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(54) Title: A MODIFIED MGLUR6 PROMOTER AND METHODS OF USE

(57) Abrégé/Abstract:

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WO 2015/138616 A1

A MODIFIED MGLUR6 PROMOTER AND METHODS OF USE

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of provisional application USSN 61/951,360, filed March 11, 2014.

GOVERNMENT SUPPORT

[0001] This invention was made with U.S. Government support under the National Institutes of Health/National Eye Institute grant NIH EY 17130. The Government has certain rights in the invention.

FIELD OF THE INVENTION

[0002] This invention relates generally to the field of molecular biology. The invention features modified metabotropic glutamate receptor 6 (mGluR6) promoters for increased expression in the retina. The modified mGluR6 promoters described herein are useful in ocular gene therapy for the improvement and/or restoration of vision.

BACKGROUND OF THE INVENTION

[0003] Gene therapy is a promising approach for improving and restoring vision. Particularly, delivery of genes, such as photosensitive proteins, to diseased or damaged retinas in mice have been recently shown to improve and restore photosensitivity and visual signals. However, challenges still remain for the efficient targeting and expression of such genes in specific inner retinal neurons for ocular gene therapy. Accordingly, there is a pressing need for improved regulatory elements and expression vectors for the delivery and expressing of transgenes for ocular gene therapy.

SUMMARY OF THE INVENTION

[0004] The invention provides a solution for the long-felt need for improved promoters and nucleic acid expression vectors for the delivery and expression of transgenes to the eye. Specifically, the promoters and vectors described herein comprise or consist essentially of a modified metabotropic glutamate receptor 6 (mGluR6) promoter that contains sequences from regulatory elements that direct the expression of the mGluR6 protein to ON bipolar cells, or retinal rod bipolar cells.

[0005] The present invention features an isolated nucleic acid molecule or a nucleic acid expression vector comprising an mGluR6 enhancer or a variant thereof and an mGluR6 promoter or a variant thereof. Optionally, the nucleic acid molecule further comprises intron 3 of the mGluR6 gene or a variant thereof. Optionally, the nucleic acid molecule further comprises intron 4 of the mGluR6 gene or a variant thereof.

[0006] The present invention features an isolated nucleic acid molecule or a nucleic acid expression vector comprising an mGluR6 enhancer or a variant thereof, an mGluR6 promoter or a variant thereof, an intron 3 of the mGluR6 gene or a variant thereof, and an intron 4 of the mGluR6 gene or a variant thereof.

[0007] A nucleic acid expression vector of the present invention may be a viral vector, preferably an adeno-associated virus vector or a recombinant adeno-associated virus (rAAV) vector. In other embodiments, the AAV vectors used with the present invention, *e.g.*, a packaging vector, comprise a capsid protein.

[0008] The present invention also provides a pharmaceutical composition comprising one or more of the nucleic acid expression vectors described herein and a pharmaceutically acceptable excipient. In some embodiments, AAV vectors of more than one serotype may be combined into a single composition for administration, or may be administered sequentially over suitable time periods.

[0009] The present invention further provides a method for expressing a transgene in the eye comprising introducing into the eye the nucleic acid expression vector described herein. The nucleic acid expression vector may be introduced to the eye by subretinal, intraocular, or

intravitreal injection. The methods described herein may be useful for increasing light sensitivity, increasing light detection, increasing photosensitivity, increasing visual evoked potential, or improving or restoring vision in a retina of a subject. For example, the subject suffers from an ocular disorder or disease associated with photoreceptor degeneration.

[0010] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention pertains. Although methods and materials similar or equivalent to those described herein can be used in the practice of the present invention, suitable non-limiting exemplary methods and materials are described below. In cases of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples described herein are illustrative only and are not intended to be limiting.

[0011] Other features and advantages of the invention will be apparent from and are encompassed by the following detailed description and claims.

BRIEF DESCRIPTION OF THE FIGURES

[0012] **Figure 1** shows a schematic diagram of an rAAV2 genome vector.

[0013] **Figure 2** shows a schematic diagram of an rAAV2 packaging vector, with a mutation in the viral capsid gene (Y444F).

[0014] **Figure 3** shows a schematic diagram of an optimized promoter construct I4 comprising a 200 bp mGluR6 enhancer and a 500bp fragment of the mGluR6 promoter upstream of a transgene (mCherry).

[0015] **Figure 4** shows a schematic diagram of an optimized promoter construct I1a comprising an intron 4 from mGluR6, intron 3 from mGluR6, a 200 bp mGluR6 enhancer and a 500bp fragment of the mGluR6 promoter upstream of a transgene (mCherry).

[0016] **Figure 5** is a series of immunofluorescence images demonstrating rAAV-mediated expression of transgene (mCherry) by two optimized mGluR6 promoter constructs (I4 and I1a). (A, D) Images were taken at the ganglion cell layer in retinal

whole-mounts to visualize the axon terminals of rod bipolar cells. (B, E) Images were taken in inner nuclear layer in retinal whole-mounts to visualize the somas of rod bipolar cells. (C, F) The expression of transgene (mCherry) in rod bipolar cells is depicted in retinal vertical sections. [0017] **Figure 6** is a series of immunofluorescence images demonstrating the expression of a transgene targeted to rod bipolar cells. The rAAV2/2 (Y444F)-mediated expression of mCherry driven by an optimized mChluR6 promoter is depicted in retinal whole-mount (A) and in retinal vertical section (D). Co-staining mCherry with anti-PKC (a rod bipolar cell marker) is also shown in retinal whole-mounts (A-C) and vertical sections (D-F).

DETAILED DESCRIPTION

[0018] The metabotropic glutamate receptor (mGluR6, also known as Grm6) mediates the synaptic transmission in the nervous system and mediates the ON-response in the ON-pathway of the vertebrate retina. Expression of mGluR6 is found on ON-type retinal bipolar cells, which has made the promoter region of mGluR6 a good candidate for cell-specific promoter-driven expression in bipolar cells (Ueda et al., Journal Neurosci., 1997).

Modified mGluR6 promoters

[0019] Previous studies have utilized a basal SV40 promoter with a mGluR6 enhancer sequence (*e.g.*, 200 bp) for AAV-mediated targeting and expression in the eye (Doroudchi et al., Mol. Ther., 2011, 19:1220-1229). However, the transgene expression by these constructs was weak, and not fully selective. The invention described herein is based upon the surprising discovery that use of a fragment of the mGluR6 promoter region (*e.g.*, about 1 kb or 500 bp) results in increased expression compared to SV40-based constructs, and, importantly, more selective expression in specific cell populations.

[0020] The instant invention features modified mGluR6 promoters with increased efficiency and targeting transgene expression to bipolar cells. The promoters described herein were discovered by constructing various constructs with different combinations of regulatory elements present in the mGluR6 gene or predicted to be present. A modified mGluR6 promoter useful to

achieve AAV-mediated selective and highly efficient expression in retinal bipolar cells was identified. As used herein, the term “modified mGluR6 promoter” refers to the combination of regulatory elements described herein that includes at least a 200 bp mGluR6 enhancer sequence and at least a fragment of the promoter region from mGluR6 gene (*e.g.*, about a 1 kb or a 500 bp fragment). Optionally, the modified mGluR6 promoter also includes the intron sequences of the mGluR6 gene, *e.g.*, intron 3 and intron 4. Exemplary modified mGluR6 constructs are shown in Figures 3 and 4.

[0021] The present invention provides a modified mGluR6 promoter that comprises at least a 200 bp mGluR6 enhancer. A preferred nucleic acid sequence for the murine 200 bp mGluR6 enhancer is provided below: (SEQ ID NO: 1)

```
gattctccagatggctaaacttttaaatcatgaatgaagtagatattaccaaattgcttttcagcatccatttagataatcatgtttttgcctttaatct
gttaatgtagtgaattacagaaatacatttcctaaatcattacatccccaaatcgtaaatctgctaaagtacatctctggctcaaacaagactggt
tgtg
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[0022] A preferred nucleic acid sequence for the human 198 bp mGluR6 enhancer (corresponding to the mouse 200 bp enhancer) is provided below in SEQ ID NO: 2:

