

GENE THERAPY DELIVERY COMPOSITIONS AND METHODS FOR TREATING HEARING LOSS

CROSS-REFERENCE TO RELATED APPLICATIONS

- [0001]** This application claims the benefit of U.S. Provisional Application No. 63/188,450, filed May 13, 2021, U.S. Provisional Application No. 63/251,025, filed September 30, 2021, and U.S. Provisional Application No. 63/277,549, filed November 9, 2021, which are hereby incorporated by reference in their entirety.

REFERENCE TO SEQUENCE LISTING SUBMITTED ELECTRONICALLY

- [0002]** The content of the electronically submitted sequence listing in ASCII text file (Name: 4833_008CP03_Seqlisting_ST25.TXT; Size: 269,049 bytes; and Date of Creation: May 9, 2022) filed with the application is incorporated herein by reference in its entirety.

BACKGROUND

- [0003]** 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 nonsyndromic 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).
- [0004]** Sensorineural hearing loss (SNHL) is the most common congenital sensory impairment, with the most common genetic cause being mutations in the gap junction β 2 gene (GJB2) encoding the connexin 26 (Cx26) protein.

SUMMARY

- [0005]** Certain aspects of the disclosure are directed to promoters, e.g., cell specific promoters, which are derived from portions of GDF6, PARM1, MMP15, or VIM promoters, and are capable of directing transcription of the coding sequence (e.g., encoding Connexin 26 polypeptide or functional fragment thereof) in an inner ear support cell.

- [0006] Certain aspects of the disclosure are directed to polynucleotide comprising a sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NOs: 16, 28, 40, 57, or 90-99. In some aspects, the polynucleotide is a promoter.
- [0007] Certain aspects of the disclosure are directed to a polynucleotide comprising a sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NOs: 40, 90, 96, or 99.
- [0008] In some aspects, the polynucleotide comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 90.
- [0009] In some aspects, the polynucleotide comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 40.
- [0010] In some aspects, the polynucleotide comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 96.
- [0011] In some aspects, the polynucleotide comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 99.
- [0012] In some aspects, the polynucleotide is capable of directing transcription of a coding sequence for a Connexin 26 polypeptide or a functional fragment thereof.
- [0013] Certain aspects of the disclosure are directed to construct comprising the polynucleotide disclosed herein and a nucleic acid sequence comprising the coding sequence for a/the Connexin 26 polypeptide or functional fragment thereof. In some aspects, the construct is an expression cassette.
- [0014] In some aspects, the polynucleotide of the construct is a promoter and is operably linked to a/the coding sequence. In some aspects, the polynucleotide is capable of directing transcription of the coding sequence in an inner ear support cell.
- [0015] In some aspects, polypeptide of the construct is a Connexin 26 polypeptide or functional fragment thereof.
- [0016] Certain aspects of the disclosure are directed to a construct comprising a construct comprising the polynucleotide. In some aspects, the construct further comprises a nucleic acid sequence encoding a polypeptide. In some aspects, the polynucleotide is operably

linked to the nucleic acid sequence encoding the polypeptide. In some aspects, the polynucleotide promotes expression of the nucleic acid in an inner ear support cell.

- [0017] Certain aspects of the disclosure are directed to a construct comprising a polynucleotide encoding a therapeutic polypeptide operably linked to a promoter which expresses the polynucleotide in an inner ear support cell. In some aspects, the polynucleotide encodes a therapeutic polypeptide or a reporter polypeptide. In some aspects, the promoter selectively expresses the polynucleotide in an inner ear support cell.
- [0018] Certain aspects of the disclosure are directed to a construct comprising a polynucleotide encoding a polypeptide operably linked to a promoter which expresses the polynucleotide in an inner ear support cell, wherein the promoter is heterologous to the polynucleotide.
- [0019] Certain aspects of the disclosure are directed to an expression construct comprising a coding sequence for a Connexin 26 polypeptide or a functional fragment thereof operably linked to a promoter, wherein the promoter comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NOs: 40, 90, 96, or 99, wherein the promoter is capable of directing transcription of the coding sequence.
- [0020] In some aspects, the promoter of the expression construct comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 90.
- [0021] In some aspects, the promoter of the expression construct comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 40.
- [0022] In some aspects, the promoter of the expression construct comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 96.
- [0023] In some aspects, the promoter of the expression construct comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 99.
- [0024] In some aspects, the expression construct further comprises a second promoter operably linked to the coding sequence, wherein the second promoter is heterologous or homologous to the coding sequence.

- [0025] In some aspects, the promoter of the expression construct is capable of directing transcription of the coding sequence in an inner ear support cell.
- [0026] In some aspects, the inner ear support cell is selected from one or more of inner phalangeal cells/border cells (IPhC), inner pillar cells (IPC), outer pillar cells (OPC), Deiters' cells rows 1 and 2 (DC1/2), Deiters' cells row 3 (DC3), Hensen's cells (Hec), Claudius cells/outer sulcus cells (CC/OSC), interdental cells (Idc), inner sulcus cells (ISC), Kölliker's organ cells (KO), greater ridge epithelial ridge cells (GER) (including lateral greater epithelial ridge cells (LGER)), and OC90+ cells (OC90), fibroblasts, and other cells of the lateral wall.
- [0027] In some aspects, the polynucleotide, construct, or the expression construct disclosed herein, further comprises a minimal GJB2 promoter which is operably linked to the coding sequence for the Connexin 26 polypeptide or functional fragment thereof.
- [0028] In some aspects, the construct or the expression construct disclosed herein comprises a GJB2 nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NOs: 117-126.
- [0029] Certain aspects of the disclosure are directed to an expression construct comprising a coding sequence for a Connexin 26 polypeptide or functional fragment thereof operably linked to an inner ear supporting cell selective promoter and a minimal GJB2 promoter, wherein the polynucleotide is expressed in an inner ear support cell. In some aspects, the inner ear supporting cell selective promoter is heterologous to the coding sequence for the Connexin 26 polypeptide or functional fragment thereof.
- [0030] In some aspects, the inner ear supporting cell selective promoter comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NOs: 40, 90, 96, or 99.
- [0031] In some aspects, the promoter comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 90.
- [0032] In some aspects, the promoter comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 40.

- [0033]** In some aspects, the promoter comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 96.
- [0034]** In some aspects, the promoter comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 99.
- [0035]** In some aspects, the inner ear supporting cell selective promoter comprises a nucleic acid sequence having having at least 95% identity to a sequence is selected from one or more of SEQ ID NO: 90, 40, 96, or 99.
- [0036]** In some aspects, the inner ear support cell is selected from one or more of inner phalangeal cells/border cells (IPhC), inner pillar cells (IPC), outer pillar cells (OPC), Deiters' cells rows 1 and 2 (DC1/2), Deiters' cells row 3 (DC3), Hensen's cells (Hec), Claudius cells/outer sulcus cells (CC/OSC), interdental cells (Idc), inner sulcus cells (ISC), Kölliker's organ cells (KO), greater ridge epithelial ridge cells (GER) (including lateral greater epithelial ridge cells (LGER)), and OC90+ cells (OC90), fibroblasts, and other cells of the lateral wall.
- [0037]** In some aspects, the polynucleotide, the construct, or expression construct of the disclosure comprises a minimal GJB2 promoter comprising a nucleic acid sequence with at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% at least 99%, or 100% identity to SEQ ID NO: 86.
- [0038]** In some aspects, the expression construct comprises a GJB2 nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NOs: 117-126.
- [0039]** Certain aspects of the disclosure is directed to a viral vector construct comprising: (i) a 5' inverted terminal repeat (ITR), (ii) a coding sequence for a Connexin 26 polypeptide or functional fragment thereof operably linked to a promoter which is capable of directing transcription of the coding sequence in an inner ear support cell, and (iii) a 3' ITR, wherein the promoter is heterologous to the coding sequence. In some aspects, the viral construct promoter comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NOs: 40, 90, 96, or 99.

- [0040] In some aspects, the viral construct promoter comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 90.
- [0041] In some aspects, the viral construct promoter comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 40.
- [0042] In some aspects, the viral construct promoter comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 96.
- [0043] In some aspects, the viral construct promoter comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 99.
- [0044] In some aspects, the viral vector construct further comprises a 5' untranslated region (UTR).
- [0045] In some aspects, the viral vector construct further comprises a 3' untranslated region (UTR).
- [0046] In some aspects, the viral vector construct comprises: (i) the 5' inverted terminal repeat (ITR), (ii) a 5' untranslated region (UTR), (iii) the coding sequence for the Connexin 26 polypeptide or functional fragment thereof operably linked to a promoter which expresses the polynucleotide in an inner ear support cell, (iv) a 3' UTR, and (v) the 3' ITR.
- [0047] In some aspects, the viral vector construct comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NOs: 117-126.
- [0048] In some aspects, the viral vector construct comprises: (i) a 5' inverted terminal repeat (ITR), (ii) a coding sequence for a Connexin 26 polypeptide or functional fragment thereof operably linked to an inner ear supporting cell selective promoter and a minimal GJB2 promoter, and (iii) a 3' ITR, wherein the inner ear supporting cell selective promoter is heterologous to the coding sequence.
- [0049] In some aspects, the viral vector construct the inner ear supporting cell selective promoter comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NOs: 40, 90, 96, or 99.

- [0050] In some aspects, the viral vector construct comprises: (i) the 5' inverted terminal repeat (ITR), (ii) a 5' untranslated region (UTR), (iii) the coding sequence for the Connexin 26 polypeptide or functional fragment thereof operably linked to an inner ear supporting cell selective promoter and a minimal GJB2 promoter, (iv) a 3' UTR, and (v) the 3' ITR.
- [0051] In some aspects, the viral vector construct comprises a GJB2 nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NOs: 117-126.
- [0052] In some aspects, the viral vector construct comprises a minimal GJB2 promoter comprising a nucleic acid sequence with at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% at least 99%, or 100% identity to SEQ ID NO: 86.
- [0053] In some aspects, the promoter is capable of expressing the coding sequence for the Connexin 26 polypeptide or functional fragment thereof in an inner ear support cell selected from one or more of inner phalangeal cells/border cells (IPhC), inner pillar cells (IPC), outer pillar cells (OPC), Deiters' cells rows 1 and 2 (DC1/2), Deiters' cells row 3 (DC3), Hensen's cells (Hec), Claudius cells/outer sulcus cells (CC/OSC), interdental cells (Idc), inner sulcus cells (ISC), Kölliker's organ cells (KO), greater ridge epithelial ridge cells (GER) (including lateral greater epithelial ridge cells (LGER)), and OC90+ cells (OC90), fibroblasts, and other cells of the lateral wall.
- [0054] In some aspects, the 5' UTR comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 100% identity to the sequence of any one of SEQ ID NOs: 20, 21, or 66.
- [0055] In some aspects, the 3' UTR comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 100% identity to the sequence of any one of SEQ ID NOs: 22, 67, 68, or 69.
- [0056] In some aspects, the polynucleotide, the construct, the expression construct, or viral vector construct disclosed herein, further comprises a polyA tail. In some aspects, the polyA tail is a bovine growth hormone, mouse- β -globin, mouse- α -globin, human collagen, polyoma virus, the Herpes simplex virus thymidine kinase gene (HSV TK), IgG heavy-chain gene, human growth hormone, or a SV40 late and early poly(A). In some aspects, the polyA tail is a bovine growth hormone polyA.
- [0057] In some aspects, the viral vector construct disclosed herein, further comprises a 5' and a 3' inverted terminal repeat (ITR). In some aspects, the 5' ITR and the 3' ITR flank

the promoter and coding sequence. In some aspects, the 5' ITR and the 3' ITR are AAV ITRs are derived from a serotype selected from AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, and AAV Anc80 ITRs. In some aspects, the AAV ITRs are derived from serotype AAV2.

[0058] In some aspects, the 5' AAV ITR comprises the nucleic acid sequence of SEQ ID NO: 8 or SEQ ID NO: 52.

[0059] In some aspects, the 3' AAV ITR comprises the nucleic acid sequence of SEQ ID NO: 9 or SEQ ID NO: 53.

[0060] In some aspects, the viral vector construct disclosed herein comprises: a) the 5' ITR comprises a nucleic acid sequence according to SEQ ID NO: 8 and the 3' ITR comprises a nucleic acid sequence according to SEQ ID NO: 9; and/or b) the 5' ITR comprises a nucleic acid sequence according to SEQ ID NO: 52 and the 3' ITR comprises a nucleic acid sequence according to SEQ ID NO: 53.

[0061] In some aspects, the viral vector comprises (i) the 5' ITR comprises the nucleic acid sequence of SEQ ID NOs: 8 or 52, (ii) the 5' UTR comprises the nucleic acid of any one of SEQ ID NOs: 20, 21, or 66, (iii) the promoter comprises the nucleic acid sequence of any one of SEQ ID NOs: 10-16, 28, 40, 57, 90-99, (iv) the 3' UTR comprises the nucleic acid sequence of SEQ ID NOs: 22, 67, 68, or 69, and (v) the 3' ITR comprises the nucleic acid sequence of SEQ ID NOs: 9 or 53.

[0062] In some aspects, the viral vector comprises (i) the 5' ITR comprises the nucleic acid sequence of SEQ ID NOs: 8 or 52, (ii) the 5' UTR comprises the nucleic acid of any one of SEQ ID NOs: 20, 21, or 66, (iii) the inner ear supporting cell selective promoter comprises the nucleic acid sequence of any one of SEQ ID NOs: 10-16, 28, 40, 57, 90-99, the minimal GJB2 promoter comprises the sequence of SEQ ID NO: 86, (v) the 3' UTR comprises the nucleic acid sequence of SEQ ID NOs: 22, 67, 68, or 69, and (vi) the 3' ITR comprises the nucleic acid sequence of SEQ ID NOs: 9 or 53.

[0063] In some aspects, the construct, the expression construct, or viral vector construct disclosed herein comprises a nucleic acid sequence according to any one of SEQ ID NOs: 7, 17, 38, 45-51, 54, 61, 82-84, 87-88, and 100-107.

[0064] In some aspects, the construct, the expression construct, or viral vector construct is selectively expressed in an inner ear supporting cell.

[0065] In some aspects, the construct, the expression construct, or viral vector construct comprises nucleotides 12-4557 of SEQ ID NO: 7, nucleotides 12-4338 of SEQ ID NO:

17, nucleotides 12-3976 of SEQ ID NO: 38, nucleotides 12-4754 of SEQ ID NO: 54, nucleotides 12-4429 of SEQ ID NO: 61, nucleotides 12-4645 of SEQ ID NO: 100, nucleotides 12-4708 of SEQ ID NO: 101, nucleotides 12-4993 of SEQ ID NO: 102, nucleotides 12-4496 of SEQ ID NO: 103, nucleotides 12-4253 of SEQ ID NO: 104, nucleotides 12-4320 of SEQ ID NO: 105, nucleotides 12-4464 of SEQ ID NO: 106, or nucleotides 12-4328 of SEQ ID NO: 107.

[0066] Certain aspects of the disclosure are directed to a viral vector or AAV particle comprising the polynucleotide, construct, expression construct, or viral vector construct disclosed herein. In some aspects, the viral vector is selected from the group consisting of an adeno-associated viral (AAV), adenovirus, or lentiviral vector. In some aspects, the viral vector is an AAV vector.

[0067] In some aspects, the viral vector or AAV particle comprises an AAV capsid, wherein the AAV capsid is or is derived from an AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV-rh8, AAV-rh10, AAV-rh39, AAV-rh43 or AAV Anc80 serotype capsid. In some aspects, the AAV vector or AAV particle comprises an AAV capsid which an AAV Anc80 capsid.

[0068] Certain aspects of the disclosure are directed to a composition comprising the polynucleotide, the construct, the expression construct, viral vector construct, or AAV particle disclosed herein. In some aspects, the composition is a pharmaceutical composition further comprising a pharmaceutically acceptable carrier. In some aspects, the pharmaceutical composition is a synthetic perilymph solution.

[0069] Certain aspects of the disclosure are directed to ex vivo cell comprising the polynucleotide, the construct, the expression construct, the viral vector construct, the viral vector, or the AAV particle disclosed herein.

[0070] In some aspects, the ex vivo cell is an inner ear cell. In some aspects, the ex vivo cell is an inner ear supporting cell. In some aspects, the supporting cell is selected from one or more of inner phalangeal cells/border cells (IPhC), inner pillar cells (IPC), outer pillar cells (OPC), Deiters' cells rows 1 and 2 (DC1/2), Deiters' cells row 3 (DC3), Hensen's cells (Hec), Claudius cells/outer sulcus cells (CC/OSC), interdental cells (Idc), inner sulcus cells (ISC), Kölliker's organ cells (KO), greater ridge epithelial ridge cells (GER) (including lateral greater epithelial ridge cells (LGER)), and OC90+ cells (OC90), fibroblasts, and other cells of the lateral wall.

- [0071] Certain aspects of the disclosure are directed to a method comprising, transducing an ex vivo cell with: a. the polynucleotide, the construct, the expression construct, the viral vector construct, the viral vector, or the AAV particle disclosed herein; and b. one or more helper plasmids collectively comprising an AAV Rep gene, AAV Cap gene, AAV VA gene, AAV E2a gene, and AAV E4 gene.
- [0072] Certain aspects of the disclosure are directed to a method of expressing the Connexin 26 polypeptide or functional fragment thereof in an inner ear supporting cell, comprising administering the polynucleotide, the construct, the expression construct, the viral vector construct, the viral vector, the AAV particle, or the ex vivo cell disclosed herein.
- [0073] Certain aspects of the disclosure are directed to a method of increasing expression of the Connexin 26 polypeptide or functional fragment thereof in an inner ear supporting cell, comprising administering the polynucleotide, the construct, the expression construct, the viral vector construct, the viral vector, the AAV particle, or the ex vivo cell disclosed herein to the subject.
- [0074] In some aspects, the expression of the Connexin 26 polypeptide or functional fragment thereof in the inner ear supporting cell is increased relative to endogenous expression of the polypeptide in the inner ear supporting cell.
- [0075] Certain aspects of the disclosure are directed to a method of treating hearing loss in a subject suffering from or at risk of hearing loss, comprising administering the polynucleotide, the construct, the expression construct, the viral vector construct, the viral vector, the AAV particle, or the ex vivo cell disclosed herein to the subject.
- [0076] In some aspects, (i) the Connexin 26 polypeptide or functional fragment thereof is predominately expressed in inner ear supporting cells, (ii) the Connexin 26 polypeptide or functional fragment thereof is selectively expressed at a higher level in inner ear supporting cells than in inner ear hair cells, (iii) the Connexin 26 polypeptide or functional fragment thereof not expressed at levels sufficient to cause toxicity in inner ear hair cells, or (iv) or any combination thereof.
- [0077] In some aspects, the inner ear supporting cells are selected from one or more of inner phalangeal cells/border cells (IPhC), inner pillar cells (IPC), outer pillar cells (OPC), Deiters' cells rows 1 and 2 (DC1/2), Deiters' cells row 3 (DC3), Hensen's cells (Hec), Claudius cells/outer sulcus cells (CC/OSC), interdental cells (Idc), inner sulcus cells (ISC), Kölliker's organ cells (KO), and OC90+ cells (OC90).

- [0078] In some aspects, the administration is to the inner ear of the subject.
- [0079] In some aspects, the administration is to the cochlea of the subject.
- [0080] In some aspects, the administration is via a round window membrane injection.
- [0081] Certain aspects are directed to the use of the polynucleotide, the construct, the expression construct, the viral vector construct, the viral vector, the AAV particle, or the ex vivo cell disclosed herein, for the treatment of hearing loss in a subject suffering from or at risk of hearing loss.
- [0082] Certain aspects are directed to the use of polynucleotide, the construct, the expression construct, the viral vector construct, the viral vector, the AAV particle, or the ex vivo cell disclosed herein, in the manufacture of a medicament for the treatment of hearing loss.
- [0083] In some aspects, the polynucleotide, the construct, the expression construct, the viral vector construct, the viral vector, the AAV particle, or the ex vivo cell disclosed herein, for use as a medicament.
- [0084] In some aspects, the polynucleotide, the construct, the expression construct, the viral vector construct, the viral vector, the AAV particle, or the ex vivo cell disclosed herein, for use in the treatment of hearing loss.
- [0085] In some aspects, the construct, vector, AAV particle, composition or ex vivo cell is pre-loaded in a device for administration. In some aspects, the device is a microcatheter. In some aspects, 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 aspects, a distal end of the microcatheter is comprised of at least one microneedle with diameter of between 10 and 1,000 microns. In some aspects, the kit further comprises a device. In some aspects, the device is a device described in any one of FIGS. 5-8. In some aspects, the device comprises a needle comprising a bent portion and an angled tip.
- [0086] Certain aspects are directed to a kit comprising the polynucleotide, the construct, the expression construct, the viral vector construct, the viral vector, the AAV particle, or the ex vivo cell disclosed herein. In some aspects, the kit further comprises a device disclosed herein.
- [0087] Certain aspects of the disclosure are directed to a construct comprising a polynucleotide encoding a polypeptide operably linked to a promoter, wherein the promoter comprises a nucleic acid sequence having at least 80%, at least 85%, at least

90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NOs: 16, 28, 40, 57, 90-99. In some aspects, the promoter is heterologous to the polynucleotide.

[0088] Certain aspects of the disclosure are directed to a construct comprising a polynucleotide encoding a polypeptide, an inner ear supporting cell selective promoter and a minimal GJB2 promoter, wherein the polynucleotide is operably linked to the inner ear supporting cell selective promoter and the minimal GJB2 promoter such that the polynucleotide is expressed in an inner ear support cell, wherein the inner ear supporting cell selective promoter is heterologous to the polynucleotide.

[0089] Certain aspects of the disclosure are directed to a construct comprising a polynucleotide encoding a polypeptide, an inner ear supporting cell selective promoter and a minimal GJB2 promoter, wherein the polynucleotide is operably linked to the inner ear supporting cell selective promoter and the minimal GJB2 promoter, wherein the inner ear supporting cell selective promoter comprises a nucleic acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NOs: 16, 28, 40, 57, 90-99. In some aspects, the inner ear supporting cell selective promoter is heterologous to the polynucleotide. In some aspects, the minimal GJB2 promoter comprises a nucleic acid sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 86.

[0090] Certain aspects of the disclosure are directed to a construct comprising: (i) a 5' inverted terminal repeat (ITR), (ii) a polynucleotide encoding a polypeptide operably linked to a promoter which expresses the polynucleotide in an inner ear support cell, and (iii) a 3' ITR, wherein the promoter is heterologous to the polynucleotide.

[0091] Certain aspects of the disclosure are directed to a construct comprising: (i) a 5' inverted terminal repeat (ITR), (ii) a polynucleotide encoding a polypeptide operably linked to a promoter, and (iii) a 3' ITR, wherein the promoter comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NOs: 16, 28, 40, 57, or 90-99. In some aspects, the construct further comprises a minimal GJB2 promoter. In some aspects, the minimal GJB2 promoter comprises a nucleic acid sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 86.

- [0092] In some aspects, the promoter is selected from one or more a GJB6 promoter, a GDF6 promoter, a IGFBP2 promoter, a RBP7 promoter, a PARM1 promoter, a GFAP promoter, a BACE2 promoter, a DBI2 promoter, a FABP3 promoter, a KLHL14 promoter, a MMP15 promoter, a SPARC promoter, a TSPAN8 promoter, a VIM promoter, derivatives thereof, or fragments thereof.
- [0093] In some aspects, the promoter is a GJB2 promoter or a minimal GJB2 promoter.
- [0094] In some aspects, the construct comprises two or more promoters. In some aspects, the first promoter is selected from a GJB6 promoter, a GDF6 promoter, a IGFBP2 promoter, a RBP7 promoter, a PARM1 promoter, a GFAP promoter, a BACE2 promoter, a DBI2 promoter, a FABP3 promoter, a KLHL14 promoter, a MMP15 promoter, a SPARC promoter, a TSPAN8 promoter, a VIM promoter, or any combination thereof. In some aspects, the second promoter is selected from a GJB2 promoter or a minimal GJB2 promoter.
- [0095] Certain aspects of the disclosure are directed to a construct comprising: (i) a 5' inverted terminal repeat (ITR), (ii) a polynucleotide encoding a polypeptide operably linked to an inner ear supporting cell selective promoter and a minimal GJB2 promoter, wherein the polynucleotide is expressed in an inner ear support cell, and (iii) a 3' ITR, wherein the inner ear supporting cell selective promoter is heterologous to the polynucleotide.
- [0096] Certain aspects of the disclosure are directed to a construct comprising: (i) a 5' inverted terminal repeat (ITR), (ii) a polynucleotide encoding a polypeptide operably linked to a inner ear supporting cell selective promoter and a minimal GJB2 promoter, and (iii) a 3' ITR, wherein the inner ear supporting cell selective promoter comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NOs: 16, 28, 40, 57, or 90-99.
- [0097] In some aspects, inner ear supporting cells include, but are not limited to, inner phalangeal cells/border cells (IPhC), inner pillar cells (IPC), outer pillar cells (OPC), Deiters' cells rows 1 and 2 (DC1/2), Deiters' cells row 3 (DC3), Hensen's cells (Hec), Claudius cells/outer sulcus cells (CC/OSC), interdental cells (Idc), inner sulcus cells (ISC), Kölliker's organ cells (KO), greater ridge epithelial ridge cells (GER) (including lateral greater epithelial ridge cells (LGER)), and OC90+ cells (OC90), fibroblasts, and other cells of the lateral wall.

- [0098] In some aspects, the promoter comprises a nucleic acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NOs: 16, 28, 40, 57, 90-99.
- [0099] In some aspects, the construct comprises a miRNA regulatory target site (miRTS) for a microRNA expressed in an inner ear cell. In some aspects, the microRNA is expressed in an inner ear hair cell. In some aspects, the microRNA is one or more of miR-194, miR-140, miR-18a, miR-99a, miR-30b, miR-15a, miR182, miR-183, or any combination thereof.
- [0100] Certain aspects of the disclosure are directed to a construct comprising a polynucleotide encoding a polypeptide operably linked to a promoter, wherein the construct comprises a miRNA regulatory target site (miRTS) for a microRNA expressed in an inner ear cell.
- [0101] In some aspects, the polynucleotide encodes a therapeutic polypeptide (e.g., a Connexin 26 polypeptide) or a reporter polypeptide.
- [0102] In some aspects, the microRNA is expressed in one or more of inner ear hair cells, spiral ganglion cells, lateral supporting cells, basilar membrane cells, medial supporting cells, spiral limbus cells, or inner sulcus cells.
- [0103] In some aspects, the microRNA is expressed in inner ear hair cells.
- [0104] In some aspects, the microRNA is one or more of miR-194, miR-140, miR-18a, miR-99a, miR-30b, miR-15a, miR182, or miR-183.
- [0105] In some aspects, the construct comprises a 5' and a 3' inverted terminal repeat (ITR). In some aspects, the construct comprises a 5' untranslated region (UTR). In some aspects, the construct comprises a 3' untranslated region (UTR).
- [0106] Certain aspects of the disclosure are directed to vectors, viral particles (e.g., AAV), ex vivo cells, and compositions comprising the constructs disclosed herein.
- [0107] Certain aspects of the disclosure are directed to an adeno-associated virus (AAV) particle comprising a construct disclosed herein.
- [0108] Certain aspects are directed to an adeno-associated virus (AAV) particle comprising a construct comprising: (i) a 5' inverted terminal repeat (ITR), (ii) a polynucleotide encoding a polypeptide operably linked to a promoter which expresses the polynucleotide in an inner ear support cell, and (iii) a 3' ITR, wherein the promoter is heterologous to the polynucleotide. In some aspects, the promoter comprises a nucleic acid sequence at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least

98%, at least 99%, or 100% identical to any one of SEQ ID NOs: 16, 28, 40, 57, or 90-99.

In some aspects, the construct further comprises a minimal GJB2 promoter. In some aspects, the minimal GJB2 promoter comprises a nucleic acid sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO: 86.

- [0109] Certain aspects are directed to an adeno-associated virus (AAV) particle comprising a construct comprising: (i) a 5' inverted terminal repeat (ITR), (ii) a 5' untranslated region (UTR), (iii) a polynucleotide encoding a polypeptide operably linked to a promoter which expresses the polynucleotide in an inner ear support cell, (iv) a 3' UTR, and (v) a 3' ITR, wherein the promoter is heterologous to the polynucleotide.
- [0110] Certain aspects of the disclosure are directed to an adeno-associated virus (AAV) particle comprising a construct comprising: (i) a 5' inverted terminal repeat (ITR), (ii) a 5' untranslated region (UTR), (iii) a polynucleotide encoding a polypeptide operably linked to an inner ear supporting cell selective promoter and a minimal GJB2 promoter, wherein the polynucleotide is expressed in an inner ear support cell, (iv) a 3' UTR, and (v) a 3' ITR, wherein the inner ear supporting cell selective promoter is heterologous to the polynucleotide
- [0111] Certain aspects of the disclosure are directed to an adeno-associated virus (AAV) particle comprising a construct comprising: (i) a 5' inverted terminal repeat (ITR), (ii) a 5' untranslated region (UTR), (iii) a polynucleotide encoding a polypeptide operably linked to a promoter, (iv) a 3' UTR, and (v) a 3' ITR, wherein the promoter comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NOs: 16, 28, 40, 57, or 90-99.
- [0112] Certain adeno-associated virus (AAV) particle comprising a construct comprising: (i) a 5' inverted terminal repeat (ITR), (ii) a 5' untranslated region (UTR), (iii) a polynucleotide encoding a polypeptide operably linked to a promoter, (iv) a miRNA regulatory target site (miRTS) for a microRNA expressed in an inner ear cell, (v) a 3' UTR, and (vi) a 3' ITR.
- [0113] Certain aspects of the disclosure are directed to an adeno-associated virus (AAV) particle comprising a construct comprising: (i) a 5' inverted terminal repeat (ITR), (ii) a 5' untranslated region (UTR), (iii) a polynucleotide encoding a polypeptide operably linked to an inner ear supporting cell selective promoter and minimal GJB2 promoter, (iv) a 3'

UTR, and (v) a 3' ITR, wherein the inner ear supporting cell selective promoter comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NOs: 16, 28, 40, 57, or 90-99.

[0114] Certain adeno-associated virus (AAV) particle comprising a construct comprising:

(i) a 5' inverted terminal repeat (ITR), (ii) a 5' untranslated region (UTR), (iii) a polynucleotide encoding a polypeptide operably linked to an inner ear supporting cell selective promoter and a minimal GJB2 promoter, (iv) a miRNA regulatory target site (miRTS) for a microRNA expressed in an inner ear cell, (v) a 3' UTR, and (vi) a 3' ITR.

[0115] In some aspects, the inner ear supporting cell selective promoter is selected from one or more a GJB6 promoter, a GDF6 promoter, a IGFBP2 promoter, a RBP7 promoter, a PARM1 promoter, a GFAP promoter, a BACE2 promoter, a DBI2 promoter, a FABP3 promoter, a KLHL14 promoter, a MMP15 promoter, a SPARC promoter, a TSPAN8 promoter, a VIM promoter, derivatives thereof, or fragments thereof.

[0116] In some aspects, the minimal GJB2 promoter comprises a nucleic acid sequence with at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% at least 99%, or 100% identity to SEQ ID NO: 86.

[0117] Certain aspects of the disclosure are directed to methods of using the constructs, vectors, viral particles (e.g., AAV), ex vivo cells, and compositions disclosed herein for expressing a polypeptide in an inner ear cell (e.g., a supporting cells).

[0118] Certain aspects of the disclosure are directed to methods of using the constructs, vectors, viral particles (e.g., AAV), ex vivo cells, and compositions disclosed herein for increasing expression of a polypeptide (e.g., a therapeutic polypeptide, a Connexin 26 polypeptide) in an inner ear cell (e.g., a supporting cells). In some aspects, the increasing expression is relative to the corresponding endogenous polypeptide expression in the inner ear cell (e.g., a supporting cells).

[0119] Certain aspects of the disclosure are directed to methods of using the constructs, vectors, viral particles (e.g., AAV), ex vivo cells, and compositions disclosed herein for decreasing expression of a polypeptide (e.g., a therapeutic polypeptide) in non-inner ear supporting cells (e.g., inner ear hair cells). In some aspects, the decreasing expression is relative to the corresponding endogenous polypeptide expression in the non-inner ear cell supporting cells (e.g., inner ear hair cells).

- [0120] Certain aspects of the disclosure are directed to methods of using the constructs, vectors, viral particles (e.g., AAV), ex vivo cells, and compositions disclosed herein for reducing toxicity associated with expression of a polypeptide, (e.g., a therapeutic polypeptide) in an inner ear cell.
- [0121] Certain aspects of the disclosure are directed to methods of using the constructs, vectors, viral particles (e.g., AAV), ex vivo cells, and compositions disclosed herein for treating hearing loss in a subject suffering from or at risk of hearing loss.

BRIEF DESCRIPTION OF THE DRAWINGS

- [0122] **FIG. 1A-1B** panel (1A) depicts a simplified endogenous AAV genome; panel (1B) depicts a simplified recombinant AAV (rAAV) construct capable of expressing a therapeutic polypeptide (e.g., a GJB2 gene).
- [0123] **FIGs. 2A-2H** depict alternative exemplary rAAV constructs comprising a therapeutic polypeptide FIG. 2A depicts an exemplary rAAV construct comprising a 5' ITR, a CAG promoter, a nucleic acid encoding a therapeutic polypeptide (a hGJB2 gene), a bGH polyA, and a 3' ITR. FIG. 2B depicts an exemplary rAAV construct comprising a 5' ITR, a CAG promoter, a nucleic acid encoding a therapeutic polypeptide (a hGJB2 gene), a 3' UTR, a bGH polyA, and a 3' ITR. FIG. 2C depicts an exemplary rAAV construct comprising a 5' ITR, a CAG promoter, a 5' UTR, a nucleic acid encoding a therapeutic polypeptide (a hGJB2 gene), a FLAG tag, a 3' UTR, a bGH polyA, and a 3' ITR. FIG. 2D depicts an exemplary rAAV construct comprising a 5' ITR, a smCBA promoter, a 5' UTR, a nucleic acid encoding a therapeutic polypeptide (a hGJB2 gene), a FLAG tag, a 3' UTR, a bGH polyA, and a 3' ITR. FIG. 2E depicts an exemplary rAAV construct comprising a 5' ITR, a promoter comprising a CMV promoter and a hGJB2 promoter, a 5' UTR, a nucleic acid encoding a hGJB2 gene, a FLAG tag, a 3' UTR, a bGH polyA, and a 3' ITR. FIG. 2F depicts an exemplary rAAV construct comprising a 5' ITR, a CAG promoter, a 5' UTR, a hGJB2 promoter, a FLAG tag, a microRNA regulatory target site, a 3' UTR, a bGH polyA, and a 3' ITR. FIG. 2G depicts an exemplary rAAV construct comprising a 5' ITR, a promoter comprising an inner ear supporting cell selective promoter and a hGJB2 minimal promoter, a nucleic acid encoding a therapeutic polypeptide (a hGJB2 gene), a FLAG tag, a 5' UTR, a bGH polyA, and a 3' ITR. FIG. 2H depicts an exemplary rAAV construct comprising a 5' ITR, a CAG promoter, a nucleic acid encoding a therapeutic polypeptide (a hGJB2 gene), a FLAG tag, a T2A element, a nucleic acid encoding eGFP, a bGH polyA, and a 3' ITR.

[0124] **FIGs. 3A-3Q** depict *in vitro* or *ex vivo* expression of a transgene in HEK293FT cells transfected or transduced with a construct with a microRNA targeting site (miRTS) in the presence or absence of a microRNA recognizing that site. FIG. 3A is a schematic that represents construct comprising a gene of interest and a miRTS. FIG. 3B is a Venn diagram representing selection of miRTS based on expression of microRNAs expressed in the different inner ear cell types. FIG. 3C is a graph showing the GFP expression in cells transfected with miRNA-expressing plasmid (pITR.CAG.mScarlet.miRNA) and a plasmid comprising a gene-of-interest and microRNA target site (pITR.CAG.GOI.miRTS). FIG. 3D is a graph showing GFP expression as measured by flow cytometry in HEK293FT cells transduced with an AAVAnc80 vector comprising GFP and a microRNA target site (AAVAnc0-CAG.GOI.miRTS) and transfected with a plasmid expressing miRNA targeting the miRTS (pITR.CAG.mScarlet.miRNA). FIG. 3E is a graph showing a gene of interest expression measured by RT-qPCR in cells transduced with an AAVAnc80 expressing the gene of interest with a microRNA targeting site (AAVAnc80-CAG.GOI.miRTS) following transfection with either of two amounts of plasmid expressing a plasmid encoding a miRNA targeting the miRTS (pITR.CAG.mScarlet.miRNA). FIG. 3F is a western blot of protein showing expression of the gene of interest in cells transduced with an AAVAnc80 comprising the gene of interest and a microRNA targeting site (AAVAnc80-CAG.GOI.miRTS) following transfection with either of two amounts of plasmid expressing a miRNA targeting the miRTS (pITR.CAG.mScarlet.miRNA). FIG. 3G is a graph showing the quantification of proteins levels determined from the western blot in FIG. 3F. FIG. 3H is a heat map of gene expression due to *in vitro* transduction of the gene-of-interest with the microRNA targeting site compared to transduction with the gene-of-interest alone. FIG. 3I is a volcano plot displaying differential gene expression between the samples. FIG. 3J shows expression of a gene of interest in an untreated cochlear explant (left panel) and after transduction with an AAV encoding a FLAG-tagged gene of interest without a microRNA target site (right panel). Immunostaining of the FLAG tag is shown in green. Immunostaining of MYO7A was used to label hair cells in red. White arrowheads indicate hair cells expressing the Connexin 26-FLAG. FIG. 3K shows a cochlear explant transduced with AAVAnc80-CAG-GOI.miRTS1 comprising a FLAG-tagged gene of interest and a microRNA targeting site for a microRNA expressed in hair cells. FIG. 3L shows a cochlear explant transduced with AAVAnc80-CAG-GOI.miRTS1 comprising a

FLAG-tagged gene of interest and a microRNA targeting site for a microRNA expressed in hair cells. FIG. 3M shows a cochlear explant transduced with AAVAnc80-CAG-GOI.miRTS2 comprising FLAG-tagged gene of interest and a microRNA targeting site recognized by a microRNA expressed in hair cells. FIG. 3N shows a cochlear explant transduced with AAVAnc80-CAG-GOI.miRTS3 comprising FLAG-tagged gene of interest and a microRNA targeting site recognized by a microRNA expressed in hair cells. FIG. 3O shows a cochlear explant transduced with AAVAnc80-CAG-GOI.miRTS4 comprising a FLAG-tagged gene of interest and a microRNA targeting for a microRNA expressed in hair cells. FIG. 3P and 3Q depict *in vitro* expression of GJB2 protein in HEK293FT cells transfected with CAG.5UTR.hGJB2.FLAG.miRTS.3UTR (SEQ ID NO: 87), CAG.5UTR.hGJB2.FLAG.3UTR (SEQ ID NO: 82), or CAG.5UTR.hGJB2.FLAG.GFP constructs. CAG.5UTR.hGJB2.FLAG.miRTS.3UTR comprises miRNA targeting sites (miRTS) for miR-182 and miR-183 in the 3UTR to permit exogenous hGJB2 knockdown in the presence of regulatory miR-182 and/or miR-183. To confirm miRNA regulation of constructs, HEK293FT cells were transfected with hGJB2 comprising plasmids and optionally co-transfected with (+) or without (-) plasmids expressing miR-182 and miR-183. 72h post transfection the cells were harvested for protein and RNA analysis. FIG. 3P depicts exemplary GJB2 protein levels analyzed using western blot. FIG. 3Q depicts exemplary GJB2 mRNA levels analyzed using qPCR.

[0125] FIG. 4A-4C depicts FLAG protein expression in mouse cochlear explants transduced at P2 with exemplary rAAVAnc80 particles comprising constructs driven by CAG, CMVe-GJB2p, or smCBA promoter/enhancer sequences as noted, explants were fixed after 72h, immunostaining for FLAG is noted in green, immunostaining for hair cell marker Myo7a is noted in red, and nuclear marker DAPI is noted in blue. Panel (4A) depicts exemplary explants transduced with AAVAnc80-CAG.5UTR.hGJB2.3F.3UTR (SEQ ID NO: 82) at 5.8E9 vg/explant. Panel (4B) depicts exemplary explants transduced with AAVAnc80-smCBA.5UTR.hGJB2.3F.3UTR (SEQ ID NO: 83) at 1.4E10 vg/explant. Panel (4C) depicts exemplary explants transduced with AAVAnc80-CMVeGFAPp.5UTR.hGJB2.3F.3UTR (SEQ ID NO: 84) at 1.8E10 vg/explant.

[0126] FIG. 5 illustrates a perspective of a device for delivering fluid to an inner ear, according to aspects of the present disclosure.

[0127] FIG. 6 illustrates a sideview of a bent needle sub-assembly, according to aspects of the present disclosure.

- [0128] **FIG. 7** illustrates a perspective view of a device for delivering fluid to an inner ear, according to aspects of the present disclosure.
- [0129] **FIG. 8** illustrates a perspective view of a bent needle sub-assembly coupled to the distal end of a device, according to aspects of the present disclosure.
- [0130] **FIGs. 9A-9O** depicts *in vivo* expression of Connexin 26 in wild-type mice. Wild type mice (p20) were administered rAAVAnc80 particles comprising CAG.hGJB2.FLAG.GFP (schematic provided in Fig. 2H) to the cochlea (FIG. 9A). Expression of Connexin 26 in the supporting cells and inner hair cells was detected 10 days after administration. Immunostaining of actin filaments and hair cell stereocilia bundles by phalloidin is noted in blue, GFP is noted in green, FLAG is noted in purple, and endogenous Connexin 26 is noted in red. SC – supporting cells; IHC – inner hair cells; OHC – outer hair cells. Juvenile mice were administered rAAVAnc80 particles comprising AAVAnc80-CMV.eGFAPp.mGJB2p.5UTR.hGJB2.FLAG.3UTR (FIG. 9B), AAVAnc80-GDF6p.mGJB2p.5UTR.hGJB2.FLAG.3UTR (FIGs. 9C and 9I) (schematic provided in Fig. 2G), AAVAnc80-IGFBP2p.mGJB2p.5UTR.hGJB2.FLAG.3UTR (FIG. 9D) (schematic provided in Fig. 2G), AAVAnc80-PARM1p.mGJB2p.5UTR.hGJB2.FLAG.3UTR (FIGs. 9E and 9J) (schematic provided in Fig. 2G), AAVAnc80-GFAPp.mGJB2p.hGJB2 (FIG. 9F), AAVAnc80-MMP15p.mGJB2p.hGJB2 (FIGs. 9G and 9L), AAVAnc80-VIMp.mGJB2p-hGJB2 (FIGs. 9H and 9K) to the cochlea. (VIM is also referred to as VIM1 in Figure 9K.) Expression of Connexin 26 was detected two weeks after administration. Immunostaining of actin filaments and hair cell stereocilia bundles by phalloidin is noted in blue, FLAG is noted in green, and endogenous Connexin 26 or Myo7a is noted in red. FIG. 9M depicts *in vivo* expression of Connexin 26 in wild-type mice administered AAVAnc80 particles comprising AAVAnc80.CMV.eGFAP.mGJB2p.hGJB2.FLAG. Endogenous Connexin 26 is shown in white, flag-tagged Connexin 26 is shown in green, and hair cells are shown by phalloidin staining in blue. FIGs. 9N-9O) depicts *in vivo* expression of Connexin 26 in wild-type mice administered AAVAnc80 particles comprising AAVAnc80.CMV.eGDF6.mGJB2p.hGJB2.FLAG or AAVAnc80.CMV.e.PARM1.mGJB2p.hGJB2.FLAG. Flag-tagged Connexin 26 is shown in green, phalloidin staining in blue, and Myo7a marking hair cells is shown in red.
- [0131] **FIGs. 10A-10C** depicts *in vitro* expression of GJB2 mRNA and detection of Connexin 26 protein from constructs including supporting cell selective promoters. FIG.

10A shows Connexin 26-FLAG protein levels (“GJB2-FLAG”) in HEK293FT cells transduced with exemplary rAAVAnc80 particles comprising constructs driven by GJB6, IGFBP2, RPB7, PARM1, or GDF6 promoters in combination with a minimal GJB2 promoter. GAPDH is shown as a loading control. FIG. 10B shows GJB2 mRNA levels in HEK293FT cells transduced with rAAVAnc80 particles comprising constructs driven by GFAP and a minimal GJB2 promoter, CMV enhancer/GFAP, GJB2 enhancer/GJB2, CMV enhancer/GJB2, or CAG promoters. FIG. 10C shows Connexin 26-FLAG protein levels (GJB2-FLAG) in HEK293FT cells transfected with plasmids comprising constructs driven by FABP3, KLHL14, DBI2, TSPAN8, MMP15, SPARC, or VIM promoters in combination with a minimal GJB2 promoter. FLAG was used to distinguish protein levels between endogenous and transduced Connexin 26 expression. GAPDH is shown as a loading control.

[0132] FIG. 11 shows GJB2 mRNA levels in mouse cochlear explants transduced with rAAVAnc80 particles comprising constructs driven by a CAG promoter, a CMV enhancer/GFAP promoter, or a GFAP and a minimal GJB2 promoter. GJB2 mRNA levels were determined by qPCR.

DEFINITIONS

[0133] The scope of the present disclosure is defined by the claims appended hereto and is not limited by certain aspects described herein. Those skilled in the art, reading the present specification, will be aware of various modifications that may be equivalent to such described aspects, or otherwise within the scope of the claims. In general, terms used herein are in accordance with their understood meaning in the art, unless clearly indicated otherwise. Explicit definitions of certain terms are provided below; meanings of these and other terms in particular instances throughout this specification will be clear to those skilled in the art from context.

[0134] Use of ordinal terms such as “first,” “second,” “third,” etc., in the claims to modify a claim element does not by itself connote any priority, precedence, or order of one claim element over another or the temporal order in which acts of a method are performed, but are used merely as labels to distinguish one claim element having a certain name from another element having a same name (but for use of the ordinal term) to distinguish the claim elements.

[0135] The articles “a” and “an,” as used herein, should be understood to include the plural referents unless clearly indicated to the contrary. Claims or descriptions that

include “or” between one or more members of a group are considered satisfied if one, more than one, or all of the group members are present in, employed in, or otherwise relevant to a given product or process unless indicated to the contrary or otherwise evident from the context. In some aspects, exactly one member of a group is present in, employed in, or otherwise relevant to a given product or process. In some aspects, more than one, or all group members are present in, employed in, or otherwise relevant to a given product or process. It is to be understood that the present disclosure encompasses all variations, combinations, and permutations in which one or more limitations, elements, clauses, descriptive terms, etc., from one or more of the listed claims is introduced into another claim dependent on the same base claim (or, as relevant, any other claim) unless otherwise indicated or unless it would be evident to one of ordinary skill in the art that a contradiction or inconsistency would arise. Where elements are presented as lists (e.g., in Markush group or similar format), it is to be understood that each subgroup of the elements is also disclosed, and any element(s) can be removed from the group. It should be understood that, in general, where aspects or aspects are referred to as “comprising” particular elements, features, etc., certain aspects or aspects “consist,” or “consist essentially of,” such elements, features, etc. For purposes of simplicity, those aspects have not in every case been specifically set forth in so many words herein. It should also be understood that any embodiment or aspect can be explicitly excluded from the claims, regardless of whether the specific exclusion is recited in the specification.

[0136] Throughout the specification, whenever a polynucleotide or polypeptide is represented by a sequence of letters (e.g., A, C, G, and T, which denote adenosine, cytidine, guanosine, and thymidine, respectively in the case of a polynucleotide), such polynucleotides or polypeptides are presented in 5' to 3' or N-terminus to C-terminus order, from left to right.

[0137] *Administration:* As used herein, the term “administration” typically refers to administration of a construct or composition to a subject or system to achieve delivery of an agent to a subject or system. In some aspects, an agent is, or is included in, a composition; in some aspects, an agent is generated through metabolism of a composition or one or more components thereof. Those of ordinary skill in the art will be aware of a variety of routes that may, in appropriate circumstances, be utilized for administration to a subject, for example a human. For example, in some aspects, administration may be systematic or local. In some aspects, a systematic administration can be intravenous. In

some aspects, administration can be local. Local administration can involve delivery to cochlear perilymph via, e.g., injection through a round-window membrane or into scala-tympani, a scala-media injection through endolymph, perilymph and/or endolymph following canalostomy. In some aspects, administration may involve only a single dose. In some aspects, administration may involve application of a fixed number of doses. In some aspects, administration may involve dosing that is intermittent (e.g., a plurality of doses separated in time) and/or periodic (e.g., individual doses separated by a common period of time) dosing. In some aspects, administration may involve continuous dosing (e.g., perfusion) for at least a selected period of time.

[0138] *Allele*: As used herein, the term “allele” refers to one of two or more existing genetic variants of a specific polymorphic genomic locus.

[0139] *Amelioration*: As used herein, the term “amelioration” refers to prevention, reduction or palliation of a state, or improvement of a state of a subject. Amelioration may include, but does not require, complete recovery or complete prevention of a disease, disorder or condition.

[0140] *Amino acid*: In its broadest sense, as used herein, the term “amino acid” refers to any compound and/or substance that can be incorporated into a polypeptide chain, e.g., through formation of one or more peptide bonds. In some aspects, an amino acid has a general structure, e.g., $\text{H}_2\text{N}-\text{C}(\text{H})(\text{R})-\text{COOH}$. In some aspects, an amino acid is a naturally-occurring amino acid. In some aspects, an amino acid is a non-natural amino acid; in some aspects, an amino acid is a D-amino acid; in some aspects, an amino acid is an L-amino acid. “Standard amino acid” refers to any of the twenty standard L-amino acids commonly found in naturally occurring peptides. “Nonstandard amino acid” refers to any amino acid, other than standard amino acids, regardless of whether it is prepared synthetically or obtained from a natural source. In some aspects, an amino acid, including a carboxy- and/or amino-terminal amino acid in a polypeptide, can contain a structural modification as compared with general structure as shown above. For example, in some aspects, an amino acid may be modified by methylation, amidation, acetylation, pegylation, glycosylation, phosphorylation, and/or substitution (e.g., of an amino group, a carboxylic acid group, one or more protons, and/or a hydroxyl group) as compared with a general structure. In some aspects, such modification may, for example, alter circulating half-life of a polypeptide containing a modified amino acid as compared with one containing an otherwise identical unmodified amino acid. In some aspects, such

modification does not significantly alter a relevant activity of a polypeptide containing a modified amino acid, as compared with one containing an otherwise identical unmodified amino acid.

[0141] *Approximately or About:* As used herein, the terms “approximately” or “about” may be applied to one or more values of interest, including a value that is similar to a stated reference value. In some aspects, the term “approximately” or “about” refers to a range of values that fall within $\pm 10\%$ (greater than or less than) of a stated reference value unless otherwise stated or otherwise evident from context (except where such number would exceed 100% of a possible value). For example, in some aspects, the term “approximately” or “about” may encompass a range of values that within 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, or less of a reference value.

[0142] *Associated:* As used herein, the term “associated” describes two events or entities as “associated” with one another, if the presence, level and/or form of one is correlated with that of the other. For example, a particular entity (e.g., polypeptide, genetic signature, metabolite, microbe, etc.) is considered to be associated with a particular disease, disorder, or condition, if its presence, level and/or form correlates with incidence of and/or susceptibility to the disease, disorder, or condition (e.g., across a relevant population). In some aspects, two or more entities are physically “associated” with one another if they interact, directly or indirectly, so that they are and/or remain in physical proximity with one another. In some aspects, two or more entities that are physically associated with one another are covalently linked to one another; in some aspects, two or more entities that are physically associated with one another are not covalently linked to one another but are non-covalently associated, for example by means of hydrogen bonds, van der Waals interaction, hydrophobic interactions, magnetism, and combinations thereof.

[0143] *Biologically active:* As used herein, the term “biologically active” refers to an observable biological effect or result achieved by an agent or entity of interest. For example, in some aspects, a specific binding interaction is a biological activity. In some aspects, modulation (e.g., induction, enhancement, or inhibition) of a biological pathway or event is a biological activity. In some aspects, presence or extent of a biological activity is assessed through detection of a direct or indirect product produced by a biological pathway or event of interest.

- [0144] ***Cell Selective Promoter:*** As used herein, the term "cell selective promoter" refers to a promoter that is predominately active in certain cell types (e.g., transcription of a specific gene occurs only within cells expressing transcription regulatory and/or control proteins that bind to the tissue-specific promoter). In some aspects, an inner ear supporting cell selective promoter is a promoter that is predominately active in one or more supporting cells of the inner ear.
- [0145] ***Characteristic portion:*** As used herein, the term "characteristic portion," in the broadest sense, refers to a portion of a substance whose presence (or absence) correlates with presence (or absence) of a particular feature, attribute, or activity of the substance. In some aspects, a characteristic portion of a substance is a portion that is found in a given substance and in related substances that share a particular feature, attribute or activity, but not in those that do not share the particular feature, attribute or activity. In some aspects, a characteristic portion shares at least one functional characteristic with the intact substance. For example, in some aspects, a "characteristic portion" of a protein or polypeptide is one that contains a continuous stretch of amino acids, or a collection of continuous stretches of amino acids, that together are characteristic of a protein or polypeptide. In some aspects, each such continuous stretch generally contains at least 2, 5, 10, 15, 20, 50, or more amino acids. In general, a characteristic portion of a substance (e.g., of a protein, antibody, etc.) is one that, in addition to a sequence and/or structural identity specified above, shares at least one functional characteristic with the relevant intact substance. In some aspects, a characteristic portion may be biologically active.
- [0146] ***Characteristic sequence:*** As used herein, the term "characteristic sequence" is a sequence that is found in all members of a family of polypeptides or nucleic acids, and therefore can be used by those of ordinary skill in the art to define members of the family.
- [0147] ***Characteristic sequence element:*** As used herein, the phrase "characteristic sequence element" refers to a sequence element found in a polymer (e.g., in a polypeptide or nucleic acid) that represents a characteristic portion of that polymer. In some aspects, presence of a characteristic sequence element correlates with presence or level of a particular activity or property of a polymer. In some aspects, presence (or absence) of a characteristic sequence element defines a particular polymer as a member (or not a member) of a particular family or group of such polymers. A characteristic sequence element typically comprises at least two monomers (e.g., amino acids or nucleotides). In some aspects, a characteristic sequence element includes at least 2, 3, 4, 5, 6, 7, 8, 9, 10,

11, 12, 13, 14, 15, 20, 25, 30, 35, 40, 45, 50, or more monomers (e.g., contiguously linked monomers). In some aspects, a characteristic sequence element includes at least first and second stretches of contiguous monomers spaced apart by one or more spacer regions whose length may or may not vary across polymers that share a sequence element.

[0148] ***Combination therapy:*** As used herein, the term “combination therapy” refers to those situations in which a subject is simultaneously exposed to two or more therapeutic regimens (e.g., two or more therapeutic agents). In some aspects, two or more agents may be administered simultaneously. In some aspects, two or more agents may be administered sequentially. In some aspects, two or more agents may be administered in overlapping dosing regimens.

[0149] ***Comparable:*** As used herein, the term “comparable” refers to two or more agents, entities, situations, sets of conditions, subjects, populations, etc., that may not be identical to one another but that are sufficiently similar to permit comparison therebetween so that one skilled in the art will appreciate that conclusions may reasonably be drawn based on differences or similarities observed. In some aspects, comparable sets of agents, entities, situations, sets of conditions, subjects, populations, etc. are characterized by a plurality of substantially identical features and one or a small number of varied features. Those of ordinary skill in the art will understand, in context, what degree of identity is required in any given circumstance for two or more such agents, entities, situations, sets of conditions, subjects, populations, etc. to be considered comparable. For example, those of ordinary skill in the art will appreciate that sets of agents, entities, situations, sets of conditions, subjects, populations, etc. are comparable to one another when characterized by a sufficient number and type of substantially identical features to warrant a reasonable conclusion that differences in results obtained or phenomena observed under or with different sets of circumstances, stimuli, agents, entities, situations, sets of conditions, subjects, populations, etc. are caused by or indicative of the variation in those features that are varied.

[0150] ***Construct:*** As used herein, the term “construct” refers to a composition including a polynucleotide capable of carrying at least one heterologous polynucleotide. In some aspects, a construct can be a plasmid, 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)) or a viral vector, capsid, viral particle and any Gateway® plasmids. A construct can, e.g.,

include sufficient cis-acting elements for expression; other elements for expression can be supplied by the host primate cell or in an in vitro expression system. A construct may include any genetic element (e.g., a plasmid, a transposon, a cosmid, an artificial chromosome, or a viral vector, capsid, viral particle etc.) that is capable of replicating when associated with proper control elements. Thus, in some aspects, “construct” may include a cloning and/or expression construct and/or a viral construct (e.g., an adeno-associated virus (AAV) construct, an adenovirus construct, a lentivirus construct, or a retrovirus construct).

[0151] *Conservative:* As used herein, the term “conservative” refers to instances describing a conservative amino acid substitution, including a substitution of an amino acid residue by another amino acid residue having a side chain R group with similar chemical properties (e.g., charge or hydrophobicity). In general, a conservative amino acid substitution will not substantially change functional properties of interest of a protein, for example, ability of a receptor to bind to a ligand. Examples of groups of amino acids that have side chains with similar chemical properties include: aliphatic side chains such as glycine (Gly, G), alanine (Ala, A), valine (Val, V), leucine (Leu, L), and isoleucine (Ile, I); aliphatic-hydroxyl side chains such as serine (Ser, S) and threonine (Thr, T); amide-containing side chains such as asparagine (Asn, N) and glutamine (Gln, Q); aromatic side chains such as phenylalanine (Phe, F), tyrosine (Tyr, Y), and tryptophan (Trp, W); basic side chains such as lysine (Lys, K), arginine (Arg, R), and histidine (His, H); acidic side chains such as aspartic acid (Asp, D) and glutamic acid (Glu, E); and sulfur-containing side chains such as cysteine (Cys, C) and methionine (Met, M). Conservative amino acids substitution groups include, for example, valine/leucine/isoleucine (Val/Leu/Ile, V/L/I), phenylalanine/tyrosine (Phe/Tyr, F/Y), lysine/arginine (Lys/Arg, K/R), alanine/valine (Ala/Val, A/V), glutamate/aspartate (Glu/Asp, E/D), and asparagine/glutamine (Asn/Gln, N/Q). In some aspects, a conservative amino acid substitution can be a substitution of any native residue in a protein with alanine, as used in, for example, alanine scanning mutagenesis. In some aspects, a conservative substitution is made that has a positive value in the PAM250 log-likelihood matrix disclosed in Gonnet et al., 1992, Science 256:1443-1445, which is incorporated herein by reference in its entirety. In some aspects, a substitution is a moderately conservative substitution wherein the substitution has a nonnegative value in the PAM250 log-likelihood matrix. One skilled in the art would appreciate that a change

(e.g., substitution, addition, deletion, etc.) of amino acids that are not conserved between the same protein from different species is less likely to have an effect on the function of a protein and therefore, these amino acids should be selected for mutation. Amino acids that are conserved between the same protein from different species should not be changed (e.g., deleted, added, substituted, etc.), as these mutations are more likely to result in a change in function of a protein. Exemplary conservative amino acid substitutions are shown in Table 1.

[0152] **Table 1.** Conservative Amino Acid Substitutions

CONSERVATIVE AMINO ACID SUBSTITUTIONS		
For Amino Acid	Code	Replace With
Alanine	A	D-ala, Gly, Aib, β -Ala, Acp, L-Cys, D-Cys
Arginine	R	D-Arg, Lys, D-Lys, homo-Arg, D-homo-Arg, Met, Ile, D-Met, D-Ile, Orn, D-Orn
Asparagine	N	D-Asn, Asp, D-Asp, Glu, D-Glu, Gln, D-Gln
Aspartic Acid	D	D-Asp, D-Asn, Asn, Glu, D-Glu, Gln, D-Gln
Cysteine	C	D-Cys, S-Me-Cys, Met, D-Met, Thr, D-Thr
Glutamine	Q	D-Gln, Asn, D-Asn, Glu, D-Glu, Asp, D-Asp
Glutamic Acid	E	D-Glu, D-Asp, Asp, Asn, D-Asn, Gln, D-Gln
Glycine	G	Ala, D-Ala, Pro, D-Pro, Aib, β -Ala, Acp
Isoleucine	I	D-Ile, Val, D-Val, AdaA, AdaG, Leu, D-Leu, Met, D-Met
Leucine	L	D-Leu, Val, D-Val, AdaA, AdaG, Leu, D-Leu, Met, D-Met
Lysine	K	D-Lys, Arg, D-Arg, homo-Arg, D-homo-Arg, Met, D-Met, Ile, D-Ile, Orn, D-Orn
Methionine	M	D-Met, S-Me-Cys, Ile, D-Ile, Leu, D-Leu, Val, D-Val
Phenylalanine	F	D-Phe, Tyr, D-Thr, L-Dopa, His, D-His, Trp, D-Trp, Trans-3,4 or 5-phenylproline, AdaA, AdaG, cis-3,4 or 5-phenylproline, Bpa, D-Bpa
Proline	P	D-Pro, L-I-thioazolidine-4-carboxylic acid, D-or-L-1-oxazolidine-4-carboxylic acid (Kauer, U.S. Pat. No. 4,511,390)
Serine	S	D-Ser, Thr, D-Thr, allo-Thr, Met, D-Met, Met (O), D-Met (O), L-Cys, D-Cys
Threonine	T	D-Thr, Ser, D-Ser, allo-Thr, Met, D-Met, Met (O), D-Met (O), Val, D-Val
Tyrosine	Y	D-Tyr, Phe, D-Phe, L-Dopa, His, D-His
Valine	V	D-Val, Leu, D-Leu, Ile, D-Ile, Met, D-Met, AdaA, AdaG

- [0153] **Control:** As used herein, the term “control” refers to the art-understood meaning of a “control” being a standard against which results are compared. Typically, controls are used to augment integrity in experiments by isolating variables in order to make a conclusion about such variables. In some aspects, a control is a reaction or assay that is performed simultaneously with a test reaction or assay to provide a comparator. For example, in one experiment, a “test” (i.e., a variable being tested) is applied. In a second experiment, a “control,” the variable being tested is not applied. In some aspects, a control is a historical control (e.g., of a test or assay performed previously, or an amount or result that is previously known). In some aspects, a control is or comprises a printed or otherwise saved record. In some aspects, a control is a positive control. In some aspects, a control is a negative control.
- [0154] **Determining, measuring, evaluating, assessing, assaying and analyzing:** As used herein, the terms “determining,” “measuring,” “evaluating,” “assessing,” “assaying,” and “analyzing” may be used interchangeably to refer to any form of measurement, and include determining if an element is present or not. These terms include both quantitative and/or qualitative determinations. Assaying may be relative or absolute. For example, in some aspects, “Assaying for the presence of” can be determining an amount of something present and/or determining whether or not it is present or absent.
- [0155] **Endogenous:** As used herein in reference to a substances or process refers to a naturally occurring substances or processes that originates from within a system such as an organism, tissue, or cell.
- [0156] **Engineered:** In general, as used herein, the term “engineered” refers to an aspect of having been manipulated by the hand of man. For example, a cell or organism is considered to be “engineered” if it has been manipulated so that its genetic information is altered (e.g., new genetic material not previously present has been introduced, for example by transformation, mating, somatic hybridization, transfection, transduction, or other mechanism, or previously present genetic material is altered or removed, for example by substitution or deletion mutation, or by mating protocols). As is common practice and is understood by those in the art, progeny of an engineered polynucleotide or cell are typically still referred to as “engineered” even though the actual manipulation was performed on a prior entity.
- [0157] **Excipient:** As used herein, the term “excipient” refers to an inactive (e.g., non-therapeutic) agent that may be included in a pharmaceutical composition, for example to

provide or contribute to a desired consistency or stabilizing effect. In some aspects, suitable pharmaceutical excipients may include, for example, starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like.

[0158] ***Expression:*** As used herein, the term “expression” of a nucleic acid sequence refers to generation of any gene product (e.g., transcript, e.g., mRNA, e.g., polypeptide, etc.) from a nucleic acid sequence. In some aspects, a gene product can be a transcript. In some aspects, a gene product can be a polypeptide. In some aspects, expression of a nucleic acid sequence involves one or more of the following: (1) production of an RNA template from a DNA sequence (e.g., by transcription); (2) processing of an RNA transcript (e.g., by splicing, editing, 5' cap formation, and/or 3' end formation); (3) translation of an RNA into a polypeptide or protein; and/or (4) post-translational modification of a polypeptide or protein.

[0159] ***Flanked:*** As used herein, the term "flanked" refers to a position relative to ends of a reference item. More specifically, in referring to reference nucleic acid sequence(s), “flanked” refers to having a sequences upstream and downstream of the reference nucleic acid sequence(s). In some aspects, a flanked referenced nucleic acid sequence has a first sequence or series of nucleotide residues positioned adjacent to the 5' end of the referenced nucleic acid and a second sequence or series of nucleotide residues positioned adjacent to the 3' end of the referenced nucleic acid. In some aspects, the upstream and/or downstream flanking sequences are immediately adjacent to the referenced nucleic acid sequence. In some aspects, there are intervening nucleic acids between the upstream and/or downstream flanking sequences and the referenced nucleic acid sequence.

[0160] ***Functional:*** As used herein, the term “functional” describes something that exists in a form in which it exhibits a property and/or activity by which it is characterized. For example, in some aspects, a “functional” biological molecule is a biological molecule in a form in which it exhibits a property and/or activity by which it is characterized. In some such aspects, a functional biological molecule is characterized relative to another biological molecule which is non-functional in that the “non-functional” version does not exhibit the same or equivalent property and/or activity as the “functional” molecule. A biological molecule may have one function, two functions (i.e., bifunctional) or many functions (i.e., multifunctional).

- [0161] **Gene:** As used herein, the term “gene” refers to a DNA sequence in a chromosome that codes for a gene product (e.g., an RNA product, e.g., a polypeptide product). In some aspects, a gene includes coding sequence (i.e., sequence that encodes a particular product). In some aspects, a gene includes non-coding sequence. In some particular aspects, a gene may include both coding (e.g., exonic) and non-coding (e.g., intronic) sequence. In some aspects, a gene may include one or more regulatory sequences (e.g., promoters, enhancers, etc.) and/or intron sequences that, for example, may control or impact one or more aspects of gene expression (e.g., cell-type-specific expression, inducible expression, etc.). As used herein, the term “gene” generally refers to a portion of a nucleic acid that encodes a polypeptide or fragment thereof; the term may optionally encompass regulatory sequences, as will be clear from context to those of ordinary skill in the art. This definition is not intended to exclude application of the term “gene” to non-protein-coding expression units but rather to clarify that, in most cases, the term as used in this document refers to a polypeptide-coding nucleic acid. In some aspects, a gene may encode a polypeptide, but that polypeptide may not be functional, e.g., a gene variant may encode a polypeptide that does not function in the same way, or at all, relative to the wild-type gene. In some aspects, a gene may encode a transcript which, in some aspects, may be toxic beyond a threshold level. In some aspects, a gene may encode a polypeptide, but that polypeptide may not be functional and/or may be toxic beyond a threshold level.
- [0162] **Hearing loss:** As used herein, the term “hearing loss” may be used to a partial or total inability of a living organism to hear. In some aspects, hearing loss may be acquired. In some aspects, hearing loss may be hereditary. In some aspects, hearing loss may be genetic. In some aspects, hearing loss may be as a result of disease or trauma (e.g., physical trauma, treatment with one or more agents resulting in hearing loss, etc.). In some aspects, hearing loss may be due to one or more known genetic causes and/or syndromes. In some aspects, hearing loss may be of unknown etiology. In some aspects, hearing loss may or may not be mitigated by use of hearing aids or other treatments.
- [0163] **Heterologous:** As used herein, the term “heterologous” the relationship between two or more nucleic acid or protein sequences that are derived from different sources. In some aspects, the promoter operably linked to the nucleic acid encoding the therapeutic protein may be derived from a different gene other than the gene encoding the therapeutic protein.

[0164] *Identity*: As used herein, the term “identity” refers to overall relatedness between polymeric molecules, e.g., between nucleic acid molecules (e.g., DNA molecules and/or RNA molecules) and/or between polypeptide molecules. In some aspects, polymeric molecules are considered to be “substantially identical” to one another if their sequences are at least 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 99% identical. Calculation of percent identity of two nucleic acid or polypeptide sequences, for example, can be performed by aligning two sequences for optimal comparison purposes (e.g., gaps can be introduced in one or both of a first and a second sequences for optimal alignment and non-identical sequences can be disregarded for comparison purposes). In some aspects, a length of a sequence aligned for comparison purposes is at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or substantially 100% of length of a reference sequence; nucleotides at corresponding positions are then compared. When a position in the first sequence is occupied by the same residue (e.g., nucleotide or amino acid) as a corresponding position in the second sequence, then the two molecules (i.e., first and second) are identical at that position. Percent identity between two sequences is a function of the number of identical positions shared by the two sequences being compared, taking into account the number of gaps, and the length of each gap, which needs to be introduced for optimal alignment of the two sequences. Comparison of sequences and determination of percent identity between two sequences can be accomplished using a mathematical algorithm. For example, percent identity between two nucleotide sequences can be determined using the algorithm of Meyers and Miller (CABIOS, 1989, 4: 11-17, which is herein incorporated by reference in its entirety), which has been incorporated into the ALIGN program (version 2.0). In some aspects, nucleic acid sequence comparisons made with the ALIGN program use a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4.

[0165] *Improve, increase, enhance, inhibit or reduce*: As used herein, the terms “improve,” “increase,” “enhance,” “inhibit,” “reduce,” or grammatical equivalents thereof, indicate values that are relative to a baseline or other reference measurement. In some aspects, a value is statistically significantly difference that a baseline or other reference measurement. In some aspects, an appropriate reference measurement may be or comprise a measurement in a particular system (e.g., in a single individual) under otherwise comparable conditions absent presence of (e.g., prior to and/or after) a

particular agent or treatment, or in presence of an appropriate comparable reference agent. In some aspects, an appropriate reference measurement may be or comprise a measurement in comparable system known or expected to respond in a particular way, in presence of the relevant agent or treatment. In some aspects, an appropriate reference is a negative reference; in some aspects, an appropriate reference is a positive reference.

[0166] ***Knockdown:*** As used herein, the term “knockdown” refers to a decrease in expression of one or more gene products. In some aspects, an inhibitory nucleic acid achieve knockdown. In some aspects, a genome editing system described herein achieves knockdown.

[0167] ***Knockout:*** As used herein, the term “knockout” refers to ablation of expression of one or more gene products. In some aspects, a genome editing system described herein achieve knockout.

[0001] ***Minimal Promoter:*** As used herein, the term “minimal promoter”, unless indicated otherwise, refers to a promoter that includes less than the full naturally occurring promoter sequence, which is still capable of directing transcription of a coding sequence (e.g., a heterogenous or homogenous coding sequence).

[0002] In some aspects, the minimal promoter can comprise one or more regions (including all regions) of the fully naturally occurring promoter that can direct transcription of a coding sequence.

[0003] In some aspects, the minimal promoter can comprise a portion or portions of the region(s) of the fully naturally occurring promoter that can direct transcription of a coding sequence.

[0004] ***microRNA:*** As used herein, the term “microRNA” or “miRNA” refers to a class of biomolecules involved in control of gene expression. A mature miRNA is typically an 18-25 nucleotide non-coding RNA that regulates expression of an mRNA including sequences complementary to the miRNA. These small RNA molecules are known to control gene expression by regulating the stability and/or translation of mRNAs. For example, miRNAs bind to the 3' UTR of target mRNAs and suppress translation. MiRNAs may also bind to target mRNAs and mediate gene silencing through the RNAi pathway. MiRNAs may also regulate gene expression by causing chromatin condensation.

[0005] In some aspects, a microRNA is between about 10 nucleotides to about 30 nucleotides in length (e.g., about 10 nucleotides to about 28 nucleotides, about 10

nucleotides to about 26 nucleotides, about 10 nucleotides to about 24 nucleotides, about 10 nucleotides to about 22 nucleotides, about 10 nucleotides to about 20 nucleotides, about 10 nucleotides to about 18 nucleotides, about 10 nucleotides to about 16 nucleotides, about 10 nucleotides to about 14 nucleotides, about 10 nucleotides to about 12 nucleotides, about 12 nucleotides to about 30 nucleotides, about 12 nucleotides to about 28 nucleotides, about 12 nucleotides to about 26 nucleotides, about 12 nucleotides to about 24 nucleotides, about 12 nucleotides to about 22 nucleotides, about 12 nucleotides to about 20 nucleotides, about 12 nucleotides to about 18 nucleotides, about 12 nucleotides to about 16 nucleotides, about 12 nucleotides to about 14 nucleotides, about 16 nucleotides to about 30 nucleotides, about 16 nucleotides to about 28 nucleotides, about 16 nucleotides to about 26 nucleotides, about 16 nucleotides to about 24 nucleotides, about 16 nucleotides to about 22 nucleotides, about 16 nucleotides to about 20 nucleotides, about 16 nucleotides to about 18 nucleotides, about 18 nucleotides to about 30 nucleotides, about 18 nucleotides to about 28 nucleotides, about 18 nucleotides to about 26 nucleotides, about 18 nucleotides to about 24 nucleotides, about 18 nucleotides to about 22 nucleotides, about 18 nucleotides to about 20 nucleotides, about 20 nucleotides to about 30 nucleotides, about 20 nucleotides to about 28 nucleotides, about 20 nucleotides to about 26 nucleotides, about 20 nucleotides to about 24 nucleotides, about 20 nucleotides to about 22 nucleotides, about 22 nucleotides to about 30 nucleotides, about 22 nucleotides to about 28 nucleotides, about 22 nucleotides to about 26 nucleotides, about 22 nucleotides to about 24 nucleotides, about 24 nucleotides to about 30 nucleotides, about 24 nucleotides to about 28 nucleotides, about 24 nucleotides to about 26 nucleotides, about 26 nucleotides to about 30 nucleotides, about 26 nucleotides to about 28 nucleotides, about 28 nucleotides to about 30 nucleotides, or 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30 nucleotides).

[0006] *microRNA regulatory target site*: As used herein, the term "microRNA regulatory target site" or "miRTS" refers to a sequence that directly interacts with a miRNA on the mRNA transcript. Often, the miRTS is present in the 3' untranslated region (UTR) of the mRNA, but it may also be present in the coding sequence, or in the 5' UTR. miRTS are not necessarily perfect complements to miRNAs, usually having only a few bases of complementarity to the miRNA, and often containing one or more mismatches. The miRTS may be any sequence capable of being bound by a miRNA sufficiently that the

translation of a gene to which the miRTS is operably linked is repressed by a miRNA silencing mechanism such as the RISC. In some aspects, inclusion of a miRTS into a nucleic acid construct comprising a polynucleotide (e.g., a therapeutic polynucleotide) can result in degradation of the therapeutic polynucleotide after transcription.

[0007] ***Nucleic acid:*** As used herein, the term “nucleic acid”, in its broadest sense, refers to any compound and/or substance that is or can be incorporated into an oligonucleotide chain. In some aspects, a nucleic acid is a compound and/or substance that is or can be incorporated into an oligonucleotide chain via a phosphodiester linkage. As will be clear from context, in some aspects, “nucleic acid” refers to an individual nucleic acid residue (e.g., a nucleotide and/or nucleoside); in some aspects, “nucleic acid” refers to an oligonucleotide chain comprising individual nucleic acid residues. In some aspects, a “nucleic acid” is or comprises RNA; in some aspects, a “nucleic acid” is or comprises DNA. In some aspects, a nucleic acid is, comprises, or consists of one or more natural nucleic acid residues. In some aspects, a nucleic acid is, comprises, or consists of one or more nucleic acid analogs. In some aspects, a nucleic acid analog differs from a nucleic acid in that it does not utilize a phosphodiester backbone. Alternatively or additionally, in some aspects, a nucleic acid has one or more phosphorothioate and/or 5'-N-phosphoramidite linkages rather than phosphodiester bonds. In some aspects, a nucleic acid is, comprises, or consists of one or more natural nucleosides (e.g., adenosine, thymidine, guanosine, cytidine, uridine, deoxyadenosine, deoxythymidine, deoxyguanosine, and deoxycytidine). In some aspects, a nucleic acid is, comprises, or consists of one or more nucleoside analogs (e.g., 2-aminoadenosine, 2-thiothymidine, inosine, pyrrolo-pyrimidine, 3-methyl adenosine, 5-methylcytidine, C-5 propynyl-cytidine, C-5 propynyl-uridine, 2-aminoadenosine, C5-bromouridine, C5-fluorouridine, C5-iodouridine, C5-propynyl-uridine, C5-propynyl-cytidine, C5-methylcytidine, 2-aminoadenosine, 7-deazaadenosine, 7-deazaguanosine, 8-oxoadenosine, 8-oxoguanosine, 0(6)-methylguanine, 2-thiocytidine, methylated bases, intercalated bases, and combinations thereof). In some aspects, a nucleic acid comprises one or more modified sugars (e.g., 2'-fluororibose, ribose, 2'-deoxyribose, arabinose, and hexose) as compared with those in natural nucleic acids. In some aspects, a nucleic acid has a nucleotide sequence that encodes a functional gene product such as an RNA or protein. In some aspects, a nucleic acid includes one or more introns. In some aspects, nucleic acids are prepared by one or more of isolation from a natural source, enzymatic synthesis by

polymerization based on a complementary template (in vivo or in vitro), reproduction in a recombinant cell or system, and chemical synthesis. In some aspects, a nucleic acid is at least 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500, 600, 700, 800, 900, 1000, 1500, 2000, 2500, 3000, 3500, 4000, 4500, 5000 or more residues long. In some aspects, a nucleic acid is partly or wholly single stranded; in some aspects, a nucleic acid is partly or wholly double stranded. In some aspects, a nucleic acid has a nucleotide sequence comprising at least one element that encodes, or is complementary to a sequence that encodes, a polypeptide. In some aspects, a nucleic acid has enzymatic activity.

[0008] ***Operably linked:*** As used herein, refers to a juxtaposition wherein the components described are in a relationship permitting them to function in their intended manner. A control element “operably linked” to a functional element is associated in such a way that expression and/or activity of the functional element is achieved under conditions compatible with the control element. In some aspects, “operably linked” control elements are contiguous (e.g., covalently linked) with coding elements of interest; in some aspects, control elements act in trans to or otherwise at a distance from the functional element of interest. In some aspects, “operably linked” refers to functional linkage between a regulatory sequence and a heterologous nucleic acid sequence resulting in expression of the latter. For example, a first nucleic acid sequence is operably linked with a second nucleic acid sequence when the first nucleic acid sequence is placed in a functional relationship with the second nucleic acid sequence. In some aspects, for example, a functional linkage may include transcriptional control. For instance, a promoter is operably linked to a coding sequence if the promoter affects the transcription or expression of the coding sequence. Operably linked DNA sequences can be contiguous with each other and, e.g., where necessary to join two protein coding regions, are in the same reading frame.

[0009] ***Pharmaceutical composition:*** As used herein, the term “pharmaceutical composition” refers to a composition in which an active agent is formulated together with one or more pharmaceutically acceptable carriers. In some aspects, an active agent is present in unit dose amount appropriate for administration in a therapeutic regimen that shows a statistically significant probability of achieving a predetermined therapeutic effect when administered to a relevant population. In some aspects, a pharmaceutical composition may be specially formulated for administration in solid or liquid form,

including those adapted for, e.g., administration, for example, an injectable formulation that is, e.g., an aqueous or non-aqueous solution or suspension or a liquid drop designed to be administered into an ear canal. In some aspects, a pharmaceutical composition may be formulated for administration via injection either in a particular organ or compartment, e.g., directly into an ear, or systemic, e.g., intravenously. In some aspects, a formulation may be or comprise drenches (aqueous or non-aqueous solutions or suspensions), tablets, boluses, powders, granules, pastes, capsules, powders, etc. In some aspects, an active agent may be or comprise an isolated, purified, or pure compound.

[0010] ***Pharmaceutically acceptable:*** As used herein, the term “pharmaceutically acceptable” which, for example, may be used in reference to a carrier, diluent, or excipient used to formulate a pharmaceutical composition as disclosed herein, means that a carrier, diluent, or excipient is compatible with other ingredients of a composition and not deleterious to a recipient thereof.

[0011] ***Pharmaceutically acceptable carrier:*** As used herein, the term “pharmaceutically acceptable carrier” means a pharmaceutically-acceptable material, composition or vehicle, such as a liquid or solid filler, diluent, excipient, or solvent encapsulating material, involved in carrying or transporting a subject compound from one organ, or portion of a body, to another organ, or portion of a body. Each carrier must be is “acceptable” in the sense of being compatible with other ingredients of a formulation and not injurious to a patient. Some examples of materials which can serve as pharmaceutically-acceptable carriers include: sugars, such as lactose, glucose and sucrose; starches, such as corn starch and potato starch; cellulose, and its derivatives, such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; powdered tragacanth; malt; gelatin; talc; excipients, such as cocoa butter and suppository waxes; oils, such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil; glycols, such as propylene glycol; polyols, such as glycerin, sorbitol, mannitol and polyethylene glycol; esters, such as ethyl oleate and ethyl laurate; agar; buffering agents, such as magnesium hydroxide and aluminum hydroxide; alginic acid; pyrogen-free water; isotonic saline; Ringer’s solution; ethyl alcohol; pH buffered solutions; polyesters, polycarbonates and/or polyanhydrides; and other non-toxic compatible substances employed in pharmaceutical formulations.

[0012] ***Polyadenylation:*** 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. In some aspects, a 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, a poly(A) tail can be added onto transcripts that contain a specific sequence, the polyadenylation signal or "poly(A) sequence." A poly(A) tail and proteins bound to it aid in protecting mRNA from degradation by exonucleases. Polyadenylation can be affect transcription termination, export of the mRNA from the nucleus, and translation. Typically, polyadenylation occurs in the nucleus immediately after transcription of DNA into RNA, but additionally can also occur later in the cytoplasm. After transcription has been terminated, the mRNA chain can be cleaved through the action of an endonuclease complex associated with RNA polymerase. The cleavage site can be characterized by the presence of the base sequence AAUAAA near the cleavage site. After mRNA has been cleaved, adenosine residues can be added to the free 3' end at the cleavage site. As used herein, a "poly(A) sequence" is a sequence that triggers the endonuclease cleavage of an mRNA and the additional of a series of adenosines to the 3' end of the cleaved mRNA.

[0013] ***Polypeptide:*** As used herein, the term "polypeptide" refers to any polymeric chain of residues (e.g., amino acids) that are typically linked by peptide bonds. In some aspects, a polypeptide has an amino acid sequence that occurs in nature. In some aspects, a polypeptide has an amino acid sequence that does not occur in nature. In some aspects, a polypeptide has an amino acid sequence that is engineered in that it is designed and/or produced through action of the hand of man. In some aspects, a polypeptide may comprise or consist of natural amino acids, non-natural amino acids, or both. In some aspects, a polypeptide may include one or more pendant groups or other modifications, e.g., modifying or attached to one or more amino acid side chains, at a polypeptide's N-terminus, at a polypeptide's C-terminus, or any combination thereof. In some aspects, such pendant groups or modifications may be acetylation, amidation, lipidation, methylation, pegylation, etc., including combinations thereof. In some aspects, polypeptides may contain L-amino acids, D-amino acids, or both and may contain any of a variety of amino acid modifications or analogs known in the art. In some aspects, useful modifications may be or include, e.g., terminal acetylation, amidation, methylation, etc. In some aspects, a protein may comprise natural amino acids, non-natural amino acids, synthetic amino acids, and combinations thereof. The term "peptide" is generally

used to refer to a polypeptide having a length of less than about 100 amino acids, less than about 50 amino acids, less than 20 amino acids, or less than 10 amino acids. In some aspects, a polypeptide can be a therapeutic polypeptide (e.g., a Connexin 26 polypeptide). In some aspects, a polypeptide can be a supporting cell polypeptide (e.g., a Connexin 26 polypeptide). In some aspects, a polypeptide can be a reporter polypeptide.

[0014] *Polynucleotide:* As used herein, the term “polynucleotide” refers to any polymeric chain of nucleic acids. In some aspects, a polynucleotide is or comprises RNA; in some aspects, a polynucleotide is or comprises DNA. In some aspects, a polynucleotide is, comprises, or consists of one or more natural nucleic acid residues. In some aspects, a polynucleotide is, comprises, or consists of one or more nucleic acid analogs. In some aspects, a polynucleotide analog differs from a nucleic acid in that it does not utilize a phosphodiester backbone. Alternatively or additionally, in some aspects, a polynucleotide has one or more phosphorothioate and/or 5'-N-phosphoramidite linkages rather than phosphodiester bonds. In some aspects, a polynucleotide is, comprises, or consists of one or more natural nucleosides (e.g., adenosine, thymidine, guanosine, cytidine, uridine, deoxyadenosine, deoxythymidine, deoxy guanosine, and deoxycytidine). In some aspects, a polynucleotide is, comprises, or consists of one or more nucleoside analogs (e.g., 2-aminoadenosine, 2-thiothymidine, inosine, pyrrolo-pyrimidine, 3-methyl adenosine, 5-methylcytidine, C-5 propynyl-cytidine, C-5 propynyl-uridine, 2-aminoadenosine, C5-bromouridine, C5-fluorouridine, C5-iodouridine, C5-propynyl-uridine, C5-propynyl-cytidine, C5-methylcytidine, 2-aminoadenosine, 7-deazaadenosine, 7-deazaguanosine, 8-oxoadenosine, 8-oxoguanosine, 0(6)-methylguanine, 2-thiocytidine, methylated bases, intercalated bases, and combinations thereof). In some aspects, a polynucleotide comprises one or more modified sugars (e.g., 2'-fluororibose, ribose, 2'-deoxyribose, arabinose, and hexose) as compared with those in natural nucleic acids. In some aspects, a polynucleotide has a nucleotide sequence that encodes a functional gene product such as an RNA or protein. In some aspects, a polynucleotide includes one or more introns. In some aspects, a polynucleotide is prepared by one or more of isolation from a natural source, enzymatic synthesis by polymerization based on a complementary template (in vivo or in vitro), reproduction in a recombinant cell or system, and chemical synthesis. In some aspects, a polynucleotide is at least 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 225, 250, 275, 300, 325, 350,

375, 400, 425, 450, 475, 500, 600, 700, 800, 900, 1000, 1500, 2000, 2500, 3000, 3500, 4000, 4500, 5000 or more residues long. In some aspects, a polynucleotide is partly or wholly single stranded; in some aspects, a polynucleotide is partly or wholly double stranded. In some aspects, a polynucleotide has a nucleotide sequence comprising at least one element that encodes, or is the complement of a sequence that encodes, a polypeptide. In some aspects, a polynucleotide has enzymatic activity.

[0015] ***Promoter:*** As used herein, the term "promoter" refers to a nucleic acid sequence that functions to control the transcription of one or more coding sequences (e.g., a gene or transgene, e.g., encoding a polypeptide (e.g., a therapeutic polypeptide), located upstream with respect to the direction of transcription of the transcription initiation site of the coding sequence. In some aspects, the promoter is structurally identified by the presence of a binding site for DNA-dependent RNA polymerase, transcription initiation sites or other DNA sequence (e.g., a transcription factor binding site, a repressor and/or activator protein binding site, or other sequences of nucleotides that act directly or indirectly to regulate the amount of transcription from the promoter). In some aspects, the promoter can comprise a naturally occurring promoter sequence, a functional fragment thereof, or a mutant of the naturally occurring promoter sequence or a functional fragment thereof.

[0016] ***Protein:*** As used herein, the term "protein" refers to a polypeptide (i.e., a string of at least two amino acids linked to one another by peptide bonds). Proteins may include moieties other than amino acids (e.g., may be glycoproteins, proteoglycans, etc.) and/or may be otherwise processed or modified. Those of ordinary skill in the art will appreciate that a "protein" can be a complete polypeptide chain as produced by a cell (with or without a signal sequence), or can be a characteristic portion thereof. Those of ordinary skill will appreciate that a protein can sometimes include more than one polypeptide chain, for example linked by one or more disulfide bonds or associated by other means.

[0017] ***Recombinant:*** As used herein, the term "recombinant" is intended to refer to polypeptides that are designed, engineered, prepared, expressed, created, manufactured, and/or isolated by recombinant means, such as polypeptides expressed using a recombinant expression construct transfected into a host cell; polypeptides isolated from a recombinant, combinatorial human polypeptide library; polypeptides isolated from an animal (e.g., a mouse, rabbit, sheep, fish, etc.) that is transgenic for or otherwise has been manipulated to express a gene or genes, or gene components that encode and/or direct expression of the polypeptide or one or more component(s), portion(s), element(s), or

domain(s) thereof; and/or polypeptides prepared, expressed, created or isolated by any other means that involves splicing or ligating selected nucleic acid sequence elements to one another, chemically synthesizing selected sequence elements, and/or otherwise generating a nucleic acid that encodes and/or directs expression of a polypeptide or one or more component(s), portion(s), element(s), or domain(s) thereof. In some aspects, one or more of such selected sequence elements is found in nature. In some aspects, one or more of such selected sequence elements is designed in silico. In some aspects, one or more such selected sequence elements results from mutagenesis (e.g., in vivo or in vitro) of a known sequence element, e.g., from a natural or synthetic source such as, for example, in the germline of a source organism of interest (e.g., of a human, a mouse, etc).

[0018] *Reference:* As used herein, the term “reference” describes a standard or control relative to which a comparison is performed. For example, in some aspects, an agent, animal, individual, population, sample, sequence or value of interest is compared with a reference or control agent, animal, individual, population, sample, sequence or value. In some aspects, a reference or control is tested and/or determined substantially simultaneously with the testing or determination of interest. In some aspects, a reference or control is a historical reference or control, optionally embodied in a tangible medium. Typically, as would be understood by those skilled in the art, a reference or control is determined or characterized under comparable conditions or circumstances to those under assessment. Those skilled in the art will appreciate when sufficient similarities are present to justify reliance on and/or comparison to a particular possible reference or control. In some aspects, a reference is a negative control reference; in some aspects, a reference is a positive control reference. In some aspects, the reference can be a compound, a protein, a polypeptide, or a polynucleotide disclosed in the present disclosure.

[0019] *Regulatory Element:* As used herein, the term “regulatory element” or “regulatory sequence” refers to non-coding regions of DNA that regulate, in some way, expression of one or more particular genes. In some aspects, such genes are apposed or “in the neighborhood” of a given regulatory element. In some aspects, such genes are located quite far from a given regulatory element. In some aspects, a regulatory element impairs or enhances transcription of one or more genes. In some aspects, a regulatory element may be located in cis to a gene being regulated. In some aspects, a regulatory element may be located in trans to a gene being regulated. For example, in some aspects,

a regulatory sequence refers to a nucleic acid sequence which is regulates expression of a gene product operably linked to a regulatory sequence. In some such aspects, this sequence may be an enhancer sequence and other regulatory elements which regulate expression of a gene product.

[0020] *Sample:* As used herein, the term “sample” typically refers to an aliquot of material obtained or derived from a source of interest. In some aspects, a source of interest is a biological or environmental source. In some aspects, a source of interest may be or comprise a cell or an organism, such as a microbe (e.g., virus), a plant, or an animal (e.g., a human). In some aspects, a source of interest is or comprises biological tissue or fluid. In some aspects, a biological tissue or fluid may be or comprise amniotic fluid, aqueous humor, ascites, bile, bone marrow, blood, breast milk, cerebrospinal fluid, cerumen, chyle, chime, ejaculate, endolymph, exudate, feces, gastric acid, gastric juice, lymph, mucus, pericardial fluid, perilymph, peritoneal fluid, pleural fluid, pus, rheum, saliva, sebum, semen, serum, smegma, sputum, synovial fluid, sweat, tears, urine, vaginal secretions, vitreous humour, vomit, and/or combinations or component(s) thereof. In some aspects, a biological fluid may be or comprise an intracellular fluid, an extracellular fluid, an intravascular fluid (blood plasma), an interstitial fluid, a lymphatic fluid, and/or a transcellular fluid. In some aspects, a biological fluid may be or comprise a plant exudate. In some aspects, a biological tissue or sample may be obtained, for example, by aspirate, biopsy (e.g., fine needle or tissue biopsy), swab (e.g., oral, nasal, skin, or vaginal swab), scraping, surgery, washing or lavage (e.g., bronchioalveolar, ductal, nasal, ocular, oral, uterine, vaginal, or other washing or lavage). In some aspects, a biological sample is or comprises cells obtained from an individual. In some aspects, a sample is a “primary sample” obtained directly from a source of interest by any appropriate means. In some aspects, as will be clear from context, the term “sample” refers to a preparation that is obtained by processing (e.g., by removing one or more components of and/or by adding one or more agents to) a primary sample. For example, filtering using a semi-permeable membrane. Such a “processed sample” may comprise, for example nucleic acids or proteins extracted from a sample or obtained by subjecting a primary sample to one or more techniques such as amplification or reverse transcription of nucleic acid, isolation and/or purification of certain components, etc.

[0021] *Selective expression:* As used herein, the term "selective expression" or "selectively expresses" refers to expression of a gene or polypeptide of interest

predominately in certain specific cell types (e.g., inner ear cells, e.g., inner ear supporting cells).

[0022] ***Subject***: As used herein, the term “subject” refers to an organism, typically a mammal (e.g., a human, in some aspects including prenatal human forms). In some aspects, a subject is suffering from a relevant disease, disorder or condition. In some aspects, a subject is susceptible to a disease, disorder, or condition. In some aspects, a subject displays one or more symptoms or characteristics of a disease, disorder or condition. In some aspects, a subject does not display any symptom or characteristic of a disease, disorder, or condition. In some aspects, a subject is someone with one or more features characteristic of susceptibility to or risk of a disease, disorder, or condition. In some aspects, a subject is a patient. In some aspects, a subject is an individual to whom diagnosis and/or therapy is and/or has been administered.

[0023] ***Substantially***: As used herein, the term “substantially” refers to a qualitative condition of exhibiting total or near-total extent or degree of a characteristic or property of interest. One of ordinary skill in the art will understand that biological and chemical phenomena rarely, if ever, go to completion and/or proceed to completeness or achieve or avoid an absolute result. The term “substantially” is therefore used herein to capture a potential lack of completeness inherent in many biological and chemical phenomena.

[0024] ***Supporting cell***: As used herein, the term "support cell," "supporting cell," "inner ear support cell," or "inner ear supporting cell" refers to cells of the inner ear that maintain the structure of the inner ear and maintain the environment of the sensory epithelium of the inner ear. In some aspects, inner ear supporting cells include, but are not limited to, inner phalangeal cells/border cells (IPhC), inner pillar cells (IPC), outer pillar cells (OPC), Deiters' cells rows 1 and 2 (DC1/2), Deiters' cells row 3 (DC3), Hensen's cells (Hec), Claudius cells/outer sulcus cells (CC/OSC), interdental cells (Idc), inner sulcus cells (ISC), Kölliker's organ cells (KO), greater ridge epithelial ridge cells (GER) (including lateral greater epithelial ridge cells (LGER)), and OC90+ cells (OC90), fibroblasts, and other cells of the lateral wall.

[0025] ***Supporting cell polypeptide***: As used herein, the term "supporting cell polypeptide" or "support cell polypeptide" refers to a polypeptide that is endogenously expressed in a supporting cell of the inner ear.

[0026] ***Reporter polypeptide***: As used herein, the term "reporter polypeptide" refers to a polypeptide that confers onto an organism or cell, a detectable or selectable phenotype.

The detectable phenotype can be colorimetric, fluorescent or luminescent, for example. Reporter polypeptides can include enzymes mediating luminescence reactions (luxA, luxB, luxAB, luc, ruc, nluc), enzymes mediating colorimetric reactions (lacZ, HRP), fluorescent proteins (GFP, eGFP, YFP, RFP, CFP, BFP, mCherry, near-infrared fluorescent proteins), affinity peptides (His-tag, 3X-FLAG), and selectable markers (ampC, tet(M), CAT, erm). The reporter polypeptide can be used as a marker for successful uptake of a nucleic acid molecule or exogenous sequence (plasmid) into a cell. The reporter polypeptide can also be used to indicate the presence of a target gene, target nucleic acid molecule, target polypeptide, target intracellular molecule, or a cell, as described herein.

[0027] ***Therapeutic polypeptide:*** As used herein, the term "therapeutic polypeptide" refers to a polypeptide possessing biological activity that can be used for the prevention and/or treatment of disease (e.g., hearing loss). Examples of therapeutic polypeptides include those capable of preventing, inhibiting, stabilizing or reversing an inherited or noninherited genetic defect in metabolism, immune regulation, hormonal regulation, enzymatic or membrane associated structural function. For example, therapeutic protein can replace an absent or defective cellular protein or enzyme, or supplement production of a defective or low expressed cellular protein or enzyme

[0028] ***Treatment:*** As used herein, the term "treatment" (also "treat" or "treating") refers to any administration of a therapy that partially or completely alleviates, ameliorates, eliminates, reverses, relieves, inhibits, delays onset of, reduces severity of, and/or reduces incidence of one or more symptoms, features, and/or causes of a particular disease, disorder, and/or condition. In some aspects, such treatment may be of a subject who does not exhibit signs of the relevant disease, disorder and/or condition and/or of a subject who exhibits only early signs of the disease, disorder, and/or condition. Alternatively, or additionally, such treatment may be of a subject who exhibits one or more established signs of the relevant disease, disorder and/or condition. In some aspects, treatment may be of a subject who has been diagnosed as suffering from the relevant disease, disorder, and/or condition. In some aspects, treatment may be of a subject known to have one or more susceptibility factors that are statistically correlated with increased risk of development of a given disease, disorder, and/or condition.

[0029] ***Variant:*** As used herein, the term "variant" refers to a version of something, e.g., a gene sequence, that is different, in some way, from another version. To determine if

something is a variant, a reference version is typically chosen and a variant is different relative to that reference version. In some aspects, a variant can have the same or a different (e.g., increased or decreased) level of activity or functionality than a wild type sequence. For example, in some aspects, a variant can have improved functionality as compared to a wild-type sequence if it is, e.g., codon-optimized to resist degradation, e.g., by an inhibitory nucleic acid, e.g., miRNA. Such a variant is referred to herein as a gain-of-function variant. In some aspects, a variant has a reduction or elimination in activity or functionality or a change in activity that results in a negative outcome (e.g., increased electrical activity resulting in chronic depolarization that leads to cell death). Such a variant is referred to herein as a loss-of-function variant. In some aspects, a gain-of-function variant is a codon-optimized sequence which encodes a transcript or polypeptide that may have improved properties (e.g., less susceptibility to degradation, e.g., less susceptibility to miRNA mediated degradation) than its corresponding wild type (e.g., non-codon optimized) version. In some aspects, a loss-of-function variant has one or more changes that result in a transcript or polypeptide that is defective in some way (e.g., decreased function, non-functioning) relative to the wild type transcript and/or polypeptide.

DETAILED DESCRIPTION

- [0030] In certain aspects, the present disclosure is directed to promoters for selective transgene expression, e.g., preferential expression in inner ear supporting cells.
- [0031] In some aspects, the present disclosure is directed to constructs comprising a polynucleotide encoding a therapeutic polypeptide (e.g., a Connexin 26 polypeptide) and compositions comprising the same which are designed for selective transgene expression, e.g., preferential expression in inner ear supporting cells and/or reduced expression in other inner ear cells such as hair cells.
- [0032] In some aspects, the present disclosure is also directed to constructs comprising a polynucleotide encoding a polypeptide and compositions comprising the same which are designed for selective transgene expression, e.g., preferential expression in inner ear supporting cells and/or reduced expression in other inner ear cells such as hair cells.
- [0033] In some aspects, the present disclosure is directed to constructs comprising a polynucleotide encoding a therapeutic polypeptide (e.g., a Connexin 26 polypeptide) and compositions comprising the same which are designed for transgene expression in inner ear supporting cells, e.g., preferential expression in inner ear supporting cells and/or

reduced expression in other inner ear cells such as hair cells. In some aspects, the preferential expression and/or reduced expression is relative to the corresponding endogenous expression.

