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METHODS OF TREATING CLRN1-ASSOCIATED HEARING LOSS AND/OR VISION LOSS

ABSTRACT

Provided herein are compositions that include a single nucleic acid vector or two different nucleic acid vectors, and the use of these compositions to treat hearing loss and/or vision loss in a subject.

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METHODS OF TREATING CLRN1-ASSOCIATED HEARING LOSS AND/OR VISION LOSS

CROSS-REFERENCE TO RELATED APPLICATIONS

This is a divisional application of Australian patent application No. 2019294603, the entire contents of which are incorporated herein by reference.

TECHNICAL FIELD

The present disclosure relates generally to the use of nucleic acids to treat hearing loss, vision loss, or both, in a human subject.

BACKGROUND OF THE INVENTION

Current treatments for hearing loss consist mainly of hearing amplification for mild to severe hearing loss and cochlear implants for severe to profound hearing loss; however, a long-felt need remains for agents and methods for preventing or reversing syndromic deafness.

Hearing loss can be conductive (arising from the ear canal or middle ear), sensorineural (arising from the inner ear or auditory nerve), or mixed. Most forms of syndromic deafness are associated with permanent hearing loss caused by damage to structures in the inner ear (sensorineural deafness), although some forms may involve changes in the middle ear (conductive hearing loss). The great majority of human sensorineural hearing loss is caused by abnormalities in the hair cells of the organ of Corti in the cochlea (poor hair cell function). The hair cells may be abnormal at birth, or may be damaged during the lifetime of an individual (e.g., as a result of noise trauma or infection).

SUMMARY

The present invention relates to a composition including at least two different nucleic acid vectors, where each of the at least two different vectors includes a coding sequence that encodes a different portion of a CLRN1 protein, can be used to generate a sequence encoding an active CLRN1 protein (e.g., a full-length CLRN1 protein) in a mammalian cell, and thereby treat CLRN1-associated hearing loss and/or vision loss in a

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subject in need thereof. The invention also related to compositions including a single nucleic acid vector that includes a coding sequence for a first and/or second isoform of CLRN1 protein.

Provided herein are compositions including at least two different nucleic acid vectors, where: each of the at least two different vectors includes a coding sequence that encodes a different portion of a CLRN1 protein, each of the encoded portions being at least 30 amino acid residues in length, wherein the amino acid sequence of each of the encoded portions may optionally partially overlap with the amino acid sequence of a different one of the encoded portions; no single vector of the at least two different vectors encodes a full-length CLRN1 protein; at least one of the coding sequences includes a nucleotide sequence spanning two consecutive exons of CLRN1 genomic DNA, and lacking an intronic sequence between the two consecutive exons; and when introduced into a mammalian cell, the at least two different vectors undergo homologous recombination with each other, thereby forming a recombined nucleic acid that encodes a full-length CLRN1 protein. In some embodiments of any of the compositions provided herein, each of the at least two different vectors includes a coding sequence that encodes a different portion of a first isoform of the CLRN1 protein. In some embodiments of any of the compositions provided herein, the first isoform of the CLRN1 protein includes a sequence that is at least 95% identical to SEQ ID NO: 3. In some embodiments of any of the compositions provided herein, the first isoform of the CLRN1 protein includes SEQ ID NO: 3. In some embodiments of any of the compositions provided herein, the first isoform of the CLRN1 protein consists of SEQ ID NO: 3. In some embodiments of any of the compositions provided herein, one of the at least two different nucleic acid vectors further includes a sequence that encodes a second isoform of the CLRN1 protein. In some embodiments of any of the compositions provided herein, the second isoform of the CLRN1 protein includes a sequence that is at least 95% identical to SEQ ID NO: 5. In some embodiments of any of the compositions provided herein, the second isoform of the CLRN1 protein includes SEQ ID NO: 5. In some embodiments of any of the compositions provided herein, the second isoform of the CLRN1 protein consists of SEQ ID NO: 5. In some embodiments of any of the compositions provided herein, each of the at least two different vectors includes a coding sequence that encodes a different portion

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of a second isoform of the CLRN1 protein. In some embodiments of any of the compositions provided herein, the second isoform of the CLRN1 protein includes a sequence that is at least 95% identical to SEQ ID NO: 5. In some embodiments of any of the compositions provided herein, the second isoform of the CLRN1 protein includes SEQ ID NO: 5. In some embodiments of any of the compositions provided herein, the second isoform of the CLRN1 protein consists of SEQ ID NO: 5. In some embodiments of any of the compositions provided herein, one of the at least two different nucleic acid vectors further includes a sequence that encodes a first isoform of the CLRN1 protein. In some embodiments of any of the compositions provided herein, the first isoform of the CLRN1 protein includes a sequence that is at least 95% identical to SEQ ID NO: 3. In some embodiments of any of the compositions provided herein, the first isoform of the CLRN1 protein includes SEQ ID NO: 3. In some embodiments of any of the compositions provided herein, the first isoform of the CLRN1 protein consists of SEQ ID NO: 3.

In some embodiments of any of the compositions provided herein, at least one of the at least two different vectors includes a 5' untranslated region (UTR), a 3' UTR, or both. In some embodiments of any of the compositions provided herein, the 5' UTR comprises at least 10 contiguous nucleotides from anywhere within SEQ ID NO: 12. In some embodiments of any of the compositions provided herein, the 5' UTR includes at least 20 contiguous nucleotides from anywhere within SEQ ID NO: 12. In some embodiments of any of the compositions provided herein, the 5' UTR includes at least 50 contiguous nucleotides from anywhere within SEQ ID NO: 12. In some embodiments of any of the compositions provided herein, the 5' UTR includes at least 80 contiguous nucleotides from anywhere within SEQ ID NO: 12. In some embodiments of any of the compositions provided herein, the 5'UTR includes a sequence that is at least 80% identical to SEQ ID NO: 12. In some embodiments of any of the compositions described herein, the 3' UTR includes at least 10 contiguous nucleotides from anywhere within SEQ ID NO: 15. In some embodiments of any of the compositions described herein, the 3' UTR includes at least 20 contiguous nucleotides from anywhere within SEQ ID NO: 15. In some embodiments of any of the compositions provided herein, the 3' UTR includes at least 50 contiguous nucleotides from anywhere within SEQ ID NO: 15. In

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some embodiments of any of the compositions provided herein, the 3' UTR includes at least 80 contiguous nucleotides from anywhere within SEQ ID NO: 15. In some embodiments of any of the compositions provided herein, the 3' UTR includes a sequence that is at least 80% identical to SEQ ID NO: 15.

In some embodiments of any of the compositions provided herein, each of the at least two different vectors is a plasmid, a transposon, a cosmid, an artificial chromosome, or a viral vector. In some embodiments of any of the compositions provided herein, each of the at least two different vectors is a human artificial chromosome (HAC), yeast artificial chromosome (YAC), bacterial artificial chromosome (BAC), or a P1-derived artificial chromosome (PAC). In some embodiments of any of the compositions provided herein, each of the at least two different vectors is a viral vector selected from an adeno-associated virus (AAV) vector, an adenovirus vector, a lentivirus vector, or a retrovirus vector. In some embodiments of any of the compositions provided herein, each of the at least two different vectors is an AAV vector.

In some embodiments of any of the compositions provided herein, the amino acid sequence of none of the encoded portions overlaps with the amino acid sequence of a different one of the encoded portions. In some embodiments of any of the compositions provided herein, the amino acid sequence of each of the encoded portions partially overlaps with the amino acid sequence of a different one of the encoded portions. In some embodiments of any of the compositions provided herein, the overlapping amino acid sequence is between about 30 amino acid residues to about 202 amino acid residues in length. In some embodiments of any of the compositions provided herein, the vectors include two different vectors, each of which includes a different segment of an intron, wherein the intron includes the nucleotide sequence of an intron that is present in CLRN1 genomic DNA, and wherein the two different intron segments overlap in sequence by at least 100 nucleotides. In some embodiments of any of the compositions provided herein, the two different intron segments overlap in sequence by 100 nucleotides to about 800 nucleotides.

In some embodiments of any of the compositions provided herein, the entire nucleotide sequence of each of the at least two different vectors is between about 500 nucleotides to about 10,000 nucleotides in length. In some embodiments of any of the

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compositions provided herein, the entire nucleotide sequence of each of the at least two different vectors is between 500 nucleotides to 5,000 nucleotides in length. In some embodiments of any of the compositions provided herein, the number of different vectors in the composition is two. In some embodiments of any of the compositions provided herein, a first of the two different vectors includes a coding sequence that encodes an N-terminal portion of the CLRN1 protein. In some embodiments of any of the compositions provided herein, the N-terminal portion of the CLRN1 protein is between 30 amino acids to 202 amino acids in length. In some embodiments of any of the compositions provided herein, the N-terminal portion of the CLRN1 protein is between 60 amino acids to 170 amino acids in length. In some embodiments of any of the compositions provided herein, the first vector further includes a 5' UTR sequence. In some embodiments of any of the compositions provided herein, the 5' UTR comprises at least 10 contiguous nucleotides from anywhere within SEQ ID NO: 12. In some embodiments of any of the compositions provided herein, the 5' UTR includes at least 20 contiguous nucleotides from anywhere within SEQ ID NO: 12. In some embodiments of any of the compositions provided herein, the 5' UTR includes at least 50 contiguous nucleotides from anywhere within SEQ ID NO: 12. In some embodiments of any of the compositions provided herein, the 5' UTR includes at least 80 contiguous nucleotides from anywhere within SEQ ID NO: 12. In some embodiments of any of the compositions provided herein, the 5'UTR includes a sequence that is at least 80% identical to SEQ ID NO: 12. In some embodiments of any of the compositions provided herein, the first vector further includes one or both of a promoter and a Kozak sequence. In some embodiments of any of the compositions provided herein, the first vector includes a promoter that is an inducible promoter, a constitutive promoter, or a tissue-specific promoter. In some embodiments of any of the compositions provided herein, the second of the two different vectors includes a coding sequence that encodes a C-terminal portion of the CLRN1 protein. In some embodiments of any of the compositions provided herein, the C-terminal portion of the CLRN1 protein is between 30 amino acids to 202 amino acids in length. In some embodiments of any of the compositions provided herein, the C-terminal portion of the CLRN1 protein is between 60 amino acids to 170 amino acids in length. In some embodiments of any of the compositions provided herein,

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the second vector further includes a polyadenylation signal sequence. In some embodiments of any of the compositions provided herein, the second vector further includes a 3'UTR sequence. In some embodiments of any of the compositions provided herein, the 3' UTR includes at least 10 contiguous nucleotides from anywhere within SEQ ID NO: 15. In some embodiments of any of the compositions provided herein, the 3' UTR includes at least 20 contiguous nucleotides from anywhere within SEQ ID NO: 15. In some embodiments of any of the compositions provided herein, the 3' UTR includes at least 50 contiguous nucleotides from anywhere within SEQ ID NO: 15. In some embodiments of any of the compositions provided herein, the 3' UTR includes at least 80 contiguous nucleotides from anywhere within SEQ ID NO: 15. In some embodiments of any of the compositions provided herein, the 3' UTR includes a sequence that is at least 80% identical to SEQ ID NO: 15.

Also provided herein are compositions that include a single nucleic acid vector, where the vector includes one or both of (i) a first coding sequence encoding a first isoform of CLRN1 protein, and (ii) a second coding sequence encoding a second isoform of CLRN1 protein, where one or both of the first and second coding sequences includes a nucleotide sequence spanning two consecutive exons of a CLRN1 genomic DNA, and lacking an intronic sequence between the two consecutive introns. In some embodiments of any of the compositions provided herein, the single nucleic acid vector contains the first coding sequence and not the second coding sequence. In some embodiments of any of the compositions provided herein, the single nucleic acid vector contains the second coding sequence and not the first coding sequence. In some embodiments of any of the compositions provided herein, the single nucleic acid vector contains both the first coding sequence and the second coding sequence. In some embodiments of any of the compositions provided herein, the first isoform of the CLRN1 protein includes a sequence that is at least 95% identical to SEQ ID NO: 3. In some embodiments of any of the compositions provided herein, the first isoform of the CLRN1 protein includes SEQ ID NO: 3. In some embodiments of any of the compositions provided herein, the first isoform of the CLRN1 protein consists of SEQ ID NO: 3. In some embodiments of any of the compositions provided herein, the second isoform of the CLRN1 protein includes a sequence that is at least 95% identical to SEQ ID NO: 5. In some embodiments of any of

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the compositions provided herein, the second isoform of the CLRN1 protein includes SEQ ID NO: 5. In some embodiments of any of the compositions provided herein, the second isoform of the CLRN1 protein consists of SEQ ID NO: 5.

In some embodiments of any of the compositions provided herein, the single nucleic acid vector further includes a 5' untranslated region (UTR), a 3' UTR, or both. In some embodiments of any of the compositions provided herein, the 5' UTR includes at least 10 contiguous nucleotides from anywhere within SEQ ID NO: 12. In some embodiments of any of the compositions provided herein, the 5' UTR includes at least 20 contiguous nucleotides from anywhere within SEQ ID NO: 12. In some embodiments of any of the compositions provided herein, the 5' UTR includes at least 50 contiguous nucleotides from anywhere within SEQ ID NO: 12. In some embodiments of any of the compositions provided herein, the 5' UTR includes at least 80 contiguous nucleotides from anywhere within SEQ ID NO: 12. In some embodiments of any of the compositions provided herein, the 5' UTR includes a sequence that is at least 80% identical to SEQ ID NO: 12. In some embodiments of any of the compositions provided herein, the 3' UTR includes at least 10 contiguous nucleotides from anywhere within SEQ ID NO: 15. In some embodiments of any of the compositions provided herein, the 3' UTR includes at least 20 contiguous nucleotides from anywhere within SEQ ID NO: 15. In some embodiments of any of the compositions provided herein, the 3' UTR includes at least 50 contiguous nucleotides from anywhere within SEQ ID NO: 15. In some embodiments of any of the compositions provided herein, the 3' UTR includes at least 80 contiguous nucleotides from anywhere within SEQ ID NO: 15. In some embodiments of any of the compositions provided herein, the 3' UTR includes a sequence that is at least 80% identical to SEQ ID NO: 15.

In some embodiments of any of the compositions provided herein, the single nucleic acid vector is a plasmid, a transposon, a cosmid, an artificial chromosome, or a viral vector. In some embodiments of any of the compositions provided herein, the single nucleic acid vector is a human artificial chromosome (HAC), yeast artificial chromosome (YAC), bacterial artificial chromosome (BAC), or a P1-derived artificial chromosome (PAC). In some embodiments of any of the compositions provided herein, the single nucleic acid vector is a viral vector selected from an adeno-associated virus (AAV)

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vector, an adenovirus vector, a lentivirus vector, or a retrovirus vector. In some embodiments of any of the compositions provided herein, the single nucleic acid vector is an AAV vector. In some embodiments of any of the compositions provided herein, the single nucleic acid vector further includes one or both of a promoter and a Kozak sequence. In some embodiments of any of the compositions provided herein, the first vector includes a promoter that is an inducible promoter, a constitutive promoter, or a tissue-specific promoter. In some embodiments of any of the compositions provided herein, the single nucleic acid vector further includes a polyadenylation signal sequence. Some embodiments of any of the compositions provided herein further include a pharmaceutically acceptable excipient.

Also provided herein are kits that include any of the compositions provided herein. Some embodiments of any of the kits provided herein further include a pre-loaded syringe including or containing any of the compositions described herein.

Also provided herein are methods that include introducing into a cochlea of a mammal a therapeutically effective amount of any of the compositions provided herein. In some embodiments of any of the methods provided herein, the mammal is a human. In some embodiments of any of the methods provided herein, the mammal has been previously identified as having a defective CLRN1 gene.

Also provided herein are methods of increasing expression of a full-length CLRN1 protein in a mammalian cell that include introducing any of the compositions provided herein into the mammalian cell. In some embodiments of any of the methods provided herein, the mammalian cell is a cochlear inner hair cell or a cochlear outer hair cell. In some embodiments of any of the methods provided herein, the mammalian cell is a retinal cell. In some embodiments of any of the methods provided herein, the mammalian cell is a human cell. In some embodiments of any of the methods provided herein, the mammalian cell has previously been determined to have a defective CLRN1 gene.

Also provided herein are methods of increasing expression of a full-length CLRN1 protein in an inner hair cell, an outer hair cell, or both, in a cochlea of a mammal, that include: introducing into the cochlea of the mammal a therapeutically effective amount of any of the compositions provided herein. Also provided herein are methods of

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increasing expression of a full-length CLRN1 protein in an eye of a mammal that include: intraocularly administering to the eye of the mammal a therapeutically effective amount of any of the compositions provided herein. In some embodiments of any of the methods provided herein, the mammal has been previously identified as having a defective CLRN1 gene. In some embodiments of any of the methods provided herein, the mammal is a human.

Also provided herein are methods of treating hearing loss in a subject identified as having a defective CLRN1 gene that include: administering a therapeutically effective amount of any of the compositions provided herein into the cochlea of the subject. Also provided herein are methods of treating vision loss in a subject identified as having a defective CLRN1 gene that include: administering a therapeutically effective amount of any of the compositions provided herein into the eye of the subject. In some embodiments of any of the methods provided herein, the subject has Usher syndrome type III. In some embodiments of any of the methods provided herein, the subject is a human. Some embodiments of any of the methods provided herein further include, prior to the administering step, determining that the subject has a defective CLRN1 gene.

Also provided herein are compositions that include two different nucleic acid vectors, where: a first nucleic acid vector of the two different nucleic acid vectors includes a promoter, a first coding sequence that encodes an N-terminal portion of a CLRN1 protein positioned 3' of the promoter, and a splice donor sequence positioned at the 3' end of the first coding sequence; and a second nucleic acid vector of the two different nucleic acid vectors includes a splice acceptor sequence, a second coding sequence that encodes a C-terminal portion of a CLRN1 protein positioned at the 3' end of the splice acceptor sequence, and a polyadenylation signal sequence at the 3' end of the second coding sequence; where each of the encoded portions is at least 30 amino acid residues in length, where the amino acid sequences of the two encoded portions do not overlap with each other; where no single vector of the two different vectors encodes a full-length CLRN1 protein; and when introduced into a mammalian cell, splicing occurs between the splice donor sequence and the splice acceptor sequence, thereby forming a recombined nucleic acid that encodes a full-length CLRN1 protein. In some embodiments of any of the compositions provided herein, the first coding sequence

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encodes an N-terminal portion of a first isoform of CLRN1 protein, and the second coding sequence encodes a C-terminal portion of the first isoform of CLRN1 protein. In some embodiments of any of the compositions provided herein, the first isoform of the CLRN1 protein includes a sequence that is at least 95% identical to SEQ ID NO: 3. In some embodiments of any of the compositions provided herein, the first isoform of the CLRN1 protein includes SEQ ID NO: 3. In some embodiments of any of the compositions provided herein, the first isoform of the CLRN1 protein consists of SEQ ID NO: 3. In some embodiments of any of the compositions provided herein, the first nucleic acid vector of the second nucleic acid vector further includes a sequence that encodes a second isoform of the CLRN1 protein. In some embodiments of any of the compositions provided herein, the second isoform of the CLRN1 protein includes a sequence that is at least 95% identical to SEQ ID NO: 5. In some embodiments of any of the compositions provided herein, the second isoform of the CLRN1 protein includes SEQ ID NO: 5. In some embodiments of any of the compositions provided herein, the second isoform of the CLRN1 protein consists of SEQ ID NO: 5.

In some embodiments of any of the compositions provided herein, the first coding sequence encodes an N-terminal portion of a second isoform of CLRN1 protein, and the second coding sequence encodes a C-terminal portion of the second isoform of CLRN1 protein. In some embodiments of any of the compositions provided herein, the second isoform of the CLRN1 protein includes a sequence that is at least 95% identical to SEQ ID NO: 5. In some embodiments of any of the compositions provided herein, the second isoform of the CLRN1 protein includes SEQ ID NO: 5. In some embodiments of any of the compositions provided herein, the second isoform of the CLRN1 protein consists of SEQ ID NO: 5. In some embodiments of any of the compositions provided herein, the first nucleic acid vector or the second nucleic acid vector further includes a sequence that encodes a first isoform of the CLRN1 protein. In some embodiments of any of the compositions provided herein, the first isoform of the CLRN1 protein includes a sequence that is at least 95% identical to SEQ ID NO: 3. In some embodiments of any of the compositions provided herein, the first isoform of the CLRN1 protein includes SEQ ID NO: 3. In some embodiments of any of the compositions provided herein, the first isoform of the CLRN1 protein consists of SEQ ID NO: 3.

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In some embodiments of any of the compositions provided herein, one or both of the first nucleic acid vector and the second nucleic acid vector includes a 5' untranslated region (UTR), a 3' UTR, or both. In some embodiments of any of the compositions provided herein, the 5' UTR includes at least 10 contiguous nucleotides from anywhere within SEQ ID NO: 12. In some embodiments of any of the compositions provided herein, the 5' UTR includes at least 20 contiguous nucleotides from anywhere within SEQ ID NO: 12. In some embodiments of any of the compositions provided herein, the 5' UTR includes at least 50 contiguous nucleotides from anywhere within SEQ ID NO: 12. In some embodiments of any of the compositions provided herein, the 5' UTR includes at least 80 contiguous nucleotides from anywhere within SEQ ID NO: 12. In some embodiments of any of the compositions provided herein, the 5'UTR includes a sequence that is at least 80% identical to SEQ ID NO: 12. In some embodiments of any of the compositions provided herein, the 3' UTR includes at least 10 contiguous nucleotides from anywhere within SEQ ID NO: 15. In some embodiments of any of the compositions provided herein, the 3' UTR includes at least 20 contiguous nucleotides from anywhere within SEQ ID NO: 15. In some embodiments of any of the compositions provided herein, the 3' UTR includes at least 50 contiguous nucleotides from anywhere within SEQ ID NO: 15. In some embodiments of any of the compositions provided herein, the 3' UTR includes at least 80 contiguous nucleotides from anywhere within SEQ ID NO: 15. In some embodiments of any of the compositions provided herein, the 3' UTR includes a sequence that is at least 80% identical to SEQ ID NO: 15.

In some embodiments of any of the compositions provided herein, each of the first nucleic acid vector and the second nucleic acid vector is a plasmid, a transposon, a cosmid, an artificial chromosome, or a viral vector. In some embodiments of any of the compositions provided herein, each of the first nucleic acid vector and the second nucleic acid vector is a human artificial chromosome (HAC), yeast artificial chromosome (YAC), bacterial artificial chromosome (BAC), or a P1-derived artificial chromosome (PAC). In some embodiments of any of the compositions provided herein, each of the first nucleic acid vector and the second nucleic acid vector is a viral vector selected from an adeno-associated virus (AAV) vector, an adenovirus vector, a lentivirus vector, or a retrovirus

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vector. In some embodiments of any of the compositions provided herein, each of the first nucleic acid vector and the second nucleic acid vector is an AAV vector. In some embodiments of any of the compositions provided herein, at least one of the coding sequences includes a nucleotide sequence spanning two consecutive exons of CLRN1 genomic DNA, and lacking an intronic sequence between the two consecutive exons.

Also provided herein are compositions that include two different nucleic acid vectors, where: a first nucleic acid vector of the two different nucleic acid vectors includes a promoter, a first coding sequence that encodes an N-terminal portion of a CLRN1 protein positioned 3' of the promoter, a splice donor sequence positioned at the 3' end of the first coding sequence, and a first detectable marker gene positioned 3' of the splice donor sequence; and a second nucleic acid vector of the two different nucleic acid vectors includes a second detectable marker gene, a splice acceptor sequence positioned 3' of the second detectable marker gene, a second coding sequence that encodes a C-terminal portion of a CLRN1 protein positioned at the 3' end of the splice acceptor sequence, and a polyadenylation signal sequence positioned at the 3' end of the second coding sequence; where each of the encoded portions is at least 30 amino acid residues in length, where the amino acid sequences of the encoded portions do not overlap with each other; where no single vector of the two different vectors encodes a full-length CLRN1 protein; and when introduced into a mammalian cell, splicing occurs between the splice donor sequence and the splice acceptor sequence, thereby forming a recombined nucleic acid that encodes a full-length CLRN1 protein. In some embodiments of any of the compositions provided herein, the first coding sequence encodes an N-terminal portion of a first isoform of CLRN1 protein, and the second coding sequence encodes a C-terminal portion of the first isoform of CLRN1 protein. In some embodiments of any of the compositions provided herein, the first isoform of the CLRN1 protein includes a sequence that is at least 95% identical to SEQ ID NO: 3. In some embodiments of any of the compositions provided herein, the first isoform of the CLRN1 protein includes SEQ ID NO: 3. In some embodiments of any of the compositions provided herein, the first isoform of the CLRN1 protein consists of SEQ ID NO: 3. In some embodiments of any of the compositions provided herein, the first nucleic acid vector of the second nucleic acid vector further includes a sequence that encodes a second isoform of the CLRN1

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protein. In some embodiments of any of the compositions provided herein, the second isoform of the CLRN1 protein includes a sequence that is at least 95% identical to SEQ ID NO: 5. In some embodiments of any of the compositions provided herein, the second isoform of the CLRN1 protein includes SEQ ID NO: 5. In some embodiments of any of the compositions provided herein, the second isoform of the CLRN1 protein consists of SEQ ID NO: 5. In some embodiments of any of the compositions provided herein, the first coding sequence encodes an N-terminal portion of a second isoform of CLRN1 protein, and the second coding sequence encodes a C-terminal portion of the second isoform of CLRN1 protein. In some embodiments of any of the compositions provided herein, the second isoform of the CLRN1 protein includes a sequence that is at least 95% identical to SEQ ID NO: 5. In some embodiments of any of the compositions provided herein, thesecond isoform of the CLRN1 protein includes SEQ ID NO: 5. In some embodiments of any of the compositions provided herein, the second isoform of the CLRN1 protein consists of SEQ ID NO: 5. In some embodiments of any of the compositions provided herein,the first nucleic acid vector or the second nucleic acid vector further includes a sequence that encodes a first isoform of the CLRN1 protein. In some embodiments of any of the compositions provided herein, the first isoform of the CLRN1 protein includes a sequence that is at least 95% identical to SEQ ID NO: 3. In some embodiments of any of the compositions provided herein, the first isoform of the CLRN1 protein includes SEQ ID NO: 3. In some embodiments of any of the compositions provided herein, the first isoform of the CLRN1 protein consists of SEQ ID NO: 3.

In some embodiments of any of the compositions provided herein, one or both of the first nucleic acid vector and the second nucleic acid vector includes a 5’ untranslated region (UTR), a 3’ UTR, or both. In some embodiments of any of the compositions provided herein, the 5’ UTR includes at least 10 contiguous nucleotides from anywhere within SEQ ID NO: 12. In some embodiments of any of the compositions provided herein, the 5’ UTR includes at least 20 contiguous nucleotides from anywhere within SEQ ID NO: 12. In some embodiments of any of the compositions provided herein, the 5’ UTR includes at least 50 contiguous nucleotides from anywhere within SEQ ID NO: 12.

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In some embodiments of any of the compositions provided herein, the 5' UTR comprises at least 80 contiguous nucleotides from anywhere within SEQ ID NO: 12. In some embodiments of any of the compositions provided herein, the 5' UTR includes a sequence that is at least 80% identical to SEQ ID NO: 12. In some embodiments of any of the compositions provided herein, the 3' UTR includes at least 10 contiguous nucleotides from anywhere within SEQ ID NO: 15. In some embodiments of any of the compositions provided herein, the 3' UTR includes at least 20 contiguous nucleotides from anywhere within SEQ ID NO: 15. In some embodiments of any of the compositions provided herein, the 3' UTR includes at least 50 contiguous nucleotides from anywhere within SEQ ID NO: 15. In some embodiments of any of the compositions provided herein, the 3' UTR includes at least 80 contiguous nucleotides from anywhere within SEQ ID NO: 15. In some embodiments of any of the compositions provided herein, the 3' UTR includes a sequence that is at least 80% identical to SEQ ID NO: 15.

In some embodiments of any of the compositions provided herein, each of the first nucleic acid vector and the second nucleic acid vector is a plasmid, a transposon, a cosmid, an artificial chromosome, or a viral vector. In some embodiments of any of the compositions provided herein, each of the first nucleic acid vector and the second nucleic acid vector is a human artificial chromosome (HAC), yeast artificial chromosome (YAC), bacterial artificial chromosome (BAC), or a P1-derived artificial chromosome (PAC). In some embodiments of any of the compositions provided herein, each of the first nucleic acid vector and the second nucleic acid vector is a viral vector selected from an adeno-associated virus (AAV) vector, an adenovirus vector, a lentivirus vector, or a retrovirus vector. In some embodiments of any of the compositions provided herein, each of the first nucleic acid vector and the second nucleic acid vector is an AAV vector. In some embodiments of any of the compositions provided herein, at least one of the coding sequences comprises a nucleotide sequence spanning two consecutive exons of CLRN1 genomic DNA, and lacking an intronic sequence between the two consecutive exons. In some embodiments of any of the compositions provided herein, the first or second detectable marker gene is alkaline phosphatase.

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Also provided herein are compositions that include two different nucleic acid vectors, where: a first nucleic acid vector of the two different nucleic acid vectors includes a promoter, a first coding sequence that encodes an N-terminal portion of a CLRN1 protein positioned 3' to the promoter, a splice donor sequence positioned at the 3' end of the first coding sequence, and a F1 phage recombinogenic region positioned 3' to the splice donor sequence; and a second nucleic acid vector of the two different nucleic acid vectors includes a F1 phage recombinogenic region, a splice acceptor sequence positioned 3' of the F1 phage recombinogenic region, a second coding sequence that encodes a C-terminal portion of a CLRN1 protein positioned at the 3' end of the splice acceptor sequence, and a polyadenylation signal sequence positioned at the 3' end of the second coding sequence; where each of the two encoded portions is at least 30 amino acid residues in length, where the amino acid sequences of the two encoded portions do not overlap with each other; where no single vector of the two different vectors encodes a full-length CLRN1 protein; and when introduced into a mammalian cell, splicing occurs between the splice donor sequence and the splice acceptor sequence, thereby forming a recombined nucleic acid that encodes a full-length CLRN1 protein. In some embodiments of any of the compositions provided herein, the first coding sequence encodes an N-terminal portion of a first isoform of CLRN1 protein, and the second coding sequence encodes a C-terminal portion of the first isoform of CLRN1 protein. In some embodiments of any of the compositions provided herein, the first isoform of the CLRN1 protein includes a sequence that is at least 95% identical to SEQ ID NO: 3. In some embodiments of any of the compositions provided herein, the first isoform of the CLRN1 protein includes SEQ ID NO: 3. In some embodiments of any of the compositions provided herein, the first isoform of the CLRN1 protein consists of SEQ ID NO: 3. In some embodiments of any of the compositions provided herein, the first nucleic acid vector of the second nucleic acid vector further includes a sequence that encodes a second isoform of the CLRN1 protein. In some embodiments of any of the compositions provided herein, the second isoform of the CLRN1 protein includes a sequence that is at least 95% identical to SEQ ID NO: 5. In some embodiments of any of the compositions provided herein, the second isoform of the CLRN1 protein includes

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SEQ ID NO: 5. In some embodiments of any of the compositions provided herein, the second isoform of the CLRN1 protein consists of SEQ ID NO: 5.

In some embodiments of any of the compositions provided herein, the first coding sequence encodes an N-terminal portion of a second isoform of CLRN1 protein, and the second coding sequence encodes a C-terminal portion of the second isoform of CLRN1 protein. In some embodiments of any of the compositions provided herein, the second isoform of the CLRN1 protein includes a sequence that is at least 95% identical to SEQ ID NO: 5. In some embodiments of any of the compositions provided herein, the second isoform of the CLRN1 protein includes SEQ ID NO: 5. In some embodiments of any of the compositions provided herein, the second isoform of the CLRN1 protein consists of SEQ ID NO: 5. In some embodiments of any of the compositions provided herein, the first nucleic acid vector or the second nucleic acid vector further includes a sequence that encodes a first isoform of the CLRN1 protein. In some embodiments of any of the compositions provided herein, the first isoform of the CLRN1 protein includes a sequence that is at least 95% identical to SEQ ID NO: 3. In some embodiments of any of the compositions provided herein, the first isoform of the CLRN1 protein includes SEQ ID NO: 3. In some embodiments of any of the compositions provided herein, the first isoform of the CLRN1 protein consists of SEQ ID NO: 3.

In some embodiments of any of the compositions provided herein, one or both of the first nucleic acid vector and the second nucleic acid vector comprises a 5' untranslated region (UTR), a 3' UTR, or both. In some embodiments of any of the compositions provided herein, the 5' UTR includes at least 10 contiguous nucleotides from anywhere within SEQ ID NO: 12. In some embodiments of any of the compositions provided herein, the 5' UTR includes at least 20 contiguous nucleotides from anywhere within SEQ ID NO: 12. In some embodiments of any of the compositions provided herein, the 5' UTR includes at least 50 contiguous nucleotides from anywhere within SEQ ID NO: 12. In some embodiments of any of the compositions provided herein, the 5' UTR includes at least 80 contiguous nucleotides from anywhere within SEQ ID NO: 12. In some embodiments of any of the compositions provided herein, the 5' UTR includes a sequence that is at least 80% identical to SEQ ID NO: 12. In some embodiments of any of the compositions provided

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herein, the 3' UTR includes at least 10 contiguous nucleotides from anywhere within SEQ ID NO: 15. In some embodiments of any of the compositions provided herein, the 3' UTR includes at least 20 contiguous nucleotides from anywhere within SEQ ID NO: 15. In some embodiments of any of the compositions provided herein, the 3' UTR includes at least 50 contiguous nucleotides from anywhere within SEQ ID NO: 15. In some embodiments of any of the compositions provided herein, the 3' UTR includes at least 80 contiguous nucleotides from anywhere within SEQ ID NO: 15. In some embodiments of any of the compositions provided herein, the 3' UTR includes a sequence that is at least 80% identical to SEQ ID NO: 15.

In some embodiments of any of the compositions provided herein, each of the first nucleic acid vector and the second nucleic acid vector is a plasmid, a transposon, a cosmid, an artificial chromosome, or a viral vector. In some embodiments of any of the compositions provided herein, each of the first nucleic acid vector and the second nucleic acid vector is a human artificial chromosome (HAC), yeast artificial chromosome (YAC), bacterial artificial chromosome (BAC), or a P1-derived artificial chromosome (PAC). In some embodiments of any of the compositions provided herein, each of the first nucleic acid vector and the second nucleic acid vector is a viral vector selected from an adeno-associated virus (AAV) vector, an adenovirus vector, a lentivirus vector, or a retrovirus vector. In some embodiments of any of the compositions provided herein, each of the first nucleic acid vector and the second nucleic acid vector is an AAV vector. In some embodiments of any of the compositions provided herein, at least one of the coding sequences includes a nucleotide sequence spanning two consecutive exons of CLRN1 genomic DNA, and lacking an intronic sequence between the two consecutive exons.

Also provided herein are kits that include any of the compositions provided herein. Some embodiments of any of the kits provided herein further include a pre-loaded syringe including the composition.

Also provided herein are methods that include introducing into a cochlea of a mammal a therapeutically effective amount of any of the compositions provided herein. In some embodiments of any of the methods provided herein, the mammal is a human. In some embodiments of any of the methods provided herein, the mammal has been previously identified as having a defective CLRN1 gene.

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Also provided herein are methods of increasing expression of a full-length CLRN1 protein in a mammalian cell that include introducing any of the compositions provided herein into the mammalian cell. In some embodiments of any of the methods provided herein, the mammalian cell is a cochlear inner hair cell or a cochlear outer hair cell. In some embodiments of any of the methods provided herein, the mammalian cell is a retinal cell. In some embodiments of any of the methods provided herein, the mammalian cell is a human cell. In some embodiments of any of the methods provided herein, the mammalian cell has previously been determined to have a defective CLRN1 gene.

Also provided herein are methods of increasing expression of a full-length CLRN1 protein in an inner hair cell, an outer hair cell, or both, in a cochlea of a mammal, that include: introducing into the cochlea of the mammal a therapeutically effective amount of any of the compositions described herein. Also provided herein are methods of increasing expression of a full-length CLRN1 protein in an eye of a mammal that include: intraocularly administering to the eye of the mammal a therapeutically effective amount of any of the compositions provided herein. In some embodiments of any of the methods provided herein, the mammal has been previously identified as having a defective CLRN1 gene. In some embodiments of any of the methods provided herein, the mammal is a human.

Also provided herein are methods of treating hearing loss in a subject identified as having a defective CLRN1 gene that include: administering a therapeutically effective amount of any of the compositions provided herein into the cochlea of the subject. Also provided herein are methods of treating vision loss in a subject identified as having a defective CLRN1 gene that include: administering a therapeutically effective amount of any of the compositions provided herein into the eye of the subject. In some embodiments of any of the methods provided herein, the subject has Usher syndrome type III. In some embodiments of any of the methods provided herein, the subject is a human. Some embodiments of any of the methods provided herein further include, prior to the administering step, determining that the subject has a defective CLRN1 gene.

The term “a” and “an” refers to one or to more than one (i.e., at least one) of the grammatical object of the article. By way of example, “an element” encompasses one

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element and more than one element.

The term "mutation in a CLRN1 gene" refers to a modification in a wildtype CLRN1 gene that results in the production of a CLRN1 protein having one or more of: a deletion in one or more amino acids, one or more amino acid substitutions, and one or more amino acid insertions as compared to the wildtype CLRN1 protein, and/or results in a decrease in the expressed level of the encoded CLRN1 protein in a mammalian cell as compared to the expressed level of the encoded CLRN1 protein in a mammalian cell not having a mutation. In some embodiments, a mutation can result in the production of a CLRN1 protein having a deletion in one or more amino acids (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 16, 17, 18, 19, or 20 amino acids). In some embodiments, the mutation can result in a frameshift in the CLRN1 gene. The term "frameshift" is known in the art to encompass any mutation in a coding sequence that results in a shift in the reading frame of the coding sequence. In some embodiments, a frameshift can result in a nonfunctional protein. In some embodiments, a point mutation can be a nonsense mutation (i.e., result in a premature stop codon in an exon of the gene). A nonsense mutation can result in the production of a truncated protein (as compared to a corresponding wildtype protein) that may or may not be functional. In some embodiments, the mutation can result in the loss (or a decrease in the level) of expression of CLRN1 mRNA or CLRN1 protein or both the mRNA and protein. In some embodiments, the mutation can result in the production of an altered CLRN1 protein having a loss or decrease in one or more biological activities (functions) as compared to a wildtype CLRN1 protein.

In some embodiments, the mutation is an insertion of one or more nucleotides into a CLRN1 gene. In some embodiments, the mutation is in a regulatory sequence of the CLRN1 gene, i.e., a portion of the gene that is not coding sequence. In some embodiments, a mutation in a regulatory sequence may be in a promoter or enhancer region and prevent or reduce the proper transcription of the CLRN1 gene. The term "conservative mutation" refers to a mutation that does not change the amino acid encoded at the site of the mutation (due to codon degeneracy).

Modifications can be introduced into a nucleotide sequence by standard techniques known in the art, such as site-directed mutagenesis and PCR-mediated

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mutagenesis. Conservative amino acid substitutions are ones in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (e.g., lysine, arginine, and histidine), acidic side chains (e.g., aspartic acid and glutamic acid), uncharged polar side chains (e.g., glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine, and tryptophan), nonpolar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, and methionine), beta-branched side chains (e.g., threonine, valine, and isoleucine), and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, and histidine).

Unless otherwise specified, a "nucleotide sequence encoding an amino acid sequence" includes all nucleotide sequences that are degenerate versions of each other and thus encode the same amino acid sequence.

The term "endogenous" refers to any material originating from within an organism, cell, or tissue.

The term "exogenous" refers to any material introduced from or originating from outside an organism, cell, or tissue that is not produced or does not originate from the same organism, cell, or tissue in which it is being introduced.

The term "isolated" means altered or removed from the natural state. For example, a nucleic acid or a peptide naturally present in a living animal is not "isolated," but the same nucleic acid or peptide partially or completely separated from the coexisting materials of its natural state is "isolated." An isolated nucleic acid or protein can exist in substantially purified form, or can exist in a non-native environment such as, for example, a host cell.

The term "transfected," "transformed," or "transduced" refers to a process by which exogenous nucleic acid is transferred or introduced into a cell. A "transfected," "transformed," or "transduced" mammalian cell is one that has been transfected, transformed or transduced with exogenous nucleic acid.

The term "expression" refers to the transcription and/or translation of a particular nucleotide sequence encoding a protein.

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The term "transient expression" refers to the expression of a non-integrated coding sequence for a short period of time (e.g., hours or days). The coding sequence that is transiently expressed in a cell (e.g., a mammalian cell) is lost upon multiple rounds of cell division.

The term "subject" is intended to include any mammal. In some embodiments, the subject is a rodent (e.g., a rat or mouse), a rabbit, a sheep, a goat, a pig, a dog, a cat, a non-human primate, or a human. In some embodiments, the subject has or is at risk of hearing loss and/or vision loss. In some embodiments, the subject has been previously identified as having a mutation in a CLRN1 gene. In some embodiments, the subject has been identified as having a mutation in a CLRN1 gene and has been diagnosed with hearing loss and/or vision loss. In some embodiments, the subject has been identified as having hearing loss and/or vision loss.

A treatment is "therapeutically effective" when it results in a reduction in one or more of the number, severity, and frequency of one or more symptoms of a disease state (e.g., hearing loss or vision loss) in a subject (e.g., a human). In some embodiments, a therapeutically effective amount of a composition can result in an increase in the expression level of an active CLRN1 protein (e.g., a wildtype, full-length CLRN1 protein or a variant of a CLRN1 protein that has the desired activity) (e.g., as compared to the expression level prior to treatment with the composition). In some embodiments, a therapeutically effective amount of a composition can result in an increase in the expression level of an active CLRN1 protein (e.g., a wildtype, full-length CLRN1 protein or an active variant) in a target cell (e.g., a cochlear inner hair cell). In some embodiments, a therapeutically effective amount of a composition can result in an increase in the expression level of an active CLRN1 protein (e.g., a wildtype, full-length CLRN1 protein or active variant), and/or an increase in one or more activities of a CLRN1 protein in a target cell (e.g., as compared to a reference level, such as the level(s) in a subject prior to treatment, the level(s) in a subject having a mutation in a CLRN1 gene, or the level(s) in a subject or a population of subjects having hearing loss and/or vision loss).

The term "nucleic acid" or "polynucleotide" refers to deoxyribonucleic acid (DNA) or ribonucleic acid (RNA), or a combination thereof, in either single- or double-

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stranded form. Unless specifically limited, the term encompasses nucleic acids containing known analogues of natural nucleotides that have similar binding properties as the reference nucleotides. Unless otherwise indicated, a particular nucleic acid sequence also implicitly encompasses complementary sequences as well as the sequence explicitly indicated. In some embodiments of any of the nucleic acids described herein, the nucleic acid is DNA. In some embodiments of any of the nucleic acids described herein, the nucleic acid is RNA.

The term "active CLRN1 protein" means a protein encoded by DNA that, if substituted for both wildtype alleles encoding full-length CLRN1 protein in auditory hair cells, or ocular cells, of what is otherwise a wildtype mammal, and if expressed in the auditory hair cells, or ocular cells, of that mammal, results in that mammal's having a level of hearing, or vision, approximating the normal level of hearing, or vision, of a similar mammal that is entirely wildtype. Non-limiting examples of active CLRN1 proteins are full-length CLRN1 proteins (e.g., any of the full-length CLRN1 proteins described herein).

For example, an active CLRN1 protein can include a sequence of a wildtype, full-length CLRN1 protein (e.g., a wildtype, human, full-length CLRN1 protein) including 1 amino acid substitution to about 100 amino acid substitutions, 1 amino acid substitution to about 95 amino acid substitutions, 1 amino acid substitution to about 90 amino acid substitutions, 1 amino acid substitution to about 85 amino acid substitutions, 1 amino acid substitution to about 80 amino acid substitutions, 1 amino acid substitution to about 75 amino acid substitutions, 1 amino acid substitution to about 70 amino acid substitutions, 1 amino acid substitution to about 65 amino acid substitutions, 1 amino acid substitution to about 60 amino acid substitutions, 1 amino acid substitution to about 55 amino acid substitutions, 1 amino acid substitution to about 50 amino acid substitutions, 1 amino acid substitution to about 45 amino acid substitutions, 1 amino acid substitution to about 40 amino acid substitutions, 1 amino acid substitution to about 35 amino acid substitutions, 1 amino acid substitution to about 30 amino acid substitutions, 1 amino acid substitution to about 25 amino acid substitutions, 1 amino acid substitution to about 20 amino acid substitutions, 1 amino acid substitution to about 15 amino acid substitutions, 1 amino acid substitution to about 10 amino acid

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substitutions, 1 amino acid substitution to about 9 amino acid substitutions, 1 amino acid substitution to about 8 amino acid substitutions, 1 amino acid substitution to about 7 amino acid substitutions, 1 amino acid substitution to about 6 amino acid substitutions, 1 amino acid substitution to about 5 amino acid substitutions, 1 amino acid substitution to about 4 amino acid substitutions, 1 amino acid substitution to about 3 amino acid substitutions, about 2 amino acid substitutions to about 100 amino acid substitutions, about 2 amino acid substitutions to about 95 amino acid substitutions, about 2 amino acid substitutions to about 90 amino acid substitutions, about 2 amino acid substitutions to about 85 amino acid substitutions, about 2 amino acid substitutions to about 80 amino acid substitutions, about 2 amino acid substitutions to about 75 amino acid substitutions, about 2 amino acid substitutions to about 70 amino acid substitutions, about 2 amino acid substitutions to about 65 amino acid substitutions, about 2 amino acid substitutions to about 60 amino acid substitutions, about 2 amino acid substitutions to about 55 amino acid substitutions, about 2 amino acid substitutions to about 50 amino acid substitutions, about 2 amino acid substitutions to about 45 amino acid substitutions, about 2 amino acid substitutions to about 40 amino acid substitutions, about 2 amino acid substitutions to about 35 amino acid substitutions, about 2 amino acid substitutions to about 30 amino acid substitutions, about 2 amino acid substitutions to about 25 amino acid substitutions, about 2 amino acid substitutions to about 20 amino acid substitutions, about 2 amino acid substitutions to about 15 amino acid substitutions, about 2 amino acid substitutions to about 10 amino acid substitutions, about 2 amino acid substitutions to about 9 amino acid substitutions, about 2 amino acid substitutions to about 8 amino acid substitutions, about 2 amino acid substitutions to about 7 amino acid substitutions, about 2 amino acid substitutions to about 6 amino acid substitutions, about 2 amino acid substitutions to about 5 amino acid substitutions, about 2 amino acid substitutions to about 4 amino acid substitutions, about 3 amino acid substitutions to about 100 amino acid substitutions, about 3 amino acid substitutions to about 95 amino acid substitutions, about 3 amino acid substitutions to about 90 amino acid substitutions, about 3 amino acid substitutions to about 85 amino acid substitutions, about 3 amino acid substitutions to about 80 amino acid substitutions, about 3 amino acid substitutions to about 75 amino acid substitutions, about 3 amino acid substitutions to about 70 amino acid substitutions, about 3 amino acid

substitutions to about 65 amino acid substitutions, about 3 amino acid substitutions to about 60 amino acid substitutions, about 3 amino acid substitutions to about 55 amino acid substitutions, about 3 amino acid substitutions to about 50 amino acid substitutions, about 3 amino acid substitutions to about 45 amino acid substitutions, about 3 amino acid substitutions to about 40 amino acid substitutions, about 3 amino acid substitutions to about 35 amino acid substitutions, about 3 amino acid substitutions to about 30 amino acid substitutions, about 3 amino acid substitutions to about 25 amino acid substitutions, about 3 amino acid substitutions to about 20 amino acid substitutions, about 3 amino acid substitutions to about 15 amino acid substitutions, about 3 amino acid substitutions to about 10 amino acid substitutions, about 3 amino acid substitutions to about 9 amino acid substitutions, about 3 amino acid substitutions to about 8 amino acid substitutions, about 3 amino acid substitutions to about 7 amino acid substitutions, about 3 amino acid substitutions to about 6 amino acid substitutions, about 3 amino acid substitutions to about 5 amino acid substitutions, about 4 amino acid substitutions to about 100 amino acid substitutions, about 4 amino acid substitutions to about 95 amino acid substitutions, about 4 amino acid substitutions to about 90 amino acid substitutions, about 4 amino acid substitutions to about 85 amino acid substitutions, about 4 amino acid substitutions to about 80 amino acid substitutions, about 4 amino acid substitutions to about 75 amino acid substitutions, about 4 amino acid substitutions to about 70 amino acid substitutions, about 4 amino acid substitutions to about 65 amino acid substitutions, about 4 amino acid substitutions to about 60 amino acid substitutions, about 4 amino acid substitutions to about 55 amino acid substitutions, about 4 amino acid substitutions to about 50 amino acid substitutions, about 4 amino acid substitutions to about 45 amino acid substitutions, about 4 amino acid substitutions to about 40 amino acid substitutions, about 4 amino acid substitutions to about 35 amino acid substitutions, about 4 amino acid substitutions to about 30 amino acid substitutions, about 4 amino acid substitutions to about 25 amino acid substitutions, about 4 amino acid substitutions to about 20 amino acid substitutions, about 4 amino acid substitutions to about 15 amino acid substitutions, about 4 amino acid substitutions to about 10 amino acid substitutions, about 4 amino acid substitutions to about 9 amino acid substitutions, about 4 amino acid substitutions to about 8 amino acid substitutions, about 4 amino acid substitutions to about 7 amino acid substitutions, about

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4 amino acid substitutions to about 6 amino acid substitutions, about 5 amino acid substitutions to about 100 amino acid substitutions, about 5 amino acid substitutions to about 95 amino acid substitutions, about 5 amino acid substitutions to about 90 amino acid substitutions, about 5 amino acid substitutions to about 85 amino acid substitutions, about 5 amino acid substitutions to about 80 amino acid substitutions, about 5 amino acid substitutions to about 75 amino acid substitutions, about 5 amino acid substitutions to about 70 amino acid substitutions, about 5 amino acid substitutions to about 65 amino acid substitutions, about 5 amino acid substitutions to about 60 amino acid substitutions, about 5 amino acid substitutions to about 55 amino acid substitutions, about 5 amino acid substitutions to about 50 amino acid substitutions, about 5 amino acid substitutions to about 45 amino acid substitutions, about 5 amino acid substitutions to about 40 amino acid substitutions, about 5 amino acid substitutions to about 35 amino acid substitutions, about 5 amino acid substitutions to about 30 amino acid substitutions, about 5 amino acid substitutions to about 25 amino acid substitutions, about 5 amino acid substitutions to about 20 amino acid substitutions, about 5 amino acid substitutions to about 15 amino acid substitutions, about 5 amino acid substitutions to about 10 amino acid substitutions, about 5 amino acid substitutions to about 9 amino acid substitutions, about 5 amino acid substitutions to about 8 amino acid substitutions, about 5 amino acid substitutions to about 7 amino acid substitutions, about 6 amino acid substitutions to about 100 amino acid substitutions, about 6 amino acid substitutions to about 95 amino acid substitutions, about 6 amino acid substitutions to about 90 amino acid substitutions, about 6 amino acid substitutions to about 85 amino acid substitutions, about 6 amino acid substitutions to about 80 amino acid substitutions, about 6 amino acid substitutions to about 75 amino acid substitutions, about 6 amino acid substitutions to about 70 amino acid substitutions, about 6 amino acid substitutions to about 65 amino acid substitutions, about 6 amino acid substitutions to about 60 amino acid substitutions, about 6 amino acid substitutions to about 55 amino acid substitutions, about 6 amino acid substitutions to about 50 amino acid substitutions, about 6 amino acid substitutions to about 45 amino acid substitutions, about 6 amino acid substitutions to about 40 amino acid substitutions, about 6 amino acid substitutions to about 35 amino acid substitutions, about 6 amino acid substitutions to about 30 amino acid substitutions, about 6 amino acid substitutions to about 25 amino

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acid substitutions, about 6 amino acid substitutions to about 20 amino acid substitutions, about 6 amino acid substitutions to about 15 amino acid substitutions, about 6 amino acid substitutions to about 10 amino acid substitutions, about 6 amino acid substitutions to about 9 amino acid substitutions, about 6 amino acid substitutions to about 8 amino acid substitutions, about 7 amino acid substitutions to about 100 amino acid substitutions, about 7 amino acid substitutions to about 95 amino acid substitutions, about 7 amino acid substitutions to about 90 amino acid substitutions, about 7 amino acid substitutions to about 85 amino acid substitutions, about 7 amino acid substitutions to about 80 amino acid substitutions, about 7 amino acid substitutions to about 75 amino acid substitutions, about 7 amino acid substitutions to about 70 amino acid substitutions, about 7 amino acid substitutions to about 65 amino acid substitutions, about 7 amino acid substitutions to about 60 amino acid substitutions, about 7 amino acid substitutions to about 55 amino acid substitutions, about 7 amino acid substitutions to about 50 amino acid substitutions, about 7 amino acid substitutions to about 45 amino acid substitutions, about 7 amino acid substitutions to about 40 amino acid substitutions, about 7 amino acid substitutions to about 35 amino acid substitutions, about 7 amino acid substitutions to about 30 amino acid substitutions, about 7 amino acid substitutions to about 25 amino acid substitutions, about 7 amino acid substitutions to about 20 amino acid substitutions, about 7 amino acid substitutions to about 15 amino acid substitutions, about 7 amino acid substitutions to about 10 amino acid substitutions, about 7 amino acid substitutions to about 9 amino acid substitutions, about 8 amino acid substitutions to about 100 amino acid substitutions, about 8 amino acid substitutions to about 95 amino acid substitutions, about 8 amino acid substitutions to about 90 amino acid substitutions, about 8 amino acid substitutions to about 85 amino acid substitutions, about 8 amino acid substitutions to about 80 amino acid substitutions, about 8 amino acid substitutions to about 75 amino acid substitutions, about 8 amino acid substitutions to about 70 amino acid substitutions, about 8 amino acid substitutions to about 65 amino acid substitutions, about 8 amino acid substitutions to about 60 amino acid substitutions, about 8 amino acid substitutions to about 55 amino acid substitutions, about 8 amino acid substitutions to about 50 amino acid substitutions, about 8 amino acid substitutions to about 45 amino acid substitutions, about 8 amino acid substitutions to about 40 amino acid substitutions, about 8 amino acid substitutions to

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about 35 amino acid substitutions, about 8 amino acid substitutions to about 30 amino acid substitutions, about 8 amino acid substitutions to about 25 amino acid substitutions, about 8 amino acid substitutions to about 20 amino acid substitutions, about 8 amino acid substitutions to about 15 amino acid substitutions, about 8 amino acid substitutions to about 10 amino acid substitutions, about 10 amino acid substitutions to about 100 amino acid substitutions, about 10 amino acid substitutions to about 95 amino acid substitutions, about 10 amino acid substitutions to about 90 amino acid substitutions, about 10 amino acid substitutions to about 85 amino acid substitutions, about 10 amino acid substitutions to about 80 amino acid substitutions, about 10 amino acid substitutions to about 75 amino acid substitutions, about 10 amino acid substitutions to about 70 amino acid substitutions, about 10 amino acid substitutions to about 65 amino acid substitutions, about 10 amino acid substitutions to about 60 amino acid substitutions, about 10 amino acid substitutions to about 55 amino acid substitutions, about 10 amino acid substitutions to about 50 amino acid substitutions, about 10 amino acid substitutions to about 45 amino acid substitutions, about 10 amino acid substitutions to about 40 amino acid substitutions, about 10 amino acid substitutions to about 35 amino acid substitutions, about 10 amino acid substitutions to about 30 amino acid substitutions, about 10 amino acid substitutions to about 25 amino acid substitutions, about 10 amino acid substitutions to about 20 amino acid substitutions, about 10 amino acid substitutions to about 15 amino acid substitutions, about 15 amino acid substitutions to about 100 amino acid substitutions, about 15 amino acid substitutions to about 95 amino acid substitutions, about 15 amino acid substitutions to about 90 amino acid substitutions, about 15 amino acid substitutions to about 85 amino acid substitutions, about 15 amino acid substitutions to about 80 amino acid substitutions, about 15 amino acid substitutions to about 75 amino acid substitutions, about 15 amino acid substitutions to about 70 amino acid substitutions, about 15 amino acid substitutions to about 65 amino acid substitutions, about 15 amino acid substitutions to about 60 amino acid substitutions, about 15 amino acid substitutions to about 55 amino acid substitutions, about 15 amino acid substitutions to about 50 amino acid substitutions, about 15 amino acid substitutions to about 45 amino acid substitutions, about 15 amino acid substitutions to about 40 amino acid substitutions, about 15 amino acid substitutions to about 35 amino acid substitutions, about 15 amino acid substitutions to about 30 amino acid substitutions,

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about 15 amino acid substitutions to about 25 amino acid substitutions, about 15 amino acid substitutions to about 20 amino acid substitutions, about 20 amino acid substitutions to about 100 amino acid substitutions, about 20 amino acid substitutions to about 95 amino acid substitutions, about 20 amino acid substitutions to about 90 amino acid substitutions, about 20 amino acid substitutions to about 85 amino acid substitutions, about 20 amino acid substitutions to about 80 amino acid substitutions, about 20 amino acid substitutions to about 75 amino acid substitutions, about 20 amino acid substitutions to about 70 amino acid substitutions, about 20 amino acid substitutions to about 65 amino acid substitutions, about 20 amino acid substitutions to about 60 amino acid substitutions, about 20 amino acid substitutions to about 55 amino acid substitutions, about 20 amino acid substitutions to about 50 amino acid substitutions, about 20 amino acid substitutions to about 45 amino acid substitutions, about 20 amino acid substitutions to about 40 amino acid substitutions, about 20 amino acid substitutions to about 35 amino acid substitutions, about 20 amino acid substitutions to about 30 amino acid substitutions, about 20 amino acid substitutions to about 25 amino acid substitutions, about 25 amino acid substitutions to about 100 amino acid substitutions, about 25 amino acid substitutions to about 95 amino acid substitutions, about 25 amino acid substitutions to about 90 amino acid substitutions, about 25 amino acid substitutions to about 85 amino acid substitutions, about 25 amino acid substitutions to about 80 amino acid substitutions, about 25 amino acid substitutions to about 75 amino acid substitutions, about 25 amino acid substitutions to about 70 amino acid substitutions, about 25 amino acid substitutions to about 65 amino acid substitutions, about 25 amino acid substitutions to about 60 amino acid substitutions, about 25 amino acid substitutions to about 55 amino acid substitutions, about 25 amino acid substitutions to about 50 amino acid substitutions, about 25 amino acid substitutions to about 45 amino acid substitutions, about 25 amino acid substitutions to about 40 amino acid substitutions, about 25 amino acid substitutions to about 35 amino acid substitutions, about 25 amino acid substitutions to about 30 amino acid substitutions, about 30 amino acid substitutions to about 100 amino acid substitutions, about 30 amino acid substitutions to about 95 amino acid substitutions, about 30 amino acid substitutions to about 90 amino acid substitutions, about 30 amino acid substitutions to about 85 amino acid substitutions, about 30 amino acid substitutions to about 80 amino acid substitutions,

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about 30 amino acid substitutions to about 75 amino acid substitutions, about 30 amino acid substitutions to about 70 amino acid substitutions, about 30 amino acid substitutions to about 65 amino acid substitutions, about 30 amino acid substitutions to about 60 amino acid substitutions, about 30 amino acid substitutions to about 55 amino acid substitutions, about 30 amino acid substitutions to about 50 amino acid substitutions, about 30 amino acid substitutions to about 45 amino acid substitutions, about 30 amino acid substitutions to about 40 amino acid substitutions, about 30 amino acid substitutions to about 35 amino acid substitutions, about 35 amino acid substitutions to about 100 amino acid substitutions, about 35 amino acid substitutions to about 95 amino acid substitutions, about 35 amino acid substitutions to about 90 amino acid substitutions, about 35 amino acid substitutions to about 85 amino acid substitutions, about 35 amino acid substitutions to about 80 amino acid substitutions, about 35 amino acid substitutions to about 75 amino acid substitutions, about 35 amino acid substitutions to about 70 amino acid substitutions, about 35 amino acid substitutions to about 65 amino acid substitutions, about 35 amino acid substitutions to about 60 amino acid substitutions, about 35 amino acid substitutions to about 55 amino acid substitutions, about 35 amino acid substitutions to about 50 amino acid substitutions, about 35 amino acid substitutions to about 45 amino acid substitutions, about 35 amino acid substitutions to about 40 amino acid substitutions, about 40 amino acid substitutions to about 100 amino acid substitutions, about 40 amino acid substitutions to about 95 amino acid substitutions, about 40 amino acid substitutions to about 90 amino acid substitutions, about 40 amino acid substitutions to about 85 amino acid substitutions, about 40 amino acid substitutions to about 80 amino acid substitutions, about 40 amino acid substitutions to about 75 amino acid substitutions, about 40 amino acid substitutions to about 70 amino acid substitutions, about 40 amino acid substitutions to about 65 amino acid substitutions, about 40 amino acid substitutions to about 60 amino acid substitutions, about 40 amino acid substitutions to about 55 amino acid substitutions, about 40 amino acid substitutions to about 50 amino acid substitutions, about 40 amino acid substitutions to about 45 amino acid substitutions, about 45 amino acid substitutions to about 100 amino acid substitutions, about 45 amino acid substitutions to about 95 amino acid substitutions, about 45 amino acid substitutions to about 90 amino acid substitutions, about 45 amino acid substitutions to about 85 amino acid substitutions,

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amino acids.

