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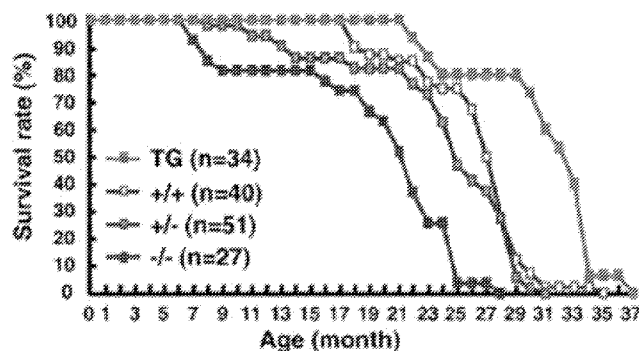


FIG. 1A

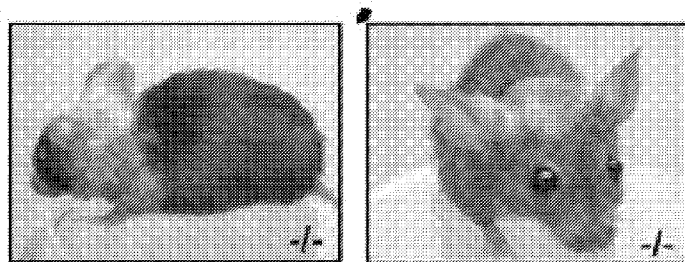


FIG. 1B

(57) Abstract: The disclosure provides viral vector delivery systems for use in treating diseases or disorders in a subject to whom the viral vector delivery systems are administered, as well as to methods of making and using the viral vector delivery systems.



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HIGH EFFICIENCY GENE DELIVERY SYSTEM

RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Application No. 63/016,968, filed on April 28, 2020. The entire teachings of the above applications are incorporated herein by reference.

5

BACKGROUND OF THE INVENTION

The field of aging has made great advances in the past few decades. Many pathways and genes whose modulation increases healthspan and longevity, and the first therapeutics targeting aging, are starting to emerge. However, most discoveries from aging studies cannot be translated to the clinic due to lack of appropriate small-molecule drugs, even for severe early-aging diseases. Furthermore, research into the genetics of aging using mice and other mammals has remained slow and expensive because it requires generation, breeding and aging of large cohorts of transgenic animals.

15 The lack of translatability and the time and cost of research are the main problems that are seen in the field of aging today. These problems cannot be solved using conventional methods. However, they could potentially be solved through the use of advanced gene therapy to directly perturb genes in aged animals and to deliver geroprotective genes into patients. However, this has not been achieved due to
20 current limitations in gene delivery technologies.

SUMMARY OF THE INVENTION

Described herein is a high-efficiency adeno-associated virus (AAV)-based body-wide gene therapy method to express or overexpress genes (e.g., geroprotective genes). The system is an AAV expression system for systemic expression (e.g.,
25

uniform systemic expression), e.g., a single or multi AAV expression system for uniform, systemic expression (DAEUS). It is shown herein that DAEUS can achieve overexpression of several geroprotective genes in aged wild-type mice. It is further shown herein that DAEUS can fully rescue *Cisd2* expression in Wolfram Syndrome II mice, as well as retard and reverse major progeroid morbidities in these mice.

DAEUS is a gene therapy platform that, among other uses, enables acceleration of studies into the basic biology of aging, the treatment of progerias, and the overexpression of geroprotective genes to extend healthspan and/or lifespan.

Disclosed herein is a viral vector delivery system. The viral vector delivery system comprises two or more viral serotypes engineered for delivery of a single gene (i.e., the same gene is delivered by each of the two or more viral serotypes). In some embodiments, the viral vector delivery system comprises an unlimited number of viral serotypes for delivery of the single gene. For example, the viral vector delivery system may comprise at least 5, 10, 25, 50, 75, or 100 viral serotypes, or may comprise 2 to 20 or 5 to 10 viral serotypes.

In some embodiments, the viral serotypes are adeno-associated viral serotypes (e.g., AAV1, AAV2, AAV3, AAV3B, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, Anc80, AAVrh10, AAV-DJ, AAV-DJ/8, AAV-PHP.B, AAV-PHP.S, AAV-PHP.eB, AAV.CAP-B10, AAV.CAP-B22, and AAVMYO, etc.). In some embodiments each of the two or more viral serotypes is trophic for a different cell or tissue type (i.e., a first viral serotype is trophic for a first cell or tissue type, and a second viral serotype is trophic for a second cell or tissue type). In some embodiments, at least one viral serotype is AAV9. In some embodiments, at least one viral serotype is PHP.eB. In certain embodiments, a first viral serotype is AAV9 and a second viral serotype is PHP.eB. In some embodiments, a viral serotype is selected from Table 1.

The viral vector delivery system may further comprise a miRNA target site. In some embodiments, the miRNA target site is selected based on a tissue target, e.g., aorta, endothelium, cardiac muscle skeletal muscle, tongue, esophagus, stomach, small intestine, large intestine, diaphragm, eye, optic nerve, inner ear, auditory nerve, brown fat, white fat, central nervous system, peripheral nervous system, kidney, spleen, liver, lung, heart, brain, thymus, ovaries, testes, skin, pancreas, bone marrow

cells, osteoblasts and osteoclasts, blood cells, hematopoietic stem cells, or muscle satellite cells, or more specifically, cardiac, liver, muscle, or brain tissue. In some embodiments, miRNA target site is selected from the group consisting of miRNA-1, miRNA-24, miRNA-29, miRNA-30c, miRNA-33, miRNA-122, miRNA-124, miRNA-128, miRNA-133, miRNA-144, miRNA-148a, miRNA-208a, miRNA-208b, miRNA-223, and miRNA-499. For example, a target tissue may be cardiac tissue and the miRNA target site may be miRNA-1, miRNA-133, miRNA-208a, miRNA-208b, or miRNA-499. In some embodiments, a target tissue is liver tissue and the miRNA target site is selected from the group consisting of miRNA-24, miRNA-29, miRNA-30c, miRNA-33, miRNA-122, miRNA-144, miRNA-148a, and miRNA-223. In some embodiments, a target tissue is muscle tissue and the miRNA target site is miRNA-1 or miRNA-133. In some embodiments, a target tissue is brain tissue and the miRNA target site is miRNA-124 or miRNA-128.

The viral vector delivery system may further comprise a non-silencing promoter. In some embodiments, the non-silencing promoter leads to RNA expression of at least 30%, or optionally at least 50%, of CMV promoter expression. In some embodiments, the promoter is selected from the group consisting of Cbh, CAG, CB7, and CBA. In certain embodiments, the promoter is Cbh.

In some embodiments, the viral vector delivery system optionally further comprises a self-complementary vector backbone.

In some embodiments, the gene to be delivered is selected from Table 2. In certain embodiments, the gene is selected from the group consisting of Cisd2, Atg5, and PTEN. In some embodiments, the gene is a geroprotective gene. In some embodiments, the gene is a gene associated with a disease or disorder in need of treatment in a subject, e.g., a gene whose expression is absent or reduced in a disease or disorder to be treated.

Also disclosed herein are pharmaceutical compositions comprising the viral vector delivery systems disclosed herein. Also disclosed herein are methods of treating or preventing a disease or disorder in a subject comprising administering the pharmaceutical compositions or viral vector delivery systems disclosed herein.

Disclosed herein are methods of delivering to and expressing in multiple (two or more) cell or tissue types of a subject the same gene relatively simultaneously, as

well as methods of treating or preventing a disease or disorder. The methods comprise administering to a subject a viral vector delivery system comprising at least one viral serotype, at least two viral serotypes, at least three viral serotypes, at least four viral serotypes, or at least five viral serotypes engineered for delivery of a single gene. In some embodiments, the viral vector delivery system comprises an unlimited number of viral serotypes for delivery of the single gene.

In some embodiments, the disease or disorder is an aging related disease or disorder, e.g., progeria syndrome, Wolfram Syndrome, neurodegenerative disorder, neurovascular disorder, skeletal muscle conditions, Cowden syndrome, Bannayan-Riley-Ruvalcaba syndrome, Proteus syndrome, Proteus-like syndrome and other PTEN-opathies. Werner syndrome, Bloom syndrome, Rothmund-Thomson syndrome, Cockayne syndrome, xeroderma pigmentosum, trichothiodystrophy, combined xeroderma pigmentosum-Cockayne syndrome, restrictive dermopathy, diabetes, obesity, cardiovascular disease, cancer, ocular degeneration, liver failure, and age-related macular degeneration. In some embodiments, the disease or disorder would benefit from administration of the gene to two or more tissue targets. In certain embodiments, the disease or disorder is Wolfram Syndrome II.

In some embodiments, the gene is expressed in two or more tissues in the subject. The gene may be uniformly expressed or overexpressed across two or more tissues in the subject. In some embodiments, the gene is delivered to at least 50% of tissues in the subject, and in some embodiments, is expressed for at least 4 months in the subject.

Also disclosed herein is a viral vector delivery system comprising two or more AAV serotypes engineered for delivery of a single gene, a non-silencing promoter, at least one miRNA target site, the gene, and optionally a self-complementary backbone.

In some embodiments, the AAV serotypes are AAV9 and PHP.eB. In some embodiments, the gene is selected from the group consisting of Cisd2, Atg5, and PTEN, and preferably is Cisd2.

Methods of treating a disease or disorder, e.g., Wolfram Syndrome II, are also disclosed herein, comprising administering to a subject the viral vector delivery system disclosed herein.

Also disclosed herein are methods of extending the lifespan of a subject. For example, lifespan may be extended by administering the viral vector delivery system described herein or a pharmaceutical composition comprising the viral vector delivery system described herein (e.g., a viral vector delivery system comprising at least one, at least two, at least three, at least four, or more viral serotypes engineered for delivery of a single gene).

Further described herein are methods of treating Wolfram Syndrome II comprising administering an effective amount of *Cisd2* to a subject suffering from Wolfram Syndrome II.

10 In some embodiments, *Cisd2* is administered to the subject via gene therapy, e.g., via a viral vector delivery system or any other gene therapy known to those of skill in the art. In some embodiments, the viral vector delivery system comprises at least one viral serotype, at least two viral serotypes, at least three viral serotypes, at least four viral serotypes, at least five viral serotypes.

15 Also described herein are methods of identifying a pre-determined level of gene transfer in one or more target tissues of a subject comprising: obtaining a dose-response curve characterizing the relationship between an amount of a vector administered to the subject and a resulting gene transfer level in the one or more target tissues; obtaining a linear or non-linear equation characterizing the relationship
20 between the amount of vector administered to the subject and the resulting gene transfer level in the one or more target tissues; and interpolating or extrapolating a required dose of a gene delivery system to achieve a defined level of gene transfer in the one or more target tissues.

Further described herein are methods of identifying a pre-determined level of
25 transgene expression in one or more target tissues of a subject comprising: obtaining a dose-response curve characterizing the relationship between an amount of a vector administered to the subject and a resulting transgene expression level in the one or more target tissues; obtaining a linear or non-linear equation characterizing the relationship between the amount of vector administered to the subject and the
30 resulting transgene expression level in the one or more target tissues; and interpolating or extrapolating a required dose of a gene delivery system to achieve a defined level of transgene expression in the one or more target tissues.

In some embodiments, the gene delivery system comprises at least one viral serotype, at least two viral serotypes, at least three viral serotypes, at least four viral serotypes, at least five viral serotypes. In some embodiments, the viral serotype is an adeno-associated viral serotype (e.g., AAV1, AAV2, AAV3, AAV3B, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, Anc80, AAVrh10, AAV-DJ, AAV-DJ/8, AAV-PHP.B, AAV-PHP.S, AAV-PHP.eB, AAV.CAP-B10, AAV.CAP-B22, AAVMYO, etc.). In some embodiments, the viral serotype is selected from Table 1. In some embodiments, the one or more target tissues comprise a single tissue or two or more tissues. In some embodiments, the one or more target tissues are selected from the group consisting of aorta, endothelium, cardiac muscle skeletal muscle, tongue, esophagus, stomach, small intestine, large intestine, diaphragm, eye, optic nerve, inner ear, auditory nerve, brown fat, white fat, central nervous system, peripheral nervous system, kidney, spleen, liver, lung, heart, brain, thymus, ovaries, testes, skin, pancreas, bone marrow cells, osteoblasts and osteoclasts, blood cells, hematopoietic stem cells, and muscle satellite cells.

BRIEF DESCRIPTION OF THE DRAWINGS

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

FIGS. 1A-1B demonstrates the results of *Cisd2* deficiency in mice. FIG. 1A shows dose-dependent modulation of lifespan by *Cisd2* in male mice. *Cisd2* deficiency shortens the lifespan and causes premature aging in *Cisd2* KO mice. In contrast, a persistent level of *Cisd2* expression prolongs lifespan and increases the survival rate of *Cisd2* TG mice. See Wu, *et al. Hum. Mol. Genet.* 21, 3956–3968 (2012). FIG. 1B provides images showing the decreased body weight, shortened life span, and the ocular and cutaneous symptoms of aging in *Cisd2*^{-/-} mice. Early depigmentation and gray hair are seen on the top of the head and on the shoulders, and the prominent eyes and protruding ears of the *Cisd2*^{-/-} mice are also shown. See Chen, *et al. Genes Dev.* 23, 1183–1194 (2009).

FIGS. 2A-2D provide an overview of ssAAV9. FIG. 2A provides an ssAAV9 vector overview. FIG. 2B shows ssAAV9 DNA biodistribution at a dose of

~1e12vg/mouse (ssAAV9-Atg5 and ssAAV9-Cisd2 denoted as ssAAV9). FIGS. 2C-2D show lack of global overexpression on the protein level for Atg5 (FIG. 2C) or Cisd2 (FIG. 2D). 8 week old wild-type C57BL6/J mice were injected and euthanized 28 days post-injection. Cisd2 and Atg5 levels were determined via Simple Wes.

5 FIGS. 3A-3E demonstrate poor systemic overexpression of rejuvenation genes Oct4-Sox2-Klf4 using conventional ssAAV9 vectors. (*See* Lu et al 2019). FIG. 3A shows Sox2 expression in the liver of WT mice post-intravenous delivery of OSK-AAV9 and OSK transgenic (TG) mice. FIG. 3B shows body weight of WT mice and AAV-mediated OSK-expressing mice (1.0×10^{12} gene copies total) with or without
10 doxycycline in the following 9 months after 4 weeks of monitoring (n=5,3,6,4 respectively). FIG. 3C shows AAV-UBC-rtTA and AAV-TRE-Luc vectors used for measuring tissue distribution. FIG. 3D shows Luciferase imaging of WT mice at 2 months after retroorbital injections of AAV9-UBC-rtTA and AAV9-TRE-Luc (1.0×10^{12} gene copies total). Doxycycline was delivered in drinking water (1
15 mg/mL) for 7 days to the mouse shown on the right. FIG. 3E shows Luciferase imaging of eye (Ey), brain (Br), pituitary gland (Pi), heart (He), thymus (Th), lung (Lu), liver (Li), kidney (Ki), spleen (Sp), pancreas (Pa), testis (Te), adipose (Ad), muscle (Mu), spinal cord (SC), stomach (St), small intestine (In), and cecum(Ce) 2
20 months after retro-orbital injection of AAV9-UBC-rtTA and AAV9-TRE-Luc followed by treatment with doxycycline for 7 days. The luciferase signal is primarily in liver. Imaging the same tissues with a longer exposure time (FIG. 3E cont.) revealed lower levels of luciferase signal in pancreas (liver was removed).

 FIGS. 4A-4B demonstrate viral DNA and luciferase expression in different tissues using single-stranded backbone and various AAV serotypes. All serotypes
25 show large variability of more than 100-fold in DNA load and expression levels between major tissues (*See* Zincarelli et al 2008). FIG. 4A provides luciferase protein expression profiles of adeno-associated virus (AAV) serotypes 1–9. The levels of luciferase activity [in relative light units (RLU) per mg protein] were determined in selected tissue at 100 days after intravenous injection of 1×10^{11} particles of
30 AAV1–9 into adult mice. The data are presented as mean values $\pm$ SEM. FIG. 4B provides vector genome copy numbers in selected tissues. Luciferase genome copy numbers/ μ g of genomic DNA. Persistence of viral genomes in selected tissues 100

days after tail vein injection of 1×10^{11} particles of adeno-associated virus (AAV) serotypes 1–9. Genomic DNA was isolated from the indicated tissues and 100 ng of each was used in triplicate to determine vector genome copies. Levels of significance were determined using one-way analysis of variance. The data are shown as mean values $\pm$ SEM. * $P < 0.05$ versus AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV8. # $P < 0.05$ versus all. ** $P < 0.05$ versus all.

FIGS. 5A-5C provide an overview of the DAEUS system. FIG. 5A shows the vector delivery system. FIG. 5B shows AAV DNA biodistribution and FIG. 5C shows GFP expression at a dose of 2×10^{12} vg per mouse using AAV9, PHP.eB or AAV9+PHP.eB together. Note the high tissue-to-tissue variability in viral DNA and GFP expression when AAV9 and PHP.eB are used separately. 18-month old male C57BL/6J mice were injected and euthanized 28 days post-injection. Viral DNA and GFP protein levels were measured via qPCR and Simple Wes respectively.

FIG. 6 shows alanine aminotransferase (ALT) levels 7 days post ssAAV9 (left panel) or scAAV9-miR122 injection (right panel). Elevated ALT levels are indicative of liver damage. Left: elevated ALT levels in ssAAV9-Cisd2 injected mice indicated the need for a strategy of lowering expression in the liver to avoid toxicity. Note no elevation of ALT in ssAAV9-Atg5 injected mice, because Atg5 failed to overexpress with this vector. Right: Addition of miR122 target site to the expression vector reduces ALT increase despite the use of more frail aged mice and more potent vector. Left: 8-week old wild-type male C57BL/6J mice were injected retro-orbitally at a dose of 8×10^{11} vg/mouse (Atg5) and 5×10^{11} vg/mouse (Cisd2). Right: 18-month old wild-type male C57BL/6J mice were retro-orbitally injected with 4×10^{11} vg/mouse of scAAV9-Atg5/Cisd2/GFP. Vehicle used was Final Formulation Buffer. ALT serum levels were measured 7 days post-injection by UMass Mouse Metabolic Phenotyping Center.

FIG. 7 shows scAAV9 vs DAEUS overexpression of Cisd2. AAV9 alone is insufficient to achieve systemic overexpression. Left: 8-week old male C57BL/6J mice were retro-orbitally injected with 4×10^{11} vg/mouse of scAAV9-Cisd2. Right: 18-month old mice were retro-orbitally injected with a total of 4×10^{11} or 2×10^{12} vg/mouse of DAEUS-Cisd2. Mice were euthanized 28 days post-injection and Cisd2 levels measured using Simple Wes.

FIG. 8 shows scAAV9 vs DAEUS overexpression of Atg5. AAV9 alone is insufficient to achieve systemic overexpression. Left: 18 month old male C57BL/6J mice were retro-orbitally injected with 2×10^{12} vg/mouse of scAAV9-Atg5. Right: 18-month old mice were retro-orbitally injected with a total of 8×10^{12} vg/mouse of DAEUS-Atg5. Mice were euthanized 28 days post-injection and Atg5 levels measured using Simple Wes.

FIG. 9 demonstrates DAEUS overexpression of PTEN. 18-month old male and female mice (50:50 ratio) were retro-orbitally injected with a total of 4×10^{11} or 2×10^{12} vg/mouse of DAEUS-PTEN. Mice were euthanized 28 days post-injection and PTEN levels measured using Simple Wes.

FIG. 10 provides dose-response curves of AAV dose to AAV gene transfer for the brain, heart, liver, and tibialis anterior. 5-week old male C57BL6-J mice were injected with doses of approximately 5×10^{12} , 2×10^{13} , 5×10^{13} and 2×10^{14} AAV vector genomes per kg, at N=3 mice per group with the following serotypes and their combinations: (1) scAAV9-Cbh-GFP-miR122; (2) scAAV9-Cbh-GFP-miRScr (where miRNA target site is scrambled to remove its function); (3) scPHP.eB-Cbh-GFP-miR122; and (4) scPHP.eB-Cbh-GFP-miR122 together with scAAV9-Cbh-GFP-miRScr.

FIG. 11 provides a regression analysis of expected vs observed gene transfer levels. The gene transfer levels observed in the mice of group (1) and group (3) from FIG. 10 were summed for each tissue individually and compared to the observed gene transfer levels in the mice of group (4) of FIG. 10. If no interaction is present between AAV9 and PHP.eB, the sum of gene transfer from groups 1 and 3 for every tissue (Expected) should closely match gene transfer levels in group 4 for every tissue respectively (Observed). The regression analysis of the expected vs observed gene transfer levels indicated that the expected values matched to and correlated highly with the observed values.

FIG. 12 provides a comparison of predicated and observed gene transfer patterns for the brain, heart, liver, and tibialis anterior (TA). 5 groups of 5-week old male C57BL6-J mice were injected retro-orbitally with N=3 mice per group, with different combinations of AAV9 and PHP.eB: (1) 1.4×10^{14} GC/kg scAAV9-Cbh-GFP-miR122 + 1.9×10^{13} GC/kg scPHP.eB-Cbh-GFP-miR122; (2) 1.9×10^{14} GC/kg scAAV9-

Cbh-GFP-miR122 + 4.8e12 GC/kg scPHP.eB-Cbh-GFP-miR122; (3) 4.8e13 GC/kg scAAV9-Cbh-GFP-miR122 + 1.9e14 GC/kg scPHP.eB-Cbh-GFP-miR122; (4) 2.4e13 GC/kg scAAV9-Cbh-GFP-miR122 + 9.5e12 GC/kg scPHP.eB-Cbh-GFP-miR122; and (5) 4.8e12 GC/kg scAAV9-Cbh-GFP-miR122 + 4.8e13 GC/kg scPHP.eB-Cbh-GFP-miR122. A high match between predicted and observed gene transfer patterns was observed.

FIG. 13 provides a linear regression analysis showing a high correlation of predicted and observed gene transfer levels in the brain, heart, liver, and tibialis anterior (TA) for the different combinations of AAV9 and PHP.eB identified in FIG. 12.

FIG. 14 shows Cisd2 KO mice and their symptoms at 5 months of age. Statistical significance was assessed via two-way ANOVA with Tukey's post-hoc tests.

FIGS. 15A-15D demonstrate effects of DAEUS-Cisd2. Uniform transduction (FIG. 15A) and rescue of Cisd2 expression (FIG. 15B) in Cisd2 knockout Wolfram Syndrome II mice is shown. Rescue of weight (FIG. 15C) and protection against frailty (FIG. 15D) in 2-4 month old Cisd2 knockout mice injected with 4e11 total dose of DAEUS-Cisd2 is shown. Weight was assayed for 155 days post-injection and normalized to weight pre-injection for each mouse. Frailty was assayed 4 months post-injection for Cisd2 knockout mice, Cisd2 knockout mice injected with DAEUS-Cisd2 and their wild-type littermates. Male and female mice were used at approximately 1:1 ratio. Statistical significance was assessed via two-way (left) and one-way (right) ANOVA with Tukey's post-hoc tests.

FIG. 16 shows timelines for assessing effects from administration of DAEUS-Cisd2 on Cisd2 KO mice of various ages (aged (7 months), young (2-4 months), and neonatal (P5-P8)).

FIG. 17 provides results of administering DAEUS-Cisd2 or a vehicle to Cisd2 KO mice aged about P5-P8 days (neonatal) compared to administering a vehicle to WT mice. The data measures survival post-injection, frailty, weight change, speed, and time in movement of mice. The neonatal mice were further observed for corneal scarring or opacity.

FIG. 18 provides results of administering DAEUS-Cisd2 or a vehicle to Cisd2 KO mice aged about 2-4 months (young) compared to administering a vehicle to WT mice. The data measures survival post-injection, frailty, weight change, grid hang ability, and challenging beam crossing of mice.

5 FIG. 19 provides results of administering DAEUS-Cisd2 or a vehicle to Cisd2 knockout (KO) mice aged about 7 months (aged). Photographs show the mice 40, 64, and 125 days post infection (DPI) and graphs show weight gain and survival of mice who were administered DAEUS-Cisd2 compared to mice that were administered the vehicle (FFB) only. Mice were injected retro-orbitally with a total of 3×10^{11} of
10 DAEUS-Cisd2, then followed for 125 days post-injection (DPI). Vehicle injected mouse died 23 days post-injection.

 FIG. 20 shows results of overexpressing DAEUS-PTEN, DAEUS-Atg5, and DAEUS-Cisd2 in WT mice. 18 month old wild-type male and female (1:1 ratio) C57BL6/J mice were injected with either 1×10^{12} vg/mouse of DAEUS-PTEN,
15 2×10^{12} vg/mouse of DAEUS-Cisd2 or 8×10^{12} vg/mouse of DAEUS-Atg5. Mice were euthanized 1 month post-injection and PTEN, Cisd2 and Atg5 protein levels were measured respectively using Simple Wes. Two separate experiments were performed for each and are shown in individual graphs.

 FIG. 21 shows the lifespan of 24 month old wild-type C57BL/6J mice treated
20 with DAEUS-PTEN/Cisd2/GFP or vehicle. Equal numbers of male and female mice were injected retro-orbitally either with vehicle (FFB) (N=14), DAEUS-PTEN at 1×10^{12} vg/mouse (PTEN) (N=8), DAEUS-GFP at 1×10^{12} vg/mouse (GFP) (N=5) or DAEUS-Cisd2 at 2×10^{12} vg/mouse (Cisd2) (N=6). Survival plotted as with all groups together (top left panel) or individually compared to FFB group (rest). DAEUS-PTEN
25 treated mice showed a 7% increase in overall median survival and 37% increase in post-injection median survival compared to vehicle treated mice. DAEUS-Cisd2 treated mice showed a 7% increase in overall median survival and 38% increase in post-injection median survival compared to FFB treated mice.

30 DETAILED DESCRIPTION OF THE INVENTION

Disclosed herein are gene therapy methods that allow for long-term, efficient, and body wide gene expression. Also disclosed herein are viral vector delivery

systems for delivery of one or more genes. The viral vector delivery systems described herein deliver genes into the majority of tissues within a subject, provide uniform gene expression across these tissues, provide long-term and stable gene expression, provide strong and efficient expression of the genes so as to achieve
5 overexpression above wild-type levels, and provide evenly distributed gene expression between individual cells. Also disclosed herein are methods of treating or preventing one or more diseases (e.g., Wolfram Syndrome II) or extending the lifespan of a subject by utilizing gene therapy (e.g., a viral vector delivery system) to deliver a gene (e.g., Cisd2, Atg5, of PTEN) to one or more tissues of a subject.

10 Unless defined otherwise, all technical and scientific terms used herein have the meaning commonly understood by a person skilled in the art. The following references provide one of skill with a general definition of many of the terms used herein: Singleton et al., Dictionary of Microbiology and Molecular Biology (2nd ed. 1994); The Cambridge Dictionary of Science and Technology (Walker ed., 1988);
15 The Glossary of Genetics, 5th Ed., R. Rieger et al. (eds.), Springer Verlag (1991); and Hale & Marham, The Harper Collins Dictionary of Biology (1991).

Standard techniques may be used for recombinant DNA, oligonucleotide synthesis, tissue culture and transformation, protein purification, etc. Enzymatic reactions and purification techniques may be performed according to the
20 manufacturer's specifications or as commonly accomplished in the art or as described herein. The following procedures and techniques may be generally performed according to conventional methods well known in the art and as described in various general and more specific references that are cited and discussed throughout the specification. See, e.g., Sambrook et al., 2001, Molecular Cloning: A Laboratory
25 Manual, 3rd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., which is incorporated herein by reference for any purpose. Unless specific definitions are provided, the nomenclature used in connection with, and the laboratory procedures and techniques of, analytic chemistry, organic chemistry, and medicinal and pharmaceutical chemistry described herein are those well-known and commonly
30 used in the art. Standard techniques may be used for chemical synthesis, chemical analyses, pharmaceutical preparation, formulation, and delivery and treatment of patients.

The present application provides viral vector delivery systems capable of delivering genes to a target environment, for example, a cell, a population of cells, a tissue, an organ, or a combination thereof, in a subject transduced with the viral vector delivery system. For example, the viral vector delivery system can be used to deliver genes to the aorta, endothelium, cardiac muscle, skeletal muscle, tongue, esophagus, stomach, small intestine, large intestine, diaphragm, eye, optic nerve, inner ear, auditory nerve, brown fat, white fat, central nervous system, peripheral nervous system, kidney, spleen, liver, lung, heart, brain, thymus, ovaries, testes, skin, pancreas, bone marrow cells, osteoblasts and osteoclasts, blood cells, hematopoietic stem cells, and muscle satellite cells of a subject. In certain aspects, the viral vector delivery system can be used to deliver genes to the brain, heart, liver, and/or muscle (e.g., transverse abdominal muscle or quadricep muscle) of a subject. Also disclosed herein are peptides capable of directing viral vectors to a target environment (e.g., the brain, the heart, the liver, muscles, or the combination thereof) in a subject, viral vector capsid proteins comprising the peptides, compositions (e.g., pharmaceutical compositions) comprising viral vectors having capsid proteins comprising the peptides, and the nucleic acid sequences encoding the peptides and viral vector capsid proteins. In addition, methods of making and using the viral vectors are also disclosed. In some embodiments, the viral vectors are used to prevent and/or treat one or more diseases and disorders, for example diseases and disorders related to aging.

Disclosed herein are vector delivery systems (e.g., viral vector delivery systems). The viral vector delivery systems may comprise one or more viral serotypes for delivery of a single gene, and in certain aspects may comprise two or more viral serotypes for delivery of a single gene. A viral vector delivery system may comprise an unlimited number of viral serotypes for delivery of a single transgene to a subject. In some embodiments, the viral vector delivery system comprises at least 1, 2, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, or 100 viral serotypes. In some embodiments, the viral vector delivery system comprises at least one, two, three, four, five, six, seven, eight, nine, or ten viral serotypes. In some embodiments, the viral vector delivery system comprises one to ten, two to eight, five to ten, or five to eight viral serotypes. In some embodiments, the viral vector delivery system comprises one viral serotype. In some embodiments, the viral vector delivery

system comprises two viral serotypes. In some embodiments, a first viral serotype delivers a gene to a first target tissue and a second viral serotype delivers the same gene to the first target tissue and/or to a second target tissue. In some aspects, a third, fourth, fifth, sixth, seventh, eighth, ninth, and/or tenth viral serotype delivers the gene to one or more tissues. In some embodiments, the viral serotypes are administered concurrently, proximately, or sequentially.

Suitable viruses for use in the viral vector delivery system described herein include, e.g., adenoviruses, adeno-associated viruses, retroviruses (e.g., lentiviruses), vaccinia virus and other poxviruses, herpesviruses (e.g., herpes simplex virus), and others. The virus may or may not contain sufficient viral genetic information for production of infectious virus when introduced into host cells, i.e., viral vectors may be replication-competent or replication-defective.

In some embodiments, the virus is adeno-associated virus. Adeno-associated virus (AAV) is a small (20 nm) replication-defective, nonenveloped virus. The AAV genome a single-stranded DNA (ssDNA) about 4.7 kilobase long. The genome comprises inverted terminal repeats (ITRs) at both ends of the DNA strand, and two open reading frames (ORFs): rep and cap. The AAV genome integrates most frequently into a particular site on chromosome 19. Random incorporations into the genome take place with a negligible frequency. The integrative capacity may be eliminated by removing at least part of the rep ORF from the vector resulting in vectors that remain episomal and provide sustained expression at least in non-dividing cells. To use AAV as a gene transfer vector, a nucleic acid comprising a nucleic acid sequence encoding a desired protein or RNA, e.g., encoding a polypeptide or RNA, operably linked to a promoter, is inserted between the inverted terminal repeats (ITR) of the AAV genome. Adeno-associated viruses (AAV) and their use as vectors, e.g., for gene therapy, are also discussed in Snyder, RO and Moullier, P., Adeno-Associated Virus Methods and Protocols, Methods in Molecular Biology, Vol. 807. Humana Press, 2011.

In some embodiments, the virus is AAV serotype 1, 2, 3, 3B, 4, 5, 6, 7, 8, 9, 10, 11, Anc80, or PHP.eB. (disclosed in US 2017/0166926, incorporated herein by reference). Any AAV serotype, or modified AAV serotype, may be used as appropriate and is not limited.

