

otoferlin protein) in a mammalian cell, and thereby treat non-syndromic sensorineural hearing loss in a subject in need thereof.

~~Provided~~ Disclosed herein are compositions that include at least two different nucleic acid vectors, wherein: each of the at least two different vectors includes a coding sequence that encodes a different portion of an otoferlin 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 otoferlin protein; at least one of the coding sequences includes a nucleotide sequence spanning two neighboring exons of otoferlin genomic DNA, and lacks an intronic sequence between the two neighboring exons; and when introduced into a mammalian cell the at least two different vectors undergo concatemerization or homologous recombination with each other, thereby forming a recombined nucleic acid that encodes a full-length otoferlin protein. In some ~~embodiments~~ examples of any of the compositions described 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 ~~examples~~ embodiments of any of the compositions described 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 ~~examples~~ embodiments of any of the compositions described 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~~ examples of any of the compositions described herein, each of the at least two different vectors is an AAV vector.

In some ~~examples~~ embodiments of any of the compositions described herein, the amino acid sequence of one of the encoded portions overlaps with the amino acid sequence of a different one of the encoded portions. In some ~~examples~~ embodiments of any of the compositions described herein, the amino acid sequence of each of the encoded portions partially overlaps with the amino acid sequence of a different encoded portion. In some ~~examples~~ embodiments of any of the compositions described herein, the overlapping amino acid

sequence is between about 30 amino acid residues to about 1000 amino acid residues in length.

In some ~~examples~~ ~~embodiments~~ of any of the compositions described 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 otoferlin genomic DNA, and wherein the two different segments overlap in sequence by at least 100 nucleotides. In some ~~embodiments~~ ~~examples~~ of any of the compositions described herein, the two different segments overlap in sequence by about 100 nucleotides to about 800 nucleotides. In some ~~embodiments~~ ~~examples~~ of any of the compositions described herein, the 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~~ ~~examples~~ of any of the compositions described herein, the nucleotide sequence of each of the at least two different vectors is between 500 nucleotides to 5,000 nucleotides in length.

In some ~~embodiments~~ ~~examples~~ of any of the compositions described herein, the number of different vectors in the composition is two. In some ~~embodiments~~ ~~examples~~ of any of the compositions described herein, a first of the two different vectors includes a coding sequence that encodes an N-terminal portion of the otoferlin protein. In some ~~embodiments~~ ~~examples~~ of any of the compositions described herein, the N-terminal portion of the otoferlin protein is between 30 amino acids to 1600 amino acids in length. In some ~~embodiments~~ ~~examples~~ of any of the compositions described herein, the N-terminal portion of the otoferlin protein is between 200 amino acids to 1500 amino acids in length. In some ~~embodiments~~ ~~examples~~ of any of the compositions described herein, the first vector further includes one or both of a promoter and a Kozak sequence. In some ~~embodiments~~ ~~examples~~ of any of the compositions described herein, the first vector includes a promoter that is an inducible promoter, a constitutive promoter, or a tissue-specific promoter.

In some ~~embodiments~~ ~~examples~~ of any of the compositions described herein, one of the two vectors comprises SEQ ID NO: 39 (or comprises a sequence that is at least 80%, at least 82%, at least 84%, at least 86%, at least 88%, at least 90%, at least 92%, at least 94%, at least 96%, at least 98%, or at least 99% identical to SEQ ID NO: 39) and the second of the two vectors comprises SEQ ID NO: 40 (or comprises a sequence that is at least 80%, at least 82%, at least 84%, at least 86%, at least 88%, at least 90%, at least 92%, at least

94%, at least 96%, at least 98%, or at least 99% identical to SEQ ID NO: 40). In some ~~embodiments~~examples of any of the compositions described herein, one of the two vectors comprises SEQ ID NO: 41 (or comprises a sequence that is at least 80%, at least 82%, at least 84%, at least 86%, at least 88%, at least 90%, at least 92%, at least 94%, at least 96%, at least 98%, or at least 99% identical to SEQ ID NO: 41) and the second of the two vectors comprises SEQ ID NO: 42 (or comprises a sequence that is at least 80%, at least 82%, at least 84%, at least 86%, at least 88%, at least 90%, at least 92%, at least 94%, at least 96%, at least 98%, or at least 99% identical to SEQ ID NO: 42). In some ~~embodiments~~examples of any of the compositions described herein, one of the two vectors comprises SEQ ID NO:84 (or comprises a sequence that is at least 80%, at least 82%, at least 84%, at least 86%, at least 88%, at least 90%, at least 92%, at least 94%, at least 96%, at least 98%, or at least 99% identical to SEQ ID NO: 84) and the second of the two vectors comprises SEQ ID NO: 85 (or comprises a sequence that is at least 80%, at least 82%, at least 84%, at least 86%, at least 88%, at least 90%, at least 92%, at least 94%, at least 96%, at least 98%, or at least 99% identical to SEQ ID NO: 85).

In some ~~embodiments~~examples of any of the compositions described herein, wherein one of the at least two different vectors comprises a sequence encoding a NTF3 protein.

In some ~~embodiments~~examples of any of the compositions described herein, wherein the sequence encoding a NTF3 protein is at least 80%, at least 82%, at least 84%, at least 86%, at least 88%, at least 90%, at least 92%, at least 94%, at least 96%, at least 98%, or at least 99% identical to SEQ ID NO: 78. In one aspect, the present disclosure refers to a plurality of recombinant adeno-associated viral (rAAV) vectors comprising: a) a first rAAV vector comprising a nucleic acid sequence of SEQ ID NO: 96; and b) a second rAAV vector comprising a nucleic acid sequence of SEQ ID NO: 105.

In some ~~embodiments~~examples of any of the compositions described herein, the first vector further includes a coding sequence encoding a destabilization domain, wherein the destabilization domain is 3' to the coding sequence that encodes the N-terminal portion of the otoferlin protein.

In some ~~embodiments~~examples of any of the compositions described herein, the coding sequence that encodes the N-terminal portion of the otoferlin protein comprises exons 1-21 of isoform 5 of the human otoferlin gene.

