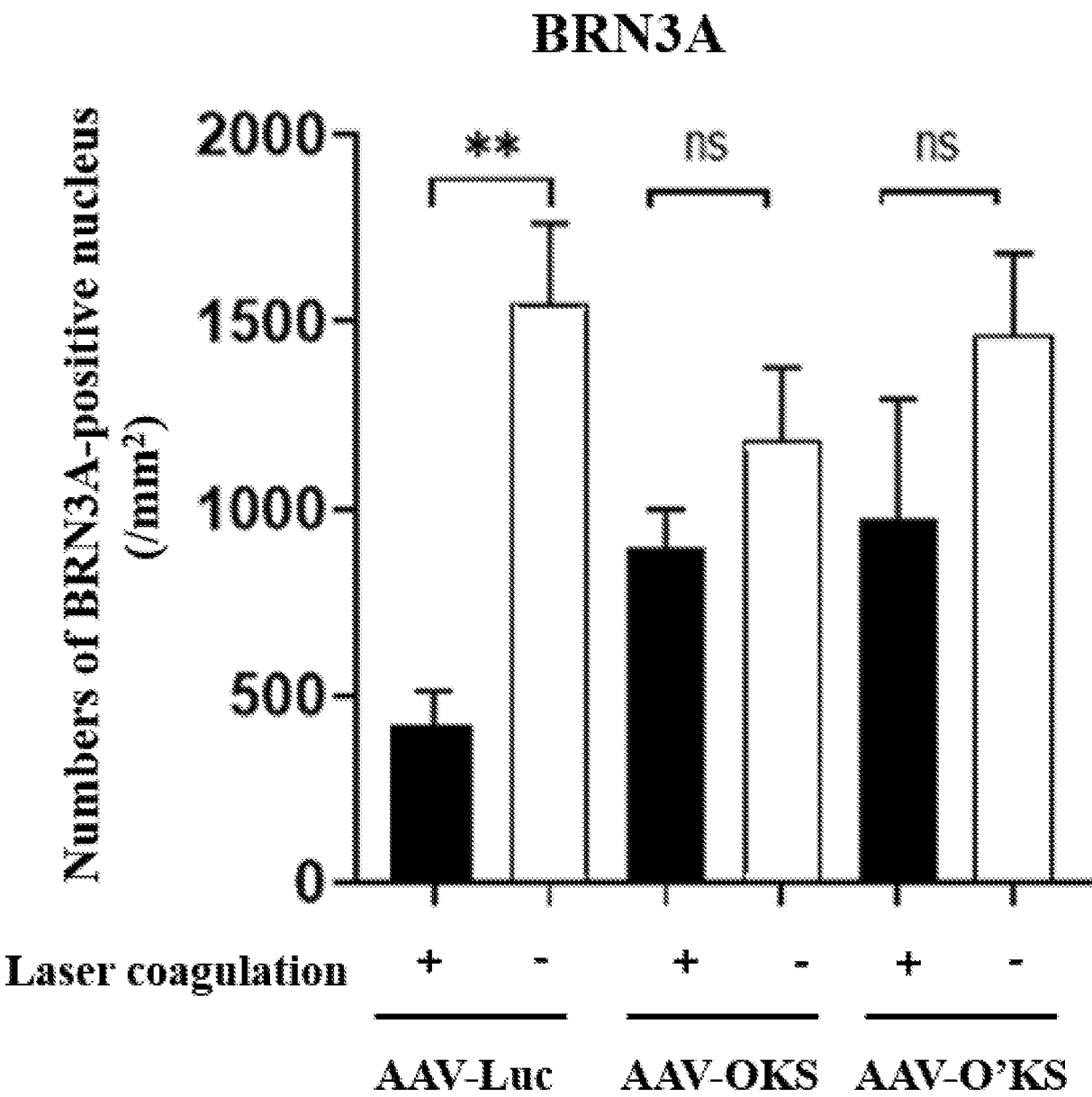


FIG. 39B

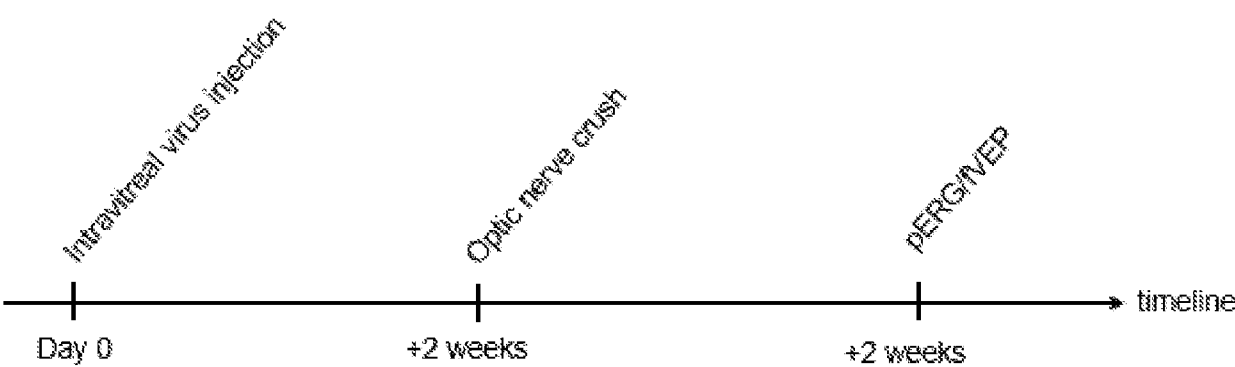


FIG. 40A

Group Number	Group	Administration Route	Dosage volume (μL/eye)	# successful eye injections
1	AAV9-CMV-eGFP	IVT	2	6
2	AAV9-CMV-eGFP (crush)	IVT	2	6
3	AAV9-CMV-MsOKS (crush)	IVT	2	6
4	AAV9-CMV-MsOmut1KS	IVT	2	6

