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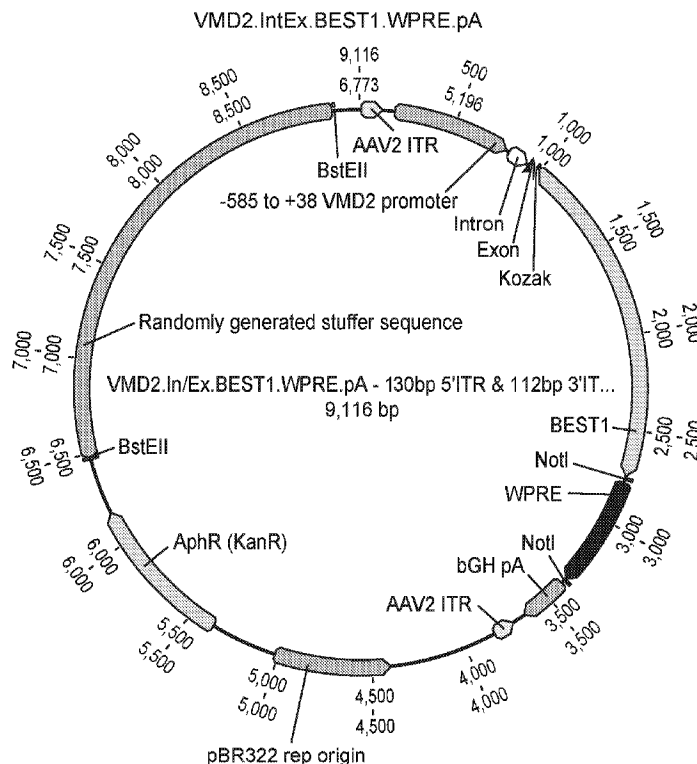
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(54) Titre : COMPOSITIONS ET PROCEDES DE TRAITEMENT DE LA DYSTROPHIE MACULAIRE
(54) Title: COMPOSITIONS AND METHODS FOR TREATING MACULAR DYSTROPHY

FIG. 1A



(57) **Abrégé/Abstract:**

The disclosure provides composition comprising a nucleic acid sequence comprising (a) a sequence encoding a vitelliform macular dystrophy-2 (VMD2) promoter, and (b) a sequence encoding a Bestrophin-1 (BEST1) protein as well as the use of these compositions for the treatment of macular dystrophy in a subject comprising administration of the composition to an eye of a subject via a subretinal or a suprachoroidal route.

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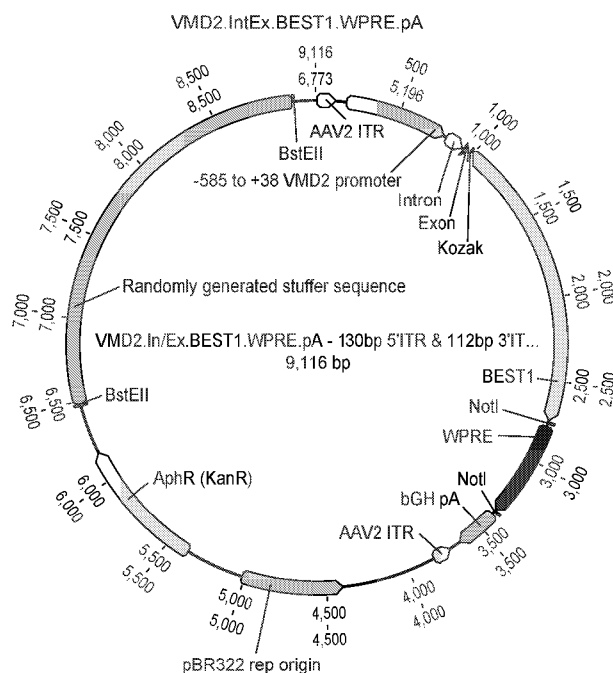
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(54) Title: COMPOSITIONS AND METHODS FOR TREATING MACULAR DYSTROPHY

FIG. 1A



(57) **Abstract:** The disclosure provides composition comprising a nucleic acid sequence comprising (a) a sequence encoding a vitelliform macular dystrophy-2 (VMD2) promoter, and (b) a sequence encoding a Bestrophin-1 (BEST1) protein as well as the use of these compositions for the treatment of macular dystrophy in a subject comprising administration of the composition to an eye of a subject via a subretinal or a suprachoroidal route.

[Continued on next page]



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COMPOSITIONS AND METHODS FOR TREATING MACULAR DYSTROPHY

RELATED APPLICATIONS

[01] This application claims the benefit of provisional application USSN 62/653,131, filed April 5, 2018, the contents of which are herein incorporated by reference in their entirety.

INCORPORATION OF SEQUENCE LISTING

[02] The contents of the text file named "NIGH-011/002WO_SeqList.txt," which was created on April 4, 2019 and is 72 KB in size, are hereby incorporated by reference in their entirety.

FIELD OF THE DISCLOSURE

[03] The invention relates to the fields of molecular biology, neurobiology and gene therapy treatments for degenerative eye diseases.

BACKGROUND

[04] Macular degeneration is a medical condition, which may result in blurred or no vision in the center of the visual field. In macular degeneration, the photoreceptors in the part of the retina called the macula, which is responsible for central vision, degenerate or die. In some cases, macular degeneration is caused by mutations in the Bestrophin-1 gene (BEST1, also called VMD2). There is currently no treatment for this devastating disease. There is thus a long felt need in the art for additional therapeutic approaches to treat macular degeneration. The disclosure provides compositions and methods of treatment for macular degeneration.

SUMMARY

[05] The disclosure provides a composition comprising a nucleic acid sequence comprising: (a) a sequence encoding a vitelliform macular dystrophy-2 (VMD2) promoter, and (b) a sequence encoding a Bestrophin-1 (BEST1) protein. In some embodiments, the sequence encoding the VMD2 promoter encodes a human VMD2 promoter. In some embodiments, the sequence encoding the BEST1 protein encodes a human BEST1 protein. In some embodiments, the sequence encoding the BEST1 protein comprises a coding sequence. In some embodiments, the sequence encoding the BEST1 protein comprises a cDNA sequence.

[06] The disclosure provides a composition comprising a nucleic acid sequence comprising: (a) a sequence encoding a ubiquitous promoter, and (b) a sequence encoding a

Bestrophin-1 (BEST1) protein. In some embodiments, the sequence encoding the BEST1 protein encodes a human BEST1 protein. In some embodiments, the sequence encoding the BEST1 protein comprises a coding sequence. In some embodiments, the sequence encoding the BEST1 protein comprises a cDNA sequence. In some embodiments, the sequence encoding a ubiquitous promoter comprises a sequence encoding a CAG promoter.

[07] In some embodiments of the compositions of the disclosure, the nucleic acid sequence further comprises: (c) a sequence encoding a posttranscriptional regulatory element (PRE). In some embodiments, the sequence encoding the PRE comprises a sequence isolated or derived from a naturally occurring sequence. In some embodiments, the sequence encoding the PRE comprises a sequence isolated or derived from a non-naturally-occurring sequence. In some embodiments, the sequence encoding the PRE comprises a sequence isolated or derived from a viral sequence. In some embodiments, the sequence encoding the PRE comprises a sequence isolated or derived from a woodchuck hepatitis virus (WPRE).

[08] In some embodiments of the compositions of the disclosure, the nucleic acid sequence further comprises: (d) a sequence encoding a polyadenylation (polyA) signal. In some embodiments, the sequence encoding the polyA signal comprises a sequence isolated or derived from a naturally occurring sequence. In some embodiments, the sequence encoding the polyA signal comprises a sequence isolated or derived from a non-naturally-occurring sequence. In some embodiments, the sequence encoding the polyA signal comprises a sequence isolated or derived from a mammalian sequence. In some embodiments, the sequence encoding the polyA signal comprises a sequence isolated or derived from a human sequence. In some embodiments, the sequence encoding the polyA signal comprises a sequence isolated or derived from a mammalian Bovine Growth Hormone (BGH) gene.

[09] In some embodiments of the compositions of the disclosure, the nucleic acid sequence further comprises: (e) a sequence encoding a 5' untranslated region (UTR). In some embodiments, the sequence encoding the 5' UTR comprises a sequence isolated or derived from a naturally occurring sequence. In some embodiments, the sequence encoding the 5' UTR comprises a sequence isolated or derived from a non-naturally-occurring sequence. In some embodiments, the sequence encoding the 5' UTR comprises a sequence isolated or derived from a mammalian sequence. In some embodiments, the sequence encoding the 5' UTR comprises a sequence isolated or derived from a human sequence. In some embodiments, the sequence encoding the 5' UTR comprises a sequence isolated or derived from a viral sequence.

In some embodiments of the compositions of the disclosure, the nucleic acid sequence further comprises: (f) a sequence encoding an intron, and (g) a sequence encoding an exon, wherein

the sequence encoding the intron and the sequence encoding the exon are operably linked. In some embodiments, the intron is located between the sequence encoding the VMD2 promoter and the sequence encoding the exon, wherein the sequence encoding the exon is located between the sequence encoding the intron and the sequence encoding the 5' UTR, and wherein the sequence encoding the intron is spliced by a mammalian cell. In some embodiments, the sequence encoding the exon comprises a sequence isolated or derived from a mammalian gene. In some embodiments, the sequence encoding the exon comprises a sequence isolated or derived from a rabbit (*Oryctolagus cuniculus*) beta globin gene. In some embodiments, the sequence encoding the intron comprises a non-naturally occurring sequence. In some embodiments, the sequence encoding the intron comprises a fusion sequence. In some embodiments, the sequence encoding the intron comprises a sequence encoding a splice donor site, and a sequence encoding a splice branch point and acceptor site. In some embodiments, the sequence encoding the splice donor site comprises a sequence isolated or derived from a vertebrate gene. In some embodiments, the sequence encoding the splice donor site comprises a sequence isolated or derived from a chicken (*Gallus gallus*) beta actin gene (CBA). In some embodiments, the sequence encoding the splice branch point and acceptor site comprises a sequence isolated or derived from a vertebrate gene. In some embodiments, the sequence encoding the splice branch point and acceptor site comprises a sequence isolated or derived from a rabbit (*Oryctolagus cuniculus*) beta globin gene.

[010] In some embodiments of the compositions of the disclosure, the sequence encoding the 5' UTR comprises a sequence encoding a Kozak sequence or a portion thereof. In some embodiments, the sequence encoding a Kozak sequence has at least 50% identity to the nucleic acid sequence of GCCRCCATGG. In some embodiments, the sequence encoding a Kozak sequence comprises or consists of the nucleic acid sequence of GGCACCATGA.

[011] In some embodiments of the compositions of the disclosure, the sequence encoding the human VMD2 promoter comprises or consists of

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1  AATTCTGTCA TTTTACTAGG GTGATGAAAT TCCCAAGCAA CACCATCCTT TTCAGATAAG
61 GGCACCTGAGG CTGAGAGAGG AGCTGAAACC TACCCGGGGT CACCACACAC AGGTGGCAAG
121 GCTGGGACCA GAAACCAGGA CTGTTGACTG CAGCCCGGTA TTCATTCTTT CCATAGCCCA
181 CAGGGCTGTC AAAGACCCCA GGGCCTAGTC AGAGGCTCCT CCTTCCTGGA GAGTTCCTGG
241 CACAGAAGTT GAAGCTCAGC ACAGCCCCCT AACCCCAAC TCTCTCTGCA AGGCCTCAGG
301 GGTGAGAACA CTGGTGGAGC AGATCCTTTA GCCTCTGGAT TTAGGGCCA TGGTAGAGGG
361 GGTGTTGCCC TAAATTCCAG CCCTGGTCTC AGCCCAACAC CCTCCAAGAA GAAATTAGAG
421 GGGCCATGGC CAGGCTGTGC TAGCCGTTGC TTCTGAGCAG ATTACAAGAA GGGACTAAGA
481 CAAGGACTCC TTTGTGGAGG TCCTGGCTTA GGGAGTCAAG TGACGGCGGC TCAGCACTCA
541 CGTGGGCAGT GCCAGCCTCT AAGAGTGGGC AGGGGCACTG GCCACAGAGT CCCAGGGAGT
601 CCCACCAGCC TAGTCGCCAG ACC (SEQ ID NO: 1) .

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[012] In some embodiments of the compositions of the disclosure, the sequence encoding the CAG promoter comprises or consists of

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1  CCATTGACGT  CAATAATGAC  GTATGTTCCC  ATAGTAACGC  CAATAGGGAC  TTTCCATTGA
61  CGTCAATGGG  TGGAGTATTT  ACGGTAAACT  GCCCACTTGG  CAGTACATCA  AGTGTATCAT
121  ATGCCAAGTA  CGCCCCCTAT  TGACGTCAAT  GACGGTAAAT  GGCCCGCCTG  GCATTATGCC
181  CAGTACATGA  CCTTATGGGA  CTTTCCTACT  TGGCAGTACA  TCTACGTATT  AGTCATCGCT
241  ATTACCATGG  TCGAGGTGAG  CCCCACGTTT  TGCTTCACTC  TCCCCATCTC  CCCCCCTCC
301  CCACCCCAA  TTTTGTATTT  ATTTATTTT  TAATTATTTT  GTGCAGCGAT  GGGGGCGGGG
361  GGGGGGGGGG  GGCGCGCGCC  AGGCGGGGCG  GGGCGGGGCG  AGGGCGGGG  CGGGGCGAGG
421  CGGAGAGGTG  CGGCGGCAGC  CAATCAGAGC  GGCGCGCTCC  GAAAGTTTCC  TTTTATGGCG
481  AGGCGGCGGC  GGCGGCGGCC  CTATAAAAAG  CGAAGCGCGC  GGCGGGCGGG  AGTCGCTGCG
541  CGCTGCCTTC  GCCCCGTGCC  CCGCTCCGCC  GCCGCTCGC  GCCGCGGCC  CCGGCTCTGA
601  CTGACCGCGT  TACTCCCACA  GGTGAGCGGG  CGGGACGGCC  CTTCTCCTCC  GGGCTGTAAT
661  TAGCGCTTGG  TTTAATGACG  GCTTGTTTCT  TTTCTGTGGC  TCGGTGAAAG  CTTTGAGGGG
721  CTCCGGGAGG  GCCCTTTGTG  CGGGGGGAGC  GGCTCGGGGC  TGTCGCGGG  GGGACGGCTG
781  CCTTCGGGGG  GGACGGGGCA  GGGCGGGGTT  CGGCTTCTGG  CGTGTGACCG  GCGGCTCTAG
841  AGCCTCTGCT  AACCATGTTC  ATGCCTTCTT  CTTTTTCCTA  CAGCTCCTGG  GCAACGTGCT
901  GGTATTGTG  CTGTCTCATC  ATTTTGCCAA  AGAATTGGAT  C (SEQ ID NO: 2) .

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[013] In some embodiments of the compositions of the disclosure, the sequence encoding the human BEST1 protein comprises or consists of

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1  ATGACCATCA  CTTACACAAG  CCAAGTGGCT  AATGCCCGCT  TAGGCTCCTT  CTCCCGCCTG
61  CTGCTGTGCT  GGCGGGGCAG  CATCTACAAG  CTGCTATATG  GCGAGTTCTT  AATCTTCCTG
121  CTCTGCTACT  ACATCATCCG  CTTTATTTAT  AGGCTGGCCC  TCACGGAAGA  ACAACAGCTG
181  ATGTTTGAGA  AACTGACTCT  GTATTGCGAC  AGCTACATCC  AGCTCATCCC  CATTTCCTTC
241  GTGCTGGGCT  TCTACGTGAC  GCTGGTCGTG  ACCCGCTGGT  GGAACCAGTA  CGAGAACCTG
301  CCGTGGCCCG  ACCGCCTCAT  GAGCCTGGTG  TCGGGCTTCG  TCGAAGGCAA  GGACGAGCAA
361  GGCCGGCTGC  TGCGGCGCAC  GCTCATCCGC  TACGCCAACC  TGGGCAACGT  GCTCATCCTG
421  CGCAGCGTCA  GCACCGCAGT  CTACAAGCGC  TTCCCCAGCG  CCCAGCACCT  GGTGCAAGCA
481  GGCTTTATGA  CTCCGGCAGA  ACACAAGCAG  TTGGAGAAAC  TGAGCCTACC  ACACAACATG
541  TTCTGGGTGC  CCTGGGTGTG  GTTGGCCAAC  CTGTCAATGA  AGGCGTGGCT  TGGAGGTCGA
601  ATCCGGGACC  CTATCCTGCT  CCAGAGCCTG  CTGAACGAGA  TGAACACCTT  GCGTACTCAG
661  TGTGGACACC  TGTATGCCTA  CGACTGGATT  AGTATCCAC  TGGTGTATAC  ACAGGTGGTG
721  ACTGTGGCGG  TGTACAGCTT  CTTCTGACT  TGTCTAGTTG  GGCGGCAGTT  TCTGAACCCA
781  GCCAAGGCCT  ACCCTGGCCA  TGAGTGGGAC  CTCGTTGTGC  CCGTCTTCAC  GTTCCTGCAG
841  TTCTTCTTCT  ATGTTGGCTG  GCTGAAGGTG  GCAGAGCAGC  TCATCAACCC  CTTTGGAGAG
901  GATGATGATG  ATTTTGAGAC  CAACTGGATT  GTCGACAGGA  ATTTGCAGGT  GTCCCTGTTG
961  GCTGTGGATG  AGATGCACCA  GGACCTGCCT  CGGATGGAGC  CGGACATGA  CTGGAATAAG
1021  CCCGAGCCAC  AGCCCCCTA  CACAGCTGCT  TCCGCCAGT  TCCGTGAGC  CTCCTTTATG
1081  GGCTCCACCT  TCAACATCAG  CCTGAACAAA  GAGGAGATGG  AGTTCCAGCC  CAATCAGGAG
1141  GACGAGGAGG  ATGCTCACGC  TGGCATCATT  GGCGCTTCC  TAGGCCTGCA  GTCCCATGAT
1201  CACCATCCTC  CCAGGGCAAA  CTCAAGGACC  AAATACTGT  GGCCCAAGAG  GGAATCCCTT
1261  CTCCACGAGG  GCCTGCCCAA  AAACCACAAG  GCAGCCAAAC  AGAACGTTAG  GGGCCAGGAA
1321  GACAACAAGG  CCTGGAAGCT  TAAGGCTGTG  GACGCCTTCA  AGTCTGCCCC  ACTGTATCAG
1381  AGGCCAGGCT  ACTACAGTGC  CCCACAGACG  CCCCTCAGCC  CCACTCCCAT  GTTCTTCCCC
1441  CTAGAACCAT  CAGCGCCGTC  AAAGCTTCAC  AGTGTACAG  GCATAGACAC  CAAAGACAAA
1501  AGCTTAAAGA  CTGTGAGTTC  TGGGGCCAA  AAAAGTTTTG  AATTGCTCTC  AGAGACCGAT
1561  GGGGCCTTGA  TGGAGACCC  AGAAGTATCT  CAAGTGAGGA  GGAAAACGTG  GGAGTTTAAC
1621  CTGACGGATA  TGCCAGAGAT  CCCCAGAAAT  CACCTCAAAG  AACCTTTGGA  ACAATACCCA
1681  ACCAACATAC  AACTACACT  CAAAGATCAC  ATGGATCCTT  ATTGGGCCTT  GGAAAACAGG
1741  GATGAAGCAC  ATTCTTAA  (SEQ ID NO: 3) .

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[014] The disclosure provides a vector comprising a composition of the disclosure. In some embodiments, the vector is a plasmid.

[015] The disclosure provides a delivery vector comprising the vector of the disclosure. In some embodiments, the delivery vector is a viral delivery vector. In some embodiments, the delivery vector comprises a single stranded viral genome. In some embodiments, the delivery vector comprises a double stranded viral genome. In some embodiments, the delivery vector comprises an RNA molecule.

[016] The disclosure provides a delivery vector comprising the vector of the disclosure. In some embodiments, the delivery vector comprises a sequence isolated or derived from an adeno-associated virus (AAV) vector. In some embodiments, the delivery vector comprises a sequence isolated or derived from an AAV vector of serotype AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11 or any combination thereof. In some embodiments, the delivery vector comprises a sequence isolated or derived from an AAV vector of serotype AAV2. In some embodiments, the delivery vector comprises a sequence isolated or derived from an AAV vector of serotype AAV8. In some embodiments, the delivery vector comprises a sequence encoding a first inverted terminal repeat (ITR) and a second ITR isolated or derived from an AAV vector of serotype AAV2 and a sequence encoding a viral gene isolated or derived from an AAV vector of serotype AAV2. In some embodiments, the delivery vector comprises a sequence encoding a first inverted terminal repeat (ITR) and a second ITR isolated or derived from an AAV vector of serotype AAV8 and a sequence encoding a viral gene isolated or derived from an AAV vector of serotype AAV8. In some embodiments, the delivery vector comprises a sequence encoding a first inverted terminal repeat (ITR) and a second ITR isolated or derived from an AAV vector of serotype AAV2 and a sequence encoding a viral gene isolated or derived from an AAV vector of serotype AAV8.

[017] The disclosure provides a pharmaceutical composition comprising a composition of the disclosure and a pharmaceutically-acceptable carrier. In some embodiments, the pharmaceutically-acceptable carrier comprises TMN200.

[018] The disclosure provides a pharmaceutical composition comprising a vector of the disclosure a pharmaceutically acceptable carrier. In some embodiments, the pharmaceutically acceptable carrier comprises TMN200.

[019] The disclosure provides a pharmaceutical composition comprising a delivery vector of the disclosure and a pharmaceutically acceptable carrier. In some embodiments, the pharmaceutically acceptable carrier comprises TMN200.

[020] The disclosure provides a cell comprising a composition of the disclosure. The disclosure provides a cell comprising a vector of the disclosure. The disclosure provides a cell comprising a delivery vector of the disclosure. The disclosure provides a cell comprising a pharmaceutical composition of the disclosure. In some embodiments, the cell is a mammalian cell. In some embodiments, the mammalian cell is a non-human primate cell, a rodent cell, a mouse cell, a rat cell or a rabbit cell. In some embodiments, the cell is a human cell. In some embodiments, the human cell is a neuronal cell, a glial cell, a retinal cell, a photoreceptor cell, a rod cell, a cone cell or a cuboidal cell of the retinal pigment epithelium

(RPE). In some embodiments, the human cell is a photoreceptor cell. In some embodiments, the human cell is an HEK293 cell or an ARPE19 cell. In some embodiments, the human cell is isolated or derived from an RPE of a human retina. In some embodiments, the cell is *in vivo*, *in vitro*, *ex vivo* or *in situ*.

[021] The disclosure provides a method of treating macular dystrophy in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of a composition of the disclosure.

[022] The disclosure provides a method of treating macular dystrophy in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of a composition comprising the vector of the disclosure.

[023] The disclosure provides a method of treating macular dystrophy in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of a composition comprising the delivery vector of the disclosure.

[024] In some embodiments of the methods of the disclosure, the subject is a human. In some embodiments, the subject is a non-human primate, a dog, a cat, a rodent, a mouse, a rat, or a rabbit. In some embodiments, the subject has macular dystrophy.

[025] In some embodiments of the methods of the disclosure, the subject has a mutation in one or both copies of a BEST1 gene. In some embodiments, the mutation is heritable as a dominant mutation. In some embodiments, the dominant mutation causes Best Vitelliform Macular Dystrophy (BVMD) in the subject. In some embodiments, the mutation is heritable as a recessive mutation. In some embodiments, the recessive mutation causes Autosomal Recessive Bestrophinopathy (ARB) in the subject. In some embodiments, the mutation occurs in a coding sequence of one or both copies of a BEST1 gene. In some embodiments, the mutation occurs in a non-coding sequence of one or both copies of a BEST1 gene. In some embodiments, the mutation comprises a substitution, an insertion, a deletion, an inversion, a translocation, a frameshift, or a combination thereof in one both copies of a BEST1 gene.

[026] In some embodiments of the methods of the disclosure, administering comprises an injection or an infusion via a subretinal, a suprachoroidal or an intravitreal route. In some embodiments, administering comprises an injection or an infusion via a subretinal route. In some embodiments, administering comprises a two-step injection or a two-step infusion via a subretinal route.

[027] In some embodiments of the methods of the disclosure, the therapeutically effective amount is formulated in a volume of between 10 and 200 μ L, inclusive of the endpoints. In some embodiments, the therapeutically effective amount is formulated in a volume of

between 10 and 50 μL , between 50 and 100 μL , between 100 and 150 μL or between 150 and 200 μL , inclusive of the endpoints, for each range. In some embodiments, the therapeutically effective amount is formulated in a volume of between 70 and 120 μL , inclusive of the endpoints, and wherein the administering comprises an injection or an infusion via a subretinal route. In some embodiments, the therapeutically effective amount is formulated in a volume of 100 μL and wherein the administering comprises an injection or an infusion via a subretinal route.

[028] In some embodiments of the methods of the disclosure, the therapeutically effective amount comprises a concentration of an AAV delivery vector of at least 1×10^{10} DRP/mL, at least 1×10^{11} DRP/mL, at least 1×10^{12} DRP/mL, at least 2×10^{12} DRP/mL, at least 5×10^{12} DRP/mL or at least 1.5×10^{13} DRP/mL. In some embodiments, the therapeutically effective amount comprises a concentration of an AAV delivery vector of at least 2×10^{12} DRP/mL, at least 5×10^{12} DRP/mL or at least 1.5×10^{13} DRP/mL. In some embodiments, the therapeutically effective amount comprises a concentration of an AAV delivery vector of at least 5×10^{12} DRP/mL. In some embodiments, the therapeutically effective amount comprises a concentration of an AAV delivery vector of at least 1.5×10^{13} DRP/mL.

[029] In some embodiments of the methods of the disclosure, the therapeutically effective amount comprises a dose of 2×10^8 genome particles (gp), 5×10^8 gp, 1.5×10^9 gp, 2×10^9 gp, 5×10^9 gp, 2×10^{10} gp, 5×10^{10} gp, 6×10^{10} gp, 1.2×10^{11} gp, 1.5×10^{11} gp, 2×10^{11} gp, 4.5×10^{11} gp, 5×10^{11} gp, 1.2×10^{12} gp, 1.5×10^{12} gp, 2×10^{12} gp or 5×10^{12} gp. In some embodiments, the subject is a mouse and wherein the therapeutically effective amount comprises a dose of 5×10^8 gp, 1.5×10^9 gp or 5×10^9 gp. In some embodiments, the subject is a non-human primate and wherein the therapeutically effective amount comprises a dose of 1.2×10^{11} gp, 4.5×10^{11} gp or 1.2×10^{12} gp of AAV viral particles. In some embodiments, the subject is human and wherein the therapeutically effective amount comprises a dose of 5×10^{10} gp, 1.5×10^{11} gp, 5×10^{11} gp or 1.5×10^{12} gp of AAV viral particles.

[030] In some embodiments of the methods of the disclosure, the composition further comprises a TMN200 buffer.

[031] The disclosure provides a composition of the disclosure for use in treating macular dystrophy in a subject in need thereof.

[032] The disclosure provides a vector of the disclosure for use in treating macular dystrophy in a subject in need thereof.

[033] The disclosure provides a delivery vector of the disclosure for use in treating macular dystrophy in a subject in need thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

[034] The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

[035] **FIG. 1A-B** are a pair of maps of a plasmid encoding VMD2.IntEx.BEST1.WPRE.pA construct with AAV2 ITRs.

[036] **FIG. 2A-B** are a pair of maps of a plasmid encoding VMD2.BEST1.WPRE.pA construct with AAV2 ITRs.

[037] **FIG. 3A-C** are a series of three maps of two plasmids encoding CAG.BEST1.WPRE.pA with AAV2 ITRs. FIG.3A and FIG.3B are two maps of a CAG.BEST.WPRE.pA plasmid with an AmpR selectable marker. FIG. 3C is a map of a CAG.BEST.WPRE.pA plasmid with a KanR selectable marker and a stuffer sequence.

[038] **FIG. 4** is a map of a plasmid encoding VMD2.GFP.WPRE.pA with AAV2 ITRs.

[039] **FIG. 5** is a map of a plasmid encoding VMD2.Int.Ex.GFP.WPRE.pA with AAV2 ITRs.

[040] **FIG. 6A-B** are each a series of images, 6 images and 3 images, respectively, showing BEST1 expression in HEK293 cells transduced with AAV.CAG.BEST1.pA, AAV.CAG.BEST1.WPRE.pA and an untransduced control. Cells are stained with Hoechst blue dye and anti-hBestrophin-1. Bestrophin-1 protein is localized throughout the cytosol

[041] **FIG. 7A-B** is a picture of a Western Blot (7A) and a bar graph (7B), respectively, showing the expression of Bestrophin-1 protein and a beta-actin control in HEK293 cells transduced with AAV.CAG.BEST1.pA (sample 1) or AAV.CAG.BEST1.WPRE.pA (sample 2) or a negative control (sample 3). Plasmid-transfected HEK293 cells were used as a positive control. FIG. 7B shows the quantification of Bestrophin-1 protein expression in HEK293 cells transduced with AAV.CAG.BEST1.pA (n=9) or AAV.CAG.BEST1.WPRE.pA (n=9) or an untransduced negative control (n=8). The Y-axis shows the normalized LiCor Value. Error bars are $\pm$ SEM. *** indicates $p < 0.001$ when compared to the un-transduced control.

[042] **FIG. 8A-B** is a single plot (8A) and a series of four plots (8B), respectively, showing whole-cell patch clamp recording data from HEK293 cells transduced with AAV2/2 CAG.BEST1.pA, AAV2/2 CAG.BEST1.WPRE.pA or AAV2/2 CAG.GFP.WPRE.pA vectors, as well as an untransduced control. In FIG. 8A, Current (pA) is plotted on the X-axis from -140 to 500 in increments of 20, while Voltage (in mV) is plotted on the Y-axis from -200 to 500 in units of 100. In FIG. 8B, the current waveforms are shown. Current (I)/Voltage

(V) plots of HEK293 transduced with the different vectors and an untransduced control are shown, clockwise from the top left: AAV2/2 CAG.BEST1.pA, AAV2/2 CAG.BEST1.WPRE.pA, untransduced control and AAV2/2 CAG.GFP.WPRE.pA. The inset scale bar (center) shows 250 pA on the Y-axis and 100 milliseconds on the X-axis.

[043] FIG. 9A-B is a pair of plots showing the chord conductance of HEK293 cells transduced with AAV2/2 CAG.BEST1.pA (n = 10), AAV2/2 CAG.BEST1.WPRE.pA (n = 10), AAV2/2 CAG.GFP.WPRE.pA (n = 11) and an untransduced control (n = 10). Chord conductance is plotted on the Y-axis from 0 to 10 in units of 2. **** indicates $p < 0.0001$, * indicates $p < 0.05$, ns stands for not significant.

[044] FIG. 10A-B are a pair of flow charts showing two embodiments of an experimental procedure for assaying BEST1 expression in differentiated ARPE19 cells. FIG. 10A show an experimental procedure for assaying BEST1 expression in transfected differentiated ARPE19 cells. FIG. 10B shows an experimental procedure for assaying BEST1 expression in transfected and/or transduced differentiated ARPE19 cells.

[045] FIG. 11A-B is a series of 16 images (A) and 6 images (B) showing BEST1 and ZO-1 immunostaining of transfected ARPE19 cells that were differentiated for 1 month. (A) The rows, top to bottom show ARPE19 cells with the following constructs: untransfected control, CAG.BEST1.WPRE, VMD2.BEST1.WPRE and VMD2.IntEx.BEST1.WPRE. The columns from left to right show: nuclei stained with Hoechst in blue, ZO-1 staining in green (ZO-1 is a marker of the cytoplasmic membrane surface of intercellular tight junctions), BEST1 in red, and a merged image (Hoechst, ZO-1, BEST1). Scale bars show 100 microns (μm). (B) Shown in the top row are ARPE19 cells transfected with VMD2.BEST1.WPRE.pA. Shown in the bottom row are ARPE19 cells transfected with VMD2.IntEx.BEST1.WPRE.pA. The images from left to right show ZO-1 (green) and BEST1 (red), and a merged image (Hoechst, ZO-1 and BEST1). The scale bar in the merged images indicates 25 μm .

[046] FIG. 12A-B is a series of 16 images (A) and 9 images (B) showing BEST1 and ZO-1 immunostaining of transfected ARPE19 cells that were differentiated for 3 months. (A) The rows, top to bottom show ARPE19 cells with the following constructs: untransfected control, CAG.BEST1.WPRE, VMD2.BEST1.WPRE and VMD2.IntEx.BEST1.WPRE. The columns from left to right show: nuclei stained with Hoechst in blue, ZO-1 staining in green, BEST1 in red, and a merged image (Hoechst, ZO-1, BEST1). Scale bars show 100 microns (μm). (B) Representative images of Fig. 12A at higher magnification. The rows from top to bottom show CAG.BEST1.WPRE, VMD2.BEST1.WPRE and VMD2.IntEx.BEST1.WPRE. The columns from left to right show staining for ZO-1 in green, BEST1 in red and a merged image including Hoechst in blue. The scale bar in the merged images indicates 25 μm .

[047] **FIG. 13A-B** shows two series of 8 images each showing GFP fluorescence in ARPE19 cells differentiated for 4 months, pre-treated with 400 nM doxorubicin and transduced with (FIG. 13A) AAV2/2.CAG.GFP.WPRE or (FIG. 13B) AAV2/2.VMD2.InEx.GFP.WPRE at 3 different multiplicities of infection (MOI). The MOIs used were 2, 4 and 8×10^4 genome particles (gp)/cell. The scale bars in the negative control (untransduced and untreated cells) indicates 50 μ m. The top row in each panel indicates untreated control cells, the bottom row are cells pre-treated with 400 nM doxorubicin.

[048] **FIG. 14** is a series of 20 images showing BEST1 and ZO-1 immunostaining of ARPE19 cells differentiated for 4 months, pre-treated with 400 nM doxorubicin and transduced with AAV2/2.CAG.BEST1.WPRE and AAV2/2.VMD2.InEx.BEST1.WPRE at two different MOIs: 1 and 4×10^4 gp/cell. The rows, top to bottom show ARPE19 cells with the following viral vectors: untransduced control, AAV2/2.CAG.BEST1.WPRE at a MOI 10,000 gp/cell, AAV2/2.CAG.BEST1.WPRE at a MOI 40,000 gp/cell, AAV2/2.VMD2.InEx.BEST1.WPRE at a MOI 10,000 gp/cell and AAV2/2.VMD2.InEx.BEST1.WPRE at a MOI 40,000 gp/cell. The columns from left to right show: nuclei stained with Hoechst in blue, ZO-1 staining in green, BEST1 in red, and a merged image (Hoechst, ZO-1, BEST1). Scale bars show 50 μ m.

[049] **FIG. 15** is a table outlining a 4/8 week *in vivo* pilot study protocol in mice.

[050] **FIG. 16** is a series of 6 optical coherence tomography (OCT) images of mouse eyes four weeks after being injected with sham, VMD2.BEST1.WPRE or VMD2.IntEx.BEST1.WPRE AAV constructs. The columns, from left to right, show mice injected with a sham, with VMD2.BEST1.WPRE and with VMD2.IntEx.BEST1.WPRE AAV constructs.

[051] **FIG. 17** is a series of 3 OCT images of mouse eyes four weeks after being injected with, from left to right: sham, VMD2.BEST1.WPRE or VMD2.IntEx.BEST1.WPRE AAV constructs. Indicated morphological structures are the retinal ganglion cell (RGC), inner plexiform layer (IPL), the inner nuclear layer (INL), the outer plexiform layer (OPL), the outer nuclear layer (ONL), the retinal pigmented epithelium (RPE). Blue and red arrows indicate retinal thicknesses.

[052] **FIG. 18** is a series of 12 OCT images of mouse eyes four and eight weeks after being injected with sham, VMD2.BEST1.WPRE or VMD2.IntEx.BEST1.WPRE AAV constructs (columns, from left to right). Mid-sagittal and off-center views are shown in alternating rows. The top two rows are animals imaged at 4 weeks post injection, and the bottom two rows are animals imaged at 8 weeks post injection.

[053] **FIG. 19** is a series of 12 fluorescent microscopy images of mouse eyes four weeks after being injected with sham, VMD2.BEST1.WPRE or VMD2.IntEx.BEST1.WPRE AAV constructs and stained with anti BEST1 (green), anti Rhodopsin (red) and DAPI (blue). The rows show, from top to bottom, sham injected eyes, eyes injected VMD2.BEST1.WPRE or VMD2.IntEx.BEST1.WPRE AAV particles. The columns, from left to right, show anti BEST1 (green), anti Rhodopsin (red), DAPI (blue) and a merged image. The retinal pigment epithelium (RPE), photoreceptors (PR) and retinal ganglion cells (RGC) are indicated at bottom.

[054] **FIG. 20** is a series of 12 images of mouse eyes eight weeks after being injected with, in columns from left to right: sham, VMD2.BEST1.WPRE or VMD2.IntEx.BEST1.WPRE AAV particles and stained for BEST1 (green), Rhodopsin (red) and DAPI (blue). The rows, from top to bottom, are a merged image, anti-BEST1 (also called huBEST1), anti-Rhodopsin and a bright field image.

[055] **FIG. 21** is an image of a western blot showing BEST1 protein expression in dissected mouse RPE and choroid complex four weeks after injection with sham, CAG.BEST1.WPRE, VMD2.BEST1.WPRE or VMD2.IntEx.BEST1.WPRE AAV constructs. The blue arrow indicates a recombinant human Bestrophin-1 protein, while the red arrow indicates the suggested size of the BEST1 protein. CAG.BEST1.WPRE was used as a control. CAG is a strong promoter with a constitutive expression in mammalian cells. It is an hybrid between the cytomegalovirus (CMV) enhancer element, the chicken beta-actin promoter (CBA) and the splice acceptor of the rabbit beta-globin gene.

[056] **FIG. 22** is a table outlining a 4/13 week *in vivo* proof of concept (PoC) study protocol in mice.

[057] **FIG. 23** is a series of 20 OCT images of mouse eyes four weeks and 13 weeks after being injected with sham, VMD2.IntEx.BEST1.WPRE or VMD2.BEST1.WPRE AAV constructs at two different dosages (1×10^8 GC/ μ L/eye and 1×10^9 GC/ μ L/eye). Mid-sagittal (top row) and off-center (bottom row) views are shown in alternating rows.

[058] **FIG. 24** is a series of 20 microscopy images of mouse eyes four weeks after being injected with sham, VMD2.IntEx.BEST1.WPRE or VMD2.BEST1.WPRE AAV constructs at two different dosages (1×10^8 GC/ μ L/eye and 1×10^9 GC/ μ L/eye), and stained with anti-BEST1 (huBEST1, green), anti-Rhodopsin (red) and DAPI (blue). Also shown are bright field images (bottom row). The columns, from left to right, show VMD2.IntEx.BEST1.WPRE at 1×10^8 GC/ μ L/eye, VMD2.IntEx.BEST1.WPRE at 1×10^9 GC/ μ L/eye, VMD2.BEST1.WPRE at 1×10^8 GC/ μ L/eye and VMD2.BEST1.WPRE at 1×10^9 GC/ μ L/eye. Rows, from top to bottom show: a merged image, anti-BEST1, anti-Rhodopsin

and bright field. Anatomical structures indicated in the upper left image are the inner nuclear layer (INL), the outer nuclear layer (ONL), the outer segment (OS), the retinal pigment epithelium (RPE) and the choroid.

[059] **FIG. 25A-B** are a pair of images of western blots looking at BEST1 protein expression in cells or injected mouse RPE and choroid complex. FIG. 25A is a western blot showing the expression of Bestrophin-1 protein and a beta-actin control in HEK293 and ARPE-19 cells transfected with pCAG.BEST1.WPRE, pVMD2.BEST1.WPRE and pVMD2.InEx.BEST1.WPRE or an untransfected sample as negative control. FIG. 25B is a western blot showing BEST1 protein in isolated RPE and choroid samples from mice injected with either a high dose (1×10^9 GC/ μ L/eye) or low dose (1×10^8 GC/ μ L/eye) of either VMD2.IntEx.BEST1.WPRE or VMD2.BEST1.WPRE AAV particles.

[060] **FIG. 26** is a table showing a study design for assaying human BEST1 expression by immunohistochemistry and western blot in mice injected with AAV2/2.VMD2.InEx.BEST1.WPRE.

[061] **FIG. 27** is a table showing a protocol for a proposed good laboratory practice (GLP) study to assess potential toxicity in mice.

[062] **FIG. 28** is a table showing a protocol for the evaluation of toxicity assessment study materials at 4 weeks.

[063] **FIG. 29** is a table showing a protocol for a proposed good laboratory practice (GLP) study to assess potential toxicity in non-human primates.

[064] **FIG. 30** is a table showing a dosing regimen in mouse, non-human primate and human equivalent doses in genome particles (gp) using a BEST1 AAV viral vector of the disclosure. The BEST1 AAV viral vector for the proposed doses is at a concentration of 2×10^{12} DRP/mL and made according to current good manufacturing practice (GMP) standards.

[065] **FIG. 31** is a table showing a dosing regimen and the required concentrations of DNase resistant particles (DRP) and number of genome particles (gp) per dose in mouse, non-human primate and human of a BEST1 AAV viral vector of the disclosure.

DETAILED DESCRIPTION

[066] The disclosure relates to the finding that in many cases macular degeneration may be caused by mutations in or the abnormal function of the protein Bestrophin-1 (BEST1, also known as VMD2). The macula is a region near the center of the retina, and is responsible for central, high-resolution color vision. The fovea, located near the center of the macula, contains the largest concentration of cone cell photoreceptors in the eye. Mutations in a gene called Bestrophin-1 (BEST1, or human BEST1 (hBEST1), also known as VMD2) are

associated with at least five distinct retinal degeneration diseases, called bestrinopathies. Bestrinopathies comprise best vitelliform macular dystrophy (BVMD), autosomal recessive bestrophinopathy, adult-onset vitelliform macular dystrophy, autosomal dominant vitreoretinopathopathy and retinitis pigmentosa. These mutations can be either dominant (for example, BVMD) or recessive. Best Vitelliform Macular Dystrophy (BVMD) and Autosomal Recessive Bestrophinopathy may cause macular degeneration with an onset in late childhood or adolescence. However, in some cases, macular degeneration begins in adulthood. However, regardless of age of onset, bestrinopathies can have a devastating effect on vision, and there is currently no known effective treatment. Given the key role that BEST1 function plays in bestrophinopathies, one approach to the treatment of bestrophinopathy is to deliver a functional BEST1 protein to the affected cells of the patient.

Bestrophin -1 (BEST1)

[067] Bestrophin-1 (BEST1) is an integral membrane protein found primarily in the retinal pigment epithelium of the eye (RPE) and predominantly localizes to the basolateral plasma membrane. BEST1 protein is thought to function as an ion channel and a regulator of intracellular calcium signaling. Human BEST1 can be found in the NCBI database with accession numbers NP_004174.1 and NM_004183.3, the contents of which are incorporated by reference in their entirety herein.