In some ~~embodiments~~examples of any of the compositions described herein, the second of the two different vectors includes a coding sequence that encodes a C-terminal portion of the otoferlin protein. In some ~~embodiments~~examples of any of the compositions described herein,

the C-terminal portion of the otoferlin protein is between 30 amino acids to 1600 amino acids in length. In some ~~embodiments~~examples of any of the compositions described herein, the C-terminal portion of the otoferlin protein is between 200 amino acids to 1500 amino acids in length. In some ~~embodiments~~examples of any of the compositions described herein, the second vector further includes a poly(dA) signal sequence. In some ~~embodiments~~examples of any of the compositions described herein, the coding sequence that encodes the C-terminal portion of the otoferlin protein comprises exons 22-48 of isoform 5 of the human otoferlin gene. In some ~~embodiments~~examples of any of the compositions described herein, the second vector further includes sequences for mRNA stabilization. Some ~~embodiments~~examples of any of the compositions described herein further include a pharmaceutically acceptable excipient. Also ~~provided~~described herein are kits that include any of the compositions described herein. Some ~~embodiments~~examples of any of the kits described herein further include a pre-loaded syringe containing the composition. Also ~~described~~provided herein are methods that include introducing into a cochlea of a mammal a therapeutically effective amount of any of the compositions described herein. In some ~~embodiments~~examples of any of the compositions described herein, the mammal is a human. In some ~~embodiments~~examples of any of the compositions described herein, the mammal has been previously identified as having a defective otoferlin gene. In another aspect, the present disclosure refers to the use of a plurality of recombinant adeno-associated viral (rAAV) vectors in the manufacture of a medicament for expressing a recombinant full-length otoferlin protein in a mammalian cell, wherein the plurality of recombinant adeno-associated viral (rAAV) vectors comprises: a) a first rAAV vector comprising a nucleic acid sequence of SEQ ID NO: 96; and b) a second rAAV vector comprising a nucleic acid sequence of SEQ ID NO: 105; and wherein the mammalian cell has reduced expression, lack of expression or dysfunction of otoferlin. Also provided herein are methods of increasing expression of an active otoferlin protein, e.g., a full-length otoferlin protein, in a mammalian cell that include introducing any of the compositions described herein into the mammalian cell. In some ~~embodiments~~examples of any of the methods described herein, the mammalian cell is a cochlear inner hair cell. In some ~~embodiments~~examples of any of the methods described herein, the mammalian cell is a human cell. In some ~~embodiments~~examples of any of the methods described herein, the mammalian cell has previously been determined to have a defective otoferlin gene. Also ~~provided~~disclosed herein are methods of increasing expression of an active otoferlin protein, e.g., a full-length otoferlin protein in an inner hair cell 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. In some ~~embodiments~~examples of any of the methods described herein, the mammal has been previously identified as having a

defective otoferlin gene. In some ~~embodiments~~examples of any of the methods described herein, the mammal is a human.

In yet another aspect, the present disclosure refers to the use of a plurality of recombinant adeno-associated viral (rAAV) vectors in the manufacture of a medicament for treating hearing loss in a subject having otoferlin related hearing loss, wherein the plurality of recombinant adeno-associated viral (rAAV) vectors comprises: a) a first rAAV vector comprising a nucleic acid sequence of SEQ ID NO: 96; and b) a second rAAV vector comprising a nucleic acid sequence of SEQ ID NO: 105; and wherein the subject has reduced expression, lack of expression, or dysfunction of otoferlin. Also ~~provided~~disclosed herein are methods of treating non-syndromic sensorineural hearing loss in a subject identified as having a defective otoferlin gene that include administering a therapeutically effective amount of a composition of any one of the compositions described herein into the cochlea of the subject. In some ~~embodiments~~examples of any of the methods described herein, the subject is a human. Some ~~embodiments~~examples of any of the methods described herein further include, prior to the administering step, determining that the subject has a defective otoferlin gene.

Also ~~described~~provided herein are compositions that include two different nucleic acid vectors, wherein: 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 an otoferlin protein positioned 3' of the promoter, and a splicing donor signal 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 splicing acceptor signal sequence, a second coding sequence that encodes a C-terminal portion of an otoferlin protein positioned at the 3' end of the splicing acceptor signal sequence, and a polyadenylation 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 encoded portions do not overlap, wherein no single vector of the two different vectors encodes a full-length otoferlin protein, and, when the coding sequences are transcribed in a mammalian cell, to produce RNA transcripts, splicing occurs between the splicing donor signal sequence on one transcript and the splicing acceptor signal sequence on the other transcript, thereby forming a recombined RNA molecule that encodes a full-length otoferlin protein. In some ~~embodiments~~examples of any of the compositions described herein, the coding sequence of at least one of the vectors includes a nucleotide sequence spanning two neighboring exons of otoferlin genomic DNA, and lacks an intronic sequence between the two neighboring exons.

Also ~~described~~provided herein are compositions that include: a first nucleic acid vector including a promoter, a first coding sequence that encodes an N-terminal portion of an otoferlin protein positioned 3' of the promoter, a splicing donor signal sequence

positioned at the 3' end of the first coding sequence, and a first detectable marker gene positioned 3' of the splicing donor signal sequence; and a second nucleic acid vector, different from the first nucleic acid vector, including a second detectable marker gene, a splicing acceptor signal sequence positioned 3' of the second detectable marker gene, a second coding sequence that encodes a C-terminal portion of an otoferlin protein positioned at the 3' end of the splicing acceptor signal sequence, and a polyadenylation 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 respective 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 otoferlin protein, and, when the coding sequences are transcribed in a mammalian cell to produce RNA transcripts, splicing occurs between the splicing donor signal on one transcript and the splicing acceptor signal on the other transcript, thereby forming a recombined RNA molecule that encodes a full-length otoferlin protein. In some ~~embodiments~~examples of any of the compositions described herein, the coding sequence of at least one of the vectors includes a nucleotide sequence spanning two neighboring exons of otoferlin genomic DNA, and lacks an intronic sequence between the two neighboring exons. In some ~~embodiments~~examples of any of the compositions described herein, the first or second detectable marker gene encodes alkaline phosphatase. In some ~~embodiments~~examples of any of the compositions described herein, the first and second detectable marker genes are the same.

Also ~~described provided~~ herein are compositions that include a first nucleic acid vector including a promoter, a first coding sequence that encodes an N-terminal portion of an otoferlin protein positioned 3' to the promoter, a splicing donor signal sequence positioned at the 3' end of the first coding sequence, and a highly recombinogenic sequence (e.g., a F1 phage recombinogenic region, e.g., SEQ ID NO: 66) positioned 3' to the splicing donor signal sequence; and a second nucleic acid vector, different from the first nucleic acid vector, including a second highly recombinogenic sequence (e.g., a F1 phage recombinogenic region, e.g., SEQ ID NO: 67, or an alkaline phosphatase recombinogenic region, e.g., SEQ ID NO: 89), a splicing acceptor signal sequence positioned 3' of the second highly recombinogenic sequence (e.g., a F1 phage recombinogenic region or an alkaline phosphatase recombinogenic region), a second

coding sequence that encodes a C-terminal portion of an otoferlin protein positioned at the 3' end of the splicing acceptor signal sequence, and a polyadenylation 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 respective 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 otoferlin protein, and, when the coding sequences are transcribed in a mammalian cell to produce RNA transcripts, splicing occurs between the splicing donor signal on one transcript and the splicing acceptor signal on the other transcript, thereby forming a recombined RNA molecule that encodes a full-length otoferlin protein.

10 In some ~~embodiments~~examples of any of the compositions described herein, the coding sequence of at least one of the vectors includes a nucleotide sequence spanning two neighboring exons of otoferlin genomic DNA, and lacks an intronic sequence between the two neighboring exons.

Also provided herein are kits that include any of the compositions described herein.
15 Some ~~embodiments~~examples of any of the kits described herein further include a pre-loaded syringe containing the composition.

