

ON-bipolar cell-specific promoters for ocular gene delivery

The present invention relates to synthetic retinal ON-bipolar cell-specific promoter sequences and their use in therapeutic transgene delivery to the eye for the improvement and/or restoration of vision. The invention features metabotropic glutamate receptor 6 (mGluR6)
5 promoters for an increased and more specific expression in ON-bipolar cells. In particular, in efficient expression in cone ON-bipolar cells exclusively present in the human macula.

DescriptionBackground

Many causes for blindness have only limited treatment possibilities or no cure at all. Most
10 prevalent among them are age-related macular degeneration (AMD) and inherited retinal diseases (IRDs) like retinitis pigmentosa (RP). These degenerative diseases are characterized by the progressive loss of photoreceptors (PRs) which eventually leads to complete blindness. Gene therapies, delivering curative DNA or RNA, replacing or silencing defect genes or encoding an exogenous curative gene are aimed at slowing disease
15 progression, ameliorating symptoms or introducing lost function.

Optogenetic gene therapy is one of the most promising emerging technologies which could be employed for the treatment of blindness caused by retinal degeneration. Ongoing clinical trials of optogenetic therapies unspecifically target retinal ganglion cells (RGCs) with channelrhodopsins to reintroduce light sensitivity to the retina. In the future, next-generation
20 cell-tailored optogenetic gene therapies will prove superior to these unspecific therapies. These next-generation therapies employ cell type-specific promoters to deliver novel and effective optogenetic tools to specific cell types of the retina. Most promising among cell type targets are retinal bipolar cells (BCs), the first interneurons of the retina that naturally receive direct input from the PRs. BCs are divided into ON- and OFF-type BCs, responding to either
25 light increments or decrements, respectively, and expressing either mGluR6 or AMPA/Kainate glutamate receptors. ON-bipolar cells (OBCs) are particularly interesting targets for gene therapy. Mutations in OBC specific genes such as *NYX*, *GRM6*, *GPR179* or *TRPM1* all lead to complete blindness (congenital stationary night blindness) since these genes are involved in the mGluR6 signalling cascade and OBCs consequently become non-
30 functional. More recently, expression of optogenetic proteins in OBCs has proven to restore vision in photoreceptor degenerative mouse models suffering from late stages of degeneration. Channelrhodopsin-2 (Lagali et al., Nat Neurosci 2008. **11** :p. 667-675), rhodopsin (Cehajic-Kapetanovic et al., Curr Biol 2015. **25** : p. 2111-2122) and chimeric Opto-mGluR6 (van Wyk et al., PLoS Biol 2015. **13**: p. e1002143) have been successfully

expressed in the OBCs of blind mice and restored functional vision at the retinal, cortical and behavioural levels. For all-above mentioned approaches the OBC type needs to be targeted specifically, in particular in the case of optogenetic approaches to avoid controversial signalling from off-target cells corrupting the retinal code. In addition, specific OBC targeting also allows for lower and thus safer AAV dosing. The lack of a functional and OBC-specific promoter hitherto prevented the clinical application of OBC-targeted gene therapies.

Short enhancer promoter sequences were typically employed in the field to achieve OBC-specific targeting in combination with an AAV-based gene therapy. This, since the packaging capacity of an AAV is limited to 4.7 kb and does typically not accommodate endogenous promoters of several kb in length. In this respect, enhancer promoter sequences derived from the OBC-specific mGluR6 glutamate receptor, exclusively expressed in the OBCs of the retina, have proven most successful. Until recently, a 200 bp long enhancer sequence derived from the murine Grm6 gene and in combination with an SV40 viral core promoter (Kim et al. J Neurosci, 2008. **28**: p. 7748-7764.), abbreviated as 200En-SV40, was standardly used. However, the inventors recently showed that a variant thereof, 4x200En-SV40, which carries the enhancer sequence in quadruple (Cronin et al. EMBO Mol Med 2014. **6**: p. 1175-1190) is neither OBC-specific nor functional in advanced degenerated retina (van Wyk et al. Front Neurosci 2017. **11**: p. 161). More recently, an entirely murine Grm6 gene based short enhancer/promoter was designed (200En-mGluR500P) that expresses in the wild type C57BL/6 mouse retina with relatively good OBC specificity (Lu et al. Gene Ther, 2016. **23**: p. 680-9.). Nonetheless, expression in OBCs of the degenerated retina was not shown and expression was almost exclusively driven in the rod-type OBC. Therefore, the cone-type OBCs found exclusively in the macula of the retina of foveated animals – including humans – and connecting to foveal cones mediating high-acuity colour vision are virtually not targeted by 200En-mGluR500P, rendering this promoter not suited for restoration of high-acuity central human vision. In addition, a human GRM6 gene based promoter is favourable since it will be fully controlled by the human transcriptomic machinery, regulating gene expression – i.e. expression of protein levels mediating function but devoid of inducing cytotoxicity.

Based on the above-mentioned state of the art, the objective of the present invention is to provide means and methods to provide novel synthetic OBC-specific human promoters. This objective is attained by the subject-matter of the independent claims of the present specification.

Summary of the invention

A first aspect of the invention relates to an isolated nucleic acid molecule of 850 base pairs (bp) to 1500 bp length comprising

- a. an enhancer sequence element selected from SEQ ID NO 1 to 6, and
- b. a promoter sequence element selected from SEQ ID NO 7 to 10.

An alternative of the first aspect of the invention relates to an isolated nucleic acid molecule of 850 base pairs (bp) to 1500 bp length comprising

- 5 a. an enhancer sequence element being at least ($\geq$) 70%, particularly $\geq 75\%$, more particularly $\geq 80\%$, more particularly $\geq 85\%$, more particularly $\geq 90\%$, more particularly $\geq 95\%$, even more particularly $\geq 98\%$, most particularly 100% identical to a sequence selected from SEQ ID NO 1 and 2; and
- 10 b. a promoter sequence element being $\geq 70\%$, particularly $\geq 75\%$, more particularly $\geq 80\%$, more particularly $\geq 85\%$, more particularly $\geq 90\%$, more particularly $\geq 95\%$, even more particularly $\geq 98\%$, most particularly 100% identical to a sequence of SEQ ID NO 7;
- 15 and said isolated nucleic acid molecule has $\geq 40\%$, particularly $\geq 50\%$, more particularly $\geq 60\%$, even more particularly $\geq 70\%$, more particularly $\geq 80\%$, even more particularly $\geq 90\%$, most particularly 100% of the cone ON bipolar cell-specificity from a sequence of SEQ ID NO 13 and a cone ON bipolar cell preference of $\geq 20\%$, particularly $\geq 25\%$, more particularly $\geq 30\%$, even more particularly $\geq 35\%$, more particularly $\geq 40\%$, most particularly $\geq 50\%$.

20 A second aspect of the invention relates to a nucleic acid expression vector comprising a nucleic acid molecule according to the first aspect.

A third aspect of the invention relates to the transgene driven by the promoter.

A fourth aspect of the invention relates to an adeno-associated virion particle comprising the isolated nucleic acid molecule according to the first aspect, the nucleic acid expression vector according to the second aspect or the transgene according to the third aspect.

25 A fifth aspect of the invention relates to an agent selected from the isolated nucleic acid molecule according to the first aspect, the nucleic acid expression vector according to the second aspect, the transgene according to the third aspect and the adeno-associated virion particle according to the fourth aspect for use as a medicament.

30 Administration forms comprising the agents of the invention are further aspects of the invention.

Terms and definitions

The term OBC in the context of the present specification relates to ON-bipolar cell.

The term RBC in the context of the present specifications relates to rod bipolar cell.

The term cOBC in the context of the present specifications relates to cone ON-bipolar cell.

The term RGC in the context of the present specification relates to retinal ganglion cell.

The term PR in the context of the present specification relates to photoreceptor.

5 The abbreviation AAV in the context of the present specification relates to adeno-associated virus. Except otherwise stated, AAV refers to all subtypes or serotypes and both replication-competent and recombinant forms.

10 The terms *AAV virion* and *AAV viral particle* in the context of the present specification relate to a viral particle composed of at least one AAV capsid protein and an encapsidated nucleic acid. Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art (e.g., in cell culture, molecular genetics, nucleic acid chemistry, hybridization techniques and biochemistry). Standard techniques are used for molecular, genetic and biochemical methods (see generally, Sambrook et al., *Molecular Cloning: A Laboratory Manual*, 2d ed. (1989) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. and Ausubel et al., *Short Protocols in Molecular Biology* (1999) 4th Ed, John Wiley & Sons, Inc.) and chemical methods.

15 The term *AAV capsid* in the context of the present specification relates to synthetic capsid (*cap*) genes. The *AAV capsid* disclosed herein may be used to package recombinant adeno-associated viruses for gene therapy.

20 The term *homologous* in the context of the present specification relates to sequences sharing a large part of their sequence, but differ in some positions by insertion, deletion or substitution of nucleic acids or amino acids.

25 The term *transgene* in the context of the present specification relates to a gene or genetic material that has been transferred from one organism to another. In the present context, the term may also refer to transfer of the natural or physiologically intact variant of a genetic sequence into tissue of a patient where it is missing. It may further refer to transfer of a natural encoded sequence the expression of which is driven by a promoter absent or silenced in the targeted tissue. The term *transgene* as used herein refers to a polynucleotide encoding a polypeptide of interest, which, when expressed in the damaged or diseased retina may be useful for improving or restoring vision. Transgenes of particular interest for restoration of photosensitivity or vision include photosensitive proteins, such as opsin genes, i.e. Channelrhodopsins, vertebrate opsins and variants thereof.

30 The term *recombinant* in the context of the present specification relates to a nucleic acid, which is the product of one or several steps of cloning, restriction and/or ligation and which is different from the naturally occurring nucleic acid. A recombinant virus particle comprises a recombinant nucleic acid.

The term *intravitreal administration* in the context of the present specification relates to a route of administration of a pharmaceutical agent, for example a virus, in which the agent is delivered into the vitreous body of the eye. Intravitreal administration is a procedure to place a medication directly into the space in the back of the eye called the vitreous cavity, which is filled with a jelly-like fluid called the vitreous humour gel.

The term *subretinal administration* in the context of the present specification relates to a route of administration of a pharmaceutical agent, particularly a virus in the context of this specification, into the space between retinal pigment epithelium (RPE) cells and photoreceptors.

10 “Nucleotides” in the context of the present specification are nucleic acid or nucleic acid analogue building blocks, oligomers of which are capable of forming selective hybrids with RNA or DNA oligomers on the basis of base pairing. The term nucleotides in this context includes the classic ribonucleotide building blocks adenosine, guanosine, uridine (and ribosylthymine), cytidine, the classic deoxyribonucleotides deoxyadenosine, deoxyguanosine, thymidine, deoxyuridine and deoxycytidine.

In the context of the present specifications the terms *sequence identity* and *percentage of sequence identity* refer to the values determined by comparing two aligned sequences. Methods for alignment of sequences for comparison are well-known in the art. Alignment of sequences for comparison may be conducted by the local homology algorithm of Smith and Waterman, Adv. Appl. Math. 2:482 (1981), by the global alignment algorithm of Needleman and Wunsch, J. Mol. Biol. 48:443 (1970), by the search for similarity method of Pearson and Lipman, Proc. Nat. Acad. Sci. 85:2444 (1988) or by computerized implementations of these algorithms, including, but not limited to: CLUSTAL, GAP, BESTFIT, BLAST, FASTA and TFASTA. Software for performing BLAST analyses is publicly available, e.g., through the National Center for Biotechnology-Information (<http://blast.ncbi.nlm.nih.gov/>).

One such example for comparison of nucleic acid sequences is the BLASTN algorithm that uses the default settings: Expect threshold: 10; Word size: 28; Max matches in a query range: 0; Match/Mismatch Scores: 1.-2; Gap costs: Linear. Unless stated otherwise, sequence identity values provided herein refer to the value obtained using the BLAST suite of programs (Altschul et al., J. Mol. Biol. 215:403-410 (1990)) using the above identified default parameters for protein and nucleic acid comparison, respectively.

In the context of the present specification, the term *upstream* refers to a direction towards the 5' end. For enhancer and promoter sequences, single-stranded sequences are given in this application and when the enhancer is upstream of the promoter, this means that the enhancer is in 5'-direction of the promoter. Analogously, the term *downstream* refers to a direction towards the 3' end.

In the context of the present specification, the term *spacer sequence* refers to a nucleic acid of variable length that is used to connect the enhancer and the promoter in order to generate a single chain nucleic acid molecule. Exemplary embodiments of linkers useful for practicing the invention specified herein are oligo nucleic acid chains consisting of 1 to 1000 nucleic acids.

The **cone ON bipolar cell (cOBC) -specificity** in human retinal explants is measured using the following protocol.