[068] In some embodiments of the compositions of the disclosure, a sequence encoding a BEST1 protein of the disclosure comprises or consists of an amino acid sequence having at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identity to the sequence of:

```

1  MTITYTSQVA  NARLGSFSRL  LLCWRGSIYK  LLYGEFLIFL  LCYYIIRFIY  RALATEEQQL
61  MFEKLTLYCD  SYIQLIPISF  VLGFYVTLVV  TRWWNQYENL  PWPDRMLSLV  SGFVEGKDEQ
121  GRLLRRTLIR  YANLGNVLIL  RSVSTAVYKR  FPSAQHLVQA  GFMTPEAEHKQ  LEKLSLPHNM
181  FWVPWVWFAN  LSMKAWLGGR  IRDPILLQSL  LNEMNTLRTQ  CGHLYAYDWI  SIPLVYTQVV
241  TVAVYSFFLT  CLVGRQFLNP  AKAYPGHELD  LVVPVFTFLQ  FFFYVGWLKV  AEQLINPFGE
301  DDDDFETNWI  VDRNLQVSL  AVDEMHQDLP  RMEPDMYWNK  PEPQPPYTAA  SAQFRRASF
361  GSTFNISLNK  EEMEFQPNQE  DEEDAHAGII  GRFLGLQSHD  HHPPRANSRT  KLLWPKRESL
421  LHEGLPKNHK  AAKQNVRGQE  DNKAWKLKAV  DAFKSAPLYQ  RPYYSAPQT  PLSPTPMFFP
481  LEPSAPSKLH  SVTGIDTKDK  SLKTVSSGAK  KSFELLSESD  GALMEHPEVS  QVRRKTVFEN
541  LTDMPEIPEN  HLKEPLEQSP  TNIHTTLKDH  MDPYWALENR  DEAHS (SEQ ID NO: 4).
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[069] In some embodiments of the compositions of the disclosure, a sequence encoding a BEST1 protein of the disclosure comprises or consists of the amino acid sequence:

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1  MTITYTSQVA  NARLGSFSRL  LLCWRGSIYK  LLYGEFLIFL  LCYYIIRFIY  RALATEEQQL
61  MFEKLTLYCD  SYIQLIPISF  VLGFYVTLVV  TRWWNQYENL  PWPDRMLSLV  SGFVEGKDEQ
121  GRLLRRTLIR  YANLGNVLIL  RSVSTAVYKR  FPSAQHLVQA  GFMTPEAEHKQ  LEKLSLPHNM
181  FWVPWVWFAN  LSMKAWLGGR  IRDPILLQSL  LNEMNTLRTQ  CGHLYAYDWI  SIPLVYTQVV
241  TVAVYSFFLT  CLVGRQFLNP  AKAYPGHELD  LVVPVFTFLQ  FFFYVGWLKV  AEQLINPFGE
301  DDDDFETNWI  VDRNLQVSL  AVDEMHQDLP  RMEPDMYWNK  PEPQPPYTAA  SAQFRRASF
361  GSTFNISLNK  EEMEFQPNQE  DEEDAHAGII  GRFLGLQSHD  HHPPRANSRT  KLLWPKRESL
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421 LHEGLPKNHK AAKQNVRGQE DNKAWKLKAV DAFKSAPLYQ RPYYSAPQT PLSPTPMFFP
 481 LEPSAPSKLH SVTGIDTKDK SLKTVSSGAK KSFELLSESD GALMEHPEVS QVRRKTVEFN
 541 LTDMPEIPEN HLKEPLEQSP TNIHTTLKDH MDPYWALENR DEAHs (SEQ ID NO: 4).

[070] In some embodiments of the compositions of the disclosure, a nucleic acid sequence encoding a BEST1 protein of the disclosure comprises or consists of a nucleic acid having at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identity to the nucleic acid sequence of:

```

1 ATGACCATCA CTTACACAAG CCAAGTGGCT AATGCCCGCT TAGGCTCCTT CTCCCGCCTG
61 CTGCTGTGCT GCGGGGGCAG CATCTACAAG CTGCTATATG GCGAGTTCTT AATCTTCCTG
121 CTCTGCTACT ACATCATCCG CTTTATTTAT AGGCTGGCCC TCACGGAAGA ACAACAGCTG
181 ATGTTTGAGA AACTGACTCT GTATTGCGAC AGCTACATCC AGCTCATCCC CATTTCCTTC
241 GTGCTGGGCT TCTACGTGAC GCTGGTCGTG ACCCGCTGGT GGAACCAAGTA CGAGAACCCTG
301 CCGTGGCCCG ACCGCCTCAT GAGCCTGGTG TCGGGCTTCG TCGAAGGCAA GGACGAGCAA
361 GGCCGGCTGC TGCGGCGCAC GCTCATCCGC TACGCCAACC TGGGCAACGT GCTCATCCTG
421 CGCAGCGTCA GCACCGCAGT CTACAAGCGC TTCCCCAGCG CCCAGCACCT GGTGCAAGCA
481 GGCTTTATGA CTCCGGCAGA ACACAAGCAG TTGGAGAAAC TGAGCCTACC ACACAACATG
541 TTCTGGGTGC CCTGGGTGTG GTTTGCCAAC CTGTCAATGA AGGCGTGGCT TGGAGGTCGA
601 ATCCGGGACC CTATCCTGCT CCAGAGCCTG CTGAACGAGA TGAACACCTT GCGTACTCAG
661 TGTGGACACC TGTATGCCTA CGACTGGATT AGTATCCAC TGGTGATATAC ACAGGTGGTG
721 ACTGTGGCGG TGTACAGCTT CTTCTGACT TGTCTAGTTG GCGGCAGTT TCTGAACCCA
781 GCCAAGGCCT ACCCTGGCCA TGAGCTGGAC CTCGTTGTGC CCGTCTTAC GTTCCTGCAG
841 TTCTTCTTCT ATGTTGGCTG GCTGAAGGTG GCAGAGCAGC TCATCAACCC CTTTGGAGAG
901 GATGATGATG ATTTTGAGAC CAACTGGATT GTCGACAGGA ATTTGCAGGT GTCCCTGTTG
961 GCTGTGGATG AGATGCACCA GGACCTGCCT CGGATGGAGC CGGACATGTA CTGGAATAAG
1021 CCCGAGCCAC AGCCCCCTA CACAGCTGCT TCCGCCAGT TCCGTGAGC CTCCTTTATG
1081 GGCTCCACCT TCAACATCAG CCTGAACAAA GAGGAGATGG AGTTCAGCC CAATCAGGAG
1141 GACGAGGAGG ATGCTCACGC TGGCATCATT GGCCGCTTCC TAGGCCTGCA GTCCCATGAT
1201 CACCATCCTC CCAGGGCAAA CTCAGGACC AAACACTGT GGCCTAAGAG GGAATCCCTT
1261 CTCCACGAGG GCCTGCCCAA AAACCACAAG GCAGCCAAAC AGAACGTTAG GGGCCAGGAA
1321 GACAACAAGG CCTGGAAGCT TAAGGCTGTG GACGCCTTCA AGTCTGCCCC ACTGTATCAG
1381 AGGCCAGGCT ACTACAGTGC CCCACAGAGC CCCCTCAGCC CCACTCCCCT GTTCTTCCCC
1441 CTAGAACCAT CAGCGCCGTC AAAGCTTCAC AGTGTACAG GCATAGACAC CAAAGACAAA
1501 AGCTTAAAGA CTGTGAGTTC TGGGGCCAAAG AAAAGTTTTG AATTGCTCTC AGAGAGCGAT
1561 GGGGCTTGA TGGAGCACC AGAAGTATCT CAAGTGAGGA GGAAAACGT GGAGTTTAAC
1621 CTGACGGATA TGCCAGAGAT CCCCAGAAAT CACCTCAAAG AACCTTTGGA ACAATCACCA
1681 ACCAACATAC AACTACACT CAAAGATCAC ATGGATCCTT ATTGGGCCTT GGAAAACAGG
1741 GATGAAGCAC ATTCTAA (SEQ ID NO: 3).
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[071] In some embodiments of the compositions of the disclosure, a nucleic acid sequence encoding a BEST1 protein of the disclosure comprises or consists of the nucleic acid sequence:

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1 ATGACCATCA CTTACACAAG CCAAGTGGCT AATGCCCGCT TAGGCTCCTT CTCCCGCCTG
61 CTGCTGTGCT GCGGGGGCAG CATCTACAAG CTGCTATATG GCGAGTTCTT AATCTTCCTG
121 CTCTGCTACT ACATCATCCG CTTTATTTAT AGGCTGGCCC TCACGGAAGA ACAACAGCTG
181 ATGTTTGAGA AACTGACTCT GTATTGCGAC AGCTACATCC AGCTCATCCC CATTTCCTTC
241 GTGCTGGGCT TCTACGTGAC GCTGGTCGTG ACCCGCTGGT GGAACCAAGTA CGAGAACCCTG
301 CCGTGGCCCG ACCGCCTCAT GAGCCTGGTG TCGGGCTTCG TCGAAGGCAA GGACGAGCAA
361 GGCCGGCTGC TGCGGCGCAC GCTCATCCGC TACGCCAACC TGGGCAACGT GCTCATCCTG
421 CGCAGCGTCA GCACCGCAGT CTACAAGCGC TTCCCCAGCG CCCAGCACCT GGTGCAAGCA
481 GGCTTTATGA CTCCGGCAGA ACACAAGCAG TTGGAGAAAC TGAGCCTACC ACACAACATG
541 TTCTGGGTGC CCTGGGTGTG GTTTGCCAAC CTGTCAATGA AGGCGTGGCT TGGAGGTCGA
601 ATCCGGGACC CTATCCTGCT CCAGAGCCTG CTGAACGAGA TGAACACCTT GCGTACTCAG
661 TGTGGACACC TGTATGCCTA CGACTGGATT AGTATCCAC TGGTGATATAC ACAGGTGGTG
721 ACTGTGGCGG TGTACAGCTT CTTCTGACT TGTCTAGTTG GCGGCAGTT TCTGAACCCA
781 GCCAAGGCCT ACCCTGGCCA TGAGCTGGAC CTCGTTGTGC CCGTCTTAC GTTCCTGCAG
841 TTCTTCTTCT ATGTTGGCTG GCTGAAGGTG GCAGAGCAGC TCATCAACCC CTTTGGAGAG
901 GATGATGATG ATTTTGAGAC CAACTGGATT GTCGACAGGA ATTTGCAGGT GTCCCTGTTG
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961 GCTGTGGATG AGATGCACCA GGACCTGCCT CGGATGGAGC CGGACATGTA CTGGAATAAG
1021 CCCGAGCCAC AGCCCCCCTA CACAGCTGCT TCCGCCCAGT TCCGTGAGC CTCCTTTATG
1081 GGCTCCACCT TCAACATCAG CCTGAACAAA GAGGAGATGG AGTTCCAGCC CAATCAGGAG
1141 GACGAGGAGG ATGCTCACGC TGGCATCATT GGCCGCTTCC TAGGCCTGCA GTCCCATGAT
1201 CACCATCCTC CCAGGGCAAA CTCAAGGACC AAACCTACTGT GGCCCAAGAG GGAATCCCTT
1261 CTCCACGAGG GCCTGCCCAA AAACCACAAG GCAGCCAAAC AGAACGTTAG GGGCCAGGAA
1321 GACAACAAGG CCTGGAAGCT TAAGGCTGTG GACGCCTTCA AGTCTGCCCC ACTGTATCAG
1381 AGGCCAGGCT ACTACAGTGC CCCACAGACG CCCCTCAGCC CCACTCCCAT GTTCTTCCCC
1441 CTAGAACCAT CAGCGCCGTC AAAGCTTCAC AGTGTACACG GCATAGACAC CAAAGACAAA
1501 AGCTTAAAGA CTGTGAGTTC TGGGGCCAAG AAAAGTTTTG AATTGCTCTC AGAGAGCGAT
1561 GGGGCCTTGA TGGAGCACCC AGAAGTATCT CAAGTGAGGA GGAAACTGT GGAGTTTAAC
1621 CTGACGGATA TGCCAGAGAT CCCCAGAAAAT CACCTCAAAG AACCTTTGGA ACAATCACCA
1681 ACCAACATAC ACACTACACT CAAAGATCAC ATGGATCCTT ATTGGGCCTT GGAAAACAGG
1741 GATGAAGCAC ATTCTTAA (SEQ ID NO: 3).

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[072] In some embodiments of the compositions of the disclosure, a nucleic acid sequence encoding a BEST1 protein of the disclosure comprises a codon optimized sequence. In some embodiments, the sequence has been codon optimized for expression in a mammalian cell. In some embodiments, the sequence has been codon optimized for expression in a human cell.

BEST1 Expression

[073] In some embodiments of the compositions of the disclosure, a nucleic acid sequence encoding a BEST1 protein of the disclosure further comprises a sequence encoding a regulatory element that enhances or increases BEST1 transcript or BEST1 protein expression. Exemplary regulatory element that enhances or increases BEST1 transcript or BEST1 protein expression include, but are not limited to, a promoter, an enhancer, a superenhancer, an intron, an exon, a combination of an intron and exon, a sequence encoding an untranslated region (e.g. a 5' untranslated region (UTR) or a 3' UTR), a sequence comprising a polyadenylation (polyA) signal, and a posttranscriptional regulatory element (PRE).

[074] Exemplary promoters of the disclosure include, but are not limited to, those promoters capable of expressing a sequence encoding a BEST1 protein or a BEST1 protein in a mammalian cell. Exemplary promoters of the disclosure include, but are not limited to, those promoters capable of expressing a sequence encoding a BEST1 protein or a BEST1 protein in a human cell. In some embodiments, the mammalian or the human cell may be *in vivo*, *ex vivo*, *in vitro* or *in situ*. In some embodiments, the promoter may be constitutively active. In some embodiments, the promoter may be cell-type specific. In some embodiments, the promoter may be inducible.

[075] Exemplary constitutively active promoters of the disclosure include, but are not limited to, a viral promoter. Viral promoters of the disclosure may include, but are not limited to, a simian virus 40 (SV40) promoter, a cytomegalovirus (CMV) promoter, ubiquitin C (UBC) promoter, elongation factor-1 alpha (EF1A) promoter, phosphoglycerate kinase 1 (PGK) promoter and a CAG promoter (a combination of a (C) the cytomegalovirus (CMV)

early enhancer element, (A) the promoter comprising the first exon and the first intron of chicken beta-actin gene, and (G) the splice acceptor of the rabbit beta-globin gene). In some embodiments, a CMV promoter is used to control expression of a nucleic acid sequence encoding a BEST1 protein of the disclosure. In some embodiments, a CAG promoter is used to control expression of a nucleic acid sequence encoding a BEST1 protein of the disclosure. Non-viral promoters of the disclosure may include, but are not limited to, a chicken beta actin (CBA) promoter. In some embodiments, the CBA promoter comprises the chicken beta actin the first exon and intron of the CBA gene. In some embodiments, the promoter comprises the chicken beta actin promoter and the cytomegalovirus early enhancer elements. In some embodiments, the promoter further comprises a rabbit beta globin splice acceptor sequence (the CAG promoter). In some embodiments, the CAG promoter comprises or consists of a nucleic acid sequence having at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identity to the nucleic acid sequence of:

```

1  CCATTGACGT  CAATAATGAC  GTATGTTCCC  ATAGTAACGC  CAATAGGGAC  TTTCCATTGA
61  CGTCAATGGG  TGGAGTATTT  ACGGTAAACT  GCCCACTTGG  CAGTACATCA  AGTGTATCAT
121  ATGCCAAGTA  CGCCCCCTAT  TGACGTCAAT  GACGGTAAAT  GGCCCGCCTG  GCATTATGCC
181  CAGTACATGA  CCTTATGGGA  CTTTCCTACT  TGGCAGTACA  TCTACGTATT  AGTCATCGCT
241  ATTACCATGG  TCGAGGTGAG  CCCACGTTT  TGCTTCACTC  TCCCCATCTC  CCCCCCTCC
301  CCACCCCAA  TTTTGTATTT  ATTTATTTT  TAATTATTTT  GTGCAGCGAT  GGGGGCGGGG
361  GGGGGGGGGG  GGC GCGCGCC  AGGCGGGGCG  GGGCGGGGCG  AGGGCGGGG  CGGGGCGAGG
421  CGGAGAGGTG  CGGCGGCAGC  CAATCAGAGC  GGC GCGCTCC  GAAAGTTTCC  TTTTATGGCG
481  AGGCGGCGGC  GGC GCGCGCC  CTATAAAAAG  CGAAGCGCGC  GGC GGGCGGG  AGTCGCTGCG
541  CGCTGCCTTC  GCCCCGTGCC  CCGCTCCGCC  GCCGCTCGC  GCCGCCCCGC  CCGGCTCTGA
601  CTGACCGCGT  TACTCCCACA  GGTGAGCGGG  CGGGACGGCC  CTTCTCCTCC  GGGCTGTAAT
661  TAGCGCTTGG  TTTAATGACG  GCTTGTTTCT  TTTCTGTGGC  TCGGTGAAAG  CTTGAGGGG
721  CTCCGGGAGG  GCCCTTTGTG  CGGGGGGAGC  GGCTCGGGGC  TGTCCGCGGG  GGGACGGCTG
781  CCTTCGGGGG  GGACGGGGCA  GGGCGGGGTT  CGGCTTCTGG  CGTGTGACCG  GCGGCTCTAG
841  AGCCTCTGCT  AACCATGTTC  ATGCCTTCTT  CTTTTTCCTA  CAGCTCCTGG  GCAACGTGCT
901  GGTATTGTG  CTGTCTCATC  ATTTTGCAA  AGAATTGGAT  C (SEQ ID NO: 2) .

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[076] Exemplary cell-type specific promoters of the disclosure include, but are not limited to, a promoter capable of expressing a nucleic acid or a protein in a neuron, a promoter capable of expressing a nucleic acid or a protein in a retinal cell, a promoter capable of expressing a nucleic acid or a protein in a photoreceptor, a promoter capable of expressing a nucleic acid or a protein in a rod cell, and a promoter capable of expressing a nucleic acid or a protein in a cone cell. In some embodiments, a sequence encoding a tissue specific promoter comprises a sequence encoding a human VMD2 gene (also known as Bestrophin-1). In some embodiments, a tissue specific promoter comprises a human VMD2 promoter (also known as Bestrophin-1). In some embodiments, the human VMD2 promoter comprises or consists of a nucleic acid sequence having at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity to the nucleic acid sequence of:

```

1  AATTCTGTCA  TTTTACTAGG  GTGATGAAAT  TCCCAAGCAA  CACCATCCTT  TTCAGATAAG
61  GGCAGTGAGG  CTGAGAGAGG  AGCTGAAACC  TACCCGGGGT  CACCACACAC  AGGTGGCAAG
121  GCTGGGACCA  GAAACCAGGA  CTGTTGACTG  CAGCCCGGTA  TTCATTCTTT  CCATAGCCCA

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181 CAGGGCTGTC AAAGACCCCA GGGCCTAGTC AGAGGCTCCT CCTTCCTGGA GAGTTCCTGG
241 CACAGAAGTT GAAGCTCAGC ACAGCCCCCT AACCCCAAC TCTCTCTGCA AGGCCTCAGG
301 GGTGAGAACA CTGGTGGAGC AGATCCTTTA GCCTCTGGAT TTTAGGGCCA TGGTAGAGGG
361 GGTGTTGCC TAAATTCCAG CCCTGGTCTC AGCCCAACAC CCTCCAAGAA GAAATTAGAG
421 GGGCCATGGC CAGGCTGTGC TAGCCGTTGC TTCTGAGCAG ATTACAAGAA GGGACTAAGA
481 CAAGGACTCC TTTGTGGAGG TCCTGGCTTA GGGAGTCAAG TGACGGCGGC TCAGCACTCA
541 CGTGGGCAGT GCCAGCCTCT AAGAGTGGGC AGGGGCACTG GCCACAGAGT CCCAGGGAGT
601 CCCACCAGCC TAGTCGCCAG ACC (SEQ ID NO: 1) .

```

[077] In some embodiments, the human VMD2 promoter comprises or consists of a nucleic acid sequence having 100% identity to the nucleic acid sequence of:

```

1 AATTCTGTCA TTTTACTAGG GTGATGAAAT TCCCAAGCAA CACCATCCTT TTCAGATAAG
61 GGGACTGAGG CTGAGAGAGG AGCTGAAACC TACCCGGGGT CACCACACAC AGGTGGCAAG
121 GCTGGGACCA GAAACCAGGA CTGTTGACTG CAGCCCGGTA TTCATTCTTT CCATAGCCCA
181 CAGGGCTGTC AAAGACCCCA GGGCCTAGTC AGAGGCTCCT CCTTCCTGGA GAGTTCCTGG
241 CACAGAAGTT GAAGCTCAGC ACAGCCCCCT AACCCCAAC TCTCTCTGCA AGGCCTCAGG
301 GGTGAGAACA CTGGTGGAGC AGATCCTTTA GCCTCTGGAT TTTAGGGCCA TGGTAGAGGG
361 GGTGTTGCC TAAATTCCAG CCCTGGTCTC AGCCCAACAC CCTCCAAGAA GAAATTAGAG
421 GGGCCATGGC CAGGCTGTGC TAGCCGTTGC TTCTGAGCAG ATTACAAGAA GGGACTAAGA
481 CAAGGACTCC TTTGTGGAGG TCCTGGCTTA GGGAGTCAAG TGACGGCGGC TCAGCACTCA
541 CGTGGGCAGT GCCAGCCTCT AAGAGTGGGC AGGGGCACTG GCCACAGAGT CCCAGGGAGT
601 CCCACCAGCC TAGTCGCCAG ACC (SEQ ID NO: 1) .

```

[078] In some embodiments of the compositions of the disclosure, the nucleic acid sequence comprising a sequence encoding a BEST1 protein and a sequence encoding a promoter, further comprises an intron and an exon. The presence of an intron and an exon increases levels of protein expression. In some embodiments, the intron is positioned between the VMD2 promoter and the exon. In some embodiments, including those embodiments wherein the intron is positioned between the VMD2 promoter and the exon, the exon is positioned 5' of the BEST coding sequence.

[079] The exon may comprise a coding sequence, a non-coding sequence, or a combination of both. In some embodiments, the exon comprises non-coding sequence. In some embodiments, the exon is isolated or derived from a mammalian gene. In embodiments, the mammal is a rabbit (*Oryctolagus cuniculus*). In some embodiments, the mammalian gene comprises a rabbit beta globin gene. In some embodiments, the exon comprises a nucleic acid sequence having at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to the nucleic acid sequence of:

```
CTCCTGGGCA ACGTGCTGGT TATTGTGCTG TCTCATCATT TTGGCAAAGA ATT (SEQ ID NO: 6) .
```

[080] In some embodiments, the exon comprises a nucleic acid sequence having 100% identity to the nucleic acid sequence of:

```
CTCCTGGGCA ACGTGCTGGT TATTGTGCTG TCTCATCATT TTGGCAAAGA ATT (SEQ ID NO: 6) .
```

[081] Introns may comprise a splice donor site, a splice acceptor site or a branch point.

Introns may comprise a splice donor site, a splice acceptor site and a branch point. Exemplary splice acceptor sites comprise nucleotides “GT” (“GU” in the pre-mRNA) at the 5' end of the intron. Exemplary splice acceptor sites comprise an “AG” at the 3' end of the intron. In some

embodiments, the branch point comprises an adenosine (A) between 20 and 40 nucleotides, inclusive of the endpoints, upstream of the 3' end of the intron. The intron may be an artificial or non-naturally occurring sequence. Alternatively, the intron may be isolated or derived from a vertebrate gene. The intron may comprise a sequence encoding a fusion of two sequences, each of which may be isolated or derived from a plurality of vertebrate genes. In some embodiments, a vertebrate gene contributing to the intron nucleic acid sequence comprises a chicken (*Gallus gallus*) gene. In some embodiments, the chicken gene comprises the chicken beta actin gene. In some embodiments, a vertebrate gene contributing to the intron nucleic acid sequence comprises a rabbit (*Oryctolagus cuniculus*) gene. In some embodiments, the rabbit gene comprises the rabbit beta globin gene. In some embodiments, the intron comprises a nucleic acid sequence having at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to the nucleic acid sequence of:

```

      1 GTGCCGCAGG GGGACGGCTG CCTTCGGGGG GGACGGGGCA GGGCGGGGTT
CGGCTTCTGG
     61 CGTGTGACCG GCGGCTCTAG AGCCTCTGCT AACCATGTTC ATGCCTTCTT
CTTTTTCCTA
    121 CAG (SEQ ID NO: 7) .

```

[082] In some embodiments, the intron comprises a nucleic acid sequence having 100% identity to the nucleic acid sequence of:

```

      1 GTGCCGCAGG GGGACGGCTG CCTTCGGGGG GGACGGGGCA GGGCGGGGTT
CGGCTTCTGG
     61 CGTGTGACCG GCGGCTCTAG AGCCTCTGCT AACCATGTTC ATGCCTTCTT
CTTTTTCCTA
    121 CAG (SEQ ID NO: 7) .

```

[083] Kozak sequences are short sequence motifs that are recognized by the ribosome as the translation start site. Kozak sequences may be positioned immediately upstream, or surrounding the translational start site. In vertebrates, the Kozak consensus sequence comprises a sequence of having at least 50% identity to the consensus sequence of gccRcc**ATGG**, where R represents an A or G, and the ATG encoding the start methionine is bolded. An exemplary Kozak sequence of the disclosure comprises a sequence of GGCACCATGA. In some embodiments, the nucleic acid comprising a nucleic acid sequence encoding BEST1, further comprises a sequence encoding a 5' untranslated sequence (5' UTR). In some embodiments, the 5' UTR comprises a Kozak sequence. In some embodiments, the 5' UTR comprises a portion of a Kozak sequence. In some embodiments, the 5' UTR comprises at least 50%, at least 60%, at least 70% or at least 80% of a Kozak sequence.

[084] In some embodiments, the nucleic acid comprising a nucleic acid sequence encoding BEST1, further comprises a nucleic acid sequence encoding transcriptional response element (PRE). Exemplary PREs comprise a Woodchuck PRE (WPPE), which is derived from the

Woodchuck hepatitis virus. In some embodiments, a sequence encoding a WPRE is positioned 3' of the nucleic acid sequence encoding BEST1. In some embodiments, a sequence encoding a WPRE is positioned between the nucleic acid sequence encoding BEST1 and the sequence encoding a polyA signal. In some embodiments, a sequence encoding a WPRE comprises a nucleic acid sequence having at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to the nucleic acid sequence of:

```

1  ATCGATAATC AACCTCTGGA TTACAAAATT TGTGAAAGAT TGA CTGGTAT TCTTAACTAT
61 GTTGCTCCTT TTACGCTATG TGGATACGCT GCTTTAATGC CTTTGTATCA TGCTATTGCT
121 TCCCGTATGG CTTTCATTTT CTCTCCTTG TATAAATCCT GGTGCTGTC TCTTTATGAG
181 GAGTTGTGGC CCGTTGTGAG GCAACGTGGC GTGGTGTGCA CTGTGTTTGC TGACGCAACC
241 CCCACTGGTT GGGGCATTGC CACCACCTGT CAGCTCCTTT CCGGGACTTT CGCTTTCCCC
301 CTCCCTATTG CCACGGCGGA ACTCATCGCC GCCTGCCTTG CCCGCTGCTG GACAGGGGCT
361 CGGCTGTTGG GCACTGACAA TTCCGTGGTG TTGTCGGGGA AATCATCGTC CTTTCCTTGG
421 CTGCTCGCCT GTGTTGCCAC CTGGATTCTG CGCGGGACGT CTTCTGCTA CGTCCCTTCG
481 GCCCTCAATC CAGCGGACCT TCCTTCCCGC GGCCTGCTGC CGGCTCTGCG GCCTCTTCCG
541 CGTCTTCGCC TTCGCCCTCA GACGAGTCGG ATCTCCCTTT GGGCCGCCTC CCC (SEQ ID
NO: 8) .

```

[085] In some embodiments, a sequence encoding a WPRE comprises a nucleic acid sequence having 100% identity to the nucleic acid sequence of:

```

1  ATCGATAATC AACCTCTGGA TTACAAAATT TGTGAAAGAT TGA CTGGTAT TCTTAACTAT
61 GTTGCTCCTT TTACGCTATG TGGATACGCT GCTTTAATGC CTTTGTATCA TGCTATTGCT
121 TCCCGTATGG CTTTCATTTT CTCTCCTTG TATAAATCCT GGTGCTGTC TCTTTATGAG
181 GAGTTGTGGC CCGTTGTGAG GCAACGTGGC GTGGTGTGCA CTGTGTTTGC TGACGCAACC
241 CCCACTGGTT GGGGCATTGC CACCACCTGT CAGCTCCTTT CCGGGACTTT CGCTTTCCCC
301 CTCCCTATTG CCACGGCGGA ACTCATCGCC GCCTGCCTTG CCCGCTGCTG GACAGGGGCT
361 CGGCTGTTGG GCACTGACAA TTCCGTGGTG TTGTCGGGGA AATCATCGTC CTTTCCTTGG
421 CTGCTCGCCT GTGTTGCCAC CTGGATTCTG CGCGGGACGT CTTCTGCTA CGTCCCTTCG
481 GCCCTCAATC CAGCGGACCT TCCTTCCCGC GGCCTGCTGC CGGCTCTGCG GCCTCTTCCG
541 CGTCTTCGCC TTCGCCCTCA GACGAGTCGG ATCTCCCTTT GGGCCGCCTC CCC (SEQ ID
NO: 8) .

```

[086] In some embodiments, the nucleic acid comprising a nucleic acid sequence encoding BEST1, further comprises a sequence encoding a polyadenylation (polyA) signal. The polyA signal facilitates nuclear export, enhances translation and increases mRNA stability. In some embodiments, the sequence encoding the polyA signal comprises a synthetic or an artificial sequence. In some embodiments, the sequence encoding the polyA signal comprises a sequence isolated or derived from a mammalian gene. In some embodiments, the mammalian gene is a human gene. In some embodiments, the mammalian gene is a bovine growth hormone gene (BGH). In some embodiments, the sequence encoding the polyA signal comprises a nucleic acid sequence having at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity to the nucleic acid sequence of:

```

1  CGCTGATCAG CCTCGACTGT GCCTTCTAGT TGCCAGCCAT CTGTTGTTT CCCCTCCCC
61 GTGCCTTCCT TGACCCTGGA AGGTGCCACT CCCACTGTCC TTTCCTAATA AAATGAGGAA
121 ATTGATCGCG ATTGTCTGAG TAGGTGTCAT TCTATTCTGG GGGGTGGGGT GGGGCAGGAC

```

181 AGCAAGGGGG AGGATTGGGA AGACAATAGC AGGCATGCTG GGGATGCGGT GGGCTCTATG
 241 GCTTCTGAGG CGGAAAGAAC CAGCTGGGG (SEQ ID NO: 9).

[087] In some embodiments, the sequence encoding the polyA signal comprises a nucleic acid sequence having 100% identity to the nucleic acid sequence of:

1 CGCTGATCAG CCTCGACTGT GCCTTCTAGT TGCCAGCCAT CTGTTGTTTG CCCCTCCCCC
 61 GTGCCTTCCT TGACCCTGGA AGGTGCCACT CCCACTGTCC TTCCTAATA AAATGAGGAA
 121 ATTGCATCGC ATTGTCTGAG TAGGTGTCAT TCTATTCTGG GGGGTGGGGT GGGGCAGGAC
 181 AGCAAGGGGG AGGATTGGGA AGACAATAGC AGGCATGCTG GGGATGCGGT GGGCTCTATG
 241 GCTTCTGAGG CGGAAAGAAC CAGCTGGGG (SEQ ID NO: 9).

AAV vectors

[088] A vector may comprise the nucleic acid comprising a nucleic acid sequence encoding BEST1. In some embodiments of the compositions of the disclosure, the vector may be a viral delivery vector. Viral delivery vectors of the disclosure may contain sequences necessary for packaging a nucleic acid sequence of the disclosure into a viral delivery system for delivery to a target cell or tissue. Typical viral delivery vectors of the disclosure include, but are not limited to, lentiviral, retroviral or adeno-associated viral (AAV) vectors.

[089] An AAV viral delivery system of the disclosure may be in the form of a mature AAV particle or virion, i.e. nucleic acid surrounded by an AAV protein capsid. In some embodiments, the AAV viral delivery vector may comprise an AAV genome or a derivative thereof.

[090] An AAV genome is a nucleic acid sequence which encodes functions needed for production of an AAV particle. These functions include those operating in the replication and packaging cycle of AAV in a host cell, including encapsidation of the AAV genome into an AAV particle. Naturally occurring AAVs are replication-deficient and rely on the provision of helper functions in trans for completion of a replication and packaging cycle. In preferred embodiments, an AAV genome of a vector of the disclosure is replication-deficient.

[091] The AAV genome may be in single-stranded form, either positive or negative-sense, or alternatively in double-stranded form. The use of a double-stranded form allows bypass of the DNA replication step in the target cell and so can accelerate transgene expression. The AAV genome of a vector of the disclosure may be single-stranded form.

[092] The AAV genome may be from any naturally derived serotype, isolate or clade of AAV. Thus, the AAV genome may be the full genome of a naturally occurring AAV. As is known to the person skilled in the art, AAVs occurring in nature may be classified according to various biological systems.

[093] AAVs are referred to in terms of their serotype. A serotype corresponds to a variant subspecies of AAV which, owing to its profile of expression of capsid surface antigens, has a distinctive reactivity which can be used to distinguish it from other variant subspecies. A

virus having a particular AAV serotype does not efficiently cross- react with neutralizing antibodies specific for any other AAV serotype. AAV serotypes include AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10 and AAV11, and also recombinant serotypes, such as Rec2 and Rec3, recently identified from primate brain. Any of these AAV serotypes may be used in the invention. Thus, in some embodiments, an AAV vector of the invention may be derived from an AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, Rec2 or Rec3 AAV.

[094] Reviews of AAV serotypes may be found in Choi et al. (2005) *Cur. Gene Ther.* 5: 299-310 and Wu et al. (2006) *Molecular Therapy* 14: 316-27. The sequences of AAV genomes or of elements of AAV genomes including ITR sequences, rep or cap genes may be derived from the following accession numbers for AAV whole genome sequences: Adeno-associated virus 1 NC_002077, AF063497; Adeno-associated virus 2 NC_001401; Adeno-associated virus 3 NC_001729; Adeno-associated virus 3B NC_001863; Adeno-associated virus 4 NC_001829; Adeno-associated virus 5 Y18065, AF085716; Adeno-associated virus 6 NC_001862; Avian AAV ATCC VR-865 AY186198, AY629583, NC_004828; Avian AAV strain DA-1 NC_006263, AY629583; Bovine AAV NC_005889, AY388617.

[095] AAV may also be referred to in terms of clades or clones. This refers to the phylogenetic relationship of naturally derived AAVs, as well as to a phylogenetic group of AAVs which can be traced back to a common ancestor, and includes all descendants thereof. Additionally, AAVs may be referred to in terms of a specific isolate, i.e. a genetic isolate of a specific AAV found in nature. The term genetic isolate describes a population of AAVs which has undergone limited genetic mixing with other naturally occurring AAVs, thereby defining a recognizably distinct population at a genetic level.

[096] The AAV serotype determines the tissue specificity of infection (or tropism) of an AAV virus. Accordingly, preferred AAV serotypes for use in AAVs administered to patients in accordance with the invention are those which have natural tropism for or a high efficiency of infection of target cells within the eye. In one embodiment, AAV serotypes for use in the invention are those which infect cells of the neurosensory retina, retinal pigment epithelium and/or macula.

[097] The AAV genome of a naturally derived serotype, isolate or clade of AAV comprises at least one inverted terminal repeat sequence (ITR). An ITR sequence acts in cis to provide a functional origin of replication and allows for integration and excision of the vector from the genome of a cell.

[098] An AAV viral delivery vector may include at least one inverted terminal repeat sequence (ITR), preferably more than one ITR, such as two ITRs or more. One or more of the

ITRs may be derived from AAV genomes having different serotypes, or may be a chimeric or mutant ITR. A preferred mutant ITR is one having a deletion of a trs (terminal resolution site). This deletion allows for continued replication of the genome to generate a single-stranded genome which contains both coding and complementary sequences, i.e. a self-complementary AAV genome. This allows for bypass of DNA replication in the target cell, and so enables accelerated transgene expression.

[0099] The inclusion of one or more ITRs is preferred to aid concatamer formation of a viral delivery vector of the invention in the nucleus of a host cell, for example following the conversion of single-stranded vector DNA into double-stranded DNA by the action of host cell DNA polymerases. The formation of such episomal concatamers protects the vector construct during the life of the host cell, thereby allowing for prolonged expression of the transgene *in vivo*.

[0100] In some embodiments, ITR elements are the only sequences retained from the native AAV genome in the viral delivery vector. Thus, in some embodiments, a viral delivery vector does not include either the rep or cap genes of the native genome and, furthermore, lacks any other sequences of the native genome. This is preferred for the reasons described above, and also to reduce the possibility of integration of the vector into the host cell genome.

Additionally, reducing the size of the AAV genome allows for increased flexibility in incorporating other sequence elements (such as regulatory elements) within the vector in addition to the transgene. In some embodiments, the viral delivery vector of the disclosure comprises sequences encoding AAV2 ITRs. In some embodiments, the sequences encoding the two AAV2 ITRs may comprise or consist of a nucleic acid sequence of:

```

1 ctgcgcgctc gctcgtcac tgaggccgcc cgggcaaagc cggggcgctc ggcgaccttt
61 ggtcgcccg cctcagtgag cgagcgagcg cgcagagagg gaggggccaa ctccatcact
121 aggggttcct tgtagttaat gatt (SEQ ID NO: 10).

```

and/or

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1 tgcgcgctcg ctcgctcact gaggccgggc gaccaaaggt cgcccgacgc cggggcctttg
61 cccgggcggc ctcagtgagc gagcgagcgc gcagagcttt ttgcaaaagc ctaggcctcc
121 aaaaaagcct ctcactact tctgg (SEQ ID NO: 11).

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[0101] The AAV genome may comprise a nucleic acid sequence of about 4.7 kb in length. Thus, in those embodiments where the nucleic acid sequence to be delivered by an AAV viral vector is less than 4.7 kb in length, a stuffer or filler sequence may be used. The presence of a stuffer sequence can, in some embodiments, aid in AAV viral vector packaging into the viral particle. In some embodiments, the stuffer sequence comprises a random sequence. An exemplary stuffer sequence of the disclosure may comprise or consist of the nucleic acid sequence of:

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1 GATGTAACCA TATACTTAGG CTGGATCTTC TCCGCGAAT TTAAACCTC ACCAACTACG

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61 AGATATGAGG TAAGCCAAAA AAGCACGTAG TGGCGCTCTC CGACTGTTCC CAAATTGTAA
121 CTTATCGTTC CGTGAAGGCC AGAGTACTT CCCGGCCCTT TCCATGCGCG CACCATACCC
181 TCCTAGTTCC CCGGTTATCT TTCCGAAAGT GGAGTGAGCG AACCTCCGTT TACGTCCTGT
241 TACCAATGAT GTAGCTATGC ACTTTGTACA GGGTGCCAAC GGGTTTCACA ATTCACAGAT
301 AGTGGGGATC CCGGCAAAGG GCCTATATTT GCGGTCCAAC TTAGGCGTAA ACCTCGATGC
361 TACCTACTCA GACCCACCTC GCGCGGGGTA AATAAGGCAC TCATCCCAGC TGGTTCCTGG
421 CGTTCTACGC AGCGACATGT TTATTAACAG TTGTCTGGCA GCACAAAAC TTTACCATGG
481 TCGTAGAAGC CCCCCAGAGT TAGTTCATAC CTAATGCCAC AAATGTGACA GGACGCCGAT
541 GGGTACCGGA CTTTAGGTCG AGCACAGTTC GGTAACGGAG AGACCCTGCG GCGTACTTCA
601 TTATGTATAT GGAACGTGCC CAAGTGACGC CAGGCAAGTC TCAGCTGGTT CCTGTGTTAG
661 CTCGAGGGTA GACATACGAG CTGATGAAC ATGGGTTGGG GGCTCGAAC CGTCGAGGAC
721 CCCATAGTAC CTCGGAGACC AAGTAGGGCA GCCTATAGTT TGAAGCAGAA CTATTTCCGG
781 GGGCGAGCCC TCATCGTCTC TTCTGCGGAT GACTCAACAC GCTAGGGACG TGAAGTCGAT
841 TCCTTCGATG GTTATAAATC AAAGACTCAG AGTGCTGTCT GGAGCGTGAA TCTAACGGTA
901 CGTATCTCGA TTGCTCGGTC GCTTTTCGCA CTCCGCGAAA GTTCGTACCG CTCATTTACT
961 AGGTTGCGAA GCCTATGCTG ATATATGAAT CCAAAC TAGA GCAGGGCTCT TAAGATTCCG
1021 AGTTGTAAAT ACTTAATACT CCAATCGGCT TTTACGTGCA CCACCGCGGG CGGCTGACAA
1081 GGGTCTCACA TCGAGAAACA AGACAGTTCC GGGCTGGAAG TAGCGCCGGC TAAGGAAGAC
1141 GCCTGGTACG GCAGGACTAT GAAACCAGTA CAAAGGCAAC ATCCTCACTT GGGTGAACGG
1201 AAACGCAGTA TTATGGTTAC TTTTGGGATA CGTGAAACAT ATCCCATGGT AGTCCTTAGA
1261 CTTGGGAGTC TATCACCCCT AGGGCCCAT TCTGGAAATA GACGCCAGGT TGAATCCGTA
1321 TTTGGAGGTA CGATGGAACA GTCTGGGTGG GACGTGCTTC ATTTATACCC TGCGCAGGCT
1381 GGACCGAGGA CCGCAAGGTG CGGCGGTGCA CAAGCAATTG ACACTAACC ACCGTGTATT
1441 CATTATGTTA CCAGGAAGTT TAAGCCGAGT CAATGAAGCT CGCATTACAG GTTTTACCGC
1501 ATCTTGCCGT TACTCACAAA CTGTGATCCA CCACAAGTCA AGCCATTGCC TCTCTGACAC
1561 GCCGTAAGAA TTAATATGTA AACTTTGCGC GGGTTGACTG CGATCCGTTC AGTCTCGTCC
1621 GAGGGCACAA TCCTATTCCC ATTTGTATGT TCAGCTAACT TCTACCCATC CCCCAGAGTT
1681 AAGTAGGTCG TGAGATGCCA TGGAGGCTCT CGTTTCATCC GTGGGACATC AAGCTTCCCC
1741 TTGATAAAGC ACCCCGCTCG GGTGTAGCAG AGAAGACGCC TTCTGAATTG TGCAATCCCT
1801 CCACCTTATC TAAGCTTGCT ACCAATAATT AGCATTTTTG CCTTGCGACA GACCTCCTAC
1861 TTAGATTGCC ACACATTGAG CTAGTCAGTG AGCGATAAGC TTGACGCGCT TTCAAGGGTC
1921 GCGAGTACGT GAACCTAAGG TCCGGACAGG ACTATATACT TGGGTTTGAT CTCGCCCCGA
1981 CAACTGCAAA CCTCAACTTT TTTAGATTAT ATGGTTAGCC GAAGTTGCAC GAGGTGGCGT
2041 CCGCGGACTG CTCCCCGAGT GTGGCTCTTT CATCTGACAA CGTGCAACCC CTATCGCGGC
2101 CGATTGTTTC TGCGGACGAT GTTGCTCTCA TAGTTTGGGC ATGTTTCCCT TGTAGGTGTG
2161 AAACCACTTA GCTTCGCGCC GTAGTCCCAA TGAAAAACCT ATGGACTTTG TTTTGGGTAG
2221 CACCAGGAAT CTGAACCGTG TGAATGTGGA CGTCGCGCGC GTAGACCTTT ATCTCCGTTT
2281 CAAGCTAGGG ATGTGGCTGC ATGCTACGTT GTCACACCTA CACTGCTCGA AGTAAATATG
2341 CGAAGCGCGC GGCCTGGCCG GAGGCGTTCC GCGCCGCCAC GTGTTCTGTT ACTGTTGATT
2401 GGTGGCACAT AAGCAATATC GTAGTCCGTC AAATTGAGCT CTGTTATCCC GGGCGTTATG
2461 TGTCAAATGG CGTAGAACGG GATTGACTGT TTGACGGTAG (SEQ ID NO: 12).

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[0102] In some embodiments, the AAV viral delivery vector comprises a nucleic acid sequence comprising a sequence encoding a VMD2 promoter, a sequence encoding a BEST1 protein, and a sequence encoding a WPRE. An exemplary AAV viral delivery vector of the disclosure comprising this nucleic acid sequence (VMD2.BEST1.WPRE.pA) comprises or consists of the nucleic acid sequence of:

```

1 TAGCTGCGCG CTCGCTCGCT CACTGAGGCC GCCCGGGCAA AGCCCGGGCG TCGGGCGACC
61 TTTGGTCGCC CGGCCTCAGT GAGCGAGCGA GCGCGCAGAG AGGGAGTGGC CAACTCCATC
121 ACTAGGGGTT CTTGTAGATT AATGATTAAC CCGCCATGCT ACTTATCTAC GTAGCCATGC
181 TCTAGGTAAT TTCTGTCATT TTACTAGGGT GATGAAATTC CCAAGCAACA CCATCCTTTT
241 CAGATAAGGG CACTGAGGCT GAGAGAGGAG CTGAAACCTA CCCGGGGTCA CCACACACAG
301 GTGGCAAGGC TGGGACCAGA AACCAGGACT GTTGAAGTCA GCCCGGTATT CATTCTTTCC
361 ATAGCCACA GGGCTGTCAA AGACCCAGG GCCTAGTCAG AGGCTCCTCC TTCCTGGAGA
421 GTTCCTGACA CAGAAGTTGA AGCTCAGCAC AGCCCCCTAA CCCCCAACTC TCTCTGCAAG
481 GCCTCAGGGG TCAGAACACT GGTGGAGCAG ATCCTTTAGC CTCTGGATTT TAGGGCCATG
541 GTAGAGGGGG TGTTGCCCTA AATTCCAGCC CTGGTCTCAG CCCAACACCC TCCAAGAAGA
601 AATTAGAGGG GCCATGGCCA GGCTGTGCTA GCCGTTGCTT CTGAGCAGAT TACAAGAAGG
661 GACTAAGACA AGGACTCCTT TGTGGAGGTC CTGGCTTAGG GAGTCAAGTG ACGGCGGCTC