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

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

Also ~~provided~~described herein are methods of increasing expression of a full-length
30 otoferlin protein in an inner hair cell in a cochlea of a mammal that include introducing

into the cochlea a therapeutically effective amount of any of the compositions described herein. In some ~~embodiments~~examples of any of the methods described herein, the mammal has been previously identified as having a defective otoferlin gene. In some ~~embodiments~~examples of any of the methods described herein, the mammal is a human.

5 Also ~~provided~~described herein are methods of treating non-symptomatic sensorineural hearing loss in a subject identified as having a defective otoferlin gene that include administering a therapeutically effective amount of any of the compositions described herein into a cochlea of the subject. In some ~~embodiments~~examples of any of the methods described herein, the subject is a human. Some ~~embodiments~~examples of any of the
10 methods described herein further include, prior to the administering step, determining that the subject has a defective otoferlin gene.

~~Provided~~Described herein are therapeutic compositions including a plurality of adeno-associated viral (AAV) vectors, wherein the plurality of AAV vectors are capable of constituting an auditory polypeptide messenger RNA in a target cell of a human subject to
15 whom the therapeutic composition is administered.

In some ~~embodiments~~examples of any of the therapeutic compositions described herein, the plurality of AAV vectors are capable of constituting a full-length auditory polypeptide messenger RNA in a target cell of a human subject to whom the therapeutic composition is administered.

20 Some ~~embodiments~~examples of any of the therapeutic composition described herein further include a first AAV vector and a second AAV vector, wherein the first and second AAV vectors independently contain packaging capacity of less than about 6kb.

In some ~~embodiments~~examples of any of the therapeutic compositions described herein, the auditory polypeptide messenger RNA encodes an auditory polypeptide selected
25 from the group of otoferlin and an ortholog or homolog thereof.

Some ~~embodiments~~examples of any of the therapeutic compositions described herein, can further include a nucleic acid (e.g., a vector) including a nucleic acid sequence encoding an auditory polypeptide messenger RNA encodes an auditory polypeptide selected from the group consisting of Cav1.3, a scaffold protein selected from bassoon, piccolo, ribeye, and
30 harmonin, Vglut3, synaptotagmin, a vesicle tethering / docking protein, a vesicle priming protein, a vesicle fusion proteins, GluA2/3, and GluA4.

In some ~~embodiments~~examples of any of the therapeutic compositions described herein, the first AAV vector further includes at least one promoter sequence selected from a CBA, a CMV, or a CB7 promoter.

In some ~~embodiments~~examples of any of the therapeutic compositions described herein, the first AAV vector further includes at least one promoter sequence selected from Cochlea-specific promoters.

In some ~~embodiments~~examples of any of the therapeutic compositions described herein, the therapeutic composition is formulated for intra-cochlear administration. In some

~~embodiments~~examples of any of the therapeutic compositions described herein, the

therapeutic composition is formulated to include a lipid nanoparticle. In some

~~embodiments~~examples of any of the therapeutic compositions described herein, the therapeutic composition is formulated to include a polymeric nanoparticle. In some

~~embodiments~~examples of any of the therapeutic compositions described herein, the therapeutic composition is formulated to include a mini-circle DNA. In some

~~embodiments~~examples of any of the therapeutic compositions described herein, the therapeutic composition is formulated to include a CELiD DNA. In some

~~embodiments~~examples of any of the compositions described herein, the therapeutic composition is formulated to include a synthetic perilymph solution. In some

~~embodiments~~examples of any of the therapeutic compositions described herein, the therapeutic composition is formulated to include a synthetic perilymph solution including 20-200mM NaCl; 1-5 mM KCl; 0.1-10mM CaCl₂; 1-10mM glucose; and 2-50 mM HEPES; and having a pH of between about 6 and about 9.

Also ~~provided~~described herein are therapeutic compositions that include an auditory polypeptide messenger RNA.

Also ~~provided~~described herein are therapeutic compositions that include one or a plurality of adenoviral (AV) vectors, where the one or the plurality of AV vectors are capable of constituting an auditory polypeptide messenger RNA in a target cell of a human subject to whom the therapeutic composition is administered.

In some ~~embodiments~~examples of any of the therapeutic compositions described herein, the one or the plurality of AV vectors are capable of constituting a full-length auditory polypeptide messenger RNA in a target cell of a human subject to whom the therapeutic composition is administered.

Also ~~provided~~described herein are therapeutic compositions including one or a plurality of lentiviral vectors, where the one or the plurality of lentiviral vectors are capable of constituting an auditory polypeptide messenger RNA in a target cell of a human subject to whom the therapeutic composition is administered.

5 In some ~~embodiments~~examples of any of the therapeutic compositions described herein, the one or the plurality of lentiviral vectors are capable of constituting an active, e.g., full-length auditory polypeptide messenger RNA in a target cell of a human subject to whom the therapeutic composition is administered.

Also ~~provided~~described herein are surgical methods that include the steps of: i) 10 introducing into a cochlea of a human subject a first incision at a first incision point; ii) administering intra-cochlearly an effective dose of a therapeutic composition (e.g., any of the therapeutic compositions described herein).

In some ~~embodiments~~examples of any of the methods described herein, the therapeutic composition is administered to the subject at the first incision point. In some 15 ~~embodiments~~examples of any of the methods described herein, the therapeutic composition is administered to the subject into or through the first incision.

In some ~~embodiments~~examples of any of the methods described herein, the therapeutic composition is administered to the subject into or through the cochlea oval window membrane. In some ~~embodiments~~examples of any of the methods described herein, 20 the therapeutic composition is administered to the subject into or through the cochlea round window membrane.

In some ~~embodiments~~examples of any of the methods described herein, the therapeutic composition is administered using a medical device capable of creating a plurality of incisions in the round window membrane.

25 In some ~~embodiments~~examples of any of the methods described herein, the medical device includes a plurality of micro-needles. In some ~~embodiments~~examples of any of the methods described herein, 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.

30 In some ~~embodiments~~examples of any of the methods described herein, the medical device includes a base and/or a reservoir capable of holding the therapeutic composition. In

some ~~embodiments~~examples of any of the methods described herein, the medical device includes a plurality of hollow micro-needles individually including a lumen capable of transferring the therapeutic composition.

In some ~~embodiments~~examples of any of the methods described herein, the medical device includes a means for generating at least a partial vacuum.

Also ~~provided~~described herein are therapeutic delivery systems, that include i) a medical device capable of creating 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 therapeutic composition including a plurality of adeno-associated viral (AAV) vectors, wherein the plurality of AAV vectors are capable of constituting an active, e.g., full-length, auditory polypeptide messenger RNA in a target cell of the inner ear.

Also ~~provided~~described herein are means for performing a surgical method that includes the steps of: i) administering intra-cochlearly to a human subject in need thereof an effective dose of the therapeutic composition (e.g., any of the therapeutic composition described herein), where the therapeutic composition is capable of being administered by using a medical device including a) means for creating a plurality of incisions in the round window membrane and b) the effective dose of the therapeutic composition.

In some ~~embodiments~~examples of any of the means for performing a surgical method described herein, the medical device includes a plurality of micro-needles.

