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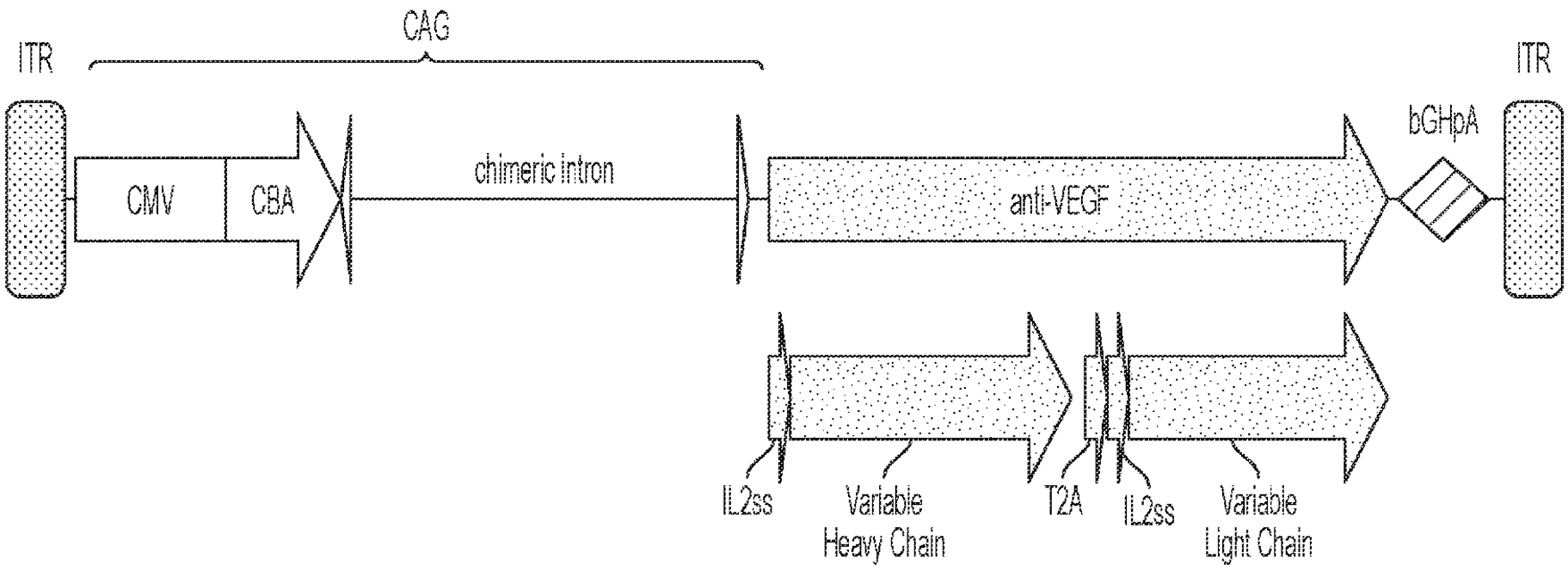


FIG. 6A

(57) Abstract: The present disclosure provides a construct comprising a coding sequence operably linked to a promoter, wherein the coding sequence encodes a vascular endothelial growth factor (VEGF) binding agent or a portion thereof. In some embodiments, a construct is an AAV construct. In some embodiments, an AAV construct is a part of an AAV particle. Compositions comprising constructs and AAV particles described herein can be useful in treating hearing loss, for example, hearing loss associated with vestibular schwannoma.

[Continued on next page]

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ANTI-VEGF ANTIBODY CONSTRUCTS AND RELATED METHODS FOR TREATING VESTIBULAR SCHWANNOMA ASSOCIATED SYMPTOMS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Patent Applications 63/120,189 filed on December 1, 2020, and 63/152,832 filed on February 23, 2021, the entire contents of each of which is hereby incorporated by reference.

SEQUENCE LISTING

[0002] The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on November 19, 2021, is named 2013615-0503_(AK020_WO)_SL.txt and is 328,540 bytes in size.

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. Sensorineural hearing loss includes hearing loss that is caused by a malfunction of the cells (e.g., hair cells) in an inner ear of a mammal. Non-limiting causes of sensorineural hearing loss include exposure to loud noise, head trauma, viral infection, autoimmune inner ear disease, genetic hearing loss, aging, malformations in the inner ear, Meniere's disease, otosclerosis, and tumors. As discussed herein, another cause of hearing loss can be vestibular schwannoma (VS), which is, e.g., a tumor that develops on the nerves leading from the inner ear to the brain.

SUMMARY

[0004] The present disclosure provides the recognition that administration of anti-VEGF proteins (e.g., ranibizumab, bevacizumab, and/or aflibercept) to a subject can be useful in treating conditions, diseases, or disorders associated with neovascularization. The present disclosure further recognizes that administration of anti-VEGF proteins may not always be straightforward. For example, administration of anti-VEGF proteins should be achieved in such a way that provides

the proper levels of anti-VEGF proteins locally at cells and tissues associated with neovascularization.

[0005] The present disclosure provides that administration of anti-VEGF constructs, which can express anti-VEGF proteins (e.g., ranibizumab, bevacizumab, and/or aflibercept) can be useful in treating conditions, diseases, or disorders associated with neovascularization. In particular, recombinant AAV (rAAV) constructs encoding anti-VEGF proteins (e.g., ranibizumab, bevacizumab, and/or aflibercept) can be particularly useful in treating conditions, diseases, or disorders associated with neovascularization in the ears and eyes, particularly when used with an rAAVAnc80 capsid to form an rAAVAnc80-antiVEGF particle.

[0006] Among other things, the present disclosure provides a construct comprising a coding sequence operably linked to a promoter, where the coding sequence encodes a vascular endothelial growth factor (VEGF) binding agent or portion thereof (also collectively referred to herein as an anti-VEGF protein).

[0007] In some embodiments, a promoter is an inducible promoter, a constitutive promoter, or a tissue-specific promoter. In some embodiments, a promoter is a CAG promoter, a CBA promoter, a CMV promoter, or a CB7 promoter. In some embodiments, a promoter comprises a nucleic acid sequence according to SEQ ID NO: 49 or 50, SEQ ID NO: 64, and/or SEQ ID NO: 65.

[0008] In some embodiments, a coding sequence is or comprises a primate coding sequence. In some embodiments, a coding sequence is or comprises a human coding sequence. In some embodiments, a coding sequence is or comprises an engineered coding sequence.

[0009] In some embodiments, a VEGF binding agent or portion thereof is a primate VEGF binding agent. In some embodiments, a VEGF binding agent is or comprises a human VEGF binding agent. In some embodiments, a VEGF binding agent is or comprises a humanized VEGF binding agent.

[0010] In some embodiments, a VEGF binding agent is capable of binding to at least one VEGF protein. In some embodiments, at least one VEGF protein is VEGF-A, VEGF-B, VEGF-C, VEGF-D, or a combination thereof. In some embodiments, at least one VEGF protein is VEGF-A.

[0011] In some embodiments, a VEGF binding agent comprises at least one polypeptide. In some embodiments, a VEGF binding agent is or comprises an antibody or fragment thereof. In some embodiments, an antibody fragment is a Fab fragment, a Fab' fragment, a F(ab')2 fragment, a Fd fragment, a Fd' fragment, a complementarity determining region (CDR), a single chain Fv, or an Fc domain. In some embodiments, a VEGF binding agent is or comprises an immunoglobulin heavy chain, an immunoglobulin light chain, or a combination thereof.

[0012] In some embodiments, a VEGF binding agent comprises a polypeptide that comprises an amino sequence according to SEQ ID NO: 16. In some embodiments, a VEGF binding agent comprises a polypeptide that comprises an amino sequence according to SEQ ID NO: 20. In some embodiments, a VEGF binding agent comprises a polypeptide that comprises an amino sequence according to SEQ ID NO: 16 and a polypeptide that comprises an amino sequence according to SEQ ID NO: 20.

[0013] In some embodiments, a VEGF binding agent is or comprises ranibizumab.

[0014] In some embodiments, a coding sequence comprises a nucleic acid sequence according to SEQ ID NO: 13. In some embodiments, a coding sequence comprises a nucleic acid sequence according to SEQ ID NO: 19. In some embodiments, a coding sequence comprises a nucleic acid sequence according to SEQ ID NO: 13 and a nucleic acid sequence according to SEQ ID NO: 19.

[0015] In some embodiments, a coding sequence is or comprises a nucleic acid sequence according to SEQ ID NO: 103.

