(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
03 January 2019 (03.01.2019)

WIPO | PCT

(10) International Publication Number
WO 2019/006182 A1

(51) International Patent Classification:
A61K 48/00 (2006.01) *C07K 14/075* (2006.01)
A61P 27/00 (2006.01) *C12N 15/62* (2006.01)
A61P 27/02 (2006.01) *C12N 15/63* (2006.01)

(21) International Application Number:
PCT/US2018/040115

(22) International Filing Date:
28 June 2018 (28.06.2018)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
62/527,871 30 June 2017 (30.06.2017) US
62/535,042 20 July 2017 (20.07.2017) US

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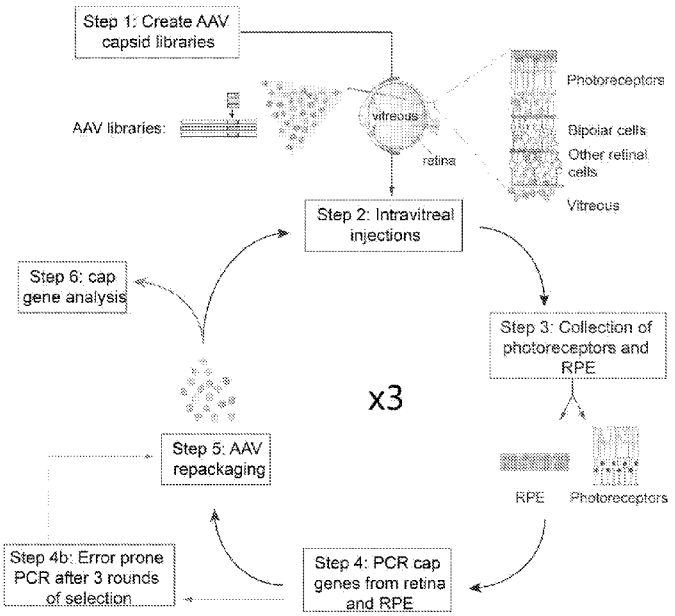
(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH,

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

FIG. 1



(57) Abstract: The present disclosure provides recombinant adeno-associated virus (AAV) virions with altered capsid protein, where the recombinant AAV (rAAV) virions exhibit greater ability to cross barriers between intravitreal fluid and retinal cells, and thus greater infectivity of a retinal cell compared to wild-type AAV, and where the rAAV virions comprise a heterologous nucleic acid. The present disclosure provides methods of delivering a gene product to a retinal cell in an individual.

[Continued on next page]

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GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*
— *with sequence listing part of description (Rule 5.2(a))*

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

CROSS-REFERENCE

[0001] This application claims the benefit of U.S. Provisional Patent Application No. 62/527,871, filed June 30, 2017, and 62/535,042, filed July 20, 2017, which applications are incorporated herein by reference in their entirety.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH

[0002] This invention was made with government support under Grant No. 1R01EY022975-01A1 awarded by the National Institutes of Health. The government has certain rights in the invention.

INTRODUCTION

[0003] Vision is mediated by cells located in the retina, a thin, layered structure lining the back of the eye. Photoreceptors, which lie at the back of the retina, respond to the absorption of photons, initiating a stream of signal processing that passes through second and third order neurons in the retina, including bipolar, horizontal and amacrine cells. Retinal pigment epithelium (RPE) cells, which lie underneath photoreceptors, promote the regeneration of the photon-detecting molecule, 11-cis retinal, via the visual cycle pathway and hence are essential for promoting this photoreceptor function. Retinal ganglion cells (RGCs) in the inner retina receive visual signals from third order neurons, and communicate the visual signals in the form of action potentials to the brain.

[0004] Mutations in genes expressed in retinal cells, including transcripts in photoreceptors, RPE, bipolar cells and other cells, result in a breakdown of visual signal processing and retinal degeneration. Many of the mutations underlying retinal degenerative disease result in the death of photoreceptor and RPE cells.

[0005] 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 4.7 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

[0006] The present disclosure provides recombinant adeno-associated virus (AAV) virions with altered capsid protein, where the recombinant AAV (rAAV) virions exhibit greater ability to cross barriers between intravitreal fluid and retinal cells, and thus greater infectivity of a retinal cell compared to wild-type AAV, and where the rAAV virions comprise a heterologous nucleic acid. The present disclosure provides methods of delivering a gene product to a retinal cell in an individual.

BRIEF DESCRIPTION OF THE DRAWINGS

[0007] **FIG. 1** provides a schematic depiction of the directed evolution methodology used to develop primate retinal AAV variants.

[0008] **FIG. 2** provides a table of peptide insertions and peptide replacements in variant AAV capsids.

[0009] **FIG. 3A-3C** provide amino acid sequences of exemplary guide-RNA-directed endonucleases.

[0010] **FIG. 4** provides an amino acid sequence of AAV2 capsid protein VP1. Amino acids 587 and 588 (NP) are in bold and underlined.

[0011] **FIG. 5** provides amino acid sequences corresponding to amino acids 570-610 of AAV capsid protein VP1 of various AAV serotypes.

[0012] **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.

[0013] **FIG. 7A-7V** provide amino acid sequences of exemplary heterologous gene products.

[0014] **FIG. 8A-8B** provide amino acid sequences of AAV4 capsid (FIG. 8A) and an ancestral AAV capsid (FIG. 8B).

[0015] **FIG. 9** provides Table 1. Table 1 provides a ranking of primate-derived variants and controls recovered from photoreceptors following injection of a green fluorescent protein (GFP)-Barcode library.

[0016] **FIG. 10** provides Table 2. Table 2 provides a ranking of primate-derived variants and controls recovered from RPE cells following injection of a GFP-Barcode library.

[0017] **FIG. 11** depicts GFP expression of GFP-barcoded libraries in primate retina.

[0018] **FIG. 12A-12F** depict directed evolution of AAV in primate retina. The sequences in FIG. 12F from top to bottom are set forth in SEQ ID NOs:117-135.

[0019] **FIG. 13A-13Q** depict validation of evolved AAV variants in primate retina.

DEFINITIONS

[0020] 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.

[0021] "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), AAV type 10 (AAV-10), AAV type 11 (AAV-11), avian AAV, bovine AAV, canine AAV, equine AAV, primate AAV, non-primate AAV, and ovine AAV. See, e.g., Mori et al. (2004) *Virology* 330:375. The term "AAV" also includes chimeric 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.

[0022] 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.

[0023] 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.

[0024] "Packaging" refers to a series of intracellular events that result in the assembly and encapsidation of an AAV particle.

[0025] 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."

[0026] 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.

[0027] "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.

[0028] 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.

[0029] 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).

[0030] 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.

[0031] 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)

[0032] 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.

[0033] 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:

[0034] Mismatch Penalty: 1.00;

[0035] Gap Penalty: 1.00;

[0036] Gap Size Penalty: 0.33; and

[0037] Joining Penalty:30.0.

[0038] 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.

[0039] 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”).

[0040] A "small interfering" or "short interfering RNA" or siRNA is an RNA duplex of nucleotides that is targeted to a gene interest (a “target gene”). An "RNA duplex" refers to the structure formed by the complementary pairing between two regions of an 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.

[0041] 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.

[0042] "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.

[0043] 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.

[0044] "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.

[0045] 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.

[0046] "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.

[0047] 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.

[0048] 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.

[0049] 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.

[0050] 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.

[0051] 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.

[0052] 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.

[0053] 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.

[0054] 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.

[0055] 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.

[0056] 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 rAAV virion" includes a plurality of such virions and reference to "the variant capsid protein" includes reference to one or more variant capsid proteins 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.

[0057] 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.

[0058] 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

[0059] The present disclosure provides recombinant adeno-associated virus (AAV) virions with altered capsid protein, where the recombinant AAV (rAAV) virions exhibit greater ability to cross barriers between intravitreal fluid and retinal cells, and thus greater infectivity of a retinal cell compared to wild-type AAV, and where the rAAV virions comprise a heterologous nucleic acid. The present disclosure provides methods of delivering a gene product to a retinal cell in an individual. The present disclosure also provides methods of modifying a target nucleic acid present in a retinal cell.

[0060] 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 increased ability to cross a barrier between intravitreal fluid and retinal cells. 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

[0061] The present disclosure provides a variant AAV capsid protein. As noted above, a variant AAV capsid protein of the present disclosure is altered, compared to a wild-type or other reference AAV capsid protein. Alterations include insertions and swaps (e.g., replacements of a contiguous stretch of amino acids with a different contiguous stretch of amino acids).

[0062] In some cases, 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, particularly when the AAV virion is injected intravitreally. Thus, a variant AAV capsid protein of the present disclosure, when present in an AAV virion, confers increased ability of the AAV virion to cross a barrier between the intravitreal fluid ("vitreous") and a retinal cell, where such barriers include, e.g., the inner limiting membrane (ILM), the extracellular matrix of the retina, the cell membranes of the retinal cells themselves, inner nuclear layer, the outer nuclear layer, the photoreceptor layer, the ganglion cell layer, and the retinal pigment epithelium. In some 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 a heterologous

peptide insertion. In some 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.

[0063] An alteration in an AAV capsid can also be a swap, e.g., a replacement of a contiguous stretch of amino acids with a heterologous peptide. Thus, a replacement is an insertion of a heterologous peptide in place of a contiguous stretch of amino acids. In some cases, a variant AAV capsid protein of the present disclosure comprises replacement of a contiguous stretch of amino acids with a heterologous peptide of from 5 amino acids to 20 amino acids in length in a 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, particularly when the AAV virion is injected intravitreally. Thus, a variant AAV capsid protein of the present disclosure, when present in an AAV virion, confers increased ability of the AAV virion to cross a barrier between the intravitreal fluid ("vitreous") and a retinal cell, where such barriers include, e.g., ILM, the extracellular matrix of the retina, the cell membranes of the retinal cells themselves, inner nuclear layer, the outer nuclear layer, the photoreceptor layer, the ganglion cell layer, and the retinal pigment epithelium. In some cases, the retinal cell is a Müller cell. Other retinal cells include amacrine cells, bipolar cells, and horizontal cells. A "replacement of from about 5 amino acids to about 20 amino acids" is also referred to herein as a "peptide swap" (e.g., a replacement of a contiguous stretch of amino acids with a heterologous peptide). A "corresponding parental AAV capsid protein" refers to an AAV capsid protein of the same AAV serotype, without a heterologous peptide. In some instances, the variant AAV capsid comprises a single heterologous peptide replacement 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.

[0064] For purposes of the following discussion, "insertion" refers to both insertion of a heterologous peptide without replacement of a contiguous stretch of amino acids, and to insertion of a heterologous peptide that replaces a contiguous stretch of amino acids.

[0065] 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. In some cases, the insertion site is between amino acids 575 and 576 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 584 and 585 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 590 and 591 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 584 and 585 of an AAV4 capsid protein, or a corresponding insertion site in an AAV of a different serotype. In some cases, the insertion site is between amino acids 575 and 576 of an AAV5 capsid protein, or a corresponding insertion site in an AAV of a different serotype. In some cases, the site for replacement is between amino acids 584 and 598 of an AAV2 capsid protein, or a corresponding site in an AAV of a different serotype.

[0066] 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 between amino acids 588 and 589 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. NP_044927 for AAV4; 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; GenBank Accession

No. AAT46337 for AAV10; and GenBank Accession No. AAO88208 for AAVrh10. See, e.g., Santiago-Ortiz et al. (2015) *Gene Ther.* 22:934 for ancestral AAV capsid.

[0067] 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, between amino acids 588 and 589 of AAV10, or between amino acids 585 and 586 of AAV4. The insertion sites are underlined in FIG. 5; the amino acid numbering is based on the numbering depicted in FIG. 5.

[0068] In some cases, 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.

[0069] In some cases, a variant AAV capsid protein of the present disclosure comprises a replacement, or substitution, of a segment, or sequence of consecutive amino acids, in a surface-accessible (e.g., solvent-accessible) portion of a parental AAV capsid, 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, particularly when the AAV virion is injected intravitreally. Thus, a subject variant AAV capsid protein comprising the sequence substitution, when present in an AAV virion, confers increased ability of the AAV virion to cross a barrier between the vitreous and a retinal cell, where such barriers include, e.g., the inner limiting membrane, the extracellular matrix of the retina, and the cell membranes of the retinal cells themselves. A "replacement of from about 5 consecutive amino acids to about 25 consecutive amino acids" is also referred to herein as a "loop swap" (i.e. a heterologous peptide substitution). A "corresponding parental AAV capsid protein" in such instances refers to an AAV capsid protein of the same AAV serotype, without the subject loop swap. In some instances, the variant AAV capsid comprises a heterologous peptide substitution of from 5 contiguous amino acids to 25 contiguous amino acids, e.g. from 5 to 9, from 9 to 11, from 10 to 15, from 15 to 20, or from 20 to 25 amino acids in length.

[0070] In some cases, a heterologous peptide of from about 5 amino acids to about 25 amino acids (e.g., from 5 to 9, from 9 to 11, from 10 to 15, from 15 to 20, or from 20 to 25 amino acids) in length is substituted in for an equivalent number of consecutive amino acids in a corresponding parental AAV capsid protein. In some embodiments, the substitution begins at around amino acid 588 of AAV2, or the corresponding position of the capsid subunit of another AAV serotype, and ends at

around amino acid 598 of AAV2 or the corresponding position of the capsid subunit of another AAV serotype. It should be noted that the residues 588-598 are based on an AAV2 VP1 capsid protein. A heterologous peptide of 5 amino acids to about 25 amino acids in length can be substituted into 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 a substitution site "corresponding to amino acids 588-598 of AAV2" would be in a capsid protein of any given AAV serotype. The amino acid residue corresponding to amino acids 588-598 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. NP_044927 for AAV4; 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, GenBank Accession No. AAT46337 for AAV10, and GenBank Accession No. AAO88208 for AAVrh10.

[0071] In some cases, a heterologous peptide of from about 5 amino acids to about 25 amino acids (e.g., from 5 to 9, from 9 to 11, from 10 to 15, from 15 to 20, or from 20 to 25 amino acids) in length is substituted in for an equivalent number of consecutive amino acids in a corresponding parental AAV capsid protein. In some embodiments, the substitution begins at around amino acid 585 of AAV2, or the corresponding position of the capsid subunit of another AAV serotype, and ends at around amino acid 598 of AAV2 or the corresponding position of the capsid subunit of another AAV serotype. It should be noted that the residues 585-598 are based on an AAV2 VP1 capsid protein. A heterologous peptide of 5 amino acids to about 25 amino acids in length can be substituted into 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 a substitution site "corresponding to amino acids 585-598 of AAV2" would be in a capsid protein of any given AAV serotype. The amino acid residue corresponding to amino acids 585-598 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. NP_044927 for AAV4; 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, GenBank Accession No. AAT46337 for AAV10, and GenBank Accession No. AAO88208 for AAVrh10.

Insertion/replacement peptides

[0072] 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, or replaces an equivalent number of consecutive amino acids in the GH loop of an AAV capsid. For simplicity, the term "insertion peptide" is used below to describe both a peptide that is inserted into a parental AAV capsid and a peptide that replaces a segment of contiguous amino acids in 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.

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

[0074] LA(L/N)(I/Q)(Q/E)(D/H)(S/V)(M/K)(R/N)A. (SEQ ID NO: 136)

[0075] In some cases, a peptide of Formula I comprises the following amino acid sequence: (21) LALIQDSMRA (SEQ ID NO: 35). In some cases, a peptide of Formula I comprises the following amino acid sequence: (22) LANQEHVKNA (SEQ ID NO:2).

[0076] The peptide insert is, in some cases, a peptide of **Formula II**:

[0077] $TX_1X_2X_3X_4X_5X_6X_7X_8GLX_9$ (SEQ ID NO: 137), where:

[0078] X_1 is G, V, or S;

[0079] X_2 is V, E, P, G, D, M, A, or S;

[0080] X_3 is M, V, Y, H, G, S, or D;

[0081] X_4 is R, D, S, G, V, Y, T, H, or M;

[0082] X_5 is S, L, G, T, Q, P, or A;

[0083] X_6 is T, A, S, M, D, Q, or H;

[0084] X_7 is N, G, S, L, M, P, G, or A;

[0085] X_8 is S, G, D, N, A, I, P, or T; and

[0086] X_9 is **S** or N.

[0087] Peptide inserts of Formula II include, but are not limited to: (1) TGVMRSTNSGLN (SEQ ID NO: 6); (2) TGEVDLAGGGLS (SEQ ID NO: 7); (3) TSPYSGSSDGLS (SEQ ID NO: 8); (4) TGGHDSSLDGLS (SEQ ID NO: 9); (5) TGDGGTTMNGLS (SEQ ID NO: 98); (6) TGGHGSAPDGLS (SEQ ID NO: 99); (7) TGMHVTMMAGLN (SEQ ID NO: 100); (8) TGASYLDNSGLS (SEQ ID NO: 101); (9) TVVSTQAGIGLS (SEQ ID NO: 135); (10) TGVMHSQASGLS (SEQ ID NO: 21); (11) TGDGSPAAPGLS (SEQ ID NO: 22); and (12) TGSDMAHGTGLS (SEQ ID NO: 23). In some cases, the peptide insert is (1) TGVMRSTNSGLN (SEQ ID NO: 6). In some cases, the peptide insert is (2) TGEVDLAGGGLS (SEQ ID NO: 7). In some cases, the peptide insert is (3) TSPYSGSSDGLS (SEQ ID NO: 8). In some cases, the peptide insert is (4) TGGHDSSLDGLS (SEQ ID NO: 9). In some cases, the peptide insert is (5) TGDGGTTMNGLS (SEQ ID NO: 98). In some cases, the peptide insert is (6) TGGHGSAPDGLS (SEQ ID NO: 99). In some cases, the peptide insert is (7) TGMHVTMMAGLN (SEQ ID NO: 100). In some cases, the peptide insert is (8) TGASYLDNSGLS (SEQ ID NO: 101). In some cases, the peptide insert is (9) TVVSTQAGIGLS (SEQ ID NO: 20). In some cases, the peptide insert is (10) TGVMHSQASGLS (SEQ ID NO: 21). In some cases, the peptide insert is (11) TGDGSPAAPGLS (SEQ ID NO: 22). In some cases, the peptide insert is (12) TGSDMAHGTGLS (SEQ ID NO: 23).

[0088] The peptide insert is, in some cases, a peptide of **Formula III**:

[0089] $TGX_1X_2X_3X_4X_5X_6X_7GLS$ (SEQ ID NO: 138), where:

[0090] X_1 is V, E, P, G, D, M, A, or S;

[0091] X_2 is M, V, Y, H, G, S, or D;

[0092] X_3 is R, D, S, G, V, Y, T, H, or M;

[0093] X_4 is S, L, G, T, Q, P, or A;

[0094] X_5 is T, A, S, M, D, Q, or H;

[0095] X_6 is N, G, S, L, M, P, G, or A; and

[0096] X_7 is S, G, D, N, A, I, P, or T.

[0097] Peptide inserts of Formula III include, but are not limited to: (2) TGEVDLAGGGLS (SEQ ID NO: 7); (4) TGGHDSSLDGLS (SEQ ID NO: 9); (5) TGDGGTTMNGLS (SEQ ID NO: 98); (6) TGGHGSAPDGLS (SEQ ID NO: 99); (8) TGASYLDNSGLS (SEQ ID NO: 101); (10) TGVMHSQASGLS (SEQ ID NO: 21); (11) TGDGSPAAPGLS (SEQ ID NO: 22); and (12) TGSDMAHGTGLS (SEQ ID NO: 23).

[0098] The peptide insert is, in some cases, a peptide of **Formula IV**:

[0099] $X_1GX_2X_3X_4X_5X_6X_7X_8GLSPX_9TX_{10}X_{11}$ (SEQ ID NO: 139), where

[00100] X_1 is **T** or N;

[00101] X_2 is L, S,A, or G;

[00102] X_3 is **D** or V;

[00103] X_4 is A, G, or P;

[00104] X_5 is **T** or D;

[00105] X_6 is **R** or Y;

[00106] X_7 is D, T, or G;

[00107] X_8 is H, R, or T;

[00108] X_9 is **V** or A;

[00109] X_{10} is **G** or W; and

[00110] X_{11} is **T** or A.

[00111] Peptide inserts of Formula IV include, but are not limited to: (13) TGLDATRDHGLSPVTGT (SEQ ID NO: 24); (14) TGSDGTRDHGLSPVTWT (SEQ ID NO: 25); (15) NGAVADYTRGLSPATGT (SEQ ID NO: 26); and (16) TGGDPTRGTGLSPVTGA (SEQ ID NO: 27). In some cases, the peptide insert is (13) TGLDATRDHGLSPVTGT (SEQ ID NO: 24). In some cases, the peptide insert is (14) TGSDGTRDHGLSPVTWT (SEQ ID NO: 25). In some cases, the peptide insert is (15) NGAVADYTRGLSPATGT (SEQ ID NO: 26). In some cases, the peptide insert is (16) TGGDPTRGTGLSPVTGA (SEQ ID NO: 27).

[00112] The peptide insert is, in some cases, a peptide of **Formula V**:

[00113] $TGX_1DX_2TRX_3X_4GLSPVTGT$ (SEQ ID NO: 140), where

[00114] X_1 is L, S, A, or G;

[00115] X_2 is A, G, or P;

[00116] X_3 is D, T, or G; and

[00117] X_4 is H, R, or T.

[00118] Peptide inserts of Formula V include, but are not limited to: (13) TGLDATRDHGLSPVTGT (SEQ ID NO: 24); (14) TGSDGTRDHGLSPVTWT (SEQ ID NO: 25); and (16) TGGDPTRGTGLSPVTGA (SEQ ID NO: 27).

[00119] The peptide insert is, in some cases, a peptide of **Formula VI**:

[00120] $LQX_1X_2X_3RX_4X_5X_6X_7X_8X_9VNX_{10}Q$ (SEQ ID NO: 141), where

[00121] X_1 is K or R;

[00122] X_2 is N, G, or A;

[00123] X_3 is A, V, N, or D;

[00124] X_4 is P, I, or Q;

[00125] X_5 is A, P, or V;

[00126] X_6 is S, T, or G;

[00127] X_7 is T or V;

[00128] X_8 is E, L, A, or V;

[00129] X_9 is S, E, D, or V; and

[00130] X_{10} is F, G, T, or C.

[00131] Peptides of Formula VI include, but are not limited to: (17) LQKNARPASTESVNFQ (SEQ ID NO: 28); (18) LQRGVRIPSVLEVNGQ (SEQ ID NO: 29); (19) LQRGNRPVTTADVNTQ (SEQ ID NO: 30); and (20) LQKADRQPGVVVVNCQ (SEQ ID NO: 31). In some cases, the peptide insert is (17) LQKNARPASTESVNFQ (SEQ ID NO: 28). In some cases, the peptide insert is (18) LQRGVRIPSVLEVNGQ (SEQ ID NO: 29). In some cases, the peptide insert is (19) LQRGNRPVTTADVNTQ (SEQ ID NO: 30). In some cases, the peptide insert is (20) LQKADRQPGVVVVNCQ (SEQ ID NO: 31).

[00132] Any of the above-described peptide inserts can replace an equal number of contiguous amino acids in the GH loop of an AAV capsid polypeptide. For example, in some cases, a peptide of **Formula VI**:

[00133] $LQX_1X_2X_3RX_4X_5X_6X_7X_8X_9VNX_{10}Q$ (SEQ ID NO: 141), where

[00134] X_1 is K or R;

[00135] X_2 is N, G, or A;

[00136] X_3 is A, V, N, or D;

[00137] X_4 is P, I, or Q;

[00138] X_5 is A, P, or V;

[00139] X_6 is S, T, or G;

[00140] X_7 is T or V;

[00141] X_8 is E, L, A, or V;

[00142] X_9 is S, E, D, or V; and

[00143] X_{10} is F, G, T, or C,

[00144] replaces a contiguous stretch of from 5 amino acids to 20 amino acids in the GH loop of an AAV capsid polypeptide. In other words, in some cases, an "insert peptide" replaces an endogenous peptide (e.g., a contiguous stretch of from 5 amino acids to 20 amino acids) present in in the GH loop of an AAV capsid polypeptide, resulting in a variant AAV capsid comprising a heterologous peptide in the GH loop. In some cases, the "insert peptide" replaces an endogenous

contiguous stretch of amino acids of the same length as the insert peptide. Thus, for example, where the "insert peptide" has a length of 16 amino acids, in some cases, an endogenous contiguous stretch of 16 amino acids is replaced by the insert peptide.

