

(19) **DANMARK**



Patent- og
Varemærkestyrelsen

(12)

Oversættelse af europæisk patentskrift

(10) **DK/EP 3445773 T3**

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- (51) Int.Cl.: **C 07 K 14/075 (2006.01)** **C 12 N 15/62 (2006.01)** **C 12 N 15/63 (2006.01)**
C 12 N 15/861 (2006.01) **C 12 Q 1/70 (2006.01)**
- (45) Oversættelsen bekendtgjort den: **2023-03-20**
- (80) Dato for Den Europæiske Patentmyndigheds bekendtgørelse om meddelelse af patentet: **2022-12-28**
- (86) Europæisk ansøgning nr.: **17797002.7**
- (86) Europæisk indleveringsdag: **2017-05-12**
- (87) Den europæiske ansøgnings publiceringsdag: **2019-02-27**
- (86) International ansøgning nr.: **US2017032542**
- (87) Internationalt publikationsnr.: **WO2017197355**
- (30) Prioritet: **2016-05-13 US 201662336441 P** **2016-08-22 US 201662378106 P**
2016-09-07 US 201662384590 P **2017-02-03 US 201762454612 P**
- (84) Designerede stater: **AL AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HR HU IE IS IT LI LT LU LV MC MK MT NL NO PL PT RO RS SE SI SK SM TR**
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- (54) Benævnelse: **Adeno-associerede virusvariantcapsider og fremgangsmåder til anvendelse deraf**
- (56) Fremdragne publikationer:
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DESCRIPTION

FIELD OF THE INVENTION

[0001] The present invention is defined by the claims. The invention disclosed herein relates generally to the field of adeno-associated virus (AAV) virions comprising variant capsid proteins as claimed.

BACKGROUND OF THE DISCLOSURE

[0002] Inherited retinal diseases encompass a large group of heterogeneous genetic diseases that affect approximately 1 in 3000 people (greater than 2 million people worldwide) and are a major source of severe vision loss or blindness. Complex, multifactorial retinal diseases such as wet age related macular degeneration (wAMD) and diabetic retinopathy (DR) impact even more individuals, with 1.7 million Americans currently living with severe central vision loss associated with wAMD and almost one-third of adults over age 40 years with diabetes being visually impaired. These diseases are typically associated with the dysfunction or death of one or more types of cell of the retina, in some instances due to the absence of expression or function of a key protein, e.g. RPE65 in LCA2, in other instances due to gene mutations that create toxic gene products, e.g. dominant mutations that affect rhodopsin protein folding, or in yet other instances due to changes in retinal physiology induced by the ectopic expression of a protein, e.g. VEGF in wAMD.

[0003] One approach to addressing this great unmet medical need is gene-based adeno-associated virus (AAV)-mediated therapy, in which a recombinant adeno associated virus (rAAV) is used to deliver a gene to one or more types of cells in the retina, for example to replace a missing gene, to correct a dominant defective gene, or to provide a template for continuous protein therapy. While AAV-based clinical gene therapy has been increasingly successful, it is still fraught with shortcomings with regard to viral vector properties, including, for example, targeting the desired cells of the retina with high efficiency. For example, multiple homologous primate AAV serotypes and numerous nonhuman primate serotypes have been identified and characterized, with AAV2 being the best characterized among the AAV serotypes and the first to be adapted as a gene delivery vehicle in the eye. However, these AAVs (including AAV2) have not been reported to be effective at transducing the deeper cell types of the retina when delivered via intravitreal administration. Accordingly, there is a need in the art for new AAV variants with superior transduction capabilities that will provide for more effective gene-based delivery to the cells of the retina for the treatment of ocular disease. There is a need in the art for such AAV variants which exhibit an enhanced retinal transduction profile -- in some instances broadly, in other instances preferentially to certain retinal cell types - as compared to wild-type AAVs and AAV variants as known in the art.

[0004] Naturally occurring AAV is a single stranded DNA virus that contains three open reading frames, *rep*, *cap*, and *aap*. The first gene, *rep*, encodes four proteins necessary for genome replication (Rep78, Rep68, Rep52, and Rep40), the second, *cap*, expresses three structural proteins (VP1-3) that assemble to form the viral capsid, and the third expresses the assembly activating protein (AAP) that is essential for capsid assembly. AAV is dependent upon the presence of a helper virus, such as an adenovirus or herpesvirus, for active replication. In the absence of a helper virus, AAV establishes a latent state in which its genome is maintained episomally or integrated into the host chromosome in the AAVS1 locus.

[0005] *In vitro* and *in vivo*-directed evolution techniques may be used to select for AAV variants that offer an improvement over current AAV-based gene delivery vectors. Such directed evolution techniques are known in the art and described, e.g., in PCT publication WO 2014/194132 and Kotterman & Schaffer (Nature Review Genetics, AOP, published online 20 May 2014; doi: 10.1038/nrg3742). Directed evolution is a capsid engineering approach that emulates natural evolution through iterative rounds of genetic diversification and selection processes, thereby enabling the accumulation of beneficial mutations that progressively improve the function of a biomolecule such as an AAV-based virion. In this approach, wild-type AAV *cap* genes are diversified to create large genetic libraries that are packaged to generate libraries of viral particles, and selective pressure is applied to isolate unique variants with superior phenotypes that can overcome gene delivery barriers.

[0006] AAV variants have been disclosed in, for example, in United States Patent Numbers 9,193,956; 9,186,419; 8,632,764; 8,663,624; 8,927,514; 8,628,966; 8,263,396; 8,734,809; 8,889,641; 8,632,764; 8,691,948; 8,299,295; 8,802,440; 8,445,267; 8,906,307; 8,574,583; 8,067,015; 7,588,772; 7,867,484; 8,163,543; 8,283,151; 8,999,678; 7,892,809; 7,906,111; 7,259,151; 7,629,322; 7,220,577; 8,802,080; 7,198,951; 8,318,480; 8,962,332; 7,790,449; 7,282,199; 8,906,675; 8,524,446; 7,712,893; 6,491,907; 8,637,255; 7,186,522; 7,105,345; 6,759,237; 6,984,517; 6,962,815; 7,749,492; 7,259,151; and 6,156,303; United States Publication Numbers 2013/0295614; 2015/0065562; 2014/0364338; 2013/0323226; 2014/0359799; 2013/0059732; 2014/0037585; 2014/0056854; 2013/0296409; 2014/0335054; 2013/0195801; 2012/0070899; 2011/0275529; 2011/0171262; 2009/0215879; 2010/0297177; 2010/0203083; 2009/0317417; 2009/0202490; 2012/0220492; 2006/0292117; and 2004/0002159; European Publication Numbers 2692731 A1; 2383346 B1; 2359865 B1; 2359866 B1; 2359867 B1; and 2357010 B1; 1791858 B1; 1668143 B1; 1660678 B1; 1664314 B1; 1496944 B1; 1456383 B1; 2341068 B1; 2338900 B1; 1456419 B1; 1310571 B1; 1456383 B1; 1633772 B1; and 1135468 B1; and International (PCT) Publication Numbers WO 2014/124282; WO 2013/170078; WO 2014/160092; WO 2014/103957; WO 2014/052789; WO 2013/174760; WO 2013/123503; WO 2011/038187; and WO 2008/124015; WO 2003/054197; however, none of these references disclose the embodiments and/or features and/or composition of matter structures of the AAV variants disclosed herein.

[0007] WO2017218842A1 describes variant adeno-associated viruses and methods of using the same. WO2009012176A2 describes methods and compositions for treating brain diseases.

SUMMARY OF THE INVENTION

[0008] The present invention is defined by the claims. Provided herein are variant adeno-associated virus (AAV) capsid proteins as claimed having one or more modifications in amino acid sequence relative to a parental AAV capsid protein, which, when present in an AAV virion, confer increased infectivity of one or more types of retinal cells as compared to the infectivity of the retinal cells by an AAV virion comprising an unmodified parental AAV capsid protein. Also provided are recombinant AAV virions and pharmaceutical compositions thereof comprising a variant AAV capsid protein as described herein, methods of making variant rAAV capsid proteins and virions, and methods for using these rAAV capsid proteins and virions in research and in clinical practice, for example in the delivery of nucleic acid sequences to one or more cells of the retina for the treatment of retinal disorders and diseases.

[0009] In some aspects of the disclosure, variant adeno-associated virus (AAV) capsid proteins as defined in the claims are provided, these variant AAV capsid proteins having one or more modifications in amino acid sequence relative to a parental AAV capsid, which, when present in an AAV virion, confer increased infectivity of one or more types of retinal cells (e.g. a photoreceptor cell (e.g. rods; cones), a retinal ganglion cell (ROC), a glial cell (e.g. a Müller glial cell, a microglial cell), a bipolar cell, an amacrine cell, a horizontal cell, and/or a retinal pigmented epithelium (RPE) cell) as compared to the infectivity of the retinal cells by an AAV virion comprising a parental AAV capsid protein that does not comprise the amino acid sequence modification.

[0010] In some aspects of the disclosure, recombinant AAV (rAAV) virions as defined in the claims are provided, these rAAV virions comprising a variant capsid protein as described herein, wherein the rAAV virions exhibit increased infectivity of one or more types of retinal cells (e.g. a photoreceptor cell (e.g. rods; cones), a retinal ganglion cell (RGC), a glial cell (e.g. a Müller glial cell, a microglial cell), a bipolar cell, an amacrine cell, a horizontal cell, and/or a retinal pigmented epithelium (RPE) cell) relative to the infectivity of the retinal cell by an AAV virion comprising a corresponding unmodified parental AAV capsid protein. In some embodiments, the rAAV virion exhibits increased infectivity of all retinal cells relative to the AAV virion comprising the parental AAV capsid protein. In other embodiments, the rAAV virion exhibits increased infectivity of certain cell types of the retina but not others relative of the AAV virion comprising the parental AAV capsid protein. Put another way, the rAAV virion exhibits increased infectivity that is preferential for certain cell types of the retina but not others, e.g. the rAAV demonstrates a preferentially increased infectivity of one or more cell types selected from photoreceptor cells, retinal ganglion cells, glial cells, bipolar cells, amacrine cells horizontal cell, and/or retinal pigmented epithelium (RPE) cell, but does not demonstrate increased infectivity of all cell types.

[0011] In some embodiments, the rAAV virion comprises a heterologous nucleic acid. In some such embodiments, the heterologous nucleic acid encodes an RNA that encodes a

polypeptide. In other such embodiments, the heterologous nucleic acid sequence encodes an RNA that does not encode a polypeptide, e.g. the heterologous nucleic acid sequence an RNA interference agent, a guide RNA for a nuclease, etc.

[0012] Also provided herein are pharmaceutical compositions comprising the subject infectious rAAV virions and a pharmaceutically acceptable carrier.

[0013] Also provided is an rAAV virion comprising a variant capsid protein as herein described for use in a method of delivering a heterologous nucleic acid to a target cell (such as a retinal cell) by contacting the target cell with the rAAV virion. In some embodiments, the target cell is *in vivo*, such as in the eye of an individual in need of treatment for an ocular disease. In other embodiments, the target cell is *in vitro*.

[0014] Also provided is an rAAV virion as claimed for use in methods of treating an ocular disease by administering to a subject in need of such treatment an effective amount of rAAV virions comprising a variant capsid protein as herein described or a pharmaceutical composition comprising an effective amount of the rAAV virions.

[0015] Also provided is an isolated nucleic acid comprising a sequence encoding a variant AAV capsid protein as described herein and a host cell comprising the isolated nucleic acid. In yet other embodiments, the isolated nucleic acid and/or isolated host cell comprises the rAAV.

[0016] The variant AAV capsid protein comprises an insertion as defined in the claims, for example when present in an AAV virion, confers increased infectivity of a retinal cell compared to the infectivity of a retinal cell by an AAV virion comprising the corresponding parental AAV capsid protein. The peptide comprises the sequence defined in the claims.

[0017] In some embodiments, the peptide consists essentially of the sequence ISDQTKH (SEQ ID NO:14).

[0018] In some aspects, the variant AAV capsid protein comprises one or more amino acid substitutions relative to a corresponding parental AAV capsid protein, wherein the variant capsid protein, when present in an AAV virion, confers increased infectivity of a retinal cell compared to the infectivity of a retinal cell by an AAV virion comprising the corresponding parental AAV capsid protein.

[0019] In related aspects, the variant AAV capsid protein comprises a peptide insertion and one or more amino acid substitutions relative to a corresponding parental AAV capsid protein, wherein the variant capsid protein, when present in an AAV virion, confers increased infectivity of a retinal cell compared to the infectivity of a retinal cell by an AAV virion comprising the corresponding parental AAV capsid protein.

[0020] Also disclosed herein is a variant AAV capsid protein comprising the heterologous peptide LAISDQTKHA (SEQ ID NO:28) and a P34A substitution relative to AAV2.

[0021] Also disclosed herein is a variant AAV capsid protein comprising the heterologous peptide LAISDQTKHA (SEQ ID NO:28) and amino acid substitutions N312K, N449D, N551S, I698V, and L735Q relative to AAV2.

[0022] Also disclosed herein are methods for manufacture and/or delivery of an rAAV comprising a variant AAV capsid as disclosed herein. In addition, provided herein are kits comprising an rAAV comprising a variant AAV capsid as disclosed herein and for use in methods described herein.

[0023] In other embodiments, the AAV virion comprising the variant capsid protein in the preceding paragraphs may incorporate any of the preceding or subsequently disclosed embodiments. Indeed, it is appreciated that certain features of the invention, which are, for clarity, described in the context of separate embodiments, may also be provided in combination in a single embodiment. Conversely, various features of the invention, which are, for brevity, described in the context of a single embodiment, may also be provided separately or in any suitable sub-combination. All combinations of the embodiments pertaining to the invention are specifically embraced by the invention and are disclosed herein just as if each and every combination was individually and explicitly disclosed. In addition, all subcombinations of the various embodiments and elements thereof are also specifically embraced by the invention and are disclosed herein just as if each and every such sub-combination was individually and explicitly disclosed herein.

[0024] The present invention is defined by the claims.

[0025] Other features and advantages of the invention disclosed herein will be apparent from the following Figures, Detailed Description, and the Claims.

BRIEF DESCRIPTION OF THE FIGURES

[0026] The invention is best understood from the following detailed description when read in conjunction with the accompanying drawings. The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee. It is emphasized that, according to common practice, the various features of the drawings are not to-scale. On the contrary, the dimensions of the various features are arbitrarily expanded or reduced for clarity. Included in the drawings are the following figures.

Figure 1 depicts embodiments of a directed evolution methodology. Step (a) depicts the generation of a viral capsid library comprising combinations of DNA mutation techniques and *cap* genes. Step (b) depicts the packaging of the viruses such that each viral particle is composed of a mutant capsid surrounding the *cap* gene encoding that capsid and purified. The capsid library is then placed under selective pressure *in vitro* or *in vivo*. In this aspect of the

directed evolution technology, tissues or cellular material of interest are harvested for isolation of AAV variants that have successfully infected that target, and the successful viruses are recovered. Step (c) depicts the Stage 1 enrichment of successful clones through repeated selection. Step (d) depicts the Stage 2 enrichment of selected *cap* genes which undergo re-diversification and further selection steps to iteratively increase viral fitness. Step (e) depicts the variants, identified as hits during Vector Selection Stages 1 and 2, which will be manufactured as recombinant AAV vectors and characterized for the level of transduction of various cell types and tissue targets. By the nature of the AAV directed evolution process, variants that are disclosed herein have already demonstrated the ability to transduce retinal cells and deliver a genome (the genome encoding the variant *cap* gene) during the selection process.

Figure 2 provides a retinal flat mount schematic showing where samples from which viral genomes are amplified, are collected across a broad area of the retina.

Figure 3 shows a PCR amplification of viral genomes from the ganglion cell layer (GCL), inner nuclear layer (INL), photoreceptor/outer nuclear layer (ONL), and retinal pigment epithelia (RPE) layer retinal tissue from a representative round of selection. Both the right eye (top image) and left eye (bottom image) were injected with library. Inner retina (in), middle retina (mid), and outer/peripheral retina (out) were sampled. Bands within red boxes represent successful amplification of viral genomes.

Figure 4A-4D shows frequency of motifs within sequencing analysis. Figure 4A provides Round 3 sequencing analysis. Figure 4B provides Round 4 sequencing analysis. Figure 4C provides Round 5 sequencing analysis. Figure 4D provides Round 6 sequencing analysis.

Figure 5 provides a representative three-dimensional model of AAV2 containing a random heptamer following amino acid 587.

Figure 6 provides an alignment of wild-type AAV SEQ ID NOS:1-11 showing amino acid locations between and across the wild-type (naturally occurring) serotypes AAV1, AAV2, AAV3A, AAV3B, and AAV4-10.

Figure 7 provides fundus fluorescence images taken with a Heidelberg Spectralis™ of the retina of an African Green monkey following intravitreal administration of 2×10^{11} vector genomes (vg) of AAV2 delivering a GFP transgene under the control of the CMV promoter (AAV2.CMV.GFP). Images were taken at baseline (A) and at 14 days (B), 28 days (C), and 42 days (D) after injection.

Figure 8 provides fundus fluorescence images taken with a Heidelberg Spectralis™ the retina of an African Green monkey following intravitreal administration of 2×10^{11} vector genomes (vg) of the novel AAV variant LAISDQTKHA+P34A delivering a GFP transgene under the control of the CMV promoter (LAISDQTKHA+P34A.CMV.GFP). Images taken at baseline (A) and at 14 days (B), 28 days (C), and 42 days (D) after injection.

Figure 9 provides fundus fluorescence images taken with a Heidelberg Spectralis™ of the

retinas of Cynomolgus monkeys following intravitreal administration of the novel AAV variant LAISDQTKHA+P34A delivering a GFP transgene under the control of the CAG promoter (LAISDQTKHA+P34A.CAG.EGFP). (A) The retina of a monkey injected intravitreally with 2×10^{11} vg of vector, imaged 14 days (A1), 21 days (A2), and 28 days (A3) after injection. (B) The retina of a monkey injected intravitreally with 1×10^{12} vg of vector, imaged 14 days (B1) and 21 days (B2) after injection.

Figures 10A-10E provide the results of immunohistochemical analysis of the retina of a monkey injected intravitreally with 1×10^{12} vg of the novel AAV variant LAISDQTKHA+P34A delivering a GFP transgene under the control of the CAG promoter, analyzed three weeks after injection. All immunohistochemistry is provided alongside the corresponding fundus fluorescence image, with a red box to denote approximately where in the retina the analysis was performed. Figure 10A: Robust retinal pigment epithelium (RPE) and photoreceptor transduction was observed using a GFP-specific antibody (red). Cone photoreceptor immunostaining using an M/L opsin antibody is shown in white. Figures 10B and 10C: Robust rod and cone photoreceptor (Figure 10B) and RPE (Figure 10C) transduction was observed by direct EGFP fluorescence (green) and by immunohistochemistry using a GFP-specific antibody (red). Melanosomes in RPE appear black in the image. Figure 10D: Transduction of cone photoreceptors (identified by M/L opsin, white) and retinal ganglion cells (RGC) in and around the fovea was observed by direct EGFP fluorescence (green) and by immunohistochemistry using a GFP-specific antibody (red). Images in the middle panels are a higher magnification (63X) of the area denoted by a white box in the left panel. Figure 10E: Transduction of retinal ganglion cells (RGC) and the retinal ganglion cell layer was observed by direct EGFP fluorescence (right panels, green; lower right panel is a 63X magnification of the upper right panel); top left panel shows the region under brightfield illumination.

Figures 11A-11F provides data on the transduction of human retinal pigment epithelial (RPE) cells in vitro by recombinant AAV virus comprising the novel AAV variant LAISDQTKHA+P34A capsid and a GFP transgene under the control of the CAG promoter. Cells that were differentiated into RPE cells from a human embryonic stem cell line (Figures 11A and 11C) or from human fibroblast-derived induced pluripotent stem cells (FB-iPSC) (Figures 11B and 11D) were infected with novel AAV variant LAISDQTKHA+P34A.CAG.GFP or wild type control AAV2.CAG.GFP. Figures 11A and 11B: Immunofluorescence imaging of the cell cultures 7 days after infection at an MOI of 500 demonstrates that the novel AAV variant capsid (left panels) transduces RPE cells better than wild type AAV2 capsid (right panels). Figures 11C and 11D: Quantification of the percent of GFP-positive RPE cells in each culture by flow cytometry reveals that the novel AAV variant capsid provides for a significant, dose-dependent improvement in the number of cells transduced over wild type AAV2 capsid regardless of cell source. Figures 11E and 11F: Quantification of the amount of GFP in each culture by Western blot reveals that the novel AAV variant provides for significant improvement in expression of the transgene over wild type capsid regardless of cell source.

DETAILED DESCRIPTION

[0027] Before the present methods and compositions are described, it is to be understood that this invention is not limited to a particular method or composition described and as such may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting, since the scope of the present invention will be limited only by the appended claims.

[0028] The invention disclosed herein is illustrated in the figures and description. However, while particular embodiments are illustrated in the figures, there is no intention to limit the invention to the specific embodiment or embodiments illustrated and/or disclosed. The present invention is defined by the claims.

[0029] As such, the figures are intended to be illustrative and not restrictive.

[0030] Where a range of values is provided, it is understood that each intervening value, to the tenth of the unit of the lower limit unless the context clearly dictates otherwise, between the upper and lower limits of that range is also specifically disclosed. Each smaller range between any stated value or intervening value in a stated range and any other stated or intervening value in that stated range is encompassed within the invention. The upper and lower limits of these smaller ranges may independently be included or excluded in the range, and each range where either, neither or both limits are included in the smaller ranges is also encompassed within the invention, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either or both of those included limits are also included in the invention.

[0031] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, some potential and preferred methods and materials are now described.

[0032] Any recited method can be carried out in the order of events recited or in any other order which is logically possible.

[0033] It is noted that as used herein and in the appended claims, the singular forms "a," "an," and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a recombinant AAV virion" includes a plurality of such virions and reference to "the photoreceptor cell" includes reference to one or more photoreceptor cells and equivalents thereof known to those skilled in the art, and so forth. It is further noted that the claims may be drafted to exclude any optional element. As such, this statement is intended to serve as antecedent basis for use of such exclusive terminology as "solely," "only" and the like

in connection with the recitation of claim elements, or use of a "negative" limitation.

[0034] The publications discussed herein are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that the present invention is not entitled to antedate such publication by virtue of prior invention. Further, the dates of publication provided may be different from the actual publication dates which may need to be independently confirmed.

DEFINITIONS

[0035] Unless otherwise defined, all scientific and technical terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this technology belongs.

[0036] Adeno-associated virus is a nonpathogenic parvovirus composed of a 4.7 kb single-stranded DNA genome within a non-enveloped, icosahedral capsid. The genome contains three open reading frames (ORF) flanked by inverted terminal repeats (ITR) that function as the viral origin of replication and packaging signal. The *rep* ORF encodes four nonstructural proteins that play roles in viral replication, transcriptional regulation, site-specific integration, and virion assembly. The *cap* ORF encodes three structural proteins (VP 1-3) that assemble to form a 60-mer viral capsid. Finally, an ORF present as an alternate reading frame within the *cap* gene produces the assembly-activating protein (AAP), a viral protein that localizes AAV capsid proteins to the nucleolus and functions in the capsid assembly process.

[0037] There are several naturally occurring ("wild-type") serotypes and over 100 known variants of AAV, each of which differs in amino acid sequence, particularly within the hypervariable regions of the capsid proteins, and thus in their gene delivery properties. No AAV has been associated with any human disease, making recombinant AAV attractive for clinical applications.

[0038] For the purposes of the disclosure herein, the terminology "AAV" is an abbreviation for adeno-associated virus, including, without limitation, the virus itself and derivatives thereof. Except where otherwise indicated, the terminology refers to all subtypes or serotypes and both replication-competent and recombinant forms. The term "AAV" includes, without limitation, AAV type 1 (AAV-1 or AAV1), AAV type 2 (AAV-2 or AAV2), AAV type 3A (AAV-3A or AAV3A), AAV type 3B (AAV-3B or AAV3B), AAV type 4 (AAV-4 or AAV4), AAV type 5 (AAV-5 or AAV5), AAV type 6 (AAV-6 or AAV6), AAV type 7 (AAV-7 or AAV7), AAV type 8 (AAV-8 or AAV8), AAV type 9 (AAV-9 or AAV9), AAV type 10 (AAV-10 or AAV10 or AAVrh10), avian AAV, bovine AAV, canine AAV, caprine AAV, equine AAV, primate AAV, non-primate AAV, and ovine AAV. "Primate AAV" refers to AAV that infect primates, "non-primate AAV" refers to AAV that infect non-primate mammals, "bovine AAV" refers to AAV that infect bovine mammals, *etc.*

[0039] The genomic sequences of various serotypes of AAV, as well as the sequences of the

native terminal repeats (TRs), Rep proteins, and capsid subunits are known in the art. Such sequences may be found in the literature or in public databases such as GenBank. See, e.g., GenBank Accession Numbers NC_002077.1 (AAV1), AF063497.1 (AAV1), NC_001401.2 (AAV2), AF043303.1 (AAV2), J01901.1 (AAV2), U48704.1 (AAV3A), NC_001729.1 (AAV3A), AF028705.1 (AAV3B), NC_001829.1 (AAV4), U89790.1 (AAV4), NC_006152.1 (AA5), AF085716.1 (AAV-5), AF028704.1 (AAV6), NC_006260.1 (AAV7), AF513851.1 (AAV7), AF513852.1 (AAV8) NC_006261.1 (AAV-8), AY530579.1 (AAV9), AAT46337 (AAV10) and AA088208 (AAVrh10).

[0040] See also, e.g., Srivistava et al. (1983) J. Virology 45:555; Chiorini et al. (1998) J. Virology 71:6823; Chiorini et al. (1999) J. Virology 73: 1309; Bantel-Schaal et al. (1999) J. Virology 73:939; Xiao et al. (1999) J. Virology 73:3994; Muramatsu et al. (1996) Virology 221:208; Shade et. al. (1986) J. Virol. 58:921; Gao et al. (2002) Proc. Nat. Acad. Sci. USA 99: 11854; Moris et al. (2004) Virology 33:375-383; international patent publications WO 00/28061, WO 99/61601, WO 98/11244; and U.S. Pat. No. 6,156,303.

[0041] The sequences of naturally existing cap (capsid) proteins associated with AAV serotypes are known in the art and include those disclosed herein as AAV1 (SEQ ID NO:1), AAV2 (SEQ ID NO:2), AAV3A (SEQ ID NO:3), AAV3B (SEQ ID NQ:4), AAV4 (SEQ ID NO:5), AAV5 (SEQ ID NO:6), AAV6 (SEQ ID NO:7), AAV7 (SEQ ID NO:8), AAV8 (SEQ ID NO:9), AAV9 (SEQ ID NO:10), AAV10 (SEQ ID NO:11), and AAVrh10 (SEQ ID NO:12). The terms "variant AAV capsid protein" or "AAV variant" refer to an AAV capsid protein comprising an amino acid sequence that includes at least one modification or substitution (including deletion, insertion, point mutation, etc.) relative to a naturally existing or "wild-type" AAV capsid protein sequences, e.g. as set forth in SEQ ID NO:1-12 herein. A variant AAV capsid protein may have about 80% identity or more to the amino acid sequence of a wild type capsid protein, for example, 85% identity or more, 90% identity or more, or 95% identity or more to the amino acid sequence of the wild type capsid protein, for example, 98% or 99% identity to the wild type capsid protein. A variant AAV capsid protein may not be a wild type capsid protein.

[0042] For the purposes of the disclosure herein, "AAV virion" or "AAV viral particle" refers to a viral particle composed of at least one AAV capsid protein and an encapsidated AAV polynucleotide.

[0043] For the purposes of the disclosure herein, the terminology "rAAV" is an abbreviation that refers to recombinant adeno-associated virus. "Recombinant," as applied to a polynucleotide means that the polynucleotide is the product of various combinations of cloning, restriction or ligation steps, and other procedures that result in a construct that is distinct from a polynucleotide found in nature. A recombinant virus is a viral particle comprising a recombinant polynucleotide. The terms respectively include replicates of the original polynucleotide construct and progeny of the original virus construct.

[0044] The term "rAAV vector" encompasses rAAV virions (i.e., rAAV viral particles) (e.g., an infectious rAAV virion), which by definition include an rAAV polynucleotide; and also

encompasses polynucleotides encoding rAAV (e.g., a single stranded polynucleotide encoding rAAV (ss-rAAV); a double stranded polynucleotide encoding rAAV (ds- rAAV), e.g., plasmids encoding rAAV; and the like).

[0045] If an AAV virion comprises a heterologous polynucleotide (i.e. a polynucleotide other than a wild-type AAV genome, e.g., a transgene to be delivered to a target cell, an RNAi agent or CRISPR agent to be delivered to a target cell, etc.), it is typically referred to as a "recombinant AAV (rAAV) virion" or an "rAAV viral particle." In general, the heterologous polynucleotide is flanked by at least one, and generally by two, AAV inverted terminal repeat sequences (ITRs).

[0046] The term "packaging" refers to a series of intracellular events that result in the assembly and encapsidation of an AAV particle. AAV "*rep*" and "*cap*" genes refer to polynucleotide sequences encoding replication and encapsidation proteins of adeno-associated virus. AAV *rep* and *cap* are referred to herein as AAV "packaging genes."

[0047] The terminology "helper virus" for AAV refers to a virus that allows AAV (e.g. wild-type AAV) to be replicated and packaged by a mammalian cell. A variety of such helper viruses for AAV are known in the art, including adenoviruses, herpesviruses and poxviruses such as vaccinia. The adenoviruses encompass a number of different subgroups, although Adenovirus type 5 of subgroup C is most commonly used. Numerous adenoviruses of human, non-human mammalian and avian origin are known and available from depositories such as the ATCC. Viruses of the herpes family include, for example, herpes simplex viruses (HSV) and Epstein-Barr viruses (EBV), as well as cytomegaloviruses (CMV) and pseudorabies viruses (PRV); which are also available from depositories such as ATCC.

[0048] The terminology "helper virus function(s)" refers to function(s) encoded in a helper virus genome which allow AAV replication and packaging (in conjunction with other requirements for replication and packaging described herein). As described herein, "helper virus function" may be provided in a number of ways, including by providing helper virus or providing, for example, polynucleotide sequences encoding the requisite function(s) to a producer cell in trans. For example, a plasmid or other expression vector comprising nucleotide sequences encoding one or more adenoviral proteins is transfected into a producer cell along with an rAAV vector.

[0049] The terminology "infectious" virus or viral particle is one that comprises a competently assembled viral capsid and is capable of delivering a polynucleotide component into a cell for which the viral species is tropic. The term does not necessarily imply any replication capacity of the virus. Assays for counting infectious viral particles are described elsewhere in this disclosure and in the art. Viral infectivity can be expressed as the ratio of infectious viral particles to total viral particles. Methods of determining the ratio of infectious viral particle to total viral particle are known in the art. See, e.g., Grainger et al. (2005) Mol. Ther. 11: S337 (describing a TCID50 infectious titer assay); and Zolotukhin et al. (1999) Gene Ther. 6:973. See also the Examples.

[0050] The term "tropism" as used herein refers to the preferential targeting by a virus (e.g., an AAV) of cells of a particular host species or of particular cell types within a host species. For example, a virus that can infect cells of the heart, lung, liver, and muscle has a broader (i.e., increased) tropism relative to a virus that can infect only lung and muscle cells. Tropism can also include the dependence of a virus on particular types of cell surface molecules of the host. For example, some viruses can infect only cells with surface glycosaminoglycans, while other viruses can infect only cells with sialic acid (such dependencies can be tested using various cells lines deficient in particular classes of molecules as potential host cells for viral infection). In some cases, the tropism of a virus describes the virus's relative preferences. For example, a first virus may be able to infect all cell types but is much more successful in infecting those cells with surface glycosaminoglycans. A second virus can be considered to have a similar (or identical) tropism as the first virus if the second virus also prefers the same characteristics (e.g., the second virus is also more successful in infecting those cells with surface glycosaminoglycans), even if the absolute transduction efficiencies are not similar. For example, the second virus might be more efficient than the first virus at infecting every given cell type tested, but if the relative preferences are similar (or identical), the second virus can still be considered to have a similar (or identical) tropism as the first virus. In some embodiments, the tropism of a virion comprising a subject variant AAV capsid protein is not altered relative to a naturally occurring virion. In some embodiments, the tropism of a virion comprising a subject variant AAV capsid protein is expanded (i.e., broadened) relative to a naturally occurring virion. In some embodiments, the tropism of a virion comprising a subject variant AAV capsid protein is reduced relative to a naturally occurring virion.

[0051] The terminology "replication-competent" virus (e.g. a replication-competent AAV) refers to a phenotypically wild-type virus that is infectious, and is also capable of being replicated in an infected cell (i.e. in the presence of a helper virus or helper virus functions). In the case of AAV, replication competence generally requires the presence of functional AAV packaging genes. In general, rAAV vectors as described herein are replication-incompetent in mammalian cells (especially in human cells) by virtue of the lack of one or more AAV packaging genes. Typically, such rAAV vectors lack any AAV packaging gene sequences in order to minimize the possibility that replication competent AAV are generated by recombination between AAV packaging genes and an incoming rAAV vector. In many embodiments, rAAV vector preparations as described herein are those which contain few if any replication competent AAV (rcAAV, also referred to as RCA) (e.g., less than about 1 rcAAV per 10^2 rAAV particles, less than about 1 rcAAV per 10^4 rAAV particles, less than about 1 rcAAV per 10^6 rAAV particles, less than about 1 rcAAV per 10^{12} rAAV particles, or no rcAAV).

[0052] The term "polynucleotide" refers to a polymeric form of nucleotides of any length, including deoxyribonucleotides or ribonucleotides, or analogs thereof. A polynucleotide may comprise modified nucleotides, such as methylated nucleotides and nucleotide analogs, and may be interrupted by nonnucleotide components. If present, modifications to the nucleotide structure may be imparted before or after assembly of the polymer. The term polynucleotide, as used herein, refers interchangeably to double- and single- stranded molecules. Unless

otherwise specified or required, any embodiment herein that comprises a polynucleotide encompasses both the double-stranded form and each of two complementary single-stranded forms known or predicted to make up the double-stranded form.