Another suitable AAV may be, e.g., Anc80 (i.e., Anc80L65) (WO2015054653) or rh10 (WO 2003/042397). Still other AAV sources may include, e.g., PHP.B, PHP.S, hu37 (see, e.g. US 7,906,111; US 2011/0236353), AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV6.2, AAV7, AAV8, (US 7,790,449; US 7,282,199), AAV9 (US 7,906,111; US 2011/0236353), AAVrh10, AAV-DJ, AAV-DJ/8, AAV.CAP-B10, AAV.CAP-B22, AAVMYO, and others. See, e.g., WO 2003/042397; WO 2005/033321, WO 2006/110689; US 7,790,449; US 7,282,199; US 7,588,772 for sequences of these and other suitable AAV, as well as for methods for generating AAV vectors. Other examples of AAVs include those listed in Table 1. Still other AAVs may be selected, optionally taking into consideration tissue preferences of the selected AAV capsid. In certain embodiments, a viral vector delivery system comprises viral serotypes AAV9 and PHP.eB.

Table 1:

Serotype name	Reference
AAV1	ACS Synth. Biol. 8, 194-206 (2019).
AAV1A1	<i>Nat Commun</i> 11, 5432 (2020).
AAV1A2	<i>Nat Commun</i> 11, 5432 (2020).
AAV1A6	<i>Nat Commun</i> 11, 5432 (2020).
AAV1P2	<i>Nat Commun</i> 11, 5432 (2020).
AAV1P4	<i>Nat Commun</i> 11, 5432 (2020).
AAV1P5	<i>Nat Commun</i> 11, 5432 (2020).
AAV2	ACS Synth. Biol. 8, 194-206 (2019).
AAV2-7m8	Sci. Transl. Med. 5, 189ra176 (2013).
AAV2A1	<i>Nat Commun</i> 11, 5432 (2020).
AAV2A2	<i>Nat Commun</i> 11, 5432 (2020).
AAV2A6	<i>Nat Commun</i> 11, 5432 (2020).
AAV2-BR1	EMBO Mol. Med. 8, 609-625 (2016).
AAV2-ESGHGYF	Mol. Ther. 24, 1050-1061 (2016).
AAV2-ESGHGYFmut1	Mol. Ther. 24, 1050-1061 (2016).
AAV2-ESGHGYFmut2	Mol. Ther. 24, 1050-1061 (2016).
AAV587MTP	Gene Ther. 16, 953-962 (2009)
AAV2P2	<i>Nat Commun</i> 11, 5432 (2020).
AAV2P4	<i>Nat Commun</i> 11, 5432 (2020).
AAV2P5	<i>Nat Commun</i> 11, 5432 (2020).
AAV2HBKO	J. Virol. 77, 6995-7006 (2003).
AAV2YF	Hum. Gene Ther. 21, 1527-1543 (2010).
AAV3b	ACS Synth. Biol. 8, 194-206 (2019).

AAV3bA1	<i>Nat Commun</i> 11 , 5432 (2020).
AAV3bA2	<i>Nat Commun</i> 11 , 5432 (2020).
AAV3bA6	<i>Nat Commun</i> 11 , 5432 (2020).
AAV3bP2	<i>Nat Commun</i> 11 , 5432 (2020).
AAV3bP4	<i>Nat Commun</i> 11 , 5432 (2020).
AAV3bP5	<i>Nat Commun</i> 11 , 5432 (2020).
AAV4	<i>ACS Synth. Biol.</i> 8 , 194-206 (2019).
AAV4A1	<i>Nat Commun</i> 11 , 5432 (2020).
AAV4A2	<i>Nat Commun</i> 11 , 5432 (2020).
AAV4A6	<i>Nat Commun</i> 11 , 5432 (2020).
AAV4L1	<i>Nat Commun</i> 11 , 5432 (2020).
AAV4P2	<i>Nat Commun</i> 11 , 5432 (2020).
AAV4P4	<i>Nat Commun</i> 11 , 5432 (2020).
AAV4P5	<i>Nat Commun</i> 11 , 5432 (2020).
AAV4mut	<i>Nat Commun</i> 11 , 5432 (2020).
AAV4mutA1	<i>Nat Commun</i> 11 , 5432 (2020).
AAV4mutA2	<i>Nat Commun</i> 11 , 5432 (2020).
AAV4mutA6	<i>Nat Commun</i> 11 , 5432 (2020).
AAV4mutP2	<i>Nat Commun</i> 11 , 5432 (2020).
AAV4mutP4	<i>Nat Commun</i> 11 , 5432 (2020).
AAV4mutP5	<i>Nat Commun</i> 11 , 5432 (2020).
AAV5	<i>ACS Synth. Biol.</i> 8 , 194-206 (2019).
AAV5A1	<i>Nat Commun</i> 11 , 5432 (2020).
AAV5A2	<i>Nat Commun</i> 11 , 5432 (2020).
AAV5A6	<i>Nat Commun</i> 11 , 5432 (2020).
AAV5P2	<i>Nat Commun</i> 11 , 5432 (2020).
AAV5P4	<i>Nat Commun</i> 11 , 5432 (2020).
AAV5P5	<i>Nat Commun</i> 11 , 5432 (2020).
AAV6	<i>ACS Synth. Biol.</i> 8 , 194-206 (2019).
AAV6A1	<i>Nat Commun</i> 11 , 5432 (2020).
AAV6A2	<i>Nat Commun</i> 11 , 5432 (2020).
AAV6A6	<i>Nat Commun</i> 11 , 5432 (2020).
AAV6P2	<i>Nat Commun</i> 11 , 5432 (2020).
AAV6P4	<i>Nat Commun</i> 11 , 5432 (2020).
AAV6P5	<i>Nat Commun</i> 11 , 5432 (2020).
AAV6.2	<i>Mol. Ther.</i> 17 , 294-301 (2009).
AAV7	<i>ACS Synth. Biol.</i> 8 , 194-206 (2019).
AAV7A1	<i>Nat Commun</i> 11 , 5432 (2020).
AAV7A2	<i>Nat Commun</i> 11 , 5432 (2020).
AAV7A6	<i>Nat Commun</i> 11 , 5432 (2020).
AAV7P2	<i>Nat Commun</i> 11 , 5432 (2020).
AAV7P4	<i>Nat Commun</i> 11 , 5432 (2020).
AAV7P5	<i>Nat Commun</i> 11 , 5432 (2020).

AAV8	ACS Synth. Biol. 8, 194-206 (2019).
AAV8A1	<i>Nat Commun</i> 11, 5432 (2020).
AAV8A2	<i>Nat Commun</i> 11, 5432 (2020).
AAV8A6	<i>Nat Commun</i> 11, 5432 (2020).
AAV8P2	<i>Nat Commun</i> 11, 5432 (2020).
AAV8P4	<i>Nat Commun</i> 11, 5432 (2020).
AAV8P5	<i>Nat Commun</i> 11, 5432 (2020).
AAV9	ACS Synth. Biol. 8, 194-206 (2019).
AAV9A1	<i>Nat Commun</i> 11, 5432 (2020).
AAV9A2	<i>Nat Commun</i> 11, 5432 (2020).
AAV9A6	<i>Nat Commun</i> 11, 5432 (2020).
AAV9BR1	<i>Nat Commun</i> 11, 5432 (2020).
AAV9-SLRSPPS	Gene Ther. 19, 800-809 (2012).
AAV9-RGDLRVS	Gene Ther. 19, 800-809 (2012).
AAVMYO	<i>Nat Commun</i> 11, 5432 (2020).
AAV9P2	<i>Nat Commun</i> 11, 5432 (2020).
AAV9P3	<i>Nat Commun</i> 11, 5432 (2020).
AAV9P4	<i>Nat Commun</i> 11, 5432 (2020).
AAV9P5	<i>Nat Commun</i> 11, 5432 (2020).
AAV-PHP.A	Nat. Biotechnol. 34, 204-209 (2016).
AAV-PHP.B	Nat. Biotechnol. 34, 204-209 (2016).
AAV-PHP.eB	Nat. Neurosci. 20, 1172-1179 (2017).
AAV-PHP.S	Nat. Neurosci. 20, 1172-1179 (2017).
AAV9BI	<i>Nat Commun</i> 11, 5432 (2020).
AAV9LD	Nat. Commun. 5, 3075 (2014).
AAVrh.10	ACS Synth. Biol. 8, 194-206 (2019).
AAVrh.10A1	<i>Nat Commun</i> 11, 5432 (2020).
AAVrh.10A2	<i>Nat Commun</i> 11, 5432 (2020).
AAVrh.10A6	<i>Nat Commun</i> 11, 5432 (2020).
AAVrh.10P2	<i>Nat Commun</i> 11, 5432 (2020).
AAVrh.10P4	<i>Nat Commun</i> 11, 5432 (2020).
AAVrh.10P5	<i>Nat Commun</i> 11, 5432 (2020).
AAVpo.1	ACS Synth. Biol. 8, 194-206 (2019).
AAVpo.1A1	<i>Nat Commun</i> 11, 5432 (2020).
AAVpo.1A2	<i>Nat Commun</i> 11, 5432 (2020).
AAVpo.1A6	<i>Nat Commun</i> 11, 5432 (2020).
AAVpo.1P2	<i>Nat Commun</i> 11, 5432 (2020).
AAVpo.1P4	<i>Nat Commun</i> 11, 5432 (2020).
AAVpo.1P5	<i>Nat Commun</i> 11, 5432 (2020).
AAV12	ACS Synth. Biol. 8, 194-206 (2019).
AAV12A1	<i>Nat Commun</i> 11, 5432 (2020).
AAV12A2	<i>Nat Commun</i> 11, 5432 (2020).
AAV12A6	<i>Nat Commun</i> 11, 5432 (2020).

AAV12P2	<i>Nat Commun</i> 11 , 5432 (2020).
AAV12P4	<i>Nat Commun</i> 11 , 5432 (2020).
AAV12P5	<i>Nat Commun</i> 11 , 5432 (2020).
AAV-Anc80L65	Cell Reports, 12(6), 1056–1068.
AAV-B1	Mol. Ther. 24, 1247-1257 (2016).
AAV-DJ	J. Virol. 82, 5887-5911 (2008).
AAV-DJYF	<i>Nat Commun</i> 11 , 5432 (2020).
AAV-LK03	Nature 506, 382-386 (2014).
AAVM41	Proc. Natl. Acad. Sci. U S A 106, 3946-3951 (2009).
AAV-ShH10	PLoS One 4, e7467 (2009).
AAVAH	<i>Nat Commun</i> 11 , 5432 (2020).
AAVJEA	<i>Nat Commun</i> 11 , 5432 (2020).
AAVHSC1	PLoS One. 2019 Nov 26;14(11):e0225582.
AAVHSC2	PLoS One. 2019 Nov 26;14(11):e0225582.
AAVHSC3	PLoS One. 2019 Nov 26;14(11):e0225582.
AAVHSC4	PLoS One. 2019 Nov 26;14(11):e0225582.
AAVHSC5	PLoS One. 2019 Nov 26;14(11):e0225582.
AAVHSC6	PLoS One. 2019 Nov 26;14(11):e0225582.
AAVHSC7	PLoS One. 2019 Nov 26;14(11):e0225582.
AAVHSC8	PLoS One. 2019 Nov 26;14(11):e0225582.
AAVHSC9	PLoS One. 2019 Nov 26;14(11):e0225582.
AAVHSC10	PLoS One. 2019 Nov 26;14(11):e0225582.
AAVHSC11	PLoS One. 2019 Nov 26;14(11):e0225582.
AAVHSC12	PLoS One. 2019 Nov 26;14(11):e0225582.
AAVHSC13	PLoS One. 2019 Nov 26;14(11):e0225582.
AAVHSC14	PLoS One. 2019 Nov 26;14(11):e0225582.
AAVHSC15	PLoS One. 2019 Nov 26;14(11):e0225582.
AAVHSC16	PLoS One. 2019 Nov 26;14(11):e0225582.
AAVHSC17	PLoS One. 2019 Nov 26;14(11):e0225582.
AAV9-OFF	
AAV3B-ON	
Anc126	Cell Reports, 12(6), 1056–1068.
Anc127	Cell Reports, 12(6), 1056–1068.
Anc113	Cell Reports, 12(6), 1056–1068.
Anc110	Cell Reports, 12(6), 1056–1068.
Anc81	Cell Reports, 12(6), 1056–1068.
Anc82	Cell Reports, 12(6), 1056–1068.
Anc83	Cell Reports, 12(6), 1056–1068.
Anc84	Cell Reports, 12(6), 1056–1068.
AAV rh32.33	Journal of Virology Nov 2009, 83 (24) 12738-12750
AAV.CAP-B10	bioRxiv 2020.06.16.152975
AAV.CAP-B1	bioRxiv 2020.06.16.152975
AAV.CAP-B2	bioRxiv 2020.06.16.152975

AAV.CAP-B8	bioRxiv 2020.06.16.152975
AAV.CAP-B10	bioRxiv 2020.06.16.152975
AAV.CAP-B18	bioRxiv 2020.06.16.152975
AAV.CAP-B22	bioRxiv 2020.06.16.152975
AAV2-retro	<i>Sci Rep</i> 10 , 6970 (2020).
AAV2-QuadYF	<i>Gene Ther</i> 21 , 96–105 (2014).

A recombinant AAV vector (AAV viral particle) may comprise, packaged within an AAV capsid, a nucleic acid molecule containing a 5' AAV ITR, the expression cassettes described herein and a 3' AAV ITR. As described herein, an expression cassette may contain regulatory elements for an open reading frame(s) within each expression cassette and the nucleic acid molecule may optionally contain additional regulatory elements.

The AAV vector may contain a full-length AAV 5' inverted terminal repeat (ITR) and a full-length 3' ITR. A shortened version of the 5' ITR, termed AITR, has been described in which the D-sequence and terminal resolution site (trs) are deleted. The abbreviation "sc" refers to self-complementary. "Self-complementary AAV" refers to a construct in which a coding region carried by a recombinant AAV nucleic acid sequence has been designed to form an intra-molecular double-stranded DNA template. Upon infection, rather than waiting for cell mediated synthesis of the second strand, the two complementary halves of scAAV will associate to form one double stranded DNA (dsDNA) unit that is ready for immediate replication and transcription. See, e.g., D M McCarty et al, "Self- complementary recombinant adeno-associated virus (scAAV) vectors promote efficient transduction independently of DNA synthesis", *Gene Therapy*, (August 2001), Vol 8, Number 16, Pages 1248-1254. Self-complementary AAVs are described in, e.g., U.S. Patent Nos. 6,596,535; 7,125,717; and 7,456,683, each of which is incorporated herein by reference in its entirety.

Where a pseudotyped AAV is to be produced, the ITRs are selected from a source which differs from the AAV source of the capsid. For example, AAV2 ITRs may be selected for use with an AAV capsid having a particular efficiency for a selected cellular receptor, target tissue or viral target. In one embodiment, the ITR sequences from AAV2, or the deleted version thereof (AITR), are used for convenience and to accelerate regulatory approval. However, ITRs from other AAV sources may be selected. Where the source of the ITRs is from AAV2 and the AAV

capsid is from another AAV source, the resulting vector may be termed pseudotyped. However, other sources of AAV ITRs may be utilized.

Methods for generating and isolating AAV viral vectors suitable for delivery to a subject are known in the art. See, e.g., US Patent 7790449; US Patent 7282199; WO 2003/042397; WO 2005/033321, WO 2006/110689; and US 7588772 B2. In one system, a producer cell line is transiently transfected with a construct that encodes the transgene flanked by ITRs and a construct(s) that encodes rep and cap. In a second system, a packaging cell line that stably supplies rep and cap is transfected (transiently or stably) with a construct encoding the transgene flanked by ITRs. In each of these systems, AAV virions are produced in response to infection with helper adenovirus or herpesvirus, requiring the separation of the rAAVs from contaminating virus. More recently, systems have been developed that do not require infection with helper virus to recover the AAV - the required helper functions (i.e., adenovirus E1, E2a, VA, and E4 or herpesvirus UL5, UL8, UL52, and UL29, and herpesvirus polymerase) are also supplied, in trans, by the system. In these newer systems, the helper functions can be supplied by transient transfection of the cells with constructs that encode the required helper functions, or the cells can be engineered to stably contain genes encoding the helper functions, the expression of which can be controlled at the transcriptional or posttranscriptional level. In yet another system, the transgene flanked by ITRs and rep/cap genes are introduced into insect cells by infection with baculovirus-based vectors. For reviews on these production systems, see generally, e.g., Zhang et al, 2009, "Adenovirus- adeno-associated virus hybrid for large-scale recombinant adeno-associated virus production," Human Gene Therapy 20:922-929, the contents of each of which is incorporated herein by reference in its entirety. Methods of making and using these and other AAV production systems are also described in the following U.S. patents, the contents of which is incorporated herein by reference in its entirety: 5,139,941; 5,741,683; 6,057,152; 6,204,059; 6,268,213; 6,491,907; 6,660,514; 6,951,753; 7,094,604; 7,172,893; 7,201,898; 7,229,823; and 7,439,065.

In another embodiment, other viral vectors may be used, including integrating viruses, e.g., herpesvirus or lentivirus, although other viruses may be selected. Suitably, where one of these other vectors is generated, it is produced as a replication-defective viral vector. A "replication-defective virus" or "viral vector" refers to a

synthetic or artificial viral particle in which an expression cassette containing a gene of interest is packaged in a viral capsid or envelope, where any viral genomic sequences also packaged within the viral capsid or envelope are replication-deficient; i.e., they cannot generate progeny virions but retain the ability to infect target cells. In one embodiment, the genome of the viral vector does not include genes encoding the enzymes required to replicate (the genome can be engineered to be "gutless" - containing only the transgene of interest flanked by the signals required for amplification and packaging of the artificial genome), but these genes may be supplied during production.

10 The one or more viruses may contain a promoter capable of directing expression in mammalian cells, such as a suitable viral promoter, e.g., from a cytomegalovirus (CMV), retrovirus, simian virus (e.g., SV40), papilloma virus, herpes virus or other virus that infects mammalian cells, or a mammalian promoter from, e.g., a gene such as EF1alpha, ubiquitin (e.g., ubiquitin B or C), globin, actin, phosphoglycerate kinase (PGK), etc., or a composite promoter such as a CAG promoter (combination of the CMV early enhancer element and chicken beta-actin promoter). In some embodiments a human promoter may be used. In some embodiments, the promoter directs expression in a particular cell type (e.g., a targeted population of cells). In some embodiments, the promoter selectively directs expression in any population of cells described herein. In some embodiments, the promoter is a non-silencing promoter. In some embodiments, the promoter is selected from the group consisting chicken β -actin hybrid (Cbh), CAG, CB7, and CBA. In certain embodiments, a non-silencing promoter is Cbh. In some embodiments, the non-silencing promoter directs expression that is high, long-term, and uniform across the cells. For example, the non-silencing promoter, e.g., Cbh, may direct expression that is at least 30%, 40%, 50%, 60%, or 70% of CMV and continues for at least one, two, three, four, five, six, or seven months.

 In some embodiments, the viral vector comprises a microRNA (miRNA) target site. In some embodiments, the miRNA target site is engineered into the vector to detarget particular tissues by reducing or silencing expression of the transgene in selected tissues. For example, liver toxicity may be reduced by including a liver-specific miRNA122 target site within the viral vector. In some embodiments, an

miRNA target site is selected based on the particular tissues in which expression is to be silenced or reduced. In some embodiments, a viral vector comprises liver specific (e.g., miRNA-33, miRNA-223, miRNA-30c, miRNA-144, miRNA-148a, miRNA-24, miRNA-29, and miRNA-122) (see, e.g., Willeit, et al., *Eur Heart J* 37, 3260-3266 (2016)), muscle specific (e.g., miRNA-1 and miRNA-133) (see, e.g., Xu et al., *J. Cell Sci.* 120, 3045-3052 (2007)), cardiac specific (e.g., miRNA-1, miRNA-133, miRNA-208a, miRNA-208b, and miRNA-499) (see, e.g., Xu et al., *J. Cell Sci.* 120, 3045-3052 (2007), Chistiakov, et al., *J. Mol. Cell. Cardiol.* 94, 107-121 (2016)), and/or brain specific miRNAs (e.g., miRNA-124 and miRNA-128) (see, e.g., Cao, et al., *Genes Dev.* 21, 531-536 (2007); Adlakha, et al., *Molecular Cancer* 13, 33 (2014)). In some embodiments, a viral vector comprises an miRNA target site selected from the group of miRNA-1, miRNA-24, miRNA-29, miRNA-30c, miRNA-33, miRNA-122, miRNA-124, miRNA-128, miRNA-133, miRNA-144, miRNA-148a, miRNA-208a, miRNA-208b, miRNA-223, and miRNA-499. Additional examples of miRNA target sites are available at mirbase.org. See Kozomara A, et al. *Nucleic Acids Res* 2019 47:D155-D162. In some embodiments, an miRNA target site is an miRNA that is specific (e.g., expressed in a specific tissue at least 10-fold higher than other tissues) and/or highly expressed (e.g., present at levels at least 5X higher than the average levels of all miRNAs in the target tissue). For example, the miRNA can be identified using FANTOM (see De Rie, et al., *Nat. Biotechnol.* 35, 872-878 (2017)) or other databases known to those of skill in the art.

In some embodiments, a viral vector comprises a self-complementary (self comp) vector backbone. For example, a viral vector may comprise codon-optimized gene coding sequences. In some aspects, a viral vector comprising a self-complementary backbone exhibits increased expression, e.g., at least 2X, 5X, 10X, or 15X greater expression.

In some embodiments, the gene is any gene to be delivered to a tissue. In some embodiments, the gene is associated with a monogenic disease or disorder. In some embodiments, the gene is an aging-related gene or a geroprotective gene. For example, the gene may be any gene listed in Table 2. In some embodiments, the gene is associated with neurological disorders, oncological disorders, retinal disorders, musculoskeletal disorders, hematology/blood disorders, infectious diseases,

immunological disorders, etc. Genes may be identified utilizing the OMIM database available at omim.org. In some embodiments, the gene is selected from the group consisting of Cisd2, Atg5, and PTEN.

Table 2:

From GenAge Database (Nucleic Acids Res. 2018 Jan 4;46(D1):D1083-D1090. doi: 10.1093/nar/gkx1042.)		
HGNC Symbol	HAGRID	Common name
A2M	139	alpha-2-macroglobulin
ABL1	78	ABL proto-oncogene 1, non-receptor tyrosine kinase
ADCY5	255	adenylate cyclase 5
AGPAT2	187	1-acylglycerol-3-phosphate O-acyltransferase 2
AGTR1	264	angiotensin II receptor, type 1
AIFM1	135	apoptosis-inducing factor, mitochondrion-associated, 1
AKT1	35	v-akt murine thymoma viral oncogene homolog 1
APEX1	195	APEX nuclease (multifunctional DNA repair enzyme) 1
APOC3	102	apolipoprotein C-III
APOE	138	apolipoprotein E
APP	137	amyloid beta (A4) precursor protein
APTX	95	aprataxin
AR	110	androgen receptor
ARHGAP1	249	Rho GTPase activating protein 1
ARNTL	251	aryl hydrocarbon receptor nuclear translocator-like
ATF2	193	activating transcription factor 2
ATM	9	ATM serine/threonine kinase
ATP5O	146	ATP synthase, H ⁺ transporting, mitochondrial F1 complex, O subunit
ATR	231	ATR serine/threonine kinase
BAK1	278	BCL2-antagonist/killer 1
BAX	119	BCL2-associated X protein
BCL2	69	B-cell CLL/lymphoma 2
BDNF	191	brain-derived neurotrophic factor
BLM	68	Bloom syndrome, RecQ helicase-like
BMI1	188	BMI1 proto-oncogene, polycomb ring finger
BRCA1	61	breast cancer 1, early onset
BRCA2	79	breast cancer 2, early onset
BSCL2	186	Berardinelli-Seip congenital lipodystrophy 2 (seipin)
BUB1B	197	BUB1 mitotic checkpoint serine/threonine kinase B
BUB3	240	BUB3 mitotic checkpoint protein
C1QA	287	complement component 1, q subcomponent, A chain
CACNA1A	133	calcium channel, voltage-dependent, P/Q type, alpha 1A subunit
CAT	107	catalase

CCNA2	177	cyclin A2
CDC42	250	cell division cycle 42
CDK1	201	cyclin-dependent kinase 1
CDK7	299	cyclin-dependent kinase 7
CDKN1A	284	cyclin-dependent kinase inhibitor 1A (p21, Cip1)
CDKN2A	226	cyclin-dependent kinase inhibitor 2A
CDKN2B	288	cyclin-dependent kinase inhibitor 2B (p15, inhibits CDK4)
CEBPA	88	CCAAT/enhancer binding protein (C/EBP), alpha
CEBPB	89	CCAAT/enhancer binding protein (C/EBP), beta
CETP	262	cholesteryl ester transfer protein, plasma
CHEK2	247	checkpoint kinase 2
CISD2	265	CDGSH iron sulfur domain 2
CLOCK	252	clock circadian regulator
CLU	220	clusterin
CNR1	282	cannabinoid receptor 1 (brain)
COQ7	132	coenzyme Q7 homolog, ubiquinone (yeast)
CREB1	192	cAMP responsive element binding protein 1
CREBBP	64	CREB binding protein
CSNK1E	244	casein kinase 1, epsilon
CTF1	304	cardiotrophin 1
CTGF	206	connective tissue growth factor
CTNNB1	223	catenin (cadherin-associated protein), beta 1, 88kDa
DBN1	228	drebrin 1
DDIT3	203	DNA-damage-inducible transcript 3
DGAT1	291	diacylglycerol O-acyltransferase 1
DLL3	225	delta-like 3 (Drosophila)
E2F1	18	E2F transcription factor 1
EEF1A1	189	eukaryotic translation elongation factor 1 alpha 1
EEF1E1	266	eukaryotic translation elongation factor 1 epsilon 1
EEF2	103	eukaryotic translation elongation factor 2
EFEMP1	260	EGF containing fibulin-like extracellular matrix protein 1
EGF	48	epidermal growth factor
EGFR	40	epidermal growth factor receptor
EGR1	76	early growth response 1
EIF5A2	289	eukaryotic translation initiation factor 5A2
ELN	230	elastin
EMD	121	emerin
EP300	94	E1A binding protein p300
EPOR	52	Erythropoietin receptor
EPS8	267	epidermal growth factor receptor pathway substrate 8
ERBB2	41	erb-b2 receptor tyrosine kinase 2
ERCC1	149	excision repair cross-complementation group 1
ERCC2	11	excision repair cross-complementation group 2

ERCC3	104	excision repair cross-complementation group 3
ERCC4	261	excision repair cross-complementation group 4
ERCC5	109	excision repair cross-complementation group 5
ERCC6	92	excision repair cross-complementation group 6
ERCC8	12	excision repair cross-complementation group 8
ESR1	216	estrogen receptor 1
FAS	115	Fas cell surface death receptor
FEN1	114	flap structure-specific endonuclease 1
FGF21	293	fibroblast growth factor 21
FGF23	259	fibroblast growth factor 23
FGFR1	169	fibroblast growth factor receptor 1
FLT1	171	fms-related tyrosine kinase 1
FOS	50	FBJ murine osteosarcoma viral oncogene homolog
FOXM1	131	forkhead box M1
FOXO1	124	forkhead box O1
FOXO3	123	forkhead box O3
FOXO4	183	forkhead box O4
GCLC	237	glutamate-cysteine ligase, catalytic subunit
GCLM	238	glutamate-cysteine ligase, modifier subunit
GDF11	309	growth differentiation factor 11
GH1	26	growth hormone 1
GHR	1	growth hormone receptor
GHRH	2	growth hormone releasing hormone
GHRHR	222	growth hormone releasing hormone receptor
GPX1	153	glutathione peroxidase 1
GPX4	257	glutathione peroxidase 4
GRB2	122	growth factor receptor-bound protein 2
GRN	300	granulin
GSK3A	295	glycogen synthase kinase 3 alpha
GSK3B	97	glycogen synthase kinase 3 beta
GSR	154	glutathione reductase
GSS	155	glutathione synthetase
GSTA4	156	glutathione S-transferase alpha 4
GSTP1	157	glutathione S-transferase pi 1
GTF2H2	111	general transcription factor IIH, polypeptide 2, 44kDa
H2AFX	212	H2A histone family, member X
HBP1	196	HMG-box transcription factor 1
HDAC1	151	histone deacetylase 1
HDAC2	207	histone deacetylase 2
HDAC3	25	histone deacetylase 3
HELLS	101	helicase, lymphoid-specific
HESX1	184	HESX homeobox 1
HIC1	253	hypermethylated in cancer 1

HIF1A	65	hypoxia inducible factor 1, alpha subunit (basic helix-loop-helix transcription factor)
HMGB1	176	high mobility group box 1
HMGB2	178	high mobility group box 2
HOXB7	213	homeobox B7
HOXC4	214	homeobox C4
HRAS	38	Harvey rat sarcoma viral oncogene homolog
HSF1	125	heat shock transcription factor 1
HSP90AA1	74	heat shock protein 90kDa alpha (cytosolic), class A member 1
HSPA1A	160	heat shock 70kDa protein 1A
HSPA1B	161	heat shock 70kDa protein 1B
HSPA8	199	heat shock 70kDa protein 8
HSPA9	152	heat shock 70kDa protein 9 (mortalin)
HSPD1	159	heat shock 60kDa protein 1 (chaperonin)
HTRA2	294	HtrA serine peptidase 2
HTT	98	huntingtin
IFNB1	308	Interferon beta
IGF1	28	insulin-like growth factor 1 (somatomedin C)
IGF1R	15	insulin-like growth factor 1 receptor
IGF2	29	insulin-like growth factor 2
IGFBP2	279	insulin-like growth factor binding protein 2, 36kDa
IGFBP3	73	insulin-like growth factor binding protein 3
IKBKB	297	inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase beta
IL2	46	interleukin 2
IL2RG	49	interleukin 2 receptor, gamma
IL6	144	interleukin 6
IL7	167	interleukin 7
IL7R	27	interleukin 7 receptor
INS	30	insulin
INSR	42	insulin receptor
IRS1	32	insulin receptor substrate 1
IRS2	34	insulin receptor substrate 2
JAK2	215	Janus kinase 2
JUN	172	jun proto-oncogene
JUND	45	jun D proto-oncogene
KCNA3	268	potassium channel, voltage gated shaker related subfamily A, member 3
KL	17	klotho
LEP	217	leptin
LEPR	218	leptin receptor
LMNA	14	lamin A/C
LMNB1	181	lamin B1

LRP2	134	low density lipoprotein receptor-related protein 2
MAP3K5	179	mitogen-activated protein kinase kinase kinase 5
MAPK14	168	mitogen-activated protein kinase 14
MAPK3	175	mitogen-activated protein kinase 3
MAPK8	163	mitogen-activated protein kinase 8
MAPK9	174	mitogen-activated protein kinase 9
MAPT	205	microtubule-associated protein tau
MAX	208	MYC associated factor X
MDM2	210	MDM2 proto-oncogene, E3 ubiquitin protein ligase
MED1	173	mediator complex subunit 1
MIF	290	macrophage migration inhibitory factor (glycosylation-inhibiting factor)
MLH1	243	mutL homolog 1
MSRA	127	methionine sulfoxide reductase A
MT-CO1	158	mitochondrially encoded cytochrome c oxidase I
MT1E	292	metallothionein 1E
MTOR	221	mechanistic target of rapamycin (serine/threonine kinase)
MXD1	209	MAX dimerization protein 1
MXI1	90	MAX interactor 1, dimerization protein
MYC	39	v-myc avian myelocytomatosis viral oncogene homolog
NBN	44	nibrin
NCOR1	43	nuclear receptor corepressor 1
NCOR2	276	nuclear receptor corepressor 2
NFE2L1	307	nuclear factor, erythroid 2-like 1
NFE2L2	283	nuclear factor, erythroid 2-like 2
NFKB1	82	nuclear factor of kappa light polypeptide gene enhancer in B-cells 1
NFKB2	20	nuclear factor of kappa light polypeptide gene enhancer in B-cells 2 (p49/p100)
NFKBIA	219	nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha
NGF	31	nerve growth factor (beta polypeptide)
NGFR	37	nerve growth factor receptor
NOG	229	noggin
NR3C1	75	nuclear receptor subfamily 3, group C, member 1 (glucocorticoid receptor)
NRG1	24	neuregulin 1
NUDT1	296	nudix (nucleoside diphosphate linked moiety X)-type motif 1
PAPPA	254	pregnancy-associated plasma protein A, pappalysin 1
PARP1	60	poly (ADP-ribose) polymerase 1
PCK1	248	phosphoenolpyruvate carboxykinase 1 (soluble)
PCMT1	162	protein-L-isoaspartate (D-aspartate) O-methyltransferase
PCNA	113	proliferating cell nuclear antigen
PDGFB	47	platelet-derived growth factor beta polypeptide