[00145] Peptides of Formula VI include, but are not limited to: (17) LQKNARPASTESVNFQ (SEQ ID NO: 28); (18) LQRGVRIPSVLEVNGQ (SEQ ID NO: 29); (19) LQRGNRPVTTADVNTQ (SEQ ID NO:30); and (20) LQKADRQPGVVVVNCQ (SEQ ID NO: 31). In some cases, the peptide that replaces an endogenous amino acid sequence in the GH loop of an AAV capsid is (17) LQKNARPASTESVNFQ (SEQ ID NO: 28). In some cases, the peptide insert is (18) LQRGVRIPSVLEVNGQ (SEQ ID NO: 29). In some cases, the peptide that replaces an endogenous amino acid sequence in the GH loop of an AAV capsid is (19) LQRGNRPVTTADVNTQ (SEQ ID NO: 30). In some cases, the peptide that replaces an endogenous amino acid sequence in the GH loop of an AAV capsid is (20) LQKADRQPGVVVVNCQ (SEQ ID NO: 31).

[00146] In some cases, a peptide insert of any one of Formulas I-VI further includes one or two linker amino acids at the N-terminus of the peptide and/or one or more amino acids at the C-terminus of the peptide. For example, in some cases, a peptide insert comprises: Thr-Gly-[peptide of any one of Formulas I-VI]-Gly-Leu-Ser (SEQ ID NO: 142). As another example, in some cases, a peptide insert comprises: Leu-Ala-[peptide of any one of Formulas I-VI]-Ala (SEQ ID NO: 143). As another example, in some cases, a peptide insert comprises: Leu-Gln-[peptide of any one of Formulas I-VI]-Gln. In some cases, a peptide insert does not include any linker amino acids.

[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. In certain embodiments, the deletion of one or more amino acids (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 amino acids) compared to the parental AAV capsid protein occurs at the site of peptide insertion.

[00148] In some cases, a variant AAV capsid polypeptide of the present disclosure does not include one, two, three, or four, of the following amino acid substitutions: Y273F, Y444F, Y500F, and Y730F.

[00149] In some cases, a variant AAV capsid polypeptide of the present disclosure 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 variant AAV capsid polypeptide of the present disclosure 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.

RECOMBINANT AAV VIRIONS

[00151] The present disclosure provides a recombinant AAV (rAAV) virion comprising: i) a variant AAV capsid polypeptide of the present disclosure; and ii) a heterologous nucleic acid comprising a nucleotide sequence encoding a heterologous polypeptide (i.e., a non-AAV polypeptide).

[00152] In some cases, an rAAV virion of the present disclosure 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.

[00153] In some cases, an rAAV virion of the present disclosure 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 cases, 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 585 and 598 relative to the amino acid sequence depicted in FIG. 4, or at a corresponding site relative to a corresponding parental AAV capsid protein.

[00154] 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.

[00155] 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.

[00156] An rAAV virion of the present disclosure 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.

[00157] Whether a given rAAV virion exhibits increased infectivity of a retinal cell can be determined by detecting expression in a retinal cell of a heterologous gene product encoded by the rAAV virion, following intravitreal administration of the rAAV virion. For example, an rAAV virion of the present disclosure that comprises: a) a variant capsid of the present disclosure comprising a peptide insert or a peptide replacement, as described above; and b) a heterologous nucleotide sequence encoding a heterologous gene product, when administered intravitreally, results in a level of the heterologous gene product in a retinal cell, that is at least 2-fold, at least 5-fold, at least 10-fold, at least 15-fold, at least 20-fold, at least 25-fold, at least 50-fold, or more than 50-fold, greater than the level of the gene product in the retinal cell that results when a control rAAV virion that comprises: a) a control AAV capsid that does not comprises the peptide insert or the peptide replacement; and b) heterologous nucleotide sequence encoding the heterologous gene product is administered intravitreally.

[00158] Whether a given rAAV virion exhibits increased infectivity of a retinal cell can be determined by assessing a therapeutic effect of a therapeutic gene product encoded by the rAAV virion in a retinal cell. Therapeutic effects can include, e.g., a) a decrease in the rate of loss of visual function, e.g. visual field, visual acuity; b) an improvement in visual function, e.g. an improvement in visual field or visual acuity; c) a decrease in sensitivity to light, i.e. photophobia; a decrease in nystagmus; etc. For example, an rAAV virion of the present disclosure that comprises: a) a variant capsid of the present disclosure comprising a peptide insert or a peptide replacement, as described above; and b) a heterologous nucleotide sequence encoding a heterologous therapeutic gene product, when administered intravitreally, results in a therapeutic effect of the therapeutic gene product in a retinal cell, that is at least 2-fold, at least 5-fold, at least 10-fold, at least 15-fold, at least 20-fold, at least 25-fold, at least 50-fold, or more than 50-fold, greater than the therapeutic effect in the retinal cell that results when a control rAAV virion that comprises: a) a control AAV capsid that does not comprises the peptide insert or the peptide replacement; and b) heterologous nucleotide sequence encoding the heterologous therapeutic gene product is administered intravitreally. Tests for visual function are known in the art; and any such test can be used to determine whether an rAAV virion of the present disclosure exhibits increased infectivity of a retinal cell.

[00159] An rAAV virion of the present disclosure 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 a barrier between the intravitreal fluid and a retinal cell, compared to the ability of a control rAAV virion comprising the corresponding parental AAV capsid protein (i.e., the AAV capsid protein without the insert peptide or replacement peptide).

[00160] 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.

[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 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.

[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 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.

[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 an RGC, compared to the infectivity of the RGC by an AAV virion comprising the corresponding parental AAV capsid protein.

[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 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.

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

[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 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.

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

[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 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.

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

[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 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.

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

[00172] 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.

[00173] 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.

[00174] 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.

[00175] 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.

[00176] 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.

[00177] 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 extracellular matrix (ECM) of the retina, compared to the ability of an AAV virion comprising the corresponding parental AAV capsid protein to cross the ECM of the retina.

[00178] 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 extracellular matrix (ECM) of the retina, compared to the ability of an AAV virion comprising the corresponding parental AAV capsid protein to cross the ECM of the retina when administered via intravitreal injection.

[00179] 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.

[00180] 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.

[00181] 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.

[00182] 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

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.

[00183] 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.

[00184] 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.

[00185] 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.

[00186] 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

[00187] An rAAV virion of the present disclosure comprises a heterologous nucleic acid comprising a nucleotide sequence encoding one or more gene products (one or more heterologous gene products). In some cases, the gene product is a polypeptide. In some cases, the gene product is an RNA. In some cases, an rAAV virion of the present disclosure comprises a heterologous nucleotide sequence encoding both a heterologous nucleic acid gene product and a heterologous polypeptide gene product. Where the gene product is an RNA, in some cases, the RNA gene product encodes a polypeptide. Where the gene product is an RNA, in some cases, the RNA gene product does not encode 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. Where the single heterologous nucleic acid encodes two heterologous gene products, in some cases, nucleotide sequences encoding the two heterologous gene products are operably linked to the same promoter. Where the single heterologous nucleic acid encodes two heterologous gene products, in some cases, nucleotide sequences encoding the two heterologous gene products are operably linked to two different promoters. In some cases, an rAAV virion of the present disclosure comprises a single heterologous nucleic acid comprising a nucleotide sequence encoding three heterologous gene products. Where the single heterologous nucleic acid encodes three heterologous gene products, in some cases, nucleotide sequences encoding the three heterologous gene products are operably linked to the same promoter. Where the single heterologous nucleic acid encodes three

heterologous gene products, in some cases, nucleotide sequences encoding the three heterologous gene products are operably linked to two or three different promoters. 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.

[00188] In some cases, the gene product is a polypeptide-encoding RNA. In some cases, the gene product is an interfering RNA. In some cases, the gene product is an aptamer. In some cases, the gene product is a polypeptide. In some cases, the gene product is a therapeutic polypeptide, e.g., a polypeptide that provides clinical benefit. 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. In some cases, the gene products are: i) an RNA-guided endonuclease that provides for modification of a target nucleic acid; and ii) a guide RNA that comprises a first segment that binds to a target sequence in a target nucleic acid and a second segment that binds to the RNA-guided endonuclease. In some cases, the gene products are: i) an RNA-guided endonuclease that provides for modification of a target nucleic acid; ii) a first guide RNA that comprises a first segment that binds to a first target sequence in a target nucleic acid and a second segment that binds to the RNA-guided endonuclease; and iii) a first guide RNA that comprises a first segment that binds to a second target sequence in the target nucleic acid and a second segment that binds to the RNA-guided endonuclease.

Interfering RNA

[00189] 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.

[00190] 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

[00191] 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:3). 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).

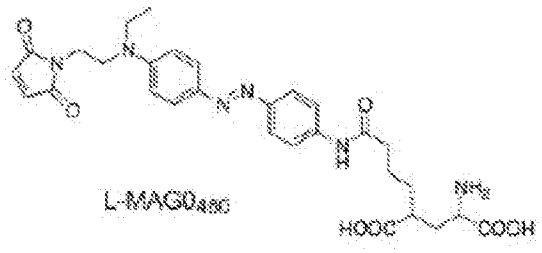
Polypeptides

[00192] Where the gene product is a polypeptide, in some cases, the polypeptide is 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.

[00193] 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.

[00194] 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:



[00195] 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; Rab escort protein 1 (REP1) (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.

[00196] 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.

[00197] 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.

[00198] 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.

[00199] 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.

[00200] 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

[00201] 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.

[00202] 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.

[00203] 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 meganucleases and 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 Structural Biology* 4:468).

RNA-guided endonucleases

[00204] 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."

[00205] 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). A suitable genome editing nuclease is 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; also referred to as a "Cas13a" protein). Also suitable for use is a CasX protein. Also suitable for use is a CasY protein.

[00206] 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.).

[00207] In some cases, the genome-editing endonuclease is a Type II CRISPR/Cas 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. 3A. 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. 3B.

[00208] 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. 3A 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. 3A, where amino acids N497, R661, Q695, and Q926 are substituted, e.g., with alanine.

[00209] In some cases, a suitable Cas9 polypeptide exhibits altered PAM specificity. See, e.g., Kleinstiver et al. (2015) *Nature* 523:481.

[00210] 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. 3C.

[00211] In some cases, the genome-editing endonuclease is a CasX or a CasY polypeptide. CasX and CasY polypeptides are described in Burstein et al. (2017) *Nature* 542:237.

Enzymatically inactive RNA-guided endonucleases

[00212] Also suitable for use is an RNA-guided endonuclease with reduced enzymatic activity. Such an RNA-guided endonuclease is referred to as a "dead" RNA-guided endonuclease; for example, a Cas9 polypeptide that comprises certain amino acid substitutions such that it exhibits substantially no endonuclease activity, but such that it still binds to a target nucleic acid when complexed with a guide RNA, is referred to as a "dead" Cas9 or "dCas9." In some cases, a "dead" Cas9 protein has a reduced ability to cleave both the complementary and the non-complementary strands of a double stranded target nucleic acid. For example, a "nuclease defective" Cas9 lacks a functioning RuvC domain (i.e., does not cleave the non-complementary strand of a double stranded target DNA) and lacks a functioning HNH domain (i.e., does not cleave the complementary strand of a double stranded target DNA). As a non-limiting example, in some cases, the nuclease defective Cas9 protein harbors mutations at amino acid positions corresponding to residues D10 and H840 (e.g., D10A and H840A) of SEQ ID NO: 15 (or the corresponding residues of a homolog of Cas9) such that the polypeptide has a reduced ability to cleave (e.g., does not cleave) both the complementary and the non-complementary strands of a target nucleic acid. Such a Cas9 protein has a reduced ability to cleave a target nucleic acid (e.g., a single stranded or double stranded target nucleic acid) but retains the ability to bind a target

nucleic acid. A Cas9 protein that cannot cleave target nucleic acid (e.g., due to one or more mutations, e.g., in the catalytic domains of the RuvC and HNH domains) is referred to as a "nuclease defective Cas9", "dead Cas9" or simply "dCas9." Other residues can be mutated to achieve the above effects (i.e. inactivate one or the other nuclease portions). As non-limiting examples, residues D10, G12, G17, E762, H840, N854, N863, H982, H983, A984, D986, and/or A987 of *Streptococcus pyogenes* Cas9 (or the corresponding amino acids of a Cas9 homolog) can be altered (i.e., substituted). In some cases, two or more of D10, E762, H840, N854, N863, and D986 of *Streptococcus pyogenes* Cas9 (or the corresponding amino acids of a Cas9 homolog) are substituted. In some cases, D10 and N863 of *Streptococcus pyogenes* Cas9 (or the corresponding amino acids of a Cas9 homolog) are substituted with Ala. Also, mutations other than alanine substitutions are suitable.

[00213] In some cases, the genome-editing endonuclease is an RNA-guided endonuclease (and it corresponding guide RNA) known as Cas9-synergistic activation mediator (Cas9-SAM). The RNA-guided endonuclease (e.g., Cas9) of the Cas9-SAM system is a "dead" Cas9 fused to a transcriptional activation domain (wherein suitable transcriptional activation domains include, e.g., VP64, p65, MyoD1, HSF1, RTA, and SET7/9) or a transcriptional repressor domain (where suitable transcriptional repressor domains include, e.g., a KRAB domain, a NuE domain, an NcoR domain, a SID domain, and a SID4X domain). The guide RNA of the Cas9-SAM system comprises a loop that binds an adapter protein fused to a transcriptional activator domain (e.g., VP64, p65, MyoD1, HSF1, RTA, or SET7/9) or a transcriptional repressor domain (e.g., a KRAB domain, a NuE domain, an NcoR domain, a SID domain, or a SID4X domain). For example, in some cases, the guide RNA is a single-guide RNA comprising an MS2 RNA aptamer inserted into one or two loops of the sgRNA; the dCas9 is a fusion polypeptide comprising dCas9 fused to VP64; and the adaptor/functional protein is a fusion polypeptide comprising: i) MS2; ii) p65; and iii) HSF1. See, e.g., U.S. Patent Publication No. 2016/0355797.

[00214] Also suitable for use is a chimeric polypeptide comprising: a) a dead RNA-guided endonuclease; and b) a heterologous fusion polypeptide. Examples of suitable heterologous fusion polypeptides include a polypeptide having, e.g., methylase activity, demethylase activity, transcription activation activity, transcription repression activity, transcription release factor activity, histone modification activity, RNA cleavage activity, DNA cleavage activity, DNA integration activity, or nucleic acid binding activity.

Guide RNA

[00215] 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.

[00216] 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."

[00217] 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).

[00218] 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).

[00219] In some cases, the gene products are: i) an RNA-guided endonuclease; and ii) one guide RNA. In some cases, the guide RNA is a single-molecule (or "single guide") guide RNA (an "sgRNA"). In some cases, the guide RNA is a dual-molecule (or "dual-guide") guide RNA ("dgRNA").

[00220] In some cases, the gene products are: i) an RNA-guided endonuclease; and ii) 2 separate sgRNAs, where the 2 separate sgRNAs provide for deletion of a target nucleic acid via non-homologous end joining (NHEJ). In some cases, the guide RNAs are sgRNAs. In some cases, the guide RNAs are dgRNAs.

[00221] In some cases, the gene products are: i) a Cpf1 polypeptide; and ii) a guide RNA precursor; in these cases, the precursor can be cleaved by the Cpf1 polypeptide to generate 2 or more guide RNAs.

[00222] 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.

[00223] 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

[00224] 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

[00225] 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.

[00226] 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

[00227] 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.

[00228] 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.

[00229] 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, e.g. by intravitreal injection, by subretinal injection, by suprachoroidal injection, or by any other convenient mode or route of administration. Other convenient modes or routes of administration include, e.g., intravenous, intranasal, etc.

[00230] 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 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 viral genomes (vg) to about 10^{15} vg of the rAAV virions, e.g., from about 10^8 vg to 10^{12} vg. 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. For example, 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} vg of the rAAV virions. As another example, for *in vitro* transduction, an effective amount of rAAV virions to be delivered to cells will be on the order of from about 10 vg/cell to about 10^4 vg/cell. Other effective dosages can be readily established by one of ordinary skill in the art through routine trials establishing dose response curves.

[00231] 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.

[00232] 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.

[00233] The present disclosure provides methods of treating retinal disease. The methods generally involve administering an rAAV virion of the present disclosure, or a composition comprising an rAAV virion of the present disclosure, to an eye of an individual in need thereof. Non-limiting methods for assessing treatment of retinal diseases include measuring functional changes, e.g. changes in visual acuity (e.g. BCVA), visual field (e.g. visual field perimetry), electrophysiological responsiveness to light and dark (e.g. ERG, VEP), color vision, and/or contrast sensitivity; measuring changes in anatomy or health using anatomical and/or photographic measures, e.g. OCT, fundus photography, and/or autofluorescence; and measuring ocular motility (e.g. nystagmus, fixation preference, and stability).

[00234] For example, one of ordinary skill in the art could readily determine an effective amount of rAAV virions by testing for an effect on one or more parameters, e.g. visual acuity, visual field, electrophysiological responsiveness to light and dark, color vision, contrast sensitivity, anatomy, retinal health and vasculature, ocular motility, fixation preference, and stability. In some cases, administering an effective amount of an rAAV virion of the present disclosure results in a decrease in the rate of loss of retinal function, anatomical integrity, or retinal health, e.g. a 2-fold, 3-fold, 4-fold, or 5-fold or more decrease in the rate of loss and hence progression

of disease, e.g. a 10-fold decrease or more in the rate of loss and hence progression of disease. In some cases, administering an effective amount of an rAAV virion of the present disclosure results in a gain in retinal function, an improvement in retinal anatomy or health, and/or a stabilization in ocular motility, e.g. a 2-fold, 3-fold, 4-fold or 5-fold improvement or more in retinal function, retinal anatomy or health, and/or stability of the orbital, e.g. a 10-fold improvement or more in retinal function, retinal anatomy or health, and/or stability of the orbital.

NUCLEIC ACIDS AND HOST CELLS

[00235] 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, or where the variant AAV capsid protein comprises a replacement 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 with a heterologous peptide of from about 5 amino acids to about 20 amino acids; 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

[00236] 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-VI, as described above. The insertion of the insertion peptide into a parental AAV capsid will in some cases replace an endogenous stretch of from about 5 amino acids to about 20 amino acids in the GH loop or loop IV. Thus, in some cases, a variant AAV capsid protein encoded by a subject nucleic acid comprises a replacement 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 with a heterologous peptide of from about 5 amino acids to about 20 amino acids, where suitable heterologous peptides include a peptide of any one of Formulas I-VI, as described above.

[00237] 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.

[00238] 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.

[00239] 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.

[00240] 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, 293T 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)

[00241] 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

[00242] 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 1-63 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:

[00243] 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 of any one of Formulas I-VI, 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.

[00244] 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.

[00245] 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.

[00246] Aspect 4. The rAAV virion of aspect 1, wherein the insertion of the heterologous peptide replaces a contiguous stretch of from 5 amino acids to 20 amino acids of the parental AAV capsid protein.

[00247] Aspect 5. The rAAV virion of aspect 1, 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.

[00248] Aspect 6. The rAAV virion of aspect 4, 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; or wherein the insertion site is located between amino acids corresponding to amino acids 585 and 598 of VP1 of AAV2, or the corresponding position in the capsid protein of another AAV serotype.

[00249] Aspect 7. The rAAV virion of any one of aspects 1-6, wherein gene product is an interfering RNA or an aptamer.

[00250] Aspect 8. The rAAV virion of any one of aspects 1-6, wherein the gene product is a polypeptide.

[00251] 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.

[00252] Aspect 10. The rAAV virion of aspect 8, wherein the polypeptide is an RNA-guided endonuclease selected from a type II CRISPR/Cas polypeptide, a type V CRISPR/Cas polypeptide, and a type VI CRISPR/Cas polypeptide.

[00253] Aspect 11. The rAAV virion of aspect 10, wherein the RNA-guided endonuclease is an enzymatically inactive type II CRISPR/Cas polypeptide.

[00254] Aspect 12. The rAAV virion of aspect 10, wherein the gene product is an RNA-guided endonuclease and a guide RNA.

[00255] Aspect 13. The rAAV virion of any one of aspects 1-12, wherein the heterologous peptide is a peptide of Formula I: LA(L/N)(I/Q)(Q/E)(D/H)(S/V)(M/K)(R/N)A (SEQ ID NO: 136).

[00256] Aspect 14. The rAAV virion of any one of aspects 1-12, wherein the heterologous peptide comprises (21) LALIQDSMRA (SEQ ID NO: 35) or (22) LANQEHVKNA (SEQ ID NO: 2).

[00257] Aspect 15. The rAAV virion of any one of aspects 1-12, wherein the heterologous peptide is a peptide of Formula II: $TX_1X_2X_3X_4X_5X_6X_7X_8GLX_9$ (SEQ ID NO: 137), where:

[00258] X_1 is G, V, or S;

[00259] X_2 is V, E, P, G, D, M, A, or S;

[00260] X_3 is M, V, Y, H, G, S, or D;

[00261] X_4 is R, D, S, G, V, Y, T, H, or M;

[00262] X_5 is S, L, G, T, Q, P, or A;

[00263] X_6 is T, A, S, M, D, Q, or H;

[00264] X_7 is N, G, S, L, M, P, G, or A;

[00265] X_8 is S, G, D, N, A, I, P, or T; and

[00266] X_9 is **S** or N.

[00267] Aspect 16. The rAAV virion of any one of aspects 1-12, wherein the heterologous peptide comprises: (1) TGVMRSTNSGLN (SEQ ID NO: 6); (2) TGEVDLAGGGLS (SEQ ID No: 7); (3) TSPYSGSSDGLS (SEQ ID NO: 8); (4) TGGHDSSLDGLS (SEQ ID NO: 9); (5) TGDGGTTMNGLS (SEQ ID NO: 98); (6) TGGHGSAPDGLS (SEQ ID NO: 99); (7) TGMHVTMMAGLN (SEQ ID NO: 100); (8) TGASYLDNSGLS (SEQ ID NO: 101); (9)

TVVSTQAGIGLS (SEQ ID NO: 135); (10) TGVMHSQASGLS (SEQ ID NO: 21); (11) TGDGSPAAPGLS (SEQ ID NO: 22); or (12) TGSDMAHGTGLS (SEQ ID NO: 23)

[00268] Aspect 17. The rAAV virion of any one of aspects 1-12, wherein the heterologous peptide is a peptide of Formula III: $TGX_1X_2X_3X_4X_5X_6X_7GLS$ (SEQ ID NO: 138), where:

[00269] X_1 is V, E, P, G, D, M, A, or S;

[00270] X_2 is M, V, Y, H, G, S, or D;

[00271] X_3 is R, D, S, G, V, Y, T, H, or M;

[00272] X_4 is S, L, G, T, Q, P, or A;

[00273] X_5 is T, A, S, M, D, Q, or H;

[00274] X_6 is N, G, S, L, M, P, G, or A; and

[00275] X_7 is S, G, D, N, A, I, P, or T.

