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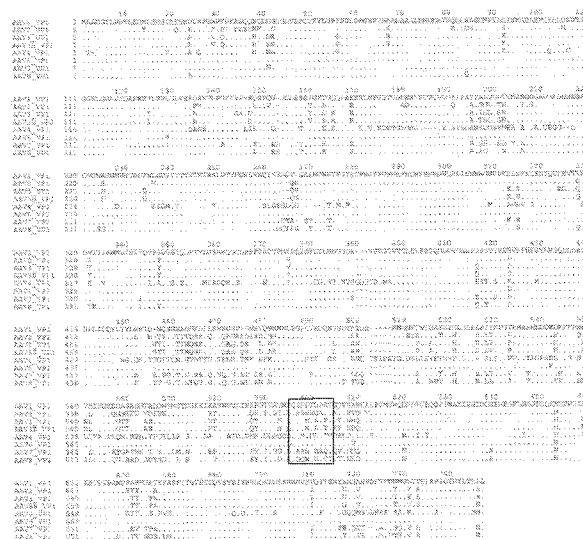
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FIGURE 1



(57) Abstract: Described herein are capsid proteins and adeno-associated viruses capable of targeting various types of ocular cells including bipolar and horizontal cells. Also described herein are methods of treating various ocular disorders in a subject in need thereof by administering to the subject an effective concentration of a composition comprising the recombinant adeno-associated virus (AAV) of the invention.

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ENHANCED AAV-MEDIATED GENE TRANSFER FOR RETINAL THERAPIES

BACKGROUND OF THE INVENTION

Recombinant Adeno-Associated Virus (rAAV) vectors have been instrumental in achieving restoration of vision in humans with Leber's congenital amaurosis due to RPE65 mutations and in a number of animal models (reviewed by Stieger, K. et al, Adeno-associated virus mediated gene therapy for retinal degenerative diseases. *Methods Mol Biol* 807, 179-218 (2011) which is incorporated by reference herein). The first retinal gene therapy clinical trials for retinal degenerative disease were initiated in humans in 2007 using AAV2 to deliver test the safety of therapeutic gene expression in children and adults with an early form of blindness called Leber's Congenital Amaurosis. Now, five years later, there are 7 Phase I/II gene therapy trials in progress involving inherited blindness. The first Phase III clinical trial initiated enrollment in the fourth quarter of 2012 (<http://clinicaltrials.gov>) and the results to date have been excellent. More than 125 individuals have already participated in retinal gene AAV-mediated gene therapy clinical trials and the safety records in these studies have been excellent.

Given the excellent safety and efficacy data relating to AAV-mediated retinal gene therapy and given the large number of inherited and acquired diseases that lead to blindness, there are many additional targets and strategies that are under consideration for application to the retina. One challenge, however, is to identify vectors which can efficiently and stably transduce a large variety of retinal cell types. Many of the rAAVs that have been characterized to date target retinal pigment epithelium (RPE) cells efficiently, and several of them target rod photoreceptors efficiently. One (AAV2) targets ganglion cells, a few target Muller cells (AAV2, AAV5, AAV8), and another (AAV9) targets cone photoreceptors. However, few AAVs have been found that target a majority of ocular cells with high efficiency.

In a normal eye, photoreceptors form the outermost layer of the retina. They convert light into electrical signals, which are sent to neurons in the retina's middle layer known as bipolar cells. Bipolar cells send visual information to the inner layer, made up of ganglion cells, which then connect to the brain via the optic nerve. In optogenetic therapy, artificial photoreceptors are constructed by gene delivery of light-activated channels or pumps to surviving cell types in the remaining retinal circuit. Because the bipolar cells are involved in processing visual signals, they have become the focus of attention from those interested in optogenetic therapies for the

retina. However, so far, no recombinant virus has been identified which transduces bipolar cells of the retina.

Horizontal cells present an interesting target. Although, to date, no horizontal cell-specific genes are known that contribute to retinal disease, 40 genes are still unidentified from the 232 loci that are currently linked to retinal disease, and disease genes may yet be discovered in horizontal cells (<https://sph.uth.edu/retnet/sum-dis.htm>). Further, horizontal cells adjust photoreceptor output through feedback signals and are crucial to vision perception. More information is needed about horizontal cells to fully understand visual perception. Thus, the ability to express desired transgenes in these, and other ocular cell types, is needed.

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SUMMARY OF THE INVENTION

In one aspect, a recombinant AAV capsid protein is provided. The AAV capsid protein is characterized by a mutation in aa 587-595 as compared to the wild type AAV8 capsid sequence, or a mutation in the analogous region of another AAV capsid as compared to the corresponding AAV wild type capsid sequence. In one embodiment, the AAV capsid protein is characterized by a mutation in nt 1759-1785 of the sequence coding for the AAV8 capsid protein as compared to the AAV8 wild type sequence, or a mutation in the analogous region of the sequence coding for another AAV capsid as compared to the corresponding AAV wild type capsid sequence. In another embodiment, the recombinant AAV capsid protein is characterized by at least one mutation in the HI loop as compared to the wild type sequence. In another embodiment, the recombinant AAV capsid protein comprises SEQ ID NO: 1 or SEQ ID NO: 2. In another embodiment, the recombinant AAV capsid protein comprises SEQ ID NO: 3 or SEQ ID NO: 4.

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In another aspect, nucleic acid sequences are provided encoding any of the recombinant AAV capsid proteins as described herein.

In another aspect, recombinant adeno-associated viruses (AAV) are provided. In one embodiment, the AAV has a recombinant AAV capsid having a mutation in aa 587-595 of the AAV8 capsid protein sequence as compared to the AAV8 wild type capsid sequence or a mutation in a corresponding region of another AAV capsid protein as compared to the corresponding wild type capsid sequence. The AAV further has a minigene having AAV inverted terminal repeats and a heterologous nucleic acid sequence operably linked to regulatory sequences which direct expression of a product encoded by the heterologous nucleic acid

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sequence in a target cell or tissue. In one embodiment, the AAV has a capsid protein comprising the sequence of SEQ ID NO: 1 or SEQ ID NO: 2. In another embodiment, the AAV has a capsid protein having the sequence of SEQ ID NO: 3 or SEQ ID NO: 4.

5 In yet another aspect, a method of preventing, arresting progression of, or ameliorating vision loss associated with an ocular disorder in a subject is provided. The method includes administering to the subject an effective concentration of a composition comprising any recombinant adeno-associated virus (AAV) described herein and a pharmaceutically acceptable carrier.

10 In another aspect, a method of targeting bipolar cells for optogenetic therapy in a subject in need thereof is provided. The method includes administering to the subject an effective concentration of a composition comprising any of the recombinant adeno-associated virus (AAV) described herein and a pharmaceutically acceptable carrier.

15 In another aspect, a method of targeting one or more type of ocular cells for optogenetic therapy in a subject in need thereof is provided. The method includes administering to the subject an effective concentration of a composition comprising any of the recombinant adeno-associated virus (AAV) described herein and a pharmaceutically acceptable carrier.

20 In another aspect, a method of targeting one or more type of ocular cells for gene augmentation therapy in a subject in need thereof is provided. The method includes administering to the subject an effective concentration of a composition comprising any of the recombinant adeno-associated virus (AAV) described herein and a pharmaceutically acceptable carrier.

25 In yet another aspect, a method of targeting one or more type of ocular cells for gene suppression therapy in a subject in need thereof is provided. The method includes administering to the subject an effective concentration of a composition comprising any of the recombinant adeno-associated virus (AAV) described herein and a pharmaceutically acceptable carrier.

30 In another aspect, a method of targeting one or more type of ocular cells for gene knockdown/augmentation therapy in a subject in need thereof is provided. The method includes administering to the subject an effective concentration of a composition comprising any of the recombinant adeno-associated virus (AAV) described herein and a pharmaceutically acceptable carrier.

In another aspect, a method of targeting one or more type of ocular cells for gene correction therapy in a subject in need thereof is provided. The method includes administering to the subject an effective concentration of a composition comprising any of the recombinant adeno-associated virus (AAV) described herein and a pharmaceutically acceptable carrier.

5 In yet another aspect, a method of targeting one or more type of ocular cells for neurotropic factor gene therapy in a subject in need thereof is provided. The method includes administering to the subject an effective concentration of a composition comprising any of the recombinant adeno-associated virus (AAV) described herein and a pharmaceutically acceptable carrier.

10 In another aspect, a method of targeting all ocular cells for optogenetic therapy in a subject in need thereof is provided. The method includes administering to the subject an effective concentration of a composition comprising any of the recombinant adeno-associated virus (AAV) described herein and a pharmaceutically acceptable carrier.

In another aspect, a method of generating a recombinant adeno-associated virus (rAAV) having an AAV capsid is provided. The method includes the steps of culturing a host cell containing: (a) a molecule encoding an AAV capsid protein as described herein; (b) a functional rep gene; (c) a minigene comprising AAV inverted terminal repeats (ITRs) and a heterologous nucleic acid sequence operably linked to regulatory sequences which direct expression of a product encoded by the heterologous nucleic acid sequence in a target cell; and (d) sufficient
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20 helper functions to permit packaging of the minigene into the AAV capsid protein.

In another aspect, a host or target cell transfected with an AAV or nucleic acid molecule as described herein is provided.

In another aspect, a composition is provided comprising an AAV as described herein and a physiologically compatible carrier.

25 In another aspect, a method of delivering a transgene to a cell is provided. The method includes the step of contacting the cell with an AAV according as described herein, wherein said AAV comprises the minigene.

In yet another aspect, the use of the AAV or a nucleic acid molecule as described herein is provided for preparing a medicament for delivery of a heterologous nucleic acid molecule
30 encoding the product to a cell.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 is an alignment of the variable protein 1 (vp1) capsid protein for AAV1, AAV2, AAV3, AAV3B, AAV4, AAV6, AAV7 and AAV8 taken from Gao G et al. PNAS, September 2002; 99:11854-11859. The region of mutation (aa 587-595 of AAV8 wt sequence) is boxed.

5 Figure 2 shows the protein structure of the AAV8 capsid protein taken from Nam, H.J. et al (2007) J.Virol. 81: 12260-12271. The HI (or, alternatively GH) loop, which is the subject of mutation in this invention, is circled.

Figure 3 is a map of the p-Red-Caps-Stuffer plasmid used for production of the library discussed in Example 1.

10 Figure 4 shows the results of the FACS sorting described in Example 2. The upper right quadrant corresponds to ON-bipolar cells transfected with AAV. The upper left quadrant corresponds to all non-bipolar cells transfected with AAV.

Figure 5 shows the sequences of aa 587-595 of the 5 “double positive” variants sequenced as discussed in Example 2. The wild type sequence of AAV8 was replaced with each variant sequence. The top line of each sequence shows the DNA coding sequence, the middle row shows the corresponding amino acid and the bottom row provides information regarding the characteristics of the amino acid. P=Polar, N=Non-polar, A=acidic, B=basic, * = very hydrophobic, and ^ = hydrophilic. Variant 5 encodes a premature stop codon and was used as a negative control. Variant 6 failed to produce high titer AAV, and no additional testing was done on this mutant.

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Figure 6A-E are photographs of 20X images from mouse retinal sections 3 weeks after subretinal injection with selected AAV variants as shown in Figure 5. These viruses express EGFP under the human EF1alpha promoter (promoter described in J Virol. 2005 Mar;79(6):3606-14). Variant 1 shows fluorescence in a diverse range of cell types with the sparsest fluorescence between the outer (OPL) and inner (IPL) plexiform layers where the bipolar cell subtypes are found (Fig. 6A). This layering is identified using a marker for choline acetyl transferase (anti-ChAT). The transduction pattern for variant 1 is qualitatively similar to that seen with the wild-type AAV8 capsid. By contrast strong fluorescence staining is found for variant 2 (AAV8b) in the outer-inner plexiform region (Fig. 6B, shown by an arrow) with many bipolar and amacrine cells stained. Bipolar cell staining is identified here with the marker anti-PKC α . In addition the photoreceptor cell bodies in the outer nuclear layer and their outer

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segments are strongly stained. The variant 3 virus shows a more distinctive staining pattern with dense staining along the outer plexiform layer with labeling of the horizontal cell bodies and their dense network of spreading dendrites (Fig. 6C, shown by an arrow). The lack of staining in the outer nuclear layer suggests that this variant selects against transduction of the photoreceptor cells. Variant 4 staining is sparse with only a few horizontal and amacrine cells labeled (Fig. 6D). Variant 5 was a negative control (Fig. 6E). POS=Photoreceptor outer segments, ONL=Outer nuclear layer, OPL= outer plexiform layer, IPL= inner plexiform layer, GCL=Ganglion cell layer.

Figure 7 are two photographs of 20X images from mouse retinal sections 3 weeks after subretinal injection with either AAV8wt-EF1 α .EGFP (left) or AAV8-b-EF1 α .EGFP (right). The various retinal sections are labeled. Abbreviations the same as in Fig. 6. Fluorescence staining shows a strong transduction of the Bruch's membrane and the inner limiting membrane of mouse retina by AAV8wt- EF1 α .EGFP. These membranes are not fluorescent in retinas transduced with AAV8b-EF1 α .EGFP. This may account for the greater diffusion of the AAV8b virus through the retina with stronger fluorescence in the region between the inner and outer plexiform layers for AAV8b compared to AAV8wt. Note that these retinas were also labeled for acetylcholine transferase to identify the region between the inner and outer plexiform layers.

Figure 8 is a fundus photograph of canine eye that had received subretinal injection of AAV8-b. Photo was taken 22 days after injection. The view shows the tapetal half of the retina. Black arrowheads demarcate the region of retina that is EGFP-positive due to exposure to the AAV8-b virus. The white arrowhead indicates the retinotomy site. The optic disc is at the bottom of the image.

Figures 9A and B are photographs showing a section of canine retina from the GFP-positive region shown in Figure 8, viewed with fluorescence microscopy. Nuclei are counterstained with DAPI. Fig. 9A shows strongly GFP-positive cone photoreceptors, inner retinal cells, and ganglion cells. Additional positive inner nuclear and ganglion cells are appreciated that have less intense GFP expression. The ONL is the same thickness as that in the untreated retina, demonstrating that there are no retinal degenerative changes. There are few if any GFP-positive RPE cells. RPE, retinal pigment epithelium; ONL, outer nuclear layer; INL, inner nuclear layer; GCL, ganglion cell layer. Fig. 9B is a higher magnification view of image

shown in Fig. 9A. Strongly GFP positive cone photoreceptors are apparent as well as a few GFP-positive rod photoreceptors. GFP-positive inner retinal cells are also apparent.

Figure 10 is a photograph showing a section through the intravitreally-injected retina in the region of the optic nerve, viewed with fluorescence microscopy. Nuclei are counterstained with DAPI. Strongly GFP-positive ganglion cell axons are appreciated entering and spanning the optic nerve head. The ONL has normal thickness, demonstrating that there are no retinal degenerative changes. There are no other GFP-positive cells in the retina with the potential exception of some RPE cells at the junction of the optic nerve. It is noted that this may be autofluorescence, and not fluorescence specific to GFP, however. Abbreviations, same as Fig. 9.

Figure 11A is a photograph of a retinal section of a WT mouse injected subretinally with AAV8-b-mGluR6-ChR2-EGFP. The ON-bipolar cell subtype is distinguished from the OFF-bipolar cell using the ChAT staining which identifies the specific sublamina of the inner plexiform layer. The OFF-bipolar cells terminate in sublamina 1 and 2 and have shorter axons than the ON-type which terminates in sublamina 4 and 5. POS=Photoreceptor outer segments, ONL=Outer nuclear layer, OPL= outer plexiform layer, IPL= inner plexiform layer, GCL=Ganglion cell layer. Fig. 11B is a diagram showing the structure of the inner retina including the bipolar cells and sublamina of the inner plexiform layer.

Figure 12 shows analysis of transduction properties of AAV8BP2. Titre analysis of the yield of purified AAV2/8BP2 compared to AAV2/8 (12a). Retinas from WT mice subretinally injected with AAV2/8 (EF1 α -EGFP) or AAV2/8BP2(EF1 α -EGFP) were collected three weeks post-injection and used for FACS analysis (n=4) (12b). QrtPCR to identify bipolar cell gene expression level was carried out on RNA from the GFP pool for each group (12c) and showed 120% increase in Grm6 expression (p=0.05) and 67% increase in trpM1L expression (p<0.05). Equivalent expression levels are determined for the cone photoreceptor genes Opn mwl and Opn swl (12d).

