GJB2 REGULATORY ELEMENTS AND USES THEREOF

Background

Hearing loss is a major public health issue that is estimated to affect nearly 15% of school-age children and one out of three people by age sixty-five. The most common type of hearing loss is sensorineural hearing loss, a type of hearing loss caused by defects in the cells of the inner ear, such as cochlear hair cells and cochlear supporting cells, or the neural pathways that project from the inner ear to the brain. Sensorineural hearing loss is often acquired, and has a variety of causes, including acoustic trauma, disease or infection, head trauma, ototoxic drugs, and aging. There are also genetic causes of sensorineural hearing loss, such as mutations in genes involved in the development and function of the inner ear. Mutations in over 90 such genes have been identified, including mutations inherited in an autosomal recessive, autosomal dominant, and X-linked pattern.

In recent years, efforts to treat hearing loss have increasingly focused on gene therapy as a possible solution. There remain, however, few approaches to target specific cell populations in the cochlea. Gene therapy approaches for hearing loss that induce expression of an exogenous gene in all cells of the inner ear may have off-target effects or result in toxicity. Accordingly, there is a need for new approaches to target specific cell populations in the cochlea for the treatment of hearing loss.

Summary

The disclosure provides compositions and methods for promoting the expression of a gene of interest, such as a gene that is endogenously expressed in GJB2-expressing cells, a gene that can induce the differentiation of cochlear supporting cells into cochlear hair cells, or a gene expressed in cochlear supporting cells that is mutated in subjects with hearing loss, in specific cell types. The compositions and methods described herein relate to polynucleotides that can induce expression of a transgene in GJB2-expressing cells (e.g., GJB2-expressing inner ear cells). The polynucleotides described herein may be operably linked, e.g., to a polynucleotide encoding an expression product such as a protein or an inhibitory RNA, and may be administered to a subject, such as a human subject, to treat or prevent hearing loss (e.g., sensorineural hearing loss, such as GJB2-related hearing loss).

In a first example, the disclosure provides a polynucleotide including a GJB2 promoter containing a first region having at least 85% sequence identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) to SEQ ID NO: 1 or a functional portion or derivative thereof including at least one of (e.g., one or more of) SEQ ID NOs: 3-12 operably linked to a second region having at least 85% sequence identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) to SEQ ID NO: 2 or a functional portion or derivative thereof including at least one of (e.g., one of more of) SEQ ID NOs: 20-24, in which the distance between the first region and the second region in the polynucleotide (e.g., the distance between the 3' end of the first region and the 5' end of the second region) is no more than 1 kb, optionally including a linker containing one to one hundred nucleotides between the first region and the second region. In some examples, the distance between the first region and the second region in the polynucleotide is no more than 0.5 kb. In some examples, the distance between the first region and the second region in the polynucleotide is no more than 0.25 kb.

In one aspect, the present disclosure refers to a polynucleotide comprising a GJB2 promoter comprising:

(a) a first region having (i) at least 85% sequence identity to SEQ ID NO: 3; or (ii) at least 85% sequence identity to SEQ ID NO: 1 or a functional portion or derivative thereof comprising at least one of SEQ ID NOs: 3-12, operably linked to

(b) a second region having (i) at least 85% sequence identity to SEQ ID NO: 20; or (ii) at least 85% sequence identity to SEQ ID NO: 2 or a functional portion or derivative thereof comprising at least one of SEQ ID NOs: 20-24,

wherein the distance between the first region and the second region in the polynucleotide is no more than 1 kilobase (kb).

In another example, the disclosure provides a polynucleotide including a GJB2 promoter containing a first region having at least 85% sequence identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) to SEQ ID NO: 1 or a functional portion or derivative thereof including at least one of (e.g., one or more of) SEQ ID NOs: 3-12 operably linked to a second region having at least 85% sequence identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) to SEQ ID NO: 2 or a functional portion or derivative thereof including at least one of (e.g., one of more of) SEQ ID NOs: 20-24, in which when the first region and the second region not joined directly (e.g., fused) the first region is operably linked to the second region by a nucleic acid sequence that differs from the intervening genomic sequence.

In another example, the disclosure provides a polynucleotide including a GJB2 promoter containing a first region having at least 85% sequence identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) to SEQ ID NO: 1 or a functional portion or derivative thereof including at least one of (e.g., one or more of) SEQ ID NOs: 3-12 operably linked to a second region having at least 85% sequence identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) to SEQ ID NO: 2 or a functional portion or derivative thereof including at least one of (e.g., one of more of) SEQ ID NOs: 20-24, in which the GJB2 promoter is operably linked to a heterologous polynucleotide, optionally including a linker containing one to one hundred nucleotides between the first region and the second region. In some examples, the heterologous polynucleotide is a codon-optimized nucleic acid sequence encoding wild-type GJB2 (e.g., the heterologous polynucleotide has a sequence set forth in SEQ ID NOs: 41-44). In some examples, the heterologous polynucleotide encodes a Gjb2 protein including one or more conservative amino acid substitutions relative to SEQ ID NO: 38 that retains the therapeutic function of wild-type Gjb2. In some examples, the heterologous polynucleotide encodes Gjb6 (e.g., encodes SEQ ID NO: 47). In some examples, the heterologous polynucleotide encodes brain derived neurotrophic factor (BDNF) or neurotrophin 3 (NTF3). In some examples, the heterologous polynucleotide is a polynucleotide encoding a protein or inhibitory RNA that can induce differentiation of a cochlear supporting cell into a cochlear hair cell or induce or increase cochlear supporting cell proliferation (e.g., a polynucleotide listed in Table 5), or a transgene corresponding to a wild-type form of a gene that is expressed in cochlear supporting cells and mutated in a subject with hearing loss (e.g., a transgene corresponding to a wild-type form of a gene listed in Table 6). In some examples, the heterologous polynucleotide encodes (e.g., can be transcribed to produce) a short hairpin RNA (shRNA), an antisense oligonucleotide (ASO), a component of a gene editing system (e.g., a nuclease, such as a CRISPR Associated Protein 9 (Cas9), Transcription Activator-Like Effector Nuclease (TALEN), or Zinc Finger Nuclease (ZFN), or a guide RNA (gRNA)), or a microRNA.