```
gattccttagattatgaacatttacaattatgaatgaatattagatgttatcaaatgctttttctgcatccatttagataatcatgtttttcctttaatctgt
taatgcggtgaattacattaatagatttcctaaagtcattaatctgctaaagtcatttctgggacaaaccagacttggttatgacattgtatgta
```

[0023] The modified mGluR6 promoter further comprises at least a fragment of the mGluR6 promoter region. A preferred example of this promoter region sequence consists of 11023 nucleotides (GenBank Accession No. BC041684). The original Ueda *et al.*, study employed a 10 kb promoter, but the actual length of the promoter and the sequence that comprises control elements of mGluR6 can be adjusted by increasing or decreasing the fragment length. For example, a fragment of the promoter about 1 kb in length can be used as a mGluR6 promoter sequence. Promoter analysis can be used to identify promoter functional fragments and derivatives (McGowen et al. Mol. Vision 1998, 4:2; Bookstein et al. PNAS 1990, 87 (19) 7762-66).

[0024] The sequences provided herein are meant to be exemplary and in no way limit the scope of the invention. A person of ordinary skill in the art, in view of the instant disclosure,

would be enabled to identify further suitable enhancer, promoter, transgene, and vector sequences.

[0025] For example, the mGluR6 promoter sequence used herein is a 1095 bp fragment of the mGluR6 promoter region. A nucleic acid sequence of the 1095 bp (about 1kb) fragment of the murine mGluR6 promoter region is provided below in SEQ ID NO: 3:

taaccatgcacgctcgcacacgatagataatacatcaccaatatctgaaaagagaaaaaggttctagtggtcaggacagagaatgaaaacg
gcaggaaggcaagaaagtgtgagaacgtaggggggtggggtaggagagacactacgagtggaaataagccacgtttggagaacgtctaggc
agatacagaaatgcagaacacagagagaccgagaccagagcagcgtcagaccggctgcaaggctctgttaggggctttagaaacact
gtgtgctctcccgaagcctggtgcagtcagagaggaagcttctccagacagagatgacacagttcacaaacctgtcagaccacctg
caggagagactgaacccagcaaccagaaccacttggctatgcatgtccttttctgtttaaacctaaagtctctgaagaccgaccaggggagt
ccctggactctttgttctcttctcggggtggcgggactgattgtgtaaatctcttatctccaactttcactcttatctgtctttaaaccgcatatt
gaggatgagtggccaagcttattggtgttctggtgagacaaatfaaggcagctctaggggagaagcagaccagggagtcagagagg
cagagagagaagagagcccttctccactctcaagctctggagggggtctctgccctcacctcatccctcccagaatccttaaatcctcta
gactgtagctctgattttacagctgtcacagactcgtctactagccagaggttggctcaggttaagcaccactggggaggtagcctagggtg
cgctgggggtgggtccagaggaagagctgccagaactgtgggggaaggagcgggaccgaccatcaacaggggggacttttcaggggaga
atgagagcaatcctctggaggcctgggagaggtctgtgagttgctggtgcgcgagtcaccaacttttctgcgtctcgggttcggccag
aatcccgaagtggcagctgagcacgggggtggcagcttcgtccgccggtctcaaggcgtcccgttaactcctttcccgagtcaggag
ca

[0026] A preferred nucleic acid sequence of the human 1784 promoter (corresponding to the mouse mGluR 1095 bp promoter) fragment of the human mGluR6 promoter region provided below in SEQ ID NO: 4:

caagcaggaggctgctgtgtgctgggagctgtcaggtcgtctgaacagggaaggggcccatccacctccaaaaccagtttatgcagtc
cttcgcaatgtcaggctcagggcctggcaccagcaagctccccacccttccactgttaaatggataggagcagggttagggccagcc
tgttgactctgggcttccaccaggagaagtgttctggcagtagaaactatcggggcctgggagaggcgggggaagagagaaaggtggc
atgtttcttctgtctccctctaccagcctgttccaaatccccgcagccaccctaatccagcctgtctaatggagcccaagccggctcaggcc
ctcgacgaggagcctgctaaccctgtggctaggagctcaccacctgtctccaggacgcccttgcctcttggcatcagagagccaaatc
ctgggcctcgatgggggatgataaaagcatctttggccaagccccctcaccttggcctccacgatgagatggggaggttaggtgcagag
agcgttggcacagtgcagccgcagctcagtggtgcctcagaccagagcccagaggagactttatcggagccagaacgacccgc
gggggtccatcctccaagcaataggcgggagtgaggagctgcgaggaaagccggccccctccccctccatccaaggcagtggtgggt
gtttgttcatgccattctgggtgtgaatcctgatgccacacatgccagctgcagtcacttgggcaactcaactcactcctcaggggctgttct
tcgactgcagggtgtgttaagttcgtaataactaaaggcttctccctctggccccctcctgccccctgcttctcctcttcttaggcctcc
cagctcaggcagccccctgccccctgcaggggttctgcaaggagaagctggggaataccttaggcaactgcagtcaggagcactggtggc
caggacagagacagagacagaaaaaggggtcaggagacagagagataaccgaggagagacaggaagggacagagacagaa
aagattccaagaagaggacagaggcagaaagccaggacagagactgagaacagagacctagaggcagaagaagactgagatag
agatggacagagattgtgtcagacacagccccagagacagccagacagctgagtcagacgcaaaccaaagacaagaaaacaggaaaa
cagaccagagattgggagagggagggggaaggagatgcggggagagccagcaccgccccccacactcaggaggggtctccacc

ctcggagcgggtctctcatccctccctagaaatccttaaatcctctctcgtcagggcctcgccgcacatctgtcacagacttgtcctgaaccgaca
 ggggtctcgtcaggtgactggcttggggcgggagcctgggtgtgcgtggggatggaccccgaggaagaggggccaagctgtcggg
 aagcggcagggctggaggggtggagggcagtggtcgggaccccgggcgacaggggttcggcgcttgaagagcgagacggagg
 cccgggagggcggctgagtaactcccagagccgaagtggaggcgccccgagcgcttctcccaggaccccggtgtccctcc
 ccgccccgagccccgcgtctctcttccccgcctcagagcgctccccgccccctgtctccccgcagccgctagacgagccg

[0027] Preferably, the mGluR6 promoter sequence used herein is a 500 bp fragment of the mGluR6 promoter region. The 500 bp fragment described in SEQ ID NO: 5 is encompassed by the 1095 bp fragment described above (SEQ ID NO: 3). The 500 bp fragment is preferred because it results in higher expression than the 1095 bp fragment. A nucleic acid sequence of the 500 bp murine mGluR6 promoter is provided below in SEQ ID NO: 5:

ttaaaggcagcttaggggagaagcagaccagggagtcagagaggcagagagagaagagagcccttctccacttcaagctctggag
 ggggtctctgccctaccctcatccctcccagaatccttaaatcctctagactgtagctctgattttacagctgtcacagactcgtctactagc
 cagaggttggctcaggtaaacacactggggaggtagcctaggggtgcgtgggggtgggtccagaggaagagctgccagaactgtggg
 ggaaggagcgggaccgacctcaacagggggacttttcaggagagaatgagagcaatcctctggaggcctgggagaggctgctgagttg
 ctggtgcgagtcaccaacttttctcgcgtctcgggtgtccggccagaatcccgaagtggcagctgagcacgggggtggcagcttcgtccg
 ccggtctcaaggcgtcccgtaacttcttcccgcagtcaggagca

[0028] A preferred nucleic acid sequence of the 547 bp human mGluR6 promoter, is encompassed by the 1784 bp fragment described above (SEQ ID NO: 4), and is provided below in SEQ ID NO: 6:

ccaaagacaagaaaacaggaaaacagaccagagattgggagagggagggggaaggagatgcggggagagccagcacccgccacccc
 ccacactcaggaggggtctccaccctcgagcgggtctctcatccctccctagaaatccttaaatcctctctcgtcagggcctcgccgcacat
 gtcacagacttgcctgaaccgacagcggctggcgaggtgactggcttggggcgggagcctgggtgtgcgtggggatggaccccgag
 ggaagaggggccaagctgtcgggaagcggcagggctggaggggtggaggcagtggtcgggcgggaccccgggcgacaggggtcgg
 cgcttgaagagcgagacggagggcccgggcagggcggtgagtaactcccagagccgaagtggaggcgccccgagcgcttc
 tcccaggaccccggtgtccctccccgcgccccgagcccgctctcttccccgcctcagagcgctccccgccccctgtctccccg
 cagccgctagacgagccg

[0029] The modified mGluR6 promoters described herein may also include intron sequences from the mGluR6 gene. Preferably, introns 3 and 4 are included. The nucleic acid sequence of intron 4 of the murine mGluR6 gene is provided below in SEQ ID NO: 7:

ggtagtccccacccactcatcctccctgatgcttctgtgtgggatgtcatttcacatttgtctcgaggtccacatgctgagtaactct
 gagatttgccttaaatgccatgcaggtaatatgggaaggctgtatcaagtgtgaagtgcagccttgatagcatgcttctccgcctc
 ccacaggttccatctttgtgggtgcccacctccacaccttttcttttagctagagtgggtcaagtggacaagctggctcattagcaatcaaggc

gtttcagatctggaagtgggtggccattatggatcagtgagccctgtatttttgtgcctctgcacaaggtgggtagtgaagccctgtccatt
acataacatggcatcccctagccatgacataaagggcagtgaaaaattctttaaggatgccagagctgcttttccatttgtgtatgcgtgc
aggtgtgtgtgtacatgacacaagtgtatgtgtgtgcctgtggagccctgaggttgattcaggaatcatcctcaattctttctacctattcac
tgaggcagggctgtgtggagagatcacgatatggctactgtgggattccccctgtctctgccttcagagccactcctggatacacagtacact
ggctcagatggtcaccacctct

[0030] The nucleic acid sequence of intron 4 of the human mGluR6 gene (corresponding to the mouse intron 4) is provided below in SEQ ID NO: 8:

tccccacccccacagctctcccagcacccccctctggggatgctcttttctgcatctgtgtaattgggtgccataatccagatcccttctcagat
cccagatcctatgagtcgcatcatctgtggacgcgtccctccaagcagcgttgactgggatggcgtgcgaggaaagcacgctgacggg
gaggaattgttgggttttgggttttcttaatttagtgctgtatttctagaaccaaggatacgggaatataccatcctgggtgatcagagtgtg
aggcaggaacagatggctcactttacaataggattgctcattttcccaagttgatgacatccttcagggtattttatgaaacacattaggggg
atatataccatcacgcctgggtgcaggtggcagcctggactgagagtagttttagcgataaaggcatctcatcacaagacaggaggtctgaga
gttgggtgactttaggcatggggcagtgagtgtgtgtgcttgggtcttctgcatagtcacaagatggctgctgaggtccaccattacat
cttcacagcagcatcctaacaggaaagagaggtctgtgatttgcacgcatgtaagtgccaccaatcagaacttggctcacctggccacg
cctagctcacctggctcacctggccacgctaactcacctggccactcctagctcacctggccatgcctagttcacctggccactcctagctc
acctggccactcctagctgaaagtgcagcaagtggcagaacacacctccccctctt

[0031] The nucleic acid sequence of intron 3 of the mGluR6 gene is provided below in SEQ ID NO: 9:

actccaggccatgagcaactcctcacatctcctaagcccttctgtgcctcttgagtttttcttgggaatgagacaggcttgactggc
tgaaggttctccgggctggcctgggaacacagggaaaacacgactattttattgttcattgtgggagagagaactggtaggcaaacccaa
gagcagaaaatgtaccgtgagggacactgccccagtaaacctgaaacctacattatcctaagccagccaaggctctttccagcctgggaa
gttgagcgtgacattgggtgctgaattttagacagaatggcttctgagtgccctgacattccccaaaaggaggctctctgcattaatccatt
gtctttattataataaaatatccaagtcagggcggttttaaggaaaagacttattttaacatcaactcttgagggtgaaagttcaggcagcgtga
caccagctctgctgaggacctagcttgcacacattttgacaaatgttatggaaagagggagtagagaaggaaagagtgcatggagagaag
gaacatcagaaagaagagggacaggggtcactctttgatagctattcaccttcacagaattacctcacccttcagagggtcaagagcaacatc
cccagtgacccaataacctgcactaagccacacctctttttatttttatgagacagggcctcactctgtatccctgaactgctatgtggacca
agctgtcctcttgagtgtgtgggattaaaggcattcagtatcagggtggtttataa

[0032] The nucleic acid sequence of intron 3 of the human mGluR6 gene (corresponding to the mouse intron 3) is provided below in SEQ ID NO: 10:

gggacagtggcacaatcacggggccctggggctctcctgaggggtccacccctccttgcacatcgccaccttacacactctccctgc
ctccctttccctgtccccgcacccccaccttccctgctgctgctgctgagctggtgccaggctcagaagcagcctggctgtggggaga
tgccacaagcccagactgaatagcgacagaacatgtgtctgtttacagctcctgggggtgcggggagggaacctgtgggcagaacct
gtgcagaaaatgagctgtgaggggaaggagccaggggtccatacagaggctgcggacccagcccagaccaccggcccgagttcat
ctccagcctaggaggctgggtgtgacgctggtggagggcatgtggggaggggctggcttcgaactggattttccccaaggaggtccctctg

aaccccctgaacagtgggttacagtgggcagagcaagtgggcaggcctagggtcagaggaaagcccaggggaaggtgccctaaatgcc
 ccggccccgttttctgagaaacaaggatctggtgagtgattatagagcaggggagatggatagagtgggaaggatggggccgagct
 ggaggaggctcccagccctccctcaccagccctgctctacc

[0033] Variants of the regulatory elements encompassed by the mGluR6 promoters described herein are also encompassed by the present invention. For example, a variant may have 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to the nucleic acid sequences described herein. The term "% identity," in the context of two or more nucleic acid or polypeptide sequences, refer to two or more sequences or subsequences that are the same or have a specified percentage of amino acid residues or nucleotides that are the same, when compared and aligned for maximum correspondence, as measured using one of the following sequence comparison algorithms or by visual inspection. For example, % identity is relative to the entire length of the coding regions of the sequences being compared.