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to various compositions and treatment methods utilizing the same comprising an effective concentration of a recombinant adeno-associated virus (rAAV) characterized by a mutation in aa 587-595 of the capsid protein sequence as compared to the wild type AAV8 capsid sequence, or a mutation in the analogous region of another AAV capsid as

compared to the corresponding AAV wild type capsid sequence. The methods are directed to use of the AAV to treat ocular disorders and associated conditions related thereto.

Unless defined otherwise, 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 and by reference to published texts, which provide one skilled in the art with a general guide to many of the terms used in the present application. The following definitions are provided for clarity only and are not intended to limit the claimed invention. As used herein, the terms “a” or “an”, refers to one or more, for example, “an ocular cell” is understood to represent one or more ocular cells. As such, the terms “a” (or “an”), “one or more,” and “at least one” are used interchangeably herein. As used herein, the term “about” means a variability of 10 % from the reference given, unless otherwise specified. While various embodiments in the specification are presented using “comprising” language, under other circumstances, a related embodiment is also intended to be interpreted and described using “consisting of” or “consisting essentially of” language.

With regard to the following description, it is intended that each of the compositions herein described, is useful, in another embodiment, in the methods of the invention. In addition, it is also intended that each of the compositions herein described as useful in the methods, is, in another embodiment, itself an embodiment of the invention.

As used herein, the term “ocular cells” refers to any cell in, or associated with the function of, the eye. The term may refer to any one or more of photoreceptor cells, including rod, cone and photosensitive ganglion cells, retinal pigment epithelium (RPE) cells, Mueller cells, bipolar cells, horizontal cells, amacrine cells. In one embodiment, the ocular cells are bipolar cells. In another embodiment, the ocular cells are horizontal cells. In another embodiment, the ocular cells are ganglion cells.

As used herein, the term “mammalian subject” or “subject” includes any mammal in need of the methods of treatment described herein or prophylaxis, including particularly humans. Other mammals in need of such treatment or prophylaxis include dogs, cats, or other domesticated animals, horses, livestock, laboratory animals, including non-human primates, etc. The subject may be male or female.

In one embodiment, the subject has, or is at risk of developing an ocular disorder. The term “ocular disorder” includes, without limitation, retinitis pigmentosa, rod-cone dystrophy,

Leber's congenital amaurosis, Usher's syndrome, Bardet-Biedl Syndrome, Best disease, retinoschisis, Stargardt disease (autosomal dominant or autosomal recessive), untreated retinal detachment, pattern dystrophy, cone-rod dystrophy, achromatopsia, ocular albinism, enhanced S cone syndrome, diabetic retinopathy, age-related macular degeneration, retinopathy of
5 prematurity, sickle cell retinopathy, Congenital Stationary Night Blindness, glaucoma, or retinal vein occlusion. In another embodiment, the subject has, or is at risk of developing glaucoma, Leber's hereditary optic neuropathy, lysosomal storage disorder, or peroxisomal disorder. In another embodiment, the subject is in need of optogenetic therapy.

As used herein, the term "host cell" may refer to the packaging cell line in which the
10 rAAV is produced from the plasmid. In the alternative, the term "host cell" may refer to the target cell in which expression of the transgene is desired.

In another embodiment, the subject has shown clinical signs of an ocular disorder. Clinical signs of an ocular disorder include, but are not limited to, decreased peripheral vision, decreased central (reading) vision, decreased night vision, loss of color perception, reduction in
15 visual acuity, decreased photoreceptor function, and pigmentary changes. In one embodiment, the subject shows degeneration of the outer nuclear layer (ONL). In another embodiment, the subject has been diagnosed with an ocular disorder. In yet another embodiment, the subject has not yet shown clinical signs of an ocular disorder.

In one embodiment, the subject has become symptomatic for the ocular disorder. In
20 another embodiment, the subject has 10% or more photoreceptor damage/loss. In another embodiment, the subject has 20% or more photoreceptor damage/loss. In another embodiment, the subject has 30% or more photoreceptor damage/loss. In another embodiment, the subject has 40% or more photoreceptor damage/loss. In another embodiment, the subject has 50% or more photoreceptor damage/loss. In another embodiment, the subject has 60% or more photoreceptor
25 damage/loss. In another embodiment, the subject has 70% or more photoreceptor damage/loss. In another embodiment, the subject has 80% or more photoreceptor damage/loss. In another embodiment, the subject has 90% or more photoreceptor damage/loss. In another embodiment, the subject's bipolar cell circuitry to ganglion cells and optic nerve remains intact.

30 A. The AAV capsid

A recombinant AAV capsid protein as described herein is characterized by a mutation in aa 587-595 as compared to the wild type full length (vp1) AAV8 capsid sequence, or a mutation in the analogous region of another AAV capsid as compared to the corresponding AAV wild type capsid sequence. An alignment of the amino acid predicted amino acid sequences for capsid protein vp1 of AAV1, -2, -3A, -3B, -4, -6, -7, and -8 is shown in Fig. 1, with a box around the region of mutation of the capsid described herein (Gao G et al. PNAS, September 2002; 99:11854-11859). This region is relatively homologous across AAV serotypes, and corresponds to a region in the capsid protein which is circled in Fig. 2. This loop has been alternatively identified as the HI loop (Nam, H.J. et al (2007) J.Virol. 81: 12260-12271), or the GH loop (Asokan et al, (January 2010), Nat Biotechnol., 28(1): 79–82).

Also encompassed by the invention are nucleic acid sequences encoding the novel AAV, capsids, and fragments thereof which are described herein. In one embodiment, the recombinant AAV capsid protein is characterized by a mutation in nt 1759-1785 of the sequence coding for the AAV8 capsid protein as compared to the AAV8 wild type sequence, or a mutation in the analogous region of the sequence coding for another AAV capsid as compared to the corresponding AAV wild type capsid sequence.

Although, for convenience, reference is made herein to AAV8, it is to be understood that mutations in the homologous region of other AAV serotype capsids are also encompassed by the invention. As used herein, the term “wild type” refers to the native AAV sequence without mutation in aa 587-595 (using AAV8 numbering) of the capsid protein. However it is not intended that only naturally occurring AAV be the source of the wild type sequence. Useful herein are non-naturally occurring AAV, including, without limitation, recombinant, modified or altered, chimeric, hybrid, synthetic, artificial, etc., AAV. This includes AAV with mutations in regions of the capsid other than in aa 587-595, provided they are used as the “starting sequence” for generating the mutant capsid described herein.

The AAV capsid consists of three overlapping coding sequences, which vary in length due to alternative start codon usage. These variable proteins are referred to as VP1, VP2 and VP3, with VP1 being the longest and VP3 being the shortest. The AAV particle consists of all three capsid proteins at a ratio of ~1:1:10 (VP1:VP2:VP3). VP3, which is comprised in VP1 and VP2 at the N-terminus, is the main structural component that builds the particle. The capsid protein can be referred to using several different numbering systems. For convenience, as used

herein, the AAV sequences are referred to using VP1 numbering, which starts with aa 1 for the first residue of VP1. However, the capsid proteins described herein include VP1, VP2 and VP3 (used interchangeably herein with vp1, vp2 and vp3) with mutations in the corresponding region of the protein. In AAV8, the variable proteins correspond to VP1 (aa 1 to 738), VP2 (aa 138 to 738), and VP3 (aa 203 to 738) using the numbering of the full length VP1. For clarity, when referring to the mutation, it is meant aa 587-595 of the VP1, aa 450-458 of the VP2, and aa 385-393 of the VP3 of AAV8, if using the first amino acid of the particular vp capsid sequence as aa 1.

In one embodiment, AAV8 is the wild type sequence used when generating the mutant. The amino acid sequence of wild type AAV8 is shown as SEQ ID NO: 8. In one embodiment, the wild type sequence is SEQ ID NO: 8. In other embodiments any other AAV serotype is useful as the starting wild type sequence. In another embodiment, the AAV wild type sequence is a Clade E AAV. A clade is a group of AAV which are phylogenetically related to one another as determined using a Neighbor-Joining algorithm by a bootstrap value of at least 75% (of at least 1000 replicates) and a Poisson correction distance measurement of no more than 0.05, based on alignment of the AAV vp1 amino acid sequence. The Neighbor-Joining algorithm has been described extensively in the literature. See, e.g., M. Nei and S. Kumar, *Molecular Evolution and Phylogenetics* (Oxford University Press, New York (2000)).

Clade E is characterized by containing the previously described AAV8 (G. Gao et al, PNAS, Sep 2002), 43.1/rh.2; 44.2/rh.10; rh. 25; 29.3/bb.1; and 29.5/bb.2 [US Patent Publication No. US 2003/0138772 A1 (Jul 24 2003)]. Additional clade E sequences are described in US Patent No. 7,096,111, which is incorporated herein by reference. Other AAV which are useful as wild type sequences include, without limitation, including, e.g., pi.1, pi.2, pi.3, rh.38; rh.40; rh.43; rh.49; rh.50, rh.51; rh.52; rh.53; rh.57; rh.58; rh.61; rh.64; hu.6; hu.17; hu.37; hu.39; hu.40; hu.41; hu.42; hu. 66; and hu.67. Still other AAV which are useful as wild type sequences include AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, and AAV9.

AAV-b

Of the many cell types in the retina, the bipolar cells are the least amenable to transduction by AAV. This may be due to a lack of appropriate receptor expression on the cell surface to mediate uptake, or to intrinsic factors governing viral trafficking and processing in the cell. Not wishing to be bound by theory, the inventors assumed that the lack of AAV-mediated

expression in the bipolar cells is largely due to failure of the capsid to bind and transduce this cell rather than to features of the cell machinery that might inhibit expression from the viral genome. When injected subretinally AAV2/8 is shown to penetrate deeper into the retinal layers compared to other serotypes (Vandenberghe *et al.*, 2011), therefore as it already offered potential to 'reach' the bipolar cell layer the AAV8 capsid was chosen as the template for modification. A 9 amino acid stretch of a conformationally variable region of AAV8 capsid from 585-594 was specifically selected for potential to alter receptor attachment and cellular transduction properties of the virus. This was based on information from 3D models and targeted mutagenesis studies of the capsid (Gurda *et al.*, 2012; Nam *et al.*, 2007; Padron *et al.*, 2005; Xie *et al.*, 2002) (Fig. 2).

10 The AAV shell is assembled from 60 copies of viral proteins (VP), VP1 (87 kDa), VP2 (73 kDa), and VP3 (61 kDa). The conserved core of each VP subunit consists of an eight-stranded, β -barrel motif and an α -helix (Xie *et al.*, 2002). The outer surface of the capsid is formed by large loops that connect the strands of the β -barrel. For example, the residues from amino acid 585-594 encompass finger-like loops in one VP subunit. The amino acid sequences and structural

15 topology of these loops are reported to facilitate tissue tropism and transduction efficiency (Agbandje-McKenna and Kleinschmidt, 2011). Furthermore, these residues contribute to the top of the protrusions that surround the icosahedral 3-fold axes, formed through symmetric interactions between the VPs. Thus this sequence holds a prominent position on the capsid. This region includes sites shown in some serotypes (notably AAV2) to be critical for heparan-sulfate-binding and cellular uptake (Kern *et al.*, 2003; Opie *et al.*, 2003). The residues are not conserved

20 in the AAV8 capsid suggestive of the fact that AAV8 does not show affinity for heparan-sulfate (Wobus *et al.*, 2000, Wu *et al.*, 2006). Furthermore, an analogous domain was previously interchanged between AAV serotypes and shown to alter tropism profiles dramatically (Asokan *et al.*, 2010). It is possible that this region in AAV8 is less subject to the evolutionary pressure

25 that would prevent alternative capsid formation in other serotypes, while nonetheless producing a surface-exposed epitope that may influence tissue tropism and transduction characteristics.

As shown in the examples below, the inventors have shown that the AAV termed AAV-b effectively transduces bipolar, ganglion, and other ocular cells. In addition, a primary benefit of AAV-b is its strength. Injection of AAV-b results in efficient and early onset transduction of

30 cone photoreceptors, inner retinal cells and ganglion cells. It was noted that AAV8-b targets cone photoreceptors and bipolar cells extremely efficiently. In addition, AAV-b is able to

transduce these cells using a relatively low titer of virus. A further recognized benefit of AAV-b is the early onset of gene expression in some tissues.

AAV-b comprises the amino acid sequence of Pro-Glu-Arg-Thr-Ala-Met-Ser-Leu-Pro (SEQ ID NO: 1) at amino acid positions 587-595. In one embodiment, a capsid comprising SEQ ID NO: 1 is provided. In another embodiment, the capsid protein comprises SEQ ID NO: 1 at aa 587-595 of the AAV8 capsid protein or at a corresponding region of another AAV capsid sequence. In another embodiment, the capsid protein comprises the amino acid sequence of SEQ ID NO: 3. In this embodiment, the capsid sequence is referred to as AAV-8b. In other embodiments, the capsid sequence is derived from another wild type sequence, but replaces the sequence at the corresponding region of aa 587-595 with SEQ ID NO: 1.

In another embodiment, nucleic acid sequences encoding the AAV-b viruses, capsids and fragments described herein are provided. In one embodiment, a polynucleotide is provided comprising the sequence nt 1759-CCT GAG CGG ACG GCG ATG AGT CTT CCG-1785 (SEQ ID NO: 9). In one embodiment, a nucleic acid sequence comprising SEQ ID NO: 9 is provided. In another embodiment, a nucleic acid sequence encoding a capsid protein is provided, comprising the sequence SEQ ID NO: 9 at nt 1759-1785 of the sequence encoding the wild type AAV8, or at a corresponding region of another sequence encoding another AAV capsid. In another embodiment, the sequence encoding the capsid protein is SEQ ID NO: 5. In this embodiment, the capsid sequence is referred to as AAV-8b.

AAV-h

As shown in the examples herein, the inventors have shown that the AAV termed AAV-h effectively transduces horizontal, and other, ocular cells. AAV-h has an amino acid sequence of 587-Ser-Phe-Ser-Arg-Ala-Val-Leu-Cys-Asp-595 (SEQ ID NO: 2). In one embodiment, a capsid comprising SEQ ID NO: 2 is provided. In another embodiment, the capsid protein comprises SEQ ID NO: 2 at aa 587-595 of the AAV8 capsid protein or at a corresponding region of another AAV capsid sequence. In another embodiment, the capsid protein has the amino acid sequence of SEQ ID NO: 4. In this embodiment, the capsid sequence is referred to as AAV-8h. In other embodiments, the capsid sequence is derived from another wild type sequence, but replaces the sequence at the corresponding region of aa 587-595 with SEQ ID NO: 2.

In another embodiment, nucleic acid sequences are provided which encode the AAV-h viruses, capsids and fragments described herein. In one embodiment, a polynucleotide

comprising the sequence nt 1759- AGT TTT AGT CGT GCG GTT CTT TGT GAT -1785 (SEQ ID NO: 10) is provided. In one embodiment, a nucleic acid sequence comprising SEQ ID NO: 10 is provided. In another embodiment, a nucleic acid sequence encoding a capsid protein is provided, comprising the sequence SEQ ID NO: 10 at nt 1759-1785 of the sequence encoding the wild type AAV8, or at a corresponding region of another sequence encoding another AAV capsid. In another embodiment, the sequence encoding the capsid protein is SEQ ID NO: 6. In this embodiment, the capsid sequence is referred to as AAV-8h.

B. rAAV Vectors and Compositions

In another aspect, described herein are molecules which utilize the AAV sequences described herein, including fragments thereof, for production of viral vectors useful in delivery of a heterologous gene or other nucleic acid sequences to a target cell. In one embodiment, the vectors useful in compositions and methods described herein contain, at a minimum, sequences encoding a selected AAV capsid as described herein, e.g., an AAVb or AAVh capsid, or a fragment thereof. In another embodiment, useful vectors contain, at a minimum, sequences encoding a selected AAV serotype rep protein, e.g., AAV8 rep protein, or a fragment thereof. Optionally, such vectors may contain both AAV cap and rep proteins. In vectors in which both AAV *rep* and *cap* are provided, the AAV *rep* and AAV *cap* sequences can both be of one serotype origin, e.g., all AAV8 origin. Alternatively, vectors may be used in which the *rep* sequences are from an AAV which differs from the wild type AAV providing the *cap* sequences. In one embodiment, the *rep* and *cap* sequences are expressed from separate sources (e.g., separate vectors, or a host cell and a vector). In another embodiment, these *rep* sequences are fused in frame to *cap* sequences of a different AAV serotype to form a chimeric AAV vector, such as AAV2/8 described in US Patent No. 7,282,199, which is incorporated by reference herein. Optionally, the vectors further contain a minigene comprising a selected transgene which is flanked by AAV 5' ITR and AAV 3' ITR. In another embodiment, the AAV is a self-complementary AAV (sc-AAV) (See, US 2012/0141422 which is incorporated herein by reference). Self-complementary vectors package an inverted repeat genome that can fold into dsDNA without the requirement for DNA synthesis or base-pairing between multiple vector genomes. Because scAAV have no need to convert the single-stranded DNA (ssDNA) genome into double-stranded DNA (dsDNA) prior to expression, they are more efficient vectors. However, the trade-off for this efficiency is the loss of half the coding capacity of the vector,

ScAAV are useful for small protein-coding genes (up to ~55 kd) and any currently available RNA-based therapy.