One skilled in the art would appreciate that amino acids that are not conserved between wildtype CLRN1 proteins from different species can be mutated without losing activity, while those amino acids that are conserved between wildtype CLRN1 proteins from different species should not be mutated as they are more likely (than amino acids that are not conserved between different species) to be involved in activity.

An active CLRN1 protein can include, e.g., a sequence of a wildtype, full-length CLRN1 protein (e.g., a wildtype, human, full-length CLRN1 protein) that has about 1 amino acid to about 100 amino acids, about 1 amino acid to about 95 amino acids, about 1 amino acid to about 90 amino acids, about 1 amino acid to about 85 amino acids, about 1 amino acid to about 80 amino acids, about 1 amino acid to about 75 amino acids, about 1 amino acid to about 70 amino acids, about 1 amino acid to about 65 amino acids, about 1 amino acid to about 60 amino acids, about 1 amino acid to about 55 amino acids, about 1 amino acid to about 50 amino acids, about 1 amino acid to about 45 amino acids, about 1 amino acid to about 40 amino acids, about 1 amino acid to about 35 amino acids, about 1 amino acid to about 30 amino acids, about 1 amino acid to about 25 amino acids, about 1 amino acid to about 20 amino acids, about 1 amino acid to about 15 amino acids, about 1 amino acid to about 10 amino acids, about 1 amino acid to about 9 amino acids, about 1 amino acid to about 8 amino acids, about 1 amino acid to about 7 amino acids, about 1 amino acid to about 6 amino acids, about 1 amino acid to about 5 amino acids, about 1 amino acid to about 4 amino acids, about 1 amino acid to about 3 amino acids, about 2 amino acids to about 100 amino acids, about 2 amino acids to about 95 amino acids, about 2 amino acids to about 90 amino acids, about 2 amino acids to about 85 amino acids, about 2 amino acids to about 80 amino acids, about 2 amino acids to about 75 amino acids, about 2 amino acids to about 70 amino acids, about 2 amino acids to about 65 amino acids, about 2 amino acids to about 60 amino acids, about 2 amino acids to about 55 amino acids, about 2 amino acids to about 50 amino acids, about 2 amino acids to about 45 amino acids, about 2 amino acids to about 40 amino acids, about 2 amino acids to about 35 amino acids, about 2 amino acids to about 30 amino acids, about 2 amino acids to about 25 amino acids, about 2 amino acids to about 20 amino acids, about 2 amino acids to about 15 amino acids, about 2 amino acids to about 10 amino acids,

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acids to about 95 amino acids, about 50 amino acids to about 90 amino acids, about 50 amino acids to about 85 amino acids, about 50 amino acids to about 80 amino acids, about 50 amino acids to about 75 amino acids, about 50 amino acids to about 70 amino acids, about 50 amino acids to about 65 amino acids, about 50 amino acids to about 60 amino acids, or about 50 amino acids to about 55 amino acids, deleted. In some embodiments where two or more amino acids are deleted from the sequence of a wildtype, full-length CLRN1 protein, at least two of the two or more deleted amino acids can be contiguous in the sequence of the wildtype, full-length protein. In other examples where two or more amino acids are deleted from the sequence of a wildtype, full-length CLRN1 protein, some or all of the two or more deleted amino acids are not contiguous in the sequence of the wildtype, full-length protein. One skilled in the art would appreciate that amino acids that are not conserved between wildtype, full-length CLRN1 proteins from different species can be deleted without losing activity, while those amino acids that are conserved between wildtype, full-length CLNRN1 proteins from different species should not be deleted as they are more likely (than amino acids that are not conserved between different species) to be involved in activity.

In some examples, an active CLRN1 protein can, e.g., include a sequence of a wildtype, full-length CLRN1 protein that has between 1 amino acid to about 100 amino acids, 1 amino acid to about 95 amino acids, 1 amino acid to about 90 amino acids, 1 amino acid to about 85 amino acids, 1 amino acid to about 80 amino acids, 1 amino acid to about 75 amino acids, 1 amino acid to about 70 amino acids, 1 amino acid to about 65 amino acids, 1 amino acid to about 60 amino acids, 1 amino acid to about 55 amino acids, 1 amino acid to about 50 amino acids, 1 amino acid to about 45 amino acids, 1 amino acid to about 40 amino acids, 1 amino acid to about 35 amino acids, 1 amino acid to about 30 amino acids, 1 amino acid to about 25 amino acids, 1 amino acid to about 20 amino acids, 1 amino acid to about 15 amino acids, 1 amino acid to about 10 amino acids, 1 amino acid to about 9 amino acids, 1 amino acid to about 8 amino acids, 1 amino acid to about 7 amino acids, 1 amino acid to about 6 amino acids, 1 amino acid to about 5 amino acids, 1 amino acid to about 4 amino acids, 1 amino acid to about 3 amino acids, about 2 amino acids to about 100 amino acids, about 2 amino acid to about 95 amino acids, about 2 amino acids to about 90 amino acids, about 2 amino acids to about 85

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amino acids, about 2 amino acids to about 80 amino acids, about 2 amino acids to about 75 amino acids, about 2 amino acids to about 70 amino acids, about 2 amino acids to about 65 amino acids, about 2 amino acids to about 60 amino acids, about 2 amino acids to about 55 amino acids, about 2 amino acids to about 50 amino acids, about 2 amino acids to about 45 amino acids, about 2 amino acids to about 40 amino acids, about 2 amino acids to about 35 amino acids, about 2 amino acids to about 30 amino acids, about 2 amino acids to about 25 amino acids, about 2 amino acids to about 20 amino acids, about 2 amino acids to about 15 amino acids, about 2 amino acids to about 10 amino acids, about 2 amino acids to about 9 amino acids, about 2 amino acids to about 8 amino acids, about 2 amino acids to about 7 amino acids, about 2 amino acids to about 6 amino acids, about 2 amino acids to about 5 amino acids, about 2 amino acids to about 4 amino acids, about 3 amino acids to about 100 amino acids, about 3 amino acid to about 95 amino acids, about 3 amino acids to about 90 amino acids, about 3 amino acids to about 85 amino acids, about 3 amino acids to about 80 amino acids, about 3 amino acids to about 75 amino acids, about 3 amino acids to about 70 amino acids, about 3 amino acids to about 65 amino acids, about 3 amino acids to about 60 amino acids, about 3 amino acids to about 55 amino acids, about 3 amino acids to about 50 amino acids, about 3 amino acids to about 45 amino acids, about 3 amino acids to about 40 amino acids, about 3 amino acids to about 35 amino acids, about 3 amino acids to about 30 amino acids, about 3 amino acids to about 25 amino acids, about 3 amino acids to about 20 amino acids, about 3 amino acids to about 15 amino acids, about 3 amino acids to about 10 amino acids, about 3 amino acids to about 9 amino acids, about 3 amino acids to about 8 amino acids, about 3 amino acids to about 7 amino acids, about 3 amino acids to about 6 amino acids, about 3 amino acids to about 5 amino acids, about 4 amino acids to about 100 amino acids, about 4 amino acid to about 95 amino acids, about 4 amino acids to about 90 amino acids, about 4 amino acids to about 85 amino acids, about 4 amino acids to about 80 amino acids, about 4 amino acids to about 75 amino acids, about 4 amino acids to about 70 amino acids, about 4 amino acids to about 65 amino acids, about 4 amino acids to about 60 amino acids, about 4 amino acids to about 55 amino acids, about 4 amino acids to about 50 amino acids, about 4 amino acids to about 45 amino acids, about 4 amino acids to about 40 amino acids, about 4 amino acids to about 35 amino

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about 70 amino acids to about 75 amino acids, about 80 amino acids to about 100 amino acids, about 80 amino acid to about 95 amino acids, about 80 amino acids to about 90 amino acids, about 80 amino acids to about 85 amino acids, about 90 amino acids to about 100 amino acids, about 90 amino acids to about 95 amino acids, or about 95 amino acids to about 100 amino acids, removed from its N-terminus and/or 1 amino acid to 100 amino acids (or any of the subranges of this range described herein) removed from its C-terminus.

In some embodiments, an active CLRN1 protein can, e.g., include the sequence of a wildtype, full-length CLRN1 protein where 1 amino acid to 50 amino acids, 1 amino acid to 45 amino acids, 1 amino acid to 40 amino acids, 1 amino acid to 35 amino acids, 1 amino acid to 30 amino acids, 1 amino acid to 25 amino acids, 1 amino acid to 20 amino acids, 1 amino acid to 15 amino acids, 1 amino acid to 10 amino acids, 1 amino acid to 9 amino acids, 1 amino acid to 8 amino acids, 1 amino acid to 7 amino acids, 1 amino acid to 6 amino acids, 1 amino acid to 5 amino acids, 1 amino acid to 4 amino acids, 1 amino acid to 3 amino acids, about 2 amino acids to 50 amino acids, about 2 amino acids to 45 amino acids, about 2 amino acids to 40 amino acids, about 2 amino acids to 35 amino acids, about 2 amino acids to 30 amino acids, about 2 amino acids to 25 amino acids, about 2 amino acids to 20 amino acids, about 2 amino acids to 15 amino acids, about 2 amino acids to 10 amino acids, about 2 amino acids to 9 amino acids, about 2 amino acids to 8 amino acids, about 2 amino acids to 7 amino acids, about 2 amino acids to 6 amino acids, about 2 amino acids to 5 amino acids, about 2 amino acids to 4 amino acids, about 3 amino acids to 50 amino acids, about 3 amino acids to 45 amino acids, about 3 amino acids to 40 amino acids, about 3 amino acids to 35 amino acids, about 3 amino acids to 30 amino acids, about 3 amino acids to 25 amino acids, about 3 amino acids to 20 amino acids, about 3 amino acids to 15 amino acids, about 3 amino acids to 10 amino acids, about 3 amino acids to 9 amino acids, about 3 amino acids to 8 amino acids, about 3 amino acids to 7 amino acids, about 3 amino acids to 6 amino acids, about 3 amino acids to 5 amino acids, about 4 amino acids to 50 amino acids, about 4 amino acids to 45 amino acids, about 4 amino acids to 40 amino acids, about 4 amino acids to 35 amino acids, about 4 amino acids to 30 amino acids, about 4 amino acids to 25 amino acids, about 4 amino acids to 20 amino acids, about 4 amino acids to 15 amino acids, about 4

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amino acids to 10 amino acids, about 4 amino acids to 9 amino acids, about 4 amino acids to 8 amino acids, about 4 amino acids to 7 amino acids, about 4 amino acids to 6 amino acids, about 5 amino acids to 50 amino acids, about 5 amino acids to 45 amino acids, about 5 amino acids to 40 amino acids, about 5 amino acids to 35 amino acids, about 5 amino acids to 30 amino acids, about 5 amino acids to 25 amino acids, about 5 amino acids to 20 amino acids, about 5 amino acids to 15 amino acids, about 5 amino acids to 10 amino acids, about 5 amino acids to 9 amino acids, about 5 amino acids to 8 amino acids, about 5 amino acids to 7 amino acids, about 6 amino acids to 50 amino acids, about 6 amino acids to 45 amino acids, about 6 amino acids to 40 amino acids, about 6 amino acids to 35 amino acids, about 6 amino acids to 30 amino acids, about 6 amino acids to 25 amino acids, about 6 amino acids to 20 amino acids, about 6 amino acids to 15 amino acids, about 6 amino acids to 10 amino acids, about 6 amino acids to 9 amino acids, about 6 amino acids to 8 amino acids, about 7 amino acids to 50 amino acids, about 7 amino acids to 45 amino acids, about 7 amino acids to 40 amino acids, about 7 amino acids to 35 amino acids, about 7 amino acids to 30 amino acids, about 7 amino acids to 25 amino acids, about 7 amino acids to 20 amino acids, about 7 amino acids to 15 amino acids, about 7 amino acids to 10 amino acids, about 7 amino acids to 9 amino acids, about 8 amino acids to 50 amino acids, about 8 amino acids to 45 amino acids, about 8 amino acids to 40 amino acids, about 8 amino acids to 35 amino acids, about 8 amino acids to 30 amino acids, about 8 amino acids to 25 amino acids, about 8 amino acids to 20 amino acids, about 8 amino acids to 15 amino acids, about 8 amino acids to 10 amino acids, about 10 amino acids to 50 amino acids, about 10 amino acids to 45 amino acids, about 10 amino acids to 40 amino acids, about 10 amino acids to 35 amino acids, about 10 amino acids to 30 amino acids, about 10 amino acids to 25 amino acids, about 10 amino acids to 20 amino acids, about 10 amino acids to 15 amino acids, about 15 amino acids to 50 amino acids, about 15 amino acids to 45 amino acids, about 15 amino acids to 40 amino acids, about 15 amino acids to 35 amino acids, about 15 amino acids to 30 amino acids, about 15 amino acids to 25 amino acids, about 15 amino acids to 20 amino acids, about 20 amino acids to 50 amino acids, about 20 amino acids to 45 amino acids, about 20 amino acids to 40 amino acids, about 20 amino acids to 35 amino acids, about 20 amino acids to 30 amino acids, about 20 amino acids to 25 amino

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acids, about 25 amino acids to 50 amino acids, about 25 amino acids to 45 amino acids, about 25 amino acids to 40 amino acids, about 25 amino acids to 35 amino acids, about 25 amino acids to 30 amino acids, about 30 amino acids to 50 amino acids, about 30 amino acids to 45 amino acids, about 30 amino acids to 40 amino acids, about 30 amino acids to 35 amino acids, about 35 amino acids to 50 amino acids, about 35 amino acids to 45 amino acids, about 35 amino acids to 40 amino acids, about 40 amino acids to 50 amino acids, about 40 amino acids to 45 amino acids, or about 45 amino acids to about 50 amino acids, are inserted. In some examples, the 1 amino acid to 50 amino acids (or any subrange thereof) can be inserted as a contiguous sequence into the sequence of a wildtype, full-length protein. In some examples, the 1 amino acid to 50 amino acids (or any subrange thereof) are inserted in multiple, non-contiguous places in the sequence of a wildtype, full-length protein. As can be appreciated in the art, the 1 amino acid to 50 amino acids can be inserted into a portion of the sequence of a wildtype, full-length protein that is not well-conserved between species.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Methods and materials are described herein for use in the present invention; other suitable methods and materials known in the art can also be used. The materials, methods, and examples are illustrative only and not intended to be limiting. All publications, patent applications, patents, sequences, database entries, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control.

Other features and advantages of the invention will be apparent from the following detailed description and figures, and from the claims

BRIEF DESCRIPTION OF DRAWINGS

FIG. 1 is an exemplary schematic representation of a genetic map of a CLRN-1 vector (SEQ ID NO: 40; 3397 basepairs (bp)) that can be used in any of the present methods described herein. The vector includes an AAV2 ITR, CMV enhancer (SEQ ID NO: 17), chicken β-actin promoter, chimeric intron (SEQ ID NO: 16), CLRN1 isoform 1

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(SEQ ID NO: 1), T2A sequence (SEQ ID NO: 31), CLRN1 isoform 2 (SEQ ID NO: 6), bGH poly(A) signal (SEQ ID NO: 20) and an AAV2 ITR.

FIG. 2 is an exemplary schematic representation of a genetic map of a CLRN-2GFPvector (SEQ ID NO: 41; 4177 bp) that can be used in any of the present methods described herein. The vector includes an AAV2 ITR, CMV enhancer (SEQ ID NO: 17), chicken β-actin promoter, chimeric intron (SEQ ID NO: 16), CLRN1 isoform 1 (SEQ ID NO: 1), T2A sequence (SEQ ID NO: 31), CLRN1 isoform 2 (SEQ ID NO: 6), T2A sequence (SEQ ID NO: 31), an eGFP (SEQ ID NO: 32), bGH poly(A) signal (SEQ ID NO: 20) and an AAV2 ITR.

FIG. 3 is an exemplary schematic representation of a genetic map of a CLRN-3 vector (SEQ ID NO: 42; 4607 bp) that can be used in any of the present methods described herein. The vector includes an AAV2 ITR, CMV enhancer (SEQ ID NO: 17), chicken β-actin promoter, chimeric intron (SEQ ID NO: 16), 5'UTR-291, CLRN1 isoform 1 (SEQ ID NO: 1), 3'UTR-1357 (SEQ ID NO: 36), bGH poly(A) signal (SEQ ID NO: 20) and an AAV2 ITR.

FIG. 4 is an exemplary schematic representation of a genetic map of a CLRN-4 vector (SEQ ID NO: 43; 4796 bp) that can be used in any of the present methods described herein. The vector includes an AAV2 ITR, CMV enhancer (SEQ ID NO: 17), chicken β-actin promoter, chimeric intron (SEQ ID NO: 16), CLRN1 isoform 1 (SEQ ID NO: 1), T2A sequence (SEQ ID NO: 31), CLRN1 isoform 2 (SEQ ID NO: 6), 3'UTR-1406 (SEQ ID NO: 37), bGH poly(A) signal (SEQ ID NO: 20) and an AAV2 ITR.

FIG. 5 is an exemplary schematic representation of a genetic map of a pITR-CBA-5'UTR-tGFP-3'UTR vector (5026 bp) that can be used in any of the present methods described herein. The vector includes an AAV2 ITR, CMV enhancer (SEQ ID NO: 17), chicken β-actin promoter, chimeric intron (SEQ ID NO: 16), 5'UTR-291, tGFP (SEQ ID NO: 19), 3'UTR-1595, bGH poly(A) signal (SEQ ID NO: 20) and an AAV2 ITR.

FIG. 6 is an exemplary schematic representation of a genetic map of a CLRN-6eGFP vector (SEQ ID NO: 44; 4756 bp) that can be used in any of the present methods described herein. The vector includes an AAV2 ITR, CMV enhancer (SEQ ID NO: 17), chicken β-actin promoter, chimeric intron (SEQ ID NO: 16), eGFP (SEQ ID NO: 32),

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3'UTR-1406 (SEQ ID NO: 37), bGH poly(A) signal (SEQ ID NO: 20) and an AAV2 ITR.

FIG. 7 is an exemplary schematic representation of a genetic map of a pITR-CBA-3'UTR-600A vector (3982 bp) that can be used in any of the present methods described herein. The vector includes an AAV2 ITR, CMV enhancer (SEQ ID NO: 17), chicken β-actin promoter, chimeric intron (SEQ ID NO: 16), CLRN1 isoform 1 (SEQ ID NO: 4), T2A sequence (SEQ ID NO: 31), CLRN1 isoform 2 (SEQ ID NO: 6), 3'UTR-600 (SEQ ID NO: 27), bGH poly(A) signal (SEQ ID NO: 20) and an AAV2 ITR.

FIG. 8 is an exemplary schematic representation of a genetic map of a CLRN-8 vector (SEQ ID NO: 46; 3982 bp) that can be used in any of the present methods described herein. The vector includes an AAV2 ITR, CMV enhancer (SEQ ID NO: 17), chicken β-actin promoter, chimeric intron (SEQ ID NO: 16), CLRN1 isoform 1 (SEQ ID NO: 1), T2A sequence (SEQ ID NO: 31), CLRN1 isoform 2 (SEQ ID NO: 6), 3'UTR-600B (SEQ ID NO: 28), bGH poly(A) signal (SEQ ID NO: 20) and an AAV2 ITR.

FIG. 9 is an exemplary schematic representation of a genetic map of a CLRN-9 vector (SEQ ID NO: 47; 3982 bp) that can be used in any of the present methods described herein. The vector includes an AAV2 ITR, CMV enhancer (SEQ ID NO: 17), chicken β-actin promoter, chimeric intron (SEQ ID NO: 16), CLRN1 isoform 1 (SEQ ID NO: 1), T2A sequence (SEQ ID NO: 31), CLRN1 isoform 2 (SEQ ID NO: 6), 3'UTR-600C (SEQ ID NO: 29), bGH poly(A) signal (SEQ ID NO: 20) and an AAV2 ITR.

FIG. 10 is an exemplary schematic representation of a genetic map of a CLRN-0 vector (SEQ ID NO: 39; 4732 bp) that can be used in any of the present methods described herein. The vector includes an AAV2 ITR, CMV enhancer (SEQ ID NO: 17), chicken β-actin promoter, a chimeric intron (SEQ ID NO: 16), CLRN1 isoform 1 (SEQ ID NO: 1), a 3'UTR 1773 (SEQ ID NO: 15), bGH poly(A) signal (SEQ ID NO: 20) and an AAV2 ITR.

FIG. 11 is an exemplary schematic representation of a genetic map of a CLRN-7eGFP vector (SEQ ID NO: 45; 3580 bp) that can be used in any of the present methods described herein. The vector includes an AAV2 ITR, CMV enhancer (SEQ ID NO: 17), chicken β-actin promoter, chimeric intron (SEQ ID NO: 16), an eGFP sequence (SEQ ID NO: 32), bGH poly(A) signal (SEQ ID NO: 20) and an AAV2 ITR.

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FIG. 12 is an exemplary schematic representation of a genetic map of a CLRN-10 (SEQ ID NO: 48; 3511 bp) that can be used in any of the present methods described herein. The vector includes an AAV2 ITR, CMV enhancer (SEQ ID NO: 17), chicken β-actin promoter, chimeric intron (SEQ ID NO: 16), CLRN1 isoform 1 (SEQ ID NO: 4), a HA sequence (SEQ ID NO: 34), a FP sequence (SEQ ID NO: 30), T2A sequence (SEQ ID NO: 31), CLRN1 isoform 2 (SEQ ID NO: 5), 3x FLAG tag sequence (SEQ ID NO: 35), bGH poly(A) signal (SEQ ID NO: 20) and an AAV2 ITR.

FIG. 13 is an exemplary schematic representation of a genetic map of a CLRN-10myc vector (SEQ ID NO: 49; 3574 bp) that can be used in any of the present methods described herein. The vector includes an AAV2 ITR, CMV enhancer (SEQ ID NO: 17), chicken β-actin promoter, chimeric intron (SEQ ID NO: 16), CLRN1 isoform 1 (SEQ ID NO: 1), a myc sequence (SEQ ID NO: 33), a FP sequence (SEQ ID NO: 30), T2A sequence (SEQ ID NO: 31), 3x FLAG tag sequence (SEQ ID NO: 35), bGH poly(A) signal (SEQ ID NO: 20) and an AAV2 ITR.

FIG. 14 is an exemplary schematic representation of a genetic map of a CLRN-10NF vector (SEQ ID NO: 50; 3499 bp) that can be used in any of the present methods described herein. The vector includes an AAV2 ITR, CMV enhancer (SEQ ID NO: 17), chicken β-actin promoter, chimeric intron (SEQ ID NO: 16), CLRN1 isoform 1 (SEQ ID NO: 1), T2A sequence (SEQ ID NO: 31), an HA sequence (SEQ ID NO: 34), CLRN-1 isoform 2 (SEQ ID NO: 5), 3x FLAG tag sequence (SEQ ID NO: 35), bGH poly(A) signal (SEQ ID NO: 20) and an AAV2 ITR.

FIG. 15 is an exemplary schematic representation of a genetic map of a CLRN-11 vector (SEQ ID NO: 51; 4908 bp) that can be used in any of the present methods described herein. The vector includes an AAV2 ITR, CMV enhancer (SEQ ID NO: 17), chicken β-actin promoter, chimeric intron (SEQ ID NO: 16), CLRN1 isoform 1 (SEQ ID NO: 1), a HA sequence (SEQ ID NO: 34), a FP sequence (SEQ ID NO: 30), T2A sequence (SEQ ID NO: 31), CLRN-1 isoform 2 (SEQ ID NO: 5), 3x FLAG tag sequence (SEQ ID NO: 35), 3'UTR-1406 (SEQ ID NO: 37), bGH poly(A) signal (SEQ ID NO: 20) and an AAV2 ITR.

FIG. 16 is an exemplary schematic representation of a genetic map of a CLRN-11myc vector (SEQ ID NO: 52; 4971 bp) that can be used in any of the present methods

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described herein. The vector includes an AAV2 ITR, CMV enhancer (SEQ ID NO: 17), chicken β-actin promoter, chimeric intron (SEQ ID NO: 16), CLRN1 isoform 1 (SEQ ID NO: 1), a myc sequence (SEQ ID NO: 33), a FP sequence (SEQ ID NO: 30), T2A sequence (SEQ ID NO: 31), CLRN1 isoform 2 (SEQ ID NO: 5), 3x FLAG tag sequence (SEQ ID NO: 35), 3'UTR-1406 (SEQ ID NO: 37), bGH poly(A) signal (SEQ ID NO: 20) and an AAV2 ITR.

FIG. 17 is an exemplary schematic representation of a genetic map of a CLRN-11NF vector (SEQ ID NO: 53; 4896 bp) that can be used in any of the present methods described herein. The vector includes an AAV2 ITR, CMV enhancer (SEQ ID NO: 17), chicken β-actin promoter, chimeric intron (SEQ ID NO: 16), CLRN1 isoform 1 (SEQ ID NO: 1), an HA sequence (SEQ ID NO: 34), T2A sequence (SEQ ID NO: 31), CLRN1 isoform 2 (SEQ ID NO: 5), 3x FLAG sequence (SEQ ID NO: 33), 3'UTR-1406 (SEQ ID NO: 37), bGH poly(A) signal (SEQ ID NO: 20) and an AAV2 ITR.

FIG. 18 is an exemplary schematic representation of a genetic map of a CLRN-12 vector (SEQ ID NO: 54; 4640 bp) that can be used in any of the present methods described herein. The vector includes an AAV2 ITR, CMV enhancer (SEQ ID NO: 17), chicken β-actin promoter, chimeric intron (SEQ ID NO: 16), a 5'UTR-291 sequence (SEQ ID NO: 12), CLRN1 isoform 1 (SEQ ID NO: 1), an HA sequence (SEQ ID NO: 34), 3'UTR-1357 (SEQ ID NO: 36), bGH poly(A) signal (SEQ ID NO: 20) and an AAV2 ITR.

FIG. 19 is an exemplary schematic representation of a genetic map of a CLRN-13 vector (SEQ ID NO: 55; 4291 bp) that can be used in any of the present methods described herein. The vector includes an AAV2 ITR, CMV enhancer (SEQ ID NO: 17), chicken β-actin promoter, chimeric intron (SEQ ID NO: 16), CLRN1 isoform 4 (SEQ ID NO: 7), 3x FLAG tag sequence (SEQ ID NO: 35), T2A sequence (SEQ ID NO: 31), CLRN1 isoform 1 (SEQ ID NO: 1), an HA sequence (SEQ ID NO: 34), 3'UTR-600 (SEQ ID NO: 27), bGH poly(A) signal (SEQ ID NO: 20) and an AAV2 ITR.

FIG. 20 is an exemplary schematic representation of a genetic map of a CLRN-14 vector (SEQ ID NO: 56; 4192 bp) that can be used in any of the present methods described herein. The vector includes an AAV2 ITR, CMV enhancer (SEQ ID NO: 17), chicken β-actin promoter, chimeric intron (SEQ ID NO: 16), CLRN1 isoform 4 (SEQ ID

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NO: 7), T2A sequence (SEQ ID NO: 31), CLRN1 isoform 1 (SEQ ID NO: 1), 3'UTR-600 (SEQ ID NO: 27), bGH poly(A) signal (SEQ ID NO: 20) and an AAV2 ITR.

FIG. 21 is an exemplary schematic representation of a genetic map of a CLRN-15 vector (SEQ ID NO: 57; 3505 bp) that can be used in any of the present methods described herein. The vector includes an AAV2 ITR, CMV enhancer (SEQ ID NO: 17), chicken β-actin promoter, chimeric intron (SEQ ID NO: 16), CLRN1 isoform 4 (SEQ ID NO: 7), 3x FLAG tag sequence (SEQ ID NO: 35), 3'UTR-600 (SEQ ID NO: 27), bGH poly(A) signal (SEQ ID NO: 20) and an AAV2 ITR.

FIG. 22 is an exemplary schematic representation of a genetic map of a CLRN-16 vector (SEQ ID NO: 58; 3439 bp) that can be used in any of the present methods described herein. The vector includes an AAV2 ITR, CMV enhancer (SEQ ID NO: 17), chicken β-actin promoter, chimeric intron (SEQ ID NO: 16), CLRN1 isoform 4 (SEQ ID NO: 7), 3'UTR-600 (SEQ ID NO: 27), bGH poly(A) signal (SEQ ID NO: 20) and an AAV2 ITR.

FIG. 23 is an exemplary schematic representation of a genetic map of a CLRN-17 vector (SEQ ID NO: 59; 130 bp) that can be used in any of the present methods described herein. The vector includes an AAV2 ITR, CMV enhancer (SEQ ID NO: 17), chicken β-actin promoter, a sh-chimeric intron (SEQ ID NO: 26), 5'UTR-291 (SEQ ID NO: 12), CLRN1 isoform 1 (SEQ ID NO: 1), an HA sequence (SEQ ID NO: 34), 3'UTR-1773 (SEQ ID NO: 15), bGH poly(A) signal (SEQ ID NO: 20) and an AAV2 ITR.

FIG. 24 is an exemplary schematic representation of a genetic map of a CLRN-18 vector (SEQ ID NO: 60; 4277 bp) that can be used in any of the present methods described herein. The vector includes an AAV2 ITR, CMV enhancer (SEQ ID NO: 17), chicken β-actin promoter, a sh-chimeric intron (SEQ ID NO: 26), CLRN1 isoform 1 (SEQ ID NO: 1), 3'UTR-1773 (SEQ ID NO: 15), bGH poly(A) signal (SEQ ID NO: 20) and an AAV2 ITR.

FIG. 25 is an image of an immunoblot of CLRN1 protein levels from transfected HEK293FT cells 48-hours post-transfection using anti-HA and anti-FLAG antibodies. Lane 1 – PageRuler Plus Prestained; Lane 2 – CLRN-10 (37 ^{o}C denaturing); Lane 3 – CLRN-11 (37 ^{o}C denaturing); Lane 4 – CLRN-12 (37 ^{o}C denaturing); Lane 5 – negative control; Lane 6 – CLRN-10 (56 ^{o}C denaturing); Lane 7 – CLRN-11 (56 ^{o}C denaturing);

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Lane 8 – CLRN-12 (56 ºC denaturing); Lane 9 – negative control. CLRN-1 isoform protein is a glycosylated protein and often migrates as smear bands.

FIG. 26 is an image of an immunoblot of CLRN1 protein levels from transfected HEK293FT cells 48-hours post-transfection using an anti- FLAG antibody. Lane 1 – PageRuler Plus Prestained; Lane 2 – CLRN-10; Lane 3 – CLRN-11; Lane 4 – CLRN-12; Lane 5 – CLRN-13; Lane 6 – CLRN-15; Lane 7 – negative control

FIG. 27 is an image of an immunoblot of CLRN1 protein levels from transfected HEK293FT cells 48-hours post-transfection using an anti- FLAG antibody. Lane 1 – PageRuler Plus Prestained; Lane 2 – CLRN-10; Lane 3 – CLRN-10NF; Lane 4 – CLRN-10myc; Lane 5 – CLRN-11NF; Lane 6 – CLRN-11myc; Lane 7 – negative control. All samples were kept at room temperature.

FIG. 28 is an image of an immunoblot of CLRN1 protein levels from transfected HEK293FT cells 48-hours post-transfection using an anti-myc antibody. Lane 1 – PageRuler Plus Prestained; Lane 2 – CLRN-10; Lane 3 – CLRN-10NF; Lane 4 – CLRN-10myc; Lane 5 – CLRN-11NF; Lane 6 – CLRN-11myc; Lane 7 – negative control.

FIG. 29 is an image of an immunoblot of CLRN1 protein levels harvested from transfected HEK293FT cells 48 hours post-transfection using anti-HA and anti-FLAG antibodies. Lane 1 – PageRuler Plus Prestained; Lane 2 – CLRN-13; Lane 3 – CLRN-10; Lane 4 – CLRN-10NF; Lane 5 – CLRN-10myc; Lane 6 – CLRN-11NF; Lane 7 – CLRN-11myc; Lane 8 – negative control.

FIG. 30 is an image of an immunoblot using anti-CLRN (EKIANYKEGTYVYKTQSEKY; SEQ ID NO: 38) rabbit polyclonal antibody of CLRN1 isoform 1 protein levels harvested from HEK239FT cells transfected with plasmids described herein 48 hours post-transfection. Lane 1 – PageRuler Plus Prestained; Lane 2 – CLRN-10; Lane 3 – CLRN-1; Lane 4- CLRN-2; Lane 5 – CLRN-3; Lane 6 – CLRN-4; Lane 7 – CLRN-8; Lane 8 – CLRN-9; Lane 9 – CLRN-10; Lane 10 – CLRN-11; Lane 11 – CLRN-12; Lane 12 – CLRN-13, Lane 13 – CLRN-14; Lane 14 – CLRN-15; Lane 15 – CLRN-16; Lane 16 – negative control; Lane 17 – negative control.

FIG. 31 is an image of an immunoblot CLRN1 protein levels harvested from transfected HEK293FT cells 48 hours post-transfection using an anti-CLRN rabbit polyclonal antibody. Lane 1 – PageRuler Plus Prestained; Lane 2 – Anc80-CLRN-0;

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Lane 3 – Anc80-CLRN-0 +PNGase F; Lane 4- Anc80-CLRN-3; Lane 5 – Anc80-CLRN-3 +PNGase F; Lane 6 – Anc80-CLRN-6eGFP; Lane 7 – Anc80-CLRN-6eGFP +PNGase F; Lane 8 – Anc80-CLRN-13; Lane 9 – Anc80-CLRN-13 +PNGase F; Lane 10 – no vector; Lane 11 – no vector +PNGase F. CLRN-1 isoform protein is a glycosylated protein and often migrates as smear bands (lanes 2, 4 and 8). After treatment with PNGase F, the smeared bands disappeared and shifted to distinct bands (alnes 3, 5 and 8).

FIG. 32 is a set of immunofluorescent images of AAVanc80-CLRN6eGFP transduced HEK293FT cells taken 24 hours and 48 hours post-transfection at MOI 8.41E+04 and MOI 2.53E+05, respetively.

FIG. 33 is a bar graph showing the relative CLRN1 and GFP expression in HEK293FT cells transduced with AAVanc80-CLRN-6eGFP (at MOI 1.05E+05 and MOI 3.15E+05), AAVanc80-CLRN-0 (at MOI 8.23E+04 and MOI 2.47E+05), AAVanc80-CLRN-3 (at MOI 8.41E+04 and MOI 2.53E+04), and AAVanc80-CLRN-13 (at MOI 8.33E+04 and MOI 2.50E+05), respectively.

FIG. 34 is a bar graph showing the relative CLRN1 and GFP expression in P2 cochlear explants from WT mice infected 16-hours with AAVanc80-CLRN-6eGFP (at MOI 2.0E+05), AAVanc80-CLRN-0 (at MOI 2.5E+05 and MOI 7.6E+0.5), AAVanc80-CLRN-3 (at MOI 2.0E+05 and 6.03E+05) and AAVanc80-CLRN-13 (at MOI 2.0E+05 and MOI 6.0E+05), respectively.

FIG. 35 is a set of fluorescent images of P2 cochlear explants from WT mice infected 72-hours with 1.3E10 AAVanc80-CLRN-0 VG/cochlea, 9.9E9 AAVanc80-CLRN-3 VG/cochlea and 1.0E10 AAVanc80-CLRN-13 VG/cochlea showing Myo7a and DAPI staining.

FIG. 36 is a set of fluorescent images of P2 cochlear explants from WT mice infected 72-hours with 1E09 VG/cochlea AAV Anc80.CAG.eGFP.3'UTR showing eGFP, Myo7a and DAPI staining.

DETAILED DESCRIPTION

Deficiency or mutations in "clarin 1," the protein encoded by the CLRN1 gene, causes hearing loss and vision loss. For example, mutations in CLRN1 lead to Usher syndrome type III and retinitis pigmentosa.

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Provided herein are compositions that include at least two different nucleic acid vectors, wherein: each of the at least two different vectors comprises a coding sequence that encodes a different portion of a CLRN1 protein, each of the encoded portions being at least 30 amino acid residues in length, wherein the amino acid sequence of each of the encoded portions may optionally partially overlap with the amino acid sequence of a different one of the encoded portions; no single vector of the at least two different vectors encodes a full-length CLRN1 protein; at least one of the coding sequences comprises a nucleotide sequence spanning two consecutive exons of CLRN1 genomic DNA, and lacking an intronic sequence between the two consecutive exons; and when introduced into a mammalian cell, the at least two different vectors undergo homologous recombination with each other, thereby forming a recombined nucleic acid that encodes a full-length CLRN1 protein.

Provided herein are compositions that include a single nucleic acid vector, wherein the vector comprises one or both of (i) a first coding sequence encoding a first isoform of CLRN1 protein; and (ii) a second coding sequence encoding a second isoform of CLRN1 protein, where one or both of the first and second coding sequences comprises a nucleotide sequence spanning two consecutive exons of a CLRN1 genomic DNA, and lacking an intronic sequence between the two consecutive introns.

Provided herein are compositions that include two different nucleic acid vectors, wherein: a first nucleic acid vector of the two different nucleic acid vectors comprises a promoter, a first coding sequence that encodes an N-terminal portion of a CLRN1 protein positioned 3’ of the promoter, and a splice donor sequence positioned at the 3’ end of the first coding sequence; and a second nucleic acid vector of the two different nucleic acid vectors comprises a splice acceptor sequence, a second coding sequence that encodes a C-terminal portion of a CLRN1 protein positioned at the 3’ end of the splice acceptor sequence, and a polyadenylation signal sequence at the 3’ end of the second coding sequence; wherein each of the encoded portions is at least 30 amino acid residues in length, wherein the amino acid sequences of the two encoded portions do not overlap with each other; wherein no single vector of the two different vectors encodes a full-length CLRN1 protein; and when introduced into a mammalian cell, splicing occurs

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between the splice donor sequence and the splice acceptor sequence, thereby forming a recombined nucleic acid that encodes a full-length CLRN1 protein.

Provided herein are compositions that include two different nucleic acid vectors, wherein: a first nucleic acid vector of the two different nucleic acid vectors comprises a promoter, a first coding sequence that encodes an N-terminal portion of a CLRN1 protein positioned 3' of the promoter, a splice donor sequence positioned at the 3' end of the first coding sequence, and a first detectable marker gene positioned 3' of the splice donor sequence; and a second nucleic acid vector of the two different nucleic acid vectors comprises a second detectable marker gene, a splice acceptor sequence positioned 3' of the second detectable marker gene, a second coding sequence that encodes a C-terminal portion of a CLRN1 protein positioned at the 3' end of the splice acceptor sequence, and a polyadenylation signal sequence positioned at the 3' end of the second coding sequence; wherein each of the encoded portions is at least 30 amino acid residues in length, wherein the amino acid sequences of the encoded portions do not overlap with each other; wherein no single vector of the two different vectors encodes a full-length CLRN1 protein; and when introduced into a mammalian cell, splicing occurs between the splice donor sequence and the splice acceptor sequence, thereby forming a recombined nucleic acid that encodes a full-length CLRN1 protein.

Also provided herein are compositions that include two different nucleic acid vectors, wherein: a first nucleic acid vector of the two different nucleic acid vectors comprises a promoter, a first coding sequence that encodes an N-terminal portion of a CLRN1 protein positioned 3' to the promoter, a splice donor sequence positioned at the 3' end of the first coding sequence, and a F1 phage recombinogenic region positioned 3' to the splice donor sequence; and a second nucleic acid vector of the two different nucleic acid vectors comprises a F1 phage recombinogenic region, a splice acceptor sequence positioned 3' of the F1 phage recombinogenic region, a second coding sequence that encodes a C-terminal portion of a CLRN1 protein positioned at the 3' end of the splice acceptor sequence, and a polyadenylation signal sequence positioned at the 3' end of the second coding sequence; wherein each of the two encoded portions is at least 30 amino acid residues in length, wherein the amino acid sequences of the two encoded portions do not overlap with each other; wherein no single vector of the two different vectors encodes

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a full-length CLRN1 protein; and when introduced into a mammalian cell, splicing occurs between the splice donor sequence and the splice acceptor sequence, thereby forming a recombined nucleic acid that encodes a full-length CLRN1 protein.

Provided herein are methods that include: introducing into a cochlea of a mammal a therapeutically effective amount of any of the compositions described herein.

Provided herein are methods of increasing expression of a full-length CLRN1 protein in a mammalian cell, that include: introducing any of the compositions described herein into the mammalian cell.

Provided herein are methods of increasing expression of a full-length CLRN1 protein in an inner hair cell, an outer hair cell, or both, in a cochlea of a mammal that include: introducing into the cochlea of the mammal a therapeutically effective amount of any of the compositions described herein.

Provided herein are methods of increasing expression of a full-length CLRN1 protein in an eye of a mammal that include: intraocularly administering to the eye of the mammal a therapeutically effective amount of any of the compositions described herein.

Provided herein are methods of treating hearing loss in a subject identified as having a defective CLRN1 gene that include: administering a therapeutically effective amount of any of the compositions described herein into the cochlea of the subject.

Also provided herein are methods of treating vision loss in a subject identified as having a defective CLRN1 gene that include: administering a therapeutically effective amount of any of the compositions described herein into the eye of the subject.

Additional non-limiting aspects of the compositions, kits, and methods are described herein and can be used in any combination without limitation.

CLRN1

The CLRN1 gene encodes “clarin 1” (CLRN1), a protein that is expressed in hair cells of the inner ear (e.g., inner ear hair cells, outer ear hair cells) and in the retina.

The human CLRN1 gene is located on chromosome 3q25.1. It contains 7 exons encompassing ~ 47 kilobases (kb) (Vastinsalo et al. (2011) Eur J Hum Genet 19(1): 30-35; NCBI Accession No. NG_009168.1).

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Various mutations in the CLRN1 genes have been associated with Usher syndrome type III (e.g., Usher syndrome type IIIA (MIM#606397) (see, e.g., Fields et al. (2002) Am J Hum Genet 71: 607-617, and Joensuu et al. (2001) Am J Hum Genet 69: 673-684) and retinitis pigmentosa (see, e.g., Khan et al. (2011) Ophthalmology 118: 1444-1448). Usher syndrome type III-causing mutations have been predominantly found in exon 3 of CLRN1. Usher syndrome type III-deafness can be modeled by generating CLRN1- deficient mice (see, e.g.,Geng et al. (2017) Sci Rep 7(1): 13480). Exemplary mutations CLRN1-associated with Usher syndrome type III include: T528G, M120K, M44K, N48K, and C40G.

Exemplary mutations CLRN1-associated with retinitis pigmentosa include L154W and P31L (see, e.g., Khan et al. (2011) Ophthalmology 118: 1444-1448).

Additional exemplary mutations in a CLRN1 gene that have been detected in subjects having hearing loss and methods of sequencing a nucleic acid encoding CLRN1 are described in, e.g., Fields et al. (2002) Am J Hum Genet 71: 607-617, Joensuu et al. (2001) Am J Hum Genet 69: 673-684, Adato et al. (2002) Europ J Hum Genet 10: 339-350, Aller et al. (2004), Clin Genet 66: 525-529. Methods of detecting mutations in a gene are well-known in the art. Non-limiting examples of such techniques include: real-time polymerase chain reaction (RT-PCR), PCR, sequencing, Southern blotting, and Northern blotting.

An exemplary human wildtype CLRN1 protein is or includes the sequence of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5 and SEQ ID NO: 7. Non-limiting examples of nucleotide sequences encoding a wildtype CLRN1 protein are or include SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6 and SEQ ID NO: 8.

In some embodiments of any of the compositions described herein, the CLRN1 protein comprises a sequence that is at least 75% (e.g., at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%) identical to SEQ ID NO: 1.

In some embodiments of any of the compositions described herein, the CLRN1 protein comprises a sequence that is at least 75% (e.g., at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%) identical to SEQ ID NO: 3.

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In some embodiments of any of the compositions described herein, the CLRN1 protein comprises a sequence that is at least 75% (e.g., at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%) identical to SEQ ID NO: 5.

In some embodiments of any of the compositions described herein, the CLRN1 protein comprises a sequence that is at least 75% (e.g., at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%) identical to SEQ ID NO: 7.

Human Full-length Wildtype CLRN1 Protein Isoform D (SEQ ID NO: 1)

MPSQQKKIIFCMAGVFSFACALGVVTALGTPLWIKATVLCKTGALLVNASGQELDKFMGEMQYGLFHGEGV
RQCGLGARPFRFSFFPDLLKAIPVSIHVNVILFSAILIVLTMVGTAFFMYNAFGKPFETLHGPLGLYLLSF
ISVALWLPATRHQAQGSCGCLVMILFASEVKIHHLSEKIANYKEGTYVYKTQSEKYTTSFWVIFFCFFVHF
LNGLLIRLAGFQFPFAKSKDAETTNVAADLMY

Human Wildtype CLRN1 Isoform D cDNA (SEQ ID NO: 2)

atgccaagc caacagaaga aaatcatttt ttgcatggcc ggagtgttca gttttgcatg
tgccctcgga gttgtgacag ccttggggac accgttgtgg atcaaagcca ctgtcctctg
caaaacggga gctctgctcg tcaatgcctc agggcaggag ctggacaagt ttatgggtga
aatgcagtac gggcttttcc acggagaggg tgtgaggcag tgtgggttgg gagcaaggcc
ctttcggttc tcatttttc cagatttgct caaagcaatc ccagtgagca tccacgtcaa
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gagcttcatt tcagttgccc tttggctgcc agctaccagg caccaggctc aaggctcctg
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ctcattctgg gtcattttct tttgcttttt tgttcatttt ctgaatgggc tcctaatacg
acttgctgga tttcagttcc cttttgcaaa atctaaagac gcagaaacaa ctaatgtagc
tgcagatcta atgtactga

Human Full-length Wildtype CLRN1 Protein Isoform A (SEQ ID NO: 3)

MPSQQKKIIFCMAGVFSFACALGVVTALGTPLWIKATVLCKTGALLVNASGQELDKFMGEMQYGLFHGEGV
RQCGLGARPFRFSFFPDLLKAIPVSIHVNVILFSAILIVLTMVGTAFFMYNAFGKPFETLHGPLGLYLLSF

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ISGSCGCLVMILFASEVKIHHLSEKIANYKEGTYVYKTQSEKYTTSFWVIFFCFFVHFLNGLLIRLAGFQF
PFAKS KDAETTNVAADLMY

Human Wildtype CLRN1 Isoform A cDNA (SEQ ID NO: 4)

atgccaagc caacagaaga aaatcatttt ttgcatggcc ggagtgttca gttttgcatg
tgccctcgga gttgtgacag ccttggggac accgttgtgg atcaaagcca ctgtcctctg
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gcaaagtgaa aaatatacca cctcattctg ggtcattttc ttttgctttt ttgttcattt
tctgaatggg ctcctaatac gacttgctgg atttcagttc ccttttgcaa aatctaaaga
cgcagaaaca actaatgtag ctgcagatct aatgtactga

Human Full-length Wildtype CLRN1 Protein Isoform C (SEQ ID NO: 5)

MQALQQQPVFPDLLKAIPVSIHVNVILFSAILIVLTMVGTAFFMYNAFGKPFETLHGPLGLYLLSFISGSC
GCLVMILFASEVKIHHLSEKIANYKEGTYVY KTQSEKYTTSFWLTKGHS

Human Wildtype CLRN1 Isoform C cDNA (SEQ ID NO: 6)

atgcaggc cctgcagcag caaccagttt ttccagattt gctcaaagca atcccagtga
gcatccacgt caatgtcatt ctcttctctg ccatccttat tgtgttaacc atggtgggga
cagccttctt catgtacaat gcttttggaa aacctttga aactctgcat ggtcccctag
ggctgtacct tttgagcttc atttcaggct cctgtggctg tcttgtcatg atattgtttg
cctctgaagt gaaaatccat cacctctcag aaaaaattgc aaattataaa gaagggactt
atgtctacaa aacgcaaagt gaaaaatata ccacctcatt ctggctgact aaaggccaca gctga

Human Full-length Wildtype CLRN1 Protein Isoform E (SEQ ID NO: 7)

MPSQQKKIIFCMAGVFSFACALGVVTALGTPLWIKATVLCKTGALLVNASGQELDKFMGEMQYGLFHGEGV
RQCGLGARPFRFSCYFLDPFMGLPTGVPHLLSLPCSTSCRREHTSERVQEPAGCFSAVRSKLHAGPAAATS
FSRFAQSNPSEHPRQCHS LLCHPYCVNHGGDSLLHVQCFWKTF

Human Wildtype CLRN1 Isoform E cDNA (SEQ ID NO: 8)

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atgccaagc caacagaaga aaatcatttt ttgcatggcc ggagtgttca gttttgcatg
tgccctcgga gttgtgacag ccttggggac accgttgtgg atcaaagcca ctgtcctctg
caaaacggga gctctgctcg tcaatgcctc agggcaggag ctggacaagt ttatgggtga
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aaccagtttt tccagatttg ctcaaagcaa tcccagtgag catccacgtc aatgtcattc
tcttctctgc catccttatt gtgttaacca tggtggggac agccttcttc atgtacaatg
cttttggaaa acctttga

Human Wildtype CLRN1 Genomic sequence (SEQ ID NO: 9)

aggagatact tgaaggcagt ttgaaagact tgttttacag attcttagtc caaagatttc 61
caattaggga gaagaagcag cagaaaagga gaaaagccaa gtatgagtga tgatgaggcc 121
ttcatctact gacatttaac ctggcgagaa ccgtcgatgg tgaagttgcc ttttcagctg 181
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gaaaaatcta atgaggaact aagaggcaaa cccacca

A non-limiting example of a human wildtype CLRN1 genomic DNA sequence is SEQ ID NO: 9. The exons in SEQ ID NO: 9 are: nucleotide positions 1-544 (exon 1), nucleotide positions 28764-29180 (exon 2), nucleotide positions 31239-31418 (exon 3),

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nucleotide positions 32481-32519 (exon 4), nucleotide positions 44799-46433 (exon 5), nucleotide positions 44799-44935 (exon 6), and nucleotide positions 46128-46837 (exon 7). The introns are located between each pair of these exons in SEQ ID NO: 9, i.e., at nucleotide positions 545-28763 (intron 1), nucleotide positions 29181-31238 (intron 2), nucleotide positions 31419-32480 (intron 3), nucleotide positions 32520-44798 (intron 4), and nucleotide positions 44936-46127 (intron 7).

