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(54) **Title:** CRISPR/RNA-GUIDED NUCLEASE-RELATED METHODS AND COMPOSITIONS FOR TREATING RHO-ASSOCIATED AUTOSOMAL-DOMINANT RETINITIS PIGMENTOSA (ADRP)

(57) **Abstract:** CRISPR/RNA-guided nuclease-related compositions and methods for treatment of RHO-associated retinitis pigmentosa, e.g., autosomal-dominant retinitis pigmentosa (adRP).

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CRISPR/RNA-GUIDED NUCLEASE-RELATED METHODS AND COMPOSITIONS FOR TREATING RHO-ASSOCIATED AUTOSOMAL-DOMINANT RETINITIS PIGMENTOSA (ADRP)

PRIORITY CLAIM

The present application claims the benefit of United States Provisional Patent Application No. 62/810,320, filed February 25, 2019, the subject matter of which is hereby incorporated by reference in its entirety, as if fully set forth herein.

FIELD

The disclosure relates to CRISPR/RNA-guided nuclease-related methods and components for editing a target nucleic acid sequence, and applications thereof in connection with autosomal dominant retinitis pigmentosa (ADRP).

BACKGROUND

Retinitis pigmentosa (RP), an inherited retinal dystrophy that affects photoreceptors and retinal pigment epithelium cells, is characterized by progressive retinal deterioration and atrophy, resulting in a gradual loss of vision and ultimately leading to blindness in affected patients. RP can be caused by both homozygous and heterozygous mutations and can present in various forms, for example, as autosomal-dominant RP (adRP), autosomal recessive RP (arRP) or X-linked RP (X-LRP). Treatment options for RP are limited, and no approved treatment that can arrest or reverse RP progression is currently available.

SUMMARY

Some aspects of the strategies, methods, compositions, and treatment modalities provided herein address a key unmet need in the field by providing new and effective means of delivering genome editing systems to the affected cells and tissues of subjects suffering from autosomal-dominant retinitis pigmentosa (adRP). Some aspects of this disclosure provide strategies, methods, and compositions for the introduction of genome editing systems targeted to the adRP associated gene rhodopsin into retinal cells. Such strategies, methods, and compositions are useful, in some embodiments, for editing adRP associated variants of the rhodopsin gene, e.g., for inducing gene editing events that result in loss-of-function of such rhodopsin variants. In some embodiments, such strategies, methods, and compositions are useful as treatment modalities for administration to a subject in need thereof, e.g., to a

subject having an autosomal-dominant form of RP. The strategies, methods, compositions, and treatment modalities provided herein thus represent an important step forward in the development of clinical interventions for the treatment of RP, e.g., for the treatment of adRP.

The *RHO* gene encodes the rhodopsin protein and is expressed in retinal photoreceptor (PR) rod cells. Rhodopsin is a G protein-coupled receptor expressed in the outer segment of rod cells and is a critical element of the phototransduction cascade. Defects in the *RHO* gene are typically characterized by decreased production of wild-type rhodopsin and/or expression of mutant rhodopsin which lead to interruptions in photoreceptor function and corresponding vision loss. Mutations in *RHO* typically result in degeneration of PR rod cells first, followed by degeneration of PR cone cells as the disease progresses. Subjects with *RHO* mutations experience progressive loss of night vision, as well as loss of peripheral visual fields followed by loss of central visual fields. Exemplary *RHO* mutations are provided in **Table A**.

Some aspects of the present disclosure provide strategies, methods, compositions, and treatment modalities for altering a *RHO* gene sequence, e.g., altering the sequence of a wild type and/or of a mutant *RHO* gene, e.g., in a cell or in a patient having adRP, by insertion or deletion of one or more nucleotides mediated by an RNA-guided nuclease (e.g., Cas9 or Cpf1 molecule) and one or more guide RNAs (gRNAs), resulting in loss of function of the *RHO* gene sequence. This type of alteration is also referred to as "knocking out" the *RHO* gene. Some aspects of the present disclosure provide strategies, methods, compositions, and treatment modalities for expressing exogenous *RHO*, e.g., in a cell subjected to an RNA-guided nuclease-mediated knock-out of *RHO*, e.g., by delivering an exogenous *RHO* complementary DNA (cDNA) sequence encoding a functional rhodopsin protein (e.g., a wild-type rhodopsin protein).

In certain embodiments, a 5' region of the *RHO* gene (e.g., 5' untranslated region (UTR), exon 1, exon 2, intron 1, the exon 1/intron 1 border or the exon 2/intron 1 border) is targeted by an RNA-guided nuclease to alter the gene. In certain embodiments, any region of the *RHO* gene (e.g., a promoter region, a 5'untranslated region, a 3' untranslated region, an exon, an intron, or an exon/intron border) is targeted by an RNA-guided nuclease to alter the gene. In certain embodiments, a non-coding region of the *RHO* gene (e.g., an enhancer region, a promoter region, an intron, 5' UTR, 3'UTR, polyadenylation signal) is targeted to alter the gene. In certain embodiments, a coding region of the *RHO* gene (e.g., early coding region, an exon) is targeted to alter the gene. In certain embodiments, a region spanning an exon/intron border of the *RHO* gene (e.g., exon 1/intron 1, exon 2/intron 1) is targeted to alter

the gene. In certain embodiments, a region of the *RHO* gene is targeted which, when altered, results in a stop codon and knocking out the *RHO* gene. In certain embodiments, alteration of the mutant *RHO* gene occurs in a mutation-independent manner, which provides the benefit of circumventing the need to develop therapeutic strategies for each *RHO* mutation set forth in **Table A**.

In an embodiment, after treatment, one or more symptoms associated with adRP (e.g., nyctalopia, abnormal electroretinogram, cataract, visual field defect, rod-cone dystrophy, or other symptom(s) known to be associated with adRP) is ameliorated, e.g., progression of adRP is delayed, inhibited, prevented or halted, PR cell degeneration is delayed, inhibited, prevented and/or halted, and/or visual loss is ameliorated, e.g., progression of visual loss is delayed, inhibited, prevented, or halted. In an embodiment, after treatment, progression of adRP is delayed, e.g., PR cell degeneration is delayed. In an embodiment, after treatment, progression of adRP is reversed, e.g., function of existing PR rod cells and cone cells and/or birth of new PR rod cells and cone cells is increased/enhanced and/or visual loss e.g., progression of visual loss is delayed, inhibited, prevented, or halted.

In an embodiment, CRISPR/RNA-guided nuclease-related methods and components and compositions of the disclosure provide for the alteration (e.g., knocking out) of a mutant *RHO* gene associated with adRP, by altering the sequence at a *RHO* target position, e.g., by creating an indel resulting in loss-of-function of the affected *RHO* gene or allele, e.g., a nucleotide substitution resulting in a truncation, nonsense mutation, or other type of loss-of-function of an encoded *RHO* gene product, e.g., of the encoded *RHO* mRNA or RHO protein; a deletion of one or more nucleotides resulting in a truncation, nonsense mutation, or other type of loss-of-function of an encoded *RHO* gene product, e.g., of the encoded *RHO* mRNA or RHO protein, e.g., a single nucleotide, double nucleotide, or other frame-shifting deletion, or a deletion resulting in a premature stop codon; or an insertion resulting in a truncation, nonsense mutation, or other type of loss-of-function of an encoded *RHO* gene product, e.g., of the encoded *RHO* mRNA or RHO protein e.g., a single nucleotide, double nucleotide, or other frame-shifting insertion, or an insertion resulting in a premature stop codon. In some embodiments, CRISPR/RNA-guided nuclease-related methods and components and compositions of the disclosure provide for the alteration (e.g., knocking out) of a mutant *RHO* gene associated with adRP, by altering the sequence at a *RHO* target position, e.g., creating an indel that results in nonsense-mediated decay of an encoded gene product, e.g., an encoded *RHO* transcript.

In one aspect, disclosed herein is a gRNA molecule, e.g., an isolated or non-naturally occurring gRNA molecule, comprising a targeting domain which is complementary with a target domain from the *RHO* gene.

In an embodiment, the targeting domain of the gRNA molecule is configured to provide a cleavage event, e.g., a double strand break or a single strand break, sufficiently close to an *RHO* target position, in the *RHO* gene to allow alteration in the *RHO* gene, resulting in disruption (e.g., knocking out) of the *RHO* gene activity, e.g., a loss-of-function of the *RHO* gene, for example, characterized by reduced or abolished expression of a *RHO* gene product (e.g., a *RHO* transcript or a RHO protein), or by expression of a dysfunctional or non-functional *RHO* gene product (e.g., a truncated RHO protein or transcript). In an embodiment, the targeting domain is configured such that a cleavage event, e.g., a double strand or single strand break, is positioned within 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90, 100, 150 or 200 nucleotides of an *RHO* target position. The break, e.g., a double strand or single strand break, can be positioned upstream or downstream of an *RHO* target position, in the *RHO* gene.

In an embodiment, a second gRNA molecule comprising a second targeting domain is configured to provide a cleavage event, e.g., a double strand break or a single strand break, sufficiently close to the *RHO* target position, in the *RHO* gene, to allow alteration in the *RHO* gene, either alone or in combination with the break positioned by said first gRNA molecule. In an embodiment, the targeting domains of the first and second gRNA molecules are configured such that a cleavage event, e.g., a double strand or single strand break, is positioned, independently for each of the gRNA molecules, within 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90, 100, 150 or 200 nucleotides of the target position. In an embodiment, the breaks, e.g., double strand or single strand breaks, are positioned on both sides of a nucleotide of a *RHO* target position, in the *RHO* gene. In an embodiment, the breaks, e.g., double strand or single strand breaks, are positioned on one side, e.g., upstream or downstream, of a nucleotide of a *RHO* target position, in the *RHO* gene.

In an embodiment, a single strand break is accompanied by an additional single strand break, positioned by a second gRNA molecule, as discussed below. For example, the targeting domains are configured such that a cleavage event, e.g., the two single strand breaks, are positioned within 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90, 100, 150 or 200 nucleotides of a *RHO* target position. In an embodiment, the first and second gRNA molecules are configured such, that when guiding a Cas9 nickase, a single strand break will be accompanied by an additional single strand break, positioned by a second

gRNA, sufficiently close to one another to result in alteration of a *RHO* target position, in the *RHO* gene. In an embodiment, the first and second gRNA molecules are configured such that a single strand break positioned by said second gRNA is within 10, 20, 30, 40, or 50 nucleotides of the break positioned by said first gRNA molecule, e.g., when the Cas9 is a nickase. In an embodiment, the two gRNA molecules are configured to position cuts at the same position, or within a few nucleotides of one another, on different strands, e.g., essentially mimicking a double strand break.

In an embodiment, a double strand break can be accompanied by an additional double strand break, positioned by a second gRNA molecule, as is discussed below. For example, the targeting domain of a first gRNA molecule is configured such that a double strand break is positioned upstream of a *RHO* target position, in the *RHO* gene, e.g., within 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90, 100, 150 or 200 nucleotides of the target position; and the targeting domain of a second gRNA molecule is configured such that a double strand break is positioned downstream of a *RHO* target position, in the *RHO* gene, e.g., within 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90, 100, 150 or 200 nucleotides of the target position.

In an embodiment, a double strand break can be accompanied by two additional single strand breaks, positioned by a second gRNA molecule and a third gRNA molecule. For example, the targeting domain of a first gRNA molecule is configured such that a double strand break is positioned upstream of a *RHO* target position, in the *RHO* gene, e.g., within 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90, 100, 150 or 200 nucleotides of the target position; and the targeting domains of a second and third gRNA molecule are configured such that two single strand breaks are positioned downstream of a *RHO* target position, in the *RHO* gene, e.g., within 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90, 100, 150 or 200 nucleotides of the target position. In an embodiment, the targeting domain of the first, second and third gRNA molecules are configured such that a cleavage event, e.g., a double strand or single strand break, is positioned, independently for each of the gRNA molecules.

In an embodiment, a first and second single strand breaks can be accompanied by two additional single strand breaks positioned by a third gRNA molecule and a fourth gRNA molecule. For example, the targeting domain of a first and second gRNA molecule are configured such that two single strand breaks are positioned upstream of a *RHO* target position, in the *RHO* gene, e.g., within 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90, 100, 150 or 200 nucleotides of the target position; and the targeting domains of a third

and fourth gRNA molecule are configured such that two single strand breaks are positioned downstream of a *RHO* target position, in the *RHO* gene, e.g., within 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90, 100, 150 or 200 nucleotides of the target position.

It is contemplated herein that when multiple gRNAs are used to generate (1) two single stranded breaks in close proximity (2) one double stranded break and two paired nicks flanking a *RHO* target position (e.g., to remove a piece of DNA) or (3) four single stranded breaks, two on each side of a *RHO* target position, that they are targeting the same *RHO* target position. It is further contemplated herein that multiple gRNAs may be used to target more than one *RHO* target position in the same gene.

In some embodiments, the targeting domain of the first gRNA molecule and the targeting domain of the second gRNA molecules are complementary to opposite strands of the target nucleic acid molecule. In some embodiments, the gRNA molecule and the second gRNA molecule are configured such that the PAMs are oriented outward.

In an embodiment, the targeting domain of a gRNA molecule is configured to avoid unwanted target chromosome elements, such as repeat elements, e.g., Alu repeats, in the target domain. The gRNA molecule may be a first, second, third and/or fourth gRNA molecule.

In an embodiment, the *RHO* target position is a target position located in exon 1 or exon 2 of the *RHO* gene and the targeting domain of a gRNA molecule comprises a sequence that is the same as, or differs by no more than 1, 2, 3, 4, or 5 nucleotides from, a targeting domain sequence from **Table 1**. In some embodiments, the targeting domain is selected from those in **Table 1**. In an embodiment, the *RHO* target position is a target position located in the 5' UTR region of the *RHO* gene and the targeting domain of a gRNA molecule comprises a sequence that is the same as, or differs by no more than 1, 2, 3, 4, or 5 nucleotides from, a targeting domain sequence from any one of **Table 2**. In some embodiments, the targeting domain is selected from those in **Table 2**. In an embodiment, the target position is a target position located in intron 1 of the *RHO* gene and the targeting domain of a gRNA molecule comprises a sequence that is the same as, or differs by no more than 1, 2, 3, 4, or 5 nucleotides from, a targeting domain sequence from any one of **Table 3**. In some embodiments, the targeting domain is selected from those in **Table 3**. In an embodiment, the target position is a target position located in the *RHO* gene and the targeting domain of a gRNA molecule comprises a sequence that is the same as, or differs by no more than 1, 2, 3, 4, or 5 nucleotides from, a targeting domain sequence from any one of **Table 18**. In some embodiments, the targeting domain is selected from those in **Table 18**. In an embodiment,

the gRNA, e.g., a gRNA comprising a targeting domain, which is complementary with the *RHO* gene, is a modular gRNA. In other embodiments, the gRNA is a unimolecular or chimeric gRNA.

In an embodiment, the targeting domain which is complementary with the *RHO* gene is 17 nucleotides or more in length. In an embodiment, the targeting domain is 17 nucleotides in length. In other embodiments, the targeting domain is 18 nucleotides in length. In still other embodiments, the targeting domain is 19 nucleotides in length. In still other embodiments, the targeting domain is 20 nucleotides in length. In still other embodiments, the targeting domain is 21 nucleotides in length. In still other embodiments, the targeting domain is 22 nucleotides in length. In still other embodiments, the targeting domain is 23 nucleotides in length. In still other embodiments, the targeting domain is 24 nucleotides in length. In still other embodiments, the targeting domain is 25 nucleotides in length. In still other embodiments, the targeting domain is 26 nucleotides in length.

A gRNA as described herein may comprise from 5' to 3': a targeting domain (comprising a "core domain", and optionally a "secondary domain"); a first complementarity domain; a linking domain; a second complementarity domain; a proximal domain; and a tail domain. In some embodiments, the proximal domain and tail domain are taken together as a single domain.

In an embodiment, a gRNA comprises a linking domain of no more than 25 nucleotides in length; a proximal and tail domain, that taken together, are at least 20 nucleotides in length; and a targeting domain of 17, 18, 19 or 20 nucleotides in length.

In another embodiment, a gRNA comprises a linking domain of no more than 25 nucleotides in length; a proximal and tail domain, that taken together, are at least 30 nucleotides in length; and a targeting domain of 17, 18, 19 or 20 nucleotides in length.

In another embodiment, a gRNA comprises a linking domain of no more than 25 nucleotides in length; a proximal and tail domain, that taken together, are at least 30 nucleotides in length; and a targeting domain of 17, 18, 19 or 20 nucleotides in length.

In another embodiment, a gRNA comprises a linking domain of no more than 25 nucleotides in length; a proximal and tail domain, that taken together, are at least 40 nucleotides in length; and a targeting domain of 17, 18, 19 or 20 nucleotides in length.

A cleavage event, e.g., a double strand or single strand break, is generated by an RNA-guided nuclease (e.g., a Cas9 or Cpf1 molecule). The Cas9 molecule may be an enzymatically active Cas9 (eaCas9) molecule, e.g., an eaCas9 molecule that forms a double strand break in a target nucleic acid or an eaCas9 molecule forms a single strand break in a

target nucleic acid (e.g., a nickase molecule). In certain embodiments, the RNA-guided nuclease may be a Cpf1 molecule.

In some embodiments, the RNA-guided nuclease (e.g., eaCas9 molecule or Cpf1 molecule) catalyzes a double strand break.

In some embodiments, the eaCas9 molecule comprises HNH-like domain cleavage activity but has no, or no significant, N-terminal RuvC-like domain cleavage activity. In this case, the eaCas9 molecule is an HNH-like domain nickase, e.g., the eaCas9 molecule comprises a mutation at D10, e.g., D10A. In other embodiments, the eaCas9 molecule comprises N-terminal RuvC-like domain cleavage activity but has no, or no significant, HNH-like domain cleavage activity. In this instance, the eaCas9 molecule is an N-terminal RuvC-like domain nickase, e.g., the eaCas9 molecule comprises a mutation at H840, e.g., H840A.

In certain embodiments, the Cas9 molecule may be a self-inactivating Cas9 molecule designed for transient expression of the Cas9 protein.

In an embodiment, a single strand break is formed in the strand of the target nucleic acid to which the targeting domain of said gRNA is complementary. In another embodiment, a single strand break is formed in the strand of the target nucleic acid other than the strand to which the targeting domain of said gRNA is complementary.

In another aspect, disclosed herein is a nucleic acid, e.g., an isolated or non-naturally occurring nucleic acid, e.g., DNA, that comprises (a) a sequence that encodes a gRNA molecule comprising a targeting domain, as disclosed herein.

In an embodiment, the nucleic acid encodes a gRNA molecule, e.g., a first gRNA molecule, comprising a targeting domain configured to provide a cleavage event, e.g., a double strand break or a single strand break, sufficiently close to a *RHO* target position, in the *RHO* gene to allow alteration in the *RHO* gene. In an embodiment, the nucleic acid encodes a gRNA molecule, e.g., the first gRNA molecule, comprising a targeting domain comprising a sequence that is the same as, or differs by no more than 1, 2, 3, 4, or 5 nucleotides from, a targeting domain sequence selected from those set forth in **Tables 1-3** and **18**. In an embodiment, the nucleic acid encodes a gRNA molecule comprising a targeting domain sequence selected from those set forth in **Tables 1-3** and **18**.

In an embodiment, the nucleic acid encodes a modular gRNA, e.g., one or more nucleic acids encode a modular gRNA. In other embodiments, the nucleic acid encodes a chimeric gRNA. The nucleic acid may encode a gRNA, e.g., the first gRNA molecule, comprising a targeting domain comprising 17 nucleotides or more in length. In one

embodiment, the nucleic acid encodes a gRNA, e.g., the first gRNA molecule, comprising a targeting domain that is 17 nucleotides in length. In other embodiments, the nucleic acid encodes a gRNA, e.g., the first gRNA molecule, comprising a targeting domain that is 18 nucleotides in length. In still other embodiments, the nucleic acid encodes a gRNA, e.g., the first gRNA molecule, comprising a targeting domain that is 19 nucleotides in length. In still other embodiments, the nucleic acid encodes a gRNA, e.g., the first gRNA molecule, comprising a targeting domain that is 20 nucleotides in length.

In an embodiment, a nucleic acid encodes a gRNA comprising from 5' to 3': a targeting domain (comprising a "core domain", and optionally a "secondary domain"); a first complementarity domain; a linking domain; a second complementarity domain; a proximal domain; and a tail domain. In some embodiments, the proximal domain and tail domain are taken together as a single domain.

In an embodiment, a nucleic acid encodes a gRNA e.g., the first gRNA molecule, comprising a linking domain of no more than 25 nucleotides in length; a proximal and tail domain, that taken together, are at least 20 nucleotides in length; and a targeting domain of 17, 18, 19 or 20 nucleotides in length.

In an embodiment, a nucleic acid encodes a gRNA e.g., the first gRNA molecule, comprising a linking domain of no more than 25 nucleotides in length; a proximal and tail domain, that taken together, are at least 30 nucleotides in length; and a targeting domain of 17, 18, 19 or 20 nucleotides in length.

In an embodiment, a nucleic acid encodes a gRNA e.g., the first gRNA molecule, comprising a linking domain of no more than 25 nucleotides in length; a proximal and tail domain, that taken together, are at least 30 nucleotides in length; and a targeting domain of 17, 18, 19 or 20 nucleotides in length.

In an embodiment, a nucleic acid encodes a gRNA comprising e.g., the first gRNA molecule, a linking domain of no more than 25 nucleotides in length; a proximal and tail domain, that taken together, are at least 40 nucleotides in length; and a targeting domain of 17, 18, 19 or 20 nucleotides in length.

In an embodiment, a nucleic acid comprises (a) a sequence that encodes a gRNA molecule e.g., the first gRNA molecule, comprising a targeting domain that is complementary with a *RHO* target domain in the *RHO* gene as disclosed herein, and further comprising (b) a sequence that encodes an RNA-guided nuclease (e.g., Cas9 or Cpf1 molecule).

The Cas9 molecule may be an enzymatically active Cas9 (eaCas9) molecule, e.g., an eaCas9 molecule that forms a double strand break in a target nucleic acid or an eaCas9 molecule forms a single strand break in a target nucleic acid (e.g., a nickase molecule).

A nucleic acid disclosed herein may comprise (a) a sequence that encodes a gRNA molecule comprising a targeting domain that is complementary with a *RHO* target domain in the *RHO* gene as disclosed herein; (b) a sequence that encodes an RNA-guided nuclease (e.g., Cas9 or Cpf1 molecule); (c) a *RHO* cDNA molecule; and further comprises (d)(i) a sequence that encodes a second gRNA molecule described herein having a targeting domain that is complementary to a second target domain of the *RHO* gene, and optionally, (ii) a sequence that encodes a third gRNA molecule described herein having a targeting domain that is complementary to a third target domain of the *RHO* gene; and optionally, (iii) a sequence that encodes a fourth gRNA molecule described herein having a targeting domain that is complementary to a fourth target domain of the *RHO* gene.

In an embodiment, the *RHO* cDNA molecule is a double stranded nucleic acid. In some embodiments, the *RHO* cDNA molecule comprises a nucleotide sequence, e.g., of one or more nucleotides, encoding rhodopsin protein. In certain embodiments, the *RHO* cDNA molecule is not codon modified. In certain embodiments, the *RHO* cDNA molecule is codon modified to provide resistance to hybridization with a gRNA molecule. In certain embodiments, the *RHO* cDNA molecule is codon modified to provide improved expression of the encoded RHO protein (e.g., SEQ ID NOs:13-18). In certain embodiments, the *RHO* cDNA molecule may include a nucleotide sequence comprising exon 1, exon 2, exon 3, exon 4, and exon 5 of the *RHO* gene. In certain embodiments, the *RHO* cDNA may include an intron (e.g., SEQ ID NOs:4-7). In certain embodiments, the *RHO* cDNA molecule may include a nucleotide sequence comprising exon 1, intron 1, exon 2, exon 3, exon 4, and exon 5 of the *RHO* gene. In certain embodiments, the *RHO* cDNA molecule may include one or more of a nucleotide sequence comprising or consisting of the sequences selected from exon 1, intron 1, exon 2, intron 2, exon 3, intron 3, exon 4, intron 4, and exon 5 of the *RHO* gene. In certain embodiments, the intron comprises one or more truncations at a 5' end of intron 1, a 3' end of intron 1, or both.

In an embodiment, a nucleic acid encodes a second gRNA molecule comprising a targeting domain configured to provide a cleavage event, e.g., a double strand break or a single strand break, sufficiently close to a *RHO* target position, in the *RHO* gene, to allow alteration in the *RHO* gene, either alone or in combination with the break positioned by said first gRNA molecule.

In an embodiment, a nucleic acid encodes a third gRNA molecule comprising a targeting domain configured to provide a cleavage event, e.g., a double strand break or a single strand break, sufficiently close to a *RHO* target position, in the *RHO* gene to allow alteration in the *RHO* gene, either alone or in combination with the break positioned by the first and/or second gRNA molecule.

In an embodiment, a nucleic acid encodes a fourth gRNA molecule comprising a targeting domain configured to provide a cleavage event, e.g., a double strand break or a single strand break, sufficiently close to a *RHO* target position, in the *RHO* gene to allow alteration either alone or in combination with the break positioned by the first gRNA molecule, the second gRNA molecule and the third gRNA molecule.

In an embodiment, the nucleic acid encodes a second gRNA molecule. The second gRNA is selected to target the same *RHO* target position, as the first gRNA molecule. Optionally, the nucleic acid may encode a third gRNA, and further optionally, the nucleic acid may encode a fourth gRNA molecule. The third gRNA molecule and the fourth gRNA molecule are selected to target the same *RHO* target position, as the first and second gRNA molecules.

In an embodiment, the nucleic acid encodes a second gRNA molecule comprising a targeting domain comprising a sequence that is the same as, or differs by no more than 1, 2, 3, 4, or 5 nucleotides from, a targeting domain sequence selected from those set forth in **Tables 1-3** and **18**. In an embodiment, the nucleic acid encodes a second gRNA molecule comprising a targeting domain selected from those set forth in **Tables 1-3** and **18**. In an embodiment, when a third or fourth gRNA molecule are present, the third and fourth gRNA molecules may independently comprise a targeting domain comprising a sequence that is the same as, or differs by no more than 1, 2, 3, 4, or 5 nucleotides from, a targeting domain sequence selected from those set forth in **Tables 1-3** and **18.** In a further embodiment, when a third or fourth gRNA molecule are present, the third and fourth gRNA molecules may independently comprise a targeting domain selected from those set forth in **Tables 1-3** and **18**.

In an embodiment, the nucleic acid encodes a second gRNA which is a modular gRNA, e.g., wherein one or more nucleic acid molecules encode a modular gRNA. In other embodiments, the nucleic acid encoding a second gRNA is a chimeric gRNA. In other embodiments, when a nucleic acid encodes a third or fourth gRNA, the third and fourth gRNA may be a modular gRNA or a chimeric gRNA. When multiple gRNAs are used, any combination of modular or chimeric gRNAs may be used.

A nucleic acid may encode a second, a third, and/or a fourth gRNA comprising a targeting domain comprising 17 nucleotides or more in length. In an embodiment, the nucleic acid encodes a second gRNA comprising a targeting domain that is 17 nucleotides in length. In other embodiments, the nucleic acid encodes a second gRNA comprising a targeting domain that is 18 nucleotides in length. In still other embodiments, the nucleic acid encodes a second gRNA comprising a targeting domain that is 19 nucleotides in length. In still other embodiments, the nucleic acid encodes a second gRNA comprising a targeting domain that is 20 nucleotides in length.

In an embodiment, a nucleic acid encodes a second, a third, and/or a fourth gRNA comprising from 5' to 3': a targeting domain; a first complementarity domain; a linking domain; a second complementarity domain; a proximal domain; and a tail domain. In some embodiments, the proximal domain and tail domain are taken together as a single domain.

In an embodiment, a nucleic acid encodes a second, a third, and/or a fourth gRNA comprising a linking domain of no more than 25 nucleotides in length; a proximal and tail domain, that taken together, are at least 20 nucleotides in length; and a targeting domain of 17, 18, 19 or 20 nucleotides in length.

In an embodiment, a nucleic acid encodes a second, a third, and/or a fourth gRNA comprising a linking domain of no more than 25 nucleotides in length; a proximal and tail domain, that taken together, are at least 30 nucleotides in length; and a targeting domain of 17, 18, 19 or 20 nucleotides in length.

In an embodiment, a nucleic acid encodes a second, a third, and/or a fourth gRNA comprising a linking domain of no more than 25 nucleotides in length; a proximal and tail domain, that taken together, are at least 30 nucleotides in length; and a targeting domain of 17, 18, 19 or 20 nucleotides in length.

In an embodiment, a nucleic acid encodes a second, a third, and/or a fourth gRNA comprising a linking domain of no more than 25 nucleotides in length; a proximal and tail domain, that taken together, are at least 40 nucleotides in length; and a targeting domain of 17, 18, 19 or 20 nucleotides in length.

As described above, a nucleic acid may comprise (a) a sequence encoding a gRNA molecule comprising a targeting domain that is complementary with a target domain in the *RHO* gene, (b) a sequence encoding an RNA-guided nuclease (e.g., Cas9 or Cpf1 molecule), and (c) a *RHO* cDNA molecule sequence. In some embodiments, (a), (b), and (c) are present on the same nucleic acid molecule, e.g., the same vector, e.g., the same viral vector, e.g., the same adeno-associated virus (AAV) vector. In an embodiment, the nucleic acid molecule is

an AAV vector. Exemplary AAV vectors that may be used in any of the described compositions and methods include an AAV5 vector, a modified AAV5 vector, AAV2 vector, a modified AAV2 vector, an AAV3 vector, a modified AAV3 vector, an AAV6 vector, a modified AAV6 vector, an AAV8 vector and an AAV9 vector.

In other embodiments, (a) is present on a first nucleic acid molecule, e.g. a first vector, e.g., a first viral vector, e.g., a first AAV vector; and (b) and (c) are present on a second nucleic acid molecule, e.g., a second vector, e.g., a second vector, e.g., a second AAV vector. The first and second nucleic acid molecules may be AAV vectors.

In other embodiments, (a) and (b) are present on a first nucleic acid molecule, e.g. a first vector, e.g., a first viral vector, e.g., a first AAV vector; and (c) is present on a second nucleic acid molecule, e.g., a second vector, e.g., a second vector, e.g., a second AAV vector. The first and second nucleic acid molecules may be AAV vectors.

In other embodiments, (a) and (c) are present on a first nucleic acid molecule, e.g. a first vector, e.g., a first viral vector, e.g., a first AAV vector; and (b) is present on a second nucleic acid molecule, e.g., a second vector, e.g., a second vector, e.g., a second AAV vector. The first and second nucleic acid molecules may be AAV vectors.

In other embodiments, (a) is present on a first nucleic acid molecule, e.g. a first vector, e.g., a first viral vector, e.g., a first AAV vector; (b) is present on a second nucleic acid molecule, e.g., a second vector, e.g., a second vector, e.g., a second AAV vector; and (c) is present on a third nucleic acid molecule, e.g., a third vector, e.g., a third vector, e.g., a third AAV vector. The first, second, and third nucleic acid molecules may be AAV vectors.

In other embodiments, the nucleic acid may further comprise (d)(i) a sequence that encodes a second gRNA molecule as described herein. In some embodiments, the nucleic acid comprises (a), (b), (c), and (d)(i). Each of (a), (b), (c), and (d)(i) may be present on the same nucleic acid molecule, e.g., the same vector, e.g., the same viral vector, e.g., the same adeno-associated virus (AAV) vector. In an embodiment, the nucleic acid molecule is an AAV vector.

In other embodiments, (a) and (d)(i) are on different vectors. For example, (a) may be present on a first nucleic acid molecule, e.g. a first vector, e.g., a first viral vector, e.g., a first AAV vector; and (d)(i) may be present on a second nucleic acid molecule, e.g., a second vector, e.g., a second vector, e.g., a second AAV vector. In an embodiment, the first and second nucleic acid molecules are AAV vectors.

In other embodiments, (b) and (d)(i) are on different vectors. For example, (b) may be present on a first nucleic acid molecule, e.g. a first vector, e.g., a first viral vector, e.g., a

first AAV vector; and (d)(i) may be present on a second nucleic acid molecule, e.g., a second vector, e.g., a second vector, e.g., a second AAV vector. In an embodiment, the first and second nucleic acid molecules are AAV vectors.

In other embodiments, (c) and (d)(i) are on different vectors. For example, (c) may be present on a first nucleic acid molecule, e.g. a first vector, e.g., a first viral vector, e.g., a first AAV vector; and (d)(i) may be present on a second nucleic acid molecule, e.g., a second vector, e.g., a second vector, e.g., a second AAV vector. In an embodiment, the first and second nucleic acid molecules are AAV vectors.

In another embodiment, (a) and (d)(i) are present on the same nucleic acid molecule, e.g., the same vector, e.g., the same viral vector, e.g., an AAV vector. In an embodiment, the nucleic acid molecule is an AAV vector. In an alternate embodiment, (a) and (d)(i) are encoded on a first nucleic acid molecule, e.g., a first vector, e.g., a first viral vector, e.g., a first AAV vector; and a second and third of (a) and (d)(i) are encoded on a second nucleic acid molecule, e.g., a second vector, e.g., a second vector, e.g., a second AAV vector. The first and second nucleic acid molecule may be AAV vectors.

In another embodiment, (b) and (d)(i) are present on the same nucleic acid molecule, e.g., the same vector, e.g., the same viral vector, e.g., an AAV vector. In an embodiment, the nucleic acid molecule is an AAV vector. In an alternate embodiment, (b) and (d)(i) are encoded on a first nucleic acid molecule, e.g., a first vector, e.g., a first viral vector, e.g., a first AAV vector; and a second and third of (b) and (d)(i) are encoded on a second nucleic acid molecule, e.g., a second vector, e.g., a second vector, e.g., a second AAV vector. The first and second nucleic acid molecule may be AAV vectors.

In another embodiment, (c) and (d)(i) are present on the same nucleic acid molecule, e.g., the same vector, e.g., the same viral vector, e.g., an AAV vector. In an embodiment, the nucleic acid molecule is an AAV vector. In an alternate embodiment, (c) and (d)(i) are encoded on a first nucleic acid molecule, e.g., a first vector, e.g., a first viral vector, e.g., a first AAV vector; and a second and third of (c) and (d)(i) are encoded on a second nucleic acid molecule, e.g., a second vector, e.g., a second vector, e.g., a second AAV vector. The first and second nucleic acid molecule may be AAV vectors.

In another embodiment, each of (a), (b), and (d)(i) are present on the same nucleic acid molecule, e.g., the same vector, e.g., the same viral vector, e.g., an AAV vector. In an embodiment, the nucleic acid molecule is an AAV vector. In an alternate embodiment, one of (a), (b), and (d)(i) is encoded on a first nucleic acid molecule, e.g., a first vector, e.g., a first viral vector, e.g., a first AAV vector; and a second and third of (a), (b), and (d)(i) is

encoded on a second nucleic acid molecule, e.g., a second vector, e.g., a second vector, e.g., a second AAV vector. The first and second nucleic acid molecule may be AAV vectors.

In another embodiment, each of (b), (c), and (d)(i) are present on the same nucleic acid molecule, e.g., the same vector, e.g., the same viral vector, e.g., an AAV vector. In an embodiment, the nucleic acid molecule is an AAV vector. In an alternate embodiment, one of (b), (c), and (d)(i) is encoded on a first nucleic acid molecule, e.g., a first vector, e.g., a first viral vector, e.g., a first AAV vector; and a second and third of (b), (c), and (d)(i) is encoded on a second nucleic acid molecule, e.g., a second vector, e.g., a second vector, e.g., a second AAV vector. The first and second nucleic acid molecule may be AAV vectors.

In another embodiment, each of (a), (c), and (d)(i) are present on the same nucleic acid molecule, e.g., the same vector, e.g., the same viral vector, e.g., an AAV vector. In an embodiment, the nucleic acid molecule is an AAV vector. In an alternate embodiment, one of (a), (c), and (d)(i) is encoded on a first nucleic acid molecule, e.g., a first vector, e.g., a first viral vector, e.g., a first AAV vector; and a second and third of (a), (c), and (d)(i) is encoded on a second nucleic acid molecule, e.g., a second vector, e.g., a second vector, e.g., a second AAV vector. The first and second nucleic acid molecule may be AAV vectors.

In an embodiment, (a) is present on a first nucleic acid molecule, e.g., a first vector, e.g., a first viral vector, a first AAV vector; and (b), (c), and (d)(i) are present on a second nucleic acid molecule, e.g., a second vector, e.g., a second vector, e.g., a second AAV vector. The first and second nucleic acid molecule may be AAV vectors.

In other embodiments, (b) is present on a first nucleic acid molecule, e.g., a first vector, e.g., a first viral vector, e.g., a first AAV vector; and (a), (c), and (d)(i) are present on a second nucleic acid molecule, e.g., a second vector, e.g., a second vector, e.g., a second AAV vector. The first and second nucleic acid molecule may be AAV vectors.

In other embodiments, (c) is present on a first nucleic acid molecule, e.g., a first vector, e.g., a first viral vector, e.g., a first AAV vector; and (a), (b), and (d)(i) are present on a second nucleic acid molecule, e.g., a second vector, e.g., a second vector, e.g., a second AAV vector. The first and second nucleic acid molecule may be AAV vectors.

In other embodiments, (d)(i) is present on a first nucleic acid molecule, e.g., a first vector, e.g., a first viral vector, e.g., a first AAV vector; and (a), (b), and (c) are present on a second nucleic acid molecule, e.g., a second vector, e.g., a second vector, e.g., a second AAV vector. The first and second nucleic acid molecule may be AAV vectors.

In another embodiment, each of (a), (b), (c), and (d)(i) are present on different nucleic acid molecules, e.g., different vectors, e.g., different viral vectors, e.g., different AAV vector.

For example, (a) may be on a first nucleic acid molecule, (b) on a second nucleic acid molecule, (c) on a third nucleic acid molecule, and (d)(i) on a fourth nucleic acid molecule. The first, second, third, and fourth nucleic acid molecule may be AAV vectors.

In another embodiment, when a third and/or fourth gRNA molecule are present, each of (a), (b), (c), (d)(i), (d)(ii) and (d)(iii) may be present on the same nucleic acid molecule, e.g., the same vector, e.g., the same viral vector, e.g., an AAV vector. In an embodiment, the nucleic acid molecule is an AAV vector. In an alternate embodiment, each of (a), (b), (c), (d)(i), (d)(ii) and (d)(iii) may be present on the different nucleic acid molecules, e.g., different vectors, e.g., the different viral vectors, e.g., different AAV vectors. In further embodiments, each of (a), (b), (c), (d)(i), (d)(ii) and (d)(iii) may be present on more than one nucleic acid molecule, but fewer than six nucleic acid molecules, e.g., AAV vectors.

The nucleic acids described herein may comprise a promoter operably linked to the sequence that encodes the gRNA molecule of (a), e.g., a promoter described herein. The nucleic acid may further comprise a second promoter operably linked to the sequence that encodes the second, third and/or fourth gRNA molecule of (d), e.g., a promoter described herein. The promoter and second promoter differ from one another. In some embodiments, the promoter and second promoter are the same.

The nucleic acids described herein may further comprise a promoter operably linked to the sequence that encodes the RNA-guided nuclease (e.g., Cas9 or Cpf1 molecule) of (b), e.g., a promoter described herein. In certain embodiments, the promoter operably linked to the sequence that encodes the RNA-guided nuclease of (b) comprises a rod-specific promoter. In certain embodiments, the rod-specific promoter may be a human *RHO* promoter. In certain embodiments, the human *RHO* promoter may be a minimal *RHO* promoter (e.g., SEQ ID NO:44).

The nucleic acids described herein may further comprise a promoter operably linked to the *RHO* cDNA molecule of (c), e.g., a promoter described herein. In certain embodiments, the promoter operably linked to the *RHO* cDNA molecule of (c) comprises a rod-specific promoter. In certain embodiments, the rod-specific promoter may be a human *RHO* promoter. In certain embodiments, the human *RHO* promoter may be a minimal *RHO* promoter (e.g., SEQ ID NO:44). In certain embodiments, the nucleic acids may further comprise a 3' UTR nucleotide sequence downstream of the *RHO* cDNA molecule. In certain embodiments, the 3' UTR nucleotide sequence downstream of the *RHO* cDNA molecule may comprise a *RHO* gene 3' UTR nucleotide sequence. In certain embodiments, the 3' UTR nucleotide sequence downstream of the *RHO* cDNA molecule may comprise a 3' UTR

nucleotide sequence of an mRNA encoding a highly expressed protein. For example, in certain embodiments, the 3' UTR nucleotide sequence downstream of the *RHO* cDNA molecule may comprise an α-globin 3' UTR nucleotide sequence. In certain embodiments, the 3' UTR nucleotide sequence downstream of the *RHO* cDNA molecule may comprise a β-globin 3' UTR nucleotide sequence. In certain embodiments, the 3' UTR nucleotide sequence comprises one or more truncations at a 5' end of said 3' UTR nucleotide sequence, a 3' end of said 3' UTR nucleotide sequence, or both.

In another aspect, disclosed herein is a composition comprising (a) a gRNA molecule comprising a targeting domain that is complementary with a target domain in the *RHO* gene, as described herein. The composition of (a) may further comprise (b) an RNA-guided nuclease (e.g., Cas9 or Cpf1 molecule as described herein). Cpf1 is also sometimes referred to as Cas12a. A composition of (a) and (b) may further comprise (c) a *RHO* cDNA molecule. A composition of (a), (b), and (c) may further comprise (d) a second, third and/or fourth gRNA molecule, e.g., a second, third and/or fourth gRNA molecule described herein.

In another aspect, disclosed herein is a method of altering a cell, e.g., altering the structure, e.g., altering the sequence, of a target nucleic acid of a cell, comprising contacting said cell with: (a) a gRNA that targets the *RHO* gene, e.g., a gRNA as described herein; (b) an RNA-guided nuclease (e.g., Cas9 or Cpf1 molecule as described herein); and (c) a *RHO* cDNA molecule; and optionally, (d) a second, third and/or fourth gRNA that targets *RHO* gene, e.g., a gRNA.

In some embodiments, the method comprises contacting said cell with (a) and (b).

In some embodiments, the method comprises contacting said cell with (a), (b), and (c).

In some embodiments, the method comprises contacting said cell with (a), (b), (c) and (d).

The gRNA of (a) and optionally (d) may comprise a targeting domain sequence selected from those set forth in **Tables 1-3** and **18**, or may comprise a targeting domain sequence that differs by no more than 1, 2, 3, 4, or 5 nucleotides from a targeting domain sequence set forth in any of **Tables 1-3** and **18**.

In some embodiments, the method comprises contacting a cell from a subject suffering from or likely to develop adRP. The cell may be from a subject having a mutation at a *RHO* target position.

In some embodiments, the cell being contacted in the disclosed method is a cell from the eye of the subject, e.g., a retinal cell, e.g., a photoreceptor cell. The contacting may be

performed *ex vivo* and the contacted cell may be returned to the subject's body after the contacting step. In other embodiments, the contacting step may be performed *in vivo*.

In some embodiments, the method of altering a cell as described herein comprises acquiring knowledge of the presence of a mutation in the *RHO* gene, in said cell, prior to the contacting step. Acquiring knowledge of a mutation in the *RHO* gene, in the cell may be by sequencing the *RHO* gene, or a portion of the *RHO* gene.

In some embodiments, the contacting step of the method comprises contacting the cell with a nucleic acid, e.g., a vector, e.g., an AAV vector, that expresses at least one of (a), (b), and (c). In some embodiments, the contacting step of the method comprises contacting the cell with a nucleic acid, e.g., a vector, e.g., an AAV vector, that expresses each of (a), (b), and (c). In another embodiment, the contacting step of the method comprises delivering to the cell an RNA-guided nuclease (e.g., Cas9 or Cpf1 molecule) of (b) and a nucleic acid which encodes a gRNA (a), a *RHO* cDNA (c), and optionally, a second gRNA (d)(i), and further optionally, a third gRNA (d)(iv) and/or fourth gRNA (d)(iii).

In some embodiments, the contacting step of the method comprises contacting the cell with a nucleic acid, e.g., a vector, e.g., an AAV vector, that expresses at least one of (a), (b), (c) and (d). In some embodiments, the contacting step of the method comprises contacting the cell with a nucleic acid, e.g., a vector, e.g., an AAV vector, that expresses each of (a), (b), and (c). In another embodiment, the contacting step of the method comprises delivering to the cell an RNA-guided nuclease (e.g., Cas9 or Cpf1 molecule) of (b), a nucleic acid which encodes a gRNA (a) and a *RHO* cDNA molecule (c), and optionally, a second gRNA (d)(i), and further optionally, a third gRNA (d)(iv) and/or fourth gRNA (d)(iii).

In an embodiment, contacting comprises contacting the cell with a nucleic acid, e.g., a vector, e.g., an AAV vector, e.g., an AAV5 vector, a modified AAV5 vector, an AAV2 vector, a modified AAV2 vector, an AAV3 vector, a modified AAV3 vector, an AAV6 vector, a modified AAV6 vector, an AAV8 vector or an AAV9 vector.

In an embodiment, contacting comprises delivering to the cell an RNA-guided nuclease (e.g., Cas9 or Cpf1 molecule) of (b), as a protein or an mRNA, and a nucleic acid which encodes (a) and (c) and optionally (d).

In an embodiment, contacting comprises delivering to the cell an RNA-guided nuclease (e.g., Cas9 or Cpf1 molecule) of (b), as a protein or an mRNA, said gRNA of (a), as an RNA, and optionally said second gRNA of (d), as an RNA, and the *RHO* cDNA molecule (c) as a DNA.

In an embodiment, contacting comprises delivering to the cell a gRNA of (a) as an RNA, optionally said second gRNA of (d) as an RNA, and a nucleic acid that encodes the RNA-guided nuclease (e.g., Cas9 or Cpf1 molecule) of (b), and the *RHO* cDNA molecule (c) as a DNA.

In another aspect, disclosed herein is a method of treating a subject suffering from or likely to develop adRP, e.g., altering the structure, e.g., sequence, of a target nucleic acid of the subject, comprising contacting the subject (or a cell from the subject) with:

(a) a gRNA that targets the *RHO* gene, e.g., a gRNA disclosed herein;

(b) an RNA-guided nuclease, e.g., a Cas9 or Cpf1 molecule disclosed herein; and

(c) a *RHO* cDNA molecule; and

optionally, (d)(i) a second gRNA that targets the *RHO* gene, e.g., a second gRNA disclosed herein, and

further optionally, (d)(ii) a third gRNA, and still further optionally, (d)(iii) a fourth gRNA that target the *RHO* gene, e.g., a third and fourth gRNA disclosed herein.

In some embodiments, contacting comprises contacting with (a) and (b).

In some embodiments, contacting comprises contacting with (a), (b), and (c).

In some embodiments, contacting comprises contacting with (a), (b), (c), and (d)(i).

In some embodiments, contacting comprises contacting with (a), (b), (c), (d)(i) and (d)(ii).

In some embodiments, contacting comprises contacting with (a), (b), (c), (d)(i), (d)(ii) and (d)(iii).

The gRNA of (a) or (d) (e.g., (d)(i), (d)(ii), or (d)(iii) may comprise a targeting domain sequence selected from any of those set forth in **Tables 1-3** and **18**, or may comprise a targeting domain sequence that differs by no more than 1, 2, 3, 4, or 5 nucleotides from a targeting domain sequence set forth in any of **Tables 1-3** and **18**.

In an embodiment, the method comprises acquiring knowledge of the presence of a mutation in the *RHO* gene, in said subject.

In an embodiment, the method comprises acquiring knowledge of the presence of a mutation in the *RHO* gene, in said subject by sequencing the *RHO* gene or a portion of the *RHO* gene.

In an embodiment, the method comprises altering a *RHO* target position in a *RHO* gene resulting in knocking out the *RHO* gene and providing exogenous *RHO* cDNA.

When the method comprises altering a *RHO* target position and providing exogenous *RHO* cDNA, an RNA-guided nuclease (e.g., Cas9 or Cpf1 molecule) of (b), at least one guide

RNA (e.g., a guide RNA of (a) and a *RHO* cDNA molecule (c) are included in the contacting step.

In an embodiment, a cell of the subject is contacted *ex vivo* with (a), (b), (c) and optionally (d). In an embodiment, said cell is returned to the subject's body.

In an embodiment, a cell of the subject is contacted is *in vivo* with (a), (b), (c) and optionally (d).

In an embodiment, the cell of the subject is contacted *in vivo* by intravenous delivery of (a), (b), (c) and optionally (d).

In an embodiment, contacting comprises contacting the subject with a nucleic acid, e.g., a vector, e.g., an AAV vector, described herein, e.g., a nucleic acid that encodes at least one of (a), (b), (c) and optionally (d).

In an embodiment, contacting comprises delivering to said subject said RNA-guided nuclease (e.g., Cas9 or Cpf1 molecule) of (b), as a protein or mRNA, and a nucleic acid which encodes (a), a *RHO* cDNA molecule of (c) and optionally (d).

In an embodiment, contacting comprises delivering to the subject the RNA-guided nuclease (e.g., Cas9 or Cpf1 molecule) of (b), as a protein or mRNA, the gRNA of (a), as an RNA, a *RHO* cDNA molecule of (c) and optionally the second gRNA of (d), as an RNA.

In an embodiment, contacting comprises delivering to the subject the gRNA of (a), as an RNA, optionally said second gRNA of (d), as an RNA, a nucleic acid that encodes the RNA-guided nuclease (e.g., Cas9 or Cpf1 molecule) of (b), and a *RHO* cDNA molecule of (c).

In an embodiment, a cell of the subject is contacted *ex vivo* with (a), (b), (c), and optionally (d). In an embodiment, said cell is returned to the subject's body.

In an embodiment, a cell of the subject is contacted is *in vivo* with (a), (b), (c) and optionally (d). In an embodiment, the cell of the subject is contacted *in vivo* by intravenous delivery of (a), (b), (c) and optionally (d).

In an embodiment, contacting comprises contacting the subject with a nucleic acid, e.g., a vector, e.g., an AAV vector, described herein, e.g., a nucleic acid that encodes at least one of (a), (b), (c) and optionally (d).

In an embodiment, contacting comprises delivering to said subject said RNA-guided nuclease (e.g., Cas9 or Cpf1 molecule) of (b), as a protein or mRNA, and a nucleic acid which encodes (a), (c) and optionally (d).

In an embodiment, contacting comprises delivering to the subject the RNA-guided nuclease (e.g., Cas9 or Cpf1 molecule) of (b), as a protein or mRNA, the gRNA of (a), as an

RNA, and optionally the second gRNA of (d), as an RNA, and further optionally the *RHO* cDNA molecule of (c) as a DNA.

In an embodiment, contacting comprises delivering to the subject the gRNA of (a), as an RNA, optionally said second gRNA of (d), as an RNA, and a nucleic acid that encodes the RNA-guided nuclease (e.g., Cas9 or Cpf1 molecule) of (b), and the *RHO* cDNA molecule of (c) as a DNA.

In another aspect, disclosed herein is a reaction mixture comprising a, gRNA, a nucleic acid, or a composition described herein, and a cell, e.g., a cell from a subject having, or likely to develop adRP, or a subject having a mutation in the *RHO* gene.

In another aspect, disclosed herein is a kit comprising, (a) gRNA molecule described herein, or nucleic acid that encodes the gRNA, and one or more of the following:

(b) an RNA-guided nuclease molecule, e.g., a Cas9 or Cpf1 molecule described herein, or a nucleic acid or mRNA that encodes the RNA-guided nuclease;

(c) a *RHO* cDNA molecule;

(d)(i) a second gRNA molecule, e.g., a second gRNA molecule described herein or a nucleic acid that encodes (d)(i);

(d)(ii) a third gRNA molecule, e.g., a second gRNA molecule described herein or a nucleic acid that encodes (d)(ii);

(d)(iii) a fourth gRNA molecule, e.g., a second gRNA molecule described herein or a nucleic acid that encodes (d)(iii).

In an embodiment, the kit comprises nucleic acid, e.g., an AAV vector, that encodes one or more of (a), (b), (c), (d)(i), (d)(ii), and (d)(iii).

In certain embodiments, the vector or nucleic acid may include a sequence set forth in one or more of SEQ ID NOs:8-11.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present disclosure, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

Headings, including numeric and alphabetical headings and subheadings, are for organization and presentation and are not intended to be limiting.

Other features and advantages of the disclosure will be apparent from the detailed description, drawings, and from the claims.

DESCRIPTION OF THE DRAWINGS

The accompanying drawings exemplify certain aspects and embodiments of the present disclosure. The depictions in the drawings are intended to provide illustrative, and schematic rather than comprehensive, examples of certain aspects and embodiments of the present disclosure. The drawings are not intended to be limiting or binding to any particular theory or model, and are not necessarily to scale. Without limiting the foregoing, nucleic acids and polypeptides may be depicted as linear sequences, or as schematic, two- or three dimensional structures; these depictions are intended to be illustrative, rather than limiting or binding to any particular model or theory regarding their structure.

Fig. 1 illustrates the genome editing strategy implemented in certain embodiments of the disclosure. Step 1 includes knocking out ("KO") or alteration of the *RHO* gene, for example, in the *RHO* target position of exon 1. Knocking out the *RHO* gene results in loss of function of the endogenous *RHO* gene (e.g., a mutant *RHO* gene). Step 2 includes replacing the *RHO* gene with an exogenous *RHO* cDNA including a minimal *RHO* promoter and a *RHO* cDNA.

Fig. 2 is a schematic of an exemplary dual AAV delivery system that may be used for a variety of applications, including without limitation, the alteration of the *RHO* target position, according to certain embodiments of the disclosure. Vector 1 shows an AAV5 genome, which encodes ITRs, a GRK1 promoter, and a Cas9 molecule flanked by NLS sequences. Vector 2 shows an AAV5 genome, which encodes ITRs, a minimal *RHO* promoter, a *RHO* cDNA molecule, a U6 promoter, and a gRNA. In certain embodiments, the AAV vectors may be delivered via subretinal injection.

Fig. 3 is a schematic of an exemplary dual AAV delivery system that may be used for a variety of applications, including without limitation, the alteration of the *RHO* target position, according to certain embodiments of the disclosure. Vector 1 shows an AAV5 genome, which encodes a minimal *RHO* promoter and a Cas9 molecule. Vector 2 shows an AAV5 genome, which encodes a minimal *RHO* promoter, a *RHO* cDNA molecule, a U6 promoter, and a gRNA. In certain embodiments, the AAV vectors may be delivered via subretinal injection.

Fig. 4 depicts indels of the *RHO* gene in HEK293 cells formed by dose-dependent gene editing using ribonucleoproteins (RNPs) comprising RHO-3, RHO-7, or RHO-10

gRNAs (**Table 17**) and Cas9. Increasing concentrations of RNP were delivered to HEK293 cells. Indels of the *RHO* gene were assessed using next generation sequencing (NGS). Data from RNP comprising RHO-3 gRNA, RHO-10 gRNA, or RHO-7 gRNA are represented by circles, squares, and triangles, respectively. Data from control plasmid (expressing Cas9 with scrambled gRNA that does not target a sequence within the human genome) are represented by X.

Fig. 5 shows details characterizing the predicted gRNA *RHO* alleles generated by RHO-3, RHO-7, or RHO-10 gRNAs (**Table 17**). As shown in the schematic of the human *RHO* cDNA and corresponding exons at the bottom of **Fig. 5**, RHO-3, RHO-10, and RHO-7 gRNAs are predicted to cut the *RHO* cDNA at Exon 1, the Exon 2/Intron 2 border, and the Exon 1/Intron 1 border, respectively. The target site positions for RHO-3, RHO-10, and RHO-7 gRNAs are located at bases encoding amino acids (AA) 96, 174, and 120 of the RHO protein, respectively. The protein lengths for each resulting construct for the predicted -1, -2, and -3 frame shifts are set forth. For RHO-3, a 1 base deletion at position 96 results in a truncated protein that is 95 amino acids long, a 2 base deletion at position 96 results in a truncated protein that is 120 amino acids long, a 3 base deletion at position 96 results in a truncated protein that is 347 amino acids long. For RHO-10, a 1 base deletion at position 174 results in a truncated protein that is 215 amino acids long, a 2 base deletion at position 174 results in a truncated protein that is 328 amino acids long, a 3 base deletion at position 174 results in a truncated protein that is 347 amino acids long. For RHO-7, a 1 base deletion at position 120 results in a truncated protein that is 142 amino acids long, a 2 base deletion at position 120 results in a truncated protein that is 142 amino acids long, a 3 base deletion at position 120 results in a truncated protein that is 347 amino acids long. **Fig 6.** provides schematics of the predicted truncated proteins.

Fig. 6 shows schematics of the predicted *RHO* alleles generated by RHO-3, RHO-7, or RHO-10 gRNAs (**Table 17**). *RHO* alleles were predicted based on deletions of 1, 2, or 3 base pairs at the RHO-3, RHO-7, or RHO-10 cut sites. RHO Exons are represented by dark grey, stop codons are represented by black, missense protein is represented by stripes, deletions are represented by light grey.

Figs. 7A and **7B** show the viability of HEK293 cells expressing wild-type or mock-edited *RHO* alleles. Schematics of *RHO* alleles predicted to be generated by RHO-3, RHO-7, and RHO-10 gRNAs (**Table 17**) having 1 base pair (bp), 2bp or 3bp deletions are illustrated in **Fig. 6**. RHO mutations predicted to be generated from RHO-3, RHO-7, and RHO-10 gRNAs (i.e., mock-edited RHO alleles) were generated using either WT-RHO cDNA or

RHO cDNA expressing the P23H RHO variant. Wild-type RHO, mock-edited RHO alleles, or RHO alleles expressing the P23H RHO variant were cloned into mammalian expression plasmids, lipofected into HEK293 cells and assessed for cell viability after 48 hours using the ATPLite Luminescence Assay by Perkin Elmer. **Fig. 7A** shows viability depicted by luminescence of cells with modified WT RHO alleles. **Fig. 7B** shows viability depicted by luminescence of cells with modified P23H RHO alleles. The upper dotted line represents the level of luminescence from WT RHO alleles and the lower dotted line represents the level of luminescence from the P23H RHO alleles.

Fig. 8 shows editing of rod photoreceptors in non-human primate (NHP) explants using RHO-9 gRNA (**Table 1**). RNA from a rod-specific mRNA (neural retina leucine zipper (NRL)) was extracted from the explants and measured to determine the percentage of rods present in the explants. RNA from beta actin (ACTB) was also measured to determine the total number of cells. The x-axis shows the delta between ACTB and NRL RNA levels as measured by RT-PCR, which is a measure for the percentage of rods in the explant at the time of lysing the explants. Indels of the *RHO* gene were assessed using next generation sequencing (NGS). Each circle represents data from a different explant.

Fig. 9 shows a schematic of the plasmid for the dual luciferase system used for optimizing the RHO replacement vector.

Fig. 10 depicts the ratio of firefly/renilla luciferase luminescence using the dual luciferase system to test the effects of different lengths of the RHO promoter on RHO expression. The lengths of the RHO promoter that were tested ranged from 3.0 Kb to 250 bp.

Figs. 11A and **11B** depict the effects on RHO mRNA and RHO protein expression of adding various 3' UTRs to the RHO replacement vector. The HBA1 3' UTR (SEQ ID NO:38), short HBA1 3' UTR (SEQ ID NO:39), TH 3' UTR (SEQ ID NO:40), COL1A1 3'UTR (SEQ ID NO:41), ALOX15 3'UTR (SEQ ID NO:42), and minUTR (SEQ ID NO:56) were tested. **Fig. 11A** shows results using RT-qPCR to measure RHO mRNA expression. **Fig. 11B** shows results using a RHO ELISA assay to measure RHO protein expression.

Fig. 12 depicts the effects on RHO protein expression of inserting different RHO introns into RHO cDNA in the RHO replacement vector. The various RHO cDNA sequences with inserted introns (i.e, Introns 1-4) are set forth in SEQ ID NOs: 4-7, respectively.

Fig. 13 depicts the effects on RHO protein expression of using wild-type or different codon optimized RHO constructs in the RHO replacement vector. The various codon optimized RHO cDNA sequences (i.e., Codon 1-6) are set forth in SEQ ID NOs: 13-18, respectively. The RHO cDNAs were under the control of a CMV or EFS promoter.

Figs. 14A and **14B** depict *in vivo* editing of the RHO gene and knock down of Cas9 using a self-limiting Cas9 vector system ("SD"). **Fig. 14A** shows successful knockdown of Cas9 levels using the self-limiting Cas9 vector system (i.e., "SD Cas9 + Rho"). **Fig. 14B** shows successful editing using the self-limiting Cas9 vector system (i.e., "SD Cas9").

Fig. 15 depicts RHO expression in human explants. Explants were transduced with "shRNA": transduction of retinal explants with shRNA targeting the RHO gene and a replacement vector providing a RHO cDNA (as published in Cideciyan 2018); "Vector A": a two-vector system (Vector 1 comprising saCas9 driven by the minimal RHO promoter (250 bp), and Vector 2 comprising a codon-optimized RHO cDNA (codon-6) and comprising a HBA1 3' UTR under the control of the minimal 250 bp RHO promoter, as well as as the RHO-9 gRNA (**Table 1**) under the control of a U6 promoter); "Vector B": a two-vector system identical to "Vector A" except for Vector 2 comprising a wt RHO cDNA; and "UTC": untransduced control.

Fig. 16 is a schematic of an exemplary AAV vector (SEQ ID NO:11) according to certain embodiments of the disclosure. The schematic shows an AAV5 genome comprising and encoding an ITR (SEQ ID NO:92), a first U6 promoter (SEQ ID NO:78), a first RHO-7 gRNA (comprising a RHO-7 gRNA targeting domain (SEQ ID NO:606) (DNA) and SEQ ID NO:12), a second U6 promoter (SEQ ID NO:78), a second RHO-7 gRNA (comprising a RHO-7 gRNA targeting domain (SEQ ID NO:606) (DNA) and SEQ ID NO:12), a minimum RHO Promoter (250 bp) (SEQ ID NO:44), an SV40 Intron (SEQ ID NO:94), a codon optimized RHO cDNA (SEQ ID NO:18), HBA1 3' UTR (SEQ ID NO:38), a minipolyA (SEQ ID NO:56), and a right ITR (SEQ ID NO:93). In certain embodiments, the AAV vector may be delivered via subretinal injection.

Fig. 17 is a schematic of an exemplary AAV vector (SEQ ID NO:10) according to certain embodiments of the disclosure. The schematic shows an AAV5 genome comprising and encoding an ITR (SEQ ID NO:92), a minimum RHO Promoter (250 bp) (SEQ ID NO:44), an SV40 Intron (SEQ ID NO:94), an NLS sequence, an *S. aureus* Cas9 sequence, an SV40 NLS, an HBA1 3' UTR (SEQ ID NO:38), and a right ITR (SEQ ID NO:93). In certain embodiments, the AAV vector may be delivered via subretinal injection.