In one aspect, the vectors described herein contain nucleic acid sequences encoding an intact AAV capsid as described herein. In one embodiment, the capsid comprises amino acids 1 to 738 of SEQ ID NO: 3 or 4. In another embodiment, the AAV has a recombinant AAV capsid comprising a mutation in aa 587-595 of the AAV8 capsid protein sequence as compared to the AAV8 wild type capsid sequence or a mutation in a corresponding region of another AAV capsid protein as compared to the corresponding wild type capsid sequence. In another embodiment, the AAV capsid comprises the sequence of SEQ ID NO: 1 or SEQ ID NO: 2. In another embodiment, the capsid is encoded by the sequence of SEQ ID NO: 5 or SEQ ID NO: 6. In another embodiment, the rAAV may have a capsid protein comprising one or more of the capsid regions selected from the vp2 and/or vp3, or from vp1, as described above.

Pseudotyped vectors, wherein the capsid of one AAV is replaced with a heterologous capsid protein, are useful herein. For illustrative purposes, AAV2/8-b (or AAV2/8-h) is used in the examples described below. See, Mussolino et al, cited above. Unless otherwise specified, the AAV ITRs, and other selected AAV components described herein, may be individually selected from among any AAV serotype, including, without limitation, AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9 or other known and unknown AAV serotypes. In one desirable embodiment, the ITRs of AAV serotype 2 are used. However, ITRs from other suitable serotypes may be selected. These ITRs or other AAV components may be readily isolated using techniques available to those of skill in the art from an AAV serotype. Such AAV may be isolated or obtained from academic, commercial, or public sources (e.g., the American Type Culture Collection, Manassas, VA). Alternatively, the AAV sequences may be obtained through synthetic or other suitable means by reference to published sequences such as are available in the literature or in databases such as, e.g., GenBank, PubMed, or the like. In one embodiment, the AAV comprises the sequence of SEQ ID NO: 5, which corresponds to the full length DNA sequence of AAV8-b. In another embodiment, the AAV comprises the sequence of SEQ ID NO: 6, which corresponds to the full length DNA sequence of AAV8-h.

The rAAV described herein also comprise a minigene. The minigene is composed of, at a minimum, a heterologous nucleic acid sequence (the transgene), as described below, and its

regulatory sequences, and 5' and 3' AAV inverted terminal repeats (ITRs). It is this minigene which is packaged into a capsid protein and delivered to a selected target cell.

The transgene is a nucleic acid sequence, heterologous to the vector sequences flanking the transgene, which encodes a polypeptide, protein, or other product, of interest. The nucleic acid coding sequence is operatively linked to regulatory components in a manner which permits
5 transgene transcription, translation, and/or expression in a target cell. The heterologous nucleic acid sequence (transgene) can be derived from any organism. The AAV may comprise one or more transgenes.

The composition of the transgene sequence will depend upon the use to which the
10 resulting vector will be put. In one desirable embodiment, the transgene is selected to provide optogenetic therapy. In optogenetic therapy, artificial photoreceptors are constructed by gene delivery of light-activated channels or pumps to surviving cell types in the remaining retinal circuit. This is particularly useful for patients who have lost a significant amount of
15 photoreceptor function, but whose bipolar cell circuitry to ganglion cells and optic nerve remains intact. In one embodiment, the heterologous nucleic acid sequence (transgene) is an opsin. The opsin sequence can be derived from any suitable single- or multicellular- organism, including human, algae and bacteria. In one embodiment, the opsin is rhodopsin, photopsin, L/M wavelength (red/green) -opsin, or short wavelength (S) opsin (blue). In another embodiment, the opsin is channelrhodopsin or halorhodopsin.

In another embodiment, the transgene is selected for use in gene augmentation therapy,
20 i.e., to provide replacement copy of a gene that is missing or defective. In this embodiment, the transgene may be readily selected by one of skill in the art to provide the necessary replacement gene. In one embodiment, the missing/defective gene is related to an ocular disorder. In another embodiment, the transgene is NYX, GRM6, TRPM1L or GPR179 and the ocular disorder is
25 Congenital Stationary Night Blindness. See, e.g., Zeitz et al, Am J Hum Genet. 2013 Jan 10;92(1):67-75. Epub 2012 Dec 13 which is incorporated herein by reference.

In another embodiment, the transgene is selected for use in gene suppression therapy, i.e.,
expression of one or more native genes is interrupted or suppressed at transcriptional or
translational levels. This can be accomplished using short hairpin RNA (shRNA) or other
30 techniques well known in the art. See, e.g., Sun et al, Int J Cancer. 2010 Feb 1;126(3):764-74 and O'Reilly M, et al. Am J Hum Genet. 2007 Jul;81(1):127-35, which are incorporated herein

by reference. In this embodiment, the transgene may be readily selected by one of skill in the art based upon the gene which is desired to be silenced.

In another embodiment, the transgene comprises more than one transgene. This may be accomplished using a single vector carrying two or more heterologous sequences, or using two
5 or more AAV each carrying one or more heterologous sequences. In one embodiment, the AAV is used for gene suppression (or knockdown) and gene augmentation co-therapy. In knockdown/augmentation co-therapy, the defective copy of the gene of interest is silenced and a non-mutated copy is supplied. In one embodiment, this is accomplished using two or more co-administered vectors. See, Millington-Ward et al, *Molecular Therapy*, April 2011, 19(4):642–
10 649 which is incorporated herein by reference. The transgenes may be readily selected by one of skill in the art based on the desired result.

In another embodiment, the transgene is selected for use in gene correction therapy. This may be accomplished using, e.g., a zinc-finger nuclease (ZFN)-induced DNA double-strand break in conjunction with an exogenous DNA donor substrate. See, e.g., Ellis et al, *Gene
15 Therapy* (epub January 2012) 20:35-42 which is incorporated herein by reference. The transgenes may be readily selected by one of skill in the art based on the desired result.

In another embodiment, the transgene is selected for use in neurotrophic factor gene therapy, i.e., providing exogenous neurotrophic factors to provide neuroprotection. In this embodiment, the transgene may be any neurotrophic factor, including ciliary-derived neurotrophic
20 factor (CNTF), fibroblast growth factor (FGF), glial-derived neurotrophic factor (GDNF), Rod-derived Cone Viability Factor (RdCVF) (Yang et al, *Mol Ther.* 2009 May;17(5):787-95) and brain-derived neurotrophic factor (BDNF). See, e.g., Schlichtenbrede et al, *Gene Therapy* (2003) 10, 523–527. The neurotrophic factor may be readily selected by one of skill in the art. These documents are incorporated herein by reference.

In another embodiment, the transgenes useful herein include reporter sequences, which
25 upon expression produce a detectable signal. Such reporter sequences include, without limitation, DNA sequences encoding β -lactamase, β -galactosidase (LacZ), alkaline phosphatase, thymidine kinase, green fluorescent protein (GFP), red fluorescent protein (RFP), chloramphenicol acetyltransferase (CAT), luciferase, membrane bound proteins including, for
30 example, CD2, CD4, CD8, the influenza hemagglutinin protein, and others well known in the art, to which high affinity antibodies directed thereto exist or can be produced by conventional

means, and fusion proteins comprising a membrane bound protein appropriately fused to an antigen tag domain from, among others, hemagglutinin or Myc.

These coding sequences, when associated with regulatory elements which drive their expression, provide signals detectable by conventional means, including enzymatic, radiographic, colorimetric, fluorescence or other spectrographic assays, fluorescent activating cell sorting assays and immunological assays, including enzyme linked immunosorbent assay (ELISA), radioimmunoassay (RIA) and immunohistochemistry. For example, where the marker sequence is the LacZ gene, the presence of the vector carrying the signal is detected by assays for beta-galactosidase activity. Where the transgene is green fluorescent protein or luciferase, the vector carrying the signal may be measured visually by color or light production in a luminometer.

Desirably, the transgene encodes a product which is useful in biology and medicine, such as proteins, peptides, RNA, enzymes, or catalytic RNAs. Desirable RNA molecules include shRNA, tRNA, dsRNA, ribosomal RNA, catalytic RNAs, and antisense RNAs. One example of a useful RNA sequence is a sequence which extinguishes expression of a targeted nucleic acid sequence in the treated animal.

The transgene may be used to correct or ameliorate gene deficiencies (as in the applications discussed above), which may include deficiencies in which normal genes are expressed at less than normal levels or deficiencies in which the functional gene product is not expressed. A preferred type of transgene sequence encodes a therapeutic protein or polypeptide which is expressed in a target cell. The invention further includes using multiple transgenes, e.g., to correct or ameliorate a gene defect caused by a multi-subunit protein. In certain situations, a different transgene may be used to encode each subunit of a protein, or to encode different peptides or proteins. This is desirable when the size of the DNA encoding the protein subunit is large. In order for the cell to produce the multi-subunit protein, a cell is infected with the recombinant virus containing each of the different subunits. Alternatively, different subunits of a protein may be encoded by the same transgene. In this case, a single transgene includes the DNA encoding each of the subunits, with the DNA for each subunit separated by an internal ribozyme entry site (IRES). This is desirable when the size of the DNA encoding each of the subunits is small, e.g., the total size of the DNA encoding the subunits and the IRES is less than five kilobases. As an alternative to an IRES, the DNA may be separated by sequences encoding a

2A peptide, which self-cleaves in a post-translational event. See, e.g., M.L. Donnelly, et al, J. Gen. Virol., 78(Pt 1):13-21 (Jan 1997); Furler, S., et al, Gene Ther., 8(11):864-873 (June 2001); Klump H., et al., Gene Ther., 8(10):811-817 (May 2001). This 2A peptide is significantly smaller than an IRES, making it well suited for use when space is a limiting factor. However, 5 the selected transgene may encode any biologically active product or other product, e.g., a product desirable for study.

The regulatory sequences include conventional control elements which are operably linked to the transgene in a manner which permits its transcription, translation and/or expression in a cell transfected with the vector or infected with the virus produced as described herein. As 10 used herein, "operably linked" sequences include both expression control sequences that are contiguous with the gene of interest and expression control sequences that act in *trans* or at a distance to control the gene of interest.

Expression control sequences include appropriate transcription initiation, termination, promoter and enhancer sequences; efficient RNA processing signals such as splicing and 15 polyadenylation (polyA) signals; sequences that stabilize cytoplasmic mRNA; sequences that enhance translation efficiency (*i.e.*, Kozak consensus sequence); sequences that enhance protein stability; and when desired, sequences that enhance secretion of the encoded product. A great number of expression control sequences, including promoters, are known in the art and may be utilized.

20 The regulatory sequences useful in the constructs provided herein may also contain an intron, desirably located between the promoter/ enhancer sequence and the gene. One desirable intron sequence is derived from SV-40, and is a 100 bp mini-intron splice donor/splice acceptor referred to as SD-SA. Another suitable sequence includes the woodchuck hepatitis virus post-transcriptional element. (See, e.g., L. Wang and I. Verma, 1999 Proc. Natl. Acad. Sci., USA, 25 96:3906-3910). PolyA signals may be derived from many suitable species, including, without limitation SV-40, human and bovine.

Another regulatory component of the rAAV useful in the methods described herein is an internal ribosome entry site (IRES). An IRES sequence, or other suitable systems, may be used to produce more than one polypeptide from a single gene transcript. An IRES (or other suitable 30 sequence) is used to produce a protein that contains more than one polypeptide chain or to express two different proteins from or within the same cell. An exemplary IRES is the

poliovirus internal ribosome entry sequence, which supports transgene expression in photoreceptors, RPE and ganglion cells. Preferably, the IRES is located 3' to the transgene in the rAAV vector.

In one embodiment, the AAV comprises a promoter (or a functional fragment of a promoter). The selection of the promoter to be employed in the rAAV may be made from among a wide number of constitutive or inducible promoters that can express the selected transgene in the desired target cell. In one embodiment, the target cell is an ocular cell. The promoter may be derived from any species, including human. Desirably, in one embodiment, the promoter is "cell specific". The term "cell-specific" means that the particular promoter selected for the recombinant vector can direct expression of the selected transgene in a particular cell or ocular cell type. In one embodiment, the promoter is specific for expression of the transgene in photoreceptor cells. In another embodiment, the promoter is specific for expression in the rods and/or cones. In another embodiment, the promoter is specific for expression of the transgene in RPE cells. In another embodiment, the promoter is specific for expression of the transgene in ganglion cells. In another embodiment, the promoter is specific for expression of the transgene in Mueller cells. In another embodiment, the promoter is specific for expression of the transgene in bipolar cells. In another embodiment, the promoter is specific for expression of the transgene in ON-bipolar cells. In one embodiment, the promoter is metabotropic glutamate receptor 6 (mGluR6) promoter (see, Vardi et al, mGluR6 Transcripts in Non-neuronal Tissues, J Histochem Cytochem. 2011 December; 59(12): 1076–1086, which is incorporated herein by reference). In another embodiment, the promoter is an enhancer-linked mGluR6 promoter. In another embodiment, the promoter is specific for expression of the transgene in OFF-bipolar cells. In another embodiment, the promoter is specific for expression of the transgene in horizontal cells. In another embodiment, the promoter is specific for expression of the transgene in amacrine cells. In another embodiment, the transgene is expressed in any of the above noted ocular cells.

In another embodiment, promoter is the native promoter for the gene to be expressed. Useful promoters include, without limitation, the rod opsin promoter, the red-green opsin promoter, the blue opsin promoter, the cGMP- β -phosphodiesterase promoter, the mouse opsin promoter (Beltran et al 2010 cited above), the rhodopsin promoter (Mussolino et al, Gene Ther, July 2011, 18(7):637-45); the alpha-subunit of cone transducin (Morrissey et al, BMC Dev, Biol, Jan 2011, 11:3); beta phosphodiesterase (PDE) promoter; the retinitis pigmentosa (RP1)

promoter (Nicord et al, J. Gene Med, Dec 2007, 9(12):1015-23); the NXNL2/NXNL1 promoter (Lambard et al, PLoS One, Oct. 2010, 5(10):e13025), the RPE65 promoter; the retinal degeneration slow/peripherin 2 (*Rds/perph2*) promoter (Cai et al, Exp Eye Res. 2010 Aug;91(2):186-94); and the VMD2 promoter (Kachi et al, Human Gene Therapy, 2009 (20:31-9)). Each of these documents is incorporated by reference herein.

Other useful promoters include transcription factor promoters including, without limitation, promoters for the neural retina leucine zipper (Nrl), photoreceptor-specific nuclear receptor Nr2e3, and basic-leucine zipper (bZIP). In one embodiment, the promoter is of a small size, under 1000 bp, due to the size limitations of the AAV vector. In another embodiment, the promoter is under 400 bp.

Other promoters useful herein include ubiquitous or constitutive promoters, when universal expression of the transgene is desired. In one embodiment, the promoter is selected from the human EF1 α promoter, phosphoglycerate kinase-1 (PGK) promoter, and cytomegalovirus (CMV) promoter (optionally with the CMV enhancer). Other examples of constitutive promoters useful herein include, without limitation, the retroviral Rous sarcoma virus (RSV) LTR promoter (optionally with the RSV enhancer), the SV40 promoter, the dihydrofolate reductase promoter, the chicken β -actin (CBA) promoter, and the immediate early CMV enhancer coupled with the CBA promoter.

Inducible promoters allow regulation of gene expression and can be regulated by exogenously supplied compounds, environmental factors such as temperature, or the presence of a specific physiological state, e.g., acute phase, a particular differentiation state of the cell, or in replicating cells only. In one embodiment, the inducible promoter is selected from rapamycin/rapalog promoter, the ecdysone promoter, the estrogen-responsive promoter, and the tetracycline-responsive promoter. Examples of other inducible promoters regulated by exogenously supplied compounds which are useful herein, include, the zinc-inducible sheep metallothionine (MT) promoter, the dexamethasone (Dex)-inducible mouse mammary tumor virus (MMTV) promoter, the T7 polymerase promoter system. Other types of inducible promoters which may be useful in this context are those which are regulated by a specific physiological state, e.g., temperature, acute phase, a particular differentiation state of the cell, or in replicating cells only. In one embodiment, any type of inducible promoter which is tightly regulated and is specific for the particular target ocular cell type may be used.