[0034] For sequence comparison, typically one sequence acts as a reference sequence, to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are input into a computer, subsequence coordinates are designated, if necessary, and sequence algorithm program parameters are designated. The sequence comparison algorithm then calculates the percent sequence identity for the test sequence(s) relative to the reference sequence, based on the designated program parameters. Percent identity is determined using search algorithms such as BLAST and PSI-BLAST (Altschul et al., 1990, J. Mol. Biol. 215:3, 403-410; Altschul et al., 1997, Nucleic Acids Res. 25:17, 3389-402).

[0035] For example, the mGluR6 enhancer is at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95% identical to SEQ ID NO: 1 or SEQ ID NO: 2. The mGluR6 promoter variant is at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95% identical to SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, or SEQ ID NO: 6. The intron 4 of the mGluR6 gene variant is at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95% identical to SEQ ID NO: 7 or SEQ ID NO: 8. The intron 3 of the mGluR6 gene variant is at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95% identical to SEQ ID NO: 9 or SEQ ID NO: 10.

[0036] In one embodiment, the mGluR6 enhancer comprises the nucleic acid sequence of SEQ ID NO: 1 or SEQ ID NO: 2. The mGluR6 promoter comprises the nucleic acid sequence of

SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, or SEQ ID NO: 6. The intron 4 of the mGluR6 gene comprises the nucleic acid sequence of SEQ ID NO: 7 or SEQ ID NO: 8. The intron 3 of the mGluR6 gene comprises the nucleic acid sequence of SEQ ID NO: 9 or SEQ ID NO: 10.

[0037] In other embodiments, the intron 4 of the mGluR6 gene is located upstream of the intron 3 of the mGluR6 gene in the present invention. The intron 3 of the mGluR6 gene is located upstream of the mGluR6 enhancer in the present invention. The mGluR6 enhancer is located upstream of the mGluR6 promoter in the present invention.

[0038] For example, the mGluR6 enhancer is at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95% identical to SEQ ID NO: 1 or SEQ ID NO: 2. The mGluR6 promoter variant is at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95% identical to SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, or SEQ ID NO: 6. The intron 4 of the mGluR6 gene variant is at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95% identical to SEQ ID NO: 7 or SEQ ID NO: 8. The intron 3 of the mGluR6 gene variant is at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95% identical to SEQ ID NO: 9 or SEQ ID NO: 10.

[0039] In one embodiment, the mGluR6 enhancer comprises the nucleic acid sequence of SEQ ID NO: 1 or SEQ ID NO: 2. The mGluR6 promoter comprises the nucleic acid sequence of SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, or SEQ ID NO: 6. The intron 4 of the mGluR6 gene comprises the nucleic acid sequence of SEQ ID NO: 7 or SEQ ID NO: 8. The intron 3 of the mGluR6 gene comprises the nucleic acid sequence of SEQ ID NO: 9 or SEQ ID NO: 10.

[0040] In other embodiments, the intron 4 of the mGluR6 gene is located upstream of the intron 3 of the mGluR6 gene in the present invention. The intron 3 of the mGluR6 gene is located upstream of the mGluR6 enhancer in the present invention. The mGluR6 enhancer is located upstream of the mGluR6 promoter in the present invention.

[0041] In a preferred embodiment, the 200 bp mGluR6 enhancer is located upstream of the sequence from the mGluR6 promoter region. In another preferred embodiment, the intron 4 of the mGluR6 gene is upstream of the intron 3 of the mGluR6 gene, the intron 3 of the mGluR6 gene is upstream of the mGluR6 enhancer, and the mGluR6 enhancer is upstream of the mGluR6 promoter. The entire modified mGluR6 promoter (*e.g.*, including the mGluR6 enhancer, mGluR6 promoter, and mGluR6 intron sequences) is located upstream of a particular transgene

of interest to drive its expression. Alternatively, other orders or combinations of the regulatory elements may be advantageous for improving expression or targeting of the transgene.

[0042] The present invention further provides expression cassettes comprising the modified mGluR6 promoters described herein.

Transgenes

[0043] The expression of transgenes in the damaged or diseased retinas may be useful for improving or restoring vision. Transgenes of particular interest for restoration of photosensitivity or vision include photosensitive proteins, such as opsin genes or rhodopsin genes. As used herein, “transgene” refers to a polynucleotide encoding a polypeptide of interest, wherein the polynucleotide is encapsidated in a viral vector (e.g., rAAV).

[0044] The opsin family of genes includes vertebrate (animal) and invertebrate opsins. Animal opsins are G-protein coupled receptors (GPCRs) with 7-transmembrane helices which regulate the activity of ion channels. Invertebrate rhodopsins are usually not GPCRs but are light-sensitive or light-activated ion pumps or ion channels.

[0045] As referred to herein, an opsin gene or light-sensitive protein includes channelrhodopsins (e.g., ChR1, ChR2, vChR1 from *Volvox carteri*, vChR2, and other variants identified from any vertebrate, invertebrate, or microbe), halorhodopsins (NpHR), melanopsins, pineal opsins, bacteriorhodopsin, and functional variants, active binding fragments, or chimeras thereof. A light-sensitive protein of this invention can occur naturally in plant, animal, archaeobacterial, algal, or bacterial cells, or can alternatively be created through laboratory techniques. Examples of opsin genes are discussed in further detail below.

[0046] Examples of channelrhodopsins as transgenes in the present invention include channelrhodopsins Chop1 (also known as ChR1) (GenBank accession number AB058890/AF385748) and Chop2 (also known as ChR2) (GenBank accession number AB058891/AF461397), as well as mutant ChR2 C128A, ChR2 C128S, ChR2 C128T, ChR1-ChR2 hybrids/chimeras, and variants thereof.

[0047] Chop1 and Chop2 are two rhodopsins from the green alga *Chlamydomonas*

reinhardtii (Nagel, 2002; Nagel, 2003). Both are light-sensitive channels that, when expressed and activated in neural tissue, allow for a cell to be depolarized when stimulated with light (Boyden, 2005). The full length amino acid sequence of Chop1 is provided below in SEQ ID NO: 11:

[0048] MSRRPWLLALALAVALAAGSAGASTGSDATVPVATQDGPDYVFHRAHERM
LFQTSYTLNNGSVICIPNNGQCFCLAWLKSNGTNAEKLAANILQWITFALSALCLMFY
YQTWKSTCGWEEIYVATIEMIKFIIYFHEFDEPAVIYSSNGNKTWVLRYAEWLLTCPVI
LIHLSNLTGLANDYNKRTMGLLVSDIGTIVWGTTAALSKGYVRVIFFLMGLCYGIYTFFN
AAKVYIEAYHTVPKGICRDLVRYLAWLYFCSWAMFPVLFLGPEGFGHINQFNSAIAHA
ILDASKNAWSMMGHFLRVKIHILLYGDIRKKQKVNVAQEMEVEVETMVHEEDDET
QKVPTAKYANRDSFIIMRDLKEKGFETRSLDGDPNGDAEANAAGGKPGMEMGKM
TGMGMGMGAGMGMATIDSGRVILAVPDISMVDFFREQFARLPVPYELVPALGAENTLQ
LVQQAQSLGGCDFVLMHPEFLRDRSPTGLLPRLKMGGQRAAAGFWAAIGPMRDLIEGS
GVDGWLEGPSFGAGINQQALVALINRMQQAQKMGMMGGMGMGMGGGMGMGMGMGM
GMGMAPSMNAGMTGGMGGASMGGA VMGMGMGMQPMQQAMPAMSPMMTQQPSM
MSQPSAMSAGGAMQAMGGVMPSPAPGGRVGTNPLFGSAPSPLSSQPGISPGMATPPAA
TAAPAAGGSEAEMLQQLMSEINRLKNELGE

[0049] The full length amino acid sequence of Chop2 is provided below in SEQ ID NO: 12:

[0050] MDYGGALSAVGRELLFVTNPVVVNGSVLVPEDQCYCAGWIESRG
[0051] TNGAQTASNVLQWLAAGFSILLMFYAYQTWKSTCGWEEIYVCAIEMVKVI
LEFFFEFKNPSMLYLATGHRVQWLRYAEWLLTCPVILHLSNLTGLSNDYSRRTMGLLV
SDIGTIVWGATSAMATGYVKVIFFCGLGCLCYGANTFFHAAKAYIEGYHTVPKGRCRQVVT
GMAWLVFFVSWGMFPILFILGPEGFGVLSVYGSTVGHTIIDLMSKNCWGLLGHYLRVLIH
EHLIHGDIRKTTKLNIGGTEIEVETLVEDEAEAGAVNKGKTGKYASRESFLVMRDKMKE
KGIDVRASLDNSKEVEQEQAARAAMMMMNNGNGMGMGMGMNGMNGMGMNGMNGMA
GGAKPGLELTPQLQPGRVILAVPDISMVDFFREQFAQLSVTYELVPALGADNTLALVTQ
AQNLGGVDFVLIHPEFLRDRSSTSILSRLRGAGQRVAAFGWAQLGPMRDLIESANLDGW
LEGPSFGQGILPAHIVALVAKMQQMRKMQQMQQIGMMTGGMNMGGMGGGMGGGMNG
MGGGNGMNNMNGMGMGGGMNGMGGNGMNGMGGGNGMNNMGGNGMAGNGMGG
GMGGNGMGGSMNGMSSGVVANVTPSAAGGMGGMMNGGMAAPQSPGMNGGRLGTN
PLFNAAPSPLSSQLGAEAGMGSMMGGMSGMGGMGGMGGMGGMGGAGAATTQAAGGN
AEAEMLQNLNMNEINRLKRELGE

[0052] A Chop2 fragment (315 amino acids) has been shown to efficiently increase photosensitivity and vision in murine models of photoreceptor degeneration (Bi et al., Neuron, 2006, and U.S. Patent 8,470,790). The amino acid sequence of this fragment is provided in

below in SEQ ID NO: 13:

MDYGGALSAVGRELLFVTNPVVVNGSVLVPEDQCYCAGWIESRGTNGAQTASNVLQW
LAAGFSILLMFYAYQTWKSTCGWEEIYVCAIEMVKVILEFFFEFKNPSMLYLATGHRV
QWLRYAEWLLTCPVILIHLSNLTGLSNDYSRRTMGLLVSDIGTIVWGATSAMATGYVK
VIFFLGLCYGANTFFHAAKAYIEGYHTVPGKRCRQVVTGMAWLFFVSWGMFPILFILG
PEGFGVLSVYGSTVGHTIIDLMSKNCWGLLGHYLRVLIHEHILIHGDIRKTTKLNIGGTEI
EVETLVEDEAEAGAVN KGTGK

[0053] Chop2 mutants and variants as described in PCT Publication WO 2013/134295 may also be expressed using the promoters described herein. Any ChRs, or microbial opsins, or other genetically encoded light sensors or switches, presently known or as yet undiscovered, are useful in generating the compositions and practicing the methods of the invention.

[0054] Other suitable transgenes include *Volvox carteri* channelrhodopsins (e.g., vChR1 and vChR2). The amino acid sequence of vChR1, GenBank Accession No. EU285658.1, is provided in below in SEQ ID NO: 14:

MDYPVARSLIVRYPTDLGNGTVCMPRGQCYCEGWLRSRGTSIEKTIAITLQWVVFALSV
ACLGWYAYQAWRATCGWEEVYVALIEMMKSIIEAFHEFDSPATLWLSSGNGVVMRY
GEWLLTCPVLLIHLSNLTGLKDDYSKRTMGLLVSDVGCIVWGATSAMCTGWTILFFLI
SLSYGMYTYFHAARKVYIEAFHTVPGKICRELVRVMAWTFVAVWGMFPVLFLLGTEGFG
HISPYGSAIGHSILDIAKNMWGVLGNYLRVKIHEHILLYGDIRKKQKITIAGQEMEVELL
VAEEEDDTVKQSTAKYASRDSFITMRNRMREKGLEVRASLDAGGGDSGMEAGGGGAA
HAQPHMAKPGTELGKTMSASFTNGAATSLEPGRVILAVPDISMVDFFREQFAQLPVPYE
VVPALGAENTVQLVQQAAMLGGCDFVLMHPEFLRDRGPTGLLPQVKMMGQRTAAFG
WSQMGPMDRLIESSGVGAWLEGPSFGSGISQAALQQLVVKMQQAKRMAAMGSMMGG
GMGMNGMGMGMGMGMGMGMGMNGMGMNGMGMGMNGMGMGMNGMGMGMNGMGMGMGN
GMGMNGMGMGMGMGMGMGMGMNGMGMNGMGMGMNGMGMGMNGMGMGMNGMGMGNM
GNGMGMGMNGMGMGMNGMGMGMNGMGMNGMGMGMNGMGMGMNGMGMNGMGMGNLA
AAAGNAMYGGGGGGGGSTMGSGNAAMMTGLVMGGGNGVGAGPGGVVANLGSSAL
QPQSQMMGGGNVGMSSPQLQLQQSSSMPLGGLAPNRIGNNPLFGAAPSPLHSQPGAS
PTGLSSPQLGMGAMLPAGTSVGAGGGSVGPTETDMLQQLMTEINRLKDELGE

The amino acid sequence of vChR2 is provided below in SEQ ID NO: 15:

MDHPVARSLIGSSYTNLNNGSIVIPSDACFCMKWLKSKGSPVALKMANALQWAAFALS
VILIIYYAYATWRTTCGWEEVYVCCVELTKVVEFFHEFDEPGMLYLANGNRVLWLRY
GEWLLTCPVILIHLSNLTGLKDDYNKRTMRLLVSDVGTIVWGATAAMSTGYIKVIFLL