In another example, the disclosure provides a polynucleotide including a GJB2 promoter having at least 85% sequence identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) to any one of SEQ ID NOs: 13-19. In some examples, the GJB2 promoter has at least 85% sequence identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) to SEQ ID NO: 13. In some examples, the GJB2 promoter has the sequence of SEQ ID NO: 13. In some examples, the GJB2 promoter has at least 85% sequence identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%,

93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) to SEQ ID NO: 14. In some examples, the GJB2 promoter has the sequence of SEQ ID NO: 14. In some examples, the GJB2 promoter has at least 85% sequence identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) to SEQ ID NO: 15. In some examples, the GJB2 promoter has the sequence of SEQ ID NO: 15. In some examples, the GJB2 promoter has at least 85% sequence identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) to SEQ ID NO: 16. In some examples, the GJB2 promoter has the sequence of SEQ ID NO: 16. In some examples, the GJB2 promoter has at least 85% sequence identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) to SEQ ID NO: 17. In some examples, the GJB2 promoter has the sequence of SEQ ID NO: 17. In some examples, the GJB2 promoter has at least 85% sequence identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) to SEQ ID NO: 18. In some examples, the GJB2 promoter has the sequence of SEQ ID NO: 18. In some examples, the GJB2 promoter has at least 85% sequence identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) to SEQ ID NO: 19. In some examples, the GJB2 promoter has the sequence of SEQ ID NO: 19.

In another example, the disclosure provides a polynucleotide including a GJB2 promoter having at least 85% sequence identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) to any one of SEQ ID NOs: 25-28. In some examples, the GJB2 promoter has at least 85% sequence identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) to SEQ ID NO: 25. In some examples, the GJB2 promoter has the sequence of SEQ ID NO: 25. In some examples, the GJB2 promoter has at least 85% sequence identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) to SEQ ID NO: 26. In some examples, the GJB2 promoter has the sequence of SEQ ID NO: 26. In some examples, the GJB2 promoter has at least 85% sequence identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) to SEQ ID NO: 27. In some examples, the GJB2 promoter has the sequence of SEQ ID NO: 27. In some examples, the GJB2 promoter has at least 85% sequence identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) to SEQ ID NO: 28. In some examples, the GJB2 promoter has the sequence of SEQ ID NO: 28.

In another example, the disclosure provides a polynucleotide containing a GJB2 promoter having at least 85% sequence identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) to SEQ ID NO: 37. In some examples, GJB2 promoter has the sequence of SEQ ID NO: 37.

In another example, the disclosure provides a polynucleotide containing a GJB2 promoter including a first region having at least 85% sequence identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) to SEQ ID NO: 2 or a functional portion or derivative thereof including at least one of (e.g., one of more of) SEQ ID Nos: 20-24 operably linked to a second region having at least 85% sequence identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) to SEQ ID NO: 1 or a

functional portion or derivative thereof including at least one of (e.g., one or more of) SEQ ID NOs: 3-12, optionally including a linker containing one to one hundred nucleotides between the first region and the second region.

In another example, the disclosure provides a nucleic acid vector containing the polynucleotide of any of the foregoing aspects and examples. In one aspect, the present disclosure refers to a nucleic acid vector comprising any one of the polynucleotides disclosed herein.

In another example, the disclosure provides a nucleic acid vector containing a polynucleotide including a GJB2 promoter having at least 85% sequence identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) to SEQ ID NO: 36. In some examples, the GJB2 promoter has the sequence of SEQ ID NO: 36.

In another example, the disclosure provides a nucleic acid vector containing a polynucleotide including a GJB2 promoter having at least 85% sequence identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) to any one of SEQ ID NOs: 3-7. In some examples, the GJB2 promoter has at least 85% sequence identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) to SEQ ID NO: 3. In some examples, the GJB2 promoter has the sequence of SEQ ID NO: 3. In some examples, GJB2 promoter has at least 85% sequence identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) to SEQ ID NO: 4. In some examples, the GJB2 promoter has the sequence of SEQ ID NO: 4. In some examples, the GJB2 promoter has at least 85% sequence identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) to SEQ ID NO: 5. In some examples, the GJB2 promoter has the sequence of SEQ ID NO: 5. In some examples, the GJB2 promoter has at least 85% sequence identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) to SEQ ID NO: 6. In some examples, the GJB2 promoter has the sequence of SEQ ID NO: 6. In some examples, the GJB2 promoter has at least 85% sequence identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) to SEQ ID NO: 7. In some examples, GJB2 promoter has the sequence of SEQ ID NO: 7.