[00276] Aspect 18. The rAAV virion of any one of aspects 1-12, wherein the heterologous peptide comprises: (2) TGEVDLAGGGLS (SEQ ID NO: 7); (4) TGGHDSSLDGLS (SEQ ID NO: 9); (5) TGDGGTTMNGLS (SEQ ID NO: 98); (6) TGGHGSAPDGLS (SEQ ID NO: 99); (8) TGASYLDNSGLS (SEQ ID NO: 101); (10) TGVMHSQASGLS (SEQ ID NO: 21); (11) TGDGSPAAPGLS (SEQ ID NO: 22); or (12) TGSDMAHGTGLS (SEQ ID NO: 23).

[00277] Aspect 19. The rAAV virion of any one of aspects 1-12, wherein the heterologous peptide is a peptide of Formula IV: $X_1GX_2X_3X_4X_5X_6X_7X_8GLSPX_9TX_{10}X_{11}$ (SEQ ID NO: 139), where

[00278] X_1 is **T** or N;

[00279] X_2 is L, S,A, or G;

[00280] X_3 is **D** or V;

[00281] X_4 is A, G, or P;

[00282] X_5 is **T** or D;

[00283] X_6 is **R** or Y;

[00284] X_7 is D, T, or G;

[00285] X_8 is H, R, or T;

[00286] X_9 is **V** or A;

[00287] X_{10} is **G** or W; and

[00288] X_{11} is **T** or A.

[00289] Aspect 20. The rAAV virion of any one of aspects 1-12, wherein the heterologous peptide comprises: (13) TGLDATRDHGLSPVTGT (SEQ ID NO: 24); (14) TGSDGTRDHGLSPVTWT (SEQ ID NO: 25); (15) NGAVADYTRGLSPATGT (SEQ ID NO: 26); or (16) TGGDPTRGTGLSPVTGA (SEQ ID NO: 27).

[00290] Aspect 21. The rAAV virion of any one of aspects 1-12, wherein the heterologous peptide is a peptide of Formula V: $TGX_1DX_2TRX_3X_4GLSPVTGT$ (SEQ ID NO: 140), where

[00291] X_1 is L, S,A, or G;

[00292] X_2 is A, G, or P;

[00293] X_3 is D, T, or G; and

[00294] X_4 is H, R, or T

[00295] Aspect 22. The rAAV virion of any one of aspects 1-12, wherein the heterologous peptide is a peptide of Formula VI: $LQX_1X_2X_3RX_4X_5X_6X_7X_8X_9VNX_{10}Q$ (SEQ ID NO: 141), where

[00296] X_1 is K or R;

[00297] X_2 is N, G, or A;

[00298] X_3 is A, V, N, or D;

[00299] X_4 is P, I, or Q;

[00300] X_5 is A, P, or V;

[00301] X_6 is S, T, or G;

[00302] X_7 is T or V;

[00303] X_8 is E, L, A, or V;

[00304] X_9 is S, E, D, or V; and

[00305] X_{10} is F, G, T, or C.

[00306] Aspect 23. The rAAV virion of any one of aspects 1-12, wherein the heterologous peptide comprises: (17) LQKNARPASTESVNFQ (SEQ ID NO: 28); (18) LQRGVRIPSVLEVNGQ (SEQ ID NO: 29); (19) LQRGNRPVTTADVNTQ (SEQ ID NO: 30); or (20) LQKADRQPGVVVVNCQ (SEQ ID NO: 31).

[00307] Aspect 24. A pharmaceutical composition comprising:

[00308] a) a recombinant adeno-associated virus virion of any one of aspects 1-23; and

[00309] b) a pharmaceutically acceptable excipient.

[00310] Aspect 25. 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 1-23 or the composition of aspect 24.

[00311] Aspect 26. The method of aspect 25, wherein the gene product is a polypeptide.

[00312] Aspect 27. The method of aspect 25, wherein the gene product is a short interfering RNA or an aptamer.

[00313] Aspect 28. The method of aspect 26, 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.

[00314] Aspect 29. The method of aspect 26, 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.

[00315] Aspect 30. The method of aspect 26, wherein the polypeptide is an RNA-guided endonuclease.

[00316] Aspect 31. 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 1-23 or the composition of aspect 24.

[00317] Aspect 32. The method of aspect 31, wherein said administering is by intraocular injection.

[00318] Aspect 33. The method of aspect 31, wherein said administering is by intravitreal injection or by suprachoroidal injection.

[00319] Aspect 34. The method of any one of aspects 31-33, wherein the ocular disease is glaucoma, retinitis pigmentosa, macular degeneration, retinoschisis, Leber's Congenital Amaurosis, diabetic retinopathy, achromotopsia, or color blindness.

[00320] Aspect 35. 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-VI.

[00321] Aspect 36. The isolated nucleic acid of aspect 35, wherein the insertion site is between amino acids 587 and 588 of AAV2, between amino acids 585 and 598 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.

[00322] Aspect 37. An isolated, genetically modified host cell comprising the nucleic acid of aspect 35 or aspect 36.

[00323] Aspect 38. 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-VI.

[00324] Aspect 39. A recombinant adeno-associated virus (rAAV) virion comprising:

[00325] a) a variant AAV capsid protein, wherein the variant AAV capsid protein comprises an insertion of a heterologous peptide of Formula VI, 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

[00326] b) a heterologous nucleic acid comprising a nucleotide sequence encoding a heterologous gene product.

[00327] Aspect 40. The rAAV virion of aspect 39, 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.

[00328] Aspect 41. The rAAV virion of aspect 39, 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.

[00329] Aspect 42. The rAAV virion of any one of aspects 39-41, wherein the insertion of the heterologous peptide replaces a contiguous stretch of from 5 amino acids to 20 amino acids of the parental AAV capsid protein.

[00330] Aspect 43. The rAAV virion of any one of aspects 39-42, 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.

[00331] Aspect 44. The rAAV virion of aspect 43, 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; or wherein the insertion site is located between amino acids corresponding to amino acids 585 and 598 of VP1 of AAV2, or the corresponding position in the capsid protein of another AAV serotype.

[00332] Aspect 45. The rAAV virion of any one of aspects 39-44, wherein gene product is an interfering RNA.

[00333] Aspect 46. The rAAV virion of any one of aspects 39-44, wherein gene product is an aptamer.

[00334] Aspect 47. The rAAV virion of any one of aspects 39-44, wherein the gene product is a polypeptide.

[00335] Aspect 48. The rAAV virion of aspect 47, wherein the polypeptide is a neuroprotective polypeptide, an anti-angiogenic polypeptide, or a polypeptide that enhances function of a retinal cell.

[00336] Aspect 49. The rAAV virion of aspect 47, wherein the polypeptide is an RNA-guided endonuclease selected from a type II CRISPR/Cas polypeptide, a type V CRISPR/Cas polypeptide, and a type VI CRISPR/Cas polypeptide.

[00337] Aspect 50. The rAAV virion of aspect 49, wherein the RNA-guided endonuclease is an enzymatically inactive type II CRISPR/Cas polypeptide.

[00338] Aspect 51. The rAAV virion of one of aspects 39-44, wherein the gene product is an RNA-guided endonuclease and a guide RNA.

[00339] Aspect 52. The rAAV virion of any one of aspects 39-51, wherein the heterologous peptide comprises: (17) LQKNARPASTESVNFQ (SEQ ID NO: 28); (18) LQRGVRIPSVLEVNGQ (SEQ ID NO: 29); (19) LQRGNRPVTTADVNTQ (SEQ ID NO: 30); or (20) LQKADRQPGVVVVNCQ (SEQ ID NO: 31).

[00340] Aspect 53. A pharmaceutical composition comprising:

[00341] a) a recombinant adeno-associated virus virion of any one of aspects 39-52; and

[00342] b) a pharmaceutically acceptable excipient.

[00343] Aspect 54. 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 39-52 or the composition of aspect 53.

[00344] Aspect 55. The method of aspect 54, wherein the gene product is a polypeptide.

[00345] Aspect 56. The method of aspect 54, wherein the gene product is a short interfering RNA or an aptamer.

[00346] Aspect 57. The method of aspect 55, 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.

[00347] Aspect 58. The method of aspect 57, 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.

[00348] Aspect 59. The method of aspect 55, wherein the polypeptide is an RNA-guided endonuclease.

[00349] Aspect 60. 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 39-52 or the composition of aspect 53.

[00350] Aspect 61. The method of aspect 60, wherein said administering is by intraocular injection.

[00351] Aspect 62. The method of aspect 60, wherein said administering is by intravitreal injection or by suprachoroidal injection.

[00352] Aspect 63. The method of any one of aspects 60-62, wherein the ocular disease is glaucoma, retinitis pigmentosa, macular degeneration, retinoschisis, Leber's Congenital Amaurosis, diabetic retinopathy, achromotopsia, or color blindness.

EXAMPLES

[00353] 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: AAV virions comprising variant AAV capsids

[00354] A number of variants of AAV capsids were derived through a directed evolution approach; AAV virions comprising the variant AAV capsids infect the primate retina, e.g., when administered via intravitreal injection. Primates are an important preclinical model for human retinal disease, with a fovea for high acuity vision, similar to humans.

AAV packaging

[00355] AAV virions comprising variant AAV capsids were identified by screening. Five libraries were used for this screen: 1) a 7mer peptide display library based on AAV2, containing

a 7mer peptide insertion at amino acid ~588, and surrounded by a 5' LA linker and a 3'A linker; 2) a 7mer peptide display library based on AAV4, with a 7mer peptide insertion at amino acid ~584, with a 5'TG linker and a 3'GLS linker; 3) a 7mer peptide display library based on AAV5 with a 7mer peptide insertion at amino acid ~575 with 5'TG linker and a 3'GLS linker; 4) a library based on an ancestral AAV sequence (Santiago-Ortiz et al., 2015) and containing a 7mer peptide display library at position amino acid ~591 with a 5'TG linker and a 3'GLS linker; and 5) an AAV2-based library with semi-random mutations at surface exposed position amino acid ~588 (Koerber, Jang, & Schaffer, 2008). Virus was packaged such that each viral genome was encapsidated within the capsid protein shell that that genome encoded, as previously described Koerber et al. (2008) *supra*; Fowler et al. *Nat Protoc* **9**, 2267–2284 (2014). Therefore functional improvements identified through selection can be linked to the genome sequence contained within the viral capsid. Briefly, AAV vectors were produced by triple transient transfection of HEK293T cells, purified via iodixanol density centrifugation, and buffer exchanged into PBS by Amicon filtration. DNase-resistant viral genomic titers were measured by quantitative real time PCR using a BioRad iCycler. From this library, an iterative *in vivo* screening selection process was used to identify variants with the ability to infect the primate retina from the vitreous (FIG. 1). Primate eyes were injected in each round with ~250 µL of 1 x 10^13 (1E13) – 1 x 10^14 (1E14) vg/mL titer virus. Three weeks after injection, eyes were enucleated, and retinal punches were taken from central and peripheral regions of the retina (FIG.1). DNA from various retinal layers was assayed, and the capsid inserts were identified. After each round of injection, capsid sequences were recovered by PCR from harvested cells using primers HindIII_F1 and NotI_R1, AscI_R1, or SpeI_R1, with reverse primers being specific to unique AAV backbones, in order to maintain separation of groups of libraries. PCR amplicons were then digested, and recloned into the backbone. RPE cells were separated from retinal tissue, and tissue was frozen. Retinal tissue was embedded and sectioned on a cryostat to isolate photoreceptors in the outer nuclear layer. DNA was then collected from the isolated photoreceptors or RPE, and cap genes were PCR amplified. Recovered cap genes were used for subsequent AAV packaging.

[00356] FIG. 1. Illustration of the directed evolution methodology used to develop primate retinal AAV variants. Peptide display libraries were created, packaged into AAV vectors, and injected into the primate 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.

Deep sequencing of AAV libraries from rounds of selection

[00357] 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 primate retina from the vitreous. A ~75-85 base pair region containing the 7mer insertion or Loop Swap mutation site was PCR amplified from harvested DNA. Primers included Illumina adapter sequences containing unique barcodes to allow for multiplexing of amplicons from multiple rounds of selection. PCR amplicons were purified and sequenced with a 100-cycle single-read run on an Illumina HiSeq 2500. Custom Python code was written to translate DNA sequences into amino acid sequences, and to identify and count reads containing unique 7mer insert sequences. Read counts were normalized by the total number of reads in the run. Python and Pandas were used to analyze dynamics of directed evolution and create plots.

Deep sequencing analysis

[00358] Out of a library of ~1 x 10^7 (~1E7) variants per library, top variants were selected. Best performing variants were chosen as ones with the greatest fold increase in the final round of selection relative to the initial plasmid library (# reads in final round, normalized to total number of reads in the round / # of reads in library, normalized to total number of reads in the round). A pseudo-count of 1 was added before normalization to each individual variant to allow analysis of variants not appearing in sequencing of the plasmid library. Fowler et al. (2014) *supra*. Amino acid sequences of the peptide insertions are shown in FIG. 2.

[00359] The variants generated through this approach enable non-invasive panretinal gene therapy strategies in the primate retina using intravitreal injections. These AAV vectors can be used for gene augmentation therapies for retinal degenerative diseases including retinitis pigmentosa, Leber Congenital Amaurosis, Rod-cone dystrophy, cone dystrophy, achromatopsia, X-linked retinoschisis, CRB1, optogenetic therapies, expression of trophic and survival factors such as GDNF, BDNF, FGF, RdCVF, RdCVFL, XIAP, and expression of blockers of neovascularization such as sFLT. The vectors can also be used to deliver gene editing tools such as CRISPR/Cas9 for gene correction or the creation of additional models of retinal disease.

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Example 2: Methods for construction and sequencing of GFP-barcode libraries

GFP barcode library construction

[00360] Unique 25 bp DNA barcodes were cloned behind an AAV ITR construct containing a self-complementary CAG promoter driving eGFP (CAG-GFP-Barcode-pA). Individual variants were packaged separately with constructs containing different barcodes. Variants were then titer matched and mixed in equal ratios before injection into mice, dogs, and primates.

Deep sequencing of GFP-barcode libraries

[00361] Barcodes were PCR amplified directly from DNA or cDNA (created from mRNA using Superscript III reverse transcriptase), which was harvested from dog or primate retinal tissue. Samples were collected from areas across the retina, and from ONL or RPE. Primers amplified a ~50 bp region surrounding the GFP barcode and contained Illumina adapter sequences and secondary barcodes to allow for multiplexing of multiple samples. PCR amplicons were purified and sequenced with a 100-cycle single-read run on a MiSeq. Read counts were normalized by total number of reads in the run. Analysis of barcode abundance was performed using custom code written in Python, followed by creation of plots in Pandas. Best performing variants were selected based on the fold increase in the percent of total library, relative to the injected library (% of total in recovered sample / % of total in injected library). Analysis was performed on n=1 primate.

[00362] FIG. 9 provides Table 1; FIG. 10 provides Table 2.

[00363] Table 1 provides a ranking of primate-derived variants and controls recovered from photoreceptors following injection of a GFP-Barcode library. Table 2 provides a ranking of primate-derived variants and controls recovered from RPE cells following injection of a GFP-Barcode library. The library contained individual variants packaged with GFP fused to a unique

DNA barcode. Polymerase chain reaction (PCR) was used to amplify barcodes from DNA recovered from specific cell types in the retina. "Region" in Tables 1 and 2 indicates the region from which the DNA was recovered. The fold increase of reads of each of the variants was calculated by dividing number of reads for each unique barcode in the recovered cells (corresponding to each unique variant), by the number of reads for each variant in the injected library. This table indicates the average of the fold increase across multiple locations in the retina. Variants were ranked by fold increase of the barcode.

[00364] FIG. 11. GFP expression of GFP-barcoded libraries in primate retina. GFP expression resulting from intravitreal injection of pooled, GFP-barcoded library (which contains all the tested viruses) was located primarily in the outer retina, with a tropism that was directed more toward the outer retina than expression of AAV24YF.

Example 3

Primate studies

[00365] Cynomolgous monkeys between 4-10 years old were used for all studies, and intravitreal injections were made. The monkey used for fluorophore expression received daily subcutaneous injections of cyclosporine at a dose of 6 mg/kg for immune suppression, and adjusted based on blood trough levels to within a 150-200 ng/ml target range. Confocal scanning laser ophthalmoscopic images (Spectralis HRA, Heidelberg Engineering) were obtained from the two retinas at 3 weeks after injection, with autofluorescence settings, which leads to effective tdTomato and GFP visualization. For histology, the monkey was euthanized, both retinas were lightly fixed in 4% paraformaldehyde, and tissue was examined by confocal microscopy. At the conclusion of the experiment, euthanasia was achieved by administering an IV overdose of sodium pentobarbital (75 mg kg−1), as recommended by the Panel on Euthanasia of the American Veterinary Medical Association. Pieces of primate retina were then prepared in 30% sucrose, embedded in OCT media, flash frozen, and sectioned at 20 μm for confocal microscopy imaging of native fluorophore expression. Antibodies for labeling were: anti-GFP (A11122, Thermo, 1:250) anti-vimentin (Dako, 1:1000), peanut agglutinin (PNA) (Molecular Probes, 1:200), and anti-cone arrestin (7G6, 1:50). The procedures were conducted according to the ARVO Statement for the Use of Animals and the guidelines of the Office of Laboratory Animal Care at the University of Rochester.

RESULTS

Directed evolution of AAV in primate retina

[00366] In addition to canine, the nonhuman primate is a critical preclinical model for human therapeutic development, as it is most closely related to, and has a retinal anatomy similar to that of humans. In particular, primates are the only large animal model that possesses a fovea, the

specialized high acuity area of the retina that is most important for daily activities such as reading, is critical to quality of life, and is lost in numerous retinal degenerations. The species specificity observed in the canine study motivated us to pursue an additional course of directed evolution in primate retina. Nine libraries were packaged and included in the primate screen: EP2, EP5, EP6, EP8, EP9, EP-Ancestral, AAV2-7mer, Ancestral-7mer (Santiago-Ortiz et al. *Gene Ther* **22**, 934–946 (2015)) and LoopSwap (Koerber et al. *Mol Ther* **17**, 2088–2095 (2009)). Libraries were injected, harvested, and repackaged for 5 sequential rounds of selection, with one round of error prone PCR performed after round 3. AAV cap genes were PCR amplified from ONL, and in parallel from overlying RPE. EP libraries were abandoned at round 3, as no variants from these libraries were recovered from retinal tissue. At round 4, additional libraries (AAV4-7mer and AAV5-7mer) were added to the selection, using a separate backbone that was isolated from other libraries by separate PCR annealing sites and restriction sites.

[00367] Deep sequencing revealed that, similar to observations from the canine screen, libraries contained ~1E+6 - ~1E+7 individual variants, which converged to ~1E+4 - ~1E+5 variants over 6 rounds of selection, a diversity not possible to observe through Sanger sequencing (Fig. 12A). As observed in the canine screen, in each of the libraries analyzed, a small portion of library members were over-represented in the initial plasmid library (Fig. 12B). Analysis of results from high throughput sequencing over the rounds of selection revealed, for each of the libraries, a subset of variants that increased significantly in their representation during rounds of selection (Fig. 12C).

Secondary barcoded-GFP library screening in primate retina

[00368] Sixteen variants, from these 5 libraries (Fig. 12C), were selected to be included in a secondary round of selection with GFP-barcoded libraries, along with AAV2, AAV2-4YF+TV, AAV4 and AAV5 as controls. This new library was injected in both eyes of a primate, and 3 weeks after injection, biopsies were collected from locations across the retina (Fig. 12D). GFP expression resulting from injection of the GFP-barcode libraries was primarily found in photoreceptors, as well as some inner retinal cells, a tropism that is shifted from AAV2 or 7m8, which yielded stronger inner retinal expression (Fig. 12E).

[00369] **Fig. 12A-12F. Directed evolution of AAV in primate retina. (A)** Deep sequencing of variant libraries revealed convergence of variants over rounds of selection. **(B)** In each of the libraries evaluated, a small proportion of variants are overrepresented in the plasmid library. **(C)** Scatterplots illustrate the behavior of individual variants at the final round of selection for each of the libraries injected in primate retinas. Variants overrepresented in the original library are colored blue. Variants that had the greatest fold increase in representation in the final round of selection are shown in magenta. Variants that were overrepresented in the original library and

increased significantly in representation over rounds of selection are colored orange. **(D)** A map of the primate retina shows the distribution of samples that were collected for rounds of selection and the GFP-barcode library. Color coding of variants is the same as in Fig. 2. **(E)** GFP expression resulting from the barcoded library revealed that expression was shifted to an outer retinal tropism in selected variants**. (F)** GFP-barcode library injection results, for primate outer retina. The lists of variants are ordered from best (top) to worst (bottom) performing vectors, along with a value indicating the extent to which the variant competed with other vectors, expressed as: % of total in AAV library / % of total in recovered library.

Validation of the top-performing primate variants

[00370] Quantification of vector performance in outer retina revealed that AAV2-based variants outperformed viruses based on other serotypes. One vector, Loop Swap variant AAV2 588~LQRGVRIPSVLEVNGQ (SEQ ID NO:116), outperformed other variants, though it yielded lower viral titers (~5E+11 vg/mL).

[00371] AAV2-LALIQDSMRA (SEQ ID NO:117; designated NHP#9), the second ranking variant from the GFP-barcode screen, which packaged at high titers (~5E+13 vg/mL), was therefore selected for a first round of validation studies focusing on ganglion cells of the inner retina and cones of the outer retina. Cone photoreceptors are involved in adult macular degeneration (AMD), the most common cause of blindness in developed countries that are predicted to affect 288 million people worldwide by the year 2040, and are therefore a primary target for retinal gene therapy. NHP#9 was packaged with an SNCG promoter driving tdTomato in RGCs and the pR1.7 promoter driving expression of GFP in cones. Vectors encoding both these constructs were mixed in equal ratios (~1.5E+12 vg/construct/eye, and injected intravitreally in a cynomolgous monkey. A previously described variant, 7m8 (Dalkara et al. (2013) *supra*), packaged with equal titers of the same constructs was injected into the vitreous of the contralateral eye. Expression of tdTomato reporter in RGC's was lower in NHP#9-injected eyes compared to 7m8, which infected ganglion cells across the expanse of the retina efficiently; however, expression in foveal cones was greatly increased relative to 7m8, indicating a shift in tropism away from the inner retina towards photoreceptors in the outer retina. qRT-PCR, performed using the ddCT method, revealed an 11.71 (10.37 - 13.22) fold increase of GFP expression in foveal cones relative to 7m8. Counting of labeled cells, performed with Imaris software on images collected from flatmounted retinas, also confirmed a substantial decrease in numbers of transduced ganglion cells and an increase in the number of cones targeted with NHP#9.

[00372] Next, the top-ranking variant from the GFP barcode screen, Loopswap variant ~588-LQRGVRIPSVLEVNGQ (SEQ ID NO:118; designated NHP#26) was also tested for validation,

although low numbers of viral particles were produced. ~5E+10 particles of NHP#26-scCAG-eGFP were injected intravitreally into one eye of a cynomolgous monkey. Although the number of particles injected was low, efficient expression of GFP was observed in the fovea and across the retina (Fig. 13G). In contrast to the foveal-spot-and-ring pattern of expression that was observed with 7m8, NHP#9 (Fig 13A), and other naturally occurring serotypes, fundus imaging of NHP#26 resulted in a disc of GFP expression centered on the foveola (Fig. 13G). Confocal imaging of the flatmounted retina confirmed this disc pattern of expression around the fovea (Fig. 13H), with very few GFP positive ganglion cell axons. Punctate regions of GFP expression were often strongest around retinal blood vessels (Fig. 13I), and were located across the expanse of the retina. Imaging of cryostat sections taken from the retina confirmed that there was little GFP expression in ganglion cells, as indicated by the lack of GFP+ ganglion cell axons, while high levels of GFP expression were found in Müller cells, additional cells in the inner nuclear layer, foveal cones and rods across the retina (Fig. 13J-13Q).

