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(71) Applicant(s)
The Regents of the University of California;The Trustees of the University of Pennsylvania

(72) Inventor(s)
Schaffer, David V.;Flannery, John G.;Beltran, William A.;Byrne, Leah C.;Visel, Meike

(74) Agent / Attorney
FB Rice Pty Ltd, Level 14 90 Collins Street, Melbourne, VIC, 3000, AU

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(71) Applicants: **THE REGENTS OF THE UNIVERSITY OF CALIFORNIA** [US/US]; 1111 Franklin Street, 12th Floor, Oakland, California 94607 (US). **THE TRUSTEES OF THE UNIVERSITY OF PENNSYLVANIA** [US/US]; 3160 Chestnut Street, Suite 200, Philadelphia, Pennsylvania 19104 (US).

(72) Inventors: **SCHAFFER, David, V.**; 19 Rima Court, Danville, California 94526 (US). **FLANNERY, John, G.**; 1054 Cragmont Avenue, Berkeley, California 94708 (US). **BELTRAN, William, A.**; 530 South 2nd Street, Apt #811, Philadelphia, Pennsylvania 19147 (US). **BYRNE, Leah, C.**; 32 Dorland St #3, San Francisco, California 94110 (US). **VISEL, Meike**; 6532 Knott Ave, El Cerrito, California 94530 (US).

(74) Agent: **BORDEN, Paula, A.**; 201 Redwood Shores Parkway, Suite 200, Redwood City, California 94065 (US).

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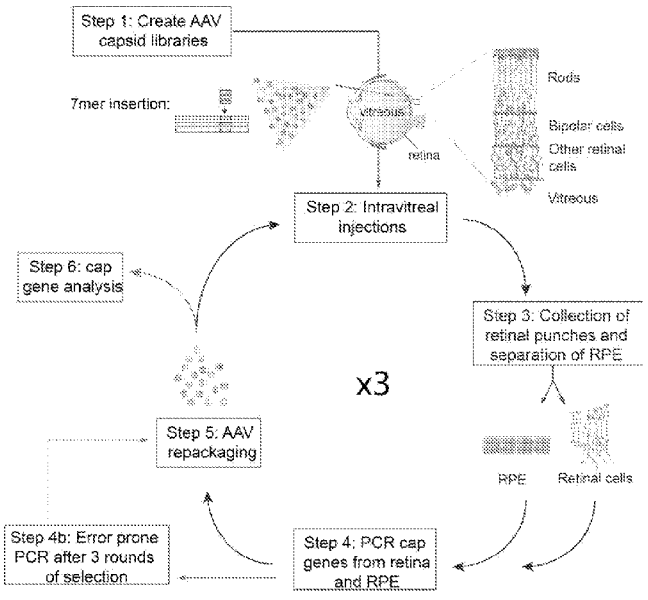
(54) Title: ADENO-ASSOCIATED VIRUS VIRIONS WITH VARIANT CAPSID AND METHODS OF USE THEREOF

FIG. 1



(57) Abstract: The present disclosure provides adeno-associated virus (AAV) virions with altered capsid protein, where the AAV virions exhibit greater infectivity of retinal cells compared to wild-type AAV. The present disclosure further provides methods of delivering a gene product to a retinal cell in an individual, and methods of treating ocular disease.

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ADENO-ASSOCIATED VIRUS VIRIONS WITH VARIANT CAPSID AND METHODS OF USE THEREOF

CROSS-REFERENCE

[0001] This application claims the benefit of U.S. Provisional Patent Application No. 62/368,929, filed July 29, 2016, which application is incorporated herein by reference in its entirety.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH

[0002] This invention was made with government support under Contract/Grant Nos. EY022975, EY018241 and EY06855 awarded by the National Institutes of Health. The government has certain rights in the invention.

INTRODUCTION

[0003] Photoreceptors are the first neurons in the retina to receive and process visual information, converting visible electromagnetic radiation into hyperpolarized responses through phototransduction. The overwhelming majority of inherited retinal diseases result in the loss of these cells, either directly, such as in dominant mutations that affect rhodopsin protein folding, or indirectly, such as in recessive mutations that affect retinal recycling pathways in the retinal pigment epithelium (RPE).

[0004] Adeno-associated virus (AAV) belongs to the *Parvoviridae* family and Dependovirus genus, whose members require co-infection with a helper virus such as adenovirus to promote replication, and AAV establishes a latent infection in the absence of a helper. Virions are composed of a 25 nm icosahedral capsid encompassing a 4.9 kb single-stranded DNA genome with two open reading frames: *rep* and *cap*. The non-structural *rep* gene encodes four regulatory proteins essential for viral replication, whereas *cap* encodes three structural proteins (VP1–3) that assemble into a 60-mer capsid shell. This viral capsid mediates the ability of AAV vectors to overcome many of the biological barriers of viral transduction–including cell surface receptor binding, endocytosis, intracellular trafficking, and unpackaging in the nucleus.

SUMMARY

[0005] The present disclosure provides recombinant adeno-associated virus (AAV) virions with altered capsid protein, where the recombinant AAV (rAAV) virions exhibit greater infectivity of a retinal cell compared to wild-type AAV, and where the rAAV virions comprise a heterologous

nucleic acid. The present disclosure further provides methods of delivering a gene product to a retinal cell in an individual, and methods of treating ocular disease. The present disclosure provides an rAAV virion, where the rAAV virion exhibits at least 5-fold increased localization to one or more of the inner nuclear layer, the outer nuclear layer, the photoreceptor layer, the ganglion cell layer, and the retinal pigment epithelium, compared to the extent of localization to the inner nuclear layer, the outer nuclear layer, the photoreceptor layer, the ganglion cell layer, or the retinal pigment epithelium, by an AAV virion comprising the corresponding parental AAV capsid protein.

[0005A] In an aspect, the present invention provides a recombinant adeno-associated virus (rAAV) virion comprising:

a) a variant AAV capsid protein, wherein the variant AAV capsid protein comprises an insertion of a heterologous peptide having a length of from about 9 amino acids to about 20 amino acids in the capsid protein GH loop relative to a corresponding parental AAV capsid protein, and wherein the variant capsid protein confers increased infectivity of a retinal cell compared to the infectivity of the retinal cell by a control AAV virion comprising the corresponding parental AAV capsid protein, and wherein the heterologous peptide comprises an amino acid sequence selected from:

PAPQDTTKKA (SEQ ID NO:57), LATTSQNKPA (SEQ ID NO:50), LAKGTELKPA (SEQ ID NO:54), LAVDGAQRSA (SEQ ID NO:56), LAKSDQSKPA (SEQ ID NO:61) and LAANQPSKPA (SEQ ID NO:63); and

b) a heterologous nucleic acid comprising a nucleotide sequence encoding a heterologous gene product.

[0005B] In another aspect, the present invention provides a pharmaceutical composition comprising:

a) a recombinant adeno-associated virus (AAV) virion described herein; and

b) a pharmaceutically acceptable excipient.

[0005C] In another aspect, the present invention provides a method of delivering a gene product to a retinal cell in an individual, the method comprising administering to the individual a recombinant adeno-associated virus (rAAV) virion described herein.

[0005D] In another aspect, the present invention provides a method of treating an ocular disease, the method comprising administering to an individual in need thereof an effective amount of a recombinant adeno-associated virus (rAAV) virion described herein.

[0005E] In another aspect, the present invention provides use of an effective amount of a recombinant adeno-associated virus (rAAV) virion described herein in the preparation of a medicament for treating an ocular disease.

[0005F] In another aspect, the present invention provides an isolated nucleic acid comprising a nucleotide sequence that encodes a variant adeno-associated virus (AAV) capsid protein, wherein the variant AAV capsid protein comprises an insertion of from about 9 amino acids to about 20 amino acids in the capsid protein GH loop relative to a corresponding parental AAV capsid protein, and wherein the variant capsid protein, when present in an AAV virion, provides for increased infectivity of the AAV virion of a retinal cell, and wherein the amino acid insertion is in the GH loop of a native AAV capsid,

and wherein the heterologous peptide comprises an amino acid sequence selected from:

PAPQDTTKKA (SEQ ID NO:57), LATTSQNKPA (SEQ ID NO:50), LAKGTELKPA (SEQ ID NO:54), LAVDGAQRSA (SEQ ID NO:56), LAKSDQSKPA (SEQ ID NO:61) and LAANQPSKPA (SEQ ID NO:63).

[0005G] In another aspect, the present invention provides an isolated, genetically modified host cell comprising a nucleic acid described herein.

[0005H] In another aspect, the present invention provides a variant adeno-associated virus (AAV) capsid protein, wherein the variant AAV capsid protein comprises an insertion

of a heterologous peptide having a length of from about 9 amino acids to about 20 amino acids wherein the amino acid insertion is in the GH loop of a native AAV capsid, and wherein the heterologous peptide comprises an amino acid sequence selected from:

PAPQDTTKKA (SEQ ID NO:57), LATTSQNKPA (SEQ ID NO:50), LAKGTELKPA (SEQ ID NO:54), LAVDGAQRSA (SEQ ID NO:56), LAKSDQSKPA (SEQ ID NO:61) and LAANQPSKPA (SEQ ID NO:63).

[0005I] Throughout this specification the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element, integer or step, or group of elements, integers or steps, but not the exclusion of any other element, integer or step, or group of elements, integers or steps.

[0005J] Any discussion of documents, acts, materials, devices, articles or the like which has been included in the present specification is not to be taken as an admission that any or all of these matters form part of the prior art base or were common general knowledge in the field relevant to the present disclosure as it existed before the priority date of each of the appended claims.

BRIEF DESCRIPTION OF THE DRAWINGS

[0006] **FIG. 1** is a schematic depiction of a directed evolution method used to develop AAV variants that exhibit increased infectivity of an ocular cell (e.g., a retinal cell), compared to the parental AAV.

[0007] **FIG. 2** is a schematic depiction of deep sequencing of AAV variants containing green fluorescent protein (GFP)-barcode constructs.

[0008] **FIG. 3** depicts infection of cells in the ganglion cell layer, the inner nuclear layer, the photoreceptor layer, and the retinal pigment epithelium (RPE) layer, by an 18-member AAV variant library.

[0009] **FIG. 4** provides an amino acid sequence of AAV2 capsid protein VP1. Amino acids 587 and 588 (NP) are in bold and underlined.

[0010] **FIG. 5** provides amino acid sequences corresponding to amino acids 570-610 of AAV capsid protein VP1 of various AAV serotypes.

[0011] **FIG. 6A-6C** provide an alignment of amino acid sequences of AAV capsid protein loop IV (GH loop) regions. Insertion sites are shown in bold and underlining. AAV1: SEQ ID NO:35; AAV6: SEQ ID NO:36; AAV3: SEQ ID NO:37; AAV2: SEQ ID NO:38; AAV8: SEQ ID NO:39; AAV8.1: SEQ ID NO:40; AAV8 rh8: SEQ ID NO:41; AAV10: SEQ ID NO:42; AAV7: SEQ ID NO:43; AAV9: SEQ ID NO:44; AAV 9.1: SEQ ID NO:45; AAV5: SEQ ID NO:46.

[0012] **FIG. 7A-7V** provide amino acid sequences of exemplary heterologous gene products.

[0013] **FIG. 8A-8C** provide amino acid sequences of exemplary guide-RNA-directed endonucleases.

DEFINITIONS

[0014] The term "retinal cell" can refer herein to any of the cell types that comprise the retina, such as retinal ganglion cells; amacrine cells; horizontal cells; bipolar cells; photoreceptor cells

including rods and cones; Müller glial cells; astrocytes (e.g., a retinal astrocyte); and retinal pigment epithelium.

[0015] "AAV" is an abbreviation for adeno-associated virus, and may be used to refer to the virus itself or derivatives thereof. The term covers all subtypes and both naturally occurring and recombinant forms, except where required otherwise. The abbreviation "rAAV" refers to recombinant adeno-associated virus, also referred to as a recombinant AAV vector (or "rAAV vector"). The term "AAV" includes AAV type 1 (AAV-1), AAV type 2 (AAV-2), AAV type 3 (AAV-3), AAV type 4 (AAV-4), AAV type 5 (AAV-5), AAV type 6 (AAV-6), AAV type 7 (AAV-7), AAV type 8 (AAV-8), AAV type 9 (AAV-9), avian AAV, bovine AAV, canine AAV, equine AAV, primate AAV, non-primate AAV, and ovine AAV. "Primate AAV" refers to AAV isolated from a primate, "non-primate AAV" refers to AAV isolated from a non-primate mammal, "bovine AAV" refers to AAV isolated from a bovine mammal (e.g., a cow), etc.

[0016] An "rAAV vector" as used herein refers to an AAV vector comprising a polynucleotide sequence not of AAV origin (i.e., a polynucleotide heterologous to AAV), typically a sequence of interest for the genetic transformation of a cell. In general, the heterologous polynucleotide is flanked by at least one, and generally by two AAV inverted terminal repeat sequences (ITRs). The term rAAV vector encompasses both rAAV vector particles and rAAV vector plasmids.

[0017] An "AAV virus" or "AAV viral particle" or "rAAV vector particle" refers to a viral particle composed of at least one AAV capsid protein (typically by all of the capsid proteins of a wild-type AAV) and an encapsidated polynucleotide rAAV vector. If the particle comprises a heterologous polynucleotide (i.e. a polynucleotide other than a wild-type AAV genome, such as a transgene to be delivered to a mammalian cell), it is typically referred to as an "rAAV vector particle" or simply an "rAAV vector". Thus, production of rAAV particle necessarily includes production of rAAV vector, as such a vector is contained within an rAAV particle.

[0018] "Packaging" refers to a series of intracellular events that result in the assembly and encapsidation of an AAV particle.

[0019] 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."

[0020] A "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.

[0021] "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.

[0022] An "infectious" virus or viral particle is one that comprises a polynucleotide component which it is capable of delivering into a cell for which the viral species is tropic. The term does not necessarily imply any replication capacity of the virus. As used herein, an "infectious" virus or viral particle is one that can access a target cell, can infect a target cell, and can express a heterologous nucleic acid in a target cell. Thus, "infectivity" refers to the ability of a viral particle to access a target cell, infect a target cell, and express a heterologous nucleic acid in a target cell. Infectivity can refer to *in vitro* infectivity or *in vivo* infectivity. 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. Total viral particles can be expressed as the number of viral genome (vg) copies. The ability of a viral particle to express a heterologous nucleic acid in a cell can be referred to as "transduction." The ability of a viral particle to express a heterologous nucleic acid in a cell can be assayed using a number of techniques, including assessment of a marker gene, such as a green fluorescent protein (GFP) assay (e.g., where the virus comprises a nucleotide sequence encoding GFP), where GFP is produced in a cell infected with the viral particle and is detected and/or measured; or the measurement of a produced protein, for example by an enzyme-linked immunosorbent assay (ELISA). 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.

[0023] A "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 10^8 rAAV particles, less than about 1 rcAAV per 10^{12} rAAV particles, or no rcAAV).

[0024] 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 non-nucleotide 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 of the invention described herein that is 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.

[0025] 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)

[0026] Of interest is the BestFit program using the local homology algorithm of Smith Waterman (Advances in Applied Mathematics 2: 482-489 (1981) to determine sequence identity. The gap generation penalty will generally range from 1 to 5, usually 2 to 4 and in many embodiments will be 3. The gap extension penalty will generally range from about 0.01 to 0.20 and in many instances will be 0.10. The program has default parameters determined by the sequences inputted

to be compared. Preferably, the sequence identity is determined using the default parameters determined by the program. This program is available also from Genetics Computing Group (GCG) package, from Madison, Wisconsin, USA.

[0027] Another program of interest is the FastDB algorithm. FastDB is described in Current Methods in Sequence Comparison and Analysis, Macromolecule Sequencing and Synthesis, Selected Methods and Applications, pp. 127-149, 1988, Alan R. Liss, Inc. Percent sequence identity is calculated by FastDB based upon the following parameters:

[0028] Mismatch Penalty: 1.00;

[0029] Gap Penalty: 1.00;

[0030] Gap Size Penalty: 0.33; and

[0031] Joining Penalty:30.0.

[0032] A "gene" refers to a polynucleotide containing at least one open reading frame that is capable of encoding a particular protein after being transcribed and translated.

[0033] The term “guide RNA”, as used herein, refers to an RNA that comprises: i) an “activator” nucleotide sequence that binds to a guide RNA-directed endonuclease (e.g., a class 2 CRISPR/Cas endonuclease such as a type II, type V, or type VI CRISPR/Cas endonuclease) and activates the RNA-directed endonuclease; and ii) a “targeter” nucleotide sequence that comprises a nucleotide sequence that hybridizes with a target nucleic acid. The “activator” nucleotide sequence and the “targeter” nucleotide sequence can be on separate RNA molecules (e.g., a “dual-guide RNA”); or can be on the same RNA molecule (a “single-guide RNA”).

[0034] A "small interfering" or "short interfering RNA" or siRNA is a 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. 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. The RNA duplex portion of the siRNA can be part of a hairpin structure. 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.

[0035] As used herein, the term "microRNA" refers to any type of interfering RNAs, including but not limited to, endogenous microRNAs and artificial microRNAs (e.g., synthetic miRNAs). Endogenous microRNAs are small RNAs naturally encoded in the genome which are capable of modulating the productive utilization of mRNA. An artificial microRNA can be any type of RNA sequence, other than endogenous microRNA, which is capable of modulating the activity of an mRNA. A microRNA sequence can be an RNA molecule composed of any one or more of these sequences. MicroRNA (or "miRNA") sequences have been described in publications such as Lim, et al., 2003, Genes & Development, 17, 991-1008, Lim et al., 2003, Science, 299, 1540, Lee and Ambrose, 2001, Science, 294, 862, Lau et al., 2001, Science 294, 858-861, Lagos-Quintana et al., 2002, Current Biology, 12, 735-739, Lagos-Quintana et al., 2001, Science, 294, 853-857, and Lagos-Quintana et al., 2003, RNA, 9, 175-179. Examples of microRNAs include any RNA that is a fragment of a larger RNA or is a miRNA, siRNA, stRNA, sncRNA, tncRNA, snoRNA, smRNA, shRNA, snRNA, or other small non-coding RNA. See, e.g., US Patent Applications 20050272923, 20050266552, 20050142581, and 20050075492. A "microRNA precursor" (or "pre-miRNA") refers to a nucleic acid having a stem-loop structure with a microRNA sequence incorporated therein. A "mature microRNA" (or "mature miRNA") includes a microRNA that has been cleaved from a microRNA precursor (a "pre-miRNA"), or that has been synthesized (e.g., synthesized in a laboratory by cell-free synthesis), and has a length of from about 19 nucleotides to about 27 nucleotides, e.g., a mature microRNA can have a length of 19 nt, 20 nt, 21 nt, 22 nt, 23 nt, 24 nt, 25 nt, 26 nt, or 27 nt. A mature microRNA can bind to a target mRNA and inhibit translation of the target mRNA.

[0036] "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.

[0037] A "control element" or "control sequence" is 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.

[0038] "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.

[0039] An "expression vector" is a vector comprising a 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 also comprises 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.

[0040] "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 encoding a heterologous gene product is an rAAV that includes a nucleic acid 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. As another example, a variant AAV capsid protein that comprises a heterologous peptide inserted into the GH loop of the capsid protein is a variant AAV capsid protein that includes an insertion of a peptide not normally included in a naturally-occurring, wild-type AAV.

[0041] The terms "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.

[0042] 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. 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.

[0043] 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.

[0044] An "isolated" plasmid, nucleic acid, vector, virus, virion, host cell, 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 invention 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.

[0045] 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 affect 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 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, i.e., arresting its development; and (c) relieving the disease, i.e., causing regression of the disease.

[0046] The terms "individual," "host," "subject," and "patient" are used interchangeably herein, and refer to a mammal, including, but not limited to, human and non-human primates, including simians and humans; mammalian sport animals (e.g., horses, camels, etc.); mammalian farm animals (e.g., sheep, goats, cows, etc.); mammalian pets (dogs, cats, etc.); and rodents (e.g., mice, rats, etc.). In some cases, the individual is a human.

[0047] Before the present invention is further described, it is to be understood that this invention is not limited to particular embodiments described, as such may, of course, 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.

[0048] 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 limit of that 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 in the smaller ranges, and are 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.

[0049] 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 also be used in the practice or testing of the present invention, the preferred methods and materials are now described. All publications mentioned herein are incorporated herein by reference to disclose and describe the methods and/or materials in connection with which the publications are cited.

[0050] It must be 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 "an AAV capsid" includes a plurality of such capsids and reference to "the AAV virion" includes reference to one or more AAV virions 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.

[0051] 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 present invention and are disclosed herein just as if each and every combination was individually and explicitly disclosed. In addition, all sub-combinations of the various embodiments and elements thereof are also specifically embraced by the present invention and are disclosed herein just as if each and every such sub-combination was individually and explicitly disclosed herein.

[0052] 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.

DETAILED DESCRIPTION

[0053] The present disclosure provides recombinant adeno-associated virus (AAV) virions with altered capsid protein, where the recombinant AAV (rAAV) virions exhibit greater infectivity of a retinal cell compared to wild-type AAV; and where the rAAV virions comprise a heterologous nucleic acid. The rAAV virions exhibit greater infectivity of a retinal cell, compared to the infectivity of a corresponding wild-type AAV for the retinal cell. The retinal cell can be a photoreceptor (e.g., rods; cones), a retinal ganglion cell (RGC), a Müller cell (a Müller glial cell), an astrocyte (e.g., a retinal astrocyte), a bipolar cell, an amacrine cell, a horizontal cell, or a retinal pigment epithelium (RPE) cell. The present disclosure further provides methods of delivering a gene product to a retinal cell in an individual, and methods of treating an ocular disease. The present disclosure provides an rAAV virion with an altered capsid protein, where the rAAV virion exhibits at least 5-fold increased localization to one or more of the inner nuclear layer, the outer nuclear layer, the photoreceptor layer, the ganglion cell layer, and the retinal pigment epithelium, compared to the extent of localization to the inner nuclear layer, the outer nuclear layer, the photoreceptor layer, the ganglion cell layer, or the retinal pigment epithelium, by an AAV virion comprising the corresponding parental AAV capsid protein; and where the rAAV virions comprise a heterologous nucleic acid.

VARIANT AAV CAPSID POLYPEPTIDES

[0054] The present disclosure provides a variant AAV capsid protein. A variant AAV capsid protein of the present disclosure comprises an insertion of a heterologous peptide of from 5 amino acids to 20 amino acids in length in an insertion site in a surface-accessible (e.g., solvent-accessible) portion of a parental AAV capsid protein, such that the variant capsid protein, when present in an AAV virion, confers increased infectivity of a retinal cell compared to the infectivity of the retinal cell by an AAV virion comprising the corresponding parental AAV capsid protein. In other cases, the retinal cell is a Müller cell. Other retinal cells include amacrine cells, bipolar cells, and horizontal cells. An "insertion of from about 5 amino acids to about 20 amino acids" is also referred to herein as a "peptide insertion" (e.g., a heterologous peptide insertion). A "corresponding parental AAV capsid protein" refers to an AAV capsid protein of the same AAV serotype, without the peptide insertion. In many instances, the variant AAV capsid comprises a single heterologous peptide insert of from 5 amino acids to 20 amino acids (e.g., from 5 to 7, from 7 to 10, from 10 to 12, from 12 to 15, or from 15 to 20 amino acids) in length.

[0055] The insertion site is in the GH loop, or loop IV, of the AAV capsid protein, e.g., in a solvent-accessible portion of the GH loop, or loop IV, of the 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. For example, the insertion site can be within amino acids 411-650 of an AAV capsid protein, as depicted in FIG. 6A-6C. For example, the insertion site can be within amino acids 570-611 of AAV2, within amino acids 571-612 of AAV1, within amino acids 560-601 of AAV5, within amino acids 571 to 612 of AAV6, within amino acids 572 to 613 of AAV7, within amino acids 573 to 614 of AAV8, within amino acids 571 to 612 of AAV9, or within amino acids 573 to 614 of AAV10, as depicted in FIG. 5. In some cases, the insertion site is between amino acids 588 and 589 of an AAV2 capsid protein, or a corresponding insertion site in an AAV of a different serotype. In some cases, the insertion site is between amino acids 587 and 588 of an AAV2 capsid protein, or a corresponding insertion site in an AAV of a different serotype.

[0056] In some cases, a heterologous peptide of from about 5 amino acids to about 20 amino acids (e.g., from 5 to 7, from 7 to 10, from 10 to 12, from 12 to 15, or from 15 to 20 amino acids) in length is inserted in an insertion site in the GH loop or loop IV of the capsid protein relative to a corresponding parental AAV capsid protein. For example, the insertion site can be between amino acids 587 and 588 of AAV2, or the corresponding positions of the capsid subunit of another AAV serotype. It should be noted that the insertion site 587/588 is based on an AAV2 capsid protein. A heterologous peptide of 5 amino acids to about 20 amino acids (e.g., from 5 to 7, from 7 to 10, from 10 to 12, from 12 to 15, or from 15 to 20 amino acids) in length can be

inserted in a corresponding site in an AAV serotype other than AAV2 (e.g., AAV8, AAV9, etc.). 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 587-588 of AAV2" would be in a capsid protein of any given AAV serotype. Sequences corresponding to amino acids 570-611 of capsid protein VP1 of AAV2 (see FIG. 4) in various AAV serotypes are shown in FIG. 5. See, e.g., GenBank Accession No. NP_049542 for AAV1; GenBank Accession No. AAD13756 for AAV5; GenBank Accession No. AAB95459 for AAV6; GenBank Accession No. YP_077178 for AAV7; GenBank Accession No. YP_077180 for AAV8; GenBank Accession No. AAS99264 for AAV9 and GenBank Accession No. AAT46337 for AAV10.