In another example, the disclosure provides a nucleic acid vector containing a polynucleotide including a GJB2 promoter having at least 85% sequence identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) to any one of SEQ ID NOs: 2 and 20-24. In some examples, the GJB2 promoter has at least 85% sequence identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) to SEQ ID NO: 2. In some examples, the GJB2 promoter has the sequence of SEQ ID NO: 2. In some examples, the GJB2 promoter has at least 85% sequence identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) to SEQ ID NO: 20. In some examples, the GJB2 promoter has the sequence of SEQ ID NO: 20. In some examples, the GJB2 promoter has at least 85% sequence identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) to SEQ ID NO: 21. In some examples, the GJB2 promoter has the sequence of SEQ ID NO: 21. In some examples, the GJB2 promoter has at least 85% sequence identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) to SEQ ID NO: 22. In some examples, the GJB2 promoter has the sequence of SEQ ID NO: 22. In some examples,

the GJB2 promoter has at least 85% sequence identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) to SEQ ID NO: 23. In some examples, the GJB2 promoter has the sequence of SEQ ID NO: 23. In some examples, the GJB2 promoter has at least 85% sequence identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) to SEQ ID NO: 24. In some examples, the GJB2 promoter has the sequence of SEQ ID NO: 24.

In another example, the disclosure provides a nucleic acid vector containing a polynucleotide including a GJB2 promoter containing a first region having at least 85% sequence identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) to SEQ ID NO: 1 or a functional portion or derivative thereof including at least one of (e.g., one or more of) SEQ ID NOs: 3-12 operably linked to a second region having at least 85% sequence identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) to SEQ ID NO: 2 or a functional portion or derivative thereof including at least one of (e.g., one or more of) SEQ ID NOs: 20-24, optionally including a linker containing one to one hundred nucleotides between the first region and the second region.

In some examples of any of the foregoing aspects, the first region has at least 85% sequence identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) to SEQ ID NO: 1. In some examples, the first region has the sequence of SEQ ID NO: 1.

In some examples of any of the foregoing aspects, the first region has at least 85% sequence identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) to any one of SEQ ID NOs: 3-12. In some examples, the first region has at least 85% sequence identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) to SEQ ID NO: 3. In some examples, the first region has the sequence of SEQ ID NO: 3. In some examples, the first region has at least 85% sequence identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) to SEQ ID NO: 4. In some examples, the first region has the sequence of SEQ ID NO: 4. In some examples, the first region has at least 85% sequence identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) to SEQ ID NO: 5. In some examples, the first region has the sequence of SEQ ID NO: 5. In some examples, the first region has at least 85% sequence identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) to SEQ ID NO: 6. In some examples, the first region has the sequence of SEQ ID NO: 6. In some examples, the first region has at least 85% sequence identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) to SEQ ID NO: 7. In some examples, the first region has the sequence of SEQ ID NO: 7. In some examples, the first region has at least 85% sequence identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) to SEQ ID NO: 8. In some examples, the first region has the sequence of SEQ ID NO: 8. In some examples, the first region has at least 85% sequence identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) to SEQ ID NO: 9. In some examples, the first region has the sequence of SEQ ID NO: 9. In some examples, the first region has at least 85% sequence identity (e.g., 85%, 86%, 87%,

88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) to SEQ ID NO: 10. In some examples, the first region has the sequence of SEQ ID NO: 10. In some examples, the first region has at least 85% sequence identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) to SEQ ID NO: 11. In some examples, the first region has the sequence of SEQ ID NO: 11. In some examples, the first region has at least 85% sequence identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) to SEQ ID NO: 12. In some examples, the first region has the sequence of SEQ ID NO: 12.

In some examples of any of the foregoing aspects, the functional portion of SEQ ID NO: 1 comprises the sequence of SEQ ID NO: 4 and the sequence of SEQ ID NO: 12. In some examples, the first region has at least 85% sequence identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) to SEQ ID NO: 13. In some examples, the first region has the sequence of SEQ ID NO: 13.

In some examples of any of the foregoing aspects, the functional portion of SEQ ID NO: 1 comprises the sequence of SEQ ID NO: 5 and the sequence of SEQ ID NO: 12. In some examples, the first region has at least 85% sequence identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) to SEQ ID NO: 14. In some examples, the first region has the sequence of SEQ ID NO: 14.

In some examples of any of the foregoing aspects, the functional portion of SEQ ID NO: 1 comprises the sequence of SEQ ID NO: 6 and the sequence of SEQ ID NO: 12. In some examples, the first region has at least 85% sequence identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) to SEQ ID NO: 15. In some examples, the first region has the sequence of SEQ ID NO: 15.

In some examples of any of the foregoing aspects, the functional portion of SEQ ID NO: 1 comprises the sequence of SEQ ID NO: 7 and the sequence of SEQ ID NO: 12. In some examples, the first region has at least 85% sequence identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) to SEQ ID NO: 16. In some examples, the first region has the sequence of SEQ ID NO: 16.