Fig. 18 is a schematic of an exemplary AAV vector (SEQ ID NO:9) according to certain embodiments of the disclosure. The schematic shows an AAV5 genome comprising and encoding an ITR (SEQ ID NO:92), a minimum RHO Promoter, an SV40 SA/SD, an NLS, an *S. aureus* Cas9 sequence, an SV40 NLS, a minipolyA (SEQ ID NO:56), and a right

ITR (SEQ ID NO:93). In certain embodiments, the AAV vector may be delivered via subretinal injection.

DETAILED DESCRIPTION

Definitions

"Domain", as used herein, is used to describe segments of a protein or nucleic acid. Unless otherwise indicated, a domain is not required to have any specific functional property.

Calculations of homology or sequence identity between two sequences (the terms are used interchangeably herein) are performed as follows. The sequences are aligned for optimal comparison purposes (e.g., gaps can be introduced in one or both of a first and a second amino acid or nucleic acid sequence for optimal alignment and non-homologous sequences can be disregarded for comparison purposes). The optimal alignment is determined as the best score using the GAP program in the GCG software package with a Blossum 62 scoring matrix with a gap penalty of 12, a gap extend penalty of 4, and a frame shift gap penalty of 5. The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position. The percent identity between the two sequences is a function of the number of identical positions shared by the sequences.

"Modulator", as used herein, refers to an entity, e.g., a drug, that can alter the activity (e.g., enzymatic activity, transcriptional activity, or translational activity), amount, distribution, or structure of a subject molecule or genetic sequence. In an embodiment, modulation comprises cleavage, e.g., breaking of a covalent or non-covalent bond, or the forming of a covalent or non-covalent bond, e.g., the attachment of a moiety, to the subject molecule. In an embodiment, a modulator alters the, three dimensional, secondary, tertiary, or quaternary structure, of a subject molecule. A modulator can increase, decrease, initiate, or eliminate a subject activity.

"Polypeptide", as used herein, refers to a polymer of amino acids having less than 100 amino acid residues. In an embodiment, it has less than 50, 20, or 10 amino acid residues.

"Replacement", or "replaced", as used herein with reference to a modification of a molecule does not require a process limitation but merely indicates that the replacement entity is present.

"*RHO* target position," as that term is used herein, refers to a target position, e.g., one or more nucleotides, in or near the *RHO* gene, that are targeted for alteration using the

methods described herein. In certain embodiments, alteration of the *RHO* target position, e.g., by substitution, deletion, or insertion, may result in disruption (e.g., "knocking out") of the *RHO* gene. In certain embodiments, the *RHO* target position may be located in a 5' region of the *RHO* gene (e.g., 5' UTR, exon 1, exon 2, intron 1, the exon 1/intron 1 border, or the exon 2/intron 1 border), a non-coding region of the *RHO* gene (e.g., an enhancer region, a promoter region, an intron, 5' UTR, 3'UTR, polyadenylation signal), or a coding region of the *RHO* gene (e.g., early coding region, an exon (e.g., exon 1, exon 2, exon 3, exon 4, exon 5), or an exon/intron border (e.g., exon 1/intron1, exon 2/intron 1) of the *RHO* gene.

"Small molecule", as used herein, refers to a compound having a molecular weight less than about 2 kD, e.g., less than about 2 kD, less than about 1.5 kD, less than about 1 kD, or less than about 0.75 kD.

"Subject", as used herein, may mean either a human or non-human animal. The term includes, but is not limited to, mammals (e.g., humans, other primates, pigs, rodents (e.g., mice and rats or hamsters), rabbits, guinea pigs, cows, horses, cats, dogs, sheep, and goats). In an embodiment, the subject is a human. In other embodiments, the subject is poultry.

"Treat", "treating" and "treatment", as used herein, mean the treatment of a disease in a mammal, e.g., in a human, including (a) inhibiting the disease, i.e., arresting or preventing its development; (b) relieving the disease, i.e., causing regression of the disease state; and (c) curing the disease.

"X" as used herein in the context of an amino acid sequence, refers to any amino acid (e.g., any of the twenty natural amino acids) unless otherwise specified.

Autosomal-dominant retinitis pigmentosa (adRP)

Retinitis pigmentosa (RP) affects between 50,000 and 100,000 people in the United States. RP is a group of inherited retinal dystrophies that affect photoreceptors and retinal pigment epithelium cells. The disease causes retinal deterioration and atrophy, and is characterized by progressive deterioration of vision, ultimately resulting in blindness.

Typical disease onset is during the teenage years, although some subjects may present in early adulthood. Subjects initially present with poor night vision and declining peripheral vision. In general, visual loss proceeds from the peripheral visual field inwards. The majority of subjects are legally blind by the age of 40. The central visual field may be spared through the late stages of the disease, so that some subjects may have normal visual acuity within a small visual field into their 70's. However, the majority of subjects lose their central vision as well between the age of 50 and 80 (Berson 1990). Upon examination, a subject

may have one or more of bone spicule pigmentation, narrowing of the visual fields and retinal atrophy.

There are over 60 genes and hundreds of mutations that cause RP. Autosomal dominant RP (adRP), accounts for 15-25% of RP. Autosomal recessive RP (arRP) accounts for 5-20% of RP. X-linked RP (X-LRP) accounts for 5-15% of RP (Daiger 2007). In general, adRP often has the latest presentation, arRP has a moderate presentation and X-LRP has the earliest presentation.

Autosomal-dominant retinitis pigmentosa (adRP) is caused by heterozygous mutations in the rhodopsin (*RHO*) gene. Mutations in the *RHO* gene account for 25-30% of cases of adRP.

The *RHO* gene encodes the rhodopsin protein. Rhodopsin is a G protein-coupled receptor expressed in the outer segment of retinal photoreceptor (PR) rod cells and is a critical element of the phototransduction cascade. Light absorbed by rhodopsin causes 11-cis retinal to isomerize into all-trans retinal. This conformational change allows rhodopsin to couple with transducin, which is the first step in the visual signaling cascade. Heterozygous mutations in the *RHO* gene cause a decreased production of wild-type rhodopsin and/or expression of mutant rhodopsin. This leads to poor function of the phototransduction cascade and declining function in rod PR cells. Over time, there is atrophy of rod PR cells and eventually atrophy of cone PR cells as well. This causes the typical phenotypic progression of cumulative vision loss experienced by RP subjects. Subjects with *RHO* mutations experience progressive loss of peripheral visual fields followed by loss of central visual fields (the latter measured by decreases in visual acuity).

Exemplary *RHO* mutations are provided in **Table A**.

Table A: RHO Mutations (Group A Mutations)

Number	Mutation
1	Pro23His
2	Pro23Leu
3	Thr58Arg
4	Pro347Thr
5	Pro347Ala
6	Pro347Ser
7	Pro347Gly
8	Pro347Leu
9	Pro347Arg
10	Thr 4 Lys
11	Asn 15 Ser

12	Thr 17 Met
13	Gln 28 His
14	Leu 40 Arg
15	Met 44 Thr
16	Phe 45 Leu
17	Leu 46 Arg
18	Gly 51 Arg
19	Gly 51 Val
20	Gly 51 Ala
21	Pro 53 Arg
22	Thr 58 Arg
23	Gln 64 stop
24	Val 87 Asp
25	Gly 89 Asp
26	Gly 106 Arg
27	Gly 106 Trp
28	Gly 109 Arg
29	Cys 110 Tyr
30	Cys 110 Phe
31	Gly 114 Asp
32	Gly 114 Val
33	Leu 125 Arg
34	Ser 127 Phe
35	Leu 131 Pro
36	Arg 135 Gly
37	Arg 135 Trp
38	Arg 135 Leu
39	Arg 135 Pro
40	Tyr 136 stop
41	Val 137 Met
42	Cys 140 Ser
43	Ala 164 Val
44	Ala 164 Glu
45	Cys 167 Arg
46	Cys 167 Trp
47	Pro 171 Glu
48	Pro 171 Ser
49	Pro 171 Leu
50	Pro 171 Gln
51	Tyr 178 Asn
52	Tyr 178 Cys
53	Pro 180 Ala
54	Glu 181 Lys
55	Gly 182 Ser
56	Gln 184 Pro
57	Ser 186 Pro
58	Ser 186 Trp
59	Cys 187 Tyr

60	Gly 188 Arg
61	Gly 188 Glu
62	Asp 190 Asn
63	Asp 190 Tyr
64	Asp 190 Gly
65	Thr 193 Met
66	Met 207 Arg
67	Val 209 Met
68	His 211 Arg
69	His 211 Pro
70	Pro 215 Thr
71	Met 216 Arg
72	Met 216 Lys
73	Phe 220 Cys
74	Cys 222 Arg
75	Pro 267 Leu
76	Pro 267 Arg
77	Ser 270 Arg
78	Thr 289 Pro
79	Lys 296 Glu
80	Lys 296 Met
81	Ser 297 Arg
82	Gln 312 stop
83	Leu 328 Pro
84	Thr 342 Met
85	Gln 344 stop
86	Val 345 Leu
87	Val 345 Met
88	Ala 346 Pro
89	stop 349 Glu
90	Glu 150 Lys
91	Gly 174 Ser
92	Glu 249 ter
93	Gly 284 Ser

Treatment for RP is limited and there is currently no approved treatment that substantially reverses or halts the progression of disease in adRP. In an embodiment, Vitamin A supplementation may delay onset of disease and slow progression. The Argus II retinal implant was approved for use in the United States in 2013. The Argus II retinal implant is an electrical implant that offers minimal improvement in vision in subjects with RP. For example, the best visual acuity achieved in trials by the device was 20/1260. However, legal blindness is defined as 20/200 vision.

Overview

As provided herein, the inventors have designed a therapeutic strategy that provides an alteration that comprises disrupting the mutant *RHO* gene by the insertion or deletion of one or more nucleotides mediated by an RNA-guided nuclease (e.g., Cas9 or Cpf1) as described below and providing a functional *RHO* cDNA. This type of alteration is also referred to as "knocking out" the mutant *RHO* gene and results in a loss of function of the mutant *RHO* gene. While not wishing to be bound by theory, knocking out the mutant *RHO* gene and providing a functional exogenous *RHO* cDNA maintains appropriate levels of rhodopsin protein in PR rod cells. This therapeutic strategy has the benefit of disrupting all known mutant alleles related to adRP, for example, the *RHO* mutations in **Table A**.

In certain embodiments, the 5' UTR region (e.g., 5' UTR, exon 1, exon 2, intron 1, exon 1/intron 1, or exon 2/intron 1 border) of a mutant *RHO* gene, is targeted to alter (i.e., knockout (e.g., eliminate expression of)) the mutant *RHO* gene.

In certain embodiments, the coding region (e.g., an exon, e.g., an early coding region) of the mutant *RHO* gene, is targeted to alter (i.e., knockout (e.g., eliminate expression of)) the mutant *RHO* gene. For example, the early coding region of the mutant *RHO* gene includes the sequence immediately following a start codon, within a first exon of the coding sequence, or within 500 bp of the start codon (e.g., less than 500, 450, 400, 350, 300, 250, 200, 150, 100 or 50 bp).

In certain embodiments, a non-coding region of the mutant *RHO* gene (e.g., an enhancer region, a promoter region, an intron, 5' UTR, 3'UTR, polyadenylation signal) is targeted to alter (i.e., knockout (e.g., eliminate expression of)) the mutant *RHO* gene.

In certain embodiments, an exon/intron border of the mutant *RHO* gene (e.g., exon 1/intron 1, exon 2/intron 1) is targeted to alter (i.e., knockout (e.g., eliminate expression of)) the mutant *RHO* gene. In certain embodiments, targeting an exon/intron border provides the benefit of being able to use an exogenous *RHO* cDNA molecule that is not codon-modified to be resistant to cutting by a gRNA.

Fig. 1 shows a schematic of one embodiment of a therapeutic strategy to knockout an endogenous *RHO* gene and provide an exogenous *RHO* cDNA. In one embodiment, CRISPR/RNA-guided nuclease genome editing systems may be used to alter (i.e., knockout (e.g., eliminate expression of)) exon 1 or exon 2 of the *RHO* gene. In certain embodiments, the *RHO* gene may be mutated *RHO* gene. In certain embodiments, the mutated *RHO* gene may comprise one or more *RHO* mutations in **Table A**. Alteration of exon 1 or exon 2 of the *RHO* gene results in disruption of the endogenous mutated *RHO* gene.

In certain embodiments, the therapeutic strategy may be accomplished using a dual-vector system. In certain aspects, the disclosure focuses on AAV vectors encoding CRISPR/RNA-guided nuclease genome editing systems and a replacement *RHO* cDNA, and on the use of such vectors to treat adRP disease. Exemplary vector genomes are schematized in **Fig. 2**, which illustrates certain fixed and variable elements of these vectors: inverted terminal repeats (ITRs), at least one gRNA sequence and a promoter sequences to drive its expression, an RNA-guided nuclease (e.g., Cas9) coding sequence and another promoter to drive its expression, nuclear localization signal (NLS) sequences, and a *RHO* cDNA sequence and another promoter to drive its expression. Each of these elements is discussed in detail herein. Additional exemplary vector genomes are schematized in **Fig. 3**, which illustrates certain fixed and variable elements of these vectors: at least one gRNA sequence and a promoter sequence to drive its expression (e.g., U6 promoter), an RNA-guided nuclease (e.g., *S. aureus* Cas9) coding sequence and another promoter to drive its expression (e.g., minimal *RHO* promoter), and a *RHO* cDNA sequence and another promoter to drive its expression (e.g., minimal *RHO* promoter). Additional exemplary vectors and sequences for use with the strategies described herein are set forth in **Figs. 16-18** and SEQ ID NOs:8-11.

In certain embodiments, the AAV vector used herein may be a self-limiting vector system as described in WO2018/106693, published on June 14, 2018, and entitled Systems and Methods for One-Shot guide RNA (ogRNA) Targeting of Endogenous and Source DNA, the entire contents of which are incorporated herein by reference.

As shown in **Fig. 1**, in certain embodiments, a dual vector system may be used to knockout expression of mutant *RHO* gene and deliver an exogenous *RHO* cDNA to restore expression of wild-type rhodopsin protein. In certain embodiments, one AAV vector genome may comprise ITRs and an RNA-guided nuclease coding sequence and promoter sequence to drive its expression and one or more NLS sequences. In certain embodiments, a second AAV vector genome may comprise ITRs, a *RHO* cDNA sequence and a promoter to drive its expression, one gRNA sequence and promoter sequence to drive its expression.

While not wishing to be bound by theory, knocking out the *RHO* gene and replacing it with functional exogenous *RHO* cDNA maintains appropriate levels of rhodopsin protein in PR rod cells. Restoring appropriate levels of functional rhodopsin protein in rod PR cells maintains the phototransduction cascade and may delay or prevent PR cell death in subjects with adRP.

In some embodiments, a method disclosed herein is characterized by knocking out a variant of the *RHO* gene that is associated with adRP, e.g., a *RHO* mutant gene or allele

described herein, and restoring wild-type *RHO* protein expression in a subject in need thereof, e.g., in a subject suffering from or predisposed to adRP. For example, in some embodiments, the methods provided herein are characterized by knocking out a mutant *RHO* allele in a subject having a mutant and a wild-type *RHO* allele, and restoring expression of wild-type rhodopsin protein in rod PR cells. In some embodiments, such methods feature knocking out the mutant allele while leaving the wild-type allele intact. In other embodiments, such methods feature knocking out both the mutant and the wild-type allele. In some embodiments, the methods are characterized by knocking out a mutant allele of the *RHO* gene and providing an exogenous wild-type protein, e.g., via expression of a cDNA encoding wild-type RHO protein. In some embodiments, knocking out expression of a mutant allele (and, optionally, a wild-type allele), and restoring wild-type RHO protein expression, e.g., via expression of an exogenous *RHO* cDNA, in a subject in need thereof, e.g., a subject suffering from or predisposed to adRP, ameliorates at least one symptom associated with adRP. In some embodiments, such an amelioration includes, for example, improving the subject's vision. In some embodiments, such an amelioration includes, for example, delaying adRP disease progression, e.g., as compared to an expected progression without clinical intervention. In some embodiments, such an amelioration includes, for example, arresting adRP disease progression. In some embodiments, such an amelioration includes, for example, preventing or delaying the onset of adRP disease in a subject.

In an embodiment, a method described herein comprises treating allogenic or autologous retinal cells *ex vivo*. In an embodiment, *ex vivo* treated allogenic or autologous retinal cells are introduced into the subject.

In an embodiment, a method described herein comprises treating an embryonic stem cell, an induced pluripotent stem cell or a cell derived from an iPS cell, a hematopoietic stem cell, a neuronal stem cell or a mesenchymal stem cell *ex vivo*. In an embodiment, *ex vivo* treated embryonic stem cells, induced pluripotent stem cells, hematopoietic stem cells, neuronal stem cells or a mesenchymal stem cells are introduced into the subject. In an embodiment, the cell is an induced pluripotent stem cells (iPS) cell or a cell derived from an iPS cell, e.g., an iPS cell generated from the subject, modified to knock out one or more mutated *RHO* genes and express functional exogenous *RHO* DNA and differentiated into a retinal progenitor cell or a retinal cell, e.g., retinal photoreceptor cell, and injected into the eye of the subject, e.g., subretinally, e.g., in the submacular region of the retina.

In an embodiment, a method described herein comprises treating autologous stem cells *ex vivo*. In an embodiment, *ex vivo* treated autologous stem cells are returned to the subject.

In an embodiment, the subject is treated *in vivo*, e.g., by a viral (or other mechanism) that targets cells from the eye (e.g., a retinal cell, e.g., a photoreceptor cell, e.g., a cone photoreceptor cell, e.g., a rod photoreceptor cell, e.g., a macular cone photoreceptor cell).

In an embodiment, the subject is treated *in vivo*, e.g., by a viral (or other mechanism) that targets a stem cell (e.g., an embryonic stem cell, an induced pluripotent stem cell or a cell derived from an iPS cell, a hematopoietic stem cell, a neuronal stem cell or a mesenchymal stem cell).

In an embodiment, treatment is initiated in a subject prior to disease onset. In a particular embodiment, treatment is initiated in a subject who has tested positive for one or more mutations in the *RHO* gene.

In an embodiment, treatment is initiated in a subject after disease onset.

In an embodiment, treatment is initiated in an early stage of adRP disease. In an embodiment, treatment is initiated after a subject presents with gradually declining vision. In an embodiment, repair of the *RHO* gene after adRP onset but early in the disease course will prevent progression of the disease.

In an embodiment, treatment is initiated in a subject in an advanced stage of disease. While not wishing to be bound by theory, it is held that advanced stage treatment will likely preserve a subject's visual acuity (in the central visual field), which is important for subject function and performance of activities of daily living.

In an embodiment, treatment of a subject prevents disease progression. While not wishing to be bound by theory, it is held that initiation of treatment for subjects at all stages of disease (e.g., prophylactic treatment, early stage adRP, and advanced stage adRP) will prevent RP disease progression and be of benefit to subjects.

In an embodiment, treatment is initiated after determination that the subject, e.g., an infant or newborn, teenager, or adult, is positive for a mutation in the *RHO* gene, e.g., a mutation described herein.

In an embodiment, treatment is initiated after determination that the subject is positive for a mutation in the *RHO* gene, e.g., a mutation described herein, but prior to manifestation of a symptom of the disease.

In an embodiment, treatment is initiated after determination that the subject is positive for a mutation in the *RHO* gene, e.g., a mutation described herein, and after manifestation of a symptom of the disease.

In an embodiment, treatment is initiated in a subject at the appearance of a decline in visual fields.

In an embodiment, treatment is initiated in a subject at the appearance of declining peripheral vision.

In an embodiment, treatment is initiated in a subject at the appearance of poor night vision and/or night blindness.

In an embodiment, treatment is initiated in a subject at the appearance of progressive visual loss.

In an embodiment, treatment is initiated in a subject at the appearance of progressive constriction of the visual field.

In an embodiment, treatment is initiated in a subject at the appearance of one or more indications consistent with adRP upon examination of a subject. Exemplary indications include, but are not limited to, bone spicule pigmentation, narrowing of the visual fields, retinal atrophy, attenuated retinal vasculature, loss of retinal pigment epithelium, pallor of the optic nerve, and/or combinations thereof.

In an embodiment, a method described herein comprises subretinal injection, submacular injection, suprachoroidal injection, or intravitreal injection, of gRNA or other components described herein, e.g., an RNA-guided nuclease (e.g., Cas9 or Cpf1 molecule) and a *RHO* cDNA molecule.

In an embodiment, a gRNA or other components described herein, e.g., an RNA-guided nuclease (e.g., Cas9 or Cpf1 molecule) and a *RHO* cDNA molecule are delivered, e.g., to a subject, by AAV, lentivirus, nanoparticle, or parvovirus, e.g., a modified parvovirus designed to target cells from the eye (e.g., a retinal cell, e.g., a photoreceptor cell, e.g., a cone photoreceptor cell, e.g., a rod photoreceptor cell, e.g., a macular cone photoreceptor cell).

In an embodiment, a gRNA or other components described herein, e.g., an RNA-guided nuclease (e.g., Cas9 or Cpf1 molecule) and a *RHO* cDNA molecule are delivered, e.g., to a subject, by AAV, lentivirus, nanoparticle, or parvovirus, e.g., a modified parvovirus designed to target stem cells (e.g., an embryonic stem cell, an induced pluripotent stem cell or a cell derived from an iPS cell, a hematopoietic stem cell, a neuronal stem cell or a mesenchymal stem cell).

In an embodiment, a gRNA or other components described herein, e.g., an RNA-guided nuclease (e.g., Cas9 or Cpf1 molecule) and a *RHO* cDNA molecule are delivered, *ex vivo*, by electroporation.

In an embodiment, CRISPR/RNA-guided nuclease components are used to knock out the mutant *RHO* gene which gives rise to the disease.

I. gRNA Molecules

The terms guide RNA and gRNA refer to any nucleic acid that promotes the specific association (or "targeting") of an RNA-guided nuclease such as a Cas9 or a Cpf1 to a target sequence such as a genomic or episomal sequence in a cell. gRNAs can be unimolecular (comprising a single RNA molecule, and referred to alternatively as chimeric), or modular (comprising more than one, and typically two, separate RNA molecules, such as a crRNA and a tracrRNA, which are usually associated with one another, for example by duplexing). gRNAs and their component parts are described throughout the literature (see, e.g., Briner 2014, which is incorporated by reference; see also Cotta-Ramusino).

In bacteria and archea, type II CRISPR systems generally comprise an RNA-guided nuclease protein such as Cas9, a CRISPR RNA (crRNA) that includes a 5' region that is complementary to a foreign sequence, and a trans-activating crRNA (tracrRNA) that includes a 5' region that is complementary to, and forms a duplex with, a 3' region of the crRNA. While not intending to be bound by any theory, it is thought that this duplex facilitates the formation of — and is necessary for the activity of — the RNA-guided nuclease/gRNA complex. As type II CRISPR systems were adapted for use in gene editing, it was discovered that the crRNA and tracrRNA could be joined into a single unimolecular or chimeric gRNA, for example by means of a four nucleotide (e.g., GAAA) "tetraloop" or "linker" sequence bridging complementary regions of the crRNA (at its 3' end) and the tracrRNA (at its 5' end) (Mali 2013; Jiang 2013; Jinek 2012; all incorporated by reference herein).

Guide RNAs, whether unimolecular or modular, include a targeting domain that is fully or partially complementary to the target domain within a target sequence (e.g., a double-stranded DNA sequence in the genome of a cell where editing is desired). In certain embodiments, a *RHO* target sequence encompasses, comprises, or is proximal to a *RHO* target position. Targeting domains are referred to by various names in the literature, including without limitation "guide sequences" (Hsu 2013, incorporated by reference herein), "complementarity regions" (Cotta-Ramusino), "spacers" (Briner 2014), and generically as "crRNAs" (Jiang 2013). Irrespective of the names they are given, targeting domains are

typically 10-30 nucleotides in length, preferably 16-24 nucleotides in length (for example, 16, 17, 18, 19, 20, 21, 22, 23 or 24 nucleotides in length), and are at or near the 5' terminus of in the case of a Cas9 gRNA, and at or near the 3' terminus in the case of a Cpf1 gRNA. The nucleic acid sequence complementary to the target domain, i.e., the nucleic acid sequence on the complementary DNA strand of the double-stranded DNA that comprises the target domain, is referred to herein as the "protospacer."

The "protospacer-adjacent motif" (PAM) sequence takes its name from its sequential relationship to the "protospacer" sequence. Together with protospacer sequences, PAM sequences define target sequences and/or target positions for specific RNA-guided nuclease/gRNA combinations. Various RNA-guided nucleases may require different sequential relationships between PAMs and protospacers.

For example, in general, Cas9 nucleases recognize PAM sequences that are 3' of the protospacer:

```
5'----[  protospacer   ][PAM]--------3'
3'----[  target domain ]--------------5'
```

For another example, in general, Cpf1 recognizes PAM sequences that are 5' of the protospacer:

```
5'----[PAM][  protospacer   ]--------3'
3'---------[  target domain ]--------5'
```

In some embodiments described herein, *RHO* protospacers and exemplary suitable targeting domains are described. Those of ordinary skill in the art will be aware of additional suitable guide RNA targeting domains that can be used to target an RNA-guided nuclease to a given protospacer, e.g., targeting domains that comprise additional or less nucleotides, or that comprise one or more nucleotide mismatches when hybridized to a target domain.

In addition to the targeting domains, gRNAs typically (but not necessarily, as discussed below) include a plurality of domains that influence the formation or activity of gRNA/Cas9 complexes. For example, as mentioned above, the duplexed structure formed by first and secondary complementarity domains of a gRNA (also referred to as a repeat:anti-repeat duplex) interacts with the recognition (REC) lobe of Cas9 and may mediate the formation of Cas9/gRNA complexes (Nishimasu 2014; Nishimasu 2015; both incorporated by reference herein). It should be noted that the first and/or second complementarity domains can contain one or more poly-A tracts, which can be recognized by RNA polymerases as a termination signal. The sequence of the first and second complementarity domains are, therefore, optionally modified to eliminate these tracts and promote the complete in vitro

transcription of gRNAs, for example through the use of A-G swaps as described in Briner 2014, or A-U swaps. These and other similar modifications to the first and second complementarity domains are within the scope of the present disclosure.

Along with the first and second complementarity domains, Cas9 gRNAs typically include two or more additional duplexed regions that are necessary for nuclease activity in vivo but not necessarily in vitro (Nishimasu 2015). A first stem-loop near the 3' portion of the second complementarity domain is referred to variously as the "proximal domain," (Cotta-Ramusino) "stem loop 1" (Nishimasu 2014; Nishimasu 2015) and the "nexus" (Briner 2014). One or more additional stem loop structures are generally present near the 3' end of the gRNA, with the number varying by species: *S. pyogenes* gRNAs typically include two 3' stem loops (for a total of four stem loop structures including the repeat:anti-repeat duplex), while *S. aureus* and other species have only one (for a total of three). A description of conserved stem loop structures (and gRNA structures more generally) organized by species is provided in Briner 2014.

Skilled artisans will appreciate that gRNAs can be modified in a number of ways, some of which are described below, and these modifications are within the scope of disclosure. For economy of presentation in this disclosure, gRNAs may be presented by reference solely to their targeting domain sequences.

gRNA modifications

The activity, stability, or other characteristics of gRNAs can be altered through the incorporation of chemical and/or sequential modifications. As one example, transiently expressed or delivered nucleic acids can be prone to degradation by, e.g., cellular nucleases. Accordingly, the gRNAs described herein can contain one or more modified nucleosides or nucleotides which introduce stability toward nucleases. While not wishing to be bound by theory it is also believed that certain modified gRNAs described herein can exhibit a reduced innate immune response when introduced into a population of cells, particularly the cells of the present invention. As noted above, the term "innate immune response" includes a cellular response to exogenous nucleic acids, including single stranded nucleic acids, generally of viral or bacterial origin, which involves the induction of cytokine expression and release, particularly the interferons, and cell death.

One common 3' end modification is the addition of a poly A tract comprising one or more (and typically 5-200) adenine (A) residues. The poly A tract can be contained in the nucleic acid sequence encoding the gRNA, or can be added to the gRNA during chemical

synthesis, or following in vitro transcription using a polyadenosine polymerase (e.g., E. coli Poly(A)Polymerase). In vivo, poly-A tracts can be added to sequences transcribed from DNA vectors through the use of polyadenylation signals. Examples of such signals are provided in Maeder.

Some exemplary gRNA modifications useful in the context of the present RNA-guided nuclease technology are provided herein, and the skilled artisan will be able to ascertain additional suitable modifications that can be used in conjunction with the gRNAs and treatment modalities disclosed herein based on the present disclosure. Suitable gRNA modifications include, without limitations, those described in U.S. Patent Application No. US 2017/0073674 A1 and International Publication No. WO 2017/165862 A1, the entire contents of each of which are incorporated by reference herein.

II. Methods for Designing gRNAs

Methods for designing gRNAs are described herein, including methods for selecting, designing and validating target domains. Exemplary targeting domains are also provided herein. Targeting domains discussed herein can be incorporated into the gRNAs described herein.

Methods for selection and validation of target sites as well as off-target analyses are described, e.g., in Mali 2013; Hsu 2013; Fu 2014; Heigwer 2014; Bae 2014; Xiao 2014.

For example, a software tool can be used to optimize the choice of gRNA within a user's target site, e.g., to minimize total off-target activity across the genome. Off target activity may be other than cleavage. For each possible gRNA choice using *S. pyo*genes Cas9, the tool can identify all off-target sites (preceding either NAG or NGG PAMs) across the genome that contain up to certain number (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) of mismatched base-pairs. The cleavage efficiency at each off-target site can be predicted, e.g., using an experimentally-derived weighting scheme. Each possible gRNA is then ranked according to its total predicted off-target cleavage; the top-ranked gRNAs represent those that are likely to have the greatest on-target and the least off-target cleavage. Other functions, e.g., automated reagent design for CRISPR construction, primer design for the on-target Surveyor assay, and primer design for high-throughput detection and quantification of off-target cleavage via next-gen sequencing, can also be included in the tool.

The targeting domains discussed herein can be incorporated into the gRNAs described herein.

Exemplary Protospacers and Targeting Domains

Guide RNAs targeting various positions within the *RHO* gene for use with *S. aureus* Cas9 were identified. Following identification, the gRNAs were ranked into three tiers. The gRNAs in tier 1 were selected based on cutting in exon 1 and exon 2 of the *RHO* gene. Tier 1 guides exhibited > 9% editing in T-cells. For selection of tier 2 gRNAs, selection was based on cutting in the 5' UTR of the *RHO* gene. Tier 2 gRNAs exhibited > 10% editing in T-cells. Tier 3 gRNAs were selected based cutting in intron 1 of the *RHO* gene. Tier 3 gRNAs exhibit > 10% editing in T-cells.

Table 1 provides targeting domains for an exon 1 or exon 2 *RHO* target position in the *RHO* gene selected according to the first-tier parameters. The targeting domains were selected based on cutting in exon 1 or exon 2 of the *RHO* gene and exhibiting > 9% editing in T-cells. It is contemplated herein that the targeting domain hybridizes to the strand complementary to the target domain sequence provided through complementary base pairing. Any of the targeting domains in the table can be used with a *S. aureus* Cas9 molecule that gives double stranded cleavage. Any of the targeting domains in the table can be used with a *S. aureus* Cas9 single-stranded break nucleases (nickases).

Table 1

Tier 1				
Location in *RHO* gene	**gRNA ID**	**Indel Fraction Window**	**Targeting Domain (RNA)**	**Targeting Domain (DNA)/ Protospacer**
utr5_0; cds_0	RHO-1	0.2284375	GUCAGCCACAAGG GCCACAGCC (SEQ ID NO:100)	GTCAGCCACAAGG GCCACAGCC (SEQ ID NO:600)
cds_0	RHO-2	0.134454179	CCGAAGACGAAGU AUCCAUGCA (SEQ ID NO:101)	CCGAAGACGAAGT ATCCATGCA (SEQ ID NO:601)
cds_0	RHO-3	0.174725089	AGUAUCCAUGCAG AGAGGUGUA (SEQ ID NO:102)	AGTATCCATGCAG AGAGGTGTA (SEQ ID NO:602)
cds_0	RHO-4	0.093809401	CUAGGUUGAGCAG GAUGUAGUU (SEQ ID NO:103)	CTAGGTTGAGCAG GATGTAGTT (SEQ ID NO:603)
cds_0	RHO-5	0.109343522	CAUGGCUCAGCCA GGUAGUACU (SEQ ID NO:104)	CATGGCTCAGCCA GGTAGTACT (SEQ ID NO:604)
cds_0	RHO-6	0.112374147	ACGGGUGUGGUAC GCAGCCCCU (SEQ ID NO:105)	ACGGGTGTGGTAC GCAGCCCCT (SEQ ID NO:605)

cds_0; intron_0	RHO-7	0.297946972	CCCACACCCGGCU CAUACCGCC (SEQ ID NO:106)	CCCACACCCGGCT CATACCGCC (SEQ ID NO:606)
cds_0; intron_0	RHO-8	0.118235744	CCCUGGGCGGUAU GAGCCGGGU (SEQ ID NO:107)	CCCTGGGCGGTAT GAGCCGGGT (SEQ ID NO:607)
cds_1	RHO-9	0.270630335	CCAUCAUGGGCGU UGCCUUCAC (SEQ ID NO:108)	CCATCATGGGCGT TGCCTTCAC (SEQ ID NO:608)
cds_1; intron_1	RHO-10	0.567902679	GUGCCAUUACCUG GACCAGCCG (SEQ ID NO:109)	GTGCCATTACCTG GACCAGCCG (SEQ ID NO:609)
cds_1; intron_1	RHO-11	0.106516652	UUACCUGGACCAG CCGGCGAGU (SEQ ID NO:110)	TTACCTGGACCAG CCGGCGAGT (SEQ ID NO:610)

Table 2 provides targeting domains for a 5'UTR *RHO* target position in the *RHO* gene selected according to the second-tier parameters. The targeting domains were selected based on cutting in the 5' UTR region of the *RHO* gene and exhibiting > 10% editing in T-cells. It is contemplated herein that the targeting domain hybridizes to the target domain through complementary base pairing. Any of the targeting domains in the table can be used with a *S. aureus* Cas9 molecule that gives double stranded cleavage. Any of the targeting domains in the table can be used with a *S. aureus* Cas9 single-stranded break nucleases (nickases).

Table 2

Tier 2				
Location in *RHO* gene	**gRNA ID**	**Indel Fraction Window**	**Targeting Domain (RNA)**	**Targeting Domain (DNA)/Protospacer**
utr5_0	RHO-12	0.459024462	GCAUUCUUGGGUGG GAGCAGCC (SEQ ID NO:111)	GCATTCTTGGGTGG GAGCAGCC (SEQ ID NO:611)
utr5_0	RHO-13	0.20572897	GCUCAGCCACUCAG GGCUCCAG (SEQ ID NO:112)	GCTCAGCCACTCAG GGCTCCAG (SEQ ID NO:612)
utr5_0	RHO-14	0.409641098	UGACCCGUGGCUGC UCCCACCC (SEQ ID NO:113)	TGACCCGTGGCTGC TCCCACCC (SEQ ID NO:613)
utr5_0	RHO-15	0.134736551	AGCUCAGGCCUUCG CAGCAUUC (SEQ ID NO:114)	AGCTCAGGCCTTCG CAGCATTC (SEQ ID NO:614)

Table 3 provides targeting domains for an intron 1 *RHO* target position in the *RHO* gene selected according to the third-tier parameters. The targeting domains were selected based on cutting in intron 1 of the *RHO* gene and exhibiting > 10% editing in T-cells. It is contemplated herein that the targeting domain hybridizes to the target domain through complementary base pairing. Any of the targeting domains in the table can be used with a *S. aureus* Cas9 molecule that gives double stranded cleavage. Any of the targeting domains in the table can be used with a *S. aureus* Cas9 single-stranded break nucleases (nickases).

Table 3

Tier 3				
Location in *RHO* gene	**gRNA ID**	**Indel Fraction Window Average**	**Targeting Domain (RNA)**	**Targeting Domain/Protospacer (DNA)**
intron_0	RHO-16	0.107449452	UAGCAGAAGAAUG CAUCCUAAU (SEQ ID NO:115)	TAGCAGAAGAATG CATCCTAAT (SEQ ID NO:615)
intron_0	RHO-17	0.107559427	ACACGCUGAGGAG AGCUGGGCA (SEQ ID NO:116)	ACACGCTGAGGAG AGCTGGGCA (SEQ ID NO:616)
intron_0	RHO-18	0.116786532	GCAAAUAACUUCC CCCAUUCCC (SEQ ID NO:117)	GCAAATAACTTCCC CCATTCCC (SEQ ID NO:617)
intron_0	RHO-19	0.129975835	AGACCCAGGCUGG GCACUGAGG (SEQ ID NO:118)	AGACCCAGGCTGG GCACTGAGG (SEQ ID NO:618)
intron_0	RHO-20	0.130270513	CUAGGUCUCCUGG CUGUGAUCC (SEQ ID NO:119)	CTAGGTCTCCTGGC TGTGATCC (SEQ ID NO:619)
intron_0	RHO-21	0.132448578	CCAGAAGGUGGGU GUGCCACUU (SEQ ID NO:120)	CCAGAAGGTGGGT GTGCCACTT (SEQ ID NO:620)
intron_0	RHO-22	0.140129895	AACAAGGAACUCU GCCCCACAU (SEQ ID NO:121)	AACAAGGAACTCT GCCCCACAT (SEQ ID NO:621)
intron_0	RHO-23	0.142141636	CAGGAUUGAACUG GGAACCCGG (SEQ ID NO:122)	CAGGATTGAACTG GGAACCCGG (SEQ ID NO:622)
intron_0	RHO-24	0.147082642	GGGCGUCACACAG GGACGGGTG (SEQ ID NO:123)	GGGCGTCACACAG GGACGGGTG (SEQ ID NO:623)
intron_0	RHO-25	0.14820997	CUGUGAUCCAGGA AUAUCUCUG	CTGTGATCCAGGA ATATCTCTG

			(SEQ ID NO:124)	(SEQ ID NO:624)
intron_0	RHO-26	0.150900653	UUGCAUUUAACAG GAAAACAGA (SEQ ID NO:125)	TTGCATTTAACAGG AAAACAGA (SEQ ID NO:625)
intron_0	RHO-27	0.151929784	GGAGUGCACCCUC CUUAGGCAG (SEQ ID NO:126)	GGAGTGCACCCTCC TTAGGCAG (SEQ ID NO:626)
intron_0	RHO-28	0.152980769	CAUCUGUCCUGCU CACCACCCC (SEQ ID NO:127)	CATCTGTCCTGCTC ACCACCCC (SEQ ID NO:627)
intron_0	RHO-29	0.156913097	GAGGGGAGGCAGA GGAUGCCAG (SEQ ID NO:128)	GAGGGGAGGCAGA GGATGCCAG (SEQ ID NO:628)
intron_0	RHO-30	0.166237876	CUCAGGGAAUCUC UGGCCAUUG (SEQ ID NO:129)	CTCAGGGAATCTCT GGCCATTG (SEQ ID NO:629)
intron_0	RHO-31	0.166367333	UGCACUCCCCCCU AGACAGGGA (SEQ ID NO:130)	TGCACTCCCCCCTA GACAGGGA (SEQ ID NO:630)
intron_0	RHO-32	0.172983706	UGCUGUUUGUGCA GGGCUGGCA (SEQ ID NO:131)	TGCTGTTTGTGCAG GGCTGGCA (SEQ ID NO:631)
intron_0	RHO-33	0.185512517	ACUGGGACAUUCC UAACAGUGA (SEQ ID NO:132)	ACTGGGACATTCCT AACAGTGA (SEQ ID NO:632)
intron_0	RHO-34	0.190420346	AUCAGGGGGUCAG GAUUGAACU (SEQ ID NO:133)	ATCAGGGGGTCAG GATTGAACT (SEQ ID NO:633)
intron_0	RHO-35	0.194765615	CUCCUCUCUGGGG GCCCAAGCU (SEQ ID NO:134)	CTCCTCTCTGGGGG CCCAAGCT (SEQ ID NO:634)
intron_0	RHO-36	0.197589827	CUGCAUCUCAGCA GAGAUAUUC (SEQ ID NO:135)	CTGCATCTCAGCAG AGATATTC (SEQ ID NO:635)
intron_0	RHO-37	0.199499884	UGUUUCCCUUGGA GCAGCUGUG (SEQ ID NO:136)	TGTTTCCCTTGGAG CAGCTGTG (SEQ ID NO:636)
intron_0	RHO-38	0.212418288	GCGCUCUGGGCCC AUAAGGGAC (SEQ ID NO:137)	GCGCTCTGGGCCCA TAAGGGAC (SEQ ID NO:637)
intron_0	RHO-39	0.215235707	AGGAUUGAACUGG GAACCCGGU (SEQ ID NO:138)	AGGATTGAACTGG GAACCCGGT (SEQ ID NO:638)
intron_0	RHO-40	0.21710799	CCUAGGAGAGGCC CCCACAUGU (SEQ ID NO:139)	CCTAGGAGAGGCC CCCACATGT (SEQ ID NO:639)
intron_0	RHO-41	0.217881646	AUCACUCAGUUCU GGCCAGAAG (SEQ ID NO:140)	ATCACTCAGTTCTG GCCAGAAG (SEQ ID NO:640)

intron_0	RHO-42	0.227315789	AGAGCUGGGCAAAGAAAUUCCA (SEQ ID NO:141)	AGAGCTGGGCAAAGAAATTCCA (SEQ ID NO:641)
intron_0	RHO-43	0.230358178	CCACCCCAUGAAGUUCCAUAGG (SEQ ID NO:142)	CCACCCCATGAAGTTCCATAGG (SEQ ID NO:642)
intron_0	RHO-44	0.231888098	CCACCCUGAGCUUGGGCCCCCA (SEQ ID NO:143)	CCACCCTGAGCTTGGGCCCCCA (SEQ ID NO:643)
intron_0	RHO-45	0.234285631	CAGAGGAAGAAGAAGGAAAUGA (SEQ ID NO:144)	CAGAGGAAGAAGAAGGAAATGA (SEQ ID NO:644)
intron_0	RHO-46	0.240341645	AAACAGCAGCCCGGCUAUCACC (SEQ ID NO:145)	AAACAGCAGCCCGGCTATCACC (SEQ ID NO:645)
intron_0	RHO-47	0.242233765	GGAUUGAACUGGGAACCCGGUA (SEQ ID NO:146)	GGATTGAACTGGGAACCCGGTA (SEQ ID NO:646)
intron_0	RHO-48	0.242660421	UGUGUGUGUGUGUGUUUAGCAG (SEQ ID NO:147)	TGTGTGTGTGTGTGTTTAGCAG (SEQ ID NO:647)
intron_0	RHO-49	0.251755576	UCACACAGGGACGGGUGCAGAG (SEQ ID NO:148)	TCACACAGGGACGGGTGCAGAG (SEQ ID NO:648)
intron_0	RHO-50	0.252241304	GUGUGUGUGUGUGUGUUUAG (SEQ ID NO:149)	GTGTGTGTGTGTGTGTGTTTAG (SEQ ID NO:649)
intron_0	RHO-51	0.255029622	UGAGCUUGGGCCCCCAGAGAGG (SEQ ID NO:150)	TGAGCTTGGGCCCCCAGAGAGG (SEQ ID NO:650)
intron_0	RHO-52	0.263525952	AAUAUCUCUGCUGAGAUGCAGG (SEQ ID NO:151)	AATATCTCTGCTGAGATGCAGG (SEQ ID NO:651)
intron_0	RHO-53	0.2666129	GGAGAGGGGAAGAGACUCAUUU (SEQ ID NO:152)	GGAGAGGGGAAGAGACTCATTT (SEQ ID NO:652)
intron_0	RHO-54	0.287053205	AGAACUGAGUGAUCUGUGAUUA (SEQ ID NO:153)	AGAACTGAGTGATCTGTGATTA (SEQ ID NO:653)
intron_0	RHO-55	0.291326632	CCACUCUCCCUAUGGAACUUCA (SEQ ID NO:154)	CCACTCTCCCTATGGAACTTCA (SEQ ID NO:654)
intron_0	RHO-56	0.292218928	AUAAGGGACACGAAUCAGAUCA (SEQ ID NO:155)	ATAAGGGACACGAATCAGATCA (SEQ ID NO:655)
intron_0	RHO-57	0.305482452	UGGAUUUUCCAUUCUCCAGUCA (SEQ ID NO:156)	TGGATTTTCCATTCTCCAGTCA (SEQ ID NO:656)

intron_0	RHO-58	0.310447227	GUGCAGGAGCCCG GGAGCAUGG (SEQ ID NO:157)	GTGCAGGAGCCCG GGAGCATGG (SEQ ID NO:657)
intron_0	RHO-59	0.31581459	GGGUGGUGAGCAG GACAGAUGU (SEQ ID NO:158)	GGGTGGTGAGCAG GACAGATGT (SEQ ID NO:658)
intron_0	RHO-60	0.329433399	CAGCUCUCCCUCA GUGCCCAGC (SEQ ID NO:159)	CAGCTCTCCCTCAG TGCCCAGC (SEQ ID NO:659)
intron_0	RHO-61	0.337601649	CCUGCUGGGGCGU CACACAGGG (SEQ ID NO:160)	CCTGCTGGGGCGTC ACACAGGG (SEQ ID NO:660)
intron_0	RHO-62	0.341369802	CACACACACACAA AACUCCCUA (SEQ ID NO:161)	CACACACACACAA AACTCCCTA (SEQ ID NO:661)
intron_0	RHO-63	0.342930279	ACUUACGGGUGGU UGUUCUCUG (SEQ ID NO:162)	ACTTACGGGTGGTT GTTCTCTG (SEQ ID NO:662)
intron_0	RHO-64	0.347123022	CACAGGGAAGACC CAAUGACUG (SEQ ID NO:163)	CACAGGGAAGACC CAATGACTG (SEQ ID NO:663)
intron_0	RHO-65	0.3604802	AGCACAGACCCCA CUGCCUAAG (SEQ ID NO:164)	AGCACAGACCCCA CTGCCTAAG (SEQ ID NO:664)
intron_0	RHO-66	0.396256305	ACCUGAGGACAGG GGCUGAGAG (SEQ ID NO:165)	ACCTGAGGACAGG GGCTGAGAG (SEQ ID NO:665)
intron_0	RHO-67	0.397224629	CAACAAUGGCCAG AGAUUCCCU (SEQ ID NO:166)	CAACAATGGCCAG AGATTCCCT (SEQ ID NO:666)
intron_0	RHO-68	0.40353484	UGCUGCCUCGGUC CCAUUCUCA (SEQ ID NO:167)	TGCTGCCTCGGTCC CATTCTCA (SEQ ID NO:667)
intron_0	RHO-69	0.416729506	UGCUGCCUGGCCA CAUCCCUAA (SEQ ID NO:168)	TGCTGCCTGGCCAC ATCCCTAA (SEQ ID NO:668)

III. RNA-Guided Nucleases

RNA-guided nucleases according to the present disclosure include, without limitation, naturally-occurring Class 2 CRISPR nucleases such as Cas9, and Cpf1, as well as other nucleases derived or obtained therefrom. In functional terms, RNA-guided nucleases are defined as those nucleases that: (a) interact with (e.g., complex with) a gRNA; and (b) together with the gRNA, associate with, and optionally cleave or modify, a target region of a DNA that includes (i) a sequence complementary to the targeting domain of the gRNA and, optionally, (ii) an additional sequence referred to as a "protospacer adjacent motif," or

"PAM," which is described in greater detail below. As the following examples will illustrate, RNA-guided nucleases can be defined, in broad terms, by their PAM specificity and cleavage activity, even though variations may exist between individual RNA-guided nucleases that share the same PAM specificity or cleavage activity. Skilled artisans will appreciate that some aspects of the present disclosure relate to systems, methods and compositions that can be implemented using any suitable RNA-guided nuclease having a certain PAM specificity and/or cleavage activity. For this reason, unless otherwise specified, the term RNA-guided nuclease should be understood as a generic term, and not limited to any particular type (e.g., Cas9 vs. Cpf1), species (e.g., *S. pyogenes* vs. *S. aureus*) or variation (e.g., full-length vs. truncated or split; naturally-occurring PAM specificity vs. engineered PAM specificity).

Turning to the PAM sequence, this structure takes its name from its sequential relationship to the "protospacer" sequence that is complementary to gRNA targeting domains (or "spacers"). Together with protospacer sequences, PAM sequences define target regions or sequences for specific RNA-guided nuclease / gRNA combinations.

Various RNA-guided nucleases may require different sequential relationships between PAMs and protospacers. In general, Cas9s recognize PAM sequences that are 5' of the protospacer as visualized relative to the top or complementary strand.

In addition to recognizing specific sequential orientations of PAMs and protospacers, RNA-guided nucleases generally recognize specific PAM sequences. *S. aureus* Cas9, for example, recognizes a PAM sequence of NNGRRT, wherein the N sequences are immediately 3' of the region recognized by the gRNA targeting domain. *S. pyogenes* Cas9 recognizes NGG PAM sequences. It should also be noted that engineered RNA-guided nucleases can have PAM specificities that differ from the PAM specificities of similar nucleases (such as the naturally occurring variant from which an RNA-guided nuclease is derived, or the naturally occurring variant having the greatest amino acid sequence homology to an engineered RNA-guided nuclease). Modified Cas9s that recognize alternate PAM sequences are described below.

RNA-guided nucleases are also characterized by their DNA cleavage activity: naturally-occurring RNA-guided nucleases typically form DSBs in target nucleic acids, but engineered variants have been produced that generate only SSBs (discussed above; see also Ran 2013, incorporated by reference herein), or that do not cut at all.

The terms "RNA-guided nuclease" and "RNA-guided nuclease molecule" are used interchangeably herein. In some embodiments, the RNA-guided nuclease is a RNA-guided DNA endonuclease enzyme. In some embodiments, the RNA-guided nuclease is a CRISPR

nuclease. Examples of RNA-guided nucleases suitable for use in the context of the methods, strategies, and treatment modalities provided herein are listed in **Table 4** below, and the methods, compositions, and treatment modalities disclosed herein can, in some embodiments, make use of any combination of RNA-guided nucleases disclosed herein, or known to those of ordinary skill in the art.

Table 4. RNA-Guided Nucleases

Nuclease	Length (a.a.)	PAM	Reference
SpCas9	1368	NGG	Cong *et al.*, Science. 2013;339(6121):819-23
SaCas9	1053	NNGRRT	Ran *et al.*, Nature. 2015;520(7546):186-91.
(KKH) SaCas9	1067	NNNRRT	Kleinstiver *et al.*, Nat Biotechnol. 2015;33(12):1293-1298
AsCpf1 (AsCas12a)	1353	TTTV	Zetsche *et al.*, Nat Biotechnol. 2017;35(1):31-34.
LbCpf1 (LbCas12a)	1274	TTTV	Zetsche *et al.*, Cell. 2015;163(3):759-71.
CasX	980	TTC	Burstein *et al.*, Nature. 2017;542(7640):237-241.
CasY	1200	TA	Burstein *et al.*, Nature. 2017;542(7640):237-241.
Cas12h1	870	RTR	Yan *et al.*, Science. 2019;363(6422):88-91.
Cas12i1	1093	TTN	Yan *et al.*, Science. 2019;363(6422):88-91.
Cas12c1	unknown	TG	Yan *et al.*, Science. 2019;363(6422):88-91.
Cas12c2	unknown	TN	Yan *et al.*, Science. 2019;363(6422):88-91.
eSpCas9	1423	NGG	Chen *et al.*, Nature. 2017;550(7676):407-410.
Cas9-HF1	1367	NGG	Chen *et al.*, Nature. 2017;550(7676):407-410.
HypaCas9	1404	NGG	Chen *et al.*, Nature. 2017;550(7676):407-410.
dCas9-Fokl	1623	NGG	U.S. Patent No. 9,322,037
Sniper-Cas9	1389	NGG	Lee *et al.*, Nat Commun. 2018;9(1):3048.
xCas9	1786	NGG, NG, GAA, GAT	Wang *et al.*, Plant Biotechnol J. 2018; pbi.13053.
AaCas12b	1129	TTN	Teng *et al.* Cell Discov. 2018;4:63.
evoCas9	1423	NGG	Casini *et al.*, Nat Biotechnol. 2018;36(3):265-271.
SpCas9-NG	1423	NG	Nishimasu *et al.*, Science. 2018;361(6408):1259-1262.
VRQR	1368	NGA	Li *et al.*, The CRISPR Journal, 2018; 01:01
VRER	1372	NGCG	Kleinstiver *et al.*, Nature. 2016;529(7587):490-5.

NmeCas9	1082	NNNNGATT	Amrani *et al.*, Genome Biol. 2018;19(1):214.
CjCas9	984	NNNNRYAC	Kim *et al.*, Nat Commun. 2017;8:14500.
BhCas12b	1108	ATTN	Strecker *et al.*, Nat Commun. 2019 Jan 22;10(1):212.
BhCas12b V4	1108	ATTN	Strecker *et al.*, Nat Commun. 2019 Jan 22;10(1):212.

In one embodiment, the RNA-guided nuclease is a *Acidaminococcus sp.* Cpf1 RR variant (AsCpf1-RR). In another embodiment, the RNA-guided nuclease is a Cpf1 RVR variant

Exemplary suitable methods for designing targeting domains and guide RNAs, as well as for the use of the various Cas nucleases in the context of genome editing approaches, are known to those of skill in the art. Some exemplary methods are disclosed herein, and additional suitable methods will be apparent to the skilled artisan based on the present disclosure. The disclosure is not limited in this respect.

IV. *RHO* genomic sequence and complementary DNA sequences

The *RHO* genomic sequence is known to those of ordinary skill in the art. An exemplary *RHO* genomic sequence is provided below for ease of reference:

AGAGTCATCCAGCTGGAGCCCTGAGTGGCTGAGCTCAGGCCTTCGCAGCATTCTTGGGTGGG
AGCAGCCACGGGTCAGCCACAAGGGCCACAGCCATGAATGGCACAGAAGGCCCTAACTTCTA
CGTGCCCTTCTCCAATGCGACGGGTGTGGTACGCAGCCCCTTCGAGTACCCACAGTACTACC
TGGCTGAGCCATGGCAGTTCTCCATGCTGGCCGCCTACATGTTTCTGCTGATCGTGCTGGGC
TTCCCCATCAACTTCCTCACGCTCTACGTCACCGTCCAGCACAAGAAGCTGCGCACGCCTCT
CAACTACATCCTGCTCAACCTAGCCGTGGCTGACCTCTTCATGGTCCTAGGTGGCTTCACCA
GCACCCTCTACACCTCTCTGCATGGATACTTCGTCTTCGGGCCCACAGGATGCAATTTGGAG
GGCTTCTTTGCCACCCTGGGCGGTATGAGCCGGGTGTGGGTGGGGTGTGCAGGAGCCCGGGA
GCATGGAGGGGTCTGGGAGAGTCCCGGGCTTGGCGGTGGTGGCTGAGAGGCCTTCTCCCTTC
TCCTGTCCTGTCAATGTTATCCAAAGCCCTCATATATTCAGTCAACAAACACCATTCATGGT
GATAGCCGGGCTGCTGTTTGTGCAGGGCTGGCACTGAACACTGCCTTGATCTTATTTGGAGC
AATATGCGCTTGTCTAATTTCACAGCAAGAAAACTGAGCTGAGGCTCAAAGAAGTCAAGCGC
CCTGCTGGGGCGTCACACAGGGACGGGTGCAGAGTTGAGTTGGAAGCCCGCATCTATCTCGG
GCCATGTTTGCAGCACCAAGCCTCTGTTTCCCTTGGAGCAGCTGTGCTGAGTCAGACCCAGG

CTGGGCACTGAGGGAGAGCTGGGCAAGCCAGACCCCTCCTCTCTGGGGGCCCAAGCTCAGGG
TGGGAAGTGGATTTTCCATTCTCCAGTCATTGGGTCTTCCCTGTGCTGGGCAATGGGCTCGG
TCCCCTCTGGCATCCTCTGCCTCCCCTCTCAGCCCCTGTCCTCAGGTGCCCCTCCAGCCTCC
CTGCCGCGTTCCAAGTCTCCTGGTGTTGAGAACCGCAAGCAGCCGCTCTGAAGCAGTTCCTT
TTTGCTTTAGAATAATGTCTTGCATTTAACAGGAAAACAGATGGGGTGCTGCAGGGATAACA
GATCCCACTTAACAGAGAGGAAAACTGAGGCAGGGAGAGGGGAAGAGACTCATTTAGGGATG
TGGCCAGGCAGCAACAAGAGCCTAGGTCTCCTGGCTGTGATCCAGGAATATCTCTGCTGAGA
TGCAGGAGGAGACGCTAGAAGCAGCCATTGCAAAGCTGGGTGACGGGGAGAGCTTACCGCCA
GCCACAAGCGTCTCTCTGCCAGCCTTGCCCTGTCTCCCCCATGTCCAGGCTGCTGCCTCGGT
CCCATTCTCAGGGAATCTCTGGCCATTGTTGGGTGTTTGTTGCATTCAATAATCACAGATCA
CTCAGTTCTGGCCAGAAGGTGGGTGTGCCACTTACGGGTGGTTGTTCTCTGCAGGGTCAGTC
CCAGTTTACAAATATTGTCCCTTTCACTGTTAGGAATGTCCCAGTTTGGTTGATTAACTATA
TGGCCACTCTCCCTATGGAACTTCATGGGGTGGTGAGCAGGACAGATGTCTGAATTCCATCA
TTTCCTTCTTCTTCCTCTGGGCAAAACATTGCACATTGCTTCATGGCTCCTAGGAGAGGCCC
CCACATGTCCGGGTTATTTCATTTCCCGAGAAGGGAGAGGGAGGAAGGACTGCCAATTCTGG
GTTTCCACCACCTCTGCATTCCTTCCCAACAAGGAACTCTGCCCCACATTAGGATGCATTCT
TCTGCTAAACACACACACACACACACACACACACACAACACACACACACACACACACACACA
CACACACAAAACTCCCTACCGGGTTCCCAGTTCAATCCTGACCCCCTGATCTGATTCGTGTC
CCTTATGGGCCCAGAGCGCTAAGCAAATAACTTCCCCCATTCCCTGGAATTTCTTTGCCCAG
CTCTCCTCAGCGTGTGGTCCCTCTGCCCCTTCCCCCTCCTCCCAGCACCAAGCTCTCTCCTT
CCCCAAGGCCTCCTCAAATCCCTCTCCCACTCCTGGTTGCCTTCCTAGCTACCCTCTCCCTG
TCTAGGGGGGAGTGCACCCTCCTTAGGCAGTGGGGTCTGTGCTGACCGCCTGCTGACTGCCT
TGCAGGTGAAATTGCCCTGTGGTCCTTGGTGGTCCTGGCCATCGAGCGGTACGTGGTGGTGT
GTAAGCCCATGAGCAACTTCCGCTTCGGGGAGAACCATGCCATCATGGGCGTTGCCTTCACC
TGGGTCATGGCGCTGGCCTGCGCCGCACCCCCACTCGCCGGCTGGTCCAGGTAATGGCACTG
AGCAGAAGGGAAGAAGCTCCGGGGGCTCTTTGTAGGGTCCTCCAGTCAGGACTCAAACCCAG
TAGTGTCTGGTTCCAGGCACTGACCTTGTATGTCTCCTGGCCCAAATGCCCACTCAGGGTAG
GGGTGTAGGGCAGAAGAAGAAACAGACTCTAATGTTGCTACAAGGGCTGGTCCCATCTCCTG
AGCCCCATGTCAAACAGAATCCAAGACATCCCAACCCTTCACCTTGGCTGTGCCCCTAATCC
TCAACTAAGCTAGGCGCAAATTCCAATCCTCTTTGGTCTAGTACCCCGGGGGCAGCCCCCTC
TAACCTTGGGCCTCAGCAGCAGGGGAGGCCACACCTTCCTAGTGCAGGTGGCCATATTGTGG
CCCCTTGGAACTGGGTCCCACTCAGCCTCTAGGCGATTGTCTCCTAATGGGGCTGAGATGAG
ACACAGTGGGGACAGTGGTTTGGACAATAGGACTGGTGACTCTGGTCCCCAGAGGCCTCATG
TCCCTCTGTCTCCAGAAAATTCCCACTCTCACTTCCCTTTCCTCCTCAGTCTTGCTAGGGTC

CATTTCTTACCCCTTGCTGAATTTGAGCCCACCCCCTGGACTTTTTCCCCATCTTCTCCAAT
CTGGCCTAGTTCTATCCTCTGGAAGCAGAGCCGCTGGACGCTCTGGGTTTCCTGAGGCCCGT
CCACTGTCACCAATATCAGGAACCATTGCCACGTCCTAATGACGTGCGCTGGAAGCCTCTAG
TTTCCAGAAGCTGCACAAAGATCCCTTAGATACTCTGTGTGTCCATCTTTGGCCTGGAAAAT
ACTCTCACCCTGGGGCTAGGAAGACCTCGGTTTGTACAAACTTCCTCAAATGCAGAGCCTGA
GGGCTCTCCCCACCTCCTCACCAACCCTCTGCGTGGCATAGCCCTAGCCTCAGCGGGCAGTG
GATGCTGGGGCTGGGCATGCAGGGAGAGGCTGGGTGGTGTCATCTGGTAACGCAGCCACCAA
ACAATGAAGCGACACTGATTCCACAAGGTGCATCTGCATCCCCATCTGATCCATTCCATCCT
GTCACCCAGCCATGCAGACGTTTATGATCCCCTTTTCCAGGGAGGGAATGTGAAGCCCCAGA
AAGGGCCAGCGCTCGGCAGCCACCTTGGCTGTTCCCAAGTCCCTCACAGGCAGGGTCTCCCT
ACCTGCCTGTCCTCAGGTACATCCCCGAGGGCCTGCAGTGCTCGTGTGGAATCGACTACTAC
ACGCTCAAGCCGGAGGTCAACAACGAGTCTTTTGTCATCTACATGTTCGTGGTCCACTTCAC
CATCCCCATGATTATCATCTTTTTCTGCTATGGGCAGCTCGTCTTCACCGTCAAGGAGGTAC
GGGCCGGGGGGTGGGCGGCCTCACGGCTCTGAGGGTCCAGCCCCCAGCATGCATCTGCGGCT
CCTGCTCCCTGGAGGAGCCATGGTCTGGACCCGGGTCCCGTGTCCTGCAGGCCGCTGCCCAG
CAGCAGGAGTCAGCCACCACACAGAAGGCAGAGAAGGAGGTCACCCGCATGGTCATCATCAT
GGTCATCGCTTTCCTGATCTGCTGGGTGCCCTACGCCAGCGTGGCATTCTACATCTTCACCC
ACCAGGGCTCCAACTTCGGTCCCATCTTCATGACCATCCCAGCGTTCTTTGCCAAGAGCGCC
GCCATCTACAACCCTGTCATCTATATCATGATGAACAAGCAGGTGCCTACTGCGGGTGGGAG
GGCCCCAGTGCCCCAGGCCACAGGCGCTGCCTGCCAAGGACAAGCTACTTCCCAGGGCAGGG
GAGGGGGCTCCATCAGGGTTACTGGCAGCAGTCTTGGGTCAGCAGTCCCAATGGGGAGTGTG
TGAGAAATGCAGATTCCTGGCCCCACTCAGAACTGCTGAATCTCAGGGTGGGCCCAGGAACC
TGCATTTCCAGCAAGCCCTCCACAGGTGGCTCAGATGCTCACTCAGGTGGGAGAAGCTCCAG
TCAGCTAGTTCTGGAAGCCCAATGTCAAAGTCAGAAGGACCCAAGTCGGGAATGGGATGGGC
CAGTCTCCATAAAGCTGAATAAGGAGCTAAAAAGTCTTATTCTGAGGGGTAAAGGGGTAAAG
GGTTCCTCGGAGAGGTACCTCCGAGGGGTAAACAGTTGGGTAAACAGTCTCTGAAGTCAGCT
CTGCCATTTTCTAGCTGTATGGCCCTGGGCAAGTCAATTTCCTTCTCTGTGCTTTGGTTTCC
TCATCCATAGAAAGGTAGAAAGGGCAAAACACCAAACTCTTGGATTACAAGAGATAATTTAC
AGAACACCCTTGGCACACAGAGGGCACCATGAAATGTCACGGGTGACACAGCCCCCTTGTGC
TCAGTCCCTGGCATCTCTAGGGGTGAGGAGCGTCTGCCTAGCAGGTTCCCTCCAGGAAGCTG
GATTTGAGTGGATGGGGCGCTGGAATCGTGAGGGGCAGAAGCAGGCAAAGGGTCGGGGCGAA
CCTCACTAACGTGCCAGTTCCAAGCACACTGTGGGCAGCCCTGGCCCTGACTCAAGCCTCTT
GCCTTCCAGTTCCGGAACTGCATGCTCACCACCATCTGCTGCGGCAAGAACCCACTGGGTGA
CGATGAGGCCTCTGCTACCGTGTCCAAGACGGAGACGAGCCAGGTGGCCCCGGCCTAAGACC

TGCCTAGGACTCTGTGGCCGACTATAGGCGTCTCCCATCCCCTACACCTTCCCCCAGCCACA
GCCATCCCACCAGGAGCAGCGCCTGTGCAGAATGAACGAAGTCACATAGGCTCCTTAATTTT
TTTTTTTTTTTTAAGAAATAATTAATGAGGCTCCTCACTCACCTGGGACAGCCTGAGAAGGG
ACATCCACCAAGACCTACTGATCTGGAGTCCCACGTTCCCCAAGGCCAGCGGGATGTGTGCC
CCTCCTCCTCCCAACTCATCTTTCAGGAACACGAGGATTCTTGCTTTCTGGAAAAGTGTCCC
AGCTTAGGGATAAGTGTCTAGCACAGAATGGGGCACACAGTAGGTGCTTAATAAATGCTGGA
TGGATGCAGGAAGGAATGGAGGAATGAATGGGAAGGGAGAACATATCTATCCTCTCAGACCC
TCGCAGCAGCAGCAACTCATACTTGGCTAATGATATGGAGCAGTTGTTTTTCCCTCCCTGGG
CCTCACTTTCTTCTCCTATAAAATGGAAATCCCAGATCCCTGGTCCTGCCGACACGCAGCTA
CTGAGAAGACCAAAAGAGGTGTGTGTGTGTCTATGTGTGTGTTTCAGCACTTTGTAAATAGC
AAGAAGCTGTACAGATTCTAGTTAATGTTGTGAATAACATCAATTAATGTAACTAGTTAATT
ACTATGATTATCACCTCCTGATAGTGAACATTTTGAGATTGGGCATTCAGATGATGGGGTTT
CACCCAACCTTGGGGCAGGTTTTTAAAAATTAGCTAGGCATCAAGGCCAGACCAGGGCTGGG
GGTTGGGCTGTAGGCAGGGACAGTCACAGGAATGCAGAATGCAGTCATCAGACCTGAAAAAA
CAACACTGGGGGAGGGGGACGGTGAAGGCCAAGTTCCCAATGAGGGTGAGATTGGGCCTGGG
GTCTCACCCCTAGTGTGGGGCCCCAGGTCCCGTGCCTCCCCTTCCCAATGTGGCCTATGGAG
AGACAGGCCTTTCTCTCAGCCTCTGGAAGCCACCTGCTCTTTTGCTCTAGCACCTGGGTCCC
AGCATCTAGAGCATGGAGCCTCTAGAAGCCATGCTCACCCGCCCACATTTAATTAACAGCTG
AGTCCCTGATGTCATCCTTATCTCGAAGAGCTTAGAAACAAAGAGTGGGAAATTCCACTGGG
CCTACCTTCCTTGGGGATGTTCATGGGCCCCAGTTTCCAGTTTCCCTTGCCAGACAAGCCCA
TCTTCAGCAGTTGCTAGTCCATTCTCCATTCTGGAGAATCTGCTCCAAAAAGCTGGCCACAT
CTCTGAGGTGTCAGAATTAAGCTGCCTCAGTAACTGCTCCCCCTTCTCCATATAAGCAAAGC
CAGAAGCTCTAGCTTTACCCAGCTCTGCCTGGAGACTAAGGCAAATTGGGCCATTAAAAGCT
CAGCTCCTATGTTGGTATTAACGGTGGTGGGTTTTGTTGCTTTCACACTCTATCCACAGGAT
AGATTGAAACTGCCAGCTTCCACCTGATCCCTGACCCTGGGATGGCTGGATTGAGCAATGAG
CAGAGCCAAGCAGCACAGAGTCCCCTGGGGCTAGAGGTGGAGGAGGCAGTCCTGGGAATGGG
AAAAACCCCA (SEQ ID NO:1)

The *RHO* genomic sequence can be annotated as follows:

mRNA 1..456,2238..2406,3613..3778,3895..4134,4970..6706

CDS 96..456,2238..2406,3613..3778,3895..4134,4970..5080

Exemplary target domains, described in more detail elsewhere herein, are provided below in **Table 5** for the purpose of illustration:

Table 5

Reference ID	Position of target domain in *RHO* genomic sequence (SEQ ID NO:1)
RHO-1	74..95
RHO-2	391..412
RHO-3	381..402
RHO-4	312..333
RHO-5	178..199
RHO-6	144..165
RHO-7	453..474
RHO-8	448..469
RHO-9	2334..2355
RHO-10	2395..2416
RHO-11	2389..2410

A variety of *RHO* cDNA sequences may be used herein. In certain embodiments, the *RHO* cDNA may be delivered to provide an exogenous functional *RHO* cDNA.