[0016] In some embodiments, a VEGF binding agent comprises a polypeptide that comprises an amino sequence according to SEQ ID NO: 24. In some embodiments, a VEGF binding agent comprises a polypeptide that comprises an amino sequence according to SEQ ID NO: 25. In some embodiments, a VEGF binding agent comprises a polypeptide that comprises an amino sequence according to SEQ ID NO: 24 and a polypeptide that comprises an amino sequence according to SEQ ID NO: 25.

[0017] In some embodiments, a VEGF binding agent is or comprises bevacizumab.

[0018] In some embodiments, a coding sequence comprises a nucleic acid sequence according to SEQ ID NO: 108. In some embodiments, a coding sequence comprises a nucleic acid sequence according to SEQ ID NO: 109. In some embodiments, a coding sequence comprises a nucleic acid

sequence according to SEQ ID NO: 108 and a nucleic acid sequence according to SEQ ID NO: 109.

[0019] In some embodiments, a coding sequence is or comprises a nucleic acid sequence according to SEQ ID NO: 22.

[0020] In some embodiments, a coding sequence comprises one or more nucleic acid sequences that each encode a signal peptide. In some embodiments, at least one nucleic acid sequence encodes an interleukin 2 (IL2) signal peptide.

[0021] In some embodiments, a coding sequence comprises one or more sequences encoding a self-cleaving peptide. In some embodiments, a self-cleaving peptide is a thosea asigna virus 2A (T2A) peptide.

[0022] In some embodiments, a VEGF binding agent comprises a Fc domain. In some embodiments, an Fc domain comprises an amino acid sequence according to SEQ ID NO: 111.

[0023] In some embodiments, a coding sequence comprises a nucleic acid sequence according to SEQ ID NO: 110.

[0024] In some embodiments, a VEGF binding agent comprises one or more extracellular domains of VEGF receptors. In some embodiments, one or more extracellular domains of VEGF receptors comprise an extracellular domain comprising an amino sequence according to SEQ ID NO: 112.

[0025] In some embodiments, a VEGF binding agent comprises two extracellular domains of VEGF receptors.

[0026] In some embodiments, a coding sequence comprises one or more nucleic acid sequences each encoding a signal peptide. In some embodiments, at least one nucleic acid sequence encodes an IL2 signal peptide.

[0027] In some embodiments, a construct comprises two AAV inverted terminal repeats (ITRs). In some embodiments, two AAV ITRs flank a coding sequence and promoter.

[0028] In some embodiments, two AAV ITRs are or are derived from AAV2 ITRs.

[0029] In some embodiments, two AAV ITRs comprise a 5' ITR comprising a nucleic acid sequence according to SEQ ID NO: 45 or 47 and a 3' ITR comprising a nucleic acid sequence according to SEQ ID NO: 46 or 48.

[0030] In some embodiments, a construct comprises a nucleic acid sequence according to any of SEQ ID NOs: 90, 91, 92, 93, 94, 106, or 107.

[0031] In some embodiments, a construct comprises a nucleic acid sequence according to any of SEQ ID NOs: 95 or 96.

[0032] In some embodiments, a construct as described herein is for use in the treatment of an otological disease characterized by neovascularization and/or one or more symptoms associated with the otological disease. In some embodiments, use of a construct as described herein is provided for the manufacture of a medicament to treat an otological disease characterized by neovascularization and/or one or more symptoms associated with the otological disease. In some embodiments, one or more symptoms associated with the otological disease comprise hearing loss, degeneration of hair cells, alteration of biochemical milieu of inner ear fluids, elevated intralabyrinthine protein, endolymphatic hydrops, cochlear aperture obstruction, intralabyrinthine hemorrhage, disruption of cochlear vascular supply, tinnitus, dizziness, intractable headache, facial neuropathy, trigeminal neuropathy, facial paralysis, facial paresthesia, hydrocephalus, cerebellar herniation, or death.

[0033] The present disclosure further provides an AAV particle comprising a construct as described herein.

[0034] In some embodiments, an rAAV particle comprises an rAAV capsid, where the rAAV 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. In some embodiments, an rAAV capsid is an rAAV Anc80 capsid. In some embodiments, an rAAV Anc80 capsid is an rAAV Anc80L65 capsid.

[0035] In some embodiments, an AAV particle as described herein is for use in the treatment of an otological disease characterized by neovascularization and/or one or more symptoms associated with the otological disease. In some embodiments, use of an AAV particle as described herein for the manufacture of a medicament to treat an otological disease characterized by

neovascularization and/or one or more symptoms associated with the otological disease. In some embodiments, one or more symptoms associated with the otological disease comprise hearing loss, degeneration of hair cells, alteration of biochemical milieu of inner ear fluids, elevated intralabyrinthine protein, endolymphatic hydrops, cochlear aperture obstruction, intralabyrinthine hemorrhage, disruption of cochlear vascular supply, tinnitus, dizziness, intractable headache, facial neuropathy, trigeminal neuropathy, facial paralysis, facial paresthesia, hydrocephalus, cerebellar herniation, or death.

[0036] The present disclosure provides a composition comprising a construct as described herein and/or an AAV particle as described herein.

[0037] In some embodiments, a composition is a pharmaceutical composition. In some embodiments, a composition comprises a pharmaceutically acceptable carrier.

[0038] In some embodiments, a composition (e.g., a pharmaceutical composition) comprising an AAV particle described herein, e.g., rAAV- antiVEGF particle, is administered at a dose (e.g., amount) of about 1 x 10^{11} vg/mL to about 1 x 10^{15} vg/mL. In some embodiments, a composition (e.g., a pharmaceutical composition) comprising an AAV particle described herein, e.g., rAAV-antiVEGF particle, is administered at a dose (e.g., amount) of 2.5 x 10^{12} vg/mL +/- 10%. In some embodiments, a composition (e.g., a pharmaceutical composition) comprising an AAV particle described herein, e.g., rAAV- antiVEGF particle, is administered at a dose (e.g., amount) of 5 x 10^{12} vg/mL +/- 10%. In some embodiments, a composition (e.g., a pharmaceutical composition) comprising an AAV particle described herein, e.g., rAAV- antiVEGF particle, is administered at a dose (e.g., amount) of 1 x 10^{13} vg/mL +/- 10%.

[0039] In some embodiments, a composition (e.g., a pharmaceutical composition) comprising an AAV particle described herein, e.g., rAAV- antiVEGF particle, is administered at a dose of about 1 x 10^{10} to about 1 x 10^{13} vg/cochlea. In some embodiments, a composition (e.g., a pharmaceutical composition) comprising an AAV particle described herein, e.g., rAAV-antiVEGF particle, is administered at a dose of about 2.3 x 10^{11} vg/cochlea. In some embodiments, a composition (e.g., a pharmaceutical composition) comprising an AAV particle described herein, e.g., rAAV- antiVEGF particle, is administered at a dose of about 4.5 x 10^{11} vg/cochlea. In some embodiments, a composition (e.g., a pharmaceutical composition) comprising an AAV particle

described herein, e.g., rAAV- antiVEGF particle, is administered at a dose of about 9 x 10^{11} vg/cochlea.

[0040] In some embodiments, a composition (e.g., a pharmaceutical composition) comprising an AAV particle described herein, e.g., rAAV- antiVEGF particle, is administered to a subject at a volume of about 0.01mL to 0.1 mL. In some embodiments, a composition (e.g., a pharmaceutical composition) comprising an AAV particle described herein, e.g., rAAV- antiVEGF particle, is administered to a subject at a volume of about 0.09 mL.

[0041] In some embodiments, a composition as described herein is for use in the treatment of an otological disease, e.g., in a mammal, which otologial disease is characterized by neovascularization and/or one or more symptoms associated with the otological disease. In some embodiments, use of a construct as described herein is provided for the manufacture of a medicament to treat an otological disease, e.g., in a mammal, which otologial disease is characterized by neovascularization and/or one or more symptoms associated with the otological disease. In some embodiments, one or more symptoms associated with the otological disease comprises hearing loss, degeneration of hair cells, alteration of biochemical milieu of inner ear fluids, elevated intralabyrinthine protein, endolymphatic hydrops, cochlear aperture obstruction, intralabyrinthine hemorrhage, disruption of cochlear vascular supply, tinnitus, dizziness, intractable headache, facial neuropathy, trigeminal neuropathy, facial paralysis, facial paresthesia, hydrocephalus, cerebellar herniation, death, or a combination thereof.