Mouse CLRN1 Protein Isoform 1 (SEQ ID NO: 10)

MPSQQKKIIFCMAGVLSFLCALGVVTAVGTPLWVKATILCKTGALLVNASGKELDKFMGEMQYGLFHGEGV
RQCGLGARPFRFSSRSMKERYSLYEDKGETAVFPDLVQAIPVSIHINIILFSMILVVLTMVGTAFFMYNAF
GKPFETLHGPLGLYLVSFISGSCGCLVMILFASEVKVHRLSEKIANFKEGTYAYRTQNENYTTSFWVVFIC
FFVHF LNGLLIRLAGFQFPFTKSKETETTNVASDLMY

Dog CLRN1 Protein (SEQ ID NO: 11)

MPNQQKKVVFCTAGVLSFVCALGVVTALGTPLWIKATFLCKTGALLVNASGQELDKFMGEMQYGLFHGEGI
RQCGLGARPFRFSLFPDLLKVIPVSIHVNVILFSTILVVLTMVGTAFFMYNAFGKPFETLHGPLGLYLLSF
ISGSCGCLVMILFASEVKIHHLSEKIANYKEGTYAYKTQSEKYTTSFWVVFICFLVHLLNGLLIRLAGFQF
PFAKS KDTETTNVAADLMY

Vectors

The compositions provided herein include at least two (e.g., two, three, four, five, or six) nucleic acid vectors, where: each of the at least two different vectors includes a coding sequence that encodes a different portion of a CLRN1 protein, each of the encoded portions being at least 30 amino acids (e.g., between about 30 amino acids to about 202 amino acids, about 30 amino acids to about 200 amino acids, about 30 amino acids to about 180 amino acids, about 30 amino acids to about 170 amino acids, about 30 amino acids to about 160 amino acids, about 30 amino acids to about 150 amino acids, about 30 amino acids to about 140 amino acids, about 30 amino acids to about 130 amino acids, about 30 amino acids to about 120 amino acids, about 30 amino acids to about 110 amino acids, about 30 amino acids to about 100 amino acids, about 30 amino acids to about 90 amino acids, about 30 amino acids to about 80 amino acids, about 30 amino acids to about 70 amino acids, about 30 amino acids to about 60 amino acids, about 30 amino acids to about 50 amino acids, about 30 amino acids to about 40 amino acids, about 60 amino acids to about 202 amino acids, about 60 amino acids to about 200 amino

acids, about 60 amino acids to about 180 amino acids, about 60 amino acids to about 170 amino acids, about 60 amino acids to about 160 amino acids, about 60 amino acids to about 150 amino acids, about 60 amino acids to about 140 amino acids, about 60 amino acids to about 130 amino acids, about 60 amino acids to about 120 amino acids, about 60 amino acids to about 110 amino acids, about 60 amino acids to about 100 amino acids, about 60 amino acids to about 90 amino acids, about 60 amino acids to about 80 amino acids, about 60 amino acids to about 70 amino acids, about 90 amino acids to about 202 amino acids, about 90 amino acids to about 200 amino acids, about 90 amino acids to about 180 amino acids, about 90 amino acids to about 170 amino acids, about 90 amino acids to about 160 amino acids, about 90 amino acids to about 150 amino acids, about 90 amino acids to about 140 amino acids, about 90 amino acids to about 130 amino acids, about 90 amino acids to about 120 amino acids, about 90 amino acids to about 110 amino acids, about 90 amino acids to about 100 amino acids, about 100 amino acids to about 202 amino acids, about 100 amino acids to about 200 amino acids, about 100 amino acids to about 180 amino acids, about 100 amino acids to about 170 amino acids, about 100 amino acids to about 160 amino acids, about 100 amino acids to about 150 amino acids, about 100 amino acids to about 140 amino acids, about 100 amino acids to about 130 amino acids, about 100 amino acids to about 120 amino acids, about 90 amino acids to about 110 amino acids, about 120 amino acids to about 202 amino acids, about 120 amino acids to about 200 amino acids, about 120 amino acids to about 180 amino acids, about 120 amino acids to about 170 amino acids, about 120 amino acids to about 160 amino acids, about 120 amino acids to about 150 amino acids, about 120 amino acids to about 140 amino acids, about 120 amino acids to about 130 amino acids, about 150 amino acids to about 202 amino acids, about 150 amino acids to about 200 amino acids, about 150 amino acids to about 180 amino acids, about 150 amino acids to about 170 amino acids, about 150 amino acids to about 160 amino acids, about 170 amino acids to about 202 amino acids, about 170 amino acids to about 200 amino acids, about 170 amino acids to about 180 amino acids, about 190 amino acids to about 202 amino acids, or about 190 amino acids to about 200 amino acids) in length.

In some embodiments of these compositions, at least one of the coding sequences

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includes a nucleotide sequence spanning two consecutive exons of CLRN1 genomic DNA (e.g., exons 1 and 2, or exons 5 and 6), and lacking the intronic sequence that naturally occurs between the two consecutive exons.

In some embodiments, the amino acid sequence of none of the encoded portions overlaps even in part with the amino acid sequence of a different one of the encoded portions. In some embodiments, the amino acid sequence of one or more of the encoded portions partially overlaps with the amino acid sequence of a different one of the encoded portions. In some embodiments, the amino acid sequence of each of the encoded portions partially overlaps with the amino acid sequence of a different one of the encoded portions.

In some embodiments, the overlapping amino acid sequence is between about 30 amino acid residues to about 202 amino acids (e.g., or any of the subranges of this range described herein) in length.

In some examples, the vectors include two different vectors, each of which comprises a different segment of an intron, wherein the intron includes the nucleotide sequence of an intron that is present in a CLRN1 genomic DNA (e.g., any of the exemplary introns in SEQ ID NO: 9 described herein), and wherein the two different segments overlap in sequence by at least 100 nucleotides (e.g., about 100 nucleotides to about 10,000 nucleotides, about 100 nucleotides to about 5,000 nucleotides, about 100 nucleotides to about 4,500 nucleotides, about 100 nucleotides to about 4,000 nucleotides, about 100 nucleotides to about 3,500 nucleotides, about 100 nucleotides to about 3,000 nucleotides, about 100 nucleotides to about 2,500 nucleotides, about 100 nucleotides to about 2,000 nucleotides, about 100 nucleotides to about 1,500 nucleotides, about 100 nucleotides to about 1,000 nucleotides, about 100 nucleotides to about 800 nucleotides, about 100 nucleotides to about 600 nucleotides, about 100 nucleotides to about 400 nucleotides, about 100 nucleotides to about 200 nucleotides, about 200 nucleotides to about 10,000 nucleotides, about 200 nucleotides to about 5,000 nucleotides, about 200 nucleotides to about 4,500 nucleotides, about 200 nucleotides to about 4,000 nucleotides, about 200 nucleotides to about 3,500 nucleotides, about 200 nucleotides to about 3,000 nucleotides, about 200 nucleotides to about 2,500 nucleotides, about 200 nucleotides to about 2,000 nucleotides, about 200 nucleotides to about 1,500 nucleotides, about 200

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nucleotides to about 1,000 nucleotides, about 200 nucleotides to about 800 nucleotides, about 200 nucleotides to about 600 nucleotides, about 200 nucleotides to about 400 nucleotides, about 400 nucleotides to about 10,000 nucleotides, about 400 nucleotides to about 5,000 nucleotides, about 400 nucleotides to about 4,500 nucleotides, about 400 nucleotides to about 4,000 nucleotides, about 400 nucleotides to about 3,500 nucleotides, about 400 nucleotides to about 3,000 nucleotides, about 400 nucleotides to about 2,500 nucleotides, about 400 nucleotides to about 2,000 nucleotides, about 400 nucleotides to about 1,500 nucleotides, about 400 nucleotides to about 1,000 nucleotides, about 400 nucleotides to about 800 nucleotides, about 400 nucleotides to about 600 nucleotides, about 600 nucleotides to about 10,000 nucleotides, about 600 nucleotides to about 5,000 nucleotides, about 600 nucleotides to about 4,500 nucleotides, about 600 nucleotides to about 4,000 nucleotides, about 600 nucleotides to about 3,500 nucleotides, about 600 nucleotides to about 3,000 nucleotides, about 600 nucleotides to about 2,500 nucleotides, about 600 nucleotides to about 2,000 nucleotides, about 600 nucleotides to about 1,500 nucleotides, about 600 nucleotides to about 1,000 nucleotides, about 600 nucleotides to about 800 nucleotides, about 800 nucleotides to about 10,000 nucleotides, about 800 nucleotides to about 5,000 nucleotides, about 800 nucleotides to about 4,500 nucleotides, about 800 nucleotides to about 4,000 nucleotides, about 800 nucleotides to about 3,500 nucleotides, about 800 nucleotides to about 3,000 nucleotides, about 800 nucleotides to about 2,500 nucleotides, about 800 nucleotides to about 2,000 nucleotides, about 800 nucleotides to about 1,500 nucleotides, about 800 nucleotides to about 1,000 nucleotides, about 1,000 nucleotides to about 10,000 nucleotides, about 1,000 nucleotides to about 5,000 nucleotides, about 1,000 nucleotides to about 4,500 nucleotides, about 1,000 nucleotides to about 4,000 nucleotides, about 1,000 nucleotides to about 3,500 nucleotides, about 1,000 nucleotides to about 3,000 nucleotides, about 1,000 nucleotides to about 2,500 nucleotides, about 1,000 nucleotides to about 2,000 nucleotides, about 1,000 nucleotides to about 1,500 nucleotides, about 1,500 nucleotides to about 10,000 nucleotides, about 1,500 nucleotides to about 5,000 nucleotides, about 1,500 nucleotides to about 4,500 nucleotides, about 1,500 nucleotides to about 4,000 nucleotides, about 1,500 nucleotides to about 3,500 nucleotides, about 1,500 nucleotides to about 3,000 nucleotides, about 1,500 nucleotides to about 2,500 nucleotides, about 1,500 nucleotides

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to about 2,000 nucleotides, about 2,000 nucleotides to about 10,000 nucleotides, about 2,000 nucleotides to about 5,000 nucleotides, about 2,000 nucleotides to about 4,500 nucleotides, about 2,000 nucleotides to about 4,000 nucleotides, about 2,000 nucleotides to about 3,500 nucleotides, about 2,000 nucleotides to about 3,000 nucleotides, about 2,000 nucleotides to about 2,500 nucleotides, about 2,500 nucleotides to about 10,000 nucleotides, about 2,500 nucleotides to about 5,000 nucleotides, about 2,500 nucleotides to about 4,500 nucleotides, about 2,500 nucleotides to about 4,000 nucleotides, about 2,500 nucleotides to about 3,500 nucleotides, about 2,500 nucleotides to about 3,000 nucleotides, about 3,000 nucleotides to about 10,000 nucleotides, about 3,000 nucleotides to about 5,000 nucleotides, about 3,000 nucleotides to about 4,500 nucleotides, about 3,000 nucleotides to about 4,000 nucleotides, about 3,000 nucleotides to about 3,500 nucleotides, about 3,500 nucleotides to about 10,000 nucleotides, about 3,500 nucleotides to about 5,000 nucleotides, about 3,500 nucleotides to about 4,500 nucleotides, about 3,500 nucleotides to about 4,000 nucleotides, about 4,000 nucleotides to about 10,000 nucleotides, about 4,000 nucleotides to about 5,000 nucleotides, about 4,000 nucleotides to about 4,500 nucleotides, about 4,500 nucleotides to about 10,000 nucleotides about 4,500 nucleotides to about 5,000 nucleotides, or about 5,000 nucleotides to about 10,000 nucleotides) in length.

The overlapping nucleotide sequence in any two of the different vectors can include part or all of one or more exons of a CLRN1 gene (e.g., any one or more of the exemplary exons in SEQ ID NO: 9 described herein).

In some embodiments, the number of different vectors in the composition is two, three, four, or five. In compositions where the number of different vectors in the composition is two, the first of the two different vectors can include a coding sequence that encodes an N-terminal portion of the CLRN1 protein. In some examples, the N-terminal portion of the CLRN1 gene is between about 30 amino acids to about 202 amino acids (or any of the subranges of this range described above) in length. In some examples, the first vector further includes one or both of a promoter (e.g., any of the promoters described herein or known in the art) and a Kozak sequence (e.g., any of the exemplary Kozak sequences described herein or known in the art). In some examples, the first vector includes a promoter that is an inducible promoter, a constituitive

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promoter, or a tissue-specific promoter. In some examples, the second of the two different vectors includes a coding sequence that encodes a C-terminal portion of the CLRN1 protein. In some examples, the C-terminal portion of the CLRN1 protein is between 30 amino acids to about 202 amino acids (or any of the subranges of this range described above) in length. In some examples, the second vector further includes a polyadenylation signal sequence.

In some examples where the number of different vectors in the composition is two, the N-terminal portion encoded by one of the two vectors can include a portion comprising amino acid position 1 to any of the following: about amino acid position 202, about amino acid position 200, about amino acid 190, about amino acid position 180, about amino acid position 170, about amino acid position 160, about amino acid position 150, about amino acid position 140, about amino acod position 130, about amino acid position 120, about amino acid position 110, about amino acid position 100, about amino acid position 90, about amino acid position 80, about amino acid position 70, about amino acid position 60, about amino acid position 50, or about amino acid position 40 of a wildtype CLRN1 protein (e.g., SEQ ID NO: 1, 3, 5, or 7).

In some examples where the number of different vectors in the composition is two, the N-terminal portion of the precursor CLRN1 protein can include a portion comprising amino acid position 1 to amino acid position 202, amino acid position 1 to about amino acid position 200, amino acid position 1 to about amino acid position 190, amino acid position 1 to about amino acid position 180, amino acid position 1 to about amino acid position 170, amino acid position 1 to about amino acid position 160, amino acid position 1 to about amino acid position 150, amino acid position 1 to about amino acid position 140, amino acid position 1 to about amino acid position 130, amino acid position 1 to about amino acid position 120, amino acid position 1 to about amino acid position 110, amino acid position 1 to about amino acid position 100, amino acid position 1 to about amino acid position 90, amino acid position 1 to about amino acid position80, amino acid position 1 to about amino acid position 70, amino acid position 1 to about amino acid position 60, amino acid position 1 to about amino acid position 50, amino acid position 1 to about amino acid position 40, amino acid position 1 to about amino acid position 30 of a wildtype CLRN1 protein (e.g., SEQ ID NO: 1, 3, 5, or 7).

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As used herein, the term "vector" means a composition including a polynucleotide capable of carrying at least one exogenous nucleic acid fragment, e.g., a plasmid vector, a transposon, a cosmid, an artificial chromosome (e.g., a human artificial chromosome (HAC), a yeast artificial chromosome (YAC), a bacterial artificial chromosome (BAC), or a P1-derived artificial chromosome (PAC)), a viral vector (e.g., any adenoviral vectors (e.g., pSV or pCMV vectors) or any retroviral vectors as described herein), and any Gateway® vectors. A vector can, e.g., include sufficient cis-acting elements for expression; other elements for expression can be supplied by the host mammalian cell or in an *in vitro* expression system. The term "vector" includes any genetic element (e.g., a plasmid, a transposon, a cosmid, an artificial chromosome, a viral vector, etc.) that is capable of replicating when associated with the proper control elements. Thus, the term includes cloning and expression vectors, as well as viral vectors (e.g., an adeno-associated virus (AAV) vector, an adenovirus vector, a lentivirus vector, or a retrovirus vector).

Vectors include all those known in the art, including cosmids, plasmids (e.g., naked or contained in liposomes) and viruses (e.g., lentiviruses, retroviruses, adenoviruses, and adeno-associated viruses) that incorporate the recombinant polynucleotide. Skilled practitioners will be capable of selecting suitable vectors and mammalian cells for making any of the nucleic acids described herein.

In some embodiments, the vector is a plasmid (i.e. a circular DNA molecule that can autonomously replicate inside a cell). In some embodiments, the vector can be a cosmid (e.g., pWE and sCos series (Wahl et al. (1987), Evans et al. (1989)).

In some embodiments, the vector(s) is an artificial chromosome. An artificial chromosome is a genetically engineered chromosome that can be used as a vector to carry large DNA inserts. In some embodiments, the artificial chromosome is human artificial chromosome (HAC) (see, e.g., Kouprina et al., *Expert Opin. Drug Deliv* 11(4): 517-535, 2014; Basu et al., *Pediatr. Clin. North Am.* 53: 843-853, 2006; Ren et al., *Stem. Cell Rev.* 2(1):43-50, 2006; Kazuki et al., *Mol. Ther.* 19(9):1591-1601, 2011; Kazuki et al., *Gen. Ther.* 18: 384-393, 2011; and Katoh et al., *Biochem. Biophys. Res. Commun.* 321:280-290, 2004).

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In some embodiments, the vector(s) is a yeast artificial chromosome (YAC) (see, e.g., Murray et al., *Nature* 305: 189-193, 1983; Ikeno et al. (1998) *Nat. Biotech.* 16:431-439, 1998). In some embodiments, the vector(s) is a bacterial artificial chromosome (BAC) (e.g., pBeloBAC11, pECBAC1, and pBAC108L). In some embodiments, the vector(s) is a P1-derived artificial chromosome (PAC). Examples of artificial chromosome are known in the art.

In some embodiments, the vector(s) is a viral vector (e.g., adeno-associated virus, adenovirus, lentivirus, and retrovirus). Non-limiting examples of viral vectors are described herein. In some embodiments, the vector(s) is an adeno-associated viral vector (AAV) (see, e.g., Asokan et al., *Mol. Ther.* 20: 699-7080, 2012). Recombinant AAV vectors or "rAAVs" are typically composed of, at a minimum, a transgene or a portion thereof and a regulatory sequence, and optionally 5' and 3' AAV inverted terminal repeats (ITRs). Such a recombinant AAV vector is packaged into a capsid and delivered to a selected target cell (e.g., a cochlear hair cell).

The AAV sequences of the vector typically comprise the cis-acting 5' and 3' ITR sequences (See, e.g., B. J. Carter, in "Handbook of Parvoviruses", ed., P. Tijsser, CRC Press, pp. 155 168, 1990). Typical AAV ITR sequences are about 145 nucleotides in length. In some embodiments, at least 75% of a typical ITR sequence (e.g., at least 80%, at least 85%, at least 90%, or at least 95%) is incorporated into the AAV vector. The ability to modify these ITR sequences is within the skill of the art. (See, e.g., texts such as Sambrook et al., "Molecular Cloning. A Laboratory Manual", 2d ed., Cold Spring Harbor Laboratory, New York, 1989; and K. Fisher et al., *J Virol.* 70:520 532, 1996). In some embodiments, any of the coding sequences described herein is flanked by 5' and 3' AAV ITR sequences in the AAV vectors. The AAV ITR sequences may be obtained from any known AAV, including presently identified AAV types.

AAV vectors as described herein may include any of the regulatory elements described herein (e.g., one or more of a promoter, a polyadenylation (poly(A)) signal sequence, and an IRES).

In some embodiments, the AAV vector is selected from the group consisting of: an AAV1 vecotr, an AAV2 vector, an AAV3 vector, an AAV4 vector, an AAV5 vector, an AAV6 vector, an AAV7 vector, an AAV8 vector, an AAV9 vector, an AAV2.7m8

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vector, an AAV8BP2 vector, and an AAV293 vector. Additional exemplary AAV vectors that can be used herein are known in the art. See, e.g., Kanaan et al., *Mol. Ther. Nucleic Acids* 8:184-197, 2017; Li et al., *Mol. Ther.* 16(7): 1252-1260; Adachi et al., *Nat. Commun.* 5: 3075, 2014; Isgrig et al., *Nat. Commun.* 10(1): 427, 2019; and Gao et al., *J. Virol.* 78(12): 6381-6388.

In some embodiments, an AAV vector provided herein includes or consists of a sequence that is at least 80% identical (e.g., at least 82%, at least 84%, at least 85%, at least 86%, at least 88%, at least 90%, at least 92%, at least 94%, at least 95%, at least 96%, at least 98%, at least 99%, or 100% identical) to SEQ ID NO: 40, 41, 42, 43, 44, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59 or 60. In some embodiments, the vector(s) is an adenovirus (see, e.g., Dmitriev et al. (1998) *J. Virol.* 72: 9706-9713; and Poulin et al., *J. Virol* 8: 10074-10086, 2010). In some embodiments, the vector(s) is a retrovirus (see, e.g., Maier et al. (2010) *Future Microbiol* 5: 1507-23).

In some embodiments, the vector(s) is a lentivirus (see, e.g., Matrai et al. (2010) *Mol Ther.* 18: 477-490; Banasik et al. (2010) *Gene Ther.* 17:150-7; and Wanisch et al. (2009) *Mol. Ther.* 17: 1316-32). A lentiviral vector refers to a vector derived from at least a portion of a lentivirus genome, including especially a self-inactivating lentiviral vector as described in Milone et al., Mol. Ther. 17(8): 1453-1464 (2009). Non-limiting lentivirus vectors that may be used in the clinic include the LENTIVECTOR® gene delivery technology from Oxford BioMedica, the LENTIMAX™ vector system from Lentigen, and the like. Other types of lentiviral vectors are also available and would be known to one skilled in the art.

The vectors provided herein can be of different sizes. The choice of vector that is used in any of the compositions, kits, and methods described herein may depend on the size of the vector.

In some embodiments, the vector(s) is a plasmid and can include a total length of up to about 1 kb, up to about 2 kb, up to about 3 kb, up to about 4 kb, up to about 5 kb, up to about 6 kb, up to about 7 kb, up to about 8kb, up to about 9 kb, up to about 10 kb, up to about 11 kb, up to about 12 kb, up to about 13 kb, up to about 14 kb, or up to about 15 kb. In some embodiments, the vector(s) is a plasmid and can have a total length in a range of about 1 kb to about 2 kb, about 1 kb to about 3 kb, about 1 kb to about 4 kb,

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about 1 kb to about 5 kb, about 1 kb to about 6 kb, about 1 kb to about 7 kb, about 1 kb to about 8 kb, about 1 kb to about 9 kb, about 1 kb to about 10 kb, about 1 kb to about 11 kb, about 1 kb to about 12 kb, about 1 kb to about 13 kb, about 1 kb to about 14 kb, or about 1 kb to about 15 kb.

In some embodiments, the vector(s) is a transposon (e.g., PiggyBac™ transposon) and can include greater than 200 kb. In some examples, the vector(s) is a transposon having a total length in the range of about 1 kb to about 10 kb, about 1 kb to about 20 kb, about 1 kb to about 30 kb, about 1 kb to about 40 kb, about 1 kb to about 50 kb, about 1 kb to about 60 kb, about 1 kb to about 70 kb, about 1 kb to about 80 kb, about 1 kb to about 90 kb, about 10 kb to about 20 kb, about 10 kb to about 30 kb, about 10 kb to about 40 kb, about 10 kb to about 50 kb, about 10 kb to about 60 kb, about 10 kb to about 70 kb, about 10 kb to about 90 kb, about 10 kb to about 100 kb, about 20 kb to about 30 kb, about 20 kb to about 40 kb, about 20 kb to about 50 kb, about 20 kb to about 60 kb, about 20 kb to about 70 kb, about 20 kb to about 80 kb, about 20 kb to about 90 kb, about 20 kb to about 100 kb, about 30 kb to about 40 kb, about 30 kb to about 50 kb, about 30 kb to about 60 kb, about 30 kb to about 70 kb, about 30 kb to about 80 kb, about 30 kb to about 90 kb, about 30 kb to about 100 kb, about 40 kb to about 50 kb, about 40 kb to about 60 kb, about 40 kb to about 70 kb, about 40 kb to about 80 kb, about 40 kb to about 90 kb, about 40 kb to about 100 kb, about 50 kb to about 60 kb, about 50 kb to about 70 kb, about 50 kb to about 80 kb, about 50 kb to about 90 kb, about 50 kb to about 100 kb, about 60 kb to about 70 kb, about 60 kb to about 80 kb, about 60 kb to about 90 kb, about 60 kb to about 100 kb, about 70 kb to about 80 kb, about 70 kb to about 90 kb, about 70 kb to about 100 kb, about 80 kb to about 90 kb, about 80 kb to about 100 kb, about 90 kb to about 100 kb, about 1 kb to about 100 kb, about 100 kb to about 200 kb, about 100 kb to about 300 kb, about 100 kb to about 400 kb, or about 100 kb to about 500 kb.

In some embodiments, the vector is a cosmid and can have a total length of up to 55 kb. In some examples, the vector is a cosmid and has a total number of nucleotides of about 1 kb to about 10 kb, about 1 kb to about 20 kb, about 1 kb to about 30 kb, about 1 kb to about 40 kb, about 1 kb to about 50 kb, about 1 kb to about 55 kb, about 10 kb to about 20 kb, about 10 kb to about 30 kb, about 10 kb to about 40 kb, about 10 kb to about

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50 kb, about 10 kb to about 55 kb, about 15 kb to about 55 kb, about 15 kb to about 50 kb, about 15 kb to about 40 kb, about 15 kb to about 30 kb, about 15 kb to about 20 kb, about 20 kb to about 55 kb, about 20 kb to about 50 kb, about 20 kb to about 40 kb, about 20 kb to about 30 kb, about 25 kb to about 55 kb, about 25 kb to about 50 kb, about 25 kb to about 40 kb, about 25 kb to about 30 kb, about 30 kb to about 55 kb, about 30 kb to about 50 kb, about 30 kb to about 40 kb, about 35 kb to about 55 kb, about 40 kb to about 55 kb, about 40 kb to about 50 kb, or about 45 kb to about 55 kb.

In some embodiments, the vector(s) is an artificial chromosome and can have a total number of nucleotides of about 100 kb to about 2000 kb. In some embodiments, the artificial chromosome(s) is a human artificial chromosome (HAC) and can have a total number of nucleotides in the range of about 1 kb to about 10 kb, 1 kb to about 20 kb, about 1 kb to about 30 kb, about 1 kb to about 40 kb, about 1 kb to about 50 kb, about 1 kb to about 60 kb, about 10 kb to about 20 kb, about 10 kb to about 30 kb, about 10 kb to about 40 kb, about 10 kb to about 50 kb, about 10 kb to about 60 kb, about 20 kb to about 30 kb, about 20 kb to about 40 kb, about 20 kb to about 50 kb, about 20 kb to about 60 kb, about 30 kb to about 40 kb, about 30 kb to about 50 kb, about 30 kb to about 60 kb, about 40 kb to about 50 kb, about 40 kb to about 60 kb, or about 50 kb to about 60 kb.

In some embodiments, the artificial chromosome(s) is a yeast artificial chromosome (YAC) and can have a total number of nucleotides up to 1000 kb. In some embodiments, the articial chromosome(s) is a YAC having a total number of nucleotides in the range of about 100 kb to about 1,000 kb, about 100 kb to about 900 kb, about 100 kb to about 800 kb, about 100 kb to about 700 kb, about 100 kb to about 600 kb, about 100 kb to about 500 kb, about 100 kb to about 400 kb, about 100 kb to about 300 kb, about 100 kb to about 200 kb, about 200 kb to about 1,000 kb, about 200 kb to about 900 kb, about 200 kb to about 800 kb, about 200 kb to about 700 kb, about 200 kb to about 600 kb, about 200 kb to about 500 kb, about 200 kb to about 400 kb, about 200 kb to about 300 kb, about 300 kb to about 1,000 kb, about 300 kb to about 900 kb, about 300 kb to about 800 kb, about 300 kb to about 700 kb, about 300 kb to about 600 kb, about 300 kb to about 500 kb, about 300 kb to about 400 kb, about 400 kb to

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about 1,000 kb, about 400 kb to about 900 kb, about 400 kb to about 800 kb, about 400 kb to about 700 kb, about 400 kb to about 600 kb, about 400 kb to about 500 kb, about 500 kb to about 1,000 kb, about 500 kb to about 900 kb, about 500 kb to about 800 kb, about 500 kb to about 700 kb, about 500 kb to about 600 kb, about 600 kb to about 1,000 kb, about 600 kb to about 900 kb, about 600 kb to about 800 kb, about 600 kb to about 700 kb, about 700 kb to about 1,000 kb, about 700 kb to about 900 kb, about 700 kb to about 800 kb, about 800 kb to about 1,000 kb, about 800 kb to about 900 kb, or about 900 kb to about 1,000 kb.

In some embodiments, the artificial chromosome(s) is a bacterial artificial chromosome (BAC) and can have a total number of nucleotides of up to 750 kb. In some embodiments, the artificial chrosome(s) is a BAC and can have a total number of nucleotides in the range of about 100 kb to about 750 kb, about 100 kb to about 700 kb, about 100 kb to about 600 kb, about 100 kb to about 500 kb, about 100 kb to about 400 kb, about 100 kb to about 300 kb, about 100 kb to about 200 kb, about 150 kb to about 750 kb, about 150 kb to about 700 kb, about 150 kb to about 600 kb, about 150 kb to about 500 kb, about 150 kb to about 400 kb, about 150 kb to about 300 kb, about 150 kb to about 200 kb, about 200 kb to about 750 kb, about 200 kb to about 700 kb, about 200 kb to about 600 kb, about 200 kb to about 500 kb, about 200 kb to about 400 kb, about 200 kb to about 300 kb, about 250 kb to about 750 kb, about 250 kb to about 700 kb, about 250 kb to about 600 kb, about 250 kb to about 500 kb, about 250 kb to about 400 kb, about 250 kb to about 300 kb, about 300 kb to about 750 kb, about 300 kb to about 700 kb, about 300 kb to about 600 kb, about 300 kb to about 500 kb, about 300 kb to about 400 kb, about 350 kb to about 750 kb, about 350 kb to about 700 kb, about 350 kb to about 600 kb, about 350 kb to about 500 kb, about 350 kb to about 400 kb, about 400 kb to about 750 kb, about 400 kb to about 700 kb, about 450 kb to about 600 kb, about 450 kb to about 500 kb, about 500 kb to about 750 kb, about 500 kb to about 700 kb, about 500 kb to about 600 kb, about 550 kb to about 750 kb, about 550 kb to about 700 kb, about 550 kb to about 600 kb, about 600 kb to about 750 kb, about 600 kb to about 700 kb, or about 650 kb to about 750 kb.

In some embodiments, the artificial chromosome(s) is a P1-derived artificial chromosome (PAC) and can have a total number of nucleotides of up to 300 kb. In some

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embodiments, the P1-derived artificial chromosome(s) can have a total number of nucleotides in the range of about 100 kb to about 300 kb, about 100 kb to about 200 kb, or about 200 kb to about 300 kb.

In some embodiments, the vector(s) is a viral vector and can have a total number of nucleotides of up to 10 kb. In some embodiments, the viral vector(s) can have a total number of nucleotides in the range of about 1 kb to about 2 kb, 1 kb to about 3 kb, about 1 kb to about 4 kb, about 1 kb to about 5 kb, about 1 kb to about 6 kb, about 1 kb to about 7 kb, about 1 kb to about 8 kb, about 1 kb to about 9 kb, about 1 kb to about 10 kb, about 2 kb to about 3 kb, about 2 kb to about 4 kb, about 2 kb to about 5 kb, about 2 kb to about 6 kb, about 2 kb to about 7 kb, about 2 kb to about 8 kb, about 2 kb to about 9 kb, about 2 kb to about 10 kb, about 3 kb to about 4 kb, about 3 kb to about 5 kb, about 3 kb to about 6 kb, about 3 kb to about 7 kb, about 3 kb to about 8 kb, about 3 kb to about 9 kb, about 3 kb to about 10 kb, about 4 kb to about 5 kb, about 4 kb to about 6 kb, about 4 kb to about 7 kb, about 4 kb to about 8 kb, about 4 kb to about 9 kb, about 4 kb to about 10 kb, about 5 kb to about 6 kb, about 5 kb to about 7 kb, about 5 kb to about 8 kb, about 5 kb to about 9 kb, about 5 kb to about 10 kb, about 6 kb to about 7 kb, about 6 kb to about 8 kb, about 6 kb to about 9 kb, about 6 kb to about 10 kb, about 7 kb to about 8 kb, about 7 kb to about 9 kb, about 7 kb to about 10 kb, about 8 kb to about 9 kb, about 8 kb to about 10 kb, or about 9 kb to about 10 kb.

In some embodiments, the vector(s) is a lentivirus and can have a total number of nucleotides of up to 8 kb. In some examples, the lentivirus(es) can have a total number of nucleotides of about 1 kb to about 2 kb, about 1 kb to about 3 kb, about 1 kb to about 4 kb, about 1 kb to about 5 kb, about 1 kb to about 6 kb, about 1 kb to about 7 kb, about 1 kb to about 8 kb, about 2 kb to about 3 kb, about 2 kb to about 4 kb, about 2 kb to about 5 kb, about 2 kb to about 6 kb, about 2 kb to about 7 kb, about 2 kb to about 8 kb, about 3 kb to about 4 kb, about 3 kb to about 5 kb, about 3 kb to about 6 kb, about 3 kb to about 7 kb, about 3 kb to about 8 kb, about 4 kb to about 5 kb, about 4 kb to about 6 kb, about 4 kb to about 7 kb, about 4 kb to about 8 kb, about 5 kb to about 6 kb, about 5 kb to about 7 kb, about 5 kb to about 8 kb, about 6 kb to about 8kb, about 6 kb to about 7 kb, or about 7 kb to about 8 kb.

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In some embodiments, the vector(s) is an adenovirus and can have a total number of nucleotides of up to 8 kb. In some embodiments, the adenovirus(es) can have a total number of nucleotides in the range of about 1 kb to about 2 kb, about 1 kb to about 3 kb, about 1 kb to about 4 kb, about 1 kb to about 5 kb, about 1 kb to about 6 kb, about 1 kb to about 7 kb, about 1 kb to about 8 kb, about 2 kb to about 3 kb, about 2 kb to about 4 kb, about 2 kb to about 5 kb, about 2 kb to about 6 kb, about 2 kb to about 7 kb, about 2 kb to about 8 kb, about 3 kb to about 4 kb, about 3 kb to about 5 kb, about 3 kb to about 6 kb, about 3 kb to about 7 kb, about 3 kb to about 8 kb, about 4 kb to about 5 kb, about 4 kb to about 6 kb, about 4 kb to about 7 kb, about 4 kb to about 8 kb, about 5 kb to about 6 kb, about 5 kb to about 7 kb, about 5 kb to about 8 kb, about 6 kb to about 7 kh, about 6 kb to about 8 kb, or about 7 kb to about 8 kb.

In some embodiments, the vector(s) is an adeno-associated virus (AAV vector) and can include a total number of nucleotides of up to 5 kb. In some embodiments, the AAV vector(s) can include a total number of nucleotides in the range of about 1 kb to about 2 kb, about 1 kb to about 3 kb, about 1 kb to about 4 kb, about 1 kb to about 5 kb, about 2 kb to about 3 kb, about 2 kb to about 4 kb, about 2 kb to about 5kb, about 3 kb to about 4 kb, about 3 kb to about 5 kb, or about 4 kb to about 5 kb.

In some embodiments, the vector(s) is a Gateway® vector and can include a total number of nucleotides of up to 5 kb. In some embodiments, each Gateway® vector(s) includes a total number of nucleotides in the range of about 1 kb to about 2 kb, about 1 kb to about 3 kb, about 1 kb to about 4 kb, about 1 kb to about 5 kb, about 2 kb to about 3 kb, about 2 kb to about 4 kb, about 2 kb to about 5 kb, about 3 kb to about 4 kb, about 3 kb to about 5 kb, or about 4 kb to about 5 kb.

In some embodiments of any of the compositions, kits, and methods provided herein, the at least two different vectors can be substantially the same type of vector and may differ in size. In some embodiments, the at least two different vectors can be different types of vector, and may have substantially the same size or have different sizes.

In some embodiments, any of the at least two vectors can have a total number of nucleotides in the range of about 500 nucleotides to about 15,000 nucleotides, about 500 nucleotides to about 14,500 nucleotides, about 500 nucleotides to about 14,000 nucleotides, about 500 nucleotides to about 13,500 nucleotides, about 500 nucleotides to

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about 13,000 nucleotides, about 500 nucleotides to about 12,500 nucleotides, about 500 nucleotides to about 12,000 nucleotides, about 500 nucleotides to about 11,500 nucleotides, about 500 nucleotides to about 11,000 nucleotides, about 500 nucleotides to about 10,500 nucleotides, about 500 nucleotides to about 10,000 nucleotides, about 500 nucleotides to about 9,500 nucleotides, about 500 nucleotides to about 9,000 nucleotides, about 500 nucleotides to about 8,500 nucleotides, about 500 nucleotides to about 8,000 nucleotides, about 500 nucleotides to about 7,800 nucleotides, about 500 nucleotides to about 7,600 nucleotides, about 500 nucleotides to about 7,400 nucleotides, about 500 nucleotides to about 7,200 nucleotides, about 500 nucleotides to about 7,000 nucleotides, about 500 nucleotides to about 6,800 nucleotides, about 500 nucleotides to about 6,600 nucleotides, about 500 nucleotides to about 6,400 nucleotides, about 500 nucleotides to about 6,200 nucleotides, about 500 nucleotides to about 6,000 nucleotides, about 500 nucleotides to about 5,800 nucleotides, about 500 nucleotides to about 5,600 nucleotides, about 500 nucleotides to about 5,400 nucleotides, about 500 nucleotides to about 5,200 nucleotides, about 500 nucleotides to about 5,000 nucleotides, about 500 nucleotides to about 4,800 nucleotides, about 4,600 nucleotides, about 500 nucleotides to about 4,400 nucleotides, about 500 nucleotides to about 4,200 nucleotides, about 500 nucleotides to about 4,000 nucleotides, about 500 nucleotides to about 3,800 nucleotides, about 500 nucleotides to about 3,600 nucleotides, about 500 nucleotides to about 3,400 nucleotides, about 500 nucleotides to about 3,200 nucleotides, about 500 nucleotides to about 3,000 nucleotides, about 500 nucleotides to about 2,800 nucleotides, about 500 nucleotides to about 2,600 nucleotides, about 500 nucleotides to about 2,400 nucleotides, about 500 nucleotides to about 2,200 nucleotides, about 500 nucleotides to about 2,000 nucleotides, about 500 nucleotides to about 1,800 nucleotides, about 500 nucleotides to about 1,600 nucleotides, about 500 nucleotides to about 1,400 nucleotides, about 500 nucleotides to about 1,200 nucleotides, about 500 nucleotides to about 1,000 nucleotides, about 500 nucleotides to about 800 nucleotides, about 800 nucleotides to about 15,000 nucleotides, about 800 nucleotides to about 14,500 nucleotides, about 800 nucleotides to about 14,000 nucleotides, about 800 nucleotides to about 13,500 nucleotides, about 800 nucleotides to about 13,000 nucleotides, about 800 nucleotides to about 12,500 nucleotides, about 800 nucleotides to about 12,000 nucleotides, about 800 nucleotides to about 11,500

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Provided herein are exemplary vectors that can be used in any of the compositions and methods described herein. See, e.g., FIGs. 1-24.

A variety of different methods known in the art can be used to introduce any of vectors disclosed herein into a mammalian cell (e.g., a cochlear inner hair cell, a cochlear outer hair cell, a retinal cell). Non-limiting examples of methods for introducing nucleic acid into a mammalian cell include: lipofection, transfection (e.g., calcium phosphate transfection, transfection using highly branched organic compounds, transfection using cationic polymers, dendrimer-based transfection, optical transfection, particle-based transfection (e.g., nanoparticle transfection), or transfection using liposomes (e.g., cationic liposomes)), microinjection, electroporation, cell squeezing, sonoporation, protoplast fusion, impalefection, hydrodynamic delivery, gene gun, magnetofection, viral transfection, and nucleofection.

Skilled practitioners will appreciate that any of the vectors described herein can be introduced into a mammalian cell by, for example, lipofection, and can be stably integrated into an endogenous gene locus (e.g., a CLRN1 gene locus). In some embodiments, the vectors provided herein stably integrate into an endogenous defective CLRN1 gene locus, and thereby replace the defective CLRN1 gene with a nucleic acid encoding a functioning (e.g., wildtype) CLRN1 protein.

Various molecular biology techniques that can be used to introduce a mutation(s) and/or a deletion(s) into an endogenous gene are also known in the art. Non-limiting examples of such techniques include site-directed mutagenesis, CRISPR (e.g., CRISPR/Cas9-induced knock-in mutations and CRISPR/Cas9-induced knock-out mutations), and TALENs. These methods can be used to correct the sequence of a defective endogenous gene present in a chromosome of a target cell.

Any of the vectors described herein can further include a control sequence, e.g., a control sequence selected from the group of a transcription initiation sequence, a transcription termination sequence, a promoter sequence, an enhancer sequence, an RNA splicing sequence, a polyadenylation (polyA) signal, and a Kozak consensus sequence. Non-limiting examples of these control sequences are described herein. In some embodiments, a promoter can be a native promoter, a constitutive promoter, an inducible promoter, and/or a tissue-specific promoter.

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Promoters

The term "promoter" means a DNA sequence recognized by enzymes/proteins in a mammalian cell required to initiate the transcription of a specific gene (e.g., a CLRN1 gene). A promoter typically refers to, e.g., a nucleotide sequence to which an RNA polymerase and/or any associated factor binds and at which transcription is initiated. Non-limiting examples of promoters are described herein. Additional examples of promoters are known in the art.

In some embodiments, a vector encoding an N-terminal portion of a CLRN1 protein (e.g., a human CLRN1 protein) can include a promoter and/or an enhancer. The vector encoding the N-terminal portion of the CLRN1 protein can include any of the promoters and/or enhancers described herein or known in the art.

In some embodiments, the promoter is an inducible promoter, a constitutive promoter, a mammalian cell promoter, a viral promoter, a chimeric promoter, an engineered promoter, a tissue-specific promoter, or any other type of promoter known in the art. In some embodiments, the promoter is a RNA polymerase II promoter, such as a mammalian RNA polymerase II promoter. In some embodiments, the promoter is a RNA polymerase III promoter, including, but not limited to, a H1 promoter, a human U6 promoter, a mouse U6 promoter, or a swine U6 promoter. The promoter will generally be one that is able to promote transcription in an inner hair cell In some examples, the promoter is a cochlea-specific promoter or a cochlea-oriented promoter.

A variety of promoters are known in the art that can be used herein. Non-limiting examples of promoters that can be used herein include: human EF1a, human cytomegalovirus (CMV) (US Patent No. 5,168,062), human ubiquitin C (UBC), mouse phosphoglycerate kinase 1, polyoma adenovirus, simian virus 40 (SV40), β-globin, β-actin, α-fetoprotein, γ-globin, β-interferon, γ-glutamyl transferase, mouse mammary tumor virus (MMTV), Rous sarcoma virus, rat insulin, glyceraldehyde-3-phosphate dehydrogenase, metallothionein II (MT II), amylase, cathepsin, MI muscarinic receptor, retroviral LTR (e.g. human T-cell leukemia virus HTLV), AAV ITR, interleukin-2, collagenase, platelet-derived growth factor, adenovirus 5 E2, stromelysin, murine MX gene, glucose regulated proteins (GRP78 and GRP94), α-2-macroglobulin, vimentin, MHC class I gene H-2κ b, HSP70, proliferin, tumor necrosis factor, thyroid stimulating

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hormone α gene, immunoglobulin light chain, T-cell receptor, HLA DQα and DQβ, interleukin-2 receptor, MHC class II, MHC class II HLA-DRα, muscle creatine kinase, prealbumin (transthyretin), elastase I, albumin gene, c-fos, c-HA-ras, neural cell adhesion molecule (NCAM), H2B (TH2B) histone, rat growth hormone, human serum amyloid (SAA), troponin I (TN I), duchenne muscular dystrophy, human immunodeficiency virus, and Gibbon Ape Leukemia Virus (GALV) promoters. Additional examples of promoters are known in the art. See, e.g., Lodish, Molecular Cell Biology, Freeman and Company, New York 2007. In some embodiments, the promoter is the CMV immediate early promoter. In some embodiments, the promoter is a CAG promoter or a CAG/CBA promoter. In some embodiments, the promoter is a CBA promoter, e.g., a CBA promoter comprising or consisting of SEQ ID NO: 18.

The term "constitutive" promoter refers to a nucleotide sequence that, when operably linked with a nucleic acid encoding a protein (e.g., a CLRN1 protein), causes RNA to be transcribed from the nucleic acid in a mammalian cell under most or all physiological conditions.

Examples of constitutive promoters include, without limitation, the retroviral Rous sarcoma virus (RSV) LTR promoter, the cytomegalovirus (CMV) promoter (see, e.g., Boshart et al, *Cell* 41:521-530, 1985), the SV40 promoter, the dihydrofolate reductase promoter, the beta-actin promoter, the phosphoglycerol kinase (PGK) promoter, and the EF1-alpha promoter (Invitrogen).

Inducible promoters allow regulation of gene expression and can be regulated by exogenously supplied compounds, environmental factors such as temperature, or the presence of a specific physiological state, e.g., acute phase, a particular differentiation state of the cell, or in replicating cells only. Inducible promoters and inducible systems are available from a variety of commercial sources, including, without limitation, Invitrogen, Clontech, and Ariad. Additional examples of inducible promoters are known in the art.

Examples of inducible promoters regulated by exogenously supplied compounds include the zinc-inducible sheep metallothionine (MT) promoter, the dexamethasone (Dex)-inducible mouse mammary tumor virus (MMTV) promoter, the T7 polymerase promoter system (WO 98/10088); the ecdysone insect promoter (No et al, *Proc. Natl.*

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Acad. Sci. U.S.A. 93:3346-3351, 1996), the tetracycline-repressible system (Gossen et al, *Proc. Natl. Acad. Sci. U.S.A.* 89:5547-5551, 1992), the tetracycline-inducible system (Gossen et al, *Science* 268:1766-1769, 1995, see also Harvey et al, *Curr. Opin. Chem. Biol.* 2:512-518, 1998), the RU486-inducible system (Wang et al, *Nat. Biotech.* 15:239-243, 1997) and Wang et al, *Gene Ther.* 4:432-441, 1997), and the rapamycin-inducible system (Magari et al. *J. Clin. Invest.* 100:2865-2872, 1997).

The term "tissue-specific" promoter refers to a promoter that is active only in certain specific cell types and/or tissues (e.g., transcription of a specific gene occurs only within cells expressing transcription regulatory proteins that bind to the tissue-specific promoter).

In some embodiments, the regulatory sequences impart tissue-specific gene expression capabilities. In some cases, the tissue-specific regulatory sequences bind tissue-specific transcription factors that induce transcription in a tissue-specific manner.

Exemplary tissue-specific promoters include but are not limited to the following: a liver-specific thyroxin binding globulin (TBG) promoter, an insulin promoter, a glucagon promoter, a somatostatin promoter, a pancreatic polypeptide (PPY) promoter, a synapsin-1 (Syn) promoter, a creatine kinase (MCK) promoter, a mammalian desmin (DES) promoter, an alpha-myosin heavy chain (a-MHC) promoter, and a cardiac Troponin T (cTnT) promoter. Additional exemplary promoters include Beta-actin promoter, hepatitis B virus core promoter (Sandig et al., *Gene Ther.* 3:1002-1009, 1996), alpha-fetoprotein (AFP) promoter (Arbuthnot et al., *Hum. Gene Ther.* 7:1503-1514, 1996), bone osteocalcin promoter (Stein et al., *Mol. Biol. Rep.* 24:185-196, 1997); bone sialoprotein promoter (Chen et al., *J. Bone Miner. Res.* 11:654-664, 1996), CD2 promoter (Hansal et al., *J. Immunol.* 161:1063-1068, 1998); immunoglobulin heavy chain promoter; T cell receptor alpha-chain promoter, neuronal such as neuron-specific enolase (NSE) promoter (Andersen et al., *Cell. Mol. Neurobiol.* 13:503-515, 1993), neurofilament light-chain gene promoter (Piccioli et al., *Proc. Natl. Acad. Sci. U.S.A.* 88:5611-5615, 1991), and the neuron-specific vgf gene promoter (Piccioli et al., *Neuron* 15:373-384, 1995).

In some embodiments, the tissue-specific promoter is a cochlea-specific promoter. In some embodiments, the tissue-specific promoter is a cochlear hair cell-specific

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promoter. Non-limiting examples of cochlear hair cell-specific promoters include but are not limited to: a ATOH1 promoter, a POU4F3 promoter, a LHX3 promoter, a MYO7A promoter, a MYO6 promoter, a α9ACHR promoter, and a α10ACHR promoter. In some embodiments, the promoter is an cochlear hair cell-specific promoter such as a PRESTIN promoter or an ONCOMOD promoter. See, e.g., Zheng et al., *Nature* 405:149-155, 2000; Tian et al. *Dev. Dyn.* 231:199-203, 2004; and Ryan et al., *Adv. Otorhinolaryngol*. 66: 99-115, 2009.

Enhancers

In some instances, a vector can include an enhancer sequence. The term "enhancer" refers to a nucleotide sequence that can increase the level of transcription of a nucleic acid encoding a protein of interest (e.g., a CLRN1 protein). Enhancer sequences (50-1500 basepairs in length) generally increase the level of transcription by providing additional binding sites for transcription-associated proteins (e.g., transcription factors). In some embodiments, an enhancer sequence is found within an intronic sequence. Unlike promoter sequences, enhancer sequences can act at much larger distance away from the transcription start site (e.g., as compared to a promoter). Non-limiting examples of enhancers include a RSV enhancer, a CMV enhancer, and a SV40 enhancer. In some embodiments, the CMV enhancer sequence comprises or consists of SEQ ID NO: 17.

Poly(A) Signal

In some embodiments, any of the vectors provided herein can include a polyadenylation (poly(A)) signal sequence. Most nascent eukaryotic mRNAs possess a poly(A) tail at their 3' end which is added during a complex process that includes cleavage of the primary transcript and a coupled polyadenylation reaction driven by the poly(A) signal sequence (see, e.g., Proudfoot et al., *Cell* 108:501-512, 2002). The poly(A) tail confers mRNA stability and transferability (Molecular Biology of the Cell, Third Edition by B. Alberts et al., Garland Publishing, 1994). In some embodiments, the poly(A) signal sequence is positioned 3' to the nucleic acid sequence encoding the C-terminus of the CLRN1 protein.

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As used herein, “polyadenylation” refers to the covalent linkage of a polyadenylyl moiety, or its modified variant, to a messenger RNA molecule. In eukaryotic organisms, most messenger RNA (mRNA) molecules are polyadenylated at the 3' end. The 3' poly(A) tail is a long sequence of adenine nucleotides (e.g., 50, 60, 70, 100, 200, 500, 1000, 2000, 3000, 4000, or 5000) added to the pre-mRNA through the action of an enzyme, polyadenylate polymerase. In higher eukaryotes, the poly(A) tail is added onto transcripts that contain a specific sequence, the polyadenylation (or poly(A)) signal. The poly(A) tail and the protein bound to it aid in protecting mRNA from degradation by exonucleases. Polyadenylation is also important for transcription termination, export of the mRNA from the nucleus, and translation. Polyadenylation occurs in the nucleus immediately after transcription of DNA into RNA, but also can occur later in the cytoplasm. After transcription has been terminated, the mRNA chain is cleaved through the action of an endonuclease complex associated with RNA polymerase. The cleavage site is usually characterized by the presence of the base sequence AAUAAA near the cleavage site. After the mRNA has been cleaved, adenosine residues are added to the free 3' end at the cleavage site.

As used herein, a “poly(A) signal sequence” or “polyadenylation signal sequence” is a sequence that triggers the endonuclease cleavage of an mRNA and the addition of a series of adenosines to the 3’ end of the cleaved mRNA.

There are several poly(A) signal sequences that can be used, including those derived from bovine growth hormone (bgh) (Woychik et al., *Proc. Natl. Acad. Sci. U.S.A.* 81(13):3944-3948, 1984; U.S. Patent No. 5,122,458), mouse-β-globin, mouse-α-globin (Orkin et al., *EMBO J.* 4(2):453-456, 1985; Thein et al., *Blood* 71(2):313-319, 1988), human collagen, polyoma virus (Batt et al., *Mol. Cell Biol.* 15(9):4783-4790, 1995), the Herpes simplex virus thymidine kinase gene (HSV TK), IgG heavy-chain gene polyadenylation signal (US 2006/0040354), human growth hormone (hGH) (Szymanski et al., *Mol. Therapy* 15(7):1340-1347, 2007), the group consisting of SV40 poly(A) site, such as the SV40 late and early poly(A) site (Schek et al., *Mol. Cell Biol.* 12(12):5386-5393, 1992).

The poly(A) signal sequence can be AATAAA. The AATAAA sequence may be substituted with other hexanucleotide sequences with homology to AATAAA and that

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are capable of signaling polyadenylation, including ATTAAA, AGTAAA, CATAAA, TATAAA, GATAAA, ACTAAA, AATATA, AAGAAA, AATAAT, AAAAAA, AATGAA, AATCAA, AACAAA, AATCAA, AATAAC, AATAGA, AATTAA, or AATAAG (see, e.g., WO 06/12414).

In some embodiments, the poly(A) signal sequence can be a synthetic polyadenylation site (see, e.g., the pCl-neo expression vector of Promega that is based on Levitt el al, *Genes Dev.* 3(7):1019-1025, 1989). In some embodiments, the poly(A) signal sequence is the polyadenylation signal of bovine growth hormone (CTGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGCCCCTCCCCCGTGCCTTCC TTGACCCTGGAAGGTGCCACTCCCACTGTCCTTTCCTAATAAAATGAGGAAAT TGCATCGCATTGTCTGAGTAGGTGTCATTCTATTCTGGGGGGTGGGGTGGGGC AGGACAGCAAGGGGGAGGATTGGGAAGACAATAGCAGGCATGCTGGGGATG CGGTGGGCTCTATGG (SEQ ID NO: 20)). In some embodiments, the poly(A) signal sequence is the polyadenylation signal of soluble neuropilin-1 (sNRP) (AAATAAAATACGAAATG (SEQ ID NO: 21)) (see, e.g., WO 05/073384). Additional examples of poly(A) signal sequences are known in the art.

Internal Ribosome Entry Site (IRES)

In some embodiments, a vector encoding the C-terminal portion of the CLRN1 protein can include a polynucleotide internal ribosome entry site (IRES). An IRES sequence is used to produce more than one polypeptide from a single gene transcript. An IRES forms a complex secondary structure that allows translation initiation to occur from any position with an mRNA immediately downstream from where the IRES is located (see, e.g., Pelletier and Sonenberg, *Mol. Cell. Biol.* 8(3):1103-1112, 1988).