Provided below is an exemplary nucleic acid sequence of a wild-type *RHO* cDNA:

ATGAATGGCACAGAAGGCCCTAACTTCTACGTGCCCTTCTCCAATGCGACGGGTGTGGTACG
CAGCCCCTTCGAGTACCCACAGTACTACCTGGCTGAGCCATGGCAGTTCTCCATGCTGGCCG
CCTACATGTTTCTGCTGATCGTGCTGGGCTTCCCCATCAACTTCCTCACGCTCTACGTCACC
GTCCAGCACAAGAAGCTGCGCACGCCTCTCAACTACATCCTGCTCAACCTAGCCGTGGCTGA
CCTCTTCATGGTCCTAGGTGGCTTCACCAGCACCCTCTACACCTCTCTGCATGGATACTTCG
TCTTCGGGCCCACAGGATGCAATTTGGAGGGCTTCTTTGCCACCCTGGGCGGTGAAATTGCC
CTGTGGTCCTTGGTGGTCCTGGCCATCGAGCGGTACGTGGTGGTGTGTAAGCCCATGAGCAA
CTTCCGCTTCGGGGAGAACCATGCCATCATGGGCGTTGCCTTCACCTGGGTCATGGCGCTGG
CCTGCGCCGCACCCCCACTCGCCGGCTGGTCCAGGTACATCCCCGAGGGCCTGCAGTGCTCG
TGTGGAATCGACTACTACACGCTCAAGCCGGAGGTCAACAACGAGTCTTTTGTCATCTACAT
GTTCGTGGTCCACTTCACCATCCCCATGATTATCATCTTTTTCTGCTATGGGCAGCTCGTCT
TCACCGTCAAGGAGGCCGCTGCCCAGCAGCAGGAGTCAGCCACCACACAGAAGGCAGAGAAG
GAGGTCACCCGCATGGTCATCATCATGGTCATCGCTTTCCTGATCTGCTGGGTGCCCTACGC
CAGCGTGGCATTCTACATCTTCACCCACCAGGGCTCCAACTTCGGTCCCATCTTCATGACCA
TCCCAGCGTTCTTTGCCAAGAGCGCCGCCATCTACAACCCTGTCATCTATATCATGATGAAC
AAGCAGTTCCGGAACTGCATGCTCACCACCATCTGCTGCGGCAAGAACCCACTGGGTGACGA
TGAGGCCTCTGCTACCGTGTCCAAGACGGAGACGAGCCAGGTGGCCCCGGCCTAA (SEQ
ID NO:2)

In certain embodiments, the *RHO* cDNA may be codon-optimized to increase expression. In certain embodiments, the *RHO* cDNA may be codon-modified to be resistant

to hybridization with a gRNA targeting domain. In certain embodiments, the *RHO* cDNA is not codon-modified to be resistant to hybridization with a gRNA targeting domain.

Provided below are exemplary nucleic acid sequences of codon optimized *RHO* cDNA:

Codon optimized RHO-encoding sequence 1 (Codon 1):

ATGAACGGCACCGAGGGCCCCAACTTCTACGTCCCCTTCAGCAACGCCACCGGCGTCGTCCG
CAGCCCCTTCGAGTACCCCCAGTACTACCTGGCCGAGCCCTGGCAGTTCAGCATGCTGGCCG
CCTACATGTTCCTGCTGATCGTCCTGGGCTTCCCCATCAACTTCCTGACCCTGTACGTCACC
GTCCAGCACAAGAAGCTGCGCACCCCCCTGAACTACATCCTGCTGAACCTGGCCGTCGCCGA
CCTGTTCATGGTCCTGGGCGGCTTCACCAGCACCCTGTACACCAGCCTGCACGGCTACTTCG
TCTTCGGCCCCACCGGCTGCAACCTGGAGGGCTTCTTCGCCACCCTGGGCGGCGAGATCGCC
CTGTGGAGCCTGGTCGTCCTGGCCATCGAGCGCTACGTCGTCGTCTGCAAGCCCATGAGCAA
CTTCCGCTTCGGCGAGAACCACGCCATCATGGGCGTCGCCTTCACCTGGGTCATGGCCCTGG
CCTGCGCCGCCCCCCCCCTGGCCGGCTGGAGCCGCTACATCCCCGAGGGCCTGCAGTGCAGC
TGCGGCATCGACTACTACACCCTGAAGCCCGAGGTCAACAACGAGAGCTTCGTCATCTACAT
GTTCGTCGTCCACTTCACCATCCCCATGATCATCATCTTCTTCTGCTACGGCCAGCTGGTCT
TCACCGTCAAGGAGGCCGCCGCCCAGCAGCAGGAGAGCGCCACCACCCAGAAGGCCGAGAAG
GAGGTCACCCGCATGGTCATCATCATGGTCATCGCCTTCCTGATCTGCTGGGTCCCCTACGC
CAGCGTCGCCTTCTACATCTTCACCCACCAGGGCAGCAACTTCGGCCCCATCTTCATGACCA
TCCCCGCCTTCTTCGCCAAGAGCGCCGCCATCTACAACCCCGTCATCTACATCATGATGAAC
AAGCAGTTCCGCAACTGCATGCTGACCACCATCTGCTGCGGCAAGAACCCCCTGGGCGACGA
CGAGGCCAGCGCCACCGTCAGCAAGACCGAGACCAGCCAGGTCGCCCCCGCCTAA (SEQ
ID NO:13)

Codon optimized RHO-encoding sequence 2 (Codon 2):

ATGAACGGCACCGAGGGCCCCAACTTCTACGTGCCCTTCTCCAACGCCACCGGCGTGGTGCG
CTCCCCCTTCGAGTACCCCCAGTACTACCTGGCCGAGCCCTGGCAGTTCTCCATGCTGGCCG
CCTACATGTTCCTGCTGATCGTGCTGGGCTTCCCCATCAACTTCCTGACCCTGTACGTGACC
GTGCAGCACAAGAAGCTGCGCACCCCCCTGAACTACATCCTGCTGAACCTGGCCGTGGCCGA
CCTGTTCATGGTGCTGGGCGGCTTCACCTCCACCCTGTACACCTCCCTGCACGGCTACTTCG
TGTTCGGCCCCACCGGCTGCAACCTGGAGGGCTTCTTCGCCACCCTGGGCGGCGAGATCGCC
CTGTGGTCCCTGGTGGTGCTGGCCATCGAGCGCTACGTGGTGGTGTGCAAGCCCATGTCCAA
CTTCCGCTTCGGCGAGAACCACGCCATCATGGGCGTGGCCTTCACCTGGGTGATGGCCCTGG
CCTGCGCCGCCCCCCCCCTGGCCGGCTGGTCCCGCTACATCCCCGAGGGCCTGCAGTGCTCC
TGCGGCATCGACTACTACACCCTGAAGCCCGAGGTGAACAACGAGTCCTTCGTGATCTACAT
GTTCGTGGTGCACTTCACCATCCCCATGATCATCATCTTCTTCTGCTACGGCCAGCTGGTGT
TCACCGTGAAGGAGGCCGCCGCCCAGCAGCAGGAGTCCGCCACCACCCAGAAGGCCGAGAAG
GAGGTGACCCGCATGGTGATCATCATGGTGATCGCCTTCCTGATCTGCTGGGTGCCCTACGC
CTCCGTGGCCTTCTACATCTTCACCCACCAGGGCTCCAACTTCGGCCCCATCTTCATGACCA
TCCCCGCCTTCTTCGCCAAGTCCGCCGCCATCTACAACCCCGTGATCTACATCATGATGAAC
AAGCAGTTCCGCAACTGCATGCTGACCACCATCTGCTGCGGCAAGAACCCCCTGGGCGACGA
CGAGGCCTCCGCCACCGTGTCCAAGACCGAGACCTCCCAGGTGGCCCCCGCCTAA (SEQ
ID NO:14)

Codon Optimized RHO-encoding sequence 3 (Codon 3):

ATGAACGGCACCGAGGGCCCCAACTTCTACGTCCCCTTCAGCAACGCCACCGGCGTCGTCCG

CAGCCCCTTCGAGTACCCCCAGTACTACCTGGCCGAGCCCTGGCAGTTCTCTATGCTGGCCG
CCTACATGTTCCTGCTGATCGTCCTGGGCTTCCCTATCAACTTCCTCACCCTCTACGTCACC
GTCCAGCACAAGAAGCTCCGCACCCCTCTCAACTACATCCTCCTTAACCTTGCCGTCGCCGA
CCTTTTCATGGTCCTTGGCGGCTTCACCTCTACTCTTTACACTTCTTTGCACGGGTACTTCG
TGTTCGGTCCTACTGGTTGCAACTTGGAGGGTTTCTTCGCCACTTTGGGTGGTGAGATCGCC
TTGTGGTCGTTGGTGGTGTTAGCTATCGAGCGATACGTGGTGGTGTGCAAGCCTATGTCGAA
CTTCCGGTTCGGTGAGAATCATGCTATCATGGGAGTGGCTTTTACTTGGGTGATGGCTTTAG
CTTGCGCTGCTCCTCCGTTAGCTGGATGGTCGCGTTATATCCCGGAGGGATTACAGTGCTCA
TGCGGAATCGACTATTATACTCTAAAGCCGGAAGTTAATAATGAATCATTTGTTATTTATAT
GTTTGTTGTTCATTTTACAATTCCGATGATTATTATTTTTTTTTGTTATGGACAGCTAGTTT
TTACAGTTAAGGAAGCAGCAGCACAGCAACAAGAATCAGCAACAACACAAAAGGCAGAAAAA
GAAGTTACAAGGATGGTTATTATTATGGTAATTGCATTTCTAATATGTTGGGTACCGTATGC
ATCCGTAGCATTTTATATATTTACACATCAAGGGTCCAATTTTGGGCCAATATTTATGACGA
TACCAGCGTTTTTTGCGAAATCCGCGGCGATATATAATCCAGTAATATATATAATGATGAAT
AAACAATTTAGAAATTGTATGCTAACGACGATATGTTGTGGGAAAAATCCACTAGGGGATGA
TGAAGCGAGTGCGACGGTAAGTAAAACGGAAACGAGTCAAGTAGCGCCAGCGTAA (SEQ
ID NO:15)

Codon Optimized RHO-encoding sequence 4 (Codon 4):

ATGAACGGCACCGAGGGTCCCAATTTCTACGTCCCATTTTCCAACGCCACGGGGGTGGTACG
CAGCCCTTTCGAATATCCGCAGTACTATCTGGCTGAGCCCTGGCAGTTTTCTATGCTCGCAG
CGTACATGTTCTTGCTAATCGTTCTGGGATTTCCAATTAATTTCCTCACATTGTATGTCACC
GTGCAGCACAAGAAGCTACGGACGCCTCTGAACTACATCCTCTTGAATCTAGCCGTCGCTGA
CCTGTTTATGGTTCTCGGCGGTTTCACATCGACCTTGTATACGTCACTACATGGGTACTTTG
TCTTCGGACCGACAGGCTGCAACCTGGAAGGTTTTTTCGCAACCCTCGGGGGAGAGATTGCG
TTGTGGTCCCTAGTGGTACTGGCCATCGAAAGGTATGTTGTCGTGTGTAAGCCCATGAGCAA
TTTTCGCTTCGGCGAGAACCACGCTATTATGGGTGTAGCATTTACGTGGGTTATGGCGCTCG
CCTGCGCTGCACCACCTTTGGCGGGGTGGTCTCGGTACATCCCGGAAGGACTACAGTGTTCG
TGCGGCATTGATTATTACACACTGAAGCCCGAGGTCAATAACGAATCATTCGTGATCTATAT
GTTTGTAGTTCATTTCACCATTCCAATGATCATTATCTTTTTCTGTTACGGTCAGCTCGTCT
TTACGGTGAAGGAGGCCGCTGCACAGCAGCAGGAATCCGCGACAACCCAGAAGGCCGAGAAG
GAAGTAACGAGGATGGTTATTATCATGGTCATTGCTTTCTTGATCTGCTGGGTGCCTTATGC
AAGCGTAGCGTTTTACATTTTCACACACCAGGGGTCTAATTTTGGACCGATCTTCATGACCA
TTCCCGCCTTTTTCGCTAAGTCGGCAGCGATCTATAACCCAGTTATTTACATCATGATGAAT
AAGCAGTTTCGCAACTGTATGCTAACGACAATTTGCTGTGGCAAGAATCCTCTGGGTGACGA
TGAGGCCTCAGCTACCGTCTCCAAGACGGAAACAAGCCAGGTGGCACCGGCGTAA (SEQ
ID NO:16)

Codon Optimized RHO-encoding sequence 5 (Codon 5):

ATGAATGGGACTGAAGGACCTAATTTCTATGTGCCATTTAGCAATGCTACTGGCGTTGTCAG
AAGCCCCTTCGAATATCCACAATACTATCTGGCCGAACCTTGGCAGTTCAGCATGCTCGCTG
CCTATATGTTTCTGCTGATTGTGCTGGGCTTTCCCATAAATTTCCTCACCCTGTATGTTACT
GTTCAACACAAAAAGCTGCGGACGCCTCTGAACTACATACTGCTGAACCTGGCCGTCGCCGA
CCTGTTTATGGTCCTGGGAGGCTTTACAAGCACTCTGTATACAAGCCTGCACGGCTACTTCG
TGTTCGGCCCCACAGGCTGCAACCTCGAAGGCTTCTTTGCCACCCTCGGAGGAGAGATTGCC
CTGTGGAGCCTGGTGGTGCTGGCCATCGAAAGGTATGTGGTGGTGTGTAAACCCATGTCCAA
TTTTCGGTTCGGCGAGAACCACGCTATTATGGGAGTGGCTTTCACTTGGGTGATGGCCCTGG
CCTGCGCCGCCCCACCACTGGCCGGGTGGAGCCGGTACATCCCAGAGGGGCTGCAATGTAGC

TGCGGAATCGACTATTATACCCTGAAACCAGAGGTGAACAACGAGAGCTTTGTGATTTATAT
GTTTGTGGTGCATTTTACAATTCCTATGATTATCATTTTCTTCTGTTACGGGCAACTGGTGT
TTACCGTGAAGGAAGCCGCCGCTCAACAGCAGGAGAGCGCCACAACCCAAAAGGCCGAGAAG
GAGGTGACCAGAATGGTGATTATTATGGTGATCGCTTTTCTGATTTGCTGGGTGCCATACGC
TAGCGTCGCTTTCTATATTTTCACTCACCAGGGGAGCAACTTCGGCCCCATTTTCATGACAA
TCCCTGCCTTTTTTGCTAAAAGCGCCGCCATCTATAACCCAGTGATCTACATCATGATGAAC
AAACAGTTTAGGAACTGTATGCTCACAACAATCTGCTGTGGAAAGAACCCCCTCGGCGATGA
CGAAGCCAGCGCCACCGTCAGCAAGACAGAAACAAGCCAGGTGGCCCCTGCCTAA (SEQ
ID NO:17)

Codon Optimized RHO-encoding sequence 6 (Codon 6):

ATGAATGGCACAGAGGGCCCTAACTTCTACGTGCCCTTTAGCAATGCCACAGGCGTCGTGCG
GAGCCCTTTTGAGTACCCTCAGTACTATCTGGCCGAGCCTTGGCAGTTTAGCATGCTGGCCG
CCTACATGTTCCTGCTGATCGTGCTGGGCTTCCCCATCAACTTTCTGACCCTGTACGTGACC
GTGCAGCACAAGAAGCTGCGGACCCCTCTGAACTACATCCTGCTGAATCTGGCCGTGGCCGA
CCTGTTTATGGTGCTCGGCGGCTTTACCAGCACACTGTACACAAGCCTGCACGGCTACTTCG
TGTTTGGCCCCACCGGCTGCAATCTGGAAGGCTTTTTTGCCACACTCGGCGGCGAAATTGCT
CTGTGGTCACTGGTGGTGCTGGCCATCGAGAGATACGTGGTCGTGTGCAAGCCCATGAGCAA
CTTCAGATTCGGCGAGAACCACGCCATCATGGGCGTCGCCTTTACATGGGTTATGGCCCTGG
CTTGTGCAGCTCCTCCTCTTGCCGGCTGGTCCAGATATATTCCTGAGGGCCTGCAGTGCAGC
TGCGGCATCGATTACTACACCCTGAAGCCTGAAGTGAACAACGAGAGCTTCGTGATCTACAT
GTTTGTGGTGCACTTCACGATCCCCATGATCATCATATTCTTTTGCTACGGCCAGCTGGTGT
TCACCGTGAAAGAAGCCGCTGCTCAGCAGCAAGAGAGCGCCACAACACAGAAAGCCGAGAAA
GAAGTGACCCGGATGGTCATTATCATGGTTATCGCCTTTCTGATCTGTTGGGTGCCCTACGC
CAGCGTGGCCTTCTACATCTTTACCCACCAAGGCAGCAACTTCGGCCCCATCTTTATGACAA
TCCCCGCCTTCTTTGCCAAGAGCGCCGCCATCTACAACCCCGTGATCTATATCATGATGAAC
AAGCAGTTCCGCAACTGCATGCTGACCACCATCTGCTGCGGAAAGAACCCTCTGGGAGATGA
TGAGGCCAGCGCCACCGTGTCTAAGACCGAAACATCTCAGGTGGCCCCTGCATGA (SEQ
ID NO: 18)

In certain embodiments, the *RHO* cDNA may include a modified 5' UTR, a modified 3'UTR, or a combination thereof. For example, in certain embodiments, the *RHO* cDNA may include a truncated 5' UTR, a truncated 3'UTR, or a combination thereof. In certain embodiments, the *RHO* cDNA may include a 3'UTR from a known stable messenger RNA (mRNA). For example, in certain embodiments, the *RHO* cDNA may include a heterologous 3'-UTR downstream of the *RHO* coding sequence. For example, in some embodiments, the *RHO* cDNA may include an α-globin 3' UTR. In certain embodiments, the *RHO* cDNA may include a β-globin 3' UTR. In certain embodiments, the *RHO* cDNA may include one or more introns. In certain embodiments, the *RHO* cDNA may include a truncation of one or more introns.

Exemplary suitable heterologous 3'-UTRs that can be used to stabilize the transcript of the *RHO* cDNA include, but are not limited, to the following:

HBA1 3'UTR:
GCTGGAGCCTCGGTGGCCATGCTTCTTGCCCCTTGGGCCTCCCCCCAGCCCCTCCTCCCCTT
CCTGCACCCGTACCCCCGTGGTCTTTGAATAAAGTCTGAGTGGGCGGCA (SEQ ID
NO:38)

short HBA1 3'UTR:
GCTGGAGCCTCGGTGGCCATGCTTCTTGCCCCTTGGGCCTCCCCCCAGCCCCTCCTCCCCTT
CCTGCACCCGTACCCCCGTGGTCTTTGAATAAAGTCTGA (SEQ ID NO:39)

TH 3'UTR:
GTGCACGGCGTCCCTGAGGGCCCTTCCCAACCTCCCCTGGTCCTGCACTGTCCCGGAGCTCA
GGCCCTGGTGAGGGGCTGGGTCCCGGGTGCCCCCCATGCCCTCCCTGCTGCCAGGCTCCCAC
TGCCCCTGCACCTGCTTCTCAGCGCAACAGCTGTGTGTGCCCGTGGTGAGGTTGTGCTGCCT
GTGGTGAGGTCCTGTCCTGGCTCCCAGGGTCCTGGGGGCTGCTGCACTGCCCTCCGCCCTTC
CCTGACACTGTCTGCTGCCCCAATCACCGTCACAATAAAAGAAACTGTGGTCTCTA (SEQ
ID NO:40)

COL1A1 3'UTR:
ACTCCCTCCATCCCAACCTGGCTCCCTCCCACCCAACCAACTTTCCCCCCAACCCGGAAACA
GACAAGCAACCCAAACTGAACCCCCTCAAAAGCCAAAAAATGGGAGACAATTTCACATGGAC
TTTGGAAAATATTTTTTTCCTTTGCATTCATCTCTCAAACTTAGTTTTTATCTTTGACCAAC
CGAACATGACCAAAAACCAAAAGTGCATTCAACCTTACCAAAAAAAAAAAAAAAAAAAGAAT
AAATAAATAACTTTTTAAAAAAGGAAGCTTGGTCCACTTGCTTGAAGACCCATGCGGGGGTA
AGTCCCTTTCTGCCCGTTGGGCTTATGAAACCCCAATGCTGCCCTTTCTGCTCCTTTCTCCA
CACCCCCCTTGGGGCCTCCCCTCCACTCCTTCCCAAATCTGTCTCCCCAGAAGACACAGGAA
ACAATGTATTGTCTGCCCAGCAATCAAAGGCAATGCTCAAACACCCAAGTGGCCCCCACCCT
CAGCCCGCTCCTGCCCGCCCAGCACCCCCAGGCCCTGGGGGACCTGGGGTTCTCAGACTGCC
AAAGAAGCCTTGCCATCTGGCGCTCCCATGGCTCTTGCAACATCTCCCCTTCGTTTTTGAGG
GGGTCATGCCGGGGGAGCCACCAGCCCCTCACTGGGTTCGGAGGAGAGTCAGGAAGGGCCAC
GACAAAGCAGAAACATCGGATTTGGGGAACGCGTGTCAATCCCTTGTGCCGCAGGGCTGGGC
GGGAGAGACTGTTCTGTTCCTTGTGTAACTGTGTTGCTGAAAGACTACCTCGTTCTTGTCTT
GATGTGTCACCGGGGCAACTGCCTGGGGGCGGGGATGGGGGCAGGGTGGAAGCGGCTCCCCA
TTTTATACCAAAGGTGCTACATCTATGTGATGGGTGGGGTGGGGAGGGAATCACTGGTGCTA
TAGAAATTGAGATGCCCCCCCAGGCCAGCAAATGTTCCTTTTTGTTCAAAGTCTATTTTTAT
TCCTTGATATTTTTCTTTTTTTTTTTTTTTTTTTGTGGATGGGGACTTGTGAATTTTTCTAA
AGGTGCTATTTAACATGGGAGGAGAGCGTGTGCGGCTCCAGCCCAGCCCGCTGCTCACTTTC
CACCCTCTCTCCACCTGCCTCTGGCTTCTCAGGCCTCTGCTCTCCGACCTCTCTCCTCTGAA
ACCCTCCTCCACAGCTGCAGCCCATCCTCCCGGCTCCCTCCTAGTCTGTCCTGCGTCCTCTG
TCCCCGGGTTTCAGAGACAACTTCCCAAAGCACAAAGCAGTTTTTCCCCCTAGGGGTGGGAG
GAAGCAAAAGACTCTGTACCTATTTTGTATGTGTATAATAATTTGAGATGTTTTTAATTATT
TTGATTGCTGGAATAAAGCATGTGGAAATGACCCAAACATAA (SEQ ID NO:41)

ALOX15 3'UTR:
GCGTCGCCACCCTTTGGTTATTTCAGCCCCCATCACCCAAGCCACAAGCTGACCCCTTCGTG
GTTATAGCCCTGCCCTCCCAAGTCCCACCCTCTTCCCATGTCCCACCCTCCCTAGAGGGGCA
CCTTTTCATGGTCTCTGCACCCAGTGAACACATTTTACTCTAGAGGCATCACCTGGGACCTT
ACTCCTCTTTCCTTCCTTCCTCCTTTCCTATCTTCCTTCCTCTCTCTCTTCCTCTTTCTTCA
TTCAGATCTATATGGCAAATAGCCACAATTATATAAATCATTTCAAGACTAGAATAGGGGGA
TATAATACATATTACTCCACACCTTTTATGAATCAAATATGATTTTTTTGTTGTTGTTAAGA
CAGAGTCTCACTTTGACACCCAGGCTGGAGTGCAGTGGTGCCATCACCACGGCTCACTGCAG
CCTCAGCGTCCTGGGCTCAAATGATCCTCCCACCTCAGCCTCCTGAGTAGCTGGGACTACAG

GCTCATGCCATCATGCCCAGCTAATATTTTTTTATTTTCGTGGAGACGGGGCCTCACTATGT
TGCCTAGGCTGGAAATAGGATTTTGAACCCAAATTGAGTTTAACAATAATAAAAAGTTGTTT
TACGCTAAAGATGGAAAAGAACTAGGACTGAACTATTTTAAATAAAATATTGGCAAAAGAA
(SEQ ID NO:42)

In certain embodiments, the *RHO* cDNA may include one or more introns. In certain embodiments, the *RHO* cDNA may include a truncation of one or more introns.

Table 6 below provides exemplary sequences of *RHO* cDNA containing introns.

Table 6

cDNA Identifier	*RHO* cDNA sequence
***RHO* cDNA with intron 1**	ATGAATGGCACAGAAGGCCCTAACTTCTACGTGCCCTTCTCCAATGCGACGGGTGTGG TACGCAGCCCCTTCGAGTACCCACAGTACTACCTGGCTGAGCCATGGCAGTTCTCCAT GCTGGCCGCCTACATGTTTCTGCTGATCGTGCTGGGCTTCCCCATCAACTTCCTCACG CTCTACGTCACCGTCCAGCACAAGAAGCTGCGCACGCCTCTCAACTACATCCTGCTCA ACCTAGCCGTGGCTGACCTCTTCATGGTCCTAGGTGGCTTCACCAGCACCCTCTACAC CTCTCTGCATGGATACTTCGTCTTCGGGCCCACAGGATGCAATTTGGAGGGCTTCTTT GCCACCCTGGGCGGTATGAGCCGGGTGTGGGTGGGGTGTGCAGGAGCCCGGGAGCATG GAGGGGTCTGGGAGAGTCCCGGGCTTGGCGGTGGTGGCTGAGAGGCCTTCTCCCTTCT CCTGTCCTGTCAATGTTATCCAAAGCCCTCATATATTCAGTCAACAAACACCATTCAT GGTGATAGCCGGGCTGCTGTTTGTGCAGGGCTGGCACTGAACACTGCCTTGATCTTAT TTGGAGCAATATGCGCTTGTCTAATTTCACAGCAAGAAAACTGAGCTGAGGCTCAAAG AAGTCAAGCGCCCTGCTGGGGCGTCACACAGGGACGGGTGCAGAGTTGAGTTGGAAGC CCGCATCTATCTCGGGCCATGTTTGCAGCACCAAGCCTCTGTTTCCCTTGGAGCAGCT GTGCTGAGTCAGACCCAGGCTGGGCACTGAGGGAGAGCTGGGCAAGCCAGACCCCTCC TCTCTGGGGGCCCAAGCTCAGGGTGGGAAGTGGATTTTCCATTCTCCAGTCATTGGGT CTTCCCTGTGCTGGGCAATGGGCTCGGTCCCCTCTGGCATCCTCTGCCTCCCCTCTCA GCCCCTGTCCTCAGGTGCCCCTCCAGCCTCCCTGCCGCGTTCCAAGTCTCCTGGTGTT GAGAACCGCAAGCAGCCGCTCTGAAGCAGTTCCTTTTTGCTTTAGAATAATGTCTTGC ATTTAACAGGAAAACAGATGGGGTGCTGCAGGGATAACAGATCCCACTTAACAGAGAG GAAAACTGAGGCAGGGAGAGGGGAAGAGACTCATTTAGGGATGTGGCCAGGCAGCAAC AAGAGCCTAGGTCTCCTGGCTGTGATCCAGGAATATCTCTGCTGAGATGCAGGAGGAG ACGCTAGAAGCAGCCATTGCAAAGCTGGGTGACGGGGAGAGCTTACCGCCAGCCACAA GCGTCTCTCTGCCAGCCTTGCCCTGTCTCCCCCATGTCCAGGCTGCTGCCTCGGTCCC ATTCTCAGGGAATCTCTGGCCATTGTTGGGTGTTTGTTGCATTCAATAATCACAGATC ACTCAGTTCTGGCCAGAAGGTGGGTGTGCCACTTACGGGTGGTTGTTCTCTGCAGGGT CAGTCCCAGTTTACAAATATTGTCCCTTTCACTGTTAGGAATGTCCCAGTTTGGTTGA TTAACTATATGGCCACTCTCCCTATGGAACTTCATGGGGTGGTGAGCAGGACAGATGT CTGAATTCCATCATTTCCTTCTTCTTCCTCTGGGCAAAACATTGCACATTGCTTCATG GCTCCTAGGAGAGGCCCCCACATGTCCGGGTTATTTCATTTCCCGAGAAGGGAGAGGG AGGAAGGACTGCCAATTCTGGGTTTCCACCACCTCTGCATTCCTTCCCAACAAGGAAC TCTGCCCCACATTAGGATGCATTCTTCTGCTAAACACACACACACACACACACACACA CAACACACACACACACACACACACACACACACACACACAAAACTCCCTACCGGGTTCCCA GTTCAATCCTGACCCCCTGATCTGATTCGTGTCCCTTATGGGCCCAGAGCGCTAAGCA AATAACTTCCCCCATTCCCTGGAATTTCTTTGCCCAGCTCTCCTCAGCGTGTGGTCCC TCTGCCCCTTCCCCCTCCTCCCAGCACCAAGCTCTCTCCTTCCCCAAGGCCTCCTCAA ATCCCTCTCCCACTCCTGGTTGCCTTCCTAGCTACCCTCTCCCTGTCTAGGGGGGAGT GCACCCTCCTTAGGCAGTGGGGTCTGTGCTGACCGCCTGCTGACTGCCTTGCAGGTGA AATTGCCCTGTGGTCCTTGGTGGTCCTGGCCATCGAGCGGTACGTGGTGGTGTGTAAG CCCATGAGCAACTTCCGCTTCGGGGAGAACCATGCCATCATGGGCGTTGCCTTCACCT GGGTCATGGCGCTGGCCTGCGCCGCACCCCCACTCGCCGGCTGGTCCAGGTACATCCC CGAGGGCCTGCAGTGCTCGTGTGGAATCGACTACTACACGCTCAAGCCGGAGGTCAAC AACGAGTCTTTTGTCATCTACATGTTCGTGGTCCACTTCACCATCCCCATGATTATCA TCTTTTTCTGCTATGGGCAGCTCGTCTTCACCGTCAAGGAGGCCGCTGCCCAGCAGCA GGAGTCAGCCACCACACAGAAGGCAGAGAAGGAGGTCACCCGCATGGTCATCATCATG

	GTCATCGCTTTCCTGATCTGCTGGGTGCCCTACGCCAGCGTGGCATTCTACATCTTCA CCCACCAGGGCTCCAACTTCGGTCCCATCTTCATGACCATCCCAGCGTTCTTTGCCAA GAGCGCCGCCATCTACAACCCTGTCATCTATATCATGATGAACAAGCAGTTCCGGAAC TGCATGCTCACCACCATCTGCTGCGGCAAGAACCCACTGGGTGACGATGAGGCCTCTG CTACCGTGTCCAAGACGGAGACGAGCCAGGTGGCCCCGGCCTAA (SEQ ID NO:4)
***RHO* cDNA with intron 2**	ATGAATGGCACAGAAGGCCCTAACTTCTACGTGCCCTTCTCCAATGCGACGGGTGTG GTACGCAGCCCCTTCGAGTACCCACAGTACTACCTGGCTGAGCCATGGCAGTTCTCC ATGCTGGCCGCCTACATGTTTCTGCTGATCGTGCTGGGCTTCCCCATCAACTTCCTC ACGCTCTACGTCACCGTCCAGCACAAGAAGCTGCGCACGCCTCTCAACTACATCCTG CTCAACCTAGCCGTGGCTGACCTCTTCATGGTCCTAGGTGGCTTCACCAGCACCCTC TACACCTCTCTGCATGGATACTTCGTCTTCGGGCCCACAGGATGCAATTTGGAGGGC TTCTTTGCCACCCTGGGCGGTGAAATTGCCCTGTGGTCCTTGGTGGTCCTGGCCATC GAGCGGTACGTGGTGGTGTGTAAGCCCATGAGCAACTTCCGCTTCGGGGAGAACCAT GCCATCATGGGCGTTGCCTTCACCTGGGTCATGGCGCTGGCCTGCGCCGCACCCCCA CTCGCCGGCTGGTCCAGGTAATGGCACTGAGCAGAAGGGAAGAAGCTCCGGGGGCTC TTTGTAGGGTCCTCCAGTCAGGACTCAAACCCAGTAGTGTCTGGTTCCAGGCACTGA CCTTGTATGTCTCCTGGCCCAAATGCCCACTCAGGGTAGGGGTGTAGGGCAGAAGAA GAAACAGACTCTAATGTTGCTACAAGGGCTGGTCCCATCTCCTGAGCCCCATGTCAA ACAGAATCCAAGACATCCCAACCCTTCACCTTGGCTGTGCCCCTAATCCTCAACTAA GCTAGGCGCAAATTCCAATCCTCTTTGGTCTAGTACCCCGGGGGCAGCCCCCTCTAA CCTTGGGCCTCAGCAGCAGGGGAGGCCACACCTTCCTAGTGCAGGTGGCCATATTGT GGCCCCTTGGAACTGGGTCCCACTCAGCCTCTAGGCGATTGTCTCCTAATGGGGCTG AGATGAGACACAGTGGGGACAGTGGTTTGGACAATAGGACTGGTGACTCTGGTCCCC AGAGGCCTCATGTCCCTCTGTCTCCAGAAAATTCCCACTCTCACTTCCCTTTCCTCC TCAGTCTTGCTAGGGTCCATTTCTTACCCCTTGCTGAATTTGAGCCCACCCCCTGGA CTTTTTCCCCATCTTCTCCAATCTGGCCTAGTTCTATCCTCTGGAAGCAGAGCCGCT GGACGCTCTGGGTTTCCTGAGGCCCGTCCACTGTCACCAATATCAGGAACCATTGCC ACGTCCTAATGACGTGCGCTGGAAGCCTCTAGTTTCCAGAAGCTGCACAAAGATCCC TTAGATACTCTGTGTGTCCATCTTTGGCCTGGAAAATACTCTCACCCTGGGGCTAGG AAGACCTCGGTTTGTACAAACTTCCTCAAATGCAGAGCCTGAGGGCTCTCCCCACCT CCTCACCAACCCTCTGCGTGGCATAGCCCTAGCCTCAGCGGGCAGTGGATGCTGGGG CTGGGCATGCAGGGAGAGGCTGGGTGGTGTCATCTGGTAACGCAGCCACCAAACAAT GAAGCGACACTGATTCCACAAGGTGCATCTGCATCCCCATCTGATCCATTCCATCCT GTCACCCAGCCATGCAGACGTTTATGATCCCCTTTTCCAGGGAGGGAATGTGAAGCC CCAGAAAGGGCCAGCGCTCGGCAGCCACCTTGGCTGTTCCCAAGTCCCTCACAGGCA GGGTCTCCCTACCTGCCTGTCCTCAGGTACATCCCCGAGGGCCTGCAGTGCTCGTGT GGAATCGACTACTACACGCTCAAGCCGGAGGTCAACAACGAGTCTTTTGTCATCTAC ATGTTCGTGGTCCACTTCACCATCCCCATGATTATCATCTTTTTCTGCTATGGGCAG CTCGTCTTCACCGTCAAGGAGGCCGCTGCCCAGCAGCAGGAGTCAGCCACCACACAG AAGGCAGAGAAGGAGGTCACCCGCATGGTCATCATCATGGTCATCGCTTTCCTGATC TGCTGGGTGCCCTACGCCAGCGTGGCATTCTACATCTTCACCCACCAGGGCTCCAAC TTCGGTCCCATCTTCATGACCATCCCAGCGTTCTTTGCCAAGAGCGCCGCCATCTAC AACCCTGTCATCTATATCATGATGAACAAGCAGTTCCGGAACTGCATGCTCACCACC ATCTGCTGCGGCAAGAACCCACTGGGTGACGATGAGGCCTCTGCTACCGTGTCCAAG ACGGAGACGAGCCAGGTGGCCCCGGCCTAA (SEQ ID NO:5)
***RHO* cDNA with intron 3**	ATGAATGGCACAGAAGGCCCTAACTTCTACGTGCCCTTCTCCAATGCGACGGGTGTG GTACGCAGCCCCTTCGAGTACCCACAGTACTACCTGGCTGAGCCATGGCAGTTCTCC ATGCTGGCCGCCTACATGTTTCTGCTGATCGTGCTGGGCTTCCCCATCAACTTCCTC ACGCTCTACGTCACCGTCCAGCACAAGAAGCTGCGCACGCCTCTCAACTACATCCTG CTCAACCTAGCCGTGGCTGACCTCTTCATGGTCCTAGGTGGCTTCACCAGCACCCTC TACACCTCTCTGCATGGATACTTCGTCTTCGGGCCCACAGGATGCAATTTGGAGGGC TTCTTTGCCACCCTGGGCGGTGAAATTGCCCTGTGGTCCTTGGTGGTCCTGGCCATC GAGCGGTACGTGGTGGTGTGTAAGCCCATGAGCAACTTCCGCTTCGGGGAGAACCAT GCCATCATGGGCGTTGCCTTCACCTGGGTCATGGCGCTGGCCTGCGCCGCACCCCCA CTCGCCGGCTGGTCCAGGTACATCCCCGAGGGCCTGCAGTGCTCGTGTGGAATCGAC TACTACACGCTCAAGCCGGAGGTCAACAACGAGTCTTTTGTCATCTACATGTTCGTG GTCCACTTCACCATCCCCATGATTATCATCTTTTTCTGCTATGGGCAGCTCGTCTTC ACCGTCAAGGAGGTACGGGCCGGGGGGGTGGGCGGCCTCACGGCTCTGAGGGTCCAGC

	CCCCAGCATGCATCTGCGGCTCCTGCTCCCTGGAGGAGCCATGGTCTGGACCCGGGT CCCGTGTCCTGCAGGCCGCTGCCCAGCAGCAGGAGTCAGCCACCACACAGAAGGCAG AGAAGGAGGTCACCCGCATGGTCATCATCATGGTCATCGCTTTCCTGATCTGCTGGG TGCCCTACGCCAGCGTGGCATTCTACATCTTCACCCACCAGGGCTCCAACTTCGGTC CCATCTTCATGACCATCCCAGCGTTCTTTGCCAAGAGCGCCGCCATCTACAACCCTG TCATCTATATCATGATGAACAAGCAGTTCCGGAACTGCATGCTCACCACCATCTGCT GCGGCAAGAACCCACTGGGTGACGATGAGGCCTCTGCTACCGTGTCCAAGACGGAGA CGAGCCAGGTGGCCCCGGCCTAA (SEQ ID NO:6)
***RHO* cDNA with intron 4**	ATGAATGGCACAGAAGGCCCTAACTTCTACGTGCCCTTCTCCAATGCGACGGGTGTG GTACGCAGCCCCTTCGAGTACCCACAGTACTACCTGGCTGAGCCATGGCAGTTCTCC ATGCTGGCCGCCTACATGTTTCTGCTGATCGTGCTGGGCTTCCCCATCAACTTCCTC ACGCTCTACGTCACCGTCCAGCACAAGAAGCTGCGCACGCCTCTCAACTACATCCTG CTCAACCTAGCCGTGGCTGACCTCTTCATGGTCCTAGGTGGCTTCACCAGCACCCTC TACACCTCTCTGCATGGATACTTCGTCTTCGGGCCCACAGGATGCAATTTGGAGGGC TTCTTTGCCACCCTGGGCGGTGAAATTGCCCTGTGGTCCTTGGTGGTCCTGGCCATC GAGCGGTACGTGGTGGTGTGTAAGCCCATGAGCAACTTCCGCTTCGGGGAGAACCAT GCCATCATGGGCGTTGCCTTCACCTGGGTCATGGCGCTGGCCTGCGCCGCACCCCCA CTCGCCGGCTGGTCCAGGTACATCCCCGAGGGCCTGCAGTGCTCGTGTGGAATCGAC TACTACACGCTCAAGCCGGAGGTCAACAACGAGTCTTTTGTCATCTACATGTTCGTG GTCCACTTCACCATCCCCATGATTATCATCTTTTTCTGCTATGGGCAGCTCGTCTTC ACCGTCAAGGAGGCCGCTGCCCAGCAGCAGGAGTCAGCCACCACACAGAAGGCAGAG AAGGAGGTCACCCGCATGGTCATCATCATGGTCATCGCTTTCCTGATCTGCTGGGTG CCCTACGCCAGCGTGGCATTCTACATCTTCACCCACCAGGGCTCCAACTTCGGTCCC ATCTTCATGACCATCCCAGCGTTCTTTGCCAAGAGCGCCGCCATCTACAACCCTGTC ATCTATATCATGATGAACAAGCAGGTGCCTACTGCGGGTGGGAGGGCCCCAGTGCCC CAGGCCACAGGCGCTGCCTGCCAAGGACAAGCTACTTCCCAGGGCAGGGGAGGGGGC TCCATCAGGGTTACTGGCAGCAGTCTTGGGTCAGCAGTCCCAATGGGGAGTGTGTGA GAAATGCAGATTCCTGGCCCCACTCAGAACTGCTGAATCTCAGGGTGGGCCCAGGAA CCTGCATTTCCAGCAAGCCCTCCACAGGTGGCTCAGATGCTCACTCAGGTGGGAGAA GCTCCAGTCAGCTAGTTCTGGAAGCCCAATGTCAAAGTCAGAAGGACCCAAGTCGGG AATGGGATGGGCCAGTCTCCATAAAGCTGAATAAGGAGCTAAAAAGTCTTATTCTGA GGGGTAAAGGGGTAAAGGGTTCCTCGGAGAGGTACCTCCGAGGGGTAAACAGTTGGG TAAACAGTCTCTGAAGTCAGCTCTGCCATTTTCTAGCTGTATGGCCCTGGGCAAGTC AATTTCCTTCTCTGTGCTTTGGTTTCCTCATCCATAGAAAGGTAGAAAGGGCAAAAC ACCAAACTCTTGGATTACAAGAGATAATTTACAGAACACCCTTGGCACACAGAGGGC ACCATGAAATGTCACGGGTGACACAGCCCCCTTGTGCTCAGTCCCTGGCATCTCTAG GGGTGAGGAGCGTCTGCCTAGCAGGTTCCCTCCAGGAAGCTGGATTTGAGTGGATGG GGCGCTGGAATCGTGAGGGGCAGAAGCAGGCAAAGGGTCGGGGCGAACCTCACTAAC GTGCCAGTTCCAAGCACACTGTGGGCAGCCCTGGCCCTGACTCAAGCCTCTTGCCTT CCAGTTCCGGAACTGCATGCTCACCACCATCTGCTGCGGCAAGAACCCACTGGGTGA CGATGAGGCCTCTGCTACCGTGTCCAAGACGGAGACGAGCCAGGTGGCCCCGGCCTA A (SEQ ID NO:7)

V. Genome Editing Approaches

In some embodiments, the *RHO* gene is altered using one of the approaches discussed herein.

5

NHEJ-mediated knock-out of *RHO*

Some aspects of this disclosure provide strategies, methods, compositions, and treatment modalities that are characterized by targeting an RNA-guided nuclease, e.g., a Cas9 or Cpf1 nuclease to a *RHO* target sequence, e.g., a target sequence described herein and/or

using a guide RNA described herein, wherein the RNA-guided nuclease cuts the *RHO* genomic DNA at or near the *RHO* target sequence, resulting in NHEJ-mediated repair of the cut genomic DNA. The outcome of this NHEJ-mediated repair is typically the creation of an indel at the cut site, which in turn results in a loss-of-function of the cut *RHO* gene. A loss-of-function can be characterized by a decrease or a complete abolishment of expression of a gene product, e.g., in the case of the *RHO* gene: a *RHO* gene product, for example, a *RHO* transcript or a RHO protein, or by expression of a gene product that does not exhibit a function of the wild-type gene product. In some embodiments, a loss-of-function of the *RHO* gene is characterized by expression of a lower level of functional RHO protein. In some embodiments, a loss-of-function of the *RHO* gene is characterized by abolishment of expression of RHO protein from the *RHO* gene. In some embodiments, a loss-of-function of a mutant *RHO* gene or allele is characterized by decreased expression, or abolishment of expression, of the encoded mutant RHO protein.

As described herein, nuclease-induced non-homologous end-joining (NHEJ) can be used to introduce indels at a target position. Nuclease-induced NHEJ can also be used to remove (e.g., delete) genomic sequence including the mutation at a target position in a gene of interest.

While not wishing to be bound by theory, it is believed that, in an embodiment, the genomic alterations associated with the methods described herein rely on nuclease-induced NHEJ and the error-prone nature of the NHEJ repair pathway. NHEJ repairs a double-strand break in the DNA by joining together the two ends; however, generally, the original sequence is restored only if two compatible ends, exactly as they were formed by the double-strand break, are perfectly ligated. The DNA ends of the double-strand break are frequently the subject of enzymatic processing, resulting in the addition or removal of nucleotides, at one or both strands, prior to rejoining of the ends. This results in the presence of insertion and/or deletion (indel) mutations in the DNA sequence at the site of the NHEJ repair.

The indel mutations generated by NHEJ are unpredictable in nature; however, at a given break site certain indel sequences are favored and are over represented in the population, likely due to small regions of microhomology. The lengths of deletions can vary widely; most commonly in the 1-50 bp range, but they can easily reach greater than 100-200 bp. Insertions tend to be shorter and often include short duplications of the sequence immediately surrounding the break site. However, it is possible to obtain large insertions, and in these cases, the inserted sequence has often been traced to other regions of the genome or to plasmid DNA present in the cells.

Because NHEJ is a mutagenic process, it can also be used to delete small sequence motifs as long as the generation of a specific final sequence is not required. If a double-strand break is targeted near to a specific sequence motif, the deletion mutations caused by the NHEJ repair often span, and therefore remove, the unwanted nucleotides. For the deletion of larger DNA segments, introducing two double-strand breaks, one on each side of the sequence, can result in NHEJ between the ends with removal of the entire intervening sequence. Both of these approaches can be used to delete specific DNA sequences; however, the error-prone nature of NHEJ may still produce indel mutations at the site of deletion.

Both double strand cleaving RNA-guided nucleases and single strand, or nickase, RNA-guided nucleases can be used in the methods and compositions described herein to generate break-induced indels.

Some exemplary methods featuring NHEJ-mediated knock-out of the *RHO* gene are provided herein, as are some exemplary suitable guide RNAs, RNA-guided nucleases, delivery methods, and other aspects related to such methods. Additional suitable methods, guide RNAs, RNA-guided nucleases, delivery methods, etc., will be apparent to those of ordinary skill in the art based on the present disclosure.

HDR Repair and Template Nucleic Acids

As described herein, in certain embodiments, nuclease-induced homology directed repair (HDR) can be used to alter a target position of a mutant *RHO* gene (e.g., knock out) and replace the mutant *RHO* gene with a wild-type *RHO* sequence. While not wishing to be bound by theory, it is believed that alteration of the target position occurs by homology-directed repair (HDR) with a donor template or template nucleic acid. For example, the donor template or the template nucleic acid provides for alteration of the target position. It is contemplated that a plasmid donor can be used as a template for homologous recombination. It is further contemplated that a single stranded donor template can be used as a template for alteration of the target position by alternate methods of homology directed repair (e.g., single strand annealing) between the cut sequence and the donor template. Donor template-effected alteration of a target sequence depends on cleavage by an RNA-guided nuclease molecule. Cleavage by RNA-guided nuclease molecule can comprise a double strand break or two single strand breaks.

Mutant *RHO* genes that can be replaced with wild-type *RHO* by HDR using a template nucleic acid include mutant *RHO* genes comprising point mutations, mutation hotspots or sequence insertions. In an embodiment, a mutant *RHO* gene having a point

mutation or a mutation hotspot (e.g., a mutation hotspot of less than about 30 bp, e.g., less than 25, 20, 15, 10 or 5 bp) can be altered (e.g., knocked out) by either a single double-strand break or two single strand breaks. In an embodiment, a mutant *RHO* gene having a point mutation or a mutation hotspot (e.g., a mutation hotspot greater than about 30 bp, e.g., more than 35, 40, 45, 50, 75, 100, 150, 200, 250, 300, 400 or 500 bp) or an insertion can be altered (e.g., knocked out) by (1) a single double-strand break, (2) two single strand breaks, (3) two double stranded breaks with a break occurring on each side of the target position, or (4) four single stranded breaks with a pair of single stranded breaks occurring on each side of the target position.

Mutant *RHO* genes that can be altered (e.g., knocked out) by HDR and replaced with a template nucleic acid include, but are not limited to, those in **Table A**, such as P23, e.g., P23H or P23L, T58, e.g., T58R and P347, e.g., P347T, P347A, P347S, P347G, P347L or P347R.

Double strand break mediated alteration

In an embodiment, double strand cleavage is affected by an RNA-guided nuclease. In certain embodiments, the RNA-guided nuclease may be a Cas9 molecule having cleavage activity associated with an HNH-like domain and cleavage activity associated with anRuvC-like domain, e.g., an N-terminal RuvC-like domain, e.g., a wild type Cas9. Such embodiments require only a single gRNA.

Single strand break mediated alteration

In other embodiments, two single strand breaks, or nicks, are affected by a Cas9 molecule having nickase activity, e.g., cleavage activity associated with an HNH-like domain or cleavage activity associated with an N-terminal RuvC-like domain. Such embodiments require two gRNAs, one for placement of each single strand break. In an embodiment, the Cas9 molecule having nickase activity cleaves the strand to which the gRNA hybridizes, but not the strand that is complementary to the strand to which the gRNA hybridizes. In an embodiment, the Cas9 molecule having nickase activity does not cleave the strand to which the gRNA hybridizes, but rather cleaves the strand that is complementary to the strand to which the gRNA hybridizes.

In an embodiment, the nickase has HNH activity, e.g., a Cas9 molecule having the RuvC activity inactivated, e.g., a Cas9 molecule having a mutation at D10, e.g., the D10A mutation. D10A inactivates RuvC; therefore, the Cas9 nickase has (only) HNH activity and

will cut on the strand to which the gRNA hybridizes (the complementary strand, which does not have the NGG PAM on it). In other embodiments, a Cas9 molecule having an H840, e.g., an H840A, mutation can be used as a nickase. H840A inactivates HNH; therefore, the Cas9 nickase has (only) RuvC activity and cuts on the non-complementary strand (the strand that has the NGG PAM and whose sequence is identical to the gRNA).

In an embodiment, in which a nickase and two gRNAs are used to position two single strand nicks, one nick is on the + strand and one nick is on the – strand of the target nucleic acid. The PAMs are outwardly facing. The gRNAs can be selected such that the gRNAs are separated by, from about 0-50, 0-100, or 0-200 nucleotides. In an embodiment, there is no overlap between the target domains that are complementary to the targeting domains of the two gRNAs. In an embodiment, the gRNAs do not overlap and are separated by as much as 50, 100, or 200 nucleotides. In an embodiment, the use of two gRNAs can increase specificity, e.g., by decreasing off-target binding (Ran 2013).

In an embodiment, a single nick can be used to induce HDR. It is contemplated herein that a single nick can be used to increase the ratio of HR to NHEJ at a given cleavage site.

Placement of the double strand break or a single strand break relative to the target position

The double strand break or single strand break in one of the strands should be sufficiently close to the target position such that alteration occurs. In an embodiment, the distance is not more than 50, 100, 200, 300, 350 or 400 nucleotides. While not wishing to be bound by theory, it is believed that the break should be sufficiently close to the target position such that the break is within the region that is subject to exonuclease-mediated removal during end resection.

In an embodiment, in which a gRNA (unimolecular (or chimeric) or modular gRNA) and RNA-guided nuclease induce a double strand break for the purpose of inducing HDR-mediated replacement, the cleavage site is between 0-200 bp (e.g., 0-175, 0 to 150, 0 to 125, 0 to 100, 0 to 75, 0 to 50, 0 to 25, 25 to 200, 25 to 175, 25 to 150, 25 to 125, 25 to 100, 25 to 75, 25 to 50, 50 to 200, 50 to 175, 50 to 150, 50 to 125, 50 to 100, 50 to 75, 75 to 200, 75 to 175, 75 to 150, 75 to 125, 75 to 100 bp) away from the target position. In an embodiment, the cleavage site is between 0-100 bp (e.g., 0 to 75, 0 to 50, 0 to 25, 25 to 100, 25 to 75, 25 to 50, 50 to 100, 50 to 75 or 75 to 100 bp) away from the target position.

In an embodiment, in which two gRNAs (independently, unimolecular (or chimeric) or modular gRNA) complexing with Cas9 nickases induce two single strand breaks for the

purpose of inducing HDR-mediated replacement, the closer nick is between 0-200 bp (e.g., 0-175, 0 to 150, 0 to 125, 0 to 100, 0 to 75, 0 to 50, 0 to 25, 25 to 200, 25 to 175, 25 to 150, 25 to 125, 25 to 100, 25 to 75, 25 to 50, 50 to 200, 50 to 175, 50 to 150, 50 to 125, 50 to 100, 50 to 75, 75 to 200, 75 to 175, 75 to 150, 75 to 125, 75 to 100 bp) away from the target position and the two nicks will ideally be within 25-55 bp of each other (e.g., 25 to 50, 25 to 45, 25 to 40, 25 to 35, 25 to 30, 30 to 55, 30 to 50, 30 to 45, 30 to 40, 30 to 35, 35 to 55, 35 to 50, 35 to 45, 35 to 40, 40 to 55, 40 to 50, 40 to 45 bp) and no more than 100 bp away from each other (e.g., no more than 90, 80, 70, 60, 50, 40, 30, 20, 10 or 5 bp away from each other). In an embodiment, the cleavage site is between 0-100 bp (e.g., 0 to 75, 0 to 50, 0 to 25, 25 to 100, 25 to 75, 25 to 50, 50 to 100, 50 to 75 or 75 to 100 bp) away from the target position.

In one embodiment, two gRNAs, e.g., independently, unimolecular (or chimeric) or modular gRNA, are configured to position a double-strand break on both sides of a target position. In an alternate embodiment, three gRNAs, e.g., independently, unimolecular (or chimeric) or modular gRNA, are configured to position a double strand break (i.e., one gRNA complexes with a cas9 nuclease) and two single strand breaks or paired single stranded breaks (i.e., two gRNAs complex with Cas9 nickases) on either side of the target position. In another embodiment, four gRNAs, e.g., independently, unimolecular (or chimeric) or modular gRNA, are configured to generate two pairs of single stranded breaks (i.e., two pairs of two gRNAs complex with Cas9 nickases) on either side of the target position. The double strand break(s) or the closer of the two single strand nicks in a pair will ideally be within 0-500 bp of the target position (e.g., no more than 450, 400, 350, 300, 250, 200, 150, 100, 50 or 25 bp from the target position). When nickases are used, the two nicks in a pair are within 25-55 bp of each other (e.g., between 25 to 50, 25 to 45, 25 to 40, 25 to 35, 25 to 30, 50 to 55, 45 to 55, 40 to 55, 35 to 55, 30 to 55, 30 to 50, 35 to 50, 40 to 50 , 45 to 50, 35 to 45, or 40 to 45 bp) and no more than 100 bp away from each other (e.g., no more than 90, 80, 70, 60, 50, 40, 30, 20 or 10 bp).

Length of the homology arms

The homology arm should extend at least as far as the region in which end resection may occur, e.g., in order to allow the resected single stranded overhang to find a complementary region within the donor template. The overall length could be limited by parameters such as plasmid size or viral packaging limits. In an embodiment, a homology arm does not extend into repeated elements, e.g., ALU repeats, LINE repeats.

Exemplary homology arm lengths include a least 50, 100, 250, 500, 750 or 1000 nucleotides.

Target position, as used herein, refers to a site on a target nucleic acid (e.g., the *RHO* gene) that is modified by a Cas9 molecule-dependent process. For example, the target position can be a modified Cas9 molecule cleavage of the target nucleic acid and template nucleic acid directed modification, e.g., alteration, of the target position. In an embodiment, a target position can be a site between two nucleotides, e.g., adjacent nucleotides, on the target nucleic acid into which one or more nucleotides is added. The target position may comprise one or more nucleotides that are altered, e.g., knocked out, by a template nucleic acid. In an embodiment, the target position is within a target domain (e.g., the sequence to which the gRNA binds). In an embodiment, a target position is upstream or downstream of a target domain (e.g., the sequence to which the gRNA binds).