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721	AGCACTCAGC	TGGGCAGTGC	CAGCCTCTAA	GAGTGGGCAG	GGGCACTGGC	CACAGAGTCC
781	CAGGGAGTCC	CACCAGCCTA	GTCGCCAGAC	CGGCACCATG	ACCATCACTT	ACACAAGCCA
841	AGTGGCTAAT	GCCCGCTTAG	GCTCCTTCTC	CCGCCTGCTG	CTGTGCTGGC	GGGGCAGCAT
901	CTACAAGCTG	CTATATGGCG	AGTTCTTAAT	CTTCCTGCTC	TGCTACTACA	TCATCCGCTT
961	TATTTATAGG	CTGGCCCTCA	CGGAAGAACA	ACAGCTGATG	TTTGAGAAAC	TGACTCTGTA
1021	TTGCGACAGC	TACATCCAGC	TCATCCCCAT	TTCTTTCGTG	CTGGGCTTCT	ACGTGACGCT
1081	GGTCGTGACC	CGCTGGTGGA	ACCAGTACGA	GAACCTGCCG	TGGCCCGACC	GCCTCATGAG
1141	CCTGGTGTGC	GGCTTCGTGC	AAGGCAAGGA	CGAGCAAGGC	CGGCTGCTGC	GGCGCACGCT
1201	CATCCGCTAC	GCCAACCTGG	GCAACGTGCT	CATCCTGCGC	AGCGTCAGCA	CCGCAGTCTA
1261	CAAGCGCTTC	CCCAGCGCCC	AGCACCTGGT	GCAAGCAGGC	TTTATGACTC	CGGCAGAACCA
1321	CAAGCAGTTG	GAGAAACTGA	GCCTACCACA	CAACATGTTT	TGGGTGCCCT	GGGTGTGGTT
1381	TGCCAACCTG	TCAATGAAGG	CGTGGCTTGG	AGGTCGAATC	CGGGACCCTA	TCCTGCTCCA
1441	GAGCCTGTGC	AACGAGATGA	ACACCTTGCG	TACTCAGTGT	GGACACCTGT	ATGCCTACGA
1501	CTGGATTAGT	ATCCCCTGCT	TGTATACACA	GGTGGTGACT	GTGGCGGTGT	ACAGCTTCTT
1561	CCTGACTTGT	CTAGTTGGGC	GGCAGTTTCT	GAACCCAGCC	AAGGCCTACC	CTGGCCATGA
1621	GCTGGACCTC	GTTGTGCCCG	TCTTCACGTT	CCTGCAGTTC	TTCTTCTATG	TTGGCTGGCT
1681	GAAGGTGGCA	GAGCAGCTCA	TCAACCCCTT	TGGAGAGGAT	GATGATGATT	TTGAGACCAA
1741	CTGGATTGTC	GACAGGAATT	TGCAGGTGTC	CCTGTTGGCT	GTGGATGAGA	TGCACCAGGA
1801	CCTGCCTCGG	ATGGAGCCGG	ACATGTACTG	GAATAAGCCC	GAGCCACAGC	CCCCCTACAC
1861	AGCTGCTTCC	GCCCAGTTCC	GTCGAGCCTC	CTTTATGGGC	TCCACCTTCA	ACATCAGCCT
1921	GAACAAAGAG	GAGATGGAGT	TCCAGCCCAA	TCAGGAGGAC	GAGGAGGATG	CTCACGCTGG
1981	CATCATTGGC	CGCTTCCTAG	GCCTGCAGTC	CCATGATCAC	CATCCTCCCA	GGGCAAACCT
2041	AAGGACCAAA	CTACTGTGGC	CCAAGAGGGA	ATCCCTTCTC	CACGAGGGCC	TGCCCAAAAA
2101	CCACAAGGCA	GCCAAACAGA	ACGTTAGGGG	CCAGGAAGAC	AACAAGGCTT	GGAAGCTTAA
2161	GGCTGTGGAC	GCCTTCAAGT	CTGCCCACT	GTATCAGAGG	CCAGGCTACT	ACAGTGCCCC
2221	ACAGACGCCC	CTCAGCCCCA	CTCCCATGTT	CTTCCCCCTA	GAACCATCAG	CGCCGTCAAA
2281	GCTTCACAGT	GTCACAGGCA	TAGACACCAA	AGACAAAAGC	TTAAAGACTG	TGAGTTCTGG
2341	GGCCAAGAAA	AGTTTTGAAT	TGCTCTCAGA	GAGCGATGGG	GCCTTGATGG	AGCACCCAGA
2401	AGTATCTCAA	GTGAGGAGGA	AAACTGTGGA	GTTTAACTTG	ACGGATATGC	CAGAGATCCC
2461	CGAAAATCAC	CTCAAAGAAC	CTTTGGAACA	ATCACCACC	AACATACACA	CTACACTCAA
2521	AGATCACATG	GATCCTTATT	GGGCCCTTGA	AAACAGGGAT	GAAGCACATT	CCTAATCTAG
2581	CGGCCGCGAA	TTGATATCA	AGCTTATCGA	TAATCAACCT	CTGGATTACA	AAATTTGTGA
2641	AAGATTGACT	GGTATTCTTA	ACTATGTTGC	TCCTTTTACG	CTATGTGGAT	ACGCTGCTTT
2701	AATGCCTTTG	TATCATGCTA	TTGCTTCCCG	TATGGCTTTC	ATTTTCTCCT	CCTTGTATAA
2761	ATCCTGGTTG	CTGTCTCTTT	ATGAGGAGTT	GTGGCCCGTT	GTCAGGCAAC	GTGGCGTGGT
2821	GTGCACTGTG	TTTGCTGACG	CAACCCAC	TGGTTGGGGC	ATTGCCACCA	CCTGTCAGCT
2881	CCTTTCCGGG	ACTTTTCGCT	TCCCCCTCCC	TATTGCCACG	GCGGAACCTA	TCGCCGCCTG
2941	CCTTGCCCGC	TGCTGGACAG	GGGCTCGGCT	GTTGGGCACT	GACAATTCCG	TGGTGTGTGC
3001	GGGGAAATCA	TCGTCTTTTC	CTTGCTGCT	CGCCTGTGTT	GCCACCTGGA	TTCTGCGCGG
3061	GACGTCTTTC	TGCTACGTCC	CTTCGGCCCT	CAATCCAGCG	GACCTTCCTT	CCCGCGGCCCT
3121	GCTGCCGGCT	CTCGGCCCTC	TTCCGCGTCT	TCGCCTTCGC	CCTCAGACGA	GTCGGATCTC
3181	CCTTTGGGCC	GCCTCCCCGG	CGGCCGCGCA	CCGTCGACTC	GCTGATCAGC	CTCGACTGTG
3241	CCTTCTAGTT	GCCAGCCATC	TGTTGTTTGC	CCCTCCCCCG	TGCCTTCCTT	GACCCTGGAA
3301	GGTGCCACTC	CCACTGTCTT	TTCTTAATAA	AATGAGGAAA	TTGCATCGCA	TTGTCTGAGT
3361	AGGTGTCATT	CTATTCTGGG	GGGTGGGGTG	GGGCAGGACA	GCAAGGGGGA	GGATTGGGAA
3421	GACAATAGCA	GGCATGCTGG	GGATGCGGTG	GGCTCTATGG	CTTCTGAGGC	GGAAAGAACC
3481	AGCTGGGGCT	CGACTAGAGC	ATGGCTACGT	AGATAAGTAG	CATGGCGGGT	TAATCATTAA
3541	CTACAAGGAA	CCCCTAGTGA	TGGAGTTGGC	CACTCCCTCT	CTGCGCGCTC	GCTCGCTCAC
3601	TGAGGCCGGG	CGACCAAAGG	TCGCCCAGAC	CCCGGGCGGC	CTCAGTGAGC	GAGCGAGCGC
3661	CGAGAGCTTT	TTGCAAAAGC	CTAGGCCTCC	AAAAAAGCCT	CCTCACTACT	TCTGGAATAG
3721	CTCAGAGGCC	GAGGCGGCCT	CGGCCTCTGC	ATAAATAAAA	AAAATTAGTC	AGCCATGGGG
3781	CGGAGAAATG	GCGGAACTGG	GCGGAGTTAG	GGGCGGGATG	GGCGGAGTTA	GGGGCGGGAC
3841	TATGGTTGCT	GACTAATTGA	GATGCATGCT	TTGCATACTT	CTGCCTGCTG	GGGAGCCTGG
3901	GGACTTTCCA	CACCTGGTTG	CTGACTAATT	GAGATGCATG	CTTTGCATAC	TTCTGCCTGC
3961	TGGGGAGCCT	GGGGACTTTC	CACACCTTAA	CTGACACACA	TCCACAGCT	GCATTAATGA
4021	ATCGGCCAAC	GCGCGGGGAG	AGGCGGTTTG	CGTATTGGGC	GCTCTTCCGC	TTCTCGCTC
4081	ACTGACTCGC	TGCGCTCGGT	CGTTCCGGCT	CGGCGAGCGG	TATCAGCTCA	CTCAAAGGCG
4141	GTAATACGGT	TATCCACAGA	ATCAGGGGAT	AACGCAGGAA	AGAACATGTG	AGCAAAAGGC
4201	CACCAAAAGG	CCAGGAACCG	TAAAAAGGCC	CGGTTGCTGG	CGTTTTTCCA	TAGGCTCCGC
4261	CCCCCTGACG	AGCATCACAA	AAATCGACGC	TCAAGTCAGA	GGTGGCGAAA	CCCGACAGGA
4321	CTATAAAGAT	ACCAGGCGTT	TCCCCCTGGA	AGCTCCCTCG	TGCGCTCTCC	TGTTCCGACC
4381	CTGCCGCTTA	CCGATACCTT	GTCCGCCTTT	CTCCCTTCGG	GAAGCGTGGC	GCTTTCTCAT
4441	AGCTCACGCT	GTAGGTATCT	CAGTTCGGTG	TAGGTCGTTT	GCTCCAAGCT	GGGCTGTGTG
4501	CACGAACCCC	CCGTTACAGC	CGACCGCTGC	GCCTTATCCG	GTAACATATC	TCTTGAGTCC

4561	AACCCGGTAA	GACACGACTT	ATCGCCACTG	GCAGCAGCCA	CTGGTAACAG	GATTAGCAGA
4621	GCGAGGTATG	TAGGCGGTGC	TACAGAGTTC	TTGAAGTGGT	GGCCTAACTA	CGGCTACACT
4681	AGAAGAACAG	TATTTGGTAT	CTGCCTCTG	CTGAAGCCAG	TTACCTTCGG	AAAAAGAGTT
4741	GGTAGTCTT	GATCCGGCAA	ACAAACCACC	GCTGGTAGCG	GTGGTTTTTT	TGTTTGCAAG
4801	CAGCAGATTA	CGCGCAGAAA	AAAAGGATCT	CAAGAAGATC	CTTTGATCTT	TTCTACGGGG
4861	TC TGACGCTC	AGTGGAACGA	AAACTCACGT	TAAGGGATT	TGGTCATGAG	ATTATCAAAA
4921	AGGATCTTCA	CCTAGATCCT	TTTAAATTAA	AAATGAAGTT	TTAAATCAAT	CTAAAGTATA
4981	TATGAGTAAA	CTTGGTCTGA	CAGTTACCAA	TGCTTAATCA	GTGAGGCACC	TATCTCAGCG
5041	ATCTGTCTAT	TTCGTTCATC	CATAGTTGCC	TGACTCCTGC	AAACCACGTT	GTGTCTCAAA
5101	ATCTCTGATG	TTACATTGCA	CAAGATAAAA	ATATATCATC	ATGAACAATA	AAACTGTCTG
5161	CTTACATAAA	CAGTAATACA	AGGGGTGTTA	TGAGCCATAT	TCAACGGGAA	ACGTCTTGCT
5221	CGAGGCCGCG	ATTAAATTCC	AACATGGATG	CTGATTTATA	TGGGTATAAA	TGGGCTCGCG
5281	ATAATGTCGG	GCAATCAGGT	GCGACAATCT	ATCGATTGTA	TGGGAAGCCC	GATGCGCCAG
5341	AGTTGTTTCT	GAAACATGGC	AAAGGTAGCG	TTGCCAATGA	TGTTACAGAT	GAGATGGTCA
5401	GACTAAACTG	GCTGACGGAA	TTTATGCCTC	TTCCGACCAT	CAAGCATTTT	ATCCGTACTC
5461	CTGATGATGC	ATGGTTACTC	ACCACTGCGA	TCCCCGGGAA	AACAGCATTC	CAGGTATTAG
5521	AAGAATATCC	TGATTTCAGGT	GAAAAATATTG	TTGATGCGCT	GGCAGTGTTT	CTGCGCCGGT
5581	TGCATTTCGAT	TCCTGTTTGT	AATTGTCCTT	TTAACAGCGA	TCGCGTATTT	CGTCTCGCTC
5641	AGGCGCAATC	ACGAATGAAT	AACGGTTTGG	TTGATGCGAG	TGATTTTGAT	GACGAGCGTA
5701	ATGGCTGGCC	TGTTGAACAA	GTCTGAAAAG	AAATGCATAA	GCTTTTGCCA	TTCTCACCGG
5761	ATT CAGTCGT	CACTCATGGT	GATTTCTCAC	TTGATAACCT	TATTTTTGAC	GAGGGGAAAT
5821	TAATAGGTTG	TATTGATGTT	GGACGAGTCG	GAATCGCAGA	CCGATACCAG	GATCTTGCCA
5881	TCCTATGGAA	CTGCCTCGGT	GAGTTTCTC	CTTCATTACA	GAAACGGCTT	TTTCAAAAT
5941	ATGGTATTGA	TAATCCTGAT	ATGAATAAAT	TGCAGTTTCA	TTGATGCTC	TTGATGTTT
6001	TCTAAGGCG	GCCTGCCACC	ATACCCACGC	CGAAACAAGC	GCTCATGAGC	CCGAAGTGGC
6061	GAGCCCGATC	TTCCCATCG	GTGATGTCGG	CGATATAGGC	GCCAGCAACC	GCACCTGTGG
6121	CGCCGGTGAT	GCCGGCCACG	ATGCGTCCGG	CGTAGAGGAT	CTGGCTAGCG	ATGACCCTGC
6181	TGATTGGTTC	GCTGACCATT	TCCGGGTGCG	GGACGGCGTT	ACCAGAAACT	CAGAAGGTTT
6241	GTCCAACCAA	ACCGACTCTG	ACGGCAGTTT	ACGAGAGAGA	TGATAGGGTC	TGCTTCAGGG
6301	TGACCGATGT	AACCATATAC	TTAGGCTGGA	TCTTCTCCCG	CGAATTTTAA	CCCTCACCAA
6361	CTACGAGATA	TGAGGTAAGC	CAAAAAAGCA	CGTAGTGGCG	CTCTCCGACT	GTTCCCAAAT
6421	TGTAACCTAT	CGTTCCGTGA	AGGCCAGAGT	TACTTCCCGG	CCCTTTCCAT	GCGCGACCA
6481	TACCCTCCTA	GTTCCCGGT	TATCTTTCCG	AAGTGGGAGT	GAGCGAACCT	CCGTTTACGT
6541	CTTGTTACCA	ATGATGTAGC	TATGCACTTT	GTACAGGGTG	CCAACGGGTT	TCACAATTCA
6601	CAGATAGTGG	GGATCCCGGC	AAAGGGCCTA	TATTTGCGGT	CCAACCTTAG	CGTAAACCTC
6661	GATGCTACCT	ACTCAGACCC	ACCTCGCGCG	GGGTAAATAA	GGCACTCATC	CCAGCTGGTT
6721	CTTGGCGTTC	TACGCAGCGA	CATGTTTATT	AACAGTTGTC	TGGCAGCACA	AAACTTTTAC
6781	CATGGTCGTA	GAAGCCCCC	AGAGTTAGTT	CATACCTAAT	GCCACAAATG	TGACAGGACG
6841	CCGATGGGTA	CCGGACTTTA	GGTCGAGCAC	AGTTCGGTAA	CGGAGAGACC	CTGCGGCGTA
6901	CTTCATTATG	TATATGGAAC	GTGCCCAAGT	GACGCCAGGC	AAGTCTCAGC	TGGTTCCTGT
6961	GTTAGTCTGA	GGGTAGACAT	ACGAGCTGAT	TGAACATGGG	TTGGGGGCTT	CGAACCCTCG
7021	AGGACCCCAT	AGTACCTCGG	AGACCAAGTA	GGGCAGCCTA	TAGTTTGAAG	CAGAAGTATT
7081	TCGGGGGGCG	AGCCCTCATC	GTCTCTTCTG	CGGATGACTC	AACACGCTAG	GGACGTGAAG
7141	TCGATTCCCT	CGATGGTTAT	AAATCAAAGA	CTCAGAGTGC	TGTCTGGAGC	GTGAATCTAA
7201	CGGTACGTAT	CTCGATTGCT	CGGTCGCTTT	TCGCACTCCG	CGAAAGTTTC	TACCGCTCAT
7261	TCACTAGGTT	GCGAAGCCTA	TGCTGATATA	TGAATCCAAA	CTAGAGCAGG	GCTCTTAAGA
7321	TTCGGAGTTG	TAAATACTTA	ATACTCCAAT	CGGCTTTTAC	GTGCACCACC	GCGGGCGGCT
7381	GACAAGGGTC	TCACATCGAG	AAACAAGACA	GTTCCGGGCT	GGAAGTAGCG	CCGGCTAAGG
7441	AAGACGCCCTG	GTACGGCAGG	ACTATGAAAC	CAGTACAAAG	GCAACATCCT	CACTTGGGTG
7501	AACGGAAACG	CAGTATTATG	GTTACTTTTT	GGATACGTGA	AACATATCCC	ATGGTAGTCC
7561	TTAGACTTGG	GAGTCTATCA	CCCCTAGGGC	CCATATCTGG	AAATAGACGC	CAGGTTGAAT
7621	CCGTATTTGG	AGGTACGATG	GAACAGTCTG	GGTGGGACGT	GCTTCATTTA	TACCCTGCGC
7681	AGGCTGGACC	GAGGACCGCA	AGGTGCGGCG	GTGCACAAGC	AATTGACAAC	TAACCACCGT
7741	GTATTCATTA	TGGTACCAGG	AACTTTAAGC	CGAGTCAATG	AAGCTCGCAT	TACAGTGTTT
7801	ACCGCATCTT	GCCGTTACTC	ACAAACTGTG	ATCCACCACA	AGTCAAGCCA	TTGCCTCTCT
7861	GACACGCCGT	AAGAATTAAT	ATGTAAACTT	TGCGCGGGTT	GACTGCGATC	CGTTCAGTCT
7921	CGTCCGAGGG	CACAATCCTA	TTCCCATTTG	TATGTTTCAGC	TAACTTCTAC	CCATCCCCCG
7981	AAGTTAAAGTA	GGTCGTGAGA	TGCCATGGAG	GCTCTCGTTC	ATCCCGTGCG	ACATCAAGCT
8041	TCCCTTTGAT	AAAGCACCCC	GCTCGGTTGT	AGCAGAGAAG	ACGCCCTCTG	AATTGTGCAA
8101	TCCCTCCACC	TTATCTAAGC	TTGCTACCAA	TAATTAGCAT	TTTTGCCTTG	CGACAGACCT
8161	CCTACTTAGA	TTGCCACACA	TTGAGCTAGT	CAGTGAGCGA	TAAGCTTGAC	GCGCTTTCAA
8221	GGGTCGCGAG	TACGTGAACT	AAGGCTCCGG	ACAGGACTAT	ATACTTGGGT	TTGATCTCGC
8281	CCCACAACCT	GCAAACCTCA	ACTTTTTTAG	ATTATATGGT	TAGCCGAAGT	TGCACGAGGT
8341	GGCGTCCGCG	GACTGCTCCC	CGAGTGTTGG	TCTTTCATCT	GACAACGTGC	AACCCCTATC

8401 GCGGCCGATT GTTCTGCGG ACGATGTTGT CCTCATAGTT TGGGCATGTT TCCCTTGTAG
 8461 GTGTGAAACC ACTTAGCTTC GCGCCGTAGT CCCAATGAAA AACCTATGGA CTTTGTITTTG
 8521 GGTAGCACCA GGAATCTGAA CCGTGTGAAT GTGGACGTCG CGCGCGTAGA CCTTTATCTC
 8581 CGGTTCAAGC TAGGGATGTG GCTGCATGCT ACGTTGTCAC ACCTACACTG CTCGAAGTAA
 8641 ATATGCGAAG CGCGCGGCCT GGCCGGAGGC GTTCCGCGCC GCCACGTGTT CGTTAACTGT
 8701 TGATTGGTGG CACATAAGCA ATATCGTAGT CCGTCAAATT CAGCTCTGTT ATCCCGGGCG
 8761 TTATGTGTCA AATGGCGTAG AACGGGATTG ACTGTTTGAC GGTAGGGTGA CCTAAGCCAG
 8821 ATGCTACACA ATTAGGCTTG TACATATTGT CGTTAGAACG CGGCTACAAT TAATACATAA
 8881 CCTTATGTAT CATAACATA CGATTTAGGT GACACTATAG AATACACGGA ATTAATTC
 (SEQ ID NO: 13).

[0103] Table 1. Features of the VMD2.BEST1.WPRE.pA plasmid sequence

Name	Type	Minimum	Maximum	Length	Direction
130bp AAV2 5'ITR	LTR	4	133	130	forward
VMD2 promoter	promoter	189	811	623	forward
Kozak	Kozak	812	821	10	forward
BEST1	CDS	818	2,575	1,758	forward
WPRE	WPRE	2,606	3,198	593	forward
bGH pA	polyA_signal	3,220	3,488	269	forward
112bp AAV2 3'ITR	LTR	3,546	3,657	112	reverse
pBR322 rep origin	rep_origin	4,230	4,849	620	reverse
AphR (KanR)	CDS	5,190	6,005	816	forward
Randomly generated stuffer sequence	Stuffer	6,306	8,805	2,500	none

[0104] In some embodiments, the AAV viral delivery vector comprising a nucleic acid sequence comprising a sequence encoding a VMD2 promoter, a sequence encoding a BEST1 protein, a sequence encoding an intron, a sequence encoding an exon and a sequence encoding a WPRE. An exemplary AAV viral delivery vector of the disclosure comprises a nucleic acid sequence encoding a VMD2.IntEx.BEST1.WPRE.pA sequence comprising or consisting of the nucleic acid sequence of:

1 TAGCTGCGCG CTCGCTCGCT CACTGAGGCC GCCCGGGCAA AGCCCGGGCG TCGGGCGACC
 61 TTTGGTCGCC CGGCCTCAGT GAGCGAGCGA GCGCGCAGAG AGGGAGTGGC CAACTCCATC
 121 ACTAGGGGTT CCTTGTAGTT AATGATTAAC CCGCCATGCT ACTTATCTAC GTAGCCATGC
 181 TCTAGGTAAA TTCTGTCAAT TTACTAGGGT GATGAAATTC CCAAGCAACA CCATCCTTTT
 241 CAGATAAGGG CACTGAGGCT GAGAGAGGAG CTGAAACCTA CCCGGGGTCA CCACACACAG
 301 GTGGCAAGGC TGGGACCAGA AACCAGGACT GTTGAAGTCA GCCCGGTATT CATTCTTTCC
 361 ATAGCCCAACA GGGCTGTCAA AGACCCAGG GCCTAGTCAG AGGCTCCTCC TTCCTGGAGA
 421 GTTCCTGGCA CAGAAGTTGA AGCTCAGCAC AGCCCCCTAA CCCCCAACTC TCTCTGCAAG
 481 GCCTCAGGGG TCAGAACACT GGTGGAGCAG ATCCTTTAGC CTCTGGATTT TAGGGCCATG
 541 GTAGAGGGGG TGTGCCCCTA AATTCCAGCC CTGGTCTCAG CCCAACACCC TCCAAGAAGA
 601 AATTAGAGGG GCCATGGCCA GGCTGTGCTA GCCGTTGCTT CTGAGCAGAT TACAAGAAGG
 661 GACTAAGACA AGGACTCCTT TGTGGAGGTC CTGGCTTAGG GAGTCAAGTG ACGGCGGCTC
 721 AGCACTACAG TGGGCAGTGC CAGCCTCTAA GAGTGGGCAG GGGCACTGGC CACAGAGTCC
 781 CAGGGAGTCC CACCAGCCTA GTCGCCAGAC CGGGTGCCGC AGGGGGACGG CTGCCTTCGG
 841 GGGGGACGGG GCAGGGCGGG GTTCGGCTTC TGGCGTGTGA CCGGCGGCTC TAGAGCCTCT
 901 GCTAACCATG TTCATGCCTT CTTCCTTTTC CTACAGCTCC TGGGCAACGT GCTGGTTATT
 961 GTGCTGTCTC ATCATTTTGG CAAAGAATTG GCACCATGAC CATCACTTAC ACAAGCCAAG
 1021 TGGCTAATGC CCGCTTAGGC TCCTTCTCCC GCCTGCTGCT GTGCTGGCGG GGCAGCATCT
 1081 ACAAGCTGCT ATATGGCGAG TTCTTAATCT TCCTGCTCTG CTACTACATC ATCCGCTTTA
 1141 TTTATAGGCT GGCCCTCACG GAAGAACAAC AGCTGATGTT TGAGAAACTG ACTCTGTATT

1201	GCGACAGCTA	CATCCAGCTC	ATCCCCATTT	CCTTCGTGCT	GGGCTTCTAC	GTGACGCTGG
1261	TCGTGACCCG	CTGGTGGAAC	CAGTACGAGA	ACCTGCCGTG	GCCCGACCCG	CTCATGAGCC
1321	TGGTGTGGG	CTTCGTGCGA	GGCAAGGACG	AGCAAGGCCG	GCTGCTGCGG	CGCACGCTCA
1381	TCCGCTACGC	CAACCTGGGC	AACGTGCTCA	TCCTGCGCAG	CGTCAGCACC	GCAGTCTACA
1441	AGCGCTTCCC	CAGCGCCCAG	CACCTGGTGC	AAGCAGGCTT	TATGACTCCG	GCAGAACACA
1501	AGCAGTTGGA	GAAACTGAGC	CTACCACACA	ACATGTTCTG	GGTGCCCTGC	GTGTGGTTTG
1561	CCAACCTGTC	AATGAAGGCG	TGGCTTGGAG	GTCGAATCCG	GGACCCCTATC	CTGCTCCAGA
1621	GCCTGCTGAA	CGAGATGAAC	ACCTTGCGTA	CTCAGTGTGG	ACACCTGTAT	GCCTACGACT
1681	GGATTAGTAT	CCCACTGGTG	TATACACAGG	TGGTGAAGTG	GGCGGTGTAC	AGCTTCTTCC
1741	TGACTTGTCT	AGTTGGGCGG	CAGTTTCTGA	ACCCAGCCAA	GGCCTACCCT	GGCCATGAGC
1801	TGGACCTCGT	TGTGCCCCTC	TTCAGGTTCC	TGCAGTTCTT	CTTCTATGTT	GGCTGGCTGA
1861	AGGTGGCAGA	GCAGCTCATC	AACCCCTTTG	GAGAGGATGA	TGATGATTTT	GAGACCAACT
1921	GGATTGTCGA	CAGGAATTTG	CAGGTGTCCC	TGTTGGCTGT	GGATGAGATG	CACCAGGACC
1981	TGCCCTCGAT	GGAGCCGGAC	ATGTAAGTGA	ATAAGCCCGA	GCCACAGCCC	CCCTACACAG
2041	CTGCTTCCGC	CCAGTTCCGT	CGAGCCTCCT	TTATGGGCTC	CACCTTCAAC	ATCAGCTTGA
2101	ACAAAGAGGA	GATGGAGTTC	CAGCCCAATC	AGGAGGACGA	GGAGGATGCT	CACGCTGGCA
2161	TCATTGGCCG	CTTCTTAGGC	CTGCACTCCC	ATGATCACCA	TCCTCCAGAG	GCAAACCTCAA
2221	GGACCAAACCT	ACTGTGGCCC	AAGAGGGAAT	CCCTTCTCCA	CGAGGGCCTG	CCCAAAAACC
2281	ACAAGGCAGC	CAAACAGAAC	GTTAGGGGCC	AGGAAGACAA	CAAGGCCTGG	AAGCTTAAGG
2341	CTGTGGACGC	CTTCAAGTCT	GCCCCACTGT	ATCAGAGGCC	AGGCTACTAC	AGTGCCCCAC
2401	AGACGCCCCCT	CAGCCCCACT	CCCATGTTCT	TCCCCCTAGA	ACCATCAGCG	CCGTCAAAGC
2461	TTACAGTGT	CACAGGCATA	GACACCAAAG	ACAAAAGCTT	AAAGACTGTG	AGTTCTGGGG
2521	CCAAGAAAAG	TTTTGAATTG	CTCTCAGAGA	GCGATGGGGC	CTTGATGGAG	CACCCAGAAG
2581	TATCTCAAGT	GAGGAGGAAA	ACTGTGGAGT	TTAACCTGAC	GGATATGCCA	GAGATCCCCG
2641	AAAATCACCT	CAAAGAACCT	TTGGAACAAT	CACCAACCAA	CATACACACT	ACACTCAAAG
2701	ATCACATGGA	TCCTTATTGG	GCCTTGAAAA	ACAGGGATGA	AGCACATTCC	TAATCTAGCG
2761	GCCGCGAATT	CGATATCAAG	CTTATCGATA	ATCAACCTCT	GGATTACAAA	ATTTGTGAAA
2821	GATTGACTGG	TATTCTTAAC	TATGTTGCTC	CTTTTACGCT	ATGTGGATAC	GCTGCTTTAA
2881	TGCCTTTGTA	TCATGCTATT	GCTTCCCGTA	TGGCTTTCAT	TTTCTCCTCC	TTGTATAAAT
2941	CCTGGTTGCT	GTCTCTTTAT	GAGGAGTTGT	GGCCCGTTGT	CAGGCAACGT	GGCGTGGTGT
3001	GCACTGTGTT	TGCTGACGCA	ACCCCCACTG	GTTGGGGCAT	TGCCACCACC	TGTCAGCTCC
3061	TTTCCGGGAC	TTTCGCTTTC	CCCCTCCCTA	TTGCCACGGC	GGAACCTATC	GCCGCTGCC
3121	TTGCCCGCTG	CTGGACAGGG	GCTCGCTGT	TGGGCACTGA	CAATTCCGTG	TGTTGTCTCG
3181	GGAAATCATC	GTCTTTTCTT	TGGCTGCTCG	CCTGTGTTGC	CACCTGGATT	CTGCGCGGGA
3241	CGTCTTCTG	CTACGTCCCT	TCGGCCCTCA	ATCCAGCGGA	CCTTCTTCTC	CGCGGCTGCG
3301	TGCCGCTCT	GCGGCTCTT	CCGCGTCTTC	GCCTTCGCCC	TCAGACGAGT	CGGATCTCCC
3361	TTTGGGCGCG	CTCCCCGGCG	GCCGCGCACC	GTCGACTCGC	TGATCAGCCT	CGACTGTGCC
3421	TTCTAGTTGC	CAGCCATCTG	TTGTTTGCCC	CTCCCCCGTG	CCTTCTTGA	CCCTGGAAGG
3481	TGCCACTCCC	ACTGTCCTTT	CCTAATAAAA	TGAGGAAATT	GCATCGCATT	GTCTGAGTAG
3541	GTGTCAATTCT	ATTCTGGGGG	GTGGGGTGGG	GCAGGACAGC	AAGGGGGAGG	ATTGGGAAGA
3601	CAATAGCAGG	CATGCTGGGG	ATGCGGTGGG	CTCTATGGCT	TCTGAGGCGG	AAAGAACCAG
3661	CTGGGGCTCG	ACTAGAGCAT	GGCTACGTAG	ATAAGTAGCA	TGGCGGGTTA	ATCATTAACT
3721	ACAAGGAACC	CCTAGTGATG	GAGTTGGCCA	CTCCCTCTCT	GCGCGCTCGC	TCGCTCACTG
3781	AGGCCGGGCG	ACCAAAGGTC	GCCCGACGCC	CGGGCGGCCT	CAGTGAGCGA	GCGAGCGCGC
3841	AGAGCTTTTT	GCAAAAGCCT	AGGCCTCCAA	AAAAGCCTCC	TCACTACTTC	TGGAATAGCT
3901	CAGAGGCCGA	GGCGGCCTCG	GCCTCTGCAT	AAATAAAAAA	AATTAGTCAG	CCATGGGGCG
3961	GAGAATGGGC	GGAAGTGGGC	GGAGTTAGGG	GCGGGATGGG	CGGAGTTAGG	GGCGGGACTA
4021	TGGTTGCTGA	CTAATTGAGA	TGCATGCTTT	GCATACTTCT	GCCTGTCTGG	GAGCCTGGGG
4081	ACTTTCCACA	CCTGGTTGCT	GACTAATTGA	GATGCATGCT	TTGCATACTT	CTGCCTGCTG
4141	GGGAGCCTGG	GGACTTTCCA	CACCCTAACT	GACACACATT	CCACAGCTTC	ATTAATGAAT
4201	CGGCCAACGC	GCGGGGAGAG	GCGGTTTGGC	TATTGGGCGC	TCTTCCGCTT	CCTCGCTCAC
4261	TGACTCGCTG	CGCTCGGTCT	TTCGGCTGCG	GCGAGCGGTA	TCAGCTCACT	CAAAGGCGGT
4321	AATACGGTTA	TCCACAGAAT	CAGGGGATAA	CGCAGGAAAG	AACATGTGAG	CAAAGGCCCA
4381	GCAAAAGGCC	AGGAACCGTA	AAAAGGCCGC	GTTGCTGGCG	TTTTTCCATA	GGCTCCGCCC
4441	CCCTGACGAG	CATCACAATA	ATCGACGCTC	AAGTCAGAGG	TGGCGAAACC	CGACAGGACT
4501	ATAAAGATAC	CAGGCGTTTT	CCCTTGGAAG	CTCCCTCGTG	CGCTCTCTCT	TTCCGACCCCT
4561	GCCGCTTACC	GGATACCTGT	CCGCTTTTCT	CCCTTCGGGA	AGCGTGGCGC	TTTCTCATAG
4621	CTCACGCTGT	AGGTATCTCA	GTTGCGGTGA	GGTCGTTTCG	TCCAAGCTGG	GCTGTGTGCA
4681	CGAACCCCGC	GTTACGCCCG	ACCGCTGCGC	CTTATCCGGT	AACTATCGTC	TTGAGTCCAA
4741	CCCGGTAAGA	CACGACTTAT	CGCCACTGGC	AGCAGCCACT	GGTAACAGGA	TTAGTACAGC
4801	GAGGTATGTA	GGCGGTGCTA	CAGAGTTCTT	GAAGTGGTGG	CCTAACTACG	GCTACACTAG
4861	AAGAACAGTA	TTTGGTATCT	GCGCTCTGCT	GAAGCCAGTT	ACCTTCGGAA	AAAGAGTTGG
4921	TAGCTCTTGA	TCCGGCAAAC	AAACCACCGC	TGGTAGCGGT	GGTTTTTTTG	TTTGCAAGCA
4981	GCAGATTACG	CGCAGAAAAA	AAGGATCTCA	AGAAGATCCT	TTGATCTTTT	CTACGGGGTC

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5041 TGACGCTCAG TGAACGAAA ACTCACGTTA AGGGATTTTG GTCATGAGAT TATCAAAAAG
5101 GATCTTCACC TAGATCCTTT TAAATAAAA ATGAAGTTTT AAATCAATCT AAAGTATATA
5161 TGAGTAAACT TGGTCTGACA GTTACCAATG CTTAATCAGT GAGGCACCTA TCTCAGCGAT
5221 CTGTCTATTT CGTTCATCCA TAGTTGCCTG ACTCCTGCAA ACCACGTTGT GTCTCAAAAT
5281 CTCTGATGTT ACATTGCACA AGATAAAAA ATATCATCAT GAACAATAAA ACTGTCTGCT
5341 TACATAAACA GTAATACAAG GGGTGTATG AGCCATATT AACGGGAAAC GTCTTGCTCG
5401 AGGCCGCGAT TAAATCCAA CATGGATGCT GATTTATATG GGTATAAATG GGCTCGCGAT
5461 AATGTCGGGC AATCAGGTGC GACAATCTAT CGATTGTATG GGAAGCCCGA TCGCCAGAG
5521 TTGTTTCTGA AACATGGCAA AGGTAGCGTT GCCAATGATG TTACAGATGA GATGGTCAGA
5581 CTAAACTGGC TGACGGAATT TATGCCTCTT CCGACCATCA AGCATTTTAT CCGTACTCCT
5641 GATGATGCAT GGTTACTCAC CACTGCGATC CCCGGGAAAA CAGCATTCCA GGTATTAGAA
5701 GAATATCCTG ATTCAGGTGA AAATATTGTT GATGCGCTGG CAGTGTTTCT GCGCCGGTTG
5761 CATTGATTC CTGTTTGTA TTTGCTTTT AACAGCGATC GCGTATTTCT TCTCGCTCAG
5821 GCGCAATCAC GAATGAATAA CGGTTTGTT GATGCGAGTG ATTTTGATGA CGAGCGTAAT
5881 GGCTGGCCTG TTGAACAAGT CTGGAAAGAA ATGCATAAGC TTTTGCCATT CTCACCGGAT
5941 TCAGTCGTCA CTCATGGTGA TTTCTCACTT GATAACCTTA TTTTGACGA GGGGAAATTA
6001 ATAGGTTGTA TTGATGTTGG ACGAGTCGGA ATCGCAGACC GATACCAGGA TCTTGCCATC
6061 CTATGGAAC TCGCTCGGTGA GTTTTCTCCT TCATTACAGA AACGGCTTTT TCAAAAATAT
6121 GGTATTGATA ATCCTGATAT GAATAAATTG CAGTTTCATT TGATGCTCGA TGAGTTTTTC
6181 TAAGGGCGCG CTGCCACCAT ACCCACGCCG AAACAAGCGC TCATGAGCCC GAAGTGGCGA
6241 GCCCGATCTT CCCCATCGGT GATGTCGGCG ATATAGGCGC CAGCAACCGC ACCTGTGGCG
6301 CCGGTGATGC CGGCCACGAT GCGTCCGGCG TAGAGGATCT GGCTAGCGAT GACCCTGCTG
6361 ATTGGTTGCG TGACCATTTC CGGGTGC GCGGCTTAC CAGAACTCA GAAGTTTCGT
6421 CCAACCAAAC CGACTCTGAC GGCAGTTTAC GAGAGAGATG ATAGGGTCTG CTCAGGGTG
6481 ACCGATGTAA CCATATACTT AGGCTGGATC TTCTCCCGCG AATTTTAAAC CTCACCAACT
6541 ACGAGATATG AGGTAAGCCA AAAAAGCACG TAGTGGCGCT CTCCGACTGT TCCCAAATTG
6601 TAACTTATCG TTCCGTGAAG GCCAGAGTTA CTTCCCGGCC CTTTCCATGC GCGCACCATA
6661 CCCTCCTAGT TCCCCGGTTA TCTTTCGAA GTGGGAGTGA GCGAACCTCC GTTTACGTCT
6721 TGTTACCAAT GATGTAGCTA TGCATTTGT ACAGGGTGCC AACGGGTTTC ACAATTACCA
6781 GATAGTGGGG ATCCCGGCAA AGGGCCTATA TTTGCGGTCC AACTTAGGCG TAAACCTCGA
6841 TGCTACCTAC TCAGACCCAC CTCGCGCGGG GTAAATAAGG CACTCATCCC AGCTGGTTCT
6901 TGGCGTTCTA CGCAGCGACA TGTTTATTA CAGTTGTCTG GCAGCACAAA ACTTTTACCA
6961 TGGTCGTAGA AGCCCCCAG AGTTAGTTCA TACCTAATGC CACAAATGTG ACAGACGCC
7021 GATGGGTACC GGACTTTAGG TCGAGCACAG TTCGGTAACG GAGAGACCCT GCGGCGTACT
7081 TCATTATGTA TATGGAACGT GCCCAAGTGA CGCCAGGCAA GTCTCAGCTG GTTCTGTGT
7141 TAGCTCGAGG GTAGACATAC GAGCTGATTG AACATGGGTT GGGGGCCTCG AACCGTCGAG
7201 GACCCCATAG TACCTCGGAG ACCAAGTAGG GCAGCCTATA GTTTGAAGCA GAACTATTTT
7261 GGGGGCGGAG CCCTCATCGT CTCTTCTGCG GATGACTCAA CACGCTAGGG ACGTGAAGTC
7321 GATTCTTTCG ATGGTTATAA ATCAAAGACT CAGAGTGCTG TCTGGAGCGT GAATCTAACC
7381 GTACGTATCT CGATTGCTCG GTCGCTTTTC GCACTCCGCG AAAGTTCGTA CCGTCTATTC
7441 ACTAGTTGTC GAAGCCTATG CTGATATATG AATCCAACT AGAGCAGGGC TCTTAAGATT
7501 CCGAGTTGTA AATACTTAAT ACTCCAATCG GCTTTTACGT GCACCACCGC GGGCGGCTGA
7561 CAAGGGTCTC ACATCGAGAA ACAAGACAGT TCCGGGCTGG AAGTAGCGCC GGCTAAGGAA
7621 GACGCCTGGT ACGGCAGGAC TATGAAACCA GTACAAAGGC AACATCCTCA CTTGGGTGAA
7681 CGGAAACGCA GTATTATGGT TACTTTTTGG ATACGTGAAA CATATCCCAT GGTAGTCCTT
7741 AGACTTGGA GTCTATCACC CCTAGGGCCC ATATCTGGAA ATAGACGCA GGTGAATCC
7801 GTATTTGGAG GTACGATGGA ACAGTCTGGG TGGGACGTGC TTCATTTATA CCCTGCGCAG
7861 GCTGGACCGA GGACCGCAAG GTGCGGCGGT GCACAAGCAA TTGACAACTA ACCACCGTGT
7921 ATTCAATTAT GTACCAGGAA CTTTAAGCCG AGTCAATGAA GCTCGCATT CAGTGTTTAC
7981 CGCATCTTGC CGTTACTCAC AAAGTGTGAT CCACCACAAG TCAAGCCATT CCCTCTCTGA
8041 CACGCCGTAA GAATTAATAT GTAAACTTTG CGCGGGTTGA CTGCGATCCG TTCAGTCTCG
8101 TCCGAGGGCA CAATCCTATT CCCATTTGTA TGTTGAGCTA ACTTCTACCC ATCCCCGAA
8161 GTTAAGTAGG TCGTGAGATG CCATGGAGGC TCTCGTTCAT CCCGTGGGAC ATCAAGCTTC
8221 CCCTTGATAA AGCACCCCGC TCGGGTGTAG CAGAGAAGAC GCCTTCTGAA TTGTGCAATC
8281 CCTCCACCTT ATCTAAGCTT GCTACCAATA ATTAGCATTT TTGCTTGCG ACAGACCTCC
8341 TACTTAGATT GCCACACATT GAGCTAGTCA GTGAGCGATA AGCTTGACGC GCTTTCAAGG
8401 GTCGCGAGTA CGTGAACATA GGCTCCGGAC AGGACTATAT ACTTGGGTTT GATCTCGCCC
8461 CGACAACTGC AAACCTCAAC TTTTTAGAT TATATGGTTA GCCGAAGTTG CACGAGGTGG
8521 CGTCCGCGGA CTGCTCCCGG AGTGTGGCTC TTTTCTGTA CAACGTGCAA CCCCTATCGC
8581 GGCCGATTGT TTCTGCGGAC GATGTTGTCC TCATAGTTTG GGCATGTTTC CTTGTAGGT
8641 GTGAAACCAC TTAGCTTCGC GCCGTAGTCC CAATGAAAAA CCTATGGACT TTGTTTTGGG
8701 TAGCACCAGG AATCTGAACC GTGTGAATGT GGACGTCGCG CGCGTAGACC TTTATCTCCG
8761 GTTCAAGCTA GGGATGTGGC TGCATGCTAC GTTGTACAC CTACTGCT CGAAGTAAAT
8821 ATGCGAAGCG CGCGGCCTGG CCGGAGCGCT TCCGCGCCGC CACGTGTTCT TTAAGTGTG

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8881 ATTGGTGGCA CATAAGCAAT ATCGTAGTCC GTCAAATTCA GCTCTGTTAT CCCGGGCGTT
 8941 ATGTGTCAAA TGGCGTAGAA CGGGATTGAC TGTTTGACGG TAGGGTGACC TAAGCCAGAT
 9001 GCTACACAAT TAGGCTTGTA CATAATTGTCG TTAGAACGCG GCTACAATTA ATACATAACC
 9061 TTATGTATCA TACACATACG ATTTAGGTGA CACTATAGAA TACACGGAAT TAATTC (SEQ
 ID NO: 14).