Other regulatory sequences useful herein include enhancer sequences. Enhancer sequences useful herein include the IRBP enhancer (Nicord 2007, cited above), immediate early cytomegalovirus enhancer, one derived from an immunoglobulin gene or SV40 enhancer, the cis-acting element identified in the mouse proximal promoter, etc.

5 Selection of these and other common vector and regulatory elements are conventional and many such sequences are available. See, e.g., Sambrook et al, and references cited therein at, for example, pages 3.18-3.26 and 16.17-16.27 and Ausubel et al., Current Protocols in Molecular Biology, John Wiley & Sons, New York, 1989). Of course, not all vectors and expression control sequences will function equally well to express all of the transgenes as
10 described herein. However, one of skill in the art may make a selection among these, and other, expression control sequences without departing from the scope of this invention.

In another embodiment, a method of generating a recombinant adeno-associated virus is provided. A suitable recombinant adeno-associated virus (AAV) is generated by culturing a host cell which contains a nucleic acid sequence encoding an AAV capsid protein as described herein,
15 or fragment thereof; a functional rep gene; a minigene composed of, at a minimum, AAV inverted terminal repeats (ITRs) and a heterologous nucleic acid sequence encoding a desirable transgene; and sufficient helper functions to permit packaging of the minigene into the AAV capsid protein. The components required to be cultured in the host cell to package an AAV minigene in an AAV capsid may be provided to the host cell in *trans*. Alternatively, any one or
20 more of the required components (e.g., minigene, *rep* sequences, *cap* sequences, and/or helper functions) may be provided by a stable host cell which has been engineered to contain one or more of the required components using methods known to those of skill in the art.

Most suitably, such a stable host cell will contain the required component(s) under the control of an inducible promoter. However, the required component(s) may be under the control
25 of a constitutive promoter. Examples of suitable inducible and constitutive promoters are provided herein, in the discussion below of regulatory elements suitable for use with the transgene. In still another alternative, a selected stable host cell may contain selected component(s) under the control of a constitutive promoter and other selected component(s) under the control of one or more inducible promoters. For example, a stable host cell may be generated
30 which is derived from 293 cells (which contain E1 helper functions under the control of a

constitutive promoter), but which contains the *rep* and/or *cap* proteins under the control of inducible promoters. Still other stable host cells may be generated by one of skill in the art.

The minigene, *rep* sequences, *cap* sequences, and helper functions required for producing the rAAV described herein may be delivered to the packaging host cell in the form of any genetic element which transfers the sequences carried thereon. The selected genetic element may be delivered by any suitable method, including those described herein. The methods used to construct any embodiment of this invention are known to those with skill in nucleic acid manipulation and include genetic engineering, recombinant engineering, and synthetic techniques. See, e.g., Sambrook et al, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Press, Cold Spring Harbor, NY. Similarly, methods of generating rAAV virions are well known and the selection of a suitable method is not a limitation on the present invention. See, e.g., K. Fisher et al, 1993 *J. Virol.*, 70:520-532 and US Patent 5,478,745, among others. These publications are incorporated by reference herein.

15 C. Pharmaceutical Compositions and Administration

In one embodiment, the recombinant AAV containing the desired transgene and cell-specific promoter for use in the target ocular cells as detailed above is optionally assessed for contamination by conventional methods and then formulated into a pharmaceutical composition intended for subretinal or intravitreal injection. Such formulation involves the use of a pharmaceutically and/or physiologically acceptable vehicle or carrier, particularly one suitable for administration to the eye, e.g., by subretinal injection, such as buffered saline or other buffers, e.g., HEPES, to maintain pH at appropriate physiological levels, and, optionally, other medicinal agents, pharmaceutical agents, stabilizing agents, buffers, carriers, adjuvants, diluents, etc. For injection, the carrier will typically be a liquid. Exemplary physiologically acceptable carriers include sterile, pyrogen-free water and sterile, pyrogen-free, phosphate buffered saline. A variety of such known carriers are provided in US Patent Publication No. 7,629,322, incorporated herein by reference. In one embodiment, the carrier is an isotonic sodium chloride solution. In another embodiment, the carrier is balanced salt solution. In one embodiment, the carrier includes tween. If the virus is to be stored long-term, it may be frozen in the presence of glycerol or Tween20. In another embodiment, the pharmaceutically acceptable carrier comprises a surfactant, such as perfluorooctane (Perfluron liquid).

In certain embodiments of the methods described herein, the pharmaceutical composition described above is administered to the subject by subretinal injection. In other embodiments, the pharmaceutical composition is administered by intravitreal injection. Other forms of administration that may be useful in the methods described herein include, but are not limited to, 5 direct delivery to a desired organ (e.g., the eye), oral, inhalation, intranasal, intratracheal, intravenous, intramuscular, subcutaneous, intradermal, and other parental routes of administration. Routes of administration may be combined, if desired.

Furthermore, in certain embodiments it is desirable to perform non-invasive retinal imaging and functional studies to identify areas of specific ocular cells to be targeted for therapy.

10 In these embodiments, clinical diagnostic tests are employed to determine the precise location(s) for one or more subretinal injection(s). These tests may include ophthalmoscopy, electroretinography (ERG) (particularly the b-wave measurement), perimetry, topographical mapping of the layers of the retina and measurement of the thickness of its layers by means of confocal scanning laser ophthalmoscopy (cSLO) and optical coherence tomography (OCT), 15 topographical mapping of cone density via adaptive optics (AO), functional eye exam, etc. These, and other desirable tests, are described in International Patent Application No. PCT/US2013/022628. In view of the imaging and functional studies, in some embodiments, one or more injections are performed in the same eye in order to target different areas of retained bipolar cells. The volume and viral titer of each injection is determined individually, as further 20 described below, and may be the same or different from other injections performed in the same, or contralateral, eye. In another embodiment, a single, larger volume injection is made in order to treat the entire eye. In one embodiment, the volume and concentration of the rAAV composition is selected so that only a specific region of ocular cells is impacted. In another embodiment, the volume and/or concentration of the rAAV composition is a greater amount, in 25 order reach larger portions of the eye, including non-damaged ocular cells.

The composition may be delivered in a volume of from about 0.1 μ L to about 1 mL, including all numbers within the range, depending on the size of the area to be treated, the viral titer used, the route of administration, and the desired effect of the method. In one embodiment, the volume is about 50 μ L. In another embodiment, the volume is about 70 μ L. In another 30 embodiment, the volume is about 100 μ L. In another embodiment, the volume is about 125 μ L. In another embodiment, the volume is about 150 μ L. In another embodiment, the volume is

about 175 μL . In yet another embodiment, the volume is about 200 μL . In another embodiment, the volume is about 250 μL . In another embodiment, the volume is about 300 μL . In another embodiment, the volume is about 450 μL . In another embodiment, the volume is about 500 μL . In another embodiment, the volume is about 600 μL . In another embodiment, the volume is about 750 μL . In another embodiment, the volume is about 850 μL . In another embodiment, the volume is about 1000 μL . An effective concentration of a recombinant adeno-associated virus carrying a nucleic acid sequence encoding the desired transgene under the control of the cell-specific promoter sequence desirably ranges from about 10^7 and 10^{13} vector genomes per milliliter (vg/mL) (also called genome copies/mL (GC/mL)). The rAAV infectious units are measured as described in S.K. McLaughlin et al, 1988 J. Virol., 62:1963, which is incorporated herein by reference. Preferably, the concentration in the retina is from about 1.5×10^9 vg/mL to about 1.5×10^{12} vg/mL, and more preferably from about 1.5×10^9 vg/mL to about 1.5×10^{11} vg/mL. In one embodiment, the effective concentration is about 1.4×10^8 vg/mL. In one embodiment, the effective concentration is about 3.5×10^{10} vg/mL. In another embodiment, the effective concentration is about 5.6×10^{11} vg/mL. In another embodiment, the effective concentration is about 5.3×10^{12} vg/mL. In yet another embodiment, the effective concentration is about 1.5×10^{12} vg/mL. In another embodiment, the effective concentration is about 1.5×10^{13} vg/mL. In one embodiment, the effective dosage (total genome copies delivered) is from about 10^7 to 10^{13} vector genomes. It is desirable that the lowest effective concentration of virus be utilized in order to reduce the risk of undesirable effects, such as toxicity, retinal dysplasia and detachment. Still other dosages and administration volumes in these ranges may be selected by the attending physician, taking into account the physical state of the subject, preferably human, being treated, the age of the subject, the particular ocular disorder and the degree to which the disorder, if progressive, has developed. For extra-ocular delivery, the dosage will be increased according to the scale-up from the retina. Intravenous delivery, for example may require doses on the order of 1.5×10^{13} vg/kg.

D. Methods of treatment/prophylaxis

Described herein are various methods of preventing, treating, arresting progression of or ameliorating the above-described ocular disorders and retinal changes associated therewith. Generally, the methods include administering to a mammalian subject in need thereof, an

effective amount of a composition comprising a recombinant adeno-associated virus (AAV) described above, carrying a nucleic acid sequence encoding transgene, or fragment thereof, under the control of regulatory sequences which express the product of the gene in the subject's ocular cells, and a pharmaceutically acceptable carrier. Any of the AAV described herein are
5 useful in the methods described below.

The retinal diseases described above are associated with various retinal changes. These may include a loss of photoreceptor structure or function; thinning or thickening of the outer nuclear layer (ONL); thinning or thickening of the outer plexiform layer (OPL); disorganization followed by loss of rod and cone outer segments; shortening of the rod and cone inner segments;
10 retraction of bipolar cell dendrites; thinning or thickening of the inner retinal layers including inner nuclear layer, inner plexiform layer, ganglion cell layer and nerve fiber layer; opsin mislocalization; overexpression of neurofilaments; thinning of specific portions of the retina (such as the fovea or macula); loss of ERG function; loss of visual acuity and contrast sensitivity; loss of optokinetic reflexes; loss of the pupillary light reflex; and loss of visually
15 guided behavior. In one embodiment, a method of preventing, arresting progression of or ameliorating any of the retinal changes associated with these retinal diseases is provided. As a result, the subject's vision is improved, or vision loss is arrested and/or ameliorated.

In a particular embodiment, a method of preventing, arresting progression of or ameliorating vision loss associated with an ocular disorder in the subject is provided. Vision loss
20 associated with an ocular disorder refers to any decrease in peripheral vision, central (reading) vision, night vision, day vision, loss of color perception, loss of contrast sensitivity, or reduction in visual acuity.

In another embodiment, a method of targeting one or more ocular cells for optogenetic therapy in a subject in need thereof is provided. As discussed above, optogenetic therapy is a
25 method of providing "substitute" photoreceptor function in subjects who have significant photoreceptor damage. In one embodiment, the cells targeted for optogenetic therapy are bipolar cells. In another embodiment, the cells targeted for optogenetic therapy are ganglion cells. In another embodiment, all ocular cells are targeted.

In another embodiment, a method of targeting one or more type(s) of ocular cells for gene
30 augmentation therapy in a subject in need thereof is provided. In another embodiment, a method of targeting one or more type of ocular cells for gene suppression therapy in a subject in need

thereof is provided. In yet another embodiment, a method of targeting one or more type of ocular cells for gene knockdown/augmentation therapy in a subject in need thereof is provided. In another embodiment, a method of targeting one or more type of ocular cells for gene correction therapy in a subject in need thereof is provided. In still another embodiment, a method of targeting one or more type of ocular cells for neurotropic factor gene therapy in a subject in need thereof is provided.

In any of the methods described herein, the targeted cell may be an ocular cell. In one embodiment, the targeted cell is an RPE cell. In another embodiment, the targeted cell is a photoreceptor. In another embodiment, the photoreceptor is a cone cell. In another embodiment, the targeted cell is a Mueller cell. In another embodiment, the targeted cell is a bipolar cell. In yet another embodiment, the targeted cell is a horizontal cell. In another embodiment, the targeted cell is an amacrine cell. In still another embodiment, the targeted cell is a ganglion cell. In still another embodiment, the gene may be expressed and delivered to an intracellular organelle, such as a mitochondrion or a lysosome.

As used herein “photoreceptor function loss” means a decrease in photoreceptor function as compared to a normal, non-diseased eye or the same eye at an earlier time point. As used herein, “increase photoreceptor function” means to improve the function of the photoreceptors or increase the number or percentage of functional photoreceptors as compared to a diseased eye (having the same ocular disease), the same eye at an earlier time point, a non-treated portion of the same eye, or the contralateral eye of the same patient. Photoreceptor function may be assessed using the functional studies described above and in the examples below, e.g., ERG or perimetry, which are conventional in the art.

For each of the described methods, the treatment may be used to prevent the occurrence of retinal damage or to rescue eyes having mild or advanced disease. As used herein, the term “rescue” means to prevent progression of the disease to total blindness, prevent spread of damage to uninjured ocular cells, improve damage in injured ocular cells, or to provide enhanced vision. In one embodiment, the composition is administered before the disease becomes symptomatic or prior to photoreceptor loss. By symptomatic is meant onset of any of the various retinal changes described above or vision loss. In another embodiment, the composition is administered after disease becomes symptomatic. In yet another embodiment, the composition is administered after initiation of photoreceptor loss. In another embodiment, the composition is

administered after outer nuclear layer (ONL) degeneration begins. In some embodiments, it is desirable that the composition is administered while bipolar cell circuitry to ganglion cells and optic nerve remains intact. In one embodiment, the bipolar cells are targeted for optogenetic therapy. By ensuring that the composition is administered while the circuitry to ganglion cells and optic nerve remains intact, it is possible to provide “substitute” photoreceptor cells, and increase vision.

In another embodiment, the composition is administered after initiation of photoreceptor loss. In yet another embodiment, the composition is administered when less than 90% of the photoreceptors are functioning or remaining, as compared to a non-diseased eye. In another embodiment, the composition is administered when less than 80% of the photoreceptors are functioning or remaining. In another embodiment, the composition is administered when less than 70% of the photoreceptors are functioning or remaining. In another embodiment, the composition is administered when less than 60% of the photoreceptors are functioning or remaining. In another embodiment, the composition is administered when less than 50% of the photoreceptors are functioning or remaining. In another embodiment, the composition is administered when less than 40% of the photoreceptors are functioning or remaining. In another embodiment, the composition is administered when less than 30% of the photoreceptors are functioning or remaining. In another embodiment, the composition is administered when less than 20% of the photoreceptors are functioning or remaining. In another embodiment, the composition is administered when less than 10% of the photoreceptors are functioning or remaining. In one embodiment, the composition is administered only to one or more regions of the eye. In another embodiment, the composition is administered to the entire eye.

In another embodiment, the method includes performing functional and imaging studies to determine the efficacy of the treatment. These studies include ERG and in vivo retinal imaging, as described in the examples below. In addition visual field studies, perimetry and microperimetry, pupillometry, mobility testing, visual acuity, contrast sensitivity, color vision testing may be performed.

In yet another embodiment, any of the above described methods is performed in combination with another, or secondary, therapy. The therapy may be any now known, or as yet unknown, therapy which helps prevent, arrest or ameliorate any of the described retinal changes and/or vision loss. In one embodiment, the secondary therapy is encapsulated cell therapy (such

as that delivering Ciliary Neurotrophic Factor (CNTF)). See, Sieving, P.A. et al, 2006. Proc Natl Acad Sci USA, 103(10):3896-3901, which is hereby incorporated by reference. In another embodiment, the secondary therapy is a neurotrophic factor therapy (such as pigment epithelium-derived factor, PEDF; ciliary neurotrophic factor 3; rod-derived cone viability factor (RdCVF) or glial-derived neurotrophic factor). In another embodiment, the secondary therapy is anti-apoptosis therapy (such as that delivering X-linked inhibitor of apoptosis, XIAP). In yet another embodiment, the secondary therapy is rod derived cone viability factor 2. The secondary therapy can be administered before, concurrent with, or after administration of the rAAV described above.

10 In another embodiment, a method of delivering a transgene to a cell is provided, said method comprising the step of contacting the cell with an AAV described herein, wherein said AAV comprises the minigene.

In another embodiment, a method of delivering a protein to the brain is provided, said method comprising contacting a ganglion cell with an AAV described herein, wherein said AAV 15 comprises the minigene. In one embodiment, the AAV is AAV-b. In another embodiment, the AAV is AAV-8b.