A template nucleic acid, as that term is used herein, refers to a nucleic acid sequence which can be used in conjunction with an RNA-guided nuclease molecule and a gRNA molecule to alter the structure of a target position. In an embodiment, the target nucleic acid is modified to have some or all of the sequence of the template nucleic acid, typically at or near cleavage site(s). In an embodiment, the template nucleic acid is single stranded. In an alternate embodiment, the template nucleic acid is double stranded. In an embodiment, the template nucleic acid is DNA, e.g., double stranded DNA. In an alternate embodiment, the template nucleic acid is single stranded DNA. In an embodiment, the template nucleic acid is encoded on the same vector backbone, e.g. AAV genome, plasmid DNA, as the Cas9 and gRNA. In an embodiment, the template nucleic acid is excised from a vector backbone *in vivo*, e.g., it is flanked by gRNA recognition sequences.

In an embodiment, the template nucleic acid alters the structure of the target position by participating in a homology directed repair event. In an embodiment, the template nucleic acid alters the sequence of the target position. In an embodiment, the template nucleic acid results in the incorporation of a modified, or non-naturally occurring base into the target nucleic acid.

Typically, the template sequence undergoes a breakage-mediated or -catalyzed recombination with the target sequence. In an embodiment, the template nucleic acid includes a sequence that corresponds to a site on the target sequence that is cleaved by an eaCas9 mediated cleavage event. In an embodiment, the template nucleic acid includes a sequence that corresponds to both, a first site on the target sequence that is cleaved in a first

Cas9 mediated event, and a second site on the target sequence that is cleaved in a second Cas9 mediated event.

In an embodiment, the template nucleic acid can include sequence which results in an alteration in the coding sequence of a translated sequence, e.g., one which results in the substitution of one amino acid for another in a protein product, e.g., transforming a mutant allele into a wild type allele, transforming a wild type allele into a mutant allele, and/or introducing a stop codon, insertion of an amino acid residue, deletion of an amino acid residue, or a nonsense mutation.

In other embodiments, the template nucleic acid can include sequence which results in an alteration in a non-coding sequence, e.g., an alteration in an exon or in a 5' or 3' non-translated or non-transcribed region. Such alterations include an alteration in a control element, e.g., a promoter, enhancer, and an alteration in a cis-acting or trans-acting control element.

A template nucleic acid having homology with a target position in the *RHO* gene can be used to alter the structure of a target sequence. The template sequence can be used to alter an unwanted structure, e.g., an unwanted or mutant nucleotide.

A template nucleic acid comprises the following components:

[5' homology arm]-[replacement sequence]-[3' homology arm].

The homology arms provide for recombination into the chromosome, thus replacing the undesired element, e.g., a mutation or signature, with the replacement sequence. In an embodiment, the homology arms flank the most distal cleavage sites.

In an embodiment, the 3' end of the 5' homology arm is the position next to the 5' end of the replacement sequence. In an embodiment, the 5' homology arm can extend at least 10, 20, 30, 40, 50, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1500, or 2000 nucleotides 5' from the 5' end of the replacement sequence.

In an embodiment, the 5' end of the 3' homology arm is the position next to the 3' end of the replacement sequence. In an embodiment, the 3' homology arm can extend at least 10, 20, 30, 40, 50, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1500, or 2000 nucleotides 3' from the 3' end of the replacement sequence.

Exemplary Template Nucleic Acids

Exemplary template nucleic acids (also referred to herein as donor constructs) comprise one or more nucleotides of a *RHO* gene. In certain embodiments, the template nucleic acid comprises a *RHO* cDNA molecule. In certain embodiments, the template

nucleic acid sequence may be codon modified to be resistant to hybridization with a gRNA molecule.

Table 7 below provides exemplary template nucleic acids. In an embodiment, the template nucleic acid includes the 5' homology arm and the 3' homology arm of a row from **Table 7**. In other embodiments, a 5' homology arm from the first column can be combined with a 3' homology arm from **Table 7**. In each embodiment, a combination of the 5' and 3' homology arms include a replacement sequence, e.g., a cytosine (C) residue.

Table 7

5' homology arm (the number of nucleotides from SEQ ID NO: 5'H, beginning at the 3' end of SEQ ID NO: 5'H)	**Replacement Sequence=C**	**3' homology arm (the number of nucleotides from SEQ ID NO: 3'H, beginning at the 5' end of SEQ ID NO: 3'H)**
10 or more		10 or more
20 or more		20 or more
50 or more		50 or more
100 or more		100 or more
150 or more		150 or more
200 or more		200 or more
250 or more		250 or more
300 or more		300 or more
350 or more		350 or more
400 or more		400 or more
450 or more		450 or more
500 or more		500 or more
550 or more		550 or more
600 or more		600 or more
650 or more		650 or more
700 or more		700 or more
750 or more		750 or more
800 or more		800 or more
850 or more		850 or more
900 or more		900 or more

1000 or more		1000 or more
1100 or more		1100 or more
1200 or more		1200 or more
1300 or more		1300 or more
1400 or more		1400 or more
1500 or more		1500 or more
1600 or more		1600 or more
1700 or more		1700 or more
1800 or more		1800 or more
1900 or more		1900 or more
1200 or more		1200 or more
At least 50 but not long enough to include a repeated element.		At least 50 but not long enough to include a repeated element.
At least 100 but not long enough to include a repeated element.		At least 100 but not long enough to include a repeated element.
At least 150 but not long enough to include a repeated element.		At least 150 but not long enough to include a repeated element.
5 to 100 nucleotides		5 to 100 nucleotides
10 to 150 nucleotides		10 to 150 nucleotides
20 to 150 nucleotides		20 to 150 nucleotides

Examples of gRNAs in Genome Editing Methods

gRNA molecules as described herein can be used with RNA-guided nuclease molecules (e.g., Cas9 or Cpf1 molecules) that generate a double strand break or a single strand break to alter the sequence of a target nucleic acid, e.g., a target position or target genetic signature. The skilled artisan will be able to ascertain additional suitable gRNA molecules that can be used in conjunction with the methods and treatment modalities disclosed herein based on the present disclosure. Suitable gRNA molecules include, without limitations, those described in U.S. Patent Application No. US 2017/0073674 A1 and International Publication No. WO 2017/165862 A1, the entire contents of each of which are incorporated by reference herein.

VI. Target Cells

RNA-guided nuclease molecules (e.g., Cas9 or Cpf1 molecules) and gRNA molecules, e.g., a Cas9 or Cpf1 molecule/gRNA molecule complex can be used to manipulate a cell, e.g., to edit a target nucleic acid, in a wide variety of cells

In some embodiments, a cell is manipulated by editing (e.g., altering) one or more target genes, e.g., as described herein. In some embodiments, the expression of one or more target genes (e.g., one or more target genes described herein) is modulated, e.g., *in vivo*. In other embodiments, the expression of one or more target genes (e.g., one or more target genes described herein) is modulated, e.g., *ex vivo*.

The RNA-guided nuclease molecules (e.g., Cas9 or Cpf1 molecules), gRNA molecules, and *RHO* cDNA molecules described herein can be delivered to a target cell. In an embodiment, the target cell is a cell from the eye, e.g., a retinal cell, e.g., a photoreceptor cell. In an embodiment, the target cell is a cone photoreceptor cell or cone cell. In an embodiment, the target cell is a rod photoreceptor cell or rod cell. In an embodiment, the target cell is a macular cone photoreceptor cell. In an exemplary embodiment, cone photoreceptors in the macula are targeted, i.e., cone photoreceptors in the macula are the target cells.

A suitable cell can also include a stem cell such as, by way of example, an embryonic stem cell, an induced pluripotent stem cell, a hematopoietic stem cell, a neuronal stem cell and a mesenchymal stem cell. In an embodiment, the cell is an induced pluripotent stem cells (iPS) cell or a cell derived from an iPS cell, e.g., an iPS cell generated from the subject, modified to alter (e.g., knock out) the mutant *RHO* gene and deliver exogenous *RHO* cDNA to the cell and differentiated into a retinal progenitor cell or a retinal cell, e.g., retinal photoreceptor, and injected into the eye of the subject, e.g., subretinally, e.g., in the submacular region of the retina.

VII. Delivery, Formulations and Routes of Administration

The components, e.g., an RNA-guided nuclease molecule (e.g., Cas9 or Cpf1 molecule), gRNA molecule, and *RHO* cDNA molecule can be delivered or formulated in a variety of forms, see, e.g., **Tables 8-9**. In an embodiment, one RNA-guided nuclease molecule (e.g., Cas9 or Cpf1 molecule), one or more (e.g., 1, 2, 3, 4, or more) gRNA molecules, and the sequence of the *RHO* cDNA molecule are delivered, e.g., by an AAV vector. In an embodiment, the sequence encoding the RNA-guided nuclease molecule (e.g., Cas9 or Cpf1 molecule), the sequence(s) encoding the one or more (e.g., 1, 2, 3, 4, or more)

gRNA molecules, and the sequence of the *RHO* cDNA molecule are present on the same nucleic acid molecule, e.g., an AAV vector. In an embodiment, the sequence encoding the RNA-guided nuclease molecule (e.g., Cas9 or Cpf1 molecule) is present on a first nucleic acid molecule, e.g., an AAV vector, and the sequence(s) encoding the one or more (e.g., 1, 2, 3, 4, or more) gRNA molecules and the sequence of the *RHO* cDNA molecule are present on a second nucleic acid molecule, e.g., an AAV vector. In an embodiment, the sequence encoding the RNA-guided nuclease molecule (e.g., Cas9 or Cpf1 molecule) is present on a first nucleic acid molecule, e.g., an AAV vector, and the sequence(s) encoding the one or more (e.g., 1, 2, 3, 4, or more) gRNA molecules are present on a second nucleic acid molecule, e.g., an AAV vector, and the sequence of the *RHO* cDNA molecule is present on a third nucleic acid molecule, e.g., an AAV vector.

When an RNA-guided nuclease molecule (e.g., Cas9 or Cpf1 molecule), gRNA, or *RHO* cDNA component is delivered encoded in DNA the DNA will typically include a control region, e.g., comprising a promoter, to effect expression. Useful promoters for RNA-guided nuclease molecule (e.g., Cas9 or Cpf1 molecule) sequences include CMV, EFS, EF-1a, MSCV, PGK, CAG, hGRK1, hCRX, hNRL, and hRCVRN control promoters. Useful promoters for gRNAs include H1, EF-1a and U6 promoters. Useful promoters for *RHO* cDNA sequences include CMV, EFS, EF-1a, MSCV, PGK, CAG, hGRK1, hCRX, hNRL, and hRCVRN control promoters. In certain embodiments, useful promoters for *RHO* cDNA and RNA-guided nuclease molecule sequences include a *RHO* promoter sequence. In certain embodiments, the *RHO* promoter sequence may be a minimal *RHO* promoter sequence. In certain embodiments, a minimal *RHO* promoter sequence may comprise the sequence set forth in SEQ ID NO:44. In some embodiments, a minimal *RHO* promoter comprises no more than 100 bp, no more than 200 bp, no more than 250 bp, no more than 300 bp, no more than 400 bp, no more than 500 bp, no more than 600 bp, no more than 700 bp, no more than 800 bp, no more than 900bp, or no more than 1000 bp of the endogenous *RHO* promoter region, e.g., the region of up to 3000 bp upstream from the *RHO* transcription start site. In some embodiments, the minimal *RHO* promoter comprises no more than 100 bp, no more than 200 bp, no more than 250 bp, no more than 300 bp, no more than 400 bp, no more than 500 bp, or no more than 600 bp of the sequence proximal to the transcription start site of the endogenous *RHO* gene, and the distal enhancer region of the *RHO* promoter, or a fragment thereof. In certain embodiments, the minimal *RHO* cDNA promoter may be a rod-specific promoter. In certain embodiments, the *RHO* cDNA promoter may be a human opsin promoter. *RHO* promoters, and engineered promoter variants, suitable for use in the context of the methods,

compositions, and treatment modalities provided herein include, for example, those described in Pellissier 2014; and those described in International Patent Applications PCT/NL2014/050549, PCT/US2016/050809, and PCT/US2016/019725, the entire contents of each of which are incorporated by reference herein.

In an embodiment, the promoter is a constitutive promoter. In another embodiment, the promoter is a tissue specific promoter. Promoters with similar or dissimilar strengths can be selected to tune the expression of components. Sequences encoding an RNA-guided nuclease molecule can comprise a nuclear localization signal (NLS), e.g., an SV40 NLS. In an embodiment, the sequence encoding an RNA-guided nuclease molecule comprises at least two nuclear localization signals. In an embodiment, a promoter for an RNA-guided nuclease molecule, a gRNA molecule, or a *RHO* cDNA molecule can be, independently, inducible, tissue specific, or cell specific. To detect the expression of an RNA-guided nuclease, an affinity tag can be used. Useful affinity tag sequences include, but are not limited to, 3xFlag tag, single Flag tag, HA tag, Myc tag or HIS tag. Exemplary affinity tag sequences are disclosed in **Table 12**. To regulate RNA-guided nuclease expression, e.g., in mammalian cells, polyadenylation signals (poly(A) signals) can be used. Exemplary polyadenylation signals are disclosed in **Table 13**.

Table 8 provides examples of the form in which the components can be delivered to a target cell.

Table 8

Elements			
RNA-guided nuclease molecule(s)	gRNA molecule(s)	*RHO* cDNA	Comments
DNA	DNA	DNA	In this embodiment, an RNA-guided nuclease and a gRNA are transcribed from DNA. In this embodiment, they are encoded on separate molecules. In this embodiment, the *RHO* cDNA is provided as a separate DNA molecule.
DNA	DNA		In this embodiment, an RNA-guided nuclease and a gRNA are transcribed from DNA. In this embodiment, they are encoded on separate molecules. In this embodiment, the *RHO* cDNA is provided on the same DNA molecule that encodes the gRNA.

DNA		DNA	In this embodiment, an RNA-guided nuclease and a gRNA are transcribed from DNA, here from a single molecule. In this embodiment, the *RHO* cDNA is provided as a separate DNA molecule.
DNA	DNA	DNA	In this embodiment, an RNA-guided nuclease and a gRNA are transcribed from DNA. In this embodiment, they are encoded on separate molecules. In this embodiment, the *RHO* cDNA is provided on the same DNA molecule that encodes the RNA-guided nuclease.
DNA	RNA	DNA	In this embodiment, an RNA-guided nuclease, is transcribed from DNA, and a gRNA is provided as in vitro transcribed or synthesized RNA. In this embodiment, the *RHO* cDNA is provided as a separate DNA molecule.
DNA	RNA	DNA	In this embodiment, an RNA-guided nuclease is transcribed from DNA, and a gRNA is provided as in vitro transcribed or synthesized RNA. In this embodiment, the *RHO* cDNA is provided on the same DNA molecule that encodes the RNA-guided nuclease.
mRNA	RNA	DNA	In this embodiment, an RNA-guided nuclease is translated from in vitro transcribed mRNA, and a gRNA is provided as in vitro transcribed or synthesized RNA. In this embodiment, the *RHO* cDNA is provided as a DNA molecule.
mRNA	DNA	DNA	In this embodiment, an RNA-guided nuclease is translated from in vitro transcribed mRNA, and a gRNA is transcribed from DNA. In this embodiment, the *RHO* cDNA is provided as a separate DNA molecule.
mRNA	DNA		In this embodiment, an RNA-guided nuclease is translated from in vitro transcribed mRNA, and a gRNA is transcribed from DNA. In this embodiment, the *RHO* cDNA is provided on the same DNA molecule that encodes the gRNA.
Protein	DNA	DNA	In this embodiment, an RNA-guided nuclease is provided as a protein, and a gRNA is transcribed from DNA. In this embodiment, the *RHO* cDNA is provided as a separate DNA molecule.
Protein	DNA		In this embodiment, an RNA-guided nuclease is provided as a protein, and a

			gRNA is transcribed from DNA. In this embodiment, the *RHO* cDNA is provided on the same DNA molecule that encodes the gRNA.
Protein	RNA	DNA	In this embodiment, an RNA-guided nuclease is provided as a protein, and a gRNA is provided as transcribed or synthesized RNA. In this embodiment, the *RHO* cDNA is provided as a DNA molecule.

Table 9 summarizes various delivery methods for the components of an RNA-guided nuclease system, e.g., the Cas9 or Cpf1 molecule component, the gRNA molecule component, and the *RHO* cDNA molecule component as described herein.

Table 9

Delivery Vector/Mode		**Delivery into Non-Dividing Cells**	**Duration of Expression**	**Genome Integration**	**Type of Molecule Delivered**
Physical (e.g., electroporation, particle gun, Calcium Phosphate transfection)		YES	Transient	NO	Nucleic Acids and Proteins
Viral	**Retrovirus**	NO	Stable	YES	RNA
	Lentivirus	YES	Stable	YES/NO with modifications	RNA
	Adenovirus	YES	Transient	NO	DNA
	Adeno-Associated Virus (AAV)	YES	Stable	NO	DNA
	Vaccinia Virus	YES	Very Transient	NO	DNA
	Herpes Simplex Virus	YES	Stable	NO	DNA
Non-Viral	**Cationic Liposomes**	YES	Transient	Depends on what is delivered	Nucleic Acids and Proteins
	Polymeric Nanoparticles	YES	Transient	Depends on what is delivered	Nucleic Acids and Proteins
Biological Non-Viral	**Attenuated Bacteria**	YES	Transient	NO	Nucleic Acids

Delivery Vehicles	**Engineered Bacteriophages**	YES	Transient	NO	Nucleic Acids
	Mammalian Virus-like Particles	YES	Transient	NO	Nucleic Acids
	Biological liposomes: Erythrocyte Ghosts and Exosomes	YES	Transient	NO	Nucleic Acids

Table 10 describes exemplary promoter sequences that can be used in AAV vectors for RNA-guided nuclease (e.g., Cas9 or Cpf1) expression.

Table 10. RNA-Guided Nuclease Promoter Sequences

Promoter	Length (bp)	DNA Sequence
CMV	617	CATTGATTATTGACTAGTTATTAATAGTAATCAAT TACGGGGTCATTAGTTCATAGCCCATATATGGAGT TCCGCGTTACATAACTTACGGTAAATGGCCCGCCT GGCTGACCGCCCAACGACCCCCGCCCATTGACGTC AATAATGACGTATGTTCCCATAGTAACGCCAATAG GGACTTTCCATTGACGTCAATGGGTGGACTATTTA CGGTAAACTGCCCACTTGGCAGTACATCAAGTGTA TCATATGCCAAGTACGCCCCCTATTGACGTCAATG ACGGTAAATGGCCCGCCTGGCATTATGCCCAGTAC ATGACCTTATGGGACTTTCCTACTTGGCAGTACAT CTACGTATTAGTCATCGCTATTACCATGGTGATGC GGTTTTGGCAGTACATCAATGGGCGTGGATAGCGG TTTGACTCACGGGGATTTCCAAGTCTCCACCCCAT TGACGTCAATGGGAGTTTGTTTTGGCACCAAAATC AACGGGACTTTCCAAAATGTCGTAACAACTCCGCC CCATTGACGCAAATGGGCGGTAGGCGTGTACGGTG GGAGGTCTATATAAGCAGAGCTGGTTTAGTGAACC GTCAGATCCGCTAGAGATCCGC (SEQ ID NO:45)
EFS	252	TCGAGTGGCTCCGGTGCCCGTCAGTGGGCAGAGCG CACATCGCCCACAGTCCCCGAGAAGTTGGGGGGAG GGGTCGGCAATTGAACCGGTGCCTAGAGAAGGTGG CGCGGGGTAAACTGGGAAAGTGATGTCGTGTACTG GCTCCGCCTTTTTCCCGAGGGTGGGGGAGAACCGT ATATAAGTGCAGTAGTCGCCGTGAACGTTCTTTTT CGCAACGGGTTTGCCGCCAGAACACAGGTGTCGTG ACCGCGG (SEQ ID NO:46)
Human GRK1 (rhodopsin kinase)	292	GGGCCCCAGAAGCCTGGTGGTTGTTTGTCCTTCTC AGGGGAAAAGTGAGGCGGCCCCTTGGAGGAAGGGG CCGGGCAGAATGATCTAATCGGATTCCAAGCAGCT CAGGGGATTGTCTTTTTCTAGCACCTTCTTGCCAC TCCTAAGCGTCCTCCGTGACCCCGGCTGGGATTTC

		GCCTGGTGCTGTGTCAGCCCCGGTCTCCCAGGGGC TTCCCAGTGGTCCCCAGGAACCCTCGACAGGGCCC GGTCTCTCTCGTCCAGCAAGGGCAGGGACGGGCCA CAGGCCAAGGGC (SEQ ID NO:47)
Human CRX (cone rod homeobox transcription factor)	113	GCCTGTAGCC TTAATCTCTC CTAGCAGGGG GTTTGGGGGA GGGAGGAGGA GAAAGAAAGG GCCCCTTATG GCTGAGACAC AATGACCCAG CCACAAGGAG GGATTACCGG GCG (SEQ ID NO:48)
Human NRL (neural retina leucine zipper transcription factor enhance upstream of the human TK terminal promoter)	281	AGGTAGGAAG TGGCCTTTAA CTCCATAGAC CCTATTTAAA CAGCTTCGGA CAGGTTTAAA CATCTCCTTG GATAATTCCT AGTATCCCTG TTCCCACTCC TACTCAGGGA TGATAGCTCT AAGAGGTGTT AGGGGATTAG GCTGAAAATG TAGGTCACCC CTCAGCCATC TGGGAACTAG AATGAGTGAG AGAGGAGAGA GGGGCAGAGA CACACACATT CGCATATTAA GGTGACGCGT GTGGCCTCGA ACACCGAGCG ACCCTGCAGC GACCCGCTTA A (SEQ ID NO:49)
Human RCVRN (recoverin)	235	ATTTTAATCT CACTAGGGTT CTGGGAGCAC CCCCCCCCAC CGCTCCCGCC CTCCACAAAG CTCCTGGGCC CCTCCTCCCT TCAAGGATTG CGAAGAGCTG GTCGCAAATC CTCCTAAGCC ACCAGCATCT CGGTCTTCAG CTCACACCAG CCTTGAGCCC AGCCTGCGGC CAGGGGACCA CGCACGTCCC ACCCACCCAG CGACTCCCCA GCCGCTGCCC ACTCTTCCTC ACTCA (SEQ ID NO:50)
Human rhodopsin promoter	516	CCACGTCAGA ATCAAACCCT CACCTTAACC TCATTAGCGT TGGGCATAAT CACCAGGCCA AGCGCCTTAA ACTACGAGAG GCCCCATCCC ACCCGCCCTG CCTTAGCCCT GCCACGTGTG CCAAACGCTG TTAGACCCAA CACCACCCAG GCCAGGTAGG GGGCTGGAGC CCAGGTGGGC ATTTGAGTCA CCAACCCCCA GGCAGTCTCC CTTTTCCTGG ATCCTGAGTA CCTCTCCTCC CTGACCTCAG GCTTCCTCCT AGTGTCACCT TGGCCCCTCT TAGAAGCCAA TTAGGCCCTC AGTTTCTGCA GCGGGGATTA ATATGATTAT GAACACCCCC AATCTCCCAG ATGCTGATTC AGCCAGGAGC TTAGGAGGGG GAGGTCACTT TATAAGGGTC TGGGGGGGTC AGAACCCAGA GTCATCCAGC TGGAGCCCTG AGTGGCTGAG CTCAGGCCTT CGCAGCATTC TTGGGTGGGA GCAGCCACGG GTCAGCCACA AGGGCCACCA CCATGG (SEQ ID NO:43)
Minimal Human rhodopsin promoter	249	GTCACCTTGGCCCCTCTTAGAAGCCAATTAGGCCC TCAGTTTCTGCAGCGGGGATTAATATGATTATGAA

		CACCCCCAATCTCCCAGATGCTGATTCAGCCAGGA GCTTAGGAGGGGGAGGTCACTTTATAAGGGTCTGG GGGGGTCAGAACCCAGAGTCATCCAGCTGGAGCCC TGAGTGGCTGAGCTCAGGCCTTCGCAGCATTCTTG GGTGGGAGCAGCCACGGGTCAGCCACAAGGGCCAC AGCC (SEQ ID NO:44)

Table 11 describes exemplary promoter sequences that can be used in AAV vectors for *RHO* cDNA.

Table 11. *RHO* cDNA Promoter Sequences

Promoter	Length (bp)	DNA Sequence
CMV	617	CATTGATTATTGACTAGTTATTAATAGTAATCAATTA CGGGGTCATTAGTTCATAGCCCATATATGGAGTTCCG CGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGA CCGCCCAACGACCCCCGCCCATTGACGTCAATAATGA CGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCA TTGACGTCAATGGGTGGACTATTTACGGTAAACTGCC CACTTGGCAGTACATCAAGTGTATCATATGCCAAGTA CGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGC CTGGCATTATGCCCAGTACATGACCTTATGGGACTTT CCTACTTGGCAGTACATCTACGTATTAGTCATCGCTA TTACCATGGTGATGCGGTTTTGGCAGTACATCAATGG GCGTGGATAGCGGTTTGACTCACGGGGATTTCCAAGT CTCCACCCCATTGACGTCAATGGGAGTTTGTTTTGGC ACCAAAATCAACGGGACTTTCCAAAATGTCGTAACAA CTCCGCCCCATTGACGCAAATGGGCGGTAGGCGTGTA CGGTGGGAGGTCTATATAAGCAGAGCTGGTTTAGTGA ACCGTCAGATCCGCTAGAGATCCGC (SEQ ID NO:45)
EFS	252	TCGAGTGGCTCCGGTGCCCGTCAGTGGGCAGAGCGCA CATCGCCCACAGTCCCCGAGAAGTTGGGGGGAGGGGT CGGCAATTGAACCGGTGCCTAGAGAAGGTGGCGCGGG GTAAACTGGGAAAGTGATGTCGTGTACTGGCTCCGCC TTTTTCCCGAGGGTGGGGGAGAACCGTATATAAGTGC AGTAGTCGCCGTGAACGTTCTTTTTCGCAACGGGTTT GCCGCCAGAACACAGGTGTCGTGACCGCGG (SEQ ID NO:46)
Human GRK1 (rhodopsin kinase)	292	GGGCCCCAGAAGCCTGGTGGTTGTTTGTCCTTCTCAG GGGAAAAGTGAGGCGGCCCCTTGGAGGAAGGGGCCGG GCAGAATGATCTAATCGGATTCCAAGCAGCTCAGGGG ATTGTCTTTTTTCTAGCACCTTCTTGCCACTCCTAAGC GTCCTCCGTGACCCCGGCTGGGATTTCGCCTGGTGCT GTGTCAGCCCCGGTCTCCCAGGGGCTTCCCAGTGGTC CCCAGGAACCCTCGACAGGGCCCGGTCTCTCTCGTCC AGCAAGGGCAGGGACGGGCCACAGGCCAAGGGC (SEQ ID NO:47)

Human CRX (cone rod homeobox transcription factor)	113	GCCTGTAGCC TTAATCTCTC CTAGCAGGGG GTTTGGGGGA GGGAGGAGGA GAAAGAAAGG GCCCCTTATG GCTGAGACAC AATGACCCAG CCACAAGGAG GGATTACCGG GCG (SEQ ID NO:48)
Human NRL (neural retina leucine zipper transcription factor enhance upstream of the human TK terminal promoter)	281	AGGTAGGAAG TGGCCTTTAA CTCCATAGAC CCTATTTAAA CAGCTTCGGA CAGGTTTAAA CATCTCCTTG GATAATTCCT AGTATCCCTG TTCCCACTCC TACTCAGGGA TGATAGCTCT AAGAGGTGTT AGGGGATTAG GCTGAAAATG TAGGTCACCC CTCAGCCATC TGGGAACTAG AATGAGTGAG AGAGGAGAGA GGGGCAGAGA CACACACATT CGCATATTAA GGTGACGCGT GTGGCCTCGA ACACCGAGCG ACCCTGCAGC GACCCGCTTA A (SEQ ID NO:49)
Human RCVRN (recoverin)	235	ATTTTAATCT CACTAGGGTT CTGGGAGCAC CCCCCCCCAC CGCTCCCGCC CTCCACAAAG CTCCTGGGCC CCTCCTCCCT TCAAGGATTG CGAAGAGCTG GTCGCAAATC CTCCTAAGCC ACCAGCATCT CGGTCTTCAG CTCACACCAG CCTTGAGCCC AGCCTGCGGC CAGGGGACCA CGCACGTCCC ACCCACCCAG CGACTCCCCA GCCGCTGCCC ACTCTTCCTC ACTCA (SEQ ID NO:50)
Human rhodopsin promoter	516	CCACGTCAGA ATCAAACCCT CACCTTAACC TCATTAGCGT TGGGCATAAT CACCAGGCCA AGCGCCTTAA ACTACGAGAG GCCCCATCCC ACCCGCCCTG CCTTAGCCCT GCCACGTGTG CCAAACGCTG TTAGACCCAA CACCACCCAG GCCAGGTAGG GGGCTGGAGC CCAGGTGGGC ATTTGAGTCA CCAACCCCCA GGCAGTCTCC CTTTTCCTGG ATCCTGAGTA CCTCTCCTCC CTGACCTCAG GCTTCCTCCT AGTGTCACCT TGGCCCCTCT TAGAAGCCAA TTAGGCCCTC AGTTTCTGCA GCGGGGATTA ATATGATTAT GAACACCCCC AATCTCCCAG ATGCTGATTC AGCCAGGAGC TTAGGAGGGG GAGGTCACTT TATAAGGGTC TGGGGGGGTC AGAACCCAGA GTCATCCAGC TGGAGCCCTG AGTGGCTGAG CTCAGGCCTT CGCAGCATTC TTGGGTGGGA GCAGCCACGG GTCAGCCACA AGGGCCACCA CCATGG (SEQ ID NO:43)

Minimal Human rhodopsin promoter	249	GTCACCTTGGCCCCTCTTAGAAGCCAATTAGGCCCTC AGTTTCTGCAGCGGGGATTAATATGATTATGAACACC CCCAATCTCCCAGATGCTGATTCAGCCAGGAGCTTAG GAGGGGGAGGTCACTTTATAAGGGTCTGGGGGGGTCA GAACCCAGAGTCATCCAGCTGGAGCCCTGAGTGGCTG AGCTCAGGCCTTCGCAGCATTCTTGGGTGGGAGCAGC CACGGGTCAGCCACAAGGGCCACAGCC (SEQ ID NO:44)

Table 12 describes exemplary affinity tag sequences that can be used in AAV vectors, e.g., for RNA-guided nuclease (e.g., Cas9 or Cpf1) expression.

Table 12. Exemplary Affinity Tag Sequences

Affinity tag	Amino Acid Sequence
3XFlag tag	DYKDHDGDYKDHDIDYKDDDDK (SEQ ID NO:51)
Flag tag (single)	DYKDDDDK (SEQ ID NO:52)
HA tag	YPYDVPDYA (SEQ ID NO:53)
Myc tag	EQKLISEEDL (SEQ ID NO:54)
HIS tag	HHHHHH (SEQ ID NO:55)

Table 13 describes exemplary polyadenylation (polyA) sequences that can be used in AAV vectors, e.g., for RNA-guided nuclease (e.g., Cas9 or Cpf1) expression.

Table 13. Exemplary PolyA Sequences

PolyA	DNA sequence
Mini polyA	TAGCAATAAA GGATCGTTTA TTTTCATTGG AAGCGTGTGT TGGTTTTTTG ATCAGGCGCG (SEQ ID NO:56)
bGH polyA	GCTGCAGGAT GACCGGTCAT CATCACCATC ACCATTGAGT TTAAACCCGC TGATCAGCCT CGACTGTGCC TTCTAGTTGC CAGCCATCTG TTGTTTGCCC CTCCCCCGTG CCTTCCTTGA CCCTGGAAGG TGCCACTCCC ACTGTCCTTT CCTAATAAAA TGAGGAAATT GCATCGCATT GTCTGAGTAG GTGTCATTCT ATTCTGGGGG GTGGGGTGGG GCAGGACA (SEQ ID NO:57)
SV40 polyA	ATGCTTTATT TGTGAAATTT GTGATGCTAT TGCTTTATTT GTAACCATTA TAAGCTGCAA TAAACAAGTT AACAACAACA ATTGCATTCA TTTTATGTTT CAGGTTCAGG GGGAGGTGTG GGAGGTTTTT TAAA (SEQ ID NO:58)

Table 14 describes exemplary Inverted Terminal Repeat (ITR) sequences that can be used in AAV vectors.

Table 14. Sequences of ITRs from Exemplary AAV Serotypes

AAV Serotype	Left ITR Sequence	Right ITR Sequence
AAV1	TTGCCCACTC CCTCTCTGCG CGCTCGCTCG CTCGGTGGGG CCTGCGGACC AAAGGTCCGC AGACGGCAGA GCTCTGCTCT GCCGGCCCCA CCGAGCGAGC GAGCGCGCAG AGAGGGAGTG GGCAACTCCA TCACTAGGGG TAA (SEQ ID NO:59)	TTACCCCTAG TGATGGAGTT GCCCACTCCC TCTCTGCGCG CTCGCTCGCT CGGTGGGGCC GGCAGAGCAG AGCTCTGCCG TCTGCGGACC TTTGGTCCGC AGGCCCCACC GAGCGAGCGA GCGCGCAGAG AGGGAGTGGG CAA (SEQ ID NO:68)
AAV2	TTGGCCACTC CCTCTCTGCG CGCTCGCTCG CTCACTGAGG CCGGGCGACC AAAGGTCGCC CGACGCCCGG GCTTTGCCCG GGCGGCCTCA GTGAGCGAGC GAGCGCGCAG AGAGGGAGTG GCCAACTCCA TCACTAGGGG TTCCT (SEQ ID NO: 60)	AGGAACCCCT AGTGATGGAG TTGGCCACTC CCTCTCTGCG CGCTCGCTCG CTCACTGAGG CCGCCCGGGC AAAGCCCGGG CGTCGGGCGA CCTTTGGTCG CCCGGCCTCA GTGAGCGAGC GAGCGCGCAG AGAGGGAGTG GCCAA (SEQ ID NO:69)
AAV3B	TGGCCACTCC CTCTATGCGC ACTCGCTCGC TCGGTGGGGC CTGGCGACCA AAGGTCGCCA GACGGACGTG CTTTGCACGT CCGGCCCCAC CGAGCGAGCG AGTGCGCATA GAGGGAGTGG CCAACTCCAT CACTAGAGGT AT (SEQ ID NO:61)	ATACCTCTAG TGATGGAGTT GGCCACTCCC TCTATGCGCA CTCGCTCGCT CGGTGGGGCC GGACGTGCAA AGCACGTCCG TCTGGCGACC TTTGGTCGCC AGGCCCCACC GAGCGAGCGA GTGCGCATAG AGGGAGTGGC CA (SEQ ID NO:70)
AAV4	TTGGCCACTC CCTCTATGCG CGCTCGCTCA CTCACTCGGC CCTGGAGACC AAAGGTCTCC AGACTGCCGG CCTCTGGCCG GCAGGGCCGA GTGAGTGAGC GAGCGCGCAT AGAGGGAGTG GCCAACTCCA TCATCTAGGT TTGCCC (SEQ ID NO:62)	GGGCAAACCT AGATGATGGA GTTGGCCACT CCCTCTATGC GCGCTCGCTC ACTCACTCGG CCCTGCCGGC CAGAGGCCGG CAGTCTGGAG ACCTTTGGTC TCCAGGGCCG AGTGAGTGAG CGAGCGCGCA TAGAGGGAGT GGCCAA (SEQ ID NO:71)
AAV5	CTCTCCCCCC TGTCGCGTTC GCTCGCTCGC TGGCTCGTTT GGGGGGGTGG CAGCTCAAAG AGCTGCCAGA CGACGGCCCT CTGGCCGTCG CCCCCCCAAA CGAGCCAGCG AGCGAGCGAA	TTGCTTGAGA GTGTGGCACT CTCCCCCCTG TCGCGTTCGC TCGCTCGCTG GCTCGTTTGG GGGGGCGACG GCCAGAGGGC CGTCGTCTGG CAGCTCTTTG AGCTGCCACC CCCCCAAACG

	CGCGACAGGG GGGAGAGTGC CACACTCTCA AGCAA (SEQ ID NO:63)	AGCCAGCGAG CGAGCGAACG CGACAGGGGG GAGAG (SEQ ID NO:72)
AAV6	ATACCCCTAG TGATGGAGTT GCCCACTCCC TCTATGCGCG CTCGCTCGCT CGGTGGGGCC GGCAGAGCAG AGCTCTGCCG TCTGCGGACC TTTGGTCCGC AGGCCCCACC GAGCGAGCGA GCGCGCATAG AGGGAGTGGG CAA (SEQ ID NO:64)	TTGCCCACTC CCTCTATGCG CGCTCGCTCG CTCGGTGGGG CCTGCGGACC AAAGGTCCGC AGACGGCAGA GCTCTGCTCT GCCGGCCCCA CCGAGCGAGC GAGCGCGCAT AGAGGGAGTG GGCAACTCCA TCACTAGGGG TAT (SEQ ID NO:73)
AAV7	TTGGCCACTC CCTCTATGCG CGCTCGCTCG CTCGGTGGGG CCTGCGGACC AAAGGTCCGC AGACGGCAGA GCTCTGCTCT GCCGGCCCCA CCGAGCGAGC GAGCGCGCAT AGAGGGAGTG GCCAACTCCA TCACTAGGGG TACCG (SEQ ID NO:65)	CGGTACCCCT AGTGATGGAG TTGGCCACTC CCTCTATGCG CGCTCGCTCG CTCGGTGGGG CCGGCAGAGC AGAGCTCTGC CGTCTGCGGA CCTTTGGTCC GCAGGCCCCA CCGAGCGAGC GAGCGCGCAT AGAGGGAGTG GCCAA (SEQ ID NO:74)
AAV8	CAGAGAGGGA GTGGCCAACT CCATCACTAG GGGTAGCGCG AAGCGCCTCC CACGCTGCCG CGTCAGCGCT GACGTAAATT ACGTCATAGG GGAGTGGTCC TGTATTAGCT GTCACGTGAG TGCTTTTGCG GCATTTTGCG ACACC (SEQ ID NO:66)	GGTGTCGCAA AATGCCGCAA AAGCACTCAC GTGACAGCTA ATACAGGACC ACTCCCCTAT GACGTAATTT ACGTCAGCGC TGACGCGGCA GCGTGGGAGG CGCTTCGCGC TACCCCTAGT GATGGAGTTG GCCACTCCCT CTCTG (SEQ ID NO:75)
AAV9	CAGAGAGGGA GTGGCCAACT CCATCACTAG GGGTAATCGC GAAGCGCCTC CCACGCTGCC GCGTCAGCGC TGACGTAGAT TACGTCATAG GGGAGTGGTC CTGTATTAGC TGTCACGTGA GTGCTTTTGC GACATTTTGC GACAC (SEQ ID NO:67)	GTGTCGCAAA ATGTCGCAAA AGCACTCACG TGACAGCTAA TACAGGACCA CTCCCCTATG ACGTAATCTA CGTCAGCGCT GACGCGGCAG CGTGGGAGGC GCTTCGCGAT TACCCCTAGT GATGGAGTTG GCCACTCCCT CTCTG (SEQ ID NO:76)
AAV	TGCAGGCAGCTGCGCGCTCGCTCG CTCACTGAGGCCGCCCGGGCAAAG CCCGGGCGTCGGGCGACCTTTGGT CGCCCGGCCTCAGTGAGCGAGCGA GCGCGCAGAGAGGGAGTGGCCAAC TCCATCACTAGGGGTTCCT (SEQ ID NO:92)	AGGAACCCCTAGTGATGGAGTTGG CCACTCCCTCTCTGCGCGCTCGCT CGCTCACTGAGGCCGGGCGACCAA AGGTCGCCCGACGCCCGGGCTTTG CCCGGGCGGCCTCAGTGAGCGAGC GAGCGCGCAGCTGCCTGCA (SEQ ID NO:93)

Additional exemplary sequences for the recombinant AAV genome components described herein are provided below.

Exemplary U6 promoter sequence:
AAGGTCGGGCAGGAAGAGGGCCTATTTCCCATGATTCCTTCATATTTGCATATACGATACAA
GGCTGTTAGAGAGATAATTAGAATTAATTTGACTGTAAACACAAAGATATTAGTACAAAATA
CGTGACGTAGAAAGTAATAATTTCTTGGGTAGTTTGCAGTTTTAAAATTATGTTTTAAAATG
GACTATCATATGCTTACCGTAACTTGAAAGTATTTCGATTTCTTGGCTTTATATATCTTGTG
GAAAGGACGAAACACC (SEQ ID NO:78).

Exemplary gRNA targeting domain sequences are described herein, e.g., in **Tables 1-3**, and **18**.

Skilled artisans will understand that it may be advantageous in some embodiments to add a 5' G to a gRNA targeting domain sequence, e.g., when the gRNA is driven by a U6 promoter.

Exemplary gRNA scaffold domain sequences:
GTTTTAGTACTCTGGAAACAGAATCTACTAAAACAAGGCAAAATGCCGTGTTTATCTCGTCA
ACTTGTTGGCGAGATTTTTT (SEQ ID NO:79);
GTTATAGTACTCTGGAAACAGAATCTACTATAACAAGGCAAAATGCCGTGTTTATCTCGTCA
ACTTGTTGGCGAGA (SEQ ID NO:12).

Exemplary N-ter NLS nucleotide sequence:
CCGAAGAAAAAGCGCAAGGTCGAAGCGTCC (SEQ ID NO:81).

Exemplary N-ter NLS amino acid sequence: PKKKRKV (SEQ ID NO:82).

Exemplary Cas9 nucleotide sequences as described herein.

Exemplary Cas9 amino acid sequences as described herein.

Exemplary Cpf1 nucleotide sequences as described herein.

Exemplary Cpf1 amino acid sequences as described herein.

Exemplary C-ter NLS sequence: CCCAAGAAGAAGAGGAAAGTC (SEQ ID NO:83).

Exemplary C-ter NLS amino acid sequence: PKKKRKV (SEQ ID NO:84).

Exemplary poly(A) signal sequence:
TAGCAATAAAGGATCGTTTATTTTCATTGGAAGCGTGTGTTGGTTTTTTGATCAGGCGCG
(SEQ ID NO:56).

Exemplary 3xFLAG nucleotide sequence:
GACTACAAAGACCATGACGGTGATTATAAAGATCATGACATCGATTACAAGGATGACGATGA
CAAG (SEQ ID NO:86).

Exemplary 3xFLAG amino acid sequence:
DYKDHDGDYKDHDIDYKDDDDK (SEQ ID NO:51).

Exemplary spacer sequences:

CAGATCTGAATTCGGTACC (SEQ ID NO:77);

GGTACCGCTAGCGCTTAAGTCGCGATGTACGGGCCAGATATACGCGTTGA (SEQ ID NO:80);

TCCAAGCTTCGCAGGAAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCGTTAACTCTAGATT TAAATGCATGCTGGGGAGAGATCT (SEQ ID NO:85);

CGACTTAGTTCGATCGAAGG (SEQ ID NO:87).

Exemplary SV40 intron sequence:

TCTAGAGGATCCGGTACTCGAGGAACTGAAAAACCAGAAAGTTAACTGGTAAGTTTAGTCTT TTTGTCTTTTATTTCAGGTCCCGGATCCGGTGGTGGTGCAAATCAAAGAACTGCTCCTCAGT GGATGTTGCCTTTACTTCTAGGCCTGTACGGAAGTGTTAC (SEQ ID NO:94).

In certain aspects, the present disclosure focuses on AAV vectors encoding CRISPR/RNA-guided nuclease genome editing systems and a *RHO* cDNA molecule, and on the use of such vectors to treat adRP. Exemplary AAV vector genomes are schematized in **Fig. 2**, which illustrate certain fixed and variable elements of these vectors: a first AAV vector comprising ITRs, an RNA-guided nuclease (e.g., Cas9) coding sequence and a promoter to drive its expression, with the RNA-guided nuclease coding sequence flanked by NLS sequences; and a second AAV vector comprising ITRs, one *RHO* cDNA sequence and a minimal *RHO* promoter to drive its expression and one gRNA sequence and promoter sequences to drive its expression. Additional exemplary AAV vector genomes are also set forth in **Figs. 3** and **16-18**. Exemplary AAV vector genome sequences are set forth in SEQ ID NOs: 8-11.

Turning first to the gRNA utilized in the nucleic acids or AAV vectors of the present disclosure, one or more gRNAs may be used to cut the 5' region of a mutant *RHO* gene (e.g., 5' UTR, exon 1, exon 2, intron 1, exon 1/intron border). In certain embodiments, cutting in the 5' region of the mutant *RHO* gene results in knocking out or loss of function of the mutant *RHO* gene. In certain embodiments, one or more gRNAs may be used to cut the coding region of a mutant *RHO* gene (e.g., exon 1, exon 2, exon 3, exon 4, exon 5) or the non-coding region of a mutant *RHO* gene (e.g., 5' UTR, introns, 3' UTR). In certain embodiments, cutting in the coding region or non-coding region of the mutant *RHO* gene may result in knocking out or loss of function of the mutant *RHO* gene.

Targeting domain sequences of exemplary guides (both DNA and RNA sequences) are presented in **Tables 1-3** and **18**.

In some embodiments, the gRNAs used in the present disclosure may be derived from *S. aureus* gRNAs and can be unimolecular or modular, as described below. Exemplary DNA and RNA sequences corresponding to unimolecular *S. aureus* gRNAs are shown below:

DNA: [N]$_{16-24}$GTTTTAGTACTCTGGAAACAGAATCTACTAAAACAAGGCAAAATGCCGTGTTTATCTCGTCAACTTGTTGGCGAGATTTTTT (SEQ ID NO:88) and

RNA: [N]$_{16-24}$GUUUUAGUACUCUGGAAACAGAAUCUACUAAAACAAGGCAAAAUGCCGUGUUUAUCUCGUCAACUUGUUGGCGAGAUUUUUU (SEQ ID NO:89).

DNA: [N]$_{16-24}$GTTATAGTACTCTGGAAACAGAATCTACTATAACAAGGCAAAATGCCGTGTTTATCTCGTCAACTTGTTGGCGAGATTTTTT (SEQ ID NO:90) and

RNA: [N]$_{16-24}$GUUAUAGUACUCUGGAAACAGAAUCUACUAUAACAAGGCAAAAUGCCGUGUUUAUCUCGUCAACUUGUUGGCGAGAUUUUUU (SEQ ID NO:91).

It should be noted that the targeting domain can have any suitable length. gRNAs used in the various embodiments of this disclosure preferably include targeting domains of between 16 and 24 (inclusive) bases in length at their 5' ends, and optionally include a 3' U6 termination sequence as illustrated.

In some instances, modular guides can be used. In the exemplary unimolecular gRNA sequences above, a 5' portion corresponding to a crRNA (underlined) is connected by a GAAA linker to a 3' portion corresponding to a tracrRNA (double underlined). Skilled artisans will appreciate that two-part modular gRNAs can be used that correspond to the underlined and double underlined sections.

Skilled artisans will appreciate that the exemplary gRNA designs set forth herein can be modified in a variety of ways, which are described below or are known in the art; the incorporation of such modifications is within the scope of this disclosure.

Expression of the one or more gRNAs in the AAV vector may be driven by a pair of U6 promoters, such as a human U6 promoter. An exemplary U6 promoter sequence, as set forth in Maeder, is SEQ ID NO:78.

Turning next to RNA-guided nucleases, in some embodiments the RNA-guided nuclease may be a Cas9 or Cpf1 protein. In certain embodiments, the Cas9 protein is *S.*

*pyo*genes Cas9. In certain embodiments, the Cas9 protein is *S. aureus* Cas9. In further embodiments of this disclosure an Cas9 sequence is modified to include two nuclear localization sequences (NLSs) at the C- and N-termini of the Cas9 protein, and a mini-polyadenylation signal (or Poly-A sequence). Exemplary Cas9 sequences and Cpf1 sequences are provided herein. These sequences are exemplary in nature and are not intended to be limiting. The skilled artisan will appreciate that modifications of these sequences may be possible or desirable in certain applications; such modifications are described below, or are known in the art, and are within the scope of this disclosure.

Skilled artisans will also appreciate that polyadenylation signals are widely used and known in the art, and that any suitable polyadenylation signal can be used in the embodiments of this disclosure. Exemplary polyadenylation signals are set forth in SEQ ID NOs:56-58.

Cas9 expression may be driven, in certain vectors of this disclosure, by one of three promoters: cytomegalovirus (CMV) (i.e., SEQ ID NO:45), elongation factor-1 (EFS) (i.e., SEQ ID NO:46), or human g-protein receptor coupled kinase-1 (hGRK1) (i.e., SEQ ID NO:47), which is specifically expressed in retinal photoreceptor cells. Modifications of the sequences of the promoters may be possible or desirable in certain applications, and such modifications are within the scope of this disclosure. In certain embodiments, Cas9 expression may be driven by a *RHO* promoter described herein (e.g., a minimum RHO Promoter (250 bp) SEQ ID NO:44).

Turning next to *RHO* cDNA, in some embodiments the *RHO* cDNA molecule may be wild-type *RHO* cDNA (e.g., SEQ ID NO:2). In certain embodiments, the *RHO* cDNA molecule may be a codon-modified cDNA to be resistant to hybridizing with a gRNA. In certain embodiments, the *RHO* cDNA molecule is not codon-modified to be resistant to hybridizing with a gRNA. In certain embodiments, the *RHO* cDNA molecule may be a codon-optimized cDNA to provide increased expression of rhodopsin protein (e.g., SEQ ID NOs:13-18). In certain embodiments, the *RHO* cDNA may comprise a modified 3' UTR, for example, a 3' UTR from a highly expressed, stable transcript, such as alpha- or beta-globin. Exemplarly 3' UTRs are set forth in SEQ ID NOs:38-42. In certain embodiments, the *RHO* cDNA may include one or more introns (e.g., SEQ ID NOs:4-7). In certain embodiments, the *RHO* cDNA may include a truncation of one or more introns.

In certain embodiments, *RHO* cDNA expression may be driven by a rod-specific promoter. In certain embodiments, *RHO* cDNA expression may be driven by a *RHO* promoter described herein (e.g., a minimum RHO Promoter (250 bp) SEQ ID NO:44).

AAV genomes according to the present disclosure generally incorporate inverted terminal repeats (ITRs) derived from the AAV5 serotype. Exemplary left and right ITRs are SEQ ID NO:63 (AAV5 Left ITR) and SEQ ID NO:72 (AAV5 Right ITR), respectively. In certain embodiments, exemplary left and right ITRs are SEQ ID NO:92 (AAV Left ITR) and SEQ ID NO:93 (AAV Right ITR), respectively. It should be noted, however, that numerous modified versions of the AAV5 ITRs are used in the field, and the ITR sequences shown herein are exemplary and are not intended to be limiting. Modifications of these sequences are known in the art, or will be evident to skilled artisans, and are thus included in the scope of this disclosure.

The gRNA, RNA-guided nuclease, and *RHO* cDNA promoters are variable and can be selected from the lists presented herein. For clarity, this disclosure encompasses nucleic acids and/or AAV vectors comprising any combination of these elements, though certain combinations may be preferred for certain applications.

In various embodiments, a first nucleic acid or AAV vector may encode the following: left and right AAV ITR sequences (e.g., AAV5 ITRs), a promoter (e.g., CMV, hGRK1, EFS, *RHO* promoter) to drive expression of an RNA-guided nuclease (e.g., Cas9 encoded by a Cas9 nucleic acid molecule or Cpf1 encoded by a Cpf1 nucleic acid), NLS sequences flanking the RNA-guided nuclease nucleic acid molecule, and a second nucleic acid or AAV vector may encode the following: left and right AAV ITR sequences (e.g., AAV5 ITRs), a U6 promoter to drive expression of a guide RNA comprising a targeting domain sequence (e.g., a sequence according to a sequence in **Tables 1-3** or **18**), and a *RHO* promoter (e.g., minimal *RHO* promoter) to drive expression of a *RHO* cDNA molecule.

The nucleic acid or AAV vector may also comprise a Simian virus 40 (SV40) splice donor/splice acceptor (SD/SA) sequence element. In certain embodiments, the SV40 SD/SA element may be positioned between the promoter and the RNA-guided nuclease gene (e.g., Cas9 or Cpf1 gene). In certain embodiments, a Kozak consensus sequence may precede the start codon of the RNA-guided nuclease (e.g., Cas9 or Cpf1) to ensure robust RNA-guided nuclease (e.g., Cas9 or Cpf1) expression.

In some embodiments, the nucleic acid or AAV vector shares at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or greater sequence identity with one of the nucleic acids or AAV vectors recited above.

It should be noted that these sequences described above are exemplary and can be modified in ways that do not disrupt the operating principles of elements they encode. Such modifications, some of which are discussed below, are within the scope of this disclosure.

Without limiting the foregoing, skilled artisans will appreciate that the DNA, RNA or protein sequences of the elements of this disclosure may be varied in ways that do not interrupt their function, and that a variety of similar sequences that are substantially similar (e.g., greater than 90%, 95%, 96%, 97%, 98% or 99% sequence similarity, or in the case of short sequences such as gRNA targeting domains, sequences that differ by no more than 1, 2 or 3 nucleotides) can be utilized in the various systems, methods and AAV vectors described herein. Such modified sequences are within the scope of this disclosure.

The AAV genomes described above can be packaged into AAV capsids (for example, AAV5 capsids), which capsids can be included in compositions (such as pharmaceutical compositions) and/or administered to subjects. An exemplary pharmaceutical composition comprising an AAV capsid according to this disclosure can include a pharmaceutically acceptable carrier such as balanced saline solution (BSS) and one or more surfactants (e.g., Tween20) and/or a thermosensitive or reverse-thermosensitive polymer (e.g., pluronic). Other pharmaceutical formulation elements known in the art may also be suitable for use in the compositions described here.

Compositions comprising AAV vectors according to this disclosure can be administered to subjects by any suitable means, including without limitation injection, for example, subretinal injection. The concentration of AAV vector within the composition is selected to ensure, among other things, that a sufficient AAV dose is administered to the retina of the subject, taking account of dead volume within the injection apparatus and the relatively limited volume that can be safely administered to the retina. Suitable doses may include, for example, $1x10^{11}$ viral genomes (vg)/mL, $2x10^{11}$ viral genomes (vg)/mL, $3x10^{11}$ viral genomes (vg)/mL, $4x10^{11}$ viral genomes (vg)/mL, $5x10^{11}$ viral genomes (vg)/mL, $6x10^{11}$ viral genomes (vg)/mL, $7x10^{11}$ viral genomes (vg)/mL, $8x10^{11}$ viral genomes (vg)/mL, $9x10^{11}$ viral genomes (vg)/mL, $1x10^{12}$ vg/mL, $2x10^{12}$ viral genomes (vg)/mL, $3x10^{12}$ viral genomes (vg)/mL, $4x10^{12}$ viral genomes (vg)/mL, $5x10^{12}$ viral genomes (vg)/mL, $6x10^{12}$ viral genomes (vg)/mL, $7x10^{12}$ viral genomes (vg)/mL, $8x10^{12}$ viral genomes (vg)/mL, $9x10^{12}$ viral genomes (vg)/mL, $1x10^{13}$ vg/mL, $2x10^{13}$ viral genomes (vg)/mL, $3x10^{13}$ viral genomes (vg)/mL, $4x10^{13}$ viral genomes (vg)/mL, $5x10^{13}$ viral genomes (vg)/mL, $6x10^{13}$ viral genomes (vg)/mL, $7x10^{13}$ viral genomes (vg)/mL, $8x10^{13}$ viral genomes (vg)/mL, or $9x10^{13}$ viral genomes (vg)/mL. Any suitable volume of the composition may be delivered to the subretinal space. In some instances, the volume is selected to form a bleb in the subretinal space, for example 1 microliter, 10 microliters, 50 microliters, 100 microliters, 150 microliters, 200 microliters, 250 microliters, 300 microliters, etc.

Any region of the retina may be targeted, though the fovea (which extends approximately 1 degree out from the center of the eye) may be preferred in certain instances due to its role in central visual acuity and the relatively high concentration of cone photoreceptors there relative to peripheral regions of the retina. Alternatively or additionally, injections may be targeted to parafoveal regions (extending between approximately 2 and 10 degrees off center), which are characterized by the presence of both rod and cone photoreceptor cells. In addition, injections into the parafoveal region may be made at comparatively acute angles using needle paths that cross the midline of the retina. For instance, injection paths may extend from the nasal aspect of the sclera near the limbus through the vitreal chamber and into the parafoveal retina on the temporal side, from the temporal aspect of the sclera to the parafoveal retina on the nasal side, from a portion of the sclera located superior to the cornea to an inferior parafoveal position, and/or from an inferior portion of the sclera to a superior parafoveal position. The use of relatively small angles of injection relative to the retinal surface may advantageously reduce or limit the potential for spillover of vector from the bleb into the vitreous body and, consequently, reduce the loss of the vector during delivery. In other cases, the macula (inclusive of the fovea) can be targeted, and in other cases, additional retinal regions can be targeted, or can receive spillover doses.

To mitigate ocular inflammation and associated discomfort, one or more corticosteroids may be administered before, during, and/or after administration of the composition comprising AAV vectors. In certain embodiments, the corticosteroid may be an oral corticosteroid. In certain embodiments, the oral corticosteroid may be prednisone. In certain embodiments, the corticosteroid may be administered as a prophylactic, prior to administration of the composition comprising AAV vectors. For example, the corticosteroid may be administered the day prior to administration, or 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 8 days, 9 days, 10 days, 11 days, 12 days, 13 days, or 14 days prior to administration of the composition comprising AAV vectors. In certain embodiments, the corticosteroid may be administered for 1 week to 10 weeks after administration of the composition comprising AAV vectors (e.g., 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 7 weeks, 8 weeks, 9 weeks, or 10 weeks after administration of the composition comprising AAV vectors). In certain embodiments, the corticosteroid treatment may be administered prior to (e.g., the day prior to administration, or 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 8 days, 9 days, 10 days, 11 days, 12 days, 13 days, or 14 days prior to administration) and after administration of the composition comprising AAV vectors (e.g., 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 7 weeks, 8 weeks, 9 weeks, or 10 weeks after administration). For example,

the corticosteroid treatment may be administered beginning 3 days prior to until 6 weeks after administration of the AAV vector.

Suitable doses of corticosteroids may include, for example, 0.1 mg/kg/day to 10 mg/kd/day (e.g., 0.1 mg/kg/day, 0.2 mg/kg/day, 0.3 mg/kg/day, 0.4 mg/kg/day, 0.5 mg/kg/day, 0.6 mg/kg/day, 0.7 mg/kg/day, 0.8 mg/kg/day, 0.9 mg/kg/day, or 1.0 mg/kg/day). In certain embodiments, the corticosteroid may be administered at an elevated dose during the corticosteroid treatment, followed by a tapered dose of the corticosteroid. For example, 0.5 mg/kg/day corticosteroid may be administered for 4 weeks, followed by a 15-day taper (0.4 mg/kg/day for 5 days, and then 0.2 mg/kg/day for 5 days, and then 0.1 mg/kg/day for 5 days). The corticosteroid dose may be increased if there is an increase in vitreous inflammation by 1+ on the grading scale following surgery (e.g., within 4 weeks after surgery). For example, if there is an increase in vitreous inflammation by 1+ on the grading scale while the patient is receiving a 0.5 mg/kg/day dose (e.g., within 4 weeks after surgery), the corticosteroid dose may be may be increased to 1 mg/kg/day. If any inflammation is present within 4 weeks after surgery, the taper may be delayed.

For pre-clinical development purposes, systems, compositions, nucleotides and vectors according to this disclosure can be evaluated ex vivo using a retinal explant system, or in vivo using an animal model such as a mouse, rabbit, pig, nonhuman primate, etc. Retinal explants are optionally maintained on a support matrix, and AAV vectors can be delivered by injection into the space between the photoreceptor layer and the support matrix, to mimic subretinal injection. Tissue for retinal explantation can be obtained from human or animal subjects, for example mouse.

Explants are particularly useful for studying the expression of gRNAs, RNA-guided nucleases, and rhodopsin protein following viral transduction, and for studying genome editing over comparatively short intervals. These models also permit higher throughput than may be possible in animal models and can be predictive of expression and genome editing in animal models and subjects. Small (mouse, rat) and large animal models (such as rabbit, pig, nonhuman primate) can be used for pharmacological and/or toxicological studies and for testing the systems, nucleotides, vectors and compositions of this disclosure under conditions and at volumes that approximate those that will be used in clinic. Because model systems are selected to recapitulate relevant aspects of human anatomy and/or physiology, the data obtained in these systems will generally (though not necessarily) be predictive of the behavior of AAV vectors and compositions according to this disclosure in human and animal subjects.

DNA-based Delivery of an RNA-guided nuclease molecule, a gRNA molecule, and/or a *RHO* expression cassette

DNA encoding RNA-guided nuclease molecules (e.g., Cas9 or Cpf1 molecules), gRNA molecules, and/or *RHO* cDNA molecules can be administered to subjects or delivered into cells by art-known methods or as described herein. For example, RNA-guided nuclease (e.g., Cas9 or Cpf1) encoding DNA, gRNA-encoding DNA, and/or *RHO* cDNA can be delivered, e.g., by vectors (e.g., viral or non-viral vectors), non-vector based methods (e.g., using naked DNA or DNA complexes), or a combination thereof.

In some embodiments, the RNA-guided nuclease (e.g., Cas9 or Cpf1)-encoding DNA, gRNA-encoding DNA, and/or *RHO* cDNA is delivered by a vector (e.g., viral vector/virus or plasmid).

A vector can comprise a sequence that encodes an RNA-guided nuclease-encoding DNA, gRNA-encoding DNA, and/or *RHO* cDNA molecule. A vector can also comprise a sequence encoding a signal peptide (*e.g.*, for nuclear localization, nucleolar localization, mitochondrial localization), fused, e.g., to an RNA-guided nuclease sequence. For example, a vector can comprise a nuclear localization sequence (e.g., from SV40) fused to the sequence encoding the RNA-guided nuclease (e.g., Cas9 or Cpf1) molecule.

One or more regulatory/control elements, e.g., a promoter, an enhancer, an intron, a polyadenylation signal, a Kozak consensus sequence, internal ribosome entry sites (IRES), a 2A sequence, and splice acceptor or donor can be included in the vectors. In some embodiments, the promoter is recognized by RNA polymerase II (e.g., a CMV promoter). In other embodiments, the promoter is recognized by RNA polymerase III (e.g., a U6 promoter). In some embodiments, the promoter is a regulated promoter (e.g., inducible promoter). In other embodiments, the promoter is a constitutive promoter. In some embodiments, the promoter is a tissue specific promoter. In some embodiments, the promoter is a viral promoter. In other embodiments, the promoter is a non-viral promoter.