PDGFRA	285	platelet-derived growth factor receptor, alpha polypeptide
PDGFRB	51	platelet-derived growth factor receptor, beta polypeptide
PDPK1	87	3-phosphoinositide dependent protein kinase 1
PEX5	58	peroxisomal biogenesis factor 5
PIK3CA	286	phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha
PIK3CB	36	phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit beta
PIK3R1	185	phosphoinositide-3-kinase, regulatory subunit 1 (alpha)
PIN1	62	peptidylprolyl cis/trans isomerase, NIMA-interacting 1
PLAU	10	plasminogen activator, urokinase
PLCG2	57	phospholipase C, gamma 2 (phosphatidylinositol-specific)
PMCH	242	pro-melanin-concentrating hormone
PML	96	promyelocytic leukemia
POLA1	204	polymerase (DNA directed), alpha 1, catalytic subunit
POLB	236	polymerase (DNA directed), beta
POLD1	118	polymerase (DNA directed), delta 1, catalytic subunit
POLG	72	polymerase (DNA directed), gamma
PON1	142	paraoxonase 1
POU1F1	4	POU class 1 homeobox 1
PPARA	55	peroxisome proliferator-activated receptor alpha
PPARG	263	peroxisome proliferator-activated receptor gamma
PPARGC1A	256	peroxisome proliferator-activated receptor gamma, coactivator 1 alpha
PPM1D	246	protein phosphatase, Mg ²⁺ /Mn ²⁺ dependent, 1D
PPP1CA	227	protein phosphatase 1, catalytic subunit, alpha isozyme
PRDX1	141	peroxiredoxin 1
PRKCA	99	protein kinase C, alpha
PRKCD	54	protein kinase C, delta
PRKDC	106	protein kinase, DNA-activated, catalytic polypeptide
PROP1	5	PROP paired-like homeobox 1
PSEN1	224	presenilin 1
PTEN	63	phosphatase and tensin homolog
PTGS2	198	prostaglandin-endoperoxide synthase 2 (prostaglandin G/H synthase and cyclooxygenase)
PTK2	166	protein tyrosine kinase 2
PTK2B	165	protein tyrosine kinase 2 beta
PTPN1	33	protein tyrosine phosphatase, non-receptor type 1
PTPN11	19	protein tyrosine phosphatase, non-receptor type 11
PYCR1	280	pyrroline-5-carboxylate reductase 1
RAD51	84	RAD51 recombinase
RAD52	147	RAD52 homolog, DNA repair protein
RAE1	241	ribonucleic acid export 1
RB1	120	retinoblastoma 1

RECQL4	128	RecQ helicase-like 4
RELA	143	v-rel avian reticuloendotheliosis viral oncogene homolog A
RET	56	ret proto-oncogene
RGN	145	regucalcin
RICTOR	303	RPTOR independent companion of MTOR, complex 2
RPA1	67	replication protein A1, 70kDa
S100B	70	S100 calcium binding protein B
SDHC	182	succinate dehydrogenase complex, subunit C, integral membrane protein, 15kDa
SERPINE1	301	serpin peptidase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1), member 1
SHC1	3	SHC (Src homology 2 domain containing) transforming protein 1
SIN3A	200	SIN3 transcription regulator family member A
SIRT1	150	sirtuin 1
SIRT3	275	sirtuin 3
SIRT6	239	sirtuin 6
SIRT7	269	sirtuin 7
SLC13A1	270	solute carrier family 13 (sodium/sulfate symporter), member 1
SNCG	140	synuclein, gamma (breast cancer-specific protein 1)
SOCS2	271	suppressor of cytokine signaling 2
SOD1	130	superoxide dismutase 1, soluble
SOD2	129	superoxide dismutase 2, mitochondrial
SP1	170	Sp1 transcription factor
SPRTN	302	SprT-like N-terminal domain
SQSTM1	298	sequestosome 1
SST	53	somatostatin
SSTR3	100	somatostatin receptor 3
STAT3	22	signal transducer and activator of transcription 3 (acute-phase response factor)
STAT5A	23	signal transducer and activator of transcription 5A
STAT5B	21	signal transducer and activator of transcription 5B
STK11	93	serine/threonine kinase 11
STUB1	245	STIP1 homology and U-box containing protein 1, E3 ubiquitin protein ligase
SUMO1	211	small ubiquitin-like modifier 1
SUN1	277	Sad1 and UNC84 domain containing 1
TAF1	180	TAF1 RNA polymerase II, TATA box binding protein (TBP)-associated factor, 250kDa
TBP	194	TATA box binding protein
TCF3	59	transcription factor 3
TERC	7	telomerase RNA component
TERF1	105	telomeric repeat binding factor (NIMA-interacting) 1
TERF2	116	telomeric repeat binding factor 2

TERT	8	telomerase reverse transcriptase
TFAP2A	190	transcription factor AP-2 alpha (activating enhancer binding protein 2 alpha)
TFDP1	202	transcription factor Dp-1
TGFB1	91	transforming growth factor, beta 1
TNF	86	tumor necrosis factor
TOP1	83	topoisomerase (DNA) I
TOP2A	80	topoisomerase (DNA) II alpha
TOP2B	81	topoisomerase (DNA) II beta
TOP3B	148	topoisomerase (DNA) III beta
TP53	6	tumor protein p53
TP53BP1	274	tumor protein p53 binding protein 1
TP63	234	tumor protein p63
TP73	281	tumor protein p73
TPP2	273	tripeptidyl peptidase II
TRAP1	305	TNF receptor-associated protein 1
TRPV1	306	transient receptor potential cation channel subfamily V member 1
TXN	16	thioredoxin
UBB	66	ubiquitin B
UBE2I	85	ubiquitin-conjugating enzyme E2I
UCHL1	136	ubiquitin carboxyl-terminal esterase L1 (ubiquitin thiolesterase)
UCP1	258	uncoupling protein 1 (mitochondrial, proton carrier)
UCP2	235	uncoupling protein 2 (mitochondrial, proton carrier)
UCP3	232	uncoupling protein 3 (mitochondrial, proton carrier)
VCP	71	valosin containing protein
VEGFA	77	vascular endothelial growth factor A
WRN	13	Werner syndrome, RecQ helicase-like
XPA	126	xeroderma pigmentosum, complementation group A
XRCC5	112	X-ray repair complementing defective repair in Chinese hamster cells 5 (double-strand-break rejoining)
XRCC6	117	X-ray repair complementing defective repair in Chinese hamster cells 6
YWHAZ	164	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, zeta
ZMPSTE24	233	zinc metalloproteinase STE24
Dsup		Tardigrade radiation resistance DNA repair protein

In some embodiments, a viral vector delivery system comprises an AAV9 serotype and/or a PHP.eB serotype for delivery of the Cisd2 gene to a subject. In some embodiments, the viral vector delivery system comprises a miRNA target site, e.g., a miRNA-122 target site. In some embodiments, the viral vector delivery system

comprises a non-silencing promoter, e.g., Cbh, and optionally further comprises a self-complementary backbone.

The viral vector delivery system may result in overexpression of a native gene by at least 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%,
5 or 75% of wild-type levels in a target tissue (e.g., in at least 70% of fat free, blood free body mass). In some embodiments, the viral vector delivery system may result in overexpression of a native gene by at least 100%, 200%, 300%, 400%, 500%, 600%, 700%, 800%, 900%, 1000%, 1500%, 2000%, 2500%, 5000%, 7500%, 10000%, 50000%, 100000% of wild-type levels in a target tissue. In some embodiments, the
10 viral vector delivery system delivers a native gene resulting in overexpression of the native gene by about 10%-90%, 20%-80%, 30%-70%, or 40%-60% of wild-type levels in a tissue. In some embodiments, the viral vector delivery system results in overexpression of a native gene by at least 30%, or by about 25-50%, of wild-type levels. The viral vector delivery system may result in detectable expression (e.g.,
15 greater than trace expression) of a non-native gene in a target tissue (e.g., in at least 70% of fat free, blood free body mass). In some embodiments, expression of the delivered gene is stable and long-term (e.g., expression is maintained for at least 1 month, 2 months, 3 months, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, 12 months, 15 months, 18 months, 21 months, 24
20 months, 3 years, 4 years, 5 years, 10 years, 15 years, 20 years, 30 years, 40 years, 50 years, 60 years, 70 years, 80 years, 90 years).

In some embodiments, the viral vector delivery system delivers a gene of interest to a tissue of interest (e.g., aorta, endothelium, cardiac muscle skeletal muscle, tongue, esophagus, stomach, small intestine, large intestine, diaphragm, eye, optic
25 nerve, inner ear, auditory nerve, brown fat, white fat, central nervous system, peripheral nervous system, kidney, spleen, liver, lung, heart, brain, thymus, ovaries, testes, skin, pancreas, bone marrow cells, osteoblasts and osteoclasts, blood cells, hematopoietic stem cells, and/or muscle satellite cells). In some embodiments, the viral vector delivery system delivers a gene of interest to multiple tissues of interest in
30 a subject. For example, the viral vector delivery system may deliver a gene of interest to at least 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% of tissues in a subject. In some embodiments, the viral vector delivery system delivers a gene to about 10%-

90%, 20%-80%, 30%-70%, or 40%-60% of tissues in the subject. The viral vector delivery system may provide uniform or limited variable delivery of a gene across multiple tissues within a subject.

Some embodiments of the present invention relate to methods of treatment or prevention for a disease or condition, such as an aging-related disease or disorder, by the delivery of a pharmaceutical composition comprising an effective amount of the viral vector delivery system described herein. An effective amount of the pharmaceutical composition is an amount sufficient to prevent, slow, inhibit, or ameliorate a disease or disorder in a subject to whom the composition is administered.

5 In some embodiments, the delivery of a pharmaceutical composition comprising an effective amount of the viral vector delivery system described herein extends the life expectancy or lifespan of a subject.

In some embodiments, the viral vector delivery system is administered to a subject. The viral vector delivery system may deliver a gene to a subject, e.g., to one or more tissues of a subject. In some embodiments, the subject is expected to suffer from a disease or disorder based on family history or genetic analysis but is not currently suffering from the disease or disorder. In some embodiments, the subject is suffering from a disease or disorder. In some embodiments, the subject lacks an effective amount of active Cisd2. For example, the Cisd2 gene may be mutated or otherwise inactive in a subject. The gene may be delivered using the viral vector delivery system to treat or ameliorate the disease or disorder in the subject.

15 20

As used herein, a “subject” means a human or animal. Usually the animal is a vertebrate such as a primate, rodent, domestic animal or game animal. Primates include chimpanzees, cynomologous monkeys, spider monkeys, and macaques, e.g., Rhesus. Rodents include mice, rats, woodchucks, ferrets, rabbits and hamsters. Domestic and game animals include cows, horses, pigs, deer, bison, buffalo, feline species, e.g., domestic cat, canine species, e.g., dog, fox, wolf, avian species, e.g., chicken, emu, ostrich, and fish, e.g., trout, catfish and salmon. Patient or subject includes any subset of the foregoing, e.g., all of the above, but excluding one or more groups or species such as humans, primates or rodents. In certain embodiments, the subject is a mammal, e.g., a primate, e.g., a human. The terms, “patient”, “individual” and “subject” are used interchangeably herein. Preferably, the subject is a mammal.

25 30

The mammal can be a human, non-human primate, mouse, rat, dog, cat, horse, or cow, but are not limited to these examples. In addition, the methods described herein can be used to treat domesticated animals and/or pets. A subject can be male or female. A subject can be one who has been previously diagnosed with or identified as
5 suffering from or having a condition in need of treatment or one or more complications related to such a condition, and optionally, but need not have already undergone treatment for a condition or the one or more complications related to the condition. Alternatively, a subject can also be one who has not been previously
10 diagnosed as having a condition in need of treatment or one or more complications related to such a condition. Rather, a subject can include one who exhibits one or more risk factors for a condition or one or more complications related to a condition. A “subject in need” of treatment for a particular condition can be a subject having that condition, diagnosed as having that condition, or at increased risk of developing that condition relative to a given reference population.

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

In some embodiments, the viral vector delivery system is administered for immunological purposes, e.g., for vaccination or tolerance induction.

30 The efficacy of a given treatment for a disorder or disease can be determined by the skilled clinician. However, a treatment is considered “effective treatment,” as the term is used herein, if any one or all of the signs or symptoms of a disorder are

altered in a beneficial manner, other clinically accepted symptoms are improved or ameliorated, e.g., by at least 10% following treatment with an agent or composition as described herein. Efficacy can also be measured by a failure of an individual to worsen as assessed by hospitalization or need for medical interventions (i.e.,
5 progression of the disease is halted). Methods of measuring these indicators are known to those of skill in the art and/or described herein.

In accordance with methods of the invention, treatment comprises contacting one or more tissues with a composition according to the invention. The routes of administration will vary and include, e.g., intradermal, transdermal, parenteral,
10 intravenous, intramuscular, intranasal, subcutaneous, regional, percutaneous, intratracheal, intraperitoneal, intraarterial, intravesical, intraocular, intratumoral, inhalation, perfusion, lavage, and oral administration and formulation. Treatment regimens may vary as well, and often depend on disease type, disease location, disease progression, and health and age of the patient.

The treatments may include various "unit doses" defined as containing a predetermined-quantity of the therapeutic composition. The quantity to be administered, and the particular route and formulation, are within the skill of those in the clinical arts. A unit dose need not be administered as a single injection but may comprise continuous infusion over a specified period of time. The dosage ranges for
20 the agent depends upon the potency, and are amounts large enough to produce the desired effect. The dosage should not be so large as to cause unacceptable adverse side effects.

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

The pharmaceutical compositions disclosed herein may be administered intratumorally, parenterally, intravenously, intradermally, intramuscularly, transdermally or even intraperitoneally as described in U.S. Pat. Nos. 5,543,158; 5,641,515 and 5,399,363.

5 Injection of the viral vector delivery system may be delivered by syringe or any other method used for injection of a solution, as long as the expression construct can pass through the particular gauge of needle required for injection and the dosage can be administered with the required level of precision.

10 For parenteral administration in an aqueous solution, for example, the solution should be suitably buffered if necessary and the liquid diluent first rendered isotonic with sufficient saline or glucose. These particular aqueous solutions are especially suitable for intravenous, intramuscular, subcutaneous, intratumoral and intraperitoneal administration. In this connection, sterile aqueous media that can be employed will be known to those of skill in the art in light of the present disclosure. For example, one
15 dosage may be dissolved in 1 ml of isotonic NaCl solution and either added to 1000 ml of hypodermoclysis fluid or injected at the proposed site of infusion, (see for example, "Remington's Pharmaceutical Sciences" 15th Edition, pages 1035-1038 and 1570-1580). Some variation in dosage will necessarily occur depending on the condition of the subject being treated. The person responsible for administration will,
20 in any event, determine the appropriate dose for the individual subject. Moreover, for human administration, preparations should meet sterility, pyrogenicity, general safety and purity standards as required by FDA Office of Biologics standards.

25 The phrase "pharmaceutically-acceptable" or "pharmacologically-acceptable" refers to molecular entities and compositions that do not produce an allergic or similar untoward reaction when administered to a subject. As used herein, "carrier" includes any and all solvents, dispersion media, vehicles, coatings, diluents, antibacterial and antifungal agents, isotonic and absorption delaying agents, buffers, carrier solutions, suspensions, colloids, and the like. The use of such media and agents for
30 pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the viral agent, its use in the therapeutic compositions is contemplated. Supplementary active ingredients can also be incorporated into the compositions.

In some embodiments, the methods further comprise administering the pharmaceutical composition described herein along with one or more additional agents, biologics, drugs, or treatments beneficial to a subject suffering from a disorder or disease.

- 5 In some embodiments, the viral vector delivery system or pharmaceutical compositions comprising the viral vector delivery system are administered to a subject to treat a disease or condition. The disease or condition may be an aging-related disease or condition. In some embodiments, the disease or condition is a progeria syndrome, (e.g., Hutchinson–Gilford progeria syndrome (HGPS), Wolfram
- 10 Syndrome (e.g., Wolfram Syndrome I or II), Werner Syndrome, Cockayne syndrome, Myotonic Dystrophy type 1, MDPL syndrome, Dyskeratosis congenital disorder, etc.), connective tissue disorder (e.g., Marfan syndrome, Loeys-Dietz syndrome, Ehlers-Danlos syndrome, Osteogenesis Imperfecta, etc.), metabolic disorders (e.g., Methylmalonic Acidemia, Wilson’s disease, etc.), tumor suppressor and DNA
- 15 replication deficiency disorders (e.g., PTENopathies (Cowden syndrome, Proteus-like syndromes), Bloom syndrome, RASopathies (Noonan syndrome, Costello syndrome)), neurodegenerative disorder (e.g., Alzheimer’s disease, dementia, mild cognitive decline, etc.), neurovascular disorder (e.g., stroke), skeletal muscle conditions (e.g., sarcopenia, frailty), Cowden syndrome, Bannayan-Riley-Ruvalcaba
- 20 syndrome, Proteus syndrome, Proteus-like syndrome and other PTEN-opathies. Werner syndrome, Bloom syndrome, Rothmund–Thomson syndrome, Cockayne syndrome, xeroderma pigmentosum, trichothiodystrophy, combined xeroderma pigmentosum-Cockayne syndrome, restrictive dermopathy, diabetes, obesity, cardiovascular disease, cancer, ocular degeneration, liver failure, and age-related
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In some embodiments, the neurodegenerative disorder is one of polyglutamine expansion disorders (e.g., HD, dentatorubropallidoluysian atrophy, Kennedy's disease (also referred to as spinobulbar muscular atrophy), and spinocerebellar ataxia (e.g., type 1, type 2, type 3 (also referred to as Machado-Joseph disease), type 6, type 7, and type 17)), other trinucleotide repeat expansion disorders (e.g., fragile X syndrome, fragile XE mental retardation, Friedreich's ataxia, myotonic dystrophy, spinocerebellar ataxia type 8, and spinocerebellar ataxia type 12), Alexander disease, Alper's disease, Alzheimer disease, amyotrophic lateral sclerosis (ALS), ataxia telangiectasia, Batten disease (also referred to as Spielmeyer-Vogt-Sjogren-Batten disease), Canavan disease, Cockayne syndrome, corticobasal degeneration, Creutzfeldt-Jakob disease, Guillain-Barré syndrome, ischemia stroke, Krabbe disease, kuru, Lewy body dementia, multiple sclerosis, multiple system atrophy, non-Huntingtonian type of Chorea, Parkinson's disease, Pelizaeus-Merzbacher disease, Pick's disease, primary lateral sclerosis, progressive supranuclear palsy, Refsum's disease, Sandhoff disease, Schilder's disease, spinal cord injury, spinal muscular atrophy (SMA), SteeleRichardson-Olszewski disease, and Tabes dorsalis.

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

In some embodiments, the skeletal muscle condition is selected from the group consisting of atrophy, bony fractures associated with muscle wasting or weakness, cachexia, denervation, diabetes, dystrophy, exercise-induced skeletal muscle fatigue, fatigue, frailty, inflammatory myositis, metabolic syndrome, neuromuscular disease, 5 obesity, post-surgical muscle weakness, post-traumatic muscle weakness, sarcopenia, toxin exposure, wasting, and weakness.

In some embodiments, a vector delivery system or a pharmaceutical composition comprising the vector delivery system is administered (e.g., intravenously) to a subject. The vector delivery system may deliver a gene, e.g., 10 *Cisd2*, to the subject to treat a disease or condition associated with mutated *Cisd2* (e.g., Wolfram Syndrome II or related condition, i.e., loss of vision or cataracts, diabetes, deafness, kidney failure, etc.).

The description of embodiments of the disclosure is not intended to be 15 exhaustive or to limit the disclosure to the precise form disclosed. While specific embodiments of, and examples for, the disclosure are described herein for illustrative purposes, various equivalent modifications are possible within the scope of the disclosure, as those skilled in the relevant art will recognize. For example, while method steps or functions are presented in a given order, alternative embodiments 20 may perform functions in a different order, or functions may be performed substantially concurrently. The teachings of the disclosure provided herein can be applied to other procedures or methods as appropriate. The various embodiments described herein can be combined to provide further embodiments. Aspects of the disclosure can be modified, if necessary, to employ the compositions, functions and 25 concepts of the above references and application to provide yet further embodiments of the disclosure. These and other changes can be made to the disclosure in light of the detailed description.

Specific elements of any of the foregoing embodiments can be combined or substituted for elements in other embodiments. Furthermore, while advantages 30 associated with certain embodiments of the disclosure have been described in the context of these embodiments, other embodiments may also exhibit such advantages,

and not all embodiments need necessarily exhibit such advantages to fall within the scope of the disclosure.

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

One skilled in the art readily appreciates that the present invention is well adapted to carry out the objects and obtain the ends and advantages mentioned, as
15 well as those inherent therein. The details of the description and the examples herein are representative of certain embodiments, are exemplary, and are not intended as limitations on the scope of the invention. Modifications therein and other uses will occur to those skilled in the art. These modifications are encompassed within the spirit of the invention. It will be readily apparent to a person skilled in the art that varying
20 substitutions and modifications may be made to the invention disclosed herein without departing from the scope and spirit of the invention.

The articles “a” and “an” as used herein in the specification and in the claims, unless clearly indicated to the contrary, should be understood to include the plural referents. Claims or descriptions that include “or” between one or more members of a
25 group are considered satisfied if one, more than one, or all of the group members are present in, employed in, or otherwise relevant to a given product or process unless indicated to the contrary or otherwise evident from the context. The invention includes embodiments in which exactly one member of the group is present in, employed in, or otherwise relevant to a given product or process. The invention also
30 includes embodiments in which more than one, or all of the group members are present in, employed in, or otherwise relevant to a given product or process. Furthermore, it is to be understood that the invention provides all variations,

combinations, and permutations in which one or more limitations, elements, clauses, descriptive terms, etc., from one or more of the listed claims is introduced into another claim dependent on the same base claim (or, as relevant, any other claim) unless otherwise indicated or unless it would be evident to one of ordinary skill in the art that a contradiction or inconsistency would arise. It is contemplated that all embodiments described herein are applicable to all different aspects of the invention where appropriate. It is also contemplated that any of the embodiments or aspects can be freely combined with one or more other such embodiments or aspects whenever appropriate. Where elements are presented as lists, e.g., in Markush group or similar format, it is to be understood that each subgroup of the elements is also disclosed, and any element(s) can be removed from the group. It should be understood that, in general, where the invention, or aspects of the invention, is/are referred to as comprising particular elements, features, etc., certain embodiments of the invention or aspects of the invention consist, or consist essentially of, such elements, features, etc.

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

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

Where ranges are given herein, the invention includes embodiments in which the endpoints are included, embodiments in which both endpoints are excluded, and

embodiments in which one endpoint is included and the other is excluded. It should be assumed that both endpoints are included unless indicated otherwise. Furthermore, it is to be understood that unless otherwise indicated or otherwise evident from the context and understanding of one of ordinary skill in the art, values that are expressed as ranges can assume any specific value or subrange within the stated ranges in different embodiments of the invention, to the tenth of the unit of the lower limit of the range, unless the context clearly dictates otherwise. It is also understood that where a series of numerical values is stated herein, the invention includes embodiments that relate analogously to any intervening value or range defined by any two values in the series, and that the lowest value may be taken as a minimum and the greatest value may be taken as a maximum. Numerical values, as used herein, include values expressed as percentages. For any embodiment of the invention in which a numerical value is prefaced by “about” or “approximately”, the invention includes an embodiment in which the exact value is recited. For any embodiment of the invention in which a numerical value is not prefaced by “about” or “approximately”, the invention includes an embodiment in which the value is prefaced by “about” or “approximately”.

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

EXEMPLIFICATION

Example 1:

Aging is the single biggest factor in the most burdensome diseases today. The
5 major concerns of current health-care systems are diabetes, obesity, cardiovascular
disease, cancers, frailty, age-related macular degeneration and neurodegenerative
diseases. All of them share advanced age as the common and largest risk factor. [1]
The dominant paradigm in medicine now is to treat these diseases individually after
they appear and continue managing the symptoms through multiple treatment
10 modalities. However, this is a downhill battle, as most aged patients never recover
and instead develop further morbidities with advanced age, all of which require
chronic treatment. The average patient is now being treated for decades for multiple
chronic diseases, at a cost that is often considerably higher than that patient's lifetime
contribution to the healthcare system. [2] This is non-sustainable.

15 From fundamental studies into the biology of aging, over 50 genes which
influence lifespan in the mouse have been identified. [3] Based on these findings, a
new class of drugs termed geroprotectors has emerged, which target conserved aging
pathways (e.g., proteostasis, autophagy, insulin-IGF signaling, mitochondrial
metabolism and other pathways) at a systemic level. They have gone through an
20 explosive growth in number and investment over the past 5 years, with over 200
different drugs now existing. [4]

These drugs are important first steps towards preventing age-related diseases
at their source and will likely go on to have a large patient population. However,
small-molecule drugs are fundamentally limited as geroprotectors due to three
25 aspects. Firstly, they have side-effects. Side-effects are caused by off-target effects
and on-target effects in tissues where perturbation of the target is unwanted. While
side-effects are tolerated for other drugs, these drugs are expected to treat healthy
people, and will thus have to have very mild side-effects (if at all) to justify their
usage. Secondly, they require continuous, life-long administration. While this may be
30 possible for cheap drugs such as metformin, for many others this is prohibitively
costly or cumbersome (e.g., for drugs that require injections). Finally, these drugs can

only achieve limited efficacy, as they cannot perturb the function of their targets as fully as is possible via genetic methods.

Therefore, successful therapies that aim to prevent age-related diseases need to be long-acting, tissue-specific, and be able to perturb intracellular networks precisely and completely. This is not achievable with small-molecule drugs or biologics but is
5 achievable with gene therapies. In fact, gene therapy seems to be the only viable method in the long term that meets these requirements.

Gene therapies are also the main contestants for treatment of progerias. An example of one such disease is Wolfram Syndrome II – a progeria characterized by
10 diabetes, deafness, cataracts, loss of vision and hearing, atrophy of optic nerves, kidney and GI failure, and a number of other health problems, with average lifespan of about 30 years [5,6]. Wolfram Syndrome II was found to be caused by homozygous loss-of-function mutation in *Cisd2* – a small protein active in the mitochondrial membrane and endoplasmic reticulum (ER) [7,8]. *Cisd2* loss in mice
15 leads to decreased lifespan and phenocopy of most human Wolfram Syndrome II symptoms (FIGS. 1A-1B) [9]. Levels of *Cisd2* decrease with age in mice [9], whereas overexpression of *Cisd2* increases health and lifespan in mice (FIG. 1A) [10] and possibly humans [7,11]. As such, *Cisd2* gene therapy is both a potential treatment of Wolfram Syndrome II and geroprotector to increase healthspan in the general
20 population.

In summary, considering the limitations of small-molecule drugs as geroprotectors, the need for treatment of progerias, and the current major trends of increasing burden of age-related disease, increased knowledge and investment into biology of aging, and increasing efficiency and cost-effectiveness of adeno-associated
25 viruses (AAVs), AAV-based geroprotective gene therapies are on track to become a major part of healthcare.

DAEUS and the shortcomings of current gene therapy methods

From the various gene therapy methods, adeno-associated viruses (AAVs) are
30 by far the most efficacious and commonly used vectors. From AAVs, one of the most commonly used vectors in both research and new clinical trials are single-stranded AAV9 based vectors (ssAAV9). This is because ssAAV9 can be produced at high

titers and can transduce various tissues of the body, with highest expression present in the liver and lowest (by about 100-1000x) in the brain. While there is now a flurry of new engineered and discovered AAV serotypes, ssAAV9 has remained the method of choice as new vectors have either been more difficult to produce (Anc80) or are more efficacious towards a specific tissue only (PHP.B). Similarly to AAV9, other currently existing AAV serotypes result in highly variable gene transfer levels between various tissues. While ssAAV9 is sufficient for some applications, the attempts to use them for aging studies, which require gene delivery to a broad set of tissues, quickly shows that they are not suitable for this purpose. Empirically, it was found that for several geroprotective genes, even optimized ssAAV9 vectors (FIG. 2A) resulted in none or only modest overexpression in aged mice, with high tissue-to-tissue variability (FIGS. 2B-2D). This observation as well as high tissue-to tissue variability has been reported by several others (FIGS. 3-4) [12,13]. This presented a critical roadblock to using AAVs in aging research and therapeutics, and as a result, only three manuscripts using AAVs for aging research appear to have ever been published, all limited to genes that do not require long-term expression or can be secreted or studied in a confined tissue.

To generalize the use of AAVs to effectively deliver most genes involved in aging, a number of technical advances were needed. Firstly, because aging affects the whole body, it is necessary to be able to deliver genes into most tissues of the body, as opposed to a single or a few tissues. Secondly, gene expression must be uniform across these tissues, as opposed to varying multiple orders of magnitude. Third, gene expression must be long-term and stable. Fourth, expression must be strong and efficient to achieve overexpression above wild-type levels (most gene therapies restore expression to only a fraction of wild-type levels). Finally, gene expression must be evenly distributed between individual cells (as opposed to having high cell-to-cell variation as with ssAAV9 vectors).

After multiple iterations of testing and development spanning five years and combining resources from two labs, a system that meets these requirements has been developed: DAEUS (Different AAV Expression system for Uniform, Systemic expression). To achieve this, DAEUS employs a newly designed vector architecture using self-complementary vector backbone, two or more AAV serotypes, one or more

microRNA target sites, and a strong non-silencing promoter. Specifically, in one example, it uses the chicken β -actin hybrid (Cbh) promoter to provide expression that is high, long-term and uniform across cells, the liver-specific microRNA 122 target sequence to normalize expression in the liver, codon-optimized gene coding sequences to increase expression further, and two viral serotypes simultaneously (AAV9 and PHP.eB) to deliver genes to most tissues of the body (FIG. 5A). The resulting DAEUS system provided uniform gene transfer and gene expression across major tissues of the body, unlike their components AAV9 and PHP.eB alone (FIGS. 5B-5C). miRNA target sites are included to dampen too high expression in unwanted tissues. In particular, the liver-specific miRNA122 target site was included as the experiments with non-dampened ssAAV9 vectors demonstrated liver toxicity apparent from elevated alanine transaminase (ALT) levels (FIG. 6A). Addition of miR-122 target site decreased toxicity despite the use of more potent vectors (FIG. 6B). Furthermore, at least two serotypes (AAV9 and PHP.eB) were included because the experiments using a single serotype alone, even with an optimized self-complementary backbone containing the Cbh promoter and miR122 target sites showed highly unequal or unsatisfactory expression (FIGS. 5B-5C, FIGS. 7-9). In contrast, using DAEUS, fairly uniform, high level and long-term overexpression of several geroprotective genes in aged wild-type mice was demonstrated (FIGS. 7-9).

Achieving defined levels of gene transfer and transgene expression using DAEUS

To achieve optimal therapeutic efficacy, a defined level of transgene expression across various tissues is often required. The methods described herein employ DAEUS (consisting of multiple different AAV serotypes, such as AAV9, PHP.eB, AAV8, AAV2, etc. in a single cocktail, possibly in conjunction with miRNA target sites on the vector genome, such as miR122 target site, miR182 target site, etc.) to achieve target levels of gene transfer and expression across multiple tissues of the body.

To achieve a defined pattern of gene transfer and gene expression in a subject of a target species, first standard curves of the relationship between injected dose of a specific AAV serotype and the resulting gene transfer level and gene expression at the RNA and/or the protein level are created. To achieve this, individuals of the target

species are injected with a specific AAV serotype with doses ranging anywhere between 1×10^10 to 1×10^{18} AAV vector genomes copies (GC) per kg and the resulting gene transfer and gene expression at the RNA and/or protein levels are measured. This process is repeated singly for every serotype used in the AAV cocktail. This process is also repeated for every miRNA target site used. Additionally, this process is repeated for each pair of AAV serotypes used to estimate possible interaction effects.