First, the promoter is combined with the reporter transgene mCitrine and packaged into the self-complementary (sc) AAV vector scAAV2(7m8) (Dalkara et al. Sci Transl Med 2013. 5: p. 189ra76). Approximately 10^{10} vg (vector genomes) are added to the RGC side of cultured post-mortem human retinal explants at day 0 as described in detail in (van Wyk et al. Front Neurosci 2017. 11: p.161). Retinas are fixed at day 7 of culture with 4% PFA and subsequently cryoprotected (10/20/30% sucrose in PBS) and frozen. Retinal cryosections are triple-stained with antibodies against the transgene mCitrine (Invitrogen, A11122, 1:500), the ubiquitous OBC marker Gao (EMD, MAB3073, 1:750) and the RBC specific antibody PKC α (Santa Cruz, sc8393, 1:750). Expressing RBCs are identified as [mCitrine(+), PKC α (+)], whereas [mCitrine(+), PKC α (-), Gao(+)] cells are identified as expressing cOBCs. cOBC type specificity is determined by the ratio of expressing cOBCs of all expressing OBCs:

$$\left[\frac{N\{\text{mCitrine}(+), \text{PKC}\alpha(-), \text{Gao}(+)\}}{N\{\text{mCitrine}(+), \text{Gao}(+)\}} \right] \quad (1)$$

With N being the number of cells with the staining characteristics given in brackets.

The **cone ON bipolar cell preference** is subsequently determined as follows.

The amount of RBCs and cOBCs is not identical and varies in different retinal regions. The explants are produced from the mid-periphery of the retina where the ratio of RBCs to cOBCs

$$\left[\frac{N\{\text{PKC}\alpha(+)\}}{N\{\text{PKC}\alpha(-), \text{Gao}(+)\}} \right] \quad (3)$$

is approximately constant over the small area of the explant. Consequently, the ratio of expressing cOBCs to expressing RBCs

$$\left[\frac{N\{\text{mCitrine}(+), \text{PKC}\alpha(-), \text{Gao}(+)\}}{N\{\text{mCitrine}(+), \text{PKC}\alpha(+), \text{Gao}(+)\}} \right] \quad (4)$$

can be assumed to be constant in the explant as well. This allows the calculation of the cOBC over RBCs preference ratio factor

$$x = \frac{N\{\text{PKC}\alpha(+)\} * N\{\text{mCitrine}(+), \text{PKC}\alpha(-), \text{Gao}(+)\}}{N\{\text{PKC}\alpha(-), \text{Gao}(+)\} * N\{\text{mCitrine}(+), \text{PKC}\alpha(+), \text{Gao}(+)\}} \quad (5)$$

which accounts for the specific distribution of cOBCs and RBCs in the explant, by multiplying (3) with (4). The cOBC preference in percent is then calculated with

$$\frac{100\% \cdot x}{1+x} \quad (6)$$

As used herein, the term *treating* or *treatment* of any disease or disorder (e.g. loss of vision) refers in one embodiment, to ameliorating the disease or disorder (e.g. slowing or arresting or reducing the development of the disease or at least one of the clinical symptoms thereof). In another embodiment "treating" or "treatment" refers to alleviating or ameliorating at least one physical parameter including those which may not be discernible by the patient. In yet another embodiment, "treating" or "treatment" refers to modulating the disease or disorder, either physically, (e.g., stabilization of a discernible symptom), physiologically, (e.g., stabilization of a physical parameter), or both. In yet another embodiment, "treating" or "treatment" refers to introducing an exogenous, therapeutic function into a target cell type. Methods for assessing treatment and/or prevention of disease are generally known in the art, unless specifically described herein below.

15 Detailed description of the invention

This invention discloses human GRM6 enhancer promoter sequences with enhanced OBC-specificity and far enhanced cOBC-induced protein expression compared to 200En-mGluR500P, in mouse and human post-mortem retina. The promoters described herein consist of a modified metabotropic glutamate receptor 6 (mGluR6) promoter that contains sequences from regulatory elements that direct the expression of the mGluR6 protein to OBCs, in particular RBCs and cOBCs. The invention features an isolated nucleic acid molecule or a nucleic acid expression vector comprising an mGluR6 enhancer or a variant thereof and an mGluR6 promoter or a variant thereof. This novel GRM6 enhancer promoter sequence drives efficient transgene expression for the first time in cOBCs of the human retina, in particular of the human parafovea. Further, the novel human GRM6 enhancer promoter sequences in combination with an optogene (MWOPN_mGluR6, SEQ ID NO: 16) led to widespread OBC-specific expression in the degenerated murine (*rd1*, C3HHe/OuJ) retina and restored functional vision (optomotor response) in otherwise blind, photoreceptor degenerated mice. The novel human GRM6 enhancer/promoter showed highly efficient, widespread and specific OBC targeting in mouse and human retina.

A first aspect of the invention relates to an isolated nucleic acid molecule comprising

- a. an enhancer sequence element selected from SEQ ID NO 1 to 6, and
- b. a promoter sequence element selected from SEQ ID NO 7 to 10.

An alternative of the first aspect of the invention relates to an isolated nucleic acid molecule of 850 base pairs (bp) to 1500 bp length comprising

- a. an enhancer sequence element selected from SEQ ID NO 1 to 6, and
- a promoter sequence element selected from SEQ ID NO 7 to 10.

5 Another alternative of the first aspect of the invention relates to an isolated nucleic acid molecule comprising

- a. an enhancer sequence element being $\geq 70\%$, particularly $\geq 75\%$, more particularly $\geq 80\%$, more particularly $\geq 85\%$, more particularly $\geq 90\%$, more particularly $\geq 95\%$, even more particularly $\geq 98\%$, most particularly 100% identical to a sequence selected from SEQ ID
10 NO 1 and 2; and
- b. a promoter sequence element being $\geq 70\%$, particularly $\geq 75\%$, more particularly $\geq 80\%$, more particularly $\geq 85\%$, more particularly $\geq 90\%$, more particularly $\geq 95\%$, even more particularly $\geq 98\%$, most particularly 100% identical to a sequence of SEQ ID NO 7;
15 and said isolated nucleic acid molecule has $\geq 40\%$, particularly $\geq 50\%$, more particularly $\geq 60\%$, even more particularly $\geq 70\%$, more particularly $\geq 80\%$, even more particularly $\geq 90\%$, most particularly 100% of the cone ON bipolar cell-specificity from a sequence of SEQ ID NO 13 and a cone ON bipolar cell preference of $\geq 20\%$, particularly $\geq 25\%$, more particularly $\geq 30\%$, even more particularly $\geq 35\%$, more particularly $\geq 40\%$, most particularly $\geq 50\%$.

20 Another alternative of the first aspect of the invention relates to an isolated nucleic acid molecule of 850 base pairs (bp) to 1500 bp length comprising

- a. an enhancer sequence element being $\geq 70\%$, particularly $\geq 75\%$, more particularly $\geq 80\%$, more particularly $\geq 85\%$, more particularly $\geq 90\%$, more particularly $\geq 95\%$, even more particularly $\geq 98\%$, most particularly 100%
25 identical to a sequence selected from SEQ ID NO 1 and 2; and
- b. a promoter sequence element being $\geq 70\%$, particularly $\geq 75\%$, more particularly $\geq 80\%$, more particularly $\geq 85\%$, more particularly $\geq 90\%$, more particularly $\geq 95\%$, even more particularly $\geq 98\%$, most particularly 100% identical to a sequence of SEQ ID NO 7;
30 and said isolated nucleic acid molecule has $\geq 40\%$, particularly $\geq 50\%$, more particularly $\geq 60\%$, even more particularly $\geq 70\%$, more particularly $\geq 80\%$, even more particularly $\geq 90\%$, most particularly 100% of the cone ON bipolar cell-specificity from a sequence of SEQ ID NO 13 and a cone ON bipolar cell

preference of $\geq 20\%$, particularly $\geq 25\%$, more particularly $\geq 30\%$, even more particularly $\geq 35\%$, more particularly $\geq 40\%$, most particularly $\geq 50\%$.

The cOBC specificity and the cOBC expression level is measured as described above.

5 The inventors have shown that a combination of SEQ ID NO 1 or 2 with SEQ ID NO 7 results in a high cone ON bipolar cell-specificity and a high cone ON bipolar cell expression level. The skilled person in the art is able to find similar sequences with equal cone ON bipolar cell-specificity and cone ON bipolar cell expression level based on the disclosure of this invention.

10 In certain embodiments, the enhancer sequence element is upstream of the promoter sequence element.

In certain embodiments, the isolated nucleic acid molecule additionally comprises a spacer sequence of length 1 to 1000 basepairs, particularly 1 to 394 basepairs. In certain embodiments, the spacer is located between the enhancer and the promoter. In certain embodiments, the isolated nucleic acid molecule additionally comprises a spacer sequence
15 of length 1 to 1000 basepairs, particularly 1 to 394 basepairs, and the spacer is located between the enhancer and the promoter.

In certain embodiments, the isolated nucleic acid molecule comprises a sequence selected from SEQ ID NO 11 - SEQ ID NO 15.

20 In certain embodiments, the isolated nucleic acid molecule comprises the sequence SEQ ID NO 11 or SEQ ID NO 13.

A second aspect of the invention relates to a nucleic acid expression vector comprising a nucleic acid molecule according to the first aspect.

In certain embodiments, the viral vector is a viral genome.

25 In certain embodiments, the vector is an adeno-associated virus vector or a recombinant adeno-associated vector (rAAV).

In certain embodiments, the AAV vector is either a single-stranded vector (ssAAV) or a self-complementary vector (scAAV).

30 In certain embodiments, the vector is a recombinant AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, or AAV12 vector. In certain embodiments, the vector is a recombinant AAV2 vector.

In certain embodiments, the nucleic acid expression vector additionally comprises

- a. a sequence encoding a capsid protein, and
- b. a transgene.

From 5'-end to 3'-end, the isolated nucleic acid molecule comprises first the enhancer, then optionally the spacer and then the promoter. The transgene is located in 3'-direction of the promoter. In certain embodiments, the transgene is preceded by an optimized KOZAK sequence.

- 5 The KOZAK sequence has the consensus (gcc)gccAccAUGG (SEQ ID NO 24) or (gcc)gccGccAUGG (SEQ ID NO 25) and is important in the initiation of the translation.

In certain embodiments, the nucleic acid expression vector also comprises a WPRE (Woodchuck hepatitis virus post-transcriptional regulatory element) regulatory sequence. The WPRE is a DNA sequence that, when transcribed, creates a tertiary structure enhancing expression. In certain embodiments, the nucleic acid expression vector also comprises a polyA tail, which is inserted downstream of the transgene. The polyA tail promotes translation of the transgene.

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In certain embodiments, the capsid protein is AAV2, AAV2(7m8) or AAV8(BP2).

A third aspect of the invention relates to the transgene driven by the promoter.

- 15 In certain embodiments, the transgene is *NYX*, *GRM6*, *GPR179* or *TRPM1* to restore light sensitivity or vision in congenital stationary night blindness.

In certain embodiments, the transgene comprises or essentially consists of the sequence of SEQ ID NO 16.

In certain embodiments, the transgene is an opsin gene restoring light detection or vision.

- 20 In certain embodiments, the opsin gene is selected from the group consisting of channelrhodopsin, melanopsin, rhodopsin, cone opsins, pineal opsin, photopsins, halorhodopsin, bacteriorhodopsin, proteorhodopsin, jellyfish opsin, jumping spider opsin or any functional variant or fragment thereof.

In certain embodiments, the opsin gene is a chimeric protein between an opsin and the metabotropic glutamate receptor mGluR6 of retinal OBCs.

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In certain embodiments, the chimeric protein is Opto-mGluR6.

In certain embodiments, the chimeric protein is murine or human MWOPN_mGluR6 (SEQ ID NO: 16).

- A fourth aspect of the invention relates to an adeno-associated virion particle comprising the isolated nucleic acid molecule according to the first aspect or the nucleic acid expression vector according to the second aspect.
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A fifth aspect of the invention relates to an agent selected from the isolated nucleic acid molecule according to the first aspect or the nucleic acid expression vector according to the

second aspect, and the adeno-associated virion particle according to the third and fourth aspects for use as a medicament.

5 A further aspect relates to an agent selected from the isolated nucleic acid molecule according to the first aspect, the nucleic acid expression vector according to the second aspect, the transgene according to the third aspect and the adeno-associated virion particle according to the fourth aspect for use in treatment of a condition affecting a retinal bipolar cell.

10 A further aspect relates to an agent selected from the isolated nucleic acid molecule according to the first aspect, the nucleic acid expression vector according to the second aspect, the transgene according to the third aspect and the adeno-associated virion particle according to the fourth aspect for use in treatment of congenital stationary night blindness or rod-cone and cone-rod dystrophies, in particular of retinitis pigmentosa and macular degeneration.

15 A further aspect relates to an agent selected from the isolated nucleic acid molecule according to the first aspect, the nucleic acid expression vector according to the second aspect, the transgene according to the third aspect and the adeno-associated virion particle according to the fourth aspect, wherein the agent is administered by

- a. intravitreal administration, particularly by intravitreal injection, or by
- b. subretinal injection.

20 A further aspect relates to a method of treatment administering the agent of the invention to a patient in need thereof.

25 Wherever alternatives for single separable features such as, for example, a promoter sequence or medical indication are laid out herein as "embodiments", it is to be understood that such alternatives may be combined freely to form discrete embodiments of the invention disclosed herein. Thus, any of the alternative embodiments for a promoter sequence may be combined with any medical indication compromising OBC function and any DNA delivery vehicle or method, including alternative viruses, nanoparticles, liposomes or "naked" DNA delivery by using, for example, a gene gun or electroporation.