FIG. 40B

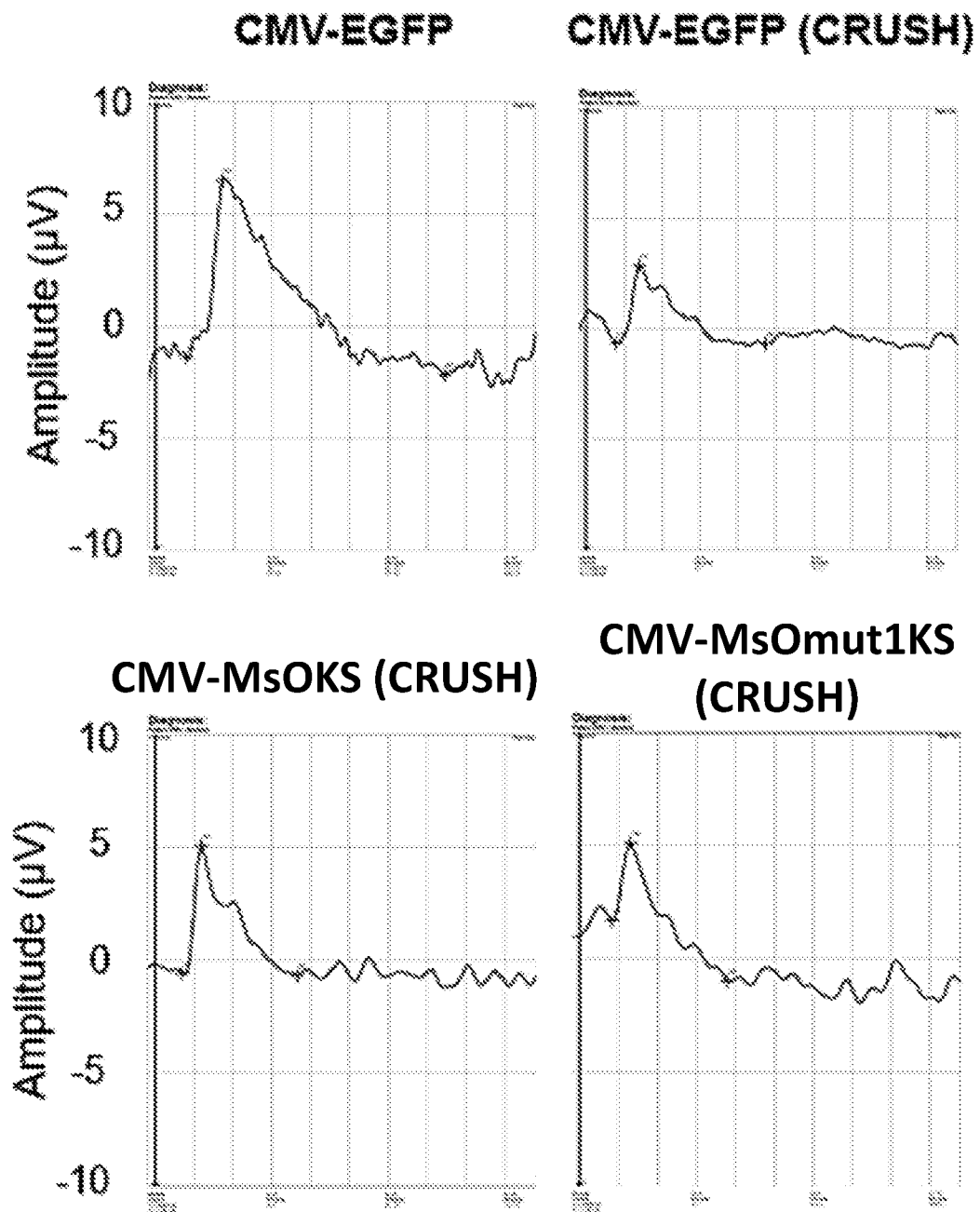


FIG. 41A

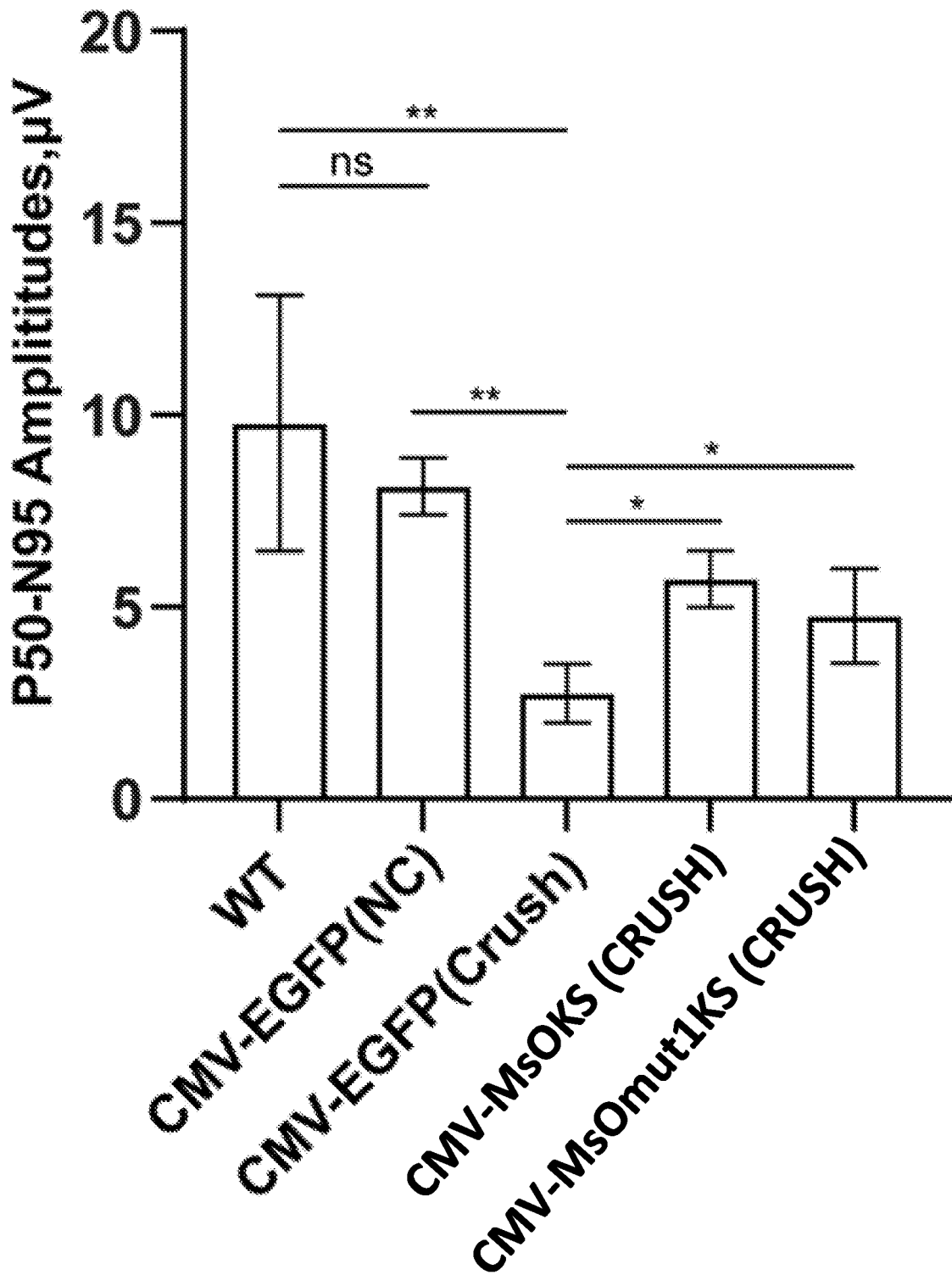


FIG. 41B

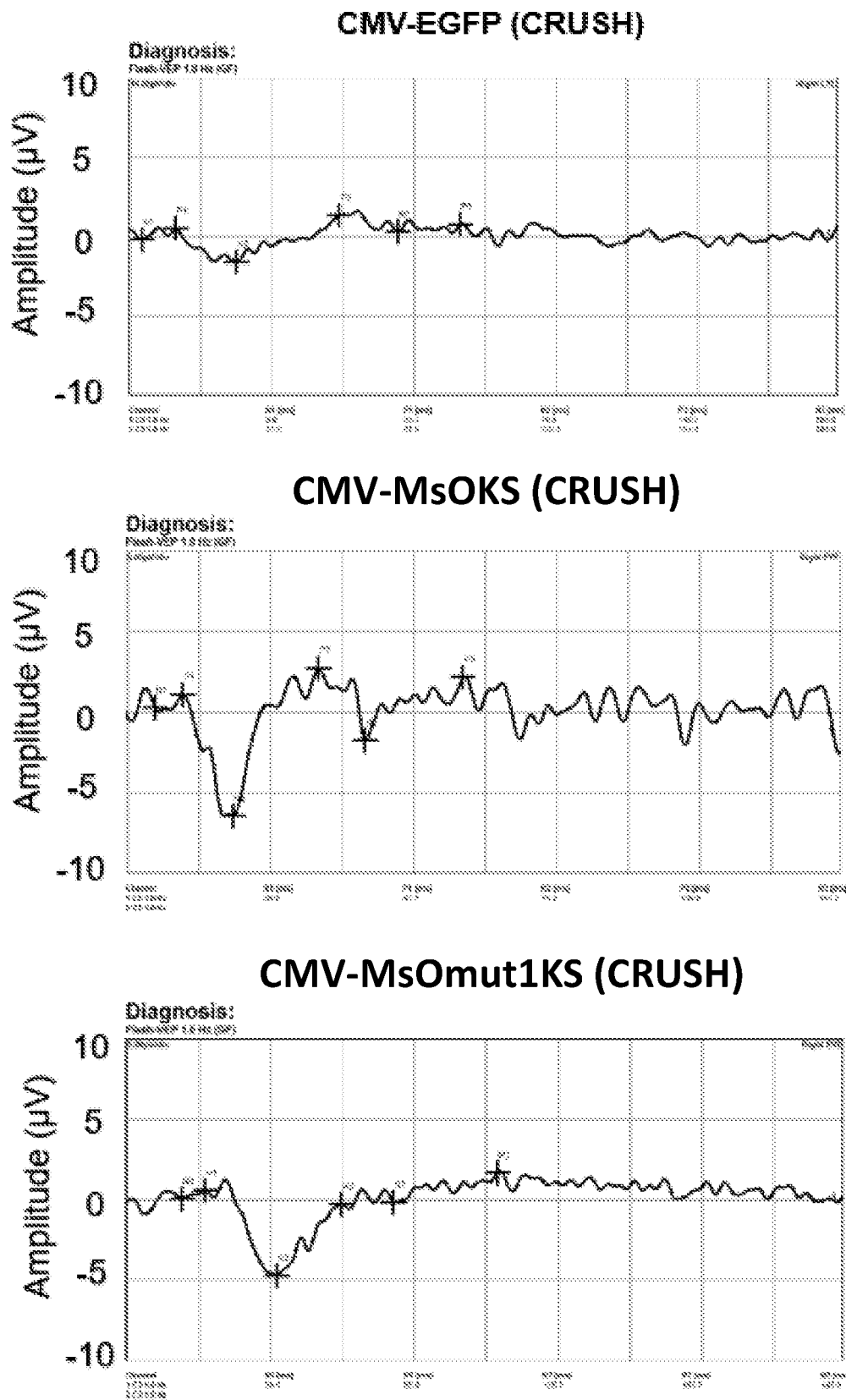