In some embodiments, the vector or delivery vehicle is a viral vector (e.g., for generation of recombinant viruses). In some embodiments, the virus is a DNA virus (e.g., dsDNA or ssDNA virus). In other embodiments, the virus is an RNA virus (e.g., an ssRNA virus). Exemplary viral vectors/viruses include, e.g., retroviruses, lentiviruses, adenovirus, adeno-associated virus (AAV), vaccinia viruses, poxviruses, and herpes simplex viruses.

In some embodiments, the virus infects dividing cells. In other embodiments, the virus infects non-dividing cells. In some embodiments, the virus infects both dividing and non-dividing cells. In some embodiments, the virus can integrate into the host genome. In

some embodiments, the virus is engineered to have reduced immunity, e.g., in human. In some embodiments, the virus is replication-competent. In other embodiments, the virus is replication-defective, e.g., having one or more coding regions for the genes necessary for additional rounds of virion replication and/or packaging replaced with other genes or deleted. In some embodiments, the virus causes transient expression of the RNA-guided nuclease molecule, the gRNA molecule, and/or the *RHO* cDNA molecule. In other embodiments, the virus causes long-lasting, e.g., at least 1 week, 2 weeks, 1 month, 2 months, 3 months, 6 months, 9 months, 1 year, 2 years, or permanent expression, of the RNA-guided nuclease molecule, the gRNA molecule, and/or the *RHO* cDNA molecule. The packaging capacity of the viruses may vary, e.g., from at least about 4 kb to at least about 30 kb, e.g., at least about 5 kb, 10 kb, 15 kb, 20 kb, 25 kb, 30 kb, 35 kb, 40 kb, 45 kb, or 50 kb.

In some embodiments, the RNA-guided nuclease-encoding DNA, gRNA-encoding DNA, and/or *RHO* cDNA is delivered by a recombinant retrovirus. In some embodiments, the retrovirus (e.g., Moloney murine leukemia virus) comprises a reverse transcriptase, e.g., that allows integration into the host genome. In some embodiments, the retrovirus is replication-competent. In other embodiments, the retrovirus is replication-defective, e.g., having one of more coding regions for the genes necessary for additional rounds of virion replication and packaging replaced with other genes, or deleted.

In some embodiments, the RNA-guided nuclease-encoding DNA, gRNA-encoding DNA, and/or *RHO* cDNA is delivered by a recombinant lentivirus. For example, the lentivirus is replication-defective, e.g., does not comprise one or more genes required for viral replication.

In some embodiments, the RNA-guided nuclease-encoding DNA, gRNA-encoding DNA, and/or *RHO* cDNA is delivered by a recombinant adenovirus. In some embodiments, the adenovirus is engineered to have reduced immunity in human.

In some embodiments, the RNA-guided nuclease-encoding DNA, gRNA-encoding DNA, and/or *RHO* cDNA is delivered by a recombinant AAV. In some embodiments, the AAV can incorporate its genome into that of a host cell, e.g., a target cell as described herein. In some embodiments, the AAV is a self-complementary adeno-associated virus (scAAV), e.g., a scAAV that packages both strands which anneal together to form double stranded DNA. AAV serotypes that may be used in the disclosed methods, include AAV1, AAV2, modified AAV2 (e.g., modifications at Y444F, Y500F, Y730F and/or S662V), AAV3, modified AAV3 (e.g., modifications at Y705F, Y731F and/or T492V), AAV4, AAV5, AAV6, modified AAV6 (e.g., modifications at S663V and/or T492V), AAV8, AAV 8.2,

AAV9, AAV rh 10, and pseudotyped AAV, such as AAV2/8, AAV2/5 and AAV2/6 can also be used in the disclosed methods.

In some embodiments, the RNA-guided nuclease-encoding DNA, gRNA-encoding DNA, and/or *RHO* cDNA is delivered by a hybrid virus, e.g., a hybrid of one or more of the viruses described herein.

A Packaging cell is used to form a virus particle that is capable of infecting a host or target cell. Such a cell includes a 293 cell, which can package adenovirus, and a ψ2 cell or a PA317 cell, which can package retrovirus. A viral vector used in gene therapy is usually generated by a producer cell line that packages a nucleic acid vector into a viral particle. The vector typically contains the minimal viral sequences required for packaging and subsequent integration into a host or target cell (if applicable), with other viral sequences being replaced by an expression cassette encoding the protein to be expressed. For example, an AAV vector used in gene therapy typically only possesses inverted terminal repeat (ITR) sequences from the AAV genome which are required for packaging and gene expression in the host or target cell. The missing viral functions are supplied in trans by the packaging cell line. Henceforth, the viral DNA is packaged in a cell line, which contains a helper plasmid encoding the other AAV genes, namely rep and cap, but lacking ITR sequences. The cell line is also infected with adenovirus as a helper. The helper virus promotes replication of the AAV vector and expression of AAV genes from the helper plasmid. The helper plasmid is not packaged in significant amounts due to a lack of ITR sequences. Contamination with adenovirus can be reduced by, e.g., heat treatment to which adenovirus is more sensitive than AAV.

In an embodiment, the viral vector has the ability of cell type and/or tissue type recognition. For example, the viral vector can be pseudotyped with a different/alternative viral envelope glycoprotein; engineered with a cell type-specific receptor (e.g., genetic modification of the viral envelope glycoproteins to incorporate targeting ligands such as a peptide ligand, a single chain antibody, a growth factor); and/or engineered to have a molecular bridge with dual specificities with one end recognizing a viral glycoprotein and the other end recognizing a moiety of the target cell surface (e.g., ligand-receptor, monoclonal antibody, avidin-biotin and chemical conjugation).

In an embodiment, the viral vector achieves cell type specific expression. For example, a tissue-specific promoter can be constructed to restrict expression of the transgene (Cas 9 and gRNA) in only the target cell. The specificity of the vector can also be mediated by microRNA-dependent control of transgene expression. In an embodiment, the viral vector has increased efficiency of fusion of the viral vector and a target cell membrane. For

example, a fusion protein such as fusion-competent hemagglutin (HA) can be incorporated to increase viral uptake into cells. In an embodiment, the viral vector has the ability of nuclear localization. For example, a virus that requires the breakdown of the cell wall (during cell division) and therefore will not infect a non-diving cell can be altered to incorporate a nuclear localization peptide in the matrix protein of the virus thereby enabling the transduction of non-proliferating cells.

In some embodiments, the RNA-guided nuclease-encoding DNA, gRNA-encoding DNA, and/or *RHO* cDNA is delivered by a non-vector based method (e.g., using naked DNA or DNA complexes). For example, the DNA can be delivered, e.g., by organically modified silica or silicate (Ormosil), electroporation, gene gun, sonoporation, magnetofection, lipid-mediated transfection, dendrimers, inorganic nanoparticles, calcium phosphates, or a combination thereof.

In some embodiments, the RNA-guided nuclease-encoding DNA, gRNA-encoding DNA, and/or *RHO* cDNA is delivered by a combination of a vector and a non-vector based method. For example, a virosome comprises a liposome combined with an inactivated virus (e.g., HIV or influenza virus), which can result in more efficient gene transfer, e.g., in a respiratory epithelial cell than either a viral or a liposomal method alone.

In an embodiment, the delivery vehicle is a non-viral vector. In an embodiment, the non-viral vector is an inorganic nanoparticle (e.g., attached to the payload to the surface of the nanoparticle). Exemplary inorganic nanoparticles include, e.g., magnetic nanoparticles (e.g., Fe_3MnO_2), or silica. The outer surface of the nanoparticle can be conjugated with a positively charged polymer (e.g., polyethylenimine, polylysine, polyserine) which allows for attachment (e.g., conjugation or entrapment) of payload. In an embodiment, the non-viral vector is an organic nanoparticle (e.g., entrapment of the payload inside the nanoparticle). Exemplary organic nanoparticles include, e.g., SNALP liposomes that contain cationic lipids together with neutral helper lipids which are coated with polyethylene glycol (PEG) and protamine and nucleic acid complex coated with lipid coating.

Exemplary lipids for gene transfer are shown below in **Table 15**.

Table 15: Lipids Used for Gene Transfer

Lipid	Abbreviation	Feature
1,2-Dioleoyl-sn-glycero-3-phosphatidylcholine	DOPC	Helper
1,2-Dioleoyl-sn-glycero-3-phosphatidylethanolamine	DOPE	Helper
Cholesterol		Helper
N-[1-(2,3-Dioleyloxy)prophyl]*N,N,N*-trimethylammonium chloride	DOTMA	Cationic
1,2-Dioleoyloxy-3-trimethylammonium-propane	DOTAP	Cationic
Dioctadecylamidoglycylspermine	DOGS	Cationic
N-(3-Aminopropyl)-*N,N*-dimethyl-2,3-bis(dodecyloxy)-1-propanaminium bromide	GAP-DLRIE	Cationic
Cetyltrimethylammonium bromide	CTAB	Cationic
6-Lauroxyhexyl ornithinate	LHON	Cationic
1-(2,3-Dioleoyloxypropyl)-2,4,6-trimethylpyridinium	2Oc	Cationic
2,3-Dioleyloxy-*N*-[2(sperminecarboxamido-ethyl]-*N,N*-dimethyl-1-propanaminium trifluoroacetate	DOSPA	Cationic
1,2-Dioleyl-3-trimethylammonium-propane	DOPA	Cationic
N-(2-Hydroxyethyl)-*N,N*-dimethyl-2,3-bis(tetradecyloxy)-1-propanaminium bromide	MDRIE	Cationic
Dimyristooxypropyl dimethyl hydroxyethyl ammonium bromide	DMRI	Cationic
3β-[*N*-(*N',N'*-Dimethylaminoethane)-carbamoyl]cholesterol	DC-Chol	Cationic
Bis-guanidium-tren-cholesterol	BGTC	Cationic
1,3-Diodeoxy-2-(6-carboxy-spermyl)-propylamide	DOSPER	Cationic
Dimethyloctadecylammonium bromide	DDAB	Cationic
Dioctadecylamidoglicylspermidin	DSL	Cationic
rac-[(2,3-Dioctadecyloxypropyl)(2-hydroxyethyl)]-dimethylammonium chloride	CLIP-1	Cationic
rac-[2(2,3-Dihexadecyloxypropyl-oxymethyloxy)ethyl]trimethylammonium bromide	CLIP-6	Cationic
Ethyldimyristoylphosphatidylcholine	EDMPC	Cationic
1,2-Distearyloxy-*N,N*-dimethyl-3-aminopropane	DSDMA	Cationic
1,2-Dimyristoyl-trimethylammonium propane	DMTAP	Cationic
O,O'-Dimyristyl-N-lysyl aspartate	DMKE	Cationic
1,2-Distearoyl-sn-glycero-3-ethylphosphocholine	DSEPC	Cationic
N-Palmitoyl D-erythro-sphingosyl carbamoyl-spermine	CCS	Cationic
N-t-Butyl-*N*0-tetradecyl-3-tetradecylaminopropionamidine	diC14-amidine	Cationic
Octadecenolyoxy[ethyl-2-heptadecenyl-3 hydroxyethyl] imidazolinium chloride	DOTIM	Cationic
*N*1-Cholesteryloxycarbonyl-3,7-diazanonane-1,9-diamine	CDAN	Cationic
2-(3-[Bis(3-amino-propyl)-amino]propylamino)-*N*-ditetradecylcarbamoylme-ethyl-acetamide	RPR209120	Cationic

Exemplary polymers for gene transfer are shown below in **Table 16**.

Table 16: Polymers Used for Gene Transfer

Polymer	Abbreviation
Poly(ethylene)glycol	PEG
Polyethylenimine	PEI
Dithiobis(succinimidylpropionate)	DSP
Dimethyl-3,3'-dithiobispropionimidate	DTBP
Poly(ethylene imine) biscarbamate	PEIC
Poly(L-lysine)	PLL
Histidine modified PLL	
Poly(*N*-vinylpyrrolidone)	PVP
Poly(propylenimine)	PPI
Poly(amidoamine)	PAMAM
Poly(amido ethylenimine)	SS-PAEI
Triethylenetetramine	TETA
Poly(β-aminoester)	
Poly(4-hydroxy-L-proline ester)	PHP
Poly(allylamine)	
Poly(α-[4-aminobutyl]-L-glycolic acid)	PAGA
Poly(D,L-lactic-co-glycolic acid)	PLGA
Poly(*N*-ethyl-4-vinylpyridinium bromide)	
Poly(phosphazene)s	PPZ
Poly(phosphoester)s	PPE
Poly(phosphoramidate)s	PPA
Poly(*N*-2-hydroxypropylmethacrylamide)	pHPMA
Poly (2-(dimethylamino)ethyl methacrylate)	pDMAEMA
Poly(2-aminoethyl propylene phosphate)	PPE-EA
Chitosan	
Galactosylated chitosan	
N-Dodacylated chitosan	
Histone	
Collagen	
Dextran-spermine	D-SPM

In an embodiment, the vehicle has targeting modifications to increase target cell update of nanoparticles and liposomes, e.g., cell specific antigens, monoclonal antibodies, single chain antibodies, aptamers, polymers, sugars, and cell penetrating peptides. In an embodiment, the vehicle uses fusogenic and endosome-destabilizing peptides/polymers. In an embodiment, the vehicle undergoes acid-triggered conformational changes (e.g., to accelerate endosomal escape of the cargo). In an embodiment, a stimuli-cleavable polymer is used, e.g., for release in a cellular compartment. For example, disulfide-based cationic polymers that are cleaved in the reducing cellular environment can be used.

In an embodiment, the delivery vehicle is a biological non-viral delivery vehicle. In an embodiment, the vehicle is an attenuated bacterium (e.g., naturally or artificially engineered to be invasive but attenuated to prevent pathogenesis and expressing the transgene (e.g., *Listeria monocytogenes*, certain *Salmonella strains*, *Bifidobacterium longum*, and modified *Escherichia coli*), bacteria having nutritional and tissue-specific tropism to target specific tissues, bacteria having modified surface proteins to alter target tissue specificity). In an embodiment, the vehicle is a genetically modified bacteriophage (e.g., engineered phages having large packaging capacity, less immunogenic, containing mammalian plasmid maintenance sequences and having incorporated targeting ligands). In an embodiment, the vehicle is a mammalian virus-like particle. For example, modified viral particles can be generated (e.g., by purification of the "empty" particles followed by *ex vivo* assembly of the virus with the desired cargo). The vehicle can also be engineered to incorporate targeting ligands to alter target tissue specificity. In an embodiment, the vehicle is a biological liposome. For example, the biological liposome is a phospholipid-based particle derived from human cells (e.g., erythrocyte ghosts, which are red blood cells broken down into spherical structures derived from the subject (e.g., tissue targeting can be achieved by attachment of various tissue or cell-specific ligands), or secretory exosomes –subject (i.e., patient) derived membrane-bound nanovesicle (30 -100 nm) of endocytic origin (e.g., can be produced from various cell types and can therefore be taken up by cells without the need of for targeting ligands).

In an embodiment, one or more nucleic acid molecules (e.g., DNA molecules) other than the components of an RNA-guided nuclease system, e.g., the Cas9 or Cpf1 molecule component, the gRNA molecule component, and/or the *RHO* cDNA molecule component described herein, are delivered. In an embodiment, the nucleic acid molecule is delivered at the same time as one or more of the components of the RNA-guided nuclease system are delivered. In an embodiment, the nucleic acid molecule is delivered before or after (e.g., less than about 30 minutes, 1 hour, 2 hours, 3 hours, 6 hours, 9 hours, 12 hours, 1 day, 2 days, 3 days, 1 week, 2 weeks, or 4 weeks) one or more of the components of the RNA-guided nuclease system are delivered. In an embodiment, the nucleic acid molecule is delivered by a different means than one or more of the components of the RNA-guided nuclease system, e.g., the Cas9 or Cpf1 molecule component, the gRNA molecule component, and/or the *RHO* cDNA molecule component are delivered. The nucleic acid molecule can be delivered by any of the delivery methods described herein. For example, the nucleic acid molecule can be delivered by a viral vector, e.g., an integration-deficient lentivirus, and the RNA-guided

nuclease molecule component, the gRNA molecule component, and/or the *RHO* cDNA molecule component can be delivered by electroporation, e.g., such that the toxicity caused by nucleic acids (e.g., DNAs) can be reduced. In an embodiment, the nucleic acid molecule encodes a therapeutic protein, e.g., a protein described herein. In an embodiment, the nucleic acid molecule encodes an RNA molecule, e.g., an RNA molecule described herein.

Delivery of RNA encoding an RNA-guided nuclease molecule

RNA encoding RNA-guided nuclease molecules (e.g., Cas9 or Cpf1 molecules described herein), gRNA molecules, and/or *RHO* cDNA molecules can be delivered into cells, e.g., target cells described herein, by art-known methods or as described herein. For example, RNA-guided nuclease molecules (e.g., Cas9 or Cpf1 molecules described herein), gRNA molecules, and/or *RHO* cDNA molecules can be delivered, e.g., by microinjection, electroporation, lipid-mediated transfection, peptide-mediated delivery, or a combination thereof.

Delivery RNA-guided nuclease molecule protein

RNA-guided nuclease molecules (e.g., Cas9 or Cpf1 molecules described herein) can be delivered into cells by art-known methods or as described herein. For example, RNA-guided nuclease protein molecules can be delivered, e.g., by microinjection, electroporation, lipid-mediated transfection, peptide-mediated delivery, or a combination thereof. Delivery can be accompanied by DNA encoding a gRNA and/or *RHO* cDNA or by a gRNA and/or *RHO* cDNA.

Routes of Administration

Systemic modes of administration include oral and parenteral routes. Parenteral routes include, by way of example, intravenous, intraarterial, intraosseous, intramuscular, intradermal, subcutaneous, intranasal and intraperitoneal routes. Components administered systemically may be modified or formulated to target the components to the eye.

Local modes of administration include, by way of example, intraocular, intraorbital, subconjuctival, intravitreal, subretinal or transscleral routes. In an embodiment, significantly smaller amounts of the components (compared with systemic approaches) may exert an effect when administered locally (for example, intravitreally) compared to when administered systemically (for example, intravenously). Local modes of administration can reduce or eliminate the incidence of potentially toxic side effects that may occur when therapeutically

effective amounts of a component are administered systemically.

In an embodiment, components described herein are delivered by subretinally, e.g., by subretinal injection. Subretinal injections may be made directly into the macular, e.g., submacular injection.

In an embodiment, components described herein are delivered by intravitreal injection. Intravitreal injection has a relatively low risk of retinal detachment risk. In an embodiment, nanoparticle or viral, e.g., AAV vector, e.g., an AAV5 vector, e.g., a modified AAV5 vector, an AAV2 vector, e.g., a modified AAV2 vector, is delivered intravitreally.

Methods for administration of agents to the eye are known in the medical arts and can be used to administer components described herein. Exemplary methods include intraocular injection (e.g., retrobulbar, subretinal, submacular, intravitreal and intrachoridal), iontophoresis, eye drops, and intraocular implantation (e.g., intravitreal, sub-Tenons and sub-conjunctival).

Administration may be provided as a periodic bolus (for example, subretinally, intravenously or intravitreally) or as continuous infusion from an internal reservoir (for example, from an implant disposed at an intra- or extra-ocular location (see, U.S. Pat. Nos. 5,443,505 and 5,766,242)) or from an external reservoir (for example, from an intravenous bag). Components may be administered locally, for example, by continuous release from a sustained release drug delivery device immobilized to an inner wall of the eye or via targeted transscleral controlled release into the choroid (see, for example, PCT/US00/00207, PCT/US02/14279, Ambati 2000a, and Ambati 2000b. A variety of devices suitable for administering components locally to the inside of the eye are known in the art. See, for example, U.S. Pat. Nos. 6,251,090, 6,299,895, 6,416,777, 6,413,540, and PCT/US00/28187.

In addition, components may be formulated to permit release over a prolonged period of time. A release system can include a matrix of a biodegradable material or a material which releases the incorporated components by diffusion. The components can be homogeneously or heterogeneously distributed within the release system. A variety of release systems may be useful, however, the choice of the appropriate system will depend upon rate of release required by a particular application. Both non-degradable and degradable release systems can be used. Suitable release systems include polymers and polymeric matrices, non-polymeric matrices, or inorganic and organic excipients and diluents such as, but not limited to, calcium carbonate and sugar (for example, trehalose). Release systems may be natural or synthetic. However, synthetic release systems are preferred because generally they are more reliable, more reproducible and produce more defined release profiles. The release

system material can be selected so that components having different molecular weights are released by diffusion through or degradation of the material.

Representative synthetic, biodegradable polymers include, for example: polyamides such as poly(amino acids) and poly(peptides); polyesters such as poly(lactic acid), poly(glycolic acid), poly(lactic-co-glycolic acid), and poly(caprolactone); poly(anhydrides); polyorthoesters; polycarbonates; and chemical derivatives thereof (substitutions, additions of chemical groups, for example, alkyl, alkylene, hydroxylations, oxidations, and other modifications routinely made by those skilled in the art), copolymers and mixtures thereof. Representative synthetic, non-degradable polymers include, for example: polyethers such as poly(ethylene oxide), poly(ethylene glycol), and poly(tetramethylene oxide); vinyl polymers-polyacrylates and polymethacrylates such as methyl, ethyl, other alkyl, hydroxyethyl methacrylate, acrylic and methacrylic acids, and others such as poly(vinyl alcohol), poly(vinyl pyrolidone), and poly(vinyl acetate); poly(urethanes); cellulose and its derivatives such as alkyl, hydroxyalkyl, ethers, esters, nitrocellulose, and various cellulose acetates; polysiloxanes; and any chemical derivatives thereof (substitutions, additions of chemical groups, for example, alkyl, alkylene, hydroxylations, oxidations, and other modifications routinely made by those skilled in the art), copolymers and mixtures thereof.

Poly(lactide-co-glycolide) microsphere can also be used for intraocular injection. Typically the microspheres are composed of a polymer of lactic acid and glycolic acid, which are structured to form hollow spheres. The spheres can be approximately 15-30 microns in diameter and can be loaded with components described herein.

Bi-Modal or Differential Delivery of Components

Separate delivery of the components of an RNA-guided nuclease system, e.g., the RNA-guided nuclease molecule component (e.g., Cas9 or Cpf1 molecule component), the gRNA molecule component, and the *RHO* cDNA molecule component, and more particularly, delivery of the components by differing modes, can enhance performance, e.g., by improving tissue specificity and safety.

In an embodiment, the RNA-guided nuclease molecule component, the gRNA molecule component, and the *RHO* cDNA molecule component, are delivered by different modes, or as sometimes referred to herein as differential modes. Different or differential modes, as used herein, refer modes of delivery that confer different pharmacodynamic or pharmacokinetic properties on the subject component molecule, e.g., n RNA-guided nuclease molecule, gRNA molecule, or *RHO* cDNA molecule. For example, the modes of delivery

can result in different tissue distribution, different half-life, or different temporal distribution, e.g., in a selected compartment, tissue, or organ.

Some modes of delivery, e.g., delivery by a nucleic acid vector that persists in a cell, or in progeny of a cell, e.g., by autonomous replication or insertion into cellular nucleic acid, result in more persistent expression of and presence of a component. Examples include viral, e.g., adeno- associated virus or lentivirus, delivery.

By way of example, the components, e.g., an RNA-guided nuclease molecule, a gRNA molecule, and a *RHO* cDNA molecule can be delivered by modes that differ in terms of resulting half-life or persistent of the delivered component the body, or in a particular compartment, tissue or organ. In an embodiment, a gRNA molecule can be delivered by such modes. The RNA-guided nuclease molecule component can be delivered by a mode which results in less persistence or less exposure to the body or a particular compartment or tissue or organ. The *RHO* cDNA molecule component may be delivered by a mode that difference from that mode of the gRNA molecule component and the RNA-guided nuclease molecule component.

More generally, in an embodiment, a first mode of delivery is used to deliver a first component and a second mode of delivery is used to deliver a second component. The first mode of delivery confers a first pharmacodynamic or pharmacokinetic property. The first pharmacodynamic property can be, e.g., distribution, persistence, or exposure, of the component, or of a nucleic acid that encodes the component, in the body, a compartment, tissue or organ. The second mode of delivery confers a second pharmacodynamic or pharmacokinetic property. The second pharmacodynamic property can be, e.g., distribution, persistence, or exposure, of the component, or of a nucleic acid that encodes the component, in the body, a compartment, tissue or organ.

In an embodiment, the first pharmacodynamic or pharmacokinetic property, e.g., distribution, persistence or exposure, is more limited than the second pharmacodynamic or pharmacokinetic property.

In an embodiment, the first mode of delivery is selected to optimize, e.g., minimize, a pharmacodynamic or pharmacokinetic property, e.g., distribution, persistence or exposure.

In an embodiment, the second mode of delivery is selected to optimize, e.g., maximize, a pharmacodynamic or pharmcokinetic property, e.g., distribution, persistence or exposure.

In an embodiment, the first mode of delivery comprises the use of a relatively persistent element, e.g., a nucleic acid, e.g., a plasmid or viral vector, e.g., an AAV or

lentivirus. As such vectors are relatively persistent product transcribed from them would be relatively persistent.

In an embodiment, the second mode of delivery comprises a relatively transient element, e.g., an RNA or protein.

In an embodiment, the first component comprises gRNA, and the delivery mode is relatively persistent, e.g., the gRNA is transcribed from a plasmid or viral vector, e.g., an AAV or lentivirus. Transcription of these genes would be of little physiological consequence because the genes do not encode for a protein product, and the gRNAs are incapable of acting in isolation. The second component, an RNA-guided nuclease molecule, is delivered in a transient manner, for example as mRNA or as protein, ensuring that the full RNA-guided nuclease molecule/gRNA molecule complex is only present and active for a short period of time.

Furthermore, the components can be delivered in different molecular form or with different delivery vectors that complement one another to enhance safety and tissue specificity.

Use of differential delivery modes can enhance performance, safety and efficacy. E.g., the likelihood of an eventual off-target modification can be reduced. Delivery of immunogenic components, e.g., RNA-guided nuclease molecules, by less persistent modes can reduce immunogenicity, as peptides from the bacterially-derived Cas enzyme are displayed on the surface of the cell by MHC molecules. A two-part delivery system can alleviate these drawbacks.

Differential delivery modes can be used to deliver components to different, but overlapping target regions. The formation active complex is minimized outside the overlap of the target regions. Thus, in an embodiment, a first component, e.g., a gRNA molecule is delivered by a first delivery mode that results in a first spatial, e.g., tissue, distribution. A second component, e.g., an RNA-guided nuclease molecule is delivered by a second delivery mode that results in a second spatial, e.g., tissue, distribution. In an embodiment, the first mode comprises a first element selected from a liposome, nanoparticle, e.g., polymeric nanoparticle, and a nucleic acid, e.g., viral vector. The second mode comprises a second element selected from the group. In an embodiment, the first mode of delivery comprises a first targeting element, e.g., a cell specific receptor or an antibody, and the second mode of delivery does not include that element. In embodiment, the second mode of delivery comprises a second targeting element, e.g., a second cell specific receptor or second antibody.

When the RNA-guided nuclease molecule is delivered in a virus delivery vector, a liposome, or polymeric nanoparticle, there is the potential for delivery to and therapeutic activity in multiple tissues, when it may be desirable to only target a single tissue. A two-part delivery system can resolve this challenge and enhance tissue specificity. If the gRNA molecule and the RNA-guided nuclease molecule are packaged in separated delivery vehicles with distinct but overlapping tissue tropism, the fully functional complex is only formed in the tissue that is targeted by both vectors.

Ex vivo delivery

In some embodiments, components described in **Table 8** are introduced into cells which are then introduced into the subject. Methods of introducing the components can include, e.g., any of the delivery methods described in **Table 9**.

VIII. Modified Nucleosides, Nucleotides, and Nucleic Acids

In some embodiments of the present disclosure, modified nucleosides and/or modified nucleotides can be present in nucleic acids, e.g., in a gRNA molecule provided herein. Some exemplary nucleoside, nucleotide, and nucleic acid modifications useful in the context of the present RNA-guided nuclease technology are provided herein, and the skilled artisan will be able to ascertain additional suitable modifications that can be used in conjunction with the nucleosides, nucleotides, and nucleic acids and treatment modalities disclosed herein based on the present disclosure. Suitable nucleoside, nucleotide, and nucleic acid modifications include, without limitation, those described in U.S. Patent Application No. US 2017/0073674 A1 and International Publication No. WO 2017/165862 A1, the entire contents of each of which are incorporated by reference herein.

Examples

The following Examples are merely illustrative and are not intended to limit the scope or content of the disclosure in any way.

Example 1: Screening of gRNAs for editing *RHO* alleles in T cells

Approximately 430 gRNAs targeting various positions within the *RHO* gene for use with Cas9 were designed and screened for editing activity in T cells. Briefly, SA Cas9 and guide RNA were complexed at a 1:2 ratio (RNP complex) and delivered to T cells via

electroporation. Three days after electroporation, gDNA was extracted from T cells and the target site was PCR amplified from the gDNA. Sequencing analysis of the *RHO* PCR gene product was evaluated by next generation sequencing (NGS). **Table 18** below provides the RNA and DNA sequences of the targeting domains of the gRNAs that exhibited > 0.1% editing in T cells. These data indicate that gRNA comprising targeting domains set forth in **Table 18** and Cas9 support editing of the *RHO* gene.

Example 2: Dose-dependent editing of *RHO* alleles in HEK293 cells

Three gRNAs whose target sites are predicted to be within exon 1 or exon 2 of the *RHO* gene, RHO-3, RHO-7, and RHO-10 (**Table 17**), were selected for further optimization and testing for dose-dependent editing with Cas9. Briefly, increasing concentrations of control plasmid (expressing Cas9 with scrambled gRNA that does not target a sequence within the human genome) or plasmids expressing Cas9 and gRNA were delivered to HEK293 cells by electroporation. Three days after electroporation, gDNA was extracted from HEK293 cells and the gRNA target site was PCR amplified from the gDNA. Sequencing analysis of the *RHO* PCR gene product was evaluated by NGS. The increasing concentration of Cas9/gRNA plasmid supported an increase in indels at the *RHO* gene to 80% (**Fig. 4**). Sequencing analysis indicated that increasing the plasmid concentration resulted in an increase in indels.

Table 17: gRNAs Targeting *RHO* Gene

gRNA ID	Targeting Domain (RNA)	Targeting Domain (DNA)/ Protospacer
RHO-3	AGUAUCCAUGCAGAGAGGUGUA (SEQ ID NO:102)	AGTATCCATGCAGAGAGGTGTA (SEQ ID NO:602)
RHO-7	CCCACACCCGGCUCAUACCGCC (SEQ ID NO:106)	CCCACACCCGGCTCATACCGCC (SEQ ID NO:606)
RHO-10	GUGCCAUUACCUGGACCAGCCG (SEQ ID NO:109)	GTGCCATTACCTGGACCAGCCG (SEQ ID NO:609)

Specificity of the gRNA (*i.e.*, RHO-3, RHO-7, RHO-10) and Cas9 ribonucleoprotein complexes was evaluated using two different assays that are well-known to skilled artisans for profiling CRISPR-Cas9 specificity, the Digenome-seq (digested genome sequencing) and GUIDE-seq assays. No apparent off target editing was detected under physiological conditions for RNP comprising RHO-3, RHO-7, or RHO-10 gRNA complexed with Cas9 (data not shown).

Example 3: Characterization of novel *RHO* alleles generated by simulation of on-targeted editing by RHO-3, RHO-7, and RHO-10 gRNAs

The cut sites generated by on-targeted editing of RHO-3, RHO-7, or RHO-10 gRNA (see targeting domains in **Table 17**) of *RHO* alleles were predicted. **Fig. 5** illustrates the predicted cutting locations of RHO-3, RHO-7, or RHO-10 gRNAs on the *RHO* human cDNA and resulting lengths of RHO protein. RHO-3 is predicted to target Exon 1, RHO-10 is predicted to target the boundary of Exon 2 and Intron 2, and RHO-7 is predicted to target the boundary of Exon 1 and Intron 1 of *RHO* cDNA. Deletions of 1 or 2 base pairs at the RHO-3, RHO-10, or RHO-7 target sites are predicted to cause frameshifts in the *RHO* cDNA resulting in abnormal RHO proteins. **Fig. 6** shows schematics of the predicted *RHO* alleles resulting from editing by RHO-3, RHO-10, or RHO-7 gRNAs.

The effects of the alleles generated by on-targeted editing by RHO-3, RHO-7, or RHO-10 gRNA were characterized to determine whether editing using these gRNAs could result in potentially deleterious *RHO* alleles. Briefly, wild-type (WT) or mock-edited *RHO* alleles were cloned into mammalian expression plasmids under the control of a CMV promoter and lipofected into HEK293 cells. Mock-edited *RHO* alleles included each of the mutated alleles shown in **Fig. 6** (i.e., RHO-3 (-1, -2, or -3 bp), RHO-10 (-1, -2, or -3 bp), or RHO-7 (-1 bp, -2 bp, -3 bp)). The well-known P23H *RHO* variant leading to a dominant form of retinitis pigmentosa was also cloned and tested. After 48 hours of overexpression, cell viability for WT and each mock-edited allele was assessed using ATPLite Luminescence Assay (Perkin Elmer).

While WT RHO overexpression induced relatively no cytotoxicity with respect to the vector control (pUC19 plasmid, upper dotted line), P23H RHO resulted in 50% cell death (lower dotted line), as expected (**Fig. 7A**). Furthermore, expression of the frameshifting of one- or two-base pair deletions at the RHO-3, RHO-7, or RHO-10 gRNA target sites did not induce significant loss in cell viability with respect to WT RHO (**Fig. 7A,** *see* RHO-3 1 and 2 bp del; RHO-10 1 and 2 bp del; and RHO-7 1 and 2 bp del). However, for in-frame three-base pair deletions at RHO-3 and RHO-10 target sites, there was a significant loss in cell viability, resulting in levels of cell death comparable to that of P23H RHO (**Fig. 7A,** *see* RHO-3 3 bp del and RHO-10 3 bp del). This was not the case for all gRNAs as a three-base pair deletion at the RHO-7 sequence resulted in a non-cytotoxic RHO allele (**Fig. 7A,** *see* RHO-7 3bp del).

Next, to determine whether the RHO-3, RHO-7, and RHO-10 mock-edited *RHO* alleles could reduce toxicity of the P23H variant of *RHO*, mock-edited RHO-3, RHO-7, and RHO-10 *RHO* alleles shown in **Fig. 6** and containing the P23H mutation were cloned into mammalian expression plasmids under the control of a CMV promoter and lipofected into HEK293 cells. After 48 hours of overexpression, cell viability for WT and each mock-edited allele was assessed using ATPLite Luminescence Assay (Perkin Elmer).

Expression of the frameshifting of one- or two-base pair deletions at the RHO-3, RHO-7, or RHO-10 gRNA target sites reduced toxicity of the P23H variant of *RHO* and did not induce significant loss in cell viability with respect to WT RHO (**Fig. 7B,** *see* RHO-3 1 and 2 bp del, RHO-10 1 and 2 bp del and RHO-7 1 and 2 bp del). The in-frame three-base pair deletions at RHO-3 and RHO-10 target sites did not reduce toxicity of the P23H variant of *RHO* as there was a significant loss in cell viability, resulting in levels of cell death comparable to that of P23H RHO (**Fig. 7B,** *see* RHO-3 3 bp del and RHO-10 3 bp del). However, the three-base pair deletion at the RHO-7 target sequence reduced toxicity of the P23H variant of *RHO* and resulted in a non-cytotoxic RHO allele (**Fig. 7B,** *see* RHO-7 3bp del).

These data indicate that out-of-frame *RHO* edits produced by RHO-3, RHO-7, or RHO-10 gRNA were productive and non-toxic while the effect of in-frame edits were gRNA/locus dependent.

Example 4: Editing of non-human primate explants by ribonucleoproteins comprising Cas9 and gRNA targeting the *RHO* gene

The ability of ribonucleoproteins comprising RHO-9 gRNA targeting the *RHO* gene and Cas9 to edit explants from non-human primates (NHP) was assessed. The RHO-9 gRNA (comprising the targeting domain sequence set forth in SEQ ID NO:108 (RNA) (SEQ ID NO:608 (DNA), **Table 1**) is cross-reactive and can edit both human and NHP *RHO* sequences.

Briefly, retinal explants from NHP donors were harvested and transferred to a membrane on a trans-well chamber in a 24 well plate. 300 µl of retinal media was added to the 24 well plate (i.e., Neurobasal-A media (no phenol red) (470 mL) containing B27 (with VitA) 50X (20 mL), Antibiotic- Antimycotic (5 mL), and GlutaMAX 1% (5 mL)). Transduction with dual AAV comprising RHO-9 gRNA, SA Cas9, and Replacement RHO occurred after 24-48 hours. AAVs were diluted to the desired titer (10^{12} vg/ml)) with the retinal media to obtain the final concentration in a total of 100 µl. The diluted/titered AAV

was added dropwise on top of the explant in the 24 well plate. 300 µl of retinal media was replenished every 72 hours. After 2-4 weeks, explants were lysed to obtain DNA, RNA and protein for molecular biology analysis. To measure the percentage of rods in the explants, a rod-specific mRNA (neural retina leucine zipper (NRL)) was extracted from the explants and measured. The housekeeping RNA (beta actin (ACTB)) was also measured to determine the total number of cells.

As shown in **Fig. 8**, each data point represents a single explant, which can contain differing numbers of rod photoreceptors. The x-axis shows the delta between ACTB and NRL RNA levels as measured by RT-qPCR, which is a measure for the percentage of rods in the explant at the time of lysing the explants. A correlation between significant editing and high percentage of rods was shown, demonstrating that robust editing levels can be achieved in explants with a substantial number of rods (**Fig. 8**). These data show that gRNA targeting RHO can efficiently edit non-human primate explants.

Example 5: Optimization of RHO replacement vector

Various components of the RHO replacement vector (e.g., promoter, UTRs, RHO sequence) were optimized to identify the optimal RHO replacement vector for maximal expression of RHO mRNA and RHO protein. First, a dual luciferase system was designed to test the impact that different lengths of the RHO promoter have on RHO expression. The components of the luciferase system included a Renilla luciferase driven by CMV in the backbone to normalize for plasmid concentrations and transfection efficiencies (**Fig. 9**).

Briefly, plasmids containing different lengths of the RHO promoter and the *RHO* gene tagged with a firefly luciferase separated by a self-cleaving T2A peptide (100 ng/10,000 cells) were transfected into HEK293 cells along with a plasmid expressing NRL, CRX, and NONo (100 ng/10,000) to turn on expression from the RHO promoters (see Yadav 2014, the entire contents of which are incorporated herein by reference). 72 hours later the cells were lysed and both transfection efficiency (Firefly) and experimental variable (NanoLuc) were analyzed. The Nano-Glo® Dual-Luciferase® Reporter Assay System (Promega Corporation, Cat# N1521) was used to measure luminescence. Luminescence from both Firefly and NanoLuc were measured. As shown in **Fig. 10**, promoters of different lengths were shown to be functional, including the minimal 250 bp RHO promoter (SEQ ID NO:44).

Next, varying 3' UTRs were tested to determine whether 3' UTRs can improve expression of RHO mRNA and RHO protein. Briefly, 3' UTRs from highly stable transcripts and genes were cloned downstream of CMV RHO (i.e., HBA1 3' UTR (SEQ ID NO:38),

short HBA1 3' UTR (SEQ ID NO:39), TH 3' UTR (SEQ ID NO:40), COL1A1 3'UTR (SEQ ID NO:41), ALOX15 3'UTR (SEQ ID NO:42), and minUTR (SEQ ID NO:56)). Vectors (500 ng) were transfected into HEK293 cells (80,000 cells/well). 72 hours later the cells were lysed, and RHO mRNA and protein expression levels were determined using RHO RT-qPCR and RHO ELISA assays, respectively. **Fig. 11A** shows that incorporation of 3' UTRs from stable transcripts into the RHO replacement vector improved RHO mRNA expression levels. **Fig. 11B** shows that incorporation of 3' UTRs from stable transcripts into the RHO replacement vector also improved RHO protein expression levels.

Next, incorporation of sequences of RHO introns 1, 2, 3, or 4 were added to RHO cDNA (i.e., SEQ ID NOs:4-7, respectively) in the RHO replacement vector to determine the impact on RHO protein expression. Vectors (500 and 250 ng) were transfected into HEK293 cells (80,000/well). 72 hours later the cells were lysed, and RHO protein expression was determined using RHO ELISA. **Fig. 12** shows that addition of introns affects RHO protein expression.

Lastly, different codon optimized RHO cDNA constructs (i.e., SEQ ID NOs:13-18) were tested to determine the impact of codon optimization on RHO expression. Vectors (500 and 250 ng) were transfected into HEK293 cells (80,000/well). 72 hours later the cells were lysed and RHO protein expression was determined using a RHO ELISA. **Fig. 13** shows that codon optimization of the RHO cDNA impacts RHO protein expression.

Example 6: *In vivo* editing using self-limiting Cas9 vector system to reduce Cas9 levels after successful editing

The ability of a dual vector system expressing Cas9 and gRNAs to edit the RHO genome and to render Cas9 vector expression non-functional was tested *in vivo*. The self-limiting vector system has previously been published (see WO2018/106693, published on June 14, 2018, and entitled Systems and Methods for One-Shot guide RNA (ogRNA) Targeting of Endogenous and Source DNA, the entire contents of which are incorporated herein by reference). Briefly, a Cas9 vector system was generated in which the Cas9 vector comprised a target site for the RHO gRNA within the Cas9 cDNA (SD Cas9). Six weeks after administration of the SD Cas9 and RHO vectors, Cas9 protein levels, Cas9 AAV, and editing of *RHO* was assessed.

Fig. 14A indicates that the SD Cas9 vector system demonstrated successful silencing of Cas9 levels. **Fig. 14B** indicates that the vector system carrying the SD Cas9 system

resulted in robust editing at the *RHO* locus, albeit at slightly lower levels as compared to a vector system encoding a wild-type Cas9 sequence.

Example 7: Editing of human explants by ribonucleoproteins comprising gRNA targeting the *RHO* gene and Cas9

The ability of ribonucleoproteins comprising RHO-9 gRNA (**Table 1**) targeting the *RHO* gene and Cas9 to edit human explants was assessed. Briefly, retinal explants from one human donor were harvested and transferred to a membrane on a trans-well chamber in a 24 well plate. 300 µl of retinal media was added to the 24 well plate (i.e., Neurobasal-A media (no phenol red) (470 mL) containing B27 (with VitA) 50X (20 mL), Antibiotic- Antimycotic (5 mL), and GlutaMAX 1% (5 mL)). Different "knock-down and replace" strategies were compared: "shRNA": transduction of retinal explants with shRNA targeting the RHO gene and a replacement vector providing a RHO cDNA (as published in Cideciyan 2018); "Vector A": a two-vector system (Vector 1 comprising saCas9 driven by the minimal RHO promoter (250 bp), and Vector 2 comprising a codon-optimized RHO cDNA (Codon 6 (SEQ ID NO:18)) and comprising a HBA1 3' UTR under the control of the minimal 250 bp RHO promoter, as well as a the RHO-9 gRNA under the control of a U6 promoter); "Vector B": a two-vector system identical to "Vector A" except for Vector 2 comprising a wt RHO cDNA; and "UTC": untransduced control. The respective AAVs were diluted to the desired titer (1 x 10^{12} vg/ml) with the retinal media to obtain the final concentration in a total of 100 µl. The diluted/titered AAV was added dropwise on top of the explant in the 24 well plate. 300 µl of retinal media was replenished every 72 hours. After 4 weeks, explants were lysed to obtain protein for molecular biology analysis. The ratio of RHO protein:total protein was measured. Data indicate that Vector A (comprising the minimal 250 bp promoter, RHO cDNA, HBA1 3' UTR, and RHO-9 gRNA), resulted in robust expression of RHO protein (**Fig. 15**).

Example 8: Administration of a gene editing system to a patient in need thereof

A human patient presenting with adRP is administered a gene editing system comprising two AAV5-based expression vectors.

Vector 1 comprises a nucleic acid sequence encoding an *S. aureus* Cas9 protein, flanked on each site by a nuclear localization sequence under the control of a GRK1 promoter or under the control of a *RHO* minimal promoter (e.g., 250 bp RHO promoter).

Vector 2 comprises a nucleic acid sequence encoding one or more guide RNAs, each under the control of a U6 promoter. The targeting domain of the one or more guide RNAs, independently, is selected from the following sequences:

RHO-1: GUCAGCCACAAGGGCCACAGCC (SEQ ID NO:100)

RHO-2: CCGAAGACGAAGUAUCCAUGCA (SEQ ID NO:101)

RHO-3: AGUAUCCAUGCAGAGAGGUGUA (SEQ ID NO:102)

RHO-4: CUAGGUUGAGCAGGAUGUAGUU (SEQ ID NO:103)

RHO-5: CAUGGCUCAGCCAGGUAGUACU (SEQ ID NO:104)

RHO-6: ACGGGUGUGGUACGCAGCCCCU (SEQ ID NO:105)

RHO-7: CCCACACCCGGCUCAUACCGCC (SEQ ID NO:106)

RHO-8: CCCUGGGCGGUAUGAGCCGGGU (SEQ ID NO:107)

RHO-9: CCAUCAUGGGCGUUGCCUUCAC (SEQ ID NO:108)

RHO-10: GUGCCAUUACCUGGACCAGCCG (SEQ ID NO:109)

RHO-11: UUACCUGGACCAGCCGGCGAGU (SEQ ID NO:110)

The nucleic acid sequence encoding the guide RNA is under the control of a U6 promoter. Vector 2 further comprises a nucleic acid comprising an upstream sequence encoding a *RHO* 5'-UTR, a *RHO* cDNA, and a downstream sequence encoding an HBA1 3'-UTR under the control of a minimal *RHO* promoter sequence that comprises a portion of the *RHO* distal enhancer and a portion of the *RHO* proximal promoter region. The [promoter]-[5'UTR]-[cDNA]-[3'UTR] sequence of Vector 2 is as follows:

CCACGTCAGAATCAAACCCTCACCTTAACCTCATTAGCGTTGGGCATAATCACCAGGCCAAG
CGCCTTAAACTACGAGAGGCCCCATCCCACCCGCCCTGCCTTAGCCCTGCCACGTGTGCCAA
ACGCTGTTAGACCCAACACCACCCAGGCCAGGTAGGGGGCTGGAGCCCAGGTGGGCATTTGA
GTCACCAACCCCCAGGCAGTCTCCCTTTTCCTGGATCCTGAGTACCTCTCCTCCCTGACCTC
AGGCTTCCTCCTAGTGTCACCTTGGCCCCTCTTAGAAGCCAATTAGGCCCTCAGTTTCTGCA
GCGGGGATTAATATGATTATGAACACCCCCAATCTCCCAGATGCTGATTCAGCCAGGAGCTT
AGGAGGGGGAGGTCACTTTATAAGGGTCTGGGGGGGGTCAGAACCCAGAGTCATCCAGCTGGA
GCCCTGAGTGGCTGAGCTCAGGCCTTCGCAGCATTCTTGGGTGGGAGCAGCCACGGGTCAGC
CACAAGGGCCACCACC
ATGAATGGCACAGAAGGCCCTAACTTCTACGTGCCCTTCTCCAATGCGACGGGTGTGGTACG
CAGCCCCTTCGAGTACCCACAGTACTACCTGGCTGAGCCATGGCAGTTCTCCATGCTGGCCG
CCTACATGTTTCTGCTGATCGTGCTGGGCTTCCCCATCAACTTCCTCACGCTCTACGTCACC
GTCCAGCACAAGAAGCTGCGCACGCCTCTCAACTACATCCTGCTCAACCTAGCCGTGGCTGA
CCTCTTCATGGTCCTAGGTGGCTTCACCAGCACCCTCTACACCTCTCTGCATGGATACTTCG
TCTTCGGGCCCACAGGATGCAATTTGGAGGGCTTCTTTGCCACCCTGGGCGGTGAAATTGCC
CTGTGGTCCTTGGTGGTCCTGGCCATCGAGCGGTACGTGGTGGTGTGTAAGCCCATGAGCAA
CTTCCGCTTCGGGGAGAACCATGCCATCATGGGCGTTGCCTTCACCTGGGTCATGGCGCTGG
CCTGCGCCGCACCCCCACTCGCCGGCTGGTCCAGGTACATCCCCGAGGGCCTGCAGTGCTCG
TGTGGAATCGACTACTACACGCTCAAGCCGGAGGTCAACAACGAGTCTTTTGTCATCTACAT
GTTCGTGGTCCACTTCACCATCCCCATGATTATCATCTTTTTCTGCTATGGGCAGCTCGTCT

TCACCGTCAAGGAGGCCGCTGCCCAGCAGCAGGAGTCAGCCACCACACAGAAGGCAGAGAAG
GAGGTCACCCGCATGGTCATCATCATGGTCATCGCTTTCCTGATCTGCTGGGTGCCCTACGC
CAGCGTGGCATTCTACATCTTCACCCACCAGGGCTCCAACTTCGGTCCCATCTTCATGACCA
TCCCAGCGTTCTTTGCCAAGAGCGCCGCCATCTACAACCCTGTCATCTATATCATGATGAAC
AAGCAGTTCCGGAACTGCATGCTCACCACCATCTGCTGCGGCAAGAACCCACTGGGTGACGA
TGAGGCCTCTGCTACCGTGTCCAAGACGGAGACGAGCCAGGTGGCCCCGGCCTAAGCTGGAG
CCTCGGTGGCCATGCTTCTTGCCCCTTGGGCCTCCCCCCAGCCCCTCCTCCCCTTCCTGCAC
CCGTACCCCCGTGGTCTTTGAATAAAGTCTGAGTGGGCGGCA (SEQ ID NO:8)

Where a guide RNA is used that comprises a targeting domain that binds to a wild-type *RHO* sequence present in the *RHO* cDNA, a codon-modified version of the *RHO* cDNA may be substituted for the *RHO* cDNA comprised in the nucleic acid construct above.

Vector 1 and Vector 2 are packaged into viral particles according to methods known in the art, and delivered to the patient via subretinal injection at a dose of about 300 microliters of $1x10^{11}$ - $3x10^{11}$ viral genomes (vg)/mL. The patient is monitored post-administration, and periodically subjected to an assessment of one or more symptoms associated with adRP. For example, the patient is periodically subjected to an assessment of rod photoreceptor function, e.g., by scotopic microperimetry. About one year after administration of Vector 1 and Vector 2, the patient shows an amelioration of at least one adRP associated symptom, e.g. a stabilization of rod function, characterized by improved rod function compared to the expected level of rod function in the patient, or in an appropriate control group, in the absence of a clinical intervention.

Table 18: gRNAs Providing > 0.1% Editing of *RHO* Alleles in HEK293T Cells

gRNA ID	Targeting Domain (RNA)	Targeting Domain (DNA)/ Protospacer
RHO-1	GUCAGCCACAAGGGCCACAGCC (SEQ ID NO:100)	GTCAGCCACAAGGGCCACAGCC (SEQ ID NO:600)
RHO-2	CCGAAGACGAAGUAUCCAUGCA (SEQ ID NO:101)	CCGAAGACGAAGTATCCATGCA (SEQ ID NO:601)
RHO-3	AGUAUCCAUGCAGAGAGGUGUA (SEQ ID NO:102)	AGTATCCATGCAGAGAGGTGTA (SEQ ID NO:602)
RHO-4	CUAGGUUGAGCAGGAUGUAGUU (SEQ ID NO:103)	CTAGGTTGAGCAGGATGTAGTT (SEQ ID NO:603)
RHO-5	CAUGGCUCAGCCAGGUAGUACU (SEQ ID NO:104)	CATGGCTCAGCCAGGTAGTACT (SEQ ID NO:604)
RHO-6	ACGGGUGUGGUACGCAGCCCCU (SEQ ID NO:105)	ACGGGTGTGGTACGCAGCCCCT (SEQ ID NO:605)
RHO-7	CCCACACCCGGCUCAUACCGCC (SEQ ID NO:106)	CCCACACCCGGCTCATACCGCC (SEQ ID NO:606)
RHO-8	CCCUGGGCGGUAUGAGCCGGGU (SEQ ID NO:107)	CCCTGGGCGGTATGAGCCGGGT (SEQ ID NO:607)
RHO-9	CCAUCAUGGGCGUUGCCUUCAC (SEQ ID NO:108)	CCATCATGGGCGTTGCCTTCAC (SEQ ID NO:608)

RHO-10	GUGCCAUUACCUGGACCAGCCG (SEQ ID NO:109)	GTGCCATTACCTGGACCAGCCG (SEQ ID NO:609)
RHO-11	UUACCUGGACCAGCCGGCGAGU (SEQ ID NO:110)	TTACCTGGACCAGCCGGCGAGT (SEQ ID NO:610)
RHO-12	GCAUUCUUGGGUGGGAGCAGCC (SEQ ID NO:111)	GCATTCTTGGGTGGGAGCAGCC (SEQ ID NO:611)
RHO-13	GCUCAGCCACUCAGGGCUCCAG (SEQ ID NO:112)	GCTCAGCCACTCAGGGCTCCAG (SEQ ID NO:612)
RHO-14	UGACCCGUGGCUGCUCCCACCC (SEQ ID NO:113)	TGACCCGTGGCTGCTCCCACCC (SEQ ID NO:613)
RHO-15	AGCUCAGGCCUUCGCAGCAUUC (SEQ ID NO:114)	AGCTCAGGCCTTCGCAGCATTC (SEQ ID NO:614)
RHO-17	ACACGCUGAGGAGAGCUGGGCA (SEQ ID NO:116)	ACACGCTGAGGAGAGCTGGGCA (SEQ ID NO:616)
RHO-18	GCAAAUAACUUCCCCCAUUCCC (SEQ ID NO:117)	GCAAATAACTTCCCCCATTCCC (SEQ ID NO:617)
RHO-19	AGACCCAGGCUGGGCACUGAGG (SEQ ID NO:118)	AGACCCAGGCTGGGCACTGAGG (SEQ ID NO:618)
RHO-20	CUAGGUCUCCUGGCUGUGAUCC (SEQ ID NO:119)	CTAGGTCTCCTGGCTGTGATCC (SEQ ID NO:619)
RHO-21	CCAGAAGGUGGGUGUGCCACUU (SEQ ID NO:120)	CCAGAAGGTGGGTGTGCCACTT (SEQ ID NO:620)
RHO-24	GGGCGUCACACAGGGACGGGUG (SEQ ID NO:123)	GGGCGTCACACAGGGACGGGTG (SEQ ID NO:623)
RHO-25	CUGUGAUCCAGGAAUAUCUCUG (SEQ ID NO:124)	CTGTGATCCAGGAATATCTCTG (SEQ ID NO:624)
RHO-26	UUGCAUUUAACAGGAAAACAGA (SEQ ID NO:125)	TTGCATTTAACAGGAAAACAGA (SEQ ID NO:625)
RHO-27	GGAGUGCACCCUCCUUAGGCAG (SEQ ID NO:126)	GGAGTGCACCCTCCTTAGGCAG (SEQ ID NO:626)
RHO-28	CAUCUGUCCUGCUCACCACCCC (SEQ ID NO:127)	CATCTGTCCTGCTCACCACCCC (SEQ ID NO:627)
RHO-29	GAGGGGAGGCAGAGGAUGCCAG (SEQ ID NO:128)	GAGGGGAGGCAGAGGATGCCAG (SEQ ID NO:628)
RHO-30	CUCAGGGAAUCUCUGGCCAUUG (SEQ ID NO:129)	CTCAGGGAATCTCTGGCCATTG (SEQ ID NO:629)
RHO-31	UGCACUCCCCCCUAGACAGGGA (SEQ ID NO:130)	TGCACTCCCCCCTAGACAGGGA (SEQ ID NO:630)
RHO-32	UGCUGUUUGUGCAGGGCUGGCA (SEQ ID NO:131)	TGCTGTTTGTGCAGGGCTGGCA (SEQ ID NO:631)
RHO-33	ACUGGGACAUUCCUAACAGUGA (SEQ ID NO:132)	ACTGGGACATTCCTAACAGTGA (SEQ ID NO:632)
RHO-35	CUCCUCUCUGGGGGCCCAAGCU (SEQ ID NO:134)	CTCCTCTCTGGGGGCCCAAGCT (SEQ ID NO:634)
RHO-36	CUGCAUCUCAGCAGAGAUAUUC (SEQ ID NO:135)	CTGCATCTCAGCAGAGATATTC (SEQ ID NO:635)
RHO-37	UGUUUCCCUUGGAGCAGCUGUG (SEQ ID NO:136)	TGTTTCCCTTGGAGCAGCTGTG (SEQ ID NO:636)
RHO-40	CCUAGGAGAGGCCCCCACAUGU (SEQ ID NO:139)	CCTAGGAGAGGCCCCCACATGT (SEQ ID NO:639)

RHO-41	AUCACUCAGUUCUGGCCAGAAG (SEQ ID NO:140)	ATCACTCAGTTCTGGCCAGAAG (SEQ ID NO:640)
RHO-42	AGAGCUGGGCAAAGAAAUUCCA (SEQ ID NO:141)	AGAGCTGGGCAAAGAAATTCCA (SEQ ID NO:641)
RHO-43	CCACCCCAUGAAGUUCCAUAGG (SEQ ID NO:142)	CCACCCCATGAAGTTCCATAGG (SEQ ID NO:642)
RHO-44	CCACCCUGAGCUUGGGCCCCCA (SEQ ID NO:143)	CCACCCTGAGCTTGGGCCCCCA (SEQ ID NO:643)
RHO-45	CAGAGGAAGAAGAAGGAAAUGA (SEQ ID NO:144)	CAGAGGAAGAAGAAGGAAATGA (SEQ ID NO:644)
RHO-46	AAACAGCAGCCCGGCUAUCACC (SEQ ID NO:145)	AAACAGCAGCCCGGCTATCACC (SEQ ID NO:645)
RHO-49	UCACACAGGGACGGGUGCAGAG (SEQ ID NO:148)	TCACACAGGGACGGGTGCAGAG (SEQ ID NO:648)
RHO-51	UGAGCUUGGGCCCCCAGAGAGG (SEQ ID NO:150)	TGAGCTTGGGCCCCCAGAGAGG (SEQ ID NO:650)
RHO-52	AAUAUCUCUGCUGAGAUGCAGG (SEQ ID NO:151)	AATATCTCTGCTGAGATGCAGG (SEQ ID NO:651)
RHO-53	GGAGAGGGGAAGAGACUCAUUU (SEQ ID NO:152)	GGAGAGGGGAAGAGACTCATTT (SEQ ID NO:652)
RHO-54	AGAACUGAGUGAUCUGUGAUUA (SEQ ID NO:153)	AGAACTGAGTGATCTGTGATTA (SEQ ID NO:653)
RHO-55	CCACUCUCCCUAUGGAACUUCA (SEQ ID NO:154)	CCACTCTCCCTATGGAACTTCA (SEQ ID NO:654)
RHO-57	UGGAUUUUCCAUUCUCCAGUCA (SEQ ID NO:156)	TGGATTTTCCATTCTCCAGTCA (SEQ ID NO:656)
RHO-58	GUGCAGGAGCCCGGGAGCAUGG (SEQ ID NO:157)	GTGCAGGAGCCCGGGAGCATGG (SEQ ID NO:657)
RHO-59	GGGUGGUGAGCAGGACAGAUGU (SEQ ID NO:158)	GGGTGGTGAGCAGGACAGATGT (SEQ ID NO:658)
RHO-60	CAGCUCUCCCUCAGUGCCCAGC (SEQ ID NO:159)	CAGCTCTCCCTCAGTGCCCAGC (SEQ ID NO:659)
RHO-61	CCUGCUGGGGCGUCACACAGGG (SEQ ID NO:160)	CCTGCTGGGGCGTCACACAGGG (SEQ ID NO:660)
RHO-63	ACUUACGGGUGGUUGUUCUCUG (SEQ ID NO:162)	ACTTACGGGTGGTTGTTCTCTG (SEQ ID NO:662)
RHO-64	CACAGGGAAGACCCAAUGACUG (SEQ ID NO:163)	CACAGGGAAGACCCAATGACTG (SEQ ID NO:663)
RHO-65	AGCACAGACCCCACUGCCUAAG (SEQ ID NO:164)	AGCACAGACCCCACTGCCTAAG (SEQ ID NO:664)
RHO-66	ACCUGAGGACAGGGGCUGAGAG (SEQ ID NO:165)	ACCTGAGGACAGGGGCTGAGAG (SEQ ID NO:665)
RHO-67	CAACAAUGGCCAGAGAUUCCCU (SEQ ID NO:166)	CAACAATGGCCAGAGATTCCCT (SEQ ID NO:666)
RHO-68	UGCUGCCUCGGUCCCAUUCUCA (SEQ ID NO:167)	TGCTGCCTCGGTCCCATTCTCA (SEQ ID NO:667)
RHO-69	UGCUGCCUGGCCACAUCCCUAA (SEQ ID NO:168)	TGCTGCCTGGCCACATCCCTAA (SEQ ID NO:668)
RHO-70	GCCACUCUCCCUAUGGAACUUC (SEQ ID NO:169)	GCCACTCTCCCTATGGAACTTC (SEQ ID NO:669)