[0042] In some embodiments, a composition as described herein is for use in the treatment of an inner ear disorder, e.g. in a mammal. In some embodiments, use of a construct as described herein is provided for the manufacture of a medicament to treat an inner ear disorder, e.g., in a mammal. In some embodiments, an inner ear disorder comprises acoustic neuroma, vestibular schwannoma, or neurofibromatosis type II. In some embodiments, an inner ear disorder is or comprises acoustic neuroma. In some embodiments, an inner ear disorder is or comprises vestibular schwannoma. In some embodiments, an inner ear disorder is or comprises neurofibromatosis type II.

[0043] In some embodiments, a composition as described herein is for use in the treatment of vestibular schwannoma, e.g. in a mammal. In some embodiments, use of a construct as described

herein is provided for the manufacture of a medicament to treat vestibular schwannoma, e.g., in a mammal.

[0044] In some embodiments of any of the methods or uses disclosed herein, the mammal is a human.

[0045] The present disclosure also provides a cell. In some embodiments, a cell comprises a construct as described herein, an rAAV particle as described herein, and/or a composition as described herein.

[0046] In some embodiments, a cell is in vivo, ex vivo, or in vitro.

[0047] In some embodiments, a cell is a mammalian cell. In some embodiments, a cell is a human cell. In some embodiments, a human cell is in the ear of a subject.

[0048] In some embodiments, a cell is immortalized to generate a stable cell line.

[0049] The present disclosure provides a system. A system comprises a construct as described herein, an rAAV particle as described herein, a composition as described herein, and/or a cell as described herein.

[0050] The present disclosure provides a method. In some embodiments, a method comprises contacting a cell with a construct as described herein, an rAAV particle as described herein, and/or a composition as described herein.

[0051] In some embodiments, a cell is a cell of a subject.

[0052] In some embodiments, a cell is an ear cell. In some embodiments, a cell is an inner ear cell. In some embodiments, an inner ear cell is an outer hair cell. In some embodiments, an inner ear cell is an inner hair cell.

[0053] In some embodiments, an inner ear cell is in vitro or ex vivo.

[0054] In some embodiments, a method comprises introducing a construct as described herein, an rAAV particle as described herein, and/or a composition as described herein into the inner ear of a subject.

[0055] In some embodiments, a construct, rAAV particle, or composition is introduced into a cochlea of a subject. In some embodiments, a construct, rAAV particle, or composition is introduced via a round window membrane injection.

[0056] In some embodiments, a method comprises measuring a hearing level of a subject. In some embodiments, a hearing level is measured by performing an auditory brainstem response (ABR) test.

[0057] In some embodiments, a method comprises comparing a hearing level of a subject to a reference hearing level. In some embodiments, a reference hearing level is a published or historical reference hearing level.

[0058] In some embodiments, a hearing level of a subject is measured after a construct as described herein, an rAAV particle as described herein, and/or a composition as described herein is introduced, and a reference hearing level is a hearing level of a subject that was measured before a construct as described herein, an rAAV particle as described herein, and/or a composition as described herein was introduced.

[0059] In some embodiments, a method comprises measuring a level of a vascular endothelial growth factor (VEGF) binding agent or portion thereof in a subject.

[0060] In some embodiments, a level of a vascular endothelial growth factor (VEGF) binding agent or portion thereof is measured in an inner ear of a subject. In some embodiments, a level of a vascular endothelial growth factor (VEGF) binding agent or portion thereof is measured in a cochlea of a subject.

[0061] In some embodiments, a method comprises comparing a level of a vascular endothelial growth factor (VEGF) binding agent or portion thereof in a subject to a reference level of vascular endothelial growth factor (VEGF) binding agent or portion thereof.

[0062] In some embodiments, a reference level of vascular endothelial growth factor (VEGF) binding agent or portion thereof is a published or historical reference level of vascular endothelial growth factor (VEGF) binding agent or portion thereof.

[0063] In some embodiments, a level of the vascular endothelial growth factor (VEGF) binding agent or portion thereof in a subject is measured after a construct as described herein, an AAV particle as described herein, and/or a composition as described herein is introduced, and a reference level of vascular endothelial growth factor (VEGF) binding agent or portion thereof is a level a vascular endothelial growth factor (VEGF) binding agent or portion thereof in a subject

that was measured before a construct as described herein, an AAV particle as described herein, and/or a composition as described herein was introduced.

[0064] In some embodiments, a method comprises measuring a dimension or volume of a tumor in a subject. In some embodiments, a dimension is a maximum diameter or length across a tumor.

[0065] In some embodiments, a method comprises comparing a dimension or volume of a tumor in the subject to a reference tumor dimension or volume, respectively.

[0066] In some embodiments, a reference tumor dimension or volume is a published or historical reference tumor dimension or volume.

[0067] In some embodiments, a dimension or volume of a tumor in a subject is measured after a construct as described herein, an AAV particle as described herein, and/or a composition as described herein is introduced, and a reference tumor dimension or volume is the dimension or volume of the tumor in the subject that was measured before a construct as described herein, an AAV particle as described herein, and/or a composition as described herein was introduced.

[0068] In some embodiments, a method is a method of treating hearing loss comprising administering a construct as described herein, an rAAV particle as described herein, and/or a composition as described herein to a subject in need thereof.

[0069] In some embodiments, a subject is suffering from or is at risk of an otological disease characterized by neovascularization. In some embodiments, an otological disease is or comprises an acoustic neuroma. In some embodiments, an otological disease is or comprises a vestibular schwannoma.

[0070] In some embodiments, a method is a method of treating an inner ear disorder comprising administering a construct as described herein, an rAAV particle as described herein, and/or a composition as described herein to a subject in need thereof. In some embodiments, an inner ear disorder is acoustic neuroma, vestibular schwannoma, or neurofibromatosis type II.

[0071] In some embodiments of any of the methods or uses disclosed herein, a subject is a human.

[0072] In some embodiments, one or more symptoms associated with an otological disease is alleviated or ameliorated following administration of a construct as described herein, an AAV

particle as described herein, and/or a composition as described herein. In some embodiments, one or more symptoms comprise hearing loss, degeneration of hair cells, alteration of biochemical milieu of inner ear fluids, elevated intralabyrinthine protein, endolymphatic hydrops, cochlear aperture obstruction, intralabyrinthine hemorrhage, disruption of cochlear vascular supply, tinnitus, dizziness, intractable headache, facial neuropathy, trigeminal neuropathy, facial paralysis, facial paresthesia, hydrocephalus, cerebellar herniation, or death.

[0073] In some embodiments, a method is a method of treating vestibular schwannoma.

[0074] In some embodiments, a method is a method of modulating the level of VEGF.

[0075] In some embodiments, a method is a method of modulating the level of active VEGF.

[0076] In some embodiments, a method is a method of decreasing the activity of VEGF.

[0077] The present disclosure provides a method comprising contacting a cell with a construct as described herein, and one or more constructs comprising an AAV Rep gene, AAV Cap gene, AAV VA gene, AAV E2a gene, and AAV E4 gene.

[0078] In some embodiments, a cell is an inner ear cell. In some embodiments, an inner ear cell is an outer hair cell. In some embodiments, an inner ear cell is an inner hair cell. In some embodiments, an inner ear cell is in an ear of a subject. In some embodiments, an inner ear cell is in vitro or ex vivo.

[0079] The present disclosure provides a population of cells comprising one or more cells as described herein, where the population is or comprises a stable cell line.

[0080] Methods and materials are described herein for use in the present invention; other suitable methods and materials known in the art can also be used. The materials, methods, and examples are illustrative only and not intended to be limiting. All publications, patent applications, patents, sequences, database entries, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control.

DEFINITIONS

[0081] The scope of the present disclosure is defined by the claims appended hereto and is not limited by certain embodiments described herein. Those skilled in the art, reading the present specification, will be aware of various modifications that may be equivalent to such described embodiments, 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.

[0082] 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.

[0083] 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 embodiments, exactly one member of a group is present in, employed in, or otherwise relevant to a given product or process. In some embodiments, 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 embodiments or aspects are referred to as "comprising" particular elements, features, etc., certain embodiments or aspects "consist," or "consist essentially of," such elements, features, etc. For purposes of simplicity, those embodiments 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.

[0084] 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.

[0085] ***Administration***: As used herein, the term "administration" typically refers to administration of a composition to a subject or system to achieve delivery of an agent to a subject or system. In some embodiments, an agent is, or is included in, a composition; in some embodiments, 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 embodiments, administration may be systematic or local. In some embodiments, a systematic administration can be intravenous. In some embodiments, 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 embodiments, administration may involve only a single dose. In some embodiments, administration may involve application of a fixed number of doses. In some embodiments, 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 embodiments, administration may involve continuous dosing (e.g., perfusion) for at least a selected period of time.