30 A non-limiting list of retinal diseases that may benefit from the methods described herein include congenital night blindness, macular degeneration, age-related macular degeneration, congenital cone dystrophies and a large group of retinitis pigmentosa (RP)-related disorders.

Items

1. An isolated nucleic acid molecule of 850 base pairs (bp) to 1500 bp length, comprising

- a. an enhancer sequence element selected from SEQ ID NO 1 to 6, and
 - b. a promoter sequence element selected from SEQ ID NO 7 to 10.
2. An isolated nucleic acid molecule of 850 base pairs (bp) to 1500 bp length, comprising
 - 5 a. an enhancer sequence element being at least ($\geq$) 70%, particularly $\geq$ 75%, $\geq$ 80%, more particularly $\geq$ 85%, more particularly $\geq$ 90%, more particularly $\geq$ 95%, even more particularly $\geq$ 98%, most particularly 100% identical to a sequence selected from SEQ ID NO 1 and 2; and
 - 10 b. a promoter sequence element being $\geq$ 70%, particularly $\geq$ 75%, more particularly $\geq$ 80%, more particularly $\geq$ 85%, more particularly $\geq$ 90%, more particularly $\geq$ 95%, even more particularly $\geq$ 98%, most particularly 100% identical to a sequence of SEQ ID NO 7;
 - 15 and said isolated nucleic acid molecule has $\geq$ 40%, particularly $\geq$ 50%, more particularly $\geq$ 60%, even more particularly $\geq$ 70%, more particularly $\geq$ 80%, even more particularly $\geq$ 90%, most particularly 100% of the cone ON bipolar cell-specificity from a sequence of SEQ ID NO 13 and a cone ON bipolar cell preference of $\geq$ 20%, particularly $\geq$ 25%, more particularly $\geq$ 30%, even more particularly $\geq$ 35%, more particularly $\geq$ 40%, most particularly $\geq$ 50%.
- 20 3. The isolated nucleic acid molecule according to item 1 or 2, wherein the isolated molecule consists of one and only one of said enhancer sequence elements, one and only one of said promoter sequence elements and optionally, a spacer separating the enhancer sequence element from the promoter sequence element.
- 25 4. The isolated nucleic acid molecule according to any one of the preceding items comprising or consisting of a sequence selected from SEQ ID NO 11 - SEQ ID NO 15, or comprising or consisting of a sequence characterized by $\geq$ 98% identity to a sequence selected from SEQ ID NO 11 - SEQ ID NO 15.
- 30 5. The isolated nucleic acid molecule according to any one of the preceding items comprising or consisting of the sequence SEQ ID NO 11 or SEQ ID NO 13, or comprising or consisting of a sequence characterized by $\geq$ 98% identity to SEQ ID NO 11 or SEQ ID NO 13, particularly comprising or consisting of the sequence SEQ ID NO 13, or comprising or consisting of a sequence characterized by $\geq$ 98% identity to SEQ ID NO 13.
6. A nucleic acid expression vector comprising a nucleic acid molecule according to any one of the previous items.

7. The nucleic acid expression vector according to item 6, wherein the nucleic acid expression vector is an adeno-associated virus vector or a recombinant adeno-associated vector (rAAV), particularly wherein the nucleic acid expression vector is a recombinant AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, or AAV12 vector, more particularly wherein the nucleic acid expression vector is a recombinant AAV2 vector.
8. The nucleic acid expression vector according to any one of items 6 to 7, additionally comprising
- a sequence encoding a capsid protein, and
 - a transgene.
9. The nucleic acid expression vector according to item 8, wherein the transgene comprises the sequence of SEQ ID NO 16.
10. An adeno-associated virion particle comprising the isolated nucleic acid molecule according to any one of items 1 to 5 or the nucleic acid expression vector according to any one of items 6 to 9.
11. An agent selected from the isolated nucleic acid molecule according to any one of items 1 to 5 or the nucleic acid expression vector according to any one of items 6 to 9, and the adeno-associated virion particle according to item 10 for use as a medicament.
12. An agent selected from the isolated nucleic acid molecule according to any one of items 1 to 5, the nucleic acid expression vector according to any one of items 6 to 9, and the adeno-associated virion particle according to item 10 for use in treatment of a condition affecting a retinal bipolar cell, particularly in treatment of congenital stationary night blindness (CSBN1) or rod-cone and cone-rod dystrophies, more particularly of retinitis pigmentosa and macular degeneration.
13. An agent selected from the isolated nucleic acid molecule according to any one of items 1 to 5, the nucleic acid expression vector according to any one of items 6 to 9, and the adeno-associated virion particle according to item 10, wherein the agent is administered by
- intravitreal administration, particularly by intravitreal injection, or by

b. subretinal injection.

The invention is further illustrated by the following examples and figures, from which further embodiments and advantages can be drawn. These examples are meant to illustrate the invention but not to limit its scope.

5 Brief description of the figures

Fig. 1 **Genome browser view of the human GRM6 sequences chosen for promoter design.** (A) Distal enhancer region of human GRM6 (positioned at approx. -14 kb rel. to translation start site (TLSS)), indicating the three selected enhancer elements including the 310 bp conserved region between murine Grm6 and human GRM6 (horizontally striped) as well as the 188 bp syntenic region to Kim's murine 200En(Grm6) (horizontally striped and shaded section). (B) Promoter sequence of human GRM6, including the transcription start site (TSS) and translation start site (TLSS), latter defined as position 0 by the inventors. The two promoters selected are also indicated and the 167 bp conserved region between mouse and human genes shown by the horizontally striped section. The graphs were downloaded from the UCSC Genome Browser at <https://genome.ucsc.edu/> and modified. Grey shaded stretches illustrate potentially relevant cis-regulatory regions, including transcription factor binding sites, interspecies conserved regions (Vert. Cons) and Dnase hypersensitivity clusters (Dnase clusters). Additionally, the track for the H3K27Ac Mark signal peak and Chip-seq peaks were also considered.

Fig. 2 **Promoter driven mCitrine expression strength in OBCs of post-mortem human retinal explants.** Human retinal explants were transduced with scAAV2(7m8)-407En_566P(hGRM6)-mCitrine (n=3), scAAV2(7m8)-444En_454P(hGRM6)-mCitrine (n=3), scAAV2(7m8)-770En_454P(hGRM6)-mCitrine (n=5), scAAV2(7m8)-407En_454P(hGRM6)-mCitrine (n=3) and scAAV(7m8)-200En-mGluR500P-mCitrine (n=4). mCitrine was immunohistochemically labeled and the fluorescence intensity in expressing ON-bipolar cells determined as a measure of transgene expression strength. The 566P promoter element mediated much weaker mCitrine expression in OBCs compared to promoter combinations with proximal element 454P. 454P was therefore selected for all subsequent experiments. 770En_454P(hGRM6) ($F = 5.42 \pm 0.9$; mean $\pm$ s.d.) and 444En_454P(hGRM6) ($F = 5.59 \pm 0.51$; mean $\pm$ s.d.) performed equally well in terms of transgene expression strength and significantly better than the murine genome-derived 200En-mGluR500P ($F = 3.94 \pm 0.45$; mean $\pm$ s.d.). * represents $P \leq 0.05$, ** represents $P \leq 0.01$, *** represents $P \leq 0.001$ and n.s. represents non-significant differences (one-way ANOVA with Tukey's honest significance test).

Fig. 3 **Promoter specificities for cone ON-bipolar cells (cOBCs) in human retinal explants.** Human retinal explants were transduced with scAAV2(7m8)-444En_454P(hGRM6)-mCitrine, scAAV2(7m8)-770En_454P(hGRM6)-mCitrine, scAAV2(7m8)-407En_454P(hGRM6)-mCitrine or scAAV(7m8)-200En-mGluR500P-mCitrine. Vertical cryo-sections were labeled against mCitrine, PKC α (for rod bipolar cells (RBCs) and G α 0 (ubiquitous marker for OBCs) and mCitrine expressing cells were counted. (A) From cell counting, 770En_454P(hGRM6) drove expression in significantly more cOBCs than 200En-mGluR500P. (B) Normalizing the amount of targeted cOBCs and RBCs to their overall number within the particular retinal regions from where the counts were taken visualizes that 770En_454P(hGRM6) and 407En_454P(hGRM6) drive expression much more efficiently in cOBCs than 200En-mGluR500P, which has a clear preference for RBCs. 770En_454P(hGRM6) even shows equal preference for cOBCs and RBCs of 50% each, an important hallmark for foveal gene therapy where only cOBCs exist. (C) The cOBC type preference of promoter 444En_454P(hGRM6) (n=5) was not significantly different to that of promoter 770En_454P(hGRM6). Shown are mean $\pm$ s.d., * represents $P \leq 0.05$, ** represents $P \leq 0.01$ (one-way ANOVA with Tukey's honest significance test). Only significant differences are indicated.

Fig. 4 **OBC expression efficacy and specificity of 770En_454P(hGRM6)-driven mCitrine expression in human retinal explants.** (A) Comparing 770En_454P(hGRM6)-driven mCitrine expression to 200En-mGluR500P driven mCitrine expression when packaged into scAAV2(7m8). 770En_454P(hGRM6) shows a much higher OBC preference compared to 200En-mGluR500P and latter has in addition a significantly higher off-target expression in amacrine cells. Shown are the % of expressing cells of a particular cell type as means $\pm$ s.d., ** represents $P \leq 0.01$ and *** represents $P \leq 0.001$ (Student's T-Test).

Fig. 5 **Promoter 770En_454P(hGRM6) drives transgene expression reliably and wide-spread in the degenerated *rd1* mouse retina.** *rd1* mice were injected with 3×10^9 vg of AAV carrying the transgene MWOPN_mGluR6_IRES2_TurboFP635 (SEQ ID NO: 16, plasmid map Fig. 9) at 22 weeks of age. The sketches in A-D represent the transduced retinal areas in retinal whole mounts in grey. A) ssAAV2(7m8) in combination with 200En-mGluR500P also expresses in the *rd1* retina, but only in restricted areas (as opposed to 4xGrm6-SV40). B) ssAAV(7m8) in combination with 770En_454P(hGRM6) leads to more extensive and widespread transduction of the degenerated retina compared to 200En-mGluR500P, probably due to an increased expression strength which overcomes the threshold for expression when degeneration-caused Grm6 downregulation has occurred. C) Example laser scanning

micrograph of a retinal whole-mount of a treated retina from a *rd1* mouse undergoing OKR testing (see Example 7 and Fig. 6), where TurboFP635 was labelled immunocytochemically (depicted in sketch B). (D) Mean expression specificity (% expressing OBCs of all cells expressing, $66.6 \pm 8.5\%$) and efficiency (% of expressing OBCs of all OBCs, 54.7 ± 8.3) in the *rd1* degenerated retina. Mean $\pm$ s.d., N=9 (Student's T-test).

Fig. 6 **Vision restoration determined by the optomotor reflex in completely photoreceptor-less *rd1* mice intravitreally and bilaterally treated with ssAAV(7m8)-MWOPN_mGluR6_IRES2_TurboFP635 at 22 weeks of age.** Visual acuity was measured 41, 47, 55, 82 and 112 days after transduction by determining the threshold of spatial frequencies at which the optokinetic response was still elicited in a virtual optomotor system. The treated mice (n=3) showed a significant increase in visual acuity compared to non-injected control *rd1* littermates (n=7) but still had significantly lower visual acuity than the wild type control mice (C57BL/6J, n=10). Shown mean (over all trials and individuals) $\pm$ s.d., ** represents $P \leq 0.01$ and *** represents $P \leq 0.001$ (Student's T-test).

Fig. 7 **Highly efficient mCitrine expression in the cOBCs of an explanted human macula by promoter 770En_454P(hGRM6).** Example explant of a human macula transduced with scAAV2(7m8)-770En_454P(hGRM6)-mCitrine and immunohistochemically labeled against the transgene mCitrine as well as for OBCs (Gao) and cellular nuclei (DAPI). (A) is a sketch of the human macula and the areas from where the photomicrographs in B and C were taken. The foveola only contains M- and L-cone photoreceptors and neither OBCs or RGCs, since their cell bodies are pushed aside for the light to pass without diffraction to the photoreceptors. The fovea contains only cones (M, L and S) and is the area of highest acuity vision with the midget system, where each cone connects to one BPC and to one RGC. (B) shows a section through the parafovea, with DAPI (top micrograph) showing a clear layering of photoreceptors (ONL), BPCs and amacrine cells (INL) and the 3-dimensional layering of RGCs (GCL) indicative of the macula. The bottom micrograph shows exclusively the transgene labeling, indicative of mCitrine expression exclusively in the INL where the OBCs are located. (C) shows a section through the fovea. The photomicrograph on the left solely shows Gao labeling of the OBCs, which are all devoid of PKC labeling (not shown here) and therefore clearly identified as cOBCs. The right photomicrograph depicts in addition mCitrine labeling within the cytoplasm, indicating that virtually each cOBC of the fovea is expressing mCitrine (arrowheads). This is a very clear evidence that 770En_454P(hGRM6) drives excellent expression in cOBCs,

in particular cOBCs of the human macula, and is therefore well suited for high acuity vision restoration in human patients.