[0053] A polynucleotide or polypeptide has a certain percent "sequence identity" to another polynucleotide or polypeptide, meaning that, when aligned, that percentage of bases or amino acids are the same when comparing the two sequences. Sequence similarity can be determined in a number of different manners. To determine sequence identity, sequences can be aligned using the methods and computer programs, including BLAST, available over the world wide web at ncbi.nlm.nih.gov/BLAST/. Another alignment algorithm is FASTA, available in the Genetics Computing Group (GCG) package, from Madison, Wisconsin, USA, a wholly owned subsidiary of Oxford Molecular Group, Inc. Other techniques for alignment are described in *Methods in Enzymology*, vol. 266: *Computer Methods for Macromolecular Sequence Analysis* (1996), ed. Doolittle, Academic Press, Inc., a division of Harcourt Brace & Co., San Diego, California, USA. Of particular interest are alignment programs that permit gaps in the sequence. The Smith- Waterman is one type of algorithm that permits gaps in sequence alignments. See *Meth. Mol. Biol.* 70: 173-187 (1997). Also, the GAP program using the Needleman and Wunsch alignment method can be utilized to align sequences. See *J. Mol. Biol.* 48: 443- 453 (1970).

[0054] The term "gene" refers to a polynucleotide that performs a function of some kind in the cell. For example, a gene can contain an open reading frame that is capable of encoding a gene product. One example of a gene product is a protein, which is transcribed and translated from the gene. Another example of a gene product is an RNA, e.g. a functional RNA product, e.g., an aptamer, an interfering RNA, a ribosomal RNA (rRNA), a transfer RNA (tRNA), a non-coding RNA (ncRNA), a guide RNA for nucleases, etc., which is transcribed but not translated.

[0055] The terminology "gene expression product" or "gene product" is a molecule resulting from expression of a particular gene, as defined above. Gene expression products include, e.g., a polypeptide, an aptamer, an interfering RNA, a messenger RNA (mRNA), an rRNA, a tRNA, a non-coding RNA (ncRNA), and the like.

[0056] The term "siRNA agent" ("small interfering" or "short interfering RNA" (or siRNA)) is an RNA duplex of nucleotides that is targeted to a gene interest (a "target gene"). An "RNA duplex" refers to the structure formed by the complementary pairing between two regions of a RNA molecule, forming a region of double stranded RNA (dsRNA). siRNA is "targeted" to a gene in that the nucleotide sequence of the duplex portion of the siRNA is complementary to a nucleotide sequence of the targeted gene. In some embodiments, the length of the duplex of siRNAs is less than 30 nucleotides. In some embodiments, the duplex can be 29, 28, 27, 26, 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11 or 10 nucleotides in length. In some embodiments, the length of the duplex is 19-25 nucleotides in length. In some embodiments, siRNA-mediated gene targeting is accomplished through the use of DNA-directed RNA interference (ddRNAi) which is a gene-silencing technique that utilizes DNA constructs to activate an animal cell's endogenous RNA interference (RNAi) pathways. Such DNA constructs

are designed to express selfcomplementary double-stranded RNAs, typically short-hairpin RNAs (shRNA), that once processed bring about silencing of a target gene or genes. Any RNA, including endogenous mRNAs or viral RNAs, can be silenced by designing constructs to express double-stranded RNA complementary to the desired mRNA target. As such, the RNA duplex portion of an siRNA agent can be part of a short hairpin structure referred to as shRNA. In addition to the duplex portion, the hairpin structure may contain a loop portion positioned between the two sequences that form the duplex. The loop can vary in length. In some embodiments the loop is 5, 6, 7, 8, 9, 10, 11, 12 or 13 nucleotides in length. The hairpin structure can also contain 3' or 5' overhang portions. In some embodiments, the overhang is a 3' or a 5' overhang 0, 1, 2, 3, 4 or 5 nucleotides in length. In general, the level of expression product (e.g., mRNA, polypeptide, etc.) of a target gene is reduced by an siRNA agent (e.g., an siRNA, an shRNA, etc.) that contains specific double stranded nucleotide sequences that are complementary to at least a 19-25 nucleotide long segment (e.g., a 20-21 nucleotide sequence) of the target gene transcript, including the 5' untranslated (UT) region, the ORF, or the 3' UT region. In some embodiments, short interfering RNAs are about 19-25nt in length. See, e.g., PCT applications WOO/44895, WO99/32619, WO01/75164, WO01/92513, WO01/29058, WO01/89304, WO02/16620, and WO02/29858; and U.S. Patent Publication No. 20040023390 for descriptions of siRNA technology. The siRNA and/or shRNA can be encoded by a nucleic acid sequence, and the nucleic acid sequence can also include a promoter. The nucleic acid sequence can also include a polyadenylation signal. In some embodiments, the polyadenylation signal is a synthetic minimal polyadenylation signal.

[0057] The terminology "antisense RNA" encompasses RNA that is complementary to a gene expression product. For example, an antisense RNA targeted to a specific mRNA is an RNA-based agent (or can be a modified RNA) that is complementary to the mRNA, where hybridization of the antisense RNA to the mRNA alters the expression of the mRNA (e.g., via altering the stability of the RNA, altering the translation of the RNA, etc.). Also included in "antisense RNA" are nucleic acids encoding an antisense RNA.

[0058] With regards to "CRISPR/Cas9 agents", the term "CRISPR" encompasses Clustered regularly interspaced short palindromic repeats/CRISPR-associated (Cas) systems that evolved to provide bacteria and archaea with adaptive immunity against viruses and plasmids by using CRISPR RNAs (crRNAs) to guide the silencing of invading nucleic acids. The Cas9 protein (or functional equivalent and/or variant thereof, i.e., Cas9-like protein) naturally contains DNA endonuclease activity that depends on association of the protein with two naturally occurring or synthetic RNA molecules called crRNA and tracrRNA (also called guide RNAs). In some cases, the two molecules are covalently linked to form a single molecule (also called a single guide RNA ("sgRNA")). Thus, the Cas9 or Cas9-like protein associates with a DNA-targeting RNA (which term encompasses both the two-molecule guide RNA configuration and the single-molecule guide RNA configuration), which activates the Cas9 or Cas9-like protein and guides the protein to a target nucleic acid sequence.

[0059] If the Cas9 or Cas9-like protein retains its natural enzymatic function, it will cleave target DNA to create a double-strand break, which can lead to genome alteration (i.e., editing:

deletion, insertion (when a donor polynucleotide is present), replacement, etc.), thereby altering gene expression. Some variants of Cas9 (which variants are encompassed by the term Cas9-like) have been altered such that they have a decreased DNA cleaving activity (in some cases, they cleave a single strand instead of both strands of the target DNA, while in other cases, they have severely reduced to no DNA cleavage activity). Cas9-like proteins with decreased DNACleavage activity (even no DNA-cleaving activity) can still be guided to a target DNA to block RNA polymerase activity. Alternatively, the Cas9 or Cas9-like protein may be modified by fusing a VP64 transcription activation domain to the Cas9 protein and codelivering the fusion protein with a MS2-P65-HSF1 helper protein and a single guide RNA comprising MS2 RNA aptamers at the tetraloop and stem-loop to form a Synergistic Activation Mediator (Cas9-SAM) complex in the cell that activates transcription. Thus enzymatically inactive Cas9-like proteins can be targeted to a specific location in a target DNA by a DNA-targeting RNA in order to block or activate transcription of the target DNA. The term "CRISPR/Cas9 agents" as used herein encompasses all forms of CRISPR/Cas9 as described above or as known in the art.

[0060] Detailed information regarding CRISPR agents can be found, for example in (a) Jinek et al., *Science*. 2012 Aug 17;337(6096):816-21: "A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity"; (b) Qi et al., *Cell*. 2013 Feb 28; 152(5): 1173-83: "Repurposing CRISPR as an RNA-guided platform for sequence- specific control of gene expression", and (c) US patent application number 13/842,859 and PCT application number PCT/US13/32589;

[0061] Thus, the term "CRISPR agent" as used herein encompasses any agent (or nucleic acid encoding such an agent), comprising naturally occurring and/or synthetic sequences, that can be used in the Cas9-based system (e.g., a Cas9 or Cas9-like protein; any component of a DNA-targeting RNA, e.g., a crRNA-like RNA, a tracrRNA-like RNA, a single guide RNA, etc.; a donor polynucleotide; and the like).

[0062] By "Zinc-finger nucleases" (ZFNs) it is meant artificial DNA endonucleases generated by fusing a zinc finger DNA binding domain to a DNA cleavage domain. ZFNs can be engineered to target desired DNA sequences and this enables zinc-finger nucleases to cleave unique target sequences. When introduced into a cell, ZFNs can be used to edit target DNA in the cell (e.g., the cell's genome) by inducing double strand breaks. For more information on the use of ZFNs, see, for example: Asuri et al., *Mol Ther*. 2012 Feb;20(2):329-38; Bibikova et al. *Science*. 2003 May 2;300(5620):764; Wood et al. *Science*. 2011 Jul 15;333(6040):307; Ochiai et al. *Genes Cells*. 2010 Aug;15(8):87S-85; Takasu et. al., *Insect Biochem Mol Biol*. 2010 Oct;40(10):759-65; Ekker et al, *Zebrafish* 2008 Summer;5(2): 121-3; Young et al, *Proc Natl Acad Sci U S A*. 2011 Apr 26;108(17):7052-7; Goldberg et al, *Cell*. 2010 Mar 5;140(5):678-91; Geurts et al, *Science*. 2009 Jul 24;325(5939):433; Flisikowska et al, *PLoS One*. 2011;6(6):e21045. doi: 10.1371/journal.pone.0021045. Epub 2011 Jun 13; Hauschild et al, *Proc Natl Acad Sci U S A*. 2011 Jul 19;108(29): 12013-7; and Yu et al, *Cell Res*. 2011 Nov;21(1 1): 1638-40.

[0063] The term "ZFN agent" encompasses a zinc finger nuclease and/or a polynucleotide comprising a nucleotide sequence encoding a zinc finger nuclease.

[0064] The terminology "Transcription activator-like effector nuclease" or "TALEN" agents refers to Transcription activator-like effector nucleases (TALENs) are artificial DNA endonucleases generated by fusing a TAL (Transcription activator-like) effector DNA binding domain to a DNA cleavage domain. TALENS can be quickly engineered to bind practically any desired DNA sequence and when introduced into a cell, TALENs can be used to edit target DNA in the cell (e.g., the cell's genome) by inducing double strand breaks. For more information on the use of TALENs, see, for example: Hockemeyer et al. Nat Biotechnol. 2011 Jul 7;29(8):731-4; Wood et al. Science. 2011 Jul 15;333(6040):307; Tesson et al. Nat Biotechnol. 2011 Aug 5;29(8):695-6; and Huang et. al., Nat Biotechnol. 2011 Aug 5;29(8):699-700.

[0065] The term "TALEN agent" encompasses a TALEN and/or a polynucleotide comprising a nucleotide sequence encoding a TALEN.

[0066] The terminology "control element" or "control sequence" refers to a nucleotide sequence involved in an interaction of molecules that contributes to the functional regulation of a polynucleotide, including replication, duplication, transcription, splicing, translation, or degradation of the polynucleotide. The regulation may affect the frequency, speed, or specificity of the process, and may be enhancing or inhibitory in nature. Control elements known in the art include, for example, transcriptional regulatory sequences such as promoters and enhancers. A promoter is a DNA region capable under certain conditions of binding RNA polymerase and initiating transcription of a coding region usually located downstream (in the 3' direction) from the promoter. Promoters may be ubiquitously acting, i.e. active in many cell types, e.g. CAG or CMV promoters; or tissue or cell specific, e.g. the rho promoter, which is active in rods, or the opsin promoter, which is active in cones.

[0067] The terminology "operatively linked" or "operably linked" refers to a juxtaposition of genetic elements, wherein the elements are in a relationship permitting them to operate in the expected manner. For instance, a promoter is operatively linked to a coding region if the promoter helps initiate transcription of the coding sequence. There may be intervening residues between the promoter and coding region so long as this functional relationship is maintained.

[0068] The terminology "expression vector" encompasses a vector comprising a polynucleotide region which encodes a polypeptide of interest, and is used for effecting the expression of the protein in an intended target cell. An expression vector may also comprise control elements operatively linked to the encoding region to facilitate expression of the protein in the target. The combination of control elements and a gene or genes to which they are operably linked for expression is sometimes referred to as an "expression cassette," a large number of which are known and available in the art or can be readily constructed from components that are available in the art.

[0069] The term "heterologous" means derived from a genotypically distinct entity from that of the rest of the entity to which it is being compared. For example, a polynucleotide introduced by genetic engineering techniques into a plasmid or vector derived from a different species is a heterologous polynucleotide. A promoter removed from its native coding sequence and operatively linked to a coding sequence with which it is not naturally found linked is a heterologous promoter. Thus, for example, an rAAV that includes a heterologous nucleic acid sequence encoding a heterologous gene product is an rAAV that includes a polynucleotide not normally included in a naturally-occurring, wild-type AAV, and the encoded heterologous gene product is a gene product not normally encoded by a naturally-occurring, wild type AAV.

[0070] The terminology "genetic alteration" and "genetic modification" (and grammatical variants thereof), are used interchangeably herein to refer to a process wherein a genetic element (e.g., a polynucleotide) is introduced into a cell other than by mitosis or meiosis. The element may be heterologous to the cell, or it may be an additional copy or improved version of an element already present in the cell. Genetic alteration may be effected, for example, by transfecting a cell with a recombinant plasmid or other polynucleotide through any process known in the art, such as electroporation, calcium phosphate precipitation, or contacting with a polynucleotide-liposome complex. Genetic alteration may also be effected, for example, by transduction or infection with a DNA or RNA virus or viral vector. Generally, the genetic element is introduced into a chromosome or mini-chromosome in the cell; but any alteration that changes the phenotype and/or genotype of the cell and its progeny is included in this term.

[0071] With regards to cell modification, the terminology "genetically modified" or "transformed" or "transfected" or "transduced" by exogenous DNA (e.g. via a recombinant virus) refers to when such DNA has been introduced inside the cell. The presence of the exogenous DNA results in permanent or transient genetic change. The transforming DNA may or may not be integrated (covalently linked) into the genome of the cell. A "clone" is a population of cells derived from a single cell or common ancestor by mitosis. A "cell line" is a clone of a primary cell that is capable of stable growth *in vitro* for many generations.

[0072] As used herein, a cell is said to be "stably" altered, transduced, genetically modified, or transformed with a genetic sequence if the sequence is available to perform its function during extended culture of the cell *in vitro* and/or for an extended period of time *in vivo*. Generally, such a cell is "heritably" altered (genetically modified) in that a genetic alteration is introduced which is also inheritable by progeny of the altered cell.

[0073] The terms "polypeptide," "peptide," and "protein" are used interchangeably herein to refer to polymers of amino acids of any length. The terms also encompass an amino acid polymer that has been modified; for example, disulfide bond formation, glycosylation, lipidation, phosphorylation, or conjugation with a labeling component. Polypeptides such as anti-angiogenic polypeptides, neuroprotective polypeptides, and the like, when discussed in the context of delivering a gene product to a mammalian subject, and compositions therefor, refer to the respective intact polypeptide, or any fragment or genetically engineered derivative

thereof, which retains the desired biochemical function of the intact protein. Similarly, references to nucleic acids encoding anti-angiogenic polypeptides, nucleic acids encoding neuroprotective polypeptides, and other such nucleic acids for use in delivery of a gene product to a mammalian subject (which may be referred to as "transgenes" to be delivered to a recipient cell), include polynucleotides encoding the intact polypeptide or any fragment or genetically engineered derivative possessing the desired biochemical function.

[0074] As used herein, an "isolated" plasmid, nucleic acid, vector, virus, virion, host cell, protein, or other substance refers to a preparation of the substance devoid of at least some of the other components that may also be present where the substance or a similar substance naturally occurs or is initially prepared from. Thus, for example, an isolated substance may be prepared by using a purification technique to enrich it from a source mixture. Enrichment can be measured on an absolute basis, such as weight per volume of solution, or it can be measured in relation to a second, potentially interfering substance present in the source mixture. Increasing enrichments of the embodiments of this disclosure are increasingly more isolated. An isolated plasmid, nucleic acid, vector, virus, host cell, or other substance is in some embodiments purified, e.g., from about 80% to about 90% pure, at least about 90% pure, at least about 95% pure, at least about 98% pure, or at least about 99%, or more, pure.

[0075] As used herein, the terms "treatment," "treating," and the like, refer to obtaining a desired pharmacologic and/or physiologic effect. The effect may be prophylactic in terms of completely or partially preventing a disease or symptom thereof and/or may be therapeutic in terms of a partial or complete cure for a disease and/or adverse effect attributable to the disease. "Treatment," as used herein, covers any treatment of a disease in a mammal, particularly in a human, and includes: (a) preventing the disease (and/or symptoms caused by the disease) from occurring in a subject which may be predisposed to the disease or at risk of acquiring the disease but has not yet been diagnosed as having it; (b) inhibiting the disease (and/or symptoms caused by the disease), i.e., arresting its development; and (c) relieving the disease (and/or symptoms caused by the disease), i.e., causing regression of the disease (and/or symptoms caused by the disease), i.e., ameliorating the disease and/or one or more symptoms of the disease. For example, the subject compositions and methods may be directed towards the treatment of retinal disease. Nonlimiting methods for assessing retinal diseases and the treatment thereof include measuring retinal function and changes thereof, e.g. changes in visual acuity (e.g. best-corrected visual acuity [BCVA], ambulation, navigation, object detection and discrimination), changes in visual field (e.g. static and kinetic visual field perimetry), clinical examination (e.g. slit lamp examination of the anterior and posterior segments of the eye), electrophysiological responsiveness to all wavelengths of light and dark (e.g. all forms of electroretinography (ERG) [full-field, multifocal and pattern], all forms of visual evoked potential (VEP), electrooculography (EOG), color vision, dark adaptation and/or contrast sensitivity; measuring changes in anatomy or health using anatomical and/or photographic measures, e.g. Optical Coherence Tomography (OCT), fundus photography, adaptive optics scanning laser ophthalmoscopy, fluorescence and/or autofluorescence; measuring ocular motility and eye movements (e.g. nystagmus, fixation preference, and stability), measuring reported outcomes (patient-reported changes in visual and non-visually-

guided behaviors and activities, patient-reported outcomes [PRO], questionnaire-based assessments of quality-of-life, daily activities and measures of neurological function (e.g. functional Magnetic Resonance Imaging (MRI)).

[0076] The terms "individual," "host," "subject," and "patient" are used interchangeably herein, and refer to a mammal, including, but not limited to, humans; non-human primates, including simians; mammalian sport animals (e.g., horses); mammalian farm animals (e.g., sheep, goats, etc.); mammalian pets (dogs, cats, etc.); and rodents (e.g., mice, rats, etc.).

[0077] In some embodiments, the individual is a human who has previously been naturally exposed to AAV and as a result harbors anti-AAV antibodies (i.e., AAV neutralizing antibodies). In some embodiments, the individual is a human who has previously been administered an AAV vector (and as a result may harbor anti-AAV antibodies) and needs re-administration of vector for treatment of a different condition or for further treatment of the same condition. Based on positive results in clinical trials involving AAV gene delivery to, for example, liver, muscle, and retina - all tissues affected by neutralizing antibodies against this vehicle - there are many such therapeutic applications/disease targets.

[0078] The term "effective amount" as used herein is an amount sufficient to effect beneficial or desired clinical results. An effective amount can be administered in one or more administrations. For purposes of this disclosure, an effective amount of a compound (e.g., an infectious rAAV virion) is an amount that is sufficient to palliate, ameliorate, stabilize, reverse, prevent, slow or delay the progression of (and/or symptoms associated with) a particular disease state (e.g., a retinal disease). Accordingly, an effective amount of an infectious rAAV virion is an amount of the infectious rAAV virion that is able to effectively deliver a heterologous nucleic acid to a target cell (or target cells) of the individual. Effective amounts may be determined preclinically by, e.g., detecting in the cell or tissue the gene product (RNA, protein) that is encoded by the heterologous nucleic acid sequence using techniques that are well understood in the art, e.g. RT-PCR, western blotting, ELISA, fluorescence or other reporter readouts, and the like. Effective amounts may be determined clinically by, e.g. detecting a change in the onset or progression of disease using methods known in the art, e.g. fundus autofluorescence, fluorescein angiography, OCT, microperimetry, adaptive optics, etc. and the like, as described herein and as known in the art.

[0079] The terminology "retinal cell" refers herein to any of the cell types that comprise the retina, such as, without limitation, retinal ganglion (RG) cells, amacrine cells, horizontal cells, bipolar cells, photoreceptor cells, Müller glial cells, microglial cells, and retinal pigmented epithelium (RPE). The terminology "photoreceptor cells" refers herein to, without limitation, rod cells or "rods" and cone cells or "cones". The terminology "Müller cells" or "Müller glia" refers to glial cells that support neurons in the vertebrate retina.

[0080] The terminology "directed evolution" refers to a capsid engineering methodology, *in vitro* and/or *in vivo*, which emulates natural evolution through iterative rounds of genetic diversification and selection processes, thereby accumulating beneficial mutations that

progressively improve the function of a biomolecule. Directed evolution often involves an *in vivo* method referred to as "biopanning" for selection of AAV variants from a library which variants possess a more efficient level of infectivity of a cell or tissue type of interest.

DETAILED DESCRIPTION

[0081] Adeno-associated viruses (AAVs) are a family of parvoviruses with a 4.7 kb single-stranded DNA genome contained inside a non-enveloped capsid. The viral genome of a naturally occurring AAV has 2 inverted terminal repeats (ITR) - which function as the viral origin of replication and packaging signal - flanking 2 primary open reading frames (ORF): *rep* (encoding proteins that function in viral replication, transcriptional regulation, site-specific integration, and virion assembly) and *cap*. The *cap* ORF codes for 3 structural proteins that assemble to form a 60-mer viral capsid. Many naturally occurring AAV variants and serotypes have been isolated, and none have been associated with human disease.

[0082] Recombinant versions of AAV can be used as gene delivery vectors, where a marker or therapeutic gene of interest is inserted between the ITRs in place of *rep* and *cap*. These vectors have been shown to transduce both dividing and non-dividing cells *in vitro* and *in vivo* and can result in stable transgene expression for years in post-mitotic tissue. See e.g., Knipe DM, Howley PM. Fields' Virology. Lippincott Williams & Wilkins, Philadelphia, PA, USA, 2007; Gao G-P, Alvira MR, Wang L, Calcedo R, Johnston J, Wilson JM. Novel adeno-associated viruses from rhesus monkeys as vectors for human gene therapy. Proc Natl Acad Sci U S A 2002; 99: 11854-9; Atchison RW, Casto BC, Hammon WM Adenovirus-Associated Defective Virus Particles. Science 1965; 149: 754-6; Hoggan MD, Blacklow NR, Rowe WP. Studies of small DNA viruses found in various adenovirus preparations: physical, biological, and immunological characteristics. Proc Natl Acad Sci U S A 1966; 55: 1467-74; Blacklow NR, Hoggan MD, Rowe WP. Isolation of adenovirus-associated viruses from man. Proc Natl Acad Sci U S A 1967; 58: 1410-5; Bantel-Schaal U, zur Hausen H. Characterization of the DNA of a defective human parvovirus isolated from a genital site. Virology 1984; 134: 52-63; Mayor HD, Melnick JL. Small deoxyribonucleic acid-containing viruses (picodnavirus group). Nature 1966; 210: 331-2; Mori S, Wang L, Takeuchi T, Kanda T. Two novel adeno-associated viruses from cynomolgus monkey: pseudotyping characterization of capsid protein. Virology 2004; 330: 375-83; Flotte TR. Gene therapy progress and prospects: recombinant adeno-associated virus (rAAV) vectors. Gene Ther 2004; 11: 805-10.

[0083] Recombinant AAV (referred to herein simply as "AAV") has yielded promising results in an increasing number of clinical trials. However, there are impediments to gene delivery that may limit AAV's utility, such as anti-capsid immune responses, low transduction of certain tissues, an inability for targeted delivery to specific cell types and a relatively low carrying capacity. In many situations, there is insufficient mechanistic knowledge to effectively empower rational design with the capacity to improve AAV. As an alternative, directed evolution has emerged as a strategy to create novel AAV variants that meet specific biomedical needs. Directed evolution strategies harness genetic diversification and selection processes to enable

the accumulation of beneficial mutations that progressively improve the function of a biomolecule. In this process, wild-type AAV *cap* genes are diversified by several approaches to create large genetic libraries that are packaged to generate libraries of viral particles, and selective pressure is then applied to isolate novel variants that can overcome gene delivery barriers. Importantly, the mechanistic basis underlying a gene delivery problem does not need to be known for directed evolution of function, which can thus accelerate the development of enhanced vectors.

[0084] Typically, the variants disclosed herein were generated through use of an AAV library and/or libraries. Such an AAV library or libraries is/are generated by mutating the *cap* gene, the gene which encodes the structural proteins of the AAV capsid, by a range of directed evolution techniques known by and readily available to the skilled artisan in the field of viral genome engineering. See e.g., Bartel et al. Am. Soc. Gene Cell Ther. 15th Annu. Meet. 20, S140 (2012); Bowles, D. et al. J. Virol. 77, 423-432 (2003); Gray et al. Mol. Ther. 18, 570-578 (2010); Grimm, D. et al. J. Virol. 82, 5887-5911; Koerber, J. T. et al. Mol. Ther. 16, 1703-1709 (2008); Li W. et al. Mol. Ther. 16, 1252-1260 (2008); Koerber, J. T. et al. Methods Mol. Biol. 434, 161-170 (2008); Koerber, J. T. et al. Hum. Gene Ther. 18, 367-378 (2007); and Koerber, J. T. et al. Mol. Ther. 17, 2088-2095 (2009). Such techniques, without limitation, are as follows: i) Error-prone PCR to introduce random point mutations into the AAV *cap* open reading frame (ORF) at a predetermined, modifiable rate; ii) *In vitro* or *in vivo* viral recombination or "DNA shuffling" to generate random chimeras of AAV *cap* genes to yield a gene library with multiple AAV serotypes; iii) Random peptide insertions at defined sites of the capsid by ligation of degenerate oligonucleotides in the *cap* ORF; iv) Defined insertions of peptide-encoding sequences into random locations of the AAV *cap* ORF using transposon mutagenesis; v) Replacing surface loops of AAV capsids with libraries of peptide sequences bioinformationally designed based on the level of conservation of each amino acid position among natural AAV serotypes and variants to generate "loop-swap" libraries; vi) Random amino acid substitution at positions of degeneracy between AAV serotypes to generate libraries of ancestral variants (Santiago-Ortiz et al., 2015); and a combination of such techniques thereof.

[0085] DNA shuffling generates chimeras which combine their parental properties in unique and, often beneficial, ways; however, some may be incapable of packaging which, in effect, reduces the diversity of the library. Diversity concentration of the library is achieved through peptide insertion techniques such as, without limitation, iii-iv) above. Diversity of the library is also concentrated in techniques such as v) above, and such concentration is directed onto multiple hypervariable regions, which lie on surface exposed loops, of the AAV capsid. While many of the techniques generate variant capsids with only a small area of the capsid mutated, these techniques can be paired with additional mutagenesis strategies to modify the full capsid.

[0086] Once the AAV library or libraries is/are generated, viruses are then packaged, such that each AAV particle is comprised of a mutant capsid surrounding a *cap* gene encoding that capsid, and purified. Variants of the library are then subjected to *in vitro* and/or *in vivo* selective pressure techniques known by and readily available to the skilled artisan in the field of AAV. See e.g., Maheshri, N. et al. Nature Biotech. 24, 198-204 (2006); Dalkara, D. et al. Sci. Transl.

Med. 5, 189ra76 (2013); Lisowski, L. et al. Nature. 506, 382-286 (2013); Yang, L. et al. PNAS. 106, 3946-3951 (2009); Gao, G. et al. Mol. Ther. 13, 77-87 (2006); and Bell, P. et al. Hum. Gene. Ther. 22, 985-997 (2011). For example, without limitation, AAV variants can be selected using i) affinity columns in which elution of different fractions yields variants with altered binding properties; ii) primary cells - isolated from tissue samples or immortal cells lines that mimic the behavior of cells in the human body - which yield AAV variants with increased efficiency and/or tissue specificity; iii) animal models - which mimic a clinical gene therapy environment - which yield AAV variants that have successfully infected target tissue; iv) human xenograft models which yield AAV variants that have infected grafted human cells; and/or a combination of selection techniques thereof.

[0087] Once viruses are selected, they may be recovered by known techniques such as, without limitation, adenovirus-mediated replication, PCR amplification, Next Generation sequencing and cloning, and the like. Virus clones are then enriched through repeated rounds of the selection techniques and AAV DNA is isolated to recover selected variant *cap* genes of interest. Such selected variants can be subjected to further modification or mutation and as such serve as a new starting point for further selection steps to iteratively increase AAV viral fitness. However, in certain instances, successful capsids have been generated without additional mutation.

[0088] The AAV variants disclosed herein were generated at least in part through the use of *in vivo* directed evolution methodology, such as the techniques described above, involving the use of primate retinal screens following intravitreal administration. As such, the AAV variant capsids disclosed herein comprise one or more modifications in amino acid sequence that confer more efficient transduction of primate retinal cells than a corresponding parental AAV capsid protein. As used herein, a "corresponding parental AAV capsid protein" refers to an AAV capsid protein of the same wild-type or variant AAV serotype as the subject variant AAV capsid protein but that does not comprise the one or more amino acid sequence modifications of the subject variant AAV capsid protein.

[0089] The subject variant AAV capsid protein comprises a heterologous peptide with a length of 7, 8, 9, 10 or 11 amino acids covalently inserted in the GH-loop of the capsid protein relative to a corresponding parental AAV capsid protein, wherein the peptide insertion comprises the amino acid sequence ISDQTKH (SEQ ID NO:14). By the "GH loop," or loop IV, of the AAV capsid protein it is meant the solvent-accessible portion referred to in the art as the GH loop, or loop IV, of AAV capsid protein. For the GH loop/loop IV of AAV capsid, see, e.g., van Vliet et al. (2006) Mol. Ther. 14:809; Padron et al. (2005) J. Virol. 79:5047; and Shen et al. (2007) Mol. Ther. 15:1955. Thus, for example, the insertion site can be within about amino acids 411-650 of an AAV VP1 capsid protein. For example, the insertion site can be within amino acids 571-612 of AAV1 VP1, amino acids 570-611 of AAV2 VP1, within amino acids 571-612 of AAV3A VP1, within amino acids 571-612 of AAV3B VP1, within amino acids 569-610 of AAV4 VP1, within amino acids 560-601 of AAV5 VP1, within amino acids 571 to 612 of AAV6 VP1, within amino acids 572 to 613 of AAV7 VP1, within amino acids 573 to 614 of AAV8 VP1, within amino acids 571 to 612 of AAV9 VP1, or within amino acids 573 to 614 of AAV10 VP1, or the

corresponding amino acids of any variant thereof. Those skilled in the art would know, based on a comparison of the amino acid sequences of capsid proteins of various AAV serotypes, where an insertion site "corresponding to amino acids of AAV2" would be in a capsid protein of any given AAV serotype. See also Figure 6 for an alignment of wild-type AAV SEQ ID NOS:1-11 which provides amino acid locations between and across the wild-type (naturally occurring) serotypes AAV1, AAV2, AAV3A, AAV3B, and AAV4-10.

[0090] In certain embodiments, the insertion site is a single insertion site between two adjacent amino acids located between amino acids 570-614 of VP1 of any wild-type AAV serotype or AAV variant, e.g., the insertion site is between two adjacent amino acids located in amino acids 570-610, amino acids 580-600, amino acids 570-575, amino acids 575-580, amino acids 580-585, amino acids 585-590, amino acids 590-600, or amino acids 600-614, of VP1 of any AAV serotype or variant. For example, the insertion site can be between amino acids 580 and 581, amino acids 581 and 582, amino acids 583 and 584, amino acids 584 and 585, amino acids 585 and 586, amino acids 586 and 587, amino acids 587 and 588, amino acids 588 and 589, or amino acids 589 and 590. The insertion site can be between amino acids 575 and 576, amino acids 576 and 577, amino acids 577 and 578, amino acids 578 and 579, or amino acids 579 and 580. The insertion site can be between amino acids 590 and 591, amino acids 591 and 592, amino acids 592 and 593, amino acids 593 and 594, amino acids 594 and 595, amino acids 595 and 596, amino acids 596 and 597, amino acids 597 and 598, amino acids 598 and 599, or amino acids 599 and 600. For example, the insertion site can be between amino acids 587 and 588 of AAV2, between amino acids 590 and 591 of AAV1, between amino acids 588 and 589 of AAV3A, between amino acids 588 and 589 of AAV3B, between amino acids 584 and 585 of AAV4, between amino acids 575 and 576 of AAV5, between amino acids 590 and 591 of AAV6, between amino acids 589 and 590 of AAV7, between amino acids 590 and 591 of AAV8, between amino acids 588 and 589 of AAV9, or between amino acids 588 and 589 of AAV10.

[0091] The peptide insertion disclosed herein has a length as defined in the claims.

[0092] In another embodiment, a peptide insertion disclosed herein comprises from 1 to 4 spacer amino acids at the amino terminus (N-terminus) and/or at the carboxyl terminus (C-terminus) of any one of the peptide insertions disclosed herein. Exemplary spacer amino acids include, without limitation, leucine (L), alanine (A), glycine (G), serine (S), threonine (T), and proline (P). In certain embodiments, a peptide insertion comprises 2 spacer amino acids at the N-terminus and 2 spacer amino acids at the C-terminus. In other embodiments, a peptide insertion comprises 2 spacer amino acids at the N-terminus and 1 spacer amino acids at the C-terminus.

[0093] The peptide insertions disclosed herein have not been previously described and/or inserted into an AAV capsid. Without wishing to be bound by theory, the presence of any of the disclosed peptide insertions may act to lower the variant capsid's affinity for heparin sulfate which likely reduces binding to the extracellular matrix in the front of the primate retina. In addition, the peptide insertion motifs disclosed herein may confer enhanced transduction of

primate retinal cells through the addition of a cell surface receptor binding domain.