In some examples of any of the foregoing aspects, the functional portion of SEQ ID NO: 1 comprises the sequence of SEQ ID NO: 9 and the sequence of SEQ ID NO: 12. In some examples, the first region has at least 85% sequence identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) to SEQ ID NO: 17. In some examples, the first region has the sequence of SEQ ID NO: 17.

In some examples of any of the foregoing aspects, the functional portion of SEQ ID NO: 1 comprises the sequence of SEQ ID NO: 10 and the sequence of SEQ ID NO: 12. In some examples, the first region has at least 85% sequence identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) to SEQ ID NO: 18. In some examples, the first region has the sequence of SEQ ID NO: 18.

In some examples of any of the foregoing aspects, the functional portion of SEQ ID NO: 1 comprises the sequence of SEQ ID NO: 11 and the sequence of SEQ ID NO: 12. In some examples, the first region has at least 85% sequence identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%,

93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) to SEQ ID NO: 19. In some examples, the first region has the sequence of SEQ ID NO: 19.

In some examples of any of the foregoing aspects, the sequence of SEQ ID NO: 12 precedes the sequence of SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 10, or SEQ ID NO: 11.

In some examples of any of the foregoing aspects, the second region has at least 85% sequence identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) to SEQ ID NO: 2. In some examples, the second region has the sequence of SEQ ID NO: 2.

In some examples of any of the foregoing aspects, the second region has at least 85% sequence identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) to any one of SEQ ID NOs: 20-24. In some examples, the second region has at least 85% sequence identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) to SEQ ID NO: 20. In some examples, the second region has the sequence of SEQ ID NO: 20. In some examples, the second region has at least 85% sequence identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) to SEQ ID NO: 21. In some examples, the second region has the sequence of SEQ ID NO: 21. In some examples, the second region has at least 85% sequence identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) to SEQ ID NO: 22. In some examples, the second region has the sequence of SEQ ID NO: 22. In some examples, the second region has at least 85% sequence identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) to SEQ ID NO: 23. In some examples, the second region has the sequence of SEQ ID NO: 23. In some examples, the second region has at least 85% sequence identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) to SEQ ID NO: 24. In some examples, the second region has the sequence of SEQ ID NO: 24.

In some examples of any of the foregoing aspects, the functional portion of SEQ ID NO: 2 comprises the sequence of SEQ ID NO: 23 and the sequence of SEQ ID NO: 20. In some examples, the second region has at least 85% sequence identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) to SEQ ID NO: 25. In some examples, the second region has the sequence of SEQ ID NO: 25.

In some examples of any of the foregoing aspects, the functional portion of SEQ ID NO: 2 comprises the sequence of SEQ ID NO: 24 and the sequence of SEQ ID NO: 20. In some examples, the second region has at least 85% sequence identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) to SEQ ID NO: 26. In some examples, the second region has the sequence of SEQ ID NO: 26.

In some examples of any of the foregoing aspects, the functional portion of SEQ ID NO: 2 comprises the sequence of SEQ ID NO: 23 and the sequence of SEQ ID NO: 22. In some examples, the second region has at least 85% sequence identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) to SEQ ID NO: 27. In some examples, the second region has the sequence of SEQ ID NO: 27.

In some examples of any of the foregoing aspects, the functional portion of SEQ ID NO: 2 comprises the sequence of SEQ ID NO: 24 and the sequence of SEQ ID NO: 22. In some examples, the second region has at least 85% sequence identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) to SEQ ID NO: 28. In some examples, the second region has the sequence of SEQ ID NO: 28.

In some examples of any of the foregoing aspects, the sequence of SEQ ID NO: 20 precedes the sequence of SEQ ID NO: 23 or SEQ ID NO: 24. In some examples of any of the foregoing aspects, the sequence of SEQ ID NO: 22 precedes the sequence of SEQ ID NO: 23 or SEQ ID NO: 24.

In some examples of any of the foregoing aspects, the first region is joined directly to the second region without a linker (e.g., the 3' end of the first region directly precedes the 5' end of the second region). In some examples of any of the foregoing aspects, the GJB2 promoter has at least 85% sequence identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) to SEQ ID NO: 29. In some examples, the GJB2 promoter has the sequence of SEQ ID NO: 29. In some examples of any of the foregoing aspects, the GJB2 promoter has at least 85% sequence identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) to SEQ ID NO: 30. In some examples, the GJB2 promoter has the sequence of SEQ ID NO: 30. In some examples of any of the foregoing aspects, the GJB2 promoter has at least 85% sequence identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) to SEQ ID NO: 31. In some examples, the GJB2 promoter has the sequence of SEQ ID NO: 31. In some examples of any of the foregoing aspects, the GJB2 promoter has at least 85% sequence identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) to SEQ ID NO: 32. In some examples, the GJB2 promoter has the sequence of SEQ ID NO: 32. In some examples of any of the foregoing aspects, the GJB2 promoter has at least 85% sequence identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) to SEQ ID NO: 33. In some examples, the GJB2 promoter has the sequence of SEQ ID NO: 33. In some examples of any of the foregoing aspects, the GJB2 promoter has at least 85% sequence identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) to SEQ ID NO: 34. In some examples, the GJB2 promoter has the sequence of SEQ ID NO: 34. In some examples of any of the foregoing aspects, the GJB2 promoter has at least 85% sequence identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) to SEQ ID NO: 35. In some examples, the GJB2 promoter has the sequence of SEQ ID NO: 35.