[0034] In some aspects, the present disclosure is directed to AAV particles comprising the promoters or constructs disclosed herein.

[0035] In some aspects, the present disclosure is directed to methods of using the promoters, constructs, and AAV particles disclosed herein for treating hearing loss.

Hearing Loss

[0036] Generally, an ear can be described as including: an outer ear, middle ear, inner ear, hearing (acoustic) nerve, and auditory system (which processes sound as it travels from the ear to the brain). In addition to detecting sound, ears also help to maintain balance. Thus, in some aspects, disorders of the inner ear can cause hearing loss, tinnitus, vertigo, imbalance, or combinations thereof.

[0037] Hearing loss can be the result of genetic factors, environmental factors, or a combination of genetic and environmental factors. About half of all people who have tinnitus--phantom noises in their auditory system (ringing, buzzing, chirping, humming, or beating)--also have an over-sensitivity to/reduced tolerance for certain sound frequency and volume ranges, known as hyperacusis (also spelled hyperacousis). A variety of nonsyndromic and syndromic-related hearing losses will be known to those of skill in the art (e.g., DFNB1 and DFNA3; and Bart-Pumphrey syndrome, hystrix-like ichthyosis with deafness (HID), palmoplantar keratoderma with deafness, keratitis-ichthyosis-deafness (KID) syndrome and Vohwinkel syndrome, respectively). Environmental causes of hearing impairment or loss may include, e.g., certain medications, specific infections before or after birth, and/or exposure to loud noise over an extended period. In some aspects, hearing loss can result from noise, ototoxic agents, presbycusis, disease, infection or cancers that affect specific parts of the ear. In some aspects, ischemic damage can cause hearing loss via pathophysiological mechanisms. In some aspects, intrinsic abnormalities, like congenital mutations to genes that play an important role in cochlear anatomy or physiology, or genetic or anatomical changes in supporting and/or hair cells can be responsible for or contribute to hearing loss.

[0038] Hearing loss and/or deafness is one of the most common human sensory deficits, and can occur for many reasons. In some aspects, a subject may be born with hearing loss or without hearing, while others may lose hearing slowly over time. Approximately

36 million American adults report some degree of hearing loss, and one in three people older than 60 and half of those older than 85 experience hearing loss. Approximately 1.5 in 1,000 children are born with profound hearing loss, and another two to three per 1,000 children are born with partial hearing loss (Smith et al., 2005, Lancet 365:879-890, which is incorporated in its entirety herein by reference). More than half of these cases are attributed to a genetic basis (Di Domenico, et al., 2011, J. Cell. Physiol. 226:2494-2499, which is incorporated in its entirety herein by reference).

[0039] Treatments for hearing loss currently consist of hearing amplification for mild to severe losses and cochlear implantation for severe to profound losses (Kral and O'Donoghue, 2010, N. Engl. J. Med. 363:1438-1450, which is incorporated in its entirety herein by reference). Recent research in this arena has focused on cochlear hair cell regeneration, applicable to the most common forms of hearing loss, including presbycusis, noise damage, infection, and ototoxicity. There remains a need for effective treatments, such as gene therapy, which can repair and/or mitigate a source of a hearing problem (see e.g., WO 2018/039375, WO 2019/165292, and PCT filing application US2019/060328, each of which is incorporated in its entirety herein by reference).

[0040] In some aspects, nonsyndromic hearing loss and/or deafness is not associated with other signs and symptoms. In some aspects, syndromic hearing loss and/or deafness occurs in conjunction with abnormalities in other parts of the body. Approximately 70 percent to 80 percent of genetic hearing loss and/or deafness cases are nonsyndromic; remaining cases are often caused by specific genetic syndromes. Nonsyndromic deafness and/or hearing loss can have different patterns of inheritance, and can occur at any age. Types of nonsyndromic deafness and/or hearing loss are generally named according to their inheritance patterns. For example, autosomal dominant forms are designated DFNA, autosomal recessive forms are DFNB, and X-linked forms are DFN. Each type is also numbered in the order in which it was first described. For example, DFNA1 was the first described autosomal dominant type of nonsyndromic deafness. Between 75 percent and 80 percent of genetically causative hearing loss and/or deafness cases are inherited in an autosomal recessive pattern, which means both copies of the gene in each cell have mutations. Usually, each parent of an individual with autosomal recessive hearing loss and/or deafness is a carrier of one copy of the mutated gene, but is not affected by this form of hearing loss. Another 20 percent to 25 percent of nonsyndromic hearing loss and/or deafness cases are autosomal dominant, which means one copy of the altered gene

in each cell is sufficient to result in deafness and/or hearing loss. People with autosomal dominant deafness and/or hearing loss most often inherit an altered copy of the gene from a parent who is deaf and/or has hearing loss. Between 1 to 2 percent of cases of deafness and/or hearing loss show an X-linked pattern of inheritance, which means the mutated gene responsible for the condition is located on the X chromosome (one of the two sex chromosomes). Males with X-linked nonsyndromic hearing loss and/or deafness tend to develop more severe hearing loss earlier in life than females who inherit a copy of the same gene mutation. A characteristic of X-linked inheritance is that fathers cannot pass X-linked traits to their sons. Mitochondrial nonsyndromic deafness, which results from changes to mitochondrial DNA, occurs in less than one percent of cases in the United States. The altered mitochondrial DNA is passed from a mother to all of her sons and daughters. This type of deafness is not inherited from fathers. The causes of syndromic and nonsyndromic deafness and/or hearing loss are complex. Researchers have identified more than 30 genes that, when altered, are associated with syndromic and/or nonsyndromic deafness and/or hearing loss; however, some of these genes have not been fully characterized. Different mutations in the same gene can be associated with different types of deafness and/or hearing loss, and some genes are associated with both syndromic and nonsyndromic deafness and/or hearing loss.

[0041] In some aspects, deafness and/or hearing loss can be conductive (arising from the ear canal or middle ear), sensorineural (arising from the inner ear or auditory nerve), or mixed. In some aspects, nonsyndromic deafness and/or hearing loss is associated with permanent hearing loss caused by damage to structures in the inner ear (sensorineural deafness). In some aspects, sensorineural hearing loss can be due to poor hair cell function. In some aspects, sensorineural hearing impairments involve the eighth cranial nerve (the vestibulocochlear nerve) or the auditory portions of the brain. In some such aspects, only the auditory centers of the brain are affected. In such a situation, cortical deafness may occur, where sounds may be heard at normal thresholds, but quality of sound perceived is so poor that speech cannot be understood. Hearing loss that results from changes in the middle ear is called conductive hearing loss. Some forms of nonsyndromic deafness and/or hearing loss involve changes in both the inner ear and the middle ear, called mixed hearing loss. Hearing loss and/or deafness that is present before a child learns to speak can be classified as prelingual or congenital. Hearing loss and/or deafness that occurs after the development of speech can be classified as postlingual.

Most autosomal recessive loci related to syndromic or nonsyndromic hearing loss cause prelingual severe-to-profound hearing loss.

[0042] As is known to those of skill in the art, hair cells are sensory receptors for both auditory and vestibular systems of vertebrate ears. Hair cells detect movement in the environment and, in mammals, hair cells are located within the cochlea of the ear, in the organ of Corti. Mammalian ears are known to have two types of hair cells – inner hair cells and outer hair cells. Outer hair cells can amplify low level sound frequencies, either through mechanical movement of hair cell bundles or electrically-driven movement of hair cell soma. Inner hair cells transform vibrations in cochlear fluid into electrical signals that the auditory nerve transmits to the brain. In some aspects, hair cells may be abnormal at birth, or damaged during the lifetime of an individual. In some aspects, outer hair cells may be able to regenerate. In some aspects, inner hair cells are not capable of regeneration after illness or injury. In some aspects, sensorineural hearing loss is due to abnormalities in hair cells.

[0043] As is known to those of skill in the art, hair cells do not occur in isolation, and their function is supported by a wide variety of cells which can collectively be referred to as supporting cells. Supporting cells may fulfill numerous functions, and include a number of cell types, including but not limited to inner phalangeal cells/border cells (IPhC), inner pillar cells (IPC), outer pillar cells (OPC), Deiters' cells rows 1 and 2 (DC1/2), Deiters' cells row 3 (DC3), Hensen's cells (Hec), Claudius cells/outer sulcus cells (CC/OSC), interdental cells (Idc), inner sulcus cells (ISC), Kölliker's organ cells (KO), greater ridge epithelial ridge cells (GER) (including lateral greater epithelial ridge cells (LGER)), and OC90+ cells (OC90), fibroblasts, and other cells of the lateral wall.

[0044] In some aspects, sensorineural hearing loss is due to abnormalities in supporting cells. In some aspects, supporting cells may be abnormal at birth, or damaged during the lifetime of an individual. In some aspects, supporting cells may be able to regenerate. In some aspects, certain supporting cells may not be capable of regeneration.

Polypeptides

[0045] Certain aspects of the disclosure are directed to polynucleotides encoding a polypeptide. The polynucleotide can encode a polypeptide that is capable of being expressed in a cell (e.g., an inner ear cell). The polynucleotide can encode a full length polypeptide or a functional fragment thereof.

- [0046]** Exemplary polypeptides encoded by the polynucleotide include, but are not limited to, transmembrane proteins, enzymes, growth factors, cytokines, receptors, receptor ligands, hormones, membrane proteins, membrane-associated proteins, antigens, and antibodies.
- [0047]** Exemplary polynucleotides encoding polypeptides include, but are not limited to, ATPase Plasma Membrane Ca²⁺ Transporting 2 (ATP2B2), Cholinergic Receptor Nicotinic Alpha 9 Subunit (CHRNA9), Cadherin 23 (CDH23), Coiled-coil Glutamate Rich Protein 2 (CCER2), Clarin 1 (CLRN1), Clarin 2 (CLRN2), cochlin (COCH or DFNA9), Dystrotelin (DYTN), Epidermal Growth Factor Receptor Pathway Substrate 8 (EPS8), EPS8 Like 2 (EPS8L2), Espin (ESPN), Espin Like (ESPNL), Gap junction protein beta 2 (GJB2), Gap junction protein beta 6 (GJB6), Gap junction protein beta 3 (GJB3), gasdermin E protein (GSDME or DFNA5), Insulinoma-associated 1 (INSM1), Ikaros family zinc finger 2 (IKZF2), LIM Homeobox Protein 3 (LHX3), Myosin 7A (MYO7A), Myosin 11 (MYO3A), Norrin cystine knot growth factor (NDP), Protocadherin 15 (PCDH15), Protein Tyrosine Phosphatase, Receptor Type Q (PTPRQ), Stereocilin (STRC), Protein Network Component Harmonin (USH1C), Usherin (USH2A), and Spectrin repeat containing nuclear envelope family member 4 (SYNE4).
- [0048]** In some aspects, the polynucleotide can comprise a GJB2 gene. In some aspects, the polynucleotide can comprise a nucleic acid encoding a Connexin 26 polypeptide. In some aspects, the nucleic acid comprises a coding sequence for a Connexin 26 polypeptide.
- [0049]** In some aspects, the polynucleotide or nucleic acid comprises a gap junction beta-2 (GJB2) gene. The GJB2 gene is highly conserved across the mammalian class and encodes connexin 26 (Cx26) (also referred to as gap junction beta-2 (GJB2) protein). Connexin 26 is a member of the gap junction protein family, which is also known as the connexin family. Gap junction proteins are specialized proteins, involved in intracellular communication. Mutations in the human GJB2 gene have been associated with hearing loss and deafness (Amorini et al., *Ann. Hum. Genet.* 79(5):341-349, 2015; Qing et al., *Genet. Test Mol. Biomarkers* 19(1):52-58, 2015).
- [0050]** The human GJB2 gene is located on chromosome 13q12. It contains two transcriptional isoforms beginning from alternative transcriptional start sites, both of which contain two exons and a single intron encompassing a total of about 5 kilobases (kb) (approximately 5,469 or 4,675 nucleotides respectively) (NCBI Gene ID 2706, NCBI

Reference Sequence: NG_008358.1). Both human GJB2 mRNA isoforms comprise a second exon, which completely encodes a full-length connexin 26 in exon two. This coding sequence is approximately 681 nucleotides, and encodes a connexin 26 that is 226 amino acids in length.

- [0051]** A monomer of connexin 26 includes four transmembrane helices linked by two extracellular loops and one shorter intracellular loop, with N- and C-termini on the cytosolic side of the plasma membrane. Gap junctions between cells can be formed in a homomeric and/or heteromeric manner. Connexin 26 has been shown to form functional homomeric channels, as well as functional heteromeric channels with at least connexin 30, connexin 32, connexin 46, and connexin 50. In some aspects, GJB2 gene associated sensorineural hearing loss (e.g., nonsyndromic or syndromic) may be due to compound heterozygous mutations in GJB2 and in an alternative connexin protein encoding gene. The gap junctions created with connexin 26 transport potassium ions and certain other small molecules across cells. Connexin 26 helps maintain the correct level of intracellular potassium ions, and is required for the maturation of certain cells in the cochlea.
- [0052]** A human GJB2 gene is expressed in a number of tissues, but is known to be involved in important cellular homeostasis functions in the epidermis and inner ear. Within the inner ear, connexin 26 is synthesized by all supporting cell types within the organ of corti, including the inner phalangeal cells/border cells (IPhC), inner pillar cells (IPC), outer pillar cells (OPC), Deiters' cells rows 1 and 2 (DC1/2), Deiters' cells row 3 (DC3), Hensen's cells (Hec), Claudius cells/outer sulcus cells (CC/OSC), interdental cells (Idc), inner sulcus cells (ISC), Kölliker's organ cells (KO), greater ridge epithelial ridge cells (GER) (including lateral greater epithelial ridge cells (LGER)), and OC90+ cells (OC90), root cells, fibrocytes, fibroblasts, basal and intermediate cells from the stria vascularis, and other cells of the lateral wall. In addition, connexin 26 is known to be present in mesenchymal cells in the lateral wall, and type 1 neurons in the spiral ganglion.
- [0053]** The human GJB2 gene has a defined 128bp long basal/minimal promoter just upstream of the canonical first exon in the most abundant isoform. This sequence includes a TATA box and two GC boxes, which are known to be bound by the Sp1 and Sp3 TFs.
- [0054]** There are over 200 defined mutations of GJB2, which show some level of pathogenicity, and various mutations in the GJB2 gene have been associated with hearing loss (e.g., non-syndromic sensorineural hearing loss or syndromic sensorineural hearing loss). For example, the c.35delG allele was found on 65.5% of patients from Eastern

Sicily (Amorini et al., *Ann. Hum. Genet.* 79(5):341-349, 2015). Additional exemplary mutations in a GJB2 gene detected in subjects having nonsyndromic sensorineural hearing loss or syndromic sensorineural hearing loss, and methods of sequencing a nucleic acid encoding GJB2 are described in, e.g., Snoeckx et al., *Am. J. Hum. Genet* 77: 945-957, 2005; Welch et al., *Am. J. Med. Genet A* 143: 1567- 1573, 2007; Zelante et al., *Hum. Mol. Genet.* 6:1605-1609, 1997; and Tsukada et al., *Annals of Otology, Rhinology & Laryngology*. 2015, Vol. 124(5S) 61S-76S, each of which is incorporated in its entirety herein by reference. 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, Sanger sequencing, Next-generation sequencing, Southern blotting, and Northern blotting. Multiple disease states associated with sensorineural hearing loss with either nonsyndromic or syndromic manifestations have been linked with specific mutations of the human GJB2 gene (see Nickel & Forge, *Curr Opin Otolaryngol Head Neck Surg.* 2008 Oct;16(5):452-7, which is incorporated in its entirety herein by reference). Human GJB2 gene mutations which lead to syndromic or nonsyndromic hearing loss vary from large deletions that remove either the entirety of GJB2 or GJB2 gene regulatory regions, to hundreds of small scale alterations including nonsense, missense, indels (leading to phase shifting), and splice-site point mutations.

[0055] In some aspects, GJB2 gene mutations such as Gly59Ser, and Asn52Lys are associated with Bart-Pumphrey syndrome. A syndrome defined by manifestations of thickened skin, wart-like growths, and generally congenital moderate to profound sensorineural hearing loss. In other aspects, GJB2 gene mutations such as Aspn50Asn are associated with Hystrix-like Ichthyosis with deafness & Keratitisichthyosis- deafness syndrome. These syndromes are associated with dry scaly skin, generally congenital profound sensorineural hearing loss, and in Keratitis-ichthyosisdeafness syndrome, additional inflammation of the cornea.

[0056] In some aspects, GJB2 gene missense mutations are associated with Palmoplantar keratoderma with deafness. A syndrome associated with thick skin on the palms of the hands and soles of the feet, and mild to profound sensorineural hearing loss which begins in early childhood and gets worse over time, affected individuals may have particular trouble hearing high-pitched sounds. While in other aspects, GJB2 gene missense mutations are associated with Vohwinkel syndrome. A syndrome associated with skin abnormalities (e.g., thick bands of fibrous tissue around their fingers and toes that may

cut off the circulation to the digits and result in spontaneous amputation) and sensorineural hearing loss.

[0057] In some aspects, GJB2 gene mutations are associated with nonsyndromic hearing loss, which may be inherited in either a dominant (e.g., DFNA3) or recessive manner (DFNB1). In some aspects, loss of function GJB2 gene mutations are associated with nonsyndromic DFNB1 which is inherited in an autosomal recessive manner and presents as mild to profound hearing loss that is generally prelingual and does not become more severe over time. It is estimated that DFNB1 is present in approximately 14 out of every 100,000 live births in the US and EU5. It has been postulated that an early but not always congenital onset of DFNB1 hearing impairment could be followed by a quick progression of the hearing loss. In general, DFNB1 patients treatment options include education, hearing aids, and cochlear implants. Patients generally do not have additional symptoms, and live a normal lifespan. It is estimated that DFNB1 accounts for about 50% of congenital severe-to-profound autosomal recessive non-syndromic hearing loss in many first world countries (e.g., US, France, Britain, and Australia).

[0058] In some aspects, sensorineural hearing loss due to GJB2 gene mutations are inherited in an autosomal dominant manner as nonsyndromic DFNA3. These mutations are generally dominant negative missense mutations that prevent the formation of necessary functional gap junctions. This disease state presents with hearing loss that can be either prelingual or postlingual, ranging from mild to profound, which generally becomes more severe over time.

[0059] Among other things, the present disclosure provides polynucleotides, e.g., polynucleotides comprising a GJB2 gene or characteristic portion thereof, as well as compositions including such polynucleotides and methods utilizing such polynucleotides and/or compositions.

[0060] In some aspects, a polynucleotide comprising a GJB2 gene or characteristic portion thereof can be DNA or RNA. In some aspects, DNA can be genomic DNA or cDNA. In some aspects, RNA can be an mRNA. In some aspects, a polynucleotide comprises exons and/or introns of a GJB2 gene.

[0061] In some aspects, a gene product is expressed from a polynucleotide comprising a GJB2 gene or characteristic portion thereof. In some aspects, expression of such a polynucleotide can utilize one or more control elements (e.g., promoters, enhancers,

splice sites, poly-adenylation sites, translation initiation sites, etc.). Thus, in some aspects, a polynucleotide provided herein can include one or more control elements.

[0062] In some aspects, a GJB2 gene is a mammalian GJB2 gene. In some aspects, a GJB2 gene is a murine GJB2 gene. In some aspects, a GJB2 gene is a primate GJB2 gene. In some aspects, a GJB2 gene is a human GJB2 gene. In some aspects, a GJB2 gene is codon optimized. An exemplary human GJB2 coding cDNA sequence is or includes the sequence of SEQ ID NO: 117 or SEQ ID NO: 118. An exemplary human GJB2 spliced cDNA sequence with untranslated regions is or includes the sequence of SEQ ID NO: 119. An alternative transcriptional start site exemplary human GJB2 spliced cDNA sequence with untranslated regions is or includes the sequence of SEQ ID NO: 120. An exemplary human GJB2 genomic DNA sequence can be found in SEQ ID NO: 121. Exemplary codon optimized GJB2 DNA sequences can be found in SEQ ID NOs: 123-126.

Exemplary Human GJB2 cDNA coding Sequence (SEQ ID NO: 117)

ATGGATTGGGGCACGCTGCAGACGATCCTGGGGGGTGTGAACAAACACTCCA
CCAGCATTGGAAAGATCTGGCTCACCGTCCTCTTCATTTTTCGCATTATGATCC
TCGTTGTGGCTGCAAAGGAGGTGTGGGGAGATGAGCAGGCCGACTTTGTCTG
CAACACCCTGCAGCCAGGCTGCAAGAACGTGTGCTACGATCACTACTTCCCC
ATCTCCCACATCCGGCTATGGGCCCTGCAGCTGATCTTCGTGTCCACGCCAGC
GCTCCTAGTGGCCATGCACGTGGCCTACCGGAGACATGAGAAGAAGAGGAAG
TTCATCAAGGGGGAGATAAAGAGTGAATTTAAGGACATCGAGGAGATCAAA
ACCCAGAAGGTCCGCATCGAAGGCTCCCTGTGGTGGACCTACACAAGCAGCA
TCTTCTTCCGGGTCATCTTCGAAGCCGCCTTCATGTACGTCTTCTATGTCATGT
ACGACGGCTTCTCCATGCAGCGGCTGGTGAAGTGCAACGCCTGGCCTTGTCCC
AACACTGTGGACTGCTTTGTGTCCCGGCCACGGAGAAGACTGTCTTCACAGT
GTTTCATGATTGCAGTGTCTGGAATTTGCATCCTGCTGAATGTCACTGAATTGT
GTTATTTGCTAATTAGATATTGTTCTGGGAAGTCAAAAAAGCCAGTT

Exemplary Human GJB2 cDNA coding Sequence (SEQ ID NO: 118)

ATGGATTGGGGCACGCTGCAGACGATCCTGGGGGGTGTGAACAAACACTCCA
CCAGCATTGGAAAGATCTGGCTCACCGTCCTCTTCATTTTTCGCATTATGATCC
TCGTTGTGGCTGCAAAGGAGGTGTGGGGAGATGAGCAGGCCGACTTTGTCTG

CAACACCCTGCAGCCAGGCTGCAAGAACGTGTGCTACGATCACTACTTCCCC
ATCTCCCACATCCGGCTATGGGCCCTGCAGCTGATCTTCGTGTCCACGCCAGC
GCTCCTAGTGGCCATGCACGTGGCCTACCGGAGACATGAGAAGAAGAGGAAG
TTCATCAAGGGGGAGATAAAGAGTGAATTTAAGGACATCGAGGAGATCAAA
ACCCAGAAGGTCCGCATCGAAGGCTCCCTGTGGTGGACCTACACAAGCAGCA
TCTTCTTCCGGGTCATCTTCGAAGCCGCCTTCATGTACGTCTTCTATGTCATGT
ACGACGGCTTCTCCATGCAGCGGCTGGTGAAGTGCAACGCCTGGCCTTGTCCC
AACACTGTGGACTGCTTTGTGTCCCGGCCACGGAGAAGACTGTCTTCACAGT
GTTTCATGATTGCAGTGTCTGGAATTTGCATCCTGCTGAATGTCAGTGAATTGT
GTTATTTGCTAATTAGATATTGTTCTGGGAAGTCAAAAAAGCCAGTTTAA

Exemplary spliced Human GJB2 isoform 1 cDNA including untranslated regions
Sequence (SEQ ID NO: 119)

GTTGCGGCCCCGCAGCGCCCGCGCGCTCCTCTCCCCGACTCGGAGCCCCTCGG
CGGCGCCCGGCCAGGACCCGCCTAGGAGCGCAGGAGCCCCAGCGCAGAGA
CCCCAACGCCGAGACCCCGCCCCGGCCCCGCGCGCTTCTCCCGACGCAG
AGCAAACCGCCCAGAGTAGAAGATGGATTGGGGCACGCTGCAGACGATCCTG
GGGGGTGTGAACAAACTCCACCAGCATTGGAAAGATCTGGCTCACCGTCC
TCTTCATTTTTTCGCATTATGATCCTCGTTGTGGCTGCAAAGGAGGTGTGGGGA
GATGAGCAGGCCGACTTTGTCTGCAACACCCTGCAGCCAGGCTGCAAGAACG
TGTGCTACGATCACTACTTCCCCATCTCCACATCCGGCTATGGGCCCTGCAG
CTGATCTTCGTGTCCACGCCAGCGCTCCTAGTGGCCATGCACGTGGCCTACCG
GAGACATGAGAAGAAGAGGAAGTTCATCAAGGGGGAGATAAAGAGTGAATT
TAAGGACATCGAGGAGATCAAAACCCAGAAGGTCCGCATCGAAGGCTCCCTG
TGGTGGACCTACACAAGCAGCATCTTCTTCCGGGTCATCTTCGAAGCCGCCTT
CATGTACGTCTTCTATGTCATGTACGACGGCTTCTCCATGCAGCGGCTGGTGA
AGTGCAACGCCTGGCCTTGTCCCAAACTGTGGACTGCTTTGTGTCCCGGCC
ACGGAGAAGACTGTCTTCACAGTGTTTCATGATTGCAGTGTCTGGAATTTGCAT
CCTGCTGAATGTCAGTGAATTGTGTTATTTGCTAATTAGATATTGTTCTGGGA
AGTCAAAAAAGCCAGTTTAAACGCATTGCCAGTTGTTAGATTAAGAAATAGA
CAGCATGAGAGGGATGAGGCAACCCGTGCTCAGCTGTCAAGGCTCAGTCGCT
AGCATTTCCCAACACAAAGATTCTGACCTTAAATGCAACCATTTGAAACCCCT
GTAGGCCTCAGGTGAAACTCCAGATGCCACAATGGAGCTCTGCTCCCCTAAA

GCCTCAAAACAAAGGCCTAATTCTATGCCTGTCTTAATTTTCTTTCACTTAAGT
TAGTTCCACTGAGACCCCAGGCTGTTAGGGGTATTGGTGTAAGGTACTTTCA
TATTTTAAACAGAGGATATCGGCATTTGTTTCTTTCTCTGAGGACAAGAGAAA
AAAGCCAGGTTCCACAGAGGACACAGAGAAGGTTTGGGTGTCCTCCTGGGGT
TCTTTTGGCCAACCTTTCCCCACGTTAAAGGTGAACATTGGTTCCTTCATTGCT
TTGGAAGTTTTAATCTCTAACAGTGGACAAAGTTACCAGTGCCTTAAACTCTG
TTACACTTTTTGGAAGTGAAAACCTTTGTAGTATGATAGGTTATTTTGATGTAA
AGATGTTCTGGATACCATTATATGTTCCCCCTGTTTCAGAGGCTCAGATTGTA
ATATGTAAATGGTATGTCATTTCGCTACTATGATTTAATTTGAAATATGGTCTTT
TGGTTATGAATACTTTGCAGCACAGCTGAGAGGCTGTCTGTTGTATTTCATTGT
GGTCATAGCACCTAACAACATTGTAGCCTCAATCGAGTGAGACAGACTAGAA
GTTCTAGTGATGGCTTATGATAGCAAATGGCCTCATGTCAAATATTTAGATG
TAATTTTGTGTAAGAAATACAGACTGGATGTACCACCACTACTACCTGTAAT
GACAGGCCTGTCCAACACATCTCCCTTTTCCATGACTGTGGTAGCCAGCATCG
GAAAGAACGCTGATTTAAAGAGGTCGCTTGGGAATTTTATTGACACAGTACC
ATTTAATGGGGAGGACAAAATGGGGCAGGGGAGGGAGAAGTTTCTGTCGTTA
AAAACAGATTTGGAAAGACTGGACTCTAAAGTCTGTTGATTAAAGATGAGCT
TTGTCTACTTCAAAGTTTGTGTTGCTTACCCCTTCAGCCTCCAATTTTTTAAGT
GAAAATATAGCTAATAACATGTGAAAAGAATAGAAGCTAAGGTTTAGATAAA
TATTGAGCAGATCTATAGGAAGATTGAACCTGAATATTGCCATTATGCTTGAC
ATGGTTTCCAAAAAATGGTACTCCACATATTTTCAGTGAGGGTAAGTATTTTCC
TGTTGTCAAGAATAGCATTGTAAAAGCATTTTGTAAATAATAAAGAATAGCTTT
AATGATATGCTTGTAATAAATAATTTTGTAAATGTATCAAATACATTTAAAA
CATTAATAATAATCTCTATAATAA

**Exemplary spliced Human GJB2 isoform X1 cDNA including untranslated regions
Sequence (SEQ ID NO: 120)**

TTTAGGACCCTTGTTTCGCGAAGAGGTGGTGTGCGGCTGAGACCCGCGTCCTCA
GGACGGTTCCATCAGTGCCTCGATCCTGCCCCACTGGAGGAGGAAGGCAGCC
CGAACAGCGCTCACCTAACTAACAGCTGCTGAGAGCTGGGTTCCGTGGCCAT
GCACCTGGGACTGCCTTGAGAAGCGTGAGCAAACCGCCCAGAGTAGAAGATG
GATTGGGGCAGCTGCAGACGATCCTGGGGGGTGTGAACAAACACTCCACCA
GCATTGGAAAGATCTGGCTCACCGTCCTCTTCATTTTTTCGATTATGATCCTCG

TTGTGGCTGCAAAGGAGGTGTGGGGAGATGAGCAGGCCGACTTTGTCTGCAA
CACCTTGCAGCCAGGCTGCAAGAACGTGTGCTACGATCACTACTTCCCCATCT
CCCACATCCGGCTATGGGCCCTGCAGCTGATCTTCGTGTCCACGCCAGCGCTC
CTAGTGGCCATGCACGTGGCCTACCGGAGACATGAGAAGAAGAGGAAGTTCA
TCAAGGGGGAGATAAAGAGTGAATTTAAGGACATCGAGGAGATCAAAACCC
AGAAGGTCCGCATCGAAGGCTCCCTGTGGTGGACCTACACAAGCAGCATCTT
CTTCCGGGTCATCTTCGAAGCCGCCTTCATGTACGTCTTCTATGTCATGTACGA
CGGCTTCTCCATGCAGCGGCTGGTGAAGTGCAACGCCTGGCCTTGTCCCAACA
CTGTGGACTGCTTTGTGTCCCGGCCACGGAGAAGACTGTCTTCACAGTGTTT
ATGATTGCAGTGTCTGGAATTTGCATCCTGCTGAATGTCACTGAATTGTGTTA
TTTGCTAATTAGATATTGTTCTGGGAAGTCAAAAAAGCCAGTTTAACGCATTG
CCCAGTTGTTAGATTAAGAAATAGACAGCATGAGAGGGATGAGGCAACCCGT
GCTCAGCTGTCAAGGCTCAGTCGCTAGCATTTCCTAACACAAAGATTCTGACC
TTAAATGCAACCATTTGAAACCCCTGTAGGCCTCAGGTGAAACTCCAGATGCC
ACAATGGAGCTCTGCTCCCCTAAAGCCTCAAAACAAAGGCCTAATTCTATGCC
TGTCTTAATTTTCTTTCACTTAAGTTAGTTCCACTGAGACCCCAGGCTGTTAGG
GGTTATTGGTGTAAGGTACTTTTCATATTTTAAACAGAGGATATCGGCATTTGT
TTCTTTCTCTGAGGACAAGAGAAAAAAGCCAGGTTCACAGAGGACACAGAG
AAGGTTTGGGTGTCCTCCTGGGGTTCTTTTTGCCAACTTTCCCCACGTAAAG
GTGAACATTGGTTCTTTCAATTGCTTTGGAAGTTTAAATCTCTAACAGTGGACA
AAGTTACCAGTGCCTTAAACTCTGTTACACTTTTTGGAAGTGAAAACCTTTGTA
GTATGATAGGTTATTTTGATGTAAAGATGTTCTGGATACCATTATATGTTCCC
CCTGTTTCAGAGGCTCAGATTGTAATATGTAAATGGTATGTCATTCGCTACTA
TGATTTAATTTGAAATATGGTCTTTTGGTTATGAATACTTTGCAGCACAGCTG
AGAGGCTGTCTGTTGTATTCATTGTGGTCATAGCACCTAACAACATTGTAGCC
TCAATCGAGTGAGACAGACTAGAAGTTCCTAGTGATGGCTTATGATAGCAAA
TGGCCTCATGTCAAATATTTAGATGTAATTTTGTGTAAGAAATACAGACTGGA
TGTACCACCAACTACTACCTGTAATGACAGGCCTGTCCAACACATCTCCCTTT
TCCATGACTGTGGTAGCCAGCATCGGAAAGAACGCTGATTTAAAGAGGTCGC
TTGGGAATTTTATTGACACAGTACCATTTAATGGGGAGGACAAAATGGGGCA
GGGGAGGGAGAAGTTTCTGTCGTTAAAAACAGATTTGGAAAGACTGGACTCT
AAAGTCTGTTGATTAAAGATGAGCTTTGTCTACTTCAAAAGTTTGTGTTGCTTA
CCCCTTCAGCCTCCAATTTTTTAAGTGAAAATATAGCTAATAACATGTGAAAA

GAATAGAAGCTAAGGTTTAGATAAATATTGAGCAGATCTATAGGAAGATTGA
ACCTGAATATTGCCATTATGCTTGACATGGTTTCCAAAAAATGGTACTCCACA
TATTTCAGTGAGGGTAAGTATTTTCCTGTTGTCAAGAATAGCATTGTAAAAGC
ATTTTGTAAATAATAAAGAATAGCTTTAATGATATGCTTGTAATACTAAAATAATT
TTGTAATGTATCAAATACATTTAAAACATTAAAATATAATCTCTATAATAA

Exemplary Human GJB2 Genomic DNA Sequence (SEQ ID NO: 121)

GTTGCGGCCCCGCAGCGCCCGCGCGCTCCTCTCCCCGACTCGGAGCCCCCTCGG
CGGCGCCCGGCCAGGACCCGCCTAGGAGCGCAGGAGCCCCAGCGCAGAGA
CCCCAACGCCGAGACCCCGCCCCGGCCCCGCCGCGCTTCTCCCGACGCAG
GTGAGCCCGCCGGCCCCGGACTGCCCCGGCCAGGAACCTGGCGCGGGGAGGG
ACCGCGAGACCCAGAGCGGTTGCCCGGCCGCGTGGGTCTCGGGGAACCGGGG
GGCTGGACCAACACACGTCTTGGGCCGGGGGGCGGGGGCCGCCTTCTGGAG
CGGGCGTTTCTGCGGCCGAGCTCCGGAGCTGGAATGGGGCGGCCGGGGAAGT
GGACGCGATGGCACCGCCCGGGGTGCGAGTGGGGCCGGGCGCGCGCGGGAG
GGGAAAAAGGCGCGGGCGAGCCGCCAGCGCGAGGTTTGTGGTGTGCGCGATG
TCCCTTCGGGGTACTCTAGCGCAGCCGCCTGGCTACTTGACCCACTGCCACCA
AACGTTTTTAATTACCGAAAGCTTAGCTTCGAAGCAAAGCTCCGTTTCGCCG
GTGAAGCAGGAAGCCTTCGCTGCAGGAAGTACCTTTACCTCTTGGAGCGGC
TTCTGCAGAAAAATCCCCGGGCAGAGATTTGGGCGGAGTTTGCCTAGAACTA
ACGCGGAGCCAGCCGATCCCGGCCTACCCCGGGGCCAAGATTTCAAGTGGCTT
CCCTTTTTTCTAAACACTTCACGAGGGTCTGTTTCCGGGCTGTGCTCCCCGCCT
AGAAGGAAAAATTTTAGGACCCTTGTTTCGCGAAGAGGTGGTGTGCGGCTGAG
ACCCGCGTCCTCAGGACGGTTCCATCAGTGCCTCGATCCTGCCCCACTGGAGG
AGGAAGGCAGCCCGAACAGCGCTCACCTAACTAACAGCTGCTGAGAGCTGGG
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CCATGTGACCTTAGAGTCCCTTTTCGAAACTGCTGTGCACAGTCGGTCACAATT
TCAGACACTGGTGAGAAGGGTGGAGGAACCCTCTGGGGACAGCCAGGCAAG
GTCGACCACCCATCACCTAAGGGTGGAGAAATTTAAGGGGTGAAGAGTCCCT
TTTGCCTTTTCTGGATCCTGGTGATTCACCTAGTGTCTTCCCTAAGGAACTGAA
CCAACTCCTCCGCTGGCCTCTGGCAGCCCTCCAGGCGGTGCAGGATGGCGTG
GGCCCGGTAGGAAGCTGCATGTAACCGCCCAGGGTCGGGAGGCCAGGAGGG
CAGCTCCTCCTCTGACTTGAATATTGAAAACAACCTTCGTCCTGCTTCTGAGCC

CCTCTTAACCCATGACCCCCTAGCCCATTGGGGAGTAAATCTTAATTTACTCC
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ACAGGTCTGTAGAAGGGTGTATGACCTGGGTTTGGCAGGTGGAAGAGGGCAA
AGGATAAACCCCTCCTCCTGCAGCCCCATATTCTTGGCCAGGTGTATTGTTGT
AAACCAGGAGAGAGTTTACTTCGGGGAGTATCCTGTTTTCCACTCAGTGAGG
GCCAATGAAGAATGTCTAATTCATAAGATGCTTTTGTAAAATCGGAATGTT
GCTGTCCTCGGTGGTTCTGCTGTTGGGACGGGACTGGCCTGAGCTGTGGGTGC
TGTAGCAGGACAACCAGCTCACCTAAGGGCCTCCCAGTCTGGATTATCAATG
GGTCAGTGCTGAACCTGGGCTAAAATATTGTTTTTCCAATGATGTTGTCTTTC
CCAAGCTCAGTGAAGCTAAATGTTTACAGGCCTATGTCAATCTGATGTAAC
TTCGTGGCCACCTCTCTCCTGTTAGCCTCTGACCAAGGTGGCACTGGATGGTT
TCTGCCTGACCTTGGTGCCCCGTGGCAGCGACTGTGGGTCATGAAAGACATTC
ACTACGAGCCTGCTTCTGGAGTCCATCAGAAAACGGGATGCAACTTGCCTAA
AATGAGGAGAGGAGGATGCTTTTAAGAAAAAGAAGAAGGAGGATTCAC
CAGCTCTGAAGGGTGGAAAAGAGATGATTCATCCGATTGTGGAGAGGGTGG
AATCTTGTTTAGGAGAGCGTTGGTTGTGGCAGGCAGGGTGTAACATGAATC
AGTGAAGACAATTCACATCCTGGGATGAAAAGAAGGCCATGGGCTCACAGGA
GATTATCCACTGGCCTCTCCACATCCGCTTGCAGTAAGGAGTGTGGGACTCTC
CCAAGCTTCAGCGCTGAACTGCAATGCAGTGACGTCGCTTAGCTGGGCCAGT
AACCGAGGGAGTTGAATTTTCTGTCAATTTTAAAATAATGTGTCTTTTAAGAAA
CACTTTGAAATTTAAACCACAGCCCACAATTATAATGCACTGTTGCAGCACTT
ATCAAAACAGATATGCTAACTGAGCCATCAGTGCCAGCCTGACAGTGAGGCC
ACCAAGCCATCCACAAAGCCTACACGAAAGTCTGTGCTCACAGTGGCTTTTCT
CCATGAAGAGGGCATTCCTAACCTCTTCCTTTACGTAGGAGGAAGCAAGGT
CCTTTGTAAAATTTTAACTCGGGGTGCCTCAAATGTAACTTAACCACTGGTA
ACAACAGTTTCACTGCTACATGCCACGTCTGTGAAAATTCATTCAAGACATTA
AGGAAAGTGGCTCAGCAGAGAGACTAGACATCTTATCCTCACGGTTCTCCTGT
ACTTGGCCTCTCAGCCTTTGAGCAAGGTTGGCCCAAGCTAGTATCGGCCCCAG
TGGTACAGCCAAAACCTGAGACTGCAAATGGATGCAGCTGTTGAACGCTGAG
TAACTTCTGCAGAGTCAGGAAGACCCAAGGAAGCTCTGCAGAGGATGCAGGG
GTACGGTCAGAACCCTGAGTGCCTTTACGCTAACGAGGACTTTATGACACTC
CCCAGCACAGCAAATTTTATGATGTGTTTAAAGATTGGGTGAATTACTCAGG

TGAACAAGCTACTTTTTATCAGAGAACACCTAAAAACACGTTCAAGAGGGTT
TGGGAACATAACATTTAATCCTATGACAAACTAAGTTGGTTCTGTCTTCACCT
GTTTTGGTGAGGTTGTGTAAGAGTTGGTGTGTTGCTCAGGAAGAGATTTAAGCA
TGCTTGCTTACCCAGACTCAGAGAAGTCTCCCTGTTCTGTCCTAGCTAGTGAT
TCCTGTGTTGTGTGCATTTCGTCTTTTCCAGAGCAAACCGCCCAGAGTAGAAGA
TGGATTGGGGCACGCTGCAGACGATCCTGGGGGGTGTGAACAAACACTCCAC
CAGCATTGGAAGATCTGGCTCACCGTCCTCTTCATTTTTTCGCATTATGATCCT
CGTTGTGGCTGCAAAGGAGGTGTGGGGAGATGAGCAGGCCGACTTTGTCTGC
AACACCCTGCAGCCAGGCTGCAAGAACGTGTGCTACGATCACTACTTCCCCAT
CTCCCACATCCGGCTATGGGCCCTGCAGCTGATCTTCGTGTCCACGCCAGCGC
TCCTAGTGGCCATGCACGTGGCCTACCGGAGACATGAGAAGAAGAGGAAGTT
CATCAAGGGGGAGATAAAGAGTGAATTTAAGGACATCGAGGAGATCAAAAC
CCAGAAGGTCCGCATCGAAGGCTCCCTGTGGTGGACCTACACAAGCAGCATC
TTCTTCCGGGTCATCTTCGAAGCCGCCTTCATGTACGTCTTCTATGTCATGTAC
GACGGCTTCTCCATGCAGCGGCTGGTGAAGTGCAACGCCTGGCCTTGTCCCAA
CACTGTGGACTGCTTTGTGTCCCGGCCACGGAGAAGACTGTCTTCACAGTGT
TCATGATTGCAGTGTCTGGAATTTGCATCCTGCTGAATGTCACTGAATTGTGT
TATTTGCTAATTAGATATTGTTCTGGGAAGTCAAAAAAGCCAGTTTAACGCAT
TGCCCAGTTGTTAGATTAAGAAATAGACAGCATGAGAGGGATGAGGCAACCC
GTGCTCAGCTGTCAAGGCTCAGTCGCTAGCATTTCCCAACACAAAGATTCTGA
CCTTAAATGCAACCATTTGAAACCCCTGTAGGCCTCAGGTGAAACTCCAGATG
CCACAATGGAGCTCTGCTCCCTTAAAGCCTCAAAACAAAGGCCTAATTCTATG
CCTGTCTTAATTTTCTTTCACTTAAGTTAGTTCCACTGAGACCCCAGGCTGTTA
GGGGTTATTGGTGTAAAGGTACTTTCATATTTTAAACAGAGGATATCGGCATTT
GTTTCTTTCTCTGAGGACAAGAGAAAAAAGCCAGGTTCCACAGAGGACACAG
AGAAGGTTTGGGTGTCCCTCCTGGGGTTCTTTTTGCCAACTTTCCCCACGTTAA
AGGTGAACATTGGTTCTTTCATTTGCTTTGGAAGTTTAAATCTCTAACAGTGG
ACAAAGTTACCAGTGCCTTAAACTCTGTTACACTTTTTGGAAGTGAAAACCTT
GTAGTATGATAGGTTATTTTGATGTAAAGATGTTCTGGATACCATTATATGTT
CCCCCTGTTTCAGAGGCTCAGATTGTAATATGTAAATGGTATGTCATTTCGCTA
CTATGATTTAATTTGAAATATGGTCTTTTGGTTATGAATACTTTGCAGCACAG
CTGAGAGGCTGTCTGTTGTATTTCATTGTGGTCATAGCACCTAACAAACATTGTA
GCCTCAATCGAGTGAGACAGACTAGAAGTTCCTAGTGATGGCTTATGATAGC

AAATGGCCTCATGTCAAATATTTAGATGTAATTTTGTGTAAGAAATACAGACT
GGATGTACCACCACTACTACCTGTAATGACAGGCCTGTCCAACACATCTCCC
TTTTCCATGACTGTGGTAGCCAGCATCGGAAAGAACGCTGATTTAAAGAGGT
CGCTTGGGAATTTTATTGACACAGTACCATTTAATGGGGAGGACAAAATGGG
GCAGGGGAGGGAGAAGTTTCTGTCGTTAAAAACAGATTTGGAAAGACTGGAC
TCTAAAGTCTGTTGATTAAAGATGAGCTTTGTCTACTTCAAAAGTTTGTGTTGCT
TACCCCTTCAGCCTCCAATTTTTTAAGTGAAAATATAGCTAATAACATGTGAA
AAGAATAGAAGCTAAGGTTTAGATAAATATTGAGCAGATCTATAGGAAGATT
GAACCTGAATATTGCCATTATGCTTGACATGGTTTCCAAAAAATGGTACTCCA
CATATTTTCAGTGAGGGTAAGTATTTTCCTGTTGTCAAGAATAGCATTGTAAAA
GCATTTTGTAAATAATAAAGAATAGCTTTAATGATATGCTTGTAACATAAATAA
TTTTGTAATGTATCAAATACATTTAAAACATTAAAATATAATCTCTATAATAA

**Exemplary expanded Human GJB2 Genomic DNA Sequence including certain
regulatory regions (SEQ ID NO: 122)**

GACTGTGAACTTAAGGCACAGCAGAGCTGGGGCTGCTCTTAAGGCCCTGCTG
TCTCTCCTCTTAGTAACAACACCATTTACATGAAGTGACAGTGGTATCTTTT
GTTGCCCTGGAAATGGAATACAACAATGGCTTTCCAACCTTTTCTGTGGCAGAG
ACCTACAGACAGAAGTACATTTTACACTGGATCCAGGACACACATCAGTCTG
AAAACACACACATGAACCAAACGTTTCCTAAAGCATTACTTATCCTTGCTAAT
AGCAACACATTCTCATATTCTTTTATACTTCATTTAATTTTCATATAAAAAAGA
AAAGGAAAGGAAAGAAATCTATTTCTCAGCCCATTAATAAGGTCAGGAGCAG
CAACACCAGACTAGAAGAAAAGCTTACCTATAGATTTTTCTGCCACCTCTTGA
GTGCGTCCAGCTTTCCGACAAGTCTCAGTGCCATCTACTGTGCGCTCTGGGTA
TTGCAATTGCTTTTTTTTTTTTTTTTTTTTTTTTTTTTGAATGAGACTAAGTCAG
AGAACACAAAGAACTTCTTTCCCCACAGTGGAGATGGCTCTGAAAGCGTTTA
AGGAATAGCTTAGATGAGTGGCTAACACATTCTCCCGGTTCTGAATTCTAAGA
CCACAGACTCCATGTCCAGTCCCCAAAGAGAGGCTTTGCAAGCTACAGAATA
CCCCTCTGACTGGGACCTCAGGAGCTAACTGACCACGTAATTGGTTCTAGAA
AGTGAAACGTTTTAATTTGAAACATCCAAATGAGCATTTTGTGAAAAGCTACT
GCCGTCCATCAAATACAACACAGCCAGGGAGTCATCGCTCTATTGCCCTTGTC
AATCCTACATCTATAGTTTTTTTTTGCTACAGCAGTTCATGAGTGTGACTCTAT
TCTAACTTGTTCCAGAAGCCCTTCAAGATGATAGATAGCACAAATTTTTTGTA

GCCAGAGCTAGAATGTAGAGCTCTTTTTGGCTTCCTTGTGAATGATCCAGAAT
TTCCATGTTGGCAAGCCACCATAATTTACAGAATTTACTTTTTATATTCAATAG
AAGTAAAAAAATTTACCTATTTAAGGAGTTATAGCTCTGGATTCATTTCTGA
CCAAAATGTGCTTTTTGACACAAATACAATTGGAAATGTCTTTGTAATTTATC
CACAGTCTGCCTAGATAATCATAAAAGAACTGCATGGATATATTTGTGAGTA
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CCTTGAAACGGAGACACATCTAGCAGTTTGAATTTCCCCCAAAGATTGTATG
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GAACAATTAAATTATTGAAGGCTGGAATTTTTTTTTTAACCTTTGAATGGAAC
AACAGATTTTCCCCAAAAGATTTGACTTTAACAATTTTCAGAAAACATAAGTC
AGGGTGTGGTTCAATTACACAGAGAGAAATTGTAGTGAAATAGTGTTCCCTG
TAATAATTACCCACAAAGGAGCACAGTGGAGCCACTCCTGCATTAAAATTAC
AGTATCATATGTAAGTTATTATTAATTAACCAGAGATGCCAGGAGCTTGTCAG
TTTCCAACCTGCTATTTTGAGGAGAGCTAAAGTTTCTCTTTTTTTGCCAGTTATT
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AAGTGGTAGGACAGTCTCTTCGCGTCTTGCATCATTTTTCTGCCATCACCTACG
TGTGATTCGTGAGTCGGAAATTCAACCAAGACATGTTTAATGTATATTTAGAG
CATTCTTCCCGGCGGGAATTCACGGTGCCATTCCATCAGGCAGTTGGCAAGCA
GTCACCTTGAAATATTAAGAAATATGATTTGTGTCACACTGATTTATTGCAAAA
CAGCAACTTCTTTCTTTTTGGTTCATTTATAAAACAACTGTCAAATTAATATGC
CAAATAGCTTTAAACATTAGCATTTTACCTTATAACCTTACAAGTGCATCAC
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GCATGGTACTAAGCATATATGTAAAAATATTACTGATGGGTATCTCTGGCACT
CTGAAGTGACAAAGTGTAGCCTTCACAGATCTTTGTCAGTTAATCATCAATAG
TTACCTGAAAAGTGCCCACTTGCCATCATTCAGATCAACCAGGCAGACACC
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GTCCTCACCATGGGGCTGGGTGGCTGGGGTGGAGGAACACAGGATTGGGCTT
CAGCTTCTCTAGGGACTGGTACATTAAGAGATGAAGACATAAAAGGTGAGAA
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GAAAAAAAATGCAGCAATATAAGTGTGTAATTTACAAAATAATTTACAGGATT

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CTCTCCTCCTTCTCCTCCCCTACACTGGAGGACACTATGTACATGCATATAAT
GTCCTGCCCTAGAGGAGTCCTGAGCCTACTTGGGAAGAAAACACCAACTCAC
AGGAAAACAGCAGAAATCACACAAAACAGAATAAAAGCAAGCGCTGATCTG
TAAGTGAAGACTTAAGTGCTATAGGACTTCCAGCTACAAATCCTGAAAACAC
GGAGTGGCTGTGATAATACGACTAGCCAACATCACACAGTAATTTTGCACAT
AAGGAGAATAAATCAAAGAAAACAAGGAAAAGAAAGTTGAGCCTATAATC
GTGATACAGGCACTAAAATCTCAGGTGACATTTTCAATGGGGGAAAGTCAG
TCAACTTCCGATCTCCAAACCATCTTTACTAGCGAGCTTCCCACAATGGTTCT
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ACAGCCTTTGAGAAATTAAAGGGAGAACCCACCAACCGGCGCCCCACTCCCC
ACCCCAAGTCACCTCTGGCTCAACCAAGATGCGCTCAGGCCAAGAAAGCTGC
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TCAGATGGGCCATGCAAGGCTTTTCGCAGCTCCTGGGGCTTTGCCTCTTCATG
AGCAGACACTCCCTCTTAGACTAAGACCTGGAGCTGGAAAGTAGGTGGTAAC
CGCGGTACAAAACCTCACGCTCGTCCCTGCAGAACTGCCTAGGTGCGCCCAT
GGCCACGGGGCGCCAATTTTCAAGGAAAAGTCAATGCTAATAATGGTGGCA
ATCACGGGAAATCCATTCTGAGGCCAGATCTGACTTGTGAGGATTAATCATCA
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AGGCTGCACCTCTGACCTGGCTCCCCTCTGCAAAAATCGCGAAGTGGGTGCCC
GAGGTGGGGCGGGGGTTGGGGGAGACCTCCCCGGGAGTCCCCACCCAGCCTG
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ATTTGTATCGCAAGACATCAAAATCCCCAACCAATGCAAATACTGAGACCT
CATAATCTGAGACAAAGTTTCACGGTATCCAGAAAGCCCCCAGCAGGTGTGC
AGTGCAGAGCCAGCCCCCAGCGGTCTTCCGCAGAATCCTATCAGTTTCCCCC
TTTCGTGCTGTGTGCATCGAGCAGGAAGGGGCTTGGCAGGTTTTACCTGCCCT
CTTTCCTTTCTGAAAAGTCTGGGCCTCCTCACCCCGAAAGGAGTCACCTCCTT
GCAGTTCCCCAGTTGCGAAAAGAGGAGGAAGTTGGCTGGGCCGGGGGCCGCG
GGGGGCACCTCCGCAGATGGCGGGACCCCCCTGCCGGCCATGGCAAAAACG
AGGCTTGTCTCTCCACCGCCCCCAACCTTAGTCCTTGGCACATTGTTGAAAG
TAATTGAATAAAAATCGGAAATTCGAGAAGGCGTTCGTTCGGATTGGTGAGAT
TTTGAGGGGAGAAAGAAGCGGGGACTTCGCCGGCACACAGCGGCGCCCCCTCC

TCGGCCACCGTTAACCCCCATTCCAGAGGGCACTGCCCCGCCACCCAGCCTAG
GTCCCCCTGCGAGAGCCTCGCGGGCCCGCGCAGCCTCCGCGACTCGAACAGA
TCTTCAGTCCTTGGAGGAATGCCTGTTTCTCTAACAATAAAAAATTAAAGAAG
CGCTCATAAATGCCAAGTCCTCTCGCACTATGCGGAGTACAGAGGACAACGA
CCACAGCCATCCCTGAACCCCGCCACGGCACAGCGCCGAGCCGGGGTCTG
GGGCGCCGCTTCTGGGGGGTCCCGACTCTCAGCCGCCCCCGCTTCACCCGGG
CCGCCAAGGGGCTGGGGGAGGCGGCGCTCGGGGTAACCGGGGGAGACTCAG
GGCGCTGGGGGCACTTGGGGAACATCGGGGGCTCAAAGGAACTAGGAGATC
GGGACCTCGAAGGGGACTTGGGGGGTTCGGGGCTTTCGGGGGCGGTTCGGGGG
TTCGCGGACCCGGGAAGCTCTGAGGACCCAGAGGCCGGGCGCGCTCCGCCCG
CGGCGCCGCCCCCTCCGTAACCTTCCCAGTCTCCGAGGGAAGAGGCGGGGTG
TGGGGTGCGGTTAAAAGGCGCCACGGCGGGAGACAGGTGTTGCGGCCCCGCA
GCGCCCGCGCGCTCCTCTCCCCGACTCGGAGCCCCTCGGCGGCGCCCGGCCA
GGACCCGCCTAGGAGCGCAGGAGCCCCAGCGCAGAGACCCCAACGCCGAGA
CCCCCGCCCCGGCCCCGCGCGCTTCTCCCGACGCAGGTGAGCCCGCCGGCC
CCGACTGCCCGGCCAGGAACCTGGCGCGGGGAGGGACCGCGAGACCCAGA
GCGGTTGCCCCGCCGCGTGGGTCTCGGGGAACCGGGGGGCTGGACCAACACA
CGTCTTGGGCCGGGGGCGGGGGCCGCCTTCTGGAGCGGGCGTTTCTGCGG
CCGAGCTCCGGAGCTGGAATGGGGCGGCCGGGGAAGTGGACGCGATGGCAC
CGCCCGGGGTGCGAGTGGGGCCGGGCGCGCGCGGGAGGGGAAAAAGGCGCG
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CTAGCGCAGCCGCCTGGCTACTTGACCCACTGCCACCAAACGTTTTAAATTCA
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ACCTGGGACTGCCTTGAGAAGCGTGGTACGGCCGTGTCCCATGTGACCTTAG
AGTCCCTTTCGAAACTGCTGTGCACAGTCGGTCACAATTCAGACACTGGTGA
GAAGGGTGGAGGAACCCTCTGGGGACAGCCAGGCAAGGTCGACCACCCATC

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TCCTGGTGATTACCTAGTGTCTTCCCTAAGGAACTGAACCAACTCCTCCGCT
GGCCTCTGGCAGCCCTCCAGGCGGTGCAGGATGGCGTGGGCCCCGGTAGGAAG
CTGCATGTAACCGCCCAGGGTCGGGAGGCCAGGAGGGCAGCTCCTCCTCTGA
CTTGAATATTGAAAACAACCTTCGTCCTGCTTCTGAGCCCCCTCTTAACCCATGA
CCCCCTAGCCCATTTGGGGAGTAAATCTTAATTTACTCCTCTTCCTGAAAAAGG
ATCTTTAAAACAGGTAGCTTCAACTCAAGCTTTATAAAAATAACAATATAGGGT
TTCTCGGAACTGTATTTTTCTCAGCTGATGGTAACTGGACAGGTCTGTAGAAG
GGTGTATGACCTGGGTTTGGCAGGTGGAAGAGGGCAAAGGATAAACCCCTCC
TCCTGCAGCCCCATATTCTTGGCCAGGTGTATTGTTGTAAACCAGGAGAGAGT
TTACTTCGGGGAGTATCCTGTTTTCCACTCAGTGAGGGCCAATGAAGAATGTC
TAATTCATAAGATGCTTTTGTTAAAATCGGAATGTTGCTGTCCTCGGTGGTT
CTGCTGTTGGGACGGGACTGGCCTGAGCTGTGGGTGCTGTAGCAGGACAACC
AGCTCACCTAAGGGCCTCCCAGTCTGGATTATCAATGGGTCAGTGCTGAACCT
GGGCTAAAATATTGTTTTTTCCAATGATGTTGTCTTTCCCAAGCTCAGTGAAG
CTAAATGTTTCACAGGCCTATGTCAATCTGATGTAACCTTCGTGGCCACCTCT
CTCCTGTTAGCCTCTGACCAAGGTGGCACTGGATGGTTTCTGCCTGACCTTGG
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TCTCCACATCCGCTTGCAGTAAGGAGTGTGGGACTCTCCCAAGCTTCAGCGCT
GAACTGCAATGCAGTGACGTCGCTTAGCTGGGCCAGTAACCGAGGGAGTTGA
ATTTTCTGTCATTTTAAAATAATGTGTCTTTTAAAGAAACACTTTGAAATTTAA
ACCACAGCCCACAATTATAATGCACTGTTGCAGCACTTATCAAAACAGATAT
GCTAACTGAGCCATCAGTGCCAGCCTGACAGTGAGGCCACCAAGCCATCCAC
AAAGCCTACACGAAAGTCTGTGCTCACAGTGGCTTTTCTCCATGAAGAGGGC
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TAACTCGGGGTGCCTCAAATGTAACTTAACCACTGGTAACAACAGTTTCACT
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AGCAGAGAGACTAGACATCTTATCCTCACGGTTCTCCTGTACTTGGCCTCTCA

GCCTTTGAGCAAGGTTGGCCCAAGCTAGTATCGGCCCCAGTGGTACAGCCAA
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CATTTAATCCTATGACAACTAAGTTGGTTCTGTCTTCACCTGTTTTGGTGAGG
TTGTGTAAGAGTTGGTGTTTGCTCAGGAAGAGATTTAAGCATGCTTGCTTACC
CAGACTCAGAGAAGTCTCCCTGTTCTGTCCTAGCTAGTGATTCCCTGTGTTGTG
TGCATTTCGTCTTTTCCAGAGCAAACCGCCCAGAGTAGAAGATGGATTGGGGC
ACGCTGCAGACGATCCTGGGGGGTGTGAACAAACACTCCACCAGCATTGGAA
AGATCTGGCTCACCGTCCTCTTCATTTTTTCGATTATGATCCTCGTTGTGGCTG
CAAAGGAGGTGTGGGGAGATGAGCAGGCCGACTTTGTCTGCAACACCCTGCA
GCCAGGCTGCAAGAACGTGTGCTACGATCACTACTTCCCCATCTCCCACATCC
GGCTATGGGCCCTGCAGCTGATCTTCGTGTCCACGCCAGCGCTCCTAGTGGCC
ATGCACGTGGCCTACCGGAGACATGAGAAGAAGAGGAAGTTCATCAAGGGG
GAGATAAAGAGTGAATTTAAGGACATCGAGGAGATCAAAACCCAGAAGGTC
CGCATCGAAGGCTCCCTGTGGTGGACCTACACAAGCAGCATCTTCTTCCGGGT
CATCTTCGAAGCCGCCTTCATGTACGTCTTCTATGTCATGTACGACGGCTTCTC
CATGCAGCGGCTGGTGAAGTGCAACGCCTGGCCTTGTCCTCAACACTGTGGAC
TGCTTTGTGTCCCGGCCACGGAGAAGACTGTCTTCACAGTGTTTCATGATTGC
AGTGTCTGGAATTTGCATCCTGCTGAATGTCACTGAATTGTGTTATTTGCTAAT
TAGATATTGTTCTGGGAAGTCAAAAAAGCCAGTTTAACGCATTGCCCAGTTGT
TAGATTAAGAAATAGACAGCATGAGAGGGATGAGGCAACCCGTGCTCAGCTG
TCAAGGCTCAGTCGCTAGCATTTCCTCAACACAAAGATTCTGACCTTAAATGCA
ACCATTTGAAACCCCTGTAGGCCTCAGGTGAACTCCAGATGCCACAATGGA
GCTCTGCTCCCCTAAAGCCTCAAAACAAAGGCCTAATTCTATGCCTGTCTTAA
TTTTCTTTCACTTAAGTTAGTTCCACTGAGACCCAGGCTGTTAGGGGTTATTG
GTGTAAGGTACTTTCATATTTTAAACAGAGGATATCGGCATTTGTTTCTTCTC
TGAGGACAAGAGAAAAAGCCAGGTTCACAGAGGACACAGAGAAGGTTTG
GGTGTCTCCTGGGGTTCTTTTTGCCAACTTTCCCCACGTAAAGGTGAACATT
GGTTCTTTCATTTGCTTTGGAAGTTTAAATCTCTAACAGTGGAACAAAGTTACC
AGTGCCTTAACTCTGTTACACTTTTTGGAAGTGAAACTTTGTAGTATGATA

GGTTATTTTGATGTAAAGATGTTCTGGATACCATTATATGTTCCCCCTGTTTCA
GAGGCTCAGATTGTAATATGTAAATGGTATGTCATTTCGCTACTATGATTTAAT
TTGAAATATGGTCTTTTGGTTATGAATACTTTGCAGCACAGCTGAGAGGCTGT
CTGTTGTATTTCATTGTGGTCATAGCACCTAACAAACATTGTAGCCTCAATCGAG
TGAGACAGACTAGAAGTTCCTAGTGATGGCTTATGATAGCAAATGGCCTCAT
GTCAAATATTTAGATGTAATTTTGTGTAAGAAATACAGACTGGATGTACCACC
AACTACTACCTGTAATGACAGGCCTGTCCAACACATCTCCCTTTTCCATGACT
GTGGTAGCCAGCATCGGAAAGAACGCTGATTTAAAGAGGTTCGCTTGGGAATT
TTATTGACACAGTACCATTTAATGGGGAGGACAAAATGGGGCAGGGGAGGGA
GAAGTTTCTGTTCGTTAAAAACAGATTTGGAAAGACTGGACTCTAAAGTCTGTT
GATTAAAGATGAGCTTTGTCTACTTCAAAAGTTTGTGTTGCTTACCCCTTCAGCC
TCCAATTTTAAAGTGAAAATATAGCTAATAACATGTGAAAAGAATAGAAGC
TAAGGTTTAGATAAATATTGAGCAGATCTATAGGAAGATTGAACCTGAATAT
TGCCATTATGCTTGACATGGTTTCCAAAAAATGGTACTCCACATATTTTCAGTG
AGGGTAAGTATTTTCCTGTTGTCAAGAATAGCATTGTAAAAGCATTGTTGTAAT
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TATGGTTTTAATAGAACAGCAAATTTTAATTTTCATCTATCACTTTTTATATAAA
TACATTAATGTTTTATATTTTCATAACACCAATGGGTAAGTTGCCAGAGTGTCT
GACCCCATCTGCCCCAGTTACAGAAAAGCTTCTGTCCAGAGAAAGTTTGGTG
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AGGTTTTTGGAGGCATATCAGTCTATGGACAATGTGGTGTTTGGTCTGGAAACG
TACCTTGGTGAATGCTGAGTTGGCTGGACATGACCCGTTTAGCTCCTGGATGA
ATCCCAGAAGTGGACCTTCAAAATGTTACTCATAGCATGACCTTGGCTCACTG
CAACCTCTGCCTCCCAGGCTCAAGCGATCCTCCCACCTCAGCGTCCCAAGTAG
CTGGGACCACTGGAGTGTGCCACCACACTCCACTAATTTTTTTCATTTTTTGTAG
AAACGAGGTCCCACTATATTGCCCAGTCTGGTCTCGAACTCCTGGGCTGAAGG
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GCACCTGGCCTGAACTGCTTTTTATTCTCAGTGCCCACTTCCATGGGAAAT
AAGCCTGCCAGGTCAGCCTGTCCCCATGGGAGTGACTGCCTGCTACCCCCACA
GGCTTGCCCGGCCCTCGTGAGCCTCTCCAGAGACACCACCAACAGTTCTGTT
CTTTCATGGTACAAGATTTCCATCCAAGGATTTCAAAGCATTTACACATCAA
TAATTAGAAGTATTTTCATAGAGGACCATACACTTTTAAATGGATTTCAAAG

AACAAAAACCAGTCAACTATCACCCAGGTAATAGAAAATGGGAAATGGTTTC
TACCTGACTTCCAAAATGCTCTGCACATAGACTGTGAAAATAGGATTTTTTAA
GCTGGGTGCAGAGGCTTATACCTATAATCCCAACACTTTGGGAGGCTGAGAC
GAGAGGATCACTTGAGCCCAGGAGTTCAAAACCAGCCTGGGCAATATAGGGA
GACATTGTTTCTATAAAAAATAAAAAATGTTAGCCAGGCAGGCGTGGTAACAT
GTGCCTGTAGTCTCAGCTACTCAGGAGGCTGAGGTGGGAAGATTGCTTGAAC
CTGGGAGGTCCATGCTGCAGTGAGCTGAGATTGTGCCACTGCACTCCAGCCTA
GGCGACAGCAAGATCCTGTCCCAAACAACAACATCAAAAAACACAGAA
CTTTTAAAATAAGTACATTCACTTCTACAAGCTATGTAGATTATTACTCTCAA
GCTATTAAAAGACCAAGCCAAAATAATTATGGGCTACTCTCGACCACTTGTA
GGAATGGATAGAGAGGTCTGGTCACATGCCTGGAAATTAGAGCTTGAGCTCT
GAAAATGATAATCCTGACTATATCTCAAAGCATCAGTCTGCACTTTGTATGGA
GCAAGAAAAAGCCTTGTGGAAGCGGCCTCCCACCCAGCCGAGCCCTCGGCGT
GGACAAGCTCTGCTTTTTATGAGCAGTGGGTGCAGCCTCGCTGCTCCCTCCTC
CTGTCAAAAGACAGTCACAGCTGGGGTGAGCAGATCGGGCCCACTTGGGAGG
CCCCAAGGAATATGCTGCAGGGGTGCGGCCTGAGCCACCCCAAGGGTTGGT
CTTTGACAAGTAGAGAGCAGCTGAGAGGTGGGTAAAAGCTCACTCACTTACC
CTGACCTCAGTGTCTCATCTTAAAATGGGTTTCCTGAATCTTTCCCGGCTTA
GTGGCAATGAAATAAGATAATTTATGTAAACGTTCTCCACATAGTAAAGCAC
TAAGTAACATATGACTGTCATCTGTTTTCCACTAGACAGATCCCAACCTGGAA
GAGTGACAGATGGTATTTTCAAGTACAAGTCAAGCAAAGCTTGATAAAC
TGGGGGCTGGAAAAAATGCACATTTACACAAAGCCTGGAGTAACTGC

[0063] In some aspects, the GJB2 gene is codon optimized. In some aspects, the codon optimized GJB2 gene as at least 70%, 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%, at least 99%, or 100 identity to any one of SEQ ID NOs: 123-126. In some aspects, the codon optimized GJB2 gene has the sequence of any one of SEQ ID NOs: 123-126.

Exemplary codon optimized Human GJB2 DNA Sequence (SEQ ID NO: 123)

ATGGACTGGGGCACCTGCAGACTATCCTGGGGGGCGTCAATAAGCATTCAA
CTAGCATCGGAAAGATTTGGCTGACTGTCCTGTTTATCTTTTCGGATCATGATC

CTGGTGGTGGCAGCAAAGGAAGTGTGGGGCGACGAGCAGGCCGATTTCGTGT
GCAACACACTGCAGCCAGGCTGCAAGAACGTGTGCTACGACCACTATTTTCC
CATCTCTCACATCAGGCTGTGGGCCCTGCAGCTGATCTTCGTGAGCACCCCTG
CCCTGCTGGTGGCAATGCACGTGGCCTATCGGAGACACGAGAAGAAGCGCAA
GTTTATCAAGGGCGAGATCAAGAGCGAGTTCAAGGATATCGAGGAGATCAAG
ACACAGAAGGTGAGGATCGAGGGCTCCCTGTGGTGGACCTACACAAGCTCCA
TCTTCTTTCGCGTGATCTTCGAGGCCGCCTTTATGTACGTGTTCTATGTGATGT
ACGACGGCTTTTCTATGCAGCGGCTGGTGAAGTGCAACGCCTGGCCCTGTCTT
AATACAGTGGATTGTTTCGTGTCCAGACCCACCGAGAAGACAGTGTTACCGT
GTTTATGATCGCCGTGTCTGGCATCTGCATCCTGCTGAACGTGACCGAGCTGT
GCTATCTGCTGATCCGGTACTGTAGTGGAAAGAGCAAAAAACCCGTG

Exemplary codon optimized Human GJB2 DNA Sequence (SEQ ID NO: 124)

ATGGACTGGGGAACATTGCAAACATTTTTGGGAGGAGTCAACAAGCATTCAA
CTAGCATCGGGAAGATCTGGCTGACCGTGCTGTTTCATCTTTCGCATCATGATT
CTCGTGGTGGCCGCTAAGGAAGTCTGGGGCGATGAACAGGCCGACTTCGTGT
GTAACACGCTGCAGCCCGGTTGCAAAAACGTCTGCTACGATCACTACTTCCCC
ATCTCACACATTAGACTGTGGGCGCTGCAGCTGATTTTCGTGTCCACCCGGC
ACTTCTTGTGGCGATGCACGTGGCCTACCGGCGGCACGAGAAGAAAAGGAAG
TTCATTAAGGGCGAAATCAAGTCCGAGTTCAAGGACATCGAAGAAATCAAGA
CCCAGAAGGTCCGCATTGAGGGCTCCCTCTGGTGGACCTACACCTCGTCCATC
TTCTTCCGGGTCATATTCGAGGCCGCCTTTATGTACGTGTTTTACGTGATGTAC
GACGGTTTCAGCATGCAAAGACTCGTCAAGTGCAACGCTTGGCCTTGCCCCA
ATACCGTGGATTGCTTCGTGTCCCGCCCGACCGAGAAAACGTGTGTTCACTGTG
TTCATGATCGCCGTGTCCGGCATCTGCATCCTGCTGAACGTGACCGAGCTGTG
CTATCTCCTGATCCGGTACTGTAGCGGAAAGTCGAAGAAGCCTGTG

Exemplary codon optimized Human GJB2 DNA Sequence (SEQ ID NO: 125)

ATGGATTGGGGGACGCTCCAGACTATACTTGGCGGGGTAAACAAACATTCCA
CCTCAATTGGCAAAATCTGGCTCACAGTCCTCTTCATCTTCAGAATAATGATA
CTCGTGGTTGCCGCTAAAGAAGTTTGGGGTGACGAGCAAGCCGATTTTCGTCTG
TAACACCCCTCCAACCAGGTTGCAAAAATGTCTGTTACGATCACTACTTTCCTA
TTAGCCATATTAGACTCTGGGCCCTGCAACTTATCTTCGTTTCCACTCCTGCTC

TGCTCGTCGCTATGCACGTTGCCTATCGCCGCCATGAAAAAAAAACGGAAATTC
ATTAAGGGAGAGATTAAGAGTGAATTC AAGGATATTGAAGAGATTAAAACGC
AAAAAGTTAGAATTGAGGGATCACTGTGGTGGACTTATACCAGTAGCATCTTT
TTTAGGGTCATTTTCGAAGCTGCTTTCATGTATGTTTTCTATGTAATGTACGAC
GGTTTCTCCATGCAACGCTTGGTTAAATGTAACGCCTGGCCATGCCCTAATAC
GGTTGATTGCTTTGTCTCCCGCCCTACTGAAAAGACAGTGTTTACCGTTTTTCAT
GATCGCCGTAAGTGGAATTTGTATCCTTCTTAACGTGACCGAGTTGTGCTATC
TCCTTATTCGCTACTGTTTCAGGAAAAAGTAAAAAACAGTA

Exemplary codon optimized Human GJB2 DNA Sequence (SEQ ID NO: 126)

ATGGACTGGGGCACGCTGCAGACTATCCTGGGGGGTGTCAACAAGCATTCAA
CTAGCATCGGAAAGATCTGGCTGACCGTCCTGTTTCATCTTTCGCATCATGATC
CTCGTGGTGGCCGCTAAGGAAGTGTGGGGCGACGAGCAGGCCGATTTTCGTGT
GTAACACCCTGCAGCCAGGTTGCAAAAACGTCTGCTACGATCACTACTTTCCC
ATCTCCCACATTAGACTGTGGGCCCTGCAGCTGATCTTCGTGTCCACCCCTGC
GCTGCTAGTGGCCATGCACGTGGCCTATCGGCGACACGAGAAGAAACGGAAG
TTCATTAAGGGCGAGATCAAGAGCGAGTTCAAGGATATCGAAGAGATCAAGA
CCCAGAAGGTCCGCATTGAGGGCTCCCTGTGGTGGACCTACACCAGCTCCATC
TTCTTTCGGGTCATCTTCGAGGCCGCCTTTATGTACGTGTTCTATGTGATGTAC
GACGGTTTCTCCATGCAACGGCTGGTGAAGTGCAACGCCTGGCCTTGCCCTAA
TACTGTGGATTGCTTCGTGTCCCGCCCCACCGAGAAGACAGTGTTACCGTGT
TCATGATCGCCGTGTCTGGCATCTGCATCCTGCTGAACGTGACCGAGCTGTGC
TATCTCCTGATCCGGTACTGTAGTGGAAGTCAAAAAAACAGTGTA

[0064] The present disclosure recognizes that certain changes to a polynucleotide sequence will not impact its expression or a protein encoded by said polynucleotide. In some aspects, a polynucleotide comprises a GJB2 gene having one or more silent mutations. In some aspects, the disclosure provides a polynucleotide that comprises a GJB2 gene having one or more silent mutations, e.g., a GJB2 gene having a sequence different from SEQ ID NOs: 117-126 but encoding the same amino acid sequence as a functional GJB2 gene. In some aspects, the disclosure provides a polynucleotide that comprises a GJB2 gene having a sequence different from SEQ ID NO: 117-126 that encodes an amino acid sequence including one or more mutations (e.g., a different amino

acid sequence when compared to that produced from a functional GJB2 gene), where the one or more mutations are conservative amino acid substitutions.

[0065] In some aspects, the disclosure provides a polynucleotide that comprises a GJB2 gene having a sequence different from SEQ ID NO: 117-126 that encodes an amino acid sequence including one or more mutations (e.g., a different amino acid sequence when compared to that produced from a functional GJB2 gene), where the one or more mutations are not within a characteristic portion of a GJB2 gene or an encoded connexin 26 protein. In some aspects, a polynucleotide in accordance with the present disclosure comprises a GJB2 gene that is at least 70%, 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 a sequence of SEQ ID NO: 117-126. In some aspects, a polynucleotide in accordance with the present disclosure comprises a GJB2 gene that is identical to the sequence of SEQ ID NO: 117-126. As can be appreciated in the art, SEQ ID NO: 117-126 can be optimized (e.g., codon optimized) to achieve increased or optimal expression in an animal, e.g., a mammal, e.g., a human.

[0066] Among other things, the present disclosure provides polypeptides encoded by a GJB2 gene or characteristic portion thereof. In some aspects, a GJB2 gene is a mammalian GJB2 gene. In some aspects, a GJB2 gene is a murine GJB2 gene. In some aspects, a GJB2 gene is a primate GJB2 gene. In some aspects, a GJB2 gene is a human GJB2 gene.

[0067] In some aspects, a polypeptide comprises a connexin 26 protein or characteristic portion thereof. In some aspects, a connexin 26 protein or characteristic portion thereof is mammalian connexin 26 protein or characteristic portion thereof, e.g., primate connexin 26 protein or characteristic portion thereof. In some aspects, a connexin 26 protein or characteristic portion thereof is a human connexin 26 protein or characteristic portion thereof.

[0068] In some aspects, a polypeptide provided herein comprises post-translational modifications. In some aspects, a connexin 26 protein or characteristic portion thereof provided herein comprises post-translational modifications. In some aspects, post-translational modifications can comprise but is not limited to glycosylation (e.g., N-linked glycosylation, O-linked glycosylation), phosphorylation, acetylation, amidation, hydroxylation, methylation, ubiquitylation, sulfation, and/or a combination thereof. An

exemplary human connexin 26 protein sequence is or includes the sequence of SEQ ID NO: 127.

Exemplary Human Connexin 26 Protein Sequence (SEQ ID NO: 127)

MDWGTLQTLGGVNVKHSTSIGKIWLTVLFIFRIMILVVAAKEVWGDEQADFVCNT
LQPGCKNVCDHYFPISHIRLWALQLIFVSTPALLVAMHVAYRRHEKKRKFIDGE
IKSEFKDIEEIKTQKVRIEGLWWTYTSSIFFRVIFEAAAFMYVFYVMYDGFMSQRL
VKCNAWPCPNTVDCFVSRPTEKTVFTVFMIAVSGICILLNVTELCYLLIRYCSGKS
KKPV

[0069] The present disclosure recognizes that certain mutations in an amino acid sequence of a polypeptide described herein (e.g., including connexin 26 or a characteristic portion thereof) will not impact the expression, folding, or activity of the polypeptide. In some aspects, a polypeptide (e.g., including connexin 26 or a characteristic portion thereof) includes one or more mutations, where the one or more mutations are conservative amino acid substitutions. In some aspects, a polypeptide in accordance with the present disclosure comprises a connexin 26 or a characteristic portion thereof that is at least 70%, 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 a sequence of SEQ ID NO: 127. In some aspects, a polypeptide in accordance with the present disclosure comprises a connexin 26 or a characteristic portion thereof that is identical to the sequence of SEQ ID NO: 127. In some aspects, a polypeptide in accordance with the present disclosure comprises a connexin 26 or a characteristic portion thereof that is at least 70%, 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 a sequence of SEQ ID NO: 127. In some aspects, a polypeptide in accordance with the present disclosure comprises a connexin 26 protein or a characteristic portion thereof that is identical to the sequence of SEQ ID NO: 127.

[0070] In some aspects, the polypeptide is a therapeutic polypeptide (e.g., a Connexin 26 polypeptide). In some aspects, the polypeptide is a supporting cell polypeptide (e.g., a Connexin 26 polypeptide). In some aspects, the polypeptide is a reporter polypeptide.

Supporting Cell Polypeptides

- [0071]** Certain aspects of the disclosure are directed to polynucleotides encoding a supporting cell polypeptide (e.g., a Connexin 26 polypeptide). The polynucleotide can encode a polypeptide that is capable of being expressed in a cell (e.g., an inner ear cell). In some aspects, the supporting cell polypeptide (e.g., a Connexin 26 polypeptide) is a polypeptide that is endogenously expressed in supporting cells of the inner ear. In some aspects, the inner ear supporting cells are selected from one or more of inner phalangeal cells/border cells (IPhC), inner pillar cells (IPC), outer pillar cells (OPC), Deiters' cells rows 1 and 2 (DC1/2), Deiters' cells row 3 (DC3), Hensen's cells (Hec), Claudius cells/outer sulcus cells (CC/OSC), interdental cells (Idc), inner sulcus cells (ISC), Kölliker's organ cells (KO), greater ridge epithelial ridge cells (GER) (including lateral greater epithelial ridge cells (LGER)), and OC90+ cells (OC90), fibroblasts, and other cells of the lateral wall. The polynucleotide can encode a full length polypeptide or a functional fragment thereof.
- [0072]** Exemplary supporting cell polypeptides encoded by the polynucleotide include, but are not limited to, transmembrane proteins, enzymes, growth factors, cytokines, receptors, receptor ligands, hormones, membrane proteins, membrane-associated proteins, antigens, and antibodies.
- [0073]** Exemplary supporting cell polynucleotides encoding polypeptides include, but are not limited to, ATPase Plasma Membrane Ca²⁺ Transporting 2 (ATP2B2), Cholinergic Receptor Nicotinic Alpha 9 Subunit (CHRNA9), Cadherin 23 (CDH23), Coiled-coil Glutamate Rich Protein 2 (CCER2), Clarin 1 (CLRN1), Clarin 2 (CLRN2), cochlin (COCH or DFNA9), Dystrotelin (DYTN), Epidermal Growth Factor Receptor Pathway Substrate 8 (EPS8), EPS8 Like 2 (EPS8L2), Espin (ESPN), Espin Like (ESPNL), Gap junction protein beta 2 (GJB2), Gap junction protein beta 6 (GJB6), Gap junction protein beta 3 (GJB3), gasdermin E protein (GSDME or DFNA5), Insulinoma-associated 1 (INSM1), Ikaros family zinc finger 2 (IKZF2), LIM Homeobox Protein 3 (LHX3), Myosin 7A (MYO7A), Myosin 11 (MYO3A), Norrin cystine knot growth factor (NDP), Protocadherin 15 (PCDH15), Protein Tyrosine Phosphatase, Receptor Type Q (PTPRQ), Stereocilin (STRC), Protein Network Component Harmonin (USH1C), Usherin (USH2A), and Spectrin repeat containing nuclear envelope family member 4 (SYNE4). In some aspects, the polynucleotide comprises a gap junction protein beta 2 (GJB2) gene. In some aspects, the polynucleotide encodes a gap junction protein beta 2 polypeptide. In

some aspects, the polynucleotide encodes a Connexin 26 polypeptide. In some aspects, the supporting cell polypeptide is a gap junction protein beta 2 polypeptide. In some aspects, the supporting cell polypeptide is a Connexin 26 polypeptide.

[0074] In some aspects, a polynucleotide in accordance with the present disclosure comprises a GJB2 gene that is at least 70%, 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 a sequence of SEQ ID NO: 117-126. In some aspects, a polynucleotide in accordance with the present disclosure comprises a GJB2 gene that is identical to the sequence of SEQ ID NO: 117-126. As can be appreciated in the art, SEQ ID NO: 117-126 can be optimized (e.g., codon optimized) to achieve increased or optimal expression in an animal, e.g., a mammal, e.g., a human.

[0075] In some aspects, a polypeptide in accordance with the present disclosure comprises a connexin 26 or a characteristic portion thereof that is at least 70%, 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 a sequence of SEQ ID NO: 127. In some aspects, a polypeptide in accordance with the present disclosure comprises a connexin 26 or a characteristic portion thereof that is identical to the sequence of SEQ ID NO: 127. In some aspects, a polypeptide in accordance with the present disclosure comprises a connexin 26 or a characteristic portion thereof that is at least 70%, 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 a sequence of SEQ ID NO: 127. In some aspects, a polypeptide in accordance with the present disclosure comprises a connexin 26 protein or a characteristic portion thereof that is identical to the sequence of SEQ ID NO: 127.

[0076]

Therapeutic Polypeptides

[0077] Certain aspects of the disclosure are directed to polynucleotides encoding a polypeptide (e.g., a therapeutic polypeptide, a Connexin 26 polypeptide). The polynucleotide can encode a polypeptide that is capable of being expressed in a cell (e.g., an inner ear cell). The polynucleotide can encode a full length polypeptide or a functional fragment thereof.

[0078] Exemplary polypeptides encoded by the polynucleotide include, but are not limited to, transmembrane proteins, enzymes, growth factors, cytokines, receptors,

receptor ligands, hormones, membrane proteins, membrane-associated proteins, antigens, and antibodies.

[0079] Exemplary polynucleotides encoding therapeutic polypeptides (e.g., a Connexin 26 polypeptide) include, but are not limited to, ATPase Plasma Membrane Ca²⁺ Transporting 2 (ATP2B2), Cholinergic Receptor Nicotinic Alpha 9 Subunit (CHRNA9), Cadherin 23 (CDH23), Coiled-coil Glutamate Rich Protein 2 (CCER2), Clarin 1 (CLRN1), Clarin 2 (CLRN2), cochlin (COCH or DFNA9), Dystrotelin (DYTN), Epidermal Growth Factor Receptor Pathway Substrate 8 (EPS8), EPS8 Like 2 (EPS8L2), Espin (ESPN), Espin Like (ESPNL), Gap junction protein beta 2 (GJB2), Gap junction protein beta 6 (GJB6), Gap junction protein beta 3 (GJB3), gasdermin E protein (GSDME or DFNA5), Insulinoma-associated 1 (INSM1), Ikaros family zinc finger 2 (IKZF2), LIM Homeobox Protein 3 (LHX3), Myosin 7A (MYO7A), Myosin 11 (MYO3A), Norrin cystine knot growth factor (NDP), Protocadherin 15 (PCDH15), Protein Tyrosine Phosphatase, Receptor Type Q (PTPRQ), Stereocilin (STRC), Protein Network Component Harmonin (USH1C), Usherin (USH2A), and Spectrin repeat containing nuclear envelope family member 4 (SYNE4). In some aspects, the polynucleotide comprises a gap junction protein beta 2 (GJB2) gene. In some aspects, the polynucleotide encodes a gap junction protein beta 2 polypeptide. In some aspects, the polynucleotide encodes a Connexin 26 polypeptide. In some aspects, the therapeutic polypeptide is a gap junction protein beta 2 polypeptide. In some aspects, the therapeutic polypeptide is a Connexin 26 polypeptide.

[0080] In some aspects, a polynucleotide in accordance with the present disclosure comprises a GJB2 gene that is at least 70%, 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 a sequence of SEQ ID NO: 117-126. In some aspects, a polynucleotide in accordance with the present disclosure comprises a GJB2 gene that is identical to the sequence of SEQ ID NO: 117-126. As can be appreciated in the art, SEQ ID NO: 117-126 can be optimized (e.g., codon optimized) to achieve increased or optimal expression in an animal, e.g., a mammal, e.g., a human.

[0081] In some aspects, a polypeptide in accordance with the present disclosure comprises a connexin 26 or a characteristic portion thereof that is at least 70%, 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 a sequence of SEQ ID NO: 127. In some aspects, a polypeptide in accordance with the present disclosure comprises a connexin 26 or a characteristic portion thereof that is identical to the sequence of SEQ ID NO: 127. In some aspects, a polypeptide in accordance with the present disclosure comprises a connexin 26 or a characteristic portion thereof that is at least 70%, 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 a sequence of SEQ ID NO: 127. In some aspects, a polypeptide in accordance with the present disclosure comprises a connexin 26 protein or a characteristic portion thereof that is identical to the sequence of SEQ ID NO: 127.

Constructs

[0082] Among other things, the present disclosure provides that some polynucleotides as described herein are polynucleotide constructs. Polynucleotide constructs according to the present disclosure include all those known in the art, including cosmids, plasmids (e.g., naked or contained in liposomes) and viral constructs (e.g., lentiviral, retroviral, adenoviral, and adeno-associated viral constructs) that incorporate a polynucleotide comprising a nucleic acid sequence (e.g., GJB2 gene) or characteristic portion thereof encoding a polypeptide (e.g., Connexin 26). Those of skill in the art will be capable of selecting suitable constructs, as well as cells, for making any of the polynucleotides described herein. In some aspects, a construct is a plasmid (i.e., a circular DNA molecule that can autonomously replicate inside a cell). In some aspects, a construct can be a cosmid (e.g., pWE or sCos series). In some aspects, the construct is a mammalian or a viral vector.

[0083] In some aspects, a construct is a viral construct. In some aspects, a viral construct is a lentivirus, retrovirus, adenovirus, or adeno-associated virus construct. In some aspects, a construct is an adeno-associated virus (AAV) construct (see, e.g., Asokan et al., Mol. Ther. 20: 699-7080, 2012, which is incorporated in its entirety herein by reference). In some aspects, the construct is a viral vector. In some aspects, the construct is a lentivirus, retrovirus, adenovirus, or adeno-associated virus vector. In some aspects, the construct is an AAV vector. In some aspects, a viral construct is an adenovirus construct. In some aspects, a viral construct may also be based on or derived from an alphavirus. Alphaviruses include Sindbis (and VEEV) virus, Aura virus, Babanki virus, Barmah Forest virus, Bebaru virus, Cabassou virus, Chikungunya virus, Eastern equine encephalitis virus, Everglades virus, Fort Morgan virus, Getah virus, Highlands J virus,

Kyzylagach virus, Mayaro virus, Me Tri virus, Middelburg virus, Mosso das Pedras virus, Mucambo virus, Ndumu virus, O'nyong-nyong virus, Pixuna virus, Rio Negro virus, Ross River virus, Salmon pancreas disease virus, Semliki Forest virus, Southern elephant seal virus, Tonate virus, Trocara virus, Una virus, Venezuelan equine encephalitis virus, Western equine encephalitis virus, and Whataroa virus. Generally, the genome of such viruses encode nonstructural (e.g., replicon) and structural proteins (e.g., capsid and envelope) that can be translated in the cytoplasm of the host cell. Ross River virus, Sindbis virus, Semliki Forest virus (SFV), and Venezuelan equine encephalitis virus (VEEV) have all been used to develop viral constructs for coding sequence delivery. Pseudotyped viruses may be formed by combining alphaviral envelope glycoproteins and retroviral capsids. Examples of alphaviral constructs can be found in U.S. Publication Nos. 20150050243, 20090305344, and 20060177819; constructs and methods of their making are incorporated herein by reference to each of the publications in its entirety.

[0084] Constructs provided herein can be of different sizes. In some aspects, a construct 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 8 kb, 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 aspects, a construct 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, 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.

[0085] In some aspects, a construct is a viral construct and can have a total number of nucleotides of up to 10 kb. In some aspects, a viral construct 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.

[0086] In some aspects, a construct is a lentivirus construct and can have a total number of nucleotides of up to 8 kb. In some examples, a lentivirus construct 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 8 kb, about 6 kb to about 7 kb, or about 7 kb to about 8 kb.

[0087] In some aspects, a construct is an adeno-associated virus construct and can have a total number of nucleotides of up to 8 kb. In some aspects, an adeno-associated virus construct 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 kb, about 6 kb to about 8 kb, or about 7 kb to about 8 kb.

[0088] In some aspects, a construct is an adenovirus construct and can have a total number of nucleotides of up to 8 kb. In some aspects, an adenovirus construct 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 kb, about 6 kb to about 8 kb, or about 7 kb to about 8 kb.

[0089] Any of the constructs 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 (poly(A)) sequence, a Kozak consensus sequence, and/or additional untranslated regions which may house pre- or post-transcriptional regulatory and/or control elements. In some aspects, a promoter can be a native promoter, a constitutive promoter, an inducible promoter, and/or a tissue-specific promoter. Non-limiting examples of control sequences are described herein.

[0090] In some aspects, the construct comprises a polynucleotide encoding a therapeutic polypeptide (e.g., a Connexin 26 polypeptide) operably linked to a promoter which selectively expresses the polynucleotide in an inner ear support cell. In some aspects, the construct comprise a 5' ITR, a promoter which selectively expresses the polynucleotide in an inner ear support cell, a 5' UTR, a polynucleotide encoding a therapeutic polypeptide (e.g., a Connexin 26 polypeptide), a 3' UTR, a polyA, and a 3' ITR. In some aspects, the construct comprise a 5' ITR, a promoter which selectively expresses the polynucleotide in an inner ear support cell, a 5' UTR, a polynucleotide encoding a therapeutic polypeptide (e.g., a Connexin 26 polypeptide), a tag, a 3' UTR, a polyA, and a 3' ITR. In some aspects, the construct comprise a 5' ITR, a promoter which selectively expresses the polynucleotide in an inner ear support cell, a 5' UTR, a polynucleotide encoding a therapeutic polypeptide (e.g., a Connexin 26 polypeptide), a tag, a 3' UTR, a microRNA regulatory target site, a polyA, and a 3' ITR.

[0091] In some aspects, the construct comprises a polynucleotide encoding a polypeptide operably linked to a promoter which selectively expresses the polynucleotide in an inner ear support cell. In some aspects, the construct comprise a 5' ITR, a promoter which selectively expresses the polynucleotide in an inner ear support cell, a 5' UTR, a polynucleotide encoding a polypeptide (e.g., a Connexin 26 polypeptide), a 3' UTR, a polyA, and a 3' ITR. In some aspects, the construct comprise a 5' ITR, a promoter which

selectively expresses the polynucleotide in an inner ear support cell, a 5' UTR, a polynucleotide encoding a polypeptide (e.g., a Connexin 26 polypeptide), a tag, a 3' UTR, a polyA, and a 3' ITR. In some aspects, the construct comprises a 5' ITR, a promoter which selectively expresses the polynucleotide in an inner ear support cell, a 5' UTR, a polynucleotide encoding a polypeptide (e.g., a Connexin 26 polypeptide), a tag, a 3' UTR, a microRNA regulatory target site, a polyA, and a 3' ITR.

[0092] In some aspects, the construct comprises a polynucleotide encoding a therapeutic polypeptide (e.g., a Connexin 26 polypeptide) operably linked to a promoter, wherein the construct comprises a miRNA regulatory target site for a microRNA expressed in an inner ear cell (e.g., a hair cell). In some aspects, the construct comprises a 5' ITR, a promoter which selectively expresses the polynucleotide in an inner ear support cell, a 5' UTR, a polynucleotide encoding a therapeutic polypeptide (e.g., a Connexin 26 polypeptide), a 3' UTR, a microRNA regulatory target site, a polyA, and a 3' ITR. In some aspects, the construct comprises a 5' ITR, a promoter which selectively expresses the polynucleotide in an inner ear support cell, a 5' UTR, a polynucleotide encoding a therapeutic polypeptide (e.g., a Connexin 26 polypeptide), a tag, a 3' UTR, a microRNA regulatory target site, a polyA, and a 3' ITR. In some aspects, the construct comprises a 5' ITR, a constitutive promoter, a 5' UTR, a polynucleotide encoding a therapeutic polypeptide (e.g., a Connexin 26 polypeptide), a 3' UTR, a microRNA regulatory target site, a polyA, and a 3' ITR. In some aspects, the construct comprises a 5' ITR, a constitutive promoter, a 5' UTR, a polynucleotide encoding a therapeutic polypeptide (e.g., a Connexin 26 polypeptide), a tag, a 3' UTR, a microRNA regulatory target site, a polyA, and a 3' ITR.

[0093] In some aspects, the construct comprises a polynucleotide encoding a polypeptide operably linked to a promoter, wherein the construct comprises a miRNA regulatory target site for a microRNA expressed in an inner ear cell (e.g., a hair cell). In some aspects, the construct comprises a 5' ITR, a promoter which selectively expresses the polynucleotide in an inner ear support cell, a 5' UTR, a polynucleotide encoding a polypeptide (e.g., a Connexin 26 polypeptide), a 3' UTR, a microRNA regulatory target site, a polyA, and a 3' ITR. In some aspects, the construct comprises a 5' ITR, a promoter which selectively expresses the polynucleotide in an inner ear support cell, a 5' UTR, a polynucleotide encoding a polypeptide (e.g., a Connexin 26 polypeptide), a tag, a 3' UTR, a microRNA regulatory target site, a polyA, and a 3' ITR. In some aspects, the construct

comprises a 5' ITR, a constitutive promoter, a 5' UTR, a polynucleotide encoding a polypeptide (e.g., a Connexin 26 polypeptide), a 3' UTR, a microRNA regulatory target site, a polyA, and a 3' ITR. In some aspects, the construct comprises a 5' ITR, a constitutive promoter, a 5' UTR, a polynucleotide encoding a polypeptide (e.g., a Connexin 26 polypeptide), a tag, a 3' UTR, a microRNA regulatory target site, a polyA, and a 3' ITR.

[0094] In some aspects, the construct comprises (i) a 5' inverted terminal repeat (ITR), (ii) a polynucleotide encoding a polypeptide (e.g., a Connexin 26 polypeptide) operably linked to a promoter which expresses the polynucleotide in an inner ear support cell, and (iii) a 3' ITR, wherein the promoter is heterologous to the polynucleotide.

[0095] In some aspects, the construct comprises (i) a 5' inverted terminal repeat (ITR), (ii) a polynucleotide encoding a polypeptide (e.g., a Connexin 26 polypeptide) operably linked to a promoter, and (iii) a 3' ITR, wherein the promoter comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NOs: 16, 28, 40, 57, or 90-99.

[0096] In some aspects, the construct comprises (i) a 5' inverted terminal repeat (ITR), (ii) a polynucleotide encoding a polypeptide (e.g., a Connexin 26 polypeptide) operably linked to a promoter, and (iii) a 3' ITR, wherein the promoter comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NO: 40. In some aspects, the construct comprises (i) a 5' inverted terminal repeat (ITR), (ii) a polynucleotide encoding a polypeptide (e.g., a Connexin 26 polypeptide) operably linked to a promoter, and (iii) a 3' ITR, wherein the promoter comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NO: 90. In some aspects, the construct comprises (i) a 5' inverted terminal repeat (ITR), (ii) a polynucleotide encoding a polypeptide (e.g., a Connexin 26 polypeptide) operably linked to a promoter, and (iii) a 3' ITR, wherein the promoter comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NO: 96. In some aspects, the construct comprises (i) a 5' inverted terminal repeat (ITR), (ii) a polynucleotide encoding a polypeptide (e.g., a Connexin 26 polypeptide) operably linked to a promoter, and (iii) a 3' ITR, wherein the promoter comprises a

nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NO: 99.

[0097] In some aspects, the construct comprises (i) a 5' inverted terminal repeat (ITR), (ii) a polynucleotide encoding a polypeptide (e.g., a Connexin 26 polypeptide) operably linked to a promoter, and (iii) a 3' ITR, wherein the promoter comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NO: 16. In some aspects, the construct comprises (i) a 5' inverted terminal repeat (ITR), (ii) a polynucleotide encoding a polypeptide (e.g., a Connexin 26 polypeptide) operably linked to a promoter, and (iii) a 3' ITR, wherein the promoter comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NO: 28. In some aspects, the construct comprises (i) a 5' inverted terminal repeat (ITR), (ii) a polynucleotide encoding a polypeptide (e.g., a Connexin 26 polypeptide) operably linked to a promoter, and (iii) a 3' ITR, wherein the promoter comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NO: 57. In some aspects, the construct comprises (i) a 5' inverted terminal repeat (ITR), (ii) a polynucleotide encoding a polypeptide (e.g., a Connexin 26 polypeptide) operably linked to a promoter, and (iii) a 3' ITR, wherein the promoter comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NO: 91. In some aspects, the construct comprises (i) a 5' inverted terminal repeat (ITR), (ii) a polynucleotide encoding a polypeptide (e.g., a Connexin 26 polypeptide) operably linked to a promoter, and (iii) a 3' ITR, wherein the promoter comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NO: 92. In some aspects, the construct comprises (i) a 5' inverted terminal repeat (ITR), (ii) a polynucleotide encoding a polypeptide (e.g., a Connexin 26 polypeptide) operably linked to a promoter, and (iii) a 3' ITR, wherein the promoter comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NO: 93. In some aspects, the construct comprises (i) a 5' inverted terminal repeat (ITR), (ii) a polynucleotide encoding a polypeptide (e.g., a Connexin 26 polypeptide) operably linked to a promoter, and (iii) a 3' ITR, wherein the promoter

comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NO: 94. In some aspects, the construct comprises (i) a 5' inverted terminal repeat (ITR), (ii) a polynucleotide encoding a polypeptide (e.g., a Connexin 26 polypeptide) operably linked to a promoter, and (iii) a 3' ITR, wherein the promoter comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NO: 95. In some aspects, the construct comprises (i) a 5' inverted terminal repeat (ITR), (ii) a polynucleotide encoding a polypeptide (e.g., a Connexin 26 polypeptide) operably linked to a promoter, and (iii) a 3' ITR, wherein the promoter comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NO: 97. In some aspects, the construct comprises (i) a 5' inverted terminal repeat (ITR), (ii) a polynucleotide encoding a polypeptide (e.g., a Connexin 26 polypeptide) operably linked to a promoter, and (iii) a 3' ITR, wherein the promoter comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NO: 98.