Here, gene transfer is defined as AAV vector genome DNA per host cell nuclear genome DNA in a target tissue. RNA expression is defined as transgene RNA counts per million based on next generation sequencing or as transgene RNA levels normalized to host housekeeping gene levels as determined by reverse quantitative PCR or other quantitative RNA assay in a target tissue. Protein expression is defined as levels of transgene protein expression normalized to weight of input tissue, total protein or housekeeping gene protein levels, as assayed by Western Blot, Simple Western, ELISA, or other quantitative protein expression assays in a target tissue.

From these data, standard dose-response curves of AAV dose vs gene transfer and gene expression are estimated using linear or non-linear regression methods for each target tissue. Finally, for each target tissue, the equations derived from regression are summed, including interaction terms, for every AAV serotype and miRNA target site used, providing a model which consists of a set of equations, that allows prediction of the individual doses of AAV serotypes used in the cocktail to achieve target level of gene transfer and gene expression pattern. Any target species, target tissue, AAV serotype and miRNA target site can optionally be used in this method.

A prototype system, based on the methods described above, to achieve target levels of gene transfer in brain, tibialis anterior, heart, liver, and other organs and tissues of house mice (*Mus musculus*) was engineered. For this end, one embodiment of the DAEUS system employing serotypes AAV9 and PHP.eB and miR122 target site was used.

5-week old male C57BL6-J mice were injected with doses of approximately 5×10^{12} , 2×10^{13} , 5×10^{13} and 2×10^{14} AAV vector genomes per kg, at N=3 mice per group with the following serotypes and their combinations:

- 1) scAAV9-Cbh-GFP-miR122,
- 2) scAAV9-Cbh-GFP-miRScr (where miRNA target site is scrambled to remove its function),
- 3) scPHP.eB-Cbh-GFP-miR122
- 5 4) scPHP.eB-Cbh-GFP-miR122 together with scAAV9-Cbh-GFP-miRScr

From this, equations of dose-response curves of AAV dose to AAV gene transfer for brain, heart, liver and tibialis anterior were estimated using linear regression (FIG. 10). The specific equations for each serotype and for each tissue are listed in FIG. 10.

- 10 Interaction effects were estimated by summing the gene transfer levels observed in groups 1 and 3 for every tissue individually, and then comparing them to the observed gene transfer levels in group 4. If no interaction is present between AAV9 and PHP.eB, the sum of gene transfer from groups 1 and 3 for every tissue (Expected) should closely match gene transfer levels in group 4 for every tissue
- 15 respectively (Observed). Regression analysis of expected vs observed gene transfer levels indicated that Expected values matched to and correlated highly with Observed values ($r^2 = 0.9 \dots 0.999$) (FIG. 11). A two-way ANOVA analysis of interactions indicated no significant interaction between Expected and Observed (FIG. 11). These data indicate that interaction effects between AAV9 and PHP.eB at the doses tested
- 20 are very minimal or non-existent.

- It was then sought to achieve 5 different gene transfer levels of interest. Gene expression patterns were predicted using the model described above of 5 different combinations of AAV9 and PHP.eB doses and 5 groups of 5-week old male C57BL6-J mice were injected retro-orbitally with N=3 mice per group, with the following
- 25 cocktails:

- 1) 1.4×10^{14} GC/kg scAAV9-Cbh-GFP-miR122 + 1.9×10^{13} GC/kg scPHP.eB-Cbh-GFP-miR122
- 2) 1.9×10^{14} GC/kg scAAV9-Cbh-GFP-miR122 + 4.8×10^{12} GC/kg scPHP.eB-Cbh-GFP-miR122
- 30 3) 4.8×10^{13} GC/kg scAAV9-Cbh-GFP-miR122 + 1.9×10^{14} GC/kg scPHP.eB-Cbh-GFP-miR122
- 4) 2.4×10^{13} GC/kg scAAV9-Cbh-GFP-miR122 + 9.5×10^{12} GC/kg scPHP.eB-Cbh-

GFP-miR122

5) 4.8e12 GC/kg scAAV9-Cbh-GFP-miR122 + 4.8e13 GC/kg scPHP.eB-Cbh-GFP-miR122

The results indicated a high match between predicted (Predicted) and observed (Observed) gene transfer patterns (FIG. 12). The results also indicated a high correlation of predicted and observed gene transfer levels using linear regression (FIG. 13). This indicates that the DAEUS system, employed in a manner described above, accurately allows pre-determined levels of gene transfer to be achieved.

10 Use of DAEUS to treat Wolfram Syndrome II

To test the efficacy of DAEUS for treating progerias, lines of Cisd2 knockout mice were established in house (FIG. 14). These are the only non-transgenic Cisd2 knockout models in existence, as they were generated via CRISPR (as opposed to insertional mutagenesis for other models). This model was chosen because as stated above, loss of Cisd2 causes Wolfram Syndrome II, while overexpression of Cisd2 increases healthspan and lifespan in mice and possibly humans [2]. Therefore, Cisd2 gene therapy is potentially both a treatment for Wolfram Syndrome II (WSII) and a geroprotective gene therapy for the general population. With the goal of restoring uniform levels of Cisd2 expression, Cisd2 KO mice were treated with DAEUS-Cisd2 at a total dose of 2e13 vector genomes/kg across various stages of the disease. Treatment of mice with DAEUS-Cisd2 at this dose indeed resulted in uniform restoration of Cisd2 gene transfer (FIG. 15A) and Cisd2 protein expression to physiological levels across multiple tissues (FIG. 15B). This significantly decreased morbidity and mortality across all age groups tested (e.g., mice injected as neonates, at 2-4 months old, or at 7 months old) (FIGS. 15-16).

In mice injected as neonates, frailty, weight loss, activity, and vision (assayed as looming spot) were maintained at wild-type levels by DAEUS-Cis2 treatment in comparison to the untreated Cisd2 knockout mice, which saw increased morbidity in all of these functions (FIG. 17). Additionally, lifespan of DAEUS-Cisd2 treated mice was extended approximately two-fold compared to untreated controls (FIG. 17). In mice treated at 2-4 months old, frailty, weight loss, muscle strength (assayed as grid hand), and coordination (assayed as challenging beam crossing) were improved

compared to untreated controls (FIG. 18). In addition, lifespan increased by about two-fold (FIG. 18). Strikingly, in mice with advanced disease, DAEUS-Cisd2 treatment reversed weight loss, hair loss, kyphosis and other morbidities and extends lifespan by three-fold (FIG. 19). This data demonstrates that DAEUS-Cisd2 is a highly effective therapy for the prevention and treatment of Wolfram Syndrome II.

Use of DAEUS to extend lifespan of wild-type mice

Next, a DAEUS system was engineered to overexpress geroprotective genes Cisd2, Atg5, and PTEN in wild-type (not progeroid) mice with the goal of extending the lifespan of treated mice. For this purpose, the ability to overexpress Cisd2, Atg5, and PTEN above wild-type levels in wild-type mice was verified by delivering DAEUS-Atg5, DAEUS-PTEN, and DAEUS-Cisd2 at optimized doses into 18 month old wild-type mice, and measuring the resulting protein expression 1 month post-injection. In two sets of experiments, overexpression of all three genes using optimized doses of DAEUS across multiple major tissues of the body were demonstrated (FIG. 20). Then the effect of DAEUS-Cisd2 and DAEUS-PTEN treatment on the lifespan of wild-type C57BL6/J mice was tested. For this end, equal numbers of male and female 24 month old mice were injected retro-orbitally with 2e12vg/mouse of DAEUS-Cisd2, 1e12vg/mouse of DAEUS-PTEN, or 1e12vg/mouse of DAEUS-GFP or vehicle (FFB) as controls. The lifespans of the treated groups were then measured and the results indicated that DAEUS-Cisd2 and DAEUS-PTEN treated mice did show longer lifespans compared to DAEUS-GFP or vehicle treated mice (DAEUS-Cisd2: 7% increase in overall median lifespan and 38% increase in post-injection lifespan; DAEUS-PTEN: 7% increase in overall median lifespan and 37% increase in post-injection lifespan) (FIG. 21). The results demonstrate that the DAEUS system described herein can be used to overexpress the geroprotective genes and extend the lifespan of treated subjects.

Materials and Methods

Construction of plasmids and plasmid sequences

ssAAV9 and DAEUS vectors were constructed by DNA synthesis and cloning. The ITR to ITR sequence of DAEUS vectors were fully synthesized and

cloned into pAAV\SC\CMV\EGFP\WPRE\bGH-2 backbone (received from Vandenberghe lab) using standard molecular cloning. ssAAV9 vectors were partially synthesized and cloned into the AAV pCAG-FLEX2-tTA2-WPRE-bGHpA backbone (Addgene). For ssAAV9 vectors, native *Mus musculus* coding sequences were used.

- 5 For DAEUS vectors, Atg5 and PTEN coding sequences were codon optimized.

AAV production and purification

- HEK293 cells at 80% confluency from four 15cm dishes were seeded to a hyperflask, grown to 80% confluency and triple-transfected with AAV vector, Rep/Cap for AAV8 or AAV9 (Addgene 112864 and 112865) and pAd Δ F6 at 130ug :130ug :260ug per hyperflask respectively. Four days post-transfection, supernatant from a hyperflask was decanted into a 1L flask and 3ml Triton-X 100 (8787-100ML Millipore Sigma), 2.5 mg RNase A at 1mg/ml concentration (10109142001 Millipore Sigma), 25U/mL of Turbonuclease (ACGC80007 VitaScientific) and 56 μ l of 10% Pluronic F68 (24040032 Thermo Fisher) was added to the supernatant. The supernatant was then mixed, poured back into the hyperflask, and shaken on an orbital shaker at 150rpm at 37 °C for 1 hour to lyse the cells and remove plasmid DNA. Lysate was then decanted from the hyperflask, and the hyperflask washed with 140mL of DPBS (10010072 Life Tech) which was added to the rest of the lysate. The total lysate was then centrifuged at 4000g, 4 °C for 30 min, and the supernatant was filtered through a 0.45 μ m PES bottle-top filter (295-4545 Thermo Fisher) before loading onto HPLC.

- AAV purification was performed using AAVX POROS CaptureSelect (ThermoFisher Scientific) resin with 6.6mm X 100mm column (Glass, Omnifit, kinesis-USA) in an Akta Pure HPLC system containing an auxiliary sample pump (GE LifeSciences). The machine was setup at room temperature and all purifications were performed at room temperature (approximately 21 °C). Column volume [CV] for each purification was 1 mL. The chromatography column was pre-equilibrated with 10 [CV] of wash buffer 1X Tris-buffered Saline (1X TBS) (Boston Bioproducts), before application of the AAV lysate. Equilibration and all subsequent washes of the column were performed at a rate of 2ml/minute.

The clarified/filtered lysate containing the AAV virions was loaded at a rate of 1mL/minute onto AAVX POROS column, with total loading time ranging from 30 minutes for small-scale preparations to 700 minutes (overnight) for hyperflasks. In later purifications a loading rate of 1.5mL/min was also used to decrease total run
5 time and no decrease in purification efficiency was observed. The column containing bound AAV was then washed with 10 [CV] of 1X TBS, followed by washes of 5 [CV] of 2X TBS, 10 [CV] 20% EtOH and 10 [CV] 1X TBS wash. The bound AAV was eluted using a low-pH (pH 2.5...2.9) buffer of 0.2M Glycine in 1X TBS at a rate of 1ml/minute. Elution fractions were taken as 0.25 – 1 mL volumes per fraction. The
10 eluted virus solution was neutralized by adding 1M Tris-HCL (pH 8.0) at 1/10th of the fraction volume directly into the fraction collection tube prior to elution. Peak fractions based on UV (280 nm) absorption graphs were collected and buffer exchanged in final formulation buffer (FFB: 1X PBS, 172mM NaCl, 0.001% pluronic F68) and concentrated using an Amicon filter with a molecular weight cut-off of
15 50kDa (UFC905008 EMD Millipore) prior to virus titration.

In brief, viral titer and the genomic titer was determined by a quantitative PCR (TaqMan, Life Technologies). Real-time qPCR (7500 Real-Time PCR System; Applied Biosystems, Foster City, CA, USA) with BghpA-targeted primer-probes (GCCAGCCATCTGTTGT (SEQ ID NO: 1), GGAGTGGCACCTTCCA (SEQ ID
20 NO: 2), 6FAM-TCCCCCGTGCCTTCCTTGACC-TAMRA (SEQ ID NO: 3)) was used. Linearized CBA-EGFP DNA was used at a series of dilutions of known concentration as a standard. After 95 °C holding stage for 10 seconds, two-step PCR cycling stage was performed at 95 °C for 5 seconds, followed by 60 °C for 5 seconds for 40 cycles. Genomic vector titers were interpolated from the standard and
25 expressed as vector genomes per milliliter.

DNA and protein quantification

Tissues were homogenized by disrupting 30mg of tissue in 1mL of RLT+ buffer for DNA and RNA and 1mL of RIPA buffer containing 1X Halt protease and
30 phosphatase inhibitors for protein (78444 Thermo Fisher Sci). For disruption, samples, buffer and 1mm Zirconia/Silica beads (11079110z Biospec) were loaded into XXTuff vials (330TX BioSpec) and disrupted using Mini Beadbeater 24 (112011

BioSpec) at max speed for 3 minutes. Vials were then placed on ice for 2-5 minutes for RNA and 1 hour for protein, centrifuged at 10,000g for 3 min and supernatant used for further procedures.

For DNA/RNA, 700 µL of supernatant was loaded onto AllPrep DNA Mini Spin Columns and purified using AllPrep DNA/RNA/miRNA Universal Kit (80224 Qiagen) for quadriceps and Allprep DNA/RNA mini kit (80204 Qiagen) for brain and liver. Purification was performed on Qiacube Connect (9002864 Qiagen).

Total AAV copy number was assessed using BghpA primers and linearized CBA-GFP plasmid dilution series as standard for AAV copy number (GCCAGCCATCTGTTGT (SEQ ID NO: 1), GGAGTGGCACCTTCCA (SEQ ID NO: 2), 6FAM-TCCCCCGTGCCTTCCTTGACC-TAMRA (SEQ ID NO: 3)). Total genome copy number was estimated using RPII primers-probes (GTTTTTCATCACTGTTCATGATGC (SEQ ID NO: 4), TCATGGGCATTACTATTCCTAC (SEQ ID NO: 5), probe: VIC-AGGACCAGCTTCTCTGCATTATCATCGTTGAAGAT-3IABkFQ (SEQ ID NO: 6)) along with a standard of gDNA dilution series of known concentration. AAV copy number per diploid genome was then calculated as

$$\text{copy number per diploid genome} = 2 * \left(\frac{\text{total AAV copy number}}{\text{total genome copy number}} \right).$$

Efficiency and specificity of amplification for both primer-probe sets was previously established, and amplification was performed using Luna Universal Probe qPCR Master Mix (M3004L NEB) at thermocycling conditions recommended by the manufacturer.

For quantification of protein expression, protein lysate was first diluted 5x twice in fresh RIPA+Halt inhibitors buffer and all dilutions were assayed for total protein content using Pierce™ BCA Protein Assay Kit (23225 Thermo Fisher). For each tissue type, lysates were then diluted in RIPA+Halt inhibitors buffer to the concentration where they would be at the lower end of the linear range. For GFP, anti-GFP antibody ab290 (ab290 Abcam) was used. For Cisd2, PTEN and Atg5, anti-Cisd2 (13318-1-AP Proteintech), anti-Atg5 (NB110-53818 Novus) and anti-PTEN D4.3 (Cell Signaling) antibodies, respectively, were used. Linear range for protein quantification was previously determined by assaying each protein separately using 12-230 kDa Jess or Wes Separation Module (SM-W004 Protein Simple) on Wes with

ab290 for dilutions ranging from 3µg/µl...0.03µg/µl for each tissue. Linear range for total protein was also previously determined by assaying total protein in the range of 4µg/µl...0.1µg/µl using Total Protein Detection Module (DM-TP01 Protein Simple) (linear range: <1µg/µl for all tissues tested). GFP, Atg5, Cisd2 and PTEN as well as
5 total protein levels were then assayed and GFP and total protein quantified using Compass for SW 4.1 (Protein Simple). Finally, GFP was normalized to total protein to arrive at the final value.

Animal experiments

10 Mice were housed in standard ventilated racks at a maximum density of 5 mice per cage. Room temperature was maintained at 22 °C with 30%–70% humidity. Mice were kept on a 12-hour light/dark cycle and provided food and water ad libitum. Breeder mice were kept on irradiated PicoLab Mouse Diet 20 5058 (LabDiet, St. Louis, MO), and non-breeder mice were kept on irradiated LabDiet Prolab Isopro
15 RMH 3000 5P75 (LabDiet, St. Louis, MO). Cages were filled with 1/4 inch Anderson's Bed o Cob bedding (The Andersons, Inc., Maumee, OH) and every cage contained three nestlet (2 3 2'' compressed cotton square, Ancare, Bellmore, NY) and one red mouse hut (certified polycarbonate; 3 3/4'' wide x 1 7/8'' tall x 3'' long, BioServ, Flemington, NJ). Cage changes were performed at least every 14 days, and
20 more frequently if necessary. Animal health surveillance was performed quarterly by PCR testing of index animals and through swabs from rack plenums
AAV was injected retro-orbitally under isofluorane anesthesia in a volume of 150 µl per mouse at various total doses as described in text and in figures. AAV9 and PHP.eB were used in 1:1 ratios for injections of DAEUS-Atg5, DAEUS-Cisd2,
25 DAEUS-GFP and DAEUS-PTEN, 8-week old or 18-month old wild-type C57BL/6J mice were used as described in text and in figures. Mice were CO2 euthanized 28 days post-injection and tissues and serum collected for analysis, except as otherwise noted in the text and in figures. Serum ALT levels were quantified by UMass Mouse Metabolic Phenotyping Center.

30

Generation of Cisd2 KO mice and frailty measurements

Cisd2 knockout mice were generated via microinjection of C57BL6/J fertilized oocytes with SpCas9 protein and three guide RNAs targeting Exon 2 of Cisd2 (AGCGCAAGTACCCCGAGGAA (SEQ ID NO: 7), CCCCGAGGAAGGGCAGTAGG (SEQ ID NO: 8), TGCTGTGTTTCAGTTTCAGAC (SEQ ID NO: 9)). Founders were then genotyped and Sanger sequenced (primers AGCCCTAAGTTTCTCCGAGTTC (SEQ ID NO: 10), GTGACATGTGGTGCTGTAGAAC (SEQ ID NO: 11)), and founders with loss-of-function mutation bred to WT C57BL6/J. Pups were then backcrossed further to WT C57BL6/J mice. Heterozygous pups of this backcross were then bred to arrive at homozygous Cisd2 knockout mice. Two lines were bred further (Line 6: deletion of 780bp, deletion of whole exon 2 and Line 14: deletion of 261bp, frameshift due to deletion of most of exon 2, 4bp left at 3' of exon 2). Loss of Cisd2 expression was confirmed via Simple Wes (not shown). Mice were then weighed at intervals and frailty assessed 4 months post-injection. Frailty was assessed blinded as the weighted sum of 31 morbidity related measures as described in Whitehead et al. [14], with the exception that non-informative measures (measures that were 0 or 1 across all mice) were excluded from final analysis.

Statistical analysis

All data was visualized and statistical analysis was performed in GraphPad Prism (GraphPad). Specific statistical tests used are listed in figure legends for each test, and all tests were performed with default settings unless otherwise specified.

Exemplary viral vectors

LOCUS scAAV-CbhM-Atg5(GS)-miR 5237 bp ds-DNA circular
DEFINITION .

FEATURES Location/Qualifiers

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        /ApEinfo_fwdcolor=#f8d3a9
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 15 /ApEinfo_fwdcolor=#b7e6d7
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 20 misc_feature 1472..1700
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 misc_feature 1713..2540

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 promoter 1194..1471
 25 /label="chicken beta-actin promoter"
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LOCUS scAAV-CbhM-Cisd2-miR122 4817 bp ds-DNA circular

DEFINITION .

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		/ApEinfo_fwdcolor=#ff9ccd

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CDS 2751..3611

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15

LOCUS scAAV-CbhM-GFP-miR122-8 5129 bp ds-DNA circular

DEFINITION

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LOCUS scAAV-CbhM-PTEN-miR122- 5283 bp ds-DNA circular

DEFINITION

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 30 5221 cctcgtctg ctaatcctgt taccagtggc tgctgccagt ggcgataagt cgtgtcttac
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LOCUS AAV_pCAG-Atg5-WPRE-bGHp 6325 bp ds-DNA circular

DEFINITION

KEYWORDS "accession:addgene_65455_110978"

FEATURES Location/Qualifiers

- 5 misc_feature 2957..3050
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/ApEinfo_fwdcolor=#75c6a9
- misc_feature 2930..2956
- 15 /label="oMF209"
/ApEinfo_revcolor=#c7b0e3
/ApEinfo_fwdcolor=#c7b0e3
- misc_feature 4064..4088
- 20 /label="oMF65"
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/ApEinfo_fwdcolor=#d6b295
- misc_feature 1267..1295
- 25 /label="AmpR promtoer"
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- misc_feature 2958..4027
- 30 /label="chicken beta actin exon-intron-rabbit beta globin intron"
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- 30 /label="Atg5 STS"
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ORIGIN

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References

1. Niccoli, T. *et al.* Ageing as a risk factor for disease. *Curr. Biol.* 22, R741-52
 30 (2012). PMID:22975005
2. CDC. The State of Aging and Health in America 2013. (2013).

3. de Magalhães, J. P. *et al.* The Human Ageing Genomic Resources: online databases and tools for biogerontologists. *Aging Cell* 8, 65–72 (2009). PMID:18986374

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CLAIMS

What is claimed is:

1. A viral vector delivery system comprising two or more viral serotypes engineered for delivery of a single gene.
2. The viral vector delivery system of claim 1, comprising 5-10 viral serotypes for delivery of the gene.
3. The viral vector delivery system of claim 1, wherein the two or more viral serotypes are adeno-associated viral serotypes.
4. The viral vector delivery system of claim 1, wherein the two or more viral serotypes are selected from the group consisting of AAV1, AAV2, AAV3, AAV3B, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, Anc80, AAVrh10, AAV-DJ, AAV-DJ/8, AAV-PHP.B, AAV-PHP.S, AAV-PHP.eB, AAV.CAP-B10, AAV.CAP-B22, and AAVMYO.
5. The viral vector delivery system of claim 1, wherein the two or more viral serotypes are selected from the group consisting of AAV1, AAV2, AAV3, AAV3B, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, Anc80, and PHP.eB.
6. The viral vector delivery system of claim 1, wherein the two or more viral serotypes are selected from Table 1.
7. The viral vector delivery system of claim 1, wherein at least one viral serotype is AAV9.
8. The viral vector delivery system of claim 1, wherein at least one viral serotype is PHP.eB.

9. The viral vector delivery system of claim 1, wherein the two or more viral serotypes include AAV9 and PHP.eB.
10. The viral vector delivery system of any one of claims 1-9, further comprising a miRNA target site.
11. The viral vector delivery system of claim 10, wherein the miRNA target site is selected based on a tissue target.
12. The viral vector delivery system of claim 11, wherein the tissue target is selected from the group consisting of aorta, endothelium, cardiac muscle skeletal muscle, tongue, esophagus, stomach, small intestine, large intestine, diaphragm, eye, optic nerve, inner ear, auditory nerve, brown fat, white fat, central nervous system, peripheral nervous system, kidney, spleen, liver, lung, heart, brain, thymus, ovaries, testes, skin, pancreas, bone marrow cells, osteoblasts and osteoclasts, blood cells, hematopoietic stem cells, and muscle satellite cells.
13. The viral vector delivery system of claim 11, wherein the tissue target is selected from the group consisting of cardiac, liver, muscle, and brain.
14. The viral vector delivery system of any one of claims 10-13, wherein the miRNA target site is selected from the group consisting of miRNA-1, miRNA-24, miRNA-29, miRNA-30c, miRNA-33, miRNA-122, miRNA-124, miRNA-128, miRNA-133, miRNA-144, miRNA-148a, miRNA-208a, miRNA-208b, miRNA-223, and miRNA-499.
15. The viral vector delivery system of any one of claims 10-14, wherein a target tissue is cardiac tissue and the miRNA target site is selected from the group consisting of miRNA-1, miRNA-133, miRNA-208a, miRNA-208b, and miRNA-499.
16. The viral vector delivery system of any one of claims 10-14, wherein a target tissue is liver tissue and the miRNA target site is selected from the group

consisting of miRNA-24, miRNA-29, miRNA-30c, miRNA-33, miRNA-122, miRNA-144, miRNA-148a, and miRNA-223.

17. The viral vector delivery system of any one of claims 10-14, wherein a target tissue is muscle tissue and the miRNA target site is miRNA-1 or miRNA-133.
18. The viral vector delivery system of any one of claims 10-14, wherein a target tissue is brain tissue and the miRNA target site is miRNA-124 or miRNA-128.
19. The viral vector delivery system of any one of claims 1-18, further comprising a non-silencing promoter.
20. The viral vector delivery system of claim 19, wherein the non-silencing promoter leads to RNA expression of at least 30% of CMV promoter expression.
21. The viral vector delivery system of claim 19 or claim 20, wherein the non-silencing promoter leads to RNA expression of at least 50% of CMV promoter expression.
22. The viral vector delivery system of any one of claims 19-21, wherein the promoter is selected from the group consisting of Cbh, CAG, CB7, and CBA.
23. The viral vector delivery system of any one of claims 1-22 further comprising a self-complementary vector backbone.
24. The viral vector delivery system of any one of claims 1-23, wherein the gene is selected from Table 2.
25. The viral vector delivery system of any one of claims 1-24, wherein the gene is selected from the group consisting of Cisd2, Atg5, and PTEN.
26. A pharmaceutical composition comprising the viral vector delivery system of any one of claims 1-25.
27. A method of treating a disease or disorder in a subject comprising administering the pharmaceutical composition of claim 26 to the subject.

28. A method of extending the lifespan of a subject comprising administering the pharmaceutical composition of claim 26 to the subject.
29. A method of treating or preventing a disease or disorder comprising administering to a subject a viral vector delivery system comprising at least two viral serotypes engineered for delivery of a single gene.
30. The method of claim 29, wherein the viral vector delivery system comprises 5-10 viral serotypes for delivery of the gene.
31. The method of claim 29, wherein the two or more viral serotypes are adeno-associated viral serotypes.
32. The method of claim 29, wherein the two or more viral serotypes are selected from the group consisting of AAV1, AAV2, AAV3, AAV3B, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, Anc80, AAVrh10, AAV-DJ, AAV-DJ/8, AAV-PHP.B, AAV-PHP.S, AAV-PHP.eB, AAV.CAP-B10, AAV.CAP-B22, and AAVMYO.
33. The method of claim 29, wherein the two or more viral serotypes are selected from the group consisting of AAV1, AAV2, AAV3, AAV3B, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, Anc80, and PHP.eB.
34. The method of claim 29, wherein the two or more viral serotypes are selected from Table 1
35. The method of claim 29, wherein at least one viral serotype is AAV9.
36. The method of claim 29, wherein at least one viral serotype is PHP.eB.
37. The method of claim 29, wherein the two or more viral serotypes include AAV9 and PHP.eB.
38. The method of any one of claims 29-37, wherein the viral vector delivery system further comprises an miRNA target site.

39. The method of claim 38, wherein the miRNA target site is selected based on a tissue target.
40. The method of claim 39, wherein the tissue target is selected from the group consisting of aorta, endothelium, cardiac muscle skeletal muscle, tongue, esophagus, stomach, small intestine, large intestine, diaphragm, eye, optic nerve, inner ear, auditory nerve, brown fat, white fat, central nervous system, peripheral nervous system, kidney, spleen, liver, lung, heart, brain, thymus, ovaries, testes, skin, pancreas, bone marrow cells, osteoblasts and osteoclasts, blood cells, hematopoietic stem cells, and muscle satellite cells.
41. The method of claim 39, wherein the tissue target is selected from the group consisting of cardiac, liver, muscle, and brain.
42. The method of any one of claims 38-41, wherein the miRNA target site is selected from the group consisting of miRNA-1, miRNA-24, miRNA-29, miRNA-30c, miRNA-33, miRNA-122, miRNA-124, miRNA-128, miRNA-133, miRNA-144, miRNA-148a, miRNA-208a, miRNA-208b, miRNA-223, and miRNA-499.
43. The method of any one of claims 38-42, wherein a target tissue is cardiac tissue and the miRNA target site is selected from the group consisting of miRNA-1, miRNA-133, miRNA-208a, miRNA-208b, and miRNA-499.
44. The method of any one of claims 38-42, wherein a target tissue is liver tissue and the miRNA target site is selected from the group consisting of miRNA-24, miRNA-29, miRNA-30c, miRNA-33, miRNA-122, miRNA-144, miRNA-148a, and miRNA-223.
45. The method of any one of claims 38-42, wherein a target tissue is muscle tissue and the miRNA target site is miRNA-1 or miRNA-133.
46. The method of any one of claims 38-42, wherein a target tissue is brain tissue and the miRNA target site is miRNA-124 or miRNA-128.

47. The method of any one of claims 29-46, wherein the viral vector delivery system further comprises a non-silencing promoter.
48. The method of claim 47, wherein the non-silencing promoter leads to RNA expression of at least 30% of CMV promoter expression.
49. The method of claim 47 or claim 48, wherein the non-silencing promoter leads to RNA expression of at least 50% of CMV promoter expression.
50. The method of any one of claims 47-49, wherein the promoter is selected from the group consisting of Cbh, CAG, CB7, and CBA.
51. The method of any one of claims 29-50, wherein the viral vector delivery system further comprises a self-complementary vector backbone.
52. The method of any one of claims 29-51, wherein the gene is selected from Table 2.
53. The method of any one of claims 29-52, wherein the gene is selected from the group consisting of Cisd2, Atg5, and PTEN.
54. The method of any one of claims 29-53, wherein the disease or disorder is an aging related disease or disorder.
55. The method of any one of claims 29-54, wherein the disease or disorder is selected from the group consisting of progeria syndrome, Wolfram Syndrome, neurodegenerative disorder, neurovascular disorder, skeletal muscle conditions, Cowden syndrome, Bannayan-Riley-Ruvalcaba syndrome, Proteus syndrome, Proteus-like syndrome and other PTEN-opathies. Werner syndrome, Bloom syndrome, Rothmund–Thomson syndrome, Cockayne syndrome, xeroderma pigmentosum, trichothiodystrophy, combined xeroderma pigmentosum-Cockayne syndrome, Hutchinson-Gilford Progeria syndrome, restrictive dermopathy, diabetes, obesity, cardiovascular disease, cancer, ocular degeneration, liver failure, and age-related macular degeneration.

56. The method of any one of claims 29-54, wherein the disease or disorder would benefit from administration of the gene to two or more tissue targets.
57. The method of any one of claims 29-54, wherein the disease or disorder is Wolfram Syndrome II.
58. The method of any one of claims 29-57, wherein the gene is expressed in two or more tissues in the subject.
59. The method of any one of claims 29-58, wherein the gene is uniformly expressed across two or more tissues in the subject.
60. The method of any one of claims 29-58, wherein the gene is uniformly overexpressed across two or more tissues in the subject.
61. The method of any one of claims 29-58, wherein the gene is delivered to at least 50% of tissues in the subject.
62. The method of any one of claims 29-61, wherein the gene is expressed for at least 4 months in the subject.
63. A viral vector delivery system comprising two or more AAV serotypes engineered for delivery of a single gene, a non-silencing promoter, at least one miRNA target site, the gene, and optionally a self-complementary backbone.
64. The viral vector delivery system of claim 63, wherein the AAV serotypes are AAV9 and PHP.eB.
65. The viral vector delivery system of claim 63 or claim 64, wherein the gene is selected from the group consisting of Cisd2, Atg5, and PTEN.
66. A method of treating a disease or disorder comprising administering to a subject the viral vector delivery system of any one of claims 63-65.
67. The method of claim 66, wherein the disease or disorder is Wolfram Syndrome II.