[00373] Fig. 13A-13Q. Validation of evolved AAV variants in primate retina. (A-F) Co-injection of ~1.5E+12 particles of SCNG-tdTomato and ~1.5E+12 pR1.7-eGFP packaged in 7m8 and variant NHP#9 in primate retina. Intravitreal injection of 7m8 (A,C,E) resulted in robust tdTomato expression in ganglion cells and expression of GFP in foveal cones. In contrast, injection of equal number of particles of NHP#9 resulted in reduced ganglion cell expression, and increased GFP expression in cones relative to 7m8 (B,D,F). (G) Fundus imaging in a primate eye following injection of 5E+10 particles of NHP#26-scCAG-GFP resulted in a disc of GFP expression centered on the fovea, and a punctate pattern of GFP expression across the retina. (H) Confocal imaging of native GFP expression in the flatmounted fovea. (I) Confocal imaging of native GFP expression in the area outside of the vascular arcade. (J) Confocal imaging of native GFP expression in a cryostat section through the fovea. (K) Native GFP expression in inferior retina, outside the vascular arcade, shows little GFP expression in ganglion cells, but high levels of expression in Müller cells and in photoreceptors in outer retina. Autofluorescence was also observed in RPE. (L) Anti-GFP labeling in a cryostat section revealed GFP expression in photoreceptors, evident by their outer segments, Müller cells, evident by their retina-spanning processes, as well as cells in the inner nuclear layer with horizontal processes that are likely interneurons. (M) Anti-GFP labeling in a foveal section reveals additional transfected cones, Müller glia and interneurons. (N) Co-labeling with anti-cone arrestin and anti-GFP reveals GFP expression in rod photoreceptors, as well as cells in the inner nuclear layer, in a section taken next to the optic nerve head. (O) Co-labeling with anti-cone arrestin and anti-GFP antibodies in an area of low expression reveals GFP expression in inner nuclear layer cells. (P,Q) Montages of

confocal images from cryostat sections collected outside the vascular arcade show efficient expression of GFP in the inner nuclear layer and outer retina.

[00374] 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.

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 of any one of Formulas I-VI, 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.

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.

3. The rAAV virion of claim 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.

4. The rAAV virion of claim 1, wherein the insertion of the heterologous peptide replaces a contiguous stretch of from 5 amino acids to 20 amino acids of the parental AAV capsid protein.

5. The rAAV virion of claim 1, 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.

6. The rAAV virion of claim 4, 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; or wherein the insertion site is located between amino acids corresponding to amino acids 585 and 598 of VP1 of AAV2, or the corresponding position in the capsid protein of another AAV serotype.

7. The rAAV virion of any one of claims 1-6, wherein gene product is an interfering RNA or an aptamer.

8. The rAAV virion of any one of claims 1-6, wherein the gene product is a polypeptide.

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

10. The rAAV virion of claim 8, wherein the polypeptide is an RNA-guided endonuclease selected from a type II CRISPR/Cas polypeptide, a type V CRISPR/Cas polypeptide, and a type VI CRISPR/Cas polypeptide.

11. The rAAV virion of claim 10, wherein the RNA-guided endonuclease is an enzymatically inactive type II CRISPR/Cas polypeptide.

12. The rAAV virion of claim 10, wherein the gene product is an RNA-guided endonuclease and a guide RNA.

13. The rAAV virion of any one of claims 1-12, wherein the heterologous peptide is a peptide of Formula I: LA(L/N)(I/Q)(Q/E)(D/H)(S/V)(M/K)(R/N)A (SEQ ID NO: 136).

14. The rAAV virion of any one of claims 1-12, wherein the heterologous peptide comprises (21) LALIQDSMRA (SEQ ID NO: 35) or (22) LANQEHVKNA (SEQ ID NO: 2).

15. The rAAV virion of any one of claims 1-12, wherein the heterologous peptide is a peptide of Formula II: $TX_1X_2X_3X_4X_5X_6X_7X_8GLX_9$ (SEQ ID NO: 137), where:

X_1 is G, V, or S;

X_2 is V, E, P, G, D, M, A, or S;

X_3 is M, V, Y, H, G, S, or D;

X_4 is R, D, S, G, V, Y, T, H, or M;

X_5 is S, L, G, T, Q, P, or A;

X_6 is T, A, S, M, D, Q, or H;

X_7 is N, G, S, L, M, P, G, or A;

X_8 is S, G, D, N, A, I, P, or T; and

X_9 is **S** or N.

16. The rAAV virion of any one of claims 1-12, wherein the heterologous peptide comprises: (1) TGVMRSTNSGLN (SEQ ID NO: 6); (2) TGEVDLAGGGLS (SEQ ID NO: 7); (3) TSPYSGSSDGLS (SEQ ID NO: 8); (4) TGGHDSSLDGLS (SEQ ID NO: 9); (5) TGDGGTTMNGLS (SEQ ID NO: 98); (6) TGGHGSAPDGLS (SEQ ID NO: 99); (7) TGMHVTMMAGLN (SEQ ID NO: 100); (8) TGASYLDNSGLS (SEQ ID NO: 101); (9) TVVSTQAGIGLS (SEQ ID NO: 20); (10) TGVMHSQASGLS (SEQ ID NO: 21); (11) TGDGSPAAPGLS (SEQ ID NO: 22); or (12) TGSDMAHGTGLS (SEQ ID NO: 23)

17. The rAAV virion of any one of claims 1-12, wherein the heterologous peptide is a peptide of Formula III: $TGX_1X_2X_3X_4X_5X_6X_7GLS$ (SEQ ID NO: 138), where:

X_1 is V, E, P, G, D, M, A, or S;

X_2 is M, V, Y, H, G, S, or D;

X_3 is R, D, S, G, V, Y, T, H, or M;

X_4 is S, L, G, T, Q, P, or A;

X_5 is T, A, S, M, D, Q, or H;

X_6 is N, G, S, L, M, P, G, or A; and

X_7 is S, G, D, N, A, I, P, or T.

18. The rAAV virion of any one of claims 1-12, wherein the heterologous peptide comprises: (2) TGEVDLAGGGLS (SEQ ID NO: 7); (4) TGGHDSSLDGLS (SEQ ID NO: 9); (5) TGDGGTTMNGLS (SEQ ID NO: 98); (6) TGGHGSAPDGLS (SEQ ID NO: 99); (8) TGASYLDNSGLS (SEQ ID NO: 101); (10) TGVMHSQASGLS (SEQ ID NO: 21); (11) TGDGSPAAPGLS (SEQ ID NO: 22); or (12) TGSDMAHGTGLS (SEQ ID NO: 23).

19. The rAAV virion of any one of claims 1-12, wherein the heterologous peptide is a peptide of Formula IV: $X_1GX_2X_3X_4X_5X_6X_7X_8GLSPX_9TX_{10}X_{11}$ (SEQ ID NO: 139), where

X_1 is **T** or N;

X_2 is L, S, A, or G;

X_3 is **D** or V;

X_4 is A, G, or P;

X_5 is **T** or D;

X_6 is **R** or Y;

X_7 is D, T, or G;

X_8 is H, R, or T;

X_9 is **V** or A;

X_{10} is **G** or W; and

X_{11} is **T** or A.

20. The rAAV virion of any one of claims 1-12, wherein the heterologous peptide comprises: (13) TGLDATRDHGLSPVTGT (SEQ ID NO: 24); (14) TGSDGTRDHGLSPVTWT (SEQ ID NO: 25); (15) NGAVADYTRGLSPATGT (SEQ ID NO: 26); or (16) TGGDPTRGTGLSPVTGA (SEQ ID NO: 27).

21. The rAAV virion of any one of claims 1-12, wherein the heterologous peptide is a peptide of Formula V: $TGX_1DX_2TRX_3X_4GLSPVTGT$ (SEQ ID NO: 140), where

X_1 is L, S, A, or G;

X_2 is A, G, or P;

X_3 is D, T, or G; and

X_4 is H, R, or T

22. The rAAV virion of any one of claims 1-12, wherein the heterologous peptide is a peptide of Formula VI: $LQX_1X_2X_3RX_4X_5X_6X_7X_8X_9VNX_{10}Q$ (SEQ ID NO: 141), where

X_1 is K or R;

X_2 is N, G, or A;

X_3 is A, V, N, or D;

X_4 is P, I, or Q;

X_5 is A, P, or V;

X_6 is S, T, or G;

X_7 is T or V;

X_8 is E, L, A, or V;

X_9 is S, E, D, or V; and

X_{10} is F, G, T, or C.

23. The rAAV virion of any one of claims 1-12, wherein the heterologous peptide comprises: (17) LQKNARPASTESVNFQ (SEQ ID NO: 28); (18) LQRGVRIPSVLEVNGQ (SEQ ID NO: 29); (19) LQRGNRPVTTADVNTQ (SEQ ID NO: 30); or (20) LQKADRQPGVVVVNCQ (SEQ ID NO: 31).

24. A pharmaceutical composition comprising:

a) a recombinant adeno-associated virus virion of any one of claims 1-23; and

b) a pharmaceutically acceptable excipient.

25. 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 1-23 or the composition of claim 24.

26. The method of claim 25, wherein the gene product is a polypeptide.

27. The method of claim 25, wherein the gene product is a short interfering RNA or an aptamer.

28. The method of claim 26, 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.

29. The method of claim 26, 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.

30. The method of claim 26, wherein the polypeptide is an RNA-guided endonuclease.

31. 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 1-23 or the composition of claim 24.

32. The method of claim 31, wherein said administering is by intraocular injection.

33. The method of claim 31, wherein said administering is by intravitreal injection or by suprachoroidal injection.

34. The method of any one of claims 31-33, wherein the ocular disease is glaucoma, retinitis pigmentosa, macular degeneration, retinoschisis, Leber's Congenital Amaurosis, diabetic retinopathy, achromotopsia, or color blindness.

35. 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-VI.

36. The isolated nucleic acid of claim 35, wherein the insertion site is between amino acids 587 and 588 of AAV2, between amino acids 585 and 598 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.

37. An isolated, genetically modified host cell comprising the nucleic acid of claim 35 or 36.

38. 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-VI.

39. 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 of Formula VI, 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.

40. The rAAV virion of claim 39, 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.

41. The rAAV virion of claim 39, 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.

42. The rAAV virion of any one of claims 39-41, wherein the insertion of the heterologous peptide replaces a contiguous stretch of from 5 amino acids to 20 amino acids of the parental AAV capsid protein.

43. The rAAV virion of any one of claims 39-42, 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.

44. The rAAV virion of claim 43, 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; or wherein the insertion site is located between amino acids corresponding to amino acids 585 and 598 of VP1 of AAV2, or the corresponding position in the capsid protein of another AAV serotype.

45. The rAAV virion of any one of claims 39-44, wherein gene product is an interfering RNA.

46. The rAAV virion of any one of claims 39-44, wherein gene product is an aptamer.

47. The rAAV virion of any one of claims 39-44, wherein the gene product is a polypeptide.

48. The rAAV virion of claim 47, wherein the polypeptide is a neuroprotective polypeptide, an anti-angiogenic polypeptide, or a polypeptide that enhances function of a retinal cell.

49. The rAAV virion of claim 47, wherein the polypeptide is an RNA-guided endonuclease selected from a type II CRISPR/Cas polypeptide, a type V CRISPR/Cas polypeptide, and a type VI CRISPR/Cas polypeptide.

50. The rAAV virion of claim 49, wherein the RNA-guided endonuclease is an enzymatically inactive type II CRISPR/Cas polypeptide.

51. The rAAV virion of one of claims 39-44, wherein the gene product is an RNA-guided endonuclease and a guide RNA.

52. The rAAV virion of any one of claims 39-51, wherein the heterologous peptide comprises: (17) LQKNARPASTESVNFQ (SEQ ID NO: 28); (18) LQRGVRIPSVLEVNGQ (SEQ ID NO: 29); (19) LQRGNRPVTTADVNTQ (SEQ ID NO: 30); or (20) LQKADRQPGVVVVNCQ (SEQ ID NO: 31).

53. A pharmaceutical composition comprising:

a) a recombinant adeno-associated virus virion of any one of claims 39-52; and

b) a pharmaceutically acceptable excipient.

54. 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 39-52 or the composition of claim 53.

55. The method of claim 54, wherein the gene product is a polypeptide.

56. The method of claim 54, wherein the gene product is a short interfering RNA or an aptamer.

57. The method of claim 55, 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.

58. The method of claim 57, 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.

59. The method of claim 55, wherein the polypeptide is an RNA-guided endonuclease.

60. 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 39-52 or the composition of claim 5.

61. The method of claim 60, wherein said administering is by intraocular injection.

62. The method of claim 60, wherein said administering is by intravitreal injection or by suprachoroidal injection.

63. The method of any one of claims 60-62, wherein the ocular disease is glaucoma, retinitis pigmentosa, macular degeneration, retinoschisis, Leber's Congenital Amaurosis, diabetic retinopathy, achromotopsia, or color blindness.

FIG. 1

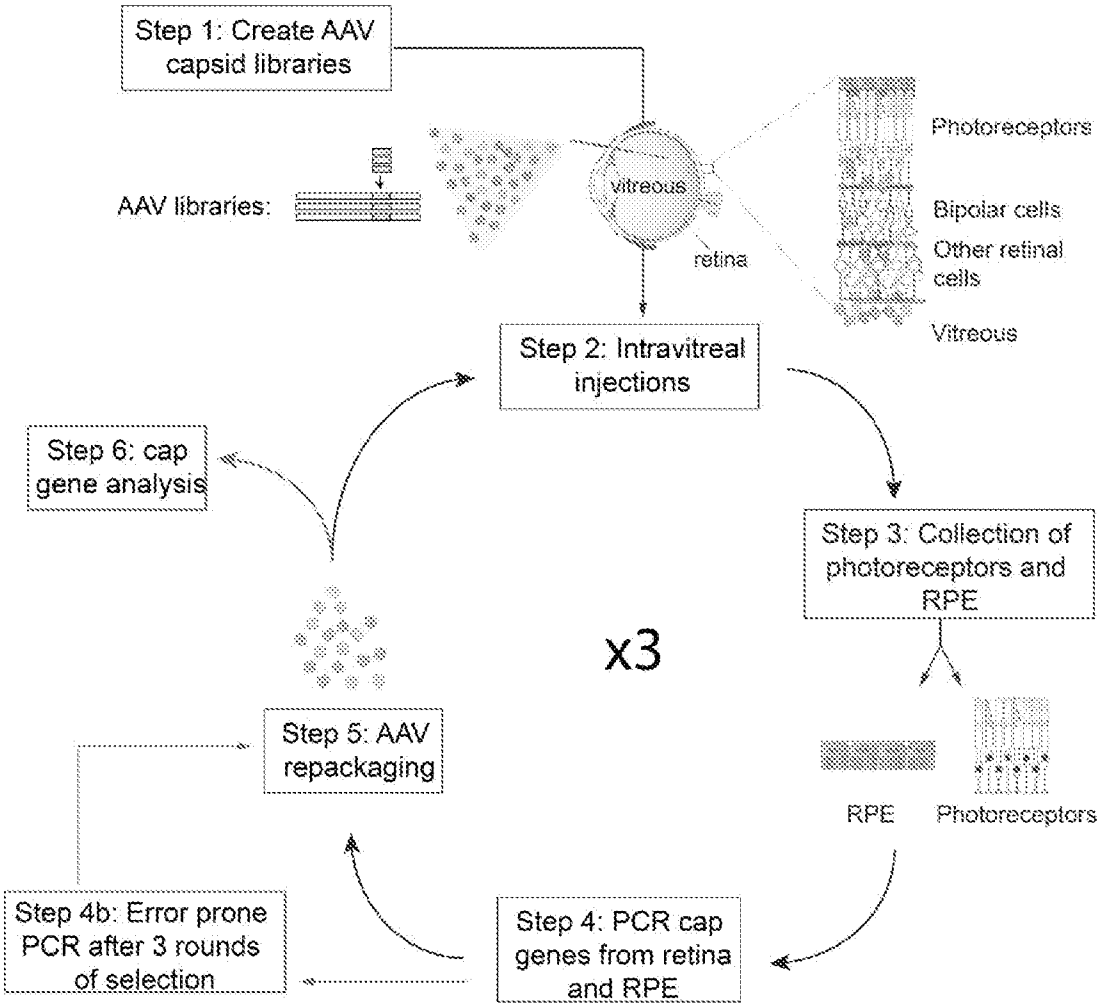

Step 1: Create AAV capsid libraries
AAV libraries:
vitreous
retina
Photoreceptors
Bipolar cells
Other retinal cells
Vitreous
Step 2: Intravitreal injections
Step 3: Collection of photoreceptors and RPE
RPE
Photoreceptors
x3
Step 4: PCR cap genes from retina and RPE
Step 4b: Error prone PCR after 3 rounds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

FIG. 2

Source library	peptide insertion	Peptide No.	SEQ ID NO:
AAV2-7mer	LALIQDSMRA	21	35
AAV2-7mer	LTHQDTTKNA		4
AAV2-7mer	LANQEHVKNA	22	2
AAV2-7mer	QAHQDTTKNA		5
AAV4-7mer	TGVMRSTNSGLN	1	6
AAV4-7mer	TGEVDLAGGGLS	2	7
AAV4-7mer	TSPYSGSSDGLS	3	8
AAV4-7mer	TGGHDSSLDGLS	4	9
AAV4-7mer	TGDGGTTMNGLS	5	98
AAV4-7mer	TGGHGSAPDGLS	6	99
AAV5-7mer	TGMHVTMMAGLN	7	100
AAV5-7mer	TGASYLDNSGLS	8	101
AAV5-7mer	TVVSTQAGIGLS	9	20
AAV5-7mer	TGVMHSQASGLS	10	21
AAV5-7mer	TGDGSPAAPGLS	11	22
AAV5-7mer	TGSDMAHGTGLS	12	23
Anc-7mer	TGLDATRDHGLSPVTGT	13	24
Anc-7mer	TGSDGTRDHGLSPVTWT	14	25
Anc-7mer	NGAVADYTRGLSPATGT	15	26
Anc-7mer	TGGDPTRGTGLSPVTGA	16	27
LoopSwap588	LQKNARPASTESVNFQ	17	28
LoopSwap588	LQRGVRIPSVLEVNGQ	18	29
LoopSwap588	LQRGNRPVTTADVNTQ	19	30
LoopSwap588	LQKADRQPGVVVVNCQ	20	31

FIG. 3A

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 lialslgltp nfksnfdlae daklqlskdt ydddldnlla qigdqyadlf laaknlsdai
 301 llsdilrvnt eitkaplsas mikrydehhq dltllkalvr 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 enediledіv ltltlfedre mieerlktya hlfddkvmkq lkrrrytgwg
 661 rlsrklingi rdkqsgktil dflksdgfan rnfmqlihdd sltfkediqk aqvsgqgdsl
 721 hehianlags paikkgilqt vkvvdelvkv mgrhkpeniv iemarenqtt qkgqknsrer
 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. 3B
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 kqllkvqkay hqldqsfidt yidlletrrt yyegpgegsp fgwkdikewy emlmghctyf
 241 peelrsvkya ynadlynaln dlnnlvitrd enekleyyek fqiienvfkq kkkptlkqia
 301 keilvneedi kgyrvtstgk peftnlkvyh dikditarke iienaelldq iakiltiygs
 361 sediqeeltn lnseltqeei eqisnlkgyt gthnlslkai nlildelwht ndnqiaifnr
 421 lklvpkkvdl sqqkeipttl vddfilspvv krsfiqsikv inaiikkygl pndiiielar
 481 eknskdaqkm inemqkrnrq tnerieeiir ttgkenakyl iekiklhdmq egkclyslea
 541 ipledllnnp fnyevdhiip rsvsfdnsfn nkvlvkqeen skkgnrtpfq ylsssdskis
 601 yetfkkhiln lakgkgrisk tkkeylleer dinrfsvqkd finrnlvdtr yatrglmnll
 661 rsyfrvnnld vkvksinggf tsflrrkwkf kkernkgykh haedalilan adfifkewkk
 721 ldkakkvmen qmfeekqaes mpeietegey keifitphqi khikdfkdyk yshrvdkkpn
 781 relindtlys trkddkgntl ivnnlnglyd kdndklkkli nkspekllmy hhdpqtyqkl
 841 klimeqygde knplykyyee tgnyltkysk kdngpvikki kyygnklnah lditddypns
 901 rnkvvklslk pyrfdvyldn gvykfvtvkn ldvikkenyy evnskcyeea kklkkisnqa
 961 efiasfynnd likingelyr vigvnndlln rievnmnidit yreylenmnd krppriikti
1021 asktqsikky stdilgnlye vkskkhpqii kkg (SEQ ID NO: 33)
```

FIG. 3C
Francisella tularensis Cpf1

```
   1 msiyqefvnk yslsktlrfe lipqgktlen ikarglildd ekrakdykka kqiidkyhqf
  61 fieeilssvc isedllqnys dvyfklkksd ddnlqkdfks akdtikkqis eyikdsekfk
 121 nlfnqnlida kkgqesdlil 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 ketlslfdd 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 nlllkekand vhilsidrge rhlayytlvd gkgniikqdt fniignderme tnyhdklaai
 961 ekdrdsarkd wkkinnikem kegylsqvvh eiaklvieyn aivvfedlnf gfkrgrfkve
1021 kqvyqklekm lieklnylvf kdnefdktgg vlrayqltap fetfkkmgkq tgiiyyvpag
1081 ftskicpvtg fvnqlypkye svsksqeffs kfdkicynld kgyfefsfdy knfgdkaakg
1141 kwtiasfgsr linfrnsdkn hnwdtrevyp tkelekllkd ysieyghgec ikaaicgesd
1201 kkffakltsv lntilqmrns ktgteldyli spvadvngnf fdsrqapknm pqdadangay
1261 higlkglmll griknnqegk klnlviknee yfefvqnrnn (SEQ ID NO: 34)
```

Figure 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 PVATEQYGSVSTNLQRGNRQAATADVNTQGVLPGMVWQDRDV 611 (SEQ ID NO: 36)
AAV-1      571 PVATERFGTVAVNFQSSSTDPATGDVHAMGALPGMVWQDRDV 612 (SEQ ID NO: 37)
AAV-5      560 RVAYNVGGQMATNNQSSTTAPATGTYNLQEIVPGSVWMERDV 601 (SEQ ID NO: 38)
AAV-6      571 PVATERFGTVAVNLQSSSTDPATGDVHVMGALPGMVWQDRDV 612 (SEQ ID NO: 39)
AAV-7      572 PVATEEYGIVSSNLQAANTAAQTQVVNNQGALPGMVWQNRDV 613 (SEQ ID NO: 40)
AAV-8      573 PVATEEYGIVADNLQQQNTAPQIGTVNSQGALPGMVWQNRDV 614 (SEQ ID NO: 41)
AAV-9      571 PVATESYGQVATNHQSAQAQAQTGWVQNQGILPGMVWQDRDV 612 (SEQ ID NO: 42)
AAV-10     573 PVATEQYGVVADNLQQANTGPIVGNVNSQGALPGMVWQNRDV 614 (SEQ ID NO: 43)
AAV-4      569 ATDTDMWGNLPGGDQSNSNLPTVDRLTALGAVPGMVWQNRDI 610 (SEQ ID NO: 44)
Ancestral  573 PVATEXYGVVAXNLQSSNTAPXTGXVNSQGALPGMVWQNRDV 613 (SEQ ID NO: 45)
```