GCMYGANTFFHAAKVYIESYHTVPKGLCRQLVRAMAWLFFVSWGMFPVLFLLGPEGF
 GHLSVYGSTIGHTIIDLLSKNCWGLLGHLRLKIHEHILLYGDIRKVQKIRVAGEELEVET
 LMTEEAPDTVKKSTAQYANRESFLTMRDKLKEKGFEVRASLDNSGIDAVINHNHNNYNN
 ALANAAAAGVKPGMELSKLDHVAANAAGMGGIADHVATTSGAISPGRVILAVPDISMV
 DYFREQFAQLPVQYEVVPALGADNAVQLVVQAAGLGGCDFVLLHPEFLRDKSSTSLPA
 RLRSIGQRVAAFGWSPVGPVRDLIESAGLDGWLEGPSFGLGISLPNLASLVLRMQHARK
 MAAMLGGMGMLGSNLMSGSGGVGLMGAGSPGGGGGAMGVGMTGMMVGTNAM
 GRGAVGNSVANASMGGGSAGMGMGMMGMVGAGVGGQQQMGANGMGPTSFQLGSN
 PLYNTAPSPLSSQPGGDASAAAAAAAAAAAAATGAASNSMNAMQAGGSVRNSGILAGGL
 GSMMGPPGAPAAPTAAATAAPAVTMGAPGGGGAAASEAEMLQQLMAEINRLKSELGE

[0055] NpHR (Halorhodopsin) (GenBank accession number EF474018) is from the haloalkaliphilic archaeon *Natronomonas pharaonis*. The amino acid sequence of NpHR is provided below in SEQ ID NO: 16:

MTETLPPVTESAVALQAEVTQRELFEFVLNDPLLASSLYINIALAGLSILLFVFMTRGLDD
 PRAKLIIVSTILVPVVSIASTGLASGLTISVLEMPAGHFAEGSSVMLGGEEVDGVVTM
 WGRYLTWALSTPMILLALGLLAGSNATKLFTAITFDIAMCVTGLAAALTTSSHLMRWF
 WYAISCACFLVVLVYILLVEWAQDAKAAGTADMFTLTKLLTVVMWLGYPIVWALGVEG
 IAVLPVGVTSWGYSFLDIVAKYIFAFLLNLYLTSNESVVSIGSILDVPSASGTPADD

[0056] In certain embodiments variants of NpHR can be created. In specific embodiments single or multiple point mutations to the NpHR protein can result in NpHR variants. In specific embodiments a mammalian codon optimized version of NpHR can be utilized. In one embodiment NpHR variants are utilized. In one specific embodiment eNpHR (enhanced NpHR) is utilized. Addition of the amino acids FCYENEV (SEQ ID NO: 38) to the NpHR C-terminus along with the signal peptide from a β subunit of the nicotinic acetylcholine receptor to the NpHR N-terminus results in the construction of eNpHR.

[0057] Melanopsin (GenBank accession number 6693702) is a photopigment found in specialized photosensitive ganglion cells of the retina that are involved in the regulation of circadian rhythms, pupillary light reflex, and other non-visual responses to light. In structure, melanopsin is an opsin, a retinylidene protein variety of G-protein-coupled receptor. Melanopsin resembles invertebrate opsins in many respects, including its amino acid sequence and downstream signaling cascade. Like invertebrate opsins, melanopsin appears to be a bistable photopigment, with intrinsic photoisomerase activity. In certain embodiments variants of

melanopsin can be created and used in the invention. In specific embodiments single or multiple point mutations to the melanopsin protein can result in melanopsin variants. The amino acid sequence of *Mus musculus* melanopsin (GenBank accession number 6693702) is provided below in SEQ ID NO: 17:

MDSPSGPRVLSSLTQDPSFTTSPALQGIWNGTQNVSVRAQLLSVSPTTSAHQAAAWVPF
PTVDVPDHAHYTLGTVILLVGLTGMLGNLTVIYTFCSRNRGLRTPANMFIINLAVSDFLMS
VTQAPVFFASSLYKKWLFGETGCEFYAFCGAVFGITSMITLTAIAMDRLVITRPLATIG
RGSKRRTALVLLGVWLYALAWSLPPFFGWSAYVPEGLLTSCSWDYMTFTTPQVRAYTM
LLFCFVFFLPLLIIFCYIFIFRAIRETGRACEGCGESPLRQRRQWQRLQSEWKMAKVALIV
ILLFVLSWAPYSTVALVAFAGYSHILTPYMSSVPAVIAKASAIHNPIIYAITHPKYRVAIAQ
HLPCLGVLLGVSGQRSHPSLSYRSTHRSTLSSQSSDLSWISGRKRQESLGSESEVGWTD
ETTAAGWAAQQASGQSFCNQLEDGELKASSSPQVQRSKTPKVPGPSTCRPMKGQGAR
PSSLRGDQKGRLAVCTGLSECPHPHTSQFPLAFLEDDVTLRHL

[0058] The amino acid sequence of *Homo sapiens* melanopsin (GenBank accession number BC113558.1) is provided below in SEQ ID NO: 18:

MNPPSGPRVLPSPTQEPSCMATPAPPSWWDSSQSSISLGRLPISPTAPGTWAAA WVPL
PTVDVPDHAHYTLGTVILLVGLTGMLGNLTVIYTFCSRSLRTPANMFIINLAVSDFLMS
FTQAPVFFTSSLYKQWLFGETGCEFYAFCGALFGISSMITLTAIALDRLVITRPLATFGV
ASKRRAAFVLLGVWLYALAWSLPPFFGWSAYVPEGLLTSCSWDYMSFTPAVRAYTML
LCCFVFFLPLLIICYIFIFRAIRETGRALQTFGACKGNESLWQRQRLQSECKMAKIML
LVILLFVLSWAPYSAVALVAFAGYAHVLTTPYMSSVPAVIAKASAIHNPIIYAITHPKYRV
AIAQHLPCLGVLLGVSRHRSRPYSYRSTHRSTLTSHTSNLSWISIRRRQESLGSESEVGW
THMEAAAVWGAAQQANGRSLYGQGLEDEAKAPPRPQGHEAETPGKTKGLIPSQDPR
M

[0059] Other suitable channel proteins of the invention include ChD, ChEF, ChF, ChIEF, and variants thereof.

[0060] Light-sensitive proteins may also include proteins that are at least about 10%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 95%, or at least about 99% identical to any of the light-sensitive proteins described herein (*e.g.*, ChR1, ChR2, vChR1, vChR2, NpHR and melanopsin). The light-sensitive proteins of the present invention may also include proteins that have at least one mutation. The mutation may be a point mutation.

[0061] In some embodiments, light-sensitive proteins can modulate signaling within neural circuits and bidirectionally control behavior of ionic conductance at the level of a single neuron. In some embodiments the neuron is a retinal neuron, a retinal bipolar cell (e.g. ON or OFF retinal bipolar cells; rod and cone bipolar cells), a retinal ganglion cell, a photoreceptor cell, or a retinal amacrine cell.

[0062] In some embodiments, a polyA tail can be inserted downstream of the transgene in an expression cassette or nucleic acid expression vector of the present invention. Suitable polyA tails are known in the art, and include, for example, human growth hormone poly A tail (hGHpA), bovine growth hormone polyA tail (bGHpA), bovine polyA, SV40 polyA, and AV40pA. The nucleic acid sequence of hGHpA is provided below in SEQ ID NO: 19:

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gtcgagagatctacgggtggcatccctgtgacccctcccagtcgctctcctggccctggaagttgccactccagtgcccaccagccttgct
ctaataaaattaagttgcatcattttgtctgactaggtgtccttctataatattatgggggtggaggggggtggtatggagcaaggggcaagttgg
gaagacaacctgtagggcctgcgggggtctattgggaaccaagctggagtgcatggcacaatcttggtcactgcaatctccgctcctgg
gttcaagcgattctcctgcctcagcctcccagttgttgggattccaggcatgcatgaccaggctcagctaattttgtttttggtagagacgg
ggtttcaccatattggccaggctgggtctcaactcctaattctcaggtgatctaccaccttggtcctccaaattgctgggattacaggcgtgaa
ccactgtccctccctgtccttctgattttgtaggtaaccacgtg
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Vectors

[0063] The modified mGluR6 promoter sequences may be inserted into various different nucleic acid expression vectors. Vectors for use in the present invention can include various viral vectors, such as plasmids and recombinant viruses, *e.g.*, recombinant adeno-associated virus (rAAV), recombinant adenoviruses, recombinant retroviruses, recombinant lentiviruses, and other viruses known in the art.

[0064] Adeno-associated viruses are small, single-stranded DNA viruses which require helper virus to facilitate efficient replication. The 4.7 kb genome of AAV is characterized by two inverted terminal repeats (ITR) and two open reading frames which encode the Rep proteins and Cap proteins, respectively. The Rep reading frame encodes four proteins of molecular weight 78 kD, 68 kD, 52 kD and 40 kD. These proteins function mainly in regulating AAV replication and rescue and integration of the AAV into a host cell's chromosomes. The Cap reading frame encodes three structural proteins of molecular weight 85 kD (VP 1), 72 kD (VP2)

and 61 kD (VP3) (Berns, cited above) which form the virion capsid. More than 80% of total proteins in AAV virion comprise VP3.

[0065] The genome of rAAV generally comprises: (1) a 5'adeno-associated virus ITR, (2) a coding sequence (e.g., transgene) for the desired gene product (e.g., a light-sensitive protein) operatively linked to a sequence which regulates its expression in a cell (e.g., a modified mGluR6 promoter sequence), and (3) a 3'adeno-associated virus inverted terminal repeat. In addition, the rAAV vector may preferably contain a polyadenylation sequence.

[0066] Generally, rAAV vectors have one copy of the AAV ITR at each end of the transgene or gene of interest, in order to allow replication, packaging, and efficient integration into cell chromosomes. The ITR consists of nucleotides 1 to 145 at the 5'end of the AAV DNA genome, and nucleotides 4681 to 4536 (e.g., the same sequence) at the 3'end of the AAV DNA genome. The rAAV vector may also include at least 10 nucleotides following the end of the ITR (e.g., a portion of the "D region").

[0067] The transgene sequence (e.g., the polynucleotide encoding a light-sensitive protein) can be of about 2 to 5 kb in length (or alternatively, the transgene may additionally contain a "stuffer" or "filler" sequence to bring the total size of the nucleic acid sequence between the two ITRs to between 2 and 5 kb). Alternatively, the transgene may be composed of repeated copies of the same or similar heterologous sequence several times (e.g., two nucleic acid molecules which encode one or more light-sensitive proteins separated by a ribosome readthrough, or alternatively, by an Internal Ribosome Entry Site or "IRES"), or several different heterologous sequences.

[0068] In one embodiment the vector comprises a recombinant AAV of a particular serotype, either naturally occurring or engineered. Adeno-associated viruses have been found in many animal species, including primates, canines, fowl and human. Presently, there are 12 different known serotypes, e.g., AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, and AAV12, all of which are appropriate for use in the present invention. Recombinant AAV vectors of the present invention may be generated from a variety of adeno-associated viruses, including for example, any of serotypes 1 through 12, as described herein.

For example, ITRs from any AAV serotype are expected to have similar structures and functions with regard to replication, integration, excision, and transcriptional mechanisms.

[0069] For example, the nucleic acid expression vector of the present invention is an adeno-associated virus vector or a recombinant adeno-associated virus (rAAV) vector. In preferred embodiments, the vector is a recombinant AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, or AAV12 vector. In certain embodiments the AAV vector is of a wild-type serotype or variant of any of AAV1-AAV12, or a mutant, hybrid, or fragment thereof. In other embodiments, the AAV vector is of a natural serotype or variant/mutant thereof that has yet to be discovered.

[0070] Viral and virus-like particles contemplated by the invention may be prepared by methods known to those of skill in the art, as well as those developed in the future. Virus producing cell lines and virus-like particle producing cell lines having the ability to produce sufficiently large quantities of virus are transfected with the vectors of the invention. Preferred production methods include, but are not limited to, the baculovirus expression vector system/insect cells and HEK 293 host cells. Other methods and host cell are suitable, such as those described in the art. See, e.g., Vicente, T. "Virus production for clinical gene therapy," Methods Mol. Biol., vol. 542:447-70 (2009).