In another example, the disclosure provides a polynucleotide including a GJB2 enhancer having at least 85% sequence identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) to any one of SEQ ID NOs: 52-63 (e.g., any one of SEQ ID NOs: 52-59) operably linked to a promoter, in which the distance between the enhancer and the promoter in the polynucleotide is less than 3 kilobases (3 kb). In some examples, the distance between the enhancer and the promoter in the polynucleotide is less than 2 kb. In some examples, the distance between the enhancer and the promoter in the polynucleotide is less than 1 kb. In some examples, the distance between the enhancer and the promoter in the polynucleotide is less than 0.5 kb. In some examples, the polynucleotide including the GJB2 enhancer is contained in a nucleic acid vector. In

some examples, the promoter is an inner ear cell type-specific promoter (e.g., a cochlear supporting cell-specific promoter). In some examples, the inner ear cell type-specific promoter is a promoter listed in Table 9 (e.g., a promoter that can induce expression in a GJB2-expressing inner ear cell). In some examples, the promoter is a GJB2 promoter. In some examples, the GJB2 promoter is a promoter described herein above (e.g., a polynucleotide that contains a first region having at least 85% sequence identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) to SEQ ID NO: 1 or a functional portion or derivative thereof and/or a second region having at least 85% sequence identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) to SEQ ID NO: 2 or a functional portion or derivative thereof, optionally containing a linker joining the first region and the second region, such as a GJB2 promoter of any one of SEQ ID NOs: 29-35). In some examples, the GJB2 promoter has at least 85% sequence (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) identity to any one of SEQ ID NOS: 1 and 8-11. In some examples, the GJB2 promoter has the sequence of any one of SEQ ID NOs: 1 and 8-11. In some examples, the GJB2 promoter has at least 85% sequence (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) identity to a promoter listed in Table 8 (SEQ ID NOs: 66-68). In some examples, the GJB2 promoter has the sequence of a promoter listed in Table 8.

In another example, the disclosure provides a nucleic acid vector containing a polynucleotide including a GJB2 enhancer having at least 85% sequence identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) to any one of SEQ ID NOs: 52-63 (e.g., any one of SEQ ID NOs: 52-59). In some examples, the enhancer is operably linked to a promoter. In some examples, the promoter is an inner ear cell type-specific promoter (e.g., a cochlear supporting cell-specific promoter). In some examples, the inner ear cell type-specific promoter is a promoter listed in Table 9 (e.g., a promoter that can induce expression in a GJB2-expressing inner ear cell). In some examples, the promoter is a GJB2 promoter. In some examples, the GJB2 promoter is a promoter described herein above (e.g., a polynucleotide that contains a first region having at least 85% sequence identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) to SEQ ID NO: 1 or a functional portion or derivative thereof and/or a second region having at least 85% sequence identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) to SEQ ID NO: 2 or a functional portion or derivative thereof, optionally containing a linker joining the first region and the second region, such as a GJB2 promoter of any one of SEQ ID NOs: 30-32). In some examples, the GJB2 promoter has at least 85% sequence (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) identity to any one of SEQ ID NOS: 1 and 8-11. In some examples, the GJB2 promoter has the sequence of any one of SEQ ID NOs: 1 and 8-11. In some examples, the GJB2 promoter has at least 85% sequence (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) identity to a promoter listed in Table 8 (SEQ ID NOs: 66-68). In some examples, the GJB2 promoter has the sequence of a promoter listed in Table 8.

In some examples of any of the foregoing aspects, the promoter (e.g., the GJB2 promoter) is operably linked to a polynucleotide encoding an expression product. In some examples, the

expression product is a heterologous expression product. In some examples, the heterologous expression product is BDNF or NTF3. In some examples, the expression product is a protein, a short hairpin RNA (shRNA), an antisense oligonucleotide (ASO), a component of a gene editing system (e.g., a nuclease, such as a CRISPR Associated Protein 9 (Cas9), Transcription Activator-Like Effector Nuclease (TALEN), or Zinc Finger Nuclease (ZFN), or a guide RNA (gRNA)), or a microRNA. In some examples, the expression product is an expression product that is endogenously expressed in a GJB2-expressing cell. In some examples, the expression product is an expression product that is endogenously expressed in a GJB2-expressing inner ear cell. In some examples, the expression product is Gjb2 or Gjb6. In some examples, the polynucleotide encoding an expression product is a polynucleotide encoding a protein or inhibitory RNA that can induce differentiation of a cochlear supporting cell into a cochlear hair cell, a polynucleotide encoding a protein or inhibitory RNA that can induce or increase cochlear supporting cell proliferation, or a transgene corresponding to a wild-type form of a gene that is expressed in cochlear supporting cells and mutated in a subject with hearing loss. In some examples, the polynucleotide encoding an expression product is a polynucleotide listed in Table 5 or a transgene corresponding to a wild-type form of a gene listed in Table 6.

In some examples of any of the foregoing aspects, the polynucleotide further comprises a GJB2 enhancer operably linked to the GJB2 promoter.