[0086] ***Allele***: As used herein, the term "allele" refers to one of two or more existing genetic variants of a specific polymorphic genomic locus.

[0087] ***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.

[0088] ***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 embodiments, an amino acid has a general

structure, e.g., H_2N–C(H)(R)–COOH. In some embodiments, an amino acid is a naturally-occurring amino acid. In some embodiments, an amino acid is a non-natural amino acid; in some embodiments, an amino acid is a D-amino acid; in some embodiments, 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 embodiments, 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 embodiments, 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 embodiments, 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 embodiments, 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.

[0089] ***Antibody:*** As used herein, the term "antibody" refers to a polypeptide that includes canonical immunoglobulin sequence elements sufficient to confer specific binding to a particular target antigen. As is known in the art, intact antibodies as produced in nature are approximately 150 kD tetrameric agents comprised of two identical heavy chain polypeptides (about 50 kD each) and two identical light chain polypeptides (about 25 kD each) that associate with each other into what is commonly referred to as a "Y-shaped" structure. Each heavy chain is comprised of at least four domains (each about 110 amino acids long)– an amino-terminal variable (VH) domain (located at the tips of the Y structure), followed by three constant domains: CH1, CH2, and the carboxy-terminal CH3 (located at the base of the Y's stem). A short region, known as the "switch", connects the heavy chain variable and constant regions. The "hinge" connects CH2 and CH3 domains to the rest of the antibody. Two disulfide bonds in this hinge region connect the two heavy chain polypeptides to one another in an intact antibody. Each light chain is comprised of two domains – an amino-terminal variable (VL) domain, followed by a carboxy-terminal constant (CL) domain, separated from one another by another "switch". Intact antibody tetramers are

comprised of two heavy chain-light chain dimers in which the heavy and light chains are linked to one another by a single disulfide bond; two other disulfide bonds connect the heavy chain hinge regions to one another, so that the dimers are connected to one another and the tetramer is formed. Naturally-produced antibodies are also glycosylated, typically on the CH2 domain. Each domain in a natural antibody has a structure characterized by an "immunoglobulin fold" formed from two beta sheets (e.g., 3-, 4-, or 5-stranded sheets) packed against each other in a compressed antiparallel beta barrel. Each variable domain contains three hypervariable loops known as "complement determining regions" (CDR1, CDR2, and CDR3) and four somewhat invariant "framework" regions (FR1, FR2, FR3, and FR4). When natural antibodies fold, the FR regions form the beta sheets that provide the structural framework for the domains, and the CDR loop regions from both the heavy and light chains are brought together in three-dimensional space so that they create a single hypervariable antigen binding site located at the tip of the Y structure. The Fc region of naturally-occurring antibodies binds to elements of the complement system, and also to receptors on effector cells, including for example effector cells that mediate cytotoxicity. As is known in the art, affinity and/or other binding attributes of Fc regions for Fc receptors can be modulated through glycosylation or other modification. In some embodiments, antibodies produced and/or utilized in accordance with the present invention include glycosylated Fc domains, including Fc domains with modified or engineered such glycosylation. For purposes of the present invention, in certain embodiments, any polypeptide or complex of polypeptides that includes sufficient immunoglobulin domain sequences as found in natural antibodies can be referred to and/or used as an "antibody", whether such polypeptide is naturally produced (e.g., generated by an organism reacting to an antigen), or produced by recombinant engineering, chemical synthesis, or other artificial system or methodology. In some embodiments, an antibody is polyclonal; in some embodiments, an antibody is monoclonal. In some embodiments, an antibody has constant region sequences that are characteristic of mouse, rabbit, primate, or human antibodies. In some embodiments, antibody sequence elements are humanized, primatized, chimeric, etc, as is known in the art. Moreover, the term "antibody" as used herein, can refer in appropriate embodiments (unless otherwise stated or clear from context) to any of the art-known or developed constructs or formats for utilizing antibody structural and functional features in alternative presentation. For example, in some embodiments, an antibody utilized in accordance with the present invention is in a format selected from, but not limited to, intact IgA, IgG, IgE or IgM antibodies; bi- or multi-

specific antibodies (e.g., Zybodies®, etc); antibody fragments such as Fab fragments, Fab' fragments, F(ab')2 fragments, Fd' fragments, Fd fragments, and isolated CDRs or sets thereof; single chain Fvs; polypeptide-Fc fusions; single domain antibodies (e.g., shark single domain antibodies such as IgNAR or fragments thereof); cameloid antibodies; masked antibodies (e.g., Probodies®); Small Modular ImmunoPharmaceuticals ("SMIPs™"); single chain or Tandem diabodies (TandAb®); VHHs; Anticalins®; Nanobodies® minibodies; BiTE®s; ankyrin repeat proteins or DARPINs®; Avimers®; DARTs; TCR-like antibodies;, Adnectins®; Affilins®; Trans-bodies®; Affibodies®; TrimerX®; MicroProteins; Fynomers®, Centyrins®; and KALBITOR®s. In some embodiments, an antibody can include a heavy and/or light chain variable domain. In some embodiments, an antibody may not include a constant domain. In some embodiments, an antibody may lack a covalent modification (e.g., attachment of a glycan) that it would have if produced naturally. In some embodiments, an antibody may contain a covalent modification (e.g., attachment of a glycan, a payload [e.g., a detectable moiety, a therapeutic moiety, a catalytic moiety, etc], or other pendant group [e.g., poly-ethylene glycol, etc.].

[0090] ***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 embodiments, the term "approximately" or "about" refers to a range of values that fall within ±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 embodiments, 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.

[0091] ***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 embodiments, 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 embodiments, two or more

entities that are physically associated with one another are covalently linked to one another; in some embodiments, 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.

[0092] ***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 embodiments, a specific binding interaction is a biological activity. In some embodiments, modulation (e.g., induction, enhancement, or inhibition) of a biological pathway or event is a biological activity. In some embodiments, 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.

[0093] ***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 embodiments, 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 embodiments, a characteristic portion shares at least one functional characteristic with the intact substance. For example, in some embodiments, 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 embodiments, 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 embodiments, a characteristic portion may be biologically active.

[0094] ***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.

[0095] ***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 embodiments, presence of a characteristic sequence element correlates with presence or level of a particular activity or property of a polymer. In some embodiments, 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 embodiments, 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 embodiments, 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.

[0096] ***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 embodiments, two or more agents may be administered simultaneously. In some embodiments, two or more agents may be administered sequentially. In some embodiments, two or more agents may be administered in overlapping dosing regimens.

[0097] ***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 embodiments, 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.

[0098] ***Construct:*** As used herein, the term "construct" refers to a composition including a polynucleotide capable of carrying at least one heterologous polynucleotide. In some embodiments, 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 construct, 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 construct, etc.) that is capable of replicating when associated with proper control elements. Thus, in some embodiments, "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).

[0099] ***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 embodiments, 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 embodiments, a conservative substitution is made that has a positive value in the PAM250 log-likelihood matrix disclosed in Gonnet, G.H. et al., 1992, Science 256:1443-1445, which is incorporated herein in its entirety by reference. In some embodiments, 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.

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, incorporated herein in its entirety by reference)
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

[0100] ***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 embodiments, 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 embodiments, 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 embodiments, a control is or comprises a printed or otherwise saved record. In some embodiments, a control is a positive control. In some embodiments, a control is a negative control.

[0101] ***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

embodiments, "Assaying for the presence of" can be determining an amount of something present and/or determining whether or not it is present or absent.

[0102] ***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. In some embodiments, "engineering" comprises "humanization" of a coding sequence. In some embodiments, "humanization" can include introducing human non-coding sequences, such as introns and regulatory elements, into a non-human sequence. In some embodiments, "humanization" can include codon optimizing a nucleotide sequence for human usage. In some embodiments, "humanization" can include replacing a portion of a polypeptide (such as a domain, e.g., a framework region or a complementarity domain region) or a nucleotide sequence (e.g., coding or non-coding) with a human polypeptide or nucleotide sequence.

[0103] ***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 embodiments, 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.

[0104] ***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 embodiments, a gene product can be a transcript. In some embodiments, a gene product can be a polypeptide. In some embodiments, 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.

[0105] ***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 embodiments, 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 embodiments, 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).