Fig. 8 **Plasmid map of the AAV plasmid** encoding a cone opsin and mGluR6 chimeric optogenetic protein, MWOPN_mGluR6-IRES2-TurboFP635, under the novel 770En_454P(hGRM6) promoter. TurboFP635 is a red fluorescent protein marker for identification of expression, WPRE and BGHpA are regulatory sequences and the 5' and 3' ITRs (internal repeats) the regions used by the AAV machinery to package the transgene (in between the ITRs) into the capsid. This plasmid was used for the transduction of *rd1* degenerated mouse retinas (Figures and Examples 5 & 6). In-Fusion Primers for cloning are also given.

Fig. 9 **Example of OBC expression specificity and efficacy of 770En_454P(hGRM6) and 444En_454P(hGRM6) driving mCitrine expression in human retinal explants.** Vertical cryosections through human retinal explants transduced with scAAV2(7m8)-770En_454P(hGRM6)-mCitrine (A) and scAAV2(7m8)-444En_454P(hGRM6)-mCitrine (B), respectively. Cryosections were labelled with the nuclear stain DAPI (grey, shown only on the far left of the micrographs for orientation) and against the transgene mCitrine marker (white). 770En_454P(hGRM6) has an OBC efficacy (percent of bright cells in the INL) of $85.2\% \pm 12.3\%$ (n=4) and 444En_454P(hGRM6) of $87.9\% \pm 6.5\%$ (n=5). ONL outer nuclear layer, INL inner nuclear layer, GCL ganglion cell layer. The bipolar cells are located in the peripheral INL.

Examples

Example 1: Analysis of *Grm6* gene expression changes in the *rd1* mouse model

The inventors selected the gene (*Grm6* in mouse and *GRM6* in human) encoding the metabotropic glutamate receptor 6 (mGluR6) selectively expressed in ON-bipolar cells (OBCs) of the retina as a template for promoter design. This was because mGluR6's expression is selective to OBCs, which was recently confirmed by a single-cell transcriptome analyses of adult mouse retina (Siegert et al. Nat Neurosci 2012. 15: p. 487-95) and also clearly obvious in a transgenic mouse line previously generated by the inventors where the full-length *Grm6* promoter drives transgene expression specifically in retinal OBCs (van Wyk et al., PloS Biol 2015. 13: p. e1002143). Further, short promoter versions derived from the murine *Grm6* gene have been successfully constructed and shown to drive preferential expression in OBCs (Cronin et al., EMBO Mol Med, 2014. 6(9): p. 1175-1190; Kim et al. J Neurosci 2008. 28: p. 7748-7764; Lagali et al. Nat Neurosci 2008. 11 p: 667-675). Kim et al. initially chose a distal 200 bp enhancer sequence in the promoter of the murine *Grm6* gene,

which enhanced OBC-specific expression in the wildtype mouse retina. This enhancer sequence was subsequently employed in the 4xGRM6-SV40 promoter [Cronin, T., et al., EMBO Mol Med, 2014. 6(9): p. 1175-1190], which contains four of these 200 bp enhancer sequences in tandem. However, the inventors showed recently that the 4xGRM6-SV40 promoter [Cronin, T., et al., EMBO Mol Med, 2014. 6(9): p. 1175-1190] was completely downregulated in the degenerated *rd1* (C3H/HeOu) mouse retina, even when gene therapy was performed at 3.5 weeks of age before completed photoreceptor degeneration (van Wyk et al. Front Neurosci 2017. 11: p. 161). This makes 4xGRM6-SV40 (and equally GRM6-SV40) not suited to treat degenerated retina. In addition, the SV40 basal viral promoter is inflicted with issues such as silencing under chronic activation and protein overexpression leading to cellular cytotoxicity. In order to design better-suited OBC-specific promoters, the inventors first investigated if *Grm6* expression remained upregulated during the degeneration process in the *rd1* degeneration mouse model. The inventors employed the *rd1* mouse model under the rationale that promoters active in this severe and rapid degeneration model are likely to be active in most, less severe degenerative diseases of the retina. This was previously exemplified by the inventors comparing transgene expression in the slower degenerating *rd10* (B6.CXB1-Pde6brd10) mouse model where 4xGRM6-SV40 was able to still drive some expression [van Wyk, M., et al., Front Neurosci, 2017. 11(161): p. 161.]. The inventors quantified *Grm6* gene expression in *rd1* mouse retinas by real-time quantitative PCR at time points P14, P21, P28 and P54 and compared expression levels to wildtype C57BL/6J mouse retinas. Ribosomal protein L8 (*Rpl8*) expression was used for normalization of expression levels. *Grm6* expression remained constant during degeneration ($P = 0.8795$), with the exception of a small downregulation (0.59-fold) between P21 and P28. From this, the inventors concluded that the severe downregulation observed for the 4xGRM6-SV40 promoter [van Wyk, M., et al., Front Neurosci, 2017. 11(161): p. 161.] was not likely due to a downregulation of *Grm6* enhancer elements, but probably the consequence of a diminished functionality of the SV40 basal promoter with progressing degeneration. Consequently, the *Grm6* gene was used by the inventors as a template for OBC-specific promoter design.

30 Example 2: Design of GRM6-based promoters

To align with the human transcription machinery in light of a future use in a human therapy, the inventors employed the human *GRM6* sequence and not the murine *Grm6* sequence as a template.

The inventors used the Basic Local Alignment Search Tool (BLAST, <https://blast.ncbi.nlm.nih.gov/Blast.cgi>) optimized for “somewhat similar” sequences (blastn) [Altschul et al., J Mol Biol, 1990. 215(3): p. 403-10; Coordinators, Nucleic Acids Res, 2018.

46(D1): p. D8-D13] to align murine *Grm6* and human *GRM6* gene sequences. For enhancer specification, the inventors aligned 1500 bp around the murine 200En enhancer sequence identified by Kim et al. [Kim et al., J Neurosci, 2008. 28(31): p. 7748-64.]. The inventors found a 310 bp long conserved sequence between mouse and human genomes [-13819 to -13510 rel. to the translation start site (TLSS) of *GRM6*] extending beyond the 200En sequence defined by Kim et al. in both 3' and 5' direction (Figure 1A, horizontally striped sections). To identify promoter sequences, the inventors focused on the *GRM6* sequences 5' of the translation start site (TLSS, defined as position 0 by the inventors) (Figure 1B). After alignment, the inventors used the Genome Browser of the Genomics Institute of the University of California Santa Cruz (UCSC Genome Browser) [Church et al., PloS Biol, 2011. 9(7): p. e1001091.], [Kent et al., Genome Res, 2002. 12(6): p. 996-1006.; Kuhn et al. Brief Bioinform, 2013. 14(2): p. 144-61.] [<https://genome.ucsc.edu/>; genome assembly Feb. 2009 (GRCh37/hg19)] to identify potential regulatory sequences of the *GRM6* gene, such as inter-species conserved sequences, active chromatin regions (DnaseI hypersensitivity clusters or H3K27Ac Mark tracks) or transcription factor binding sites. The Gene Transcription Regulation Database (GTRD) [Yevshin et al., Nucleic Acids Res, 2017. 45(D1): p. D61-D67] was further used for identification of chromatin immunoprecipitation-DNA-sequencing (ChIP-seq) peaks, providing experimentally verified transcription factor binding sites. Together, this information enabled the inventors to highlight sequences in the above-identified regions with likely functional importance in *GRM6* gene expression.

The inventors then selected three possible enhancer regions [407En(h*GRM6*), 444En(h*GRM6*) and 770En(h*GRM6*)] and two possible promoter regions [566P(h*GRM6*) and 454P(*GRM6*)] (Figure 1 and Table 1) according to the following rationales: 407En(h*GRM6*) (-13873 to -13467 rel. TLSS *GRM6*) consists of the 300 bp conserved sequence between the murine and human genomes (horizontally striped in Fig. 1A). 770En(h*GRM6*) (-14236 to -13467 rel. TLSS *GRM6*) in addition to 407En(h*GRM6*) also contains the 3' ChIP-seq peaks and Dnase hypersensitivity cluster (-13990 to -13816 rel. TLSS *GRM6*). The 444En(h*GRM6*) (-14033 to -13590 rel. TLSS *GRM6*) is a 3' and 5' truncated version of 770En(h*GRM6*) including 3' and 5' only the ChIP-seq peaks.

When aligning the sequences -1000 to -1 (rel. TLSS) of *GRM6* the inventors identified a 167 bp conserved region (-425 to -259 rel. TLSS *GRM6*) (Fig. 1B, horizontally striped in Fig. 1B). With this conserved sequence included, the inventors designed two promoters: 566P(h*GRM6*) (-691 to -126 rel. TLSS *GRM6*) containing in addition the 5' transcription start site (TSS, -179 rel. TLSS *GRM6*) and the 5' H3K27Ac Mark signal peak (-656 to -405 rel. TLSS *GRM6*) and the second 3' ERG ChIP-Seq peak. ERG is known as an activator that interacts with FLI1 contained in 770En and 444En. The second selected promoter sequence 454P(h*GRM6*) (-453 to +1 rel. TLSS *GRM6*) extends further 5' compared to 566P(h*GRM6*)

including the TLSS and additional potentially regulatory sequences located between TLSS and TSS, such as the TCF7L1 and MYC ChIP-Seq peaks.

Five possible combinations of enhancer and promoter sequences (Table 1) preceding a reporter transgene were cloned between the ITR sequences of an adeno-associated viral (AAV) vector as detailed in the examples below using standard molecular methods:

Table 1: Selected enhancer/promoter combinations.

Name	Length	Sequence N°	
407En_454P(hGRM6)	867 bp	11	
444En_454P(hGRM6)	917 bp	15	
407En_566P(hGRM6)	978 bp	12	
770En_454P(hGRM6)	1243 bp	13	10
770En_566P(hGRM6)	1354 bp	14	

Example 3: Functional promoter evaluation in the human retina

Having the promoter designed on the human *GRM6* gene in light of therapeutic use in human patients, all promoters were evaluated in post-mortem human retinal explants. For this, promoters were combined with a mCitrine transgene and packaged into self-complementary (sc) AAV capsids, in particular scAAV2(7m8) (Dalkara et al. Sci Transl Med 2013. **5**: p. 189ra76).

Approximately 5×10^6 vg (vector genomes) were added to the RGC side of cultured post-mortem human retinal explants at day 1 as described in detail in [van Wyk, M., et al., Front Neurosci, 2017. **11**(161): p. 161.]. Retinas were frozen at day 7 of culture when transgene expression from scAAVs was visible. The inventors stained cryo-sections for the reporter protein mCitrine and the OBC marker $\text{Go}\alpha$ to visualize localization of expression and to compare expression strengths. Up to 85% of OBCs were expressing mCitrine in well transduced areas (Fig. 9). To compare performance to state-of-the-art, Lu's 200En-mGluR500P promoter (Lu et al. Gene Ther, 2016. **23**: p. 680-9.) was also packaged into scAAV2(7m8) and used in human retinal explant transduction. For each promoter, cultures from three human eyes were transduced with the promoter constructs and cryo-sections throughout the retina histologically stained and analysed. To control for variation between experiments, processing and immunohistochemistry were conducted for all samples in parallel. Fluorescence images were acquired with a ZEISS LSM 880 with Airyscan and ZEN 2.1 software. Confocal photomicrographs (z-stacks) were taken under a 20x objective with identical microscope settings. To determine average transduction efficacy, cytoplasmic Alexa488 fluorescence (secondary antibody for mCitrine) was determined from transduced OBC cell bodies [mCitrine(+) and $\text{Go}\alpha$ (+)]. In particular, arbitrary fluorescence values from a $4.15 \mu\text{m}$ diameter OBC somatic area were determined using the luminance function of Fiji image

processing software. Image analyses, including fluorescence quantification and cell counting were performed with Fiji 21ulfil21 (version 2.0.0, <https://fiji.sc/>, Schindelin et al., Nat Methods, 2012. 9(7): p. 676-82). For normalization, the fluorescence value from each expressing OBC [mCitrine(+) and Gao(+)] was divided by the average background fluorescence value determined from measurements from non-expressing OBCs [mCitrine(-) and Gao(+)]. As evident from Figure 2, the 566P basal promoter mediated weakest mCitrine expression in OBCs, whereas expression from 454P was always significantly stronger than that from Lu's 200En-mGluR500P, independent of the enhancer element employed. 454P was therefore selected for all subsequent experiments. 770En_454P(hGRM6) ($F = 6.03 \pm 1.53$; mean $\pm$ s.d.) and 407En_454P(hGRM6) ($F = 5.65 \pm 0.09$; mean $\pm$ s.d.) performed equally well in terms of efficacy and significantly better than Lu's mouse derived 200En-mGluR500P ($F = 4.49 \pm 0.22$; mean $\pm$ s.d.) and both were therefore analyzed in more detail.