[0057] 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 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. The insertion sites are underlined in FIG. 5; the amino acid numbering is based on the numbering depicted in FIG. 5.

[0058] In some embodiments, a subject capsid protein includes a GH loop comprising an amino acid sequence having at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity to an amino acid sequence set forth in FIG. 6A-6C; and having an insertion of a heterologous peptide of from 5 to 20 amino acids (e.g., from 5 to 7, from 7 to 10, from 10 to 12, from 12 to 15, or from 15 to 20 amino acids) in length.

Insertion peptides

[0059] As noted above, a heterologous peptide of from about 5 amino acids to about 20 amino acids in length is inserted into the GH loop of an AAV capsid. In some cases, the insertion peptide has a length of from 5 amino acids to 20 amino acids. In some cases, the insertion peptide has a length of from 7 amino acids to 15 amino acids. In some cases, the insertion peptide has a length of from 9 amino acids to 15 amino acids. In some cases, the insertion peptide has a length of from 9 amino acids to 12 amino acids. The insertion peptide has a length of 5 amino acids, 6 amino acids, 7 amino acids, 8 amino acids, 9 amino acids, 10 amino acids, 11 amino acids, 12 amino acids, 13 amino acids, 14 amino acids, 15 amino acids, 16 amino acids, 17 amino acids, 18 amino acids, 19 amino acids, or 20 amino acids. In some cases, the insertion peptide has a length of 7 amino acids. In some cases, the insertion peptide has a length of 8 amino acids. In some cases, the insertion peptide has a length of 9 amino acids. In some cases, the insertion peptide has a length of 10 amino acids. In some cases, the insertion peptide has a length of 11 amino acids. In some cases, the insertion peptide has a length of 12 amino acids. In some cases, the

insertion peptide has a length of 13 amino acids. In some cases, the insertion peptide has a length of 14 amino acids. In some cases, the insertion peptide has a length of 15 amino acids.

[0060] The peptide insert is, in some cases, a peptide of **Formula I**:

[0061] $X_1X_2X_3X_4X_5X_6X_7X_8X_9X_{10}$, where:

[0062] X_1 is Leu, Ile, Pro, or Gln;

[0063] X_2 is Ala, Pro, Ser, Asp, Gly, Thr, or Val;

[0064] X_3 is Lys, His, Thr, Ile, Pro, Val, Arg, Ala, Asp, Glu, Asn, Gln, or Tyr;

[0065] X_4 (if present) is Gln, Asp, Ser, Gly, Thr, Ile, Asn, Glu, Lys, or Arg;

[0066] X_5 is Asp, Ser, Gln, Val, Thr, Gly, Ala, Asn, Lys, or Tyr;

[0067] X_6 is Thr, Ala, Gln, Ser, Glu, Pro, or Ile;

[0068] X_7 is Thr, Ser, Asn, Pro, Leu, Gln, Lys, Ala, or Cys;

[0069] X_8 is Lys, Ser, Arg, Thr, Ala, Glu, Ile, or Asn;

[0070] X_9 is Asn, Pro, Ser, Lys, His, Ile, Thr, or Ala; and

[0071] X_{10} is Ala, Phe, Asp, Thr, Val, or Met.

[0072] Peptide inserts of **Formula I** include, but are not limited to, (1) LAKDATKNA (SEQ ID NO:47); (2) PAHQDTTKNA (SEQ ID NO:48); (3) LAHQDTTKNA (SEQ ID NO:49); (4) LATTSQNKPA (SEQ ID NO:50); (5) LAISDQTKHA (SEQ ID NO:51); (6) IARGVAPSSA (SEQ ID NO:52); (7) LAPDSTTRSA (SEQ ID NO:53); (8) LAKGTELKPA (SEQ ID NO:54); (9) LAIIDATKNA (SEQ ID NO:55); (10) LAVDGAQRSA (SEQ ID NO:56); (11) PAPQDTTKKA (SEQ ID NO:57); (12) LPHQDTTKNA (SEQ ID NO:58); (13) LAKDATKTIA (SEQ ID NO:59); (14) LAKQQSASTA (SEQ ID NO:60); (15) LAKSDQSKPA (SEQ ID NO:61); (16) LSHQDTTKNA (SEQ ID NO:62); (17) LAANQPSKPA (SEQ ID NO:63); (18) LAVSDSTKAA (SEQ ID NO:64); (19) LAAQGTAKKPA (SEQ ID NO:65); (20) LAPDQTTRNA (SEQ ID NO:66); (21) LAASDSTKAA (SEQ ID NO:67); (22) LAPQDTTKNA (SEQ ID NO:68); (23) LAKADETRPA (SEQ ID NO:69); (24) LAHQDTAKNA (SEQ ID NO:70); (25) LAHQDTKKNA (SEQ ID NO:71); (26) LAHQDTTKHA (SEQ ID NO:72); (27) LAHQDTTKKA (SEQ ID NO:73); (28) LAHQDTTRNA (SEQ ID NO:74); (29) LAHQDTTNA (SEQ ID NO:75); (30) LAHQGTTKNA (SEQ ID NO:76); (31) LAHQVTTKNA (SEQ ID NO:77); (32) LAISDQSKPA (SEQ ID NO:78); (33) LADATKTA (SEQ ID NO:79); (34) LAKDTTKNA (SEQ ID NO:80); (35) LAKSDQSRPA (SEQ ID NO:81); (36) LAPQDTKKNA (SEQ ID NO:82); (37) LATSDSTKAA (SEQ ID NO:83); (38) LAVDGSQRSA (SEQ ID NO:84); (39) LPISDQTKHA (SEQ ID NO:85); (40) LPKDATKTIA (SEQ ID NO:86); (41) LPPQDTTKNA (SEQ ID NO:87); (42) PAPQDTTKNA (SEQ ID NO:88); (43) QAHQDTTKNA (SEQ ID NO:89); (44)

LAHETSPRPA (SEQ ID NO:90); (45) LAKSTSTAPA (SEQ ID NO:91); (46) LADQDTTKNA (SEQ ID NO:92); (47) LAESDQSKPA (SEQ ID NO:93); (48) LAHKDTTKNA (SEQ ID NO:94); (49) LAHKTQQKM (SEQ ID NO:95); (50) LAHQDTTENA (SEQ ID NO:96); (51) LAHQDTTINA (SEQ ID NO:97); (52) LAHQDTTKKT (SEQ ID NO:98); (53) LAHQDTTKND (SEQ ID NO:99); (54) LAHQDTTKNT (SEQ ID NO:100); (55) LAHQDTTKNV (SEQ ID NO:101); (56) LAHQDTTKTM (SEQ ID NO:102); (57) LAHQNTTKNA (SEQ ID NO:103); (58) LAHRDTTKNA (SEQ ID NO:104); (59) LAISDQTNHA (SEQ ID NO:105); (60) LAKQKSASTA (SEQ ID NO:106); (61) LAKSDQCKPA (SEQ ID NO:107); (62) LAKSDQSKPD (SEQ ID NO:108); (63) LAKSDQSNPA(SEQ ID NO:109); (64) LAKSYQSKPA (SEQ ID NO:110); (65) LANQDTTKNA (SEQ ID NO:111); (66) LAPQNTTKNA (SEQ ID NO:112); (67) LAPSSIQKPA (SEQ ID NO:113); (68) LAQQDTTKNA (SEQ ID NO:114); (69) LAYQDTTKNA (SEQ ID NO:115); (70) LDHQDTTKNA (SEQ ID NO:116); (71) LDHQDTTKSA (SEQ ID NO:117); (72) LGHQDTTKNA (SEQ ID NO:118); (73) LPHQDTTKND (SEQ ID NO:119); (74) LPHQDTTKNT (SEQ ID NO:120); (75) LPHQDTTNNA (SEQ ID NO:121); (76) LTHQDTTKNA (SEQ ID NO:122); (77) LTKDATKTIA (SEQ ID NO:123); (78) LTPQDTTKNA (SEQ ID NO:124); and (79) LVHQDTTKNA (SEQ ID NO:125).

[0073] The peptide insert is, in some cases, a peptide of **Formula II**:

[0074] $X_1X_2X_3X_4X_5X_6X_7X_8X_9X_{10}$, where:

[0075] X_1 is Leu, Ile, or Pro;

[0076] X_2 is Ala, Pro, or Ser;

[0077] X_3 is Lys, His, Thr, Ile, Pro, Val, Arg, or Ala;

[0078] X_4 (if present) is Gln, Asp, Ser, Gly, Thr, Ile, or Asn;

[0079] X_5 is Asp, Ser, Gln, Val, Thr, Gly, or Ala;

[0080] X_6 isThr, Ala, Gln, Ser, Glu, or Pro;

[0081] X_7 is Thr, Ser, Asn, Pro, Leu, Gln, Lys, or Ala;

[0082] X_8 is Lys, Ser, Arg, or Thr;

[0083] X_9 is Asn, Pro, Ser, Lys, His, Ile, Thr, or Ala; and

[0084] X_{10} is Ala.

[0085] Peptide inserts of **Formula II** include, but are not limited to, (1) LAKDATKNA (SEQ ID NO:47); (2) PAHQDTTKNA (SEQ ID NO:48); (3) LAHQDTTKNA (SEQ ID NO:49); (4) LATTSQNKPA (SEQ ID NO:50); (5) LAISDQTKHA (SEQ ID NO:51); (6) IARGVAPSSA (SEQ ID NO:52); (7) LAPDSTTRSA (SEQ ID NO:53); (8) LAKGTELKPA (SEQ ID NO:54);

(9) LAIIDATKNA (SEQ ID NO:55); (10) LAVDGAQRSA (SEQ ID NO:56); (11) PAPQDTTKKA (SEQ ID NO:57); (12) LPHQDTTKNA (SEQ ID NO:58); (13) LAKDATKTIA (SEQ ID NO:59); (14) LAKQQSASTA (SEQ ID NO:60); (15) LAKSDQSKPA (SEQ ID NO:61); (16) LSHQDTTKNA(SEQ ID NO:62); (17) LAANQPSKPA (SEQ ID NO:63); (18) LAVSDSTKAA (SEQ ID NO:64); (19) LAAQGTAKKPA (SEQ ID NO:65); (20) LAPDQTTRNA (SEQ ID NO:66); (21) LAASDSTKAA (SEQ ID NO:67); (22) LAPQDTTKNA (SEQ ID NO:68); (23) LAKADETRPA (SEQ ID NO:69); (24) LAHQDTAKNA (SEQ ID NO:70); (25) LAHQDTKKNA (SEQ ID NO:71); (26) LAHQDTTKHA (SEQ ID NO:72); (27) LAHQDTTKKA (SEQ ID NO:73); (28) LAHQDTTRNA (SEQ ID NO:74); (29) LAHQDTTNA (SEQ ID NO:75); (30) LAHQGTTKNA (SEQ ID NO:76); (31) LAHQVTTKNA (SEQ ID NO:77); (32) LAISDQSKPA (SEQ ID NO:78); (33) LADATKTA (SEQ ID NO:79); (34) LAKDTTKNA (SEQ ID NO:80); (35) LAKSDQSRPA (SEQ ID NO:81); (36) LAPQDTKKNA (SEQ ID NO:82); (37) LATSDSTKAA (SEQ ID NO:83); (38) LAVDGSQRSA (SEQ ID NO:84); (39) LPISDQTKHA (SEQ ID NO:85); (40) LPKDATKTIA (SEQ ID NO:86); (41) LPPQDTTKNA (SEQ ID NO:87); and (42) PAPQDTTKNA (SEQ ID NO:88).

[0086] Peptides of **Formula II** include, but are not limited to: (1) LAKDATKNA (SEQ ID NO:47); (2) PAHQDTTKNA (SEQ ID NO:48); (3) LAHQDTTKNA (SEQ ID NO:49); (4) LATTSQNKPA (SEQ ID NO:50); (5) LAISDQTKHA (SEQ ID NO:51); (6) IARGVAPSSA (SEQ ID NO:52); (7) LAPDSTTRSA (SEQ ID NO:53); (8) LAKGTELKPA (SEQ ID NO:54); (9) LAIIDATKNA (SEQ ID NO:55); (10) LAVDGAQRSA (SEQ ID NO:56); (11) PAPQDTTKKA (SEQ ID NO:57); (12) LPHQDTTKNA (SEQ ID NO:58); (13) LAKDATKTIA (SEQ ID NO:59); (14) LAKQQSASTA (SEQ ID NO:60); (15) LAKSDQSKPA (SEQ ID NO:61); (16) LSHQDTTKNA (SEQ ID NO:62); (17) LAANQPSKPA (SEQ ID NO:63); and (18) LAVSDSTKAA (SEQ ID NO:64). In some cases, the peptide insert is (1) LAKDATKNA (SEQ ID NO:47). In some cases, the peptide insert is (2) PAHQDTTKNA (SEQ ID NO:48). In some cases, the peptide insert is (3) LAHQDTTKNA (SEQ ID NO:49). In some cases, the peptide insert is (4) LATTSQNKPA (SEQ ID NO:50). In some cases, the peptide insert is (5) LAISDQTKHA (SEQ ID NO:51). In some cases, the peptide insert is (6) IARGVAPSSA (SEQ ID NO:52). In some cases, the peptide insert is (7) LAPDSTTRSA (SEQ ID NO:53). In some cases, the peptide insert is (8) LAKGTELKPA (SEQ ID NO:54). In some cases, the peptide insert is (9) LAIIDATKNA (SEQ ID NO:55). In some cases, the peptide insert is (10) LAVDGAQRSA (SEQ ID NO:56). In some cases, the peptide insert is (11) PAPQDTTKKA (SEQ ID NO:57). In some cases, the peptide insert is (12) LPHQDTTKNA (SEQ ID NO:58). In some cases, the peptide insert is (13) LAKDATKTIA (SEQ ID NO:59). In some cases, the

peptide insert is (14) LAKQQSASTA (SEQ ID NO:60). In some cases, the peptide insert is (15) LAKSDQSKPA (SEQ ID NO:61). In some cases, the peptide insert is (16) LSHQDTTKNA (SEQ ID NO:62). In some cases, the peptide insert is (17) LAANQPSKPA (SEQ ID NO:63). In some cases, the peptide insert is (18) LAVSDSTKAA (SEQ ID NO:64).

[0087] The peptide insert is, in some cases, a peptide of **Formula III**:

[0088] $X_1X_2X_3X_4X_5X_6X_7X_8X_9X_{10}$, where:

[0089] X_1 is Leu, Ile, or Pro;

[0090] X_2 is Ala, Pro, or Ser;

[0091] X_3 is Lys, His, Thr, Ile, Pro, Val, Arg, or Ala;

[0092] X_4 (if present) is Gln, Asp, Ser, Gly, Thr, Ile, or Asn;

[0093] X_5 is Asp, Ser, Gln, Val, Thr, Gly, or Ala;

[0094] X_6 isThr, Ala, Gln, Ser, Glu, or Pro;

[0095] X_7 is Thr, Ser, Asn, Pro, Leu, Gln, Lys, or Ala;

[0096] X_8 is Lys, Ser, Arg, or Thr;

[0097] X_9 is Asn, Pro, Ser, Lys, His, Ile, Thr, or Ala; and

[0098] X10 is Ala, Thr, Asp Val, or Met.

[0099] Peptide inserts of **Formula III** include, but are not limited to, (1) LAKDATKNA (SEQ ID NO:47); (2) PAHQDTTKNA (SEQ ID NO:48); (3) LAHQDTTKNA (SEQ ID NO:49); (6) IARGVAPSSA (SEQ ID NO:52);(7) LAPDSTTRSA (SEQ ID NO:53); (8) LAKGTELKPA (SEQ ID NO:54); (9) LAIIDATKNA (SEQ ID NO:55); (10) LAVDGAQRSA (SEQ ID NO:56); (11) PAPQDTTKKA (SEQ ID NO:57); (12) LPHQDTTKNA (SEQ ID NO:58); (13) LAKDATKTIA (SEQ ID NO:59); (14) LAKQQSASTA (SEQ ID NO:60);(16) LSHQDTTKNA (SEQ ID NO:62); (17) LAANQPSKPA (SEQ ID NO:63); (18) LAVSDSTKAA (SEQ ID NO:64); (19) LAAQGTAKPA (SEQ ID NO:65); (20) LAPDQTTRNA (SEQ ID NO:66); (24) LAHQDTAKNA (SEQ ID NO:70); (25) LAHQDTKKNA (SEQ ID NO:71); (26) LAHQDTTKHA (SEQ ID NO:72); (27) LAHQDTTKKA (SEQ ID NO:73); (28) LAHQDTTRNA (SEQ ID NO:74); (29) LAHQDTTTNA (SEQ ID NO:75); (30) LAHQGTTKNA (SEQ ID NO:76); (21) LAASDSTKAA (SEQ ID NO:67); (22) LAPQDTTKNA (SEQ ID NO:68); (31) LAHQVTTKNA (SEQ ID NO:77); (33) LAKDATKTA (SEQ ID NO:79); (34) LAKDTTKNA (SEQ ID NO:80); (36) LAPQDTKKNA (SEQ ID NO:82); (37) LATSDSTKAA (SEQ ID NO:83); (38) LAVDGSQRSA (SEQ ID NO:84); (41) LPPQDTTKNA (SEQ ID NO:87); (42) PAPQDTTKNA (SEQ ID NO:88); (52) LAHQDTTKKT (SEQ ID NO:98); (53) LAHQDTTKND (SEQ ID NO:99); (54) LAHQDTTKNT (SEQ ID NO:100); (55) LAHQDTTKNV (SEQ ID NO:101); (56)

LAHQDTTKTM (SEQ ID NO:102); (73) LPHQDTTKND (SEQ ID NO:119); and (74) LPHQDTTKNT (SEQ ID NO:120).

[00100] The peptide insert is, in some cases, a peptide of **Formula IV**:

[00101] $X_1X_2X_3X_4X_5X_6X_7X_8X_9X_{10}$, where:

[00102] X_1 is Leu;

[00103] X_2 is Ala;

[00104] X_3 is Lys, His, Thr, Ile, Pro, or Val;

[00105] X_4 (if present) is Gln, Asp, Ser, or Gly;

[00106] X_5 is Asp, Ser, or Gln;

[00107] X_6 is Thr, Ala, Gln, or Ser;

[00108] X_7 is Thr or Ser;

[00109] X_8 is Lys, Ser, or Arg;

[00110] X_9 is Asn, Pro, or Ser; and

[00111] X_{10} is Ala.

[00112] Peptide inserts of **Formula IV** include, but are not limited to, (1) LAKDATKNA (SEQ ID NO:47); (3) LAHQDTTKNA (SEQ ID NO:49); (7) LAPDSTTRSA (SEQ ID NO:53); (15) LAKSDQSKPA (SEQ ID NO:61); (20) LAPDQTTRNA (SEQ ID NO:66); (22) LAPQDTTKNA (SEQ ID NO:68) ;(28) LAHQDTTRNA (SEQ ID NO:74); (32) LAISDQSKPA (SEQ ID NO:78); (34) LAKDTTKNA (SEQ ID NO:80); and (35) LAKSDQSRPA (SEQ ID NO:81).

[00113] The peptide insert is, in some cases, a peptide of **Formula V**:

[00114] $X_1X_2X_3X_4X_5X_6X_7X_8X_9X_{10}$, where:

[00115] X_1 is Leu;

[00116] X_2 is Ala;

[00117] X_3 is Lys or His;

[00118] X_4 (if present) is Gln, Asp, Ser, or Gly;

[00119] X_5 is Asp, Ser, or Gln;

[00120] X_6 is Thr, Ala, Gln, or Ser;

[00121] X_7 is Thr or Ser;

[00122] X_8 is Lys, Ser, or Arg;

[00123] X_9 is Asn, Pro, or Ser; and

[00124] X_{10} is Ala.

[00125] Peptide inserts of **Formula V** include, but are not limited to, (1) LAKDATKNA (SEQ ID NO:47); (15) LAKSDQSKPA (SEQ ID NO:51); (34) LAKDTTKNA (SEQ ID NO:80); and (35) LAKSDQSRPA (SEQ ID NO:81).

[00126] The peptide insert is, in some cases, a peptide of **Formula VI**:

[00127] $X_1X_2X_3X_4X_5X_6X_7X_8X_9X_{10}$, where:

[00128] X_1 is Leu;

[00129] X_2 is Ala;

[00130] X_3 is Asn, Lys, Thr, Gln, Ser, Ile, or Leu;

[00131] X_4 is Ser, Ala, Thr, Glu, Gln, Gly, Lys, or Pro;

[00132] X_5 is Asp, Pro, Glu, Thr, Asn, or Arg;

[00133] X_6 is Ile, His, Thr, Gln, Asn, Tyr, Asp, or Glu;

[00134] X_7 is Gln, Thr, Asn, Ala, or Lys;

[00135] X_8 is Lys, Thr, Arg, or Asp;

[00136] X_9 is Pro, Asn, Thr, Arg, Lys, or Ser; and

[00137] X_{10} is Ala.

[00138] Peptides of **Formula VI** include, but are not limited to: (80) LAKANQNTPA (SEQ ID NO:126); (81) LATTPITKPA (SEQ ID NO:127); (82) LATTPIAKPA (SEQ ID NO:128); (83) LAIEDHTKSA (SEQ ID NO:129); (84) LAQSEHQRPA (SEQ ID NO:130); (85) LAKSPNKDNA (SEQ ID NO:131); (86) LANQDYTKTA (SEQ ID NO:132); (87) LANSTDQTRA (SEQ ID NO:133); (88) LALGETTRPA (SEQ ID NO:134); (89) LANSTEQTRA (SEQ ID NO:135); (90) LAQADTTKNA (SEQ ID NO:136); (91) LASKDITKTA (SEQ ID NO:137); and (92) LASPRHNKKC (SEQ ID NO:138).

[00139] In some cases, the peptide insert is a peptide of **Formula VII**: $LAHQDTTKX_1X_2X_3$ (SEQ ID NO:148), where X_1 is Lys, Thr, Asn, or His; X_2 is Ala, Thr, Val, Ile, Met, or Asp; and X_3, if present, is Ala. Peptides of **Formula VII** include, but are not limited to: (26) LAHQDTTKHA (SEQ ID NO:72); (27) LAHQDTTKKA (SEQ ID NO:73); (52) LAHQDTTKKT (SEQ ID NO:98); (53) LAHQDTTKND (SEQ ID NO:99); (54) LAHQDTTKNT (SEQ ID NO:100); (55) LAHQDTTKNV (SEQ ID NO:101); (56) LAHQDTTKTM (SEQ ID NO:102); and (93) LAHQDTTKTIA (SEQ ID NO:139).

[00140] In some cases, the peptide insert is a peptide of **Formula VIII**: $LAX_1QX_2TX_3X_4X_5X_6$ (SEQ ID NO:149), where X_1 is Ala, Pro, Asp, or His; X_2 is Gly or Asp; X_3 is Ala, Thr, or Lys; X_4 is Asn, Glu, Lys, Arg, or Thr; X_5 is Leu, Asn, Lys, or Thr; and X_6, if present, is Ala, Thr, Asp, Val, or Met. Peptides of **Formula VIII** include, but are not limited to, (94) LAAQGTANL (SEQ ID NO:140); (22) LAPQDTTKNA (SEQ ID NO:68); (46) LADQDTTKNA (SEQ ID

NO:92); (24) LAHQDTAKNA (SEQ ID NO:70); (25) LAHQDTKKNA (SEQ ID NO:71); (26) LAHQDTTKHA (SEQ ID NO:72); (27) LAHQDTTKKA (SEQ ID NO:73); (28) LAHQDTTRNA (SEQ ID NO:74); (29) LAHQDTTTNA (SEQ ID NO:75); (50) LAHQDTTENA (SEQ ID NO:96); (51) LAHQDTTINA (SEQ ID NO:97); (52) LAHQDTTKKT (SEQ ID NO:98); (53) LAHQDTTKND (SEQ ID NO:99); (54) LAHQDTTKNT (SEQ ID NO:100); (55) LAHQDTTKNV (SEQ ID NO:101); and (56) LAHQDTTKTM (SEQ ID NO:102).

[00141] In some cases, the peptide insert is a peptide of **Formula IX**: $X_1AX_2X_3DX_4TKX_5A$ (SEQ ID NO:150), where X_1 is Val or Leu; X_2 is Ile, Val, His, or Asp; X_3 is Glu, Ser, Lys, or Gln; X_4 is His, Ser, or Thr; and X_5 is Ser, Ala, Asn, His, or Lys. Peptides of **Formula IX** include, but are not limited to, (95) VAIEDHTKSA (SEQ ID NO:141); (18) LAVSDSTKAA (SEQ ID NO:64); (46) LADQDTTKNA (SEQ ID NO:92); (48) LAHKDTTKNA (SEQ ID NO:94); (26) LAHQDTTKHA (SEQ ID NO:72); and (27) LAHQDTTKKA (SEQ ID NO:73).