[0105] Table 2. Features of the VMD2.IntEx.BEST1.WPRE.pA plasmid sequence

Name	Type	Minimum	Maximum	Length	Direction
AAV2 ITR	LTR	4	133	130	forward
-585 to +38 VMD2 promoter	promoter	189	811	623	forward
Intron	intron	814	936	123	forward
Exon	exon	937	989	53	forward
Kozak	Kozak	990	999	10	forward
BEST1	CDS	996	2753	1758	forward
NotI	RBS	2758	2765	8	none
WPRE	WPRE	2784	3376	593	forward
NotI	RBS	3378	3385	8	none
bGH pA	polyA_signal	3398	3666	269	forward
AAV2 ITR	LTR	3724	3844	121	reverse
pBR322 rep origin	rep_origin	4408	5027	620	reverse
AphR (KanR)	CDS	5368	6183	816	forward
BstEII	RBS	6477	6483	7	none
Randomly generated stuffer sequence	Stuffer	6484	8983	2500	none
BstEII	RBS	8984	8990	7	none

[0106] In some embodiments, the AAV viral delivery vector comprises a nucleic acid sequence comprising a sequence encoding a CAG promoter, a sequence encoding a BEST1 protein and a sequence encoding a WPRE. An exemplary AAV viral delivery vector of the disclosure comprising a nucleic acid sequence encoding a CAG.BEST1.WPRE.pA sequence comprises or consists of the nucleic acid sequence of:

1 TAGCTGCGCG CTCGCTCGCT CACTGAGGCC GCCCGGGCAA AGCCCGGGCG TCGGGCGACC
 61 TTTGGTCCGC CGGCCTCAGT GAGCGAGCGA GCGCGCAGAG AGGGAGTGGC CAACTCCATC
 121 ACTAGGGGTT CCTTGTAGTT AATGATTAAAC CCGCCATGCT ACTTATCTAC GTAGCCATGC
 181 TCTAGGTACC ATTGACGTCA ATAATGACGT ATGTTCCCAT AGTAACGCCA ATAGGGACTT
 241 TCCATTGACG TCAATGGGTG GAGTATTTAC GGTAAACTGC CCACTTGGCA GTACATCAAG
 301 TGTATCATAT GCCAAGTACG CCCCTATTG ACGTCAATGA CGGTAAATGG CCCGCCTGGC
 361 ATTATGCCCC GTACATGACC TTATGGGACT TTCCTACTTG GCAGTACATC TACGTATTAG
 421 TCATCGCTAT TACCATGGTC GAGGTGAGCC CCACGTTCTG CTTCCTCTC CCCATCTCCC
 481 CCCCTCCCC ACCCCCAATT TTGTATTTAT TTATTTTTTA ATTATTTTGT GCAGCGATGG
 541 GGGCGGGGGG GGGGGGGGGG CGCGCGCCAG GCGGGGCGGG GCGGGGCGAG GGGCGGGGCG
 601 GGGCGAGGCG GAGAGGTGCG GCGGCAGCCA ATCAGAGCGG CGCGCTCCGA AAGTTTCCTT
 661 TTATGGCGAG GCGGCGGGCG CGGCGGCCCT ATAAAAAGCG AAGCGCGCGG CGGGCGGGAG
 721 TCGCTGCGCG CTGCCTTCGC CCCGTGCCCC GCTCCGCGCG CGCCTCGCGC CGCCCGCCCC
 781 GGCTCTGACT GACCGCGTTA CTCCACAGG TGAGCGGGCG GGACGGCCCT TCTCCTCCGG
 841 GCTGTAATTA GCGCTTGTT TAATGACGG TTGTTTCTT TCTGTGGCTG CGTGAAAGCC
 901 TTGAGGGGCT CCGGGAGGGC CCTTTGTGCG GGGGGAGCGG CTCGGGGCTG TCCGCGGGGG
 961 GACGGTGCC TTCGGGGGGG ACGGGGCGAG GCGGGGTTG GCTTCTGGCG TGTGACGGGC
 1021 GGCTCTAGAG CCTCTGCTAA CCATGTTTCAT GCCTTCTTCT TTTTCTACA GCTCCTGGGC
 1081 AACGTGCTGG TTATTGTGCT GTCTCATCAT TTTGGCAAAG AATTGGATCC GCGGCCGAG

1141	CTTGGTACCG	CCACCATGAC	CATCACTTAC	ACAAGCCAAG	TGGCTAATGC	CCGCTTAGGC
1201	TCCTTCTCCC	GCCTGCTGCT	GTGCTGGCGG	GGCAGCATCT	ACAAGCTGCT	ATATGGCGAG
1261	TTCTTAATCT	TCCTGCTCTG	CTACTACATC	ATCCGCTTTA	TTTATAGGCT	GGCCCTCACG
1321	GAAGAACAAAC	AGCTGATGTT	TGAGAAACTG	ACTCTGTATT	GCGACAGTTA	CATCCAGCTC
1381	ATCCCCATTT	CCTTCGTGCT	GGGCTTCTAC	GTGACGCTGG	TCGTGACCCG	CTGGTGGAAC
1441	CAGTACGAGA	ACCTGCCGTG	GCCCGACCGC	CTCATGAGCC	TGGTGTCGGG	CTTCGTCGAA
1501	GGCAAGGACG	AGCAAGGCCG	GCTGCTGCGG	CGCACGCTCA	TCCGCTACGC	CAACCTGGGC
1561	AACGTGCTCA	TCCTGCGCAG	CGTCAGCACC	GCAGTCTACA	AGCGCTTCCC	CAGCGCCAG
1621	CACCTGGTGC	AAGCAGGCTT	TATGACTCCG	GCAGAACACA	AGCAGTTGGA	GAAACTGAGC
1681	CTACCACACA	ACATGTTCTG	GGTGCCCTGG	GTGTGGTTTG	CCAACCTGTC	AATGAAGGCG
1741	TGGCTTGAG	GTCGAATCCG	GGACCTATC	CTGCTCCAGA	GCCTGCTGAA	CGAGATGAAC
1801	ACCTTGCGTA	CTCAGTGTGG	ACACCTGTAT	GCCTACGACT	GGATTAGTAT	CCCCTGGTG
1861	TATACACAGG	TGGTGACTGT	GGCGGTGTAC	AGCTTCTTCC	TGACTTGTCT	AGTTGGGCGG
1921	CAGTTTCTGA	ACCCAGCCAA	GGCCTACCTT	GGCCATGAGC	TGGACCTCGT	TGTGCCCGTC
1981	TTACGTTCC	TGCAGTTCTT	CTTCTATGTT	GGCTGGCTGA	AGGTGGCAGA	GCAGCTCATC
2041	AACCCCTTTG	GAGAGGATGA	TGATGATTTT	GAGACCAACT	GGATTGTGCA	CAGGAATTTG
2101	CAGGTGTCCC	TGTTGGCTGT	GGATGAGATG	CACCAGGACC	TGCCTCGGAT	GGAGCCGGAC
2161	ATGTACTGGA	ATAAGCCCGA	GCCACAGCCC	CCCTACACAG	CTGCTTCCGC	CCAGTTCCGT
2221	CGAGCCTCCT	TTATGGGCTC	CACCTTCAAC	ATCAGCCTGA	ACAAAGAGGA	GATGGAGTTC
2281	CAGCCCAATC	AGGAGGACGA	GGAGGATGCT	CACGCTGGCA	TCATTGGCCG	CTTCCTAGGC
2341	CTGCAGTCCC	ATGATCACCA	TCCTCCCAGG	GCAAACTCAA	GGACCAAAC	ACTGTGGCCC
2401	AAGAGGGAAT	CCCTTCTCCA	CGAGGGCCTG	CCCAAAAACC	ACAAGGCAGC	CAAACAGAAC
2461	GTTAGGGGCC	AGGAAGACAA	CAAGGCCTGG	AAGCTTAAGG	CTGTGGACCG	CTTCAAGTCT
2521	GGCCCACTGT	ATCAGAGGCC	AGGCTACTAC	AGTGCCCCAC	AGACGCCCTC	CAGCCCACTC
2581	CCCATGTTCT	TCCCCCTAGA	ACCATCAGCG	CCGTCAAAGC	TTACAGTGT	CACAGGCATA
2641	GACACCAAAG	ACAAAAGCTT	AAAGACTGTG	AGTTCTGGGG	CCAAGAAAAG	TTTTGAATTG
2701	CTCTCAGAGA	GCGATGGGGC	CTTGATGGAG	CACCCAGAAG	TATCTCAAGT	GAGGAGGAAA
2761	ACTGTGGAGT	TTAACCTGAC	GGATATGCCA	GAGATCCCCG	AAAATCACCT	CAAAGAACCT
2821	TTGGAACAAAT	CACCAACCAA	CATACACACT	ACACTCAAAG	ATCACATGGA	TCCTTATTGG
2881	GCCTTGAAAA	ACAGGGATGA	AGCACATTCC	TAAGAGCTCA	AGCTTATCGA	TAATCAACCT
2941	CTGGATTACA	AAATTTGTGA	AAGATTGACT	GGTATTCTTA	ACTATGTTGC	TCCTTTTACG
3001	CTATGTGGAT	ACGCTGCTTT	AATGCCTTTG	TATCATGCTA	TTGCTTCCCG	TATGGCTTTC
3061	ATTTTCTCCT	CCTTGATAAA	ATCCTGGTTG	CTGTCTCTTT	ATGAGGAGTT	TGCGCCCGTT
3121	GTCAGGCAAC	GTGGCGTGGT	GTGCACTGTG	TTTGCTGACG	CAACCCCCAC	TGGTTGGGGC
3181	ATTGCCACCA	CCTGTACAGT	CCTTTCGGGG	ACTTTCGCTT	TCCCCCTCCC	TATTGCCACG
3241	GCGGAACCTA	TCGCCGCCTG	CCTTGCCCGC	TGCTGGACAG	GGGCTCGGCT	GTTGGGCACT
3301	GACAATTCCG	TGGTGTTGTC	GGGGAATCA	TCGTCTTTTC	CTTGCTGCT	CGCCTGTGTT
3361	GCCACCTGGA	TTCTGCGCGG	GACGTCTTTC	TGCTACGTCC	CTTCGGCCCT	CAATCCAGCG
3421	GACCTTCCCT	CCCGCGGCCT	GCTGCCGGCT	CTGCGGCCCT	TTCCGCGTCT	TCGCCTTCGC
3481	CCTCAGACGA	GTCGGATCTC	CCTTTGGGGC	GCCTCCCCGC	ATCGATAACC	TCGACTCGCT
3541	GATCAGCCTC	GACTGTGCCT	TCTAGTTGCC	AGCCATCTGT	TGTTTGCCCC	TCCCCCGTGC
3601	CTTCCCTGAC	CCTGGAAGGT	GCCACTCCCA	CTGTCTTTTC	CTAATAAAAT	GAGGAAATTG
3661	CATCGCATTG	TCTGAGTAGG	TGTCATTCTA	TTCTGGGGGG	TGGGGTGGGG	CAGGACAGCA
3721	AGGGGGAGGA	TTGGGAAGAC	AATAGCAGGC	ATGCTGGGGA	TGCGGTGGGC	TCTATGGCTT
3781	CTGAGGCGGA	AAGAACCAGC	TGGGGCTCGA	CTAGAGCATG	GCTACGTAGA	TAAGTAGCAT
3841	GGCGGGTTAA	TCATTAACCTA	CAAGGAACCC	CTAGTGATGG	AGTTGGCCAC	TCCCTCTCTG
3901	CGCGCTCGCT	CGCTCACTGA	GGCCGGGCGA	CCAAAGGTCG	CCCGACGCCC	GGGCGGCCTC
3961	AGTGAGCGAG	CGAGCGCGCA	GAGCTTTTTG	CAAAAGCCTA	GGCCTCCAAA	AAAGCCTCCT
4021	CACTACTTCT	GGAATAGCTC	AGAGGCCGAG	GCGGCCTCGG	CCTCTGCATA	AATAAAAAAA
4081	ATTAGTCAGC	CATGGGGCGG	AGAATGGGCG	GAACCTGGCG	GAGTTAGGGG	CGGGTAGGGC
4141	GGAGTTAGGG	GCGGGACTAT	GGTTGCTGAC	TAATTGAGAT	GCATGCTTTG	CATACTTCTG
4201	CCTGCTGGGG	AGCCTGGGGA	CTTTCACAC	CTGGTTGCTG	ACTAATTGAG	ATGCATGCTT
4261	TGCATACTTC	TGCCTGCTGG	GGAGCCTGGG	GACTTTCCAC	ACCCTAACTG	ACACACATTC
4321	CACAGCTGCA	TTAATGAATC	GGCCAACGCG	CGGGGAGAGG	CGGTTTGCGT	ATTGGGCGCT
4381	CTTCCGCTTC	CTCGCTCACT	GACTCGCTGC	GCTCGGTCTG	TCGCTGCGG	CGAGCGGTAT
4441	CAGCTCACTC	AAAGGCGGTA	ATACGGTTAT	CCACAGAATC	AGGGGATAAC	GCAGGAAAGA
4501	ACATGTGAGC	AAAAGGCCAG	CAAAAGGCCA	GGAACCGTAA	AAAAGCCGCG	TTGCTGGCGT
4561	TTTTCCATAG	GCTCCGCCCC	CCTGACGAGC	ATCACAAAAA	TCGACGCTCA	AGTCAGAGGT
4621	GGCGAAACCC	GACAGGACTA	TAAAGATACC	AGGCGTTTCC	CCCTGGAAGC	TCCCTGTGTC
4681	GCTCTCTGT	TCCGACCCTG	CCGCTTACCG	GATACCTGTC	CGCCTTTCTC	CCCTCGGGAA
4741	GCGTGCGGCT	TTCTCATAGC	TCACGCTGTA	GGTATCTCAG	TTGCGGTGAG	GTCGTTTCGCT
4801	CCAAGCTGGG	CTGTGTGCAC	GAACCCCCCG	TTCAGCCCGA	CCGCTGCGCC	TTATCCGGTA
4861	ACTATCGTCT	TGAGTCCAAC	CCGGTAAGAC	ACGACTTATC	GCCACTGGCA	GCAGCCACTG
4921	GTAACAGGAT	TAGCAGAGCG	AGGTATGTAG	GCGGTGCTAC	AGAGTTCTTG	AAGTGGTGCC

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4981 CTAACCTACGG CTACACTAGA AGAACAGTAT TTGGTATCTG CGCTCTGCTG AAGCCAGTTA
5041 CCTTCGGAAA AAGAGTTGGT AGCTCTTGAT CCGGCAAACA AACCACCGCT GGTAGCGGTG
5101 GTTTTTTTGT TTGCAAGCAG CAGATTACGC GCAGAAAAAA AGGATCTCAA GAAGATCCTT
5161 TGATCTTTTC TACGGGGTCT GACGCTCAGT GGAACGAAAA CTCACGTTAA GGGATTTTGG
5221 TCATGAGATT ATCAAAAAGG ATCTTCACCT AGATCCTTTT AAATTAATAA TGAAGTTTTA
5281 AATCAATCTA AAGTATATAT GAGTAAACTT GGTCTGACAG TTACCAATGC TTAATCAGTG
5341 AGGCACCTAT CTCAGCGATC TGTCTATTTT GTTCATCCAT AGTTGCCTGA CTCCCCGTCTG
5401 TGTAGATAAC TACGATACGG GAGGGCTTAC CATCTGGCCC CAGTGCTGCA ATGATACCGC
5461 GAGACCCACG CTCACCGGCT CCAGATTAT CAGCAATAAA CCAGCCAGCC GGAAGGGCCG
5521 AGCGCAGAAG TGGTCCTGCA ACTTTATCCG CCTCCATCCA GTCTATTAAT TGTGCGCGG
5581 AAGCTAGAGT AAGTAGTTCG CCAGTTAATA GTTTGCGCAA CGTTGTTGCC ATTGCTACAG
5641 GCATCGTGGT GTCACGCTCG TCGTTTGGTA TGGCTTCATT CAGCTCCGGT TCCCAACGAT
5701 CAAGGCGAGT TACATGATCC CCCATGTTGT GCAAAAAGC GGTTAGCTCC TTCGGTCTC
5761 CGATCGTTGT CAGAAGTAAG TTGGCCGCGC TGTTATCACT CATGGTTATG GCAGCACTGC
5821 ATAATTCTCT TACTGTCATG CCATCCGTAA GATGCTTTT TGTGACTGTG GAGTACTCAA
5881 CCAAGTCATT CTGAGAATAG TGTATGCGGC GACCGAGTTG CTCTTGCCCG GCGTCAATAC
5941 GGGATAATAC CGCGCCACAT AGCAGAACTT TAAAAGTGCT CATCATTGGA AAACGTTCTT
6001 CGGGGCGAAA ACTCTCAAGG ATCTTACCGC TGTTGAGATC CAGTTCGATG TAACCCACTC
6061 GTGCACCCAA CTGATCTTCA GCATCTTTTA CTTTCACCAG CGTTTCTGGG TGAGCAAAAA
6121 CAGGAAGGCA AAATGCCGCA AAAAAGGGAA TAAGGGCGAC ACGGAAATGT TGAATACTCA
6181 TACTCTTCCT TTTTCAATAT TATTGAAGCA TTTATCAGGG TTATTGTCTC ATGAGCGGAT
6241 ACATATTTGA ATGTATTTAG AAAAATAAAC AAATAGGGGT TCCGCGCACA TTTCCCCGAA
6301 AAGTGCCACC TGACGTCTAA GAAACCATTA TTATCATGAC ATTAACCTAT AAAATAGGCG
6361 GTATCACGAG GCCCTTTCGT CTCGCGCGTT TCGGTGATGA CGGTGAAAAC CTCTGACACA
6421 TGCAGCTCCC GGAGACGGTC ACAGCTTGTC TGTAAGCGGA TGCCGGGAGC AGACAAGCCC
6481 GTCAGGCGCG GTCAGCGGGT GTTGGCGGGT GTCGGGGCTG GCTTAAGTAT GCGGCATCAG
6541 AGCAGATTGT ACTGAGAGTG CACCATTCTGA CGCTCTCCCT TATGCGACTC CTGCATTAGG
6601 AAGCAGCCCA GTAGTAGGTT GAGGCCGTTG AGCACCGCCG CCGCAAGGAA TGGTGCATGC
6661 AAGGAGATGG CGCCCAACAG TCCCCGGGCC ACGGGGCCTG CCACCATACC CACGCCGAAA
6721 CAAGCGCTCA TGAGCCCGAA GTGGCGAGCC CGATCTTCCC CATCGGTGAT GTCGGCGATA
6781 TAGGCGCCAG CAACCGCACC TGTGGCGCCG GTGATGCCGG CCACGATGCG TCCGGCGTAG
6841 AGGATCTGGC TAGCGATGAC CTTGCTGATT GGTTCGCTGA CCATTTCGGG GTGCGGGACG
6901 GCGTTACGAG AAACCTCAGAA GGTTCTGTTA ACCAAACCGA CTCTGACGGC AGTTTACGAG
6961 AGAGATGATA GGGTCTGCTT CAGTAAGCCA GATGCTACAC AATTAGGCTT GTACATATTG
7021 TCGTTAGAAC GCGGCTACAA TTAATACATA ACCTTATGTA TCATACACAT ACGATTTAGG
7081 TGACACTATA GAATACACGG AATTAATTC (SEQ Id NO: 15).

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[0107] Table 3. Features of the CAG.BEST1.WPRE.PA plasmid sequence

Name	Type	Minimum	Maximum	Length	Direction
AAV2 ITR	repeat_region	7,066	133	177	forward
amp prom	promoter	6,223	6,251	29	reverse
AmpR gene	gene	5,321	6,181	861	reverse
Bla gene	gene	5,321	5,983	663	reverse
ColE1 origin rep origin	rep_origin	4,503	5,176	674	forward
SV40 origin	origin of replication	4,059	4,136	78	reverse
AAV2 ITR	repeat_region	3,864	4,000	137	reverse
bGH_PA term	terminator	3,550	3,777	228	forward
WPRE	misc_feature	2,932	3,520	589	forward
Exon 10	exon	2,895	2,913	19	forward
Exon 9	exon	2,256	2,894	639	forward
Exon 8	exon	2,104	2,255	152	forward
Exon 7	exon	2,023	2,103	81	forward
Exon 6	exon	1,870	2,022	153	forward
Exon 5	exon	1,792	1,869	78	forward
Exon 4	exon	1,637	1,791	155	forward

Exon 3	exon	1,403	1,636	234	forward
C>T	modified_base	1,368	1,368	1	forward
Exon 2	exon	1,308	1,402	95	forward
hBEST1 CDS	CDS	1,156	2,913	1,758	forward
Exon 1	exon	1,156	1,307	152	forward
Kozak	unsure	1,147	1,155	9	forward
	Editing History Insertion	<1133	1,138	>6	none
CAG promoter	promoter	189	1,129	941	forward
5'ITR on REP1 official sequence file	LTR	64	183	120	forward

[0108] In some embodiments of the compositions of the disclosure, a vector may comprise a sequence encoding a marker, which may be expressed in a cell when the cell is either *in vitro* or *in vivo*. For example, in a vector or nucleic acid sequence of the disclosure, a sequence encoding a marker may be used in place of or may replace a sequence encoding a BEST1 protein of the disclosure (e.g. a sequence comprising a coding sequence of a BEST1 gene). Exemplary markers of the disclosure include, but are not limited to, fluorophore proteins such as GFP, YFP or dsRED as well as various epitope tags such as FLAG, HA, His or Myc. The fluorophore or epitope tag may be fused to the BEST1 coding sequence, for example as an N or C terminal fusion, or may be used in place of BEST1 to characterize a vector of the disclosure. Exemplary uses for a vector containing a marker of the disclosure include, but are not limited to characterizing gene expression, for example levels of expression, or characterizing the cell type specificity of a vector of the disclosure.

[0109] An exemplary a vector of the disclosure comprising a marker includes VMD2.GFP.WPRE.pA. A nucleic acid sequence encoding a VMD2.GFP.WPRE.pA construct comprises or consists of :

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1 TAGCTGCGCG CTCGCTCGCT CACTGAGGCC GCCCGGGCAA AGCCCGGGCG TCGGGCGACC
61 TTTGGTCGCC CGGCCTCAGT GAGCGAGCGA GCGCGCAGAG AGGGAGTGGC CAACTCCATC
121 ACTAGGGGTT CCTTGTAAGT AATGATTAAC CCGCCATGCT ACTTATCTAC GTAGCCATGC
181 TCTAGGTAAA TTCTGTCATT TTACTAGGGT GATGAAATTC CCAAGCAACA CCATCCTTTT
241 CAGATAAGGG CACTGAGGCT GAGAGAGGAG CTGAAACCTA CCCGGGGTCA CCACACACAG
301 GTGGCAAGGC TGGGACCAGA AACCAGGACT GTTGACTGCA GCCCGGTATT CATTCTTTCC
361 ATAGCCCAACA GGGCTGTCAA AGACCCAGG GCCTAGTCAG AGGCTCCTCC TTCCTGGAGA
421 GTTCCTGGCA CAGAAGTTGA AGCTCAGCAC AGCCCCCTAA CCCCCAACTC TCTCTGCAAG
481 GCCTCAGGGG TCAGAACACT GGTGGAGCAG ATCCTTTAGC CTCTGGATT TAGGGCCATG
541 GTAGAGGGGG TGTTGCCCTA AATTCCAGCC CTGGTCTCAG CCCAACACCC TCCAAGAAGA
601 AATTAGAGGG GCCATGGCCA GGCTGTGCTA GCCGTTGCTT CTGAGCAGAT TACAAGAAGG
661 GACTAAGACA AGGACTCCTT TGTGGAGGTC CTGGCTTAGG GAGTCAAGTG ACGGCGGCTC
721 AGCACTCACG TGGGCAGTGC CAGCCTCTAA GAGTGGGCAG GGGCACTGGC CACAGAGTCC
781 CAGGGAGTCC CACCAGCCTA GTCGCCAGAC CGGCACCATG AGCAAGGGCG AGGAACGTGT
841 CACTGGCGTG GTCCCAATTC TCGTGGAAC TGGATGGCGAT GTGAATGGGC ACAAATTTTC
901 TGTCAGCGGA GAGGGTGAAG GTGATGCCAC ATACGGAAG CTCACCCTGA AATTATCTG
961 CACCACTGGA AAGCTCCCTG TGCCATGGCC AACACTGGTC ACTACCCTGA CCTATGGCGT
1021 GCAGTGCTTT TCCAGATACC CAGACCATAT GAAGCAGCAT GACTTTTTC AAGAGCGCCAT
1081 GCCCGAGGGC TATGTGCAGG AGAGAACCAT CTTTTTCAAA GATGACGGGA ACTACAAGAC

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1141 CCGCGCTGAA GTCAAGTTCTG AAGGTGACAC CCTGGTGAAT AGAATCGAGC TGAAGGGCAT
1201 TGACTTTAAG GAGGATGGAA ACATTCTCGG CCACAAGCTG GAATACAACAT ATAAC TCCCA
1261 CAATGTGTAC ATCATGGCCG ACAAGCAAAA GAATGGCATC AAGGTCAACT TCAAGATCAG
1321 ACACAACATT GAGGATGGAT CCGTGCAGCT GGCCGACCAT TATCAACAGA AACTTCCAAT
1381 CGGCGACGGC CCTGTGCTCC TCCAGACAAA CCATTACCTG TCCACCCAGT CTGCCCTGTC
1441 TAAAGATCCC AACGAAAAGA GAGACCACAT GGTCCTGCTG GAGTTTGTGA CCGCTGCTGG
1501 GATCACACAT GGCATGGACG AGCTGTACAA GTGAAAGCTT ATCGATAATC AACCTCTGGA
1561 TTACAAAATT TGTGAAAGAT TGA CTGGTAT TCTTAACTAT GTTGCTCCTT TTACGCTATG
1621 TGGATACGCT GCTTTAATGC CTTTGTATCA TGCTATTGCT TCCCGTATGG CTTTCATTTT
1681 CTCCTCCTTG TATAAATCCT GGTGCTGCTC TCTTTATGAG GAGTTGTGGC CCGTTGTGAG
1741 GCAACGTGGC GTGGTGTGCA CTGTGTTTGC TGACGCAACC CCCACTGGTT GGGGCATTGC
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1861 ACTCATCGCC GCCTGCCTTG CCCGCTGCTG GACAGGGGCT CGGCTGTTGG GCACTGACAA
1921 TTCCGTGGTG TTGTCGGGGA AATCATCGTC CTTTCCTTGG CTGCTCGCCT GTGTTGCCAC
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2101 GACGAGTCGG ATCTCCCTTT GGGCCGCTC CCCGGCGGCC GCGCACCGTC GACTCGCTGA
2161 TCAGCCTCGA CTGTGCCTTC TAGTTGCCAG CCATCTGTTG TTTGCCCTC CCCCCTGCTC
2221 TCCTTGACCC TGGAAGGTGC CACTCCCACT GTCCTTTTCT AATAAAATGA GGAAATTGCA
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2581 TGAGCGAGCG AGCGCGCAGA GCTTTTGTGA AAAGCCTAGG CCTCCAAAAA AGCCTCCTCA
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2761 AGTTAGGGGC GGGACTATGG TTGCTGACTA ATTGAGATGC ATGCTTTGCA TACTTCTGCC
2821 TGCTGGGGAG CCTGGGGACT TTCCACACCT GGTGCTGAC TAATTGAGAT GCATGCTTTG
2881 CATACTTCTG CCTGCTGGGG AGCCTGGGGA CTTTCCACAC CCTAACTGAC ACACATTCCA
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3001 TCCGCTTCTC CGCTCACTGA CTCGCTGCGC TCGGTCGTTT GGCTGCGGCG AGCGGTATCA
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3961 GCACCTATCT CAGCGATCTG TCTATTTCTG TCATCCATAG TTGCCTGACT CCTGCAAACC
4021 ACGTTGTGTC TCAAAATCTC TGATGTTACA TTGCACAAGA TAAAAATATA TCATCATGAA
4081 CAATAAAACT GTCTGCTTAC ATAAACAGTA ATACAAGGGG TGTTATGAGC CATATTCAAC
4141 GGGAAACGTC TTGCTCGAGG CCGCGATTAA ATTCCAACAT GGATGCTGAT TTATATGGGT
4201 ATAAATGGGC TCGCGATAAT GTCGGGCAAT CAGGTGCGAC AATCTATCGA TTGTATGGGA
4261 AGCCCGATGC GCCAGAGTTG TTTCTGAAAC ATGGCAAAGG TAGCGTTGCC AATGATGTTA
4321 CAGATGAGAT GGTCAGACTA AACTGGCTGA CGGAATTTAT GCCTCTTCCG ACCATCAAGC
4381 ATTTTATCCG TACTCCTGAT GATGCATGGT TACTACCAC TCGATCCCC GGGAAAACAG
4441 CATTCCAGGT ATTAGAAGAA TATCTGATT CAGGTGAAAA TATTGTTGAT GCGCTGGCAG
4501 TGTTCTTGCG CCGGTTGCA TCGATTCTTG TTTGTAATTG TCCTTTTAAAC AGCGATCGCG
4561 TATTTCTGCT CGCTCAGGCG CAATCACGAA TGAATAACGG TTTGGTTGAT GCGAGTGATT
4621 TTGATGACGA GCGTAATGGC TGGCTGTTG AACAAGTCTG GAAAGAAATG CATAAGCTTT
4681 TGCCATTCTC ACCGATTCA GTCGTCACTC ATGGTGATTT CTCACTTGAT AACCTTATT
4741 TTGACGAGGG GAAATTAATA GGTGTATTG ATGTTGGACG AGTCGGAATC GCAGACCGAT
4801 ACCAGGATCT TGCCATCCTA TGGAACTGCC TCGGTGAGTT TTCTCCTTCA TTACAGAAAC
4861 GGCTTTTTTCA AAAATATGGT ATTGATAATC CTGATATGAA TAAATTGCAG TTTCAATTTGA
4921 TGCTCGATGA GTTTTTCTAA GGGCGGCTG CCACCATACC CACCCGAAA CAAGCGCTCA

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4981 TGAGCCCGAA GTGGCGAGCC CGATCTTCCC CATCGGTGAT GTCGGCGATA TAGGCGCCAG
 5041 CAACCGCACC TGTGGCGCCG GTGATGCCGG CCACGATGCG TCCGGCGTAG AGGATCTGGC
 5101 TAGCGATGAC CCTGCTGATT GGTTCGCTGA CCATTTCCGG GTGCGGGACG GCGTTACCAG
 5161 AAACCTCAGAA GGTTTCGTCCA ACCAAACCGA CTCTGACGGC AGTTTACGAG AGAGATGATA
 5221 GGGTCTGCTT CAGGGTGACC GATGTAACCA TATACTTAGG CTGGATCTTC TCCGCGCAAT
 5281 TTTAACCTTC ACCAACTACG AGATATGAGG TAAGCCAAAA AAGCACGTAG TGGCGCTCTC
 5341 CGACTGTTCC CAAATTGTAA CTTATCGTTC CGTGAAGGCC AGAGTTACTT CCCGGCCCTT
 5401 TCCATGCGCG CACCATACCC TCCTAGTTCC CCGGTTATCT TTCCGAAGTG GGAGTGAGCG
 5461 AACCTCCGTT TACGTCTTGT TACCAATGAT GTAGCTATGC ACTTTGTACA GGGTGCCAAC
 5521 GGGTTTCACA ATTCACAGAT AGTGGGGATC CCGGCAAAGG GCCTATATTT GCGGTCCAAC
 5581 TTAGGCGTAA ACCTCGATGC TACCTACTCA GACCCACCTC GCGCGGGGTA AATAAGGCAC
 5641 TCATCCAGC TGGTTCTTGG CGTTCTACGC AGCGACATGT TTATTAACAG TTGTCTGGCA
 5701 GCACAAAAC TTTACCATGG TCGTAGAAGC CCCCCAGAGT TAGTTCATAC CTAATGCCAC
 5761 AAATGTGACA GGACGCCGAT GGGTACCGGA CTTTAGGTCG AGCACAGTTC GGTAACGGAG
 5821 AGACCTGCG GCGTACTTCA TTATGTATAT GGAACGTGCC CAAGTGACGC CAGGCAAGTC
 5881 TCAGCTGGTT CCTGTGTTAG CTCGAGGGTA GACATACGAG CTGATTGAAC ATGGGTTGGG
 5941 GGCCTCGAAC CGTCGAGGAC CCCATAGTAC CTCGAGAGC AAGTAGGGCA GCCTATAGTT
 6001 TGAAGCAGAA CTATTTCCGG GGGCGAGCCC TCATCGTCTC TTCTGCGGAT GACTCAACAC
 6061 GCTAGGGACG TGAAGTCGAT TCCTTCGATG GTTATAAATC AAAGACTCAG AGTGCTGTCT
 6121 GGAGCGTGAA TCTAACGGTA CGTATCTCGA TTGCTCGGTC GCTTTTCGCA CTCCGCGAAA
 6181 GTTCGTACCG CTCATTCACT AGGTTGCGAA GCCTATGCTG ATATATGAAT CCAAAC TAGA
 6241 GCAGGGCTCT TAAGATTCGG AGTTGTAAAT ACTTAATACT CCAATCGGCT TTTACGTGCA
 6301 CCACCGCGGG CGGCTGACAA GGGTCTCACA TCGAGAAACA AGACAGTTCC GGGCTGGAAG
 6361 TAGCGCCGGC TAAGGAAGAG GCCTGGTACG GCAGGACTAT GAAACCAAC CAGGCAAC
 6421 ATCCTCATT GGGTGAACGG AAACCCAGTA TTATGGTTAC TTTTGGATA CGTGAAACAT
 6481 ATCCCATGGT AGTCCTTAGA CTTGGGAGTC TATCACCCCT AGGGCCATA TCTGGAATA
 6541 GACGCCAGGT TGAATCCGTA TTTGGAGGTA CGATGGAACA GTCTGGGTGG GACGTGCTTC
 6601 ATTTATACCC TGCGCAGGCT GGACCGAGGA CCGCAAGGTG CCGCGGTGCA CAAGCAATTG
 6661 ACAACTAACC ACCGTGTATT CATTATGGTA CCAGGAACCT TAAGCCGAGT CAATGAAGCT
 6721 CGCATTACAG TGTTTACCGC ATCTTGCCGT TACTCACAAA CTGTGATCCA CCACAAGTCA
 6781 AGCCATTGCC TCTCTGACAC GCCGTAAGAA TTAATATGTA AACTTTGCGC GGGTTGACTG
 6841 CGATCCGTT AGTCTCGTCC GAGGGCACAA TCCTATTCCC ATTTGTATGT TCAGCTAAT
 6901 TCTACCCATC CCCCAGAGTT AAGTAGGTCG TGAGATGCCA TGGAGGCTCT CGTTCATCCC
 6961 GTGGGACATC AAGCTTCCCC TTGATAAAGC ACCCCGCTCG GGTGTAGCAG AGAAGACGCC
 7021 TTCTGAATTG TGCAATCCCT CCACCTTATC TAAGCTTGCT ACCAATAATT AGCATTTTTG
 7081 CTTGCGACA GACCTCCTAC TTAGATTGCC ACACATTGAG CTAGTCAGTG AGCGATAAGC
 7141 TTGACGCGCT TTCAAGGGTC GCGAGTACGT GAACTAAGGC TCCGACAGG ACTATATACT
 7201 TGGGTTTGAT CTCGCCCCGA CAACTGCAAA CCTCAACTTT TTAGATTAT ATGGTTAGCC
 7261 GAAGTTGCAC GAGGTGGCGT CCGCGGACTG CTCCCCGAGT GTGGCTCTTT CATCTGACAA
 7321 CGTGCAACCC CTATCGCGGC CGATTGTTTC TGCGGACGAT GTTGTCCTCA TAGTTTGGGC
 7381 ATGTTTCCCT TGTAGGTGTG AAACCACTTA GCTTCGCGCC GTAGTCCCAA TGAAAAACCT
 7441 ATGGACTTGT TTTTGGGTAG CACCAGGAAT CTGAACCGTG TGAATGTGGA CGTCGCGCGC
 7501 GTAGACCTTT ATCTCCGGTT CAAGCTAGGG ATGTGGCTGC ATGCTACGTT GTCACACCTA
 7561 CACTGCTCGA AGTAAATATG CGAAGCGCGC GGCCTGGCCG GAGGCGTTCC GCGCCGCCAC
 7621 GTGTTCTGTA ACTGTTGATT GGTGGCACAT AAGCAATATC GTAGTCCGTC AAATTCAGCT
 7681 CTGTTATCCC GGGCGTTATG TGTCAAATGG CGTAGAACGG GATTGACTGT TTGACGGTAG
 7741 GGTGACCTAA GCCAGATGCT ACACAATTAG GCTTGACAT ATTGTCTGTA GAACGCGGCT
 7801 ACAATTAATA CATAACCTTA TGTATCATAC ACATACGATT TAGGTGACAC TATAGAATAC
 7861 ACGGAATTAA TTC (SEQ ID NO: 16).

[0110] Table 4. Features of the VMD2.GFP.WPRE.pA plasmid sequence

Name	Type	Minimum	Maximum	Length	Direction
Randomly generated stuffer sequence	Stuffer	5,241	7,740	2,500	none
AphR (KanR)	CDS	4,125	4,940	816	forward
pBR322 rep origin	rep_origin	3,165	3,784	620	reverse
AAV2 ITR	LTR	2,481	2,601	121	reverse
bGH pA	polyA_signal	2,155	2,423	269	forward
WPRE	WPRE	1,547	2,136	590	forward
GFP	misc_feature	818	1,534	717	forward

Kozak	Kozak	812	817	6	forward
-585 to +38 VMD2 promoter	promoter	189	811	623	forward
AAV2 ITR	LTR	4	133	130	forward

[0111] An exemplary a vector of the disclosure comprising a marker includes

VMD.IntEx.GFP.WPRE.pA. A nucleic acid sequence encoding a

VMD.IntEx.GFP.WPRE.pA construct comprises or consists of :

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1 TAGCTGCGCG CTCGCTCGCT CACTGAGGCC GCCCGGGCAA AGCCCGGGCG TCGGGCGACC
61 TTTGGTCGCC CGGCCTCAGT GAGCGAGCGA GCGCGCAGAG AGGGAGTGGC CAACTCCATC
121 ACTAGGGGTT CTTGTAGTT AATGATTAAC CCGCCATGCT ACTTATCTAC GTAGCCATGC
181 TCTAGGTAAT TTCTGTCATT TTTACTAGGGT GATGAAATTC CCAAGCAACA CCATCCTTTT
241 CAGATAAGGG CACTGAGGCT GAGAGAGGAG CTGAAACCTA CCCGGGGTCA CCACACACAG
301 GTGGCAAGGC TGGGACCAGA AACCAGGACT GTTGAATGCA GCCCGGTATT CATTCTTTCC
361 ATAGCCACCA GGGCTGTCAA AGACCCAGG GCCTAGTCAG AGGCTCCTCC TTCCTGGAGA
421 GTTCTTGGCA CAGAAGTTGA AGCTCAGCAC AGCCCCCTAA CCCCCAACTC TCTCTGCAAG
481 GCCTCAGGGG TCAGAACACT GGTGAGCAGC ATCCTTTAGC CTCTGGATTG TAGGGCCATG
541 GTAGAGGGGG TGTTGCCCTA AATTCCAGCC CTGGTCTCAG CCAACACACC TCCAAGAAGA
601 AATTAGAGGG GCCATGGCCA GGCTGTGCTA GCCGTTGCTT CTGAGCAGAT TACAAGAAGG
661 GACTAAGACA AGGACTCCTT TGTGGAGGTC CTGGCTTAGG GAGTCAAGTG ACGGCGGCTC
721 AGCACTACAG TGGGCAGTGC CAGCCTCTAA GAGTGGGCAG GGGCACTGGC CACAGAGTCC
781 CAGGGAGTCC CACCAGCCTA GTCGCCAGAC CGGGTGCCGC AGGGGGACGG CTGCCTTCGG
841 GGGGGACGGG GCAGGGCGGG GTTCGGCTTC TGGCGTGTGA CCGGCGGCTC TAGAGCCTCT
901 GCTAACCATG TTCATGCCTT CTTCTTTTTC CTACAGCTCC TGGGCAACGT GCTGGTTATT
961 GTGCTGTCTC ATCATTTTTG CAAAGAATTG GCACCATGAG CAAGGGCGAG GAATGTTTCA
1021 CTGGCGTGGT CCCAATTCTC GTGGAATGAT ATGGCGATGT GAATGGGCAC AAATTTTCTG
1081 TCAGCGGAGA GGGTGAAGGT GATGCCACAT ACGGAAAGCT CACCCTGAAA TTCATCTGCA
1141 CCACTGAAAA GCTCCCTGTG CCATGGCCAA CACTGGTCAC TACCCTGACC TATGGCGTGC
1201 AGTGCTTTTC CAGATACCCA GACCATATGA AGCAGCATGA CTTTTTCAAG AGCGCCATGC
1261 CCGAGGGCTA TGTGCAGGAG AGAACCATCT TTTTCAAAGA TGACGGGAAC TACAAGACCC
1321 GCGCTGAAGT CAAGTTCGAA GGTGACACCC TGGTGAATAG AATCGAGCTG AAGGGCATTG
1381 ACTTTAAGGA GGATGGAAC ATTCCTCGGC ACAAGCTGGA ATACAACAT AACTCCCACA
1441 ATGTGTACAT CATGGCCGAC AAGCAAAAGA ATGGCATCAA GGTCAACTTC AAGATCAGAC
1501 ACAACATTGA GGATGGATCC GTGCAGCTGG CCGACCATT TCAACAGAAC ACTCCAATCG
1561 GCGACGGCCC TGTGCTCCTC CCAGACAACC ATTACCTGTC CACCCAGTCT GCCCTGTCTA
1621 AAGATCCCAA CGAAAAGAGA GACCACATGG TCCTGCTGGA GTTTGTGACC GCTGCTGGGA
1681 TCACACATGG CATGGACGAG CTGTACAAGT GAAAGCTTAT CGATAATCAA CCTCTGGATT
1741 ACAAATTTG TGAAAGATTG ACTGGTATTC TTAATATGT TGCTCCTTTT ACGCTATGTG
1801 GATACGCTGC TTTAATGCCT TTGTATCATG CTATTGCTTC CCGTATGGCT TTCATTTTCT
1861 CCTCCTTGTA TAAATCCTGG TTGCTGTCTC TTTATGAGGA GTTGTGGCCC GTTGTAGGCG
1921 AACGTGGCGT GGTGTGCACT GTGTTTGCTG ACGCAACCCC CACTGGTTGG GGCATTGCCA
1981 CCACCTGTCA GCTCCTTTCC GGGACTTTTC CTTTCCCCCT CCCTATTGCC ACGGCGGAAC
2041 TCATCGCCGC CTGCCTTGCC CGCTGCTGGA CAGGGGCTCG GCTGTTGGG ACTGACAATT
2101 CCGTGGTGTG TCGGGGAAA TCATCGTCTT TCTCTGGCT GCTCGCCTGT GTTGCCACCT
2161 GGATTCGCG CGGGACGTCC TTCTGCTACG TCCCTTCGGC CCTCAATCCA GCGGACCTTC
2221 CTTCCGCGCG CCTGCTGCCG GCTCTGCGGC CTCTTCGCG TCTTCGCTT CGCCCTCAGA
2281 CGAGTCGGAT CTCCCTTTGG GCCGCTTCCC CGGCGGCCGC GCACCGTCGA CTCGCTGATC
2341 AGCCTCGACT GTGCCTTCTA GTTGCCAGCC ATCTGTTGTT TGCCCTCCC CCGTGCCTTC
2401 CTTGACCTG GAAGGTGCCA CTCCCACTGT CTTTTCCTAA TAAAATGAGG AAATTGCATC
2461 GCATTGTCTG AGTAGGTGTC ATTCTATTCT GGGGGGTGGG GTGGGGCAGG ACAGCAAGGG
2521 GGAGGATTGG GAAGACAATA GCAGGCATGC TGGGGATGCG GTGGGCTCTA TGGCTTCTGA
2581 GCGGAAAAGA ACCAGCTGGG GCTCGACTAG AGCATGGCTA CGTAGATAAG TAGCATGGCG
2641 GGTTAATCAT TAACTACAAG GAACCCCTAG TGATGGAGTT GGCCACTCCC TCTCTGCGCG
2701 CTCGCTCGCT CACTGAGGCC GGGCGACCAA AGGTCGCCC GCGCCCGGGC GGCCTCAGTG
2761 AGCGAGCGAG CGCGCAGAGC TTTTTCGAAA AGCCTAGGCC TCCAAAAAAG CCTCCTCACT
2821 ACTTCTGGAA TAGCTCAGAG GCCGAGGCGG CCTCGGCCTC TGCATAAATA AAAAAAATTA
2881 GTCAGCCATG GGGCGGAGAA TGGGCGGAAC TGGGCGGAGT TAGGGGCGGG ATGGGCGGAG
2941 TTAGGGGCGG GACTATGGTT GCTGACTAAT TGAGATGCAT GCTTTCGATA CTTCTGCCTG
3001 CTGGGGAGCC TGGGGACTTT CCACACCTGG TTGCTGACTA ATTGAGATGC ATGCTTTGCA
3061 TACTTCTGCC TGCTGGGGAG CCTGGGGACT TTCCACACCC TAACTGACAC ACATTCCACA
3121 GCTGCATTAA TGAATCGGCC AACGCGCGGG GAGAGGCGGT TTGCGTATTG GGCGCTCTTC

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3181 CGCTTCCTCG CTCACTGACT CGCTGCGCTC GGTCTGTTCCG CTGCGGCGAG CGGTATCAGC
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3301 GTGAGCAAAA GGCCAGCAAA AGGCCAGGAA CCGTAAAAAG GCCGCGTTGC TGGCGTTTTT
3361 CCATAGGCTC CGCCCCCTG ACGAGCATCA CAAAAATCGA CGCTCAAGTC AGAGGTGGCG
3421 AAACCCGACA GGAATAAAA GATACCAGGC GTTTCCCCCT GGAAGCTCCC TCGTGCGCTC
3481 TCCTGTTCCG ACCCTGCCGC TTACCGGATA CCTGTCCGCC TTTCTCCCTT CGGGAAGCGT
3541 GGCCTTTCT CATAGCTCAC GCTGTAGGTA TCTCAGTTCG GTGTAGGTCG TTCGCTCCAA
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3841 CGGAAAAAGA GTTGGTAGCT CTTGATCCGG CAAACAAACC ACCGCTGGTA GCGGTGGTTT
3901 TTTTGTTCG AAGCAGCAGA TTACGCGCAG AAAAAAAGGA TCTCAAGAAG ATCCTTTGAT
3961 CTTTTCTACG GGGTCTGACG CTCAGTGGAA CGAAAACTCA CGTTAAGGGA TTTTGGTCAT
4021 GAGATTATCA AAAAGGATCT TCACCTAGAT CCTTTTAAAT TAAAAATGAA GTTTTAAATC
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4141 ACCTATCTCA GCGATCTGTC TATTTCTGTT ATCCATAGTT GCCTGACTCC TGCAAACAC
4201 GTTGTGTCTC AAAATCTCTG ATGTTACATT GCACAAGATA AAAATATATC ATCATGAACA
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5461 TAACCTCAC CAACTACGAG ATATGAGGTA AGCCAAAAAA GCACGTAGTG GCGCTCTCCG
5521 ACTGTTCCCA AATTGTAAC TATCGTTCCG TGAAGGCCAG AGTTACTTCC CGGCCCTTTC
5581 CATGCGCGCA CCATACCTC CTAGTTCCCC GGTATCTTTT CCGAAGTGGG AGTGACGAA
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5701 GTTTCACAAT TCACAGATAG TGGGGATCCC GGCAAAGGGC CTATATTTGC GGTCCAACCT
5761 AGGCGTAAAC CTCGATGCTA CCTACTCAGA CCCACCTCGC GCGGGGTAAA TAAGGCACTC
5821 ATCCCAGCTG GTTCTTGGCG TTCTACGCAG CGACATGTTT ATTAACAGTT GTCTGGCAGC
5881 ACAAACTTT TACCATGGTC GTAGAAGCCC CCCAGAGTTA GTTCATACCT AATGCCACAA
5941 ATGTGACAGG ACGCCGATGG GTACCGGACT TTAGGTCGAG CACAGTTCGG TAACGGAGAG
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6061 AGCTGGTTCC TGTGTTAGCT CGAGGGTAGA CACACGAGCT GATTGAACAT GGGTTGGGGG
6121 CCTCGAACCG TCGAGGACCC CATAGTACCT CGGAGACCAA GTAGGGCAGC CTATAGTTTG
6181 AAGCAGAACT ATTTCCGGGG GCGAGCCCTC ATCGTCTCTT CTGCGGAATG CTCAACACGC
6241 TAGGGACGTG AAGTCGATTC CTTGATGGT TATAAATCAA AGACTCAGAG TGCTGTCTGG
6301 AGCGTGAATC TAACGGTACG TATCTCGATT GCTCGGTCGC TTTTCGCACT CCGCGAAAGT
6361 TCGTACCGCT CATTCACTAG GTTGCGAAGC CTATGCTGAT ATATGAATCC AAAC TAGAGC
6421 AGGGCTCTTA AGATTGCGAG TTGTAAATAC TTAATACTCC AATCGGCTTT TACGTGCACC
6481 ACCGCGGGCG GCTGACAAGG GTCTCACATC GAGAAACAAG ACAGTTCCGG GCTGGAAGTA
6541 GCGCCGGCTA AGGAAGACGC CTGGTACGGC AGGACTATGA AACCAGTACA AAGGCAACAT
6601 CCTCACTTGG GTGAACGGAA ACGCAGTATT ATGGTTACTT TTTGGATACG TGAAACATAT
6661 CCTAGGTAG TCCTTAGACT TGGGAGTCTA TCACCCCTAG GGCCCATATC TGGAAATAGA
6721 CCCAGGTTG AATCCGATT TGGAGGTACG ATGGAACAGT CTGGGTGGGA CGTGCTTCAT
6781 TTATACCTCG CGCAGGCTGG ACCGAGGACC GCAAGGTGCG GCGGTGCACA AGCAATTGAC
6841 AACTAACCAC CGTGTATTCA TTATGGTACC AGGAACTTTA AGCCGAGTCA ATGAAGCTCG
6901 CATTACAGTG TTTACCGCAT CTTGCCGTTA CTCACAAACT GTGATCCACC ACAAGTCAAG
6961 CCATTGCCCTC TCTGACACGC CGTAAGAATT AATATGTAAA CTTTGCGCGG GTTGACTGCG

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7021 ATCCGTTTCTG TCTCGTCCGA GGGCACAATC CTATTCCCAT TTGTATGTTT AGCTAACTTC
7081 TACCCATCCC CCGAAGTTAA GTAGTTCGTG AGATGCCATG GAGGCTCTCG TTCATCCCGT
7141 GGGACATCAA GCTTCCCCTT GATAAAGCAC CCCGCTCGGG TGTAGCAGAG AAGACGCCTT
7201 CTGAATTGTG CAATCCCTCC ACCTTATCTA AGCTTGCTAC CAATAATTAG CATTTTTGCC
7261 TTGCGACAGA CCTCCTACTT AGATTGCCAC ACATTGAGCT AGTCAGTGAG CGATAAGCTT
7321 GACGCGCTTT CAAGGGTCGC GAGTACGTGA ACTAAGGCTC CGGACAGGAC TATATACTTG
7381 GGTTTGTACT CGCCCCGACA ACTGCAAACC TCAACTTTTT TAGATTATAT GGTTAGCCGA
7441 AGTTGCACGA GGTGGCGTCC GCGGACTGCT CCCCAGAGTG GGCTCTTTCA TCTGACAACG
7501 TGCAACCCCT ATCGCGGCCG ATTGTTTCTG CGGACGATGT TGTCTCATA GTTTGGGCAT
7561 GTTTCCTTG TAGGTGTGAA ACCACTTAGC TTCGCGCCGT AGTCCCAATG AAAAACCTAT
7621 GGACTTTGTT TTGGGTAGCA CCAGGAATCT GAACCGTGTG AATGTGGACG TCGCGCGCGT
7681 AGACCTTTAT CTCCGGTTCA AGCTAGGGAT GTGGCTGCAT GCTACGTTGT CACACCTACA
7741 CTGCTCGAAG TAAATATGCG AAGCGCGCGG CCTGGCCGGA GCGGTTCCGC GCCGCCACGT
7801 GTTCGTTAAC TGTTGATTGG TGGCACAATA GCAATATCGT AGTCCGTCAA ATTACAGTCT
7861 GTTATCCCGG GCGTTATGTG TCAAATGGCG TAGAACGGGA TTGACTGTTT GACGGTAGGG
7921 TGACCTAAGC CAGATGCTAC ACAATTAGGC TTGTACATAT TGTGTTAGA ACGCGGCTAC
7981 AATTAATACA TAACCTTATG TATCATAAC ATACGATTTA GGTGACACTA TAGAATACAC
8041 GGAATTAATT C (SEQ ID NO: 17) .