Example 4: Significantly enhanced cone ON-bipolar cell preference in human retinal explants

In a next step, sections were analysed for OBC cell-type specificity of expression. For this purpose, sections were stained with antibodies against the transgene mCitrine, the ubiquitous OBC markers Gao and the rod bipolar cell specific antibody PKC α . [mCitrine(+), PKC α (+), Gao(+)] cells were clearly identified as expressing rod ON-bipolar cells (RBCs), whereas [mCitrine(+), PKC α (-), Gao(+)] cells were clearly identified as expressing cone ON-bipolar cells (cOBCs). Accordingly, [mCitrine(-), PKC α (+)] cells were identified as non-expressing RBCs and [mCitrine(-), PKC α (-), Gao (+)] cells as non-expressing cOBCs. The results shown in Figure 3 indicate clearly that both, 770En_454P(hGRM6) and 407En_454P(hGRM6) drive significantly higher transgene expression in cOBCs compared to 200En-mGluR500P. A measure for cOBC preference (Fig. 3B) was determined by normalizing the amount of expressing cOBCs and RBCs to the overall number of cOBCs and RBCs in the analysed retinal area. Such normalization revealed that 770En_454P(hGRM6) (cOBC preference of 49.5%) and 407En_454P(hGRM6) (cOBC preference of 36.4%) possessed a highly enhanced ability to drive expression in the cOBC cell type compared to 200En-mGluR500P (cOBC preference of 16.3%). Of particular note, 770En_454P(hGRM6) shows equal preference for RBCs and cOBCs (~50% each, Fig. 3B).

Also the maculas of explanted human retinas were transduced with scAAV2(7m8)-770En_454P(hGRM6)-mCitrine. Immunolabeling with mCitrine, PKC α and Gao clearly showed that the fovea contains exclusively cOBCs and that 770En_454P(hGRM6) drives mCitrine expression in virtually all cOBCs (Fig. 7). The ability to drive transgene expression efficiently in the cOBC of the human fovea is of major importance for a human therapy, since the fovea mediates high acuity vision and thus represents the primary target for a retinal gene therapy restoring vision – the fovea contains only cOBCs.

Example 5: OBC specificity of 770En_454P(hGRM6) in comparison to 200En-mGluR500P

A high preference for OBCs is needed in order to avoid off-target effects such as corrupted retinal signaling. Human retinal sections were labelled with antibodies against mCitrine (transgene), Go α (general OBC marker) and the nuclear stain DAPI to differentiate cell layers.

- 5 From this the identity of the expressing cell type could be derived: photoreceptors (PRs, mCitrine(+), located in the outer nuclear layer), OBCs [mCitrine(+),Go α (+) and located in the inner nuclear layer], amacrine cells [ACs, mCitrine(+),Go α (-) and located in the inner nuclear layer] and ganglion cells (GCs, mCitrine(+), located in the ganglion cell layer). Figure 4A clearly shows that the novel promoter 770En_454P(hGRM6) has a significantly increased preference
- 10 for OBCs ($88.3 \pm 7.8 \%$) compared to 200En-mGluR500P ($70.1 \pm 12.2 \%$). In addition, off-target expression of 200En-mGluR500P was generally higher, in particular in ACs ($16.9 \pm 9.1\%$) as compared to the novel 770En_454P(hGRM6) promoter ($4.1 \pm 3.2 \%$). Latter is of particular importance for optogenetic vision restoration where off-target expression corrupts retinal signaling.

15 Example 6: Promoter evaluation in the degenerating mouse retina

- Important for a retinal therapy is the tissue's accessibility to treatment. This can be challenging in a degenerative process with anatomical, functional and transcriptional changes. The inventors had previously shown that Kim's murine 200En-SV40 promoter is no longer functional in the rapid degeneration *rd1* mouse model (van Wyk et al. Front Neurosci 2017. 11: p. 161). Therefore, the performance of 770En_454P(hGRM6) [and for comparison its murine counterpart 200En-mGluR500P [Lu et al. Gene Ther 2016. 23 p: 680-689] were tested in the degenerative *rd1* mouse model. The promoters were combined with the optogenetic MWOPN_-mGluR6-IRES2-TurboFP635 (SEQ ID NO: 16, plasmid map Fig. 8) transgene and packaged into ssAAV2(7m8) (Dalkara et al. Sci Transl Med 2013. 5: p. 189ra76). 3×10^9 vg
- 20 were intravitreally injected as described in (van Wyk et al. Front Neurosci 2017. 11: p. 161; van Wyk et al., PloS Biol 2015. 13: p. e1002143) into the eyes of late degenerated, 22 weeks old *rd1* mice. 4 weeks post injections, mice were euthanized and retinas extracted for immunohistochemical analysis as previously described [van Wyk, M., et al., Front Neurosci, 2017. 11(161): p. 161 ;van Wyk et al., PloS Biol 2015. 13: p. e1002143]. The inventors labelled
- 25 the sections against the transgene TurboFP635, the OBC-specific Go α marker and the nuclear stain DAPI. As opposed to 200En-SV40, 770En_454P(hGRM6) as well as Lu's *Grm6*-derived 200En-mGluR500P were functional in the OBCs of the *rd1* retina (Figure 5). However, 200En-mGluR500P led to expression only in restricted retinal areas, as exemplified in figure 5A, whereas 770En_454P(hGRM6) led to far more extensive and widespread transduction of the
- 30 degenerated retina (Fig. 5B), probably due to its increased expression strength which overcomes expression threshold when some *Grm6* downregulation has occurred. To analyze
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the specificity and efficacy of transgene (mCitrine) expression driven by 770En_454P(hGRM6) in a degenerated retina, the inventors injected an additional 9 *rd1* mice at even later time points (29-33 weeks of age). After 4 weeks, animals were euthanized and eyes frozen and sectioned. Cryosections were again labeled with antibodies against mCitrine and Go α and analyzed under a confocal microscope. Even here, in these fully degenerated retinas, ~67% of all cells expressing the transgene [mCitrine(+), Go α (-)] were OBCs [mCitrine(+), Go α (+)]. Equally, ~55% of all OBCs [mCitrine(-), Go α (+)] expressed the transgene [mCitrine(+), Go α (+)] (Figs. 5D). Widespread and specific expression of the therapeutic transgene in a degenerated retina is fundamental for an efficient gene therapy.

10 Example 7: Optogenetic gene therapy and vision restoration in *rd1* mice

To see whether the favourable properties of 770En_454P(hGRM6) support functional optogenetic vision restoration targeted at the OBCs, the inventors performed a proof-of-principle experiment with the *rd1* degeneration mouse model. The inventors injected 3×10^9 vg of ssAAV(7m8)- 770En_454P(hGRM6)-MWOPN_mGluR6-IRES2-TurboFP635-WPRE-BGHpA (plasmid map Fig. 8) bilaterally into three completely photoreceptor-less *rd1* mice of 15 22 weeks of age. MWOPN_mGluR6 (SEQ ID NO 16) is a chimeric protein between murine cone middle-wavelength opsin (MWOPN) and murine mGluR6 that operates analogously to Opto-mGluR6 mediating OBC activity and with this visual restoration (van Wyk et al., PloS Biol 2015. 13: p. e1002143). The inventors measured visual acuity by detecting optomotor responses (OMRs) (after [Prusky et al., Invest Ophthalmol Vis Sci, 2004. 45(12): p. 4611-6.]) at different time points (days 41, 47, 55, 82 and 112) post injection in an automated virtual OptoDrum (Striatech®) containing small chamber (54 x 54 x 30 cm) with four screens (23.8" full-HD IPS panels) surrounding a platform where an animal can be placed. The brightness of the screens was adjusted to 250 cd/m² and the bottom and the top of the chamber were 25 covered with mirrors. A compact industrial camera (IR-sensitive 1/3" CMOS sensor with global shutter and wide-angle lens, F1.6) tracked the head movements of mice from above while a rotating pattern of black and white non-sinusoidal vertical bars were displayed on the screen at different spatial frequencies. The software Optodrum analyzed the recorded head movements and controlled thickness, contrast and speed of the applied stimuli. The velocity of the moving bars was set to 12°/s and the contrast to 100%. As shown in figure 6, the median visual acuity for each mouse was determined from all measured visual acuities of the different trials. The median visual acuity of the treated mice was 0.29 ± 0.03 cycles / ° (n=3, $\pm$ s.d., $P=0.0048$), significantly better than that of the un-injected *rd1* control mice with 0.15 ± 0.06 cycles / ° (n=7, $P \geq 194$) but still worse than that of the seeing C57BL/6 positive control, 35 which had an average visual acuity of 0.43 ± 0.05 cycles / ° (n=10, s.d., $P=0.0006$). The optogenetic OBC targeted therapy, however, resulted in a marked improvement in the optomotor response. Together, the results prove that 770En_454P(hGRM6) fulfils all the

requirements for an OBC-targeted human gene therapy, being a short (1243 bp), human gene based promoter, which in degeneration is still highly efficient and specific for OBCs, including cOBCs.

Materials and methods

5 *Bioactivity Assays*

Bioactivity assays are described in the above Example sections. Culturing and AAV transduction of human retinal explants as well as intravitreal AAV injection into mouse eyes and subsequent immunohistochemical processing of frozen retinal sections is described in detail elsewhere [van Wyk, M., et al., Front Neurosci, 2017. 11(161): p. 161.].

10 *Determination of cone ON-bipolar cell specificity*

For this purpose, retinal cryosections were triple-stained with antibodies against the transgene mCitrine (Invitrogen, A11122, 1:500), the ubiquitous OBC marker Gao (EMD, MAB3073, 1:750) and the RBC specific antibody PKC α (Santa Cruz, sc8393, 1:750). [mCitrine(+), PKC α (+), Gao(+)] cells were clearly identified as expressing RBCs, whereas [mCitrine(+), PKC α (-), Gao(+)] cells were clearly identified as expressing cOBCs. OBC type preference depicted in figure 3A was determined by the ratios of expressing cOBCs to all OBCs [mCitrine(+), PKC α (-), Gao(+)] / [Gao(+)] and RBCs type preference by the ratio of expressing RBCs to all OBCs [mCitrine(+), PKC α (+), Gao(+)] / [Gao (+)]. To have a measure of cOBC preference of expression, in other words the chance that a cOBC expresses the inventors related the number of expressing cOBCs and RBCs, respectively, to the amount of cOBCs and RBCs in this particular retinal area of analysis. This normalization was possible, since [mCitrine(-), PKC α (+), Gao(+)] cells can be clearly identified as non-expressing RBCs and [mCitrine(-), PKC α (-), Gao(+)] as non-expressing cOBCs. The ratio [mCitrine(+), PKC α (+), Gao(+)] / [mCitrine(-), PKC α (+), Gao(+)] therefore represents the percentage of transduced and expressing RBCs, whereas the ratio [mCitrine(+), PKC α (-), Gao(+)] / [mCitrine(-), PKC α (-), Gao(+)] represents the percentage of transduced and expressing cOBCs in the respective retinal area of analysis. Therefore, the resulting percentages shown in Fig. 3B indicate the chance that a cOBC gets transduced.

Software used for molecular engineering

30 The inventors used the Genome Browser of the Genomics Institute of the University of California Santa Cruz (UCSC Genome Browser, <https://genome.ucsc.edu/>) [Kent et al., Genome Res, 2002. 12(6): p. 996-1006; Kuhn et al., Brief Bioinform, 2013. 14(2): p. 144-61] to study genomic promoter sequences and genome annotations.

To identify transcription factors and transcription factor binding sites that are likely involved in the regulation of gene expression produced by the novel GRM6-based promoters the inventors employed ChIP-seq data from the Gene Transcription Regulation Database (GTRD, gtrd.biouml.org/) [Yevshin et al., Nucleic Acids Res, 2017. 45(D1): p. D61-D67]

- 5 Plasmid maps were generated in Vector NTI Advance (version 11.5.2)

Antibodies

Table 3: Antibodies used for immunohistochemistry

Primary antibodies	1_1	anti-GFP	Rabbit	Invitrogen	A-11122	1:500
	1_2	anti-GFP	Chicken	Abcam	ab13970	1:500
	1_3	anti-tRFP	Rabbit	Evrogen	AB234	1:500
	1_4	anti-PKC α	Mouse	Santa Cruz Biotechnology	sc-8393	1:750
	1_5	anti-Go α	Mouse	EMD Millipore	MAB3073	1:750
	1_6	anti-Gy13	Rabbit	Santa Cruz Biotechnology	sc-368324	1:500
Secondary antibodies	2_1	anti-Rabbit Alexa Fluor 488	Goat	Invitrogen	A-11008	1:400
	2_2	anti-Mouse Cyanine 3	Goat	Invitrogen	A-10521	1:400
	2_3	anti-Rabbit Alexa Fluor 488	Donkey	Invitrogen	A-21206	1:400
	2_4	anti-Chicken Cyanine 5	Donkey	Jackson Imm. Research Laboratories	703-175-155	1:400

Confocal imaging hardware and software

- 10 A ZEISS LSM 880 with Airyscan and ZEN 2.1 software was used to take confocal images with either a 20x or a 40x objective lens. Images were processed and evaluated in Fiji [Schindelin et al., Nat Methods, 2012. 9(7): p. 676-82.]. The cell counter plugin was used for cell counting and standard Fiji tools for image processing. The Stitch plugin [Preibisch et al., Bioinformatics, 2009. 25(11): p. 1463-5.] was used in cases where Fiji failed to automatically
- 15 combine tile scan pictures.

Statistics

If not stated otherwise, values were compared with a two-tailed Student's t-test and gave average values with $\pm$ the standard deviation across biological samples throughout this work. Significance levels are indicated by stars: * represents $P \leq 0.05$, ** represents $P \leq 0.01$ and ***

20 represents $P \leq 0.001$.

Other methods

Remaining methods not described above and in examples 1 and 2 can be found in [van Wyk, M., et al., Front Neurosci, 2017. **11**(161): p. 161.].