[0106] ***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 embodiments, a gene includes coding sequence (i.e., sequence that encodes a particular product). In some embodiments, a gene includes non-coding sequence. In some particular embodiments, a gene may include both coding (e.g., exonic) and non-coding (e.g., intronic) sequence. In some embodiments, 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 embodiments, 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 embodiments, a gene may encode a transcript which, in some embodiments, may be toxic beyond a threshold level. In some embodiments, a gene may encode a polypeptide, but that polypeptide may not be functional and/or may be toxic beyond a threshold level.

[0107] ***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 embodiments, hearing loss may be acquired. In

some embodiments, hearing loss may be hereditary. In some embodiments, hearing loss may be genetic. In some embodiments, 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 embodiments, hearing loss may be due to one or more known genetic causes and/or syndromes. In some embodiments, hearing loss may be of unknown etiology. In some embodiments, hearing loss may or may not be mitigated by use of hearing aids or other treatments.

[0108] ***Heterologous***: As used herein, the term "heterologous" may be used in reference to one or more regions of a particular molecule as compared to another region and/or another molecule. For example, in some embodiments, heterologous polypeptide domains, refers to the fact that polypeptide domains do not naturally occur together (e.g., in the same polypeptide). For example, in fusion proteins generated by the hand of man, a polypeptide domain from one polypeptide may be fused to a polypeptide domain from a different polypeptide. In such a fusion protein, two polypeptide domains would be considered "heterologous" with respect to each other, as they do not naturally occur together.

[0109] ***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 embodiments, 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 embodiments, 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 embodiments, 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.

[0110] ***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 embodiments, a value is statistically significantly difference that a baseline or other reference measurement. In some embodiments, 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 embodiments, 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 embodiments, an appropriate reference is a negative reference; in some embodiments, an appropriate reference is a positive reference.

[0111] ***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 embodiments, 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 embodiments, "nucleic acid" refers to an individual nucleic acid residue (e.g., a nucleotide and/or nucleoside); in some embodiments, "nucleic acid" refers to an oligonucleotide chain comprising individual nucleic acid residues. In some embodiments, a "nucleic acid" is or comprises RNA; in some embodiments, a "nucleic acid" is or comprises DNA. In some embodiments, a nucleic acid is, comprises, or consists of one or more natural nucleic acid residues. In some embodiments, a nucleic acid is, comprises, or consists of one or more nucleic acid analogs. In some embodiments, a nucleic acid analog differs from a nucleic acid in that it does not utilize a phosphodiester backbone. Alternatively or additionally, in some embodiments, a nucleic acid has one or more

phosphorothioate and/or 5`-N-phosphoramidite linkages rather than phosphodiester bonds. In some embodiments, a nucleic acid 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 embodiments, 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 embodiments, 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 embodiments, a nucleic acid has a nucleotide sequence that encodes a functional gene product such as an RNA or protein. In some embodiments, a nucleic acid includes one or more introns. In some embodiments, 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 embodiments, 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, 1 10, 120, 130, 140, 150, 160, 170, 180, 190, 20, 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 embodiments, a nucleic acid is partly or wholly single stranded; in some embodiments, a nucleic acid is partly or wholly double stranded. In some embodiments, 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 embodiments, a nucleic acid has enzymatic activity.

[0112] ***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 embodiments, "operably linked" control elements are contiguous (e.g., covalently linked) with coding elements of interest; in some embodiments, control elements act in

trans to or otherwise at a from the functional element of interest. In some embodiments, "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 embodiments, 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.

[0113] ***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 embodiments, 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 embodiments, 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 embodiments, 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 embodiments, a formulation may be or comprise drenches (aqueous or non-aqueous solutions or suspensions), tablets, boluses, powders, granules, pastes, capsules, powders, etc. In some embodiments, an active agent may be or comprise an isolated, purified, or pure compound.

[0114] ***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.

[0115] ***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.

[0116] ***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 embodiments, 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)(SEQ ID NO: 117) 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.

[0117] ***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 embodiments, a polypeptide has an amino acid sequence that occurs in nature. In some embodiments, a polypeptide has an amino acid sequence that does not occur in nature. In some embodiments, 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 embodiments, a polypeptide may comprise or consist of natural amino acids, non-natural amino acids, or both. In some embodiments, 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 embodiments, such pendant groups or modifications may be acetylation, amidation, lipidation, methylation, pegylation, etc., including combinations thereof. In some embodiments, 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 embodiments, useful modifications may be or include, e.g., terminal acetylation, amidation, methylation, etc. In some embodiments, 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 embodiments, a protein is antibodies, antibody fragments, biologically active portions thereof, and/or characteristic portions thereof.

[0118] ***Polynucleotide:*** As used herein, the term "polynucleotide" refers to any polymeric chain of nucleic acids. In some embodiments, a polynucleotide is or comprises RNA; in some embodiments, a polynucleotide is or comprises DNA. In some embodiments, a polynucleotide is, comprises, or consists of one or more natural nucleic acid residues. In some embodiments, a polynucleotide is, comprises, or consists of one or more nucleic acid analogs. In some embodiments, a polynucleotide analog differs from a nucleic acid in that it does not utilize a phosphodiester backbone. Alternatively or additionally, in some embodiments, a polynucleotide has one or more phosphorothioate and/or 5`-N-phosphoramidite linkages rather than phosphodiester bonds. In some embodiments, 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 embodiments,

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 embodiments, 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 embodiments, a polynucleotide has a nucleotide sequence that encodes a functional gene product such as an RNA or protein. In some embodiments, a polynucleotide includes one or more introns. In some embodiments, 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 embodiments, 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, 1 10, 120, 130, 140, 150, 160, 170, 180, 190, 20, 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 embodiments, a polynucleotide is partly or wholly single stranded; in some embodiments, a polynucleotide is partly or wholly double stranded. In some embodiments, 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 embodiments, a polynucleotide has enzymatic activity.

[0119] ***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.

[0120] ***Recombinant***: As used herein, the term "recombinant" is intended to refer to polypeptides that are designed, engineered, prepared, expressed, created, manufactured, and/or 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 embodiments, one or more of such selected sequence elements is found in nature. In some embodiments, one or more of such selected sequence elements is designed in silico. In some embodiments, 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).

[0121] ***Reference***: As used herein, the term "reference" describes a standard or control relative to which a comparison is performed. For example, in some embodiments, 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 embodiments, a reference or control is tested and/or determined substantially simultaneously with the testing or determination of interest. In some embodiments, 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 embodiments, a reference is a negative control reference; in some embodiments, a reference is a positive control reference.

[0122] ***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 embodiments, such genes are apposed or "in the neighborhood" of a given regulatory element. In some embodiments, such genes are located quite far from a given regulatory element. In some embodiments, a regulatory element impairs or enhances transcription of one or more genes. In some embodiments, a regulatory element may be located in cis to a gene being regulated. In some embodiments, a regulatory element may be located in trans to a gene being regulated. For example, in some embodiments, 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 embodiments, this sequence may be an enhancer sequence and other regulatory elements which regulate expression of a gene product.

[0123] ***Sample***: As used herein, the term "sample" typically refers to an aliquot of material obtained or derived from a source of interest. In some embodiments, a source of interest is a biological or environmental source. In some embodiments, 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 embodiments, a source of interest is or comprises biological tissue or fluid. In some embodiments, 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 embodiments, 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 embodiments, a biological fluid may be or comprise a plant exudate. In some embodiments, 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 embodiments, a biological sample is or comprises cells obtained from an individual. In some embodiments, a sample is a "primary sample" obtained directly from a source of interest by any appropriate means. In some embodiments, 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.

[0124] ***Subject***: As used herein, the term "subject" refers an organism, typically a mammal (e.g., a human, in some embodiments including prenatal human forms). In some embodiments, a subject is suffering from a relevant disease, disorder or condition. In some embodiments, a subject is susceptible to a disease, disorder, or condition. In some embodiments, a subject displays one or more symptoms or characteristics of a disease, disorder or condition. In some embodiments, a subject does not display any symptom or characteristic of a disease, disorder, or condition. In some embodiments, a subject is someone with one or more features characteristic of susceptibility to or risk of a disease, disorder, or condition. In some embodiments, a subject is a patient. In some embodiments, a subject is an individual to whom diagnosis and/or therapy is and/or has been administered.

[0125] ***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.

[0126] ***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 embodiments, 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 embodiments, treatment may be of a subject who has been diagnosed as suffering from the relevant disease, disorder, and/or condition. In some embodiments, 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.