In another embodiment, the use of an AAV or a molecule as described herein is provided in preparing a medicament for delivery of a heterologous nucleic acid molecule encoding the product to a cell. In still another embodiment, a composition is provided comprising an AAV as 20 described herein, for use in treating an ocular condition.

As is demonstrated in the examples below, exemplary AAV-8b and AAV-8h were employed in *in vivo* experiments to provide evidence of the utility and efficacy of the methods and compositions described herein. The examples demonstrated targeting of ocular cells in mice and dog.

25 Further, these results show that subretinal injection of AAV8-b in the eye of a large animal model results in efficient and early onset transduction of cone photoreceptors, inner retinal cells and ganglion cells. It was noted that AAV8-b targets cone photoreceptors extremely efficiently. This provides a useful tool for delivering halorhodopsin-mediated optogenetic therapy. In addition, AAV8-b is also useful for treating diseases which originate in cone 30 photoreceptors, such as achromatopsia.

Notably there is little if any transduction of RPE cells with AAV8-b even if the vector is injected subretinally. To the inventors' knowledge, there is no other vector that has this feature. All other AAV serotypes and all of the different pseudotyped lentiviruses which target the eye, target RPE cells efficiently. There is a great need for AAV vectors that do not target RPE cells
5 to treat neural retinal disorders (and not those of the RPE). Further, a lack of transduction of the RPE attenuates side effects/toxicity from targeting of these cells.

In addition, AAV-b targets inner retinal cells efficiently and can be used to deliver optogenetic therapy, including therapy mediated by modified channelopsin proteins. The early onset of expression mediated by this vector allows for rapid reversal of sensorineural deficits.

10 In addition, AAV-b targets ganglion cells extremely efficiently after either subretinal or intravitreal injection. Thus, this vector may be used through intravitreal injection (an office procedure) to efficiently deliver secretable proteins including, but not limited to, neurotrophic factors, antineovascular factors, molecules that serve as decoy receptors to block function of unwanted proteins. Further, AAV-b, due to its ability to target ganglion cells, is useful in the
15 treatment of glaucoma (a disease which causes loss of ganglion cells).

Further, because AAV-b targets ganglion cells efficiently, this vector may be used to deliver protein to the brain. This approach is useful in developing treatments for degenerative diseases which affect the brain, including lysosomal storage diseases and metabolic diseases.

This data allow one of skill in the art to readily anticipate that these methods may be
20 similarly used in treatment of retinal disease in other subjects, including humans.

E. Examples

Example 1: Generation of mutants

To find recombinant AAV capable of transducing bipolar cells, the divergent VP3 loop
25 HI of the AAV8 capsid gene was targeted for mutation (Fig. 2). Briefly, a library of mutations across 9 amino acids (aa 587-595 of AAV8 vp1), was engineered into the AAV capsid and this library cloned into the plasmid pRFP-AAV8cap (Fig. 3). The AAV viral library was then produced by triple transfection of 293T cells using standard methods. See, e.g., Flannery and Visel, *Methods in Molecular Biology*, 935:351-69 (September 2012), which is incorporated by
30 reference herein.

From crystallography studies carried out at 2.6-Å resolution Nam *et al* have contributed the lack of heparan sulfate binding by AAV8 to be in part due to the structural differences in the region utilized for receptor recognition by AAV2 (highlighted in red in figure 1A, from PDB 2QA0, (Nam *et al.*, 2007)). Two critical residues, R585 and R588 are particularly necessary for the ability of AAV2 to bind heparan sulfate (Kern *et al.*, 2003; Opie *et al.*, 2003). These positions align with Q588 and T591, respectively, in AAV8 (Lochrie *et al.*, 2006). Random mutagenesis was carried out on residues from amino acid 585 to 594.

This mutated capsid region was cloned into a vector designed for AAV library production, the red fluorescently labelled plasmid, pAAV8Caps-Lib (Fig. 3). This plasmid which has the AAV2 ITRs flanking the minimal rep/cap gene of AAV8 expressed in reverse orientation to turbo RFP was used to produce the AAV viral library. The plasmid was cotransfected with helper plasmid into 293T cells as described in the methods section. Up to 2µl of the viral library was subretinally injected into Grm6-EGFP transgenic mice, which have GFP labelling of the ON-bipolar cells. After 3 weeks, cell dissociation and FACS was used to isolate the ON-bipolar cells. These cells included a subset of cells double-labelled with EGFP and dsRED, i.e. library-transfected bipolar cells. The sorted EGFP cells were lysed and the mutant region of the capsid was amplified by PCR and recloned back into the pAAV8Caps-Lib plasmid for a second round of viral library production and injection. It was desirable to select for viruses that could effectively compete with the wild-type capsid. Therefore in the second round, the viral library was spiked with AAV2/8, which in effect served as a negative competing selective force. From this round unmutated and mutant sequences were found in double-positive EGFP/dsRED-labelled cells (transduced ON-bipolar cells) with only unmutated AAV8 capsid sequences isolated from the dsRed-positive, EGFP-negative cells (transduced non-bipolar cells) (Fig. 4). From the double positive red/green cells, the AAVs were isolated and sequenced. Six variants were identified based on their appearance more than once in DNA samples from 50 colonies. These variants were processed for further analysis.

A. Production of mutated region by DNA2.0

The region of the AAV8 rep/cap gene between the sbf1 and mlu1 sites was synthesized by the company DNA2.0 (<https://www.dna20.com/>). This region includes the amino acids 587-595 (counting from the first amino acid of AAV8 vp1) and mutations were incorporated at each

position of these 9 amino acids. A combinatorial NNK strategy was used to generate maximum diversity while eliminating two of the three possible stop codons (Muranaka et al, Nucleic Acids Res. 2006 Jan 5;34, which is incorporated by reference herein).

B. Cloning of the AAV plasmid library:

5 The plasmid pRed-Caps-Stuffer was linearized with *sbf1* and *mlu1* (an *sbf1* site further upstream was mutated using a site-directed mutagenesis kit prior to cloning to render the extra *sbf1* site inactive). We cloned the synthesized mutated region in the *sbf1/mlu1* digested backbone using T4 DNA ligase (New England Biolabs). This was electroporated into DH10b electrocompetent bacteria (Invitrogen). It was crucial that no uncut backbone from the WT
10 plasmid escape through the cloning as it would contaminate and saturate the library. The absence of backbone was confirmed by absence of growth on agar-Kanamycin plates.

Up to 20ul of the transformation broth was plated on agar-Ampicillin plates to estimate the number of transformants. The remainder of the broth was used to inoculate 2 litres of broth to prepare rep/cap plasmid library DNA for AAV production.

15 C. AAV library production

The AAV was prepared according to standard triple transfection protocols with some specifications:

To avoid the packaging of multiple genomes into each capsid a low plasmid concentration was used for the triple transfection – 150ng of rep/cap library plasmid was used
20 per 150mm confluent HEK293 cell culture plate. By Avogadro's number and the molecular weight of the plasmid it was estimated that 200 DNA capsid molecules were present per HEK293 cell. Ultimately the transfected cell lysates and broths from 70 confluent 150mm transfected plates were used to prepare the library. This broth and benzonase-treated lysates were concentrated 20-fold using a tangential flow filtration system before virus production according
25 to state of the art.

Example 2: Mutant selection

The viral library (2 µl) was subretinally injected into 10 adult transgenic mice with GFP-labeled ON-bipolar cells (mGluR6-EGFP mice, as described in Dhingra et al, J Comp Neurol.
30 2008 Oct 10;510(5):484-96 which is incorporated by reference herein). After 3 weeks, the GFP-labeled cells were isolated after retinal cell dissociation FACS sorting. Viruses were isolated

from the bipolar cells and the mutant region of the capsid was amplified by PCR and sequenced. These capsid sequences were then reincorporated into the pRFP-AAV8 cap plasmid for a second round of library production and injection.

After 3 weeks, FACS sorting was again performed. ON-bipolar cells expressed a double
5 positive for dsRED and GFP. Non-bipolar cells transfected with AAV were positive for dsRED alone. The ON-bipolar cells accounted for about 1% of the parent population, while the transfected non-bipolars accounted for about 9.1% (Fig. 4). It was noted that a large range of mutants were isolated from both GFP positive cells (bipolar cells) and GFP negative cells (non-bipolar cells) in the first round of selection. Thus, in the second round, the library was spiked
10 with WT AAV8 to serve as a negative competing selective force. From this round mutant sequences were only found in GFP-positive cells (bipolar cells, “double positive” cells) with only WT sequences isolated from the GFP-negative cells.

From these “double positive” cells, the AAV were isolated and sequenced. Five (5) variants were sequenced (Fig. 5). AAV vectors were then created using each of these mutant
15 sequences in place of the wild type AAV8 capsid gene in an AAV(EF1 α -EGFP) vector. This virus expresses EGFP under the control of human EF1 α promoter (Nakai et al, J. Virology, 79(6):3606-14 (March 2005), which is hereby incorporated by reference). One additional mutant, carrying a premature stop codon, was also used to create a virus (Variant 5, Fig. 5). These viruses were subretinally injected (1.5 μ l) into 10 week old C57Bl6 (WT) mice. After
20 three weeks the mice were sacrificed, the retinas removed and 150 μ m vibratome sections were made of the tissue (see, e.g., http://www.immunostar.com/content/pdf/instruction_manual.pdf) Figs. 6A-E show expression of EGFP in retina injected with AAV variants 1-5. Variant 1 shows fluorescence in a diverse range of cell types with the sparsest fluorescence between the outer (OPL) and inner (IPL) plexiform layers where the bipolar cell subtypes are found (Fig. 6A). This
25 layering is identified using a marker for choline acetyl transferase (anti-ChAT). The transduction pattern for variant 1 is qualitatively similar to that seen with the wild-type AAV8 capsid. By contrast strong fluorescence staining is found for variant 2 (AAV8b) in this outer-inner plexiform region (fig. 6B, shown by an arrow) with many bipolar and amacrine cells stained. Bipolar cell staining is identified here with the marker anti-PKC α . In addition the
30 photoreceptor cell bodies in the outer nuclear layer and their outer segments are strongly stained. The variant 3 virus shows a more distinctive staining pattern with dense staining along the outer

plexiform layer with labeling of the horizontal cell bodies and their dense network of spreading dendrites (Fig. 6C, shown by an arrow). The lack of staining in the outer nuclear layer suggests that this variant selects against transduction of the photoreceptor cells. Variant 4 staining is sparse with only a few horizontal and amacrine cells labeled (Fig. 6D). Variant 5 carried a premature stop codon and effectively served as a negative control (Fig. 6E).

Variant 2, preferentially transduced bipolar cells and was termed AAV8-b. Variant 3 preferentially transduced horizontal cells and was termed AAV8-h. Based upon these results, variants 2 and 3 were selected for further analysis.

10 Example 3: Expression from AAV8-b compared to AAV8wt using the ubiquitous promoter EF1 α

1.5 μ l of AAV8-b(EF1 α -EGFP) at a titer of 5.3×10^{12} gc/ml was subretinally injected into 10 week old C57Bl6 (WT) mice (n=5). For comparison, 1.5 μ l of AAV8wt-(EF1 α -EGFP) at a titer of 6.4×10^{13} gc/ml was subretinally injected into 10 week old C57Bl6 (WT) mice (n=5).

15 After three weeks the mice were sacrificed, the retinas removed and 150 μ m vibratome sections were made of the tissue. Immunostaining was performed to identify bipolar cell strata (anti-ChAT). This requires a 7-day incubation causing a loss in fluorescence, therefore GFP was also labeled (anti-GFP). Sections were imaged by confocal microscopy with equivalent laser intensities, gains and step sizes for a 40X z-stack. Fluorescent bipolar cells are marked with an arrow to distinguish them from amacrine cells (Fig. 7). The amacrine cell bodies lie just beneath the bipolar cell bodies and have a dendritic tree spreading horizontally across the retinal plane directly from the cell body. The bipolar cell body has a 'true' axon descending through the inner plexiform layer to the terminal boutons at the ganglion cell layer (see also Figure 11: AAV-8b-mG6-EGFP).

25 Fluorescence staining shows a strong transduction of the Bruch's membrane and the inner limiting membrane of mouse retina by AAV8wt- EF1 α .EGFP. In contrast, these membranes are not fluorescent in retinas transduced with AAV8b-EF1 α .EGFP. This may account for the greater diffusion of the AAV8b virus through the retina with stronger fluorescence in the region between the inner and outer plexiform layers for AAV8b compared to AAV8wt. Note that these retinas were also labeled for acetyl choline transferase to identify the region between the inner and outer plexiform layers.

Example 4: Quantitative analysis of AAV2/8BP2 in the retina using a non-cell-specific promoter

In vitro and in vivo transduction of AAV2/8BP2 was assayed using pure large-scale preparation. BP2 was confirmed to transduce HEK293 cells in vitro similarly to the parental AAV2/8 suggesting that the modified epitope was not having a significant negative impact on viral packaging (Fig. 12a). Adult C57bl6 mice were subretinally injected with titre-matched AAVs expressing EGFP under the EF1 α promoter and made using either the unmutated AAV8 capsid or the mutant AAV8BP2. After three weeks the retinas were dissociated and processed for FACS analysis. Cell counts (Fig. 12b) show 20% transduction of retinas injected with AAV2/8 versus 32% transduction of retina injected with AAV2/8BP2. A pool of 100,000 cells was taken from each sorted fraction and the relative expression of retinal genes in the green and non-green cell populations was tested by qRT-PCR. Increased expression from bipolar-cell-specific genes, Grm6 and the long form of a transient receptor potential cation channel, TrpM1L was found for the AAV2/8BP2-injected retinas. A 120% increase in Grm6 expression and 67% increase in TrpM1L expression were determined (Fig. 12c). By contrast, equivalent expression levels of cone opsin genes were measured between the pools for AAV2/8 versus AAV2/8BP2 retinas (Fig. 12d), suggesting targeting to the bipolar cells is being achieved even with a strong constitutive promoter such as EF1 α .

Example 5: Transduction of wild-type dog retina with AAV8-b

Both eyes of a normal-sighted, approximately 14 month old male dog were injected with AAV8-b. The right eye received a subretinal injection of 1.66×10^{10} genome copies (GC) in 0.2 ml. The left eye received an intravitreal injection of 1.66×10^{10} in 0.2 ml. The dog was examined by ophthalmoscopy daily after injection to monitor for potential inflammation and to determine when transgene expression first became apparent. Thereafter the animal was evaluated at weekly intervals. Fundus photographs were made 22 days after injection. The animal was euthanized 33 days after injection. The ophthalmoscopic appearance had not changed from that noted on day 22.

No inflammation was noted at any timepoint following injection. EGFP was first identified by ophthalmoscopy 5 days after injection in the subretinally injected eye. Levels of EGFP detectable by ophthalmoscopy increased through the two week timepoint and plateaued

(Figure 8). No EGFP was detectable by ophthalmoscopy in the intravitreally injected eye . It was noted that the dog remained healthy and active throughout the study. At necropsy, no abnormalities were noted in gross pathology.

Histologic analyses of the subretinally injected eye revealed no evidence of inflammation (cell infiltrate, immune cells) (Figure 9) or retinal degeneration (there was normal thickness of photoreceptor layers in injected and uninjected regions of the retina) (Figure 9A). GFP-positive cells were noted only in the region of subretinal injection (and in the region that appeared GFP-positive by ophthalmoscopy) (Figure 9A). GFP-positive cells were tentatively identified by their morphology and location in the retina and included cone photoreceptors (Figures 9A-9B), inner nuclear cells, and ganglion cells. The inner nuclear cells (Fig. 9) and ganglion cells (Fig. 10) included cells with high levels of EGFP and also cells with lower levels of GFP.

Histologic analyses of the intravitreally injected eye revealed no evidence of inflammation (cell infiltrate, immune cells)(Figure 10) or retinal degeneration. EGFP was detectable in ganglion cells by fluorescence microscopy.

15

Example 6: Expression of channelrhodopsin using a bipolar cell-specific promoter

To test for bipolar cell-specific expression of an ocular protein, WT mice were injected subretinally with AAV8-b carrying ON-bipolar cell-specific promoter driving channelrhodopsin (ChR2)-EGFP. The ON-bipolar cell-specific promoter was the metabotropic glutamate receptor 6 (mGluR6) promoter. The OFF-bipolar cells terminate in sublamina 1 and 2 and have shorter axons than the ON-type which terminates in sublamina 4 and 5 (Fig. 11b). Thus, the ON-bipolar cell subtype can be distinguished from the OFF-bipolar cell using the ChAT staining which identifies the specific sublamina of the inner plexiform layer.