68. A viral vector delivery system comprising at least one viral serotype engineered for delivery of a single gene selected from the group consisting of Cisd2, Atg5, and PTEN.
69. The viral vector delivery system of claim 68, wherein the at least one viral serotype is selected from the group consisting of AAV1, AAV2, AAV3, AAV3B, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, Anc80, AAVrh10, AAV-DJ, AAV-DJ/8, AAV-PHP.B, AAV-PHP.S, AAV-PHP.eB, AAV.CAP-B10, AAV.CAP-B22, and AAVMYO.
70. The viral vector delivery system of claim 68, wherein the at least one viral serotype is selected from the group consisting of AAV1, AAV2, AAV3, AAV3B, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, Anc80, and PHP.eB.
71. The viral vector delivery system of claim 68, wherein the at least one viral serotype is selected from Table 1.
72. The viral vector delivery system of claim 68, wherein at least one viral serotype is AAV9.
73. The viral vector delivery system of claim 68, wherein at least one viral serotype is PHP.eB.
74. The viral vector delivery system of any one of claims 68-73, further comprising a miRNA target site.
75. The viral vector delivery system of 74, wherein the miRNA target site is selected based on a tissue target.
76. The viral vector delivery system of claim 75, wherein the tissue target is selected from the group consisting of aorta, endothelium, cardiac muscle, skeletal muscle, tongue, esophagus, stomach, small intestine, large intestine, diaphragm, eye, optic nerve, inner ear, auditory nerve, brown fat, white fat, central nervous system, peripheral nervous system, kidney, spleen, liver, lung, heart, brain, thymus, ovaries, testes, skin, pancreas, bone marrow cells,

osteoblasts and osteoclasts, blood cells, hematopoietic stem cells, and muscle satellite cells.

77. The viral vector delivery system of claim 75, wherein the tissue target is selected from the group consisting of cardiac, liver, muscle, and brain.
78. The viral vector delivery system of any one of claims 74-77, wherein the miRNA target site is selected from the group consisting of miRNA-1, miRNA-24, miRNA-29, miRNA-30c, miRNA-33, miRNA-122, miRNA-124, miRNA-128, miRNA-133, miRNA-144, miRNA-148a, miRNA-208a, miRNA-208b, miRNA-223, and miRNA-499.
79. The viral vector delivery system of any one of claims 74-78, wherein a target tissue is cardiac tissue and the miRNA target site is selected from the group consisting of miRNA-1, miRNA-133, miRNA-208a, miRNA-208b, and miRNA-499.
80. The viral vector delivery system of any one of claims 74-78, wherein a target tissue is liver tissue and the miRNA target site is selected from the group consisting of miRNA-24, miRNA-29, miRNA-30c, miRNA-33, miRNA-122, miRNA-144, miRNA-148a, and miRNA-223.
81. The viral vector delivery system of any one of claims 74-78, wherein a target tissue is muscle tissue and the miRNA target site is miRNA-1 or miRNA-133.
82. The viral vector delivery system of any one of claims 74-78, wherein a target tissue is brain tissue and the miRNA target site is miRNA-124 or miRNA-128.
83. The viral vector delivery system of any one of claims 68-82, further comprising a non-silencing promoter.
84. The viral vector delivery system of claim 83, wherein the non-silencing promoter leads to RNA expression of at least 30% of CMV promoter expression.

85. The viral vector delivery system of claim 83 or claim 84, wherein the non-silencing promoter leads to RNA expression of at least 50% of CMV promoter expression.
86. The viral vector delivery system of any one of claims 83-85, wherein the promoter is selected from the group consisting of Cbh, CAG, CB7, and CBA.
87. The viral vector delivery system of any one of claims 68-86 further comprising a self-complementary vector backbone.
88. A pharmaceutical composition comprising the viral vector delivery system of any one of claims 68-87.
89. A method of treating a disease or disorder in a subject comprising administering the pharmaceutical composition of claim 88 to the subject.
90. The method of claim 89, wherein the disease is Wolfram Syndrome II.
91. A method of extending the lifespan of a subject comprising administering the pharmaceutical composition of claim 88 to the subject.
92. A method of extending the lifespan of a subject comprising administering to a subject a viral vector delivery system comprising at least one viral serotype engineered for delivery of a single gene.
93. A method of treating or preventing a disease or disorder comprising administering to a subject a viral vector delivery system comprising at least one viral serotype engineered for delivery of a single gene.
94. The method of claim 92 or claim 93, wherein the at least one viral serotype is an adeno-associated viral serotype.
95. The method of claim 92 or claim 93, wherein the at least one viral serotype is selected from the group consisting of AAV1, AAV2, AAV3, AAV3B, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, Anc80, AAVrh10,

AAV-DJ, AAV-DJ/8, AAV-PHP.B, AAV-PHP.S, AAV-PHP.eB, AAV.CAP-B10, AAV.CAP-B22, and AAVMYO.

96. The method of claim 92 or claim 93, wherein the at least one viral serotype is selected from the group consisting of AAV1, AAV2, AAV3, AAV3B, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, Anc80, and PHP.eB.
97. The method of claim 92 or claim 93, wherein the at least one viral serotype is selected from Table 1
98. The method of claim 92 or claim 93, wherein the at least one viral serotype is AAV9.
99. The method of claim 92 or claim 93, wherein the at least one viral serotype is PHP.eB.
100. The method of any one of claims 92-99, wherein the viral vector delivery system further comprises an miRNA target site.
101. The method of claim 100, wherein the miRNA target site is selected based on a tissue target.
102. The method of claim 101, wherein the tissue target is selected from the group consisting of aorta, endothelium, cardiac muscle skeletal muscle, tongue, esophagus, stomach, small intestine, large intestine, diaphragm, eye, optic nerve, inner ear, auditory nerve, brown fat, white fat, central nervous system, peripheral nervous system, kidney, spleen, liver, lung, heart, brain, thymus, ovaries, testes, skin, pancreas, bone marrow cells, osteoblasts and osteoclasts, blood cells, hematopoietic stem cells, and muscle satellite cells.
103. The method of claim 101, wherein the tissue target is selected from the group consisting of cardiac, liver, muscle, and brain.
104. The method of any one of claims 100-103, wherein the miRNA target site is selected from the group consisting of miRNA-1, miRNA-24, miRNA-29, miRNA-30c, miRNA-33, miRNA-122, miRNA-124, miRNA-128, miRNA-

133, miRNA-144, miRNA-148a, miRNA-208a, miRNA-208b, miRNA- 223, and miRNA-499.

105. The method of any one of claims 100-104, wherein a target tissue is cardiac tissue and the miRNA target site is selected from the group consisting of miRNA-1, miRNA-133, miRNA-208a, miRNA-208b, and miRNA-499.
106. The method of any one of claims 100-104, wherein a target tissue is liver tissue and the miRNA target site is selected from the group consisting of miRNA-24, miRNA-29, miRNA-30c, miRNA-33, miRNA-122, miRNA-144, miRNA-148a, and miRNA-223.
107. The method of any one of claims 100-104, wherein a target tissue is muscle tissue and the miRNA target site is miRNA-1 or miRNA-133.
108. The method of any one of claims 100-104, wherein a target tissue is brain tissue and the miRNA target site is miRNA-124 or miRNA-128.
109. The method of any one of claims 92-108, wherein the viral vector delivery system further comprises a non-silencing promoter.
110. The method of claim 109, wherein the non-silencing promoter leads to RNA expression of at least 30% of CMV promoter expression.
111. The method of claim 109 or claim 110, wherein the non-silencing promoter leads to RNA expression of at least 50% of CMV promoter expression.
112. The method of any one of claims 109-111, wherein the promoter is selected from the group consisting of Cbh, CAG, CB7, and CBA.
113. The method of any one of claims 92-112, wherein the viral vector delivery system further comprises a self-complementary vector backbone.
114. The method of any one of claims 92-113, wherein the gene is selected from Table 2.

115. The method of any one of claims 92-114, wherein the gene is selected from the group consisting of Cisd2, Atg5, and PTEN.
116. The method of any one of claims 93-115, wherein the disease or disorder is an aging related disease or disorder.
117. The method of any one of claims 93-116, wherein the disease or disorder is selected from the group consisting of progeria syndrome, Wolfram Syndrome, neurodegenerative disorder, neurovascular disorder, skeletal muscle conditions, Cowden syndrome, Bannayan-Riley-Ruvalcaba syndrome, Proteus syndrome, Proteus-like syndrome and other PTEN-opathies. Werner syndrome, Bloom syndrome, Rothmund–Thomson syndrome, Cockayne syndrome, xeroderma pigmentosum, trichothiodystrophy, combined xeroderma pigmentosum-Cockayne syndrome, Hutchinson-Gilford Progeria syndrome, restrictive dermopathy, diabetes, obesity, cardiovascular disease, cancer, ocular degeneration, liver failure, and age-related macular degeneration.
118. The method of any one of claims 93-117, wherein the disease or disorder is Wolfram Syndrome II.
119. A method of treating Wolfram Syndrome II comprising administering an effective amount of Cisd2 to a subject suffering from Wolfram Syndrome II.
120. The method of claim 119, wherein Cisd2 is administered to the subject via gene therapy.
121. The method of claim 119 or claim 120, wherein Cisd2 is administered to the subject via a viral vector delivery system.
122. The method of claim 121, wherein the viral vector delivery system comprises at least one viral serotype.
123. The method of claim 121, wherein the viral vector delivery system comprises two or more viral serotypes.

124. A method of identifying a pre-determined level of gene transfer in one or more target tissues of a subject comprising:
- obtaining a dose-response curve characterizing the relationship between an amount of a vector administered to the subject and a resulting gene transfer level in the one or more target tissues;
 - obtaining a linear or non-linear equation characterizing the relationship between the amount of vector administered to the subject and the resulting gene transfer level in the one or more target tissues; and
 - interpolating or extrapolating a required dose of a gene delivery system to achieve a defined level of gene transfer in the one or more target tissues.
125. A method of identifying a pre-determined level of transgene expression in one or more target tissues of a subject comprising:
- obtaining a dose-response curve characterizing the relationship between an amount of a vector administered to the subject and a resulting transgene expression level in the one or more target tissues;
 - obtaining a linear or non-linear equation characterizing the relationship between the amount of vector administered to the subject and the resulting transgene expression level in the one or more target tissues; and
 - interpolating or extrapolating a required dose of a gene delivery system to achieve a defined level of transgene expression in the one or more target tissues.
126. The method of claim 124 or claim 125, wherein the gene delivery system comprises at least one viral serotype.
127. The method of claim 124 or claim 125, wherein the gene delivery system comprises two or more viral serotypes.
128. The method of claim 126 or claim 127, wherein the viral serotype is an adeno-associated viral serotype.
129. The method of any one of claims 126-128, wherein the viral serotype is selected from the group consisting of AAV1, AAV2, AAV3, AAV3B, AAV4,

AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, Anc80, AAVrh10, AAV-DJ, AAV-DJ/8, AAV-PHP.B, AAV-PHP.S, AAV-PHP.eB, AAV.CAP-B10, AAV.CAP-B22, and AAVMYO.

130. The method of any one of claims 126-128, wherein the viral serotype is selected from the group consisting of AAV1, AAV2, AAV3, AAV3B, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, Anc80, and PHP.eB.
131. The method of any one of claims 126-128, wherein the viral serotype is selected from Table 1.
132. The method of any one of claims 124-131, wherein the one or more target tissues comprise a single tissue.
133. The method of any one of claims 124-131, wherein the one or more target tissues comprise two or more tissues.
134. The method of any one of claims 124-133, wherein the one or more target tissues are selected from the group consisting of aorta, endothelium, cardiac muscle skeletal muscle, tongue, esophagus, stomach, small intestine, large intestine, diaphragm, eye, optic nerve, inner ear, auditory nerve, brown fat, white fat, central nervous system, peripheral nervous system, kidney, spleen, liver, lung, heart, brain, thymus, ovaries, testes, skin, pancreas, bone marrow cells, osteoblasts and osteoclasts, blood cells, hematopoietic stem cells, and muscle satellite cells.
135. The method of any one of claims 124-133, wherein the one or more target tissues are selected from the group consisting of cardiac, liver, muscle, and brain.

FIG. 1A

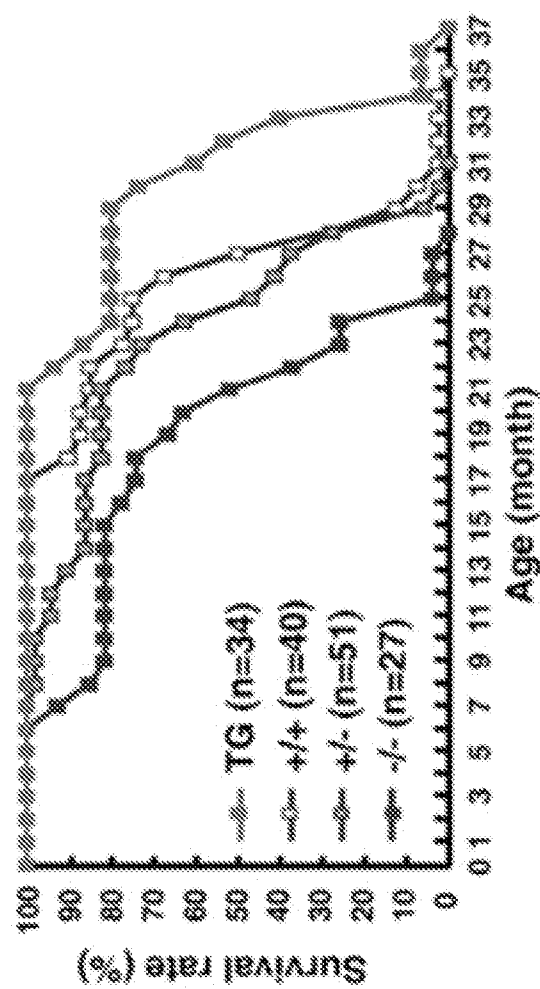
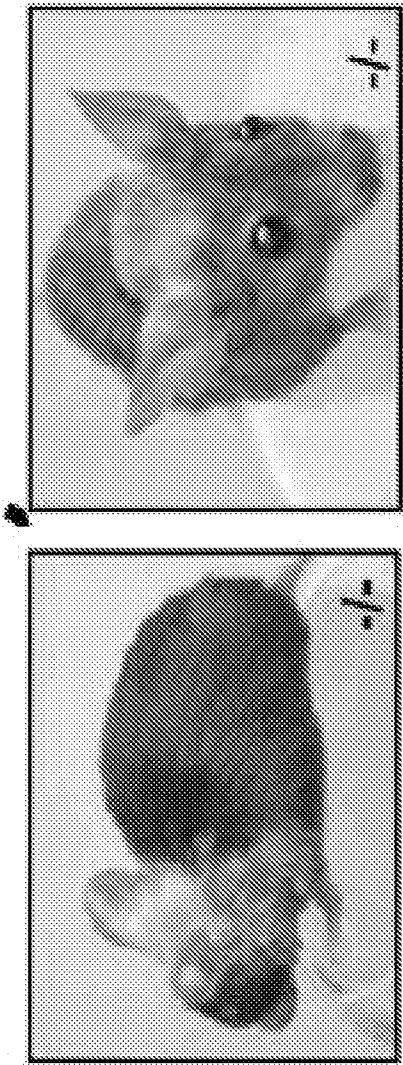