Also ~~provided~~described herein are therapeutic compositions that include a single adeno-associated viral (AAV) vector, where the AAV vector is capable of constituting an auditory polypeptide messenger RNA in a target cell of a human subject to whom the therapeutic composition is administered.

In some ~~embodiments~~examples of any of the therapeutic compositions described herein, the single AAV vector is capable of constituting a full-length auditory polypeptide messenger RNA in a target cell of a human subject to whom the therapeutic composition is administered. In some ~~embodiments~~examples of any of the methods described herein, a single vector (e.g., any of the vectors described herein) that includes a sequence encoding an active otoferlin protein (e.g., any of the full-length or truncated active otoferlin proteins described herein) can be administered to the subject.

In some ~~embodiments~~examples of any of the therapeutic compositions described herein, the therapeutic composition further includes a second vector other than an AAV vector, where the single AAV vector and the second vector independently contain packaging capacity of less than about 6kb.

5 In some ~~embodiments~~examples of any of the therapeutic compositions described herein, the auditory polypeptide messenger RNA encodes an auditory polypeptide selected from the group of otoferlin and an ortholog or homolog thereof.

In some ~~embodiments~~examples of any of the therapeutic compositions described herein, the auditory polypeptide messenger RNA encodes an auditory polypeptide selected
10 from the group of otoferlin and truncation mutant thereof.

In some ~~embodiments~~examples of any of the therapeutic compositions described herein, the otoferlin truncation mutant includes at least a single C2 domain of the following:

C2 Domain	a.a. Designation (from Pangrsic et al. 2012 <i>Trends Neurosci</i> 35(11):671-680
C2A	1-121
C2B	256-378
C2C	419-542
C2D	962-1095
C2E	1494-1622
C2F	1734-1895

15 In some ~~embodiments~~examples of any of the therapeutic compositions described herein, the otoferlin truncation mutant does not include an endogenous otoferlin polypeptide C-terminal region.

In some ~~embodiments~~examples of any of the therapeutic compositions described herein, the auditory polypeptide messenger RNA encodes an auditory polypeptide selected
20 from the group consisting of Cav1.3, a scaffold protein selected from bassoon, piccolo, ribeye, and harmonin, Vglut3, synaptotagmin, a vesicle tethering / docking protein, a vesicle priming protein, a vesicle fusion proteins, GluA2/3, and GluA4.

In some ~~embodiments~~examples of any of the therapeutic compositions described herein, the single AAV vector further includes at least one promoter sequence selected from a CBA, a CMV, or a CB7 promoter.

In some ~~embodiments~~examples of any of the therapeutic compositions described herein, the single AAV vector further includes at least one promoter sequence selected from a Cochlea-specific promoters.

In some ~~embodiments~~examples of any of the therapeutic compositions described herein, the therapeutic composition is formulated for intra-cochlear administration.

In some ~~embodiments~~examples of any of the therapeutic compositions described herein, the therapeutic composition is formulated to include a lipid nanoparticle.

In some ~~embodiments~~examples of any of the therapeutic compositions described herein, the therapeutic composition is formulated to include a polymeric nanoparticle.

In some ~~embodiments~~examples of any of the therapeutic compositions described herein, the therapeutic composition is formulated to include a mini-circle DNA.

In some ~~embodiments~~examples of any of the therapeutic compositions described herein, the therapeutic composition is formulated to include a CELiD DNA.

In some ~~embodiments~~examples of any of the therapeutic compositions described herein, the therapeutic composition is formulated to include a synthetic perilymph solution.

In some ~~embodiments~~examples of any of the therapeutic compositions described herein, the therapeutic composition is formulated to include a synthetic perilymph solution including 20-200mM NaCl; 1-5 mM KCl; 0.1-10mM CaCl₂; 1-10mM glucose; 2-50 mM HEPES; having a pH of between about 6 and about 9.

Also ~~provided~~described herein are therapeutic compositions that include an auditory polypeptide messenger RNA encoding an otoferlin truncation mutant.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the disclosure pertains.

The terms “a” and “an” refer 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 element and more than one element.

The term “about” when referring to a measurable value such as an amount, a

including a variable region of a light chain and at least one antibody fragment including a variable region of a heavy chain, wherein the light and heavy chain variable regions are contiguously linked via a short flexible polypeptide linker, and capable of being expressed as a single chain polypeptide, and wherein the scFv retains the specificity of the intact antibody from which it is derived. Unless specified, as used herein an scFv may have the VL and VH variable regions in either order, e.g., with respect to the N-terminal and C-terminal ends of the polypeptide, the scFv may comprise VL-linker-VH or may comprise VH-linker-VL.

The term “recombinant polypeptide” refers to a polypeptide which is generated using recombinant DNA technology, such as, for example, a polypeptide expressed by a viral vector expression system. The term should also be construed to mean a polypeptide which has been generated by the synthesis of a DNA molecule encoding the polypeptide and which DNA molecule expresses a protein, or an amino acid sequence specifying the polypeptide, wherein the DNA or amino acid sequence has been obtained using recombinant DNA or amino acid sequence technology which is available and well known in the art.

The term “mutation in an otoferlin gene” refers to a modification in a wildtype otoferlin gene that results in the production of an otoferlin protein having one or more of: a deletion of one or more amino acids, one or more amino acid substitutions, and one or more amino acid insertions, as compared to the wildtype otoferlin protein, and/or results in a decrease in the expressed level of the encoded otoferlin protein in a mammalian cell as compared to the expressed level of the encoded otoferlin protein in a mammalian cell not having the mutation. In some ~~embodiments~~examples, a mutation can result in the production of an otoferlin protein having a deletion of 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~~examples, the mutation can result in a frameshift in the otoferlin 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~~examples, a frameshift can result in a nonfunctional protein. In some ~~embodiments~~examples, 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~~examples, the mutation can result in the loss (or a decrease in the level) of expression of otoferlin mRNA or otoferlin protein or both the mRNA and protein. In some ~~embodiments~~examples, the mutation can result in the production of an altered otoferlin protein having a loss or decrease in one or more biological activities (functions) as compared to a wildtype otoferlin protein.

In some ~~embodiments~~examples, the mutation is an insertion of one or more nucleotides into an otoferlin gene. In some ~~embodiments~~examples, the mutation is in a regulatory sequence of the otoferlin gene, i.e., a portion of the gene that is not coding sequence. In some ~~embodiments~~examples, a mutation in a regulatory sequence may be in a promoter or enhancer region and prevent or reduce the proper transcription of the otoferlin gene.

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

The term “conservative sequence modifications” refers to amino acid modifications that do not significantly affect or alter the binding characteristics of the antibody or antibody fragment containing the amino acid sequence. Such conservative modifications include amino acid substitutions, additions, and deletions. Modifications can be introduced into an antibody or antibody fragment of the disclosure by standard techniques known in the art, such as site-directed mutagenesis and PCR-mediated 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).

The term “encoding” refers to the inherent property of specific sequences of

example, a host cell.

The term “substantially purified cell” refers to a cell that is essentially free of other cell types.