In some examples of any of the foregoing aspects, the GJB2 enhancer has at least 85% sequence identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) to any one of SEQ ID NOs: 52-63. In some examples, the GJB2 enhancer has at least 85% sequence identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) to SEQ ID NO: 52. In some examples, the GJB2 enhancer has at least 85% sequence identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) to SEQ ID NO: 53. In some examples, the GJB2 enhancer has at least 85% sequence identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) to SEQ ID NO: 54. In some examples, the GJB2 enhancer has at least 85% sequence identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) to SEQ ID NO: 55. In some examples, the GJB2 enhancer has at least 85% sequence identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) to SEQ ID NO: 56. In some examples, the GJB2 enhancer has at least 85% sequence identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) to SEQ ID NO: 57. In some examples, the GJB2 enhancer has at least 85% sequence identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) to SEQ ID NO: 58. In some examples, the GJB2 enhancer has at least 85% sequence identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) to SEQ ID NO: 59. In some examples, the GJB2 enhancer has at least 85% sequence identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) to SEQ ID NO: 60. In some examples, the GJB2 enhancer has at least 85% sequence identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) to SEQ ID NO: 61. In some examples, the GJB2 enhancer has at least 85% sequence identity (e.g., 85%, 86%, 87%, 88%, 89%,

90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) to SEQ ID NO: 62. In some examples, the GJB2 enhancer has at least 85% sequence identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) to SEQ ID NO: 63. In some examples, the GJB2 enhancer has the sequence of any one of SEQ ID NOs: 52-63. In some examples, the GJB2 enhancer has the sequence of SEQ ID NO: 52. In some examples, the GJB2 enhancer has the sequence of SEQ ID NO: 53. In some examples, the GJB2 enhancer has the sequence of SEQ ID NO: 54. In some examples, the GJB2 enhancer has the sequence of SEQ ID NO: 55. In some examples, the GJB2 enhancer has the sequence of SEQ ID NO: 56. In some examples, the GJB2 enhancer has the sequence of SEQ ID NO: 57. In some examples, the GJB2 enhancer has the sequence of SEQ ID NO: 58. In some examples, the GJB2 enhancer has the sequence of SEQ ID NO: 59. In some examples, the GJB2 enhancer has the sequence of SEQ ID NO: 60. In some examples, the GJB2 enhancer has the sequence of SEQ ID NO: 61. In some examples, the GJB2 enhancer has the sequence of SEQ ID NO: 62. In some examples, the GJB2 enhancer has the sequence of SEQ ID NO: 63.

In some examples of any of the foregoing aspects, the GJB2 enhancer is located 5' of the promoter.

In some examples of any of the foregoing aspects, the GJB2 enhancer is located 3' of the promoter.

In some examples of any of the foregoing aspects, the polynucleotide comprises two or more different GJB2 enhancers, in which each enhancer is independently selected from an enhancer having at least 85% sequence identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) to any one of SEQ ID NOs: 52-63 (e.g., any one of SEQ ID NOS: 52-59). In some examples, each different GJB2 enhancer is independently selected from an enhancer having the sequence of one of SEQ ID NOs: 52-63 (e.g., any one of SEQ ID NOS: 52-59). In some examples, the polynucleotide comprises four different GJB2 enhancers. In some examples, the four enhancers are SEQ ID NO: 52, SEQ ID NO: 57, SEQ ID NO: 58, and SEQ ID NO: 59. In some examples, the four enhancers are SEQ ID NO: 53, SEQ ID NO: 54, SEQ ID NO: 55, and SEQ ID NO: 56.

In some examples of any of the foregoing aspects, the polynucleotide comprises two or more copies of a GJB2 enhancer having at least 85% sequence identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) to any one of SEQ ID NOs: 52-63 (e.g., any one of SEQ ID NOS: 52-59). In some examples, each copy of the two or more copies of the enhancer has the sequence of one of SEQ ID NOs: 52-63 (e.g., any one of SEQ ID NOS: 52-59).

In some examples of any of the foregoing aspects, the nucleic acid vector is a viral vector, plasmid, cosmid, or artificial chromosome. In some examples, the nucleic acid vector is a viral vector. In some examples, the viral vector is an adeno-associated virus (AAV) vector, an adenovirus vector, or a lentivirus vector. In some examples, the viral vector is an AAV vector. In some examples, the AAV vector has an AAV1, AAV2, AAV2quad(Y-F), AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, rh10, rh39, rh43, rh74, Anc80, Anc80L65, DJ, DJ/8, DJ/9, 7m8, PHP.B, PHP.eB, or PHP.S capsid. In some examples, the AAV vector has an AAV1 capsid. In some examples, the AAV vector has an AAV9 capsid. In some examples, the AAV vector has a 7m8 capsid. In some

examples, the AAV vector has a PHP.S capsid. In some examples, the AAV vector has a DJ capsid. In some examples, the AAV vector has an Anc80 capsid. In some examples, the AAV vector has an Anc80L65 capsid. In some examples, the AAV vector has an AAV2 capsid. In some examples, the AAV vector has an AAV2quad(Y-F) capsid. In some examples, the AAV vector has a PHP.eB capsid. In some examples, the AAV vector has an AAV3 capsid. In some examples, the AAV vector has an AAV4 capsid. In some examples, the AAV vector has an AAV5 capsid. In some examples, the AAV vector has an AAV6 capsid. In some examples, the AAV vector has an AAV7 capsid. In some examples, the AAV vector has an AAV8 capsid. In some examples, the AAV vector has a PHP.B capsid.

