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**BRAIN-CELL SPECIFIC PARTIAL CELLULAR REPROGRAMMING TREATMENT AND
PREVENTION METHODS FOR ALZHEIMER'S DISEASE AND PROGERIA, COMPOSITIONS
THEREFORE, AND USES THEREOF**

RELATED APPLICATIONS

[0001] This application claims the benefit of US patent application 63/544,689 filed on October 18, 2023, herein incorporated by reference.

FIELD

[0002] The present disclosure relates to vectors that provide for brain cell-specific expression of reprogramming genes useful for the treatment of Alzheimer's disease and progeria and methods and uses of the same to treat Alzheimer's disease or progeria.

BACKGROUND

[0003] Alzheimer's disease and progeria (Hutchinson-Gilford progeria syndrome; HGPS), a rare and rapid aging disorder, pose significant challenges for modern medicine. Existing treatments offer limited effectiveness and primarily focus on managing symptoms rather than preventing or reversing these conditions. Improved treatments are desired.

[0004] The concept of cellular reprogramming, wherein differentiated somatic cells are converted back to a pluripotent or multipotent state, has paved the way for transformative advancements in regenerative medicine. The discovery of this phenomenon was centered on the use of a combination of transcription factors, primarily Oct4, Sox2, Klf4, and c-Myc (OSKM). Beyond this canonical set, other factors like Lin28 and Nanog have also been identified to possess reprogramming potential.

[0005] While the full reprogramming approach holds promise for creating induced pluripotent stem cells (iPSCs) for therapeutic applications, there is a growing interest in partial reprogramming for rejuvenating cells while preserving their identity. Partial reprogramming has already demonstrated its ability to prolong lifespan in both progeric (Ocampo et al. 2016) and normally aging mice (Macip et al. 2023) when applied *in vivo*. Partial reprogramming was also demonstrated to rejuvenate cells *in vitro* and tissues *in vivo*, as well as shift epigenetics and transcriptomics of the cells in which it

is induced to a younger profile (Sarkar et al. 2020, Roux et al. 2022, Chondronasiou et al. 2022).

[0006] Alzheimer's Disease (AD) has strong epigenetic etiology (Nativio et al. 2020, Shireby et al. 2020). The Zheng et al. AD study showed that broad epigenetic modulation can reverse AD symptoms (Zheng et al. 2019).

[0007] Besides using the canonical OSKM factors for partial reprogramming, several groups demonstrated that subsets of OSKM or additional factors can also produce rejuvenation while preserving cellular identity. For instance, Lu et al. (2020) demonstrated the rejuvenating potential of just the OSK factors, without the inclusion of c-Myc. Sarkar et al. (2020), on the contrary, used a combination of OSKM, Lin28, and Nanog, while Roux et al. (2022) illustrated that any 2- or 3-factor subset of the OSKM factors could produce some degree of rejuvenation in the cells they tested.

[0008] Loss of cell identity due to prolonged expression of reprogramming genes is a key reason why *in vivo* reprogramming for rejuvenating purposes has to be partial and needs to be periodically repeated. If reprogramming genes are induced for too long *in vivo*, this can lead to teratomas, organ failure, and death, as shown by Abad et al. 2013 and Ocampo et al. 2016.

[0009] Some cell types are too permissive/prone to reprogramming and can quickly lose their cellular identity which can cause loss of their function and failure of the organs that comprise them. In support of this, Parras et al. 2022 showed that the liver and intestine are the two key tissues that can cause premature death from reprogramming, and avoiding them extends from 4 to 10 days the duration of consecutive days of OSKM (i.e., the four reprogramming transcription factors OCT4, SOX2, KLF4, and MYC) expression that 100% of tested mice can safely tolerate.

SUMMARY

[0010] To avoid loss of cellular identity and increase therapeutic safety margins, the present inventors tested partial reprogramming in specific cells. This way, reprogramming gene expression was avoided in permissive cell types and organs prone to dedifferentiation, and could prolong the safe duration of induction of reprogramming genes in other cell types and organs which could benefit from rejuvenation through shifting epigenetic and transcriptomic profiles to a younger state without reverting to

pluripotency. In particular, the present inventors have shown that brain cell-specific expression of cellular reprogramming provides positive results in cognitive tests and postmortem histopathological assessments in a mouse model of Alzheimer's Disease. The present inventors have also shown that brain specific expression of cellular reprogramming provides benefit in a mouse model of Hutchinson-Gilford Progeria Syndrome (HGPS; Progeria).

[0011] Accordingly, herein provided is a method of preparing a subject for treating Alzheimer's disease comprising administering

- a. a first nucleic acid molecule comprising at least 2 reprogramming genes under the control of an inducible promoter; and
- b. a second nucleic acid molecule comprising a gene encoding an inducer moiety under the control of a brain cell-specific promoter;

wherein the inducer moiety induces expression of the at least 2 reprogramming genes under the control of the inducible promoter in the presence of an inducer.

[0012] Also herein provided is a method of preparing a subject for treating progeria comprising administering

- a. a first nucleic acid molecule comprising at least 2 reprogramming genes under the control of an inducible promoter; and
- b. a second nucleic acid molecule comprising a gene encoding an inducer moiety under the control of a brain cell-specific promoter;

wherein the inducer moiety induces expression of the at least 2 reprogramming genes under the control of the inducible promoter in the presence of an inducer.

[0013] In an embodiment, the at least 2 reprogramming genes comprise at least 2 of Oct4, Sox2, Klf4 and c-Myc. In another embodiment, the method comprises at least 3 of the reprogramming genes. In yet another embodiment, the method comprises at least 4 reprogramming genes.

[0014] In an embodiment,

- a) the inducible promoter is a tetracycline response element (TRE) and the inducer moiety is a reverse tetracycline-controlled transactivator (rtTA);

- b) the inducible promoter is an ecdysteroid response element (EcRE) and the inducer moiety is an ecdysone receptor-derived transactivator, optionally VpEcR; or
- c) the inducible promoter is a GAL4 binding site-containing promoter and the inducer moiety is
 - i) an RSL1-activated two-hybrid transactivator comprised of VP16-RXR and GAL4-EcR chimeric constructs;
 - ii) a Mifepristone-responsive regulator (pGL-VP); or
 - iii) rapamycin-induced transactivator.

[0015] In an embodiment, the first and second nucleic acid molecules are on the same vector, optionally a lentiviral vector. In another embodiment, the first and second nucleic acid molecules are on different vectors, optionally lentiviral vectors.

[0016] In an embodiment, the brain cell-specific promoter is a neuron-specific promoter, optionally a Synapsin 1 (SYN1) promoter, a Ca²⁺/calmodulin-dependent kinase subunit α (CaMKII) promoter, or a neuron-specific enolase (NSE) promoter for neuron-specific expression, or a tyrosine hydroxylase promoter for expression specific to dopamine neurons.

[0017] In another embodiment, the brain cell-specific promoter is an astrocyte-specific promoter, optionally a glial fibrillary acidic protein (GFAP) promoter, a GfaABC1D promoter, or an ALDH1L1 promoter for astrocyte-specific expression.

[0018] In an embodiment, the administration is by stereotactic injection into a brain region. In another embodiment, the brain region is the hippocampus or hypothalamus.

[0019] In an embodiment, the administration is by intravenous injection and wherein the first and second nucleic acid molecules are contained in an adeno-associated vector(s) that is able to cross the blood-brain barrier.

[0020] Also herein provided is a method of treating Alzheimer's disease in a subject in need thereof comprising:

- i) preparing the subject for treating the Alzheimer's disease according to a method herein disclosed; and
- ii) administering the inducer to the subject.

[0021] Further herein provided is a method of treating progeria in a subject in need thereof comprising:

- i) preparing the subject for treating the progeria according to a method herein disclosed; and
- ii) administering the inducer to the subject.

[0022] In an embodiment, the administering ii) begins at least 2 weeks following the preparing the subject i).

[0023] In another embodiment, the administering ii) comprises a cycle of 1 to 7 consecutive days of inducer administration followed by at least 7 days without inducer administration.

[0024] In an embodiment, the cycle is repeated on a bimonthly or monthly basis. In another embodiment, the cycle is repeated indefinitely.

[0025] In an embodiment, the inducer is administered orally.

[0026] In another embodiment, the subject is a human.

[0027] Also provided herein is a vector comprising:

- a. a first nucleic acid molecule comprising at least 2 reprogramming genes under the control of an inducible promoter; and
- b. a second nucleic acid molecule comprising a gene encoding an inducer moiety under the control of a brain cell-specific promoter;

wherein the inducer moiety induces expression of the at least 2 reprogramming genes under the control of the inducible promoter in the presence of an inducer.

[0028] In an embodiment, the vector is a lentiviral vector.

[0029] In another embodiment, the vector is an adeno-associated vector that is able to cross the blood-brain barrier.

[0030] Also provided herein is a composition comprising:

- a. a first vector comprising a first nucleic acid molecule comprising at least 2 reprogramming genes under the control of an inducible promoter; and

- b. a second vector comprising a second nucleic acid molecule comprising a gene encoding an inducer moiety under the control of a brain cell-specific promoter;

wherein the inducer moiety induces expression of the at least 2 reprogramming genes under the control of the inducible promoter in the presence of an inducer.

[0031] In an embodiment, the first vector and/or the second vector is a lentiviral vector.

[0032] In another embodiment, the first vector and/or the second vector is an adeno-associated vector that is able to cross the blood-brain barrier.

[0033] In an embodiment, the vector or the composition comprises at least 2 reprogramming genes comprising at least 2 of Oct4, Sox2, Klf4 and c-Myc. In another embodiment, the vector or the composition comprises at least 3 of the reprogramming genes. In yet another embodiment, the vector or the composition comprises at least 4 of the reprogramming genes.

[0034] In an embodiment,

- a) the inducible promoter is a tetracycline response element (TRE) and the inducer moiety is a reverse tetracycline-controlled transactivator (rtTA);
- b) the inducible promoter is an ecdysteroid response element (EcRE) and the inducer moiety is an ecdysone receptor-derived transactivator, optionally VpEcR; or
- c) the inducible promoter is a GAL4 binding site-containing promoter and the inducer moiety is:
 - i) an RSL1-activated two-hybrid transactivator comprised of VP16-RXR and GAL4-EcR chimeric constructs;
 - ii) a Mifepristone-responsive regulator (pGL-VP); or
 - iii) a rapamycin-induced transactivator.

[0035] In an embodiment, the brain cell-specific promoter is a neuron-specific promoter, optionally a Synapsin 1 (SYN1) promoter, a Ca²⁺/calmodulin-dependent kinase subunit α (CaMKII) promoter, or a neuron-specific enolase (NSE) promoter for neuron-specific expression, or a tyrosine hydroxylase promoter for expression specific to dopamine neurons.

[0036] In another embodiment, the brain cell-specific promoter is an astrocyte-specific promoter, optionally a glial fibrillary acidic protein (GFAP) promoter, a GfaABC1D promoter, or a ALDH1L1 promoter for astrocyte-specific expression.

[0037] Also herein provided is a pharmaceutical composition comprising a vector or composition herein disclosed and a pharmaceutically acceptable carrier or buffer.

[0038] Further provided herein is a kit comprising a vector, composition, or pharmaceutical composition herein disclosed and an inducer.

[0039] Also provided herein is a use of a vector, a composition, a pharmaceutical composition, or a kit herein disclosed in preparing a subject for treating Alzheimer's disease.

[0040] Further provided herein is a use of an inducer or kit provided herein for treating Alzheimer's disease in a subject that has previously been prepared for treating the Alzheimer's disease according to a method provided herein. Even further provided herein is a use of an inducer or kit disclosed herein for treating Alzheimer's disease in a subject that has brain-cell specific expression of a first nucleic acid molecule comprising at least 2 reprogramming genes under the control of an inducible promoter; and a second nucleic acid molecule comprising a gene encoding an inducer moiety under the control of a brain cell-specific promoter; wherein the inducer moiety induces expression of the at least 2 reprogramming genes under the control of the inducible promoter in the presence of the inducer.

[0041] Also provided herein is a use of a vector, a composition, a pharmaceutical composition, or a kit herein disclosed in preparing a subject for treating progeria.

[0042] Further provided herein is a use of an inducer or kit provided herein for treating progeria in a subject that has previously been prepared for treating the progeria according to a method provided herein. Even further provided herein is a use of an inducer or kit disclosed herein for treating progeria in a subject that has brain-cell specific expression of a first nucleic acid molecule comprising at least 2 reprogramming genes under the control of an inducible promoter; and a second nucleic acid molecule comprising a gene encoding an inducer moiety under the control of a brain cell-specific promoter; wherein the inducer moiety induces expression of the at least 2

reprogramming genes under the control of the inducible promoter in the presence of an inducer.

[0043] Other features and advantages of the present disclosure will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples, while indicating embodiments of the disclosure, are given by way of illustration only and the scope of the claims should not be limited by these embodiments but should be given the broadest interpretation consistent with the description as a whole.

BRIEF DESCRIPTION OF DRAWINGS

[0044] The embodiments of the disclosure will now be described in greater detail with reference to the attached drawings in which:

[0045] FIG. 1 shows one version of the lentivector construct in an exemplary embodiment of the disclosure.

[0046] FIG. 2A shows results of female ARTE10 mice of the indicated groups in the Elevated Plus Maze test in an exemplary embodiment of the disclosure. * represents $p < 0.05$ and ** represents $p < 0.01$ in an independent t-test compared to Group 5.

[0047] FIG. 2B shows results of male ARTE10 mice of the indicated groups in the Elevated Plus Maze test in an exemplary embodiment of the disclosure.

[0048] FIG. 3A shows results of male ARTE10 mice of the indicated groups in the Three Chamber test in an exemplary embodiment of the disclosure. * represents $p < 0.05$, ** represents $p < 0.01$, and **** represents $p < 0.0001$ in a t-test within a group. # represents $p < 0.05$, and ## represents $p < 0.01$ compared to group G4 in a one-way ANOVA with Dunnett's post-hoc correction.

[0049] FIG. 3B shows results of female ARTE10 mice of the indicated groups in the Three Chamber test in an exemplary embodiment of the disclosure. * represents $p < 0.05$ in a t-test within a group. # represents $p < 0.05$ compared to group G4 in a one-way ANOVA with Dunnett's post-hoc correction.

[0050] FIG. 4 shows the levels of soluble amyloid beta post-mortem from the indicated groups of ARTE10 mice in an exemplary embodiment of the disclosure. * represents $p < 0.05$ compared to group G5 in a one-way ANOVA with Dunnett's post-hoc

correction. # represents $p < 0.05$ compared to group G4 in a one-way ANOVA with Dunnett's post-hoc correction.

[0051] FIG. 5 shows the quantification of plaque area from the indicated groups of ARTE10 mice in an exemplary embodiment of the disclosure. * represents $p < 0.05$ and ** represents $p < 0.01$ compared to group G4 in a one-way ANOVA with Dunnett's post-hoc correction.

[0052] FIG. 6 shows *in vitro* validation of delivered gene expression in murine primary cortical neurons from *Lmna*^{G609G/G609G} mice in an exemplary embodiment of the disclosure.

[0053] FIG. 7A-B show the *in vivo* treatment protocol in an exemplary embodiment of the disclosure. FIG. 7A shows a schematic representation of the study. Six-week-old *Lmna*^{G609G/G609G} mice were injected bilaterally in the hypothalamus with lentiviral vectors encoding for the OSKM overexpression. Two weeks after the surgery, (injection) animals were submitted to a doxycycline administration protocol (see FIG. 7B). FIG. 7B shows a schematic representation of the cyclic doxycycline administration protocol. The *in vivo* OSKM cyclic induction took place for six weeks. The phase 1 of the study of 3 weeks included 3 days of doxycycline (+Dox) administration followed by 4 days of doxycycline withdrawal (-Dox). The phase 2, also of 3 weeks, included 4 days of doxycycline administration (+Dox) followed by 3 days of doxycycline withdrawal (-Dox). The doxycycline administration was achieved through Diet 4RF25 medicated with doxycycline at a dosage of 625 mg/Kg. wk=week; d=day; Dox=Doxycycline.

[0054] FIG. 8A-B shows that treatment with the SYN-OSKM lentiviral vector (LV) and doxycycline directly impacts the *Lmna*^{G609G/G609G} phenotype, preventing the progressive loss of weight at later stages of the disease in an exemplary embodiment of the disclosure. FIG. 8A) Cumulative body weight gain/loss of control and treated female *Lmna*^{G609G/G609G} mice represented as the percentage of weight gain from treatment start until the end of the study (left) and total body weight (in grams) before surgery and at the end of the study for female *Lmna*^{G609G/G609G} mice, comparing treated and untreated mice (right). FIG. 8B) Cumulative body weight gain/loss of control and treated male and female *Lmna*^{G609G/G609G} mice represented as the percentage of weight

gain/loss from treatment start until the end of the study (left) and total body weight (in grams) before surgery and at the end of the study for male and female *Lmna*^{G609G/G609G} mice, comparing treated and untreated mice (right).