[0094] In some embodiments, the subject variant AAV capsid protein does not include any other amino acid sequence modifications other than a peptide insertion as defined in the claims in the GH loop, or loop IV. For example, in some embodiments, the subject variant AAV capsid protein comprises a peptide insertion comprising an amino acid sequence as defined in the claims, and the variant AAV capsid does not include any other amino acid substitutions, insertions, or deletions (i.e., the variant AAV capsid protein comprises said insertion and is otherwise identical to the corresponding AAV capsid protein). Put another way, the variant AAV capsid protein comprising said insertion is otherwise identical to the parental AAV capsid protein into which the peptide has been inserted. As another example, the subject variant AAV capsid protein comprises a peptide insertion having an amino acid sequence as defined in the claims, wherein the peptide insertion is located between amino acids 587 and 588 of the VP1 of the AAV2 capsid or the corresponding amino acids of a VP1 of another parental AAV, e.g. between amino acids 588 and 589 of VP1 of AAV1, AAV3A, AAV3B, AAV6 or AAV9, between amino acids 586 and 587 of VP1 of AAV4, between amino acids 577 and 578 of VP1 of AAV5, between amino acids 589 and 590 of VP1 of AAV7, between amino acids 590 to 591 of VP1 of AAV8 or AAV10, etc. wherein the variant AAV capsid protein sequence is otherwise identical to the corresponding parental AAV capsid protein sequence, e.g. any one of SEQ ID NOs:1-12.

[0095] In other embodiments, the subject variant AAV capsid protein, in addition to comprising a peptide insertion, e.g. as disclosed herein or as known in the art, in the GH loop, comprises from about 1 to about 100 amino acid substitutions or deletions, e.g. 1 to about 5, from about 2 to about 4, from about 2 to about 5, from about 5 to about 10, from about 10 to about 15, from about 15 to about 20, from about 20 to about 25, from about 25-50, from about 50-100 amino acid substitutions or deletions compared to the parental AAV capsid protein. Thus, in some embodiments, a subject variant capsid protein comprises an amino acid sequence having a sequence identity of 85% or more, 90% or more, 95% or more, or 98% or more, e.g. or 99% identity to the corresponding parental AAV capsid, e.g. a wild type capsid protein as set forth in SEQ ID NOs:1-12.

[0096] In a further embodiment, the one or more amino acid substitutions are at amino acid residue(s) 1, 15, 34, 57, 66, 81, 101, 109, 144, 164, 176, 188, 196, 226, 236, 240, 250, 312, 363, 368, 449, 456, 463, 472, 484, 524, 535, 551, 593, 698, 708, 719, 721, and/or 735 of AAV2 VP1 capsid protein as numbered prior to insertion of the peptide, or the corresponding amino acid residue(s) of another AAV capsid protein. In some such embodiments, the one or more amino acid substitutions are selected from the group consisting of M1L, L15P, P34A, N57D, N66K, R81Q, Q101R, S109T, R144K, R144M, Q164K, T176P, L188I, S196Y, G226E, G236V, I240T, P250S, N312K, P363L, D368H, N449D, T456K, S463Y, D472N, R484C, A524T, P535S, N551S, A593E, I698V, V708I, V719M, S721L, and L735Q of AAV2 VP1 capsid protein as numbered prior to the insertion of the peptide, or the corresponding amino acid residue(s) of another AAV capsid protein .

[0097] In a preferred embodiment, a variant AAV capsid protein is provided comprising a) a

peptide insertion in the GH-loop of the capsid protein, wherein the peptide insertion comprises an amino acid sequence selected from ISDQTKH (SEQ ID NO:14), LGISDQTKHA (SEQ ID NO:29) and LAISDQTKHA (SEQ ID NO:28), and b) one or more of the following amino acid substitutions compared to the amino acid sequence of AAV2 (SEQ ID NO:2) or the corresponding substitution in another AAV parental serotype (i.e. other than AAV2), wherein the substituted amino acid(s) do not naturally occur at the corresponding positions: M1L, L15P, P34A, N57D, N66K, R81Q, Q101R, S109T, R144K, R144M, Q164K, T176P, L188I, S196Y, G226E, G236V, I240T, P250S, N312K, P363L, D368H, N449D, T456K, S463Y, D472N, R484C, A524T, P535S, N551S, A593E, I698V, V708I, V719M, S721L, L735Q and a combination thereof. In some embodiments, the one or more amino acid substitutions are selected from the group consisting of: M1L+L15P+P535S, P34A, P34A+S721L, N57D, N66K, R81Q, Q101R, S109T, R144K, R144M, Q164K, Q164K+V708I, T176P, L188I, S196Y, G226E, G236V, I240T, N312K, N312K+N449D+D472N+N551S+I698V+L735Q, P363L, R484C+V708I, T456K and V708I. Preferably, the peptide insertion site is located between amino acids 587 and 588 of AAV2 capsid or the corresponding position in the capsid protein of another AAV serotype.

[0098] In a particularly preferred embodiment, the variant AAV capsid comprises a peptide insertion comprising the amino acid sequence ISDQTKH (SEQ ID NO:14) or comprising, consisting essentially of, or consisting of the amino acid sequence LAISDQTKHA (SEQ ID NO:28) or LGISDQTKHA (SEQ ID NO:29) between amino acids 587 and 588 of VP1 of AAV2 or the corresponding amino acids of another AAV capsid, and further comprises a P34A amino acid substitution at residue 34 relative to the amino acid sequence of AAV2 capsid (SEQ ID NO:2) or the corresponding residue of another AAV capsid. The variant AAV capsid may have at least about 85%, at least about 90%, at least about 95%, at least about 98%, or at least about 99%, or greater, amino acid sequence identity to the entire length of the amino acid sequence set forth in SEQ ID NO:2 or the corresponding parental AAV capsid. In a particularly preferred embodiment, the variant AAV capsid has an amino acid sequence having at least about 85%, at least about 90%, at least about 95%, at least about 98% sequence identity to or is 100% identical to the following amino acid sequence:

MAADGYLPDWLEDTLSEGIRQWWKLKPGPPPKA~~A~~AERHKDDSRGLVLPGYK
 YLGPENGLDKGEPVNEADAAALEHDKAYDRQLDSGDNPYLKYNHADAEEFQ
 ERLKEDTSFGGNLGRAVFQAKKRVLEPLGLVEEPVKTAPGKKRPVEHSPVEP
 DSSSGTGKAGQQPARKRLNFGQTGDADSVDPQPPLGQPPAAPSGLGTTNTMAT
 GSGAPMADNNEGADGVGNSSGNWHCDSTWMGDRVITTTSTRTWALPTYNNH
 LYKQISSQSGASNDNHYFGYSTPWGYFDNRFHCHFSRWDQRLINNNWGFR
 PKRLNFKLFNIQVKEVTQNDGTTTIANNLTSTVQVFTDSEYQLPYVLGSAHQG
 CLPPFPADVFMVPQYGYLTLNNGSQAVGRSSFYCLEYFPSQMLRTGNNFTFS
 YTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYLSRTNTPSGTTTQSRLQFSQA
 GASDIRDQSRNWLPGPCYRQQRVSKTSADNNNSEYSWTGATKYHLNGRDSL
 VNPGPAMASHKDDEEKFFPQSGVLIFGKQGSEKTNVDIEKVMITDEEEIIRTTN
 PVATEQYGSVSTNLQRGNLAISDQTKHARQAATADVNTQGVLPGMVWQDR
 DVYLQGPIWAKIPHTDGHFHPSPLMGGFGLKHPPPQILIKNTPVPANPSTTFS
 AKFASFITQYSTGQVSVEIEWELQKENS KRWNPEIQYTSNYNKS VNVDFTVDT
 NGVYSEPRPIGTRYLTRNI. (SEQ ID NO:42)

[0099] In another particularly preferred embodiment, the variant AAV capsid comprises a peptide insertion comprising the amino acid sequence ISDQTKH (SEQ ID NO:14) or comprising, consisting essentially of, or consisting of the amino acid sequence LAISDQTKHA (SEQ ID NO:28) or LGISDQTKHA (SEQ ID NO:29) between amino acids 587 and 588 of AAV2 capsid protein or the corresponding position in the capsid protein of another AAV serotype and comprises an N312K amino acid substitution compared to the amino acid sequence of AAV2 capsid (SEQ ID NO:2) or the corresponding substitution in another AAV parental serotype and optionally further comprises N449D, D472N, N551S, I698V and/or L735Q amino acid substitutions compared to the amino acid sequence of AAV2 capsid or the corresponding substitutions in another AAV parental serotype. In another particularly preferred embodiment, the variant AAV capsid comprises a peptide insertion comprising the amino acid sequence ISDQTKH (SEQ ID NO:14) or comprising, consisting essentially of, or consisting of the amino acid sequence LAISDQTKHA (SEQ ID NO:28) or LGISDQTKHA (SEQ ID NO:29) between amino acids 587 and 588 of AAV2 capsid or the corresponding position in the capsid protein of another AAV serotype and comprises N312K, N449D, D472N, N551S, I698V and L735Q amino acid substitutions compared to the amino acid sequence of AAV2 capsid (SEQ ID NO:2) or substitutions at the corresponding residues in another AAV parental serotype. The variant AAV capsid may have at least about 85%, at least about 90%, at least about 95%, at least about 98%, or greater, amino acid sequence identity to the entire length of the amino acid sequence set forth in SEQ ID NO:2. In a particularly preferred embodiment, the variant AAV capsid has an amino acid sequence having at least about 85%, at least about 90%, at least about 95%, at least about 98% sequence identity to or is 100% identical to the following amino acid sequence:

MAADGYLPDWLEDTLSEGIRQWWKLKPGPPPPKPAERHKDDSRGLVLPGYK
 YLGPFNGLDKGEPVNEADAAALEHDKAYDRQLDSGDNPYLKYNHADAEFQ
 ERLKEDTSFGGNLGRAVFQAKKRVLEPLGLVEEPVKTAPGKKRPVEHSPVEP
 DSSSGTGKAGQQPARKRLNFGQTGDADSVDPQPPLGQPPAAPSGLGTNTMAT
 GSGAPMADNNEGADGVGNSSGNWHCDSTWMGDRVITTSTRTWALPTYNNH
 LYKQISSQSGASNDNHYFGYSTPWGYFDNFHCHFSRPDWQRLINNNWGFR
 PKRLKFKLFNIQVKEVTQNDGTTTIANNLTSTVQVFTDSEYQLPYVLGSAHQG
 CLPPFPADVFMVPQYGYLTLNNGSQAVGRSSFYCLEYFPSQMLRTGNNFTFS
 YTFEDVPHSSYAHSQSLDRLMNPLIDQYLYLSRTDTPSGTTTQSRLQFSQA
 GASDIRNQSRNWLPGPCYRQQRVSKTSADNNNSEYSWTGATKYHLNGRDSL
 VNPGPAMASHKDDEEKFFPQSGVLIFGKQGSEKTQVDIEKVMITDEEEIRTTNP
 VATEQYGSVSTNLQRGNLAISDOTKHARQAATADVNTQGVLPGMVWQDRD
 VYLQGPWAKIPHTDGHFHPSPLMGGFGLKHPPQILIKNTPVPANPSTTFSA
 KFAFITQYSTGQVSVEIEWELQKENS KRWNPEVQYTSNYNKSVNVDFTVDT
 NGVYSEPRPIGTRYLTRNQ (SEQ ID NO:43)

[0100] In another embodiment, a variant AAV capsid protein is provided comprising a) a

peptide insertion located between amino acids 588 and 589 of VP1 of AAV1, AAV3A, AAV3B, AAV6 or AAV9, amino acids 586 and 587 of AAV4, amino acids 577 and 578 of AAV5, amino acids 589 and 590 of AAV7, or amino acids 590 to 591 of AAV8 or AAV10, the peptide insertion comprising an amino acid sequence selected from ISDQTKH (SEQ ID NO:14), LGISDQTKHA (SEQ ID NO:29) and LAISDQTKHA (SEQ ID NO:28), and b) a valine to isoleucine substitution at amino acid 709 of AAV3A or AAV3B, an alanine to isoleucine substitution at position 709 of AAV1 or AAV6, an asparagine to isoleucine substitution at amino acid 707 of AAV4 or amino acid 709 of AAV9 or a threonine to isoleucine substitution at amino acid 710 of AAV7 or amino acid 711 of AAV8 or AAV10 or a glutamine to isoleucine substitution at amino acid 697 of AAV5 and is optionally otherwise identical to any one of SEQ ID NOs: 1 and 3-12. In preferred embodiments, the variant capsid protein comprises a) a peptide insertion comprising the amino acid sequence ISDQTKH (SEQ ID NO:14) or comprising, consisting essentially of, or consisting of the amino acid sequence LAISDQTKHA (SEQ ID NO:28) or LGISDQTKHA (SEQ ID NO:29) between amino acids 587 and 588 of AAV2 capsid and b) a valine to isoleucine amino acid substitution at amino acid 708 compared to the amino acid sequence of AAV2, wherein the variant capsid protein comprises from 2 to 5, from 5 to 10, or from 10 to 15 amino acid substitutions.

[0101] In yet another embodiment, the variant capsid protein comprises a) a peptide insertion comprising the amino acid sequence ISDQTKH (SEQ ID NO:14) or comprising, consisting essentially of, or consisting of the amino acid sequence LAISDQTKHA (SEQ ID NO:28) or LGISDQTKHA (SEQ ID NO:29) between amino acids 587 and 588 of AAV2 capsid and b) a valine to isoleucine amino acid substitution at amino acid 708 compared to the amino acid sequence of AAV2 and is otherwise identical to the amino acid sequence of SEQ ID NO:2.

[0102] In yet another embodiment, the variant capsid protein comprises a) a peptide insertion comprising the amino acid sequence ISDQTKH (SEQ ID NO:14) or comprising, consisting essentially of, or consisting of the amino acid sequence LAISDQTKHA (SEQ ID NO:28) or LGISDQTKHA (SEQ ID NO:29) between amino acids 587 and 588 of AAV2 capsid and is otherwise identical to the amino acid sequence of SEQ ID NO:2. In some embodiments, the variant AAV capsid has an amino acid sequence having at least about 85%, at least about 90%, at least about 95%, at least about 98% sequence identity to or is 100% identical to the following amino acid sequence:

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MAADGYLPDWLEDTLSEGIRQWWKLKPGPPPPKPAERHKDDSRGLVLPGYK
YLGPFNGLDKGEPVNEADAAALEHDKAYDRQLDSGDNPYLKYNHADAEFQ
ERLKEDTSFGGNLGRAVFQAKKRVLEPLGLVEEPVKTAPGKKRPVEHSPVEP
DSSSGTGKAGQQPARKRLNFGQTGDADSVDPDQPLGQPPAAPSGLGTNTMAT
GSGAPMADNNEGADGVGNSSGNWHCDSTWMGDRVITSTRTWALPTYNNH
LYKQISSQSGASNDNHYFGYSTPWGYFDFNRFHCHFSPRDWQRLINNNWGFR
PKRLNFKLFNIQVKEVTQNDGTTTIANNLTSTVQVFTDSEYQLPYVLGSAHQG
CLPPFPADVFMVPQYGYLTLNNGSQAVGRSSFYCLEYFPSQMLRTGNNFTFS
YTFEDVPFHSSYAHQSGLDRMLNPLIDQYLYLSRTNTPSGTTTQSRLQFSQA
GASDIRDQSRNWLPGPCYRQQRVSKTSADNNNSEYSWTGATKYHLNGRDSL
VNPGPAMASHKDDEEKFFPQSGVLIFGKQGSEKTNVDIEKVMITDEEEIRTTN
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PVATEQYGSVSTNLQRGNLGISDOTKHARQAATADVNTQGVLPGMVWQDR
 DVYLQGPWAKIPHTDGHFHPSPMLMGFGFLKHPPQILIKNTPVPANPSTTFSA
 AKFASFITQYSTGQVSVEIEWELQKENSkrwnPEIQYTSNynKSVNVDFTVDT
 NGVYSEPRPIGTRYLTRNL (SEQ ID NO:44)

[0103] In several aspects, a variant AAV capsid protein as defined in the claims is provided comprising one or more amino acid substitutions relative to a corresponding parental AAV capsid protein, wherein the variant capsid protein, when present in an AAV virion, confers increased infectivity of a retinal cell compared to the infectivity of a retinal cell by an AAV virion comprising the corresponding parental AAV capsid protein.

[0104] In some embodiments, a variant AAV capsid protein comprises a P34A amino acid substitution compared to the amino acid sequence of AAV2 capsid (SEQ ID NO:2) or a P33A amino acid substitution compared to the amino acid sequence of AAV5 capsid (SEQ ID NO:6). In some preferred embodiments, the variant capsid protein comprises an amino acid sequence having at least about 85%, at least about 90%, at least about 95%, at least about 98%, or at least about 99%, or greater, amino acid sequence identity to the entire length of the amino acid sequence set forth in SEQ ID NO:2 or SEQ ID NO:6 and comprises a P34A or P33A amino acid substitution compared to the amino acid sequence of AAV2 or AAV5 capsid respectively. In some preferred embodiments, the variant capsid protein comprises an amino acid sequence comprising a P34A amino acid substitution compared to the amino acid sequence set forth in SEQ ID NO 2 and is otherwise identical to the amino acid sequence set forth in SEQ ID NO:2. In related embodiments, the variant capsid protein comprises a P34A amino acid substitution compared to the amino acid sequence of SEQ ID NO:2, wherein the variant capsid protein comprises from 1 to 5, from 5 to 10, or from 10 to 15 amino acid substitutions compared to the amino acid sequence of an AAV2 capsid protein set forth in SEQ ID NO:2.

[0105] In other embodiments a variant AAV capsid protein comprises an amino acid substitution at amino acid 164 compared to the amino acid sequence of AAV2 capsid (SEQ ID NO:2) or the corresponding position in another AAV parental serotype (i.e. other than AAV2), wherein the substituted amino acid does not naturally occur at the corresponding position. In some preferred embodiments, the variant capsid protein comprises an amino acid sequence having at least about 85%, at least about 90%, at least about 95%, at least about 98%, or at least about 99%, or greater, amino acid sequence identity to the entire length of the amino acid sequence set forth in SEQ ID NO 2 and comprises an amino acid substitution at amino acid 164 compared to the amino acid sequence of AAV2 capsid (SEQ ID NO:2). In some embodiments, the rAAV virion comprises a glutamine to lysine amino acid substitution at amino acid 164 compared to amino acid sequence of AAV1, AAV2 or AAV6 or at amino acid 165 compared to the amino acid sequence of AAV7, AAV8, or AAV10; or comprises a serine to lysine substitution at amino acid 160 of AAV5 or comprises an alanine to lysine substitution at amino acid 164 of AAV9. In related embodiments, the variant capsid protein comprises an amino acid substitution at amino acid 164 (e.g. Q164K) compared to the amino acid sequence of AAV2 capsid (SEQ ID NO:2), wherein the variant capsid protein comprises from 1 to 5, from

5 to 10, or from 10 to 15 amino acid substitutions compared to the amino acid sequence of an AAV2 capsid protein set forth in SEQ ID NO:2. In some preferred embodiments, the variant capsid protein comprises an amino acid sequence comprising a Q164K amino acid substitution compared to the amino acid sequence set forth in SEQ ID NO:2 and is otherwise identical to the amino acid sequence set forth in SEQ ID NO:2. In other embodiments the variant capsid protein comprises Q164K and V708I amino acid substitutions compared to the amino acid sequence of AAV2 capsid (SEQ ID NO:2) or the corresponding substitutions in another AAV parental serotype (i.e. other than AAV2) and is at least about 85%, at least about 90%, at least about 95%, at least about 98%, or at least about 99%, or greater, amino acid sequence identity to the entire length of the amino acid sequence set forth in SEQ ID NO:2.

[0106] In other embodiments a variant AAV capsid protein comprises an amino acid substitution at amino acid 698 compared to the amino acid sequence of AAV2 capsid (SEQ ID NO:2) or the corresponding position in another AAV parental serotype (i.e. other than AAV2), wherein the substituted amino acid does not naturally occur at the corresponding position. In some preferred embodiments, the variant capsid protein comprises an amino acid sequence having at least about 85%, at least about 90%, at least about 95%, at least about 98%, or at least about 99%, or greater, amino acid sequence identity to the entire length of the amino acid sequence set forth in SEQ ID NO 2 and comprises an amino acid substitution at amino acid 698 compared to the amino acid sequence of AAV2 capsid (SEQ ID NO:2). In some embodiments, the rAAV virion comprises an isoleucine to valine amino acid substitution at amino acid 698 compared to amino acid sequence of AAV2, or at amino acid 699 compared to the amino acid sequence of AAV3A, AAV3B, or AAV9, or at amino acid 687 of AAV5, or at amino acid 700 of AAV7, or at amino acid 701 of AAV8 or AAV10. In related embodiments, the variant capsid protein comprises an amino acid substitution at amino acid 699 (e.g. I698V) compared to the amino acid sequence of AAV2 capsid (SEQ ID NO:2), wherein the variant capsid protein comprises from 1 to 5, from 5 to 10, or from 10 to 15 amino acid substitutions compared to the amino acid sequence of an AAV2 capsid protein set forth in SEQ ID NO:2. In some preferred embodiments, the variant capsid protein comprises an amino acid sequence comprising an I698V amino acid substitution compared to the amino acid sequence set forth in SEQ ID NO:2 and is otherwise identical to the amino acid sequence set forth in SEQ ID NO:2.

[0107] In other embodiments a variant AAV capsid protein comprises an amino acid substitution at amino acid 109 compared to the amino acid sequence of AAV2 capsid (SEQ ID NO:2) or the corresponding position in another AAV parental serotype (i.e. other than AAV2). In some preferred embodiments, the variant capsid protein comprises an amino acid sequence having at least about 85%, at least about 90%, at least about 95%, at least about 98%, or at least about 99%, or greater, amino acid sequence identity to the entire length of the amino acid sequence set forth in SEQ ID NO 2 and comprises an amino acid substitution at amino acid 109 compared to the amino acid sequence of AAV2 capsid (SEQ ID NO:2). In some embodiments, the variant capsid protein comprises a serine to threonine amino acid substitution at position 109 compared to the amino acid sequence of AAV1, AAV3A, AAV3B, AAV4, AAV7, AAV8, AAV9, or AAV10 or at position 108 compared to the amino acid sequence of AAV5 or AAV6. In related embodiments, the variant capsid protein comprises an S109T

amino acid substitution compared to the amino acid sequence AAV2, wherein the variant capsid protein comprises from 1 to 5, from 5 to 10, or from 10 to 15 amino acid substitutions. In other related embodiments, the variant capsid protein comprises an S109T amino acid substitution and an A593E amino acid substitution compared to the amino acid sequence of AAV2. In some embodiments the variant capsid protein comprises S109T and A493V and optionally A593E and/or V708I amino acid substitutions compared to the amino acid sequence of AAV2 capsid (SEQ ID NO:2) or the corresponding substitutions in another AAV parental serotype (i.e. other than AAV2) and has at least about 85%, at least about 90%, at least about 95%, at least about 98%, or at least about 99%, or greater, amino acid sequence identity to the entire length of the amino acid sequence set forth in SEQ ID NO 2. In some preferred embodiments the variant capsid protein comprises S109T, A493V, A593E and V708I amino acid substitutions compared to the amino acid sequence of AAV2 capsid (SEQ ID NO:2) or the corresponding substitutions in another AAV parental serotype (i.e. other than AAV2) and is at least about 85%, at least about 90%, at least about 95%, at least about 98%, or at least about 99%, or greater, amino acid sequence identity to the entire length of the amino acid sequence set forth in SEQ ID NO 2. In other preferred embodiments, the variant capsid protein comprises S109T and V708I amino acid substitutions compared to the amino acid sequence of AAV2 capsid and has at least about 85%, at least about 90%, at least about 95%, at least about 98%, or at least about 99%, or greater, amino acid sequence identity to the entire length of the amino acid sequence set forth in SEQ ID NO 2 or is otherwise identical to the amino acid sequence of SEQ ID NO:2.

[0108] In other embodiments a variant AAV capsid protein comprises an amino acid substitution at amino acid 593 compared to the amino acid sequence of AAV2 capsid (SEQ ID NO:2) or the corresponding position in another AAV parental serotype (i.e. other than AAV2). In some preferred embodiments, the variant capsid protein comprises an amino acid sequence having at least about 85%, at least about 90%, at least about 95%, at least about 98%, or at least about 99%, or greater, amino acid sequence identity to the entire length of the amino acid sequence set forth in SEQ ID NO 2 and comprises an amino acid substitution at amino acid 593 compared to the amino acid sequence of AAV2 capsid (SEQ ID NO:2). In some embodiments, the variant capsid protein comprises a glycine to glutamate amino acid substitution at amino acid 594 compared to the amino acid sequence of AAV1, AAV3A, AAV6, or AAV9, or at amino acid 583 of AAV5, or at amino acid 596 of AAV8 or AAV10, or an arginine to glutamate amino acid substitution at amino acid 594 of AAV3B, or an aspartate to glutamate amino acid substitution at amino acid 592 of AAV4 or a glutamine to glutamate amino acid substitution at position 595 of AAV7. In other embodiments, the variant capsid protein comprises an A593E amino acid substitution compared to the amino acid sequence of AAV2 and does not comprise one or more of the following amino acid substitutions compared to the amino acid sequence of AAV2: I19V, V369A, K26R, N215D, G355S, V46A and S196P. In related embodiments, the variant capsid protein comprises A593E and N596D amino acid substitutions compared to the amino acid sequence of AAV2 and has at least about 85%, at least about 90%, at least about 95%, at least about 98% or at least about 99% identity to the entire length of the amino acid sequence set forth in SEQ ID NO 2. In other embodiments, the variant capsid comprises A593E and N596D amino acid substitutions compared to the amino

acid sequence of AAV2 and is otherwise identical to the amino acid sequence of AAV2. In other embodiments, the variant capsid comprises A593E and V708I amino acid substitutions compared to the amino acid sequence of AAV2 and has at least about 85%, at least about 90%, at least about 95%, at least about 98% or at least about 99% identity to the entire length of the amino acid sequence set forth in SEQ ID NO 2. In other embodiments, the variant capsid comprises A593E and V708I amino acid substitutions compared to the amino acid sequence of AAV2 and is otherwise identical to the amino acid sequence of AAV2.

[0109] In other embodiments a variant AAV capsid protein comprises an amino acid substitution at amino acid 708 compared to the amino acid sequence of AAV2 capsid (SEQ ID NO:2) or the corresponding position in another AAV parental serotype (i.e. other than AAV2) wherein the substituted amino acid does not naturally occur at the corresponding position. Preferably, the rAAV virion does not comprise a proline to serine substitution at amino acid 250 compared to AAV2 or a corresponding amino acid in another AAV parental serotype. In some embodiments, the variant capsid protein comprises an amino acid sequence having at least about 85%, at least about 90%, at least about 95%, at least about 98%, or at least about 99%, or greater, amino acid sequence identity to the entire length of the amino acid sequence set forth in SEQ ID NO 2 and comprises an amino acid substitution at amino acid 708 compared to the amino acid sequence of AAV2 capsid (SEQ ID NO:2). In preferred embodiments, the variant capsid protein comprises a valine to isoleucine (V708I) substitution at amino acid 708 compared to the amino acid sequence of AAV2 capsid and has at least about 85%, at least about 90%, at least about 95%, at least about 98%, or at least about 99%, or greater, amino acid sequence identity to the entire length of the amino acid sequence set forth in SEQ ID NO 2 or is otherwise identical to the amino acid sequence of SEQ ID NO:2, wherein the variant capsid protein does not comprise a P250S amino acid substitution. In some embodiments, the variant capsid protein comprises a valine to isoleucine substitution at amino acid 709 of AAV3A or AAV3B, an alanine to isoleucine substitution at position 709 of AAV1 or AAV6, an asparagine to isoleucine substitution at amino acid 707 of AAV4 or amino acid 709 of AAV9 or a threonine to isoleucine substitution at amino acid 710 of AAV7 or amino acid 711 of AAV8 or AAV10 or a glutamine to isoleucine substitution at amino acid 697 of AAV5. In related embodiments, the variant capsid protein comprises a V708I amino acid substitution compared to the amino acid sequence of AAV2, wherein the variant capsid protein comprises from 2 to 5, from 5 to 10, or from 10 to 15 amino acid substitutions and wherein the variant capsid protein does not comprise a P250S amino acid substitution. In other embodiments, the variant capsid protein comprises a V708I amino acid substitution and also comprises an A593E and/or an S109T amino acid substitution compared to the amino acid sequence of AAV2. In other related embodiments, the variant capsid comprises V708I and A593E amino acid substitutions compared to the amino acid sequence of AAV2, wherein the variant capsid protein is otherwise identical to the amino acid sequence of AAV2. In other related embodiments, the variant capsid comprises V708I and S109T amino acid substitutions compared to the amino acid sequence of AAV2, wherein the variant capsid protein is otherwise identical to the amino acid sequence of AAV2. In other embodiments, the variant capsid protein comprises V708I and V719M amino acid substitutions compared to the amino acid sequence of AAV2 and has at least about 85%, at least about 90%, at least about 95%, at least about 98%, or at least about 99%, or greater,

amino acid sequence identity to the entire length of the amino acid sequence set forth in SEQ ID NO 2 or is otherwise identical to the amino acid sequence of SEQ ID NO:2. In other embodiments, the variant capsid protein comprises V708I and R733C amino acid substitutions compared to the amino acid sequence of AAV2 and has at least about 85%, at least about 90%, at least about 95%, at least about 98%, or at least about 99%, or greater, amino acid sequence identity to the entire length of the amino acid sequence set forth in SEQ ID NO 2 or is otherwise identical to the amino acid sequence of SEQ ID NO:2. In other embodiments, the variant capsid protein comprises V708I and G727D amino acid substitutions compared to the amino acid sequence of AAV2 and has at least about 85%, at least about 90%, at least about 95%, at least about 98%, or at least about 99%, or greater, amino acid sequence identity to the entire length of the amino acid sequence set forth in SEQ ID NO 2 or is otherwise identical to the amino acid sequence of SEQ ID NO:2.

[0110] In other embodiments a variant AAV capsid protein comprises an amino acid substitution at amino acid 196 compared to the amino acid sequence of AAV2 capsid (SEQ ID NO:2) or the corresponding position in another AAV parental serotype (i.e. other than AAV2), wherein the substituted amino acid does not naturally occur at the corresponding position and is optionally other than proline. In some preferred embodiments, the variant capsid protein comprises an amino acid sequence having at least about 85%, at least about 90%, at least about 95%, at least about 98%, or at least about 99%, or greater, amino acid sequence identity to the entire length of the amino acid sequence set forth in SEQ ID NO 2 and comprises an amino acid substitution at amino acid 196 compared to the amino acid sequence of AAV2 capsid (SEQ ID NO:2) and is optionally other than an S196P substitution. In preferred embodiments, the variant capsid protein comprises a serine to tyrosine amino acid substitution at amino acid 196 of AAV2 or AAV9 or at amino acid 197 of AAV7, AAV8 or AAV10 or at amino acid 186 of AAV5; or an alanine to tyrosine substitution at amino acid 196 of AAV1 or AAV6; or a methionine to tyrosine substitution at amino acid 191 of AAV4; or a threonine to tyrosine substitution at amino acid 196 of AAV3A or AAV3B. In a related embodiment, the variant capsid protein comprises an amino acid sequence comprising an S196Y amino acid substitution compared to the amino acid sequence set forth in SEQ ID NO:2 and is otherwise identical to the amino acid sequence set forth in SEQ ID NO:2. In related embodiments, the variant capsid protein comprises an amino acid substitution at amino acid 196 other than an S196P substitution (e.g. comprises an S196Y substitution) compared to the amino acid sequence of AAV2 capsid (SEQ ID NO:2), wherein the variant capsid protein comprises from 1 to 5, from 5 to 10, or from 10 to 15 amino acid substitutions compared to the amino acid sequence of an AAV2 capsid protein set forth in SEQ ID NO:2.

[0111] In other embodiments a variant AAV capsid protein comprises an amino acid substitution at amino acid 175 compared to the amino acid sequence of AAV2 capsid (SEQ ID NO:2) or the corresponding position in another AAV parental serotype (i.e. other than AAV2), wherein the substituted amino acid does not naturally occur at the corresponding position. In some preferred embodiments, the variant capsid protein comprises an amino acid sequence having at least about 85%, at least about 90%, at least about 95%, at least about 98%, or at least about 99%, or greater, amino acid sequence identity to the entire length of the amino acid

sequence set forth in SEQ ID NO 2 and comprises an amino acid substitution at amino acid 175 compared to the amino acid sequence of AAV2 capsid (SEQ ID NO:2). In some embodiments, the variant capsid comprises a Q175H amino acid substitution compared to the amino acid sequence of AAV2 as set forth in SEQ ID NO:2 or a glutamine to histidine substitution at the corresponding position in another AAV parental serotype. In related embodiments, the variant capsid protein comprises an amino acid substitution at amino acid 175 (e.g. Q175H) compared to the amino acid sequence of AAV2 capsid (SEQ ID NO:2), wherein the variant capsid protein comprises from 1 to 5, from 5 to 10, or from 10 to 15 amino acid substitutions compared to the amino acid sequence of an AAV2 capsid protein set forth in SEQ ID NO:2.

[0112] In other embodiments a variant AAV capsid protein comprises an amino acid substitution at amino acid 64 compared to the amino acid sequence of AAV2 capsid (SEQ ID NO:2) or the corresponding position in another AAV parental serotype (i.e. other than AAV2), wherein the substituted amino acid does not naturally occur at the corresponding position. In some preferred embodiments, the variant capsid protein comprises an amino acid sequence having at least about 85%, at least about 90%, at least about 95%, at least about 98%, or at least about 99%, or greater, amino acid sequence identity to the entire length of the amino acid sequence set forth in SEQ ID NO 2 and comprises an amino acid substitution at amino acid 64 compared to the amino acid sequence of AAV2 capsid (SEQ ID NO:2). In some embodiments, the rAAV virion comprises a P64S amino acid substitution compared to the amino acid sequence of AAV2 as set forth in SEQ ID NO:2 or a proline to serine substitution at the corresponding position in another AAV parental serotype. In related embodiments, the variant capsid protein comprises an amino acid substitution at amino acid 64 (e.g. P64S) compared to the amino acid sequence of AAV2 capsid (SEQ ID NO:2), wherein the variant capsid protein comprises from 1 to 5, from 5 to 10, or from 10 to 15 amino acid substitutions compared to the amino acid sequence of an AAV2 capsid protein set forth in SEQ ID NO:2.

[0113] In other embodiments, a variant AAV capsid protein comprises an amino acid sequence at least 85%, at least 90%, at least 95% or at least 98% identical to a wild-type AAV capsid sequence selected from the group consisting of SEQ ID NOS: 1, 2, 3, 4, 5, 6, 7, 8, 10, 11 and 12 and also comprises i) one or more amino acid substitutions selected from the group consisting of P34A, S109T+V708I, A593E+N596D, V708I+V719M, V708I+G727D, S109T+A493V+A593E+V708I, V708I+R733C, Q164K, and I698V and/or (ii) a peptide insertion as defined in the claims.