There are several IRES sequences known to those in skilled in the art, including those from, e.g., foot and mouth disease virus (FMDV), encephalomyocarditis virus (EMCV), human rhinovirus (HRV), cricket paralysis virus, human immunodeficiency virus (HIV), hepatitis A virus (HAV), hepatitis C virus (HCV), and poliovirus (PV). See e.g., Alberts, Molecular Biology of the Cell, Garland Science, 2002; and Hellen et al., *Genes Dev.* 15(13):1593-612, 2001.

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In some embodiments, the IRES sequence that is incorporated into the vector that encodes the C-terminal portion of a CLRN1 protein is the foot and mouth diseause virus (FMDV) 2A sequence. The Foot and Mouth Disease Virus 2A sequence is a small peptide (approximately 18 amino acids in length) that has been shown to mediate the cleavage of polyproteins (Ryan, M D et al., *EMBO* 4:928-933, 1994; Mattion et al., *J. Virology* 70:8124-8127, 1996; Furler et al., *Gene Therapy* 8:864-873, 2001; and Halpin et al., *Plant Journal* 4:453-459, 1999). The cleavage activity of the 2A sequence has previously been demonstrated in artificial systems including plasmids and gene therapy vectors (AAV and retroviruses) (Ryan et al., *EMBO* 4:928-933, 1994; Mattion et al., *J. Virology* 70:8124-8127, 1996; Furler et al., *Gene Therapy* 8:864-873, 2001; and Halpin et al., *Plant Journal* 4:453-459, 1999; de Felipe et al., *Gene Therapy* 6:198-208, 1999; de Felipe et al., *Human Gene Therapy* 11:1921-1931, 2000; and Klump et al., *Gene Therapy* 8:811-817, 2001).

Reporter Sequences

Any of the vectors provided herein can optionally include a sequence encoding a reporter protein ("a reporter sequence"). Non-limiting examples of reporter sequences include DNA sequences encoding: a beta-lactamase, a beta-galactosidase (LacZ), an alkaline phosphatase, a thymidine kinase, a green fluorescent protein (GFP), a red fluorescent protein, an mCherry fluorescent protein, a yellow fluorescent protein, a chloramphenicol acetyltransferase (CAT), and a luciferase. Additional examples of reporter sequences are known in the art. When associated with regulatory elements which drive their expression, the reporter sequence can provide signals detectable by conventional means, including enzymatic, radiographic, colorimetric, fluorescence, or other spectrographic assays; fluorescent activating cell sorting (FACS) assays; immunological assays (e.g., enzyme linked immunosorbent assay (ELISA), radioimmunoassay (RIA), and immunohistochemistry).

In some embodiments, the reporter sequence is tGFP (SEQ ID NO: 19). In some embodiments, the reporter sequence is the LacZ gene, and the presence of a vector carrying the LacZ gene in a mammalian cell (e.g., a cochlear hair cell, an ocular cell, such as a retinal cell) is detected by assays for beta-galactosidase activity. When the

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reporter is a fluorescent protein (e.g., green fluorescent protein) or luciferase, the presence of a vector carrying the fluorescent protein or luciferase in a mammalian cell (e.g., a cochlear hair cell, an ocular cell, such as a retinal cell) may be measured by fluorescent techniques (e.g., fluorescent microscopy or FACS) or light production in a luminometer (e.g., a spectrophotometer or an IVIS imaging instrument). In some embodiments, the reporter sequence can be used to verify the tissue-specific targeting capabilities and tissue-specific promoter regulatory activity of any of the vectors described herein.

Flanking Regions Untranslated Regions (UTRs)

In some embodiments, any of the vectors described herein (e.g., any of the at least two different vectors) can include an untranslated region, such as a 5’ UTR or a 3’ UTR.

Untranslated regions (UTRs) of a gene are transcribed but not translated. The 5' UTR starts at the transcription start site and continues to the start codon but does not include the start codon. The 3' UTR starts immediately following the stop codon and continues until the transcriptional termination signal. There is growing body of evidence about the regulatory roles played by the UTRs in terms of stability of the nucleic acid molecule and translation. The regulatory features of a UTR can be incorporated into any of the vectors, compositions, kits, or methods as described herein to enhance the expression of a CLRN1 protein.

Natural 5' UTRs include a sequence that plays a role in translation initiation. They harbor signatures like Kozak sequences, which are commonly known to be involved in the process by which the ribosome initiates translation of many genes. Kozak sequences have the consensus sequence CCR(A/G)CCAUGG, where R is a purine (A or G) three bases upstream of the start codon (AUG), and the start codon is followed by another “G”. The 5' UTRs have also been known to form secondary structures that are involved in elongation factor binding.

In some embodiments, a 5’ UTR is included in any of the vectors described herein. Non-limiting examples of 5’ UTRs, including those from the following genes: albumin, serum amyloid A, Apolipoprotein A/B/E, transferrin, alpha fetoprotein,

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erythropoietin, and Factor VIII, can be used to enhance expression of a nucleic acid molecule, such as a mRNA.

In some embodiments, a 5’ UTR from a mRNA that is transcribed by a cell in the cochlea or retina can be included in any of the vectors, compositions, kits, and methods described herein.

3' UTRs are known to have stretches of adenosines and uridines (in the RNA form) or thymidines (in the DNA form) embedded in them. These AU-rich signatures are particularly prevalent in genes with high rates of turnover. Based on their sequence features and functional properties, the AU-rich elements (AREs) can be separated into three classes (Chen et al., *Mol. Cell. Biol.* 15:5777-5788, 1995; Chen et al., *Mol. Cell Biol.* 15:2010-2018, 1995): Class I AREs contain several dispersed copies of an AUUUA motif within U-rich regions. For example, c-Myc and MyoD mRNAs contain class I AREs. Class II AREs possess two or more overlapping UUAUUUA(U/A) (U/A) nonamers. GM-CSF and TNF-alpha mRNAs are examples that contain class II AREs. Class III AREs are less well defined. These U-rich regions do not contain an AUUUA motif. Two well-studied examples of this class are c-Jun and myogenin mRNAs.

Most proteins binding to the AREs are known to destabilize the messenger, whereas members of the ELAV family, most notably HuR, have been documented to increase the stability of mRNA. HuR binds to AREs of all the three classes. Engineering the HuR specific binding sites into the 3' UTR of nucleic acid molecules will lead to HuR binding and thus, stabilization of the message *in vivo*.

An exemplary human wildtype 5’ UTR is or includes the sequence of SEQ ID NO: 12 or SEQ ID NO: 13. An exemplary human wildtype 5’ UTR is or includes the sequence of SEQ ID NO: 14 or SEQ ID NO: 15.

In some embodiments of any of the compositions described herein, a 5’ untranslated region (UTR), a 3’ UTR, or both are included in a vector (e.g., any of the vectors described herein). For example, any of the 5’-UTRs described herein can be operatively linked to the start codon in any of the coding sequences described herein. For example, any of the 3’-UTR’s can be operately linked to the 3’-terminal codon (last codon) in any of the coding sequences described herein.

In some embodiments of any of the compositions described herein, the 5’ UTR

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comprises at least 10 contiguous (e.g., at least 15 contiguous, at least 20 contiguous, at least 25 contiguous, at least 30 contiguous, at least 35 contiguous, at least 40 contiguous, at least 45 contiguous, at least 50 contiguous, at least 55 contiguous, at least 60 contiguous, at least 65 contiguous, at least 70 contiguous, at least 75 contiguous, at least 80 contiguous, at least 85 contiguous, at least 90 contiguous, at least 100 contiguous, at least 105 contiguous, at least 110 contiguous, at least 115 contiguous, at least 120 contiguous, at least 125 contiguous, atleast 130 contiguous, at least 135 contiguous, at least 140 contiguous, at least 145 contiguous, at least 150 contiguous, at least 155 contiguous, at least 160 contiguous, at least 165 contiguous, at least 170 contiguous, at least 175 contiguous, at least 180 contiguous, at least 185 contiguous, at least 190 contiguous, at least 195 contiguous, at least 200 contiguous, at least 205 contiguous, at least 210 contiguous, at least 215 contiguous, at least 220 contiguous, at least 225 contiguous, at least 230 contiguous, at least 235 contiguous, at least 240 contiguous, at least 245 contiguous, at least 250 contiguous, at least 255 contiguous, or at least 260 contiguous) nucleotides from anywhere within SEQ ID NO: 12 or SEQ ID NO: 13.

For example, a 5' UTR can include or consist of one or more of: nucleotide positions 1 to 291, nucleotide positions 1 to 290, nucleotide positions 1 to 280, nucleotide positions 1 to 270, nucleotide positions 1 to 260, nucleotide positions 1 to 250, nucleotide positions 1 to 240, nucleotide positions 1 to 230, nucleotide positions 1 to 220, nucleotide positions 1 to 210, nucleotide positions 1 to 200, nucleotide positions 1 to 190, nucleotide positions 1 to 180, nucleotide positions 1 to 170, nucleotide positions 1 to 160, nucleotide positions 1 to 150, nucleotide positions 1 to 140, nucleotide positions 1 to 130, nucleotide positions 1 to 120, nucleotide positions 1 to 110, nucleotide positions 1 to 100, nucleotide positions 1 to 90, nucleotide positions 1 to 80, nucleotide positions 1 to 70, nucleotide positions 1 to 60, nucleotide positions 1 to 50, nucleotide positions 1 to 40, nucleotide positions 1 to 30, nucleotide positions 1 to 20, nucleotide positions 1 to 10, nucleotide positions 10 to 291, nucleotide positions 10 to 290, nucleotide positions 10 to 280, nucleotide positions 10 to 270, nucleotide positions 10 to 260, nucleotide positions 10 to 250, nucleotide positions 10 to 240, nucleotide positions 10 to 230, nucleotide positions 10 to 220, nucleotide positions 10 to 210, nucleotide positions 10 to 200, nucleotide positions 10 to 190, nucleotide positions 10 to 180, nucleotide positions 10 to 170,

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nucleotide positions 10 to 160, nucleotide positions 10 to 150, nucleotide positions 10 to 140, nucleotide positions 10 to 130, nucleotide positions 10 to 120, nucleotide positions 10 to 110, nucleotide positions 10 to 100, nucleotide positions 10 to 90, nucleotide positions 10 to 80, nucleotide positions 10 to 70, nucleotide positions 10 to 60, nucleotide positions 10 to 50, nucleotide positions 10 to 40, nucleotide positions 10 to 30, nucleotide positions 10 to 20, nucleotide positions 20 to 291, nucleotide positions 20 to 290, nucleotide positions 20 to 280, nucleotide positions 20 to 270, nucleotide positions 20 to 260, nucleotide positions 20 to 250, nucleotide positions 20 to 240, nucleotide positions 20 to 230, nucleotide positions 20 to 220, nucleotide positions 20 to 210, nucleotide positions 20 to 200, nucleotide positions 20 to 190, nucleotide positions 20 to 180, nucleotide positions 20 to 170, nucleotide positions 20 to 160, nucleotide positions 20 to 150, nucleotide positions 20 to 140, nucleotide positions 20 to 130, nucleotide positions 20 to 120, nucleotide positions 20 to 110, nucleotide positions 20 to 100, nucleotide positions 20 to 90, nucleotide positions 20 to 80, nucleotide positions 20 to 70, nucleotide positions 20 to 60, nucleotide positions 20 to 50, nucleotide positions 20 to 40, nucleotide positions 20 to 30, nucleotide positions 30 to 291, nucleotide positions 30 to 290, nucleotide positions 30 to 280, nucleotide positions 30 to 270, nucleotide positions 30 to 260, nucleotide positions 30 to 250, nucleotide positions 30 to 240, nucleotide positions 30 to 230, nucleotide positions 30 to 220, nucleotide positions 30 to 210, nucleotide positions 30 to 200, nucleotide positions 30 to 190, nucleotide positions 30 to 180, nucleotide positions 30 to 170, nucleotide positions 30 to 160, nucleotide positions 30 to 150, nucleotide positions 30 to 140, nucleotide positions 30 to 130, nucleotide positions 30 to 120, nucleotide positions 30 to 110, nucleotide positions 30 to 100, nucleotide positions 30 to 90, nucleotide positions 30 to 80, nucleotide positions 30 to 70, nucleotide positions 30 to 60, nucleotide positions 30 to 50, nucleotide positions 30 to 40, nucleotide positions 40 to 291, nucleotide positions 40 to 290, nucleotide positions 40 to 280, nucleotide positions 40 to 270, nucleotide positions 40 to 260, nucleotide positions 40 to 250, nucleotide positions 40 to 240, nucleotide positions 40 to 230, nucleotide positions 40 to 220, nucleotide positions 40 to 210, nucleotide positions 40 to 200, nucleotide positions 40 to 190, nucleotide positions 40 to 180, nucleotide positions 40 to 170, nucleotide positions 40 to 160, nucleotide positions 40 to 150, nucleotide positions 40 to

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140, nucleotide positions 40 to 130, nucleotide positions 40 to 120, nucleotide positions 40 to 110, nucleotide positions 40 to 100, nucleotide positions 40 to 90, nucleotide positions 40 to 80, nucleotide positions 40 to 70, nucleotide positions 40 to 60, nucleotide positions 40 to 50, nucleotide positions 50 to 291, nucleotide positions 50 to 290, nucleotide positions 50 to 280, nucleotide positions 50 to 270, nucleotide positions 50 to 260, nucleotide positions 50 to 250, nucleotide positions 50 to 240, nucleotide positions 50 to 230, nucleotide positions 50 to 220, nucleotide positions 50 to 210, nucleotide positions 50 to 200, nucleotide positions 50 to 190, nucleotide positions 50 to 180, nucleotide positions 50 to 170, nucleotide positions 50 to 160, nucleotide positions 50 to 150, nucleotide positions 50 to 140, nucleotide positions 50 to 130, nucleotide positions 50 to 120, nucleotide positions 50 to 110, nucleotide positions 50 to 100, nucleotide positions 50 to 90, nucleotide positions 50 to 80, nucleotide positions 50 to 70, nucleotide positions 50 to 60, nucleotide positions 60 to 291, nucleotide positions 60 to 290, nucleotide positions 60 to 280, nucleotide positions 60 to 270, nucleotide positions 60 to 260, nucleotide positions 60 to 250, nucleotide positions 60 to 240, nucleotide positions 60 to 230, nucleotide positions 60 to 220, nucleotide positions 60 to 210, nucleotide positions 60 to 200, nucleotide positions 60 to 190, nucleotide positions 60 to 180, nucleotide positions 60 to 170, nucleotide positions 60 to 160, nucleotide positions 60 to 150, nucleotide positions 60 to 140, nucleotide positions 60 to 130, nucleotide positions 60 to 120, nucleotide positions 60 to 110, nucleotide positions 60 to 100, nucleotide positions 60 to 90, nucleotide positions 60 to 80, nucleotide positions 60 to 70, nucleotide positions 70 to 291, nucleotide positions 70 to 290, nucleotide positions 70 to 280, nucleotide positions 70 to 270, nucleotide positions 70 to 260, nucleotide positions 70 to 250, nucleotide positions 70 to 240, nucleotide positions 70 to 230, nucleotide positions 70 to 220, nucleotide positions 70 to 210, nucleotide positions 70 to 200, nucleotide positions 70 to 190, nucleotide positions 70 to 180, nucleotide positions 70 to 170, nucleotide positions 70 to 160, nucleotide positions 70 to 150, nucleotide positions 70 to 140, nucleotide positions 70 to 130, nucleotide positions 70 to 120, nucleotide positions 70 to 110, nucleotide positions 70 to 100, nucleotide positions 70 to 90, nucleotide positions 70 to 80, nucleotide positions 80 to 291, nucleotide positions 80 to 290, nucleotide positions 80 to 280, nucleotide positions 80 to 270, nucleotide positions 80 to

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260, nucleotide positions 80 to 250, nucleotide positions 80 to 240, nucleotide positions 80 to 230, nucleotide positions 80 to 220, nucleotide positions 80 to 210, nucleotide positions 80 to 200, nucleotide positions 80 to 190, nucleotide positions 80 to 180, nucleotide positions 80 to 170, nucleotide positions 80 to 160, nucleotide positions 80 to 150, nucleotide positions 80 to 140, nucleotide positions 80 to 130, nucleotide positions 80 to 120, nucleotide positions 80 to 110, nucleotide positions 80 to 100, nucleotide positions 80 to 90, nucleotide positions 90 to 291, nucleotide positions 90 to 290, nucleotide positions 90 to 280, nucleotide positions 90 to 270, nucleotide positions 90 to 260, nucleotide positions 90 to 250, nucleotide positions 90 to 240, nucleotide positions 90 to 230, nucleotide positions 90 to 220, nucleotide positions 90 to 210, nucleotide positions 90 to 200, nucleotide positions 90 to 190, nucleotide positions 90 to 180, nucleotide positions 90 to 170, nucleotide positions 90 to 160, nucleotide positions 90 to 150, nucleotide positions 90 to 140, nucleotide positions 90 to 130, nucleotide positions 90 to 120, nucleotide positions 90 to 110, nucleotide positions 90 to 100, nucleotide positions 100 to 291, nucleotide positions 100 to 290, nucleotide positions 100 to 280, nucleotide positions 100 to 270, nucleotide positions 100 to 260, nucleotide positions 100 to 250, nucleotide positions 100 to 240, nucleotide positions 100 to 230, nucleotide positions 100 to 220, nucleotide positions 100 to 210, nucleotide positions 100 to 200, nucleotide positions 100 to 190, nucleotide positions 100 to 180, nucleotide positions 100 to 170, nucleotide positions 100 to 160, nucleotide positions 100 to 150, nucleotide positions 100 to 140, nucleotide positions 100 to 130, nucleotide positions 100 to 120, nucleotide positions 100 to 110, nucleotide positions 110 to 291, nucleotide positions 110 to 290, nucleotide positions 110 to 280, nucleotide positions 110 to 270, nucleotide positions 110 to 260, nucleotide positions 110 to 250, nucleotide positions 110 to 240, nucleotide positions 110 to 230, nucleotide positions 110 to 220, nucleotide positions 110 to 210, nucleotide positions 110 to 200, nucleotide positions 110 to 190, nucleotide positions 110 to 180, nucleotide positions 110 to 170, nucleotide positions 110 to 160, nucleotide positions 110 to 150, nucleotide positions 110 to 140, nucleotide positions 110 to 130, nucleotide positions 110 to 120, nucleotide positions 120 to 291, nucleotide positions 120 to 290, nucleotide positions 120 to 280, nucleotide positions 120 to 270, nucleotide positions 120 to 260, nucleotide positions 120 to 250, nucleotide positions 120

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to 240, nucleotide positions 120 to 230, nucleotide positions 120 to 220, nucleotide positions 120 to 210, nucleotide positions 120 to 200, nucleotide positions 120 to 190, nucleotide positions 120 to 180, nucleotide positions 120 to 170, nucleotide positions 120 to 160, nucleotide positions 120 to 150, nucleotide positions 120 to 140, nucleotide positions 120 to 130, nucleotide positions 130 to 291, nucleotide positions 130 to 290, nucleotide positions 130 to 280, nucleotide positions 130 to 270, nucleotide positions 130 to 260, nucleotide positions 130 to 250, nucleotide positions 130 to 240, nucleotide positions 130 to 230, nucleotide positions 130 to 220, nucleotide positions 130 to 210, nucleotide positions 130 to 200, nucleotide positions 130 to 190, nucleotide positions 130 to 180, nucleotide positions 130 to 170, nucleotide positions 130 to 160, nucleotide positions 130 to 150, nucleotide positions 130 to 140, nucleotide positions 140 to 291, nucleotide positions 140 to 290, nucleotide positions 140 to 280, nucleotide positions 140 to 270, nucleotide positions 140 to 260, nucleotide positions 140 to 250, nucleotide positions 140 to 240, nucleotide positions 140 to 230, nucleotide positions 140 to 220, nucleotide positions 140 to 210, nucleotide positions 140 to 200, nucleotide positions 140 to 190, nucleotide positions 140 to 180, nucleotide positions 140 to 170, nucleotide positions 140 to 160, nucleotide positions 140 to 150, nucleotide positions 150 to 291, nucleotide positions 150 to 290, nucleotide positions 150 to 280, nucleotide positions 150 to 270, nucleotide positions 150 to 260, nucleotide positions 150 to 250, nucleotide positions 150 to 240, nucleotide positions 150 to 230, nucleotide positions 150 to 220, nucleotide positions 150 to 210, nucleotide positions 150 to 200, nucleotide positions 150 to 190, nucleotide positions 150 to 180, nucleotide positions 150 to 170, nucleotide positions 150 to 160, nucleotide positions 160 to 291, nucleotide positions 160 to 290, nucleotide positions 160 to 280, nucleotide positions 160 to 270, nucleotide positions 160 to 260, nucleotide positions 160 to 250, nucleotide positions 160 to 240, nucleotide positions 160 to 230, nucleotide positions 160 to 220, nucleotide positions 160 to 210, nucleotide positions 160 to 200, nucleotide positions 160 to 190, nucleotide positions 160 to 180, nucleotide positions 160 to 170, nucleotide positions 170 to 291, nucleotide positions 170 to 290, nucleotide positions 170 to 280, nucleotide positions 170 to 270, nucleotide positions 170 to 260, nucleotide positions 170 to 250, nucleotide positions 170 to 240, nucleotide positions 170 to 230, nucleotide positions 170 to 220, nucleotide

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positions 170 to 210, nucleotide positions 170 to 200, nucleotide positions 170 to 190, nucleotide positions 170 to 180, nucleotide positions 180 to 291, nucleotide positions 180 to 290, nucleotide positions 180 to 280, nucleotide positions 180 to 270, nucleotide positions 180 to 260, nucleotide positions 180 to 250, nucleotide positions 180 to 240, nucleotide positions 180 to 230, nucleotide positions 180 to 220, nucleotide positions 180 to 210, nucleotide positions 180 to 200, nucleotide positions 180 to 190, nucleotide positions 190 to 291, nucleotide positions 190 to 290, nucleotide positions 190 to 280, nucleotide positions 190 to 270, nucleotide positions 190 to 260, nucleotide positions 190 to 250, nucleotide positions 190 to 240, nucleotide positions 190 to 230, nucleotide positions 190 to 220, nucleotide positions 190 to 210, nucleotide positions 190 to 200, nucleotide positions 200 to 291, nucleotide positions 200 to 290, nucleotide positions 200 to 280, nucleotide positions 200 to 270, nucleotide positions 200 to 260, nucleotide positions 200 to 250, nucleotide positions 200 to 240, nucleotide positions 200 to 230, nucleotide positions 200 to 220, nucleotide positions 200 to 210, nucleotide positions 210 to 291, nucleotide positions 210 to 290, nucleotide positions 210 to 280, nucleotide positions 210 to 270, nucleotide positions 210 to 260, nucleotide positions 210 to 250, nucleotide positions 210 to 240, nucleotide positions 210 to 230, nucleotide positions 210 to 220, nucleotide positions 220 to 291, nucleotide positions 220 to 290, nucleotide positions 220 to 280, nucleotide positions 220 to 270, nucleotide positions 220 to 260, nucleotide positions 220 to 250, nucleotide positions 220 to 240, nucleotide positions 220 to 230, nucleotide positions 230 to 291, nucleotide positions 230 to 290, nucleotide positions 230 to 280, nucleotide positions 230 to 270, nucleotide positions 230 to 260, nucleotide positions 230 to 250, nucleotide positions 230 to 240, nucleotide positions 240 to 291, nucleotide positions 240 to 290, nucleotide positions 240 to 280, nucleotide positions 240 to 270, nucleotide positions 240 to 260, nucleotide positions 240 to 250, nucleotide positions 250 to 291, nucleotide positions 250 to 290, nucleotide positions 250 to 280, nucleotide positions 250 to 270, nucleotide positions 250 to 260, nucleotide positions 260 to 291, nucleotide positions 260 to 290, nucleotide positions 260 to 280, nucleotide positions 260 to 270, nucleotide positions 270 to 291, nucleotide positions 270 to 290, nucleotide positions 270 to 280, nucleotide positions 280 to 291, or nucleotide positions 280 to 290, of SEQ ID NO: 12 or 13.

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In some embodiments of any of the compositions described herein, the 5’ UTR comprises a sequence that is at least 70% (e.g., at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical to SEQ ID NO: 12 or 13.

In some embodiments of any of the compositions described herein, the 3’ UTR comprises at least 10 contiguous (e.g., at least 15 contiguous, at least 20 contiguous, at least 25 contiguous, at least 30 contiguous, at least 35 contiguous, at least 40 contiguous, at least 45 contiguous, at least 50 contiguous, at least 55 contiguous, at least 60 contiguous, at least 65 contiguous, at least 70 contiguous, at least 75 contiguous, at least 80 contiguous, at least 85 contiguous, at least 90 contiguous, at least 100 contiguous, at least 105 contiguous, at least 110 contiguous, at least 115 contiguous, at least 120 contiguous, at least 125 contiguous, at least 130 contiguous, at least 135 contiguous, at least 140 contiguous, at least 145 contiguous, at least 150 contiguous, at least 155 contiguous, at least 160 contiguous, at least 165 contiguous, at least 170 contiguous, at least 175 contiguous, at least 180 contiguous, at least 185 contiguous, at least 190 contiguous, at least 195 contiguous, at least 200 contiguous, at least 205 contiguous, at least 210 contiguous, at least 215 contiguous, at least 220 contiguous, at least 225 contiguous, at least 230 contiguous, at least 235 contiguous, at least 240 contiguous, at least 245 contiguous, at least 250 contiguous, at least 255 contiguous, at least 260 contiguous, at least 265 contiguous, at least 270 contiguous, at least 275 contiguous, at least 280 contiguous, at least 285 contiguous, at least 290 contiguous, at least 295 contiguous, at least 300 contiguous, at least 305 contiguous, at least 310 contiguous, at least 315 contiguous, at least 320 contiguous, at least 325 contiguous, at least 330 contiguous, at least 335 contiguous, at least 340 contiguous, at least 345 contiguous, at least 350 contiguous, at least 355 contiguous, at least 360 contiguous, at least 365 contiguous, at least 370 contiguous, at least 375 contiguous, at least 380 contiguous, at least 385 contiguous, at least 390 contiguous, at least 395 contiguous, at least 400 contiguous, at least 450 contiguous, at least 500 contiguous, at least 550 contiguous, at least 600 contiguous, at least 650 contiguous, at least 700 contiguous, at least 750 contiguous, at least 800 contiguous, at least 850 contiguous, at least 900 contiguous, at least 950 contiguous, at least 1000 contiguous, at least 1050 contiguous, at least 1100

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contiguous, at least 1150 contiguous, at least 1200 contiguous, at least 1250 contiguous, at least 1300 contiguous, at least 1350 contiguous, at least 1400 contiguous, at least 1450 contiguous, at least 1500 contiguous, at least 1550 contiguous, at least 1600 contiguous, at least 1650 contiguous, at least 1700 contiguous, or at least 1750 contiguous) nucleotides from anywhere within SEQ ID NO: 14 or 15.

For example, a 5' UTR can include or consist of one or more of: nucleotide positions 1 to 1773, nucleotide positions 1 to 1770, nucleotide positions 1 to 1750, nucleotide positions 1 to 1700, nucleotide positions 1 to 1650, nucleotide positions 1 to 1600, nucleotide positions 1 to 1550, nucleotide positions 1 to 1500, nucleotide positions 1 to 1450, nucleotide positions 1 to 1400, nucleotide positions 1 to 1350, nucleotide positions 1 to 1300, nucleotide positions 1 to 1250, nucleotide positions 1 to 1200, nucleotide positions 1 to 1150, nucleotide positions 1 to 1100, nucleotide positions 1 to 1050, nucleotide positions 1 to 1000, nucleotide positions 1 to 950, nucleotide positions 1 to 900, nucleotide positions 1 to 850, nucleotide positions 1 to 800, nucleotide positions 1 to 750, nucleotide positions 1 to 700, nucleotides positions 1 to 650, nucleotide positions 1 to 600, nucleotide positions 1 to 550, nucleotide positions 1 to 500, nucleotide positions 1 to 450, nucleotide positions 1 to 400, nucleotide positions 1 to 350, nucleotide positions 1 to 300, nucleotide positions 1 to 250, nucleotide positions 1 to 200, nucleotide positions 1 to 150, nucleotide positions 1 to 100, nucleotide positions 1 to 50, nucleotide position 1 to 25, nucleotide positions 25 to 1773, nucleotide positions 25 to 1770, nucleotide positions 25 to 1750, nucleotide positions 25 to 1700, nucleotide positions 25 to 1650, nucleotide positions 25 to 1600, nucleotide positions 25 to 1550, nucleotide positions 25 to 1500, nucleotide positions 25 to 1450, nucleotide positions 25 to 1400, nucleotide positions 25 to 1350, nucleotide positions 25 to 1300, nucleotide positions 25 to 1250, nucleotide positions 25 to 1200, nucleotide positions 25 to 1150, nucleotide positions 25 to 1100, nucleotide positions 25 to 1050, nucleotide positions 25 to 1000, nucleotide positions 25 to 950, nucleotide positions 25 to 900, nucleotide positions 25 to 850, nucleotide positions 25 to 800, nucleotide positions 25 to 750, nucleotide positions 25 to 700, nucleotides positions 25 to 650, nucleotide positions 25 to 600, nucleotide positions 25 to 550, nucleotide positions 25 to 500, nucleotide positions 25 to 450, nucleotide positions 25 to 400, nucleotide positions 25 to 350, nucleotide positions 25 to 300,

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nucleotide positions 25 to 250, nucleotide positions 25 to 200, nucleotide positions 25 to 150, nucleotide positions 25 to 100, nucleotide positions 25 to 50, nucleotide positions 50 to 1773, nucleotide positions 50 to 1770, nucleotide positions 50 to 1750, nucleotide positions 50 to 1700, nucleotide positions 50 to 1650, nucleotide positions 50 to 1600, nucleotide positions 50 to 1550, nucleotide positions 50 to 1500, nucleotide positions 50 to 1450, nucleotide positions 50 to 1400, nucleotide positions 50 to 1350, nucleotide positions 50 to 1300, nucleotide positions 50 to 1250, nucleotide positions 50 to 1200, nucleotide positions 50 to 1150, nucleotide positions 50 to 1100, nucleotide positions 50 to 1050, nucleotide positions 50 to 1000, nucleotide positions 50 to 950, nucleotide positions 50 to 900, nucleotide positions 50 to 850, nucleotide positions 50 to 800, nucleotide positions 50 to 750, nucleotide positions 50 to 700, nucleotides positions 50 to 650, nucleotide positions 50 to 600, nucleotide positions 50 to 550, nucleotide positions 50 to 500, nucleotide positions 50 to 450, nucleotide positions 50 to 400, nucleotide positions 50 to 350, nucleotide positions 50 to 300, nucleotide positions 50 to 250, nucleotide positions 50 to 200, nucleotide positions 50 to 150, nucleotide positions 50 to 100, nucleotide positions 100 to 1773, nucleotide positions 100 to 1770, nucleotide positions 100 to 1750, nucleotide positions 100 to 1700, nucleotide positions 100 to 1650, nucleotide positions 100 to 1600, nucleotide positions 100 to 1550, nucleotide positions 100 to 1500, nucleotide positions 100 to 1450, nucleotide positions 100 to 1400, nucleotide positions 100 to 1350, nucleotide positions 100 to 1300, nucleotide positions 100 to 1250, nucleotide positions 100 to 1200, nucleotide positions 100 to 1150, nucleotide positions 100 to 1100, nucleotide positions 100 to 1050, nucleotide positions 100 to 1000, nucleotide positions 100 to 950, nucleotide positions 100 to 900, nucleotide positions 100 to 850, nucleotide positions 100 to 800, nucleotide positions 100 to 750, nucleotide positions 100 to 700, nucleotides positions 100 to 650, nucleotide positions 100 to 600, nucleotide positions 100 to 550, nucleotide positions 100 to 500, nucleotide positions 100 to 450, nucleotide positions 100 to 400, nucleotide positions 100 to 350, nucleotide positions 100 to 300, nucleotide positions 100 to 250, nucleotide positions 100 to 200, nucleotide positions 100 to 150, nucleotide positions 150 to 1773, nucleotide positions 150 to 1770, nucleotide positions 150 to 1750, nucleotide positions 150 to 1700, nucleotide positions 150 to 1650, nucleotide positions 150 to 1600, nucleotide positions

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150 to 1550, nucleotide positions 150 to 1500, nucleotide positions 150 to 1450, nucleotide positions 150 to 1400, nucleotide positions 150 to 1350, nucleotide positions 150 to 1300, nucleotide positions 150 to 1250, nucleotide positions 150 to 1200, nucleotide positions 150 to 1150, nucleotide positions 150 to 1100, nucleotide positions 150 to 1050, nucleotide positions 150 to 1000, nucleotide positions 150 to 950, nucleotide positions 150 to 900, nucleotide positions 150 to 850, nucleotide positions 150 to 800, nucleotide positions 150 to 750, nucleotide positions 150 to 700, nucleotides positions 150 to 650, nucleotide positions 150 to 600, nucleotide positions 150 to 550, nucleotide positions 150 to 500, nucleotide positions 150 to 450, nucleotide positions 150 to 400, nucleotide positions 150 to 350, nucleotide positions 150 to 300, nucleotide positions 150 to 250, nucleotide positions 150 to 200, nucleotide positions 200 to 1773, nucleotide positions 200 to 1770, nucleotide positions 200 to 1750, nucleotide positions 200 to 1700, nucleotide positions 200 to 1650, nucleotide positions 200 to 1600, nucleotide positions 200 to 1550, nucleotide positions 200 to 1500, nucleotide positions 200 to 1450, nucleotide positions 200 to 1400, nucleotide positions 200 to 1350, nucleotide positions 200 to 1300, nucleotide positions 200 to 1250, nucleotide positions 200 to 1200, nucleotide positions 200 to 1150, nucleotide positions 200 to 1100, nucleotide positions 200 to 1050, nucleotide positions 200 to 1000, nucleotide positions 200 to 950, nucleotide positions 200 to 900, nucleotide positions 200 to 850, nucleotide positions 200 to 800, nucleotide positions 200 to 750, nucleotide positions 200 to 700, nucleotides positions 200 to 650, nucleotide positions 200 to 600, nucleotide positions 200 to 550, nucleotide positions 200 to 500, nucleotide positions 200 to 450, nucleotide positions 200 to 400, nucleotide positions 200 to 350, nucleotide positions 200 to 300, nucleotide positions 200 to 250, nucleotide positions 250 to 1773, nucleotide positions 250 to 1770, nucleotide positions 250 to 1750, nucleotide positions 250 to 1700, nucleotide positions 250 to 1650, nucleotide positions 250 to 1600, nucleotide positions 250 to 1550, nucleotide positions 250 to 1500, nucleotide positions 250 to 1450, nucleotide positions 250 to 1400, nucleotide positions 250 to 1350, nucleotide positions 250 to 1300, nucleotide positions 250 to 1250, nucleotide positions 250 to 1200, nucleotide positions 250 to 1150, nucleotide positions 250 to 1100, nucleotide positions 250 to 1050, nucleotide positions 250 to 1000, nucleotide positions 250 to 950,

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nucleotide positions 250 to 900, nucleotide positions 250 to 850, nucleotide positions 250 to 800, nucleotide positions 250 to 750, nucleotide positions 250 to 700, nucleotides positions 250 to 650, nucleotide positions 250 to 600, nucleotide positions 250 to 550, nucleotide positions 250 to 500, nucleotide positions 250 to 450, nucleotide positions 250 to 400, nucleotide positions 250 to 350, nucleotide positions 250 to 300, nucleotide positions 300 to 1773, nucleotide positions 300 to 1770, nucleotide positions 300 to 1750, nucleotide positions 300 to 1700, nucleotide positions 300 to 1650, nucleotide positions 300 to 1600, nucleotide positions 300 to 1550, nucleotide positions 300 to 1500, nucleotide positions 300 to 1450, nucleotide positions 300 to 1400, nucleotide positions 300 to 1350, nucleotide positions 300 to 1300, nucleotide positions 300 to 1250, nucleotide positions 300 to 1200, nucleotide positions 300 to 1150, nucleotide positions 300 to 1100, nucleotide positions 300 to 1050, nucleotide positions 300 to 1000, nucleotide positions 300 to 950, nucleotide positions 300 to 900, nucleotide positions 300 to 850, nucleotide positions 300 to 800, nucleotide positions 300 to 750, nucleotide positions 300 to 700, nucleotides positions 300 to 650, nucleotide positions 300 to 600, nucleotide positions 300 to 550, nucleotide positions 300 to 500, nucleotide positions 300 to 450, nucleotide positions 300 to 400, nucleotide positions 300 to 350, nucleotide positions 350 to 1773, nucleotide positions 350 to 1770, nucleotide positions 350 to 1750, nucleotide positions 350 to 1700, nucleotide positions 350 to 1650, nucleotide positions 350 to 1600, nucleotide positions 350 to 1550, nucleotide positions 350 to 1500, nucleotide positions 350 to 1450, nucleotide positions 350 to 1400, nucleotide positions 350 to 1350, nucleotide positions 350 to 1300, nucleotide positions 350 to 1250, nucleotide positions 350 to 1200, nucleotide positions 350 to 1150, nucleotide positions 350 to 1100, nucleotide positions 350 to 1050, nucleotide positions 350 to 1000, nucleotide positions 350 to 950, nucleotide positions 350 to 900, nucleotide positions 350 to 850, nucleotide positions 350 to 800, nucleotide positions 350 to 750, nucleotide positions 350 to 700, nucleotides positions 350 to 650, nucleotide positions 350 to 600, nucleotide positions 350 to 550, nucleotide positions 350 to 500, nucleotide positions 350 to 450, nucleotide positions 350 to 400, nucleotide positions 400 to 1773, nucleotide positions 400 to 1770, nucleotide positions 400 to 1750, nucleotide positions 400 to 1700, nucleotide positions 400 to 1650, nucleotide positions 400 to 1600, nucleotide positions

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400 to 1550, nucleotide positions 400 to 1500, nucleotide positions 400 to 1450, nucleotide positions 400 to 1400, nucleotide positions 400 to 1350, nucleotide positions 400 to 1300, nucleotide positions 400 to 1250, nucleotide positions 400 to 1200, nucleotide positions 400 to 1150, nucleotide positions 400 to 1100, nucleotide positions 400 to 1050, nucleotide positions 400 to 1000, nucleotide positions 400 to 950, nucleotide positions 400 to 900, nucleotide positions 400 to 850, nucleotide positions 400 to 800, nucleotide positions 400 to 750, nucleotide positions 400 to 700, nucleotides positions 400 to 650, nucleotide positions 400 to 600, nucleotide positions 400 to 550, nucleotide positions 400 to 500, nucleotide positions 400 to 450, nucleotide positions 450 to 1773, nucleotide positions 450 to 1770, nucleotide positions 450 to 1750, nucleotide positions 450 to 1700, nucleotide positions 450 to 1650, nucleotide positions 450 to 1600, nucleotide positions 450 to 1550, nucleotide positions 450 to 1500, nucleotide positions 450 to 1450, nucleotide positions 450 to 1400, nucleotide positions 450 to 1350, nucleotide positions 450 to 1300, nucleotide positions 450 to 1250, nucleotide positions 450 to 1200, nucleotide positions 450 to 1150, nucleotide positions 450 to 1100, nucleotide positions 450 to 1050, nucleotide positions 450 to 1000, nucleotide positions 450 to 950, nucleotide positions 450 to 900, nucleotide positions 450 to 850, nucleotide positions 450 to 800, nucleotide positions 450 to 750, nucleotide positions 450 to 700, nucleotides positions 450 to 650, nucleotide positions 450 to 600, nucleotide positions 450 to 550, nucleotide positions 450 to 500, nucleotide positions 500 to 1773, nucleotide positions 500 to 1770, nucleotide positions 500 to 1750, nucleotide positions 500 to 1700, nucleotide positions 500 to 1650, nucleotide positions 500 to 1600, nucleotide positions 500 to 1550, nucleotide positions 500 to 1500, nucleotide positions 500 to 1450, nucleotide positions 500 to 1400, nucleotide positions 500 to 1350, nucleotide positions 500 to 1300, nucleotide positions 500 to 1250, nucleotide positions 500 to 1200, nucleotide positions 500 to 1150, nucleotide positions 500 to 1100, nucleotide positions 500 to 1050, nucleotide positions 500 to 1000, nucleotide positions 500 to 950, nucleotide positions 500 to 900, nucleotide positions 500 to 850, nucleotide positions 500 to 800, nucleotide positions 500 to 750, nucleotide positions 500 to 700, nucleotides positions 500 to 650, nucleotide positions 500 to 600, nucleotide positions 500 to 550, nucleotide positions 550 to 1773, nucleotide positions 550 to 1770, nucleotide positions

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550 to 1750, nucleotide positions 550 to 1700, nucleotide positions 550 to 1650, nucleotide positions 550 to 1600, nucleotide positions 550 to 1550, nucleotide positions 550 to 1500, nucleotide positions 550 to 1450, nucleotide positions 550 to 1400, nucleotide positions 550 to 1350, nucleotide positions 550 to 1300, nucleotide positions 550 to 1250, nucleotide positions 550 to 1200, nucleotide positions 550 to 1150, nucleotide positions 550 to 1100, nucleotide positions 550 to 1050, nucleotide positions 550 to 1000, nucleotide positions 550 to 950, nucleotide positions 550 to 900, nucleotide positions 550 to 850, nucleotide positions 550 to 800, nucleotide positions 550 to 750, nucleotide positions 550 to 700, nucleotides positions 550 to 650, nucleotide positions 550 to 600, nucleotide positions 600 to 1773, nucleotide positions 600 to 1770, nucleotide positions 600 to 1750, nucleotide positions 600 to 1700, nucleotide positions 600 to 1650, nucleotide positions 600 to 1600, nucleotide positions 600 to 1550, nucleotide positions 600 to 1500, nucleotide positions 600 to 1450, nucleotide positions 600 to 1400, nucleotide positions 600 to 1350, nucleotide positions 600 to 1300, nucleotide positions 600 to 1250, nucleotide positions 600 to 1200, nucleotide positions 600 to 1150, nucleotide positions 600 to 1100, nucleotide positions 600 to 1050, nucleotide positions 600 to 1000, nucleotide positions 600 to 950, nucleotide positions 600 to 900, nucleotide positions 600 to 850, nucleotide positions 600 to 800, nucleotide positions 600 to 750, nucleotide positions 600 to 700, nucleotides positions 600 to 650, nucleotide positions 650 to 1773, nucleotide positions 650 to 1770, nucleotide positions 650 to 1750, nucleotide positions 650 to 1700, nucleotide positions 650 to 1650, nucleotide positions 650 to 1600, nucleotide positions 650 to 1550, nucleotide positions 650 to 1500, nucleotide positions 650 to 1450, nucleotide positions 650 to 1400, nucleotide positions 650 to 1350, nucleotide positions 650 to 1300, nucleotide positions 650 to 1250, nucleotide positions 650 to 1200, nucleotide positions 650 to 1150, nucleotide positions 650 to 1100, nucleotide positions 650 to 1050, nucleotide positions 650 to 1000, nucleotide positions 650 to 950, nucleotide positions 650 to 900, nucleotide positions 650 to 850, nucleotide positions 650 to 800, nucleotide positions 650 to 750, nucleotide positions 650 to 700, nucleotide positions 700 to 1773, nucleotide positions 700 to 1770, nucleotide positions 700 to 1750, nucleotide positions 700 to 1700, nucleotide positions 700 to 1650, nucleotide positions 700 to 1600, nucleotide positions

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700 to 1550, nucleotide positions 700 to 1500, nucleotide positions 700 to 1450, nucleotide positions 700 to 1400, nucleotide positions 700 to 1350, nucleotide positions 700 to 1300, nucleotide positions 700 to 1250, nucleotide positions 700 to 1200, nucleotide positions 700 to 1150, nucleotide positions 700 to 1100, nucleotide positions 700 to 1050, nucleotide positions 700 to 1000, nucleotide positions 700 to 950, nucleotide positions 700 to 900, nucleotide positions 700 to 850, nucleotide positions 700 to 800, nucleotide positions 700 to 750, nucleotide positions 750 to 1773, nucleotide positions 750 to 1770, nucleotide positions 750 to 1750, nucleotide positions 750 to 1700, nucleotide positions 750 to 1650, nucleotide positions 750 to 1600, nucleotide positions 750 to 1550, nucleotide positions 750 to 1500, nucleotide positions 750 to 1450, nucleotide positions 750 to 1400, nucleotide positions 750 to 1350, nucleotide positions 750 to 1300, nucleotide positions 750 to 1250, nucleotide positions 750 to 1200, nucleotide positions 750 to 1150, nucleotide positions 750 to 1100, nucleotide positions 750 to 1050, nucleotide positions 750 to 1000, nucleotide positions 750 to 950, nucleotide positions 750 to 900, nucleotide positions 750 to 850, nucleotide positions 750 to 800, nucleotide positions 800 to 1773, nucleotide positions 800 to 1770, nucleotide positions 800 to 1750, nucleotide positions 800 to 1700, nucleotide positions 800 to 1650, nucleotide positions 800 to 1600, nucleotide positions 800 to 1550, nucleotide positions 800 to 1500, nucleotide positions 800 to 1450, nucleotide positions 800 to 1400, nucleotide positions 800 to 1350, nucleotide positions 800 to 1300, nucleotide positions 800 to 1250, nucleotide positions 800 to 1200, nucleotide positions 800 to 1150, nucleotide positions 800 to 1100, nucleotide positions 800 to 1050, nucleotide positions 800 to 1000, nucleotide positions 800 to 950, nucleotide positions 800 to 900, nucleotide positions 800 to 850, nucleotide positions 850 to 1773, nucleotide positions 850 to 1770, nucleotide positions 850 to 1750, nucleotide positions 850 to 1700, nucleotide positions 850 to 1650, nucleotide positions 850 to 1600, nucleotide positions 850 to 1550, nucleotide positions 850 to 1500, nucleotide positions 850 to 1450, nucleotide positions 850 to 1400, nucleotide positions 850 to 1350, nucleotide positions 850 to 1300, nucleotide positions 850 to 1250, nucleotide positions 850 to 1200, nucleotide positions 850 to 1150, nucleotide positions 850 to 1100, nucleotide positions 850 to 1050, nucleotide positions 850 to 1000, nucleotide positions 850 to 950, nucleotide positions

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850 to 900, nucleotide positions 900 to 1773, nucleotide positions 900 to 1770, nucleotide positions 900 to 1750, nucleotide positions 900 to 1700, nucleotide positions 900 to 1650, nucleotide positions 900 to 1600, nucleotide positions 900 to 1550, nucleotide positions 900 to 1500, nucleotide positions 900 to 1450, nucleotide positions 900 to 1400, nucleotide positions 900 to 1350, nucleotide positions 900 to 1300, nucleotide positions 900 to 1250, nucleotide positions 900 to 1200, nucleotide positions 900 to 1150, nucleotide positions 900 to 1100, nucleotide positions 900 to 1050, nucleotide positions 900 to 1000, nucleotide positions 900 to 950, nucleotide positions 950 to 1773, nucleotide positions 950 to 1770, nucleotide positions 950 to 1750, nucleotide positions 950 to 1700, nucleotide positions 950 to 1650, nucleotide positions 950 to 1600, nucleotide positions 950 to 1550, nucleotide positions 950 to 1500, nucleotide positions 950 to 1450, nucleotide positions 950 to 1400, nucleotide positions 950 to 1350, nucleotide positions 950 to 1300, nucleotide positions 950 to 1250, nucleotide positions 950 to 1200, nucleotide positions 950 to 1150, nucleotide positions 950 to 1100, nucleotide positions 950 to 1050, nucleotide positions 950 to 1000, nucleotide positions 1000 to 1773, nucleotide positions 1000 to 1770, nucleotide positions 1000 to 1750, nucleotide positions 1000 to 1700, nucleotide positions 1000 to 1650, nucleotide positions 1000 to 1600, nucleotide positions 1000 to 1550, nucleotide positions 1000 to 1500, nucleotide positions 1000 to 1450, nucleotide positions 1000 to 1400, nucleotide positions 1000 to 1350, nucleotide positions 1000 to 1300, nucleotide positions 1000 to 1250, nucleotide positions 1000 to 1200, nucleotide positions 1000 to 1150, nucleotide positions 1000 to 1100, nucleotide positions 1000 to 1050, nucleotide positions 1050 to 1773, nucleotide positions 1050 to 1770, nucleotide positions 1050 to 1750, nucleotide positions 1050 to 1700, nucleotide positions 1050 to 1650, nucleotide positions 1050 to 1600, nucleotide positions 1050 to 1550, nucleotide positions 1050 to 1500, nucleotide positions 1050 to 1450, nucleotide positions 1050 to 1400, nucleotide positions 1050 to 1350, nucleotide positions 1050 to 1300, nucleotide positions 1050 to 1250, nucleotide positions 1050 to 1200, nucleotide positions 1050 to 1150, nucleotide positions 1050 to 1100, nucleotide positions 1100 to 1773, nucleotide positions 1100 to 1770, nucleotide positions 1100 to 1750, nucleotide positions 1100 to 1700, nucleotide positions 1100 to 1650, nucleotide positions 1100 to 1600, nucleotide positions 1100 to

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1550, nucleotide positions 1100 to 1500, nucleotide positions 1100 to 1450, nucleotide positions 1100 to 1400, nucleotide positions 1100 to 1350, nucleotide positions 1100 to 1300, nucleotide positions 1100 to 1250, nucleotide positions 1100 to 1200, nucleotide positions 1100 to 1150, nucleotide positions 1150 to 1773, nucleotide positions 1150 to 1770, nucleotide positions 1150 to 1750, nucleotide positions 1150 to 1700, nucleotide positions 1150 to 1650, nucleotide positions 1150 to 1600, nucleotide positions 1150 to 1550, nucleotide positions 1150 to 1500, nucleotide positions 1150 to 1450, nucleotide positions 1150 to 1400, nucleotide positions 1150 to 1350, nucleotide positions 1150 to 1300, nucleotide positions 1150 to 1250, nucleotide positions 1150 to 1200, nucleotide positions 1200 to 1773, nucleotide positions 1200 to 1770, nucleotide positions 1200 to 1750, nucleotide positions 1200 to 1700, nucleotide positions 1200 to 1650, nucleotide positions 1200 to 1600, nucleotide positions 1200 to 1550, nucleotide positions 1200 to 1500, nucleotide positions 1200 to 1450, nucleotide positions 1200 to 1400, nucleotide positions 1200 to 1350, nucleotide positions 1200 to 1300, nucleotide positions 1200 to 1250, nucleotide positions 1250 to 1773, nucleotide positions 1250 to 1770, nucleotide positions 1250 to 1750, nucleotide positions 1250 to 1700, nucleotide positions 1250 to 1650, nucleotide positions 1250 to 1600, nucleotide positions 1250 to 1550, nucleotide positions 1250 to 1500, nucleotide positions 1250 to 1450, nucleotide positions 1250 to 1400, nucleotide positions 1250 to 1350, nucleotide positions 1250 to 1300, nucleotide positions 1300 to 1773, nucleotide positions 1300 to 1770, nucleotide positions 1300 to 1750, nucleotide positions 1300 to 1700, nucleotide positions 1300 to 1650, nucleotide positions 1300 to 1600, nucleotide positions 1300 to 1550, nucleotide positions 1300 to 1500, nucleotide positions 1300 to 1450, nucleotide positions 1300 to 1400, nucleotide positions 1300 to 1350, nucleotide positions 1350 to 1773, nucleotide positions 1350 to 1770, nucleotide positions 1350 to 1750, nucleotide positions 1350 to 1700, nucleotide positions 1350 to 1650, nucleotide positions 1350 to 1600, nucleotide positions 1350 to 1550, nucleotide positions 1350 to 1500, nucleotide positions 1350 to 1450, nucleotide positions 1350 to 1400, nucleotide positions 1400 to 1773, nucleotide positions 1400 to 1770, nucleotide positions 1400 to 1750, nucleotide positions 1400 to 1700, nucleotide positions 1400 to 1650, nucleotide positions 1400 to 1600, nucleotide positions 1400 to 1550, nucleotide positions 1400 to 1500, nucleotide positions 1400 to 1450, nucleotide

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positions 1450 to 1773, nucleotide positions 1450 to 1770, nucleotide positions 1450 to 1750, nucleotide positions 1450 to 1700, nucleotide positions 1450 to 1650, nucleotide positions 1450 to 1600, nucleotide positions 1450 to 1550, nucleotide positions 1450 to 1500, nucleotide positions 1500 to 1773, nucleotide positions 1500 to 1770, nucleotide positions 1500 to 1750, nucleotide positions 1500 to 1700, nucleotide positions 1500 to 1650, nucleotide positions 1500 to 1600, nucleotide positions 1500 to 1550, nucleotide positions 1550 to 1773, nucleotide positions 1550 to 1770, nucleotide positions 1550 to 1750, nucleotide positions 1550 to 1700, nucleotide positions 1550 to 1650, nucleotide positions 1550 to 1600, nucleotide positions 1600 to 1773, nucleotide positions 1600 to 1770, nucleotide positions 1600 to 1750, nucleotide positions 1600 to 1700, nucleotide positions 1600 to 1650, nucleotide positions 1650 to 1773, nucleotide positions 1650 to 1770, nucleotide positions 1650 to 1750, nucleotide positions 1650 to 1700, nucleotide positions 1700 to 1773, nucleotide positions 1700 to 1770, nucleotide positions 1700 to 1750, nucleotide positions 1750 to 1773, or nucleotide positions 1750 to 1770, of SEQ ID NO: 14 or 15.

In some embodiments of any of the compositions described herein, the 3' UTR comprises a sequence that is at least 70% (e.g., at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical to SEQ ID NO: 14 or 15.