FIG. 1B



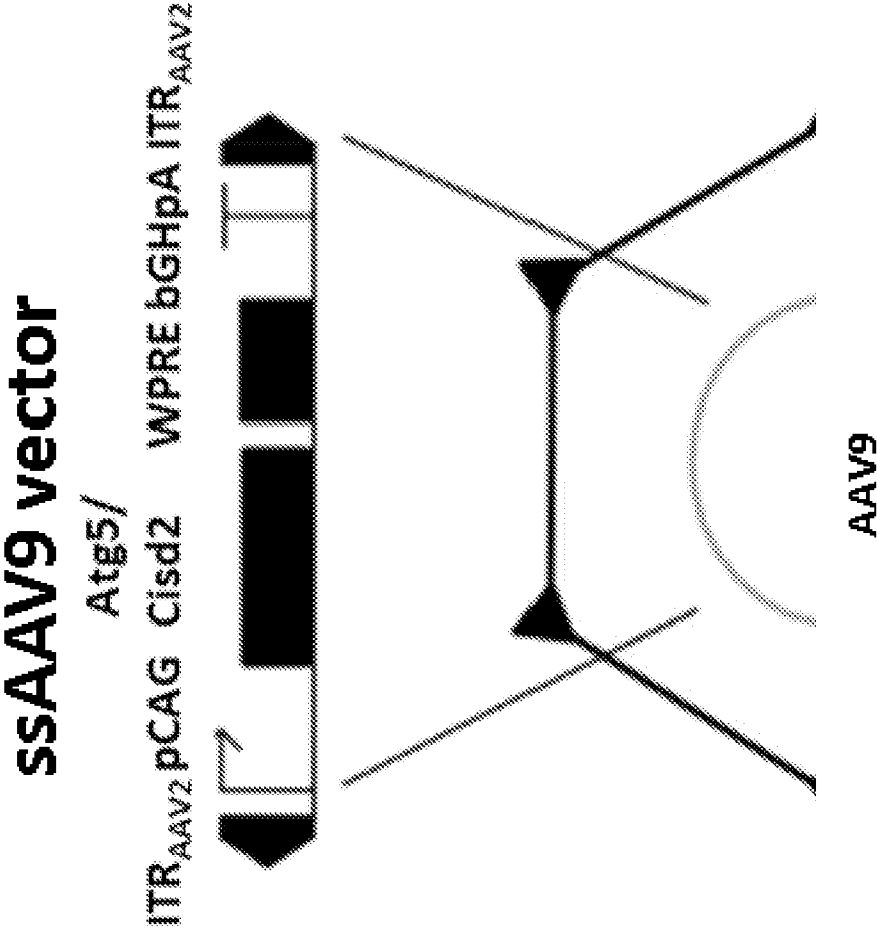


FIG. 2A

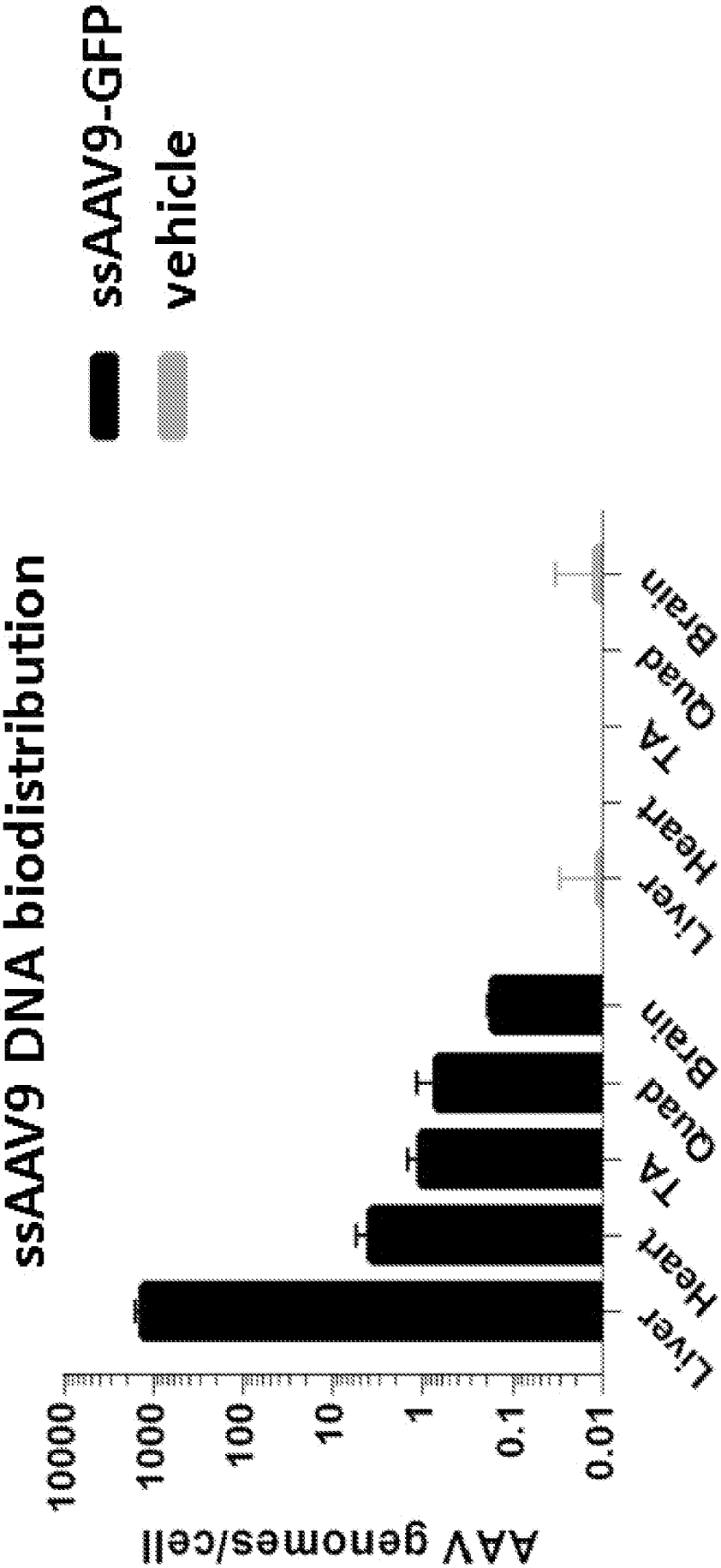


FIG. 2B

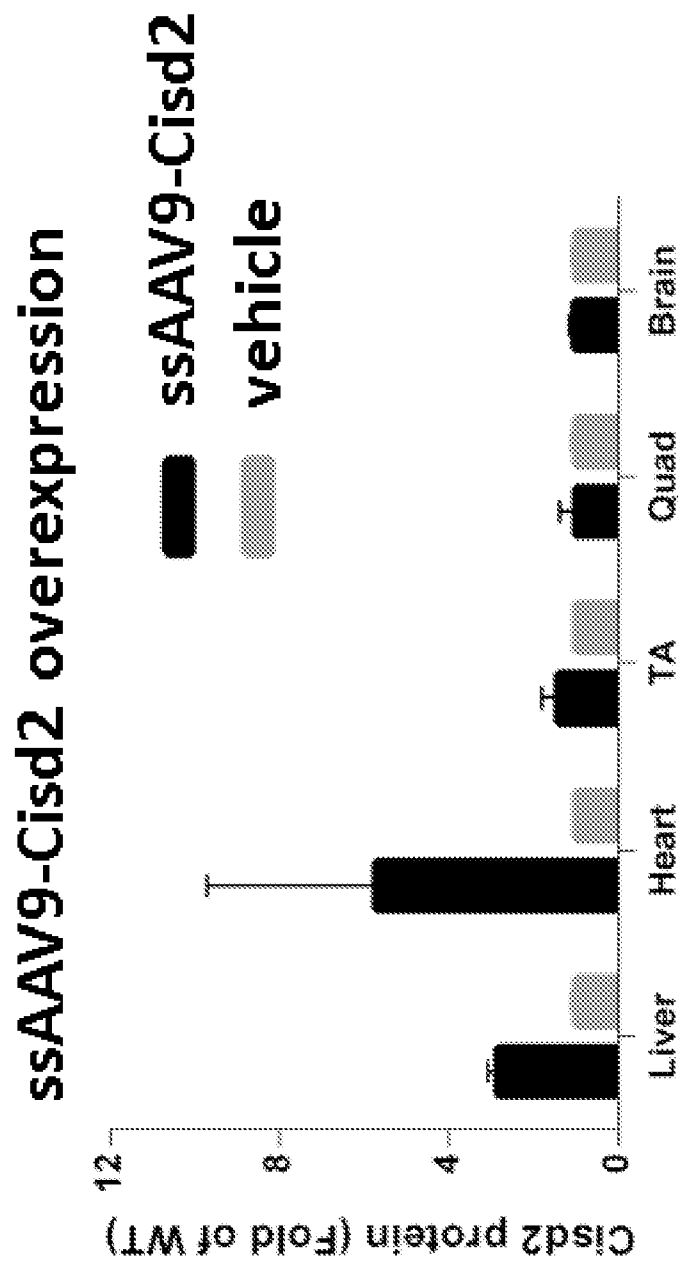


FIG. 2C

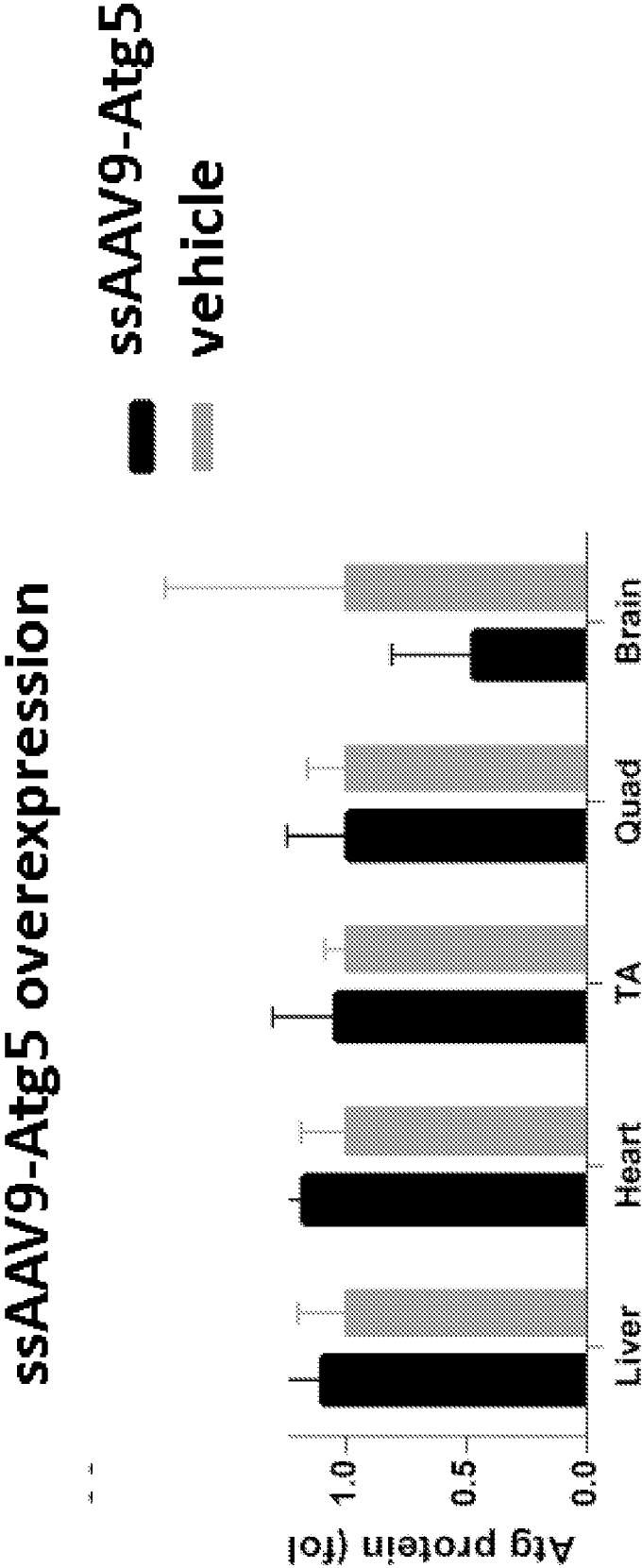


FIG. 2D

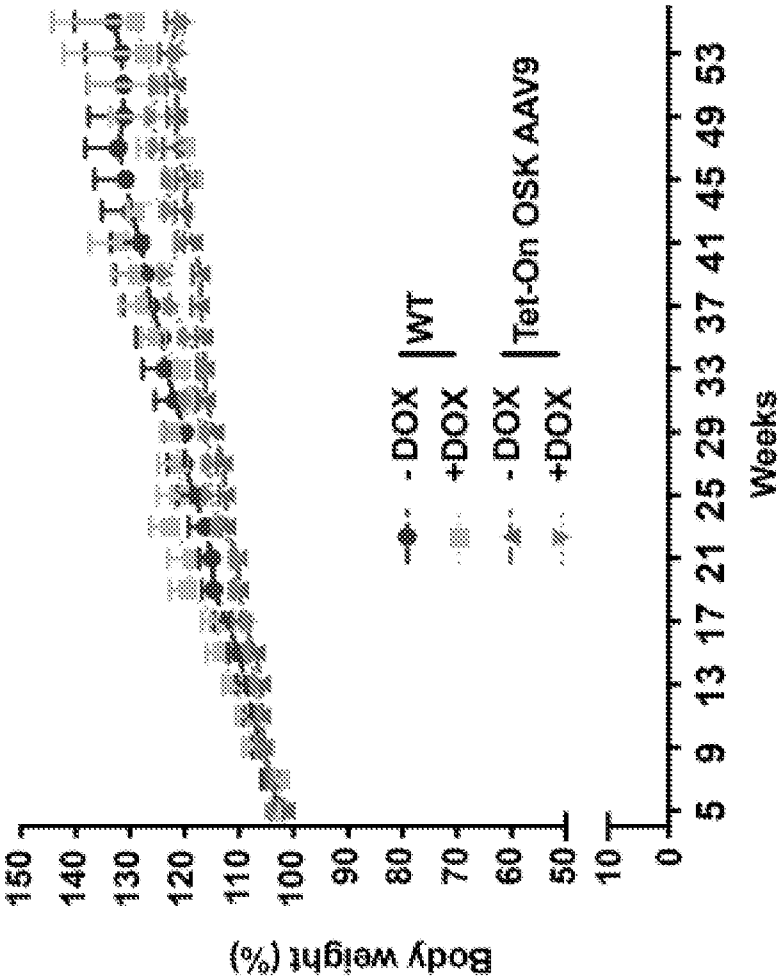


FIG. 3B

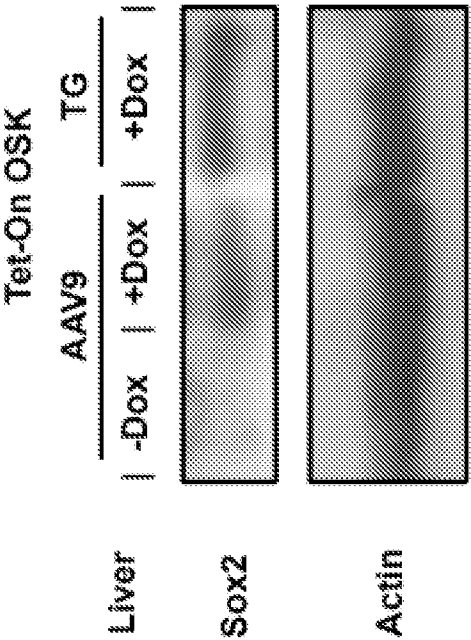


FIG. 3A

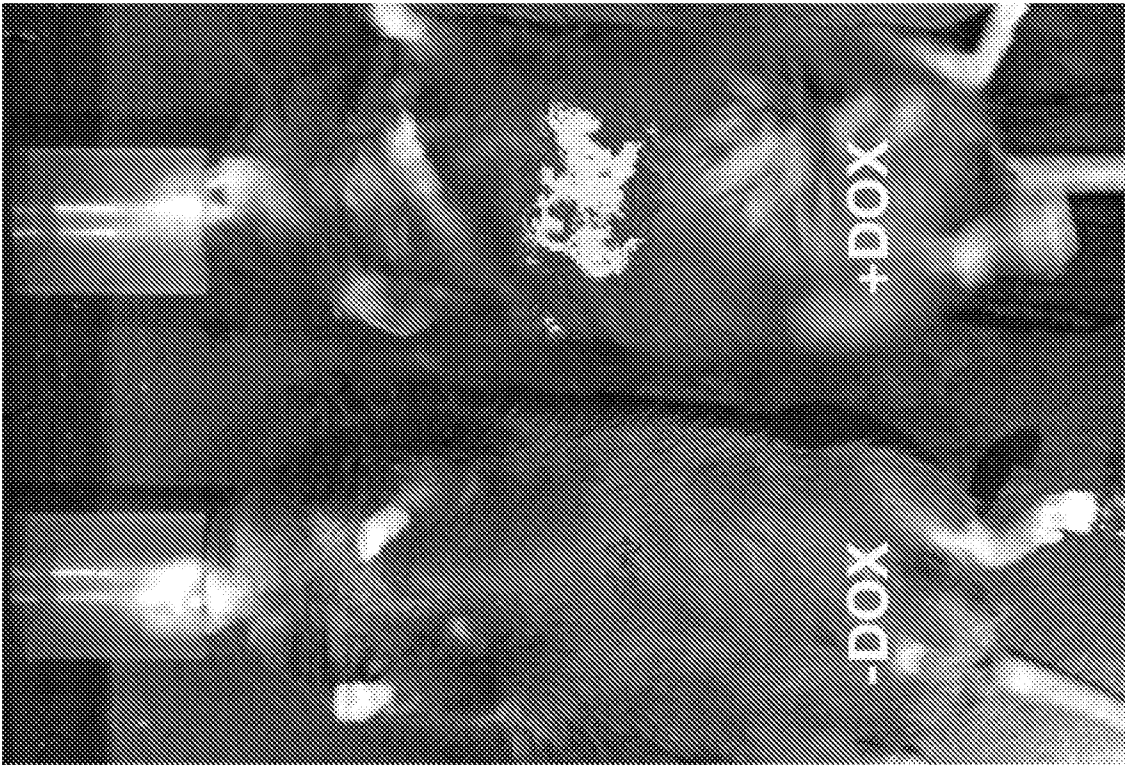


FIG. 3D

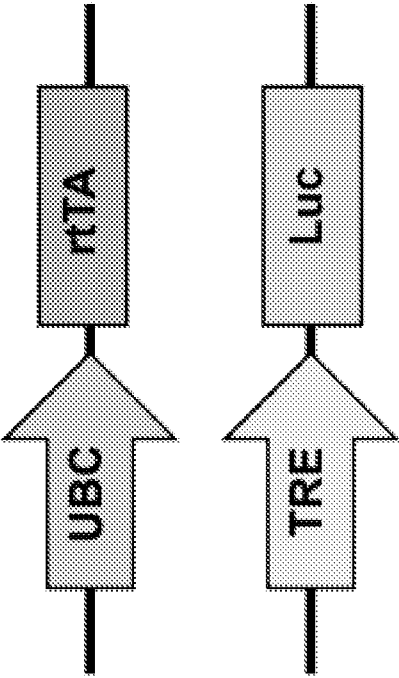


FIG. 3C

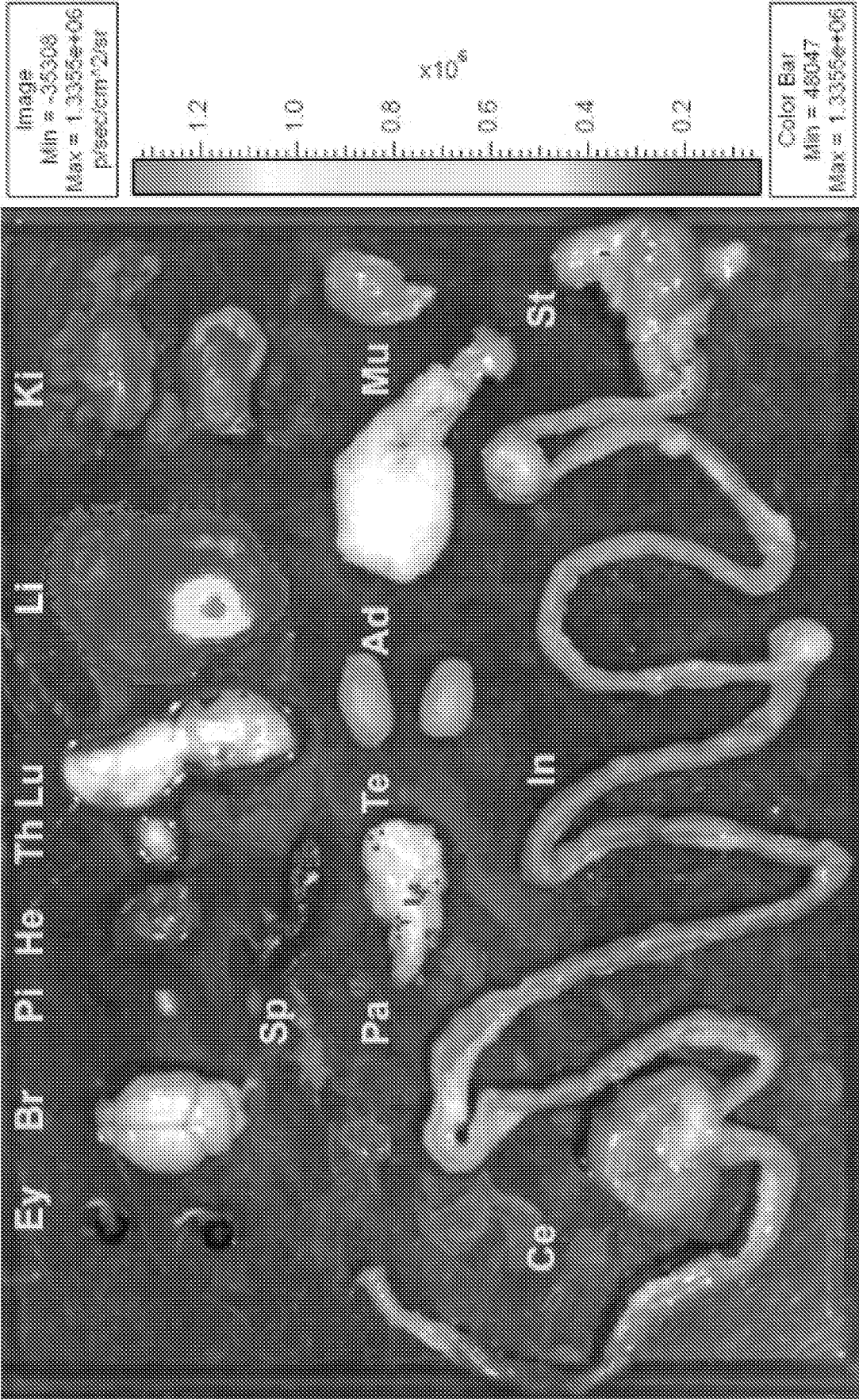


FIG. 3E

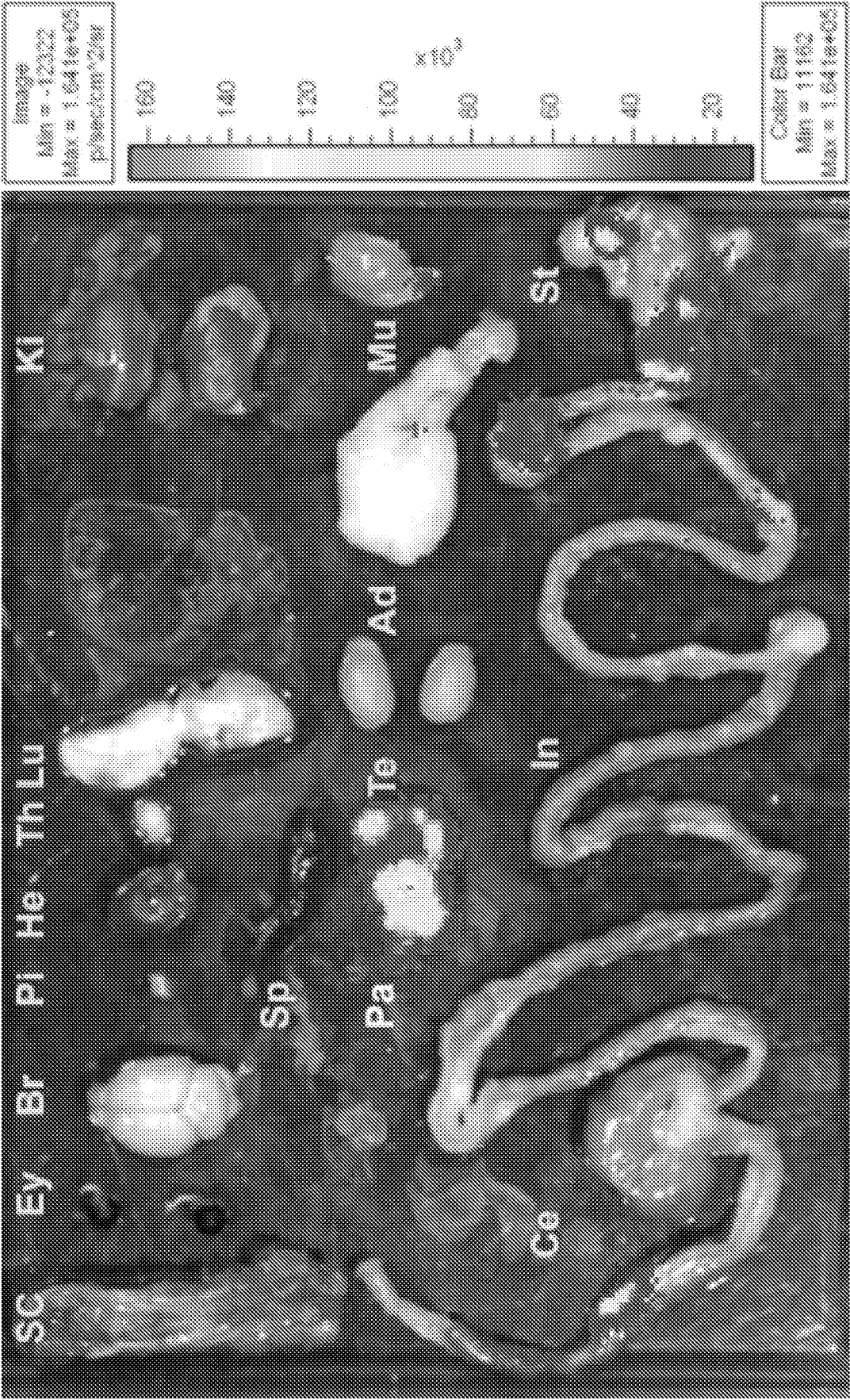


FIG. 3E cont.