[0114] In some embodiments, the variant AAV capsid comprises the specified one or more amino acid substitutions and/or peptide insertions and is otherwise identical to a sequence selected from the group consisting of SEQ ID NOS: 1-12.

[0115] In some embodiments, a variant AAV capsid protein is an ancestral capsid protein. By an ancestral capsid protein it is meant an evolutionary ancestor of a capsid protein that is found in nature today, e.g. AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAVrh10, AAV11, AAV12, AAV13, which is generated in silico by random amino acid

substitution at positions of degeneracy between AAV capsid proteins that are found in nature today. One nonlimiting example of an ancestral capsid is provided below, wherein the positions of degeneracy (residues 264, 266, 268, 448, 459, 460, 467, 470, 471, 474, 495, 516, 533, 547, 551, 555, 557, 561, 563, 577, 583, 593, 596, 661, 662, 664, 665, 710, 717, 718, 719, 723) are marked as an "X":

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MAADGYLPDWLEDNLSEGIREWWDLKPGAPKPKANQQ
KQDDGRGLVLPGYKYLGPFNGLDKGEPVNAADAAALEHDKAYDQQLKAGD
NPYLRYNHADADEFQERLQEDTSFGGNLGRAVFQAKKRVLEPLGLVEEGAKT
APGKKRPVEPSPQSPDSSTGIGKKKGQPAKKRLNFGQTGDSESVDPDQPLGE
PPAGPSGLSGTMAAGGGAPMADNNEGADGVGNASGNWHCDSTWLGDRVI
TTSTRTWALPTYNNHLYKQISSXSXGXTNDNHYFGYSTPWGYFDNRFHCHF
SPRDWQRLINNNWGFPRKRLNFKLFNIQVKEVTTNDGVTIANNLSTSTVQVFS
DSEYQLPYVLGSAHQGCLPPFPADVFMPQYGYLTLNNGSQAVGRSSFYCLE
YFPSQMLRTGNNFTFSYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYLXRTQ
STGGTAGXXELLFSQXGPXXMSXQAKNWLPGPCYRQQRVSKTLXQNNNSNF
AWTGATKYHLNGRXSLVNPGVAMATHKDDEXRFFPSSGVLIFGKXGAGXNN
TXLXNVMXTXEEIEIKTTNPVATEXYGVVAXNLQSSNTAPXTGXVNSQGALP
GMVWQNRDVYLQGPiWAKIPHTDGNFHPSPLMGGFGLKHPPPQILIKNTPVP
ANPPXXFXXAKFASFITQYSTGQVSVEIEWELQKENSkrwnPEIQYTSNYAKS
XNVDFAVXXXGVYXEPPIGTRYLTRNL (SEQ ID NO:58)

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[0116] In some embodiments, the ancestral capsid protein comprises an amino acid sequence having at least about 85%, at least about 90%, at least about 95%, at least about 98%, or at least about 99%, or greater, amino acid sequence identity to the entire length of the amino acid sequence set forth in SEQ ID NO:58. In some embodiments, the ancestral capsid protein comprises an amino acid sequence having at least about 85%, at least about 90%, at least about 95%, at least about 98%, or at least about 99%, or greater, amino acid sequence identity to the entire length of the amino acid sequence of AAV2, e.g. as set forth in SEQ ID NO 2. In some embodiments, the ancestral capsid protein comprises an amino acid sequence having at least about 85%, at least about 90%, at least about 95%, at least about 98%, or at least about 99%, or greater, amino acid sequence identity to the entire length of the amino acid sequence of the ancestral sequence disclosed in SEQ ID NO:58 or in SEQ ID NO:2 and comprises one or more amino acid residues selected from the group consisting of: Alanine (A) at 264, Alanine (A) at 266, Serine (S) at 268, Alanine (A) at 448, Threonine (T) at 459, Arginine (R) at 460, Alanine (A) at 467, Serine (S) at 470, Asparagine (N) at 471, Alanine (A) at 474, Serine (S) at 495, Asparagine (D) at 516, Asparagine (D) at 533, Glutamine (Q) at 547, Alanine (A) at 551, Alanine (A) at 555, Glutamic acid (E) at 557, Methionine (M) at 561, Serine (S) at 563, Glutamine (Q) at 577, Serine (S) at 583, Valine (V) at 593, Threonine (T) at 596, Alanine (A) at 661, Valine (V) at 662, Threonine (T) at 664, Proline (P) at 665, Threonine (T) at 710, Aspartic Acid (D) at 717, Asparagine (N) at 718, Glutamic acid (E) at 719, and Serine (S) at 723. In some preferred embodiments, the variant capsid protein comprises an amino acid sequence having at least about 85%, at least about 90%, at least about 95%, at least about 98%, or at

least about 99%, in some instances 100% amino acid sequence identity to the entire length of the following amino acid sequence and comprises one or more amino acid residues selected from the group consisting of: Alanine (A) at 264, Alanine (A) at 266, Serine (S) at 268, Alanine (A) at 448, Threonine (T) at 459, Arginine (R) at 460, Alanine (A) at 467, Serine (S) at 470, Asparagine (N) at 471, Alanine (A) at 474, Serine (S) at 495, Asparagine (D) at 516, Asparagine (D) at 533, Glutamine (Q) at 547, Alanine (A) at 551, Alanine (A) at 555, Glutamic acid (E) at 557, Methionine (M) at 561, Serine (S) at 563, Glutamine (Q) at 577, Serine (S) at 583, Valine (V) at 593, Threonine (T) at 596, Alanine (A) at 661, Valine (V) at 662, Threonine (T) at 664, Proline (P) at 665, Threonine (T) at 710, Aspartic Acid (D) at 717, Asparagine (N) at 718, Glutamic acid (E) at 719, and Serine (S) at 723:

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MAADGYLPDWLEDNLSEGIREWWDLKPGAPKPKANQQKQ
DDGRGLVLPGYKYLGPFNGLDKGEPVNAADAAALEHDKAYDQQLKAGDNP
YLRYNHADAEFQERLQEDTSFGGNLGRAVFQAKKRVLEPLGLVEEGAKTAP
GKKRPVEPSPQSPDSSTGIGKKGQPAKKRLNFGQTGDSVSPDPQPLGEPP
AGPSGLSGTMAAGGGAPMADNNEGADGVGNASGNWHCDSTWLGDRVITT
STRTWALPTYNNHLYKQISSASAGSTNDNHYFGYSTPWGYFDENRFHCHFSP
RDWQRLINNNWGFPRPKRLNFKLFNIQVKEVTTNDGVTTIANNLTSTVQVFSD
SEYQLPYVLGSAHQGCLPPFPADVFMIPQYGYLTLNNGSQAVGRSSFYCLEYF

PSQMLRTGNNTFSYTFEDVPPFHSSYAHSQSLDRLMNPLIDQYLYLARTQST
GGTACTRELLFSQAGPSNMSAQAKNWLPGPCYRQQRVSKTLSQNNNSNFAW
TGATKYHLNGRDSLVPNGVAMATHKDDDEDRFFPSSGVLIFGKQGAGANNTA
LENVMMTSEEEIKTTNPVATEQYGVVASNLQSSNTAPVTGTVNSQGALPGM
VWQNRDVYLGPIWAKIPHDTGDNFHPSPLMGGFGLKHPPPQILIKNTPVPANP
PAVFTPAKFASFITQYSTGQVSVEIEWELQKENS KRWNPEIQYTSNYAKSTNV
DFAVDNEG VYSEPRPIGTRYLTRNL (SEQ ID NO:59).

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[0117] In other embodiments, a variant AAV capsid protein comprises an amino acid sequence at least 85%, at least 90%, at least 95% or at least 98% identical to a wild-type AAV capsid sequence selected from the group consisting of the ancestral variant disclosed herein as SEQ ID NO:58, comprises one or more amino acid residues selected from the group consisting of: Alanine (A) at 264, Alanine (A) at 266, Serine (S) at 268, Alanine (A) at 448, Threonine (T) at 459, Arginine (R) at 460, Alanine (A) at 467, Serine (S) at 470, Asparagine (N) at 471, Alanine (A) at 474, Serine (S) at 495, Asparagine (D) at 516, Asparagine (D) at 533, Glutamine (Q) at 547, Alanine (A) at 551, Alanine (A) at 555, Glutamic acid (E) at 557, Methionine (M) at 561, Serine (S) at 563, Glutamine (Q) at 577, Serine (S) at 583, Valine (V) at 593, Threonine (T) at 596, Alanine (A) at 661, Valine (V) at 662, Threonine (T) at 664, Proline (P) at 665, Threonine (T) at 710, Aspartic Acid (D) at 717, Asparagine (N) at 718, Glutamic acid (E) at 719, and Serine (S) at 723; and also comprises i) one or more amino acid substitutions selected from the group consisting of P34A, S109T+V708I, A593E+N596D, V708I+V719M, V708I+G727D, S109T+A493V+A593E+V708I, V708I+R733C, Q164K, and I698V and/or (ii) a peptide insertion as defined in the claims.

[0118] In some embodiments, the variant AAV capsid comprises the specified one or more amino acid substitutions and/or peptide insertions and is otherwise identical to SEQ ID NO:59.

[0119] The AAV variants disclosed herein were generated through the use of *in vivo* directed evolution involving the use of primate retinal screens following intravitreal administration. In some embodiments, the variant capsid proteins disclosed herein, when present in an AAV virion, confer increased transduction of a retinal cell compared to the transduction of the retinal cell by an AAV virion comprising the corresponding parental AAV capsid protein or wild-type AAV. For example, in some embodiments, the variant capsid proteins disclosed herein, when present in an AAV virion, confer more efficient transduction of primate retinal cells than AAV virions comprising the corresponding parental AAV capsid protein or wild-type AAV capsid protein, e.g. the retinal cells take up more AAV virions comprising the subject variant AAV capsid protein than AAV virions comprising the parental AAV capsid protein or wild-type AAV. In some such embodiments, the AAV variant virion or variant rAAV exhibits at least 2-fold, at least 5-fold, at least 10-fold, at least 15-fold, at least 20-fold, at least 25-fold, at least 50-fold, or more than 50-fold, increased transduction of a retinal cell, compared to the transduction of the retinal cell by a wild-type AAV virion or rAAV comprising the corresponding parental AAV capsid protein. In certain such embodiments, the variant capsid proteins disclosed herein, when present in an AAV virion, confer broader transduction of the primate retinal cells than AAV virions comprising the corresponding parental AAV capsid protein or wild type AAV capsid protein. In other words, the variant AAV virion transduces cell types not transduced by virions comprising the corresponding parental AAV capsid protein, and hence more types of cells in the retina than the corresponding parental AAV virion. In some embodiments, the AAV variant virion preferentially transduces a retinal cell, e.g., a subject rAAV virion infects a retinal cell with 2-fold, 5- fold, 10-fold, 15-fold, 20-fold, 25-fold, 50-fold, or more than 50-fold, specificity than another retinal cell or a non-retinal cell, e.g., a cell outside the eye. In some embodiments, the transduced retinal cell is a photoreceptor cell (e.g., rods; cones). In some embodiments, the retinal cell is a retinal ganglion cell (RGC). In some embodiments, the retinal cell is a retinal epithelial cell (RPE cell). In some embodiments, the retinal cell is a Müller glial cell. In some embodiments, the retinal cell is a microglial cell. In some embodiments, the retinal cell is an amacrine cell. In some embodiments, the retinal cell is a bipolar cell. In some embodiments, the retinal cell is a horizontal cell. An increase in transduction of a retinal cell, e.g. increased efficiency of transduction, broader transduction, more preferential transduction, etc. may be readily assessed *in vitro* or *in vivo* by any number of methods in the art for measuring gene expression. For example, the AAV may be packaged with a genome comprising an expression cassette comprising a reporter gene, e.g. a fluorescent protein, under the control of a ubiquitous or tissue specific promoter, and the extent of transduction assessed by detecting the fluorescent protein by, e.g., fluorescence microscopy. As another example, the AAV may be packaged with a genome comprising a bar coded nucleic acid sequence, and the extent of transduction assessed by detecting the nucleic acid sequence by, e.g., PCR. As another example, the AAV may be packaged with a genome comprising an expression cassette comprising a therapeutic gene for the treatment of a retinal disease, and the extent of transduction assessed by detecting the treatment of the retinal disease in an afflicted patient that was administered the AAV.

[0120] Also provided is a variant rAAV vector as claimed for use in treating ocular diseases that include, but are not limited to, monogenic diseases, complex genetic diseases, acquired diseases, and traumatic injuries. Examples of monogenic diseases include, but are not limited to, Bardet-Biedl syndrome; Batten's Disease; Bietti's Crystalline Dystrophy; choroideremia; chorioretinal atrophy; chorioretinal degeneration; cone or cone-rod dystrophies (autosomal dominant, autosomal recessive, and X-linked); congenital stationary night blindness (autosomal dominant, autosomal recessive, and X-linked); disorders of color vision, including achromatopsia (including ACHM2, ACHM3, ACHM4, and ACHM5), protanopia, deuteranopia, and tritanopia; Friedreich's ataxia; Leber's congenital amaurosis (autosomal dominant and autosomal recessive), including, but not limited to, LCA1, LCA2, LCA3, LCA4, LCA6, LCA7, LCA8, LCA12, and LCA15; Leber's Hereditary Optic Neuropathy; macular dystrophy (autosomal dominant and autosomal recessive), including, but not limited to, acute macular degeneration, Best vitelliform macular dystrophy, pattern dystrophy, North Carolina Macular Dystrophy, inherited drusen, Sorsby's fundus dystrophy, malattia levantanese, and genetically-determined retinopathy of prematurity; ocular-retinal developmental disease; ocular albinism; optic atrophies (autosomal dominant, autosomal recessive, and X-linked); retinitis pigmentosa (autosomal dominant, autosomal recessive, X-linked, and mitochondrially-inherited traits), examples of which include RP1, RP2, RP3, RP10, RP20, RP38, RP40, and RP43; X-linked retinoschisis; Stargardt disease; and Usher syndrome, including, but not limited to, USH1B, USH1C, USH1D, USH1F, USH1G, USH2A, USH2C, USH2D, AND USH3. Examples of complex genetic diseases include, but are not limited to, glaucoma (open angle, angle-closure, low-tension, normal-tension, congenital, neovascular, pigmentary, pseudoexfoliation); age-related and other forms of macular degeneration, both exudative and non-exudative forms (autosomal dominant and autosomal recessive), such as acute macular degeneration, vitelliform macular degeneration; retinopathy of prematurity; and Vogt Koyanagi-Harada (VKH) syndrome. Examples of acquired diseases include, but are not limited to, acute macular neuroretinopathy; anterior ischemic optic neuropathy and posterior ischemic optic neuropathy; Behcet's disease; branch retinal vein occlusion; choroidal neovascularization; diabetic retinopathy, including proliferative diabetic retinopathy and associated complications; diabetic uveitis; edema, such as macular edema, cystoid macular edema and diabetic macular edema; epiretinal membrane disorders; macular telangiectasia; multifocal choroiditis; non-retinopathy diabetic retinal dysfunction; ocular tumors; optic atrophies; retinal detachment; retinal disorders, such as central retinal vein occlusion, proliferative vitreoretinopathy (PVR), retinal arterial and venous occlusive disease, vascular occlusion, uveitic retinal disease; uveal effusion; retinal infective and infiltrative disease; optic nerve diseases such as acquired optic atrophy. Examples of traumatic injuries include, but are not limited to, histoplasmosis; optic nerve trauma; ocular trauma which affects a posterior ocular site or location; retinal trauma; viral infection of the eye; viral infection of the optic nerve; a posterior ocular condition caused by or influenced by an ocular laser treatment; posterior ocular conditions caused by or influenced by a photodynamic therapy; photocoagulation; radiation retinopathy; and sympathetic ophthalmia.

[0121] In another embodiment, a variant capsid disclosed herein comprises a heterologous nucleic acid comprising a nucleotide sequence encoding a gene product such as, without

limitation, an interfering RNA, a long non-coding RNA, a short non-coding RNA, an antisense RNA, an aptamer, a polypeptide, a secreted antibody, a single chain antibody, a V_{HH} domain, a soluble receptor, an affibody, a knottin, a DARPin, a centurin, a chaperone, a site-specific nuclease that provides for site-specific knock-down of gene function or a modified site-specific nuclease that provides for gene-specific activation of transcription.

[0122] A rAAV variant virion disclosed herein comprises a heterologous nucleic acid comprising a nucleotide sequence encoding a gene product. In some embodiments, the gene product is an interfering RNA. In some embodiments, the gene product is a long non-coding RNA. In some embodiments, the gene product is a short non-coding RNA. In some embodiments, the gene product is an antisense RNA. In some embodiments, the gene product is an aptamer. In some embodiments, the gene product is a polypeptide. In some embodiments, the gene product is a secreted antibody. In some embodiments, the gene product is a single chain antibody. In some embodiments, the gene product is a V_{HH} domain. In some embodiments, the gene product is a soluble receptor. In some embodiments, the gene product is an affibody. In some embodiments, the gene product is a knottin. In some embodiments, the gene product is a DARPin. In some embodiments, the gene product is a centurin. In some embodiments, the gene product is a chaperone. In some embodiments, the gene product is a site-specific nuclease that provide for site-specific knock-down of gene function.

[0123] The uses of the gene product include, but are not limited to, enhancing the level of a factor in a cell, enhancing the level of a factor in a neighboring cell through secretion of a factor, decreasing the level of a factor in a cell, or decreasing the level of a factor in a neighboring cell through secretion of a factor. The gene product can be designed to supplement the level of a defective or missing gene product, decrease the level of a defective or missing gene product, introduce a new supporting gene product, supplement the level of a supporting gene product, decrease the level of a hindering gene product, or both decrease the level of a hindering gene product and introduce or supplement the level of a supporting gene product.

[0124] Gene products delivered by the subject AAV variants can be used to alter the level of gene products or gene product activity directly or indirectly linked to retinal diseases and trauma. Genes whose gene products are directly or indirectly linked to genetic diseases include, e.g., ADP-ribosylation factor-like 6 (ARL6); BBSome interacting protein 1 (BBIP1); BBSome protein 1 (BBS1); BBSome protein 2 (BBS2); BBSome protein 4 (BBS4); BBSome protein 5 (BBS5); BBSome protein 7 (BBS7); BBSome protein 9 (BBS9); BBSome protein 10 (BBS10); BBSome protein 12 (BBS12); centrosomal protein 290 kDa (CEP290); intraflagellar transport protein 172 (IFT172); intraflagellar transport protein 27 (IFT27); inositol polyphosphate-5-phosphatase E (INPP5E); inwardly-rectifying potassium channel subfamily J member 13 (KCNJ13); leucine zipper transcription factor like-1 (LZTFL1); McKusick-Kaufman syndrome protein (MKKS); Meckel syndrome type 1 protein (MKS1); nephronophthisis 3 protein (NPHP1); serologically-defined colon cancer antigen 8 (SDCCAG8); tripartite motif-containing protein 32 (TRIM32); tetratricopeptide repeat domain 8 (TTC8); Batten disease protein (CLN3); cytochrome P450 4V2 (CYP4V2); Rab escort protein 1 (CHM); PR (positive

regulatory) domain-containing 13 protein (PRDM13); RPE-retinal G protein-coupled receptor (RGR); TEA domain family member 1 (TEAD1); arylhydrocarbon-interacting receptor protein-like 1 (AIPL1); cone-rod otx-like photoreceptor homeobox transcription factor (CRX); guanylate cyclase activating protein 1A (GUCA1A); retinal-specific guanylate cyclase (GUCY2D); phosphatidylinositol transfer membrane-associated family member 3 (PITPNM3); prominin 1 (PROM1); peripherin (PRPH); peripherin 2 (PRPH2); regulating synaptic membrane exocytosis protein 1 (R1MS1); semaphorin 4A (SEMA4A); human homolog of *C. elegans* unc119 protein (UNC119); ATP-binding cassette transporter - retinal (ABCA4); ADAM metalloproteinase domain 9 (ADAM9); activating transcription factor 6 (ATF6); chromosome 21 open reading frame 2 (C21orf2); chromosome 8 open reading frame 37 (C8orf37); calcium channel; voltage-dependent; alpha 2/delta subunit 4 (CACNA2D4); cadherin-related family member 1 (protocadherin 21) (CDHR1); ceramide kinase-like protein (CERKL); cone photoreceptor cGMP-gated cation channel alpha subunit (CNGA3); cone cyclic nucleotide-gated cation channel beta 3 subunit (CNGB3); cyclin M4 (CNNM4); guanine nucleotide binding protein (G protein); alpha transducing activity polypeptide 2 (GNAT2); potassium channel subfamily V member 2 (KCNV2); Phosphodiesterase 6C (PDE6C); Phosphodiesterase 6H (PDE6H); proteome of centriole 1 centriolar protein B (POC1B); RAB28 member of RAS oncogene family (RAB28); retina and anterior neural fold homeobox 2 transcription factor (RAX2); 11-cis retinol dehydrogenase 5 (RDH5); RP GTPase regulator-interacting protein 1 (RPGRIP1); tubulin tyrosine ligase-like family member 5 (TTLL5); L-type voltage-gated calcium channel alpha-1 subunit (CACNA1F); retinitis pigmentosa GTPase regulator (RPGR); rod transducin alpha subunit (GNAT1); rod cGMP phosphodiesterase beta subunit (PDE6B); rhodopsin (RHO); calcium binding protein 4 (CABP4); G protein-coupled receptor 179 (OPR179); rhodopsin kinase (GRK1); metabotropic glutamate receptor 6 (GRM6); leucine-rich repeat immunoglobulin-like transmembrane domains protein 3 (LRIT3); arrestin (s-antigen) (SAG); solute carrier family 24 (SLC24A1); transient receptor potential cation channel, subfamily M, member 1 (TRPM1); nyctalopin (NYX); green cone opsin (OPN1LW); red cone opsin (OPN1MW); blue cone opsin (OPN1SW); frataxin (FXN); inosine monophosphate dehydrogenase 1 (IMPDH1); orthodenticle homeobox 2 protein (OTX2); crumbs homolog 1 (CRB1); death domain containing protein 1 (DTHD1); growth differentiation factor 6 (GDF6); intraflagellar transport 140 *Chlamydomonas* homolog protein (IFT140); IQ motif containing B1 protein (IQCB1); lebercilin (LCA5); lecithin retinol acyltransferase (LRAT); nicotinamide nucleotide adenyltransferase 1 (NMNAT1); RD3 protein (RD3); retinol dehydrogenase 12 (RDH12); retinal pigment epithelium-specific 65 kD protein (RPE65); spermatogenesis associated protein 7 (SPATA7); tubby-like protein 1 (TULP1); mitochondrial genes (KSS, LHON, MT-ATP6, MT-TH, MT-TL1, MT-TP, MT-TS2, mitochondrially encoded NADH dehydrogenases [MT-ND]); bestrophin 1 (BEST1); C1q and tumor necrosis-related protein 5 collagen (C1QTNF5); EGF-containing fibrillin-like extracellular matrix protein 1 (EFEMP1); elongation of very long fatty acids protein (ELOVL4); retinal fascin homolog 2, actin bundling protein (FSCN2); guanylate cyclase activating protein 1B (GUCA1B); hemiscentin 1 (HMCN1); interphotoreceptor matrix proteoglycan 1 (IMPG1); retinitis pigmentosa 1-like protein 1 (RP1L1); tissue inhibitor of metalloproteinases-3 (TIMP3); complement factor H (CFH); complement factor D (CFD); complement component 2 (C2); complement component 3 (C3); complement factor B (CFB); DNA-damage regulated autophagy modulator 2 (DRAM2);

chondroitin sulfate proteoglycan 2 (VCAN); mitofusin 2 (MFN2); nuclear receptor subfamily 2 group F member 1 (NR2F1); optic atrophy 1 (OPA1); transmembrane protein 126A (TMEM126A); inner mitochondrial membrane translocase 8 homolog A (TIMM8A); carbonic anhydrase IV (CA4); hexokinase 1 (HK1); kelch-like 7 protein (KLHL7); nuclear receptor subfamily 2 group E3 (NR2E3); neural retina luciferase zipper (NRL); olfactory receptor family 2 subfamily W member 3 (OR2W3); pre-mRNA processing factor 3 (PRPF3); pre-mRNA processing factor 4 (PRPF4); pre-mRNA processing factor 6 (PRPF6); pre-mRNA processing factor 8 (PRPF8); pre-mRNA processing factor 31 (PRPF31); retinal outer segment membrane protein 1 (ROM1); retinitis pigmentosa protein 1 (RP1); PIM1-kinase associated protein 1 (RP9); small nuclear ribonucleoprotein 200kDa (SNRNP200); secreted phosphoprotein 2 (SPP2); topoisomerase I binding arginine/serine rich protein (TOPORS); ADP-ribosylation factor-like 2 binding protein (ARL2BP); chromosome 2 open reading frame 71 (C2orf71); clarin-1 (CLRN1); rod cGMP-gated channel alpha subunit (CNGA1); rod cGMP-gated channel beta subunit (CNGB1); cytochrome P450 4V2 (CYP4V2); dehydrolipoyl diphosphate synthetase (DHDDS); DEAH box polypeptide 38 (DHX38); ER membrane protein complex subunit 1 (EMC1); eyes shut/spacemaker homolog (EYS); family with sequence similarity 161 member A (FAM161A); G protein-coupled receptor 125 (GPR125); heparan-alpha-glucosaminide N-acetyltransferase (HGSNAT); NAD(+)-specific isocitrate dehydrogenase 3 beta (IDH3B); interphotoreceptor matrix proteoglycan 2 (IMPG2); KIAA1549 protein (KIAA1549); kizuna centrosomal protein (KIZ); male germ-cell associated kinase (MAK); c-met protooncogene receptor tyrosine kinase (MERTK); mevalonate kinase (MVK); NIMA (never in mitosis gene A)-related kinase 2 (NEK2); neuronal differentiation protein 1 (NEUROD1); cGMP phosphodiesterase alpha subunit (PDE6A); phosphodiesterase 6G cGMP-specific rod gamma (PDE6G); progressive rod-cone degeneration protein (PRCD); retinol binding protein 3 (RBP3); retinaldehyde-binding protein 1 (RLBP1); solute carrier family 7 member 14 (SLC7A14); usherin (USH2A); zinc finger protein 408 (ZNF408); zinc finger protein 513 (ZNF513); oral-facial-digital syndrome 1 protein (OFD1); retinitis pigmentosa 2 (RP2); retinoschisin (RS1); abhydrolase domain containing protein 12 (ABHD12); cadherin-like gene 23 (CDH23); centrosomal protein 250 kDa (CEP250); calcium and integrin binding family member 2 (CIB2); whirlin (DFNB31); monogenic audiogenic seizure susceptibility 1 homolog (GPR98); histidyl-tRNA synthetase (HARS); myosin VIIA (MYO7A); protocadherin 15 (PCDH15); harmonin (USH1C); human homolog of mouse scaffold protein containing ankyrin repeats and SAM domain (USH1G); dystrophin (DMD); norrin (NDP); phosphoglycerate kinase (PGK1); calpain 5 (CAPN5); frizzled-4 Wnt receptor homolog (FZD4); integral membrane protein 2B (ITM2B); low density lipoprotein receptor-related protein 5 (LRP5); micro RNA 204 (MIR204); retinoblastoma protein 1 (RB1); tetraspanin 12 (TSPAN12); chromosome 12 open reading frame 65 (C12orf65); cadherin 3 (CDH3); membrane-type frizzled-related protein (MFRP); ornithine aminotransferase (OAT); phospholipase A2 group V (PLA2G5); retinol-binding protein 4 (RBP4); regulator of G-protein signaling 9 (RGS9); regulator of G-protein signaling 9-binding protein (RGS9BP); ARMS2; excision repair cross-complementing rodent repair deficiency complementation group 6 protein (ERCC6); fibulin 5 (FBLN5); HtrA serine peptidase 1 (HTRA1); toll-like receptor 3 (TLR3); and toll-like receptor 4 (TLR4).

[0125] Genes whose gene products induce or promote apoptosis are referred to herein as

"pro-apoptotic genes" and the products of those genes (mRNA; protein) are referred to as "pro-apoptotic gene products." Pro-apoptotic targets include, e.g., Bax gene products; Bid gene products; Bak gene products; Bad gene products; Bcl-2; Bcl-X1. Anti-apoptotic gene products include X-linked inhibitor of apoptosis.

[0126] Genes whose gene products induce or promote angiogenesis are referred to herein as "pro-angiogenic genes" and the products of those genes (mRNA; protein) are referred to as "pro-angiogenic gene products." Pro-angiogenic targets include, e.g., vascular endothelial growth factor (VEGFa, VEGFb, VEGFc, VEGFd); vascular endothelial growth factor receptor 1 (VEGFR1); vascular endothelial growth factor receptor 2 (VEGFR2); Fms-Related Tyrosine Kinase 1 (Flt1); placenta growth factor (PGF); Platelet-derived growth factor (PDGF); angiopoietins; sonic hedgehog. Genes whose gene products inhibit angiogenesis are referred to herein as "anti-angiogenic genes" and the products of those genes (mRNA; protein) are referred to as "anti-angiogenic gene products." Anti-angiogenic gene products include endostatin; tumstatin; angiostatin; pigment epithelium-derived factor (PEDF), and fusion proteins or antibodies that are specific for pro-angiogenic targets and/or their receptors, e.g. the anti-VEGF fusion proteins sFLT1 or Eylea, the VEGF-specific antibodies Lucentis[™] and Avastin[™], etc.

[0127] Genes whose gene products function as immune modulators, e.g., complement factors, toll-like receptors, are called "immunomodulatory genes". Exemplary immunomodulatory genes include cytokines, chemokines, and the fusion proteins or antibodies that are specific for them and/or their receptors, e.g. the anti-IL-6 fusion protein Rilonacept[™], the Complement Factor H-specific antibody lampalizumab, etc. Genes whose gene products function as neuroprotective factors, e.g., platelet derived growth factor receptor (PDGFR); glial derived neurotrophic factor (GDNF); rod-derived con viability factor (RdCVF); fibroblast growth factor (FGF); neurturin (NTN); ciliary neurotrophic factor (CNTF); nerve growth factor (NGF); neurotrophin-4 (NT4); brain derived neurotrophic factor (BDNF); epidermal growth factor. Genes whose gene products function as light responsive opsins, e.g., opsin; rhodopsin; channel rhodopsin; halo rhodopsin.

[0128] In some cases, a gene product of interest is a site-specific endonuclease that provide for site-specific knock-down of gene function, e.g., where the endonuclease knocks out an allele associated with a retinal disease. For example, where a dominant allele encodes a defective copy of a gene that, when wild-type, is a retinal structural protein and/or provides for normal retinal function, a site-specific endonuclease can be targeted to the defective allele and knock out the defective allele.

[0129] In addition to knocking out a defective allele, a site-specific nuclease can also be used to stimulate homologous recombination with a donor DNA that encodes a functional copy of the protein encoded by the defective allele. Thus, e.g., a subject rAAV virion can be used to deliver both a site-specific endonuclease that knocks out a defective allele, and can be used to deliver a functional copy of the defective allele, resulting in repair of the defective allele, thereby

providing for production of a functional retinal protein (e.g., functional retinoschisin, functional RPE65, functional peripherin, etc.). See, e.g., Li et al. (2011) *Nature* 475:217. In some embodiments, a rAAV virion disclosed herein comprises a heterologous nucleotide sequence that encodes a site-specific endonuclease; and a heterologous nucleotide sequence that encodes a functional copy of a defective allele, where the functional copy encodes a functional retinal protein. Functional retinal proteins include, e.g., retinoschisin, RPE65, retinitis pigmentosa GTPase regulator (RGPR)-interacting protein-1, peripherin, peripherin-2, and the like.

[0130] Site-specific endonucleases that are suitable for use include, e.g., meganucleases; zinc finger nucleases (ZFNs); transcription activator-like effector nucleases (TALENs); and Clustered regularly interspaced short palindromic repeats/CRISPR-associated (Cas), where such site-specific endonucleases are non-naturally occurring and are modified to target a specific gene. Such site-specific nucleases can be engineered to cut specific locations within a genome, and non-homologous end joining can then repair the break while inserting or deleting several nucleotides. Such site-specific endonucleases (also referred to as "INDELs") then throw the protein out of frame and effectively knock out the gene. See, e.g., U.S. Patent Publication No. 2011/0301073.