Human 5' UTR of CLRN1 (SEQ ID NO: 12)

AGGAGATACTTGAAGGCAGTTTGAAAGACTTGTTTTACAGATTCTTAGTCCAAAGATTTCCAATTAGGGAG
AAGAAGCAGCAGAAAAGGAGAAAAGCCAAGTATGAGTGATGATGAGGCCTTCATCTACTGACATTTAACCT
GGCGAGAACCGTCGATGGTGAAGTTGCCTTTTCAGCTGGGAGCTGTCCGTTCAGCTTCCGTAATAAATGCA
GTCAAAGAGGCAGTCCCTTCCCATTGCTCACAAAGGTCTTGTTTTTGAACCTCGCCCTCACAGAAGCCGTT
TCTCATC

Human 5'UTR of CLRN1 (SEQ ID NO: 13)

CCTGCAGGCAGCTGCGCGCTCGCTCGCTCACTGAGGCCGCCCGGGCGTCGGGCGACCTTTGGTCGCCCGGC
CTCAGTGAGCGAGCGAGCGCGCAGAGAGGGAGTGGCCAACTCCATCACTAGGGGTTCCT

Human 3' UTR of CLRN1 (SEQ ID NO: 14)

AGGAACCCCTAGTGATGGAGTTGGCCACTCCCTCTCTGCGCGCTCGCTCGCTCACTGAGGCCGGGCGACCA
AAGGTCGCCCGACGCCCGGGCTTTGCCCGGGCGGCCTCAGTGAGCGAGCGAGCGCGCAGCTGCCTGCAGG

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Human 3' UTR of CLRN1 (SEQ ID NO: 15)

AAGGCAAACCTTTCTATAATTTTACAAGGGAGTAGACTTGCTTTGGTCACTTTTAGATGTGGTTAATTTTG
CATATCCTTTTAGTCTGCATATATTAAAGCATCAGGACCCTTCGTGACAATGTTTACAAATTACGTACTAA
GGATACAGGCTGGAAAGTAAGGGAAGCAGAAGGAAGGCTTTGAAAAGTTGTTTTATCTGGTGGGAAATTGC
TTGACCCAGGTAGTCAAAGGCAGTTGACTAGAATCGACAAATTGTTACTCCATATATATATATGTGTGTGT
GTGTGTGTGTGTGTGTGTGTAAGATGTCTTCCTATCAAAAAGATATCAAAGGCACATGGAATATATTTTAA
TAAAAACAAATAATATCTCTAATATATCCACACATTTGTTGCCAGATTTCAGAAAACTGAGCTGCAATCGC
TTTCCTAAAACAGTAGTGTATTAAATGAACATCTATAAAATGTATCAACACACATTTTAAAAAATTTGTTT
AAAGTATACTCTTAGGCCAGGCGTGGTGACTCACACCTGTAATTCCAGCACTTCAGGAGGCCAAGGTGGGA
AGATCATTTGAGTTCAGGAGTTCGAGTTACAGCCTGGGCAATAAAGTGAGACCCTGTCACTAACAAAATTA
AAAAATAAAATAAATATAAAATATAGGCTTTAAAAAAGCATAGTCTTATTAACCATGTCTGTTGGTCAAAA
TCTGCAAACTCTAAAAGAAGAAAAGAAGAAAAAACCAAGCTTAGGGTATTTTTCCTCCCGTGCCTGAGTCC
CAATTACATTCACGACAGTACTTTCAATGAACATAATTGTTAGGACCACTGAGGAATCATGAAAAATGATC
TCTGCTTAGTACATTTGATGCAAAATGACTTATTAGGGGCTGTTTTTCTAGCTATAGTGTCTCGAGTACTA
ATATGCAATTATGAAAATTATATTAAATCTGGGATTATGACGGTATCACTGTATCATCTTGGTCTTGTTCT
GGCTGTCACCAAGCATGACCCAGGTCAACTTTTTTTTTCCCCTGAATTACCCATCAAATTGATCTGCAGCT
GACTAAAGGCCACAGCTGAGCCTGGAACTGACCCTTCCTTCATCCTCAACCTGCTGTCCTCCAGAAAGCAC
CAAGGAAAAAGCAGAGAATGACAGCAAACAGATCACTAGGCCTCTGACCACAGGTGCTGAGTACTCAGCAG
CCCTCATATAATAGGTTTGAAAGTACTCCTTAAAATAAAACACTGTTTCCCTTTGGAACTATTTACAAGGA
TGAAACAACCGTATACCTGAGAAATAACTTGCTCTGGTGTCAATTCGCTATTCGCCAGCAGACATCAGAAC
ACACCGAGTTTCCAGATGCTGGTTTTTCCCCTTAAATCAGGAAATACACCTGGACAATTTCTAGAAGACTA
CAATTCAGTCTAGCCACAAAGGGGATTTTTTTTTTTTGGTAACAGGCTAGAGCCCGGTTCTGTAAGTCTTT
AGCTGAAATGGTCCAGTACAAAAGCACTGGAAATGAGTGGGCTAGGAGGACAAGGACCGTCTCCTGCGTGA
GGAGTTGGTTGGAGGTCCCCAAGGCCAGGTACCCCCTGCACTCTTATTGGATTCCTCTCTGTCTTCTTGGA
GTTTTGAAAAACTCCTTCGAACACCAGGCTTTTTTCTTTAGAAAACAAGTCTCCAATCGTTCTCTGTTCCG
TAGAAAGAGAAAGAAAACCTGGAGCAGCTGCTGAAAAATCTAATGAGGAACTAAGAGGCAAACCCACCA

In some embodiments, the introduction, removal, or modification of 3' UTR AREs can be used to modulate the stability of an mRNA encoding a CLRN1 protein. In other embodiments, AREs can be removed or mutated to increase the intracellular stability and thus increase translation and production of a CLRN1 protein.

In other embodiments, non-ARE sequences may be incorporated into the 5' or 3' UTRs. In some embodiments, introns or portions of intron sequences may be incorporated into the flanking regions of the polynucleotides in any of the vectors,

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compositions, kits, and methods provided herein. Incorporation of intronic sequences may increase protein production as well as mRNA levels.

In some embodiments of any of the vectors described herein, the vector includes a chimeric intron sequence (SEQ ID NO: 16).

Mammalian Cells

Also provided herein is a cell (e.g., a mammalian cell) that includes any of the nucleic acids, vectors (e.g., at least two different vectors described herein), or compositions described herein. Skilled practitioners will appreciate that the nucleic acids and vectors described herein can be introduced into any mammalian cell. Non-limiting examples of vectors and methods for introducing vectors into mammalian cells are described herein.

In some embodiments, the cell is a human cell, a mouse cell, a porcine cell, a rabbit cell, a dog cell, a cat cell, a rat cell, or a non-human primate cell. In some embodiments, the cell is a specialized cell of the cochlea. In some embodiments, the cell is a cochlear hair cell, such as a cochlear inner hair cell or a cochlear out hair cell. In some embodiments, the cell is an ocular cell (e.g. a retinal cell, a retinal ganglion cell, an amacrine cell, a hortizontal cell, a bipolar cell, a photoreceptor cell).

In some embodiments, the mammalian cell is *in vitro*. In some embodiments, the mammalian cell is present in a mammal. In some embodiments, the mammalian cell is an autologous cell obtained from a subject and cultured *ex vivo*.

Methods

Also provided herein are methods that include: introducing into a cochlea of a mammal (e.g., a human) a therapeutically effective amount of any of the compositions described herein.

Also provided herein are methods of increasing expression of a full-length CLRN1 protein in a mammalian cell, that include: introducing any of the compositions described herein into the mammalian cell.

Also provided herein are methods of increasing expression of a full-length CLRN1 protein in an inner hair cell, an outer hair cell, or both, in a cochlea of a mammal

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(e.g., a human) that include: introducing into the cochlea of the mammal a therapeutically effective amount of any of the compositions described herein.

Also provided herein are methods of increasing expression of a full-length CLRN1 protein in an eye of a mammal (e.g., a human) that include: intraocularly administering to the eye of the mammal a therapeutically effective amount of any of the compositions described herein.

Also provided herein are methods of treating hearing loss in a subject identified as having a defective CLRN1 gene that include: administering a therapeutically effective amount of any of the compositions described herein into the cochlea of the subject.

Also provided herein are methods of treating vision loss in a subject identified as having a defective CLRN1 gene that include: administering a therapeutically effective amount of any of the compositions described herein into the eye of the subject.

In some embodiments of any of these methods, the mammal has been previously identified as having a defective CLRN1 gene (e.g., a CLRN1 gene having a mutation that results in a decrease in the expression and/or activity of a CLRN1 protein encoded by the gene). Some embodiments of any of these methods further include, prior to the introducing or administering step, determining that the subject has a defective CLRN1 gene. Some embodiments of any of these methods can further include detecting a mutation in a CLRN1 gene in a subject. Some embodiments of any of the methods can further include identifying or diagnosing a subject as having hearing loss and/or vision loss.

In some embodiments of any of these methods, two or more doses of any of the compositions described herein are introduced or administered into the cochlea of the mammal or subject. Some embodiments of any of these methods can include introducing or administering a first dose of the composition into the cochlea of the mammal or subject, assessing hearing function of the mammal or subject following the introducing or the administering of the first dose, and administering an additional dose of the composition into the cochlea of the mammal or subject found not to have a hearing function within a normal range (e.g., as determined using any test for hearing known in the art).

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In some embodiments of any of the methods described herein, the composition can be formulated for intra-cochlear administration. In some embodiments of any of the methods described herein, the compositions described herein can be administered via intra-cochlear administration or local administration. In some embodiments of any of the methods described herein, the compositions are administered through the use of a medical device (e.g., any of the exemplary medical devices described herein).

In some embodiments, intra-cochlear administration can be performed using any of the methods described herein or known in the art. For example, a composition can be administered or introduced into the cochlea using the following surgical technique: first using visualization with a 0 degree, 2.5-mm rigid endoscope, the external auditory canal is cleared and a round knife is used to sharply delineate an approximately 5-mm tympanomeatal flap. The tympanomeatal flap is then elevated and the middle ear is entered posteriorly. The chorda tympani nerve is identified and divided, and a currette is used to remove the scutal bone, exposing the round window membrane. To enhance apical distribution of the administered or introduced composition, a surgical laser may be used to make a small 2-mm fenestration in the oval window to allow for perilymph displacement during trans-round window membrane infusion of the composition. The microinfusion device is then primed and brought into the surgical field. The device is maneuvered to the round window, and the tip is seated within the bony round window overhang to allow for penetration of the membrane by the microneedle(s). The footpedal is engaged to allow for a measured, steady infusion of the composition. The device is then withdrawn and the round window and stapes foot plate are sealed with a gelfoam patch.

In some embodiments of any of these methods, two or more doses of any of the compositions described herein are introduced or administered into the eye of the mammal or subject. Some embodiments of any of these methods can include introducing or administering a first dose of the composition into the eye (e.g., intraocular space) of the mammal or subject, assessing hearing function of the mammal or subject following the introducing or the administering of the first dose, and administering an additional dose of the composition into the eye of the mammal or subject found not to have a vision within a normal range (e.g., as determined using any test for vision known in the art).

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In some embodiments of any of the methods described herein, the composition can be formulated for intra-ocular administration. In some embodiments of any of the methods described herein, the compositions described herein can be administered via intra-ocular administration or local administration.

In some embodiments, intra-ocular administration can be performed using any of the methods described herein or known in the art.

In some embodiments of any of the methods described herein, the subject or mammal is a rodent, a non-human primate, or a human. In some embodiments of any of the methods described herein, the subject or mammal is an adult, a teenager, a juvenile, a child, a toddler, an infant, or a newborn. In some embodiments of any of the methods described herein, the subject or mammal is 1-5, 1-10, 1-20, 1-30, 1-40, 1-50, 1-60, 1-70, 1-80, 1-90, 1-100, 1-110, 2-5, 2-10, 10-20, 20-30, 30-40, 40-50, 50-60, 60-70, 70-80, 80-90, 90-100, 100-110, 10-30, 10-40, 10-50, 10-60, 10-70, 10-80, 10-90, 10-100, 10-110, 20-40, 20-50, 20-60, 20-70, 20-80, 20-90, 20-100, 20-110, 30-50, 30-60, 30-70, 30-80, 30-90, 30-100, 40-60, 40-70, 40-80, 40-90, 40-100, 50-70, 50-80, 50-90, 50-100, 60-80, 60-90, 60-100, 70-90, 70-100, 70-110, 80-100, 80-110, or 90-110 years of age. In some embodiments of any of the methods described herein, the subject or mammal is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or 11 months of age.

In some embodiments of any of the methods described herein, the subject or mammal has or is at risk of developing hearing loss and/or vision loss (e.g., Usher syndrome type III, retinitis pigmentosa). In some embodiments of any of the methods described herein, the subject or mammal has been previously identified as having a mutation in a CLRN1 gene. In some embodiments of any of the methods described herein, the subject or mammal has any of the mutations in a CLRN1 gene that are described herein or are known in the art to be associated with hearing loss and/or vision loss.

In some embodiments of any of the methods described herein, the subject or mammal has been identified as being a carrier of a mutation in a CLRN1 gene (e.g., via genetic testing). In some embodiments of any of the methods described herein, the subject or human has been identified as having a mutation in a CLRN1 gene and has been diagnosed with hearing loss and/or vision loss (e.g., Usher syndrome type III, retinitis

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pigmentosa). In some embodiments of any of the methods described herein, the subject or human has been identified as having hearing loss and/or vision loss (e.g., Usher syndrome type III, retinitis pigmentosa).

In some embodiments, successful treatment of hearing loss (e.g., Usher syndrome type III) can be determined in a subject using any of the conventional functional hearing tests known in the art. Non-limiting examples of functional hearing tests are various types of audiometric assays (e.g., pure-tone testing, speech testing, test of the middle ear, auditory brainstem response, and otoacoustic emissions).

In some embodiments, successful treatment of vision loss can be determined in a subject using any of the conventional functional vision tests known in the art. Non-limiting examples of functional retinal and vision tests are acuity testing, intraocular pressure (IOP) testing, and an electroretinogram (ERG).

Also provided herein are methods of increasing expression of an active CLRN1 protein (e.g., a full-length CLRN1 protein) in a mammalian cell that include introducing any of the compositions described herein into the mammalian cell. In some embodiments of these methods, the mammalian cell is a cochlear hair cell (e.g., an inner hair cell, an outer hair cell) or an ocular cell (e.g., a retinal cell). In some embodiments of these methods, the mammalian cell is a human cell (e.g., a human cochlear hair cell). In some embodiments of these methods, the mammalian cell is *in vitro*. In some embodiments of these methods, the mammalian cell is in a mammal. In some embodiments of these methods, the mammalian cell is originally obtained from a mammal and is cultured *ex vivo*. In some embodiments, the mammalian cell has previously been determined to have a defective CLRN1 gene.

Methods for introducing any of the compositions described herein into a mammalian cell are known in the art (e.g., via lipofection or through the use of a viral vector, e.g., any of the viral vectors described herein).

An increase in expression of an active CLRN1 protein (e.g., a full-length CLRN1 protein) as described herein is, e.g., as compared to a control or to the level of expression of an active CLRN1 protein (e.g., a full-length CLRN1 protein) prior to the introduction of the vector(s).

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Methods of detecting expression and/or activity of CLRN1 are known in the art. In some embodiments, the level of expression of a CLRN1 protein can be detected directly (e.g., detecting CLRN1 protein or detecting CLRN1 mRNA). Non-limiting examples of techniques that can be used to detect expression and/or activity of CLRN1 directly include: real-time PCR, Western blotting, immunoprecipitation, immunohistochemistry, or immunofluorescence. In some embodiments, expression of a CLRN1 protein can be detected indirectly (e.g., through functional hearing tests, functional retinal and vision tests).

Pharmaceutical Compositions and Kits

In some embodiments, any of the compositions described herein can further include one or more agents that promote the entry of a nucleic acid or any of the vectors described herein into a mammalian cell (e.g., a liposome or cationic lipid).

In some embodiments, any of the vectors described herein can be formulated using natural and/or synthetic polymers. Non-limiting examples of polymers that may be included in any of the compositions described herein can include, but are not limited to, DYNAMIC POLYCONJUGATE® (Arrowhead Research Corp., Pasadena, Calif.), formulations from Mirus Bio (Madison, Wis.) and Roche Madison (Madison, Wis.), PhaseRX polymer formulations such as, without limitation, SMARTT POLYMER TECHNOLOGY® (PhaseRX, Seattle, Wash.), DMRI/DOPE, poloxamer, VAXFECTIN® adjuvant from Vical (San Diego, Calif.), chitosan, cyclodextrin from Calando Pharmaceuticals (Pasadena, Calif.), dendrimers and poly (lactic-co-glycolic acid) (PLGA) polymers, RONDEL™ (RNAi/Oligonucleotide Nanoparticle Delivery) polymers (Arrowhead Research Corporation, Pasadena, Calif.), and pH responsive co-block polymers, such as, but not limited to, those produced by PhaseRX (Seattle, Wash.). Many of these polymers have demonstrated efficacy in delivering oligonucleotides *in vivo* into a mammalian cell (see, e.g., deFougerolles, *Human Gene Ther.* 19:125-132, 2008; Rozema et al., *Proc. Natl. Acad. Sci. U.S.A.* 104:12982-12887, 2007; Rozema et al., *Proc. Natl. Acad. Sci. U.S.A.* 104:12982-12887, 2007; Hu-Lieskovan et al., *Cancer Res.* 65:8984-8982, 2005; Heidel et al., *Proc. Natl. Acad. Sci. U.S.A.* 104:5715-5721, 2007).

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Any of the compositions described herein can be, e.g., a pharmaceutical composition. A pharmaceutical composition can include any of the compositions described herein and one or more pharmaceutically or physiologically acceptable carriers, diluents, or excipients. Such compositions may comprise one or more buffers, such as neutral-buffered saline, phosphate-buffered saline, and the like; one or more carbohydrates, such as glucose, mannose, sucrose, and dextran; mannitol; one or more proteins, polypeptides, or amino acids, such as glycine; one or more antioxidants; one or more chelating agents, such as EDTA or glutathione; and/or one or more preservatives.

In some embodiments, the composition includes a pharmaceutically acceptable carrier (e.g., phosphate buffered saline, saline, or bacteriostatic water). Upon formulation, solutions will be administered in a manner compatible with the dosage formulation and in such amount as is therapeutically effective. The formulations are easily administered in a variety of dosage forms such as injectable solutions, injectable gels, drug-release capsules, and the like.

As used herein, the term "pharmaceutically acceptable carrier" includes solvents, dispersion media, coatings, antibacterial agents, antifungal agents, and the like that are compatible with pharmaceutical administration. Supplementary active compounds can also be incorporated into any of the compostions described herein.

In some embodiments, a single dose of any of the compositions described herein can include a total sum amount of the at least two different vectors of at least 1 ng, at least 2 ng, at least 4 ng, about 6 ng, about 8 ng, at least 10 ng, at least 20 ng, at least 30 ng, at least 40 ng, at least 50 ng, at least 60 ng, at least 70 ng, at least 80 ng, at least 90 ng, at least 100 ng, at least 200 ng, at least 300 ng, at least 400 ng, at least 500 ng, at least 1 μg, at least 2 μg, at least 4 μg, at least 6 μg, at least 8 μg, at least 10 μg, at least 12 μg, at least 14 μg, at least 16 μg, at least 18 μg, at least 20 μg, at least 22 μg, at least 24 μg, at least 26 μg, at least 28 μg, at least 30 μg at least 32 μg, at least 34 μg, at least 36 μg, at least 38 μg, at least 40 μg, at least 42 μg, at least 44 μg, at least 46 μg, at least 48 μg, at least 50 μg, at least 52 μg, at least 54 μg, at least 56 μg, at least 58 μg, at least 60 μg, at least 62 μg, at least 64 μg, at least 66 μg, at least 68 μg, at least 70 μg, at least 72 μg, at least 74 μg, at least 76 μg, at least 78 μg, at least 80 μg, at least 82 μg, at least 84 μg, at least 86 μg, at least 88 μg, at least 90 μg, at least 92 μg, at least 94 μg, at least

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96 μg, at least 98 μg, at least 100 μg, at least 102 μg, at least 104 μg, at least 106 μg, at least 108 μg, at least 110 μg, at least 112 μg, at least 114 μg, at least 116 μg, at least 118 μg, at least 120 μg, at least 122 μg, at least 124 μg, at least 126 μg, at least 128 μg, at least 130 μg at least 132 μg, at least 134 μg, at least 136 μg, at least 138 μg, at least 140 μg, at least 142 μg, at least 144 μg, at least 146 μg, at least 148 μg, at least 150 μg, at least 152 μg, at least 154 μg, at least 156 μg, at least 158 μg, at least 160 μg, at least 162 μg, at least 164 μg, at least 166 μg, at least 168 μg, at least 170 μg, at least 172 μg, at least 174 μg, at least 176 μg, at least 178 μg, at least 180 μg, at least 182 μg, at least 184 μg, at least 186 μg, at least 188 μg, at least 190 μg, at least 192 μg, at least 194 μg, at least 196 μg, at least 198 μg, or at least 200 μg, e.g., in a buffered solution.

The compositions provided herein can be, e.g., formulated to be compatible with their intended route of administration. A non-limiting example of an intended route of administration is local administration (e.g., intra-cochlear administration).

In some embodiments, the therapeutic compositions are formulated to include a lipid nanoparticle. In some embodiments, the therapeutic compositions are formulated to include a polymeric nanoparticle. In some embodiments, the therapeutic compositions are formulated to comprise a mini-circle DNA. In some embodiments, the therapeutic compositions are formulated to comprise a CELiD DNA. In some embodiments, the therapeutic compositions are formulated to comprise a synthetic perilymph solution. An exemplary synthetic perilymph solution includes 20-200 mM NaCl; 1-5 mM KCl; 0.1-10 mM $CaCl_2$; 1-10 mM glucose; 2-50 mM HEPES, having a pH of between about 6 and about 9.

Also provided are kits including any of the compositions described herein. In some embodiments, a kit can include a solid composition (e.g., a lyophilized composition including the at least two different vectors described herein) and a liquid for solubilizing the lyophilized composition. In some embodiments, a kit can include a pre-loaded syringe including any of the compositions described herein.

In some embodiments, the kit includes a vial comprising any of the compositions described herein (e.g., formulated as an aqueous composition, e.g., an aqueous pharmaceutical composition).

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In some embodiments, the kits can include instructions for performing any of the methods described herein.

Devices and Surgical Methods

Provided herein are therapeutic delivery systems for treating hearing loss and/or vision loss (e.g., Usher syndrome type III, retinitis pigmentosa). In one aspect, the therapeutic delivery systems include i) a medical device capable of creating one or a plurality of incisions in a round window membrane of an inner ear of a human subject in need thereof, and ii) an effective dose of a composition (e.g., any of the compositions described herein). In some embodiments, the medical device includes a plurality of micro-needles.

Also provided herein are surgical methods for treatment of hearing loss (e.g., Usher syndrome type III). In some embodiments, the methods include the steps of: introducing into a cochlea of a human subject a first incision at a first incision point; and administering intra-cochlearly a therapeutically effective amount of any of the compositions provided herein. In some embodiments, the composition is administered to the subject at the first incision point. In some embodiments, the composition is administered to the subject into or through the first incision.

In some embodiments of any of the methods described herein, any of the compositions described herein is administered to the subject into or through the cochlea oval window membrane. In some embodiments of any of the methods described herein, any of the compositions described herein is administered to the subject into or through the cochlea round window membrane. In some embodiments of any of the methods described herein, the composition is administered using a medical device capable of creating a plurality of incisions in the round window membrane. In some embodiments, the medical device includes a plurality of micro-needles. In some embodiments, the medical device includes a plurality of micro-needles including a generally circular first aspect, where each micro-needle has a diameter of at least about 10 microns. In some embodiments, the medical device includes a base and/or a reservoir capable of holding the composition. In some embodiments, the medical device includes a plurality of hollow micro-needles individually including a lumen capable of transferring the

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composition. In some embodiments, the medical device includes a means for generating at least a partial vacuum.

Also provided herein are surgical methods for treatment of vision loss (e.g., retinitis pigmentosa). In some embodiments, the methods include the steps of: administering intra-ocularly a therapeutically effective amount of any of the compositions provided herein.

The invention is further described in detail by reference to the following experimental examples. These examples are provided for purposes of illustration only, and are not intended to be limiting unless otherwise specified. Thus, the invention should in no way be construed as being limited to the following examples, but rather should be construed to encompass any and all variations that become evident as a result of the teaching provided herein.

Without further description, it is believed that one of ordinary skill in the art can, using the preceding description and the following illustrative examples, make and utilize the compounds of the present invention and practice the claimed methods. The following working examples specifically point out various aspects of the present invention, and are not to be construed as limiting in any way the remainder of the disclosure.

EXAMPLES

Example 1: Construction of Viral Vectors

Recombinant AAV is generated by transfection with an adenovirus-free method as used by Xiao et al. *J. Virol.* 73(5):3994-4003, 1999. The cis plasmids with AAV ITRs, the trans plasmid with AAV Rep and Cap genes, and a helper plasmid with an essential region from an adenovirus genome are co-transfected in 293 cells in a ratio of 1:1:2. The AAV vectors used here express human CLRN1 or mouse CLRN1 under multiple dual vector strategies using the constructs described below. AAV serotypes 1, 2, 3, 4, 5, 6, 7, 8, 9, rh8, rh10, rh39, rh43, and Anc80 are each prepared to encapsulate three sets of CLRN1 constructs to test (i) a concatemerization-transplicing strategy, (ii) a hybrid intronic-homologous recombination-transplicing strategy, and (iii) an exonic homologous recombination strategy, as summarized by Pryadkina et al., *Meth. Clin. Devel.* 2:15009, 2015.

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Example 2: Generating and Purifying Viral Particles

Recombinant AAV-1 is produced using a triple transfection protocol and purified by two sequential cesium chloride (CsCl) density gradients, as described by Pryadkina et al., *Mol. Ther.* 2:15009, 2015. At the end of second centrifugation, 11 fractions of 500 µl are recovered from the CsCl density gradient tube and purified through dialysis in 1× PBS. The fractions are analyzed by dot blot to determine those containing rAAV genomes. The viral genome number (vg) of each preparation is determined by a quantitative real-time PCR-based titration method using primers and probe corresponding to the ITR region of the AAV vector genome (Bartoli et al. *Gene. Ther.* 13:20-28, 2006).

Example 3: Formulation of Viral Particles

AAV produced at a titer of 1e14 vg / mL is prepared at dilutions of 3.2e13, 1.0e13, 3.2e12, 1.0e12 vg/mL in artificial perilymph. Artificial perilymph is prepared by combining the following reagents: NaCl, 120 mM; KCl, 3.5 mM; $CaCl_2$, 1.5 mM; glucose, 5.5 mM; HEPES, 20 mM. The artificial perilymph is titrated with NaOH to adjust its pH to 7.5 (total Na^+ concentration of 130 mM) (Chen et al., *J. Controlled Rel.* 110:1-19, 2005).

Example 4: Device Description

The AAV- CLRN1 formulation is delivered to the cochlea using a specialized microcatheter designed for consistent and safe penetration of the round window membrane (RWM). The microcatheter is shaped such that the surgeon performing the delivery procedure can enter the middle ear cavity via the external auditory canal and contact the end of the microcatheter with the RWM. The distal end of the microcatheter is comprised of at least one microneedle with diameter between 10 and 1,000 microns, which produces perforations in the RWM that are sufficient to allow AAV- CLRN1 to enter the cochlear perilymph of the scala tympani at a rate of approximately 1 uL/min, but small enough to heal without surgical repair. The remaining portion of the microcatheter, proximal to the microneedle(s), is loaded with the AAV- CLRN1 /artificial perilymph formulation at a titer of approximately 1e13 vg/mL. The proximal

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end of the microcatheter is connected to a micromanipulator that allows for precise, low volume infusions of approximately 1 TL / min.

Example 5: Animal Model 1A: Surgical Method in Aged Mice

AAV- CLRN1 prepared in artificial perilymph is administered to the scala tympani in mice as described by Shu et al. (*Human Gene Therapy*, doi:10.1089/hum.2016.053, June 2016,). Six-week-old male mice are anesthetized using an intraperitoneal injection of xylazine (20 mg/kg) and ketamine (100 mg/kg). Body temperature is maintained at 37 ˚C using an electric heating pad. An incision is made from the right post-auricular region and the tympanic bulla is exposed. The bulla is perforated with a surgical needle and the small hole is expanded to provide access to the cochlea. The bone of the cochlear lateral wall of the scala tympani is thinned with a dental drill so that the membranous lateral wall is left intact. A Nanoliter Microinjection System in conjunction with glass micropipette is used to deliver a total of approximately 300 nL of AAV- CLRN1 in artificial perilymph to the scala tympani at a rate of 2 nL/second. The glass micropipette is left in place for 5 minutes post-injection. Following cochleostomy and injection, the opening in the tympanic bulla is sealed with dental cement, and the muscle and skin are sutured. The mice are allowed to awaken from anesthesia and their pain is controlled with 0.15 mg/kg buprenorphine hydrochloride for 3 days.

Example 6: Animal Model 2: Reciprocating Micropump in Guinea Pig

Surgical Procedure

AAV- CLRN1 prepared in artificial perilymph is administered to guinea pigs to assess distribution and toxicity following intracochlear delivery with a reciprocating micropump as descrbied by Tandon et al., *Lab Chip*, DOI: 10.1039/c5lc01396h, 2015. Male guinea pigs weighing approximately 350 g each (n=16) are anesthetized with a combination of pentobarbital sodium (Nembutal; 25 mg kg−1, injected intraperitoneally), fentanyl (0.2 mg kg−1, intramuscularly), and haloperidol (10 mg kg−1, intramuscularly). Lidocaine with epinephrine is given subcutaneously at the incision site as a topical anesthetic. Using a dorsal approach, a 5 mm diameter hole is made in the bulla and a

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cochleostomy is created approximately 0.5 mm distal to the round window membrane. The cannula of the micropump (described below) is inserted into the cochleostomy, threaded into the cochlea 3 mm apically, and glued to the bulla with a common cyanoacrylate glue. For compound action potential (CAP) measurements, a perfluoroalkoxy-alkane-insulated silver wire electrode (203 μm uncoated diameter) is inserted near the round window niche and glued to the bulla.

Procedures for measurement of distortion product otoacoustic emissions (DPOAEs) and CAPs are performed as previously described in Tandon et al. *Biomed Microdevices* 17:3-21, 2015. DPOAEs are measured before and after the cochleostomy procedure at the characteristic frequencies: 32, 24, 16, 12, 8, 5.6, 4, and 2.78 kHz in order to monitor any damage that occurs as a result of the surgery.

AAV- CLRN1 at a maximum titer of 1e14 vg/mL is administered to the guinea pig using a micropump as described by Tandon et al. *Lab Chip*, DOI: 10.1039/c5lc01396h, 2015. The micropump system has 4 selectable ports. These ports are connected to: (i) a large fluidic capacitor used for artificial perilymph storage; (ii) an outlet that connects to the cochlea; (iii) the outlet from an integrated AAV- CLRN1 reservoir; (iv) the inlet to the integrated AAV- CLRN1 reservoir. Each port is fluidically connected to a central pump chamber, and each is individually addressed with a valve. The sequence of events for reciprocating AAV- CLRN1 delivery is as follows: (i) an internal AAV- CLRN1 -refresh loop is run, transferring AAV- CLRN1 from the AAV-CLRN1 reservoir into the main infuse–withdraw line; (ii) AAV- CLRN1 is infused into the cochlea and some artificial perilymph is drained from the artificial perilymph storage capacitor; (iii) the first two steps can be repeated several times for additional doses; (iv) after the AAV- CLRN1 has been allowed to diffuse for some time, a volume of perilymph is withdrawn from the cochlea that is equal to the volume infused in steps (i)–(iii), refilling the artificial perilymph storage capacitor. This process results in net delivery of drug with zero net fluid volume added to the cochlea.

The fluidic capacitors in the micropump are cylindrical chambers whose ceilings are a thin (25.4 μm), flexible, polyimide membrane. The pump chamber has a diameter of 3.5 mm, the fluidic storage capacitor has a diameter of 14 mm, and all of the remaining capacitors have diameters of 4 mm. The same membrane is deflected to block flow at

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each of the valves. The valve chambers have diameters of 3.1 mm. The serpentine channel that comprises the drug reservoir has a square cross section of width 762 μm and a length of 410 mm for a total volume of 238 μL. All of the other microchannels in the pump have a width of 400 μm and a height of 254 μm.

Acute drug delivery in guinea pigs

The micropump is loaded with AAV- CLRN1 and artificial perilymph, and the cannula inserted into a cochleostomy made in the region of the cochlea between the locations with characteristic frequency sensitivity of 24 and 32 kHz, and threaded apically 3 mm, terminating in the 12–16 kHz region. Baseline DPOAE and CAP hearing tests are performed prior to the start of AAV- CLRN1 /artificial perilymph infusion. The pump is then activated and approximately 1 μL of artificial perilymph is infused every 5 min until a total of approximately 10 μL of artificial perilymph is delivered to the cochlea. After a 20 min wait time, approximately 10 μL of perilymph is withdrawn from the cochlea. AAV- CLRN1 delivery is then initiated at a rate of approximately 1 μL every 5 min until a total of approximately 10 μL of fluid delivered.

Animals are sacrificed at 1 week, 1 month, 3 months, and 6 months post-treatment (n=4 per group) and their cochleae extracted. Extent of AAV transduction and CLRN1 expression along the organ of Corti is assessed via immunostaining with anti-CLRN1 antibodies. Antibodies against markers for hair cells (Myo7a) and supporting cells (Sox2) are used to quantify IHCs, OHCs, supporting cells and stereocilia morphology. Annexin V staining is used to assess evidence of apoptosis in cells along the cochlear sensory epithelium.

Example 7: Animal Model 3: Sheep

AAV- CLRN1 prepared in artificial perilymph is administered to juvenile sheep to assess distribution and toxicity following delivery to the cochlea via trans-RWM infusion. Baseline auditory brainstem response (ABR) and distortion product optoacoustic emissions (DPOAEs) are measured in female sheep at 3 months of age (n=40), bilaterally, to assess pre-treatment inner hair cell (IHC) and outer hair cell (OHC) function. Following baseline ABR and DPOAE measurements, 20 uL of AAV1- CLRN1

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at titers of 1.0e14, 3.2e13, 1.0e13 and 3.2e12 vg/mL is injected into the left scala tympani of the sheep (n=10 per group). Each animal's right ear is left as an untreated control. ABR and DPOAE measurements are taken again bilaterally 1, 5 and 10 days following the surgical procedure. At 6 months post-procedure, additional bilateral ABR and DPOAE measurements are taken from all animals, and the animals are subsequently sacrificed and their cochleae removed.

In half of the sacrificed animals (n=5 from each of the dose cohorts), immunostaining is performed to identify hair cell structures and to assess CLRN1 protein expression along the cochlear sensory epithelium. Antibodies against markers for hair cells (Myo7a), supporting cells (Sox2) and CLRN1 are used as described previously (Duncker et al. 2013, *J Neurosci* 33(22):9508-9519). At the basal, middle and apical turns of the organ of corti, total numbers of hair cells and those hair cells expressing CLRN1 are counted within 200 um regions.

In the remaining half of the sacrificed animals (remaining 5 animals from each dose cohort), cochlear tissue samples are collected from the same basal, middle and apical regions as described above, and assayed for CLRN1 mRNA transcript.

Example 8: Human Clinical Example (Pediatric Treatment)

The patient is put under general anesthesia. The surgeon approaches the tympanic membrane from external auditory canal, makes a small incision at the inferior edge of the external auditory canal where it meets the tympani membrane, and lifts the tympanic membrane as a flap to expose the middle ear space. A surgical laser is used to make a small opening (approximately 2 mm) in the stapes footplate. The surgeon then penetrates the round window membrane with a microcatheter loaded with a solution of AAV-CLRN1 prepared in artificial perilymph at a titer of 1e13 vg/mL. The microcatheter is connected to a micromanipulator that infuses approximately 20 uL of the AAV- CLRN1 solution at a rate of approximately 1 uL / min. At the conclusion of the AAV- CLRN1 infusion, the surgeon withdraws the microcatheter and patches the holes in the stapes foot plate and RWM with a gel foam patch. The procedure concludes with replacement of the tympanic membrane flap.

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Example 9: Non-Invasive Prenatal Testing of Maternal Blood to Detect CLRN1 Mutation

Maternal blood samples (20-40 mL) are collected into Cell-free DNA tubes. At least 7 mL of plasma is isolated from each sample via a double centrifugation protocol of 2,000 g for 20 minutes, followed by 3,220 g for 30 minutes, with supernatant transfer following the first spin. cfDNA is isolated from 7-20 mL plasma using a QIAGEN QIAmp® Circulating Nuclei Acid kit and eluted in 45 μL TE buffer. Pure maternal genomic DNA is isolated from the buffy coat obtained following the first centrifugation.

By combining thermodynamic modeling of the assays to select probes with minimized likelihood of probe-probe interaction with amplification approaches described previously (Stiller et al., *Genome Res.* 19(10):1843-1848, 2009), multiplexing of 11,000 assays can be achieved. Maternal cfDNA and maternal genomic DNA samples are pre-amplified for 15 cycles using 11,000 target-specific assays and an aliquot is transferred to a second PCR reaction of 15 cycles using nested primers. Samples are prepared for sequencing by adding barcoded tags in a third 12-cycle round of PCR. The amplicons are then sequenced using an Illumina HiSeq sequencer. Genome sequence alignment is performed using commercially available software.

Example 10: Adenovirus (AAV) Trans-Splicing Strategy

At least two different nucleic acid vectors (e.g., AAV vectors) can be used to reconstitute an active CLRN1 gene (e.g., a full-length CLRN1 gene) within a cell following intermolecular concatamerization and trans-splicing. See, e.g., Yan et al., *Proc. Natl. Acad. Sci. U.S.A.* 97:12; 6716-6721, 2000, incorporated in its entirety herein.

In some examples, two different nucleic acid vectors will be used. A first nucleic acid vector can include a promoter (e.g., any of the promoters described herein), a first coding sequence that encodes an N-terminal portion of a CLRN1 protein positioned 3' of the promoter (e.g., any of the sizes of a portion of a CLRN1 protein described herein and/or any of the N-terminal portions of a CLRN1 protein described herein), and a splice donor sequence positioned at the 3' end of the first coding sequence. A second nucleic acid vector can include a splice acceptor sequence, a second coding sequence that encodes a C-terminal portion of a CLRN1 protein (i.e., the entire portion of the CLRN1

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protein that is not included in the N-terminal portion) positioned at the 3' end of the splice acceptor sequence (e.g., any of the sizes of a portion of a CLRN1 protein described herein and/or any of the C-terminal portions of a CLRN1 protein described herein), and a polyadenylation sequence at the 3' end of the second coding sequence (e.g., any of the polyadenylation seqences described herein). In some embodiments, each of the encoded portions is at least 30 amino acid residues in length (e.g., at least 50 amino acids, at least 75 amino acids, or at least 100 amino acids in length), the amino acid sequence of each of the encoded portions does not overlap with the sequence of the other encoded portion, and no single vector of the two different vectors encodes an active CLRN1 protein (e.g., a full-length CLRN1 protein). When introduced into a mammalian cell (e.g., any of the mammalian cells described herein) splicing occurs between the splice donor sequence and the splice acceptor sequence, thereby forming a recombined nucleic acid that encodes an active CLRN1 protein (e.g., a full-length CLRN1 protein).

In another example, three different nucleic acid vectors can be used. A first nucleic acid vector can include a portion of a promoter sequence (e.g., any of the promoter sequences described herein), a first coding sequence of a CLRN1 gene that encodes a first portion of a CLRN1 protein (e.g., any of the CLRN1 coding sequences described herein) positioned 3' of the promoter, and a first splice donor sequence positioned at the 3' end of the first coding sequence. A second nucleic acid vector can include a first splice acceptor sequence, a second coding sequence of a CLRN1 gene that encodes a second portion of a CLRN1 protein positioned at the 3' end of the first splice acceptor sequence, and a second splice donor sequence positioned at the 3' end of the second coding sequence (e.g., any of the splicedonor sequences described herein). A feature of the second nucleic acid vector will be that self-splicing cannot occur (i.e., splicing will not occur between the second splice donor sequence and the first splice acceptor sequence of the second nucleic acid vector). In some embodiments, the splice donor sequence of the first nucleic acid vector and the second splice donor sequence of the second nucleic acid vector are the same (e.g., any of the splice donor sequences described herein or known in the art). In some embodiments, the first splice donor sequence of the first nucleic acid vector and the second splice donor sequence of the second nucleic acid vector are different (e.g., any of the splice donor sequences described

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herein or known in the art). A third nucleic acid vector will include a second splice acceptor sequence, a third coding sequence of a CLRN1 gene that encodes a third portion of a CLRN1 protein positioned at the 3' end of the second splice acceptor sequence, and a polyadenylation sequence positioned at the 3' end of the third coding sequence (e.g., any of the polyadenylation sequences described herein). In such methods where three nucleic acid vectors are used, the first splice donor sequence and the first splice acceptor sequence can assemble together (recombine) and the second splice donor sequence and the second splice acceptor sequence can assemble together (recombine), and the portion of CLRN1 protein encoded by the first, second, and third coding sequences do not overlap, and when introduced into a mammalian cell (e.g., any of the mammalian cells described herein), splicing occurs between the first splice donor sequence and the first splice acceptor sequence, and between the second splice donor sequence and the second splice acceptor sequence, to form a recombined nucleic acid that encodes an active CLRN1 protein (e.g., a full-length CLRN1 protein). Based on the strategies provided above, one skilled in the art would understand how to develop a strategy using four, five, or six different nucleic acid vectors.

In any of the examples of these methods, none of the amino acid sequences of the encoded portions overlap with any other encoded portion, and no single vector encodes an active CLRN1 protein (e.g., a full-length CLRN1 protein).

Each of the at least two different vectors includes a coding sequence that encodes a different portion of a CLRN1 protein, and each of the encoded portions can be at least 30 amino acids (e.g., between about 30 amino acids to about 1200 amino acids, or any of the other subranges of this range described herein).

In some embodiments, each of the coding sequences can include at least one exon and at least one intron of SEQ ID NO: 9 (e.g., at least two exons and at least one intron, at least two exons and at least two introns, at least three exons and at least one intron, at least three exons and at least two introns, or at least three exons and at least three introns). In some embodiments, each of the at least two different vectors includes a coding sequence that encodes a different portion of a CLRN1 protein, and each of the encoded portions can encode up to 80% of the amino acid sequence of SEQ ID NO: 1 (e.g., up to 10%, up to 20%, up to 30%, up to 40%, up to 50%, up to 60%, or up to 70%

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of SEQ ID NO: 1) such that each of the encoded portions is non-overlapping. In some embodiments, each of the at least two different vectors includes a coding sequence that encodes a different portion of a CLRN1 protein, each of the encoded portions encoding up to 80% of the amino acid sequence of SEQ ID NO: 3 (e.g., up to 10%, up to 20%, up to 30%, up to 40%, up to 50%, up to 60%, or up to 70% of SEQ ID NO: 3), provided that each of the encoded portions is non-overlapping with any other. In some embodiments, each of the at least two different vectors includes a coding sequence that encodes a different portion of a CLRN1 protein, each of the encoded portions encoding up to 80% of the amino acid sequence of SEQ ID NO: 5 (e.g., up to 10%, up to 20%, up to 30%, up to 40%, up to 50%, up to 60%, or up to 70% of SEQ ID NO: 5), provided that each of the encoded portions is non-overlapping with any other. In some embodiments, each of the at least two different vectors includes a coding sequence that encodes a different portion of a CLRN1 protein, each of the encoded portions encoding up to 80% of the amino acid sequence of SEQ ID NO: 7 (e.g., up to 10%, up to 20%, up to 30%, up to 40%, up to 50%, up to 60%, or up to 70% of SEQ ID NO: 7), provided that each of the encoded portions is non-overlapping with any other.

Each of the at least two nucleic acid vectors may further include an inverted terminal repeat (ITR) to allow head-to-tail recombination. The ITR will be subsequently removed via splicing. For example, the ITR could be a palindromic double-D ITR as described in Yan et al., *Proc. Natl. Acad. Sci. U.S.A.* 97(12):6716-6721, 2000, incorporated in its entirety herein, or an AAV serotype-2 ITR as described in Gosh et al., *Mol. Ther.* 16:124-130, 2008, and Gosh et al., *Human Gene Ther.* 22: 77-83, 2011. Non-limiting examples of splice acceptor and/or donor sequences are known in the art. See, e.g., Reich et al., *Human Gene Ther.* 14(1):37-44, 2003, and Lai et al. (2005) *Nat. Biotechnol.* 23(11):1435-1439, 2005, 2005. The splice donor and acceptor sequences can be any endogenous intron splice donor/acceptor sequence of a gene (e.g., a CLRN1 gene). For example, the splice donor sequence can be: 5'-GTAAGTATCAAGGTTACAAGACAGGTTTAAGGAGACCAATAGA AACTGGGCTTGTCGAGACAGAGAAGACTCTTGCGTTTCT-3' (SEQ ID NO: 22) and the splice acceptor sequence can be 5'-GATAGGCACCTATTGGTCTTACTG ACATCCACTTTGCCTTTCTCTCCACAG-3' (SEQ ID NO: 23) (see, e.g., Trapani et

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al., *EMBO Mol. Med.* 6(2):194-211, 2014). Methods of evaluating splicing and splicing efficiency are known in the art (see, e.g., Lai et al., *Nat. Biotechnol.* 23(11): 1435-1439, 2005).

Example 11: Hybrid Vector Trans-Splicing Strategy using an alkaline phosphatase (AP) highly recombinogenic exogenous gene region

At least two (e.g., two, three, four, five, or six) different nucleic acid vectors (e.g., AAV vectors) can also be used in any of the methods described herein to reconstitute an active CLRN1 gene (e.g., a full-length CLRN1 gene) within a cell following intermolecular concatamerization, marker gene-mediated recombination, and trans-splicing. This strategy is a hybrid strategy as it will include homologous recombination and/or trans-splicing. See, e.g., Gosh et al., *Mol. Ther.* 16: 124-130, 2008; Gosh et al., *Human Gene Ther.* 22: 77-83, 2011; and Duan et al., *Mol. Ther.* 4: 383-391, 2001, each incorporated in its entirety herein. As used herein, a detectable marker gene can be a highly recombinogenic DNA sequence that will allow for coding sequence-independent recombination. An non-limiting example of a detectable marker gene is an alkaline phosphatase (AP) gene. For example, the detectable marker gene can be the middle one-third of the human placental AP complementary DNA, which is 872 bp in length (see, e.g., Gosh et al., 2008). At least two different nucleic acid vectors will contain a detectable marker gene (e.g., any of the detectable marker genes described herein). Since the hybrid vector will be constructed based on a trans-splicing vector as described in Example 10, an active CLRN1 gene (e.g., a full-length CLRN1 gene) may be reconstituted using either ITR-mediated recombination and trans-splicing or detectable marker gene-mediated (e.g., AP-gene mediated) recombination and trans-splicing. After trans-splicing, an active CLRN1 gene (e.g., a full-length CLRN1 gene) will be reconstituted in the genomic DNA of a mammalian cell (e.g., any mammalian cell described herein).

In one example, two different nucleic acid vectors will be used. A first nucleic acid vector can include a promoter (e.g., any of the promoters described herein), a first coding sequence that encodes an N-terminal portion of a CLRN1 protein positioned 3' of the promoter (e.g., any of the sizes of a portion of a CLRN1 protein described herein

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and/or any of the N-terminal portions of a CLRN1 protein described herein), a splice donor sequence positioned at the 3' end of the first coding sequence, and a first detectable marker gene positioned 3' of the splice donor sequence. A second nucleic acid vector can include a second detectable marker gene, a splice acceptor sequence positioned 3' of the second detectable marker gene, a second coding sequence that encodes a C-terminal portion of a CLRN1 protein positioned at the 3' end of the splice acceptor sequence (e.g., any of the sizes of a portion of a CLRN1 protein described herein and/or any of the C-terminal portions of a CLRN1 protein described herein), and a polyadenylation sequence at the 3' end of the second coding sequence (e.g., any of the polyadenylation sequences described herein). In some embodiments, each of the encoded portions is at least 30 amino acid residues in length (e.g., at least 50 amino acids, at least 75 amino acids, or at least 100 amino acids in length), the amino acid sequences of the encoded portions do not overlap, and no single vector of the two different vectors encodes an active CLRN1 protein (e.g., a full-length CLRN1 protein). When introduced into a mammalian cell (e.g., any of the mammalian cells described herein) splicing occurs between the splice donor sequence and the splice acceptor sequence, thereby forming a recombined nucleic acid that encodes an active CLRN1 protein (e.g., a full-length CLRN1 protein).

In another example, three different nucleic acid vectors can be used. A first nucleic acid vector can include a portion of promoter sequence (e.g., any of the promoter sequences described herein), a first coding sequence of a CLRN1 gene that encodes a first portion of a CLRN1 protein (e.g., any of the CLRN1 coding sequences described herein) positioned 3' of the promoter, a first splice donor sequence positioned at the 3' end of the first coding sequence, and a first detectable marker gene. A second nucleic acid vector can include a second detectable marker gene, a first splice acceptor sequence positioned 3' of the second detectable marker gene, a second coding sequence of a CLRN1 gene that encodes a second portion of a CLRN1 protein positioned at the 3' end of the first splice acceptor sequence, a second splice donor sequence positioned at the 3' end of the second coding sequence (e.g., any of the splice donor sequences described herein), and a third detectable marker gene. A feature of the second nucleic acid vector will be that self-splicing cannot occur (i.e., splicing will not occur between the second splice donor sequence and the first splice acceptor sequence of the second nucleic acid

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vector). In some embodiments, the splice donor sequence of the first nucleic acid vector and the second splice donor sequence of the second nucleic acid vector are the same (e.g., any of the splice donor sequences described herein or known in the art). In some embodiments, the first splice donor sequence of the first nucleic acid vector and the second splice donor sequence of the second nucleic acid vector are different (e.g., any of the splice donor sequences described herein or known in the art). A third nucleic acid vector can include a fourth detectable marker gene, a second splice acceptor sequence positioned 3' of the fourth detectable marker gene, a third coding sequence of a CLRN1 gene that encodes a third portion of a CLRN1 protein positioned at the 3' end of the second splice acceptor sequence, and a polyadenylation sequence positioned at the 3' end of the third coding sequence (e.g., any of the polyadenylation sequences described herein). In such methods where three nucleic acid vectors are used, the first splice donor sequence and the first splice acceptor sequence can assemble together (recombine) and the second splice donor sequence and the second splice acceptor sequence can assemble together (recombine), and the portions of CLRN1 protein encoded by the first, second, and third coding sequences do not overlap with each other, and when introduced into a mammalian cell (e.g., any of the mammalian cells described herein), splicing occurs between the first splice donor sequence and the first splice acceptor sequence, and between the second splice donor sequence and the second splice acceptor sequence, to form a recombined nucleic acid that encodes an active CLRN1 protein (e.g., a full-length CLRN1 protein). As can be appreciated in the art, when three nucleic acid vectors are used, two of the at least two different nucleic acid vectors can include a detectable marker gene (e.g., an AP marker gene) and one of the at least two different nucleic acid vectors may include a splice acceptor sequence that is complementary to a splice donor sequence in a nucleic acid vector that includes a detectable marker gene. For example, in some embodiments, the first and second nucleic acid vectors can include a detectable marker gene (e.g., an AP marker gene), and the third nucleic acid vector will include a splice acceptor sequence that is complementary to the splice donor sequence in the second nucleic acid vector, and the third nucleic acid vector will not include a detectable marker gene (e.g., an AP marker gene). In other examples, the second and third nucleic acid vector can include a detectable marker gene (e.g., an AP marker gene), and the first

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nucleic acid vector will include a splice donor sequence that is complementary to the splice acceptor sequence in the second nucleic acid vector and the first nucleic acid vector will not include a detectable marker gene (e.g., an AP marker gene).

Based on the strategies provided above, one skilled in the art would understand how to develop a strategy using four, five, or six vectors.

The CLRN1 coding sequences provided in the at least two nucleic acid vectors (e.g., two, three, four, five or six) will not be overlapping. Each of the at least two different vectors can include a coding sequence that encodes a different portion of a CLRN1 protein, each of the encoded portions being, e.g., at least 30 amino acids (e.g., about 30 amino acids to about 1600 amino acids, or any of the other subranges of this range described herein).

In some embodiments, each of the at least two different vectors includes a coding sequence that encodes a different portion of a CLRN1 protein, each of the encoded portions encoding at least one exon and at least one intron of SEQ ID NO: 9 (e.g., at least two exons and at least one intron, at least two exons and at least two introns, at least three exons at least one intron, at least three exons and at least two introns, or at least three exons and at least three introns). In some embodiments, each of the at least two different vectors include a coding sequence that encodes a different portion of a CLRN1 protein, each of the encoded portions encoding up to 80% of SEQ ID NO: 1 (e.g., up to 10%, up to 20%, up to 30%, up to 40%, up to 50%, up to 60%, up to 70% of SEQ ID NO: 1), provided that each of the encoded portions is non-overlapping with any other. In some embodiments, each of the at least two different vectors include a coding sequence that encodes a different portion of a CLRN1 protein, each of the encoded portions encoding up to 80% of SEQ ID NO: 3 (e.g., up to 10%, up to 20%, up to 30%, up to 40%, up to 50%, up to 60%, up to 70% of SEQ ID NO: 3), provided that each of the encoded portions is non-overlapping with any other. In some embodiments, each of the at least two different vectors include a coding sequence that encodes a different portion of a CLRN1 protein, each of the encoded portions encoding up to 80% of SEQ ID NO: 5 (e.g., up to 10%, up to 20%, up to 30%, up to 40%, up to 50%, up to 60%, up to 70% of SEQ ID NO: 5), provided that each of the encoded portions is non-overlapping with any other. In some embodiments, each of the at least two different vectors include a coding

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sequence that encodes a different portion of a CLRN1 protein, each of the encoded portions encoding up to 80% of SEQ ID NO: 7 (e.g., up to 10%, up to 20%, up to 30%, up to 40%, up to 50%, up to 60%, up to 70% of SEQ ID NO: 7), provided that each of the encoded portions is non-overlapping with any other.

As described in Example 10, each of the at least two nucleic acid vectors may further include an inverted terminal repeat (ITR) to allow head-to-tail recombination. The ITR will be subsequently removed via splicing. Examples of ITRs and splice acceptor sequences and/or splice donor sequences are known in the art and have been described in Example 10.

Example 12: Hybrid Vector Trans-Splicing Strategy using a F1 phage highly recombinogenic exogenous gene region (AK)

At least two (e.g., two, three, four, five, or six) different nucleic acid vectors (e.g., AAV vectors) can also be used in any of the methods described herein to reconstitute an active CLRN1 gene (e.g., a full-length CLRN1 gene) within a cell following intermolecular concatamerization, marker gene-mediated recombination, and trans-splicing. This strategy is a hybrid strategy as it will include homologous recombination and/or trans-splicing. See, e.g., Trapani et al., *EMBO Mol. Med.* 6(2):194-211, 2014, incorporated in its entirety herein. As used herein, an F1 phage recombinogenic region (AK) will be used to allow coding sequence-independent recombination. The F1 phage recombinogenic region may be a 77 bp recombinogenic region from the F1 phage genome as described in Trapani et al. (2014). At least two different nucleic acid vectors will contain an F1 phage recombinogenic region. Since the hybrid vector will be constructed based on a trans-splicing vector as described in Example 10, a nucleic acid encoding an active CLRN1 protein (e.g., a full-length CLRN1 protein) may be generated using F1 phage recombinogenic region-induced recombination and trans-splicing. After trans-splicing, a nucleic acid encoding an active CLRN1 protein (e.g., a full-length CLRN1 protein) will be generated in a mammalian cell (e.g., any of the mammalian cells described herein).

In one example, two different nucleic acid vectors will be used. A first nucleic acid vector can include a promoter (e.g., any of the promoters described herein), a first

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coding sequence that encodes an N-terminal portion of a CLRN1 protein positioned 3' of the promoter (e.g., any of the sizes of a portion of a CLRN1 protein described herein and/or any of the N-terminal portions of a CLRN1 protein described herein), a splice donor sequence positioned at the 3' end of the first coding sequence, and an F1 phage recombinogenic region positioned 3' of the splice donor sequence. A second nucleic acid vector can include an F1 phage recombinogenic region, a splice acceptor sequence positioned 3' of the F1 phage recombinogenic region, a second coding sequence that encodes a C-terminal portion of a CLRN1 protein positioned at the 3' end of the splice acceptor sequence (e.g., any of the sizes of a portion of a CLRN1 protein described herein and/or any of the C-terminal portions of a CLRN1 protein described herein), and a polyadenylation sequence at the 3' end of the second coding sequence (e.g., any of the polyadenylation sequences described herein). In some embodiments, each of the encoded portions is at least 30 amino acid residues in length (e.g., at least 50 amino acids, at least 75 amino acids, or at least 100 amino acids in length), the amino acid sequence of each of the encoded portions do not overlap, and no single vector of the two different vectors encodes an active CLRN1 protein (e.g., a full-length CLRN1 protein). When introduced into a mammalian cell (e.g., any of the mammalian cells described herein) splicing occurs between the splice donor sequence and the splice acceptor sequence, thereby forming a recombined nucleic acid that encodes an active CLRN1 protein (e.g., a full-length CLRN1 protein).