[0055] FIG. 9A-B show that treatment with the vector and doxycycline mitigates pathological changes in fat distribution in *Lmna*^{G609G/G609G} mice in an exemplary embodiment of the disclosure. FIG. 9A) Representative images of Haematoxylin-Eosin-stained sections of gonadal white adipose tissue (WAT) in *wild-type* and progeria mice – *Lmna*^{G609G/G609G} – of 3 months old (left) and WAT size, expressed as a percentage of body weight, in 4 months-old female *Lmna*^{G609G/G609G} mice, comparing to non-treated and LV+Dox-treated groups (right). FIG. 9B) Representative images of Haematoxylin-Eosin-stained sections of WAT in non-treated and LV+Dox-treated female *Lmna*^{G609G/G609G} mice- of 4 months old (left) and quantification of adipocyte area (μm^2) in female *Lmna*^{G609G/G609G} mice, comparing non-treated and LV+Dox-treated animals (right). *N*=2-4 *per* group.

[0056] FIG. 10 shows that treatment with the vector and doxycycline rescued white adipose tissue dysfunction in the skin of female *Lmna*^{G609G/G609G} mice, reversing lipodystrophy resulting in an increased subcutaneous fat layer in an exemplary embodiment of the disclosure. Representative images of Haematoxylin-eosin-stained sections of dorsal skin of non-treated and LV+Dox-treated female *Lmna*^{G609G/G609G} mice. *N*=2-4 *per* group.

[0057] FIG. 11 shows aorta media thickness in female *Lmna*^{G609G/G609G} mice with or without treatment in an exemplary embodiment of the disclosure. Histological images (left) and bar graph (right) demonstrate that Dox treatment in female *Lmna*^{G609G/G609G} mice with brain-cell specific expression of partial reprogramming genes increased aortic media thickness compared to no treatment. * represents $p < 0.05$ in an unpaired t-test

DETAILED DESCRIPTION

[0058] The following is a detailed description provided to aid those skilled in the art in practicing the present disclosure. Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs. The terminology used in the description

herein is for describing particular embodiments only and is not intended to be limiting of the disclosure. All publications, patent applications, patents, figures and other references mentioned herein are expressly incorporated by reference in their entirety.

[0059] Further, the definitions and embodiments described in particular sections are intended to be applicable to other embodiments herein described for which they are suitable as would be understood by a person skilled in the art. For example, in the following passages, different aspects of the disclosure are defined in more detail. Each aspect so defined may be combined with any other aspect or aspects unless clearly indicated to the contrary. In particular, any feature described herein may be combined with any other feature or features described herein.

I. Definitions

[0060] Unless otherwise indicated, the definitions and embodiments described in this and other sections are intended to be applicable to all embodiments and aspects of the present disclosure herein described for which they are suitable as would be understood by a person skilled in the art.

[0061] As used herein, the following terms may have meanings ascribed to them below, unless specified otherwise. However, it should be understood that other meanings that are known or understood by those having ordinary skill in the art are also possible, and within the scope of the present disclosure. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In the case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

[0062] In understanding the scope of the present disclosure, the term “comprising” and its derivatives, as used herein, are intended to be open ended terms that specify the presence of the stated features, elements, components, groups, integers, and/or steps, but do not exclude the presence of other unstated features, elements, components, groups, integers and/or steps. The foregoing also applies to words having similar meanings such as the terms, “including”, “having” and their derivatives. The term “consisting” and its derivatives, as used herein, are intended to be closed terms that specify the presence of the stated features, elements, components, groups, integers,

and/or steps, but exclude the presence of other unstated features, elements, components, groups, integers and/or steps. The term “consisting essentially of”, as used herein, is intended to specify the presence of the stated features, elements, components, groups, integers, and/or steps as well as those that do not materially affect the basic and novel characteristic(s) of features, elements, components, groups, integers, and/or steps.

[0063] Terms of degree such as “substantially”, “about” and “approximately” as used herein mean a reasonable amount of deviation of the modified term such that the end result is not significantly changed. These terms of degree should be construed as including a deviation of at least $\pm 5\%$ of the modified term if this deviation would not negate the meaning of the word it modifies.

[0064] As used in this disclosure, the singular forms “a”, “an” and “the” include plural references unless the content clearly dictates otherwise.

[0065] In embodiments comprising an “additional” or “second” component, the second component as used herein is chemically different from the other components or first component. A “third” component is different from the other, first, and second components, and further enumerated or “additional” components are similarly different.

[0066] The phrase “and/or,” as used herein in the specification and in the claims, should be understood to mean “either or both” of the elements so conjoined, i.e., elements that are conjunctively present in some cases and disjunctively present in other cases. Multiple elements listed with “and/or” should be construed in the same fashion, i.e., “one or more” of the elements so conjoined. Other elements may optionally be present other than the elements specifically identified by the “and/or” clause, whether related or unrelated to those elements specifically identified.

[0067] As used herein, “or” should be understood to have the same meaning as “and/or” as defined above. For example, when separating items in a list, “or” or “and/or” shall be interpreted as being inclusive, i.e., the inclusion of at least one, but also including more than one, of a number or list of elements, and, optionally, additional unlisted items. Only terms clearly indicated to the contrary, such as “only one of” or “exactly one of” or, when used in the claims, “consisting of” will refer to the inclusion of exactly one element of a number or list of elements. In general, the term “or” as used herein

shall only be interpreted as indicating exclusive alternatives (i.e., "one or the other but not both") when preceded by terms of exclusivity, such as "either," "one of," "only one of," or "exactly one of."

[0068] It should also be understood that, in certain methods described herein that include more than one step or act, the order of the steps or acts of the method is not necessarily limited to the order in which the steps or acts of the method are recited unless the context indicates otherwise.

[0069] The term "sample" or "test sample" as used herein may refer to any material in which the presence or amount of a target analyte is unknown and can be determined in an assay. The sample may be from any source, for example, any biological (e.g. human or animal samples, including clinical samples), environmental (e.g. water, soil or air) or natural (e.g. plants) source, or from any manufactured or synthetic source (e.g. food or drinks). The sample may be comprised or is suspected of comprising one or more analytes. The sample may be a "biological sample" comprising cellular and non-cellular material, including, but not limited to, tissue samples, saliva, sputum, urine, blood, serum, other bodily fluids and/or secretions. In some embodiments, the sample comprises saliva, sputum, oropharyngeal and/or nasopharyngeal secretions.

[0070] The term "target", "analyte" or "target analyte" as used herein may refer to any agent, including, but not limited to, a small inorganic molecule, small organic molecule, metal ion, biomolecule, toxin, biopolymer (such as a nucleic acid, carbohydrate, lipid, peptide, protein), cell, tissue, microorganism and virus, for which one would like to sense or detect. The analyte may be either isolated from a natural source or synthetic. The analyte may be a single compound or a class of compounds, such as a class of compounds that share structural or functional features. The term analyte also includes combinations (e.g. mixtures) of compounds or agents such as, but not limited, to combinatorial libraries and samples from an organism or a natural environment.

[0071] The term "nucleic acid" as used herein may refer to a biopolymer comprising monomers of nucleotides, such as deoxyribonucleic acid (DNA), ribonucleic acid (RNA) and other polynucleotides of modified nucleotides and/or nucleotide derivatives, and may be either double stranded (ds) or single stranded (ss). "Modified" bases include, for example, tritiated bases and unusual bases such as inosine. A variety

of modifications can be made to DNA and RNA; thus “nucleic acid molecule”, “DNA molecule”, and “RNA molecule” embrace chemically, enzymatically, or metabolically modified forms. Examples of modified nucleotides which can be used to generate the nucleic acids disclosed herein include xanthine, hypoxanthine, 2-aminoadenine, 6-methyl, 2-propyl and other alkyl adenines, 5-halo uracil, 5-halo cytosine, 6-aza uracil, 6-aza cytosine and 6-aza thymine, pseudo uracil, 4-thiouracil, 8-halo adenine, 8-aminoadenine, 8-thiol adenine, 8-thiolalkyl adenines, 8-hydroxyl adenine and other 8-substituted adenines, 8-halo guanines, 8 amino guanine, 8-thiol guanine, 8-thiolalkyl guanines, 8-hydroxyl guanine and other 8-substituted guanines, other aza and deaza uracils, thymidines, cytosines, adenines, or guanines, 5-trifluoromethyl uracil and 5-trifluoro cytosine or fluorophore and quencher conjugated nucleotides. Alternatively, the nucleic acid molecules can be produced biologically using an expression vector. In some embodiments, modified nucleotides comprise one or more modified bases (e.g. unusual bases such as inosine, and functional modifications to the bases such as amino modifications), modified backbones (e.g. peptide nucleic acid, PNA) and/or other chemically, enzymatically, or metabolically modified forms. The term “functional fragment” as used herein refers to a fragment of the nucleic acid that retains the functional property of the full-length nucleic acid, for example, the ability encode factors that induce partial reprogramming.

[0072] The recitation of numerical ranges by endpoints herein includes all numbers and fractions subsumed within that range (*e.g.* 1 to 5 includes for example 1, 1.5, 2, 2.75, 3, 3.90, 4, and 5). It is also to be understood that all numbers and fractions thereof are presumed to be modified by the term “about”.

[0073] It will be understood that any component defined herein as being included can be explicitly excluded by way of proviso or negative limitation, such as any specific compounds or method steps, whether implicitly or explicitly defined herein.

II. Vectors and Compositions

[0074] Disclosed herein are vectors that provide for brain cell-specific expression of reprogramming genes useful for the treatment of Alzheimer’s disease and progeria.

[0075] In one embodiment, there is provided a vector comprising:

- a. a first nucleic acid molecule comprising at least 2 reprogramming genes under the control of an inducible promoter and
- b. a second nucleic acid molecule comprising a gene encoding an inducer moiety under the control of a brain cell-specific promoter;
wherein the inducer moiety induces expression of the at least 2 reprogramming genes under the control of the inducible promoter in the presence of the inducer.

[0076] Also provided are compositions comprising a first and a second vector that provide for brain cell-specific expression of reprogramming genes useful for the treatment of Alzheimer's disease and progeria.

[0077] In one embodiment, there is provided a composition comprising:

- a. a first vector comprising a first nucleic acid molecule comprising at least 2 reprogramming genes under the control of an inducible promoter and
- b. a second vector comprising a second nucleic acid molecule comprising a gene encoding an inducer moiety under the control of a brain cell-specific promoter;
wherein the inducer moiety induces expression of the at least 2 reprogramming genes under the control of the inducible promoter in the presence of the inducer.

[0078] The term "vector" as used herein refers to any intermediary vehicle for a nucleic acid molecule which enables said nucleic acid molecule, for example, to be introduced into eukaryotic cells and/or integrated into a genome. The vector can be a viral vector, for example a retrovirus such as a lentiviral vector, an adeno-associated virus (AAV), an adenovirus, or a herpes simplex virus (HSV)-based vector. The vector can also be a non-viral vector, for example a lipid nanoparticle (LNP)-based vector for use in methods such as the Fusogenix platform from Entos Pharmaceuticals.

[0079] Accordingly, in one embodiment, a vector disclosed herein is a lentiviral vector. In yet another embodiment, a vector disclosed herein is an adeno-associated vector. In a further embodiment, the adeno-associated vector is able to cross the blood-brain

barrier. In yet another embodiment, the adeno-associated vector is AAV9, AAV-rh10, or AAV-PHP.eB.

[0080] A vector disclosed herein may integrate a first or a second nucleic acid disclosed herein into the genome of a transfected or transduced brain cell. A vector disclosed herein may also be incapable of integrating a first or a second nucleic acid disclosed herein into the genome of a transfected or transduced brain cell, for example, if the vector is integrase deficient. In an embodiment, the vector is integrase deficient. In another embodiment, the vector is an integrase deficient lentiviral vector.

[0081] Vectors can be pseudotyped with ligands that are preferentially or exclusively tropic to brain cell-specific receptors to provide or increase cell type specificity of the expression of a first or second nucleic acid herein disclosed. For example, FUG-B2 and canonical VSV-G lentiviral pseudotyping can be used to provide a lentiviral construct with preferential tropism for neurons and astrocytes and to take advantage of retrograde neuronal transport for wider distribution of the first and the second nucleic acid. Vectors can also be pseudotyped with ligands that are preferentially or exclusively tropic to receptors that are specific to a subtype of brain cells, for example a neuron as opposed to an astrocyte. In an embodiment, the vector is pseudotyped with a ligand that is preferentially or exclusively tropic to receptors that are brain cell-specific. In another embodiment, the vector is FUG-B2 or VSV-G pseudotyped. In yet another embodiment, the vector is FUG-B2 pseudotyped. In a further embodiment, the vector is VSV-G pseudotyped.

[0082] The term “partial reprogramming” as used herein refers to a process whereby somatic cells such as neurons and astrocytes are exposed to transient expression of reprogramming genes that can, for a short period of time, restore a more youthful epigenetic and/or transcriptomic signature (i.e., provide youthful rejuvenation) to the cells, and can thereby benefit the subject comprising the cells, such as by extending its lifespan.

[0083] The term “reprogramming genes” as used herein refers to genes capable of inducing a more youthful epigenetic and/or transcriptomic signature in somatic cells.

[0084] In an embodiment, the at least 2 reprogramming genes are selected from the Yamanaka factors: Oct4, Sox2, Klf4 and c-Myc (OSKM). Oct4 can be from any

organism or source and optionally as shown in GenBank Accession Number NM_203289. Sox2 can be from any organism or source and optionally as shown in GenBank Accession Number NM_003106. Klf4 can be from any organism or source and optionally as shown in GenBank Accession Number NM_004235. c-Myc can be from any organism or source and optionally as shown in GenBank Accession Number NM_002467.

[0085] Each Yamanaka factor can be substituted for a functional fragment thereof or for a reprogramming gene that is its close family member (see e.g., **Table 1**, below). Each Yamanaka factor can also be substituted for other partial reprogramming genes known in the art. For example, Nr5a2 (also called LRH-1) can be substituted for Oct4. Nr5a2 can be from any organism or source and optionally as shown in GenBank Accession Number NM_004489. In another example, Sox1 or Sox3 can be substituted for Sox2. Sox1 can be from any organism or source and optionally as shown in GenBank Accession Number NM_005986. Sox3 can be from any organism or source and optionally as shown in GenBank Accession Number NM_005634. In yet another example, Esrrb, Klf2, or Klf5 can be substituted for Klf4. Esrrb can be from any organism or source and optionally as shown in GenBank Accession Number NM_004452. Klf2 can be from any organism or source and optionally as shown in GenBank Accession Number NM_016270. Klf5 can be from any organism or source and optionally as shown in GenBank Accession Number NM_001730. In a further example, Lin28 (Lin28a or Lin28b), Nanog, L-Myc, or N-Myc can be substituted for c-Myc. Lin28a can be from any organism or source and optionally as shown in GenBank Accession Number NM_024674. Lin28b can be from any organism or source and optionally as shown in GenBank Accession Number NM_001004317. Nanog can be from any organism or source and optionally as shown in GenBank Accession Number NM_024865. L-Myc can be from any organism or source and optionally as shown in GenBank Accession Number NM_005376. N-Myc can be from any organism or source and optionally as shown in GenBank Accession Number NM_005378.

[0086] Other suitable reprogramming genes can include DNMT3B, CROC4, H2AFX, HIST1H2AB, HIST1H4J, HMGB2, LEFTB, MYBL2, SALL4, TERF1, and ZNF206 (**Table 1**, below). DNMT3B can be from any organism or source and optionally as shown in GenBank Accession Number NM_006892. CROC4 can be from any

organism or source and optionally as shown in GenBank Accession Number NR_135260. H2AFX can be from any organism or source and optionally as shown in GenBank Accession Number NM_002105. HIST1H2AB can be from any organism or source and optionally as shown in GenBank Accession Number NM_003519. HIST1H4J can be from any organism or source and optionally as shown in GenBank Accession Number NM_003546. HMGB2 can be from any organism or source and optionally as shown in GenBank Accession Number NM_002128. LEFTB can be from any organism or source and optionally as shown in GenBank Accession Number NM_020997. MYBL2 can be from any organism or source and optionally as shown in GenBank Accession Number NM_002466. SALL4 can be from any organism or source and optionally as shown in GenBank Accession Number NM_020436. TERF1 can be from any organism or source and optionally as shown in GenBank Accession Number NM_003218. ZNF206 can be from any organism or source and optionally as shown in GenBank Accession Number NM_015884.

Table 1. Examples of Partial Reprogramming Genes

Yamanaka Factors	O	S	K	M
Canonical	Oct4	Sox2	Klf4	c-Myc
Substitutes	Nr5a2 (LRH-1)	Sox1 Sox3	Esrrb Klf2 Klf5	Lin28a Lin28b Nanog L-Myc N-Myc
Other Suitable Examples	DNMT3B; CROC4; H2AFX; HIST1H2AB; HIST1H4J; HMGB2; LEFTB; MYBL2; SALL4; TERF1; and ZNF206.			