[0127] ***Tumor***: As used herein, the term "tumor" refers to an abnormal growth of cells or tissue. In some embodiments, a tumor may comprise cells that are precancerous (*e.g.*, benign), malignant, pre-metastatic, metastatic, and/or non-metastatic. In some embodiments, a tumor is associated with, or is a manifestation of, a cancer. In some embodiments, a tumor may be a disperse tumor or a liquid tumor. In some embodiments, a tumor may be a solid tumor.

[0128] ***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 embodiments, 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 embodiments, 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 embodiments, 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. For example, in some embodiments, a gene sequence is a wild-type sequence, which encodes a functional protein and exists in a majority of members of species with genomes containing the gene. In some such embodiments, a gain-of-function variant can be a gene sequence that contains one or more nucleotide differences relative to a wild-type gene sequence. In some embodiments, 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 embodiments, 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.

[0129] ***VEGF inhibitor***: As used herein, the term "VEGF inhibitor" is used interchangeably with the term "anti-VEGF protein".

BRIEF DESCRIPTION OF THE DRAWING

[0130] **FIG. 1** is a schematic of a representative anatomy of the human ear, including common areas for vestibular schwannoma (VS) occurrence.

[0131] **FIG. 2** is a graphical representation of VS (less than 5 mm width) locations within the internal auditory canal. In this analysis, the majority of small, intracanalicular VS (those located entirely in the internal auditory canal) were positioned close to the fundus of the internal auditory canal (e.g., within millimeters of the base of the cochlea), based on measurements from MRI scans (Koen 2020, incorporated herein in its entirety by reference). When the origin of these 38 tumors was localized to lateral, middle, and medial thirds of the internal auditory canal length, 60% originated within the lateral third (inner ear-adjacent) (Koen 2020, which is incorporated herein in its entirety by reference); when analyzing the distribution of these tumors as a function of percent of internal auditory canal length (normalized for modest internal auditory canal length [mm] variations between individuals), ~85% of the area under the curve was within the lateral (inner ear-adjacent) half of the internal auditory canal (Koen 2020, which is incorporated herein in its entirety by reference).

[0132] **FIG.s 3A-3B** is a schematic representation of an inner ear, indicating fluid continuity of perilymph between the vestibular system, on the left, and the cochlea (scala tympani, scala vestibuli), on the right. As shown in **FIGS. 3A-3B** of U.S. Provisional patent application 63/152,832 (the entire contents of which is incorporated herein by reference), perilymph is shown in light purple. **FIG. 3A** is a schematic of a coiled cochlea. The number of cochlear turns shown is representative of a mouse inner ear. **FIG. 3B** is a schematic showing a cross-section of the cochlea. In the schematic, scala tympani and scala vestibuli are filled with perilymph, while scala media is filled with endolymph (Talaei 2019, incorporated herein in its entirety by reference).

[0133] **FIGs. 4A-4B** is a schematic representation of an administration method as described herein. **FIG. 4A** includes an image of a delivery device as described herein (Appendix A, which is incorporated herein in its entirety by reference). A delivery device as shown is intended for intracochlear administration of injected fluid through the round window membrane, with a stopper to guide insertion depth. The stopper is shown in green in **FIG. 4A** of U.S. Provisional patent application 63/152,832, the entire contents of which is incorporated herein by reference. **FIG. 4B**

includes an image showing an expected flow of injected fluid through scala tympani to scala vestibuli (via communication at the helicotrema at the cochlear apex) and then out of the cochlea through a vent placed in the stapes footplate of a delivery device within the oval window (Talei 2019, which is incorporated herein in its entirety by reference).

[0134] **FIGs. 5A-5B** are schematic representations of a simplified endogenous AAV construct **(FIG. 5A)** and a simplified recombinant AAV (rAAV) construct **(FIG. 5B)**.

[0135] **FIGs. 6A-6D** are a series of schematic representations of exemplary rAAV constructs as described herein. **FIG. 6A** is an exemplary rAAV-AntiVEGF construct that comprises, *inter alia*, sequences encoding an immunoglobulin heavy chain variable domain and an immunoglobulin light chain variable domain separated by a sequence encoding a self-cleaving peptide. Such a construct is referred to herein as a "V_H/V_L construct," or an "rAAV-V_H/V_L construct." Exemplary rAAV-V_H/V_L include rAAV-ranibizumab and rAAV-ranibizumab-PC, which are rAAV-V_H/V_Ls that encode ranibizumab. **FIG. 6B** is an exemplary rAAV-AntiVEGF construct that comprises, *inter alia*, sequences encoding an immunoglobulin heavy chain variable domain, an optional immunoglobulin heavy chain constant domain, an immunoglobulin light chain variable domain, an optional immunoglobulin light chain variable domain, and a green florescent protein (GFP). Each of these components may be separated by a sequence encoding a self-cleaving peptide. Such a construct may be referred to as an "ABGFP construct," or "rAAV-ABGFP construct." Exemplary rAAV-ABGFP include rAAV-ranibizumab-GFP and rAAV-bevacizumab-GFP, which are rAAV-ABGFPs that encode ranibizumab or bevacizumab. **FIG. 6C** is an exemplary rAAV-AntiVEGF construct that comprises, *inter alia*, sequences encoding an immunoglobulin heavy chain (comprising an immunoglobulin heavy chain variable domain and an immunoglobulin heavy chain constant domain), and immunoglobulin light chain (comprising an immunoglobulin light chain variable domain and an immunoglobulin light chain constant domain), with the chains separated by a sequence encoding a self-cleaving peptide. Such a construct is referred to herein as an "AB construct," or an "rAAV-AB construct." Exemplary rAAV-AB include rAAV-bevacizumab and rAAV-bevacizumab-PC, which are rAAV-ABs that encode bevacizumab. **FIG. 6D** is an exemplary rAAV construct that comprises, *inter alia,* sequences encoding a portion of VEGF Receptor Extracellular domain 1, VEGF Receptor Extracellular domain 2, and human immunoglobulin gamma (IgG) Fc. Such a construct may be referred to as a "VEGF TRAP construct," or "rAAV-TRAP construct." Exemplary rAAV-TRAP

include rAAV-aflibercept and rAAV-aflibercept-PC, which are rAAV-TRAPs that encode aflibercept.

[0136] **FIG. 7** includes a Western blot showing HEK cell expression of different anti-VEGF proteins, ranibizumab and bevacizumab, using transfection or transduction of exemplary rAAV-AntiVEGF constructs described herein. Lanes are noted along the top of the figure, with predicted protein sizes noted on the left of the figure. Lanes 2-8 contain reduced proteins, while lanes 10-16 contain non-reduced proteins. Lane 1: pre-stained PageRuler™ protein ladder. Lane 2: untransfected/negative control. Lane 3: transfection with an rAAV-bevacizumab-PC construct. Lane 4: transfection with an rAAV-ranibizumab-GFP construct. Lane 5: transfection with rAAV-ranibizumab-PC construct. Lane 6: transduction with an rAAVAnc80-bevacizumab-PC particle with a multiplicity of infection (MOI) of 7.5×10^4. Lane 7: transduction with an rAAVAnc80-bevacizumab-PC particle with a MOI of 2.2×10^5. Lane 8: transduction with an rAAVAnc80-bevacizumab-PC particle with an MOI of 5.5×10^5. Lane 9: prestained PageRuler™ protein ladder. Lane 10: untransfected/negative control. Lane 11: transfection with an rAAV-bevacizumab-PC construct. Lane 12: transfection with an rAAV-ranibizumab-GFP construct. Lane 13: transfection with rAAV-ranibizumab-PC construct. Lane 14: transduction with an rAAVAnc80-bevacizumab-PC particle with a MOI of 7.5×10^4. Lane 15: transduction with an rAAVAnc80-bevacizumab-PC particle with a MOI of 2.2×10^5. Lane 16: transduction with an rAAVAnc80-bevacizumab-PC particle with a MOI of 5.5×10^5.

[0137] **FIGs. 8A-8D** are a series of graphs showing affinity of certain anti-VEGF proteins described herein as measured by Octet® HTX biosensor instrument using the Octet® analysis software, Data Analysis HT10.0. **FIG. 8A** is a graph showing the affinity of a control mouse anti-human VEGF monoclonal antibody (anti-hVEGF MmAb) in a buffer using recombinant human VEGF as the binding agent. In this assay, anti-hVEGF MmAb was prepared in CM at 100 µg/mL, then diluted to a final concentration of 10 µg/mL in 1x kinetics buffer. **FIG 8B** is a graph showing the affinity of secreted proteins in culture medium from HEK cells transfected with rAAV-ranibizumab-PC construct corresponding to SEQ ID NO: 90 using recombinant human VEGF as the binding agent. **FIG. 8C** is a graph showing the affinity of secreted proteins in culture medium from HEK cells transfected with an rAAV-bevacizumab-PC construct corresponding to SEQ ID NO: 93 using recombinant human VEGF as the binding agent. **FIG. 8D** is a graph showing the

affinity of secreted proteins in control culture medium (CM) from HEK cells that were not transfected with recombinant human VEGF as the binding agent.