[0071] The rAAV vector may also contain additional sequences, for example from an adenovirus, which assist in effecting a desired function for the vector. Such sequences include, for example, those which assist in packaging the rAAV vector into virus particles. Packaging cell lines suitable for producing adeno-associated viral vectors may be accomplished given available techniques (U.S. Pat. No. 5,872,005). Methods for constructing and packaging rAAV1 vectors are described in, for example, WO 00/54813.

[0072] In other embodiments, the AAV vectors used with the present invention, *e.g.*, a packaging vector, comprise a capsid protein. Suitable capsid proteins include, but are not limited to:

an AAV1 capsid protein comprising the amino acid sequence of SEQ ID NO: 23:

AADGYLPDWLEDNLSEGIREWDLKPGAPKPKANQQKQDDGRGLVLPGYKYLGPFGN
LDKGEPVNAADAAALEHDKAYDQQLKAGDNPYLRYNHADAERLQEDTSFGGNLGR

AVFQAKKRVLEPLGLVEEGAKTAPGKKRPVEQSPQEPDSSSGIGKTGQQPAKKRLNFGQ
 TGDSSEVPDPQPLGEPPATPAAVGTTMASGGGAPMADNNEGADGVGNASGNWHCDST
 WLGD RVITTSTRTWALPTYNNHLYKQISSASTGASNDNHYFGYSTPWGYFDFNRFHCH
 FSPRDWQLINNNWGFRPKRLNFKLFNIQVKEVTTNDGVTTIANNLTSTVQVFS DSEYQL
 PYVLGSAHQGCLPPFPADVFMIPQYGYLTLNNGSQAVGRSSFYCEYFPSQMLRTGNNFT
 FSYTFEEVPFHSSYAHSQSLDRLMNPLIDQYLYLNRTQNQSGSAQNKDLLFSRGSPAG
 MSVQPKNWLPGPCYRQQRVSKTKTNNNSNFTWTGASKYNLNGRESIINPGTAMASHKD
 DEDKFFPMSGVMIFGKESAGASNTALDNVMITDEEEIKATNPVATERFGTVAVNFQSST
 DPATGDVHAMGALPGMVWQDRDVYLQGPWAKIPHTDGHFHPSPLMGGFGLKNPPPQI
 LIKNTVPANPPAEFSATKFASFITQYSTGQVSVEIEWEQKENS KRWNPEVQYTSNYAKS
 ANVDFTVDNNGLYTEPRPIGTRYLTRPL

an AAV2 capsid protein comprising the amino acid sequence of SEQ ID NO: 24:

AADGYLPDWLEDTLSEGIRQWWKLKPGPPPKPAERHKDDSRGLVLPGYKYLGPFNGL
 DKGEPVNEADAAALEHDKAYDRQLDSGDNPYLKYNHADAEEFERLKEDTSFGGNLGRA
 VFQAKKRVLEPLGLVEEPVKTAPGKKRPVEHSPVEPDSSSGTGKAGQQPARKRLNFGQT
 GDADSVDPDPQLGQPPAAPSGLGNTMATGSGAPMADNNEGADGVGNSSGNWHCDST
 WMGDRVITTSTRTWALPTYNNHLYKQISSQSGASNDNHYFGYSTPWGYFDFNRFHCHF
 SPRDWQLINNNWGFRPKRLNFKLFNIQVKEVTQNDGTTTIANNLTSTVQVFTDSEYQLP
 YVLGSAHQGCLPPFPADVFMVPQYGYLTLNNGSQAVGRSSFYCEYFPSQMLRTGNNFT
 FSYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYLSRTNTPSGTTTQSRLQFSQAGASDIR
 DQSRNWLPGPCYRQQRVSKTSANNNSEYSWTGATKYHLNGRDSLVPNGPAMASHKDD
 EEKFFPQSGVLIFGKQGSEKTNVDIEKVMITDEEEIRTTPVATEQYGSVSTNLQRNRQA
 ATADVNTQGVLPGMVWQDRDVYLQGPWAKIPHTDGHFHPSPLMGGFGLKHPPPQILI
 KNTVPANPSTTFSAKFASFITQYSTGQVSVEIEWEQKENS KRWNPEIQYTSNYNKS VN
 VDFTVDNTNGVYSEPRPIGTRYLTRNL

an AAV3 capsid protein comprising the amino acid sequence of SEQ ID NO: 25:

AADGYLPDWLEDNLSEGIREWWALKPGVPQPKANQQHQDNRRGLVLPGYKYLGPNG
 LDKGEPVNEADAAALEHDKAYDQQLKAGDNPYLKYNHADAEEFERLQEDTSFGGNLGR
 AVFQAKKRILEPLGLVEEAAKTAPGKKGAVDQSPQEPDSSSGVGKSGKQPARKRLNFG
 QTGDSESVDPDPQLGEPPAAPTSLGNTMASGGGAPMADNNEGADGVGNSSGNWHCDS
 QWLGD RVITTSTRTWALPTYNNHLYKQISSQSGASNDNHYFGYSTPWGYFDFNRFHCH
 FSPRDWQLINNNWGFRPKKLSFKLFNIQVRGVTQNDGTTTIANNLTSTVQVFTDSEYQL
 PYVLGSAHQGCLPPFPADVFMVPQYGYLTLNNGSQAVGRSSFYCEYFPSQMLRTGNNF
 QFSYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYLNRTQGTTS GTTNQSRLLSQAGPQ
 SMSLQARNWLPGPCYRQQRLSKTANNNNNSNFPWTAASKYHLNGRDSLVPNGPAMASH
 KDDEEKFFPMHGNLIFGKEGTTASNAELDNVMITDEEEIRTTPVATEQYGTVANLQS
 NTAPTTGTVNHQGALPGMVWQDRDVYLQGPWAKIPHTDGHFHPSPLMGGFGLKHPPP
 QIMIKNTVPANPPTTFSPAKFASFITQYSTGQVSVEIEWEQKENS KRWNPEIQYTSNYNK
 SVNVDFTVDNTNGVYSEPRPIGTRYLTRNL

an AAV4 capsid protein comprising the amino acid sequence of SEQ ID NO: 26:

MTDGYLPDWLEDNLSEGVREWWALQPGAPKPKANQQHQDNARGLVLPGYKYLGPNGN
GLDKGEPVNAADAAALEHDKAYDQQLKAGDNPYLKYNHADADEFQRLQGDTSFGGNL
GRAVFQAKKRVLEPLGLVEQAGETAPGKKRPLIESPQQPDSSTGIGKKKGKQPAKKKLVF
EDETGAGDGPPEGSTSGAMSDDSMRAAAGGAAVEGGQGADGVGNASGDWHCDSTWS
EGHVTTTSTRTWVLPTYNNHLYKRLGESLQSN TYNGFSTPWGYFDFNRFHCHFSRPRDW
QLINNNWGMRPKAMRVKIFNIQVKEVTTSNGETTVANNLTSTVQIFADSSYELPYVMDA
GQEGSLPPFPNDVFMVPQYGYCGLVTGNTSQQQTDRNAFYCEYFPSQMLRTGNNFEITY
SFEKVPFHSMYAHQS LDR LMNPLIDQYLWGLQSTTTGTTLNAGTATTNFTKL RPTNFS
NFKKNWLPGPSIKQQGFSKTANNYKIPATGSDSLIKYETHSTLDGRWSALTPGPPMATA
GPADSKFSNSQLIFAGPKQNGNTATVPGLTIFTSEEELAA TNATD TDMWGNLPGGDQSS
NLPTVDRLTALGAVPGMVWQN RDIYYQGPIWAKIPHTDGHFHPSPLIGGFGLKHPPPQIF
IKNTPVPANPATTFSSSTPVNSFITQYSTGQVSVQIDWEQKERSKRWNPEVQFTSNY GQQN
SLLWAPDAAGKYTEPRAIGTRYLTHHL

an AAV5 capsid protein comprising the amino acid sequence of SEQ ID NO: 27:

SFVDHPPDWLEEVGEGLEFLGLEAGPPKPKPNQQHQDQARGLVLPGYNYLGPNGNGLD
RGEPVNRADDEVAREHDISYNEQLEAGDNPYLKYNHADADEFKLADDTSFGGNLGKAVF
QAKKRVLEPFGLVEEGAKTAPTGKRIDDHFPRKKARTEEDSKPSTSSDAEAGPSGSQQ
LQIPAQPASSLGDTMSAGGGGGLGDNNQGADGVGNASGDWHCDSTWMGDRVVTKST
RTWVLPSYNNHQQYREIKSGSV DGSNANAYFGYSTPWGYFDFNRFHSHWSRPRDWQLINN
YWGFRPRSLRVKIFNIQVKEVTVQDSTTTIANNLTSTVQVFTDDDDYQLPYVVGNGTEGC
LPAFPQVFTLPQYGYATLNRDNTENPTERSSFFCEYFPSKMLRTGNNFEITYNFEEVPF
HSSFAPSQNLFKLANPLVDQYLRFVSTNNTGGVQFNKNLAGRYANTYKNWFPGPMG
RTQGWNLGSGNRASVSFAATTNRMELEGASYQVPPQPNGMTNNLQGSNTYALENTMIF
NSQPANPGTTATYLEGNMLITSESETQPVNRVAYNVGGQMATNNQSTTAPATGTYNLQ
EIVPGSVWMERDVYLQGP IWAKIPETGAHFHPSAMGGFGLKHPPPMMLIKNTPVPGNI
TSFSDVPVSSFITQYSTGQVTVEMEWEKKENSKRWNPEIQYTNNYNDPQFVDFAPDSTG
EYRTTRPIGTRYLTRPL

an AAV6 capsid protein comprising the amino acid sequence of SEQ ID NO: 28:

AADGYLPDWLEDNLSEGI REWWDLKPGAPKPKANQQKQDDGRGLVLPGYKYLGPFGN
LDKGEPVNAADAAALEHDKAYDQQLKAGDNPYLRYNHADADEFERLQEDTSFGGNLGR
AVFQAKKRVLEPFGLVEEGAKTAPGKKRPVEQSPQEPDSSSGIGKTGQQPAKKRLNFGQ
TGDSSEVPDPQPLGEPPATPAAVGTTMASGGGAPMADNNEGADGVGNASGNWHCDST
WLGD RVITTSTRTWALPTYNNHLYKQISSASTGASNDNH YFGYSTPWGYFDFNRFHCH
FSRPRDWQLINNNWGFPRKRLNFKLFNIQVKEVTTNDGVTTIANNLTSTVQVFS DSEYQL
PYVLGSAHQGCLPPFPADVFMIPQYGYLTLNNGSQAVGRSSFYCEYFPSQMLRTGNNFT
FSYTFEDVPFHSSYAHQS LDR LMNPLIDQYL YLNR TQNQSGSAQNKDLLFSRGSAG
MSVQPKNWLPGPCYRQQRVSKTKTNNNSNFTWTGASKYNLNGRESIINPGTAMASHKD

DKDKFFPMSGVMIFGKESAGASNTALDNVMITDEEEIKATNPVATERFGTVAVNLSST
DPATGDVHVMGALPGMVWQDRDVYLQGPWAKIPHTDGHFHPSPLMGGFGLKHPPPQI
LIKNTVPANPPAEFSATKFASFITQYSTGQVSVEIEWEQKENSKRWNPEVQYTSNYAKS
ANVDFTVDNNGLYTEPRPIGTRYLTRPL

an AAV7 capsid protein comprising the amino acid sequence of SEQ ID NO: 29:

AADGYLPDWLEDNLSEGIREWWDLKPGAPKPKANQQKQDNGRGLVLPGYKYLGPFG
LDKGEPVNAADAAALEHDKAYDQQLKAGDNPYLRYNHADAEEFERLQEDTSFGGNLGR
AVFQAKKRVLEPLGLVEEGAKTAPAKKRPVEPSPQRSPTSSTGIGKKGQQPARKRLNFG
QTGDSESVDPDPQPLGEPPAAPSSVGGTVAAGGGAPMADNNEGADGVGNASGNWHCDS
TWLGDRVITTSTRTWALPTYNNHLYKQISSETAGSTNDNTYFGYSTPWGYFDFNRHCH
FSPRDWQLINNNWGFRPKKLRFKLFNIQVKEVTTNDGVTTIANNLTSTIQVFSSEYQLP
YVLGSAHQGCLPPFPADVFMIPQYGYLTLNNGSQSVGRSSFYCEYFPSQMLRTGNNFEF
SYSFEDVPFHSSYAHQSGLDRLMNPLIDQYLYLARTQSNPGGTAGNRELQFYQGGPST
MAEQAKNWLPGPCFRQQRVSKTLDNNNSNFAWTGATKYHLNGRNSLVNPGVAMATH
KDDedrFFPSSGVLIFGKTGATNKTTLNVLMTNEEEIRPTNPVATEEYGIVSSNLQANT
AAQTQVVNNQALPGMVWQNRDVYLQGPWAKIPHTDGNFHPSPLMGGFGLKHPPPQI
LIKNTVPANPPEVFTPAKFASFITQYSTGQVSVEIEWEQKENSKRWNPEIQYTSNFEKQT
GVDFAVDSQGVYSEPRPIGTRYLTRNL

an AAV8 capsid protein comprising the amino acid sequence of SEQ ID NO: 30:

AADGYLPDWLEDNLSEGIREWWALKPGAPKPKANQQKQDDGRGLVLPGYKYLGPFG
LDKGEPVNAADAAALEHDKAYDQQLQAGDNPYLRYNHADAEEFERLQEDTSFGGNLGR
AVFQAKKRVLEPLGLVEEGAKTAPGKKRPVEPSPQRSPTSSTGIGKKGQQPARKRLNFG
QTGDSESVDPDPQPLGEPPAAPSGVGNTMAAGGGAPMADNNEGADGVGSSSGNWHCDS
TWLGDRVITTSTRTWALPTYNNHLYKQISNGTSGGATNDNTYFGYSTPWGYFDFNRH
CHFSRPDWQLINNNWGFRPKRLSFKLFNIQVKEVTQNEGKTIANNLTSTIQVFTDSEYQ
LPYVLGSAHQGCLPPFPADVFMIPQYGYLTLNNGSQAVGRSSFYCEYFPSQMLRTGNNF
QFTYTFEDVPFHSSYAHQSGLDRLMNPLIDQYLYLSRTQTTGGTANTQTLGFSQGGPN
TMANQAKNWLPGPCYRQQRVSTTTGNNSNFAWTAGTKYHLNGRNSLANPGIAMAT
HKDDERFFPSNGILIFGKQNAARDNADYSDVMLTSEEEIKTTNPVATEEYGIVADNLQQ
NTAPQIGTVNSQALPGMVWQNRDVYLQGPWAKIPHTDGNFHPSPLMGGFGLKHPPP
QILIKNTVPADPPTTFNQSKLNSFITQYSTGQVSVEIEWEQKENSKRWNPEIQYTSNYYK
STSVDFAVNTEGVYSEPRPIGTRYLTRNL

an AAV9 capsid protein comprising the amino acid sequence of SEQ ID NO: 31:

AADGYLPDWLEDNLSEGIREWWALKPGAPQPKANQQHQDNARGLVLPGYKYLGPFG
GLDKGEPVNAADAAALEHDKAYDQQLKAGDNPYLKYNHADAEEFERLKEDTSFGGNLGR
RAVFQAKKRLLEPLGLVEEAAKTAPGKKRPVEQSPQEPDSSAGIGKSGAQAQKRLNFG
QTGDTESVDPDPQPIGEPPAAPSGVGLTMAAGGGAPVADNNEGADGVGSSSGNWHCDSQ
WLGDRVITTSTRTWALPTYNNHLYKQISNSTSGGSSNDNAYFGYSTPWGYFDFNRHCH

HFSPRDWQLINNNWGFRPKRLNFKLFNIQVKEVTDNNGVKTIANNLTSTVQVFTDSYQ
 LPYVLGSAHEGCLPPFPADVFMIPQYGYLTLNDGSQAVGRSSFYCEYFPSQMLRTGNNF
 QFSYEFENVPFHSSYAHQSQSLDRLMNPLIDQYLYYLSKTINGSGQNQQTTLKFSVAGPSN
 MAVQGRNYIPGPSYRQQRVSTTVTNNNSEFAWPGASSWALNGRNSLMNPGPAMASHK
 EGEDRFFPLSGSLIFGKQGTGRDNVDADKVMITNEEEIKTTNPVATESYQQVATNHQSQ
 AQAQTGWVQNQGILPGMVWQDRDVYLQGPIWAKIPHTDGNFHPSPLMGGFGMKHPPP
 QILIKNTPVPADPPTAFNKDKLNSFITQYSTGQVSVEIEWEQKENS KRWNPEIQYTSNYY
 KSNNVEFAVNTEGVYSEPRPIGTRYLTRNL

an AAV10 capsid protein comprising the amino acid sequence of SEQ ID NO: 32:

MAADGYLPDWLEDNLSEGIREWWDLKPGAPKPKANQQKQDDGRGLVLPGYKYLGPF
 NGLDKGEPVNAADAAALEHDKAYDQQLKAGDNPYLRYNHADADEFQERLQEDTSFGGN
 LGRAVFQAKKRVLEPLGLVEEGAKTAPGKKRPVEPSQSPDSSTGIGKKGQQPAKKRL
 NFGQTGDSESVDPDPQIGEPAGPSGLSGTMAAGGGAPMADNNEGADGVGSSSGNWH
 CDSTWLGDREVTTSTRTWALPTYNNHLYKQISNGTSGGSTNDNTYFGYSTPWGYFDEN
 RFHCHFSRPDWQRLINNNWGFRPKRLNFKLFNIQVKEVTQNEGKTIANNLTSTIQVFTD
 SEYQLPYVLGSAHQGCLPPFPADVFMIPQYGYLTLNNGSQAVGRSSFYCLEYFPSQMLR
 TGNNFEFSYQFEDVPFHSSYAHQSQSLDRLMNPLIDQYLYYLSRTQSTGGTAGTQQLFSQ
 AGPNNMSAQAKNWLPGPCYRQQRVSTTSLQNNNSNFAWTGATKYHLNGRDSL VNPG
 VAMATHKDDDEERFFPSSGVLMFGKQGAGKDNVDYSSVMLTSEEEIKTTNPVATEQYGV
 VADNLQQQNAAPIVGAVNSQGALPGMVWQNRDVYLQGPIWAKIPHTDGNFHPSPLMG
 GFGLKHPPPQILIKNTPVPADPPTTFSQAKLASFITQYSTGQVSVEIEWELQKENS KRWN
 PEIQYTSNYYKSTNVDFAVNTDGTYSERPIGTRYLTRNL

an AAV11 capsid protein comprising the amino acid sequence of SEQ ID NO: 33:

MAADGYLPDWLEDNLSEGIREWWDLKPGAPKPKANQQKQDDGRGLVLPGYKYLGPF
 NGLDKGEPVNAADAAALEHDKAYDQQLKAGDNPYLRYNHADADEFQERLQEDTSFGGN
 LGRAVFQAKKRVLEPLGLVEEGAKTAPGKKRPLESPQEPDSSSGIGKKGKQPARKRLNF
 EEDTGAGDGPPEGSDTSAMSSDIEMRAAPGGNAVDAGQGS DGVGNASGDWHCDSTWS
 EGKVTTTSTRTWLPTYNHLYLRLGTTSSSNTYNGFSTPWGYFDENRFHCHFSRPDW
 QRLINNNWGLRPKAMRVKIFNIQVKEVTTSNGETTVANNLTSTVQIFADSSYELPYVMD
 AGQEGSLPPFPNDVFMVPQYGYCGIVTGENQNQTDRNAFYCLEYFPSQMLRTGNNFEM
 AYNFEKVPFHSMYAHQSQSLDRLMNPLLDQYLWHLQSTTSGETLNQGNAAATTFGKIRSG
 DFAFYRKNWLPGPCVKQQRFSKTASQNYKIPASGGNALLKYDTHYTLNNRWSNIAPGP
 PMATAGPSDGD FSNAQLIFGPSVTGNTTTSANNLLFTSEEEIAATNPRD TDMFGQIADN
 NQNATTAPITGNVTAMGVLPGMVWQNRDIYYQGPIWAKIPHADGHFHPSP LIGGFGLK
 HPPPQIFIKNTPVPANPATTTAARVDSFITQYSTGQVAVQIEWEIEKERSKRWNPEVQFT
 SNYGNQSSMLWAPDTTGKYTEPRVIGSRYLTNHL

an AAV12 capsid protein comprising the amino acid sequence of SEQ ID NO: 34:

MAADGYLPDWLEDNLSEGIREWWALKPGAPQPKANQQHQDNGRGLVLPGYKYLGP
 NGLDKGEPVNEADAAALEHDKAYDKQLEQGDNPYLKYNHADADEFQQRLATDTSFGGN
 LGRAVFQAKKRILEPLGLVEEGVKTA PGKKRPLEKTPNRPTNPDSGKAPAKKKQKDGE
 ADSARRTLDFEDSGAGDGPPEGSSSGEMSHDAEMRAAPGGNAVEAGQGADGVGNASG
 DWHCDSTWSEGRVTTTSTRTWVLPTYNNHLYLRIGTTANSNTYNGFSTPWGYFDFNRF