Figure 6A

```
AAV1            --TFSYTFEEVPFHSSYAHSQSLDRLMNPLIDQYLYYLNRTQ-NQSGSAQNKDLLFSRGS 467 (SEQ ID NO: 46)
AAV6            --TFSYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYLNRTQ-NQSGSAQNKDLLFSRGS 467 (SEQ ID NO: 47)
AAV3            ---FSYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYLNRTQGTTSGTTNQSRLLFSQAG 467 (SEQ ID NO: 48)
AAV2            ---FSYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYLSRTN-TPSGTTTQSRLQFSQAG 466 (SEQ ID NO: 49)
AAV8            NFQFTYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYLSRTQTT-GGTANTQTLGFSQGG 469 (SEQ ID NO: 50)
AAV8.1          NFQFTYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYLSRTQTT-GGTANTQTLGFSQGG 469 (SEQ ID NO: 51)
AAV8 rh8         FQFSYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYLVRTQTTGTGGTQTLAFSQAGPS 469 (SEQ ID NO: 52)
AAV10           NFEFSYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYLSRTQST-GGTQGTQQLLFSQAG 469 (SEQ ID NO: 53)
AAV7            -FEFSYSFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYLARTQSNPGGTAGNRELQFYQGG 469 (SEQ ID NO: 54)
AAV9            -FQFSYEFENVPFHSSYAHSQSLDRLMNPLIDQYLYYLSKTI--NGSGQNQQTLKFSVAG 467 (SEQ ID NO: 55)
AAV9.1          -FQFSYEFENVPFHSSYAHSQSLDRLMNPLIDQYLYYLSKTI--NGSGQNQQTLKFSVAG 467 (SEQ ID NO: 56)
AAV5            NFEFTYNFEEVPFHSSFAPSQNLFKLANPLVDQYLYRFVSTN-------NTGGVQFNKNL 453 (SEQ ID NO: 57)
                   *:* **:******:* **.* :* ***:***** :  *           : *

AAV1            PAGMSVQPKNWLPGPCYRQQRVSKTKTDNNNSNFTWTGASKYNLNGRESIINPGTAMASH 527 (SEQ ID NO: 58)
AAV6            PAGMSVQPKNWLPGPCYRQQRVSKTKTDNNNSNFTWTGASKYNLNGRESIINPGTAMASH 527 (SEQ ID NO: 59)
AAV3            PQSMSLQARNWLPGPCYRQQRLSKTANDNNNSNFPWTAASKYHLNGRDSLVNPGPAMASH 527 (SEQ ID NO: 60)
AAV2            ASDIRDQSRNWLPGPCYRQQRVSKTSADNNNSEYSWTGATKYHLNGRDSLVNPGPAMASH 526 (SEQ ID NO: 61)
AAV8            PNTMANQAKNWLPGPCYRQQRVSTTTGQNNNSNFAWTAGTKYHLNGRNSLANPGIAMATH 529 (SEQ ID NO: 62)
AAV8.1          PNTMANQAKNWLPGPCYRQQRVSTTTGQNNNSNFAWTAGTKYHLNGRNSLANPGIAMATH 529 (SEQ ID NO: 63)
AAV8 rh8        S--MANQARNWVPGPCYRQQRVSTTTNQNNNSNFAWTGAAKFKLNGRDSLMNPGVAMASH 527 (SEQ ID NO: 64)
AAV10           PANMSAQAKNWLPGPCYRQQRVSTTLSQNNNSNFAWTGATKYHLNGRDSLVNPGVAMATH 529 (SEQ ID NO: 65)
AAV7            PSTMAEQAKNWLPGPCFRQQRVSKTLDQNNNSNFAWTGATKYHLNGRNSLVNPGVAMATH 529 (SEQ ID NO: 66)
AAV9            PSNMAVQGRNYIPGPSYRQQRVSTTVTQNNNSEFAWPGASSWALNGRNSLMNPGPAMASH 527 (SEQ ID NO: 67)
AAV9.1          PSNMAVQGRNYIPGPSYRQQRVSTTVTQNNNSEFAWPGASSWALNGRNSLMNPGPAMASH 527 (SEQ ID NO: 68)
AAV5            AGRYANTYKNWFPGPMGRTQGWNLGSGVNRASVSAFATTNRMELEGASYQVPPQPNGMTN 513 (SEQ ID NO: 69)
                .       :*::***  * *  .     *. *  .:.  .   *:* .    *     ::
```

Figure 6B

```
AAV1          KDDEDKFFPMSGVMIFGK--ESAGASNTALD-NVMITDEEEIKATNPVATERFGTVAVNF 584 (SEQ ID NO: 70)
AAV6          KDDKDKFFPMSGVMIFGK--ESAGASNTALD-NVMITDEEEIKATNPVATERFGTVAVNL 584 (SEQ ID NO: 71)
AAV3          KDDEEKFFPMHGNLIFGK--EGTTASNAELD-NVMITDEEEIRTTNPVATEQYGTVANNL 584 (SEQ ID NO: 72)
AAV2          KDDEEKFFPQSGVLIFGK--QGSEKTNVDIE-KVMITDEEEIRTTNPVATEQYGSVSTNL 583 (SEQ ID NO: 73)
AAV8          KDDEERFFPSNGILIFGK--QNAARDNADYS-DVMLTSEEEIKTTNPVATEYGIVADNL 586 (SEQ ID NO: 74)
AAV8.1        KDDEERFFPSNGILIFGK--QNAARDNADYS-DVMLTSEEEIKTTNPVATEEYGIVADNL 586 (SEQ ID NO: 75)
AAV8 rh8      KDDDRFFPSSGVLIFGK--QGAGNDGVDYS-QVLITDEEEIKATNPVATEEYGAVAINN 584 (SEQ ID NO: 76)
AAV10         KDDEERFFPSSGVLMFGK--QGAGRDNVDYS-SVMLTSEEEIKTTNPVATEQYGVVADNL 586 (SEQ ID NO: 77)
AAV7          KDDEDRFFPSSGVLIFGK--TGAT-NKTTLE-NVLMTNEEEIRPTNPVATEEYGIVSSNL 585 (SEQ ID NO: 78)
AAV9          KEGEDRFFPLSGSLIFGK--QGTGRDNVDAD-KVMITNEEEIKTTNPVATESYGQVATNH 584 (SEQ ID NO: 79)
AAV9.1        KEGEDRFFPLSGSLIFGK--QGTGRDNVDAD-KVMITNEEEIKTTNPVATESYGQVATNH 584 (SEQ ID NO: 80)
AAV5          NLQGSNTYALENTMIFNSQPANPGTTATYLEGNMLITSESETQPVNRVAYNVGGQMATNN 573 (SEQ ID NO: 81)
              :   .. :.  . ::*..   ..    .  . .:::*.*.* :..* ** :  * :: *

AAV1          QSSSTDPATGDVHAMGALPGMVWQDRDVYLQGPIWAKIPHTDGHFHPSPLMGGFGLKNPP 644 (SEQ ID NO: 82)
AAV6          QSSSTDPATGDVHVMGALPGMVWQDRDVYLQGPIWAKIPHTDGHFHPSPLMGGFGLKHPP 644 (SEQ ID NO: 83)
AAV3          QSSNTAPTTGTVNHQGALPGMVWQDRDVYLQGPIWAKIPHTDGHFHPSPLMGGFGLKHPP 644 (SEQ ID NO: 84)
AAV2          QRGNRQAATADVNTQGVLPGMVWQDRDVYLQGPIWAKIPHTDGHFHPSPLMGGFGLKHPP 643 (SEQ ID NO: 85)
AAV8          QQQNTAPQIGTVNSQGALPGMVWQNRDVYLQGPIWAKIPHTDGNFHPSPLMGGFGLKHPP 646 (SEQ ID NO: 86)
AAV8.1        QGQRQAAQIGTVNSQGALPGMVWQNRDVYLQGPIWAKIPHTDGNFHPSPLMGGFGLKHPP 646 (SEQ ID NO: 87)
AAV8 rh8      QAANTQAQTGLVHNQGVIPGMVWQNRDVYLQGPIWAKIPHTDGNFHPSPLMGGFGLKHPP 644 (SEQ ID NO: 88)
AAV10         QQANTGPIVGNVNSQGALPGMVWQNRDVYLQGPIWAKIPHTDGNFHPSPLMGGFGLKHPP 646 (SEQ ID NO: 89)
AAV7          QAANTAAQTQVVNNQGALPGMVWQNRDVYLQGPIWAKIPHTDGNFHPSPLMGGFGLKHPP 645 (SEQ ID NO: 90)
AAV9          QSAQAQAQTGWVQNQGILPGMVWQDRDVYLQGPIWAKIPHTDGNFHPSPLMGGFGMKHPP 644 (SEQ ID NO: 91)
AAV9.1        QSGQAQAATGWVQNQGILPGMVWQDRDVYLQGPIWAKIPHTDGNFHPSPLMGGFGMKHPP 644 (SEQ ID NO: 92)
AAV5          QSSTTAPATGTYNLQEIVPGSVWMERDVYLQGPIWAKIPETGAHFHPSPAMGGFGLKHPP 633 (SEQ ID NO: 93)
              *     .      :  :** ** :*********************.*..:*****  *****:*:**
```

Figure 6C

```
AAV1          PQILIK- 650 (SEQ ID NO: 94)
AAV6          PQILIK- 650 (SEQ ID NO: 94)
AAV3          PQIMIK- 650 (SEQ ID NO: 95)
AAV2          PQILIKN 650 (SEQ ID NO: 96)
AAV8          PQILIKN 653 (SEQ ID NO: 96)
AAV8.1        PQILIKN 653 (SEQ ID NO: 96)
AAV8 rh8      PQILIKN 651 (SEQ ID NO: 96)
AAV10         PQILIKN 653 (SEQ ID NO: 96)
AAV7          PQILIKN 652 (SEQ ID NO: 96)
AAV9          PQILIK- 650 (SEQ ID NO: 94)
AAV9.1        PQILIK- 650 (SEQ ID NO: 94)
AAV5          PMMLIKN 640 (SEQ ID NO: 97)
              * ::**
```

FIG. 7A
Retinoschisin-1
Homo sapiens

```
  1 msrkiegfll lllfgyeatl glsstedege dpwyqkackc dcqggpnalw sagatsldci
 61 pecpyhkplg fesgevtpdq itcsnpeqyv gwysswtank arlnsqgfgc awlskfqdss
121 qwlqidlkei kvisgiltqg rcdidewmtk ysvqyrtder lnwiyykdqt gnnrvfygns
181 drtstvqnll rppiisrfir liplgwhvri airmellecv skca (SEQ ID NO:10)
```

FIG. 7B
BDNF
Homo sapiens

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

FIG. 7C
RPE65
Homo sapiens

```
   1 msiqvehpag gykklfetve elsspltahv tgriplwltg sllrcgpglf evgsepfyhl
  61 fdgqallhkf dfkeghvtyh rrfirtdayv ramtekrivi tefgtcafpd pcknifsrff
 121 syfrgvevtd nalvnvypvg edyyactetn fitkinpetl etikqvdlcn yvsvngatah
 181 phiendgtvy nigncfgknf siaynivkip plqadkedpi skseivvqfp csdrfkpsyv
 241 hsfgltpnyi vfvetpvkin lfkflsswsl wganymdcfe snetmgvwlh iadkkrkkyl
 301 nnkyrtspfn lfhhintyed ngflivdlcc wkgfefvyny lylanlrenw eevkknarka
 361 pqpevrryvl plnidkadtg knlvtlpntt atailcsdet iwlepevlfs gprqafefpq
 421 inyqkycgkp ytyayglgln hfvpdrlckl nvktketwvw qepdsypsep ifvshpdale
 481 eddgvvlsvv vspgaggkpa yllilnakdl sevaraevei nipvtfhglf kks (SEQ ID NO:12)
```

FIG. 7D
Peripherin-2
Homo sapiens

```
   1 mallkvkfdq kkrvklaqgl wlmnwfsvla giiifslglf lkielrkrsd vmnnseshfv
  61 pnsligmgvl scvfnslagk icydaldpak yarwkpwlkp ylaicvlfni illflvalccf
 121 llrgslentl gqglkngmky yrdtdtpgrc fmkktidmlq iefkccgnng frdwfeiqwi
 181 snryldfssk evkdriksnv dgrylvdgvp fsccnpsspr pciqyqitnn sahysydhqt
 241 eelnlwvrgc raallsyyss lmnsmgvvtl liwlfevtit iglrylqtsl dgvsnpeese
 301 sesqgwller svpetwkafl esvkklgkgn qveaegadag qapeag (SEQ ID NO:13)
```

FIG. 7E
Peripherin
Homo sapiens

```
   1 mshhpsglra gfsstsyrrt fgpppslspg afsyssssrf sssrllgsas psssvrlgsf
  61 rspragagal lrlpserldf smaealnqef latrsnekqe lqelndrfan fiekvrfleq
 121 qnaalrgels qargqepara dqlcqqelre lrrelellgr erdrvqverd glaedlaalk
 181 qrleeetrkr edaehnlvlf rkdvddatls rlelerkies lmdeieflkk lheeelrdlq
 241 vsvesqqvqq veveatvkpe ltaalrdira qyesiaaknl qeaeewyksk yadlsdaanr
 301 nhealrqakq emnesrrqiq sltcevdglr qtneallrql 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 aeeaaplset 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 lklevtnilq khkqevellq naatisqppd rqsepathpa
 481 vlqentqiep sepknqeekk lsqvlnelqv shaettlele ktrdmlilqr kinvcyqeel
 541 eammtkadnd nrdhkekler ltrlldlknn rikqlegilr shdlptseql kdvaygtrpl
 601 slcletlpah gdedkvdisl lhqgenlfel hihqafltsa alaqagdtqp ttfctysfyd
 661 fethctplsv qpqplydfts qyvmetdslf lhylqeasar ldihqamase hstlaagwic
 721 fdrvletvek vhglatliga ggeefgvley wmrlrfpikp slqacnkrkk aqvylstdvl
 781 ggrkaqeeef rseswepqne lwieitkccg lrsrwlgtqp spyavyrfft fsdhdtaiip
 841 asnnpyfrdq arfpvlvtsd ldhylrreal sihvfddedl epgsylgrar vpllplakne
 901 sikgdfnltd paekpngsiq vqldwkfpyi ppesflkpea qtkgkdtkds skisseeeka
 961 sfpsqdqmas pevpieaggy rskrkpphgg erkekehqvv sysrrkhgkr igvqgknrme
1021 ylslnilngn tpeqvnytew kfsetnsfig dgfknqheee emtlshsalk qkeplhpvnd
1081 kesseqgsev seaqttdsdd vivppmsqky pkadsekmci eivslafype aevmsdenik
1141 qvyveykfyd lplsetetpv slrkpragee ihfhfskvid ldpqeqqgrr 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 grrvlhvdsr syyggnwasf sfsgllswlk
  61 eyqensdivs dspvwqdqil eneeaialsr kdktiqhvev fcyasqdlhe dveeagalqk
 121 nhalvtsans teaadsaflp tedeslstms cemlteqtps sdpenalevn gaevtgeken
 181 hcddktcvps tsaedmsenv piaedtteqp kknritysqi ikegrrfnid lvskllysrg
 241 llidlliksn vsryaefkni trilafregr veqvpcsrad vfnskqltmv ekrmlmkflt
 301 fcmeyekypd eykgyeeitf yeylktqklt pnlqyivmhs iamtsetass tidglkatkn
 361 flhclgrygn tpflfplygq gelpqcfcrm cavfggiycl rhsvqclvvd kesrkckaii
 421 dqfgqriise hflvedsyfp enmcsrvqyr qisravlitd rsvlktdsdq qisiltvpae
 481 epgtfavrvi elcsstmtcm kgtylvhltc tssktaredl esvvqklfvp ytemeieneq
 541 vekprilwal yfnmrdssdi srscyndlps nvyvcsgpdc glgndnavkq aetlfqeicp
 601 nedfcppppn pediildgds lqpeasessa ipeansetfk estnlgnlee sse (SEQ ID NO:16)
```

FIG. 7H
212-amino acid isoform of RdCVF

```
  1 maslfsgril irnnsdqdel 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_00431

```
  1 mgevtaeeve kfldsnigfa kqyynlhyra klisdllgak eaavdfsnyh spssmeesei
 61 ifdllrdfqe nlqtekcifn vmkklcfllq adrmslfmyr trngiaelat rlfnvhkdav
121 ledclvmpdq eivfpldmgi vghvahskki anvpnteede hfcdfvdilt eyktknilas
181 pimngkdvva iimavnkvdg shftkrdeei llkylnfanl imkvyhlsyl hncetrrgqi
241 llwsgskvfe eltdierqfh kalytvrafl ncdrysvgll dmtkqkeffd vwpvlmgevp
301 pysgprtpdg reinfykvid yilhgkedik vipnpppdhw alvsglpayv aqnglicnim
361 napaedffaf qkepldesgw miknvlsmpi vnkkeeivgv atfynrkdgk pfdemdetlm
421 esltqflgws vlnpdtyesm nklenrkdif qdivkyhvkc dneeiqkilk trevygkepw
481 eceeeelaei lqaelpdadk yeinkfhfsd lpltelelvk cgiqmyyelk vvdkfhipqe
541 alvrfmysls kgyrkityhn wrhgfnvgqt mfsllvtgkl kryftdleal amvtaafchd
601 idhrgtnnly qmksqnplak lhgssilerh hlefgktllr deslnifqnl nrrqhehaih
661 mmdiaiiatd lalyfkkrtm fqkivdqskt yeseqewtqy mmleqtrkei vmammmtacd
721 lsaitkpwev qsqvallvaa efweqgdler tvlqqnpipm mdrnkadelp klqvgfidfv
781 ctfvykefsr fheeitpmld gitnnrkewk aladeydakm kvqeekkqkq qsaksaaagn
841 qpggnpspgg attskscciq (SEQ ID NO:102)
```

FIG. 7L

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

```
  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 fytvraylnc erysvglldm tkekeffdvw svlmgesqpy
301 sgprtpdgre ivfykvidyi lhgkeeikvi ptpsadhwal asglpsyvae sgficnimna
361 sademfkfqe galddsgwli knvlsmpivn kkeeivgvat fynrkdgkpf deqdevlmes
421 ltqflgwsvm ntdtydkmnk lenrkdiaqd mvlyhvkcdr deiqlilptr arlgkepadc
481 dedelgeilk eelpgpttfd iyefhfsdle cteldlvkcg iqmyyelgvv rkfqipqevl
541 vrflfsiskg yrrityhnwr hgfnvaqtmf tllmtgklks yytdleafam vtaglchdid
601 hrgtnnlyqm ksqnplaklh gssilerhhl efgkfllsee tlniyqnlnr rqhehvihlm
661 diaiiatdla lyfkkramfq kivdesknyq dkkswveyls lettrkeivm ammmtacdls
721 aitkpwevqs kvallvaaef weqgdlertv ldqqpipmmd rnkaaelpkl qvgfidfvct
781 fvykefsrfh eeilpmfdrl qnnrkewkal adeyeakvka leekeeeerv aakkvgteic
841 nggpapksst ccil (SEQ ID NO: 103)
```

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 fytvraylnc erysvglldm tkekeffdvw svlmgesqpy
301 sgprtpdgre ivfykvidyi lhgkeeikvi ptpsadhwal asglpsyvae sgficnimna
361 sademfkfqe galddsgwli knvlsmpivn kkeeivgvat fynrkdgkpf deqdevlmes
421 ltqflgwsvm ntdtydkmnk lenrkdiaqd mvlyhvkcdr deiqlilptr arlgkepadc
481 dedelgeilk eelpgpttfd iyefhfsdle cteldlvkcg iqmyyelgvv rkfqipqevl
541 vrflfsiskg yrrityhnwr hgfnvaqtmf tllmtgklks yytdleafam vtaglchdid
601 hrgtnnlyqm ksqnplaklh gssilerhhl efgkfllsee tlniyqnlnr rqhehvihlm
661 diaiiatdla lyfkkramfq kivdesknyq dkkswveyls lettrkeivm ammmtacdls
721 aitkpwevqs kvallvaaef weqgdlertv ldqqpipmmd rnkaaelpkl qvgfidfvct
781 fvykefsrfh eeilpmfdrl qnnrkewkal adeyeakvka leekeeeerv aakkgteicn
841 ggpapksstc cil (SEQ ID NO: 104)
```

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 lasglpsyva esgficnimn asademfkfq egalddsgwl iknvlsmpiv nkkeeivgva
121 tfynrkdgkp fdeqdevlme sltqflgwsv mntdtydkmn klenrkdiaq dmvlyhvkcd
181 rdeiqlilpt rarlgkepad cdedelgeil keelpgpttf diyefhfsdl ecteldlvkc
241 giqmyyelgv vrkfqipqev lvrflfsisk gyrrityhnw rhgfnvaqtm ftllmtgklk
301 syytdleafa mvtaglchdi dhrgtnnlyq mksqnplakl hgssilerhh lefgkfllse
361 etlniyqnln rrqhehvihl mdiaiiatdl alyfkkramf qkivdeskny qdkkswveyl
421 slettrkeiv mammmtacdl saitkpwevq skvallvaae fweqgdlert vldqqpipmm
481 drnkaaelpk lqvgfidfvc tfvykefsrf heeilpmfdr lqnnrkewka ladeyeakvk
541 aleekeeeer vaakkvgtei cnggpapkss tccil (SEQ ID NO: 105)
```

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 gqgsftgqgi arlsrlifll rrwaarhvhh qdqgpdsfpd rfrgaelkev ssqesnaqan
121 vgsqepadrg rsawplakcn tntsnnteee kktkkkdaiv vdpssnlyyr wltaialpvf
181 ynwyllicra cfdelqseyl mlwlvldysa dvlyvldvlv rartgfleqg lmvsdtnrlw
241 qhyktttqfk ldvlslvptd laylkvgtny pevrfnrllk fsrlfeffdr tetrtnypnm
301 frignlvlyi liiihwnaci yfaiskfigf gtdswvypni sipehgrlsr kyiyslywst
361 ltlttigetp ppvkdeeylf vvvdflvgvl ifativgnvg smisnmnasr 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 eqlgssldtl qtrfarllae ynatqmkmkq
661 rlsqlesqvk gggdkpladg evpgdatkte dkqq (SEQ ID NO: 106)
```

FIG. 7P

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

```
  1 makintqysh psrthlkvkt sdrdlnraen glsrahssse etssvlqpgi ametrglads
 61 gqgsftgqgi arlsrlifll rrwaarhvhh qdqgpdsfpd rfrgaelkev ssqesnaqan
121 vgsqepadrg rrkktkkkda ivvdpssnly yrwltaialp vfynwyllic racfdelqse
181 ylmlwlvldy sadvlyvldv lvrartgfle qglmvsdtnr lwqhykttтq fkldvlslvp
241 tdlaylkvgt nypevrfnrl lkfsrlfeff drtetrtnyp nmfrignlvl yiliiihwna
301 ciyfaiskfi gfgtdswvyp nisipehgrl srkyiyslyw stltlttige tpppvkdeey
361 lfvvvdflvg vlifativgn vgsmisnmna sraefqakid sikqymqfrk vtkdletrvi
421 rwfdylwank ktvdekevlk slpdklkaei ainvhldtlk kvrifqdcea gllvelvlkl
481 rptvfspgdy ickkgdigke myiinegkla vvaddgvtqf vvlsdgsyfg eisilnikgs
541 ksgnrrtani rsigysdlfc lskddlmeal teypeakkal eekgrqilmk dnlideelar
601 agadpkdlee kveqlgssld tlqtrfarll aeynatqmkm kqrlsqlesq vkgggdkpla
661 dgevpgdatk tedkqq (SEQ ID NO: 107)
```