[00142] In some cases, the peptide insert is a peptide of **Formula X**: $X_1X_2X_3AX_4QX_5TX_6KNA$ (SEQ ID NO:151), where X_1, if present, is Leu; X_2, if present, is Ala; X_3 is Lys, Leu, or Pro; X_4 is Asn, His, Pro, or Tyr; X_5 is Asn, Gly, Val, or Asp; and X_6 is Pro or Thr. Peptides of **Formula X** include, but are not limited to, (96) LAKANQNTPKNA (SEQ ID NO:142); (57) LAHQNTTKNA (SEQ ID NO:103); (66) LAPQNTTKNA (SEQ ID NO:112); (69) LAYQDTTKNA (SEQ ID NO:115); (30) LAHQGTTKNA (SEQ ID NO:76); (31) LAHQVTTKNA (SEQ ID NO:77); and (42) PAPQDTTKNA (SEQ ID NO:88).

[00143] In some cases, the peptide insert is LAHQDTTKKX (SEQ ID NO:143), where X is any amino acid. In some cases, the peptide insert is LAHQDTTKKX (SEQ ID NO:143), where X is Ala, Thr, Asp, Val, or Met. In some cases, the peptide insert is (27) LAHQDTTKKA (SEQ ID NO:73). In some cases, the peptide insert is (52) LAHQDTTKKT (SEQ ID NO:98). In some cases, the peptide insert is LAHQDTTKKD (SEQ ID NO:144). In some cases, the peptide insert is LAHQDTTKKV (SEQ ID NO:145). In some cases, the peptide insert is LAHQDTTKKM (SEQ ID NO:146).

[00144] In some cases, the peptide insert is not (88) LALGETTRPA (SEQ ID NO:134). In some cases, the peptide insert is not LGETTRP (SEQ ID NO:147).

[00145] Suitable peptide inserts include, but are not limited to, (1) LAKDATKNA (SEQ ID NO:47); (2) PAHQDTTKNA (SEQ ID NO:48); (3) LAHQDTTKNA (SEQ ID NO:49); (4) LATTSQNKPA (SEQ ID NO:50); (5) LAISDQTKHA (SEQ ID NO:51); (6) IARGVAPSSA (SEQ ID NO:52); (7) LAPDSTTRSA (SEQ ID NO:53); (8) LAKGTELKPA (SEQ ID NO:54); (9) LAIIDATKNA (SEQ ID NO:55); (10) LAVDGAQRSA (SEQ ID NO:56); (11)

PAPQDTTKKA (SEQ ID NO:57); (12) LPHQDTTKNA (SEQ ID NO:58); (13) LAKDATKTIA (SEQ ID NO:59); (14) LAKQQSASTA (SEQ ID NO:60); (15) LAKSDQSKPA (SEQ ID NO:61); (16) LSHQDTTKNA (SEQ ID NO:62); (17) LAANQPSKPA (SEQ ID NO:63); (18) LAVSDSTKAA (SEQ ID NO:64); (19) LAAQGTAKKPA (SEQ ID NO:65); (20) LAPDQTTRNA (SEQ ID NO:66); (21) LAASDSTKAA (SEQ ID NO:67); (22) LAPQDTTKNA (SEQ ID NO:68); (23) LAKADETRPA (SEQ ID NO:69); (24) LAHQDTAKNA (SEQ ID NO:70); (25) LAHQDTKKNA (SEQ ID NO:71); (26) LAHQDTTKHA (SEQ ID NO:72); (27) LAHQDTTKKA (SEQ ID NO:73); (28) LAHQDTTRNA (SEQ ID NO:74); (29) LAHQDTTNA (SEQ ID NO:75); (30) LAHQGTTKNA (SEQ ID NO:76); (31) LAHQVTTKNA (SEQ ID NO:77); (32) LAISDQSKPA (SEQ ID NO:78); (33) LADATKTA (SEQ ID NO:79); (34) LAKDTTKNA (SEQ ID NO:80); (35) LAKSDQSRPA (SEQ ID NO:81); (36) LAPQDTKKNA (SEQ ID NO:82); (37) LATSDSTKAA (SEQ ID NO:83); (38) LAVDGSQRSA (SEQ ID NO:84); (39) LPISDQTKHA (SEQ ID NO:85); (40) LPKDATKTIA (SEQ ID NO:86); (41) LPPQDTTKNA (SEQ ID NO:87); (42) PAPQDTTKNA (SEQ ID NO:88); (43) QAHQDTTKNA (SEQ ID NO:89); (44) LAHETSPRPA (SEQ ID NO:90); (45) LAKSTSTAPA (SEQ ID NO:91); (46) LADQDTTKNA (SEQ ID NO:92); (47) LAESDQSKPA (SEQ ID NO:93); (48) LAHKDTTKNA (SEQ ID NO:94); (49) LAHKTQQKM (SEQ ID NO:95); (50) LAHQDTTENA (SEQ ID NO:96); (51) LAHQDTTINA (SEQ ID NO:97); (52) LAHQDTTKKT (SEQ ID NO:98); (53) LAHQDTTKND (SEQ ID NO:99); (54) LAHQDTTKNT (SEQ ID NO:100); (55) LAHQDTTKNV (SEQ ID NO:101); (56) LAHQDTTKTM (SEQ ID NO:102); (57) LAHQNTTKNA (SEQ ID NO:103); (58) LAHRDTTKNA (SEQ ID NO:104); (59) LAISDQTNHA (SEQ ID NO:105); (60) LAKQKSASTA (SEQ ID NO:106); (61) LAKSDQCKPA (SEQ ID NO:107); (62) LAKSDQSKPD (SEQ ID NO:108); (63) LAKSDQSNPA(SEQ ID NO:109); (64) LAKSYQSKPA (SEQ ID NO:110); (65) LANQDTTKNA (SEQ ID NO:111); (66) LAPQNTTKNA (SEQ ID NO:112); (67) LAPSSIQKPA (SEQ ID NO:113); (68) LAQQDTTKNA (SEQ ID NO:114); (69) LAYQDTTKNA (SEQ ID NO:115); (70) LDHQDTTKNA (SEQ ID NO:116); (71) LDHQDTTKSA (SEQ ID NO:117); (72) LGHQDTTKNA (SEQ ID NO:118); (73) LPHQDTTKND (SEQ ID NO:119); (74) LPHQDTTKNT (SEQ ID NO:120); (75) LPHQDTTNNA (SEQ ID NO:121); (76) LTHQDTTKNA (SEQ ID NO:122); (77) LTKDATKTIA (SEQ ID NO:123); (78) LTPQDTTKNA (SEQ ID NO:124); (79) LVHQDTTKNA (SEQ ID NO:125); (80) LAKANQNTPA (SEQ ID NO:126); (81) LATTPITKPA (SEQ ID NO:127); (82) LATTPIAKPA (SEQ ID NO:128); (83) LAIEDHTKSA (SEQ ID NO:129); (84) LAQSEHQRPA (SEQ ID NO:130); (85) LAKSPNKDNA (SEQ ID

NO:131); (86) LANQDYTKTA (SEQ ID NO:132); (87) LANSTDQTRA (SEQ ID NO:133); (88) LALGETTRPA (SEQ ID NO:134); (89) LANSTEQTRA (SEQ ID NO:135); (90) LAQADTTKNA (SEQ ID NO:136); (91) LASKDITKTA (SEQ ID NO:137); (92) LASPRHNKKC (SEQ ID NO:138); (93) LAHQDTTKTIA (SEQ ID NO:139); (94) LAAQGTANL (SEQ ID NO:140); (95) VAIEDHTKSA (SEQ ID NO:141); and (96) LAKANQNTPKNA (SEQ ID NO:142).

[00146] In some cases, the peptide insert is (11) PAPQDTTKKA (SEQ ID NO:57). In some cases, the peptide insert is (7) LAPDSTTRSA (SEQ ID NO:53).

[00147] In some embodiments, a subject rAAV virion capsid does not include any other amino acid substitutions, insertions, or deletions, other than an insertion of from about 5 amino acids to about 20 amino acids (e.g., 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 amino acids; e.g., 9 amino acids, 10 amino acids, 11 amino acids, or 12 amino acids) in the GH loop or loop IV relative to a corresponding parental AAV capsid protein. In other embodiments, a subject rAAV virion capsid includes from 1 to about 25 amino acid insertions, deletions, or substitutions, compared to the parental AAV capsid protein, in addition to an insertion of from about 5 amino acids to about 20 amino acids (e.g., 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 amino acids; e.g., 9 amino acids, 10 amino acids, 11 amino acids, or 12 amino acids) in the GH loop or loop IV relative to a corresponding parental AAV capsid protein. For example, in some embodiments, a subject rAAV virion capsid includes from 1 to about 5, from about 5 to about 10, from about 10 to about 15, from about 15 to about 20, or from about 20 to about 25 amino acid insertions, deletions, or substitutions, compared to the parental AAV capsid protein, in addition to an insertion of from about 5 amino acids to about 20 amino acids (e.g., 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 amino acids; e.g., 9 amino acids, 10 amino acids, 11 amino acids, or 12 amino acids) in the GH loop or loop IV relative to a corresponding parental AAV capsid protein.

[00148] In some cases, a subject rAAV virion capsid does not include one, two, three, or four, of the following amino acid substitutions: Y273F, Y444F, Y500F, and Y730F.

[00149] In some cases, a subject variant capsid polypeptide comprises, in addition to an insertion peptide as described above, one, two, three, or four, of the following amino acid substitutions: Y273F, Y444F, Y500F, and Y730F.

[00150] In some cases, a subject rAAV virion capsid is a chimeric capsid, e.g., the capsid comprises a portion of an AAV capsid of a first AAV serotype and a portion of an AAV capsid of a second serotype; and comprises an insertion of from about 5 amino acids to about 20 amino acids (e.g., 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 amino acids; e.g., 9 amino

acids, 10 amino acids, 11 amino acids, or 12 amino acids) in the GH loop or loop IV relative to a corresponding parental AAV capsid protein.

[00151] In some embodiments, a subject rAAV virion comprises a capsid protein comprising 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%, amino acid sequence identity to the amino acid sequence provided in FIG. 4; and an insertion of from about 5 amino acids to about 20 amino acids (e.g., 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 amino acids; e.g., 9 amino acids, 10 amino acids, 11 amino acids, or 12 amino acids) in the GH loop or loop IV relative to a corresponding parental AAV capsid protein. In some embodiments, a subject rAAV virion comprises a capsid protein comprising 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%, amino acid sequence identity to the amino acid sequence provided in FIG. 4; and an insertion of from about 5 amino acids to about 20 amino acids (e.g., 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 amino acids; e.g., 9 amino acids, 10 amino acids, 11 amino acids, or 12 amino acids) between amino acids 587 and 588 relative to the amino acid sequence depicted in FIG. 4, or at a corresponding site relative to a corresponding parental AAV capsid protein.

[00152] In some embodiments, a subject rAAV virion comprises a capsid protein that includes a GH loop comprising an amino acid sequence having at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity to an amino acid sequence set forth in FIG. 5, and comprising an insertion of from about 5 amino acids to about 20 amino acids (e.g., 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 amino acids; e.g., 9 amino acids, 10 amino acids, 11 amino acids, or 12 amino acids) between the bolded and underlined amino acids.

[00153] In some embodiments, a subject rAAV virion comprises a capsid protein comprising 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%, amino acid sequence identity to any one of the amino acid sequences provided in FIG. 6A-6C; and an insertion of from about 5 amino acids to about 20 amino acids (e.g., 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 amino acids; e.g., 9 amino acids, 10 amino acids, 11 amino acids, or 12 amino acids) between amino acids 587 and 588 of AAV2, or at a corresponding site relative to another AAV genotype. In some cases, the corresponding insertion site is a site as indicated by bold text and underlining in FIG. 6B.

[00154] A subject rAAV virion exhibits 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 infectivity of a retinal cell, compared to the infectivity of the retinal cell by an AAV virion comprising the corresponding parental AAV capsid protein.

[00155] In some cases, a subject rAAV virion exhibits 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 infectivity of a retinal cell, when administered via intravitreal injection, compared to the infectivity of the retinal cell by an AAV virion comprising the corresponding parental AAV capsid protein, when administered via intravitreal injection.

[00156] In some embodiments, a subject rAAV virion exhibits 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 infectivity of a photoreceptor (rod or cone) cell, compared to the infectivity of the photoreceptor cell by an AAV virion comprising the corresponding parental AAV capsid protein.

[00157] In some embodiments, a subject rAAV virion exhibits 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 infectivity of a photoreceptor (rod or cone) cell, when administered via intravitreal injection, compared to the infectivity of the photoreceptor cell by an AAV virion comprising the corresponding parental AAV capsid protein, when administered via intravitreal injection.

[00158] In some embodiments, a subject rAAV virion exhibits 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 infectivity of an RGC, compared to the infectivity of the RGC by an AAV virion comprising the corresponding parental AAV capsid protein.

[00159] In some embodiments, a subject rAAV virion exhibits 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 infectivity of an RGC, when administered via intravitreal injection, compared to the infectivity of the RGC by an AAV virion comprising the corresponding parental AAV capsid protein, when administered via intravitreal injection.

[00160] In some embodiments, a subject rAAV virion exhibits 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 infectivity of an RPE cell, compared to the infectivity of the RPE cell by an AAV virion comprising the corresponding parental AAV capsid protein.

[00161] In some embodiments, a subject rAAV virion exhibits 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 infectivity of an RPE cell, when administered via intravitreal injection, compared to the infectivity of the RPE cell by an AAV virion comprising the corresponding parental AAV capsid protein, when administered via intravitreal injection.

[00162] In some embodiments, a subject rAAV virion exhibits 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

infectivity of a Müller cell, compared to the infectivity of the Müller cell by an AAV virion comprising the corresponding parental AAV capsid protein.

[00163] In some embodiments, a subject rAAV virion exhibits 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 infectivity of a Müller cell, when administered via intravitreal injection, compared to the infectivity of the Müller cell by an AAV virion comprising the corresponding parental AAV capsid protein, when administered via intravitreal injection.

[00164] In some embodiments, a subject rAAV virion exhibits 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 infectivity of a bipolar cell, compared to the infectivity of the bipolar cell by an AAV virion comprising the corresponding parental AAV capsid protein.

[00165] In some embodiments, a subject rAAV virion exhibits 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 infectivity of a bipolar cell, when administered via intravitreal injection, compared to the infectivity of the bipolar cell by an AAV virion comprising the corresponding parental AAV capsid protein, when administered via intravitreal injection.

[00166] In some embodiments, a subject rAAV virion exhibits 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 infectivity of an amacrine cell, compared to the infectivity of the amacrine cell by an AAV virion comprising the corresponding parental AAV capsid protein.

[00167] In some embodiments, a subject rAAV virion exhibits 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 infectivity of an amacrine cell, when administered via intravitreal injection, compared to the infectivity of the amacrine cell by an AAV virion comprising the corresponding parental AAV capsid protein, when administered via intravitreal injection.

[00168] In some embodiments, a subject rAAV virion exhibits 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 infectivity of a horizontal cell, compared to the infectivity of the horizontal cell by an AAV virion comprising the corresponding parental AAV capsid protein.

[00169] In some embodiments, a subject rAAV virion exhibits 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 infectivity of a horizontal cell, when administered via intravitreal injection, compared to the infectivity of the horizontal cell by an AAV virion comprising the corresponding parental AAV capsid protein, when administered via intravitreal injection.

[00170] In some embodiments, a subject rAAV virion exhibits 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 infectivity of a retinal astrocyte, compared to the infectivity of the retinal astrocyte by an AAV virion comprising the corresponding parental AAV capsid protein.

[00171] In some embodiments, a subject rAAV virion exhibits 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 infectivity of a retinal astrocyte, when administered via intravitreal injection, compared to the infectivity of the retinal astrocyte by an AAV virion comprising the corresponding parental AAV capsid protein, when administered via intravitreal injection.

[00172] In some cases, a subject rAAV virion exhibits 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 ability to cross the internal limiting membrane (ILM), compared to the ability of an AAV virion comprising the corresponding parental AAV capsid protein to cross the ILM.

[00173] In some cases, a subject rAAV virion exhibits 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 ability, when administered via intravitreal injection, to cross the ILM, compared to the ability of an AAV virion comprising the corresponding parental AAV capsid protein to cross the ILM when administered via intravitreal injection.

[00174] A subject rAAV virion can cross the ILM, and can also traverse cell layers, including Müller cells, amacrine cells, etc., to reach the photoreceptor cells and or RPE cells. For example, a subject rAAV virion, when administered via intravitreal injection, can cross the ILM, and can also traverse cell layers, including Müller cells, amacrine cells, etc., to reach the photoreceptor cells and or RPE cells.

[00175] In some cases, a subject rAAV virion, when injected intravitreally, exhibits 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 localization past the ILM, compared to the extent of localization past the ILM by an intravitreally injected control AAV virion comprising the corresponding parental AAV capsid protein. For example, in some cases, a subject rAAV virion, when injected intravitreally, exhibits 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 localization to the retinal pigment epithelium (RPE), compared to the extent of localization to the RPE layer by an intravitreally injected control AAV virion comprising the corresponding parental AAV capsid protein. As another example, in some cases, a subject rAAV virion, when injected intravitreally, exhibits 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 localization to the photoreceptor (PR) layer, compared to the extent of localization to the PR layer by an intravitreally injected control AAV virion comprising the corresponding parental AAV capsid protein. As another example, in some cases, a subject rAAV virion, when injected intravitreally, exhibits 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 localization to the inner nuclear layer, compared to the extent of localization to the inner nuclear layer by an intravitreally injected control AAV virion comprising the corresponding parental AAV capsid protein. As another example, in some cases, a subject rAAV virion, when injected intravitreally, exhibits 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 localization to the outer nuclear layer, compared to the extent of localization to the outer nuclear layer by an intravitreally injected control AAV virion comprising the corresponding parental AAV capsid protein. As another example, in some cases, a subject rAAV virion, when injected intravitreally, exhibits 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 localization to the ganglion cell layer, compared to the extent of localization to the ganglion cell layer by an intravitreally injected control AAV virion comprising the corresponding parental AAV capsid protein.

[00176] In some embodiments, a subject rAAV virion selectively infects a retinal cell, e.g., a subject rAAV virion infects a retinal cell with 10-fold, 15-fold, 20-fold, 25-fold, 50-fold, or more than 50-fold, specificity than a non-retinal cell, e.g., a cell outside the eye. For example, in some embodiments, a subject rAAV virion selectively infects a retinal cell, e.g., a subject rAAV virion infects a photoreceptor cell with 10-fold, 15-fold, 20-fold, 25-fold, 50-fold, or more than 50-fold, specificity than a non-retinal cell, e.g., a cell outside the eye.

[00177] In some embodiments, a subject rAAV virion selectively infects a photoreceptor cell, e.g., a subject rAAV virion infects a photoreceptor cell with 10-fold, 15-fold, 20-fold, 25-fold, 50-fold, or more than 50-fold, specificity than a non-photoreceptor cell present in the eye, e.g., a retinal ganglion cell, a Müller cell, etc.

[00178] In some embodiments, a subject rAAV virion exhibits 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 infectivity of a photoreceptor cell, when administered via intravitreal injection, compared to the infectivity of the photoreceptor cell by an AAV virion comprising the corresponding parental AAV capsid protein, when administered via intravitreal injection.

Gene products

[00179] A subject rAAV virion comprises a heterologous nucleic acid comprising a nucleotide sequence encoding a gene product (a heterologous gene product. In some cases, the gene product

is a polypeptide. In some cases, the gene product is an RNA. Where the gene product is an RNA, in some cases, the RNA gene product encodes a polypeptide. In some cases, an rAAV virion of the present disclosure comprises a single heterologous nucleic acid comprising a nucleotide sequence encoding a single heterologous gene product. In some cases, an rAAV virion of the present disclosure comprises a single heterologous nucleic acid comprising a nucleotide sequence encoding two heterologous gene products. In some cases, an rAAV virion of the present disclosure comprises two heterologous nucleic acids, each comprising a nucleotide sequence encoding a heterologous gene product.

[00180] In some embodiments, the gene product is an interfering 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 site-specific nuclease that provide for site-specific knock-down of gene function. In some embodiments, the gene product is an RNA-guided endonuclease that provides for modification of a target nucleic acid.

Interfering RNA

[00181] Where the gene product is an interfering RNA (RNAi), suitable RNAi include RNAi that decrease the level of an apoptotic or angiogenic factor in a cell. For example, an RNAi can be an shRNA or siRNA that reduces the level of a gene product that induces or promotes apoptosis in a cell. 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 gene products include, e.g., *Bax*, *Bid*, *Bak*, and *Bad* gene products. See, e.g., U.S. Patent No. 7,846,730.

[00182] Interfering RNAs could also be against an angiogenic product, for example vascular endothelial growth factor (VEGF) (e.g., Cand5; see, e.g., U.S. Patent Publication No. 2011/0143400; U.S. Patent Publication No. 2008/0188437; and Reich et al. (2003) *Mol. Vis.* 9:210); VEGF receptor-1 (VEGFR1) (e.g., Sirna-027; see, e.g., Kaiser et al. (2010) *Am. J. Ophthalmol.* 150:33; and Shen et al. (2006) *Gene Ther.* 13:225); or VEGF receptor-2 (VEGFR2) (Kou et al. (2005) *Biochem.* 44:15064). See also, U.S. Patent Nos. 6,649,596, 6,399,586, 5,661,135, 5,639,872, and 5,639,736; and U.S. Patent Nos. 7,947,659 and 7,919,473.

Aptamers

[00183] Where the gene product is an aptamer, exemplary aptamers of interest include an aptamer against VEGF. See, e.g., Ng et al. (2006) *Nat. Rev. Drug Discovery* 5:123; and Lee et al. (2005) *Proc. Natl. Acad. Sci. USA* 102:18902. For example, a VEGF aptamer can comprise the nucleotide sequence 5'-cgcaaucagugaaugcuuauacauccg-3' (SEQ ID NO://). Also suitable for use is a platelet-derived growth factor (PDGF)-specific aptamer, e.g., E10030; see, e.g., Ni and Hui (2009) *Ophthalmologica* 223:401; and Akiyama et al. (2006) *J. Cell Physiol.* 207:407).

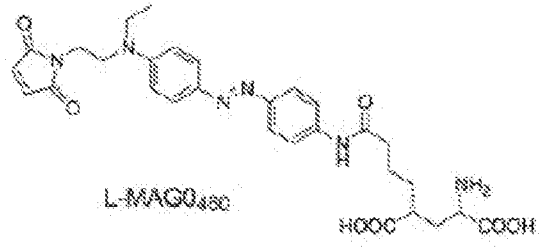
Polypeptides

[00184] Where the gene product is a polypeptide, the polypeptide is generally a polypeptide that enhances function of a retinal cell, e.g., the function of a rod or cone photoreceptor cell, a retinal ganglion cell, a Müller cell, a bipolar cell, an amacrine cell, a horizontal cell, or a retinal pigment epithelial cell. Exemplary polypeptides include neuroprotective polypeptides (e.g., glial cell derived neurotrophic factor (GDNF), ciliary neurotrophic factor (CNTF), neurotrophin-4 (NT4), nerve growth factor (NGF), and neurturin (NTN)); anti-angiogenic polypeptides (e.g., a soluble VEGF receptor; a VEGF-binding antibody; a VEGF-binding antibody fragment (e.g., a single chain anti-VEGF antibody); endostatin; tumstatin; angiostatin; a soluble Flt polypeptide (Lai et al. (2005) *Mol. Ther.* 12:659); an Fc fusion protein comprising a soluble Flt polypeptide (see, e.g., Pechan et al. (2009) *Gene Ther.* 16:10); pigment epithelium-derived factor (PEDF); a soluble Tie-2 receptor; etc.); tissue inhibitor of metalloproteinases-3 (TIMP-3); a light-responsive opsin, e.g., a rhodopsin; anti-apoptotic polypeptides (e.g., Bcl-2, Bcl-Xl; XIAP); and the like. Suitable polypeptides include, but are not limited to, glial derived neurotrophic factor (GDNF); fibroblast growth factor; fibroblast growth factor 2; neurturin (NTN); ciliary neurotrophic factor (CNTF); nerve growth factor (NGF); neurotrophin-4 (NT4); brain derived neurotrophic factor (BDNF; e.g., a polypeptide comprising an amino acid sequence having at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity to a contiguous stretch of from about 200 amino acids to 247 amino acids of the amino acid sequence depicted in Figure 7B (SEQ ID NO:11)); epidermal growth factor; rhodopsin; X-linked inhibitor of apoptosis; and Sonic hedgehog.

[00185] Suitable light-responsive opsins include, e.g., a light-responsive opsin as described in U.S. Patent Publication No. 2007/0261127 (e.g., channelrhodopsin-2; ChR2; Chop2); U.S. Patent Publication No. 2001/0086421; U.S. Patent Publication No. 2010/0015095; U.S. Patent Publication No. 2016/0002302; U.S. Patent Publication No. 2013/0347137; U.S. Patent Publication No. 2013/0019325; and Diester et al. (2011) *Nat. Neurosci.* 14:387. See, Thyagarajan et al. (2010) J Neurosci. 30(26):8745–8758; Lagali et al. (2008) Nat Neurosci. 11(6):667–675; Doroudchi et al. (2011) Mol Ther. 19(7):1220–1229; Henriksen et al. (2014) *J. Ophthalmic Vis. Res.* 9:374; Tomita et al. (2014) *Mol. Ther.* 22:1434.