[0087] Accordingly, in an embodiment, the reprogramming genes comprise Oct4. In another embodiment, the reprogramming genes comprise Sox2. In another embodiment, the reprogramming genes comprise Klf4. In another embodiment, the reprogramming genes comprise c-Myc. In another embodiment, the reprogramming genes comprise Nr5a2. In another embodiment, the reprogramming genes comprise Sox1. In another embodiment, the reprogramming genes comprise Sox3. In another embodiment, the reprogramming genes comprise Esrrb. In another embodiment, the reprogramming genes comprise Klf2. In another embodiment, the reprogramming

genes comprise Klf5. In another embodiment, the reprogramming genes comprise Lin28a. In another embodiment, the reprogramming genes comprise Lin28b. In another embodiment, the reprogramming genes comprise Nanog. In another embodiment, the reprogramming genes comprise L-Myc. In another embodiment, the reprogramming genes comprise N-Myc. In another embodiment, the reprogramming genes comprise DNMT3B. In another embodiment, the reprogramming genes comprise CROC4. In another embodiment, the reprogramming genes comprise H2AFX. In another embodiment, the reprogramming genes comprise HIST1H2AB. In another embodiment, the reprogramming genes comprise HIST1H4J. In another embodiment, the reprogramming genes comprise HMGB2. In another embodiment, the reprogramming genes comprise LEFTB. In another embodiment, the reprogramming genes comprise MYBL2. In another embodiment, the reprogramming genes comprise SALL4. In another embodiment, the reprogramming genes comprise TERF1. In yet another embodiment, the reprogramming genes comprise ZNF206.

[0088] In an embodiment, the first nucleic acid molecule comprises at least 2 of the reprogramming genes. In another embodiment, the first nucleic acid molecule comprises at least 3 of the reprogramming genes. In yet another embodiment, the first nucleic acid molecule comprises at least 4 of the reprogramming genes. In an embodiment, the reprogramming genes are Oct4, Sox2, Klf4 and c-Myc. In an embodiment, the first nucleic acid molecule comprises at least 5 of the reprogramming genes. In another embodiment, the first nucleic acid molecule comprises at least 6 reprogramming genes. In yet another embodiment, the reprogramming genes are Oct4, Sox2, Klf4, c-Myc, Nanog, and Lin28.

[0089] In an embodiment, the reprogramming genes comprise Oct4, Sox2, Klf4 and c-Myc. In another embodiment, the reprogramming genes comprise Oct4, Sox2, and Klf4. In another embodiment, the reprogramming genes comprise Sox2, Klf4, and c-Myc. In another embodiment, the reprogramming genes comprise Oct4, Klf4, and c-Myc. In another embodiment, the reprogramming genes comprise Oct4, Sox2, and c-Myc. In another embodiment, the reprogramming genes comprise Oct4 and Sox2. In another embodiment, the reprogramming genes comprise Oct4 and Klf4. In another embodiment, the reprogramming genes comprise Oct4 and c-Myc. In another embodiment, the reprogramming genes comprise Sox2 and Klf4. In yet another

embodiment, the reprogramming genes comprise Sox2 and c-Myc. In a further embodiment, the reprogramming genes comprise Klf4 and c-Myc.

[0090] In an embodiment, the reprogramming genes comprise a substitute of each of Oct4, Sox2, Klf4 and c-Myc. In another embodiment, the reprogramming genes comprise a substitute of each of Oct4, Sox2, and Klf4. In another embodiment, the reprogramming genes comprise a substitute of each of Sox2, Klf4, and c-Myc. In another embodiment, the reprogramming genes comprise a substitute of each of Oct4, Klf4, and c-Myc. In another embodiment, the reprogramming genes comprise a substitute of each of Oct4, Sox2, and c-Myc. In another embodiment, the reprogramming genes comprise a substitute of each of Oct4 and Sox2. In another embodiment, the reprogramming genes comprise a substitute of each of Oct4 and Klf4. In another embodiment, the reprogramming genes comprise a substitute of each of Oct4 and c-Myc. In another embodiment, the reprogramming genes comprise a substitute of each of Sox2 and Klf4. In yet another embodiment, the reprogramming genes comprise a substitute of each of Sox2 and c-Myc. In a further embodiment, the reprogramming genes comprise a substitute of each of Klf4 and c-Myc.

[0091] In an embodiment, the reprogramming genes comprise Oct4 or a substitute thereof, Sox2 or a substitute thereof, Klf4 or a substitute thereof, and c-Myc or a substitute thereof. In another embodiment, the reprogramming genes comprise Oct4 or a substitute thereof, Sox2 or a substitute thereof, and Klf4 or a substitute thereof. In another embodiment, the reprogramming genes comprise Sox2 or a substitute thereof, Klf4 or a substitute thereof, and c-Myc or a substitute thereof. In another embodiment, the reprogramming genes comprise Oct4 or a substitute thereof, Klf4 or a substitute thereof, and c-Myc or a substitute thereof. In another embodiment, the reprogramming genes comprise Oct4 or a substitute thereof, Sox2 or a substitute thereof, and c-Myc or a substitute thereof. In another embodiment, the reprogramming genes comprise Oct4 or a substitute thereof and Sox2 or a substitute thereof. In another embodiment, the reprogramming genes comprise Oct4 or a substitute thereof and Klf4 or a substitute thereof. In another embodiment, the reprogramming genes comprise Oct4 or a substitute thereof and c-Myc or a substitute thereof. In another embodiment, the reprogramming genes comprise Sox2 or a substitute thereof and Klf4 or a substitute thereof. In yet another embodiment, the reprogramming genes comprise Sox2 or a

substitute thereof and c-Myc or a substitute thereof. In a further embodiment, the reprogramming genes comprise Klf4 or a substitute thereof and c-Myc or a substitute thereof.

[0092] The term “inducible promoter” as used herein refers to a portion of nucleic acids that precede a gene and that can turn on gene expression in the presence of an inducer moiety.

[0093] The term “inducer moiety” as used herein refers to a moiety that is capable of binding to a promoter to turn on gene expression when in the presence of an inducer.

[0094] The term “inducer” refers to a product that activates an inducer moiety to bind to a promoter to turn on gene expression.

[0095] In one embodiment, the inducible promoter is a tetracycline response element (TRE), the inducer moiety is a reverse tetracycline-controlled transactivator (rtTA) and the inducer is tetracycline or a tetracycline derivative such as doxycycline. In another embodiment, the inducible promoter is an ecdysteroid response element (EcRE), the inducer moiety is an ecdysone receptor-derived transactivator such as VpEcR and the inducer is ecdysone or an ecdysone analog such as muristerone A. In yet another embodiment, the inducible promoter is a GAL4 binding site-containing promoter, the inducer moiety is an RSL1-activated two-hybrid transactivator comprised of VP16-RXR and GAL4-EcR chimeric constructs and the inducer is RSL1 (diacylhydrazine) or ponasterone A. In a further embodiment, the inducible promoter is a GAL4 binding site-containing promoter, the inducer moiety is a Mifepristone-responsive regulator (pGL-VP) and the inducer is mifepristone. In yet a further embodiment, the inducible promoter is a GAL4 binding site-containing promoter, the inducer moiety is a rapamycin-induced transactivator and the inducer is rapamycin.

[0096] The term “brain cell-specific” as used herein refers to a promoter which induces expression with cell-type specificity, wherein the cell-type is a brain cell. For example, the brain-cell specific promoter may only induce transcription of the transducer moiety in a brain cell such as a neuron or a glial cell, for example an astrocyte, oligodendrocyte, or microglia, but is silent in all other cell types.

[0097] In an embodiment, the brain cell-specific promoter is a neuron-specific promoter, such as Synapsin 1(SYN1) promoter, Ca²⁺/calmodulin dependent kinase

subunit α (CaMKII) promoter, or neuron specific enolase (NSE) promoter. In another embodiment, the brain cell-specific promoter is a dopamine neuron specific promoter such as tyrosine hydroxylase promoter.

[0098] In an embodiment, the brain cell-specific promoter is an astrocyte-specific promoter, such as glial fibrillary acidic protein (GFAP) promoter, GfaABC1D promoter, or ALDH1L1 promoter.

[0099] In another embodiment, the brain cell-specific promoter is an oligodendrocyte-specific promoter, such as Myelin Basic Protein (MBP) promoter, Proteolipid Protein (PLP) promoter, 2',3'-Cyclic Nucleotide 3'-Phosphodiesterase (CNP) promoter, or Myelin-Associated Glycoprotein (MAG) promoter.

[00100] In yet another embodiment, the brain cell-specific promoter is a microglia-specific promoter, such as ionized calcium-binding adaptor molecule 1 (Iba1) promoter, TMEM119 (transmembrane protein 119) promoter, or P2RY12 (Purinergic Receptor P2Y12) promoter.

[00101] Also provided herein is a pharmaceutical composition comprising a vector or composition herein disclosed, optionally comprising a pharmaceutically acceptable carrier or buffer. In an embodiment, the pharmaceutical composition comprises a vector and a pharmaceutically acceptable carrier or buffer. In another embodiment, the pharmaceutical composition comprises a composition and a pharmaceutically acceptable carrier or buffer.

[00102] Suitable buffers and carriers are described, for example, in Remington's Pharmaceutical Sciences, 22nd Edition (Pharmaceutical Press and Philadelphia College of Pharmacy at the University of the Sciences, 2012). On this basis, the pharmaceutical compositions include, albeit not exclusively, solutions of a vector or composition herein disclosed in association with one or more pharmaceutically acceptable vehicles or diluents, and contained in buffered solutions with a suitable pH and iso-osmotic with physiological fluids.

[00103] The term "pharmaceutically acceptable" means compatible with the treatment of animals, in particular, humans. Each carrier or buffer must also be compatible with the other ingredients of the pharmaceutical composition.

III. Kits

[00104] Disclosed herein are kits that provide for brain cell-specific expression of reprogramming genes useful for the treatment of Alzheimer's disease and progeria.

[00105] In one embodiment, the kit comprises a vector, composition, or pharmaceutical composition herein disclosed and an inducer. In another embodiment, the kit comprises a vector or a composition herein disclosed and an inducer. In another embodiment, the kit comprises a vector herein disclosed and an inducer. In yet another embodiment, the kit comprises a composition herein disclosed and an inducer. In a further embodiment, the kit comprises a pharmaceutical composition herein disclosed and an inducer.

[00106] The kit may further comprise suitable additional components, for example a pharmaceutically acceptable carrier or buffer, instructions for use, or vials to contain the vector, composition, pharmaceutical composition, inducer and/or pharmaceutically acceptable carrier or buffer. In one embodiment, the kit further comprises a pharmaceutically acceptable carrier or buffer. In another embodiment, the kit further comprises instructions for use. In yet another embodiment, the kit further comprises vials to contain the vector, composition, pharmaceutical composition, inducer and/or pharmaceutically acceptable carrier or buffer.

IV. Methods and Uses

[00107] Also disclosed herein are methods for preparing a subject for treating Alzheimer's disease and progeria by providing for brain cell-specific expression of reprogramming genes.

[00108] In one embodiment, there is provided a method of preparing a subject for treating Alzheimer's disease comprising administering:

- a. a first nucleic acid molecule comprising at least 2 reprogramming genes under the control of an inducible promoter; and
- b. a second nucleic acid molecule comprising a gene encoding an inducer moiety under the control of a brain cell-specific promoter;

wherein the inducer moiety induces expression of the at least 2 reprogramming genes under the control of the inducible promoter in the presence of an inducer.

[00109] In another embodiment, herein provided is a use of a first nucleic acid molecule and a second nucleic acid molecule disclosed herein for preparing a subject

for treating Alzheimer's disease. In a further embodiment, there is provided use of a first nucleic acid molecule and a second nucleic acid molecule disclosed herein in the manufacture of a medicament for preparing a subject for treating Alzheimer's disease. In yet another embodiment, there is provided a first nucleic acid molecule and a second nucleic acid molecule disclosed herein for use in preparing a subject for treating Alzheimer's disease.

[00110] In another embodiment, there is provided a method of preparing a subject for treating progeria comprising administering:

- a. a first nucleic acid molecule comprising at least 2 reprogramming genes under the control of an inducible promoter; and
- b. a second nucleic acid molecule comprising a gene encoding an inducer moiety under the control of a brain cell-specific promoter;

wherein the inducer moiety induces expression of the at least 2 reprogramming genes under the control of the inducible promoter in the presence of an inducer.

[00111] In another embodiment, herein provided is a use of a first nucleic acid molecule and a second nucleic acid molecule disclosed herein for preparing a subject for treating progeria. In a further embodiment, there is provided use of a first nucleic acid molecule and a second nucleic acid molecule disclosed herein in the preparation of a medicament for preparing a subject for treating progeria. In yet another embodiment, there is provided a first nucleic acid molecule and a second nucleic acid molecule disclosed herein for use in preparing a subject for treating progeria.

[00112] The term "preparing a subject" as used herein refers to providing the subject the prerequisites such that subsequent administration of an inducer to the subject can induce expression of the inducer moiety and the at least 2 reprogramming genes in the brain cells of the subject.

[00113] The term "Alzheimer's Disease" or "AD" as used herein refers to a neurodegenerative disorder with progressive cognitive deficits, such as gradually increasing short-term memory impairments and executive dysfunction. AD also comprises progressive functional deficits and behavioural changes. Anatomical and histopathological characteristics of AD can include ventricular enlargement, hippocampal atrophy, axonal demyelination, and beta amyloid deposits.

[00114] The terms “progeria”, “Hutchinson-Gilford Progeria Syndrome”, and “HGPS” as used herein refer to a rare autosomal dominant genetic disorder caused by a mutation to the lamin A gene that is characterized by increased speed of aging and a significantly reduced lifespan. It can be associated with severe cardiovascular complications.

[00115] In an embodiment, the first and the second nucleic acid molecules are on the same vector. In another embodiment, the first and the second nucleic acid molecules are on different vectors.

[00116] The term “administering” or “administration” as used herein refers to the placement of a vector, composition, or pharmaceutical composition as disclosed herein into a subject by a method or route which results in at least partial delivery to a desired site. The vectors, compositions, or inducers disclosed herein can be administered by any appropriate route which results in effective expression of the genes encoded by the first and the second nucleic acid in the subject, or that results in effective treatment in the subject. Possible routes of administration of the vectors, compositions, and inducers disclosed herein include, but are not limited to, intravenous, intraperitoneal, intramuscular, subcutaneous, transdermal, oral, buccal, sublingual, intranasal, intrathecal, intravitreal, stereotactic, or rectal routes of administration, or a combination thereof.

[00117] Accordingly, in one embodiment, the administration or use is by stereotactic injection into a brain region or formulated for stereotactic injection into a brain region. Stereotaxis-based methods are well known in the art and can comprise three-dimensional coordinates representative of the location of a given brain region or structure, often with reference to at least one static landmark, for example Bregma. Detailed maps or atlases of the brain can assist in determining the correct coordinates for a given brain region. Suitable atlases exist for stereotactic surgery in several species, including mouse (e.g., Paxinos and Kenneth, 2001) and human (e.g., Mai et al., 2015). Detailed imaging (e.g., MRI or CT scans) and computer algorithms can also be used to refine stereotactic coordinates, for example in humans.

[00118] In another embodiment, the administration or use is by intrathecal injection or formulated for intrathecal injection. In yet another embodiment, the administration or use is by intranasal delivery or formulated for intranasal delivery.

[00119] The blood-brain barrier (BBB) can impede delivery of some vectors to the brain when the vector is administered systemically or by another route outside of the central nervous system (CNS), unless the BBB is leaky or is otherwise permeable to the vector. Some vectors can be naturally capable of crossing the BBB, such as AAV9 and AAV-rh10. Other vectors have been engineered for increased ability to cross the BBB, such as AAV-PHP.eB (Kimura and Harashima, 2022). Suitable non-viral vectors and routes of administration can comprise systemic nanoparticle delivery systems that include brain targeting ligands. Alternatively, methods that temporarily disrupt the BBB, such as via temporary disruption of BBB tight junctions, can be used to permit vectors that can otherwise not cross the BBB to enter the CNS. These methods can include, for example, intra-arterial infusion of hyperosmotic agents such as mannitol. Accordingly, in an embodiment, the vector is able to cross the blood brain barrier. In another embodiment, the vector is an adeno associated virus (AAV) that is able to cross the blood brain barrier, optionally AAV9, AAV-rh10, or AAV-PHP.eB. In yet another embodiment, the vector is a herpes simplex virus-based vector that is able to cross the blood brain barrier. In a further embodiment, the vector comprises a nanoparticle that is able to cross the blood brain barrier. In yet another embodiment, the BBB is temporarily disrupted to permit a vector to cross the BBB.

[00120] In an embodiment, the administration or use is by intravenous injection or formulated for intravenous injection. In another embodiment, the administration or use is by intravitreal injection or formulated for intravitreal injection.

[00121] The brain region can be any brain region associated with Alzheimer's disease or progeria that can benefit from partial reprogramming. The brain region can also be any brain region that contributes to symptoms associated with Alzheimer's disease or progeria and that benefits from partial reprogramming. Further, the brain region can be any brain region wherein its partial reprogramming alleviates a symptom of Alzheimer's disease or progeria despite the brain region not contributing to the symptom. In an embodiment, the brain region is the hippocampus. In another

embodiment, the brain region is the hypothalamus. In yet another embodiment, the brain region is the hippocampus and/or the hypothalamus.