[0131] In some embodiments of the variant rAAV vector disclosed herein, a nucleotide sequence encoding a gene product of interest is operably linked to a constitutive promoter. Suitable constitutive promoters include e.g. cytomegalovirus promoter (CMV) (Stinski et al. (1985) *Journal of Virology* 55(2): 431-441), CMV early enhancer/chicken β -actin (CBA) promoter/rabbit β -globin intron (CAG) (Miyazaki et al. (1989) *Gene* 79(2): 269-277, CB^{SB} (Jacobson et al. (2006) *Molecular Therapy* 13(6): 1074-1084), human elongation factor 1 α promoter (EF1 α) (Kim et al. (1990) *Gene* 91(2): 217-223), human phosphoglycerate kinase promoter (PGK) (Singer-Sam et al. (1984) *Gene* 32(3): 409-417, mitochondrial heavy-strand promoter (Loderio et al. (2012) *PNAS* 109(17): 6513-6518), ubiquitin promoter (Wulff et al. (1990) *FEBS Letters* 261: 101-105). In other embodiments, a nucleotide sequence encoding a gene product of interest is operably linked to an inducible promoter. In some instances, a nucleotide sequence encoding a gene product of interest is operably linked to a tissue-specific or cell type-specific regulatory element. For example, in some instances, a nucleotide sequence encoding a gene product of interest is operably linked to a photoreceptor-specific regulatory element (e.g., a photoreceptor-specific promoter), e.g., a regulatory element that confers selective expression of the operably linked gene in a photoreceptor cell. Suitable photoreceptor-specific regulatory elements include, e.g., a rhodopsin promoter; a rhodopsin kinase promoter (Young et al. (2003) *Ophthalmol. Vis. Sci.* 44:4076); a beta phosphodiesterase gene promoter (Nicoud et al. (2007) *J. Gene Med.* 9:1015); a retinitis pigmentosa gene promoter (Nicoud et al. (2007) *supra*); an interphotoreceptor retinoid-binding protein (IRBP) gene enhancer (Nicoud et al. (2007) *supra*); an IRBP gene promoter (Yokoyama et al. (1992) *Exp Eye Res.* 55:225), an opsin gene promoter (Tucker et al. (1994) *PNAS* 91:2611-2615), a retinoschisin gene promoter (Park et al. (2009) *Gene Therapy* 16(7): 916-926), a CRX homeodomain protein gene promoter (Furukawa et al. (2002) *The Journal of Neuroscience* 22(5): 1640-1647), a guanine nucleotide binding protein alpha transducing

activity polypeptide 1 (ONAT1) gene promoter (Lee et al. (2010) *Gene Therapy* 17:1390-1399), a neural retina-specific leucine zipper protein (NRL) gene promoter (Akimoto et al. (2006) *PNAS* 103(10): 3890-3895), human cone arrestin (hCAR) promoter (Li et al. (2002) *Biochemistry and Molecular Biology* 43: 1375-1383), and the PR2.1, PR1.7, PR1.5, and PR1.1 promoters (Ye et al. (2016) *Human Gene Therapy* 27(1): 72-82)). In some instances, a nucleotide sequence encoding a gene product of interest is operably linked to a retinal pigment epithelia (RPE) cell-specific regulatory element (e.g., a RPE-specific promoter), e.g., a regulatory element that confers selective expression of the operably linked gene in a RPE cell. Suitable RPE-specific regulatory elements include, e.g., an RPE65 gene promoter (Meur et al. (2007) *Gene Therapy* 14: 292-303), a cellular retinaldehyde-binding protein (CRALBP) gene promoter (Kennedy et al. (1998) *Journal of Biological Chemistry* 273: 5591-5598), a pigment epithelium-derived factor (PEDF aka serpin F1) gene promoter (Kojima et al. (2006) *Molecular and Cellular Biochemistry* 293(1-2): 63-69), and a vitelliform macular dystrophy (VMD2) promoter (Esumi et al. (2004) *The Journal of Biological Chemistry* 279(18): 19064-19073). In some instances, a nucleotide sequence encoding a gene product of interest is operably linked to a Müller glia cell-specific regulatory element (e.g., a glial-specific promoter), e.g., a regulatory element that confers selective expression of the operably linked gene in a retinal glial cell. Suitable glial-specific regulatory elements include, e.g., a glial fibrillary acidic protein (GFAP) promoter (Besnard et al. (1991) *Journal of Biological Chemistry* 266(28): 18877-18883). In some instances, a nucleotide sequence encoding a gene product of interest is operably linked to a bipolar cell-specific regulatory element (e.g., a bipolar-specific promoter), e.g., a regulatory element that confers selective expression of the operably linked gene in a bipolar cell. Suitable bipolar-specific regulatory elements include, e.g., a GRM6 promoter (Cronin et al. (2014) *EMBO Molecular Medicine* 6(9): 1175-1190).

[0132] For the purposes of the invention, the disclosure herein provides an isolated nucleic acid comprising a nucleotide sequence that encodes a variant AAV capsid protein as described above. An isolated nucleic acid can be an AAV vector, e.g., a recombinant AAV vector.

[0133] The disclosure herein also provides a rAAV variant virion as claimed for use in a method of treating a retinal disease, the method comprising administering to an individual in need thereof an effective amount of a rAAV variant virion comprising a transgene of interest as described above and disclosed herein. One of ordinary skill in the art would be readily able to determine an effective amount of the subject rAAV virion and that the disease had been treated by testing for a change in one or more functional or anatomical parameters, e.g. visual acuity, visual field, electrophysiological responsiveness to light and dark, color vision, contrast sensitivity, anatomy, retinal health and vasculature, ocular motility, fixation preference, and stability.

[0134] Nonlimiting methods for assessing retinal function and changes thereof include assessing visual acuity (e.g. best-corrected visual acuity [BCVA], ambulation, navigation, object detection and discrimination), assessing visual field (e.g. static and kinetic visual field perimetry), performing a clinical examination (e.g. slit lamp examination of the anterior and posterior segments of the eye), assessing electrophysiological responsiveness to all

wavelengths of light and dark (e.g. all forms of electroretinography (ERG) [full-field, multifocal and pattern], all forms of visual evoked potential (VEP), electrooculography (EOG), color vision, dark adaptation and/or contrast sensitivity). Nonlimiting methods for assessing anatomy and retinal health and changes thereof include Optical Coherence Tomography (OCT), fundus photography, adaptive optics scanning laser ophthalmoscopy (AO-SLO), fluorescence and/or autofluorescence; measuring ocular motility and eye movements (e.g. nystagmus, fixation preference, and stability), measuring reported outcomes (patient-reported changes in visual and non-visually-guided behaviors and activities, patient-reported outcomes [PRO], questionnaire-based assessments of quality-of-life, daily activities and measures of neurological function (e.g. functional Magnetic Resonance Imaging (MRI)).

[0135] In some embodiments, an effective amount of the subject rAAV virion results in a decrease in the rate of loss of retinal function, anatomical integrity, or retinal health, e.g. a 2-fold, 3-fold, 4-fold, or 5-fold or more decrease in the rate of loss and hence progression of disease, for example, a 10-fold decrease or more in the rate of loss and hence progression of disease. In some embodiments, the effective amount of the subject rAAV virion results in a gain in visual function, retinal function, an improvement in retinal anatomy or health, and/or an improvement in ocular motility and/or improvement in neurological function, e.g. a 2-fold, 3-fold, 4-fold or 5-fold improvement or more in retinal function, retinal anatomy or health, and/or improvement in ocular motility, e.g. a 10-fold improvement or more in retinal function, retinal anatomy or health, and/or improvement in ocular motility. As will be readily appreciated by the ordinarily skilled artisan, the dose required to achieve the desired treatment effect will typically be in the range of 1×10^8 to about 1×10^{15} recombinant virions, typically referred to by the ordinarily skilled artisan as 1×10^8 to about 1×10^{15} "vector genomes".

[0136] A subject rAAV virion can be administered via intraocular injection, for example by intravitreal injection, by subretinal injection, by suprachoroidal injection, or by any other convenient mode or route of administration that will result in the delivery of the rAAV virion to the eye. Other convenient manners or routes of administration include, without limitation, intravenous, intra-arterial, peri-ocular, intracameral, subconjunctival and sub-tenons injections and topical administration and intranasal. When administered via intravitreal injection, the subject rAAV virion is able to move through the vitreous and traverse the internal limiting membrane (also referred to herein as an inner limiting membrane, or "ILM"; a thin, transparent acellular membrane on the surface of the retina forming the boundary between the retina and the vitreous body, formed by astrocytes and the end feet of Müller cells), and/or moves through the layers of the retina more efficiently, compared to the capability of an AAV virion comprising the corresponding parental AAV capsid protein.

[0137] A variant capsid protein disclosed herein is isolated, e.g., purified. In some embodiments, a variant capsid protein disclosed herein is included in an AAV vector or a recombinant AAV (rAAV) virion. In other embodiments, such AAV variant vectors and/or AAV variant virions are used in an *in vivo* or *ex vivo* method of treating ocular disease in the primate retina.

[0138] The disclosure herein further provides host cells such as, without limitation, isolated (genetically modified) host cells comprising a subject nucleic acid. A host cell according to the invention disclosed herein, can be an isolated cell, such as a cell from an *in vitro* cell culture. Such a host cell is useful for producing a subject rAAV variant virion, as described herein. In one embodiment, such a host cell is stably genetically modified with a nucleic acid. In other embodiments, a host cell is transiently genetically modified with a nucleic acid. Such a nucleic acid is introduced stably or transiently into a host cell, using established techniques, including, but not limited to, electroporation, calcium phosphate precipitation, liposome-mediated transfection, and the like. For stable transformation, a nucleic acid will generally further include a selectable marker, e.g., any of several well-known selectable markers such as neomycin resistance, and the like. Such a host cell is generated by introducing a nucleic acid into any of a variety of cells, e.g., mammalian cells, including, e.g., murine cells, and primate cells (e.g., human cells). Exemplary mammalian cells include, but are not limited to, primary cells and cell lines, where exemplary cell lines include, but are not limited to, 293 cells, COS cells, HeLa cells, Vero cells, 3T3 mouse fibroblasts, C3H10T1/2 fibroblasts, CHO cells, and the like. Exemplary host cells include, without limitation, HeLa cells (e.g., American Type Culture Collection (ATCC) No. CCL-2), CHO cells (e.g., ATCC Nos. CRL9618, CCL61, CRL9096), 293 cells (e.g., ATCC No. CRL-1573), Vero cells, NIH 3T3 cells (e.g., ATCC No. CRL-1658), Huh-7 cells, BHK cells (e.g., ATCC No. CCL10), PC12 cells (ATCC No. CRL1721), COS cells, COS-7 cells (ATCC No. CRL1651), RAT1 cells, mouse L cells (ATCC No. CCL1.3), human embryonic kidney (HEK) cells (ATCC No. CRL1573), HLHepG2 cells, and the like. A host cell can also be made using a baculovirus to infect insect cells such as Sf9 cells, which produce AAV (see, e.g., U.S. Pat. No. 7,271,002; U.S. patent application Ser. No. 12/297,958). In some embodiments, a genetically modified host cell includes, in addition to a nucleic acid comprising a nucleotide sequence encoding a variant AAV capsid protein, as described above, a nucleic acid that comprises a nucleotide sequence encoding one or more AAV rep proteins. In other embodiments, a host cell further comprises an rAAV variant vector. An rAAV variant virion can be generated using such host cells. Methods of generating an rAAV virion are described in, e.g., U.S. Patent Publication No. 2005/0053922 and U.S. Patent Publication No. 2009/0202490.

[0139] The disclosure herein additionally provides a pharmaceutical composition comprising: a) the rAAV variant virion, as described above and disclosed herein; and b) a pharmaceutically acceptable carrier, diluent, excipient, or buffer. In some embodiments, the pharmaceutically acceptable carrier, diluent, excipient, or buffer is suitable for use in a human or non-human patient. Such excipients, carriers, diluents, and buffers include any pharmaceutical agent that can be administered without undue toxicity. Pharmaceutically acceptable excipients include, but are not limited to, liquids such as water, saline, glycerol and ethanol. Pharmaceutically acceptable salts can be included therein, for example, mineral acid salts such as hydrochlorides, hydrobromides, phosphates, sulfates, and the like; and the salts of organic acids such as acetates, propionates, malonates, benzoates, and the like. Additionally, auxiliary substances, such as wetting or emulsifying agents, surfactants, pH buffering substances, and the like, may be present in such vehicles. A wide variety of pharmaceutically acceptable excipients are known in the art and need not be discussed in detail herein. Pharmaceutically

acceptable excipients have been amply described in a variety of publications, including, for example, A. Gennaro (2000) "Remington: The Science and Practice of Pharmacy," 20th edition, Lippincott, Williams, & Wilkins; Pharmaceutical Dosage Forms and Drug Delivery Systems (1999) H. C. Ansel et al., eds., 7th ed., Lippincott, Williams, & Wilkins; and Handbook of Pharmaceutical Excipients (2000) A. H. Kibbe et al., eds., 3rd ed. Amer. Pharmaceutical Assoc. In some aspects of the present invention, the present invention provides a pharmaceutical composition comprising about 1×10^8 to about 1×10^{15} recombinant viruses or 1×10^8 to about 1×10^{15} vector genomes, wherein each said recombinant virus comprises a genome encoding one or more gene products.

[0140] The following examples are set forth to provide the ordinarily skilled artisan with a complete disclosure and description for guidance as to how to make and use the variant AAV capsids disclosed herein, and are not intended to limit the scope of the invention disclosed herein. In addition, the following examples are not intended to represent that the experiments below are all or the only experiments performed.

EXAMPLES

[0141] The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the present invention, and are not intended to limit the scope of what the inventors regard as their invention nor are they intended to represent that the experiments below are all or the only experiments performed. Efforts have been made to ensure accuracy with respect to numbers used (e.g. amounts, temperature, etc.) but some experimental errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, molecular weight is weight average molecular weight, temperature is in degrees Centigrade, and pressure is at or near atmospheric.

[0142] General methods in molecular and cellular biochemistry can be found in such standard textbooks as Molecular Cloning: A Laboratory Manual, 3rd Ed. (Sambrook et al., Harbor Laboratory Press 2001); Short Protocols in Molecular Biology, 4th Ed. (Ausubel et al. eds., John Wiley & Sons 1999); Protein Methods (Bollag et al., John Wiley & Sons 1996); Nonviral Vectors for Gene Therapy (Wagner et al. eds., Academic Press 1999); Viral Vectors (Kaplift & Loewy eds., Academic Press 1995); Immunology Methods Manual (I. Lefkovits ed., Academic Press 1997); and Cell and Tissue Culture: Laboratory Procedures in Biotechnology (Doyle & Griffiths, John Wiley & Sons 1998).

[0143] Reagents, cloning vectors, and kits for genetic manipulation referred to in this disclosure are available from commercial vendors such as BioRad, Stratagene, Invitrogen, Sigma-Aldrich, and ClonTech.

Example 1

[0144] Intravitreal Injection and Tissue Harvesting. A single male cynomolgus macaque (*macaca fascicularis*) age 4-10 years old and weighing at least 4 kg was dosed via intravitreal injection through the sclera (approximately 3 mm behind the limbus using a procedure and delivery device suitable for human use). The animal was anesthetized and given the topical anesthetic 100 µL of the library was administered to each eye

[0145] Euthanasia was performed by trained veterinary staff using 100 mg/kg pentobarbital sodium intravenous injection on day 14±3. Eyes were nucleated and stored at 4°C until dissection.

[0146] Tissue Dissection. Eyes were cut along the ora serrata with a scalpel, and the anterior segment was removed. Relief cuts were made into the retina around the fovea to enable flat mounting of the retina, and the vitreous was removed. Six samples of the retina from each quadrant (superior, inferior, nasal, and temporal) were collected, as shown in Figure 2, and cellular material corresponding to RPE cells, photoreceptors, biopolar cells, amacrine cells, horizontal cells, and/or ganglion cells was isolated.

[0147] Directed Evolution. The directed evolution process is shown in Figure 1A-1E. Briefly, a viral capsid library comprising 20+ proprietary combinations of DNA mutation technique and *cap* genes is created (Figure 1A). Viruses are then packaged (Figure 1B) - such that each particle is composed of a mutant capsid surrounding the *cap* gene encoding that capsid - and purified. The capsid library is placed under selective pressure *in vivo*. The tissue or cellular material of interest is harvested to isolate AAV variants that have successfully infected that target, and the successful viruses are recovered. Successful clones are enriched through repeated selection (Stage 1 - Figure 1D). Selected *cap* genes then undergo proprietary re-diversification and are enriched through further selection steps to iteratively increase viral fitness (Stage 2 - Figure 1D). Variants identified during Vector Selection Stages 1 and 2 demonstrate the ability to transduce primate retina cells (Figure 1E).

[0148] Successful Recovery of AAV Capsid Genomes: Rounds 1-6. The capsids recovered from each round of selection were used to package the library injected to initiate the subsequent round of selection. Recovery of capsid genes from tissue represents successful internalization of library vectors into the tissue of interest. Following Round 4, additional re-diversification of the library was incorporated prior to library packaging and injection for Round 5. Recovery of viral genomes from RPE, PR, inner nuclear layer (INL), and ganglion cell layer (GCL) retinal tissue from a representative round of selection are shown in Figure 3. Bands within boxes represent successful recovery of viral genomes.

[0149] Sequencing Analysis: Rounds 3-6. During rounds 3-6, sequencing was performed on individual clones within the library to determine the frequency of variants within the population. Variants were evaluated for the presence of motifs within the sequencing data. Variants were grouped into motifs based on the presence of a unifying variation (for example, a specific point

mutation or specific peptide insertion sequence in a consistent location within the capsid) that occurred in multiple sequences. Motifs representing at least 5% of the sequenced population in two or more rounds of the selection or at least 10% of the sequenced population in one or more rounds of the selection are represented in Figure 4A (Round 3 sequencing analysis), 4B (Round 4 sequencing analysis), 4C (Round 5 sequencing analysis), and 4D (Round 6 sequencing analysis).

[0150] Several representative clones that were identified as conferring increased infectivity of retinal cells are listed in Table 1 below (each clone contains the identified substitution(s) and/or peptide insertion and is otherwise identical to SEQ ID NO:2; the selection round, number of sequences and frequency (in parentheses) are listed for each clone):

Table 1. Amino acid sequence modifications to the AAV VP1 capsid protein that confer increased infectivity of one or more cells of the retina. Substitutions listed in column 2 are based on the amino acid sequence for wild type AAV2, i.e. in the absence of inserted peptide.

Insertion	Substitution	Pan-Retinal	RPE	Photo-receptor
588~LAISDQTKHA~ (SEQ ID NO:28)	None	Round 4, 5, 6	Round 3, 5,6	Round 4, 5
588~LAISDQTKHA~ (SEQ ID NO:28)	+M1L+L15P +P535S	Round 6 1 (1.61%)		
588~LAISDQTKHA~ +P34A (SEQ ID NO:28)	+P34A	Round 5 1 (1.89%)		
588-LAISDQTKHA~ (SEQ ID NO:28)	+P34A+S731L			Round 5 1(1.82%)
588-LAISDQTKHA~ (SEQ ID NO:28)	+N57D	Round 4 1(1.33%)		
588-LAISDQTKHA~ (SEQ ID NO:28)	+N66K			Round 5 1(1.82%)
588-LAISDQTKHA~ (SEQ ID NO:28)	+R81Q	Round 6 1(1.61%)		
588-LAISDQTKHA~ (SEQ ID NO:28)	+Q101R			Round 4(2.27%)
588-LAISDQTKHA~ (SEQ ID NO:28)	+S109T1	Round 3 1(1.85%)		
588-LAISDQTKHA~ (SEQ ID NO:28)	+R144K			Round 5 1(1.82%)
588-LAISDQTKHA~ (SEQ ID NO:28)	+R144M			Round 5 1(1.82%)
588-LAISDQTKHA~ (SEQ ID NO:28)	+Q164K			Round 4 1(2.27%)
588-LAISDQTKHA~ (SEQ ID NO:28)	+Q164K+V708I	Rounds 3 and 4		

Insertion	Substitution	Pan-Retinal	RPE	Photo-receptor
		(3.7%)		
		(1.33%)		
588-LAISDQTKHA~ (SEQ ID NO:28)	+T176P	Round 4 1 (1.33%)		
588-LAISDQTKHA~ (SEQ ID NO:28)	+L188I		Round 5 1(2.27%)	
588-LAISDQTKHA~ (SEQ ID NO:28)	+S196Y1	Round 4 1(1.33%)		
588-LAISDQTKHA~ (SEQ ID NO:28)	+G226E			Round 4 1(2.27%)
588~LAISDQTKHA~ (SEQ ID NO:28)	+G236V	Round 4 1(1.33%)		
588~LAISDOTKHA~ (SEQ ID NO:28)	+I240T	Round 3 (1.85%)		
588~LAISDQTKHA~ (SEQ ID NO:28)	+N312K	Round 4 (11.33%)		
588~LAISDQTKHA~ (SEQ ID NO:28)	+P363L	Round 6(1.61%)		
588~LAISDQTKHA~ (SEQ ID NO:28)	+T456K	Round 6(1.61%)		
588-LAISDQTKHA~ (SEQ ID NO:28) 588~LAISDQTKHA~ (SEQ ID NO:28)	+I698V	Round 5(1.89%)		
(SEQ ID NO:28)	+V708I	Round 4, 5,6	Round 3,4,5	Round 4,5
588~LAISDQTKHA~ (SEQ ID NO:28)	+V708I+R484C		Round 5 1(2.27%)	Engineered
588~LAISDQTKHA~ (SEQ ID NO:28) :	N312K+N449EH+N 551S+I698V+L73 5Q	Engineered Round		
588~LGISDQTKHA~ (SEQ ID NO:29)	None	Round 5 1 (1.89%)		
588~LAQADTTKNA~ (SEQ ID NO:27)	+E36D	Round 3,4,5,6	Round 3,4,5	Round 4,5
588~LAQADTTKNA~ (SEQ ID NO:27)	+P250S		Round 5 1 (2.27%)	
588~LAQADTTKNA~ (SEQ ID NO:27)	+A524T	Round 6 1 (1.61%)		

Insertion	Substitution	Pan-Retinal	RPE	Photo-receptor
588~LAQADTTKNA~ (SEQ ID NO:27)	+A593E	Round 3 1 (1.85%)		
588~LAQADTTKNA~ (SEQ ID NO:27)	+A593E	Round 4 1 (1.33%)		
588~LAQADTTKNA~ + (SEQ ID NO:27)	+I698V	Rounds 5 and 6		
		1(1.61%)		
		1(1.89%) Round	Round 3,	
588~LAQADTTKNA~ (SEQ IDNO:27)	+V708I	3, 4, 5,6	4,5	
588~LAQADTTKNA~ (SEQ ID NO:27)	+V708I+V719M		Rounds 3 and 4	
		1	(2.08%)	
		2	(4.55%)	
588~LAQADTTKNA~ (SEQ ID NO:27)	+V719M		Round 4	
			1 (2.27%)	
588~LAHQDTTKNA~ (SEQ ID NO:34)	None	Round 5		
		2 (3.77%) Rounds 4	Rounds 3,	Round 5
588~LANQDYTKTA~ (SEQ ID NO:31)	None	3, and 5	4 and 5	1 (1.82%)
		5 (9.26%)	2 (4.17%)	
		1 (1.33%)	2 (4.55%)	
		3(5.66%)	2 (2.27%)	
588~LANQDYTKTA~ (SEQ ID NO:31)	+S109T+S463Y	Round 3		
		1 (1.85%)		
588~LANQDYTKTA~ (SEQ ID NO:31)	+D368H	Round 3		
		1 (1.85%)		
588~LANQDYTKTA~ (SEQ ID NO:31)	+V708I	Round 4	Round 3	
		1 (1.33%)	1 (2.08%)	
588~LAHDITKNIA~ (SEQ ID NO:32)	None	Rounds 3, 4 5 and 6		
		1 (1.85%)		
		1 (1.33%)		
		1 (1.89%)		
		2 (3.23%) 4		

Insertion	Substitution	Pan-Retinal	RPE	Photo-receptor
588~LAHDITKNIA~ (SEQ ID NO:32)	+S109T	Round		
		1 (1.33%) 5		
588~LAHDITkNIA~ (SEQ ID NO:32)	+R389S	Round		
	1	(1.89%) Round 3		
588~LAHDITKNIA~ (SEQ ID NO:32)	+A593E			
		1 (1.85%)		
588~LAHDITKNIA~ (SEQ ID NO:32)	+V708I	Round 4, 5		
588~LAHDTKNIA~ (SEQ ID NO: 60)	+V708I		Round 3	
			1 (2.08%)	
588~LAPNSTHGSA~ (SEQ ID NO:40)	+V708I	Round 3		
		1 (1.85%)		
588~LANKTTNKDA~ (SEQ ID NO:35)	None		Round 5	
			1 (2.27%)	
589~LANKTTNKDA~ (SEQ ID NO:35)	+N449D			Round 41
				(2.17%)
589~LAHPDTTKNA~ (SEQ ID NO:33)		Round 6		
		1 (1.61%) Round		
58~LATNRTSPDA~ (SEQ ID NO:39)		Round 5		
		1 (1.61%)		
588~LPQANANENA~ (SEQ ID NO:37)		Round 5		
		1 (1.89%)		
588~LAASDSTKAA~ (SEQ ID NO:30)		Rounds 3and 4		
		1 (1.85%)		
		1 (1.33%)		
588~LAKDRAPSTA~ (SEQ ID NO:41)		Round 3		
		1 (1.85%)		
588~LPISNENEHA~ (SEQ ID NO:36)				Round 4
				1 (2.17%)
599~ILAGKSKVIDA~ (SEQ ID NO:38)				Round 5
				1 (1.82%)
NONE	P34A	Round 5	Rounds 4,	Round 4

Insertion	Substitution	Pan-Retinal	RPE	Photo-receptor
		1 (1.89%)	5	(2.17%)
			3 (6.82%)	
			1 (2.27%)	
NONE	P64S	Round 3	Round 4	
		1 (1.85%)	1 (2.27%)	
NONE	S109T	Round 4	Rounds 3, 4, 5	
		2 (2.67%)		
			4 (8.33%)	
			1 (2.27%)	
			1 (2.27%)	
NONE	S109T+P8L		Round 3	
			1 (2.08%)	
NONE	S109T+Q120R		Round 3	
			1 (2.08%)	
NONE	S109T+A493V	Round 3		
	+A593E+V708I	1 (1.85%)		
NONE	Q164K	Rounds 4 and 5	Round 4	
			1 (2.27%)	
		2 (2.67%)		
		1 (1.89%)		
NONE	Q175H	Round 3		Round 4
		1 (1.85%)		1 (2.17%)
NONE	S196Y	Round 3	Round 4	
		1 (1.85%)	1 (2.27%)	
NONE	A593E	Rounds 3, 4, 5	Rounds 3, 4, 5	Round 4
				1 (2.17%)
		3 (5.56%)	12 (25%)	
		7 (9.33%)	7 (15.9%)	
		1 (1.89%)	14(31.8%)	
NONE	A593E+Q464R		Round 3	
			1 (2.08%)	
NONE	A593E+N596D		Round 4	
			1 (2.27%)	
NONE	A593E+N596D		Round 3	

Insertion	Substitution	Pan-Retinal	RPE	Photo-receptor
	+T491A		2 (4.17%)	
NONE	A593E+V708I	Rounds 3, 4	Rounds 3, 4, 5	
		2 (3.7%)		
		2 (2.67%)	4 (8.33%)	
			1 (2.27%)	
			1 (2.27%)	
NONE	1698V	Round 5	Round 5	
		1 (1.89%)	1 (2.27%)	
NONE	V708I	Rounds 3, 4,	Rounds 3, 4, 5	
		2 (3.7%)		
		5 (6.67%)	1 (2.08%)	
			4 (9.09%)	
			4 (9.09%)	
NONE	V708I+V719M		Round 4	
			2 (4.55%)	
NONE	V708I-G5727D		Round 5	
			1 (2.27%)	
NONE	V708I+R733C			Round 4
				1 (2.17%)

[0151] Also identified as a capsid having increased infectivity of one or more cells of the retina was a clone having the following ancestral VP1 capsid sequence:

MAADGYLPDWLEDNLSEGIREWDLKPGAPKPKANQQKQ
DDGRGLVLPGYKYLGPFNGLDKGEPVNAADAAALEHDKAYDQQLKAGDNP

YLRYNHADAEFQERLQEDTSFGGNLGRAVFQAKKRVLEPLGLVEEGAKTAP
GKKRPVEPSPQRSPDSSTGIGKKGQPAKKRLNFGQTGDSESVDPQPPLGEPP
AGPSGLGSGTMAAGGGAPMADNNEGADGVGNASGNWHCDSTWLGDRVITT
STRTWALPTYNNHLYKQISSASAGSTNDNHYFGYSTPWGYFDNRFHCHFSP
RDWQRLINNNWGFRPKRLNFKLFNIQVKEVTTNDGVTTIANNLTSTVQVPSD
SEYQLPYVLGSAHQGCLPPFPADVFMIPQYGYLTLNNGSQAVGRSSFYCLEYF
PSQMLRTGNNFTFSYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYLARTQST
GGTAGTRELLFSQAGPSNMSAQAKNWLPGPCYRQQRVSKTLSQNNNSNFAW
TGATKYHLNGRDSLVPNGVAMATHKDDedrFFPSSGVLIFGKQGAGANNTA

LENVMMITSEEEIKTINPVATEQYGVVASNLQSSNTAPVTGTVNSQGALPGM
 VWQNRDVYLQGPWAKIPHTDGNFHPSPMLGGFGLKHPPQILIKNTVPANP
 PAVFTPAKFASFITQYSTGQVSVEIEWELQKENS KRWNPEIQYTSNYAKSTNV
 DFAVDNEG VYSEPRPIGTRYLTRNL. (SEQ ID NO:59)

[0152] This ancestral capsid variant is evolved from the ancestral capsid SEQ ID NO:58, in which the positions of degeneracy (residues 264, 266, 268, 448, 459, 460, 467, 470, 471, 474, 495, 516, 533, 547, 551, 555, 557, 561, 563, 577, 583, 593, 596, 661, 662, 664, 665, 710, 717, 718, 719, 723) evolved to comprise Alanine (A) at 264, Alanine (A) at 266, Serine (S) at 268, Alanine (A) at 448, Threonine (T) at 459, Arginine (R) at 460, Alanine (A) at 467, Serine (S) at 470, Asparagine (N) at 471, Alanine (A) at 474, Serine (S) at 495, Asparagine (D) at 516, Asparagine (D) at 533, Glutamine (Q) at 547, Alanine (A) at 551, Alanine (A) at 555, Glutamic acid (E) at 557, Methionine (M) at 561, Serine (S) at 563, Glutamine (Q) at 577, Serine (S) at 583, Valine (V) at 593, Threonine (T) at 596, Alanine (A) at 661, Valine (V) at 662, Threonine (T) at 664, Proline (P) at 665, Threonine (T) at 710, Aspartic Acid (D) at 717, Asparagine (N) at 718, Glutamic acid (E) at 719, and Serine (S) at 723.

[0153] The AAV variant virions disclosed herein may incorporate reasonable rational design parameters, features, modifications, advantages, and variations that are readily apparent to those skilled in the art in the field of engineering AAV viral vectors.

Example 2

[0154] Directed evolution was employed to discover novel adeno-associated virus (AAV) variants with superior gene delivery to retinal cells following intravitreal (IVT) administration, a route of administration with significant advantages over other methods of gene delivery to the human eye (Example 1). The cell tropism following intravitreal administration of the novel AAV variant comprising a P34A substitution and the peptide LAISDQTKHA (SEQ ID NO:28) inserted at amino acid 588 (LAISDQTKHA+P34A) was assessed *in vivo* in non-human primates (NHP) as a representative example of the ability of ISDQTKH (SEQ ID NO:14)-containing AAV variants to transduce retinal cells.

[0155] Recombinant AAV virions comprising either an AAV2 capsid or the novel variant capsid LAISDQTKHA+P34A and a genome comprising a green fluorescent protein (GFP) transgene operably linked to a CMV promoter (AAV2.CMV.GFP and LAISDQTKHA+P34A.CMV.GFP, respectively) or a CAG promoter (AAV2.CAG.EGFP and LAISDQTKHA+P34A.CAG.EGFP, respectively) were manufactured using standard methods. African Green Monkeys (Figures 7, 8) or Cynomolgus macaques (Figure 9) were injected intravitreally with various doses of vector ranging from 4×10^{10} vg to 1×10^{12} vg per eye (see figure legends for details) and the transduction of retinal cells was assessed *in vivo* by fundus fluorescence imaging with a Heidelberg Spectralis™.

[0156] Intravitreal delivery of AAVs comprising the novel variant LAISDQTKHA+P34A resulted in broader and more robust transgene expression across the NHP retina than AAV2 (Figures 7-9). Images reveal that the novel AAV variant capsid provides for robust expression within the center of the fovea (an area rich in cones); in the parafoveal ring (an area rich in retinal ganglion cells), and in the periphery (an area rich in many types of cells including rods, Muller glia, amacrine cells, bipolar cells) as early as 2 weeks after injection. In contrast, and consistent with results reported by others, wild type AAV2 provides for weaker expression that is primarily in the parafoveal ring and can only be detected at later time points. Immunohistochemical analysis of various regions of the retina performed 3 weeks after injection confirmed that many types of retinal cells, including retinal pigment epithelial cells, rod and cone photoreceptors, and retinal ganglion cells, had been successfully transduced throughout the retina (Figures 10A-10E).

[0157] This study illustrates superior gene delivery by the ISDQTKH-comprising variant following a clinically preferred route of administration as compared to the clinically relevant AAV2. Similar efficacy is achievable with other variants comprising this peptide insertion motif. Likewise, similar efficacy is achievable with other variants disclosed herein that were identified using the same directed evolution approach.

Example 3

[0158] The cell tropism of the novel AAV variant LAISDQTKHA+P34A for retinal pigment epithelial (RPE) cells and photoreceptor (PR) cells was assessed in vitro in use RPE cells and PR cells generated from fibroblast-derived human induced pluripotent stem cells (FB-iPSC) or human embryonic stem cells (ESC).

[0159] AAV virions comprising either an AAV2 capsid or the novel variant capsid LAISDQTKHA+P34A and a genome comprising a green fluorescent protein (EGFP) transgene operably linked to a CAG promoter (AAV2.CAG.EGFP and LA1SDQTKHA+P34A.CAG.EGFP, respectively) were manufactured using standard methods. Human RPE cell cultures were generated from the human embryonic stem cell line ESI-017 or human fibroblast-derived induced pluripotent stem cells ("FB-iPSC") using a 45-day differentiation protocol. Maturation into RPE cells was confirmed by detecting the expression of mature RPE markers including RPE65 and BEST1; the synthesis of VEGF and PEDF; and the ability to phagocytose rod outer segments. PR cultures were generated by a multi-step eye cup formation paradigm and confirmed to comprise PRs by detecting the expression of Recoverin and S Opsin after 179 days in culture.

[0160] Relative to AAV2, LAISDQTKHA+P34A provided for significantly higher transduction efficiency of and transgene expression in human RPE cultures seven days post-infection as determined by immunofluorescence (Figures 11A-B), flow cytometry (2.7-fold increase; Figures 11C-D) and Western blot analysis (Figures 11E-F). Robust transduction and expression was

likewise achieved using LAISDQTKHA+P34A.CAG.EGFP in human PR cultures by 32 days post-infection. This study illustrates the superior ability of ISDQTKH (SEQ ID NO:14)-comprising variants to deliver genes to retinal cells.

[0161] The preceding merely illustrates the principles of the invention.

[0162] Furthermore, all examples and conditional language recited herein are principally intended to aid the reader in understanding the principles of the invention and the concepts contributed by the inventors to furthering the art, and are to be construed as being without limitation to such specifically recited examples and conditions.

[0163] The present invention is defined by the claims.

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P a t e n t k r a v

5 **1.** Variant adeno-associeret virus-(AAV)-capsidprotein, der omfatter et heterologt peptid med en længde på 7, 8, 9, 10 eller 11 aminosyrer, der er kovalent indsat i GH-sløjfen af capsidproteinet i forhold til et tilsvarende parentalt AAV-capsidprotein, hvor peptidindsættelsen omfatter aminosyresekvensen ISDQTTKH (SEQ ID NO:14).