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[0112] Table 5. Features of the VMD2.IntEx.GFP.WPRE.pA plasmid sequence

Name	Type	Minimum	Maximum	Length	Direction
Randomly generated stuffer sequence	Stuffer	5,419	7,918	2,500	none
AphR (KanR)	CDS	4,303	5,118	816	forward
pBR322 rep origin	rep_origin	3,343	3,962	620	reverse
AAV2 ITR	LTR	2,659	2,779	121	reverse
bGH pA	polyA_signal	2,333	2,601	269	forward
WPRE	WPRE	1,725	2,314	590	forward
GFP	misc_feature	996	1,712	717	forward
Kozak	Kozak	990	995	6	forward
Exon	exon	937	989	53	forward
Intron	intron	814	936	123	forward
-585 to +38 VMD2 promoter	promoter	189	811	623	forward
AAV2 ITR	LTR	4	133	130	forward

AAV particles

[0113] The AAV vectors of the disclosure contain an AAV genome that has been derivatized for the purpose of administration to patients. Such derivatization is standard in the art and the invention encompasses the use of any known derivative of an AAV genome, and derivatives which could be generated by applying techniques known in the art. Derivatization of the AAV genome and of the AAV capsid are reviewed in Coura and Nardi (2007) *Virology Journal* 4: 99, and in Choi et al. and Wu et al., referenced above.

[0114] Derivatives of an AAV genome include any truncated or modified forms of an AAV genome which allow for expression of a transgene from a vector of the invention *in vivo*. It is possible to truncate the AAV genome significantly to include minimal viral sequence yet retain the above function. This is preferred for safety reasons to reduce the risk of

recombination of the vector with wild-type virus, and also to avoid triggering a cellular immune response by the presence of viral gene proteins in the target cell.

[0115] The following portions could therefore be removed in a derivative of the invention: one inverted terminal repeat (ITR) sequence, the replication (rep) and capsid (cap) genes. However, in some embodiments, derivatives may additionally include one or more rep and/or cap genes or other viral sequences of an AAV genome. Naturally occurring AAV integrates with a high frequency at a specific site on human chromosome 19, and shows a negligible frequency of random integration, such that retention of an integrative capacity in the vector may be tolerated in a therapeutic setting.

[0116] The AAV genome comprises packaging genes, such as rep and/or cap genes which encode packaging functions for an AAV particle. The rep gene encodes one or more of the proteins Rep78, Rep68, Rep52 and Rep40 or variants thereof. The cap gene encodes one or more capsid proteins such as VP1, VP2 and VP3 or variants thereof. These proteins make up the capsid of an AAV particle.

[0117] Where a derivative comprises capsid proteins i.e. VP1, VP2 and/or VP3, the derivative may be a chimeric, shuffled or capsid-modified derivative of one or more naturally occurring AAVs. In particular, the invention encompasses the provision of capsid protein sequences from different serotypes, clades, clones, or isolates of AAV within the same vector (i.e. a pseudotyped vector).

[0118] Chimeric, shuffled or capsid-modified derivatives are selected to provide one or more desired functionalities for the viral vector. Thus, these derivatives may display increased efficiency of gene delivery, decreased immunogenicity (humoral or cellular), an altered tropism range and/or improved targeting of a particular cell type compared to an AAV vector comprising a naturally occurring AAV genome, such as that of AAV2. Increased efficiency of gene delivery may be effected by improved receptor or co-receptor binding at the cell surface, improved internalization, improved trafficking within the cell and into the nucleus, improved uncoating of the viral particle and improved conversion of a single-stranded genome to double-stranded form. Increased efficiency may also relate to an altered tropism range or targeting of a specific cell population, such that the vector dose is not diluted by administration to tissues where it is not needed.

[0119] Chimeric capsid proteins include those generated by recombination between two or more capsid coding sequences of naturally occurring AAV serotypes. This may be performed for example by a marker rescue approach in which non-infectious capsid sequences of one serotype are co-transfected with capsid sequences of a different serotype, and directed selection is used to select for capsid sequences having desired properties. The

capsid sequences of the different serotypes can be altered by homologous recombination within the cell to produce novel chimeric capsid proteins.

[0120] Chimeric capsid proteins also include those generated by engineering of capsid protein sequences to transfer specific capsid protein domains, surface loops or specific amino acid residues between two or more capsid proteins, for example between two or more capsid proteins of different serotypes.

[0121] Shuffled or chimeric capsid proteins may also be generated by DNA shuffling or by error-prone PCR. Hybrid AAV capsid genes can be created by randomly fragmenting the sequences of related AAV genes e.g. those encoding capsid proteins of multiple different serotypes and then subsequently reassembling the fragments in a self-priming polymerase reaction, which may also cause crossovers in regions of sequence homology. A library of hybrid AAV genes created in this way by shuffling the capsid genes of several serotypes can be screened to identify viral clones having a desired functionality. Similarly, error prone PCR may be used to randomly mutate AAV capsid genes to create a diverse library of variants which may then be selected for a desired property.

[0122] The sequences of the capsid genes may also be genetically modified to introduce specific deletions, substitutions or insertions with respect to the native wild-type sequence. In particular, capsid genes may be modified by the insertion of a sequence of an unrelated protein or peptide within an open reading frame of a capsid coding sequence, or at the N- and/or C-terminus of a capsid coding sequence. The unrelated protein or peptide may advantageously be one which acts as a ligand for a particular cell type, thereby conferring improved binding to a target cell or improving the specificity of targeting of the vector to a particular cell population. The unrelated protein may also be one which assists purification of the viral particle as part of the production process, i.e. an epitope or affinity tag. The site of insertion will be selected so as not to interfere with other functions of the viral particle e.g. internalization, trafficking of the viral particle. The skilled person can identify suitable sites for insertion based on their common general knowledge. Particular sites are disclosed in Choi et al., referenced above.

[0123] The invention additionally encompasses the provision of sequences of an AAV genome in a different order and configuration to that of a native AAV genome. The invention also encompasses the replacement of one or more AAV sequences or genes with sequences from another virus or with chimeric genes composed of sequences from more than one virus. Such chimeric genes may be composed of sequences from two or more related viral proteins of different viral species.

[0124] AAV vectors of the invention include transcapsidated forms wherein an AAV genome or derivative having an ITR of one serotype is packaged in the capsid of a different serotype. AAV vectors of the invention also include mosaic forms wherein a mixture of unmodified capsid proteins from two or more different serotypes makes up the viral capsid. An AAV vector may also include chemically modified forms bearing ligands adsorbed to the capsid surface. For example, such ligands may include antibodies for targeting a particular cell surface receptor.

[0125] Thus, for example, AAV vectors of the invention include those with an AAV2 genome and AAV2 capsid proteins (AAV2/2), those with an AAV2 genome and AAV5 capsid proteins (AAV2/5) and those with an AAV2 genome and AAV8 capsid proteins (AAV2/8). An AAV vector of the invention may comprise a mutant AAV capsid protein. In one embodiment, an AAV vector of the invention comprises a mutant AAV8 capsid protein. Preferably the mutant AAV8 capsid protein is an AAV8 Y733F capsid protein.

[0126] Methods of making AAV viral particles of the disclosure will be known to one of skill in the art. An exemplary, but non-limiting method of preparing AAV viral particles of the disclosure is described below. For generation of a given AAV vector, three plasmids are required: one comprising the viral delivery vector encoding the nucleic acid sequence of interest to be delivered (i.e the nucleic acid sequence encoding BEST1), a plasmid encoding the *rep* and *cap* genes, and a third helper plasmid that contains the required adenoviral genes necessary for successful AAV generation. A promoter may be operably linked to each of the packaging genes. Specific examples of such promoters include the p5, p19 and p40 promoters (Laughlin et al. (1979) Proc. Natl. Acad. Sci. USA 76: 5567-5571). For example, the p5 and p19 promoters are generally used to express the rep gene, while the p40 promoter is generally used to express the cap gene. The plasmids are used to transfect suitable cells that are capable of replicating the AAV viral vector, transcribing and translating the AAV protein, and packaging the AAV viral vector into an AAV viral particle. Exemplary suitable cells comprise HEK293 cells. Post-transfection, the cells are collected and lysed. AAV particles can then be purified from the lysate through a variety of methods. Alternatively, AAV particles can be purified from the supernatant. For example, the lysate can be treated with Benzonase and clarified before applying to an iodixanol gradient comprised of 15%, 25%, 40% and 60% phases. The gradients can spun at 59,000rpm for 1 hour 30 minutes and the 40% fraction then withdrawn. This AAV phase can then purified and concentrated using an Amicon Ultra-15 100K filter unit.

Pharmaceutical compositions

[0127] The AAV vectors of the invention may be formulated into pharmaceutical compositions. These compositions may comprise, in addition to the medicament, a pharmaceutically acceptable carrier, diluent, excipient, buffer, stabilizer or other materials well known in the art. Such materials should be non-toxic and should not interfere with the efficacy of the active ingredient. The precise nature of the carrier or other material may be determined by the skilled person according to the route of administration, e.g. subretinal, direct retinal or intravitreal injection.

[0128] The pharmaceutical composition may be formulated as a liquid. Liquid pharmaceutical compositions may include a liquid carrier such as water, petroleum, animal or vegetable oils, mineral oil or synthetic oil. Physiological saline solution, magnesium chloride, dextrose or other saccharide solution, or glycols such as ethylene glycol, propylene glycol or polyethylene glycol may be included. In some cases, a surfactant, such as pluronic acid (PF68) 0.001% may be used.

[0129] For injection at the site of affliction, the active ingredient may be in the form of an aqueous solution which is pyrogen-free, and has suitable pH, isotonicity and stability. The skilled person is well able to prepare suitable solutions using, for example, isotonic vehicles such as Sodium Chloride Injection, Ringer's Injection or Lactated Ringer's Injection. Preservatives, stabilisers, buffers, antioxidants and/or other additives may be included as required.

[0130] Buffers may have an effect on the stability and biocompatibility of the viral vectors and vector particles of the disclosure following storage and passage through injection devices for AAV gene therapy. In some embodiments, the viral vectors and vector particles of the disclosure may be diluted in TMN 200 buffer to maintain biocompatibility and stability. TMN 200 buffer comprises 20 mM Tris (pH adjusted to 8.0), 1 mM MgCl₂ and 200 mM NaCl.

[0131] The determination of the physical viral genome titer comprises part of the characterization of the viral vector or viral particle. In some embodiments, determination of the physical viral genome titre comprises a step in ensuring the potency and safety of viral vectors and viral particles during gene therapy. In some embodiments, a method to determine the AAV titer comprises quantitative PCR (qPCR). There are different variables that can influence the results, such as the conformation of the DNA used as standard or the enzymatic digestion during the sample preparation. The viral vector or particle preparation whose titer may be measured may be compared against a standard dilution curve generated using a plasmid. In some embodiments, the plasmid DNA used in the standard curve is in the supercoiled conformation. In some embodiments, the plasmid DNA used in the standard

curve is in the linear conformation. Linearized plasmid can be prepared, for example by digestion with *HindIII* restriction enzyme, visualized by agarose gel electrophoresis and purified using the QIAquick Gel Extraction Kit (Qiagen) following manufacturer's instructions. Other restriction enzymes that cut within the plasmid used to generate the standard curve may also be appropriate. In some embodiments, the use of supercoiled plasmid as the standard increased the titre of the AAV vector compared to the use of linearized plasmid.

[0132] To extract the DNA from purified AAV vectors for quantification of AAV genome titer, two enzymatic methods can be used. In some embodiments, the AAV vector may be singly digested with DNase I. In some embodiments, the AAV vector may be double digested with DNase I and an additional proteinase K treatment. QPCR can then performed with the CFX Connect Real-Time PCR Detection System (BioRad) using primers and Taqman probe specific to the transgene sequence.

[0133] For delayed release, the medicament may be included in a pharmaceutical composition which is formulated for slow release, such as in microcapsules formed from biocompatible polymers or in liposomal carrier systems according to methods known in the art.

Dosages

[0134] As used herein, the term "Dnase resistant particle (DRP)" refers to AAV particles that are resistant to Dnase digestion, and are therefore thought to completely encapsulate and protect the AAV vector of the disclosure from Dnase digestion. AAV particles may also be quantified in terms of the total numbers of genome particles (gp) administered in a dose, or gp/mL, the number genome particles per milliliter (mL) of solution. As used herein, genome particle (gp) refers to AAV particles containing a copy of an AAV delivery vector (or AAV genome) of the disclosure. As used herein, the term genome content (GC) per mL refers to the number of viral genomes per mL of solution, and may be determined, for example, by qPCR as described above. The terms GC and VG (viral genomes) may be used synonymously to characterize AAV dosages and concentrations of the disclosure.

[0135] In some embodiments of the compositions of the disclosure, a composition comprising an AAV vector or an AAV vector is administered to a subject as a single dose.

[0136] In some embodiments of the compositions of the disclosure, a composition comprising an AAV vector or an AAV vector may be formulated as a liquid suspension wherein the AAV vectors are suspended in a pharmaceutically-acceptable carrier. In some embodiments, compositions of the disclosure may comprise a plurality of AAV vectors at a

concentration of $1-2 \times 10^9$, $1-2 \times 10^{10}$, $1-2 \times 10^{11}$, $1-2 \times 10^{12}$ or $1-2 \times 10^{13}$ genome particles (gp) per mL. In some embodiments, compositions of the disclosure may comprise a plurality of AAV vectors at a concentration of 5×10^{11} DRP/mL, 1.5×10^{12} DRP/mL, 5×10^{12} DRP/mL, 1.2×10^{12} DRP/mL, 4.5×10^{12} DRP/mL, 1.2×10^{13} DRP/mL, 1.5×10^{13} DRP/mL or 5×10^{13} DRP/ 1.2×10^{12} DRP/mL. In some embodiments, compositions of the disclosure may comprise a plurality of AAV vectors at a concentration of 5×10^{12} DRP per mL. In some embodiments, compositions of the disclosure may comprise a plurality of AAV vectors at a concentration of 1.5×10^{13} DRP per mL. Thus, to administer a dose of AAV vector of about 2×10^{10} gp, for example, a single injection of about 10 microliters of a pharmaceutical composition having a concentration of about 2×10^{12} gp per mL will achieve the desired dose in vivo.

[0137] In some embodiments of the compositions of the disclosure, a composition comprising an AAV vector or an AAV vector may comprise a volume of between 1 and 500 μ L, inclusive of the endpoints. In some embodiments of the compositions of the disclosure, a composition comprising an AAV vector or an AAV vector may comprise a volume of between 10-500, 50-500, 100-500, 200-500, 300-500, 400-500, 50-250, 100-250, 200-250, 50-150, 1-100 or 1-10 μ L, inclusive of the endpoints for each range. In some embodiments of the compositions of the disclosure, a composition comprising an AAV vector or an AAV vector may comprise a volume of 1, 2, 5, 10, 50, 100, 150, 200, 250, 300, 350, 400, 450, 500 μ L or any number of microliters in between. In some embodiments, a composition comprising an AAV vector or an AAV vector may comprise 100 μ L.

[0138] In some embodiments of the compositions of the disclosure, an entire volume of a composition comprising an AAV vector or an AAV vector may be injected in a single injection. In some embodiments, a portion of a volume of a composition comprising an AAV vector or an AAV vector may be injected in a single injection. In some embodiments, a first portion of a volume of a composition comprising an AAV vector or an AAV vector may be injected in a first single injection and a second portion of a volume of a composition comprising an AAV vector or an AAV vector may be injected in a second single injection.

[0139] In some embodiments of the compositions of the disclosure, a composition comprising an AAV vector or an AAV vector is administered at a dosage of at least 2×10^7 , 2×10^8 , 5×10^8 , 1.5×10^9 , 2×10^9 , 5×10^9 , 2×10^{10} , 5×10^{10} , 6×10^{10} , 1.2×10^{11} , 2×10^{11} , 4.5×10^{11} , 5×10^{11} , 1.2×10^{12} , 1.5×10^{12} , 2×10^{12} or 5×10^{12} gp per eye. In some embodiments, a composition comprising an AAV vector or an AAV vector is administered at a dosage of about 5×10^{10} , 1.5×10^{11} , 5×10^{11} or 1.5×10^{11} gp per eye. In some embodiments, a composition comprising an AAV vector or an AAV vector is administered at a dosage of about 5×10^{11} DRP per eye, by subretinal injection. In some embodiments, a composition comprising an AAV vector or

an AAV vector is administered at a dosage of about 2×10^{10} gp per eye, by subretinal injection. In some embodiments, a composition comprising an AAV vector or an AAV vector is administered at a dosage of about 5×10^{10} gp per eye, by subretinal injection. In some embodiments, the AAV vector is administered at a dosage of about 6×10^{10} gp per eye, by subretinal injection. In some embodiments, a composition comprising an AAV vector or an AAV vector is administered at a dosage of about 1.5×10^{11} gp per eye, by subretinal injection. In some embodiments, a composition comprising an AAV vector or an AAV vector is administered at a dosage of about 2×10^{11} gp per eye, by subretinal injection. In some embodiments, a composition comprising an AAV vector or an AAV vector is administered at a dosage of about 5×10^{11} gp per eye, by subretinal injection. In some embodiments, a composition comprising an AAV vector or an AAV vector is administered at a dosage of about 1.5×10^{12} gp per eye, by subretinal injection.

[0140] Dosages or volumes may be calculated based on allometric scaling between species based on vitreal volume. “Allometry”, as used herein, refers to the changes in organisms with respect to body size. Some factors to take into account when comparing species include body volume, surface area, metabolic rate, and unique anatomical, physiological or biochemical processes. The human equivalent dose can be normalized to body surface area, body weight or a combination of surface area and weight. Other factors may also be taken into account.

Delivery

[0141] The viral vectors of the invention may be administered to the eye of a subject by subretinal, direct retinal, suprachoroidal or intravitreal injection. A skilled person will be familiar with and well able to carry out individual subretinal, direct retinal or intravitreal injections.

[0142] Subretinal injections are injections into the subretinal space, i.e. underneath the neurosensory retina. During a subretinal injection, the injected material is directed into, and creates a space between, the photoreceptor cell and retinal pigment epithelial (RPE) layers. When the injection is carried out through a small retinotomy, a retinal detachment may be created. The detached, raised layer of the retina that is generated by the injected material is referred to as a “bleb”. The hole created by the subretinal injection must be sufficiently small that the injected solution does not significantly reflux back into the vitreous cavity after administration. Such reflux would be particularly problematic when a medicament is injected, because the effects of the medicament would be directed away from the target zone. Preferably, the injection creates a self-sealing entry point in the neurosensory retina, i.e. once

the injection needle is removed, the hole created by the needle reseals such that very little or substantially no injected material is released through the hole.

[0143] To facilitate this process, specialist subretinal injection needles are commercially available (e.g. DORC 41G Teflon subretinal injection needle, Dutch Ophthalmic Research Center International BV, Zuidland, The Netherlands). These are needles designed to carry out subretinal injections.

[0144] Alternatively, subretinal injections can be performed by delivering the composition comprising AAV particles under direct visual guidance using an operating microscope (Leica Microsystems, Germany). One exemplary approach is that of using a scleral tunnel approach through the posterior pole to the superior retina with a Hamilton syringe and 34-gauge needle (ESS labs, UK). Alternatively, sub-retinal injections can be performed using an anterior chamber paracentesis with a 33G needle prior to the subretinal injection using a WPI syringe and a beveled 35G-needle system (World Precision Instruments, UK). An additional alternative is a WPI Nanofil Syringe (WPI, part #NANOFIL) and a 34 gauge WBI Nanofil needle (WPI, part # NF34BL-2).

[0145] Vectors or compositions of the disclosure may be administered via suprachoroidal injection. Any means of suprachoroidal injection is envisaged as a potential delivery system for a vector or a composition of the disclosure. Suprachoroidal injections are injections into the suprachoroidal space, which is the space between the choroid and the sclera. Injection into the suprachoroidal space is thus a potential route of administration for the delivery of compositions to proximate eye structures such as the retina, retinal pigment epithelium (RPE) or macula. In some embodiments, injection into the suprachoroidal space is done in an anterior portion of the eye using a microneedle, microcannula, or microcatheter. An anterior portion of the eye may comprise or consist of an area anterior to the equator of the eye. The vector composition or AAV viral particles may diffuse posteriorly from an injection site via a suprachoroidal route. In some embodiments, the suprachoroidal space in the posterior eye is injected directly using a catheter system. In this embodiment, the suprachoroidal space may be catheterized via an incision in the pars plana. In some embodiments, an injection or an infusion via a suprachoroidal route traverses the choroid, Bruch's membrane and/or RPE layer to deliver a vector or a composition of the disclosure to a subretinal space. In some embodiments, including those in which a vector or a composition of the disclosure is delivered to a subretinal space via a suprachoroidal route, one or more injections is made into at least one of the sclera, the pars plana, the choroid, the Bruch's membrane, and the RPE layer. In some embodiments, including those in which a vector or a composition of the disclosure is delivered to a subretinal space via a suprachoroidal route, a two-step procedure

is used to create a bleb in a suprachoroidal or a subretinal space prior to delivery of a vector or a composition of the disclosure.

[0146] In those embodiments where mice are injected, animals can be anaesthetized by intraperitoneal injection containing ketamine (40-80mg/kg) and xylazine (1-10mg/kg) and pupils fully dilated with tropicamide eye drops (Mydraticum 1%, Bausch & Lomb, UK) and phenylephrine eye drops (phenylephrine hydrochloride 2.5%, Bausch & Lomb, UK). Proxymetacaine eye drops (proxymetacaine hydrochloride 0.5%, Bausch & Lomb, UK) can also applied prior to sub-retinal injection. Post-injection, chloramphenicol eye drops can applied (chloramphenicol 0.5%, Bausch & Lomb, UK) and anaesthesia reversed with atipamezole (2mg/kg) and carbomer gel applied (Viscotears, Novartis, UK) to prevent cataract formation.

[0147] Unless damage to the retina occurs during the injection, and as long as a sufficiently small needle is used, substantially all injected material remains localized between the detached neurosensory retina and the RPE at the site of the localized retinal detachment (i.e. does not reflux into the vitreous cavity). Indeed, the typical persistence of the bleb over a short time frame indicates that there is usually little escape of the injected material into the vitreous. The bleb may dissipate over a longer time frame as the injected material is absorbed.

[0148] Visualizations of the eye, in particular the retina, for example using optical coherence tomography, may be made pre-operatively.

[0149] The AAV vectors of the invention may be delivered with increased accuracy and safety by using a two-step method in which a localized retinal detachment is created by the subretinal injection of a first solution. The first solution does not comprise the vector. A second subretinal injection is then used to deliver the medicament comprising the vector into the subretinal fluid of the bleb created by the first subretinal injection. Because the injection delivering the medicament is not being used to detach the retina, a specific volume of solution may be injected in this second step. An AAV vector of the invention may be delivered by: (a) administering a solution to the subject by subretinal injection in an amount effective to at least partially detach the retina to form a subretinal bleb, wherein the solution does not comprise the vector; and (b) administering a medicament composition by subretinal injection into the bleb formed by step (a), wherein the medicament comprises the vector.

EXAMPLES

Example 1: Bestrophin-1 protein in HEK293 cells using the CAG promoter

[0150] HEK293 cells were transduced with an AAV2/2 vector containing the CAG promoter driving Best1 expression with a WPRE (AAV2/2 CAG.BEST1.WPRE.pA, Figure 3) and without a WPRE (AAV2/2 CAG.BEST1.pA), and the expression and localization of Bestrophin-1 protein was examined. In Figure 6, transduced HEK293 cells were stained with Hoechst and an anti-human Bestrophin-1 (hBEST1 or huBEST1) antibody. Bestrophin-1 protein was found throughout the cytosol when compared to untransduced control cells.

[0151] Bestrophin-1 expression in HEK293 cells was quantified from Western Blot (Figure 7). In Figure 7A, sample 1 was the AAV2/2 CAG.hBEST1.pA vector; sample 2 was the AAV2/2 CAG.hBEST1.WPRE.pA vector and sample 3 was a negative control. Plasmid-transfected HEK293 cells were used as a positive control. In Figure 7B, quantification showed that AAV2/2 CAG.BEST1.WPRE.pA (n=9) showed an approximately 4-fold increase in Bestrophin-1 expression over AAV2/2 CAG.BEST1.pA (n=9) ($p<0.01$ by One-way ANOVA with Tukey's Multiple Comparisons Test) and a statistically significant increase over un-transduced control cells (n=8) ($p<0.001$) was seen. Although Bestrophin-1 expression was seen in the AAV2/2 CAG.BEST1.pA cells, this was not statistically significant over un-transduced cells. Error bars = $\pm$ SEM, and *** indicates $p<0.001$ when compared to un-transduced control.

[0152] HEK293 cells expressing Bestrophin-1 were additionally assayed with whole-cell patch clamp recording. Figure 8A shows the Current (I) /Voltage (V) plots of HEK293 cells transduced with AAV2/2 CAG.BEST1.pA, AAV2/2 CAG.BEST1.WPRE.pA and AAV2/2 CAG.GFP.WPRE.pA vectors as well as an untransduced control. Figure 8B shows the current waveforms, and chord conductance is shown in Figure 9.

Example 2: Bestrophin-1 protein in cultured ARPE19 cells using the VMD2 promoter

[0153] Appropriately differentiated ARPE19 are known to have gene expression profiles similar to those of native retinal pigment epithelium (RPE) cells, and can be used as an alternative to native RPE cells to test gene expression. Differentiated ARPE19 cells were used to test the ability of the VMD2 and CAG promoters to drive BEST1 expression in RPE cells, and to test the effect of the intron-exon (IntEx) sequence on expression from the VMD2 promoter.

[0154] ARPE19 cells were transfected and assayed for BEST1 expression using the protocol outlined in Figure 10B. ARPE19 cells were grown in differentiation medium (DMEM with 4.5 g/l glucose, L-glutamine, and 1 mM sodium pyruvate supplemented with 1% fetal bovine serum (FBS) for 1-4 months at 37°C and 5% CO₂ in 96 well plates. Differentiated ARPE19 cells were then transfected with either pCAG.BEST1.WPRE (CAG promoter),

pVMD2.BEST1.WPRE (VMD2 promoter), or pVMD2.IntEx.BEST1.WPRE (VMD2 promoter and an intron-exon construct) at 3.8×10^{10} number of copies of each plasmid per well. Cells treated with TransIT-LT1 reagent alone and cells without transfection reagent or plasmid served as negative controls. Cells were then cultured for 2 days at 37°C, before being fixed and stained with Anti-hBest1 and Anti-ZO1 (also called ZO-1, or zona occludens-1, or tight junction protein 1, a protein located on the cytoplasmic membrane surface of intercellular tight junctions). Figures 11-13 show BEST1 expression in differentiated ARPE19 cells transfected with the three vectors encoding BEST1 and an untransfected control.

[0155] In ARPE19 cells that were differentiated for one month before transfection, the untransfected cells showed no expression. In contrast, both the pCAG.BEST1.WPRE and pVMD2.IntEx.BEST1.WPRE were able to drive the expression of BEST1 protein in differentiated ARPE19 cells (see Figure 11A, contrast the first, second and fourth rows). pVMD2.BEST1.WPRE (no exon-intron) was also able to drive the expression of BEST1 in 1 month differentiated ARPE19 cells (Figure 11B), although this construct seemed to express BEST1 at lower levels than the construct with the intron-exon sequence (pVMD2.IntEx.BEST1.WPRE). In ARPE19 cells that were differentiated for three months, similar results were obtained: pCAG.BEST1.WPRE, pVMD2.IntEx.BEST1.WPRE and pVMD2.BEST1.WPRE were all able to drive expression of BEST1 protein, although the expression with the CAG promoter was higher than with the VMD2 promoter and, with the VMD2 promoter, the intron-exon sequence improves the expression (contrast the first row of Figure 12A, the untransfected control, with Figure 12B).

[0156] ARPE19 cells were transduced and assayed for BEST1 expression using the protocol outlined in Figure 10. Differentiated ARPE19 cells were pre-treated with 400 nM doxorubicin before transduction. This drug has been proved to improve AAV2 transduction efficiency in several *in vitro* models. Four hours after the treatment, cells were transduced with the different viral constructs at different multiplicities of infection (MOIs).

[0157] ARPE19 cells differentiated for 4 months, pre-treated with 400 nM doxorubicin and transduced with AAV2/2.CAG.GFP.WPRE and AAV2/2.VMD2.InEx.GFP.WPRE at 2, 4 and 8×10^4 gp/cell showed higher GFP fluorescence compared to transduced cells without pre-treatment with doxorubicin 10 days after transduction (contrast top and bottom row of each panel of Figure 13A) AAV2/2.CAG.GFP.WPRE and B) AAV2/2.VMD2.InEx.GFP.WPRE). GFP fluorescence was not detected in untransduced cells, used as negative controls (first column of Figure 13A and B).

[0158] In ARPE19 cells differentiated for 4 months, pre-treated with 400 nM doxorubicin and transduced with AAV2/2.CAG.BEST1.WPRE and AAV2/2.VMD2.IntEx.BEST1.WPRE at 1 and 4 x 10⁴ gp/cell, BEST1 expression could be detected by immunostaining with anti-hBEST1 (red, third column, second to fifth row of Figure 14) compared to the untransduced control (first row, Figure 14) 10 days after transduction.

Example 3: 4/8 week in vivo pilot study in mice

[0159] The ability of the VMD2.BEST1.WPRE and VMD2.IntEx.BEST1.WPRE constructs to drive the expression BEST1 was assayed *in vivo*. The protocol of the 4/8 week *in vivo* pilot study is shown in Figure 15. C57BL/6 mice (6 per group) were injected bilaterally with either a sham injection, AAV2/2 VMD2.BEST1.WPRE or AAV2/2 VMD2.IntEx.BEST1.WPRE AAV viral particles. 1 µL of AAV solution was injected subretinally with a 34 gauge Nanofil needle (WPI #NF34BL-2) at 1x10⁹ GC/µL/eye. Eyes were imaged using optical coherence tomography at 4 and 8 weeks to assess for retinal thinning (toxicity), and 3 animals were sacrificed at each time point to assay BEST1 protein expression by immunohistochemistry and Western Blot.

[0160] OCT imaging at 4 and 8 weeks showed that neither VMD2 construct showed photoreceptor toxicity when compared to the sham treatment (Figures 16-18).

[0161] Three animals were sacrificed at both the 4- and 8-week time points, and BEST1 protein expression was further characterized by western blot (Figure 21) and immunohistochemistry (Figure 19). Figure 19 shows immunohistochemistry results for eyes four weeks post injection, while Figure 20 shows immunohistochemistry results for eyes eight weeks post injection. Eyes were stained with anti-BEST1 (green) and anti-Rhodopsin (red), which marks photoreceptor cells, and DAPI (Figures 19 and 20). BEST1 protein expression was observed from VMD2.BEST1.WPRE.pA and VMD2.IntEx.BEST1.WPRE.pA. VMD2 promoter driven BEST1 expression localized to the junction of the RPE layer and photoreceptor outer layer. Western Blot on dissected RPE/choroid complex tissue from four week injected eyes shows protein expression (Figure 21).

Example 4: 4/13 week in vivo proof of concept study in mice

[0162] An additional 4 and 13 week *in vivo* proof of concept (PoC) study was carried out in mice to confirm the results of the pilot study, assay the effect of AAV viral particle dosage, and look at the effects at later time points post AAV injection. An outline of the protocol for the 4/13 week Proof of Concept study is set forth in Figure 22. C57BL/6 mice (12 per cohort)

were bilaterally injected with VMD2.IntEx.BEST1.WPRE or VMD2.BEST1.WPRE.pA AAV particles at either 1×10^8 GC/ μ L/eye or 1×10^9 GC/ μ L/eye, or with a sham injection. 1 μ L of AAV solution was injected subretinally with a 34 gauge Nanofil needle (WPI #NF34BL-2). Eyes were imaged with OCT at 4 and 13 weeks post injection. Four mice were sacrificed four weeks post injection, and the remaining eight at 13 weeks post injection, and BEST1 expression was characterized by immunohistochemistry and Western blot.

[0163] OCT imaging at 4 weeks and 13 weeks showed that neither VMD2 construct (with or without the intron-exon sequence) at either the high dose (1×10^9 GC/eye) or the low dose (1×10^8 GC/eye) showed toxicity as evidenced by retinal thinning when compared to the sham control (Figure 23). Staining with anti-BEST1 (huBEST1 in Figure 24) and anti-Rhodopsin showed that VMD2 driven BEST1 localized to the RPE layer, with a trend of more BEST1 expression in VMD2.IntEx.BEST1.WPRE injected eyes. Western Blot on pooled dissected RPE/choroid complex tissue from four week injected eyes (4) shows protein expression (Figure 25B).

Example 5: Good Laboratory Practice (GLP) toxicity assessment study in mice

[0164] The safety and expression of BEST1 AAV over longer periods of time is verified in mice with a Good Laboratory Practice (GLP) toxicity study in mice. An outline of the study is set forth in Figure 27. Cohorts of 8 male and 8 female mice are injected subretinally and bilaterally with a low (5.0×10^8 GC/eye), medium (1.5×10^9 GC/eye) or high (5.0×10^9 GC/eye) dose of VMD2.IntEx.BEST1.WPRE AAV particles. Using allometric volume scaling, the high mouse dose is equivalent to a dose of 100 μ L at 5×10^{12} GC/mL/eye in humans. Mice are evaluated and sacrificed at 4 weeks and 26 weeks. Eyes are assessed with an ophthalmic examination, tonometry to measure intraocular pressure (IOP), OCT for retinal thickness (predose, and the end of 4 and 13 weeks). Post sacrifice, necropsies assess organ weights and tissues such as the left eye, brain, heart, skeletal muscle, lung, liver, kidney, testes and ovary are collected for qPCR. Histopathological evaluations are carried out, and tissues are reserved, .e.g. by storage in formalin, for additional immunohistochemistry. Alternatively, or in addition, groups of 4 mice are injected with dosages of 2×10^9 GC/eye and 5×10^9 GC/eye of VMD2.IntEx.BEST1.WPRE AAV particles and evaluated at 4 weeks to optimize protocols for the larger toxicity study (see Figure 28 for an outline).

INCORPORATION BY REFERENCE

[0165] Every document cited herein, including any cross referenced or related patent or application is hereby incorporated herein by reference in its entirety unless expressly

excluded or otherwise limited. The citation of any document is not an admission that it is prior art with respect to any invention disclosed or claimed herein or that it alone, or in any combination with any other reference or references, teaches, suggests or discloses any such invention. Further, to the extent that any meaning or definition of a term in this document conflicts with any meaning or definition of the same term in a document incorporated by reference, the meaning or definition assigned to that term in this document shall govern.

OTHER EMBODIMENTS

[0166] While particular embodiments of the disclosure have been illustrated and described, various other changes and modifications can be made without departing from the spirit and scope of the disclosure. The scope of the appended claims includes all such changes and modifications that are within the scope of this disclosure.

CLAIMS

What is claimed is:

1. A composition comprising:
a nucleic acid sequence comprising:
(a) a sequence encoding a vitelliform macular dystrophy-2 (VMD2) promoter, and
(b) a sequence encoding a Bestrophin-1 (BEST1) protein.
2. The composition of claim 1, wherein the sequence encoding the VMD2 promoter encodes a human VMD2 promoter.
3. The composition of claim 1 or 2, wherein the sequence encoding the BEST1 protein encodes a human BEST1 protein.
4. The composition of any one of claims 1-3, wherein the sequence encoding the BEST1 protein comprises a coding sequence.
5. The composition of any one of claims 1-4, wherein the sequence encoding the BEST1 protein comprises a cDNA sequence.
6. The composition of any one of claims 1-5, wherein the nucleic acid sequence further comprises:
(c) a sequence encoding a posttranscriptional regulatory element (PRE).
7. The composition of claim 7, wherein the sequence encoding the PRE comprises a sequence isolated or derived from a woodchuck hepatitis virus (WPRE).
8. The composition of any one of claims 1-7, wherein the nucleic acid sequence further comprises:
(d) a sequence encoding a polyadenylation (polyA) signal.
9. The composition of any one of claims 1-8, wherein the nucleic acid sequence further comprises:
(e) a sequence encoding a 5' untranslated region.

10. The composition of any one of claims 1-8, wherein the nucleic acid sequence further comprises:
- (f) a sequence encoding an intron, and
 - (g) a sequence encoding an exon,
- wherein the sequence encoding the intron and the sequence encoding the exon are operably linked.
11. The composition of claim 9 or 10,
- wherein the sequence encoding the intron is located between the sequence encoding the VMD2 promoter and the sequence encoding the exon,
 - wherein the sequence encoding the exon is located between the sequence encoding the intron and the sequence encoding the 5' UTR, and
 - wherein the sequence encoding the intron is spliced by a mammalian cell.
12. The composition of any one of claims 9-11, wherein the sequence encoding the 5' UTR comprises a sequence encoding a Kozak sequence or a portion thereof.
13. The composition of claim 12, wherein the sequence encoding a Kozak sequence has at least 50% identity to the nucleic acid sequence of GCCRCCATGG.
14. The composition of claim 12, wherein the sequence encoding a Kozak sequence comprises or consists of the nucleic acid sequence of GGCACCATGA.
15. The composition of any one of claims 2-14, wherein the sequence encoding the human VMD2 promoter comprises or consists of
- ```

1 AATTCTGTCA TTTTACTAGG GTGATGAAAT TCCCAAGCAA CACCATCCTT TTCAGATAAG
61 GGCAGTGAAG CTGAGAGAGG AGCTGAAACC TACCCGGGGT CACCACACAC AGGTGGCAAG
121 GCTGGGACCA GAAACCAGGA CTGTTGACTG CAGCCCGGTA TTCATTCTTT CCATAGCCCA
181 CAGGGCTGTC AAAGACCCCA GGGCCTAGTC AGAGGCTCCT CCTTCCTGGA GAGTTCCTGG
241 CACAGAAGTT GAAGCTCAGC ACAGCCCCCT AACCCCAAC TCTCTCTGCA AGGCCTCAGG
301 GGTGAGAACA CTGGTGGAGC AGATCCTTTA GCCTCTGGAT TTTAGGGCCA TGGTAGAGGG
361 GGTGTTGCCC TAAATTCCAG CCCTGGTCTC AGCCCAACAC CCTCCAAGAA GAAATTAGAG
421 GGGCCATGGC CAGGCTGTGC TAGCCGTTGC TTCTGAGCAG ATTACAAGAA GGGACTAAGA
481 CAAGGACTCC TTTGTGGAGG TCCTGGCTTA GGGAGTCAAG TGACGGCGGC TCAGCACTCA
541 CGTGGGCAGT GCCAGCCTCT AAGAGTGGGC AGGGGCACTG GCCACAGAGT CCCAGGGAGT
601 CCCACCAGCC TAGTCGCCAG ACC (SEQ ID NO: 1) .

```
16. The composition of any one of claims 3-15, wherein the sequence encoding the human BEST1 protein comprises or consists of
- ```

1 ATGACCATCA CTTACACAAG CCAAGTGGCT AATGCCCGCT TAGGCTCCTT CTCCCGCCTG
61 CTGCTGTGCT GGCGGGGCAG CATCTACAAG CTGCTATATG GCGAGTTCTT AATCTTCCTG

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121 CTCTGCTACT ACATCATCCG CTTTATTTAT AGGCTGGCCC TCACGGAAGA ACAACAGCTG
181 ATGTTTGAGA AACTGACTCT GTATTGCGAC AGCTACATCC AGCTCATCCC CATTTCCTTC
241 GTGCTGGGCT TCTACGTGAC GCTGGTCGTG ACCCGCTGGT GGAACCAGTA CGAGAACCTG
301 CCGTGGCCCG ACCGCCTCAT GAGCCTGGTG TCGGGCTTCG TCGAAGGCAA GGACGAGCAA
361 GGCCGGCTGC TGGCGGCAC GTCATCCGC TACGCCAACC TGGGCAACGT GCTCATCCTG
421 CGCAGCGTCA GCACGCGAGT CTACAAGCGC TTCCCCAGCG CCCAGCACCT GGTGCAAGCA
481 GGCTTTTATGA CTCCGGCAGA ACACAAGCAG TTGGAGAAAC TGAGCCTACC ACACAACATG
541 TTCTGGGTGC CCTGGGTGTG GTTTGCCAAC CTGTCAATGA AGGCGTGGCT TGGAGGTCGA
601 ATCCGGGACC CTATCCTGCT CCAGAGCCTG CTGAACGAGA TGAACACCTT GCGTACTCAG
661 TGTGGACACC TGTATGCCTA CGACTGGATT AGTATCCAC TGGTGTATAC ACAGGTGGTG
721 ACTGTGGCGG TGTACAGCTT CTTCTGACT TGTCTAGTTG GCGGCAGTT TCTGAACCCA
781 GCCAAGGCCT ACCCTGGCCA TGAGCTGGAC CTCGTTGTGC CCGTCTTCAC GTTCCTGCAG
841 TTCTTCTTCT ATGTTGGCTG GCTGAAGGTG GCAGAGCAGC TCATCAACCC CTTTGGAGAG
901 GATGATGATG ATTTTGAGAC CAACTGGATT GTCGACAGGA ATTTGCAGGT GTCCCTGTTG
961 GCTGTGGATG AGATGCACCA GGACCTGCCT CGGATGGAGC CGGACATGTA CTGGAATAAG
1021 CCCGAGCCAC AGCCCCCTA CACAGCTGCT TCCGCCAGT TCCGTCGAGC CTCCTTTATG
1081 GGCTCCACCT TCAACATCAG CCTGAACAAA GAGGAGATGG AGTTCCAGCC CAATCAGGAG
1141 GACGAGGAGG ATGCTCACGC TGGCATCATT GGCCGCTTCC TAGGCCTGCA GTCCCATGAT
1201 CACCATCCTC CCAGGGCAAA CTCAAGGACC AAACACTGT GGCCTAAGAG GGAATCCCTT
1261 CTCCACGAGG GCCTGCCCAA AAACCACAAG GCAGCCAAAC AGAACGTTAG GGGCCAGGAA
1321 GACAACAAGG CCTGGAAGCT TAAGGCTGTG GACGCCTTCA AGTCTGCCCC ACTGTATCAG
1381 AGGCCAGGCT ACTACAGTGC CCCACAGACG CCCCTCAGCC CCACTCCCAT GTTCTTCCCC
1441 CTAGAACCAT CAGCGCCGTC AAAGCTTCAC AGTGTACAG GCATAGACAC CAAAGACAAA
1501 AGCTTAAAGA CTGTGAGTTC TGGGGCCAAG AAAAGTTTGT AATTGCTCTC AGAGAGCGAT
1561 GGGGCCTTGA TGGAGACCC AGAAGTATCT CAAGTGAGGA GGAAAACCTGT GGAGTTTAAC
1621 CTGACGGATA TGCCAGAGAT CCCCAGAAAAT CACCTCAAAG AACCTTTGGA ACAATCACCA
1681 ACCAACATAC ACACTACACT CAAAGATCAC ATGGATCCTT ATTGGGCCTT GGAAAACAGG
1741 GATGAAGCAC ATTCCTAA (SEQ ID NO: 3) .

```

17. The composition of any one of claims 8-16, wherein the sequence encoding the poly A signal comprises a sequence isolated or derived from a mammalian gene.