Sequences

5 Table 4: Sequences used in this study

Name	SEQ ID NO.	Type	Origin
407En(hGRM6)	1	nucleic acid	human
770En(hGRM6)	2	nucleic acid	human
444En(hGRM6)	3	nucleic acid	human
429En(mGrm6)	4	nucleic acid	murine
792En(mGrm6)	5	nucleic acid	murine
460En(mGrm6)	6	nucleic acid	murine
454P(hGRM6)	7	nucleic acid	human
566P(hGRM6)	8	nucleic acid	human
454P(mGrm6)	9	nucleic acid	murine
566P(mGrm6)	10	nucleic acid	murine
407En_454P(hGRM6)	11	nucleic acid	human
407En_566P(hGRM6)	12	nucleic acid	human
770En_454P(hGRM6)	13	nucleic acid	human
770En_566P(hGRM6)	14	nucleic acid	human
444En_454P(hGRM6)	15	nucleic acid	human
MWOPN_mGluR6	16	nucleic acid	murine
IRES2	17	nucleic acid	Picornavirus
mCitrine	18	nucleic acid	Aequorea victoria
TurboFP635	19	nucleic acid	Entacmaea quadricolor
WPRE	20	nucleic acid	Woodchuck hepatitis B virus
BGH pA	21	nucleic acid	bovine
sNRP-1 pA	22	nucleic acid	human
WT capsid AAV2	23	amino acid	viral
KOZAK sequence a)	24	nucleic acid	eukaryotic

KOZAK sequence b)	25	nucleic acid	eukaryotic
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SEQ ID NO. 1: **407En(hGRM6)**

```

1  ctctgatttt aaaggaagta gataacttcaa ataattcatc atggagtgca 50
5  51  atattttctg taggctttta gtagataact tcatcagttt aaagaagatc 100
101 cttagattat gaaacattta caattatgaa tgaatattag atgttatcaa 150
151 atgctttttc tgcattccatt tagataatca tgtttttcct ttaatctggt 200
201 aatgcggtga attacattaa tagatttcct aagtcattaa tctgctaaag 250
251 tgcatttctg ggacaaacca gacttggtta tgacattgta tgtatttcag 300
10 301 tttgcaaata ttggactagg atttttgtat ctatattcct tagtttgacc 350
351 tgtaaatttt atttcttgta ctaagtatta gcctcacgaa aggcattgtc 400
401 aaatggt 407

```

15 SEQ ID NO. 2: **770En(hGRM6)**

```

1  ggggtctccaa cttgccaaact gtagatcttg gaacctttca tccttcataa 50
51  ctgcataagc caattccttc taataaatct gtataatata tctgtctata 100
101 taataaatat gtactttacat aactctgtat gttacatcta tctattctat 150
20 151 ctatctatct atctatctat ctatctatct atctatcatc tatctatcta 200
201 tttatcttatt ctctgtgtct ttggagaacc ctgacatagt aagcaatcat 250
251 atcacctgca aatgatgaaa gctgtgtatt ttccaaatca gtcgttttat 300
301 gtcttttttt cttgactga ctagtgcccc ctagagggaa tgataattgg 350
351 aattattgtc ttgctctgat tttaaaggaa gtagataact caaataattc 400
25 401 atcatggagt gcaatatatt ctgtaggctt ttagtagata acttcatcag 450
451 tttaaagaag atccttagat tatgaaacat ttacaattat gaatgaatat 500
501 tagatgttat caaatgcttt ttctgcatcc atttagataa tcatgttttt 550
551 cctttaatat gttaatgcgg tgaattacat taatagattt cctaagtcac 600
601 taatctgcta aagtgcattt ctgggacaaa ccagacttgg ttatgacatt 650
30 651 gtatgtattt cagtttgcaa atattggact aggatttttg tatctatatt 700
701 ccttagtttg acctgtaaat tttatttctt gtactaagta ttagcctcac 750
751 gaaaggcatt gtcaaagtgt 770

```

35 SEQ ID NO. 3: **444En(hGRM6)**

```

1  atctattctc tgtgtctttg gagaaccctg acatagtaag caatcatatc 50
51  acctgcaaat gatgaaagct gtgtattttc caaatcagtc gttttatgtc 100
101 tttttttctt gactgacta gtgccccta gagggaaatga taattggaat 150
40 151 tattgtcttg ctctgatttt aaaggaagta gataactcaa ataattcatc 200
201 atggagtgca atattttctg taggctttta gtagataact tcatcagttt 250
251 aaagaagatc cttagattat gaaacattta caattatgaa tgaatattag 300
301 atgttatcaa atgctttttc tgcattccatt tagataatca tgtttttcct 350
351 ttaatctggt aatgcggtga attacattaa tagatttcct aagtcattaa 400
45 401 tctgctaaag tgcatttctg ggacaaacca gacttggtta tgac 444

```

SEQ ID NO. 4: **429En(mGrM6)**, murine sequence corresponding to 407En(hGRM6)

```

50 1  aaaacatacc acttttagttt aaggactata gtgattccac actaggtaag 50
51 51  gtgctttctg taggctttta gttaatagtt ttgtcaagct aaagaagatc 100
101 101  tccagatggc taaactttta aatcatgaat gaagtagata ttaccaaatt 150
151 151  gctttttcag catccattta gataatcatg ttttttgctt ttaatctggt 200
201 201  aatgtagtga attacagaaa tacatttcct aaatcattac atccccaaa 250

```

```

251 tcgttaaatct gctaaagtac atctctggct caaacaagac tggttgtgac 300
301 aggtttgtct ctgtcagttt gtgactgttg ggctggctct tcctaccct 350
351 ctgcttcttg gtttggcctg aacattaatt ttattttatt tttttaattt 400
401 tacctacaat caatttcaca atgtgtgtt 429

```

5

SEQ ID NO. 5: **792En(mGrm6)**, murine sequence corresponding to 770En(hGRM6)

```

1 ggtctcaaca agatacaaat tatgttctct aggtagcaat taacacaagg 50
10 51 aacgccttga ggtatgggag gggtaggaa gctcacaaga tagaccctgg 100
101 tgcctggaag gaagacagcc aactaaagg catatcacag tgtcccgga 150
151 accaacttga agggcttctg ctgtacaaat gtgggagaat ttcacgtca 200
201 gaaggctctg caaaggctctg aaagtcaccg aactctgtaa gattctatcc 250
251 tgcttctatt cctgtcaaaa tataccagaa ggaatggaac taccctcc 300
15 301 aaaaaataaa taaacaaaca aaccaccaa ccacgcacag acaaagcatt 350
351 caatacacat gctaaaacat accactttag ttaaggact atagtgattc 400
401 cacactagggt aagggtgcttt ctgtaggctt ttagttaata gttttgtcaa 450
451 gctaaagaag atctccagat ggctaaactt ttaaatcatg aatgaagtag 500
501 atattaccaa attgcttttt cagcatccat ttagataatc atgttttttg 550
20 551 cctttaatct gttaatgtag tgaattacag aaatacatat cctaaatcat 600
601 tacatcccc aaatcgtaa tctgctaaag tacatctctg gctcaaaca 650
651 gactggttgt gacaggtttg tctctgtcag tttgtgactg ttgggctggc 700
701 tcttcctacc cctctgcttc ttggtttggc ctgaacatta attttatttt 750
751 atttttttta ttttacctac aatcaatttc acaatgtgtg tt 792
25

```

SEQ ID NO. 6: **460En(mGrm6)**, murine sequence corresponding to 444En(hGRM6)

```

1 ggctctgcaa aggtctgaaa gtcaccgaac tctgtaagat tctatcctgc 50
30 51 ttctattcct gtcaaaatat accagaagga atggaaactac cccctccaaa 100
101 aaataaataa acaaaacaac caccaaacca cgacagaca aagcattcaa 150
151 tacacatgct aaaacatacc actttagttt aaggactata gtgattccac 200
201 actaggtaag gtgctttctg taggctttta gttaatagtt ttgtcaagct 250
251 aaagaagatc tccagatggc taaactttta aatcatgaat gaagtagata 300
35 301 ttaccaaat gctttttcag catccattta gataatcatg tttttgcct 350
351 ttaatctgtt aatgtagtga attacagaaa tacatttcct aaatcattac 400
401 atcccccaa tcgttaatct gctaaagtac atctctggct caaacaagac 450
451 tggttgtgac 460

```

40

SEQ ID NO. 7: **454P(hGRM6)**

```

1 ggaggggtct ccaccctcgg agcgggtctct catccctccc tagaatcctt 50
45 51 aaatcctctc tcgctcaggg cctcggccgc atctgtcaca gacttgctct 100
101 gaaccgacag cggctggcgc aggtgactgg cttggggcgg gaggctgggt 150
151 gtgcgctggg gatggacccc gaggaagagg ggccaagctg tcgggaagcg 200
201 gcagggctgg aggggtggag gcagtggctg ggcgggaccc cgggagacag 250
251 ggttcggcgc ttgtaagagc gagacggagg cccgggcagg cgggctgagc 300
301 taactcccca gagccgaagt ggaaggcgcg ccccgagcgc cttctcccca 350
50 351 ggaccccggt gtccctcccc gcgccccgag cccgcgctct ccttcccccg 400
401 ccctcagagc gctccccgcc cctctgtctc cccgcagccc gctagacgag 450
451 ccga 454

```

55

SEQ ID NO. 8: **566P(hGRM6)**

	1	ccaagaagag	gacagaggca	gaaagccagg	gacagagact	gagaaacaga	50
	51	gacctagagg	cagaagaaga	ctgagataga	gatggacaga	gatttgtgtca	100
	101	gacacagccc	cagagacagc	cagacagtct	gagtcagacg	caaaccaaag	150
5	151	acaagaaaac	aggaaaacag	acccagagat	tgggagaggg	aggggaagga	200
	201	gatgcgggga	gagccagcac	cgccaccccc	cacactcagg	aggggtctcc	250
	251	accctcggag	cggctctctca	tccctccccta	gaatccttaa	atcctctctc	300
	301	gctcagggcc	tcggccgcac	ctgtcacaga	cttgtcctga	accgacagcg	350
	351	gctggcgag	gtgactggct	tggggcgagg	gcctgggtgt	gcgctgggga	400
10	401	tggaccccga	ggaagagggg	ccaagctgtc	gggaagcggc	agggctggag	450
	451	gggtggaggc	agtggtcggg	cgggaccccg	ggcgacaggg	ttcggcgctt	500
	501	gtaagagcga	gacggaggcc	cgggcaggcc	ggctgagcta	actccccaga	550
	551	gccgaagtgg	aaggcg				566

15

SEQ ID NO. 9: **454P(mGrm6)**, murine sequence corresponding to 454P(hGRM6)

	1	agagagaaga	gagcccttcc	tccactctca	agctctggag	ggggtctctg	50
	51	ccctcaccct	catccctccc	cagaatcctt	aaatcctcta	gactgtagct	100
20	101	ctgattttac	agctgtcaca	gactcgtcct	actagccaga	ggttggtctca	150
	151	ggtaagcacc	actggggagg	tagcctaggg	tgcgctgggg	tgggtccaga	200
	201	ggaagagctg	ccagaactg	tgggggaagg	agcgggaccg	accatcaaca	250
	251	gggggacttt	tcaggagagaa	tgagagcaat	cctctggagg	cctgggagag	300
	301	gctgctgagt	tgctggtgag	cgagtcacca	acttttcctg	cgctctcggt	350
25	351	gtccggccag	aatcccgaag	tggcagctga	gcacgggggtg	gcagcttcgt	400
	401	ccgccggctc	tcaaggcgctc	ccggtaactt	cctttcccgc	agtccaggag	450
	451	caga					454

30

SEQ ID NO. 10: **566P(mGrm6)**, murine sequence corresponding to 566P(hGRM6)

	1	gaccgaccag	gggagtcctt	ggacttcttt	gttcctcttc	tcgggggtggc	50
	51	gggactgatt	gtgtaaatct	cttatctcca	actttcactc	ttatctgtct	100
	101	ctttaatcgg	catattgagg	atgagtggcc	aagcttattg	gtgttgcttg	150
35	151	gtcagacaat	ttaaaggcag	tctaggggag	aagcagaccc	agggagtcag	200
	201	agaggcagag	agagaagaga	gcccttcctc	cactctcaag	ctctggaggg	250
	251	ggtctctgcc	ctcaccctca	tccctcccga	gaatccttaa	atcctctaga	300
	301	ctgtagctct	gattttacag	ctgtcacaga	ctcgtcctac	tagccagagg	350
	351	ttggctcagg	taagcaccac	tggggaggta	gcctaggggtg	cgctgggggtg	400
40	401	ggtccagagg	aagagctgcc	cagaactgtg	ggggaaggag	cgggaccgac	450
	451	catcaacagg	gggacttttc	agggagaatg	agagcaatcc	tctggaggcc	500
	501	tgggagaggc	tgctgagttg	ctggtgcgag	agtcaccaac	ttttcctgag	550
	551	ctctcgggtg	cgggcc				566