In another example, three different nucleic acid vectors will be used. A first nucleic acid vector can include a promoter sequence (e.g., any of the promoter sequences described herein), a first coding sequence that encodes a first portion of a CLRN1 protein (e.g., any of the CLRN1 coding sequences described herein) positioned 5' of the promoter, a first splice donor sequence positioned at the 3' end of the first coding sequence, and an F1 phage recombinogenic region. A second nucleic acid vector can include an F1 phage recombinogenic region, a first splice acceptor sequence positioned 3' of the F1 phage recombinogenic region, a second coding sequence that encodes a second portion of a CLRN1 protein positioned at the 3' end of the first splice acceptor sequence, a second splice donor sequence positioned at the 3' end of the second coding sequence (e.g., any of the splice donor sequences described herein), and an F1 phage

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recombinogenic region. A feature of the second nucleic acid vector will be that self-splicing cannot occur (i.e., splicing will not occur between the second splice donor sequence and the first splice acceptor sequence of the second nucleic acid vector). In some embodiments, the splice donor sequence of the first nucleic acid vector and the second splice donor sequence of the second nucleic acid vector are the same (e.g., any of the splice donor sequences described herein or known in the art). In some embodiments, the first splice donor sequence of the first nucleic acid vector and the second splice donor sequence of the second nucleic acid vector are different (e.g., any of the splice donor sequences described herein or known in the art). A third nucleic acid vector can include an F1 phage recombinogenic region, a second splice acceptor sequence positioned 3' of the F1 phage recombinogenic region, a third coding sequence that encodes a third portion of a CLRN1 protein positioned at the 3' end of the second splice acceptor sequence, and a polyadenylation sequence positioned at the 3' end of the third coding sequence (e.g., any of the polyadenylation sequences described herein). In such methods where three nucleic acid vectors are used, the first splice donor sequence and the first splice acceptor sequence can assemble together (recombine) and the second splice donor sequence and the second splice acceptor sequence can assemble together (recombine), and the portion of CLRN1 protein encoded by the first, second, and third coding sequences do not overlap, and when introduced into a mammalian cell (e.g., any of the mammalian cells described herein), splicing occurs between the first splice donor sequence and the first splice acceptor sequence, and between the second splice donor sequence and the second splice acceptor sequence, to form a recombined nucleic acid that encodes an active CLRN1 protein (e.g., a full-length CLRN1 protein). As can be appreciated in the art when three nucleic acid vectors are used, two of the different nucleic acid vectors can include an F1 phage recombinogenic region and one of the different nucleic acid vectors may include a splice acceptor sequence that is complementary to a splice donor sequence in a nucleic acid vector that includes an F1 phage recombinogenic region. For example, in some embodiments, the first and second nucleic acid vectors can include an F1 phage recombinogenic region, and the third nucleic acid vector will include a splice acceptor sequence that is complementary to the splice donor sequence in the second nucleic acid vector, and the third nucleic acid vector will not include an F1 phage recombinogenic

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region (e.g., an AP marker gene). In other examples, the second and third nucleic acid vector can include an F1 phage recombinogenic region and the first nucleic acid vector will include a splice donor sequence that is complementary to the splice acceptor sequence in the second nucleic acid vector and the first nucleic acid vector will not include an F1 phage recombinogenic region. Based on the strategies provided above, one skilled in the art would understand how to develop a strategy using four, five, or six vectors.

The CLRN1 coding sequences provided in each of the at least two nucleic acid vectors (e.g., two, three, four, five or six) will not be overlapping. Each of the at least two different vectors include a coding sequence that encodes a different portion of a CLRN1 protein, each of the encoded portions being at least 30 amino acids (e.g., about 30 amino acids to about 1600 amino acids, or any of the subranges of this range described herein).

In some embodiments, each of the at least two different vectors include a coding sequence that encodes a different portion of a CLRN1 protein, each of the encoded portions encoding at least one exon and at least one intron of SEQ ID NO: 9 (e.g., at least two exons and at least one intron, at least two exons and at least two introns, at least three exons and at least one intron, at least three exons and at least two introns, or at least three exons and at least three introns). In some embodiments, each of the at least two different vectors includes a coding sequence that encodes a different portion of a CLRN1 protein, each of the encoded portions encoding up to 80% of SEQ ID NO: 1 (e.g., up to 10%, up to 20%, up to 30%, up to 40%, up to 50%, up to 60%, or up to 70% of SEQ ID NO: 1), provided that each of the encoded portions is non-overlapping. In some embodiments, each of the at least two different vectors include a coding sequence that encodes a different portion of a CLRN1 protein, each of the encoded portions encoding up to 80% of SEQ ID NO: 3 (e.g., up to 10%, up to 20%, up to 30%, up to 40%, up to 50%, up to 60%, or up to 70% of SEQ ID NO: 3), provided that each of the encoded portions is non-overlapping. In some embodiments, each of the at least two different vectors include a coding sequence that encodes a different portion of a CLRN1 protein, each of the encoded portions encoding up to 80% of SEQ ID NO: 5 (e.g., up to 10%, up to 20%, up to 30%, up to 40%, up to 50%, up to 60%, or up to 70% of SEQ ID NO: 5), provided that

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each of the encoded portions is non-overlapping. In some embodiments, each of the at least two different vectors include a coding sequence that encodes a different portion of a CLRN1 protein, each of the encoded portions encoding up to 80% of SEQ ID NO: 7 (e.g., up to 10%, up to 20%, up to 30%, up to 40%, up to 50%, up to 60%, or up to 70% of SEQ ID NO: 7), provided that each of the encoded portions is non-overlapping.

As described in Example 10, each of the at least two nucleic acid vectors may further include an inverted terminal repeat (ITR) to allow head-to-tail recombination. The ITR will be subsequently removed via splicing. Examples of ITRs and splice acceptor sequences and/or splice donor sequences are known in the art and have been described in Example 10.

Example 13. Hybrid Vector Trans-Splicing Strategy using Two CLRN1 Isoforms

At least two different nucleic acid vectors (e.g., AAV vectors) can be used to reconstitute an active CLRN1 gene (e.g., a full-length CLRN1 gene) within a cell following intermolecular concatamerization and trans-splicing. See, e.g., Yan et al., *Proc. Natl. Acad. Sci. U.S.A.* 97:12; 6716-6721, 2000, incorporated in its entirety herein.

In some examples, two different nucleic acid vectors will be used. A first nucleic acid vector can include a promoter (e.g., any of the promoters described herein), a first coding sequence that encodes an N-terminal portion of a CLRN1 protein positioned 3' of the promoter (e.g., any of the sizes of a portion of a CLRN1 protein described herein and/or any of the N-terminal portions of a CLRN1 protein described herein), and a splice donor sequence positioned at the 3' end of the first coding sequence.

A second nucleic acid vector can include a splice acceptor sequence, a second coding sequence that encodes a C-terminal portion of a CLRN1 protein (i.e., the entire portion of the CLRN1 protein that is not included in the N-terminal portion) positioned at the 3' end of the splice acceptor sequence (e.g., any of the sizes of a portion of a CLRN1 protein described herein and/or any of the C-terminal portions of a CLRN1 protein described herein), and a polyadenylation signal sequence at the 3' end of the second coding sequence (e.g., any of the polyadenylation seqences described herein).

In some embodiments, each of the encoded portions is at least 30 amino acid residues in length (e.g., at least 50 amino acids, at least 75 amino acids, or at least 100

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amino acids in length), the amino acid sequences of the two encoded portions do not overlap with each other; and no single vector of the two different vectors encodes an active CLRN1 protein (e.g., a full-length CLRN1 protein).

In some embodiments, each of the at least two different vectors includes a coding sequence that encodes a different portion of a first isoform of the CLRN1 protein (e.g., SEQ ID NO: 3). In some embodiments, each of the at least two different vectors includes a coding sequence that encodes a different portion of a CLRN1 protein, each of the encoded portions encoding up to 80% of the amino acid sequence of SEQ ID NO: 3 (e.g., up to 10%, up to 20%, up to 30%, up to 40%, up to 50%, up to 60%, or up to 70% of SEQ ID NO: 3), provided that each of the encoded portions is non-overlapping with any other.

In some embodiments, one of the at least two different nucleic acid vectors further includes a sequence that encodes a second isoform of the CLRN1 protein (e.g., SEQ ID NO: 5). In some embodiments, each of the at least two different vectors includes a coding sequence that encodes a different portion of a CLRN1 protein, each of the encoded portions encoding up to 80% of the amino acid sequence of SEQ ID NO: 5 (e.g., up to 10%, up to 20%, up to 30%, up to 40%, up to 50%, up to 60%, or up to 70% of SEQ ID NO: 5), provided that each of the encoded portions is non-overlapping with any other.

In some embodiments, each of the at least two different vectors includes a coding sequence that encodes a different potion of a second isoform of the CLRN1 protein. In some embodiments, one of the at least two different nucleic acid vectors further incudes a sequence that encodes a first isoform of the CLRN1 protein.

When introduced into a mammalian cell (e.g., any of the mammalian cells described herein), splicing occurs between the splice donor sequence and the splice acceptor sequence, thereby forming a recombined nucleic acid that encodes an active CLRN1 protein (e.g., a full-length CLRN1 protein).

Non-limiting examples of such vectors are shown in FIGs. 1, 2, 4, 7-10, and 12-24.

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Example 14. CLRN1 Expression in HEK293FT Cells

HEK293FT cells were transfected with exemplary CLRN vectors. 48 hours post-transfection, HEK293FT cell lysates were prepared and CLRN1 protein expression was determined by Western blot. As shown in Figures 25 to 31, CLRN1 protein was detected in all tested samples. This result confirmed that CLRN1 protein can be expressed by exemplary CLRN1 vectors described herein.

Figure 32 showed that HEK293FT cells transfected with CLRN1-6eGFP vector expressed high levels of GFP as soon as 72 hours post-transfection. At 24 hours post-transfection, few HEK293FT cells expressed GFP following transfection with CLRN1-e6GFP vector at MOI 8.41E+04 and 2.53E+05. At 72 hours post-transfection, most HEK293FT cells transfected with CLRN1-e6GFP vector at MOI 2.53E+05 expressed GFP, while some HEK293FT cells transfected with CLRN1-e6GFP vector at MOI 8.41E+04 expressed GFP. As shown in Figure 33, CLRN1 was expressed at high levels in HEK293FT cells transfected with CLRN-0 vector, CLRN-3 vector, and CLRN-13 vector.

Example 15. CLRN1 Expression P2 Cochlear Mouse Explants

P2 cochlear explants from WT mice were infected 16 hours after plating and were harvested for RNA and immunofluorescence 72 hours after infection. As shown in Figure 34, CLRN1 was efficiently expressed in cochlear explants. As shown in Figure 35, outher hair cells (OHC) and inner hair cells (IHC) of P2 cochlear explants express Myo7a when transfected with CLRN-0 vector (1.3E10 VG/cochlea), CLRN-3 (9.9E09 VG/cochlea) and CLRN-13 (1.0E10 VG/cochlea). Transfection with either vector did not disrupt the structural integrity of OHCs or IHCs of the cochlea. Thus, Figure 35 shows lack of toxicity of CLRN1 constructs with viable and organized outer hair cells (OHC), inner hair cells (IHC) and stereociliary bundles. As shown in Figure 36, eGFP expression with CLRN1-3'UTR appeared to specify the transduction compared to CAG promoter alone. Cochlear explants infected with 1E09 AAV/Anc80.CAG.eGFP expressed Myo7a in the inner hair cells. Cochlear explants infected with 1E09 AAV/Anc80.CAG.eGFP.CLRN-3'UTR expressed My07a in both the OHC and IHC.

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Higher transduction and co-expression of CLRN1 was seen in OHCs of cochlear explants infected with AAV/Anc80.CAG.eGFP.CLRN-3'UTR. GFP expression in cochlear explants infected with AAV/Anc80.CAG.eGFP.CLRN-3'UTR was restricted to OHCs.

OTHER EMBODIMENTS

It is to be understood that while the invention has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.

All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control. Section headings and any descriptions of materials, methods, and examples are illustrative only and not intended to be limiting.

Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", and variations such as "comprises" and "comprising", will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

The reference in this specification to any prior publication (or information derived from it), or to any matter which is known, is not, and should not be taken as an acknowledgment or admission or any form of suggestion that that prior publication (or information derived from it) or known matter forms part of the common general knowledge in the field of endeavour to which this specification relates.

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SEQUENCE LISTING

SEQ ID NO: 1 - Human Full-length Wildtype CLRN1 Protein Isoform D

MPSQQKKIIFCMAGVFSFACALGVVTALGTPLWIKATVLCKTGALLVNASGQELDKFMGEMQYGLFHGEGV
RQCGLGARPFRFSFFPDLLKAIPVSIHVNVILFSAILIVLTMVGTAFFMYNAFGKPFETLHGPLGLYLLSF
ISVALWLPATRHQAQGSCGCLVMILFASEVKIHHLSEKIANYKEGTYVYKTQSEKYTTSFWVIFFCFFVHF
LNGLLIRLAGFQFPFAKSKDAETTNVAADLMY

SEQ ID NO: 2 - Human Wildtype CLRN1 Isoform D cDNA

atgccaagc caacagaaga aaatcatttt ttgcatggcc ggagtgttca gttttgcatg
tgccctcgga gttgtgacag ccttggggac accgttgtgg atcaaagcca ctgtcctctg
caaaacggga gctctgctcg tcaatgcctc agggcaggag ctggacaagt ttatgggtga
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tggctgtctt gtcatgatat tgtttgcctc tgaagtgaaa atccatcacc tctcagaaaa
aattgcaaat tataaagaag ggacttatgt ctacaaaacg caaagtgaaa aatataccac
ctcattctgg gtcattttct tttgcttttt tgttcatttt ctgaatgggc tcctaatacg
acttgctgga tttcagttcc cttttgcaaa atctaaagac gcagaaacaa ctaatgtagc
tgcagatcta atgtactga

SEQ ID NO: 3 - Human Full-length Wildtype CLRN1 Protein Isoform A

MPSQQKKIIFCMAGVFSFACALGVVTALGTPLWIKATVLCKTGALLVNASGQELDKFMGEMQYGLFHGEGV
RQCGLGARPFRFSFFPDLLKAIPVSIHVNVILFSAILIVLTMVGTAFFMYNAFGKPFETLHGPLGLYLLSF
ISGSCGCLVMILFASEVKIHHLSEKIANYKEGTYVYKTQSEKYTTSFWVIFFCFFVHFLNGLLIRLAGFQF
PFAKS KDAETTNVAADLMY

SEQ ID NO: 4 - Human Wildtype CLRN1 Isoform A cDNA

atgccaagc caacagaaga aaatcatttt ttgcatggcc ggagtgttca gttttgcatg
tgccctcgga gttgtgacag ccttggggac accgttgtgg atcaaagcca ctgtcctctg
caaaacggga gctctgctcg tcaatgcctc agggcaggag ctggacaagt ttatgggtga
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ctttcggttc tcatttttc cagatttgct caaagcaatc ccagtgagca tccacgtcaa
tgtcattctc ttctctgcca tccttattgt gttaaccatg gtggggacag ccttcttcat
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tctgaatggg ctcctaatac gacttgctgg atttcagttc ccttttgcaa aatctaaaga
cgcagaaaca actaatgtag ctgcagatct aatgtactga

SEQ ID NO: 5 - Human Full-length Wildtype CLRN1 Protein Isoform C

MQALQQQPVFPDLLKAIPVSIHVNVILFSAILIVLTMVGTAFFMYNAFGKPFETLHGPLGLYLLSFISGSC
GCLVMILFASEVKIHHLSEKIANYKEGTYVY KTQSEKYTTSFWLTKGHS

SEQ ID NO: 6 - Human Wildtype CLRN1 Isoform C cDNA

atgcaggc cctgcagcag caaccagttt ttccagattt gctcaaagca atcccagtga
gcatccacgt caatgtcatt ctcttctctg ccatccttat tgtgttaacc atggtgggga
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atgtctacaa aacgcaaagt gaaaaatata ccacctcatt ctggctgact aaaggccaca gctga

SEQ ID NO: 7 - Human Full-length Wildtype CLRN1 Protein Isoform E

MPSQQKKIIFCMAGVFSFACALGVVTALGTPLWIKATVLCKTGALLVNASGQELDKFMGEMQYGLFHGEGV
RQCGLGARPFRFSCYFLDPFMGLPTGVPHLLSLPCSTSCRREHTSERVQEPAGCFSAVRSKLHAGPAAATS
FSRFAQSNPSEHPRQCHS LLCHPYCVNHGGDSLLHVQCFWKTF

SEQ ID NO: 8 - Human Wildtype CLRN1 Isoform E cDNA

atgccaagc caacagaaga aaatcatttt ttgcatggcc ggagtgttca gttttgcatg
tgccctcgga gttgtgacag ccttggggac accgttgtgg atcaaagcca ctgtcctctg
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SEQ ID NO: 9 - Human Wildtype CLRN1 Genomic sequence

aggagatact tgaaggcagt ttgaaagact tgttttacag attcttagtc caaagatttc 61
caattaggga gaagaagcag cagaaaagga gaaaagccaa gtatgagtga tgatgaggcc 121
ttcatctact gacatttaac ctggcgagaa ccgtcgatgg tgaagttgcc ttttcagctg 181
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aacaagtctc caatcgttct ctgttccgta gaaagagaaa gaaaacctgg agcagctgct 46801
gaaaaatcta atgaggaact aagaggcaaa cccacca

SEQ ID NO: 10 - Mouse CLRN1 Protein Isoform 1

MPSQQKKIIFCMAGVLSFLCALGVVTAVGTPLWVKATILCKTGALLVNASGKELDKFMGEMQYGLFHGEGV
RQCGLGARPFRFSSRSMKERYSLYEDKGETAVFPDLVQAIPVSIHINIILFSMILVVLTMVGTAFFMYNAF
GKPFETLHGPLGLYLVSFISGSCGCLVMILFASEVKVHRLSEKIANFKEGTYAYRTQNENYTTSFWVVFIC
FFVHF LNGLLIRLAGFQFPFTKSKETETTNVASDLMY

SEQ ID NO: 11 - Dog CLRN1 Protein

MPNQQKKVVFCTAGVLSFVCALGVVTALGTPLWIKATFLCKTGALLVNASGQELDKFMGEMQYGLFHGEGI
RQCGLGARPFRFSLFPDLLKVIPVSIHVNVILFSTILVVLTMVGTAFFMYNAFGKPFETLHGPLGLYLLSF
ISGSCGCLVMILFASEVKIHHLSEKIANYKEGTYAYKTQSEKYTTSFWVVFICFLVHLLNGLLIRLAGFQF
PFAKS KDTETTNVAADLMY

SEQ ID NO: 12 - 5’ UTR

AGGAGATACTTGAAGGCAGTTTGAAAGACTTGTTTTACAGATTCTTAGTCCAA
AGATTTCCAATTAGGGAGAAGAAGCAGCAGAAAAGGAGAAAAGCCAAGTAT
GAGTGATGATGAGGCCTTCATCTACTGACATTTAACCTGGCGAGAACCGTCG

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ATGGTGAAGTTGCCTTTTCAGCTGGGAGCTGTCCGTTCAGCTTCCGTAATAAA
TGCAGTCAAAGAGGCAGTCCCTTCCCATTGCTCACAAAGGTCTTGTTTTTGAA
CCTCGCCCTCACAGAAGCCGTTTCTCATC

SEQ ID NO: 13 - 5' ITR

CCTGCAGGCAGCTGCGCGCTCGCTCGCTCACTGAGGCCGCCCGGGCGTCGGG
CGACCTTTGGTCGCCCGGCCTCAGTGAGCGAGCGAGCGCGCAGAGAGGGAGT
GGCCAACTCCATCACTAGGGGTTCCT

SEQ ID NO: 14 – 3' ITR

AGGAACCCCTAGTGATGGAGTTGGCCACTCCCTCTCTGCGCGCTCGCTCGCTC
ACTGAGGCCGGGCGACCAAAGGTCGCCCGACGCCCGGGCTTTGCCCGGGCGG
CCTCAGTGAGCGAGCGAGCGCGCAGCTGCCTGCAGG

SEQ ID NO: 15 – 3' UTR

AAGGCAAACCTTTCTATAATTTTACAAGGGAGTAGACTTGCTTTGGTCACTTT
TAGATGTGGTTAATTTTGCATATCCTTTTAGTCTGCATATATTAAAGCATCAG
GACCCTTCGTGACAATGTTTACAAATTACGTACTAAGGATACAGGCTGGAAA
GTAAGGGAAGCAGAAGGAAGGCTTTGAAAAGTTGTTTTATCTGGTGGGAAAT
TGCTTGACCCAGGTAGTCAAAGGCAGTTGACTAGAATCGACAAATTGTTACT
CCATATATATATATGTGTGTGTGTGTGTGTGTGTGTGTGTAAGATGTCTTC
CTATCAAAAAGATATCAAAGGCACATGGAATATATTTTAATAAAAACAAATA
ATATCTCTAATATATCCACACATTTGTTGCCAGATTTCAGAAAACTGAGCTGC
AATCGCTTTCCTAAAACAGTAGTGTATTAAATGAACATCTATAAAATGTATCA
ACACACATTTTAAAAAATTTGTTTAAAGTATACTCTTAGGCCAGGCGTGGTGA
CTCACACCTGTAATTCCAGCACTTCAGGAGGCCAAGGTGGGAAGATCATTTG
AGTTCAGGAGTTCGAGTTACAGCCTGGGCAATAAAGTGAGACCCTGTCACTA
ACAAAATTAAAAAATAAAATAAATATAAAATATAGGCTTTAAAAAAGCATA
GTCTTATTAACCATGTCTGTTGGTCAAAATCTGCAAACTCTAAAAGAAGAAA
AGAAGAAAAAACCAAGCTTAGGGTATTTTTCCTCCCGTGCCTGAGTCCCAATT
ACATTCACGACAGTACTTTCAATGAACATAATTGTTAGGACCACTGAGGAAT
CATGAAAAATGATCTCTGCTTAGTACATTTGATGCAAAATGACTTATTAGGGG
CTGTTTTTCTAGCTATAGTGTCTCGAGTACTAATATGCAATTATGAAAATTAT
ATTAAATCTGGGATTATGACGGTATCACTGTATCATCTTGGTCTTGTTCTGGC
TGTCACCAAGCATGACCCAGGTCAACTTTTTTTTTCCCCTGAATTACCCATCA
AATTGATCTGCAGCTGACTAAAGGCCACAGCTGAGCCTGGAACTGACCCTTC
CTTCATCCTCAACCTGCTGTCCTCCAGAAAGCACCAAGGAAAAAGCAGAGAA
TGACAGCAAACAGATCACTAGGCCTCTGACCACAGGTGCTGAGTACTCAGCA
GCCCTCATATAATAGGTTTGAAAGTACTCCTTAAAATAAAACACTGTTTCCCT
TTGGAACTATTTACAAGGATGAAACAACCGTATACCTGAGAAATAACTTGCT
CTGGTGTCAATTCGCTATTCGCCAGCAGACATCAGAACACACCGAGTTTCCA

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GATGCTGGTTTTTCCCCTTAAATCAGGAAATACACCTGGACAATTTCTAGAAG
ACTACAATTCAGTCTAGCCACAAAGGGGATTTTTTTTTTTTGGTAACAGGCTA
GAGCCCGGTTCTGTAAGTCTTTAGCTGAAATGGTCCAGTACAAAAGCACTGG
AAATGAGTGGGCTAGGAGGACAAGGACCGTCTCCTGCGTGAGGAGTTGGTTG
GAGGTCCCCAAGGCCAGGTACCCCCTGCACTCTTATTGGATTCCTCTCTGTCT
TCTTGGAGTTTTGAAAAACTCCTTCGAACACCAGGCTTTTTTCTTTAGAAAAC
AAGTCTCCAATCGTTCTCTGTTCCGTAGAAAGAGAAAGAAAACCTGGAGCAG
CTGCTGAAAAATCTAATGAGGAACTAAGAGGCAAACCCACCA

SEQ ID NO: 16 – Chimeric intron

GGAGTCGCTGCGTTGCCTTCGCCCCGTGCCCCGCTCCGCGCCGCCTCGCGCCG
CCCGCCCCGGCTCTGACTGACCGCGTTACTCCCACAGGTGAGCGGGCGGGAC
GGCCCTTCTCCTCCGGGCTGTAATTAGCGCTTGGTTTAATGACGGCTCGTTTC
TTTTCTGTGGCTGCGTGAAAGCCTTAAAGGGCTCCGGGAGGGCCCTTTGTGCG
GGGGGGAGCGGCTCGGGGGGTGCGTGCGTGTGTGTGTGCGTGGGGAGCGCC
GCGTGCGGCCCGCGCTGCCCGGCGGCTGTGAGCGCTGCGGGCGCGGCGCGGG
GCTTTGTGCGCTCCGCGTGTGCGCGAGGGGAGCGCGGCCGGGGGCGGTGCCC
CGCGGTGCGGGGGGGCTGCGAGGGGAACAAAGGCTGCGTGCGGGGTGTGTG
CGTGGGGGGGTGAGCAGGGGGTGTGGGCGCGGCGGTCGGGCTGTAACCCCC
CCCTGCACCCCCCTCCCCGAGTTGCTGAGCACGGCCCGGCTTCGGGTGCGGG
GCTCCGTGCGGGGCGTGGCGCGGGGCTCGCCGTGCCGGGCGGGGGGTGGCG
GCAGGTGGGGGTGCCGGGCGGGGCGGGGCCGCCTCGGGCCGGGGAGGGCTC
GGGGGAGGGGCGCGGCGGCCCCCGGAGCGCCGGCGGCTGTCGAGGCGCGGC
GAGCCGCAGCCATTGCCTTTTATGGTAATCGTGCGAGAGGGCGCAGGGACTT
CCTTTGTCCCAAATCTGTGCGGAGCCGAAATCTGGGAGGCGCCGCCGCACCC
CCTCTAGCGGGCGCGGGGCGAAGCGGTGCGGCGCCGGCAGGAAGGAAATGG
GCGGGGAGGGCCTTCGTGCGTCGCCGCGCCGCCGTCCCCTTCTCCCTCTCCAG
CCTCGGGGCTGTCCGCGGGGGGACGGCTGCCTTCGGGGGGGACGGGGCAGG
GCGGGGTTCGGCTTCTGGCGTGTGACCGGCGGCTCTAGAGCCTCTGCTAACC
ATGTTCATGCCTTCTTCTTTTTCCTACAG

SEQ ID NO: 17 – CMV Enhancer

GACATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTT
CATAGCCCATATATGGAGTTCCGCGTTACATAACTTACGGTAAATGGCCCGCC
TGGCTGACCGCCCAACGACCCCCGCCCATTGACGTCAATAATGACGTATGTT
CCCATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGACTATTT
ACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACG
CCCCCTATTGACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCCAGT
ACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATC
GCTATTACCATG

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SEQ ID NO: 18 – CBA promoter

TCGAGGTGAGCCCCACGTTCTGCTTCACTCTCCCCATCTCCCCCCCCCTCCCCA
CCCCCAATTTTGTATTTATTTATTTTTTAATTATTTTGTGCAGCGATGGGGGCG
GGGGGGGGGGGGGGCGCGCGCCAGGCGGGGCGGGGCGGGGCGAGGGGCGGG
GCGGGGCGAGGCGGAGAGGTGCGGCGGCAGCCAATCAGAGCGGCGCGCTCC
GAAAGTTTCCTTTTATGGCGAGGCGGCGGCGGCGGCGGCCCTATAAAAAGCG
AAGCGCGCGGCGGGCG

SEQ ID NO: 19 –tGFP

MESDESGLPAMEIECRITGTLNGVEFELVGGGEGTPEQGRMTNKMKSTKGALTF
SPYLLSHVMGYGFYHFGTYPSGYENPFLHAINNGGYTNTRIEKYEDGGVLHVSFS
YRYEAGRVIGDFKVMGTGFPEDSVIFTDKIIRSNATVEHLHPMGDNDLDGSFTRT
FSLRDGGYYSSVVDSHMHFKSAIHPSILQNGGPMFAFRRVEEDHSNTELGIVEYQ
HAFKTPDADAGEE

SEQ ID NO: 20 – bovine growth hormone poly-A

CTGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGCCCCTCCCCCGTGCCTTCCT
TGACCCTGGAAGGTGCCACTCCCACTGTCCTTTCCTAATAAAATGAGGAAATT
GCATCGCATTGTCTGAGTAGGTGTCATTCTATTCTGGGGGGTGGGGTGGGGC
AGGACAGCAAGGGGGAGGATTGGGAAGACAATAGCAGGCATGCTGGGGATG
CGGTGGGCTCTATGG

SEQ ID NO: 21 – polyadenylation signal of soluble neuropilin-1

AAATAAAATACGAAATG

SEQ ID NO: 22 – Splice donor sequence

GTAAGTATCAAGGTTACAAGACAGGTTTAAGGAGACCAATAGAAACTGGGCT
TGTCGAGACAGAGAAGACTCTTGCGTTTCT

SEQ ID NO: 23 - Splice acceptor sequence
GATAGGCACCTATTGGTCTTACTG ACATCCACTTTGCCTTTCTCTCCACAG

SEQ ID NO: 24 – 3'ITR
CCTGCAGGCAGCTGCGCGCTCGCTCGCTCACTGAGGCCGCCCGGGCAAAGCC
CGGGCGTCGGGCGACCTTTGGTCGCCCGGCCTCAGTGAGCGAGCGAGCGCGC
AGAGAGGGAGTGGCCAACTCCATCACTAGGGGTTCCT

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SEQ ID NO: 25 – ITR

TTGGCCACTCCCTCTCTGCGCGCTCGCTCGCTCACTGAGGCCGCCCGGGCAAA
GCCCGGGCGTCGGGCGACCTTTGGTCGCCCGGCCTCAGTGAGCGAGCGAGCG
CGCAGAGAGGGAGTGGCCAACTCCATCACTAGGGGTTCCT

SEQ ID NO: 26 – sh-chimeric intron

GGAGTCGCTGCGTTGCCTTCGCCCCGTGCCCCGCTCCGCGCCGCCTCGCGCCG
CCCGCCCCGGCTCTGACTGACCGCGTTACTCCCACAGGTGAGCGGGCGGGAC
GGCCCTTCTCCTCCGGGCTGTAATTAGCGCTTGGTTTAATGACGGCTTGTTTCT
TTTCTGTGGCTGCGTGAAAGCCTTGAGGGGCTCCGGGAGCTAGAGCCTCTGCT
AACCATGTTCATGCCTTCTTCTTTTTCCTACAG

SEQ ID NO: 27 – 3'UTR 600A

AAGGCAAACCTTTCTATAATTTTACAAGGGAGTAGACTTGCTTTGGTCACTTT
TAGATGTGGTTAATTTTGCATATCCTTTTAGTCTGCATATATTAAAGCATCAG
GACCCTTCGTGACAATGTTTACAAATTACGTACTAAGGATACAGGCTGGAAA
GTAAGGGAAGCAGAAGGAAGGCTTTGAAAAGTTGTTTTATCTGGTGGGAAAT
TGCTTGACCCAGGTAGTCAAAGGCAGTTGACTAGAATCGACAAATTGTTACT
CCATATATATATATGTGTGTGTGTGTGTGTGTGTGTGTGTAAGATGTCTTC
CTATCAAAAAGATATCAAAGGCACATGGAATATATTTTAATAAAAACAAATA
ATATCTCTAATATATCCACACATTTGTTGCCAGATTTCAGAAAACTGAGCTGC
AATCGCTTTCCTAAAACAGTAGTGTATTAAATGAACATCTATAAAATGTATCA
ACACACATTTTAAAAAATTTGTTTAAAGTATACTCTTAGGCCAGGCGTGGTGA
CTCACACCTGTAATTCCAGCACTTCAGGAGGCCAAGGTGGGAAGATCATTTG
AGTTCAGGAGTTCGAGTTACAG

SEQ ID NO: 28 – 3'UTR 600B

GAAGATCATTTGAGTTCAGGAGTTCGAGTTACAGCCTGGGCAATAAAGTGAG
ACCCTGTCACTAACAAAATTAAAAAATAAAATAAATATAAAATATAGGCTTT
AAAAAAGCATAGTCTTATTAACCATGTCTGTTGGTCAAAATCTGCAAACTCTA
AAAGAAGAAAAGAAGAAAAAACCAACGTTAGGGTATTTTTCCTCCCGTGCCT
GAGTCCCAATTACATTCACGACAGTACTTTCAATGAACATAATTGTTAGGACC
ACTGAGGAATCATGAAAAATGATCTCTGCTTAGTACATTTGATGCAAAATGA
CTTATTAGGGGCTGTTTTTCTAGCTATAGTGTCTCGAGTACTAATATGCAATT
ATGAAAATTATATTAAATCTGGGATTATGACGGTATCACTGTATCATCTTGGT
CTTGTTCTGGCTGTCACCAAGCATGACCCAGGTCAACTTTTTTTTTCCCCTGAA
TTACCCATCAAATTGATCTGCAGCTGACTAAAGGCCACAGCTGAGCCTGGAA
CTGACCCTTCCTTCATCCTCAACCTGCTGTCCTCCAGAAAGCACCAAGGAAAA
AGCAGAGAATGACAGCAAACA

SEQ ID NO: 29 – 3'UTR 600C

AGGCCTCTGACCACAGGTGCTGAGTACTCAGCAGCCCTCATATAATAGGTTT
GAAAGTACTCCTTAAAATAAAACACTGTTTCCCTTTGGAACTATTTACAAGGA
TGAAACAACCGTATACCTGAGAAATAACTTGCTCTGGTGTCAATTCGCTATTC
GCCAGCAGACATCAGAACACACCGAGTTTCCAGATGCTGGTTTTTCCCCTTAA
ATCAGGAAATACACCTGGACAATTTCTAGAAGACTACAATTCAGTCTAGCCA

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CAAAGGGGATTTTTTTTTTTTGGTAACAGGCTAGAGCCCGGTTCTGTAAGTCT
TTAGCTGAAATGGTCCAGTACAAAAGCACTGGAAATGAGTGGGCTAGGAGG
ACAAGGACCGTCTCCTGCGTGAGGAGTTGGTTGGAGGTCCCCAAGGCCAGGT
ACCCCCTGCACTCTTATTGGATTCCTCTCTGTCTTCTTGGAGTTTTGAAAAACT
CCTTCGAACACCAGGCTTTTTTCTTTAGAAAACAAGTCTCCAATCGTTCTCTG
TTCCGTAGAAAGAGAAAGAAAACCTGGAGCAGCTGCTGAAAAATCTAATGA
GGAACTAAGAGGCAAACCCACCA

SEQ ID NO: 30 – FP
KRKRRG

SEQ ID NO: 31 – T2A
EGRGSLLTCGDVEENPGP

SEQ ID NO: 32 – eGFP
MVSKGEELFTGVVPILVELDGDVNGHKFSVSGEGEGDATYGKLTLKFICTTGKLP
VPWPTLVTTLTYGVQCFSRYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNYK
TRAEVKFEGDTLVNRIELKGIDFKEDGNILGHKLEYNYNSHNVYIMADKQKNGI
KVNFKIRHNIEDGSVQLADHYQQNTPIGDGPVLLPDNHYLSTQSALSKDPNEKRD
HMVLLEFVTAAGITLGMDELYK

SEQ ID NO: 33 – Myc
EQKLISEEDLEQKLISEEDLEQKLISEEDL

SEQ ID NO: 34 – HA
YPYDVPDYA

SEQ ID NO: 35 – 3x FLAG tag
DYKDHDGDYKDHDIDYKDDDDK

SEQ ID NO: 36 – 3'UTR 1357
AAGGCAAACCTTTCTATAATTTTACAAGGGAGTAGACTTGCTTTGGTCACTTT
TAGATGTGGTTAATTTTGCATATCCTTTTAGTCTGCATATATTAAAGCATCAG
GACCCTTCGTGACAATGTTTACAAATTACGTACTAAGGATACAGGCTGGAAA
GTAAGGGAAGCAGAAGGAAGGCTTTGAAAAGTTGTTTTATCTGGTGGGAAAT
TGCTTGACCCAGGTAGTCAAAGGCAGTTGACTAGAATCGACAAATTGTTACT
CCATATATATATATGTGTGTGTGTGTGTAAGATGTCTTCCTATCAAAAAGA
TATCAAAGGCACATGGAATATATTTTAATAAAAACAAATAATATCTCTAATA
TATCCACACATTTGTTGCCAGATTTCAGAAAACTGAGCTGCAATCGCTTTCCT
AAAACAGTAGTGTATTAAATGAACATCTATAAAATGTATCAACACACATTTT
AAAAAATTTGTTTAAAGTATACTCTTAGGCCAGGCGTGGTGACTCACACCTGT
AATTCCAGCACTTCAGGAGGCCAAGGTGGGAAGATCATTTGAGTTCAGGAGT
TCGAGTTACAGTCTGGGCAATAAAGTGAGACCCTGTCACTAACAAAATTAAA
AAATAAAATAAATATAAAATATAGGCTTTAAAAAAGCATAGTCTTATTAACC
ATGTCTGTTGGTCAAAATCTGCAAACTCTAAAAGAAGAAAAGAAGAAAAAA
CCAACGTTAGGGTATTTTTCCTCCCGTGCCTGAGTCCCAATTACATTCACGAC

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AGTACTTTCAATGAACATAATTGTTAGGACCACTGAGGAATCATGAAAAATG
ATCTCTGCTTAGTACATTTGATGCAAAATGACTTATTAGGGGCTGTTTTTCTA
GCTATAGTGTCTCGAGTACTAATATGCAATTATGAAAATTATATTAAATCTGG
GATTATGACGGTATCACTGTATCATCTTGGTCTTGTTCTGGCTGTCACCAAGC
ATGACCCAGGTCAACTTTTTTTTTCCCCTGAATTACCCATCAAATTGATCTGC
AGCTGACTAAAGGCCACAGCTGAGCCTGGAACTGACCCTTCCTTCATCCTCA
ACCTGCTGTCCTCCAGAAAGCACCAAGGAAAAAGCAGAGAATGACAGCAAA
CAGATCACTAGGCCTCTGACCACAGGTGCTGAGTACTCAGCAGCCCTCATAT
AATAGGTTTGAAAGTACTCCTTAAAATAAAACACTGTTTCCCTTTGGAACTAT
TTACAAGGATGAAACAACCGTATACCTGAGAAATAACTTGCTCTGGTGTCAA
TTCGCTATTCGCCAGCAGACATCAGAACACACCGAGTTTCCAGATGCT

SEQ ID NO: 37 - 3'UTR 1406

AAGGCAAACCTTTCTATAATTTTACAAGGGAGTAGACTTGCTTTGGTCACTTT
TAGATGTGGTTAATTTTGCATATCCTTTTAGTCTGCATATATTAAAGCATCAG
GACCCTTCGTGACAATGTTTACAAATTACGTACTAAGGATACAGGCTGGAAA
GTAAGGGAAGCAGAAGGAAGGCTTTGAAAAGTTGTTTTATCTGGTGGGAAAT
TGCTTGACCCAGGTAGTCAAAGGCAGTTGACTAGAATCGACAAATTGTTACT
CCATATATATATATATGTGTGTGTGTGTGTAAGATGTCTTCCTATCAAAAAGA
TATCAAAGGCACATGGAATATATTTTAATAAAAACAAATAATATCTCTAATA
TATCCACACATTTGTTGCCAGATTTCAGAAAACTGAGCTGCAATCGCTTTCCT
AAAACAGTAGTGTATTAAATGAACATCTATAAAATGTATCAACACACATTTT
AAAAAATTTGTTTAAAGTATACTCTTAGGCCAGGCGTGGTGACTCACACCTGT
AATTCCAGCACTTCAGGAGGCCAAGGTGGGAAGATCATTTGAGTTCAGGAGT
TCGAGTTACAGCCTGGGCAATAAAGTGAGACCCTGTCACTAACAAAATTAAA
AAATAAAATAAATATAAAATATAGGCTTTAAAAAAGCATAGTCTTATTAACC
ATGTCTGTTGGTCAAAATCTGCAAACTCTAAAAGAAGAAAAGAAGAAAAAA
CCAACGTTAGGGTATTTTTCCTCCCGTGCCTGAGTCCCAATTACATTCACGAC
AGTACTTTCAATGAACATAATTGTTAGGACCACTGAGGAATCATGAAAAATG
ATCTCTGCTTAGTACATTTGATGCAAAATGACTTATTAGGGGCTGTTTTTCTA
GCTATAGTGTCTCGAGTACTAATATGCAATTATGAAAATTATATTAAATCTGG
GATTATGACGGTATCACTGTATCATCTTGGTCTTGTTCTGGCTGTCACCAAGC
ATGACCCAGGTCAACTTTTTTTTTCCCCTGAATTACCCATCAAATTGATCTGC
AGCTGACTAAAGGCCACAGCTGAGCCTGGAACTGACCCTTCCTTCATCCTCA
ACCTGCTGTCCTCCAGAAAGCACCAAGGAAAAAGCAGAGAATGACAGCAAA
CAGATCACTAGGCCTCTGACCACAGGTGCTGAGTACTCAGCAGCCCTCATAT
AATAGGTTTGAAAGTACTCCTTAAAATAAAACACTGTTTCCCTTTGGAACTAT
TTACAAGGATGAAACAACCGTATACCTGAGAAATAACTTGCTCTGGTGTCAA
TTCGCTATTCGCCAGCAGACATCAGAACACACCGAGTTTCCAGATGCTGGTTT
TTCCCCTTAAATCAGGAAATACACCTGGACAATTTCTAGAAGAC

SEQ ID NO: 38 – CLRN1 antibody epitope

EKIANYKEGTYVYKTQSEKY

SEQ ID NO: 39 – CLRN-0

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CCTGCAGGCAGCTGCGCGCTCGCTCGCTCACTGAGGCCGCCCGGGCGTCGGG
CGACCTTTGGTCGCCCGGCCTCAGTGAGCGAGCGAGCGCGCAGAGAGGGAGT
GGCCAACTCCATCACTAGGGGTTCCTGCGGCCGCACGCGTGACATTGATTATT
GACTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCCATATA
TGGAGTTCCGCGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCC
CAACGACCCCCGCCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACG
CCAATAGGGACTTTCCATTGACGTCAATGGGTGGACTATTTACGGTAAACTGC
CCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACG
TCAATGACGGTAAATGGCCCGCCTGGCATTATGCCCAGTACATGACCTTATG
GGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGG
GTCGAGGTGAGCCCCACGTTCTGCTTCACTCTCCCCATCTCCCCCCCCTCCCC
ACCCCCAATTTTGTATTTATTTATTTTTTAATTATTTTGTGCAGCGATGGGGGC
GGGGGGGGGGGGGGGCGCGCGCCAGGCGGGGCGGGGCGGGGCGAGGGGCGG
GGCGGGGCGAGGCGGAGAGGTGCGGCGGCAGCCAATCAGAGCGGCGCGCTC
CGAAAGTTTCCTTTTATGGCGAGGCGGCGGCGGCGGCGGCCCTATAAAAAGC
GAAGCGCGCGGCGGGCGGGAGTCGCTGCGTTGCCTTCGCCCCGTGCCCCGCT
CCGCGCCGCCTCGCGCCGCCCGCCCCGGCTCTGACTGACCGCGTTACTCCCAC
AGGTGAGCGGGCGGGACGGCCCTTCTCCTCCGGGCTGTAATTAGCGCTTGGT
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GGGAGGGCCCTTTGTGCGGGGGGGAGCGGCTCGGGGGGTGCGTGCGTGTGTG
TGTGCGTGGGGAGCGCCGCGTGCGGCCCGCGCTGCCCGGCGGCTGTGAGCGC
TGCGGGCGCGGCGCGGGGCTTTGTGCGCTCCGCGTGTGCGCGAGGGGAGCGC
GGCCGGGGGCGGTGCCCCGCGGTGCGGGGGGGCTGCGAGGGGAACAAAGGC
TGCGTGCGGGGTGTGTGCGTGGGGGGGTGAGCAGGGGGTGTGGGCGCGGCG
GTCGGGCTGTAACCCCCCCCTGCACCCCCCTCCCCGAGTTGCTGAGCACGGCC
CGGCTTCGGGTGCGGGGCTCCGTGCGGGGCGTGGCGCGGGGCTCGCCGTGCC
GGGCGGGGGGTGGCGGCAGGTGGGGGTGCCGGGCGGGGCGGGGCCGCCTCG
GGCCGGGGAGGGCTCGGGGGAGGGGCGCGGCGGCCCCCGGAGCGCCGGCGG
CTGTCGAGGCGCGGCGAGCCGCAGCCATTGCCTTTTATGGTAATCGTGCGAG
AGGGCGCAGGGACTTCCTTTGTCCCAAATCTGTGCGGAGCCGAAATCTGGGA
GGCGCCGCCGCACCCCCTCTAGCGGGCGCGGGGCGAAGCGGTGCGGCGCCG
GCAGGAAGGAAATGGGCGGGGAGGGCCTTCGTGCGTCGCCGCGCCGCCGTC
CCCTTCTCCCTCTCCAGCCTCGGGGCTGTCCGCGGGGGGACGGCTGCCTTCGG
GGGGGACGGGGCAGGGCGGGGTTCGGCTTCTGGCGTGTGACCGGCGGCTCTA
GAGCCTCTGCTAACCATGTTCATGCCTTCTTCTTTTTCCTACAGCTCCTGGGCA
ACGTGCTGGTTATTGTGACCGGTGCCACCATGCCTAGCCAGCAGAAGAAAAT
CATCTTCTGCATGGCCGGCGTGTTCAGCTTCGCCTGTGCTCTGGGAGTTGTGA
CAGCCCTGGGAACCCCTCTGTGGATCAAAGCCACAGTGCTGTGCAAGACAGG
CGCCCTGCTGGTTAATGCCTCTGGCCAAGAGCTGGACAAGTTCATGGGCGAG
ATGCAGTACGGCCTGTTCCATGGCGAAGGCGTCAGACAGTGTGGCCTGGGAG
CCAGACCTTTCAGATTCAGCTTCTTCCCAGACCTGCTGAAGGCTATCCCCGTG
TCCATCCACGTGAACGTGATCCTGTTCAGCGCCATCCTGATCGTGCTGACAAT
GGTCGGAACCGCCTTCTTCATGTACAACGCCTTCGGCAAGCCCTTCGAGACA
CTGCATGGACCTCTGGGCCTGTACCTGCTGAGCTTTATCAGCGGCAGCTGTGG
CTGCCTGGTCATGATTCTGTTCGCCAGCGAAGTGAAGATCCACCACCTGAGC
GAGAAGATCGCCAACTACAAAGAGGGCACCTACGTCTACAAGACCCAGTCC

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GAGAAGTACACCACCAGCTTTTGGGTTATCTTCTTCTGTTTCTTCGTGCACTTC
CTGAACGGCCTGCTGATCAGACTGGCCGGCTTCCAGTTTCCATTCGCCAAGA
GCAAGGACGCCGAAACCACAAACGTGGCCGCCGATCTGATGTACTAAGAGCT
CAAGGCAAACCTTTCTATAATTTTACAAGGGAGTAGACTTGCTTTGGTCACTT
TTAGATGTGGTTAATTTTGCATATCCTTTTAGTCTGCATATATTAAAGCATCA
GGACCCTTCGTGACAATGTTTACAAATTACGTACTAAGGATACAGGCTGGAA
AGTAAGGGAAGCAGAAGGAAGGCTTTGAAAAGTTGTTTTATCTGGTGGGAAA
TTGCTTGACCCAGGTAGTCAAAGGCAGTTGACTAGAATCGACAAATTGTTAC
TCCATATATATATATGTGTGTGTGTGTGTGTGTGTGTGTGTGTAAGATGTCTTC
CTATCAAAAAGATATCAAAGGCACATGGAATATATTTTAATAAAAACAAATA
ATATCTCTAATATATCCACACATTTGTTGCCAGATTTCAGAAAACTGAGCTGC
AATCGCTTTCCTAAAACAGTAGTGTATTAAATGAACATCTATAAAATGTATCA
ACACACATTTTAAAAAATTTGTTTAAAGTATACTCTTAGGCCAGGCGTGGTGA
CTCACACCTGTAATTCCAGCACTTCAGGAGGCCAAGGTGGGAAGATCATTTG
AGTTCAGGAGTTCGAGTTACAGCCTGGGCAATAAAGTGAGACCCTGTCACTA
ACAAAATTAAAAAATAAAATAAATATAAAATATAGGCTTTAAAAAAGCATA
GTCTTATTAACCATGTCTGTTGGTCAAAATCTGCAAACTCTAAAAGAAGAAA
AGAAGAAAAAACCAACGTTAGGGTATTTTTCCTCCCGTGCCTGAGTCCCAATT
ACATTCACGACAGTACTTTCAATGAACATAATTGTTAGGACCACTGAGGAAT
CATGAAAAATGATCTCTGCTTAGTACATTTGATGCAAAATGACTTATTAGGGG
CTGTTTTTCTAGCTATAGTGTCTCGAGTACTAATATGCAATTATGAAAATTAT
ATTAAATCTGGGATTATGACGGTATCACTGTATCATCTTGGTCTTGTTCTGGC
TGTCACCAAGCATGACCCAGGTCAACTTTTTTTTTCCCCTGAATTACCCATCA
AATTGATCTGCAGCTGACTAAAGGCCACAGCTGAGCCTGGAACTGACCCTTC
CTTCATCCTCAACCTGCTGTCCTCCAGAAAGCACCAAGGAAAAAGCAGAGAA
TGACAGCAAACAGATCACTAGGCCTCTGACCACAGGTGCTGAGTACTCAGCA
GCCCTCATATAATAGGTTTGAAAGTACTCCTTAAAATAAAACACTGTTTCCCT
TTGGAACTATTTACAAGGATGAAACAACCGTATACCTGAGAAATAACTTGCT
CTGGTGTCAATTCGCTATTCGCCAGCAGACATCAGAACACACCGAGTTTCCA
GATGCTGGTTTTTCCCCTTAAATCAGGAAATACACCTGGACAATTTCTAGAAG
ACTACAATTCAGTCTAGCCACAAAGGGGATTTTTTTTTTTTGGTAACAGGCTA
GAGCCCGGTTCTGTAAGTCTTTAGCTGAAATGGTCCAGTACAAAAGCACTGG
AAATGAGTGGGCTAGGAGGACAAGGACCGTCTCCTGCGTGAGGAGTTGGTTG
GAGGTCCCCAAGGCCAGGTACCCCCTGCACTCTTATTGGATTCCTCTCTGTCT
TCTTGGAGTTTTGAAAAACTCCTTCGAACACCAGGCTTTTTTCTTTAGAAAAC
AAGTCTCCAATCGTTCTCTGTTCCGTAGAAAGAGAAAGAAAACCTGGAGCAG
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SEQ ID NO: 47 – CLRN-9

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SEQ ID NO: 55 – CLRN-13

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SEQ ID NO: 56 – CLRN-14

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SEQ ID NO: 57 – CLRN-15

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SEQ ID NO: 58 - CLRN-16

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SEQ ID NO: 59 – CLRN-17
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SEQ ID NO: 60 – CLRN-18
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GTCGAGGTGAGCCCCACGTTCTGCTTCACTCTCCCCATCTCCCCCCCCTCCCC
ACCCCCAATTTTGTATTTATTTATTTTTTAATTATTTTGTGCAGCGATGGGGGC
GGGGGGGGGGGGGGGCGCGCGCCAGGCGGGGCGGGGCGGGGCGAGGGGCGG
GGCGGGGCGAGGCGGAGAGGTGCGGCGGCAGCCAATCAGAGCGGCGCGCTC
CGAAAGTTTCCTTTTATGGCGAGGCGGCGGCGGCGGCGGCCCTATAAAAAGC
GAAGCGCGCGGCGGGCGGGAGTCGCTGCGTTGCCTTCGCCCCGTGCCCCGCT
CCGCGCCGCCTCGCGCCGCCCGCCCCGGCTCTGACTGACCGCGTTACTCCCAC
AGGTGAGCGGGCGGGACGGCCCTTCTCCTCCGGGCTGTAATTagcgcttggtttaatgac
ggcttgtttcttttctgtggctgcgtgaaagccttgaggggctccgggagctagagcctctgctaaccatgttcatgccttcttcttttt
cctacagctcctgggcaacgtgctggttattgtgctgtctcatcattttggcaaagAGGAGATACTTGAAGGCAG
TTTGAAAGACTTGTTTTACAGATTCTTAGTCCAAAGATTTCCAATTAGGGAGA

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AGAAGCAGCAGAAAAGGAGAAAAGCCAAGTATGAGTGATGATGAGGCCTTC
ATCTACTGACATTTAACCTGGCGAGAACCGTCGATGGTGAAGTTGCCTTTTCA
GCTGGGAGCTGTCCGTTCAGCTTCCGTAATAAATGCAGTCAAAGAGGCAGTC
CCTTCCCATTGCTCACAAAGGTCTTGTTTTTGAACCTCGCCCTCACAGAAGCC
GTTTCTCATCACCGGTGCCACCATGCCTAGCCAGCAGAAGAAAATCATCTTCT
GCATGGCCGGCGTGTTCAGCTTCGCCTGTGCTCTGGGAGTTGTGACAGCCCTG
GGAACCCCTCTGTGGATCAAAGCCACAGTGCTGTGCAAGACAGGCGCCCTGC
TGGTTAATGCCTCTGGCCAAGAGCTGGACAAGTTCATGGGCGAGATGCAGTA
CGGCCTGTTCCATGGCGAAGGCGTCAGACAGTGTGGCCTGGGAGCCAGACCT
TTCAGATTCAGCTTCTTCCCAGACCTGCTGAAGGCTATCCCCGTGTCCATCCA
CGTGAACGTGATCCTGTTCAGCGCCATCCTGATCGTGCTGACAATGGTCGGA
ACCGCCTTCTTCATGTACAACGCCTTCGGCAAGCCCTTCGAGACACTGCATGG
ACCTCTGGGCCTGTACCTGCTGAGCTTTATCAGCGGCAGCTGTGGCTGCCTGG
TCATGATTCTGTTCGCCAGCGAAGTGAAGATCCACCACCTGAGCGAGAAGAT
CGCCAACTACAAAGAGGGCACCTACGTCTACAAGACCCAGTCCGAGAAGTAC
ACCACCAGCTTTTGGGTTATCTTCTTCTGTTTCTTCGTGCACTTCCTGAACGGC
CTGCTGATCAGACTGGCCGGCTTCCAGTTTCCATTCGCCAAGAGCAAGGACG
CCGAAACCACAAACGTGGCCGCCGATCTGATGTACTAAGAGCTCAAGGCAAA
CCTTTCTATAATTTTACAAGGGAGTAGACTTGCTTTGGTCACTTTTAGATGTG
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AGCAGAAGGAAGGCTTTGAAAAGTTGTTTTATCTGGTGGGAAATTGCTTGAC
CCAGGTAGTCAAAGGCAGTTGACTAGAATCGACAAATTGTTACTCCATATAT
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AAGATATCAAAGGCACATGGAATATATTTTAATAAAAACAAATAATATCTCT
AATATATCCACACATTTGTTGCCAGATTTCAGAAAACTGAGCTGCAATCGCTT
TCCTAAAACAGTAGTGTATTAAATGAACATCTATAAAATGTATCAACACACA
TTTTAAAAAATTTGTTTAAAGTATACTCTTAGGCCAGGCGTGGTGACTCACAC
CTGTAATTCCAGCACTTCAGGAGGCCAAGGTGGGAAGATCATTTGAGTTCAG
GAGTTCGAGTTACAGCCTGGGCAATAAAGTGAGACCCTGTCACTAACAAAAT
TAAAAAATAAAATAAATATAAAATATAGGCTTTAAAAAAGCATAGTCTTATT
AACCATGTCTGTTGGTCAAAATCTGCAAACTCTAAAAGAAGAAAAGAAGAAA
AAACCAACGTTAGGGTATTTTTCCTCCCGTGCCTGAGTCCCAATTACATTCAC
GACAGTACTTTCAATGAACATAATTGTTAGGACCACTGAGGAATCATGAAAA
ATGATCTCTGCTTAGTACATTTGATGCAAAATGACTTATTAGGGGCTGTTTTT
CTAGCTATAGTGTCTCGAGTACTAATATGCAATTATGAAAATTATATTAAATC
TGGGATTATGACGGTATCACTGTATCATCTTGGTCTTGTTCTGGCTGTCACCA
AGCATGACCCAGGTCAACTTTTTTTTTCCCCTGAATTACCCATCAAATTGATC
TGCAGCTGACTAAAGGCCACAGCTGAGCCTGGAACTGACCCTTCCTTCATCCT
CAACCTGCTGTCCTCCAGAAAGCACCAAGGAAAAAGCAGAGAATGACAGCA
AACAGATCACTAGGCCTCTGACCACAGGTGCTGAGTACTCAGCAGCCCTCAT
ATAATAGGTTTGAAAGTACTCCTTAAAATAAAACACTGTTTCCCTTTGGAACT
ATTTACAAGGATGAAACAACCGTATACCTGAGAAATAACTTGCTCTGGTGTC
AATTCGCTATTCGCCAGCAGACATCAGAACACACCGAGTTTCCAGATGCTGG
TTTTTCCCCTTAAATCAGGAAATACACCTGGACAATTTCTAGAAGACTACAAT
TCAGTCTAGCCACAAAGGGGATTTTTTTTTTTGGTAACAGGCTAGAGCCCGG

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TTCTGTAAGTCTTTAGCTGAAATGGTCCAGTACAAAAGCACTGGAAATGAGT
GGGCTAGGAGGACAAGGACCGTCTCCTGCGTGAGGAGTTGGTTGGAGGTCCC
CAAGGCCAGGTACCCCCTGCACTCTTATTGGATTCCTCTCTGTCTTCTTGGAG
TTTTGAAAAACTCCTTCGAACACCAGGCTTTTTTCTTTAGAAAACAAGTCTCC
AATCGTTCTCTGTTCCGTAGAAAGAGAAAGAAAACCTGGAGCAGCTGCTGAA
AAATCTAATGAGGAACTAAGAGGCAAACCCACCACTGTGCCTTCTAGTTGCC
AGCCATCTGTTGTTTGCCCCTCCCCCGTGCCTTCCTTGACCCTGGAAGGTGCC
ACTCCCACTGTCCTTTCCTAATAAAATGAGGAAATTGCATCGCATTGTCTGAG
TAGGTGTCATTCTATTCTGGGGGGTGGGGTGGGGCAGGACAGCAAGGGGGAG
GATTGGGAAGACAATAGCAGGCATGCTGGGGATGCGGTGGGCTCTATGGAA
GCTTGAATTCAGCTGACGTGCCTCGGACCGCTAGGAACCCCTAGTGATGGAG
TTGGCCACTCCCTCTCTGCGCGCTCGCTCGCTCACTGAGGCCGGGCGACCAAA
GGTCGCCCGACGCCCGGGCTTTGCCCGGGCGGCCTCAGTGAGCGAGCGAGCG
CGCAGCTGCCTGCAGG

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What is claimed is:

1. A composition comprising at least two different nucleic acid vectors, wherein:

each of the at least two different vectors comprises a coding sequence that encodes a different portion of a CLRN1 protein, each of the encoded portions being at least 30 amino acid residues in length, wherein the amino acid sequence of each of the encoded portions may optionally partially overlap with the amino acid sequence of a different one of the encoded portions;

no single vector of the at least two different vectors encodes a full-length CLRN1 protein;

at least one of the coding sequences comprises a nucleotide sequence spanning two consecutive exons of CLRN1 genomic DNA, and lacking an intronic sequence between the two consecutive exons; and

when introduced into a mammalian cell, the at least two different vectors undergo homologous recombination with each other, thereby forming a recombined nucleic acid that encodes a full-length CLRN1 protein.