[00122] The term "subject", also referred to as patient, as used herein includes all members of the animal kingdom including mammals, and suitably refers to humans. In an embodiment, the subject is a human.

[00123] Also disclosed herein are methods for treating Alzheimer's disease and progeria in a subject in need thereof by providing for brain cell-specific expression of reprogramming genes and an inducer.

[00124] In one embodiment, there is provided a method of treating Alzheimer's disease in a subject in need thereof comprising:

- i) preparing the subject for treating the Alzheimer's disease according to a method disclosed herein; and
- ii) administering an inducer disclosed herein to the subject.

[00125] In another embodiment, herein provided is a use of an inducer or kit disclosed herein for treating Alzheimer's disease in a subject that has previously been prepared for treating the Alzheimer's disease according to any method described herein. Also provided is use of an inducer or kit disclosed herein in the manufacture of a medicament for treating Alzheimer's disease in a subject that has been previously prepared for treating the Alzheimer's disease according to any method described herein. Further provided is an inducer or kit disclosed herein for use in treating Alzheimer's disease in a subject that has been previously prepared for treating the Alzheimer's disease according to any method described herein.

[00126] In another embodiment, there is provided a method of treating Alzheimer's disease in a subject in need thereof comprising administering an inducer disclosed herein to a subject that has brain-cell specific expression of a first nucleic acid molecule comprising at least 2 reprogramming genes under the control of an inducible promoter; and a second nucleic acid molecule comprising a gene encoding an inducer moiety under the control of a brain cell-specific promoter; wherein the inducer moiety induces expression of the at least 2 reprogramming genes under the control of the inducible promoter in the presence of an inducer.

[00127] In another embodiment, herein provided is a use of an inducer or kit disclosed herein for treating Alzheimer's disease in a subject that has brain-cell specific expression of a first nucleic acid molecule comprising at least 2 reprogramming genes under the control of an inducible promoter; and a second nucleic acid molecule comprising a gene encoding an inducer moiety under the control of a brain cell-specific promoter; wherein the inducer moiety induces expression of the at least 2 reprogramming genes under the control of the inducible promoter in the presence of an inducer, and optionally wherein the subject has previously been prepared for treating the Alzheimer's disease according to any method described herein. Also provided is use of an inducer or kit disclosed herein in the manufacture of a medicament for treating Alzheimer's disease in a subject that has brain-cell specific expression of a first nucleic acid molecule comprising at least 2 reprogramming genes under the control of an inducible promoter; and a second nucleic acid molecule comprising a gene encoding an inducer moiety under the control of a brain cell-specific promoter; wherein the inducer moiety induces expression of the at least 2 reprogramming genes under the control of the inducible promoter in the presence of an inducer, and optionally wherein the subject has previously been prepared for treating the Alzheimer's disease according to any method described herein. Further provided is an inducer or kit disclosed herein for use in treating Alzheimer's disease in a subject that has brain-cell specific expression of a first nucleic acid molecule comprising at least 2 reprogramming genes under the control of an inducible promoter; and a second nucleic acid molecule comprising a gene encoding an inducer moiety under the control of a brain cell-specific promoter; wherein the inducer moiety induces expression of the at least 2 reprogramming genes under the control of the inducible promoter in the presence of an inducer, and optionally wherein the subject has previously been prepared for treating the Alzheimer's disease according to any method described herein.

[00128] In another embodiment, there is provided a method of treating progeria in a subject in need thereof comprising:

- i) preparing the subject for treating progeria according to a method disclosed herein; and
- ii) administering an inducer disclosed herein to the subject.

[00129] In yet another embodiment, is a use of an inducer or kit disclosed herein for treating progeria in a subject that has previously been prepared for treating progeria according to any method described herein. Also provided is use of an inducer or kit disclosed herein in the manufacture of a medicament for treating progeria in a subject that has been previously prepared for treating the progeria according to any method described herein. Further provided is an inducer or kit disclosed herein for use in treating progeria in a subject that has been previously prepared for treating the progeria according to any method described herein.

[00130] In another embodiment, there is provided a method of treating progeria in a subject in need thereof comprising administering an inducer disclosed herein to a subject that has brain-cell specific expression of a first nucleic acid molecule comprising at least 2 reprogramming genes under the control of an inducible promoter; and a second nucleic acid molecule comprising a gene encoding an inducer moiety under the control of a brain cell-specific promoter; wherein the inducer moiety induces expression of the at least 2 reprogramming genes under the control of the inducible promoter in the presence of an inducer.

[00131] In another embodiment, herein provided is a use of an inducer or kit disclosed herein for treating progeria in a subject that has brain-cell specific expression of a first nucleic acid molecule comprising at least 2 reprogramming genes under the control of an inducible promoter; and a second nucleic acid molecule comprising a gene encoding an inducer moiety under the control of a brain cell-specific promoter; wherein the inducer moiety induces expression of the at least 2 reprogramming genes under the control of the inducible promoter in the presence of an inducer, and optionally wherein the subject has previously been prepared for treating progeria according to any method described herein. Also provided is use of an inducer or kit disclosed herein in the manufacture of a medicament for treating progeria in a subject that has brain-cell specific expression of a first nucleic acid molecule comprising at least 2 reprogramming genes under the control of an inducible promoter; and a second nucleic acid molecule comprising a gene encoding an inducer moiety under the control of a brain cell-specific promoter; wherein the inducer moiety induces expression of the at least 2 reprogramming genes under the control of the inducible promoter in the presence of an inducer, and optionally wherein the subject has previously been prepared for treating

progeria according to any method described herein. Further provided is an inducer or kit disclosed herein for use in treating progeria in a subject that has brain-cell specific expression of a first nucleic acid molecule comprising at least 2 reprogramming genes under the control of an inducible promoter; and a second nucleic acid molecule comprising a gene encoding an inducer moiety under the control of a brain cell-specific promoter; wherein the inducer moiety induces expression of the at least 2 reprogramming genes under the control of the inducible promoter in the presence of an inducer, and optionally wherein the subject has previously been prepared for treating progeria according to any method described herein.

[00132] The term “treating” or “treatment” as used herein, and as is well understood in the art, refers to an approach for obtaining beneficial or desired results, including clinical results. Beneficial or desired clinical results include, but are not limited to alleviation or amelioration of one or more symptoms or conditions, arresting development of disease, diminishment of extent of disease, stabilized (i.e. not worsening) state of disease, preventing spread of disease, delay or slowing of disease progression, amelioration or palliation of the disease state, including regression of the disease, diminishment of the reoccurrence of disease, and remission (whether partial or total), whether detectable or undetectable. “Treating” and “treatment” may also refer to prolonging survival as compared to expected survival if not receiving treatment. “Treating” and “treatment” as used herein also include prophylactic treatment. The effect may be prophylactic in terms of completely or partially preventing a disease or symptom thereof and/or may be therapeutic in terms of affecting a partial or complete cure for a disease and/or symptoms of the disease. Prophylactic treatment includes preventing the disease or a symptom of a disease from occurring in a subject which may be predisposed to the disease but has not yet been diagnosed as having it (e.g., including diseases that may be associated with or caused by a primary disease).

[00133] Treating may refer to any indicia of success in the treatment or amelioration or prevention of the disease, including any objective or subjective parameter such as abatement; remission; diminishing of symptoms; or making the disease condition more tolerable to the patient; slowing in the rate of degeneration or decline; or making the final point of degeneration less debilitating. The treatment or amelioration of symptoms is based on one or more objective or subjective parameters;

including the results of an examination by a physician. Accordingly, the term "treating" includes the administration of the methods of the present disclosure to prevent, delay, alleviate, arrest or inhibit development of the symptoms or conditions associated with Alzheimer's disease or progeria.

[00134] Administration or use of an inducer can be by any route effective to deliver the inducer to the brain. Some inducers can readily cross the BBB, for example doxycycline. Accordingly in an embodiment, the administration or the use of the inducer is by oral, buccal, or sublingual delivery. In another embodiment, the administration or use is by systemic, intramuscular, or subcutaneous injection. In another embodiment, the administration or use is by intrathecal injection. In another embodiment, the administration or use of the inducer is by intranasal delivery, for example with nasal drops or an aerosol. In another embodiment, the administration or use of the inducer is by ocular delivery. In another embodiment, the administration or use of the inducer is by transdermal delivery. In another embodiment, the administration or use of the inducer is by rectal delivery. In yet another embodiment, the administration or use of the inducer is via an inhaler. In a further embodiment, the administration or use of the inducer is by a combination of routes of delivery.

[00135] Administration or use of an inducer can begin after a delay following preparing the subject, for example to ensure sufficient time for transfection or transduction of the brain cell or to allow for integration of the first and the second nucleic molecule into the genome of the brain cell. For example, the administration or use can be after a delay of 1 week, 2 weeks, 3 weeks, 4 weeks or more. In an embodiment, the administering ii) begins at least 2 weeks following the preparing the subject i). In another embodiment, the administering ii) begins at least 3 weeks following the preparing the subject i). In yet another embodiment, the administering ii) begins at least 4 weeks following the preparing the subject i).

[00136] Administration or use of an inducer can occur once, or it can be repeated, for example every day, every other day, every 3 days, every 4 days, every 5 days, every 6 days or every 7 days. Administration or use of the inducer can also be repeated on multiple consecutive days. For example, administration or use of the inducer can be repeated on consecutive days for a period of 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 8 days, 9 days, 10 days, 1 week, 2 weeks, or more. Administration or use of the

inducer can also occur in cycles, for example 3 days of consecutive administration or use followed by 4 days without administration or use. Administration or use of the inducer can also occur for multiple consecutive days followed by a period without inducer administration. The period without inducer administration or use can, for example, last 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 8 days, 9 days, 10 days, 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 2 months, 3 months, 4 months, 5 months, 6 months or more. In an embodiment, the administering ii) comprises a cycle of 3 days of inducer administration followed by 4 days without administration. In another embodiment, the administering ii) comprises a cycle of 4 days of inducer administration followed by 3 days without administration. In yet another embodiment, the administering ii) comprises a cycle of 1 to 7 consecutive days of inducer administration followed by a period without inducer administration, optionally wherein the period without inducer administration is at least 7 days. In an embodiment, the cycle is repeated on a bimonthly basis. In another embodiment, the cycle is repeated on a monthly basis.

[00137] Administration or use of an inducer can occur for a set period, for example 12 weeks. Administration of an inducer can also occur until an indicium of success in the treatment or prevention of the disease is achieved. Administration or use of an inducer can further occur indefinitely. In an embodiment, the administration ii) continues for 12 weeks. In another embodiment, the cycle is repeated for 12 weeks. In yet another embodiment, the administering ii) comprises a single cycle. In further embodiment, the cycle is repeated indefinitely.

[00138] On days when the inducer is administered or used, the inducer can be administered or used once, twice, three times, or more. In an embodiment, on days when the inducer is administered, the inducer is administered once. In another embodiment, on days when the inducer is administered, the inducer is administered twice. In an embodiment, on days when the inducer is administered, the inducer is administered three times.

[00139] Also disclosed herein are uses of a vector, composition, pharmaceutical composition, or kit provided herein for preparing a subject for treating Alzheimer's disease and progeria.

[00140] In one embodiment, there is provided a use of a vector, composition, pharmaceutical composition, or kit provided herein for preparing a subject for treating Alzheimer's disease. In another embodiment, is a use of a vector, composition, pharmaceutical composition, or kit provided herein in the preparation of a medicament for preparing a subject for treating Alzheimer's disease. In yet another embodiment, is a vector, composition, pharmaceutical composition, or kit provided herein for use in preparing a subject for treating Alzheimer's disease.

[00141] In an embodiment, there is provided a use of a vector, composition, pharmaceutical composition, or kit provided herein for preparing a subject for treating progeria. In another embodiment, is a use of a vector, composition, pharmaceutical composition, or kit provided herein in the preparation of a medicament for preparing a subject for treating progeria. In yet another embodiment, is a vector, composition, pharmaceutical composition, or kit provided herein for use in preparing a subject for treating progeria.

[00142] The above disclosure generally describes the present application. A more complete understanding can be obtained by reference to the following specific examples. These examples are described solely for the purpose of illustration and are not intended to limit the scope of the application. Changes in form and substitution of equivalents are contemplated as circumstances might suggest or render expedient. Although specific terms have been employed herein, such terms are intended in a descriptive sense and not for purposes of limitation.

EXAMPLES

[00143] The following non-limiting examples are illustrative of the present disclosure:

Example 1. Brain cell-specific expression of reprogramming in Alzheimer's mouse model

[00144] Methods

[00145] The design of the lentiviral therapies utilized in this example involves an integrase-deficient lentivirus carrying two elements (FIG. 1). The first is a tetracycline-inducible OSKM (Oct4, Sox2, Klf4, and c-Myc) construct, separated by self-cleaving peptides (P2A, T2A or E2A) and placed under the control of a TRE3G

promoter. The second is a TetOn3G reverse Tetracycline TransActivator (rTTA3), driven by either synapsin (SYN; for neuron-specific therapies) or glial fibrillary acidic protein (GFAP; for astrocyte-specific therapies) promoters which ensures cell-type specificity: the construct is designed to only be inducible in the respective cell types (neurons or astrocytes) and silent in all other cell types.

[00146] This therapeutic construct was administered via stereotactic injections into the specific brain region: the hippocampi and hypothalami for Alzheimer's disease (as tested in APP/PS1 mice). Stereotactic injections were performed bilaterally using the following parameters: the injection coordinates were AP -2.2, ML +/-2 and DV -2 for the hippocampus and AP -2.10, ML +/- 0.3 and DV -5.6 for the hypothalamus. The injected volume was 0.5 µl of each virus per location and the titer of the virus was 1300 ng/µl for the FUG/B2 pseudotyped SYN-OSKM, 1249 ng/µl for the VSV-G pseudotyped SYN-OSKM and 799 for the VSV-G pseudotyped GFAP-OSKM. The flow rate was 32 nl per 30s.

[00147] A total of 62 animals were employed, 50 ARTE10 (APP/PS1) mice and 12 WT controls. These animals were divided into six experimental groups: Experimental groups 1, 2, and 3 were comprised of ARTE10 mice that received the vectors carrying a neuron-specific and/or astrocyte-specific Tet-inducible OSKM cassette (YBT vectors), which were subsequently periodically induced via a diet medicated with Doxycycline (Dox; pellet food with a doxycycline dose of 625 ppm).

[00148] Experimental groups 4 and 5 were comprised of ARTE10 mice that did not receive any gene therapy vectors. Group 4 then periodically received a Dox-medicated diet while group 5 did not.

[00149] Experimental group 6 was constituted by WT animals, which did not receive any gene therapy vectors or Dox-medicated diet.

[00150] Experimental groups 4, 5, and 6 served as negative control and positive control to establish baseline behavior during the behavioral studies. Each group was constituted by an approximately balanced number of males and females, in order to assess sex-specific effects.

[00151] Below are detailed gene therapy and induction protocols for each group:

[00152] **Group 1:** 10 ARTE10 animals (5 males and 5 females), injection in the hippocampus (stereotaxic injection – S.I.), **YBT vector 1** – (FUG/B2 pseudotyped Syn-OSKM), Doxycycline diet –3 days on/ 4 days off regime 12 weeks.

[00153] **Group 2:** 9 ARTE10 animals (5 males and 4 females), injection in the hippocampus (stereotaxic injection – S.I.), **YBT vector 2** – (VSV-G pseudotyped GFAP-OSKM + VSV-G pseudotyped Syn-OSKM), Doxycycline diet –3 days on/ 4 days off regime 12 weeks.

[00154] **Group 3:** 10 ARTE10 animals (5 males and 5 females), injection in the hippocampus and hypothalamus (stereotaxic injection – S.I.), **YBT vector 3** – (VSV-G pseudotyped Syn-OSKM), Doxycycline diet –3 days on/ 4 days off regime 12 weeks.

[00155] **Group 4:** 14 ARTE10 animals (7 males and 7 females), No injection, Doxycycline diet –3 days on/ 4 days off regime 12 weeks.

[00156] **Group 5:** 7 ARTE10 animals (4 males and 3 females), No injection, No diet.

[00157] **Group 6:** 12 WT animals (6 males and 6 females), No injection, No diet.

[00158] FuG/B2 and canonical VSV-G lentiviral pseudotyping were used as a means to provide the lentiviral construct with preferential tropism for neurons and astrocytes, as well as take advantage of retrograde transport for wider distribution.

[00159] Timeline: Gene therapy administration via stereotactic injection was done in groups 1-3 when mice were 7-8 months of age. Pulsed (periodically given) doxycycline administration was started 2-4 weeks after lentiviral administration and lasted for 12 weeks of repeated 3 days on, 4 days off cycles. Post-treatment behavioral assessments were started within 1 week after the completion of treatment. Histopathological assessment was performed upon mouse sacrifice after the conclusion of behavioral assessments.