[00186] Suitable polypeptides include light-gated ion channel polypeptides. See, e.g., Gaub et al. (2014) *Proc. Natl. Acad. Sci. USA* 111:E5574. For example, a suitable polypeptide is a light-gated ionotropic glutamate receptor (LiGluR). Expression of LiGluR in retinal ganglion cells and ON-bipolar cells, in the presence of a photoisomerizable compound, renders the cells responsive to light. LiGluR comprises a L439C substitution; see, Caporale et al. (2011) *Mol Ther.* 19:1212–1219; Volgraf et al. (2006) Nat Chem Biol. 2:47–52; and Gorostiza et al. (2007) Proc Natl Acad

Sci USA. 104:10865–10870. Photoisomerizable compounds include, e.g., maleimide-azobenzene-glutamate 0 with peak efficiency at 460 nm ($MAG0_{460}$). $MAG0_{460}$ has the following structure:



[00187] Suitable polypeptides also include retinoschisin (e.g., a polypeptide comprising an amino acid sequence having at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity to a contiguous stretch of from about 200 amino acids to 224 amino acids of the amino acid sequence depicted in FIG. 7A (SEQ ID NO:10). Suitable polypeptides include, e.g., retinitis pigmentosa GTPase regulator (RPGR)-interacting protein-1 (see, e.g., GenBank Accession Nos. Q96KN7, Q9EPQ2, and Q9GLM3) (e.g., a polypeptide comprising an amino acid sequence having at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity to a contiguous stretch of from about 1150 amino acids to about 1200 amino acids, or from about 1200 amino acids to 1286 amino acids, of the amino acid sequence depicted in FIG. 7F (SEQ ID NO:15); peripherin-2 (Prph2) (see, e.g., GenBank Accession No. NP_000313 (e.g., a polypeptide comprising an amino acid sequence having at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity to a contiguous stretch of from about 300 amino acids to 346 amino acids of the amino acid sequence depicted in FIG. 7D (SEQ ID NO:13); and Travis et al. (1991) *Genomics* 10:733); peripherin (e.g., a polypeptide comprising an amino acid sequence having at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity to a contiguous stretch of from about 400 amino acids to about 470 amino acids of the amino acid sequence depicted in FIG. 7E (SEQ ID NO:14); a retinal pigment epithelium-specific protein (RPE65), (e.g., a polypeptide comprising an amino acid sequence having at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity to a contiguous stretch of from about 200 amino acids to 247 amino acids of the amino acid sequence depicted in FIG. 7C (SEQ ID NO:12)) (see, e.g., GenBank AAC39660; and Morimura et al. (1998) *Proc. Natl. Acad. Sci. USA* 95:3088); rod-derived cone viability factor (RdCVF) (e.g., a polypeptide comprising an amino acid sequence having at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity to the amino acid

sequence depicted in any one of FIG. 7H, 7I, and 7J; choroideremia (e.g., a polypeptide comprising an amino acid sequence having at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity to the amino acid sequence depicted in FIG. 7G); retinitis pigmentosa GTPase regulator (RPGR) (e.g., a polypeptide comprising an amino acid sequence having at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity to the amino acid sequence depicted in one of FIG. 7S-7V); and the like. For example, in some cases, a suitable RPGR polypeptide comprises an amino acid sequence having at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity to the amino acid sequence depicted in FIG. 7S. As another example, in some cases, a suitable RPGR polypeptide comprises an amino acid sequence having at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity to the amino acid sequence depicted in FIG. 7T. example, in some cases, a suitable RPGR polypeptide comprises an amino acid sequence having at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity to the amino acid sequence depicted in FIG. 7U. example, in some cases, a suitable RPGR polypeptide comprises an amino acid sequence having at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity to the amino acid sequence depicted in FIG. 7V.

[00188] Suitable polypeptides also include: CHM (choroideremia (Rab escort protein 1 (REP1))), a polypeptide that, when defective or missing, causes choroideremia (see, e.g., Donnelly et al. (1994) *Hum. Mol. Genet.* 3:1017; and van Bokhoven et al. (1994) *Hum. Mol. Genet.* 3:1041); and Crumbs homolog 1 (CRB1), a polypeptide that, when defective or missing, causes Leber congenital amaurosis and retinitis pigmentosa (see, e.g., den Hollander et al. (1999) *Nat. Genet.* 23:217; and GenBank Accession No. CAM23328). For example, a suitable REP1 polypeptide can comprise an amino acid having at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity to the amino acid sequence set depicted in FIG. 7G.

[00189] Suitable polypeptides include Rod cGMP-specific 3',5'-cyclic phosphodiesterase subunit alpha (PDE6α), Rod cGMP-specific 3',5'-cyclic phosphodiesterase subunit beta isoform 1 (PDE6β isoform 1), Rod cGMP-specific 3',5'-cyclic phosphodiesterase subunit beta isoform 2 (PDE6β isoform 2), Rod cGMP-specific 3',5'-cyclic phosphodiesterase subunit beta isoform 3 (PDE6β isoform 3). For example, a suitable PDE6α polypeptide can comprise an amino acid having at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity to the amino acid sequence set depicted in FIG. 7K. As another example, a suitable PDE6β6 isoform 1 polypeptide can comprise an amino acid having at least

about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity to the amino acid sequence set depicted in FIG. 7L. As another example, a suitable PDE6β6 isoform 2 polypeptide can comprise an amino acid having at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity to the amino acid sequence set depicted in FIG. 7M. As another example, a suitable PDE6β6 isoform 3 polypeptide can comprise an amino acid having at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity to the amino acid sequence set depicted in FIG. 7N.

[00190] Suitable polypeptides also include polypeptides that, when defective or missing, lead to achromotopsia, where such polypeptides include, e.g., cone photoreceptor cGMP-gated channel subunit alpha (CNGA3) (see, e.g., GenBank Accession No. NP_001289; and Booij et al. (2011) *Ophthalmology* 118:160-167); cone photoreceptor cGMP-gated cation channel beta-subunit (CNGB3) (see, e.g., Kohl et al.(2005) *Eur J Hum Genet.* 13(3):302); guanine nucleotide binding protein (G protein), alpha transducing activity polypeptide 2 (GNAT2) (ACHM4); and ACHM5; and polypeptides that, when defective or lacking, lead to various forms of color blindness (e.g., L-opsin, M-opsin, and S-opsin). See Mancuso et al. (2009) *Nature* 461(7265):784–787.

[00191] For example, a suitable CNGA3 (also known as ACHM2) isoform 1 polypeptide can comprise an amino acid having at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity to the amino acid sequence set depicted in FIG. 7O. As another example, a suitable CNGA3 (also known as ACHM2) isoform 2 polypeptide can comprise an amino acid having at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity to the amino acid sequence set depicted in FIG. 7P.

[00192] As another example, a suitable CNGB3 (also known as ACHM3) polypeptide can comprise an amino acid having at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity to the amino acid sequence set depicted in FIG. 7Q. As another example, GNAT2 (also known as ACHM4) can comprise an amino acid having at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity to the amino acid sequence set depicted in FIG. 7R.

Site-specific endonucleases

[00193] 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. In some cases, a site-specific endonuclease is an RNA-guided endonuclease.

[00194] 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 subject rAAV virion 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, RdCVF, and the like.

[00195] Site-specific endonucleases that are suitable for use include, e.g., zinc finger nucleases (ZFNs); meganucleases; and transcription activator-like effector nucleases (TALENs), 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. Suitable site-specific endonucleases include engineered meganuclease re-engineered homing endonucleases. Suitable endonucleases include an I-TevI nuclease. Suitable meganucleases include I-SceI (see, e.g., Bellaiche et al. (1999) *Genetics* 152:1037); and I-CreI (see, e.g., Heath et al. (1997) *Nature Sructural Biology* 4:468).

RNA-guided endonucleases

[00196] In some cases, the gene product is an RNA-guided endonuclease. In some cases, the gene product is an RNA comprising a nucleotide sequence encoding an RNA-guided endonuclease. In some cases, the gene product is a guide RNA, e.g., a single-guide RNA. In some cases, the gene products are: 1) a guide RNA; and 2) an RNA-guided endonuclease. The guide RNA can comprise: a) a protein-binding region that binds to the RNA-guided endonuclease; and b) a region that binds to a target nucleic acid. An RNA-guided endonuclease is also referred to herein as a "genome editing nuclease."

[00197] Examples of suitable genome editing nucleases are CRISPR/Cas endonucleases (e.g., class 2 CRISPR/Cas endonucleases such as a type II, type V, or type VI CRISPR/Cas

endonucleases). Thus, a genome targeting composition can include a CRISPR/Cas endonuclease (e.g., a class 2 CRISPR/Cas endonuclease such as a type II, type V, or type VI CRISPR/Cas endonuclease). In some cases, a genome targeting composition includes a class 2 CRISPR/Cas endonuclease. In some cases, a genome targeting composition includes a class 2 type II CRISPR/Cas endonuclease (e.g., a Cas9 protein). In some cases, a genome targeting composition includes a class 2 type V CRISPR/Cas endonuclease (e.g., a Cpf1 protein, a C2c1 protein, or a C2c3 protein). In some cases, a genome targeting composition includes a class 2 type VI CRISPR/Cas endonuclease (e.g., a C2c2 protein).

[00198] In some cases, a genome editing nuclease is a fusion protein that is fused to a heterologous polypeptide (also referred to as a "fusion partner"). In some cases, a genome editing nuclease is fused to an amino acid sequence (a fusion partner) that provides for subcellular localization, i.e., the fusion partner is a subcellular localization sequence (e.g., one or more nuclear localization signals (NLSs) for targeting to the nucleus, two or more NLSs, three or more NLSs, etc.).

[00199] In some cases, the genome-editing endonuclease is a Type II CRISPR/Case endonuclease. In some cases, the genome-editing endonuclease is a Cas9 polypeptide. The Cas9 protein is guided to a target site (e.g., stabilized at a target site) within a target nucleic acid sequence (e.g., a chromosomal sequence or an extrachromosomal sequence, e.g., an episomal sequence, a minicircle sequence, a mitochondrial sequence, a chloroplast sequence, etc.) by virtue of its association with the protein-binding segment of the Cas9 guide RNA. In some cases, a Cas9 polypeptide comprises an amino acid sequence having at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, at least 99%, or more than 99%, amino acid sequence identity to the *Streptococcus pyogenes* Cas9 depicted in FIG. 8A. In some cases, the Cas9 polypeptide used in a composition or method of the present disclosure is a *Staphylococcus aureus* Cas9 (saCas9) polypeptide. In some cases, the saCas9 polypeptide comprises an amino acid sequence having at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the saCas9 amino acid sequence depicted in FIG. 8B.

[00200] In some cases, a suitable Cas9 polypeptide is a high-fidelity (HF) Cas9 polypeptide. Kleinstiver et al. (2016) *Nature* 529:490. For example, amino acids N497, R661, Q695, and Q926 of the amino acid sequence depicted in FIG. 8A are substituted, e.g., with alanine. For example, an HF Cas9 polypeptide can comprise an amino acid sequence having at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the amino acid sequence depicted in FIG. 8A, where amino acids N497, R661, Q695, and Q926 are substituted, e.g., with alanine.

[00201] In some cases, a suitable Cas9 polypeptide exhibits altered PAM specificity. See, e.g., Kleinstiver et al. (2015) *Nature* 523:481.

[00202] In some cases, the genome-editing endonuclease is a type V CRISPR/Cas endonuclease. In some cases a type V CRISPR/Cas endonuclease is a Cpf1 protein. In some cases, a Cpf1 protein comprises an amino acid sequence having at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 90%, or 100%, amino acid sequence identity to the Cpf1 amino acid sequence depicted in FIG. 8C.

[00203] A nucleic acid that binds to a class 2 CRISPR/Cas endonuclease (e.g., a Cas9 protein; a type V or type VI CRISPR/Cas protein; a Cpf1 protein; etc.) and targets the complex to a specific location within a target nucleic acid is referred to herein as a "guide RNA" or "CRISPR/Cas guide nucleic acid" or "CRISPR/Cas guide RNA." A guide RNA provides target specificity to the complex (the RNP complex) by including a targeting segment, which includes a guide sequence (also referred to herein as a targeting sequence), which is a nucleotide sequence that is complementary to a sequence of a target nucleic acid.

[00204] In some cases, a guide RNA includes two separate nucleic acid molecules: an "activator" and a "targeter" and is referred to herein as a "dual guide RNA", a "double-molecule guide RNA", a "two-molecule guide RNA", or a "dgRNA." In some cases, the guide RNA is one molecule (e.g., for some class 2 CRISPR/Cas proteins, the corresponding guide RNA is a single molecule; and in some cases, an activator and targeter are covalently linked to one another, e.g., via intervening nucleotides), and the guide RNA is referred to as a "single guide RNA", a "single-molecule guide RNA," a "one-molecule guide RNA", or simply "sgRNA."

[00205] Where the gene product is an RNA-guided endonuclease, or is both an RNA-guided endonuclease and a guide RNA, the gene product can modify a target nucleic acid. In some cases, e.g., where a target nucleic acid comprises a deleterious mutation in a defective allele (e.g., a deleterious mutation in a retinal cell target nucleic acid), the RNA-guided endonuclease/guide RNA complex, together with a donor nucleic acid comprising a nucleotide sequence that corrects the deleterious mutation (e.g., a donor nucleic acid comprising a nucleotide sequence that encodes a functional copy of the protein encoded by the defective allele), can be used to correct the deleterious mutation, e.g., via homology-directed repair (HDR).

[00206] In some cases, the gene products are an RNA-guided endonuclease and 2 separate sgRNAs, where the 2 separate sgRNAs provide for deletion of a target nucleic acid via non-homologous end joining (NHEJ).

[00207] The present disclosure provides a method of modifying a target nucleic acid in a retinal cell in an individual, where the target nucleic acid comprises a deleterious mutation, the method comprising administering to the individual (e.g., by intraocular; intravitreal; etc. administration) an rAAV virion of the present disclosure, where the rAAV virion comprises a heterologous nucleic acid comprising: i) a nucleotide sequence encoding an RNA-guided endonuclease (e.g., a Cas9 endonuclease); ii) a nucleotide sequence encoding a sgRNA that comprises a nucleotide sequence that is complementary to the target nucleic acid; and iii) a nucleotide sequence encoding a donor DNA template that comprises a nucleotide sequence that corrects the deleterious mutation. Administration of the rAAV virion results in correction of the deleterious mutation in the target nucleic acid by HDR.

[00208] The present disclosure provides a method of modifying a target nucleic acid in a retinal cell in an individual, where the target nucleic acid comprises a deleterious mutation, the method comprising administering to the individual (e.g., by intraocular; intravitreal; etc. administration) an rAAV virion of the present disclosure, where the rAAV virion comprises a heterologous nucleic acid comprising: i) a nucleotide sequence encoding an RNA-guided endonuclease (e.g., a Cas9 endonuclease); ii) a nucleotide sequence encoding a first sgRNA that comprises a nucleotide sequence that is complementary to a first sequence in the target nucleic acid; and iii) a nucleotide sequence encoding a second sgRNA that comprises a nucleotide sequence that is complementary to a second sequence in the target nucleic acid. Administration of the rAAV virion results in excision of the deleterious mutation in the target nucleic acid by NHEJ.

Regulatory sequences

[00209] In some cases, a nucleotide sequence encoding a gene product of interest is operably linked to a transcriptional control element. For example, in some cases, a nucleotide sequence encoding a gene product of interest is operably linked to a constitutive promoter. In other cases, 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 retinal cell-specific promoter. 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).

PHARMACEUTICAL COMPOSITIONS

[00210] The present disclosure provides a pharmaceutical composition comprising: a) a subject rAAV virion, as described above; 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.

[00211] 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, 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.

METHODS OF DELIVERING A GENE PRODUCT TO A RETINAL CELL AND TREATMENT METHODS

[00212] The present disclosure provides a method of delivering a gene product to a retinal cell in an individual, the method comprising administering to the individual a subject rAAV virion as described above. The gene product can be a polypeptide or an interfering RNA (e.g., an shRNA, an siRNA, and the like), an aptamer, or a site-specific endonuclease (e.g., an RNA-guided endonuclease), as described above. Delivering a gene product to a retinal cell can provide for treatment of a retinal disease. The retinal cell can be a photoreceptor, a retinal ganglion cell, a Müller cell, a bipolar cell, an amacrine cell, a horizontal cell, or a retinal pigmented epithelial cell. In some cases, the retinal cell is a photoreceptor cell, e.g., a rod or cone cell.

[00213] The present disclosure provides a method modifying a target nucleic acid in a retinal cell, the method comprising contacting the retinal cell with: 1) an rAAV virion of the present disclosure, wherein the rAAV virion comprises a heterologous nucleic acid comprising a nucleotide sequence encoding an RNA-guided endonuclease that binds a guide RNA; and 2) the

guide RNA. The present disclosure provides a method modifying a target nucleic acid in a retinal cell, the method comprising contacting the retinal cell with an rAAV virion of the present disclosure, wherein the rAAV virion comprises a heterologous nucleic acid comprising a nucleotide sequence encoding: i) an RNA-guided endonuclease that binds a guide RNA; and ii) the guide RNA. In some cases, the method comprises contacting the retinal cell with a donor DNA template. In some cases, the RNA-guided endonuclease is a Cas9 polypeptide. In some cases, the guide RNA is a single-guide RNA.

[00214] The present disclosure provides a method of treating an ocular disease (e.g., a retinal disease), the method comprising administering to an individual in need thereof an effective amount of a subject rAAV virion as described above. A subject rAAV virion can be administered via intraocular injection, by intravitreal injection, or by any other convenient mode or route of administration. Other convenient modes or routes of administration include, e.g., intravenous, intranasal, etc.

[00215] A "therapeutically effective amount" will fall in a relatively broad range that can be determined through experimentation and/or clinical trials. For example, for in vivo injection, i.e., injection directly into the eye, a therapeutically effective dose will be on the order of from about 10^6 to about 10^{15} of the rAAV virions, e.g., from about 10^8 to 10^{12} rAAV virions. For *in vitro* transduction, an effective amount of rAAV virions to be delivered to cells will be on the order of from about 10^8 to about 10^{13} of the rAAV virions. Other effective dosages can be readily established by one of ordinary skill in the art through routine trials establishing dose response curves.

[00216] In some embodiments, more than one administration (e.g., two, three, four or more administrations) may be employed to achieve the desired level of gene expression. In some cases, the more than one administration is administered at various intervals, e.g., daily, weekly, twice monthly, monthly, every 3 months, every 6 months, yearly, etc. In some cases, multiple administrations are administered over a period of time of from 1 month to 2 months, from 2 months to 4 months, from 4 months to 8 months, from 8 months to 12 months, from 1 year to 2 years, from 2 years to 5 years, or more than 5 years.

[00217] Ocular diseases that can be treated using a subject method include, but are not limited to, acute macular neuroretinopathy; Behcet's disease; choroidal neovascularization; diabetic uveitis; histoplasmosis; macular degeneration, such as acute macular degeneration, non-exudative age related macular degeneration and exudative age related macular degeneration; edema, such as macular edema, cystoid macular edema and diabetic macular edema; multifocal choroiditis; ocular trauma which affects a posterior ocular site or location; ocular tumors; retinal disorders, such as central retinal vein occlusion, diabetic retinopathy (including proliferative diabetic

retinopathy), proliferative vitreoretinopathy (PVR), retinal arterial occlusive disease, retinal detachment, uveitic retinal disease; sympathetic opthalmia; Vogt Koyanagi-Harada (VKH) syndrome; uveal diffusion; 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; epiretinal membrane disorders; branch retinal vein occlusion; anterior ischemic optic neuropathy; non-retinopathy diabetic retinal dysfunction; retinoschisis; retinitis pigmentosa; glaucoma; Usher syndrome, cone-rod dystrophy; Stargardt disease (fundus flavimaculatus); inherited macular degeneration; chorioretinal degeneration; Leber congenital amaurosis; congenital stationary night blindness; choroideremia; Bardet-Biedl syndrome; macular telangiectasia; Leber's hereditary optic neuropathy; retinopathy of prematurity; disorders of color vision, including achromatopsia, protanopia, deuteranopia, and tritanopia; and Bietti's crystalline dystrophy.

NUCLEIC ACIDS AND HOST CELLS

[00218] The present disclosure provides an isolated nucleic acid comprising a nucleotide sequence that encodes a subject variant adeno-associated virus (AAV) capsid protein as described above, where the variant AAV capsid protein comprises an insertion of from about 5 amino acids to about 20 amino acids in the GH loop or loop IV relative to a corresponding parental AAV capsid protein, and where the variant capsid protein, when present in an AAV virion, provides for increased infectivity of a retinal cell compared to the infectivity of the retinal cell by an AAV virion comprising the corresponding parental AAV capsid protein. A subject isolated nucleic acid can be an AAV vector, e.g., a recombinant AAV vector.

Insertion peptides

[00219] A variant AAV capsid protein encoded by a subject nucleic acid has an insertion peptide of from about 5 amino acids to about 20 amino acids in length is inserted into the GH loop of an AAV capsid. The insertion peptide has a length of 5 amino acids, 6 amino acids, 7 amino acids, 8 amino acids, 9 amino acids, 10 amino acids, 11 amino acids, 12 amino acids, 13 amino acids, 14 amino acids, 15 amino acids, 16 amino acids, 17 amino acids, 18 amino acids, 19 amino acids, or 20 amino acids. Suitable insertion peptides are as described above. Suitable insertion peptides include a peptide of any one of Formulas I-X, as described above.

[00220] A subject recombinant AAV vector can be used to generate a subject recombinant AAV virion, as described above. Thus, the present disclosure provides a recombinant AAV vector that, when introduced into a suitable cell, can provide for production of a subject recombinant AAV virion.

[00221] The present invention further provides host cells, e.g., isolated (genetically modified) host cells, comprising a subject nucleic acid. A subject host cell can be an isolated cell, e.g., a

cell in *in vitro* culture. A subject host cell is useful for producing a subject rAAV virion, as described below. Where a subject host cell is used to produce a subject rAAV virion, it is referred to as a "packaging cell." In some embodiments, a subject host cell is stably genetically modified with a subject nucleic acid. In other embodiments, a subject host cell is transiently genetically modified with a subject nucleic acid.

[00222] A subject 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 subject 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.

[00223] A subject host cell is generated by introducing a subject 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). Suitable mammalian cells include, but are not limited to, primary cells and cell lines, where suitable 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. Non-limiting examples of suitable host cells include, e.g., 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. CCLI.3), human embryonic kidney (HEK) cells (ATCC No. CRL1573), HLHepG2 cells, and the like. A subject 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. Patent No. 7,271,002; US patent application 12/297,958)

[00224] In some embodiments, a subject 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 subject host cell further comprises an rAAV vector. An rAAV virion can be generated using a subject host cell. 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.

Examples of Non-Limiting Aspects of the Disclosure

[00225] Aspects, including embodiments, of the present subject matter described above may be beneficial alone or in combination, with one or more other aspects or embodiments. Without limiting the foregoing description, certain non-limiting aspects of the disclosure numbered 1-34 are provided below. As will be apparent to those of skill in the art upon reading this disclosure,

each of the individually numbered aspects may be used or combined with any of the preceding or following individually numbered aspects. This is intended to provide support for all such combinations of aspects and is not limited to combinations of aspects explicitly provided below:

[00226] Aspect 1. A recombinant adeno-associated virus (rAAV) virion comprising: a) a variant AAV capsid protein, wherein the variant AAV capsid protein comprises an insertion of a heterologous peptide having a length of from about 5 amino acids to about 20 amino acids in the capsid protein GH loop relative to a corresponding parental AAV capsid protein, and wherein the variant capsid protein confers increased infectivity of a retinal cell compared to the infectivity of the retinal cell by a control AAV virion comprising the corresponding parental AAV capsid protein; and b) a heterologous nucleic acid comprising a nucleotide sequence encoding a heterologous gene product.

[00227] Aspect 2. The rAAV virion of aspect 1, wherein the rAAV virion exhibits at least 5-fold increased infectivity of a retinal cell compared to the infectivity of the retinal cell by a control AAV virion comprising the corresponding parental AAV capsid protein.

[00228] Aspect 3. The rAAV virion of aspect 1, wherein the rAAV virion exhibits at least 10-fold increased infectivity of a retinal cell compared to the infectivity of the retinal cell by an AAV virion comprising the corresponding parental AAV capsid protein.

[00229] Aspect 4. The rAAV virion of any one of aspects 1-3, wherein the rAAV virion exhibits at least 5-fold increased localization to one or more of the inner nuclear layer, the outer nuclear layer, the photoreceptor layer, the ganglion cell layer, and the retinal pigment epithelium, compared to the extent of localization to the inner nuclear layer, the outer nuclear layer, the photoreceptor layer, the ganglion cell layer, or the retinal pigment epithelium, by an AAV virion comprising the corresponding parental AAV capsid protein.

[00230] Aspect 5. The rAAV virion of any one of aspects 1-4, wherein the insertion site is between amino acids corresponding to amino acids 570 and 611 of VP1 of AAV2, or the corresponding position in the capsid protein of another AAV serotype.

[00231] Aspect 6. The rAAV virion of any one of aspects 1-5, wherein the insertion site is located between amino acids corresponding to amino acids 587 and 588 of VP1 of AAV2, or the corresponding position in the capsid protein of another AAV serotype.