FIG. 42

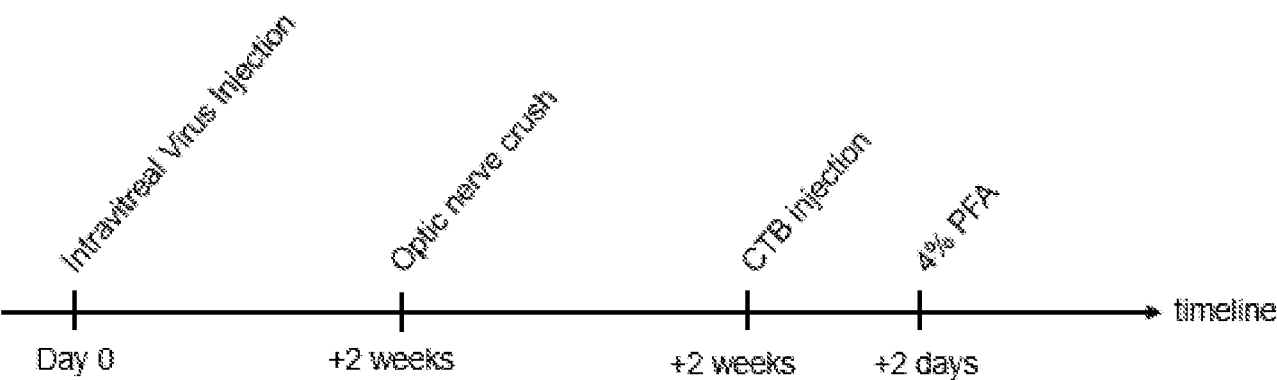


FIG. 43A

Group Number	Group	Administration Route	Dosage volume (μL/eye)	# successful eye injections
1	AAV2-CMV-eGFP	IVT	2	6
2	AAV2-CMV-eGFP (crush)	IVT	2	6
3	AAV2-CMV-MsOKS (crush)	IVT	2	6
4	AAV2-CMV-MsOmut1KS	IVT	2	6
5	AAV2-CMV-MsOmut2KS	IVT	2	6