In another example, the disclosure provides a composition containing the nucleic acid vector of any of the foregoing aspects and examples. In some examples, the composition further includes a pharmaceutically acceptable carrier, diluent, or excipient.

In another example, the disclosure provides a cell containing the polynucleotide or vector of any of the foregoing aspects and examples. In some examples, the cell is a GJB2-expressing cell. In some examples, the cell is a GJB2-expressing inner ear cell. In some examples, the cell is a mammalian cell. In some examples, the mammalian cell is a human cell. In some examples, the cell is a cochlear supporting cell.

In another example, the disclosure provides a method of expressing an expression product in a GJB2-expressing cell by contacting the GJB2-expressing cell with the nucleic acid vector of or composition of any of the foregoing aspects and examples. In some examples, the GJB2-expressing cell is a GJB2-expressing inner ear cell (e.g., a cochlear supporting cell). In some examples, the contacting is in a subject (e.g., in vivo). In one aspect, the present disclosure refers to an *in vitro* method of expressing an expression product in a GJB2-expressing cell, comprising contacting the GJB2-expressing cell with the nucleic acid vector disclosed herein. In another aspect, the present disclosure refers to use of the nucleic acid vector disclosed herein in the manufacture of a medicament for expressing an expression product in a GJB2-expressing cell in a subject.

In another aspect, the present disclosure refers to use of the nucleic acid vector disclosed herein in the manufacture of a medicament for treating a subject having or at risk of developing GJB2-related hearing loss, wherein the medicament is to be administered to an inner ear of the subject, and wherein the expression product is Gjb2 or Gjb6. In another example, the disclosure provides a method of treating a subject having or at risk of developing GJB2-related hearing loss by administering to an inner ear of the subject a therapeutically effective amount of the nucleic acid vector composition of any of the foregoing aspects and examples, in which the expression product is Gjb2 or Gjb6. In some examples, the GJB2-related hearing loss is DFNB1, DFNA3, or hearing loss associated with Bart-Pumphrey syndrome, hystrix-like ichthyosis with deafness, keratitis-ichthyosis-deafness syndrome, palmoplantar keratoderma with deafness, or Vohwinkel syndrome. In some examples, the GJB2-related hearing loss is DFNB1 or DFNA3. In some examples, the subject has a mutation in GJB2, a mutation in GJB6, or a mutation in both GJB2 and GJB6.

In another example, the disclosure provides a method of treating a subject having or at risk of developing hearing loss (e.g., sensorineural hearing loss or deafness) by administering to an inner ear of the subject an effective amount of the nucleic acid vector or composition of any of the foregoing aspects and examples.

In another example, the disclosure provides a method of treating a subject having or at risk of developing tinnitus by administering to an inner ear of the subject an effective amount of the nucleic acid vector or composition of any of the foregoing aspects and examples.

In one aspect, the present disclosure refers to use of the nucleic acid vector disclosed herein in the manufacture of a medicament for inducing or increasing cochlear hair cell regeneration in a subject in need thereof, wherein the medicament is to be administered to an inner ear of the subject, and wherein the nucleic acid vector comprises a GJB2 enhancer having at least 85% sequence identity to SEQ ID NO:59 and the expression product promotes or increases cochlear supporting cell proliferation or differentiation of cochlear supporting cells into cochlear hair cells. In another example, the disclosure provides a method of inducing or increasing cochlear hair cell regeneration in a subject in need thereof by administering to an inner ear of the subject a therapeutically effective amount of the nucleic acid vector or composition of any of the foregoing aspects and examples, in which the enhancer has at least 85% sequence identity (e.g., 85%, 86%, 87%, 88%,

89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) to SEQ ID NO: 59 and the expression product is an expression product that can promote or increase cochlear supporting cell proliferation or differentiation of cochlear supporting cells into cochlear hair cells. In some examples, the polynucleotide encoding the expression product is a polynucleotide listed in Table 5. In some examples, the expression product is an inhibitory RNA directed to LATS1 and/or LATS2.

In one aspect, the present disclosure refers to use of the nucleic acid vector disclosed herein in the manufacture of a medicament for treating a subject having or at risk of developing hearing loss associated with damage to or loss of cochlear hair cells, wherein the medicament is to be administered to an inner ear of the subject, and wherein the nucleic acid vector comprises a GJB2 enhancer having at least 85% sequence identity to SEQ ID NO: 59 and the expression product promotes or increases cochlear supporting cell proliferation or differentiation of cochlear supporting cells into cochlear hair cells. In another example, the disclosure provides a method of treating a subject having or at risk of developing hearing loss associated with damage to or loss of cochlear hair cells by administering to an inner ear of the subject a therapeutically effective amount of the nucleic acid vector or composition of any of the foregoing aspects and examples, in which the enhancer has at least 85% sequence identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) to SEQ ID NO: 59 and the expression product is an expression product that can promote or increase cochlear supporting cell proliferation or differentiation of cochlear supporting cells into cochlear hair cells. In some examples, the polynucleotide encoding the expression product is a polynucleotide listed in Table 5. In some examples, the expression product is an inhibitory RNA directed to LATS1 and/or LATS2.