RHO-71	GAGGGAGGAAGGACUGCCAAUU (SEQ ID NO:170)	GAGGGAGGAAGGACTGCCAATT (SEQ ID NO:670)
RHO-72	GAGGGUAGCUAGGAAGGCAACC (SEQ ID NO:171)	GAGGGTAGCTAGGAAGGCAACC (SEQ ID NO:671)
RHO-73	GGAAGGCAACCAGGAGUGGGAG (SEQ ID NO:172)	GGAAGGCAACCAGGAGTGGGAG (SEQ ID NO:672)
RHO-74	GCUGAGAUGCAGGAGGAGACGC (SEQ ID NO:173)	GCTGAGATGCAGGAGGAGACGC (SEQ ID NO:673)
RHO-75	AGGCUGGAGGGGCACCUGAGGA (SEQ ID NO:174)	AGGCTGGAGGGGCACCTGAGGA (SEQ ID NO:674)
RHO-76	AGGAAGGCAACCAGGAGUGGGA (SEQ ID NO:175)	AGGAAGGCAACCAGGAGTGGGA (SEQ ID NO:675)
RHO-77	CCGGGAGCAUGGAGGGGUCUGG (SEQ ID NO:176)	CCGGGAGCATGGAGGGGTCTGG (SEQ ID NO:676)
RHO-78	GGAUAACAGAUCCCACUUAACA (SEQ ID NO:177)	GGATAACAGATCCCACTTAACA (SEQ ID NO:677)
RHO-79	AGGCAGAGGAUGCCAGAGGGGA (SEQ ID NO:178)	AGGCAGAGGATGCCAGAGGGGA (SEQ ID NO:678)
RHO-80	GGGCCCAAGCUCAGGGUGGGAA (SEQ ID NO:179)	GGGCCCAAGCTCAGGGTGGGAA (SEQ ID NO:679)
RHO-81	UAACUAUAUGGCCACUCUCCCU (SEQ ID NO:180)	TAACTATATGGCCACTCTCCCT (SEQ ID NO:680)
RHO-82	UCCCACUUAACAGAGAGGAAAA (SEQ ID NO:181)	TCCCACTTAACAGAGAGGAAAA (SEQ ID NO:681)
RHO-83	GAAUGCAGAGGUGGUGGAAACC (SEQ ID NO:182)	GAATGCAGAGGTGGTGGAAACC (SEQ ID NO:682)
RHO-84	GGGAGACAGGGCAAGGCUGGCA (SEQ ID NO:183)	GGGAGACAGGGCAAGGCTGGCA (SEQ ID NO:683)
RHO-85	CACCACCCCAUGAAGUUCCAUA (SEQ ID NO:184)	CACCACCCCATGAAGTTCCATA (SEQ ID NO:684)
RHO-86	GCCAUAUAGUUAAUCAACCAAA (SEQ ID NO:185)	GCCATATAGTTAATCAACCAAA (SEQ ID NO:685)
RHO-87	GUAGCUAGGAAGGCAACCAGGA (SEQ ID NO:186)	GTAGCTAGGAAGGCAACCAGGA (SEQ ID NO:686)
RHO-88	CACAUUGCUUCAUGGCUCCUAG (SEQ ID NO:187)	CACATTGCTTCATGGCTCCTAG (SEQ ID NO:687)
RHO-89	CUGAGCUUGGGCCCCCAGAGAG (SEQ ID NO:188)	CTGAGCTTGGGCCCCCAGAGAG (SEQ ID NO:688)
RHO-90	ACCGAGCCCAUUGCCCAGCACA (SEQ ID NO:189)	ACCGAGCCCATTGCCCAGCACA (SEQ ID NO:689)
RHO-91	CUCAAAGAAGUCAAGCGCCCUG (SEQ ID NO:190)	CTCAAAGAAGTCAAGCGCCCTG (SEQ ID NO:690)
RHO-92	GCUACCCUCUCCCUGUCUAGGG (SEQ ID NO:191)	GCTACCCTCTCCCTGTCTAGGG (SEQ ID NO:691)
RHO-93	ACCCUGAGCUUGGGCCCCCAGA (SEQ ID NO:192)	ACCCTGAGCTTGGGCCCCCAGA (SEQ ID NO:692)
RHO-94	GGCAGAGGGACCACACGCUGAG (SEQ ID NO:193)	GGCAGAGGGACCACACGCTGAG (SEQ ID NO:693)
RHO-95	UCUGACUCAGCACAGCUGCUCC (SEQ ID NO:194)	TCTGACTCAGCACAGCTGCTCC (SEQ ID NO:694)

RHO-96	CUCUCAGCCACCACCGCCAAGC (SEQ ID NO:195)	CTCTCAGCCACCACCGCCAAGC (SEQ ID NO:695)
RHO-97	AGGGAUGUGGCCAGGCAGCAAC (SEQ ID NO:196)	AGGGATGTGGCCAGGCAGCAAC (SEQ ID NO:696)
RHO-98	CACCUGAGGACAGGGGCUGAGA (SEQ ID NO:197)	CACCTGAGGACAGGGGCTGAGA (SEQ ID NO:697)
RHO-99	GCCCAUGAUGGCAUGGUUCUCC (SEQ ID NO:198)	GCCCATGATGGCATGGTTCTCC (SEQ ID NO:698)
RHO-100	GAAGGGGCAGAGGGACCACACG (SEQ ID NO:199)	GAAGGGGCAGAGGGACCACACG (SEQ ID NO:699)
RHO-101	AGCACCCUCUACACCUCUCUGC (SEQ ID NO:200)	AGCACCCTCTACACCTCTCTGC (SEQ ID NO:700)
RHO-102	CUUUGGAUAACAUUGACAGGAC (SEQ ID NO:201)	CTTTGGATAACATTGACAGGAC (SEQ ID NO:701)
RHO-103	GGUGAAGCCACCUAGGACCAUG (SEQ ID NO:202)	GGTGAAGCCACCTAGGACCATG (SEQ ID NO:702)
RHO-104	UAACAUUGACAGGACAGGAGAA (SEQ ID NO:203)	TAACATTGACAGGACAGGAGAA (SEQ ID NO:703)
RHO-105	GGGAGAGGGGAAGAGACUCAUU (SEQ ID NO:204)	GGGAGAGGGGAAGAGACTCATT (SEQ ID NO:704)
RHO-106	GCUGUGCUGAGUCAGACCCAGG (SEQ ID NO:205)	GCTGTGCTGAGTCAGACCCAGG (SEQ ID NO:705)
RHO-107	UUGAGGAGGCCUUGGGGAAGGA (SEQ ID NO:206)	TTGAGGAGGCCTTGGGGAAGGA (SEQ ID NO:706)
RHO-108	GCCCGGGAGCAUGGAGGGGUCU (SEQ ID NO:207)	GCCCGGGAGCATGGAGGGGTCT (SEQ ID NO:707)
RHO-109	GUAAACUGGGACUGACCCUGCA (SEQ ID NO:208)	GTAAACTGGGACTGACCCTGCA (SEQ ID NO:708)
RHO-110	AUAACAUUGACAGGACAGGAGA (SEQ ID NO:209)	ATAACATTGACAGGACAGGAGA (SEQ ID NO:709)
RHO-111	GGCAGGGAGGCUGGAGGGGCAC (SEQ ID NO:210)	GGCAGGGAGGCTGGAGGGGCAC (SEQ ID NO:710)
RHO-112	GCAAACAUGGCCCGAGAUAGAU (SEQ ID NO:211)	GCAAACATGGCCCGAGATAGAT (SEQ ID NO:711)
RHO-113	GGACCGAGCCCAUUGCCCAGCA (SEQ ID NO:212)	GGACCGAGCCCATTGCCCAGCA (SEQ ID NO:712)
RHO-114	GCUCUACGUCACCGUCCAGCAC (SEQ ID NO:213)	GCTCTACGTCACCGTCCAGCAC (SEQ ID NO:713)
RHO-115	AGCACAGCUGCUCCAAGGGAAA (SEQ ID NO:214)	AGCACAGCTGCTCCAAGGGAAA (SEQ ID NO:714)
RHO-116	CUAAAGCAAAAAGGAACUGCUU (SEQ ID NO:215)	CTAAAGCAAAAAGGAACTGCTT (SEQ ID NO:715)
RHO-117	GAGAGGAAAACUGAGGCAGGGA (SEQ ID NO:216)	GAGAGGAAAACTGAGGCAGGGA (SEQ ID NO:716)
RHO-118	CAUUGCAAAGCUGGGUGACGGG (SEQ ID NO:217)	CATTGCAAAGCTGGGTGACGGG (SEQ ID NO:717)
RHO-119	UUGCCACCCUGGGCGGUAUGAG (SEQ ID NO:218)	TTGCCACCCTGGGCGGTATGAG (SEQ ID NO:718)
RHO-120	AGCUAGGAAGGCAACCAGGAGU (SEQ ID NO:219)	AGCTAGGAAGGCAACCAGGAGT (SEQ ID NO:719)

RHO-121	UCUCUGGGGGCCCAAGCUCAGG (SEQ ID NO:220)	TCTCTGGGGGCCCAAGCTCAGG (SEQ ID NO:720)
RHO-122	AGCACAGGGAAGACCCAAUGAC (SEQ ID NO:221)	AGCACAGGGAAGACCCAATGAC (SEQ ID NO:721)
RHO-123	GUUGACUGAAUAUAUGAGGGCU (SEQ ID NO:222)	GTTGACTGAATATATGAGGGCT (SEQ ID NO:722)
RHO-124	UUGUAAACUGGGACUGACCCUG (SEQ ID NO:223)	TTGTAAACTGGGACTGACCCTG (SEQ ID NO:723)
RHO-125	CACACCCACCUUCUGGCCAGAA (SEQ ID NO:224)	CACACCCACCTTCTGGCCAGAA (SEQ ID NO:724)
RHO-126	CCAGAGGAAGAAGAAGGAAAUG (SEQ ID NO:225)	CCAGAGGAAGAAGAAGGAAATG (SEQ ID NO:725)
RHO-127	GAGAUAUUCCUGGAUCACAGCC (SEQ ID NO:226)	GAGATATTCCTGGATCACAGCC (SEQ ID NO:726)
RHO-128	AGGGGCAGAGGGACCACACGCU (SEQ ID NO:227)	AGGGGCAGAGGGACCACACGCT (SEQ ID NO:727)
RHO-129	AACUAUAUGGCCACUCUCCCUA (SEQ ID NO:228)	AACTATATGGCCACTCTCCCTA (SEQ ID NO:728)
RHO-130	GCUGCUUGCGGUUCUCAACACC (SEQ ID NO:229)	GCTGCTTGCGGTTCTCAACACC (SEQ ID NO:729)
RHO-131	CACCAUGAAUGGUGUUUGUUGA (SEQ ID NO:230)	CACCATGAATGGTGTTTGTTGA (SEQ ID NO:730)
RHO-132	GCAGCCAUUGCAAAGCUGGGUG (SEQ ID NO:231)	GCAGCCATTGCAAAGCTGGGTG (SEQ ID NO:731)
RHO-133	UGACUCAGCACAGCUGCUCCAA (SEQ ID NO:232)	TGACTCAGCACAGCTGCTCCAA (SEQ ID NO:732)
RHO-134	CUGGGAGGAGGGGGAAGGGGCA (SEQ ID NO:233)	CTGGGAGGAGGGGGAAGGGGCA (SEQ ID NO:733)
RHO-135	GAUAACAUUGACAGGACAGGAG (SEQ ID NO:234)	GATAACATTGACAGGACAGGAG (SEQ ID NO:734)
RHO-136	CCAAACUGGGACAUUCCUAACA (SEQ ID NO:235)	CCAAACTGGGACATTCCTAACA (SEQ ID NO:735)
RHO-137	AGGAAAACAGAUGGGGUGCUGC (SEQ ID NO:236)	AGGAAAACAGATGGGGTGCTGC (SEQ ID NO:736)
RHO-138	CGGACAUGUGGGGGCCUCUCCU (SEQ ID NO:237)	CGGACATGTGGGGGCCTCTCCT (SEQ ID NO:737)
RHO-139	GCAAAGAAAUUCCAGGGAAUGG (SEQ ID NO:238)	GCAAAGAAATTCCAGGGAATGG (SEQ ID NO:738)
RHO-140	CCAGGAGACUUGGAACGCGGCA (SEQ ID NO:239)	CCAGGAGACTTGGAACGCGGCA (SEQ ID NO:739)
RHO-141	UGGUCCUUGGUGGUCCUGGCCA (SEQ ID NO:240)	TGGTCCTTGGTGGTCCTGGCCA (SEQ ID NO:740)
RHO-142	AAUGGAAAAUCCACUUCCCACC (SEQ ID NO:241)	AATGGAAAATCCACTTCCCACC (SEQ ID NO:741)
RHO-143	GCCCGAAGACGAAGUAUCCAUG (SEQ ID NO:242)	GCCCGAAGACGAAGTATCCATG (SEQ ID NO:742)
RHO-144	GUGCUGGACGGUGACGUAGAGC (SEQ ID NO:243)	GTGCTGGACGGTGACGTAGAGC (SEQ ID NO:743)
RHO-145	AGAAACAUGUAGGCGGCCAGCA (SEQ ID NO:244)	AGAAACATGTAGGCGGCCAGCA (SEQ ID NO:744)

RHO-146	CCGCUCGAUGGCCAGGACCACC (SEQ ID NO:245)	CCGCTCGATGGCCAGGACCACC (SEQ ID NO:745)
RHO-147	UCAGCACAGACCCCACUGCCUA (SEQ ID NO:246)	TCAGCACAGACCCCACTGCCTA (SEQ ID NO:746)
RHO-148	GAAUAUCUCUGCUGAGAUGCAG (SEQ ID NO:247)	GAATATCTCTGCTGAGATGCAG (SEQ ID NO:747)
RHO-149	GAGUACCCACAGUACUACCUGG (SEQ ID NO:248)	GAGTACCCACAGTACTACCTGG (SEQ ID NO:748)
RHO-150	CAACCAGGAGUGGGAGAGGGAU (SEQ ID NO:249)	CAACCAGGAGTGGGAGAGGGAT (SEQ ID NO:749)
RHO-151	UUGAGAACCGCAAGCAGCCGCU (SEQ ID NO:250)	TTGAGAACCGCAAGCAGCCGCT (SEQ ID NO:750)
RHO-152	GCAAGCCAGACCCCUCCUCUCU (SEQ ID NO:251)	GCAAGCCAGACCCCTCCTCTCT (SEQ ID NO:751)
RHO-153	GAGAGCUGGGCAAAGAAAUUCC (SEQ ID NO:252)	GAGAGCTGGGCAAAGAAATTCC (SEQ ID NO:752)
RHO-154	CGAGGCAGCAGCCUGGACAUGG (SEQ ID NO:253)	CGAGGCAGCAGCCTGGACATGG (SEQ ID NO:753)
RHO-155	AGGAAUAUCUCUGCUGAGAUGC (SEQ ID NO:254)	AGGAATATCTCTGCTGAGATGC (SEQ ID NO:754)
RHO-156	UUCCCGAGAAGGGAGAGGGAGG (SEQ ID NO:255)	TTCCCGAGAAGGGAGAGGGAGG (SEQ ID NO:755)
RHO-157	UCCUUCCUCCCCUCUCCCUUCUC (SEQ ID NO:256)	TCCTTCCTCCCCTCTCCCTTCTC (SEQ ID NO:756)
RHO-158	UGUUUUGCCCAGAGGAAGAAGA (SEQ ID NO:257)	TGTTTTGCCCAGAGGAAGAAGA (SEQ ID NO:757)
RHO-159	CCGGCUGGUCCAGGUAAUGGCA (SEQ ID NO:258)	CCGGCTGGTCCAGGTAATGGCA (SEQ ID NO:758)
RHO-160	CAGCACAGGGAAGACCCAAUGA (SEQ ID NO:259)	CAGCACAGGGAAGACCCAATGA (SEQ ID NO:759)
RHO-161	ACCAGGAGUGGGAGAGGGAUUU (SEQ ID NO:260)	ACCAGGAGTGGGAGAGGGATTT (SEQ ID NO:760)
RHO-162	GCUGGUGAAGCCACCUAGGACC (SEQ ID NO:261)	GCTGGTGAAGCCACCTAGGACC (SEQ ID NO:761)
RHO-163	GGCGGUAUGAGCCGGGUGUGGG (SEQ ID NO:262)	GGCGGTATGAGCCGGGTGTGGG (SEQ ID NO:762)
RHO-164	CAGCCAUUGCAAAGCUGGGUGA (SEQ ID NO:263)	CAGCCATTGCAAAGCTGGGTGA (SEQ ID NO:763)
RHO-165	ACAUUGACAGGACAGGAGAAGG (SEQ ID NO:264)	ACATTGACAGGACAGGAGAAGG (SEQ ID NO:764)
RHO-166	UGGGUCUUCCCUGUGCUGGGCA (SEQ ID NO:265)	TGGGTCTTCCCTGTGCTGGGCA (SEQ ID NO:765)
RHO-167	GUACGUGGUGGUGUGUAAGCCC (SEQ ID NO:266)	GTACGTGGTGGTGTGTAAGCCC (SEQ ID NO:766)
RHO-168	AGCAAAUAACUUCCCCCAUUCC (SEQ ID NO:267)	AGCAAATAACTTCCCCCATTCC (SEQ ID NO:767)
RHO-169	GGAUUUGAGGAGGCCUUGGGGA (SEQ ID NO:268)	GGATTTGAGGAGGCCTTGGGGA (SEQ ID NO:768)
RHO-170	CCCUGAGCUUGGGCCCCCAGAG (SEQ ID NO:269)	CCCTGAGCTTGGGCCCCCAGAG (SEQ ID NO:769)

RHO-171	CAGAGAUUCCCUGAGAAUGGGA (SEQ ID NO:270)	CAGAGATTCCCTGAGAATGGGA (SEQ ID NO:770)
RHO-172	GAGUUGGAAGCCCGCAUCUAUC (SEQ ID NO:271)	GAGTTGGAAGCCCGCATCTATC (SEQ ID NO:771)
RHO-173	AGUCCUUCCUCCCUCUCCCUUC (SEQ ID NO:272)	AGTCCTTCCTCCCTCTCCCTTC (SEQ ID NO:772)
RHO-174	GUUAUUUCAUUUCCCGAGAAGG (SEQ ID NO:273)	GTTATTTCATTTCCCGAGAAGG (SEQ ID NO:773)
RHO-175	AUUUCAUUUCCCGAGAAGGGAG (SEQ ID NO:274)	ATTTCATTTCCCGAGAAGGGAG (SEQ ID NO:774)
RHO-176	GACGUAGAGCGUGAGGAAGUUG (SEQ ID NO:275)	GACGTAGAGCGTGAGGAAGTTG (SEQ ID NO:775)
RHO-177	CAUUUCCCGAGAAGGGAGAGGG (SEQ ID NO:276)	CATTTCCCGAGAAGGGAGAGGG (SEQ ID NO:776)
RHO-178	GUAGAGCGUGAGGAAGUUGAUG (SEQ ID NO:277)	GTAGAGCGTGAGGAAGTTGATG (SEQ ID NO:777)
RHO-179	CAGGCCUUCGCAGCAUUCUUGG (SEQ ID NO:278)	CAGGCCTTCGCAGCATTCTTGG (SEQ ID NO:778)
RHO-180	AGGUAGUACUGUGGGUACUCGA (SEQ ID NO:279)	AGGTAGTACTGTGGGTACTCGA (SEQ ID NO:779)
RHO-181	AAACAUGUAGGCGGCCAGCAUG (SEQ ID NO:280)	AAACATGTAGGCGGCCAGCATG (SEQ ID NO:780)
RHO-182	UUUCAUUUCCCGAGAAGGGAGA (SEQ ID NO:281)	TTTCATTTCCCGAGAAGGGAGA (SEQ ID NO:781)
RHO-183	GGGAAGACCCAAUGACUGGAGA (SEQ ID NO:282)	GGGAAGACCCAATGACTGGAGA (SEQ ID NO:782)
RHO-184	AAAACUGAGGCAGGGAGAGGGG (SEQ ID NO:283)	AAAACTGAGGCAGGGAGAGGGG (SEQ ID NO:783)
RHO-185	UGAGUCAGACCCAGGCUGGGCA (SEQ ID NO:284)	TGAGTCAGACCCAGGCTGGGCA (SEQ ID NO:784)
RHO-186	GGGAUUUGAGGAGGCCUUGGGG (SEQ ID NO:285)	GGGATTTGAGGAGGCCTTGGGG (SEQ ID NO:785)
RHO-187	UCUGGGGGCCCAAGCUCAGGGU (SEQ ID NO:286)	TCTGGGGGCCCAAGCTCAGGGT (SEQ ID NO:786)
RHO-188	CGGGCCCACAGGAUGCAAUUUG (SEQ ID NO:287)	CGGGCCCACAGGATGCAATTTG (SEQ ID NO:787)
RHO-189	ACGUAGAGCGUGAGGAAGUUGA (SEQ ID NO:288)	ACGTAGAGCGTGAGGAAGTTGA (SEQ ID NO:788)
RHO-190	GACCGAGGCAGCAGCCUGGACA (SEQ ID NO:289)	GACCGAGGCAGCAGCCTGGACA (SEQ ID NO:789)
RHO-191	CAGGCUGGGCACUGAGGGAGAG (SEQ ID NO:290)	CAGGCTGGGCACTGAGGGAGAG (SEQ ID NO:790)
RHO-192	UAUUUCAUUUCCCGAGAAGGGA (SEQ ID NO:291)	TATTTCATTTCCCGAGAAGGGA (SEQ ID NO:791)
RHO-193	GUCCCGGGCUUGGCGGUGGUGG (SEQ ID NO:292)	GTCCCGGGCTTGGCGGTGGTGG (SEQ ID NO:792)
RHO-194	CUGCUGCCUCGGUCCCAUUCUC (SEQ ID NO:293)	CTGCTGCCTCGGTCCCATTCTC (SEQ ID NO:793)
RHO-195	AGCGUCUCCUCCUGCAUCUCAG (SEQ ID NO:294)	AGCGTCTCCTCCTGCATCTCAG (SEQ ID NO:794)

RHO-196	UCAGACCCAGGCUGGGCACUGA (SEQ ID NO:295)	TCAGACCCAGGCTGGGCACTGA (SEQ ID NO:795)
RHO-197	AGCUACCCUCUCCCUGUCUAGG (SEQ ID NO:296)	AGCTACCCTCTCCCTGTCTAGG (SEQ ID NO:796)
RHO-198	CAGAGAGGAAAACUGAGGCAGG (SEQ ID NO:297)	CAGAGAGGAAAACTGAGGCAGG (SEQ ID NO:797)
RHO-199	GGAGAGGGAUUUGAGGAGGCCU (SEQ ID NO:298)	GGAGAGGGATTTGAGGAGGCCT (SEQ ID NO:798)
RHO-200	GUCCUUCCUCCCUCUCCCUUCU (SEQ ID NO:299)	GTCCTTCCTCCCTCTCCCTTCT (SEQ ID NO:799)
RHO-201	AGAGAGCUUGGUGCUGGGAGGA (SEQ ID NO:300)	AGAGAGCTTGGTGCTGGGAGGA (SEQ ID NO:800)
RHO-202	CCUUCUCGGGAAAUGAAAUAAC (SEQ ID NO:301)	CCTTCTCGGGAAATGAAATAAC (SEQ ID NO:801)
RHO-203	GCGGUUCUCAACACCAGGAGAC (SEQ ID NO:302)	GCGGTTCTCAACACCAGGAGAC (SEQ ID NO:802)
RHO-204	CUCUGGGGGCCCAAGCUCAGGG (SEQ ID NO:303)	CTCTGGGGGCCCAAGCTCAGGG (SEQ ID NO:803)
RHO-205	UGUGCAGGAGCCCGGGAGCAUG (SEQ ID NO:304)	TGTGCAGGAGCCCGGGAGCATG (SEQ ID NO:804)
RHO-206	CAGAGAGGUGUAGAGGGUGCUG (SEQ ID NO:305)	CAGAGAGGTGTAGAGGGTGCTG (SEQ ID NO:805)
RHO-207	CUCCCCGAAGCGGAAGUUGCUC (SEQ ID NO:306)	CTCCCCGAAGCGGAAGTTGCTC (SEQ ID NO:806)
RHO-208	GCUAGAAGCAGCCAUUGCAAAG (SEQ ID NO:307)	GCTAGAAGCAGCCATTGCAAAG (SEQ ID NO:807)
RHO-209	CAAACACCAUUCAUGGUGAUAG (SEQ ID NO:308)	CAAACACCATTCATGGTGATAG (SEQ ID NO:808)
RHO-210	UCAUUUCCCGAGAAGGGAGAGG (SEQ ID NO:309)	TCATTTCCCGAGAAGGGAGAGG (SEQ ID NO:809)
RHO-211	UCACCACCCCAUGAAGUUCCAU (SEQ ID NO:310)	TCACCACCCCATGAAGTTCCAT (SEQ ID NO:810)
RHO-212	GGGAGUGCACCCUCCUUAGGCA (SEQ ID NO:311)	GGGAGTGCACCCTCCTTAGGCA (SEQ ID NO:811)
RHO-213	AAUGGCCAGAGAUUCCCUGAGA (SEQ ID NO:312)	AATGGCCAGAGATTCCCTGAGA (SEQ ID NO:812)
RHO-214	AGAAUGGGACCGAGGCAGCAGC (SEQ ID NO:313)	AGAATGGGACCGAGGCAGCAGC (SEQ ID NO:813)
RHO-215	GGCAAGCCAGACCCCUCCUCUC (SEQ ID NO:314)	GGCAAGCCAGACCCCTCCTCTC (SEQ ID NO:814)
RHO-216	CCCGGGCUUGGCGGUGGUGGCU (SEQ ID NO:315)	CCCGGGCTTGGCGGTGGTGGCT (SEQ ID NO:815)
RHO-217	AGCCCGGGAGCAUGGAGGGGUC (SEQ ID NO:316)	AGCCCGGGAGCATGGAGGGGTC (SEQ ID NO:816)
RHO-218	CCGGGUUAUUUCAUUUCCCGAG (SEQ ID NO:317)	CCGGGTTATTTCATTTCCCGAG (SEQ ID NO:817)
RHO-219	GGUGUUUGUUGACUGAAUAUAU (SEQ ID NO:318)	GGTGTTTGTTGACTGAATATAT (SEQ ID NO:818)
RHO-220	CCGUCCCUGUGUGACGCCCCAG (SEQ ID NO:319)	CCGTCCCTGTGTGACGCCCCAG (SEQ ID NO:819)

RHO-221	GGACAGGGGCUGAGAGGGGAGG (SEQ ID NO:320)	GGACAGGGGCTGAGAGGGGAGG (SEQ ID NO:820)
RHO-222	AGAGGGUGCUGGUGAAGCCACC (SEQ ID NO:321)	AGAGGGTGCTGGTGAAGCCACC (SEQ ID NO:821)
RHO-223	AUUGCAUCCUGUGGGCCCGAAG (SEQ ID NO:322)	ATTGCATCCTGTGGGCCCGAAG (SEQ ID NO:822)
RHO-224	CGGGUUAUUUCAUUUCCCGAGA (SEQ ID NO:323)	CGGGTTATTTCATTTCCCGAGA (SEQ ID NO:823)
RHO-225	GGAAAUGAAAUAACCCGGACAU (SEQ ID NO:324)	GGAAATGAAATAACCCGGACAT (SEQ ID NO:824)
RHO-226	CUGACUCAGCACAGCUGCUCCA (SEQ ID NO:325)	CTGACTCAGCACAGCTGCTCCA (SEQ ID NO:825)
RHO-227	GGCACCUGAGGACAGGGGCUGA (SEQ ID NO:326)	GGCACCTGAGGACAGGGGCTGA (SEQ ID NO:826)
RHO-228	GGAGAGCUGGGCAAAGAAAUUC (SEQ ID NO:327)	GGAGAGCTGGGCAAAGAAATTC (SEQ ID NO:827)
RHO-229	GGGCGGUAUGAGCCGGGUGUGG (SEQ ID NO:328)	GGGCGGTATGAGCCGGGTGTGG (SEQ ID NO:828)
RHO-230	CCUCCCUCUCCCUUCUCGGGAA (SEQ ID NO:329)	CCTCCCTCTCCCTTCTCGGGAA (SEQ ID NO:829)
RHO-231	UCCAGGUAAUGGCACUGAGCAG (SEQ ID NO:330)	TCCAGGTAATGGCACTGAGCAG (SEQ ID NO:830)
RHO-232	GUGGGGGCCUCUCCUAGGAGCC (SEQ ID NO:331)	GTGGGGGCCTCTCCTAGGAGCC (SEQ ID NO:831)
RHO-233	GAUGGCAUGGUUCUCCCCGAAG (SEQ ID NO:332)	GATGGCATGGTTCTCCCCGAAG (SEQ ID NO:832)
RHO-234	CGUCGCAUUGGAGAAGGGCACG (SEQ ID NO:333)	CGTCGCATTGGAGAAGGGCACG (SEQ ID NO:833)
RHO-235	UGGGUGGGGUGUGCAGGAGCCC (SEQ ID NO:334)	TGGGTGGGGTGTGCAGGAGCCC (SEQ ID NO:834)
RHO-236	CUGGACGGUGACGUAGAGCGUG (SEQ ID NO:335)	CTGGACGGTGACGTAGAGCGTG (SEQ ID NO:835)
RHO-237	GAGGAAAACUGAGGCAGGGAGA (SEQ ID NO:336)	GAGGAAAACTGAGGCAGGGAGA (SEQ ID NO:836)
RHO-238	CUGAACACUGCCUUGAUCUUAU (SEQ ID NO:337)	CTGAACACTGCCTTGATCTTAT (SEQ ID NO:837)
RHO-239	CAUUACCUGGACCAGCCGGCGA (SEQ ID NO:338)	CATTACCTGGACCAGCCGGCGA (SEQ ID NO:838)
RHO-240	GGAGAGAGCUUGGUGCUGGGAG (SEQ ID NO:339)	GGAGAGAGCTTGGTGCTGGGAG (SEQ ID NO:839)
RHO-241	AGAAUAAUGUCUUGCAUUUAAC (SEQ ID NO:340)	AGAATAATGTCTTGCATTTAAC (SEQ ID NO:840)
RHO-242	CUAGGAAGGCAACCAGGAGUGG (SEQ ID NO:341)	CTAGGAAGGCAACCAGGAGTGG (SEQ ID NO:841)
RHO-243	UCUCCCAGACCCCUCCAUGCUC (SEQ ID NO:342)	TCTCCCAGACCCCTCCATGCTC (SEQ ID NO:842)
RHO-244	ACAGGGGCUGAGAGGGGAGGCA (SEQ ID NO:343)	ACAGGGGCTGAGAGGGGAGGCA (SEQ ID NO:843)
RHO-245	GGGGCAGAGGGACCACACGCUG (SEQ ID NO:344)	GGGGCAGAGGGACCACACGCTG (SEQ ID NO:844)

RHO-246	AGGGGAGGCAGAGGAUGCCAGA (SEQ ID NO:345)	AGGGGAGGCAGAGGATGCCAGA (SEQ ID NO:845)
RHO-247	UGGUCCAGGUAAUGGCACUGAG (SEQ ID NO:346)	TGGTCCAGGTAATGGCACTGAG (SEQ ID NO:846)
RHO-248	CCGGACAUGUGGGGGCCUCUCC (SEQ ID NO:347)	CCGGACATGTGGGGGCCTCTCC (SEQ ID NO:847)
RHO-249	GCAGGCCAGCGCCAUGACCCAG (SEQ ID NO:348)	GCAGGCCAGCGCCATGACCCAG (SEQ ID NO:848)
RHO-250	CUAGCUACCCUCUCCCUGUCUA (SEQ ID NO:349)	CTAGCTACCCTCTCCCTGTCTA (SEQ ID NO:849)
RHO-251	GCUUUGGAUAACAUUGACAGGA (SEQ ID NO:350)	GCTTTGGATAACATTGACAGGA (SEQ ID NO:850)
RHO-252	GCCAUUGCAAAGCUGGGUGACG (SEQ ID NO:351)	GCCATTGCAAAGCTGGGTGACG (SEQ ID NO:851)
RHO-253	CCUAGGUCUCCUGGCUGUGAUC (SEQ ID NO:352)	CCTAGGTCTCCTGGCTGTGATC (SEQ ID NO:852)
RHO-254	AACAGAGAGGAAAACUGAGGCA (SEQ ID NO:353)	AACAGAGAGGAAAACTGAGGCA (SEQ ID NO:853)
RHO-255	AUUACCUGGACCAGCCGGCGAG (SEQ ID NO:354)	ATTACCTGGACCAGCCGGCGAG (SEQ ID NO:854)
RHO-256	GAGGGGCACCUGAGGACAGGGG (SEQ ID NO:355)	GAGGGGCACCTGAGGACAGGGG (SEQ ID NO:855)
RHO-257	GGGUUAUUUCAUUUCCCGAGAA (SEQ ID NO:356)	GGGTTATTTCATTTCCCGAGAA (SEQ ID NO:856)
RHO-258	AGGGUGCACUCCCCCCUAGACA (SEQ ID NO:357)	AGGGTGCACTCCCCCCTAGACA (SEQ ID NO:857)
RHO-259	CCAGGAGUGGGAGAGGGAUUUG (SEQ ID NO:358)	CCAGGAGTGGGAGAGGGATTTG (SEQ ID NO:858)
RHO-260	AGAGGGGAGGCAGAGGAUGCCA (SEQ ID NO:359)	AGAGGGGAGGCAGAGGATGCCA (SEQ ID NO:859)
RHO-261	CCGCCUGCUGACUGCCUUGCAG (SEQ ID NO:360)	CCGCCTGCTGACTGCCTTGCAG (SEQ ID NO:860)
RHO-262	GGCUUGGUGCUGCAAACAUGGC (SEQ ID NO:361)	GGCTTGGTGCTGCAAACATGGC (SEQ ID NO:861)
RHO-263	CAGGUAAUGGCACUGAGCAGAA (SEQ ID NO:362)	CAGGTAATGGCACTGAGCAGAA (SEQ ID NO:862)
RHO-264	UUGGAACGCGGCAGGGAGGCUG (SEQ ID NO:363)	TTGGAACGCGGCAGGGAGGCTG (SEQ ID NO:863)
RHO-265	UGUCCGGGUUAUUUCAUUUCCC (SEQ ID NO:364)	TGTCCGGGTTATTTCATTTCCC (SEQ ID NO:864)
RHO-266	CAGGUAGUACUGUGGGUACUCG (SEQ ID NO:365)	CAGGTAGTACTGTGGGTACTCG (SEQ ID NO:865)
RHO-267	AUAACAGAUCCCACUUAACAGA (SEQ ID NO:366)	ATAACAGATCCCACTTAACAGA (SEQ ID NO:866)
RHO-268	AGGGACGGGUGCAGAGUUGAGU (SEQ ID NO:367)	AGGGACGGGTGCAGAGTTGAGT (SEQ ID NO:867)
RHO-269	GAAGGAGAGAGCUUGGUGCUGG (SEQ ID NO:368)	GAAGGAGAGAGCTTGGTGCTGG (SEQ ID NO:868)
RHO-270	GGUCAGCCACGGCUAGGUUGAG (SEQ ID NO:369)	GGTCAGCCACGGCTAGGTTGAG (SEQ ID NO:869)

RHO-271	AUUUCACAGCAAGAAAACUGAG (SEQ ID NO:370)	ATTTCACAGCAAGAAAACTGAG (SEQ ID NO:870)
RHO-272	UCAAAGAAGUCAAGCGCCCUGC (SEQ ID NO:371)	TCAAAGAAGTCAAGCGCCCTGC (SEQ ID NO:871)
RHO-273	GCUGCUCCCACCCAAGAAUGCU (SEQ ID NO:372)	GCTGCTCCCACCCAAGAATGCT (SEQ ID NO:872)
RHO-274	GCAACAAACACCCAACAAUGGC (SEQ ID NO:373)	GCAACAAACACCCAACAATGGC (SEQ ID NO:873)
RHO-275	AAAUCCACUUCCCACCCUGAGC (SEQ ID NO:374)	AAATCCACTTCCCACCCTGAGC (SEQ ID NO:874)
RHO-276	CAGGGAGGCUGGAGGGGCACCU (SEQ ID NO:375)	CAGGGAGGCTGGAGGGGCACCT (SEQ ID NO:875)
RHO-277	GGGCAAGCCAGACCCCUCCUCU (SEQ ID NO:376)	GGGCAAGCCAGACCCCTCCTCT (SEQ ID NO:876)
RHO-278	CAGGAAAACAGAUGGGGUGCUG (SEQ ID NO:377)	CAGGAAAACAGATGGGGTGCTG (SEQ ID NO:877)
RHO-279	UUGGAGAAGGGCACGUAGAAGU (SEQ ID NO:378)	TTGGAGAAGGGCACGTAGAAGT (SEQ ID NO:878)
RHO-280	AGAGCUUGGUGCUGGGAGGAGG (SEQ ID NO:379)	AGAGCTTGGTGCTGGGAGGAGG (SEQ ID NO:879)
RHO-281	UAGCUAGGAAGGCAACCAGGAG (SEQ ID NO:380)	TAGCTAGGAAGGCAACCAGGAG (SEQ ID NO:880)
RHO-282	GGCUAGGUUGAGCAGGAUGUAG (SEQ ID NO:381)	GGCTAGGTTGAGCAGGATGTAG (SEQ ID NO:881)
RHO-283	CUCACCACCCCAUGAAGUUCCA (SEQ ID NO:382)	CTCACCACCCCATGAAGTTCCA (SEQ ID NO:882)
RHO-284	AAGCAAUGUGCAAUGUUUUGCC (SEQ ID NO:383)	AAGCAATGTGCAATGTTTTGCC (SEQ ID NO:883)
RHO-285	GGAAGACCCAAUGACUGGAGAA (SEQ ID NO:384)	GGAAGACCCAATGACTGGAGAA (SEQ ID NO:884)
RHO-286	UGGCCAGGACCACCAAGGACCA (SEQ ID NO:385)	TGGCCAGGACCACCAAGGACCA (SEQ ID NO:885)
RHO-287	AAAUAUUGUCCCUUUCACUGUU (SEQ ID NO:386)	AAATATTGTCCCTTTCACTGTT (SEQ ID NO:886)
RHO-288	CAUGAGCAACUUCCGCUUCGGG (SEQ ID NO:387)	CATGAGCAACTTCCGCTTCGGG (SEQ ID NO:887)
RHO-289	AGAGAUAUUCCUGGAUCACAGC (SEQ ID NO:388)	AGAGATATTCCTGGATCACAGC (SEQ ID NO:888)
RHO-290	CAUGGAGGGGUCUGGGAGAGUC (SEQ ID NO:389)	CATGGAGGGGTCTGGGAGAGTC (SEQ ID NO:889)
RHO-291	AUGUUUUGCCCAGAGGAAGAAG (SEQ ID NO:390)	ATGTTTTGCCCAGAGGAAGAAG (SEQ ID NO:890)
RHO-292	GUGGGUGGGGUGUGCAGGAGCC (SEQ ID NO:391)	GTGGGTGGGGTGTGCAGGAGCC (SEQ ID NO:891)
RHO-293	CCAGGUAAUGGCACUGAGCAGA (SEQ ID NO:392)	CCAGGTAATGGCACTGAGCAGA (SEQ ID NO:892)
RHO-294	CCCAACAAUGGCCAGAGAUUCC (SEQ ID NO:393)	CCCAACAATGGCCAGAGATTCC (SEQ ID NO:893)
RHO-295	GCACCUGAGGACAGGGGCUGAG (SEQ ID NO:394)	GCACCTGAGGACAGGGGCTGAG (SEQ ID NO:894)

RHO-296	GUCAGACCCAGGCUGGGCACUG (SEQ ID NO:395)	GTCAGACCCAGGCTGGGCACTG (SEQ ID NO:895)
RHO-297	GGGGCACCUGAGGACAGGGGCU (SEQ ID NO:396)	GGGGCACCTGAGGACAGGGGCT (SEQ ID NO:896)
RHO-298	AGAGGAAAACUGAGGCAGGGAG (SEQ ID NO:397)	AGAGGAAAACTGAGGCAGGGAG (SEQ ID NO:897)
RHO-299	AGGGAUAACAGAUCCCACUUAA (SEQ ID NO:398)	AGGGATAACAGATCCCACTTAA (SEQ ID NO:898)
RHO-300	CUUGGUGCUGGGAGGAGGGGGA (SEQ ID NO:399)	CTTGGTGCTGGGAGGAGGGGGA (SEQ ID NO:899)
RHO-301	AGAGGGUAGCUAGGAAGGCAAC (SEQ ID NO:400)	AGAGGGTAGCTAGGAAGGCAAC (SEQ ID NO:900)
RHO-302	UUGCACAUUGCUUCAUGGCUCC (SEQ ID NO:401)	TTGCACATTGCTTCATGGCTCC (SEQ ID NO:901)
RHO-303	GACCGAGCCCAUUGCCCAGCAC (SEQ ID NO:402)	GACCGAGCCCATTGCCCAGCAC (SEQ ID NO:902)
RHO-304	UGAACACUGCCUUGAUCUUAUU (SEQ ID NO:403)	TGAACACTGCCTTGATCTTATT (SEQ ID NO:903)
RHO-305	GGUGCACUCCCCCCUAGACAGG (SEQ ID NO:404)	GGTGCACTCCCCCCTAGACAGG (SEQ ID NO:904)
RHO-306	GCUUGGUGCUGGGAGGAGGGGG (SEQ ID NO:405)	GCTTGGTGCTGGGAGGAGGGGG (SEQ ID NO:905)
RHO-307	GGAUACUUCGUCUUCGGGCCCA (SEQ ID NO:406)	GGATACTTCGTCTTCGGGCCCA (SEQ ID NO:906)
RHO-308	AGUCAGACCCAGGCUGGGCACU (SEQ ID NO:407)	AGTCAGACCCAGGCTGGGCACT (SEQ ID NO:907)
RHO-309	AGCACCAAGCCUCUGUUUCCCU (SEQ ID NO:408)	AGCACCAAGCCTCTGTTTCCCT (SEQ ID NO:908)
RHO-310	UGGGCAAAGAAAUUCCAGGGAA (SEQ ID NO:409)	TGGGCAAAGAAATTCCAGGGAA (SEQ ID NO:909)
RHO-311	AGAGGGAUUUGAGGAGGCCUUG (SEQ ID NO:410)	AGAGGGATTTGAGGAGGCCTTG (SEQ ID NO:910)
RHO-312	GCAAUGUUUUGCCCAGAGGAAG (SEQ ID NO:411)	GCAATGTTTTGCCCAGAGGAAG (SEQ ID NO:911)
RHO-313	CAUGUCCGGGUUAUUUCAUUUC (SEQ ID NO:412)	CATGTCCGGGTTATTTCATTTC (SEQ ID NO:912)
RHO-314	AAGCCCAUGAGCAACUUCCGCU (SEQ ID NO:413)	AAGCCCATGAGCAACTTCCGCT (SEQ ID NO:913)
RHO-315	UCCCACCCUGAGCUUGGGCCCC (SEQ ID NO:414)	TCCCACCCTGAGCTTGGGCCCC (SEQ ID NO:914)
RHO-316	GAGAGAGCUUGGUGCUGGGAGG (SEQ ID NO:415)	GAGAGAGCTTGGTGCTGGGAGG (SEQ ID NO:915)
RHO-317	CUACGUGCCCUUCUCCAAUGCG (SEQ ID NO:416)	CTACGTGCCCTTCTCCAATGCG (SEQ ID NO:916)
RHO-318	CUUGCAUUUAACAGGAAAACAG (SEQ ID NO:417)	CTTGCATTTAACAGGAAAACAG (SEQ ID NO:917)
RHO-319	GAAAUGAAAUAACCCGGACAUG (SEQ ID NO:418)	GAAATGAAATAACCCGGACATG (SEQ ID NO:918)
RHO-320	CGAAGGCCUGAGCUCAGCCACU (SEQ ID NO:419)	CGAAGGCCTGAGCTCAGCCACT (SEQ ID NO:919)

RHO-321	GGAGGGUGCACUCCCCCCUAGA (SEQ ID NO:420)	GGAGGGTGCACTCCCCCCTAGA (SEQ ID NO:920)
RHO-322	CAGCACCAAGCCUCUGUUUCCC (SEQ ID NO:421)	CAGCACCAAGCCTCTGTTTCCC (SEQ ID NO:921)
RHO-323	GGGCAAAGAAAUUCCAGGGAAU (SEQ ID NO:422)	GGGCAAAGAAATTCCAGGGAAT (SEQ ID NO:922)
RHO-324	CUUCGGGGAGAACCAUGCCAUC (SEQ ID NO:423)	CTTCGGGGAGAACCATGCCATC (SEQ ID NO:923)
RHO-325	UGGGAGGAGGGGGAAGGGGCAG (SEQ ID NO:424)	TGGGAGGAGGGGGAAGGGGCAG (SEQ ID NO:924)
RHO-326	CCUAGACAGGGAGAGGGUAGCU (SEQ ID NO:425)	CCTAGACAGGGAGAGGGTAGCT (SEQ ID NO:925)
RHO-327	UAACAGAGAGGAAAACUGAGGC (SEQ ID NO:426)	TAACAGAGAGGAAAACTGAGGC (SEQ ID NO:926)
RHO-328	UCUCAGCCACCACCGCCAAGCC (SEQ ID NO:427)	TCTCAGCCACCACCGCCAAGCC (SEQ ID NO:927)
RHO-329	GUCAGCACAGACCCCACUGCCU (SEQ ID NO:428)	GTCAGCACAGACCCCACTGCCT (SEQ ID NO:928)
RHO-330	AGGAAAACUGAGGCAGGGAGAG (SEQ ID NO:429)	AGGAAAACTGAGGCAGGGAGAG (SEQ ID NO:929)
RHO-331	AGCCAUUGCAAAGCUGGGUGAC (SEQ ID NO:430)	AGCCATTGCAAAGCTGGGTGAC (SEQ ID NO:930)
RHO-332	AAAUGAAAUAACCCGGACAUGU (SEQ ID NO:431)	AAATGAAATAACCCGGACATGT (SEQ ID NO:931)
RHO-333	UAGCUACCCUCUCCCUGUCUAG (SEQ ID NO:432)	TAGCTACCCTCTCCCTGTCTAG (SEQ ID NO:932)
RHO-334	UGUGGGUGGGGUGUGCAGGAGC (SEQ ID NO:433)	TGTGGGTGGGGTGTGCAGGAGC (SEQ ID NO:933)
RHO-335	UGGGGAAGGAGAGAGCUUGGUG (SEQ ID NO:434)	TGGGGAAGGAGAGAGCTTGGTG (SEQ ID NO:934)
RHO-336	GACUUGGAACGCGGCAGGGAGG (SEQ ID NO:435)	GACTTGGAACGCGGCAGGGAGG (SEQ ID NO:935)
RHO-337	AAGGAGAGAGCUUGGUGCUGGG (SEQ ID NO:436)	AAGGAGAGAGCTTGGTGCTGGG (SEQ ID NO:936)
RHO-338	GGGAAGGAGAGAGCUUGGUGCU (SEQ ID NO:437)	GGGAAGGAGAGAGCTTGGTGCT (SEQ ID NO:937)
RHO-339	AUUUGAGGAGGCCUUGGGGAAG (SEQ ID NO:438)	ATTTGAGGAGGCCTTGGGGAAG (SEQ ID NO:938)
RHO-340	AUCCAGCUGGAGCCCUGAGUGG (SEQ ID NO:439)	ATCCAGCTGGAGCCCTGAGTGG (SEQ ID NO:939)
RHO-341	GAGAGCUUGGUGCUGGGAGGAG (SEQ ID NO:440)	GAGAGCTTGGTGCTGGGAGGAG (SEQ ID NO:940)
RHO-342	UCCUAGCUACCCUCUCCCUGUC (SEQ ID NO:441)	TCCTAGCTACCCTCTCCCTGTC (SEQ ID NO:941)
RHO-343	CCGAGGCAGCAGCCUGGACAUG (SEQ ID NO:442)	CCGAGGCAGCAGCCTGGACATG (SEQ ID NO:942)
RHO-344	GGGGAAGGAGAGAGCUUGGUGC (SEQ ID NO:443)	GGGGAAGGAGAGAGCTTGGTGC (SEQ ID NO:943)
RHO-345	UGCUGGGAGGAGGGGGAAGGGG (SEQ ID NO:444)	TGCTGGGAGGAGGGGGAAGGGG (SEQ ID NO:944)

RHO-346	CUUCUUGUGCUGGACGGUGACG (SEQ ID NO:445)	CTTCTTGTGCTGGACGGTGACG (SEQ ID NO:945)
RHO-347	UACCACACCCGUCGCAUUGGAG (SEQ ID NO:446)	TACCACACCCGTCGCATTGGAG (SEQ ID NO:946)
RHO-348	AGCAGCCUGGACAUGGGGGAGA (SEQ ID NO:447)	AGCAGCCTGGACATGGGGGAGA (SEQ ID NO:947)
RHO-349	AGCCAGGUAGUACUGUGGGUAC (SEQ ID NO:448)	AGCCAGGTAGTACTGTGGGTAC (SEQ ID NO:948)
RHO-350	GGCUGCUUGCGGUUCUCAACAC (SEQ ID NO:449)	GGCTGCTTGCGGTTCTCAACAC (SEQ ID NO:949)
RHO-351	GGACCGAGGCAGCAGCCUGGAC (SEQ ID NO:450)	GGACCGAGGCAGCAGCCTGGAC (SEQ ID NO:950)
RHO-352	CUGGGCAAAGAAAUUCCAGGGA (SEQ ID NO:451)	CTGGGCAAAGAAATTCCAGGGA (SEQ ID NO:951)
RHO-353	UGAGAGGGGAGGCAGAGGAUGC (SEQ ID NO:452)	TGAGAGGGGAGGCAGAGGATGC (SEQ ID NO:952)
RHO-354	GAGGGUGCACUCCCCCCUAGAC (SEQ ID NO:453)	GAGGGTGCACTCCCCCCTAGAC (SEQ ID NO:953)
RHO-355	CGGUUCUCAACACCAGGAGACU (SEQ ID NO:454)	CGGTTCTCAACACCAGGAGACT (SEQ ID NO:954)
RHO-356	UGUGCAAUGUUUUGCCCAGAGG (SEQ ID NO:455)	TGTGCAATGTTTTGCCCAGAGG (SEQ ID NO:955)
RHO-357	GGGGGAGACAGGGCAAGGCUGG (SEQ ID NO:456)	GGGGGAGACAGGGCAAGGCTGG (SEQ ID NO:956)
RHO-358	GCCGGGUGUGGGUGGGGUGUGC (SEQ ID NO:457)	GCCGGGTGTGGGTGGGGTGTGC (SEQ ID NO:957)
RHO-359	CUGCGUACCACACCCGUCGCAU (SEQ ID NO:458)	CTGCGTACCACACCCGTCGCAT (SEQ ID NO:958)
RHO-360	CACCCAAGAAUGCUGCGAAGGC (SEQ ID NO:459)	CACCCAAGAATGCTGCGAAGGC (SEQ ID NO:959)
RHO-361	CCUAGCUACCCUCUCCCUGUCU (SEQ ID NO:460)	CCTAGCTACCCTCTCCCTGTCT (SEQ ID NO:960)
RHO-362	CACCAGGAGACUUGGAACGCGG (SEQ ID NO:461)	CACCAGGAGACTTGGAACGCGG (SEQ ID NO:961)
RHO-363	UUGGAUAACAUUGACAGGACAG (SEQ ID NO:462)	TTGGATAACATTGACAGGACAG (SEQ ID NO:962)
RHO-364	UUCGGGCCCACAGGAUGCAAUU (SEQ ID NO:463)	TTCGGGCCCACAGGATGCAATT (SEQ ID NO:963)
RHO-365	GAAGUAUCCAUGCAGAGAGGUG (SEQ ID NO:464)	GAAGTATCCATGCAGAGAGGTG (SEQ ID NO:964)
RHO-366	GGUGUGCAGGAGCCCGGGAGCA (SEQ ID NO:465)	GGTGTGCAGGAGCCCGGGAGCA (SEQ ID NO:965)
RHO-367	GGAGCAGCCACGGGUCAGCCAC (SEQ ID NO:466)	GGAGCAGCCACGGGTCAGCCAC (SEQ ID NO:966)
RHO-368	AGCGCCCUGCUGGGGCGUCACA (SEQ ID NO:467)	AGCGCCCTGCTGGGGCGTCACA (SEQ ID NO:967)
RHO-369	GAGCCCGGGAGCAUGGAGGGGU (SEQ ID NO:468)	GAGCCCGGGAGCATGGAGGGGT (SEQ ID NO:968)
RHO-370	AGGGCCACAGCCAUGAAUGGCA (SEQ ID NO:469)	AGGGCCACAGCCATGAATGGCA (SEQ ID NO:969)

RHO-371	GCAAUGUGCAAUGUUUUGCCCA (SEQ ID NO:470)	GCAATGTGCAATGTTTTGCCCA (SEQ ID NO:970)
RHO-372	GAAGAGGUCAGCCACGGCUAGG (SEQ ID NO:471)	GAAGAGGTCAGCCACGGCTAGG (SEQ ID NO:971)
RHO-373	GGCCUUCGCAGCAUUCUUGGGU (SEQ ID NO:472)	GGCCTTCGCAGCATTCTTGGGT (SEQ ID NO:972)
RHO-374	UUAACAGAGAGGAAAACUGAGG (SEQ ID NO:473)	TTAACAGAGAGGAAAACTGAGG (SEQ ID NO:973)
RHO-375	UGAUGGCAUGGUUCUCCCCGAA (SEQ ID NO:474)	TGATGGCATGGTTCTCCCCGAA (SEQ ID NO:974)
RHO-376	ACCGAGGCAGCAGCCUGGACAU (SEQ ID NO:475)	ACCGAGGCAGCAGCCTGGACAT (SEQ ID NO:975)
RHO-377	AGGGACCACACGCUGAGGAGAG (SEQ ID NO:476)	AGGGACCACACGCTGAGGAGAG (SEQ ID NO:976)
RHO-378	UGGAACGCGGCAGGGAGGCUGG (SEQ ID NO:477)	TGGAACGCGGCAGGGAGGCTGG (SEQ ID NO:977)
RHO-379	UGCACAUUGCUUCAUGGCUCCU (SEQ ID NO:478)	TGCACATTGCTTCATGGCTCCT (SEQ ID NO:978)
RHO-380	GCGUUCCAAGUCUCCUGGUGUU (SEQ ID NO:479)	GCGTTCCAAGTCTCCTGGTGTT (SEQ ID NO:979)
RHO-381	GGGUGUGCAGGAGCCCGGGAGC (SEQ ID NO:480)	GGGTGTGCAGGAGCCCGGGAGC (SEQ ID NO:980)
RHO-382	GGCAAAGAAAUUCCAGGGAAUG (SEQ ID NO:481)	GGCAAAGAAATTCCAGGGAATG (SEQ ID NO:981)
RHO-383	GGCUGGAGGGGCACCUGAGGAC (SEQ ID NO:482)	GGCTGGAGGGGCACCTGAGGAC (SEQ ID NO:982)
RHO-384	GCGCCCUGCUGGGGCGUCACAC (SEQ ID NO:483)	GCGCCCTGCTGGGGCGTCACAC (SEQ ID NO:983)
RHO-385	GCGUACCACACCCGUCGCAUUG (SEQ ID NO:484)	GCGTACCACACCCGTCGCATTG (SEQ ID NO:984)
RHO-386	ACCAGGAGACUUGGAACGCGGC (SEQ ID NO:485)	ACCAGGAGACTTGGAACGCGGC (SEQ ID NO:985)
RHO-387	GCUGCUGCCUCGGUCCCAUUCU (SEQ ID NO:486)	GCTGCTGCCTCGGTCCCATTCT (SEQ ID NO:986)
RHO-388	GAAGCCCUCCAAAUUGCAUCCU (SEQ ID NO:487)	GAAGCCCTCCAAATTGCATCCT (SEQ ID NO:987)
RHO-389	CGUAGAGCGUGAGGAAGUUGAU (SEQ ID NO:488)	CGTAGAGCGTGAGGAAGTTGAT (SEQ ID NO:988)
RHO-390	CUGAAGCAGUUCCUUUUUGCUU (SEQ ID NO:489)	CTGAAGCAGTTCCTTTTTGCTT (SEQ ID NO:989)
RHO-391	GCUGGACGGUGACGUAGAGCGU (SEQ ID NO:490)	GCTGGACGGTGACGTAGAGCGT (SEQ ID NO:990)
RHO-392	UGAGGGCUUUGGAUAACAUUGA (SEQ ID NO:491)	TGAGGGCTTTGGATAACATTGA (SEQ ID NO:991)
RHO-393	AGCCGGGUGUGGGUGGGGUGUG (SEQ ID NO:492)	AGCCGGGTGTGGGTGGGGTGTG (SEQ ID NO:992)
RHO-394	CUCAGUUUUCCUCUCUGUUAAG (SEQ ID NO:493)	CTCAGTTTTCCTCTCTGTTAAG (SEQ ID NO:993)
RHO-395	CAAGACAUUAUUCUAAAGCAAA (SEQ ID NO:494)	CAAGACATTATTCTAAAGCAAA (SEQ ID NO:994)

RHO-396	UGGAACUUCAUGGGGUGGUGAG (SEQ ID NO:495)	TGGAACTTCATGGGGTGGTGAG (SEQ ID NO:995)
RHO-397	GAGAGGGAUUUGAGGAGGCCUU (SEQ ID NO:496)	GAGAGGGATTTGAGGAGGCCTT (SEQ ID NO:996)
RHO-398	CUUCGGGCCCACAGGAUGCAAU (SEQ ID NO:497)	CTTCGGGCCCACAGGATGCAAT (SEQ ID NO:997)
RHO-399	ACUUGGAACGCGGCAGGGAGGC (SEQ ID NO:498)	ACTTGGAACGCGGCAGGGAGGC (SEQ ID NO:998)
RHO-400	AUGGCCAGAGAUUCCCUGAGAA (SEQ ID NO:499)	ATGGCCAGAGATTCCCTGAGAA (SEQ ID NO:999)
RHO-401	CCUCAGUUUUCCUCUCUGUUAA (SEQ ID NO:500)	CCTCAGTTTTCCTCTCTGTTAA (SEQ ID NO:1000)
RHO-402	UAACAGAUCCCACUUAACAGAG (SEQ ID NO:501)	TAACAGATCCCACTTAACAGAG (SEQ ID NO:1001)
RHO-403	GGGAGAGGGAUUUGAGGAGGCC (SEQ ID NO:502)	GGGAGAGGGATTTGAGGAGGCC (SEQ ID NO:1002)

Incorporation by Reference

All publications, patents, and patent applications mentioned herein are hereby incorporated by reference in their entirety as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated by reference. In case of conflict, the present application, including any definitions herein, will control.

Equivalents

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the disclosure described herein. Such equivalents are intended to be encompassed by the following claims.