[0098] In some aspects, the construct further comprises a minimal GJB2 promoter. In some aspects, the minimal GJB2 promoter comprises a nucleic acid sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 86.

[0099] In some aspects, the construct comprises (i) a 5' inverted terminal repeat (ITR), (ii) a polynucleotide encoding a polypeptide (e.g., a Connexin 26 polypeptide) operably linked to a promoter, and (iii) a 3' ITR, wherein the promoter comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NO: 40 and a minimal GJB2 promoter comprising a nucleic acid sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 86. In some aspects, the construct comprises (i) a 5' inverted terminal repeat (ITR), (ii) a polynucleotide encoding a polypeptide (e.g., a Connexin 26 polypeptide) operably linked to a promoter, and (iii) a 3' ITR, wherein the promoter comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID

NO: 90 and a minimal GJB2 promoter comprising a nucleic acid sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 86. In some aspects, the construct comprises (i) a 5' inverted terminal repeat (ITR), (ii) a polynucleotide encoding a polypeptide (e.g., a Connexin 26 polypeptide) operably linked to a promoter, and (iii) a 3' ITR, wherein the promoter comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NO: 96 and a minimal GJB2 promoter comprising a nucleic acid sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 86. In some aspects, the construct comprises (i) a 5' inverted terminal repeat (ITR), (ii) a polynucleotide encoding a polypeptide (e.g., a Connexin 26 polypeptide) operably linked to a promoter, and (iii) a 3' ITR, wherein the promoter comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NO: 99 and a minimal GJB2 promoter comprising a nucleic acid sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 86.

[0100] In some aspects, the construct comprises (i) a 5' inverted terminal repeat (ITR), (ii) a polynucleotide encoding a polypeptide (e.g., a Connexin 26 polypeptide) operably linked to a promoter, and (iii) a 3' ITR, wherein the promoter comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NO: 16 and a minimal GJB2 promoter comprising a nucleic acid sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 86. In some aspects, the construct comprises (i) a 5' inverted terminal repeat (ITR), (ii) a polynucleotide encoding a polypeptide (e.g., a Connexin 26 polypeptide) operably linked to a promoter, and (iii) a 3' ITR, wherein the promoter comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NO: 28 and a minimal GJB2 promoter comprising a nucleic acid sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 86. In some aspects, the construct comprises (i) a 5' inverted terminal repeat (ITR), (ii) a polynucleotide encoding a polypeptide (e.g., a

Connexin 26 polypeptide) operably linked to a promoter, and (iii) a 3' ITR, wherein the promoter comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NO: 57 and a minimal GJB2 promoter comprising a nucleic acid sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 86. In some aspects, the construct comprises (i) a 5' inverted terminal repeat (ITR), (ii) a polynucleotide encoding a polypeptide (e.g., a Connexin 26 polypeptide) operably linked to a promoter, and (iii) a 3' ITR, wherein the promoter comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NO: 91 and a minimal GJB2 promoter comprising a nucleic acid sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 86. In some aspects, the construct comprises (i) a 5' inverted terminal repeat (ITR), (ii) a polynucleotide encoding a polypeptide (e.g., a Connexin 26 polypeptide) operably linked to a promoter, and (iii) a 3' ITR, wherein the promoter comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NO: 92 and a minimal GJB2 promoter comprising a nucleic acid sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 86. In some aspects, the construct comprises (i) a 5' inverted terminal repeat (ITR), (ii) a polynucleotide encoding a polypeptide (e.g., a Connexin 26 polypeptide) operably linked to a promoter, and (iii) a 3' ITR, wherein the promoter comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NO: 93 and a minimal GJB2 promoter comprising a nucleic acid sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 86. In some aspects, the construct comprises (i) a 5' inverted terminal repeat (ITR), (ii) a polynucleotide encoding a polypeptide (e.g., a Connexin 26 polypeptide) operably linked to a promoter, and (iii) a 3' ITR, wherein the promoter comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NO: 94 and a minimal GJB2 promoter comprising a nucleic acid sequence at least 80%, at least 85%, at least 90%, at least 95%,

at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 86. In some aspects, the construct comprises (i) a 5' inverted terminal repeat (ITR), (ii) a polynucleotide encoding a polypeptide (e.g., a Connexin 26 polypeptide) operably linked to a promoter, and (iii) a 3' ITR, wherein the promoter comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NO: 95 and a minimal GJB2 promoter comprising a nucleic acid sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 86. In some aspects, the construct comprises (i) a 5' inverted terminal repeat (ITR), (ii) a polynucleotide encoding a polypeptide (e.g., a Connexin 26 polypeptide) operably linked to a promoter, and (iii) a 3' ITR, wherein the promoter comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NO: 97 and a minimal GJB2 promoter comprising a nucleic acid sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 86. In some aspects, the construct comprises (i) a 5' inverted terminal repeat (ITR), (ii) a polynucleotide encoding a polypeptide (e.g., a Connexin 26 polypeptide) operably linked to a promoter, and (iii) a 3' ITR, wherein the promoter comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NO: 98 and a minimal GJB2 promoter comprising a nucleic acid sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 86.

- [0101]** In some aspects, the construct comprises (i) the 5' inverted terminal repeat (ITR), (ii) a 5' untranslated region (UTR), (iii) the polynucleotide encoding a polypeptide (e.g., a Connexin 26 polypeptide) operably linked to a promoter which expresses the polynucleotide in an inner ear support cell, and (iv) the 3' ITR.
- [0102]** In some aspects, the construct comprises (i) the 5' inverted terminal repeat (ITR), (ii) a 5' untranslated region (UTR), (iii) the polynucleotide encoding a polypeptide (e.g., a Connexin 26 polypeptide) operably linked to a promoter, and (iv) the 3' ITR.
- [0103]** In some aspects, the construct comprises (i) the 5' inverted terminal repeat (ITR), (ii) the polynucleotide encoding a polypeptide (e.g., a Connexin 26 polypeptide) operably

linked to a promoter which expresses the polynucleotide in an inner ear support cell, (iii) a 3' untranslated region (UTR), and (iv) the 3' ITR.

[0104] In some aspects, the construct comprises (i) the 5' inverted terminal repeat (ITR), (ii) the polynucleotide encoding a polypeptide (e.g., a Connexin 26 polypeptide) operably linked to a promoter, (iii) a 3' untranslated region (UTR), and (iv) the 3' ITR.

[0105] In some aspects, the construct comprises (i) the 5' inverted terminal repeat (ITR), (ii) the polynucleotide encoding a polypeptide (e.g., a Connexin 26 polypeptide) operably linked to a promoter which expresses the polynucleotide in an inner ear support cell, (iii) a miRNA regulatory target site (miRTS) for a microRNA expressed in an inner ear cell (e.g., a hair cell), (iv) a 3' untranslated region (UTR), and (v) the 3' ITR.

[0106] In some aspects, the construct comprises (i) the 5' inverted terminal repeat (ITR), (ii) the polynucleotide encoding a polypeptide (e.g., a Connexin 26 polypeptide) operably linked to a promoter, (iii) a miRNA regulatory target site (miRTS) for a microRNA expressed in an inner ear cell (e.g., a hair cell), (iv) a 3' untranslated region (UTR), and (v) the 3' ITR.

[0107] In some aspects, the construct comprises (i) the 5' inverted terminal repeat (ITR), (ii) a 5' untranslated region (UTR), (iii) the polynucleotide encoding a polypeptide (e.g., a Connexin 26 polypeptide) operably linked to a promoter which expresses the polynucleotide in an inner ear support cell, (iv) a 3' UTR, and (v) the 3' ITR.

[0108] In some aspects, the construct comprises (i) the 5' inverted terminal repeat (ITR), (ii) a 5' untranslated region (UTR), (iii) the polynucleotide encoding a polypeptide (e.g., a Connexin 26 polypeptide) operably linked to a promoter, (iv) a 3' UTR, and (v) the 3' ITR.

[0109] In some aspects, the construct comprises (i) the 5' inverted terminal repeat (ITR), (ii) a 5' untranslated region (UTR), (iii) the polynucleotide encoding a polypeptide (e.g., a Connexin 26 polypeptide) operably linked to a promoter which expresses the polynucleotide in an inner ear support cell, (iii) a miRNA regulatory target site (miRTS) for a microRNA expressed in an inner ear cell (e.g., a hair cell), (v) a 3' UTR, and (vi) the 3' ITR.

[0110] In some aspects, the construct comprises (i) the 5' inverted terminal repeat (ITR), (ii) a 5' untranslated region (UTR), (iii) the polynucleotide encoding a polypeptide (e.g., a Connexin 26 polypeptide) operably linked to a promoter, (iv) a miRNA regulatory target

site (miRTS) for a microRNA expressed in an inner ear cell (e.g., a hair cell), (v) a 3' UTR, and (vi) the 3' ITR.

[0111] In some aspects, the construct comprises a polynucleotide encoding a polypeptide (e.g., a Connexin 26 polypeptide) operably linked to a promoter, wherein the construct comprises a miRNA regulatory target site (miRTS) for a microRNA expressed in an inner ear cell (e.g., a hair cell).

[0112] In some aspects, the construct comprises (i) a 5' inverted terminal repeat (ITR), (ii) the polynucleotide encoding a polypeptide (e.g., a Connexin 26 polypeptide) operably linked to a promoter, (iii) the miRNA regulatory target site (miRTS) for a microRNA expressed in an inner ear cell (e.g., a hair cell), and (iv) a 3' ITR.

[0113] In some aspects, the construct comprises (i) a 5' inverted terminal repeat (ITR), (ii) a 5' untranslated region (UTR), (iii) the polynucleotide encoding a polypeptide (e.g., a Connexin 26 polypeptide) operably linked to a promoter, (iv) the miRNA regulatory target site (miRTS) for a microRNA expressed in an inner ear cell (e.g., a hair cell), and (v) a 3' ITR.

[0114] In some aspects, the construct comprises (i) a 5' inverted terminal repeat (ITR), (ii) the polynucleotide encoding a polypeptide (e.g., a Connexin 26 polypeptide) operably linked to a promoter, (iii) the miRNA regulatory target site (miRTS) for a microRNA expressed in an inner ear cell (e.g., a hair cell), (iv) a 3' untranslated region (UTR) and (v) a 3' ITR.

[0115] In some aspects, the construct comprises (i) a 5' inverted terminal repeat (ITR), (ii) a 5' untranslated region (UTR), (iii) the polynucleotide encoding a polypeptide (e.g., a Connexin 26 polypeptide) operably linked to a promoter, (iv) the miRNA regulatory target site (miRTS) for a microRNA expressed in an inner ear cell (e.g., a hair cell), (v) a 3' UTR, and (vi) a 3' ITR.

[0116] In some aspects, the construct comprises (i) a 5' inverted terminal repeat (ITR), (ii) a polynucleotide encoding a polypeptide (e.g., a Connexin 26 polypeptide) operably linked to an inner ear supporting cell selective promoter and a minimal GJB2 promoter which expresses the polynucleotide in an inner ear support cell, and (iii) a 3' ITR, wherein the inner ear supporting cell selective promoter is heterologous to the polynucleotide.

[0117] In some aspects, the construct comprises (i) a 5' inverted terminal repeat (ITR), (ii) a polynucleotide encoding a polypeptide (e.g., a Connexin 26 polypeptide) operably

linked to an inner ear supporting cell selective promoter and a minimal GJB2 promoter, and (iii) a 3' ITR, wherein the inner ear supporting cell selective promoter comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NOs: 16, 28, 40, 57, or 90-99.

[0118] In some aspects, the construct comprises (i) a 5' inverted terminal repeat (ITR), (ii) a polynucleotide encoding a polypeptide (e.g., a Connexin 26 polypeptide) operably linked to a promoter, and (iii) a 3' ITR, wherein the promoter comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NO: 40. In some aspects, the construct comprises (i) a 5' inverted terminal repeat (ITR), (ii) a polynucleotide encoding a polypeptide (e.g., a Connexin 26 polypeptide) operably linked to a promoter, and (iii) a 3' ITR, wherein the promoter comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NO: 90. In some aspects, the construct comprises (i) a 5' inverted terminal repeat (ITR), (ii) a polynucleotide encoding a polypeptide (e.g., a Connexin 26 polypeptide) operably linked to a promoter, and (iii) a 3' ITR, wherein the promoter comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NO: 96. In some aspects, the construct comprises (i) a 5' inverted terminal repeat (ITR), (ii) a polynucleotide encoding a polypeptide (e.g., a Connexin 26 polypeptide) operably linked to a promoter, and (iii) a 3' ITR, wherein the promoter comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NO: 99.

[0119] In some aspects, the construct comprises (i) a 5' inverted terminal repeat (ITR), (ii) a polynucleotide encoding a polypeptide (e.g., a Connexin 26 polypeptide) operably linked to a promoter, and (iii) a 3' ITR, wherein the promoter comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NO: 16. In some aspects, the construct comprises (i) a 5' inverted terminal repeat (ITR), (ii) a polynucleotide encoding a polypeptide (e.g., a Connexin 26 polypeptide) operably linked to a promoter, and (iii) a 3' ITR, wherein the promoter comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or

100% identity to any one of SEQ ID NO: 28. In some aspects, the construct comprises (i) a 5' inverted terminal repeat (ITR), (ii) a polynucleotide encoding a polypeptide (e.g., a Connexin 26 polypeptide) operably linked to a promoter, and (iii) a 3' ITR, wherein the promoter comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NO: 57. In some aspects, the construct comprises (i) a 5' inverted terminal repeat (ITR), (ii) a polynucleotide encoding a polypeptide (e.g., a Connexin 26 polypeptide) operably linked to a promoter, and (iii) a 3' ITR, wherein the promoter comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NO: 91. In some aspects, the construct comprises (i) a 5' inverted terminal repeat (ITR), (ii) a polynucleotide encoding a polypeptide (e.g., a Connexin 26 polypeptide) operably linked to a promoter, and (iii) a 3' ITR, wherein the promoter comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NO: 92. In some aspects, the construct comprises (i) a 5' inverted terminal repeat (ITR), (ii) a polynucleotide encoding a polypeptide (e.g., a Connexin 26 polypeptide) operably linked to a promoter, and (iii) a 3' ITR, wherein the promoter comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NO: 93. In some aspects, the construct comprises (i) a 5' inverted terminal repeat (ITR), (ii) a polynucleotide encoding a polypeptide (e.g., a Connexin 26 polypeptide) operably linked to a promoter, and (iii) a 3' ITR, wherein the promoter comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NO: 94. In some aspects, the construct comprises (i) a 5' inverted terminal repeat (ITR), (ii) a polynucleotide encoding a polypeptide (e.g., a Connexin 26 polypeptide) operably linked to a promoter, and (iii) a 3' ITR, wherein the promoter comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NO: 95. In some aspects, the construct comprises (i) a 5' inverted terminal repeat (ITR), (ii) a polynucleotide encoding a polypeptide (e.g., a Connexin 26 polypeptide) operably linked to a promoter, and (iii) a 3' ITR, wherein the promoter comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or

100% identity to any one of SEQ ID NO: 97. In some aspects, the construct comprises (i) a 5' inverted terminal repeat (ITR), (ii) a polynucleotide encoding a polypeptide (e.g., a Connexin 26 polypeptide) operably linked to a promoter, and (iii) a 3' ITR, wherein the promoter comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NO: 98. In some aspects, the construct comprises (i) the 5' inverted terminal repeat (ITR), (ii) a 5' untranslated region (UTR), (iii) the polynucleotide encoding a polypeptide (e.g., a Connexin 26 polypeptide) operably linked to an inner ear supporting cell selective promoter and a minimal GJB2 promoter which expresses the polynucleotide in an inner ear support cell, and (iv) the 3' ITR.

[0120] In some aspects, the construct comprises (i) the 5' inverted terminal repeat (ITR), (ii) a 5' untranslated region (UTR), (iii) the polynucleotide encoding a polypeptide (e.g., a Connexin 26 polypeptide) operably linked to an inner ear supporting cell selective promoter and a minimal GJB2 promoter, and (iv) the 3' ITR.

[0121] In some aspects, the construct comprises (i) the 5' inverted terminal repeat (ITR), (ii) the polynucleotide encoding a polypeptide (e.g., a Connexin 26 polypeptide) operably linked to an inner ear supporting cell selective promoter and a minimal GJB2 promoter which expresses the polynucleotide in an inner ear support cell, (iii) a 3' untranslated region (UTR), and (iv) the 3' ITR.

[0122] In some aspects, the construct comprises (i) the 5' inverted terminal repeat (ITR), (ii) the polynucleotide encoding a polypeptide (e.g., a Connexin 26 polypeptide) operably linked to an inner ear supporting cell selective promoter and a minimal GJB2 promoter, (iii) a 3' untranslated region (UTR), and (iv) the 3' ITR.

[0123] In some aspects, the construct comprises (i) the 5' inverted terminal repeat (ITR), (ii) the polynucleotide encoding a polypeptide (e.g., a Connexin 26 polypeptide) operably linked to an inner ear supporting cell selective promoter and a minimal GJB2 promoter which expresses the polynucleotide in an inner ear support cell, (iii) a miRNA regulatory target site (miRTS) for a microRNA expressed in an inner ear cell (e.g., hair cell), (iv) a 3' untranslated region (UTR), and (v) the 3' ITR.

[0124] In some aspects, the construct comprises (i) the 5' inverted terminal repeat (ITR), (ii) the polynucleotide encoding a polypeptide (e.g., a Connexin 26 polypeptide) operably linked to an inner ear supporting cell selective promoter and a minimal GJB2 promoter,

(iii) a miRNA regulatory target site (miRTS) for a microRNA expressed in an inner ear cell (e.g., a hair cell), (iv) a 3' untranslated region (UTR), and (v) the 3' ITR.

[0125] In some aspects, the construct comprises (i) the 5' inverted terminal repeat (ITR), (ii) a 5' untranslated region (UTR), (iii) the polynucleotide encoding a polypeptide (e.g., a Connexin 26 polypeptide) operably linked to an inner ear supporting cell selective promoter and a minimal GJB2 promoter which expresses the polynucleotide in an inner ear support cell, (iv) a 3' UTR, and (v) the 3' ITR.

[0126] In some aspects, the construct comprises (i) the 5' inverted terminal repeat (ITR), (ii) a 5' untranslated region (UTR), (iii) the polynucleotide encoding a polypeptide (e.g., a Connexin 26 polypeptide) operably linked to an inner ear supporting cell selective promoter and a minimal GJB2 promoter, (iv) a 3' UTR, and (v) the 3' ITR.

[0127] In some aspects, the construct comprises (i) the 5' inverted terminal repeat (ITR), (ii) a 5' untranslated region (UTR), (iii) the polynucleotide encoding a polypeptide (e.g., a Connexin 26 polypeptide) operably linked to an inner ear supporting cell selective promoter and a minimal GJB2 promoter which expresses the polynucleotide in an inner ear support cell, (iii) a miRNA regulatory target site (miRTS) for a microRNA expressed in an inner ear cell (e.g., a hair cell), (v) a 3' UTR, and (vi) the 3' ITR.

[0128] In some aspects, the construct comprises (i) the 5' inverted terminal repeat (ITR), (ii) a 5' untranslated region (UTR), (iii) the polynucleotide encoding a polypeptide (e.g., a Connexin 26 polypeptide) operably linked to an inner ear supporting cell selective promoter and a minimal GJB2 promoter, (iv) a miRNA regulatory target site (miRTS) for a microRNA expressed in an inner ear cell (e.g., a hair cell), (v) a 3' UTR, and (vi) the 3' ITR.

[0129] In some aspects, the construct comprises a polynucleotide encoding a polypeptide (e.g., a Connexin 26 polypeptide) operably linked to an inner ear supporting cell selective promoter and a minimal GJB2 promoter, wherein the construct comprises a miRNA regulatory target site (miRTS) for a microRNA expressed in an inner ear cell (e.g., a hair cell).

[0130] In some aspects, the construct comprises (i) a 5' inverted terminal repeat (ITR), (ii) the polynucleotide encoding a polypeptide (e.g., a Connexin 26 polypeptide) operably linked to an inner ear supporting cell selective promoter and a minimal GJB2 promoter, (iii) the miRNA regulatory target site (miRTS) for a microRNA expressed in an inner ear cell (e.g., a hair cell), and (iv) a 3' ITR.

- [0131] In some aspects, the construct comprises (i) a 5' inverted terminal repeat (ITR), (ii) a 5' untranslated region (UTR), (iii) the polynucleotide encoding a polypeptide (e.g., a Connexin 26 polypeptide) operably linked to an inner ear supporting cell selective promoter and a minimal GJB2 promoter, (iv) the miRNA regulatory target site (miRTS) for a microRNA expressed in an inner ear cell (e.g., a hair cell), and (v) a 3' ITR.
- [0132] In some aspects, the construct comprises (i) a 5' inverted terminal repeat (ITR), (ii) the polynucleotide encoding a polypeptide (e.g., a Connexin 26 polypeptide) operably linked to an inner ear supporting cell selective promoter and a minimal GJB2 promoter, (iii) the miRNA regulatory target site (miRTS) for a microRNA expressed in an inner ear cell (e.g., a hair cell), (iv) a 3' untranslated region (UTR) and (v) a 3' ITR.
- [0133] In some aspects, the construct comprises (i) a 5' inverted terminal repeat (ITR), (ii) a 5' untranslated region (UTR), (iii) the polynucleotide encoding a polypeptide (e.g., a Connexin 26 polypeptide) operably linked to an inner ear supporting cell selective promoter and a minimal GJB2 promoter, (iv) the miRNA regulatory target site (miRTS) for a microRNA expressed in an inner ear cell (e.g., a hair cell), (v) a 3' UTR, and (vi) a 3' ITR.
- [0134] In some aspects, the minimal GJB2 promoter comprises a nucleic acid sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 86.
- [0135] In some aspects, the construct comprises (i) a 5' inverted terminal repeat (ITR), (ii) a polynucleotide encoding a polypeptide (e.g., a Connexin 26 polypeptide) operably linked to a promoter, and (iii) a 3' ITR, wherein the promoter comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NO: 40 and a minimal GJB2 promoter comprising a nucleic acid sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 86. In some aspects, the construct comprises (i) a 5' inverted terminal repeat (ITR), (ii) a polynucleotide encoding a polypeptide (e.g., a Connexin 26 polypeptide) operably linked to a promoter, and (iii) a 3' ITR, wherein the promoter comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NO: 90 and a minimal GJB2 promoter comprising a nucleic acid sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least

99%, or 100% identity to SEQ ID NO: 86. In some aspects, the construct comprises (i) a 5' inverted terminal repeat (ITR), (ii) a polynucleotide encoding a polypeptide (e.g., a Connexin 26 polypeptide) operably linked to a promoter, and (iii) a 3' ITR, wherein the promoter comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NO: 96 and a minimal GJB2 promoter comprising a nucleic acid sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 86. In some aspects, the construct comprises (i) a 5' inverted terminal repeat (ITR), (ii) a polynucleotide encoding a polypeptide (e.g., a Connexin 26 polypeptide) operably linked to a promoter, and (iii) a 3' ITR, wherein the promoter comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NO: 99 and a minimal GJB2 promoter comprising a nucleic acid sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 86.

[0136] In some aspects, the construct comprises (i) a 5' inverted terminal repeat (ITR), (ii) a polynucleotide encoding a polypeptide (e.g., a Connexin 26 polypeptide) operably linked to a promoter, and (iii) a 3' ITR, wherein the promoter comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NO: 16 and a minimal GJB2 promoter comprising a nucleic acid sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 86. In some aspects, the construct comprises (i) a 5' inverted terminal repeat (ITR), (ii) a polynucleotide encoding a polypeptide (e.g., a Connexin 26 polypeptide) operably linked to a promoter, and (iii) a 3' ITR, wherein the promoter comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NO: 28 and a minimal GJB2 promoter comprising a nucleic acid sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 86. In some aspects, the construct comprises (i) a 5' inverted terminal repeat (ITR), (ii) a polynucleotide encoding a polypeptide (e.g., a Connexin 26 polypeptide) operably linked to a promoter, and (iii) a 3' ITR, wherein the promoter comprises a nucleic acid sequence having at least 85%, at least 90%, at least

95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NO: 57 and a minimal GJB2 promoter comprising a nucleic acid sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 86. In some aspects, the construct comprises (i) a 5' inverted terminal repeat (ITR), (ii) a polynucleotide encoding a polypeptide (e.g., a Connexin 26 polypeptide) operably linked to a promoter, and (iii) a 3' ITR, wherein the promoter comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NO: 91 and a minimal GJB2 promoter comprising a nucleic acid sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 86. In some aspects, the construct comprises (i) a 5' inverted terminal repeat (ITR), (ii) a polynucleotide encoding a polypeptide (e.g., a Connexin 26 polypeptide) operably linked to a promoter, and (iii) a 3' ITR, wherein the promoter comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NO: 92 and a minimal GJB2 promoter comprising a nucleic acid sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 86. In some aspects, the construct comprises (i) a 5' inverted terminal repeat (ITR), (ii) a polynucleotide encoding a polypeptide (e.g., a Connexin 26 polypeptide) operably linked to a promoter, and (iii) a 3' ITR, wherein the promoter comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NO: 93 and a minimal GJB2 promoter comprising a nucleic acid sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 86. In some aspects, the construct comprises (i) a 5' inverted terminal repeat (ITR), (ii) a polynucleotide encoding a polypeptide (e.g., a Connexin 26 polypeptide) operably linked to a promoter, and (iii) a 3' ITR, wherein the promoter comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NO: 94 and a minimal GJB2 promoter comprising a nucleic acid sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 86. In some aspects, the construct comprises (i) a 5' inverted terminal repeat (ITR), (ii) a

polynucleotide encoding a polypeptide (e.g., a Connexin 26 polypeptide) operably linked to a promoter, and (iii) a 3' ITR, wherein the promoter comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NO: 95 and a minimal GJB2 promoter comprising a nucleic acid sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO:

86. In some aspects, the construct comprises (i) a 5' inverted terminal repeat (ITR), (ii) a polynucleotide encoding a polypeptide (e.g., a Connexin 26 polypeptide) operably linked to a promoter, and (iii) a 3' ITR, wherein the promoter comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NO: 97 and a minimal GJB2 promoter comprising a nucleic acid sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO:

86. In some aspects, the construct comprises (i) a 5' inverted terminal repeat (ITR), (ii) a polynucleotide encoding a polypeptide (e.g., a Connexin 26 polypeptide) operably linked to a promoter, and (iii) a 3' ITR, wherein the promoter comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NO: 98 and a minimal GJB2 promoter comprising a nucleic acid sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO:

86.

AAV Particles

[0137] Among other things, the present disclosure provides AAV particles that comprise a construct encoding a therapeutic polypeptide (e.g., a Connexin 26 polypeptide), and a capsid described herein. Among other things, the present disclosure provides AAV particles that comprise a construct comprising a nucleic acid sequence (e.g., a gene) encoding a polypeptide, and a capsid described herein. In some aspects, AAV particles can be described as having a serotype, which is a description of the construct strain and the capsid strain. In some aspects, the AAV particle has an AAV1, AAV2, AAV3 (e.g., AAV3B), AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV2-tYF, AAV2-P2V2, AAV2-P2V3, AAV2-MeBtYFTV, AAV2-MeB, AAV2-P2V6, AAV2-DGEDF, or an AAV Anc80 serotype. In some aspects, the AAV particle has an AAVAnc80 serotype (including, for example, an AAVAnc80L65). In some aspects an

AAV particle may be described as AAV2, wherein the particle has an AAV2 capsid and a construct that comprises characteristic AAV2 Inverted Terminal Repeats (ITRs). In some aspects, an AAV particle may be described as a pseudotype, wherein the capsid and construct are derived from different AAV strains, for example, AAV2/9 would refer to an AAV particle that comprises a construct utilizing the AAV2 ITRs and an AAV9 capsid.

AAV Construct

[0138] The present disclosure provides constructs that comprise a nucleic acid sequence (e.g., a gene) encoding a polypeptide or characteristic portion thereof. In some aspects described herein, a construct comprising a nucleic acid sequence (e.g., a gene) encoding a polypeptide or characteristic portion thereof can be included in an AAV particle.

[0139] The present disclosure provides polynucleotide constructs that comprise a nucleic acid sequence (e.g., a gene) encoding a therapeutic polypeptide (e.g., a Connexin 26 polypeptide) or characteristic portion thereof. In some aspects described herein, a polynucleotide comprising a nucleic acid sequence (e.g., a gene) encoding a therapeutic polypeptide (e.g., a Connexin 26 polypeptide) or characteristic portion thereof can be included in an AAV particle.

[0140] In some aspects, a polynucleotide construct comprises one or more components derived from or modified from naturally occurring AAV genomic construct. In some aspects, a sequence derived from an AAV construct is an AAV1 construct, an AAV2 construct, an AAV3 construct, an AAV4 construct, an AAV5 construct, an AAV6 construct, an AAV7 construct, an AAV8 construct, an AAV9 construct, an AAV2.7m8 construct, an AAV8BP2 construct, an AAV293 construct, an AAV2-tYF construct, an AAV2-P2V2 construct, an AAV2-P2V3 construct, an AAV2-MeBtYFTV construct, an AAV2-MeB construct, an AAV2-P2V6 construct, an AAV2-DGEDF construct, or AAV Anc80 construct. In some aspects, the construct is derived from an AAV Anc80 construct (including, for example, an AAVAnc80L65). Additional exemplary AAV constructs 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, 2008; 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, 2004; each of which is incorporated in its entirety herein by reference.

[0141] In some aspects, provided constructs comprise coding sequence, e.g., a nucleic acid encoding polypeptide (e.g., a therapeutic polypeptide, a Connexin 26 polypeptide),

one or more regulatory and/or control sequences, and optionally 5' and 3' AAV derived inverted terminal repeats (ITRs). In some aspects wherein a 5' and 3' AAV derived ITR is utilized, the polynucleotide construct may be referred to as a recombinant AAV (rAAV) construct. In some aspects, provided rAAV constructs are packaged into an AAV capsid to form an AAV particle. In some aspects, an AAV capsid is an Anc80 capsid (e.g., an Anc80L65 capsid).

[0142] In some aspects, AAV derived sequences (which are comprised in a polynucleotide construct) typically include 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, which is incorporated herein by reference in its entirety). Typical AAV2-derived ITR sequences are about 145 nucleotides in length. In some aspects, 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 a construct provided herein. 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, each of which is incorporated in its entirety by reference). In some aspects, any of the coding sequences and/or constructs described herein are flanked by 5' and 3' AAV ITR sequences. The AAV ITR sequences may be obtained from any known AAV, including presently identified AAV types.

[0143] In some aspects, polynucleotide constructs described in accordance with this disclosure and in a pattern known to the art (see, e.g., Asokan et al., Mol. Ther. 20: 699-7080, 2012, which is incorporated herein by reference in its entirety) are typically comprised of, a coding sequence or a portion thereof, at least one and/or control sequence, and optionally 5' and 3' AAV inverted terminal repeats (ITRs). In some aspects, provided constructs can be packaged into a capsid to create an AAV particle. An AAV particle may be delivered to a selected target cell. In some aspects, provided constructs comprise an additional optional coding sequence that is a nucleic acid sequence (e.g., inhibitory nucleic acid sequence), heterologous to the construct sequences, which encodes a polypeptide, protein, functional RNA molecule (e.g., miRNA, miRNA inhibitor) or other gene product, of interest. In some aspects, a nucleic acid coding sequence is operatively linked to and/or control components in a manner that permits coding sequence transcription, translation, and/or expression in a cell of a target tissue.

[0144] As shown in FIG. 1A, an unmodified AAV endogenous genome includes two open reading frames, “cap” and “rep,” which are flanked by ITRs. As shown in FIG. 1B, exemplary rAAV constructs similarly include ITRs flanking a coding region, e.g., a coding sequence (e.g., a polynucleotide encoding a polypeptide (e.g., a therapeutic polypeptide, a Connexin 26 polypeptide)). In some aspects, a rAAV construct also comprises conventional control elements that are operably linked to the coding sequence in a manner that permits its transcription, translation and/or expression in a cell transfected with the plasmid construct or infected with the virus produced by the disclosure. In some aspects, a rAAV construct optionally comprises a promoter (shown in Figure 1, panel (B)), an enhancer, an untranslated region (e.g., a 5' UTR, 3' UTR), a Kozak sequence, an internal ribosomal entry site (IRES), splicing sites (e.g., an acceptor site, a donor site), a polyadenylation site (shown in FIG. 1, panel (B)), or any combination thereof. In some aspects, an rAAV construct comprises a promoter, a 5' UTR, and a polyadenylation site. In some aspects, an rAAV construct comprises a promoter, a 5' UTR, a 3' UTR, and a polyadenylation site. Such additional elements are described further herein.

[0145] In some aspects, a construct is an rAAV construct. In some aspects, an rAAV construct can include at least 500 bp, at least 1 kb, at least 1.5 kb, at least 2 kb, at least 2.5 kb, at least 3 kb, at least 3.5 kb, at least 4 kb, or at least 4.5 kb. In some aspects, an AAV construct can include at most 7.5 kb, at most 7 kb, at most 6.5 kb, at most 6 kb, at most 5.5 kb, at most 5 kb, at most 4.5 kb, at most 4 kb, at most 3.5 kb, at most 3 kb, or at most 2.5 kb. In some aspects, an AAV construct can include 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.

[0146] Any of the constructs described herein can further include regulatory and/or control sequences, 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 (poly(A)) sequence, a Kozak consensus sequence, and/or any combination thereof. In some aspects, a promoter can be a native promoter, a constitutive promoter, an inducible promoter, and/or a tissue-specific promoter. Non-limiting examples of control sequences are described herein.

- [0147] In some aspects, an adeno-associated virus (AAV) particle (e.g., an Anc80 particle) comprises a construct comprising: (i) a 5' inverted terminal repeat (ITR), (ii) a polynucleotide encoding a polypeptide (e.g., a Connexin 26 polypeptide) operably linked to a promoter which expresses the polynucleotide in an inner ear support cell, and (iii) a 3' ITR, wherein the promoter is heterologous to the polynucleotide. In some aspects, the promoter comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NOs: 16, 28, 40, 57, or 90-99.
- [0148] In some aspects, an adeno-associated virus (AAV) particle (e.g., an Anc80 particle) comprises a construct comprising: (i) a 5' inverted terminal repeat (ITR), (ii) a polynucleotide encoding a polypeptide (e.g., a Connexin 26 polypeptide) operably linked to a promoter having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NO: 40, and (iii) a 3' ITR, wherein the promoter is heterologous to the polynucleotide. In some aspects, an adeno-associated virus (AAV) particle (e.g., an Anc80 particle) comprises a construct comprising: (i) a 5' inverted terminal repeat (ITR), (ii) a polynucleotide encoding a polypeptide (e.g., a Connexin 26 polypeptide) operably linked to a promoter having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NO: 90, and (iii) a 3' ITR, wherein the promoter is heterologous to the polynucleotide. In some aspects, an adeno-associated virus (AAV) particle (e.g., an Anc80 particle) comprises a construct comprising: (i) a 5' inverted terminal repeat (ITR), (ii) a polynucleotide encoding a polypeptide (e.g., a Connexin 26 polypeptide) operably linked to a promoter having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NO: 96, and (iii) a 3' ITR, wherein the promoter is heterologous to the polynucleotide. In some aspects, an adeno-associated virus (AAV) particle (e.g., an Anc80 particle) comprises a construct comprising: (i) a 5' inverted terminal repeat (ITR), (ii) a polynucleotide encoding a polypeptide (e.g., a Connexin 26 polypeptide) operably linked to a promoter having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NO: 99, and (iii) a 3' ITR, wherein the promoter is heterologous to the polynucleotide.
- [0149] In some aspects, an adeno-associated virus (AAV) particle (e.g., an Anc80 particle) comprises a construct comprising: (i) a 5' inverted terminal repeat (ITR), (ii) a

polynucleotide encoding a polypeptide (e.g., a Connexin 26 polypeptide) operably linked to a promoter having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NO: 16, and (iii) a 3' ITR, wherein the promoter is heterologous to the polynucleotide. In some aspects, an adeno-associated virus (AAV) particle (e.g., an Anc80 particle) comprises a construct comprising: (i) a 5' inverted terminal repeat (ITR), (ii) a polynucleotide encoding a polypeptide (e.g., a Connexin 26 polypeptide) operably linked to a promoter having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NO: 28, and (iii) a 3' ITR, wherein the promoter is heterologous to the polynucleotide. In some aspects, an adeno-associated virus (AAV) particle (e.g., an Anc80 particle) comprises a construct comprising: (i) a 5' inverted terminal repeat (ITR), (ii) a polynucleotide encoding a polypeptide (e.g., a Connexin 26 polypeptide) operably linked to a promoter having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NO: 57, and (iii) a 3' ITR, wherein the promoter is heterologous to the polynucleotide. In some aspects, an adeno-associated virus (AAV) particle (e.g., an Anc80 particle) comprises a construct comprising: (i) a 5' inverted terminal repeat (ITR), (ii) a polynucleotide encoding a polypeptide (e.g., a Connexin 26 polypeptide) operably linked to a promoter having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NO: 91, and (iii) a 3' ITR, wherein the promoter is heterologous to the polynucleotide. In some aspects, an adeno-associated virus (AAV) particle (e.g., an Anc80 particle) comprises a construct comprising: (i) a 5' inverted terminal repeat (ITR), (ii) a polynucleotide encoding a polypeptide (e.g., a Connexin 26 polypeptide) operably linked to a promoter having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NO: 92, and (iii) a 3' ITR, wherein the promoter is heterologous to the polynucleotide. In some aspects, an adeno-associated virus (AAV) particle (e.g., an Anc80 particle) comprises a construct comprising: (i) a 5' inverted terminal repeat (ITR), (ii) a polynucleotide encoding a polypeptide (e.g., a Connexin 26 polypeptide) operably linked to a promoter having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NO: 93, and (iii) a 3' ITR, wherein the promoter is heterologous to the polynucleotide. In some aspects, an adeno-associated virus (AAV) particle (e.g., an

Anc80 particle) comprises a construct comprising: (i) a 5' inverted terminal repeat (ITR), (ii) a polynucleotide encoding a polypeptide (e.g., a Connexin 26 polypeptide) operably linked to a promoter having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NO: 94, and (iii) a 3' ITR, wherein the promoter is heterologous to the polynucleotide. In some aspects, an adeno-associated virus (AAV) particle (e.g., an Anc80 particle) comprises a construct comprising: (i) a 5' inverted terminal repeat (ITR), (ii) a polynucleotide encoding a polypeptide (e.g., a Connexin 26 polypeptide) operably linked to a promoter having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NO: 95, and (iii) a 3' ITR, wherein the promoter is heterologous to the polynucleotide. In some aspects, an adeno-associated virus (AAV) particle (e.g., an Anc80 particle) comprises a construct comprising: (i) a 5' inverted terminal repeat (ITR), (ii) a polynucleotide encoding a polypeptide (e.g., a Connexin 26 polypeptide) operably linked to a promoter having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NO: 97, and (iii) a 3' ITR, wherein the promoter is heterologous to the polynucleotide. In some aspects, an adeno-associated virus (AAV) particle (e.g., an Anc80 particle) comprises a construct comprising: (i) a 5' inverted terminal repeat (ITR), (ii) a polynucleotide encoding a polypeptide (e.g., a Connexin 26 polypeptide) operably linked to a promoter having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NO: 98, and (iii) a 3' ITR, wherein the promoter is heterologous to the polynucleotide.

[0150] In some aspects, the construct further comprises a minimal GJB2 promoter. In some aspects, the minimal GJB2 promoter comprises a nucleic acid sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 86.

[0151] In some aspects, an adeno-associated virus (AAV) particle (e.g., an Anc80 particle) comprises a construct comprising: (i) a 5' inverted terminal repeat (ITR), (ii) a polynucleotide encoding a polypeptide (e.g., a Connexin 26 polypeptide) operably linked to a promoter having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NO: 40 and a minimal GJB2 promoter comprises a nucleic acid sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity

to SEQ ID NO: 86, and (iii) a 3' ITR, wherein the promoter is heterologous to the polynucleotide. In some aspects, an adeno-associated virus (AAV) particle (e.g., an Anc80 particle) comprises a construct comprising: (i) a 5' inverted terminal repeat (ITR), (ii) a polynucleotide encoding a polypeptide (e.g., a Connexin 26 polypeptide) operably linked to a promoter having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NO: 90 and a minimal GJB2 promoter comprises a nucleic acid sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 86, and (iii) a 3' ITR, wherein the promoter is heterologous to the polynucleotide. In some aspects, an adeno-associated virus (AAV) particle (e.g., an Anc80 particle) comprises a construct comprising: (i) a 5' inverted terminal repeat (ITR), (ii) a polynucleotide encoding a polypeptide (e.g., a Connexin 26 polypeptide) operably linked to a promoter having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NO: 96 and a minimal GJB2 promoter comprises a nucleic acid sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 86, and (iii) a 3' ITR, wherein the promoter is heterologous to the polynucleotide. In some aspects, an adeno-associated virus (AAV) particle (e.g., an Anc80 particle) comprises a construct comprising: (i) a 5' inverted terminal repeat (ITR), (ii) a polynucleotide encoding a polypeptide (e.g., a Connexin 26 polypeptide) operably linked to a promoter having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NO: 99 and a minimal GJB2 promoter comprises a nucleic acid sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 86, and (iii) a 3' ITR, wherein the promoter is heterologous to the polynucleotide.

[0152] In some aspects, an adeno-associated virus (AAV) particle (e.g., an Anc80 particle) comprises a construct comprising: (i) a 5' inverted terminal repeat (ITR), (ii) a polynucleotide encoding a polypeptide (e.g., a Connexin 26 polypeptide) operably linked to a promoter having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NO: 16 and a minimal GJB2 promoter comprises a nucleic acid sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity

to SEQ ID NO: 86, and (iii) a 3' ITR, wherein the promoter is heterologous to the polynucleotide. In some aspects, an adeno-associated virus (AAV) particle (e.g., an Anc80 particle) comprises a construct comprising: (i) a 5' inverted terminal repeat (ITR), (ii) a polynucleotide encoding a polypeptide (e.g., a Connexin 26 polypeptide) operably linked to a promoter having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NO: 28 and a minimal GJB2 promoter comprises a nucleic acid sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 86, and (iii) a 3' ITR, wherein the promoter is heterologous to the polynucleotide. In some aspects, an adeno-associated virus (AAV) particle (e.g., an Anc80 particle) comprises a construct comprising: (i) a 5' inverted terminal repeat (ITR), (ii) a polynucleotide encoding a polypeptide (e.g., a Connexin 26 polypeptide) operably linked to a promoter having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NO: 57 and a minimal GJB2 promoter comprises a nucleic acid sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 86, and (iii) a 3' ITR, wherein the promoter is heterologous to the polynucleotide. In some aspects, an adeno-associated virus (AAV) particle (e.g., an Anc80 particle) comprises a construct comprising: (i) a 5' inverted terminal repeat (ITR), (ii) a polynucleotide encoding a polypeptide (e.g., a Connexin 26 polypeptide) operably linked to a promoter having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NO: 91 and a minimal GJB2 promoter comprises a nucleic acid sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 86, and (iii) a 3' ITR, wherein the promoter is heterologous to the polynucleotide. In some aspects, an adeno-associated virus (AAV) particle (e.g., an Anc80 particle) comprises a construct comprising: (i) a 5' inverted terminal repeat (ITR), (ii) a polynucleotide encoding a polypeptide (e.g., a Connexin 26 polypeptide) operably linked to a promoter having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NO: 92 and a minimal GJB2 promoter comprises a nucleic acid sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 86, and (iii) a 3' ITR, wherein the promoter is heterologous to the

polynucleotide. In some aspects, an adeno-associated virus (AAV) particle (e.g., an Anc80 particle) comprises a construct comprising: (i) a 5' inverted terminal repeat (ITR), (ii) a polynucleotide encoding a polypeptide (e.g., a Connexin 26 polypeptide) operably linked to a promoter having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NO: 93 and a minimal GJB2 promoter comprises a nucleic acid sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 86, and (iii) a 3' ITR, wherein the promoter is heterologous to the polynucleotide. In some aspects, an adeno-associated virus (AAV) particle (e.g., an Anc80 particle) comprises a construct comprising: (i) a 5' inverted terminal repeat (ITR), (ii) a polynucleotide encoding a polypeptide (e.g., a Connexin 26 polypeptide) operably linked to a promoter having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NO: 94 and a minimal GJB2 promoter comprises a nucleic acid sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 86, and (iii) a 3' ITR, wherein the promoter is heterologous to the polynucleotide. In some aspects, an adeno-associated virus (AAV) particle (e.g., an Anc80 particle) comprises a construct comprising: (i) a 5' inverted terminal repeat (ITR), (ii) a polynucleotide encoding a polypeptide (e.g., a Connexin 26 polypeptide) operably linked to a promoter having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NO: 95 and a minimal GJB2 promoter comprises a nucleic acid sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 86, and (iii) a 3' ITR, wherein the promoter is heterologous to the polynucleotide. In some aspects, an adeno-associated virus (AAV) particle (e.g., an Anc80 particle) comprises a construct comprising: (i) a 5' inverted terminal repeat (ITR), (ii) a polynucleotide encoding a polypeptide (e.g., a Connexin 26 polypeptide) operably linked to a promoter having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NO: 97 and a minimal GJB2 promoter comprises a nucleic acid sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 86, and (iii) a 3' ITR, wherein the promoter is heterologous to the polynucleotide. In some aspects, an adeno-associated virus (AAV) particle (e.g., an

Anc80 particle) comprises a construct comprising: (i) a 5' inverted terminal repeat (ITR), (ii) a polynucleotide encoding a polypeptide (e.g., a Connexin 26 polypeptide) operably linked to a promoter having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NO: 98 and a minimal GJB2 promoter comprises a nucleic acid sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 86, and (iii) a 3' ITR, wherein the promoter is heterologous to the polynucleotide. In some aspects, an adeno-associated virus (AAV) particle (e.g., an Anc80 particle) comprises a construct comprising: (i) a 5' inverted terminal repeat (ITR), (ii) a 5' untranslated region (UTR), (iii) a polynucleotide encoding a polypeptide (e.g., a Connexin 26 polypeptide) operably linked to a promoter which expresses the polynucleotide in an inner ear support cell, (iv) a 3' UTR, and (v) a 3' ITR, wherein the promoter is heterologous to the polynucleotide.

[0153] In some aspects, an adeno-associated virus (AAV) particle (e.g., an Anc80 particle) comprises a construct comprising: (i) a 5' inverted terminal repeat (ITR), (ii) a 5' untranslated region (UTR), (iii) a polynucleotide encoding a polypeptide (e.g., a Connexin 26 polypeptide) operably linked to a promoter, (iv) a 3' UTR, and (v) a 3' ITR, wherein the promoter comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NOs: 16, 28, 40, 57, or 90-99.

[0154] In some aspects, an adeno-associated virus (AAV) particle (e.g., an Anc80 particle) comprises a construct comprising: (i) a 5' inverted terminal repeat (ITR), (ii) a 5' untranslated region (UTR), (iii) a polynucleotide encoding a polypeptide (e.g., a Connexin 26 polypeptide) operably linked to a promoter, (iv) a 3' UTR, and (v) a 3' ITR, wherein the promoter comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NO: 40. In some aspects, an adeno-associated virus (AAV) particle (e.g., an Anc80 particle) comprises a construct comprising: (i) a 5' inverted terminal repeat (ITR), (ii) a 5' untranslated region (UTR), (iii) a polynucleotide encoding a polypeptide (e.g., a Connexin 26 polypeptide) operably linked to a promoter, (iv) a 3' UTR, and (v) a 3' ITR, wherein the promoter comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NO: 90. In some aspects, an adeno-associated virus (AAV) particle

particle (e.g., an Anc80 particle) comprises a construct comprising: (i) a 5' inverted terminal repeat (ITR), (ii) a 5' untranslated region (UTR), (iii) a polynucleotide encoding a polypeptide (e.g. a Connexin 26 polypeptide) operably linked to a promoter, (iv) a 3' UTR, and (v) a 3' ITR, wherein the promoter comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NO: 96. In some aspects, an adeno-associated virus (AAV) particle (e.g., an Anc80 particle) comprises a construct comprising: (i) a 5' inverted terminal repeat (ITR), (ii) a 5' untranslated region (UTR), (iii) a polynucleotide encoding a polypeptide (e.g. a Connexin 26 polypeptide) operably linked to a promoter, (iv) a 3' UTR, and (v) a 3' ITR, wherein the promoter comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NO: 99.

[0155] In some aspects, an adeno-associated virus (AAV) particle (e.g., an Anc80 particle) comprises a construct comprising: (i) a 5' inverted terminal repeat (ITR), (ii) a 5' untranslated region (UTR), (iii) a polynucleotide encoding a polypeptide (e.g. a Connexin 26 polypeptide) operably linked to a promoter, (iv) a 3' UTR, and (v) a 3' ITR, wherein the promoter comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NO: 16. In some aspects, an adeno-associated virus (AAV) particle (e.g., an Anc80 particle) comprises a construct comprising: (i) a 5' inverted terminal repeat (ITR), (ii) a 5' untranslated region (UTR), (iii) a polynucleotide encoding a polypeptide (e.g. a Connexin 26 polypeptide) operably linked to a promoter, (iv) a 3' UTR, and (v) a 3' ITR, wherein the promoter comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NO: 28. In some aspects, an adeno-associated virus (AAV) particle (e.g., an Anc80 particle) comprises a construct comprising: (i) a 5' inverted terminal repeat (ITR), (ii) a 5' untranslated region (UTR), (iii) a polynucleotide encoding a polypeptide (e.g. a Connexin 26 polypeptide) operably linked to a promoter, (iv) a 3' UTR, and (v) a 3' ITR, wherein the promoter comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NO: 57. In some aspects, an adeno-associated virus (AAV) particle (e.g., an Anc80 particle) comprises a construct comprising: (i) a 5' inverted terminal repeat (ITR), (ii) a 5' untranslated region (UTR),

(iii) a polynucleotide encoding a polypeptide (e.g. a Connexin 26 polypeptide) operably linked to a promoter, (iv) a 3' UTR, and (v) a 3' ITR, wherein the promoter comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NO: 91. In some aspects, an adeno-associated virus (AAV) particle (e.g., an Anc80 particle) comprises a construct comprising: (i) a 5' inverted terminal repeat (ITR), (ii) a 5' untranslated region (UTR), (iii) a polynucleotide encoding a polypeptide (e.g. a Connexin 26 polypeptide) operably linked to a promoter, (iv) a 3' UTR, and (v) a 3' ITR, wherein the promoter comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NO: 92. In some aspects, an adeno-associated virus (AAV) particle (e.g., an Anc80 particle) comprises a construct comprising: (i) a 5' inverted terminal repeat (ITR), (ii) a 5' untranslated region (UTR), (iii) a polynucleotide encoding a polypeptide (e.g. a Connexin 26 polypeptide) operably linked to a promoter, (iv) a 3' UTR, and (v) a 3' ITR, wherein the promoter comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NO: 93. In some aspects, an adeno-associated virus (AAV) particle (e.g., an Anc80 particle) comprises a construct comprising: (i) a 5' inverted terminal repeat (ITR), (ii) a 5' untranslated region (UTR), (iii) a polynucleotide encoding a polypeptide (e.g. a Connexin 26 polypeptide) operably linked to a promoter, (iv) a 3' UTR, and (v) a 3' ITR, wherein the promoter comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NO: 94. In some aspects, an adeno-associated virus (AAV) particle (e.g., an Anc80 particle) comprises a construct comprising: (i) a 5' inverted terminal repeat (ITR), (ii) a 5' untranslated region (UTR), (iii) a polynucleotide encoding a polypeptide (e.g. a Connexin 26 polypeptide) operably linked to a promoter, (iv) a 3' UTR, and (v) a 3' ITR, wherein the promoter comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NO: 96. In some aspects, an adeno-associated virus (AAV) particle (e.g., an Anc80 particle) comprises a construct comprising: (i) a 5' inverted terminal repeat (ITR), (ii) a 5' untranslated region (UTR), (iii) a polynucleotide encoding a polypeptide (e.g. a Connexin 26 polypeptide) operably linked to a promoter, (iv) a 3' UTR, and (v) a 3' ITR, wherein

the promoter comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NO: 97. In some aspects, an adeno-associated virus (AAV) particle (e.g., an Anc80 particle) comprises a construct comprising: (i) a 5' inverted terminal repeat (ITR), (ii) a 5' untranslated region (UTR), (iii) a polynucleotide encoding a polypeptide (e.g. a Connexin 26 polypeptide) operably linked to a promoter, (iv) a 3' UTR, and (v) a 3' ITR, wherein the promoter comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NO: 98.

[0156] In some aspects, an adeno-associated virus (AAV) particle (e.g., an Anc80 particle) comprises a construct comprising: (i) a 5' inverted terminal repeat (ITR), (ii) a 5' untranslated region (UTR), (iii) a polynucleotide encoding a polypeptide (e.g., a Connexin 26 polypeptide) operably linked to a promoter, (iv) a miRNA regulatory target site (miRTS) for a microRNA expressed in an inner ear cell (e.g., a hair cell), (v) a 3' UTR, and (vi) a 3' ITR.

[0157] In some aspects, an adeno-associated virus (AAV) particle (e.g., an Anc80 particle) comprises a construct comprising: (i) a 5' inverted terminal repeat (ITR), (ii) a 5' untranslated region (UTR), (iii) a polynucleotide encoding a polypeptide (e.g., a Connexin 26 polypeptide) operably linked to an inner ear supporting cell selective promoter and a minimal GJB2 promoter which expresses the polynucleotide in an inner ear support cell, (iv) a 3' UTR, and (v) a 3' ITR, wherein the inner ear supporting cell selective promoter is heterologous to the polynucleotide.

[0158] In some aspects, an adeno-associated virus (AAV) particle (e.g., an Anc80 particle) comprises a construct comprising: (i) a 5' inverted terminal repeat (ITR), (ii) a 5' untranslated region (UTR), (iii) a polynucleotide encoding a polypeptide (e.g., a Connexin 26 polypeptide) operably linked to an inner ear supporting cell selective promoter and a minimal GJB2 promoter, (iv) a 3' UTR, and (v) a 3' ITR, wherein the inner ear supporting cell selective promoter comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NOs: 16, 28, 40, 57, or 90-99.

[0159] In some aspects, an adeno-associated virus (AAV) particle (e.g., an Anc80 particle) comprises a construct comprising: (i) a 5' inverted terminal repeat (ITR), (ii) a 5' untranslated region (UTR), (iii) a polynucleotide encoding a polypeptide operably linked

to an inner ear supporting cell selective promoter and a minimal GJB2 promoter, (iv) a miRNA regulatory target site (miRTS) for a microRNA expressed in an inner ear cell, (v) a 3' UTR, and (vi) a 3' ITR.

[0160] In some aspects, an adeno-associated virus (AAV) particle (e.g., an Anc80 particle) comprises a construct comprising: (i) a 5' inverted terminal repeat (ITR), (ii) a 5' untranslated region (UTR), (iii) a polynucleotide encoding a polypeptide (e.g. a Connexin 26 polypeptide) operably linked to a promoter, (iv) a 3' UTR, and (v) a 3' ITR, wherein the promoter comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NO: 40 and a minimal GJB2 promoter comprises a nucleic acid sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 86. In some aspects, an adeno-associated virus (AAV) particle (e.g., an Anc80 particle) comprises a construct comprising: (i) a 5' inverted terminal repeat (ITR), (ii) a 5' untranslated region (UTR), (iii) a polynucleotide encoding a polypeptide (e.g. a Connexin 26 polypeptide) operably linked to a promoter, (iv) a 3' UTR, and (v) a 3' ITR, wherein the promoter comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NO: 90 and a minimal GJB2 promoter comprises a nucleic acid sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 86. In some aspects, an adeno-associated virus (AAV) particle (e.g., an Anc80 particle) comprises a construct comprising: (i) a 5' inverted terminal repeat (ITR), (ii) a 5' untranslated region (UTR), (iii) a polynucleotide encoding a polypeptide (e.g. a Connexin 26 polypeptide) operably linked to a promoter, (iv) a 3' UTR, and (v) a 3' ITR, wherein the promoter comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NO: 96 and a minimal GJB2 promoter comprises a nucleic acid sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 86. In some aspects, an adeno-associated virus (AAV) particle (e.g., an Anc80 particle) comprises a construct comprising: (i) a 5' inverted terminal repeat (ITR), (ii) a 5' untranslated region (UTR), (iii) a polynucleotide encoding a polypeptide (e.g. a Connexin 26 polypeptide) operably linked to a promoter, (iv) a 3' UTR, and (v) a 3' ITR, wherein

the promoter comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NO: 99 and a minimal GJB2 promoter comprises a nucleic acid sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 86.

[0161] In some aspects, an adeno-associated virus (AAV) particle (e.g., an Anc80 particle) comprises a construct comprising: (i) a 5' inverted terminal repeat (ITR), (ii) a 5' untranslated region (UTR), (iii) a polynucleotide encoding a polypeptide (e.g. a Connexin 26 polypeptide) operably linked to a promoter, (iv) a 3' UTR, and (v) a 3' ITR, wherein the promoter comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NO: 16 and a minimal GJB2 promoter comprises a nucleic acid sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 86. In some aspects, an adeno-associated virus (AAV) particle (e.g., an Anc80 particle) comprises a construct comprising: (i) a 5' inverted terminal repeat (ITR), (ii) a 5' untranslated region (UTR), (iii) a polynucleotide encoding a polypeptide (e.g. a Connexin 26 polypeptide) operably linked to a promoter, (iv) a 3' UTR, and (v) a 3' ITR, wherein the promoter comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NO: 28 and a minimal GJB2 promoter comprises a nucleic acid sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 86. In some aspects, an adeno-associated virus (AAV) particle (e.g., an Anc80 particle) comprises a construct comprising: (i) a 5' inverted terminal repeat (ITR), (ii) a 5' untranslated region (UTR), (iii) a polynucleotide encoding a polypeptide (e.g. a Connexin 26 polypeptide) operably linked to a promoter, (iv) a 3' UTR, and (v) a 3' ITR, wherein the promoter comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NO: 57 and a minimal GJB2 promoter comprises a nucleic acid sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 86. In some aspects, an adeno-associated virus (AAV) particle (e.g., an Anc80 particle) comprises a construct comprising: (i) a 5' inverted terminal repeat (ITR), (ii) a 5'

untranslated region (UTR), (iii) a polynucleotide encoding a polypeptide (e.g. a Connexin 26 polypeptide) operably linked to a promoter, (iv) a 3' UTR, and (v) a 3' ITR, wherein the promoter comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NO: 91 and a minimal GJB2 promoter comprises a nucleic acid sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 86. In some aspects, an adeno-associated virus (AAV) particle (e.g., an Anc80 particle) comprises a construct comprising: (i) a 5' inverted terminal repeat (ITR), (ii) a 5' untranslated region (UTR), (iii) a polynucleotide encoding a polypeptide (e.g. a Connexin 26 polypeptide) operably linked to a promoter, (iv) a 3' UTR, and (v) a 3' ITR, wherein the promoter comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NO: 92 and a minimal GJB2 promoter comprises a nucleic acid sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 86. In some aspects, an adeno-associated virus (AAV) particle (e.g., an Anc80 particle) comprises a construct comprising: (i) a 5' inverted terminal repeat (ITR), (ii) a 5' untranslated region (UTR), (iii) a polynucleotide encoding a polypeptide (e.g. a Connexin 26 polypeptide) operably linked to a promoter, (iv) a 3' UTR, and (v) a 3' ITR, wherein the promoter comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NO: 93 and a minimal GJB2 promoter comprises a nucleic acid sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 86. In some aspects, an adeno-associated virus (AAV) particle (e.g., an Anc80 particle) comprises a construct comprising: (i) a 5' inverted terminal repeat (ITR), (ii) a 5' untranslated region (UTR), (iii) a polynucleotide encoding a polypeptide (e.g. a Connexin 26 polypeptide) operably linked to a promoter, (iv) a 3' UTR, and (v) a 3' ITR, wherein the promoter comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NO: 94 and a minimal GJB2 promoter comprises a nucleic acid sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 86. In some aspects, an adeno-associated

virus (AAV) particle (e.g., an Anc80 particle) comprises a construct comprising: (i) a 5' inverted terminal repeat (ITR), (ii) a 5' untranslated region (UTR), (iii) a polynucleotide encoding a polypeptide (e.g. a Connexin 26 polypeptide) operably linked to a promoter, (iv) a 3' UTR, and (v) a 3' ITR, wherein the promoter comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NO: 96 and a minimal GJB2 promoter comprises a nucleic acid sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 86. In some aspects, an adeno-associated virus (AAV) particle (e.g., an Anc80 particle) comprises a construct comprising: (i) a 5' inverted terminal repeat (ITR), (ii) a 5' untranslated region (UTR), (iii) a polynucleotide encoding a polypeptide (e.g. a Connexin 26 polypeptide) operably linked to a promoter, (iv) a 3' UTR, and (v) a 3' ITR, wherein the promoter comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NO: 97 and a minimal GJB2 promoter comprises a nucleic acid sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 86. In some aspects, an adeno-associated virus (AAV) particle (e.g., an Anc80 particle) comprises a construct comprising: (i) a 5' inverted terminal repeat (ITR), (ii) a 5' untranslated region (UTR), (iii) a polynucleotide encoding a polypeptide (e.g. a Connexin 26 polypeptide) operably linked to a promoter, (iv) a 3' UTR, and (v) a 3' ITR, wherein the promoter comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NO: 98 and a minimal GJB2 promoter comprises a nucleic acid sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 86.

Exemplary Construct Components

Inverted Terminal Repeat Sequences (ITRs)

[0162] AAV derived sequences of a construct typically comprises the cis-acting 5' and 3' ITRs (See, e.g., B. J. Carter, in "Handbook of Parvoviruses", ed., P. Tijsser, CRC Press, pp. 155-168 (1990), which is incorporated in its entirety herein by reference). Generally, ITRs are able to form a hairpin. The ability to form a hairpin can contribute to an ITR's ability to self-prime, allowing primase-independent synthesis of a second DNA strand.

ITRs also play a role in integration of AAV construct (e.g., a coding sequence, e.g., a polynucleotide encoding a polypeptide (e.g., a therapeutic polypeptide, a Connexin 26 polypeptide) into a genome of a subject's cell. ITRs can also aid in efficient encapsidation of an AAV construct in an AAV particle.

[0163] An rAAV particle (e.g., an AAV2/Anc80 particle) of the present disclosure can comprise a rAAV construct comprising a coding sequence (e.g., a polynucleotide encoding a polypeptide (e.g., a therapeutic polypeptide, a Connexin 26 polypeptide)) and associated elements flanked by a 5' and a 3' AAV ITR sequences. In some aspects, an ITR is or comprises about 145 nucleic acids. In some aspects, an ITR is or comprises about 119 nucleic acids. In some aspects, an ITR is or comprises about 130 nucleic acids. In some aspects, all or substantially all of a sequence encoding an ITR is used. An AAV ITR sequence may be obtained from any known AAV, including presently identified mammalian AAV types. In some aspects an ITR is an AAV2 ITR.

[0164] An example of a construct molecule employed in the present disclosure is a "cis-acting" construct containing a transgene, in which the selected transgene sequence and associated regulatory elements are flanked by 5' or "left" and 3' or "right" AAV ITR sequences. 5' and left designations refer to a position of an ITR sequence relative to an entire construct, read left to right, in a sense direction. For example, in some aspects, a 5' or left ITR is an ITR that is closest to a promoter (as opposed to a polyadenylation sequence) for a given construct, when a construct is depicted in a sense orientation, linearly. Concurrently, 3' and right designations refer to a position of an ITR sequence relative to an entire construct, read left to right, in a sense direction. For example, in some aspects, a 3' or right ITR is an ITR that is closest to a polyadenylation sequence (as opposed to a promoter sequence) for a given construct, when a construct is depicted in a sense orientation, linearly. ITRs as provided herein are depicted in 5' to 3' order in accordance with a sense strand. Accordingly, one of skill in the art will appreciate that a 5' or "left" orientation ITR can also be depicted as a 3' or "right" ITR when converting from sense to antisense direction. Further, it is well within the ability of one of skill in the art to transform a given sense ITR sequence (e.g., a 5'/left AAV ITR) into an antisense sequence (e.g., 3'/right ITR sequence). One of ordinary skill in the art would understand how to modify a given ITR sequence for use as either a 5'/left or 3'/right ITR, or an antisense version thereof.

- [0165] For example, in some aspects an ITR (e.g., a 5' ITR) can have a sequence according to SEQ ID NO: 8. In some aspects, an ITR (e.g., a 3' ITR) can have a sequence according to SEQ ID NO: 9. In some aspects, an ITR includes one or more modifications, e.g., truncations, deletions, substitutions or insertions, as is known in the art. In some aspects, an ITR comprises fewer than 145 nucleotides, e.g., 119, 127, 130, 134 or 141 nucleotides. For example, in some aspects, an ITR comprises 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, or 145 nucleotides. In some aspects, the ITR comprises about 119 nucleotides. In some aspects, the ITR comprises about 130 nucleotides. In some aspects an ITR (e.g., a 5' ITR) can have a sequence according to SEQ ID NO: 52. In some aspects, an ITR (e.g., a 3' ITR) can have a sequence according to SEQ ID NO: 53.
- [0166] A non-limiting example of 5' AAV ITR sequences includes SEQ ID NO: 8 or 52. A non-limiting example of 3' AAV ITR sequences includes SEQ ID NO: 9 or 53. In some aspects, the 5' and a 3' AAV ITRs (e.g., SEQ ID NOs: 8 and 9, or SEQ ID NOs: 52 and 53) flank a portion of a coding sequence, e.g., all or a portion of a polynucleotide encoding a polypeptide (e.g., a therapeutic polypeptide, a Connexin 26 polypeptide). 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), each of which is incorporated in its entirety herein by reference). In some aspects, a 5' ITR sequence is at least at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99%, or 100% identical to a 5' ITR sequence represented by SEQ ID NO: 8. In some aspects, a 3' ITR sequence is at least at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% at least 99%, or 100% identical to a 3' ITR sequence represented by SEQ ID NO: 9. In some aspects, a 5' ITR sequence is at least at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% at least 99%, or 100% identical to a 5' ITR sequence represented by SEQ ID NO: 52. In some aspects, a 3' ITR sequence is at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% at least 99%, or 100% identical to a 3' ITR sequence represented by SEQ ID NO: 53.
- [0167] In some aspects, a 3' ITR sequence is at least at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% at least 99%, or 100% identical to a 3' ITR

sequence represented by SEQ ID NO: 116. In some aspects, a 3' ITR sequence is at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% at least 99%, or 100% identical to a 3' ITR sequence represented by SEQ ID NO: 116.

Exemplary 5' AAV ITR (SEQ ID NO: 8)

TTGGCCACTCCCTCTCTGCGCGCTCGCTCGCTCACTGAGGCCGCCCGGGCAAAGCCCGGGCGTCG
GGCGACCTTTGGTCGCCCCGGCCTCAGTGAGCGAGCGAGCGCGCAGAGAGGGAGTGGCCAACTCCA
TCACTAGGGGTTCCT

Exemplary 3' AAV ITR (SEQ ID NO: 9)

AGGAACCCCTAGTGATGGAGTTGGCCACTCCCTCTCTGCGCGCTCGCTCGCTCACTGAGGCCGGG
CGACCAAAGGTCGCCCCGACGCCCGGGCTTTGCCCCGGGCGGCCTCAGTGAGCGAGCGAGCGCGCAG
AGAGGGAGTGGCCAA

Exemplary 5' AAV ITR (SEQ ID NO: 52)

CTGCGCGCTCGCTCGCTCACTGAGGCCGCCCGGGCGTCGGGCGACCTTTGGTCGCCCCGGCCTCAG
TGAGCGAGCGAGCGCGCAGAGAGGGAGTGGCCAACTCCATCACTAGGGGTTCCT

Exemplary 3' AAV ITR (SEQ ID NO: 53)

AGGAACCCCTAGTGATGGAGTTGGCCACTCCCTCTCTGCGCGCTCGCTCGCTCACTGAGGCCGGG
CGACCAAAGGTCGCCCCGACGCCCGGGCTTTGCCCCGGGCGGCCTCAGTGAGCGAGCGAGCGCGCAG

Exemplary 3' ITR (SEQ ID NO: 116)

AGGAACCCCTAGTGATGGAGTTGGCCACTCCCTCTCTGCGCGCTCGCTCGCTCACTGAGG
CCGCCCGGGCAAAGCCCGGGCGTCGGGCGACCTTTGGTCGCCCCGGCCTCAGTGAGCGAGC
GAGCGCGCAGAGAGGGAGTGGCCAA

Promoters

[0168] In some aspects, the disclosure is directed to constructs comprising a cell selective promoter which can be used to regulate (e.g., increase) expression of a polynucleotide encoding a therapeutic polypeptide (e.g., a Connexin 26 polypeptide) in a cell (e.g., an inner ear cell, e.g., a supporting cell). In some aspects, the constructs provide reduced toxicity associated with expression of the therapeutic polypeptide (e.g., a Connexin 26 polypeptide) in some cells (e.g., an inner ear cell, e.g., a hair cell).

[0169] In some aspects, the disclosure is directed to constructs comprising a cell selective promoter which can be used to regulate (e.g., increase) expression of a polynucleotide

encoding a polypeptide in a cell (e.g., an inner ear cell, e.g., a supporting cell). In some aspects, the constructs provide reduced toxicity associated with expression of the polypeptide in some cells (e.g., an inner ear cell, e.g., a hair cell).

[0170] In some aspects, a construct (e.g., an rAAV construct) comprises a promoter. The term “promoter” refers to a DNA sequence recognized by enzymes/proteins that can promote and/or initiate transcription of an operably linked gene (e.g., a polynucleotide encoding a polypeptide (e.g., a therapeutic polypeptide, a Connexin 26 polypeptide)). For example, a promoter typically refers to, e.g., a nucleotide sequence to which an RNA polymerase and/or any associated factor binds and from which it can initiate transcription. Thus, in some aspects, a construct (e.g., an rAAV construct) comprises a polynucleotide operably linked to one of the non-limiting example promoters described herein.

[0171] In some aspects, a 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, a cell-selective promoter or any other type of promoter known in the art. In some aspects, a promoter is a RNA polymerase II promoter, such as a mammalian RNA polymerase II promoter. In some aspects, a promoter is a RNA polymerase III promoter, including, but not limited to, a HI promoter, a human U6 promoter, a mouse U6 promoter, or a swine U6 promoter. A promoter will generally be one that is able to promote transcription in an inner ear cell. In some aspects, a promoter is a cochlea-selective promoter or a cochlea-oriented promoter. In some aspects, a promoter is a hair cell selective promoter, or a supporting cell selective promoter. In some aspects, a promoter is an inner ear supporting cell selective promoter.

[0172] The term “constitutive” promoter refers to a nucleotide sequence that, when operably linked with a nucleic acid encoding a protein (e.g., a polypeptide (e.g., a therapeutic polypeptide, a Connexn 26 polypeptide)), causes RNA to be transcribed from the nucleic acid in a cell under most or all physiological conditions.

[0173] 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, which is incorporated in its entirety herein by reference), the SV40 promoter, the dihydrofolate reductase promoter, the beta-actin promoter, the phosphoglycerol kinase (PGK) promoter, and the EFl-alpha promoter (Invitrogen). In some aspects, the promoter is a constitutive promoter. In some aspects, the constitutive promoter is a CAG promoter, a CBA promoter, a CMV promoter, a

CMV/CBA enhancer/promoter, or a CB7 promoter. In some aspects, the a CMV/CBA enhancer/promoter comprises a nucleic acid with at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% at least 99%, or 100% identity to SEQ ID NOs: 12 or 13. In some aspects, the CMV/CBA enhancer/promoter comprises a nucleic acid of SEQ ID NO: 12. In some aspects, the CMV/CBA enhancer/promoter comprises a nucleic acid of SEQ ID NO: 13. In some aspects, the CBA promoter comprises a nucleic acid with at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% at least 99%, or 100% identity to SEQ ID NOs: 10 or 11. In some aspects, the CBA promoter comprises a nucleic acid of SEQ ID NO: 10. In some aspects, the CBA promoter comprises a nucleic acid of SEQ ID NO: 11.

[0174] In some aspects, the CAG promoter comprises a nucleic acid with at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% at least 99%, or 100% identity to SEQ ID NOs: 14 or 15. In some aspects, the CAG promoter comprises a nucleic acid of SEQ ID NO: 14. In some aspects, the CAG promoter comprises a nucleic acid of SEQ ID NO: 15.

[0175] In some aspects, regulatory and/or control sequences impart cell selective gene expression capabilities. In some cases, cell selective regulatory and/or control sequences bind cell selective transcription factors that induce transcription in a cell selective manner.

[0176] In some aspects, a cell selective promoter is an ear cell selective promoter. In some aspects, a cell selective promoter is an inner ear cell selective promoter. In some aspects, a promoter is a characteristic fragment of a cell selective promoter. In some aspects, the promoter is an inner ear supporting cell selective promoter.

[0177] In some aspects, the inner ear supporting cells are selected from one or more of inner phalangeal cells/border cells (IPhC), inner pillar cells (IPC), outer pillar cells (OPC), Deiters' cells rows 1 and 2 (DC1/2), Deiters' cells row 3 (DC3), Hensen's cells (Hec), Claudius cells/outer sulcus cells (CC/OSC), interdental cells (Idc), inner sulcus cells (ISC), Kölliker's organ cells (KO), greater ridge epithelial ridge cells (GER) (including lateral greater epithelial ridge cells (LGER)), and OC90+ cells (OC90), fibroblasts, and other cells of the lateral wall.

[0178] In some aspects, inner ear support cell selective promoters are selected from one or more of GJB2, GJB6, IGFBP2, RBP7, GDF6, PARM1, GFAP, BACE2, DBI2,

FABP3, KLHL14, MMP15, SPARC, TSPAN8, VIM, derivatives thereof, or fragments thereof.

[0179] In some aspects, the inner ear support cell selective promoter is a GDF6 promoter.

In some aspects, the inner ear support cell selective promoter is a PARM1 promoter. In some aspects, the inner ear support cell selective promoter is a MMP15 promoter. In some aspects, the inner ear support cell selective promoter is a VIM promoter.

[0180] In some aspects, the inner ear support cell selective promoter is a GJB2 promoter.

In some aspects, the inner ear support cell selective promoter is a GJB6 promoter. In some aspects, the inner ear support cell selective promoter is a IGFBP2 promoter. In some aspects, the inner ear support cell selective promoter is a RBP7 promoter. In some aspects, the inner ear support cell selective promoter is a GFAP promoter. In some aspects, the inner ear support cell selective promoter is BACE2 promoter. In some aspects, the inner ear support cell selective promoter is a DBI2 promoter. In some aspects, the inner ear support cell selective promoter is a FABP3. In some aspects, the inner ear support cell selective promoter is a KLHL14 promoter. In some aspects, the inner ear support cell selective promoter is a SPARC promoter. In some aspects, the inner ear support cell selective promoter is a TSPAN8 promoter.

[0181] In some aspects, derivatives thereof can include a modified parent sequence (e.g., a naturally occurring promoter sequence), one or more portions of a parent sequence, fragments of a parent sequence, and the like.

[0182] In some aspects, the promoter is an inner ear medial support cell selective promoter. In some aspects, inner ear medial support cells are selected from one or more of lateral greater epithelial ridge cells and inner sulcus cells. In some aspects, inner ear medial support cell selective promoters are selected from one or more of GJB6, IGFBP2, GDF6, PARM1, derivatives thereof, or fragments thereof. In some aspects, the inner ear medial support cell selective promoter is a GDF6 promoter. In some aspects, the inner ear medial support cell selective promoter is a PARM1 promoter. In some aspects, the inner ear medial support cell selective promoter is a IGFBP2 promoter. In some aspects, the inner ear medial support cell selective promoter is a GJB6 promoter.

[0183] In some aspects, the promoter is an inner ear sensory epithelial support cell selective promoter. In some aspects, sensory epithelial support cells are selected from one or more of inner pillar cells, outer pillar cells, dieter cells, and inner phalangeal cells. In some aspects, a inner ear sensory epithelial support cell selective promoters are

selected from one or more of GJB6, IGFBP2, RBP7, GDF6, PARM1, FABP3, BACE2 derivatives thereof, or fragments thereof.

[0184] In some aspects, a inner ear sensory epithelial support cell selective promoter is a GDF6 promoter. In some aspects, a inner ear sensory epithelial support cell selective promoter is a PARM1 promoter. In some aspects, a inner ear sensory epithelial support cell selective promoter is a GJB6 promoter. In some aspects, a inner ear sensory epithelial support cell selective promoter is a IGFBP2 promoter. In some aspects, a inner ear sensory epithelial support cell selective promoter is a RBP7 promoter. In some aspects, a inner ear sensory epithelial support cell selective promoter is a FABP3 promoter. In some aspects, a inner ear sensory epithelial support cell selective promoter is a BACE2 promoter.

[0185] In some aspects, the promoter is an inner phalangeal cell selective promoter. In some aspects, the inner phalangeal cell selective promoters are selected from one or more of IGFBP2, GDF6, FABP3, BACE2, derivatives thereof, or fragments thereof. In some aspects, the inner phalangeal cell selective promoter is a IGFBP2 promoter. In some aspects, the inner phalangeal cell selective promoter is a GDF6 promoter. In some aspects, the inner phalangeal cell selective promoter is a FABP3 promoter. In some aspects, the inner phalangeal cell selective promoter is a BACE2 promoter.

[0186] In some aspects, the promoter is an interdental cell selective promoter. In some aspects, the interdental cell promoter is IGFBP2, derivative thereof, or fragment thereof.

[0187] In some aspects, the inner ear supporting cell selective promoter is a GJB2 promoter. In some aspects, the GJB2 enhancer comprises a nucleic acid sequence with at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% at least 99%, or 100% identity to SEQ ID NO: 65. In some aspects, the GJB2 enhancer comprises the nucleic acid sequence of SEQ ID NO: 65. In some aspects, the GJB2 minimal promoter comprises a nucleic acid sequence with at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% at least 99%, or 100% identity to SEQ ID NO: 86. In some aspects, the GJB2 minimal promoter comprises the nucleic acid sequence of SEQ ID NO: 86.

[0188] In some aspects, the promoter is derived from a GJB2 promoter and has a length of 1000-1050 nucleotides. In some aspects, the inner ear supporting cell selective promoter is a GJB6 promoter. In some aspects, the GJB6 promoter comprises a nucleic acid sequence with at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at

least 98% at least 99%, or 100% identity to SEQ ID NO: 16. In some aspects, the GJB6 promoter comprises the nucleic acid sequence of SEQ ID NO: 16. In some aspects, the promoter is derived from a GJB6 promoter and has a length of 700-750 nucleotides.

[0189] In some aspects, the inner ear supporting cell selective promoter is an IGFBP2 promoter. In some aspects, the IGFBP2 promoter comprises a nucleic acid sequence with at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% at least 99%, or 100% identity to SEQ ID NO: 57. In some aspects, the IGFBP2 promoter comprises the nucleic acid sequence of SEQ ID NO: 57. In some aspects, the promoter is derived from an IGFBP2 promoter and has a length of 1500-1550 nucleotides.

[0190] In some aspects, the inner ear supporting cell selective promoter is a RBP7 promoter. In some aspects, the RBP7 promoter comprises a nucleic acid sequence with at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% at least 99%, or 100% identity to SEQ ID NO: 28. In some aspects, the RBP7 promoter comprises the nucleic acid sequence of SEQ ID NO: 28. In some aspects, the promoter is derived from a RBP7 promoter and has a length of 1050-1100 nucleotides.

[0191] In some aspects, the inner ear supporting cell selective promoter is a GDF6 promoter. In some aspects, the GDF6 promoter comprises a nucleic acid sequence with at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% at least 99%, or 100% identity to SEQ ID NO: 90. In some aspects, the GDF6 promoter comprises the nucleic acid sequence of SEQ ID NO: 90. In some aspects, the promoter is derived from a GDF6 promoter and has a length of 1150-1200 nucleotides.

[0192] In some aspects, the inner ear supporting cell selective promoter is a PARM1 promoter. In some aspects, the PARM1 promoter comprises a nucleic acid sequence with at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% at least 99%, or 100% identity to SEQ ID NO: 40. In some aspects, the PARM1 promoter comprises the nucleic acid sequence of SEQ ID NO: 40. In some aspects, the promoter is derived from a PARM1 promoter and has a length of 1300-1350 nucleotides.

[0193] In some aspects, the construct comprises two or more promoters. In some aspects, the first promoter is selected from a GJB6 promoter, a GDF6 promoter, a IGFBP2 promoter, a RBP7 promoter, a PARM1 promoter, a GFAP promoter, a BACE2 promoter, a DBI2 promoter, a FABP3 promoter, a KLHL14 promoter, a MMP15 promoter, a SPARC promoter, a TSPAN8 promoter, a VIM promoter, and any combination thereof.

In some aspects, the second promoter is selected from a GJB2 promoter or a minimal GJB2 promoter.

[0194] In some aspects, the first promoter is a GDF6 promoter and the second promoter is a minimal GJB2 promoter. In some aspects, the first promoter is a PARM1 promoter and the second promoter is a minimal GJB2 promoter. In some aspects, the first promoter is a MMP15 promoter and the second promoter is a minimal GJB2 promoter. In some aspects, the first promoter is a VIM promoter and the second promoter is a minimal GJB2 promoter.

[0195] In some aspects, the first promoter is a GJB6 promoter and the second promoter is a minimal GJB2 promoter. In some aspects, the first promoter is a IGFBP2 promoter and the second promoter is a minimal GJB2 promoter. In some aspects, the first promoter is a GDF6 promoter and the second promoter is a minimal RBP7 promoter. In some aspects, the first promoter is a GFAP promoter and the second promoter is a minimal GJB2 promoter. In some aspects, the first promoter is a BACE2 promoter and the second promoter is a minimal GJB2 promoter. In some aspects, the first promoter is a DBI2 promoter and the second promoter is a minimal GJB2 promoter. In some aspects, the first promoter is a FABP3 promoter and the second promoter is a minimal GJB2 promoter. In some aspects, the first promoter is a KLHL14 promoter and the second promoter is a minimal GJB2 promoter. In some aspects, the first promoter is a SPARC promoter and the second promoter is a minimal GJB2 promoter. In some aspects, the first promoter is a TSPAN8 promoter and the second promoter is a minimal GJB2 promoter. In some aspects, the inner ear supporting cell selective promoter comprises a GJB6 and a hGJB2 minimal promoter. In some aspects, the GJB6 promoter comprises a nucleic acid sequence with at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% at least 99%, or 100% identity to SEQ ID NO: 16 and the hGJB2 minimal promoter comprises a nucleic acid sequence with at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% at least 99%, or 100% to SEQ ID NO: 86. In some aspects, the GJB6 has the nucleic acid sequence of SEQ ID NO: 16 and the hGJB2 minimal promoter has the nucleic acid sequence of SEQ ID NO: 86.

[0196] In some aspects, the inner ear supporting cell selective promoter comprises a IGFBP2 promoter and a hGJB2 minimal promoter. In some aspects, the IGFBP2 promoter comprises a nucleic acid sequence with at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% at least 99%, or 100% identity to SEQ ID NO: 57

and the hGJB2 minimal promoter comprises a nucleic acid sequence with at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% at least 99%, or 100% to SEQ ID NO: 86. In some aspects, the IGFBP2 has the nucleic acid sequence of SEQ ID NO: 57 and the hGJB2 minimal promoter has the nucleic acid sequence of SEQ ID NO: 86.

[0197] In some aspects, the inner ear supporting cell selective promoter comprises a RBP7 promoter and a hGJB2 minimal promoter. In some aspects, the RBP7 promoter comprises a nucleic acid sequence with at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% at least 99%, or 100% identity to SEQ ID NO: 28 and the hGJB2 minimal promoter comprises a nucleic acid sequence with at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% at least 99%, or 100% to SEQ ID NO: 86. In some aspects, the RBP7 has the nucleic acid sequence of SEQ ID NO: 28 and the hGJB2 minimal promoter has the nucleic acid sequence of SEQ ID NO: 86.

[0198] In some aspects, the inner ear supporting cell selective promoter comprises a GJB6 promoter and a hGJB2 minimal promoter. In some aspects, the GJB6 promoter comprises a nucleic acid sequence with at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% at least 99%, or 100% identity to SEQ ID NO: 16 and the hGJB2 minimal promoter comprises a nucleic acid sequence with at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% at least 99%, or 100% to SEQ ID NO: 86. In some aspects, the GJB6 has the nucleic acid sequence of SEQ ID NO: 16 and the hGJB2 minimal promoter has the nucleic acid sequence of SEQ ID NO: 86.

[0199] In some aspects, the inner ear supporting cell selective promoter comprises a PARM1 promoter and a hGJB2 minimal promoter. In some aspects, the PARM1 promoter comprises a nucleic acid sequence with at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% at least 99%, or 100% identity to SEQ ID NO: 40 and the hGJB2 minimal promoter comprises a nucleic acid sequence with at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% at least 99%, or 100% to SEQ ID NO: 86. In some aspects, the PARM1 has the nucleic acid sequence of SEQ ID NO: 40 and the hGJB2 minimal promoter has the nucleic acid sequence of SEQ ID NO: 86.

[0200] In some aspects, the inner ear supporting cell selective promoter is a BACE2 promoter. In some aspects, the BACE2 promoter comprises a nucleic acid sequence with at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% at least

99%, or 100% identity to SEQ ID NO: 92. In some aspects, the BACE2 promoter comprises the nucleic acid sequence of SEQ ID NO: 92. In some aspects, the promoter is derived from a BACE2 promoter and has a length of 1400-1450 nucleotides.

[0201] In some aspects, the inner ear supporting cell selective promoter is a DBI2 promoter. In some aspects, the DBI2 promoter comprises a nucleic acid sequence with at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% at least 99%, or 100% identity to SEQ ID NO: 93. In some aspects, the DBI2 promoter comprises the nucleic acid sequence of SEQ ID NO: 93. In some aspects, the promoter is derived from a DBI2 promoter and has a length of 1450-1500 nucleotides.

[0202] In some aspects, the inner ear supporting cell selective promoter is a FABP3 promoter. In some aspects, the FABP3 promoter comprises a nucleic acid sequence with at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% at least 99%, or 100% identity to SEQ ID NO: 94. In some aspects, the FABP3 promoter comprises the nucleic acid sequence of SEQ ID NO: 94. In some aspects, the promoter is derived from a FABP3 promoter and has a length of 1750-1800 nucleotides.

[0203] In some aspects, the inner ear supporting cell selective promoter is a KLHL14 promoter. In some aspects, the KLHL14 promoter comprises a nucleic acid sequence with at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% at least 99%, or 100% identity to SEQ ID NO: 95. In some aspects, the KLHL14 promoter comprises the nucleic acid sequence of SEQ ID NO: 95. In some aspects, the promoter is derived from a KLHL14 promoter and has a length of 1250-1300 nucleotides.

[0204] In some aspects, the inner ear supporting cell selective promoter is a MMP15 promoter. In some aspects, the MMP15 promoter comprises a nucleic acid sequence with at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% at least 99%, or 100% identity to SEQ ID NO: 96. In some aspects, the MMP15 promoter comprises the nucleic acid sequence of SEQ ID NO: 96. In some aspects, the promoter is derived from a MMP15 promoter and has a length of 1000-1050 nucleotides.

[0205] In some aspects, the inner ear supporting cell selective promoter is a SPARC promoter. In some aspects, the SPARC promoter comprises a nucleic acid sequence with at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% at least 99%, or 100% identity to SEQ ID NO: 97. In some aspects, the SPARC promoter comprises the nucleic acid sequence of SEQ ID NO: 97. In some aspects, the promoter is derived from a SPARC promoter and has a length of 1000-1050 nucleotides.

- [0206]** In some aspects, the inner ear supporting cell selective promoter is a TSPAN8 promoter. In some aspects, the TSPAN8 promoter comprises a nucleic acid sequence with at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% at least 99%, or 100% identity to SEQ ID NO: 98. In some aspects, the TSPAN8 promoter comprises the nucleic acid sequence of SEQ ID NO: 98. In some aspects, the promoter is derived from a TSPAN8 promoter and has a length of 1200-1250 nucleotides.
- [0207]** In some aspects, the inner ear supporting cell selective promoter is a GFAP promoter. In some aspects, the GFAP promoter comprises a nucleic acid sequence with at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% at least 99%, or 100% identity to SEQ ID NO: 91. In some aspects, the GFAP promoter comprises the nucleic acid sequence of SEQ ID NO: 91. In some aspects, the promoter is derived from a GFAP promoter and has a length of 650-700 nucleotides.
- [0208]** In some aspects, the inner ear supporting cell selective promoter is a VIM promoter. In some aspects, the VIM promoter comprises a nucleic acid sequence with at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% at least 99%, or 100% identity to SEQ ID NO: 99. In some aspects, the VIM promoter comprises the nucleic acid sequence of SEQ ID NO: 99. In some aspects, the promoter is derived from a VIM promoter and has a length of 1050-1100 nucleotides.
- [0209]** In some aspects, the inner ear supporting cell selective promoter comprises a BACE2 promoter and a hGJB2 minimal promoter. In some aspects, the BACE2 promoter comprises a nucleic acid sequence with at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% at least 99%, or 100% identity to SEQ ID NO: 92 and the hGJB2 minimal promoter comprises a nucleic acid sequence with at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% at least 99%, or 100% to SEQ ID NO: 86. In some aspects, the BACE2 promoter comprises the nucleic acid sequence of SEQ ID NO: 92 and the hGJB2 minimal promoter has the nucleic acid sequence of SEQ ID NO: 86.
- [0210]** In some aspects, the inner ear supporting cell selective promoter comprises a DBI2 promoter and a hGJB2 minimal promoter. In some aspects, the DBI2 promoter comprises a nucleic acid sequence with at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% at least 99%, or 100% identity to SEQ ID NO: 93 and the hGJB2 minimal promoter comprises a nucleic acid sequence with at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% at least 99%, or 100% to SEQ

ID NO: 86. In some aspects, the DBI2 promoter comprises the nucleic acid sequence of SEQ ID NO: 93 and the hGJB2 minimal promoter has the nucleic acid sequence of SEQ ID NO: 86.

[0211] In some aspects, the inner ear supporting cell selective promoter comprises a FABP3 promoter and a hGJB2 minimal promoter. In some aspects, the FABP3 promoter comprises a nucleic acid sequence with at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% at least 99%, or 100% identity to SEQ ID NO: 94 and the hGJB2 minimal promoter comprises a nucleic acid sequence with at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% at least 99%, or 100% to SEQ ID NO: 86. In some aspects, the FABP3 promoter comprises the nucleic acid sequence of SEQ ID NO: 94 and the hGJB2 minimal promoter has the nucleic acid sequence of SEQ ID NO: 86.

[0212] In some aspects, the inner ear supporting cell selective promoter comprises a KLHL14 promoter and a hGJB2 minimal promoter. In some aspects, the KLHL14 promoter comprises a nucleic acid sequence with at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% at least 99%, or 100% identity to SEQ ID NO: 95 and the hGJB2 minimal promoter comprises a nucleic acid sequence with at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% at least 99%, or 100% to SEQ ID NO: 86. In some aspects, the KLHL14 promoter comprises the nucleic acid sequence of SEQ ID NO: 95 and the hGJB2 minimal promoter has the nucleic acid sequence of SEQ ID NO: 86.

[0213] In some aspects, the inner ear supporting cell selective promoter comprises a MMP15 promoter and a hGJB2 minimal promoter. In some aspects, the MMP15 promoter comprises a nucleic acid sequence with at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% at least 99%, or 100% identity to SEQ ID NO: 96 and the hGJB2 minimal promoter comprises a nucleic acid sequence with at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% at least 99%, or 100% to SEQ ID NO: 86. In some aspects, the MMP15 promoter comprises the nucleic acid sequence of SEQ ID NO: 96 and the hGJB2 minimal promoter has the nucleic acid sequence of SEQ ID NO: 86.

[0214] In some aspects, the inner ear supporting cell selective promoter comprises a SPARC promoter and a hGJB2 minimal promoter. In some aspects, the SPARC promoter comprises a nucleic acid sequence with at least 85%, at least 90%, at least 95%, at least

96%, at least 97%, at least 98% at least 99%, or 100% identity to SEQ ID NO: 97 and the hGJB2 minimal promoter comprises a nucleic acid sequence with at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% at least 99%, or 100% to SEQ ID NO: 86. In some aspects, the SPARC promoter comprises the nucleic acid sequence of SEQ ID NO: 97 and the hGJB2 minimal promoter has the nucleic acid sequence of SEQ ID NO: 86.

[0215] In some aspects, the inner ear supporting cell selective promoter comprises a TSPAN8 promoter and a hGJB2 minimal promoter. In some aspects, the TSPAN8 promoter comprises a nucleic acid sequence with at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% at least 99%, or 100% identity to SEQ ID NO: 98 and the hGJB2 minimal promoter comprises a nucleic acid sequence with at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% at least 99%, or 100% to SEQ ID NO: 86. In some aspects, the TSPAN8 promoter comprises the nucleic acid sequence of SEQ ID NO: 98 and the hGJB2 minimal promoter has the nucleic acid sequence of SEQ ID NO: 86.

[0216] In some aspects, the inner ear supporting cell selective promoter comprises a VIM promoter and a hGJB2 minimal promoter. In some aspects, the VIM promoter comprises a nucleic acid sequence with at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% at least 99%, or 100% identity to SEQ ID NO: 99 and the hGJB2 minimal promoter comprises a nucleic acid sequence with at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% at least 99%, or 100% to SEQ ID NO: 86. In some aspects, the VIM promoter comprises the nucleic acid sequence of SEQ ID NO: 99 and the hGJB2 minimal promoter has the nucleic acid sequence of SEQ ID NO: 86.