[0138] **FIG. 9** is a graphical representation of the phylogeny and ancestral sequence reconstruction of the AAV evolutionary lineage. The dendrogram models the evolutionary path of AAVs with early specification of AAV4 and 5 serotypes, parallel to a single node named Anc80. Open circles with solid lines represent evolutionary intermediates reconstructed through ancestral sequence reconstruction. The open circle with a dotted line represents library of probabilistic sequence space around AAVAnc80 variant. Subclades are collapsed for clarity (Zinn 2015, incorporated herein in its entirety by reference).

[0139] **FIG. 10** is a schematic representation of a structural modeling of an AAVAnc80 capsid surface. Structural mapping of amino acid changes as compared to AAV2 (left) and AAV8 (right) on VP1 trimer visualizing the external (top) and internal (bottom) of the virion. There are some divergent residues in AAVAnc80, and some ambiguous and therefore dimorphic residues in Anc80Lib (Zinn 2015, incorporated herein in its entirety by reference). As shown in **FIG. 10** of U.S. Provisional patent application 63/152,832 (the entire contents of which is incorporated herein by reference), divergent residues are shown in blue, while ambiguous and dimorphic residues are shown in red.

[0140] **FIG. 11** includes representative fluorescent images depicting *in-vivo* cochlear transduction of naturally occurring AAV serotypes and an AAVAnc80 variant in neonatal mice via round window membrane delivery. Mice (P1) were injected with different AAV capsids (AAV1, AAV2, AAV8, AAV6 [not shown], and AAVAnc80) comprising a construct encoding enhanced GFP (eGFP). Phalloidin labeled actin and is shown in red in **FIG. 11** of U.S. Provisional patent application 63/152,832, the entire contents of which is incorporated herein by reference. Quantification of eGFP-positive inner hair cells (IHCs) and outer hair cells (OHCs) showed transduction efficiency between approximately 90 to 100% from the base to the apex after delivery of rAAVAnc80 comprising a construct encoding enhanced GFP (rAAVAnc80-eGFP) (Landegger 2017, which is incorporated herein in its entirety by reference).

[0141] **FIG. 12** includes representative fluorescent images depicting *in-vivo* vestibular transduction of rAAVAnc80 particles in neonatal mice via round window membrane delivery. Mice (P1) were injected with AAVAnc80-eGFP and phalloidin staining labeled actin. eGFP is

shown in green and phalloidin is shown in red in **FIG. 12** of U.S. Provisional patent application 63/152,832, the entire contents of which is incorporated herein by reference. Transduction was observed in both type I and type II hair cells of the utricle **(Panel (A))**, as well as cells of the semicircular canal cristate **(Panel (B))** (Landegger 2017, which is incorporated herein in its entirety by reference).

[0142] **FIG. 13** includes representative fluorescent images depicting *in-vivo* cochlear transduction of rAAVAnc80 particles in adult mice via posterior semicircular canal delivery. Mice (7 weeks old) were injected with rAAVAnc80-eGFP particles. **Panel (A)** includes a low-magnification view of a mid-modiolar section of an injected cochlea, showing eGFP signal in IHCs, referred to in the Panel as (I), OHCs, referred to in the Panel as (O), spiral limbus, referred to in the Panel as (SL), Reissner's membrane, referred to in the Panel as (RM), and spiral ganglion, referred to in the Panel as (SG). **Panel (B1)** and **Panel (B2)** include high-magnification views of the organ of Corti from apical (**Panel (B1)**) and mid (**Panel (B2)**) regions of the cochlea. Quantification of eGFP-positive cells showed that approximately 100% of the IHCs were transduced, whereas the OHC transduction decreased from apex to base. **Panel (C)** is a low magnification view showing that eGFP signal was detected in a subset of cells (neurons and satellite glial cells) in the spiral ganglion (Suzuki 2017, incorporated herein in its entirety by reference). Color images of the panels provided in this figure are shown in **FIG. 13** of U.S. Provisional patent application 63/152,832, the entire contents of which is incorporated herein by reference.

[0143] **FIG. 14** includes representative fluorescent images depicting *in-vivo* vestibular transduction of rAAVAnc80-eGFP in adult mice via posterior semicircular canal delivery. Mice (7 weeks old) were injected with rAAVAnc80-eGFP. **Panel (A1)** and **Panel (A2)** include low-magnification view of a section through the vestibule, showing eGFP signal in both utricle and saccule. **Panel (B)** and **Panel (C)** include high-magnification views of sections through vestibular end-organs (**Panel (B)**: utricle; **Panel (C)**: crista ampularis), showing eGFP expression in supporting cells and hair cells. Filled arrowheads indicate example transduced supporting cells (hair cells not indicated) (Suzuki 2017, incorporated herein in its entirety by reference). Color images of the panels provided in this figure are shown in **FIG. 14** of U.S. Provisional patent application 63/152,832, the entire contents of which is incorporated herein by reference.

[0144] **FIG. 15** includes representative fluorescent images depicting *in-vivo* cochlear and vestibular transduction of naturally occurring AAV2 serotype compared to rAAVAnc80 variant in adult mice via round window membrane delivery with canal fenestration. Mice (4 weeks old) were injected with different AAV particles (AAV2 and rAAVAnc80 shown here; AAV1, AAV8, and AAV9 not shown) encoding eGFP. Compared to AAV2, rAAVAnc80 mediated transduction showed comparable rates of IHC and OHC transduction (**Panel (A1)** *vs.* **Panel (A2)**) but broader transduction of spiral ganglion cells (**Panel (B1)** *vs.* **Panel (B2)**) and hair cells of the saccule (**Panel (C1)** *vs.* **Panel (C2)**: whole mounts; **Panel (D1)** *vs.* **Panel (D2)**: sections) (Omichi 2020, incorporated herein in its entirety by reference). Color images of the panels provided in this figure are shown in **FIG. 15** of U.S. Provisional patent application 63/152,832, the entire contents of which is incorporated herein by reference.

[0145] **FIGS. 16A-16B** are graphical representations of RNA expression in cochlear explants and secreted protein expression in cochlear explant media following transduction of WT newborn (P2) mice cochlear explants with rAAVAnc80 particles comprising anti-VEGF proteins as disclosed herein (rAAVAnc80-antiVEGF). rAAVAnc80-bevacizumab-PC particles (construct according to SEQ ID NO: 93) or rAAVAnc80-ranibizumab-PC particles (construct according to SEQ ID NO: 90) transduced cells in cochlear explants of WT mice and drove expression and secretion of mRNA encoding the anti-VEGF proteins. **FIG. 16A** depicts RNA expression analysis, and demonstrates expression of the mRNA encoding ranibizumab and bevacizumab in cells of explants receiving rAAVAnc80-ranibizumab-PC or rAAVAnc80-bevacizumab-PC, respectively. No expression was detected in explants receiving vehicle. Results are presented as mean + SD. **FIG. 16B** depicts Meso Scale Discovery (MSD) mediated quantification of ranibizumab detected in the media of explants receiving various concentrations (1.4E10, 2.8E10, or 4.2E10 vg) of rAAVAnc80-ranibizumab-PC particles; ranibizumab was detected in the media of explants receiving rAAVAnc80-ranibizumab-PC particles but not of explants receiving vehicle. Open circles indicate ranibizumab concentration in individual samples (n=4/group), while bars represent the mean.