FIG. 7Q

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

```
  1 mfksltkvnk vkpigennen eqssrrneeg shpsnqsqqt taqeenkgee kslktkstpv
 61 tseephtniq dklskknssg dlttnpdpqn aaeptgtvpe qkemdpgkeg pnspqnkppa
121 apvineyada qlhnlvkrmr qrtalykkkl vegdlsspea spqtakptav ppvkesddkp
181 tehyyrllwf kvkkmpltey lkriklpnsi dsytdrlyll wlllvtlayn wnccfiplrl
241 vfpyqtadni hywliadiic diiylydmlf iqprlqfvrg gdiivdsnel rkhyrtstkf
301 qldvasiipf dicylffgfn pmfranrmlk ytsffefnhh lesimdkayi yrvirttgyl
361 lfilhinacv yywasnyegi gttrwvydge gneylrcyyw avrtlitigg lpepqtlfei
421 vfqllnffsg vfvfssligq mrdvigaata nqnyfracmd dtiaymnnys ipklvqkrvr
481 twyeytwdsq rmldesdllk tlpttvqlal aidvnfsiis kvdlfkgcdt qmiydmllrl
541 ksvlylpgdf vckkgeigke myiikhgevq vlggpdgtkv lvtlkagsvf geisllaagg
601 gnrrtanvva hgfanlltld kktlqeilvh ypdserilmk karvllkqka ktaeatpprk
661 dlallfppke etpklfktll ggtgkaslar llklkreqaa qkkensegge eegkenedkq
721 kenedkqken edkgkenedk dkgrepeekp ldrpectasp iaveeephsv rrtvlprgts
781 rqsliismap saeggeevlt ievkekakq (SEQ ID NO: 108)
```

FIG. 7R

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

```
  1 mgsgasaedk elakrskele kklqedadke aktvkllllg agesgkstiv kqmkiihqdg
 61 yspeeclefk aiiygnvlqs ilaiiramtt lgidyaepsc addgrqlnnl adsieegtmp
121 pelvevirrl wkdggvqacf eraaeyqlnd sasyylnqle ritdpeylps eqdvlrsrvk
181 ttgiietkfs vkdlnfrmfd vggqrserkk wihcfegvtc iifcaalsay dmvlveddev
241 nrmheslhlf nsicnhkffa atsivlflnk kdlfeekikk vhlsicfpey dgnnsyddag
301 nyiksqfldl nmrkdvkeiy shmtcatdtq nvkfvfdavt diiikenlkd cglf (SEQ ID NO: 109)
```

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 kikqlsagsn tsaaltedgr lfmwgdnseg qiglknvsnv
181 cvpqqvtigk pvswiscgyy hsafvttdge lyvfgepeng klglpnqllg nhrtpqlvse
241 ipekviqvac ggehtvvlte navytfglgq fgqlglgtfl fetsepkvie nirdqtisyi
301 scgenhtali tdiglmytfg dgrhgklglg lenftnhfip tlcsnflrfi vklvacggch
361 mvvfaaphrg vakeiefdei ndtclsvatf lpyssltsgn vlqrtlsarm rrrererspd
421 sfsmrrtlpp iegtlglsac flpnsvfprc sernlqesvl seqdlmqpee pdylldemtk
481 eaeidnsstv eslgettdil nmthimslns nekslklspv qkqkkqqtig eltqdtalte
541 nddsdeyeem semkegkack qhvsqgifmt qpattieafs deeveipeek egaedskgng
601 ieeqeveane envkvhggrk ekteilsddl tdkaedhefs kteelkledv deeinaenve
661 skkktvgdde svptgyhskt egaertndds saetiekkek anleeraice ynenpkgyml
721 ddadssslei lensettpsk dmkktkkifl fkrvpsinqk ivknnneplp eiksigdqii
781 lksdnkdadq nhmsqnhqni pptnterrsk sctil  (SEQ ID NO: 110)
```

FIG. 7T

RPGR – 646 amino acids
GenBank CAB54002

```
  1 mrepeelmpd sgavftfgks kfaennpgkf wfkndvpvhl scgdehsavv tgnnklymfg
 61 snnwgqlglg sksaiskptc vkalkpekvk laacgrnhtl vsteggnvya tggnnegqlg
121 lgdteerntf hvisfftseh kikqlsagsn tsaaltedgr lfmwgdnseg qiglknvsnv
181 cvpqqvtigk pvswiscgyy hsafvttdge lyvfgepeng klglpnqllg nhrtpqlvse
241 ipekviqvac ggehtvvlte navytfglgq fgqlglgtfl fetsepkvie nirdqtisyi
301 scgenhtali tdiglmytfg dgrhgklglg lenftnhfip tlcsnflrfi vklvacggch
361 mvvfaaphrg vakeiefdei ndtclsvatf lpyssltsgn vlqrtlsarm rrrererspd
421 sfsmrrtlpp iegtlglsac flpnsvfprc sernlqesvl seqdlmqpee pdylldemtk
481 eaeidnsstv eslgettdil nmthimslns nekslklspv qkqkkqqtig eltqdtalte
541 nddsdeyeem semkegkack qhvsqgifmt qpattieafs deeveipeek egaedskgng
601 ieeqeveane envkvhggrk ekteilsddl tdkaeysash sqivsv (SEQ ID NO: 111)
```

FIG. 7U

RPGR – 1152 amino acids

```
   1 mrepeelmpd sgavftfgks kfaennpgkf wfkndvpvhl scgdehsavv tgnnklymfg
  61 snnwgqlglg sksaiskptc vkalkpekvk laacgrnhtl vsteggnvya tggnnegqlg
 121 lgdteerntf hvisfftseh kikqlsagsn tsaaltedgr lfmwgdnseg qiglknvsnv
 181 cvpqqvtigk pvswiscgyy hsafvttdge lyvfgepeng klglpnqllg nhrtpqlvse
 241 ipekviqvac ggehtvvlte navytfglgq fgqlglgtfl fetsepkvie nirdqtisyi
 301 scgenhtali tdiglmytfg dgrhgklglg lenftnhfip tlcsnflrfi vklvacggch
 361 mvvfaaphrg vakeiefdei ndtclsvatf lpyssltsgn vlqrtlsarm rrrererspd
 421 sfsmrrtlpp iegtlglsac flpnsvfprc sernlqesvl seqdlmqpee pdylldemtk
 481 eaeidnsstv eslgettdil nmthimslns nekslklspv qkqkkqqtig eltqdtalte
 541 nddsdeyeem semkegkack qhvsqgifmt qpattieafs deeveipeek egaedskgng
 601 ieeqeveane envkvhggrk ekteilsddl tdkaevsegk aksvgeaedg pegrgdgtce
 661 egssgaehwq deerekgekd kgrgemerpg 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 egeeeegegk geeegeegeg egeeeegege gedgegegee eegewegeee
 961 egegegeeeg egegeegege geeeegegeg eeeegeeege eegegeeege gegeeeeege
1021 vegevegeeg egegeeeege eegeerekeg egeenrrnre eeeeeegkyq etgeeenerq
1081 dgeeykkvsk ikgsvkygkh ktyqkksvtn tqgngkeqrs kmpvqskrll kngpsgskkf
1141 wnnvlphyle lk (SEQ ID NO: 112)
```

FIG. 7V

RPGR – 1020 amino acids

```
   1 mrepeelmpd sgavftfgks kfaennpgkf wfkndvpvhl scgdehsavv tgnnklymfg
  61 snnwgqlglg sksaiskptc vkalkpekvk laacgrnhtl vsteggnvya tggnnegqlg
 121 lgdteerntf hvisfftseh kikqlsagsn tsaaltedgr lfmwgdnseg qiglknvsnv
 181 cvpqqvtigk pvswiscgyy hsafvttdge lyvfgepeng klglpnqllg nhrtpqlvse
 241 ipekviqvac ggehtvvlte navytfglgq fgqlglgtfl fetsepkvie nirdqtisyi
 301 scgenhtali tdiglmytfg dgrhgklglg lenftnhfip tlcsnflrfi vklvacggch
 361 mvvfaaphrg vakeiefdei ndtclsvatf lpyssltsgn vlqrtlsarm rrrererspd
 421 sfsmrrtlpp iegtlglsac flpnsvfprc sernlqesvl seqdlmqpee pdylldemtk
 481 eaeidnsstv eslgettdil nmthimslns nekslklspv qkqkkqqtig eltqdtalte
 541 nddsdeyeem semkegkack qhvsqgifmt qpattieafs deevgndtgq vgpqadtdge
 601 glqkevyrhe nnngvdqlda keiekesdgg hsqkeseaee idseketkla eiagmkdlre
 661 rekstkkmsp ffgnlpdrgm nteseenkdf vkkresckqd vifdseresv ekpdsymega
 721 sesqqgiadg fqqpeaiefs sgekeddeve tdqnirygrk lieqgneket kpiisksmak
 781 ydfkcdrlse ipeekegaed skgngieeqe veaneenvkv hggrkektei lsddltdkae
 841 dhefskteel kledvdeein aenveskkkt vgddesvptg yhsktegaer tnddssaeti
 901 ekkekanlee raiceynenp kgymlddads ssleilense ttpskdmkkt kkiflfkrvp
 961 sinqkivknn neplpeiksi gdqiilksdn kdadqnhmsq nhqnipptnt errsksctil (SEQ ID NO: 113)
```

FIG. 8A

AAV4 capsid
GenBank NP_044927

```
      1 mtdgylpdwl ednlsegvre wwalqpgapk pkanqqhqdn arglvlpgyk ylgpgngldk
     61 gepvnaadaa alehdkaydq qlkagdnpyl kynhadaefq qrlqgdtsfg gnlgravfqa
    121 kkrvleplgl veqagetapg kkrpliespq qpdsstgigk kgkqpakkkl vfedetgagd
    181 gppegstsga msddsemraa aggaaveggq gadgvgnasg dwhcdstwse ghvtttstrt
    241 wvlptynnhl ykrlgeslqs ntyngfstpw gyfdfnrfhc hfsprdwqrl innnwgmrpk
    301 amrvkifniq vkevttsnge ttvannltst vqifadssye lpyvmdagqe gslppfpndv
    361 fmvpqygycg lvtgntsqqq tdrnafycle yfpsqmlrtg nnfeitysfe kvpfhsmyah
    421 sqsldrlmnp lidqylwglq stttgttlna gtattnftkl rptnfsnfkk nwlpgpsikq
    481 qgfsktanqn ykipatgsds likyethstl dgrwsaltpg ppmatagpad skfsnsqlif
    541 agpkqngnta tvpgtlifts eeelaatnat dtdmwgnlpg gdqsnsnlpt vdrltalgav
    601 pgmvwqnrdi yyqgpiwaki phtdghfhps pliggfglkh pppqifiknt pvpanpattf
    661 sstpvnsfit qystgqvsvq idweiqkers krwnpevqft snygqqnsll wapdaagkyt
    721 epraigtryl thhl (SEQ ID NO: 114)
```

FIG. 8B
Ancestral AAV capsid