FIG. 43B

Crush, CTB-555

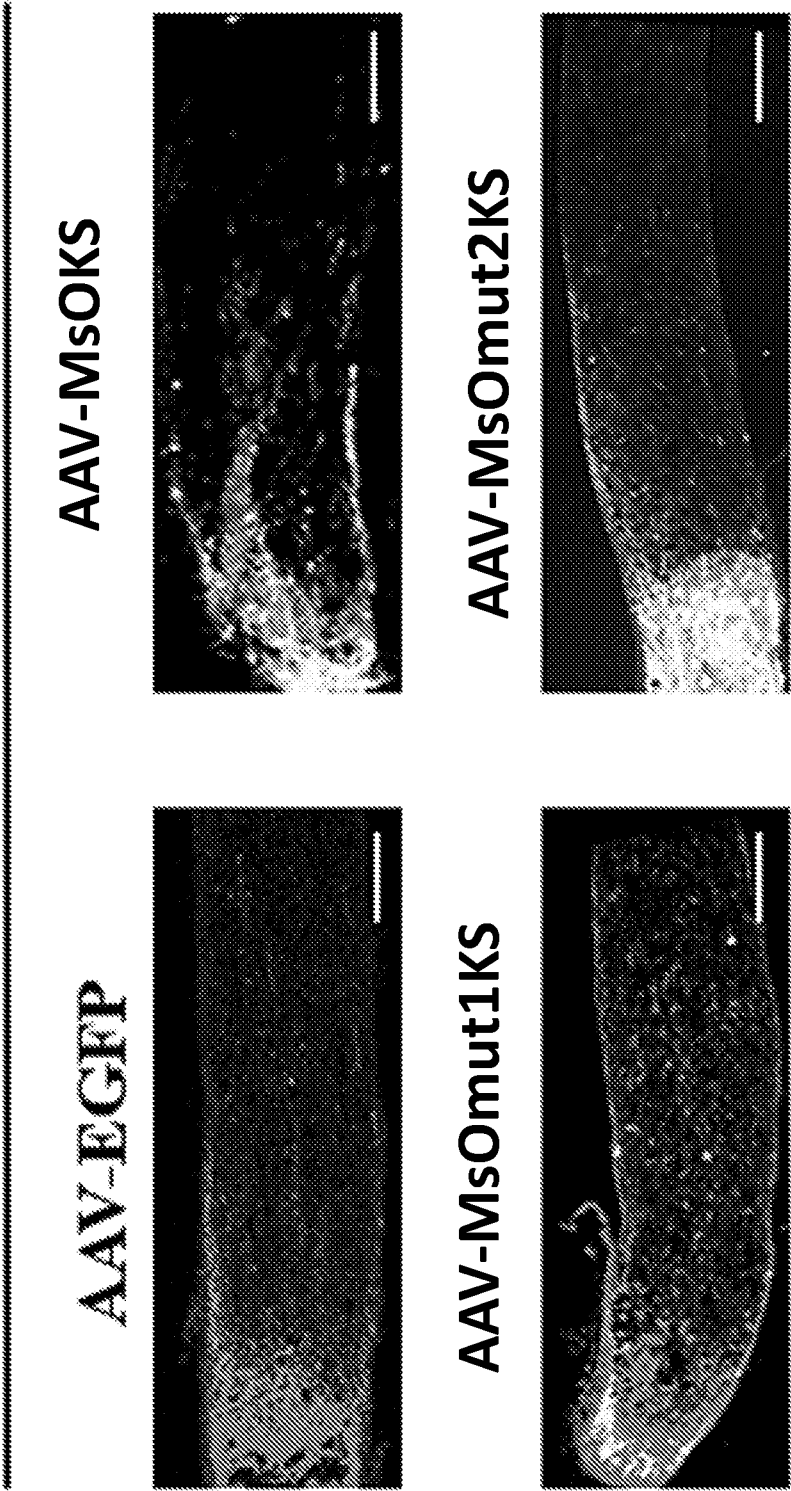


FIG. 44A

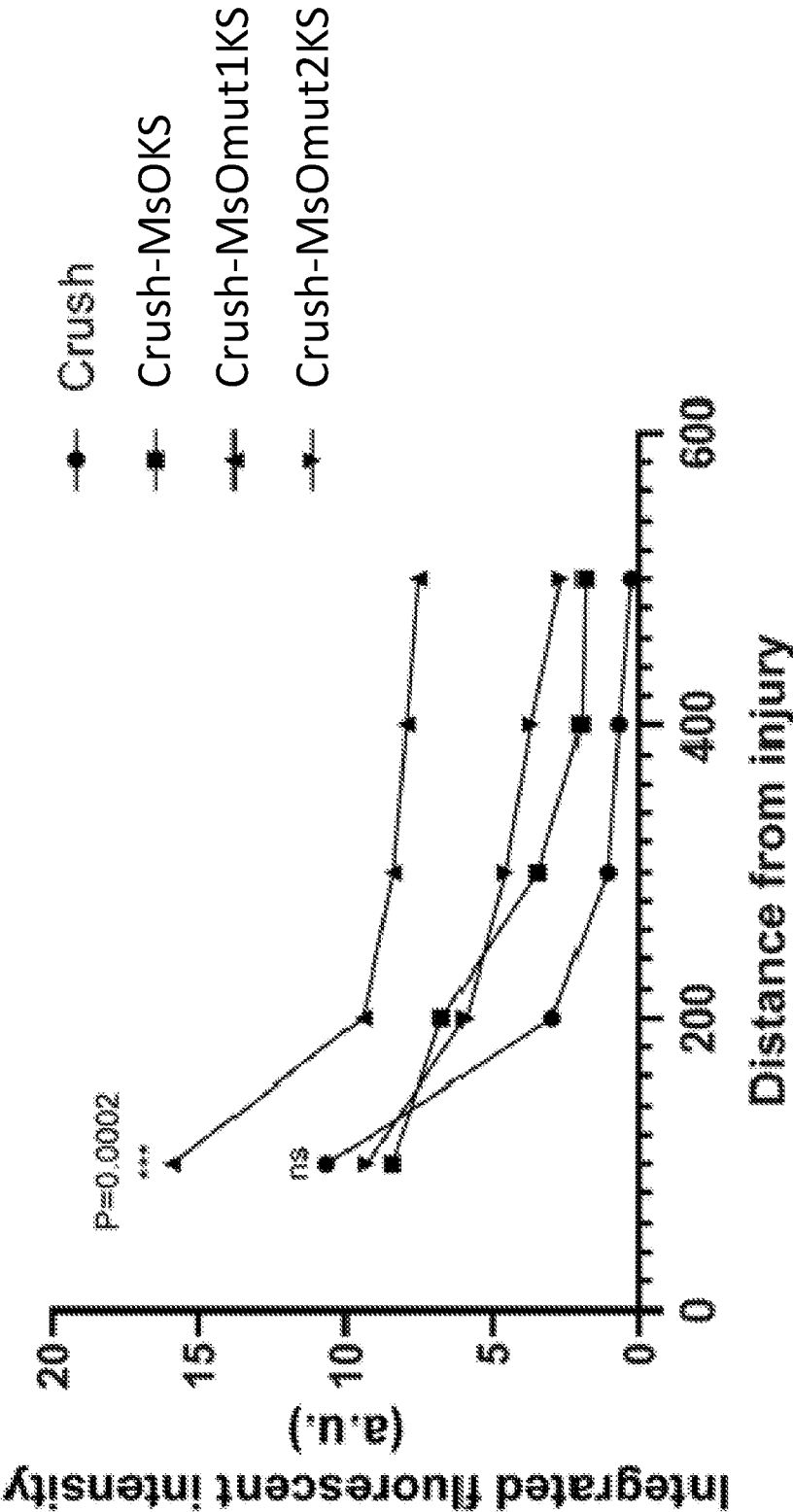


FIG. 44B

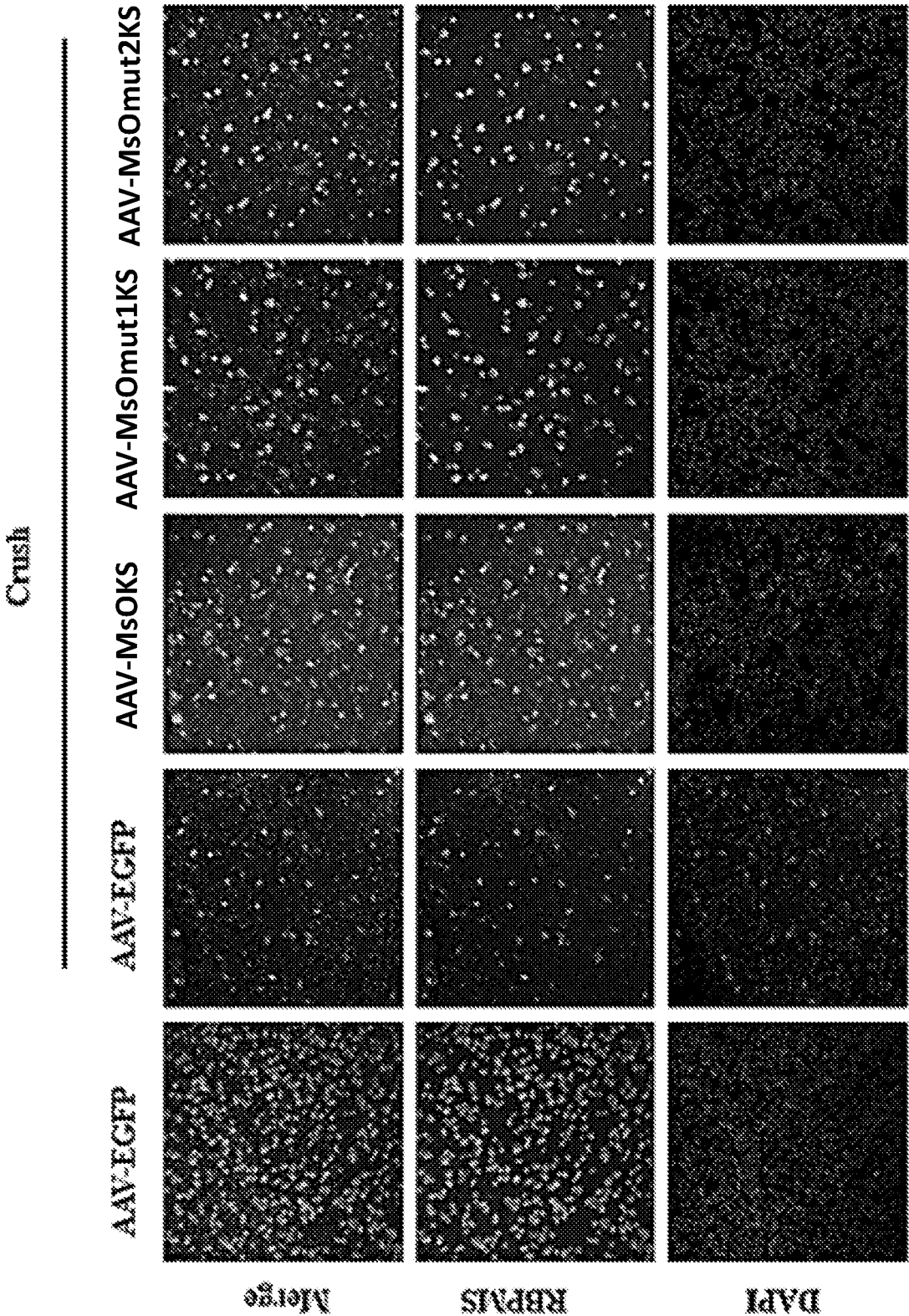


FIG. 45A

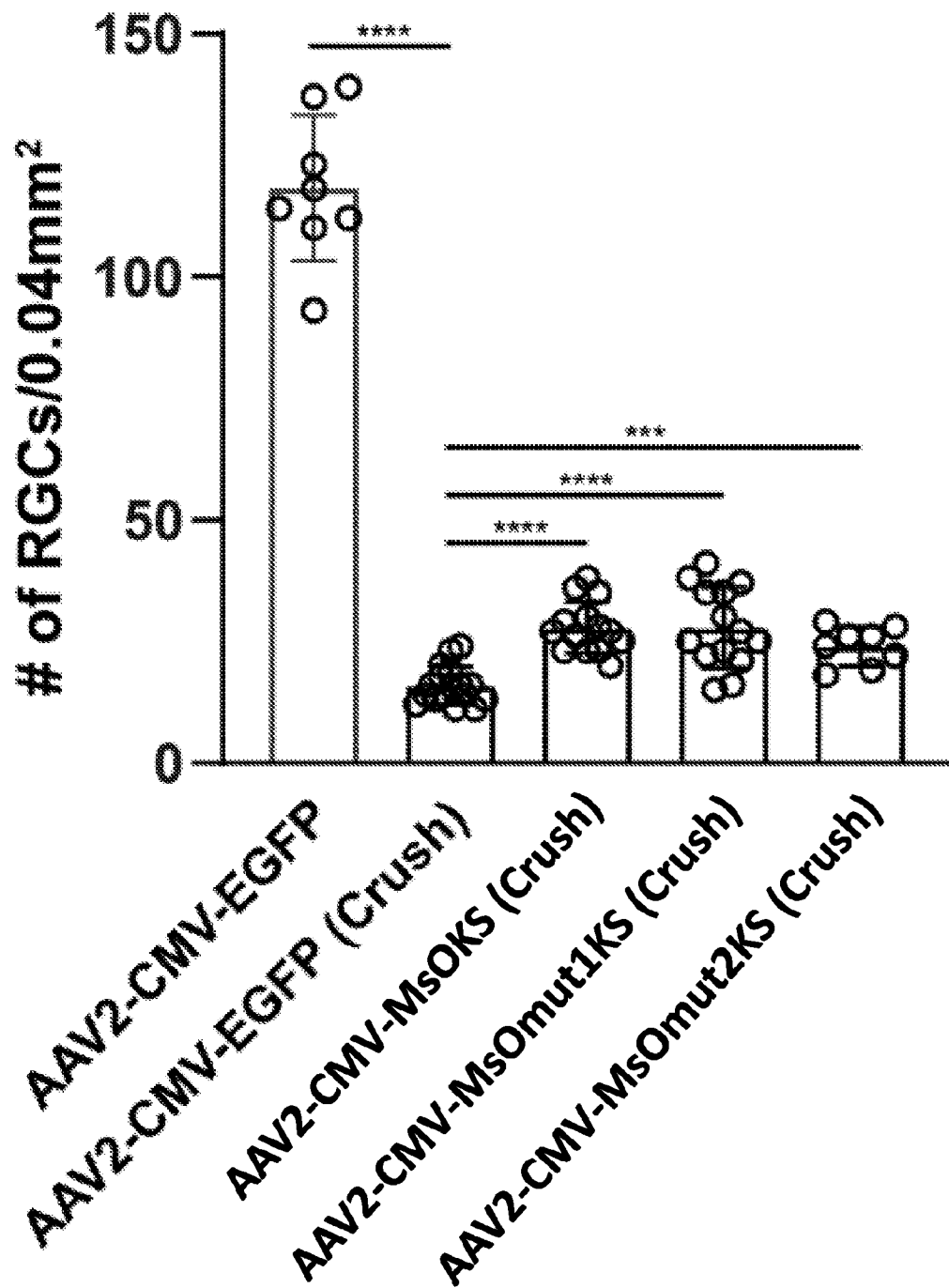


FIG. 45B

INTERNATIONAL SEARCH REPORT

International application No

PCT/CN2024/102793

A. CLASSIFICATION OF SUBJECT MATTER

INV. A61K38/18 A61K31/70 A61K48/00 A61P25/02 A61P27/02
A61Q19/08 C07K14/00 C12N15/00 A61P1/16 A61P17/00
A61P11/00

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A61K C07K C12N A61Q A61P

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2020/069373 A1 (HARVARD COLLEGE [US]) 2 April 2020 (2020-04-02) figures 7, 9, 23, 25, 28-30; examples 1-2, 9, 10, 11, 13, 14 -----	1-50
X	LU YUANCHENG ET AL: "Reprogramming to recover youthful epigenetic information and restore vision", NATURE, vol. 588, no. 7836, 2 December 2020 (2020-12-02), pages 124-129, XP037310194, DOI: 10.1038/S41586-020-2975-4 Abstract; pages 125-128; page 130, left column; figures 1-4 ----- -/-	1-50