A substantially purified cell also refers to a cell which has been separated from other cell types with which it is normally associated in its naturally occurring state. In some instances,

5 a population of substantially purified cells refers to a homogenous population of cells. In other instances, this term refers simply to cells that have been separated from the cells with which they are naturally associated in their natural state. In some ~~aspects~~examples, the cells are cultured in vitro. In other ~~aspects~~examples, the cells are not cultured in vitro.

The term “transfected” or “transformed” or “transduced” refers to a process by which
10 exogenous nucleic acid is transferred or introduced into the host cell. A “transfected” or “transformed” or “transduced” cell is one which has been transfected, transformed or transduced with exogenous nucleic acid. The cell includes the primary subject cell and its progeny.

The term “expression” refers to the transcription and/or translation of a particular
15 nucleotide sequence driven by a promoter.

As used herein, “transient” refers to expression of a non-integrated transgene for a period of hours, days or weeks, wherein the period of time of expression is less than the period of time for expression of the gene if integrated into the genome or contained within a stable plasmid replicon in the host cell.

20 The term “subject” is intended to include living organisms in which an immune response can be elicited (e.g., mammals, human). In some ~~embodiments~~examples, the subject is a rodent (e.g., a rat or mouse), a rabbit, a sheep, a dog, a cat, a horse, a non-human primate, or a human. In some ~~embodiments~~examples, the subject has or is at risk of developing non-syndromic deafness. In some ~~embodiments~~examples, the subject has been
25 previously identified as having a mutation in an otoferlin gene. In some ~~embodiments~~examples, the subject has been identified as having a mutation in an otoferlin gene and has been diagnosed with non-symptomatic sensorineural hearing loss. In some ~~embodiments~~examples, the subject has been identified as having non-symptomatic sensorineural hearing loss.

30 The term “therapeutic” as used herein means a treatment. A therapeutic effect is obtained by reduction, suppression, remission, or eradication of a disease state.

The term “prophylaxis” as used herein means the prevention of, or protective treatment for, a disease or disease state. “Prevention” in this context includes reducing the likelihood the subject will experience the disease.

The term “effective amount” or “therapeutically effective amount” are used interchangeably herein, and refer to an amount of a compound, formulation, material, or composition as described herein effective to achieve a particular biological result. In some ~~embodiments~~examples, a therapeutically effective amount of a composition can result in an increase in the expression level of an active otoferlin protein (e.g., a wildtype, full-length otoferlin protein or of a variant of an otoferlin protein that has the desired activity) (e.g., as compared to the expression level prior to treatment with the composition). In some ~~embodiments~~examples, a therapeutically effective amount of a composition can result in an increase in the expression level of an active otoferlin protein (e.g., a wildtype, full-length otoferlin protein or active variant) in a target cell (e.g., a cochlear inner hair cell). In some ~~embodiments~~examples, a therapeutically effective amount of a composition can result in a different cellular localization of an active otoferlin protein (e.g., a wildtype, full-length otoferlin protein or an active variant) in a target cell (e.g., a cochlear inner hair cell). In some ~~embodiments~~examples, a therapeutically effective amount of a composition can result in an increase in the expression level of an active otoferlin protein (e.g., a wildtype, full-length otoferlin protein or active variant), and/or an increase in one or more activities of an otoferlin 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 an otoferlin gene, or the level(s) in a subject or a population of subjects having non-symptomatic sensorineural hearing loss).

The term “parenteral” administration of a composition includes, e.g., subcutaneous (s.c.), intravenous (i.v.), intramuscular (i.m.), or intrasternal injection, or infusion techniques.

The term “nucleic acid” or “polynucleotide” refers to deoxyribonucleic acids (DNA) or ribonucleic acids (RNA) and polymers thereof in either single- or double-stranded form. Unless specifically limited, the term encompasses nucleic acids containing known analogues of natural nucleotides that have similar binding properties as the reference nucleic acid and are metabolized in a manner similar to naturally occurring

nucleotides. Unless otherwise indicated, a particular nucleic acid sequence also implicitly encompasses conservatively modified variants thereof (e.g., degenerate codon substitutions), alleles, orthologs, SNPs, and complementary sequences as well as the sequence explicitly indicated. Specifically, degenerate codon substitutions may be achieved by generating
5 sequences in which the third position of one or more selected (or all) codons is substituted with mixed-base and/or deoxyinosine residues (Batzner et al., Nucleic Acid Res. 19:5081 (1991); Ohtsuka et al., J. Biol. Chem. 260:2605-2608 (1985); and Rossolini et al., Mol. Cell. Probes 8:91-98 (1994)).

10 In some ~~embodiments~~examples of any of the nucleic acids described herein, the nucleic acid is DNA. In some ~~embodiments~~examples of any of the nucleic acids described herein, the nucleic acid is RNA.

In the context of the present disclosure, the following abbreviations for the commonly occurring nucleic acid bases are used. "A" refers to adenosine, "C" refers to cytosine, "G" refers to guanosine, "T" refers to thymidine, and "U" refers to uridine.

15 As used herein, "in vitro transcribed RNA" refers to RNA, preferably mRNA, that has been synthesized in vitro. Generally, the in vitro transcribed RNA is generated from an in vitro transcription vector. The in vitro transcription vector includes a template that is used to generate the in vitro transcribed RNA.

20 The terms "peptide," "polypeptide," and "protein" are used interchangeably, and refer to a compound comprised of amino acid residues covalently linked by peptide bonds. A protein or peptide must contain at least two amino acids, and no limitation is placed on the maximum number of amino acids that can comprise a protein's or peptide's sequence. Polypeptides include any peptide or protein including two or more amino acids joined to each other by peptide bonds. As used herein, the term refers to both short chains, which also
25 commonly are referred to in the art as peptides, oligopeptides and oligomers, for example, and to longer chains, which generally are referred to in the art as proteins, of which there are many types. "Polypeptides" include, for example, biologically active fragments, substantially homologous polypeptides, oligopeptides, homodimers, heterodimers, variants of polypeptides, modified polypeptides, derivatives, analogs, fusion proteins, among others.
30 A polypeptide includes a natural peptide, a recombinant peptide, or a combination thereof.