[00160] Behavioral Assessment: Animal wellbeing was monitored daily through direct observation by a trained researcher. Weekly weightings were performed, in order to detect abnormal weight loss due to acute toxicity. A number of behavioral tests were performed in all experimental groups, in order to assess symptoms of anxiety, cognitive

or memory dysfunction, or motor deficits. These included Elevated Plus Maze and 3 Chamber Test.

[00161] Histopathological Assessment: Following animal sacrifice and perfusion with phosphate-buffered saline (PBS), the brain was collected, which was divided into two hemispheres. One was post-fixed in paraformaldehyde (PFA) and was used for immunohistochemistry, using antibodies against the 4 Yamanaka factors, while the second half was separated into different brain regions, which were used to determine gene expression by qRT-PCR.

[00162] Immunohistochemistry against microglia and astrocyte markers (ionized calcium-binding adapter molecule 1 (Iba-1) and GFAP, respectively) are also performed, to identify any signs of neuroinflammation. In addition, plasma samples are collected to determine the levels of inflammatory mediators in the periphery using ELISA.

[00163] Statistics: Statistical analyses were performed using GraphPad Prism and Microsoft Excel with the Data Analysis ToolPak.

[00164] Results:

[00165] Positive results were observed in treated mice in cognitive tests and postmortem histopathological assessments.

[00166] In the Elevated Plus Maze test, treated ARTE10 males in Groups 1 and 3 demonstrated better performance than untreated ARTE10 control males in Groups 4 and 5 on the time in closed arms metric and the time in open arms metric, while demonstrating comparable performance to control makes on the latency to the open arm metric (FIG. 2B). Treated ARTE10 males in Group 2 demonstrated better performance than untreated ARTE10 control males in Groups 4 and 5 on the time in closed arms metric; same Group 2 males also demonstrated better performance than Group 4 males on the time in open arms metric, and comparable performance on the latency to the open arm metric (FIG. 2B).

[00167] Treated ARTE10 females in Group 2 demonstrated better performance than untreated ARTE10 control females in Group 4 on all measured metrics, as well as better performance on the latency to the open arms metric than untreated female ARTE10 control females in Group 5, and comparable performance on the other two

metrics (FIG. 2A). Treated ARTE10 females in Groups 1 and 3 demonstrated better performance than untreated ARTE10 control females in Group 4 on the time in open arms and latency to the open arm metrics (FIG. 2A).

[00168] In the Three Chamber test, treated ARTE10 males in Groups 1 and 2 demonstrated better performance than untreated ARTE10 control males in Groups 4 and 5 in the Social Preference test (FIG. 3A). In the Social Novelty test, treated ARTE10 males in Groups 1, 2 and 3 demonstrated better performance than untreated ARTE10 control males in Group 4 (FIG. 3A).

[00169] Treated ARTE10 females in Groups 1, 2 and 3 demonstrated better performance than untreated ARTE10 control females in Group 4 in the Social Preference test, while females in Groups 1 and 2 demonstrated comparable or better performance than untreated ARTE10 control females in Group 5 (FIG. 3B). In the Social Novelty test, treated ARTE10 females in Group 2 demonstrated better performance than untreated ARTE10 control females in Group 4 (FIG. 3B).

[00170] Soluble Amyloid beta levels: In the postmortem analysis of the soluble fraction of the 1-42 amyloid beta peptide, treated ARTE10 males in Groups 1, 2 and 3 demonstrated better results than untreated ARTE10 control males in Groups 4 and 5 (FIG. 4). Treated ARTE10 females in Groups 1, 2 and 3 demonstrated better results than untreated ARTE10 control females in Group 4, and treated ARTE10 females in Groups 2 and 3 demonstrated better results than untreated ARTE10 control females in Group 5 (FIG. 4).

[00171] To further validate the behavioral results, beta-amyloid plaque density was assessed in the hippocampus of the same mice that underwent the behavioral assessments, above. Hippocampal sections were immunohistochemically stained for beta-amyloid, and plaque density was quantified using ImageJ software with an in-house script that applied thresholding to distinguish plaques from the background. Plaque density was expressed as the area of plaques relative to the total area of each slice. In both female (FIG. 5, left panel) and male mice (FIG. 5, middle panel), reprogramming treatment reduced plaque density compared to the control ARTE10 mice. As demonstrated in FIG.5, right panel, this reduction in plaque density was statistically significant ($p < 0.01$) for Group 2 and Group 3 male and female mice when

combined, as compared to the Group 4 ARTE10 controls. Given that beta-amyloid plaque density is indicative of AD severity, these data further demonstrate the effectiveness of reprogramming treatment for treating AD.

Example 2. Brain cell-specific expression of reprogramming in mouse model of HGPS

[00172] Methods

[00173] The design of the lentiviral therapies utilized in this example involves the same integrase-deficient lentivirus carrying two elements (FIG. 1) as in Example 1. The first is a tetracycline-inducible OSKM (Oct4, Sox2, Klf4, and c-Myc) construct, separated by self-cleaving peptides (P2A, T2A or E2A) and placed under the control of a TRE3G promoter. The second is a TetOn3G reverse Tetracycline TransActivator (rTTA3), driven by either SYN (for neuron-specific therapies) or GFAP (for astrocyte-specific therapies) promoters which ensures cell-type specificity: the construct is designed to only be inducible in the respective cell types (neurons or astrocytes) and silent in all other cell types.

[00174] This therapeutic construct - specifically, the VSV-G pseudotyped SYN-OSKM vector - was administered via stereotactic injections into the hypothalamus of C57BL/6-Tg(LMNA*G608G)HClns/J mice, herein called *Lmna* or *Lmna*^{G609G/G609G} mice, which are a mouse model of HGPS. Stereotactic injections were performed bilaterally using the following parameters: the injection coordinates were AP -2.10, ML +/- 0.3 and DV -5.6 for the hypothalamus. Each site was injected with a volume of 0.5 µl of the virus, which had a titer of 1249 ng/µl. The flow rate was 32 nl per 30s.

[00175] In the treatment protocol described in FIG. 7A, *Lmna* mice, at six weeks of age, were subjected to bilateral stereotactic injections of the therapeutic construct into the hypothalamus. A post-injection interval of two weeks was observed prior to initiating a structured doxycycline administration regimen (FIG. 7B). The first phase, spanning three weeks, involved three consecutive days of doxycycline exposure (denoted as "+Dox") succeeded by a four-day withdrawal period (denoted as "-Dox"). Subsequently, the second phase, also extending for three weeks, instituted a regimen of four consecutive days of doxycycline exposure (+Dox) followed by a three-day

withdrawal period (-Dox). Administration of doxycycline was facilitated via the diet of 4RF25 feed medicated to achieve a concentration of 625 mg/kg of doxycycline.

[00176] Results:

[00177] Expression of delivered genes was validated in murine primary cortical neurons from Lmna mice (FIG. 6).

[00178] The progression of progeria is often marked by pronounced weight loss, a symptom indicative of disease severity. The therapeutic regimen described herein was aimed at mitigating this symptom by enhancing weight retention in Lmna mice. As depicted in FIG. 8A, female Lmna mice subjected to the treatment regimen demonstrated a reduced decline in cumulative body weight percentage over time, especially when compared to control mice. This attenuation of weight loss emphasizes the therapeutic potential of the approach in addressing this key symptom of progeria. FIG. 8A further details the total body weight of female Lmna mice both prior to surgery and at the culmination of the study, highlighting the consistency of this effect. While analogous trends were observed for male Lmna mice (FIG. 8B shows cumulative results for male and female Lmna mice), the differences were not statistically significant.

[00179] In Lmna mice, a notable reduction in white adipose tissue (WAT) is usually observed, mirroring the adipose tissue loss characteristic of human progeria patients, which contributes to metabolic dysregulation, thermogenic challenges, and an acceleration of the disease's progression. As illustrated in FIGs. 9A-9B, untreated Lmna mice exhibited pronounced WAT diminishment. Upon administration of the vector and subsequent doxycycline treatment, this adipose tissue loss was substantially reversed in female mice, indicating not just a mitigation of the pathological aspect but rejuvenation of adipose tissue. This reversal is indicative of the treatment's ability to address broader metabolic complications inherent to progeria.

[00180] The skin, notably affected in progeria, displayed changes in Lmna mice following the treatment. Specifically, the characteristic skin lipodystrophy in Lmna mice, evident through the thinning of the hypodermis layer, showed signs of mitigation. As shown in FIG. 10, female Lmna mice subjected to the treatment regimen exhibited an increased thickness of the hypodermis layer, indicating a reversal of skin lipodystrophy.

[00181] Moreover, treatment increased the aortic media thickness in female mice (FIG. 11). This result is particularly significant in the context of progeria, where cardiovascular complications, driven in part by the thinning of the aortic media, are prevalent. By thickening the aortic media, the treatment addresses a key pathological feature of the disease and thus offers a method of mitigating cardiovascular risks associated with progeria.

[00182] While the present disclosure has been described with reference to examples, it is to be understood that the scope of the claims should not be limited by the embodiments set forth in the examples but should be given the broadest interpretation consistent with the description as a whole.

[00183] All publications, patents and patent applications are herein incorporated by reference in their entirety to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated by reference in its entirety. Where a term in the present disclosure is found to be defined differently in a document incorporated herein by reference, the definition provided herein is to serve as the definition for the term.

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Claims:

1. A method of preparing a subject for treating Alzheimer's disease comprising administering
 - a. a first nucleic acid molecule comprising at least 2 reprogramming genes under the control of an inducible promoter; and
 - b. a second nucleic acid molecule comprising a gene encoding an inducer moiety under the control of a brain cell-specific promoter;wherein the inducer moiety induces expression of the at least 2 reprogramming genes under the control of the inducible promoter in the presence of an inducer.
2. The method of claim 1, wherein the at least 2 reprogramming genes comprise at least 2 of Oct4, Sox2, Klf4 and c-Myc.
3. The method of claim 2, comprising at least 3 of the reprogramming genes.
4. The method of claim 3, comprising at least 4 of the reprogramming genes.
5. The method of any one of claims 1 to 4, wherein:
 - a) the inducible promoter is a tetracycline response element (TRE) and the inducer moiety is a reverse tetracycline-controlled transactivator (rtTA);
 - b) the inducible promoter is an ecdysteroid response element (EcRE) and the inducer moiety is an ecdysone receptor-derived transactivator, optionally VpEcR; or
 - c) the inducible promoter is a GAL4 binding site-containing promoter and the inducer moiety is:
 - i) an RSL1-activated two-hybrid transactivator comprised of VP16-RXR and GAL4-EcR chimeric constructs;
 - ii) a Mifepristone-responsive regulator (pGL-VP); or
 - iii) a rapamycin-induced transactivator.
6. The method of any one of claims 1 to 5, wherein the first and second nucleic acid molecules are on the same vector, optionally a lentiviral vector.

7. The method of any one of claims 1 to 5, wherein the first and second nucleic acid molecules are on different vectors, optionally lentiviral vectors.
8. The method of any one of claims 1 to 7, wherein the brain cell-specific promoter is a neuron-specific promoter, optionally a Synapsin 1 (SYN1) promoter, a Ca²⁺/calmodulin-dependent kinase subunit α (CaMKII) promoter, or a neuron-specific enolase (NSE) promoter for neuron-specific expression, or a tyrosine hydroxylase promoter for expression specific to dopamine neurons.
9. The method of any one of claims 1 to 7, wherein the brain cell-specific promoter is an astrocyte-specific promoter, optionally a glial fibrillary acidic protein (GFAP) promoter, a GfaABC1D promoter, or an ALDH1L1 promoter for astrocyte-specific expression.
10. The method of any one of claims 1 to 9, wherein the administration is by stereotactic injection into a brain region.
11. The method of claim 10, wherein the brain region is the hippocampus or hypothalamus.
12. The method of any one of claims 1 to 9, wherein the administration is by intravenous injection and wherein the first and second nucleic acid molecules are contained in an adeno-associated vector(s) that is able to cross the blood-brain barrier.
13. A method of preparing a subject for treating progeria comprising administering
 - a. a first nucleic acid molecule comprising at least 2 reprogramming genes under the control of an inducible promoter; and
 - b. a second nucleic acid molecule comprising a gene encoding an inducer moiety under the control of a brain cell-specific promoter;wherein the inducer moiety induces expression of the at least 2 reprogramming genes under the control of the inducible promoter in the presence of an inducer.
14. The method of claim 13, wherein the at least 2 reprogramming genes comprise at least 2 of Oct4, Sox2, Klf4 and c-Myc.

15. The method of claim 14, comprising at least 3 of the reprogramming genes.
16. The method of claim 15, comprising at least 4 of the reprogramming genes.
17. The method of any one of claims 13 to 16, wherein:
 - a) the inducible promoter is a tetracycline response element (TRE) and the inducer moiety is a reverse tetracycline-controlled transactivator (rtTA);
 - b) the inducible promoter is an ecdysteroid response element (EcRE) and the inducer moiety is an ecdysone receptor-derived transactivator, optionally VpEcR; or
 - c) the inducible promoter is a GAL4 binding site-containing promoter and the inducer moiety is:
 - i) an RSL1-activated two-hybrid transactivator comprised of VP16-RXR and GAL4-EcR chimeric constructs;
 - ii) a Mifepristone-responsive regulator (pGL-VP); or
 - iii) a rapamycin-induced transactivator.
18. The method of any one of claims 13 to 17, wherein the first and second nucleic acid molecules are on the same vector, optionally a lentiviral vector.
19. The method of any one of claims 13 to 17, wherein the first and second nucleic acid molecules are on different vectors, optionally lentiviral vectors.
20. The method of any one of claims 13 to 19, wherein the brain cell-specific promoter is a neuron-specific promoter, optionally a Synapsin 1 (SYN1) promoter, a Ca²⁺/calmodulin-dependent kinase subunit α (CaMKII) promoter, or a neuron-specific enolase (NSE) promoter for neuron-specific expression, or a tyrosine hydroxylase promoter for expression specific to dopamine neurons.
21. The method of any one of claims 13 to 19, wherein the brain cell-specific promoter is an astrocyte-specific promoter, optionally a glial fibrillary acidic protein (GFAP) promoter, a GfaABC1D promoter, or a ALDH1L1 promoter for astrocyte-specific expression.

22. The method of any one of claims 13 to 21, wherein the administration is by stereotactic injection into a brain region.
23. The method of claim 22, wherein the brain region is the hypothalamus.
24. The method of any one of claims 13 to 21, wherein the administration is by intravenous injection and wherein the first and second nucleic acid molecules are contained in an adeno-associated vector(s) that is able to cross the blood-brain barrier.
25. A method of treating Alzheimer's disease in a subject in need thereof comprising:
- i) preparing the subject for treating the Alzheimer's disease according to the method of any one of claims 1 to 12; and
 - ii) administering the inducer to the subject.
26. A method of treating progeria in a subject in need thereof comprising:
- i) preparing the subject for treating the progeria according to the method of any one of claims 13 to 24; and
 - ii) administering the inducer to the subject.
27. The method of claim 25 or 26, wherein the administering ii) begins at least 2 weeks following the preparing the subject i).
28. The method of any one of claims 25 to 27, wherein the administering ii) comprises a cycle of 1 to 7 consecutive days of inducer administration followed by at least 7 days without inducer administration.
29. The method of claim 28, wherein the cycle is repeated on a bimonthly or monthly basis.
30. The method of claim 28 or 29, wherein the cycle is repeated indefinitely.
31. The method of any one of claims 25 to 30, wherein the inducer is administered orally.
32. The method of any one of claims 1 to 31, wherein the subject is a human.

33. A vector comprising:
- a. a first nucleic acid molecule comprising at least 2 reprogramming genes under the control of an inducible promoter; and
 - b. a second nucleic acid molecule comprising a gene encoding an inducer moiety under the control of a brain cell-specific promoter;
- wherein the inducer moiety induces expression of the at least 2 reprogramming genes under the control of the inducible promoter in the presence of an inducer.
34. The vector of claim 33, wherein the vector is a lentiviral vector.
35. The vector of claim 33, wherein the vector is an adeno-associated vector that is able to cross the blood-brain barrier.
36. A composition comprising:
- a. a first vector comprising a first nucleic acid molecule comprising at least 2 reprogramming genes under the control of an inducible promoter; and
 - b. a second vector comprising a second nucleic acid molecule comprising a gene encoding an inducer moiety under the control of a brain cell-specific promoter;
- wherein the inducer moiety induces expression of the at least 2 reprogramming genes under the control of the inducible promoter in the presence of an inducer.
37. The composition of claim 36, wherein the first vector and/or the second vector is a lentiviral vector.
38. The composition of claim 36, wherein the first vector and/or the second vector is an adeno-associated vector that is able to cross the blood-brain barrier.
39. The vector of any one of claims 33 to 35 or the composition of any one of claims 36 to 38, wherein the at least 2 reprogramming genes comprise at least 2 of Oct4, Sox2, Klf4 and c-Myc.
40. The vector or the composition of claim 39, comprising at least 3 of the reprogramming genes.