☒ Further documents are listed in the continuation of Box C.☒ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

18 October 2024

Date of mailing of the international search report

04/11/2024

Name and mailing address of the ISA/

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Authorized officer

Basso, Veronica

INTERNATIONAL SEARCH REPORT

International application No
PCT/CN2024/102793

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WANG JIA ET AL: "Phase separation of OCT4 controls TAD reorganization to promote cell fate transitions", CELL STEM CELL, ELSEVIER, CELL PRESS, AMSTERDAM, NL, vol. 28, no. 10, 25 May 2021 (2021-05-25), page 1868, XP086815488, ISSN: 1934-5909, DOI: 10.1016/J.STEM.2021.04.023 [retrieved on 2021-05-25] page 1877; figure 6 -----	1-50
A	PENG LIU ET AL: "From start to end: Phase separation and transcriptional regulation", BIOCHIMICA ET BIOPHYSICA ACTA. GENE REGULATORY MECHANISMS, ELSEVIER, AMSTERDAM, NL, vol. 1863, no. 12, 2 October 2020 (2020-10-02), XP086399214, ISSN: 1874-9399, DOI: 10.1016/J.BBAGRM.2020.194641 [retrieved on 2020-10-02] pages 5-6, paragraph 3.2; figure 6 -----	1-50
A	CHEN Y ET AL: "Activating transcription factor 4 mediates hyperglycaemia-induced endothelial inflammation and retinal vascular leakage through activation of STAT3 in a mouse model of type 1 diabetes", DIABETOLOGIA, SPRINGER, BERLIN, DE, vol. 55, no. 9, 4 June 2012 (2012-06-04), pages 2533-2545, XP035094584, ISSN: 1432-0428, DOI: 10.1007/S00125-012-2594-1 pages 2538-2540; figures 6-7 -----	1-50
A	WO 2019/183552 A2 (WHITEHEAD INST BIOMEDICAL RES [US]) 26 September 2019 (2019-09-26) paragraph [0810] - paragraph [0810] -----	1-50

INTERNATIONAL SEARCH REPORT

International application No.

PCT/CN2024/102793

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
 - a. ☒ forming part of the international application as filed.
 - b. ☐ furnished subsequent to the international filing date for the purposes of international search (Rule 13*ter*.1(a)).

☐ accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.
2. ☐ With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this report has been established to the extent that a meaningful search could be carried out without a WIPO Standard ST.26 compliant sequence listing.
3. Additional comments:

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/CN2024/102793

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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		US 2024261370 A1	08-08-2024
		US 2024316148 A1	26-09-2024
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		EP 3768329 A2	27-01-2021
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		TW 202003051 A	16-01-2020
		US 2022120736 A1	21-04-2022
		WO 2019183552 A2	26-09-2019