18. The composition of claim 17, wherein the sequence encoding the poly A signal comprises a sequence isolated or derived from a mammalian Bovine Growth Hormone (BGH) gene.

19. The composition of any one of claims 10-18, wherein the sequence encoding the exon comprises a sequence isolated or derived from a mammalian gene.

20. The composition of claim 19, wherein the sequence encoding the exon comprises a sequence isolated or derived from a rabbit (*Oryctolagus cuniculus*) beta globin gene.

21. The composition of any one of claims 10-20, wherein the sequence encoding the intron comprises a non-naturally occurring sequence.

22. The composition of claim 21, wherein the sequence encoding the intron comprises a fusion sequence.

23. The composition of 22, wherein the sequence encoding the intron comprises a sequence encoding a splice donor site, and a sequence encoding a splice branch point and acceptor site.
24. The composition of claim 23, wherein the sequence encoding the splice donor site comprises a sequence isolated or derived from a vertebrate gene.
25. The composition of claim 24, wherein the sequence encoding the splice donor site comprises a sequence isolated or derived from a chicken (*Gallus gallus*) beta actin gene (CBA).
26. The composition of any one of claims 23-25, wherein the sequence encoding the splice branch point and acceptor site comprises a sequence isolated or derived from a vertebrate gene.
27. The composition of claim 26, wherein the sequence encoding the splice branch point and acceptor site comprises a sequence isolated or derived from a rabbit (*Oryctolagus cuniculus*) beta globin gene.
28. A vector comprising a composition of any one of claims 1-27.
29. The vector of claim 28, wherein the vector is a plasmid.
30. A delivery vector comprising the vector of claim 28 or 29.
31. The delivery vector of claim 30, wherein the delivery vector is a viral delivery vector.
32. The delivery vector of claim 31, wherein the delivery vector comprises a single stranded viral genome.
33. The delivery vector of claim 31, wherein the delivery vector comprises a double stranded viral genome.
34. The delivery vector of any one of claims 30-33, wherein the delivery vector comprises an RNA molecule.

35. The delivery vector of any one of claims 30-34, wherein the delivery vector comprises a sequence isolated or derived from an adeno-associated virus (AAV) vector.
36. The delivery vector of claim 35, wherein the delivery vector comprises a sequence isolated or derived from an AAV vector of serotype AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11 or any combination thereof.
37. The delivery vector of claim 35, wherein the delivery vector comprises a sequence isolated or derived from an AAV vector of serotype AAV2.
38. The delivery vector of claim 35, wherein the delivery vector comprises a sequence isolated or derived from an AAV vector of serotype AAV8.
39. The delivery vector of claim 37 or 38, wherein the delivery vector comprises a sequence encoding a first inverted terminal repeat (ITR) and a second ITR isolated or derived from an AAV vector of serotype AAV2 and a sequence encoding a viral gene isolated or derived from an AAV vector of serotype AAV2.
40. The delivery vector of claim 37 or 38, wherein the delivery vector comprises a sequence encoding a first inverted terminal repeat (ITR) and a second ITR isolated or derived from an AAV vector of serotype AAV8 and a sequence encoding a viral gene isolated or derived from an AAV vector of serotype AAV8.
41. The delivery vector of claim 37 or 38, wherein the delivery vector comprises a sequence encoding a first inverted terminal repeat (ITR) and a second ITR isolated or derived from an AAV vector of serotype AAV2 and a sequence encoding a viral gene isolated or derived from an AAV vector of serotype AAV8.
42. A pharmaceutical composition comprising a composition of any one of claims 1-27 and a pharmaceutically-acceptable carrier.
43. A pharmaceutical composition comprising a vector of claim 28 or 29 and a pharmaceutically-acceptable carrier.

44. A pharmaceutical composition comprising a delivery vector of any one of claims 30-41 and a pharmaceutically-acceptable carrier.
45. A cell comprising a composition of any one of claims 1-27.
46. A cell comprising a vector of claim 28 or 29.
47. A cell comprising a delivery vector of any one of claims 30-41.
48. A cell comprising a pharmaceutical composition of any one of claims 42-44.
49. The cell of any one of claims 45-48, wherein the cell is a mammalian cell.
50. The cell of claim 49, wherein the mammalian cell is a non-human primate cell, a rodent cell, a mouse cell, a rat cell or a rabbit cell.
51. The cell of any one of claims 45-49, wherein the cell is a human cell.
52. The cell of claim 51, wherein the human cell is a neuronal cell, a glial cell, a retinal cell, a photoreceptor cell, a rod cell, a cone cell or a cuboidal cell of the retinal pigment epithelium (RPE).
53. The cell of claim 51, wherein the human cell is a photoreceptor cell.
54. The cell of claim 51, wherein the human cell is an HEK293 cell or an ARPE19 cell.
55. The cell of any one of claims 51-53, wherein the human cell is isolated or derived from an RPE of a human retina.
56. The cell of any one of claims 51-55, wherein the cell is *in vivo*, *in vitro*, *ex vivo* or *in situ*.
57. A method of treating macular dystrophy in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of a composition of any one of claims 1-27 or 42-44.

58. A method of treating macular dystrophy in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of a composition comprising the vector of claim 28 or 29.
59. A method of treating macular dystrophy in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of a composition comprising the delivery vector of any one of claims 30-41.
60. The method of any one of claims 57-59, wherein the subject is a human.
61. The method of any one of claims 57-59, wherein the subject is a non-human primate, a dog, a cat, a rodent, a mouse, a rat, or a rabbit.
62. The method of any one of claims 57-61, wherein the subject has macular dystrophy.
63. The method of any one of claims 57-62, wherein the subject has a mutation in one or both copies of a BEST1 gene.
64. The method of claim 63, wherein the mutation is heritable as a dominant mutation.
65. The method of claim 64, wherein the dominant mutation causes Best Vitelliform Macular Dystrophy (BVMD) in the subject.
66. The method of claim 63, wherein the mutation is heritable as a recessive mutation.
67. The method of claim 66, wherein the recessive mutation causes Autosomal Recessive Bestrophinopathy (ARB) in the subject.
68. The method of any one of claims 63-67, wherein the mutation occurs in a coding sequence of one or both copies of a BEST1 gene.
69. The method of any one of claims 63-67, wherein the mutation occurs in a non-coding sequence of one or both copies of a BEST1 gene.

70. The method of any one of claims 57-69, wherein administering comprises an injection or an infusion via a subretinal, a suprachoroidal or an intravitreal route.

71. The method of any one of claims 57-69, wherein administering comprises an injection or an infusion via a subretinal route.

72. The method of claim 71, wherein administering comprises a two-step injection or a two-step infusion via a subretinal route.

73. The method of any one of claims 57-72, wherein the therapeutically effective amount is formulated in a volume of between 10 and 200 μL , inclusive of the endpoints.

74. The method of claim 73, wherein the therapeutically effective amount is formulated in a volume of between 10 and 50 μL , between 50 and 100 μL , between 100 and 150 μL or between 150 and 200 μL , inclusive of the endpoints, for each range.

75. The method of any one of claims 57-72, wherein the therapeutically effective amount is formulated in a volume of between 70 and 120 μL , inclusive of the endpoints, and wherein the administering comprises an injection or an infusion via a subretinal route.

76. The method of any one of claims 57-72, wherein the therapeutically effective amount is formulated in a volume of 100 μL and wherein the administering comprises an injection or an infusion via a subretinal route.

77. The method of any one of claims 59-76, wherein the therapeutically effective amount comprises a concentration of an AAV delivery vector of at least 1×10^{10} DRP/mL, at least 1×10^{11} DRP/mL, at least 1×10^{12} DRP/mL, at least 2×10^{12} DRP/mL, at least 5×10^{12} DRP/mL or at least 1.5×10^{13} DRP/mL.

78. The method of any one of claims 59-76, wherein the therapeutically effective amount comprises a concentration of an AAV delivery vector of at least 2×10^{12} DRP/mL, at least 5×10^{12} DRP/mL or at least 1.5×10^{13} DRP/mL.

79. The method of any one of claims 59-76, wherein the therapeutically effective amount comprises a concentration of an AAV delivery vector of at least 5×10^{12} DRP/mL.

80. The method of any one of claims 59-76, wherein the therapeutically effective amount comprises a concentration of an AAV delivery vector of at least 1.5×10^{13} DRP/mL.

81. The method of any one of claims 59-80, wherein the therapeutically effective amount comprises a dose of 2×10^8 genome particles (gp), 5×10^8 gp, 1.5×10^9 gp, 2×10^9 gp, 5×10^9 gp, 2×10^{10} gp, 5×10^{10} gp, 6×10^{10} gp, 1.2×10^{11} gp, 1.5×10^{11} gp, 2×10^{11} gp, 4.5×10^{11} gp, 5×10^{11} gp, 1.2×10^{12} gp, 1.5×10^{12} gp, 2×10^{12} gp or 5×10^{12} gp.

82. The method of any one of claims 59-80, wherein the subject is a mouse and wherein the therapeutically effective amount comprises a dose of 5×10^8 gp, 1.5×10^9 gp or 5×10^9 gp.

83. The method of any one of claims 59-80, wherein the subject is a non-human primate and wherein the therapeutically effective amount comprises a dose of 1.2×10^{11} gp, 4.5×10^{11} gp or 1.2×10^{12} gp of AAV viral particles.

84. The method of any one of claims 59-80, wherein the subject is human and wherein the therapeutically effective amount comprises a dose of 5×10^{10} gp, 1.5×10^{11} gp, 5×10^{11} gp or 1.5×10^{12} gp of AAV viral particles.

85. The method of any one of claims 57-84, wherein the composition further comprises a TMN200 buffer.

FIG. 1A

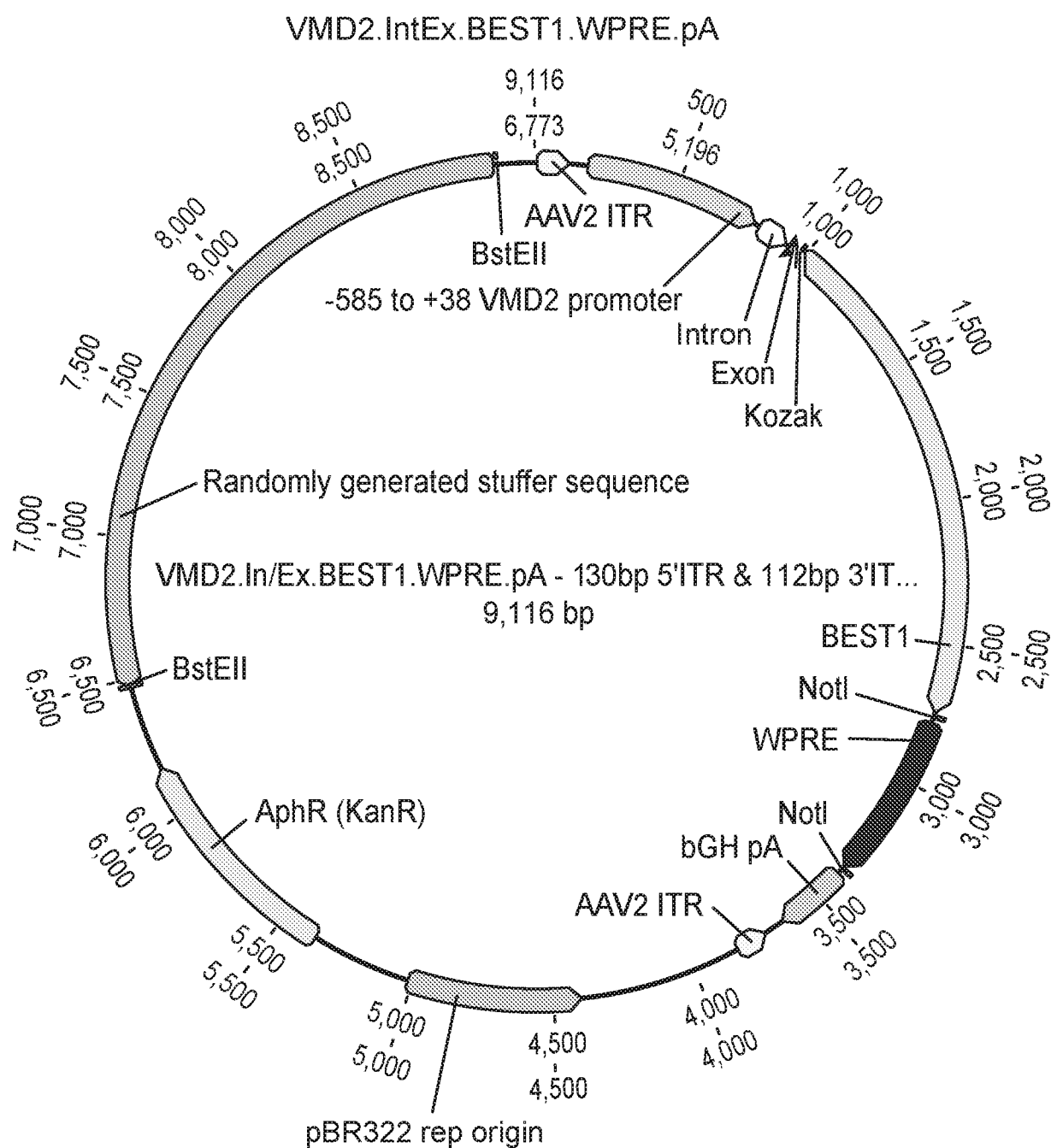


FIG. 1B

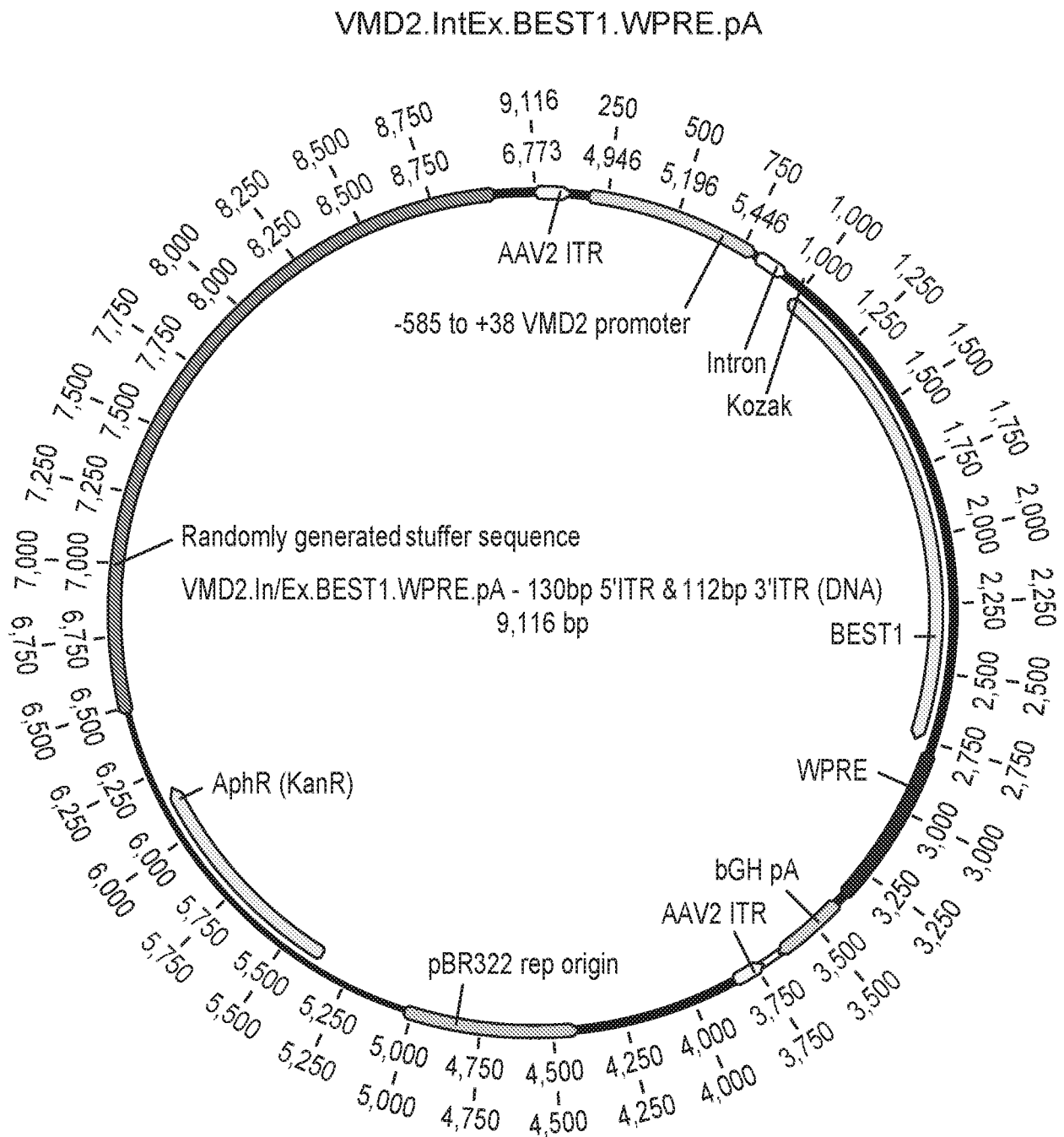


FIG. 2A

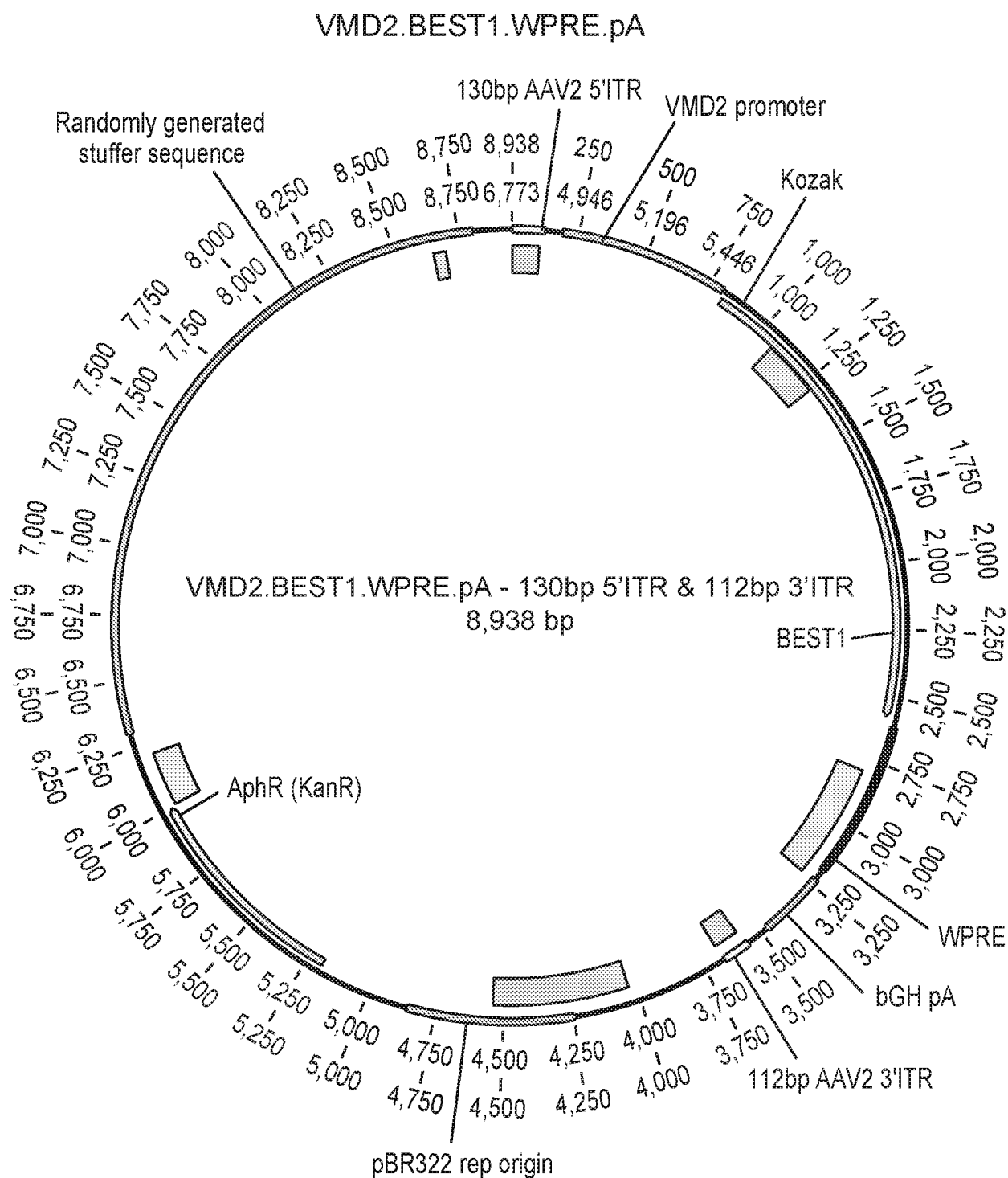
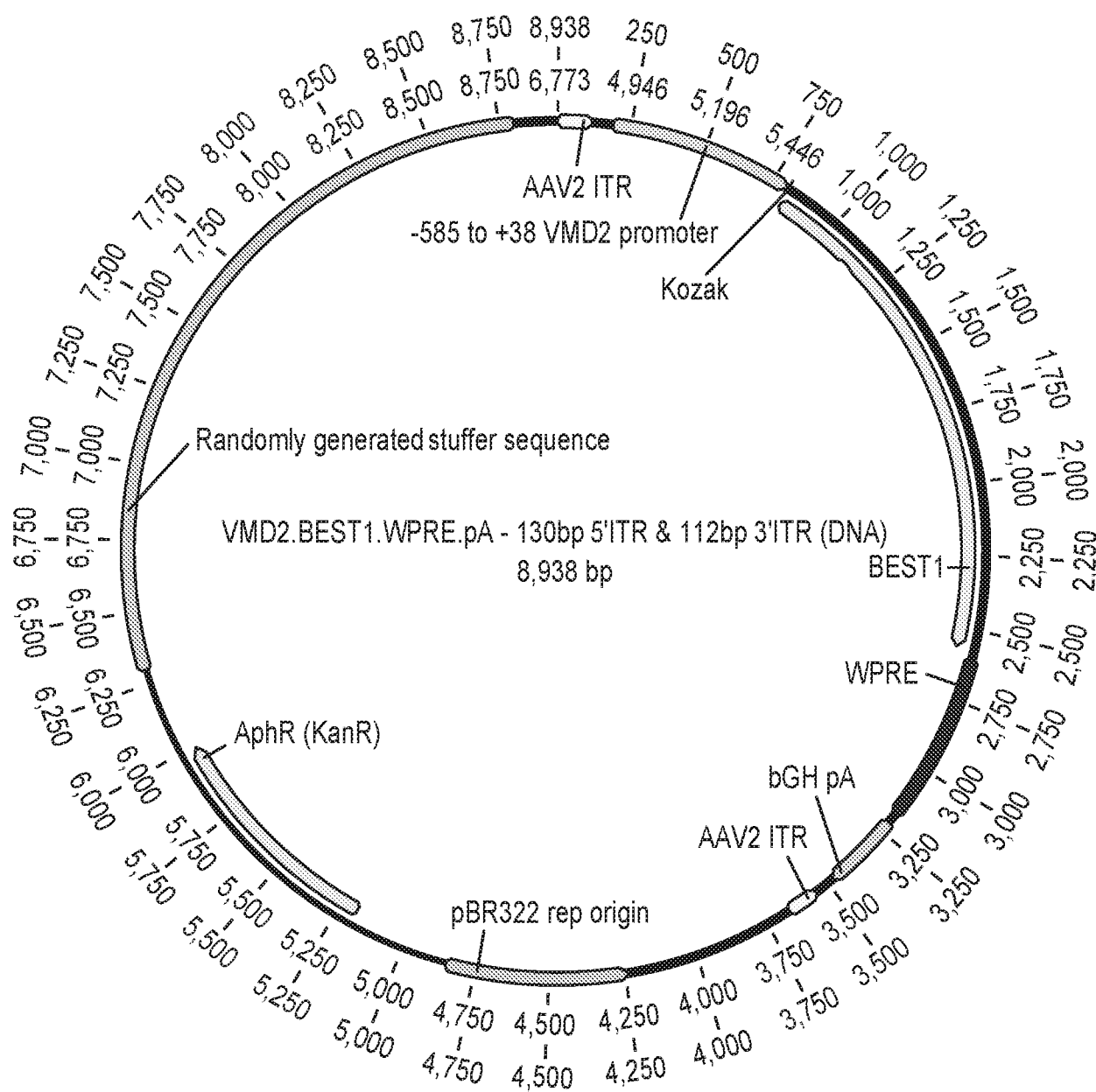


FIG. 2B

VMD2.BEST1.WPRE.pA



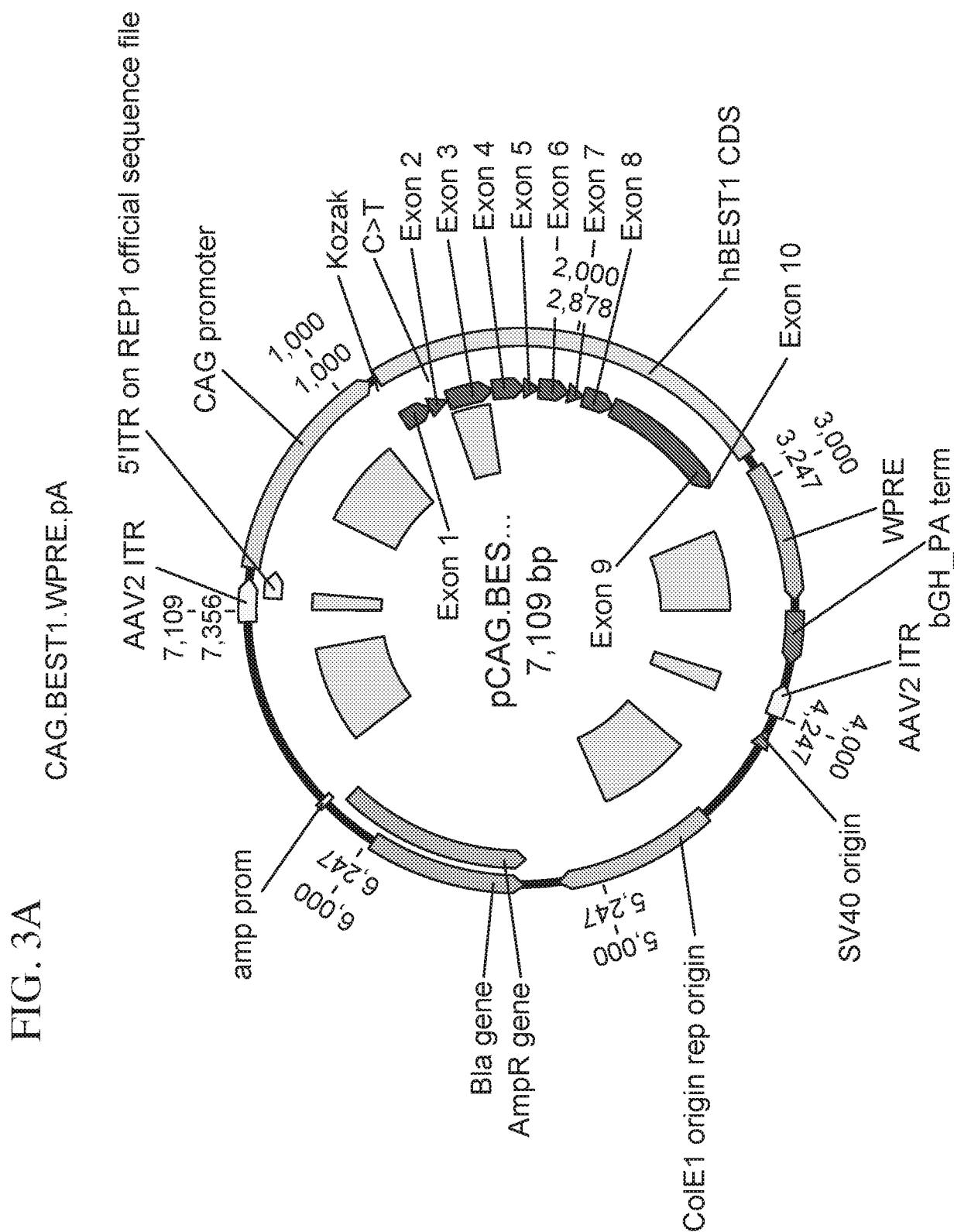


FIG. 3B

CAG.BEST1.WPRE.pA

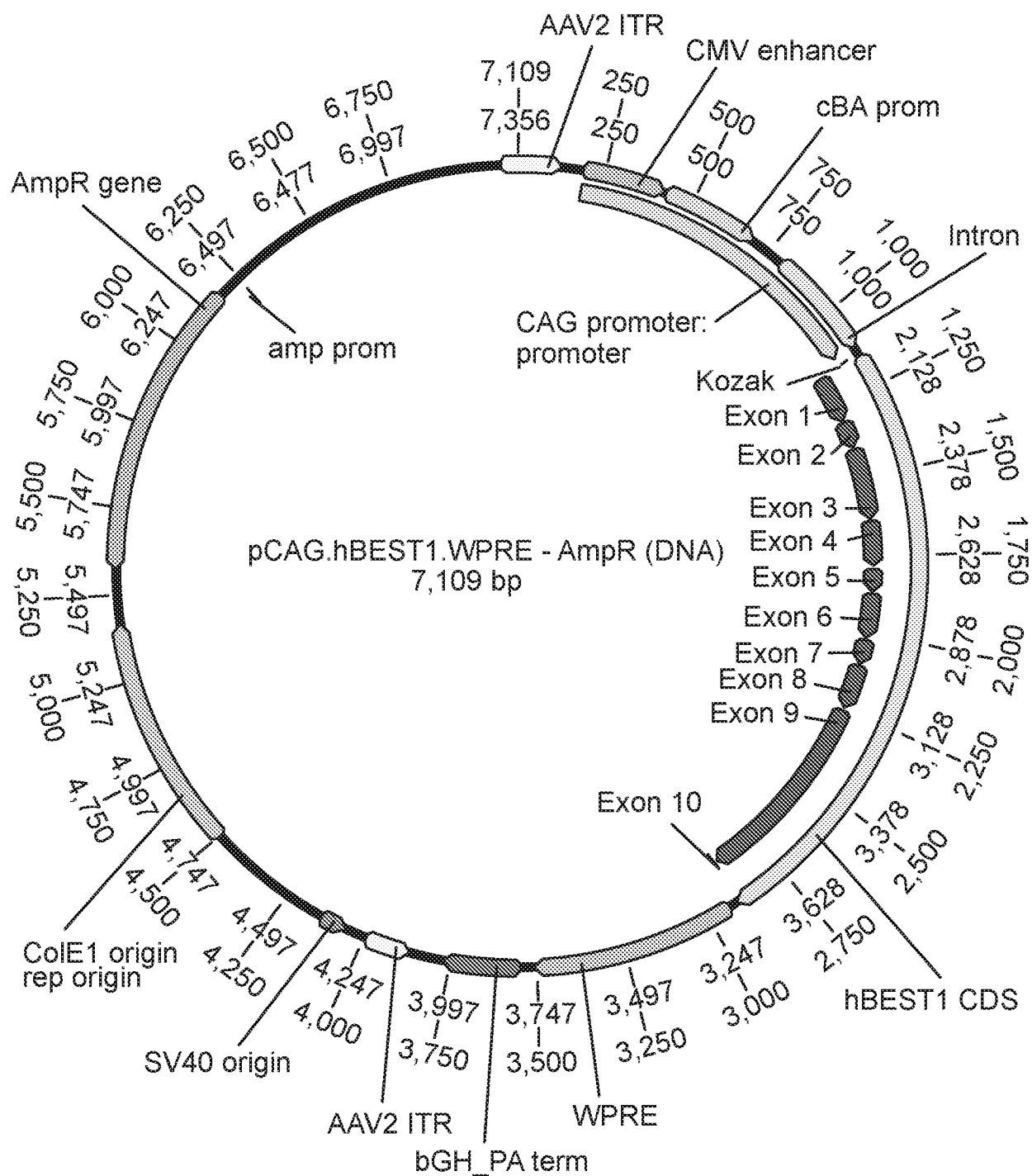


FIG. 3C

CAG.BEST1.WPRE.pA with KanR and Stuffer Sequence

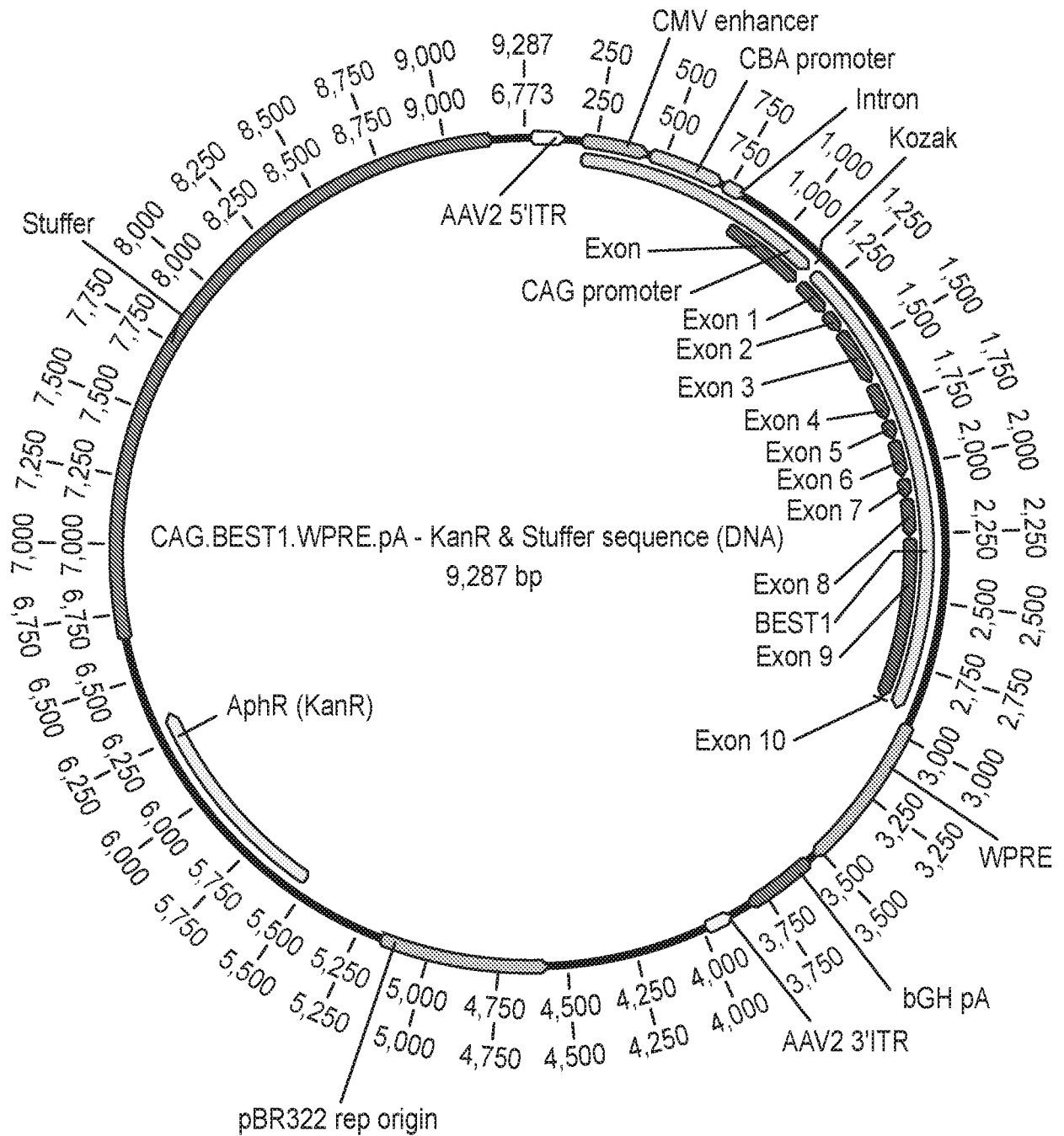


FIG. 4

VMD2.GFP.WPRE.pA Plasmid Map

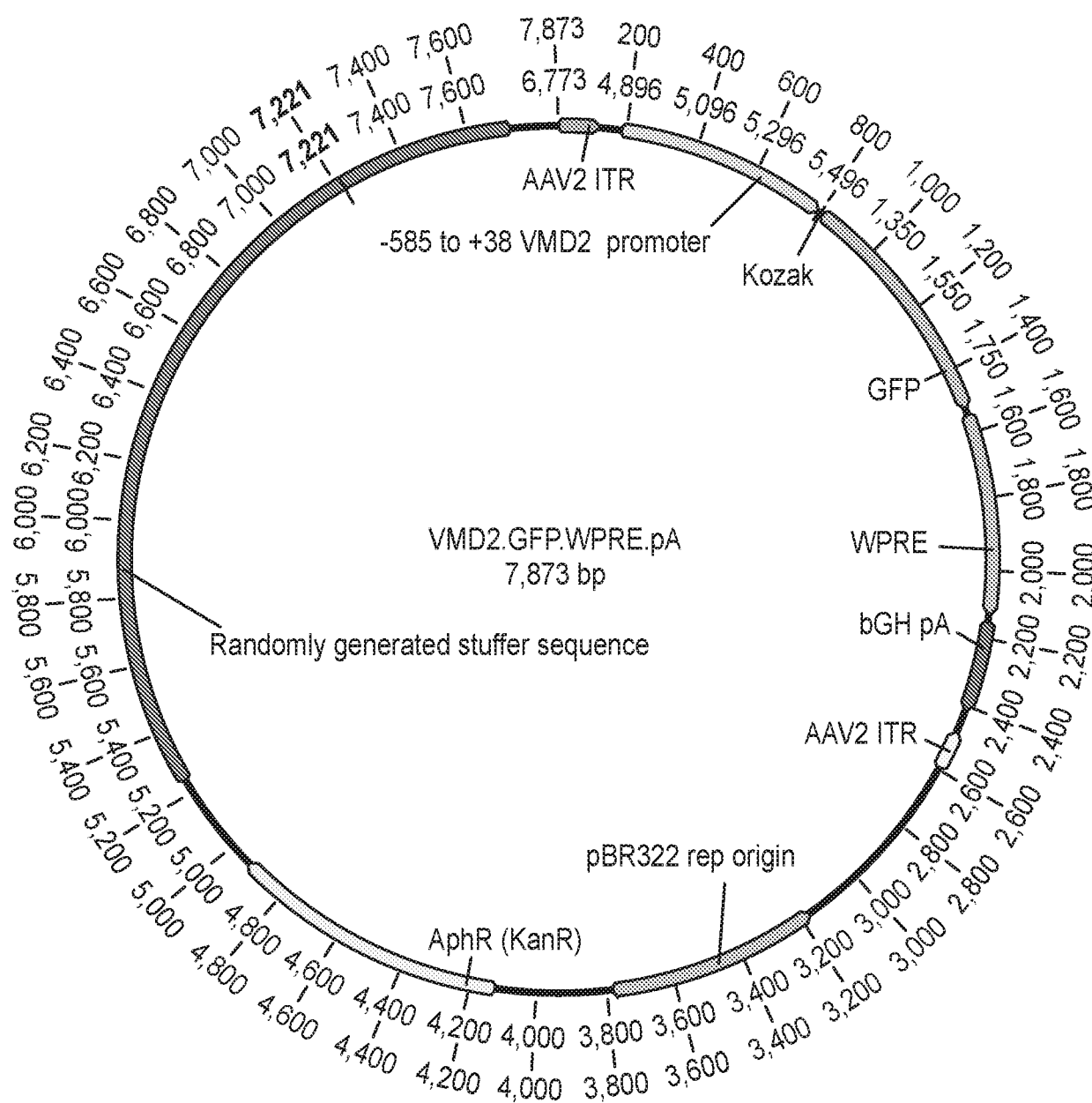


FIG. 5

VMD2.IntEx.GFP.WPRE.pA Plasmid Map

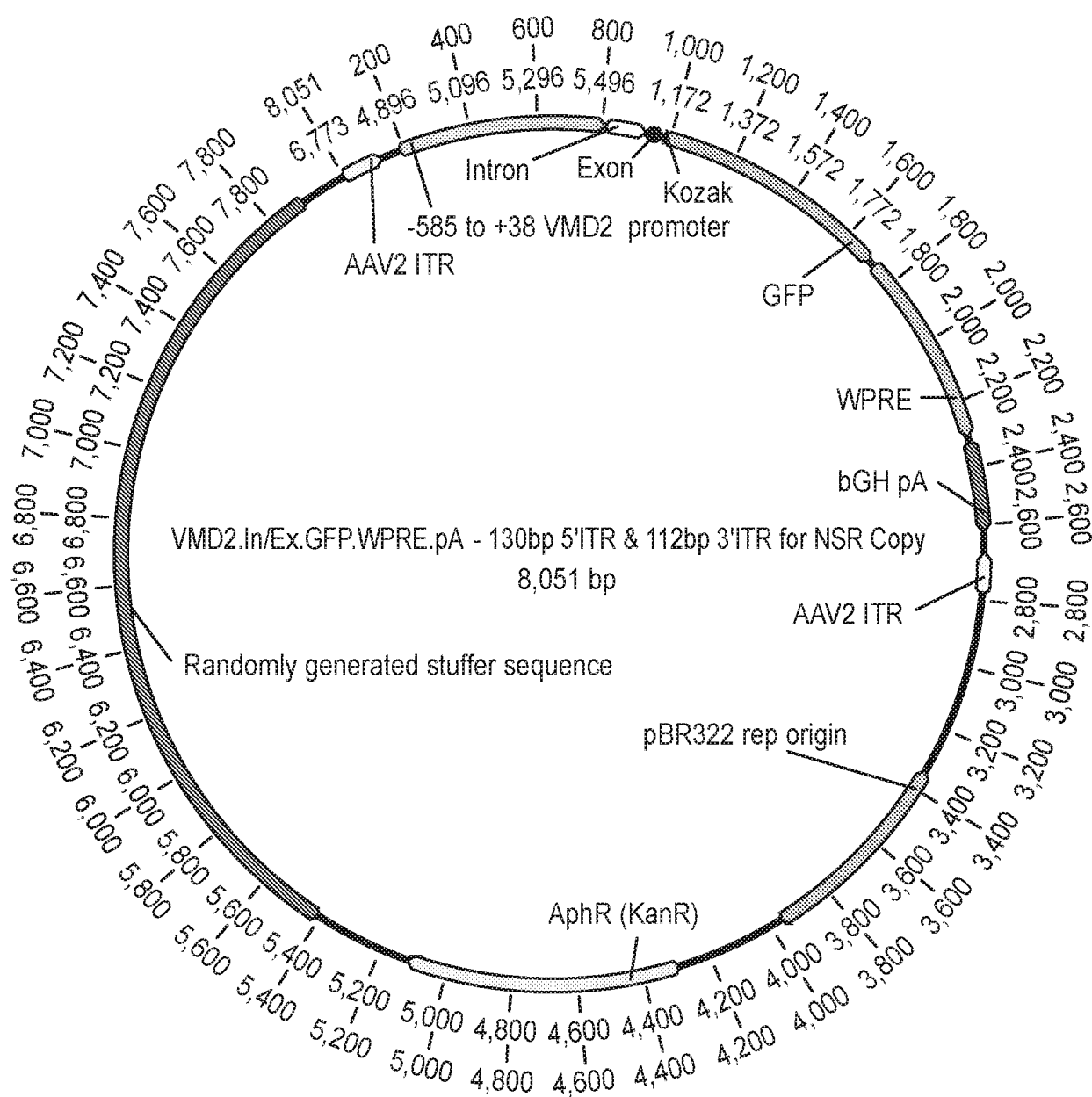


FIG. 6A

Localization of Bestrophin-1 in HEK-293 Cells Following
Transduction with AAV-CAG-BEST1