[00232] Aspect 7. The rAAV virion of any one of aspects 1-6, wherein gene product is an interfering RNA or an aptamer.

[00233] Aspect 8. The rAAV virion of any one of aspects 1-6, wherein the gene product is a polypeptide.

[00234] Aspect 9. The rAAV virion of aspect 8, wherein the polypeptide is a neuroprotective polypeptide, an anti-angiogenic polypeptide, or a polypeptide that enhances function of a retinal cell.

[00235] Aspect 10. The rAAV virion of aspect 8, wherein the polypeptide is an RNA-guided endonuclease.

[00236] Aspect 11. The rAAV virion of aspect 10, wherein the RNA-guided endonuclease is a Cas9 polypeptide.

[00237] Aspect 12. The rAAV virion of aspect 10, wherein the gene product is an RNA-guided endonuclease and a guide RNA.

[00238] Aspect 13. The rAAV virion of any one of aspects 1-12, wherein the heterologous peptide inserted into the GH loop is of any one of Formulas I-X.

[00239] Aspect 14. The rAAV virion of any one of aspects 1-12, wherein the heterologous peptide is a peptide of Formula I: $X_1X_2X_3X_4X_5X_6X_7X_8X_9X_{10}$,

[00240] wherein:

[00241] X_1 is Leu, Ile, Pro, or Gln;

[00242] X_2 is Ala, Pro, Ser, Asp, Gly, Thr, or Val;

[00243] X_3 is Lys, His, Thr, Ile, Pro, Val, Arg, Ala, Asp, Glu, Asn, Gln, or Tyr;

[00244] X_4, if present, is Gln, Asp, Ser, Gly, Thr, Ile, Asn, Glu, Lys, or Arg;

[00245] X_5 is Asp, Ser, Gln, Val, Thr, Gly, Ala, Asn, Lys, or Tyr;

[00246] X_6 is Thr, Ala, Gln, Ser, Glu, Pro, or Ile;

[00247] X_7 is Thr, Ser, Asn, Pro, Leu, Gln, Lys, Ala, or Cys;

[00248] X_8 is Lys, Ser, Arg, Thr, Ala, Glu, Ile, or Asn;

[00249] X_9 is Asn, Pro, Ser, Lys, His, Ile, Thr, or Ala; and

[00250] X_{10} is Ala, Phe, Asp, Thr, Val, or Met.

[00251] Aspect 15. The rAAV virion of aspect 14, wherein the heterologous peptide comprises one of the following amino acid sequences: (1) LAKDATKNA (SEQ ID NO:47); (2) PAHQDTTKNA (SEQ ID NO:48); (3) LAHQDTTKNA (SEQ ID NO:49); (4) LATTSQNKPA (SEQ ID NO:50); (5) LAISDQTKHA (SEQ ID NO:51); (6) IARGVAPSSA (SEQ ID NO:52); (7) LAPDSTTRSA (SEQ ID NO:53); (8) LAKGTELKPA (SEQ ID NO:54); (9) LAIIDATKNA (SEQ ID NO:55); (10) LAVDGAQRSA (SEQ ID NO:56); (11) PAPQDTTKKA (SEQ ID NO:57); (12) LPHQDTTKNA (SEQ ID NO:58); (13) LAKDATKTIA (SEQ ID NO:59); (14) LAKQQSASTA (SEQ ID NO:60); (15) LAKSDQSKPA (SEQ ID NO:61); (16) LSHQDTTKNA (SEQ ID NO:62); (17) LAANQPSKPA (SEQ ID NO:63); (18) LAVSDSTKAA (SEQ ID NO:64); (19) LAAQGTAKKPA (SEQ ID NO:65); (20)

LAPDQTTRNA (SEQ ID NO:66); (21) LAASDSTKAA (SEQ ID NO:67); (22) LAPQDTTKNA (SEQ ID NO:68); (23) LAKADETRPA (SEQ ID NO:69); (24) LAHQDTAKNA (SEQ ID NO:70); (25) LAHQDTKKNA (SEQ ID NO:71); (26) LAHQDTTKHA (SEQ ID NO:72); (27) LAHQDTTKKA (SEQ ID NO:73); (28) LAHQDTTRNA (SEQ ID NO:74); (29) LAHQDTTNA (SEQ ID NO:75); (30) LAHQGTTKNA (SEQ ID NO:76); (31) LAHQVTTKNA (SEQ ID NO:77); (32) LAISDQSKPA (SEQ ID NO:78); (33) LADATKTA (SEQ ID NO:79); (34) LAKDTTKNA (SEQ ID NO:80); (35) LAKSDQSRPA (SEQ ID NO:81); (36) LAPQDTKKNA (SEQ ID NO:82); (37) LATSDSTKAA (SEQ ID NO:83); (38) LAVDGSQRSA (SEQ ID NO:84); (39) LPISDQTKHA (SEQ ID NO:85); (40) LPKDATKTIA (SEQ ID NO:86); (41) LPPQDTTKNA (SEQ ID NO:87); (42) PAPQDTTKNA (SEQ ID NO:88); (43) QAHQDTTKNA (SEQ ID NO:89); (44) LAHETSPRPA (SEQ ID NO:90); (45) LAKSTSTAPA (SEQ ID NO:91); (46) LADQDTTKNA (SEQ ID NO:92); (47) LAESDQSKPA (SEQ ID NO:93); (48) LAHKDTTKNA (SEQ ID NO:94); (49) LAHKTQQKM (SEQ ID NO:95); (50) LAHQDTTENA (SEQ ID NO:96); (51) LAHQDTTINA (SEQ ID NO:97); (52) LAHQDTTKKT (SEQ ID NO:98); (53) LAHQDTTKND (SEQ ID NO:99); (54) LAHQDTTKNT (SEQ ID NO:100); (55) LAHQDTTKNV (SEQ ID NO:101); (56) LAHQDTTKTM (SEQ ID NO:102); (57) LAHQNTTKNA (SEQ ID NO:103); (58) LAHRDTTKNA (SEQ ID NO:104); (59) LAISDQTNHA (SEQ ID NO:105); (60) LAKQKSASTA (SEQ ID NO:106); (61) LAKSDQCKPA (SEQ ID NO:107); (62) LAKSDQSKPD (SEQ ID NO:108); (63) LAKSDQSNPA(SEQ ID NO:109); (64) LAKSYQSKPA (SEQ ID NO:110); (65) LANQDTTKNA (SEQ ID NO:111); (66) LAPQNTTKNA (SEQ ID NO:112); (67) LAPSSIQKPA (SEQ ID NO:113); (68) LAQQDTTKNA (SEQ ID NO:114); (69) LAYQDTTKNA (SEQ ID NO:115); (70) LDHQDTTKNA (SEQ ID NO:116); (71) LDHQDTTKSA (SEQ ID NO:117); (72) LGHQDTTKNA (SEQ ID NO:118); (73) LPHQDTTKND (SEQ ID NO:119); (74) LPHQDTTKNT (SEQ ID NO:120); (75) LPHQDTTNNA (SEQ ID NO:121); (76) LTHQDTTKNA (SEQ ID NO:122); (77) LTKDATKTIA (SEQ ID NO:123); (78) LTPQDTTKNA (SEQ ID NO:124); and (79) LVHQDTTKNA (SEQ ID NO:125).

[00252] Aspect 16. The rAAV virion of any one of aspects 1-12, wherein the heterologous peptide is a peptide of Formula II:

[00253] $X_1X_2X_3X_4X_5X_6X_7X_8X_9X_{10}$, wherein:

[00254] X_1 is Leu, Ile, or Pro;

[00255] X_2 is Ala, Pro, or Ser;

[00256] X_3 is Lys, His, Thr, Ile, Pro, Val, Arg, or Ala;

[00257] X_4 (if present) is Gln, Asp, Ser, Gly, Thr, Ile, or Asn;

[00258] X_5 is Asp, Ser, Gln, Val, Thr, Gly, or Ala;

[00259] X_6 isThr, Ala, Gln, Ser, Glu, or Pro;

[00260] X_7 is Thr, Ser, Asn, Pro, Leu, Gln, Lys, or Ala;

[00261] X_8 is Lys, Ser, Arg, or Thr;

[00262] X_9 is Asn, Pro, Ser, Lys, His, Ile, Thr, or Ala; and

[00263] X_{10} is Ala.

[00264] Aspect 17. The rAAV virion of aspect 16, wherein the peptide comprises one of the following amino acid sequences: (1) LAKDATKNA (SEQ ID NO:47); (2) PAHQDTTKNA (SEQ ID NO:48); (3) LAHQDTTKNA (SEQ ID NO:49); (4) LATTSQNKPA (SEQ ID NO:50); (5) LAISDQTKHA (SEQ ID NO:51); (6) IARGVAPSSA (SEQ ID NO:52); (7) LAPDSTTRSA (SEQ ID NO:53); (8) LAKGTELKPA (SEQ ID NO:54); (9) LAIIDATKNA (SEQ ID NO:55); (10) LAVDGAQRSA (SEQ ID NO:56); (11) PAPQDTTKKA (SEQ ID NO:57); (12) LPHQDTTKNA (SEQ ID NO:58); (13) LAKDATKTIA (SEQ ID NO:59); (14) LAKQQSASTA (SEQ ID NO:60); (15) LAKSDQSKPA (SEQ ID NO:61); (16) LSHQDTTKNA (SEQ ID NO:62); (17) LAANQPSKPA (SEQ ID NO:63); (18) LAVSDSTKAA (SEQ ID NO:64); (19) LAAQGTAKKPA (SEQ ID NO:65); (20) LAPDQTTRNA (SEQ ID NO:66); (21) LAASDSTKAA (SEQ ID NO:67); (22) LAPQDTTKNA (SEQ ID NO:68); (23) LAKADETRPA (SEQ ID NO:69); (24) LAHQDTAKNA (SEQ ID NO:70); (25) LAHQDTKKNA (SEQ ID NO:71); (26) LAHQDTTKHA (SEQ ID NO:72); (27) LAHQDTTKKA (SEQ ID NO:73); (28) LAHQDTTRNA (SEQ ID NO:74); (29) LAHQDTTNA (SEQ ID NO:75); (30) LAHQGTTKNA (SEQ ID NO:76); (31) LAHQVTTKNA (SEQ ID NO:77); (32) LAISDQSKPA (SEQ ID NO:78); (33) LADATKTA (SEQ ID NO:79); (34) LAKDTTKNA (SEQ ID NO:80); (35) LAKSDQSRPA (SEQ ID NO:81); (36) LAPQDTKKNA (SEQ ID NO:82); (37) LATSDSTKAA (SEQ ID NO:83); (38) LAVDGSQRSA (SEQ ID NO:84); (39) LPISDQTKHA (SEQ ID NO:85); (40) LPKDATKTIA (SEQ ID NO:86); (41) LPPQDTTKNA (SEQ ID NO:87); and (42) PAPQDTTKNA (SEQ ID NO:88);.

[00265] Aspect 18. The rAAV virion of aspect 16, wherein the peptide comprises one of the following amino acid sequences: (1) LAKDATKNA (SEQ ID NO:47); (2) PAHQDTTKNA (SEQ ID NO:48); (3) LAHQDTTKNA (SEQ ID NO:49); (4) LATTSQNKPA (SEQ ID NO:50); (5) LAISDQTKHA (SEQ ID NO:51); (6) IARGVAPSSA (SEQ ID NO:52); (7) LAPDSTTRSA (SEQ ID NO:53); (8) LAKGTELKPA (SEQ ID NO:54); (9) LAIIDATKNA (SEQ ID NO:55); (10) LAVDGAQRSA (SEQ ID NO:56); (11) PAPQDTTKKA (SEQ ID NO:57); (12)

LPHQDTTKNA (SEQ ID NO:58); (13) LAKDATKTIA (SEQ ID NO:59); (14) LAKQQSASTA (SEQ ID NO:60); (15) LAKSDQSKPA (SEQ ID NO:61); (16) LSHQDTTKNA (SEQ ID NO:62); (17) LAANQPSKPA (SEQ ID NO:63); and (18) LAVSDSTKAA (SEQ ID NO:64).

[00266] Aspect 19. A pharmaceutical composition comprising: a) a recombinant adeno-associated virus virion of any one of aspects 1-18; and b) a pharmaceutically acceptable excipient.

[00267] Aspect 20. A method of delivering a gene product to a retinal cell in an individual, the method comprising administering to the individual a recombinant adeno-associated virus (rAAV) virion according any one of aspects 1-18.

[00268] Aspect 21. The method of aspect 20, wherein the gene product is a polypeptide.

[00269] Aspect 22. The method of aspect 20, wherein the gene product is a short interfering RNA or an aptamer.

[00270] Aspect 23. The method of aspect 21, wherein the polypeptide is a neuroprotective factor, an anti-angiogenic polypeptide, an anti-apoptotic factor, or a polypeptide that enhances function of a retinal cell.

[00271] Aspect 24. The method of aspect 21, wherein the polypeptide is glial derived neurotrophic factor, fibroblast growth factor 2, neurturin, ciliary neurotrophic factor, nerve growth factor, brain derived neurotrophic factor, epidermal growth factor, rhodopsin, X-linked inhibitor of apoptosis, retinoschisin, RPE65, retinitis pigmentosa GTPase-interacting protein-1, peripherin, peripherin-2, a rhodopsin, RdCVF, retinitis pigmentosa GTPase regulator (RPGR), or Sonic hedgehog.

[00272] Aspect 25. The method of aspect 21, wherein the polypeptide is an RNA-guided endonuclease.

[00273] Aspect 26. A method of treating an ocular disease, the method comprising administering to an individual in need thereof an effective amount of a recombinant adeno-associated virus (rAAV) virion according to any one of aspects 1-18.

[00274] Aspect 27. The method of aspect 26, wherein said administering is by intraocular injection.

[00275] Aspect 28. The method of aspect 26, wherein said administering is by intravitreal injection.

[00276] Aspect 29. The method of aspect 26, wherein the ocular disease is glaucoma, retinitis pigmentosa, macular degeneration, retinoschisis, Leber's Congenital Amaurosis, diabetic retinopathy, achromotopsia, or color blindness.

[00277] Aspect 30. An isolated nucleic acid comprising a nucleotide sequence that encodes a variant adeno-associated virus (AAV) capsid protein, wherein the variant AAV capsid protein comprises an insertion of from about 5 amino acids to about 20 amino acids in the capsid protein GH loop relative to a corresponding parental AAV capsid protein, and wherein the variant capsid protein, when present in an AAV virion, provides for increased infectivity of the AAV virion of a retinal cell, and wherein the amino acid insertion is in the GH loop of a native AAV capsid, wherein the insertion is a peptide of any one of Formulas I-X.

[00278] Aspect 31. The isolated nucleic acid of aspect 30, wherein the insertion site is between amino acids 587 and 588 of AAV2, between amino acids 590 and 591 of AAV1, 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.

[00279] Aspect 32. An isolated, genetically modified host cell comprising the nucleic acid of aspect 30 or aspect 31.

[00280] Aspect 33. A variant adeno-associated virus (AAV) capsid protein, wherein the variant AAV capsid protein comprises an insertion of from about 5 amino acids to about 20 amino acids wherein the amino acid insertion is in the GH loop of a native AAV capsid, wherein the insertion is a peptide of any one of Formulas I-X.

[00281] Aspect 34. In any of aspects 1-33, the heterologous peptide that is inserted into the GH loop can be of one of Formulas I-X, where:

[00282] Formula I is $X_1X_2X_3X_4X_5X_6X_7X_8X_9X_{10}$, where:

[00283] X_1 is Leu, Ile, Pro, or Gln;

[00284] X_2 is Ala, Pro, Ser, Asp, Gly, Thr, or Val;

[00285] X_3 is Lys, His, Thr, Ile, Pro, Val, Arg, Ala, Asp, Glu, Asn, Gln, or Tyr;

[00286] X_4 (if present) is Gln, Asp, Ser, Gly, Thr, Ile, Asn, Glu, Lys, or Arg;

[00287] X_5 is Asp, Ser, Gln, Val, Thr, Gly, Ala, Asn, Lys, or Tyr;

[00288] X_6 is Thr, Ala, Gln, Ser, Glu, Pro, or Ile;

[00289] X_7 is Thr, Ser, Asn, Pro, Leu, Gln, Lys, Ala, or Cys;

[00290] X_8 is Lys, Ser, Arg, Thr, Ala, Glu, Ile, or Asn;

[00291] X_9 is Asn, Pro, Ser, Lys, His, Ile, Thr, or Ala; and

[00292] X_{10} is Ala, Phe, Asp, Thr, Val, or Met;

[00293] Formula II is $X_1X_2X_3X_4X_5X_6X_7X_8X_9X_{10}$, where:

[00294] X_1 is Leu, Ile, or Pro;

[00295] X_2 is Ala, Pro, or Ser;

[00296] X_3 is Lys, His, Thr, Ile, Pro, Val, Arg, or Ala;

[00297] X_4 (if present) is Gln, Asp, Ser, Gly, Thr, Ile, or Asn;

[00298] X_5 is Asp, Ser, Gln, Val, Thr, Gly, or Ala;

[00299] X_6 isThr, Ala, Gln, Ser, Glu, or Pro;

[00300] X_7 is Thr, Ser, Asn, Pro, Leu, Gln, Lys, or Ala;

[00301] X_8 is Lys, Ser, Arg, or Thr;

[00302] X_9 is Asn, Pro, Ser, Lys, His, Ile, Thr, or Ala; and

[00303] X_{10} is Ala;

[00304] Formula III is $X_1X_2X_3X_4X_5X_6X_7X_8X_9X_{10}$, where:

[00305] X_1 is Leu, Ile, or Pro;

[00306] X_2 is Ala, Pro, or Ser;

[00307] X_3 is Lys, His, Thr, Ile, Pro, Val, Arg, or Ala;

[00308] X_4 (if present) is Gln, Asp, Ser, Gly, Thr, Ile, or Asn;

[00309] X_5 is Asp, Ser, Gln, Val, Thr, Gly, or Ala;

[00310] X_6 isThr, Ala, Gln, Ser, Glu, or Pro;

[00311] X_7 is Thr, Ser, Asn, Pro, Leu, Gln, Lys, or Ala;

[00312] X_8 is Lys, Ser, Arg, or Thr;

[00313] X_9 is Asn, Pro, Ser, Lys, His, Ile, Thr, or Ala; and

[00314] X10 is Ala, Thr, Asp Val, or Met;

[00315] Formula IV is $X_1X_2X_3X_4X_5X_6X_7X_8X_9X_{10}$, where:

[00316] X_1 is Leu;

[00317] X_2 is Ala;

[00318] X_3 is Lys, His, Thr, Ile, Pro, or Val;

[00319] X_4 (if present) is Gln, Asp, Ser, or Gly;

[00320] X_5 is Asp, Ser, or Gln;

[00321] X_6 is Thr, Ala, Gln, or Ser;

[00322] X_7 is Thr or Ser;

[00323] X_8 is Lys, Ser, or Arg;

[00324] X_9 is Asn, Pro, or Ser; and

[00325] X_{10} is Ala;

[00326] Formula V is $X_1X_2X_3X_4X_5X_6X_7X_8X_9X_{10}$, where:

[00327] X_1 is Leu;

[00328] X_2 is Ala;

[00329] X_3 is Lys or His;

[00330] X_4 (if present) is Gln, Asp, Ser, or Gly;

[00331] X_5 is Asp, Ser, or Gln;

[00332] X_6 is Thr, Ala, Gln, or Ser;

[00333] X_7 is Thr or Ser;

[00334] X_8 is Lys, Ser, or Arg;

[00335] X_9 is Asn, Pro, or Ser; and

[00336] X_{10} is Ala;

[00337] Formula VI is $X_1X_2X_3X_4X_5X_6X_7X_8X_9X_{10}$, where:

[00338] X_1 is Leu;

[00339] X_2 is Ala;

[00340] X_3 is Asn, Lys, Thr, Gln, Ser, Ile, or Leu;

[00341] X_4 is Ser, Ala, Thr, Glu, Gln, Gly, Lys, or Pro;

[00342] X_5 is Asp, Pro, Glu, Thr, Asn, or Arg;

[00343] X_6 is Ile, His, Thr, Gln, Asn, Tyr, Asp, or Glu;

[00344] X_7 is Gln, Thr, Asn, Ala, or Lys;

[00345] X_8 is Lys, Thr, Arg, or Asp;

[00346] X_9 is Pro, Asn, Thr, Arg, Lys, or Ser; and

[00347] X_{10} is Ala;

[00348] Formula VII is LAHQDTTK$X_1X_2X_3$ (SEQ ID NO:148), where X_1 is Lys, Thr, Asn, or His; X_2 is Ala, Thr, Val, Ile, Met, or Asp; and X_3, if present, is Ala;

[00349] Formula VIII is LAX_1QX_2T$X_3X_4X_5X_6$ (SEQ ID NO:149), where X_1 is Ala, Pro, Asp, or His; X_2 is Gly or Asp; X_3 is Ala, Thr, or Lys; X_4 is Asn, Glu, Lys, Arg, or Thr; X_5 is Leu, Asn, Lys, or Thr; and X_6, if present, is Ala, Thr, Asp, Val, or Met;

[00350] Formula IX is X_1AX_2X_3DX_4TKX_5A (SEQ ID NO:150), where X_1 is Val or Leu; X_2 is Ile, Val, His, or Asp; X_3 is Glu, Ser, Lys, or Gln; X_4 is His, Ser, or Thr; and X_5 is Ser, Ala, Asn, His, or Lys; and

[00351] Formula X is $X_1X_2X_3$AX_4QX_5TX_6KNA (SEQ ID NO:151), where X_1, if present, is Leu; X_2, if present, is Ala; X_3 is Lys, Leu, or Pro; X_4 is Asn, His, Pro, or Tyr; X_5 is Asn, Gly, Val, or Asp; and X_6 is Pro or Thr.

EXAMPLES

[00352] 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 Celsius, and pressure is at or near atmospheric. Standard abbreviations may be used, e.g., bp, base pair(s); kb, kilobase(s); pl, picoliter(s); s or sec, second(s); min, minute(s); h or hr, hour(s); aa, amino acid(s); kb, kilobase(s); bp, base pair(s); nt, nucleotide(s); i.m., intramuscular(ly); i.p., intraperitoneal(ly); s.c., subcutaneous(ly); and the like.

Example 1: Generation and characterization of AAV virions with AAV capsid variants

[00353] An iterative *in vivo* screening methodology was used to create AAV with capsid variants able to overcome the significant and complex barriers preventing panretinal AAV infection in a large animal eye. Dogs are an important preclinical model for retinal degenerative disease, with an eye size and structure similar to humans, and many forms of retinal disease are naturally occurring in a variety of dog breeds. The screening method was used to identify 96 AAV variants capable of panretinal infection in the canine retina. Deep sequencing was used to quantify the performance of 18 of these variants from the pool of screened AAV variants in canine retina. Infectivity was quantified based on levels of viral DNA and mRNA in retinal cells following intravitreal injection. These variants can be used for a wide variety of gene delivery strategies in large animal and human eyes.

[00354] A peptide display library containing a random 21-nucleotide insert (surrounded by a 5' 6-nucleotide linker and a 3' 3-nucleotide linker) at a surface exposed position on the AAV capsid was created. Virus was packaged such that each viral genome was encapsidated within the capsid protein shell that that genome encoded. Therefore functional improvements identified through selection can be linked to the genome sequence contained within the viral capsid. From this library, an iterative *in vivo* screening selection process was used to identify variants with the ability to infect the canine retina from the vitreous (FIG. 1). Canine eyes were injected in each round with ~250 μL of 10E+13 - 10E+14 viral genomes/mL (vg/mL) titer virus. Three weeks after injection, eyes were enucleated, and retinal punches were taken from central and peripheral regions of the retina. RPE cells were separated from retinal tissue, and tissue was frozen. DNA was then collected from retinal cells, and cap genes were polymerase chain reaction (PCR) amplified from isolated samples. Cap genes were used for subsequent AAV packaging.

[00355] **FIG. 1**. Illustration of the directed evolution methodology used to develop canine retinal AAV variants. Peptide display libraries were created, packaged into AAV vectors, and injected into the canine eye via intravitreal injections. Iterative round of selection were used to positively select AAV variants from the pool of vectors. Three rounds of selection were followed by a round of error prone PCR, followed by additional selection rounds.

[00356] Following 5 rounds of selection, Illumina deep sequencing was used to identify variants that increased over the rounds in relative representation in the library of AAV variants. An increase of representation in the viral library indicates positive selection and ability to infect the canine retina from the vitreous. Out of a library of ~10E+7 variants, the top 96 variants that were selected for in the in vivo screen are provided in Table 1.