Exemplary CBA promoter (SEQ ID NO: 10)

GTCGAGGTGAGCCCCACGTTCTGCTTCACTCTCCCCATCTCCCCCCCCTCCCCACCCCCAATTTT
GTATTTATTTATTTTAAATTATTTTGTGCAGCGATGGGGGCGGGGGGGGGGGCGCGCGCCA
GGCGGGGCGGGGCGGGGCGAGGGGCGGGGCGGGGCGAGGCGGAGAGGTGCGGCGGCAGCCAATCA
GAGCGGCGCGCTCCGAAAGTTTCCTTTTATGGCGAGGCGGCGGCGGCGGCGGCCCTATAAAAAGC
GAAGCGCGCGGCGGGCG

Exemplary CBA promoter (SEQ ID NO: 11)

GTTCGAGGTGAGCCCCACGTTCTGCTTCACTCTCCCCATCTCCCCCCCCTCCCCACCCCCAATTTT
GTATTTATTTATTTTAAATTATTTTGTGTCAGCGATGGGGGCGGGGGGGGGGGGGGGCGCGCGC
CAGGCGGGGCGGGGCGGGGCGAGGGGCGGGGCGGGGCGAGGCGGAGAGGTGCGGCGGCAGCCAAT
CAGAGCGGCGCGCTCCGAAAGTTTCCTTTTATGGCGAGGCGGCGGCGGCGGCGGCCCTATAAAAA
GCGAAGCGCGCGGGCGGCG

Exemplary CMV/CBA enhancer/promoter (SEQ ID NO: 12)

GACATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCCATAT
ATGGAGTTCCGCGTTACATAACTTACGGTAAATGGCCCGCTGGCTGACCGCCCAACGACCCCCG
CCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGTC
AATGGGTGGACTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGT
ACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCTGGCATTATGCCCAGTACATGACCTT
ATGGGACTTTTCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGGTGAGGTG
AGCCCCACGTTCTGCTTCACTCTCCCCATCTCCCCCCCCTCCCCACCCCCAATTTTGTATTTATT
TATTTTTTAAATTATTTTGTGTCAGCGATGGGGGCGGGGGGGGGGGGGGGCGCGCGCCAGGCGGGCG
GGGCGGGGCGAGGGGCGGGGCGGGGCGAGGCGGAGAGGTGCGGCGGCAGCCAATCAGAGCGGCGC
GCTCCGAAAGTTTCCTTTTATGGCGAGGCGGCGGCGGCGGCGGCCCTATAAAAAGCGAAGCGCG
GGCGGGCG

Exemplary CMV/CBA enhancer/promoter (SEQ ID NO: 13)

GACATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCCATAT
ATGGAGTTCCGCGTTACATAACTTACGGTAAATGGCCCGCTGGCTGACCGCCCAACGACCCCCG
CCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGTC
AATGGGTGGACTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGT
ACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCTGGCATTATGCCCAGTACATGACCTT
ATGGGACTTTTCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGGTGAGGTG
AGCCCCACGTTCTGCTTCACTCTCCCCATCTCCCCCCCCTCCCCACCCCCAATTTTGTATTTATT
TATTTTTTAAATTATTTTGTGTCAGCGATGGGGGCGGGGGGGGGGGGGGGCGCGCGCCAGGCGGGG
CGGGGCGGGGCGAGGGGCGGGGCGGGGCGAGGCGGAGAGGTGCGGCGGCAGCCAATCAGAGCGGC
GCGCTCCGAAAGTTTCCTTTTATGGCGAGGCGGCGGCGGCGGCGGCCCTATAAAAAGCGAAGCGC
GCGGCGGGCG

Exemplary CAG enhancer/promoter (SEQ ID NO: 14)

GACATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCCATAT
ATGGAGTTCCGCGTTACATAACTTACGGTAAATGGCCCGCTGGCTGACCGCCCAACGACCCCCG
CCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGTC
AATGGGTGGACTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGT
ACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCTGGCATTTATGCCCAGTACATGACCTT
ATGGGACTTTCTTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGGTTCGAGGTG
AGCCCCACGTTCTGCTTCACTCTCCCCATCTCCCCCCCCCTCCCCACCCCCAATTTTGTATTTATT
TATTTTTTAAATTATTTTGTGCAGCGATGGGGGCGGGGGGGGGGGGGCGCGCGCCAGGCGGGGCG
GGGCGGGGCGAGGGGCGGGGCGGGGCGAGGCGGAGAGGTGCGGCGGCAGCCAATCAGAGCGGCGC
GCTCCGAAAGTTTCTTTTATGGCGAGGCGGCGGCGGCGGCGGCCCTATAAAAAGCGAAGCGCGC
GGCGGGCGGGAGTCGCTGCGTTGCCTTCGCCCCGTGCCCGCTCCGCGCCGCCTCGCGCCGCCCG
CCCCGGCTCTGACTGACCGCGTTACTCCACAGGTGAGCGGGCGGGACGGCCCTTCTCCTCCGGG
CTGTAATTAGCGCTTGGTTTAATGACGGCTCGTTTCTTTTCTGTGGCTGCGTGAAAGCCTTAAAG
GGCTCCGGGAGGGCCCTTTGTGCGGGGGGAGCGGCTCGGGGGGTGCGTGCGTGTGTGTGTGCGT
GGGAGCGCCGCTGCGGCCCGCGCTGCCCGGCGGCTGTGAGCGCTGCGGGCGCGGCGGGGCT
TTGTGCGCTCCGCGTGTGCGCGAGGGGAGCGCGGCCGGGGGCGGTGCCCCGCGGTGCGGGGGGGC
TGCGAGGGGAACAAAGGCTGCGTGCGGGGTGTGTGCGTGGGGGGGTGAGCAGGGGGTGTGGGCGC
GGCGGTGCGGCTGTAACCCCCCTGCACCCCCCTCCCCGAGTTGCTGAGCACGGCCCGGCTTCG
GGTGCGGGGCTCCGTGCGGGGCGTGCGCGGGGCTCGCCGTGCCGGGCGGGGGGTGGCGGCAGGT
GGGGGTGCCGGGCGGGGCGGGGCCGCTCGGGCCGGGGAGGGCTCGGGGGAGGGGCGCGCGGGCC
CCCGGAGCGCCGGCGGCTGTGAGGCGCGGCGAGCCGAGCCATTGCCTTTTATGGTAATCGTGC
GAGAGGGCGCAGGGACTTCCTTTGTCCCAAATCTGTGCGGAGCCGAAATCTGGGAGGCGCCGCCG
CACCCCCTCTAGCGGGCGGGGCGAAGCGGTGCGGCGCCGGCAGGAAGGAAATGGGCGGGGAGG
GCCTTCGTGCGTCGCCGCGCCGCGTCCCCTTCTCCCTCTCCAGCCTCGGGGCTGTCCGCGGGG
GACGGCTGCCTTCGGGGGGGACGGGGCAGGGCGGGGTTCGGCTTCTGGCGTGTGACCGGCGGCTC
TAGAGCCTCTGCTAACCATGTTTCATGCCTTCTTCTTTTCTTCTACAG

Exemplary CAG enhancer/promoter (SEQ ID NO: 15)

GACATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCCATAT
ATGGAGTTCCGCGTTACATAACTTACGGTAAATGGCCCGCTGGCTGACCGCCCAACGACCCCCG
CCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGTC
AATGGGTGGACTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGT

ACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCCAGTACATGACCTT
 ATGGGACTTTCTTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGGTCGAGGTG
 AGCCCCACGTTCTGCTTCACTCTCCCCATCTCCCCCCCCCTCCCCACCCCCAATTTTGTATTTATT
 TATTTTTTAATTATTTTGTGCAGCGATGGGGGCGGGGGGGGGGGGGGGGGGGCGCGCGCCAGGCGGGG
 CGGGGCGGGGCGAGGGGCGGGGCGGGGCGAGGCGGAGAGGTGCGGCGGCAGCCAATCAGAGCGGC
 GCGCTCCGAAAAGTTTCTTTTATGGCGAGGCGGCGGCGGCGGCGGCCCTATAAAAAGCGAAGCGC
 GCGGCGGGGCGGGAGTCGCTGCGTTGCTTCGCCCCGTGCCCCGCTCCGCGCCGCTCGCGCCGCC
 CGCCCCGGCTCTGACTGACCGCGTTACTCCACAGGTGAGCGGGCGGGACGGCCCTTCTCCTCCG
 GGCTGTAATTAGCGCTTGGTTTAATGACGGCTCGTTTTCTTTCTGTGGCTGCGTGAAAGCCTTAA
 AGGGCTCCGGGAGGGGCCCTTTGTGCGGGGGGAGCGGCTCGGGGGGTGCGTGCGTGTGTGTGTGC
 GTGGGGAGCGCCGCGTGC GGCCCGCGCTGCCCGGCGGCTGTGAGCGCTGCGGGCGCGGCGCGGGG
 CTTTGTGCGCTCCGCGTGTGCGCGAGGGGAGCGCGGCCGGGGGCGGTGCCCCGCGGTGCGGGGGG
 GCTGCGAGGGGAACAAAGGCTGCGTGCGGGGTGTGTGCGTGGGGGGGTGAGCAGGGGGTGTGGGC
 GCGGCGGTGCGGCTGTAACCCCCCTGCACCCCCCTCCCCGAGTTGCTGAGCACGGCCCCGGCTT
 CGGGTGCGGGGCTCCGTGCGGGGCGTGCGCGGGGCTCGCCGTGCCGGGCGGGGGGTGGCGGCAG
 GTGGGGGTGCCGGGCGGGGCGGGGCCGCTCGGGCCGGGGAGGGCTCGGGGAGGGGCGCGGCGG
 CCCCCGAGCGCCGGCGGCTGTGAGGCGCGGCGAGCCGAGCCATTGCCTTTTATGGTAATCGT
 GCGAGAGGGCGCAGGGACTTCCTTTGTCCCAAATCTGTGCGGAGCCGAAATCTGGGAGGCGCCGC
 CGCACCCCTCTAGCGGGCGGGGCGAAGCGGTGCGGCGCCGGCAGGAAGGAAATGGGCGGGGA
 GGGCCTTCGTGCGTCGCCGCGCCGCGTCCCCCTTCTCCCTCTCCAGCCTCGGGGCTGTCCGCGGG
 GGGACGGCTGCCTTCGGGGGGGACGGGGCAGGGCGGGGTTCGGCTTCTGGCGTGTGACCGGCGGC
 TCTAGAGCCTCTGCTAACCATGTTTCATGCCTTCTTCTTTTCTTCTACAG

[0217] In some aspects, the promoter is a GJB2 minimal promoter as set forth in SEQ ID NO: 86. In some aspects, a promoter is at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO: 86.

Exemplary Human GJB2 minimal promoter (SEQ ID NO: 86)

AAGCTCTGAGGACCCAGAGGCCGGGCGCGCTCCGCCCCGCGGCGCCGCCCCCTCCGT
 AACTTTCCAGTCTCCGAGGGAAGAGGCGGGGTGTGGGGTGCGGTTAAAAGGCGCC
 ACGGCGGGAGACAGGT

[0218] In certain aspects, the promoter is a GDF6 promoter as set forth in SEQ ID NO: 90. In some aspects, a promoter sequence is at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to a promoter

sequence represented by SEQ ID NO: 90. In some aspects, the promoter is a GDF6 promoter sequence comprising the sequence of SEQ ID NO: 90.

Exemplary Human GDF6 promoter (SEQ ID NO: 90)

CCACAGGTAAC TCCGTCGGCGTCCACAGGGGGGCAGGAGATACCATACTGCACAGTTGTACGTCT
TCCATCTGTTTGGTGTAGAAAAATCTAACCAC TACAAGAATGCCACGGGCACTGTGGCAGACAGA
AGCAGCGCTACGCCGCATCGCCTTTCAGCGTGCAGGCCAGGAATGAGCGAGGCAGTGGGCGGGG
AAGACAGGCACGGGGAATCTGGGGACAGATAAAGGAACTCGTGATGGGGCGAGGCTGGGCTGAA
GAGAAACAGATTGGGGTAGAGCTGCAAAGGGAGGGGTCCACTGGAAGGCGAGGGGGAGGCCGGG
AAGAGAGAGGGTGGGAAGGCAGTGTGAGATGGGAGGGCAGTGTGAGAAGAAAAGCAGGCTGGGGA
AGAGGGATTGGAAATGCAGAAGGAACTTGGGGAAGGAGGAAGTCTGCAGGCGGGAGGGAAAGAAG
AGAGGGGGAGCAGCTAAAGTCTGCGTCAGAAGAGGTTGGGGACTGCGAGAGGAGAGGCTGGGGCC
TGCAGGGGAGCGCAGCAGCTTTTAGCATCGATCCAACTCTAAAGACTCGTGGCCTTTGCCTGAC
CTCGAGGGTTCGGGAATAGACGCCTGTCTTTGTGGAGAGCGATAACCAACCGAGAAAATGGGGCTG
TTCCGAGCTGGGGCCCTGCGCCTGGCCCAGGGCGAGGCTTCTCTGGCTCCGGGCTGGCCCCTGAGG
GGCAGCACGCAGCCTGCAGCAGAGGCGCTGCTCCAAGCTGTCTCTTGGGGGCGCCGCCGCCGCT
TCCCTCCTCCGGGGCCGCTCGCTCCCAGGAAAGTGGAGGCGGCTGGCGAGGACCGAGAGCCGGGG
CCGCGCTGCGGAGGGACACACCTCCGGGAGTTCGAGGGGGACCTGGCGCGGCGGGCCAGCCTT
TCGGGCCGGCAGCGCCCGCTTCCCCCGGTCAGCGCTTGCGGCCCGCGCCGCGCGCACCGCCCGG
CAACCCCGCGCGCGTCCCGCGGGGGCGCTGCGTCTTCCTGCCACACCGGCGCACCGCGGCCCTC
TCCCCACACCTCCGGCCCGCACACCCGGCTCTCCTCCCACCTCCCCACCCCTCCTCTGCCCT
CCCTCCCCATTCTCCCTCCCGGCGAGGGGCGGGAGGGGGCGTGGCGGGGCCGGGGTTTGTGTG
GCTGGGACCCGGCTCCTC

[0219] In certain aspects, the promoter is a human IGFBP2 promoter as set forth in SEQ ID NO: 57. In some aspects, an promoter sequence is at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to a promoter sequence represented by SEQ ID NO: 57. In some aspects, the promoter is a human IGFBP2 promoter sequence comprised within SEQ ID NO: 57.

Exemplary Human IGFBP2 promoter (SEQ ID NO: 57)

AAGAACTTGCCCGAGTTTACACAGCTAGTAAATGGTTGCATTAGTCAGGACAGCTAGCCTATAT
TACAATAACAACCTCTCAAATCCTAATGGCTTAAACAACAGAGGTTTAATTTATACTCATTAG
CTGTTCAAGGCAGGAGGCTCTATTCTCTAATCCATACAGTCACTCAGGATCCAGGCTGGTGGAGA

CCCTGCCATATTGTAGCCTCACCATTAAAAACATGAAGAAGATAGAAAGTGAGGAGTCATGTAGG
 TTTTGTTCGGTTGCCTCAGGCTAGGAGTGACAGGTCACCTTCATCTCACTCACAGCTCACTGCCCCA
 CAACTAGTCACCTTGTGACTGTGCGAGTTAAGCTTCTGTGTGTGAAGGAAGGAAAAGAGAATGGGA
 TAAAGGTGAACATCAGCAGGCTCTACCACAGTAGTTTGAACCAAGACTTGAGCCTAGGTCATGTG
 GCTTCAGAATCTTTGCTCTTAATCACACTAAACAGCCTCTGTAAAGTCATCTTTCCTTCATCCAGT
 GCCTAAGAACATGCAGTCCAATGCCCCATCCTTCAGAAGAACTTGAGTGAACCTCAGAGAAATTG
 AGTAGAGTGCCACAGCATGCCCAAGGCCACACACCCTGAGGTTGGCAGTAGGTCCTGAGTTAGAG
 TTGTCATTTCTTGGCTCCCCTGGTAGTAGTGGAAGGTAAGGTTTTGACATACTAGTTGGATGAC
 CACGGGCAGGTCACCTAAATTGTCTAAGCATCGTTTGACCTTGTAAGAATTAAATGAAATAGCA
 CCTGTAAAAGTGCTGCACGGACTTACTGCTGTTAGTTTTGTTCCCTTCTCCTGTTGTCACCTGC
 ACTTCCCTGCCTGTTACCCAGGCCATGCAGACCAGCCAGGCCTTCGACTTACAGTGCGGATAAGA
 TTCCAAATCTCCACGGCTGGTTTTCCATGCTTTCTTCCAGGCTTCTGAGGACCCTGTGCTCTGGTT
 TCTTCTATTTCTTTTCTATTACTTTTCTGTTACTCTTGAGCACACTTGCTGGAAGCAATATGCAT
 CCAGTTCTCCCTCTCTTGCCTCATTACACTTTGCAGAACAACTCCAATCCCTTCCAACCAAGTAG
 TCCCTTTGAATTTCTTGTACCCAAGGAATCTCTCTGACAGGGGTCTTTGTTAGGGTCACACCCC
 AGGAGATGGTTGATTATGGCTGAGTCCAGCCTGGAATGATGGGGGTGGGGGCAGCTTGGGTAGA
 TGACTCAGTAAATCAAACAGAACAAATGAAAGGAGGTCATGCTTGTCCATCTGCATTATTGAAGAC
 AGCCATAAATGGCCTTACCCAGAGCGGGTCTGTGCACACCTGGAGAGCTGATCTGACCTCTCCAA
 GACCCCTGCAACTGAGTGTCTGGGATCTGTCTGCAACAAGTGCCCTCGAGATTTGTAGGTGGGG
 GCCCAGAGGGAGGGGGTCTGCAGACGAAGGGGGCAGGTTTTGCGGGGCACCTAGGGTTCTCATAG
 GTTGTAGTCACGAGCTCC

[0220] In certain aspects, the promoter is a human RBP7 promoter as set forth in SEQ ID NO: 28. In some aspects, an promoter sequence is at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% at least 99%, or 100% identical to a promoter sequence represented by SEQ ID NO: 28. In some aspects, the promoter is a human RBP7 promoter sequence comprised within SEQ ID NO: 28.

Exemplary Human RBP7 promoter (SEQ ID NO: 28)

CCCATGGCTCTGTAAAAATCAAAGAAACATCTTTTCCAACAGCCCTTTCAAACCTCCTCATCGCAT
 CTCCTGAGCTGATTCAGTCATTTAAACCTGCTTCTCCCTAAAGCTGATCACTGGCTAAGCTAATA
 GGGTTTCCGGGATTGGTTTAGCCTGATACTAATCCAGGTCTACCTTCAGGAGCCAGACCAAACCTG
 CCTATTGGCATTGCATTCTTGCAGTAGGGAGGGGAGGTATGGATGGTGTGGAGTCCACCACAAGG
 TCCATGCCAGTCTTTGCTGAACCAGCATCAGACTCCATCAAGCAACAGATGAGAGGTTCCATGAT

AAAGTGGCCCTCAGCAATCCCCATCCATTGCTGTCTAGGAAGAACAGTGCTTGTACACAGGTTTA
 GGACCTCAGTCTTGGCTGTAATCTTCTGGTTTACTTTGCCAGCACCAAACAGAAGGAAAGAAAGG
 GCTCAAATTTGACCAAATAAATTATGCTTCTCCTTCCAGAGATAACCTTGAGTCCTGTCTAGGAA
 GATATTAGAATTGTAAAGAAAAAAAAAATTACTCCTTATCCTATGGCAAGTGGAGTCTATGTCTA
 CTTTCAGCTGAAATTAAATCCTGTCCATAATAGATGACCCTTGCTCAAGCTGGCCAGAAGCCATAC
 CAACCAGCACGAAGGTTAAACTATTATTAGTTTTTTTTCTGTGATTTTTCATTTTCAGGCCAAGTTT
 TAGAACAAATAAGATTTTTAAGAATAGGAAGTAAGTAAGATTTCTGCATATCCTGTTCTCTTAGTCA
 GCTGAATTTTTTTTTTTTTTTTTTTTAGTCCTAACTCAGCCTCCCAAAGTGCTGGGATTACAGGCG
 TGAGCCACCGCACCAAGCCTGGAATCTATGTCTTACAGTTATGAGAATCAACAGCTAGCTCATTA
 TGGGCAAGGTGATGTCACTCTGGCTTCTCAATGAAAATGGCATTTCTCCCTTGAAAAAGGTCATA
 GCCAGTCAGTCAGTCAGTCACGGGAGCGCAGCGGCTTCTAGGGGTGAGTGGGACCCACGCGGCC
 CACCTGCTCCTCCCGCGCGCGGCCCCACCCCCCTGCCCCGCCCCGCCTGGTTTATAG

[0221] In certain aspects, the promoter is a human GJB6 promoter as set forth in SEQ ID NO: 16. In some aspects, an promoter sequence is at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% at least 99%, or 100% identical to promoter sequence represented by SEQ ID NO: 16. In some aspects, the promoter is a human GJB6 promoter sequence comprised within SEQ ID NO: 16.

Exemplary Human GJB6 promoter (SEQ ID NO: 16)

AAATAGCTTCCAACGTTTCCACCCCACCAGCCCTTGCACCACTCCCTGTACTGGCCCTGAGCTTT
 CTAGTCTTGA CTGAAAAGCGGGAGGCAATGTGGTCTCTCCTGGTGCAGTGTCCCGAGGAAGGCC
 TGCTCCGCTTCCCCGGAGGAGTCTTCAAAGGATGGAGGTAATTAATAAAAACAACCCCTGTACCT
 CCTCTAAGTGGTCATTAATTAATAAAGAACCCTCCAGGCTCCTATAGGAGAGGTCTGTGCACCCCG
 CGGGCTATGAGAAGGCTGGATCACCCAGAAAGACTGAGGATGTGTCCTGGCAAAAACACAGCCTG
 CCCCTCACACTGCTCCCCACGGGTGCAGTAGGGAGGAAGAGTTCCCTCGAGGGCCTGAGCAGGCG
 CCCCACACCTGCACCCGTGCAGAGGGGGCTGGGCCCCGCCCTCTGCGCTCCCGAGGGAGAGCCCTA
 CCCCCTGCATCCCCGGTACCCCGTTCCCTCCAAGGGCCGAAAGAGGGCCCCGCGCACTGTGCAC
 TTCTTAGGGGTCCCCCACCCTGCGCCCCCGCCACGGGAAAAAGGTCCCCGCTCTGCGCATCCGGC
 CCCGGAGGGACAGCCCCGGTCTGCACTCCTTGCTCCTCAGGGGGACGGTCCGCGCCCAGCGGCT
 AGTGCGCCCCGGGTAGGTGGGGGCGGGGGCTCGTCGAGTGACAGCGCTCGCCTCCCGCAGCCCG
 CCCGAGCCGCGTCAGGGCAG

[0222] In certain aspects, the promoter is a human PARM1 promoter as set forth in SEQ ID NO: 40. In some aspects, a promoter sequence is at least 85%, at least 90%, at least

95%, at least 96%, at least 97%, at least 98% at least 99%, or 100% identical to promoter sequence represented by SEQ ID NO: 40. In some aspects, the promoter is a human PARM1 promoter sequence comprised within SEQ ID NO: 40.

Exemplary Human PARM1 promoter (SEQ ID NO: 40)

TGTACAGGAGATAGTCAGGGAATTAGTAATTTTCAAAGAGGTGACTTTTGAATTCAAACTTAAATA
TCATCTTCAGCTGAAACAAAGAAGGGGTGCAGTTATGAGGAAGTGACCAGGTAAAGCATGGCAAA
CAAAGGTAAAGTTTGTATGCGTATTTAAGTCAGAGCCCTCTCCATTGATAAGAGTTTCCAGTAA
TTTAGTGCCATCCTTTTCTTGCTATAGAGTTCTCGTCTCTATCTGAGCACGCAAAAATAACATGC
TTTCTTGCTTTCTTGAAGTTGGGCATGGCCATTGACTTGCCCTTAGCCCATATTTTTCTGTGAAGT
GGTCTTCAAAAACCTATATTTCTGCCATAGAGTCACTTAACTTAACCTGCCCTATTTAAAGGGGCT
AATGCCTGATAGAATGTCGCTGCATAACTCCATCTGTGTGTGGTCCCTGCATCCATGACAACCAA
AACCCAGATGCAGAAATTGTTCCCTAATCACATAGATTACCTTAGAAACCGGAAGGGCCTTGAAGT
CAAAAGCATTCAGAGAACATGCTGAACAAATTGAATTTGCAGTTTATCTGGCCAGGGAGGATGGA
GAGGGGATGGGCACCTTGGTCTGAGTATTTTTTGTCTCATTCCAACAGAAATTACTAGATTTAC
CAAAAATCTACAAGTGGTAGTGTGATAGAGTCAGGCAGAGGAATTGACCATAGATAAGGTGCTC
AGGACTCCTAGAGTCAGCTTCTGGTATGTGAGAAAGAAGTGAGAACAGAGCCCATGGCATATGAA
GAAGATATTACAGAAAAAAGAAAGCTGCCCTTCCACGCAAATCATTTCTTTACAAAGGCTTGTTAA
CTCCTGCAGTGCCAAGAAGCTGAATGCAGCGGCAGACATCCTGGTTCGGGCCCCAGGAAGCTCAG
CCGGGTTTAATGTGGATGAGGGTTTAATGATGTACACGCAGAAGTGTTTTTGACAAATGAAGAAGG
TCCTCATTCTTGGAACATGTGCCGGTTCTCCGAGGGAACCTCTAAAAGGCTGTAAGCTCATGTAG
GAAAAGCTGAGCTAGATTCTAAGGGCAGAGATGTGCTCACATTTCTTTGCATCCCTAGTTCCCA
GCACAGTGCAAGGCGCTGCAACATTTGCTGAACCCAGGGTCTCGTGTCTTGACTGTCCAGCAGA
GGCCGCTCTGGGCCGGGGCTCTCGGGACCTGAGGGCTGAGAGAAGGAAGGCCAGGGGGTGGCCCA
GTCATCGCCGCGGGGCCGGGTGGGAGGGGTTTGGCAGCGGCAGGCGCGGCGGGCGGCGGAGG
CGGAGGCGGCCCGGG

[0223] In some aspects, the inner ear supporting cell selective promoter is a BACE2 promoter. In some aspects, the BACE2 promoter comprises a nucleic acid sequence with at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% at least 99%, or 100% identity to SEQ ID NO: 92. In some aspects, the BACE2 promoter comprises the nucleic acid sequence of SEQ ID NO: 92.

Exemplary BACE2 promoter (SEQ ID NO: 92)

TGTGCTGCGAGGGCTTCATCTCCTAAGCACTAAATGCTAAATTCCTCCCTCCACGCCC
 ATCGCCACTGTCCTCACGGATCCTCGCAGCAGCTTCCCAATCGGTCTCCCTGTCTCCA
 GCCTCACCACCCCCAACTAAGACCATTTCATGAAAACAGAGACAACCAAGGAGACAG
 TCACCCAATGCTGTCCCTTCAGCTTGCATTATTTTCTGACAAGACAGCTCTGCCATCC
 ATGGAAGCCTGTGTTTGAAGATCTCTGACATAAAGGTCCCTTGCAGAGCTAGACGTG
 ATTCTAAAATTGGGAACACAGGAATAAAAATCAAATCTTGAGTAGAAGTAGCTGAA
 AATTGCAGTGATTCGGGGAAGCTTGGCTTCTAACTCCCCACTGTTTGAAGATGGGCT
 TGTTTTGTTTTTAAAACAGCCAACATAATTCAGCTGGAGGAGGTACAAAGAATTTTC
 TATTCCTTGTTTCTGTAGAAATCGATGGACTTTAGCTTGTCTAATTGTCCCCCTGCCT
 TTAGTATCTAAAATAAAAATAACCCTCGTTGCTTGCATTACTCAACGCATTTCTGCGTC
 TTGGCGTCTATGGCTAAACGAGTATTAATTAGACAGTCCGCAGAGAGCTGGCTGGGG
 ATAGAAGGGGAGGTGGGGGAGAAGGGCAGGGATCACAGCAGGGTGGACTCGTGCC
 CCTGATTTGGGATCCTGACAGCAACTTACTAGGTGGCCTGAGGGCTGGGTGCCAGGG
 GAGGCAGCGGGTTCCAGTAGCATCTGACCTGCATTAGGGACAGGGGCGCGGCGGAG
 GGGGCGAAGGGGGCGGGGGTGGGGGAAGGTGGCTGGGGTGAAGCCAGCTTCGC
 AGCTAGCTGTGGGCAACAGAGGGAGTAAGGGGGGGCAATGAGGCTGGGGCCAGGC
 GCCAGCAGCAGCCACGCCCCCACCTCCCCGATTTTTAGGGAAAATTCTCCAAAGC
 TCTCGCATCCTCCTCTGCCTCCTTCCACCCTCCACCCTCCCAGCCTCCACTGAGACCT
 CTTTAAAACCCAGGGGCCCGGGGGATGAGGCCGGGGAACGGGCTGGACTGA
 GGGCGGGGGCTCGGGGGCAGCGGACGGGAACGCCTCGAAAGCAGCCAGACCCGG
 CGACTGAAATGAGGCGGAGGAGCTTGGCGAGGGGAGGCGCAGGCTCGGAAAGGCG
 CGCGAGGCTCCAGGCTCCTTCCCGATCCACCCTCTCCTCGCTGACCTCCGAGTCACC
 CCCGGAAGCTCCCGCCACTGCCGGGCGAATAGACCCCGCGGACCCCAAGCGCGC
 GGGGCCGGGGCCCTAGTTCAGGCCCTCGCTGCCCTTTAAGGGTTCTCGAACTTTC
 CCCCCGGTATCAGATGAGCCTCGTCACATCCGTTGGCCGTGGC

[0224] In some aspects, the inner ear supporting cell selective promoter is a DBI2 promoter. In some aspects, the DBI2 promoter comprises a nucleic acid sequence with at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% at least 99%, or 100% identity to SEQ ID NO: 93. In some aspects, the DBI2 promoter comprises the nucleic acid sequence of SEQ ID NO: 93.

Exemplary DBI2 promoter (SEQ ID NO: 93)

GAAGAAACCTGCATTTCTTACACTTCAGTGTACTTTCCCATATTTAACTCCAAGATT
 TTTGTTAATTTGTTTGGTTTTCCTTTCTCAAACAAAATTATGCTCAGACTGAAAACCC
 TAGATTTGTTCCCTATTGCATCTTCATTTCTTCCCAAACATTCATATAAACGTGACCT
 ACATTAAGTTAGCAAGTTAAGTCTGAAAGCGTCTACCTTCCCTGGGGAGGGGGAAG
 GTGTAGGCAGGGCAGAGATTTGTAGTCCAGCCCTCTTGCCACAAATTATGAATTAGA
 GAGGAATGACTTTGCTTTTTTAATGATCTCCAGAGAATTTTCCATCATTTCCTCTCTT
 CACCCAGCTCCTTTGCAACCACTGCCAGAGAAGTCTTCTTTAGCTTCTTAAACATCG
 ATCCTAAAACACTTCCAGACACCTGTGCTGCTCCTTTCAGTTCCCATGGAGATTAGGC
 TGTGTAACAATCTCGCAAAGACGTTCCCTCCGTCTCCTCATCCTCTTTTCAAACCCT
 TTTACGATTTCCCATCTCACTCAGCATGACAGTCAAAGTCCCTGTGATGGCCAACTTC
 TGCATCACCTAGCCAGTCTGCCACCGCCAAAACCTCTCCAGCCTCATCTTACACTTGTT
 CTCTGCTTGGAATCTTCCCTCCCCTCCTTGAGGAACCTTCTCAAATGTCACCTTCCCTC

AATACTCCCCCTCCTCCATTTAAAACTATAAACTTCCAACCTCTCTAAGCCCCCTAAAGT
 ACTCTATATTTAACTTATTGTATAAACTACTGTCCCTACTTGTAAGTTCCAAGATTGC
 AGGGATTACCCGCTTTGTTCACTGCTGTCTGCCAAGGTCTAGAACAGTGCAAGTTA
 CCCAACAGGAGTTCAATAAACAGCCATTCAATTAACAAATATTTGCTGAGCACTTCG
 TCCCGTCCAAGTTTGTAAATCAAGACAAATAAGACACCGTCCCTGCCTTTAACGCA
 CCAGATGGAGAAATGCACCACAGACATAAATGTGCAATACAGGCCTGACACTACGG
 CCACAAGCAAGTCAAAGAACGTGCCAAAAGTTCAGAGGAAGAAGCCTCGGCTTCGC
 CTTTCGGGAGACCAGTCCAGCTTTCCACCATCACGCTGCTCATCAGGGACCATCTCC
 GGGGGTCTCCTCTAGACCCCAAGGGAGGAGCGGGTCCCGCCCGCCATTCCCAGGTCT
 CAGAGTTTACTTGTCCAGAGATGCAACTTCCGGCCTCTTCAGGCCGGGCAAGATTTA
 AGGAAAGAAAAGAAACATAAGGACCTCCGTTCTTCGGTCTCCGTCCCCTCCCCTTCC
 CCCGCGTGCCCCACCTGTTCCCGGCGTCCCCTTCGGCTACTCCCGGCGTTTGCGCAAG
 CGGTCCCACGTGGGCTCGGGCGGGGCTAGCGCCGCGGCGGGGGCTGGGCACGCCCC
 TAGCGCATAGCTGGCTTCTGATTGGCTTTCC

[0225] In some aspects, the inner ear supporting cell selective promoter is a FABP3 promoter. In some aspects, the FABP3 promoter comprises a nucleic acid sequence with at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% at least 99%, or 100% identity to SEQ ID NO: 94. In some aspects, the FABP3 promoter comprises the nucleic acid sequence of SEQ ID NO: 94.

Exemplary FABP3 promoter (SEQ ID NO: 94)

TACCATTCTGCCTTTACCTGATGTTGCTATCCTCCTCCTCTTGTTCCTTCCACCCA
 TCCTTTCCCTCCCACATTACTCTCTTATCCCACCCTATTTTACAACCAGTAGCCTAGG
 GAAAAGAGCATAGCTCAAATGAGGAAGAAGGCAGGACAGGCAGTCATGGCTTAGCT
 GGACTGAGCTGCAGTGCTTCTCCTTCTGGGGAAGGGGGTGCAGTGTCTGCTACT
 GACACATCCCTCCAAGGCACTCAGCCCTGCAGGGAGCAACCTGATTCTATGACTGAC
 ATCTAATCTTCACATTCACCTTGCAGGAAGGCAAGAAGTGATCCCAGCCTCCAGATG
 GAAAGATCAAGGCCCAGAGAAGGTCAGTGGTGGTGGAGGCCTGAGGTCACACAGC
 AGCCAAGTCTGGAGTCACTAGTCAAGGTGACCTTGACTAGCCACCCACCTCCCCTT
 CCCTGCCCCACCATGGCCCTGGGAGATCTGTTGTCCTGTGAGGGAAAGGGGCTCCAG
 GCTGGGCTGCATCTGAAGCCCCTAGATCCAGAGACTTCATTTCTTAGGCTATCTATA
 AAATCCACCTTCCTTTCTTTTCCCAGGACCCCCATAACCCTGCTCCCAGCATCGTCTGC
 CTCAGCTAAGCCATGGGGATTGAGAGACCAGGCCTGGTGCCAGATAAACTGACCC
 TGGGTGAGGGGACAGGGGCCAGAATGGGCAGGTAGAGACTGAATACTGAAGAAG
 AATCCTCTGGAGTCTGTTAGCAGAAGCAGATGGGCCTTGCCTGACTATTGGCAGGCG
 GACCTGGTGGTCAGACCTCAGTGATCCTCAGGGACCAGTGAATATTTAGGCTGGGG
 CTGAGCATCACCTGCTCCCTTGGCCCCACTTATAGGGCAAAGGGGAGTCTACCAGCC
 TACTCACTGATGACAACTGGAAAAGTTTGTCTGTCTGCTCTGGCCCCACCTCGC
 CCTCTCCCCTACTTGGAAGTTCCTTTCTCTGAACCACTGACTGCCAAAGCTTGAGGGAT
 TAAATAAATCATCTGGCCCAACCTCCTACCATAGAGTTGGGAACACTGAAGAAAAG
 AGACTGGCCCAAGGTCACAGAGAAGGCAGGGTGAACACTGTCACAGGGAGAGCCA
 GTGTAGAATAATGGTTAAGCCACGCAAGCTCTAGAACCACTCTATCTGAGTGCAAT
 CCTGGCTGTCATCTGGTACTTGCTTCTGGAACACATCTGGCCTCAGACTCCTGAGGC
 CAAGACACACTCCCTGCCCTAAGACTTGCTGGTTCTATGGCAGGCAGAGGCAGAAA
 GAGCCCCACCATCATTTCCAGCAAATGGGAAAAGTCCCAGTTGCAGATATTAGGGG
 TGGGATGGGGCGGGGGTAGTCAGCAACCATAGACTTAGACCCTGAAGAGGCAAAAA

AGGAGGGCCATGTTCTTGGGTCAGCAGAGCTTCTACTCAGCTTCTTCAGCCTCTAGCT
 CTTTCTGGTGCTAGTAGCACATTCTCTAGTGGAGGCATCCAGATGGCAGGGAGGGT
 CCAGGAAACAGCTGAACATGCTGAGCAGGCCTCCCTTGTCCCCGCTCCCCATGGCCC
 CATGGATCATCCGGTGCTGCAGCTCATCTCATTGGCTGGCTTCTGGTTACTCATCTCT
 CCTCTTCTCCATCTTCCCAGCCTGTGGTTGCCGTGGAAACATAGAACAGTGACCTCAC
 CATAGGATGAGGGCTGGGGAGATGCTGTTCTTGGCAGGCGCT

[0226] In some aspects, the inner ear supporting cell selective promoter is a KLHL14 promoter. In some aspects, the KLHL14 promoter comprises a nucleic acid sequence with at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% at least 99%, or 100% identity to SEQ ID NO: 95. In some aspects, the KLHL14 promoter comprises the nucleic acid sequence of SEQ ID NO: 95.

Exemplary KLHL14 promoter (SEQ ID NO: 95)

GAAACAGCAGCCATTGATGTAGCTCAGGGTTCTGTGGATCTGTCATTTGGAGCATGT
 TGGTTCTCCTGTCTCAGCTGGGCTCATTCATGCATCTGAGTTCAGCTATTGGGCAATC
 TGGGGAATGTTTTGTCCATGTGATGTGTCATCTTCTACCAGGCTAGCCTGGGCTTCAT
 CACATGGTATCTGGCAGGGCTCTAAGAGGGAGAGTTGAAACACACAAGGCCTCTTG
 AAGCTTAGACTCAGAATTGGCACAAGGTCGTTCTGGCACATTCCATTGGTCAAAGC
 AAGTTACAAGGCCAGCTCACATTCAAGGATTAGGTAAGTCGATTCCACTCTTGATGA
 GAAGTCTGAAGGATTTGGAACAGTGTCCACCATGCAGTAATAAACTCAATAAGTAGT
 AGCCATTATTATTCTGTTAGAGGTTGCCAGGAAAAGTTTTATAGTGGAAGAAATCT
 GAGTTTACTCTTGAGAGGTAAGTGGAAATTTCTATTTGTAGAGAATGAAGGCCTCTCA
 AAAAGACACAGCCTAACAAATAGGTGCTGCAGTTTAAACAGTGGAGCGTGTCCAGAAC
 AGGCTGCCCTTTTAGGCAAGGGCTAGTGTCTTTCAGGACAGACCCAAACCCCAAATA
 CCAAAACAGAATAAAGTAGTGTCTTAGCATACTTTGAGATCAGACTGTTTCTGCATT
 TCACAGTGTGGGGGTGGGGGGGAGGTGTGGGGGGAAGGGAAAAGCAGCATACCA
 ATGTAGTGAAATCTGGAAACAACAGCCAAAAAAGTTTGCATATTGCACAGAGCAC
 TTGAAGATCATAAATCTATGCATGAGAAAGATGTAGTGGAAATTTTGGGGGGGATTA
 GAGTTTATTTTGTCTCTCTGTGAGACAGCTACTCATTATCCAGATCACAGCTAAG
 AAAAAAGCTGGTCACAGAAATTAGCAGTTTCAGCTCAGCAGCGAAGTCGCCAGCCT
 GTGAAGGCAGAGAGAAATTGACTAATTAGCAATGCGCACTAAAACCTTGACGGTTCTT
 TATAGAGAGAGAGAAGAGAGAGGGAGAGAGAGGGAGAGGGAGGGGGGGGCT
 CGCTTTTCCCCCTTCTTCTTCCAAAGATGTTTGAAATCGCAGTCATTTACGCTCGAC
 AATTTTACAATAGCCTTGAGCCATAATTTTGCAGTCTCTCCAGCATCCATCCCCCT
 GTATGGTCTCTCTACTGGCCAAGCACGACCGTTTCTCTCCCAACCGTGGATTTC
 TATT

[0227] In some aspects, the inner ear supporting cell selective promoter is a MMP15 promoter. In some aspects, the MMP15 promoter comprises a nucleic acid sequence with at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% at least 99%, or 100% identity to SEQ ID NO: 96. In some aspects, the MMP15 promoter comprises the nucleic acid sequence of SEQ ID NO: 96.

Exemplary MMP15 promoter (SEQ ID NO: 96)

CCTTCCTCCTCCAGGGCCCTCTGCAGACCAGGCTGAGATGGAGGAACCTGCTAAAT
 CGATGGAGATGCTTCTAGCCTCCCAGTAGGAGGCCCCAGCCATGCCTTCAACCTGGC
 AGGAGGTGTAGCCACTCCTCATCCTTGGGTTCAGGTTGGGTGCTGCTGTTGTGGTC
 CTTCCCAGAACTGCCAGTAGAGGGCAGCCTGGGCATCCTAATGCTTACTCTGGTTG
 TTACACAAAGAAAATATTGGGGTCACTGGCGAGCCCACCCACACTCACCAGAATCTC
 CACTGTAGTCCCCCTAACAAACAGCCCTTCACTTCCTCTCCCACTTCAGCAATTTGTA
 TTTTGATGCCATTGGCCTCAGATCAGAGTGTTTTAAATCATCACGCCCTGGCTTATCC
 CTGGTCGAGCCAGGACACGGGGTGGCTTCAGTGGGTCTGTCACCCTCTCTCCTTGAAG
 CATGTTGCTTTTATTTATTTACTTTTACTCTCACCTGCTCCTGTACCAGCAGGGGCCA
 CTTCAAAGCCAAGGTACAGGGTGATAACTTGTGGTCCAGCATCAGTTTTCTCCACTT
 CTTTCTCCCACTCACCCCCAGCAAGGTGCCTGGGGAGACTTGAGCAGATGTTTCATTT
 TGGCCTGGCCAGTGGCTGAAAGCCAGGCCTCCAATGCACTGTGACCTCTGGCTTCCC
 CAGCAGCTTTCCCAGAGAGGCAGAGGGAGTCTTCATTCTTCCCAGGCGGGGAGACC
 ACGCCTTCCCTGCCTCCTCCCTCCGCGGGGGGTGCGGTTGGAGGTCACCCCCGCCCC
 CTAGGCGCTGGGTGGGAGTGACGCGGGGTGGGCTGGAGAGGTTTCTGCCGTCTGG
 GAAGCGTAAACGGAACGCCACCTGTGCGGCCTCGGCCGCCCGCACCTGCTTGTGAG
 AAGCCTGCGGCTGGGGCACCGCCCCCGGTCCCCGCCCGGGTCCGCGCATTGGGAGC
 ACACTGGCCCTTTAAGAGCGCGGCGGCCGCGCGCGGG

[0228] In some aspects, the inner ear supporting cell selective promoter is a SPARC promoter. In some aspects, the SPARC promoter comprises a nucleic acid sequence with at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% at least 99%, or 100% identity to SEQ ID NO: 97. In some aspects, the SPARC promoter comprises the nucleic acid sequence of SEQ ID NO: 97.

Exemplary SPARC promoter (SEQ ID NO: 97)

CAGGCTACCTCTCAGGCTGACTGAGTCATGCAGCATAGGCTGCCACGTCTCTGGGCT
 GCGGGGCCGTCATTATTCCTGGCCTCACTGCAGCTAAATTGAAGAAACGTTTGGTT
 TGTGGGCCACGTCAAGGAATGTGTAAGAGCTGCCACGTTGTCGGGTCTGGGTATTG
 GGCTTTTCCCCTCCTTCAGAGAAGATTTCCAGGCGTGTGGGTGGGGTTTCAGAAGAA
 AATTGATGCCTGCGTGTGAGTGTCCCTGGACCTGGACCAGCAGCGCAATATTACA
 GACCCGGGGGTTGGGGCAGACTGAGCCAATCTCTGCACCGTCAAAGTTATGGATAC
 AGAGCCCTGGAAAAAGGCTGAAGGATAAGATAGCTGACATTTATGAAGTGCTTCAT
 TCATGTAGCAGTGGGCCAAATGCGTACTTTACACTTGAGGAAGCTGAGGCTGGAGGT
 TGATAACATGCCTCAAGTCTTCTAGAGTTAAATAACTTTGACCCAGGACCCAAGCCC
 AGAGTTCTGACTCAAAAAGTGGCCTCCTAAACATCCTCTTATATGAGGTTAAATTT
 CATCTTCCTCTGTTTGGCCTTGGCCTGGTTGGTGGATGCTCTGCTTCGGGGACCCAGG
 GCCAGATGACAATGGGTTCTTTGTGCCCTTCAGACAATGGGAAGGGCTGCCTGGGGA
 AAGATACAGTAACAAGGCAACAGGCTGAGTCAGCCTCCAATGTGCTTGAACCTTCTT
 AGCTTGGCAGCCTTGACATTCAGCCAGCCACACAAAGGGTATATCAAGGATGATACC
 ACTAGTAGCAGCTTGTCTTGTCTGTACCTCTGAACAAGAAAGAGGCTGTTCTGGGTC

ATCCCTCCAGGCCTGTCCAGCCCTGGCACTCTGTGAGTCGGTTTAGGCAGCAGCCCC
GGAACAGATGAGGCAGGCAGGGTTGGGACGTTTGGTCAGGACAGCCCACCAGGAGG
AAAGAAATGAAAGACAGAGACAGCTTTGGCTATGGGAGAAGGAGGAGGCCGGGGG
AAGGAGGAGACAGGAGGAGGAGGGACCGGGGTGGAGGGGAGATAGACCCAGCC
CA

[0229] In some aspects, the inner ear supporting cell selective promoter is a TSPAN8 promoter. In some aspects, the TSPAN8 promoter comprises a nucleic acid sequence with at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% at least 99%, or 100% identity to SEQ ID NO: 98. In some aspects, the TSPAN8 promoter comprises the nucleic acid sequence of SEQ ID NO: 98.

Exemplary TSPAN8 promoter (SEQ ID NO: 98)

CCAAGGACTCTTTTTCTAAACTTCCCTTCATCTTCTAGTTTGACGCCCTTGGTGGA
AAAGTGTCTGAGATAAGGAAAAGGCATCCTTTCAGTTCTCTGATACTATCTGAAGC
GAGGGATGGAGAAAGGCAAAGAGAGACACAGGAGAAGCGTATCCCCTGGGAACAG
GTGTCTAGTGGAGTCCAGTAACTCACAGTCTCTCAGTTCCGTCAGCACTGTCCCTTGG
GTCGCAAATTTCTTCCATTAGCCCTTCCACCAGCTGTATTTCAAATGGGGCTGGACAA
TAATTGTGGCCAGTGGCCTTGTGTTGTTTGTACTTGCGGACTAGTAGTTCTCACCTGT
CTTCTCTGACTCCTATTAGCCACTGGGATTTTACAGCAGCTGGTTCAGCCAATTCTACT
CAATTCAACATTAAGTTGCAGTGGGCTAGAACTCATGGGCCGATTTAACAAGTGAAA
TTCTACCAAGATACATCAAAAATAGCAACAGGACTAGATACTCAGCTCATTTTGT
TATTTGTAATATAACCAGTTGTGGCTTTAGTGCCAGTCTGATTCATCTCTCTACTACAA
AATGAGGCTCTATAAAGGAAAATATTGCAACTGGAGTGAGGAATTTGAATCTTATAG
GAAGGAATTTGTCTTCTCATGAAGACTTCAGTTTACCAGAAGTATCTATTGAGGAAG
TGTTTACAAGAAAATGTGCCATTTAGCTTTATTCTAAATTTGCATAATAACTGAACCA
AACAAAAAATATAGATAGATAGATTGTTCTATCTATAGATAGATAGGGAACATTG
GCAGTAGGTGGCAGTAAGTTCCCCTGAGCACATGGAGGACACAGTTAAATGCATTG
AGGTATGTGGGAAATGGTTTAAAGCAGAATTTTATGCCAACTTTTAGTAACGGAAGC
CTAACAAATGTTTGTCTTTTCAAGTGAGAGAAGCAAGCAATCTGGAACATTTCATAA
GCTTATTTTCTGTATCCTTAAACATATTTTATAATGAATGTATGATTTAAATAGTAAG
TTAAGTGTCTGGGGGTACTGCACACCTCCCTTGCATACAGTCAAACCTTCTTCAGGGT
GATGGGGAAGAGGAGTTATAGGCTGCCAAGCAAAATTGCCAACTGGTCTCAGAAA
TTCAGTGCATTGGAGAGCGCGGATCCTTGCAACACTGACTTTAGCAGTTAACTAG
AGTGGTTGGGGATGAGTATTCT

[0230] In some aspects, the inner ear supporting cell selective promoter is a VIM promoter. In some aspects, the VIM promoter comprises a nucleic acid sequence with at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% at least 99%, or 100% identity to SEQ ID NO: 99. In some aspects, the VIM promoter comprises the nucleic acid sequence of SEQ ID NO: 99.

Exemplary VIM promoter (SEQ ID NO: 99)

ATTCACAATGCATTCCCTCTGCCCACCACATTAATTATCAACTCCTTTTCCTGGCATT
TACTCATCCAACGCATGGCCCCACGTTAACTTTTCAGTTCCCTTTCTCCCCACAAATA
CTCCATAATCCAGCAACCCTGGGATCCCTGAGATGATGAAGAGGACCAGTGCCCAT
CCAGGAGACATCACCGCAGCCCTGAGGAATCGGCTATGGGCACCAGCAGGGCACAG
TGCCACACCTCGCCAATGCCTTGTCTCCTTTTCCATAGTGAGTCAGTCAGCAAGCGT
GTAGAAGTGAGTTCCACACTCTCTTCCTCCCATAGGGAGATCACTTTTCTCATTCTAA
GGGTTCCAGGCACACTCACAATGGTGGCATTGCTGAGCAGTGGCTTGAATAAAGGG
CTCTCAGAAAGCAAGATGTAAGTCAGAGCATAGGCTAGCCCCAGGAATGCTCTTG
GGAATGACCTGCAGCCTCCAGTGAAAGAGAGAATAAAAGAAAGCCCCAGCAGGCG
AGCTGGGCAGTAGAGAGTCTGTAAATTCACCTTGGCAAGCACCATTGCAAGAAGC
AACTGGGATAAGGTAAACAAAATATTGCCTAAAAGAGGCTTGTCCAAAGAAGTCAG
AATACGCTCTTCATTACCTCTAAATTTTCAGTACACCATAAATCTAAATACTCAAAAA
AACCTGTGCCTTTTCAATCAAGGTCAATTGCACGAATTCTTTTGGAAAACAGGACCT
ATGGCATTTCAGACAAATCACCGTGAACCCTGTACTGTGCATTGCTGCTCTAAAA
TCCAAAGATTCTGTCATTTGTGTTACATAATTGCCTTTTCATTTGAACTCATTAATCAA
ATTGGGGTTTTTAAGCAACACCTAATTAATTCTTTAACTGGCTCATCCACTGATCACT
GAGTTCTATTTTGAAGTACGGACGTCGAGTTTCTCTTTTACCCAGAAATTTTCAGAT
CTTGTTTAAAAAGTTGGGTGTGGTTTCATGGGGGGAGGGGGAAGAGCGAGAGGAGA
CCAGAGGGACGGGGGCGGGGACTCTGCAAGAAAAACCTTCCCGGTGCAATCGTGAT
CT

[0231] In some aspects, the inner ear supporting cell selective promoter is a GFAP promoter. In some aspects, the GFAP promoter comprises a nucleic acid sequence with at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% at least 99%, or 100% identity to SEQ ID NO: 91. In some aspects, the GFAP promoter comprises the nucleic acid sequence of SEQ ID NO: 91.

Exemplary GFAP promoter (SEQ ID NO: 91)

GAACATATCCTGGTGTGGAGTAGGGGACGCTGCTCTGACAGAGGCTCGGGGG
CCTGAGCTGGCTCTGTGAGCTGGGGAGGAGGCAGACAGCCAGGCCTTGTCTG
CAAGCAGACCTGGCAGCATTGGGCTGGCCGCCCCCAGGGCCTCCTCTTCATG
CCCAGTGAATGACTCACCTTGGCACAGACACAATGTTTCGGGGTGGGCACAGT
GCCTGCTTCCCGCCGCACCCCAGCCCCCTCAAATGCCTTCCGAGAAGCCCAT
TGAGCAGGGGGCTTGCATTGCACCCCAGCCTGACAGCCTGGCATCTTGGGAT
AAAAGCAGCACAGCCCCCTAGGGGCTGCCCTTGCTGTGTGGCGCCACCGGCG
GTGGAGAACAAGGCTCTATTCAGCCTGTGCCAGGAAAGGGGATCAGGGGAT
GCCCAGGCATGGACAGTGGGTGGCAGGGGGGAGAGGAGGGCTGTCTGCTTC
CCAGAAGTCCAAGGACACAAATGGGTGAGGGGAGCTCTCCCCATAGCTGGGC
TGCGGCCCAACCCACCCCTCAGGCTATGCCAGGGGGTGTGCCAGGGGCA

CCCGGGCATCGCCAGTCTAGCCCACTCCTTCATAAAGCCCTCGCATCCCAGGA
 GCGAGCAGAGCCAGAGCAGGTTGGAGAGGAGACGCATCACCTCCGCTGCTCG
 C

Table 2. Exemplary Promoters

Promoter	SEQ ID NO
PARM1 derivative	40
GJB6 derivative	16
RBP7 derivative	28
IGFBP2 derivative	57
GDF6 derivative	90
GFAP derivative	91
BACE2 derivative	92
DBI2 derivative	93
FABP3 derivative	94
KLHL14 derivative	95
MMP15 derivative	96
SPARC derivative	97
TSPAN8 derivative	98
VIM derivative	99
GJB2 minimal promoter	86
1. CAG enhancer/promoter	15

2. CAG enhancer/promoter	14
1. CMV/CBA enhancer/promoter	13
2. CMV/CBA enhancer/promoter	12
1. CBA promoter	11
2. CBA promoter	10

Enhancers

[0232] In some instances, a construct 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 polypeptide (e.g., a therapeutic polypeptide, a Connexin 26 polypeptide)). Enhancer sequences (generally 50-1500 bp in length) generally increase the level of transcription by providing additional binding sites for transcription-associated proteins (e.g., transcription factors). In some aspects, 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/or a SV40 enhancer. In some aspects, a construct comprises a CMV enhancer exemplified by SEQ ID NO: 18. In some aspects, a construct comprises a CMV enhancer exemplified by SEQ ID NO: 63. In some aspects, a construct comprises a chimeric intron enhancer exemplified by SEQ ID NO: 64. In some aspects, an enhancer sequence is at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% at least 99%, or 100% identical to the enhancer sequence represented by SEQ ID NO: 18. In some aspects, an enhancer sequence is at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% at least 99%, or 100% identical to the enhancer sequence represented by SEQ ID NO: 63. In some aspects, an enhancer sequence is at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% at least 99%, or 100% identical to the enhancer sequence represented by SEQ ID NO: 64. In some aspects, an enhancer sequence is at least 85%, at least 90%, at least 95%,

at least 96%, at least 97%, at least 98% at least 99%, or 100% identical to the enhancer sequence represented by SEQ ID NO: 65. In some aspects, an SV-40 derived enhancer is the SV-40 T intron sequence, which is exemplified by SEQ ID NO: 19. In some aspects, an enhancer sequence is at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% at least 99%, or 100% identical to the enhancer sequence represented by SEQ ID NO: 19.

[0233] In some instances, the construct does not include an enhancer sequence.

Exemplary CMV enhancer (SEQ ID NO: 18)

GACATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCCATAT
ATGGAGTTCCGCGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCCCG
CCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGTC
AATGGGTGGACTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGT
ACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCAGTACATGACCTT
ATGGGACTTTCTTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGG

Exemplary CMV enhancer (SEQ ID NO: 63)

GACATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCCATAT
ATGGAGTTCCGCGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCCCG
CCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGTC
AATGGGTGGACTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGT
ACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCAGTACATGACCTT
ATGGGACTTTCTTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGT

Exemplary SV-40 synthetic intron (SEQ ID NO: 19)

GGAGTCGCTGCGTTGCCTTCGCCCCGTGCCCCGCTCCGCGCCGCCCTCGCGCCGCCCGCCCCGGCT
CTGACTGACCGCGTTACTCCACAGGTGAGCGGGCGGGACGGCCCTTCTCCTCCGGGCTGTAATT
AGCGCTTGGTTTAATGACGGCTCGTTTCTTTCTGTGGCTGCGTGAAAGCCTTAAAGGGCTCCGG
GAGGGCCCTTTGTGCGGGGGGAGCGGCTCGGGGGGTGCGTGCGTGTGTGTGTGCGTGGGGAGCG
CCGCGTGCGGCCCGCGCTGCCCGGCGGCTGTGAGCGCTGCGGGCGCGGCGGGGCTTTGTGCGC
TCCGCGTGTGCGCGAGGGGAGCGCGGCCGGGGGCGGTGCCCCGCGGTGCGGGGGGCTGCGAGGG
GAACAAAGGCTGCGTGCGGGGTGTGTGCGTGGGGGGGTGAGCAGGGGGTGTGGGCGCGGCGGTGCG
GGCTGTAACCCCCCTGCACCCCCCTCCCGAGTTGCTGAGCACGGCCCGGCTTCGGGTGCGGG
GCTCCGTGCGGGGCGTGGCGCGGGGCTCGCCGTGCCGGGCGGGGGGTGGCGGCAGGTGGGGGTGCG

CGGGCGGGGCGGGGCCGCTCGGGCCGGGGAGGGCTCGGGGGAGGGGCGCGGGCGGGCCCCCGGAGC
 GCCGGCGGCTGTCTGAGGCGCGGGCGAGCCGAGCCATTGCCTTTTATGGTAATCGTGCGAGAGGGC
 GCAGGGACTTCCTTTGTCCCAAATCTGTGCGGAGCCGAAATCTGGGAGGCGCCGCGCACCCCCCT
 CTAGCGGGGCGCGGGGCGAAGCGGTGCGGCGCCGGCAGGAAGGAAATGGGCGGGGAGGGCCTTCGT
 GCGTCGCCGCGCCGCGTCCCCTTCTCCCTCTCCAGCCTCGGGGCTGTCCGCGGGGGGACGGCTG
 CCTTCGGGGGGGACGGGGCAGGGCGGGGTTCGGCTTCTGGCGTGTGACCGGCGGCTCTAGAGCCT
 CTGCTAACCATGTTTCATGCCTTCTTCTTTTTCCTACAG

Exemplary chimeric intron (SEQ ID NO: 64)

GGAGTCGCTGCGTTGCCTTCGCCCCGTGCCCCGCTCCGCGCCGCTCGCGCCGCCCGCCCCGGCT
 CTGACTGACCGGTTACTCCACAGGTGAGCGGGCGGGACGGCCCTTCTCCTCCGGGCTGTAATT
 AGCGCTTGGTTTAATGACGGCTTGTTTCTTTTCTGTGGCTGCGTGAAAGCCTTGAGGGGCTCCGG
 GAGCTAGAGCCTCTGCTAACCATGTTTCATGCCTTCTTCTTTTTCCTACAG

Flanking untranslated regions, 5' UTRs and 3' UTRs

[0234] In some aspects, any of the constructs described herein can include an untranslated region (UTR), such as a 5' UTR or a 3' UTR. UTRs of a gene are transcribed but not translated. A 5' UTR starts at the transcription start site and continues to the start codon but does not include the start codon. A 3' UTR starts immediately following the stop codon and continues until the transcriptional termination signal. The regulatory and/or control features of a UTR can be incorporated into any of the constructs, compositions, kits, or methods as described herein to enhance or otherwise modulate the expression of a polypeptide (e.g., a therapeutic polypeptide, a Connexin 26 polypeptide).

[0235] Natural 5' UTRs include a sequence that plays a role in translation initiation. in some aspects, a 5' UTR can comprise sequences, 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.

[0236] In some aspects, a 5' UTR is included in any of the constructs 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, erythropoietin,

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

[0237] In some aspects, a 5' UTR from an mRNA that is transcribed by a cell in the cochlea can be included in any of the constructs, compositions, kits, and methods described herein. In some aspects, a 5' UTR is derived from the endogenous GJB2 gene loci and may include all or part of the endogenous sequence exemplified by SEQ ID NO: 20, SEQ ID NO: 21, or SEQ ID NO: 66. In some aspects, a 5' UTR sequence is at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% at least 99%, or 100% identical to the 5' UTR sequence represented by SEQ ID NO: 20, SEQ ID NO: 21, or SEQ ID NO: 66.

[0238] 3' UTRs are found immediately 3' to the stop codon of the gene of interest. In some aspects, a 3' UTR from an mRNA that is transcribed by a cell in the cochlea can be included in any of the constructs, compositions, kits, and methods described herein. In some aspects, a 3' UTR is derived from the endogenous GJB2 gene loci and may include all or part of the endogenous sequence exemplified by SEQ ID NO: 22. In some aspects, a 3' UTR sequence is at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% at least 99%, or 100% identical to the 3' UTR sequence represented by SEQ ID NO: 22. In some aspects, a 3' UTR is derived from the endogenous GJB2 gene loci and may include all or part of the endogenous sequence exemplified by SEQ ID NO: 67, or SEQ ID NO: 68. In some aspects, a 3' UTR sequence is at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% at least 99%, or 100% identical to the 3' UTR sequence represented by SEQ ID NO: 67, or SEQ ID NO: 68.

[0239] 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, each of which is incorporated herein by reference in its entirety): 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.

[0240] 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.

[0241] In some aspects, the introduction, removal, or modification of 3' UTR AREs can be used to modulate the stability of an mRNA encoding a polypeptide (e.g., a therapeutic polypeptide, a Connexin 26 polypeptide). In other aspects, AREs can be removed or mutated to increase the intracellular stability and thus increase translation and production of a polypeptide (e.g., a therapeutic polypeptide, a Connexin 26 polypeptide).

[0242] In other aspects, non-ARE sequences may be incorporated into the 5' or 3' UTRs. In some aspects, introns or portions of intron sequences may be incorporated into the flanking regions of the polynucleotides in any of the constructs, compositions, kits, and methods provided herein. Incorporation of intronic sequences may increase protein production as well as mRNA levels.

Exemplary 5' UTR Sequence (SEQ ID NO: 20)

GTTCGCGCCCCGCGAGCGCCCGCGCGCTCCTCTCCCCGACTCGGAGCCCCCTCGGCGGCGCCCCGGCC
CAGGACCCGCTTAGGAGCGCAGGAGCCCCAGCGCAGAGACCCCAACGCCGAGACCCCCGCCCCGG
CCCCGCGCGCTTCTCTCCCCGACGCAGAGCAAACCGCCAGAGTAGAAG

Exemplary 5' UTR Sequence (SEQ ID NO: 21)

TTTAGGACCCTTGTTTCGCGAAGAGGTGGTGTGCGGCTGAGACCCGCGTCTCAGGACGGTTCCAT
CAGTGCCTCGATCCTGCCCCACTGGAGGAGGAAGGCAGCCCGAACAGCGCTCACCTAACTAACAG
CTGCTGAGAGCTGGGTTCCTGGCCATGCACCTGGGACTGCCTTGAGAAGCGTGAGCAAACCGCC
CAGAGTAGAAG

Exemplary 5' UTR Sequence (SEQ ID NO: 66)

GTTCGCGCCCCGCGAGCGCCCGCGCGCTCCTCTCCCCGACTCGGAGCCCCCTCGGCGGCGCCCCGGCC
CAGGACCCGCTTAGGAGCGCAGGAGCCCCAGCGCAGAGACCCCAACGCCGAGACCCCCGCCCCGG
CCCCGCGCGCTTCTCTCCCCGACGCAGTTTAGGACCCCTTGTTTCGCGAAGAGGTGGTGTGCGGCTGA
GACCCGCGTCTCAGGACGGTTCCATCAGTGCCTCGATCCTGCCCCACTGGAGGAGGAAGGCAGC

CCGAACAGCGCTCACCTAACTAACAGCTGCTGAGAGCTGGGTTCCGTGGCCATGCACCTGGGACT
GCCTTGAGAAGCGTGAGCAAACCGCCAGAGTAGAAG

Exemplary 3' UTR Sequence (SEQ ID NO: 22)

CGCATTGCCCAGTTGTTAGATTAAGAAATAGACAGCATGAGAGGGATGAGGCAACCCGTGCTCAG
CTGTCAAGGCTCAGTCGCTAGCATTTCCCAACACAAAGATTCTGACCTTAAATGCAACCATTTGA
AACCCCTGTAGGCCTCAGGTGAAACTCCAGATGCCACAATGGAGCCTCTGCTCCCCATAAGCCTC
AAAACAAAGGCCTAATTCATGCCTGTCTTAATTTTCTTTCACTTAAGTTAGTTCCACTGAGACC
CCAGGCTGTTAGGGTTATTGGTGTAAGGTACTTTTCATATTTTAAACAGAGGATATCGGCATTTG
TTTCTTTCTCTGAGGACAAGAGAAAAAGCCAGGTTCCACAGAGGACACAGAGAAGGTTTGGGTG
TCCTCCTGGGGTTCTTTTTGCCAACTTTCCCCACGTTAAAGGTGAACATTGGTTCTTTTCATTTGC
TTTGGAAGTTTTAATCTCTAACAGTGGACAAAGTTACCAGTGCCTTAAACTCTGTTACACTTTTTT
GGAAGTGAAAACCTTTGTAGTATGATAGGTTATTTTGATGTAAAGATGTTCTGGATACCATTATAT
GTTCCCCCTGTTTCAGAGGCTCAGATTGTAATATGTAAATGGTATGTCATTTCGCTACTATGATTT
AATTTGAAATATGGTCTTTTGGTTATGAATACTTTGCAGCACAGCTGAGAGGCTGTCTGTTGTAT
TCATTGTGGTCATAGCACCTAACAAACATTGTAGCCTCAATCGAGTGAGACAGACTAGAAGTTCCT
AGTGATGGCTTATGATAGCAAATGGCCTCATGTCAAATATTTAGATGTAATTTTGTGTAAGAAAT
ACAGACTGGATGTACCACCAACTACTACCTGTAATGACAGGCCTGTCCAACACATCTCCCTTTTC
CATGACTGTGGTAGCCAGCATCGGAAAGAACGCTGATTTAAAGAGGTCGCTTGGGAATTTTATTG
ACACAGTACCATTTAATGGGGAGGACAAAATGGGGCAGGGGAGGAGAGGTTTCTGTCGTTAAAA
ACAGATTTGGAAAGACTGGACTCTAAAGTCTGTTGATTAAAGATGAGCTTTGTCTACTTCAAAAG
TTTGTTTGCTTACCCCTTCAGCCTCCAATTTTTTAAAGTGAAAATATAGCTAATAACATGTGAAAA
GAATAGAAGCTAAGGTTTAGATAAAATATTGAGCAGATCTATAGGAAGATTGAACCTGAATATTGC
CATTATGCTTGACATGGTTTCCAAAAAATGGTACTCCACATATTTAGTGAGGGTAAGTATTTTC
CTGTTGTCAAGAAATAGCATTGTAAAAGCATTTTGTAAATAATAAAGAATAGCTTTAATGATATGCT
TGTAACATAAAATAATTTTGTAAATGTATCAAATACATTTAAACATTTAAATATAATCTCTATAAT
AA

Exemplary 3' UTR Sequence (SEQ ID NO: 67)

GAAATAGACAGCATGAGAGGGATGAGGCAACCCGTGCTCAGCTGTCAAGGCTCAGTCGCTAGCAT
TTCCCAACACAAAGATTCTGACCTTAAATGCAACCATTTGAAACCCCTGTAGGCCTCAGGTGAAA
CTCCAGATGCCACAATGGAGCTCTGCTCCCCATAAGCCTCAAAACAAAGGCCTAATTCATGCCT
GTCTTAATTTTCTTTCACTTAAGTTAGTTCCACTGAGACCCAGGCTGTTAGGGGTTATTGGTGT
AAGGTACTTTTCATATTTTAAACAGAGGATATCGGCATTTGTTTCTTTCTCTGAGGACAAGAGAAA

AAAGCCAGGTTCCACAGAGGACACAGAGAAGGTTTGGGTGTCCTCCTGGGGTTCTTTTTGCCAAC
TTTCCCCACGTTAAAGGTGAACATTGGTTCTTTTCATTTGCTTTGGAAGTTTAAATCTCTAACAGT
GGACAAAGTTACCAAGTGCCTTAAACTCTGTTACACTTTTTGGAAGTGAAAACCTTTGTAGTATGAT
AGGTTATTTTGATGTAAAGATGTTCTGGATACCATTATATGTTCCCCCTGTTTCAGAGGCTCAGA
TTGTAATATGTAAATGGTATGTCATTTCGCTACTATGATTTAAATTTGAAATATGGTCTTTTGGTTA
TGAATACTTTGCAGCACAGCTGAGAGGCTGTCTGTTGTATTTCATTGTGGTCATAGCACCTAACAA
CATTGTAGCCTCAATCGAGTGAGACAGACTAGAAGTTCCTAGTGATGGCTTATGATAGCAAATGG
CCTCATGTCAAATATTTAGATGTAATTTTGTGTAAAGAAATACAGACTGGATGTACCACCAACTAC
TACCTGTAATGACAGGCCTGTCCAACACATCTCCCTTTTCCATGACTGTGGTAGCCAGCATCGGA
AAGAACGCTGATTTAAAGAGGTCGCTTGGGAATTTTATTGACACAGTACCATTAAATGGGGAGGA
CAAATGGGGCAGGGGAGGGAGAAGTTTCTGTCTGTTAAAAACAGATTTGGAAAGACTGGACTCTA
AAGTCTGTTGATTAAAGATGAGCTTTGTCTACTTCAAAAGTTTGTTTGCTTACCCCTTCAGCCTC
CAATTTTTTAAGTGAAAATATAGCTAATAACATGTGAAAAGAATAGAAGCTAAGGTTTAGATAAA
TATTGAGCAGATCTATAGGAAGATTGAACCTGAATATTGCCATTATGCTTGACATGGTTTCCAAA
AAATGGTACTCCACATATTTTCAGTGAGGGTAAGTATTTTCTGTTGTCAAGAATAGCATGTGAAA
AGCATTTTGTAATAATAAAGAATAGCTTTAATGATATGCTTGTAACATAAATAATTTTGTAATGT
ATCAAATACATTTAAAACATTTAAATATAATCTCTATAATAA

Exemplary 3' UTR Sequence (SEQ ID NO: 68)

CGCATTGCCCAGTTGTTAGATTAAAGAAATAGACAGCATGAGAGGGATGAGGCAACCCGTGCTCAG
CTGTCAAGGCTCAGTCGCTAGCATTTCCCAACACAAAGATTCTGACCTTAAATGCAACCATTTGA
AACCCTGTAGGCCTCAGGTGAAACTCCAGATGCCACAATGGAGCCTCTGCTCCCCATAAGCCTC
AAAACAAAGGCCTAATTCATGCCTGTCTTAATTTTCTTTCACTTAAGTTAGTTCCACTGAGACC
CCAGGCTGTTAGGGGTTATTGGTGTAAGGTACTTTTCATATTTTAAACAGAGGATATCGGCATTTG
TTTCTTTCTCTGAGGACAAGAGAAAAAGCCAGGTTCCACAGAGGACACAGAGAAGGTTTGGGTG
TCCTCCTGGGGTTCTTTTTGCCAACTTTCCCCACGTTAAAGGTGAACATTGGTTCTTTTCATTTGC
TTTGGAAGTTTAAATCTCTAACAGTGACAAAGTTACCAAGTGCCTTAAACTCTGTTACACTTTTT
GGAAGTGAAAACCTTTGTAGTATGATAGGTTATTTTGATGTAAAGATGTTCTGGATACCATTATAT
GTTCCCCCTGTTTCAGAGGCTCAGATTGTAATATGTAAATGGTATGTCATTTCGCTACTATGATTT
AATTTGAAATATGGTCTTTTGGTTATGAATACTTTGCAGCACAGCTGAGAGGCTGTCTGTTGTAT
TCATTGTGGTCATAGCACCTAACAAACATTGTAGCCTCAATCGAGTGAGACAGACTAGAAGTTCCCT
AGTGATGGCTTATGATAGCAAATGGCCTCATGTCAAATATTTAGATGTAATTTTGTGTAAGAAAT
ACAGACTGGATGTACCACCAACTACTACCTGTAATGACAGGCCTGTCCAACACATCTCCCTTTTC
CATGACTGTGGTAGCCAGCATCGGAAAGACGCTGATTTAAAGAGGTCGCTTGGGAATTTTATTG

ACACAGTACCATTTAATGGGGAGGACAAAATGGGGCAGGGGAGGGAGAAGTTTCTGTCTGTTAAAA
ACAGATTTGGAAAAGACTGGACTCTAAAGTCTGTTGATTAAAGATGAGCTTTGTCTACTTCAAAAAG
TTTGTTTGCTTACCCCTTCAGCCTCCAATTTTTTAAAGTGAAAAATATAGCTAATAACATGTGAAAA
GAATAGAAGCTAAGGTTTAGATAAAATATTGAGCAGATCTATAGGAAGATTGAACCTGAATATTGC
CATTATGCTTGAC

Exemplary 3' UTR Sequence (SEQ ID NO: 69)

GAGCTCAGTGTGAGTTCTACCATTGCCAAACTCGAGCAGTGAATTCTACCAAGTGCCATAGGATCC
AGTGTGAGTTCTACCATTGCCAAAGGTACCCAGTGAATTCTACCAAGTGCCATAGTTAACCGCAT
GCCCAGTTGTTAGATTAAAGAAATAGACAGCATGAGAGGGATGAGGCAACCCGTGCTCAGCTGTCA
AGGCTCAGTCGCTAGCATTTCCCAACACAAAGATTCTGACCTTAAATGCAACCATTTGAAACCCC
TGTTAGGCCTCAGGTGAAACTCCAGATGCCACAATGGAGCCTCTGCTCCCCCTAAAGCCTCAAAACA
AAGGCCTAATTCTATGCCTGTCTTAATTTTCTTTTCACTTAAGTTAGTTCCACTGAGACCCAGGC
TGTTAGGGGTTATTGGTGTAAAGTACTTTTCAATTTTAAACAGAGGATATCGGCATTTGTTTCTT
TCTCTGAGGACAAGAGAAAAAGCCAGGTTCCACAGAGGACACAGAGAAGGTTTGGGTGTCTTCC
TGGGGTTCTTTTGGCCAACTTTCCCCACGTTAAAGGTGAACATTGGTTCTTTTCAATTTGCTTTGGA
AGTTTTAATCTCTAACAGTGGACAAAGTTACCAAGTGCCTTAAACTCTGTTACACTTTTTGGAAGT
GAAAACTTTGTAGTATGATAGGTTATTTTGATGTAAAGATGTTCTGGATACCATTATATGTTCCC
CCTGTTTTGAGAGGCTCAGATTGTAATATGTAAATGGTATGTCATTCGCTACTATGATTTAATTTG
AAATATGGTCTTTTGGTTATGAATACTTTGCAGCACAGCTGAGAGGCTGTCTGTTGTATTCAATTG
TGGTCATAGCACCTAACAACATTGTAGCCTCAATCGAGTGAGACAGACTAGAAGTTCCTAGTGAT
GGCTTATGATAGCAAATGGCCTCATGTCAAATATTTAGATGTAATTTTGTGTAAGAAATACAGAC
TGGATGTACCACCAACTACTACCTGTAATGACAGGCCTGTCCAACACATCTCCCTTTTCCATGAC
TGTGGTAGCCAGCATCGGAAAGAACGCTGATTTAAAGAGGTCGCTTGGGAATTTTATTGACACAG
TACCATTTAATGGGGAGGACAAAATGGGGCAGGGGAGGGAGAAGTTTCTGTCTGTTAAAAACAGAT
TTGGAAGAGCTGGACTCTAAAGTCTGTTGATTAAAGATGAGCTTTGTCTACTTCAAAAGTTTGT
TGCTTACCCCTTCAGCCTCCAATTTTTTAAAGTGAAAAATATAGCTAATAACATGTGAAAAGAATAG
AAGCTAAGGTTTAGATAAAATATTGAGCAGATCTATAGGAAGATTGAACCTGAATATTGCCATTAT
GCTTGAC

microRNA Regulatory Target Sites (miRTS)

[0243] In some aspects, the disclosure is directed to constructs comprising microRNA regulatory target site (miRTS) which can be used to regulate (e.g., reduce) expression of a polynucleotide encoding a therapeutic polypeptide (e.g., a Connexin 26 polypeptide) in a

cell (e.g., an inner ear cell, e.g., a hair cell). In some aspects, the constructs provide reduced toxicity associated with expression of the therapeutic polypeptide (e.g., a Connexin 26 polypeptide) in some cells (e.g., an inner ear cell, e.g., a hair cell). In some aspects, the construct comprising a polynucleotide encoding a therapeutic polypeptide (e.g., a Connexin 26 polypeptide) comprises a microRNA regulatory target site (miRTS). An exemplary polynucleotide construct comprising a miRTS is provided in Fig. 2F. In some aspects, a UTR may comprise miRTS. In some aspects, a 3' UTR may comprise a miRTS. In some aspects, a 5' UTR may comprise a miRTS.

[0244] In some aspects, expression of the therapeutic polypeptide (e.g., a Connexin 26 polypeptide) is reduced, suppressed, inhibited, or eliminated in cells that express the microRNA. In some aspects, the therapeutic polypeptide is predominately expressed in cells that do not express the microRNA. In some aspects, toxicity associated with the expression of the therapeutic polypeptide (e.g., a Connexin 26 polypeptide) is reduced, suppressed, inhibited, or eliminated in cells that express the microRNA.

[0245] In some aspects, the disclosure is directed to constructs comprising microRNA regulatory target site (miRTS) which can be used to regulate (e.g., reduce) expression of a polynucleotide encoding a polypeptide in a cell (e.g., an inner ear cell, e.g., a hair cell). In some aspects, the constructs provide reduced toxicity associated with expression of the polypeptide in some cells (e.g., an inner ear cell, e.g., a hair cell). In some aspects, the construct comprising a polynucleotide encoding a polypeptide comprises a microRNA regulatory target site (miRTS). An exemplary polynucleotide construct comprising a miRTS is provided in Fig. 2F. In some aspects, a UTR may comprise miRTS. In some aspects, a 3' UTR may comprise a miRTS. In some aspects, a 5' UTR may comprise a miRTS.

[0246] In some aspects, expression of the polypeptide is reduced, suppressed, inhibited, or eliminated in cells that express the microRNA (e.g., hair cells). In some aspects, the polypeptide is predominately expressed in cells that do not express the microRNA (e.g., supporting cells). In some aspects, toxicity associated with the expression of the polypeptide is reduced, suppressed, inhibited, or eliminated in cells that express the microRNA.

[0247] In some aspects, the miRTS is specific microRNAs expressed in inner ear hair cells, spiral ganglion cells, lateral supporting cells, basilar membrane cells, medial supporting cells, spiral limbus cells, or any combination thereof. In some aspects, the

miRTS is specific microRNAs expressed in inner ear hair cells. In some aspects, the miRTS is specific microRNAs expressed in spiral ganglion cells. In some aspects, the miRTS is specific microRNAs expressed in lateral supporting cells. In some aspects, the miRTS is specific microRNAs expressed in basilar membrane cells. In some aspects, the miRTS is specific microRNAs expressed in medial supporting cells. In some aspects, the miRTS is specific microRNAs expressed in spiral limbus cells.

[0248] In some aspects, the miRTS may be a human miRNA-182, miRNA-183, miRNA-194, miRNA-140, miRNA-18a, miRNA-99a, miRNA-30b, miRNA-15a target sequence. In some aspects, a miRTS may be a human miRNA-182 target sequence. In some aspects, a UTR may include all or part of the miRNA-182 target sequence. In some aspects, a UTR may contain more than one miRNA-182 target sequence. In some aspects, more than one miRNA-182 target sequences may be dispersed at multiple locations in a UTR. In some aspects, the 3' UTR may include all or part of the miRNA-182 target sequence. In some aspects, the 3' UTR may contain more than one miRNA-182 target sequence. In some aspects, more than one miRNA-182 target sequences may be dispersed at multiple locations in the 3' UTR. In some aspects, the miRNA-182 target sequence comprises the nucleic acid sequence with at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 78. In some aspects, the miRNA-182 target sequence comprises the nucleic acid sequence of SEQ ID NO: 78.

[0249] In some aspects, a miRTS may be a human miRNA-183 target sequence. In some aspects, a UTR may include all or part of the miRNA-183 target sequence. In some aspects, a UTR may contain more than one miRNA-183 target sequence. In some aspects, more than one miRNA-183 target sequences may be dispersed at multiple locations in a UTR. In some aspects, the 3' UTR may include all or part of the miRNA-183 target sequence. In some aspects, the 3' UTR may contain more than one miRNA-183 target sequence. In some aspects, more than one miRNA-183 target sequences may be dispersed at multiple locations in the 3' UTR. In some aspects, the miRNA-183 target sequence comprises the nucleic acid sequence with at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 79. In some aspects, the miRNA-183 target sequence comprises the nucleic acid sequence of SEQ ID NO: 79.

[0250] In some aspects, a miRTS may be a human miRNA-194 target sequence. In some aspects, a UTR may include all or part of the miRNA-194 target sequence. In some

aspects, a UTR may contain more than one miRNA-194 target sequence. In some aspects, more than one miRNA-194 target sequences may be dispersed at multiple locations in a UTR. In some aspects, the 3' UTR may include all or part of the miRNA-194 target sequence. In some aspects, the 3' UTR may contain more than one miRNA-194 target sequence. In some aspects, more than one miRNA-194 target sequences may be dispersed at multiple locations in the 3' UTR. In some aspects, the miRNA-194 target sequence comprises the nucleic acid sequence with at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 1. In some aspects, the miRNA-194 target sequence comprises the nucleic acid sequence of SEQ ID NO: 1.

[0251] In some aspects, a miRTS may be a human miRNA-140 target sequence. In some aspects, a UTR may include all or part of the miRNA-140 target sequence. In some aspects, a UTR may contain more than one miRNA-140 target sequence. In some aspects, more than one miRNA-140 target sequences may be dispersed at multiple locations in a UTR. In some aspects, the 3' UTR may include all or part of the miRNA-140 target sequence. In some aspects, the 3' UTR may contain more than one miRNA-140 target sequence. In some aspects, more than one miRNA-140 target sequences may be dispersed at multiple locations in the 3' UTR. In some aspects, the miRNA-140 target sequence comprises the nucleic acid sequence with at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 2. In some aspects, the miRNA-140 target sequence comprises the nucleic acid sequence of SEQ ID NO: 2.

[0252] In some aspects, a miRTS may be a human miRNA-18a target sequence. In some aspects, a UTR may include all or part of the miRNA-18a target sequence. In some aspects, a UTR may contain more than one miRNA-18a target sequence. In some aspects, more than one miRNA-18a target sequences may be dispersed at multiple locations in a UTR. In some aspects, the 3' UTR may include all or part of the miRNA-18a target sequence. In some aspects, the 3' UTR may contain more than one miRNA-18a target sequence. In some aspects, more than one miRNA-18a target sequences may be dispersed at multiple locations in the 3' UTR. In some aspects, the miRNA-18a target sequence comprises the nucleic acid sequence with at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 3. In

some aspects, the miRNA-18a target sequence comprises the nucleic acid sequence of SEQ ID NO: 3.

[0253] In some aspects, a miRTS may be a human miRNA-99a target sequence. In some aspects, a UTR may include all or part of the miRNA-99a target sequence. In some aspects, a UTR may contain more than one miRNA-99a target sequence. In some aspects, more than one miRNA-99a target sequences may be dispersed at multiple locations in a UTR. In some aspects, the 3' UTR may include all or part of the miRNA-99a target sequence. In some aspects, the 3' UTR may contain more than one miRNA-99a target sequence. In some aspects, more than one miRNA-99a target sequences may be dispersed at multiple locations in the 3' UTR. In some aspects, the miRNA-99a target sequence comprises the nucleic acid sequence with at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 4. In some aspects, the miRNA-99a target sequence comprises the nucleic acid sequence of SEQ ID NO: 4.

[0254] In some aspects, a miRTS may be a human miRNA-30b target sequence. In some aspects, a UTR may include all or part of the miRNA-30b target sequence. In some aspects, a UTR may contain more than one miRNA-30b target sequence. In some aspects, more than one miRNA-30b target sequences may be dispersed at multiple locations in a UTR. In some aspects, the 3' UTR may include all or part of the miRNA-30b target sequence. In some aspects, the 3' UTR may contain more than one miRNA-30b target sequence. In some aspects, more than one miRNA-30b target sequences may be dispersed at multiple locations in the 3' UTR. In some aspects, the miRNA-30b target sequence comprises the nucleic acid sequence with at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 5. In some aspects, the miRNA-30b target sequence comprises the nucleic acid sequence of SEQ ID NO: 5.

[0255] In some aspects, a miRTS may be a human miRNA-15a target sequence. In some aspects, a UTR may include all or part of the miRNA-15a target sequence. In some aspects, a UTR may contain more than one miRNA-15a target sequence. In some aspects, more than one miRNA-15a target sequences may be dispersed at multiple locations in a UTR. In some aspects, the 3' UTR may include all or part of the miRNA-15a target sequence. In some aspects, the 3' UTR may contain more than one miRNA-15a target sequence. In some aspects, more than one miRNA-15a target sequences may be dispersed

at multiple locations in the 3' UTR. In some aspects, the miRNA-15a target sequence comprises the nucleic acid sequence with at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 6. In some aspects, the miRNA-15a target sequence comprises the nucleic acid sequence of SEQ ID NO: 6.

[0256] In some aspects, the miRTS may be a target sequence for a miRNA that is expressed in specific cells of the inner ear. In some aspects, the miRTS may be a target sequence for a miRNA that is expressed in inner ear hair cells, spiral ganglion cells, lateral supporting cells, basilar membrane cells, medial supporting cells, spiral limbus cells, or any combination thereof.

[0257] In some aspects, the miRTS may be a target sequence for a miRNA that is expressed in inner ear hair cells. In some aspects, the miRNA that is expressed in inner ear hair cells reduces, suppresses, inhibits, or eliminates expression of the polypeptide (e.g., a therapeutic polypeptide, a Connexin 26 polypeptide) in the inner ear hair cells. In some aspects, miRNAs that are expressed in inner ear hair cells are miR-194, miR-140, miR-18a, miR-99a, miR-30b, miR-15a, miR182, or miR-183. In some aspects, the miRNA that is expressed in inner ear hair cells is miR-194. In some aspects, the miRNA-194 target sequence comprises the nucleic acid sequence with at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 1. In some aspects, the miRNA-194 target sequence comprises the nucleic acid sequence of SEQ ID NO: 1. In some aspects, the miRNA that is expressed in inner ear hair cells is miR-140. In some aspects, the miRNA-140 target sequence comprises the nucleic acid sequence with at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 2. In some aspects, the miRNA-140 target sequence comprises the nucleic acid sequence of SEQ ID NO: 2. In some aspects, the miRNA that is expressed in inner ear hair cells is miR-18a. In some aspects, the miRNA-18a target sequence comprises the nucleic acid sequence with at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 3. In some aspects, the miRNA-18a target sequence comprises the nucleic acid sequence of SEQ ID NO: 3. In some aspects, the miRNA that is expressed in inner ear hair cells is miR-99a. In some aspects, the miRNA-99a target sequence comprises the nucleic acid sequence with at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID

NO: 4. In some aspects, the miRNA-99a target sequence comprises the nucleic acid sequence of SEQ ID NO: 4. In some aspects, the miRNA that is expressed in inner ear hair cells is miR-30b. In some aspects, the miRNA-30b target sequence comprises the nucleic acid sequence with at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 5. In some aspects, the miRNA-30b target sequence comprises the nucleic acid sequence of SEQ ID NO: 5. In some aspects, the miRNA that is expressed in inner ear hair cells is miR-15a. In some aspects, the miRNA-15a target sequence comprises the nucleic acid sequence with at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 6. In some aspects, the miRNA-15a target sequence comprises the nucleic acid sequence of SEQ ID NO: 6. In some aspects, the miRNA that is expressed in inner ear hair cells is miR-182. In some aspects, the miRNA-182 target sequence comprises the nucleic acid sequence with at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 78. In some aspects, the miRNA-182 target sequence comprises the nucleic acid sequence of SEQ ID NO: 78. In some aspects, the miRNA that is expressed in inner ear hair cells is miR-183. In some aspects, the miRNA-183 target sequence comprises the nucleic acid sequence with at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 79. In some aspects, the miRNA-183 target sequence comprises the nucleic acid sequence of SEQ ID NO: 79.

[0258] In some aspects, the miRTS may be a target sequence for a miRNA that is expressed in spiral ganglion cells. In some aspects, the miRNA that is expressed in spiral ganglion cells reduces, suppresses, inhibits, or eliminates expression of the polypeptide (e.g., a therapeutic polypeptide, a Connexin 26 polypeptide) in the spiral ganglion cells. In some aspects, miRNAs that are expressed in the spiral ganglion cells are miR-194, miR-18a, miR-99a, miR-30b, miR-15a, miR182, or miR-183. In some aspects, the miRNA that is expressed in ear hair cells is miR-194. In some aspects, the miRNA-194 target sequence comprises the nucleic acid sequence with at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 1. In some aspects, the miRNA-194 target sequence comprises the nucleic acid sequence of SEQ ID NO: 1. In some aspects, the miRNA that is expressed in ear hair cells is miR-18a. In some aspects, the miRNA-18a target sequence comprises the nucleic acid sequence with at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at

least 98%, at least 99%, or 100% identity to SEQ ID NO: 3. In some aspects, the miRNA-18a target sequence comprises the nucleic acid sequence of SEQ ID NO: 3. In some aspects, the miRNA that is expressed in ear hair cells is miR-99a. In some aspects, the miRNA-99a target sequence comprises the nucleic acid sequence with at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 4. In some aspects, the miRNA-99a target sequence comprises the nucleic acid sequence of SEQ ID NO: 4. In some aspects, the miRNA that is expressed in ear hair cells is miR-30b. In some aspects, the miRNA-30b target sequence comprises the nucleic acid sequence with at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 5. In some aspects, the miRNA-30b target sequence comprises the nucleic acid sequence of SEQ ID NO: 5. In some aspects, the miRNA that is expressed in ear hair cells is miR-15a. In some aspects, the miRNA-15a target sequence comprises the nucleic acid sequence with at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 6. In some aspects, the miRNA-15a target sequence comprises the nucleic acid sequence of SEQ ID NO: 6. In some aspects, the miRNA that is expressed in ear hair cells is miR-182. In some aspects, the miRNA-182 target sequence comprises the nucleic acid sequence with at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 78. In some aspects, the miRNA-182 target sequence comprises the nucleic acid sequence of SEQ ID NO: 78. In some aspects, the miRNA that is expressed in ear hair cells is miR-183. In some aspects, the miRNA-183 target sequence comprises the nucleic acid sequence with at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 79. In some aspects, the miRNA-183 target sequence comprises the nucleic acid sequence of SEQ ID NO: 79.

[0259] In some aspects, the miRTS may be a target sequence for a miRNA that is expressed in basilar membrane cells. In some aspects, the miRNA that is expressed in basilar membrane cells reduces, suppresses, inhibits, or eliminates expression of the polypeptide (e.g., a therapeutic polypeptide, a Connexin 26 polypeptide) in the basilar membrane cells. In some aspects, miRNAs that are expressed in basilar membrane cells are miR-99a, miR-30b, and miR-15a. In some aspects, the miRNA that is expressed in ear hair cells is miR-99a. In some aspects, the miRNA-99a target sequence comprises the nucleic acid sequence with at least 85%, at least 90%, at least 95%, at least 96%, at least

97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 4. In some aspects, the miRNA-99a target sequence comprises the nucleic acid sequence of SEQ ID NO: 4. In some aspects, the miRNA that is expressed in ear hair cells is miR-30b. In some aspects, the miRNA-30b target sequence comprises the nucleic acid sequence with at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 5. In some aspects, the miRNA-30b target sequence comprises the nucleic acid sequence of SEQ ID NO: 5. In some aspects, the miRNA that is expressed in ear hair cells is miR-15a. In some aspects, the miRNA-15a target sequence comprises the nucleic acid sequence with at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 6. In some aspects, the miRNA-15a target sequence comprises the nucleic acid sequence of SEQ ID NO: 6.

[0260] In some aspects, the miRTS may be a target sequence for a miRNA that is expressed in lateral supporting cells. In some aspects, the miRNA that is expressed in lateral supporting cells reduces, suppresses, inhibits, or eliminates expression of the polypeptide (e.g., a therapeutic polypeptide, a Connexin 26 polypeptide) in the lateral supporting cells. In some aspects, miRNAs that are expressed in lateral supporting cells are miR-99a, miR-30b, and miR-15a. In some aspects, the miRNA that is expressed in ear hair cells is miR-99a. In some aspects, the miRNA-99a target sequence comprises the nucleic acid sequence with at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 4. In some aspects, the miRNA-99a target sequence comprises the nucleic acid sequence of SEQ ID NO: 4. In some aspects, the miRNA that is expressed in ear hair cells is miR-30b. In some aspects, the miRNA-30b target sequence comprises the nucleic acid sequence with at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 5. In some aspects, the miRNA-30b target sequence comprises the nucleic acid sequence of SEQ ID NO: 5. In some aspects, the miRNA that is expressed in ear hair cells is miR-15a. In some aspects, the miRNA-15a target sequence comprises the nucleic acid sequence with at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 6. In some aspects, the miRNA-15a target sequence comprises the nucleic acid sequence of SEQ ID NO: 6.

- [0261]** In some aspects, the miRTS may be a target sequence for a miRNA that is expressed in medial supporting cells. In some aspects, the miRNA that is expressed in medial supporting cells reduces, suppresses, inhibits, or eliminates expression of the polypeptide (e.g., a therapeutic polypeptide, a Connexin 26 polypeptide) in the medial supporting cells. In some aspects, miRNAs that are expressed in medial supporting cells are miR182 and miR-183. In some aspects, the miRNA that is expressed in ear hair cells is miR-182. In some aspects, the miRNA-182 target sequence comprises the nucleic acid sequence with at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 78. In some aspects, the miRNA-182 target sequence comprises the nucleic acid sequence of SEQ ID NO: 78. In some aspects, the miRNA that is expressed in ear hair cells is miR-183. In some aspects, the miRNA-183 target sequence comprises the nucleic acid sequence with at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 79. In some aspects, the miRNA-183 target sequence comprises the nucleic acid sequence of SEQ ID NO: 79.
- [0262]** In some aspects, the miRTS may be a target sequence for a miRNA that is expressed in spiral limbus cells. In some aspects, the miRNA that is expressed in spiral limbus cells reduces, suppresses, inhibits, or eliminates expression of the polypeptide (e.g., a therapeutic polypeptide, a Connexin 26 polypeptide) in the spiral limbus cells. In some aspects, miRNAs that are expressed in spiral limbus cells are miR182 and miR-183. In some aspects, the miRNA that is expressed in ear hair cells is miR-182. In some aspects, the miRNA-182 target sequence comprises the nucleic acid sequence with at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 78. In some aspects, the miRNA-182 target sequence comprises the nucleic acid sequence of SEQ ID NO: 78. In some aspects, the miRNA that is expressed in ear hair cells is miR-183. In some aspects, the miRNA-183 target sequence comprises the nucleic acid sequence with at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 79. In some aspects, the miRNA-183 target sequence comprises the nucleic acid sequence of SEQ ID NO: 79.
- [0263]** In some aspects, a non-endogenous regulatory region included in a UTR may comprise multiple miRNA regulatory target sites (miRTS). In some aspects, a UTR may comprise at least one miRNA-182 target site and at least one miRNA-183 target site. In

some aspects, a non-endogenous regulatory region included in a UTR is a destabilizing domain, and is exemplified by SEQ ID NO: 80. In some aspects, a UTR may include a sequence that is at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% at least 99%, or 100% identical to a non-endogenous regulatory region exemplified by SEQ ID NO: 80.

miRNA-182 target sequence (SEQ ID NO: 78)

AGTGTGAGTTCTACCAT TGCCAAA

miRNA-183 target sequence (SEQ ID NO: 79)

AGTGAATTCTACCA GTGCCATA

miRNA-194 target sequence (SEQ ID NO: 1)

TCCACATGGAGTTGCTGTTACA

miRNA-140 target sequence (SEQ ID NO: 2)

CCGTGGTTCTACCCTGTGGTA

miRNA-18a target sequence (SEQ ID NO: 3)

CTATCTGCACTAGATGCACCTTA

miRNA-99a target sequence (SEQ ID NO: 4)

CACAAGATCGGATCTACGGGTT

miRNA-30b target sequence (SEQ ID NO: 5)

CTGAGTGTAGGATGTTTACA

miRNA-15a target sequence (SEQ ID NO: 6)

CACAAACCATTATGTGCTGCTA

Exemplary mRNA destabilizing domain Sequence (SEQ ID NO: 80)

GAGCTCAGTGTGAGTTCTACCAT TGCCAAACTCGAGCAGTGAATTCTACCAGTGCCA
TAGGATCCAGTGTGAGTTCTACCAT TGCCAAAGGTACCCAGTGAATTCTACCAGTGC
CATAGTTAAC

Table 3. Exemplary microRNA regulatory target sites

microRNA regulatory target sites	SEQ ID NO
miRNA-182 target sequence	78
miRNA-183 target sequence	79
miRNA-194 target sequence	1
miRNA-140 target sequence	2
miRNA-18a target sequence	3
miRNA-99a target sequence	4
miRNA-30b target sequence	5
miRNA-15a target sequence	6

Internal Ribosome Entry Sites (IRES)

[0264] In some aspects, a construct encoding a polypeptide (e.g., a therapeutic polypeptide, a Connexin 26 polypeptide) can include an internal ribosome entry site (IRES). 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).

[0265] 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, each of which is incorporated in its entirety herein by reference.

[0266] In some aspects, the IRES sequence that is incorporated into a construct that encodes a polypeptide (e.g., a therapeutic polypeptide, a Connexin 26 polypeptide) is the foot and mouth disease 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, MD 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, each of which is incorporated in its entirety herein by reference). The cleavage activity of the 2A sequence has previously been demonstrated in artificial systems including plasmids and gene therapy constructs (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, each of which is incorporated in its entirety herein by reference).

Splice Sites

[0267] In some aspects, any of the constructs provided herein can include splice donor and/or splice acceptor sequences, which are functional during RNA processing occurring during transcription. In some aspects, splice sites are involved in trans-splicing.

Exemplary splice donor intron (SEQ ID NO: SEQ ID NO: 23)

GTAAGTATCAAGGTTACAAGACAGGTTTAAGGAGACCAATAGAACTGGGCTTGTCGAGACAGAG
AAGACTCTTGCGTTTCT

Exemplary splice acceptor intron (SEQ ID NO: SEQ ID NO: 24)

GATAGGCACCTATTGGTCTTACTGACATCCACTTTGCCTTTCTCTCCACAG

Polyadenylation Sequences

[0268] In some aspects, a construct 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, which is incorporated herein by reference in its entirety). A poly(A) tail confers mRNA stability and transferability (Molecular Biology of the Cell, Third Edition by B. Alberts et al., Garland Publishing, 1994, which is incorporated herein by reference in its entirety). In some aspects, a poly(A) signal sequence is positioned 3' to the coding sequence.

[0269] As used herein, "polyadenylation" refers to the covalent linkage of a polyadenyl moiety, or its modified variant, to a messenger RNA molecule. In eukaryotic organisms, most messenger RNA (mRNA) molecules are polyadenylated at the 3' end. A 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 some aspects, a poly(A) tail is added onto transcripts that contain a specific sequence, e.g., a polyadenylation (or poly(A)) signal. A poly(A) tail and associated proteins aid in protecting mRNA from degradation by exonucleases. Polyadenylation also plays a role in transcription termination, export of the mRNA from the nucleus, and translation. Polyadenylation typically occurs in the nucleus immediately after transcription of DNA into RNA, but also can occur later in the cytoplasm. After transcription has been terminated, an mRNA chain is cleaved through the action of an endonuclease complex associated with RNA polymerase. A 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.

[0270] 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.

[0271] 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, each of which is incorporated herein by reference in its entirety), 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, each of which is incorporated herein by reference in its entirety), human collagen, polyoma virus (Batt et al., *Mol. Cell Biol.* 15(9):4783-4790, 1995, which is incorporated herein by reference in its entirety), the Herpes simplex virus thymidine kinase gene (HSV TK), IgG heavy-chain gene polyadenylation signal (US 2006/0040354, which is incorporated herein by reference in its entirety), human growth hormone (hGH) (Szymanski et al., *Mol. Therapy* 15(7):1340-1347, 2007, which is incorporated herein by reference in its entirety), the group comprising a 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, which is incorporated herein by reference in its entirety).

[0272] The poly(A) signal sequence can be AATAAA. The AATAAA sequence may be substituted with other hexanucleotide sequences with homology to AATAAA and that 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, which is incorporated herein by reference in its entirety).

[0273] In some aspects, a poly(A) signal sequence can be a synthetic polyadenylation site (see, e.g., the pCI-neo expression construct of Promega that is based on Levitt et al., Genes Dev. 3(7):1019-1025, 1989, which is incorporated herein by reference in its entirety). In some aspects, a poly(A) signal sequence is the polyadenylation signal of soluble neuropilin-1 (sNRP) (AAATAAAATACGAAATG; SEQ ID NO: 89) (see, e.g., WO 05/073384, which is incorporated herein by reference in its entirety). In some aspects, a poly(A) signal sequence comprises or consists of the SV40 poly(A) site. In some aspects, a poly(A) signal comprises or consists of SEQ ID NO: 25. In some aspects, a poly(A) signal sequence comprises or consists of bGHpA. In some aspects, a poly(A) signal comprises or consists of SEQ ID NO: 26. Additional examples of poly(A) signal sequences are known in the art. In some aspects, a poly(A) sequence is at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% at least 99%, or 100% identical to the poly(A) sequence represented by SEQ ID NO: 25.

Exemplary bGH poly(A) signal sequence (SEQ ID NO: 25)

CTGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGCCCCCTCCCCGTGCCTTCCTTGACCCCTGGAA
GGTGCCACTCCCACTGTCCTTTCTAATAAAATGAGGAAATTGCATCGCATTGTCTGAGTAGGTG
TCATTCTATTCTGGGGGTGGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAGACAATAGCA
GGCATGCTGGGGATGCGGTGGGCTCTATGG

Exemplary SV40 poly(A) signal sequence (SEQ ID NO: 26)

AACTTGTTTATTGCAGCTTATAATGGTTACAAATAAAGCAATAGCATCACAAATTTACAAATAA
AGCATTTTTTTTCACTGCATTCTAGTTGTGGTTTGTCCAACTCATCAATGTATCTTA

Additional Sequences

[0274] In some aspects, constructs of the present disclosure may include one or more filler sequences. In some aspects, filler sequences may function as regulatory elements, altering construct expression. In some such aspects, filler sequences may not be fully removed prior to manufacturing for administration to a subject. In some aspects, filler sequences may have functional roles including as linker sequences, as regulatory regions,

or as stabilizing regions. As will be appreciated by those skilled in the art, filler sequences may vary significantly in primary sequence while retaining their desired function. In some aspects, constructs may contain any combination of filler sequences, exemplary filler sequences which may function as regulatory sequences are represented by SEQ ID NO: 128 or 129.

[0275] In some aspects, constructs of the present disclosure may comprise a T2A element or sequence. In some aspects, constructs of the present disclosure may include one or more cloning sites. In some such aspects, cloning sites may not be fully removed prior to manufacturing for administration to a subject. In some aspects, cloning sites may have functional roles including as linker sequences, portions of a Kozak site, or as sites encoding a stop codon. As will be appreciated by those skilled in the art, cloning sites may vary significantly in primary sequence while retaining their desired function. In some aspects, constructs may contain any combination of cloning sites, exemplary cloning sites are represented by SEQ ID NO: 29, 30, 31, 32, 33, 34, 35, 36, 37, or 85. In some aspects, constructs may contain additional cloning sites less than five nucleotides in length.