10 **2.** Variant AAV-capsidprotein ifølge krav 1, hvor indsættelsespeptidet har fra 1 til 3 spacer-aminosyrer (Y₁-Y₃) ved amino- og/eller carboxylterminus af aminosyresekvensen ISDQTKH (SEQ ID NO: 14), fortrinsvis hvor indsættelsespeptidet er LAISDQTKHA (SEQ ID NO:28).

15 **3.** Variant AAV-capsidprotein ifølge krav 1 eller 2, hvor indsættelsesstedet er placeret mellem aminosyrer, der svarer til aminosyrer 587 og 588 af VP1 af AAV2 (SEQ ID NO:2) eller den tilsvarende position i capsidproteinet af en anden AAV serotype.

20 **4.** Variant AAV-capsidprotein ifølge et hvilket som helst af kravene 1 til 3, hvor capsidproteinet omfatter en eller flere aminosyresubstitution(er) i forhold til VP1 af AAV2 (SEQ ID NO:2) eller en eller flere tilsvarende substitution(er) i capsidproteinet af en anden AAV-serotype, fortrinsvis en eller flere af følgende aminosyresubstitutioner: MIL, L15P, P34A, N57D, N66K, R81Q, Q101R, S109T, R144K, R144M, Q164K, T176P, L188I, S196Y, G226E, G236V, I240T,
25 P250S, N312K, P363L, D368H, N449D, T456K, S463Y, D472N, R484C, A524T, P535S, N551S, A593E, 1698V, V708I, V719M, S721L og L735Q, mere fortrinsvis en P34A-aminosyresubstitution.

30 **5.** Variant AAV-capsidprotein ifølge krav 4, hvor capsidproteinet omfatter en P34A-aminosyresubstitution i forhold til VP1 af AAV2 og omfatter en aminosy-

resekvens, der er mindst 90% identisk, mindst 95% identisk, mindst 98% identisk eller 100% identisk med hele længden af den aminosyresekvens, der er anført som SEQ ID NO:42, fortrinsvis hvor capsidproteinet består af den aminosyresekvens, der er anført som SEQ ID NO:42.

5

6. Variant AAV-capsidprotein ifølge et hvilket som helst af kravene 1 til 5, hvor capsidproteinet bibringer en infektiøs rekombinant AAV-(rAAV)-virion en øget infektivitet, fortrinsvis mindst en 2 gange øget infektivitet, af en retinal celle sammenlignet med infektivitet af den retinale celle med et AAV-virion, der omfatter det tilsvarende parentale AAV-capsidprotein.

10

7. Isoleret nukleinsyre omfattende en nukleotidsekvens, der koder for et variant AAV-capsidprotein ifølge et hvilket som helst af kravene 1 til 6.

15

8. rAAV-virion omfattende et variant AAV-capsidprotein ifølge et hvilket som helst af kravene 1 til 6, fortrinsvis yderligere omfattende en heterolog nukleinsyre, fortrinsvis en heterolog nukleinsyre omfattende en nukleotidsekvens, der koder for et genprodukt, fortrinsvis hvor genproduktet er et protein eller interfererende RNA.

20

9. Farmaceutisk sammensætning omfattende et rAAV-virion ifølge krav 8 og en farmaceutisk acceptabel bærer, fortyndingsmiddel, excipients eller buffer.

10. rAAV-virion ifølge krav 8 eller en farmaceutisk sammensætning ifølge krav 9 til anvendelse som et lægemiddel.

25

11. rAAV-virion eller en farmaceutisk sammensætning til anvendelse ifølge krav 10 til anvendelse i en fremgangsmåde til levering af en heterolog nukleinsyre til en retinal celle, fortrinsvis til en retinal celle valgt fra gruppen bestående af retinale ganglieceller, amacrine celler, horisontale celler, bipolære cel-

30

ler, fotoreceptorceller, Müller-gliaceller, mikrogliaceller og retinalt pigmentepitel, fortrinsvis ved intravitreal injektion.

DRAWINGS

FIGURE 1

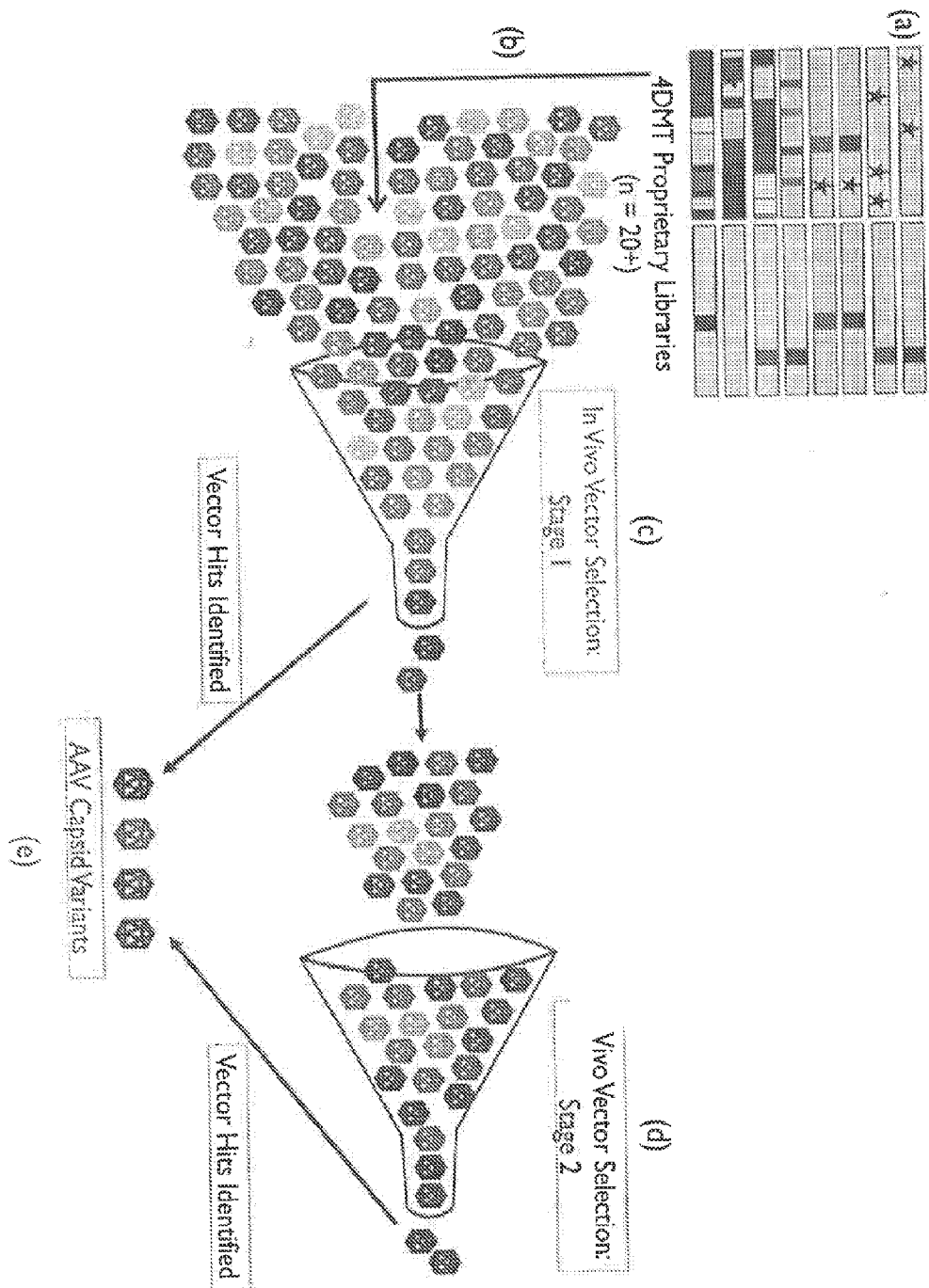


FIGURE 2

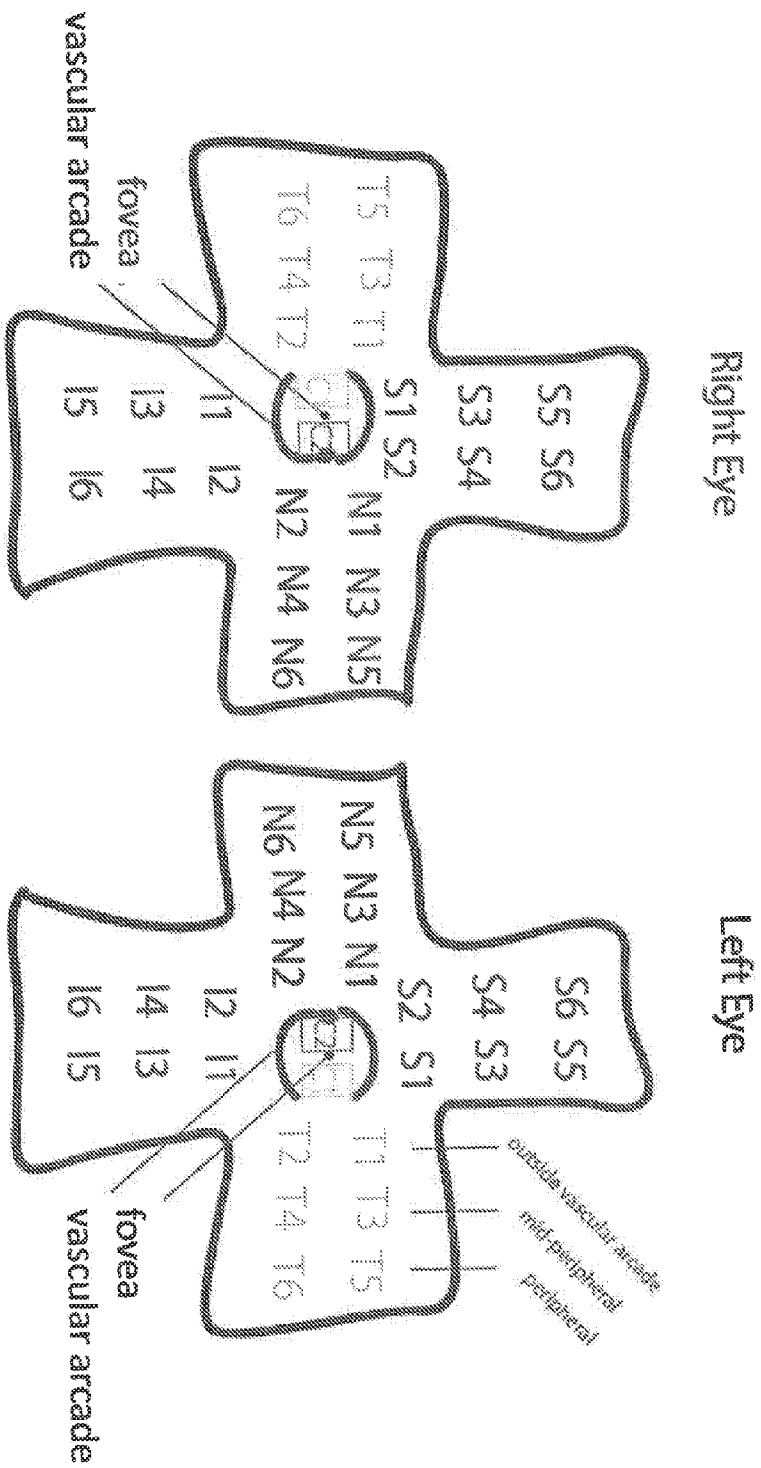


FIGURE 3

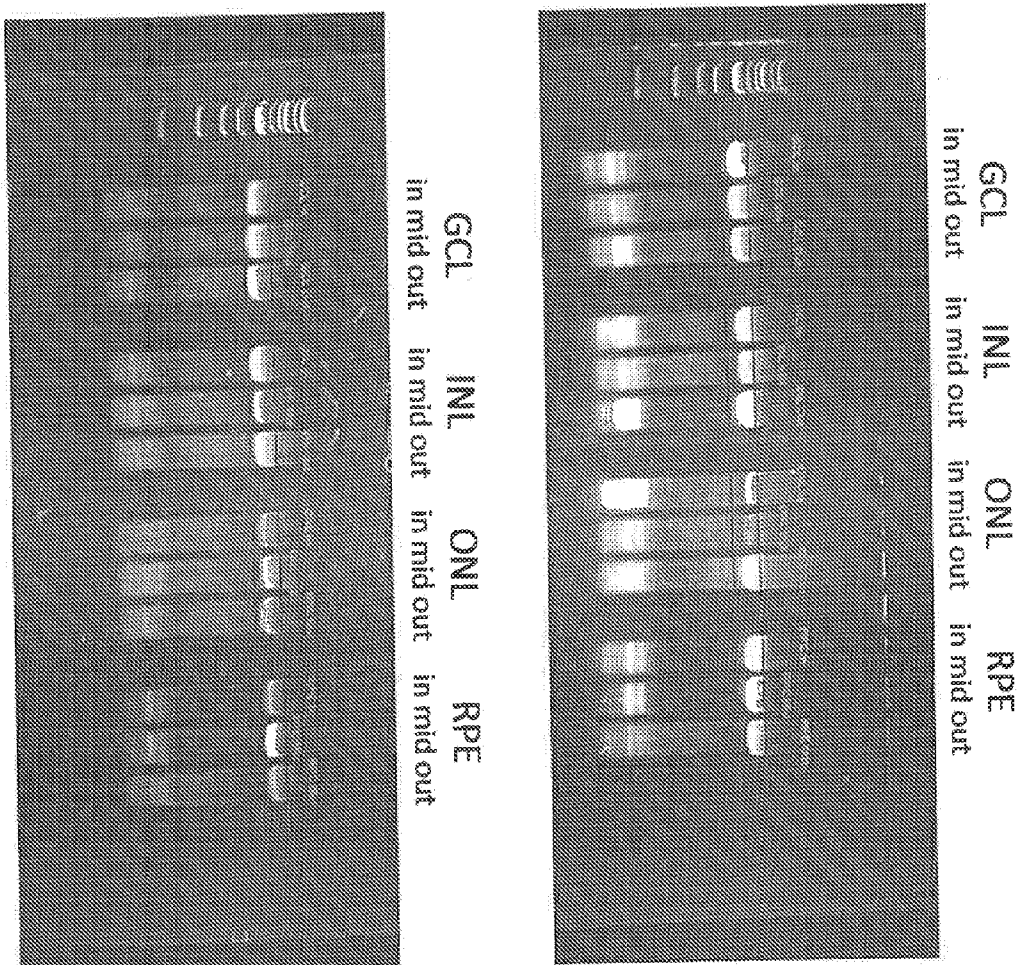


FIGURE 4

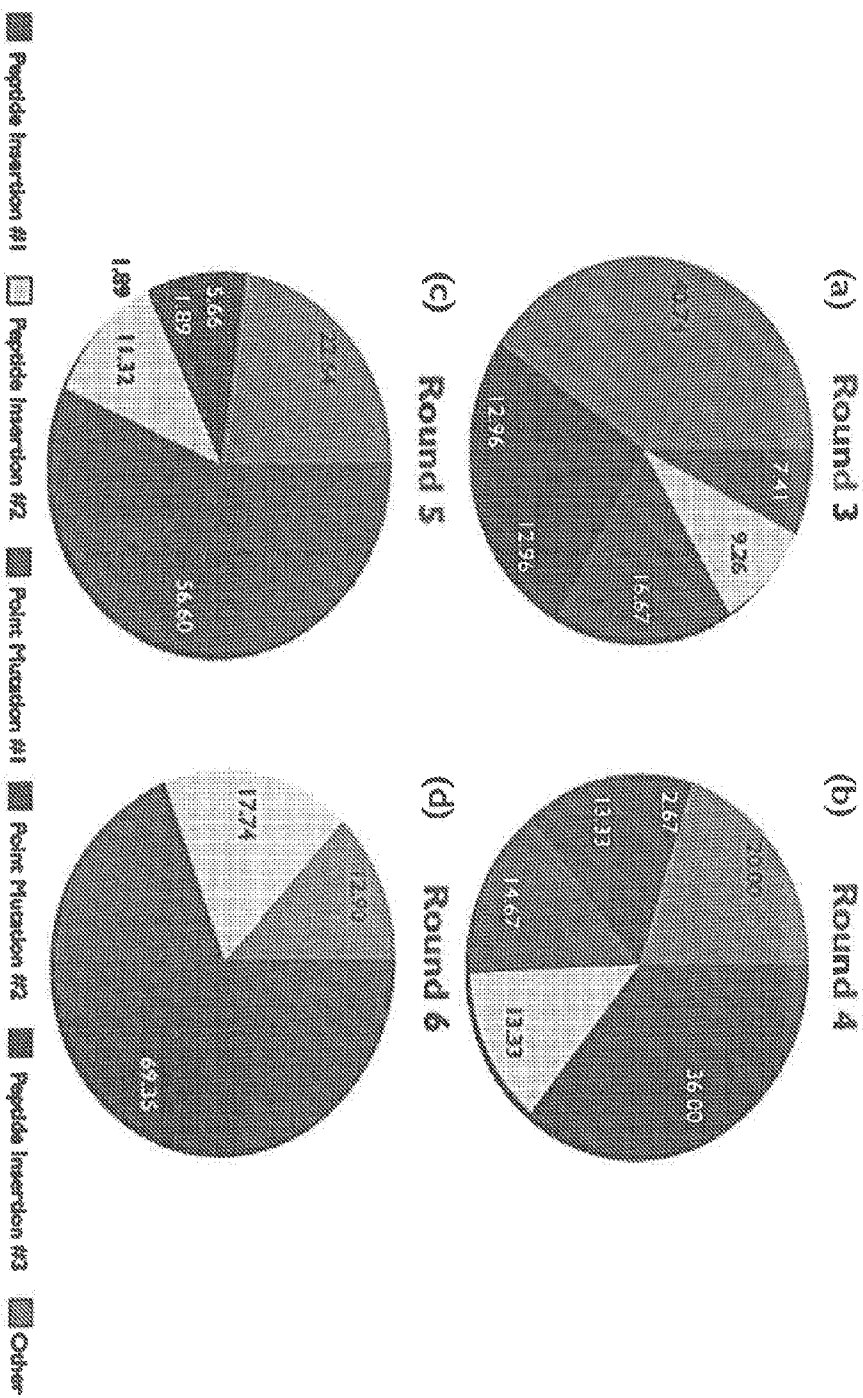


FIGURE 5

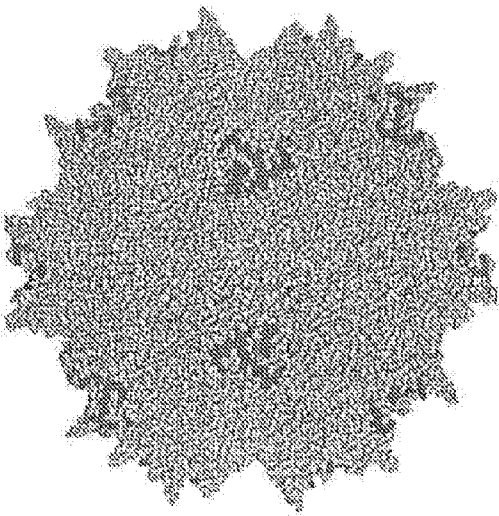


FIGURE 6

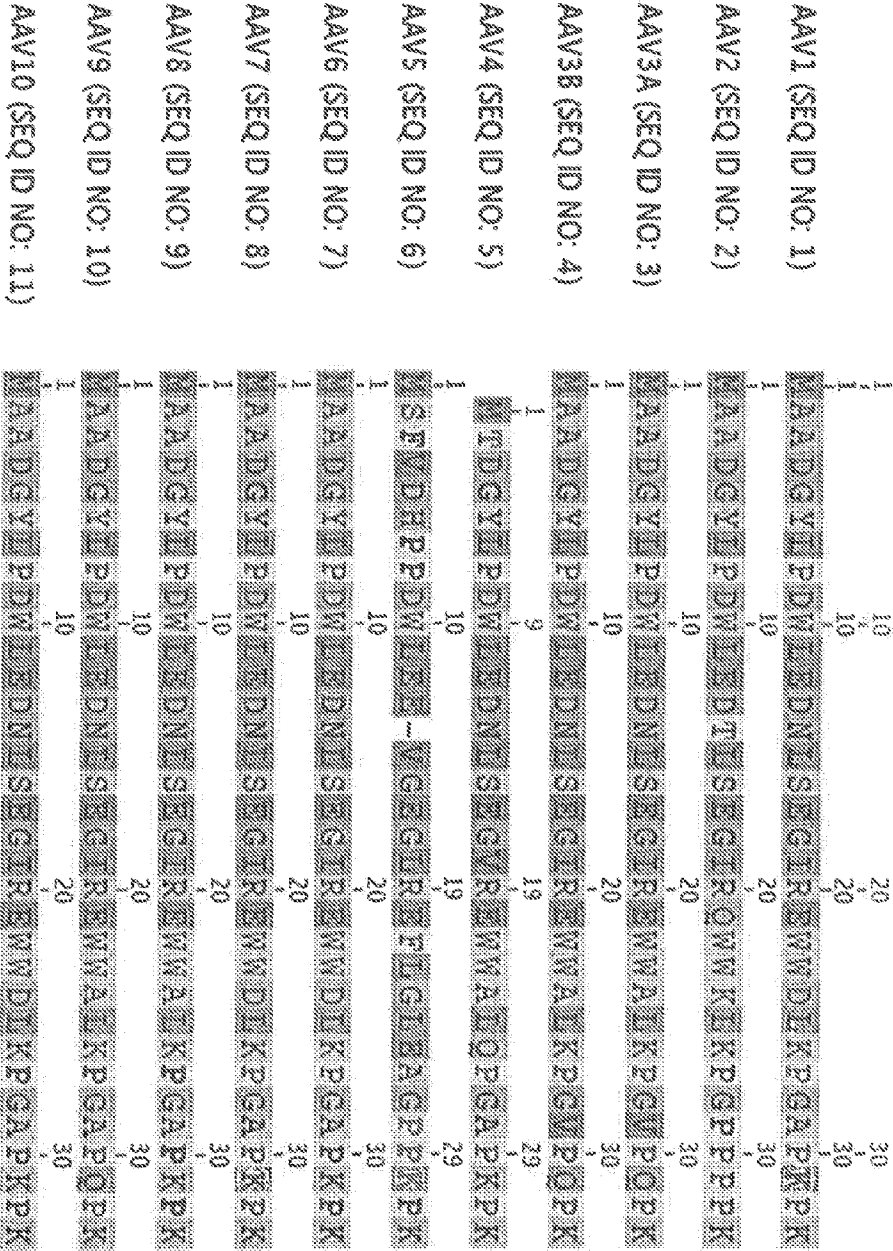


FIGURE 6 (cont'd)

AAV1 (SEQ ID NO: 1)	40	50	60
ANQOKQDDGRGLVTPGYKYLGPFGNGLDKGEPTN	40	50	60
AAV2 (SEQ ID NO: 2)	40	50	60
PARRRHDDSRGLVTPGYKYLGPFGNGLDKGEPTN	40	50	60
AAV3A (SEQ ID NO: 3)	40	50	60
ANQOHQDNRRLVTPGYKYLGPFGNGLDKGEPTN	40	50	60
AAV3B (SEQ ID NO: 4)	39	49	59
ANQOHQDNRRLVTPGYKYLGPFGNGLDKGEPTN	39	49	59
AAV4 (SEQ ID NO: 5)	39	49	59
ANQOHQDNRGLVTPGYKYLGPFGNGLDKGEPTN	39	49	59
AAV5 (SEQ ID NO: 6)	40	50	60
PNQOHQDQARGLVTPGYKYLGPFGNGLDKGEPTN	40	50	60
AAV6 (SEQ ID NO: 7)	40	50	60
ANQOKQDDGRGLVTPGYKYLGPFGNGLDKGEPTN	40	50	60
AAV7 (SEQ ID NO: 8)	40	50	60
ANQOKQDNRGLVTPGYKYLGPFGNGLDKGEPTN	40	50	60
AAV8 (SEQ ID NO: 9)	40	50	60
ANQOKQDDGRGLVTPGYKYLGPFGNGLDKGEPTN	40	50	60
AAV9 (SEQ ID NO: 10)	40	50	60
ANQOHQDNRGLVTPGYKYLGPFGNGLDKGEPTN	40	50	60
AAV10 (SEQ ID NO: 11)	40	50	60
ANQOKQDDGRGLVTPGYKYLGPFGNGLDKGEPTN	40	50	60

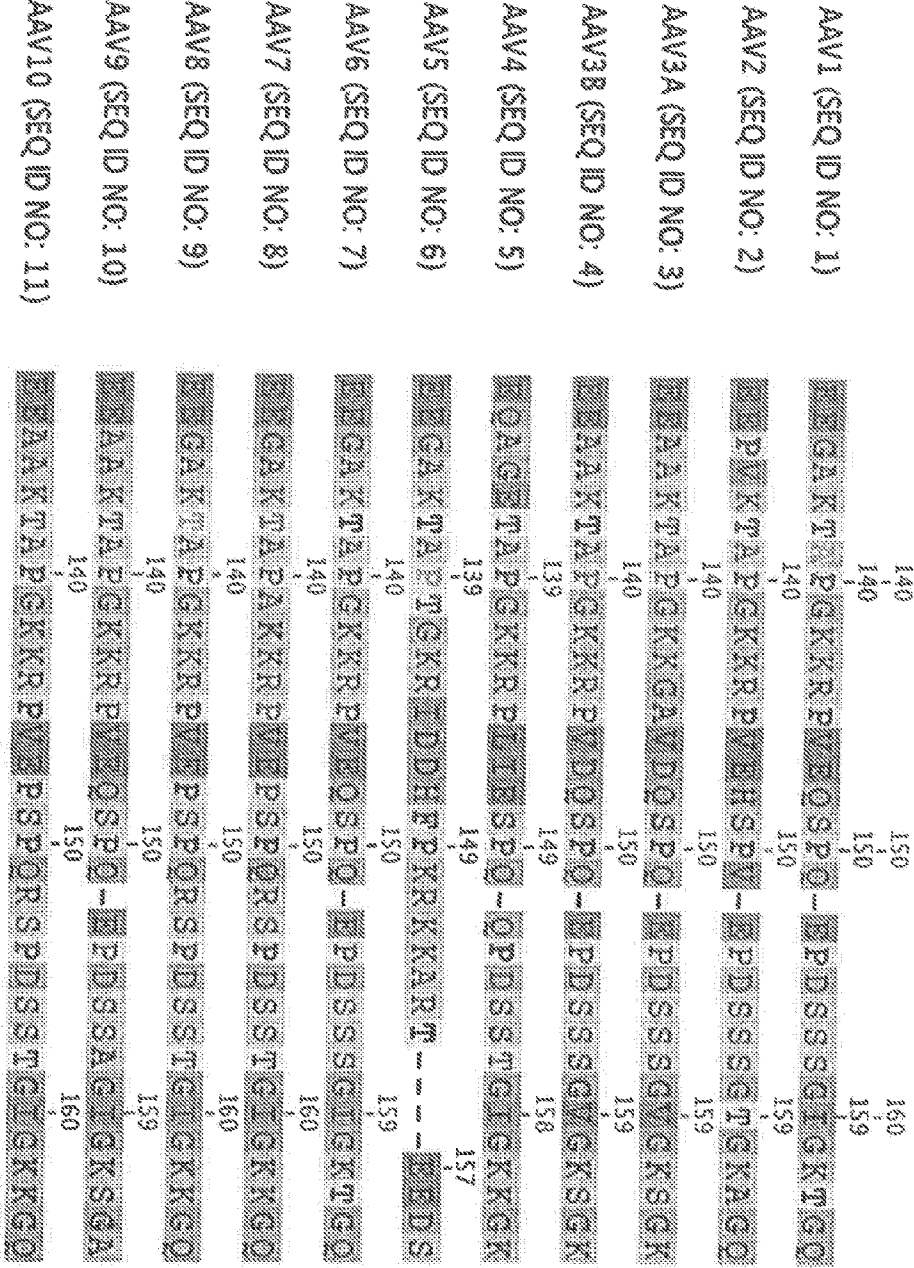
FIGURE 6 (cont'd)

AAV1 (SEQ ID NO: 1)	AAADAAALTEHDKAYDQQLKAGDNPYLRYNHADA 70 80 90
AAV2 (SEQ ID NO: 2)	AAADAAALTEHDKAYDRQLDGGDNPYLRYNHADA 70 80 90
AAV3A (SEQ ID NO: 3)	AAADAAALTEHDKAYDQQLKAGDNPYLRYNHADA 70 80 90
AAV3B (SEQ ID NO: 4)	AAADAAALTEHDKAYDQQLKAGDNPYLRYNHADA 69 79 89
AAV4 (SEQ ID NO: 5)	AAADAAALTEHDKAYDQQLKAGDNPYLRYNHADA 69 79 89
AAV5 (SEQ ID NO: 6)	RADEVAREHDSYNQQLKAGDNPYLRYNHADA 70 80 90
AAV6 (SEQ ID NO: 7)	AAADAAALTEHDKAYDQQLKAGDNPYLRYNHADA 70 80 90
AAV7 (SEQ ID NO: 8)	AAADAAALTEHDKAYDQQLKAGDNPYLRYNHADA 70 80 90
AAV8 (SEQ ID NO: 9)	AAADAAALTEHDKAYDQQLKAGDNPYLRYNHADA 70 80 90
AAV9 (SEQ ID NO: 10)	AAADAAALTEHDKAYDQQLKAGDNPYLRYNHADA 70 80 90
AAV10 (SEQ ID NO: 11)	AAADAAALTEHDKAYDQQLKAGDNPYLRYNHADA 70 80 90

FIGURE 6 (cont'd)

AAV1 (SEQ ID NO: 1)	100	110	120	130
FOERLEQEDTSFGGNLGRA	110	120	130	
FQAKKRVLLEPIGLN				
AAV2 (SEQ ID NO: 2)	100	110	120	130
FOERLEQEDTSFGGNLGRA	110	120	130	
FQAKKRVLLEPIGLN				
AAV3A (SEQ ID NO: 3)	100	110	120	130
FOERLEQEDTSFGGNLGRA	110	120	130	
FQAKKRVLLEPIGLN				
AAV3B (SEQ ID NO: 4)	99	109	119	129
FOERLEQEDTSFGGNLGRA	109	119	129	
FQAKKRVLLEPIGLN				
AAV4 (SEQ ID NO: 5)	99	109	119	129
FOERLEQEDTSFGGNLGRA	109	119	129	
FQAKKRVLLEPIGLN				
AAV5 (SEQ ID NO: 6)	100	110	120	130
FOERLEQEDTSFGGNLGRA	110	120	130	
FQAKKRVLLEPIGLN				
AAV6 (SEQ ID NO: 7)	100	110	120	130
FOERLEQEDTSFGGNLGRA	110	120	130	
FQAKKRVLLEPIGLN				
AAV7 (SEQ ID NO: 8)	100	110	120	130
FOERLEQEDTSFGGNLGRA	110	120	130	
FQAKKRVLLEPIGLN				
AAV8 (SEQ ID NO: 9)	100	110	120	130
FOERLEQEDTSFGGNLGRA	110	120	130	
FQAKKRVLLEPIGLN				
AAV9 (SEQ ID NO: 10)	100	110	120	130
FOERLEQEDTSFGGNLGRA	110	120	130	
FQAKKRVLLEPIGLN				
AAV10 (SEQ ID NO: 11)	100	110	120	130
FOERLEQEDTSFGGNLGRA	110	120	130	
FQAKKRVLLEPIGLN				

FIGURE 6 (cont'd)



AAV1 (SEQ ID NO: 1)

170 180 190
169 179 189
QPAKKRLNFGQTGDSSTPDPQPLGGPPATP--

AAV2 (SEQ ID NO: 2)

169 179 189
QPAKKRLNFGQTGDA DSTPDPQPLGGPPAAP-S

AAV3A (SEQ ID NO: 3)

169 179 189
QPAKKRLNFGQTGDSSTPDPQPLGGPPAAP-T

AAV3B (SEQ ID NO: 4)

168 175 185
QPAKKRLNFGQTGDSSTPDPQPLGGPPAAP-T

AAV4 (SEQ ID NO: 5)

165 168 178
QPAKKRLNFGQTGDSSTPDPQPLGGPPAAP-T

AAV5 (SEQ ID NO: 6)

169 179 189
KPSI-----SSDAEAGPSSGSOOLQPPAQPAS

AAV6 (SEQ ID NO: 7)

170 180 190
QPAKKRLNFGQTGDSSTPDPQPLGGPPATP-A

AAV7 (SEQ ID NO: 8)

170 180 190
QPAKKRLNFGQTGDSSTPDPQPLGGPPAAP-S

AAV8 (SEQ ID NO: 9)

169 179 189
QPAKKRLNFGQTGDSSTPDPQPLGGPPAAP-S

AAV9 (SEQ ID NO: 10)

170 180 190
QPAKKRLNFGQTGDSSTPDPQPLGGPPAAP-S

AAV10 (SEQ ID NO: 11)

170 180 190
QPAKKRLNFGQTGDSSTPDPQPLGGPPAAP-S

FIGURE 6 (cont'd)

AAV1 (SEQ ID NO: 1)	200	210	220	230
	198	208	218	228
	A V G P T T M A S G G C A P M A D N N E G A D G V G N A S G N W H			
AAV2 (SEQ ID NO: 2)	198	208	218	228
	G T G T N T M A T G S G A P M A D N N E G A D G V G N S S G N W H			
AAV3A (SEQ ID NO: 3)	198	208	218	228
	S I G S N T M A S G G G A P M A D N N E G A D G V G N S S G N W H			
AAV3B (SEQ ID NO: 4)	193	203	212	222
	S I G S N T M A S G G G A P M A D N N E G A D G V G N S S G N W H			
AAV4 (SEQ ID NO: 5)	188	198	208	218
	S D D S E R R A A A G G A A W - E G G Q G A D G V G N A S G D W H			
AAV5 (SEQ ID NO: 6)	198	208	218	228
	S I G A D T M S A G G G P L G D N N O G A D G V G N A S G D W H			
AAV6 (SEQ ID NO: 7)	199	209	219	229
	A I G P T T M A S G G G A P M A D N N E G A D G V G N A S G N W H			
AAV7 (SEQ ID NO: 8)	193	203	219	229
	S I G S G T M A A G G G A P M A D N N E G A D G V G N A S G N W H			
AAV8 (SEQ ID NO: 9)	198	208	218	228
	G I G P N T M A A G G G A P M A D N N E G A D G V G S S S G N W H			
AAV9 (SEQ ID NO: 10)	199	209	219	229
	G I G S I T M A S G G G A P M A D N N E G A D G V G S S S G N W H			
AAV10 (SEQ ID NO: 11)	199	209	219	229
	G I G S G T M A A G G G A P M A D N N E G A D G V G S S S G N W H			