Table 1

	Peptide No.	SEQ ID NO:
LAKDATKNA	1	47
PAHQDTTKNA	2	48
LAHQDTTKNA	3	49
LATTSQNKPA	4	50
LAISDQTKHA	5	51
IARGVAPSSA	6	52
LAPDSTTRSA	7	53
LAKGTELKPA	8	54
LAIIDATKNA	9	55
LAVDGAQRSA	10	56
PAPQDTTKKA	11	57
LPHQDTTKNA	12	58
LAKDATKTIA	13	59
LAKQQSASTA	14	60
LAKSDQSKPA	15	61
LSHQDTTKNA	16	62
LAANQPSKPA	17	63
LAVSDSTKAA	18	64
LAAQGTAKPA	19	65
LAPDQTTRNA	20	66
LAASDSTKAA	21	67
LAPQDTTKNA	22	68
LAKADETRPA	23	69
LAHQDTAKNA	24	70
LAHQDTKKNA	25	71
LAHQDTTKHA	26	72
LAHQDTTKKA	27	73
LAHQDTTRNA	28	74
LAHQDTTTNA	29	75
LAHQGTTKNA	30	76

	Peptide No.	SEQ ID NO:
LAHQVTTKNA	31	77
LAISDQSKPA	32	78
LAKDATKTA	33	79
LAKDTTKNA	34	80
LAKSDQSRPA	35	81
LAPQDTKKNA	36	82
LATSDSTKAA	37	83
LAVDGSQRSA	38	84
LPISDQTKHA	39	85
LPKDATKTIA	40	86
LPPQDTTKNA	41	87
PAPQDTTKNA	42	88
QAHQDTTKNA	43	89
LAHETSPRPA	44	90
LAKSTSTAPA	45	91
LADQDTTKNA	46	92
LAESDQSKPA	47	93
LAHKDTTKNA	48	94
LAHKTQQKM	49	95
LAHQDTTENA	50	96
LAHQDTTINA	51	97
LAHQDTTKKT	52	98
LAHQDTTKND	53	99
LAHQDTTKNT	54	100
LAHQDTTKNV	55	101
LAHQDTTKTM	56	102
LAHQNTTKNA	57	103
LAHRDTTKNA	58	104
LAISDQTNHA	59	105
LAKQKSASTA	60	106
LAKSDQCKPA	61	107
LAKSDQSKPD	62	108
LAKSDQSNPA	63	109
LAKSYQSKPA	64	110
LANQDTTKNA	65	111
LAPQNTTKNA	66	112
LAPSSIQKPA	67	113
LAQQDTTKNA	68	114
LAYQDTTKNA	69	115
LDHQDTTKNA	70	116
LDHQDTTKSA	71	117
LGHQDTTKNA	72	118
LPHQDTTKND	73	119
LPHQDTTKNT	74	120
LPHQDTTNNA	75	121
LTHQDTTKNA	76	122

	Peptide No.	SEQ ID NO:
LTKDATKTIA	77	123
LTPQDTTKNA	78	124
LVHQDTTKNA	79	125
LAKANQNTPA	80	126
LATTPITKPA	81	127
LATTPIAKPA	82	128
LAIEDHTKSA	83	129
LAQSEHQRPA	84	130
LAKSPNKDNA	85	131
LANQDYTKTA	86	132
LANSTDQTRA	87	133
LALGETTRPA	88	134
LANSTEQTRA	89	135
LAQADTTKNA	90	136
LASKDITKTA	91	137
LASPRHNKKC	92	138
LAHQDTTKTIA	93	139
LAAQGTANL	94	140
VAIEDHTKSA	95	141
LAKANQNTPKNA	96	142

[00357] The ability of the top 18 variants of the 96 variants depicted in Table 1 to infect the canine retina was further quantified using high throughput sequencing. Table 2 depicts the top 18 variants chosen for further quantification.

Table 2

LAKDATKNA (SEQ ID NO:47)	LAPDSTTRSA (SEQ ID NO:53)	LAKDATKTIA (SEQ ID NO:59)
PAHQDTTKNA (SEQ ID NO:48)	LAKGTELKPA (SEQ ID NO:54)	LAKQQSASTA (SEQ ID NO:60)
LAHQDTTKNA (SEQ ID NO:49)	LAIIDATKNA (SEQ ID NO:55)	LAKSDQSKPA (SEQ ID NO:61)
LATTSQNKPA (SEQ ID NO:50)	LAVDGAQRSA (SEQ ID NO:56)	LSHQDTTKNA (SEQ ID NO:62)
LAISDQTKHA (SEQ ID NO:51)	PAPQDTTKKA (SEQ ID NO:57)	LAANQPSKPA (SEQ ID NO:63)
IARGVAPSSA (SEQ ID NO:52)	LPHQDTTKNA (SEQ ID NO:58)	LAVSDSTKAA (SEQ ID NO:64)

[00358] Eighteen variants were packaged with a ubiquitous CAG promoter driving expression of GFP. The GFP cDNA was fused to a unique 25 base-pair bar code identifier. Each of the 18 variants was packaged with a unique GFP barcode. Packaged variants were mixed in equal ratios and injected into the retina, along with control AAV2-based vectors (negative controls representing the naturally occurring parental serotype). After injection, DNA and mRNA were collected from photoreceptor and RPE cells. DNA and mRNA levels were quantified to determine the ability of the canine-derived vectors to deliver DNA to the retina and lead to transgene expression (FIG. 2).

[00359] **FIG. 2**. Deep sequencing of variants containing GFP-barcode constructs. Infection of the canine retina by the canine-derived variants was quantified by deep sequencing of tagged GFP cDNA and mRNA.

[00360] Expression of the 18-member library was imaged using confocal microscopy of frozen retinal sections. GFP expression showed that retinal cells in the inner retina, and photoreceptors in the outer retina were targeted with the 18-member library (FIG. 3).

[00361] **FIG. 3**. The 18-member canine-derived AAV variant library infects cells in the ganglion cell layer, the inner nuclear layer, the photoreceptor layer, and the RPE layer.

[00362] Of the top 18 variants tested, 2 variants led to highest level of DNA and mRNA recovery. The variant leading to the highest level of DNA recovery had the insertion sequence ~588- PAPQDTTKKA (SEQ ID NO:57). The variant leading to the highest level of mRNA expression had the insertion sequence ~588- LAPDSTTRSA (SEQ ID NO:53).

[00363] While the present invention has been described with reference to the specific embodiments thereof, it should be understood by those skilled in the art that various changes may be made and equivalents may be substituted without departing from the true spirit and scope of the invention. In addition, many modifications may be made to adapt a particular situation, material, composition of matter, process, process step or steps, to the objective, spirit and scope of the present invention. All such modifications are intended to be within the scope of the claims appended hereto.

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CLAIMS

What is claimed is:

1. A recombinant adeno-associated virus (rAAV) virion comprising:

a) a variant AAV capsid protein, wherein the variant AAV capsid protein comprises an insertion of a heterologous peptide having a length of from about 9 amino acids to about 20 amino acids in the capsid protein GH loop relative to a corresponding parental AAV capsid protein, and wherein the variant capsid protein confers increased infectivity of a retinal cell compared to the infectivity of the retinal cell by a control AAV virion comprising the corresponding parental AAV capsid protein, and wherein the heterologous peptide comprises an amino acid sequence selected from:

PAPQDTTKKA (SEQ ID NO:57), LATTSQNKPA (SEQ ID NO:50), LAKGTELKPA (SEQ ID NO:54), LAVDGAQRSA (SEQ ID NO:56), LAKSDQSKPA (SEQ ID NO:61) and LAANQPSKPA (SEQ ID NO:63); and

b) a heterologous nucleic acid comprising a nucleotide sequence encoding a heterologous gene product.

2. The rAAV virion of claim 1, wherein the rAAV virion exhibits at least 5-fold increased infectivity of a retinal cell compared to the infectivity of the retinal cell by a control AAV virion comprising the corresponding parental AAV capsid protein and/or wherein the rAAV virion exhibits at least 5-fold increased localization to one or more of the inner nuclear layer, the outer nuclear layer, the photoreceptor layer, the ganglion cell layer, and the retinal pigment epithelium, compared to the extent of localization to the inner nuclear layer, the outer nuclear layer, the photoreceptor layer, the ganglion cell layer, or the retinal pigment epithelium, by an AAV virion comprising the corresponding parental AAV capsid protein.

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3. The rAAV virion of claim 1 or 2, wherein the insertion site is between amino acids corresponding to amino acids 570 and 611 of VP1 of AAV2, or the corresponding position in the capsid protein of another AAV serotype.

4. The rAAV virion of claim 3, wherein the insertion site is located between amino acids corresponding to amino acids 587 and 588 of VP1 of AAV2, or the corresponding position in the capsid protein of another AAV serotype.

5. The rAAV virion of any one of claims 1-4, wherein the heterologous gene product is:

a) an interfering RNA or an aptamer;

b) a polypeptide;

c) a neuroprotective polypeptide, an anti-angiogenic polypeptide, an anti-apoptotic polypeptide, or a polypeptide that enhances function of a retinal cell, or an RNA-guided endonuclease; or

d) an RNA-guided endonuclease and a guide RNA.

6. The rAAV virion of any one of claims 1-4, wherein the heterologous gene product is glial derived neurotrophic factor, fibroblast growth factor 2, neurturin, ciliary neurotrophic factor, nerve growth factor, brain derived neurotrophic factor, epidermal growth factor, rhodopsin, X-linked inhibitor of apoptosis, retinoschisin, RPE65, retinitis pigmentosa GTPase-interacting protein-1, peripherin, peripherin-2, RdCVF, retinitis pigmentosa GTPase regulator (RPGR), or Sonic hedgehog.

7. The rAAV virion of any one of claims 1-6, wherein the heterologous peptide comprises the following amino acid sequence: PAPQDTTKKA (SEQ ID NO:57).

8. The rAAV virion of any one of claims 1-6, wherein the heterologous peptide comprises the following amino acid sequence: LATTSQNKPA (SEQ ID NO:50).

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9. The rAAV virion of any one of claims 1-6, wherein the heterologous peptide comprises the following amino acid sequence: LAKGTELKPA (SEQ ID NO:54).

10. The rAAV virion of any one of claims 1-6, wherein the heterologous peptide comprises the following amino acid sequence: LAVDGAQRSA (SEQ ID NO:56).

11. The rAAV virion of any one of claims 1-6, wherein the heterologous peptide comprises the following amino acid sequence: LAKSDQSKPA (SEQ ID NO:61).

12. The rAAV virion of any one of claims 1-6, wherein the heterologous peptide comprises the following amino acid sequence: LAANQPSKPA (SEQ ID NO:63).

13. A pharmaceutical composition comprising:

a) a recombinant adeno-associated virus (AAV) virion of any one of claims 1-12; and

b) a pharmaceutically acceptable excipient.

14. A method of delivering a gene product to a retinal cell in an individual, the method comprising administering to the individual a recombinant adeno-associated virus (rAAV) virion according any one of claims 1-12.

15. A method of treating an ocular disease, the method comprising administering to an individual in need thereof an effective amount of a recombinant adeno-associated virus (rAAV) virion according to any one of claims 1-12.

16. The method of claim 15, wherein said administering is by intraocular injection.

17. The method of claim 15, wherein said administering is by intravitreal injection.

18. Use of an effective amount of a recombinant adeno-associated virus (rAAV) virion according to any one of claims 1-12 in the preparation of a medicament for treating an ocular disease.

19. The method of any one of claims 15-17, or the use of claim 18, wherein the ocular disease is glaucoma, retinitis pigmentosa, macular degeneration, retinoschisis, Leber's Congenital Amaurosis, diabetic retinopathy, achromotopsia, or color blindness.

20. An isolated nucleic acid comprising a nucleotide sequence that encodes a variant adeno-associated virus (AAV) capsid protein, wherein the variant AAV capsid protein comprises an insertion of from about 9 amino acids to about 20 amino acids in the capsid protein GH loop relative to a corresponding parental AAV capsid protein, and wherein the variant capsid protein, when present in an AAV virion, provides for increased infectivity of the AAV virion of a retinal cell, and wherein the amino acid insertion is in the GH loop of a native AAV capsid,

and wherein the heterologous peptide comprises an amino acid sequence selected from:

PAPQDTTKKA (SEQ ID NO:57), LATTSQNKPA (SEQ ID NO:50), LAKGTELKPA (SEQ ID NO:54), LAVDGAQRSA (SEQ ID NO:56), LAKSDQSKPA (SEQ ID NO:61) and LAANQPSKPA (SEQ ID NO:63).

21. The isolated nucleic acid of claim 20, wherein the insertion site is between amino acids 587 and 588 of AAV2, between amino acids 590 and 591 of AAV1, 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.

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22. The isolated nucleic acid of claim 20 or 21, wherein the heterologous peptide comprises the following amino acid sequence: PAPQDTTKKA (SEQ ID NO:57).

23. The isolated nucleic acid of claim 20 or 21, wherein the heterologous peptide comprises the following amino acid sequence: LATTSQNKPA (SEQ ID NO:50).

24. The isolated nucleic acid of claim 20 or 21, wherein the heterologous peptide comprises the following amino acid sequence: LAKGTELKPA (SEQ ID NO:54).

25. The isolated nucleic acid of claim 20 or 21, wherein the heterologous peptide comprises the following amino acid sequence: LAVDGAQRSA (SEQ ID NO:56).

26. The isolated nucleic acid of claim 20 or 21, wherein the heterologous peptide comprises the following amino acid sequence: LAKSDQSKPA (SEQ ID NO:61).

27. The isolated nucleic acid of claim 20 or 21, wherein the heterologous peptide comprises the following amino acid sequence: LAANQPSKPA (SEQ ID NO:63).

28. An isolated, genetically modified host cell comprising the nucleic acid of any one of claims 20-27.

29. A variant adeno-associated virus (AAV) capsid protein, wherein the variant AAV capsid protein comprises an insertion of a heterologous peptide having a length of from about 9 amino acids to about 20 amino acids wherein the amino acid insertion is in the GH loop of a native AAV capsid, and wherein the heterologous peptide comprises an amino acid sequence selected from:

PAPQDTTKKA (SEQ ID NO:57), LATTSQNKPA (SEQ ID NO:50), LAKGTELKPA (SEQ ID NO:54), LAVDGAQRSA (SEQ ID NO:56), LAKSDQSKPA (SEQ ID NO:61) and LAANQPSKPA (SEQ ID NO:63).

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30. The variant AAV capsid protein of claim 29, wherein the heterologous peptide comprises the following amino acid sequence: PAPQDTTKKA (SEQ ID NO:57).

31. The variant AAV capsid protein of claim 29, wherein the heterologous peptide comprises the following amino acid sequence: LATTSQNKPA (SEQ ID NO:50).

32. The variant AAV capsid protein of claim 29, wherein the heterologous peptide comprises the following amino acid sequence: LAKGTELKPA (SEQ ID NO:54).

33. The variant AAV capsid protein of claim 29, wherein the heterologous peptide comprises the following amino acid sequence: LAVDGAQRSA (SEQ ID NO:56).

34. The variant AAV capsid protein of claim 29, wherein the heterologous peptide comprises the following amino acid sequence: LAKSDQSKPA (SEQ ID NO:61).

35. The variant AAV capsid protein of claim 29, wherein the heterologous peptide comprises the following amino acid sequence: LAANQPSKPA (SEQ ID NO:63).

36. The variant AAV capsid protein of any one of claims 29-35, wherein the insertion site is between amino acids 587 and 588 of AAV2, between amino acids 590 and 591 of AAV1, 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.

FIG. 1

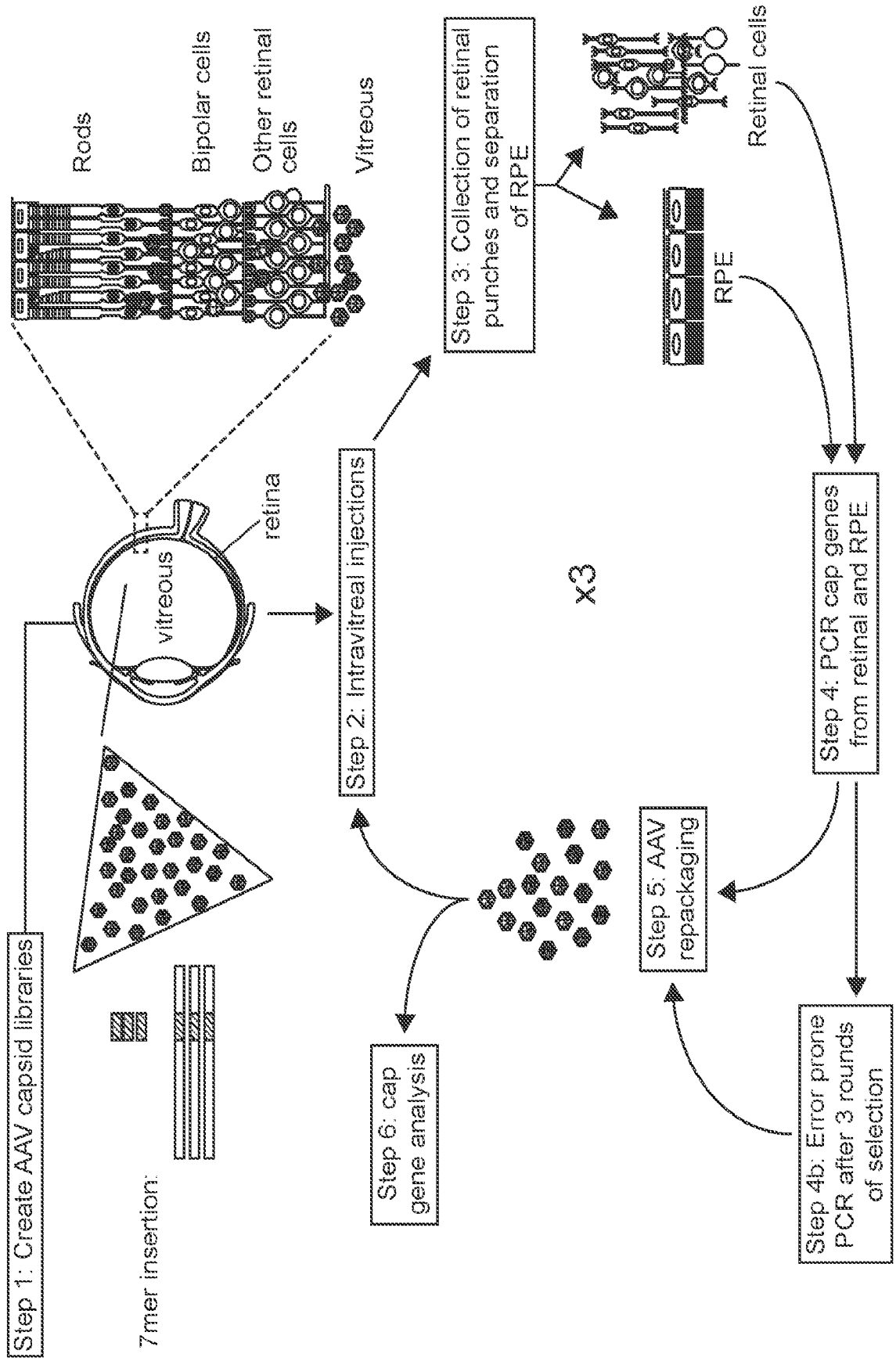
Rods
Bipolar cells
Other retinal cells
Vitreous
retina
vitreous
Step 1: Create AAV capsid libraries
7mer insertion
Step 2: Intravitreal injections
Step 3: Collection of retinal punches and separation of RPE
RPE
Retinal cells
x3
Step 4: PCR cap genes from retinal and RPE
Step 5: AAV repackaging
Step 6: cap gene analysis
Step 4b: Error prone PCR after 3 rounds of selection

FIG. 2

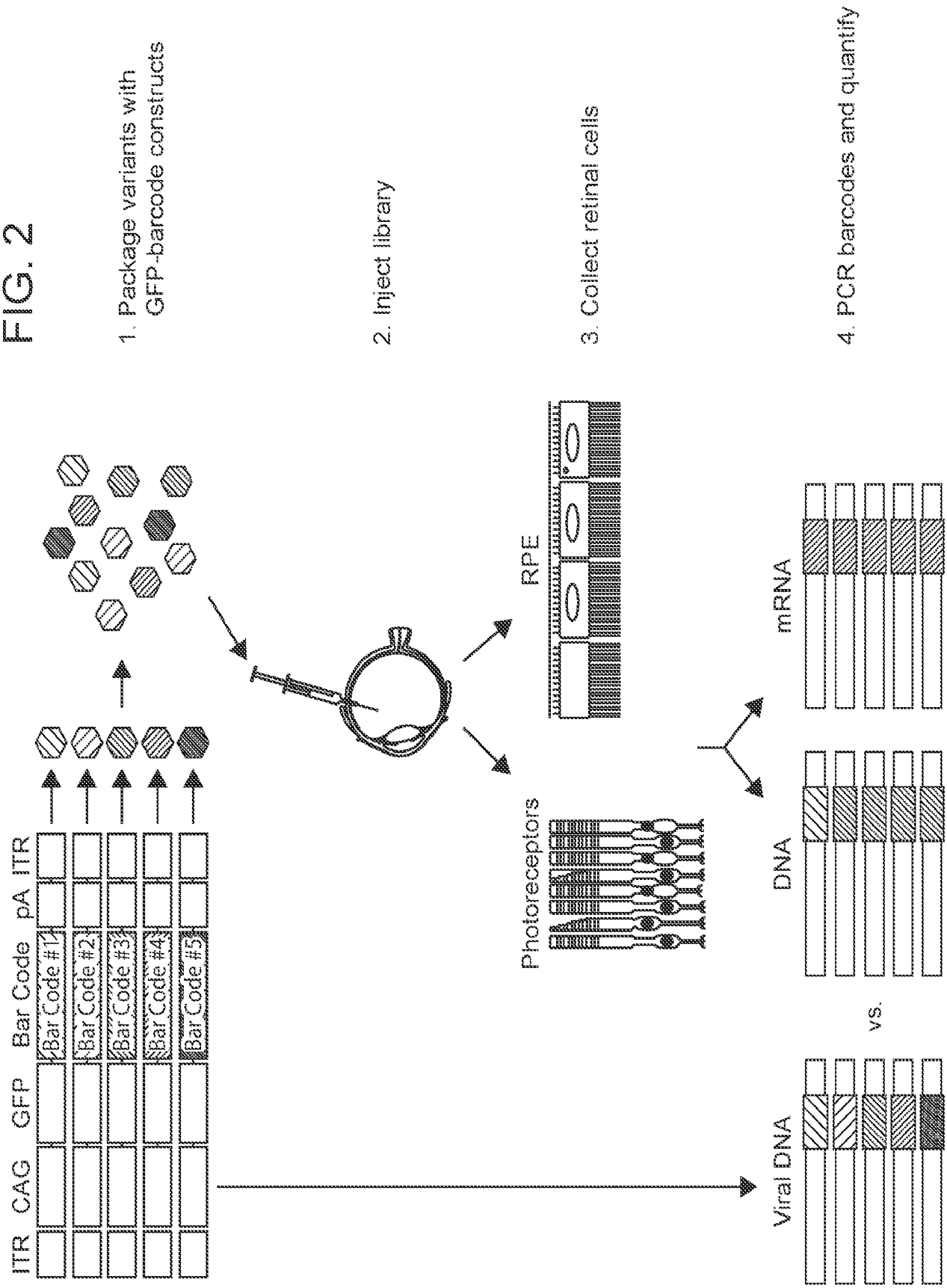
1. Package variants with GFP-barcode constructs
2. Inject library
3. Collect retinal cells
4. PCR barcodes and quantify
ITR
CAG
GFP
Bar Code
pA
ITR
Bar Code #1
Bar Code #2
Bar Code #3
Bar Code #4
Bar Code #5
RPE
Photoreceptors
mRNA
DNA
vs.
Viral DNA