 HCHFSPRDWQRLINNNWGLRPKSMRVKIFNIQVKEVTTSNGETTVANNLTSTVQIFADS
 TYELPYVMDAGQEGSFPPFPNDVFMVPQYGYCGVVTGKNQNQTDRNAFYCLEYFPSQ
 MLRTGNNFEVSYQFEKVPFHSMYAHQSLSDRMMNPLLDQYLWHLQSTTTGNSLNQGT
 ATTTYGKITTGDFAYYRKNWLPGACIKQQKFSKNANQNYKIPASGGDALLKYDTHHTL
 NGRWSNMAPGPPMATAGAGDSDFSNSQLIFAGNPSPGNTTTSSNNLLFTSEEEIATTNPR
 DTD MFGQIADNNQNATTAPHIANLDAMGIVPGMVWQNRDIYYQGPIWAKVPHTDGHF
 HPSPLMGGFGLKHPPPQIFIKNTPVPANPNTTFSAAARINSFLTQYSTGQVAVQIDWEIQKE
 HSKRWNPEVQFTSNYGTQNSMLWAPDNAGNYHELRAIGSRFLTHHL

[0073] The capsid protein may have at least one mutation, for example, an amino acid substitution. In some cases, a recombinant adeno-associated viral (rAAV) vector comprises a capsid protein with a mutated tyrosine residue which enables the vector to have improved transduction efficiency of a target cell, e.g., a retinal bipolar cell (e.g. ON or OFF retinal bipolar cells; rod and cone bipolar cells). In some cases, the rAAV further comprises a promoter (e.g., mGluR6, or fragment thereof) capable of driving the expression of a protein of interest in the target cell.

[0074] In one embodiment, a mutation may be made in any one or more of tyrosine residues of the capsid protein of AAV 1-12 or hybrid AAVs. In specific embodiments these are surface exposed tyrosine residues. In a related embodiment the tyrosine residues are part of the VP1, VP2, or VP3 capsid protein. In exemplary embodiments, the mutation may be made at one or more of the following amino acid residues of an AAV-VP3 capsid protein: Tyr252, Tyr272, Tyr444, Tyr500, Tyr700, Tyr704, Tyr730; Tyr275, Tyr281, Tyr508, Tyr576, Tyr612, Tyr673 or Tyr720. Exemplary mutations are tyrosine-to-phenylalanine mutations including, but not limited to, Y252F, Y272F, Y444F, Y500F, Y700F, Y704F, Y730F, Y275F, Y281F, Y508F, Y576F, Y612G, Y673F and Y720F. In a specific embodiment these mutations are made in the AAV2 serotype. In some cases, an AAV2 serotype comprises a Y444F mutation and/or an AAV8 serotype comprises a Y733F mutation, wherein 444 and 733 indicate the location of a point tyrosine mutation of the viral capsid. In further embodiments, such mutated AAV2 and AAV8

serotypes encode a light-sensitive protein and also comprise a modified mGluR6 promoter to drive expression of such light-sensitive protein. Such AAV vectors are described in, for example, Petrs-Silva et al., Mol. Ther., 2011 19:293-301).

[0075] In preferred embodiments, the amino acid mutation is of one or more of the surface tyrosine residues (*e.g.*, Y252, Y272, Y444, Y500, Y700, Y704, and Y730 of an AAV2 capsid protein), surface threonine residues (*e.g.*, T251, T329, T330, T454, T455, T503, T550, T592, T581, T597, T491, T671, T659, T660, T701, T713, and T716 of an AAV2 capsid protein), surface serine residues (*e.g.*, S261, S264, S267, S276, S384, S458, S468, S492, S498, S578, S658, S662, S668, S707, S721 of an AAV2 capsid protein), and/or surface lysine residues (*e.g.*, 258, K321, K459, K490, K507, K527, K572, K532, K544, K549, K556, K649, K655, K665, K706 of an AAV2 capsid protein). These residues are highly conserved between AAV1-AAV12 capsids, thus embodiments utilizing AAV1-AAV12 are encompassed herein and could be readily developed by those of ordinary skill viewing the instant disclosure. Preferred capsid mutants of the invention increase transduction efficiency compared with wild-type capsid proteins.

[0076] In one aspect, the mutation is a tyrosine (Y) to phenylalanine (F) at one or more of Y252, Y272, Y444, Y500, Y700, Y704, and Y730 of an AAV2 capsid protein or an equivalent conserved residue of AAV1 or AAV3-12. In a preferred embodiment, the Y to F mutation is at amino acid position 444 and/or position 730 of an AAV2 capsid protein or an equivalent conserved residue of AAV1 or AAV3-12. In another preferred embodiment, the mutant is a quadruple mutant with Y to F mutations at Y272, Y444, Y500, and Y730. Petrs-Silva, H. et al., "Novel Properties of Tyrosine-mutant AAV2 Vectors in the Mouse Retina," Mol. Ther., vol. 19(2): 293-301 (2011).

[0077] In another aspect, the mutation is a threonine (T) to valine (V) at one or more of T251, T329, T330, T454, T455, T503, T550, T592, T581, T597, T491, T671, T659, T660, T701, T713, and T716 of an AAV2 capsid protein or an equivalent conserved residue of AAV1 or AAV3-12. In a preferred embodiment, the T to V mutation is at amino acid position 491 of an AAV2 capsid protein or an equivalent conserved residue of AAV1 or AAV3-12. In yet another embodiment, the mutant capsid comprises Y to F mutations at Y272, Y444, Y500, and Y730, as well as a T to V mutation is at amino acid position 491 of an AAV2 capsid protein or an

equivalent conserved residue of AAV1 or AAV3-12. Kay, C. N. et al., "Targeting Photoreceptors via Intravitreal Delivery Using Novel, Capsid-Mutated AAV Vectors," PLoS One, vol. 8(4): e62097 (2013).

[0078] In another embodiment, the capsid protein is engineered to include the insert of peptide 7m8 at AAV2⁵⁸⁸, SEQ ID NO: 35: LGETTRP. Dalkara, D. et al., "In Vivo-Directed Evolution of a New Adeno-Associated Virus for Therapeutic Outer Retinal Gene Delivery from the Vitreous," Sci. Transl. Med. 5(189):ra76 (2013).

[0079] In another embodiment, the capsid protein is engineered to include a 9-amino acid stretch of a conformationally variable region of the AAV8 capsid protein between positions 585 and 593. In some embodiments, the capsid protein comprises SEQ ID NO: 36, PERTAMSLP. In preferred embodiments, SEQ ID NO: 36 is inserted between positions 585 and 593 of an AAV2 capsid protein or the equivalent conserved residues of AAV1 or AAV3-12. The capsid proteins comprising this sequence effectively transduce ocular cells, preferably bipolar and ganglion cells. In some embodiments, the capsid protein comprises SEQ ID NO: 37, SFSRAVLCD. In preferred embodiments, SEQ ID NO: 37 is inserted between positions 585 and 593 of an AAV2 capsid protein or the equivalent conserved residues of AAV1 or AAV3-12. The capsid proteins comprising this sequence effectively transduce ocular cells, preferably ON bipolar cells. Cronin, T. et al., "Efficient transduction and optogenetic stimulation of retinal bipolar cells by a synthetic adeno-associated virus capsid and promoter," EMBO Mol. Med., vol. 6(9): 1175-1190 (2014).

[0080] In any of the isolated nucleic acid molecules or nucleic acid expression vectors described herein, the modified mGluR6 promoter is upstream of a transgene to be expressed in the eye. Preferably, the transgene encodes a gene product that increases light sensitivity, increases light detection, increases photosensitivity, increases visual evoked potential, or restores vision in a retina. More preferably, the transgene is an opsin gene. Examples of opsin genes include, but are not limited to, channelrhodopsins (e.g., channelrhodopsin-1, channelrhodopsin-2, and *Volvox carteri* channelrhodopsins 1 or 2), melanopsin, pineal opsin, photopsins, halorhodopsin, bacteriorhodopsin, proteorhodopsin, or any functional variants or fragments thereof. Suitable opsin variants may be 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%

98% or 99% identical to the opsin. Preferably, the opsin variant has at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% of the functional activity of the opsin. Functional activity can be measured by any means known in the art, for example, by electroretinography.

[0081] Preferably, the mutation in the capsid protein is a tyrosine to phenylalanine at amino acid position 444. The ordinarily skilled artisan could readily design nucleic acid sequences that encode said mutated capsid protein. A nucleic acid sequence for such an exemplary capsid protein is provided below in SEQ ID NO: 20:

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tcagagagagtgtcctcagccaatctgaacaataccatcggcagccatacctgattaaatcatttattgttcaaagatgcagtcacccaat
ccacattgaccagatcgaggcagtgcaagcgtctggcacccttcccatgatgatgaatgtacacagttctgatacgcccttttgacgac
agaaacgggttgagattctgacacgggaaagcactctaaacagcttctgtccgtgagtgaaagcagatattgaattctgattcattctctcgc
attgtctgcagggaaacagcatcagattcatgcccacgtgacgagaacattgttttggtacctgtctgcgtagttgatcgaagctccgcgtct
gacgtgatggctgcgcaactgactcgcgcacccgttggggtcacttatctgcgtcactggggcggggtctttcttggtccaccctttt
gacgtagaattcatgctccacctcaaccacgtgaccccttggccaccggaaaaagctttgacttctgcttggtgacctcccaagtcagat
ccagacggcgggtgagttcaaattgaacatccggcttgcacggctgctggtgttcgaaggctgttgagttcccgtaacacggcgacac
atgttggtgttgagggtgacgatcacgggagtcgggtctatctgggcccaggacttgcatcttggtccacgcgcaccttgcttctccgaga
atggcttggccactccacgaccttggcggtcatcttccctctccaccagatcacatctgtcgacacagtcgttgagggaaggttct
cattggtccagtttacgcacccgtagaagggcacagtggtggctatggcctccgcgatgttggtcttcccggtagttgcaggccaaacagc
cagatggtgttctcttgcgaacttttctgtggccatccagaaagacggaagccgcataattggggatcgtaaccgtttagttccaaaattt
ataaatccgattgctggaatgtcctccacgggctgctggcccaccaggtagtcggggcggttttagtcaggctcataatcttcccgcat
gtccaaggcagccttgattgggaccgcgagttggaggccgcatgaaggagatgatgaggcctggtcctcctggatccactgcttctccg
aggtaatcccttgtccacgagccaccgaccagtcctgtacctggctgaagttttgatctgatcaccggcgcatcagaattgggattctg
attctcttgttctgctctgcgtctgcgacagtcgctcagatgctgcgccaccaaccgtttacgctccgtgagattcaaacaggcgctgaa
caataggaaggagtggtgatgtgtgtgctgcccggggctctgactacaggtctccccctcgcgcccgatggtgggacggtatgaat
aatccggaatatttatagggtttttattacaaaactgttacgaaaacagtaaaatacttatttttgcgagatggttatcatttaattatctcatga
tagatctctatcactgataggagtagtacttaataactgttccatattagtcacgcccactggagctcaggctgggttttggggagcaagt
aattggggatgtagcactcatccaccacctgttcccgcctccggcgccattctggtctttgtgaccgcgaaccagtttgcaaaagtcggctc
gatcccgcggtaaaattctctgaatcagtttttcgcaatctgactcaggaaacgtccaaaaccatggattcaccccggtggttccacgagc
acgtgcatgtggaagtagctctctcccttctcaattgcacaaagaaaagggcctccggggccttactcacacggcgccattccgtcagaaa
gtcgcgtgcagcttctcggccacggtcaggggtgcctgtcaatcagattcagatccatgtcagaatctggcggaactccattccttctc
ggccaccaggttcacaaagctgtcagaaatgccgggcagatgctcgtcaaggctcgtggggacctaatacacaatctgtaaaaccccg
cat
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[0082] In some embodiments, self-complementary AAV vectors may be used in the present invention. These vectors feature an inverted repeat genome that can fold into double-stranded DNA (dsDNA) without the requirement for DNA synthesis or base-pairing between multiple

vector genomes. Self-complementary vectors are particularly efficient for transduction, as the vectors hybridize for the priming of transcription, thereby effectively bypassing the step of converting single-stranded DNA (ssDNA to dsDNA). Self-complementary vectors and methods of their use are described in McCarty et al., Mol. Ther., 2008, 16:1648-1656.