Exemplary Regulatory sequence C3 (SEQ ID NO: 128)

CTTCTTCTGGAGTCTTTTCTGGAATAATTCTGGGAGTGGGCTCAGCCTGCGGGAGAGTAACATTTT
TTATAACTTGATAGATGTAGCTGAGATGCCTCCAGAGGGGAGACCCGCCTCTCCTCCGGCAGCT
GTGCACGTAGGCTTGTTCAGCAGCCTGGCCAGGGTGGTCCACCTGGTGTTCATCTTCTTT
CCCCGAGCGCTGACTCCTGCGCGTCCTCTTGGAAGACTCTTGACAGGACGGGTGTTTTATGGGT
GTGATTCACTGTCCTCTTGCATCAGTTCAATGTGGTGGTGTTCATCAACCCTGTAGCGTTAGC
AAAATTTGCTCAAGTCATTCCGCAGGAATGTCTGTGTCTTGCTTCCAAGAAAGCTTGTAAGTGCC
GGCAACAGGCCAAGCAGCTCACAAACCTGACCACAAGCCTGTGAGTAATTGTGGGGCAGCACCTTA
GCAGTCTTTTATTTTCGACTTATTAAAGTCTCATCTTGGCCTCACCTTCTCCCTGGAAGGTGGCG
TGGGTGGGAACCACTGGGTGAGATCTTTTTCACCCTTGCCGTGGAGCCAGTTTCCTGTTGCATGT
GGGGGAAGCAACATGTGGTGAAGAGTATAGAAAACGAAAACATGTGGGTACAGTATGTATAAGTG
GAGGGAACAACTCATAATTCCAAGTAGTTTCTCATGAGAGACTCATGAATCATTTGTGGTAGTTC
TCAATATAAACTTAATCTAGGCCGGATGTGGTGGCTCACACCTGTAATCTCAGCACTCTGGGTGG
ATCACTTGAGGTCAGGAGTTTGAGACCAGTCTGACCAACATGGAGAAACCCATCGCTACTAAAA
ATACAAAATTATCCAGATGTGGTGGCTCACACCTGTAATCCCAGCACTTTGGGAGGCTGAGGCGG
GTGGATCACTTGAGGTCAGGAGTTTGAGACCAGCCTGACCAACATGGAGAACTGTGTCTCTACT

AAAAATACAAAATTAGCTGGGCGTGGTGACGCATGCCTGTAATCCCAGCTATTTGGAGGCCGAAG
CAGG

Exemplary Regulatory sequence D7 (SEQ ID NO: 129)

CTTCTTCTGGAGTCTTTTCTGGAATAATTCTGGGAGTGGGCTCAGCCTGCGGGAGAGTAACATTT
TTATAACTTGATAGATGTAGCTGAGATGCCCTCCAGAGGGGAGACCCGCCTCTCCTCCGGCAGCT
GTGCACGTAGGCTTGTTCAGCAGCCTGGCCAGGGTGGTCCACCTGGTGTTCATCTTCTTT
CCCCGGAGCGCTGACTCCTGCGCGTCCTCTTGAAGACTCTTGACAGGACGGGTGTTTTATGGGT
GTGATTCAAGTGTCTTGCATCAGTTCAATGTGGTGGTGTCAATCAACCCTTGAGCGTTAGC
AAAATTTGCTCAAGTCATTCCGCAGGAATGTCTGTGTCTTGCTTCCAAGAAAGCTTGTAAGTGCC
GGCAACAGGCCAAGCAGCTCACAAACCTGACCACAAGCCTGTGAGTAATTGTGGGGCAGCACTTA
GCAGTCTTTTATTTTCGACTTATTAAAGTCTCATCTTGGCCTCACCTTCTCCCTGGAAGGTGGCG
TGGGTGGGAACCACTGGGTGAGATCTTTTACCCTTGCCGTGGAGCCAGTTTCCTGTTGCATGT
GGGGGAAGCAACATGTGGTGAAGAGTATAGAAAACGAAAACATGTGGGTACAGTATGTATAAGTG
GAGGGAACAACTCATAATTCCAAGTAGTTTCTCATGAGAGACTCATGAATCATTGTGGTAGTTC
TCAATATAAACTTAATCTAGGCCGGATGTGGTGGCTCACACCTGTAATCTCAGCACTCTGGGTGG
ATCACTTGAGGTCAGGAGTTTGAGACCAGTCTGACCAACATGGAGAAACCCCATCGCTACTAAAA
ATACAAAATTATCCAGATGTGGTGGCTCACACCTGTAATCCCAGCACTTTGGGAGGCTGAGGCGG
GTGGATCACTTGAGGTCAGGAGTTTGAGACCAGCCTGACCAACATGGAGAACTGTGTCTCTACT
AAAAATACAAAATTAGCTGGGCGTGGTGACGCATGCCTGTAATCCCAGCTATTTGGAGGCCGAAG
CAGG

Exemplary cloning site A (SEQ ID NO: 29)

TTGTGACGCGGCCGCACGCGT

Exemplary cloning site B (SEQ ID NO: 30)

CTCCTGGGCAACGTGCTGGTTATTGTGACCGGTGCCACC

Exemplary cloning site C (SEQ ID NO: 31)

TAAGAGCTCGCTGATCAGCCTCGA

Exemplary cloning site D (SEQ ID NO: 32)

AAGCTTGAATTCAGCTGACGTGCCTCGGACCGCCTAGG

Exemplary cloning site E (SEQ ID NO: 33)

TAAGAGCTC

Exemplary cloning site F (SEQ ID NO: 34)

GCTGATCAGCCTCGA

Exemplary cloning site G (SEQ ID NO: 35)

GGCATTCCGGTACTGTTGGTAAAGCCACCAGCAAACCGCCCAGAGTAGAAGACCGGTGGCCACC

Exemplary cloning site H (SEQ ID NO: 36)

AAGCTTGAATTC

Exemplary cloning site I (SEQ ID NO: 37)

AGCTGACGTGCCTCGGACCGCCTAGG

Exemplary cloning site J (SEQ ID NO: 70)

GCGGCCGCACGCGT

Exemplary cloning site K (SEQ ID NO: 71)

GCGGCCGCACGCGTGGT

Exemplary cloning site L (SEQ ID NO: 72)

CTCCTGGGCAACGTGCTGGTTATTGTGACCGGT

Exemplary cloning site M (SEQ ID NO: 73)

CGCTAGCCACC

Exemplary cloning site N (SEQ ID NO: 74)

ACCGGTCGCTAGCCACC

Exemplary cloning site O (SEQ ID NO: 75)

GAGCTCGCTGATCAGCCTCGA

Exemplary cloning site P (SEQ ID NO: 76)

AAGCTTGAATTCAGCTGACGTGCCTCGGACCGCT

Exemplary cloning site Q (SEQ ID NO: 85)

CTCACCGGT

Exemplary linker sequence (SEQ ID NO: 77)

GGATCCCGGGCT

Reporter Sequences, Elements, or Reporter Polypeptides

[0276] In some aspects, constructs provided herein can optionally include a sequence encoding a reporter polypeptide and/or 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), FLAG, and a luciferase. Additional examples of reporter sequences are known in the art. Non-limiting examples of reporter polypeptides include 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), FLAG, and a luciferase. When associated with control 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).

[0277] In some aspects, a reporter sequence is the LacZ gene, and the presence of a construct carrying the LacZ gene in a mammalian cell (e.g., a cochlear hair cell) is detected by assays for beta-galactosidase activity. When the reporter polypeptide is a fluorescent protein (e.g., green fluorescent protein) or luciferase, the presence of a construct carrying the fluorescent protein or luciferase in a mammalian cell (e.g., a cochlear hair 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 aspects, a reporter sequence can be used to verify the tissue-specific targeting capabilities and tissue-specific promoter regulatory and/or control activity of any of the constructs described herein. In some aspects, a reporter polypeptide can be used to verify the tissue-specific targeting capabilities and tissue-

specific promoter regulatory and/or control activity of any of the constructs described herein.

[0278] In some aspects, a reporter sequence is a FLAG tag (e.g., a 3xFLAG tag), and the presence of a construct carrying the FLAG tag in a mammalian cell (e.g., an inner ear cell, e.g., a cochlear hair or supporting cell) is detected by protein binding or detection assays (e.g., Western blots, immunohistochemistry, radioimmunoassay (RIA), mass spectrometry). An exemplary 3xFLAG tag sequence is provided as SEQ ID NO: 42.

Exemplary 3xFLAG tag sequence (SEQ ID NO: 42)

GGATCCCGGGCTGACTACAAAGACCATGACGGTGATTATAAAGATCATGACATCGACTACAAGGA
TGACGATGACAAG

Exemplary 3xFLAG tag sequence with stop codon (SEQ ID NO: 81)

GACTACAAAGACCATGACGGTGATTATAAAGATCATGACATCGACTACAAGGATGACGATGACAA
GTAA

Exemplary barcode tag (SEQ ID NO: 62)

GTGTCACC

Exemplary barcode tag (SEQ ID NO: 55)

CACAACCT

Exemplary barcode tag (SEQ ID NO: 27)

CGTGTGTT

Exemplary barcode tag (SEQ ID NO: 41)

TCGTGGGT

Exemplary barcode tag (SEQ ID NO: 39)

GCAAACCTG

Exemplary barcode tag (SEQ ID NO: 108)

CCTACGCT

Exemplary barcode tag (SEQ ID NO: 109)

GCCAAAGC

Exemplary barcode tag (SEQ ID NO: 110)

CCATCCAC

Exemplary barcode tag (SEQ ID NO: 111)

CCCGTTCT

Exemplary barcode tag (SEQ ID NO: 112)

TTCACTGG

Exemplary barcode tag (SEQ ID NO: 113)

ATACTCTC

Exemplary barcode tag (SEQ ID NO: 114)

GGCACTTC

Exemplary barcode tag (SEQ ID NO: 115)

TTTCAGGT

AAV Capsids

[0279] The present disclosure provides one or more polynucleotide constructs packaged into an AAV capsid. In some aspects, an AAV capsid is from or derived from an AAV capsid of an AAV2, 3, 4, 5, 6, 7, 8, 9, 10, rh8, rh10, rh39, rh43, AAV2-tYF, AAV2-P2V2, AAV2-P2V3, AAV2-MeBtYFTV, AAV2-MeB, AAV2-P2V6, AAV2-DGEDF, or Anc80 serotype, or one or more hybrids thereof. In some aspects, an AAV capsid is from an AAV ancestral serotype. In some aspects, an AAV capsid is an ancestral (Anc) AAV capsid. An Anc capsid is created from a construct sequence that is constructed using evolutionary probabilities and evolutionary modeling to determine a probable ancestral sequence. Thus, an Anc capsid/construct sequence is not known to have existed in nature. For example, in some aspects, an AAV capsid is an Anc80 capsid (e.g., an Anc80L65 capsid). In some aspects, an AAV capsid is created using a template nucleotide coding sequence comprising SEQ ID NO: 43. In some aspects, the capsid comprises a polypeptide represented by SEQ ID NO: 44. In some aspects, the capsid comprises a polypeptide with at least 85%, at least 90%, at least 95%, at least 96%, at

least 97%, at least 98% at least 99%, or 100% identical to the polypeptide represented by SEQ ID NO: 44.

[0280] As provided herein, any combination of AAV capsids and AAV constructs (e.g., comprising AAV ITRs) may be used in recombinant AAV (rAAV) particles of the present disclosure. For example, wild-type or variant AAV2 ITRs and Anc80 capsid (e.g., an Anc80L65 capsid), wild-type or variant AAV2 ITRs and AAV6 capsid, etc. In some aspects of the present disclosure, an AAV particle is wholly comprised of AAV2 components (e.g., capsid and ITRs are AAV2 serotype). In some aspects, an AAV particle is an AAV2/6, AAV2/8 or AAV2/9 particle (e.g., an AAV6, AAV8 or AAV9 capsid with an AAV construct having AAV2 ITRs). In some aspects of the present disclosure, an AAV particle is an AAV2/Anc80 particle that comprises an Anc80 capsid (e.g., comprising a polypeptide of SEQ ID NO: 44) that encapsidates an AAV construct with AAV2 ITRs (e.g., SEQ ID NOs: 8 and 9) flanking a portion of a coding sequence, for example, a nucleic acid encoding a polypeptide (e.g., a therapeutic polypeptide, a Connexin 26 polypeptide). Other AAV particles are known in the art and are described in, e.g., Sharma et al., Brain Res Bull. 2010 Feb 15; 81(2-3): 273, which is incorporated in its entirety herein by reference. In some aspects, a capsid sequence is at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% at least 99%, or 100% identical to a capsid nucleotide or amino acid sequence represented by SEQ ID NO: 43 or 44, respectively.