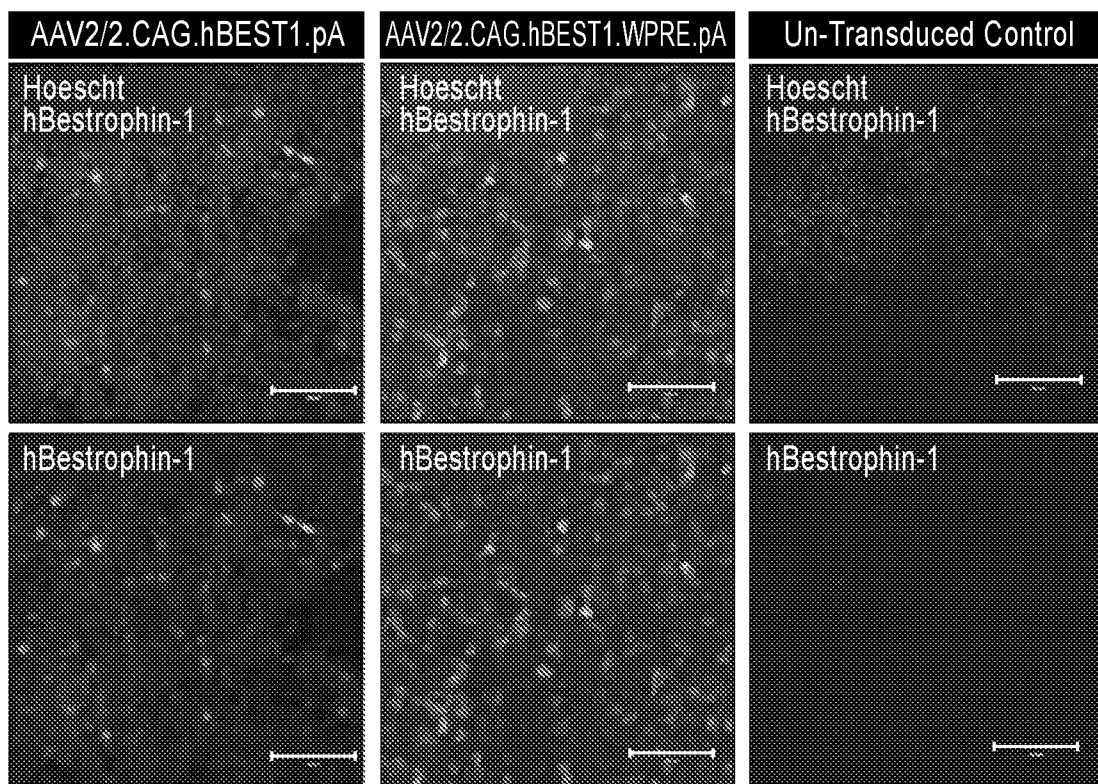
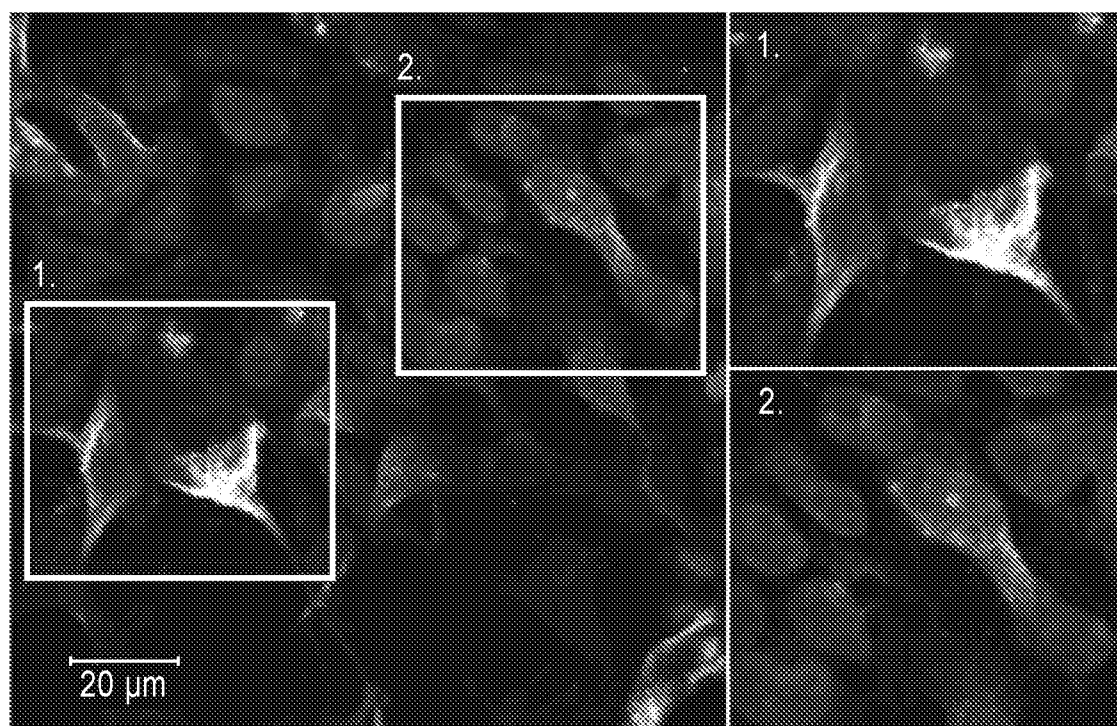
**FIG. 6B**

FIG. 7A

Expression of Bestrophin-1 in HEK-293 Cells by Western Blot

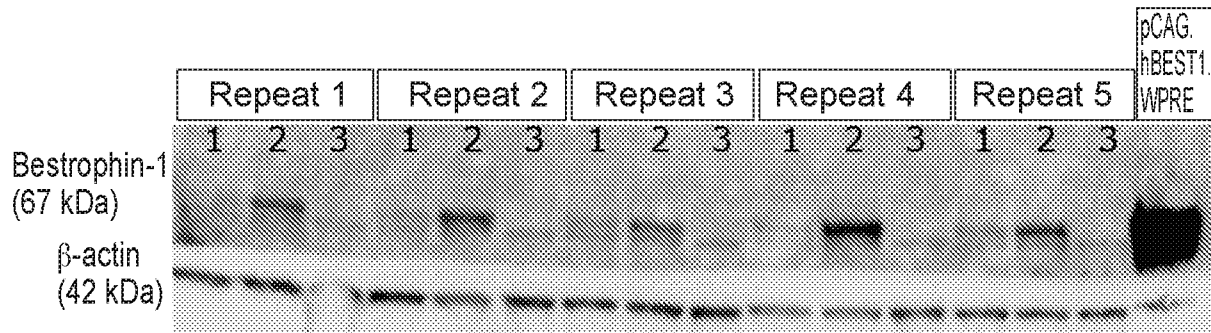


FIG. 7B

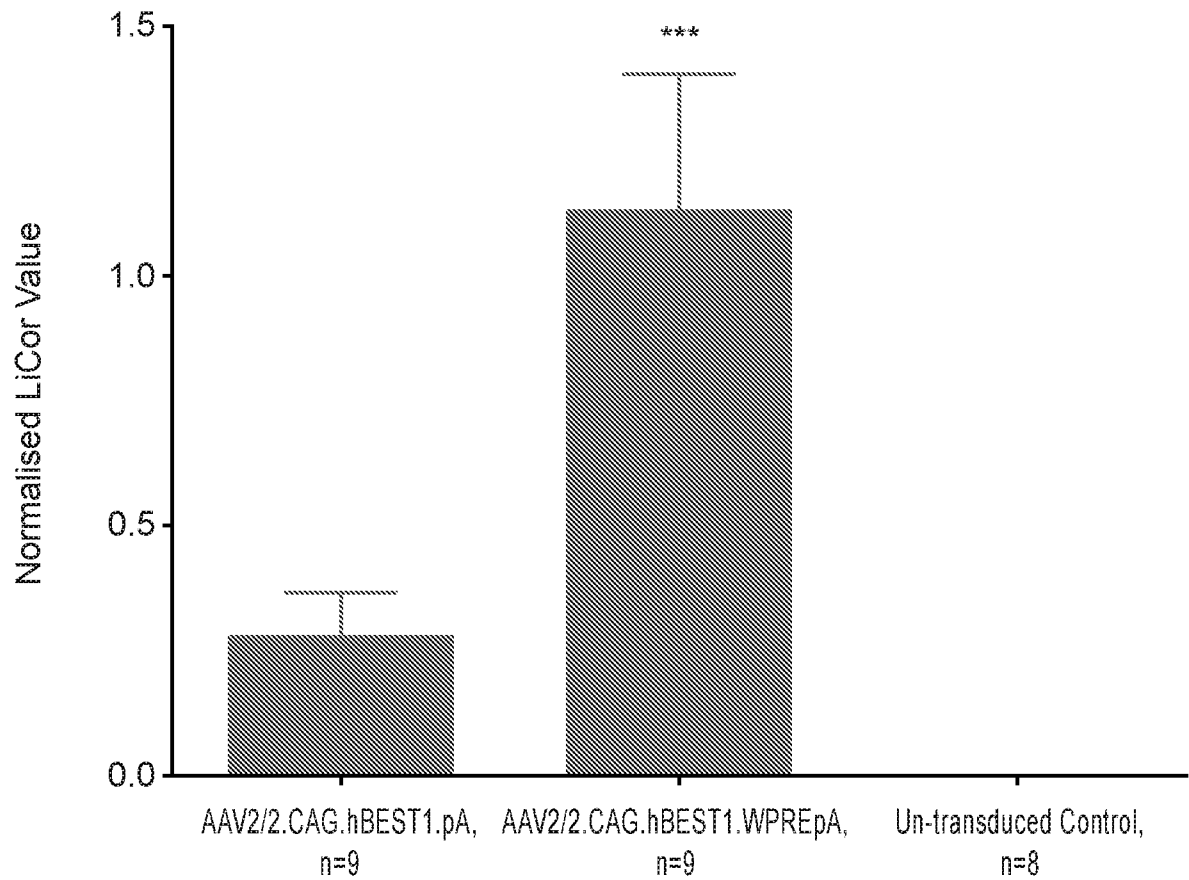


FIG. 8A

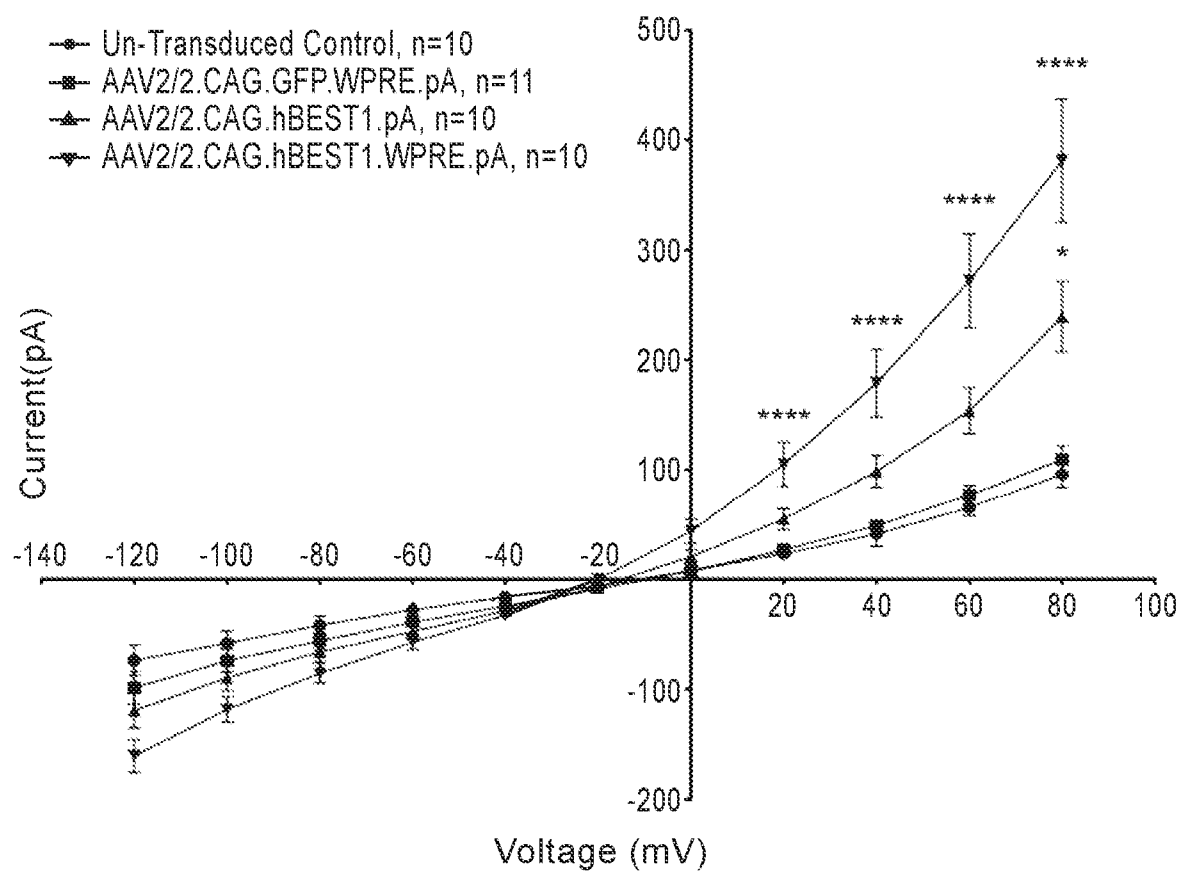
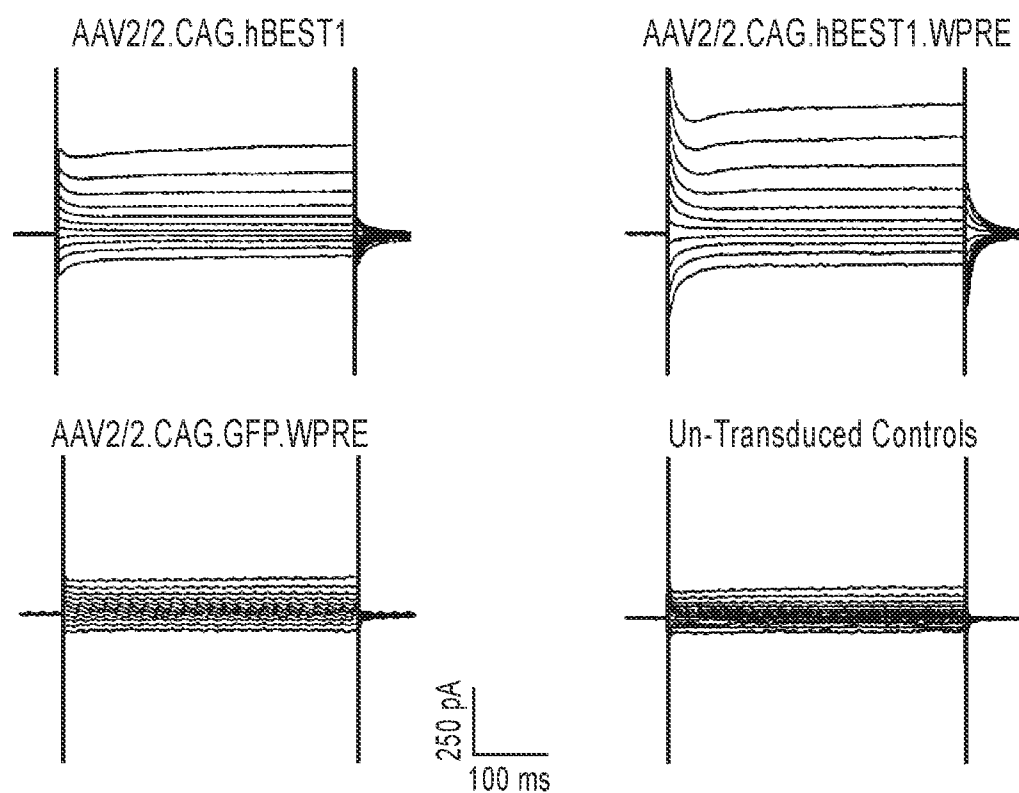


FIG. 8B



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FIG. 9A

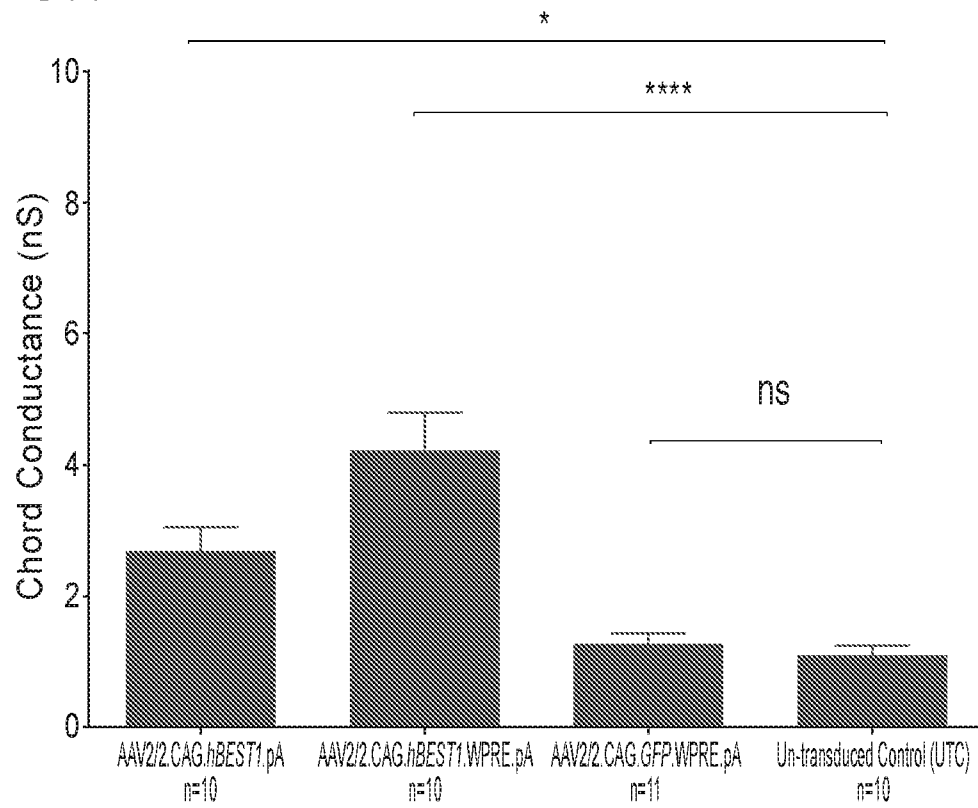


FIG. 9B

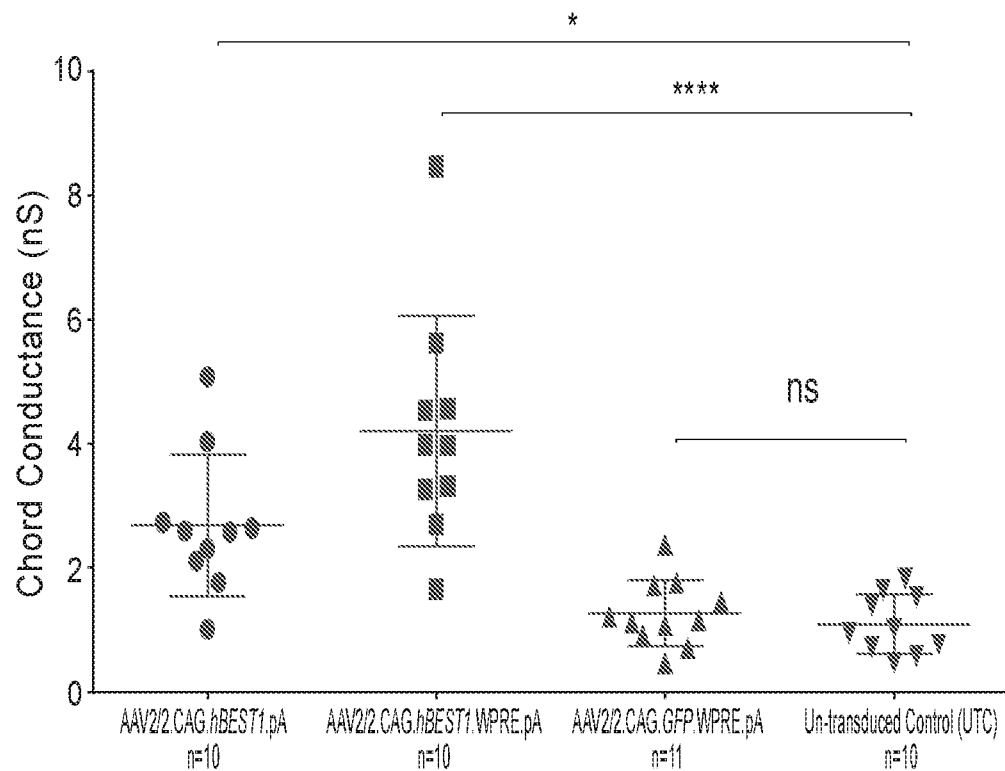


FIG. 10A

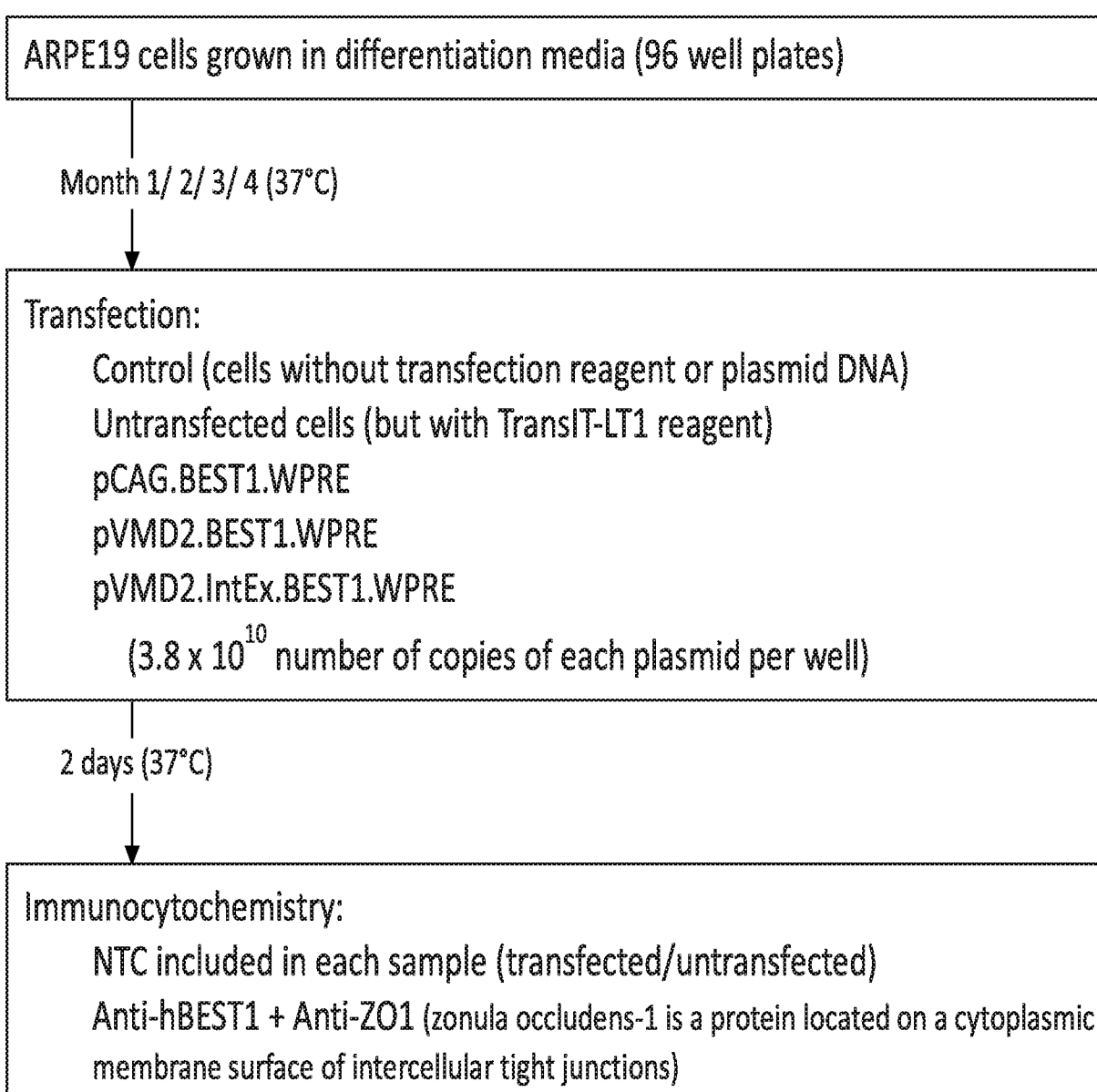
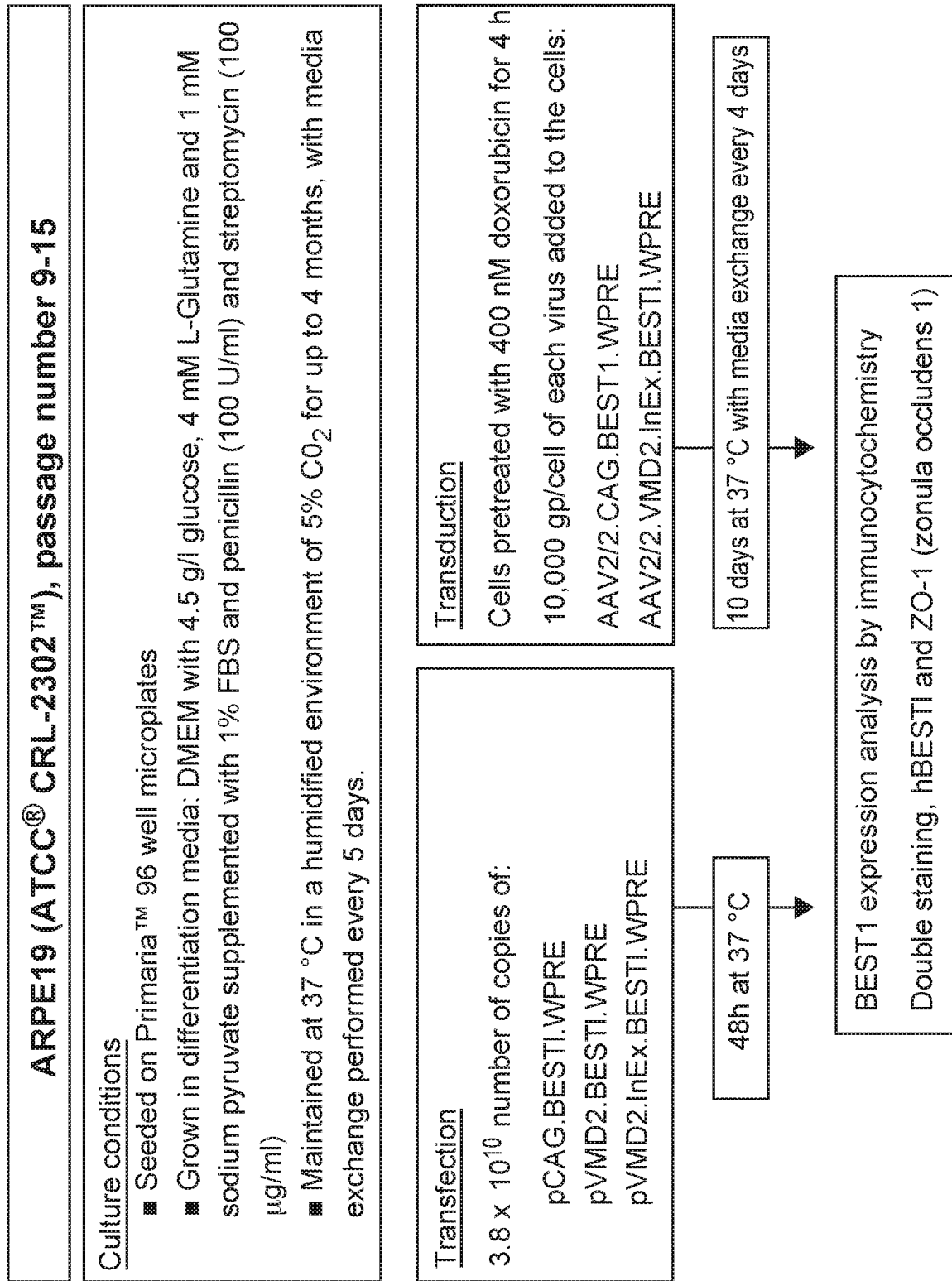
Experimental procedure

FIG. 10B



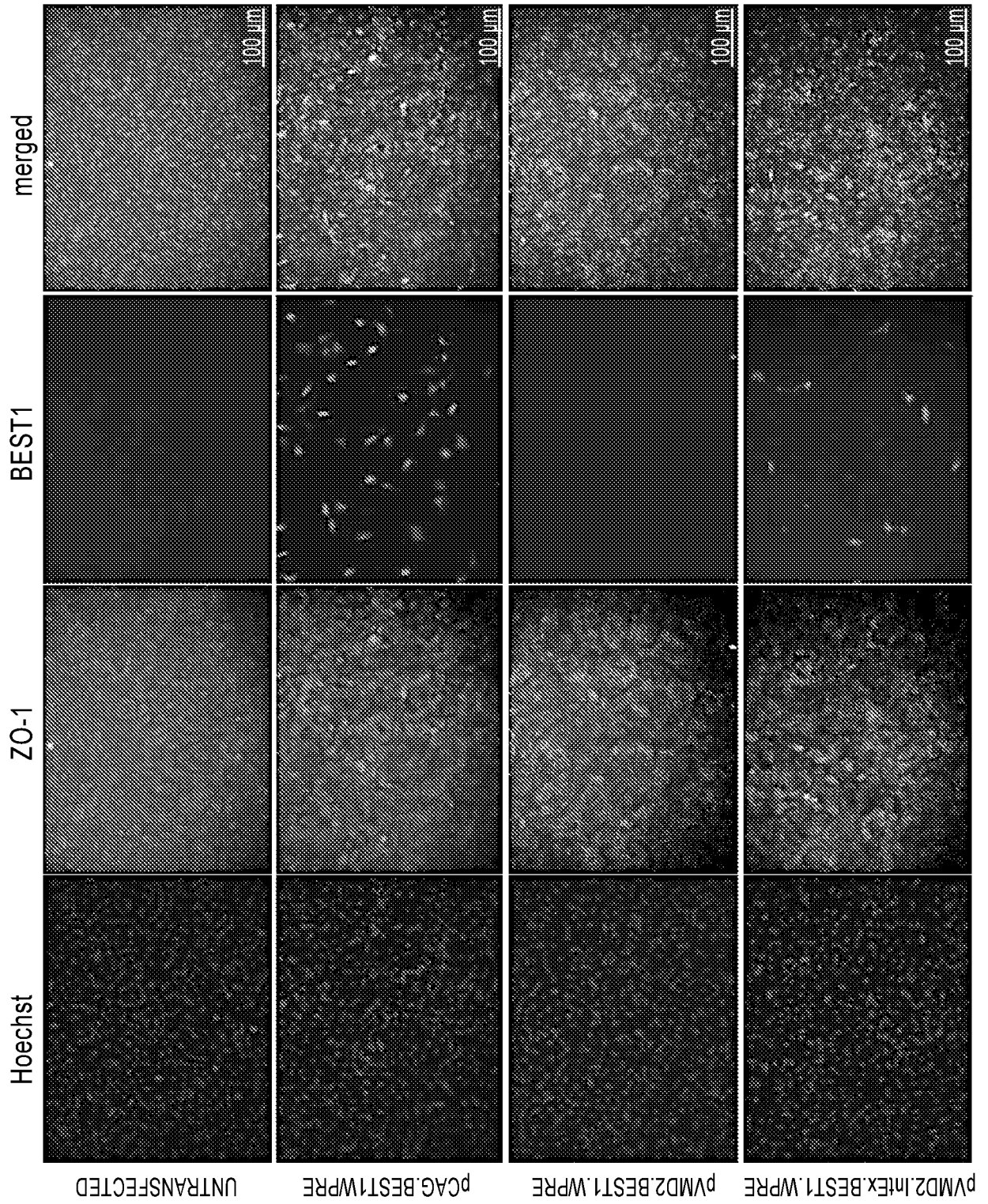
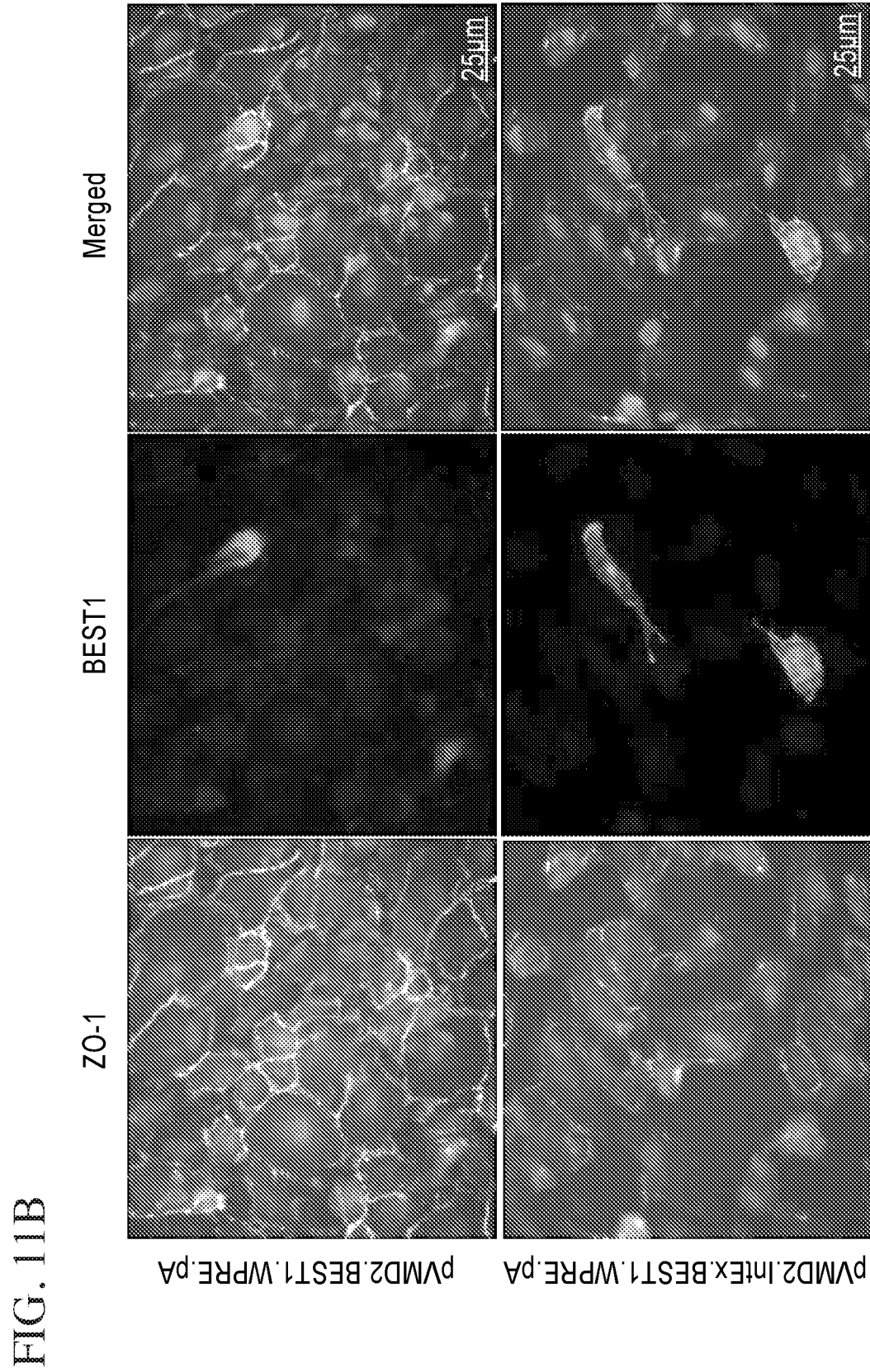


FIG. 11A



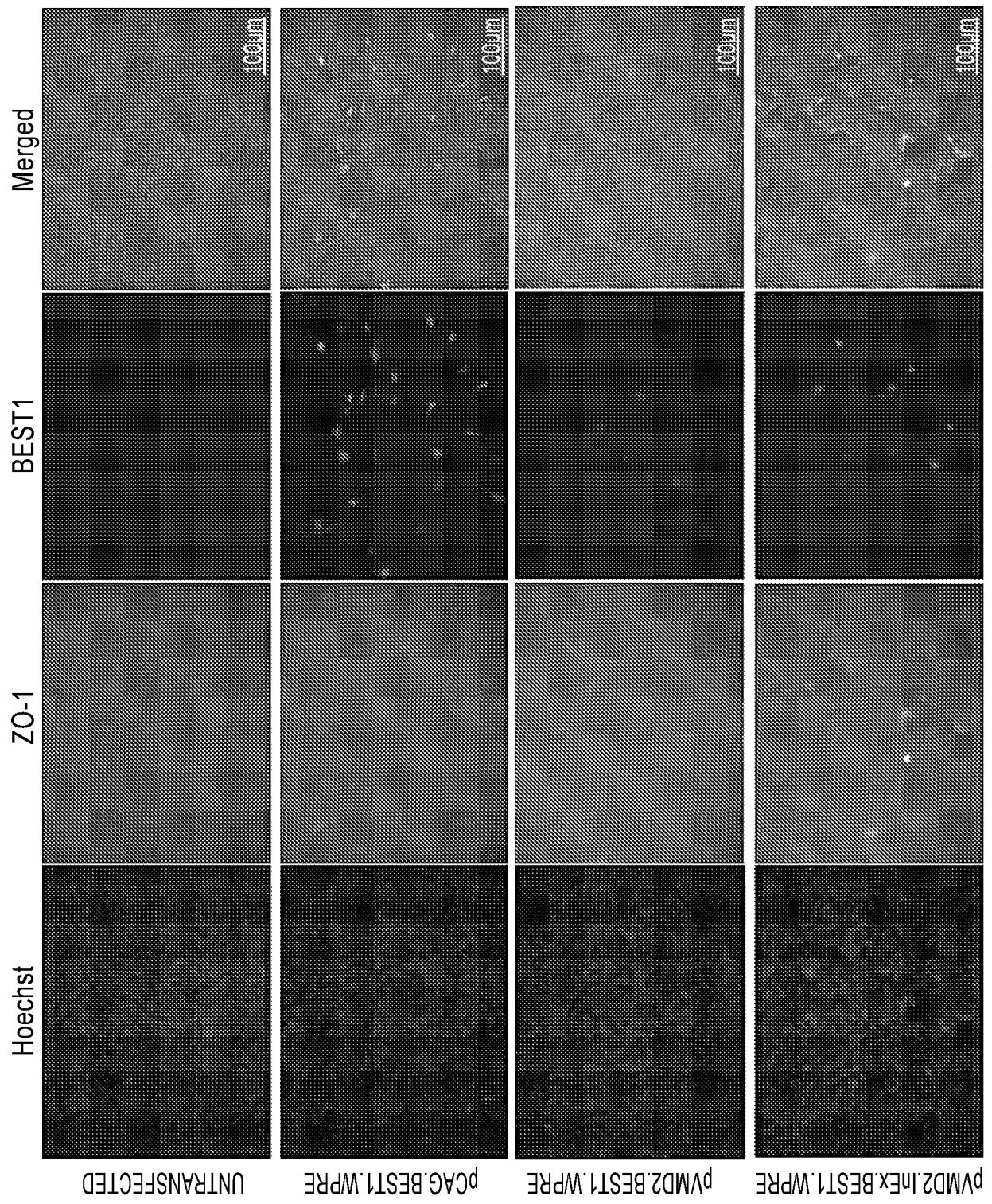


FIG. 12A

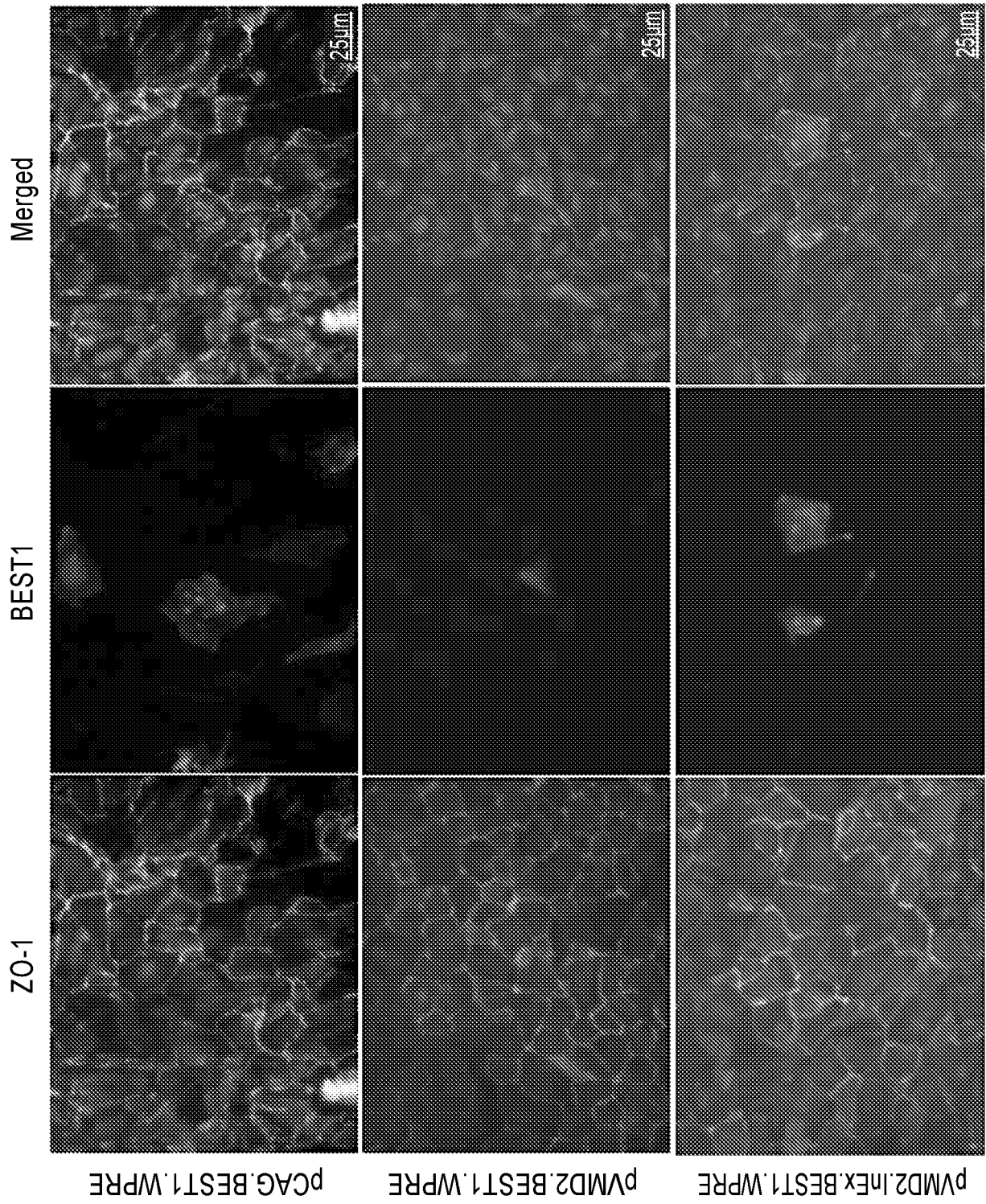


FIG. 12B

FIG. 13A

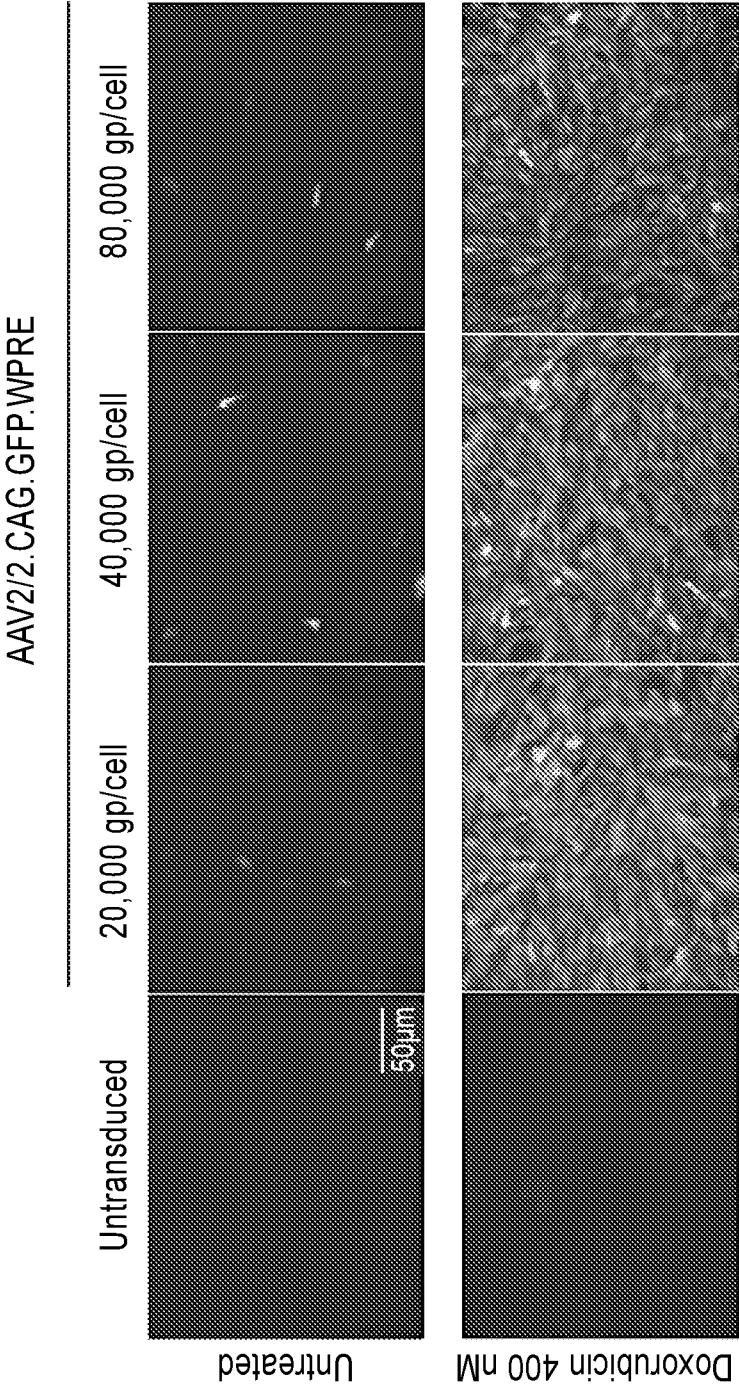
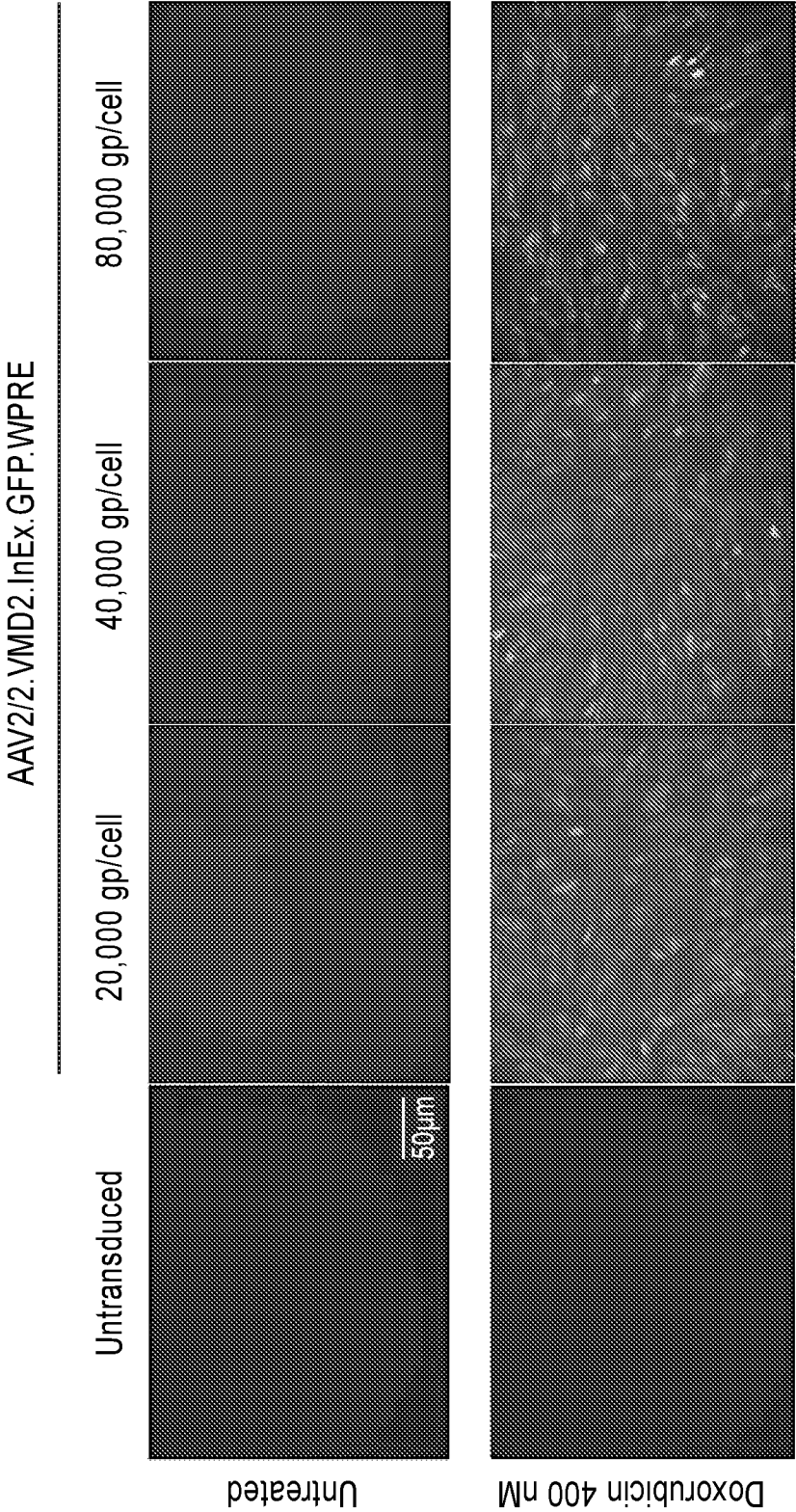