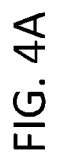


FIG. 4A

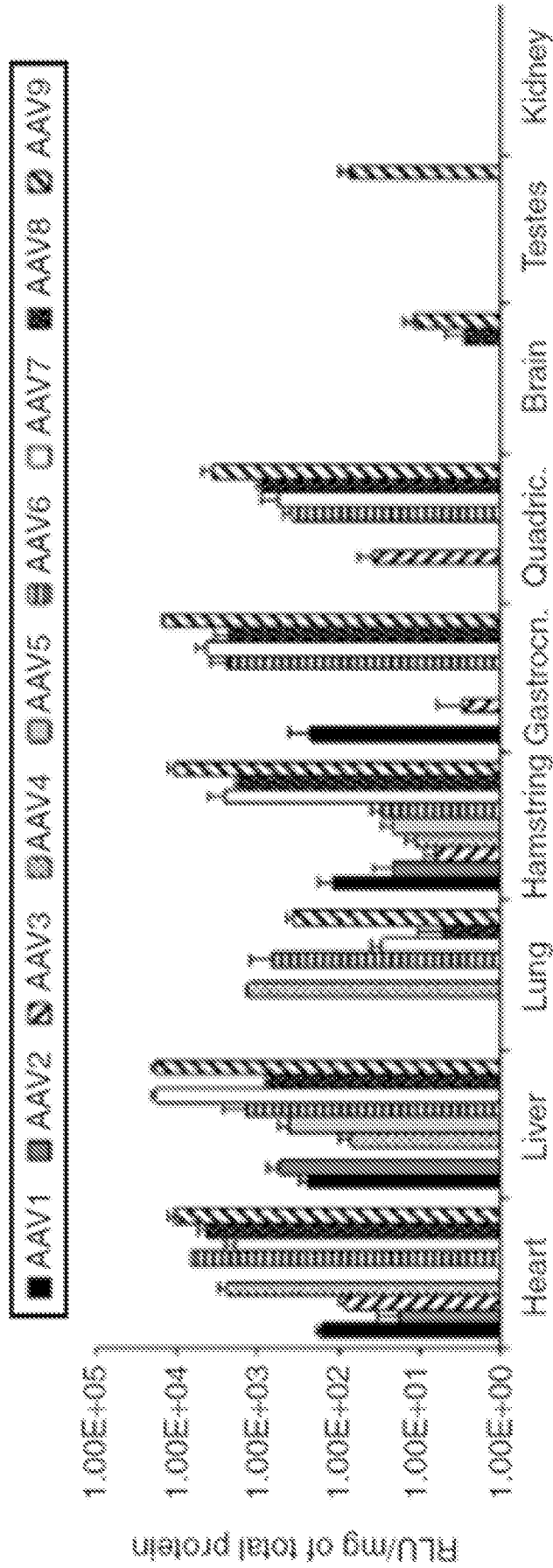


FIG. 4B

DAEUS

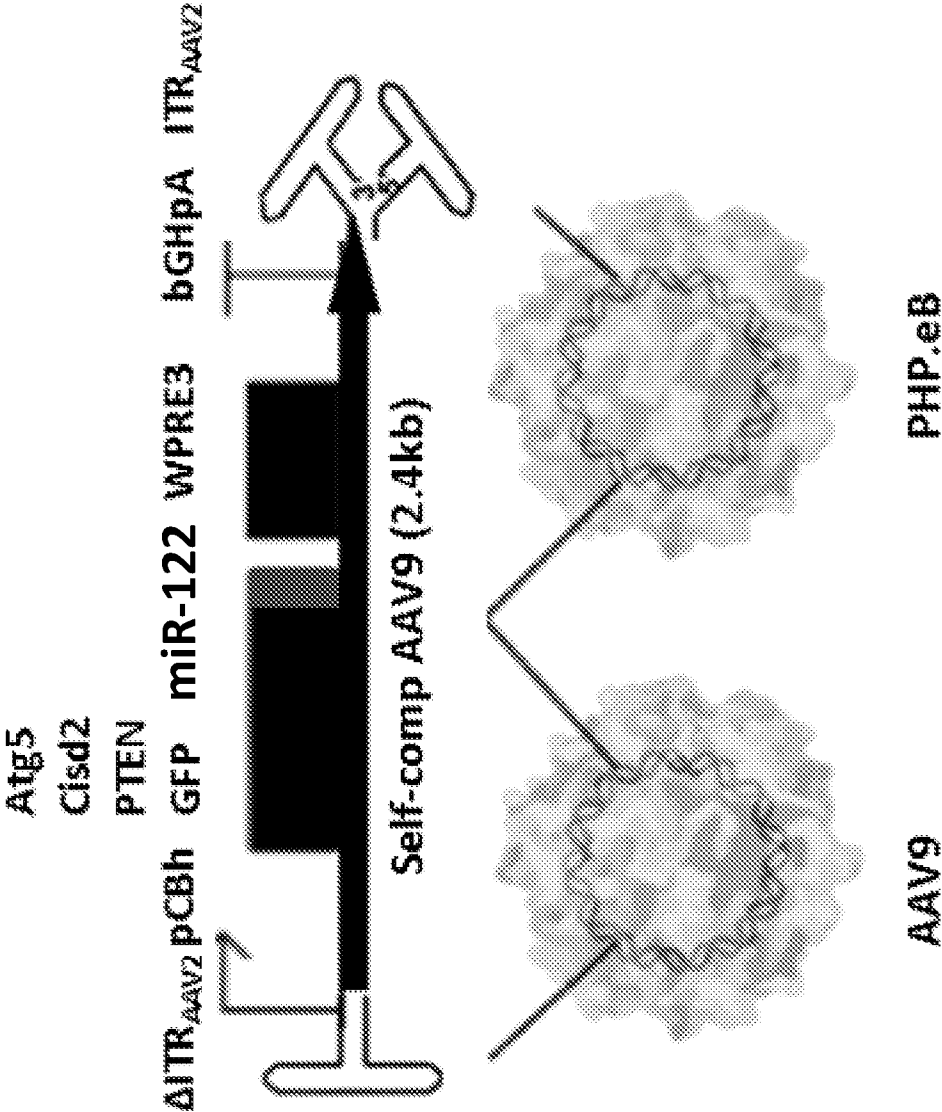


FIG. 5A

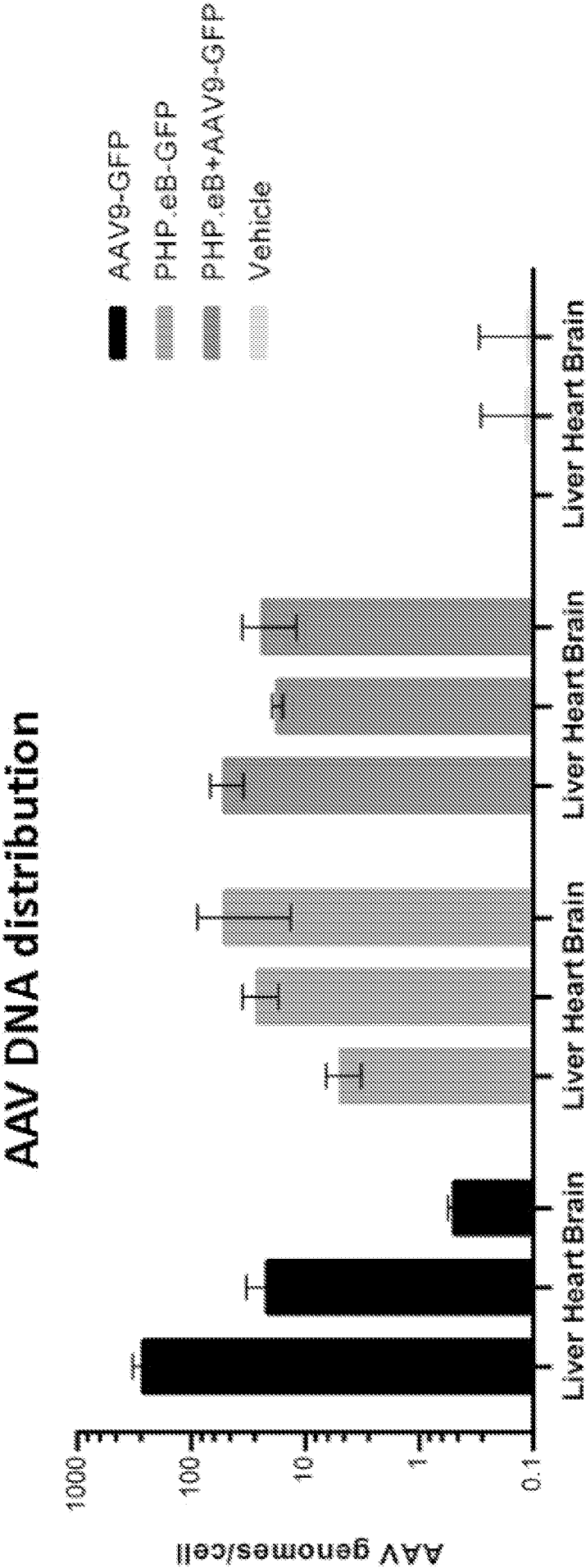


FIG. 5B

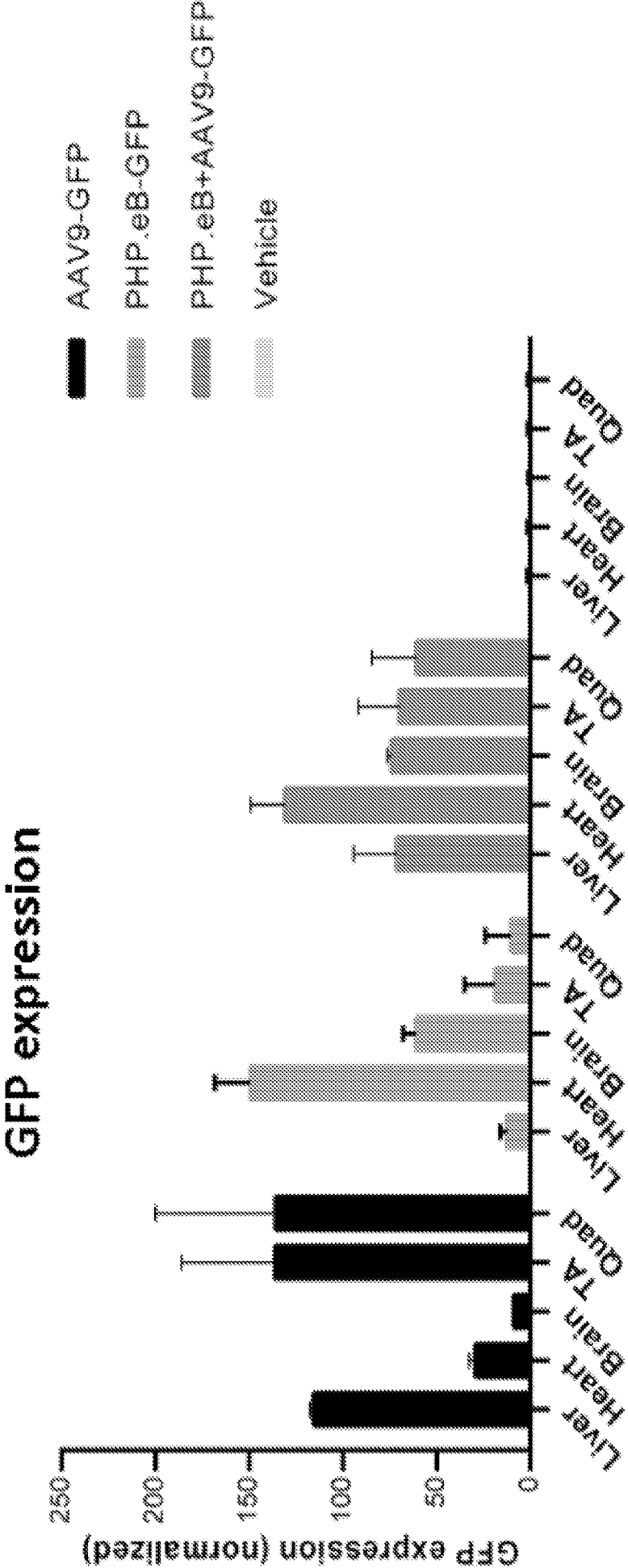


FIG. 5C

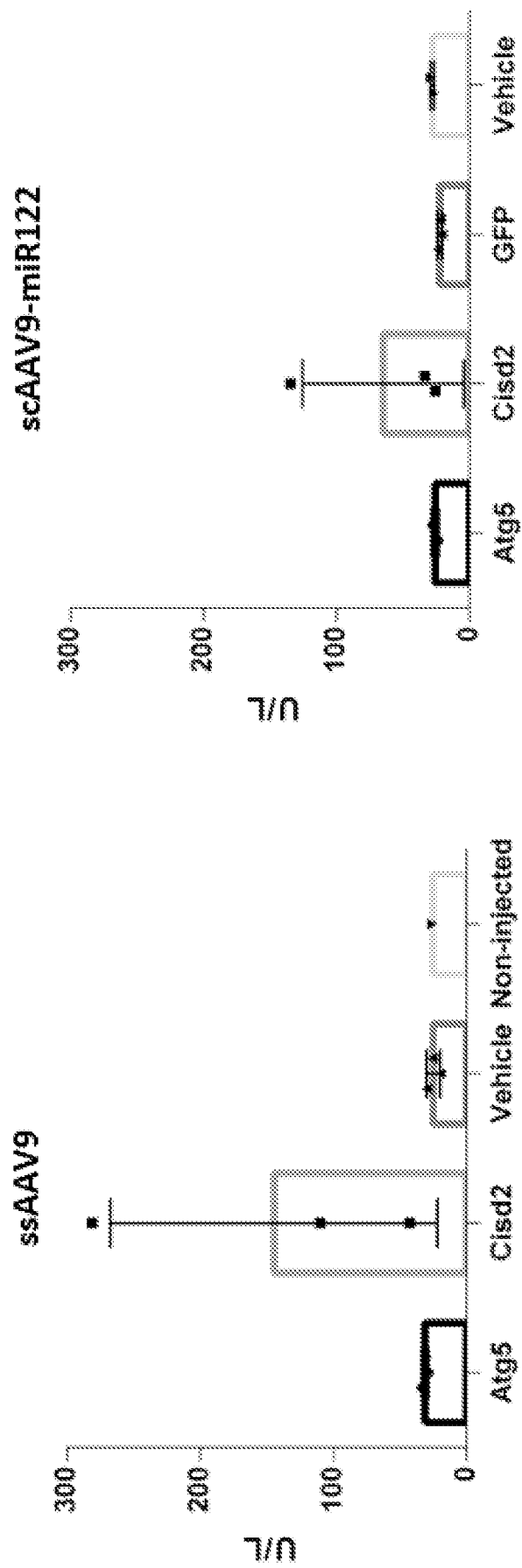


FIG. 6

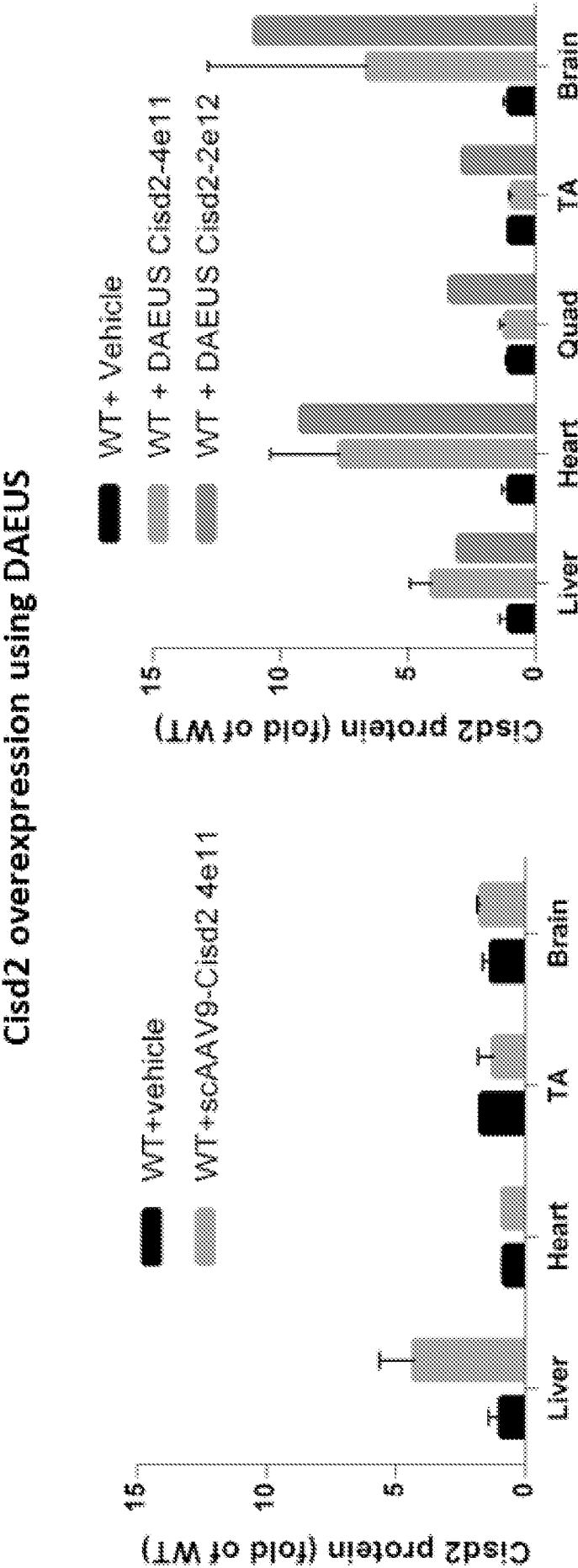


FIG. 7

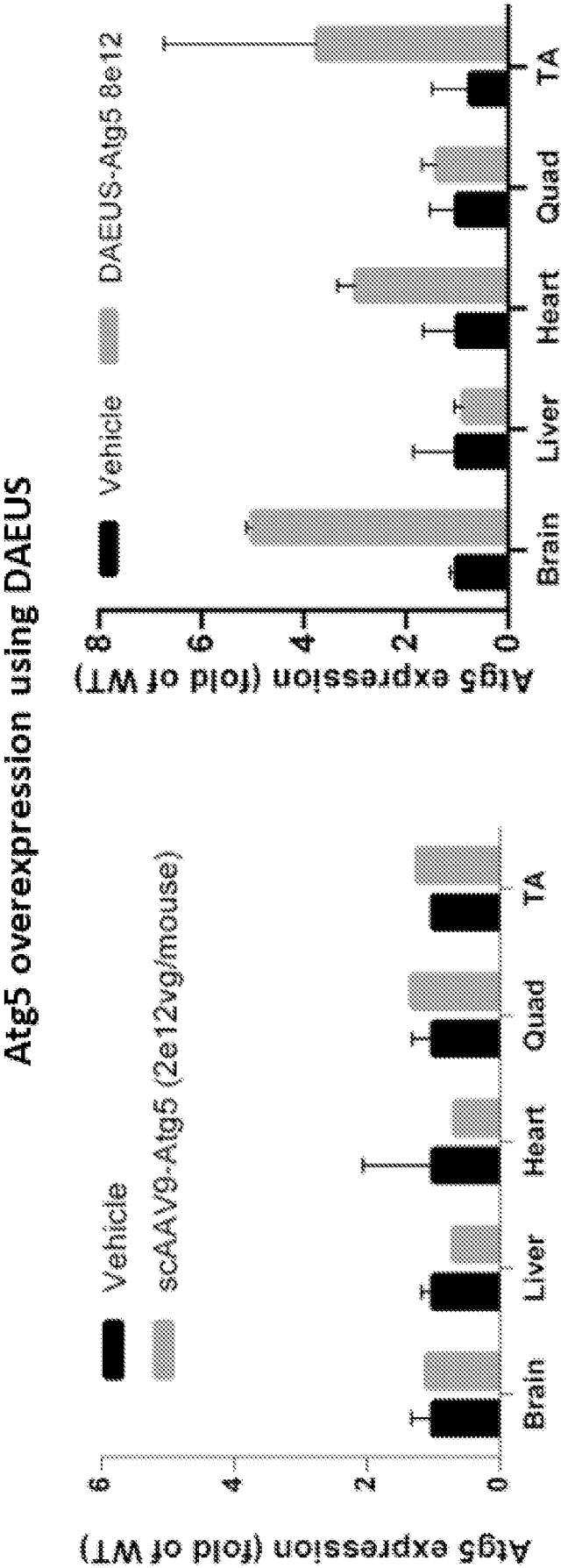


FIG. 8

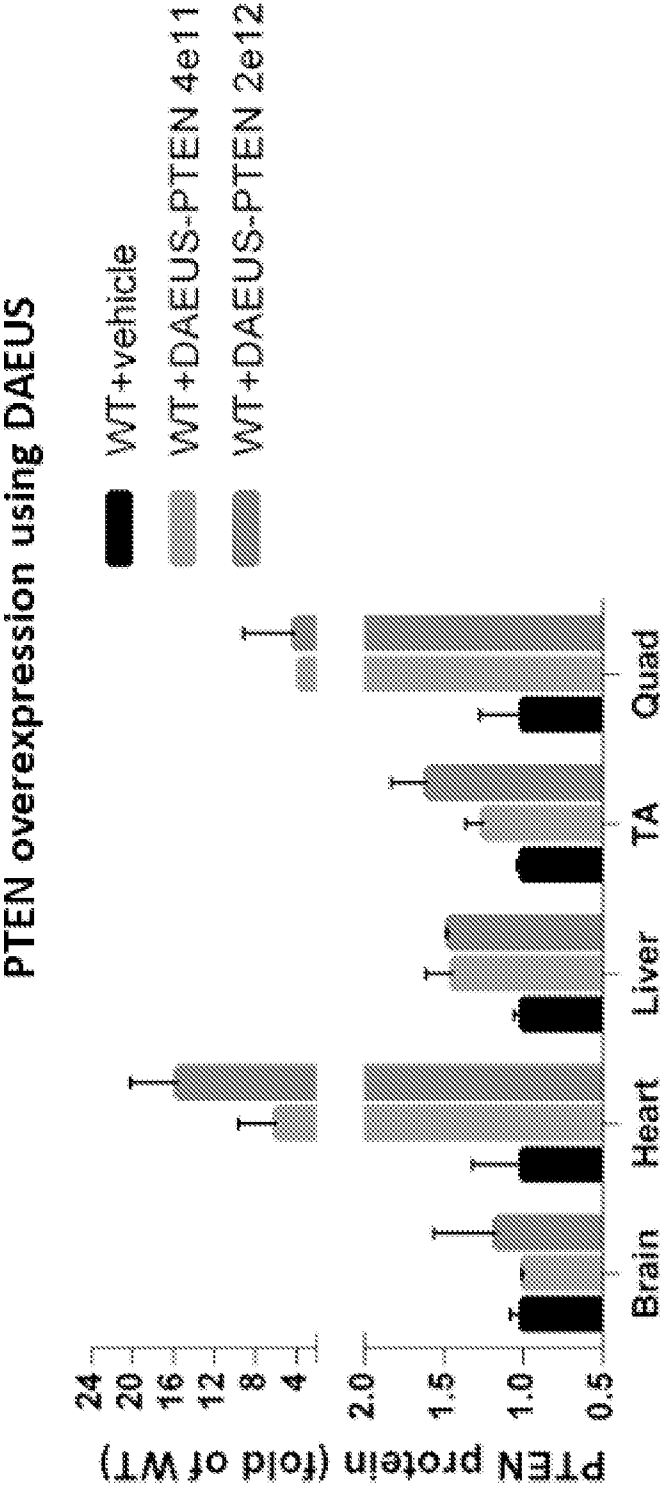


FIG. 9

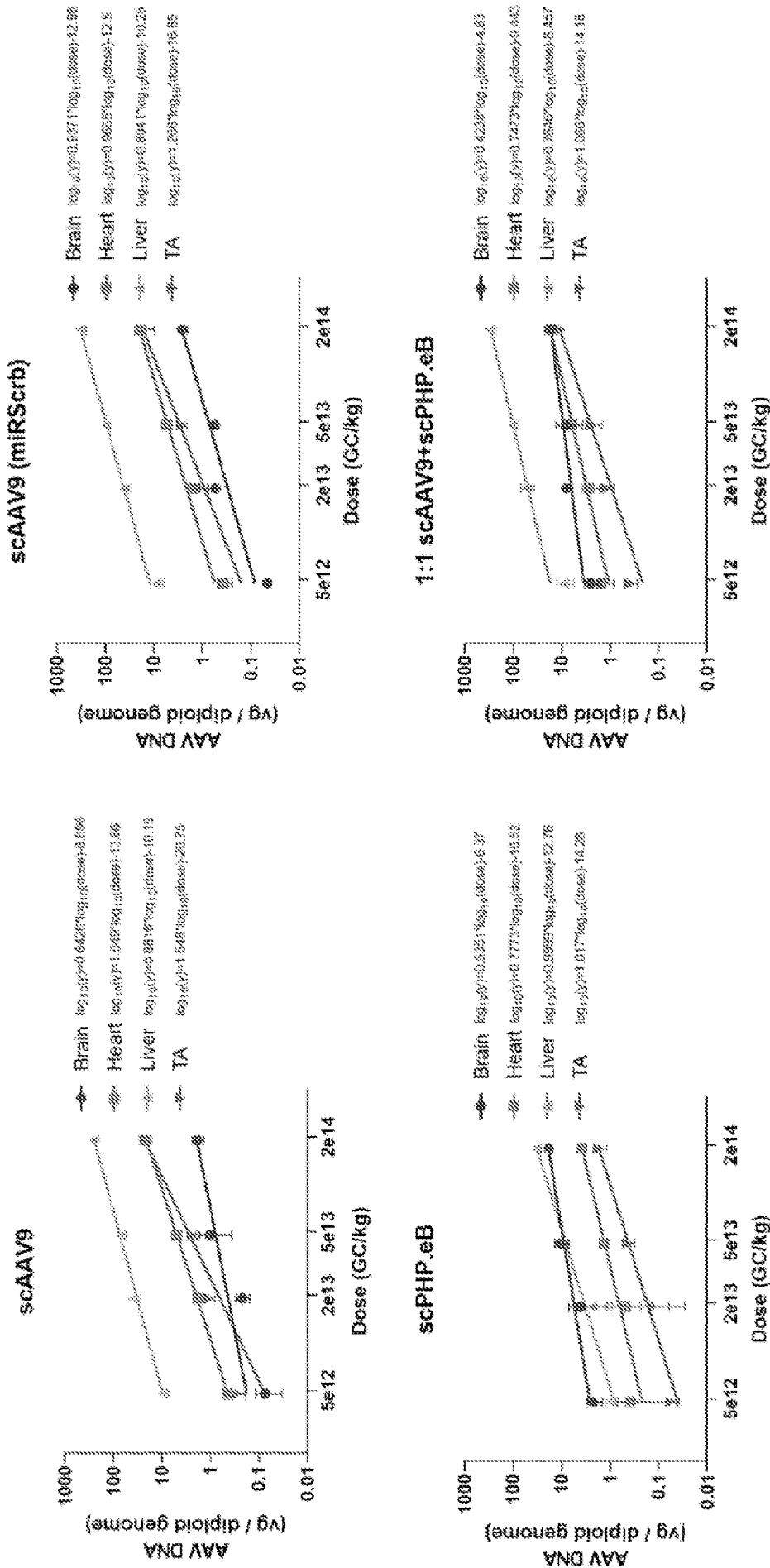


FIG. 10

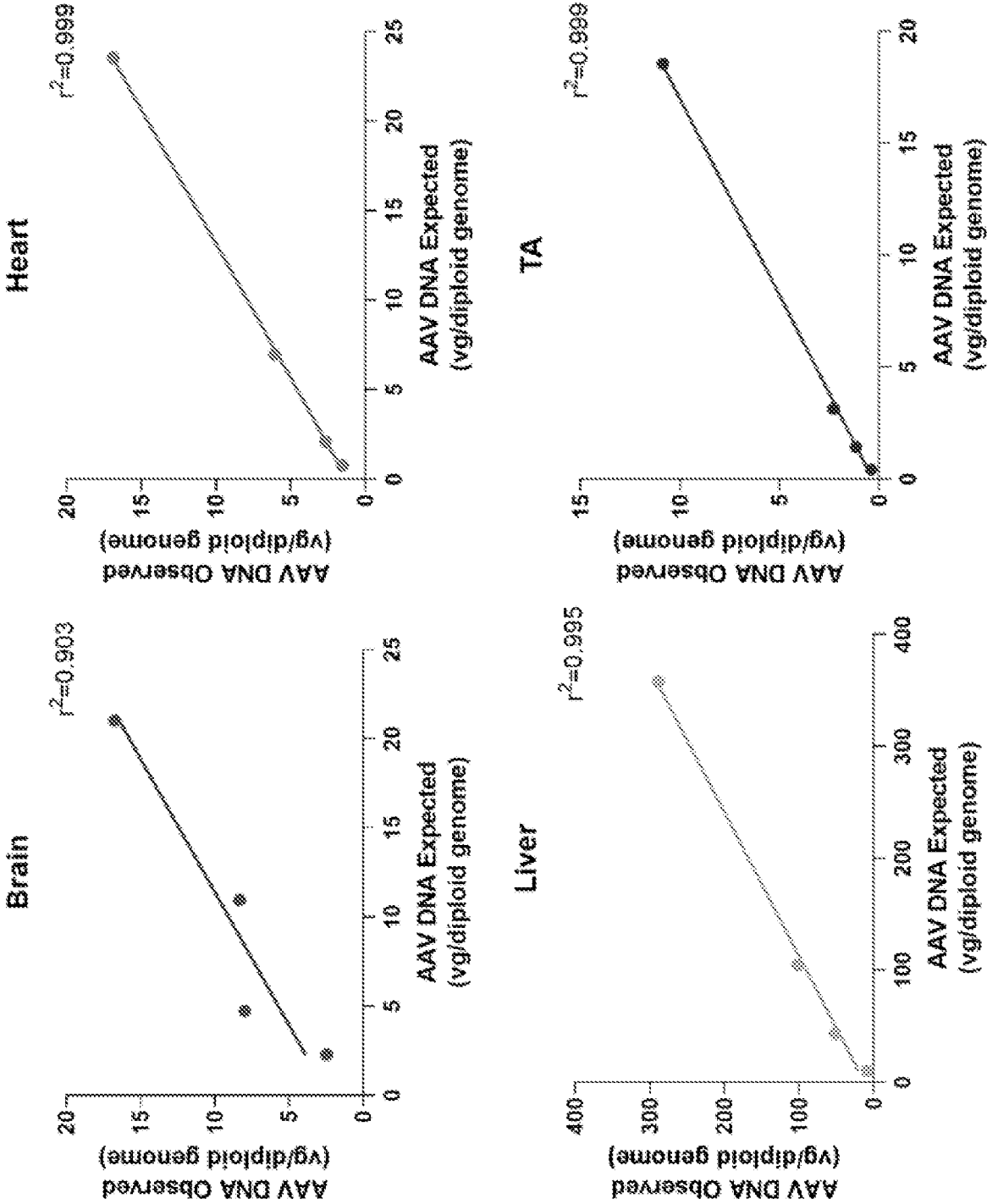


FIG. 11

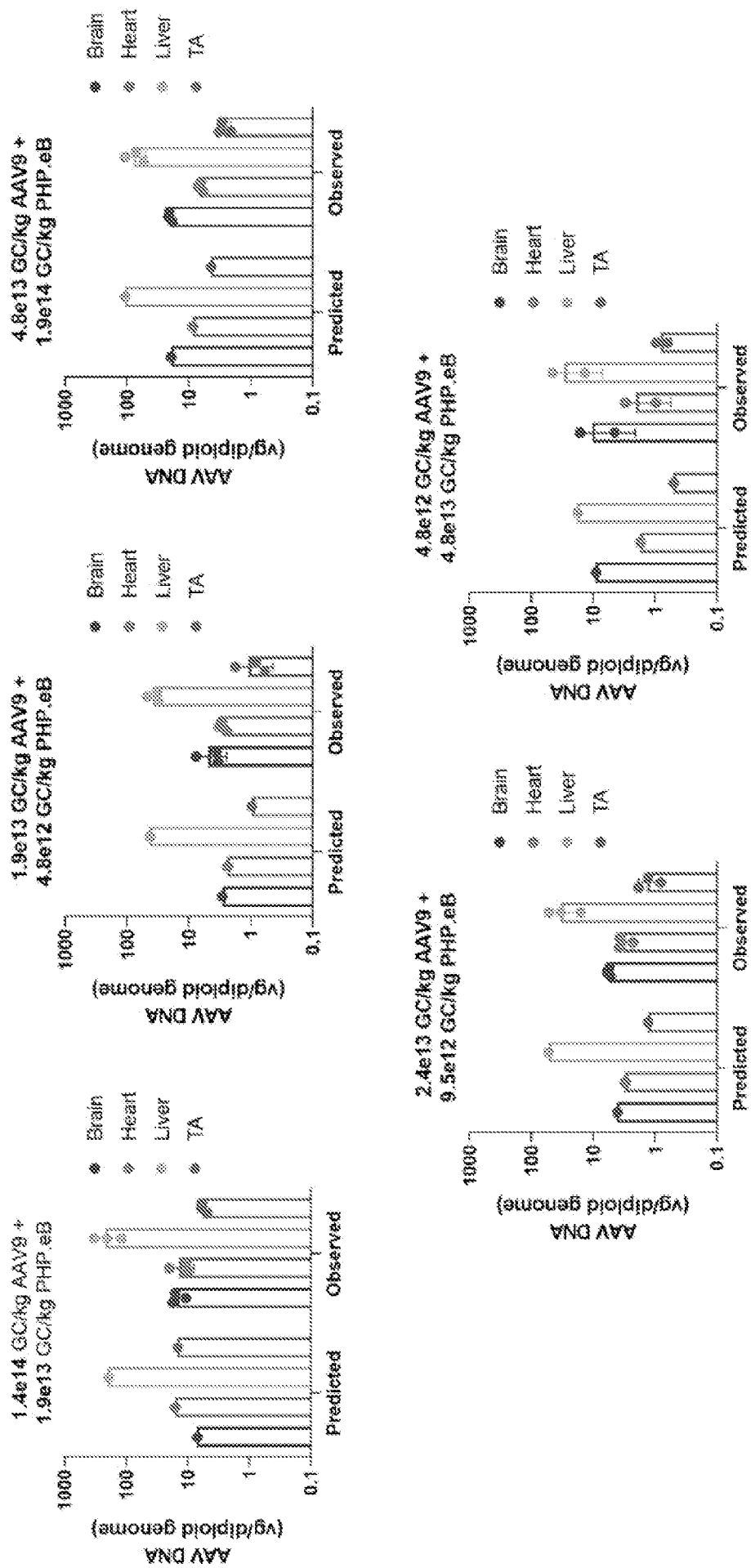


FIG. 12

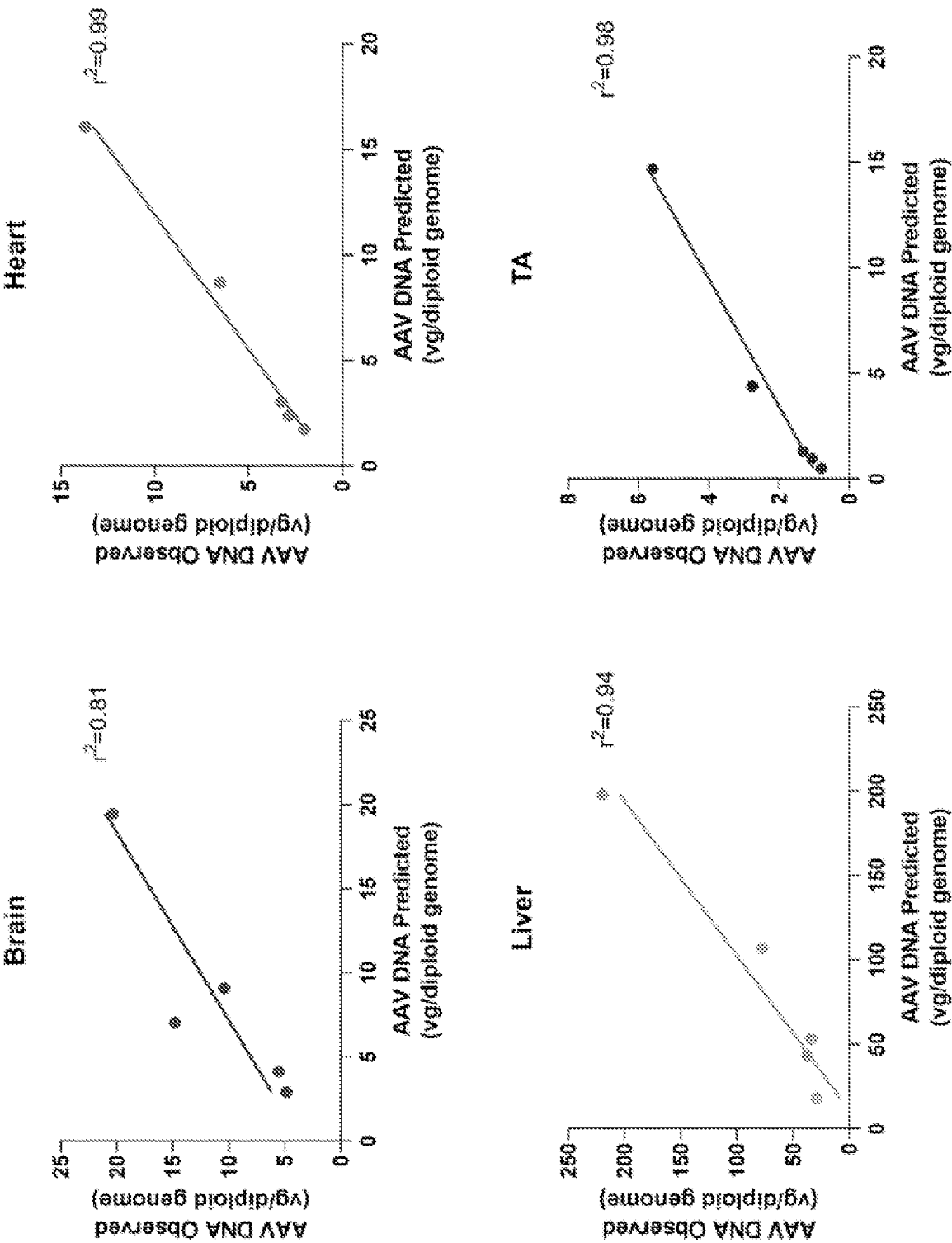


FIG. 13

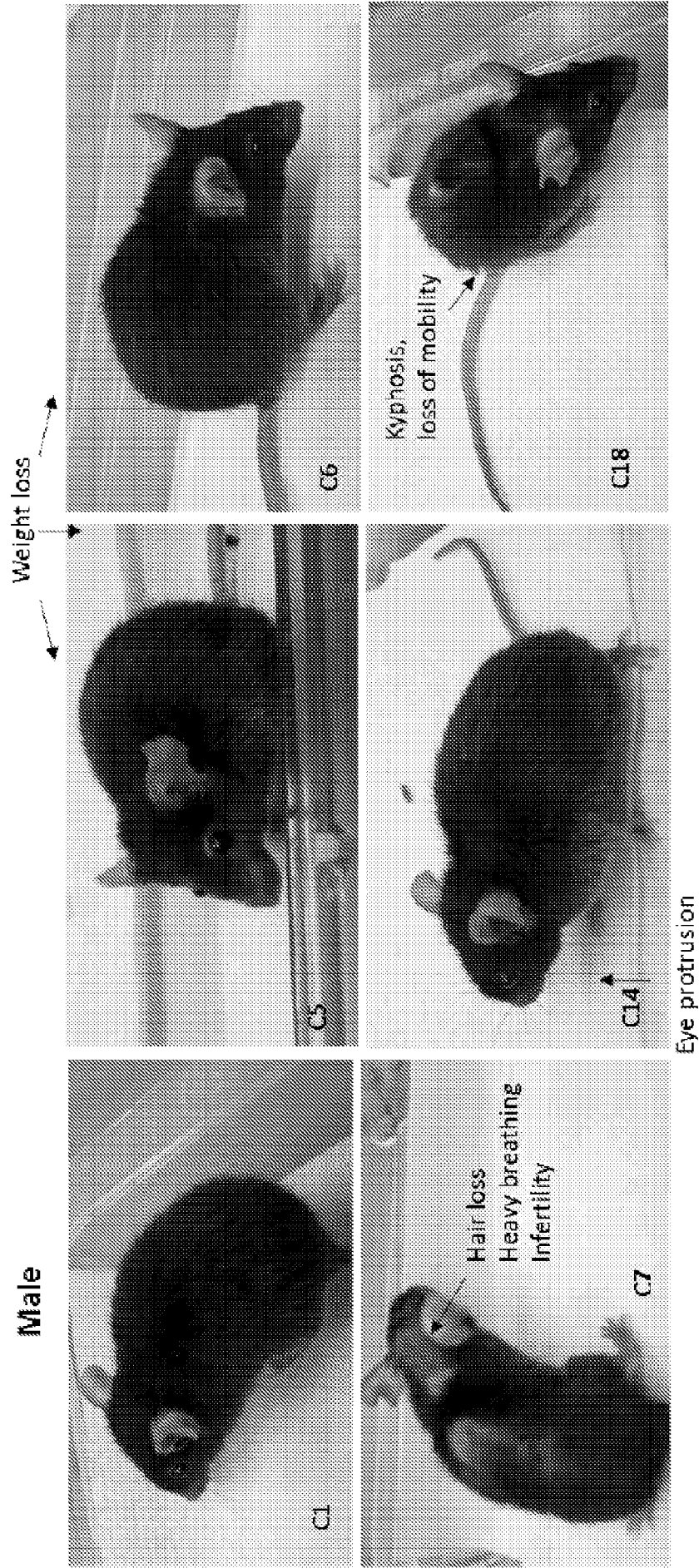


FIG. 14

Female

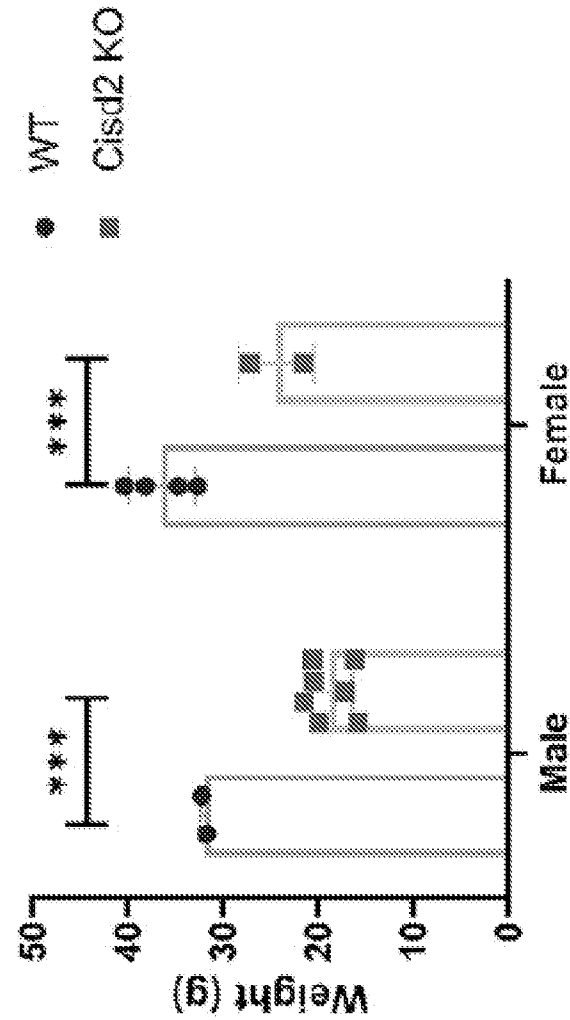
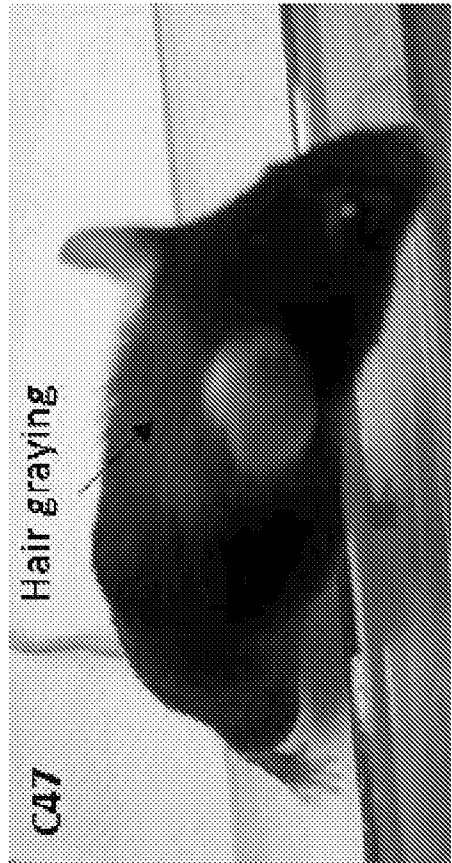


FIG. 14 cont.