FIG. 3

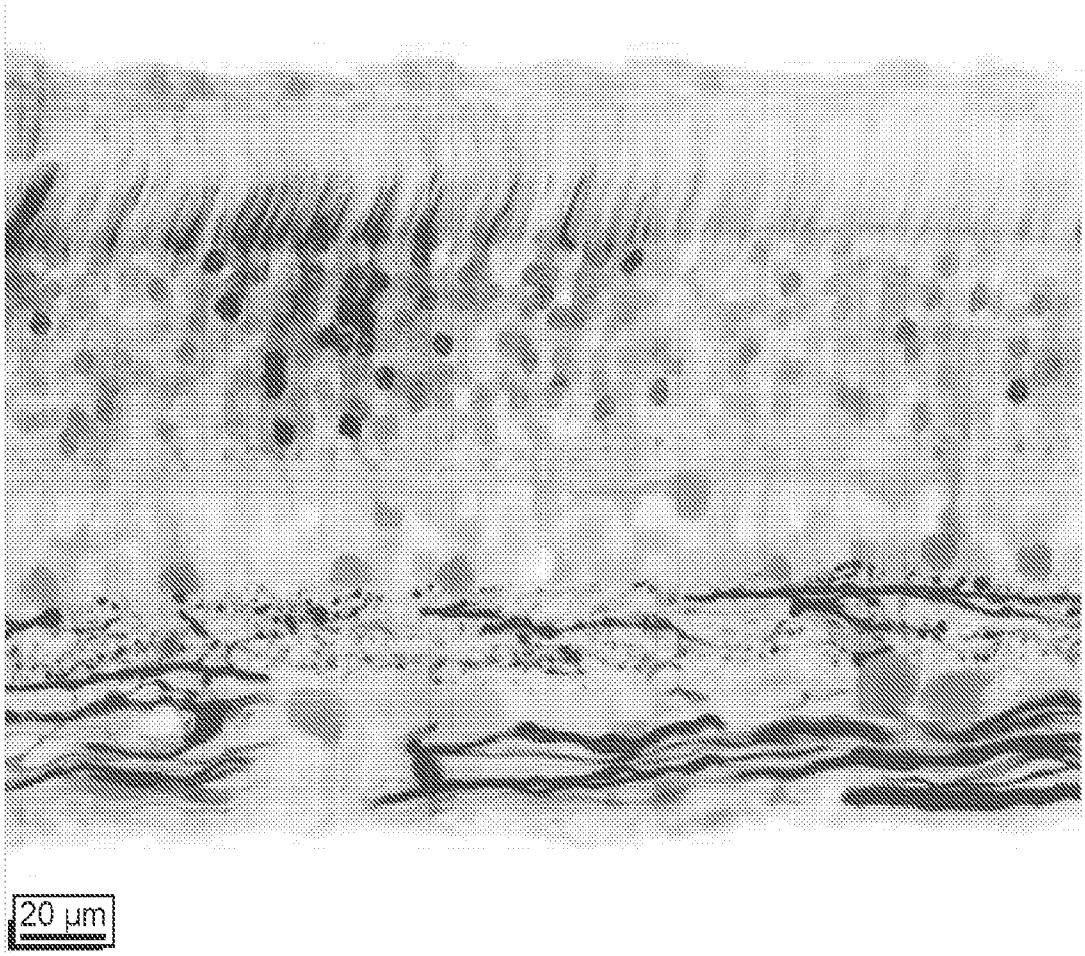

FIG. 4

```
AAV2 VP1    1 MAADGYLPDWLEDTLSEGIRQWWKLKPGPPPPKPAERHKDDSRGLVLPGYKYLGPFNGLD
AAV2 VP1   61 KGEPVNEADAAALEHDKAYDRQLDSGDNPYLKYNHADAEFQERLKEDTSFGGNLGRAVFQ
AAV2 VP1  121 AKKRVLEPLGLVEEPVKTAPGKKRPVEHSPVEPDSSSGTGKAGQQPARKRLNFGQTGDAD
AAV2 VP1  181 SVPDPQPLGQPPAAPSGLGTNTMATGSGAPMADNNEGADGVGNSSGNWHCDSTWMGDRVI
AAV2 VP1  241 TTSTRTWALPTYNNHLYKQISSQSGASNDNHYFGYSTPWGYFDFNRFHCHFSPRDWQRLI
AAV2 VP1  301 NNNWGFRPKRLNFKLFNIQVKEVTQNDGTTTIANNLTSTVQVFTDSEYQLPYVLGSAHQG
AAV2 VP1  361 CLPPFPADVFMVPQYGYLTLNNGSQAVGRSSFYCLEYFPSQMLRTGNNFTFSYTFEDVPF
AAV2 VP1  421 HSSYAHSQSLDRLMNPLIDQYLYYLSRTNTPSGTTTQSRLQFSQAGASDIRDQSRNWLPG
AAV2 VP1  481 PCYRQQRVSKTSADNNNSEYSWTGATKYHLNGRDSLVNPGPAMASHKDDEEKFFPQSGVL
AAV2 VP1  541 IFGKQGSEKTNVDIEKVMITDEEEIRTTNPVATEQYGSVSTNLQRGNRQAATADVNTQGV
AAV2 VP1  601 LPGMVWQDRDVYLQGPIWAKIPHTDGHFHPSPLMGGFGLKHPPPQILIKNTPVPANPSTT
AAV2 VP1  661 FSAAKFASFITQYSTGQVSVEIEWELQKENSKRWNPEIQYTSNYNKSVNVDFTVDTNGVY
AAV2 VP1  721 SEPRPIGTRYLTR  (SEQ ID NO:1)
```

FIG. 5

AAV-2	570	FVATEQYGSVSTNLQRG**NR**QAATADVNTQGVLPGMVWQDRDV	611	(SEQ ID NO:2)
AAV-1	571	PVATERFGTVAVNFQSSS**TDP**ATGDVHAMGALPGMVWQDRDV	612	(SEQ ID NO:3)
AAV-5	560	RVAYNVGGQMATNNQ**SST**TAPATGTYNLQEIVPGSVWMERDV	601	(SEQ ID NO:4)
AAV-6	571	PVATERFGTVAVNLQSSS**TDP**ATGDVHVMGALPGMVWQDRDV	612	(SEQ ID NO:5)
AAV-7	572	PVATEEYGIVSSNLQAA**NT**AAQTQVVNNQGALPGMVWQNRDV	613	(SEQ ID NO:6)
AAV-8	573	PVATEEYGIVADNLQQQ**NT**APQIGTVNSQGALPGMVWQNRDV	614	(SEQ ID NO:7)
AAV-9	571	PVATESYGQVATNHQSA**QA**QAQTGWVQNQGILPGMVWQDRDV	612	(SEQ ID NO:8)
AAV-10	573	PVATEQYGVVADNLQ**QA**NTGPIVGNVNSQGALPGMVWQNRDV	614	(SEQ ID NO:9)

FIG. 6A

```
AAV1        --TFSYTFEEVPFHSSYAHSQSLDRLMNPLIDQYLYYLNRTQ-NQSGSAQNKDLLFSRGS 467
AAV6        --TFSYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYLNRTQ-NQSGSAQNKDLLFSRGS 467
AAV3        ---FSYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYLNRTQGTTSGTTNQSRLLFSQAG 467
AAV2        ---FSYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYLSRTN-TPSGTTTQSRLQFSQAG 466
AAV8        NFQFTYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYLSRTQTT-GGTANTQTLGFSQGG 469
AAV8.1      NFQFTYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYLSRTQTT-GGTANTQTLGFSQGG 469
AAV8 rh8     FQFSYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYLVRTQTTGTGGTQTLAFSQAGPS 469
AAV10       NFEFSYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYLSRTQST-GGTQGTQQLLFSQAG 469
AAV7        -FEFSYSFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYLARTQSNPGGTAGNRELQFYQGG 469
AAV9        -FQFSYEFENVPFHSSYAHSQSLDRLMNPLIDQYLYYLSKTI--NGSGQNQQTLKFSVAG 467
AAV9.1      -FQFSYEFENVPFHSSYAHSQSLDRLMNPLIDQYLYYLSKTI--NGSGQNQQTLKFSVAG 467
AAV5        NFEFTYNFEEVPFHSSFAPSQNLFKLANPLVDQYLYRFVSTN-------NTGGVQFNKNL 453
                *:*  **:******:*  **.*  :*  ***:*****  :   *          :  *

AAV1        PAGMSVQPKNWLPGPCYRQQRVSKTKTDNNNSNFTWTGASKYNLNGRESIINPGTAMASH 527
AAV6        PAGMSVQPKNWLPGPCYRQQRVSKTKTDNNNSNFTWTGASKYNLNGRESIINPGTAMASH 527
AAV3        PQSMSLQARNWLPGPCYRQQRLSKTANDNNNSNFPWTAASKYHLNGRDSLVNPGPAMASH 527
AAV2        ASDIRDQSRNWLPGPCYRQQRVSKTSADNNNSEYSWTGATKYHLNGRDSLVNPGPAMASH 526
AAV8        PNTMANQAKNWLPGPCYRQQRVSTTTGQNNNSNFAWTAGTKYHLNGRNSLANPGIAMATH 529
AAV8.1      PNTMANQAKNWLPGPCYRQQRVSTTTGQNNNSNFAWTAGTKYHLNGRNSLANPGIAMATH 529
AAV8 rh8    S--MANQARNWVPGPCYRQQRVSTTTNQNNNSNFAWTGAAKFKLNGRDSLMNPGVAMASH 527
AAV10       PANMSAQAKNWLPGPCYRQQRVSTTLSQNNNSNFAWTGATKYHLNGRDSLVNPGVAMATH 529
AAV7        PSTMAEQAKNWLPGPCFRQQRVSKTLDQNNNSNFAWTGATKYHLNGRNSLVNPGVAMATH 529
AAV9        PSNMAVQGRNYIPGPSYRQQRVSTTVTQNNNSEFAWPGASSWALNGRNSLMNPGPAMASH 527
AAV9.1      PSNMAVQGRNYIPGPSYRQQRVSTTVTQNNNSEFAWPGASSWALNGRNSLMNPGPAMASH 527
AAV5        AGRYANTYKNWFPGPMGRTQGWNLGSGVNRASVSAFATTNRMELEGASYQVPPQPNGMTN 513
            .         :*::***  *  *    .       *. *   .:.   .    *:* .    *      ::
```

FIG. 6B

```
AAV1       KDDEDKFFPMSGVMIFGK--ESAGASNTALD-NVMITDEEEIKATNPVATERFGTVAVNF 584
AAV6       KDDKDKFFPMSGVMIFGK--ESAGASNTALD-NVMITDEEEIKATNPVATERFGTVAVNL 584
AAV3       KDDEEKFFPMHGNLIFGK--EGTTASNAELD-NVMITDEEEIRTTNPVATEQYGTVANNL 584
AAV2       KDDEEKFFPQSGVLIFGK--QGSEKTNVDIE-KVMITDEEEIRTTNPVATEQYGSVSTNL 583
AAV8       KDDEERFFPSNGILIFGK--QNAARDNADYS-DVMLTSEEEIKTTNPVATEEYGIVADNL 586
AAV8.1     KDDEERFFPSNGILIFGK--QNAARDNADYS-DVMLTSEEEIKTTNPVATEEYGIVADNL 586
AAV8 rh8   KDDDDRFFPSSGVLIFGK--QGAGNDGVDYS-QVLITDEEEIKATNPVATEEYGAVAINN 584
AAV10      KDDEERFFPSSGVLMFGK--QGAGRDNVDYS-SVMLTSEEEIKTTNPVATEQYGVVADNL 586
AAV7       KDDEDRFFPSSGVLIFGK--TGAT-NKTTLE-NVLMTNEEEIRPTNPVATEEYGIVSSNL 585
AAV9       KEGEDRFFPLSGSLIFGK--QGTGRDNVDAD-KVMITNEEEIKTTNPVATESYGQVATNH 584
AAV9.1     KEGEDRFFPLSGSLIFGK--QGTGRDNVDAD-KVMITNEEEIKTTNPVATESYGQVATNH 584
AAV5       NLQGSNTYALENTMIFNSQPANPGTTATYLEGNMLITSESETQPVNRVAYNVGGQMATNN 573
           :   .. :.  . ::*..    ..     .  . .:::*.*.* :..* ** :  * :: *

AAV1       QSSSTDPATGDVHAMGALPGMVWQDRDVYLQGPIWAKIPHTDGHFHPSPLMGGFGLKNPP 644
AAV6       QSSSTDPATGDVHVMGALPGMVWQDRDVYLQGPIWAKIPHTDGHFHPSPLMGGFGLKHPP 644
AAV3       QSSNTAPTTGTVNHQGALPGMVWQDRDVYLQGPIWAKIPHTDGHFHPSPLMGGFGLKHPP 644
AAV2       QRGNRQAATADVNTQGVLPGMVWQDRDVYLQGPIWAKIPHTDGHFHPSPLMGGFGLKHPP 643
AAV8       QQQNTAPQIGTVNSQGALPGMVWQNRDVYLQGPIWAKIPHTDGNFHPSPLMGGFGLKHPP 646
AAV8.1     QGQRQAAQIGTVNSQGALPGMVWQNRDVYLQGPIWAKIPHTDGNFHPSPLMGGFGLKHPP 646
AAV8 rh8   QAANTQAQTGLVHNQGVIPGMVWQNRDVYLQGPIWAKIPHTDGNFHPSPLMGGFGLKHPP 644
AAV10      QQANTGPIVGNVNSQGALPGMVWQNRDVYLQGPIWAKIPHTDGNFHPSPLMGGFGLKHPP 646
AAV7       QAANTAAQTQVVNNQGALPGMVWQNRDVYLQGPIWAKIPHTDGNFHPSPLMGGFGLKHPP 645
AAV9       QSAQAQAQTGWVQNQGILPGMVWQDRDVYLQGPIWAKIPHTDGNFHPSPLMGGFGMKHPP 644
AAV9.1     QSGQAQAATGWVQNQGILPGMVWQDRDVYLQGPIWAKIPHTDGNFHPSPLMGGFGMKHPP 644
AAV5       QSSTTAPATGTYNLQEIVPGSVWMERDVYLQGPIWAKIPETGAHFHPSPAMGGFGLKHPP 633
           *         .         :    :** ** :*************.*..:***** *****:*:**
```

FIG. 6C

```
AAV1        PQILIK- 650 (SEQ ID NO:35)
AAV6        PQILIK- 650 (SEQ ID NO:36)
AAV3        PQIMTK- 650 (SEQ ID NO:37)
AAV2        PQILIKN 650 (SEQ ID NO:38)
AAV8        PQILIKN 653 (SEQ ID NO:39)
AAV8.1      PQILIKN 653 (SEQ ID NO:40)
AAV8 rh8    PQILIKN 651 (SEQ ID NO:41)
AAV10       PQILIKN 653 (SEQ ID NO:42)
AAV7        PQILIKN 652 (SEQ ID NO:43)
AAV9        PQILIK- 650 (SEQ ID NO:44)
AAV9.1      PQILIK- 650 (SEQ ID NO:45)
AAV5        PMMLIKN 640 (SEQ ID NO:46)
            *  ::**
```

FIG. 7A

Retinoschisin-1
Homo sapiens

```
  1 msrkiegfil llifgyeatl glsstedege dpwyqkackc dcqggpnalw sagatsldci
 61 pecpyhkplg fesgevtpdq itcsnpeqyv gwysswtank arlnsqgfgc awlskfqdss
121 qwlqidlkei kvisgiltqg rcdidewmtk ysvqyrtder lnwiyykdqt gnnrvfygns
181 drtstvqnil rppiisrfir liplqwhvri airmelleev skca (SEQ ID NO:10)
```

FIG. 7B

BDNF
Homo sapiens

```
  1 mtilfltmvi syfgcmkaap mkeanirqqg glaypgvrth gtlesvngpk agsrgltsla
 61 dtfehvieel ldedhkvrpn eennkdadly tsrvmlssqv pleppllfll eeyknyldaa
121 nmsmmvlrhs dpaarqgelsv cdsisewvta adkktavdms ggtvtvlekv pvskgqlkqy
181 fyetkcnpmg ytkegcrgid krhwnsqcrt tqsyvralтm dskkrigwrf iridtscvct
241 ltikrgr (SEQ ID NO:11)
```

FIG. 7C

RPE65
Homo sapiens

```
   1 msiqvehpag gykklfetve elsspltahv tgriplwltg sllrcgpglf evqsepfyhl
  61 fdgqallhkf dfkeghvtyh rrfirtdayv ramtekrivi tefgtcafpd pcknifsrff
 121 syfrqvevtd nalvnvypvg edyyactetn fitkinpetl etikqvdlcn yvsvngatah
 181 phiendgtvy niqncfgknf siaynivkip plqadkedpi skseivvqfp csdrfkpsyv
 241 hsfgltpnyi vfvetpvkin lfkflsswsl wganymdcfe snetmgvwlh iadkkrkkyl
 301 nnkyrtspfn lfhhintyed ngflivdlcc wkgfefvyny lylanlrenw eevkkknarka
 361 pqpevrryvl pinidkadtg knlvtlpntt atailcsdet iwlepevlfs qprqafefpq
 421 inyqkycqkp ytyayglqln hfvpdrlckl nvktketwvw qepdsypsep ifvshpdale
 481 eddqvvlsvv vspgaggkpa yllilnakdl sevaraevei nipvtfhglf kks
      (SEQ ID NO:12)
```

FIG. 7D

Peripherin-2
Homo sapiens

```
   1 mallkvkfdq kkrvklaqgl wlmnwfsvla giiifsiglf lkielrkrsd vmnnseshfv
  61 pnsliqmgvl scvfnslagk icydaldpak yarwkpwlkp ylaicvlfni ilflvalccf
 121 llrgsientl gqglknqmky yrdtdtpgrc fmkktidmlq iefkccqnng frdwfeiqwi
 181 snryldfssk evkdriksnv dgrylvdqvp fsccnpsspr pciqyqitnn sahysydhqt
 241 eelnlwvrgc raallsyyss lmnsmgvvtl liwlfevtit iglrylqtsl dgvsnpeese
 301 sesqgwller svpetwkafl esvkkligkgn qveaeqadaq qapeaq (SEQ ID NO:13)
```

FIG. 7E

Peripherin
Homo sapiens

```
   1 mshhpsglra gfsstsyrrt fgpppslspg afsysssssrf sssrllgsas psssvrlgsf
  61 rspragagal lrlpserldf smaealnqef latrsnekqe lqelndrfan fiekvrfleq
 121 qmaalrgels qargqepara dqlcqqelre lrrelellgr erdrvqverd glaedlaalk
 181 qrleeetrkr edaehnlvlf rkdvddatls rlelerkies lmdeieflkk lheeelrdlq
 241 vsvesqqvqq veveatvkpe ltaalrdira qyesiaaknl qeaeewyksk yadlsdaanr
 301 nhealrqakq emmesrrqiq sltcevdgLr gtneallrql releeqfale aggyqagaar
 361 leeelrqlke emarhlreyq ellnvkmald ieiatyrkll egeesrisvp vhsfaslnik
 421 ttvpeveppq dshsrktvli ktietrngev vtesqkeqrs eldkssahsy (SEQ ID NO:14)
```

FIG. 7F

RPGR-interacting protein-1
Homo sapiens

```
   1 mshlvdptsg dlpvrdidai plvlpaskgk nmktqpplsr mnreeledsf frlredhmlv
  61 kelswkqgde ikrlrttllr ltaagrdlrv aeeaapliset arrgqkagwr qrlsmhqrpq
 121 mhrlqghfhc vgpasprraq prvqvghrql htagapvpek pkrgprdrls ytappsfkeh
 181 atnenrgeva skpselvsgs nsiisfssvi smakpiglcm pnsahimasn tmqveeppks
 241 pekmwpkden feqrssleca qkaaelrasi kekvelirlk kllhernasl vmtkaqltev
 301 qeayetllqk nqgilsaahe allkqvnelr aelkeeskka vslksqledv silqmtlkef
 361 qervedleke rkllndnydk llesmldssd sssqphwsne liaeqlqqqv sqlqdqldae
 421 ledkrkvlle lsrekaqned lklevtniiq khkqevellq naatisqppd rqsepathpa
 481 vlqentqiep sepknqeekk lsqvlnelqv shaettlele ktrdmlilqr kinvcyqeel
 541 eammtkadnd nrdhkekler ltrlldlkmn rikqlegilr shdlptseql kdvaygtrpl
 601 slcletlpah qdedkvdisl lhqqenlfel hihqafltsa alaqaqdtqp ttfctysfyd
 661 fethctplsv gpqplydfts qyvmetdslf lhylqeasar ldihqamase hstlaaqwic
 721 fdrvletvek vhglatliqa ggeefgvley wmrlrfpikp slqacnkrkk aqvylstdvl
 781 ggrkaqeeef rseswepqne lwieitkccg lrsrwlqtqp spyavyrfft fsdhdtaiip
 841 asmpyfrdq arfpvlvtsd ldhyirreal sihvfddedl epgsylgrar vpllplakne
 901 sikgdfnltd paekpngsiq vqldwkfpyi ppesflkpea qtkgkdtkds skisseeeka
 961 sfpsqdqmas pevpieaggy rskrkpphgg erkekehqvv sysrrkhgkr igvqgknrme
1021 ylslnilngn tpeqvmytew kfsetnsfig dgfknqheee emtlshsalk qkeplhpvnd
1081 kesseqgsev seaqttdsdd vivppmsqky pkadsekmci eivslafype aevmsdenik
1141 qvyveykfyd lplsetetpv slrkpragee ihfhfskvid ldpqeqggrr rflfdmlngq
1201 dpdqghlkft vvsdpldeek keceevgyay lqlwqilesg rdileqeldi vspedlatpi
1261 grlkvslqaa avlhaiykem tedlfs (SEQ ID NO:15)
```

FIG. 7G

Rab escort protein-1

```
   1 madtlpsefd vivigtglpe siiaaacsrs qrrvlhvdsr syyggnwasf sfsgllswlk
  61 eyqensdivs dspvwqdqil eneeaialsr kdktiqhvev fcyasqqdlh dveeagalqk
 121 nhalvtsans teaadsaflp tedeslstms cemlteqtps sdpenalevn gaevtgeken
 181 hcddktcvps tsaedmsenv piaedtteqp kknritysqi ikegrrfnid lvskllysrg
 241 llidllliksn vsryaefkni trilafregr veqvpcsrad vfnskqltmv ekrmlmkflt
 301 fcmeyekypd eykgyeeitf yeylktqklt pnlqyivmhs iamtsetass tidglkatkn
 361 flhclgrygn tpflfplygq gelpqcfcrm cavfggiycl rhsvqclvvd kesrkckaii
 421 dqfqgriise hflvedsyfp enmcsrvqyr qisravlitd rsvlktdsdq qisiltvpae
 481 epgtfavrvi elcsstmtcm kgtylvhltc tsskttaredl esvvqklfvp ytemeieneq
 541 vekprilwal yfnmrdssdi srscyndlps nvyyvcsgpdc glqndnavkq aetlfqeicp
 601 nedfcppppn pediildgds lqpeasessa ipeansetfk estnlqnlee sse
```

(SEQ ID NO:16)

FIG. 7H

212-amino acid isoform of RdCVF

```
  1 maslfsgril irmnsdqdel dteaevsrrl enrlvllffg agacpqcqaf vpilkdffvr
 61 ltdefyvlra aqlalvyvsq dsteeqqdlf lkdmpkkwlf lpfeddlrrd lgrqfsverl
121 pavvvlkpdg dvltrdgade iqrlgtacfa nwqeaaevld rnfqlpedle dqeprsltec
181 lrrhkyrvek aarggrdpgg gggeeggagg lf (SEQ ID NO:17)
```

FIG. 7I

156-amino acid isoform of RdCVF (isoform 1)

```
  1 mvdilgerhl vtckgatvea eaalqnkvva lyfaaarcap srdftpllcd fytalvaear
 61 rpapfevvfv sadgssqeml dfmrelhgaw lalpfhdpyr helrkrynvt aipklvivkq
121 ngevitnkgr kqirerglac fqdwveaadi fqnfsv (SEQ ID NO:18)
```

FIG. 7J

135-amino acid isoform of RdCVF (isoform 2)

```
  1 mvdilgerhl vtckgatvea eaalqnkvva lyfaaarcap srdftpllcd fytalvaear
 61 rpapfevvfv sadgssqeml dfmrelhgaw lalpfhdpyr qrslallprl ecsgvilahc
121 nlcllgssds lalas (SEQ ID NO:19)
```

FIG. 7K

Rod cGMP-specific 3',5'-cyclic phosphodiesterase subunit alpha (PDE6α)
GenBank NP_000431

```
   1 mgevtaeeve kfldsnigfa kqyynlhyra klisdilgak eaavdfsnyh spssmeesei
  61 ifdllrdfqe nlqtekcifn vmkklcfllq adrmslfmyr trngiaelat rlfnvhkdav
 121 ledclvmpdq eivfpldmgi vghvahskki anvpnteede hfcdfvdilt eyktknilas
 181 pimngkdvva iimavnkvdg shftkrdeei llkylnifanl imkvyhlsyl hncetrrgqi
 241 llwsgskvfe eltdierqfh kalytvrafl ncdrysvgll dmtkqkeffd vwpvlmgevp
 301 pysgprtpdg reinfykvid yilhgkedik vipnpppdhw alvsqlpayv aqmqlicnim
 361 napaedffaf qkepldesgw miknvlsmpi vnkkeeivgv atfynrkdqk pfdemdetlm
 421 esltqflgws vlnpddtyesm nklenrkdif qdivkyhvkc dneeiqkilk trevygkepw
 481 eceeeelaei lqaeipdadk yeinkfhfsd lpltelelvk cqiqmyyelk vvdkfhipqe
 541 alvrfmysls kgyrkityhn wrhgfnvgqt mfslivtgkl kryftdleal amvtaafchd
 601 idhrgtnnly qmksqmplak lhgssilerh hlefgktllr deslnifqnl nrrqhehaih
 661 nmdiaiiatd lalyfkkrtm fqkivdqskt yeseqewtqy mmlegtrkei vmammmtacd
 721 lsaitkpwev qsqvallvaa efweqgdler tvlqqnpipm mdrnkadelp klqvqfidfv
 781 ctfvykefsr fheeitpmld gitnnrkewk aladeydakm kvqeekkqkq qsaksaaagn
 841 qpgqnpspgg atts kscciq
```

(SEQ ID NO:20)

FIG. 7L

Rod cGMP-specific 3',5'-cyclic phosphodiesterase subunit beta isoform 1 (PDE6β isoform 1)
GenBank NP_000274

```
   1 mslseeqars fldqmpdfar qyfgkklspe nvaaacedgc ppdcdslrdl cqveestall
  61 elvqdmqesi nmervvfkvl rrlctllqad rcslfmyrqr ngvaelatrl fsvqpdsvle
 121 dclvppdsei vfpldigvvg hvaqtkkmvn vedvaecphf ssfadeltdy ktknmlatpi
 181 mngkdvvavi mavnklngpf ftsededvfl kylnfatlyl kiyhlsylhn cetrrgqvll
 241 wsankvfeel tdierqfhka fytvraylnc erysvgildm tkekeffdvw svlmgesqpy
 301 sgprtpdgre ivfykvidyi lhgkeeikvi ptpsadhwal asglpsyvae sgficnimna
 361 sademfkfqe galddsgwli knvismpivn kkeeivgvat fynrkdgkpf deqdevlmes
 421 ltqflgwsvm ntdtydkmnk lenrkdiaqd mvlyhvkcdr deiqliptr arlqkepadc
 481 dedelgeilk eelpgpttfd iyefhfsdle cteldlvkcg iqmyyvelgvv rkfqipqevl
 541 vrflfsiskg yrrityhnwr hgfnvaqtmf tllmtgklks yytdleafam vtaqlchdid
 601 hrgtnnlyqm ksqmplaklh gssilerhnl efgkfllsee tlniyqnlnr rqhehvihlm
 661 diaiiatdla lyfkkramfq kivdesknyq dkkswveyls lettrkeivm ammmtacdls
 721 aitkpwevqs kvallvaaef weqqdlertv ldqqpipmmd rnkaaelpkl qvgfidfvct
 781 fvykefsrfh eeilpmfdrl qnnrkewkal adeyeakvka leekeeeerv aakkvgteic
 841 nggpapksst ccil (SEQ ID NO:21)
```