Expression in rod (ON) bipolar cells was observed (Fig. 11a). ON-bipolar cell terminals are bright (Fig. 11a), in the optimal location for ChR2 channels.

25

All publications, patents, and patent applications cited in this application, including US Provisional Patent Application No. 61/762,775 are hereby incorporated by reference in their entireties as if each individual publication or patent application were specifically and individually indicated to be incorporated by reference. Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of

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understanding, it will be readily apparent to those of ordinary skill in the art in light of the teachings of this invention that certain changes and modifications can be made thereto without departing from the spirit or scope of the appended claims.

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What is claimed is:

1. A recombinant AAV capsid protein characterized by a mutation in aa 587-595 as compared to the wild type AAV8 vp1 capsid sequence, or a mutation in the analogous region of another AAV capsid as compared to the corresponding AAV wild type capsid sequence.
2. A recombinant AAV capsid protein characterized by a mutation in nt 1759-1785 of the sequence coding for the AAV8 vp1 capsid protein as compared to the AAV8 wild type sequence, or a mutation in the analogous region of the sequence coding for another AAV capsid as compared to the corresponding AAV wild type capsid sequence.
3. The capsid protein of any one of claims 1-2, wherein the wild type sequence is an AAV8 capsid sequence.
4. The capsid protein of claim 3, wherein the wild type sequence is SEQ ID NO: 8.
5. The capsid protein of any of claims 1-4, wherein the capsid is the vp1, vp2 or vp3 capsid protein.
6. A nucleic acid sequence encoding the recombinant AAV capsid protein of any of claims 1-5.
7. The nucleic acid sequence of claim 6, comprising the sequence of SEQ ID NO: 9 or 10.
8. A recombinant AAV capsid protein comprising SEQ ID NO: 1 or SEQ ID NO: 2.
9. The AAV capsid protein of claim 8, comprising SEQ ID NO: 1 or SEQ ID NO: 2 at aa 587-595 of the AAV8 capsid protein or at a corresponding region of another AAV capsid sequence.
10. A nucleic acid sequence encoding the recombinant AAV capsid protein of claim 9.

11. A recombinant AAV capsid protein having the amino acid sequence of SEQ ID NO: 3 or SEQ ID NO: 4.

12. A nucleic acid sequence encoding the recombinant AAV capsid protein of claim 11.

13. The nucleic acid sequence of claim 12, wherein the sequence is SEQ ID NO: 5 or SEQ ID NO: 6.

14. An adeno-associated virus (AAV) having a recombinant AAV capsid comprising a mutation in aa 587-595 of the AAV8 capsid protein sequence as compared to the AAV8 wild type capsid sequence or a mutation in a corresponding region of another AAV capsid protein as compared to the corresponding wild type capsid sequence, further comprising a minigene comprising AAV inverted terminal repeats and a heterologous nucleic acid sequence operably linked to regulatory sequences which direct expression of a product encoded by the heterologous nucleic acid sequence in a target cell.

15. An adeno-associated virus (AAV) having an AAV capsid comprising the sequence of SEQ ID NO: 1 or SEQ ID NO: 2, a minigene comprising AAV inverted terminal repeats and a heterologous nucleic acid sequence operably linked to regulatory sequences which direct expression of a product encoded by the heterologous nucleic acid sequence in a target cell.

16. The AAV according to claim 14 or 15, wherein the capsid has the sequence of SEQ ID NO: 3 or SEQ ID NO: 4.

17. The AAV according to any of claims 14-16, wherein the target cell is an ocular cell.

18. The AAV according to claim 17, wherein the ocular cell is one or more of an RPE cell, a photoreceptor, a Mueller cell, a bipolar cell, a horizontal cell, an amacrine cell, or a ganglion cell.

19. The AAV according to claim 18, wherein the ocular cell is a bipolar cell.
20. The AAV according to claim 18, wherein the ocular cell is a horizontal cell.
21. The AAV according to any of claims 14-20, wherein the regulatory sequences comprise a promoter.
22. The AAV according to claim 21, where the promoter is the human EF1 α promoter.
23. The AAV according to claim 21, wherein the promoter is a bipolar cell-specific promoter.
24. The AAV according to claim 23, wherein the bipolar cell-specific promoter is specific to ON bipolar cells.
25. The AAV according to claim 24, wherein the promoter is metabotropic glutamate receptor 6 (mGluR6) promoter.
26. The AAV according to claim 21, wherein promoter is a horizontal cell-specific promoter.
27. The AAV according to claim 21, wherein the promoter is a photoreceptor cell-specific promoter.
28. The AAV according to claim 27, wherein the promoter is selected from the rhodopsin promoter, cone opsin promoters, and transcription factor promoters (neural retina leucine zipper (Nrl) and photoreceptor-specific nuclear receptor Nr2e3, bZIP).
29. The AAV according to claim 21, wherein the promoter is a ganglion cell-specific promoter.

30. The AAV according to claim 21, wherein the promoter is an inducible promoter, a constitutive promoter or a tissue specific promoter.

31. The AAV according to claim 21, wherein the promoter is an inducible promoter selected from rapamycin/rapalog promoter, the ecdysone promoter, the estrogen-responsive promoter, and the tetracycline-responsive promoter.

32. The AAV according to any of claims 14-31, wherein the product encoded by the heterologous nucleic acid sequence is an opsin.

33. The AAV according to claim 32, where the opsin is a rhodopsin, photopsin, L/M wavelength opsin (red/green) -opsin, or short wavelength (S) opsin (blue).

34. The AAV according to claim 32, wherein the opsin is channelrhodopsin or halorhodopsin.

35. The AAV according to any of claims 14-34, wherein the product encoded by the heterologous nucleic acid sequence is NYX, GRM6, TRPM1L or GPR179.

36. An AAV comprising the capsid protein of any of claims 1-5.

37. The AAV according to claim 15, comprising the sequence of SEQ ID NO: 5 or SEQ ID NO: 6.

38. The AAV according to any of claims 14-37, wherein the AAV is pseudotyped.

39. The AAV according to claim 38, wherein the ITRs are from AAV2.

40. A method of preventing, arresting progression of, or ameliorating vision loss associated with an ocular disorder in a subject, said method comprising administering to said subject an

effective concentration of a composition comprising the recombinant adeno-associated virus (AAV) of any of claims 14-39, and a pharmaceutically acceptable carrier.

41. A method of targeting bipolar cells for optogenetic therapy in a subject in need thereof, the method comprising administering to said subject an effective concentration of a composition comprising the recombinant adeno-associated virus (AAV) of any of claims 14-39, and a pharmaceutically acceptable carrier.

42. A method of targeting one or more type of ocular cells for optogenetic therapy in a subject in need thereof, the method comprising administering to said subject an effective concentration of a composition comprising the recombinant adeno-associated virus (AAV) of any of claims 14-39, and a pharmaceutically acceptable carrier.

43. A method of targeting one or more type of ocular cells for gene augmentation therapy in a subject in need thereof, the method comprising administering to said subject an effective concentration of a composition comprising the recombinant adeno-associated virus (AAV) of any of claims 14-39, and a pharmaceutically acceptable carrier.

44. A method of targeting one or more type of ocular cells for gene suppression therapy in a subject in need thereof, the method comprising administering to said subject an effective concentration of a composition comprising the recombinant adeno-associated virus (AAV) of any of claims 14-39, and a pharmaceutically acceptable carrier.

45. A method of targeting one or more type of ocular cells for gene knockdown/augmentation therapy in a subject in need thereof, the method comprising administering to said subject an effective concentration of a composition comprising the recombinant adeno-associated virus (AAV) of any of claims 14-39, and a pharmaceutically acceptable carrier.

46. A method of targeting one or more type of ocular cells for gene correction therapy in a subject in need thereof, the method comprising administering to said subject an effective

concentration of a composition comprising the recombinant adeno-associated virus (AAV) of any of claims 14-39, and a pharmaceutically acceptable carrier.

47. A method of targeting one or more type of ocular cells for neurotropic factor gene therapy in a subject in need thereof, the method comprising administering to said subject an effective concentration of a composition comprising the recombinant adeno-associated virus (AAV) of any of claims 14-39, and a pharmaceutically acceptable carrier.

48. A method of targeting all ocular cells for optogenetic therapy in a subject in need thereof, the method comprising administering to said subject an effective concentration of a composition comprising the recombinant adeno-associated virus (AAV) of any of claims 14-39, and a pharmaceutically acceptable carrier.

49. The method according to any of claims 40 to 48, wherein the composition is administered by subretinal injection.

50. The method according to any of claims 40-49, wherein the subject has, or is at risk of developing, retinitis pigmentosa, rod-cone dystrophy, Leber's congenital amaurosis, Usher's syndrome, Bardet-Biedl Syndrome, Best disease, retinoschisis, Stargardt disease (autosomal dominant or autosomal recessive), untreated retinal detachment, pattern dystrophy, cone-rod dystrophy, achromatopsia, ocular albinism, enhanced S cone syndrome, diabetic retinopathy, age-related macular degeneration, retinopathy of prematurity, sickle cell retinopathy, Congenital Stationary Night Blindness, or retinal vein occlusion.

51. The method according to any of claims 40-50 wherein the subject has, or is at risk of developing glaucoma, Leber's hereditary optic neuropathy, lysosomal storage disorder, peroxisomal disorder.

52. The method according to any of claims 40-51, wherein the composition is administered before disease becomes symptomatic.

53. The method according to any of claims 40-51, wherein the composition is administered after disease becomes symptomatic.

54. The method according to any of claims 40-51, wherein the composition is administered after initiation of photoreceptor loss.

55. The method according to any of claims 40-51, wherein the composition is administered after outer nuclear layer (ONL) degeneration begins.

56. The method according to any of claims 40-51, wherein the composition is administered while bipolar cell circuitry to ganglion cells and optic nerve remains intact.

57. The method according to any of claims 40-51, wherein the effective concentration is about 10^9 to 10^{13} genome copies per milliliter (GC/mL).

58. The method according to any one of claims 40-51, wherein the composition is administered in a volume of from 0.1 μ L-1 mL.

59. The method according to claim 58, wherein the composition is administered in a volume of 150 μ L.

60. The method according to any one of claims 40-59, wherein the pharmaceutically acceptable carrier comprises an isotonic sodium chloride solution.

61. The method according to any one of claims 40-60 wherein the pharmaceutically acceptable carrier contains a surfactant, such as perfluorooctane (Perfluron liquid).

62. The method according to any one of claims 40-61, wherein the gene is expressed in one or more of the RPE cells, photoreceptors, Mueller cells, bipolar cells, horizontal cells, amacrine cells, or ganglion cells of the subject.

63. The method according to any one of the claims 40-62, wherein the gene is expressed and delivered to an intracellular organelle, such as a mitochondrion or a lysosome.

64. The method according to claim 62, wherein the gene is expressed in the bipolar cells or horizontal cells of the subject.

65. The method according to any one of claims 40-64, wherein the composition is administered in combination with a secondary therapy.

66. The method according to claim 65, wherein the secondary therapy is selected from one or more of neurotrophic factor therapy (such as pigment epithelium-derived factor, PEDF, RdCVF), encapsulated cell therapy (such as that delivering ciliary neurotrophic factor, CNTF), or anti-apoptosis therapy (such as that delivering X-linked inhibitor of apoptosis, XIAP), rod derived cone viability factor 2, ciliary neurotrophic factor 3, and glial-derived neurotrophic factor.

67. The method according to any one of claims 40-66, wherein administration of the composition is repeated at least once in the same eye and/or contralateral eye.

68. A method of generating a recombinant adeno-associated virus (rAAV) comprising an AAV capsid comprising culturing a host cell containing: (a) a molecule encoding the AAV capsid protein of any of claims 1-5; (b) a functional rep gene; (c) a minigene comprising AAV inverted terminal repeats (ITRs) and a heterologous nucleic acid sequence operably linked to regulatory sequences which direct expression of a product encoded by the heterologous nucleic acid sequence in the target cell; and (d) sufficient helper functions to permit packaging of the minigene into the AAV capsid protein.

69. A host or target cell transfected with an AAV according to any of claims 14-39.

70. A composition comprising an AAV according to any of claims 14-39 and a physiologically compatible carrier.

71. A method of delivering a transgene to a cell, said method comprising contacting the cell with an AAV according to any of claims 14 to 39, wherein said AAV comprises the minigene.

72. Use of an AAV according to any of claims 14 to 39 or a molecule according to any of claims 7 to 14 in preparing a medicament for delivery of a heterologous nucleic acid sequence encoding the product to a cell.

73. The AAV according to any of claims 14 to 39, wherein the AAV is a self-complementary AAV.

FIGURE 2

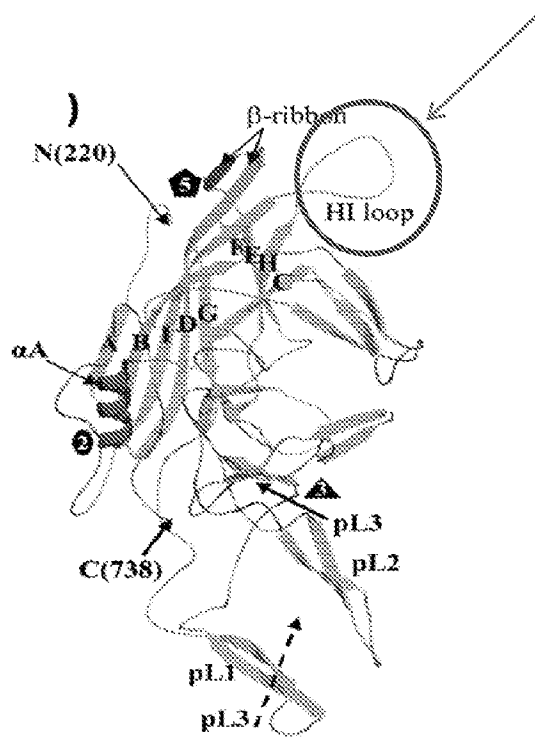


FIGURE 3

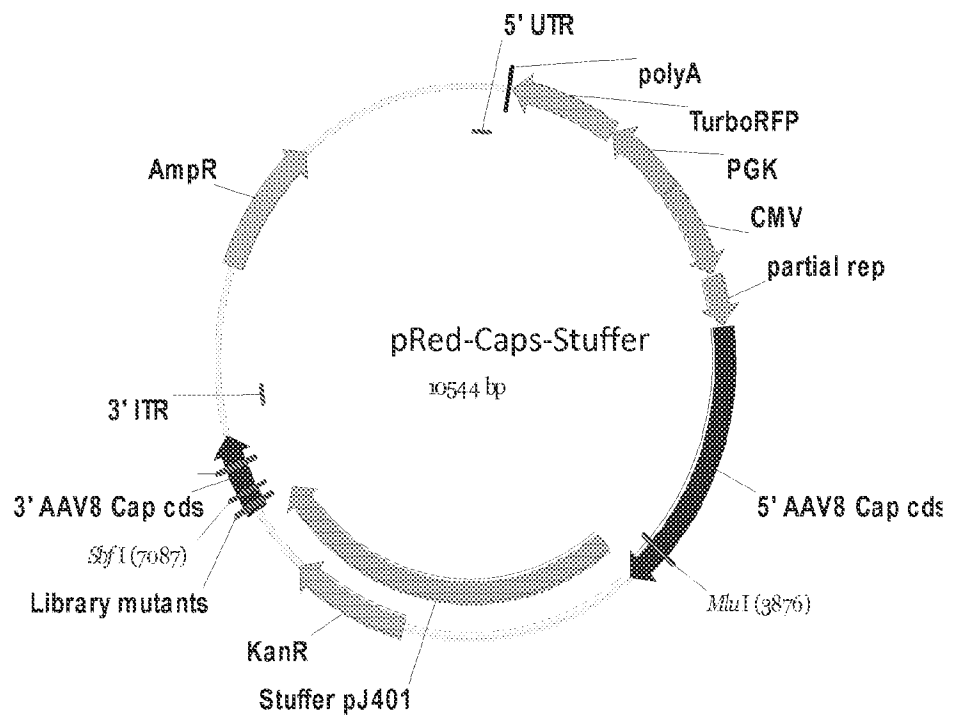
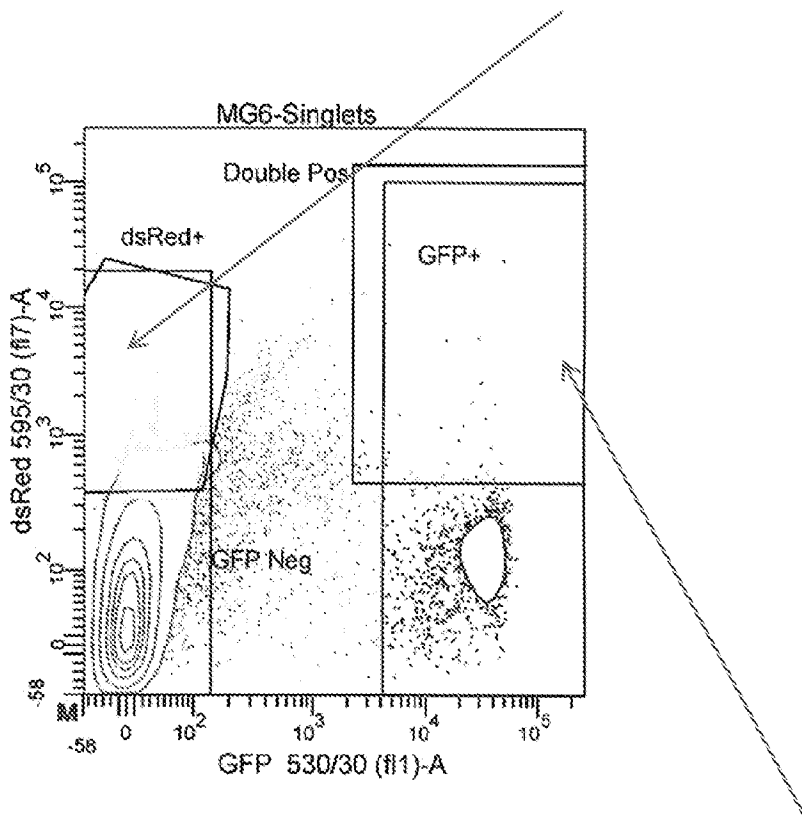