41. The vector or the composition of claim 40, comprising at least 4 of the reprogramming genes.
42. The vector of any one of claims 33-35 and 39-41 or the composition of any one of claims 36 to 41, wherein:
- a) the inducible promoter is a tetracycline response element (TRE) and the inducer moiety is a reverse tetracycline-controlled transactivator (rtTA);
 - b) the inducible promoter is an ecdysteroid response element (EcRE) and the inducer moiety is an ecdysone receptor-derived transactivator, optionally VpEcR; or
 - c) the inducible promoter is a GAL4 binding site-containing promoter and the inducer moiety is:
 - i) an RSL1-activated two-hybrid transactivator comprised of VP16-RXR and GAL4-EcR chimeric constructs;
 - ii) a Mifepristone-responsive regulator (pGL-VP); or
 - iii) a rapamycin-induced transactivator.
43. The vector of any one of claims 33-35 and 39-42 or the composition of any one of claims 36 to 42, wherein the brain cell-specific promoter is a neuron-specific promoter, optionally a Synapsin 1 (SYN1) promoter, a Ca²⁺/calmodulin-dependent kinase subunit α (CaMKII) promoter, or a neuron-specific enolase (NSE) promoter for neuron-specific expression, or a tyrosine hydroxylase promoter for expression specific to dopamine neurons.
44. The vector of any one of claims 33-35 and 39-42 or the composition of any one of claims 36 to 42, wherein the brain cell-specific promoter is an astrocyte-specific promoter, optionally a glial fibrillary acidic protein (GFAP) promoter, a GfaABC1D promoter, or a ALDH1L1 promoter for astrocyte-specific expression.
45. A pharmaceutical composition comprising the vector of any one of claims 33-35 and 39-44 or the composition of any one of claims 36-44 and a pharmaceutically acceptable carrier or buffer.

46. A kit comprising the vector of any one of claims 33-35 and 39-44, the composition of any one of claims 36 to 44, or the pharmaceutical composition of claim 45 and the inducer.

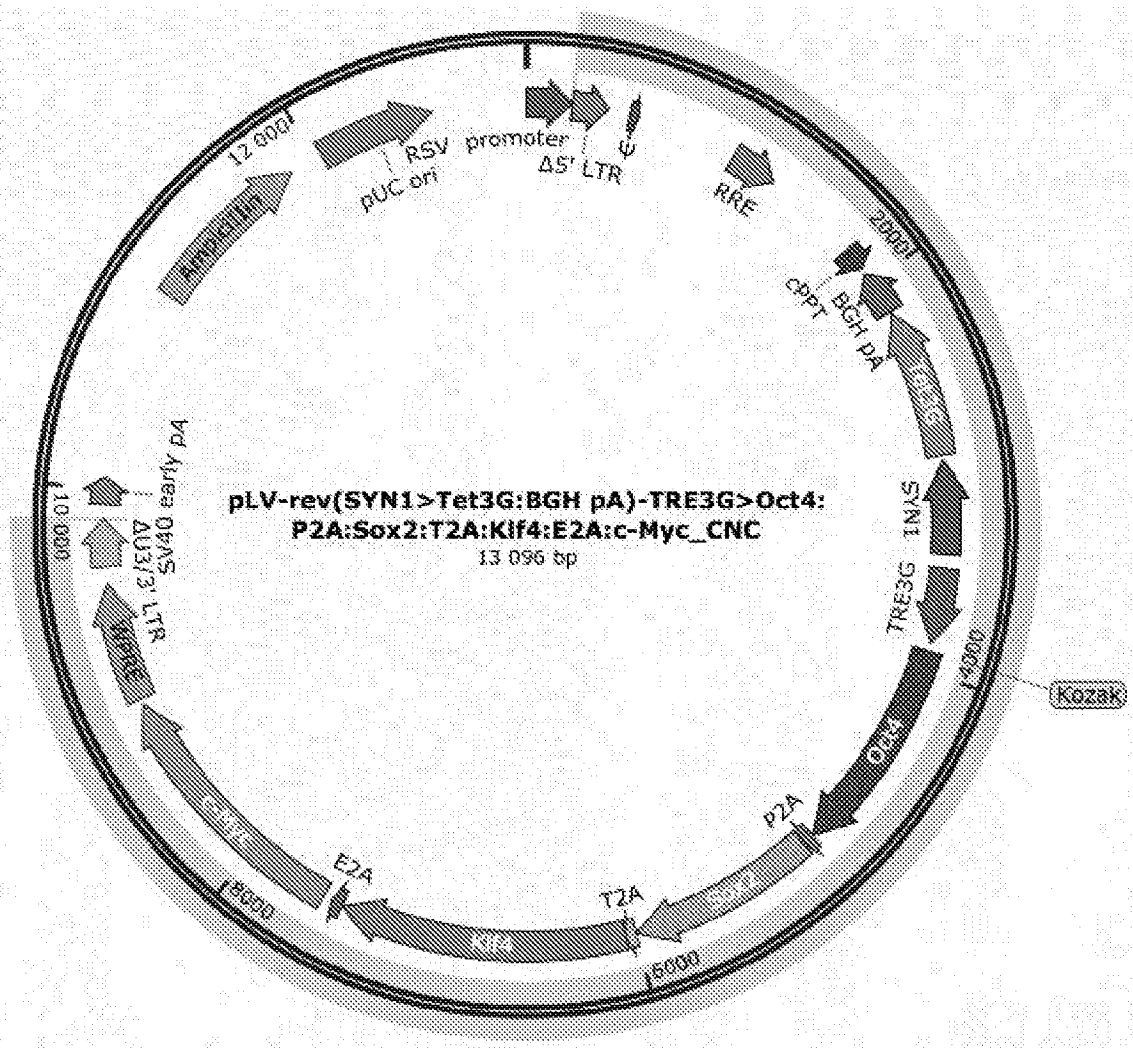
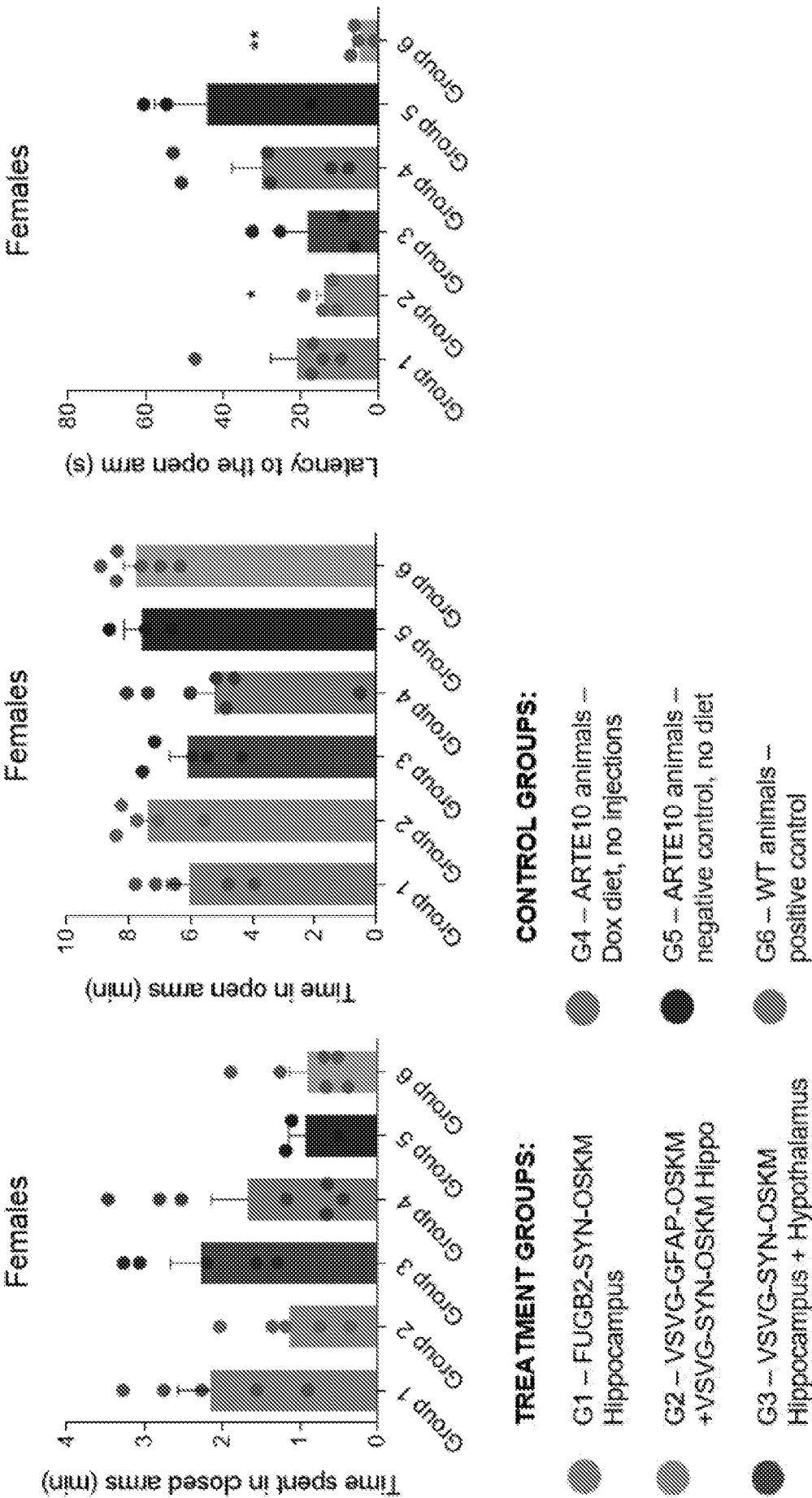
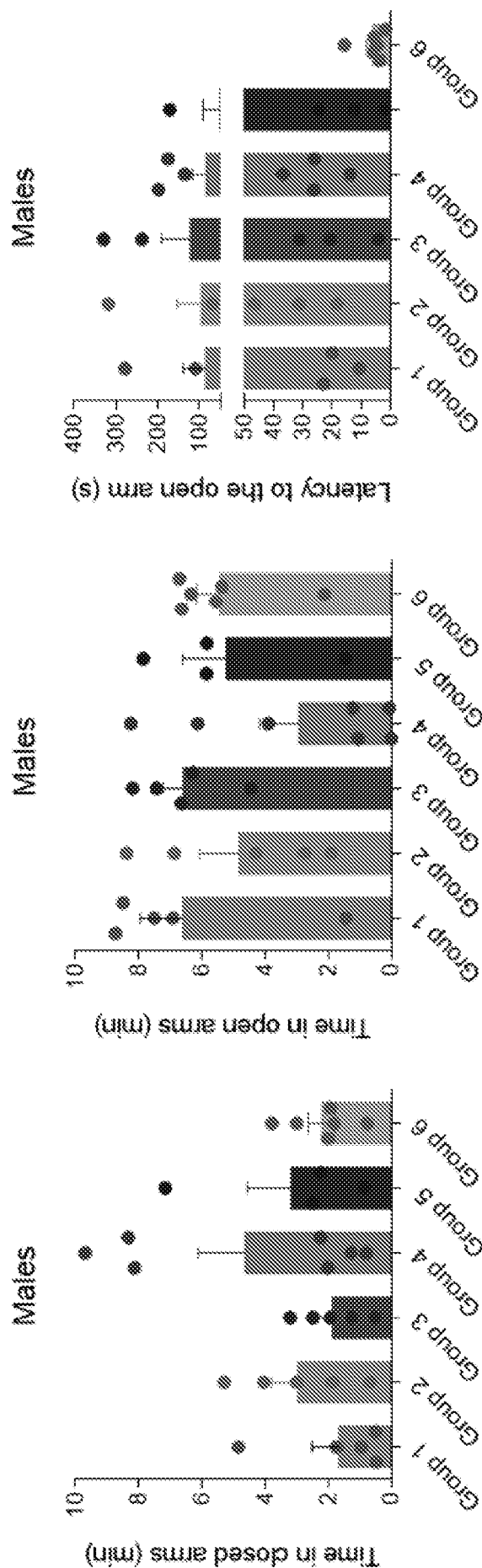


FIG. 1





CONTROL GROUPS:

- G4 - ARTE10 animals - Dox diet, no injections
- G5 - ARTE10 animals - negative control, no diet
- G6 - WT animals - positive control

TREATMENT GROUPS:

- G1 - FUGB2-SYN-OSKM Hippocampus
- G2 - VSVG-GFAP-OSKM +VSVG-SYN-OSKM Hippo
- G3 - VSVG-SYN-OSKM Hippocampus + Hypothalamus