```
MAADGYLPDWLEDNLSEGIREWWDLKPGAPKPKANQQKQDDGRGLVLPGYKYLGPFNGLDKGEPVNAADAAALEHDKAYDQQLKAGDNPYLRYNHADA
EFQERLQEDTSFGGNLGRAVFQAKKRVLEPLGLVEEGAKTAPGKKRPVEPSPQRSPDSSTGIGKKGQQPAKKRLNFGQTGDSESVPDPQPLGEPPAGP
SGLGSGTMAAGGGAPMADNNEGADGVGNASGNWHCDSTWLGDRVITTSTRTWALPTYNNHLYKQISSXSXGXTNDNHYFGYSTPWGYFDFNRFHCHFS
PRDWQRLINNNWGFRPKRLNFKLFNIQVKEVTTNDGVTTIANNLTSTVQVFSDSEYQLPYVLGSAHQGCLPPFPADVFMIPQYGYLTLNNGSQAVGRS
SFYCLEYFPSQMLRTGNNFTFSYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYLXRTQSTGGTAGXXELLFSQXGPXXMSXQAKNWLPGPCYRQQRV
SKTLXQNNNSNFAWTGATKYHLNGRXSLVNPGVAMATHKDDEXRFFPSSGVLIFGKXGAGXNNTXLXNVMXTXEEEIKTTNPVATEXYGVVAXNLQSS
NTAPXTGXVNSQGALPGMVWQNRDVYLQGPIWAKIPHTDGNFHPSPLMGGFGLKHPPPQILIKNTPVPANPPXXFXXAKFASFITQYSTGQVSVEIEW
ELQKENSKRWNPEIQYTSNYAKSXNVDFAVXXXGVYXEPRPIGTRYLTRNL (SEQ ID NO: 115)
```

(X is any amino acid)

FIG. 9 -- Table 1

	ONL Fold increase in reads	Insert	Source library	Region
(SEQ ID NO: 29)	63.79919679	LQRGVRIPSVLEVNGQ	LS588	Central
(SEQ ID NO: 30)	7.153386879	LQKADRQPGVVVVNCQ	LS588	Peripheral
(SEQ ID NO: 24)	2.181299886	TGLDATRDHGLSPVTGT	Anc-7mer	Central
(SEQ ID NO: 26)	1.975644028	NGAVADYTRGLSPATGT	Anc-7mer	Peripheral
	1.558702536	7m8	7m8	CONTROL
(SEQ ID NO: 28)	1.500800454	LQKNARPASTESVNFQ	LS588	Central
(SEQ ID NO: 27)	1.371471857	TGGDPTRGTGLSPVTGA	Anc-7mer	Peripheral
	1.181900886	k916	k916	CONTROL
	1.180138343	AAV24YF+	AAV24YF+	CONTROL
	1.096454525	AAV2	AAV2	CONTROL
(SEQ ID NO: 25)	1.040515096	TGSDGTRDHGLSPVTWT	Anc-7mer	Central
(SEQ ID NO: 30)	0.915832658	LQRGNRPVTTADVNTQ	LS588	Peripheral
(SEQ ID NO: 5)	0.821793827	QAHQDTTKNA	AAV2-7mer	Peripheral
	0.565046307	k912	k912	CONTROL
(SEQ ID NO: 21)	0.562635287	TGVMHSQASGLS	AAV5-7mer	Peripheral
	0.500833298	k91	k91	CONTROL
(SEQ ID NO: 35)	0.387792793	LALIQDSMRA	AAV2-7mer	Central
(SEQ ID NO: 20)	0.377253299	TVVSTQAGIGLS	AAV5-7mer	Peripheral
(SEQ ID NO: 23)	0.346854635	TGSDMAHGTGLS	AAV5-7mer most abundant	CONTROL
(SEQ ID NO: 22)	0.34669906	TGDGSPAAPGLS	RPE-AAV5-7mer	Central
(SEQ ID NO: 100)	0.324308359	TGMHVTMMAGLN	AAV5-7mer	Central
(SEQ ID NO: 2)	0.298540099	LANQEHVKNA	AAV2-7mer	Peripheral
	0.258738252	AAV5	AAV5	CONTROL
(SEQ ID NO: 4)	0.238979892	LTHQDTTKNA	AAV2-7mer	Central
(SEQ ID NO: 99)	0.161482878	TGGHGSAPDGLS	RPE-AAV4-7mer	Central
(SEQ ID NO: 9)	0.141133263	TGGHDSSLDGLS	AAV4-7mer	Peripheral
(SEQ ID NO: 98)	0.136923607	TGDGGTTMNGLS	AAV4-7mer most abundant	CONTROL
(SEQ ID NO: 8)	0.128082381	TSPYSGSSDGLS	AAV4-7mer	Peripheral
(SEQ ID NO: 6)	0.090871196	TGVMRSTNSGLN	AAV4-7mer	Central
	0.057446852	AAV4	AAV4	CONTROL

FIG. 10 – Table 2

RPE

	Fold increase in reads	Insert	Source library	Region
(SEQ ID NO: 29)	33.65598086	LQRGVRIPSVLEVNGQ	LS588	Central
(SEQ ID NO: 35)	4.627963274	LALIQDSMRA	AAV2-7mer	Central
(SEQ ID NO: 4)	4.155171929	LTHQDTTKNA	AAV2-7mer	Central
(SEQ ID NO: 5)	3.418111986	QAHQDTTKNA	AAV2-7mer	Peripheral
	3.307311067	k91	k91	CONTROL
(SEQ ID NO: 2)	2.250383296	LANQEHVKNA	AAV2-7mer	Peripheral
(SEQ ID NO: 26)	1.553340346	NGAVADYTRGLSPATGT	Anc-7mer	Peripheral
(SEQ ID NO: 24)	1.039956858	TGLDATRDHGLSPVTGT	Anc-7mer	Central
(SEQ ID NO: 31)	0.98426325	LQKADRQPGVVVVNCQ	LS588	Peripheral
(SEQ ID NO: 30)	0.691860699	LQRGNRPVTTADVNTQ	LS588	Peripheral
	0.584426815	k916	k916	CONTROL
	0.569675877	AAV24YF+	AAV24YF+	CONTROL
	0.563819035	AAV2	AAV2	CONTROL
(SEQ ID NO: 28)	0.515236441	LQKNARPASTESVNFQ	LS588	Central
(SEQ ID NO: 27)	0.475479014	TGGDPTRGTGLSPVTGA	Anc-7mer	Peripheral
(SEQ ID NO: 25)	0.474443207	TGSDGTRDHGLSPVTWT	Anc-7mer	Central
(SEQ ID NO: 21)	0.405199224	TGVMHSQASGLS	AAV5-7mer	Peripheral
(SEQ ID NO: 9)	0.337284091	TGGHDSSLDGLS	AAV4-7mer	Peripheral
(SEQ ID NO: 99)	0.334179068	TGGHGSAPDGLS	RPE-AAV4-7mer	Central
(SEQ ID NO: 8)	0.292104518	TSPYSGSSDGLS	AAV4-7mer	Peripheral
	0.25410362	AAV5	AAV5	CONTROL
(SEQ ID NO: 98)	0.208508888	TGDGGTTMNGLS	AAV4-7mer most abundant	CONTROL
	0.195373303	7m8	7m8	CONTROL
	0.175139543	k912	k912	CONTROL
	0.171857536	AAV4	AAV4	CONTROL
(SEQ ID NO: 23)	0.157923226	TGSDMAHGTGLS	AAV5-7mer most abundant	CONTROL
(SEQ ID NO: 20)	0.115992687	TVVSTQAGIGLS	AAV5-7mer	Peripheral
(SEQ ID NO: 6)	0.115792655	TGVMRSTNSGLN	AAV4-7mer	Central
(SEQ ID NO: 22)	0.046990066	TGDGSPAAPGLS	RPE-AAV5-7mer	Central
(SEQ ID NO: 100)	0.035004376	TGMHVTMMAGLN	AAV5-7mer	Central

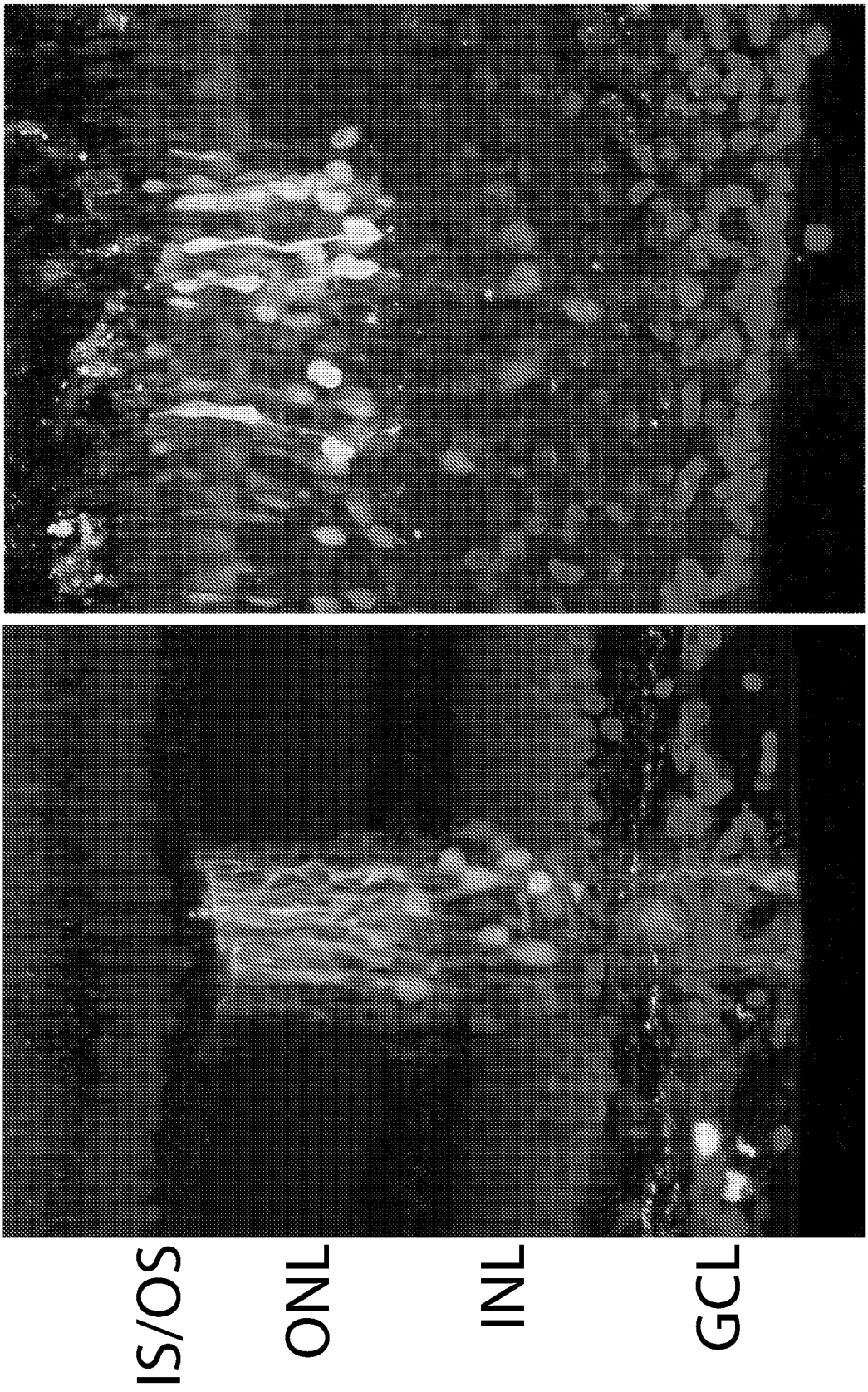


FIG. 11

FIG. 12A

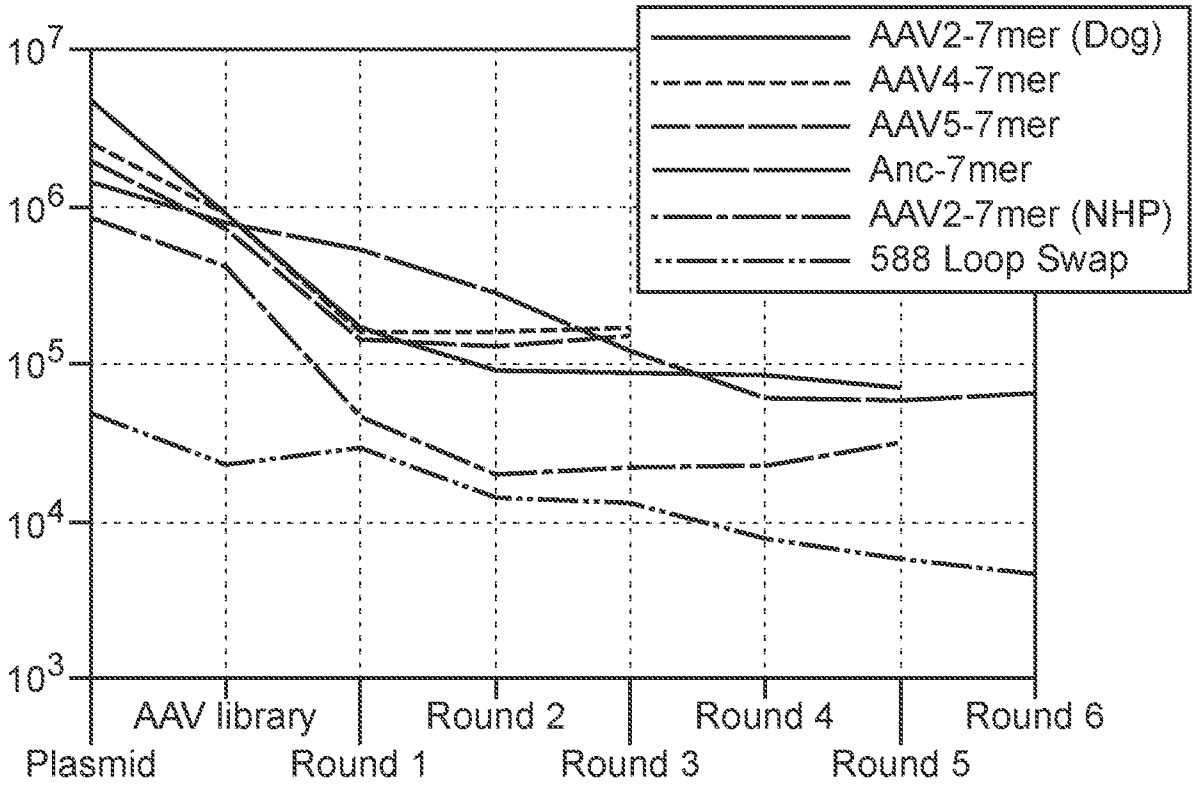


FIG. 12B

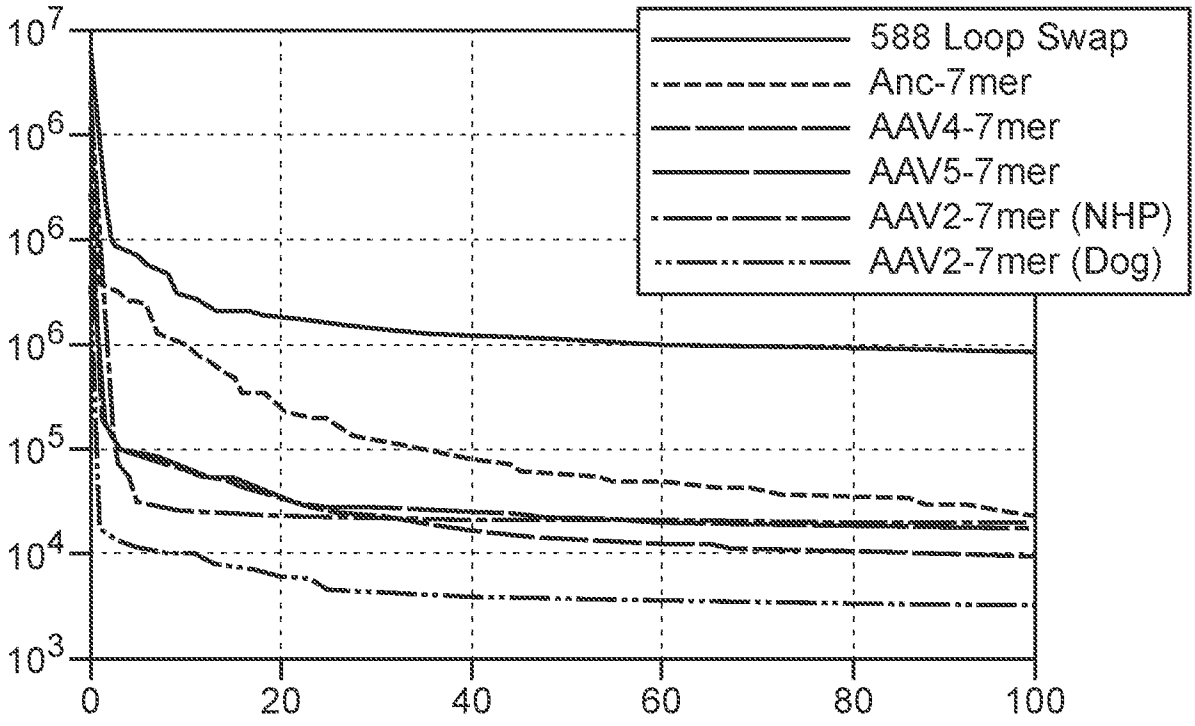
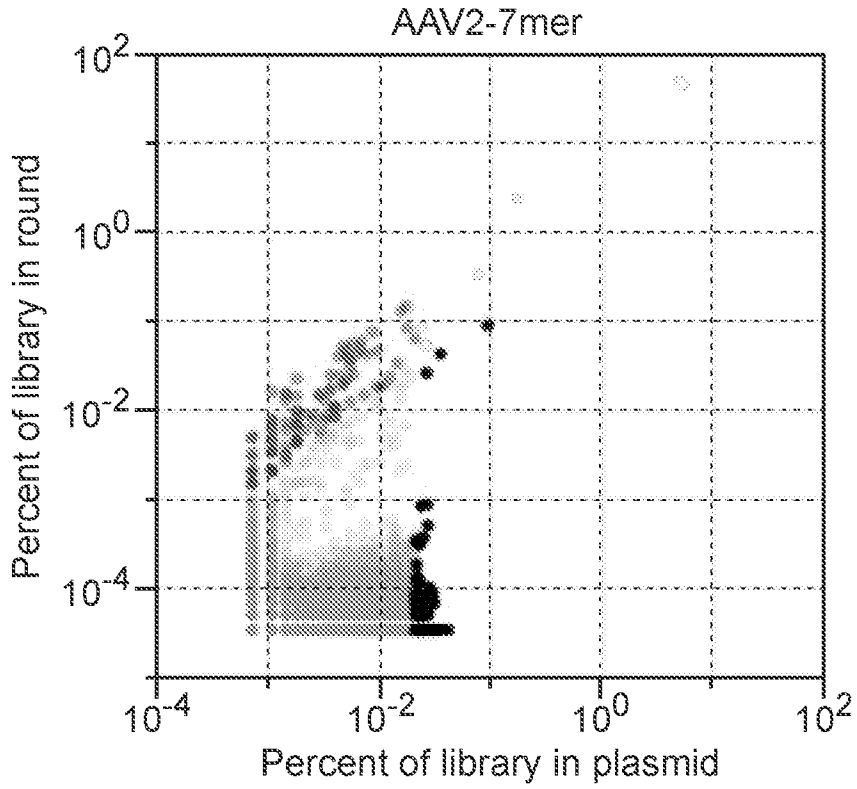


FIG. 12C


AAV2-7mer
Percent of library in round
10^{2}
10^{0}
10^{-2}
10^{-4}
10^{-4}
10^{-2}
10^{0}
10^{2}
Percent of library in plasmid

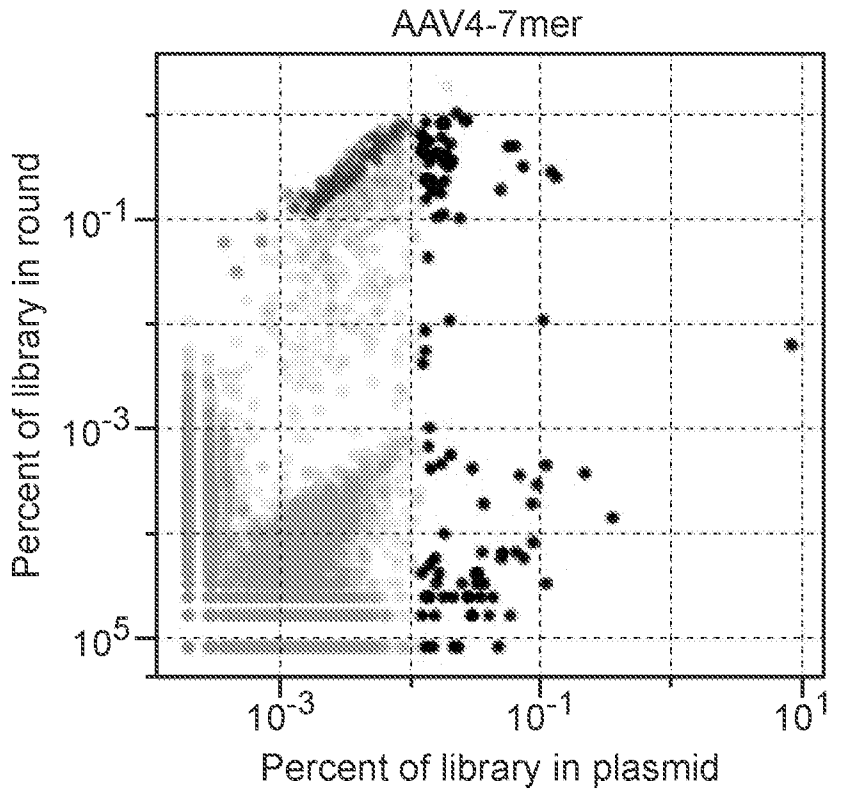
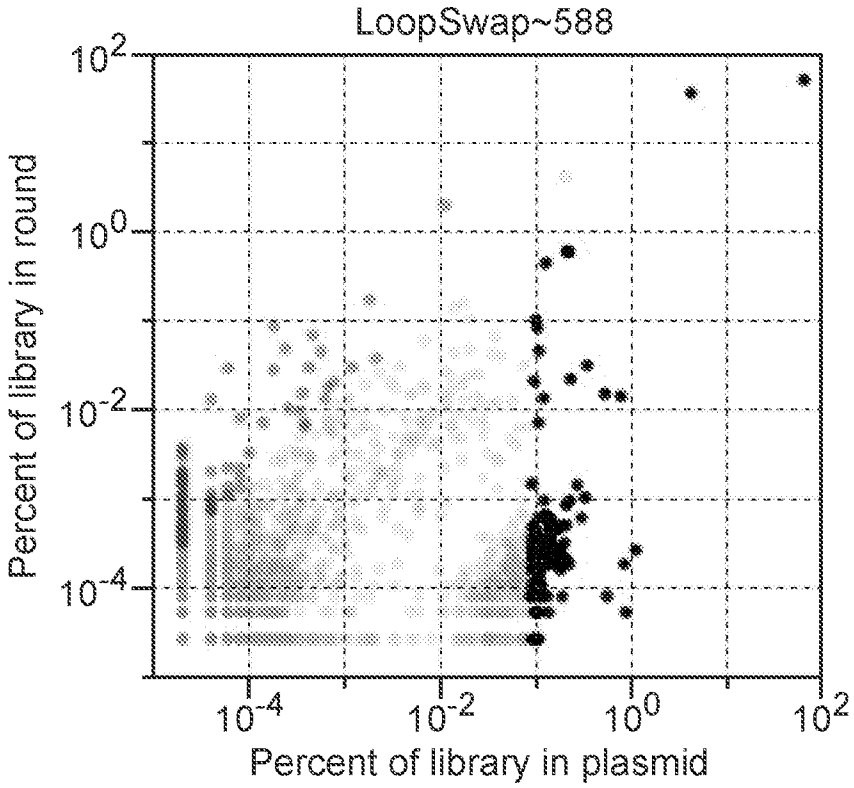

AAV4-7mer
Percent of library in round
10^{-1}
10^{-3}
10^{5}
10^{-3}
10^{-1}
10^{1}
Percent of library in plasmid

FIG. 12C (Cont.)


LoopSwap~588
Percent of library in round
Percent of library in plasmid
10^{2}
10^{0}
10^{-2}
10^{-4}

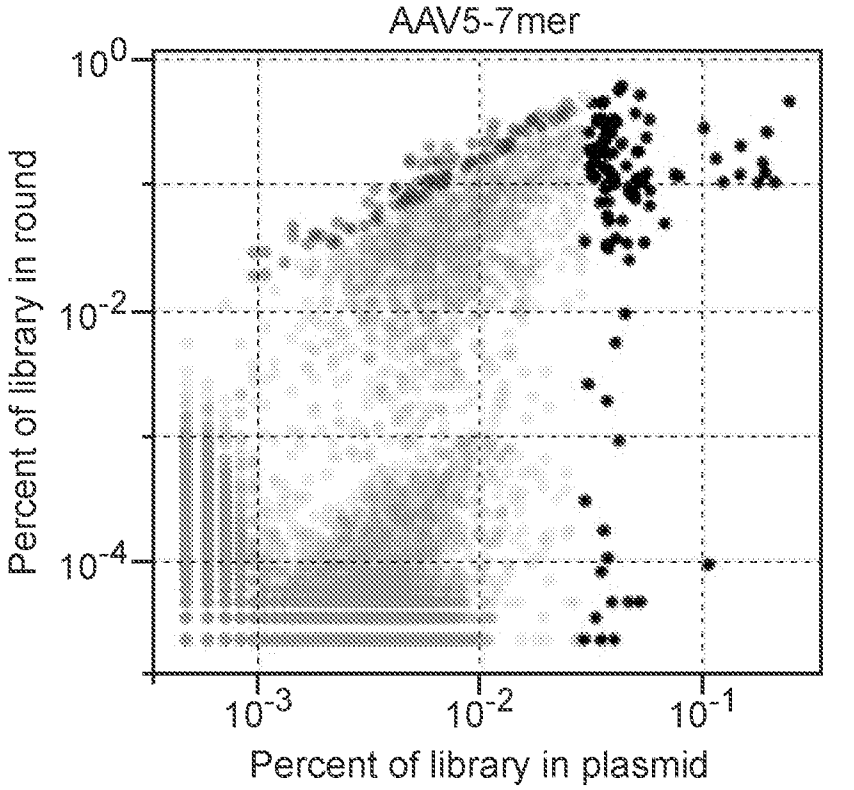

AAV5-7mer
Percent of library in round
Percent of library in plasmid
10^{0}
10^{-2}
10^{-4}
10^{-3}
10^{-2}
10^{-1}

FIG. 12C (Cont.)

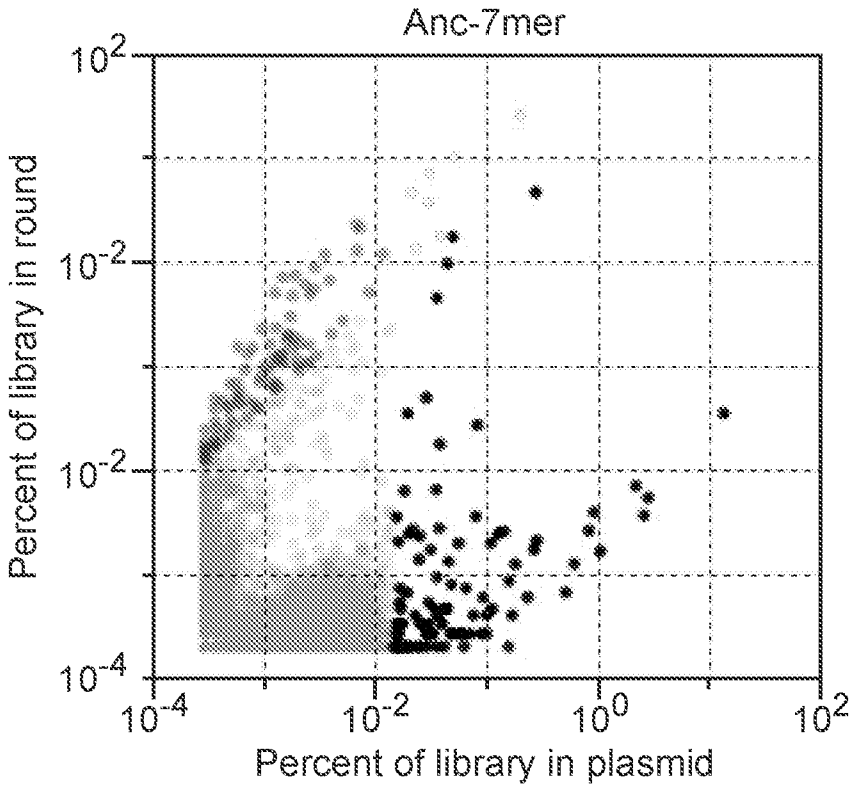


FIG. 12D

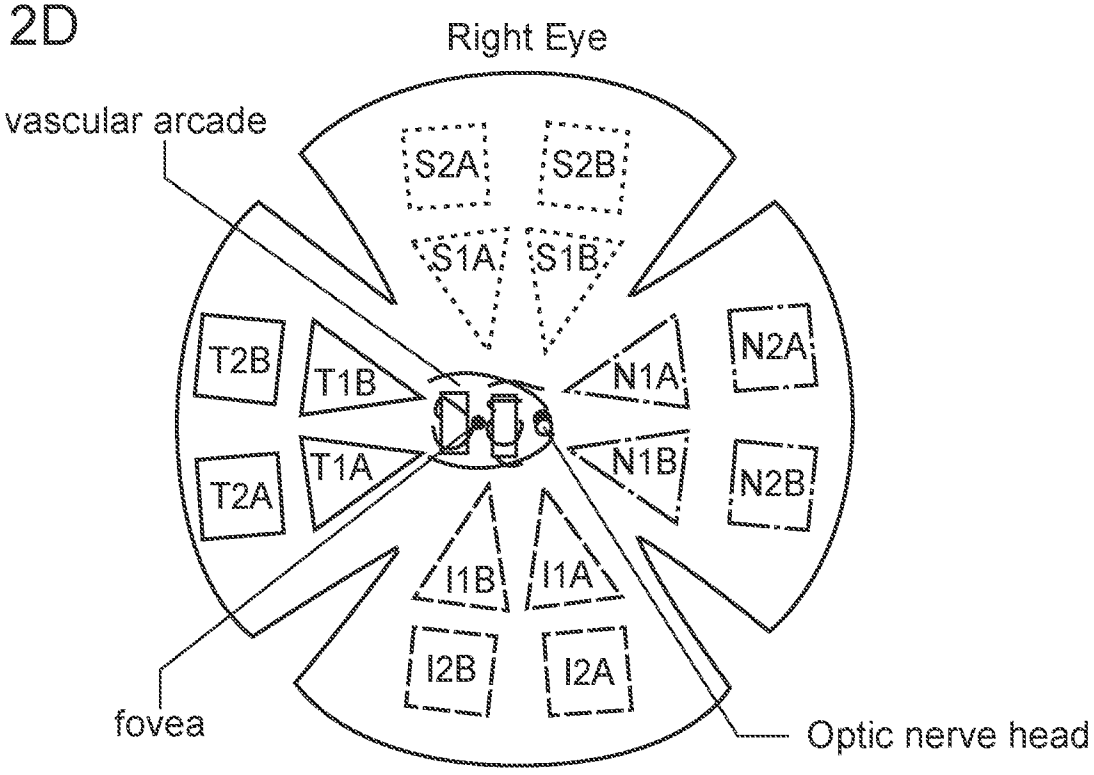


FIG. 12E

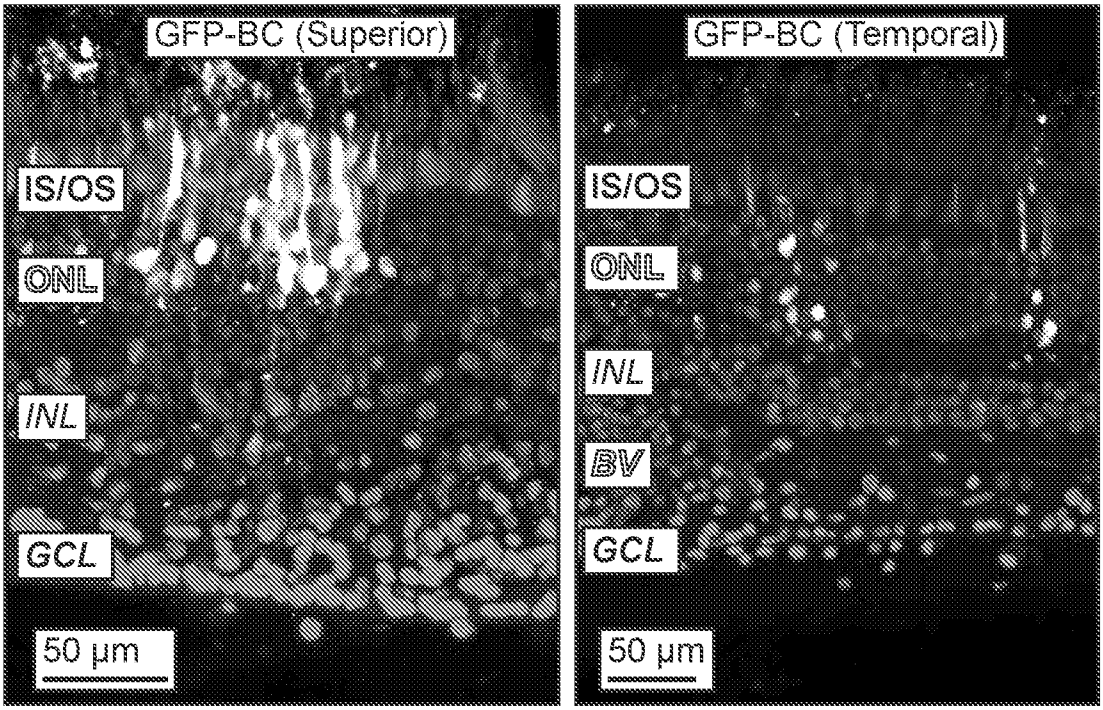


FIG. 12F

	NHP outer retina
(33.66)	LQRGVRIPSVLEVNGQ
(4.63)	LALIQDSMRA
(4.16)	LTHQDTTKNA
(3.42)	QAHQDTTKNA
(3.31)	LAHQDTTKNA
(2.25)	LANQEHVKNA
(1.55)	NGAVADYTRGLSPATGT
(1.04)	TGLDATRDHGLSPVTGT
(0.98)	LQKADRQPGVVVVNCQ
(0.69)	LQRGNRPVTTADVNTQ
(0.58)	PAPQDTTKKA
(0.57)	AAV24YF+
(0.56)	AAV2 control
(0.52)	LQKNARPASTESVNFQ
(0.48)	TGGDPTRGTGLSPVTGA
(0.47)	TGSDGTRDHGLSPVTWT
(0.41)	TGVMHSQASGLS
(0.34)	TGGHDSSLDGLS
(0.25)	AAV5 control
(0.20)	LALGETTRPA
(0.18)	LAPDSTTRSA
(0.17)	AAV4 control
(0.12)	TVVSTQAGIGLS

FIG. 13A

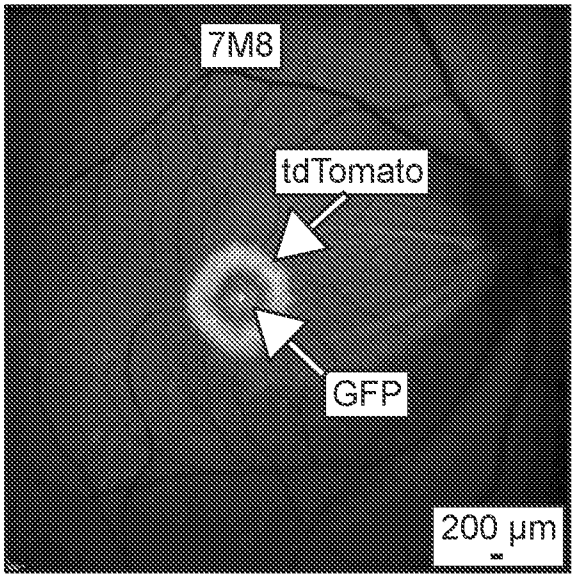


FIG. 13B

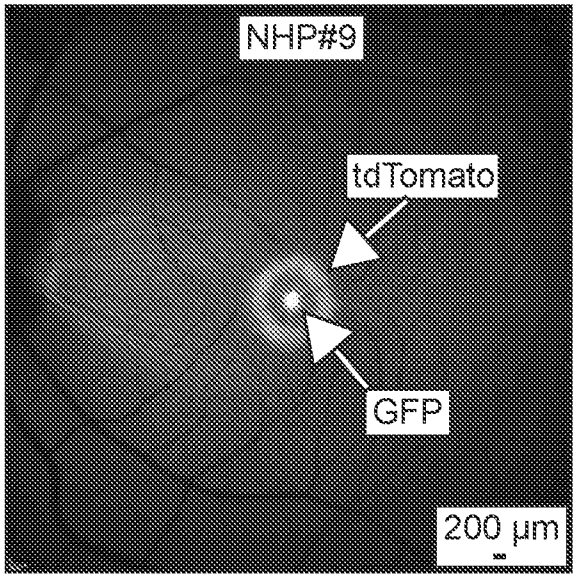


FIG. 13C

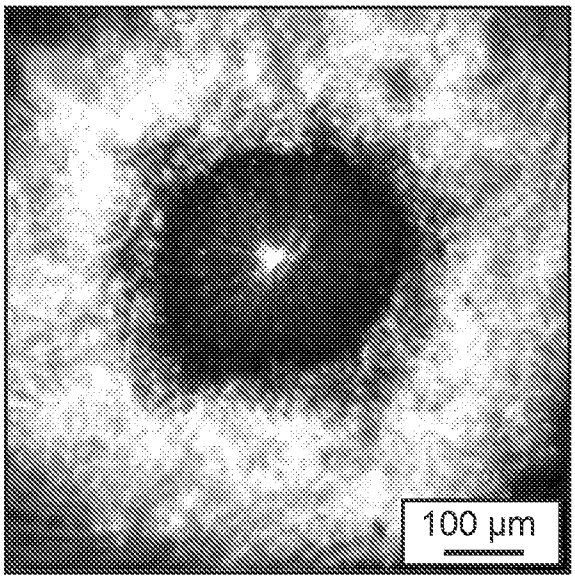


FIG. 13D

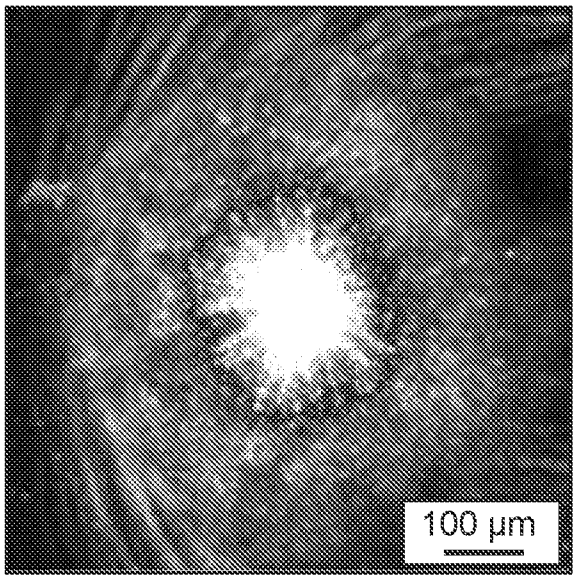


FIG. 13E

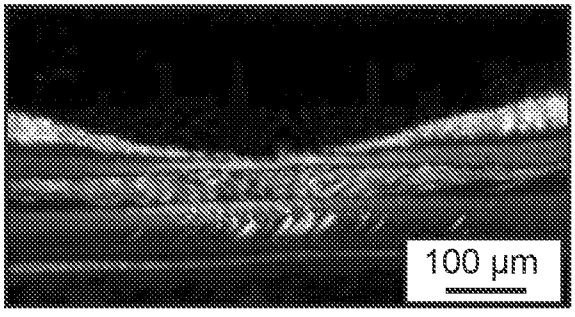


FIG. 13F

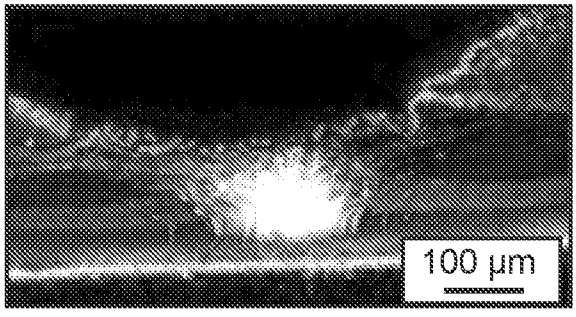


FIG. 13G

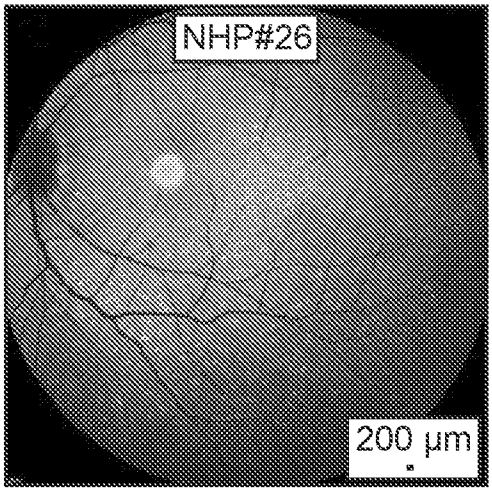


FIG. 13H

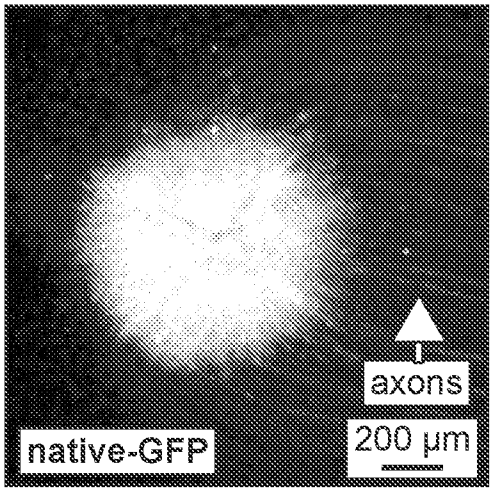


FIG. 13I

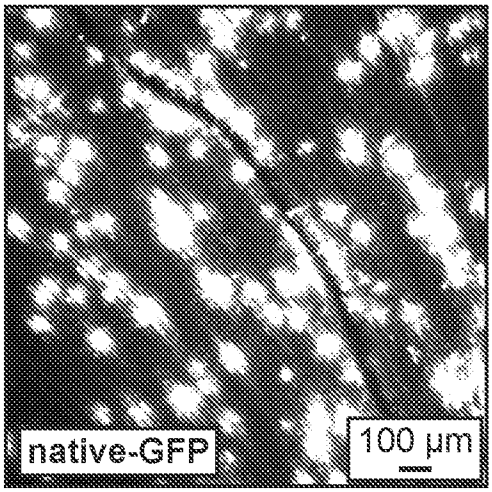


FIG. 13J

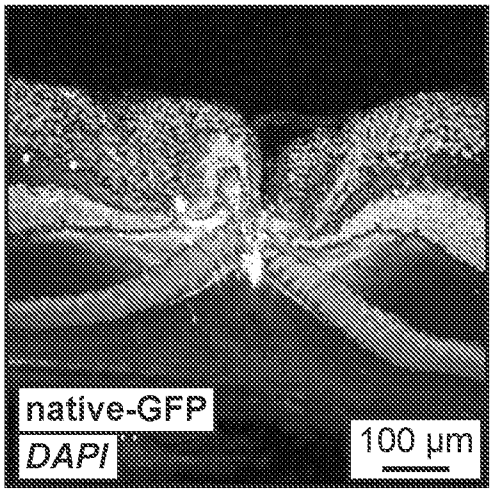


FIG. 13K

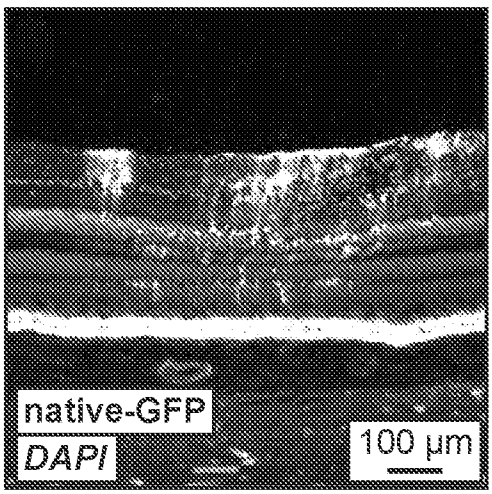


FIG. 13L

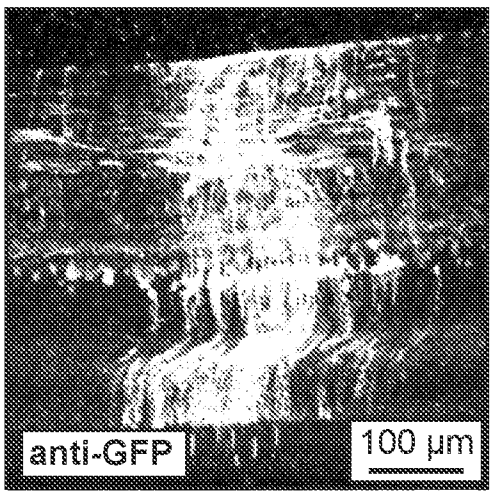


FIG. 13M

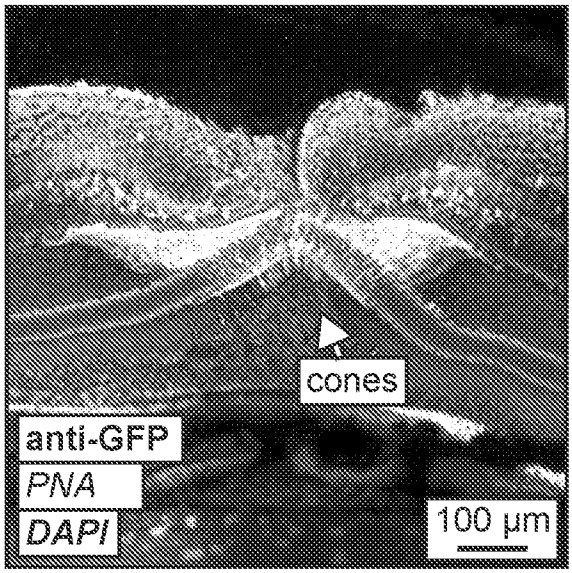


FIG. 13N

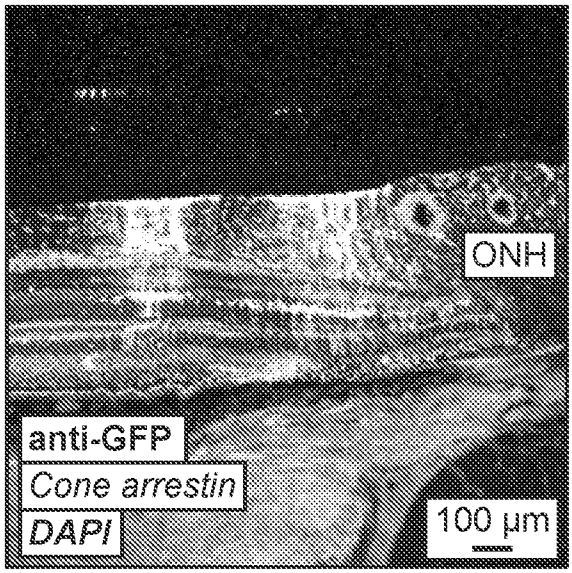


FIG. 13O

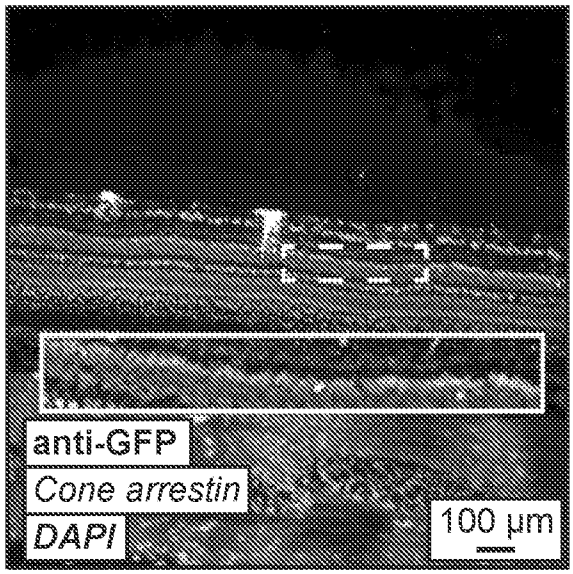


FIG. 13P

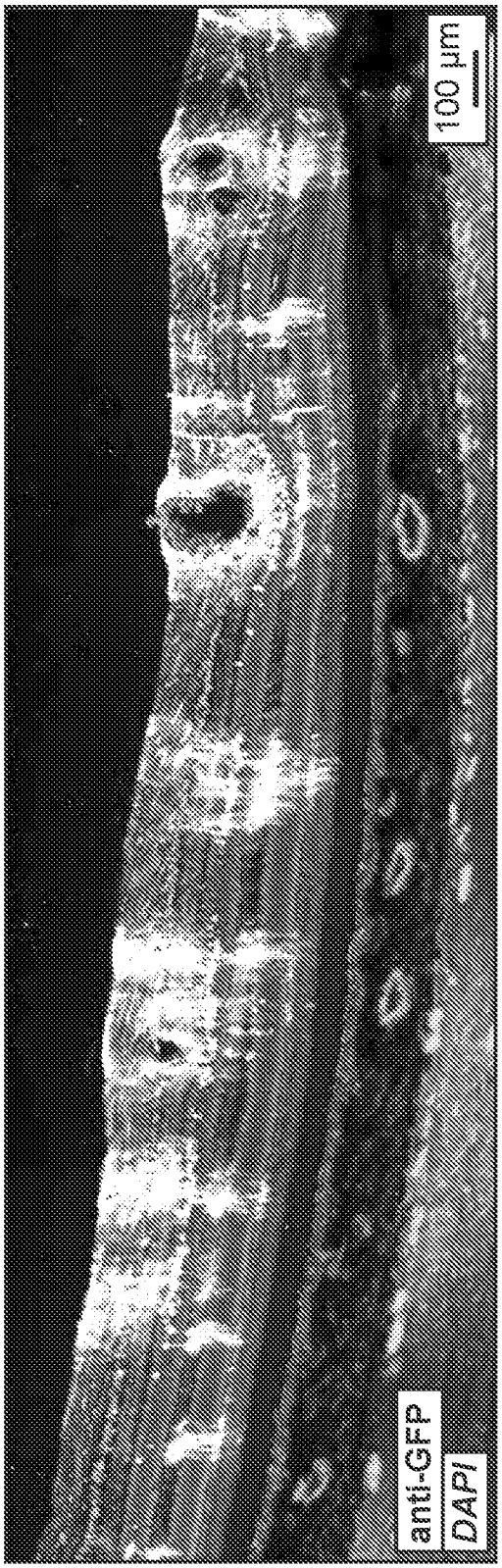
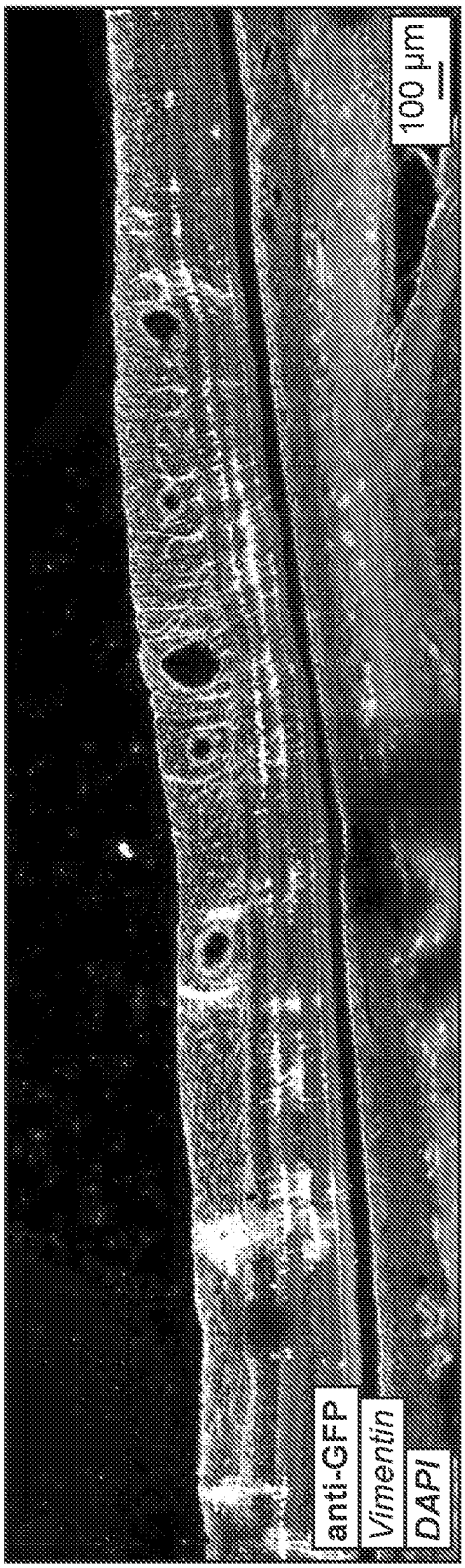


FIG. 13Q



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2018/040115

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

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. ☒ forming part of the international application as filed:

      ☒ in the form of an Annex C/ST.25 text file.

      ☐ on paper or in the form of an image file.

   b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. ☐ furnished subsequent to the international filing date for the purposes of international search only:

      ☐ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

      ☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

SEQ ID NOs: 2, 6-9, 20-31, 35, and 98-101 were searched.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2018/040115

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 13-34, 43-63
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
See extra sheet(s).

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-12 and 35-38 to the extent that they read on a heterologous peptide of SEQ ID NO:35.

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2018/040115

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61K 48/00; A61P 27/00; A61P 27/02; C07K 14/075; C12N 15/62; C12N 15/63 (2018.01)

CPC - A61K 48/0075; C07K 14/005; C07K 2319/00; C12N 15/86; C12N 2320/32; C12N 2750/14122; C12N 2750/14143; C12N 2750/14145 (2018.08)

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History document

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