FIGURE 6 (cont'd)

AAV1 (SEQ ID NO: 1)	CDSTWLGDRVITTSRTWAIPPTYNNHHYKQISS	238	248	258
		238	248	258
AAV2 (SEQ ID NO: 2)	CDSTWLGDRVITTSRTWAIPPTYNNHHYKQISS	238	248	258
		238	248	258
AAV3A (SEQ ID NO: 3)	CDSQWLGDRVITTSRTWAIPPTYNNHHYKQISS	238	248	258
		238	248	258
AAV3B (SEQ ID NO: 4)	CDSQWLGDRVITTSRTWAIPPTYNNHHYKQISS	232	242	252
		232	242	252
AAV4 (SEQ ID NO: 5)	CDSTWSEGHVTTTSRTWAIPPTYNNHHYKRGGE	228	238	248
		228	238	248
AAV5 (SEQ ID NO: 6)	CDSTWLGDRVITKSTRTWAPPTYNNHQRRTKS	238	248	258
		238	248	258
AAV6 (SEQ ID NO: 7)	CDSTWLGDRVITTSSTRTWAPPTYNNHHYKQISS	239	249	259
		239	249	259
AAV7 (SEQ ID NO: 8)	CDSTWLGDRVITTSSTRTWAPPTYNNHHYKQISS	239	249	259
		239	249	259
AAV8 (SEQ ID NO: 9)	CDSTWLGDRVITTSSTRTWAPPTYNNHHYKQISS	238	248	258
		238	248	258
AAV9 (SEQ ID NO: 10)	CDSQWLGDRVITTSSTRTWAPPTYNNHHYKQISS	239	249	259
		239	249	259
AAV10 (SEQ ID NO: 11)	CDSTWLGDRVITTSSTRTWAPPTYNNHHYKQISS	239	249	259
		239	249	259

FIGURE 6 (cont'd)

AAV1 (SEQ ID NO. 1)	A--STGASNDNH YFGYSTPWGYFDNRFFHCHFS P	270 267	280 277	290 287
AAV2 (SEQ ID NO. 2)	Q--SGASNDNH YFGYSTPWGYFDNRFFHCHFS P	266	276	286
AAV3A (SEQ ID NO. 3)	Q--SGASNDNH YFGYSTPWGYFDNRFFHCHFS P	266	276	286
AAV3B (SEQ ID NO. 4)	Q--SGASNDNH YFGYSTPWGYFDNRFFHCHFS P	266	276	286
AAV4 (SEQ ID NO. 5)	---SLOSNTYNGFS TPWG YFDNRFFHCHFS P	257 258	267 267	277 277
AAV5 (SEQ ID NO. 6)	GSNDG--SNANAYFGYSTPWGYFDNRFFHCHFS P	267	277	287
AAV6 (SEQ ID NO. 7)	A--STGASNDNH YFGYSTPWGYFDNRFFHCHFS P	268	278	288
AAV7 (SEQ ID NO. 8)	---TAGSTNDNT YFGYSTPWGYFDNRFFHCHFS P	269	279	289
AAV8 (SEQ ID NO. 9)	GTSGGATNDNT YFGYSTPWGYFDNRFFHCHFS P	268	278	288
AAV9 (SEQ ID NO. 10)	STSGGSSNDNAYFGYSTPWGYFDNRFFHCHFS P	269	279	289
AAV10 (SEQ ID NO. 11)	GTSGGSTNDNT YFGYSTPWGYFDNRFFHCHFS P			

FIGURE 6 (cont'd)

AAV1 (SEQ ID NO: 1)	300 297	310 307	320 317	330 327
AAV2 (SEQ ID NO: 2)	296	306	316	326
AAV3A (SEQ ID NO: 3)	296	306	316	326
AAV3B (SEQ ID NO: 4)	287	297	307	317
AAV4 (SEQ ID NO: 5)	287	297	307	317
AAV5 (SEQ ID NO: 6)	297	307	317	327
AAV6 (SEQ ID NO: 7)	298	308	318	328
AAV7 (SEQ ID NO: 8)	299	309	319	329
AAV8 (SEQ ID NO: 9)	298	308	318	328
AAV9 (SEQ ID NO: 10)	299	309	319	329
AAV10 (SEQ ID NO: 11)	299	309	319	329

FIGURE 6 (cont'd)

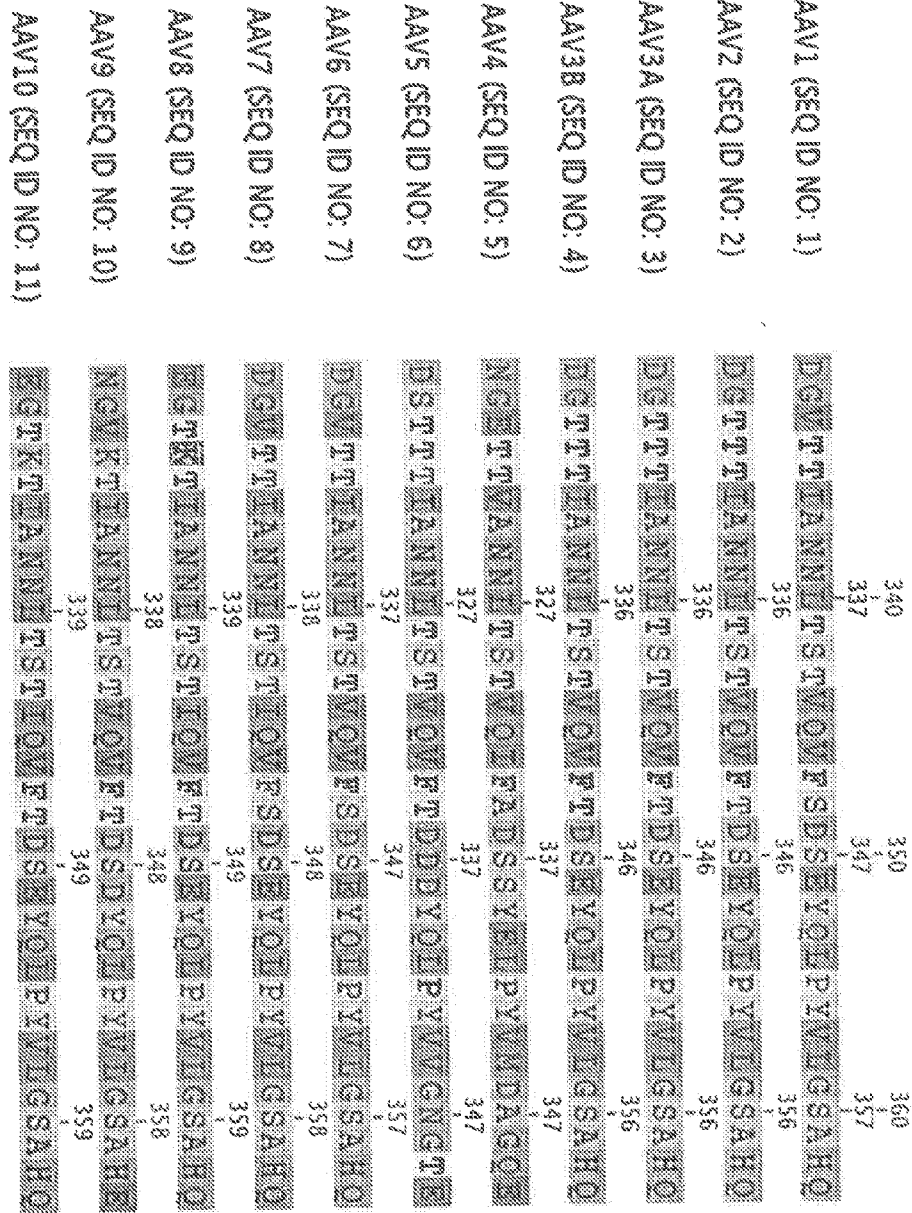


FIGURE 6 (cont'd)

AAV1 (SEQ ID NO: 1)	370380390 367377385 GCTPPFPADDTFTTPOYGXYTTNN--NGS--QAMGR
AAV2 (SEQ ID NO: 2)	366376384 GCTPPFPADDTFTTPOYGXYTTNN--NGS--QAMGR
AAV3A (SEQ ID NO: 3)	366376384 GCTPPFPADDTFTTPOYGXYTTNN--NGS--QAMGR
AAV3B (SEQ ID NO: 4)	357367377 GCTPPFPADDTFTTPOYGXYTTNN--NGS--QAMGR
AAV4 (SEQ ID NO: 5)	357367377 GCTPPFPADDTFTTPOYGXYTTNN--NGS--QAMGR
AAV5 (SEQ ID NO: 6)	367377385 GCTPPFPADDTFTTPOYGXYTTNN--NGS--QAMGR
AAV6 (SEQ ID NO: 7)	368378386 GCTPPFPADDTFTTPOYGXYTTNN--NGS--QAMGR
AAV7 (SEQ ID NO: 8)	369379387 GCTPPFPADDTFTTPOYGXYTTNN--NGS--QAMGR
AAV8 (SEQ ID NO: 9)	368378386 GCTPPFPADDTFTTPOYGXYTTNN--NGS--QAMGR
AAV9 (SEQ ID NO: 10)	369379387 GCTPPFPADDTFTTPOYGXYTTNN--NGS--QAMGR
AAV10 (SEQ ID NO: 11)	369379387 GCTPPFPADDTFTTPOYGXYTTNN--NGS--QAMGR

FIGURE 6 (cont'd)

AAV1 (SEQ ID NO: 1)	SSFYCL ³⁹³ EEYFPSSOM ⁴⁰³ IR ⁴¹⁰ TG ⁴¹⁴ NN ⁴²⁰ FT ⁴¹⁴ FSY ⁴¹⁴ T ⁴¹⁴ FE ⁴¹⁴ ED ⁴¹⁴ PP ⁴¹⁴ HS
AAV2 (SEQ ID NO: 2)	SSFYCL ³⁹³ EEYFPSSOM ⁴⁰³ IR ⁴⁰³ TG ⁴⁰³ NN ⁴¹³ FT ⁴¹³ FSY ⁴¹³ T ⁴¹³ FE ⁴¹³ ED ⁴¹³ PP ⁴¹³ HS
AAV3A (SEQ ID NO: 3)	SSFYCL ³⁹³ EEYFPSSOM ⁴⁰³ IR ⁴⁰³ TG ⁴⁰³ NN ⁴¹³ FO ⁴¹³ FSY ⁴¹³ T ⁴¹³ FE ⁴¹³ ED ⁴¹³ PP ⁴¹³ HS
AAV3B (SEQ ID NO: 4)	SSFYCL ³⁸⁷ EEYFPSSOM ³⁹⁷ IR ⁴⁰⁷ TG ⁴⁰⁷ NN ⁴⁰⁷ FO ⁴⁰⁷ FSY ⁴⁰⁷ T ⁴⁰⁷ FE ⁴⁰⁷ ED ⁴⁰⁷ PP ⁴⁰⁷ HS
AAV4 (SEQ ID NO: 5)	NAHYCL ³⁸⁶ EEYFPSSOM ³⁹⁶ IR ⁴⁰⁶ TG ⁴⁰⁶ NN ⁴⁰⁶ FT ⁴⁰⁶ SY ⁴⁰⁶ ST ⁴⁰⁶ FE ⁴⁰⁶ ED ⁴⁰⁶ PP ⁴⁰⁶ HS
AAV5 (SEQ ID NO: 6)	SSFYCL ³⁹⁴ EEYFPSSOM ⁴⁰⁴ IR ⁴¹⁴ TG ⁴¹⁴ NN ⁴¹⁴ FT ⁴¹⁴ SY ⁴¹⁴ ST ⁴¹⁴ FE ⁴¹⁴ ED ⁴¹⁴ PP ⁴¹⁴ HS
AAV6 (SEQ ID NO: 7)	SSFYCL ³⁹⁵ EEYFPSSOM ⁴⁰⁵ IR ⁴¹⁵ TG ⁴¹⁵ NN ⁴¹⁵ FT ⁴¹⁵ SY ⁴¹⁵ ST ⁴¹⁵ FE ⁴¹⁵ ED ⁴¹⁵ PP ⁴¹⁵ HS
AAV7 (SEQ ID NO: 8)	SSFYCL ³⁹⁶ EEYFPSSOM ⁴⁰⁶ IR ⁴¹⁶ TG ⁴¹⁶ NN ⁴¹⁶ FT ⁴¹⁶ SY ⁴¹⁶ ST ⁴¹⁶ FE ⁴¹⁶ ED ⁴¹⁶ PP ⁴¹⁶ HS
AAV8 (SEQ ID NO: 9)	SSFYCL ³⁹⁵ EEYFPSSOM ⁴⁰⁵ IR ⁴¹⁵ TG ⁴¹⁵ NN ⁴¹⁵ FO ⁴¹⁵ SY ⁴¹⁵ ST ⁴¹⁵ FE ⁴¹⁵ ED ⁴¹⁵ PP ⁴¹⁵ HS
AAV9 (SEQ ID NO: 10)	SSFYCL ³⁹⁶ EEYFPSSOM ⁴⁰⁶ IR ⁴¹⁶ TG ⁴¹⁶ NN ⁴¹⁶ FO ⁴¹⁶ SY ⁴¹⁶ ST ⁴¹⁶ FE ⁴¹⁶ ED ⁴¹⁶ PP ⁴¹⁶ HS
AAV10 (SEQ ID NO: 11)	SSFYCL ³⁹⁶ EEYFPSSOM ⁴⁰⁶ IR ⁴¹⁶ TG ⁴¹⁶ NN ⁴¹⁶ FT ⁴¹⁶ SY ⁴¹⁶ ST ⁴¹⁶ FE ⁴¹⁶ ED ⁴¹⁶ PP ⁴¹⁶ HS

FIGURE 6 (cont'd)

AAV1 (SEQ ID NO: 1)	430 431 432 433 434 435 436 437 438 439 440 441 442 443 444 445 446 447 448 449 450 451 452 453 454 455 456 457 458 459 460	SYAHSQSIDRINNPITDQYLYYINRTQNG--SGS
AAV2 (SEQ ID NO: 2)	423 424 425 426 427 428 429 430 431 432 433 434 435 436 437 438 439 440 441 442 443 444 445 446 447 448 449 450 451 452 453 454 455 456 457 458 459 460	SYAHSQSIDRINNPITDQYLYYINRT--NTPSGT
AAV3A (SEQ ID NO: 3)	423 424 425 426 427 428 429 430 431 432 433 434 435 436 437 438 439 440 441 442 443 444 445 446 447 448 449 450 451 452 453 454 455 456 457 458 459 460	SYAHSQSIDRINNPITDQYLYYINRTQGTSGT
AAV3B (SEQ ID NO: 4)	417 418 419 420 421 422 423 424 425 426 427 428 429 430 431 432 433 434 435 436 437 438 439 440 441 442 443 444 445 446 447 448 449 450 451 452 453 454 455 456 457 458 459 460	SYAHSQSIDRINNPITDQYLYYINRTQGTSGT
AAV4 (SEQ ID NO: 5)	416 417 418 419 420 421 422 423 424 425 426 427 428 429 430 431 432 433 434 435 436 437 438 439 440 441 442 443 444 445 446 447 448 449 450 451 452 453 454 455 456 457 458 459 460	SYAHSQSIDRINNPITDQYLYYINRTQGTSGT
AAV5 (SEQ ID NO: 6)	424 425 426 427 428 429 430 431 432 433 434 435 436 437 438 439 440 441 442 443 444 445 446 447 448 449 450 451 452 453 454 455 456 457 458 459 460	SYAHSQSIDRINNPITDQYLYYINRTQNG--SGS
AAV6 (SEQ ID NO: 7)	425 426 427 428 429 430 431 432 433 434 435 436 437 438 439 440 441 442 443 444 445 446 447 448 449 450 451 452 453 454 455 456 457 458 459 460	SYAHSQSIDRINNPITDQYLYYINRTQNG--SGS
AAV7 (SEQ ID NO: 8)	426 427 428 429 430 431 432 433 434 435 436 437 438 439 440 441 442 443 444 445 446 447 448 449 450 451 452 453 454 455 456 457 458 459 460	SYAHSQSIDRINNPITDQYLYYINRTQGT--GGT
AAV8 (SEQ ID NO: 9)	425 426 427 428 429 430 431 432 433 434 435 436 437 438 439 440 441 442 443 444 445 446 447 448 449 450 451 452 453 454 455 456 457 458 459 460	SYAHSQSIDRINNPITDQYLYYINRTQGT--GGT
AAV9 (SEQ ID NO: 10)	426 427 428 429 430 431 432 433 434 435 436 437 438 439 440 441 442 443 444 445 446 447 448 449 450 451 452 453 454 455 456 457 458 459 460	SYAHSQSIDRINNPITDQYLYYINRTQGT--GGT
AAV10 (SEQ ID NO: 11)	426 427 428 429 430 431 432 433 434 435 436 437 438 439 440 441 442 443 444 445 446 447 448 449 450 451 452 453 454 455 456 457 458 459 460	SYAHSQSIDRINNPITDQYLYYINRTQGT--GGT

FIGURE 6 (cont'd)

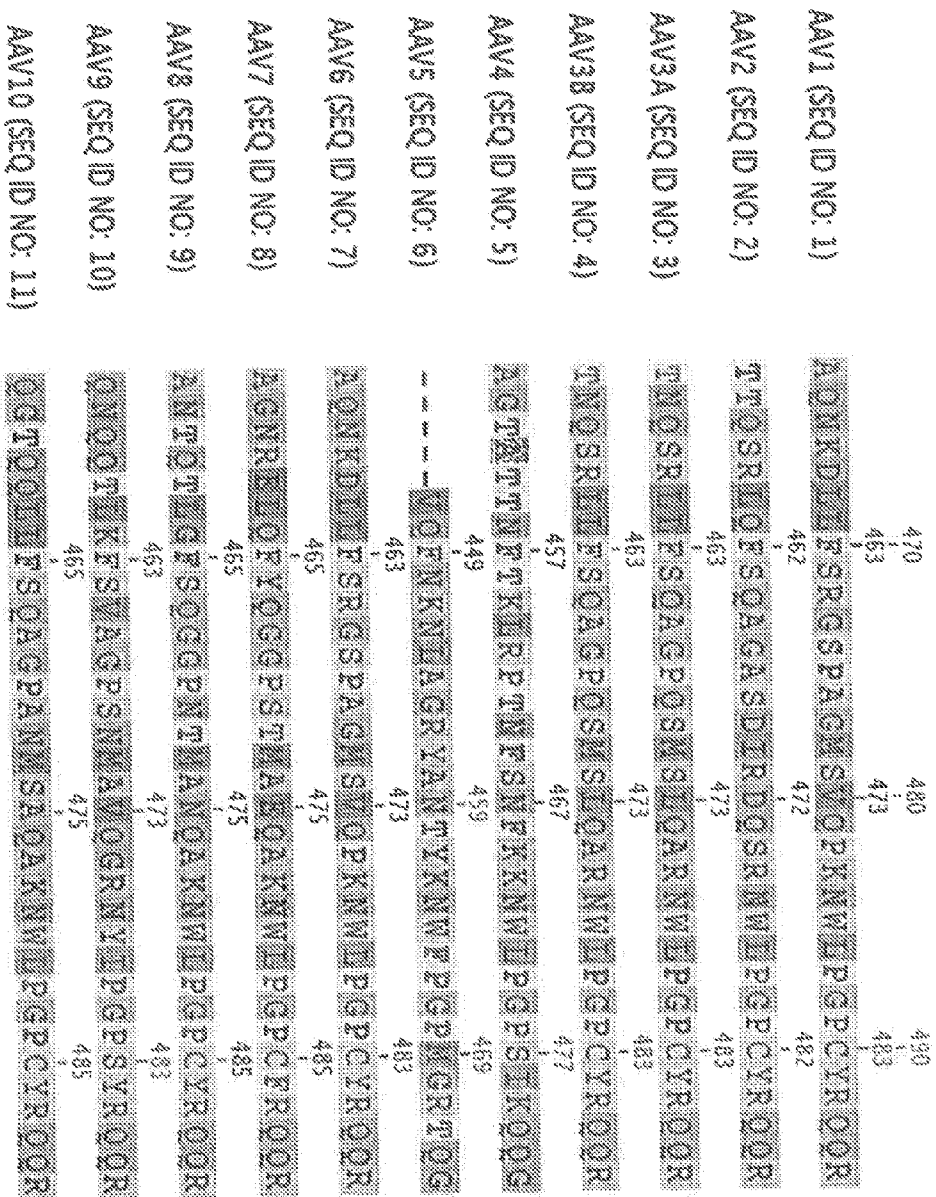


FIGURE 6 (cont'd)

AAV1 (SEQ ID NO: 1)	500 493 492 S K T S A D N N N S N F T W T G A S K Y N H N G R D S	510 498 497 N N S N F T W T G A S K Y N H N G R D S	520 508 507 N N S N F A W T G A T K Y H H N G R D S
AAV2 (SEQ ID NO: 2)	493 S K T S A D N N N S N F T W T G A S K Y N H N G R D S	498 N N S N F T W T G A S K Y N H N G R D S	508 N N S N F A W T G A T K Y H H N G R D S
AAV3A (SEQ ID NO: 3)	493 S K T A M D N N N S N F P W T A A S K Y H H N G R D S	498 N N S N F P W T A A S K Y H H N G R D S	508 N N S N F A W T G A T K Y H H N G R D S
AAV3B (SEQ ID NO: 4)	487 S K T A M D N N N S N F P W T A A S K Y H H N G R D S	497 N N S N F P W T A A S K Y H H N G R D S	507 N N S N F A W T G A T K Y H H N G R D S
AAV4 (SEQ ID NO: 5)	479 F S K T A M O N Y K P A T G S D S L K Y T H S T L D G R W S	484 A S M S A F A T T N R H E L G A S Y	494 T H S T L D G R W S
AAV5 (SEQ ID NO: 6)	493 N N S N G G N R A S M S A F A T T N R H E L G A S Y	498 A S M S A F A T T N R H E L G A S Y	508 T H S T L D G R W S
AAV6 (SEQ ID NO: 7)	495 S K T T T D N N N S N F T W T G A S K Y N H N G R D S	500 N N S N F T W T G A S K Y N H N G R D S	510 N N S N F A W T G A T K Y H H N G R D S
AAV7 (SEQ ID NO: 8)	495 S K T T T D N N N S N F A W T G A T K Y H H N G R D S	500 N N S N F A W T G A T K Y H H N G R D S	510 N N S N F A W T G A T K Y H H N G R D S
AAV8 (SEQ ID NO: 9)	493 S T T T G C N N N S N F A W T A G T K Y H H N G R D S	498 N N S N F A W T A G T K Y H H N G R D S	508 N N S N F A W T A G T K Y H H N G R D S
AAV9 (SEQ ID NO: 10)	495 S T T T T O N N N S N F A N P G A S S W A L N G R D S	500 N N S N F A N P G A S S W A L N G R D S	510 N N S N F A N P G A S S W A L N G R D S
AAV10 (SEQ ID NO: 11)	S T T T S O N N N S N F A N T G A T K Y H H N G R D S		

FIGURE 6 (cont'd)

AAV1 (SEQ ID NO: 1)	530	540	550	560
518	528	538	547	
NP	GTAA	ASHKDDDEK	FP	SGNITF - GKSSA
517	527	537	546	
NP	GPAA	ASHKDDDEK	FP	SGNITF - GKQGS
518	528	538	547	
NP	GPAA	ASHKDDDEK	FP	HGNITF - GKSGT
518	528	538	547	
NP	GPAA	ASHKDDDEK	FP	HGNITF - GKSGT
517	527	535	545	
NP	GPAA	ASHKDDDEK	FP	HGNITF - GKSGT
504	514	524	534	
NP	GPAA	TA	GPADSKE -	SN
518	528	538	547	
NP	GPAA	ASHKDDDEK	FP	SGNITF - GKSSA
520	530	540	549	
NP	GPAA	ASHKDDDEK	FP	SGNITF - GKSSA
520	530	540	549	
NP	GPAA	ASHKDDDEK	FP	SGNITF - GKSSA
518	528	538	547	
NP	GPAA	ASHKDDDEK	FP	SGNITF - GKSSA
520	530	540	549	
NP	GPAA	ASHKDDDEK	FP	SGNITF - GKSSA
520	530	540	549	
NP	GPAA	ASHKDDDEK	FP	SGNITF - GKSSA

FIGURE 6 (cont'd)

AAV1 (SEQ ID NO: 1)	QASNTAL	570	580	590
	DNVMTIDEEELKATNPVATRRFGT	555	565	575
AAV2 (SEQ ID NO: 2)	EKTNNVDL	555	564	574
	KVMTIDEEELRTTNPVATNQYGS	556	565	575
AAV3A (SEQ ID NO: 3)	TASNAEL	556	565	575
	DNVMTIDEEELRTTNPVATNQYGT	556	565	575
AAV3B (SEQ ID NO: 4)	TASNAEL	554	563	573
	DNVMTIDEEELRTTNPVATNQYGT	554	563	573
AAV4 (SEQ ID NO: 5)	GNTAITP	544	554	564
	GVLTFSERELAAATNATQTDWGN	544	554	564
AAV5 (SEQ ID NO: 6)	PQTITALEGNLITSESETPVNRWAYNVGGQ	556	565	575
AAV6 (SEQ ID NO: 7)	EASNTAL	557	566	576
	DNVMTIDEEELKATNPVATRRFGT	557	566	576
AAV7 (SEQ ID NO: 8)	TNKTTL	558	567	577
	ENVMTIDEEELRTTNPVATNQYGT	558	567	577
AAV8 (SEQ ID NO: 9)	ARDNADY	556	565	575
	SDVMTIDEEELKATNPVATNQYGT	556	565	575
AAV9 (SEQ ID NO: 10)	GRDNVDA	558	567	577
	DRVMTIDEEELKATNPVATNQYGT	558	567	577
AAV10 (SEQ ID NO: 11)	GRDNVDY	558	567	577
	SDVMTIDEEELKATNPVATNQYGT	558	567	577

FIGURE 6 (cont'd)

AAV1 (SEQ ID NO: 1)	AAV1 (SEQ ID NO: 1)	600	610	620
AAV2 (SEQ ID NO: 2)	AAV2 (SEQ ID NO: 2)	585	595	605
AAV3A (SEQ ID NO: 3)	AAV3A (SEQ ID NO: 3)	584	594	604
AAV3B (SEQ ID NO: 4)	AAV3B (SEQ ID NO: 4)	585	595	605
AAV4 (SEQ ID NO: 5)	AAV4 (SEQ ID NO: 5)	583	593	603
AAV5 (SEQ ID NO: 6)	AAV5 (SEQ ID NO: 6)	574	584	594
AAV6 (SEQ ID NO: 7)	AAV6 (SEQ ID NO: 7)	585	595	605
AAV7 (SEQ ID NO: 8)	AAV7 (SEQ ID NO: 8)	586	596	606
AAV8 (SEQ ID NO: 9)	AAV8 (SEQ ID NO: 9)	587	597	607
AAV9 (SEQ ID NO: 10)	AAV9 (SEQ ID NO: 10)	585	595	605
AAV10 (SEQ ID NO: 11)	AAV10 (SEQ ID NO: 11)	587	597	607

FIGURE 6 (cont'd)

AAV1 (SEQ ID NO: 1)	YQGPINAKIPHTDGHFRSPIMGGFGKKHP	630 635 640 645 650 660
AAV2 (SEQ ID NO: 2)	YQGPINAKIPHTDGHFRSPIMGGFGKKHP	614 615 624 625 634 644
AAV3A (SEQ ID NO: 3)	YQGPINAKIPHTDGHFRSPIMGGFGKKHP	615 625 635 645
AAV3B (SEQ ID NO: 4)	YQGPINAKIPHTDGHFRSPIMGGFGKKHP	615 625 635 645
AAV4 (SEQ ID NO: 5)	YQGPINAKIPHTDGHFRSPIMGGFGKKHP	613 623 633 643
AAV5 (SEQ ID NO: 6)	YQGPINAKIPHTGAHFRSPIMGGFGKKHP	604 614 624 634
AAV6 (SEQ ID NO: 7)	YQGPINAKIPHTDGHFRSPIMGGFGKKHP	615 625 635 645
AAV7 (SEQ ID NO: 8)	YQGPINAKIPHTDGHFRSPIMGGFGKKHP	616 626 636 646
AAV8 (SEQ ID NO: 9)	YQGPINAKIPHTDGNFRSPIMGGFGKKHP	617 627 637 647
AAV9 (SEQ ID NO: 10)	YQGPINAKIPHTDGNFRSPIMGGFGKKHP	615 625 635 645
AAV10 (SEQ ID NO: 11)	YQGPINAKIPHTDGNFRSPIMGGFGKKHP	617 627 637 647

FIGURE 6 (cont'd)

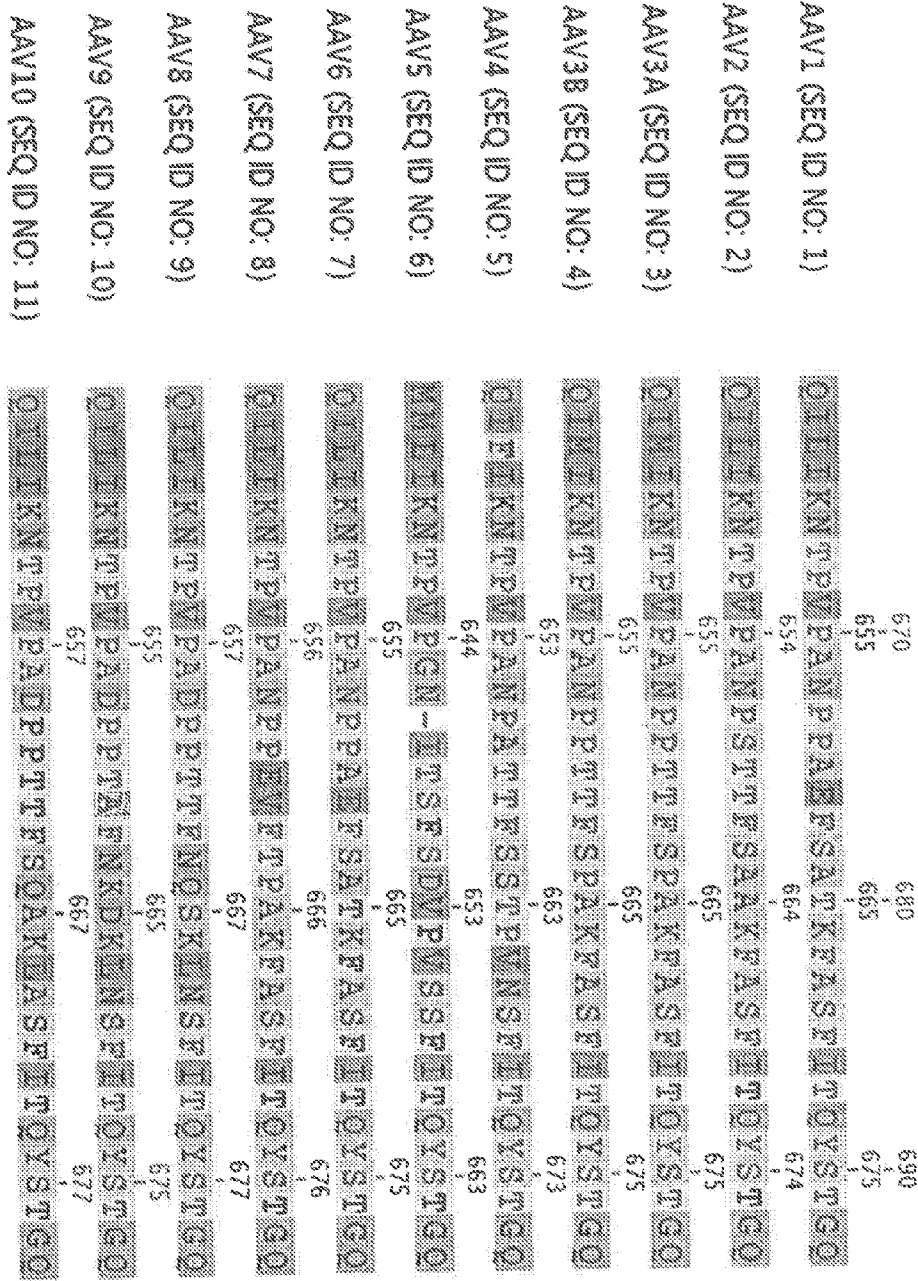


FIGURE 6 (cont'd)

AAV1 (SEQ ID NO: 1)	700 685 684 683 SVETIEWELQKENSRRWPEVQYTSN¹YAKSANN¹	710 695 694 693	720 705 704 703
AAV2 (SEQ ID NO: 2)	685 684 SVETIEWELQKENSRRWPEVQYTSN¹YKKSANN¹	695 694 693	705 704 703
AAV3A (SEQ ID NO: 3)	685 684 SVETIEWELQKENSRRWPEVQYTSN¹YKKSANN¹	695 694 693	705 704 703
AAV3B (SEQ ID NO: 4)	683 673 SVETIEWELQKENSRRWPEVQYTSN¹YKKSANN¹	693 683 693 692	703 693 692
AAV4 (SEQ ID NO: 5)	685 673 SVETIEWELQKENSRRWPEVQYTSN¹YKKSANN¹	695 685 695 694	705 695 694
AAV5 (SEQ ID NO: 6)	686 685 SVETIEWELQKENSRRWPEVQYTSN¹YKKSANN¹	696 686 696 695	706 696 695
AAV6 (SEQ ID NO: 7)	687 686 SVETIEWELQKENSRRWPEVQYTSN¹YKKSANN¹	697 687 697 696	707 697 696
AAV7 (SEQ ID NO: 8)	685 684 SVETIEWELQKENSRRWPEVQYTSN¹YKKSANN¹	695 685 695 694	705 695 694
AAV8 (SEQ ID NO: 9)	687 686 SVETIEWELQKENSRRWPEVQYTSN¹YKKSANN¹	697 687 697 696	707 697 696
AAV9 (SEQ ID NO: 10)	687 686 SVETIEWELQKENSRRWPEVQYTSN¹YKKSANN¹	697 687 697 696	707 697 696
AAV10 (SEQ ID NO: 11)	687 686 SVETIEWELQKENSRRWPEVQYTSN¹YKKSANN¹	697 687 697 696	707 697 696