FIG. 2B

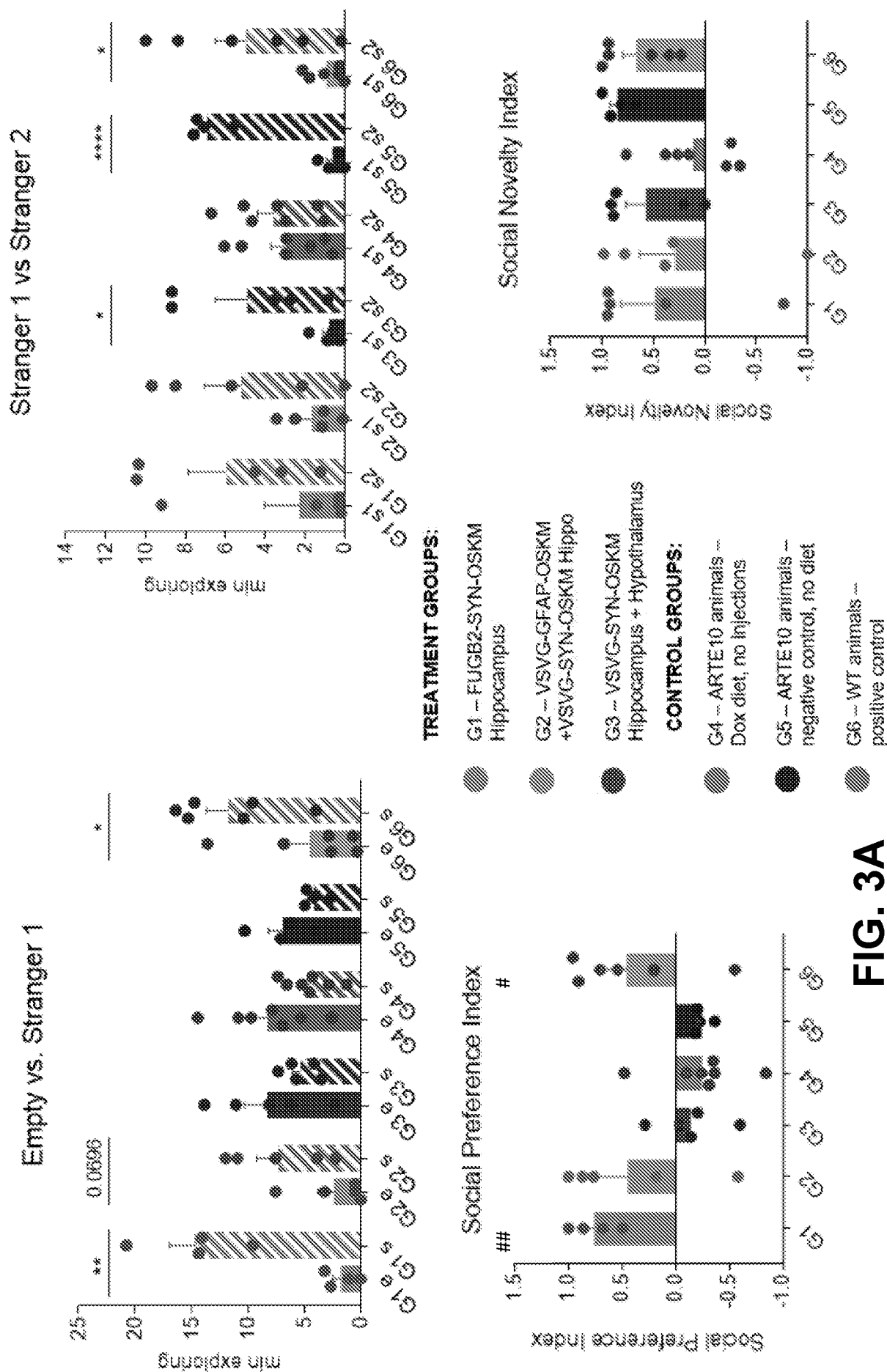


FIG. 3A

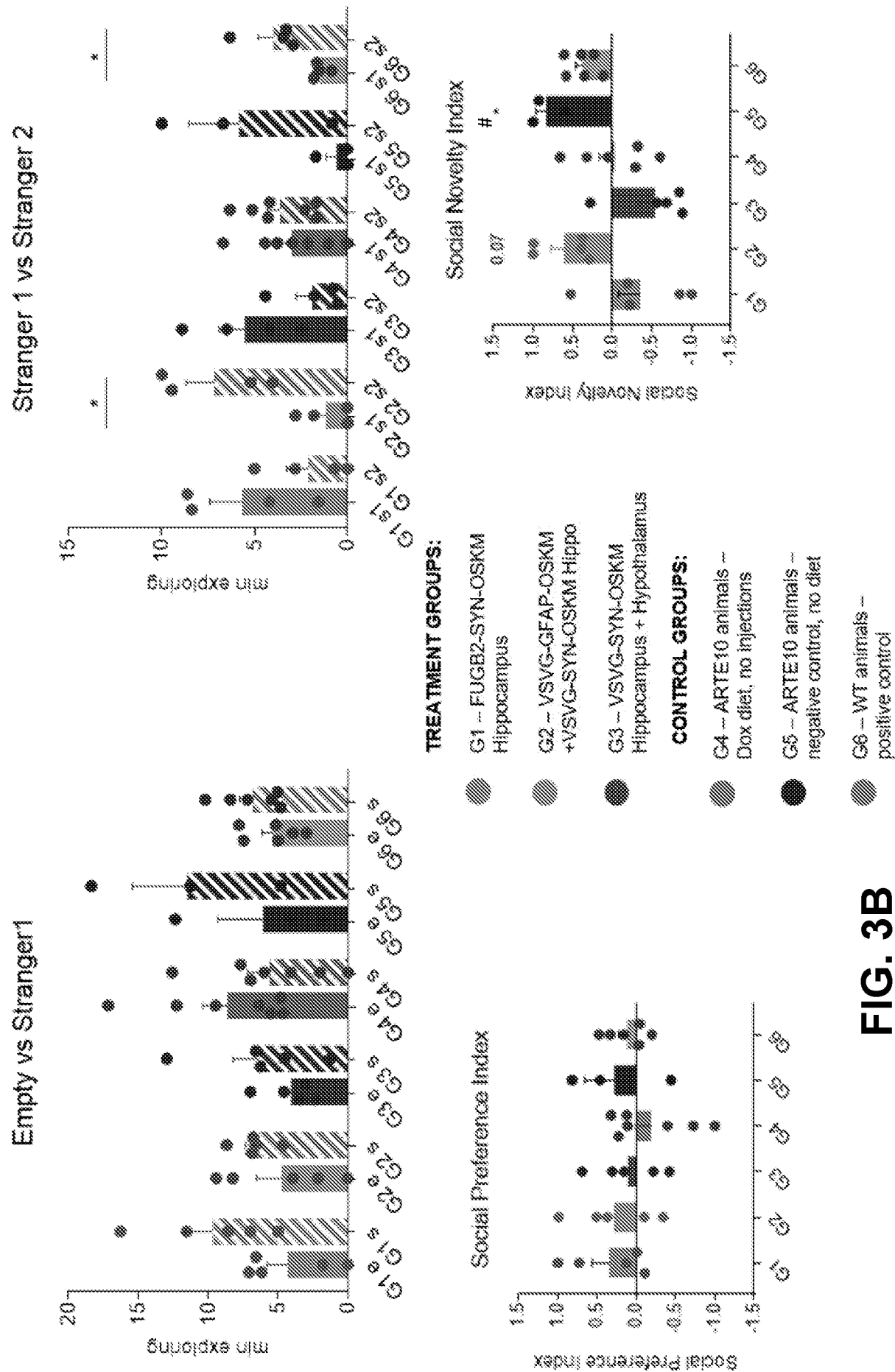


FIG. 3B

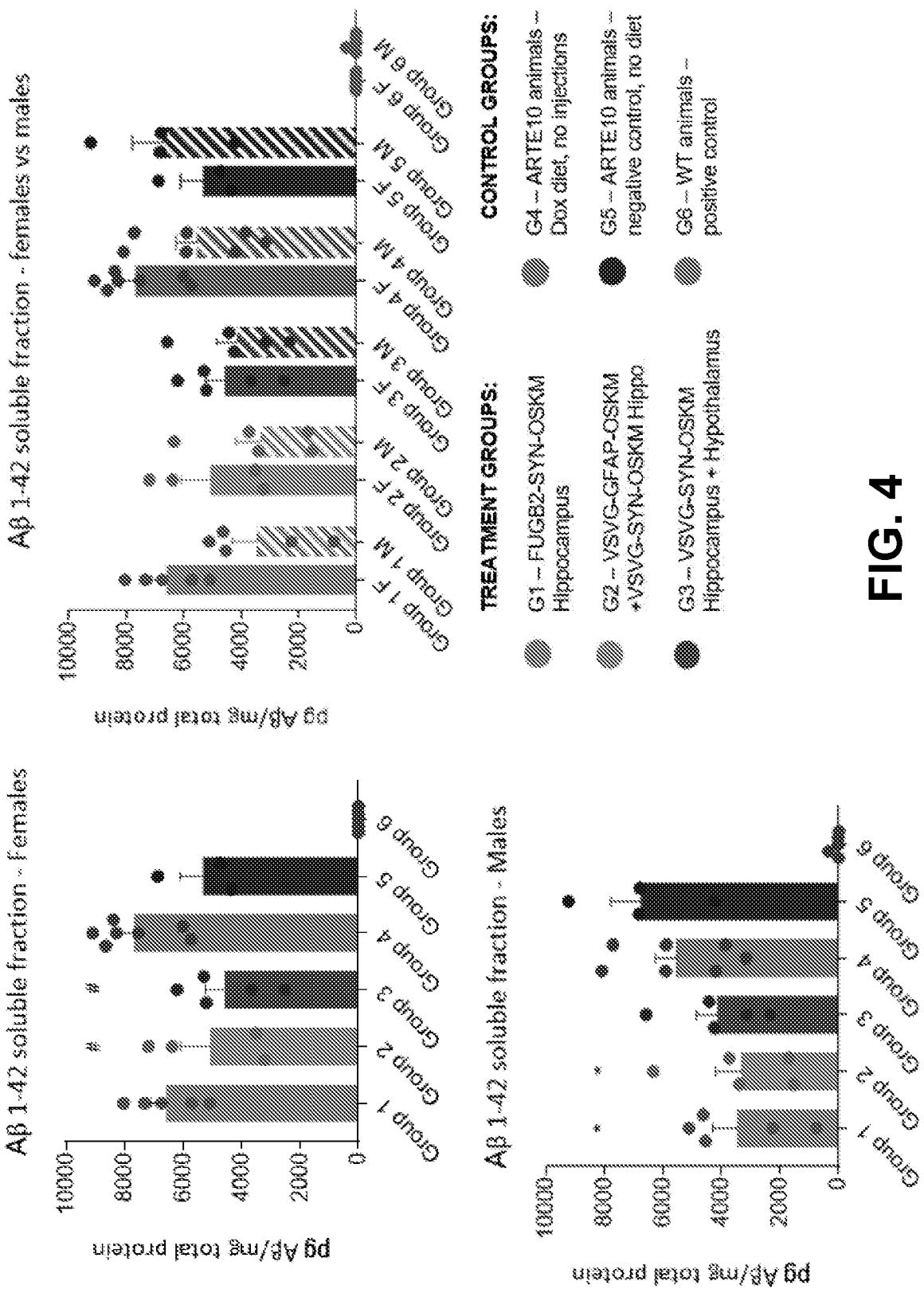


FIG. 4

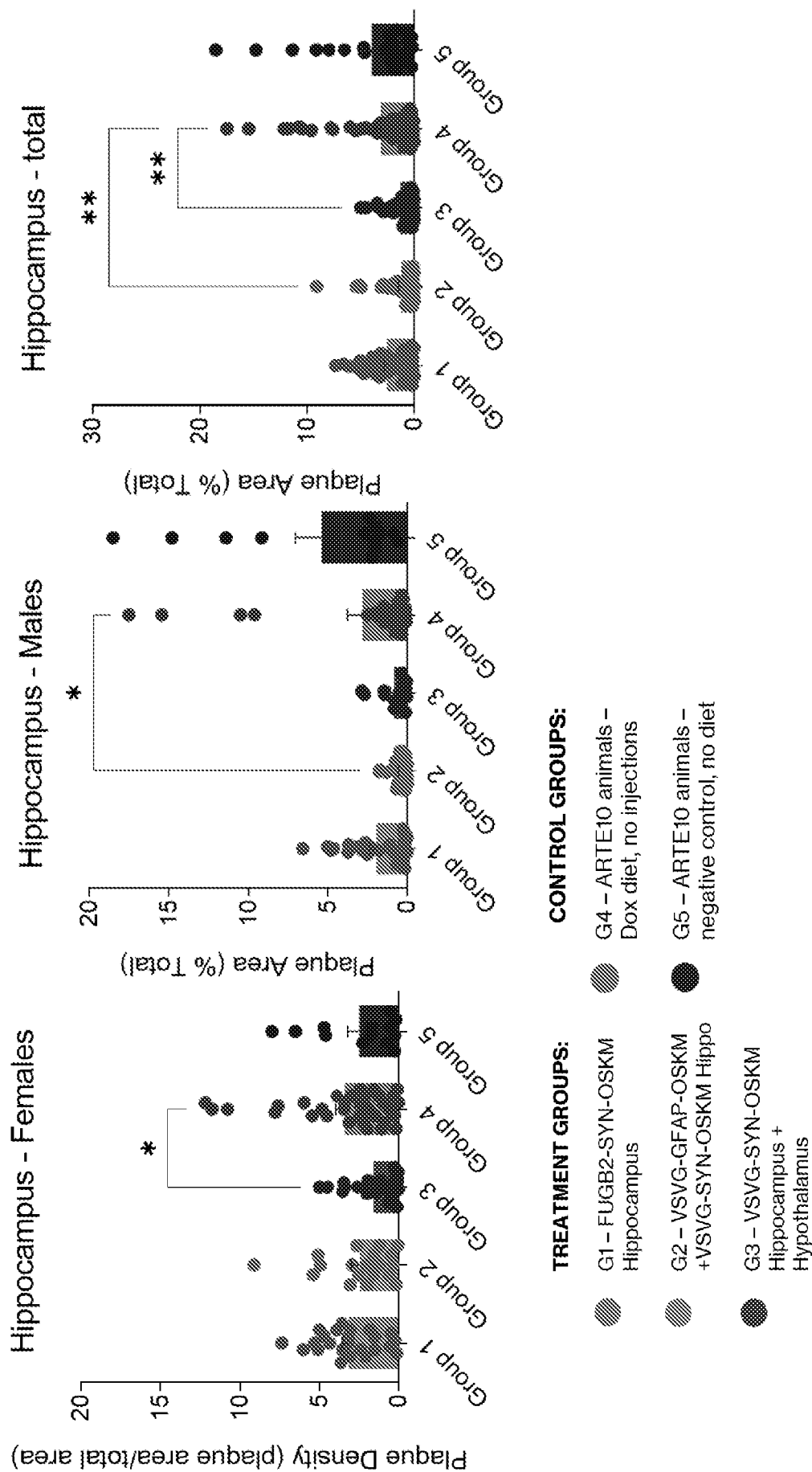


FIG. 5

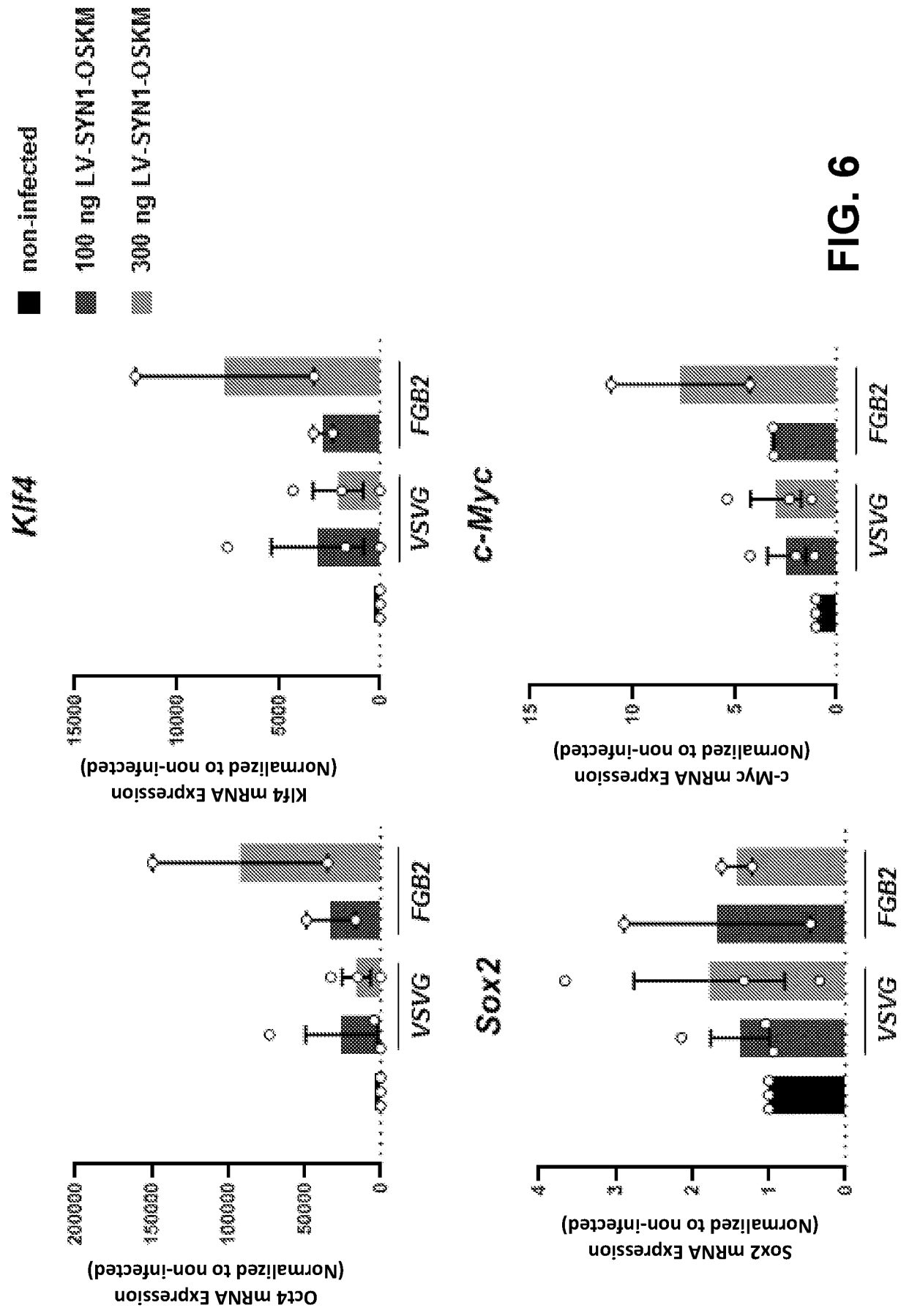


FIG. 6

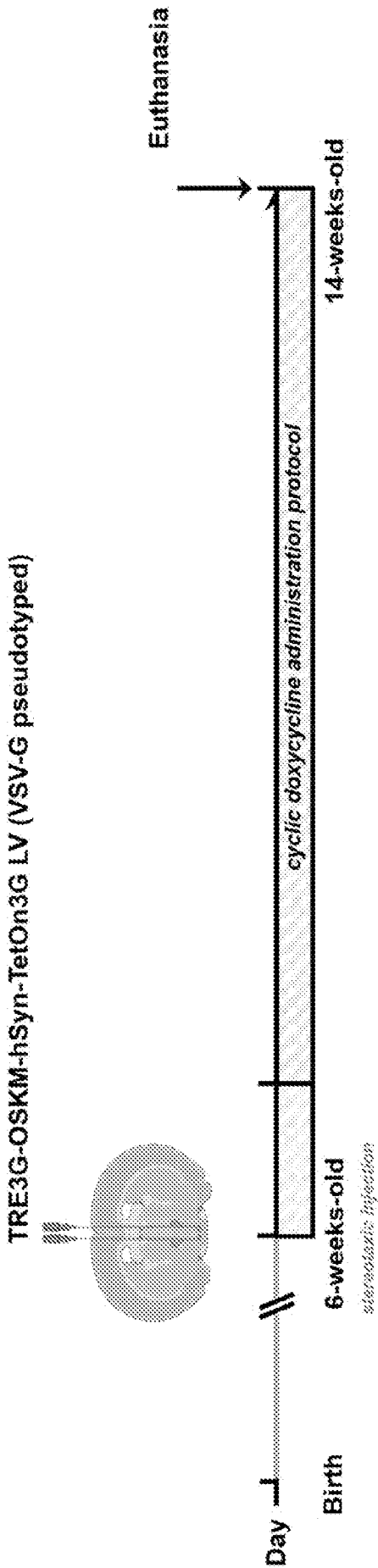


FIG. 7A

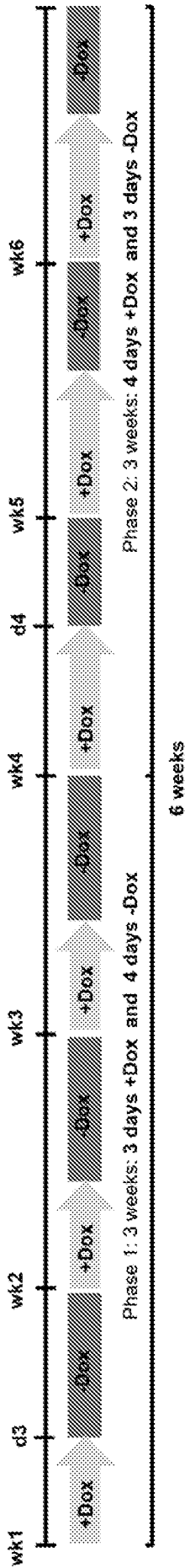


FIG. 7B

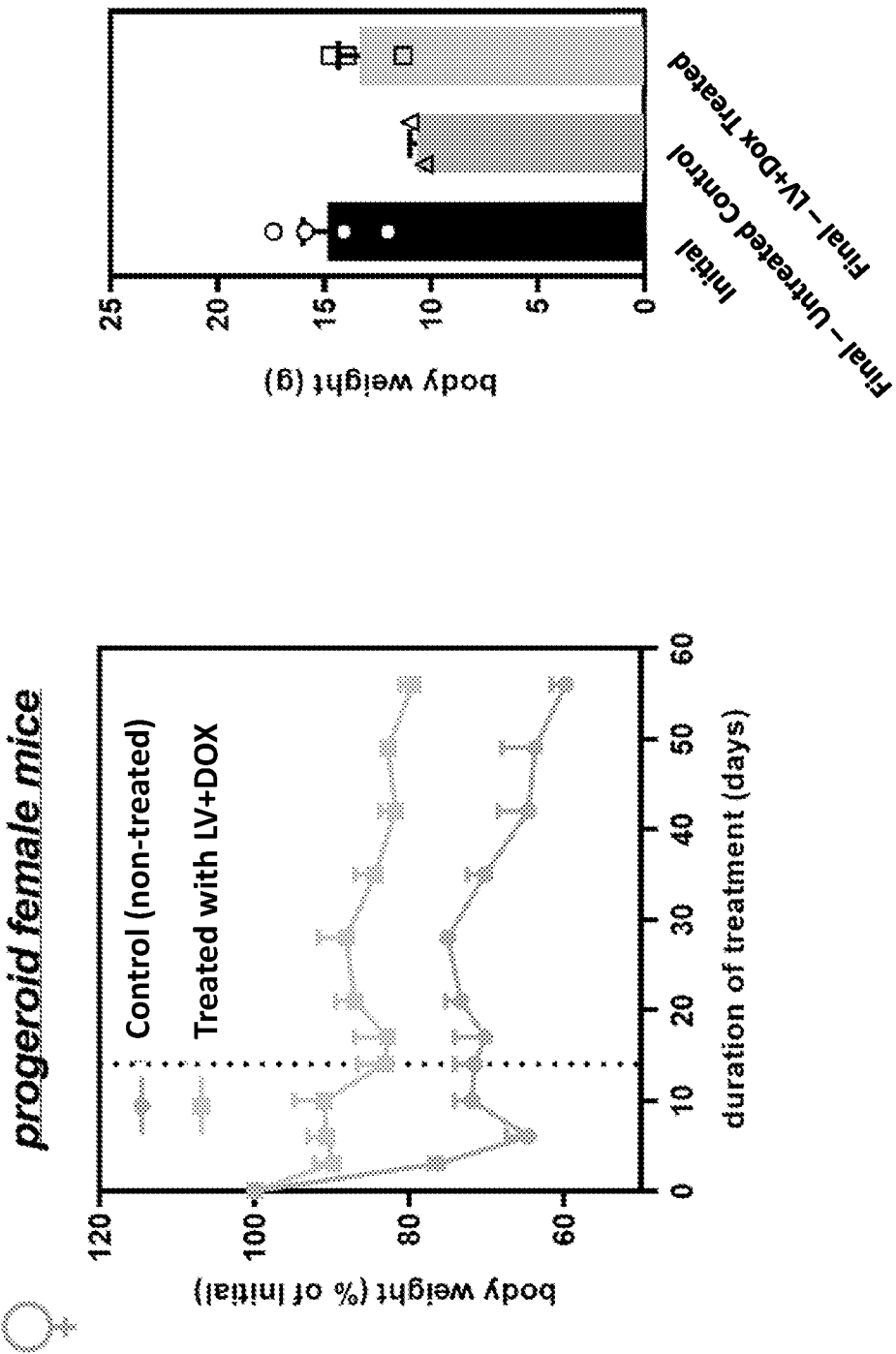


FIG. 8A

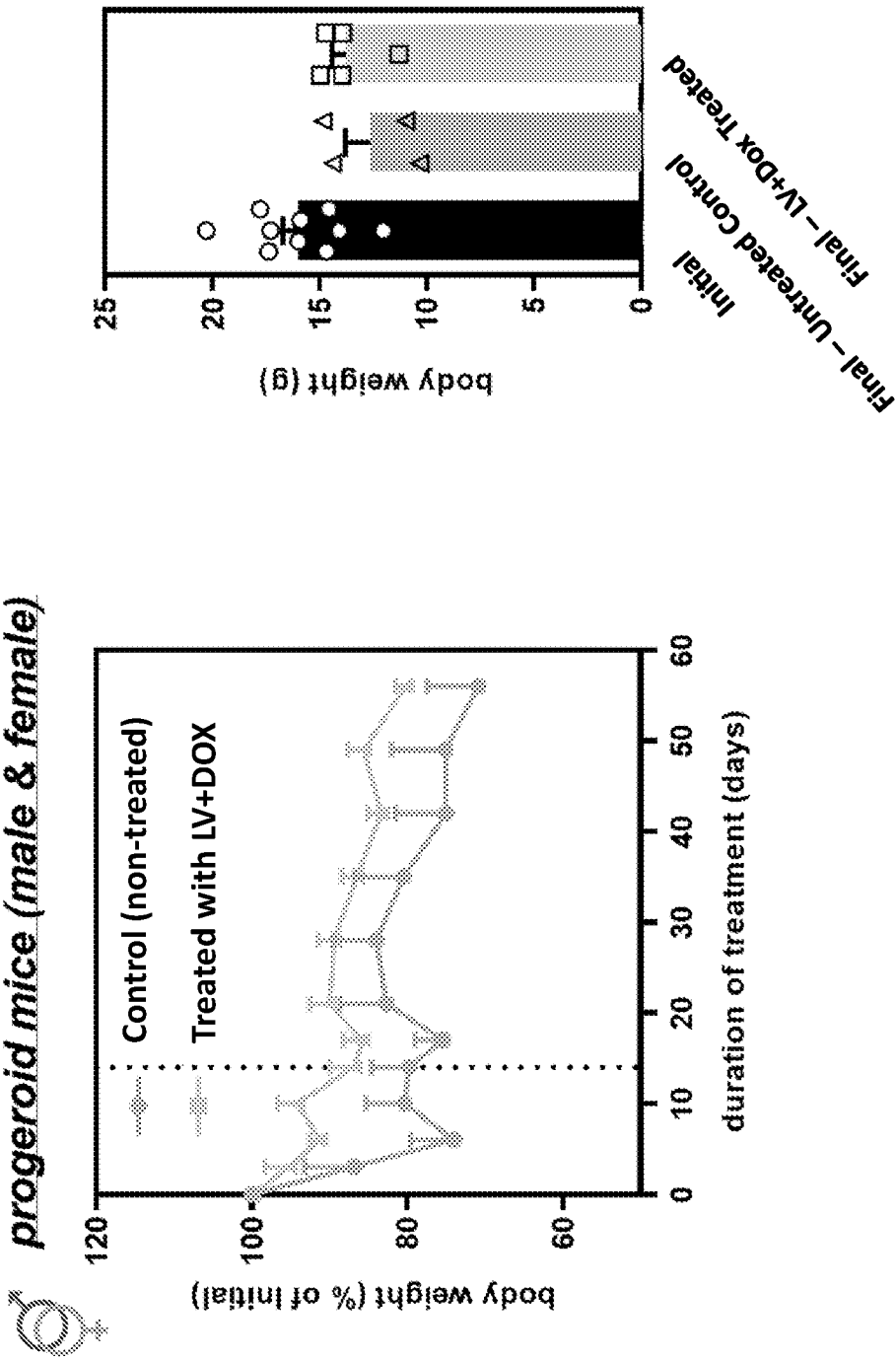


FIG. 8B

White Adipose Tissue (3 months)



Wild-type

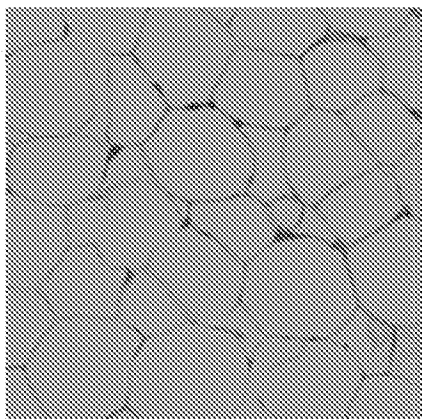