Additional Sequences

Exemplary sequences that may be used in certain embodiments are set forth below:

AAV ITR:
TGCAGGCAGCTGCGCGCTCGCTCGCTCACTGAGGCCGCCCGGGCAAAGCCCGGGCGTCGGGC
GACCTTTGGTCGCCCGGCCTCAGTGAGCGAGCGAGCGCGCAGAGAGGGAGTGGCCAACTCCA
TCACTAGGGGTTCCT (SEQ ID NO:92)

U6 Promoter:
AAGGTCGGGCAGGAAGAGGGCCTATTTCCCATGATTCCTTCATATTTGCATATACGATACAA
GGCTGTTAGAGAGATAATTAGAATTAATTTGACTGTAAACACAAAGATATTAGTACAAAATA
CGTGACGTAGAAAGTAATAATTTCTTGGGTAGTTTGCAGTTTTAAAATTATGTTTTAAAATG
GACTATCATATGCTTACCGTAACTTGAAAGTATTTCGATTTCTTGGCTTTATATATCTTGTG
GAAAGGACGAAACACC (SEQ ID NO:78)

Exemplary saCas9 gRNA protospacer:
CCCACACCCGGCTCATACCGCC (SEQ ID NO:606)

Guide RNA scaffold sequence:
GTTATAGTACTCTGGAAACAGAATCTACTATAACAAGGCAAAATGCCGTGTTTATCTCGTCA
ACTTGTTGGCGAGA (SEQ ID NO:12)

Minimal RHO Promoter (250 bp):
GTCACCTTGGCCCCTCTTAGAAGCCAATTAGGCCCTCAGTTTCTGCAGCGGGGATTAATATG
ATTATGAACACCCCCAATCTCCCAGATGCTGATTCAGCCAGGAGCTTAGGAGGGGGAGGTCA
CTTTATAAGGGTCTGGGGGGGTCAGAACCCAGAGTCATCCAGCTGGAGCCCTGAGTGGCTGA
GCTCAGGCCTTCGCAGCATTCTTGGGTGGGAGCAGCCACGGGTCAGCCACAAGGGCCACAGC
C (SEQ ID NO:44)

SV40 Intron:
TCTAGAGGATCCGGTACTCGAGGAACTGAAAAACCAGAAAGTTAACTGGTAAGTTTAGTCTT
TTTGTCTTTTATTTCAGGTCCCGGATCCGGTGGTGGTGCAAATCAAAGAACTGCTCCTCAGT
GGATGTTGCCTTTACTTCTAGGCCTGTACGGAAGTGTTAC (SEQ ID NO:94)

Codon Optimized RHO-encoding sequence 1 (Codon 1):
ATGAACGGCACCGAGGGCCCCAACTTCTACGTCCCCTTCAGCAACGCCACCGGCGTCGTCCG
CAGCCCCTTCGAGTACCCCCAGTACTACCTGGCCGAGCCCTGGCAGTTCAGCATGCTGGCCG
CCTACATGTTCCTGCTGATCGTCCTGGGCTTCCCCATCAACTTCCTGACCCTGTACGTCACC
GTCCAGCACAAGAAGCTGCGCACCCCCCTGAACTACATCCTGCTGAACCTGGCCGTCGCCGA
CCTGTTCATGGTCCTGGGCGGCTTCACCAGCACCCTGTACACCAGCCTGCACGGCTACTTCG
TCTTCGGCCCCACCGGCTGCAACCTGGAGGGCTTCTTCGCCACCCTGGGCGGCGAGATCGCC
CTGTGGAGCCTGGTCGTCCTGGCCATCGAGCGCTACGTCGTCGTCTGCAAGCCCATGAGCAA
CTTCCGCTTCGGCGAGAACCACGCCATCATGGGCGTCGCCTTCACCTGGGTCATGGCCCTGG
CCTGCGCCGCCCCCCCCCTGGCCGGCTGGAGCCGCTACATCCCCGAGGGCCTGCAGTGCAGC
TGCGGCATCGACTACTACACCCTGAAGCCCGAGGTCAACAACGAGAGCTTCGTCATCTACAT
GTTCGTCGTCCACTTCACCATCCCCATGATCATCATCTTCTTCTGCTACGGCCAGCTGGTCT
TCACCGTCAAGGAGGCCGCCGCCCAGCAGCAGGAGAGCGCCACCACCCAGAAGGCCGAGAAG
GAGGTCACCCGCATGGTCATCATCATGGTCATCGCCTTCCTGATCTGCTGGGTCCCCTACGC
CAGCGTCGCCTTCTACATCTTCACCCACCAGGGCAGCAACTTCGGCCCCATCTTCATGACCA
TCCCCGCCTTCTTCGCCAAGAGCGCCGCCATCTACAACCCCGTCATCTACATCATGATGAAC
AAGCAGTTCCGCAACTGCATGCTGACCACCATCTGCTGCGGCAAGAACCCCCTGGGCGACGA
CGAGGCCAGCGCCACCGTCAGCAAGACCGAGACCAGCCAGGTCGCCCCCGCCTAA (SEQ
ID NO: 13)

Codon Optimized RHO-encoding sequence 2 (Codon 2):
ATGAACGGCACCGAGGGCCCCAACTTCTACGTGCCCTTCTCCAACGCCACCGGCGTGGTGCG
CTCCCCCTTCGAGTACCCCCAGTACTACCTGGCCGAGCCCTGGCAGTTCTCCATGCTGGCCG
CCTACATGTTCCTGCTGATCGTGCTGGGCTTCCCCATCAACTTCCTGACCCTGTACGTGACC
GTGCAGCACAAGAAGCTGCGCACCCCCCTGAACTACATCCTGCTGAACCTGGCCGTGGCCGA
CCTGTTCATGGTGCTGGGCGGCTTCACCTCCACCCTGTACACCTCCCTGCACGGCTACTTCG
TGTTCGGCCCCACCGGCTGCAACCTGGAGGGCTTCTTCGCCACCCTGGGCGGCGAGATCGCC
CTGTGGTCCCTGGTGGTGCTGGCCATCGAGCGCTACGTGGTGGTGTGCAAGCCCATGTCCAA
CTTCCGCTTCGGCGAGAACCACGCCATCATGGGCGTGGCCTTCACCTGGGTGATGGCCCTGG
CCTGCGCCGCCCCCCCCCTGGCCGGCTGGTCCCGCTACATCCCCGAGGGCCTGCAGTGCTCC

TGCGGCATCGACTACTACACCCTGAAGCCCGAGGTGAACAACGAGTCCTTCGTGATCTACAT
GTTCGTGGTGCACTTCACCATCCCCATGATCATCATCTTCTTCTGCTACGGCCAGCTGGTGT
TCACCGTGAAGGAGGCCGCCGCCCAGCAGCAGGAGTCCGCCACCACCCAGAAGGCCGAGAAG
GAGGTGACCCGCATGGTGATCATCATGGTGATCGCCTTCCTGATCTGCTGGGTGCCCTACGC
CTCCGTGGCCTTCTACATCTTCACCCACCAGGGCTCCAACTTCGGCCCCATCTTCATGACCA
TCCCCGCCTTCTTCGCCAAGTCCGCCGCCATCTACAACCCCGTGATCTACATCATGATGAAC
AAGCAGTTCCGCAACTGCATGCTGACCACCATCTGCTGCGGCAAGAACCCCCTGGGCGACGA
CGAGGCCTCCGCCACCGTGTCCAAGACCGAGACCTCCCAGGTGGCCCCCGCCTAA (SEQ
ID NO: 14)

Codon Optimized RHO-encoding sequence 3 (Codon 3):

ATGAACGGCACCGAGGGCCCCAACTTCTACGTCCCCTTCAGCAACGCCACCGGCGTCGTCCG
CAGCCCCTTCGAGTACCCCCAGTACTACCTGGCCGAGCCCTGGCAGTTCTCTATGCTGGCCG
CCTACATGTTCCTGCTGATCGTCCTGGGCTTCCCTATCAACTTCCTCACCCTCTACGTCACC
GTCCAGCACAAGAAGCTCCGCACCCCTCTCAACTACATCCTCCTTAACCTTGCCGTCGCCGA
CCTTTTCATGGTCCTTGGCGGCTTCACCTCTACTCTTTACACTTCTTTGCACGGGTACTTCG
TGTTCGGTCCTACTGGTTGCAACTTGGAGGGTTTCTTCGCCACTTTGGGTGGTGAGATCGCC
TTGTGGTCGTTGGTGGTGTTAGCTATCGAGCGATACGTGGTGGTGTGCAAGCCTATGTCGAA
CTTCCGGTTCGGTGAGAATCATGCTATCATGGGAGTGGCTTTTACTTGGGTGATGGCTTTAG
CTTGCGCTGCTCCTCCGTTAGCTGGATGGTCGCGTTATATCCCGGAGGGATTACAGTGCTCA
TGCGGAATCGACTATTATACTCTAAAGCCGGAAGTTAATAATGAATCATTTGTTATTTATAT
GTTTGTTGTTCATTTTACAATTCCGATGATTATTATTTTTTTTTGTTATGGACAGCTAGTTT
TTACAGTTAAGGAAGCAGCAGCACAGCAACAAGAATCAGCAACAACACAAAAGGCAGAAAAA
GAAGTTACAAGGATGGTTATTATTATGGTAATTGCATTTCTAATATGTTGGGTACCGTATGC
ATCCGTAGCATTTTATATATTTACACATCAAGGGTCCAATTTTGGGCCAATATTTATGACGA
TACCAGCGTTTTTTGCGAAATCCGCGGCGATATATAATCCAGTAATATATATAATGATGAAT
AAACAATTTAGAAATTGTATGCTAACGACGATATGTTGTGGGAAAAATCCACTAGGGGATGA
TGAAGCGAGTGCGACGGTAAGTAAAACGGAAACGAGTCAAGTAGCGCCAGCGTAA (SEQ
ID NO: 15)

Codon Optimized RHO-encoding sequence 4 (Codon 4):

ATGAACGGCACCGAGGGTCCCAATTTCTACGTCCCATTTTCCAACGCCACGGGGGTGGTACG
CAGCCCTTTCGAATATCCGCAGTACTATCTGGCTGAGCCCTGGCAGTTTTCTATGCTCGCAG
CGTACATGTTCTTGCTAATCGTTCTGGGATTTCCAATTAATTTCCTCACATTGTATGTCACC
GTGCAGCACAAGAAGCTACGGACGCCTCTGAACTACATCCTCTTGAATCTAGCCGTCGCTGA
CCTGTTTATGGTTCTCGGCGGTTTCACATCGACCTTGTATACGTCACTACATGGGTACTTTG
TCTTCGGACCGACAGGCTGCAACCTGGAAGGTTTTTTCGCAACCCTCGGGGGAGAGATTGCG
TTGTGGTCCCTAGTGGTACTGGCCATCGAAAGGTATGTTGTCGTGTGTAAGCCCATGAGCAA
TTTTCGCTTCGGCGAGAACCACGCTATTATGGGTGTAGCATTTACGTGGGTTATGGCGCTCG
CCTGCGCTGCACCACCTTTGGCGGGGTGGTCTCGGTACATCCCGGAAGGACTACAGTGTTCG
TGCGGCATTGATTATTACACACTGAAGCCCGAGGTCAATAACGAATCATTCGTGATCTATAT
GTTTGTAGTTCATTTCACCATTCCAATGATCATTATCTTTTTCTGTTACGGTCAGCTCGTCT
TTACGGTGAAGGAGGCCGCTGCACAGCAGCAGGAATCCGCGACAACCCAGAAGGCCGAGAAG
GAAGTAACGAGGATGGTTATTATCATGGTCATTGCTTTCTTGATCTGCTGGGTGCCTTATGC
AAGCGTAGCGTTTTACATTTTCACACACCAGGGGTCTAATTTTGGACCGATCTTCATGACCA
TTCCCGCCTTTTTTCGCTAAGTCGGCAGCGATCTATAACCCAGTTATTTACATCATGATGAAT
AAGCAGTTTCGCAACTGTATGCTAACGACAATTTGCTGTGGCAAGAATCCTCTGGGTGACGA

TGAGGCCTCAGCTACCGTCTCCAAGACGGAAACAAGCCAGGTGGCACCGGCGTAA (SEQ ID NO: 16)

Codon Optimized RHO-encoding sequence 5 (Codon 5):

ATGAATGGGACTGAAGGACCTAATTTCTATGTGCCATTTAGCAATGCTACTGGCGTTGTCAG
AAGCCCCTTCGAATATCCACAATACTATCTGGCCGAACCTTGGCAGTTCAGCATGCTCGCTG
CCTATATGTTTCTGCTGATTGTGCTGGGCTTTCCCATAAATTTCCTCACCCTGTATGTTACT
GTTCAACACAAAAAGCTGCGGACGCCTCTGAACTACATACTGCTGAACCTGGCCGTCGCCGA
CCTGTTTATGGTCCTGGGAGGCTTTACAAGCACTCTGTATACAAGCCTGCACGGCTACTTCG
TGTTCGGCCCCACAGGCTGCAACCTCGAAGGCTTCTTTGCCACCCTCGGAGGAGAGATTGCC
CTGTGGAGCCTGGTGGTGCTGGCCATCGAAAGGTATGTGGTGGTGTGTAAACCCATGTCCAA
TTTTCGGTTCGGCGAGAACCACGCTATTATGGGAGTGGCTTTCACTTGGGTGATGGCCCTGG
CCTGCGCCGCCCCACCACTGGCCGGGTGGAGCCGGTACATCCCAGAGGGGCTGCAATGTAGC
TGCGGAATCGACTATTATACCCTGAAACCAGAGGTGAACAACGAGAGCTTTGTGATTTATAT
GTTTGTGGTGCATTTTACAATTCCTATGATTATCATTTTCTTCTGTTACGGGCAACTGGTGT
TTACCGTGAAGGAAGCCGCCGCTCAACAGCAGGAGAGCGCCACAACCCAAAAGGCCGAGAAG
GAGGTGACCAGAATGGTGATTATTATGGTGATCGCTTTTCTGATTTGCTGGGTGCCATACGC
TAGCGTCGCTTTCTATATTTTCACTCACCAGGGGAGCAACTTCGGCCCCATTTTCATGACAA
TCCCTGCCTTTTTTGCTAAAAGCGCCGCCATCTATAACCCAGTGATCTACATCATGATGAAC
AAACAGTTTAGGAACTGTATGCTCACAACAATCTGCTGTGGAAAGAACCCCCTCGGCGATGA
CGAAGCCAGCGCCACCGTCAGCAAGACAGAAACAAGCCAGGTGGCCCCTGCCTAA (SEQ ID NO: 17)

Codon Optimized RHO-encoding sequence 6 (Codon 6):

ATGAATGGCACAGAGGGCCCTAACTTCTACGTGCCCTTTAGCAATGCCACAGGCGTCGTGCG
GAGCCCTTTTGAGTACCCTCAGTACTATCTGGCCGAGCCTTGGCAGTTTAGCATGCTGGCCG
CCTACATGTTCCTGCTGATCGTGCTGGGCTTCCCCATCAACTTTCTGACCCTGTACGTGACC
GTGCAGCACAAGAAGCTGCGGACCCCTCTGAACTACATCCTGCTGAATCTGGCCGTGGCCGA
CCTGTTTATGGTGCTCGGCGGCTTTACCAGCACACTGTACACAAGCCTGCACGGCTACTTCG
TGTTTGGCCCCACCGGCTGCAATCTGGAAGGCTTTTTTGCCACACTCGGCGGCGAAATTGCT
CTGTGGTCACTGGTGGTGCTGGCCATCGAGAGATACGTGGTCGTGTGCAAGCCCATGAGCAA
CTTCAGATTCGGCGAGAACCACGCCATCATGGGCGTCGCCTTTACATGGGTTATGGCCCTGG
CTTGTGCAGCTCCTCCTCTTGCCGGCTGGTCCAGATATATTCCTGAGGGCCTGCAGTGCAGC
TGCGGCATCGATTACTACACCCTGAAGCCTGAAGTGAACAACGAGAGCTTCGTGATCTACAT
GTTTGTGGTGCACTTCACGATCCCCATGATCATCATATTCTTTTGCTACGGCCAGCTGGTGT
TCACCGTGAAAGAAGCCGCTGCTCAGCAGCAAGAGAGCGCCACAACACAGAAAGCCGAGAAA
GAAGTGACCCGGATGGTCATTATCATGGTTATCGCCTTTCTGATCTGTTGGGTGCCCTACGC
CAGCGTGGCCTTCTACATCTTTACCCACCAAGGCAGCAACTTCGGCCCCATCTTTATGACAA
TCCCCGCCTTCTTTGCCAAGAGCGCCGCCATCTACAACCCCGTGATCTATATCATGATGAAC
AAGCAGTTCCGCAACTGCATGCTGACCACCATCTGCTGCGGAAAGAACCCTCTGGGAGATGA
TGAGGCCAGCGCCACCGTGTCTAAGACCGAAACATCTCAGGTGGCCCCTGCATGA (SEQ ID NO: 18)

Hemoglobin A1 (HBA1) 3' UTR:

GCTGGAGCCTCGGTGGCCATGCTTCTTGCCCCTTGGGCCTCCCCCCAGCCCCTCCTCCCCTT
CCTGCACCCGTACCCCCGTGGTCTTTGAATAAAGTCTGAGTGGGCGGCA (SEQ ID NO:38)

Minimal UTR (minPolyA):
TAGCAATAAAGGATCGTTTATTTTCATTGGAAGCGTGTGTTGGTTTTTTGATCAGGCGCG
(SEQ ID NO:56)

Inverted ITR sequence:
AGGAACCCCTAGTGATGGAGTTGGCCACTCCCTCTCTGCGCGCTCGCTCGCTCACTGAGGCC
GGGCGACCAAAGGTCGCCCGACGCCCGGGCTTTGCCCGGGCGGCCTCAGTGAGCGAGCGAGC
GCGCAGCTGCCTGCA (SEQ ID NO:93)

Exemplary replacement vector (250 bp minimal RHO promoter driving codon-optimized RHO cDNA; U6 promoter driving gRNA targeting RHO) (see **Fig. 16** for feature annotation):
TGCAGGCAGCTGCGCGCTCGCTCGCTCACTGAGGCCGCCCGGGCAAAGCCCGGGCGTCGGGC
GACCTTTGGTCGCCCGGCCTCAGTGAGCGAGCGAGCGCGCAGAGAGGGAGTGGCCAACTCCA
TCACTAGGGGTTCCTGCGGCCGCGGTTCCTCAGATCTGAATTCGGTACCAAGGTCGGGCAGG
AAGAGGGCCTATTTCCCATGATTCCTTCATATTTGCATATACGATACAAGGCTGTTAGAGAG
ATAATTAGAATTAATTTGACTGTAAACACAAAGATATTAGTACAAAATACGTGACGTAGAAA
GTAATAATTTCTTGGGTAGTTTGCAGTTTTAAAATTATGTTTTAAAATGGACTATCATATGC
TTACCGTAACTTGAAAGTATTTCGATTTCTTGGCTTTATATATCTTGTGGAAAGGACGAAAC
ACCGCCCACACCCGGCTCATACCGCCGTTATAGTACTCTGGAAACAGAATCTACTATAACAA
GGCAAAATGCCGTGTTTATCTCGTCAACTTGTTGGCGAGATTTTTTCGACTTAGTTCGATCG
AAGGAAGGTCGGGCAGGAAGAGGGCCTATTTCCCATGATTCCTTCATATTTGCATATACGAT
ACAAGGCTGTTAGAGAGATAATTAGAATTAATTTGACTGTAAACACAAAGATATTAGTACAA
AATACGTGACGTAGAAAGTAATAATTTCTTGGGTAGTTTGCAGTTTTAAAATTATGTTTTAA
AATGGACTATCATATGCTTACCGTAACTTGAAAGTATTTCGATTTCTTGGCTTTATATATCT
TGTGGAAAGGACGAAACACCGCCCACACCCGGCTCATACCGCCGTTATAGTACTCTGGAAAC
AGAATCTACTATAACAAGGCAAAATGCCGTGTTTATCTCGTCAACTTGTTGGCGAGATTTTT
TGGTACCGCTAGCGCTGTCACCTTGGCCCCTCTTAGAAGCCAATTAGGCCCTCAGTTTCTGC
AGCGGGGATTAATATGATTATGAACACCCCCAATCTCCCAGATGCTGATTCAGCCAGGAGCT
TAGGAGGGGGAGGTCACTTTATAAGGGTCTGGGGGGGTCAGAACCCAGAGTCATCCAGCTGG
AGCCCTGAGTGGCTGAGCTCAGGCCTTCGCAGCATTCTTGGGTGGGAGCAGCCACGGGTCAG
CCACAAGGGCCACAGCCTCTAGAGGATCCGGTACTCGAGGAACTGAAAAACCAGAAAGTTAA
CTGGTAAGTTTAGTCTTTTTGTCTTTTATTTCAGGTCCCGGATCCGGTGGTGGTGCAAATCA
AAGAACTGCTCCTCAGTGGATGTTGCCTTTACTTCTAGGCCTGTACGGAAGTGTTACTCCGC
CACCATGAATGGCACAGAGGGCCCTAACTTCTACGTGCCCTTTAGCAATGCCACAGGCGTCG
TGCGGAGCCCTTTTGAGTACCCTCAGTACTATCTGGCCGAGCCTTGGCAGTTTAGCATGCTG
GCCGCCTACATGTTCCTGCTGATCGTGCTGGGCTTCCCCATCAACTTTCTGACCCTGTACGT
GACCGTGCAGCACAAGAAGCTGCGGACCCCTCTGAACTACATCCTGCTGAATCTGGCCGTGG
CCGACCTGTTTATGGTGCTCGGCGGCTTTACCAGCACACTGTACACAAGCCTGCACGGCTAC
TTCGTGTTTGGCCCCACCGGCTGCAATCTGGAAGGCTTTTTTGCCACACTCGGCGGCGAAAT
TGCTCTGTGGTCACTGGTGGTGCTGGCCATCGAGAGATACGTGGTCGTGTGCAAGCCCATGA
GCAACTTCAGATTCGGCGAGAACCACGCCATCATGGGCGTCGCCTTTACATGGGTTATGGCC
CTGGCTTGTGCAGCTCCTCCTCTTGCCGGCTGGTCCAGATATATTCCTGAGGGCCTGCAGTG
CAGCTGCGGCATCGATTACTACACCCTGAAGCCTGAAGTGAACAACGAGAGCTTCGTGATCT
ACATGTTTGTGGTGCACTTCACGATCCCCATGATCATCATATTCTTTTGCTACGGCCAGCTG
GTGTTCACCGTGAAAGAAGCCGCTGCTCAGCAGCAAGAGAGCGCCACAACACAGAAAGCCGA
GAAAGAAGTGACCCGGATGGTCATTATCATGGTTATCGCCTTTCTGATCTGTTGGGTGCCCT
ACGCCAGCGTGGCCTTCTACATCTTTACCCACCAAGGCAGCAACTTCGGCCCCATCTTTATG

ACAATCCCCGCCTTCTTTGCCAAGAGCGCCGCCATCTACAACCCCGTGATCTATATCATGAT
GAACAAGCAGTTCCGCAACTGCATGCTGACCACCATCTGCTGCGGAAAGAACCCTCTGGGAG
ATGATGAGGCCAGCGCCACCGTGTCTAAGACCGAAACATCTCAGGTGGCCCCTGCATGAGCT
GGAGCCTCGGTGGCCATGCTTCTTGCCCCTTGGGCCTCCCCCCAGCCCCTCCTCCCCTTCCT
GCACCCGTACCCCCGTGGTCTTTGAATAAAGTCTGAGTGGGCGGCACATGCTGGGGAGAGAT
CTGCGGCCGCAGGAACCCCTAGTGATGGAGTTGGCCACTCCCTCTCTGCGCGCTCGCTCGCT
CACTGAGGCCGGGCGACCAAAGGTCGCCCGACGCCCGGGCTTTGCCCGGGCGGCCTCAGTGA
GCGAGCGAGCGCGCAGCTGCCTGCA (SEQ ID NO:11)

Cas9 Vector 2 (250 bp minimal RHO promoter driving Cas9 w/ alpha globin UTR) (see **Fig. 17** for feature annotation):

CCTGCAGGCAGCTGCGCGCTCGCTCGCTCACTGAGGCCGCCCGGGCAAAGCCCGGGCGTCGG
GCGACCTTTGGTCGCCCGGCCTCAGTGAGCGAGCGAGCGCGCAGAGAGGGAGTGGCCAACTC
CATCACTAGGGGTTCCTAAGCGGCCGCGGTTCCTCAGATCTGAATTCGGTACCTGTCACCTT
GGCCCCTCTTAGAAGCCAATTAGGCCCTCAGTTTCTGCAGCGGGGATTAATATGATTATGAA
CACCCCCAATCTCCCAGATGCTGATTCAGCCAGGAGCTTAGGAGGGGGAGGTCACTTTATAA
GGGTCTGGGGGGGGTCAGAACCCAGAGTCATCCAGCTGGAGCCCTGAGTGGCTGAGCTCAGGC
CTTCGCAGCATTCTTGGGTGGGAGCAGCCACGGGTCAGCCACAAGGGCCACAGCCTCTAGAG
GATCCGGTACTCGAGGAACTGAAAAACCAGAAAGTTAACTGGTAAGTTTAGTCTTTTTGTCT
TTTATTTCAGGTCCCGGATCCGGTGGTGGTGCAAATCAAAGAACTGCTCCTCAGTGGATGTT
GCCTTTACTTCTAGGCCTGTACGGAAGTGTTACTCCGCCACCATGGGACCGAAGAAAAAGCG
CAAGGTCGAAGCGTCCATGAAAAGGAACTACATTCTGGGGCTGGACATCGGGATTACAAGCG
TGGGGTATGGGATTATTGACTATGAAACAAGGGACGTGATCGACGCAGGCGTCAGACTGTTC
AAGGAGGCCAACGTGGAAAACAATGAGGGACGGAGAAGCAAGAGGGGAGCCAGGCGCCTGAA
ACGACGGAGAAGGCACAGAATCCAGAGGGTGAAGAAACTGCTGTTCGATTACAACCTGCTGA
CCGACCATTCTGAGCTGAGTGGAATTAATCCTTATGAAGCCAGGGTGAAAGGCCTGAGTCAG
AAGCTGTCAGAGGAAGAGTTTTCCGCAGCTCTGCTGCACCTGGCTAAGCGCCGAGGAGTGCA
TAACGTCAATGAGGTGGAAGAGGACACCGGCAACGAGCTGTCTACAAAGGAACAGATCTCAC
GCAATAGCAAAGCTCTGGAAGAGAAGTATGTCGCAGAGCTGCAGCTGGAACGGCTGAAGAAA
GATGGCGAGGTGAGAGGGTCAATTAATAGGTTCAAGACAAGCGACTACGTCAAAGAAGCCAA
GCAGCTGCTGAAAGTGCAGAAGGCTTACCACCAGCTGGATCAGAGCTTCATCGATACTTATA
TCGACCTGCTGGAGACTCGGAGAACCTACTATGAGGGACCAGGAGAAGGGAGCCCCTTCGGA
TGGAAAGACATCAAGGAATGGTACGAGATGCTGATGGGACATTGCACCTATTTTCCAGAAGA
GCTGAGAAGCGTCAAGTACGCTTATAACGCAGATCTGTACAACGCCCTGAATGACCTGAACA
ACCTGGTCATCACCAGGGATGAAAACGAGAAACTGGAATACTATGAGAAGTTCCAGATCATC
GAAAACGTGTTTAAGCAGAAGAAAAAGCCTACACTGAAACAGATTGCTAAGGAGATCCTGGT
CAACGAAGAGGACATCAAGGGCTACCGGGTGACAAGCACTGGAAAACCAGAGTTCACCAATC
TGAAAGTGTATCACGATATTAAGGACATCACAGCACGGAAAGAAATCATTGAGAACGCCGAA
CTGCTGGATCAGATTGCTAAGATCCTGACTATCTACCAGAGCTCCGAGGACATCCAGGAAGA
GCTGACTAACCTGAACAGCGAGCTGACCCAGGAAGAGATCGAACAGATTAGTAATCTGAAGG
GGTACACCGGAACACACAACCTGTCCCTGAAAGCTATCAATCTGATTCTGGATGAGCTGTGG
CATACAAACGACAATCAGATTGCAATCTTTAACCGGCTGAAGCTGGTCCCAAAAAAGGTGGA
CCTGAGTCAGCAGAAAGAGATCCCAACCACACTGGTGGACGATTTCATTCTGTCACCCGTGG
TCAAGCGGAGCTTCATCCAGAGCATCAAAGTGATCAACGCCATCATCAAGAAGTACGGCCTG
CCCAATGATATCATTATCGAGCTGGCTAGGGAGAAGAACAGCAAGGACGCACAGAAGATGAT
CAATGAGATGCAGAAACGAAACCGGCAGACCAATGAACGCATTGAAGAGATTATCCGAACTA
CCGGGAAAGAGAACGCAAAGTACCTGATTGAAAAAATCAAGCTGCACGATATGCAGGAGGGA
AAGTGTCTGTATTCTCTGGAGGCCATCCCCCTGGAGGACCTGCTGAACAATCCATTCAACTA

CGAGGTCGATCATATTATCCCCAGAAGCGTGTCCTTCGACAATTCCTTTAACAACAAGGTGC
TGGTCAAGCAGGAAGAGAACTCTAAAAAGGGCAATAGGACTCCTTTCCAGTACCTGTCTAGT
TCAGATTCCAAGATCTCTTACGAAACCTTTAAAAAGCACATTCTGAATCTGGCCAAAGGAAA
GGGCCGCATCAGCAAGACCAAAAAGGAGTACCTGCTGGAAGAGCGGGACATCAACAGATTCT
CCGTCCAGAAGGATTTTATTAACCGGAATCTGGTGGACACAAGATACGCTACTCGCGGCCTG
ATGAATCTGCTGCGATCCTATTTCCGGGTGAACAATCTGGATGTGAAAGTCAAGTCCATCAA
CGGCGGGTTCACATCTTTTCTGAGGCGCAAATGGAAGTTTAAAAAGGAGCGCAACAAAGGGT
ACAAGCACCATGCCGAAGATGCTCTGATTATCGCAAATGCCGACTTCATCTTTAAGGAGTGG
AAAAAGCTGGACAAAGCCAAGAAAGTGATGGAGAACCAGATGTTCGAAGAGAAGCAGGCCGA
ATCTATGCCCGAAATCGAGACAGAACAGGAGTACAAGGAGATTTTCATCACTCCTCACCAGA
TCAAGCATATCAAGGATTTCAAGGACTACAAGTACTCTCACCGGGTGGATAAAAAGCCCAAC
AGAGAGCTGATCAATGACACCCTGTATAGTACAAGAAAAGACGATAAGGGGAATACCCTGAT
TGTGAACAATCTGAACGGACTGTACGACAAAGATAATGACAAGCTGAAAAAGCTGATCAACA
AAAGTCCCGAGAAGCTGCTGATGTACCACCATGATCCTCAGACATATCAGAAACTGAAGCTG
ATTATGGAGCAGTACGGCGACGAGAAGAACCCACTGTATAAGTACTATGAAGAGACTGGGAA
CTACCTGACCAAGTATAGCAAAAAGGATAATGGCCCCGTGATCAAGAAGATCAAGTACTATG
GGAACAAGCTGAATGCCCATCTGGACATCACAGACGATTACCCTAACAGTCGCAACAAGGTG
GTCAAGCTGTCACTGAAGCCATACAGATTCGATGTCTATCTGGACAACGGCGTGTATAAATT
TGTGACTGTCAAGAATCTGGATGTCATCAAAAAGGAGAACTACTATGAAGTGAATAGCAAGT
GCTACGAAGAGGCTAAAAAGCTGAAAAAGATTAGCAACCAGGCAGAGTTCATCGCCTCCTTT
TACAACAACGACCTGATTAAGATCAATGGCGAACTGTATAGGGTCATCGGGGTGAACAATGA
TCTGCTGAACCGCATTGAAGTGAATATGATTGACATCACTTACCGAGAGTATCTGGAAAACA
TGAATGATAAGCGCCCCCCTCGAATTATCAAAACAATTGCCTCTAAGACTCAGAGTATCAAA
AAGTACTCAACCGACATTCTGGGAAACCTGTATGAGGTGAAGAGCAAAAAGCACCCTCAGAT
TATCAAAAAGGGCGGATCCCCCAAGAAGAAGAGGAAAGTCTCGAGCTAGGCTGGAGCCTCGG
TGGCCATGCTTCTTGCCCCTTGGGCCTCCCCCCAGCCCCTCCTCCCCTTCCTGCACCCGTAC
CCCCGTGGTCTTTGAATAAAGTCTGAGTGGGCGGCACATGCTGGGGAGAGATCTGCGGCCGC
CTAGCAATAAAGGATCGTTTATTTTCATTGGAAGCGTGTGTTGGTTTTTTGATCAGGCGCGA
GGAACCCCTAGTGATGGAGTTGGCCACTCCCTCTCTGCGCGCTCGCTCGCTCACTGAGGCCG
GGCGACCAAAGGTCGCCCGACGCCCGGGCTTTGCCCGGGCGGCCTCAGTGAGCGAGCGAGCG
CGCAGCTGCCTGCAGG (SEQ ID NO:10)

Cas9 Vector 1 (550 bp minimal RHO promoter driving wt Cas9 with SV40 polyA signal) (see **Fig. 18** for feature annotation):

CCTGCAGGCAGCTGCGCGCTCGCTCGCTCACTGAGGCCGCCCGGGCAAAGCCCGGGCGTCGG
GCGACCTTTGGTCGCCCGGCCTCAGTGAGCGAGCGAGCGCGCAGAGAGGGAGTGGCCAACTC
CATCACTAGGGGTTCCTAAGCGGCCGCGGTTCCTCAGATCTGAATTCTCATGTTACAGGCAG
GGAGACGGGCACAAAACACAAATAAAAAGCTTCCATGCTGTCAGAAGCACTATGCAAAAAGC
AAGATGCTGAGGTCATGGAGCTCCTCCTGTCAGAGGAGTGTGGGGACTGGATGACTCCAGAG
GTAACTTGTGGGGGAACGAACAGGTAAGGGGCTGTGTGACGAGATGAGAGACTGGGAGAATA
AACCAGAAAGTCTCTAGCTGTCCAGAGGACATAGCACAGAGGCCCATGGTCCCTATTTCAAA
CCCAGGCCACCAGACTGAGCTGGGACCTTGGGACAGACAAGTCATGCAGAAGTTAGGGGACC
TTCTCCTCCCTTTTCCTGGATCCTGAGTACCTCTCCTCCCTGACCTCAGGCTTCCTCCTAGT
GTCACCTTGGCCCCTCTTAGAAGCCAATTAGGCCCTCAGTTTCTGCAGCGGGGATTAATATG
ATTATGAACACCCCCAATCTCCCAGATGCTGATTCAGCCAGGAGCTTAGGAGGGGGAGGTCA
CTTTATAAGGGTCTGGGGGGGTCAGAACCCAGAGTCATCCAGCTGGAGCCCTGAGTGGCTGA
GCTCAGGCCTTCGCAGCATTCTTGGGTGGGAGCAGCCACGGGTCAGCCACAATCTAGAGGAT
CCGGTACTCGAGGAACTGAAAAACCAGAAAGTTAACTGGTAAGTTTAGTCTTTTTGTCTTTT

ATTTCAGGTCCCGGATCCGGTGGTGGTGCAAATCAAAGAACTGCTCCTCAGTGGATGTTGCC
TTTACTTCTAGGCCTGTACGGAAGTGTTACGCGGCCGCCACCATGGGACCGAAGAAAAAGCG
CAAGGTCGAAGCGTCCATGAAAAGGAACTACATTCTGGGGCTGGACATCGGGATTACAAGCG
TGGGGTATGGGATTATTGACTATGAAACAAGGGACGTGATCGACGCAGGCGTCAGACTGTTC
AAGGAGGCCAACGTGGAAAACAATGAGGGACGGAGAAGCAAGAGGGGAGCCAGGCGCCTGAA
ACGACGGAGAAGGCACAGAATCCAGAGGGTGAAGAAACTGCTGTTCGATTACAACCTGCTGA
CCGACCATTCTGAGCTGAGTGGAATTAATCCTTATGAAGCCAGGGTGAAAGGCCTGAGTCAG
AAGCTGTCAGAGGAAGAGTTTTCCGCAGCTCTGCTGCACCTGGCTAAGCGCCGAGGAGTGCA
TAACGTCAATGAGGTGGAAGAGGACACCGGCAACGAGCTGTCTACAAAGGAACAGATCTCAC
GCAATAGCAAAGCTCTGGAAGAGAAGTATGTCGCAGAGCTGCAGCTGGAACGGCTGAAGAAA
GATGGCGAGGTGAGAGGGTCAATTAATAGGTTCAAGACAAGCGACTACGTCAAAGAAGCCAA
GCAGCTGCTGAAAGTGCAGAAGGCTTACCACCAGCTGGATCAGAGCTTCATCGATACTTATA
TCGACCTGCTGGAGACTCGGAGAACCTACTATGAGGGACCAGGAGAAGGGAGCCCCTTCGGA
TGGAAAGACATCAAGGAATGGTACGAGATGCTGATGGGACATTGCACCTATTTTCCAGAAGA
GCTGAGAAGCGTCAAGTACGCTTATAACGCAGATCTGTACAACGCCCTGAATGACCTGAACA
ACCTGGTCATCACCAGGGATGAAAACGAGAAACTGGAATACTATGAGAAGTTCCAGATCATC
GAAAACGTGTTTAAGCAGAAGAAAAAGCCTACACTGAAACAGATTGCTAAGGAGATCCTGGT
CAACGAAGAGGACATCAAGGGCTACCGGGTGACAAGCACTGGAAAACCAGAGTTCACCAATC
TGAAAAGTGTATCACGATATTAAGGACATCACAGCACGGAAAGAAATCATTGAGAACGCCGAA
CTGCTGGATCAGATTGCTAAGATCCTGACTATCTACCAGAGCTCCGAGGACATCCAGGAAGA
GCTGACTAACCTGAACAGCGAGCTGACCCAGGAAGAGATCGAACAGATTAGTAATCTGAAGG
GGTACACCGGAACACACAACCTGTCCCTGAAAGCTATCAATCTGATTCTGGATGAGCTGTGG
CATACAAACGACAATCAGATTGCAATCTTTAACCGGCTGAAGCTGGTCCCAAAAAAGGTGGA
CCTGAGTCAGCAGAAAGAGATCCCAACCACACTGGTGGACGATTTCATTCTGTCACCCGTGG
TCAAGCGGAGCTTCATCCAGAGCATCAAAGTGATCAACGCCATCATCAAGAAGTACGGCCTG
CCCAATGATATCATTATCGAGCTGGCTAGGGAGAAGAACAGCAAGGACGCACAGAAGATGAT
CAATGAGATGCAGAAACGAAACCGGCAGACCAATGAACGCATTGAAGAGATTATCCGAACTA
CCGGGAAAGAGAACGCAAAGTACCTGATTGAAAAAATCAAGCTGCACGATATGCAGGAGGGA
AAGTGTCTGTATTCTCTGGAGGCCATCCCCCTGGAGGACCTGCTGAACAATCCATTCAACTA
CGAGGTCGATCATATTATCCCCAGAAGCGTGTCCTTCGACAATTCCTTTAACAACAAGGTGC
TGGTCAAGCAGGAAGAGAACTCTAAAAAGGGCAATAGGACTCCTTTCCAGTACCTGTCTAGT
TCAGATTCCAAGATCTCTTACGAAACCTTTAAAAAGCACATTCTGAATCTGGCCAAAGGAAA
GGGCCGCATCAGCAAGACCAAAAAGGAGTACCTGCTGGAAGAGCGGGACATCAACAGATTCT
CCGTCCAGAAGGATTTTATTAACCGGAATCTGGTGGACACAAGATACGCTACTCGCGGCCTG
ATGAATCTGCTGCGATCCTATTTCCGGGTGAACAATCTGGATGTGAAAGTCAAGTCCATCAA
CGGCGGGTTCACATCTTTTCTGAGGCGCAAATGGAAGTTTAAAAAGGAGCGCAACAAAGGGT
ACAAGCACCATGCCGAAGATGCTCTGATTATCGCAAATGCCGACTTCATCTTTAAGGAGTGG
AAAAAGCTGGACAAAGCCAAGAAAGTGATGGAGAACCAGATGTTCGAAGAGAAGCAGGCCGA
ATCTATGCCCGAAATCGAGACAGAACAGGAGTACAAGGAGATTTTCATCACTCCTCACCAGA
TCAAGCATATCAAGGATTTCAAGGACTACAAGTACTCTCACCGGGTGGATAAAAAGCCCAAC
AGAGAGCTGATCAATGACACCCTGTATAGTACAAGAAAAGACGATAAGGGGAATACCCTGAT
TGTGAACAATCTGAACGGACTGTACGACAAAGATAATGACAAGCTGAAAAAGCTGATCAACA
AAAGTCCCGAGAAGCTGCTGATGTACCACCATGATCCTCAGACATATCAGAAACTGAAGCTG
ATTATGGAGCAGTACGGCGACGAGAAGAACCCACTGTATAAGTACTATGAAGAGACTGGGAA
CTACCTGACCAAGTATAGCAAAAAGGATAATGGCCCCGTGATCAAGAAGATCAAGTACTATG
GGAACAAGCTGAATGCCCATCTGGACATCACAGACGATTACCCTAACAGTCGCAACAAGGTG
GTCAAGCTGTCACTGAAGCCATACAGATTCGATGTCTATCTGGACAACGGCGTGTATAAATT

TGTGACTGTCAAGAATCTGGATGTCATCAAAAAGGAGAACTACTATGAAGTGAATAGCAAGT
GCTACGAAGAGGCTAAAAAGCTGAAAAAGATTAGCAACCAGGCAGAGTTCATCGCCTCCTTT
TACAACAACGACCTGATTAAGATCAATGGCGAACTGTATAGGGTCATCGGGGTGAACAATGA
TCTGCTGAACCGCATTGAAGTGAATATGATTGACATCACTTACCGAGAGTATCTGGAAAACA
TGAATGATAAGCGCCCCCCTCGAATTATCAAAACAATTGCCTCTAAGACTCAGAGTATCAAA
AAGTACTCAACCGACATTCTGGGAAACCTGTATGAGGTGAAGAGCAAAAAGCACCCTCAGAT
TATCAAAAAGGGCGGATCCCCCAAGAAGAAGAGGAAAGTCTCGAGCTAGCAATAAAGGATCG
TTTATTTTCATTGGAAGCGTGTGTTGGTTTTTTGATCAGGCGCGTCCAAGCTTGCATGCTGG
GGAGAGATCTGCGGCCGCCTAGCAATAAAGGATCGTTTATTTTCATTGGAAGCGTGTGTTGG
TTTTTTGATCAGGCGCGAGGAACCCCTAGTGATGGAGTTGGCCACTCCCTCTCTGCGCGCTC
GCTCGCTCACTGAGGCCGGGCGACCAAAGGTCGCCCGACGCCCGGGCTTTGCCCGGGCGGCC
TCAGTGAGCGAGCGAGCGCGCAGCTGCCTGCAGG (SEQ ID NO:9)

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Other embodiments are within the following claims.

CLAIMS

What is claimed is:

1. A guide RNA ("gRNA") molecule comprising a targeting domain that binds a target sequence of a *RHO* gene.
2. The gRNA molecule of claim 1, wherein the targeting domain is complementary to a target domain of a *RHO* gene.
3. The gRNA molecule of claim 1, wherein said targeting domain is configured to provide a cleavage event selected from a double strand break and a single strand break, within 10 nucleotides of a *RHO* target position.
4. The gRNA molecule of claim 1, wherein the *RHO* target position is in the 5' region of the *RHO* gene.
5. The gRNA molecule of claim 4, wherein the 5' region of the *RHO* gene is selected from the group consisting of the 5' untranslated ("UTR") region, exon 1, the exon 1/intron 1 border, exon 2, and the exon 2/intron 1 border of the *RHO* gene.
6. The gRNA molecule of claim 1, wherein said targeting domain comprises a sequence that is the same as, or differs by no more than 3 nucleotides from, a targeting domain sequence from any of Tables 1-3 and 18.
7. The gRNA molecule of claim 1, wherein said targeting domain is selected from those in Tables 1-3 and 18.
8. The gRNA molecule of any of claims 1-7, wherein said gRNA is a modular gRNA molecule or a chimeric gRNA molecule.
9. The gRNA molecule of any of claims 1-8, comprising from 5' to 3':

   a targeting domain;
   a first complementarity domain;
   a linking domain;
   a second complementarity domain;
   a proximal domain; and
   a tail domain.

10. A nucleic acid that comprises: (a) sequence that encodes a gRNA molecule comprising a targeting domain that is complementary to a target domain in the *RHO* gene.
11. The nucleic acid of claim 10, wherein said gRNA molecule is a gRNA molecule of any of claims 1-9.

12. The nucleic acid of claim 11, wherein said targeting domain is configured to provide a cleavage event selected from a double strand break and a single strand break, within 10 nucleotides of a *RHO* target position.
13. The nucleic acid of claim 11, wherein said targeting domain comprises a sequence that is the same as, or differs by no more than 3 nucleotides from, a targeting domain sequence from any of Tables 1-3 and 18.
14. The nucleic acid of claim 11, wherein said targeting domain is selected from those in Tables 1-3 and 18.
15. The nucleic acid of any of claims 10-14, wherein said gRNA is a modular gRNA molecule or a chimeric molecule.
16. The nucleic acid of any of claims 10-15, wherein said nucleic acid comprises a promoter operably linked to the sequence that encodes said gRNA molecule of (a).
17. The nucleic acid of claim 16, wherein the promoter operably linked to the sequence that encodes said gRNA molecule of (a) is a U6 promoter.
18. The nucleic acid of any of claims 10-17, further comprising: (b) sequence that encodes an RNA-guided nuclease molecule.
19. The nucleic acid of claim 18, wherein said RNA-guided nuclease molecule forms a double strand break in a target nucleic acid.
20. The nucleic acid of claim 18, wherein said RNA-guided nuclease molecule forms a single strand break in a target nucleic acid.
21. The nucleic acid of claim 20, wherein said single strand break is formed in the strand of the target nucleic acid to which the targeting domain of said gRNA molecule is complementary.
22. The nucleic acid of claim 21, wherein said single strand break is formed in the strand of the target nucleic acid other than the strand to which to which the targeting domain of said gRNA is complementary.
23. The nucleic acid of claim 18, wherein said RNA-guided nuclease molecule is a Cas9 molecule.
24. The nucleic acid of claim 23, wherein said Cas9 molecule comprises a nickase molecule.
25. The nucleic acid of claim 18, wherein said RNA-guided nuclease molecule is a Cpf1 molecule.

26. The nucleic acid of any of claims 18-25, wherein said nucleic acid comprises a promoter operably linked to the sequence that encodes the RNA-guided nuclease molecule of (b).

27. The nucleic acid of claim 26, wherein said promoter operably linked to the sequence that encodes the RNA-guided nuclease molecule of (b) comprises a promoter selected from the group consisting of RHO, CMV, EFS, GRK1, CRX, NRL, and RCVRN promoter.

28. The nucleic acid of any of claims 10-27, further comprising: (c) a *RHO* cDNA molecule.

29. The nucleic acid of claim 24, wherein said *RHO* cDNA molecule is not codon modified to be resistant to hybridization with the gRNA molecule.

30. The nucleic acid of claim 28, wherein said *RHO* cDNA molecule comprises a nucleotide sequence comprising exon 1, exon 2, exon 3, exon 4, and exon 5 of the *RHO* gene.

31. The nucleic acid of claim 28, wherein said *RHO* cDNA molecule comprises a nucleotide sequence comprising exon 1, intron 1, exon 2, exon 3, exon 4, and exon 5 of the *RHO* gene.

32. The nucleic acid of claim 28, wherein intron 1 comprises one or more truncations at a 5' end of intron 1, a 3' end of intron 1, or both.

33. The nucleic acid of any of claims 28-32, wherein said nucleic acid comprises a 3' UTR nucleotide sequence downstream of the *RHO* cDNA molecule.

34. The nucleic acid of claim 33, wherein said 3' UTR nucleotide sequence comprises a *RHO* gene 3' UTR nucleotide sequence.

35. The nucleic acid of claim 33, wherein said 3' UTR nucleotide sequence comprises an α-globin 3' UTR nucleotide sequence.

36. The nucleic acid of claim 33, wherein said 3' UTR nucleotide sequence comprises a β-globin 3' UTR nucleotide sequence.

37. The nucleic acid of any of claims 33-36, wherein said 3' UTR nucleotide sequence comprises one or more truncations at a 5' end of said 3' UTR nucleotide sequence, a 3' end of said 3' UTR nucleotide sequence, or both.

38. The nucleic acid of any of claims 28-37, wherein said nucleic acid comprises a promoter operably linked to the *RHO* cDNA molecule (c).

39. The nucleic acid of claim 38, wherein said promoter operably linked to the *RHO* cDNA molecule (c) is a rod-specific promoter.

40. The nucleic acid of claim 39, wherein said rod-specific promoter is a human *RHO* promoter.
41. The nucleic acid of claim 40, wherein said human *RHO* promoter comprises an endogenous *RHO* promoter.
42. The nucleic acid of claim 41, further comprising: (d) sequence that encodes a second gRNA molecule having a targeting domain that is complementary to a second target domain of the *RHO* gene.
43. The nucleic acid of claim 42, wherein said targeting domain of said second gRNA is configured to provide a cleavage event selected from a double strand break and a single strand break, within 10 nucleotides of a *RHO* target position.
44. The nucleic acid of any of claims 42 or 43, wherein said second gRNA molecule is a modular gRNA molecule or chimeric gRNA molecule.
45. The nucleic acid of any of claims 42-44, wherein said second gRNA molecule is a chimeric gRNA molecule.
46. The nucleic acid of any of claims 42-45, wherein said second gRNA molecule comprises from 5' to 3':
a targeting domain;
a first complementarity domain;
a linking domain;
a second complementarity domain;
a proximal domain; and
a tail domain.
47. The nucleic acid of any of claims 42-46, further comprising a third gRNA molecule.
48. The nucleic acid of claim 47, further comprising a fourth gRNA molecule.
49. The nucleic acid of any of claims 18-27, wherein each of (a) and (b) is present on the same nucleic acid molecule.
50. The nucleic acid of claim 49, wherein said nucleic acid molecule is an AAV vector.
51. The nucleic acid of any of claims 18-27, wherein: (a) is present on a first nucleic acid molecule; and (b) is present on a second nucleic acid molecule.
52. The nucleic acid of claim 51, wherein said first and second nucleic acid molecules are AAV vectors.
53. The nucleic acid of any of claims 18-27, further comprising (c) a *RHO* cDNA molecule of any of claims 28-41.

54. The nucleic acid of claim 53, wherein each of (a) and (c) is present on the same nucleic acid molecule.
55. The nucleic acid of claim 54, wherein said nucleic acid molecule is an AAV vector.
56. The nucleic acid of claim 53, wherein: (a) is present on a first nucleic acid molecule; and (c) is present on a second nucleic acid molecule.
57. The nucleic acid of claim 56, wherein said first and second nucleic acid molecules are AAV vectors.
58. The nucleic acid of any of claims 10-17, further comprising:
(b) sequence that encodes an RNA-guided nuclease molecule of any of claims 18-27; and
(c) *RHO* cDNA molecule of any of claims 28-41.
59. The nucleic acid of claim 58, wherein each of (a), (b), and (c) are present on the same nucleic acid molecule.
60. The nucleic acid of claim 59, wherein said nucleic acid molecule is an AAV vector.
61. The nucleic acid of claim 58, wherein:
one of (a), (b), and (c) are present on a first nucleic acid molecule; and
and a second and third of (a), (b), and (c) is present on a second nucleic acid molecule.
62. The nucleic acid of claim 61, wherein said first and second nucleic acid molecules are AAV vectors.
63. The nucleic acid of claim 58, wherein: (a) is present on a first nucleic acid molecule; and (b) and (c) are present on a second nucleic acid molecule.
64. The nucleic acid of claim 63, wherein said first and second nucleic acid molecules are AAV vectors.
65. The nucleic acid of claim 58, wherein: (b) is present on a first nucleic acid molecule; and (a) and (c) are present on a second nucleic acid molecule.
66. The nucleic acid of claim 65, wherein said first and second nucleic acid molecules are AAV vectors.
67. The nucleic acid of claim 58, wherein: (c) is present on a first nucleic acid molecule; and (b) and (a) are present on a second nucleic acid molecule.
68. The nucleic acid of claim 67, wherein said first and second nucleic acid molecules are AAV vectors.

69. The nucleic acid of any of claims 51, 56, 61, 63, 65, and 67, wherein said first nucleic acid molecule is other than an AAV vector and said second nucleic acid molecule is an AAV vector.

70. A composition comprising a gRNA molecule of any of claims 1-17.

71. The composition of claim 70, further comprising (b) a Cas9 molecule of any of claims 18-27.

72. The composition of claim 71, further comprising (c) a *RHO* cDNA molecule of any of claims 28-41.

73. The composition of claim 72, further comprising a second gRNA molecule.

74. The composition of claim 73, further comprising a third gRNA molecule.

75. The composition of claim 75, further comprising a fourth gRNA molecule.

76. A method of altering a cell comprising contacting said cell with:

(a) a gRNA of any of claims 1-17;

(b) an RNA-guided nuclease molecule of any of claims 18-27;

(c) a *RHO* cDNA molecule of any of claims 28-41; and

optionally, (d) a second gRNA molecule of any of claims 42-46.

77. The method of claim 76, further comprising a third gRNA molecule.

78. The method of claim 77, further comprising a fourth gRNA molecule.

79. The method of claim 76, comprising contacting said cell with (a), (b), (c) and optionally (d).

80. The method of any of claims 76-79, wherein said cell is from a subject suffering from adRP.

81. The method of any of claims 76-80, wherein said cell is from a subject having a mutation in the *RHO* gene.

82. The method of any of claims 76-81, wherein said cell is a retinal cell.

83. The method of claim 82, wherein the retinal cell is a rod photoreceptor.

84. The method of any of claims 76-83, wherein said cell is an embryonic stem cell, an induced pluripotent stem cell, a hematopoietic stem cell, a neuronal stem cell or a mesenchymal stem cell.

85. The method of any of claims 76-83, wherein said contacting is performed *ex vivo*.

86. The method of claim 84, wherein said contacted cell is returned to said subject's body.

87. The method of any of claims 76-83, wherein said contacting is performed *in vivo*.

88. The method of any of claims 80-87, comprising acquiring knowledge of the presence of the mutation in the *RHO* gene in said cell.

89. The method of claim 88, comprising acquiring knowledge of the presence of the presence of the mutation in the *RHO* gene in said cell by sequencing a portion of the *RHO* gene.

90. The method of any of claims 76-89, comprising altering a *RHO* target position to knock-out function of the *RHO* gene.

91. The method of any of claims 76-90, wherein contacting comprises contacting said cell with a nucleic acid that encodes at least one of (a), (b), (c) and optionally (d).

92. The method of any of claims 76-90, wherein contacting comprises delivering to said cell said RNA-guided nuclease molecule of (b) and a nucleic acid which encodes (a) and (c) and optionally (d).

93. The method of any of claims 76-90, wherein contacting comprises delivering to said cell said RNA-guided nuclease molecule of (b), said gRNA molecule of (a), and said *RHO* cDNA molecule of (c).

94. The method of any of claims 76-90, wherein contacting comprises delivering to said cell said gRNA molecule of (a), said *RHO* cDNA molecule of (c) and a nucleic acid that encodes the RNA-guided nuclease molecule of (b).

95. A method of contacting a subject (or a cell from said subject) with:

(a) a gRNA of any of claims 1-17;

(b) an RNA-guided nuclease molecule of any of claims 18-27;

(c) a *RHO* cDNA molecule of any of claims 28-41; and

optionally, (d) a second gRNA of any of claims 42-46.

96. The method of claim 95, further comprising a third gRNA molecule.

97. The method of claim 96, further comprising a fourth gRNA molecule.

98. The method of claim 97, further comprising contacting said subject with (a), (b), (c) and optionally (d).

99. The method of claims any one of claims 95-98, wherein said subject is suffering from adRP.

100. The method of any of claims 95-99, wherein said subject has a mutation in the *RHO* gene.

101. The method of any of claims 95-100, comprising acquiring knowledge of the presence of the mutation in the *RHO* gene in said subject.

102. The method of claim 101, comprising acquiring knowledge of the presence of the mutation in the *RHO* gene in said subject by sequencing a portion of the *RHO* gene.

103. The method of claims 95-102, comprising altering a *RHO* target position to knock-out function of the *RHO* gene.

104. The method of any of claims 95-103, wherein a cell of said subject is contacted *ex vivo* with (a), (b), (c) and optionally (d).

105. The method of claim 104, wherein said cell is returned to the subject's body.

106. The method of any of claims 95-105, wherein treatment comprises introducing a cell into said subject's body, wherein said cell subject is contacted *ex vivo* with (a), (b), (c), and optionally (d).

107. The method of any of claims 95-106, wherein said contacting is performed *in vivo*.

108. The method of claim 107, wherein said contacting comprises intravenous delivery.

109. The method of any of claims 95-108, wherein contacting comprises contacting said subject with a nucleic acid that encodes at least one of (a), (b), and (c), and optionally (d).

110. The method of any of claims 95-108, wherein contacting comprises contacting said subject with a nucleic acid of any of claims 10-69.

111. The method of any of claims 95-108, wherein contacting comprises delivering to said subject said RNA-guided nuclease molecule of (b) and a nucleic acid which encodes and (a) and (c), and optionally (d).

112. The method of any of claims 95-108, wherein contacting comprises delivering to said subject said RNA-guided nuclease molecule of (b), said gRNA of (a) and said *RHO* cDNA molecule of (c), and optionally said second gRNA of (d).

113. The method of any of claims 95-108, wherein contacting comprises delivering to said subject said gRNA of (a), said *RHO* cDNA molecule of (c) and a nucleic acid that encodes the RNA-guided nuclease molecule of (b).

114. A reaction mixture comprising a gRNA, a nucleic acid, or a composition described herein, and a cell from a subject having adRP, or a subject having a mutation in the *RHO* gene.

115. A kit comprising, (a) gRNA molecule of any of claims 1-17, or nucleic acid that encodes said gRNA, and one or more of the following:

(b) a Cas9 molecule of any of claims 18-27;

(c) a *RHO* cDNA molecule of any of claims 28-41;

optionally, (d) a second gRNA molecule of any of claims 42-46; and

(e) nucleic acid that encodes one or more of (b) and (c).

116. The kit of claim 115, comprising nucleic acid that encodes one or more of (a), (b) (c) and (d).

117. The kit of claim 116, further comprising a third gRNA molecule targeting a *RHO* target position of the *RHO* gene.

118. The kit of claim 117, further comprising a fourth gRNA molecule targeting a *RHO* target position of the *RHO* gene.