In another example, the disclosure provides a method of inducing or increasing differentiation of a cochlear supporting cell into a cochlear hair cell by contacting the cochlear supporting cell with the nucleic acid vector or composition of any of the foregoing aspects and examples, in which the enhancer has at least 85% sequence identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) to SEQ ID NO: 59 and the expression product is Atoh1 or an Atoh1 variant, Pou4F3, Gfi1, or Ikzf2. In some examples, the contacting is in vivo (e.g., in a subject).

In another example, the disclosure provides a method of inducing or increasing cochlear supporting cell proliferation by contacting the cochlear supporting cell with the nucleic acid vector or composition of any of the foregoing aspects and examples, in which the enhancer has at least 85% sequence identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) to SEQ ID NO: 59 and the expression product is Lgr5, Yap1, Tead2, or an inhibitory RNA directed to LATS1 and/or LATS2. In some examples, the contacting is in vivo (e.g., in a subject).

In one aspect, the present disclosure refers to use of the nucleic acid vector disclosed herein in the manufacture of a medicament for treating a subject having or at risk of developing genetic hearing loss associated with a mutation in a gene that is endogenously expressed in cochlear supporting cells, wherein the medicament is to be administered to an inner ear of the subject, and wherein the enhancer has at least 85% sequence identity to SEQ ID NO: 59 and the polynucleotide encoding the expression product is a wild-type form of the gene that is mutated in cochlear supporting cells. In another example, the disclosure provides a method of treating a subject having or at risk of developing genetic hearing loss associated with a mutation in a gene that is endogenously expressed in cochlear supporting cells by administering to an inner ear of the subject a therapeutically effective amount of the nucleic acid vector or composition of any of the foregoing aspects and examples, in which the enhancer has at least 85% sequence identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) to SEQ ID NO: 59 and the polynucleotide encoding the expression product is a wild-type form of the gene that is mutated in cochlear supporting cells. In some examples, the genetic hearing loss is associated with a disease listed in Table 6 and the polynucleotide encoding the expression product is a transgene corresponding to a wild-type form of a gene that is mutated in said disease (e.g., a gene listed in the same row as the disease in Table 6).

In another example, the disclosure provides a method of improving cochlear supporting cell function or cochlear supporting cell survival by contacting the cochlear supporting cell with the nucleic acid vector

or composition of any of the foregoing aspects and examples. In some examples, the contacting is in vivo (e.g., in a subject).

In another example, the disclosure provides a method of improving cochlear supporting cell function or cochlear supporting cell survival in a subject in need thereof by administering to an inner ear of the subject a therapeutically effective amount of the nucleic acid vector or composition of any of the foregoing aspects and examples.

In some examples of any of the foregoing aspects, the subject has or is at risk of developing hearing loss (e.g., sensorineural hearing loss or deafness).

In some examples of any of the foregoing aspects, the hearing loss is acquired hearing loss. In some examples, the acquired hearing loss is noise-induced hearing loss, age-related hearing loss, disease or infection-related hearing loss, head trauma-related hearing loss, or ototoxic drug-induced hearing loss. In some examples, the ototoxic drug is an aminoglycoside, an antineoplastic drug, ethacrynic acid, furosemide, a salicylate, or quinine.

In some examples of any of the foregoing aspects, the hearing loss is genetic hearing loss. In some examples, the genetic hearing loss is autosomal dominant hearing loss, autosomal recessive hearing loss, or X-linked hearing loss.

In some examples of any of the foregoing aspects, the cochlear supporting cell is a mammalian cochlear supporting cell. In some examples, the mammalian cochlear supporting cell is a human cochlear supporting cell.

In some examples of any of the foregoing aspects, the method further comprises evaluating the hearing of the subject prior to administering the nucleic acid vector or composition.

In some examples of any of the foregoing aspects, the method further comprises evaluating the hearing of the subject after administering the nucleic acid vector or composition.

In some examples of any of the foregoing aspects, the nucleic acid vector or composition is locally administered. In some examples, the nucleic acid vector or composition is administered to the inner ear. In some examples, the nucleic acid vector or composition is administered to the middle ear. In some examples, the nucleic acid vector or composition is administered transtympanically or intratympanically. In some examples, the nucleic acid vector or composition is administered into the perilymph. In some examples, the nucleic acid vector or composition is administered into the endolymph. In some examples, the nucleic acid vector or composition is administered to or through the oval window. In some examples, the nucleic acid vector or composition is administered to or through the round window.

In some examples of any of the foregoing aspects, the nucleic acid vector or composition is administered in an amount sufficient to prevent or reduce hearing loss, prevent or reduce tinnitus, delay the development of hearing loss, slow the progression of hearing loss, improve hearing, increase or induce expression of an expression product in GJB2-expressing cells, increase cochlear hair cell numbers, increase cochlear hair cell regeneration, increase cochlear supporting cell proliferation, promote or increase cochlear supporting cell survival, induce or increase the differentiation of cochlear supporting cells into cochlear hair cells, or improve cochlear supporting cell function.

In some examples of any of the foregoing aspects, the subject is a human subject.

In another example, the disclosure provides a kit including the polynucleotide, nucleic acid vector, or composition of any of the foregoing aspects and examples.