FIG. 13B



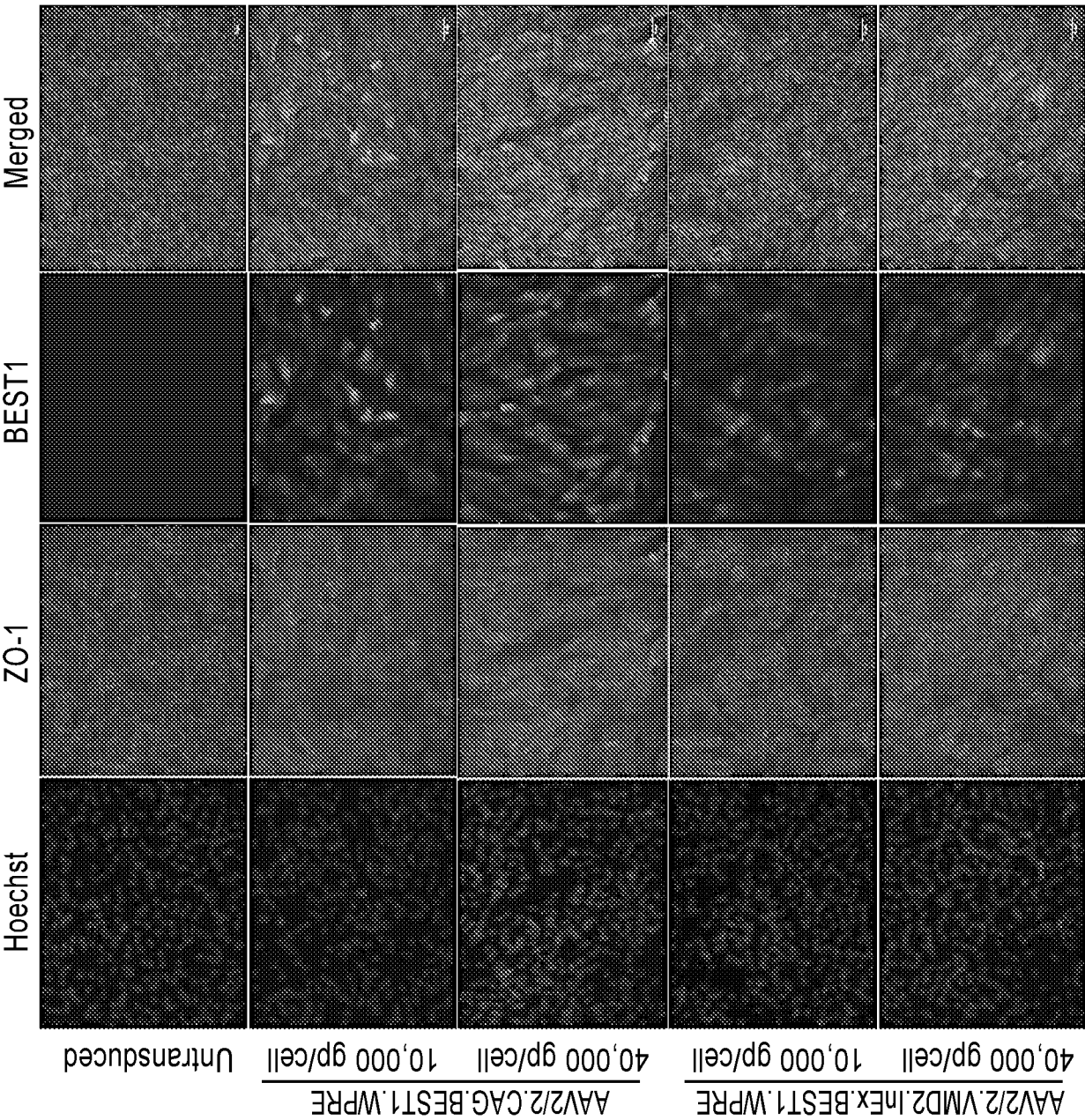


FIG. 14

FIG. 15

4/8 Week *in vivo* Pilot Study: Protocol

Animal	Group	# of Animals	Week 4		Week 8	
			OCT	Sacrificed	OCT	Sacrificed
C57BL/6 Mice (24 in total)	Sham	6	6	3 mice (1 eye-IHC; 1 eye -WB)	3	3 mice (1 eye-IHC; 1 eye -WB)
	VMD2.BEST1.WPRE 1 x 10 ⁹ GC/ μ L/eye	6	6	3 mice (3 eyes-IHC; 3 eyes -WB)	3	3 mice (3 eyes-IHC; 3 eyes -WB)
	VMD2.IntEx.BEST1.WPRE 1 x 10 ⁹ GC/ μ L/eye	6	6	3 mice (3 eyes-IHC; 3 eyes -WB)	3	3 mice (3 eyes-IHC; 3 eyes -WB)
In-life Termination						
Results of Localization (Immunohistochemistry), Expression (Western Blot) and Retinal Toxicity (OCT)						

FIG. 16

4 Week *in vivo* Pilot Study: Evaluation of Potential Toxicity

Sham VMD2.WPRE VMD2.IntEx.WPRE

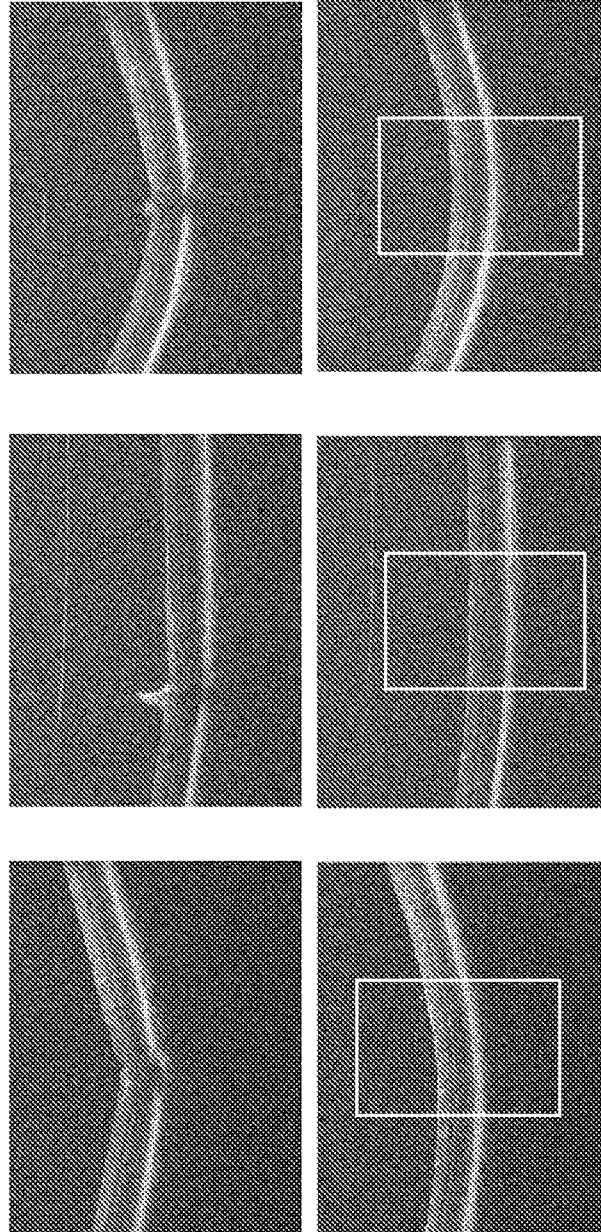
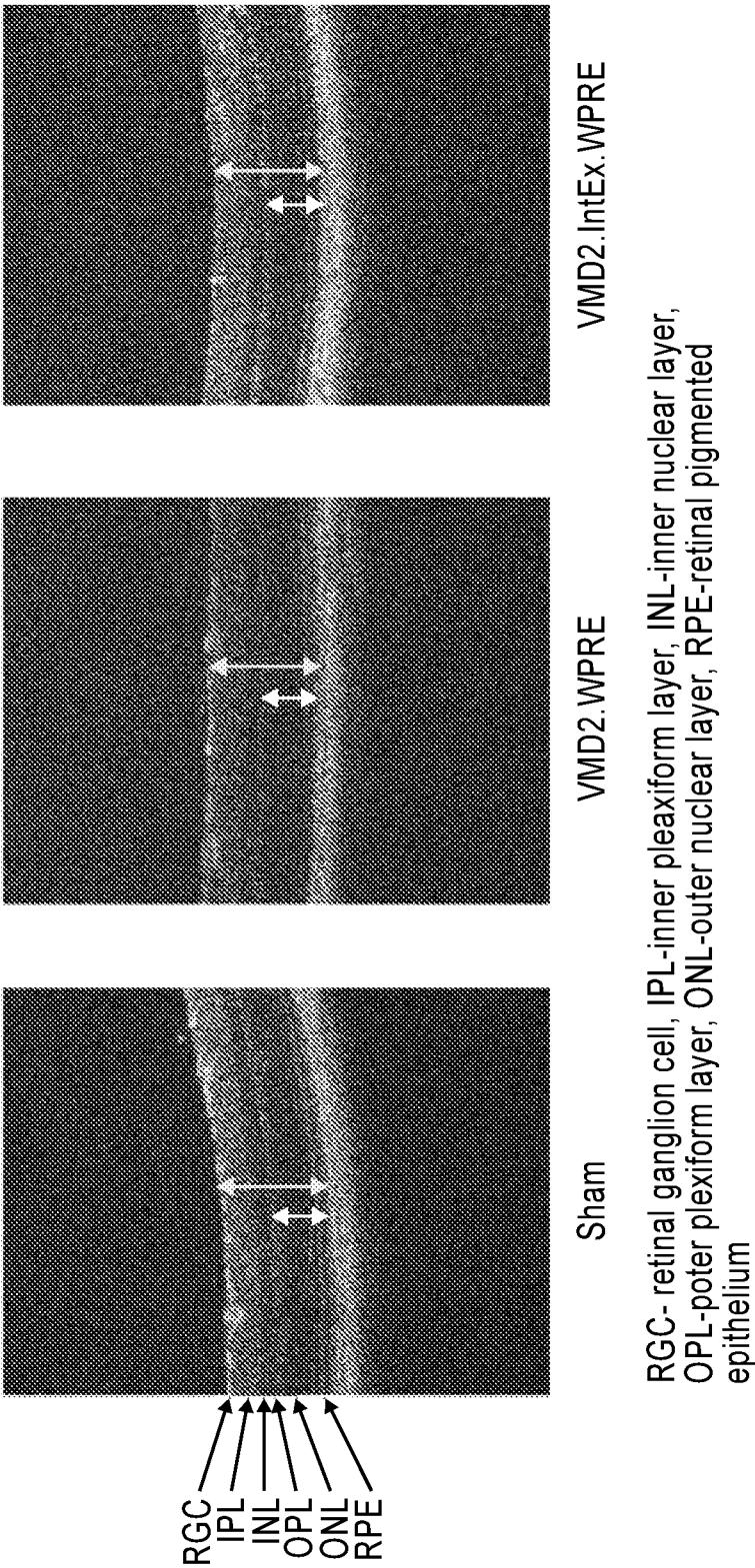
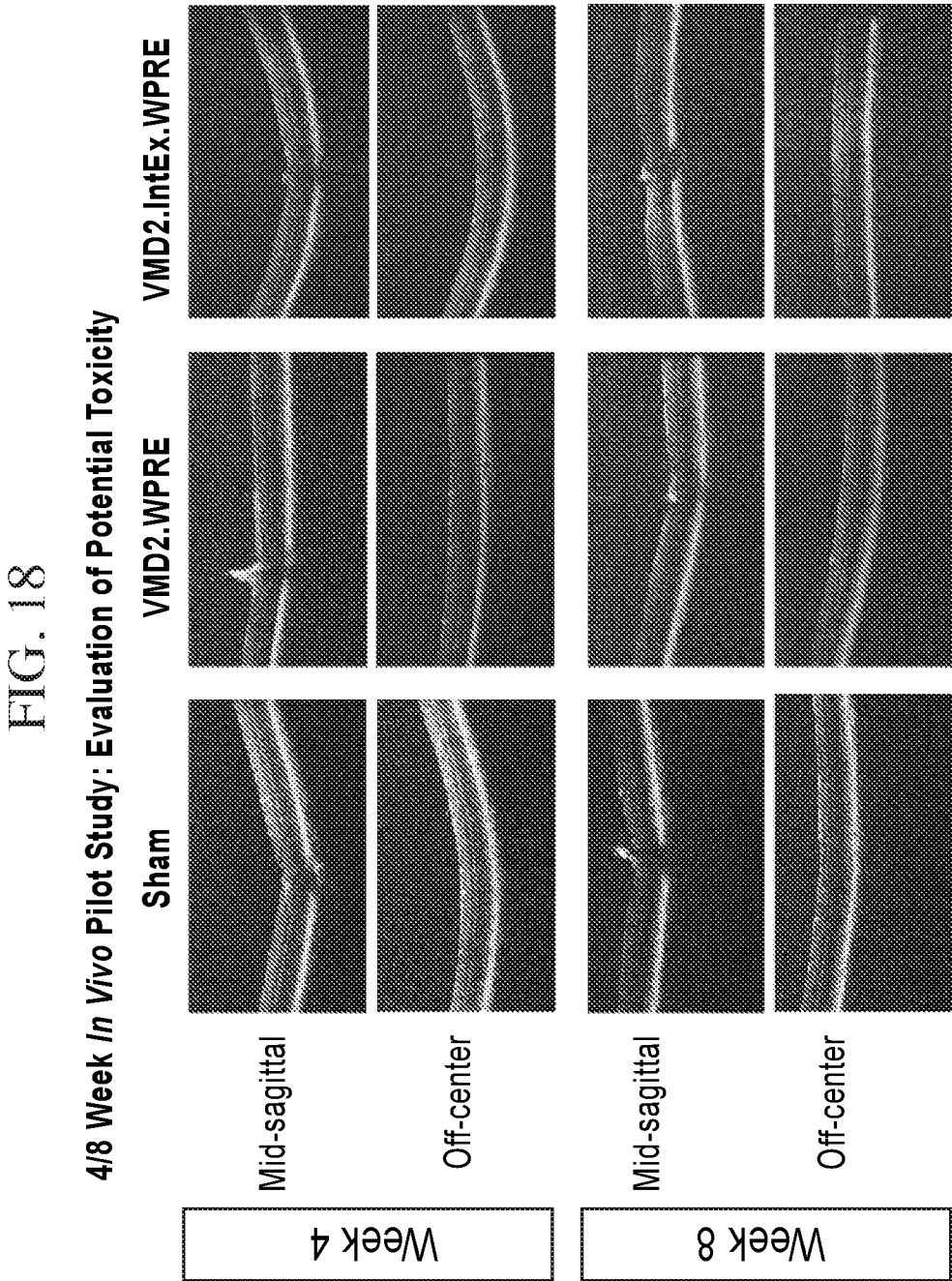


FIG. 17
4 Week *in vivo* Pilot Study: Evaluation of Potential Toxicity



RGC- retinal ganglion cell, IPL-inner plexiform layer, INL-inner nuclear layer, OPL-outer plexiform layer, ONL-outer nuclear layer, RPE-retinal pigmented epithelium

-Both VMD2 constructs did not show any toxicity of the photoreceptor when compared with sham treatment



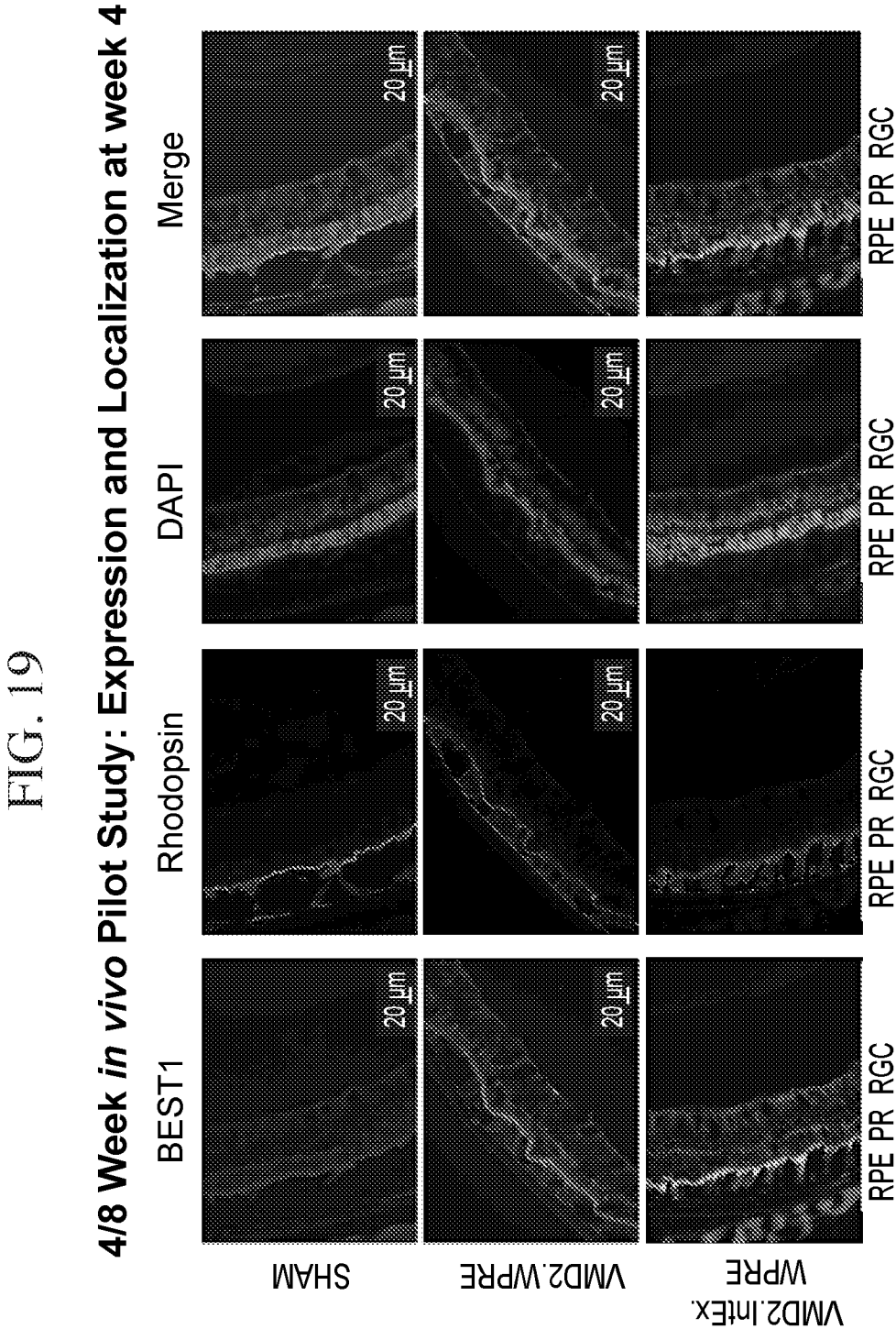


FIG. 20
4/8 Week *in vivo* Pilot Study: Expression and Localisation at Week 8

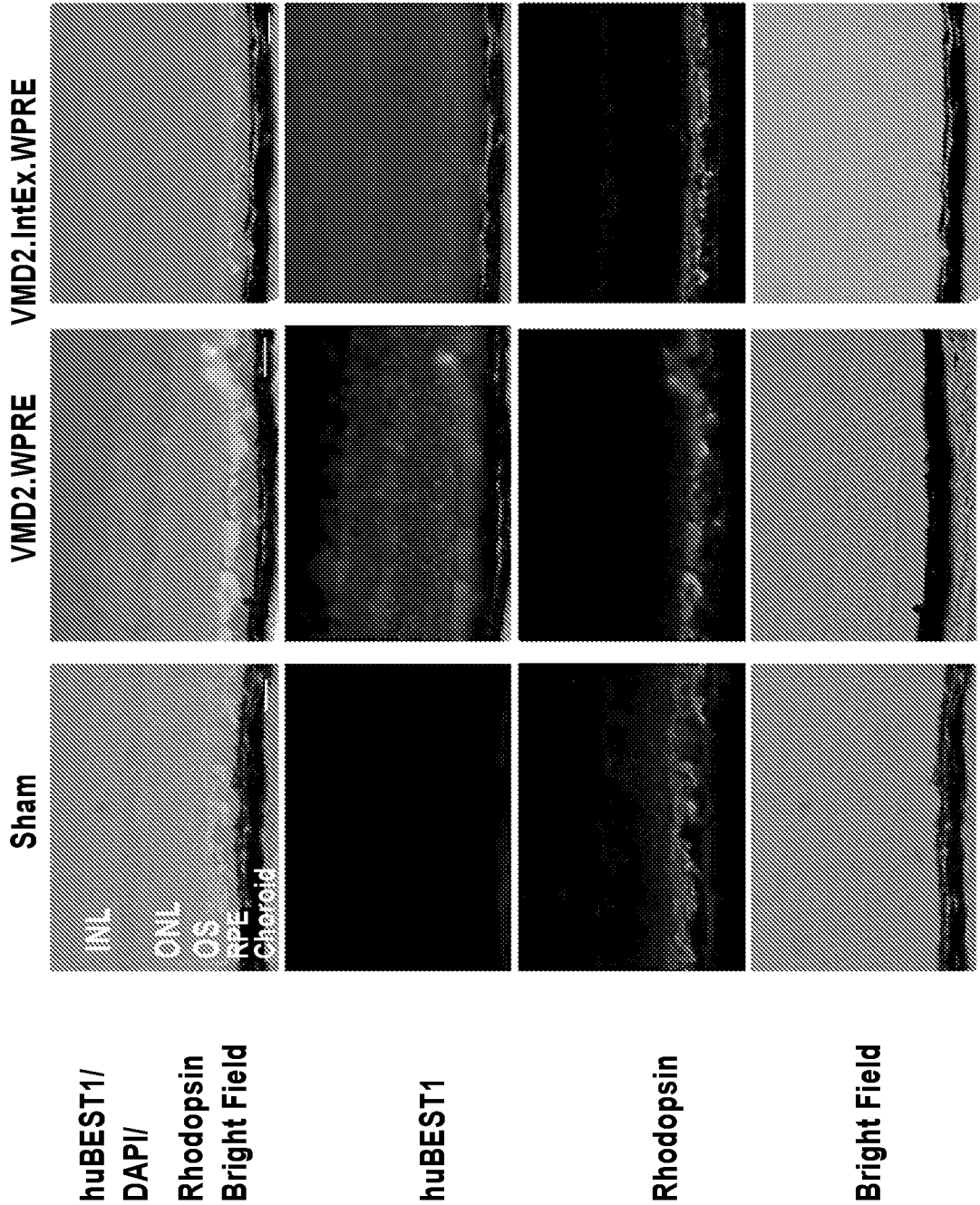


FIG. 21

4/8 Week *in vivo* Pilot Study: BEST1 Identification by Western Blot (4 Weeks)

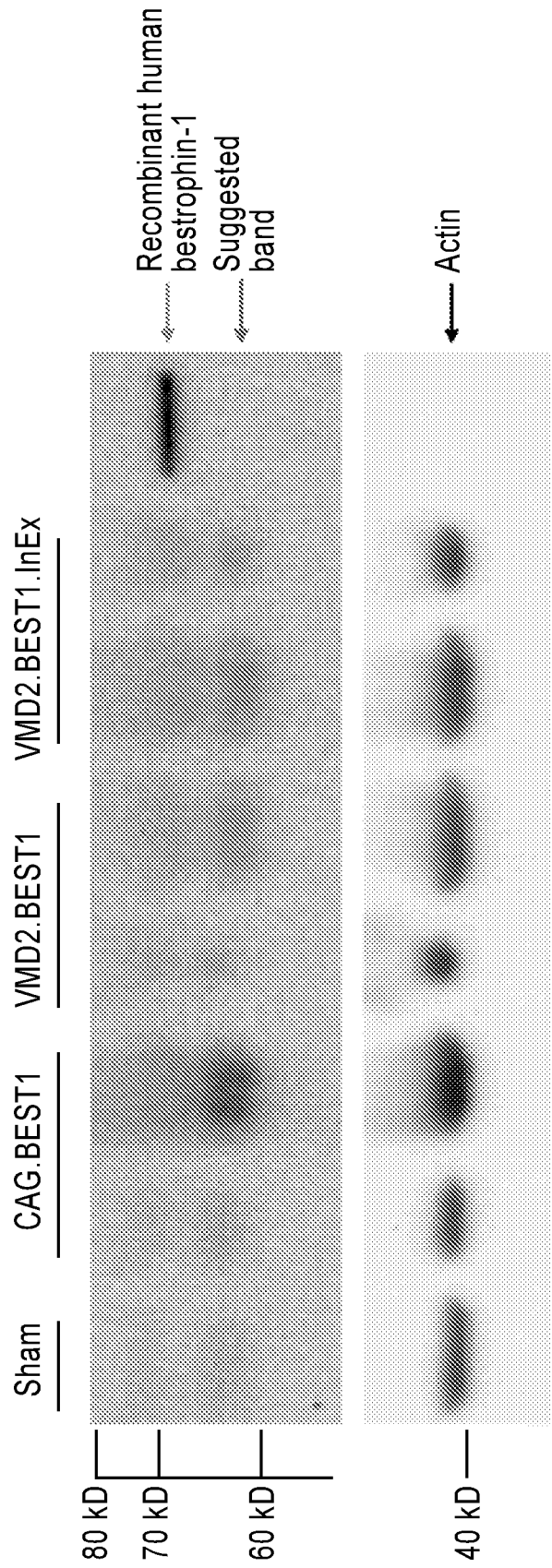


FIG. 22
4/13 Week *in vivo* Proof of Concept Study: Protocol

Animal	Group	# of Animals	Week 4		Week 13	
			OCT	Sacrifice	OCT	Sacrifice
C57BL/6 Mice (60 mice in total; Bilateral injection)	Sham	12	12 mice / 24 eyes	4 mice (1-IHC; 1-WB)	8 mice / 16 eyes	8 mice (1-IHC; 1-WB)
	VMD2.IntEx.WPRE 1 x 10 ⁸ GC/ μ L/eye	12	12 mice / 24 eyes	4 mice (4-IHC; 4-WB)	8 mice / 16 eyes	8 mice (16-IHC; 16-WB)
	VMD2.In_Ex.WPRE 1 x 10 ⁹ GC/ μ L/eye	12	12 mice / 24 eyes	4 mice (4-IHC; 4-WB)	8 mice / 16 eyes	8 mice (16-IHC; 16-WB)
	VMD2.WPRE 1 x 10 ⁸ GC/ μ L/eye	12	12 mice / 24 eyes	4 mice (4-IHC; 4-WB)	8 mice / 16 eyes	8 mice (16-IHC; 16-WB)
	VMD2.WPRE 1 x 10 ⁹ GC/ μ L/eye	12	12 mice / 24 eyes	4 mice (4-IHC; 4-WB)	8 mice / 16 eyes	8 mice (16-IHC; 16-WB)
In-life Termination						
Results (IHC = immunohistochemistry, WB = Western Blot)						

FIG. 23

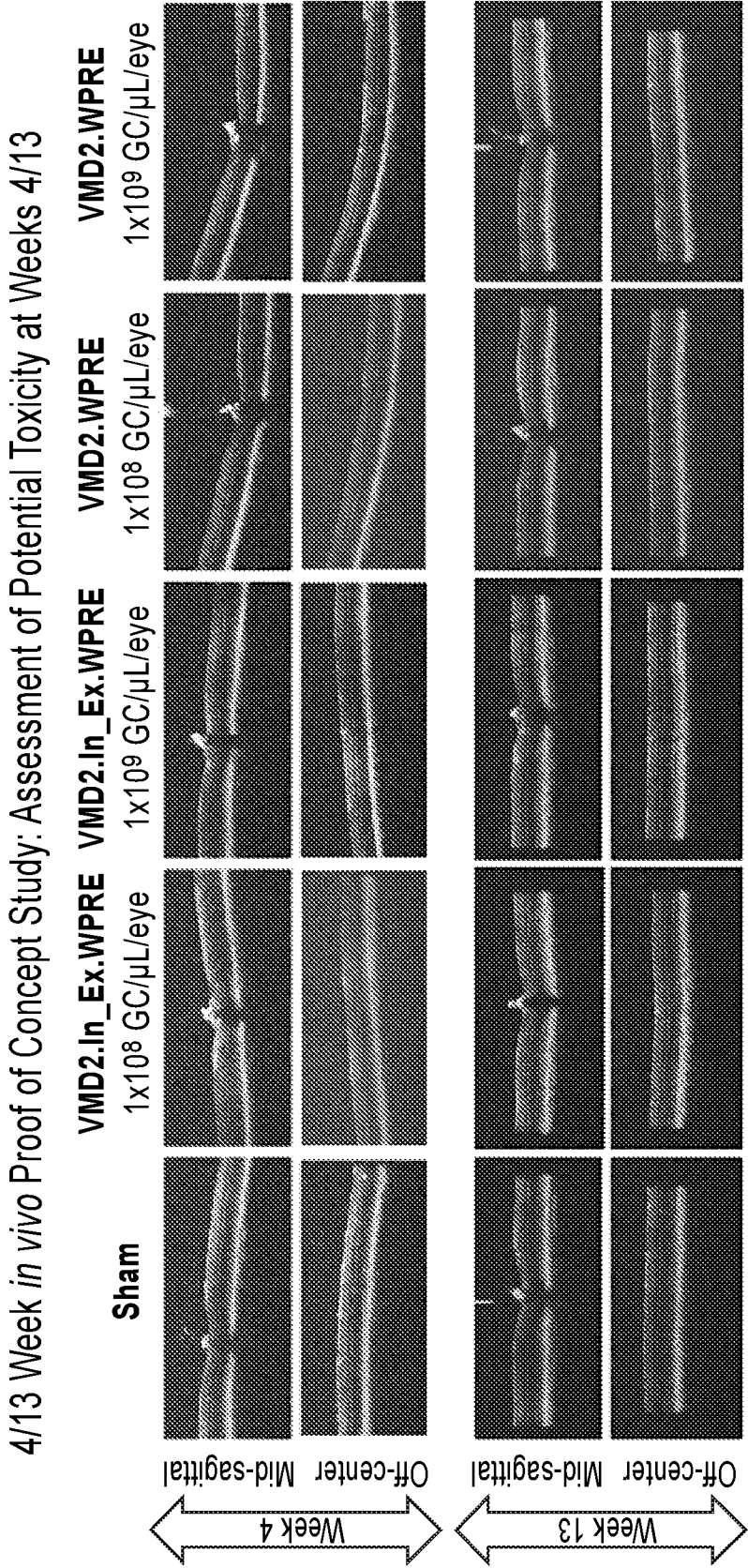


FIG. 24

4/13 Week *in vivo* Proof of Concept Study: Expression and Localization by Immunohistochemistry at Week 4

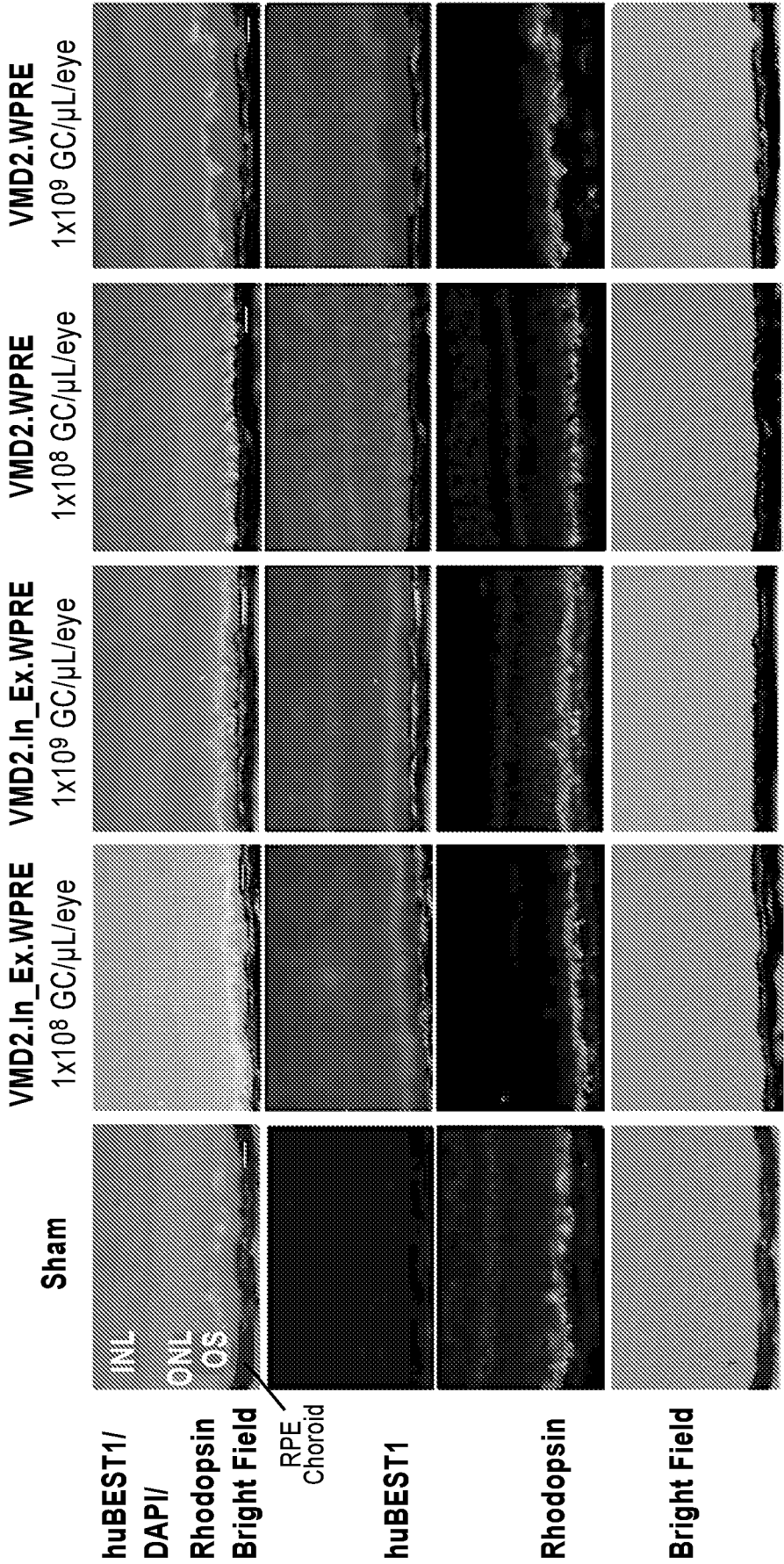


FIG. 25A

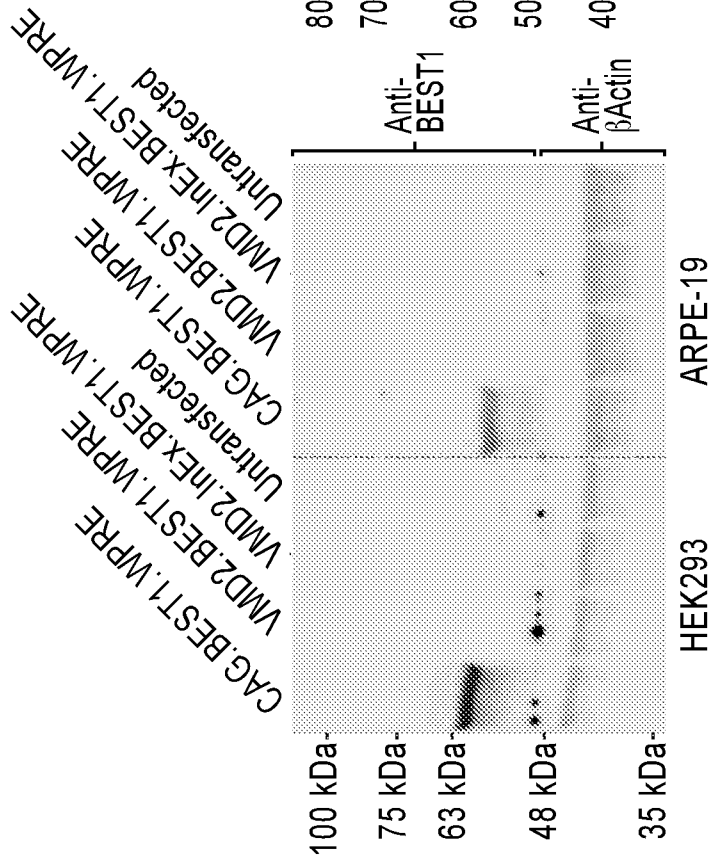
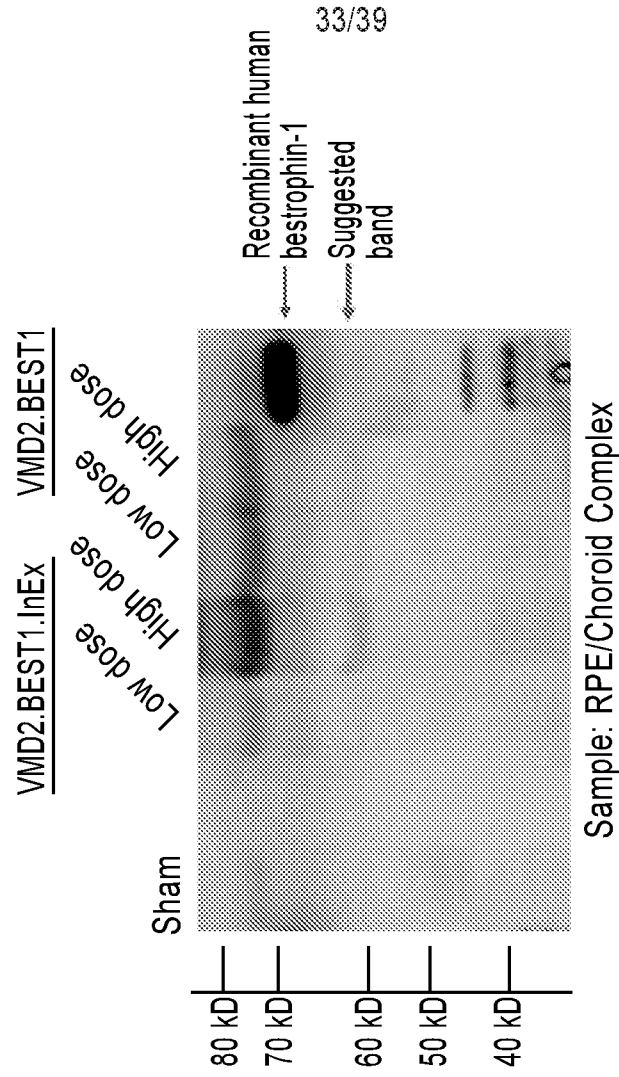


FIG. 25B



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FIG. 26

4 Weeks *in vivo* Study for Assaying Expression by Western Blot: Protocol

Animal	Group	# of Animals	Week 4	
			IHC	WB
C57BL/6 Mice (20 in total)	VMD2.In_Ex.WPRE 1 x 10 ⁹ GC/ μ L/eye	10 mice / 20 eyes	4 eyes	16 eyes
In-life Termination				
Results of Expression (Western Blot, WB) (4 eyes each group were saved for Immunohistochemistry, IHC)				

FIG. 27

Proposed Good Laboratory Practice Study in Mice:
- Evaluation of Potential Toxicity Study and
Biodistribution in Major Organs

Group	Main Study (4 weeks)		Recovery Study (26 weeks)	
	Males	Females	Males	Females
Vehicle Control	8	8	8	8
Low Dose	8	8	8	8
Mid Dose	8	8	8	8
High Dose	8	8	8	8
Total # of Animals	64		64	
Administration	Subretinal injection, bilateral, once on Day 1			
Major Evaluations	• Ophthalmic Examination • Tonometry for IOP • OCT for retinal thickness in recovery groups (predose, and at the end of 4 and 13 weeks) • Necropsy and organ weights • Tissue collection for qPCR: left eye, brain, heart, skeletal muscle, lung, liver, kidney, testes, ovary • Histopathology evaluation: - Standard tissues on control and high dose animal; right eye and gross lesions from the Low and Mid dose groups - Full Ocular histopathology Evaluation: right eye of all animals; and sections/slides save for potential IHC work • Tissue preservation: -Mid and low dose group tissues stored in formalin • qPCR (optional): In tissues with pathological findings			

FIG. 28
4 Weeks *in vivo* Toxicity Assessment Study for Material Evaluation: Protocol

Animal	Group	# of Animals	Week 4	
			OCT	Sacrifice
C57BL/6 Mice (12 in total)	Sham	4	4	4 mice/8 eyes (4-IHC; 4-WB)
	VMD2.IntEx.WPRE 2 x 10 ⁹ GC/ μ L/eye	4	4	4 mice/8 eyes (4-IHC; 4-WB)
	VMD2.IntEx.WPRE 5 x 10 ⁹ GC/ μ L/eye	4	4	4 mice/8 eyes (4-IHC; 4-WB)
In-life Termination				
Results of Localisation (Immunohistochemistry, IHC), Expression (Western Blot, WB) and Retinal Toxicity (OCT)				

FIG. 29

Proposed Good Laboratory Practice Study and Evaluation for Potential Toxicity in a Non Human Primate

Group	Main Study (26 weeks)
Vehicle Control	3 males (juvenile, ~24 months)
High Dose	3 males (juvenile, ~24 months)
Administration	Subretinal injection, bilateral, once on Day 1
Major Evaluations	• Ophthalmic Examination • Tonometry for IOP • OCT for retinal thickness in recovery groups (predose, the end of weeks 4, 8, 13 and 26) • Necropsy and organ weights • Tissue collection for qPCR: vitreous humor, aqueous humor, sample of retina with choroid in the dose site/bleb area and another outside of this area and optic nerve from the left eye; blood (plasma), heart, brain, liver, kidney, saliva, lacrimal secretion, and spleen. • Histopathology evaluation: - Standard tissues in all animals - Full Ocular histopathology Evaluation: right eye of all animals; and sections/sides save for potential IHC work • qPCR: in collected tissues mentioned above (plus the historic data of AAV2 Biodistribution data)

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FIG. 30

Doses at 2E12 GMP Concentrations for Toxicity Assessment Study

	Low Dose (gp)	Middle Dose (gp)	High Dose (gp)
Tox mouse dose (per eye, per 1 μ L)	2×10^8	2×10^9	5×10^9
Tox HED (VV)*	2×10^{11}	2×10^{12}	5×10^{12}
Proposed human dose (per 100 μ L)	2×10^{10}	6×10^{10}	2×10^{11}
Human safety margin (vs high clinical dose)	X 1	X 10	X 25

*HED - human equivalent dose based on vitreal volume (1000:1 VV difference)

FIG. 31
Doses and Concentrations

	Dose 1 (gp)	Dose 2 (gp)	Dose 3 (gp)	Dose 4 (gp)**
mouse dose (per eye, per 1 μ L)	5×10^8	1.5×10^9	5×10^9	5×10^9
NHP equiv. dose (per 100 μ L)	1.2×10^{11}	4.5×10^{11}	1.2×10^{12}	1.2×10^{12}
HED* (Vv)	5×10^{11}	1.5×10^{12}	5×10^{12}	5×10^{12}
Proposed human dose (per 100 μ L)	5×10^{10}	1.5×10^{11}	5×10^{11}	1.5×10^{12}
Human safety margin (vs. high clinical dose) Mouse/NHP	X100/X24	X30/X7.2	X10/X2.4	X3.3/X0.8
Batch Concentration Required			5×10^{12} DRP/mL	1.5×10^{13} DRP/mL

• Include at least 5×10^{12} batch concentration to reach optimal efficacy in Cohort 3 (5E11 dose)

• If MTD not reached in Cohort 3 (5E11 dose), could escalate to 1.5E12 dose in Cohort 4; however, a dose of 1.5E12 correlates to a 1.5E13 batch concentration and may carry risk of toxicity, based on preliminary data reviewed.

*HED

• Human equivalent dose based on vitreal volume (mouse) (1000:1 W difference)

• Human equivalent dose based on vitreal volume (NHP) (2.4:1 W difference)

** potential toxicity

FIG. 1A