about 165 amino acids to about 185 amino acids, about 165 amino acids to about 180 amino acids, about 165 amino acids to about 175 amino acids, about 165 amino acids to about 170 amino acids, about 170 amino acids to about 200 amino acids, about 170 amino acids to about 195 amino acids, about 170 amino acids to about 190 amino acids, about 170 amino acids to about 185 amino acids, about 170 amino acids to about 180 amino acids, about 170 amino acids to about 175 amino acids, about 175 amino acids to about 200 amino acids, about 175 amino acids to about 195 amino acids, about 175 amino acids to about 190 amino acids, about 175 amino acids to about 185 amino acids, about 175 amino acids to about 180 amino acids, about 180 amino acids to about 200 amino acids, about 180 amino acids to about 195 amino acids, about 180 amino acids to about 190 amino acids, about 180 amino acids to about 185 amino acids, about 185 amino acids to about 200 amino acids, about 185 amino acids to about 195 amino acids, about 185 amino acids to about 190 amino acids, about 190 amino acids to about 200 amino acids, about 190 amino acids to about 195 amino acids, about 195 amino acids to about 200 amino acids, deleted. In some ~~embodiments~~examples where two or more amino acids are deleted from the sequence of a wildtype, full-length otoferlin protein, 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 otoferlin protein, 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 otoferlin proteins from different species can be deleted without losing activity, while those amino acids that are conserved between wildtype, full-length otoferlin 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 otoferlin protein can, e.g., include a sequence of a wildtype, full-length otoferlin 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,

acids to about 70 amino acids, about 50 amino acids to about 65 amino acids, about 50 amino acids to about 60 amino acids, about 50 amino acids to about 55 amino acids, about 60 amino acids to about 100 amino acids, about 60 amino acid to about 95 amino acids, about 60 amino acids to about 90 amino acids, about 60 amino acids to about 85 amino acids, about 60 amino acids to about 80 amino acids, about 60 amino acids to about 75 amino acids, about 60 amino acids to about 70 amino acids, about 60 amino acids to about 65 amino acids, about 70 amino acids to about 100 amino acids, about 70 amino acid to about 95 amino acids, about 70 amino acids to about 90 amino acids, about 70 amino acids to about 85 amino acids, about 70 amino acids to about 80 amino acids, 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 from its C-terminus.

15 In some ~~embodiments~~examples, an active otoferlin protein can, e.g., include the sequence of a wildtype, full-length otoferlin 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

97-99%, 97-98% and 98-99% identity. This applies regardless of the breadth of the range.

In one ~~aspect the present disclosure provides~~ a recombinant AAV vector of SEQ ID NO: 96 is described. In one ~~aspect the present disclosure provides~~example a recombinant AAV vector of SEQ ID NO: 105 is described.

In one ~~aspect the present disclosure provides~~example a recombinant AAV vector that comprises, in order of 5' to 3': a 5' ITR sequence of SEQ ID NO: 97; a CAG promoter comprising a CMV early enhancer element of SEQ ID NO: 98, a chicken beta actin gene sequence of SEQ ID NO: 99, and a chimeric intron of SEQ ID NO: 100; a 5'OTOF coding region that comprises exons 1 to (and through) 21 of *OTOF* cDNA; a SD intron sequence of SEQ ID NO: 102; an AK recombinogenic sequence of SEQ ID NO: 103; and a 3' ITR sequence of SEQ ID NO: 104 is described. In some ~~embodiments~~examples, the 5'OTOF coding region is SEQ ID NO: 101. In some ~~embodiments~~examples, the 5'OTOF coding region is at least 70%, at least 80%, 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%, at least 99.5%, at least 99.9% or 100% identical to SEQ ID NO: 101, and encodes the same amino acid sequence as encoded by SEQ ID NO: 101.

In one ~~aspect the present disclosure provides~~example a recombinant AAV vector is described that comprises, in order of 5' to 3': a 5' ITR sequence of SEQ ID NO: 97; an AK recombinogenic sequence of SEQ ID NO: 103; a SA intron sequence of SEQ ID NO: 106; a 3' OTOF coding region that comprises exons 22 to (and through) exon 48 of *OTOF* cDNA; a bgH polyA sequence of SEQ ID NO: 108; and a 3' ITR sequence of SEQ ID NO: 104. In some ~~embodiments~~examples, the 3'OTOF coding region is SEQ ID NO: 107. In some ~~embodiments~~examples, the 3'OTOF coding region is at least 70%, at least 80%, 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%, at least 99.5%, at least 99.9% or 100% identical to SEQ ID NO: 107, and encodes the same amino acid sequence as encoded by SEQ ID NO: 107.

In one aspect the present disclosure provides an rAAV particle comprising one of the aforementioned recombinant AAV vectors encapsidated by an Anc80 capsid. In some embodiment, the Anc80 capsid comprises a polypeptide of SEQ ID NO: 109.

In one aspect the present disclosure provides a composition comprising a first rAAV particle comprising a recombinant AAV vector of SEQ ID NO: 96 and a second rAAV particle comprising a recombinant AAV vector of SEQ ID NO: 105. In some ~~embodiments~~examples the recombinant AAV vector of the first rAAV particle is encapsidated by an Anc80 capsid.

5 In some ~~embodiments~~examples the recombinant AAV vector of the second rAAV particle is encapsidated by an Anc80 capsid. In some ~~embodiments~~examples the recombinant AAV vectors of the first and second rAAV particles are each independently encapsidated by an Anc80 capsid. In some embodiment, the Anc80 capsid comprises a polypeptide of SEQ ID NO: 109.

10 In one ~~aspect the present disclosure provides~~example a composition is described comprising (a) a first rAAV particle comprising a recombinant AAV vector that comprises, in order of 5' to 3': a 5' ITR sequence of SEQ ID NO: 97; a CAG promoter comprising a CMV early enhancer element of SEQ ID NO: 98, a chicken beta actin gene sequence of SEQ ID NO: 99, and a chimeric intron of SEQ ID NO: 100; a 5'OTOF coding region that
15 comprises exons 1 to (and through) 21 of *OTOF* cDNA; a SD intron sequence of SEQ ID NO: 102; an AK recombinogenic sequence of SEQ ID NO: 103; and a 3' ITR sequence of SEQ ID NO: 104; and (b) a second rAAV particle comprising a recombinant AAV vector that comprises a 5' ITR sequence of SEQ ID NO: 97; an AK recombinogenic sequence of SEQ ID NO: 103; a SA intron sequence of SEQ ID NO: 106; a 3' OTOF coding region that
20 comprises exons 22 to (and through) exon 48 of *OTOF* cDNA; a bgH polyA sequence of SEQ ID NO: 108; and a 3' ITR sequence of SEQ ID NO: 104. In some ~~embodiments~~examples, the 5'OTOF coding region is SEQ ID NO: 101. In some ~~embodiments~~examples, the 5'OTOF coding region is at least 70% identical to SEQ ID NO: 101, and encodes the same amino acid sequence as SEQ ID NO: 101. In some
25 ~~embodiments~~examples, the 3'OTOF coding region is SEQ ID NO: 107. In some ~~embodiments~~examples, the 3'OTOF coding region is at least 70%, at least 80%, 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%, at least 99.5%, at least 99.9% or 100% identical to SEQ ID NO: 107, and encodes the same amino acid sequence as encoded by SEQ ID NO: 107. In some
30 ~~embodiments~~examples the recombinant AAV vectors in the first and second rAAV particles are each independently encapsidated by an

Anc80 capsid. In some ~~embodiment~~examples, the Anc80 capsid comprises a polypeptide of SEQ ID NO: 109.

In one ~~aspect~~example when the aforementioned composition is introduced into a human cell the recombinant AAV vectors of the first and second rAAV particles undergo concatamerization or homologous recombination with each other, thereby forming a recombined nucleic acid that encodes a full-length otoferlin protein within the cell.