FIGURE 6 (cont'd)

AAV1 (SEQ ID NO: 1)	DFTLDNNGIYTPRPFGTRYITRP	730 715 740 725 750 752 737
AAV2 (SEQ ID NO: 2)	DFTLDNNGIYSPRPFGTRYITRP	714 715 724 725 736
AAV3A (SEQ ID NO: 3)	DFTLDNNGIYSPRPFGTRYITRP	715 715 725 725 736
AAV3B (SEQ ID NO: 4)	DFTLDNNGIYSPRPFGTRYITRP	715 715 725 725 736
AAV4 (SEQ ID NO: 5)	WAPDAAGKVTQPPRAFGTRYITRP	713 703 713 725 735
AAV5 (SEQ ID NO: 6)	FAPDSTGKYRTPFGTRYITRP	715 715 725 725 737
AAV6 (SEQ ID NO: 7)	FPLDNGIYTPRPFGTRYITRP	716 716 726 726 738
AAV7 (SEQ ID NO: 8)	FALDSOGIYSPRPFGTRYITRP	717 717 727 727 739
AAV8 (SEQ ID NO: 9)	FALNTEGIYSPRPFGTRYITRP	715 715 725 725 737
AAV9 (SEQ ID NO: 10)	FALNTEGIYSPRPFGTRYITRP	717 717 727 727 738
AAV10 (SEQ ID NO: 11)	FALNTEGIYSPRPFGTRYITRP	717 717 727 727 738

FIGURE 7

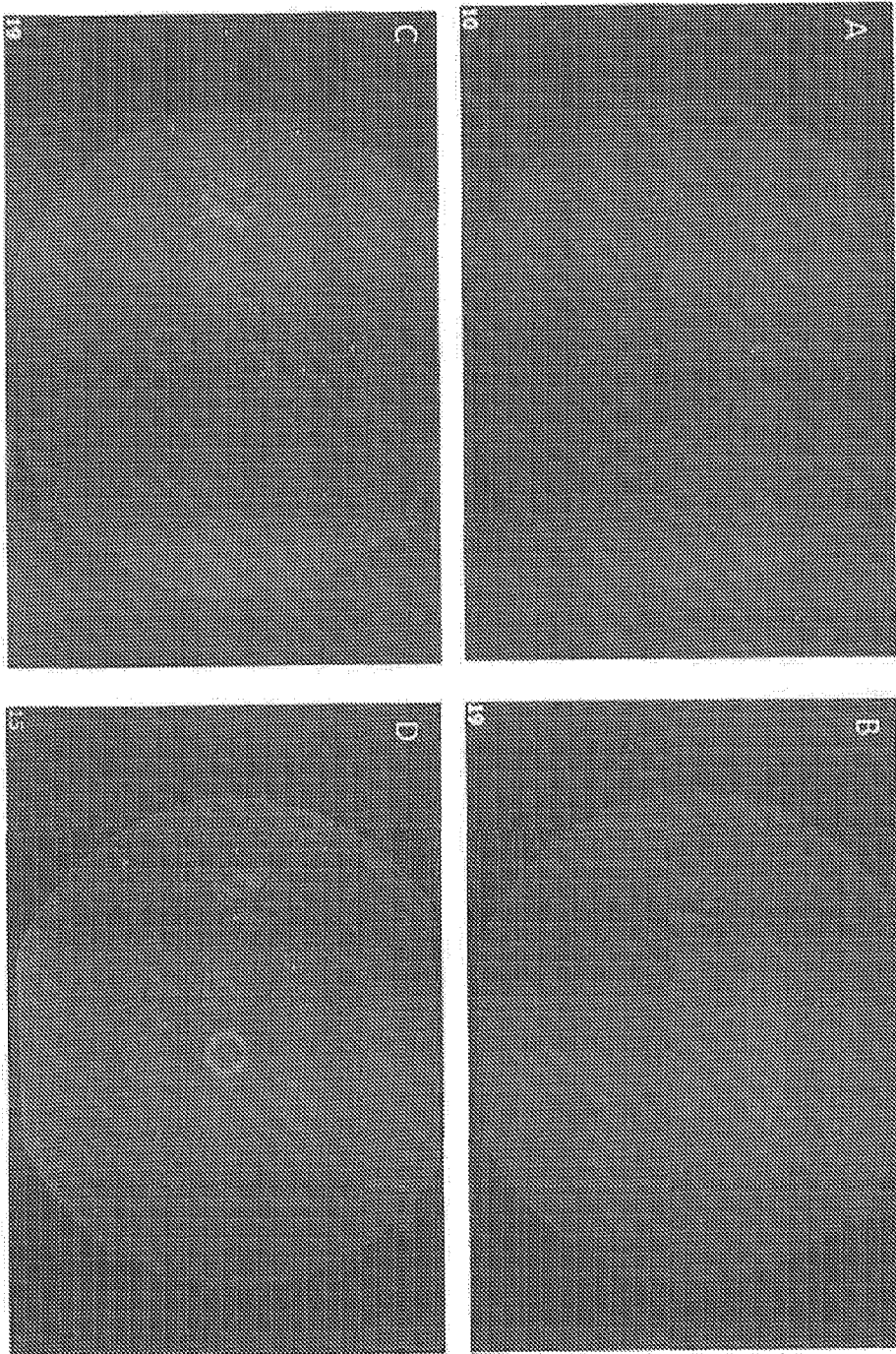


FIGURE 8

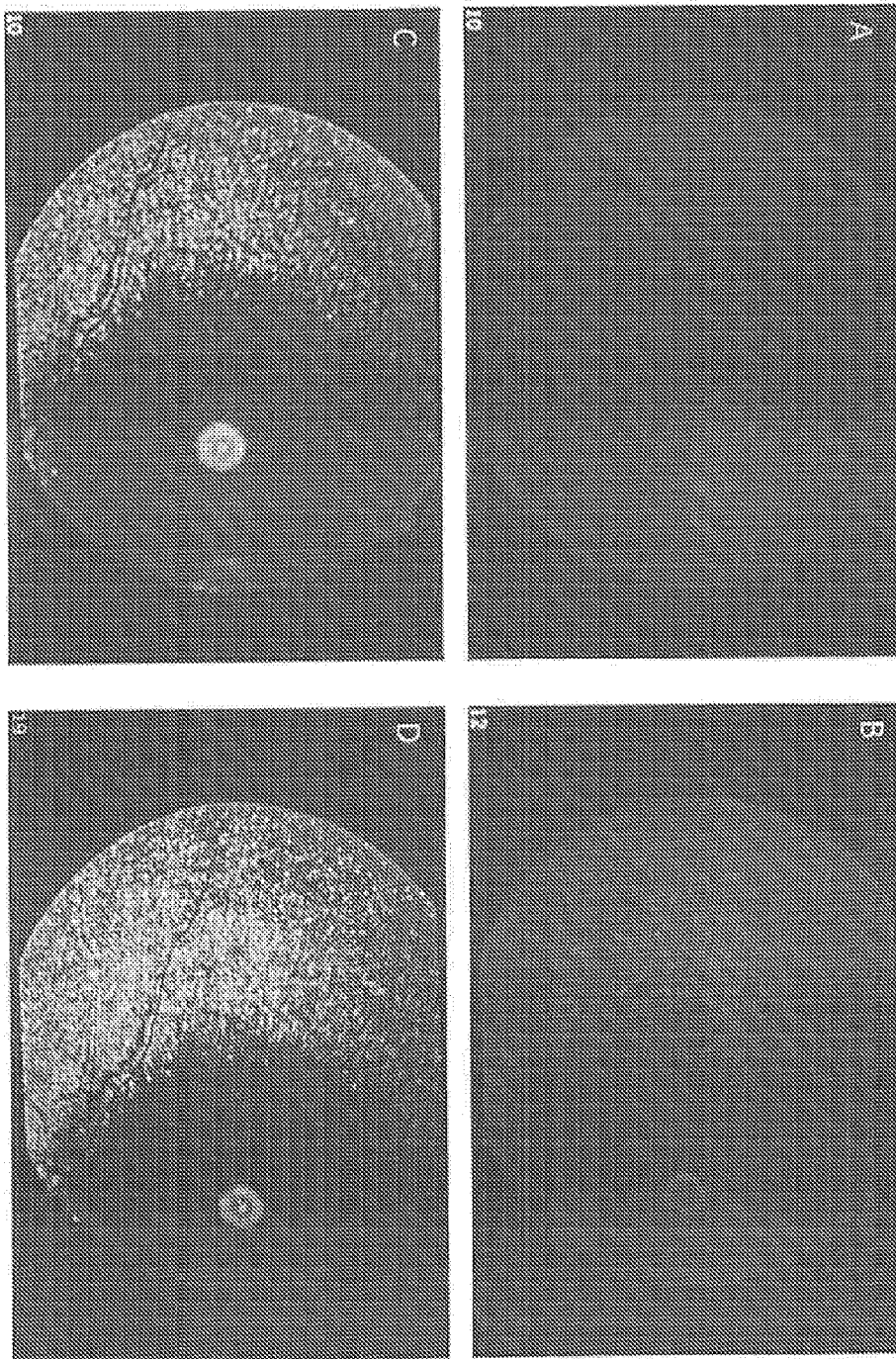


FIGURE 9

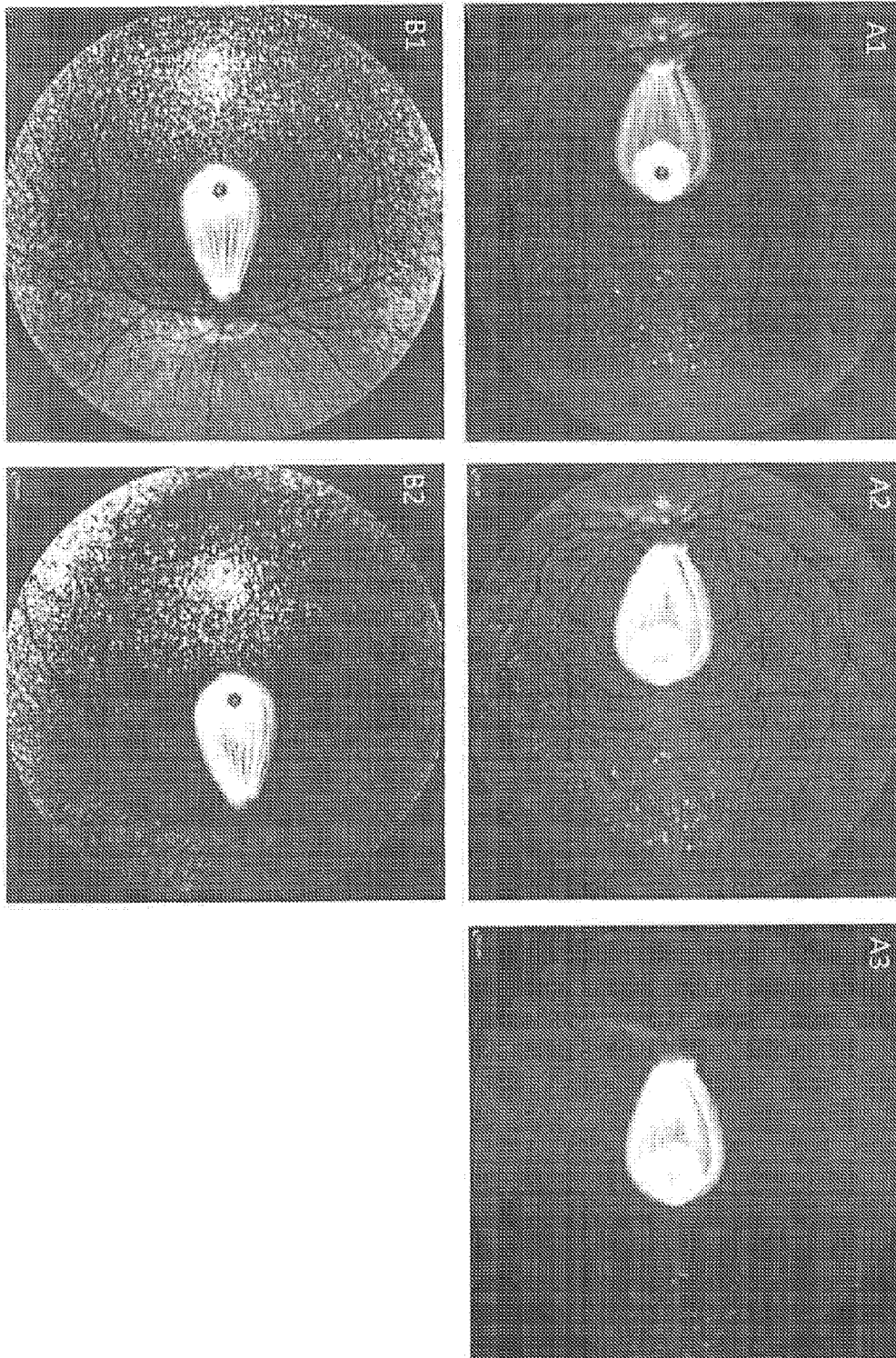


FIGURE 10A

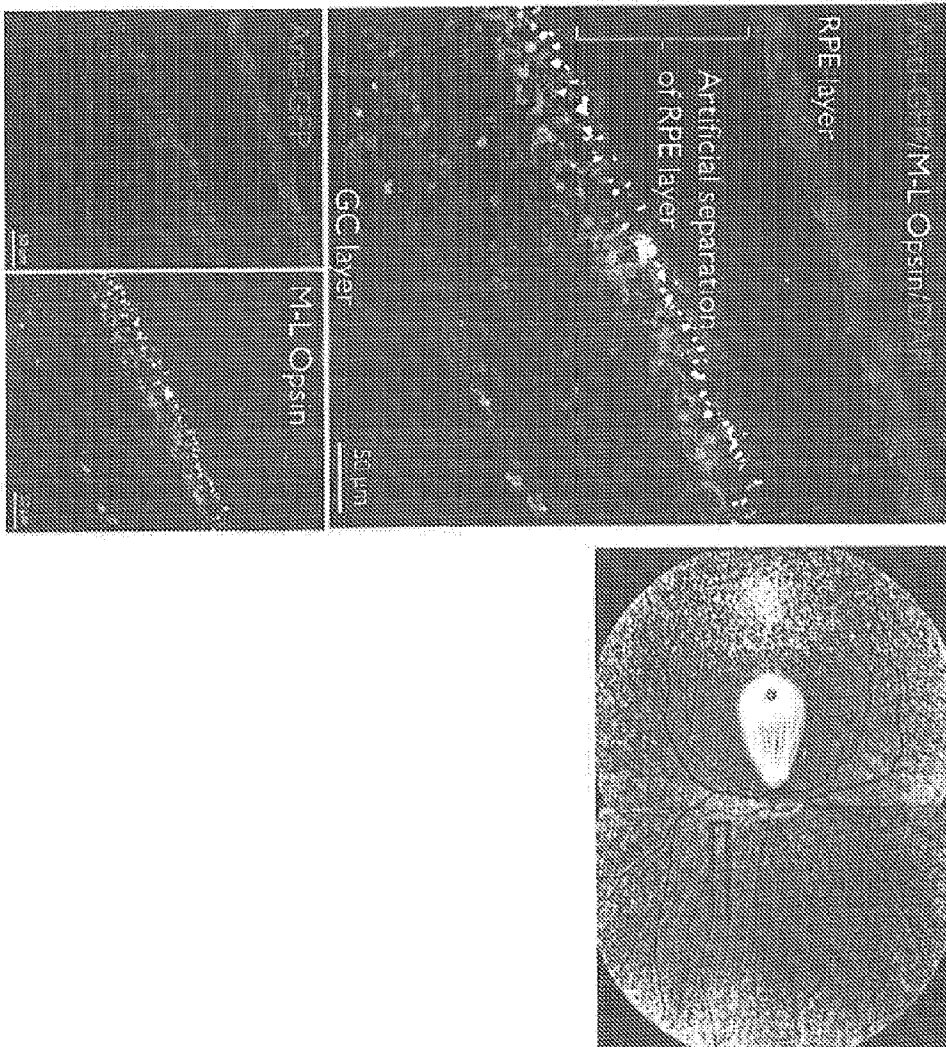


FIGURE 10B

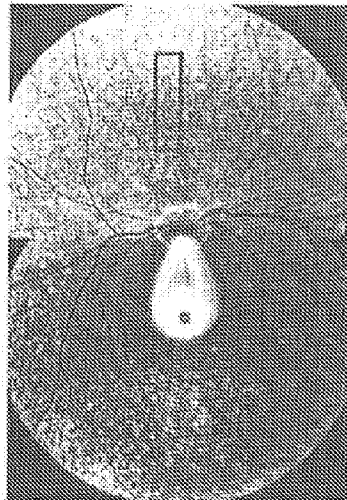
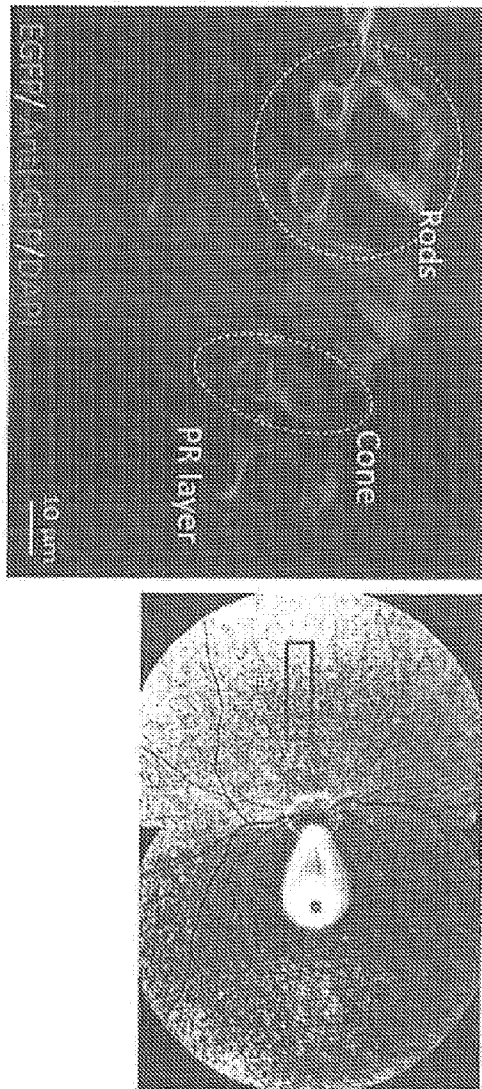
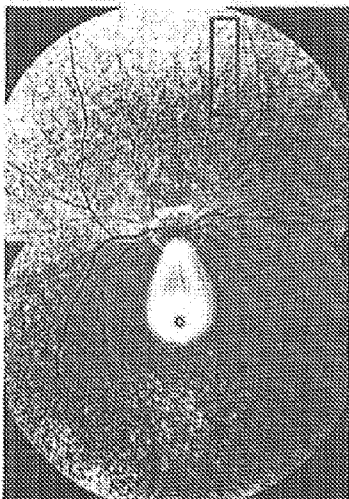
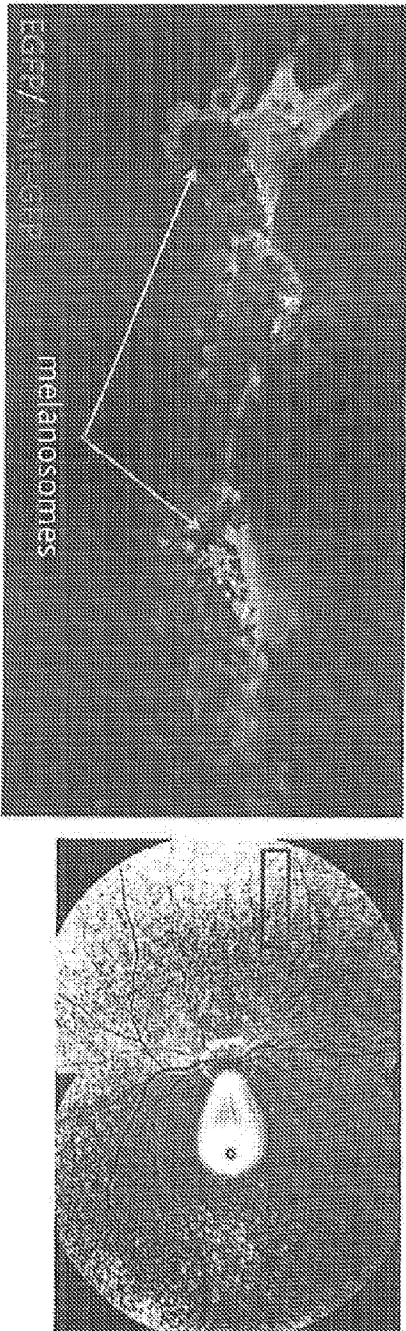


FIGURE 10C



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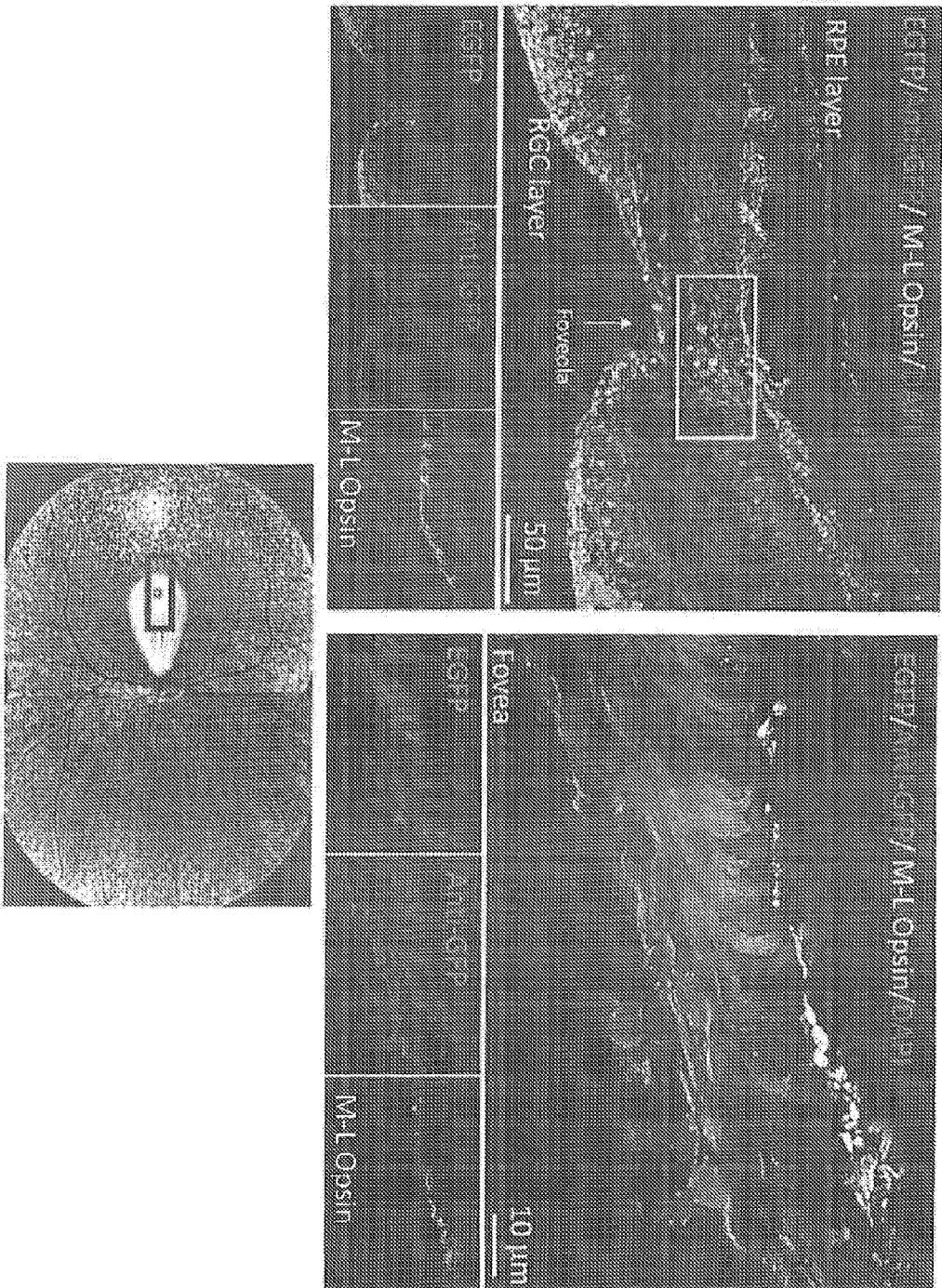


FIGURE 10E

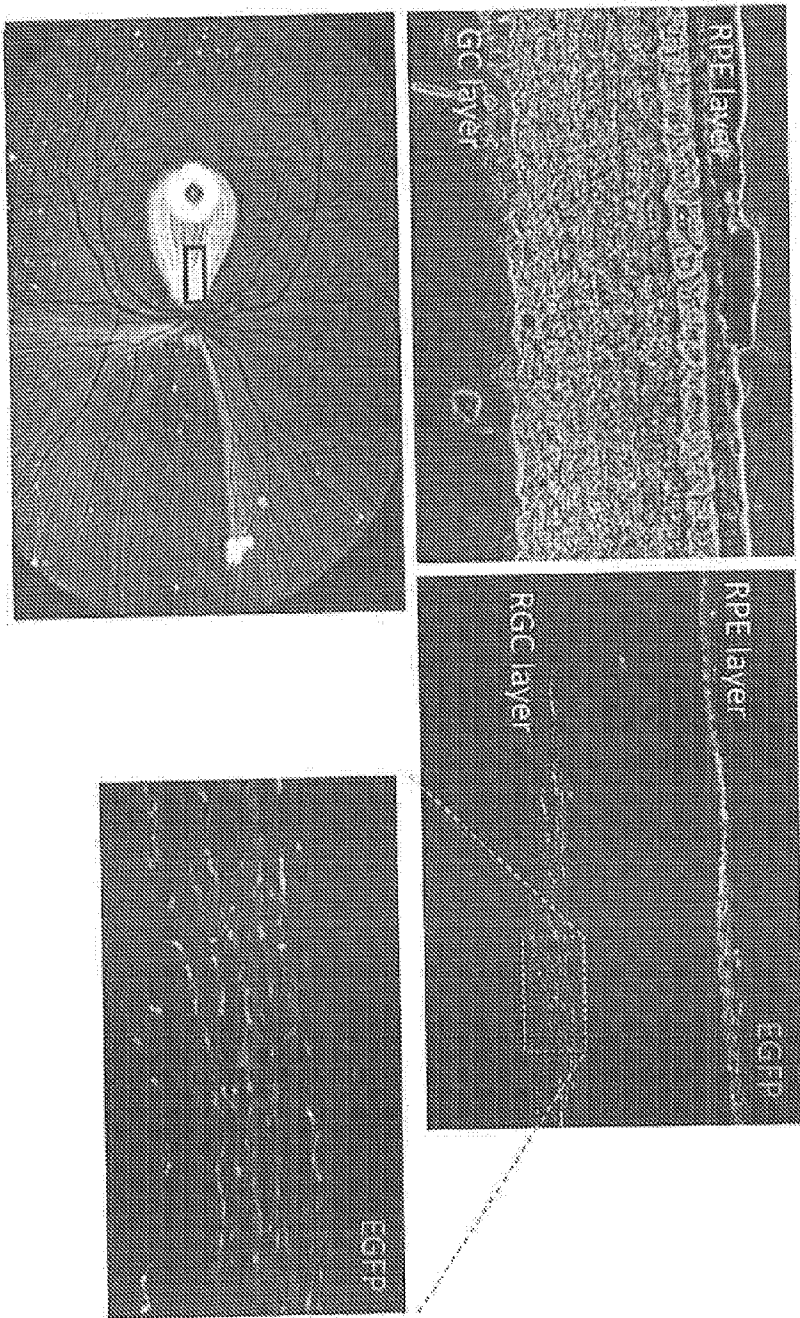


FIGURE 11A

Human ESC-derived RPEs

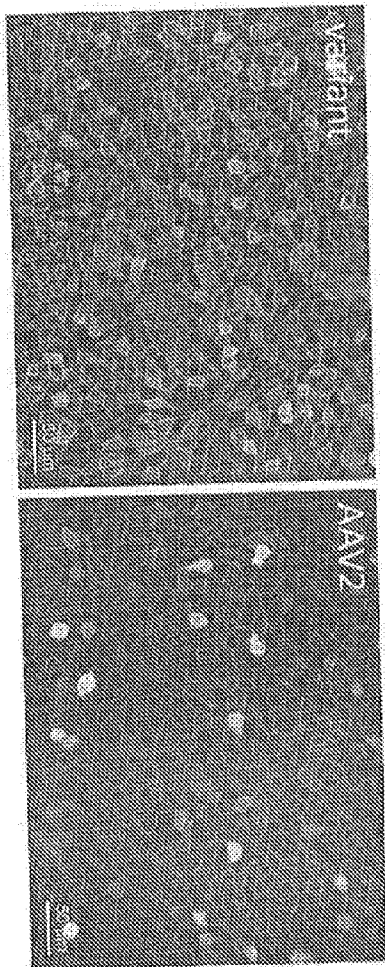


FIGURE 11B

Human FB-ipSC-derived RPEs

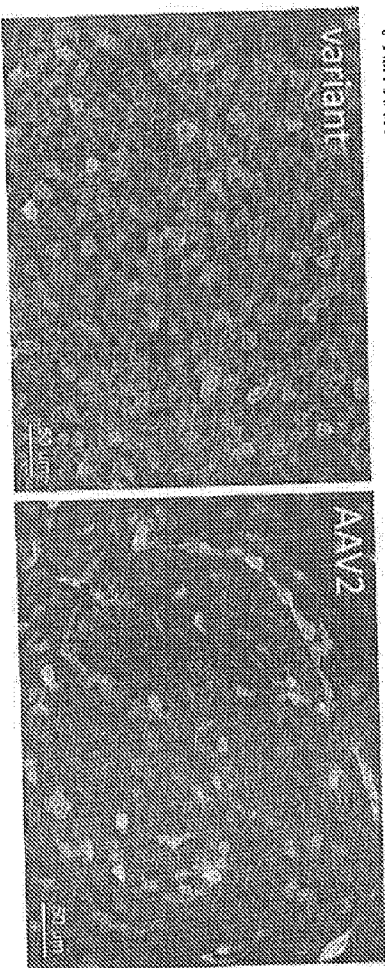


Figure 11C

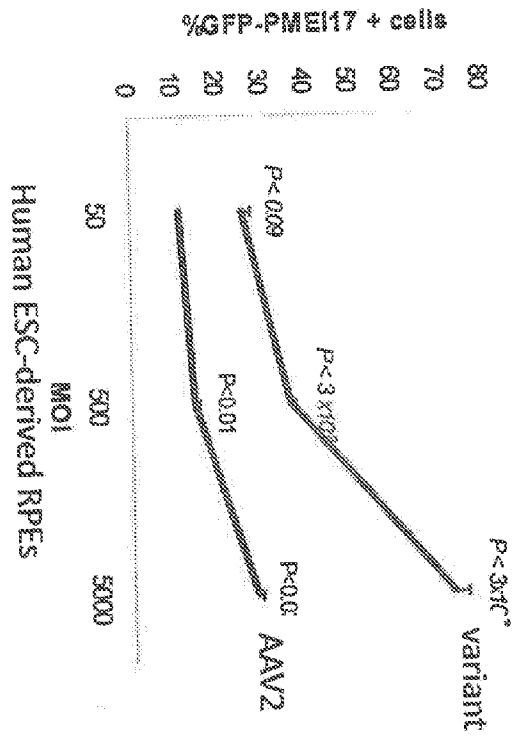


Figure 11D

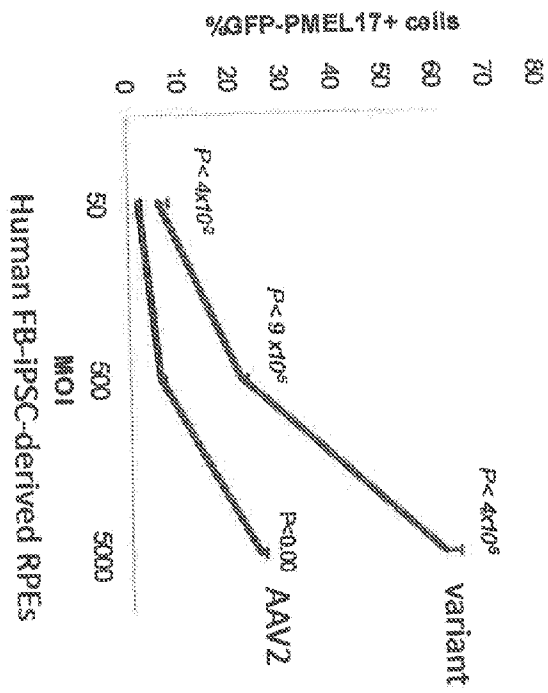


Figure 11E

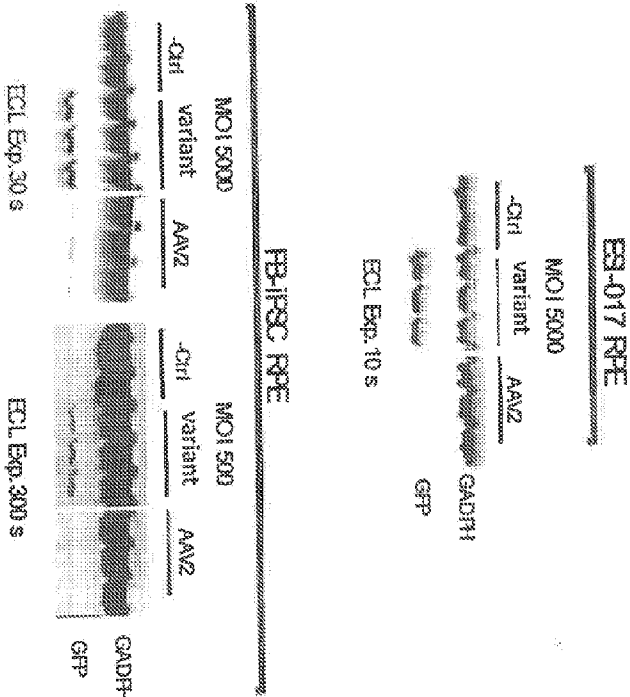


Figure 11F