[0083] Vectors of particular use for the present invention are shown in Figures 1 and 2.

Modifications can be readily made by the skilled person in the art using standard DNA recombinant techniques.

[0084] The nucleic acid sequence of vector V-032-pFB-AAV-CMV-SV40pA (depicted in Figure 1) is provided below in SEQ ID NO: 21:

gacgcgccctgtagcggcgcatgaagcgcggcggtgtggtgtgtacgcgcagcgtgaccgctacacttgccagcgccttagcgcgcgc
tcttttcgttttctcccttctttctcgcacgttcgcgggtttcccgctcaagctctaaatcgggggctcccttaggttccgatttagtcttt
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[0086] Any of a variety of other vectors adapted for expression of any light-sensitive protein in a cell of the eye, particularly within a retinal cell, more particularly within a non-photoreceptor cell (e.g. amacrine cells, retinal ganglion cells, retinal bipolar cells, (ON or OFF cone retinal bipolar cells; rod bipolar cells)), are within the scope of the present invention. Gene delivery vectors can be viral (e.g., derived from or containing sequences of viral DNA or RNA, preferably packaged within a viral particle), or non-viral (e.g., not packaged within a viral particle, including “naked” polynucleotides, nucleic acid associated with a carrier particle such as a liposome or targeting molecule, and the like).

Therapeutic Uses

[0087] The advantages of using gene regulatory elements, such as those encompassed by the present invention, that direct the expression to a specific subset of retinal eyes is to accurately recapitulate the visual processing signals from the ON and OFF pathways to improve or restore photosensitivity, and thereby improving or restoring vision.

[0088] Visual information is processed through the retina through two pathways: an ON

pathway which signals the light ON, and an OFF pathway which signals the light OFF. The existence of the ON and OFF pathway is important for the enhancement of contrast sensitivity. The visual signal in the ON pathway is relay from ON-cone bipolar cells to ON ganglion cells. Both ON-cone bipolar cells and ON-ganglion cells are depolarized in response to light. On the other hand, the visual signal in the OFF pathway is carried from OFF-cone bipolar cells to OFF ganglion cells. Both OFF-cone bipolar cells and OFF-ganglion cells are hypopolarized in response to light. Rod bipolar cells, which are responsible for the ability to see in dim light (scotopic vision), are ON bipolar cells (depolarized in response to light). Rod bipolar cells relay the vision signal through AII amacrine cells (an ON type retinal cells) to ON an OFF cone bipolar cells.

[0089] Accordingly, a dual rhodopsin system can be used to recapitulate the ON and OFF pathways integral to visual processing and acuity. Briefly, a ChR2 or Chop2 protein can be specifically targeted to ON type retinal neurons (*e.g.*, ON type ganglion cells and/or ON type bipolar cells), while a hypopolarizing light sensor (*e.g.*, halorhodopsin or other chloride pump or proton pump, preferably Arch, ArchT, Jaws known in the art, as well as variants known in the art or yet to be identified) can be targeted to OFF type retinal neurons (*e.g.* OFF type ganglion cells and/or OFF type bipolar cells) to create ON and OFF pathways. An alternative approach to restore ON and OFF pathways in the retina is achieved by, expressing a depolarizing light sensor, such as ChR2, to rod bipolar cells or AII amacrine. In this approach, the depolarization of rod bipolar cells or AII amacrine cells can lead to the ON and OFF responses at the levels of cone bipolar cells and the downstream retinal ganglion cells. Thus, the ON and OFF pathways that are inherent in the retina are restored or maintained.

[0090] The present invention can be used in methods for increasing photosensitivity, increasing phototransduction, increasing visual evoked potential (VEP), or improving or restoring vision. Tests are known in the art for quantifying photosensitivity and visual evoked potential, including electroretinography (ERG) analysis. Visual and behavior tests can be utilized to determine improvements or restoration of vision. Behavior tests include maze tests and the swimming test. Visual tests, such as the Snellen chart and visual field testing, are utilized to determine improved or restored vision. Such tests can be readily performed by a

clinical practitioner. As used herein, “increasing” is meant in reference to before treatment or in comparison to one that has not undergone treatment, *e.g.*, ocular gene therapy.

[0091] The present invention can be formulated to a pharmaceutical composition or medicament suitable for administration into a subject or patient. Suitable routes of administration include, for example, intravitreal, intraocular, or subretinal injection. Preferably, the route of administration is by intravitreal injection. All retinal neurons, including retinal ganglion cells, bipolar cells, horizontal cells, amacrine cells, and photoreceptor cells are known to be reasonably well-accessible to intravitreal injection as disclosed herein. Intravitreal and/or subretinal injection can provide the necessary access to the bipolar cells, especially in circumstances in which the photoreceptor cell layer is absent due to degeneration.

[0092] Such formulations comprise a pharmaceutically and/or physiologically acceptable vehicle, diluent, carrier, or excipient, such as buffered saline or other buffers, *e.g.*, HEPES, to maintain physiologic pH. For a discussion of such components and their formulation, see, generally, Gennaro, A.E., *Remington: The Science and Practice of Pharmacy*, Lippincott Williams & Wilkins Publishers; 2003 or latest edition). See also, WO00/15822. If the preparation is to be stored for long periods, it may be frozen, for example, in the presence of glycerol.

[0093] In one embodiment, the constructs or nucleic acid expression vectors described herein are packaged in adenoviral vectors for transgene delivery. An effective amount of rAAV virions carrying a transgene under the control of the modified mGluR6 promoter is preferably in the range of between about 10^{10} to about 10^{13} rAAV infectious units in a volume of between about 150 and about 800 μl per injection. The rAAV infectious units can be measured according to McLaughlin, SK *et al.*, 1988, *J. Virol.* 62:1963. More preferably, the effective amount is between about 10^{10} and about 10^{12} rAAV infectious units and the injection volume is preferably between about 250 and about 500 μl . Other dosages and volumes, preferably within these ranges but possibly outside them, may be selected by the treating professional, taking into account the physical state of the subject (preferably a human), who is being treated, including, age, weight, general health, and the nature and severity of the particular ocular disorder.

[0094] It may also be desirable to administer additional doses (“boosters”) of the present

nucleic acid(s) or rAAV compositions. For example, depending upon the duration of the transgene expression within the ocular target cell, a second treatment may be administered after 6 months or yearly, and may be similarly repeated. Neutralizing antibodies to AAV are not expected to be generated in view of the routes and doses used, thereby permitting repeat treatment rounds.

[0095] The need for such additional doses can be monitored by the treating professional using, for example, well-known electrophysiological and other retinal and visual function tests and visual behavior tests. The treating professional will be able to select the appropriate tests applying routine skill in the art. It may be desirable to inject larger volumes of the composition in either single or multiple doses to further improve the relevant outcome parameters.

Ocular Disorders

[0096] The term treatment includes, but is not limited to, arresting, inhibiting, or reversing the progression of an ocular disease or disorder. Preferred indicators of successful treatment are the preservation of existing vision or improvement of vision compared to vision before treatment.

[0097] The ocular disorders for which the present nucleic acids and vectors, are intended and may be used to improve one or more parameters of vision include, but are not limited to, developmental abnormalities that affect both anterior and posterior segments of the eye. Anterior segment disorders include glaucoma, cataracts, corneal dystrophy, and keratoconus. Posterior segment disorders include blinding disorders caused by photoreceptor malfunction and/or death caused by retinal dystrophies and degenerations.

[0098] A nonlimiting list of ocular diseases that may benefit from the methods described herein include, but are not limited to, retinoblastoma, ocular melanoma, diabetic retinopathy, hypertensive retinopathy, any inflammation of the ocular tissues (*i.e.*, chorioretinal inflammation, scleritis, keratitis, uveitis, etc.), or infection (*i.e.*, bacterial or viral). Angiogenesis-related eye diseases include, but are not limited to age-related macular degeneration, diabetic retinopathy, corneal neovascularizing diseases, retinal angiomatous proliferation, polypoidal choroidal

vasculopathy, ischemia-induced neovascularizing retinopathy, extreme or high myopia, and retinopathy of prematurity.

[0099] Retinal disorders include congenital stationary night blindness, macular degeneration, age-related macular degeneration, congenital cone dystrophies, and a large group of retinitis-pigmentosa (RP)-related disorders. These disorders include genetically pre-disposed death of photoreceptor cells—rods and cones in the retina—occurring at various ages. Among those are severe retinopathies, such as subtypes of RP itself that progresses with age and causes blindness in childhood and early adulthood and RP-associated diseases, such as genetic subtypes of Leber's congenital amaurosis (LCA), which frequently results in loss of vision during childhood, as early as the first year of life. The latter disorders are generally characterized by severe reduction, and often complete loss of photoreceptor cells, rods and cones. (Trabulsi, EI, ed., *Genetic Diseases of the Eye*, Oxford University Press, NY, 1998).