2. The composition of claim 1, wherein each of the at least two different vectors includes a coding sequence that encodes a different portion of a first isoform of the CLRN1 protein.

3. The composition of claim 2, wherein the first isoform of the CLRN1 protein comprises a sequence that is at least 95% identical to SEQ ID NO: 3.

4. The composition of claim 3, wherein the first isoform of the CLRN1 protein comprises SEQ ID NO: 3.

5. The composition of claim 4, wherein the first isoform of the CLRN1 protein consists of SEQ ID NO: 3.

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6. The composition of claim 2, wherein one of the at least two different nucleic acid vectors further comprises a sequence that encodes a second isoform of the CLRN1 protein.

7. The composition of claim 6, wherein the second isoform of the CLRN1 protein comprises a sequence that is at least 95% identical to SEQ ID NO: 5.

8. The composition of claim 7, wherein the second isoform of the CLRN1 protein comprises SEQ ID NO: 5.

9. The composition of claim 8, wherein the second isoform of the CLRN1 protein consists of SEQ ID NO: 5.

10. The composition of claim 1, wherein each of the at least two different vectors includes a coding sequence that encodes a different potion of a second isoform of the CLRN1 protein.

11. The composition of claim 10, wherein the second isoform of the CLRN1 protein comprises a sequence that is at least 95% identical to SEQ ID NO: 5.

12. The composition of claim 11, wherein the second isoform of the CLRN1 protein comprises SEQ ID NO: 5.

13. The composition of claim 12, wherein the second isoform of the CLRN1 protein consists of SEQ ID NO: 5.

14. The composition of claim 13, wherein one of the at least two different nucleic acid vectors further comprises a sequence that encodes a first isoform of the CLRN1 protein.

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15. The composition of claim 14, wherein the first isoform of the CLRN1 protein comprises a sequence that is at least 95% identical to SEQ ID NO: 3.

16. The composition of claim 15, wherein the first isoform of the CLRN1 protein comprises SEQ ID NO: 3.

17. The composition of claim 16, wherein the first isoform of the CLRN1 protein consists of SEQ ID NO: 3.

18. The composition of any one of claims 1-17, wherein at least one of the at least two different vectors comprises a 5' untranslated region (UTR), a 3' UTR, or both.

19. The composition of claim 18, wherein the 5' UTR comprises at least 10 contiguous nucleotides from anywhere within SEQ ID NO: 12.

20. The composition of claim 19, wherein the 5' UTR comprises at least 20 contiguous nucleotides from anywhere within SEQ ID NO: 12.

21. The composition of claim 20, wherein the 5' UTR comprises at least 50 contiguous nucleotides from anywhere within SEQ ID NO: 12.

22. The composition of claim 21, wherein the 5' UTR comprises at least 80 contiguous nucleotides from anywhere within SEQ ID NO: 12.

23. The composition of claim 18, wherein the 5'UTR comprises a sequence that is at least 80% identical to SEQ ID NO: 12.

24. The composition of claim 18, wherein the 3' UTR comprises at least 10 contiguous nucleotides from anywhere within SEQ ID NO: 15.

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25. The composition of claim 24, wherein the 3’ UTR comprises at least 20 contiguous nucleotides from anywhere within SEQ ID NO: 15.

26. The composition of claim 25, wherein the 3’ UTR comprises at least 50 contiguous nucleotides from anywhere within SEQ ID NO: 15.

27. The composition of claim 26, wherein the 3’ UTR comprises at least 80 contiguous nucleotides from anywhere within SEQ ID NO: 15.

28. The composition of claim 18, wherein the 3’ UTR comprises a sequence that is at least 80% identical to SEQ ID NO: 15.

29. The composition of claim 1, wherein each of the at least two different vectors is a plasmid, a transposon, a cosmid, an artificial chromosome, or a viral vector.

30. The composition of claim 1, wherein each of the at least two different vectors is a human artificial chromosome (HAC), yeast artificial chromosome (YAC), bacterial artificial chromosome (BAC), or a P1-derived artificial chromosome (PAC).

31. The composition of claim 1, wherein each of the at least two different vectors is a viral vector selected from an adeno-associated virus (AAV) vector, an adenovirus vector, a lentivirus vector, or a retrovirus vector.

32. The composition of claim 31, wherein each of the at least two different vectors is an AAV vector.

33. The composition of any one of claims 1-32, wherein the amino acid sequence of none of the encoded portions overlaps with the amino acid sequence of a different one of the encoded portions.

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34. The composition of any one of claims 1-32, wherein the amino acid sequence of each of the encoded portions partially overlaps with the amino acid sequence of a different one of the encoded portions.

35. The composition of claim 34, wherein the overlapping amino acid sequence is between about 30 amino acid residues to about 202 amino acid residues in length.

36. The composition of any one of claims 1-32, wherein the vectors include two different vectors, each of which comprises a different segment of an intron, wherein the intron comprises the nucleotide sequence of an intron that is present in CLRN1 genomic DNA, and wherein the two different intron segments overlap in sequence by at least 100 nucleotides.

37. The composition of claim 36, wherein the two different intron segments overlap in sequence by 100 nucleotides to about 800 nucleotides.

38. The composition of any one of claims 1-37, wherein the entire nucleotide sequence of each of the at least two different vectors is between about 500 nucleotides to about 10,000 nucleotides in length.

39. The composition of claim 38, wherein the entire nucleotide sequence of each of the at least two different vectors is between 500 nucleotides to 5,000 nucleotides in length.

40. The composition of any one of claims 1-39, wherein the number of different vectors in the composition is two.

41. The composition of claim 40, wherein a first of the two different vectors comprises a coding sequence that encodes an N-terminal portion of the CLRN1 protein.

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42. The composition of claim 41, wherein the N-terminal portion of the CLRN1 protein is between 30 amino acids to 202 amino acids in length.

43. The composition of claim 41, wherein the N-terminal portion of the CLRN1 protein is between 60 amino acids to 170 amino acids in length.

44. The composition of claim 41, wherein the first vector further comprises a 5'UTR sequence.

45. The composition of claim 44, wherein the 5' UTR comprises at least 10 contiguous nucleotides from anywhere within SEQ ID NO: 12.

46. The composition of claim 45, wherein the 5' UTR comprises at least 20 contiguous nucleotides from anywhere within SEQ ID NO: 12.

47. The composition of claim 46, wherein the 5' UTR comprises at least 50 contiguous nucleotides from anywhere within SEQ ID NO: 12.

48. The composition of claim 47, wherein the 5' UTR comprises at least 80 contiguous nucleotides from anywhere within SEQ ID NO: 12.

49. The composition of claim 44, wherein the 5'UTR comprises a sequence that is at least 80% identical to SEQ ID NO: 12.

50. The composition of any one of claims 41-49, wherein the first vector further comprises one or both of a promoter and a Kozak sequence.

51. The composition of claim 50, wherein the first vector comprises a promoter that is an inducible promoter, a constitutive promoter, or a tissue-specific promoter.

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52. The composition of any one of claims 40-51, wherein the second of the two different vectors comprises a coding sequence that encodes a C-terminal portion of the CLRN1 protein.

53. The composition of claim 52, wherein the C-terminal portion of the CLRN1 protein is between 30 amino acids to 202 amino acids in length.

54. The composition of claim 53, wherein the C-terminal portion of the CLRN1 protein is between 60 amino acids to 170 amino acids in length.

55. The composition of any one of claims 52-54, wherein the second vector further comprises a polyadenylation signal sequence.

56. The composition of any one of claims 52-55, wherein the second vector further comprises a 3'UTR sequence.

57. The composition of claim 56, wherein the 3' UTR comprises at least 10 contiguous nucleotides from anywhere within SEQ ID NO: 15.

58. The composition of claim 57, wherein the 3' UTR comprises at least 20 contiguous nucleotides from anywhere within SEQ ID NO: 15.

59. The composition of claim 58, wherein the 3' UTR comprises at least 50 contiguous nucleotides from anywhere within SEQ ID NO: 15.

60. The composition of claim 59, wherein the 3' UTR comprises at least 80 contiguous nucleotides from anywhere within SEQ ID NO: 15.

61. The composition of claim 56, wherein the 3' UTR comprises a sequence that is at least 80% identical to SEQ ID NO: 15.

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62. A composition comprising a single nucleic acid vector, wherein the vector comprises one or both of (i) a first coding sequence encoding a first isoform of CLRN1 protein, and (ii) a second coding sequence encoding a second isoform of CLRN1 protein,

wherein one or both of the first and second coding sequences comprises a nucleotide sequence spanning two consecutive exons of a CLRN1 genomic DNA, and lacking an intronic sequence between the two consecutive introns.

63. The composition of claim 62, wherein the single nucleic acid vector contains the first coding sequence and not the second coding sequence.

64. The composition of claim 62, wherein the single nucleic acid vector contains the second coding sequence and not the first coding sequence.

65. The composition of claim 62, wherein the single nucleic acid vector contains both the first coding sequence and the second coding sequence.

66. The composition of any one of claims 62, 63, and 65, wherein the first isoform of the CLRN1 protein comprises a sequence that is at least 95% identical to SEQ ID NO: 3.

67. The composition of claim 66, wherein the first isoform of the CLRN1 protein comprises SEQ ID NO: 3.

68. The composition of claim 67, wherein the first isoform of the CLRN1 protein consists of SEQ ID NO: 3.

69. The composition of any one of claims 62, 64, and 65, wherein the second isoform of the CLRN1 protein comprises a sequence that is at least 95% identical to SEQ ID NO: 5.

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70. The composition of claim 69, wherein the second isoform of the CLRN1 protein comprises SEQ ID NO: 5.

71. The composition of claim 70, wherein the second isoform of the CLRN1 protein consists of SEQ ID NO: 5.

72. The composition of any one of claims 62-71, wherein the single nucleic acid vector further comprises a 5' untranslated region (UTR), a 3' UTR, or both.

73. The composition of claim 72, wherein the 5' UTR comprises at least 10 contiguous nucleotides from anywhere within SEQ ID NO: 12.

74. The composition of claim 73, wherein the 5' UTR comprises at least 20 contiguous nucleotides from anywhere within SEQ ID NO: 12.

75. The composition of claim 74, wherein the 5' UTR comprises at least 50 contiguous nucleotides from anywhere within SEQ ID NO: 12.

76. The composition of claim 75, wherein the 5' UTR comprises at least 80 contiguous nucleotides from anywhere within SEQ ID NO: 12.

77. The composition of claim 72, wherein the 5'UTR comprises a sequence that is at least 80% identical to SEQ ID NO: 12.

78. The composition of claim 72, wherein the 3' UTR comprises at least 10 contiguous nucleotides from anywhere within SEQ ID NO: 15.

79. The composition of claim 78, wherein the 3' UTR comprises at least 20 contiguous nucleotides from anywhere within SEQ ID NO: 15.

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80. The composition of claim 79, wherein the 3’ UTR comprises at least 50 contiguous nucleotides from anywhere within SEQ ID NO: 15.

81. The composition of claim 80, wherein the 3’ UTR comprises at least 80 contiguous nucleotides from anywhere within SEQ ID NO: 15.

82. The composition of claim 72, wherein the 3’ UTR comprises a sequence that is at least 80% identical to SEQ ID NO: 15.

83. The composition of claim 62, wherein the single nucleic acid vector is a plasmid, a transposon, a cosmid, an artificial chromosome, or a viral vector.

84. The composition of claim 62, wherein the single nucleic acid vector is a human artificial chromosome (HAC), yeast artificial chromosome (YAC), bacterial artificial chromosome (BAC), or a P1-derived artificial chromosome (PAC).

85. The composition of claim 62, wherein the single nucleic acid vector is a viral vector selected from an adeno-associated virus (AAV) vector, an adenovirus vector, a lentivirus vector, or a retrovirus vector.

86. The composition of claim 85, wherein the single nucleic acid vector is an AAV vector.

87. The composition of claim 62, wherein the single nucleic acid vector further comprises one or both of a promoter and a Kozak sequence.

88. The composition of claim 87, wherein the first vector comprises a promoter that is an inducible promoter, a constitutive promoter, or a tissue-specific promoter.

89. The composition of claim 87, wherein the single nucleic acid vector further comprises a polyadenylation signal sequence.

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90. The composition of any one of claims 1-89, further comprising a pharmaceutically acceptable excipient.

91. A kit comprising a composition of any one of claims 1-90.

92. A kit of claim 91, further comprising a pre-loaded syringe comprising the composition.

93. A method comprising:

introducing into a cochlea of a mammal a therapeutically effective amount of the composition of any one of claims 1-90.

94. The method of claim 93, wherein the mammal is a human.

95. The method of claim 93 or 94, wherein the mammal has been previously identified as having a defective CLRN1 gene.

96. A method of increasing expression of a full-length CLRN1 protein in a mammalian cell, the method comprising introducing the composition of any one of claims 1-90 into the mammalian cell.

97. The method of claim 96, wherein the mammalian cell is a cochlear inner hair cell or a cochlear outer hair cell.

98. The method of claim 96, wherein the mammalian cell is a retinal cell.

99. The method of any one of claims 96-98, wherein the mammalian cell is a human cell.

100. The method of any one of claims 96-99, wherein the mammalian cell has previously been determined to have a defective CLRN1 gene.

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101. A method of increasing expression of a full-length CLRN1 protein in an inner hair cell, an outer hair cell, or both, in a cochlea of a mammal, the method comprising:

introducing into the cochlea of the mammal a therapeutically effective amount of the composition of any one of claims 1-90.

102. A method of increasing expression of a full-length CLRN1 protein in an eye of a mammal, the method comprising:

intraocularly administering to the eye of the mammal a therapeutically effective amount of the composition of any one of claims 1-90.

103. The method of claim 101 or 102, wherein the mammal has been previously identified as having a defective CLRN1 gene.

104. The method of any one of claims 101-104, wherein the mammal is a human.

105. A method of treating hearing loss in a subject identified as having a defective CLRN1 gene, the method comprising:

administering a therapeutically effective amount of a composition of any one of claims 1-90 into the cochlea of the subject.

106. A method of treating vision loss in a subject identified as having a defective CLRN1 gene, the method comprising:

administering a therapeutically effective amount of a composition of any one of claims 1-90 into the eye of the subject.

107. The method of claim 105 or 106, wherein the subject has Usher syndrome type III.

108. The method of any one claims 105-107, wherein the subject is a human.

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109. The method of any one of claims 105-108, further comprising, prior to the administering step, determining that the subject has a defective CLRN1 gene.

110. A composition comprising two different nucleic acid vectors, wherein:

a first nucleic acid vector of the two different nucleic acid vectors comprises a promoter, a first coding sequence that encodes an N-terminal portion of a CLRN1 protein positioned 3' of the promoter, and a splice donor sequence positioned at the 3' end of the first coding sequence; and

a second nucleic acid vector of the two different nucleic acid vectors comprises a splice acceptor sequence, a second coding sequence that encodes a C-terminal portion of a CLRN1 protein positioned at the 3' end of the splice acceptor sequence, and a polyadenylation signal sequence at the 3' end of the second coding sequence;

wherein each of the encoded portions is at least 30 amino acid residues in length, wherein the amino acid sequences of the two encoded portions do not overlap with each other; wherein no single vector of the two different vectors encodes a full-length CLRN1 protein; and

when introduced into a mammalian cell, splicing occurs between the splice donor sequence and the splice acceptor sequence, thereby forming a recombined nucleic acid that encodes a full-length CLRN1 protein.

111. The composition of claim 110, wherein the first coding sequence encodes an N-terminal portion of a first isoform of CLRN1 protein, and the second coding sequence encodes a C-terminal portion of the first isoform of CLRN1 protein.

112. The composition of claim 111, wherein the first isoform of the CLRN1 protein comprises a sequence that is at least 95% identical to SEQ ID NO: 3.

113. The composition of claim 112, wherein the first isoform of the CLRN1 protein comprises SEQ ID NO: 3.

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114. The composition of claim 113, wherein the first isoform of the CLRN1 protein consists of SEQ ID NO: 3.

115. The composition of claim 111, wherein the first nucleic acid vector of the second nucleic acid vector further comprises a sequence that encodes a second isoform of the CLRN1 protein.

116. The composition of claim 115, wherein the second isoform of the CLRN1 protein comprises a sequence that is at least 95% identical to SEQ ID NO: 5.

117. The composition of claim 116, wherein the second isoform of the CLRN1 protein comprises SEQ ID NO: 5.

118. The composition of claim 117, wherein the second isoform of the CLRN1 protein consists of SEQ ID NO: 5.

119. The composition of claim 110, wherein the first coding sequence encodes an N-terminal portion of a second isoform of CLRN1 protein, and the second coding sequence encodes a C-terminal portion of the second isoform of CLRN1 protein.

120. The composition of claim 119, wherein the second isoform of the CLRN1 protein comprises a sequence that is at least 95% identical to SEQ ID NO: 5.

121. The composition of claim 120, wherein the second isoform of the CLRN1 protein comprises SEQ ID NO: 5.

122. The composition of claim 121, wherein the second isoform of the CLRN1 protein consists of SEQ ID NO: 5.

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123. The composition of claim 119, wherein the first nucleic acid vector or the second nucleic acid vector further comprises a sequence that encodes a first isoform of the CLRN1 protein.

124. The composition of claim 123, wherein the first isoform of the CLRN1 protein comprises a sequence that is at least 95% identical to SEQ ID NO: 3.

125. The composition of claim 124, wherein the first isoform of the CLRN1 protein comprises SEQ ID NO: 3.

126. The composition of claim 125, wherein the first isoform of the CLRN1 protein consists of SEQ ID NO: 3.

127. The composition of any one of claims 110-126, wherein one or both of the first nucleic acid vector and the second nucleic acid vector comprises a 5' untranslated region (UTR), a 3' UTR, or both.

128. The composition of claim 127, wherein the 5' UTR comprises at least 10 contiguous nucleotides from anywhere within SEQ ID NO: 12.

129. The composition of claim 128, wherein the 5' UTR comprises at least 20 contiguous nucleotides from anywhere within SEQ ID NO: 12.

130. The composition of claim 129, wherein the 5' UTR comprises at least 50 contiguous nucleotides from anywhere within SEQ ID NO: 12.

131. The composition of claim 130, wherein the 5' UTR comprises at least 80 contiguous nucleotides from anywhere within SEQ ID NO: 12.

132. The composition of claim 127, wherein the 5'UTR comprises a sequence that is at least 80% identical to SEQ ID NO: 12.

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133. The composition of claim 127, wherein the 3’ UTR comprises at least 10 contiguous nucleotides from anywhere within SEQ ID NO: 15.

134. The composition of claim 133, wherein the 3’ UTR comprises at least 20 contiguous nucleotides from anywhere within SEQ ID NO: 15.

135. The composition of claim 134, wherein the 3’ UTR comprises at least 50 contiguous nucleotides from anywhere within SEQ ID NO: 15.

136. The composition of claim 135, wherein the 3’ UTR comprises at least 80 contiguous nucleotides from anywhere within SEQ ID NO: 15.

137. The composition of claim 127, wherein the 3’ UTR comprises a sequence that is at least 80% identical to SEQ ID NO: 15.

138. The composition of any one of claims 110-137, wherein each of the first nucleic acid vector and the second nucleic acid vector is a plasmid, a transposon, a cosmid, an artificial chromosome, or a viral vector.

139. The composition of any one of claims 110-137, wherein each of the first nucleic acid vector and the second nucleic acid vector is a human artificial chromosome (HAC), yeast artificial chromosome (YAC), bacterial artificial chromosome (BAC), or a P1-derived artificial chromosome (PAC).

140. The composition of any one of claims 110-137, wherein each of the first nucleic acid vector and the second nucleic acid vector is a viral vector selected from an adeno-associated virus (AAV) vector, an adenovirus vector, a lentivirus vector, or a retrovirus vector.

141. The composition of claim 140, wherein each of the first nucleic acid vector and the second nucleic acid vector is an AAV vector.

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142. The composition of any one of claims 110-141, wherein at least one of the coding sequences comprises a nucleotide sequence spanning two consecutive exons of CLRN1 genomic DNA, and lacking an intronic sequence between the two consecutive exons.

143. A composition comprising two different nucleic acid vectors, wherein:

a first nucleic acid vector of the two different nucleic acid vectors comprises a promoter, a first coding sequence that encodes an N-terminal portion of a CLRN1 protein positioned 3' of the promoter, a splice donor sequence positioned at the 3' end of the first coding sequence, and a first detectable marker gene positioned 3' of the splice donor sequence; and

a second nucleic acid vector of the two different nucleic acid vectors comprises a second detectable marker gene, a splice acceptor sequence positioned 3' of the second detectable marker gene, a second coding sequence that encodes a C-terminal portion of a CLRN1 protein positioned at the 3' end of the splice acceptor sequence, and a polyadenylation signal sequence positioned at the 3' end of the second coding sequence;

wherein each of the encoded portions is at least 30 amino acid residues in length, wherein the amino acid sequences of the encoded portions do not overlap with each other; wherein no single vector of the two different vectors encodes a full-length CLRN1 protein; and

when introduced into a mammalian cell, splicing occurs between the splice donor sequence and the splice acceptor sequence, thereby forming a recombined nucleic acid that encodes a full-length CLRN1 protein.

144. The composition of claim 143, wherein the first coding sequence encodes an N-terminal portion of a first isoform of CLRN1 protein, and the second coding sequence encodes a C-terminal portion of the first isoform of CLRN1 protein.

145. The composition of claim 144, wherein the first isoform of the CLRN1 protein comprises a sequence that is at least 95% identical to SEQ ID NO: 3.

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146. The composition of claim 145, wherein the first isoform of the CLRN1 protein comprises SEQ ID NO: 3.

147. The composition of claim 146, wherein the first isoform of the CLRN1 protein consists of SEQ ID NO: 3.

148. The composition of claim 144, wherein the first nucleic acid vector of the second nucleic acid vector further comprises a sequence that encodes a second isoform of the CLRN1 protein.

149. The composition of claim 148, wherein the second isoform of the CLRN1 protein comprises a sequence that is at least 95% identical to SEQ ID NO: 5.

150. The composition of claim 149, wherein the second isoform of the CLRN1 protein comprises SEQ ID NO: 5.

151. The composition of claim 150, wherein the second isoform of the CLRN1 protein consists of SEQ ID NO: 5.

152. The composition of claim 143, wherein the first coding sequence encodes an N-terminal portion of a second isoform of CLRN1 protein, and the second coding sequence encodes a C-terminal portion of the second isoform of CLRN1 protein.

153. The composition of claim 152, wherein the second isoform of the CLRN1 protein comprises a sequence that is at least 95% identical to SEQ ID NO: 5.

154. The composition of claim 153, wherein the second isoform of the CLRN1 protein comprises SEQ ID NO: 5.

155. The composition of claim 154, wherein the second isoform of the CLRN1 protein consists of SEQ ID NO: 5.

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156. The composition of claim 152, wherein the first nucleic acid vector or the second nucleic acid vector further comprises a sequence that encodes a first isoform of the CLRN1 protein.

157. The composition of claim 156, wherein the first isoform of the CLRN1 protein comprises a sequence that is at least 95% identical to SEQ ID NO: 3.

158. The composition of claim 157, wherein the first isoform of the CLRN1 protein comprises SEQ ID NO: 3.

159. The composition of claim 158, wherein the first isoform of the CLRN1 protein consists of SEQ ID NO: 3.

160. The composition of any one of claims 143-159, wherein one or both of the first nucleic acid vector and the second nucleic acid vector comprises a 5' untranslated region (UTR), a 3' UTR, or both.

161. The composition of claim 160, wherein the 5' UTR comprises at least 10 contiguous nucleotides from anywhere within SEQ ID NO: 12.

162. The composition of claim 161, wherein the 5' UTR comprises at least 20 contiguous nucleotides from anywhere within SEQ ID NO: 12.

163. The composition of claim 162, wherein the 5' UTR comprises at least 50 contiguous nucleotides from anywhere within SEQ ID NO: 12.

164. The composition of claim 163, wherein the 5' UTR comprises at least 80 contiguous nucleotides from anywhere within SEQ ID NO: 12.

165. The composition of claim 160, wherein the 5'UTR comprises a sequence that is at least 80% identical to SEQ ID NO: 12.

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166. The composition of claim 160, wherein the 3’ UTR comprises at least 10 contiguous nucleotides from anywhere within SEQ ID NO: 15.

167. The composition of claim 166, wherein the 3’ UTR comprises at least 20 contiguous nucleotides from anywhere within SEQ ID NO: 15.

168. The composition of claim 167, wherein the 3’ UTR comprises at least 50 contiguous nucleotides from anywhere within SEQ ID NO: 15.

169. The composition of claim 168, wherein the 3’ UTR comprises at least 80 contiguous nucleotides from anywhere within SEQ ID NO: 15.

170. The composition of claim 160, wherein the 3’ UTR comprises a sequence that is at least 80% identical to SEQ ID NO: 15.

171. The composition of any one of claims 143-170, wherein each of the first nucleic acid vector and the second nucleic acid vector is a plasmid, a transposon, a cosmid, an artificial chromosome, or a viral vector.

172. The composition of any one of claims 143-170, wherein each of the first nucleic acid vector and the second nucleic acid vector is a human artificial chromosome (HAC), yeast artificial chromosome (YAC), bacterial artificial chromosome (BAC), or a P1-derived artificial chromosome (PAC).

173. The composition of any one of claims 143-170, wherein each of the first nucleic acid vector and the second nucleic acid vector is a viral vector selected from an adeno-associated virus (AAV) vector, an adenovirus vector, a lentivirus vector, or a retrovirus vector.

174. The composition of claim 173, wherein each of the first nucleic acid vector and the second nucleic acid vector is an AAV vector.

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175. The composition of any one of claims 143-174, wherein at least one of the coding sequences comprises a nucleotide sequence spanning two consecutive exons of CLRN1 genomic DNA, and lacking an intronic sequence between the two consecutive exons.

176. The composition of any one of claims 143-175, wherein the first or second detectable marker gene is alkaline phosphatase.

177. A composition comprising two different nucleic acid vectors, wherein:

a first nucleic acid vector of the two different nucleic acid vectors comprises a promoter, a first coding sequence that encodes an N-terminal portion of a CLRN1 protein positioned 3' to the promoter, a splice donor sequence positioned at the 3' end of the first coding sequence, and a F1 phage recombinogenic region positioned 3' to the splice donor sequence; and

a second nucleic acid vector of the two different nucleic acid vectors comprises a F1 phage recombinogenic region, a splice acceptor sequence positioned 3' of the F1 phage recombinogenic region, a second coding sequence that encodes a C-terminal portion of a CLRN1 protein positioned at the 3' end of the splice acceptor sequence, and a polyadenylation signal sequence positioned at the 3' end of the second coding sequence;

wherein each of the two encoded portions is at least 30 amino acid residues in length, wherein the amino acid sequences of the two encoded portions do not overlap with each other; wherein no single vector of the two different vectors encodes a full-length CLRN1 protein; and

when introduced into a mammalian cell, splicing occurs between the splice donor sequence and the splice acceptor sequence, thereby forming a recombined nucleic acid that encodes a full-length CLRN1 protein.

178. The composition of claim 177, wherein the first coding sequence encodes an N-terminal portion of a first isoform of CLRN1 protein, and the second coding sequence encodes a C-terminal portion of the first isoform of CLRN1 protein.

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179. The composition of claim 178, wherein the first isoform of the CLRN1 protein comprises a sequence that is at least 95% identical to SEQ ID NO: 3.

180. The composition of claim 179, wherein the first isoform of the CLRN1 protein comprises SEQ ID NO: 3.

181. The composition of claim 180, wherein the first isoform of the CLRN1 protein consists of SEQ ID NO: 3.

182. The composition of claim 178, wherein the first nucleic acid vector of the second nucleic acid vector further comprises a sequence that encodes a second isoform of the CLRN1 protein.

183. The composition of claim 182, wherein the second isoform of the CLRN1 protein comprises a sequence that is at least 95% identical to SEQ ID NO: 5.

184. The composition of claim 183, wherein the second isoform of the CLRN1 protein comprises SEQ ID NO: 5.

185. The composition of claim 184, wherein the second isoform of the CLRN1 protein consists of SEQ ID NO: 5.

186. The composition of claim 177, wherein the first coding sequence encodes an N-terminal portion of a second isoform of CLRN1 protein, and the second coding sequence encodes a C-terminal portion of the second isoform of CLRN1 protein.

187. The composition of claim 186, wherein the second isoform of the CLRN1 protein comprises a sequence that is at least 95% identical to SEQ ID NO: 5.

188. The composition of claim 187, wherein the second isoform of the CLRN1 protein comprises SEQ ID NO: 5.

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189. The composition of claim 188, wherein the second isoform of the CLRN1 protein consists of SEQ ID NO: 5.

190. The composition of claim 186, wherein the first nucleic acid vector or the second nucleic acid vector further comprises a sequence that encodes a first isoform of the CLRN1 protein.

191. The composition of claim 190, wherein the first isoform of the CLRN1 protein comprises a sequence that is at least 95% identical to SEQ ID NO: 3.

192. The composition of claim 191, wherein the first isoform of the CLRN1 protein comprises SEQ ID NO: 3.

193. The composition of claim 192, wherein the first isoform of the CLRN1 protein consists of SEQ ID NO: 3.

194. The composition of any one of claims 177-193, wherein one or both of the first nucleic acid vector and the second nucleic acid vector comprises a 5' untranslated region (UTR), a 3' UTR, or both.

195. The composition of claim 194, wherein the 5' UTR comprises at least 10 contiguous nucleotides from anywhere within SEQ ID NO: 12.

196. The composition of claim 195, wherein the 5' UTR comprises at least 20 contiguous nucleotides from anywhere within SEQ ID NO: 12.

197. The composition of claim 196, wherein the 5' UTR comprises at least 50 contiguous nucleotides from anywhere within SEQ ID NO: 12.

198. The composition of claim 197, wherein the 5' UTR comprises at least 80 contiguous nucleotides from anywhere within SEQ ID NO: 12.

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199. The composition of claim 194, wherein the 5'UTR comprises a sequence that is at least 80% identical to SEQ ID NO: 12.

200. The composition of claim 194, wherein the 3' UTR comprises at least 10 contiguous nucleotides from anywhere within SEQ ID NO: 15.

201. The composition of claim 200, wherein the 3' UTR comprises at least 20 contiguous nucleotides from anywhere within SEQ ID NO: 15.

202. The composition of claim 201, wherein the 3' UTR comprises at least 50 contiguous nucleotides from anywhere within SEQ ID NO: 15.

203. The composition of claim 202, wherein the 3' UTR comprises at least 80 contiguous nucleotides from anywhere within SEQ ID NO: 15.

204. The composition of claim 194, wherein the 3' UTR comprises a sequence that is at least 80% identical to SEQ ID NO: 15.

205. The composition of any one of claims 177-204, wherein each of the first nucleic acid vector and the second nucleic acid vector is a plasmid, a transposon, a cosmid, an artificial chromosome, or a viral vector.

206. The composition of any one of claims 177-204, wherein each of the first nucleic acid vector and the second nucleic acid vector is a human artificial chromosome (HAC), yeast artificial chromosome (YAC), bacterial artificial chromosome (BAC), or a P1-derived artificial chromosome (PAC).

207. The composition of any one of claims 177-204, wherein each of the first nucleic acid vector and the second nucleic acid vector is a viral vector selected from an adeno-associated virus (AAV) vector, an adenovirus vector, a lentivirus vector, or a retrovirus vector.

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208. The composition of claim 207, wherein each of the first nucleic acid vector and the second nucleic acid vector is an AAV vector.

209. The composition of any one of claims 177-204, wherein at least one of the coding sequences comprises a nucleotide sequence spanning two consecutive exons of CLRN1 genomic DNA, and lacking an intronic sequence between the two consecutive exons.

210. A kit comprising a composition of any one of claims 110-209.

211. A kit of claim 210, further comprising a pre-loaded syringe comprising the composition.

212. A method comprising:

introducing into a cochlea of a mammal a therapeutically effective amount of the composition of any one of claims 110-209.

213. The method of claim 212, wherein the mammal is a human.

214. The method of claim 212 or 213, wherein the mammal has been previously identified as having a defective CLRN1 gene.

215. A method of increasing expression of a full-length CLRN1 protein in a mammalian cell, the method comprising introducing the composition of any one of claims 110-209 into the mammalian cell.

216. The method of claim 215, wherein the mammalian cell is a cochlear inner hair cell or a cochlear outer hair cell.

217. The method of claim 215, wherein the mammalian cell is a retinal cell.

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218. The method of any one of claims 215-217, wherein the mammalian cell is a human cell.

219. The method of any one of claims 215-218, wherein the mammalian cell has previously been determined to have a defective CLRN1 gene.

220. A method of increasing expression of a full-length CLRN1 protein in an inner hair cell, an outer hair cell, or both, in a cochlea of a mammal, the method comprising:

introducing into the cochlea of the mammal a therapeutically effective amount of the composition of any one of claims 110-209.

221. A method of increasing expression of a full-length CLRN1 protein in an eye of a mammal, the method comprising:

intraocularly administering to the eye of the mammal a therapeutically effective amount of the composition of any one of claims 110-209.

222. The method of claim 220 or 221, wherein the mammal has been previously identified as having a defective CLRN1 gene.

223. The method of any one of claims 220-222, wherein the mammal is a human.

224. A method of treating hearing loss in a subject identified as having a defective CLRN1 gene, the method comprising:

administering a therapeutically effective amount of a composition of any one of claims 110-209 into the cochlea of the subject.

225. A method of treating vision loss in a subject identified as having a defective CLRN1 gene, the method comprising:

administering a therapeutically effective amount of a composition of any one of claims 110-209 into the eye of the subject.

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226. The method of claim 224 or 225, wherein the subject has Usher syndrome type III.

227. The method of any one claims 224-226, wherein the subject is a human.

228. The method of any one of claims 224-227, further comprising, prior to the administering step, determining that the subject has a defective CLRN1 gene.

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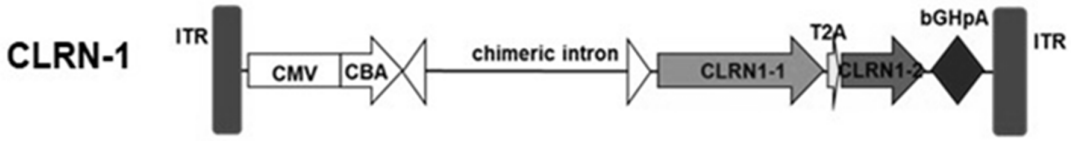


FIG. 1

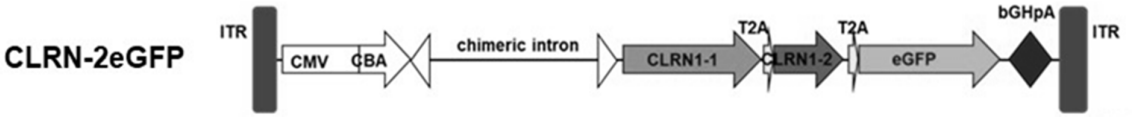


FIG. 2



FIG. 3



FIG. 4

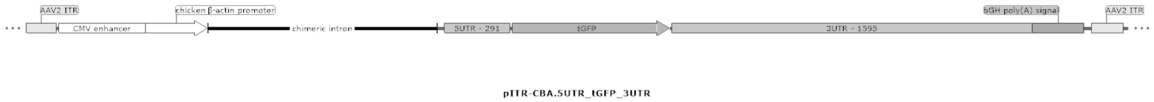


FIG. 5

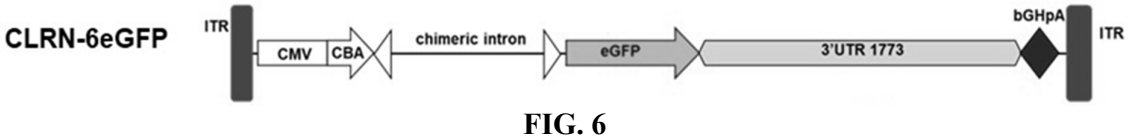


FIG. 6

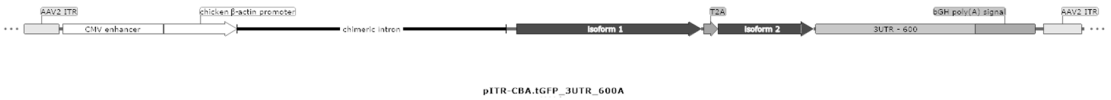


FIG. 7

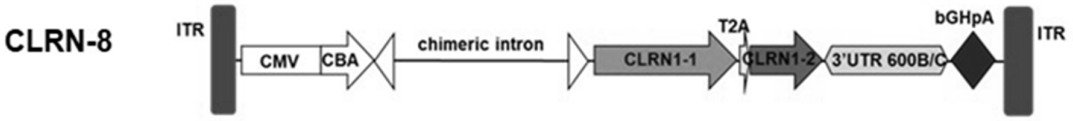


FIG. 8

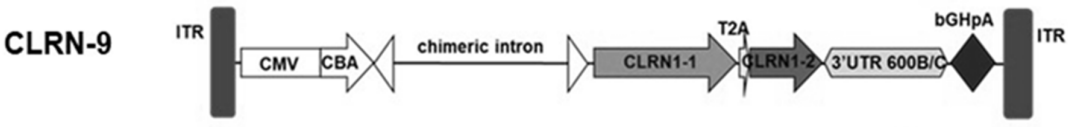


FIG. 9

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FIG. 10

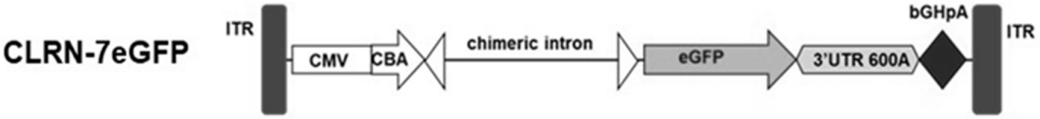


FIG. 11

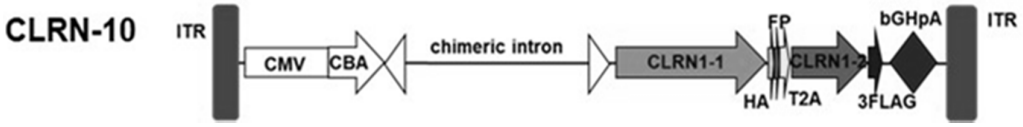


FIG. 12

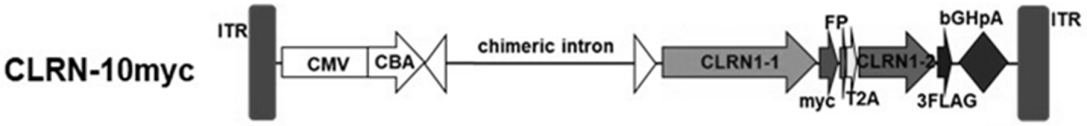


FIG. 13

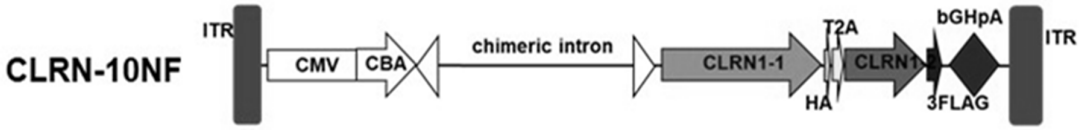


FIG. 14



FIG. 15



FIG. 16



FIG. 17



FIG. 18



FIG. 19

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FIG. 20



FIG. 21

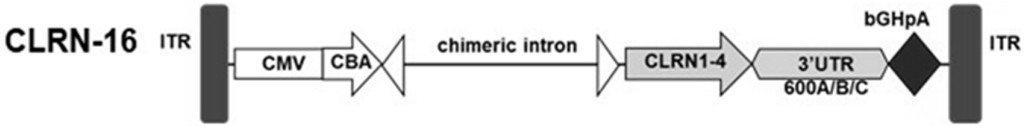


FIG. 22

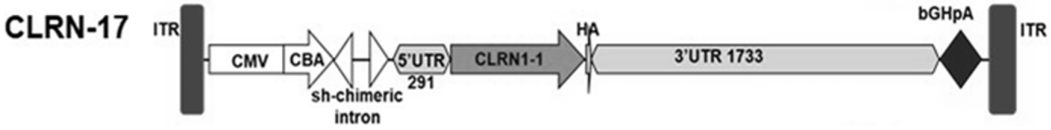


FIG. 23

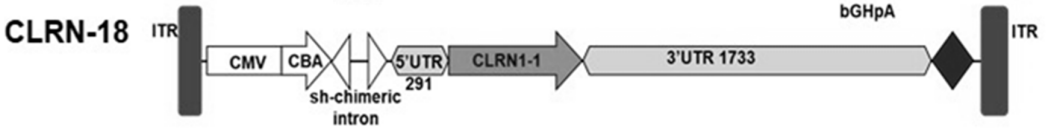


FIG. 24

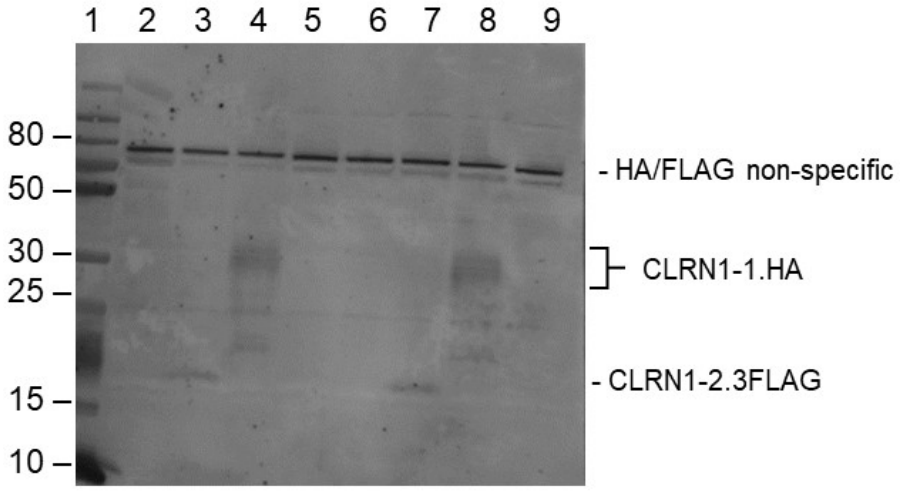


FIG. 25

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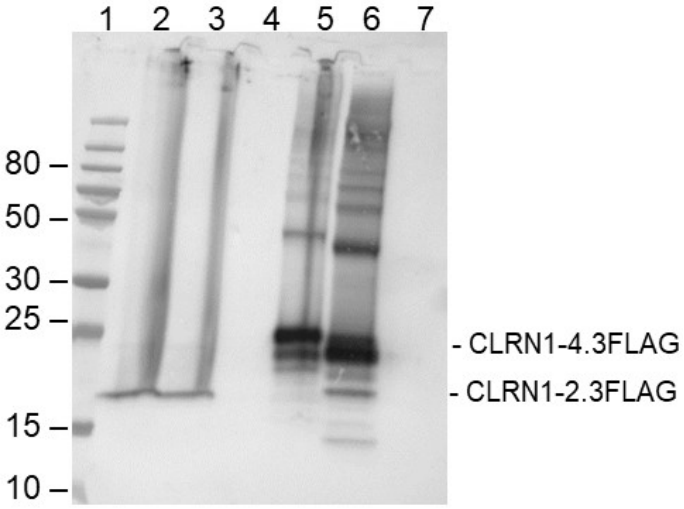


FIG. 26

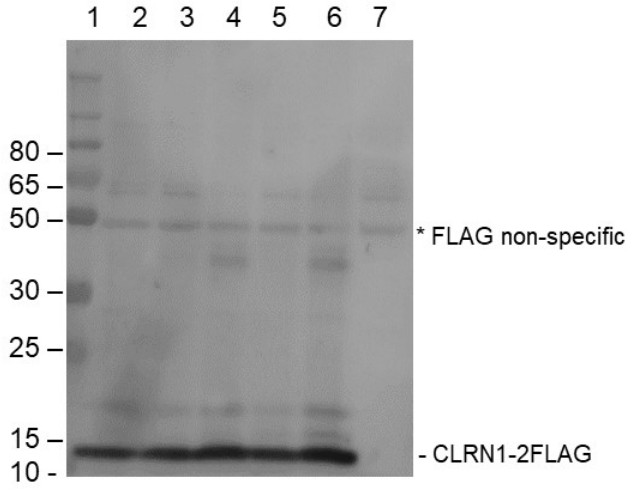


FIG. 27

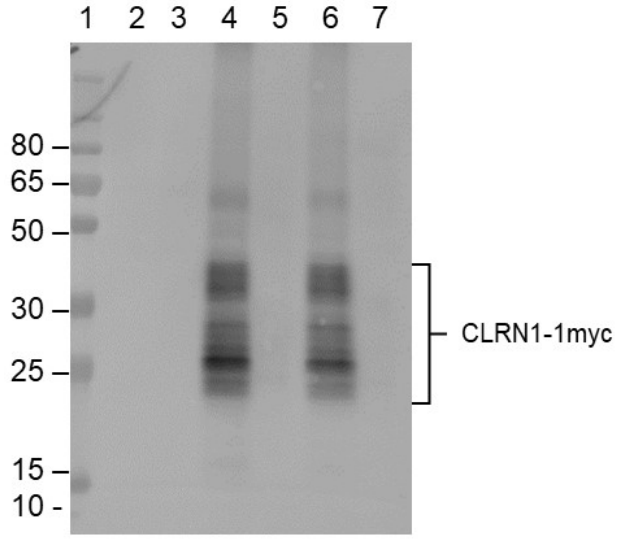


FIG. 28

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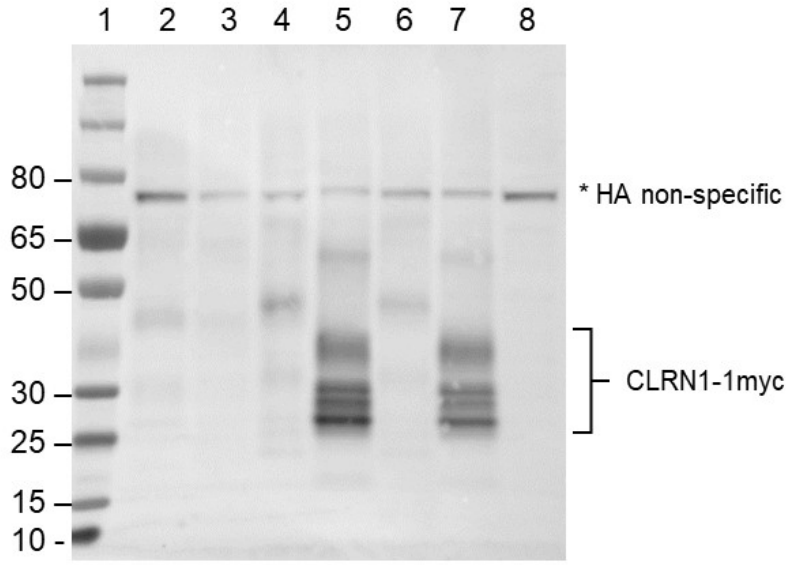


FIG. 29

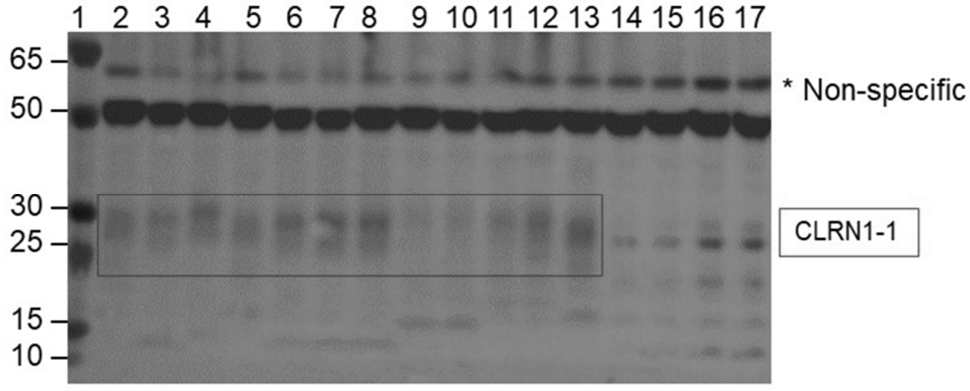


FIG. 30

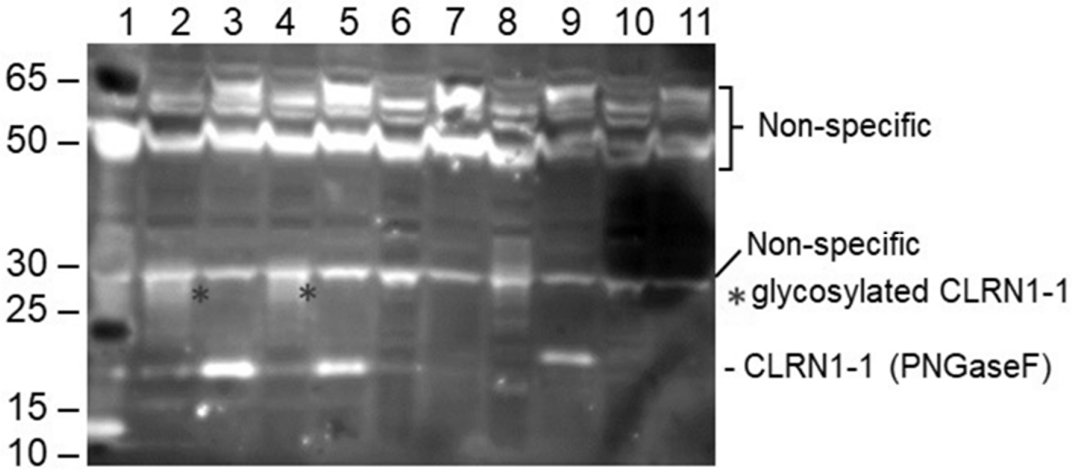


FIG. 31

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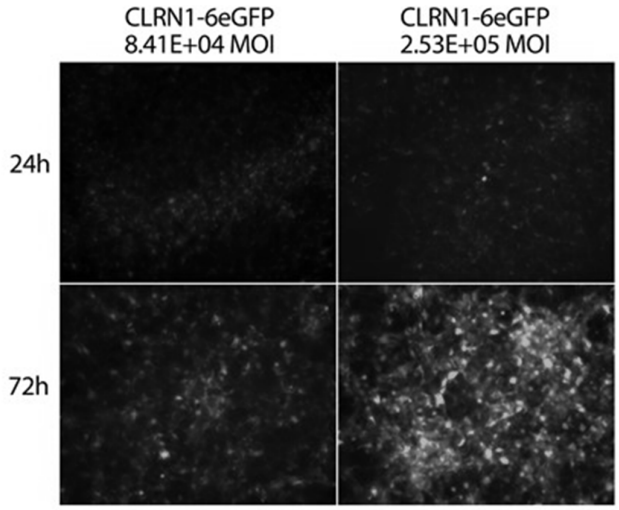