In one ~~aspect the present disclosure provides~~example a method comprising introducing into a cochlea of a mammal (e.g., a human) a therapeutically effective amount of any of the aforementioned composition is described. In some ~~embodiments~~examples, the mammal has been previously identified as having a defective otoferlin gene.

In one ~~aspect the present disclosure provides~~example a method of increasing expression of a full-length otoferlin protein in a mammalian cell is described, the method comprising introducing any of the aforementioned compositions into the mammalian cell, e.g., an inner hair cell, e.g., a human cell. In some ~~embodiments~~examples, the mammalian cell has previously been determined to have a defective otoferlin gene.

In one ~~aspect the present disclosure provides~~example a method of increasing expression of a full-length otoferlin protein in an inner hair cell in a cochlea of a mammal, e.g., a human, is described, the method comprising introducing into the cochlea of the mammal a therapeutically effective amount of any of the aforementioned compositions. In some ~~embodiments~~examples, the mammal has been previously identified as having a defective otoferlin gene.

In one ~~aspect the present disclosure provides~~example a method of treating non-symptomatic sensorineural hearing loss in a subject, e.g., a human identified as having a defective otoferlin gene, is described, the method comprising administering a therapeutically effective amount of any of the aforementioned compositions into the cochlea of the subject. In some ~~embodiments~~examples, the method further comprises, prior to the administering step, determining that the subject has a defective otoferlin gene. In some ~~embodiments~~examples the composition is administered to the cochlea using a microcatheter. In some ~~embodiments~~examples the microcatheter is shaped such that it can enter the middle ear cavity via the external auditory canal and contact the end of the microcatheter with the RWM. In some

~~embodiments~~examples the distal end of the microcatheter is comprised of at least one microneedle with diameter of between 10 and 1,000 microns.

In one ~~aspect the present disclosure provides~~example a kit comprising any of the aforementioned compositions is described. In some ~~embodiments~~examples the composition is pre-loaded in a device, e.g., a microcatheter. In some ~~embodiments~~examples the microcatheter is shaped such that it can enter the middle ear cavity via the external auditory canal and contact the end of the microcatheter with the RWM. In some ~~embodiments~~examples the distal end of the microcatheter is comprised of at least one microneedle with diameter of between 10 and 1,000 microns.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs. Methods and materials are described herein for use in the present disclosure; 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.

DETAILED DESCRIPTION

Mutations in *OTOF* lead to DFNB9, a recessively inherited, non-syndromic prelingual hearing disorder. Deficiency in otoferlin, the protein encoded by *OTOF*, abolishes fast exocytosis from auditory inner hair cells (IHCs). Due the impairment of neurotransmission at the first auditory synapse, no sound signals are transmitted to the brain, explaining the profound deafness.

The presently claimed methods were discovered to result in expression of full-length otoferlin in inner auditory hair cells and to successfully restore hearing with ABR thresholds of 30 dB to 70 dB for click stimuli and 50 dB to 90 dB for pure tones in otoferlin knock-out mice. In view of this discovery, provided herein are compositions and methods for treating non-symptomatic sensorineural hearing loss in a subject (e.g., a human) identified as having a defective otoferlin gene.

What is claimed is:

1. ~~—A recombinant AAV vector of SEQ ID NO: 96.~~
2. ~~—A recombinant AAV vector of SEQ ID NO: 105.~~
3. ~~—A recombinant AAV vector that comprises, in order of 5' to 3':
a 5' ITR sequence of SEQ ID NO: 97;
a CAG promoter comprising a CMV early enhancer element of SEQ ID NO: 98, a
chicken beta actin gene sequence of SEQ ID NO: 99, and a chimeric intron of SEQ ID
NO: 100;
a 5' OTOF coding region that comprises exons 1 to (and through) 21 of *OTOF*
cDNA;
a SD intron sequence of SEQ ID NO: 102;
an AK recombinogenic sequence of SEQ ID NO: 103; and
a 3' ITR sequence of SEQ ID NO: 104.~~
4. ~~—The recombinant AAV vector of claim 3, wherein the 5' OTOF coding region is
SEQ ID NO: 101.~~
5. ~~—The recombinant AAV vector of claim 3 or 4, wherein the 5' OTOF coding region
is at least 70%, 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%, at least 99.5%, or at least 99.9% identical to SEQ ID NO: 101, and encodes the
same amino acid sequence as encoded by SEQ ID NO: 101.~~
6. ~~—A recombinant AAV vector that comprises, in order of 5' to 3':
a 5' ITR sequence of SEQ ID NO: 97;
an AK recombinogenic sequence of SEQ ID NO: 103;
a SA intron sequence of SEQ ID NO: 106;~~

a 3' OTOF coding region that comprises exons 22 to (and through) exon 48 of *OTOF* cDNA;

a bGH polyA sequence of SEQ ID NO: 108; and

a 3' ITR sequence of SEQ ID NO: 104.

7. — The recombinant AAV vector of claim 6, wherein the 3' OTOF coding region is SEQ ID NO: 107.

8. — The recombinant AAV vector of claim 6 or 7, wherein the 3' OTOF coding region is at least 70%, 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%, at least 99.5%, or at least 99.9% identical to SEQ ID NO: 107, and encodes the same amino acid sequence as encoded by SEQ ID NO: 107.

9. — A recombinant AAV particle (rAAV particle) comprising a recombinant AAV vector of any one of claims 1-8 encapsidated by an Anc80 capsid.

10. — The rAAV particle of claim 9, wherein the Anc80 capsid comprises a polypeptide of SEQ ID NO: 109.

11. — A composition comprising a first rAAV particle comprising a recombinant AAV vector of claim 1 and a second rAAV particle comprising a recombinant AAV vector of claim 2.

12. — A composition comprising a first rAAV particle comprising a recombinant AAV vector of any one of claims 3-5 and a second rAAV particle comprising a recombinant AAV vector of any one of claims 6-8.

13. — The composition of claim 11 or 12, wherein the first and second rAAV particles are each independently encapsidated by an Anc80 capsid.

14. — The composition of claim 13, wherein the Anc80 capsid comprises a polypeptide of SEQ ID NO: 109.

15. — The composition of claim 11, wherein when the composition is introduced into a human cell the recombinant AAV vectors of claims 1 and 2 undergo concatamerization or homologous recombination with each other, thereby forming a recombined nucleic acid that encodes a full length otoferlin protein within the cell.

16. — The composition of claim 12, wherein when the composition is introduced into a human cell the recombinant AAV vectors of any one of claims 3-5 and the recombinant AAV vectors of any one of claims 6-8 undergo concatamerization or homologous recombination with each other, thereby forming a recombined nucleic acid that encodes a full length otoferlin protein within the cell.

17. — The composition of claim 15 or 16, further comprising at least one pharmaceutically acceptable excipient.

18. — A method comprising introducing into a cochlea of a mammal a therapeutically effective amount of a composition of any one of claims 11-17.

19. — The method of claim 18, wherein the mammal is a human.

20. — The method of claim 18 or 19, wherein the mammal has been previously identified as having a defective otoferlin gene.

21. — A method of increasing expression of a full length otoferlin protein in a mammalian cell, the method comprising introducing the composition of any one of claims 11-17 into the mammalian cell.