Exemplary AAV Anc80 Capsid DNA Sequence (SEQ ID NO: 43)

```
ATGGCTGCCGATGGTTATCTTCCAGATTGGCTCGAGGACAACCTCTCTGAGGGCATTTCGCGAGTGG
GTGGGACTTGAAACCTGGAGCCCCGAAACCCAAAGCCAACCAGCAAAAGCAGGACGACGGCCGGG
GTCTGGTGCTTCTTGGCTACAAGTACCTCGGACCCTTCAACGGACTCGACAAGGGGGAGCCCGTC
AACGCGGCGGACGACGCGGCCCTCGAGCACGACAAGGCCTACGACCAGCAGCTCAAAGCGGGTGA
CAATCCGTACCTGCGGTATAACCACGCGGACGCGGAGTTTCAGGAGCGTCTGCAAGAAGATACGT
CTTTTGGGGGCAACCTCGGGCGAGCAGTCTTCCAGGCCAAGAAGCGGGTTCTCGAACCTCTCGGT
CTGGTTGAGGAAGGCGCTAAGACGGCTCCTGGAAAGAAGAGACCGGTAGAGCAATCACCCAGGA
ACCAGACTCCTCTTCGGGCATCGGCAAGAAAGGCCAGCAGCCCGCAAGAAGAGACTCAACTTTG
GGCAGACAGGCGACTCAGAGTCAGTGCCCGACCCTCAACCACTCGGAGAACCCCCCGAGCCCCC
TCTGGTGTGGGATCTAATACAATGGCAGCAGGCGGTGGCGCTCCAATGGCAGACAATAACGAAGG
CGCCGACGGAGTGGGTAACGCCTCAGGAAATTGGCATTGCGATTCCACATGGCTGGGCGACAGAG
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TCATCACCACCAGCACCCGAACCTGGGCCCCTCCCCACCTACAACAACCACCTCTACAAGCAAATC
TCCAGCCAATCGGGAGCAAGCACCAACGACAACACCTACTTCGGCTACAGCACCCCCCTGGGGGTA
TTTTGACTTTAACAGATTCCACTGCCACTTCTCACCACGTGACTGGCAGCGACTCATCAACAACA
ACTGGGGATTCCGGCCCAAGAGACTCAACTTCAAGCTCTTCAACATCCAGGTCAAGGAGGTCACG
ACGAATGATGGCACCACGACCATCGCCAATAACCTTACCAGCACGGTTCAGGTCTTTACGGACTC
GGAATACCAGCTCCCGTACGTCCTCGGCTCTGCGCACCAGGGCTGCCTGCCTCCGTTCCCGGCGG
ACGTCTTCATGATTCTCAGTACGGGTACCTGACTCTGAACAATGGCAGTCAGGCCGTGGGCCGT
TCCTCCTTCTACTGCCTGGAGTACTTTCCTTCTCAAATGCTGAGAACGGGCAACAACCTTTGAGTT
CAGCTACACGTTTGAGGACGTGCCTTTTCACAGCAGCTACGCGCACAGCCAAAGCCTGGACCGGC
TGATGAACCCCTCATCGACCAGTACCTGTACTACCTGTCTCGGACTCAGACCACGAGTGGTACC
GCAGGAAATCGGACGTTGCAATTTTCTCAGGCCGGGCCTAGTAGCATGGCGAATCAGGCCAAAAA
CTGGCTACCCGGGCCCTGCTACCGGCAGCAACGCGTCTCCAAGACAGCGAATCAAAATAACAACA
GCAACTTTGCCTGGACCGGTGCCACCAAGTATCATCTGAATGGCAGAGACTCTCTGGTAAATCCC
GGTCCCGCTATGGCAACCCACAAGGACGACGAAGACAAATTTTTTCCGATGAGCGGAGTCTTAAT
ATTTGGGAAACAGGGAGCTGGAAATAGCAACGTGGACCTTGACAACGTTATGATAACCAGTGAGG
AAGAAATTAACCACCAACCCAGTGGCCACAGAACAGTACGGCACGGTGGCCACTAACCTGCAA
TCGTCAAACACCGCTCCTGCTACAGGGACCGTCAACAGTCAAGGAGCCTTACCTGGCATGGTCTG
GCAGAACCGGGACGTGTACCTGCAGGGTCCATCTGGGCCAAGATTCCTCACACGGACGGACACT
TTCATCCCTCGCCGCTGATGGGAGGCTTTGGACTGAAACACCCGCCCTCCTCAGATCCTGATTAAG
AATACACCTGTTCCCGCGAATCCTCCAACCTACCTTCAGTCCAGCTAAGTTTTCGCTCGTTCATCAC
GCAGTACAGCACCGGACAGGTGAGCTGGAATGGAATGGGAGCTGCAGAAAGAAAACAGCAAAC
GCTGGAACCCAGAGATTCAATACACTTCCAACCTACAACAAATCTACAAATGTGGACTTTGCTGTT
GACACAAATGGCGTTTATTTCTGAGCCTCGCCCCATCGGCACCCGTTACCTCACCCGTAATCTG

Exemplary AAV Anc80 Capsid Amino Acid Sequence (SEQ ID NO: 44)

MAADGYLPDWLEDNLSEGIREWDLKPGAPKPKANQQKQDDGRGLVLPGYKYLGPFNGLDKGEPV
NAADAAALEHDKAYDQQLKAGDNPYLRYNHADAEFQERLQEDTSFGGNLGRAVFQAKKRVLEPLG
LVEEGAKTAPGKKRPVEQSPQEPDSSSGIGKKGQQPAKKRLNFGQTGDSESVDPDQPLGEPAPAP
SGVGSNTMAAGGGAPMADNNEGADGVGNASGNWHCDSTWLGDRVITTTSTRTWALPTYNNHLYKQI
SSQSGASTNDNTYFGYSTPWGYFDFNRHCHFSRPDWQRLINNNWGFPRKRLNFKLFNIQVKEVT
TNDGTTTTIANNLTSTVQVFTDSEYQLPYVLGSAHQGLPPFPADVMI PQYGYLTLNNGSQAVGR
SSFYCLEYFPSQMLRTGNNEFEFSYTFEDVPFHSSYAHQSQSLDRLMNPLIDQYLYYLSRTQTTSQT
AGNRTLQFSQAGPSSMANQAKNWLPGPCYRQQRVSKTANQNNSNFAWTGATKYHLNGRDSLVP
GPAMATHKDDDEKFFPMSGVLI FGKQGAGNSNVDLDNVMITSEEEIKTTNPVATEQYGT VATNLQ

SSNTAPATGTVNSQGALPGMVWQNRDVYLQGPIWAKIPHTDGHFHPSPLMGGFGLKHPPQILIK
 NTPVPANPPTTFSPAKFASFITQYSTGQVSVEIEWELQKENS KRWNPEIQYTSNYNKSTNVDFAV
 DTNGVYSEPRPIGTRYLTRNL

Compositions

- [0281] Among other things, the present disclosure provides compositions. In some aspects, a composition comprises a construct as described herein. In some aspects, a composition comprises one or more constructs as described herein. In some aspects, a composition comprises a plurality of constructs as described herein. In some aspects, when more than one construct is included in the composition, the constructs are each different.
- [0282] In some aspects, a composition comprises an AAV particle as described herein. In some aspects, a composition comprises one or more AAV particles as described herein. In some aspects, a composition comprises a plurality of AAV particles. In some aspects, when more than one AAV particle is included in the composition, the AAV particles are each different.
- [0283] In some aspects, a composition comprises a vector as described herein.
- [0284] In some aspects, a composition comprises a cell.
- [0285] In some aspects, a composition is or comprises a pharmaceutical composition. In some aspects, the pharmaceutical composition comprises a pharmaceutically acceptable carrier. In some aspects, a composition is or comprises a synthetic perilymph solution. In some aspects, a synthetic perilymph solution comprises 20-200mM NaCl; 1-5 mM KCl; 0.1-10mM CaCl₂; 1-10mM glucose; and 2-50 mM HEPES, with a pH between about 6 and about 9.

Dosing and Volume of Administration

- [0286] In some aspects, a composition disclosed herein, e.g., one or a plurality of AAV vectors disclosed herein, is administered as a single dose or as a plurality of doses.
- [0287] In some aspects, a composition disclosed herein is administered as a single dose. In some aspects, a composition disclosed herein is administered as a plurality of doses, e.g., 2, 3, 4, 5, 6, 7, 8, 9 or 10 doses.
- [0288] In some aspects, a composition disclosed herein (e.g., a composition comprising one or a plurality of rAAV constructs disclosed herein) is administered at a volume of about 0.01mL, about 0.02 mL, about 0.03 mL, about 0.04 mL, about 0.05 mL, about 0.06

mL, about 0.07 mL, about 0.08 mL, about 0.09 mL, about 1.00 mL, about 1.10 mL, about 1.20 mL, about 1.30 mL, about 1.40 mL, about 1.50 mL, about 1.60 mL, about 1.70 mL, about 1.80 mL, about 1.90 mL, or about 2.00 mL. In some aspects, a composition disclosed herein is administered at a volume of about 0.01 mL. In some aspects, a composition disclosed herein is administered at a volume of about 0.02 mL. In some aspects, a composition disclosed herein is administered at a volume of about 0.03 mL. In some aspects, a composition disclosed herein is administered at a volume of about 0.04 mL. In some aspects, a composition disclosed herein is administered at a volume of about 0.05 mL. In some aspects, a composition disclosed herein is administered at a volume of about 0.06 mL. In some aspects, a composition disclosed herein is administered at a volume of about 0.07 mL. In some aspects, a composition disclosed herein is administered at a volume of about 0.08 mL. In some aspects, a composition disclosed herein is administered at a volume of about 0.09 mL. In some aspects, a composition disclosed herein is administered at a volume of about 1.00 mL. In some aspects, a composition disclosed herein is administered at a volume of about 1.10 mL. In some aspects, a composition disclosed herein is administered at a volume of about 1.20 mL. In some aspects, a composition disclosed herein is administered at a volume of about 1.30 mL. In some aspects, a composition disclosed herein is administered at a volume of about 1.40 mL. In some aspects, a composition disclosed herein is administered at a volume of about 1.50 mL. In some aspects, a composition disclosed herein is administered at a volume of about 1.60 mL. In some aspects, a composition disclosed herein is administered at a volume of about 1.70 mL. In some aspects, a composition disclosed herein is administered at a volume of about 1.80 mL. In some aspects, a composition disclosed herein is administered at a volume of about 1.90 mL. In some aspects, a composition disclosed herein is administered at a volume of about 2.00 mL.

[0289] In some aspects, a composition disclosed herein (e.g., a composition comprising one or a plurality of rAAV constructs disclosed herein) is administered at a volume of about 0.01 to 2.00 mL, about 0.02 to 1.90 mL, about 0.03 to 1.8 mL, about 0.04 to 1.70 mL, about 0.05 to 1.60 mL, about 0.06 to 1.50 mL, about 0.06 to 1.40 mL, about 0.07 to 1.30 mL, about 0.08 to 1.20 mL, or about 0.09 to 1.10 mL. In some aspects a composition disclosed herein (e.g., a composition comprising one or a plurality of rAAV constructs disclosed herein) is administered at a volume of about 0.01 to 2.00 mL, about 0.02 to 2.00 mL, about 0.03 to 2.00 mL, about 0.04 to 2.00 mL, about 0.05 to 2.00 mL, about 0.06 to

2.00 mL, about 0.07 to 2.00 mL, about 0.08 to 2.00 mL, about 0.09 to 2.00 mL, about 0.01 to 1.90 mL, about 0.01 to 1.80 mL, about 0.01 to 1.70 mL, about 0.01 to 1.60 mL, about 0.01 to 1.50 mL, about 0.01 to 1.40 mL, about 0.01 to 1.30 mL, about 0.01 to 1.20 mL, about 0.01 to 1.10 mL, about 0.01 to 1.00 mL, about 0.01 to 0.09 mL.

[0290] In some aspects, a dosing regimen comprises delivery in a volume of at least 0.01 mL, at least 0.02 mL, at least 0.03 mL, at least 0.04 mL, at least 0.05 mL, at least 0.06 mL, at least 0.07 mL, at least 0.08 mL, at least 0.09 mL, at least 0.10 mL, at least 0.11 mL, at least 0.12 mL, at least 0.13 mL, at least 0.14 mL, at least 0.15 mL, at least 0.16 mL, at least 0.17 mL, at least 0.18 mL, at least 0.19 mL, or at least 0.20 mL per cochlea. In some aspects, a dosing regimen comprises delivery in a volume of at most 0.30 mL, at most 0.25 mL, at most 0.20 mL, at most 0.15 mL, at most 0.14 mL, at most 0.13 mL, at most 0.12 mL, at most 0.11 mL, at most 0.10 mL, at most 0.09 mL, at most 0.08 mL, at most 0.07 mL, at most 0.06 mL, or at most 0.05 mL per cochlea. In some aspects, the dosing regimen comprises delivery in a volume of about 0.05 mL, about 0.06 mL, about 0.07 mL, about 0.08 mL, about 0.09 mL, about 0.10 mL, about 0.11 mL, about 0.12 mL, about 0.13 mL, about 0.14 mL, or about 0.15 mL per cochlea, depending on the population.

Single AAV Construct Compositions

[0291] In some aspects, the present disclosure provides compositions or systems comprising AAV particles comprised of a single construct. In some such aspects, a single construct may deliver a polynucleotide that encodes a functional (e.g., wild-type or otherwise functional, e.g., codon optimized) polypeptide (e.g., a therapeutic polypeptide, a Connexin 26 polypeptide). In some aspects, a construct is or comprises an rAAV construct. In some aspects described herein, a single rAAV construct is capable of expressing a polypeptide (e.g., a therapeutic polypeptide, a Connexin 26 polypeptide) thereof in a target cell (e.g., an inner ear supporting cell).

[0292] In some aspects, a single construct composition or system may comprise any or all of the exemplary construct components described herein. In some aspects, the, the construct comprises a nucleic acid sequence with at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% at least 99%, or 100% identity to SEQ ID NOs: 45-51, 82-84, 88, or 100-107. In some aspects, the, the construct comprises a nucleic acid sequence with at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% at least 99%, or 100% identity to SEQ ID NO: 82. In some aspects, an exemplary

single construct is represented by SEQ ID NO: 82. In some aspects, the, the construct comprises a nucleic acid sequence with at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% at least 99%, or 100% identity to SEQ ID NO: 83. In some aspects, an exemplary single construct is represented by SEQ ID NO: 83. In some aspects, the, the construct comprises a nucleic acid sequence with at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% at least 99%, or 100% identity to SEQ ID NO: 84. In some aspects, an exemplary single construct is represented by SEQ ID NO: 84. In some aspects, the, the construct comprises a nucleic acid sequence with at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% at least 99%, or 100% identity to SEQ ID NO: 87. In some aspects, an exemplary single construct is represented by SEQ ID NO: 87.

[0293] In some aspects, the construct comprises the nucleic acid sequence of SEQ ID NO: 54. In some aspects, the, the construct comprises a nucleic acid sequence with at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% at least 99%, or 100% identity to SEQ ID NO: 54. In some aspects, the construct comprises the nucleic acid sequence of nucleotides 12-4754 of SEQ ID NO: 54. In some aspects, the, the construct comprises a nucleic acid sequence with at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% at least 99%, or 100% identity to 12-4754 of SEQ ID NO: 54.

[0294] In some aspects, the construct comprises the nucleic acid sequence of SEQ ID NO: 17. In some aspects, the, the construct comprises a nucleic acid sequence with at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% at least 99%, or 100% identity to SEQ ID NO: 17. In some aspects, the construct comprises nucleotides 12-4338 of SEQ ID NO: 17. In some aspects, the, the construct comprises a nucleic acid sequence with at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% at least 99%, or 100% identity to nucleotides 12-4338 of SEQ ID NO: 17.

[0295] In some aspects, the construct comprises the nucleic acid sequence of SEQ ID NO: 7. In some aspects, the, the construct comprises a nucleic acid sequence with at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% at least 99%, or 100% identity to SEQ ID NO: 7.

[0296] . In some aspects, the construct comprises nucleotides 12-4557 of SEQ ID NO: 7. In some aspects, the, the construct comprises a nucleic acid sequence with at least 85%, at

least 90%, at least 95%, at least 96%, at least 97%, at least 98% at least 99%, or 100% identity nucleotides 12-4557 of SEQ ID NO: 7.

[0297] In some aspects, the construct comprises a nucleic acid sequence with at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% at least 99%, or 100% identity to SEQ ID NO: 61. In some aspects, the construct comprises the nucleic acid sequence of SEQ ID NO: 61. In some aspects, the construct comprises a nucleic acid sequence with at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% at least 99%, or 100% identity to nucleotides 12-4429 of SEQ ID NO: 61. In some aspects, the construct comprises the nucleic acid sequence of nucleotides 12-4429 of SEQ ID NO: 61.

[0298] In some aspects, the construct comprises a nucleic acid sequence with at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% at least 99%, or 100% identity to SEQ ID NO: 38. In some aspects, the construct comprises the nucleic acid sequence of SEQ ID NO: 38. In some aspects, the construct comprises a nucleic acid sequence with at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% at least 99%, or 100% identity to nucleotides 12-3976 of SEQ ID NO: 38. In some aspects, the construct comprises the nucleic acid sequence of nucleotides 12-3976 of SEQ ID NO: 38.

[0299] In some aspects, the construct comprises a nucleic acid sequence with at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% at least 99%, or 100% identity to SEQ ID NO: 100. In some aspects, the construct comprises SEQ ID NO: 100. In some aspects, the construct comprises a nucleic acid sequence with at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% at least 99%, or 100% identity to nucleotides 12-4645 of SEQ ID NO: 100. In some aspects, the construct comprises nucleotides 12-4645 of SEQ ID NO: 100.

[0300] In some aspects, the construct comprises a nucleic acid sequence with at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% at least 99%, or 100% identity to SEQ ID NO: 101. In some aspects, the construct comprises SEQ ID NO: 101. In some aspects, the construct comprises a nucleic acid sequence with at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% at least 99%, or 100% identity to nucleotides 12-4708 of SEQ ID NO: 101. In some aspects, the construct comprises nucleotides 12-4708 SEQ ID NO: 101.

- [0301]** In some aspects, the construct comprises a nucleic acid sequence with at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% at least 99%, or 100% identity to SEQ ID NO: 102. In some aspects, the construct comprises SEQ ID NO: 102. In some aspects, the construct comprises a nucleic acid sequence with at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% at least 99%, or 100% identity to nucleotides 12-4993 of SEQ ID NO: 102. In some aspects, the construct comprises nucleotides 12-4993 of SEQ ID NO: 102.
- [0302]** In some aspects, the construct comprises a nucleic acid sequence with at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% at least 99%, or 100% identity to SEQ ID NO: 103. In some aspects, the construct comprises SEQ ID NO: 103. In some aspects, the construct comprises a nucleic acid sequence with at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% at least 99%, or 100% identity to nucleotides 12-4496 of SEQ ID NO: 103. In some aspects, the construct comprises nucleotides 12-4496 of SEQ ID NO: 103.
- [0303]** In some aspects, the construct comprises a nucleic acid sequence with at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% at least 99%, or 100% identity to SEQ ID NO: 104. In some aspects, the construct comprises SEQ ID NO: 104. In some aspects, the construct comprises a nucleic acid sequence with at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% at least 99%, or 100% identity to nucleotides 12-4253 of SEQ ID NO: 104. In some aspects, the construct comprises nucleotides 12-4253 of SEQ ID NO: 104.
- [0304]** In some aspects, the construct comprises a nucleic acid sequence with at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% at least 99%, or 100% identity to SEQ ID NO: 105. In some aspects, the construct comprises SEQ ID NO: 105. In some aspects, the construct comprises a nucleic acid sequence with at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% at least 99%, or 100% identity to nucleotides 12-4320 of SEQ ID NO: 105. In some aspects, the construct comprises nucleotides 12-4320 SEQ ID NO: 105.
- [0305]** In some aspects, the construct comprises a nucleic acid sequence with at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% at least 99%, or 100% identity to SEQ ID NO: 106. In some aspects, the construct comprises SEQ ID NO: 106. In some aspects, the construct comprises a nucleic acid sequence with at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% at least 99%, or 100%

identity to nucleotides 12-4464 of SEQ ID NO: 106. In some aspects, the construct comprises nucleotides 12-4464 of SEQ ID NO: 106.

[0306] In some aspects, the construct comprises a nucleic acid sequence with at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% at least 99%, or 100% identity to SEQ ID NO: 107. In some aspects, the construct comprises SEQ ID NO: 107. In some aspects, the construct comprises a nucleic acid sequence with at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% at least 99%, or 100% identity to nucleotides 12-4328 of SEQ ID NO: 107. In some aspects, the construct comprises nucleotides 12-4328 of SEQ ID NO: 107.

[0307] One skilled in the art would recognize that constructs may undergo additional modifications including codon-optimization, introduction of novel but functionally equivalent (e.g., silent mutations), addition of reporter sequences, and/or other routine modification.

[0308] In some aspects, an exemplary rAAVAnc80 particle comprises a construct represented by SEQ ID NO: 82.

[0309] In one aspect, an exemplary construct comprises: a 5' ITR exemplified by SEQ ID NO: 52, optionally a cloning site exemplified by SEQ ID NO: 70, a CAG enhancer/promoter exemplified by SEQ ID NO: 14, optionally a cloning site exemplified by SEQ ID NO: 72, a GJB2 5'UTR sequence exemplified by SEQ ID NO: 66, optionally a cloning site exemplified by SEQ ID NO: 73, a GJB2 coding region, a linker sequence exemplified by SEQ ID NO: 77, a FLAG sequence with stop codon exemplified by SEQ ID NO: 81, a 3' UTR exemplified by SEQ ID NO: 67, optionally a cloning site exemplified by SEQ ID NO: 75, a poly(A) site exemplified by SEQ ID NO: 25, optionally a cloning site exemplified by SEQ ID NO: 76, and a 3' ITR exemplified by SEQ ID NO: 53.

[0310] In some aspects, an exemplary rAAVAnc80 particle comprises a construct represented by SEQ ID NO: 83.

[0311] In one aspect, an exemplary construct comprises: a 5' ITR exemplified by SEQ ID NO: 52, optionally a cloning site exemplified by SEQ ID NO: 70, a CMV/CBA enhancer/promoter exemplified by SEQ ID NO: 12, a chimeric intron exemplified by SEQ ID NO: 64, optionally a cloning site exemplified by SEQ ID NO: 72, a GJB2 5'UTR sequence exemplified by SEQ ID NO: 66, optionally a cloning site exemplified by SEQ ID NO: 73, a GJB2 coding region, a linker sequence exemplified by SEQ ID NO: 77,

optionally a FLAG sequence with stop codon exemplified by SEQ ID NO: 81, a 3' UTR exemplified by SEQ ID NO: 67, optionally a cloning site exemplified by SEQ ID NO: 75, a poly(A) site exemplified by SEQ ID NO: 25, optionally a cloning site exemplified by SEQ ID NO: 76, and a 3' ITR exemplified by SEQ ID NO: 53.

[0312] In some aspects, an exemplary rAAVAnc80 particle comprises a construct represented by SEQ ID NO: 84.

[0313] In one aspect, an exemplary construct comprises: a 5' ITR exemplified by SEQ ID NO: 52, optionally a cloning site exemplified by SEQ ID NO: 70, a CMV enhancer exemplified by SEQ ID NO: 63, a human GJB2 promoter exemplified by SEQ ID NO: 61, optionally a cloning site exemplified by SEQ ID NO: 72, a GJB2 5'UTR sequence exemplified by SEQ ID NO: 66, optionally a cloning site exemplified by SEQ ID NO: 73, a GJB2 coding region, a linker sequence exemplified by SEQ ID NO: 77, optionally a FLAG sequence with stop codon exemplified by SEQ ID NO: 81, a 3' UTR exemplified by SEQ ID NO: 67, optionally a cloning site exemplified by SEQ ID NO: 75, a poly(A) site exemplified by SEQ ID NO: 25, optionally a cloning site exemplified by SEQ ID NO: 76, and a 3' ITR exemplified by SEQ ID NO: 53.

[0314] In some aspects, an exemplary rAAVAnc80 particle comprises a construct represented by SEQ ID NO: 87.

[0315] In one aspect, an exemplary construct comprises: a 5' ITR exemplified by SEQ ID NO: 52, optionally a cloning site exemplified by SEQ ID NO: 70, a human GFAP enhancer-promoter exemplified by SEQ ID NO: 91, optionally a cloning site exemplified by SEQ ID NO: 72, a GJB2 5'UTR sequence exemplified by SEQ ID NO: 66, optionally a cloning site exemplified by SEQ ID NO: 73, a GJB2 coding region, a linker sequence exemplified by SEQ ID NO: 77, optionally a FLAG sequence with stop codon exemplified by SEQ ID NO: 81, a destabilization domain exemplified by SEQ ID NO: 80, a 3' UTR exemplified by SEQ ID NO: 68, optionally a cloning site exemplified by SEQ ID NO: 34, a poly(A) site exemplified by SEQ ID NO: 25, optionally a cloning site exemplified by SEQ ID NO: 76, and a 3' ITR exemplified by SEQ ID NO: 53.

[0316] In some aspects, the rAAVAnc80 particle comprises a construct comprising the nucleic acid sequence of SEQ ID NO: 61.

[0317] In one aspect, the construct comprises a 5' ITR comprising the nucleic acid sequence of SEQ ID NO: 52, optionally a cloning site comprising the nucleic acid sequence of SEQ ID NO: 71, a GDF6 promoter sequence comprising the nucleic acid

sequence of SEQ ID NO: 90; a hGJB2 minimal promoter comprising the nucleic acid sequence of SEQ ID NO: 86, optionally a cloning site comprising the nucleic acid sequence of SEQ ID NO: 85; optionally a synthetic barcode comprising the nucleic acid sequence of SEQ ID NO: 62; a 5'UTR sequence comprising the nucleic acid sequence of SEQ ID NO: 66, a GJB2 coding region, a linker sequence exemplified by SEQ ID NO: 77, optionally a FLAG sequence with stop codon comprising the nucleic acid sequence of SEQ ID NO: 81, a 3' UTR comprising the nucleic acid sequence of SEQ ID NO: 67, a poly(A) comprising the nucleic acid sequence of SEQ ID NO: 25, optionally a cloning site comprising the nucleic acid sequence of SEQ ID NO: 76, and a 3' ITR comprising the nucleic acid sequence of SEQ ID NO: 53.

[0318] In some aspects, the rAAVAnc80 particle comprises a construct comprising the nucleic acid sequence of SEQ ID NO: 54.

[0319] In one aspect, the construct comprises a 5' ITR comprising the nucleic acid sequence of SEQ ID NO: 52, optionally a cloning site comprising the nucleic acid sequence of SEQ ID NO: 71, a IGFBP2 promoter sequence comprising the nucleic acid sequence of SEQ ID NO: 57; a hGJB2 minimal promoter comprising the nucleic acid sequence of SEQ ID NO: 86, optionally a cloning site comprising the nucleic acid sequence of SEQ ID NO: 85; optionally a synthetic barcode comprising the nucleic acid sequence of SEQ ID NO: 55; a 5'UTR sequence comprising the nucleic acid sequence of SEQ ID NO: 66, a GJB2 coding region comp, a linker sequence exemplified by SEQ ID NO: 77, optionally a FLAG sequence with stop codon comprising the nucleic acid sequence of SEQ ID NO: 81, a 3' UTR comprising the nucleic acid sequence of SEQ ID NO: 67, a poly(A) comprising the nucleic acid sequence of SEQ ID NO: 25, optionally a cloning site comprising the nucleic acid sequence of SEQ ID NO: 76, and a 3' ITR comprising the nucleic acid sequence of SEQ ID NO: 53.

[0320] In some aspects, the rAAVAnc80 particle comprises a construct comprising the nucleic acid sequence of SEQ ID NO: 17.

[0321] In one aspect, the construct comprises a 5' ITR comprising the nucleic acid sequence of SEQ ID NO: 52, optionally a cloning site comprising the nucleic acid sequence of SEQ ID NO: 71, a RBP7 promoter sequence comprising the nucleic acid sequence of SEQ ID NO: 28; a hGJB2 minimal promoter comprising the nucleic acid sequence of SEQ ID NO: 86, optionally a cloning site comprising the nucleic acid sequence of SEQ ID NO: 85; optionally a synthetic barcode comprising the nucleic acid

sequence of SEQ ID NO: 27; a 5'UTR sequence comprising the nucleic acid sequence of SEQ ID NO: 66, a GJB2 coding region, a linker sequence exemplified by SEQ ID NO: 77, optionally a FLAG sequence with stop codon comprising the nucleic acid sequence of SEQ ID NO: 81, a 3' UTR comprising the nucleic acid sequence of SEQ ID NO: 67, a poly(A) comprising the nucleic acid sequence of SEQ ID NO: 25, optionally a cloning site comprising the nucleic acid sequence of SEQ ID NO: 76, and a 3' ITR comprising the nucleic acid sequence of SEQ ID NO: 53.

[0322] In some aspects, the rAAVAnc80 particle comprises a construct comprising the nucleic acid sequence of SEQ ID NO: 17.

[0323] In one aspect, the construct comprises a 5' ITR comprising the nucleic acid sequence of SEQ ID NO: 52, optionally a cloning site comprising the nucleic acid sequence of SEQ ID NO: 71, a GJB6 promoter sequence comprising the nucleic acid sequence of SEQ ID NO: 16; a hGJB2 minimal promoter comprising the nucleic acid sequence of SEQ ID NO: 86, optionally a cloning site comprising the nucleic acid sequence of SEQ ID NO: 85; optionally a synthetic barcode comprising the nucleic acid sequence of SEQ ID NO: 41; a 5'UTR sequence comprising the nucleic acid sequence of SEQ ID NO: 66, a GJB2 coding region, a linker sequence exemplified by SEQ ID NO: 77, optionally a FLAG sequence with stop codon comprising the nucleic acid sequence of SEQ ID NO: 81, a 3' UTR comprising the nucleic acid sequence of SEQ ID NO: 67, a poly(A) comprising the nucleic acid sequence of SEQ ID NO: 25, optionally a cloning site comprising the nucleic acid sequence of SEQ ID NO: 76, and a 3' ITR comprising the nucleic acid sequence of SEQ ID NO: 53.

[0324] In some aspects, the rAAVAnc80 particle comprises a construct comprising the nucleic acid sequence of SEQ ID NO: 7.

[0325] In one aspect, the construct comprises a 5' ITR comprising the nucleic acid sequence of SEQ ID NO: 52, optionally a cloning site comprising the nucleic acid sequence of SEQ ID NO: 71, a PARM1 promoter sequence comprising the nucleic acid sequence of SEQ ID NO: 40; a hGJB2 minimal promoter comprising the nucleic acid sequence of SEQ ID NO: 86, optionally a cloning site comprising the nucleic acid sequence of SEQ ID NO: 85; optionally a synthetic barcode comprising the nucleic acid sequence of SEQ ID NO: 39; a 5'UTR sequence comprising the nucleic acid sequence of SEQ ID NO: 66, a GJB2 coding region, a linker sequence exemplified by SEQ ID NO: 77, optionally a FLAG sequence with stop codon comprising the nucleic acid sequence of

SEQ ID NO: 81, a 3' UTR comprising the nucleic acid sequence of SEQ ID NO: 67, a poly(A) comprising the nucleic acid sequence of SEQ ID NO: 25, optionally a cloning site comprising the nucleic acid sequence of SEQ ID NO: 76, and a 3' ITR comprising the nucleic acid sequence of SEQ ID NO: 53.

[0326] In some aspects, the rAAVAnc80 particle comprises a construct comprising the nucleic acid sequence of SEQ ID NO: 100.

[0327] In one aspect, the construct comprises a 5' ITR comprising the nucleic acid sequence of SEQ ID NO: 52, optionally a cloning site comprising the nucleic acid sequence of SEQ ID NO: 71, a BACE2 promoter sequence comprising the nucleic acid sequence of SEQ ID NO: 92; a hGJB2 minimal promoter comprising the nucleic acid sequence of SEQ ID NO: 86, optionally a cloning site comprising the nucleic acid sequence of SEQ ID NO: 85, optionally a synthetic barcode comprising the nucleic acid sequence of SEQ ID NO: 108; a 5'UTR sequence comprising the nucleic acid sequence of SEQ ID NO: 66, a GJB2 coding region, optionally a FLAG sequence with stop codon comprising the nucleic acid sequence of SEQ ID NO: 81, a 3' UTR comprising the nucleic acid sequence of SEQ ID NO: 67, a poly(A) comprising the nucleic acid sequence of SEQ ID NO: 25, optionally a cloning site comprising the nucleic acid sequence of SEQ ID NO: 76, and a 3' ITR comprising the nucleic acid sequence of SEQ ID NO: 53.

[0328] In some aspects, the rAAVAnc80 particle comprises a construct comprising the nucleic acid sequence of SEQ ID NO: 101.

[0329] In one aspect, the construct comprises a 5' ITR comprising the nucleic acid sequence of SEQ ID NO: 52, optionally a cloning site comprising the nucleic acid sequence of SEQ ID NO: 71, a DBI2 promoter sequence comprising the nucleic acid sequence of SEQ ID NO: 93; a hGJB2 minimal promoter comprising the nucleic acid sequence of SEQ ID NO: 86, optionally a cloning site comprising the nucleic acid sequence of SEQ ID NO: 85, optionally a synthetic barcode comprising the nucleic acid sequence of SEQ ID NO: 109; a 5'UTR sequence comprising the nucleic acid sequence of SEQ ID NO: 66, a GJB2 coding region, optionally a FLAG sequence with stop codon comprising the nucleic acid sequence of SEQ ID NO: 81, a 3' UTR comprising the nucleic acid sequence of SEQ ID NO: 67, a poly(A) comprising the nucleic acid sequence of SEQ ID NO: 25, optionally a cloning site comprising the nucleic acid sequence of SEQ ID NO: 76, and a 3' ITR comprising the nucleic acid sequence of SEQ ID NO: 53.

- [0330] In some aspects, the rAAVAnc80 particle comprises a construct comprising the nucleic acid sequence of SEQ ID NO: 102.
- [0331] In one aspect, the construct comprises a 5' ITR comprising the nucleic acid sequence of SEQ ID NO: 52, optionally a cloning site comprising the nucleic acid sequence of SEQ ID NO: 71, a FABP3 promoter sequence comprising the nucleic acid sequence of SEQ ID NO: 94; a hGJB2 minimal promoter comprising the nucleic acid sequence of SEQ ID NO: 86, optionally a cloning site comprising the nucleic acid sequence of SEQ ID NO: 85, optionally a synthetic barcode comprising the nucleic acid sequence of SEQ ID NO: 110; a 5'UTR sequence comprising the nucleic acid sequence of SEQ ID NO: 66, a GJB2 coding region, optionally a FLAG sequence with stop codon comprising the nucleic acid sequence of SEQ ID NO: 81, a 3' UTR comprising the nucleic acid sequence of SEQ ID NO: 67, a poly(A) comprising the nucleic acid sequence of SEQ ID NO: 25, optionally a cloning site comprising the nucleic acid sequence of SEQ ID NO: 76, and a 3' ITR comprising the nucleic acid sequence of SEQ ID NO: 53.
- [0332] In some aspects, the rAAVAnc80 particle comprises a construct comprising the nucleic acid sequence of SEQ ID NO: 103.
- [0333] In one aspect, the construct comprises a 5' ITR comprising the nucleic acid sequence of SEQ ID NO: 52, optionally a cloning site comprising the nucleic acid sequence of SEQ ID NO: 71, a KLHL14 promoter sequence comprising the nucleic acid sequence of SEQ ID NO: 95; a hGJB2 minimal promoter comprising the nucleic acid sequence of SEQ ID NO: 86, optionally a cloning site comprising the nucleic acid sequence of SEQ ID NO: 85, optionally a synthetic barcode comprising the nucleic acid sequence of SEQ ID NO: 111; a 5'UTR sequence comprising the nucleic acid sequence of SEQ ID NO: 66, a GJB2 coding region, optionally a FLAG sequence with stop codon comprising the nucleic acid sequence of SEQ ID NO: 81, a 3' UTR comprising the nucleic acid sequence of SEQ ID NO: 67, a poly(A) comprising the nucleic acid sequence of SEQ ID NO: 25, optionally a cloning site comprising the nucleic acid sequence of SEQ ID NO: 76, and a 3' ITR comprising the nucleic acid sequence of SEQ ID NO: 53.
- [0334] In some aspects, the rAAVAnc80 particle comprises a construct comprising the nucleic acid sequence of SEQ ID NO: 104.
- [0335] In one aspect, the construct comprises a 5' ITR comprising the nucleic acid sequence of SEQ ID NO: 52, optionally a cloning site comprising the nucleic acid sequence of SEQ ID NO: 71, a MMP15 promoter sequence comprising the nucleic acid

sequence of SEQ ID NO: 96; a hGJB2 minimal promoter comprising the nucleic acid sequence of SEQ ID NO: 86, optionally a cloning site comprising the nucleic acid sequence of SEQ ID NO: 85, optionally a synthetic barcode comprising the nucleic acid sequence of SEQ ID NO: 112; a 5'UTR sequence comprising the nucleic acid sequence of SEQ ID NO: 66, a GJB2 coding region, optionally a FLAG sequence with stop codon comprising the nucleic acid sequence of SEQ ID NO: 81, a 3' UTR comprising the nucleic acid sequence of SEQ ID NO: 67, a poly(A) comprising the nucleic acid sequence of SEQ ID NO: 25, optionally a cloning site comprising the nucleic acid sequence of SEQ ID NO: 76, and a 3' ITR comprising the nucleic acid sequence of SEQ ID NO: 53.

[0336] In some aspects, the rAAVAnc80 particle comprises a construct comprising the nucleic acid sequence of SEQ ID NO: 105.

[0337] In one aspect, the construct comprises a 5' ITR comprising the nucleic acid sequence of SEQ ID NO: 52, optionally a cloning site comprising the nucleic acid sequence of SEQ ID NO: 71, a SPARC promoter sequence comprising the nucleic acid sequence of SEQ ID NO: 97; a hGJB2 minimal promoter comprising the nucleic acid sequence of SEQ ID NO: 86, optionally a cloning site comprising the nucleic acid sequence of SEQ ID NO: 85, optionally a synthetic barcode comprising the nucleic acid sequence of SEQ ID NO: 113; a 5'UTR sequence comprising the nucleic acid sequence of SEQ ID NO: 66, a GJB2 coding region, optionally a FLAG sequence with stop codon comprising the nucleic acid sequence of SEQ ID NO: 81, a 3' UTR comprising the nucleic acid sequence of SEQ ID NO: 67, a poly(A) comprising the nucleic acid sequence of SEQ ID NO: 25, optionally a cloning site comprising the nucleic acid sequence of SEQ ID NO: 76, and a 3' ITR comprising the nucleic acid sequence of SEQ ID NO: 53.

[0338] In some aspects, the rAAVAnc80 particle comprises a construct comprising the nucleic acid sequence of SEQ ID NO: 106.

[0339] In one aspect, the construct comprises a 5' ITR comprising the nucleic acid sequence of SEQ ID NO: 52, optionally a cloning site comprising the nucleic acid sequence of SEQ ID NO: 71, a TSPAN8 promoter sequence comprising the nucleic acid sequence of SEQ ID NO: 98; a hGJB2 minimal promoter comprising the nucleic acid sequence of SEQ ID NO: 86, optionally a cloning site comprising the nucleic acid sequence of SEQ ID NO: 85, optionally a synthetic barcode comprising the nucleic acid sequence of SEQ ID NO: 124; a 5'UTR sequence comprising the nucleic acid sequence of SEQ ID NO: 66, a GJB2 coding region, optionally a FLAG sequence with stop codon

comprising the nucleic acid sequence of SEQ ID NO: 81, a 3' UTR comprising the nucleic acid sequence of SEQ ID NO: 67, a poly(A) comprising the nucleic acid sequence of SEQ ID NO: 25, optionally a cloning site comprising the nucleic acid sequence of SEQ ID NO: 76, and a 3' ITR comprising the nucleic acid sequence of SEQ ID NO: 53.

[0340] In some aspects, the rAAVAnc80 particle comprises a construct comprising the nucleic acid sequence of SEQ ID NO: 107.

[0341] In one aspect, the construct comprises a 5' ITR comprising the nucleic acid sequence of SEQ ID NO: 52, optionally a cloning site comprising the nucleic acid sequence of SEQ ID NO: 71, a VIM promoter sequence comprising the nucleic acid sequence of SEQ ID NO: 99; a hGJB2 minimal promoter comprising the nucleic acid sequence of SEQ ID NO: 91, optionally a cloning site comprising the nucleic acid sequence of SEQ ID NO: 85, optionally a synthetic barcode comprising the nucleic acid sequence of SEQ ID NO: 114; a 5'UTR sequence comprising the nucleic acid sequence of SEQ ID NO: 66, a GJB2 coding region, optionally a FLAG sequence with stop codon comprising the nucleic acid sequence of SEQ ID NO: 81, a 3' UTR comprising the nucleic acid sequence of SEQ ID NO: 67, a poly(A) comprising the nucleic acid sequence of SEQ ID NO: 25, optionally a cloning site comprising the nucleic acid sequence of SEQ ID NO: 76, and a 3' ITR comprising the nucleic acid sequence of SEQ ID NO: 53.

Exemplary Construct sequence (SEQ ID NO: 82)

CTGCGCGCTCGCTCGCTCACTGAGGCCGCCGGGCGTCTGGGCGACCTTTGGTTCGCCCCGGCCTCAG
TGAGCGAGCGAGCGCGCAGAGAGGGAGTGGCCAACTCCATCACTAGGGGTTTCTGCGGCCGCACG
CGTGACATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTTCATAGCCCA
TATATGGAGTTCCGCGTTACATAACTTACGGTAAATGGCCCCGCTGGCTGACCGCCCAACGACCC
CCGCCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGAC
GTCAATGGGTGGACTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCA
AGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCCGCTGGCATTTATGCCAGTACATGAC
CTTATGGGACTTTCTTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGGTCGAG
GTGAGCCCCACGTTCTGCTTCACTCTCCCCATCTCCCCCCCCCTCCCCACCCCAATTTTGTATTT
ATTTATTTTTTTAATTATTTTGTGCAGCGATGGGGGCGGGGGGGGGGGGGCGCGGCCAGGCGGG
GCGGGGCGGGGCGAGGGGCGGGGCGGGGCGAGGCGGAGAGGTGCGGCGGCAGCCAATCAGAGCGG
CGCGCTCCGAAAGTTTCTTTTATGGCGAGGCGGCGGCGGGCGGCCCTATAAAAAGCGAAGCG
CGCGGCGGGCGGGAGTCGCTGCGTTGCCCTCGCCCCGTGCCCCGCTCCGCGCCGCTCGCGCCGC
CCGCCCCGGCTCTGACTGACCGCGTTACTCCACAGGTGAGCGGGCGGGACGGCCCTTCTCCTCC

GGGCTGTAATTAGCGCTTGGTTTAATGACGGCTCGTTTCTTTTCTGTGGCTGCGTGAAAGCCTTA
AAGGGCTCCGGGAGGGCCCTTTGTGCGGGGGGAGCGGCTCGGGGGGTGCGTGCGTGCTGTGTGTG
CGTGGGGAGCGCCGCTGCGGCCCGCGCTGCCGGCGGCTGTGAGCGCTGCGGGCGCGGCGCGGG
GCTTTGTGCGCTCCGCGTGTGCGCGAGGGGAGCGCGGCCGGGGGCGGTGCCCGCGGTGCGGGGG
GGCTGCGAGGGGAACAAAGGCTGCGTGCGGGGTGTGTGCGTGGGGGGGTGAGCAGGGGGTGTGGG
CGCGGCGGTGCGGGCTGTAACCCCCCTGCACCCCCCTCCCCGAGTTGCTGAGCACGGCCCCGGCT
TCGGGTGCGGGGCTCCGTGCGGGGCGTGGCGCGGGGCTCGCCGTGCCGGGCGGGGGGTGGCGGCA
GGTGGGGGTGCCGGGCGGGGCGGGGCCCTCGGGCCGGGAGGGCTCGGGGGAGGGGCGCGGCG
GCCCCCGGAGCGCCGGCGGCTGTGAGGCGCGGCGAGCCGAGCCATTGCCTTTTATGGTAATCG
TGCGAGAGGGCGCAGGGACTTCCTTTGTCCCAAATCTGTGCGGAGCCGAAATCTGGGAGGCGCCG
CCGCACCCCCCTCTAGCGGGCGCGGGGCGAAGCGGTGCGGCGCCGGCAGGAAGGAAATGGGCGGGG
AGGGCCTTCGTGCGTCGCCGCGCCCGCTCCCCCTCTCCCTCTCCAGCCTCGGGGCTGTCCGCGG
GGGGACGGCTGCCCTTCGGGGGGGACGGGGGAGGGCGGGGTTCGGCTTCTGGCGTGTGACCGGCGG
CTCTAGAGCCTCTGCTAACCATGTTTCATGCCTTCTTCTTTTCTTACAGCTCCTGGGCAACGTGC
TGGTTATTGTGACCGGTGTTGCGGCCCCGAGCGCCCGCGCGCTCCTCTCCCCGACTCGGAGCCC
CTCGGCGGCGCCCGGCCAGGACCCGCCTAGGAGCGCAGGAGCCCCAGCGCAGAGACCCCAACGC
CGAGACCCCCGCCCCGCCCCGCGCGCTTCCTCCCGACGCAGTTTAGGACCCTTGTTGCGGAAG
AGGTGGTGTGCGGCTGAGACCCGCGTCTTCAGGACGGTTCATCAGTGCCTCGATCCTGCCCCAC
TGGAGGAGGAAGGCAGCCGAACAGCGCTCACCTAACTAACAGCTGCTGAGAGCTGGGTTCGCTG
GCCATGCACCTGGGACTGCCTTGAGAAGCGTGAGCAAACCGCCAGAGTAGAAGCGCTAGCCACC
ATGGATTGGGGCACGCTGCAGACGATCTTGGGGGTGTGAACAAACACTCCACCAGCATTTGAAA
GATCTGGCTCACCGTCCTCTTCATTTTTTCGCATTATGATCCTCGTTGTGGCTGCAAAGGAGGTGT
GGGGAGATGAGCAGGCCGACTTTGTCTGCAACACCCTGCAGCCAGGCTGCAAGAACGTGTGCTAC
GATCACTACTTCCCCATCTCCACATCCGGCTATGGGCCCTGCAGCTGATCTTCGTGTCCACGCC
AGCGCTCCTAGTGGCCATGCACGTGGCTACCGGAGACATGAGAAGAAGAGGAAGTTCATCAAGG
GGGAGATAAAGAGTGAATTTAAGGACATCGAGGAGATCAAAACCCAGAAGGTCCGCATCGAAGGC
TCCCTGTGGTGGACCTACACAAGCAGCATCTTCTTCCGGGTCACTTTCGAAGCCGCCTTCATGTA
CGTCTTCTATGTCATGTACGACGGCTTCTCCATGCAGCGGCTGGTGAAGTGCAACGCCTGGCCTT
GTCCCAACACTGTGGACTGCTTTGTGTCCCGGCCACGGAGAAGACTGTCTTCACAGTGTTTCATG
ATTGCAGTGTCTGGAATTTGCATCCTGCTGAATGTCACTGAATTGTGTTATTTGCTAATTAGATA
TTGTTCTGGGAAGTCAAAAAGCCAGTTGGATCCCGGGCTGACTACAAAGACCATGACGGTGATT
ATAAAGATCATGACATCGACTACAAGGATGACGATGACAAGTAAGAAATAGACAGCATGAGAGGG
ATGAGGCAACCCGTGCTCAGCTGTCAAGGCTCAGTCGCTAGCATTTCCCAACACAAAGATTCTGA
CCTTAAATGCAACCATTGAAACCCCTGTAGGCCTCAGGTGAAACTCCAGATGCCACAATGGAGC

TCTGCTCCCCCTAAAGCCTCAAAACAAAGGCCTAATTCTATGCCTGTCTTAATTTCTTTCACTTA
AGTTAGTTCCACTGAGACCCAGGCTGTTAGGGGTATTGGTGTAAGGTACTTTTCATATTTTAAA
CAGAGGATATCGGCATTTGTTTCTTTCTCTGAGGACAAGAGAAAAAGCCAGGTTCCACAGAGGA
CACAGAGAAGGTTTGGGTGTCCTCCTGGGGTTCCTTTTGCCAACTTTCCCCACGTTAAAGGTGAA
CATTGGTTCTTTTCATTTGCTTTGGAAGTTTAAATCTCTAACAGTGGACAAAGTTACCAGTGCCTT
AAACTCTGTTACACTTTTTGGAAGTGAAAACCTTTGTAGTATGATAGGTTATTTTGATGTAAAGAT
GTTCTGGATAACCATTTATATGTTCCCCCTGTTTCAGAGGCTCAGATTGTAATATGTAAATGGTATG
TCATTGCTACTATGATTTAATTTGAAAATAGGTCTTTTGGTTATGAATACTTTGCAGCACAGCT
GAGAGGCTGTCTGTTGTATTTCATTGTGGTCATAGCACCTAACCAACATTGTAGCCTCAATCGAGTG
AGACAGACTAGAAGTTCCTAGTGATGGCTTATGATAGCAAATGGCCTCATGTCAAATATTTAGAT
GTAATTTTGTGTAAGAAATACAGACTGGATGTACCACCACTACTACCTGTAATGACAGGCCCTGT
CCAACACATCTCCCTTTTCCATGACTGTGGTAGCCAGCATCGGAAAGAACGCTGATTTAAAGAGG
TCGCTTGGGAATTTTATTGACACAGTACCATTAAATGGGGAGGACAAAATGGGGCAGGGGAGGGA
GAAGTTTCTGTCTGTTAAAAACAGATTTGGAAGACTGGACTCTAAAGTCTGTTGATTAAAGATGA
GCTTTGTCTACTTCAAAGTTTGTGTTGCTTACCCCTTCAGCCTCCAATTTTTTAAGTGAAAATAT
AGCTAATAACATGTGAAAAGAATAGAAGCTAAGGTTTAGATAAAATATTGAGCAGATCTATAGGAA
GATTGAACCTGAATATTGCCATTATGCTTGACATGGTTTCCAAAAAATGGTACTCCACATATTTTC
AGTGAGGGTAAGTATTTTCTGTTGTCAAGAATAGCATTGTAAAAGCATTTTGTAAATAATAAGA
ATAGCTTTAATGATATGCTTGTAATAAATAATTTTGTAAATGTATCAAATACATTTAAAAACATT
AAAATATAATCTCTATAATAAGAGCTCGCTGATCAGCCTCGACTGTGCCTTCTAGTTGCCAGCCA
TCTGTTGTTTGCCCCCTCCCCCGTGCTTCCCTTGACCCTGGAAGGTGCCACTCCCCTGTCCTTTTC
CTAATAAAATGAGGAAATTGCATCGCATTTGTCTGAGTAGGTGTCATTCTATTCTGGGGGGTGGGG
TGGGGCAGGACAGCAAGGGGGAGGATTGGGAAGACAATAGCAGGCATGCTGGGGATGCGGTGGGC
TCTATGGAAGCTTGAATTCAGCTGACGTGCCTCGGACCGCTAGGAACCCCTAGTGATGGAGTTGG
CCACTCCCTCTCTGCGCGCTCGCTCGCTCACTGAGGCCGGGCGACCAAAGGTCGCCCCGACCCCCG
GGCTTTGCCCCGGCGGCCCTCAGTGAGCGAGCGAGCGCGCAG

Exemplary Construct sequence (SEQ ID NO: 83)

CTGCGCGCTCGCTCGCTCACTGAGGCCGCCGGGCGTCGGGCGACCTTTGGTCGCCCCGGCCTCAG
TGAGCGAGCGAGCGCGCAGAGAGGGAGTGGCCAACTCCATCACTAGGGGTTCCTGCGGCCGCACG
CGTGACATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCCA
TATATGGAGTTCCGCGTTACATAACTTACGGTAAATGGCCCCGCTGGCTGACCGCCCAACGACCC
CCGCCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGAC
GTCAATGGGTGGACTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCA

AGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCCGCTGGCATTATGCCCAGTACATGAC
CTTATGGGACTTTTCTACTTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGGTCGAG
GTGAGCCCCACGTTCTGCTTCACTCTCCCCATCTCCCCCCCCCTCCCCACCCCCAATTTTGTATTT
ATTTATTTTTTAATTATTTTGTGTCAGCGATGGGGGCGGGGGGGGGGGGGGGCGCGGCCAGGCGGG
GCGGGGCGGGGCGAGGGGCGGGGCGGGGCGAGGCGGAGAGGTGCGGCGGCAGCCAATCAGAGCGG
CGCGCTCCGAAAAGTTTCTTTTTATGGCGAGGCGGCGGGCGGGCGGCCCTATAAAAAAGCGAAGCG
CGCGGCGGGGCGGGAGTCGCTGCGTTGCCCTTCGCCCCGTGCCCCGCTCCGCGCCGCTCGCGCCGC
CCGCCCCGGCTCTGACTGACCGCGTTACTCCACAGGTGAGCGGGCGGGACGGCCCTTCTCCTCC
GGGCTGTAATTAGCGCTTGGTTTAATGACGGCTTGTTTCTTTTCTGTGGCTGCGTGAAAGCCTTG
AGGGGCTCCGGGAGCTAGAGCCTCTGCTAACCATGTTTCATGCCTTCTTCTTTTTCTTACAGCTCC
TGGGCAACGTGCTGCTTATTGTGACCGGTGTTGCGGCCCCGCAGCGCCCGCGCGCTCCTCTCCCC
GACTCGGAGCCCCCTCGGCGGCGCCCCGGCCCCAGGACCCGCTAGGAGCGCAGGAGCCCCAGCGCAG
AGACCCCAACGCCGAGACCCCCGCCCCGGCCCCGCGGCTTCTCCCGACGCAGTTTAGGACCC
TTGTTTCGCGAAGAGGTGGTGTGCGGCTGAGACCCGCGTCCTCAGGACGGTTCCATCAGTGCCTCG
ATCCTGCCCCACTGGAGGAGGAAGGCAGCCCGAACAGCGCTCACCTAACTAACAGCTGCTGAGAG
CTGGGTTCGGTGGCCATGCACCTGGGACTGCCTTGAGAAGCGTGAGCAAACCGCCCAGAGTAGAA
GCGCTAGCCACCATGGATTGGGGCACGCTGCAGACGATCCTGGGGGGTGTGAACAAACACTCCAC
CAGCATTGGAAAGATCTGGCTCACCGTCTCTTCATTTTTTCGCATTATGATCCTCGTTGTGGCTG
CAAAGGAGGTGTGGGGAGATGAGCAGGCCGACTTTGTCTGCAACACCCTGCAGCCAGGCTGCAAG
AACGTGTGCTACGATCACTACTTCCCCATCTCCACATCCGGCTATGGGCCCTGCAGCTGATCTT
CGTGTCCACGCCAGCGCTCCTAGTGGCCATGCACGTGGCCTACCGGAGACATGAGAAGAAGAGGA
AGTTCATCAAGGGGGAGATAAAGAGTGAATTTAAGGACATCGAGGAGATCAAAACCCAGAAGGTC
CGCATCGAAGGCTCCCTGTGGTGGACCTACACAAGCAGCATCTTCTTCCGGGTTCATCTTCAAGC
CGCCTTCATGTACGTCTTCTATGTATGTACGACGGCTTCTCCATGCAGCGGCTGGTGAAGTGCA
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ACAGTGTTTCATGATTGCAGTGTCTGGAATTTGCATCCTGCTGAATGTCACTGAATTGTGTTATTT
GCTAATTAGATATTGTTCTGGGAAGTCAAAAAAGCCAGTTGGATCCCGGGCTGACTACAAAGACC
ATGACGGTGATTATAAAGATCATGACATCGACTACAAGGATGACGATGACAAGTAAGAAATAGAC
AGCATGAGAGGGATGAGGCAACCCGTGCTCAGCTGTCAAGGCTCAGTCGCTAGCATTTCCCAACA
CAAAGATTCTGACCTTAAATGCAACCATTTGAAACCCCTGTAGGCCCTCAGGTGAAACTCCAGATG
CCACAATGGAGCTCTGCTCCCCATAAGCCTCAAAACAAAGGCCCTAATTCATGCCTGTCTTAATT
TTCTTTCACTTAAGTTAGTTCCACTGAGACCCCAGGCTGTTAGGGGTATTGGTGTAAGGTACTT
TCATATTTTAAACAGAGGATATCGGCATTTGTTTCTTTCTCTGAGGACAAGAGAAAAAGCCAGG
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GTAAATGGTATGTCATTCGCTACTATGATTTAATTTGAAATATGGTCTTTTGGTTATGAATACTT
TGCAGCACAGCTGAGAGGCTGTCTGTTGTATTTCATTGTGGTCATAGCACCTAACCAACATTGTAGC
CTCAATCGAGTGAGACAGACTAGAAGTTCCTAGTGATGGCTTATGATAGCAAATGGCCTCATGTC
AAATATTTAGATGTAATTTTGTGTAAGAAATACAGACTGGATGTACCACCACTACTACCTGTAA
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GATTTAAAGAGGTCGCTTGGAATTTTATTGACACAGTACCATTTAATGGGGAGGACAAAATGGG
GCAGGGGAGGGAGAAGTTTCTGTCTGTTAAAAACAGATTTGGAAAAGACTGGACTCTAAAGTCTGTT
GATTAAAGATGAGCTTTGTCTACTTCAAAAGTTTGTGTTGCTTACCCCTTCAGCCTCCAATTTTTT
AAGTGAAAATATAGCTAATAACATGTGAAAAGAATAGAAGCTAAGGTTTAGATAAAATATTGAGCA
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TCCACATATTTCACTGAGGGTAAGTATTTTCTGTTGTCAAGAAATAGCATTGTAAAAGCATTTTG
TAATAATAAAGAAATAGCTTTAATGATATGCTTGTAAGTAAATAATTTTGTAAATGTATCAAATAC
ATTTAAACATTAAATATAATCTCTATAATAAGAGCTCGCTGATCAGCCTCGACTGTGCCCTTCT
AGTTGCCAGCCATCTGTTGTTTGCCCCCTCCCCCGTGCCTTCCTTGACCCTGGAAGGTGCCACTCC
CACTGTCCTTTCTAATAAAATGAGGAAATGTCATCGCATTGTCTGAGTAGGTGTCATTCTATTCT
TGGGGGTGGGGTGGGGCAGGACAGCAAGGGGAGGATTGGGAAGACAATAGCAGGCATGCTGGG
GATGCGGTGGGCTCTATGGAAGCTTGAATTCAGCTGACGTGCCTCGGACCGCTAGGAACCCCTAG
TGATGGAGTTGGCCACTCCCTCTCTGCGCGCTCGCTCGCTCACTGAGGCCGGGCGACCAAAGGTC
GCCCAGCGCCCGGGCTTTGCCCGGGCGGCCTCAGTGAGCGAGCGAGCGCGCAG

Exemplary Construct sequence (SEQ ID NO: 84)

CTGCGCGCTCGCTCGCTCACTGAGGCCGCCGGGCGTGGGCGACCTTTGGTTCGCCCGGCTCAG
TGAGCGAGCGAGCGCGCAGAGAGGGAGTGGCCAACCTCCATCACTAGGGGTTCTGCGGCCGCACG
CGTGACATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCCA
TATATGGAGTTCCGCGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCC
CCGCCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGAC
GTCAATGGGTGGACTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCA
AGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCCTGGCATTTATGCCAGTACATGAC
CTTATGGGACTTTCTTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGGTAAGC
TTCCGCAGAATCCTATCAGTTTCCCCCTTTCTGCTGTGTGTCATCGAGCAGGAAGGGGCTTGGCA
GGTTTTACCTGCCCTCTTTCCTTTCTGAAAAGTCTGGGCCTCCTCACCCCGAAAGGAGTCACCTC

CTTGACAGTTCCCCAGTTGCGAAAAGAGGAGGAAGTTGGCTGGGCGGGGGCCGCGGGGGGCACCC
TCCGCAGATGGCGGGACCCCCCTGCCGGCCATGGCAAAAACGAGGCTTGTCTCTCCACCGCCCC
CAACCTTAGTCCTTGGCACATTGTTGAAAGTAATTGAATAAAAATCGGAAATTCGAGAAGGCGTTC
GTTTCGGATTGGTGAGATTTTGAGGGGAGAAAGAAGCGGGGACTTCGCCGGCACACGCGGCGCCCC
CTCCTCGGCCACCGTTAACCCCCATTCCAGAGGGCACTGCCCCGCCACCCAGCCTAGGTCCCCCT
GCGAGAGCCTCGCGGGCCCGCGCAGCCTCCGCGACTCGAACAGATCTTCAGTCCTTGGAGGAATG
CCTGTTTTCTCTAACAAATAAAAAATTAAAGAAGCGCTCATAAATGCCAAGTCCTCTCGCACTATGC
GGAGTACAGAGGACAACGACCACAGCCATCCCTGAACCCCGCCACGGCACAGCGCCGGAGCCGG
GGTCTGGGGCGCCGCTTCCTGGGGGGTCCCGACTCTCAGCCGCCCCCGCTTACCCGGGGCCGCCA
AGGGGCTGGGGGAGGCGGCGCTCGGGGTAACCGGGGAGACTCAGGGCGCTGGGGGCACTTGGGG
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ACCCGCGTCTCAGGACGGTTCATCAGTGCCTCGATCCTGCCCCACTGGAGGAGGAAGGCAGCC
CGAACAGCGCTCACCTAACTAACAGCTGCTGAGAGCTGGGTTCGTGGCCATGCACCTGGGACTG
CCTTGAGAAGCGTGAGCAAACCGCCAGAGTAGAAGCGCTAGCCACCATGGATTGGGGCACGCTG
CAGACGATCCTGGGGGGTGTGAACAAACACTCCACCAGCATTGGAAAGATCTGGCTCACCGTCCT
CTTCATTTTTTCGCATTATGATCCTCGTTGTGGCTGCAAAGGAGGTGTGGGGAGATGAGCAGGCCG
ACTTTGTCTGCAACACCCTGCAGCCAGGCTGCAAGAACGTGTGCTACGATCACTACTTCCCCATC
TCCCACATCCGGCTATGGGCCCTGCAGCTGATCTTCGTGTCCACGCCAGCGCTCCTAGTGGCCAT
GCACGTGGCCTACCGGAGACATGAGAAGAAGAGGAAGTTCATCAAGGGGGAGATAAAGAGTGAAT
TTAAGGACATCGAGGAGATCAAAACCCAGAAGGTCCGCATCGAAGGCTCCCTGTGGTGGACCTAC
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AAAGCCAGTTGGATCCCGGGCTGACTACAAAGACCATGACGGTGATTATAAAGATCATGACATCG
ACTACAAGGATGACGATGACAAGTAAGAAATAGACAGCATGAGAGGGATGAGGCAACCCGTGCTC
AGCTGTCAAGGCTCAGTCGCTAGCATTTCCCAACACAAAGATTCTGACCTTAAATGCAACCATTT
GAAACCCCTGTAGGCCTCAGGTGAAACTCCAGATGCCACAATGGAGCTCTGCTCCCCCTAAAGCCT

CAAAACAAAGGCCTAATTCTATGCCTGTCTTAATTTTCTTTCACTTAAGTTAGTTCCACTGAGAC
CCCAGGCTGTTAGGGGTTATTGGTGTAAGGTACTTTTCATATTTTAAACAGAGGATATCGGCATTT
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GTCCTCCTGGGGTTCTTTTTGCCAACTTTCCCCACGTTAAAGGTGAACATTGGTTCTTTCATTTG
CTTTGGAAGTTTAAATCTCTAACAGTGACAAAGTTACCAGTGCCTTAAACTCTGTTACACTTTT
TGGAAGTGAAAACTTTGTAGTATGATAGGTTATTTTGATGTAAAGATGTTCTGGATACCATTATA
TGTTCCCCCTGTTTCAGAGGCTCAGATTGTAATATGTAAATGGTATGTCATTGCTACTATGATT
TAATTTGAAATATGGTCTTTTGGTTATGAATACTTTGCAGCACAGCTGAGAGGCTGTCTGTTGTA
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AACAGATTTGGAAAGACTGGACTCTAAAGTCTGTTGATTAAAGATGAGCTTTGTCTACTTCAAAA
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CCATTATGCTTGACATGGTTTCCAAAAAATGGTACTCCACATATTTTCAGTGAGGGTAAGTATTTT
CCTGTTGTCAAGAATAGCATTGTAAAAGCATTTTGTAAATAATAAAGAATAGCTTTAATGATATGC
TTGTAACTAAAAATAATTTTGTAAATGTATCAAATACATTTAAAAACATTAAAATATAATCTCTATAA
TAAGAGCTCGCTGATCAGCCTCGACTGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGCCCCCTCC
CCCGTGCCTTCTTGACCCCTGGAAGGTGCCACTCCCCTGTCTTTCCTAATAAAAAATGAGGAAAT
TGCATCGCATTGTCTGAGTAGGTGTCATTCTATTCTGGGGGGTGGGGTGGGGCAGGACAGCAAGG
GGGAGGATTGGGAAGACAATAGCAGGCATGCTGGGGATGCGGTGGGCTCTATGGAAGCTTGAATT
CAGCTGACGTGCCTCGGACCGCTAGGAACCCCTAGTGATGGAGTTGGCCACTCCCTCTCTGCGCG
CTCGCTCGCTCACTGAGGCCGGGCGACCAAAGGTGCCCCGACGCCCGGGCTTTGCCCGGGCGGCC
TCAGTGAGCGAGCGAGCGCGCAG

Exemplary Construct sequence (SEQ ID NO: 87)

CTGCGCGCTCGCTCGCTCACTGAGGCCGCCGGGCGTGGGCGACCTTTGGTTCGCCCGGCCTCAG
TGAGCGAGCGAGCGCGCAGAGAGGGAGTGCCAACTCCATCACTAGGGGTTCTGCGGCCGCACG
CGTGACATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCCA
TATATGGAGTTCCGCGTTACATAACTTACGGTAAATGGCCCCCTGGCTGACCGCCCAACGACCC
CCGCCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGAC
GTCAATGGGTGGACTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCA

AGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCCCTGGCATTATGCCCAGTACATGAC
CTTATGGGACTTTCTTACTTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGGTCGAG
GTGAGCCCCACGTTCTGCTTCACTCTCCCCATCTCCCCCCCCCTCCCCACCCCCAATTTTGTATTT
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CGCGCTCCGAAAAGTTTCTTTTTATGGCGAGGCGGCGGGCGGGCGGCCCTATAAAAAAGCGAAGCG
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CGCGGCGGTGCGGCTGTAACCCCCCTGCACCCCCCTCCCCGAGTTGCTGAGCACGGCCCGGCT
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GGTGGGGGTGCCGGGCGGGGCGGGGCCGCTCGGGCCGGGGAGGGCTCGGGGGAGGGGCGCGGCG
GCCCCCGAGCGCCGGCGGCTGTGAGGCGCGGCGAGCCGAGCCATTGCCTTTTATGGTAATCG
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AGCGCTCCTAGTGGCCATGCACGTGGCTACCGGAGACATGAGAAGAAGAGGAAGTTCATCAAGG
GGGAGATAAAGAGTGAATTTAAGGACATCGAGGAGATCAAAACCCAGAAGGTCCGCATCGAAGGC

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TTGTTCTGGGAAGTCAAAAAAGCCAGTTGGATCCCGGGCTGACTACAAAGACCATGACGGTGATT
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ATTGCCAAACTCGAGCAGTGAATTCTACCAGTGCCATAGGATCCAGTGAGTTCTACCATTGCC
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CCAACACAAAGATTCTGACCTTAAATGCAACCATTTGAAACCCCTGTAGGCCTCAGGTGAACTC
CAGATGCCACAATGGAGCCTCTGCTCCCCCTAAAGCCTCAAAACAAAGGCCTAATTCTATGCCTGT
CTTAATTTTCTTTCACTTAAGTTAGTTCCACTGAGACCCAGGCTGTTAGGGGTTATTGGTGTA
GGTACTTTTCATATTTTAAACAGAGGATATCGGCATTTGTTTCTTTCTCTGAGGACAAGAGAAAA
AGCCAGGTTCCACAGAGGACACAGAGAAGGTTTGGGTGTCTCCTGGGGTTCTTTTTGCCAACTT
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ACAAAGTTACCAGTGCCTTAACTCTGTTACACTTTTTGGAAGTGAAAACCTTTGTAGTATGATAG
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AATACTTTGCAGCACAGCTGAGAGGCTGTCTGTTGTATTCAATTGTGGTCATAGCACCTAACACA
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GAACGCTGATTTAAAGAGGTGCTTGGGAATTTTATTGACACAGTACCATTTAATGGGGAGGACA
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ACTGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGGCCCTCCCCGTGCCTTCTTTGACCCTGGA
AGGTGCCACTCCCCTGTCTTTTCTAATAAAATGAGGAAATGTCATCGCATTGTCTGAGTAGGT
GTCATTCTATTCTGGGGGGTGGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAGACAATAGC
AGGCATGCTGGGGATGCGGTGGGCTCTATGGAAGCTTGAATTCAGCTGACGTGCCTCGGACCGCT
AGGAACCCCTAGTGATGGAGTTGGCCACTCCCTCTCTGCGCGCTCGCTCGCTCACTGAGGCCGGG
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Exemplary Construct sequence (SEQ ID NO: 61)

CCTGCAGGCAGCTGCGCGCTCGCTCGCTCACTGAGGCCGCCGGGCGTCGGGCGACC
TTTGGTCGCCCCGGCCTCAGTGAGCGAGCGAGCGCGCAGAGAGGGAGTGGCCAACTC
CATCACTAGGGGTTCCTGCGGGCCGCACGCGTGGTCCACAGGTAACCTCCGTCGGCGTC
CACAGGGGGGCAGGAGATACCATACTGCACAGTTGTACGTCTTCCATCTGTTTGGTG
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GCTACGCCGCATCGCCTTTCAGCGTGAGGCCAGGAATGAGCGAGGCAGTGGGCG
GGGAAGACAGGCACGGGGAATCTGGGGACAGATAAAGGAACTCGTGATGGGGCG
AGGCTGGGCTGAAGAGAAACAGATTGGGGTAGAGCTGCAAAGGGAGGGGTCCACTG
GAAGGCGAGGGGGGAGGCCGGGAAGAGAGAGGGTGGGAAGGCAGTGTGAGATGGG
AGGGCAGTGTGAGAAGAAAAGCAGGCTGGGGAAAGAGGGATTGGAATGCAGAAGGA
ACTTGGGGAAGGAGGAAGTCCTGCAGGCGGGAGGGAAAGAAGAGAGGGGGAGCAG
CTAAAGTCTGCGTCAGAAGAGGTTGGGGACTGCGAGAGGAGAGGCTGGGGCCTGCA
GGGGAGCGCAGCAGCTTTTAGCATCGATCCAACTCTAAAGACTCGTGGCCTTTGCC
TGACCTCGAGGGTCGGGAATAGACGCCTGTCTTTGTGGAGAGCGATACCCAACCGA
GAAAATGGGGCTGTTCCGAGCTGGGCCCTGCGCCTGGCCCAGGGCGAGGCTTCTCTG
GCTCCGGGCTGGCCCCTGAGGGGCAGCACGCAGCCTGCAGCAGAGGCGCCTGCTCC
AAGCTGTCTCTTGGGGGCGCCGCCGCCGCTTCCCTCCTCCGGGGCCGCTCGCTCCCA
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CAACCCCGCGCGCGTCCCGCGGGGGCGCTGCGTCTTCTGCCACACCGGCGCACCGC
GGCCCCTCTCCCCACACCTCCGGCCCGCACCAACCGGCTCTCCTCCACCTCCCCA
CCCCCTCTGCCCCTCCCTCCCCATTCTCCCTCCCGGCGAGGGGCGGGAGGGGGC
GTGGCGGGGCCGGGTTTGTGTGGCTGGGACCCGGCTCCTCAAGCTCTGAGGACCCA
GAGGCCGGGCGCGCTCCGCCCCGCGCGCCGCCCCCTCCGTAACCTTCCAGTCTCCG
AGGGAAGAGGCGGGGTGTGGGGTGCGGTTAAAAGGCGCCACGGCGGGAGACAGGT
CTACCGGTGTGTCACCGTTGCGGCCCCGCAGCGCCCGCGCGCTCCTCTCCCCGACT
CGGAGCCCCCTCGGCGGCGCCCGGCCAGGACCCGCCTAGGAGCGCAGGAGCCCCAG
CGCAGAGACCCCAACGCCGAGACCCCGCCCCGGCCCCGCGCGCTTCTCCCGACG
CAGTTTAGGACCCTTGTTTCGCGAAGAGGTGGTGTGCGGCTGAGACCCGCGTCTCAG
GACGGTTCCATCAGTGCCCTCGATCCTGCCCCACTGGAGGAGGAAGGCAGCCCGAAC
AGCGCTCACCTAACTAACAGCTGCTGAGAGCTGGGTTCCGTGGCCATGCACCTGGGA

CTGCCTTGAGAAGCGTGAGCAAACCGCCCAGAGTAGAAGCGCTAGCCACCATGGAT
TGGGGCACGCTGCAGACGATCCTGGGGGGTGTGAACAAACACTCCACCAGCATTGG
AAAGATCTGGCTCACCGTCCTCTTCATTTTTTCGATTATGATCCTCGTTGTGGCTGCA
AAGGAGGTGTGGGGAGATGAGCAGGCCGACTTTGTCTGCAACACCCTGCAGCCAGG
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CCTGCAGCTGATCTTCGTGTCCACGCCAGCGCTCCTAGTGGCCATGCACGTGGCCTA
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CCATTTGAAACCCCTGTAGGCCTCAGGTGAAACTCCAGATGCCACAATGGAGCTCTG
CTCCCCTAAAGCCTCAAAACAAAGGCCTAATTCTATGCCTGTCTTAATTTTCTTTAC
TTAAGTTAGTTCCACTGAGACCCCAGGCTGTTAGGGGTTATTGGTGTAAGGTACTTTC
ATATTTTAAACAGAGGATATCGGCATTTGTTTCTTTCTCTGAGGACAAGAGAAAAAA
GCCAGGTTCCACAGAGGACACAGAGAAGGTTTGGGTGTCCTCCTGGGGTTCTTTTTG
CCAACTTTCCCCACGTAAAGGTGAACATTGGTTCTTTTCATTTGCTTTGGAAGTTTAA
ATCTCTAACAGTGGACAAAGTTACCAGTGCCTTAAACTCTGTTACACTTTTTGGAAGT
GAAAACTTTGTAGTATGATAGGTTATTTTGATGTAAAGATGTTCTGGATACCATTATA
TGTTCCCCCTGTTTCAGAGGCTCAGATTGTAATATGTAAATGGTATGTCATTCGCTAC
TATGATTTAATTTGAAATATGGTCTTTTGGTTATGAATACTTTGCAGCACAGCTGAGA
GGCTGTCTGTTGTATTTCATTGTGGTCATAGCACCTAACAACATTGTAGCCTCAATCGA
GTGAGACAGACTAGAAGTTCCTAGTGATGGCTTATGATAGCAAATGGCCTCATGTCA
AATATTTAGATGTAATTTTGTGTAAGAAATACAGACTGGATGTACCACCAACTACTA
CCTGTAATGACAGGCCTGTCCAACACATCTCCCTTTTCCATGACTGTGGTAGCCAGC
ATCGGAAAGAACGCTGATTTAAAGAGGTCGCTTGGGAATTTTATTGACACAGTACCA
TTTAATGGGGAGGACAAAATGGGGCAGGGGAGGGAGAAGTTTCTGTGCTTAAAAAC
AGATTTGGAAAGACTGGACTCTAAAGTCTGTTGATTAAAGATGAGCTTTGTCTACTT

CAAAAGTTTGTTTGCTTACCCCTTCAGCCTCCAATTTTTTAAGTGAAAATATAGCTAA
TAACATGTGAAAAGAATAGAAGCTAAGGTTTAGATAAAATATTGAGCAGATCTATAG
GAAGATTGAACCTGAATATTGCCATTATGCTTGACATGGTTTCCAAAAAATGGTACT
CCACATATTTTCAGTGAGGGTAAGTATTTTCCTGTTGTCAAGAATAGCATTGTAAAAG
CATTTTGTAATAATAAAGAATAGCTTTAATGATATGCTTGTAATAAAATAATTTTGT
AATGTATCAAATACATTTAAAACATTAAAATATAATCTCTATAATAAGAGCTCGCTG
ATCAGCCTCGACTGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGCCCCCTCCCCGTG
CCTTCCTTGACCCTGGAAGGTGCCACTCCCCTGTCTTTTCTAATAAAAATGAGGAA
ATTGCATCGCATTGTCTGAGTAGGTGTCATTCTATTCTGGGGGGTGGGGTGGGGCAG
GACAGCAAGGGGGAGGATTGGGAAGACAATAGCAGGCATGCTGGGGATGCGGTGG
GCTCTATGGAAGCTTGAATTCAGCTGACGTGCCTCGGACCGCTAGGAACCCCTAGTG
ATGGAGTTGGCCACTCCCTCTCTGCGCGCTCGCTCGCTCACTGAGGCCGGGCGACCA
AAGGTCGCCCCGACGCCCCGGGCTTTGCCCCGGGCGGCCTCAGTGAGCGAGCGAGCGCG
CAGCTGCCTGCAGGGGGCGCCTGATGCGGTATTTTCTCCTTACGCATCTGTGCGGTATT
TCACACCGCATACGTCAAAGCAACCATAGTACGCGCCCTGTAGCGGCGCATTAAAGCG
CGGCGGGTGTGGTGGTTACGCGCAGCGTGACCGCTACACTTGCCAGCGCCCTAGCGC
CCGCTCCTTTTCGCTTTCTTCCCTTCCTTTCTCGCCACGTTTCGCCGGCTTTCCCCGTCAA
GCTCTAAATCGGGGGCTCCCTTTAGGGTTCCGATTTAGTGCTTTACGGCACCTCGACC
CCAAAAAATTGATTTGGGTGATGGTTCACGTAGTGGGCCATCGCCCTGATAGACGG
TTTTTCGCCCTTTGACGTTGGAGTCCACGTTCTTTAATAGTGGACTCTTGTTCCAAAC
TGGAACAACACTCAACCCTATCTCGGGCTATTCTTTTGATTTATAAGGGATTTTGCCG
ATTTTCGGCCTATTGGTTAAAAAATGAGCTGATTTAACAATAAATTTAACGCGAATTTT
AACAAAATATTAACGTTTACAATTTTATGGTGCCTCTCAGTACAATCTGCTCTGATG
CCGCATAGTTAAGCCAGCCCCGACACCCGCCAACACCCGCTGACGCGCCCTGACGG
GCTTGTCTGCTCCCGGCATCCGCTTACAGACAAGCTGTGACCGTCTCCGGGAGCTGC
ATGTGTCAGAGGTTTTACCGTTCATACCGAAACGCGCGAGACGAAAGGGCCTCGTG
ATACGCCTATTTTTATAGGTTAATGTCATGAACAATAAACTGTCTGCTTACATAAAC
AGTAATACAAGGGGTGTTATGAGCCATATTCAACGGGAAACGTCGAGGCCGCGATT
AAATTCCAACATGGATGCTGATTTATATGGGTATAAATGGGCTCGCGATAATGTCGG
GCAATCAGGTGCGACAATCTATCGCTTGTATGGGAAGCCCGATGCGCCAGAGTTGTT
TCTGAAACATGGCAAAGGTAGCGTTGCCAATGATGTTACAGATGAGATGGTCAGACT
AAACTGGCTGACGGAATTTATGCCTCTTCCGACCATCAAGCATTTTATCCGTACTCCT
GATGATGCATGGTTACTCACCCTGCGATCCCCGAAAAACAGCATTCAGGTATTA

GAAGAATATCCTGATTCAGGTGAAAATATTGTTGATGCGCTGGCAGTGTTCCCTGCGC
 CGGTTGCATTCGATTCCTGTTTGTAAATTGTCCTTTTAACAGCGATCGCGTATTTTCGTCT
 CGCTCAGGCGCAATCACGAATGAATAACGGTTTGGTTGATGCGAGTGATTTTGATGA
 CGAGCGTAATGGCTGGCCTGTTGAACAAGTCTGGAAAGAAATGCATAAACTTTTGCC
 ATTCTCACCGGATTCAGTCGTCACCTCATGGTGATTTCTCACTTGATAACCTTATTTTT
 GACGAGGGGAAATTAATAGGTTGTATTGATGTTGGACGAGTCGGAATCGCAGACCG
 ATACCAGGATCTTGCCATCCTATGGAAGTGCCTCGGTGAGTTTTCTCCTTCATTACAG
 AAACGGCTTTTTCAAAAATATGGTATTGATAATCCTGATATGAATAAATTGCAGTTT
 CATTTGATGCTCGATGAGTTTTTCTAATCTCATGACCAAATCCCTTAACGTGAGTTT
 TCGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCT
 TTTTTTCTGCGCGTAATCTGCTGCTTGCAAACAAAAAACCACCGCTACCAGCGGTG
 GTTTGTTTGCCGGATCAAGAGCTACCAACTCTTTTTCCGAAGGTAAGTGGCTTCAGCA
 GAGCGCAGATACCAAATACTGTCCTTCTAGTGATAGCCGTAGTTAGGCCACCACTTCA
 AGAACTCTGTAGCACCGCCTACATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGC
 TGCCAGTGGCGATAAGTCGTGTCTTACCGGGTTGGACTCAAGACGATAGTTACCGGA
 TAAGGCGCAGCGGTGCGGCTGAACGGGGGGTTCGTGCACACAGCCAGCTTGGAGC
 GAACGACCTACACCGAACTGAGATACCTACAGCGTGAGCTATGAGAAAGCGCCACG
 CTTCCCGAAGGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTCGGAACAGG
 AGAGCGCACGAGGGAGCTTCCAGGGGGAAACGCCTGGTATCTTTATAGTCCTGTGCG
 GTTTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGATGCTCGTCAGGGGGGCGGAG
 CCTATGGAAAAACGCCAGCAACGCGGCCTTTTTACGGTTCCTGGCCTTTTGCTGGCCT
 TTTGCTCACATGT

Table 4: Components of Construct Sequence (SEQ ID NO: 61)

Components	Position in construct
5'ITR	12-130
Cloning site	131-147
GDF6 promoter	148-1335
hGJB2 minimal promoter	1336-1463
Cloning site	1464-1472
Synthetic barcode	1473-1480
5'UTR	1481-1842

GJB2 (exon2)	1854-2531
3xFLAG	2544-2609
3'UTR (exon2)	2613-4019
bGHPA	4041-4265
Cloning site	4266-4299
3'ITR	4300-4429

Exemplary Construct sequence (SEQ ID NO: 54)

CCTGCAGGCAGCTGCGCGCTCGCTCGCTCACTGAGGCCGCCGGGCGTCGGGCGACC
 TTTGGTCGCCCCGGCCTCAGTGAGCGAGCGAGCGCGCAGAGAGGGAGTGGCCAACTC
 CATCACTAGGGGTTTCCTGCGGCCGCACGCGTGGTAAGAACTTGCCCGAGTTTACAC
 AGCTAGTAAATGGTTGCATTAGTCAGGACAGCTAGCCTATATTACAATAACAACCTT
 CTCAAATCCTAATGGCTTAAAACAACAGAGGTTTAATTTATACTCATTAGCTGTTCA
 AGGCAGGAGGCTCTATTCTCTAATCCATACAGTCACTCAGGATCCAGGCTGGTGGAG
 ACCCTGCCATATTGTAGCCTCACCATTAAAACATGAAGAAGATAGAAAGTGAGGA
 GTCATGTAGGTTTTTGTTCGTTGCCTCAGGCTAGGAGTGACAGGTCACCTCATCTCAC
 TCACAGCTCACTGCCCACAACCTAGTCACTTGTGACTGTGCGAGTTAAGCTTCTGTGTG
 TGAAGGAAGGAAAAGAGAATGGGATAAAGGTGAACATCAGCAGGCTCTACCACAGT
 AGTTTGAACCAAGACTTGAGCCTAGGTCATGTGGCTTCAGAATCTTTGCTCTTAATCA
 CACTAAACAGCCTCTGTAAAGTCATCTTTCCCTCATCCAGTGCCTAAGAACATGCAGTC
 CAATGCCCTCATCCTTCAGAAGAATTGAGTGAACTCAGAGAAATTGAGTAGAGTGC
 CACAGCATGCCCAAGGCCACACACCCTGAGGTTGGCAGTAGGTCCTGAGTTAGAGTT
 GTCATTTCTTGGCTCCCCTGGTAGTAGTGGAAGGTAAGGTTTTGACATACTAGTTG
 GATGACCACGGGCAGGTCACCTAAATTGTCTAAGCATCGTTTGACCCTTGTAAGAAT
 TAAATGAAATAGCACCTGTAAAAGTGTCTGCACGGACTTACTGCTGTTAGTTTTGTTT
 CTTTCTTCCTGTTGTCACTGCACTCCCTGCCTGTTACCCAGGCCATGCAGACCAGCC
 AGGCCTTCGACTTACAGTGCGGATAAGATTCCAAATCTCCACGGCTGGTTTTCCATGC
 TTTCTTCCAGGCTTCTGAGGACCCTGTGCTCTGGTTTTCTTCTATTTCTTTTCTATTACT
 TTTCTGTTACTCTTGAGCACACTTGCTGGAAGCAATATGCATCCAGTTCTCCCTCTCT

TGCCTCATTACACTTTGCAGAACAACTCCAATCCCTTCCAACCAAGTAGTCCCTTTGA
ATTTCTTGTACCCCAAGGAATCTCTCTGACAGGGGTCTTTGTTAGGGTCACACCCAG
GAGATGGTTGATTATGGCTGAGTCCAGCCTGGAATGATGGGGGTGGGGGCAGCTTG
GGTAGATGACTCAGTAAATCAAACAGAACAATGAAAGGAGGTCATGCTTGTCCATC
TGCATTATTGAAGACAGCCATAAATGGCCTTACCCAGAGCGGGTCTGTCACACCTG
GAGAGCTGATCTGACCTCTCCAAGACCCCTGCAACTGAGTGTTCTGGGATCTGTCCT
GCAACAAGTGCCTCGAGATTTGTAGGTGGGGGCCAGAGGGAGGGGGTCTGCAGAC
GAAGGGGGCAGGTTTTGCGGGGCACTTAGGGTTCTCATAGGTTGTAGTCACGAGCTC
CAAGCTCTGAGGACCCAGAGGCCGGGCGCGCTCCGCCGCGGCGCCGCCCTCCGT
AACTTTCCAGTCTCCGAGGGAAGAGGCGGGGTGTGGGGTGCGGTTAAAAGGCGCC
ACGGCGGGAGACAGGTCTCACCGGTCACAACCTGTTGCGGCCCCGACGCGCCGCG
CGCTCCTCTCCCCGACTCGGAGCCCCTCGGCGGCGCCCGGCCAGGACCCGCCTAGG
AGCGCAGGAGCCCCAGCGCAGAGACCCCAACGCCGAGACCCCGCCCCGGCCCCGC
CGCGCTTCTCCCGACGCAGTTTAGGACCTTGTTGCGGAAGAGGTGGTGTGCGGCT
GAGACCCGCGTCCTCAGGACGGTTCATCAGTGCCTCGATCCTGCCCCACTGGAGGA
GGAAGGCAGCCGAACAGCGCTCACCTAACTAACAGCTGCTGAGAGCTGGGTCCG
TGGCCATGCACCTGGGACTGCCTTGAGAAGCGTGAGCAAACCGCCCAGAGTAGAAG
CGCTAGCCACCATGGATTGGGGCACGCTGCAGACGATCCTGGGGGTGTGAACAAA
CACTCCACCAGCATTGGAAAGATCTGGCTCACCGTCCTCTTCATTTTTTCGATTATGA
TCCTCGTTGTGGCTGCAAAGGAGGTGTGGGGAGATGAGCAGGCCGACTTTGTCTGCA
ACACCCTGCAGCCAGGCTGCAAGAACGTGTGCTACGATCACTACTTCCCCATCTCCC
ACATCCGGCTATGGGCCCTGCAGCTGATCTTCGTGTCCACGCCAGCGCTCCTAGTGG
CCATGCACGTGGCCTACCGGAGACATGAGAAGAAGAGGAAGTTCATCAAGGGGGAG
ATAAAGAGTGAATTTAAGGACATCGAGGAGATCAAAACCCAGAAGGTCCGCATCGA
AGGCTCCCTGTGGTGGACCTACACAAGCAGCATCTTCTTCCGGGTCATCTTCGAAGC
CGCCTTCATGTACGTCTTCTATGTCATGTACGACGGCTTCTCCATGCAGCGGCTGGTG
AAGTGCAACGCCTGGCCTTGTCCCAACACTGTGGACTGCTTTGTGTCCCGGCCACG
GAGAAGACTGTCTTCACAGTGTTTCATGATTGCAGTGTCTGGAATTTGCATCCTGCTG
AATGTCACTGAATTGTGTTATTTGCTAATTAGATATTGTTCTGGGAAGTCAAAAAAG
CCAGTTGGATCCCGGGCTGACTACAAAGACCATGACGGTGATTATAAAGATCATGAC
ATCGACTACAAGGATGACGATGACAAGTAAGAAATAGACAGCATGAGAGGGATGA
GGCAACCCGTGCTCAGCTGTCAAGGCTCAGTCGCTAGCATTTCCCAACACAAAGATT
CTGACCTTAAATGCAACCATTTGAAACCCCTGTAGGCCTCAGGTGAAACTCCAGATG

CCACAATGGAGCTCTGCTCCCCTAAAGCCTCAAAACAAAGGCCTAATTCTATGCCTG
TCTTAATTTTCTTTCACTTAAGTTAGTTCCACTGAGACCCCAGGCTGTTAGGGGTAT
TGGTGTAAGGTACTTTCATATTTTAAACAGAGGATATCGGCATTTGTTTCTTTCTCTG
AGGACAAGAGAAAAAAGCCAGGTTCCACAGAGGACACAGAGAAGGTTTGGGTGTCC
TCCTGGGGTTCTTTTTGCCAACTTTCCCCACGTAAAGGTGAACATTGGTTCTTTCAT
TTGCTTTGGAAGTTTTAATCTCTAACAGTGGACAAAGTTACCAGTGCCTTAAACTCTG
TTACACTTTTTGGAAGTGAAAACCTTTGTAGTATGATAGGTTATTTTGATGTAAAGATG
TTCTGGATAACCATTATATGTTCCCCCTGTTTCAGAGGCTCAGATTGTAATATGTAAAT
GGTATGTCATTCGCTACTATGATTTAATTTGAAATATGGTCTTTTGGTTATGAATACT
TTGCAGCACAGCTGAGAGGCTGTCTGTTGTATTTCATTGTGGTCATAGCACCTAACAA
CATTGTAGCCTCAATCGAGTGAGACAGACTAGAAGTTCCTAGTGATGGCTTATGATA
GCAAATGGCCTCATGTCAAATATTTAGATGTAATTTTGTGTAAGAAATACAGACTGG
ATGTACCACCAACTACTACCTGTAATGACAGGCCTGTCCAACACATCTCCCTTTTCCA
TGACTGTGGTAGCCAGCATCGGAAAGAACGCTGATTTAAAGAGGTCGCTTGGGAATT
TTATTGACACAGTACCATTTAATGGGGAGGACAAAATGGGGCAGGGGAGGGAGAAG
TTTCTGTCGTTAAAAACAGATTTGGAAAGACTGGACTCTAAAGTCTGTTGATTAAAG
ATGAGCTTTGTCTACTTCAAAAGTTTGTGTTGCTTACCCCTTCAGCCTCCAATTTTTTAA
GTGAAAATATAGCTAATAACATGTGAAAAGAATAGAAGCTAAGGTTTAGATAAATA
TTGAGCAGATCTATAGGAAGATTGAACCTGAATATTGCCATTATGCTTGACATGGTT
TCCAAAAAATGGTACTCCACATATTTTCAGTGAGGGTAAGTATTTTCCTGTTGTCAAG
AATAGCATTGTAAAAGCATTTTGTAAATAATAAAGAATAGCTTTAATGATATGCTTGT
AACTAAAATAATTTTGTAAATGTATCAAATACATTTAAAACATTAAAATATAATCTCT
ATAATAAGAGCTCGCTGATCAGCCTCGACTGTGCCTTCTAGTTGCCAGCCATCTGTTG
TTTGCCCTCCCCCGTGCCTTCCTTGACCCTGGAAGGTGCCACTCCCCTGTCTTTT
CTAATAAAATGAGGAAATTGCATCGCATTGTCTGAGTAGGTGTCATTCTATTCTGGG
GGGTGGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAGACAATAGCAGGCAT
GCTGGGGATGCGGTGGGCTCTATGGAAGCTTGAATTCAGCTGACGTGCCTCGGACCG
CTAGGAACCCCTAGTGATGGAGTTGGCCACTCCCTCTCTGCGCGCTCGCTCGCTCACT
GAGGCCGGGCGACCAAAGGTCGCCCAGCGCCCGGGCTTTGCCCGGGCGGCCTCAGT
GAGCGAGCGAGCGCGCAGCTGCCTGCAGGGGCGCCTGATGCGGTATTTTCTCCTTAC
GCATCTGTGCGGTATTTACACCGCATACGTCAAAGCAACCATAGTACGCGCCCTGT
AGCGGCGCATTAAGCGCGGCGGGTGTGGTGGTTACGCGCAGCGTGACCGCTACACTT
GCCAGCGCCCTAGCGCCCGCTCCTTTTCGCTTTCTTCCCTTCCTTTCTCGCCACGTTTCG

CGGCTTTCCCCGTCAAGCTCTAAATCGGGGGCTCCCTTTAGGGTTCCGATTTAGTGCT
TTACGGCACCTCGACCCCAAAAACTTGATTTGGGTGATGGTTCACGTAGTGGGCCA
TCGCCCTGATAGACGGTTTTTCGCCCTTTGACGTTGGAGTCCACGTTCTTTAATAGTG
GACTCTTGTTCCAACTGGAACAACACTCAACCCTATCTCGGGCTATTCTTTTGATTT
ATAAGGGATTTTGCCGATTTTCGGCCTATTGGTTAAAAAATGAGCTGATTTAACAAAA
ATTTAACGCGAATTTTAACAAAATATTAACGTTTACAATTTTATGGTGCCTCTCAGT
ACAATCTGCTCTGATGCCGCATAGTTAAGCCAGCCCCGACACCCGCCAACACCCGCT
GACGCGCCCTGACGGGCTTGTCTGCTCCCGGCATCCGCTTACAGACAAGCTGTGACC
GTCTCCGGGAGCTGCATGTGTCAGAGGTTTTACCGTTCATACCGAAACGCGCGAGA
CGAAAGGGCCTCGTGATACGCCTATTTTTATAGGTAAATGTCATGAACAATAAACT
GTCTGCTTACATAAACAGTAATACAAGGGGTGTTATGAGCCATATTCAACGGGAAAC
GTCGAGGCCGCGATTAAATTCCAACATGGATGCTGATTTATATGGGTATAAATGGGC
TCGCGATAATGTCGGGCAATCAGGTGCGACAATCTATCGCTTGTATGGGAAGCCCGA
TGCGCCAGAGTTGTTTCTGAAACATGGCAAAGGTAGCGTTGCCAATGATGTTACAGA
TGAGATGGTCAGACTAACTGGCTGACGGAATTTATGCCTCTTCCGACCATCAAGCA
TTTTATCCGTACTCCTGATGATGCATGGTTACTCACCCTGCGATCCCCGGAAAAAC
AGCATTCCAGGTATTAGAAGAATATCCTGATTCAGGTGAAAATATTGTTGATGCGCT
GGCAGTGTTCTGCGCCGGTTGCATTCGATTCCTGTTTGTAAATTGTCCTTTTAACAGC
GATCGCGTATTTTCGTCTCGCTCAGGCGCAATCACGAATGAATAACGGTTTGGTTGAT
GCGAGTGATTTTGATGACGAGCGTAATGGCTGGCCTGTTGAACAAGTCTGGAAAGA
AATGCATAAACTTTTGCCATTCTCACCAGGATTCAGTCGTCCTCATGGTGATTTCTCA
CTTGATAACCTTATTTTTGACGAGGGGAAATTAATAGGTGTTGATTGATGTTGGACGA
GTCGGAATCGCAGACCGATACCAGGATCTTGCCATCCTATGGAACTGCCTCGGTGAG
TTTTCTCCTTCATTACAGAAACGGCTTTTTTCAAAAATATGGTATTGATAATCCTGATA
TGAATAAATTGCAGTTTCATTTGATGCTCGATGAGTTTTTCTAATCTCATGACCAAAA
TCCCTTAACGTGAGTTTTTCGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAG
GATCTTCTTGAGATCCTTTTTTTCTGCGCGTAATCTGCTGCTTGCAAACAAAAAACC
ACCGCTACCAGCGGTGGTTTGTGTTGCCGGATCAAGAGCTACCAACTCTTTTTCCGAA
GGTAACTGGCTTCAGCAGAGCGCAGATACCAAATACTGTCCTTCTAGTGTAGCCGTA
GTTAGGCCACCACTTCAAGAACTCTGTAGCACCGCCTACATACCTCGCTCTGCTAAT
CCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCGTGTCTTACCGGGTTGGACTC
AAGACGATAGTTACCGGATAAGGCGCAGCGGTCGGGCTGAACGGGGGGTTCTGTGA
CACAGCCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCTACAGCGTGAG

CTATGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGGCGGACAGGTATCCGGTAAG
 CGGCAGGGTCGGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGGAAACGCCTGG
 TATCTTTATAGTCCTGTCGGGTTTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGAT
 GCTCGTCAGGGGGGCGGAGCCTATGGAAAAACGCCAGCAACGCGGCCTTTTTACGG
 TTCCTGGCCTTTTGCTGGCCTTTTGCTCACATGT

Table 5: Components of Construct Sequence (SEQ ID NO: 54)

Components	Position in construct
5'ITR	12-130
Cloning site	131-147
IGFBP2 promoter	148-1660
hGJB2 minimal promoter	1661-1788
Cloning site	1789-1797
Synthetic barcode	1798-1805
5'UTR	1806-2167
GJB2 (exon2)	2179-2856
3xFLAG	2869-2934
3'UTR (exon2)	2938-4344
bGHpA	4366-4590
Cloning site	4591-4624
3'ITR	4625-4754

Exemplary Construct sequence (SEQ ID NO: 17)

CCTGCAGGCAGCTGCGCGCTCGCTCGCTCACTGAGGCCGCCGGGCGTCTGGGCGACC
TTTGGTCGCCCCGGCCTCAGTGAGCGAGCGAGCGCGCAGAGAGGGAGTGGCCAACTC
CATCACTAGGGGTTCCTGCGGGCCGCACGCGTGGTCCCATGGCTCTGTAAAAATCAAA
GAAACATCTTTTCCAACAGCCCTTTCAAACCTCATCGCATCTCACTGGCTGATTCA
GTCATTTAAACCTGCTTCTCCCTAAAGCTGATCACTGGCTAAGCTAATAGGGTTTCCG
GGATTGGTTTAGCCTGATACTAATCCAGGTCTACCTTCAGGAGCCAGACCAAACCTGC
CTATTGGCATTGCATTCTTGCAGTAGGGAGGGGAGGTATGGATGGTGTGGAGTCCAC
CACAAAGGTCCATGCCAGTCTTTGCTGAACCAGCATCAGACTCCATCAAGCAACAGAT
GAGAGGTTCCATGATAAAGTGGCCCTCAGCAATCCCCATCCATTGCTGTCTAGGAAG
AACAGTGCTTGTACACAGGTTTAGGACCTCAGTCTTGGCTGTAATCTTCTGGTTTACT
TTGCCAGCACCAAACAGAAGGAAAGAAAGGGCTCAAATTTGACCAAATAAATTATG
CTTCTCCTTCCAGAGATAACCTTGAGTCCTGTCTAGGAAGATATTAGAATTGTAAAG
AAAAAAAAAATTACTCCTTATCCTATGGCAAGTGGAGTCTATGTCTACTTCAGCTGA
AATTAATCCTGTCCATAATAGATGACCCTTGCTCAAGCTGGCCAGAAGCCATACCA
ACCAGCACGAAGGTTAAACCTATTATTAGTTTTTTCTGTGATTTTTCATTTTCAGGCCA
AGTTTTAGAACATAAGATTTTAAGAATAGGAAGTAAGTAAGATTTCTGCATATCCT
GTTCTCTTAGTCAGCTGAATTTTTTTTTTTTTTTTTTTAGTCCTAACTCAGCCTCCCAA
AGTGCTGGGATTACAGGCGTGAGCCACCGCACCAAGCCTGGAATCTATGTCTTACAG
TTATGAGAATCAACAGCTAGCTCATTATGGGCAAGGTGATGTCACTCTGGCTTCTCA
ATGAAAATGGCATTCTCTCCCTTGAAAAGGTCATAGCCAGTCAGTCAGTCAGTCACG
GGAGCGCAGCGGCTTCTAGGGGTGAGTGGGACCCACGCGGCCCCACCTGCTCCTCCC
GCGCGCGGCCCCACCCCCCTGCCCCGCCCCGCTGGTTTATAGAAGCTCTGAGGACC
CAGAGGCCGGGCGCGCTCCGCCCCGCGCGCCGCCCCCTCCGTAACCTTTCCAGTCTC
CGAGGGAAGAGGCGGGGTGTGGGGTGCGGTTAAAAGGCGCCACGGCGGGAGACAG
GTCTCACCGGTCGTGTGTTGTTGCGGCCCCGCAGCGCCCGCGCGCTCCTCTCCCCGAC
TCGGAGCCCCCTCGGCGGCGCCCGGCCAGGACCCGCCTAGGAGCGCAGGAGCCCCA
GCGCAGAGACCCCAACGCCGAGACCCCGCCCCGGCCCCGCGCGCTTCTCTCCCGAC
GCAGTTTAGGACCCTTGTTGCGGAAGAGGTGGTGTGCGGCTGAGACCCGCGTCCTCA
GGACGGTTCCATCAGTGCCTCGATCCTGCCCCACTGGAGGAGGAAGGCAGCCCGAA
CAGCGCTCACCTAACTAACAGCTGCTGAGAGCTGGGTTCCGTGGCCATGCACCTGGG
ACTGCCTTGAGAAGCGTGAGCAAACCGCCCAGAGTAGAAGCGCTAGCCACCATGGA
TTGGGGCACGCTGCAGACGATCCTGGGGGGTGTGAACAAACACTCCACCAGCATTG

GAAAGATCTGGCTCACCGTCCTCTTCATTTTTTCGCATTATGATCCTCGTTGTGGCTGC
AAAGGAGGTGTGGGGAGATGAGCAGGCCGACTTTGTCTGCAACACCCTGCAGCCAG
GCTGCAAGAACGTGTGCTACGATCACTACTTCCCCATCTCCCACATCCGGCTATGGG
CCCTGCAGCTGATCTTCGTGTCCACGCCAGCGCTCCTAGTGGCCATGCACGTGGCCT
ACCGGAGACATGAGAAGAAGAGGAAGTTCATCAAGGGGGAGATAAAGAGTGAATT
TAAGGACATCGAGGAGATCAAAACCCAGAAGGTCCGCATCGAAGGCTCCCTGTGGT
GGACCTACACAAGCAGCATCTTCTTCCGGGTCATCTTCGAAGCCGCCTTCATGTACG
TCTTCTATGTCATGTACGACGGCTTCTCCATGCAGCGGCTGGTGAAGTGCAACGCCT
GGCCTTGTCCCAACACTGTGGACTGCTTTGTGTCCCGGCCACGGAGAAGACTGTCT
TCACAGTGTTTCATGATTGCAGTGTCTGGAATTTGCATCCTGCTGAATGTCACTGAATT
GTGTTATTTGCTAATTAGATATTGTTCTGGGAAGTCAAAAAAGCCAGTTGGATCCCG
GGCTGACTACAAAGACCATGACGGTGATTATAAAGATCATGACATCGACTACAAGG
ATGACGATGACAAGTAAGAAATAGACAGCATGAGAGGGATGAGGCAACCCGTGCTC
AGCTGTCAAGGCTCAGTCGCTAGCATTTCCCAACACAAAGATTCTGACCTTAAATGC
AACCATTTGAAACCCCTGTAGGCCTCAGGTGAAACTCCAGATGCCACAATGGAGCTC
TGCTCCCCTAAAGCCTCAAAACAAAGGCCTAATTCTATGCCTGTCTTAATTTTCTTTC
ACTTAAGTTAGTTCCACTGAGACCCCAGGCTGTTAGGGGTTATTGGTGTAAGGTACT
TTCATATTTTAAACAGAGGATATCGGCATTTGTTTCTTCTCTGAGGACAAGAGAAA
AAAGCCAGGTTCCACAGAGGACACAGAGAAGGTTTGGGTGTCCTCCTGGGGTTCTTT
TTGCCAACTTTCCCCACGTAAAGGTGAACATTGGTTCTTTCATTTGCTTTGGAAGTT
TTAATCTCTAACAGTGGACAAAGTTACCAGTGCCTTAAACTCTGTTACACTTTTTGGA
AGTGAAAACTTTGTAGTATGATAGGTTATTTTGATGTAAAGATGTTCTGGATACCATT
ATATGTTCCCCCTGTTTCAGAGGCTCAGATTGTAATATGTAAATGGTATGTCATTTCG
TACTATGATTTAATTTGAAATATGGTCTTTTGGTTATGAATACTTTGCAGCACAGCTG
AGAGGCTGTCTGTTGTATTTCATTGTGGTCATAGCACCTAACAAACATTGTAGCCTCAAT
CGAGTGAGACAGACTAGAAGTTCCTAGTGATGGCTTATGATAGCAAATGGCCTCATG
TCAAATATTTAGATGTAATTTTGTGTAAGAAATACAGACTGGATGTACCACCAACTA
CTACCTGTAATGACAGGCCTGTCCAACACATCTCCCTTTTCCATGACTGTGGTAGCCA
GCATCGGAAAGAACGCTGATTTAAAGAGGTCGCTTGGGAATTTTATTGACACAGTAC
CATTTAATGGGGAGGACAAAATGGGGCAGGGGAGGGAGAAGTTTCTGTCGTTAAAA
ACAGATTTGGAAAGACTGGACTCTAAAGTCTGTTGATTAAAGATGAGCTTTGTCTAC
TTCAAAAGTTTGTGTTGCTTACCCCTTCAGCCTCCAATTTTAAAGTGAAAATATAGCT
AATAACATGTGAAAAGAATAGAAGCTAAGGTTTAGATAAATATTGAGCAGATCTAT

AGGAAGATTGAACCTGAATATTGCCATTATGCTTGACATGGTTTCCAAAAAATGGTA
CTCCACATATTTTCAGTGAGGGTAAGTATTTTCCTGTTGTCAAGAATAGCATTGTAAA
AGCATTTTGTAAATAATAAAGAATAGCTTTAATGATATGCTTGTAACATAAAATAATTTT
GTAATGTATCAAATACATTTAAAACATTAAAATATAATCTCTATAATAAGAGCTCGC
TGATCAGCCTCGACTGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGCCCCCTCCCCCG
TGCTTCCCTTGACCCTGGAAGGTGCCACTCCCCTGTCCTTTCCTAATAAAATGAGGA
AATTGCATCGCATTGTCTGAGTAGGTGTCATTCTATTCTGGGGGGTGGGGTGGGGCA
GGACAGCAAGGGGGGAGGATTGGGAAGACAATAGCAGGCATGCTGGGGATGCGGTG
GGCTCTATGGAAGCTTGAATTCAGCTGACGTGCCTCGGACCGCTAGGAACCCCTAGT
GATGGAGTTGGCCACTCCCTCTCTGCGCGCTCGCTCGCTCACTGAGGCCGGGCGACC
AAAGGTCGCCCCGACGCCCGGGCTTTGCCCCGGCGGCCTCAGTGAGCGAGCGAGCGC
GCAGCTGCCTGCAGGGGCGCCTGATGCGGTATTTTCTCCTTACGCATCTGTGCGGTAT
TTCACACCGCATACGTCAAAGCAACCATAGTACGCGCCCTGTAGCGGCGCATTAAAGC
GCGGCGGGTGTGGTGGTTACGCGCAGCGTGACCGCTACACTTGCCAGCGCCCTAGCG
CCCGCTCCTTTTCGCTTTCTTCCCTTCCTTTCTCGCCACGTTCCGCGGCTTTCCCCGTCA
AGCTCTAAATCGGGGGCTCCCTTTAGGGTTCCGATTTAGTGCTTTACGGCACCTCGAC
CCCAAAAACTTGATTTGGGTGATGGTTCACGTAGTGGGCCATCGCCCTGATAGACG
GTTTTTCGCCCTTTGACGTTGGAGTCCACGTTCTTTAATAGTGACTCTTGTTCCAAA
CTGGAACAACACTCAACCCTATCTCGGGCTATTCTTTTGATTTATAAGGGATTTTGCC
GATTTTCGGCCTATTGGTTAAAAAATGAGCTGATTTAACAAAAATTTAACGCGAATTT
TAACAAAATATTAACGTTTACAATTTTATGGTGCACTCTCAGTACAATCTGCTCTGAT
GCCGCATAGTTAAGCCAGCCCCGACACCCGCCAACACCCGCTGACGCGCCCTGACG
GGCTTGTCTGCTCCCGGCATCCGCTTACAGACAAGCTGTGACCGTCTCCGGGAGCTG
CATGTGTCAGAGGTTTTACCGTCATCACCGAAACGCGCGAGACGAAAGGGCCTCGT
GATACGCCTATTTTTATAGGTAAATGTCATGAACAATAAACTGTCTGCTTACATAA
ACAGTAATACAAGGGGTGTTATGAGCCATATTCAACGGGAAACGTCGAGGCCGCGA
TTAAATTCCAACATGGATGCTGATTTATATGGGTATAAATGGGCTCGCGATAATGTC
GGGCAATCAGGTGCGACAATCTATCGCTTGATGGGAAGCCCGATGCGCCAGAGTTG
TTTCTGAAACATGGCAAAGGTAGCGTTGCCAATGATGTTACAGATGAGATGGTCAGA
CTAAACTGGCTGACGGAATTTATGCCTCTTCCGACCATCAAGCATTTTATCCGTA
CTGATGATGCATGGTTACTCACCCTGCGATCCCCGAAAAACAGCATTCCAGGTAT
TAGAAGAATATCCTGATTCAGGTGAAAATATTGTTGATGCGCTGGCAGTGTTCCCTGC
GCCGGTTGCATTTCGATTCCTGTTTGTAATTGTCCTTTTAACAGCGATCGCGTATTTTCG

TCTCGCTCAGGCGCAATCACGAATGAATAACGGTTTGGTTGATGCGAGTGATTTTGA
TGACGAGCGTAATGGCTGGCCTGTTGAACAAGTCTGGAAAGAAATGCATAAACTTTT
GCCATTCTCACC GGATT CAGTCGTC ACTCATGGTGATTTCTCACTTGATAACCTTATT
TTTGACGAGGGGAAATTAATAGGTTGTATTGATGTTGGACGAGTCGGAATCGCAGAC
CGATACCAGGATCTTGCCATCCTATGGAAC TGCCTCGGTGAGTTTTCTCCTTCATTAC
AGAAACGGCTTTTTTCAAAAATATGGTATTGATAATCCTGATATGAATAAATTGCAGT
TTCATTTGATGCTCGATGAGTTTTTCTAATCTCATGACCAAATCCCTTAACGTGAGT
TTTCGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGATC
CTTTTTTTCTGCGCGTAATCTGCTGCTTGCAAACAAAAAACCACCGCTACCAGCGG
TGGTTTGTGGCCGGATCAAGAGCTACCAACTCTTTTCCGAAGGTAAC TGGCTTCAG
CAGAGCGCAGATACCAAATACTGTCTTCTAGTGTAGCCGTAGTTAGGCCACCACTT
CAAGAACTCTGTAGCACCGCCTACATACCTCGCTCTGCTAATCCTGTTACCAGTGGCT
GCTGCCAGTGGCGATAAGTCGTGTCTTACCGGGTTGGACTCAAGACGATAGTTACCG
GATAAGGCGCAGCGGTCGGGCTGAACGGGGGGTTCGTGCACACAGCCCAGCTTGGA
GCGAACGACCTACACCGAACTGAGATACCTACAGCGTGAGCTATGAGAAAGCGCCA
CGCTTCCCGAAGGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTCGGAACA
GGAGAGCGCACGAGGGAGCTTCCAGGGGGAAACGCCTGGTATCTTTATAGTCCTGTC
GGGTTTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGATGCTCGTCAGGGGGGCGG
AGCCTATGGAAAAACGCCAGCAACGCGGCCTTTTTACGGTTCCTGGCCTTTTGCTGG
CCTTTTGCTCACATGT

Table 6: Components of Construct Sequence (SEQ ID NO: 17)

Components	Position in construct
5' ITR	12-130
Cloning site	131-147
RBP7 promoter	148-1244
hGJB2 minimal promoter	1245-1372
Cloning site	1373-1381
Synthetic barcode	1382-1389

5'UTR	1390-1751
GJB2 (exon2)	1763-2440
3xFLAG	2453-2518
3'UTR (exon2)	2522-3928
bGHpA	3950-4174
Cloning site	4175-4208
3'ITR	4209-4338

Exemplary Construct sequence (SEQ ID NO: 38)

CCTGCAGGCAGCTGCGCGCTCGCTCGCTCACTGAGGCCGCCCGGGCGTCGGGCGACC
 TTTGGTCGCCCCGGCCTCAGTGAGCGAGCGAGCGCGCAGAGAGGGAGTGGCCAACTC
 CATCACTAGGGGTTCCTGCGGCCGCACGCGTGTTAAATAGCTTCCAACGTTTCCACC
 CCACCAGCCCTTGCACCACTCCCTGTACTGGCCCTGAGCTTTCTAGTCTTGACTGAAA
 AGCGGGGAGGCAATGTGGTCTCTCCTGGTGCCTGTCCCGAGGAAGGCCTGCTCCGC
 TTCCCCGGAGGAGTCTTCAAAGGATGGAGGTAATTAATAAAAAACAACCCCTGTACCT
 CCTCTAAGTGGTCATTAATTAATAAAGAACCTCCAGGCTCCTATAGGAGAGGTCTGT
 GCACCCCGCGGGCTATGAGAAGGCTGGATCACCCAGAAAGACTGAGGATGTGTCCT
 GGCAAAAACACAGCCTGCCCCTCACACTGCTCCCCACGGGTGCACTAGGGAGGAAG
 AGTTCCCTCGAGGGCCTGAGCAGGCGCCCCACACCTGCACCCGTGCAGAGGGGGCT
 GGGCCCCGCCCTCTGCGCTCCCGAGGGAGAGCCCTACCCCTGCATCCCCGGTACCCC
 GTTCCCTCCAAGGGCCGAAAGAGGGCCCCGCGCACTGTGCACTTCTTAGGGGTCCC
 CCACCCTGCGCCCCCGCCACGGGAAAAAGGTCCCCGCTCTGCGCATCCGGCCCCGGA
 GGGACAGCCCCGGTCTGCACTCCTTGCTCCTCAGGGGGACGGTCCGCGCCAGCGG
 CTAGTGCGCCCCGGGTAGGTGGGGGCGGGGGGCTCGTCGAGTGACAGCGCTCGCCT
 CCCGCAGCCCGCCGAGCCGCTCAGGGCAGAAGCTCTGAGGACCCAGAGGCCGGG
 CGCGCTCCGCCCCGCGCGCCGCCCTCCGTAACCTTCCCAGTCTCCGAGGGAAGAG
 GCGGGGTGTGGGGTGCGGTTAAAAGGCGCCACGGCGGGAGACAGGTCTCACCGGTT
 CGTGGGTGTTGCGGCCCCGCAGCGCCCGCGCGCTCCTCTCCCCGACTCGGAGCCCCT

CGGCGGCGCCCCGGCCCCAGGACCCGCCTAGGAGCGCAGGAGCCCCAGCGCAGAGACC
CCAACGCCGAGACCCCCGCCCCGGCCCCGCCGCGCTTCCTCCCGACGCAGTTTAGGA
CCCTTGTTTCGCGAAGAGGTGGTGTGCGGCTGAGACCCGCGTCCTCAGGACGGTTCCA
TCAGTGCCTCGATCCTGCCCCACTGGAGGAGGAAGGCAGCCCCGAACAGCGCTCACCT
AACTAACAGCTGCTGAGAGCTGGGTTCCTGGCCATGCACCTGGGACTGCCTTGAGA
AGCGTGAGCAAACCGCCCAGAGTAGAAGCGCTAGCCACCATGGATTGGGGCAGCGT
GCAGACGATCCTGGGGGGTGTGAACAAACACTCCACCAGCATTGGAAAGATCTGGC
TCACCGTCCTCTTCATTTTCGCATTATGATCCTCGTTGTGGCTGCAAAGGAGGTGTG
GGGAGATGAGCAGGCCGACTTTGTCTGCAACACCCTGCAGCCAGGCTGCAAGAACG
TGTGCTACGATCACTACTTCCCCATCTCCCACATCCGGCTATGGGCCCTGCAGCTGAT
CTTCGTGTCCACGCCAGCGCTCCTAGTGGCCATGCACGTGGCCTACCGGAGACATGA
GAAGAAGAGGAAGTTCATCAAGGGGGAGATAAAGAGTGAATTTAAGGACATCGAG
GAGATCAAAACCCAGAAGGTCCGCATCGAAGGCTCCCTGTGGTGGACCTACACAAG
CAGCATCTTCTTCCGGGTCATCTTCGAAGCCGCCTTCATGTACGTCTTCTATGTCATG
TACGACGGCTTCTCCATGCAGCGGCTGGTGAAGTGCAACGCCTGGCCTTGTCCCAAC
ACTGTGGACTGCTTTGTGTCCCGGCCACGGAGAAGACTGTCTTCACAGTGTTTCATG
ATTGCAGTGTCTGGAATTTGCATCCTGCTGAATGTCAGTGAATTGTGTTATTTGCTAA
TTAGATATTGTTCTGGGAAGTCAAAAAGCCAGTTGGATCCCGGGCTGACTACAAAG
ACCATGACGGTGATTATAAAGATCATGACATCGACTACAAGGATGACGATGACAAG
TAAGAAATAGACAGCATGAGAGGGATGAGGCAACCCGTGCTCAGCTGTCAAGGCTC
AGTCGCTAGCATTTCCCAACACAAAGATTCTGACCTTAAATGCAACCATTTGAAACC
CCTGTAGGCCTCAGGTGAAACTCCAGATGCCACAATGGAGCTCTGCTCCCCTAAAGC
CTCAAAACAAAGGCCTAATTCTATGCCTGTCTTAATTTTCTTTCACTTAAGTTAGTTC
CACTGAGACCCAGGCTGTTAGGGGTTATTGGTGTAAAGGTACTTTTCATATTTTAAAC
AGAGGATATCGGCATTTGTTTCTTTCTCTGAGGACAAGAGAAAAAAGCCAGGTTCCA
CAGAGGACACAGAGAAGGTTTGGGTGTCTCCTGGGGTTCTTTTGCCAACTTTCCC
CACGTTAAAGGTGAACATTGGTTCTTTTCATTTGCTTTGGAAGTTTAAATCTCTAACAG
TGGACAAAGTTACCAGTGCCTTAAACTCTGTTACACTTTTTGGAAGTGAAAACCTTGT
AGTATGATAGGTTATTTTGATGTAAAGATGTTCTGGATACCATTATATGTTCCCCCTG
TTTCAGAGGCTCAGATTGTAATATGTAAATGGTATGTCATTCGCTACTATGATTTAAT
TTGAAATATGGTCTTTTGGTTATGAATACTTTGCAGCACAGCTGAGAGGCTGTCTGTT
GTATTCATTGTGGTCATAGCACCTAACAACATTGTAGCCTCAATCGAGTGAGACAGA
CTAGAAGTTCCTAGTGATGGCTTATGATAGCAAATGGCCTCATGTCAAATATTTAGA

TGTAATTTTGTGTAAGAAATACAGACTGGATGTACCACCACTACTACCTGTAATGA
CAGGCCTGTCCAACACATCTCCCTTTTCCATGACTGTGGTAGCCAGCATCGGAAAGA
ACGCTGATTTAAAGAGGTCGCTTGGAATTTTATTGACACAGTACCATTTAATGGGG
AGGACAAAATGGGGCAGGGGAGGGAGAAGTTTCTGTCGTTAAAAACAGATTGGA
AGACTGGACTCTAAAGTCTGTTGATTAAAGATGAGCTTTGTCTACTTCAAAAGTTTGT
TTGCTTACCCCTTCAGCCTCCAATTTTTTAAGTGAAAATATAGCTAATAACATGTGAA
AAGAATAGAAGCTAAGGTTTAGATAAATATTGAGCAGATCTATAGGAAGATTGAAC
CTGAATATTGCCATTATGCTTGACATGGTTTCCAAAAAATGGTACTCCACATATTTCA
GTGAGGGTAAGTATTTTCTGTTGTCAAGAATAGCATTGTAAAAGCATTGTTGTAATA
ATAAAGAATAGCTTTAATGATATGCTTGTAATAAATAATTTTGTAAATGTATCAAA
TACATTTAAAACATTAAAATATAATCTCTATAATAAGAGCTCGCTGATCAGCCTCGA
CTGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGCCCTCCCCCGTGCCTTCCTTGACC
CTGGAAGGTGCCACTCCCCTGTCCTTTCTAATAAAAATGAGGAAATTGCATCGCAT
TGTCTGAGTAGGTGTCATTCTATTCTGGGGGGTGGGGTGGGGCAGGACAGCAAGGG
GGAGGATTGGGAAGACAATAGCAGGCATGCTGGGGATGCGGTGGGCTCTATGGAAG
CTTGAATTCAGCTGACGTGCCTCGGACCGCTAGGAACCCCTAGTGATGGAGTTGGCC
ACTCCCTCTCTGCGCGCTCGCTCGCTCACTGAGGCCGGGCGACCAAAGGTCGCCCCGA
CGCCCGGGCTTTGCCCGGGCGGCCTCAGTGAGCGAGCGAGCGCGCAGCTGCCTGCA
GGGGCGCCTGATGCGGTATTTTCTCCTTACGCATCTGTGCGGTATTTACACCCGCATA
CGTCAAAGCAACCATAGTACGCGCCCTGTAGCGGCGCATTAAGCGCGGGCGGGTGTG
GTGGTTACGCGCAGCGTGACCGCTACACTTGCCAGCGCCCTAGCGCCCGCTCCTTTC
GCTTTCTTCCCTTCCTTTCTCGCCACGTTCCGCCGGCTTTCCCCGTCAAGCTCTAAATCG
GGGGCTCCCTTTAGGGTTCCGATTTAGTGCTTTACGGCACCTCGACCCCAAAAACT
TGATTTGGGTGATGGTTCACGTAGTGGGCCATCGCCCTGATAGACGGTTTTTTCGCCCT
TTGACGTTGGAGTCCACGTTCTTTAATAGTGGACTCTTGTTCCAACTGGAACAACA
CTCAACCCTATCTCGGGCTATTCTTTTGATTTATAAGGGATTTTGCCGATTTTCGGCCT
ATTGGTTAAAAAATGAGCTGATTTAACAAAAATTTAACGCGAATTTTAACAAAATAT
TAACGTTTACAATTTTATGGTGCCTCTCAGTACAATCTGCTCTGATGCCGCATAGTT
AAGCCAGCCCCGACACCCGCCAACACCCGCTGACGCGCCCTGACGGGCTTGTCTGCT
CCCGGCATCCGCTTACAGACAAGCTGTGACCGTCTCCGGGAGCTGCATGTGTGAGAG
GTTTTACCGTCATCACCGAAACGCGCGAGACGAAAGGGCCTCGTGATACGCCTATT
TTTATAGGTAAATGTCATGAACAATAAACTGTCTGCTTACATAAACAGTAATACAA
GGGGTGTTATGAGCCATATTCAACGGGAAACGTCGAGGCCGCGATTAAATTCCAAC

ATGGATGCTGATTTATATGGGTATAAATGGGCTCGCGATAATGTCGGGCAATCAGGT
 GCGACAATCTATCGCTTGTATGGGAAGCCCGATGCGCCAGAGTTGTTTCTGAAACAT
 GGCAAAGGTAGCGTTGCCAATGATGTTACAGATGAGATGGTCAGACTAAACTGGCT
 GACGGAATTTATGCCTCTTCCGACCATCAAGCATTTTATCCGTACTCCTGATGATGCA
 TGGTTACTCACCCTGCGATCCCCGAAAAACAGCATTCCAGGTATTAGAAGAATAT
 CCTGATTCAGGTGAAAATATTGTTGATGCGCTGGCAGTGTTCCCTGCGCCGGTTGCATT
 CGATTCCTGTTTGTAAATTGTCCTTTTAACAGCGATCGCGTATTTCTGCTCTGCTCAGGC
 GCAATCACGAATGAATAACGGTTTGGTTGATGCGAGTGATTTTGATGACGAGCGTAA
 TGGCTGGCCTGTTGAACAAGTCTGGAAAGAAATGCATAAACTTTTGCCATTCTCACC
 GGATTCAGTCGTCACCTCATGGTGATTTCTCACTTGATAACCTTATTTTTGACGAGGGG
 AAATTAATAGGTTGTATTGATGTTGGACGAGTCGGAATCGCAGACCGATAACCAGGAT
 CTTGCCATCCTATGGAAGTGCCTCGGTGAGTTTTCTCCTTCATTACAGAAACGGCTTT
 TTCAAAAATATGGTATTGATAATCCTGATATGAATAAATTGCAGTTTCATTTGATGCT
 CGATGAGTTTTTCTAATCTCATGACCAAAATCCCTTAACGTGAGTTTTCGTTCCACTG
 AGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTTCTGCG
 CGTAATCTGCTGCTTGCAAACAAAAAACACCGCTACCAGCGGTGGTTTGTGGC
 GGATCAAGAGCTACCAACTCTTTTTCCGAAGGTAAGTGGCTTCAGCAGAGCGCAGAT
 ACCAAATACTGTCCTTCTAGTGTAGCCGTAGTTAGGCCACCACTTCAAGAACTCTGT
 AGCACCGCCTACATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTGCCAGTGG
 CGATAAGTCGTGTCTTACCGGGTTGGACTCAAGACGATAGTTACCGGATAAGGCGCA
 GCGGTCGGGCTGAACGGGGGGTTTCGTGCACACAGCCCAGCTTGAGCGAACGACCT
 ACACCGAACTGAGATACCTACAGCGTGAGCTATGAGAAAGCGCCACGCTTCCCGAA
 GGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTCGGAACAGGAGAGCGCA
 CGAGGGAGCTTCCAGGGGGAAACGCCTGGTATCTTTATAGTCCTGTGGGTTTCGCC
 ACCTCTGACTTGAGCGTCGATTTTTGTGATGCTCGTCAGGGGGGCGGAGCCTATGGA
 AAAACGCCAGCAACGCGGCCTTTTTACGGTTCCTGGCCTTTTGCTGGCCTTTTGCTCA
 CATGT

Table 7: Components of Construct Sequence (SEQ ID NO: 38)

Components	Position in construct
5' ITR	12-130

Cloning site	131-147
GJB6 promoter	148-882
hGJB2 minimal promoter	883-1010
Cloning site	1011-1019
Synthetic barcode	1020-1027
5'UTR	1028-1389
GJB2 (exon2)	1401-2078
3xFLAG	2091-2156
3'UTR (exon2)	2160-3566
bGHpA	3588-3812
Cloning site	3813-3846
3'ITR	3847-3976

Exemplary Construct sequence (SEQ ID NO: 7)

CCTGCAGGCAGCTGCGCGCTCGCTCGCTCACTGAGGCCGCCGGGCGTCGGGCGACC
 TTTGGTCGCCCCGGCCTCAGTGAGCGAGCGAGCGCGCAGAGAGGGAGTGGCCAACTC
 CATCACTAGGGGTTCTGCGGCCGCACGCGTGGTTGTACAGGAGATAGTCAGGGAAT
 TAGTAATTTTCAAAGAGGTGACTTTGAATTCAAACCTAAATATCATCTTCAGCTGAA
 ACAAAGAAGGGGTGCAGTTATGAGGAAGTGACCAGGTAAAGCATGGCAAACAAAG
 GTAAAGTTTGTTATGCGTATTTAAGTCAGAGCCCTCTCCATTGATAAGAGTTTCCAGT
 AATTTAGTGCCATCCTTTTCTTGCTATAGAGTTCTCGTCTCTATCTGAGCACGCAAAA
 ATAACATGCTTTCTTGCTTTCTTGAAGTTGGGCATGGCCATTGACTTGCCTTAGCCCA
 TATTTTCTGTGAAGTGGTCTTCAAAAACCTATATTTCTGCCATAGAGTCACTTACTT
 AACCTGCCCTATTTAAAGGGGCTAATGCCTGATAGAATGTCGCTGCATAACTCCATC
 TGTGTGTGGTCCCTGCATCCATGACAACCAAAACCCAGATGCAGAAATTGTTTCCTAA

TCACATAGATTACCCTAGAAACCGGAAGGGCCTTGAAGTCAAAGCATTTCAGAGAA
CATGCTGAACAAATTGAATTTGCAGTTTATCTGGCCAGGGAGGATGGAGAGGGGAT
GGGCACTTGGTCTGAGTATTTTTGTTTCTCATTCCAACAGAAATTACTAGATTTACC
AAAAAATCTACAAGTGGTAGTGTGATAGAGTCAGGCAGAGGAATTGACCATAGATA
AGGTGCTCAGGACTCCTAGAGTCAGCTTCTGGTATGTGAGAAAGAAGTGAGAACAG
AGCCCATGGCATATGAAGAAGATATTACAGAAAAAAGAAAGCTGCCTTCCACGCAA
ATCATTTCTTTACAAAGGCTTGTTAACTCCTGCAGTGCCAAGAAGCTGAATGCAGCG
GCAGACATCCTGGTTCGGGCCCCAGGAAGCTCAGCCGGGTTTAATGTGGATGAGGGT
TTAATGATGTACACGCAGAAGTGTTTTGACAAATGAAGAAGGTCCTCATTCTTGAA
CATGTGCCGTTCTCCGAGGGAACCTCTAAAAGGCTGTAAGCTCATGTAGGAAAAGC
TGAGCTAGATTCTAAGGGCAGAGATGTGCTCACATTTCTTTGCATCCCTAGTTCCCA
GCACAGTGCAAGGCGCTGCAAACATTTGCTGAACCCAGGGTCTCGTGTCTT7GACTG
TCCAGCAGAGGCCGCTCTGGGCCGGGGCTCTCGGGACCTGAGGGCTGAGAGAAGGA
AGGCCAGGGGGTGGCCAGTCATCGCCGCGGGGCCCGGGTGGGAGGGGTTTGGCAG
CGGCAGGCGCGGCGGCGGCGGAGGCGGAGGCGGCCCGGGGAAGCTCTGAGGA
CCCAGAGGCCGGGCGCGCTCCGCCCCGCGCGCCGCCCCCTCCGTAACCTTTCCAGTC
TCCGAGGGAAGAGGCGGGGTGTGGGGTGCGGTTAAAAGGCGCCACGGCGGGAGAC
AGGTCTCACCGGTGCAAACCTGGTTGCGGCCCGCAGCGCCCGCGCGCTCCTCTCCCC
GACTCGGAGCCCCCTCGGCGGCGCCCGGCCAGGACCCGCCTAGGAGCGCAGGAGCC
CCAGCGCAGAGACCCCAACGCCGAGACCCCGCCCCGGCCCCGCGCGCTTCTCTCC
GACGCAGTTTAGGACCCTTGTTTCGCGAAGAGGTGGTGTGCGGCTGAGACCCGCGTCC
TCAGGACGGTTCCATCAGTGCCTCGATCCTGCCCCACTGGAGGAGGAAGGCAGCCCG
AACAGCGCTCACCTAACTAACAGCTGCTGAGAGCTGGGTTCCGTGGCCATGCACCTG
GGACTGCCTTGAGAAGCGTGAGCAAACCGCCCAGAGTAGAAGCGCTAGCCACCATG
GATTGGGGCACGCTGCAGACGATCCTGGGGGGTGTGAACAAACACTCCACCAGCAT
TGGAAGATCTGGCTCACCGTCCTCTTCATTTTTTCGATTATGATCCTCGTTGTGGCT
GCAAAGGAGGTGTGGGGAGATGAGCAGGCCGACTTTGTCTGCAACACCCTGCAGCC
AGGCTGCAAGAACGTGTGCTACGATCACTACTTCCCCATCTCCACATCCGGCTATG
GGCCCTGCAGCTGATCTTCGTGTCCACGCCAGCGCTCCTAGTGGCCATGCACGTGGC
CTACCGGAGACATGAGAAGAAGAGGAAGTTCATCAAGGGGGAGATAAAGAGTGAA
TTTAAGGACATCGAGGAGATCAAAACCCAGAAGGTCCGCATCGAAGGCTCCCTGTG
GTGGACCTACACAAGCAGCATCTTCTTCGGGTTCATCTTGAAGCCGCCTTCATGTA
CGTCTTCTATGTCATGTACGACGGCTTCTCCATGCAGCGGCTGGTGAAGTGCAACGC

CTGGCCTTGTCCCAACACTGTGGACTGCTTTGTGTCCCGGCCACGGAGAAGACTGT
CTTCACAGTGTTTCATGATTGCAGTGTCTGGAATTTGCATCCTGCTGAATGTCAGTAA
TTGTGTTATTTGCTAATTAGATATTGTTCTGGGAAGTCAAAAAAGCCAGTTGGATCCC
GGGCTGACTACAAAGACCATGACGGTGATTATAAAGATCATGACATCGACTACAAG
GATGACGATGACAAGTAAGAAATAGACAGCATGAGAGGGATGAGGCAACCCGTGCT
CAGCTGTCAAGGCTCAGTCGCTAGCATTTCCTCAACACAAAGATTCTGACCTTAAATG
CAACCATTTGAAACCCCTGTAGGCCTCAGGTGAAACTCCAGATGCCACAATGGAGCT
CTGCTCCCCTAAAGCCTCAAAACAAAGGCCTAATTCTATGCCTGTCTTAATTTCTTT
CACTTAAGTTAGTTCCACTGAGACCCCAGGCTGTTAGGGGTTATTGGTGTAAGGTAC
TTTCATATTTTAAACAGAGGATATCGGCATTTGTTTCTTCTCTGAGGACAAGAGAAA
AAAGCCAGGTTCCACAGAGGACACAGAGAAGGTTTGGGTGTCCTCCTGGGGTTCTTT
TTGCCAACTTTCCCCACGTTAAAGGTGAACATTGGTTCTTTCATTTGCTTTGGAAGTT
TTAATCTCTAACAGTGGACAAAGTTACCAGTGCCTTAAACTCTGTTACACTTTTTGGA
AGTGAAAACCTTTGTAGTATGATAGGTTATTTTGATGTAAAGATGTTCTGGATACCATT
ATATGTTCCCCCTGTTTCAGAGGCTCAGATTGTAATATGTAAATGGTATGTCATTTCG
TACTATGATTTAATTTGAAATATGGTCTTTTGGTTATGAATACTTTGCAGCACAGCTG
AGAGGCTGTCTGTTGTATTTCATTGTGGTCATAGCACCTAACAACATTGTAGCCTCAAT
CGAGTGAGACAGACTAGAAGTTCCTAGTGATGGCTTATGATAGCAAATGGCCTCATG
TCAAATATTTAGATGTAATTTTGTGTAAGAAATACAGACTGGATGTACCACCAACTA
CTACCTGTAATGACAGGCCTGTCCAACACATCTCCCTTTTCCATGACTGTGGTAGCCA
GCATCGGAAAGAACGCTGATTTAAAGAGGTCGCTTGGGAATTTTATTGACACAGTAC
CATTTAATGGGGAGGACAAAATGGGGCAGGGGAGGGAGAAGTTTCTGTCGTTAAAA
ACAGATTTGGAAAGACTGGACTCTAAAGTCTGTTGATTAAAGATGAGCTTTGTCTAC
TTCAAAAGTTTGTGTTGCTTACCCCTTCAGCCTCCAATTTTAAAGTGAAAATATAGCT
AATAACATGTGAAAAGAATAGAAGCTAAGGTTTAGATAAATATTGAGCAGATCTAT
AGGAAGATTGAACCTGAATATTGCCATTATGCTTGACATGGTTTCCAAAAAATGGTA
CTCCACATATTTAGTGAGGGTAAGTATTTTCTGTTGTCAAGAATAGCATTGTAAA
AGCATTTTGTAAATAATAAAGAATAGCTTTAATGATATGCTTGTAATAAATAATTTT
GTAATGTATCAAATACATTTAAAACATTAAAATATAATCTCTATAATAAGAGCTCGC
TGATCAGCCTCGACTGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGCCCTCCCCCG
TGCCTTCCTTGACCCTGGAAGGTGCCACTCCCCTGTCCTTTCCTAATAAAATGAGGA
AATTGCATCGCATTGTCTGAGTAGGTGTCATTCTATTCTGGGGGGTGGGGTGGGGCA
GGACAGCAAGGGGGAGGATTGGGAAGACAATAGCAGGCATGCTGGGGATGCGGTG

GGCTCTATGGAAGCTTGAATTCAGCTGACGTGCCTCGGACCGCTAGGAACCCCTAGT
GATGGAGTTGGCCACTCCCTCTCTGCGCGCTCGCTCGCTCACTGAGGCCGGGCGACC
AAAGGTCGCCCCGACGCCCGGGCTTTGCCCCGGCGGCCTCAGTGAGCGAGCGAGCGC
GCAGCTGCCTGCAGGGGCGCCTGATGCGGTATTTTCTCCTTACGCATCTGTGCGGTAT
TTCACACCGCATACGTCAAAGCAACCATAGTACGCGCCCTGTAGCGGCGCATTAAAGC
GCGGCGGGTGTGGTGGTTACGCGCAGCGTGACCGCTACACTTGCCAGCGCCCTAGCG
CCCGCTCCTTTTCGCTTTCTTCCCTTCCTTTCTCGCCACGTTTCGCCGGCTTTCCCCGTCA
AGCTCTAAATCGGGGGCTCCCTTTAGGGTTCCGATTTAGTGCTTTACGGCACCTCGAC
CCCAAAAACTTGATTTGGGTGATGGTTCACGTAGTGGGCCATCGCCCTGATAGACG
GTTTTTCGCCCTTTGACGTTGGAGTCCACGTTCTTTAATAGTGGACTCTTGTTCCAAA
CTGGAACAACACTCAACCCTATCTCGGGCTATTCTTTTGATTTATAAGGGATTTTGCC
GATTTTCGGCCTATTGGTTAAAAAATGAGCTGATTTAACAAAAATTTAACGCGAATTT
TAACAAAATATTAACGTTTACAATTTTATGGTGCACTCTCAGTACAATCTGCTCTGAT
GCCGCATAGTTAAGCCAGCCCCGACACCCGCCAACACCCGCTGACGCGCCCTGACG
GGCTTGTCTGCTCCCGGCATCCGCTTACAGACAAGCTGTGACCGTCTCCGGGAGCTG
CATGTGTCAGAGGTTTTACCGTCATCACCGAAACGCGCGAGACGAAAGGGCCTCGT
GATACGCCTATTTTTATAGGTAAATGTCATGAACAATAAACTGTCTGCTTACATAA
ACAGTAATACAAGGGGTGTTATGAGCCATATTCAACGGGAAACGTCGAGGCCGCGA
TTAAATTCCAACATGGATGCTGATTTATATGGGTATAAATGGGCTCGCGATAATGTC
GGGCAATCAGGTGCGACAATCTATCGCTTGTATGGGAAGCCCGATGCGCCAGAGTTG
TTTCTGAAACATGGCAAAGGTAGCGTTGCCAATGATGTTACAGATGAGATGGTCAGA
CTAAACTGGCTGACGGAATTTATGCCTCTTCCGACCATCAAGCATTTTATCCGTA CT
CTGATGATGCATGGTTACTCACCCTGCGATCCCCGAAAAACAGCATTCCAGGTAT
TAGAAGAATATCCTGATTCAGGTGAAAATATTGTTGATGCGCTGGCAGTGTTCCCTGC
GCCGTTGCATTTCGATTCCTGTTTGTAATTGTCCTTTTAAACAGCGATCGCGTATTTTCG
TCTCGCTCAGGCGCAATCACGAATGAATAACGGTTTGGTTGATGCGAGTGATTTTGA
TGACGAGCGTAATGGCTGGCCTGTTGAACAAGTCTGGAAAGAAATGCATAAACTTTT
GCCATTCTCACCGGATTCAGTCGTCACCTCATGGTGATTTCTCACTTGATAACCTTATT
TTTGACGAGGGGAAATTAATAGGTTGTATTGATGTTGGACGAGTCGGAATCGCAGAC
CGATACCAGGATCTTGCCATCCTATGGAACCTGCCTCGGTGAGTTTTCTCCTTCATTAC
AGAAACGGCTTTTTTCAAAAATATGGTATTGATAATCCTGATATGAATAAATTGCAGT
TTCATTTGATGCTCGATGAGTTTTTCTAATCTCATGACCAAAATCCCTTAACGTGAGT
TTTCGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGATC

CTTTTTTCTGCGCGTAATCTGCTGCTTGCAAACAAAAAACCACCGCTACCAGCGG
 TGGTTTGTGGCCGATCAAGAGCTACCAACTCTTTTCCGAAGGTAAGTGGCTTCAG
 CAGAGCGCAGATACCAAATACTGTCCTTCTAGTGTAGCCGTAGTTAGGCCACCACTT
 CAAGAACTCTGTAGCACCGCCTACATACCTCGCTCTGCTAATCCTGTTACCAGTGGCT
 GCTGCCAGTGGCGATAAGTCGTGTCTTACCGGGTTGGACTCAAGACGATAGTTACCG
 GATAAGGCGCAGCGGTCGGGCTGAACGGGGGGTTCGTGCACACAGCCCAGCTTGGA
 GCGAACGACCTACACCGAACTGAGATACCTACAGCGTGAGCTATGAGAAAGCGCCA
 CGCTTCCCGAAGGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTTCGGAACA
 GGAGAGCGCACGAGGGAGCTTCCAGGGGAAACGCCTGGTATCTTTATAGTCCTGTC
 GGGTTTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGATGCTCGTCAGGGGGGCGG
 AGCCTATGGAAAAACGCCAGCAACGCGGCCTTTTTACGGTTCCTGGCCTTTTGCTGG
 CCTTTTGCTCACATGT

Table 8: Components of Construct Sequence (SEQ ID NO: 7)

Components	Position in construct
5' ITR	12-130
Cloning site	131-147
PARM1 promoter	148-1463
hGJB2 minimal promoter	1464-1591
Cloning site	1592-1600
Synthetic barcode	1601-1608
5' UTR	1609-1970
GJB2 (exon2)	1982-2659
3xFLAG	2672-2737
3' UTR (exon2)	2741-4147
bGHpA	4169-4393

Cloning site	4394-4427
3'ITR	4428-4557

Exemplary Construct sequence (SEQ ID NO: 100)

CCTGCAGGCAGCTGCGCGCTCGCTCGCTCACTGAGGCCGCCGGGCGTCGGGCGACC
 TTTGGTCGCCCCGGCCTCAGTGAGCGAGCGAGCGCGCAGAGAGGGAGTGGCCAATC
 CATCACTAGGGGTTCCTGCGGCCGCACGCGTGGTTGTGCTGCGAGGGCTTCATCTCC
 TAAGCACTAAATGCTAAATTCCCCCTCCACGCCCATCGCCACTGTCCTCACGGATC
 CTCGCAGCAGCTTCCCAATCGGTCTCCCTGTCTCCAGCCTCACCACCCCCAACTAAG
 ACCATTCATGAAAACAGAGACAACCAAGGAGACAGTCACCCAATGCTGTCCCTTCA
 GCTTGCATTATTTTCTGACAAGACAGCTCTGCCATCCATGGAAGCCTGTGTTTGAAG
 ATCTCTGACATAAAGGTCCCTTGCAAGCTAGACGTGATTCTAAAATTGGGAACACA
 GGAATAAAAATCAAATCTTGAGTAGAAGTAGCTGAAAATTGCAGTGATTCGGGGAA
 GCTTGGCTTCTAACTCCCCACTGTTTGAAGATGGGCTTGTTTGTTTTTTAAACAGCC
 AACATAATTCAGCTGGAGGAGGTACAAAGAATTTTCTATTCTTGTCTGTAGAAA
 TCGATGGACTTTAGCTTGTCTAATTGTCCCCCTGCCTTTAGTATCTAAAATAAAATA
 ACCCTCGTTGCTTGCATTACTCAACGCATTTCTGCGTCTTGGCGTCTATGGCTAAACG
 AGTATTAATTAGACAGTCCGCAGAGAGCTGGCTGGGGATAGAAGGGGAGGTGGGGG
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 GCAACTTACTAGGTGGCCTGAGGGCTGGGTGCCAGGGGAGGCAGCGGGTTCCAGTA
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 AGGGAGTAAGGGGGGGCAATGAGGCTGGGGCCAGGCGCCAGCAGCAGCCACGCCC
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 AGCGGACGGGAAACGCCTCGAAAGCAGCCAGACCCGGCGACTGAAATGAGGCGGA
 GGAGCTTGGCGAGGGGAGGCGCAGGCTCGGAAAGGCGCGCGAGGCTCCAGGCTCCT
 TCCCGATCCACCGCTCTCCTCGCTGACCTCCGAGTCACCCCCGGAAGCTCCCGCCACT
 GCCGGGCGAATAGACCCCCGCGGACCCCCAAGCGCGCGGGGCCGGGGCCCTAGTTC
 AGGCCCTCGCTGCCCCTTTAAGGGTTCTCGAAACTTTCCCCCGGTATCAGATGAGC

CTCGTCACATCCGTTGGCCGTGGCAAGCTCTGAGGACCCAGAGGCCGGGCGCGCTCC
GCCCCGCGGCGCCGCCCCCTCCGTAACCTTTCCCAGTCTCCGAGGGAAGAGGCGGGGTG
TGGGGTGCGGTTAAAAGGCGCCACGGCGGGAGACAGGTCTCACCGGTCCTACGCTG
TTGCGGCCCCGCAGCGCCCCGCGCTCCTCTCCCCGACTCGGAGCCCCCTCGGCGGCG
CCCGGCCAGGACCCGCCTAGGAGCGCAGGAGCCCCAGCGCAGAGACCCCAACGCC
GAGACCCCCGCCCCGGCCCCGCGCGCTTCTCCCCGACGCAGTTTAGGACCCTTGTT
CGCGAAGAGGTGGTGTGCGGCTGAGACCCGCGTCCTCAGGACGGTTCCATCAGTGCC
TCGATCCTGCCCCACTGGAGGAGGAAGGCAGCCCGAACAGCGCTCACCTAACTAAC
AGCTGCTGAGAGCTGGGTTCGTTGGCCATGCACCTGGGACTGCCTTGAGAAGCGTGA
GCAAACCGCCCAGAGTAGAAGCGCTAGCCACCATGGATTGGGGCACGCTGCAGACG
ATCCTGGGGGGTGTGAACAAACACTCCACCAGCATTGGAAAGATCTGGCTCACCGTC
CTCTTCATTTTTTCGATTATGATCCTCGTTGTGGCTGCAAAGGAGGTGTGGGGAGATG
AGCAGGCCGACTTTGTCTGCAACACCCTGCAGCCAGGCTGCAAGAACGTGTGCTACG
ATCACTACTTCCCCATCTCCACATCCGGCTATGGGCCCTGCAGCTGATCTTCGTGTC
CACGCCAGCGCTCCTAGTGGCCATGCACGTGGCCTACCGGAGACATGAGAAGAAGA
GGAAGTTCATCAAGGGGGAGATAAAGAGTGAATTTAAGGACATCGAGGAGATCAAA
ACCCAGAAGGTCCGCATCGAAGGCTCCCTGTGGTGGACCTACACAAGCAGCATCTTC
TTCCGGGTTCATCTTGAAGCCGCCTTCATGTACGTCTTCTATGTCATGTACGACGGCT
TCTCCATGCAGCGGCTGGTGAAGTGCAACGCCTGGCCTTGTCCCAACACTGTGGACT
GCTTTGTGTCCCGGCCACGGAGAAGACTGTCTTCACAGTGTTTCATGATTGCAGTGT
CTGGAATTTGCATCCTGCTGAATGTCACTGAATTGTGTTATTTGCTAATTAGATATTG
TTCTGGGAAGTCAAAAAGCCAGTTGGATCCCGGGCTGACTACAAAGACCATGACG
GTGATTATAAAGATCATGACATCGACTACAAGGATGACGATGACAAGTAAGAAATA
GACAGCATGAGAGGGATGAGGCAACCCGTGCTCAGCTGTCAAGGCTCAGTCGCTAG
CATTTCCCAACACAAAGATTCTGACCTTAAATGCAACCATTTGAAACCCCTGTAGGC
CTCAGGTGAAACTCCAGATGCCACAATGGAGCTCTGCTCCCCTAAAGCCTCAAAACA
AAGGCCTAATTCTATGCCTGTCTTAATTTTCTTTCACTTAAGTTAGTTCCACTGAGAC
CCCAGGCTGTTAGGGGTTATTGGTGTAAGGTACTTTCATATTTTAAACAGAGGATAT
CGGCATTTGTTTCTTTCTCTGAGGACAAGAGAAAAAAGCCAGGTTCCACAGAGGACA
CAGAGAAGGTTTGGGTGTCCTCCTGGGGTTCTTTTTGCCAACTTTCCCCACGTTAAAG
GTGAACATTGGTTCTTTCATTTGCTTTGGAAGTTTTAATCTCTAACAGTGGACAAAGT
TACCAGTGCCTTAAACTCTGTTACACTTTTTGGAAGTGAAAACCTTTGTAGTATGATAG
GTTATTTTGATGTAAAGATGTTCTGGATACCATTATATGTTCCCCCTGTTTCAGAGGC

TCAGATTGTAATATGTAAATGGTATGTCATTGCTACTATGATTTAATTTGAAATATG
GTCTTTTGGTTATGAATACTTTGCAGCACAGCTGAGAGGCTGTCTGTTGTATTTCATTG
TGGTCATAGCACCTAACAACATTGTAGCCTCAATCGAGTGAGACAGACTAGAAAGTTC
CTAGTGATGGCTTATGATAGCAAATGGCCTCATGTCAAATATTTAGATGTAATTTTGT
GTAAGAAATACAGACTGGATGTACCACCAACTACTACCTGTAATGACAGGCCTGTCC
AACACATCTCCCTTTTCCATGACTGTGGTAGCCAGCATCGGAAAGAACGCTGATTTA
AAGAGGTCGCTTGGGAATTTTATTGACACAGTACCATTTAATGGGGAGGACAAAATG
GGGCAGGGGAGGGAGAAGTTTCTGTCGTTAAAAACAGATTTGGAAAGACTGGACTC
TAAAGTCTGTTGATTAAAGATGAGCTTTGTCTACTTCAAAAGTTTGTGTTGCTTACCCC
TTCAGCCTCCAATTTTTTAAGTGAATAATAGCTAATAACATGTGAAAAGAATAGAA
GCTAAGGTTTAGATAAATATTGAGCAGATCTATAGGAAGATTGAACCTGAATATTGC
CATTATGCTTGACATGGTTTCCAAAAAATGGTACTCCACATATTTAGTGAGGGTAA
GTATTTTCCTGTTGTCAAGAATAGCATTGTAAAAGCATTTTGTAAATAATAAGAATA
GCTTTAATGATATGCTTGTAATAAAATAATTTTGTAAATGTATCAAATACATTTAAAA
CATTAAATATAATCTCTATAATAAGAGCTCGCTGATCAGCCTCGACTGTGCCTTCTA
GTTGCCAGCCATCTGTTGTTTGCCCTCCCCCGTGCCTTCCTTGACCCTGGAAGGTGC
CACTCCCACTGTCCTTTCCTAATAAAATGAGGAAATTGCATCGCATTGTCTGAGTAG
GTGTCAATTCTATTCTGGGGGGTGGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGG
AAGACAATAGCAGGCATGCTGGGGATGCGGTGGGCTCTATGGAAGCTTGAATTCAG
CTGACGTGCCTCGGACCGCTAGGAACCCCTAGTGATGGAGTTGGCCACTCCCTCTCT
GCGCGCTCGCTCGCTCACTGAGGCCGGGCGACCAAAGGTCGCCCCGACCCCCGGGCTT
TGCCCCGGGCGGCCTCAGTGAGCGAGCGAGCGCGCAGCTGCCTGCAGGGGGCGCCTGA
TGCGGTATTTTCTCCTTACGCATCTGTGCGGTATTTACACCCGCATACGTCAAAGCAA
CCATAGTACGCGCCCTGTAGCGGCGCATTAAGCGCGGCGGGTGTGGTGGTTACGCGC
AGCGTGACCGCTACACTTGCCAGCGCCCTAGCGCCCGCTCCTTTCGCTTTCTTCCCTT
CCTTTCTCGCCACGTTTCGCCGGCTTTCCCCGTCAAGCTCTAAATCGGGGGCTCCCTTT
AGGGTTCCGATTTAGTGCTTTACGGCACCTCGACCCCAAAAACTTGATTTGGGTGA
TGGTTCACGTAGTGGGCCATCGCCCTGATAGACGGTTTTTCGCCCTTTGACGTTGGAG
TCCACGTTCTTTAATAGTGGACTCTTGTTCCAAACTGGAACAACACTCAACCCTATCT
CGGGCTATTCTTTTGATTTATAAGGGATTTTGCCGATTTTCGGCCTATTGGTTAAAAAA
TGAGCTGATTTAACAAAAATTTAACGCGAATTTTAACAAAATATTAACGTTTACAAT
TTTATGGTGCCTCTCAGTACAATCTGCTCTGATGCCGCATAGTTAAGCCAGCCCCG
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ATCACCGAAACGCGCGAGACGAAAGGGCCTCGTGATACGCCTATTTTTATAGGTTAA
TGTCATGAACAATAAACTGTCTGCTTACATAAACAGTAATACAAGGGGTGTTATGA
GCCATATTCAACGGGAAACGTCGAGGCCGCGATTAAATTCCAACATGGATGCTGATT
TATATGGGTATAAATGGGCTCGCGATAATGTCGGGCAATCAGGTGCGACAATCTATC
GCTTGTATGGGAAGCCCGATGCGCCAGAGTTGTTTCTGAAACATGGCAAAGGTAGCG
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TGCGATCCCCGGAAAAACAGCATTCCAGGTATTAGAAGAATATCCTGATTCAGGTGA
AAATATTGTTGATGCGCTGGCAGTGTTCTGCGCCGGTTGCATTTCGATTCTGTTTGT
AATTGTCCTTTTAACAGCGATCGCGTATTTCTGCTCGCTCAGGCGCAATCACGAATG
AATAACGGTTTGGTTGATGCGAGTGATTTTGATGACGAGCGTAATGGCTGGCCTGTT
GAACAAGTCTGGAAAGAAATGCATAAACTTTTGCCATTCTCACCGGATTCAGTCGTC
ACTCATGGTGATTTCTCACTTGATAACCTTATTTTTGACGAGGGGAAATTAATAGGTT
GTATTGATGTTGGACGAGTCGGAATCGCAGACCGATAACCAGGATCTTGCCATCCTAT
GGAAGTGCCTCGGTGAGTTTTCTCCTTCATTACAGAAACGGCTTTTTCAAAAATATGG
TATTGATAATCCTGATATGAATAAATTGCAGTTTCATTTGATGCTCGATGAGTTTTTC
TAATCTCATGACCAAAATCCCTTAACGTGAGTTTTCGTTCCACTGAGCGTCAGACCCC
GTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTTCTGCGCGTAATCTGCTGCT
TGCAAACAAAAAAACCACCGCTACCAGCGGTGGTTTGTGTTGCCGGATCAAGAGCTAC
CAACTCTTTTTCCGAAGGTAACCTGGCTTCAGCAGAGCGCAGATACCAAATACTGTCC
TTCTAGTGTAGCCGTAGTTAGGCCACCACTTCAAGAACTCTGTAGCACCGCCTACAT
ACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCGTGTCT
TACCGGGTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTCTGGGCTGAA
CGGGGGGTTCGTGCACACAGCCCAGCTTGGAGCGAACGACCTACACCGAACTGAGA
TACCTACAGCGTGAGCTATGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGGCGGA
CAGGTATCCGGTAAGCGGCAGGGTCGGAACAGGAGAGCGCACGAGGGAGCTTCCAG
GGGGAACGCCTGGTATCTTTATAGTCCTGTCTGGGTTTCGCCACCTCTGACTTGAGC
GTCGATTTTTGTGATGCTCGTCAGGGGGGCGGAGCCTATGGAAAAACGCCAGCAAC
GCGGCCTTTTTACGGTTCCTGGCCTTTTGCTGGCCTTTTGCTCACATGT

Table 9. Components of Construct Sequence (SEQ ID NO: 100)

Components	Position in construct
5' ITR	12-130
Cloning site	121-147
BACE2 promoter	148-1551
hGJB2 minimal promoter	1552-1679
Cloning site	1680-1688
Synthetic barcode	1689-1696
5' UTR	1697-2058
GJB2 (exon2)	2070-2747
3xFLAG	2760-2825
3' UTR (exon2)	2829-4235
bGHpA	4255-4481
Cloning site	4482-4515
3' ITR	4516-4645

Exemplary Construct sequence (SEQ ID NO: 101)

CCTGCAGGCAGCTGCGCGCTCGCTCGCTCACTGAGGCCGCCGGGCGTCGGGCGACC
 TTTGGTCGCCCCGGCCTCAGTGAGCGAGCGAGCGCGCAGAGAGGGAGTGGCCAACTC
 CATCACTAGGGGTTCCTGCGGCCGCACGCGTGGTGAAGAAACCTGCATTTCTTACAC
 TTCAGTGTACTTTCCCATATTTAACTCCAAGATTTTTGTTAATTTGTTTGGTTTTCT
 TTCTCAAACAAAATTATGCTCAGACTGAAAACCCTAGATTTGTTCCCTATTGCATCTT
 CATTTCTTCCCAAACATTCCATAAAACGTGACCTACATTAAGTTAGCAAGTTAAGTCT
 GAAAGCGTCTACCTTCCCTGGGGAGGGGGAAGGTGTAGGCAGGGCAGAGATTTGTA

GTCCAGCCCTCTTGCCACAAATTATGAATTAGAGAGGAATGACTTTGCTTTTTTAATG
ATCTCCAGAGAATTTTCCATCATTTCCCTCTCTTCACCCAGCTCCTTTGCAACCACTG
CCAGAGAAGTCTTCCTTTAGCTTCTTAAACATCGATCCTAAAACACTTCCAGACACCT
GTGCTGCTCCTTTCAGTTCCTCATGAGATTAGGCTGTGTAACAATCTCGCAAAGACG
TCCCCCTCCGTCTCCTCATCCTCTTTTCAAACCCTTTTACGATTTCCCATCTCACTCAG
CATGACAGTCAAAGTCCCTGTGATGGCCAACTTCTGCATCACCTAGCCAGTCTGCCA
CCGCCAAAACCTCTCCAGCCTCATCTTACACTTGTTCTCTGCTTGGAATCTTCCCTCCC
CTCCTTGAGGAACTTTCTCAAATGTCACCTTCCCTCAATACTCCCCCTCCTCCATTTA
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CTGCTGTCTGCCAAGGTCTAGAACAGTGCAAGTTACCCAACAGGAGTTCAATAAACA
GCCATTCATTTAACAATATTTGCTGAGCACTTCGTCCCGTCCAAGTTTGTTAAATCA
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CAACTTCCGGCCTCTTCAGGCCGGGCAAGATTTAAGGAAAGAAAAGAAACATAAGG
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GCGTCCCCTTCGGCTACTCCCGGCGTTTGCGCAAGCGGTCCACGTGGGCTCGGGCG
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GGCTTTCCAAGCTCTGAGGACCCAGAGGGCCGGGCGCGCTCCGCCCGCGGCGCCGCC
CCTCCGTAACCTTTCCAGTCTCCGAGGGAAGAGGCGGGGTGTGGGGTGCGGTAAAAA
GGCGCCACGGCGGGAGACAGGTCTCACCGGTGCCAAAGCGTTGCGGCCCCGCAGCG
CCCGCGCGCTCCTCTCCCCGACTCGGAGCCCCTCGGCGGCGCCCGGCCAGGACCCG
CCTAGGAGCGCAGGAGCCCCAGCGCAGAGACCCCAACGCCGAGACCCCGCCCCGG
CCCCGCGCGCTTCTCTCCCGACGCAGTTTAGGACCCTTGTTTCGCGAAGAGGTGGTGT
GCGGCTGAGACCCGCGTCCTCAGGACGGTTCCATCAGTGCCTCGATCTGCCCCACT
GGAGGAGGAAGGCAGCCCGAACAGCGCTCACCTAACTAACAGCTGCTGAGAGCTGG
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AGAAGCGCTAGCCACCATGGATTGGGGCACGCTGCAGACGATCCTGGGGGGTGTGA
ACAAACACTCCACCAGCATTGGAAAGATCTGGCTCACCGTCCTCTTCATTTTTTCGAT
TATGATCCTCGTTGTGGCTGCAAAGGAGGTGTGGGGAGATGAGCAGGCCGACTTTGT

CTGCAACACCCTGCAGCCAGGCTGCAAGAACGTGTGCTACGATCACTACTTCCCCAT
CTCCCACATCCGGCTATGGGCCCTGCAGCTGATCTTCGTGTCCACGCCAGCGCTCCTA
GTGGCCATGCACGTGGCCTACCGGAGACATGAGAAGAAGAGGAAGTTCATCAAGGG
GGAGATAAAGAGTGAATTTAAGGACATCGAGGAGATCAAAACCCAGAAGGTCCGCA
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CAACATTGTAGCCTCAATCGAGTGAGACAGACTAGAAGTTCCTAGTGATGGCTTATG
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AAATATTGAGCAGATCTATAGGAAGATTGAACCTGAATATTGCCATTATGCTTGACA
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TTGTA ACTAAAATAATTTTGT AATGTATCAAATACATTTAAAACATTAAAATATAAT
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TTTCCTAATAAAATGAGGAAATTGCATCGCATTGTCTGAGTAGGTGTCATTCTATTCT
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CATGCTGGGGATGCGGTGGGCTCTATGGAAGCTTGAATTCAGCTGACGTGCCTCGGA
CCGCTAGGAACCCCTAGTGATGGAGTTGGCCACTCCCTCTCTGCGCGCTCGCTCGCT
CACTGAGGCCGGGCGACCAAAGGTCGCCCCGACGCCCGGGCTTTGCCCGGGCGGCCT
CAGTGAGCGAGCGAGCGCGCAGCTGCCTGCAGGGGCGCCTGATGCGGTATTTTCTCC
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AGCATTTTATCCGTACTCCTGATGATGCATGGTTACTCACCCTGCGATCCCCGGAA
AAACAGCATTCCAGGTATTAGAAGAATATCCTGATTCAGGTGAAAATATTGTTGATG
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CAGCGATCGCGTATTTCTGCTCGCTCAGGCGCAATCACGAATGAATAACGGTTTGGT
TGATGCGAGTGATTTTGATGACGAGCGTAATGGCTGGCCTGTTGAACAAGTCTGGAA

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GAGTCGGAATCGCAGACCGATAACCAGGATCTTGCCATCCTATGGAACTGCCTCGGTG
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AGTTAGGCCACCACTTCAAGAACTCTGTAGCACCGCCTACATACCTCGCTCTGCTAA
TCCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCGTGTCTTACCGGGTTGGACT
CAAGACGATAGTTACCGGATAAGGCGCAGCGGTGCGGGCTGAACGGGGGGTTTCGTGC
ACACAGCCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCTACAGCGTGA
GCTATGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGGCGGACAGGTATCCGGTAA
GCGGCAGGGTCGGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGGAAACGCCTG
GTATCTTTATAGTCCTGTGCGGGTTTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGA
TGCTCGTCAGGGGGGCGGAGCCTATGGAAAAACGCCAGCAACGCGGCCTTTTTACG
GTTCTGGCCTTTTGCTGGCCTTTTGCTCACATGT

Table 10. Components of Construct Sequence (SEQ ID NO: 101)

Components	Position in construct
5' ITR	12-130
Cloning site	131-147
DBI2 promoter	148-1614
hGJB2 minimal promoter	1615-1742
Cloning site	1743-1751
Synthetic barcode	1752-1759
5' UTR	1760-2121

GJB2 (exon2)	2133-2810
3xFLAG	2823-2888
3'UTR (exon2)	2892-4298
bGHpA	4320-4544
Cloning site	4545-4578
3'ITR	4579-4708

Exemplary Construct sequence (SEQ ID NO: 102)