USPC - 424/93.1; 424/93.21; 424/233.1; 435/320.1 (keyword delimited)

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

See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 2014/0364338 A1 (THE REGENTS OF THE UNIVERSITY OF CALIFORNIA) 11 December 2014 (11.12.2014) entire document	1-12, 35-38
A	DAY et al. "Advances in AAV Vector Development for Gene Therapy in the Retina," Retinal Degenerative Diseases: Advances in Experimental Medicine and Biology, 25 March 2014 (25.03.2014), Vol. 801. Pgs. 687-693. entire document	1-12, 35-38
A	WO 2016/141078 A1 (AVALANCHE BIOTECHNOLOGIES, INC. et al) 09 September 2016 (09.09.2016) entire document	1-12, 35-38
A	US 2017/0096683 A1 (GENZYME CORPORATION) 06 April 2017 (06.04.2017) entire document	1-12, 35-38
A	US 2015/0152142 A1 (THE UNIVERSITY OF NORTH CAROLINA AT CHAPEL HILL) 04 June 2015 (04.06.2015) entire document	1-12, 35-38
A	US 2015/0315610 A1 (TAKARA BIO INC.) 05 November 2015 (05.11.2015) entire document	1-12, 35-38
P, A	SULLIVAN et al. "Rationally designed AAV2 and AAVrh8R capsids provide improved transduction in the retina and brain," Gene Therapy, 22 May 2018 (22.05.2018), Vol. 25, Pgs. 205-219. entire document	1-12, 35-38
P, A	WO 2017/197355 A2 (4D MOLECULAR THERAPEUTICS INC.) 16 November 2017 (16.11.2017) entire document	1-12, 35-38

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

* Special categories of cited documents:

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

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

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
23 October 2018	19 NOV 2018
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, VA 22313-1450 Facsimile No. 571-273-8300	Authorized officer Blaine R. Copenheaver PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2018/040115

Continued from Box No. III Observations where unity of invention is lacking

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees need to be paid.

Group I+: claims 1-12 and 35-42 are drawn to variant AAV capsid proteins comprising heterologous peptides of any one of Formulas I-VI, and nucleic acids encoding the same.

The first invention of Group I+ is restricted to a variant AAV capsid protein, and nucleic acids encoding the same, wherein the variant AAV capsid protein comprise a heterologous peptide of Formula I, wherein the peptide of Formula I is selected to be SEQ ID NO:35. It is believed that claims 1-12 and 35-38 read on this first named invention and thus these claims will be searched without fee to the extent that they read on SEQ ID NO: 35.

Applicant is invited to elect additional variant AAV capsid proteins comprising heterologous peptides of any one of Formulas I-VI, each with specified SEQ ID NO, to be searched in a specific combination by paying an additional fee for each set of election. An exemplary election would be a variant AAV capsid protein, and nucleic acids encoding the same, wherein the variant AAV capsid protein comprise a heterologous peptide of Formula II, wherein the peptide of Formula II is selected to be SEQ ID NO:6. Additional variant AAV capsid proteins comprising heterologous peptides of any one of Formulas I-VI will be searched upon the payment of additional fees. Applicants must specify the claims that read on any additional elected inventions. Applicants must further indicate, if applicable, the claims which read on the first named invention if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched/examined.

The inventions listed in Groups I+ do not relate to a single general inventive concept under PCT Rule 13.1, because under PCT Rule 13.2 they lack the same or corresponding special technical features for the following reasons:

The Groups I+ formulas do not share a significant structural element responsible for increased retinal cell infectivity, requiring the selection of alternatives for the amino acid sequence of the heterologous peptides, where "the insertion is a peptide of any one of Formulas I-VI".

Additionally, even if Groups I+ were considered to share the technical features of 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 of any one of Formulas I-VI, 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; an isolated nucleic acid comprising a nucleotide sequence that encodes a variant adenoassociated 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-VI; 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-VI; 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 of Formula VI, 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; these shared technical features do not represent a contribution over the prior art.

Specifically, US 2014/0364338 A1 to The Regents of the University of California discloses a recombinant adeno-associated virus (rAAV) virion (The present disclosure provides adeno-associated virus (AAV) virions with altered capsid protein, Abstract) comprising: a) a variant AAV capsid protein (The present disclosure provides adeno-associated virus (AAV) virions with altered capsid protein, Para. [0006]), wherein the variant AAV capsid protein comprises an insertion of a heterologous peptide (where the variant AAV capsid protein comprises an insertion of from about 5 amino acids to about 11 amino acids in an insertion site in the capsid protein GH loop, Para. [0068]) 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 (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, Para. [0068]); and b) a heterologous nucleic acid comprising a nucleotide sequence encoding a heterologous gene product (a heterologous nucleic acid comprising a nucleotide sequence encoding a gene product, Para. [0091]); an isolated nucleic acid comprising a nucleotide sequence that encodes a variant adenoassociated virus (AAV) capsid protein (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, Para. [0159]), 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 (where the variant AAV capsid protein comprises an insertion of from about 5 amino acids to about 11 amino acids in the GH loop or loop IV relative to a corresponding parental AAV capsid protein, Para. [0159]), 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 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, Para. [0159]), and wherein the amino acid insertion is in the GH loop of a native AAV capsid (where the variant AAV capsid protein comprises an insertion of from about 5 amino acids to about 11 amino acids in the GH loop or loop IV relative to a corresponding parental AAV capsid protein, Para. [0159]), a variant adeno-associated virus (AAV) capsid protein (The present disclosure provides adeno-associated virus (AAV) virions with altered capsid protein, Abstract), 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 (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, Para. [0159]; where the variant AAV capsid protein comprises an insertion of from about 5 amino acids to about 11 amino acids in the GH loop or loop IV relative to a corresponding parental AAV capsid protein, Para. [0159]), a recombinant adeno-associated virus (rAAV) virion (The present disclosure provides a recombinant adeno-associated virus (rAAV) virion, Para. [0091]) comprising: a) a variant AAV capsid protein, wherein the variant AAV capsid protein comprises an insertion of a heterologous peptide, 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 (The present disclosure provides a variant AAV capsid protein, where the variant AAV capsid protein comprises an insertion of from about 5 amino acids to about 11 amino acids in an insertion site in the capsid protein GH loop or loop IV, relative to a corresponding parental AAV capsid, Para. [0068]); and b) a heterologous nucleic acid comprising a nucleotide sequence encoding a heterologous gene product (heterologous nucleic acid comprising a nucleotide sequence encoding a gene product. In some cases, the retinal cell is a photoreceptor cell (e.g., rods and/or cones), Para. [0091]).

The inventions listed in Groups I+ therefore lack unity under Rule 13 because they do not share a same or corresponding special technical features.