22. — The method of claim 21, wherein the mammalian cell is an inner hair cell.

23. — The method of claim 21 or 22, wherein the mammalian cell is a human cell.
24. — The method of any one of claims 21-23, wherein the mammalian cell has previously been determined to have a defective otoferlin gene.
25. — A method of increasing expression of a full-length otoferlin protein in an inner hair cell in a cochlea of a mammal, the method comprising introducing into the cochlea of the mammal a therapeutically effective amount of a composition of any one of claims 11-17.
26. — The method of claim 25, wherein the mammal has been previously identified as having a defective otoferlin gene.
27. — The method of claim 25 or 26, wherein the mammal is a human.
28. — A method of treating non-symptomatic sensorineural hearing loss in a subject identified as having a defective otoferlin gene, the method comprising administering a therapeutically effective amount of a composition of any one of claims 11-17 into the cochlea of the subject.
29. — The method of claim 28, wherein the subject is a human.
30. — The method of claim 28 or 29, further comprising, prior to the administering step, determining that the subject has a defective otoferlin gene.
31. — The method of any one of claims 28-30, wherein the composition is administered to the cochlea using a microcatheter.
32. — The method of claim 31, wherein the microcatheter is shaped such that it can enter the middle ear cavity via the external auditory canal and contact the end of the microcatheter with the RWM.

33. — The method of claim 31 or 32, wherein a distal end of the microcatheter is comprised of at least one microneedle with diameter of between 10 and 1,000 microns.
34. — The method of any one of claims 28-30, wherein the composition is administered to the cochlea using a device comprising a needle.
35. — The method of claim 34, wherein the needle comprises a bent portion and an angled tip.
36. — A kit comprising a composition of any one of claims 11-17.
37. — The kit of claim 36, wherein the composition is pre-loaded in a device.
38. — The kit of claim 37, wherein the device is a microcatheter.
39. — The kit of claim 38, wherein the microcatheter is shaped such that it can enter the middle ear cavity via the external auditory canal and contact the end of the microcatheter with the RWM.
40. — The kit of claim 38 or 39, wherein a distal end of the microcatheter is comprised of at least one microneedle with diameter of between 10 and 1,000 microns.
41. — The kit of claim 36, further comprising a device.
42. — The kit of claim 41, wherein the device is a device described in any one of FIGS. 82-87.
43. — The kit of claim 42, wherein the device comprises a needle comprising a bent portion and an angled tip.

1. A plurality of recombinant adeno-associated viral (rAAV) vectors comprising:
a) a first rAAV vector comprising a nucleic acid sequence of SEQ ID NO: 96; and
b) a second rAAV vector comprising a nucleic acid sequence of SEQ ID NO: 105.
2. The plurality of rAAV vectors of claim 1, wherein the first and second rAAV vectors are each encapsulated by an AAV capsid.
3. The plurality of rAAV vectors of claim 2, wherein the AAV capsid encapsulating the first rAAV vector is a serotype selected from any one of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAVrh8, AAVrh10, AAVrh39, AAVrh43, or Anc80.
4. The plurality of rAAV vectors of claim 2, wherein the AAV capsid encapsulating the second rAAV vector is a serotype selected from any one of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAVrh8, AAVrh10, AAVrh39, AAVrh43, or Anc80.
5. The plurality of rAAV vectors of claim 2, wherein the first rAAV vector is encapsulated by an Anc80 capsid and the second rAAV vector is encapsulated by an Anc80 capsid.
6. The plurality of rAAV vectors of claim 5, wherein the Anc80 capsids comprise the polypeptide sequence of SEQ ID NO: 109.
7. A composition comprising the plurality of rAAV vectors of claim 1, wherein the composition further comprises one or more pharmaceutically acceptable carriers, diluents, or excipients.
8. The composition of claim 7, wherein the composition is formulated for intra-cochlear administration.

9. The composition of claim 8, wherein the composition is formulated to comprise a synthetic perilymph solution.
10. Use of a plurality of recombinant adeno-associated viral (rAAV) vectors in the manufacture of a medicament for expressing a recombinant full-length otoferlin protein in a mammalian cell, wherein the plurality of recombinant adeno-associated viral (rAAV) vectors comprises:
- a) a first rAAV vector comprising a nucleic acid sequence of SEQ ID NO: 96; and
- b) a second rAAV vector comprising a nucleic acid sequence of SEQ ID NO: 105; and
- wherein the mammalian cell has reduced expression, lack of expression or dysfunction of otoferlin.
11. The use of claim 10, wherein the mammalian cell is an inner hair cell.
12. The use of claim 10, wherein the mammalian cell is a human cell.
13. The use of claim 10, wherein the mammalian cell comprises a defective otoferlin gene.
14. The use of claim 10, wherein the first and second rAAV vectors are each encapsulated by an AAV capsid.
15. The use of claim 14, wherein the AAV capsid encapsulating the first rAAV vector is a serotype selected from any one of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAVrh8, AAVrh10, AAVrh39, AAVrh43, or Anc80.
16. The use of claim 14, wherein the AAV capsid encapsulating the second rAAV vector is a serotype selected from any one of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAVrh8, AAVrh10, AAVrh39, AAVrh43, or Anc80.

17. The use of claim 14, wherein the first rAAV vector is encapsulated by an Anc80 capsid and the second rAAV vector is encapsulated by an Anc80 capsid.

18. The use of claim 17, wherein the Anc80 capsids comprise the polypeptide sequence of SEQ ID NO: 109.

19. Use of a plurality of recombinant adeno-associated viral (rAAV) vectors in the manufacture of a medicament for treating hearing loss in a subject having otoferlin related hearing loss, wherein the plurality of recombinant adeno-associated viral (rAAV) vectors comprises:

a) a first rAAV vector comprising a nucleic acid sequence of SEQ ID NO: 96; and

b) a second rAAV vector comprising a nucleic acid sequence of SEQ ID NO: 105; and wherein the subject has reduced expression, lack of expression, or dysfunction of otoferlin.

20. The use of claim 19, wherein the subject is a human.

21. The use of claim 19, wherein the first and second rAAV vectors are each encapsulated by an AAV capsid.

22. The use of claim 21, wherein the AAV capsid encapsulating the first rAAV vector is a serotype AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAVrh8, AAVrh10, AAVrh39, AAVrh43, or Anc80.

23. The use of claim 21, wherein the AAV capsid encapsulating the second rAAV vector is a serotype AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAVrh8, AAVrh10, AAVrh39, AAVrh43, or Anc80.

24. The use of claim 21, wherein the first rAAV vector is encapsulated by an Anc80 capsid and the second rAAV vector is encapsulated by an Anc80 capsid.

25. The use of claim 24, wherein the Anc80 capsids comprise the polypeptide sequence of SEQ ID NO: 109.

26. The use of claim 19, wherein the plurality of rAAV vectors is administered as a single dose.

27. The use of claim 19, wherein the plurality of rAAV vectors is administered as two or more doses.

28. The use of claim 19, wherein the subject has a defective otoferlin gene.