CCTGCAGGCAGCTGCGCGCTCGCTCGCTCACTGAGGCCGCCGGGCGTCGGGCGACC
 TTTGGTCGCCCCGGCCTCAGTGAGCGAGCGAGCGCGCAGAGAGGGAGTGGCCAACTC
 CATCACTAGGGGTTCTGCGGGCCGCACGCGTGGTTACCATCTGCCTTTCACCTGATG
 TTGCTATCCTCCTCCCTCTTGTTTCCTTCCACCCATCCTTTCCTCCACATTACTCTCT
 TATCCCACCCTATTTTACAACCAGTAGCCTAGGGAAAAGAGCATAGCTCAAATGAGG
 AAGAAGGCAGGACAGGCAGTCATGGCTTAGCTGGACTGAGCTGCAGTGCTTCTCCTT
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 CCTGCAGGGAGCAACCTGATTCTATGACTGACATCTAATCTTCACATTCACCTTGCA
 GGAAGGCAAGAAGTGATCCCAGCCTCCAGATGGAAAGATCAAGGCCCCAGAGAAGGT
 CAGTGGTGGTTGGAGGCCTGAGGTCACACAGCAGCCAAGTCTGGAGTCACTAGTCA
 AGGTGACCTTGACTAGCCACCCACCTCCCCTTCCCTGCCCCACCATGGCCCTGGGA
 GATCTGTTGTCCTGTGAGGGAAAGGGGCTCCAGGCTGGGCTGCATCTGAAGCCCCTA
 GATCCAGAGACTTCATTTCTTAGGCTATCTATAAAATCCACCTTCCTTTCTTTTCCCA
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 AGACCAGGCCTGGTGCCAGATAAACTGACCCTGGGTGAGGGGACAGGGGCCCAGA
 ATGGGCAGGTAGAGACTGAATACTGAAGAAGAATCCTCTGGAGTCTGTTAGCAGAA
 GCAGATGGGCCTTGCCTGACTATTGGCAGGCGGACCTGGTGGTCAGACCTCAGTGAT
 CCTCAGGGACCAGTGAATATTTAGGCTGGGGCTGAGCATCACCTGCTCCCTTGGCC
 CCACTTATAGGGCAAAGGGGAGTCTACCAGCCTACTCACTGATGACAACTGGAAA
 AGTTTGTCTGTCTCTGCTCTGGCCCCACCTCGCCCTCTCCCCTACTTGGAAGTTCCTT
 TCCTGAACCACTGACTGCCAAAGCTTGAGGGATTAAATAAATCATCTGGCCCAACCT

CCTACCATAGAGTTGGGAACACTGAAGAAAAGAGACTGGCCCAAGGTCACAGAGAA
GGCAGGGTGAACACTGTACAGGGAGAGCCAGTGTAGAATAATGGTTAAGCCACGC
AAGCTCTAGAACCCTCTATCTGAGTGCAAATCCTGGCTGTCATCTGGTACTTGCTTC
CTGGAACACATCTGGCCTCAGACTCCTGAGGCCAAGACACACTCCCTGCCCTAAGAC
TTGCTGGTTCTATGGCAGGCAGAGGCAGAAAGAGCCCCACCATCATTCAGCAAAT
GGGAAAAGTTCCCAGTTGCAGATATTAGGGGTGGGATGGGGCGGGGGTAGTCAGCA
ACCATAGACTTAGACCCTGAAGAGGCCAAAAAAGGAGGGCCATGTTCTTGGGTCAGC
AGAGCTTCTACTCAGCTTCTTCAGCCTCTAGCTCTTTCTGGTGCTAGTAGCACATTC
TCTAGTGGAGGCATCCAGATGGCAGGGAGGGTCCAGGAAACAGCTGAACATGCTGA
GCAGGCCTCCCTTGTCCCCGCTCCCCATGGCCCCATGGATCATCCGGTGCTGCAGCTC
ATCTCATTGGCTGGCTTCTGGTTACTCATCTCTCCTCTTCTCCATCTTCCCAGCCTGTG
GTTGCCGTGGAAACATAGAACAGTGACCTCACCATAGGATGAGGGGCTGGGGAGATG
CTGTTCTTGGCAGGCGCTAAGCTCTGAGGACCCAGAGGCCGGGCGCGCTCCGCCCGC
GGCGCCGCCCCCTCCGTAACTTTCCCAGTCTCCGAGGGAAGAGGCCGGGGTGTGGGGT
GCGGTTAAAAGGCGCCACGGCGGGAGACAGGTCTCACCGGTCCATCCACGTTGCGG
CCCCGCAGCGCCCGCGCGCTCCTCTCCCCGACTCGGAGCCCCTCGGCGGCGCCCCGC
CCAGGACCCGCCTAGGAGCGCAGGAGCCCCAGCGCAGAGACCCCAACGCCGAGACC
CCGCCCCCGGCCCGCGCGCTTCTTCCGACGCAGTTTAGGACCCTTGTTTCGCGAA
GAGGTGGTGTGCGGCTGAGACCCGCGTCCTCAGGACGGTTCCATCAGTGCCTCGATC
CTGCCCCACTGGAGGAGGAAGGCAGCCGAACAGCGCTCACCTAACTAACAGCTGC
TGAGAGCTGGGTTCGTGGCCATGCACCTGGGACTGCCTTGAGAAGCGTGAGCAAA
CCGCCCAGAGTAGAAGCGCTAGCCACCATGGATTGGGGCACGCTGCAGACGATCCT
GGGGGGTGTGAACAAACACTCCACCAGCATTGGAAAGATCTGGCTCACCGTCCTCTT
CATTTTTTCGATTATGATCCTCGTTGTGGCTGCAAAGGAGGTGTGGGGAGATGAGCA
GGCCGACTTTGTCTGCAACACCCTGCAGCCAGGCTGCAAGAACGTGTGCTACGATCA
CTACTTCCCCATCTCCCACATCCGGCTATGGGCCCTGCAGCTGATCTTCGTGTCCACG
CCAGCGCTCCTAGTGGCCATGCACGTGGCCTACCGGAGACATGAGAAGAAGAGGAA
GTTTCATCAAGGGGGAGATAAAGAGTGAATTTAAGGACATCGAGGAGATCAAAACCC
AGAAGGTCCGCATCGAAGGCTCCCTGTGGTGGACCTACACAAGCAGCATCTTCTTCC
GGGTCATCTTCGAAGCCGCCTTCATGTACGTCTTCTATGTCATGTACGACGGCTTCTC
CATGCAGCGGCTGGTGAAGTGCAACGCCTGGCCTTGTCCTAACACTGTGGACTGCTT
TGTGTCCCGGCCACGGAGAAGACTGTCTTCACAGTGTTTCATGATTGCAGTGTCTGG
AATTTGCATCCTGCTGAATGTCACTGAATTGTGTTATTTGCTAATTAGATATTGTTCT

GGGAAGTCAAAAAAGCCAGTTGGATCCCGGGCTGACTACAAAGACCATGACGGTGA
TTATAAAGATCATGACATCGACTACAAGGATGACGATGACAAGTAAGAAATAGACA
GCATGAGAGGGATGAGGCAACCCGTGCTCAGCTGTCAAGGCTCAGTCGCTAGCATT
CCCAACACAAAGATTCTGACCTTAAATGCAACCATTTGAAACCCCTGTAGGCCTCAG
GTGAAACTCCAGATGCCACAATGGAGCTCTGCTCCCCTAAAGCCTCAAAACAAAGG
CCTAATTCTATGCCTGTCTTAATTTTCTTTCACTTAAGTTAGTTCCACTGAGACCCCA
GGCTGTTAGGGGTTATTGGTGTAAAGGTACTTTCATATTTTAAACAGAGGATATCGGC
ATTTGTTTCTTTCTCTGAGGACAAGAGAAAAAGCCAGGTTCCACAGAGGACACAGA
GAAGGTTTGGGTGTCCTCCTGGGGTTCTTTTTGCCAACTTTCCCCACGTAAAGGTGA
ACATTGGTTCTTTCATTTGCTTTGGAAGTTTAAATCTCTAACAGTGGACAAAGTTACC
AGTGCCTTAAACTCTGTTACACTTTTTGGAAGTGAAAACCTTGTAGTATGATAGGTTA
TTTTGATGTAAAGATGTTCTGGATACCATTATATGTTCCCCCTGTTTCAGAGGCTCAG
ATTGTAATATGTAAATGGTATGTCATTTCGCTACTATGATTTAATTTGAAATATGGTCT
TTTGGTTATGAATACTTTGCAGCACAGCTGAGAGGCTGTCTGTTGTATTTCATTGTGGT
CATAGCACCTAACAACATTGTAGCCTCAATCGAGTGAGACAGACTAGAAGTTCCTAG
TGATGGCTTATGATAGCAAATGGCCTCATGTCAAATATTTAGATGTAATTTTGTGTAA
GAAATACAGACTGGATGTACCACCAACTACTACCTGTAATGACAGGCCTGTCCAACA
CATCTCCCTTTTCCATGACTGTGGTAGCCAGCATCGGAAAGAACGCTGATTTAAAGA
GGTCGCTTGGGAATTTTATTGACACAGTACCATTTAATGGGGAGGACAAAATGGGGC
AGGGGAGGGAGAAGTTTCTGTCGTTAAAAACAGATTTGGAAAGACTGGACTCTAAA
GTCTGTTGATTAAAGATGAGCTTTGTCTACTTCAAAAGTTTGTGTTACCCCTTCA
GCCTCCAATTTTTTAAGTGAAAATATAGCTAATAACATGTGAAAAGAATAGAAGCTA
AGGTTTAGATAAATATTGAGCAGATCTATAGGAAGATTGAACCTGAATATTGCCATT
ATGCTTGACATGGTTTCCAAAAAATGGTACTCCACATATTCAGTGAGGGTAAGTAT
TTTCCTGTTGTCAAGAATAGCATTGTAAAAGCATTTTGTAAATAATAAAGAATAGCTTT
AATGATATGCTTGTAATAAATAATTTTGTAAATGTATCAAATACATTTAAAACATT
AAAATATAATCTCTATAATAAGAGCTCGCTGATCAGCCTCGACTGTGCCTTCTAGTT
GCCAGCCATCTGTTGTTTGCCCCTCCCCCGTGCCTTCCTTGACCCTGGAAGGTGCCAC
TCCCCTGTCCCTTTCCTAATAAAAATGAGGAAATTGCATCGCATTGTCTGAGTAGGTGT
CATTCTATTCTGGGGGGTGGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAGA
CAATAGCAGGCATGCTGGGGATGCGGTGGGCTCTATGGAAGCTTGAATTCAGCTGAC
GTGCCTCGGACCGCTAGGAACCCCTAGTGATGGAGTTGGCCACTCCCTCTCTGCGCG
CTCGCTCGCTCACTGAGGCCGGGCGACCAAAGGTCGCCCCGACGCCCGGGCTTTGCC

GGGCGGCCTCAGTGAGCGAGCGAGCGCGCAGCTGCCTGCAGGGGCGCCTGATGCGG
TATTTTCTCCTTACGCATCTGTGCGGTATTTACACCCGCATACGTCAAAGCAACCATA
GTACGCGCCCTGTAGCGGCGCATTAAAGCGCGGCGGGTGTGGTGGTTACGCGCAGCGT
GACCGCTACACTTGCCAGCGCCCTAGCGCCCGCTCCTTTTCGCTTTCTTCCCTTCCTTTC
TCGCCACGTTGCGCGGCTTTCCCCGTCAAGCTCTAAATCGGGGGCTCCCTTTAGGGTT
CCGATTTAGTGCTTTACGGCACCTCGACCCCAAAAACTTGATTTGGGTGATGGTTC
ACGTAGTGGGCCATCGCCCTGATAGACGGTTTTTCGCCCTTTGACGTTGGAGTCCAC
GTTCTTTAATAGTGGACTCTTGTTCCAAACTGGAACAACACTCAACCCTATCTCGGGC
TATTCTTTTGATTTATAAGGGATTTTGCCGATTTTCGGCCTATTGGTTAAAAAATGAGC
TGATTTAACAAAAATTTAACGCGAATTTTAACAAAATATTAACGTTTACAATTTTATG
GTGCACTCTCAGTACAATCTGCTCTGATGCCGCATAGTTAAGCCAGCCCCGACACCC
GCCAACACCCGCTGACGCGCCCTGACGGGCTTGCTCTGCTCCCGGCATCCGCTTACAG
ACAAGCTGTGACCGTCTCCGGGAGCTGCATGTGTCAGAGGTTTTACCGTCATCACC
GAAACGCGCGAGACGAAAGGGCCTCGTGATACGCCTATTTTTATAGGTTAATGTCAT
GAACAATAAACTGTCTGCTTACATAAACAGTAATACAAGGGGTGTTATGAGCCATA
TTCAACGGGAAACGTGAGGCCGCGATTAAATTCCAACATGGATGCTGATTTATATG
GGTATAAATGGGCTCGCGATAATGTGCGGCAATCAGGTGCGACAATCTATCGCTTGT
ATGGGAAGCCCGATGCGCCAGAGTTGTTTCTGAAACATGGCAAAGGTAGCGTTGCC
AATGATGTTACAGATGAGATGGTCAGACTAACTGGCTGACGGAATTTATGCCTCTT
CCGACCATCAAGCATTTTATCCGTA CTCTGATGATGCATGGTTACTCACCCTGCGA
TCCCCGGAAAAACAGCATTCCAGGTATTAGAAGAATATCCTGATTCAGGTGAAAATA
TTGTTGATGCGCTGGCAGTGTTCTGCGCCGGTTGCATTTCGATTCTGTTTGTAATTG
TCCTTTTAACAGCGATCGCGTATTTTCGTCTCGCTCAGGCGCAATCACGAATGAATAA
CGGTTTGGTTGATGCGAGTGATTTTGATGACGAGCGTAATGGCTGGCCTGTTGAACA
AGTCTGGAAAGAAATGCATAAACTTTTGCCATTCTCACCGGATTCAGTCGTCACTCA
TGGTGATTTCTCACTTGATAACCTTATTTTTGACGAGGGGAAATTAATAGGTTGTATT
GATGTTGGACGAGTCGGAATCGCAGACCGGATACCAGGATCTTGCCATCCTATGGAAC
TGCTCGGTGAGTTTTCTCCTTCATTACAGAAACGGCTTTTTTCAAAAATATGGTATTG
ATAATCCTGATATGAATAAATTGCAGTTTCATTTGATGCTCGATGAGTTTTTCTAATC
TCATGACCAAAATCCCTTAACGTGAGTTTTCGTTTCCACTGAGCGTCAGACCCCGTAG
AAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTTCTGCGCGTAATCTGCTGCTTGCA
AACAAAAAAACCACCGCTACCAGCGGTGGTTTGGTTTGCCGGATCAAGAGCTACCAA
CTCTTTTTCCGAAGGTA ACTGGCTTCAGCAGAGCGCAGATACCAAATACTGTCTTCT

AGTGTAGCCGTAGTTAGGCCACCACTTCAAGAACTCTGTAGCACCGCCTACATACCT
CGCTCTGCTAATCCTGTTACCACTGGCTGCTGCCAGTGGCGATAAGTCGTGTCTTACC
GGGTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTCGGGCTGAACGGG
GGGTTTCGTGCACACAGCCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACC
TACAGCGTGAGCTATGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGGCGGACAGG
TATCCGGTAAGCGGCAGGGTCGGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGG
AAACGCCTGGTATCTTTATAGTCCTGTTCGGGTTTCGCCACCTCTGACTTGAGCGTCGA
TTTTTGTGATGCTCGTCAGGGGGGCGGAGCCTATGGAAAAACGCCAGCAACGCGGC
CTTTTACGGTTCCTGGCCTTTTGCTGGCCTTTTGCTCACATGT

Table 11. Components of Construct Sequence (SEQ ID NO: 102)

Components	Position in construct
5' ITR	12-130
Cloning site	131-147
FABP3 promoter	148-1899
hGJB2 minimal promoter	1900-2027
Cloning site	2028-2036
Synthetic barcode	2037-2044
5' UTR	2045-2406
GJB2 (exon2)	2418-3095
3xFLAG	3108-3173
3' UTR (exon2)	3177-4583
bGHpA	4605-4829
Cloning site	4830-4863
3' ITR	4864-4993

Exemplary Construct sequence (SEQ ID NO: 103)

CCTGCAGGCAGCTGCGCGCTCGCTCGCTCACTGAGGCCGCCCGGGCGTCGGGCGACC
TTTGGTCGCCCCGGCCTCAGTGAGCGAGCGAGCGCGCAGAGAGGGAGTGGCCAACTC
CATCACTAGGGGTTCTCTGCGGCCGCACGCGTGGTGAAACAGCAGCCATTGATGTAGC
TCAGGGTTCTGTGGATCTGTCAATTTGGAGCATGTTGGTTCTCCTGTCTCAGCTGGGCT
CATTCATGCATCTGAGTTCAGCTATTGGGCAATCTGGGGAATGTTTTGTCCATGTGAT
GTGTCATCTTCTACCAGGCTAGCCTGGGCTTCATCACATGGTATCTGGCAGGGCTCTA
AGAGGGAGAGTTGAAACACACAAGGCCTCTTGAAGCTTAGACTCAGAATTGGCACA
AGGTCGCTTCTGGCACATTCCATTGGTCAAAGCAAGTTACAAGGCCAGCTCACATTC
AAGGATTAGGTAAGTCGATTCCACTCTTGATGAGAAGTCTGAAGGATTTGGAACAGT
GTCCACCATGCAGTAATAAACTCAATAAGTAGTAGCCATTATTATTCTGTTAGAGGT
TGCCAGGAAAAGTTTTATAGTGGAAGAAATCTGAGTTTACTCTTGAGAGGTAAGTG
GAATTTCTATTTGTAGAGAATGAAGGCCTCTCAAAAAGACACAGCCTAACAATAGGT
GCTGCAGTTTAACAGTGGAGCGTGTCCAGAACAGGCTGCCCTTTTAGGCAAGGGCTA
GTGTCTTTCAGGACAGACCCAAACCCCAAATACCAAAAACAGAATAAAGTAGTGTCTT
AGCATACTTTGAGATCAGACTGTTTCTGCATTTACAGTGCTGGGGGTGGGGGGGAG
GTGTGGGGGGAAGGGAAAAGCAGCATACCAATGTAGTGAAATCTGGAAACAACAGC
CAAAAAAAGTTTGCATATTGCACAGAGCACTTGAAGATCATAAATCTATGCATGAGA
AAGATGTAGTGGAATTTTGGGGGGGATTAGAGTTTATTTTTGTCTCTCTGTGAGA
CAGCTACTCATTTCATCCAGATCACAGCTAAGAAAAAAGCTGGTCACAGAAATTAGC
AGTTTCAGCTCAGCAGCGAAGTCGCCAGCCTGTGAAGGCAGAGAGAAATTGACTAA
TTAGCAATGCGCACTAAACTTGACGGTTCTTTATAGAGAGAGAGAAGAGAGAGGG
AGAGAGAGGGAGAGGGAGGGAGGGGGGGCTCGCTTTTTCCCTTCTTTCTTCCAAAG
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TTTTGCGAGTCTCTCCAGCATCCATCCCCCTGTATGGTCTCTCTCTACTGGCCAAGCA
CGACCGTTTCTCTCCCAACCGTGGATTTCTTATTAAGCTCTGAGGACCCAGAGGCC
GGGCGCGCTCCGCCCCGCGCGCCGCCCCCTCCGTAACCTTCCCAGTCTCCGAGGGAA
GAGGCGGGGTGTGGGGTGCGGTTAAAAGGCGCCACGGCGGGAGACAGGTCTCACCG
GTCCCGTTCTGTTGCGGCCCCGCAGCGCCCGCGCGCTCCTCTCCCCGACTCGGAGCC
CCTCGGCGGCGCCCCGGCCAGGACCCGCCTAGGAGCGCAGGAGCCCCAGCGCAGAG
ACCCCAACGCCGAGACCCCCGCCCCGGCCCCGCGCGCTTCTCCCGACGCAGTTTA
GGACCCTTGTTGCGAAGAGGTGGTGTGCGGCTGAGACCCGCGTCTCAGGACGGTT

CCATCAGTGCCTCGATCCTGCCCCACTGGAGGAGGAAGGCAGCCCGAACAGCGCTC
ACCTAACTAACAGCTGCTGAGAGCTGGGTTCCGTGGCCATGCACCTGGGACTGCCTT
GAGAAGCGTGAGCAAACCGCCCAGAGTAGAAGCGCTAGCCACCATGGATTGGGGCA
CGCTGCAGACGATCCTGGGGGGTGTGAACAAACACTCCACCAGCATTGGAAAGATC
TGGCTCACCGTCCTCTTCATTTTTTCGCATTATGATCCTCGTTGTGGCTGCAAAGGAGG
TGTGGGGAGATGAGCAGGCCGACTTTGTCTGCAACACCCTGCAGCCAGGCTGCAAG
AACGTGTGCTACGATCACTACTTCCCCATCTCCACATCCGGCTATGGGCCCTGCAG
CTGATCTTCGTGTCCACGCCAGCGCTCCTAGTGGCCATGCACGTGGCCTACCGGAGA
CATGAGAAGAAGAGGAAGTTCATCAAGGGGGAGATAAAGAGTGAATTTAAGGACAT
CGAGGAGATCAAAACCCAGAAGGTCCGCATCGAAGGCTCCCTGTGGTGGACCTACA
CAAGCAGCATCTTCTTCCGGGTCATCTTCGAAGCCGCCTTCATGTACGTCTTCTATGT
CATGTACGACGGCTTCTCCATGCAGCGGCTGGTGAAGTGCAACGCCTGGCCTTGTCC
CAAACTGTGGACTGCTTTGTGTCCCGGCCACGGAGAAGACTGTCTTCACAGTGTT
CATGATTGCAGTGTCTGGAATTTGCATCCTGCTGAATGTCACTGAATTGTGTTATTTG
CTAATTAGATATTGTTCTGGGAAGTCAAAAAAGCCAGTTGGATCCCGGGCTGACTAC
AAAGACCATGACGGTGATTATAAAGATCATGACATCGACTACAAGGATGACGATGA
CAAGTAAGAAATAGACAGCATGAGAGGGATGAGGCAACCCGTGCTCAGCTGTCAAG
GCTCAGTCGCTAGCATTTCCCAACACAAAGATTCTGACCTTAAATGCAACCATTGTA
AACCCTGTAGGCCTCAGGTGAAACTCCAGATGCCACAATGGAGCTCTGCTCCCCTA
AAGCCTCAAAACAAAGGCCTAATTCTATGCCTGTCTTAATTTTCTTTCACTTAAGTTA
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AACAGAGGATATCGGCATTTGTTTCTTTCTCTGAGGACAAGAGAAAAAAGCCAGGTT
CCACAGAGGACACAGAGAAGGTTTGGGTGTCCTCCTGGGGTTCTTTTTGCCAACTTT
CCCCACGTTAAAGGTGAACATTGGTTCTTTCAATTTGCTTTGGAAGTTTAATCTCTAA
CAGTGGACAAAGTTACCAGTGCCTTAAACTCTGTTACACTTTTTGGAAGTGAAAAT
TTGTAGTATGATAGGTTATTTTGATGTAAAGATGTTCTGGATACCATTATATGTTCCC
CCTGTTTCAGAGGCTCAGATTGTAATATGTAAATGGTATGTCATTCGCTACTATGATT
TAATTTGAAATATGGTCTTTTGGTTATGAATACTTTGCAGCACAGCTGAGAGGCTGTC
TGTTGTATTTCATTGTGGTCATAGCACCTAACAACATTGTAGCCTCAATCGAGTGAGA
CAGACTAGAAGTTCCTAGTGATGGCTTATGATAGCAAATGGCCTCATGTCAAATATT
TAGATGTAATTTTGTGTAAGAAATACAGACTGGATGTACCACCACTACTACCTGTA
ATGACAGGCCTGTCCAACACATCTCCCTTTTCCATGACTGTGGTAGCCAGCATCGGA
AAGAACGCTGATTTAAAGAGGTCGCTTGGGAATTTTATTGACACAGTACCATTTAAT

GGGGAGGACAAAATGGGGCAGGGGAGGGAGAAGTTTCTGTCGTTAAAAACAGATTT
GGAAAGACTGGACTCTAAAGTCTGTTGATTAAAGATGAGCTTTGTCTACTTCAAAAG
TTTGTTTGCTTACCCCTTCAGCCTCCAATTTTTTAAGTGAAAATATAGCTAATAACAT
GTGAAAAGAATAGAAGCTAAGGTTTAGATAAATATTGAGCAGATCTATAGGAAGAT
TGAACCTGAATATTGCCATTATGCTTGACATGGTTTCCAAAAAATGGTACTCCACAT
ATTTTCAGTGAGGGTAAGTATTTTCCTGTTGTCAAGAATAGCATTGTAAAAGCATTTTG
TAATAATAAAGAATAGCTTTAATGATATGCTTGTAATAAATAATTTTGTAATGTA
TCAAATACATTTAAAACATTAAAATATAATCTCTATAATAAGAGCTCGCTGATCAGC
CTCGACTGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGCCCCTCCCCCGTGCCTTCCT
TGACCCTGGAAGGTGCCACTCCCCTGTCCTTTCCCTAATAAAAATGAGGAAATTGCAT
CGCATTGTCTGAGTAGGTGTCATTCTATTCTGGGGGGTGGGGTGGGGCAGGACAGCA
AGGGGGAGGATTGGGAAGACAATAGCAGGCATGCTGGGGATGCGGTGGGCTCTATG
GAAGCTTGAATTCAGCTGACGTGCCTCGGACCGCTAGGAACCCCTAGTGATGGAGTT
GGCCACTCCCTCTCTGCGCGCTCGCTCGCTCACTGAGGCCGGGCGACCAAAGGTCGC
CCGACGCCCCGGGCTTTGCCCCGGGCGGCCTCAGTGAGCGAGCGAGCGCGCAGCTGCC
TGCAGGGGCGCCTGATGCGGTATTTTCTCCTTACGCATCTGTGCGGTATTTACACCG
CATACGTCAAAGCAACCATAGTACGCGCCCTGTAGCGGCGCATTAAGCGCGGCGGG
TGTGGTGGTTACGCGCAGCGTGACCGCTACACTTGCCAGCGCCCTAGCGCCCGCTCC
TTTCGCTTTCTTCCCTTCCTTTCTCGCCACGTTGCGCCGGCTTTCCCCGTCAAGCTCTAA
ATCGGGGGCTCCCTTTAGGGTTCCGATTTAGTGCTTTACGGCACCTCGACCCCAAAA
AACTTGATTTGGGTGATGGTTCACGTAGTGGGCCATCGCCCTGATAGACGGTTTTTC
GCCCTTTGACGTTGGAGTCCACGTTCTTTAATAGTGGACTCTTGTTCCAAACTGGAAC
AACACTCAACCCTATCTCGGGCTATTCTTTTGATTTATAAGGGATTTTGCCGATTTG
GCCTATTGGTTAAAAAATGAGCTGATTTAACAAAAATTTAACGCGAATTTTAACAAA
ATATTAACGTTTACAATTTTATGGTGCCTCTCAGTACAATCTGCTCTGATGCCGCAT
AGTTAAGCCAGCCCCGACACCCGCCAACACCCGCTGACGCGCCCTGACGGGCTTGTC
TGCTCCCGGCATCCGCTTACAGACAAGCTGTGACCGTCTCCGGGAGCTGCATGTGTC
AGAGGTTTTACCGTCATCACCGAAACGCGCGAGACGAAAGGGCCTCGTGATACGC
CTATTTTTATAGGTTAATGTCATGAACAATAAACTGTCTGCTTACATAAACAGTAAT
ACAAGGGGTGTTATGAGCCATATTCAACGGGAAACGTCGAGGCCGCGATTAAATTC
CAACATGGATGCTGATTTATATGGGTATAAATGGGCTCGCGATAATGTCGGGCAATC
AGGTGCGACAATCTATCGCTTGATGGGAAGCCCGATGCGCCAGAGTTGTTTCTGAA
ACATGGCAAAGGTAGCGTTGCCAATGATGTTACAGATGAGATGGTCAGACTAACT

GGCTGACGGAATTTATGCCTCTTCCGACCATCAAGCATTTTATCCGTA CTCTGATGA
 TGCATGGTTACTCACCCTGCGATCCCCGAAAAACAGCATTCAGGTATTAGAAGA
 ATATCCTGATTCAGGTGAAAATATTGTTGATGCGCTGGCAGTGTTCTGCGCCGGTT
 GCATTCGATTCCTGTTTGTAAATTGTCCTTTTAACAGCGATCGCGTATTTCTGCTCTCGCTC
 AGGCGCAATCACGAATGAATAACGGTTTGGTTGATGCGAGTGATTTTGATGACGAGC
 GTAATGGCTGGCCTGTTGAACAAGTCTGGAAAGAAATGCATAAACTTTTGCCATTCT
 CACCGGATTCAGTCGTCACCTCATGGTGATTTCTCACTTGATAACCTTATTTTTGACGA
 GGGGAAATTAATAGGTTGTATTGATGTTGGACGAGTCGGAATCGCAGACCGGATACC
 AGGATCTTGCCATCCTATGGAAGTGCCTCGGTGAGTTTTCTCCTTCATTACAGAAACG
 GCTTTTTCAAAAATATGGTATTGATAATCCTGATATGAATAAATTGCAGTTTCATTTG
 ATGCTCGATGAGTTTTTCTAATCTCATGACCAAAATCCCTTAACGTGAGTTTTCGTTC
 CACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTT
 CTGCGCGTAATCTGCTGCTTGCAAACAAAAAAACCACCGCTACCAGCGGTGGTTTGT
 TTGCCGGATCAAGAGCTACCAACTCTTTTTCCGAAGGTAAGTGGCTTCAGCAGAGCG
 CAGATACCAAATACTGTCCTTCTAGTGTAGCCGTAGTTAGGCCACCACTTCAAGAAC
 TCTGTAGCACCGCCTACATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTGCCA
 GTGGCGATAAGTCGTGTCTTACCGGGTTGGACTCAAGACGATAGTTACCGGATAAGG
 CGCAGCGGTGCGGGCTGAACGGGGGGTTCGTGCACACAGCCCAGCTTGGAGCGAACG
 ACCTACACCGAACTGAGATACCTACAGCGTGAGCTATGAGAAAGCGCCACGCTTCCC
 GAAGGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTCGGAACAGGAGAGC
 GCACGAGGGAGCTTCCAGGGGGAAACGCCTGGTATCTTTATAGTCCTGTGCGGGTTTC
 GCCACCTCTGACTTGAGCGTCGATTTTTGTGATGCTCGTCAGGGGGGCGGAGCCTAT
 GGAAAAACGCCAGCAACGCGGCCTTTTTACGGTTCCTGGCCTTTTGCTGGCCTTTTGC
 TCACATGT

Table 12. Components of Construct Sequence (SEQ ID NO: 103)

Components	Position in construct
5'ITR	12-130
Cloning site	131-147
KLHL14 promoter	148-1402

hGJB2 minimal promoter	1403-1530
Cloning site	1531-1539
Synthetic barcode	1540-1547
5'UTR	1548-1909
GJB2 (exon2)	1921-2598
3xFLAG	2611-2676
3'UTR (exon2)	2680-4086
bGHpA	4108-4332
Cloning site	4333-4366
3'ITR	4367-4496

Exemplary Construct sequence (SEQ ID NO: 104)

CCTGCAGGCAGCTGCGCGCTCGCTCGCTCACTGAGGCCGCCCGGGCGTCGGGCGACC
 TTTGGTCGCCCCGGCCTCAGTGAGCGAGCGAGCGCGCAGAGAGGGAGTGGCCAACTC
 CATCACTAGGGGTTCTCTGCGGCCGCACGCGTGGTCCTTCCTCCTCCAGGGCCCTCTGC
 AGACCAGGCTGAGATGGAGGAACCTGCTAAAATCGATGGAGATGCTTCTAGCCTCC
 CAGTAGGAGGCCCCAGCCATGCCTTCAACCTGGCAGGAGGTGTAGCCACTCCTCATC
 CTTGGGTTGCAGGTTGGGTGCTGCTGTTGTGGTCCTTCCCAGAACTGCCAGTAGAG
 GGCAGCCTGGGCATCCTAATGCTTACTCTGGTTGTTACACAAAGAAAATATTGGGGT
 CACTGGCGAGCCCACCCACACTCACCAGAATCTCCACTGTAGTCCCCCTAACAAACA
 GCCCTTCACTTCCTCTCCCACTTCAGCAATTTGTATTTTGATGCCATTGGCCTCAGAT
 CAGAGTGTTTTAAATCATCACGCCCTGGCTTATCCCTGGTCGAGCCAGGACACGGGG
 TGCTTCAGTGGGTCTGTCACCTCTCTCCTGAAGCATGTTGCTTTTATTTATTTACTT
 TTA CTCTCACCTGCTCCTGTACCAGCAGGGGCCACTTCAAAGCCAAGGTACAGGGT
 GATAACTTGTGGTCCAGCATCAGTTTTCTCCACTTCTTTCTCCCACTCACCCCCAGCA
 AGGTGCCTGGGGAGACTTGAGCAGATGTTTCATTTTGGCCTGGCCAGTGGCTGAAAG

CCAGGCCTCCAATGCACTGTGACCTCTGGCTTCCCCAGCAGCTTTCCCAGAGAGGCA
GAGGGAGTCTTCATTCTTCCCAGGCGGGGAGACCACGCCTTCCCTGCCTCCTCCCTCC
GCGGGGGGTTCGCGTTGGAGGTCACCCCCGCCCCCTAGGCGCTGGGTGTTGGGAGTGAC
GCGGGGTGGGCTGGAGAGGTTTCTGCCGTCTGGGAAGCGTAAACGGACCGCCAC
CTGTGCGGGCCTCGGCCGCCCCGACCTGCTTGTGAGAAGCCTGCGGCTGGGGCACCGC
CCCCGGTCCCCGCCCCGGGTCCGCGCATTGGGAGCACACTGGCCCTTTAAGAGCGCGG
CGGCCGCGGCGCGCGGGAAGCTCTGAGGACCCAGAGGCCGGGCGCGCTCCGCCCGC
GGCGCCGCCCCCTCCGTAACTTTCCCAGTCTCCGAGGGAAGAGGCGGGGTGTGGGGT
GCGGTTAAAAGGCGCCACGGCGGGAGACAGGTCTCACCGGTTTCACTGGGTGTCGG
CCCCGCAGCGCCCCGCGCGCTCCTCTCCCCGACTCGGAGCCCCTCGGCGGCGCCCCGC
CCAGGACCCGCCTAGGAGCGCAGGAGCCCCAGCGCAGAGACCCCAACGCCGAGACC
CCCCCCCCGGCCCCGCGCGCTTCTCTCCCGACGCAGTTTAGGACCCTTGTTTCGCGAA
GAGGTGGTGTGCGGCTGAGACCCGCGTCCTCAGGACGGTTCCATCAGTGCCTCGATC
CTGCCCCACTGGAGGAGGAAGGCAGCCGAACAGCGCTCACCTAACTAACAGCTGC
TGAGAGCTGGGTTCGTTGGCCATGCACCTGGGACTGCCTTGAGAAGCGTGAGCAAA
CCGCCCAGAGTAGAAGCGCTAGCCACCATGGATTGGGGCACGCTGCAGACGATCCT
GGGGGGTGTGAACAAACACTCCACCAGCATTGGAAAGATCTGGCTCACCGTCCTCTT
CATTTTTTCGATTATGATCCTCGTTGTGGCTGCAAAGGAGGTGTGGGGAGATGAGCA
GGCCGACTTTGTCTGCAACACCCTGCAGCCAGGCTGCAAGAACGTGTGCTACGATCA
CTACTTCCCCATCTCCCACATCCGGCTATGGGGCCTGCAGCTGATCTTCGTGTCCACG
CCAGCGCTCCTAGTGGCCATGCACGTGGCCTACCGGAGACATGAGAAGAAGAGGAA
GTTTCATCAAGGGGGAGATAAAGAGTGAATTTAAGGACATCGAGGAGATCAAAACCC
AGAAGGTCCGCATCGAAGGCTCCCTGTGGTGGACCTACACAAGCAGCATCTTCTTCC
GGGTCATCTTCGAAGCCGCCTTCATGTACGTCTTCTATGTCATGTACGACGGCTTCTC
CATGCAGCGGCTGGTGAAGTGCAACGCCTGGCCTTGCCCAACACTGTGGACTGCTT
TGTGTCCCGGCCACGGAGAAGACTGTCTTCACAGTGTTTCATGATTGCAGTGTCTGG
AATTTGCATCCTGCTGAATGTCACTGAATTGTGTTATTTGCTAATTAGATATTGTTCT
GGGAAGTCAAAAAAGCCAGTTGGATCCCGGGCTGACTACAAAGACCATGACGGTGA
TTATAAAGATCATGACATCGACTACAAGGATGACGATGACAAGTAAGAAATAGACA
GCATGAGAGGGATGAGGCAACCCGTGCTCAGCTGTCAAGGCTCAGTCGCTAGCATTT
CCCAACACAAAGATTCTGACCTTAAATGCAACCATTTGAAACCCCTGTAGGCCTCAG
GTGAAACTCCAGATGCCACAATGGAGCTCTGCTCCCCTAAAGCCTCAAAACAAAGG
CCTAATTCTATGCCTGTCTTAATTTCTTTCACTTAAGTTAGTTCCACTGAGACCCCA

GGCTGTTAGGGGTTATTGGTGTAAGGTACTTTCATATTTTAAACAGAGGATATCGGC
ATTTGTTTCTTTCTCTGAGGACAAGAGAAAAAGCCAGGTTCCACAGAGGACACAGA
GAAGGTTTGGGTGTCCTCCTGGGGTTCCTTTTGCCAACTTTCCCCACGTAAAGGTGA
ACATTGGTTCTTTCATTTGCTTTGGAAGTTTAAATCTCTAACAGTGGACAAAGTTACC
AGTGCCTTAAACTCTGTTACACTTTTTGGAAGTGAAAACCTTTGTAGTATGATAGGTTA
TTTTGATGTAAAGATGTTCTGGATACCATTATATGTTCCCCCTGTTTCAGAGGCTCAG
ATTGTAATATGTAAATGGTATGTCATTTCGCTACTATGATTTAATTTGAAATATGGTCT
TTTGGTTATGAATACTTTGCAGCACAGCTGAGAGGCTGTCTGTTGTATTTCATTGTGGT
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TGATGGCTTATGATAGCAAATGGCCTCATGTCAAATATTTAGATGTAATTTTGTGTAA
GAAATACAGACTGGATGTACCACCAACTACTACCTGTAATGACAGGCCTGTCCAACA
CATCTCCCTTTTCCATGACTGTGGTAGCCAGCATCGGAAAGAACGCTGATTTAAAGA
GGTCGCTTGGGAATTTTATTGACACAGTACCATTTAATGGGGAGGACAAAATGGGGC
AGGGGAGGGAGAAGTTTCTGTCGTTAAAAACAGATTTGGAAAGACTGGACTCTAAA
GTCTGTTGATTAAAGATGAGCTTTGTCTACTTCAAAAGTTTGTTCCTTACCCCTTCA
GCCTCCAATTTTTTAAAGTGAAAATATAGCTAATAACATGTGAAAAGAATAGAAGCTA
AGGTTTAGATAAAATATTGAGCAGATCTATAGGAAGATTGAACCTGAATATTGCCATT
ATGCTTGACATGGTTTCCAAAAAATGGTACTCCACATATTTTCAGTGAGGGTAAGTAT
TTTCCTGTTGTCAAGAATAGCATTGTAAAAGCATTTTGTAATAATAAAGAATAGCTTT
AATGATATGCTTGTAATAAATAATTTTGTAAATGTATCAAATACATTTAAAACATT
AAAATATAATCTCTATAATAAGAGCTCGCTGATCAGCCTCGACTGTGCCTTCTAGTT
GCCAGCCATCTGTTGTTTGCCCTCCCCCGTGCCTTCCTTGACCCTGGAAGGTGCCAC
TCCCCTGTCTTTTCTAATAAAAATGAGGAAATTGCATCGCATTGTCTGAGTAGGTGT
CATTCTATTCTGGGGGGTGGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAGA
CAATAGCAGGCATGCTGGGGATGCGGTGGGCTCTATGGAAGCTTGAATTCAGCTGAC
GTGCCTCGGACCGCTAGGAACCCCTAGTGATGGAGTTGGCCACTCCCTCTCTGCGCG
CTCGCTCGCTCACTGAGGCCGGGCGACCAAAGGTCGCCCCGACGCCGGGCTTTGCCC
GGGCGGCCCTCAGTGAGCGAGCGAGCGCGCAGCTGCCTGCAGGGGGCGCCTGATGCGG
TATTTTCTCCTTACGCATCTGTGCGGTATTTACACCCGCATACGTCAAAGCAACCATA
GTACGCGCCCTGTAGCGGCGCATTAAGCGCGGCGGGTGTGGTGGTTACGCGCAGCGT
GACCGCTACACTTGCCAGCGCCCTAGCGCCCGCTCCTTTTCGCTTTCTTCCCTTCCCTTC
TCGCCACGTTCCCGGCTTTCCCCGTCAAGCTCTAAATCGGGGGCTCCCTTTAGGGTT
CCGATTTAGTGCTTTACGGCACCTCGACCCCAAAAACTTGATTTGGGTGATGGTTC

ACGTAGTGGGCCATCGCCCTGATAGACGGTTTTTCGCCCTTTGACGTTGGAGTCCAC
GTTCTTTAATAGTGGACTCTTGTTCCAAACTGGAACAACACTCAACCCTATCTCGGGC
TATTCTTTTGATTTATAAGGGATTTTGCCGATTTTCGGCCTATTGGTTAAAAAATGAGC
TGATTTAACAAAAATTTAACGCGAATTTTAACAAAATATTAACGTTTACAATTTTATG
GTGCACTCTCAGTACAATCTGCTCTGATGCCGCATAGTTAAGCCAGCCCCGACACCC
GCCAACACCCGCTGACGCGCCCTGACGGGCTTGTCTGCTCCCGGCATCCGCTTACAG
ACAAGCTGTGACCGTCTCCGGGAGCTGCATGTGTCAGAGGTTTTACCGTTCATCACC
GAAACGCGCGAGACGAAAGGGCCTCGTGATACGCCTATTTTTATAGGTTAATGTCAT
GAACAATAAACTGTCTGCTTACATAAACAGTAATACAAGGGGTGTTATGAGCCATA
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AATGATGTTACAGATGAGATGGTCAGACTAACTGGCTGACGGAATTTATGCCTCTT
CCGACCATCAAGCATTTTATCCGTACTCCTGATGATGCATGGTTACTCACCCTGCGA
TCCCCGGAAAAACAGCATTCCAGGTATTAGAAGAATATCCTGATTCAGGTGAAAATA
TTGTTGATGCGCTGGCAGTGTTCTGCGCCGGTTGCATTTCGATTCCTGTTTGTAATTG
TCCTTTTAACAGCGATCGCGTATTTTCGTCTCGCTCAGGCGCAATCACGAATGAATAA
CGGTTTGGTTGATGCGAGTGATTTTGATGACGAGCGTAATGGCTGGCCTGTTGAACA
AGTCTGGAAAGAAATGCATAAACTTTTGCCATTCTCACCGGATTCAGTCGTCCTCA
TGGTGATTTCTCACTTGATAACCTTATTTTTGACGAGGGGAAATTAATAGGTTGTATT
GATGTTGGACGAGTCGGAATCGCAGACCGGATACCAGGATCTTGCCATCCTATGGAAC
TGCTCGGTGAGTTTTCTCCTTCATTACAGAAACGGCTTTTTTCAAAAATATGGTATTG
ATAATCCTGATATGAATAAATTGCAGTTTCATTTGATGCTCGATGAGTTTTTCTAATC
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AACAAAAAAACCACCGCTACCAGCGGTGGTTTGTGTTGCCGGATCAAGAGCTACCAA
CTCTTTTTCCGAAGGTAACCTGGCTTCAGCAGAGCGCAGATACCAAATACTGTCCTTCT
AGTGTAGCCGTAGTTAGGCCACCACTTCAAGAACTCTGTAGCACCGCCTACATACCT
CGCTCTGCTAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCGTGTCTTACC
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TACAGCGTGAGCTATGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGGCGGACAGG
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 TTTTGTGATGCTCGTCAGGGGGGCGGAGCCTATGGAAAAACGCCAGCAACGCGGC
 CTTTTACGGTTCCTGGCCTTTTGCTGGCCTTTTGCTCACATGT

Table 13. Components of Construct Sequence (SEQ ID NO: 104)

Components	Position in construct
5' ITR	12-130
Cloning site	131-147
MMP15 promoter	148-1159
hGJB2 minimal promoter	1160-1287
Cloning site	1288-1296
Synthetic barcode	1297-1304
5' UTR	1305-1666
GJB2 (exon2)	1678-2355
3xFLAG	2368-2433
3' UTR (exon2)	2437-3843
bGHpA	3865-4089
Cloning site	4090-4123
3' ITR	4124-4253

Exemplary Construct sequence (SEQ ID NO: 105)

CCTGCAGGCAGCTGCGCGCTCGCTCGCTCACTGAGGCCGCCGGGCGTCGGGCGACC
 TTTGGTCGCCCGGCCCTCAGTGAGCGAGCGAGCGCGCAGAGAGGGAGTGGCCAACTC
 CATCACTAGGGGTTCTGCGGCCGCACGCGTGGTCAGGCTACCTCTCAGGCTGACTG

AGTCATGCAGCATAGGCTGCCACGTCTCTGGGCTGGCGGGGCGTCATTATTCCTGG
CCTCACTGCAGCTAAATTGAAGAAACGTTTGGTTTGTGGGCCACGTCAAGGAATGTG
TAAGAGCTGCCACGTTGTCGGGTCTGGGTATTGGGCTTTTCCCCTCCTTCAGAGAAG
ATTTCCAGGCGTGTGGGTGGGGTTTCAGAAGAAAATTGATGCCTGCGTGTGAGTGTT
CCCTGGACCTGGACCAGCAGCGGCAATATTACAGACCCGGGGGTGGGGCAGACTG
AGCCAATCTCTGCACCGTCAAAGTTATGGATACAGAGCCCTGAAAAAGGCTGAAG
GATAAGATAGCTGACATTTATGAAGTGCTTCATTCATGTAGCAGTGGGCCAAATGCG
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GGTTGGTGGATGCTCTGCTTCGGGGACCCAGGGCCAGATGACAATGGGTTCTTTGTG
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GACCACGGGGTGGAGGGGAGATAGACCCAGCCCAAAGCTCTGAGGACCCAGAGGCC
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GTATACTCTCGTTGCGGCCCCGCAGCGCCCGCGCGCTCCTCTCCCCGACTCGGAGCC
CCTCGGCGGCGCCCCGGCCCAGGACCCGCCTAGGAGCGCAGGAGCCCCAGCGCAGAG
ACCCCAACGCCGAGACCCCCGCCCCGGCCCCGCGCGCTTCCTCCCGACGCAGTTTA
GGACCCTTGTTTCGCGAAGAGGTGGTGTGCGGCTGAGACCCGCGTCCTCAGGACGGTT
CCATCAGTGCCTCGATCCTGCCCCACTGGAGGAGGAAGGCAGCCCGAACAGCGCTC
ACCTAACTAACAGCTGCTGAGAGCTGGGTTCCGTGGCCATGCACCTGGGACTGCCTT
GAGAAGCGTGAGCAAACCGCCCAGAGTAGAAGCGCTAGCCACCATGGATTGGGGCA
CGCTGCAGACGATCCTGGGGGGTGTGAACAAACACTCCACCAGCATTGGAAAGATC
TGGCTACCGTCCTCTTCATTTTCGCATTATGATCCTCGTTGTGGCTGCAAAGGAGG
TGTGGGGAGATGAGCAGGCCGACTTTGTCTGCAACACCCTGCAGCCAGGCTGCAAG
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CTGATCTTCGTGTCCACGCCAGCGCTCCTAGTGGCCATGCACGTGGCCTACCGGAGA

CATGAGAAGAAGAGGAAGTTCATCAAGGGGGAGATAAAGAGTGAATTTAAGGACAT
CGAGGAGATCAAAACCCAGAAGGTCCGCATCGAAGGCTCCCTGTGGTGGACCTACA
CAAGCAGCATCTTCTTCCGGGTCATCTTCGAAGCCGCCTTCATGTACGTCTTCTATGT
CATGTACGACGGCTTCTCCATGCAGCGGCTGGTGAAGTGCAACGCCTGGCCTTGTCC
CAACACTGTGGACTGCTTTGTGTCCCGGCCACGGAGAAGACTGTCTTCACAGTGTT
CATGATTGCAGTGTCTGGAATTTGCATCCTGCTGAATGTCACTGAATTGTGTTATTTG
CTAATTAGATATTGTTCTGGGAAGTCAAAAAAGCCAGTTGGATCCCGGGCTGACTAC
AAAGACCATGACGGTGATTATAAAGATCATGACATCGACTACAAGGATGACGATGA
CAAGTAAGAAATAGACAGCATGAGAGGGATGAGGCAACCCGTGCTCAGCTGTCAAG
GCTCAGTCGCTAGCATTTCCCAACACAAAGATTCTGACCTTAAATGCAACCATTGTA
AACCCCTGTAGGCCTCAGGTGAAACTCCAGATGCCACAATGGAGCTCTGCTCCCCTA
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CAGACTAGAAGTTCCTAGTGATGGCTTATGATAGCAAATGGCCTCATGTCAAATATT
TAGATGTAATTTTGTGTAAGAAATACAGACTGGATGTACCACCAACTACTACCTGTA
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TGACCCTGGAAGGTGCCACTCCCCTGTCCTTTCCCTAATAAAAATGAGGAAATTGCAT
CGCATTGTCTGAGTAGGTGTCATTCTATTCTGGGGGGTGGGGTGGGGCAGGACAGCA
AGGGGGAGGATTGGGAAGACAATAGCAGGCATGCTGGGGATGCGGTGGGCTCTATG
GAAGCTTGAATTCAGCTGACGTGCCTCGGACCGCTAGGAACCCCTAGTGATGGAGTT
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CCGACGCCCCGGGCTTTGCCCCGGGCGGCCTCAGTGAGCGAGCGAGCGCGCAGCTGCC
TGCAGGGGGCGCCTGATGCGGTATTTTCTCCTTACGCATCTGTGCGGTATTTACACCGG
CATACGTCAAAGCAACCATAGTACGCGCCCTGTAGCGGCGCATTAAAGCGCGGCGGG
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AACTTGATTTGGGTGATGGTTCACGTAGTGGGCCATCGCCCTGATAGACGGTTTTTC
GCCCTTTGACGTTGGAGTCCACGTTCTTTAATAGTGGACTCTTGTTCCAAACTGGAAC
AACACTCAACCCTATCTCGGGCTATTCTTTTGATTTATAAGGGATTTTGCCGATTTG
GCCTATTGGTTAAAAAATGAGCTGATTTAACAAAAATTTAACGCGAATTTTAACAAA
ATATTAACGTTTACAATTTTATGGTGCCTCTCAGTACAATCTGCTCTGATGCCGCAT
AGTTAAGCCAGCCCCGACACCCGCCAACACCCGCTGACGCGCCCTGACGGGCTTGTC
TGCTCCCGGCATCCGCTTACAGACAAGCTGTGACCGTCTCCGGGAGCTGCATGTGTC
AGAGGTTTTTACCGTCATCACCGAAACGCGCGAGACGAAAGGGCCTCGTGATACGC
CTATTTTTATAGGTTAATGTCATGAACAATAAACTGTCTGCTTACATAAACAGTAAT
ACAAGGGGTGTTATGAGCCATATTCAACGGGAAACGTCGAGGCCGCGATTAAATTC
CAACATGGATGCTGATTTATATGGGTATAAATGGGCTCGCGATAATGTCGGGCAATC
AGGTGCGACAATCTATCGCTTGATGGGAAGCCCGATGCGCCAGAGTTGTTTCTGAA
ACATGGCAAAGGTAGCGTTGCCAATGATGTTACAGATGAGATGGTCAGACTAACT
GGCTGACGGAATTTATGCCTCTTCCGACCATCAAGCATTTTATCCGTACTCCTGATGA
TGCATGGTTACTCACCCTGCGATCCCCGGAAAAACAGCATTCCAGGTATTAGAAGA
ATATCCTGATTCAGGTGAAAATATTGTTGATGCGCTGGCAGTGTTCTGCGCCGGTT
GCATTCGATTCCTGTTTGTAATTGTCCTTTTAACAGCGATCGCGTATTTGTCCTCGCTC
AGGCGCAATCACGAATGAATAACGGTTTGGTTGATGCGAGTGATTTTGATGACGAGC
GTAATGGCTGGCCTGTTGAACAAGTCTGGAAAGAAATGCATAAACTTTTGCCATTCT
CACCGGATTCAGTCGTCCTCATGGTGATTTCTCACTTGATAACCTTATTTTTGACGA
GGGGAAATTAATAGGTTGTATTGATGTTGGACGAGTCGGAATCGCAGACCGATACC

AGGATCTTGCCATCCTATGGAAGTGCCTCGGTGAGTTTTCTCCTTCATTACAGAAACG
 GCTTTTTCAAAAATATGGTATTGATAATCCTGATATGAATAAATTGCAGTTTCATTTG
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 CACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTT
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 TTGCCGGATCAAGAGCTACCAACTCTTTTTTCCGAAGGTAAGTGGCTTCAGCAGAGCG
 CAGATACCAAATACTGTCCTTCTAGTGTAGCCGTAGTTAGGCCACCACTTCAAGAAC
 TCTGTAGCACCGCCTACATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTGCCA
 GTGGCGATAAGTCGTGTCTTACCGGGTTGGACTCAAGACGATAGTTACCGGATAAGG
 CGCAGCGGTCTGGGCTGAACGGGGGGTTCGTGCACACAGCCCAGCTTGGAGCGAACG
 ACCTACACCGAACTGAGATACCTACAGCGTGAGCTATGAGAAAGCGCCACGCTTCCC
 GAAGGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTCGGAACAGGAGAGC
 GCACGAGGGAGCTTCCAGGGGGAAACGCCTGGTATCTTTATAGTCCTGTCTGGGTTTC
 GCCACCTCTGACTTGAGCGTCGATTTTTGTGATGCTCGTCAGGGGGGCGGAGCCTAT
 GGAAAAACGCCAGCAACGCGGCCTTTTTACGGTTCCTGGCCTTTTGCTGGCCTTTTGC
 TCACATGT

Tabld 14. Components of Construct Sequence (SEQ ID NO: 105)

Components	Position in construct
5'ITR	12-130
Cloning site	131-147
SPARC promoter	148-1226
hGJB2 minimal promoter	1227-1354
Cloning site	1355-1363
Synthetic barcode	1364-1371
5'UTR	1372-1733
GJB2 (exon2)	1745-2422

3xFLAG	2435-2500
3'UTR (exon2)	2504-3910
bGHpA	3932-4156
Cloning site	4157-4190
3'ITR	4191-4320

Exemplary Construct sequence (SEQ ID NO: 106)

CCTGCAGGCAGCTGCGCGCTCGCTCGCTCACTGAGGCCGCCCGGGCGTCGGGCGACC
 TTTGGTCGCCCCGGCCTCAGTGAGCGAGCGAGCGCGCAGAGAGGGAGTGGCCAACTC
 CATCACTAGGGGTTTCCTGCGGCCGCACGCGTGGTCCAAGGACTCTTTTTTCTAAACTT
 CCCTTCATCTTCTAGTTTGACGCCCTTGGTGGGAAAAGTGTCTGAGATAAGGAAAAG
 GCATCCTTTCAGTTCTCTGATACTATCTTGAAGCGAGGGATGGAGAAAGGCAAAGAG
 AGACACAGGAGAAGCGTATCCCCTGGGAACAGGTGTCTAGTGGAGTCCAGTAACTC
 ACAGTCTCTCAGTTCCGTCAGCACTGTCCCTTGGGTCGCAAATTTCTTCCATTAGCCC
 TTCCACCAGCTGTATTTCAAATGGGGCTGGACAATAATTGTGGCCAGTGGCCTTGTG
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 GATTGTTCTATCTATAGATAGATAGGGAACATTGGCAGTAGGTGGCAGTAAGTTCCC
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GATCCTTGCAACACTGACTTTAGCAGTTAACTAGAGTGGTTGGGGATGAGTATTCT
AAGCTCTGAGGACCCAGAGGCCGGGCGCGCTCCGCCCCGCGGCGCCGCCCTCCGT
AACTTTCCAGTCTCCGAGGGAAGAGGCGGGGTGTGGGGTGCGGTTAAAAGGCGCC
ACGGCGGGAGACAGGTCTCACCGGTGGCACTTCGTTGCGGCCCCGAGCGCCCGCG
CGCTCCTCTCCCCGACTCGGAGCCCCCTCGGCGGGCGCCCCGCCCCAGGACCCGCCTAGG
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CGGCTTTCCCCGTCAAGCTCTAAATCGGGGGCTCCCTTTAGGGTTCCGATTTAGTGCT
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GTCTCCGGGAGCTGCATGTGTCAGAGGTTTTACCGTCATCACCGAAACGCGCGAGA
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TGCGCCAGAGTTGTTTCTGAAACATGGCAAAGGTAGCGTTGCCAATGATGTTACAGA
TGAGATGGTCAGACTAAACTGGCTGACGGAATTTATGCCTCTTCCGACCATCAAGCA
TTTTATCCGTACTCCTGATGATGCATGGTTACTCACCACTGCGATCCCCGGAAAAAC
AGCATTCCAGGTATTAGAAGAATATCCTGATTCAGGTGAAAATATTGTTGATGCGCT
GGCAGTGTTCTGCGCCGGTTGCATTCGATTCCTGTTTGTAAATTGTCCTTTTAACAGC
GATCGCGTATTTTCGTCTCGCTCAGGCGCAATCACGAATGAATAACGGTTTGGTTGAT
GCGAGTGATTTTGATGACGAGCGTAATGGCTGGCCTGTTGAACAAGTCTGGAAAGA
AATGCATAAACTTTTGCCATTCTCACCGGATTCAGTCGTCACCTCATGGTGATTTCTCA
CTTGATAACCTTATTTTTGACGAGGGGAAATTAATAGGTTGTATTGATGTTGGACGA
GTCGGAATCGCAGACCGATACCAGGATCTTGCCATCCTATGGAACTGCCTCGGTGAG
TTTTCTCCTTCATTACAGAAACGGCTTTTTCAAAAATATGGTATTGATAATCCTGATA
TGAATAAATTGCAGTTTCATTTGATGCTCGATGAGTTTTTCTAATCTCATGACCAAAA
TCCCTTAACGTGAGTTTTTCGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAG
GATCTTCTTGAGATCCTTTTTTTCTGCGCGTAATCTGCTGCTTGCAAACAAAAAAACC
ACCGCTACCAGCGGTGGTTTGTGTTGCCGGATCAAGAGCTACCAACTCTTTTTCCGAA
GGTAACTGGCTTCAGCAGAGCGCAGATACCAAATACTGTCCTTCTAGTGTAGCCGTA
GTTAGGCCACCACTTCAAGAACTCTGTAGCACCGCCTACATACCTCGCTCTGCTAAT
CCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCGTGTCTTACCGGGTTGGACTC
AAGACGATAGTTACCGGATAAGGCGCAGCGGTCGGGCTGAACGGGGGGTTCTGTGA
CACAGCCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCTACAGCGTGAG
CTATGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGGCGGACAGGTATCCGGTAAG
CGGCAGGGTCGGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGGAAACGCCTGG
TATCTTTATAGTCCTGTCGGGTTTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGAT
GCTCGTCAGGGGGGCGGAGCCTATGGAAAAACGCCAGCAACGCGGCCTTTTTACGG
TTCTTGCCCTTTTGCTGGCCTTTTGCTCACATGT

Tabl 15. Components of Construct Sequence (SEQ ID NO: 106)

Components	Position in construct
5'ITR	12-130
Cloning site	131-147
TSPAN8 promoter	148-1370
hGJB2 minimal promoter	1371-1498
Cloning site	1499-1507
Synthetic barcode	1508-1515
5'UTR	1516-1877
GJB2 (exon2)	1889-2566
3xFLAG	2579-2644
3'UTR (exon2)	2648-4054
bGHpA	4076-4300
Cloning site	4301-4334
3'ITR	4335-4464

Exemplary Construct sequence (SEQ ID NO: 107)

CCTGCAGGCAGCTGCGCGCTCGCTCGCTCACTGAGGCCGCCGGGCGTCGGGCGACC
 TTTGGTCGCCCCGGCCTCAGTGAGCGAGCGAGCGCGCAGAGAGGGAGTGGCCAACTC
 CATCACTAGGGGTTCCTGCGGCCGACGCGTGGTATTACAATGCATTCCCTCTGCC
 CACCACATTAATTATCAACTCCTTTTCTGGCATTCTACTCATCCAACGCATGGCCCCA
 CGTTAACTTTTCAGTTCCCTTTCTCCCCTACAAATACTCCATAATCCAGCAACCCTGGG
 ATCCCTGAGATGATGAAGAGGACCAGTGCCCATTCAGGAGACATCACCGCAGCCC
 TGAGGAATCGGCTATGGGCACCAGCAGGGCACAGTGCCACACCTCGCCAATGCCTT

GTCTCCTTTTCCATAGTGAGTCAGTCAGCAAGCGTGTAGAAGTGAGTTCCACACTC
TCTTCCTCCCATAGGGAGATCACTTTTCTCATTCTAAGGGTTCCAGGCACACTCACAA
TGGTGGCATTGCTGAGCAGTGGCTTGAATAAAGGGCTCTCAGAAAGCAAGATGTA
ACTCAGAGCATAGGCTAGCCCCAGGAATGCTCTTGGGGAATGACCTGCAGCCTCCCA
GTGAAAGAGAGAATAAAAGAAAGCCCCAGCAGGCGAGCTGGGCAGTAGAGAGTCC
TGTAATTCCACCTTGGCAAGCACCATTTGCAAGAACGAACCTGGGATAAGGTAAACA
AAATATTGCCTAAAAGAGGCTTGTCCAAAGAAGTCAGAATACGCTCTTCATTTACCT
CTAAATTTTCAGTACACCATAAATCTAAATACTCAAAAAAACCTGTGCCTTTTCAATC
AAGGTCAATTGCACGAATTCTTTTGAAAAACAGGACCTATGGCATTTCAGACAAA
TCACCGTGAACCCTGTACTGTGCATTGCTGTCCTAAAATCCAAAGATTCTGTCAATTTG
TGTTACATAATTGCCTTTCATTTGAACTCATTAATCAAATTGGGGTTTTTAAGCAACA
CCTAATTAATTCTTTAACTGGCTCATCCACTGATCACTGAGTTCTATTTTGAACTAC
GGACGTCGAGTTTCCTCTTTCACCCAGAATTTTCAGATCTTGTTTAAAAAGTTGGGTG
TGGTTTCATGGGGGGAGGGGGAAGAGCGAGAGGAGACCAGAGGGACGGGGGCGGG
GACTCTGCAAGAAAAACCTTCCCGGTGCAATCGTGATCTAAGCTCTGAGGACCCAGA
GGCCGGGCGCGCTCCGCCCGCGGCGCCGCCCTCCGTAACCTTCCAGTCTCCGAG
GGAAGAGGCGGGGTGTGGGGTGCGGTTAAAAGGCGCCACGGCGGGAGACAGGTCTC
ACCGTTTTTCAGGTGTTGCGGCCCGCAGCGCCCGCGCGCTCCTCTCCCCGACTCGG
AGCCCCTCGGCGGCGCCCGGCCAGGACCCGCCTAGGAGCGCAGGAGCCCCAGCGC
AGAGACCCCAACGCCGAGACCCCCGCCCGGCCCGCGCGCTTCTCTCCCGACGCGAG
TTTAGGACCTTGTTTCGCGAAGAGGTGGTGTGCGGCTGAGACCCGCGTCCTCAGGAC
GGTTCCATCAGTGCCTCGATCCTGCCCCACTGGAGGAGGAAGGCAGCCCGAACAGC
GCTCACCTAACTAACAGCTGCTGAGAGCTGGGTTCGCTGGCCATGCACCTGGGACTG
CCTTGAGAAGCGTGAGCAAACCGCCCAGAGTAGAAGCGCTAGCCACCATGGATTGG
GGCACGCTGCAGACGATCCTGGGGGGTGTGAACAAACACTCCACCAGCATTGGAAA
GATCTGGCTCACCGTCCTCTTCATTTTTTCGATTATGATCCTCGTTGTGGCTGCAAAG
GAGGTGTGGGGAGATGAGCAGGCCGACTTTGTCTGCAACACCCTGCAGCCAGGCTG
CAAGAACGTGTGCTACGATCACTACTTCCCCATCTCCCACATCCGGCTATGGGCCCT
GCAGCTGATCTTCGTGTCCACGCCAGCGCTCCTAGTGGCCATGCACGTGGCCTACCG
GAGACATGAGAAGAAGAGGAAGTTCATCAAGGGGGAGATAAAGAGTGAATTTAAG
GACATCGAGGAGATCAAAACCCAGAAGGTCCGCATCGAAGGCTCCCTGTGGTGGAC
CTACACAAGCAGCATCTTCTCCGGGTCATCTTCGAAGCCGCCTTCATGTACGTCTTC
TATGTCATGTACGACGGCTTCTCCATGCAGCGGCTGGTGAAGTGCAACGCCTGGCCT

TGTCCCAACACTGTGGACTGCTTTGTGTCCCGGCCACGGAGAAGACTGTCTTCACA
GTGTTTCATGATTGCAGTGTCTGGAATTTGCATCCTGCTGAATGTCACTGAATTGTGTT
ATTTGCTAATTAGATATTGTTCTGGGAAGTCAAAAAAGCCAGTTGGATCCCGGGCTG
ACTACAAAGACCATGACGGTGATTATAAAGATCATGACATCGACTACAAGGATGAC
GATGACAAGTAAGAAATAGACAGCATGAGAGGGATGAGGCAACCCGTGCTCAGCTG
TCAAGGCTCAGTCGCTAGCATTTCCTCAACACAAAGATTCTGACCTTAAATGCAACCA
TTTGAAACCCCTGTAGGCCTCAGGTGAAACTCCAGATGCCACAATGGAGCTCTGCTC
CCCTAAAGCCTCAAAACAAAGGCCTAATTCTATGCCTGTCTTAATTTTCTTTCACTTA
AGTTAGTTCCACTGAGACCCCAGGCTGTTAGGGGTTATTGGTGTAAGGTACTTTTCAT
ATTTTAAACAGAGGATATCGGCATTTGTTTCTTTCTCTGAGGACAAGAGAAAAAAGC
CAGGTTCCACAGAGGACACAGAGAAGGTTTGGGTGTCCTCCTGGGGTTCTTTTTGCC
AACTTTCCCCACGTTAAAGGTGAACATTGGTTCTTTCATTTGCTTTGGAAGTTTAAAT
CTCTAACAGTGGACAAAGTTACCAGTGCCTTAAACTCTGTTACACTTTTTTGAAGTG
AAAACTTTGTAGTATGATAGGTTATTTTGATGTAAAGATGTTCTGGATACCATTATAT
GTTCCCCCTGTTTCAGAGGCTCAGATTGTAATATGTAAATGGTATGTCATTGCTACT
ATGATTTAATTTGAAATATGGTCTTTTGGTTATGAATACTTTGCAGCACAGCTGAGAG
GCTGTCTGTTGTATTTCATTGTGGTCATAGCACCTAACAAACATTGTAGCCTCAATCGAG
TGAGACAGACTAGAAGTTCCTAGTGATGGCTTATGATAGCAAATGGCCTCATGTCAA
ATATTTAGATGTAATTTTGTGTAAGAAATACAGACTGGATGTACCACCAACTACTAC
CTGTAATGACAGGCCTGTCCAACACATCTCCCTTTTCCATGACTGTGGTAGCCAGCAT
CGGAAAGAACGCTGATTTAAAGAGGTCGCTTGGGAATTTTATTGACACAGTACCATT
TAATGGGGAGGACAAAATGGGGCAGGGGAGGGAGAAGTTTCTGTGCTTAAAAACAG
ATTTGGAAAGACTGGACTCTAAAGTCTGTTGATTAAAGATGAGCTTTGTCTACTTCA
AAAGTTTGTGTTGCTTACCCCTTCAGCCTCCAATTTTTTAAGTGAAAATATAGCTAATA
ACATGTGAAAAGAATAGAAGCTAAGGTTTAGATAAATATTGAGCAGATCTATAGGA
AGATTGAACCTGAATATTGCCATTATGCTTGACATGGTTTCCAAAAAATGGTACTCC
ACATATTTAGTGAGGGTAAGTATTTTCTGTTGTCAAGAATAGCATTGTAAAAGCA
TTTTGTAATAATAAAGAATAGCTTTAATGATATGCTTGTAATAAATAATTTTGTA
TGTATCAAATACATTTAAACATTAAATATAATCTCTATAATAAGAGCTCGCTGAT
CAGCCTCGACTGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGGCCCTCCCCGTGCC
TTCCTTGACCCTGGAAGGTGCCACTCCCCTGTCCTTTCCTAATAAAATGAGGAAATT
GCATCGCATTGTCTGAGTAGGTGTCATTCTATTCTGGGGGTGGGGTGGGGCAGGAC
AGCAAGGGGGAGGATTGGGAAGACAATAGCAGGCATGCTGGGGATGCGGTGGGCTC

TATGGAAGCTTGAATTCAGCTGACGTGCCTCGGACCGCTAGGAACCCCTAGTGATGG
AGTTGGCCACTCCCTCTCTGCGCGCTCGCTCGCTCACTGAGGCCGGGCGACCAAAGG
TCGCCCCGACGCCCGGGCTTTGCCCGGGCGGCCTCAGTGAGCGAGCGAGCGCGCAGC
TGCCTGCAGGGGCGCCTGATGCGGTATTTTCTCCTTACGCATCTGTGCGGTATTTTAC
ACCGCATACGTCAAAGCAACCATAGTACGCGCCCTGTAGCGGCGCATTAAGCGCGG
CGGGTGTGGTGGTTACGCGCAGCGTGACCGCTACACTTGCCAGCGCCCTAGCGCCCCG
CTCCTTTCGCTTTCTTCCCTTCCTTTCTCGCCACGTTTCGCCGGCTTTCCCCGTCAAGCT
CTAAATCGGGGGCTCCCTTTAGGGTTCCGATTTAGTGCTTTACGGCACCTCGACCCCCA
AAAAACTTGATTTGGGTGATGGTTCACGTAGTGGGCCATCGCCCTGATAGACGGTTT
TTCGCCCTTTGACGTTGGAGTCCACGTTCTTTAATAGTGGACTCTTGTTCCAAACTGG
AACAACTCAACCCTATCTCGGGCTATTCTTTTGATTTATAAGGGATTTTGCCGATT
TCGGCCTATTGGTTAAAAAATGAGCTGATTTAACAAAAATTTAACGCGAATTTTAAC
AAAATATTAACGTTTACAATTTTATGGTGCCTCTCAGTACAATCTGCTCTGATGCCG
CATAGTTAAGCCAGCCCCGACACCCGCCAACACCCGCTGACGCGCCCTGACGGGCTT
GTCTGCTCCCGGCATCCGCTTACAGACAAGCTGTGACCGTCTCCGGGAGCTGCATGT
GTCAGAGGTTTTACCGTCATCACCGAAACGCGCGAGACGAAAGGGCCTCGTGATA
CGCCTATTTTTATAGGTTAATGTCATGAACAATAAACTGTCTGCTTACATAAACAGT
AATACAAGGGGTGTTATGAGCCATATTCAACGGGAAACGTCGAGGCCGCGATTAA
TTCCAACATGGATGCTGATTTATATGGGTATAAATGGGCTCGCGATAATGTCCGGCA
ATCAGGTGCGACAATCTATCGCTTGTATGGGAAGCCCGATGCGCCAGAGTTGTTTCT
GAAACATGGCAAAGGTAGCGTTGCCAATGATGTTACAGATGAGATGGTCAGACTAA
ACTGGCTGACGGAATTTATGCCTCTTCCGACCATCAAGCATTTTATCCGTACTCCTGA
TGATGCATGGTTACTCACCCTGCGATCCCCGAAAAACAGCATTCCAGGTATTAGA
AGAATATCCTGATTGAGGTGAAAATATTGTTGATGCGCTGGCAGTGTTCCTGCGCCG
GTTGCATTCGATTCTGTTTGTAATTGTCCTTTTAAACAGCGATCGCGTATTTCTGTCTCG
CTCAGGCGCAATCACGAATGAATAACGGTTTGGTTGATGCGAGTGATTTTGATGACG
AGCGTAATGGCTGGCCTGTTGAACAAGTCTGGAAAGAAATGCATAAACTTTTGCCAT
TCTACCGGATTGAGTCGTCATCATGGTGATTTCTCACTTGATAACCTTATTTTTGA
CGAGGGGAAATTAATAGGTTGTATTGATGTTGGACGAGTCGGAATCGCAGACCGAT
ACCAGGATCTTGCCATCCTATGGAAGTGCCTCGGTGAGTTTTCTCCTTCATTACAGAA
ACGGCTTTTTTCAAAAATATGGTATTGATAATCCTGATATGAATAAATTGCAGTTTCAT
TTGATGCTCGATGAGTTTTTCTAATCTCATGACCAAAATCCCTTAACGTGAGTTTTCG
TTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTT

TTTCTGCGCGTAATCTGCTGCTTGCAAACAAAAAAACCACCGCTACCAGCGGTGGTT
 TGTTTGCCGGATCAAGAGCTACCAACTCTTTTCCGAAGGTAAGTGGCTTCAGCAGA
 GCGCAGATACCAAATACTGTCCTTCTAGTGTAGCCGTAGTTAGGCCACCACTTCAAG
 AACTCTGTAGCACCGCCTACATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTG
 CCAGTGGCGATAAGTCGTGTCTTACCGGGTTGGACTCAAGACGATAGTTACCGGATA
 AGGCGCAGCGGTCGGGCTGAACGGGGGGTTCGTGCACACAGCCCAGCTTGGAGCGA
 ACGACCTACACCGAACTGAGATACCTACAGCGTGAGCTATGAGAAAGCGCCACGCT
 TCCCGAAGGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTCGGAACAGGA
 GAGCGCACGAGGGAGCTTCCAGGGGGAAACGCCTGGTATCTTTATAGTCCTGTCGGG
 TTTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGATGCTCGTCAGGGGGGCGGAGC
 CTATGGAAAAACGCCAGCAACGCGGCCTTTTTACGGTTCCTGGCCTTTTGCTGGCCTT
 TTGCTCACATGT

Table 16. Components of Construct Sequence (SEQ ID NO: 107)

Components	Position in construct
5' ITR	12-130
Cloning site	131-147
VIM promoter	148-1234
hGJB2 minimal promoter	1235-1362
Cloning site	1363-1371
Synthetic barcode	1372-1379
5' UTR	1380-1741
GJB2 (exon2)	1753-2430
3xFLAG	2443-2508
3' UTR (exon2)	2512-3918
bGHpA	3940-4164

Cloning site	4165-4198
3' ITR	4199-4328

Pharmaceutical Compositions

[0342] Among other things, the present disclosure provides pharmaceutical compositions. In some aspects, compositions provided herein are suitable for administration to an animal for the amelioration of symptoms associated with syndromic and/or nonsyndromic hearing loss.

[0343] In some aspects, pharmaceutical compositions of the present disclosure may comprise, e.g., a polynucleotide, e.g., one or more constructs, as described herein. In some aspects, a pharmaceutical composition may comprise one or more AAV particles, e.g., one or more rAAV construct encapsidated by one or more AAV serotype capsids, as described herein.

[0344] In some aspects, a pharmaceutical composition comprises one or more pharmaceutically or physiologically acceptable carriers, diluents or excipients. 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 compositions described herein. Such compositions may include 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 aspects, formulations are in a dosage forms, such as injectable solutions, injectable gels, drug-release capsules, and the like.

[0345] In some aspects, compositions of the present disclosure are formulated for intravenous administration. In some aspects compositions of the present disclosure are formulated for intra-cochlear administration. In some aspects, a composition (e.g., a therapeutic composition) is formulated to comprise a lipid nanoparticle, a polymeric nanoparticle, a mini-circle DNA and/or a CELiD DNA.

- [0346]** In some aspects, a composition disclosed herein is formulated as a sterile suspension for intracochlear administration. In some aspects, a composition comprises constructs in an amount of at least 1E11, at least 5E11, at least 1E12, at least 5E12, at least 1E13, at least 2E13, at least 3E13, at least 4E13, at least 5E13, at least 6E13, at least 7E13, at least 8E13, at least 9E13, or at least 1E14 vector genomes (vg) per milliliter (mL). In some aspects, a composition comprises constructs in an amount of at most 1E15, at most 5E14, at most 1E14, at most 5E13, at most 1E13, at most 9E12, at most 8E12, at most 7E12, at most 6E12, at most 5E12, at most 4E12, at most 3E12, at most 2E12, or at most 1E12 vector genomes (vg) per milliliter (mL). In some aspects, a composition comprises constructs in an amount of 1E12 to 1E13, 5E12 to 5E13, or 1E13 to 2E13 vector genomes (vg) per milliliter (mL).
- [0347]** In some aspects, a therapeutic composition is formulated to comprise a synthetic perilymph solution. For example, in some aspects, a synthetic perilymph solution includes 20-200mM NaCl; 1-5 mM KCl; 0.1-10mM CaCl₂; 1-10mM glucose; and 2-50 mM HEPES, with a pH between about 6 and about 9. In some aspects, a therapeutic composition is formulated to comprise a physiologically suitable solution. For example, in some aspects, a physiologically suitable solution comprises commercially available 1xPBS with pluronic acid F68, prepared to a final concentration of: 8.10mM Sodium Phosphate Dibasic, 1.5mM Monopotassium Phosphate, 2.7mM Potassium Chloride, 172mM Sodium Chloride, and 0.001% Pluronic Acid F68). In some aspects, alternative pluronic acids are utilized. In some aspects, alternative ion concentrations are utilized.
- [0348]** In some aspects, any of the pharmaceutical compositions described herein may further comprise one or more agents that promote the entry of a nucleic acid or any of the constructs described herein into a mammalian cell (e.g., a liposome or cationic lipid). In some aspects, any of the constructs 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, each of which is incorporated in its entirety herein by reference).

[0349] In some aspects, a 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 a dosage formulation and in such amount as is therapeutically effective. Formulations are easily administered in a variety of dosage forms such as injectable solutions, injectable gels, drug-release capsules, and the like.

[0350] In some aspects, a composition 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 aspects, a provided composition comprises one nucleic acid construct. In some aspects, a provided composition comprises two or more different constructs. In some aspects, a composition that include a single nucleic acid construct comprising a coding sequence that encodes a polypeptide (e.g., a therapeutic polypeptide, a Connexin 26 polypeptide) and/or a functional characteristic portion thereof. In some aspects, compositions comprise a single nucleic acid construct comprising a coding sequence that encodes a polypeptide (e.g., a therapeutic polypeptide, a Connexin 26 polypeptide) and/or a functional characteristic portion thereof, which, when introduced into a mammalian cell, that coding sequence is integrated into the genome of the mammalian cell.

[0351] Also provided are kits including any of the compositions described herein. In some aspects, a kit can include a solid composition (e.g., a lyophilized composition including the at least two different constructs described herein) and a liquid for solubilizing the lyophilized composition. In some aspects, a kit can include a pre-loaded syringe including any of the compositions described herein.

[0352] In some aspects, 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)..

[0353] In some aspects, the kits can include instructions for performing any of the methods described herein.

Genetically Modified Cells

[0354] The present disclosure also provides a cell (e.g., an animal cell, e.g., a mammalian cell, e.g., a primate cell, e.g., a human cell) that includes any of the nucleic acids, constructs or compositions described herein. In some aspects, an animal cell is a human cell (e.g., a human supporting cell or a human hair cell). In other aspects, an animal cell is a non-human mammal (e.g., Simian cell, Felidae cell, Canidae cell etc.). A person skilled in the art will appreciate that the nucleic acids and constructs described herein can be introduced into any animal cell (e.g., the supporting or hair cells of any animal suitable for veterinary intervention). Non-limiting examples of constructs and methods for introducing constructs into animal cells are described herein.

[0355] In some aspects, an animal cell can be any cell of the inner ear, including hair and/or supporting cells. Non-limiting examples such cells include: Hensen's cells, Deiters' cells, cells of the endolymphatic sac and duct, transitional cells in the saccule, utricle, and ampulla, inner and outer hair cells, spiral ligament cells, spiral ganglion cells, spiral prominence cells, external saccule cells, marginal cells, intermediate cells, basal cells, inner pillar cells, outer pillar cells, Claudius cells, inner border cells, inner phalangeal cells, or cells of the stria vascularis.

[0356] In some aspects, an animal cell is a specialized cell of the cochlea. In some aspects, an animal cell is a hair cell. In some aspects, an animal cell is a cochlear inner hair cell or a cochlear outer hair cell. In some aspects, an animal cell is a cochlear inner hair cell. In some aspects, an animal cell is a cochlear outer hair cell.

[0357] In some aspects, an animal cell is in vitro. In some aspects, an animal cell is of a cell type which is endogenously present in an animal, e.g., in a primate and/or human. In some aspects, an animal cell is an autologous cell obtained from an animal and cultured ex vivo. In some aspects, the ex vivo cell is an inner ear cell. In some aspects, the ex vivo cell is an inner ear supporting cell. In some aspects, the ex vivo cell is an inner phalangeal cells/border cells (IPhC), inner pillar cells (IPC), outer pillar cells (OPC), Deiters' cells rows 1 and 2 (DC1/2), Deiters' cells row 3 (DC3), Hensen's cells (Hec),

Claudius cells/outer sulcus cells (CC/OSC), interdental cells (Idc), inner sulcus cells (ISC), Kölliker's organ cells (KO), Lateral greater epithelial ridge cells (LGER), and OC90+ cells (OC90). In some aspects, the ex vivo cell is an inner phalangeal cells/border cells (IPhC), inner pillar cells (IPC), outer pillar cells (OPC), Deiters' cells rows 1 and 2 (DC1/2), Deiters' cells row 3 (DC3), Hensen's cells (Hec), Claudius cells/outer sulcus cells (CC/OSC), interdental cells (Idc), inner sulcus cells (ISC), Kölliker's organ cells (KO), greater ridge epithelial ridge cells (GER) (including lateral greater epithelial ridge cells (LGER)), and OC90+ cells (OC90), fibroblasts, and other cells of the lateral wall.

Methods

[0358] Among other things, the present disclosure provides methods. In some aspects, a method comprises introducing a composition, construct, or polynucleotide as described herein into the inner ear (e.g., a cochlea) of a subject. For example, provided herein are methods that in some aspects include administering to an inner ear (e.g., cochlea) of a subject (e.g., an animal, e.g., a mammal, e.g., a primate, e.g., a human) a therapeutically effective amount of any composition, construct, or polynucleotide described herein. In some aspects of any of these methods, the subject has been previously identified as having a defective inner ear cell target gene (e.g., a supporting and/or hearing cell target gene having a mutation that results in a decrease in the expression and/or activity of a supporting and/or hearing cell target protein encoded by the gene). Some aspects of any of these methods further include, prior to the introducing or administering step, determining that the subject has a defective inner ear cell target gene. Some aspects of any of these methods can further include detecting a mutation in an inner ear cell target gene in a subject. Some aspects of any of the methods can further include identifying or diagnosing a subject as having nonsyndromic or syndromic sensorineural hearing loss.

[0359] In some aspects, provided herein are methods of correcting an inner ear cell target gene defect in an inner ear of a subject, e.g., an animal, e.g., a mammal, e.g., a primate, e.g., a human. In some aspects, methods include administering to the inner ear of a subject a therapeutically effective amount of any of the compositions described herein, where the administering repairs and or ameliorates the inner ear cell target gene defect in any cell subset of the inner ear of a subject. In some aspects, the inner ear target cell may be a sensory cell, e.g., a hair cell, and/or a non-sensory cell, e.g., a supporting cell, and/or all or any subset of inner ear cells.

- [0360]** Also provided herein are methods of promoting expression (e.g., increasing the expression level) of an inner ear cell target protein in any subset of inner ear cells of a subject (e.g., an animal, e.g., a mammal, e.g., a primate, e.g., a human) that include: administering to the inner ear of the subject a therapeutically effective amount of any of the compositions described herein, where the administering results in an increase in the expression level of the inner ear cell target protein (e.g., a polypeptide (e.g., a therapeutic polypeptide, a Connexin 26 polypeptide)) in any cell subset of the inner ear of a subject. In some aspects, the inner ear target cell may be a sensory cell, e.g., a hair cell, and/or a non-sensory cell, e.g., a supporting cell, and/or all or any subset of inner ear cells.
- [0361]** Also provided herein are methods of treating hearing loss, e.g., nonsyndromic sensorineural hearing loss or syndromic sensorineural hearing loss, in a subject (e.g., an animal, e.g., a mammal, e.g., a primate, e.g., a human) identified as having a defective inner ear cell target gene that include: administering to the inner ear of the subject a therapeutically effective amount of any of the compositions described herein.
- [0362]** Also provided herein are methods of restoring synapses and/or preserving spiral ganglion nerves in a subject identified or diagnosed as having an inner ear disorder that include: administering to the inner ear of the subject a therapeutically effective amount of any of the compositions described herein.
- [0363]** Also provided herein are methods of reducing the size of, and/or restoring the vestibular aqueduct to an appropriate size. Also provided herein are methods of restoring endolymphatic pH to an appropriate and/or acceptable level in a subject identified or diagnosed as having an inner ear disorder that include: administering to the inner ear of the subject a therapeutically effective amount of any of the compositions described herein.
- [0364]** Also provided herein are methods of expressing a polypeptide (e.g., a therapeutic polypeptide, a Connexin 26 polypeptide) in an inner ear supporting cell of a subject in need thereof. In some aspects, the inner ear supporting cells are selected from one or more of inner phalangeal cells/border cells (IPhC), inner pillar cells (IPC), outer pillar cells (OPC), Deiters' cells rows 1 and 2 (DC1/2), Deiters' cells row 3 (DC3), Hensen's cells (Hec), Claudius cells/outer sulcus cells (CC/OSC), interdental cells (Idc), inner sulcus cells (ISC), Kölliker's organ cells (KO), Lateral greater epithelial ridge cells (LGER), and OC90+ cells (OC90). In some aspects, the inner ear supporting cells are selected from one or more of inner phalangeal cells/border cells (IPhC), inner pillar cells

(IPC), outer pillar cells (OPC), Deiters' cells rows 1 and 2 (DC1/2), Deiters' cells row 3 (DC3), Hensen's cells (Hec), Claudius cells/outer sulcus cells (CC/OSC), interdental cells (Idc), inner sulcus cells (ISC), Kölliker's organ cells (KO), greater ridge epithelial ridge cells (GER) (including lateral greater epithelial ridge cells (LGER)), and OC90+ cells (OC90), fibroblasts, and other cells of the lateral wall.

[0365] In some aspects, expression of the polypeptide (e.g., therapeutic polypeptide, a Connexin 26 polypeptide) is reduced, suppressed, or eliminated in inner ear hair cells, spiral ganglion cells, lateral supporting cells, basilar membrane cells, medial supporting cells, spiral limbus cells, or any combination thereof. In some aspects, toxicity due to expression of the therapeutic polypeptide (e.g., a Connexin 26 polypeptide) is reduced in inner ear hair cells, spiral ganglion cells, lateral supporting cells, basilar membrane cells, medial supporting cells, spiral limbus cells, or any combination thereof. In some aspects, the therapeutic protein is predominately expressed in inner ear supporting cells. In some aspects, the inner ear supporting cells are selected from one or more of inner phalangeal cells/border cells (IPhC), inner pillar cells (IPC), outer pillar cells (OPC), Deiters' cells rows 1 and 2 (DC1/2), Deiters' cells row 3 (DC3), Hensen's cells (Hec), Claudius cells/outer sulcus cells (CC/OSC), interdental cells (Idc), inner sulcus cells (ISC), Kölliker's organ cells (KO), greater ridge epithelial ridge cells (GER) (including lateral greater epithelial ridge cells (LGER)), and OC90+ cells (OC90), fibroblasts, and other cells of the lateral wall.

[0366] In some aspects, expression of the polypeptide is reduced, suppressed, or eliminated in inner ear hair cells, spiral ganglion cells, lateral supporting cells, basilar membrane cells, medial supporting cells, spiral limbus cells, or any combination thereof. In some aspects, toxicity due to expression of the polypeptide is reduced in inner ear hair cells, spiral ganglion cells, lateral supporting cells, basilar membrane cells, medial supporting cells, spiral limbus cells, or any combination thereof. In some aspects, the protein is predominately expressed in inner ear supporting cells. In some aspects, the inner ear supporting cells are selected from one or more of inner phalangeal cells/border cells (IPhC), inner pillar cells (IPC), outer pillar cells (OPC), Deiters' cells rows 1 and 2 (DC1/2), Deiters' cells row 3 (DC3), Hensen's cells (Hec), Claudius cells/outer sulcus cells (CC/OSC), interdental cells (Idc), inner sulcus cells (ISC), Kölliker's organ cells (KO), greater ridge epithelial ridge cells (GER) (including lateral greater epithelial ridge cells (LGER)), and OC90+ cells (OC90), fibroblasts, and other cells of the lateral wall.

[0367] In some aspects, administration is to the inner ear of the subject. In some aspects, the administration is to the cochlea of the subject. In some aspects, the administration is via a round window membrane injection.

[0368] Also provided herein are methods of increasing expression of a therapeutic polypeptide (e.g., a Connexin 26 polypeptide) in an inner ear supporting cell of a subject in need thereof. In some aspects, the expression of the therapeutic polypeptide (e.g., a Connexin 26 polypeptide) is reduced, suppressed, or eliminated in non-inner ear supporting cells. In some aspects, the inner ear supporting cells are selected from one or more of inner phalangeal cells/border cells (IPhC), inner pillar cells (IPC), outer pillar cells (OPC), Deiters' cells rows 1 and 2 (DC1/2), Deiters' cells row 3 (DC3), Hensen's cells (Hec), Claudius cells/outer sulcus cells (CC/OSC), interdental cells (Idc), inner sulcus cells (ISC), Kölliker's organ cells (KO), greater ridge epithelial ridge cells (GER) (including lateral greater epithelial ridge cells (LGER)), and OC90+ cells (OC90), fibroblasts, and other cells of the lateral wall. In some aspects, expression of the therapeutic polypeptide (e.g., a Connexin 26 polypeptide) is reduced, suppressed, or eliminated in inner ear hair cells, spiral ganglion cells, lateral supporting cells, basilar membrane cells, medial supporting cells, spiral limbus cells, or any combination thereof. In some aspects, toxicity due to expression of the therapeutic polypeptide (e.g., Connexin 26 polypeptide) is reduced in inner ear hair cells, spiral ganglion cells, lateral supporting cells, basilar membrane cells, medial supporting cells, spiral limbus cells, or any combination thereof. In some aspects, the therapeutic protein is predominately expressed in inner ear supporting cells. In some aspects, the inner ear supporting cells are selected from one or more of inner phalangeal cells/border cells (IPhC), inner pillar cells (IPC), outer pillar cells (OPC), Deiters' cells rows 1 and 2 (DC1/2), Deiters' cells row 3 (DC3), Hensen's cells (Hec), Claudius cells/outer sulcus cells (CC/OSC), interdental cells (Idc), inner sulcus cells (ISC), Kölliker's organ cells (KO), greater ridge epithelial ridge cells (GER) (including lateral greater epithelial ridge cells (LGER)), and OC90+ cells (OC90), fibroblasts, and other cells of the lateral wall.

[0369] Also provided herein are methods of promoting expression (e.g., increasing expression) of a polypeptide in an inner ear supporting cell of a subject in need thereof. In some aspects, the expression of the polypeptide is reduced, suppressed, or eliminated in non-inner ear supporting cells. In some aspects, the inner ear supporting cells are selected from one or more of inner phalangeal cells/border cells (IPhC), inner pillar cells

(IPC), outer pillar cells (OPC), Deiters' cells rows 1 and 2 (DC1/2), Deiters' cells row 3 (DC3), Hensen's cells (Hec), Claudius cells/outer sulcus cells (CC/OSC), interdental cells (Idc), inner sulcus cells (ISC), Kölliker's organ cells (KO), greater ridge epithelial ridge cells (GER) (including lateral greater epithelial ridge cells (LGER)), and OC90+ cells (OC90), fibroblasts, and other cells of the lateral wall. In some aspects, expression of the polypeptide is reduced, suppressed, or eliminated in inner ear hair cells, spiral ganglion cells, lateral supporting cells, basilar membrane cells, medial supporting cells, spiral limbus cells, or any combination thereof. In some aspects, toxicity due to expression of the polypeptide is reduced in inner ear hair cells, spiral ganglion cells, lateral supporting cells, basilar membrane cells, medial supporting cells, spiral limbus cells, or any combination thereof. In some aspects, the protein is predominately expressed in inner ear supporting cells. In some aspects, the inner ear supporting cells are selected from one or more of inner phalangeal cells/border cells (IPhC), inner pillar cells (IPC), outer pillar cells (OPC), Deiters' cells rows 1 and 2 (DC1/2), Deiters' cells row 3 (DC3), Hensen's cells (Hec), Claudius cells/outer sulcus cells (CC/OSC), interdental cells (Idc), inner sulcus cells (ISC), Kölliker's organ cells (KO), greater ridge epithelial ridge cells (GER) (including lateral greater epithelial ridge cells (LGER)), and OC90+ cells (OC90), fibroblasts, and other cells of the lateral wall.

[0370] In some aspects, administration is to the inner ear of the subject. In some aspects, the administration is to the cochlea of the subject. In some aspects, the administration is via a round window membrane injection.

[0371] Also provided herein are methods of decreasing expression of the therapeutic polypeptide (e.g., Connexin 26 polypeptide) in non-inner ear supporting cells. In some aspects, the inner ear supporting cells are selected from one or more of inner phalangeal cells/border cells (IPhC), inner pillar cells (IPC), outer pillar cells (OPC), Deiters' cells rows 1 and 2 (DC1/2), Deiters' cells row 3 (DC3), Hensen's cells (Hec), Claudius cells/outer sulcus cells (CC/OSC), interdental cells (Idc), inner sulcus cells (ISC), Kölliker's organ cells (KO), Lateral greater epithelial ridge cells (LGER), and OC90+ cells (OC90). In some aspects, the inner ear supporting cells are selected from one or more of inner phalangeal cells/border cells (IPhC), inner pillar cells (IPC), outer pillar cells (OPC), Deiters' cells rows 1 and 2 (DC1/2), Deiters' cells row 3 (DC3), Hensen's cells (Hec), Claudius cells/outer sulcus cells (CC/OSC), interdental cells (Idc), inner sulcus cells (ISC), Kölliker's organ cells (KO), greater ridge epithelial ridge cells (GER)

(including lateral greater epithelial ridge cells (LGER)), and OC90+ cells (OC90), fibroblasts, and other cells of the lateral wall.

[0372] Also provided herein are methods of decreasing expression of the polypeptide in non-inner ear supporting cells. In some aspects, the inner ear supporting cells are selected from one or more of inner phalangeal cells/border cells (IPhC), inner pillar cells (IPC), outer pillar cells (OPC), Deiters' cells rows 1 and 2 (DC1/2), Deiters' cells row 3 (DC3), Hensen's cells (Hec), Claudius cells/outer sulcus cells (CC/OSC), interdental cells (Idc), inner sulcus cells (ISC), Kölliker's organ cells (KO), Lateral greater epithelial ridge cells (LGER), and OC90+ cells (OC90). In some aspects, the inner ear supporting cells are selected from one or more of inner phalangeal cells/border cells (IPhC), inner pillar cells (IPC), outer pillar cells (OPC), Deiters' cells rows 1 and 2 (DC1/2), Deiters' cells row 3 (DC3), Hensen's cells (Hec), Claudius cells/outer sulcus cells (CC/OSC), interdental cells (Idc), inner sulcus cells (ISC), Kölliker's organ cells (KO), greater ridge epithelial ridge cells (GER) (including lateral greater epithelial ridge cells (LGER)), and OC90+ cells (OC90), fibroblasts, and other cells of the lateral wall.

[0373] In some aspects, expression of the therapeutic polypeptide (e.g., Connexin 26 polypeptide) is reduced, suppressed, or eliminated in inner ear hair cells, spiral ganglion cells, lateral supporting cells, basilar membrane cells, medial supporting cells, spiral limbus cells, or any combination thereof. In some aspects, toxicity due to expression of the therapeutic polypeptide (e.g., Connexin 26 polypeptide) is reduced in inner ear hair cells, spiral ganglion cells, lateral supporting cells, basilar membrane cells, medial supporting cells, spiral limbus cells, or any combination thereof. In some aspects, the therapeutic protein is predominately expressed in inner ear supporting cells. In some aspects, the inner ear supporting cells are selected from one or more of inner phalangeal cells/border cells (IPhC), inner pillar cells (IPC), outer pillar cells (OPC), Deiters' cells rows 1 and 2 (DC1/2), Deiters' cells row 3 (DC3), Hensen's cells (Hec), Claudius cells/outer sulcus cells (CC/OSC), interdental cells (Idc), inner sulcus cells (ISC), Kölliker's organ cells (KO), greater ridge epithelial ridge cells (GER) (including lateral greater epithelial ridge cells (LGER)), and OC90+ cells (OC90), fibroblasts, and other cells of the lateral wall. In some aspects, administration is to the inner ear of the subject. In some aspects, the administration is to the cochlea of the subject. In some aspects, the administration is via a round window membrane injection.

[0374] In some aspects, expression of the polypeptide is reduced, suppressed, or eliminated in inner ear hair cells, spiral ganglion cells, lateral supporting cells, basilar membrane cells, medial supporting cells, spiral limbus cells, or any combination thereof. In some aspects, toxicity due to expression of the polypeptide is reduced in inner ear hair cells, spiral ganglion cells, lateral supporting cells, basilar membrane cells, medial supporting cells, spiral limbus cells, or any combination thereof. In some aspects, the protein is predominately expressed in inner ear supporting cells. In some aspects, the inner ear supporting cells are selected from one or more of inner phalangeal cells/border cells (IPhC), inner pillar cells (IPC), outer pillar cells (OPC), Deiters' cells rows 1 and 2 (DC1/2), Deiters' cells row 3 (DC3), Hensen's cells (Hec), Claudius cells/outer sulcus cells (CC/OSC), interdental cells (Idc), inner sulcus cells (ISC), Kölliker's organ cells (KO), greater ridge epithelial ridge cells (GER) (including lateral greater epithelial ridge cells (LGER)), and OC90+ cells (OC90), fibroblasts, and other cells of the lateral wall. In some aspects, administration is to the inner ear of the subject. In some aspects, the administration is to the cochlea of the subject. In some aspects, the administration is via a round window membrane injection.

[0375] Also provided herein are methods of reducing toxicity associated with expression of the therapeutic polypeptide (e.g., Connexin 26 polypeptide) in an inner ear cell. In some aspects, the inner ear cells are inner ear hair cells, spiral ganglion cells, lateral supporting cells, basilar membrane cells, medial supporting cells, spiral limbus cells, or any combination thereof. In some aspects, expression of the therapeutic polypeptide (e.g., Connexin 26 polypeptide) is reduced, suppressed, or eliminated in inner ear hair cells, spiral ganglion cells, lateral supporting cells, basilar membrane cells, medial supporting cells, spiral limbus cells, or any combination thereof. In some aspects, toxicity due to expression of the therapeutic polypeptide (e.g., Connexin 26 polypeptide) is reduced in inner ear hair cells, spiral ganglion cells, lateral supporting cells, basilar membrane cells, medial supporting cells, spiral limbus cells, or any combination thereof. In some aspects, the therapeutic protein (e.g., Connexin 26 polypeptide) is predominately expressed in inner ear supporting cells. In some aspects, the inner ear supporting cells are selected from one or more of inner phalangeal cells/border cells (IPhC), inner pillar cells (IPC), outer pillar cells (OPC), Deiters' cells rows 1 and 2 (DC1/2), Deiters' cells row 3 (DC3), Hensen's cells (Hec), Claudius cells/outer sulcus cells (CC/OSC), interdental cells (Idc), inner sulcus cells (ISC), Kölliker's organ cells (KO), greater ridge epithelial ridge cells

(GER) (including lateral greater epithelial ridge cells (LGER)), and OC90+ cells (OC90), fibroblasts, and other cells of the lateral wall. In some aspects, administration is to the inner ear of the subject. In some aspects, the administration is to the cochlea of the subject. In some aspects, the administration is via a round window membrane injection.

[0376] Also provided herein are methods of reducing toxicity associated with expression of the polypeptide in an inner ear cell. In some aspects, the inner ear cells are inner ear hair cells, spiral ganglion cells, lateral supporting cells, basilar membrane cells, medial supporting cells, spiral limbus cells, or any combination thereof. In some aspects, expression of the polypeptide is reduced, suppressed, or eliminated in inner ear hair cells, spiral ganglion cells, lateral supporting cells, basilar membrane cells, medial supporting cells, spiral limbus cells, or any combination thereof. In some aspects, toxicity due to expression of the polypeptide is reduced in inner ear hair cells, spiral ganglion cells, lateral supporting cells, basilar membrane cells, medial supporting cells, spiral limbus cells, or any combination thereof. In some aspects, the protein is predominately expressed in inner ear supporting cells. In some aspects, the inner ear supporting cells are selected from one or more of inner phalangeal cells/border cells (IPhC), inner pillar cells (IPC), outer pillar cells (OPC), Deiters' cells rows 1 and 2 (DC1/2), Deiters' cells row 3 (DC3), Hensen's cells (Hec), Claudius cells/outer sulcus cells (CC/OSC), interdental cells (Idc), inner sulcus cells (ISC), Kölliker's organ cells (KO), greater ridge epithelial ridge cells (GER) (including lateral greater epithelial ridge cells (LGER)), and OC90+ cells (OC90), fibroblasts, and other cells of the lateral wall. In some aspects, administration is to the inner ear of the subject. In some aspects, the administration is to the cochlea of the subject. In some aspects, the administration is via a round window membrane injection.

[0377] Also provided herein are methods of treating hearing loss in a subject suffering from or at risk of hearing loss. In some aspects, expression of the therapeutic polypeptide (e.g., Connexin 26 polypeptide) is reduced, suppressed, or eliminated in inner ear hair cells, spiral ganglion cells, lateral supporting cells, basilar membrane cells, medial supporting cells, spiral limbus cells, or any combination thereof. In some aspects, toxicity due to expression of the therapeutic polypeptide (e.g., Connexin 26 polypeptide) is reduced in inner ear hair cells, spiral ganglion cells, lateral supporting cells, basilar membrane cells, medial supporting cells, spiral limbus cells, or any combination thereof. In some aspects, the therapeutic protein (e.g., Connexin 26) is predominately expressed in inner ear supporting cells. In some aspects, the inner ear supporting cells are selected

from one or more of inner phalangeal cells/border cells (IPhC), inner pillar cells (IPC), outer pillar cells (OPC), Deiters' cells rows 1 and 2 (DC1/2), Deiters' cells row 3 (DC3), Hensen's cells (Hec), Claudius cells/outer sulcus cells (CC/OSC), interdental cells (Idc), inner sulcus cells (ISC), Kölliker's organ cells (KO), greater ridge epithelial ridge cells (GER) (including lateral greater epithelial ridge cells (LGER)), and OC90+ cells (OC90), fibroblasts, and other cells of the lateral wall.

[0378] Also provided herein are methods of treating hearing loss in a subject suffering from or at risk of hearing loss. In some aspects, expression of the polypeptide is reduced, suppressed, or eliminated in inner ear hair cells, spiral ganglion cells, lateral supporting cells, basilar membrane cells, medial supporting cells, spiral limbus cells, or any combination thereof. In some aspects, toxicity due to expression of the polypeptide is reduced in inner ear hair cells, spiral ganglion cells, lateral supporting cells, basilar membrane cells, medial supporting cells, spiral limbus cells, or any combination thereof. In some aspects, the protein is predominately expressed in inner ear supporting cells. In some aspects, the inner ear supporting cells are selected from one or more of inner phalangeal cells/border cells (IPhC), inner pillar cells (IPC), outer pillar cells (OPC), Deiters' cells rows 1 and 2 (DC1/2), Deiters' cells row 3 (DC3), Hensen's cells (Hec), Claudius cells/outer sulcus cells (CC/OSC), interdental cells (Idc), inner sulcus cells (ISC), Kölliker's organ cells (KO), greater ridge epithelial ridge cells (GER) (including lateral greater epithelial ridge cells (LGER)), and OC90+ cells (OC90), fibroblasts, and other cells of the lateral wall.

[0379] In some aspects, administration is to the inner ear of the subject. In some aspects, the administration is to the cochlea of the subject. In some aspects, the administration is via a round window membrane injection.

[0380] Also provided herein are methods that include administering to an inner ear of a subject a therapeutically effective amount of any of the compositions described herein.

[0381] Also provided herein are surgical methods for treatment of hearing loss (e.g., nonsyndromic sensorineural hearing loss or syndromic sensorineural hearing loss). In some aspects, the methods include the steps of: introducing into a cochlea of a 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 aspects, the composition is administered to the subject at the first incision point. In some aspects, the composition is administered to the subject into or through the first incision.

- [0382]** In some aspects of any of the methods described herein, any composition described herein is administered to the subject into or through the cochlea oval window membrane. In some aspects 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 aspects 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 aspects, the medical device includes a plurality of micro-needles. In some aspects, 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 aspects, the medical device includes a base and/or a reservoir capable of holding the composition. In some aspects, the medical device includes a plurality of hollow micro-needles individually including a lumen capable of transferring the composition. In some aspects, the medical device includes a means for generating at least a partial vacuum.
- [0383]** In some aspects, technologies of the present disclosure are used to treat subjects with or at risk of hearing loss. In some such aspects, a pathogenic variant causes or is at risk of causing hearing loss.
- [0384]** In some aspects, a subject experiencing hearing loss will be evaluated to determine if and where one or more mutations may exist that may cause hearing loss. In some aspects of any of the methods described herein, the subject or animal is a mammal, in some aspects the mammal is a domestic animal, a farm animal, a zoo animal, a non-human primate, or a human. In some aspects of any of the methods described herein, the animal, subject, or mammal is an adult, a teenager, a juvenile, a child, a toddler, an infant, or a newborn. In some aspects of any of the methods described herein, the animal, 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 aspects 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.

- [0385] In some aspects of any of the methods described herein, the methods result in improvement in hearing (e.g., any of the metrics for determining improvement in hearing described herein) in a subject in need thereof for at least 10 days, at least 15 days, at least 20 days, at least 25 days, at least 30 days, at least 35 days, at least 40 days, at least 45 days, at least 50 days, at least 55 days, at least 60 days, at least 65 days, at least 70 days, at least 75 days, at least 80 days, at least 85 days, at least 100 days, at least 105 days, at least 110 days, at least 115 days, at least 120 days, at least 5 months, at least 6 months, at least 7 months, at least 8 months, at least 9 months, at least 10 months, at least 11 months, or at least 12 months.
- [0386] In some aspects a subject (e.g., an animal, e.g., a mammal, e.g., a human) has or is at risk of developing syndromic or nonsyndromic sensorineural hearing loss.
- [0387] In some aspects, a subject (e.g., an animal, e.g., a mammal, e.g., a human) has been identified as having syndromic or nonsyndromic sensorineural hearing loss.
- [0388] In some aspects, a subject (e.g., an animal, e.g., a mammal, e.g., a human) has been identified as being at risk of hearing loss (e.g., at risk of being a carrier of a gene mutation,). In some such aspects, a subject (e.g., an animal, e.g., a mammal, e.g., a human) may have certain risk factors of hearing loss or risk of hearing loss (e.g., known parental carrier, afflicted sibling, or symptoms of hearing loss). In some such aspects, a subject (e.g., an animal, e.g., a mammal, e.g., a human) has been identified as being a carrier of a mutation in a gene (e.g., via genetic testing) that has not previously been identified (). In some such aspects, identified mutations may be novel (i.e., not previously described in the literature), and methods of treatment for a subject suffering from or susceptible to hearing loss will be personalized to the mutation(s) of the particular patient.
- [0389] In some aspects, successful treatment of syndromic or nonsyndromic sensorineural hearing loss 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).
- [0390] In some aspects of any method provided herein, two or more doses of any composition described herein are introduced or administered into a cochlea of a subject. Some aspects of any of these methods can include introducing or administering a first dose of a composition into a cochlea of a subject, assessing hearing function of the

subject following introduction or administration of a first dose, and administering an additional dose of a composition into the cochlea of the 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).

[0391] In some aspects of any method provided herein, the composition can be formulated for intra-cochlear administration. In some aspects of any of the methods described herein, the compositions described herein can be administered via intra-cochlear administration or local administration. In some aspects 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).

[0392] In some aspects, intra-cochlear administration can be performed using any of the methods described herein or known in the art. For example, in some aspects, 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 curette 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.

[0393] In some aspects of any method provided herein, a subject has or is at risk of developing syndromic or nonsyndromic sensorineural hearing loss. In some aspects of any method provided herein, a subject has been previously identified as having a mutation in an inner ear cell target gene, a gene which may be expressed in supporting cells and/or hair cells.

- [0394]** In some aspects of any method provided herein, a subject has been identified as being a carrier of a mutation in an inner ear cell target gene (e.g., via genetic testing). In some aspects of any method provided herein, a subject has been identified as having a mutation in an inner ear cell target gene and has been diagnosed with hearing loss (e.g., nonsyndromic sensorineural hearing loss or syndromic sensorineural hearing loss, e.g., DFNB1, DFNA3). Bart-Pumphrey syndrome, hystrix-like ichthyosis with deafness (HID), palmoplantar keratoderma with deafness, keratitis-ichthyosis-deafness (KID) syndrome, or Vohwinkel syndrome, respectively). In some aspects of any of the methods described herein, the subject has been identified as having hearing loss (e.g., nonsyndromic sensorineural hearing loss or syndromic sensorineural hearing loss). In some aspects, successful treatment of hearing loss (e.g., nonsyndromic sensorineural hearing loss or syndromic sensorineural hearing loss) 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 include various types of audiometric assays (e.g., pure-tone testing, speech testing, test of the middle ear, auditory brainstem response, and otoacoustic emissions).
- [0395]** In some aspects, a subject cell is in vitro. In some aspects, a subject cell is originally obtained from a subject and is cultured ex vivo. In some aspects, the ex vivo cell is an inner ear cell. In some aspects, the ex vivo cell is an inner phalangeal cells/border cells (IPhC), inner pillar cells (IPC), outer pillar cells (OPC), Deiters' cells rows 1 and 2 (DC1/2), Deiters' cells row 3 (DC3), Hensen's cells (Hec), Claudius cells/outer sulcus cells (CC/OSC), interdental cells (Idc), inner sulcus cells (ISC), Kölliker's organ cells (KO), greater ridge epithelial ridge cells (GER) (including lateral greater epithelial ridge cells (LGER)), and OC90+ cells (OC90), fibroblasts, and other cells of the lateral wall.
- [0396]** In some aspects, a subject cell has previously been determined to have a defective inner ear cell target gene. In some aspects, a subject cell has previously been determined to have a defective hair cell target gene. In some aspects, a subject cell has previously been determined to have a defective supporting cell target gene.
- [0397]** In some aspects of these methods, following treatment e.g., one or two or more administrations of compositions described herein, there is an increase in expression of an active inner ear cell target protein (e.g., a polypeptide (e.g., a therapeutic polypeptide, a Connexin 26 polypeptide)). In some aspects, an increase in expression of an active inner

ear target protein as described herein (e.g., a polypeptide (e.g., a therapeutic polypeptide, a Connexin 26 polypeptide)) is relative to a control level, e.g., as compared to the level of expression of an inner ear cell target protein prior to introduction of the compositions comprising any construct(s) as described herein.

[0398] Methods of detecting expression and/or activity of a target protein (e.g., a polypeptide (e.g., a therapeutic polypeptide, a Connexin 26 polypeptide)) are known in the art. In some aspects, a level of expression of an inner ear cell target protein can be detected directly (e.g., detecting inner ear cell target protein or target mRNA. Non-limiting examples of techniques that can be used to detect expression and/or activity of a target RNA or protein (e.g., a polypeptide (e.g., a therapeutic polypeptide, a Connexin 26 polypeptide)) directly include: real-time PCR, Western blotting, immunoprecipitation, immunohistochemistry, mass spectrometry, or immunofluorescence. In some aspects, expression of an inner ear cell target protein can be detected indirectly (e.g., through functional hearing tests).

Devices, Administration, and Surgical Methods

[0399] Provided herein are therapeutic delivery systems for treating hearing loss (e.g., nonsyndromic sensorineural hearing loss or syndromic sensorineural hearing loss). In one aspect, a therapeutic delivery system includes: i) a medical device capable of creating one or a plurality of incisions in a round window membrane of an inner ear of a subject in need thereof, and ii) an effective dose of a composition (e.g., any of the compositions described herein). In some aspects, a medical device includes a plurality of micro-needles.

[0400] Also provided herein are surgical methods for treatment of hearing loss (e.g., nonsyndromic sensorineural hearing loss or syndromic sensorineural hearing loss). In some aspects, a method the steps of: introducing into a cochlea of a 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 aspects, a composition is administered to a subject at the first incision point. In some aspects, a composition is administered to a subject into or through the first incision.

[0401] In some aspects of any method provided herein, any of the compositions described herein is administered to the subject into or through the cochlea oval window membrane. In some aspects of any method provided herein, any of the compositions described herein is administered to the subject into or through the cochlea round window membrane. In

some aspects of any method provided herein, the composition is administered using a medical device capable of creating a plurality of incisions in the round window membrane. In some aspects, a medical device includes a plurality of micro-needles. In some aspects, a 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 aspects, a medical device includes a base and/or a reservoir capable of holding a composition. In some aspects, a medical device includes a plurality of hollow micro-needles individually including a lumen capable of transferring a composition. In some aspects, a medical device includes a means for generating at least a partial vacuum.

[0402] In some aspects, the present disclosure describes a delivery approach that utilizes a minimally invasive, well-accepted surgical technique for accessing the middle ear and/or inner ear through the external auditory canal. The procedure includes opening one of the physical barriers between the middle and inner ear at the oval window, and subsequently using a device disclosed herein, e.g., as shown in Figs. 5-8 (or microcatheter) to deliver a composition disclosed herein at a controlled flow rate and in a fixed volume, via the round window membrane.

[0403] In some aspects, surgical procedures for mammals (e.g., rodents (e.g., mice, rats, hamsters, or rabbits), primates (e.g., NHP (e.g., macaque, chimpanzees, monkeys, or apes) or humans) may include venting to increase AAV vector transduction rates along the length of the cochlea. In some aspects, absence of venting during surgery may result in lower AAV vector cochlear cell transduction rates when compared to AAV vector cochlear cell transduction rates following surgeries performed with venting. In some aspects, venting facilitates transduction rates of about 75-100% of IHCs throughout the cochlea. In some aspects, venting permits IHC transduction rates of about 50-70%, about 60-80%, about 70-90%, or about 80-100% at the base of the cochlea. In some aspects, venting permits IHC transduction rates of about 50-70%, about 60-80%, about 70-90%, or about 80-100% at the apex of the cochlea.

[0404] A delivery device described herein may be placed in a sterile field of an operating room and the end of a tubing may be removed from the sterile field and connected to a syringe that has been loaded with a composition disclosed herein (e.g., one or more AAV vectors) and mounted in the pump. After appropriate priming of the system in order to remove any air, a needle may then be passed through the middle ear under visualization (surgical microscope, endoscope, and/or distal tip camera). A needle (or microneedle)

may be used to puncture the RWM. The needle may be inserted until a stopper contacts the RWM. The device may then be held in that position while a composition disclosed herein is delivered at a controlled flow rate to the inner ear, for a selected duration of time. In some aspects, the flow rate (or infusion rate) may include a rate of about 30 $\mu\text{L}/\text{min}$, or from about 25 $\mu\text{L}/\text{min}$ to about 35 $\mu\text{L}/\text{min}$, or from about 20 $\mu\text{L}/\text{min}$ to about 40 $\mu\text{L}/\text{min}$, or from about 20 $\mu\text{L}/\text{min}$ to about 70 $\mu\text{L}/\text{min}$, or from about 20 $\mu\text{L}/\text{min}$ to about 90 $\mu\text{L}/\text{min}$, or from about 20 $\mu\text{L}/\text{min}$ to about 100 $\mu\text{L}/\text{min}$. In some aspects, the flow rate is about 20 $\mu\text{L}/\text{min}$, about 30 $\mu\text{L}/\text{min}$, about 40 $\mu\text{L}/\text{min}$, about 50 $\mu\text{L}/\text{min}$, about 60 $\mu\text{L}/\text{min}$, about 70 $\mu\text{L}/\text{min}$, about 80 $\mu\text{L}/\text{min}$, about 90 $\mu\text{L}/\text{min}$ or about 100 $\mu\text{L}/\text{min}$. In some aspects, the selected duration of time (that is, the time during which a composition disclosed herein is flowing) may be about 3 minutes, or from about 2.5 minutes to about 3.5 minutes, or from about 2 minutes to about 4 minutes, or from about 1.5 minutes to about 4.5 minutes, or from about 1 minute to about 5 minutes. In some aspects, the total volume of a composition disclosed herein that flows to the inner ear may be about 0.09 mL, or from about 0.08 mL to about 0.10 mL, or from about 0.07 mL to about 0.11 mL. In some aspects, the total volume of a composition disclosed herein equates to from about 40% to about 50% of the volume of the inner ear.

[0405] Once the delivery has been completed, the device may be removed. In some aspects, a device described herein, may be configured as a single-use disposable product. In other aspects, a device described herein may be configured as a multi-use, sterilizable product, for example, with a replaceable and/or sterilizable needle sub-assembly. Single use devices may be appropriately discarded (for example, in a biohazard sharps container) after administration is complete.

[0406] In some aspects, a composition disclosed herein comprises one or a plurality of rAAV constructs. In some aspects, when more than one rAAV construct is included in the composition, the rAAV constructs are each different. In some aspects, an rAAV construct comprises an anti-VEGF coding region, e.g., as described herein. In some aspects, a composition comprises an rAAV particle comprising an AAV construct described herein. In some aspects, the rAAV particle is encapsidated by an Anc80 capsid (e.g., an Anc80L65 capsid). In some aspects, the Anc80 capsid comprises a polypeptide of SEQ ID NO: 44.

[0407] In some aspects, a composition disclosed herein can be administered to a subject with a surgical procedure. In some aspects, administration, e.g., via a surgical procedure,

comprises injecting a composition disclosed herein via a delivery device as described herein into the inner ear. In some aspects, a surgical procedure disclosed herein comprises performing a transcanal tympanotomy; performing a laser-assisted micro-stapedotomy; and injecting a composition disclosed herein via a delivery device as described herein into the inner ear.

[0408] In some aspects, a surgical procedure comprises performing a transcanal tympanotomy; performing a laser-assisted micro-stapedotomy; injecting a composition disclosed herein via a delivery device as described herein into the inner ear; applying sealant around the round window and/or an oval window of the subject; and lowering a tympanomeatal flap of the subject to the anatomical position.

[0409] In some aspects, a surgical procedure comprises performing a transcanal tympanotomy; preparing a round window of the subject; performing a laser-assisted micro-stapedotomy; preparing both a delivery device as described herein and a composition disclosed herein for delivery to the inner ear; injecting a composition disclosed herein via the delivery device into the inner ear; applying sealant around the round window and/or an oval window of the subject; and lowering a tympanomeatal flap of the subject to the anatomical position.

[0410] In some aspects, performing a laser-assisted micro-stapedotomy includes using a KTP otologic laser and/or a CO2 otologic laser.

[0411] As another example, a composition disclosed herein is administered using a device and/or system specifically designed for intracochlear route of administration. In some aspects, design elements of a device described herein may include: maintenance of sterility of injected fluid; minimization of air bubbles introduced to the inner ear; ability to precisely deliver small volumes at a controlled rate; delivery through the external auditory canal by the surgeon; minimization of damage to the round window membrane (RWM), or to inner ear, e.g., cochlear structures beyond the RWM; and/or minimization of injected fluid leaking back out through the RWM.

[0412] The devices, systems, and methods provided herein also describe the potential for delivering a composition safely and efficiently into the inner ear, in order to treat conditions and disorders that would benefit from delivery of a composition disclosed herein to the inner ear, including, but not limited to, hearing disorders, e.g., as described herein. As another example, by placing a vent in the stapes footplate and injecting through the RWM, a composition disclosed herein is dispersed throughout the cochlea

with minimal dilution at the site of action. The development of the described devices allows the surgical administration procedure to be performed through the external auditory canal in humans. The described devices can be removed from the ear following infusion of an amount of fluid into the perilymph of the cochlea. In subjects, the device may be advanced through the external auditory canal, either under surgical microscopic control or along with an endoscope.

[0413] An exemplary device for use in any of the methods disclosed herein is described in Figs 5-8. **Fig. 5** illustrates an exemplary device 10 for delivering fluid to an inner ear. Device 10 includes a knurled handle 12, and a distal handle adhesive 14 (for example, an epoxy such as Loctite 4014) that couples to a telescoping hypotube needle support 24. The knurled handle 12 (or handle portion) may include kurling features and/or grooves to enhance the grip. The knurled handle 12 (or handle portion) may be from about 5 mm to about 15 mm thick or from about 5 mm to about 12 mm thick, or from about 6 mm to about 10 mm thick, or from about 6 mm to about 9 mm thick, or from about 7 mm to about 8 mm thick. The knurled handle 12 (or handle portion) may be hollow such that fluid may pass through the device 10 during use. The device 10 may also include a proximal handle adhesive 16 at a proximal end 18 of the knurled handle 12, a needle sub-assembly 26 (shown in **Fig. 6**) with stopper 28 (shown in Fig. 34) at a distal end 20 of the device 10, and a strain relief feature 22. Strain relief feature 22 may be composed of a Santoprene material, a Pebax material, a polyurethane material, a silicone material, a nylon material, and/or a thermoplastic elastomer. The telescoping hypotube needle support 24 surrounds and supports a bent needle 38 (shown in **Fig. 6**) disposed therewithin.

[0414] Referring still to **Fig. 5**, the stopper 28 may be composed of a thermoplastic material or plastic polymer (such as a UV-cured polymer), as well as other suitable materials, and may be used to prevent the bent needle 38 from being inserted too far into the ear canal (for example, to prevent insertion of bent needle 38 into the lateral wall or other inner ear structure). Device 10 also may include a tapered portion 23 disposed between the knurled handle 12 and the distal handle adhesive 14 that is coupled to the telescoping hypotube needle support 24. The knurled handle 12 (or handle portion) may include the tapered portion 23 at the distal end of the handle portion 12. Device 10 may also include tubing 36 fluidly connected to the proximal end 16 the device 10 and acts as a fluid inlet line connecting the device to upstream components (for example, a pump, a

syringe, and/or upstream components which, in some aspects, may be coupled to a control system and/or power supply (not shown)). In some aspects, the bent needle 38 (shown in **Fig. 6**) extends from the distal end 20, through the telescoping hypotube needle support 24, through the tapered portion 23, through the knurled handle 12, and through the strain relief feature 22 and fluidly connects directly to the tubing 36. In other aspects, the bent needle 38 fluidly connects with the hollow interior of the knurled handle (for example, via the telescoping hypotube needle support 24) which in turn fluidly connects at a proximal end 16 with tubing 36. In aspects where the bent needle 38 does not extend all the way through the interior of the device 10, the contact area (for example, between overlapping nested hypotubes 42), the tolerances, and/or sealants between interfacing components must be sufficient to prevent therapeutic fluid from leaking out of the device 10 (which operates at a relatively low pressure (for example, from about 1 Pascal to about 50 Pa, or from about 2 Pa to about 20 Pa, or from about 3 Pa to about 10 Pa)).

[0415] **Fig. 6** illustrates a sideview of the bent needle sub-assembly 26, according to aspects of the present disclosed aspects. Bent needle sub-assembly 26 includes a needle 38 that has a bent portion 32. Bent needle sub-assembly 26 may also include a stopper 28 coupled to the bent portion 32. The bent portion 32 includes an angled tip 34 at the distal end 20 of the device 10 for piercing a membrane of the ear (for example, the RWM). The needle 38, bent portion 32, and angled top 34 are hollow such that fluid may flow therethrough. The angle 46 (as shown in **Fig. 8**) of the bent portion 32 may vary. A stopper 28 geometry may be cylindrical, disk-shaped, annulus-shaped, dome-shaped, and/or other suitable shapes. Stopper 28 may be molded into place onto bent portion 32. For example, stopper 28 may be positioned concentrically around the bent portion 32 using adhesives or compression fitting. Examples of adhesives include an UV cure adhesive (such as Dymax 203A-CTH-F-T), elastomer adhesives, thermoset adhesives (such as epoxy or polyurethane), or emulsion adhesives (such as polyvinyl acetate). Stopper 28 fits concentrically around the bent portion 32 such that angled tip 34 is inserted into the ear at a desired insertion depth. The bent needle 38 may be formed from a straight needle using incremental forming, as well as other suitable techniques.

[0416] **Fig. 7** illustrates a perspective view of exemplary device 10 for delivering fluid to an inner ear. Tubing 36 may be from about 1300 mm in length (dimension 11 in **Fig. 7**) to about 1600 mm, or from about 1400 mm to about 1500 mm, or from about 1430 mm to about 1450 mm. Strain release feature 22 may be from about 25 mm to about 30 mm in

length (dimension 15 in **Fig. 7**), or from about 20 mm to about 35 mm in length. Handle 12 may be about 155.4 mm in length (dimension 13 in **Fig. 7**), or from about 150 mm to about 160 mm, or from about 140 mm to about 170 mm. The telescoping hypotube needle support 24 may have two or more nested hypotubes, for example three nested hypotubes 42A, 42B, and 42C, or four nested hypotubes 42A, 42B, 42C, and 42D. The total length of hypotubes 42A, 42B, 42C and tip assembly 26 (dimension 17 in **Fig. 7**) may be from about 25 mm to about 45 mm, or from about 30 mm to about 40 mm, or about 35 mm. In addition, telescoping hypotube needle support 24 may have a length of about 36 mm, or from about 25 mm to about 45 mm, or from about 30 mm to about 40 mm. The three nested hypotubes 42A, 42B, and 42C each may have a length of 3.5 mm, 8.0 mm, and 19.8 mm, respectively, plus or minus about 20%. The inner-most nested hypotube (or most narrow portion) of the telescoping hypotube needle support 24 may be concentrically disposed around needle 38.

[0417] **Fig. 8** illustrates a perspective view of bent needle sub-assembly 26 coupled to the distal end 20 of device 10, according to aspects of the present disclosed aspects. As shown in **Fig. 8**, bent needle sub-assembly 26 may include a needle 38 coupled to a bent portion 32. In other aspects, the bent needle 38 may be a single needle (for example, a straight needle that is then bent such that it includes the desired angle 46). Needle 38 may be a 33-gauge needle, or may include a gauge from about 32 to about 34, or from about 31 to 35. At finer gauges, care must be taken to ensure tubing 36 is not kinked or damaged. Needle 38 may be attached to handle 12 for safe and accurate placement of needle 38 into the inner ear. As shown in **Fig. 8**, bent needle sub-assembly 26 may also include a stopper 28 disposed around bent portion 32. **Fig. 8** also shows that bent portion 32 may include an angled tip 34 for piercing a membrane of the ear (for example, the RWM). Stopper 28 may have a height 48 of about 0.5 mm, or from about 0.4 mm to about 0.6 mm, or from about 0.3 mm to about 0.7 mm. Bent portion 32 may have a length 52 of about 1.45 mm, or from about 1.35 mm to about 1.55 mm, or from about 1.2 mm to about 1.7 mm. In other aspects, the bent portion 32 may have a length greater than 2.0 mm such that the distance between the distal end of the stopper 28 and the distal end of the angled tip 34 is from about 0.5 mm to about 1.7 mm, or from about 0.6 mm to about 1.5 mm, or from about 0.7 mm to about 1.3 mm, or from about 0.8 mm to about 1.2 mm. **Fig. 8** shows that stopper 28 may have a geometry that is cylindrical, disk-shaped,

and/or dome-shaped. A person of ordinary skill will appreciate that other geometries could be used.

Evaluating Hearing Loss and Recovery

[0418] In some aspects, hearing function is determined using auditory brainstem response measurements (ABR). In some aspects, hearing is tested by measuring distortion product otoacoustic emissions (DPOAEs). In some such aspects, measurements are taken from one or both ears of a subject. In some such aspects, recordings are compared to prior recordings for the same subject and/or known thresholds on such response measurements used to define, e.g., hearing loss versus acceptable hearing ranges to be defined as normal hearing. In some aspects, a subject has ABR and/or DPOAE measurements recorded prior to receiving any treatment. In some aspects, a subject treated with one or more technologies described herein will have improvements on ABR and/or DPOAE measurements after treatment as compared to before treatment. In some aspects, ABR and/or DPOAE measurements are taken after treatment is administered and at regular follow-up intervals post-treatment.

[0419] In some aspects, hearing function is determined using speech pattern recognition or is determined by a speech therapist. In some aspects, hearing function is determined by pure tone testing. In some aspects, hearing function is determined by bone conduction testing. In some aspects, hearing function is determined by acoustic reflex testing. In some aspects hearing function is determined by tympanometry. In some aspects, hearing function is determined by any combination of hearing analysis known in the art. In some such aspects, measurements are taken holistically, and/or from one or both ears of a subject. In some such aspects, recordings and/or professional analysis are compared to prior recordings and/or analysis for the same subject and/or known thresholds on such response measurements used to define, e.g., hearing loss versus acceptable hearing ranges to be defined as normal hearing. In some aspects, a subject has speech pattern recognition, pure tone testing, bone conduction testing, acoustic reflex testing and/or tympanometry measurements and/or analysis conducted prior to receiving any treatment. In some aspects a subject treated with one or more technologies described herein will have improvements on speech pattern recognition, pure tone testing, bone conduction testing, acoustic reflex testing and/or tympanometry measurements after treatment as compared to before treatment. In some aspects, speech pattern recognition, pure tone testing, bone conduction testing, acoustic reflex testing and/or tympanometry