FIG. 1

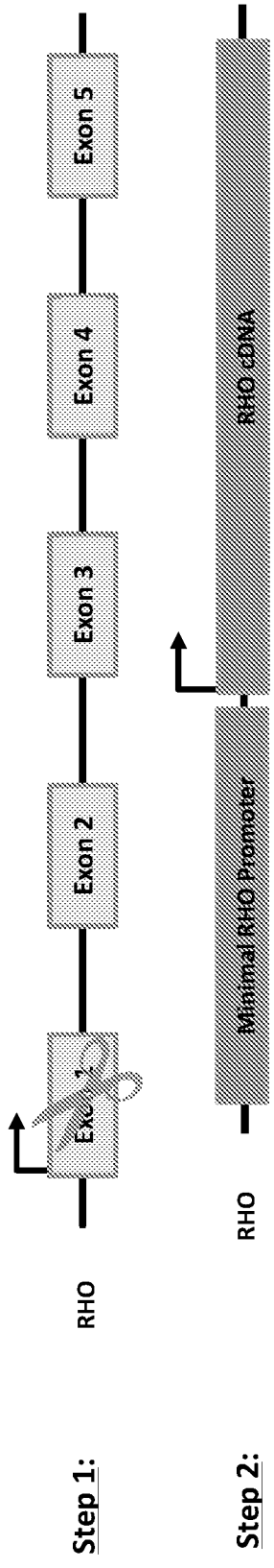
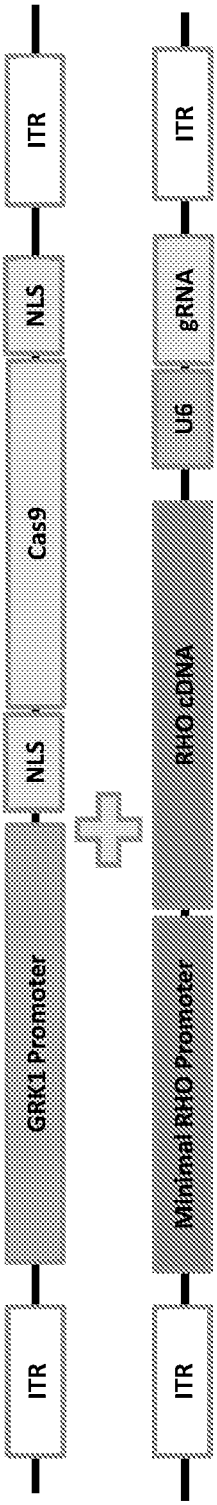

Step 1:
RHO
Exon 2
Exon 3
Exon 4
Exon 5
Step 2:
RHO
Minimal RHO Promoter
RHO cDNA

FIG. 2


ITR
GRK1 Promoter
NLS
Cas9
NLS
ITR
Vector 1
(AAV5)
ITR
Minimal RHO Promoter
RHO cDNA
U6
gRNA
ITR
Vector 2
(AAV5)

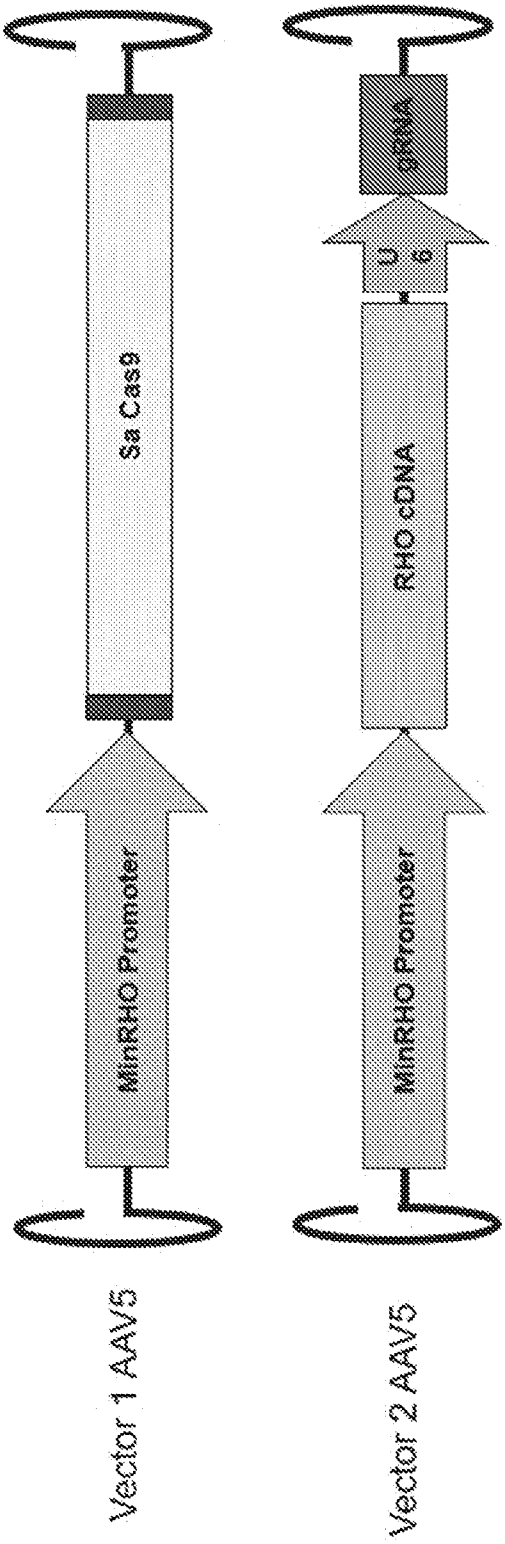

Sa Cas9
MinRHO Promoter
Vector 1 AAV5
gRNA
U 6
RHO cDNA
MinRHO Promoter
Vector 2 AAV5

FIG. 3

FIG. 4

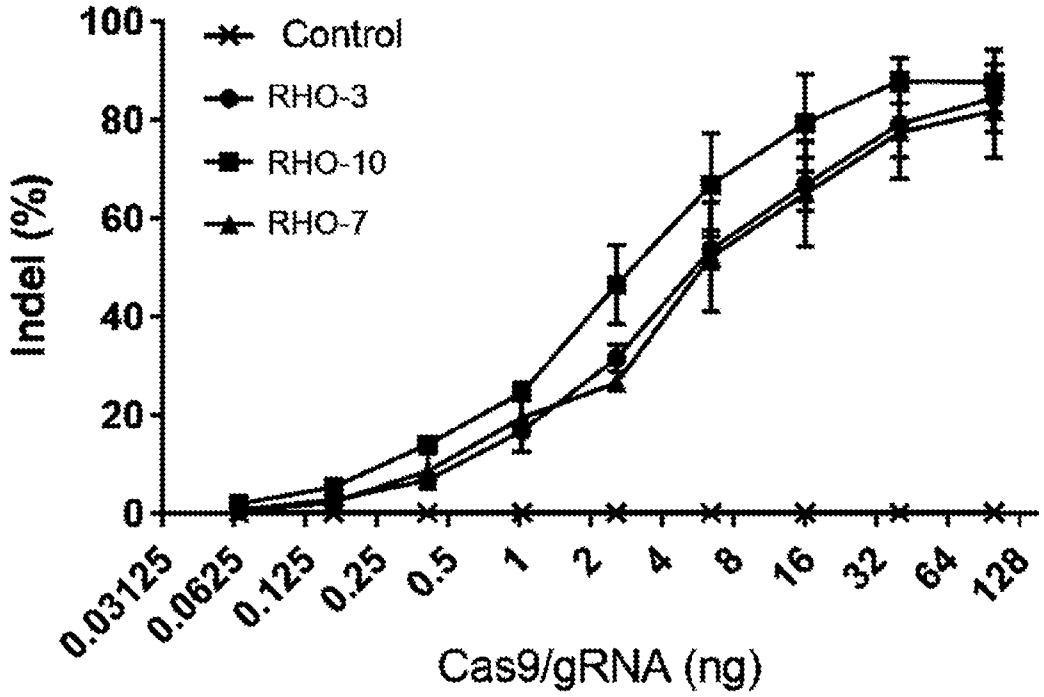

100
80
60
40
20
0
Indel (%)
Control
RHO-3
RHO-10
RHO-7
0.03125
0.0625
0.125
0.25
0.5
1
2
4
8
16
32
64
128
Cas9/gRNA (ng)

FIG. 5

Predicted gRNA-induced *rho* alleles

Guide	Location	Target Site Position (AA)	-1 Frame (AA)	-2 Frame (AA)	-3 Frame (AA)
RHO-3	Exon 1	96	95	120	347; T97del
RHO-10	Exon 2 (Intron 2)	174	215	328	347; G174del
RHO-7	Exon 1 (Intron 1)	120	142	139	347; G120del

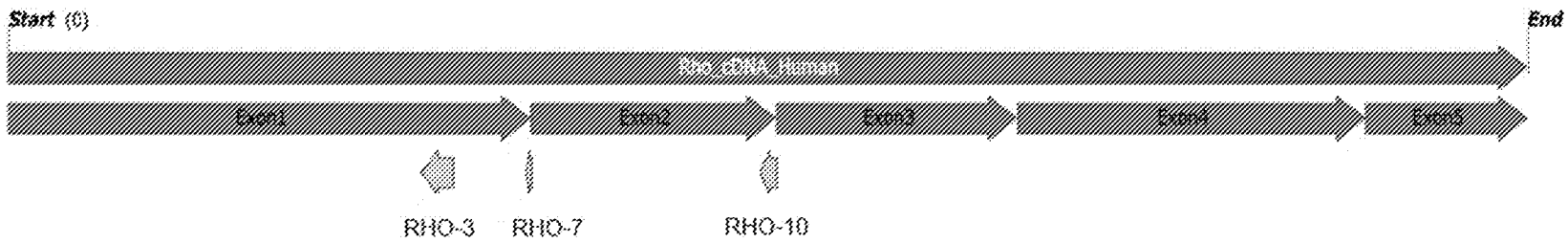


FIG. 6

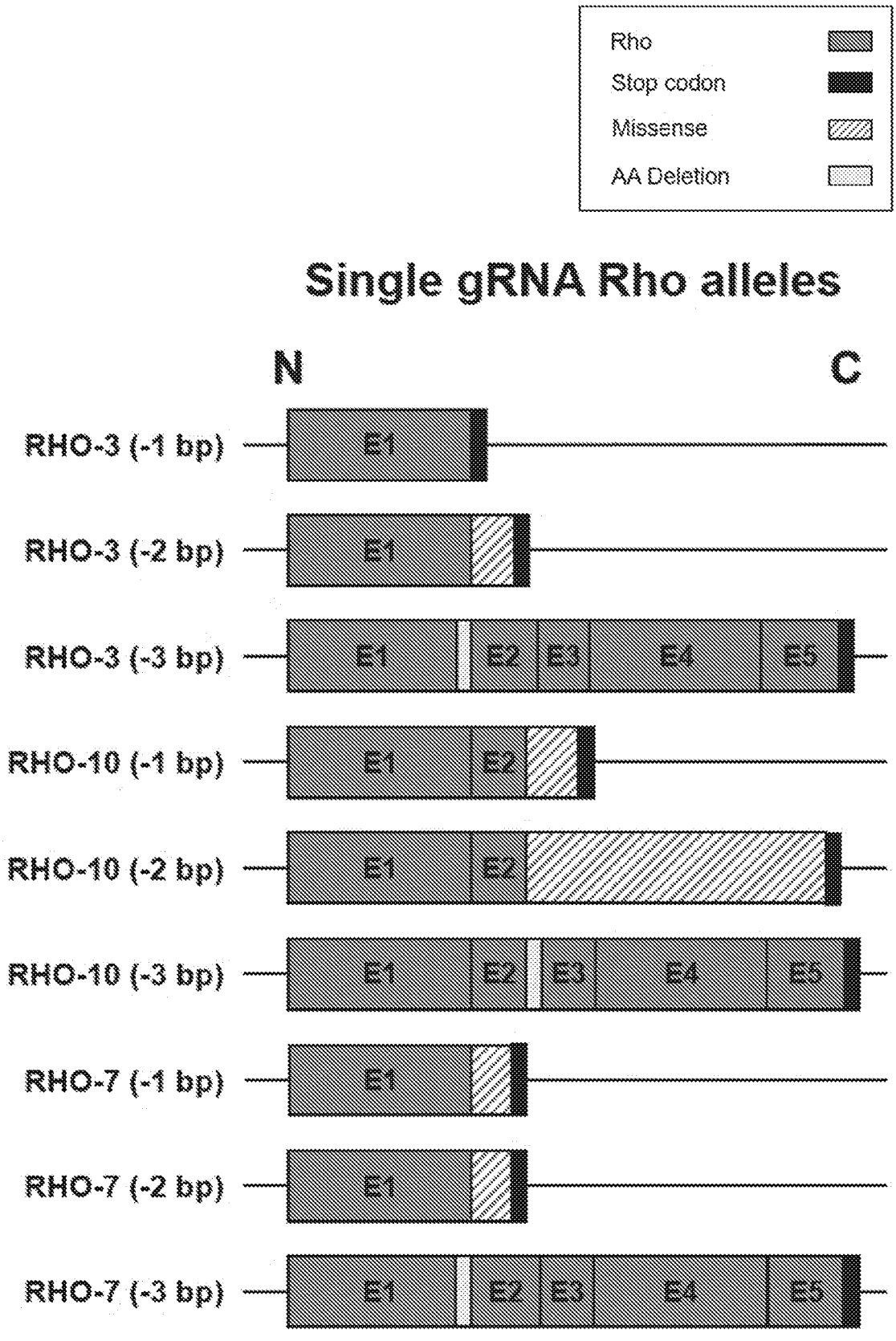

Rho
Stop codon
Missense
AA Deletion
Single gRNA Rho alleles
N
C
RHO-3 (-1 bp)
RHO-3 (-2 bp)
RHO-3 (-3 bp)
RHO-10 (-1 bp)
RHO-10 (-2 bp)
RHO-10 (-3 bp)
RHO-7 (-1 bp)
RHO-7 (-2 bp)
RHO-7 (-3 bp)
E1
E2
E3
E4
E5

FIG. 7A

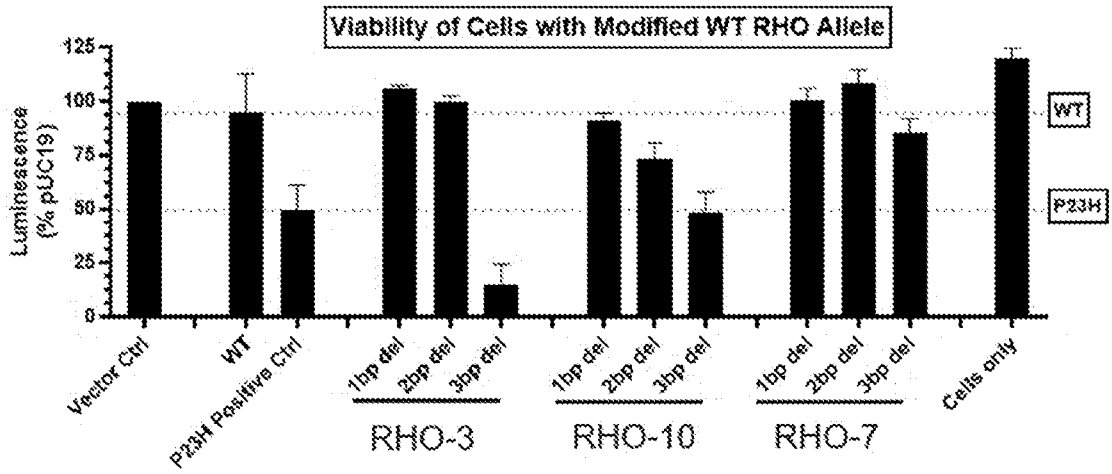

Viability of Cells with Modified WT RHO Allele
Luminescence (% pUC19)
125
100
75
50
25
0
WT
P23H
Vector Ctrl
WT
P23H Positive Ctrl
1bp del
2bp del
3bp del
1bp del
2bp del
3bp del
1bp del
2bp del
3bp del
Cells only
RHO-3
RHO-10
RHO-7

FIG. 7B

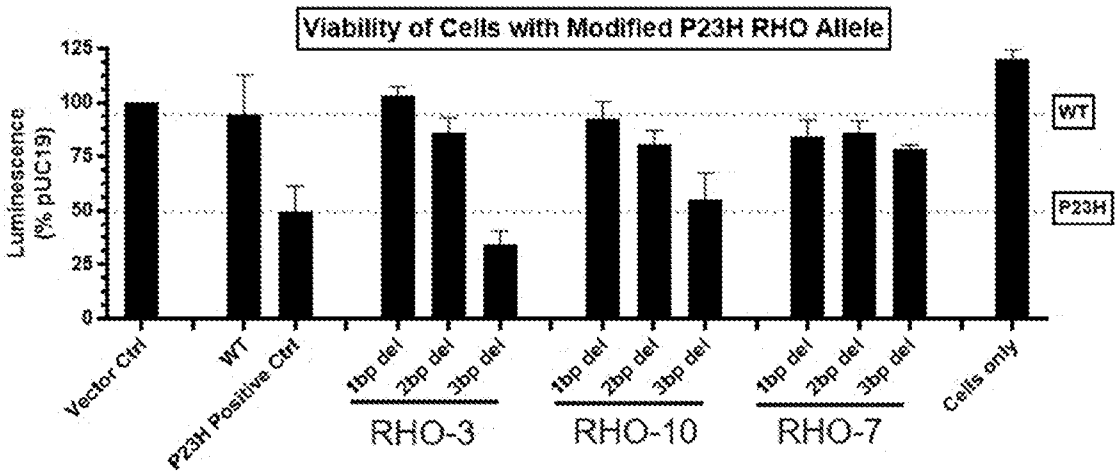

Viability of Cells with Modified P23H RHO Allele
Luminescence (% pUC19)
125
100
75
50
25
0
WT
P23H
Vector Ctrl
WT
P23H Positive Ctrl
1bp del
2bp del
3bp del
1bp del
2bp del
3bp del
1bp del
2bp del
3bp del
Cells only
RHO-3
RHO-10
RHO-7

FIG. 8

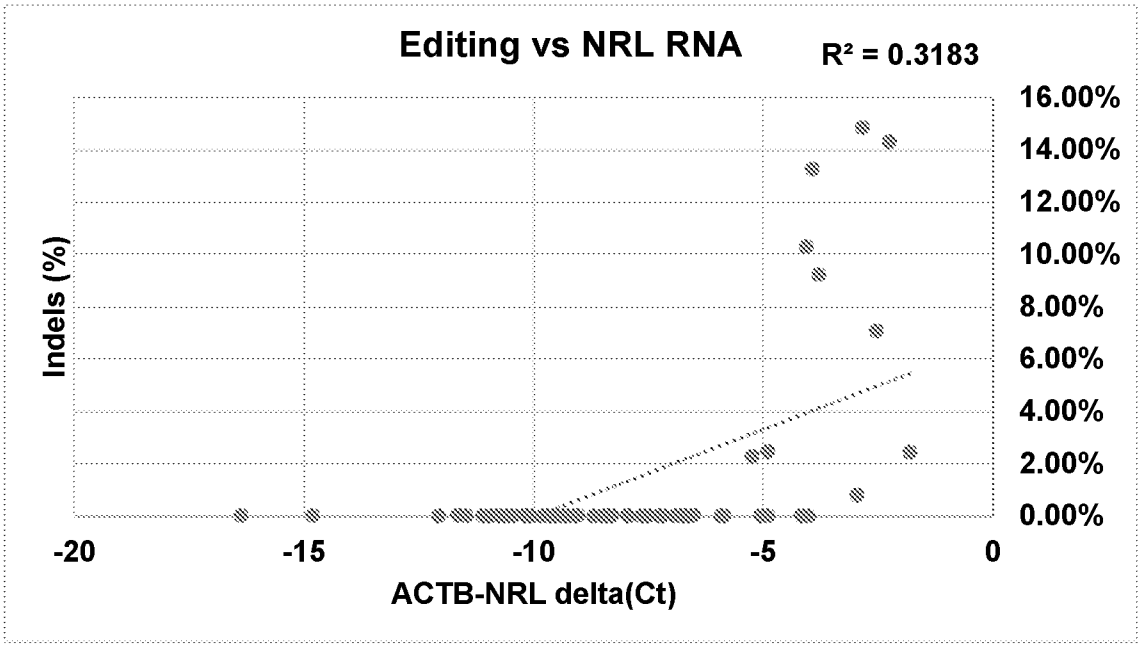

Editing vs NRL RNA
R² = 0.3183
16.00%
14.00%
12.00%
10.00%
8.00%
6.00%
4.00%
2.00%
0.00%
Indels (%)
-20
-15
-10
-5
0
ACTB-NRL delta(Ct)

FIG. 9

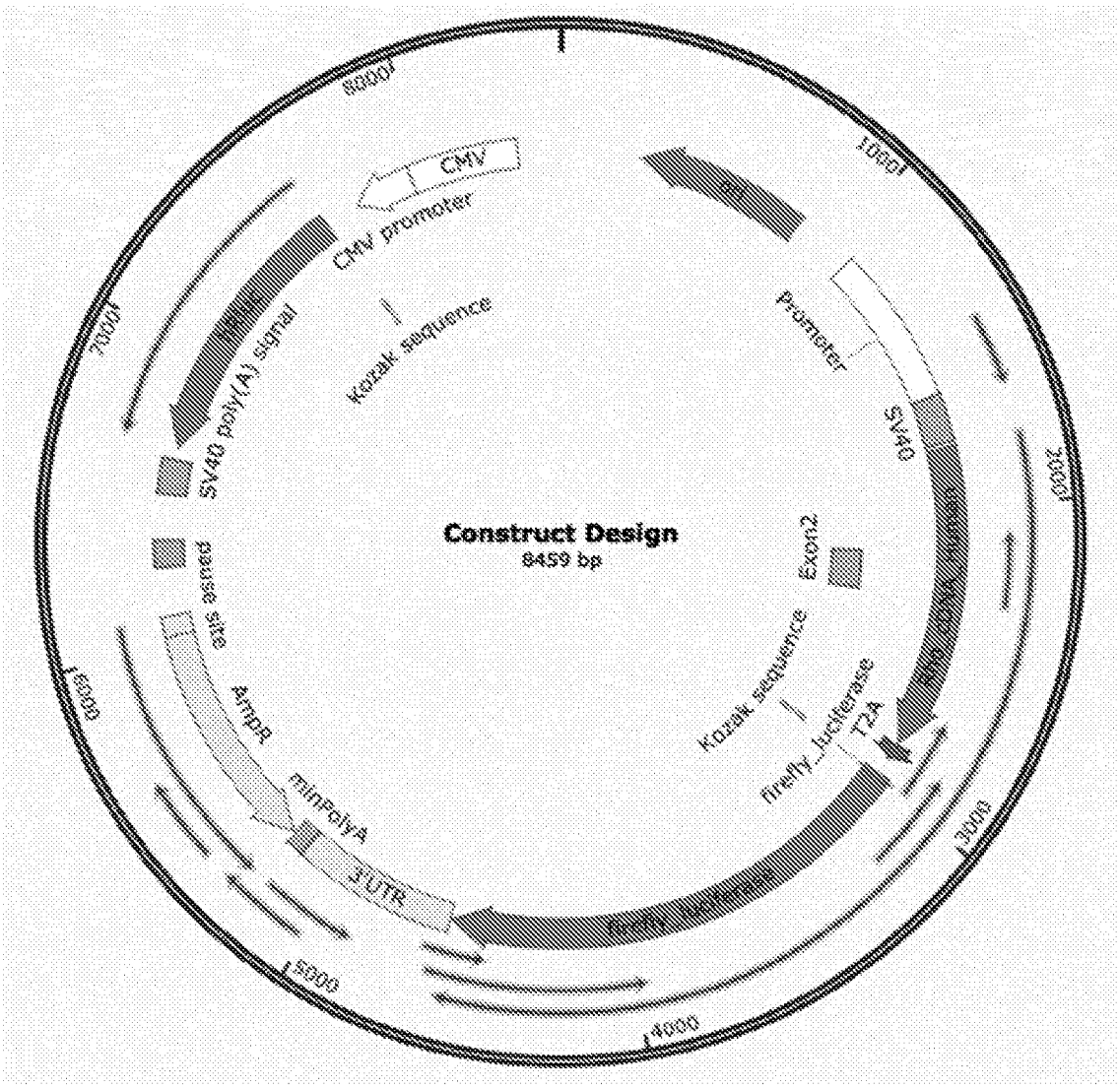

Construct Design
8459 bp
CMV
CMV promoter
Kozak sequence
SV40 poly(A) signal
SV40
Promoter
Exon2
Kozak sequence
T2A
firefly_luciferase
AmpR
minPolyA
3UTR
cause site
1000
2000
3000
4000
5000
6000
7000
8000

FIG. 10

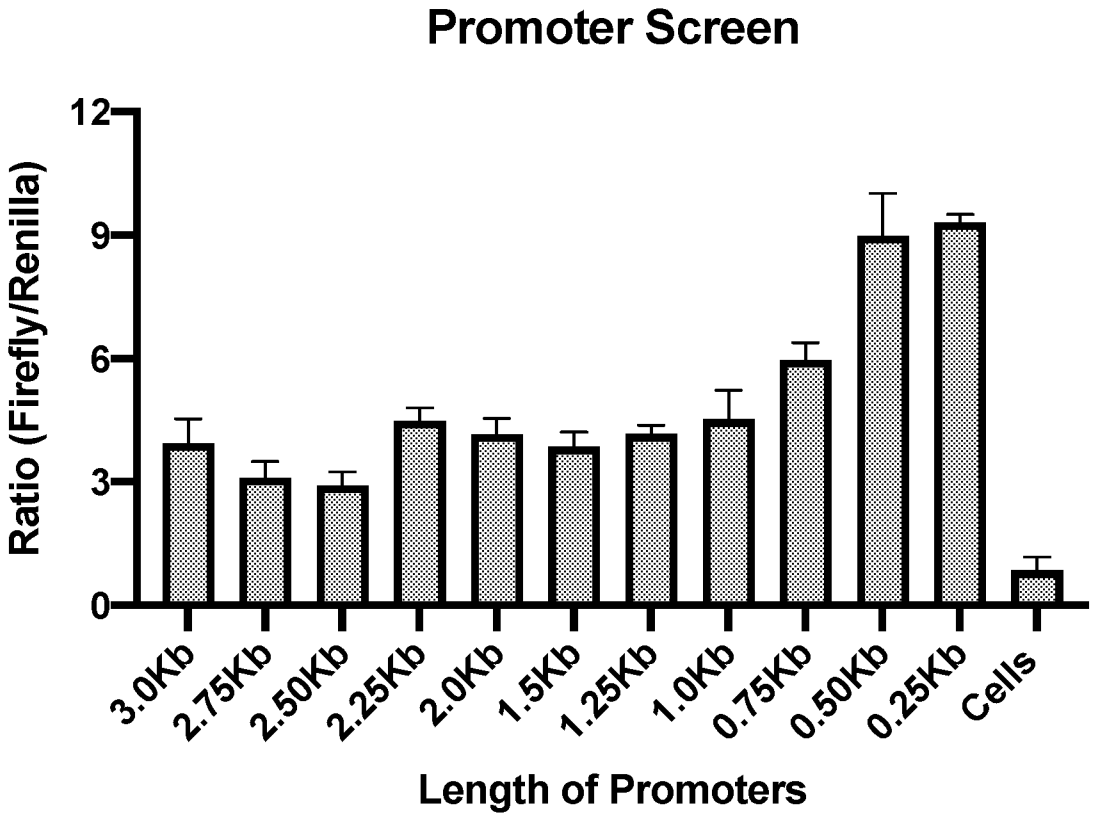

Promoter Screen
Ratio (Firefly/Renilla)
12
9
6
3
0
3.0Kb
2.75Kb
2.50Kb
2.25Kb
2.0Kb
1.5Kb
1.25Kb
1.0Kb
0.75Kb
0.50Kb
0.25Kb
Cells
Length of Promoters

FIG. 11A

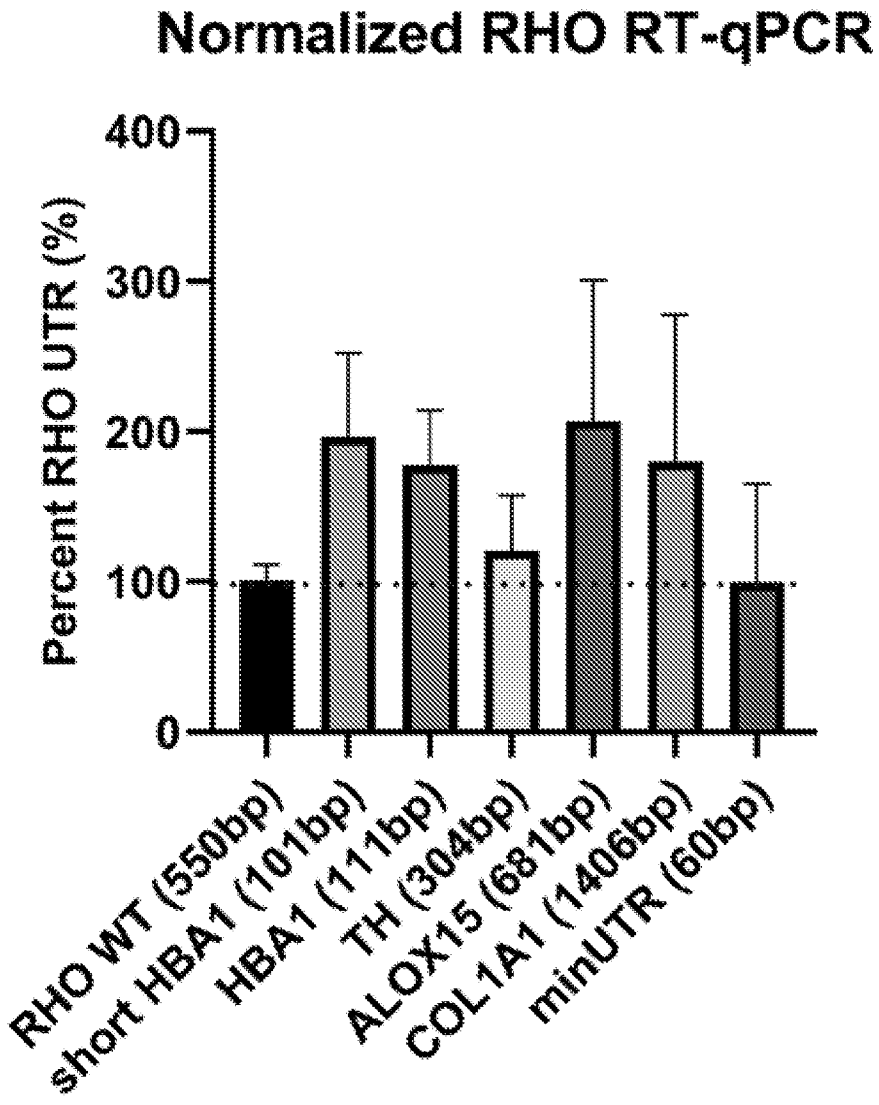

Normalized RHO RT-qPCR
Percent RHO UTR (%)
400
300
200
100
0
RHO WT (550bp)
short HBA1 (101bp)
HBA1 (111bp)
TH (304bp)
ALOX15 (681bp)
COL1A1 (1406bp)
minUTR (60bp)

FIG. 11B

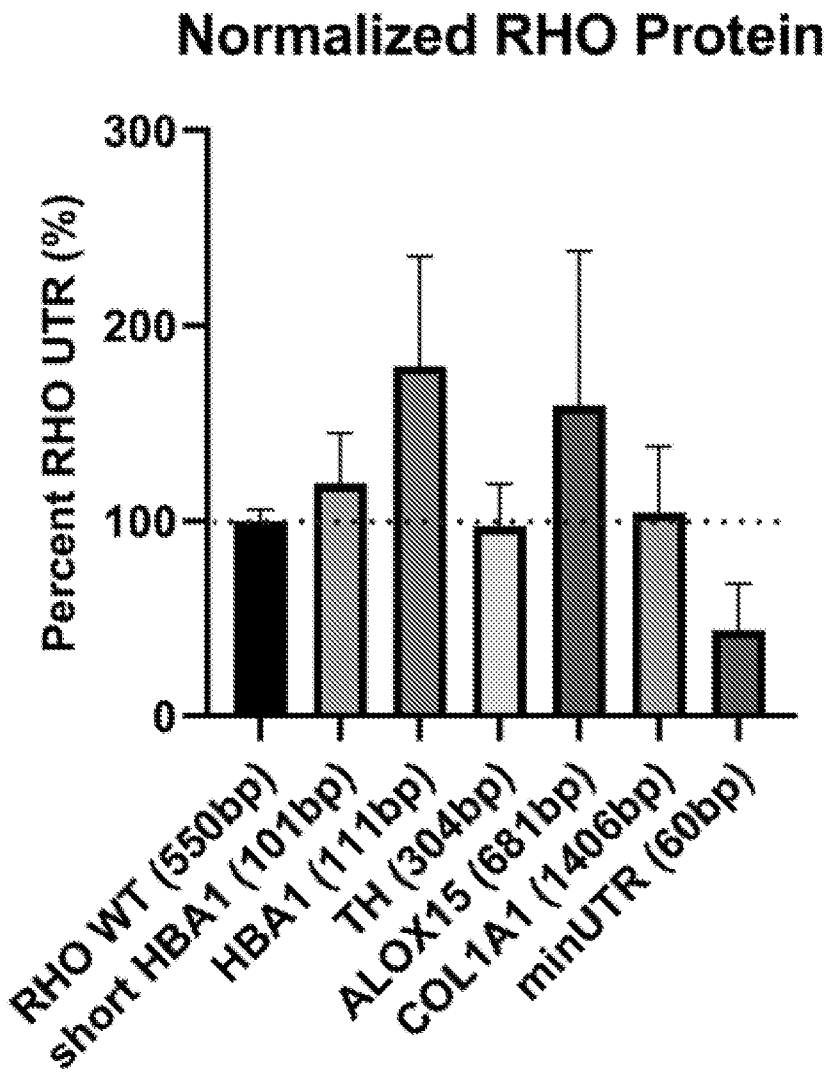

Normalized RHO Protein
Percent RHO UTR (%)
300
200
100
0
RHO WT (550bp)
short HBA1 (101bp)
HBA1 (111bp)
TH (304bp)
ALOX15 (681bp)
COL1A1 (1406bp)
minUTR (60bp)

FIG. 12

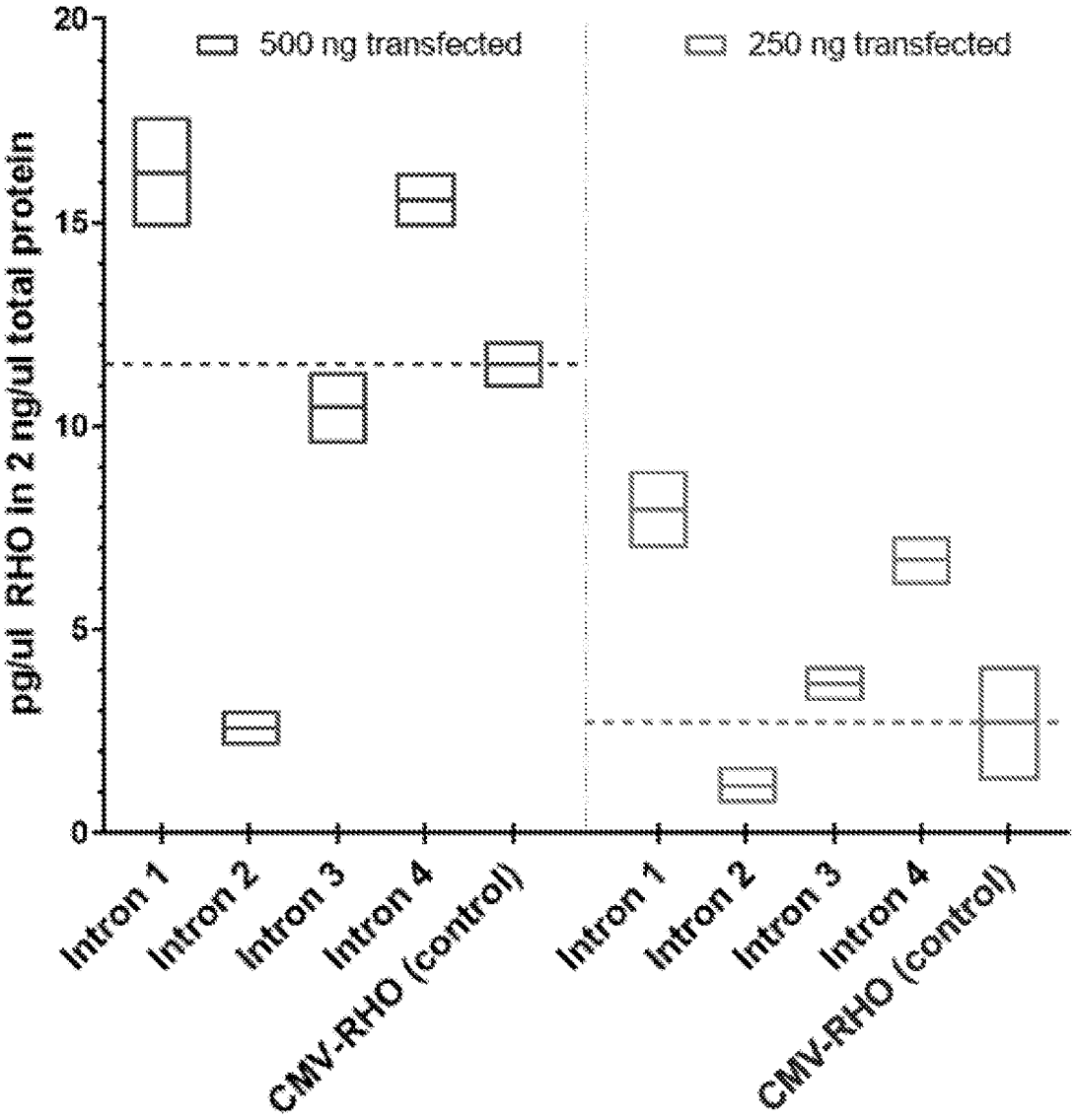

500 ng transfected
250 ng transfected
pg/ul RHO in 2 ng/ul total protein
20
15
10
5
0
Intron 1
Intron 2
Intron 3
Intron 4
CMV-RHO (control)
Intron 1
Intron 2
Intron 3
Intron 4
CMV-RHO (control)

FIG. 13

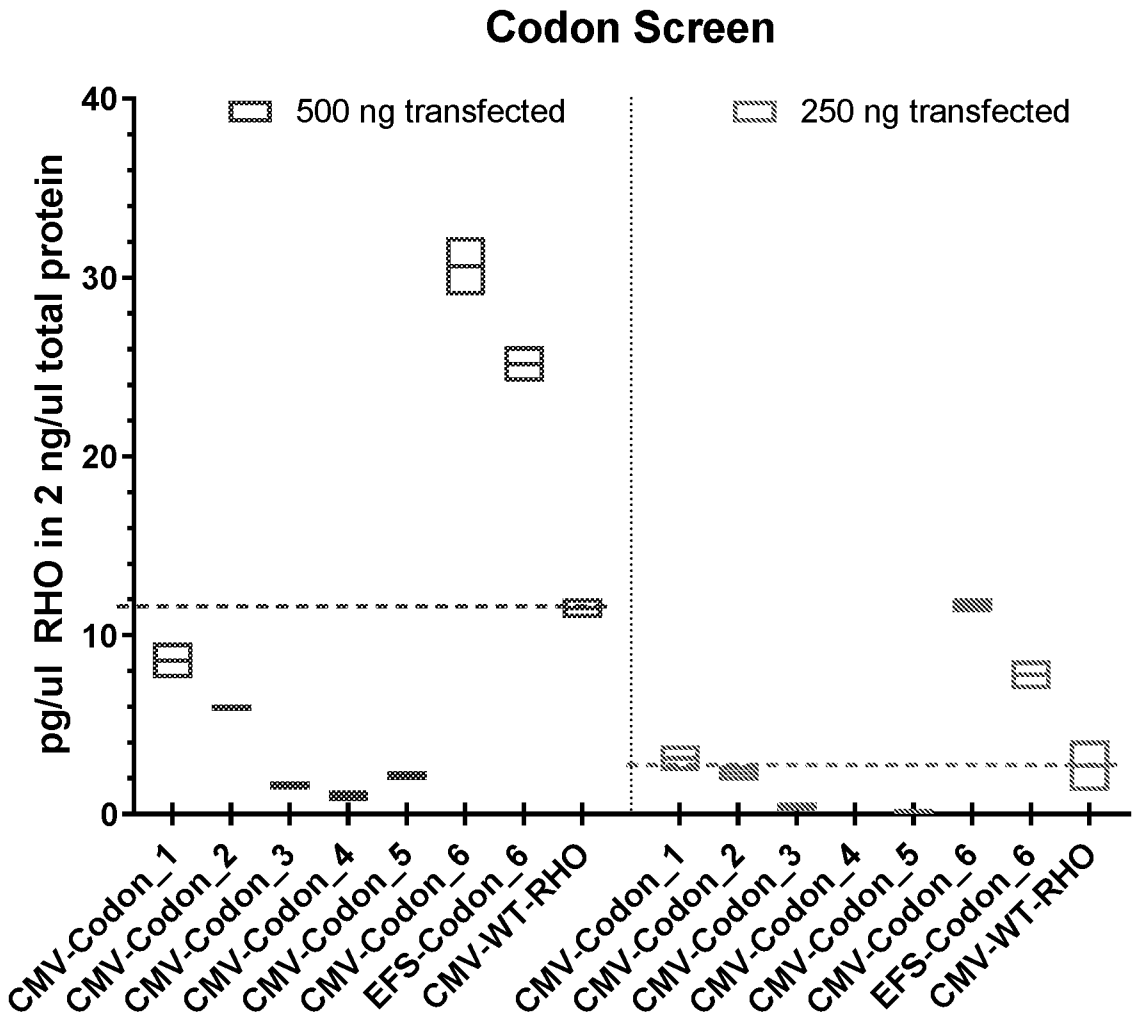

Codon Screen
500 ng transfected
250 ng transfected
pg/ul RHO in 2 ng/ul total protein
40
30
20
10
0
CMV-Codon_1
CMV-Codon_2
CMV-Codon_3
CMV-Codon_4
CMV-Codon_5
CMV-Codon_6
EFS-Codon_6
CMV-WT-RHO
CMV-Codon_1
CMV-Codon_2
CMV-Codon_3
CMV-Codon_4
CMV-Codon_5
CMV-Codon_6
EFS-Codon_6
CMV-WT-RHO

FIG. 14A

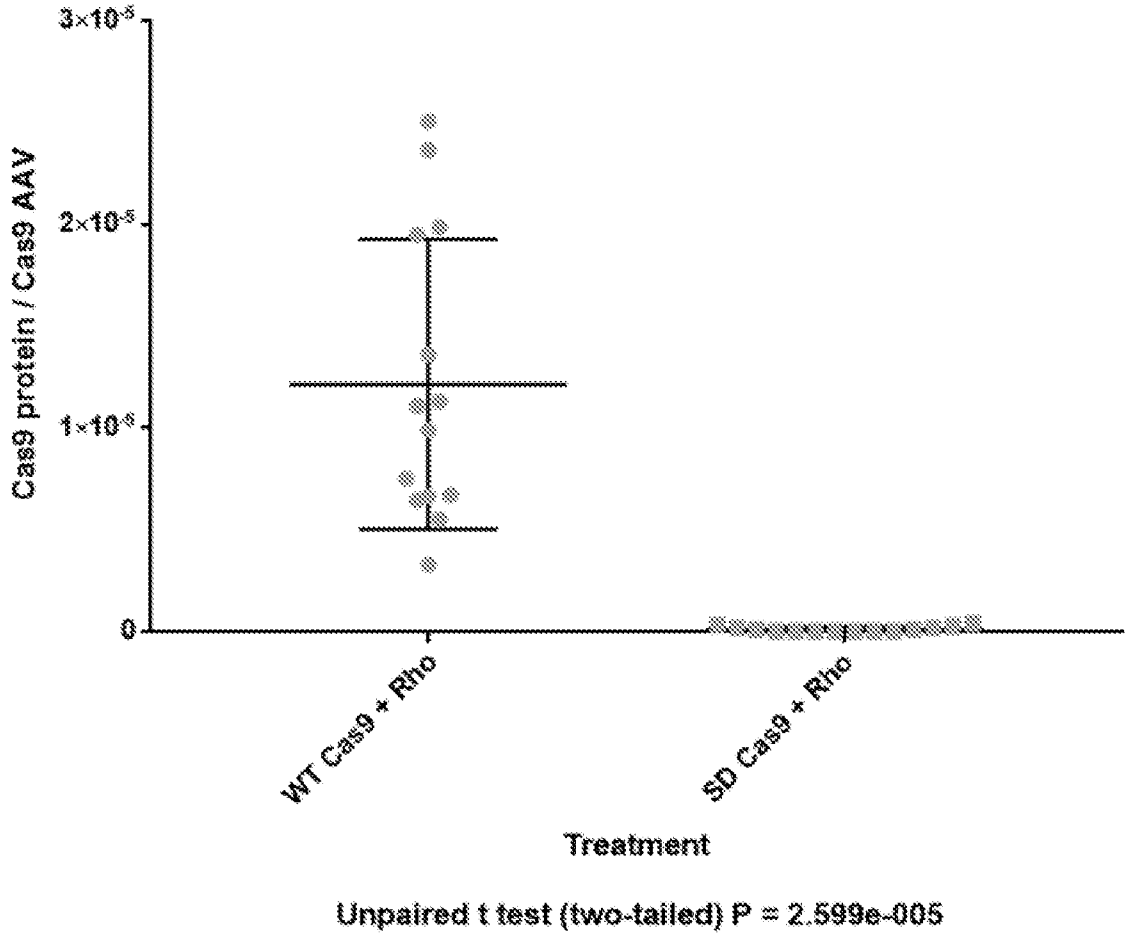

3×10⁻⁵
2×10⁻⁵
1×10⁻⁵
0
Cas9 protein / Cas9 AAV
WT Cas9 + Rho
SD Cas9 + Rho
Treatment
Unpaired t test (two-tailed) P = 2.599e-005

FIG. 14B

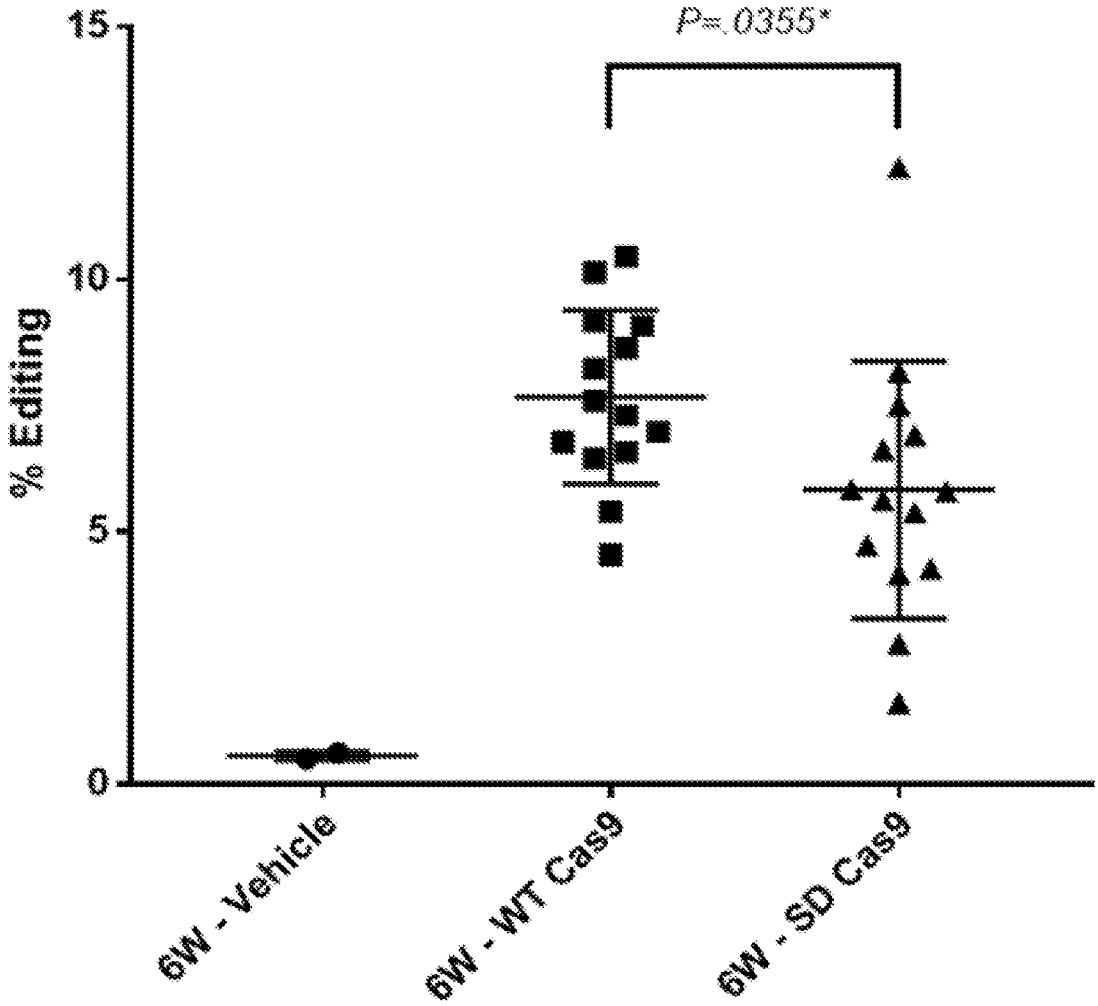


FIG. 15

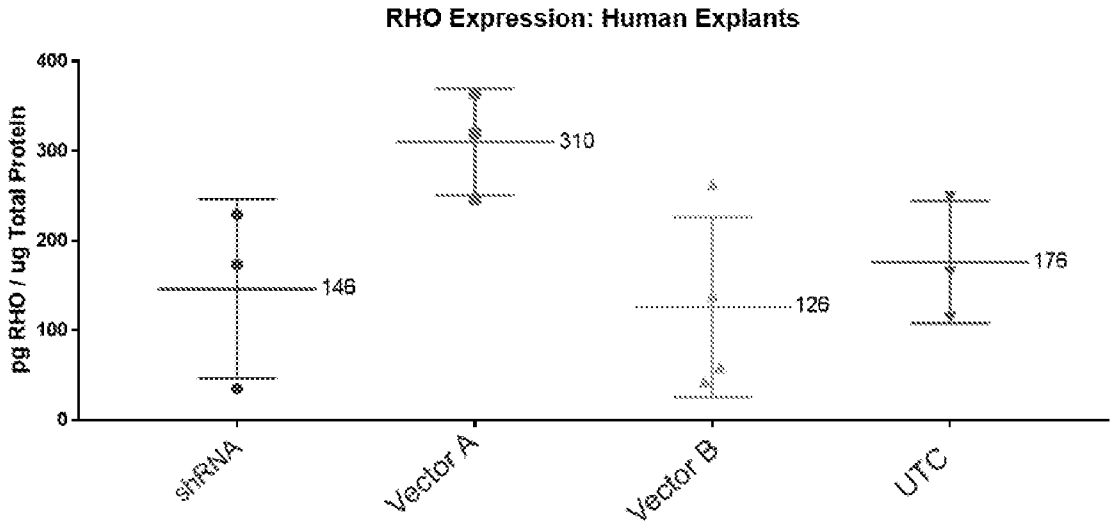

RHO Expression: Human Explants
pg RHO / ug Total Protein
400
300
200
100
0
146
310
126
176
shRNA
Vector A
Vector B
UTC

FIG. 16

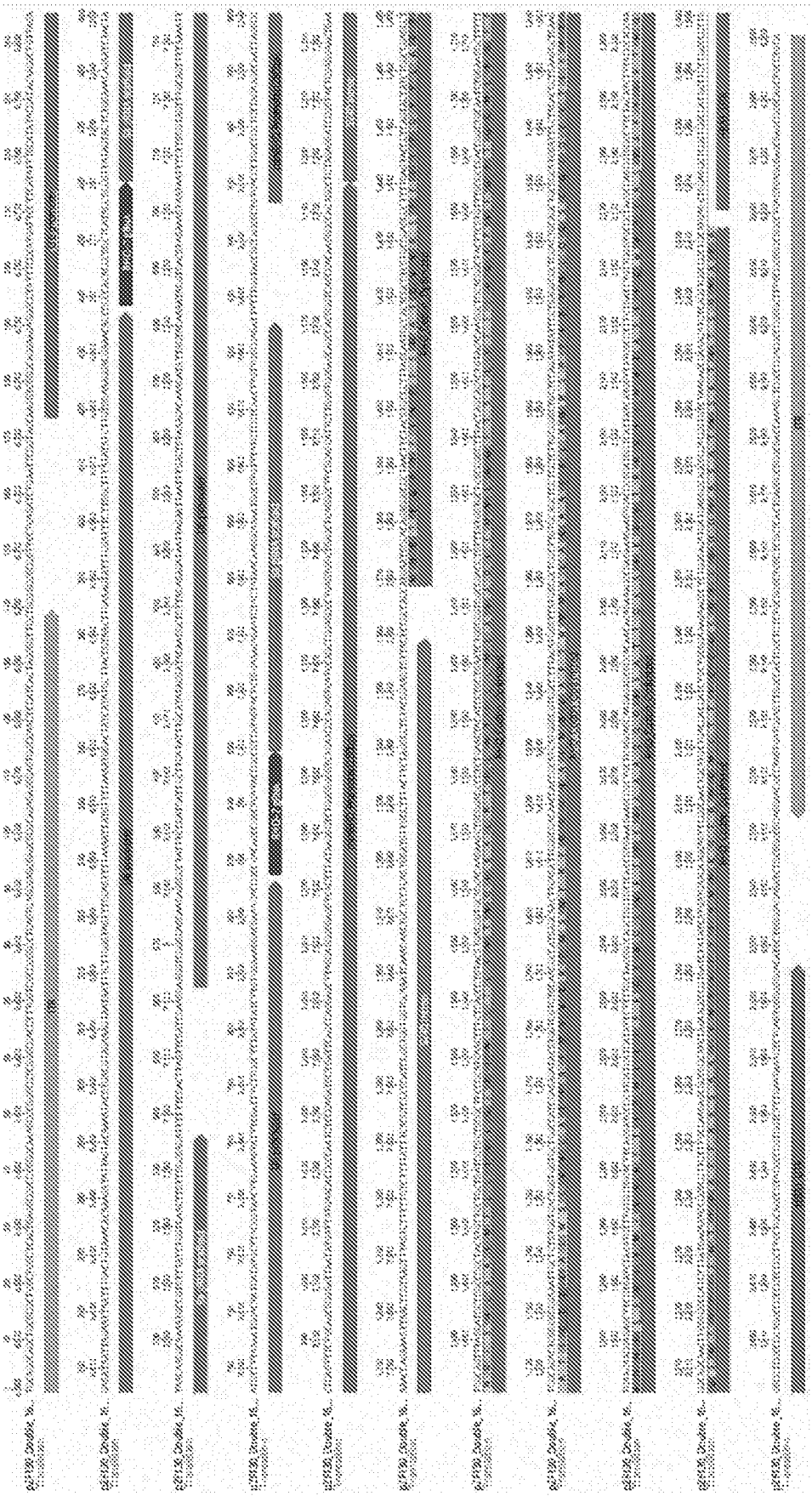


FIG. 17

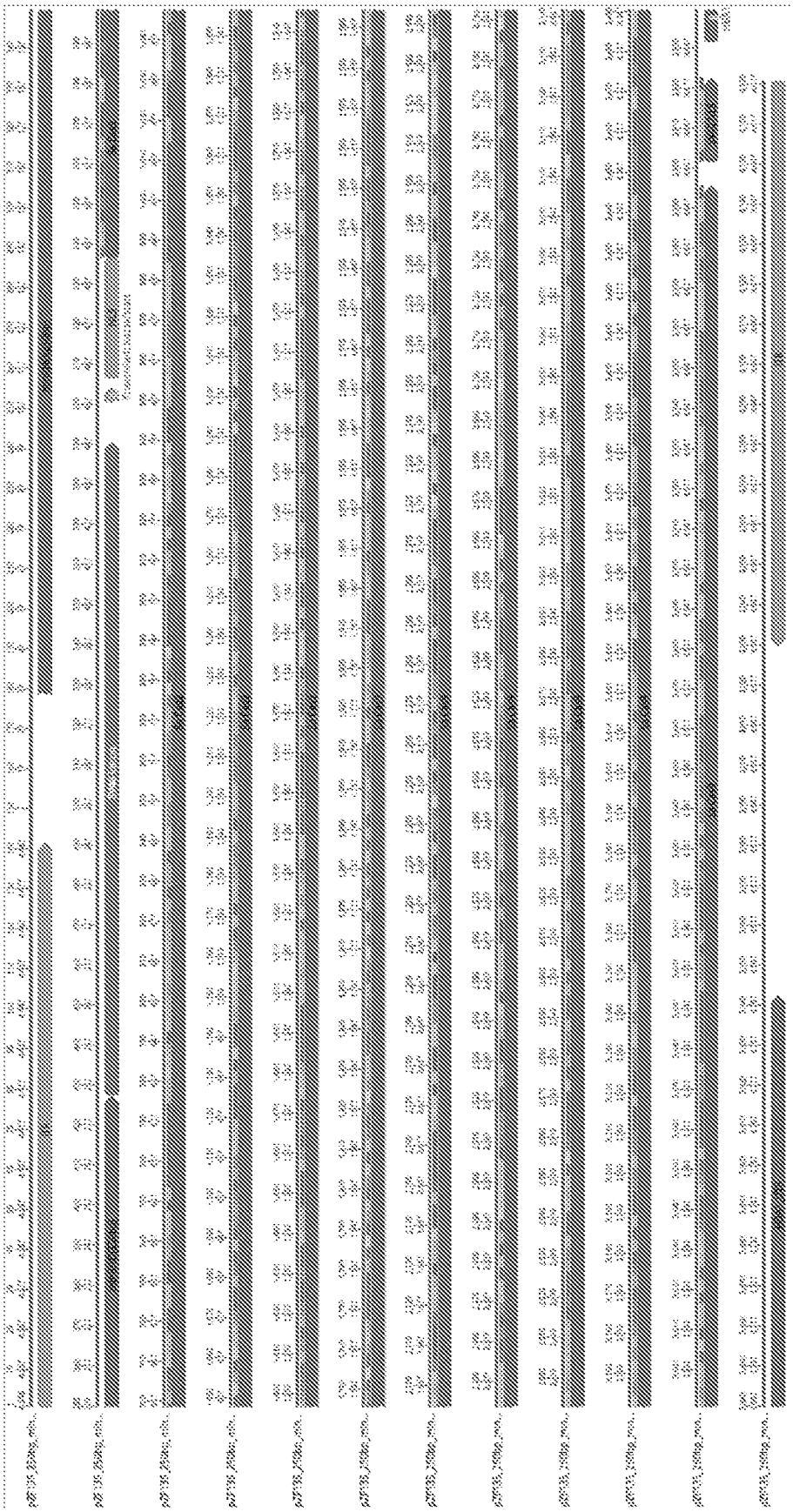
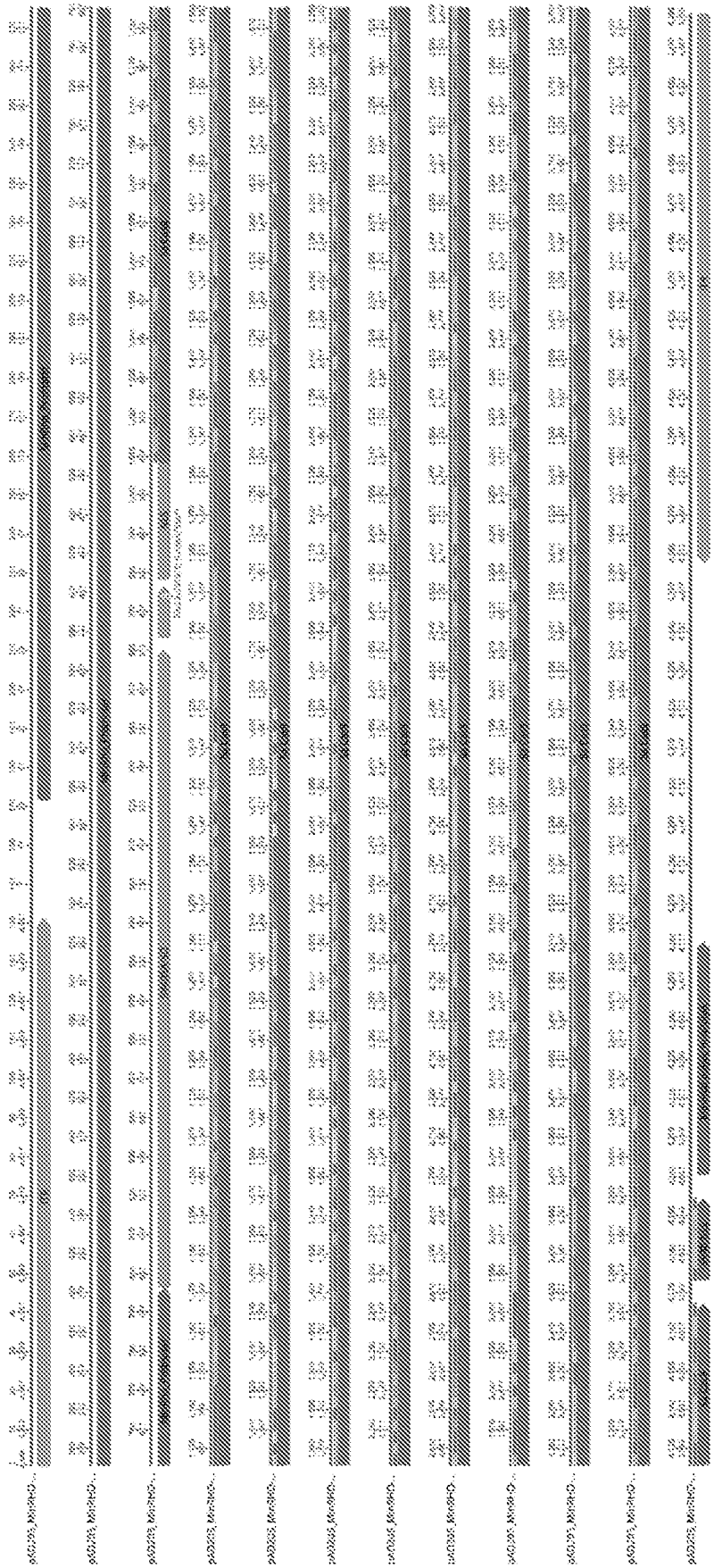


FIG. 18



INTERNATIONAL SEARCH REPORT

International application No
PCT/US2020/019766

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12N15/113 C12N15/10 A61K48/00
ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12N C40B A61K

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, BIOSIS, WPI Data, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2015/195621 A1 (THE JOHNS HOPKINS UNIVERSITY [US]) 23 December 2015 (2015-12-23) page 84 - page 97; figures 18, 19; example 4 the whole document page 16; figures 18, 19 ----- -/--	1-5, 10-12, 16,18, 19,23, 26,49, 50,70, 71,89, 102,115, 116

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
8 June 2020	17/06/2020

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Macchia, Giovanni

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Form PCT/ISA/210 (second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2020/019766

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

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

   a. [ ] forming part of the international application as filed:

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

      [ ] on paper or in the form of an image file.

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

   c. [X] furnished subsequent to the international filing date for the purposes of international search only:

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

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

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

3. Additional comments:

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ERIN R. BURNIGHT ET AL.: "Using CRISPR-Cas9 to generate gene-corrected autologous iPSCs for the treatment of inherited retinal degeneration", MOLECULAR THERAPY : THE JOURNAL OF THE AMERICAN SOCIETY OF GENE THERAPY, vol. 25, no. 9, 1 September 2017 (2017-09-01), pages 1999-2013, XP055557450, US ISSN: 1525-0016, DOI: 10.1016/j.ymthe.2017.05.015 the whole document page 2005 - page 2007; figure 4	1-5, 10-12, 16-19, 23,26, 27,70, 71, 114-116
Y	WO 2018/209158 A2 (EDITAS MEDICINE, INC. [US]) 15 November 2018 (2018-11-15) paragraphs [[0160]], [[0163]] page 68, paragraph [0202] - page 69, paragraph [0203] paragraphs [[0119]], [[0141].[0152]], [[0165]] - [[0166]], [[0175]] paragraphs [[0190]], [[0193]], [[0194]], [[0199]], [[0200]], [[0209]] paragraph [[0210]]	8,9,15, 20-22, 24,25, 44,46, 84-86, 104-106, 108
X	TSAI YI-TING ET AL.: "Clustered Regularly Interspaced Short Palindromic Repeats-based genome surgery for the treatment of Autosomal Dominant Retinitis Pigmentosa", OPHTHALMOLOGY, ELSEVIER, AMSTERDAM, NL, vol. 125, no. 9, 11 May 2018 (2018-05-11), pages 1421-1430, XP085448365, ISSN: 0161-6420, DOI: 10.1016/J.OPHTHA.2018.04.001	1-5, 10-12, 16,18, 19,23, 26-28, 30, 33-43, 45, 47-49, 51-83, 87-103, 107, 109-118
Y	the whole document	8,9,15, 20-22, 24,25, 44,46, 84-86, 104-106, 108

-/--

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	WO 2019/183630 A2 (THE TRUSTEES OF COLUMBIA UNIVERSITY IN THE CITY OF NEW YORK [US]) 26 September 2019 (2019-09-26) claim 55	1
X,P	WO 2019/102381 A1 (CASEBIA THERAPEUTICS LLP [GB]) 31 May 2019 (2019-05-31) claims	1
A	SERENA G. GIANNELLI ET AL.: "Cas9/sgRNA selective targeting of the P23H Rhodopsin mutant allele for treating retinitis pigmentosa by intravitreal AAV9.PHP.B-based delivery", HUMAN MOLECULAR GENETICS, vol. 27, no. 5, 1 March 2018 (2018-03-01), pages 761-779, XP55700284, ISSN: 0964-6906, DOI: 10.1093/hmg/ddx438 the whole document	1
A	MARIA CARMELA LATELLA ET AL.: "In vivo editing of the human mutant rhodopsin gene by electroporation of plasmid-based CRISPR/Cas9 in the mouse retina", MOLECULAR THERAPY-NUCLEIC ACIDS, vol. 5, 1 January 2016 (2016-01-01), page e389, XP055557441, US ISSN: 2162-2531, DOI: 10.1038/mtna.2016.92 the whole document	1,21,22
A	Pingjuan Li ET AL: "Allele-specific CRISPR/Cas9 genome editing of the single-base P23H mutation for rhodopsin associated dominant retinitis pigmentosa", bioRxiv, 29 January 2018 (2018-01-29), XP055557445, DOI: 10.1101/197962 Retrieved from the Internet: URL:https://www.biorxiv.org/content/biorxiv/early/2017/10/03/197962.full.pdf the whole document	1
A	WO 2016/176690 A2 (THE TRUSTEES OF COLUMBIA UNIVERSITY IN THE CITY OF NEW YORK [US]) 3 November 2016 (2016-11-03)	1

-/--

1

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	E. Burnright et al.: "Developing treatments for dominant retinal dystrophies using patient-specific induced pluripotent stem cells", Molecular Therapy, vol. 26, no. 5 Supplement 1 1 May 2018 (2018-05-01), pages 440-441, XP055700520, Retrieved from the Internet: URL:https://www.sciencedirect.com/journal/molecular-therapy/vol/28/issue/4/suppl/S1 [retrieved on 2020-06-03] the whole document	1-4
A	WO 2015/188056 A1 (SANGAMO BIOSCIENCES INC [US]) 10 December 2015 (2015-12-10) page 30, paragraph [0076] - page 31	5
A	JOANA A. VIDIGAL ET AL.: "Rapid and efficient one-step generation of paired gRNA CRISPR-Cas9 libraries", NATURE COMMUNICATIONS, vol. 6, no. 1, 17 August 2015 (2015-08-17) , XP055560017, DOI: 10.1038/ncomms9083 the whole document	17
A	US 2016/324987 A1 (CEDARS SINAI MEDICAL CENTER [US]) 10 November 2016 (2016-11-10) page 12; example 21	52
A	BENJAMIN BAKONDI ET AL.: "In vivo CRISPR/Cas9 gene editing corrects retinal dystrophy in the S334ter-3 rat model of autosomal dominant retinitis pigmentosa", MOLECULAR THERAPY : THE JOURNAL OF THE AMERICAN SOCIETY OF GENE THERAPY, vol. 24, no. 3, 1 March 2016 (2016-03-01), pages 556-563, XP055557459, US ISSN: 1525-0016, DOI: 10.1038/mt.2015.220 the whole document	1
A	LEE JIA HUI ET AL.: "Gene therapy for visual loss: opportunities and concerns", PROGRESS IN RETINAL AND EYE RESEARCH, OXFORD, GB, vol. 68, 29 August 2018 (2018-08-29), pages 31-53, XP085582549, ISSN: 1350-9462, DOI: 10.1016/J.PRETEYERES.2018.08.003 the whole document	1

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INTERNATIONAL SEARCH REPORT

International application No
PCT/US2020/019766

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2015/089462 A1 (THE BROAD INSTITUTE INC. [US]; MASSACHUSETTS INSTITUTE TECHNOLOGY [US]) 18 June 2015 (2015-06-18) example 2	1
A	WU WENYI ET AL.: "Application of CRISPR-Cas9 in eye disease", EXPERIMENTAL EYE RESEARCH, ACADEMIC PRESS LTD, LONDON, vol. 161, 12 June 2017 (2017-06-12), pages 116-123, XP085178515, ISSN: 0014-4835, DOI: 10.1016/J.EXER.2017.06.007 the whole document	1
A	WO 2018/009562 A1 (UNIV JOHNS HOPKINS [US]) 11 January 2018 (2018-01-11)	1

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2020/019766

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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WO 2019102381 A1	31-05-2019	AU 2018372763 A1	28-05-2020
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		US 2019153441 A1	23-05-2019
		WO 2019102381 A1	31-05-2019
WO 2016176690 A2	03-11-2016	EP 3289080 A2	07-03-2018
		HK 1251615 A1	01-02-2019
		US 2019275168 A1	12-09-2019
		WO 2016176690 A2	03-11-2016
WO 2015188056 A1	10-12-2015	EP 3151846 A1	12-04-2017
		EP 3152319 A1	12-04-2017
		US 2015353917 A1	10-12-2015
		WO 2015188056 A1	10-12-2015
		WO 2015188065 A1	10-12-2015
US 2016324987 A1	10-11-2016	NONE	
WO 2015089462 A1	18-06-2015	AU 2014361781 A1	16-06-2016
		BR 112016013201 A2	26-09-2017
		CA 2932478 A1	18-06-2015
		CN 106061510 A	26-10-2016
		CN 111206032 A	29-05-2020
		DK 3079725 T3	20-01-2020
		EP 3079725 A1	19-10-2016
		EP 3653229 A1	20-05-2020
		JP 2017504312 A	09-02-2017
		KR 20160089527 A	27-07-2016
		SG 10201804976Y A	30-07-2018
		US 2016340661 A1	24-11-2016
		WO 2015089462 A1	18-06-2015
WO 2018009562 A1	11-01-2018	AU 2017293773 A1	21-02-2019

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2020/019766

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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		CA 3029874 A1	11-01-2018
		CL 2019000024 A1	21-06-2019
		CN 109890424 A	14-06-2019
		EP 3481434 A1	15-05-2019
		JP 2019520391 A	18-07-2019
		KR 20190039703 A	15-04-2019
		SG 11201900049Q A	27-02-2019
		US 2020080108 A1	12-03-2020
		WO 2018009562 A1	11-01-2018