FIGURE 4

All non-bipolars transfected with AAV:
(Positive for dsRED alone: 9.1% of parent population)



ON-bipolars transfected with AAV:
(Double positive for dsRED and GFP: 1% of parent population)

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FIGURE 5

(from aa585)

WT	CAG	CAG	CAA	AAC	ACG	GCT	CCT	CAA	ATT
	Gln	Gln	Gln	Asn	Thr	Ala	Pro	Gln	Ile
	<i>P</i>	<i>P</i>	<i>P</i>	<i>P</i>	(<i>P</i>)	(<i>N</i>)	<i>P</i> [^]	<i>P</i>	(<i>N</i>) [*]
Var 1	TAT	CTT	ATG	CGT	TAT	ATT	GGT	GTT	TTT
	Tyr	Leu	Met	Arg	Tyr	Ile	Gly	Gly	Phe
	<i>P</i>	<i>N</i> [*]	<i>N</i> [*]	<i>B</i> [^]	<i>P</i>	(<i>N</i>) [*]	<i>N</i>	<i>N</i>	<i>N</i> [*]
Var 2	CCT	GAG	CGG	ACG	GCG	ATG	AGT	CTT	CCG
	Pro	Glu	Arg	Thr	Ala	Met	Ser	Leu	Pro
	<i>P</i> [^]	<i>A</i>	<i>B</i> [^]	<i>N</i>	<i>N</i>	<i>N</i> [*]	<i>P</i>	<i>N</i> [*]	<i>P</i> [^]
Var 3	AGT	TTT	AGT	CGT	GCG	GTT	CTT	TGT	GAT
	Ser	Phe	Ser	Arg	Ala	Val	Leu	Cys	Asp
	<i>P</i>	<i>N</i> [*]	<i>P</i>	<i>B</i> [^]	<i>N</i>	<i>N</i> [*]	<i>N</i> [*]	<i>P</i>	<i>A</i>
Var 4	CAT	TGT	GTG	GAT	TGT	TGT	GCG	TCT	TAT
	His	Cys	Val	Asp	Cys	Cys	Ala	Ser	Tyr
	<i>B</i> [^]	<i>P</i>	<i>N</i> [*]	<i>A</i>	<i>P</i>	<i>P</i>	<i>N</i>	<i>P</i>	<i>P</i>
Var 5	CAT	ACT	GAG	TAT	ATG	AGT	GAG	TAG	CTC
	His	Thr	Glu	Tyr	Met	Ser	Glu	!!!!	Leu
	<i>B</i> [^]	<i>N</i>	<i>A</i>	<i>P</i>	<i>N</i> [*]	<i>P</i>	<i>A</i>	<i>stop</i>	<i>N</i> [*]
Var 6	CCG	ATT	TTT	GTT	GGG	TGT	TCT	GIG	CTT
	Pro	Ile	Phe	Val	Gly	Cys	Ser	Val	Leu
	<i>P</i> [^]	(<i>N</i>) [*]	<i>N</i> [*]	<i>N</i> [*]	<i>N</i>	<i>P</i>	<i>P</i>	<i>N</i> [*]	<i>N</i> [*]

FIGURE 6

Fig. 6A

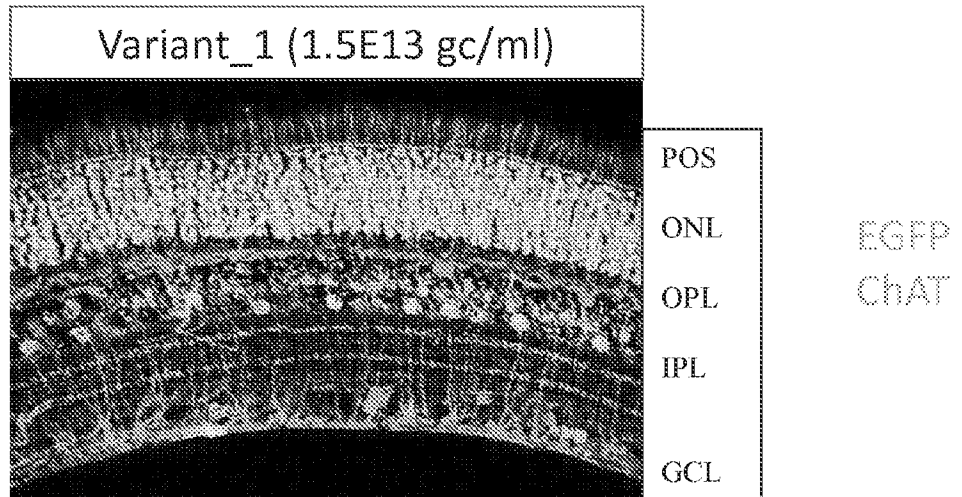


Fig. 6B

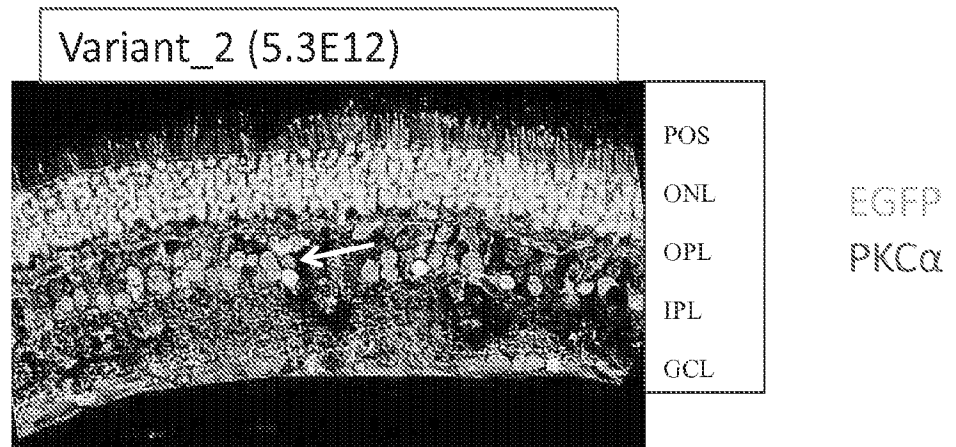


Fig. 6C

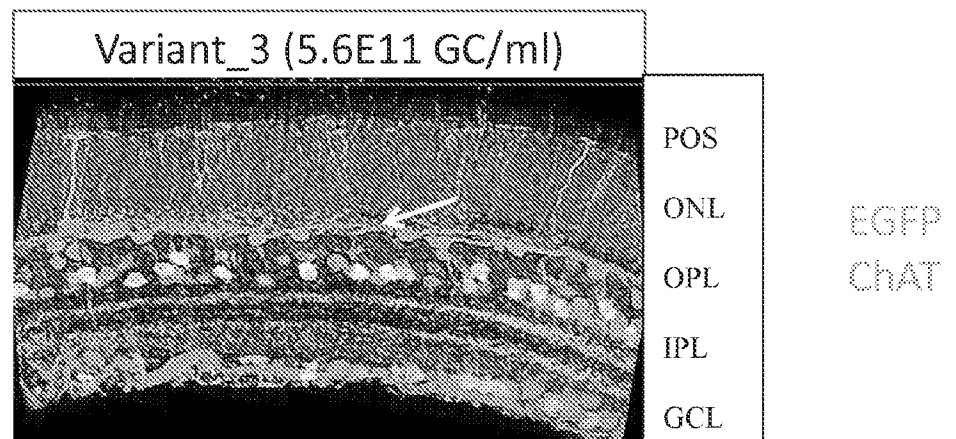


Fig. 6D

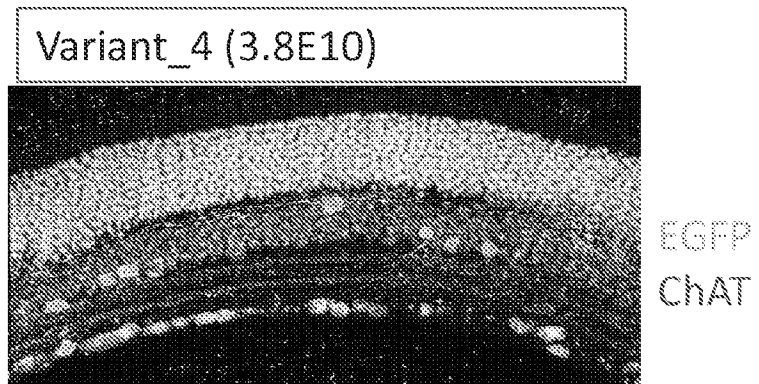
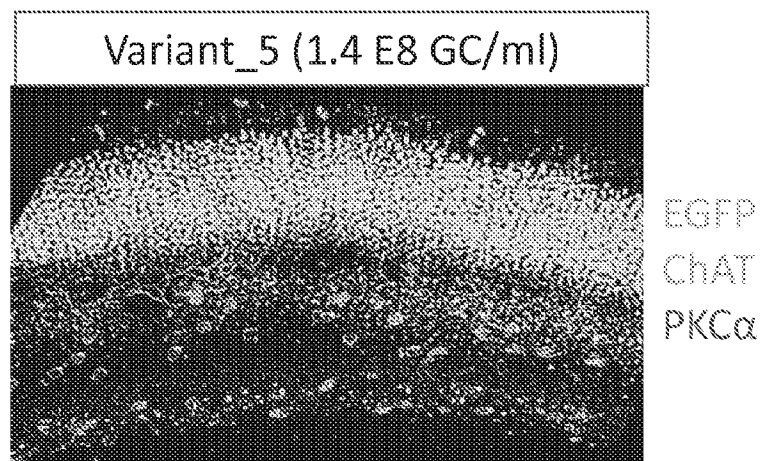


Fig. 6E



POS: Photoreceptor Outer Segments; ONL: Outer Nuclear Layer;
OPL: Outer Plexiform Layer; IPL: Inner Plexiform Layer; GCL:
Ganglion Cell Layer.