measurements are taken after treatment is administered and at regular follow-up intervals post-treatment.

Production Methods

- [0420] AAV systems are generally well known in the art (see, e.g., Kelleher and Vos, *Biotechniques*, 17(6):1110-17 (1994); Cotton et al., *P.N.A.S. U.S.A.*, 89(13):6094-98 (1992); Curiel, *Nat Immun*, 13(2-3):141-64 (1994); Muzyczka, *Curr Top Microbiol Immunol*, 158:97-129 (1992); and Asokan A, et al., *Mol. Ther.*, 20(4):699-708 (2012), each of which is incorporated in its entirety herein by reference). Methods for generating and using AAV constructs are described, for example, in U.S. Pat. Nos. 5,139,941, 4,797,368 and PCT filing application US2019/060328, each of which is incorporated in its entirety herein by reference.
- [0421] Methods for obtaining viral constructs are known in the art. For example, to produce AAV constructs, the methods typically involve culturing a host cell which contains a nucleic acid sequence encoding an AAV capsid protein or fragment thereof; a functional rep gene; a recombinant AAV construct composed of AAV inverted terminal repeats (ITRs) and a coding sequence; and/or sufficient helper functions to permit packaging of the recombinant AAV construct into the AAV capsid proteins.
- [0422] In some aspects, components to be cultured in a host cell to package an AAV construct in an AAV capsid may be provided to the host cell in trans. Alternatively, any one or more components (e.g., recombinant AAV construct, rep sequences, cap sequences, and/or helper functions) may be provided by a stable host cell that has been engineered to contain one or more such components using methods known to those of skill in the art. In some aspects, such a stable host cell contains such component(s) under the control of an inducible promoter. In some aspects, such component(s) may be under the control of a constitutive promoter. In some aspects, a selected stable host cell may contain selected component(s) under the control of a constitutive promoter and other selected component(s) under the control of one or more inducible promoters. For example, a stable host cell may be generated that is derived from HEK293 cells (which contain E1 helper functions under the control of a constitutive promoter), but that contain the rep and/or cap proteins under the control of inducible promoters. Other stable host cells may be generated by one of skill in the art using routine methods.
- [0423] Recombinant AAV construct, rep sequences, cap sequences, and helper functions required for producing an AAV of the disclosure may be delivered to a packaging host

cell using any appropriate genetic element (e.g., construct). A selected genetic element may be delivered by any suitable method known in the art, e.g., to those with skill in nucleic acid manipulation and include genetic engineering, recombinant engineering, and synthetic techniques (see, e.g., Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Press, Cold Spring Harbor, N.Y., which is incorporated in its entirety herein by reference). Similarly, methods of generating AAV particles are well known and any suitable method can be used with the present disclosure (see, e.g., K. Fisher et al., *J. Virol.*, 70:520-532 (1993) and U.S. Pat. No. 5,478,745, which are incorporated in their entirety herein by reference).

[0424] In some aspects, recombinant AAVs may be produced using a triple transfection method (e.g., as described in U.S. Pat. No. 6,001,650, which is incorporated in its entirety herein by reference). In some aspects, recombinant AAVs are produced by transfecting a host cell with a recombinant AAV construct (comprising a coding sequence) to be packaged into AAV particles, an AAV helper function construct, and an accessory function construct. An AAV helper function construct encodes “AAV helper function” sequences (i.e., rep and cap), which function in trans for productive AAV replication and encapsidation. In some aspects, the AAV helper function construct supports efficient AAV construct production without generating any detectable wild-type AAV particles (i.e., AAV particles containing functional rep and cap genes). Non-limiting examples of constructs suitable for use with the present disclosure include pHLP19 (see, e.g., U.S. Pat. No. 6,001,650, which is incorporated in its entirety herein by reference) and pRep6cap6 construct (see, e.g., U.S. Pat. No. 6,156,303, which is incorporated in its entirety herein by reference). An accessory function construct encodes nucleotide sequences for non-AAV derived viral and/or cellular functions upon which AAV is dependent for replication (i.e., “accessory functions”). Accessory functions may include those functions required for AAV replication, including, without limitation, those moieties involved in activation of AAV gene transcription, stage specific AAV mRNA splicing, AAV DNA replication, synthesis of cap expression products, and AAV capsid assembly. Viral-based accessory functions can be derived from any known helper viruses such as adenovirus, herpesvirus (other than herpes simplex virus type-1), and vaccinia virus.

[0425] Additional methods for generating and isolating AAV viral constructs suitable for delivery to a subject are described in, e.g., U.S. Pat. No. 7,790,449; U.S. Pat. No. 7,282,199; WO 2003/042397; WO 2005/033321, WO 2006/110689; and U.S. Pat. No.

7,588,772, each of which is incorporated in its entirety herein by reference. In one system, a producer cell line is transiently transfected with a construct that encodes a coding sequence flanked by ITRs and a construct(s) that encodes rep and cap. In another system, a packaging cell line that stably supplies rep and cap is transiently transfected with a construct encoding a coding sequence flanked by ITRs. In each of these systems, AAV particles are produced in response to infection with helper adenovirus or herpesvirus, and AAVs are separated from contaminating virus. Other systems do not require infection with helper virus to recover the virus particles--the helper functions (i.e., adenovirus E1, E2a, VA, and E4 or herpesvirus UL5, UL8, UL52, and UL29, and herpesvirus polymerase) are also supplied, in trans, by the system. In such systems, helper functions can be supplied by transient transfection of the cells with constructs that encode the helper functions, or the cells can be engineered to stably contain genes encoding the helper functions, the expression of which can be controlled at the transcriptional or posttranscriptional level.

[0426] In some aspects, viral construct titers post-purification are determined. In some aspects, titers are determined using quantitative PCR. In certain aspects, a TaqMan probe specific to a construct is utilized to determine construct levels. In certain aspects, the TaqMan probe is represented by SEQ ID NO: 58, while forward and reverse amplifying primers are exemplified by SEQ ID NO: 59 and 60 respectively.

Exemplary Taqman probe for quantification of constructs (SEQ ID NO: 58)

/56-FAM/TCGTGGCTCA/ZEN/CCGTCCTCTTCATTT/3IABkFQ/

Exemplary forward qPCR primer for quantification of constructs (SEQ ID NO: 59)

CAAACACTCCACCAGCATTG

Exemplary reverse qPCR primer for quantification of constructs (SEQ ID NO: 60)

CAGCCACAACGAGGATCATA

[0427] As described herein, in some aspects, a viral construct of the present disclosure is an adeno-associated virus (AAV) construct. Several AAV serotypes have been characterized, including AAV1, AAV2, AAV3 (e.g., AAV3B), AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, and AAV Anc80, as well as variants thereof. In some aspects, an AAV particle is an AAV2/6, AAV2/8, AAV2/9, or AAV2/Anc80

particle (e.g., with AAV6, AAV8, AAV9, or Anc80 capsid (e.g., an Anc80L65 capsid) and construct with AAV2 ITR). Other AAV particles and constructs are described in, e.g., Sharma et al., Brain Res Bull. 2010 Feb 15; 81(2-3): 273, which is incorporated in its entirety herein by reference. Generally, any AAV serotype may be used to deliver a coding sequence described herein. However, the serotypes have different tropisms, e.g., they preferentially infect different tissues. In some aspects, an AAV construct is a self-complementary AAV construct.

[0428] The present disclosure provides, among other things, methods of making AAV-based constructs. In some aspects, such methods include use of host cells. In some aspects, a host cell is a mammalian cell. A host cell may be used as a recipient of an AAV helper construct, an AAV minigene plasmid, an accessory function construct, and/or other transfer DNA associated with the production of recombinant AAVs. The term includes the progeny of an original cell that has been transfected. Thus, a “host cell” as used herein may refer to a cell that has been transfected with an exogenous DNA sequence. It is understood that the progeny of a single parental cell may not necessarily be completely identical in morphology or in genomic or total DNA complement as the original parent, due to natural, accidental, or deliberate mutation.

[0429] Additional methods for generating and isolating AAV particles suitable for delivery to a subject are described in, e.g., U.S. Pat. No. 7,790,449; U.S. Pat. No. 7,282,199; WO 2003/042397; WO 2005/033321, WO 2006/110689; and U.S. Pat. No. 7,588,772, each of which is incorporated in its entirety herein by reference. In one system, a producer cell line is transiently transfected with a construct that encodes a coding sequence flanked by ITRs and a construct(s) that encodes rep and cap. In another system, a packaging cell line that stably supplies rep and cap is transiently transfected with a construct encoding a coding sequence flanked by ITRs. In each of these systems, AAV particles are produced in response to infection with helper adenovirus or herpesvirus, and AAV particles are separated from contaminating virus. Other systems do not require infection with helper virus to recover the AAV particles--the helper functions (i.e., adenovirus E1, E2a, VA, and E4 or herpesvirus UL5, UL8, UL52, and UL29, and herpesvirus polymerase) are also supplied, in trans, by the system. In such systems, helper functions can be supplied by transient transfection of the cells with constructs that encode the helper functions, or the cells can be engineered to stably

contain genes encoding the helper functions, the expression of which can be controlled at the transcriptional or posttranscriptional level.

[0430] In yet another system, a coding sequence flanked by ITRs and rep/cap genes are introduced into insect host cells by infection with baculovirus-based constructs. Such production systems are known in the art (see generally, e.g., Zhang et al., 2009, Human Gene Therapy 20:922-929, which is incorporated in its entirety herein by reference). Methods of making and using these and other AAV production systems are also described in U.S. Pat. Nos. 5,139,941; 5,741,683; 6,057,152; 6,204,059; 6,268,213; 6,491,907; 6,660,514; 6,951,753; 7,094,604; 7,172,893; 7,201,898; 7,229,823; and 7,439,065, each of which is incorporated in its entirety herein by reference.

EXAMPLES

[0431] The disclosure 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 disclosure 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.

[0432] It is believed that one of ordinary skill in the art can, using the preceding description and following Examples, as well as what is known in the art, make and utilize technologies of the present disclosure.

Example 1: Construction of Viral Constructs Comprising a Polypeptide or Therapeutic Polypeptide

[0433] This example provides a description of generating a viral construct as described herein. A recombinant AAV (rAAV) particle was generated by transfection with an adenovirus-free method as used by Xiao et al., J Virol. 73(5):3994-4003, 1999, which is incorporated in its entirety herein by reference. 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 were co-transfected in HEK293 cells. The rAAV construct expressed human connexin 26 under a single construct strategy using the constructs described. AAV Anc80 capsid was prepared to encapsulate a unique rAAV connexin 26 protein encoding construct.

[0434] Those of ordinary skill in the art will readily understand that similar constructs can be made in accordance with this example. For instance, rAAV constructs that express mammalian, primate, or human connexin 26 under single, dual, or multi construct strategies can be generated. AAV serotypes 1, 2, 3, 4, 5, 6, 7, 8, 9, rh8, rh10, rh39, rh43, and Anc80 can each be prepared to encapsulate four sets of connexin 26 constructs to test (i) a concatemerization-transplicing strategy, (ii) a hybrid intronic-homologous recombination-transplicing strategy, (iii) an exonic homologous recombination strategy, as summarized by Pryadkina et al., *Meth. Clin. Devel.* 2:15009, 2015, which is incorporated in its entirety herein by reference, and (iv) a single construct strategy. In some aspects, a recombinant AAV (rAAV) particle is generated by transfection with an adenovirus-free method as used by Xiao et al., *J Virol.* 73(5):3994-4003, 1999, which is incorporated in its entirety herein by reference.

Example 2: Generating and Purifying Viral Particles

[0435] This example provides a description of purification of a viral construct. A recombinant AAV (rAAV) is produced using a triple transfection protocol and purified. 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 construct genome (Bartoli et al., *Gene. Ther.* 13:20-28, 2006, which is incorporated in its entirety herein by reference).

[0436] In some aspects of this example, a recombinant AAV (rAAV) was produced using a standard triple transfection protocol and purified by two sequential cesium chloride (CsCl) density gradients, as described by Pryadkina et al., *Mol. Ther.* 2:15009, 2015, which is incorporated in its entirety herein by reference. At the end of second centrifugation, 11 fractions of 500 µl were recovered from the CsCl density gradient tube and purified through dialysis in 1x PBS. The fractions were analyzed by dot blot to determine those containing rAAV genomes. The viral genome number (vg) of each preparation was determined by a quantitative real-time PCR-based titration method using primers and probe corresponding to the ITR region of the AAV construct genome (Bartoli et al., *Gene. Ther.* 13:20-28, 2006, which is incorporated in its entirety herein by reference).

[0437] Those of ordinary skill in the art will readily understand that similar production and purifying processes can be conducted in accordance with this example. For instance,

rAAV particles may be purified using various column chromatography methods known in the art, and/or viral genomes may be quantified using alternative primer sets.

Example 3: Formulation of Viral Particles

[0438] This example relates to the preparation of compositions comprising rAAV particles, and a physiologically acceptable solution. An rAAV particle was produced and purified to a titer of 1.2×10^{13} vg/mL and was then prepared at dilutions of 6×10^4 , 1.3×10^5 , 1.8×10^5 , 4.5×10^9 , and 1.3×10^{10} vg/mL in a physiologically acceptable solution (e.g., commercially available 1xPBS with pluronic acid F68, prepared to a final concentration of: 8.10mM Sodium Phosphate Dibasic, 1.5mM Monopotassium Phosphate, 2.7mM Potassium Chloride, 172mM Sodium Chloride, and 0.001% Pluronic Acid F68).

[0439] In alternative aspects, an rAAV is produced and purified to a known concentration (e.g., a titer of approximately 1×10^{13} vg/mL) and is then prepared at desired concentrations (e.g., dilutions of 6×10^4 , 1.3×10^5 , 1.8×10^5 , 4.5×10^9 , and 1.3×10^{10} vg/mL) in a physiologically acceptable buffer (e.g., commercially available 1xPBS with pluronic acid F68, prepared to a final concentration of: 8.10mM Sodium Phosphate Dibasic, 1.5mM Monopotassium Phosphate, 2.7mM Potassium Chloride, 172mM Sodium Chloride, and 0.001% Pluronic Acid F68; or e.g., artificial perilymph comprising NaCl, 120 mM; KCl, 3.5 mM; CaCl₂, 1.5 mM; glucose, 5.5 mM; HEPES, 20 mM. which is titrated with NaOH to adjust its pH to 7.5 (total Na⁺ concentration of 130 mM) as described in Chen et al., J Controlled Rel. 110:1-19, 2005, which is incorporated in its entirety herein by reference). Those of ordinary skill in the art will readily understand that alternative formulations can be prepared in accordance with this example. For instance, rAAV particles may be purified to an alternative titer, prepared at alternative dilutions, and suspended in alternative suitable solutions.

Example 4: Device Description

[0440] This example relates to a device suitable for the delivery of rAAV particles to the inner ear. A composition comprising rAAV particles is delivered to the cochlea of a subject 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 may include at least one microneedle with a diameter from about 10

microns to about 1,000 microns, which produces perforations in the RWM that are sufficient to allow a construct as described (e.g., an rAAV construct) to enter the cochlear perilymph of the scala tympani at a rate which does not damage the inner ear (e.g., a physiologically acceptable rate, e.g., a rate of approximately 30 $\mu\text{L}/\text{min}$ to approximately 90 $\mu\text{L}/\text{min}$), but small enough to heal without surgical repair. The remaining portion of the microcatheter, proximal to the microneedle(s), is loaded with the rAAV/artificial perilymph formulation at a defined titer (e.g., approximately 1×10^{12} to 5×10^{13} vg/mL). The proximal end of the microcatheter is connected to a micromanipulator that allows for precise, low volume infusions of approximately 30 μL to approximately 100 μL .

Example 5: In-vitro demonstration of GJB2 mRNA and Connexin 26 protein production (anti-FLAG antibody).

[0441] This example relates to the introduction, regulation, and expression analysis of rAAV constructs expressing a supporting cell protein (e.g., a hGJB2 gene) in mammalian cells grown in vitro or ex vivo.

[0442] To regulate transgene mRNA expression only in supporting cells, a construct comprising microRNA target site was created. In cell types where the transgene (e.g., a supporting cell gene or a GJB2 gene) may not be well-tolerated (e.g., hair cells), a microRNA recognizes the miRTS and degrades the mRNA, preventing its expression. A schematic of the construct is shown in FIG. 3A. The construct comprises a promoter, 5' UTR, transgene (GOI), miRTs, 3' UTR, and polyA, flanked by inverted terminal repeats. To prevent expression of a transgene in hair cells and allow expression in other cochlear cell types, such as supporting cells, microRNAs expressed in hair cells but not in supporting cells can be used (FIG. 3B).

[0443] An *in vitro* experiment was conducted with a plasmid comprising eGFP and a miRTS (eGFP-miRTS) that was co-transfected into HEK293FT cells with a plasmid expressing a microRNA targeting the miRTS (pITR.CMV.mScarlet.miRNA) at range of miRNA to target DNA ratios. eGFP expression was measured by flow cytometry and normalized to eGFP-miRTS expression in the absence of the microRNA. Increasing ratios of pITR.CMV.mScarlet.miRNA to eGFP-miRTS led to a reduction in eGFP expression (FIG. 3C). When cells were transduced with an Anc80 particle comprising a construct expressing eGFP without a miRTS (eGFP) (AAVAnc80-CAG.eGFP) alongside transfection with pITR.CMV.mScarlet.miRNA, a reduction in expression of eGFP was not observed by flow cytometry. Transduction of cells with a construct comprising eGFP

and a miRTS (AAVAnc80-CAG.eGFP.miRTS) alongside transfection of the cells with pITR.CMV.mScarlet.miRNA resulted in decreased eGFP expression (FIG. 3D).

[0444] A similar effect on expression of a gene of interest (GOI (GJB2)) was observed when HEK293FT cells were transduced with an AAVAnc80 vector comprising a gene of interest and miRTS site in its 3'UTR (AAVAnc80-CAG.GOI.miRTS) alongside transfection with pITR.CAG.mScarlet.miRNA, which expresses a microRNA targeting the miRTS. A reduction in the gene of interest mRNA was measured by real-time qPCR and western blotting when cells were transfected with either 100 ng or 200 ng of pITR.CAG.mScarlet.miRNA alongside transduction with AAVAnc80-CAG.GOI.miRTS compared to transduction of AAVAnc80-CAG.GOI.miRTS alone (FIGs. 3E and 3F).

[0445] In contrast, transfection of pITR.CAG.mScarlet-miRNA did not reduced the level of the gene of interest when cells were transduced with a construct comprising the gene of interest without the 3'UTR miRTS (AAVAnc80-CAG.GOI) (FIG. 3G). The reduction in protein level observed by Western blot following AAVAnc80-CAG.GOI.miRTS transduction and pITR.CAG.mScarlet-miRNA was quantified in FIG. 3H.

[0446] In order to determine whether there were any substantial off-target effects on gene expression caused by transduction with AAVAnc80-CAG.GOI.miRTS, bulk RNA sequencing was performed. Cluster analysis failed to detect any clear sample clustering based on the transduction with or without the miRTS (FIG. 3I). Differential expression analysis was used to identify genes that were significantly upregulated or downregulated by the expression of AAVAnc80-CAG.GOI.miRTS compared to AAVAnc80-CAG.GOI. The only genes significantly altered in expression were associated with T-cell immune response (FIG. 3J), further indicating that there were not significant off-target effects on gene expression caused by the expression of the miRTS-containing construct.

[0447] Cochlear explant cultures were transduced with an AAVAnc80 virus expressing a FLAG-tagged gene of interest (GOI (GJB2)) with a miRTS targeted by miRNAs expressed in hair cells (AAVAnc80-CAG.GOI.FLAG.miRTS1-4) or without a miRTS (AAVAnc80-CAG.GOI.FLAG). FIG. 3K (left) shows an untreated cochlear explant with hair cells labeled with MYO7A in red. Following transduction with AAVAnc80-CAG.GOI.FLAG the gene of interest is expressed in hair cells (indicated by white arrowheads) and in both lateral and medial supporting cells (FIG. 3K, right). In contrast, following transduction of AAVAnc80-CAG.GOI.FLAG.miRTS1, AAVAnc80-CAG.GOI.FLAG.miRTS2, and AAVAnc80-CAG.GOI.FLAG.miRTS3, expression of the

gene of interest was limited to supporting cells, as observed by FLAG labeling (green) (FIGs. 3L, 3M, and 3N). Of the constructs expressing the gene of interest with a miRTS in the 3'UTR, only AAVAnc80-CAG.GOI.FLAG.miRTS4 was unsuccessful in limiting expression to supporting cells. FLAG labeling of the gene of interest is colocalized with MYO7A (white arrowhead) in some hair cells (FIG. 3O). Experiments were conducted to demonstrate mRNA expression regulation from rAAV constructs transfected into HEK293FT cells. rAAV constructs comprising hGJB2.FLAG (CAG.5UTR.hGJB2.FLAG.3UTR; SEQ ID NO: 82) and optional miRNA regulatory target sites (miRTS) located in the 3'UTR (CAG.5UTR.hGJB2.FLAG.miRTS.3UTR; Fig. 2F; SEQ ID NO: 87) were transfected into HEK293FT cells at 300 ng with (+) or without (-) an additional plasmid comprising miRNA coding regions (e.g., miR-182, and miR-183) transfected at 400 ng. At 72h post transfection the cells were harvested for GJB2 protein and RNA analysis using western blot analysis (see FIG. 3P) and real-time qPCR (see FIG. 3Q). Reduction in GJB2 RNA and protein expression was detected in samples that were co-expressing the target plasmid and miR-182 and miR-183 compared to samples expressing the target plasmid alone. Similar hGJB2.FLAG comprising plasmids that did not include miR-182 and miR-183 target sites were used as control and presented similar hGJB2 protein levels with and without miR-182 and miR-183 co-expression (see FIG. 3P and FIG. 3Q).

[0448] rAAV particles comprising rAAV constructs containing a GJB2 flag tagged polynucleotide operably linked to a supporting cell selective promoter (GFAP, GJB6, IGFBP2, RPB7, PARM1, or GDF6) in combination with a minimal GJB2 promoter were transduced into HEK293FT cells. Expression by a CAG promoter was used as a positive control. Protein and RNA analysis shows that each these constructs were able to express Connexin 26 (FIGs. 10A-10B)..

[0449] Plasmids comprising a GJB2 flag tagged polynucleotide operably linked to a supporting cell selective promoter (FABP3, KLHL14, DBI2, TSPAN8, MMP15, SPARC, or VIM) in combination with a minimal GJB2 promoter were transfected into HEK293FT cells. Connexin 26 expression was observed from all constructs by western blot (FIG. 10C).

[0450] rAAV particles comprising rAAV constructs were encapsidated by Anc80 capisids and transduced into neonate cochlear explants at different doses. RNA analysis shows that GJB2 mRNA expression increased with dosing (FIG. 11).

[0451] Those of ordinary skill in the art will readily understand that there are alternative methods of conducting the experiments associated with the current example, for instance, alternative viral titers, MOIs, cell concentrations, time to cellular harvest, reagents utilized for cellular harvesting or mRNA or protein analysis, AAV serotypes, and/or standard modifications to a construct comprising an gene are practical and expected alterations of the current example.

Example 6: Preliminary hair cell tolerability assessment of transgenic GJB2 mRNA expression and connexin 26 protein production in neonate cochlear explants.

[0452] This example relates to the introduction, and expression analysis of rAAV constructs overexpressing a GJB2 gene in neonatal cochlear explants. Mock rAAV particles or rAAV particles comprising rAAV constructs (Figure 2 panels (A)-(H)) encapsidated by Anc80 capsids are prepared and transduced into neonate cochlear explants at a known MOI (e.g., approximately 4.5×10^9 or 1.3×10^{10} vg/per cochlea). Explants are grown to levels appropriate for harvest (e.g., for 72 hours post transduction), and are then prepared for immunofluorescence staining/imaging through fixation using 4% PFA or RNA extraction. RNA samples are prepared and GJB2 gene overexpression is confirmed using quantitative PCR with appropriate reagents in a manner described in a published method (e.g., appropriate according to the RNeasy Micro Kit and quantitative real-time PCR) using construct specific primers and relative to a control. Robust GJB2 mRNA production is observed in explants transduced with test rAAV when compared to mock transduction events. Tolerability and lack of hair cell toxicity is determined using immunofluorescence staining/imaging, antibodies targeting Myo7a (Proteus Biosciences) are utilized to depict inner ear hair cells, while DAPI staining is used to define nuclear positioning. No or low hair cell (Myo7) toxicity is observed after GJB2 overexpression.

[0453] rAAV Anc80 particles comprising rAAV constructs driven by CAG, CMVe-GJB2p, or smCBA promoter/enhancer combinations were prepared and transduced into mouse neonate (P2) cochlear explants at a known MOI (approximately 5.8×10^9 , 1.4×10^{10} , or 1.8×10^{10} vg/per cochlea respectively). Explants were grown to levels appropriate for harvest (e.g., for 72 hours post transduction), and were then prepared for immunofluorescence staining/imaging through fixation using 4% PFA. Explants were then DAPI stained (presented in blue) and immunostained using anti-FLAG antibodies (presented in green), and hair cell specific anti-Myo7a antibodies (presented in red), explants were subsequently imaged (exemplary data presented in Fig. 4A-4C). Robust

FLAG signal was observed in the supporting cells of the explants transduced with rAAV particles comprising AAVAnc80-CAG.5UTR.hGJB2.3F.3UTR (as depicted in Figure 2C, panel (C), SEQ ID NO: 82) at 5.8×10^9 vg/explant (see Fig. 4A, panel (A)). Robust FLAG signal was observed in the supporting cells in explants transduced with rAAV particles comprising AAVAnc80-smCBA.5UTR.hGJB2.3F.3UTR (as depicted in Figure 2D panel (D), SEQ ID NO: 83) at 1.4×10^{10} vg/explant (see FIG. 4B, panel (B)). Robust FLAG signal was observed in the supporting cells of the explants transduced with rAAV particles comprising AAVAnc80-CMVVeGFAPp.5UTR.hGJB2.3F.3UTR (as depicted in Figure 2E, panel (E), SEQ ID NO: 84) at 1.8×10^{10} vg/explant (see Fig. 4C, panel (C)). Variation in FLAG expression was detected across samples, likely the results of variability in vector titer.

Example 7: Surgical Method in Aged Mice

[0454] The current example relates to the introduction of constructs described herein to the inner ear of aged mice. rAAV particles comprising an AAV capsid and a construct encoding a connexin 26 protein or characteristic functional portion thereof are prepared in formulation buffer (e.g., artificial perilymph or 1xPBS with pluronic acid F68) and then administered to the scala tympani in mice as described by Shu et al., Human Gene Therapy, 27(9):687-699, 2016, which is incorporated in its entirety herein by reference). Male and female mice older than P15 are anesthetized using an intraperitoneal injection of xylazine (e.g., approximately 5-10 mg/kg) and ketamine (e.g., approximately 90-120 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 and posterior semicircular canal are 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 small hole is then drilled in the posterior semicircular canal (PSCC). Patency of the canalostomy is confirmed by visualization of a slow leak of perilymph. A Nanoliter Microinjection System in conjunction with glass micropipette is used to deliver a total of approximately 1 μ L of construct containing buffer (e.g., rAAV constructs described herein at approximately 4.5×10^9 to 5×10^{10} vg/per cochlea in artificial perilymph or 1xPBS with pluronic acid F68) to the scala tympani at a rate of approximately 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 and the PSCC are sealed

with small pieces of fat, 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 8: Transgenic expression and imaging of connexin 26 protein in wild-type mice.

- [0455] This example relates to the transgenic expression and analysis of transgenic connexin 26 protein in wild-type mice and GJB2 inducible conditional knockout mice. Wild-type mice were administered AAVAnc80 particles (1.2×10^{10} vg/cochlea) comprising CAG.hGJB2.FLAG.GFP (schematic provided in FIG. 2H) to the cochlea by the method described in Example 7. 10 days after administration clear and robust of exogenous Connexin 26 (FLAG; purple) was detected in the membrane of the supporting cells of the sensory epithelia (Fig. 9A, middle and right panels). Expression of exogenous Connexin 26 was also detected in the inner hair cells. Endogenous Connexin 26 (red) was detected in all supporting cells (Fig. 9A, left and right panels).
- [0456] Juvenile wild-type mice were administered 1 μ l of AAVAnc80 particles comprising AAVAnc80-CMV.eGFPp.5UTR.hGJB2.FLAG.3UTR (SEQ ID NO: 84), AAVAnc80-GDF6p.mGJB2p.5UTR.hGJB2.FLAG.3UTR (construct comprising SEQ ID NO: 61 and supporting cell selective promoter comprising SEQ ID NO: 90), AAVAnc80-IGFBP2p.mGJB2p.5UTR.hGJB2.FLAG.3UTR (construct comprising SEQ ID NO: 54 and supporting cell selective promoter comprising SEQ ID NO: 57), AAVAnc80-PARM1p.mGJB2p.5UTR.hGJB2.FLAG.3UTR (construct comprising SEQ ID NO: 7 and supporting cell selective promoter comprising SEQ ID NO: 40), AAVAnc80-GFAPP.mGJB2p.hGJB2, AAVAnc80-MMP15p.mGJB2p.hGJB2, or AAVAnc80-VIMP.mGJB2p.hGJB2. Administration of a hGJB2 construct with a promoter incorporating the CMV-enhancer resulted in supporting cell expression that colocalized with endogenous connexin 26 expression (FIG. 9B; asterisk). However, inner hair cell expression was still detected (arrowhead). In contrast, administration of a hGJB2 construct with promoters derived from supporting cell genes GDF6 (FIG. 9C), IGFBP2 (FIG. 9D), and PARM1 (FIG. 9E) in combination with a minimal GJB2 promoter resulted in supporting cell expression without detection of inner hair cell expression.
- [0457] Administration of AAVAnc80 particles comprising AAVAnc80-GFAPP.mGJB2p.hGJB2 did not result in supporting cell expression of GJB2 (FIG. 9F). Administration of AAVAnc80 particles comprising AAVAnc80-MMP15p.mGJB2p.hGJB2 or AAVAnc80-VIMP.mGJB2p.hGJB2 resulted in supporting

cell expression of flag-tagged hGJB2. Expression of flag-tagged hGJB2 not detected in hair cells as noted by Myo7a staining. Likewise, administration of a hGJB2 construct with promoters derived from supporting cell genes GDF6 (FIG. 9I), PARM1 (FIG. 9J), VIM (FIG. 9K), and MMP15p (FIG. 9L) resulted in supporting cell expression without detection of inner hair cell expression.

[0458] Juvenile WT mice were administered with AAVAnc80 particles comprising AAVAnc80.CMVe.GFAP.mGJB2p.hGJB2.FLAG, AAVAnc80.CMVe.GDF6.mGJB2p.hGJB2.FLAG, or AAVAnc80.CMVe.PARM1.mGJB2p.hGJB2.FLAG through the round window membrane with posterior semicircular canal fenestration. 4 weeks post administration, the mice were euthanized, the inner ears were harvested and fixed in PFA, and the injected (left) ear was processed for immunofluorescent staining using phalloidin to label all cells and hair-cells stereocilia bundle, anti-FLAG to label the transgene and the hair cell marker Myo7a. Anti-Cx26 antibody was also used in some of the samples to colocalize the expression of the transgene with endogenous Cx26 expression. Multiple regions from the base, middle and apex of the cochlea were imaged and representative images are presented in FIGs. 9M-9O.

[0459] Expression of the Cx26-FLAG transgene (green) was detected in supporting cells, overlapping with endogenous Cx26 expression (asterisk), and in some cases, was also observed in inner hair cells (arrowhead) (FIG. 9M). FIGs. 9N and 9O demonstrate robust FLAG expression in supporting cells (green) with no apparent expression in IHCs or hair cell loss. Further, differential expression patterns were observed between supporting cell subtypes.

WHAT IS CLAIMED IS:

1. A polynucleotide comprising a sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NOs: 40, 90, 96, or 99.
2. The polynucleotide of claim 1, which comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 90.
3. The polynucleotide of claim 1, which comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 40.
4. The polynucleotide of claim 1, which comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 96.
5. The polynucleotide of claim 1, which comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 99.
6. The polynucleotide of any one of claims 1-5, wherein the polynucleotide is capable of directing transcription of a coding sequence for a Connexin 26 polypeptide or a functional fragment thereof.
7. A construct comprising the polynucleotide of any one of claims 1-6 and a nucleic acid sequence comprising the coding sequence for a/the Connexin 26 polypeptide or functional fragment thereof.
8. The construct of claim 7, wherein the construct is an expression cassette.

9. The polynucleotide of any one of claims 1-6 or the construct of any one of claims 7-8, wherein the polynucleotide is a promoter and is operably linked to a/the coding sequence.
10. The polynucleotide of any one of claims 1-6 or the construct of any one of claims 7-9, wherein the polynucleotide is capable of directing transcription of the coding sequence in an inner ear support cell.
11. The polynucleotide of any one of claims 1-6 or the construct of any one of claims 7-10, wherein polypeptide is a Connexin 26 polypeptide or functional fragment thereof.
12. An expression construct comprising a coding sequence for a Connexin 26 polypeptide or a functional fragment thereof operably linked to a promoter, wherein the promoter comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NOs: 40, 90, 96, or 99, wherein the promoter is capable of directing transcription of the coding sequence.
13. The expression construct of claim 12, wherein the promoter comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 90.
14. The expression construct of claim 12, wherein the promoter comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 40.
15. The expression construct of claim 12, wherein the promoter comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 96.
16. The expression construct of claim 12, wherein the promoter comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 99.

17. The expression construct of any one of claims 12-16, wherein the expression construct further comprises a second promoter operably linked to the coding sequence, wherein the second promoter is heterologous or homologous to the coding sequence.
18. The expression construct of any one of claims 12-17, wherein the promoter is capable of directing transcription of the coding sequence in an inner ear support cell.
19. The polynucleotide of any one of claims 1-6, construct of any one of claims 7-11, or the expression construct of any one of claims 12-18, wherein the promoter comprises a nucleic acid sequence having having at least 95% identity to a sequence selected from one or more of SEQ ID NO: 90, 40, 96, or 99.
20. The polynucleotide or construct of any one of claims 10-11 or the expression construct of any one of claims 12-19, wherein the inner ear support cell is selected from one or more of inner phalangeal cells/border cells (IPhC), inner pillar cells (IPC), outer pillar cells (OPC), Deiters' cells rows 1 and 2 (DC1/2), Deiters' cells row 3 (DC3), Hensen's cells (Hec), Claudius cells/outer sulcus cells (CC/OSC), interdental cells (Idc), inner sulcus cells (ISC), Kölliker's organ cells (KO), greater ridge epithelial ridge cells (GER) (including lateral greater epithelial ridge cells (LGER)), and OC90+ cells (OC90), fibroblasts, and other cells of the lateral wall.
21. The polynucleotide of claim 6, construct of any one of claims 7-11, or the expression construct of any claims 12-20, further comprising a minimal GJB2 promoter which is operably linked to the coding sequence for the Connexin 26 polypeptide or functional fragment thereof.
22. The polynucleotide of claim 6, construct of any one of claims 7-11, or the expression construct of any claims 12-20, which comprises a GJB2 nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NOs: 117-126.
23. An expression construct comprising a coding sequence for a Connexin 26 polypeptide or functional fragment thereof operably linked to an inner ear supporting cell selective

promoter and a minimal GJB2 promoter, wherein the polynucleotide is expressed in an inner ear support cell.

24. The expression construct of claim 23, wherein the inner ear supporting cell selective promoter is heterologous to the coding sequence for the Connexin 26 polypeptide or functional fragment thereof.
25. The expression construct of claim 23 or 24, wherein the inner ear supporting cell selective promoter comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NOs: 40, 90, 96, or 99.
26. The expression construct of claim 25, wherein the promoter comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 90.
27. The expression construct of claim 25, wherein the promoter comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 40.
28. The expression construct of claim 25, wherein the promoter comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 96.
29. The expression construct of claim 25, wherein the promoter comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 99.
30. The expression construct of any one of claims 23-29, wherein the inner ear supporting cell selective promoter comprises a nucleic acid sequence having at least 95% identity to a sequence selected from one or more of SEQ ID NO: 90, 40, 96, or 99.
31. The expression construct of any one of claims 23-30, wherein the inner ear support cell is selected from one or more of inner phalangeal cells/border cells (IPhC), inner

pillar cells (IPC), outer pillar cells (OPC), Deiters' cells rows 1 and 2 (DC1/2), Deiters' cells row 3 (DC3), Hensen's cells (Hec), Claudius cells/outer sulcus cells (CC/OSC), interdental cells (Idc), inner sulcus cells (ISC), Kölliker's organ cells (KO), greater ridge epithelial ridge cells (GER) (including lateral greater epithelial ridge cells (LGER)), and OC90+ cells (OC90), fibroblasts, and other cells of the lateral wall.

32. The polynucleotide, construct, or expression construct of any of claims 21-31, wherein the minimal GJB2 promoter comprises a nucleic acid sequence with at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% at least 99%, or 100% identity to SEQ ID NO: 86.
33. The expression construct of any of claims 23-32, which comprises a GJB2 nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NOs: 117-126.
34. A viral vector construct comprising: (i) a 5' inverted terminal repeat (ITR), (ii) a coding sequence for a Connexin 26 polypeptide or functional fragment thereof operably linked to a promoter which is capable of directing transcription of the coding sequence in an inner ear support cell, and (iii) a 3' ITR, wherein the promoter is heterologous to the coding sequence.
35. The viral vector construct of claim 34, wherein the promoter comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NOs: 40, 90, 96, or 99.
36. The viral vector construct of claim 35, wherein the promoter comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 90.

37. The viral vector construct of claim 35, wherein the promoter comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 40.
38. The viral vector construct of claim 35, wherein the promoter comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 96.
39. The viral vector construct of claim 35, wherein the promoter comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 99.
40. The viral vector construct of any one of claims 34-39, further comprising a 5' untranslated region (UTR).
41. The viral vector construct of any one of claims 34-40, further comprising a 3' untranslated region (UTR).
42. The viral vector construct of any one of claims 34-39, comprising: (i) the 5' inverted terminal repeat (ITR), (ii) a 5' untranslated region (UTR), (iii) the coding sequence for the Connexin 26 polypeptide or functional fragment thereof operably linked to a promoter which expresses the polynucleotide in an inner ear support cell, (iv) a 3' UTR, and (v) the 3' ITR.
43. The viral vector construct of any of claims 34-39, which comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NOs: 117-126.
44. A viral vector construct comprising: (i) a 5' inverted terminal repeat (ITR), (ii) a coding sequence for a Connexin 26 polypeptide or functional fragment thereof operably linked to an inner ear supporting cell selective promoter and a minimal GJB2 promoter, and (iii) a 3' ITR, wherein the inner ear supporting cell selective promoter is heterologous to the coding sequence.

45. The viral vector construct of claim 44, wherein the inner ear supporting cell selective promoter comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NOs: 40, 90, 96, or 99.
46. The viral vector construct of claim 45, wherein the promoter comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 90.
47. The viral vector construct of claim 45, wherein the promoter comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 40.
48. The viral vector construct of claim 45, wherein the promoter comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 96.
49. The viral vector construct of claim 45, wherein the promoter comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 99.
50. The viral vector construct of claim 44-49, further comprising a 5' untranslated region (UTR).
51. The viral vector construct of claim 44-50, further comprising a 3' untranslated region (UTR).
52. The viral vector construct of claim 44-49, comprising: (i) the 5' inverted terminal repeat (ITR), (ii) a 5' untranslated region (UTR), (iii) the coding sequence for the Connexin 26 polypeptide or functional fragment thereof operably linked to an inner ear supporting cell selective promoter and a minimal GJB2 promoter, (iv) a 3' UTR, and (v) the 3' ITR.

53. The viral vector construct of any of claims 34-52, which comprises a GJB2 nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NOs: 117-126.
54. The viral vector construct of any of claims 34-53, wherein the promoter is heterologous to the coding sequence for the Connexin 26 or functional fragment thereof.
55. The viral vector construct of any one of claims 34-54, wherein the inner ear supporting cell selective promoter is selected from one or more of a sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NOs: 40, 90, 96, or 99.
56. The viral vector construct of claim 55, wherein the promoter comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 90.
57. The viral vector construct of claim 56, wherein the promoter comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 40.
58. The viral vector construct of claim 56, wherein the promoter comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 96.
59. The viral vector construct of claim 56, wherein the promoter comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 99.
60. The viral vector construct of any one of claims 44-59, wherein the minimal GJB2 promoter comprises a nucleic acid sequence with at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 86.

61. The viral vector construct of any one of claims 34-60, wherein the promoter is capable of expressing the coding sequence for the Connexin 26 polypeptide or functional fragment thereof in an inner ear support cell selected from one or more of inner phalangeal cells/border cells (IPhC), inner pillar cells (IPC), outer pillar cells (OPC), Deiters' cells rows 1 and 2 (DC1/2), Deiters' cells row 3 (DC3), Hensen's cells (Hec), Claudius cells/outer sulcus cells (CC/OSC), interdental cells (Idc), inner sulcus cells (ISC), Kölliker's organ cells (KO), greater ridge epithelial ridge cells (GER) (including lateral greater epithelial ridge cells (LGER)), and OC90+ cells (OC90), fibroblasts, and other cells of the lateral wall.
62. The polynucleotide of any one of claims 1-6, the construct of any one of claims 7-11, or the expression construct of any one of claims 12-33, wherein the construct further comprises a 5' UTR.
63. The viral vector construct of any one of claims 34-61, wherein the 5' UTR comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 100% identity to the sequence of any one of SEQ ID NOs: 20, 21, or 66.
64. The polynucleotide of any one of claims 1-6, the construct of any one of claims 7-11, or the expression construct of any one of claims 12-33, wherein the construct further comprises a 3' UTR.
65. The viral vector construct of any one of claims 34-63, wherein the 3' UTR comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 100% identity to the sequence of any one of SEQ ID NOs: 22, 67, 68, or 69.
66. The polynucleotide, construct, expression construct, or viral vector construct of any of the preceding claims, further comprising a polyA tail.
67. The polynucleotide, construct, expression construct, or viral vector construct of claim 66, wherein the polyA tail is a bovine growth hormone, mouse- β -globin, mouse- α -globin, human collagen, polyoma virus, the Herpes simplex virus thymidine kinase

gene (HSV TK), IgG heavy-chain gene, human growth hormone, or a SV40 late and early poly(A).

68. The polynucleotide, construct, expression construct, or viral vector construct of claim 67, wherein the polyA tail is a bovine growth hormone polyA.
69. The polynucleotide of any one of claims 1-6, construct of any one of claims 7-11 or the expression construct of any one of claims 12-33, further comprising a 5' and a 3' inverted terminal repeat (ITR),
70. The viral vector construct of any one of claims 34-62, wherein the 5' ITR and the 3' ITR flank the promoter and the polynucleotide.
71. The polynucleotide, construct, or the expression construct of claim 70 or the viral vector construct of claim 70, wherein the 5' ITR and the 3' ITR are AAV ITRs derived from a serotype selected from AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, and AAV Anc80 ITRs.
72. The polynucleotide, construct, expression construct, or viral vector construct of claim 71, wherein the AAV ITRs are derived from serotype AAV2.
73. The viral vector construct of any one of claims 70-72, wherein the 5' AAV ITR comprises the nucleic acid sequence of SEQ ID NO: 8 or SEQ ID NO: 52.
74. The polynucleotide, construct, or expression construct of claims 69 or 71-72, or viral vector construct of any one of claims 70-72, wherein the 3' AAV ITR comprises the nucleic acid sequence of SEQ ID NO: 9 or SEQ ID NO: 53.
75. The polynucleotide, construct, expression construct, or viral vector construct of claims 73-74, wherein
 - a. the 5' ITR comprises a nucleic acid sequence according to SEQ ID NO: 8 and the 3' ITR comprises a nucleic acid sequence according to SEQ ID NO: 9; or
 - b. the 5' ITR comprises a nucleic acid sequence according to SEQ ID NO: 52 and the 3' ITR comprises a nucleic acid sequence according to SEQ ID NO: 53.

76. The polynucleotide, construct, expression construct, or viral vector construct of claims 73-75, wherein (i) the 5' ITR comprises the nucleic acid sequence of SEQ ID NOs: 8 or 52, (ii) the 5' UTR comprises the nucleic acid of any one of SEQ ID NOs: 20, 21, or 66, (iii) the promoter comprises the nucleic acid sequence of any one of SEQ ID NOs: 10-16, 28, 40, 57, 90-99, (iv) the 3' UTR comprises the nucleic acid sequence of SEQ ID NOs: 22, 67, 68, or 69, and (v) the 3' ITR comprises the nucleic acid sequence of SEQ ID NOs: 9 or 53.
77. The polynucleotide, construct, expression construct, or viral vector construct of claims 73-76, wherein (i) the 5' ITR comprises the nucleic acid sequence of SEQ ID NOs: 8 or 52, (ii) the 5' UTR comprises the nucleic acid of any one of SEQ ID NOs: 20, 21, or 66, (iii) the inner ear supporting cell selective promoter comprises the nucleic acid sequence of any one of SEQ ID NOs: 10-16, 28, 40, 57, 90-99, the minimal GJB2 promoter comprises the sequence of SEQ ID NO: 86, (v) the 3' UTR comprises the nucleic acid sequence of SEQ ID NOs: 22, 67, 68, or 69, and (vi) the 3' ITR comprises the nucleic acid sequence of SEQ ID NOs: 9 or 53.
78. The polynucleotide, construct, expression construct, or viral vector construct of any of the preceding claims, wherein the construct comprises a nucleic acid sequence according to any one of SEQ ID NOs: 7, 17, 38, 45-51, 54, 61, 82-84, 87-88, and 100-107.
79. The polynucleotide, construct, expression construct, or viral vector construct of any of the preceding claims, wherein the construct is selectively expressed in an inner ear supporting cell.
80. The polynucleotide, construct, expression construct, or viral vector construct of any one of the preceding claims, wherein the construct comprises nucleotides 12-4557 of SEQ ID NO: 7, nucleotides 12-4338 of SEQ ID NO: 17, nucleotides 12-3976 of SEQ ID NO: 38, nucleotides 12-4754 of SEQ ID NO: 54, nucleotides 12-4429 of SEQ ID NO: 61, nucleotides 12-4645 of SEQ ID NO: 100, nucleotides 12-4708 of SEQ ID NO: 101, nucleotides 12-4993 of SEQ ID NO: 102, nucleotides 12-4496 of SEQ ID NO: 103, nucleotides 12-4253 of SEQ ID NO: 104, nucleotides 12-4320 of SEQ ID

NO: 105, nucleotides 12-4464 of SEQ ID NO: 106, or nucleotides 12-4328 of SEQ ID NO: 107.

81. A viral vector comprising the polynucleotide, construct, expression construct, or viral vector construct of any of the preceding claims.
82. The vector of claim 81, wherein the viral vector is selected from the group consisting of an adeno-associated viral (AAV), adenovirus, or lentiviral vector.
83. The vector of claim 82, wherein the viral vector is an AAV vector.
84. An AAV particle comprising the polynucleotide, construct, expression construct, or viral vector construct of any one of claims 1-80.
85. The viral vector of claim 83 or AAV particle of claim 84, which comprises an AAV capsid, wherein the AAV capsid is or is derived from an AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV-rh8, AAV-rh10, AAV-rh39, AAV-rh43 or AAV Anc80 serotype capsid.
86. The AAV particle of claim 85, wherein the AAV capsid is an AAV Anc80 capsid.
87. A composition comprising the polynucleotide, construct, expression construct, or viral vector construct of any of claims 1-80, the vector of any of claims 81-83, or the AAV particle of claim 84-86.
88. The composition of claim 88, wherein the composition is a pharmaceutical composition further comprising a pharmaceutically acceptable carrier.
89. The composition of claim 88, wherein the pharmaceutical composition is a synthetic perilymph solution.
90. An ex vivo cell comprising the polynucleotide, the construct, the expression construct, the viral vector construct, the viral vector, or the AAV particle of any of the previous claims.

91. The ex vivo cell of claim 90, wherein the ex vivo cell is an inner ear cell.
92. The ex vivo cell of claim 91, wherein the ex vivo cell is an inner ear supporting cell.
93. The ex vivo cell of claim 92, wherein the supporting cell is selected from one or more of inner phalangeal cells/border cells (IPhC), inner pillar cells (IPC), outer pillar cells (OPC), Deiters' cells rows 1 and 2 (DC1/2), Deiters' cells row 3 (DC3), Hensen's cells (Hec), Claudius cells/outer sulcus cells (CC/OSC), interdental cells (Idc), inner sulcus cells (ISC), Kölliker's organ cells (KO), greater ridge epithelial ridge cells (GER) (including lateral greater epithelial ridge cells (LGER)), and OC90+ cells (OC90), fibroblasts, and other cells of the lateral wall.
94. A method comprising, transducing an ex vivo cell with:
 - a. the polynucleotide, the construct, the expression construct, the viral vector construct, the viral vector, or the AAV particle of any of the previous claims; and
 - b. one or more helper plasmids collectively comprising an AAV Rep gene, AAV Cap gene, AAV VA gene, AAV E2a gene, and AAV E4 gene.
95. The method of claim 94, wherein the ex vivo cell is an inner ear cell.
96. The method of claim 95, wherein the ex vivo cell is an inner ear supporting cell.
97. The method of claim 96, wherein the supporting cell is selected from one or more of inner phalangeal cells/border cells (IPhC), inner pillar cells (IPC), outer pillar cells (OPC), Deiters' cells rows 1 and 2 (DC1/2), Deiters' cells row 3 (DC3), Hensen's cells (Hec), Claudius cells/outer sulcus cells (CC/OSC), interdental cells (Idc), inner sulcus cells (ISC), Kölliker's organ cells (KO), greater ridge epithelial ridge cells (GER) (including lateral greater epithelial ridge cells (LGER)), and OC90+ cells (OC90), fibroblasts, and other cells of the lateral wall.
98. A method of expressing the Connexin 26 polypeptide or functional fragment thereof in an inner ear supporting cell, comprising administering the polynucleotide, the construct, the expression construct, the viral vector construct, the viral vector, the AAV particle, or the ex vivo cell of any of the previous claims to the subject.

99. A method of increasing expression of the Connexin 26 polypeptide or functional fragment thereof in an inner ear supporting cell, comprising administering the polynucleotide, the construct, the expression construct, the viral vector construct, the viral vector, the AAV particle, or the ex vivo cell of any of the previous claims to the subject.
100. The method of claim 98 or 99, wherein the expression of the Connexin 26 polypeptide or functional fragment thereof in the inner ear supporting cell is increased relative to endogenous expression of the polypeptide in the inner ear supporting cell.
101. A method of treating hearing loss in a subject suffering from or at risk of hearing loss, comprising administering the polynucleotide, the construct, the expression construct, the viral vector construct, the viral vector, the AAV particle, or the ex vivo cell of any of the previous claims to the subject.
102. The method of any of claims 98-101, wherein (i) the Connexin 26 polypeptide or functional fragment thereof is predominately expressed in inner ear supporting cells, (ii) the Connexin 26 polypeptide or functional fragment thereof is selectively expressed at a higher level in inner ear supporting cells than in inner ear hair cells, (iii) the Connexin 26 polypeptide or functional fragment thereof not expressed at levels sufficient to cause toxicity in inner ear hair cells, or (iv) or any combination thereof.
103. The method of claims 98-102, wherein the inner ear supporting cells are selected from one or more of inner phalangeal cells/border cells (IPhC), inner pillar cells (IPC), outer pillar cells (OPC), Deiters' cells rows 1 and 2 (DC1/2), Deiters' cells row 3 (DC3), Hensen's cells (Hec), Claudius cells/outer sulcus cells (CC/OSC), interdental cells (Idc), inner sulcus cells (ISC), Kölliker's organ cells (KO), and OC90+ cells (OC90).
104. The method of any of claims 98-103, wherein the administration is to the inner ear of the subject.
105. The method of claim 104, wherein the administration is to the cochlea of the subject.

106. The method of claim 104-105, wherein the administration is via a round window membrane injection.
107. Use of the polynucleotide, the construct, the expression construct, the viral vector construct, the viral vector, the AAV particle, or the ex vivo cell of any of the previous claims, for the treatment of hearing loss in a subject suffering from or at risk of hearing loss.
108. Use of the polynucleotide, the construct, the expression construct, the viral vector construct, the viral vector, the AAV particle, or the ex vivo cell of any of the previous claims, in the manufacture of a medicament for the treatment of hearing loss.
109. The polynucleotide, the construct, the expression construct, the viral vector construct, the viral vector, the AAV particle, or the ex vivo cell of any of the previous claims, for use as a medicament.
110. The polynucleotide, the construct, the expression construct, the viral vector construct, the viral vector, the AAV particle, or the ex vivo cell of any of the previous claims, for use in the treatment of hearing loss.
111. A kit comprising the polynucleotide, the construct, the expression construct, the viral vector construct, the viral vector, the AAV particle, or the ex vivo cell of any of the previous claims.
112. The method of any one of claims 98-106, the use of any one of claims 99-108, or the kit of claim 111, wherein the construct, vector, AAV particle, composition or ex vivo cell is pre-loaded in a device.
113. The method, use, or kit of claim 112, wherein the device is a microcatheter.
114. The method, use or kit of claim 113, 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.

115. The method, use or kit of any of claims 111-114, wherein a distal end of the microcatheter is comprised of at least one microneedle with diameter of between 10 and 1,000 microns.
116. The kit of any one of claims 111-115, further comprising a device.
117. The kit of claim 116, wherein the device is a device described in any one of FIGS. 5-8.
118. The kit of any of claims 116 or 117, wherein the device comprises a needle comprising a bent portion and an angled tip.
119. The polynucleotide, the construct, the expression construct, the viral vector construct, the viral vector, the AAV particle of any of the previous claims, comprising a sequence having at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NOs: 40, 90, 96, or 99.
120. The polynucleotide, the construct, the expression construct, the viral vector construct, the viral vector, or the AAV particle of any of the previous claims, comprising a sequence having at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NOs: 40, 90, 96, or 99.
121. The polynucleotide, the construct, the expression construct, the viral vector construct, the viral vector, or the AAV particle of any of the previous claims, comprising a sequence having at least 99%, or 100% identity to any one of SEQ ID NOs: 40, 90, 96, or 99.
122. A polynucleotide comprising a sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NOs: 16, 28, 40, 57, or 90-99.
123. A construct comprising the polynucleotide of claim 122 and a nucleic acid sequence encoding a polypeptide.

124. The construct of claim 123, wherein the polynucleotide is capable of directing transcription of the nucleic acid sequence encoding the polypeptide.
125. The construct of any one of claims 123-124, wherein the polynucleotide is a promoter and is operably linked to the nucleic acid sequence encoding the polypeptide.
126. The construct of claims 123-125, wherein the construct is an expression cassette.
127. The construct of any one of claims 123-126, wherein the polynucleotide is capable of directing transcription of the nucleic acid sequence encoding a polypeptide in an inner ear support cell.
128. The construct of any one of claims 123-127, wherein polypeptide is a therapeutic polypeptide, a reporter polypeptide, or an inner ear supporting cell polypeptide.
129. An expression construct comprising a polynucleotide encoding a polypeptide operably linked to a promoter, wherein the promoter is selected from one or more of a GJB6 promoter, a GDF6 promoter, a IGFBP2 promoter, a RBP7 promoter, a PARM1 promoter, a GFAP promoter, a BACE2 promoter, a DBI2 promoter, a FABP3 promoter, a KLHL14 promoter, a MMP15 promoter, a SPARC promoter, a TSPAN8 promoter, a VIM promoter, derivatives thereof, or fragments thereof.
130. The expression construct of claim 129, wherein the promoter is heterologous to the polynucleotide.
131. The expression construct of any one of claims 129-130, wherein the promoter is capable of directing transcription of the polynucleotide in an inner ear support cell.
132. The expression construct of any one of claims 129-131, wherein the promoter comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NOs: 16, 28, 40, 57, or 90-99.

133. The expression construct of any one of claims 131-132, wherein the inner ear support cell is selected from one or more of inner phalangeal cells/border cells (IPhC), inner pillar cells (IPC), outer pillar cells (OPC), Deiters' cells rows 1 and 2 (DC1/2), Deiters' cells row 3 (DC3), Hensen's cells (Hec), Claudius cells/outer sulcus cells (CC/OSC), interdental cells (Idc), inner sulcus cells (ISC), Kölliker's organ cells (KO), greater ridge epithelial ridge cells (GER) (including lateral greater epithelial ridge cells (LGER)), and OC90+ cells (OC90), fibroblasts, and other cells of the lateral wall.
134. The expression construct of any of claims 129-133, wherein the promoter comprises a nucleic acid sequence having at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NOs: 16, 28, 40, 57, or 90-99.
135. The expression construct of any of the previous claims further comprising a miRNA regulatory target site (miRTS) for a microRNA expressed in an inner ear cell.
136. The expression construct of claim 135, wherein the microRNA is expressed in an inner ear hair cell.
137. The expression construct of claim 136, wherein the microRNA is one or more of miR-194, miR-140, miR-18a, miR-99a, miR-30b, miR-15a, miR182, or miR-183.
138. The expression construct of any of claims 129-137, further comprising a minimal GJB2 promoter which is operably linked to the nucleic acid sequence encoding the polypeptide.
139. An expression construct comprising a polynucleotide encoding a polypeptide operably linked to an inner ear supporting cell selective promoter and a minimal GJB2 promoter, wherein the polynucleotide is expressed in an inner ear support cell.
140. The expression construct of claim 139, wherein the inner ear supporting cell selective promoter is heterologous to the polynucleotide.

141. The expression construct of any one of claims 139-140, wherein the polypeptide is a therapeutic polypeptide, a reporter polypeptide, or an inner ear supporting cell polypeptide.
142. The expression construct of any one of claims 139-141, wherein the inner ear supporting cell selective promoter is selected from one or more of a GJB6 promoter, a GDF6 promoter, a IGFBP2 promoter, a RBP7 promoter, a PARM1 promoter, a GFAP promoter, a BACE2 promoter, a DBI2 promoter, a FABP3 promoter, a KLHL14 promoter, a MMP15 promoter, a SPARC promoter, a TSPAN8 promoter, or a VIM promoter.
143. The expression construct of any one of claims 139-142, wherein the inner ear supporting cell selective promoter comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NOs: 16, 28, 40, 57, or 90-99.
144. The expression construct of any one of claims 139-143, wherein the inner ear support cell is selected from one or more of inner phalangeal cells/border cells (IPhC), inner pillar cells (IPC), outer pillar cells (OPC), Deiters' cells rows 1 and 2 (DC1/2), Deiters' cells row 3 (DC3), Hensen's cells (Hec), Claudius cells/outer sulcus cells (CC/OSC), interdental cells (Idc), inner sulcus cells (ISC), Kölliker's organ cells (KO), greater ridge epithelial ridge cells (GER) (including lateral greater epithelial ridge cells (LGER)), and OC90+ cells (OC90), fibroblasts, and other cells of the lateral wall.
145. The expression construct of any one of claims 139-144, wherein the inner ear supporting cell selective promoter comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NOs: 16, 28, 40, 57, or 90-99.
146. The expression construct of any of claims 139-145, wherein the minimal GJB2 promoter comprises a nucleic acid sequence with at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% at least 99%, or 100% identity to SEQ ID NO: 86

147. The expression construct of any one of claims 139-146, further comprising a miRNA regulatory target site (miRTS) for a microRNA expressed in an inner ear cell.
148. The expression construct of claim 147, wherein the microRNA is expressed in an inner ear hair cell.
149. The expression construct of claim 148, wherein the microRNA is one or more of miR-194, miR-140, miR-18a, miR-99a, miR-30b, miR-15a, miR182, or miR-183.
150. A viral vector construct comprising: (i) a 5' inverted terminal repeat (ITR), (ii) a polynucleotide encoding a polypeptide operably linked to a promoter which is capable of driving transcription of the polynucleotide in an inner ear support cell, and (iii) a 3' ITR, wherein the promoter is heterologous to the polynucleotide.
151. The viral vector construct of claim 150, wherein the promoter comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NOs: 16, 28, 40, 57, or 90-99.
152. The viral vector construct of any one of claims 150-151, comprising: (i) the 5' inverted terminal repeat (ITR), (ii) a 5' untranslated region (UTR), (iii) the polynucleotide encoding a polypeptide operably linked to a promoter which is capable of driving transcription of the polynucleotide in an inner ear support cell, and (iv) the 3' ITR.
153. The viral vector construct of any one of claims 150-152, comprising: (i) the 5' inverted terminal repeat (ITR), (ii) the polynucleotide encoding a polypeptide operably linked to a promoter which is capable of driving transcription of the polynucleotide in an inner ear support cell, (iii) a 3' untranslated region (UTR), and (iv) the 3' ITR.
154. The viral vector construct of any one of claims 150-153, comprising: (i) the 5' inverted terminal repeat (ITR), (ii) the polynucleotide encoding a polypeptide operably linked to a promoter which is capable of driving transcription of the

polynucleotide in an inner ear support cell, (iii) a miRNA regulatory target site (miRTS) for a microRNA expressed in an inner ear cell, (iv) a 3' untranslated region (UTR), and (v) the 3' ITR.

155. The viral vector construct of any one of claims 150-154, comprising: (i) the 5' inverted terminal repeat (ITR), (ii) a 5' untranslated region (UTR), (iii) the polynucleotide encoding a polypeptide operably linked to a promoter which is capable of driving transcription of the polynucleotide in an inner ear support cell, (iv) a 3' UTR, and (v) the 3' ITR.
156. A viral vector construct comprising: (i) a 5' inverted terminal repeat (ITR), (ii) a polynucleotide encoding a polypeptide operably linked to an inner ear supporting cell selective promoter and a minimal GJB2 promoter which is capable of driving transcription of the polynucleotide in an inner ear support cell, and (iii) a 3' ITR, wherein the inner ear supporting cell selective promoter is heterologous to the polynucleotide.
157. The viral vector construct of claim 156, comprising: (i) a 5' inverted terminal repeat (ITR), (ii) a polynucleotide encoding a polypeptide operably linked to an inner ear supporting cell selective promoter and a minimal GJB2 promoter, and (iii) a 3' ITR, wherein the inner ear supporting cell selective promoter comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NOs: 16, 28, 40, 57, or 90-99.
158. The viral vector construct of any one of claims 156-157, comprising: (i) the 5' inverted terminal repeat (ITR), (ii) a 5' untranslated region (UTR), (iii) the polynucleotide encoding a polypeptide operably linked to an inner ear supporting cell selective promoter and a minimal GJB2 promoter, and (iv) the 3' ITR.
159. The viral vector construct of any one of claims 156-158, comprising: (i) the 5' inverted terminal repeat (ITR), (ii) the polynucleotide encoding a polypeptide operably linked to an inner ear supporting cell selective promoter and a minimal GJB2 promoter, (iii) a 3' untranslated region (UTR), and (iv) the 3' ITR.

160. The viral vector construct of any one of claims 156-159, comprising: (i) the 5' inverted terminal repeat (ITR), (ii) the polynucleotide encoding a polypeptide operably linked to an inner ear supporting cell selective promoter and a minimal GJB2 promoter, (iii) a miRNA regulatory target site (miRTS) for a microRNA expressed in an inner ear cell, (iv) a 3' untranslated region (UTR), and (v) the 3' ITR.
161. The viral vector construct of any one of claims 156-160, comprising: (i) the 5' inverted terminal repeat (ITR), (ii) a 5' untranslated region (UTR), (iii) the polynucleotide encoding a polypeptide operably linked to an inner ear supporting cell selective promoter and a minimal GJB2 promoter which is capable of driving transcription of the polynucleotide in an inner ear support cell, (iv) a 3' UTR, and (v) the 3' ITR.
162. The viral vector construct of any one of claims 156-161, comprising: (i) the 5' inverted terminal repeat (ITR), (ii) a 5' untranslated region (UTR), (iii) the polynucleotide encoding a polypeptide operably linked to a promoter which is capable of driving transcription of the polynucleotide in an inner ear support cell, (iii) a miRNA regulatory target site (miRTS) for a microRNA expressed in an inner ear cell, (v) a 3' UTR, and (vi) the 3' ITR.
163. The viral vector construct of any one of claims 156-162, comprising: (i) the 5' inverted terminal repeat (ITR), (ii) a 5' untranslated region (UTR), (iii) the polynucleotide encoding a polypeptide operably linked to an inner ear supporting cell selective promoter and a minimal GJB2 promoter which is capable of driving transcription of the polynucleotide in an inner ear support cell, (iii) a miRNA regulatory target site (miRTS) for a microRNA expressed in an inner ear cell, (v) a 3' UTR, and (vi) the 3' ITR.
164. A viral vector construct comprising a polynucleotide encoding a polypeptide operably linked to a promoter, wherein the construct comprises a miRNA regulatory target site (miRTS) for a microRNA expressed in an inner ear cell.
165. The viral vector construct of claim 164, comprising: (i) a 5' inverted terminal repeat (ITR), (ii) the polynucleotide encoding a polypeptide operably linked to a promoter,

- (iii) the miRNA regulatory target site (miRTS) for a microRNA expressed in an inner ear cell, and (iv) a 3' ITR.
166. The viral vector construct of any one of claims 164-165, comprising: (i) a 5' inverted terminal repeat (ITR), (ii) a 5' untranslated region (UTR), (iii) the polynucleotide encoding a polypeptide operably linked to a promoter, (iv) the miRNA regulatory target site (miRTS) for a microRNA expressed in an inner ear cell, and (v) a 3' ITR.
167. The viral vector construct of any one of claims 164-166, comprising: (i) a 5' inverted terminal repeat (ITR), (ii) the polynucleotide encoding a polypeptide operably linked to a promoter, (iii) the miRNA regulatory target site (miRTS) for a microRNA expressed in an inner ear cell, (iv) a 3' untranslated region (UTR) and (v) a 3' ITR.
168. The viral vector construct of any one of claims 164-167, comprising: (i) a 5' inverted terminal repeat (ITR), (ii) a 5' untranslated region (UTR), (iii) the polynucleotide encoding a polypeptide operably linked to a promoter, (iv) the miRNA regulatory target site (miRTS) for a microRNA expressed in an inner ear cell, (v) a 3' UTR, and (vi) a 3' ITR.
169. A viral vector construct comprising a polynucleotide encoding a polypeptide operably linked to an inner ear supporting cell selective promoter and a minimal GJB2 promoter, wherein the construct comprises a miRNA regulatory target site (miRTS) for a microRNA expressed in an inner ear cell.
170. The viral vector construct of claim 169, comprising: (i) a 5' inverted terminal repeat (ITR), (ii) the polynucleotide encoding a polypeptide operably linked to an inner ear supporting cell selective promoter and a minimal GJB2 promoter, (iii) the miRNA regulatory target site (miRTS) for a microRNA expressed in an inner ear cell, and (iv) a 3' ITR.
171. The viral vector construct of any one of claims 169-170, comprising: (i) a 5' inverted terminal repeat (ITR), (ii) a 5' untranslated region (UTR), (iii) the polynucleotide encoding a polypeptide operably linked to an inner ear supporting cell selective

promoter and a minimal GJB2 promoter, (iv) the miRNA regulatory target site (miRTS) for a microRNA expressed in an inner ear cell, and (v) a 3' ITR.

172. The viral vector construct of any one of claims 169-171, comprising: (i) a 5' inverted terminal repeat (ITR), (ii) the polynucleotide encoding a polypeptide operably linked to an inner ear supporting cell selective promoter and a minimal GJB2 promoter, (iii) the miRNA regulatory target site (miRTS) for a microRNA expressed in an inner ear cell, (iv) a 3' untranslated region (UTR) and (v) a 3' ITR.
173. The viral vector construct of any one of claims 169-172, comprising: (i) a 5' inverted terminal repeat (ITR), (ii) a 5' untranslated region (UTR), (iii) the polynucleotide encoding a polypeptide operably linked to an inner ear supporting cell selective promoter and a minimal GJB2 promoter, (iv) the miRNA regulatory target site (miRTS) for a microRNA expressed in an inner ear cell, (v) a 3' UTR, and (vi) a 3' ITR.
174. The viral vector construct of any one of claims 151-173, wherein the polypeptide is a therapeutic polypeptide, a reporter polypeptide, an inner ear supporting cell polypeptide.
175. The viral vector construct of any one of claims 60 and 160-174, wherein the microRNA is expressed in one or more of inner ear hair cells, spiral ganglion cells, lateral supporting cells, basilar membrane cells, medial supporting cells, spiral limbus cells, or inner sulcus cells.
176. The viral vector construct of claim 175, wherein the microRNA is expressed in inner ear hair cells.
177. The viral vector construct of claim 76, wherein the microRNA is one or more of miR-194, miR-140, miR-18a, miR-99a, miR-30b, miR-15a, miR182, or miR-183.
178. The viral vector construct of claim 175, wherein the microRNA is expressed in spiral ganglion cells.

179. The viral vector construct of claim 178, wherein the microRNA is selected from one or more of miR-194, miR-18a, miR-99a, miR-30b, miR-15a, miR182, or miR-183.
180. The viral vector construct of claim 175, wherein the microRNA is expressed in lateral supporting cells.
181. The viral vector construct of claim 180, wherein the microRNA is selected from one or more of miR-99a, miR-30b, or miR-15a.
182. The viral vector construct of claim 175, wherein the microRNA is expressed in basilar membrane cells.
183. The viral vector construct of claim 182, wherein the microRNA is selected from one or more of miR-99a, miR-30b, or miR-15a.
184. The viral vector construct of claim 175, wherein the microRNA is expressed in medial supporting cells.
185. The viral vector construct of claim 184, wherein the microRNA is selected from one or more of miR182 and miR-183.
186. The viral vector construct of claim 175, wherein the microRNA is expressed in spiral limbus cells.
187. The viral vector construct of claim 186, wherein the microRNA is selected from one or more of miR182 and miR-183.
188. The viral vector construct of claim 175, wherein the microRNA is expressed in inner sulcus cells.
189. The viral vector construct of any of claims 186, wherein the microRNA is selected from one or more of miR182 and miR-183.
190. The viral vector construct of any claims 160 and 162-189, wherein the microRNA regulatory target site comprises a nucleic acid sequence with least 85%, at least 90%,

at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NOs: 1-6, 78, or 79.

191. The viral vector construct of any of claims 150-190, wherein the promoter is heterologous to the polynucleotide.
192. The viral vector construct of claim 191, wherein the promoter is an inducible promoter, a constitutive promoter, a tissue-specific promoter, or a cell-selective promoter.
193. The viral vector construct of claim 192, wherein the constitutive promoter is a CAG promoter, a CBA promoter, a CMV promoter, or a CB7 promoter.
194. The viral vector construct of claims 192-193, wherein the constitutive promoter comprises a sequence having least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 100% identity to any one of SEQ ID NOs: 10-15.
195. The viral vector construct claim 192, wherein the promoter is a cell-selective promoter.
196. The viral vector construct of claim 195, wherein the cell-selective promoter is an inner ear supporting cell selective promoter.
197. The viral vector construct of claim 196, wherein the inner ear supporting cell selective promoter is selected from one or more of a GJB6 promoter, a GDF6 promoter, a IGFBP2 promoter, a RBP7 promoter, a PARM1 promoter, a GFAP promoter, a BACE2 promoter, a DBI2 promoter, a FABP3 promoter, a KLHL14 promoter, a MMP15 promoter, a SPARC promoter, a TSPAN8 promoter, a VIM promoter, derivatives thereof, or fragments thereof.
198. The viral vector construct of any one of claims 196-197, wherein the inner ear supporting cell-selective promoter has at least 85%, at least 90%, at least 95%, at least

96%, at least 97%, at least 98%, at least 99%, or at least 100% identity to any one of SEQ ID NOs: 16, 28, 40, 57, or 90-99.

199. The viral vector construct of any one of claims 169-198, wherein the minimal GJB2 promoter comprises a nucleic acid sequence with at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% at least 99%, or 100% identity to SEQ ID NO: 86.
200. The viral vector construct of any one of claims 150-199, wherein the promoter is capable of expressing the polynucleotide in an inner ear support cell selected from one or more of inner phalangeal cells/border cells (IPhC), inner pillar cells (IPC), outer pillar cells (OPC), Deiters' cells rows 1 and 2 (DC1/2), Deiters' cells row 3 (DC3), Hensen's cells (Hec), Claudius cells/outer sulcus cells (CC/OSC), interdental cells (Idc), inner sulcus cells (ISC), Kölliker's organ cells (KO), greater ridge epithelial ridge cells (GER) (including lateral greater epithelial ridge cells (LGER)), and OC90+ cells (OC90), fibroblasts, and other cells of the lateral wall.
201. The viral vector construct of claim 174, wherein the polypeptide is a therapeutic polypeptide.
202. The viral vector construct of claim 201, wherein the therapeutic polypeptide is a transmembrane protein.
203. The viral vector construct of claims 201-202, wherein the polynucleotide encoding the therapeutic polypeptide comprises a gene selected from ATP2B2, CHRNA9, CDH23, CCER2, CLRN1, CLRN2, COCH, DFNA9, DYT1, EPS8, EPS8L2, ESPN, ESPNL, GJB2, GJB6, GJB3, GSDME, DFNA5, INSM1, IKZF2, LHX3, MYO7A, MYO3A, NDP, PCDH15, PTPRQ, STRC, SLC26A5, USH1C, USH2A, SYNE4, or any combination thereof.
204. The viral vector construct of claim 174, wherein the polynucleotide encodes an inner ear supporting cell polypeptide.

205. The viral vector construct of claim 204, wherein the polynucleotide encoding an inner ear supporting cell polypeptide comprises a gene selected from ATP2B2, CHRNA9, CDH23, CCER2, CLRN1, CLRN2, COCH, DFNA9, DYT1, EPS8, EPS8L2, ESPN, ESPNL, GJB2, GJB6, GJB3, GSDME, DFNA5, INSM1, IKZF2, LHX3, MYO7A, MYO3A, NDP, PCDH15, PTPRQ, STRC, SLC26A5, USH1C, USH2A, SYNE4, or any combination thereof.
206. The viral vector construct of claim 174, wherein the polynucleotide encodes a reporter polypeptide.
207. The viral vector construct of claim 206, wherein the reporter polypeptide is one or more of 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 FLAG tag, a chloramphenicol acetyltransferase (CAT), and a luciferase.
208. The viral vector construct of claims 150-207, wherein the construct further comprises a 5' UTR.
209. The viral vector construct of any one of claims 150-208, wherein the 5' UTR comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 100% identity to the sequence of any one of SEQ ID NOs: 20, 21, or 66.
210. The viral vector construct of claims 150-209, wherein the construct further comprises a 3' UTR.
211. The viral vector construct of any one of claims 210, wherein the 3' UTR comprises a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 100% identity to the sequence of any one of SEQ ID NOs: 22, 67, 68, or 69.
212. The viral vector construct of any of claims 208-211, wherein the 3' UTR and/or the 5' UTR comprises the miRTS.

213. The viral vector construct of any of the preceding claims, further comprising a polyA tail.
214. The viral vector construct of claim 213, wherein the polyA tail is a bovine growth hormone, mouse- β -globin, mouse- α -globin, human collagen, polyoma virus, the Herpes simplex virus thymidine kinase gene (HSV TK), IgG heavy-chain gene, human growth hormone, or a SV40 late and early poly(A).
215. The viral vector construct of claim 214, wherein the polyA tail is a bovine growth hormone polyA.
216. The viral vector construct of any one of claims 150-215, further comprising a 5' and a 3' inverted terminal repeat (ITR),
217. The viral vector construct of any one of claims 216, wherein the 5' ITR and the 3' ITR flank the promoter and the polynucleotide.
218. The viral vector construct of claim 217, wherein the 5' ITR and the 3' ITR are AAV ITRs derived from a serotype selected from AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, and AAV Anc80 ITRs.
219. The viral vector construct of claim 218, wherein the AAV ITRs are derived from serotype AAV2.
220. The viral vector construct of any one of claims 216-219, wherein the 5' AAV ITR comprises the nucleic acid sequence of SEQ ID NO: 8 or SEQ ID NO: 52.
221. The viral vector construct of any one of claims 216-220, wherein the 3' AAV ITR comprises the nucleic acid sequence of SEQ ID NO: 9 or SEQ ID NO: 53.
222. The viral vector construct of claims 229 or 221, wherein
- c. the 5' ITR comprises a nucleic acid sequence according to SEQ ID NO: 8 and the 3' ITR comprises a nucleic acid sequence according to SEQ ID NO: 9; or

- d. the 5' ITR comprises a nucleic acid sequence according to SEQ ID NO: 52 and the 3' ITR comprises a nucleic acid sequence according to SEQ ID NO: 53.
223. The viral vector construct of any of claims 220-222, wherein (i) the 5' ITR comprises the nucleic acid sequence of SEQ ID NOs: 8 or 52, (ii) the 5' UTR comprises the nucleic acid of any one of SEQ ID NOs: 20, 21, or 66, (iii) the promoter comprises the nucleic acid sequence of any one of SEQ ID NOs: 10-16, 28, 40, 57, 90-99, (iv) the 3' UTR comprises the nucleic acid sequence of SEQ ID NOs: 22, 67, 68, or 69, and (v) the 3' ITR comprises the nucleic acid sequence of SEQ ID NOs: 9 or 53.
224. The viral vector construct of any of claims 220-223, wherein (i) the 5' ITR comprises the nucleic acid sequence of SEQ ID NOs: 8 or 52, (ii) the 5' UTR comprises the nucleic acid of any one of SEQ ID NOs: 20, 21, or 66, (iii) the inner ear supporting cell selective promoter comprises the nucleic acid sequence of any one of SEQ ID NOs: 10-16, 28, 40, 57, 90-99, the minimal GJB2 promoter comprises the sequence of SEQ ID NO: 86, (v) the 3' UTR comprises the nucleic acid sequence of SEQ ID NOs: 22, 67, 68, or 69, and (vi) the 3' ITR comprises the nucleic acid sequence of SEQ ID NOs: 9 or 53.
225. The viral vector construct of any one of claims 150-224, wherein the 3' UTR and/or the 5' UTR comprises the miRTS.
226. The viral vector construct of any of the preceding claims, wherein the construct comprises a nucleic acid sequence according to any one of SEQ ID NOs: 7, 17, 38, 45-51, 54, 61, 82-84, 87-88, and 100-107.
227. The viral vector construct of any of the preceding claims, wherein the construct is selectively expressed in an inner ear supporting cell.
228. The viral vector construct of any one of the preceding claims, wherein the construct comprises nucleotides 12-4557 of SEQ ID NO: 7, nucleotides 12-4338 of SEQ ID NO: 17, nucleotides 12-3976 of SEQ ID NO: 38, nucleotides 12-4754 of SEQ ID NO: 54, nucleotides 12-4429 of SEQ ID NO: 61, nucleotides 12-4645 of SEQ ID NO: 100, nucleotides 12-4708 of SEQ ID NO: 101, nucleotides 12-4993 of SEQ ID NO: 102,

nucleotides 12-4496 of SEQ ID NO: 103, nucleotides 12-4253 of SEQ ID NO: 104, nucleotides 12-4320 of SEQ ID NO: 105, nucleotides 12-4464 of SEQ ID NO: 106, or nucleotides 12-4328 of SEQ ID NO: 107.

- 229. The viral vector construct of any of the preceding claims, wherein the construct is an expression cassette.
- 230. A vector comprising the polynucleotide or construct of any of the preceding claims.
- 231. The vector of claim 230, wherein the vector is a mammalian vector or a viral vector.
- 232. The vector of claim 230 or 231, wherein the vector is a viral vector.
- 233. The vector of claim 232, wherein the viral vector is selected from the group consisting of an adeno-associated viral (AAV), adenovirus, or lentiviral vector.
- 234. The vector of claim 233, wherein the viral vector is an AAV vector.
- 235. An AAV particle comprising the polynucleotide, construct, expression construct, or viral vector construct of any one of claims 122-235.
- 236. The AAV particle of claim 235, further comprising an AAV capsid, wherein the AAV capsid is or is derived from an AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV-rh8, AAV-rh10, AAV-rh39, AAV-rh43 or AAV Anc80 capsid.
- 237. The AAV particle of claim 236, wherein the AAV capsid is an AAV Anc80 capsid.
- 238. A composition comprising the polynucleotide, construct, construct expression, or viral vector construct of any of claims 122-229, the vector of any of claims 230-234, or the AAV particle of claim 235-237.
- 239. The composition of claim 238, wherein the composition is a pharmaceutical composition further comprising a pharmaceutically acceptable carrier.

240. The composition of claims 238 or 239, wherein the pharmaceutical composition is a synthetic perilymph solution.
241. An ex vivo cell comprising the polynucleotide, construct, construct expression, or viral vector construct of any of claims 122-229, the vector of any of claims 230-234, or the AAV particle of claim 235-237.
242. The ex vivo cell of claim 241, wherein the ex vivo cell is an inner ear cell.
243. The ex vivo cell of claim 242, wherein the ex vivo cell is an inner ear supporting cell.
244. The ex vivo cell of claim 243, wherein the supporting cell is selected from one or more of inner phalangeal cells/border cells (IPhC), inner pillar cells (IPC), outer pillar cells (OPC), Deiters' cells rows 1 and 2 (DC1/2), Deiters' cells row 3 (DC3), Hensen's cells (Hec), Claudius cells/outer sulcus cells (CC/OSC), interdental cells (Idc), inner sulcus cells (ISC), Kölliker's organ cells (KO), greater ridge epithelial ridge cells (GER) (including lateral greater epithelial ridge cells (LGER)), and OC90+ cells (OC90), fibroblasts, and other cells of the lateral wall.
245. A method comprising, transducing an ex vivo cell with:
- c. the polynucleotide, construct, expression construct, or viral vector construct of any of claims 122-229 or the vector of any of claims 230-234; and
 - d. one or more helper plasmids collectively comprising an AAV Rep gene, AAV Cap gene, AAV VA gene, AAV E2a gene, and AAV E4 gene.
246. The method of claim 245, wherein the ex vivo cell is an inner ear cell.
247. The method of claim 246, wherein the ex vivo cell is an inner ear supporting cell.
248. The method of claim 247, wherein the supporting cell is selected from one or more of inner phalangeal cells/border cells (IPhC), inner pillar cells (IPC), outer pillar cells (OPC), Deiters' cells rows 1 and 2 (DC1/2), Deiters' cells row 3 (DC3), Hensen's cells (Hec), Claudius cells/outer sulcus cells (CC/OSC), interdental cells (Idc), inner sulcus cells (ISC), Kölliker's organ cells (KO), greater ridge epithelial ridge cells (GER)

(including lateral greater epithelial ridge cells (LGER)), and OC90+ cells (OC90), fibroblasts, and other cells of the lateral wall.

249. A method of expressing the therapeutic polypeptide in an inner ear supporting cell of a subject in need thereof, comprising administering the polynucleotide, construct, construct expression, or viral vector construct of any of claims 122-229, the vector of any of claims 230-234, or the AAV particle of claim 235-237, or the ex vivo cell of any one of claims 241-244 to the subject.
250. A method of expressing the polypeptide in an inner ear supporting cell, comprising administering the polynucleotide, construct, construct expression, or viral vector construct of any of claims 122-229, the vector of any of claims 230-234, or the AAV particle of claim 235-237, or the ex vivo cell of any one of claims 241-244 to the inner ear supporting cell.
251. A method of increasing expression of the therapeutic polypeptide in an inner ear supporting cell of a subject in need thereof, comprising administering the polynucleotide, construct, construct expression, or viral vector construct of any of claims 122-229, the vector of any of claims 230-234, or the AAV particle of claim 235-237, or the ex vivo cell of any one of claims 241-244 to the subject.
252. A method of increasing expression of the polypeptide in an inner ear supporting cell, comprising administering the polynucleotide, construct, construct expression, or viral vector construct of any of claims 122-229, the vector of any of claims 230-234, or the AAV particle of claim 235-237, or the ex vivo cell of any one of claims 241-244 to the inner ear supporting cell.
253. The method of claim 249 or 251, wherein the expression of the therapeutic polypeptide is reduced, suppressed, or eliminated in non-inner ear supporting cells compared to endogenous expression of the polypeptide in non-inner ear supporting cells.

254. The method of claim 250 or 252, wherein the expression of the polypeptide is reduced, suppressed, or eliminated in non-inner ear supporting cells compared to endogenous expression of the polypeptide in non-inner ear supporting cells.
255. The method of claim 252-252, wherein the expression of the polypeptide in the inner ear supporting cell is increased relative to endogenous expression of the polypeptide in the inner ear supporting cell.
256. A method of decreasing expression of the therapeutic polypeptide in non-inner ear supporting cells, comprising administering the polynucleotide, construct, construct expression, or viral vector construct of any of claims 122-229, the vector of any of claims 230-234, or the AAV particle of claim 235-237, or the ex vivo cell of any one of claims 241-244 to the subject.
257. A method of decreasing expression of the polypeptide in non-inner ear supporting cells, comprising administering the polynucleotide, construct, construct expression, or viral vector construct of any of claims 122-229, the vector of any of claims 230-234, or the AAV particle of claim 235-237, or the ex vivo cell of any one of claims 241-244 to the subject.
258. The method of claim 256 or 257, wherein the expression of the polypeptide in the non-inner ear supporting cell is decreased relative to endogenous expression of the polypeptide in the non-inner ear supporting cell.
259. The method of any of claims 249-258, wherein the inner ear supporting cells are selected from one or more of inner phalangeal cells/border cells (IPhC), inner pillar cells (IPC), outer pillar cells (OPC), Deiters' cells rows 1 and 2 (DC1/2), Deiters' cells row 3 (DC3), Hensen's cells (Hec), Claudius cells/outer sulcus cells (CC/OSC), interdental cells (Idc), inner sulcus cells (ISC), Kölliker's organ cells (KO), and OC90+ cells (OC90).
260. A method of reducing toxicity associated with expression of the therapeutic polypeptide in an inner ear cell, comprising administering comprising administering polynucleotide, construct, construct expression, or viral vector construct of any of

claims 122-229, the vector of any of claims 230-234, or the AAV particle of claim 235-237, or the ex vivo cell of any one of claims 241-244 to the subject.

261. A method of reducing toxicity associated with expression of the polypeptide in an inner ear cell, comprising administering comprising administering the polynucleotide, construct, construct expression, or viral vector construct of any of claims 122-229, the vector of any of claims 230-234, or the AAV particle of claim 235-237, or the ex vivo cell of any one of claims 241-244 to the subject.
262. The method of claims 260-261, wherein the inner ear cells are selected from inner ear hair cells, spiral ganglion cells, lateral supporting cells, basilar membrane cells, medial supporting cells, spiral limbus cells, inner sulcus cells, or any combination thereof.
263. A method of treating hearing loss in a subject suffering from or at risk of hearing loss, comprising administering the polynucleotide, construct, construct expression, or viral vector construct of any of claims 122-229, the vector of any of claims 230-234, or the AAV particle of claim 235-237, or the ex vivo cell of any one of claims 241-244 to the subject.
264. The method of claim 260, wherein expression of the therapeutic polypeptide is reduced, suppressed, or eliminated in inner ear hair cells, spiral ganglion cells, lateral supporting cells, basilar membrane cells, medial supporting cells, spiral limbus cells, inner sulcus cells, or any combination thereof.
265. The method of claim 261, wherein expression of the polypeptide is reduced, suppressed, or eliminated in inner ear hair cells, spiral ganglion cells, lateral supporting cells, basilar membrane cells, medial supporting cells, spiral limbus cells, inner sulcus cells, or any combination thereof.
266. The method of claim 260, wherein toxicity due to expression of the therapeutic polypeptide is reduced in inner ear hair cells, spiral ganglion cells, lateral supporting cells, basilar membrane cells, medial supporting cells, spiral limbus cells, inner sulcus cells, or any combination thereof.

267. The method of claim 261, wherein toxicity due to expression of the polypeptide is reduced in inner ear hair cells, spiral ganglion cells, lateral supporting cells, basilar membrane cells, medial supporting cells, spiral limbus cells, inner sulcus cells, or any combination thereof.
268. The method of any of claims 249, 251, 256 or 260, wherein (i) the therapeutic polypeptide is predominately expressed in inner ear supporting cells, (ii) the therapeutic polypeptide is selectively expressed at a higher level in inner ear supporting cells than in inner ear hair cells, (iii) the therapeutic polypeptide not expressed at levels sufficient to cause toxicity in inner ear hair cells, or (iv) or any combination thereof.
269. The method of any of claims 250, 252, 257, or 261, wherein (i) the polypeptide is predominately expressed in inner ear supporting cells, (ii) the polypeptide is selectively expressed at a higher level in inner ear supporting cells than in inner ear hair cells, (iii) the polypeptide not expressed at levels sufficient to cause toxicity in inner ear hair cells, or (iv) or any combination thereof.
270. The method of claims 250-269, wherein the inner ear supporting cells are selected from one or more of inner phalangeal cells/border cells (IPhC), inner pillar cells (IPC), outer pillar cells (OPC), Deiters' cells rows 1 and 2 (DC1/2), Deiters' cells row 3 (DC3), Hensen's cells (Hec), Claudius cells/outer sulcus cells (CC/OSC), interdental cells (Idc), inner sulcus cells (ISC), Kölliker's organ cells (KO), and OC90+ cells (OC90).
271. The method of any of claims 250-270, wherein the administration is to the inner ear of the subject.
272. The method of claim 271, wherein the administration is to the cochlea of the subject.
273. The method of claim 271-272, wherein the administration is via a round window membrane injection.

274. Use of the polynucleotide, construct, construct expression, or viral vector construct of any of claims 122-229, the vector of any of claims 230-234, or the AAV particle of claim 235-237, or the ex vivo cell of any one of claims 241-244, for the treatment of hearing loss in a subject suffering from or at risk of hearing loss.
275. Use of the polynucleotide, construct, construct expression, or viral vector construct of any of claims 122-229, the vector of any of claims 230-234, or the AAV particle of claim 235-237, or the ex vivo cell of any one of claims 241-244, in the manufacture of a medicament for the treatment of hearing loss.
276. The polynucleotide, construct, construct expression, or viral vector construct of any of claims 122-229, the vector of any of claims 230-234, or the AAV particle of claim 235-237, or the ex vivo cell of any one of claims 241-244, for use as a medicament.
277. The polynucleotide, construct, construct expression, or viral vector construct of any of claims 122-229, the vector of any of claims 230-234, or the AAV particle of claim 235-237, or the ex vivo cell of any one of claims 241-244, for use in the treatment of hearing loss.
278. A kit comprising the polynucleotide, construct, construct expression, or viral vector construct of any of claims 122-229, the vector of any of claims 230-234, or the AAV particle of claim 235-237, or the ex vivo cell of any one of claims 241-244.
279. The kit of claim 278, wherein the polynucleotide, construct, expression construct, vector, AAV particle, composition or ex vivo cell is pre-loaded in a device.
280. The kit of claim 279, wherein the device is a microcatheter.
281. The kit of claim 280, 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.

282. The kit of any of claims 278-280, wherein a distal end of the microcatheter is comprised of at least one microneedle with diameter of between 10 and 1,000 microns.
283. The kit of claim 278, further comprising a device.
284. The kit of claim 283, wherein the device is a device described in any one of FIGS. 5-8.
285. The kit of any of claims 283-284, wherein the device comprises a needle comprising a bent portion and an angled tip.
286. The polynucleotide, the construct, the expression construct, the viral vector construct, the viral vector, the AAV particle, the ex vivo cell, the method, the use or the kit of any of the previous claims, wherein the polynucleotide or the promoter is a cell selective promoter.
287. The polynucleotide, the construct, the expression construct, the viral vector construct, the viral vector, the AAV particle, the ex vivo cell, the method, the use or the kit of claim 286, wherein the cell selective promoter is a promoter that is predominately active in one or more supporting cells of the inner ear.