FIG. 7M

Rod cGMP-specific 3',5'-cyclic phosphodiesterase subunit beta isoform 2 (PDE6β isoform 2)
GenBank NP_001138763

```
   1 mslseeqars fldqnpdfar qyfgkklspe nvaaacedgc ppdcdslrdl cqveestall
  61 elvqdmqesi nmervvfkvl rrlctllqad rcslfmyrqr ngvaelatrl fsvqpdsvle
 121 dclvppdsei vfpldigvvg hvaqtkkmvn vedvaecphf ssfadeltdy ktknmlatpi
 181 mngkdvvavi mavnklngpf ftsededvfl kylnfatlyl kiyhlsylhn cetrrgqvll
 241 wsankvfeel tdierqfhka fytvrraylnc erysvglldm tkekeffdvw svlmgesqpy
 301 sgprtpdgre ivfykvidyi lhgkeeikvi ptpsadhwal asglpsyvae sgficnimna
 361 sademfkfge galddsgwli knvlsmpivn kkeeivgvat fynrkdgkpf deqdevlmes
 421 ltqflqwsvm ntdtydkmnk lenrkdiaqd mvlyhvkcdr deiqllilptr arlgkepadc
 481 dedelgeilk eelpgpttfd iyefhfsdle cteldlvkcg iqmyyeiqvv rkfqippqevl
 541 vrflfsiskg yrrityhnwr hqfnvaqtmf tllmtgkiks yytdleafam vtaglchdid
 601 hrgtnnlyqm ksqnplaklh gssilerhhl efgkfllsee tliyqnlnr rqhehvihlm
 661 diaiiatdla lyfkkramfq kivdesknyq dkkswveyls lettrkeivm ammmtacdls
 721 aitkpwevqs kvallvaaef weqgdlertv ldqqpipmmd rnkaaelpkl qvqfidfvct
 781 fvykefsrfh eeilpmfdrl qnnrkewkal adeyeakvka leekeeeerv aakkgteicn
 841 ggpapksstc cil (SEQ ID NO:22)
```

FIG. 7N

Rod cGMP-specific 3',5'-cyclic phosphodiesterase subunit beta isoform 3 (PDE6β isoform 3)
GenBank NP_001138764

```
   1 mtkekeffdv wsvlmgesqp ysgprtpdgr eivfykvidy ilhgkeeikv iptpsadhwa
  61 lasqlpsyva esqficnimn asademfkfq egaiddsgwl iknvlsmpiv nkkeeivgva
 121 tfynrkdgkp fdeqdevlme sltqflqwsv mntdtydkmn klenrkdiaq dmvlyhvkcd
 181 rdeiqlilpt rarlgkepad cdedelgeil keelpgpttf diyefhfsdl ecteldlvkc
 241 giqmyyelqv vrkfqipqev lvrflfsisk gyrrityhnw rhgfnvaqtm ftllmtgklk
 301 syytdleafa mvtaglchdi dhrgtnnlyq mksqnplakl hgssilerhh lefgkflise
 361 etlniyqnln rrqhehvihl mdiaiiatdl alyfkkramf qkivdesknv qdkkswveyl
 421 slettrkeiv mammmtacdl saitkpwevq skvallvaae fweqqdlert vldqqpipmm
 481 drnkaaelpk lqvygfidfvc tfvykefsrf heeilpmfdr lqnnrkewka ladeyeakvk
 541 aleekeeeer vaakkvgtei cnggpapkss tccil (SEQ ID NO:23)
```

FIG. 7O

Cyclic nucleotide-gated cation channel alpha-3 isoform 1 (CNGA3 isoform 1)
GenBank NP_001289

```
   1 makintqysh psrthlkvkt sdrdlnraen glsrahssse etssvlqpgi ametrglads
  61 qqgsftqqqi arlsrlifll rrwaarhvhh qdqqpdsfpd rfrgaelkev ssqesnaqan
 121 vqsqepadrg rsawplakcn tntsnnteee kktkkkdaiv vdpssnlyyr wltaialpvf
 181 ynwyllicra cfdelqseyl mlwlvldysa dvlyvldvlv rartgfleqg lmvsdtnrlw
 241 qhykttfqfk ldvlslvptd laylkvgtny pevrfnrlik fsrlfeffdr tetrtnypnm
 301 friqnlvlyi liihwnaci yfaiskfigf gtdswvypni sipehgrlsr kyiyslywst
 361 ltlttigetp ppvkdeeylf vvvdflvgvl ifativqnvg smismmasr aefqakidsi
 421 kqymqfrkvt kdletrvirw fdylwankkt vdekevlksl pdklkaeiai nvhldtlkkv
 481 rifqdceagl lvelvlklrp tvfspgdyic kkgdigkemy iinegklavv addgvtqfvv
 541 lsdgsyfgei silnikgsks gnrrtanirs igysdlfcls kddlmealte ypeakkalee
 601 kgrqilmkdn lideelarag adpkdleekv eglgssldtl qtrfarllae ynatqmkmkq
 661 rlsqlesqvk gqgdkpladg evpgdatkte dkqq (SEQ ID NO:24)
```

FIG. 7P

Cyclic nucleotide-gated cation channel alpha-3 isoform 2 (CNGA3 isoform 2)
GenBank NP_001073347

```
   1 makintqysh psrthlkvkt sdrdlnraen qlsrahssse etssvlqpgi ametrglads
  61 qqgsftqqqi arlsrlifil rrwaarhvhh qdqqppdsfpd rfrqaelkev ssqesnaqan
 121 vgsqepadrg rrkktkkkda ivvdpssnly yrwltaialp vfynwyllic racfdelqse
 181 ylmlwlvldy sadvlyvldv lvrartgfle qqilmvsdtnr lwqhykttfq fkldvlslvp
 241 tdlaylkvgt nypevrfnrl lkfsrlfeff drtetrtnyp nmfriqnlvl yiliiihwna
 301 ciyfaiskfi qfqtdswvyp nisipehgrl srkyiyslyw stltlttige tpppvkdeey
 361 lfvvvdflvg vlifativgn vqsmisnmna sraefqakid sikqymqfrk vtkdletrvi
 421 rwfdylwank ktvdekevlk slpdklkaei ainvhldtlk kvrifqdcea qllvelvlkl
 481 rptvfspgdy icKkqdigke myiinegkla vvaddgvtqf vvlsdgsyfg eisilnikgs
 541 ksgnrrtani rsigysdlfc lskddlmeal teypeakkal eekqrqilmk dnlideelar
 601 agadpkdlee kveqlgssld tlqtrfarll aeynatqmkm kqrlsqlesq vkgggdkpla
 661 dqevpgdatk tedkqq (SEQ ID NO:25)
```

FIG. 7Q

Cyclic nucleotide-gated cation channel beta-3 (CNGB3)
GenBank NP_061971

```
   1 mfksltkvnk vkpigennen eqssrrneeg shpsnqsgqt taqeenkgee kslktkstpv
  61 tseephtniq dklskknssg dlttnpdpqn aaeptqtvpe qkemdpgkeg pnspqnkppa
 121 apvineyada qlhnlvkrmr qrtalykkkl vegdlsspea spqtakptav ppvkesddkp
 181 tehyyrilwf kvkkmpltey lkrikipnsi dsytdrlyll wllivtlayn wnccfiplri
 241 vfpyqtadni hywliadlic diylylydmlf iqprlqfvrg gdiivdsnel rkhyrtstkf
 301 qldvasiipf dicylffgfn pmfranrmlk ytsffefnhh lesimdkayi yrvirttgyi
 361 lfilhinacv yywasnyegi gttrwvydge gneylrcyyw avrtlitigg lpepqtlfei
 421 vfqllnffsg vfvfssligq mrdvigaata nqnyfracmd dtiaymnnys ipklvqkrvr
 481 twyeytwdsq rmldesdllk tlpttvqlal aidvnfsiis kvdlfkgcdt qmiydmllrl
 541 ksvlylpgdf vckkkgeigke myiikhgevq vlggpdqtkv lvtlkagsvf geislaagg
 601 qnrrtanvva hqfanlitld kktlqeilvh ypdserillmk karvlikqka ktaeatpprk
 661 dlallfppke etpklfktll ggtqkaslar llklkreqaa qkkensegge eegkenedkq
 721 kenedkqken edkgkenedk dkgrepeekp ldrpectasp iaveeephsv rrtvlprqts
 781 rqslilsmap saeggeevlt ievkekakq (SEQ ID NO:26)
```

FIG. 7R

Guanine nucleotide-binding protein G(t) subunit alpha-2 (GNAT2)
GenBank NP_005263

```
   1 mgsgasaedk elakrskele kklqedadke aktvkllllg agesgkstiv kqmkiihqdg
  61 yspeeciefk aiiygnvlqs ilaiiramtt lgidyaepsc addgrqlnnl adsieegtmp
 121 pelvevirrl wkdggvqacf eraaeyqlnd sasyylnqle ritdpeylps eqdvlrsrvk
 181 ttgiietkfs vkdlnfrmfd vgggrserkk wihcfegvtc iifcaalsay dmvlveddev
 241 nrmheslhlf nsicnhkffa atsivlflnk kdlfeekikk vhlsicfpey dgnnsyddag
 301 nyiksqfldl nmrkdvkeiy shmtcatdtq nvkfvfdavt diiikenlkd cglf
```

(SEQ ID NO:27)

FIG. 7S

RPGR – 815 amino acids
GenBank NP_000319

```
   1 mrepeelmpd sgavftfgks kfaennpgkf wfkndvpvhl scgdehsavv tgnnklymfg
  61 snnwgqlglg sksaiskptc vkalkpekvk laacgrnhtl vsteggnvya tggnnegqlg
 121 lgdteerntf hvisfftseh kikqisagsn tsaaltedgr lfmwggdnseg qiglknvsnv
 181 cvpgqvtiqk pvswiscgyy hsafvttdge lyvfgepeng klglpnqlig nhrtpqlvse
 241 ipekviqvac ggehtvvlte navytfglgq fgqlglgtfl fetsepkvie nirdqtisyi
 301 scgenhtali tdiglmytfg dgrhgklglg lenftnhfip tlcsnflrfi vklvacggch
 361 mvvfaaphrg vakeiefdei ndtclsvatf lpyssltsqn vlqrtlsarm rrrererspd
 421 sfsmrrtlpp iegtiglsac flpnsvfprc sernlqesvl seqdlmqpee pdyllddemtk
 481 eaeidnsstv eslgettdil nmthimslns nekslklspv qkqkkqqtig eltqdtalte
 541 nddsdeyeem semkegkack qhvsqqgifmt qpattieafs deeveipeek egaedskgng
 601 ieeqeveane envkvhggrk ekteilsddl tdkaedhefs kteelkledv deeinaenve
 661 skkktvgdde svptgyhskt egaeertndds saetiekkek anleeraice ynenpkgyml
 721 ddadssslei lensettpsk dmkktkkifl fkrvpsinqk ivknnneplp eiksigdqni
 781 lksdnkdadq nhmsqnhqni pptnterrsk sctil (SEQ ID NO:28)
```

FIG. 7T

RPGR – 646 amino acids
GenBank CAB54002

```
   1 mrepeelmpd sgavftfgks kfaemmpgkf wfkndvpvhl scgdehsavv tgmnklymfg
  61 snnwgqlglg sksaiskptc vkalkpekvk laacgrnhtl vsteggnvya tggmnegqlg
 121 lgdteemntf hvisfftseh kikqlsagsn tsaaltedgr lfmwgdnseg qiglknvsnv
 181 cvpgqvtigk pvswiscgyy hsafvttdge lyvfgepeng klglpnqllq nhrtpqlvse
 241 ipekviqvac ggehtvvlte navytfglgq fgqlglgtfl fetsepkvie nirdqtisyi
 301 scgenhtali tdigimytfg dgrhgklglg lenftmhfip tlcsnflrfi vklvacggch
 361 mvvfaaphrg vakeiefdei ndtclsvatf lpyssltsgn vlqrtlsarm rrrererspd
 421 sfsmrrtlpp iegtlglsac flpnsvfprc sernlqesvl seqdlmqpee pdylldemtk
 481 eaeidnsstv eslgettdil nmthimslms nekslklspv qkqkkqqtig eltqdtalte
 541 nddsdeyeem semkeqkack qhvsqqifmt qpatttieafs deeveipeek egaedskgng
 601 ieeqeveane envkvhggrk ektеilsddl tdkaeysash sqivsv (SEQ ID NO:29)
```

FIG. 7U

RPGR — 1152 amino acids

```
   1 mrepeelmpd sqavftfgks kfaennpgkf wfkndvpvhl scgdehsavv tgnnklymfg
  61 snnwgqlglg sksaiskptc vkalkpekvk laacgrnhtl vsteggnvya tggnneggig
 121 lgdteernff hvisfftseh kikqisagsn tsaaltedgr lfmwgdnseg qiglknvsnv
 181 cvpgqvtiqk pvswiscgyy hsafvttdge lyvfgepeng klglpnqlig nhrtpqlvse
 241 ipekviqvac ggehtvvlte navytfglgq fgqlglgtfl fetsepkvie nirdqtisyi
 301 scgenhtali tdiglmytfg dgrhgklglg lenftnhfip tlcsnflrfi vklvacggch
 361 mvvfaaphrg vakeiefdei ndtclsvatf lpyssltsgn vlqrtlsarm rrrererspd
 421 sfsmrrtlpp ieqtiqlsac flpnsvfprc sernlqesvl seqdlmqpee pdyilddemtk
 481 eaeidnsstv eslgettdil nmthimslns nekslklspv qkqkkqqtig eltqdtalte
 541 nddsdeyeem semkegkack qhvsqqgifmt qpattieafs deeveipeek egaedskgng
 601 ieeqeveane envkvhggrk ektеilsddl tdkaevsegk aksvgeaedg pegrqddgtce
 661 egssgaehwq deerekgekd kqrgemerpg egekelaeke ewkkrdgeeq eqkereqghq
 721 kernqemeeg geeehgegee eegdreeeee kegegkeege geevegerek eegerkkeer
 781 agkeekgeee gdqgegeeee tegrgeekee ggeveggeve egkgereeee eegegeeeeg
 841 egeeeegege eeegegkgee egeegegeee geegegegee eegegegeee gegegeeeeg
 901 egegeeegeg eqeeeegegk geeegegeeg egeeeegege gedqegegee eegewegeee
 961 egegegeeeg egegeegege geeeegeeeg eeegegeeege eegegeeeege gegeeeeege
1021 vegevegeeg egegeeeege eegeerekeg egeenrrnre eeeeeegkyq etgeeeenerq
1081 dgeeykkvsk ikgsvkygkh ktyqkksvtn tqgngkeqrs kmpvqgskrll knqpsgsskkf
1141 wnnvlphyle lk (SEQ ID NO:30)
```

FIG. 7V

RPGR – 1020 amino acids

```
   1 mrepeelmpd sgavftfgks kfaennpgkf wfkndvpvhl scgdehsavv tgnnklymfg
  61 snnwgqiglg sksaiskptc vkalkpekvk laacgrnhtl vsteggnvya tggnnegqlg
 121 lgdteerntf hvisfftseh kikqlsaqsn tsaaltedqr lfmwgdnseg qiglknvsnv
 181 cvpqqvtigk pvswiscgyy hsafvttdge lyvfgepeng klglpnqlig nhrtpqlvse
 241 ipekviqvac ggehtvvlte navytfglgq fgqlglgtfl fetsepkvie nirdqtisyi
 301 scgenhtali tdiglmytfg dgrhgklgig lenftnhfip tlcsnflrfi vklvacgqch
 361 mvvfaaphrg vakeiefdei ndtclsvatf lpyssltsgn vlqrtlsarm rrrererspd
 421 sfsmrrtlpp iegtlglsac flpnsvfprc sernlqesvl seqdlmqpee pdylldemtk
 481 eaeidnsstv eslgettdil nmthimslns nekslklspv qkqkkqqtig eltqdtaite
 541 nddsdeyeem semkeqkack qhvsqqgifmt qpattieafs deevqndtgq vqpqadtdge
 601 glqkevyrhe nnngvdqlda keiekesdqg hsqkeseaee idseketkla eiaqmkdlre
 661 rekstkkmsp ffqnlpdrqm nteseenkdf vkkresckqd vifdseresv ekpdsymega
 721 sesqqgiadg fqqpeaiefs sgekeddeve tdqniryqrk lieqqneket kpiisksmak
 781 ydfkcdrlse ipeekegaed skqngqieeqe veaneenvkv hggrkektei lsddltdkae
 841 dhefskteel kledvdeein aenveskkkt vqddesvptq yhsktegaer tnddssaeti
 901 ekkekanlee raiceynenp kqymlddads sslеillense ttpskdmkkt kkiflfkrvp
 961 sinqkivknn neplpeiksi qdqiillksdn kdadqnhmsq nhqnipptnt errsksctil
```

(SEQ ID NO:31)

FIG. 8A

Streptococcus pyogenes Cas9

```
   1 mdkkysigld igtnsvgwav itdeykvpsk kfkvlgntdr hsikknliga llfdsgetae
  61 atrlkrtarr rytrrknric ylqeifsnem akvddsffhr leesflveed kkherhpifg
 121 nivdevayhe kyptiyhlrk klvdstdkad lrliylalah mikfrghfli egdlnpdnsd
 181 vdklfiqlvq tynqlfeenp inasgvdaka ilsarlsksr rlenliaqlp gekknglfgn
 241 lialslglтp nfksnfdlae daklqlskdt ydddldnlla qigdqyadlf laaknlsdai
 301 llsdilrvnt eitkaplsas mikryдehhq dltilkalvr qqlpekykei ffdqskngya
 361 gyidggasqe efykfikpil ekmdgteell vklnredllr kqrtfdngsi phqihlgelh
 421 ailrrqedfy pflkdnreki ekiltfripy yvgplargns rfawmtrkse etitpwnfee
 481 vvdkgasaqs fiermtnfdk nlpnekvlpk hsllyeyftv yneltkvkyv tegmrkpafl
 541 sgeqkkaivd llfktnrkvt vkqlkedyfk kiecfdsvei sgvedrfnas lgtyhdllki
 601 ikdkdfldne enedilediv ltltlfedre mieerlktya hlfddkvmkq lkrrrytgwg
 661 rlsrklingi rdkqsgktil dfiksdgfan rnfmqlihdd sitfkediqk aqvsggqgdsl
 721 hehianlags paikkgilqt vkvvdelvkv mgrhkpeniv iemarenqtt qkqgknsrer
 781 mkrieegike lgsqilkehp ventqlqnek lylyylqngr dmyvdqeldi nrlsdydvdh
 841 ivpqsflkdd sidnkvltrs dknrgksdnv pseevvkkmk nywrqllnak litqrkfdnl
 901 tkaergglse ldkagfikrq lvetrqitkh vaqildsrmn tkydendkli revkvitlks
 961 klvsdfrkdf qfykvreinn yhhahdayln avvgtalikk ypklesefvy gdykvydvrk
1021 miakseqeig katakyffys nimnffktei tlangeirkr plietngetg eivwdkgrdf
1081 atvrkvlsmp qvnivkktev qtggfskesi lpkrnsdkli arkkdwdpkk yggfdsptva
1141 ysvlvvakve kgkskklksv kellgitime rssfeknpid fleakgykev kkdliiklpk
1201 yslfelengr krmlasagel qkgnelalps kyvnflylas hyeklkgspe dneqkqlfve
1261 qhkhyldeii eqisefskrv iladanldkv lsaynkhrdk pireqaenii hlftltnlga
1321 paafkyfdtt idrkrytstk evldatlihq sitglyetri dlsqlggd
```

(SEQ ID NO:32)

FIG. 8B

Staphylococcus aureus Cas9

```
   1 mkrnyilgld igitsvgygi idyetrdvid agvrlfkean vennegrrsk rgarrlkrrr
  61 rhriqrvkkl lfdynlltdh selsginpye arvkglsqkl seeefsaall hlakrrgvhn
 121 vneveedtgn elstkeqisr nskaleekyv aelqlerlkk dgevrgsinr fktsdyvkea
 181 kqlkvqkay hqldqsfidt yidlletrrt yyeqpgegsp fgwkdikewy emlmghctyf
 241 peelrsvkya ynadlynaln dlnnlvitrd enekleyyek fqiienvfkq kkkptlkqia
 301 keilvneedi kgyrvtstgk peftnlkvyh dikditarke iienaelldq iakiltiyqs
 361 sediqeeltn lnseltqeei eqisnlkgyt gthnislkai nlildelwht ndnqiaifnr
 421 lklvpkkvdl sqqkeipttl vddfilspvv krsfiqsikv inaiikkygl pndiiielar
 481 ekmskdaqkm inemqkrnrq tnerieeiir ttgkenakyl iekiklhdmq egkclyslea
 541 ipledllnnp fnyevdhiip rsvsfdnsfn nkvlvkqeen skkgnrtpfq ylsssdskis
 601 yetfkkhiln lakgkgrisk tkkeylleer dinrfsvqkd finrnlvdtr yatrglmnll
 661 rsyfrvnnld vkvksinggf tsflrrkwkf kkernkgykh haedaliian adfifkewkk
 721 ldkakkvmen qmfeekqaes mpeietegey keifitphqi khikdfkdyk yshrvdkkpn
 781 relindtlys trkddkgntl ivnnlnglyd kdndklkkli nkspeklimy hhdpqtyqkl
 841 klimeqygde knplykyyee tgnyltkysk kdngpvikki kyygnklnah lditddypns
 901 rnkvvklslk pyrfdvyldn qvykfvtvkn ldvikkenyy evnskcyeea kklkkisnqa
 961 efiasfynnd likingelyr vigvnndlln rievnmidit yreylemmnd krppriikti
1021 asktqsikky stdilgnlye vkskkhpqii kkg (SEQ ID NO:33)
```

FIG. 8C

Francisella tularensis Cpf1

```
   1 msiyqefvnk yslsktlrfe lipqgktlen ikarglildd ekrakdykka kqlidkyhqf
  61 fieeilssvc isedllqnys dvyfklkksd ddnlqkdfks akdtikkqis eyikdsekfk
 121 nlfnqnlida kkqqesdlil wlkqskdngi elfkansdit didealeiik sfkgwttyfk
 181 gfhenrknvy ssndiptsii yrivddnlpk flenkakyes lkdkapeain yeqikkdlae
 241 eltfdidykt sevnqrvfsl devfeianfn nylnqsgitk fntiiggkfv ngentkrkgi
 301 neyinlysqq indktlkkyk msvlfkqils dtesksfvid kleddsdvvt tmqsfyeqia
 361 afktveeksi ketlsllfdd lkaqkldlsk iyfkndkslt dlsqqvfddy svigtavley
 421 itqqiapknl dnpskkeqel iakktekaky lsletiklal eefnkhrdid kqcrfeeila
 481 nfaaipmifd eiaqnkdnla qisikyqnqg kkdllqasae ddvkaikdll dqtnnllhkl
 541 kifhisqsed kanildkdeh fylvfeecyf elanivplyn kirnyitqkp ysdekfklnf
 601 enstlangwd knkepdntai lfikddkyyl gvmnkknnki fddkaikenk gegykkivyk
 661 llpgankmlp kvffsaksik fynpsedilr irnhsthtkn gspqkgyekf efniedcrkf
 721 idfykqsisk hpewkdfgfr fsdtqrynsi defyrevenq gykltfenis esyidsvvnq
 781 gklylfqiyn kdfsayskgr pnlhtlywka lfdernlqdv vyklngeael fyrkqsipkk
 841 ithpakeaia nknkdnpkke svfeydlikd krftedkfff hcpitinfks sgankfndei
 901 nllikekand vhilsidrge rhlayytlvd gkqniikqdt fniignddrmk tnyhdklaai
 961 ekdrdsarkd wkkinnikem kegylsqvvh eiaklvieyn aivvfedlnf gfkrgrfkve
1021 kqvyqklekm lieklnylvf kdnefdktgg vlrayqltap fetfkkmgkq tgiyyvpag
1081 ftskicpvtg fvnqlypkye svsksqeffs kfdkicynld kgyfefsfdy knfqdkaakg
1141 kwtiasfgsr linfrnsdkn hnwdtrevyp tkelekllkd ysieyghqec ikaaicgesd
1201 kkffaktsv lntilqmrns ktgteldyli spvadvnqnf fdsrqapknm pqdadangay
1261 higlkglmll grikmnqegk klnlvikneе yfefvqmrnn (SEQ ID NO:34)
```