FIGURE 7

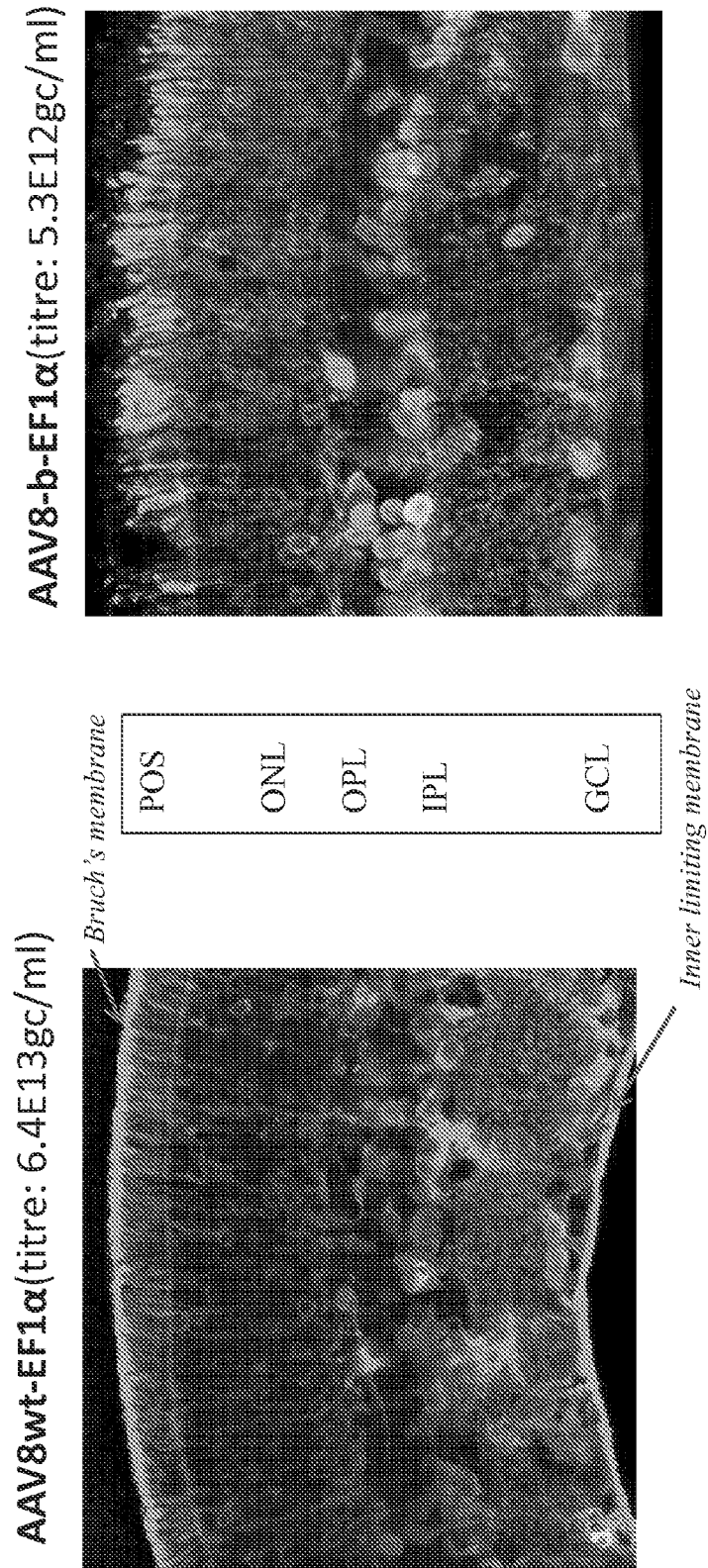


Fig. 8

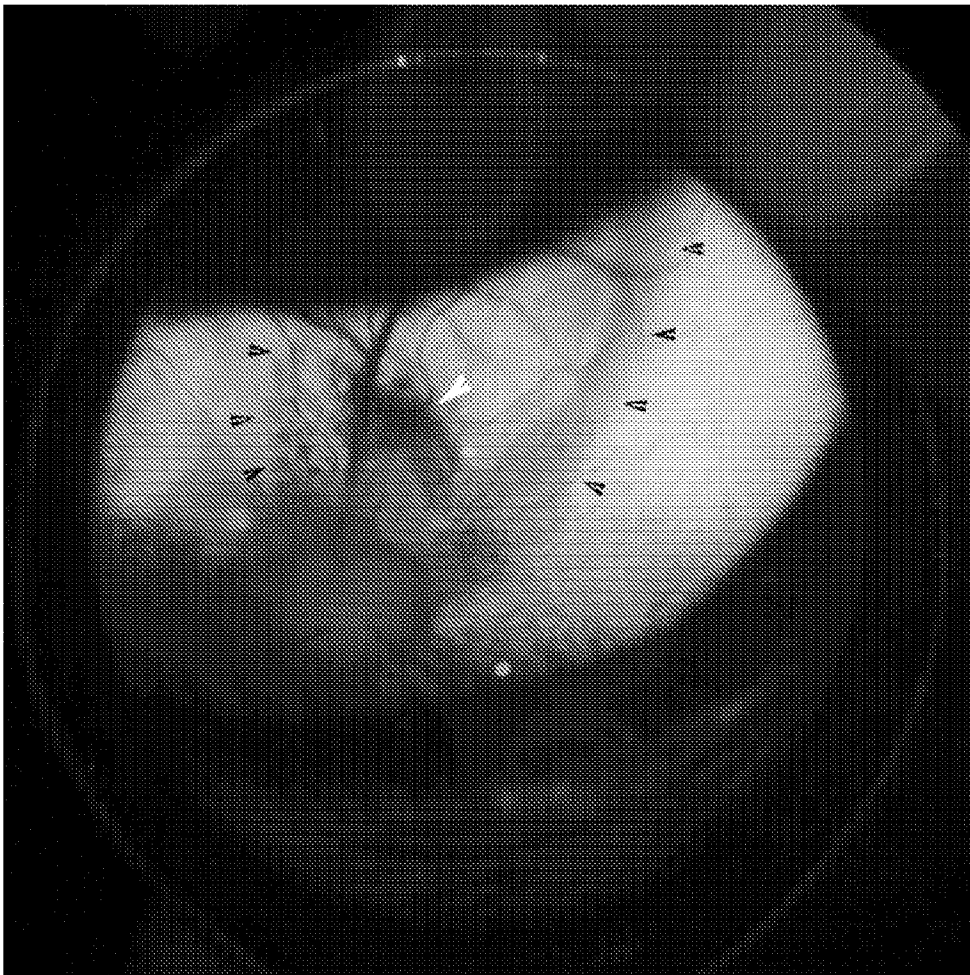


FIG. 9

Fig. 9A

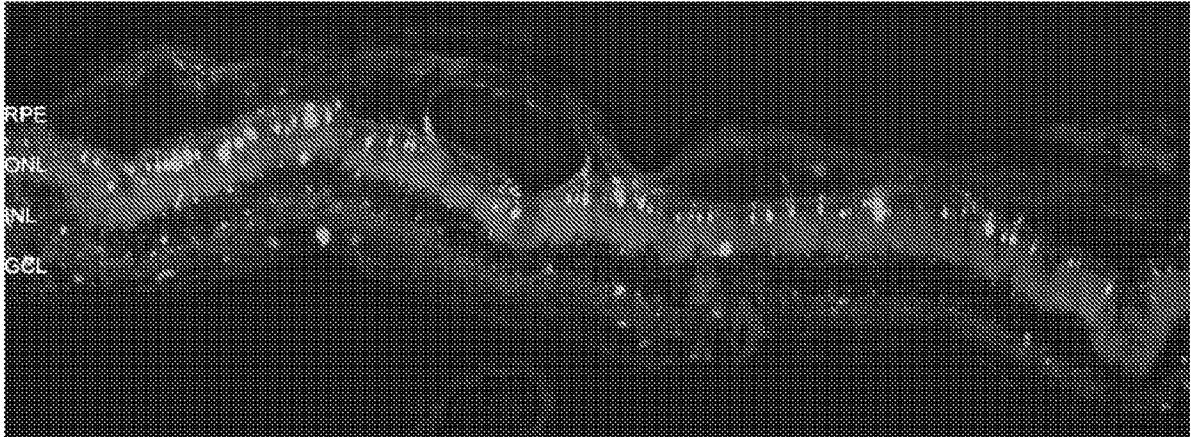


Fig. 9B

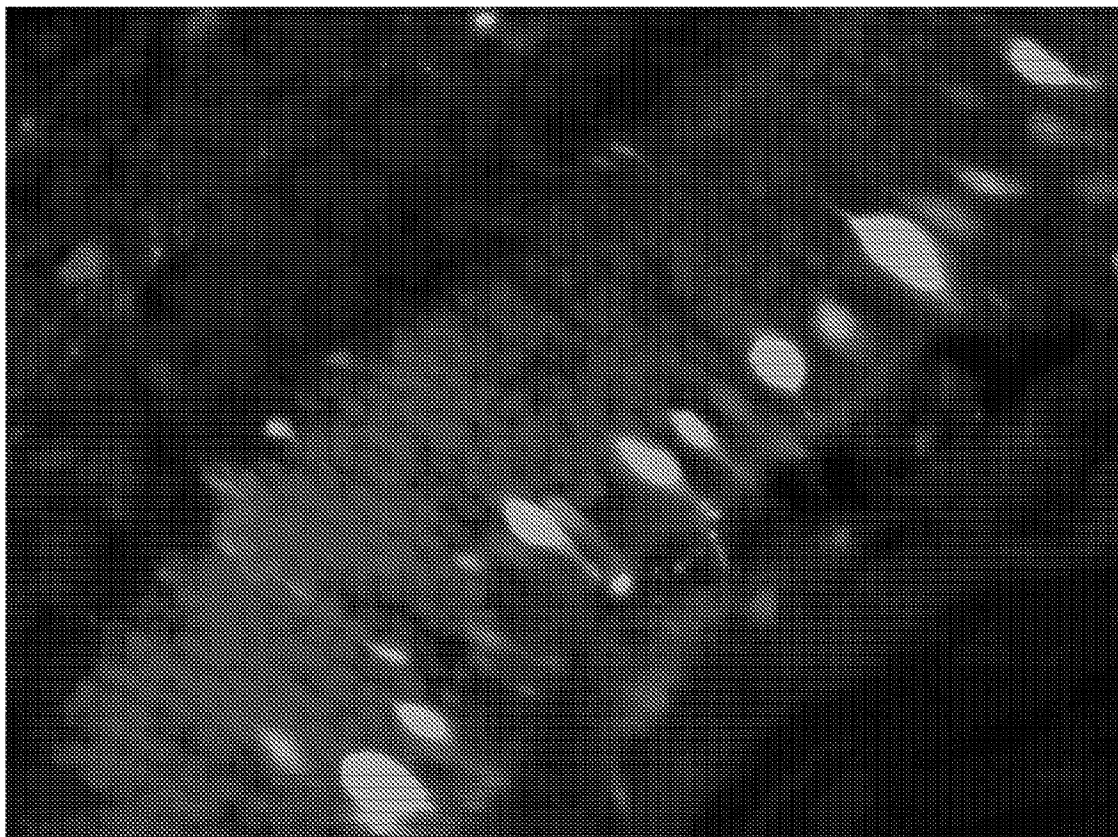


FIG. 10

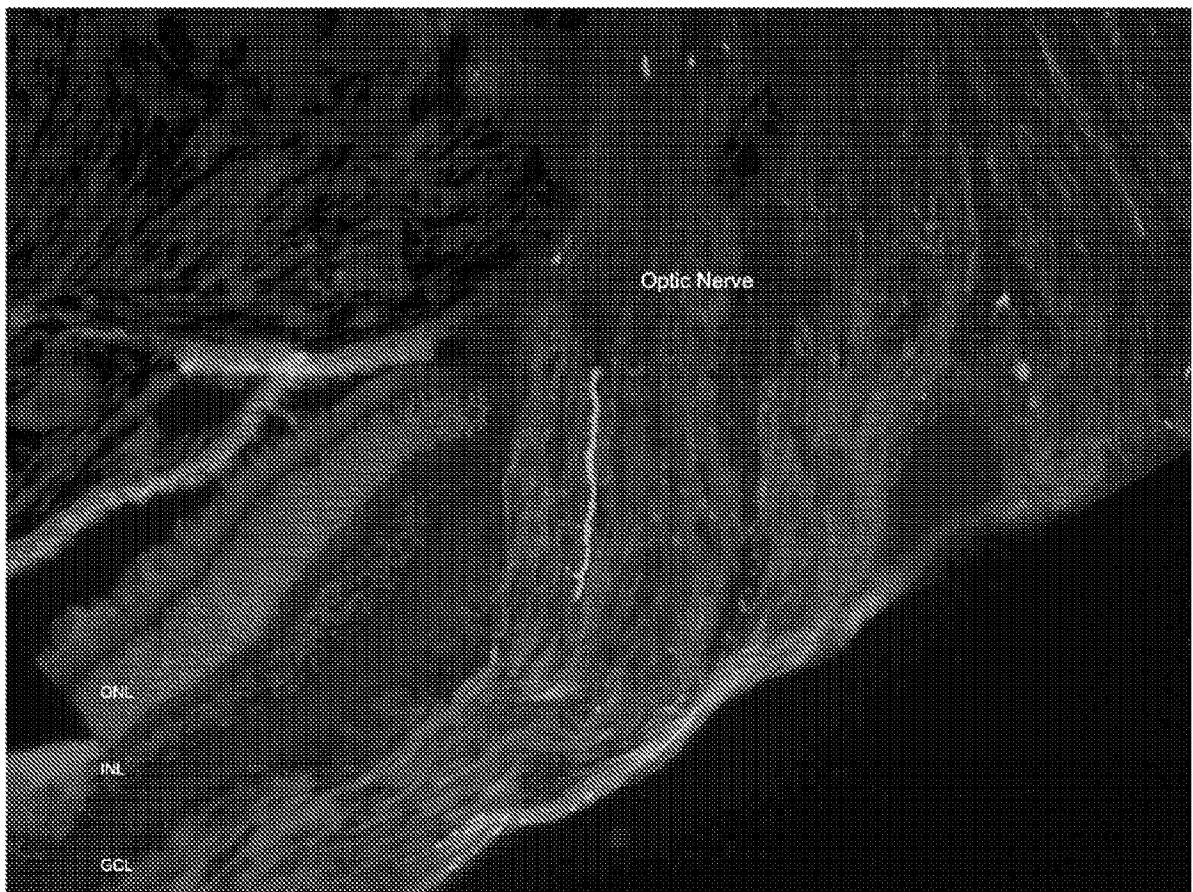


FIGURE 11

Fig. 11A

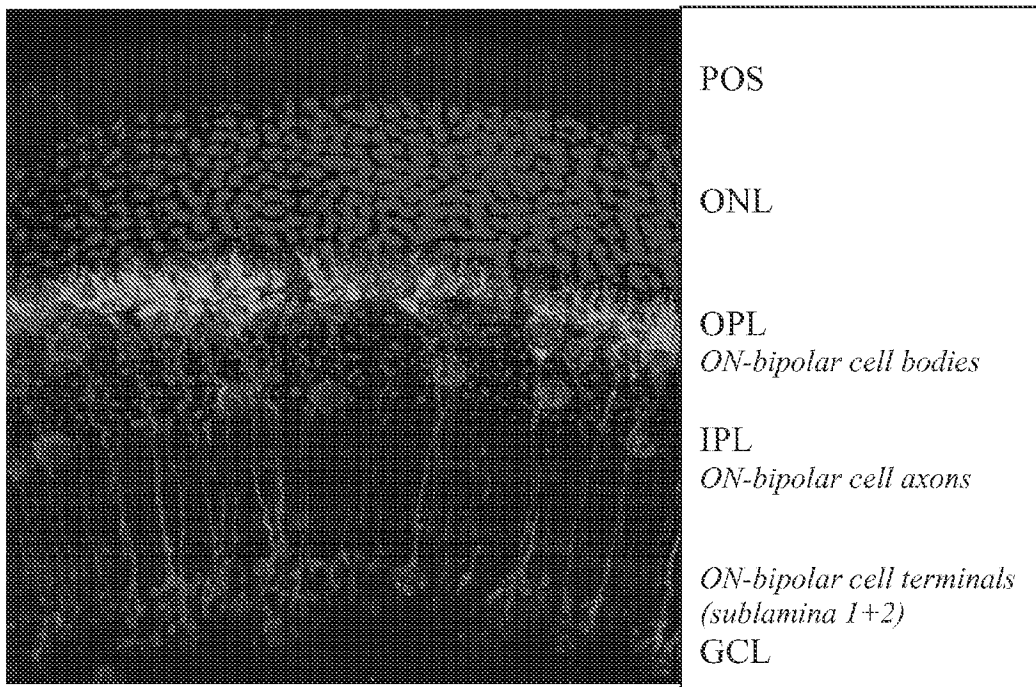


Fig. 11B

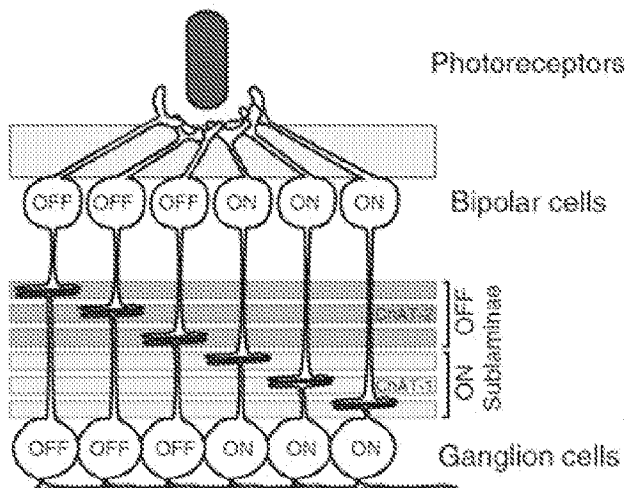


Fig. 12A

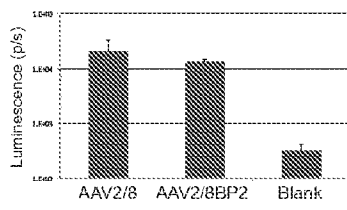


Fig. 12B

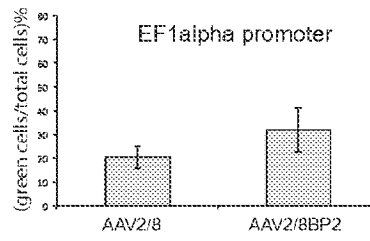


Fig. 12C

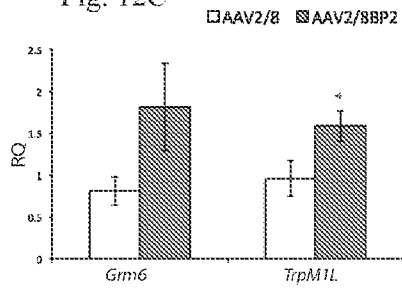
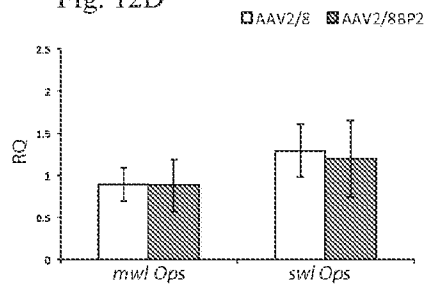


Fig. 12D



INTERNATIONAL SEARCH REPORT

International application no.
PCT/US2014/015340

A. CLASSIFICATION OF SUBJECT MATTER
 IPC(8) - C12N7/01 (2014.01)
 USPC - 424/93.2
 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)
 IPC(8) - A61K 48/00; C12N 7/00, 7/01, 15/864 (2014.01)
 USPC - 424/93.2; 435/235.1, 456; 536/23.72

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
 CPC - A61K 48/00; C07K 14/005; C12N 7/00, 2750/14122 (2014.02)

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	RAUPP et al. "The threefold protrusions of adeno-associated virus type 8 are involved in cell surface targeting as well as postattachment processing," J Virol. 20 June 2012 (20.06.2012), Vol. 86, No. 17, Pgs. 9396-9408. entire document	1-3, 14
A	US 2003/0228282 A1 (GUO et al) 11 December 2003 (11.12.2003) entire document	1-4, 8-16, 37
A	WO 2012/145601 A2 (SCHAFFER et al) 26 October 2012 (26.10.2012) entire document	1-4, 8-16, 37
A	US 2012/0066783 A1 (KAY et al) 15 March 2012 (15.03.2012) entire document	1-4, 8-16, 37
A	US 2004/0052764 A1 (HILDINGER et al) 18 March 2004 (18.03.2004) entire document	1-4, 8-16, 37

Further documents are listed in the continuation of Box C.

* 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 16 April 2014	Date of mailing of the international search report 14 MAY 2014
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Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Blaine R. Copenheaver PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774
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INTERNATIONAL SEARCH REPORT

international application no.

PCT/US2014/015340

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.: 5-7, 17-36, 38-73
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:

- 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.:

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/US2014/015340

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 filed or furnished:

a. (means)

on paper

in electronic form

b. (time)

in the international application as filed

together with the international application in electronic form

subsequently to this Authority for the purposes of search

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 in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

Specifically, SEQ ID NOs:1-6 and 8 were searched.