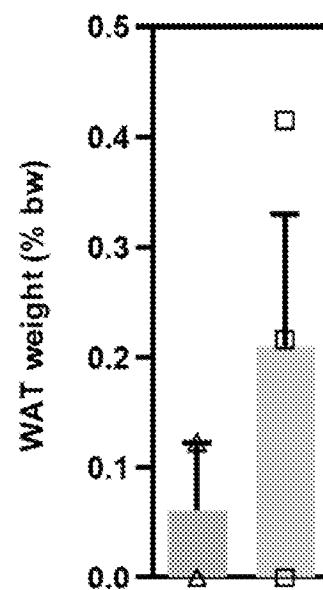
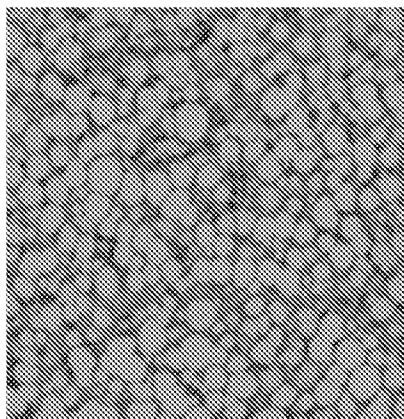
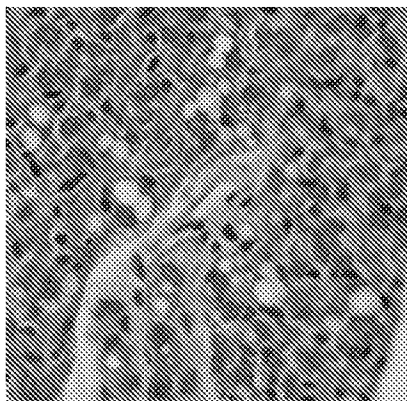
Progeria mice
(*Lmna*^{G609G/G609G})

FIG. 9A

White Adipose Tissue (4 months)



Control (non-treated)



Treated with LV+DOX

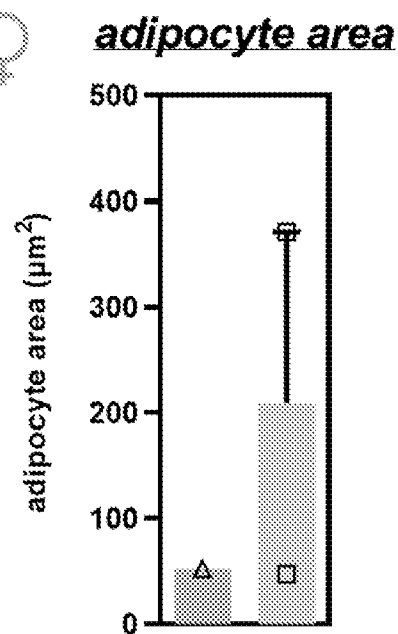
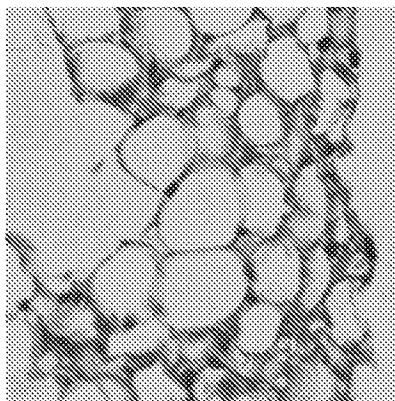


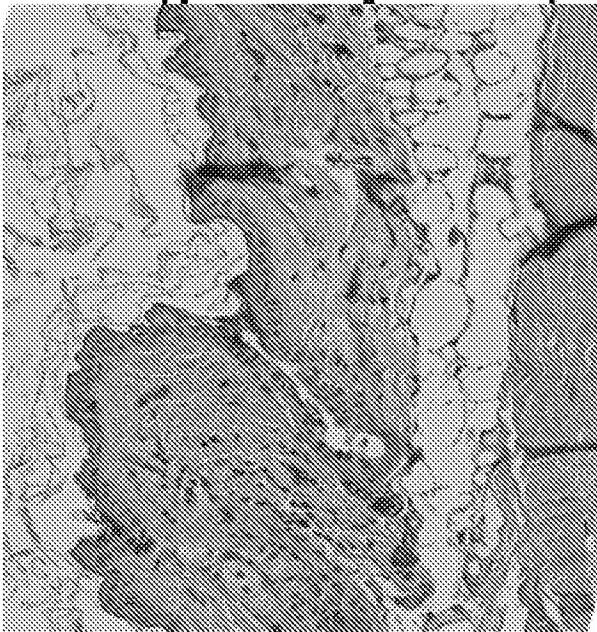
FIG. 9B

Dorsal skin

Control (non-treated)



Treated with LV+DOX



epidermis

dermis

Hypodermis
(subcutaneous layer)

FIG. 10

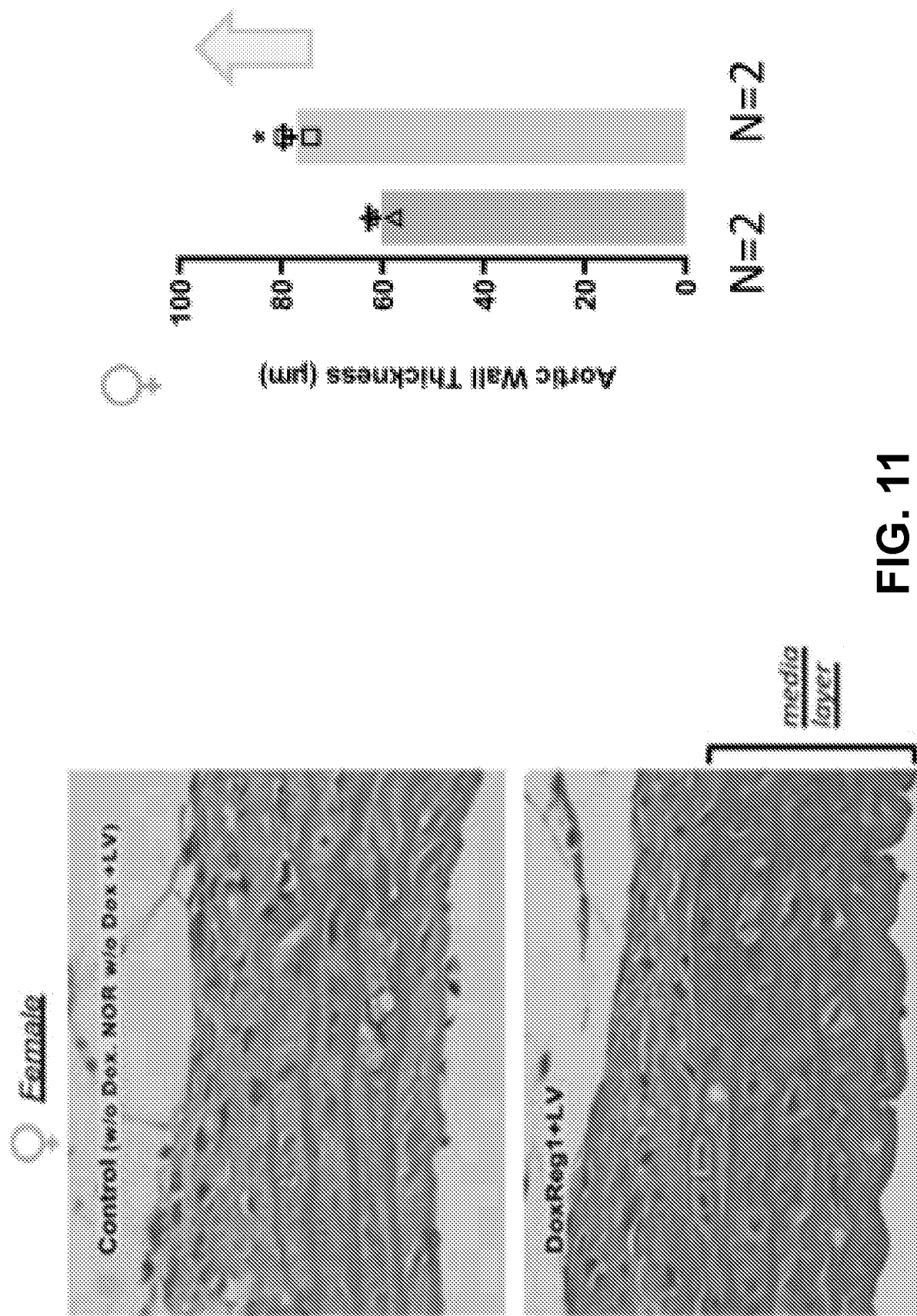


FIG. 11