45

SEQ ID NO. 11: **407En_454P(hGRM6)**

	1	ctctgatttt	aaaggaagta	gataacttcaa	ataattcatc	atggagtgcg	50
	51	atattttctg	taggctttta	gtagataact	tcatcagttt	aaagaagatc	150
50	101	cttagattat	gaaacattta	caattatgaa	tgaatattag	atgttatcaa	200
	151	atgctttttc	tgcattccatt	tagataatca	tgtttttcct	ttaatctgtt	250
	201	aatgcggtga	attacattaa	tagatttcct	aagtcattaa	tctgctaaag	300
	251	tgcatctctg	ggacaaacca	gacttggtta	tgacattgta	tgtatttcag	350
	301	tttgcaaata	ttggactagg	atttttgtat	ctatatccct	tagtttgacc	400
55	351	tgtaaatttt	atctcttgta	ctaagtatta	gcctcacgaa	aggcattgtc	450
	401	aaatgttgct	agcggagggg	tctccaccct	cggagcgggtc	tctcatccct	500

	451	ccctagaatc	cttaaatacct	ctctcgctca	gggcctcggc	cgcatctgtc	550
	501	acagacttgt	cctgaaccga	cagcggctgg	cgcaggtgac	tggcttgggg	600
	551	cgggagcctg	ggtgtgctg	ggggatggac	cccaggaag	aggggccaag	650
	601	ctgtcgggaa	gcggcagggc	tggaggggtg	gaggcagtg	tcgggcgga	700
5	651	ccccgggcga	cagggttcgg	cgcttgtaag	agcgagacgg	aggcccgggc	750
	701	aggccggctg	agctaactcc	ccagagccga	agtggaaggc	gcgccccgag	800
	751	cgctttctcc	ccaggacccc	ggtgtccctc	cccgcgcccc	gagcccgccg	850
	801	tctccttccc	ccgccctcag	agcgctcccc	gccctctgt	ctccccgcag	900
	851	cccgctagac	gagccga				867

10

SEQ ID NO. 12: 407En_566P(hGRM6)

	1	ctctgatttt	aaaggaagta	gatacttcaa	ataattcatc	atggagtgc	50
15	51	atattttctg	taggctttta	gtagataact	tcatcagttt	aaagaagatc	100
	101	cttagattat	gaaacattta	caattatgaa	tgaatattag	atgttatcaa	150
	151	atgctttttc	tgcattccatt	tagataatca	tgtttttcct	ttaatctgtt	200
	201	aatgcggtga	attacattaa	tagatttcct	aagtcattaa	tctgctaaag	250
	251	tgcattttctg	ggacaaacca	gacttggtta	tgacattgta	tgtatttcag	300
20	301	tttgcaaata	ttggactagg	atttttgtat	ctatattcct	tagtttgacc	350
	351	tgtaaatttt	atltcttgta	ctaagtatta	gcctcacgaa	aggcattgtc	400
	401	aaatgttgct	agccaagaag	aggacagagg	cagaaagcca	gggacagaga	450
	451	ctgagaaaca	gagacctaga	ggcagaagaa	gactgagata	gagatggaca	500
	501	gagatttgtgt	cagacacagc	cccagagaca	gccagacagt	ctgagtcaga	550
25	551	cgcaaaccac	agacaagaaa	acaggaaaac	agaccacag	attgggagag	600
	601	ggaggggaag	gagatgcggg	gagagccagc	accgccaccc	cccacactca	650
	651	ggaggggtct	ccaccctcgg	agcggctctc	catccctccc	tagaatcctt	700
	701	aaatcctctc	tcgctcaggg	cctcgcccg	atctgtcaca	gacttgcct	750
	751	gaaccgcacag	cggctggcgc	aggtgactgg	cttggggcgg	gagcctgggt	800
30	801	gtgcgctggg	gatggacccc	gaggaagagg	ggccaagctg	tcgggaagcg	850
	851	gcagggctgg	aggggtggag	gcagtggctg	ggcgggaccc	cgggcgacag	900
	901	ggttcggcgc	ttgtaagagc	gagacggagg	cccgggcagg	ccggctgagc	950
	951	taactcccca	gagccgaagt	ggaaggcg			978

35

SEQ ID NO. 13: 770En_454P(hGRM6)

	1	gggtctccaa	cttgccaact	gtagatcttg	gaacctttca	tccttcataa	50
	51	ctgcataagc	caattccttc	taataaatct	gtataatata	tctgtctata	100
40	101	taataaatat	gtacttacat	aactctgtat	gtacatcta	tctattctat	150
	151	ctatctatct	atctatctat	ctatctatct	atctatcatc	tatctatcta	200
	201	tttatctatt	ctctgtgtct	ttggagaacc	ctgacatagt	aagcaatcat	250
	251	atcacctgca	aatgatgaaa	gctgtgtatt	ttccaaatca	gtcgttttat	300
	301	gtcttttttt	cttgcaactga	ctagtgcctc	ctagagggaa	tgataattgg	350
45	351	aattattgtc	ttgctctgat	tttaaaggaa	gtagataact	caaataattc	400
	401	atcatggagt	gcaatatttt	ctgtaggctt	ttagtagata	acttcatcag	450
	451	tttaaagaag	atccttagat	tatgaaacat	ttacaattat	gaatgaatat	500
	501	tagatgttat	caaagtcttt	ttctgcatcc	atttagataa	tcatgttttt	550
	551	cctttaatct	gttaatgcgg	tgaattacat	taatagattt	cctaagtcac	600
50	601	taatctgcta	aagtgcattt	ctgggacaaa	ccagacttgg	ttatgacatt	650
	651	gtatgtattt	cagtttgcaa	atattggact	aggatttttg	tatctatatt	700
	701	ccttagtttg	acctgtaaat	tttatttctt	gtactaagta	ttagcctcac	750
	751	gaaaggcatt	gtcaaatgtt	caattgatat	aatgctagcg	gaggggtctc	800
	801	caccctcggg	gcggtctctc	atccctccct	agaatcctta	aatcctctct	850
55	851	cgctcagggc	ctcgcccgca	tctgtcacag	acttgtcctg	aaccgacagc	900
	901	ggctggcgca	ggtgactggc	ttggggcggg	agcctgggtg	tgcgctgggg	950

951 atggacccccg aggaagaggg gccaaagctgt cgggaagcgg cagggtgga 1000
 1001 ggggtggagg cagtggtcgg gcgggacccc gggcgacagg gttcggcgct 1050
 1051 tgtaagagcg agacggaggg ccgggcaggg cggctgagct aactccccag 1100
 1101 agccgaagtg gaaggcgcgcc ccgagcgccc ttctccccag gaccccggtg 1150
 5 1151 tccctccccg cgccccgagc ccgcgctctc cttcccccg cctcagagcg 1200
 1201 ctccccgccc ctctgtctcc ccgcagcccc ctagacgagc cga 1243

SEQ ID NO. 14: 770En_566P(hGRM6)

10 1 ggggtctccaa cttgccaaact gtagatcttg gaacctttca tccttcataa 50
 51 ctgcataagc caatttccttc taataaatct gtataatata tctgtctata 100
 101 taataaatat gtactttacat aactctgtat gttacatcta tctattctat 150
 151 ctatctatct atctatctat ctatctatct atctatcatc tatctatcta 200
 15 201 tttatctatt ctctgtgtct ttggagaacc ctgacatagt aagcaatcat 250
 251 atcacctgca aatgatgaaa gctgtgtatt ttccaaatca gtcgtttttat 300
 301 gtcttttttt cttgcactga ctagtgcccc ctagaaggaa tgataattgg 350
 351 aattattgtc ttgctctgat tttaaaggaa gtagatactt caaataattc 400
 401 atcatggagt gcaatatatt ctgtaggctt ttagtagata acttcatcag 450
 20 451 tttaaagaag atccttagat tatgaaacat ttacaattat gaatgaatat 500
 501 tagatgttat caaatgcctt ttctgcatcc atttagataa tcatgttttt 550
 551 cctttaatct gttaatgcgg tgaattacat taatagattt cctaagtcac 600
 601 taatctgcta aagtgcattt ctgggacaaa ccagacttgg ttatgacatt 650
 651 gtatgtattt cagtttgcaa atattggact aggatttttg tatctatatt 700
 25 701 ccttagtttg acctgtaaat tttatttctt gtactaagta ttagcctcac 750
 751 gaaaggcatt gtcaaagtgt caattgatat aatgctagcc aagaagagga 800
 801 cagaggcaga aagccaggga cagagactga gaaacagaga cctagaggca 850
 851 gaagaagact gagatagaga tggacagaga ttgtgtcaga cacagcccca 900
 901 gagacagcca gacagtctga gtcagacgca aaccaaagac aagaaaacag 950
 30 951 gaaaacagac ccagagattg ggagaggagg ggggaaggaga tgcggggaga 1000
 1001 gccagcaccg ccacccccca cactcaggag gggctctcac cctcggagcg 1050
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 1101 ggccgcactc gtcacagact tgtcctgaac cgacagcggc tggcgcaggg 1150
 1151 gactggcctg gggcgggagc gctgggtgtg cgtggggatg gaccccgagg 1200
 35 1201 aagagggggc aagctgtcgg gaagcggcag ggctggaggg gtggaggcag 1250
 1251 tggcggggcg ggaccccggg cgacagggtt cggcgcttgt aagagcgaga 1300
 1301 cggaggcccc ggagggccgg ctgagctaac tccccagagc cgaagtggaa 1350
 1351 ggcg 1354

40

SEQ ID NO. 15: 444En_454P(hGRM6)

1 atctattctc tgtgtctttg gagaaccctg acatagtaag caatcatatc 50
 51 acctgcaaat gatgaaagct gtgtattttc caaatcagtc gttttatgtc 100
 45 101 tttttttctt gcactgacta gtgccccta gaggaatga taattggaat 150
 151 tattgtcttg ctctgatttt aaaggaaagta gatacttcaa ataattcatc 200
 201 atggagtgca atattttctg taggctttta gtagataact tcatcagttt 250
 251 aaagaagatc cttagattat gaaacattta caattatgaa tgaatattag 300
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 50 351 ttaatctgtt aatgcgggtg attacattaa tagatttcct aagtcattaa 400
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 551 acagacttgt cctgaaccga cagcggctgg cgaggtgac tggcttgggg 600
 55 601 cgggagcctg ggtgtgcgct ggggatggac cccgaggaag aggggccaag 650
 651 ctgtcgggaa gcggcagggc tggaggggtg gaggcagtg tgggcgggga 700

	701	ccccggggcga	caggggttcgg	cgcttgtaag	agcgagacgg	aggccccgggc	750
	751	aggccggctg	agctaactcc	ccagagccga	agtggaaggc	gcgccccgag	800
	801	cgcttctcc	ccaggacccc	ggtgtccctc	cccgcgcccc	gagccccgcg	850
	851	tctccttccc	ccgccctcag	agcgctcccc	gcccctctgt	ctccccgcag	900
5	901	cccgcctagac	gagccga				917

SEQ ID NO. 16: MWOPN_mGluR6

10	1	atggcccaaa	ggcttacagg	tgaacagaca	ctggaccact	atgaggatag	50
	51	cacccatgca	agcatcttca	cctataccaa	cagcaacagc	accaaagggtc	100
	101	cctttgaagg	cccccaattat	cacattgctc	ccagggtgggt	gtaccacctc	150
	151	accagcacct	ggatgattct	tgtggtcggt	gcatctgtct	tcactaatgg	200
	201	acttggtgctg	gcagccacca	tgagattcaa	gaagctgcgc	catccactga	250
15	251	actggattct	ggtgaacttg	gcagttgctg	acctagcaga	gaccattatt	300
	301	gccagcacta	tcagtgttgt	gaaccaaata	tatggctact	tcgttctggg	350
	351	acaccctctg	tgtgtcattg	aaggctacat	tgtctcattg	tgtggaatca	400
	401	caggcctctg	gtccctggcc	atcatttcct	gggagagatg	gctgggtgggc	450
	451	tgcaagccct	ttggcaatgt	gagatttgat	gctaagctgg	ccactgtggg	500
20	501	aatcgtcttc	tcctgggtct	gggctgctat	atggacggcc	ccaccaatct	550
	551	ttgggtggag	caggtactgg	ccttatggcc	tgaagacatc	ctgtggccca	600
	601	gacgtgttca	gcggtaccta	gtaccccggt	gttcagtctt	atatgatggt	650
	651	cctcatgggtc	acgtgctgca	tcttcccact	cagcatcatc	gtgctctgct	700
	701	acctccaagt	gtggctggcc	atccgagcag	tggaagca	acagaaagaa	750
25	751	tctgagtcca	ctcagaaggc	cgagaaggag	gtgacacgca	tggtggtggg	800
	801	gatggtcttc	gcatactgcc	tctgctgggg	accctatact	ttctttgcat	850
	851	gctttgctac	tgcccaccct	ggctatgcct	tccaccctct	tgtggcctcc	900
	901	ctaccatcct	actttgcca	aagtgccact	atctacaacc	ccattatcta	950
	951	tgtctttatg	aaccggcagt	ttcgaaactg	catcttacat	ctctttggaa	1000
30	1001	agaaggttga	tgatagctct	gaactttcca	gcacctccaa	gacagaagtc	1050
	1051	tcatctgtct	cttcagtgtc	acctgcagag	cagaacgtgc	agaagcggaa	1100
	1101	gcgcagcctc	aagaagacct	ccacgatggc	ggccccgccc	aagagcgaga	1150
	1151	actcagagga	cgccaagaca	gagaccagcc	aagtggcgcc	tgccaagagc	1200
	1201	aggatcacca	gcgagggcga	gtacatcccc	ctggaccaga	tcgacatcaa	1250
35	1251	cggtgtaa					1257