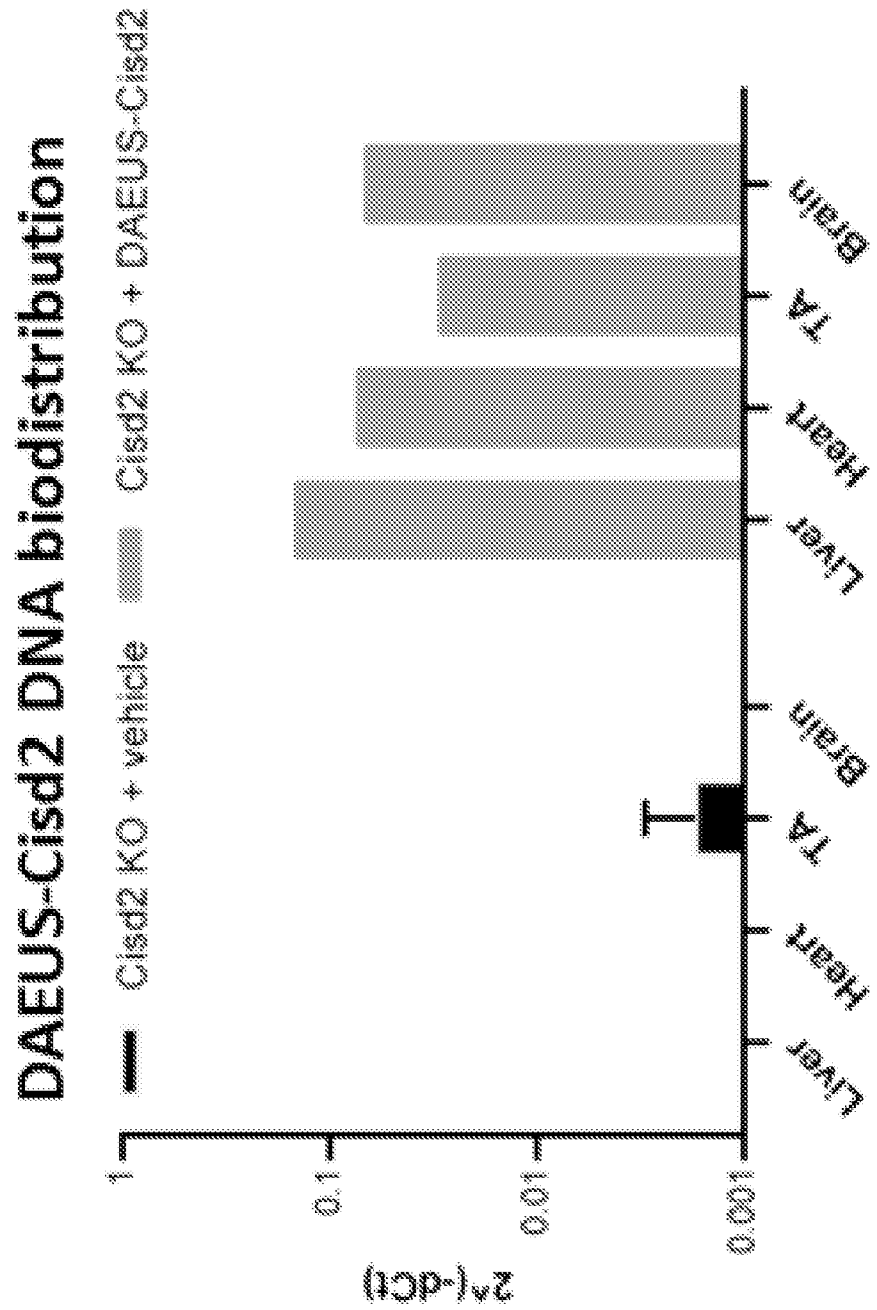


FIG. 15A

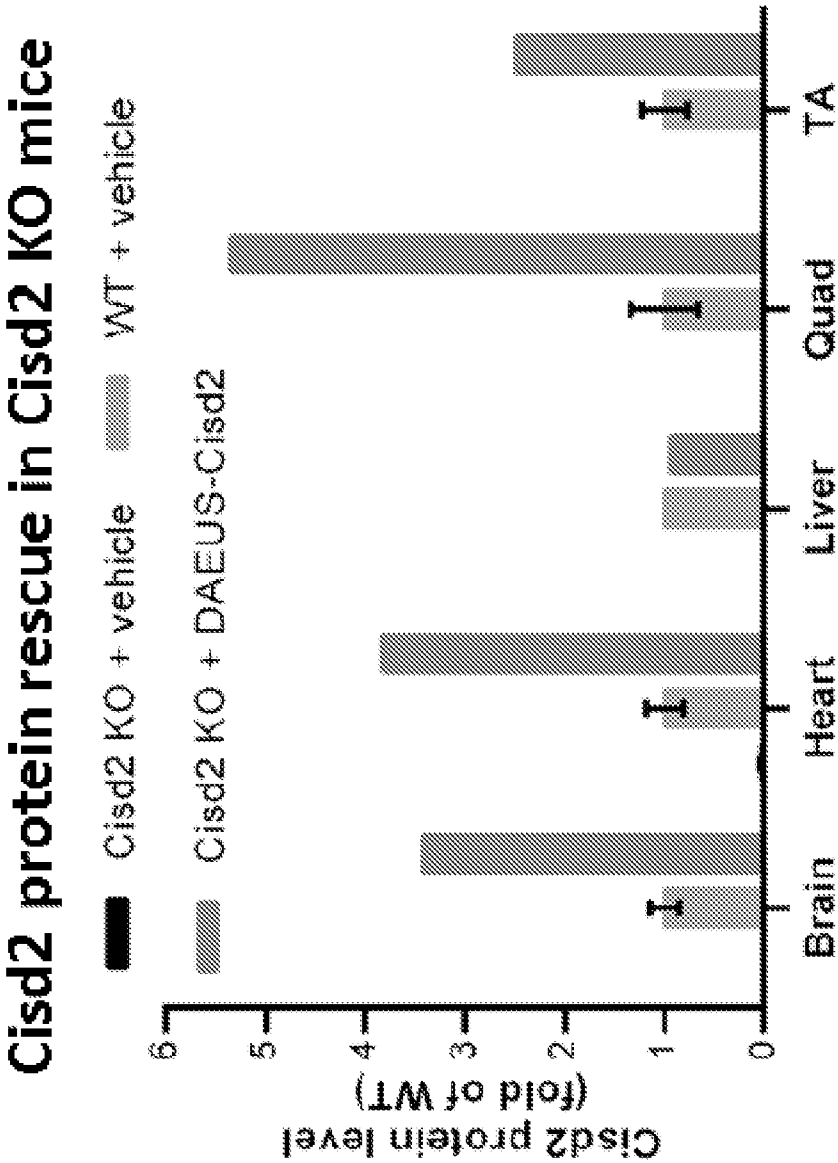


FIG. 15B

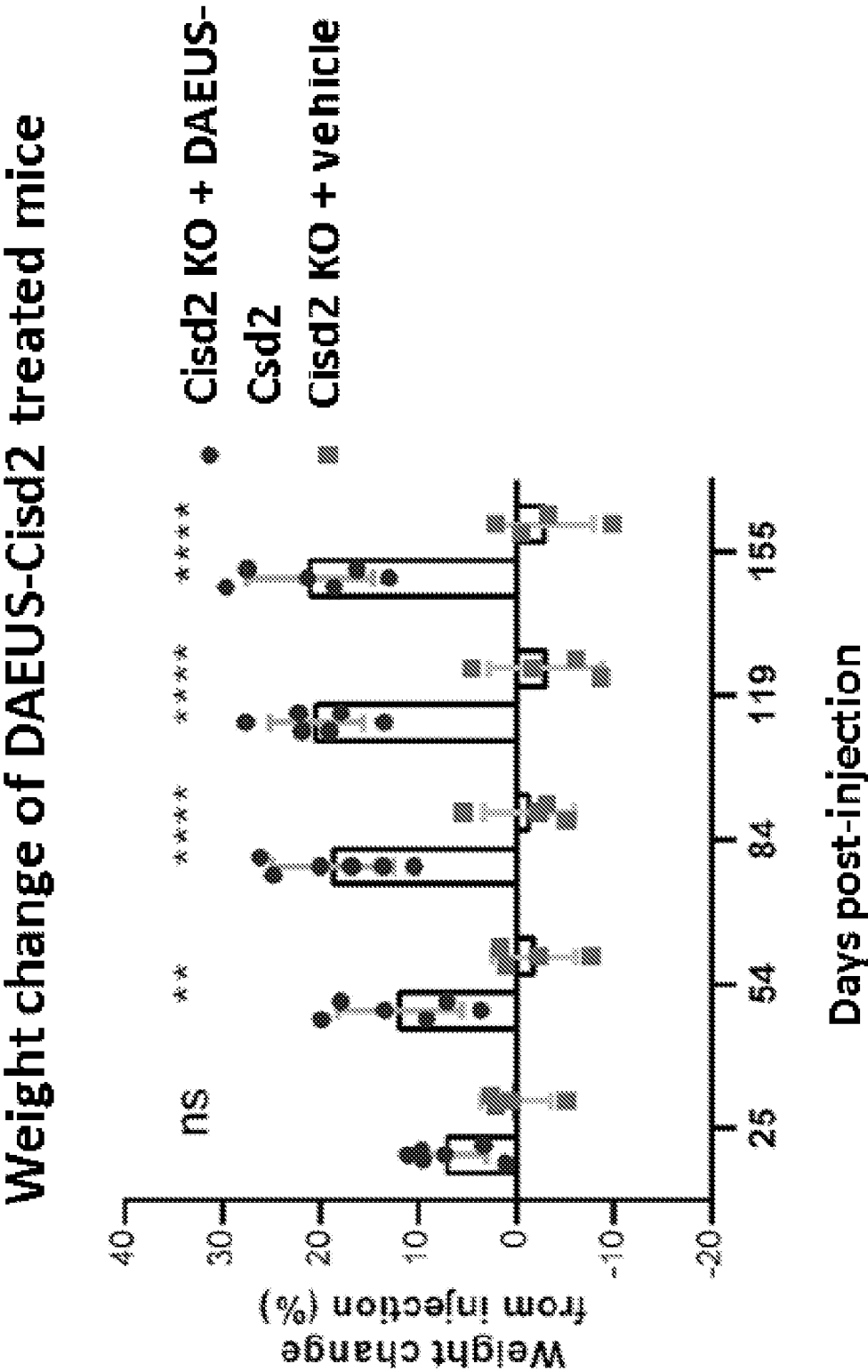


FIG. 15C

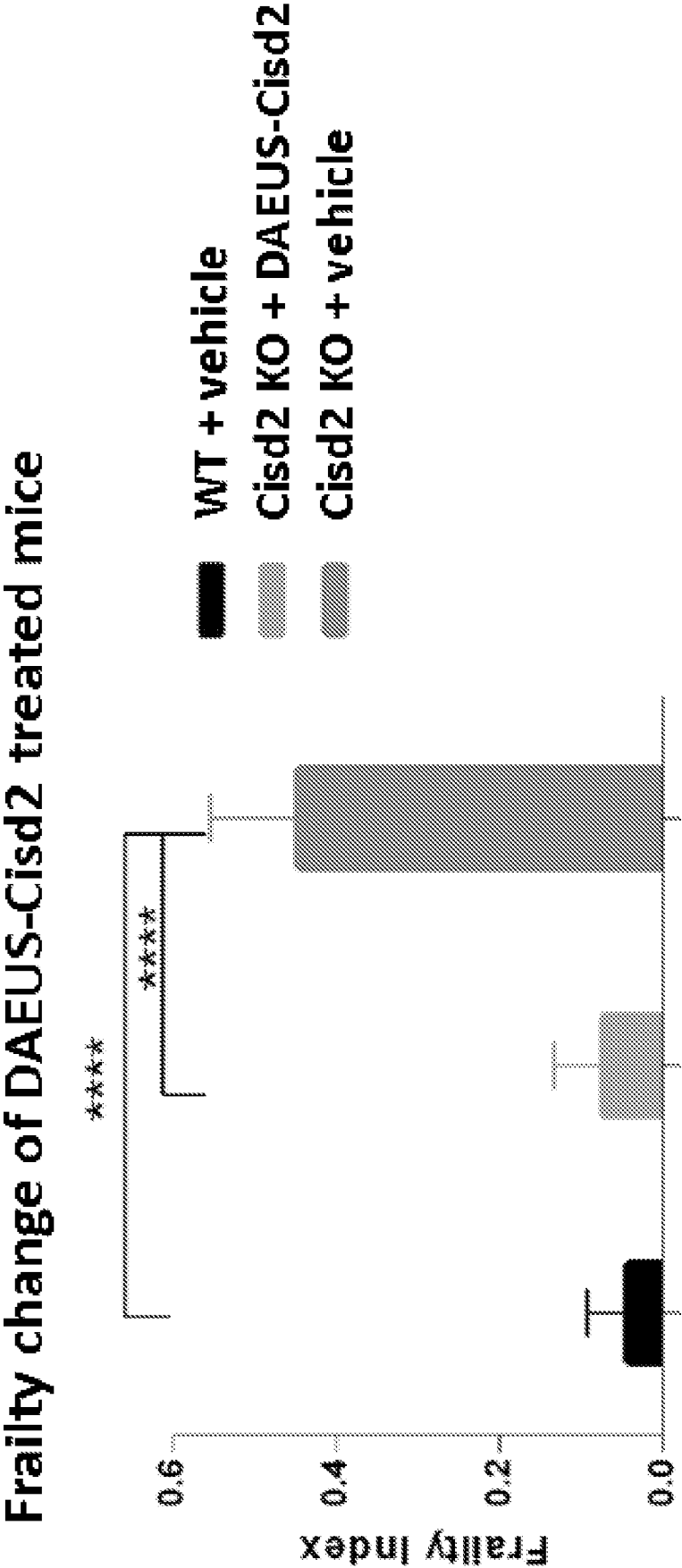


FIG. 15D

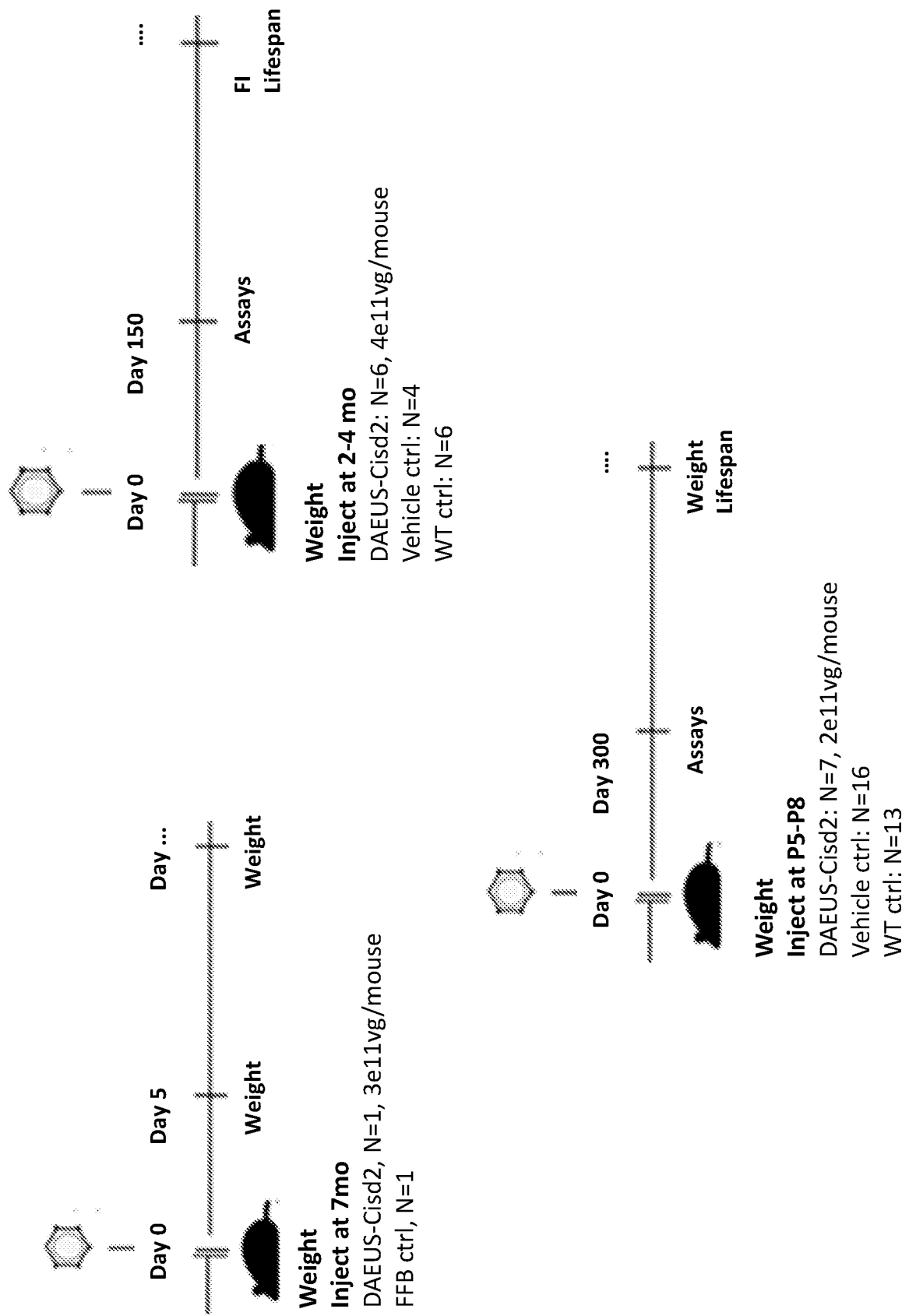


FIG. 16



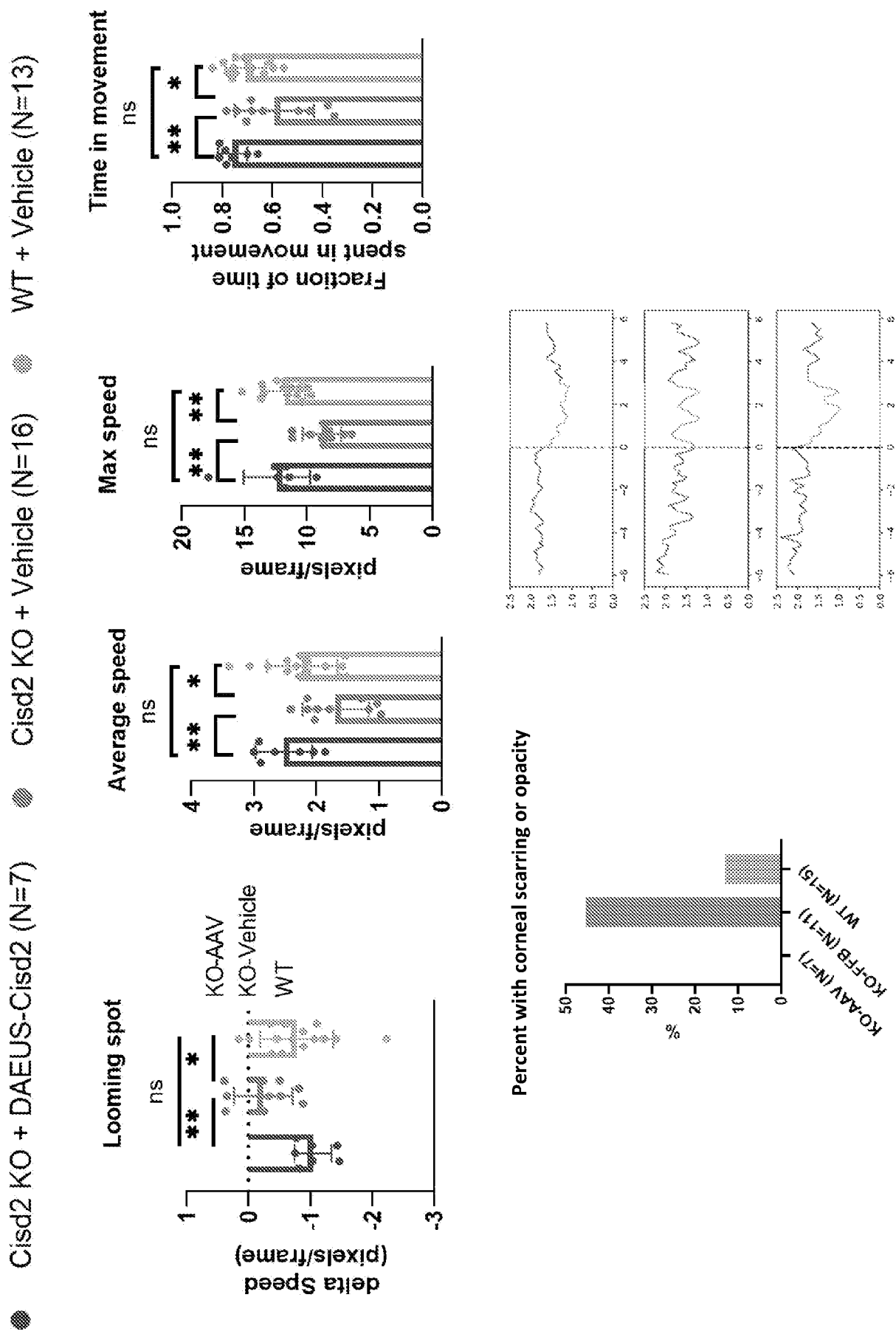


FIG. 17 cont.

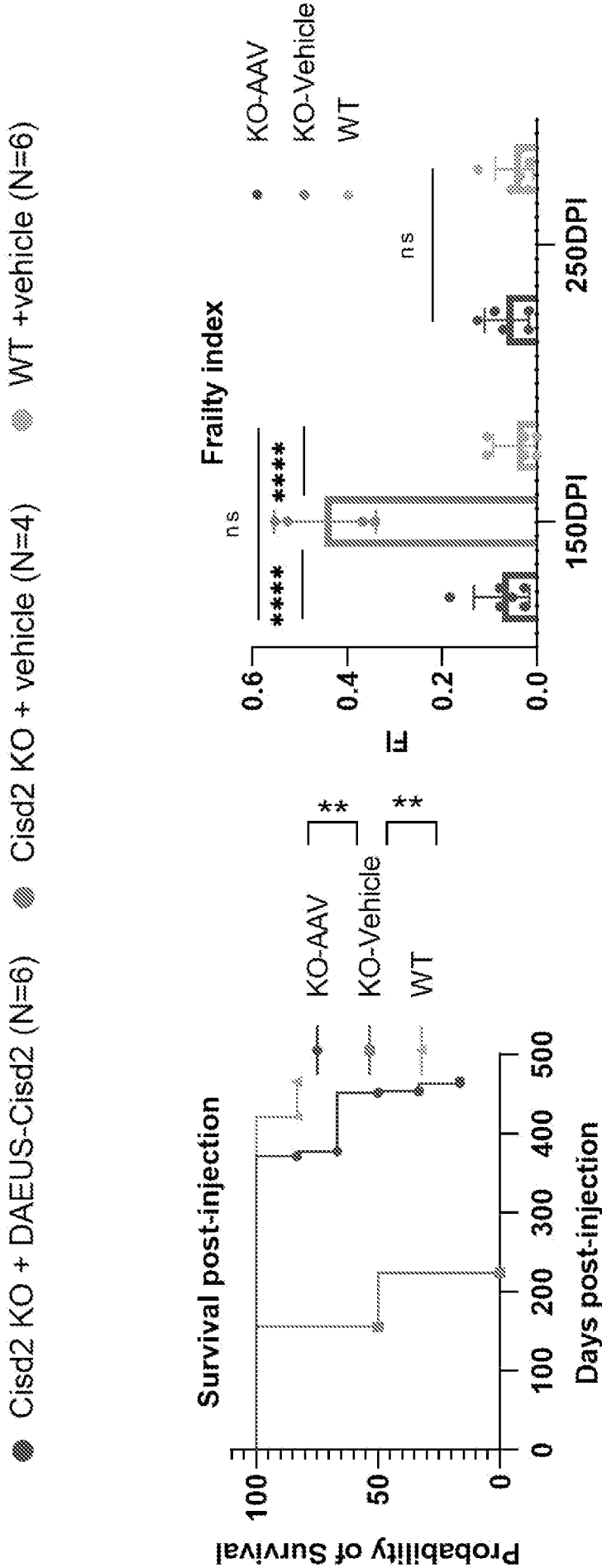


FIG. 18

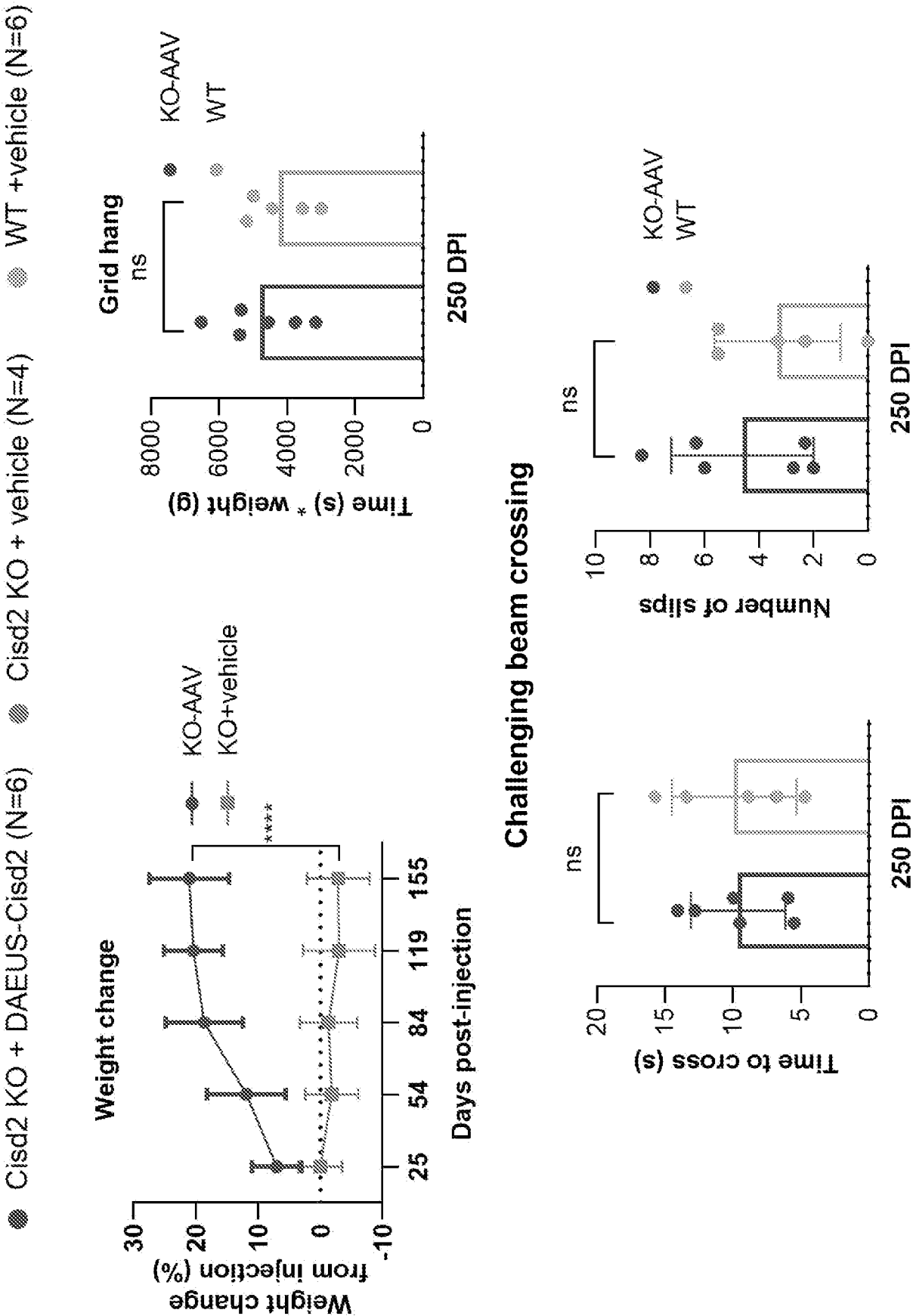


FIG. 18 cont.

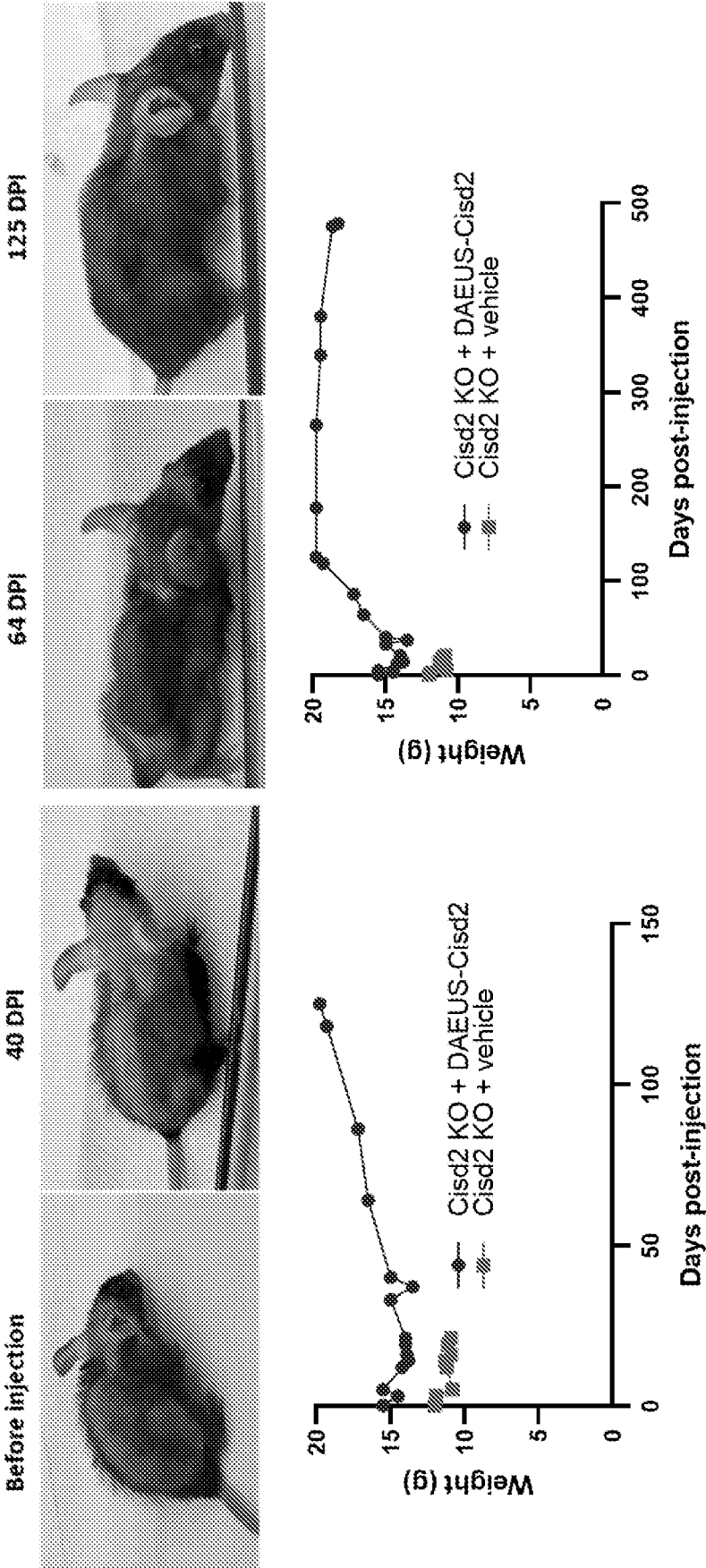


FIG. 19

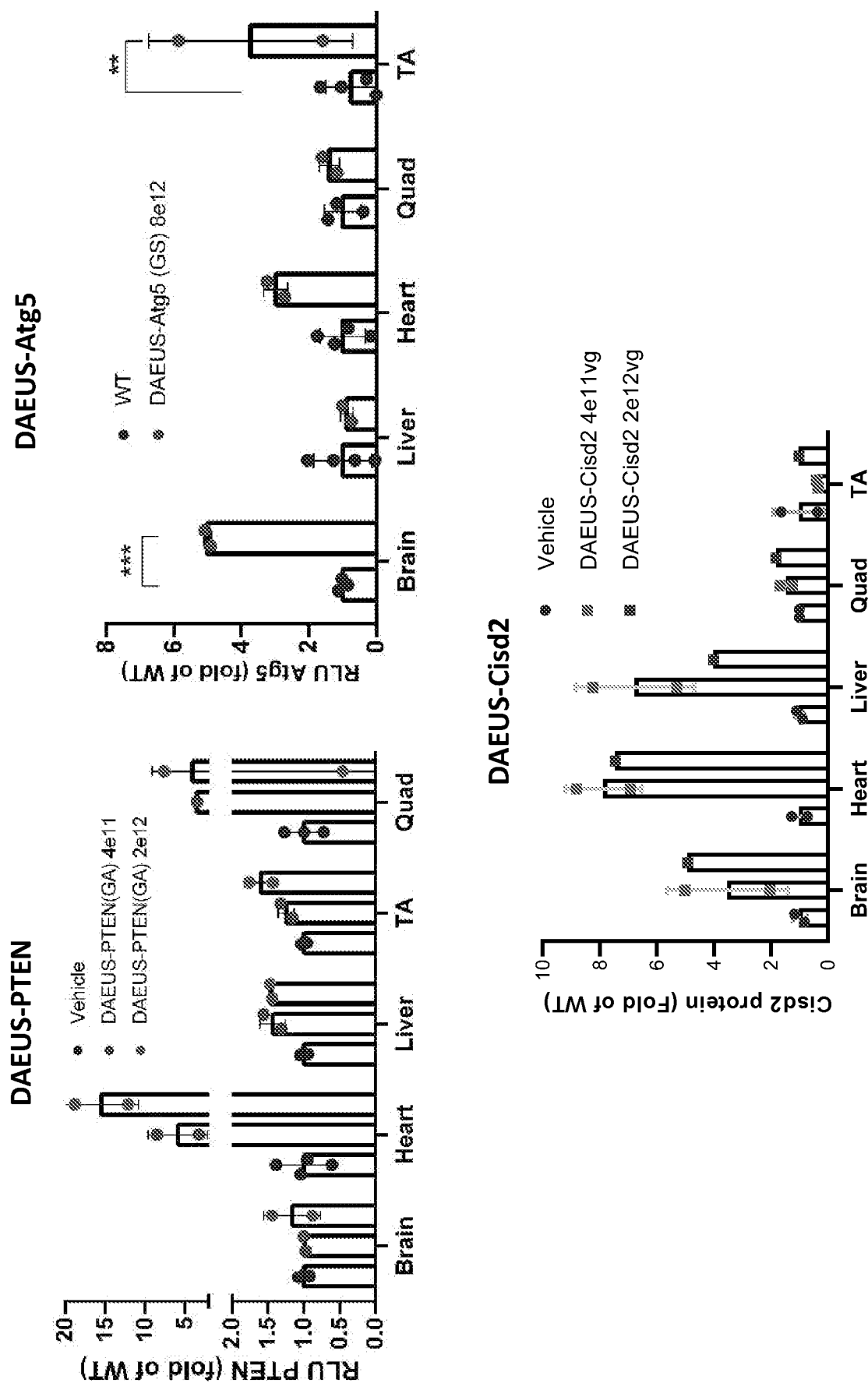


FIG. 20

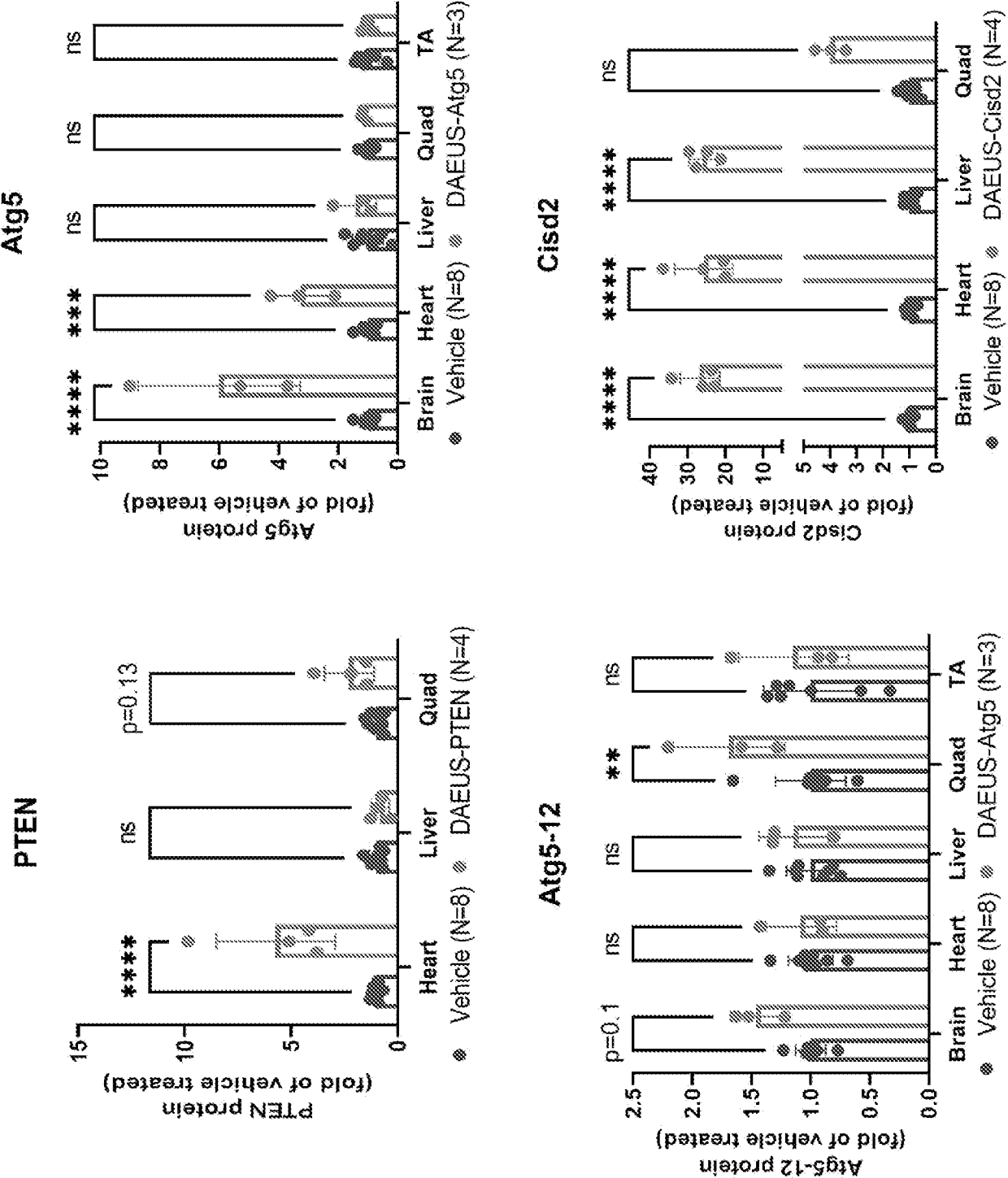


FIG. 20 cont.

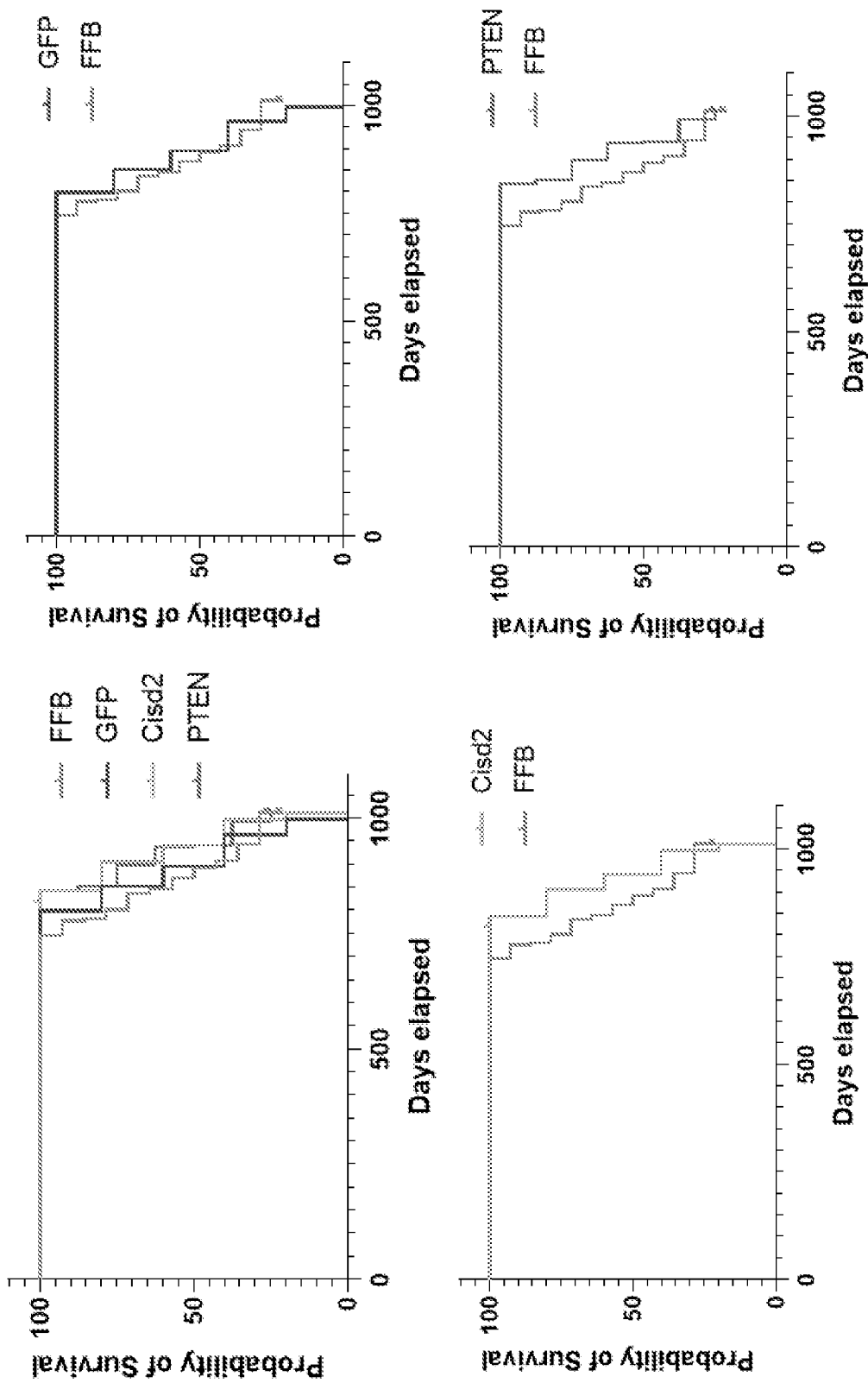


FIG. 21