FIG. 32

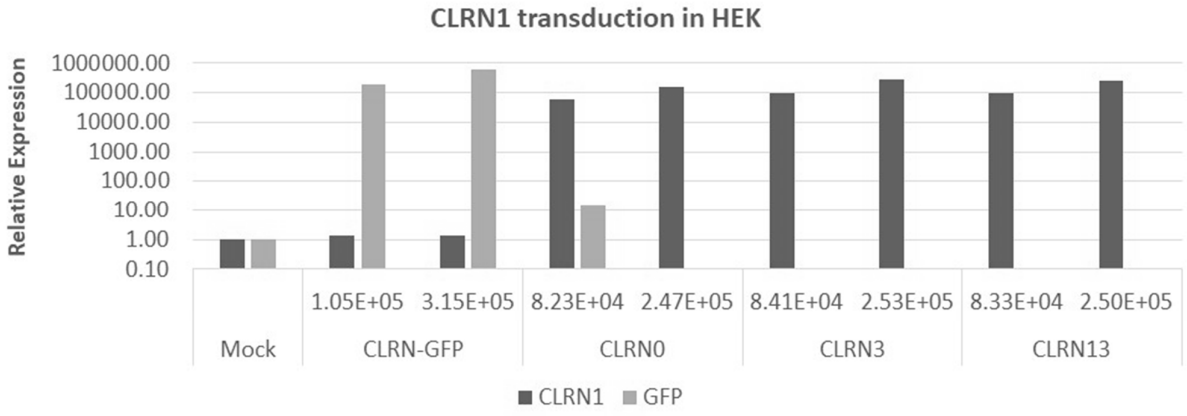


FIG. 33

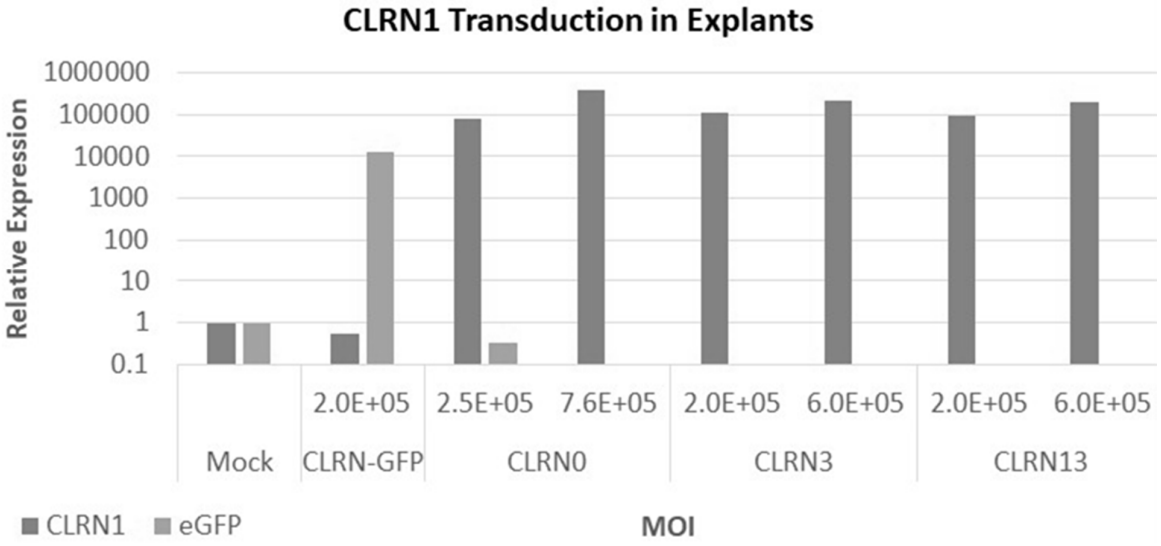


FIG. 34

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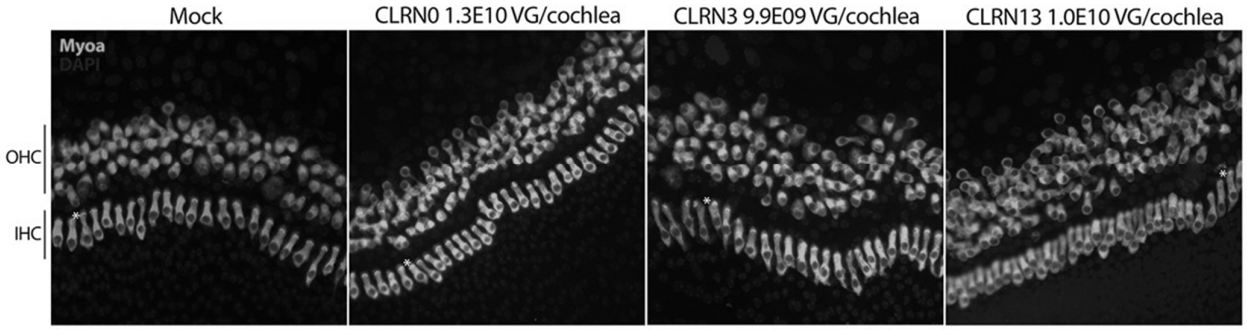


FIG. 35

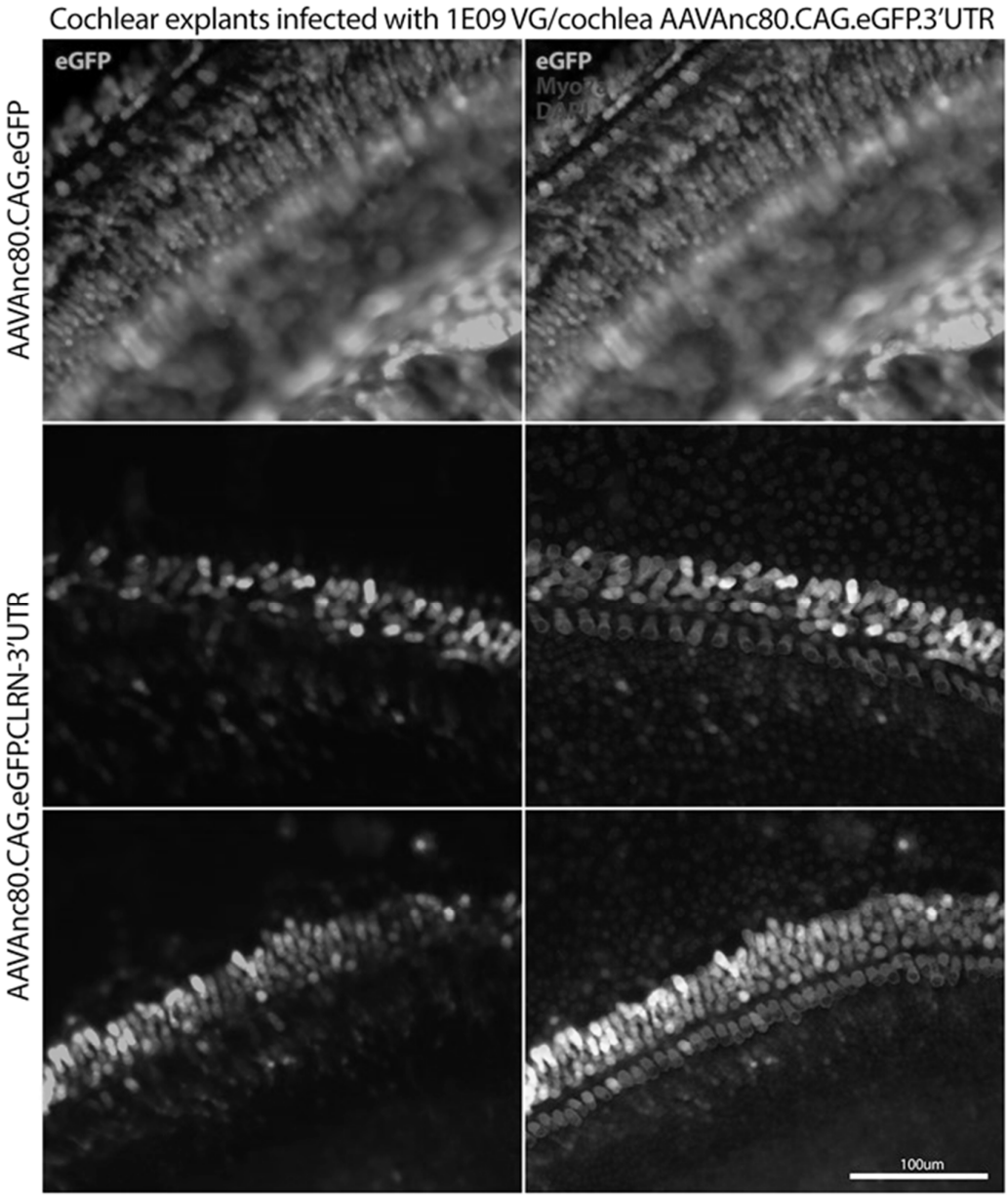


FIG. 36

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Sequence Listing

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1-2	DTD Version	V1_3
1-3	Software Name	WIPO Sequence
1-4	Software Version	2.3.0
1-5	Production Date	2026-02-23
1-6	Original free text language code	
1-7	Non English free text language code	
2	**General Information**	
2-1	Current application: IP Office	AU
2-2	Current application: Application number	
2-3	Current application: Filing date	
2-4	Current application: Applicant file reference	35644898-VPA
2-5	Earliest priority application: IP Office	US
2-6	Earliest priority application: Application number	62/689660
2-7	Earliest priority application: Filing date	2018-06-25
2-8en	Applicant name	Akouos, Inc.
2-8	Applicant name: Name Latin	
2-9en	Inventor name	
2-9	Inventor name: Name Latin	
2-10en	Invention title	Methods of treating CLRN1-associated hearing loss and/or vision loss
2-11	Sequence Total Quantity	60

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3-3-5	Residues	MPSQQKKIIF CMAGVFSFAC ALGVVTALGT PLWIKATVLC KTGALLVNAS GQELDKFMGE 60 MQYGLFHGEG VRQCGLGARP FRFSFFPDLL KAIPVSIHVN VILFSAILIV LTMVGTAFFM 120 YNAFGKPFET LHGPLGLYLL SFISGSCGCL VMILFASEVK IHHLSEKIAN YKEGTYVYKT 180 QSEKYTTSFW VIFFCFFVHF LNGLLIRLAG FQFPFAKSKD AETTNVAADL MY 232
3-4	**Sequences**	
3-4-1	Sequence Number [ID]	4
3-4-2	Molecule Type	DNA
3-4-3	Length	699
3-4-4	Features	**source 1..699**
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		organism=Homo sapiens
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3-4-5	Residues	atgccaagcc aacagaagaa aatcattttt tgcatggccg gagtgttcag ttttgcatgt 60 gccctcggag ttgtgacagc cttggggaca ccgttgtgga tcaaagccac tgtcctctgc 120 aaaacgggag ctctgctcgt caatgcctca gggcaggagc tggacaagtt tatgggtgaa 180 atgcagtacg ggcttttcca cggagagggt gtgaggcagt gtgggttggg agcaaggccc 240 tttcggttct catttttcc agatttgctc aaagcaatcc cagtgagcat ccacgtcaat 300 gtcattctct tctctgccat ccttattgtg ttaaccatgg tggggacagc cttcttcatg 360 tacaatgctt ttggaaaacc ttttgaaact ctgcatggtc ccctagggct gtacctttg 420 agcttcattt caggctcctg tggctgtctt gtcatgatat tgtttgcctc tgaagtgaaa 480 atccatcacc tctcagaaaa aattgcaaat tataaagaag ggacttatgt ctacaaaacg 540 caaagtgaaa aatataccac ctcattctgg gtcattttct tttgcttttt tgttcatttt 600 ctgaatgggc tcctaatacg acttgctgga tttcagttcc cttttgcaaa atctaaagac 660 gcagaaacaa ctaatgtagc tgcagatcta atgtactga 699
3-5	**Sequences**	
3-5-1	Sequence Number [ID]	5
3-5-2	Molecule Type	AA
3-5-3	Length	120
3-5-4	Features	**source 1..120**

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	Location/Qualifiers	mol_type=protein
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3-5-5	Residues	MQALQQQPVF PDLLKAIPVS IHVNVILFSA ILIVLTMVGT AFFMYNAFGK PFETLHGPLG 60 LYLLSFISGS CGCLVMILFA SEVKIHHLSE KIANYKEGTY VYKTQSEKYT TSFWLTKGHS 120
3-6	**Sequences**	
3-6-1	Sequence Number [ID]	6
3-6-2	Molecule Type	DNA
3-6-3	Length	363
3-6-4	Features	**source 1..363**
	Location/Qualifiers	mol_type=other DNA
		organism=Homo sapiens
	NonEnglishQualifier Value	
3-6-5	Residues	atgcaggccc tgcagcagca accagttttt ccagatttgc tcaaagcaat cccagtgagc 60 atccacgtca atgtcattct cttctctgcc atccttattg tgttaaccat ggtggggaca 120 gccttcttca tgtacaatgc ttttggaaaa ccttttgaaa ctctgcatgg tcccctaggg 180 ctgtaccttt tgagcttcat ttcaggctcc tgtggctgtc ttgtcatgat attgtttgcc 240 tctgaagtga aaatccatca cctctcagaa aaaattgcaa attataaaga agggacttat 300 gtctacaaaa cgcaaagtga aaaatatacc acctcattct ggctgactaa aggccacagc 360 tga 363
3-7	**Sequences**	
3-7-1	Sequence Number [ID]	7
3-7-2	Molecule Type	AA
3-7-3	Length	185
3-7-4	Features	**source 1..185**
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3-7-5	Residues	MPSQQKKIIF CMAGVFSFAC ALGVVTALGT PLWIKATVLC KTGALLVNAS GQELDKFMGE 60 MQYGLFHGEG VRQCGLGARP FRFSCYFLDP FMGLPTGVPH LLSLPCSTSC RREHTSERVQ 120 EPAGCFSAVR SKLHAGPAAA TSFSRFAQSN PSEHPRQCHS LLCHPYCVNH GGDSLLHVQC 180 FWKTF 185
3-8	**Sequences**	
3-8-1	Sequence Number [ID]	8
3-8-2	Molecule Type	DNA
3-8-3	Length	558
3-8-4	Features	**source 1..558**
	Location/Qualifiers	mol_type=other DNA
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	NonEnglishQualifier Value	
3-8-5	Residues	atgccaagcc aacagaagaa aatcattttt tgcatggccg gagtgttcag ttttgcatgt 60 gccctcggag ttgtgacagc cttggggaca ccgttgtgga tcaaagccac tgtcctctgc 120 aaaacgggag ctctgctcgt caatgcctca gggcaggagc tggacaagtt tatgggtgaa 180 atgcagtacg ggcttttcca cggagagggt gtgaggcagt gtgggttggg agcaaggccc 240 tttcggttct catgctattt tcttgacccc ttcatgggac tcccaacagg ggtaccccat 300 ttactcagcc tgccctgctc aacctcttgc aggagggagc acacgagtga acgagtgcag 360 gaaccagctg gctgctttag tgctgtgagg agtaaactcc atgcaggccc tgcagcagca 420 accagttttt ccagatttgc tcaaagcaat cccagtgagc atccacgtca atgtcattct 480 cttctctgcc atccttattg tgttaaccat ggtggggaca gccttcttca tgtacaatgc 540 ttttggaaaa ccttttga 558
3-9	**Sequences**	
3-9-1	Sequence Number [ID]	9
3-9-2	Molecule Type	DNA
3-9-3	Length	46837
3-9-4	Features	**source 1..46837**
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		organism=Homo sapiens
	NonEnglishQualifier Value	
3-9-5	Residues	aggagatact tgaaggcagt ttgaaagact tgttttacag attcttagtc caaagatttc 60 caattaggga gaagaagcag cagaaaagga gaaaagccaa gtatgagtga tgatgaggcc 120 ttcatctact gacatttaac ctggcgagaa ccgtcgatgg tgaagttgcc ttttcagctg 180 ggagctgtcc gttcagcttc cgtaataaat gcagtcaaag aggcagtccc ttcccattgc 240 tcacaaaggt cttgtttttg aacctcgccc tcacagaagc cgtttctcat catgccaagc 300 caacagaaga aaatcatttt ttgcatggcc ggagtgttca gttttgcatg tgccctcgga 360 gttgtgacag ccttggggac accgttgtgg atcaaagcca ctgtcctctg caaaacggga 420 gctctgctcg tcaatgcctc agggcaggag ctggacaagt ttatgggtga aatgcagtac 480 gggcttttcc acggagaggg tgtgaggcag tgtgggttgg gagcaaggcc ctttcggttc 540 tcatgtaagt agcaattgca tttgagttat ttaatgcttt aggcagactc ttcccagtgt 600 tgcgaggaat tatatttgag aattttgccg tgtttactgc aggacttttt aaatcggtgt 660 gaaccatatg aaaaacctat gactctgagc aatttcttct tcctagtttt tattatttta 720 tacttgcttt ttattataat atagagttaa ttcattgtta cataattaag gttttggaa 780 atattggcaa ttaagatgct taagtattaa tatttatgta aaaaattatg gagtcttttt 840

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3-10	**Sequences**	
3-10-1	Sequence Number [ID]	10
3-10-2	Molecule Type	AA
3-10-3	Length	250
3-10-4	Features	**source 1..250**
	Location/Qualifiers	mol_type=protein
		organism=Mus musculus
	NonEnglishQualifier Value	
3-10-5	Residues	MPSQQKKIIF CMAGVLSFLC ALGVVTAVGT PLWVKATILC KTGALLVNAS GKELDKFMGE 60 MQYGLFHGEG VRQCGLGARP FRFSSRSMKE RYSLYEDKGE TAVFPDLVQA IPVSIHINII 120 LFSMILVVLT MVGTAFFMYN AFGKPFETLH GPLGLYLVSF ISGSCGCLVM ILFASEVKVH 180 RLSEKIANFK EGTYAYRTQN ENYTTSFWVV FICFFVHFLN GLLIRLAGFQ FPFTKSKETE 240 TTNVASDLMY 250
3-11	**Sequences**	
3-11-1	Sequence Number [ID]	11
3-11-2	Molecule Type	AA
3-11-3	Length	232
3-11-4	Features	**source 1..232**
	Location/Qualifiers	mol_type=protein
		organism=Canis lupus
	NonEnglishQualifier Value	
3-11-5	Residues	MPNQQKKVVF CTAGVLSFVC ALGVVTALGT PLWIKATFLC KTGALLVNAS GQELDKFMGE 60 MQYGLFHGEG IRQCGLGARP FRFSLFPDLL KVIPVSIHVN VILFSTILVV LTMVGTAFFM 120 YNAFGKPFET LHGPLGLYLL SFISGSCGCL VMILFASEVK IHHLSEKIAN YKEGTYAYKT 180 QSEKYTTSFW VVFICFLVHL LNGLLIRLAG FQFPFAKSKD TETTNVAADL MY 232
3-12	**Sequences**	
3-12-1	Sequence Number [ID]	12
3-12-2	Molecule Type	DNA
3-12-3	Length	291
3-12-4	Features	**misc_feature 1..291**
	Location/Qualifiers	note=5' untranslated region
		source 1..291
		mol_type=other DNA
		organism=synthetic construct
	NonEnglishQualifier Value	
3-12-5	Residues	aggagatact tgaaggcagt ttgaaagact tgttttacag attcttagtc caaagatttc 60 caattaggga gaagaagcag cagaaaagga gaaaagccaa gtatgagtga tgatgaggcc 120 ttcatctact gacatttaac ctggcgagaa ccgtcgatgg tgaagttgcc ttttcagctg 180 ggagctgtcc gttcagcttc cgtaataaat gcagtcaaag aggcagtccc ttcccattgc 240 tcacaaaggt cttgtttttg aacctcgccc tcacagaagc cgtttctcat c 291
3-13	**Sequences**	
3-13-1	Sequence Number [ID]	13
3-13-2	Molecule Type	DNA
3-13-3	Length	130
3-13-4	Features	**misc_feature 1..130**
	Location/Qualifiers	note=5' inverted terminal repeat
		source 1..130
		mol_type=other DNA
		organism=synthetic construct
	NonEnglishQualifier Value	
3-13-5	Residues	cctgcaggca gctgcgcgct cgctcgctca ctgaggccgc ccgggcgtcg ggcgaccttt 60 ggtcgcccgg cctcagtgag cgagcgagcg cgcagagagg gagtggccaa ctccatcact 120 aggggttcct 130
3-14	**Sequences**	
3-14-1	Sequence Number [ID]	14
3-14-2	Molecule Type	DNA
3-14-3	Length	141
3-14-4	Features	**misc_feature 1..141**
	Location/Qualifiers	note=3' inverted terminal repeat
		source 1..141
		mol_type=other DNA
		organism=synthetic construct
	NonEnglishQualifier Value	

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3-14-5	Residues	aggaacccct agtgatggag ttggccactc cctctctgcg cgctcgctcg ctcactgagg 60 ccgggcgacc aaaggtcgcc cgacgcccgg gctttgcccg ggcggcctca gtgagcgagc 120 gagcgcgcag ctgcctgcag g 141
3-15	**Sequences**	
3-15-1	Sequence Number [ID]	15
3-15-2	Molecule Type	DNA
3-15-3	Length	1773
3-15-4	Features	**misc_feature 1..1773**
	Location/Qualifiers	note=5' untranslated region **source 1..1773** mol_type=other DNA organism=synthetic construct
	NonEnglishQualifier Value	
3-15-5	Residues	aaggcaaacc tttctataat tttacaaggg agtagacttg ctttggtcac ttttagatgt 60 ggttaatttt gcatatcctt ttagtctgca tatattaaag catcaggacc cttcgtgaca 120 atgtttacaa attacgtact aaggatacag gctggaaagt aagggaagca gaaggaaggc 180 tttgaaaagt tgttttatct ggtgggaaat tgcttgaccc aggtagtcaa aggcagttga 240 ctagaatcga caaattgtta ctccatatat atatatgtgt gtgtgtgtgt gtgtgtgtgt 300 gtgtaagatg tcttcctatc aaaaagatat caaaggcaca tggaatatat tttaataaaa 360 acaaataata tctctaatat atccacacat ttgttgccag atttcagaaa actgagctgc 420 aatcgctttc ctaaaacagt agtgtattaa atgaacatct ataaaatgta tcaacacaca 480 ttttaaaaaa tttgtttaaa gtatactctt aggccaggcg tggtgactca cacctgtaat 540 tccagcactt caggaggcca aggtgggaag atcatttgag ttcaggagtt cgagttacag 600 cctgggcaat aaagtgagac cctgtcacta acaaaattaa aaaataaaat aaatataaaa 660 tataggcttt aaaaaagcat agtcttatta accatgtctg ttggtcaaaa tctgcaaact 720 ctaaaagaag aaaagaagaa aaaaccaagc ttagggtatt tttcctcccg tgcctgagtc 780 ccaattacat tcacgacagt actttcaatg aacataattg ttaggaccac tgaggaatca 840 tgaaaaatga tctctgctta gtacatttga tgcaaaatga cttattaggg gctgtttttc 900 tagctatagt gtctcgagta ctaatatgca attatgaaaa ttatattaaa tctgggatta 960 tgacggtatc actgtatcat cttggtcttg ttctggctgt caccaagcat gacccaggtc 1020 aacttttttt ttcccctgaa ttacccatca aattgatctg cagctgacta aaggccacag 1080 ctgagcctgg aactgaccct tccttcatcc tcaacctgct gtcctccaga aagcaccaag 1140 gaaaaagcag agaatgacag caaacagatc actaggcctc tgaccacagg tgctgagtac 1200 tcagcagccc tcatataata ggtttgaaag tactccttaa aataaaacac tgtttccctt 1260 tggaactatt tacaaggatg aaacaaccgt atacctgaga aataacttgc tctggtgtca 1320 attcgctatt cgccagcaga catcagaaca caccgagttt ccagatgctg gtttttcccc 1380 ttaaatcagg aaatacacct ggacaatttc tagaagacta caattcagtc tagccacaaa 1440 ggggattttt ttttttggt aacaggctag agcccggttc tgtaagtctt tagctgaaat 1500 ggtccagtac aaaagcactg gaaatgagtg ggctaggagg acaaggaccg tctcctgcgt 1560 gaggagttgg ttggaggtcc ccaaggccag gtacccctg cactcttatt ggattcctct 1620 ctgtcttctt ggagttttga aaaactcctt cgaacaccag gctttttct ttagaaaaca 1680 agtctccaat cgttctctgt tccgtagaaa gagaaagaaa acctggagca gctgctgaaa 1740 aatctaatga ggaactaaga ggcaaaccca cca 1773
3-16	**Sequences**	
3-16-1	Sequence Number [ID]	16
3-16-2	Molecule Type	DNA
3-16-3	Length	1013
3-16-4	Features	**misc_feature 1..1013**
	Location/Qualifiers	note=Chimeric intron **source 1..1013** mol_type=other DNA organism=synthetic construct
	NonEnglishQualifier Value	
3-16-5	Residues	ggagtcgctg cgttgccttc gccccgtgcc ccgctccgcg ccgcctcgcg ccgcccgccc 60 cggctctgac tgaccgcgtt actcccacag gtgagcgggc gggacggccc ttctcctccg 120 ggctgtaatt agcgcttggt ttaatgacgg ctcgtttctt ttctgtggct gcgtgaaagc 180 cttaaagggc tccgggaggg ccctttgtgc ggggggggagc ggctcggggg gtgcgtgcgt 240 gtgtgtgtgc gtggggagcg ccgcgtgcgg cccgcgctgc ccggcggctg tgagcgctgc 300 gggcgcggcg cggggctttg tgcgctccgc gtgtgcgcga ggggagcgcg gccgggggcg 360 gtgccccgcg gtgcgggggg gctgcgaggg gaacaaaggc tgcgtgcggg gtgtgtgcgt 420 gggggggtga gcaggggggtg tgggcgcggc ggtcgggctg taacccccccc ctgcacccccc 480 ctccccgagt tgctgagcac ggcccggctt cgggtgcggg gctccgtgcg gggcgtggcg 540 cggggctcgc cgtgccgggc gggggggtggc ggcaggtggg ggtgccgggc ggggcggggc 600 cgcctcgggc cggggagggc tcgggggagg ggcgcggcgg cccccggagc gccggcggct 660 gtcgaggcgc ggcgagccgc agccattgcc ttttatggta atcgtgcgag agggcgcagg 720 gacttccttt gtcccaaatc tgtgcggagc cgaaatctgg gaggcgccgc cgcacccccct 780 ctagcgggcg cggggcgaag cggtgcggcg ccggcaggaa ggaaatgggc ggggagggcc 840 ttcgtgcgtc gccgcgccgc cgtcccccttc tccctctcca gcctcggggc tgtccgcggg 900 gggacggctg ccttcggggg ggacggggca gggcggggtt cggcttctgg cgtgtgaccg 960 gcggctctag agcctctgct aaccatgttc atgccttctt ctttttccta cag 1013
3-17	**Sequences**	
3-17-1	Sequence Number [ID]	17
3-17-2	Molecule Type	DNA

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3-17-3	Length	380
3-17-4	Features	**misc_feature 1..380**
	Location/Qualifiers	note=CMV enhancer
		source 1..380
		mol_type=other DNA
		organism=synthetic construct
	NonEnglishQualifier Value	
3-17-5	Residues	gacattgatt attgactagt tattaatagt aatcaattac ggggtcatta gttcatagcc 60 catatatgga gttccgcgtt acataactta cggtaaatgg cccgcctggc tgaccgccca 120 acgacccccg cccattgacg tcaataatga cgtatgttcc catagtaacg ccaataggga 180 ctttccattg acgtcaatgg gtggactatt tacggtaaac tgcccacttg gcagtacatc 240 aagtgtatca tatgccaagt acgccccta ttgacgtcaa tgacggtaaa tggcccgcct 300 ggcattatgc ccagtacatg accttatggg actttcctac ttggcagtac atctacgtat 360 tagtcatcgc tattaccatg 380
3-18	**Sequences**	
3-18-1	Sequence Number [ID]	18
3-18-2	Molecule Type	DNA
3-18-3	Length	276
3-18-4	Features	**misc_feature 1..276**
	Location/Qualifiers	note=CBA promoter
		source 1..276
		mol_type=other DNA
		organism=synthetic construct
	NonEnglishQualifier Value	
3-18-5	Residues	tcgaggtgag ccccacgttc tgcttcactc tccccatctc ccccccctcc ccacccccaa 60 ttttgtattt atttatttttt taattatttt gtgcagcgat gggggcgggg ggggggggggg 120 cgcgcgccag gcggggcggg gcggggcgag gggcggggcg gggcgaggcg gagaggtgcg 180 gcggcagcca atcagagcgg cgcgctccga aagtttcctt ttatggcgag gcggcggcgg 240 cggcggccct ataaaaagcg aagcgcgcgg cgggcg 276
3-19	**Sequences**	
3-19-1	Sequence Number [ID]	19
3-19-2	Molecule Type	AA
3-19-3	Length	232
3-19-4	Features	**REGION 1..232**
	Location/Qualifiers	note=tGFP
		source 1..232
		mol_type=protein
		organism=synthetic construct
	NonEnglishQualifier Value	
3-19-5	Residues	MESDESGLPA MEIECRITGT LNGVEFELVG GGEGTPEQGR MTNKMKSTKG ALTFSPYLLS 60 HVMGYGFYHF GTYPSGYENP FLHAINNGGY TNTRIEKYED GGVLHVSFSY RYEAGRVIGD 120 FKVMGTGFPE DSVIFTDKII RSNATVEHLH PMGDNDLDGS FTRTFSLRDG GYYSSVVDSH 180 MHFKSAIHPS ILQNGGPMFA FRRVEEDHSN TELGIVEYQH AFKTPDADAG EE 232
3-20	**Sequences**	
3-20-1	Sequence Number [ID]	20
3-20-2	Molecule Type	DNA
3-20-3	Length	225
3-20-4	Features	**source 1..225**
	Location/Qualifiers	mol_type=other DNA
		organism=Bos taurus
	NonEnglishQualifier Value	
3-20-5	Residues	ctgtgccttc tagttgccag ccatctgttg tttgcccctc ccccgtgcct tccttgaccc 60 tggaaggtgc cactcccact gtcctttcct aataaaatga ggaaattgca tcgcattgtc 120 tgagtaggtg tcattctatt ctgggggggtg gggtggggca ggacagcaag ggggaggatt 180 gggaagacaa tagcaggcat gctggggatg cggtgggctc tatgg 225
3-21	**Sequences**	
3-21-1	Sequence Number [ID]	21
3-21-2	Molecule Type	DNA
3-21-3	Length	17
3-21-4	Features	**source 1..17**
	Location/Qualifiers	mol_type=other DNA
		organism=Homo sapiens
	NonEnglishQualifier Value	
3-21-5	Residues	aaataaaata cgaaatg 17
3-22	**Sequences**	
3-22-1	Sequence Number [ID]	22
3-22-2	Molecule Type	DNA
3-22-3	Length	82
3-22-4	Features	**misc_feature 1..82**
	Location/Qualifiers	note=Splice donor

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		source 1..82
		mol_type=other DNA
		organism=synthetic construct
	NonEnglishQualifier Value	
3-22-5	Residues	gtaagtatca aggttacaag acaggtttaa ggagaccaat agaaactggg cttgtcgaga 60 cagagaagac tcttgcgttt ct 82
3-23	**Sequences**	
3-23-1	Sequence Number [ID]	23
3-23-2	Molecule Type	DNA
3-23-3	Length	51
3-23-4	Features	**misc_feature 1..51**
	Location/Qualifiers	note=Splice acceptor
		source 1..51
		mol_type=other DNA
		organism=synthetic construct
	NonEnglishQualifier Value	
3-23-5	Residues	gataggcacc tattggtctt actgacatcc actttgcctt tctctccaca g 51
3-24	**Sequences**	
3-24-1	Sequence Number [ID]	24
3-24-2	Molecule Type	DNA
3-24-3	Length	141
3-24-4	Features	**misc_feature 1..141**
	Location/Qualifiers	note=3' inverted terminal repeat
		source 1..141
		mol_type=other DNA
		organism=synthetic construct
	NonEnglishQualifier Value	
3-24-5	Residues	cctgcaggca gctgcgcgct cgctcgctca ctgaggccgc ccgggcaaag cccgggcgtc 60 gggcgacctt tggtcgcccg gcctcagtga gcgagcgagc gcgcagagag ggagtggcca 120 actccatcac taggggttcc t 141
3-25	**Sequences**	
3-25-1	Sequence Number [ID]	25
3-25-2	Molecule Type	DNA
3-25-3	Length	145
3-25-4	Features	**misc_feature 1..145**
	Location/Qualifiers	note=ITR
		source 1..145
		mol_type=other DNA
		organism=synthetic construct
	NonEnglishQualifier Value	
3-25-5	Residues	ttggccactc cctctctgcg cgctcgctcg ctcactgagg ccgcccgggc aaagcccggg 60 cgtcgggcga cctttggtcg cccggcctca gtgagcgagc gagcgcgcag agagggagtg 120 gccaactcca tcactagggg ttcct 145
3-26	**Sequences**	
3-26-1	Sequence Number [ID]	26
3-26-2	Molecule Type	DNA
3-26-3	Length	245
3-26-4	Features	**misc_feature 1..245**
	Location/Qualifiers	note=sh-chimeric intron
		source 1..245
		mol_type=other DNA
		organism=synthetic construct
	NonEnglishQualifier Value	
3-26-5	Residues	ggagtcgctg cgttgccttc gccccgtgcc ccgctccgcg ccgcctcgcg ccgcccgccc 60 cggctctgac tgaccgcgtt actcccacag gtgagcgggc gggacggccc ttctcctccg 120 ggctgtaatt agcgcttggt ttaatgacgg cttgtttctt ttctgtggct gcgtgaaagc 180 cttgaggggc tccgggagct agagcctctg ctaaccatgt tcatgccttc ttctttttcc 240 tacag 245
3-27	**Sequences**	
3-27-1	Sequence Number [ID]	27
3-27-2	Molecule Type	DNA
3-27-3	Length	600
3-27-4	Features	**misc_feature 1..600**
	Location/Qualifiers	note=3'UTR 600A
		source 1..600
		mol_type=other DNA
		organism=synthetic construct
	NonEnglishQualifier Value	
3-27-5	Residues	aaggcaaacc tttctataat tttacaaggg agtagacttg ctttggtcac ttttagatgt 60

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		ggttaatttt gcatatcctt ttagtctgca tatattaaag catcaggacc cttcgtgaca 120 atgtttacaa attacgtact aaggatacag gctggaaagt aagggaagca gaaggaaggc 180 tttgaaaagt tgttttatct ggtgggaaat tgcttgaccc aggtagtcaa aggcagttga 240 ctagaatcga caaattgtta ctccatatat atatatgtgt gtgtgtgtgt gtgtgtgtgt 300 gtgtaagatg tcttcctatc aaaaagatat caaaggcaca tggaatatat tttaataaaa 360 acaaataata tctctaatat atccacacat ttgttgccag atttcagaaa actgagctgc 420 aatcgctttc ctaaaacagt agtgtattaa atgaacatct ataaaatgta tcaacacaca 480 ttttaaaaaa tttgtttaaa gtatactctt aggccaggcg tggtgactca cacctgtaat 540 tccagcactt caggaggcca aggtgggaag atcatttgag ttcaggagtt cgagttacag 600
3-28	**Sequences**	
3-28-1	Sequence Number [ID]	28
3-28-2	Molecule Type	DNA
3-28-3	Length	600
3-28-4	Features	**misc_feature 1..600**
	Location/Qualifiers	note=3'UTR 600B
		source 1..600
		mol_type=other DNA
		organism=synthetic construct
	NonEnglishQualifier Value	
3-28-5	Residues	gaagatcatt tgagttcagg agttcgagtt acagcctggg caataaagtg agaccctgtc 60 actaacaaaa ttaaaaaata aaataaatat aaaatatagg ctttaaaaaa gcatagtctt 120 attaaccatg tctgttggtc aaaatctgca aactctaaaa gaagaaaaga agaaaaaacc 180 aacgttaggg tatttttcct cccgtgcctg agtcccaatt acattcacga cagtactttc 240 aatgaacata attgttagga ccactgagga atcatgaaaa atgatctctg cttagtacat 300 ttgatgcaaa atgacttatt aggggctgtt tttctagcta tagtgtctcg agtactaata 360 tgcaattatg aaaattatat taaatctggg attatgacgg tatcactgta tcatcttggt 420 cttgttctgg ctgtcaccaa gcatgaccca ggtcaacttt ttttttcccc tgaattaccc 480 atcaaattga tctgcagctg actaaaggcc acagctgagc ctggaactga cccttccttc 540 atcctcaacc tgctgtcctc cagaaagcac caaggaaaaa gcagagaatg acagcaaaca 600
3-29	**Sequences**	
3-29-1	Sequence Number [ID]	29
3-29-2	Molecule Type	DNA
3-29-3	Length	600
3-29-4	Features	**misc_feature 1..600**
	Location/Qualifiers	note=3'UTR 600C
		source 1..600
		mol_type=other DNA
		organism=synthetic construct
	NonEnglishQualifier Value	
3-29-5	Residues	aggcctctga ccacaggtgc tgagtactca gcagccctca tataataggt ttgaaagtac 60 tccttaaaat aaaacactgt ttccctttgg aactatttac aaggatgaaa caaccgtata 120 cctgagaaat aacttgctct ggtgtcaatt cgctattcgc cagcagacat cagaacacac 180 cgagtttcca gatgctggtt tttcccctta aatcaggaaa tacacctgga caatttctag 240 aagactacaa ttcagtctag ccacaaaggg gatttttttt ttttggtaac aggctagagc 300 ccggttctgt aagtctttag ctgaaatggt ccagtacaaa agcactggaa atgagtgggc 360 taggaggaca aggaccgtct cctgcgtgag gagttggttg gaggtcccca aggccaggta 420 ccccctgcac tcttattgga ttcctctctg tcttcttgga gttttgaaaa actccttcga 480 acaccaggct tttttcttta gaaaacaagt ctccaatcgt tctctgttcc gtagaaagag 540 aaagaaaacc tggagcagct gctgaaaaat ctaatgagga actaagaggc aaacccacca 600
3-30	**Sequences**	
3-30-1	Sequence Number [ID]	30
3-30-2	Molecule Type	AA
3-30-3	Length	6
3-30-4	Features	**REGION 1..6**
	Location/Qualifiers	note=FP
		source 1..6
		mol_type=protein
		organism=synthetic construct
	NonEnglishQualifier Value	
3-30-5	Residues	KRKRRG 6
3-31	**Sequences**	
3-31-1	Sequence Number [ID]	31
3-31-2	Molecule Type	AA
3-31-3	Length	18
3-31-4	Features	**REGION 1..18**
	Location/Qualifiers	note=T2A
		source 1..18
		mol_type=protein
		organism=synthetic construct
	NonEnglishQualifier Value	
3-31-5	Residues	EGRGSLLTCG DVEENPGP 18

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3-32	**Sequences**	
3-32-1	Sequence Number [ID]	32
3-32-2	Molecule Type	AA
3-32-3	Length	239
3-32-4	Features	**REGION 1..239**
	Location/Qualifiers	note=Enhanced GFP
		source 1..239
		mol_type=protein
		organism=synthetic construct
	NonEnglishQualifier Value	
3-32-5	Residues	MVSKGEELFT GVVPILVELD GDVNGHKFSV SGEGEGDATY GKLTLKFICT TGKLPVPWPT 60 LVTTLTYGVQ CFSRYPDHMK QHDFFKSAMP EGYVQERTIF FKDDGNYKTR AEVKFEGDTL 120 VNRIELKGID FKEDGNILGH KLEYNYNSHN VYIMADKQKN GIKVNFKIRH NIEDGSVQLA 180 DHYQQNTPIG DGPVLLPDNH YLSTQSALSK DPNEKRDHMV LLEFVTAAGI TLGMDELYK 239
3-33	**Sequences**	
3-33-1	Sequence Number [ID]	33
3-33-2	Molecule Type	AA
3-33-3	Length	30
3-33-4	Features	**REGION 1..30**
	Location/Qualifiers	note=Myc tag sequence
		source 1..30
		mol_type=protein
		organism=synthetic construct
	NonEnglishQualifier Value	
3-33-5	Residues	EQKLISEEDL EQKLISEEDL EQKLISEEDL 30
3-34	**Sequences**	
3-34-1	Sequence Number [ID]	34
3-34-2	Molecule Type	AA
3-34-3	Length	9
3-34-4	Features	**REGION 1..9**
	Location/Qualifiers	note=HA tag sequence
		source 1..9
		mol_type=protein
		organism=synthetic construct
	NonEnglishQualifier Value	
3-34-5	Residues	YPYDVPDYA 9
3-35	**Sequences**	
3-35-1	Sequence Number [ID]	35
3-35-2	Molecule Type	AA
3-35-3	Length	22
3-35-4	Features	**REGION 1..22**
	Location/Qualifiers	note=FLAG tag sequence
		source 1..22
		mol_type=protein
		organism=synthetic construct
	NonEnglishQualifier Value	
3-35-5	Residues	DYKDHDGDYK DHDIDYKDDD DK 22
3-36	**Sequences**	
3-36-1	Sequence Number [ID]	36
3-36-2	Molecule Type	DNA
3-36-3	Length	1357
3-36-4	Features	**misc_feature 1..1357**
	Location/Qualifiers	note=3'UTR 1357
		source 1..1357
		mol_type=other DNA
		organism=synthetic construct
	NonEnglishQualifier Value	
3-36-5	Residues	aaggcaaacc tttctataat tttacaaggg agtagacttg ctttggtcac ttttagatgt 60 ggttaatttt gcatatcctt ttagtctgca tatattaaag catcaggacc cttcgtgaca 120 atgtttacaa attacgtact aaggatacag gctggaaagt aagggaagca gaaggaaggc 180 tttgaaaagt tgttttatct ggtgggaaat tgcttgaccc aggtagtcaa aggcagttga 240 ctagaatcga caaattgtta ctccatatat atatatgtgt gtgtgtgtgt gtaagatgtc 300 ttcctatcaa aaagatatca aaggcacatg gaatatattt taataaaaac aaataatatc 360 tctaatatat ccacacattt gttgccagat ttcagaaaac tgagctgcaa tcgctttcct 420 aaaacagtag tgtattaaat gaacatctat aaaatgtatc aacacacatt ttaaaaaatt 480 tgtttaaagt atactcttag gccaggcgtg gtgactcaca cctgtaattc cagcacttca 540 ggaggccaag gtgggaagat catttgagtt caggagttcg agttacagtc tgggcaataa 600 agtgagaccc tgtcactaac aaaattaaaa aataaaataa atataaaata taggctttaa 660 aaaagcatag tcttattaac catgtctgtt ggtcaaaatc tgcaaactct aaaagaagaa 720 aagaagaaaa aaccaacgtt agggtatttt tcctcccgtg cctgagtccc aattacattc 780

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		acgacagtac tttcaatgaa cataattgtt aggaccactg aggaatcatg aaaaatgatc 840 tctgcttagt acatttgatg caaaatgact tattaggggc tgtttttcta gctatagtgt 900 ctcgagtact aatatgcaat tatgaaaatt atattaaatc tgggattatg acggtatcac 960 tgtatcatct tggtcttgtt ctggctgtca ccaagcatga cccaggtcaa ctttttttt 1020 cccctgaatt acccatcaaa ttgatctgca gctgactaaa ggccacagct gagcctggaa 1080 ctgacccttc cttcatcctc aacctgctgt cctccagaaa gcaccaagga aaaagcagag 1140 aatgacagca aacagatcac taggcctctg accacaggtg ctgagtactc agcagccctc 1200 atataatagg tttgaaagta ctccttaaaa taaaacactg tttccctttg gaactattta 1260 caaggatgaa acaaccgtat acctgagaaa taacttgctc tggtgtcaat tcgctattcg 1320 ccagcagaca tcagaacaca ccgagtttcc agatgct 1357
3-37	**Sequences**	
3-37-1	Sequence Number [ID]	37
3-37-2	Molecule Type	DNA
3-37-3	Length	1406
3-37-4	Features	**misc_feature 1..1406**
	Location/Qualifiers	note=3'UTR 1406
		source 1..1406
		mol_type=other DNA
		organism=synthetic construct
	NonEnglishQualifier Value	
3-37-5	Residues	aaggcaaacc tttctataat tttacaaggg agtagacttg ctttggtcac ttttagatgt 60 ggttaatttt gcatatcctt ttagtctgca tatattaaag catcaggacc cttcgtgaca 120 atgtttacaa attacgtact aaggatacag gctggaaagt aagggaagca gaaggaaggc 180 tttgaaaagt tgttttatct ggtgggaaat tgcttgaccc aggtagtcaa aggcagttga 240 ctagaatcga caaattgtta ctccatatat atatatatgt gtgtgtgtgt gtaagatgtc 300 ttcctatcaa aaagatatca aaggcacatg gaatatattt taataaaaac aaataatatc 360 tctaatatat ccacacattt gttgccagat ttcagaaaac tgagctgcaa tcgctttcct 420 aaaacagtag tgtattaaat gaacatctat aaaatgtatc aacacacatt ttaaaaaatt 480 tgtttaaagt atactcttag gccaggcgtg gtgactcaca cctgtaattc cagcacttca 540 ggaggccaag gtgggaagat catttgagtt caggagttcg agttacagcc tgggcaataa 600 agtgagaccc tgtcactaac aaaattaaaa aataaaataa atataaaata taggctttaa 660 aaaagcatag tcttattaac catgtctgtt ggtcaaaatc tgcaaactct aaaagaagaa 720 aagaagaaaa aaccaacgtt agggtatttt tcctcccgtg cctgagtccc aattacattc 780 acgacagtac tttcaatgaa cataattgtt aggaccactg aggaatcatg aaaaatgatc 840 tctgcttagt acatttgatg caaaatgact tattaggggc tgtttttcta gctatagtgt 900 ctcgagtact aatatgcaat tatgaaaatt atattaaatc tgggattatg acggtatcac 960 tgtatcatct tggtcttgtt ctggctgtca ccaagcatga cccaggtcaa ctttttttt 1020 cccctgaatt acccatcaaa ttgatctgca gctgactaaa ggccacagct gagcctggaa 1080 ctgacccttc cttcatcctc aacctgctgt cctccagaaa gcaccaagga aaaagcagag 1140 aatgacagca aacagatcac taggcctctg accacaggtg ctgagtactc agcagccctc 1200 atataatagg tttgaaagta ctccttaaaa taaaacactg tttccctttg gaactattta 1260 caaggatgaa acaaccgtat acctgagaaa taacttgctc tggtgtcaat tcgctattcg 1320 ccagcagaca tcagaacaca ccgagtttcc agatgctggt ttttcccctt aaatcaggaa 1380 atacacctgg acaatttcta gaagac 1406
3-38	**Sequences**	
3-38-1	Sequence Number [ID]	38
3-38-2	Molecule Type	AA
3-38-3	Length	20
3-38-4	Features	**REGION 1..20**
	Location/Qualifiers	note=CLRN1 antibody epitope
		source 1..20
		mol_type=protein
		organism=synthetic construct
	NonEnglishQualifier Value	
3-38-5	Residues	EKIANYKEGT YVYKTQSEKY 20
3-39	**Sequences**	
3-39-1	Sequence Number [ID]	39
3-39-2	Molecule Type	DNA
3-39-3	Length	4732
3-39-4	Features	**misc_feature 1..4732**
	Location/Qualifiers	note=CLRN-0 vector
		source 1..4732
		mol_type=other DNA
		organism=synthetic construct
	NonEnglishQualifier Value	
3-39-5	Residues	cctgcaggca gctgcgcgct cgctcgctca ctgaggccgc ccgggcgtcg ggcgaccttt 60 ggtcgcccgg cctcagtgag cgagcgagcg cgcagagagg gagtggccaa ctccatcact 120 aggggttcct gcggccgcac gcgtgacatt gattattgac tagttattaa tagtaatcaa 180 ttacggggtc attagttcat agcccatata tggagttccg cgttacataa cttacggtaa 240 atggcccgcc tggctgaccg cccaacgacc cccgcccatt gacgtcaata atgacgtatg 300 ttcccatagt aacgccaata gggactttcc attgacgtca atgggtggac tatttacggt 360 aaactgccca cttggcagta catcaagtgt atcatatgcc aagtacgccc cctattgacg 420

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3-40	**Sequences**	
3-40-1	Sequence Number [ID]	40
3-40-2	Molecule Type	DNA

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3-40-3	Length	3397
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3-41-2	Molecule Type	DNA
3-41-3	Length	4177
3-41-4	Features	**misc_feature 1..4177**
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3-42	**Sequences**	
3-42-1	Sequence Number [ID]	42
3-42-2	Molecule Type	DNA
3-42-3	Length	4607
3-42-4	Features	**misc_feature 1..4607**

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3-45	**Sequences**	
3-45-1	Sequence Number [ID]	45
3-45-2	Molecule Type	DNA
3-45-3	Length	3580
3-45-4	Features	**misc_feature 1..3580**
	Location/Qualifiers	note=CLRN-7eGFP vector
		source 1..3580
		mol_type=other DNA
		organism=synthetic construct
	NonEnglishQualifier Value	
3-45-5	Residues	cctgcaggca gctgcgcgct cgctcgctca ctgaggccgc ccgggcgtcg ggcgaccttt 60 ggtcgcccgg cctcagtgag cgagcgagcg cgcagagagg gagtggccaa ctccatcact 120 aggggttcct gcggccgcac gcgtgacatt gattattgac tagttattaa tagtaatcaa 180 ttacggggtc attagttcat agcccatata tggagttccg cgttacataa cttacggtaa 240 atggcccgcc tggctgaccg cccaacgacc cccgcccatt gacgtcaata atgacgtatg 300 ttcccatagt aacgccaata gggactttcc attgacgtca atgggtggac tatttacggt 360 aaactgccca cttggcagta catcaagtgt atcatatgcc aagtacgccc cctattgacg 420 tcaatgacgg taaatggccc gcctggcatt atgcccagta catgacctta tgggactttc 480 ctacttggca gtacatctac gtattagtca tcgctattac catgggtcga ggtgagcccc 540 acgttctgct tcactctccc catctccccc ccctccccac ccccaatttt gtatttattt 600 atttttttaat tattttgtgc agcgatgggg gcgggggggg ggggggcgcg cgccaggcgg 660 ggcggggcgg ggcgaggggc ggggcggggc gaggcggaga ggtgcggcgg cagccaatca 720 gagcggcgcg ctccgaaagt ttccttttat ggcgaggcgg cggcggcggc ggccctataa 780 aaagcgaagc gcgcggcggg cgggagtcgc tgcgttgcct tcgccccgtg ccccgctccg 840 cgccgcctcg cgccgcccgc cccggctctg actgaccgcg ttactcccac aggtgagcgg 900 gcgggacggc ccttctcctc cgggctgtaa ttagcgcttg gtttaatgac ggctcgtttc 960 ttttctgtgg ctgcgtgaaa gccttaaagg gctccgggag ggccctttgt gcgggggga 1020 gcggctcggg gggtgcgtgc gtgtgtgtgt gcgtggggag cgccgcgtgc ggcccgcgct 1080 gcccggcggc tgtgagcgct gcgggcgcgg cgcggggctt gtgcgctcc gcgtgtgcgc 1140 gaggggagcg cggccggggg cggtgccccg cggtgcgggg gggctgcgag gggaacaaag 1200 gctgcgtgcg gggtgtgtgc gtgggggggt gagcaggggg tgtgggcgcg gcggtcgggc 1260 tgtaaccccc ccctgcaccc ccctccccga gttgctgagc acggcccggc ttcgggtgcg 1320 gggctccgtg cggggcgtgg cgcggggctc gccgtgccgg gcgggggggtg gcggcaggtg 1380 ggggtgccgg gcggggcggg gccgcctcgg gccggggagg gctcgggga ggggcgcggc 1440 ggcccccgga gcgccggcgg ctgtcgaggc gcggcgagcc gcagccattg ccttttatgg 1500 taatcgtgcg agagggcgca gggacttcct ttgtcccaaa tctgtgcgga gccgaaatct 1560 gggaggcgcc gccgcacccc ctctagcggg cgcggggcga agcggtgcgg cgccggcagg 1620 aaggaaatgg gcggggaggg ccttcgtgcg tcgccgcgcc gccgtcccct tctccctctc 1680

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3-46	**Sequences**	
3-46-1	Sequence Number [ID]	46
3-46-2	Molecule Type	DNA
3-46-3	Length	3982
3-46-4	Features	**misc_feature 1..3982**
	Location/Qualifiers	note=CLRN-8 vector
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		mol_type=other DNA
		organism=synthetic construct
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3-47	**Sequences**	
3-47-1	Sequence Number [ID]	47
3-47-2	Molecule Type	DNA
3-47-3	Length	3982
3-47-4	Features	**misc_feature 1..3982**
	Location/Qualifiers	note=CLRN-9 vector **source 1..3982** mol_type=other DNA organism=synthetic construct
	NonEnglishQualifier Value	
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3-48-2	Molecule Type	DNA
3-48-3	Length	3511
3-48-4	Features	**misc_feature 1..3511**
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3-50	**Sequences**	
3-50-1	Sequence Number [ID]	50
3-50-2	Molecule Type	DNA
3-50-3	Length	3499
3-50-4	Features	**misc_feature 1..3499**
	Location/Qualifiers	note=CLRN-10NF vector
		source 1..3499
		mol_type=other DNA
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3-51-2	Molecule Type	DNA
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3-51-4	Features	**misc_feature 1..4908**
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		organism=synthetic construct
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3-52-2	Molecule Type	DNA
3-52-3	Length	4971
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3-53-1	Sequence Number [ID]	53
3-53-2	Molecule Type	DNA
3-53-3	Length	4896
3-53-4	Features	**misc_feature 1..4896**
	Location/Qualifiers	note=CLRN-11NF vector
		source 1..4896
		mol_type=other DNA
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20262013 71 24 Feb 2026

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3-54-2	Molecule Type	DNA
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3-55-2	Molecule Type	DNA
3-55-3	Length	4291
3-55-4	Features	**misc_feature 1..4291**
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3-56-2	Molecule Type	DNA
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3-56-4	Features	**misc_feature 1..4192**
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3-57-2	Molecule Type	DNA
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3-57-4	Features	**misc_feature 1..3505**
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3-58	**Sequences**	
3-58-1	Sequence Number [ID]	58
3-58-2	Molecule Type	DNA
3-58-3	Length	3439
3-58-4	Features	**misc_feature 1..3439**
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		source 1..3439
		mol_type=other DNA
		organism=synthetic construct
	NonEnglishQualifier Value	
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3-59	**Sequences**	
3-59-1	Sequence Number [ID]	59
3-59-2	Molecule Type	DNA
3-59-3	Length	130
3-59-4	Features	**misc_feature 1..130**
	Location/Qualifiers	note=CLRN-17 vector
		source 1..130
		mol_type=other DNA
		organism=synthetic construct
	NonEnglishQualifier Value	
3-59-5	Residues	cctgcaggca gctgcgcgct cgctcgctca ctgaggccgc ccgggcgtcg ggcgaccttt 60 ggtcgcccgg cctcagtgag cgagcgagcg cgcagagagg gagtggccaa ctccatcact 120 aggggttcct 130
3-60	**Sequences**	
3-60-1	Sequence Number [ID]	60
3-60-2	Molecule Type	DNA
3-60-3	Length	4277
3-60-4	Features	**misc_feature 1..4277**
	Location/Qualifiers	note=CLRN-18 vector
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	NonEnglishQualifier Value	
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