SEQ ID NO. 17: IRES2

40	1	gcccctctcc	ctcccccccc	cctaacgtta	ctggccgaag	ccgcttggaa	50
	51	taaggccggt	gtgcggttgt	ctatatgtta	ttttccacca	tattgccgtc	100
	101	ttttggcaat	gtgagggccc	ggaaacctgg	cctgtcttc	ttgacgagca	150
	151	ttcctagggg	tctttccctc	ctcgccaaag	gaatgcaagg	tctgttgaat	200
	201	gtcgtgaagg	aagcagttcc	tctggaagct	tcttgaagac	aaacaacgtc	250
45	251	tgtagcgacc	ctttgcaggc	agcggaaacc	cccacctggc	gacaggtgcc	300
	301	tctgcggcca	aaagccacgt	gtataagata	cacctgcaaa	ggcggcacaa	350
	351	ccccagtgcc	acgttgtgag	ttggatagtt	gtggaaagag	tcaaattggct	400
	401	ctcctcaagc	gtattcaaca	aggggctgaa	ggatgcccg	aaggtacccc	450
	451	attgtatggg	atctgatctg	gggcctcggt	gcacatgctt	tacatgtggt	500
50	501	tagtcgaggt	taaaaaaacg	tctaggcccc	ccgaaccacg	gggacgtggg	550
	551	tttcctttga	aaaacacgat	gataatatgg	ccaca		585

SEQ ID NO. 18: mCitrine

55	1	atggtgagca	agggcgagga	gctgttcacc	ggggtgggtg	ccatcctggg	50
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	51	cgagctggac	ggcgacgtaa	acggccacaa	gttcagcgtg	tccggcgagg	100
	101	gcgagggcga	tgccacctac	ggcaagctga	ccctgaagtt	catctgcacc	150
	151	accggcaagc	tgcccgtgcc	ctggcccacc	ctcgtgacca	ccttcggcta	200
	201	cggcctgatg	tgcttcgccc	gctaccccga	ccacatgaag	cagcacgact	250
5	251	tcttcaagtc	cgccatgccc	gaaggctacg	tccaggagcg	caccatcttc	300
	301	ttcaaggacg	acggcaacta	caagaccgcg	gccgaggtga	agttcgaggg	350
	351	cgacaccctg	gtgaaccgca	tcgagctgaa	gggcatcgac	ttcaaggagg	400
	401	acggcaacat	cctggggcac	aagctggagt	acaactacaa	cagccacaac	450
	451	gtctatatca	tggccgacaa	gcagaagaac	ggcatcaagg	tgaacttcaa	500
10	501	gatccggcac	aacatcgagg	acggcagcgt	gcagctcgcc	gacctacc	550
	551	agcagaacac	ccccatcggc	gacggccccg	tgctgctgcc	cgacaaccac	600
	601	tacctgagct	accagtccaa	gctgagcaaa	gaccccaacg	agaagcgcg	650
	651	tcacatggtc	ctgctggagt	tcgtgaccgc	cgccgggatc	actctcggca	700
	701	tggacgagct	gtacaagtcc	ggataa			726

15

SEQ ID NO. 19: TurboFP635

	1	atggtgggtg	aggatagcgt	gctgatcacc	gagaacatgc	acatgaaact	50
20	51	gtacatggag	ggcaccgtga	acgaccacca	cttcaagtgc	acatccgagg	150
	101	gcgaaggcaa	gccctacgag	ggcaccaga	ccatgaagat	caaggtgggtc	200
	151	gagggcgggc	ctctcccctt	cgccttcgac	atcctggcta	ccagcttcat	250
	201	gtacggcgagc	aaaaccttta	tcaaccacac	ccagggcac	cccgaacttc	300
	251	ttaagcagtc	cttccctgag	ggcttcacat	gggagaggat	caccacatac	350
25	301	gaagacgggg	gcgctgctgac	cgctaccag	gacaccagcc	tccagaacgg	400
	351	ctgcctcatc	tacaacgtca	agatcaacgg	ggtgaacttc	ccatccaacg	450
	401	gccctgtgat	gcagaagaaa	acactcggct	gggaggccag	caccgagatg	500
	451	ctgtaccccc	ctgacagcgg	cctgagaggc	catagccaga	tggccctgaa	550
	501	gctcgtgggc	ggggggtacc	tgcactgctc	cctcaagacc	acatacagat	600
30	551	ccaagaaacc	cgctaagaac	ctcaagatgc	ccggcttcta	cttcgtggac	650
	601	aggagactgg	aaagaatcaa	ggaggccgac	aaagagacct	acgtcgagca	700
	651	gcacgagatg	gctgtggcca	ggtactgcga	cctgcctagc	aaactggggc	750
	701	acagctga					708

35

SEQ ID NO. 20: WPRE

	1	aatcaacctc	tggattacaa	aatttgtgaa	agattgactg	gtattcttaa	50
	51	ctatgttgct	ccttttacgc	tatgtggata	cgctgcttta	atgcctttgt	100
40	101	atcatgctat	tgcttcccgt	atggctttca	ttttctcctc	cttgataaaa	150
	151	tcctgggttg	tgtctcttta	tgaggagtgt	tggcccggtg	tcaggcaacg	200
	201	tggcggttg	tgcaactgtg	ttgctgacgc	aacccccact	ggttggggca	250
	251	ttgccaccac	ctgtcagctc	ctttccggga	ctttcgcttt	ccccctccct	300
	301	attgccacgg	cggaaactcat	cgccgcctgc	cttgcccgct	gctggacagg	350
45	351	ggctcggctg	ttgggcactg	acaattccgt	ggtgtgtcgc	gggaaatcat	400
	401	cgctcctttc	ttggctgctc	gcctgtgttg	ccacctggat	tctgcgcggg	450
	451	acgtccttct	gctacgtccc	ttcgccctc	aatccagcgg	accttccttc	500
	501	ccgcggcctg	ctgccggctc	tgccgcctct	tccgcgtctt	cg	542

50

SEQ ID NO. 21: BGH pA

	1	gcctcgactg	tgccttctag	ttgccagcca	tctgttgttt	gccccctccc	50
	51	cgtgccttcc	ttgacctgg	aaggtgccac	tcccaactgtc	ctttcctaata	100
55	101	aaaatgagga	aattgcatcg	cattgtctga	gtaggtgtca	ttctattctg	150
	151	gggggtgggg	tggggcagga	cagcaagggg	gaggattggg	aagacaatag	200

201 caggcatgct gggga

215

SEQ ID NO. 22: sNRP-1 pA

5

1 aaataaaata cgaaatg

17

SEQ ID NO. 23: WT capsid AAV2

	MAADGYLPDW LEDTLSEGIR QWWKLKPGPP PPKPAERHKD DSRGLVLPGY KYLGPFNGLD	60
10	KGEPVNEADA AALEHDKAYD RQLDSGDNPY LKYNHADA EF QERLKEDTSF GGNLGRAVFQ	120
	AKKRVLEPLG LVEEPVKTAP GKKRPVEHSP VEPDSSSGTG KAGQQPARKR LNFGQTGDAD	180
	SVPDPQPLGQ PPAAPSGLGT NTMATGSGAP MADNNEGADG VGNSSGNWHC DSTWMGDRVI	240
	TTSTRTWALP TYNNHLYKQI SSQSGASNDN HYFGYSTPWG YFDENRFHCH FSPRDWQRLI	300
	NNNWGFRPKR LNFKLFIQV KEVTQNDGTT TIANNLTSTV QVFTDSEYQL PYVLGSAHQG	360
15	CLPPFPADVF MVPQYGYLTL NNGSQAVGRS SFYCLEYFPS QMLRTGNNFT FSYTFEDVPF	420
	HSSYAHSQSL DRLMNPLIDQ YLYYLSRTNT PSGTTTQSRL QFSQAGASDI RDQSRNWLPG	480
	PCYRQQRVSK TSADNNNSEF SWTGATKYHL NGRDSLVPNG PAMASHKDDE EKFFPQSGVL	540
	IFGKQGSEKT NVDIEKVMIT DEEEIRTTNP VATEQYGSVS TNLQRGNRQA ATADVNTQGV	600
	LPGMVWQDRD VYLQGPWAK IPHTDGHFHP SPLMGGFGLK HPPPQILIKN TPVPANPSTT	660
20	FSAAKFASFI TQYSTGQVSV EIEWELQKEN SKRWNPEIQY TSNYNKSVNV DFTVDTNQVY	720
	SEPRPIGTRY LTRNL	735

SEQ ID NO 24

gccgccAccAUGG

25 SEQ ID NO 25

gccgccGccAUGG

Claims

1. An isolated nucleic acid molecule of 850 base pairs (bp) to 1500 bp length, comprising
 - a. an enhancer sequence element selected from SEQ ID NO 1 to 3, and
 - b. a promoter sequence element of SEQ ID NO 7.
2. An isolated nucleic acid molecule of 850 base pairs (bp) to 1500 bp length, comprising
 - a. an enhancer sequence element being at least ($\geq$) 70%, particularly $\geq 75\%$, $\geq 80\%$, more particularly $\geq 85\%$, more particularly $\geq 90\%$, more particularly $\geq 95\%$, even more particularly $\geq 98\%$, most particularly 100% identical to a sequence selected from SEQ ID NO 1 to 3; and
 - b. a promoter sequence element being $\geq 70\%$, particularly $\geq 75\%$, more particularly $\geq 80\%$, more particularly $\geq 85\%$, more particularly $\geq 90\%$, more particularly $\geq 95\%$, even more particularly $\geq 98\%$, most particularly 100% identical to a sequence of SEQ ID NO 7;

and said isolated nucleic acid molecule has $\geq 40\%$, particularly $\geq 50\%$, more particularly $\geq 60\%$, even more particularly $\geq 70\%$, more particularly $\geq 80\%$, even more particularly $\geq 90\%$, most particularly 100% of the cone ON bipolar cell-specificity from a sequence of SEQ ID NO 13 and a cone ON bipolar cell preference of $\geq 20\%$, particularly $\geq 25\%$, more particularly $\geq 30\%$, even more particularly $\geq 35\%$, more particularly $\geq 40\%$, most particularly $\geq 50\%$.
3. The isolated nucleic acid molecule according to claim 1 or 2, wherein the isolated molecule consists of one and only one of said enhancer sequence elements, one and only one of said promoter sequence elements and optionally, a spacer separating the enhancer sequence element from the promoter sequence element.
4. The isolated nucleic acid molecule according to any one of the preceding claims comprising or consisting of a sequence selected from SEQ ID NO 11, SEQ ID NO 13, and SEQ ID NO 15, or comprising or consisting of a sequence characterized by $\geq 98\%$ identity to a sequence selected from SEQ ID NO 11, SEQ ID NO 13, and SEQ ID NO 15.
5. The isolated nucleic acid molecule according to any one of the preceding claims comprising or consisting of the sequence SEQ ID NO 11 or SEQ ID NO 13, or comprising or consisting of a sequence characterized by $\geq 98\%$ identity to SEQ ID NO

- 11 or SEQ ID NO 13, particularly comprising or consisting of the sequence SEQ ID NO 13, or comprising or consisting of a sequence characterized by $\geq 98\%$ identity to SEQ ID NO 13.
6. A nucleic acid expression vector comprising a nucleic acid molecule according to any one of the previous claims.
 7. The nucleic acid expression vector according to claim 6, wherein the nucleic acid expression vector is an adeno-associated virus vector or a recombinant adeno-associated vector (rAAV), particularly wherein the nucleic acid expression vector is a recombinant AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, or AAV12 vector, more particularly wherein the nucleic acid expression vector is a recombinant AAV2 vector.
 8. The nucleic acid expression vector according to any one of claims 6 to 7, additionally comprising
 - a. a sequence encoding a capsid protein, and
 - b. a transgene.
 9. The nucleic acid expression vector according to claim 8, wherein the transgene comprises the sequence of SEQ ID NO 16.
 10. An adeno-associated virion particle comprising the isolated nucleic acid molecule according to any one of claims 1 to 5 or the nucleic acid expression vector according to any one of claims 6 to 9.
 11. An agent selected from the isolated nucleic acid molecule according to any one of claims 1 to 5 or the nucleic acid expression vector according to any one of claims 6 to 9, and the adeno-associated virion particle according to claim 10 for use as a medicament.
 12. An agent selected from the isolated nucleic acid molecule according to any one of claims 1 to 5, the nucleic acid expression vector according to any one of claims 6 to 9, and the adeno-associated virion particle according to claim 10 for use in treatment of congenital stationary night blindness (CSBN1) or rod-cone and cone-rod dystrophies, particularly of retinitis pigmentosa and macular degeneration.
 13. An agent selected from the isolated nucleic acid molecule according to any one of claims 1 to 5, the nucleic acid expression vector according to any one of claims 6 to 9,

and the adeno-associated virion particle according to claim 10, wherein the agent is administered by

- a. intravitreal administration, particularly by intravitreal injection, or by
- b. subretinal injection.