[0100] Ocular neovascularization is a widespread cause of vision loss that may also be treated using the optimized enhancers, promoters, and vectors of the present invention. It can occur in a number of proliferative retinal diseases including, but not limited to, diabetic retinopathy, dry (atrophic) and wet (neovascular or exudative) age-related macular degeneration (AMD), retinal artery or vein occlusion, glaucoma, and other inherited retinal degenerations, uveitis, retinal detachment, and eye cancers (ocular melanoma and retinoblastoma), and retinopathy of prematurity (ROP).

[0101] In particular, the optimized enhancers, promoters, and vectors of the present invention useful for expressing transgenes for the treatment and/or restoration of at least partial vision to subjects that have lost vision due to ocular disorders, such as RPE-associated retinopathies, which are characterized by a long-term preservation of ocular tissue structure despite loss of function and by the association between function loss and the defect or absence of a normal gene in the ocular cells of the subject. A variety of such ocular disorders are known, such as childhood onset blinding diseases, retinitis pigmentosa, macular degeneration, and diabetic retinopathy, as well as ocular blinding diseases known in the art. It is anticipated that these other disorders, as well as blinding disorders of presently unknown causation which later are characterized by the same description as above, may also be successfully treated by the

transgenes expressed by the nucleic acids and vectors of the present invention.

[0102] Thus, the particular ocular disorder treated by the present invention may include the above-mentioned disorders and a number of diseases which have yet to be so characterized.

EXAMPLES

Example 1: Generation of Optimized mGluR6 Promoter Constructs

[0103] A series of AAV2 expression cassettes were constructed with the combination of sequences of the mGluR6 promoter, the 200 bp mGluR6 enhancer, and intron sequences of the mGluR6 gene, carrying a transgene. The transgene was either reporter mCherry or GFP-fused channelrhodopsin-2 (GFP-ChR2), ChR2 alone.

[0104] Two optimized mGluR6 promoter constructs are shown in Figure 3 (I4) and Figure 4 (I1a) for driving expression of a transgene. The transgene is, for example, a reporter gene, mCherry. The I4 construct comprises an optimized promoter with the 200bp mGluR6 enhancer sequence located upstream of a 500 bp fragment of the mGluR6 promoter. The I1a construct comprises an optimized promoter with intron 4 of the mGluR6 gene located upstream of intron 3 of the mGluR6 gene, intron 3 of the mGluR6 gene located upstream of the 200 bp mGluR6 enhancer sequence, and the mGluR6 enhancer sequence located upstream of a 500 bp fragment of the mGluR6 promoter.

[0105] AAV2 serotype 2 vectors with an Y444F capsid mutation were packaged and affinity purified at Virovek (Hayward, CA).

Example 2: *In Vivo* Expression in the Retina

[0106] Recombinant AAV-mediated expression of a transgene by the two optimized mGluR6 promoters shown in Figures 3 and 4 were tested in mice.

[0107] Briefly, 1-month-old C57BL/6J mice were anesthetized by intraperitoneal injection of a mixture of 120 mg/kg ketamine and 15 mg/kg xylazine. Under a dissecting microscope, a small perforation was made in the temporal sclera region with a needle. A total of 1 µl viral vector solution at the concentration of 1×10^{13} vg/ml was injected into the intravitreal space

through the hole with a Hamilton syringe. One month after the injection, the mice were killed to examine the expression patterns of transgene.

[0108] Retinal whole-mounts or vertical sections were blocked for 1 h in a solution containing 5% Chemiblocker (membrane-blocking agent; Chemicon, Brica, MA, USA), 0.5% Triton X-100 and 0.05% sodium azide (Sigma). The primary antibodies were diluted in the same solution and applied overnight, followed by incubation (1 h) in the secondary antibodies, which were conjugated to Alexa 594 (1:600, red fluorescence, Molecular Probes), Alexa 488 (1:600, green fluorescence, Molecular Probes). The following antibodies were used in this study: rabbit anti-mCherry (1:500, 632496, Clontech); rabbit anti-PKC (1:20000, 2056, Cell Signal).

[0109] All images were made using a Zeiss Axioplan 2 microscope with the Apotome oscillating grating to reduce out-of-focus stray light. Z-stack images were captured and image projections were made by collapsing individual z-stacks of optical sections into a single plane. To create the merged images for double labeling, the red and green or blue channels for each individual optical section were combined and the merged z-optical sections were collapsed into a single plane. The brightness and contrast were adjusted using Adobe Photoshop CS4.

[0110] Retinal whole mounts from mice injected with I4 and I1a-containing virions are shown in Figure 5. The mCherry expression at the axon terminals of bipolar cells was detected in the ganglion cell layer (Figure 5A and 5D), as well as the expression at the bipolar cell bodies in the inner nuclear layer (Figure 5B and 5D). Figures 5C and 5F demonstrate the mCherry staining viewed from retinal vertical sections. Thus, Figure 5 shows that both optimized mGluR6 promoter constructs efficiently and selectively expressed the transgene in bipolar cells in the retinas of mice. Furthermore, the expression level of I1a was shown to be two to three times higher than that of I4.

Example 3: Transgene Expression Targeting

[0111] Further immunostaining analysis demonstrated the targeting of transgene expression by the optimized mGluR6 promoters. Specifically, the retinal whole-mount sections obtained as described in Example 2 were co-stained with anti-PKC. PKC is a rod bipolar cell marker.

mCherry and PKC staining in the retinal whole mount are shown in Figure 6A and 6B, respectively, while staining in vertical sections are shown in Figure 6D and 6E, respectively. The merged images in Figures 6C and 6F show that co-labeling between the mGluR6-driven mCherry-expressing cells and the PKC-expressing cells in up to 58% of the rod bipolar cells. These results indicate that the optimized mGluR6 is able to target transgene expression to rod bipolar cells after intravitreal injection.

OTHER EMBODIMENTS

[0112] While the invention has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.

[0113] The patent and scientific literature referred to herein establishes the knowledge that is available to those with skill in the art.

[0114] While this invention has been particularly shown and described with references to preferred embodiments thereof, it will be understood by those skilled in the art that various changes in form and details may be made therein without departing from the scope of the invention encompassed by the appended claims.

CLAIMS

What is claimed is:

1. An adeno-associated virus (AAV) or a recombinant adeno-associated virus (rAAV) nucleic acid expression vector comprising an mGluR6 enhancer comprising the nucleic acid sequence of SEQ ID NO: 2, or a sequence having at least 70% identity thereto, and an mGluR6 promoter comprising the nucleic acid sequence of SEQ ID NO: 6, or a sequence having at least 70% identity thereto, operably linked to a transgene.
2. An adeno-associated virus (AAV) or a recombinant adeno-associated virus (rAAV) nucleic acid expression vector comprising an mGluR6 promoter comprising the nucleic acid sequence of SEQ ID NO: 6, or a sequence having at least 70% identity thereto, operably linked to a transgene.
3. The nucleic acid expression vector of claim 2, wherein the vector further comprises an mGluR6 enhancer comprising the nucleic acid sequence of SEQ ID NO: 2, or a sequence having at least 70% identity thereto.
4. The nucleic acid expression vector of claim 1 or 2, wherein the vector is a recombinant AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, or AAV12 vector.
5. The nucleic acid expression vector of any one of claims 1-4, wherein the vector further encodes a capsid protein and the capsid protein comprises at least one mutation.
6. The nucleic acid expression vector of claim 5, wherein said at least one mutation is selected from the group consisting of:

a tyrosine (Y) to phenylalanine (F) at amino acid position 444;
a tyrosine (Y) to phenylalanine (F) at amino acid position 730;
a tyrosine (Y) to phenylalanine (F) at amino acid positions 272, 444, 500, and 730;
a threonine (T) to valine (V) at amino acid position 491; and
a tyrosine (Y) to phenylalanine (F) at amino acid positions 272, 444, 500, 730, and a threonine (T) to valine (V) at amino acid position 491,
wherein the position is in reference to an AAV2 capsid protein or an equivalent conserved residue of AAV1 or any one of AAV3-12..

7. The nucleic acid expression vector of any one of claims 1-4, wherein the vector further encodes a capsid protein and the capsid protein comprises a peptide insert selected from the group consisting of SEQ ID NO: 35, SEQ ID NO: 36, and SEQ ID NO: 37.

8. The nucleic acid expression vector of any one of claims 1-7, wherein the transgene encodes an opsin.

9. The nucleic acid expression vector of claim 8, wherein said opsin is selected from the group consisting of channelrhodopsin, melanopsin, pineal opsin, photopsins, halorhodopsin, bacteriorhodopsin, and proteorhodopsin, or any functional variants or fragments thereof.

10. The nucleic acid expression vector of claim 9, wherein said opsin gene is encodes a channelrhodopsin.

11. The nucleic acid expression vector of any one of claims 1-10, wherein the transgene encodes a gene product that increases light sensitivity, increases light detection, increases photosensitivity, increases visual evoked potential, or restores vision in a retina.

12. A pharmaceutical composition comprising the nucleic acid expression vector of any one of claims 1-11 and a pharmaceutically acceptable carrier or excipient.
13. Use of the nucleic acid expression vector of any one of claims 1-11 for expressing a transgene in the eye of a subject.
14. Use of the pharmaceutical composition of claim 12 for expressing a transgene in the eye of a subject.

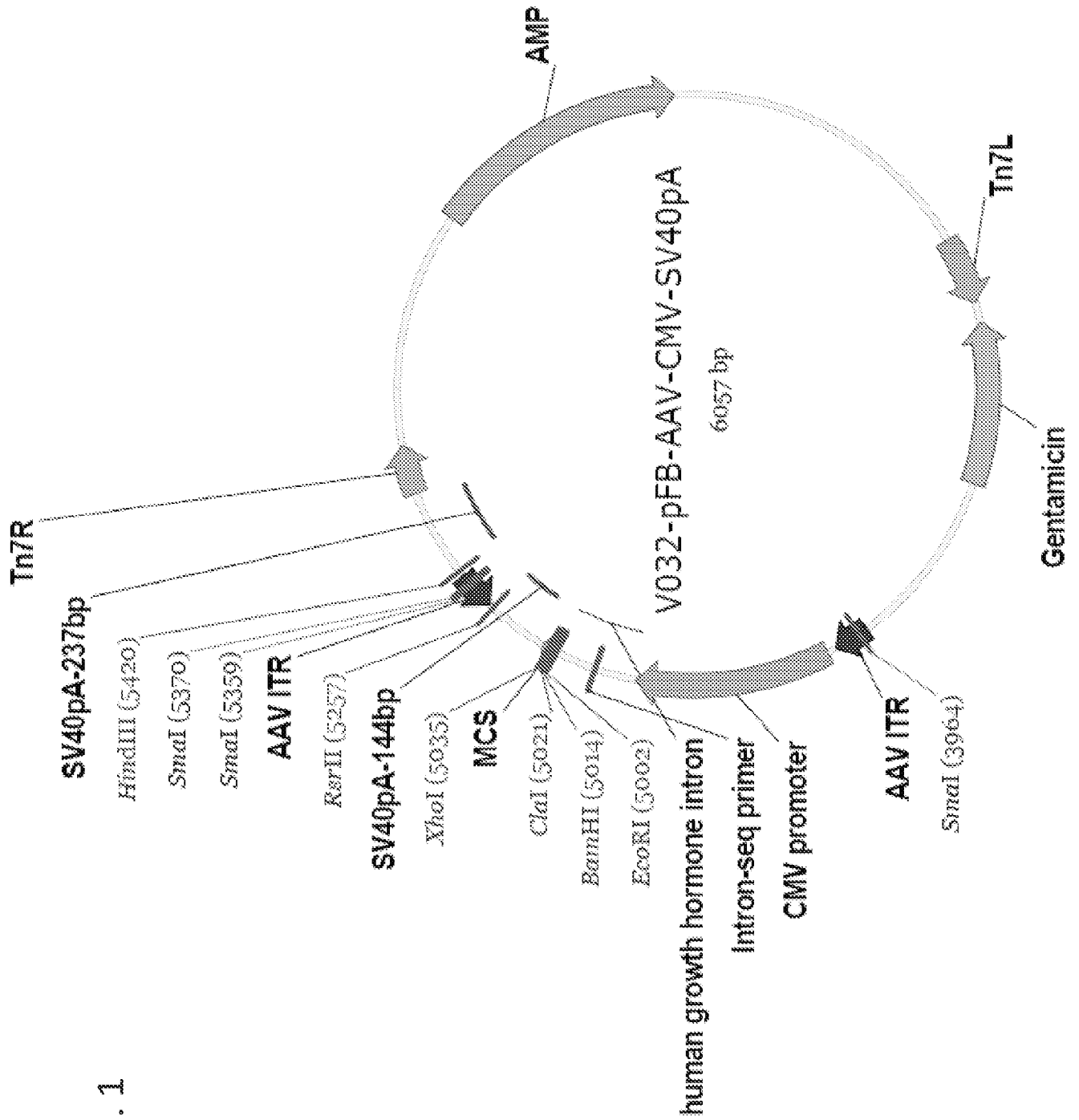


FIG. 1

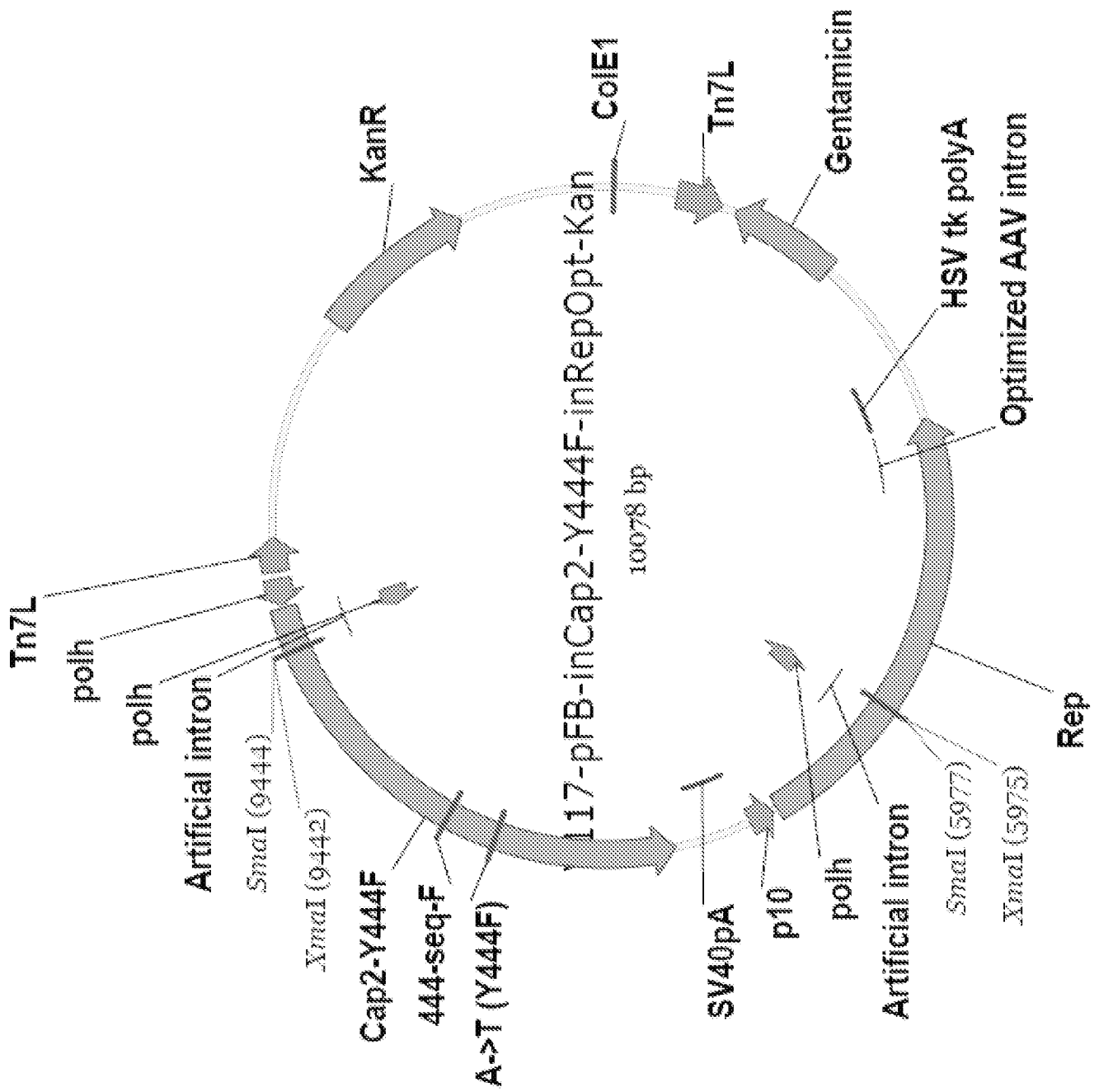
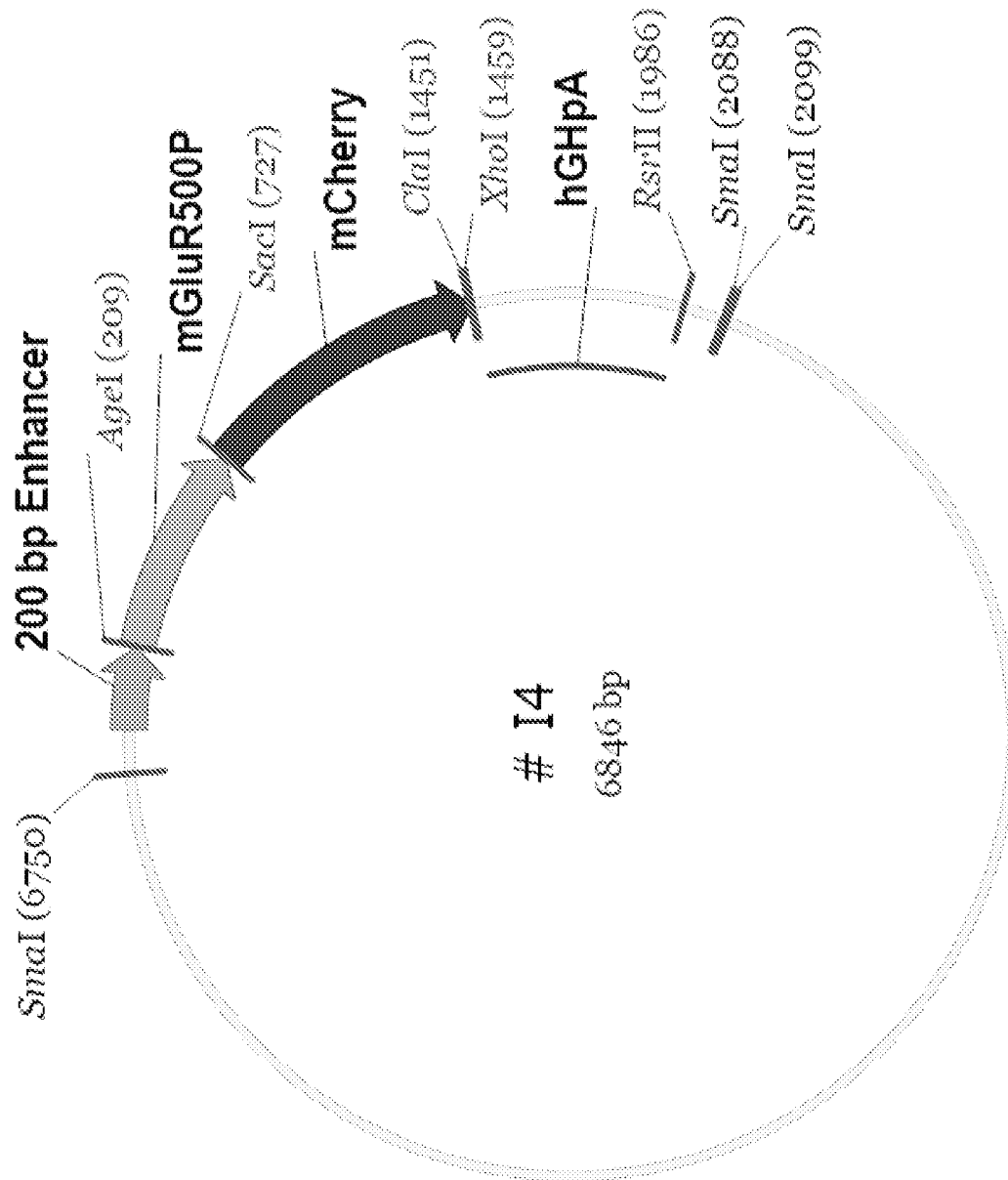


FIG. 2

FIG. 3



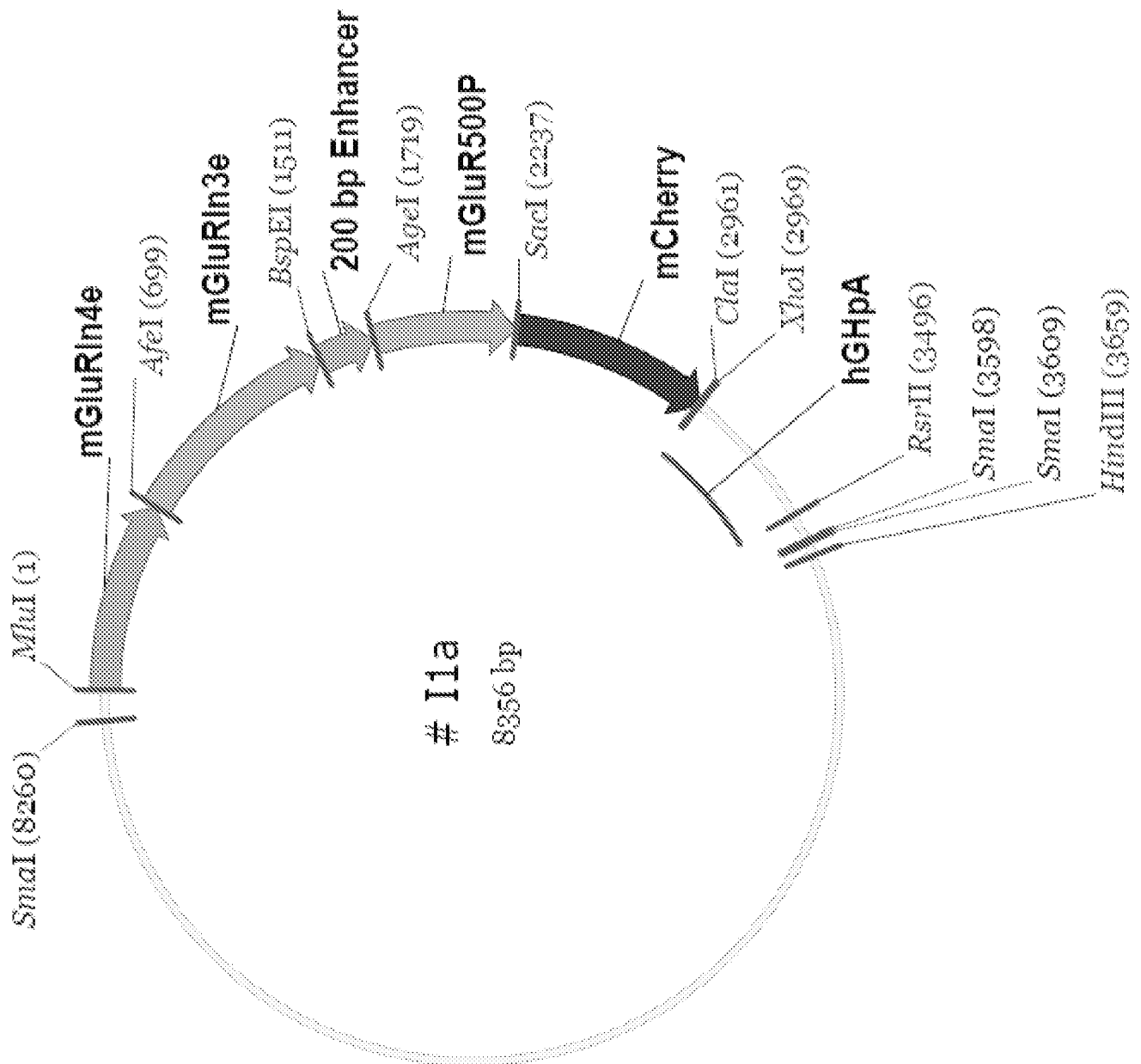


FIG. 4

FIG. 5

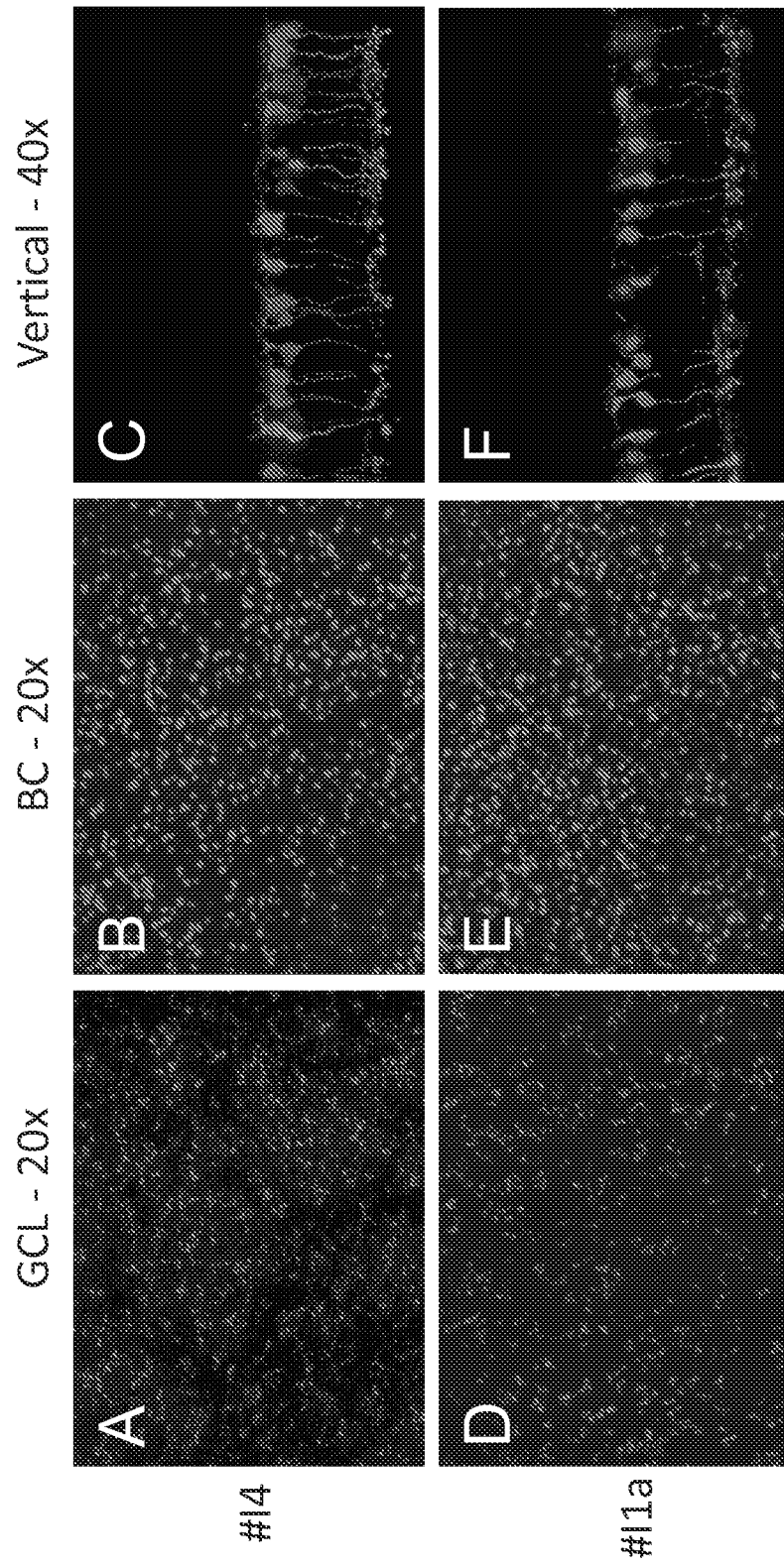


FIG. 6