[0146] **FIG. 17** includes representative low-magnification florescent staining images from a first study (referred to herein as "Study 1"), depicting the inner ear of CBA/CaJ mice transduced with rAAVAnc80-antiVEGF particles as described herein. Images are representative cochlear micrographs of the middle turn of microdissected cochleae after intracochlear administration of

either rAAVAnc80-ranibizumab-PC (construct according to SEQ ID NO: 90) particles at 1.4E10 vg/cochlea, rAAVAnc80-bevacizumab-PC (construct according to SEQ ID NO: 93) particles at 1.2E10 vg/cochlea, or vehicle control. The sensory epithelium was immunostained with primary antibodies against phalloidin, a hair-cell marker (which also shows faint non-specific labeling of the spiral limbus in these micrographs), and ranibizumab ("anti-Fab staining"), to detect anti-VEGF protein expression. As shown in **FIG. 17** of U.S. Provisional patent application 63/152,832 (the entire contents of which is incorporated herein by reference), phalloidin is shown in red and ranibizumab is shown in green. A human anti-ranibizumab antibody was used to detect the Fab segment of the proteins, which is shared between ranibizumab and bevacizumab. Clear labeling was detected in the IHCs and supporting cells lateral to the OHCs. Background staining was detected in the nerve fiber region of the cochlea (e.g., labeling of neuronal fibers was apparent for both the particle-injected and vehicle-injected cochleae), preventing reliable expression assessment in this particular area.

[0147] **FIG. 18** includes representative florescent staining images from Study 1, depicting the inner ear of CBA/CaJ mice transduced with rAAVAnc80-ranibizumab-PC (construct according to SEQ ID NO: 90) particles at 1.4E10 vg/cochlea or rAAVAnc80-bevacizumab-PC (construct according to SEQ ID NO: 93) particles at 1.2E10 vg/cochlea, compared with non-injected and/or vehicle injected controls. IHCs and OHCs were immunostained with anti-myosin VIIa antibodies and imaged at the 8, 16, and 32 kHz regions using a published cochleogram (Viberg and Canlon, 2004, which is incorporated in its entirety herein by reference). Control non-injected ear images are from ears contralateral from control vehicle injected ears. Scale bar = 20 μM. Color images of the panels provided in this figure are shown in **FIG. 18** of U.S. Provisional patent application 63/152,832, the entire contents of which is incorporated herein by reference.

[0148] **FIG. 19** includes representative confocal images from Study 1, depicting transduced hair cells and neurons for populations of CBA/CaJ mice transduced with rAAVAnc80-ranibizumab-PC (construct according to SEQ ID NO: 90). **Panel (A)** and **Panel (C)** represent transduced cells immunostained with anti-Fab antibodies. **Panel (B)** and **Panel (D)** represent neuronal projections immunostained with anti-Neurofilament 200. Both the vehicle injected samples (**Panels (A) and (B)**) and rAAVAnc80-ranibizumab-PC particle injected samples (**Panels (C) and (D)**) have transduced neuronal projections in the inner sulcus region, but not in the organ of Corti. Only the rAAVAnc80-ranibizumab-PC particle injected samples have transduced inner

and outer hair cells. Scale bar = 20 μM. Color images of the panels provided in this figure are shown in **FIG. 19** of U.S. Provisional patent application 63/152,832, the entire contents of which is incorporated herein by reference.

[0149] **FIGs. 20A-20B** are graphical representations from Study 1, depicting IHC and OHC count histograms from CBA/CaJ mice transduced with an rAAVAnc80-ranibizumab-PC (construct according to SEQ ID NO: 90) particles at 1.4E10 vg/cochlea, or rAAVAnc80-bevacizumab-PC (construct according to SEQ ID NO: 93) particles at 1.2E10 vg/cochlea, as compared with non-injected and/or vehicle injected controls. A representative population of the data sets utilized in this analysis are shown in **FIG. 18**. **FIG. 20A** depicts OHC counts for non-injected controls, vehicle injected controls, and test articles. **FIG. 20B** depicts IHC counts for non-injected controls, vehicle injected controls, and test articles. Counts for both **FIGs 20A** and **20B** were quantified and graphed as a function of treatment group and frequency region. The N for each group was either 9 or 10 animals, and the data are presented as mean +/- standard error of the mean (S.E.M). Control non-injected ear quantifications were from ears contralateral from control vehicle injected ears. *$p<0.05$, **$p<0.01$, and ***$p<0.001$ was made in comparison with the non-injected ear except for the bracket. p values were determined by a two-way ANOVA followed by a post-hoc Tukey's test.

[0150] **FIGS. 21A-21B** are graphical representations of the transduction frequency of hair cells (HCs) and non-hair cells (non-HCs). A representative population of the data sets utilized in this analysis are shown in **FIG. 18**. **FIG. 21A** depicts hair cell (HC) counts for non-injected controls, vehicle injected controls, and test articles. **FIG. 21B** depicts non-hair cell (non-HC) counts for non-injected controls, vehicle injected controls, and test articles. Data are from Study 1, and are representative of populations of CBA/CaJ mice transduced with an rAAVAnc80-ranibizumab-PC (construct according to SEQ ID NO: 90) particles at 1.4E10 vg/cochlea, or rAAVAnc80-bevacizumab-PC (construct according to SEQ ID NO: 93) particles at 1.2E10 vg/cochlea. Quantification of transduced (Fab+) hair cells **(FIG. 21A)** and non-hair cells **(FIG. 21B)** are graphed as a function of treatment group and frequency region with data combined from both genders. The N for each group was either 9 or 10 animals, and the data are presented as mean +/- standard error of the mean (S.E.M). Control non-injected ear quantifications are from ears contralateral from control vehicle injected ears. No statistical comparisons were made due to the variability across samples.

[0151] **FIG. 22** includes representative high-magnification images from Study 2, depicting florescent staining of cochlear transduction by rAAVAnc80- ranibizumab-PC (construct according to SEQ ID NO: 90) particles at 1.4E10 vg/cochlea. Cochlear micrographs from three regions of injected cochleae (63X) showing anti-ranibizumab (mAb) labeling are shown. Each column represents maximum projections through confocal image stacks acquired from an injected mouse, and each row represents a frequency region from the apex (8 kHz), middle (16 kHz), and base (32 kHz) of the cochlea. Listed to the right of each row are the primary cell types that immunostained positive. Substantial background staining was detected in the nerve fiber region of the cochlea (e.g., labeling of neuronal fibers was apparent for both the injected and uninjected cochleae [uninjected cochleae not shown]), preventing reliable expression assessment in this particular area. Color images of the panels provided in this figure with anti-ranibizumab labeling in green are shown in **FIG. 22** of U.S. Provisional patent application 63/152,832, the entire contents of which is incorporated herein by reference.

[0152] **FIG. 23** is a graphical representation of the detection and quantification of secreted anti-VEGF protein in serum from Study 2, and is following intracochlear delivery of rAAVAnc80-ranibizumab-PC(construct according to SEQ ID NO: 90) particles at 1.4E10 vg/cochlea. Anti-VEGF protein was detected using meso scale discovery in the serum of mice injected with rAAVAnc80-ranibizumab-PC particles at a higher level than in the serum of mice injected with vehicle. Open circles indicate anti-VEGF protein concentration in individual samples (vehicle, n = 7; ranibizumab, n = 9), while bars represent the mean.

[0153] **FIG. 24** is a graphical representation of the detection and quantification of secreted anti-VEGF protein in mouse cerebral spinal fluid (CSF) from Study 1, and is following intracochlear delivery of rAAVAnc80-ranibizumab-PC (construct according to SEQ ID NO: 90) particles at 1.4E10 vg/cochlea, or rAAVAnc80-bevacizumab-PC (construct according to SEQ ID NO: 93) particles at 1.2E10 vg/cochlea. Anti-VEGF protein (ranibizumab or bevacizumab) was detected using MSD in the CSF of mice administered rAAVAnc80-antiVEGF particles, but not in the CSF of mice administered vehicle. Open circles indicate an anti-VEGF protein concentration in individual samples (vehicle, n = 10; ranibizumab, n = 9; bevacizumab, n = 4), while bars represent the mean.

[0154] **FIG. 25** is a graphical representation of Study 1 mice auditory brainstem response (ABR) thresholds pre- and post-intracochlear delivery of rAAVAnc80-ranibizumab-PC (construct according to SEQ ID NO: 90) particles at 1.4E10 vg/cochlea, or rAAVAnc80-bevacizumab-PC (construct according to SEQ ID NO: 93) particles at 1.2E10 vg/cochlea. Mean ABR thresholds were elevated in all groups following intracochlear surgery, including vehicle- and rAAVAnc80 particle-injected mice. Mean ABR thresholds were elevated relative to baseline ABRs measured prior to surgery in rAAVAnc80 particle- and vehicle-injected ears. Error bars represent standard deviation.

[0155] **FIGs. 26A-26B** are graphical representations of Study 2 mice Distortion Product Otoacoustic Emissions (DPOAE) and ABR thresholds post-intracochlear delivery of rAAVAnc80-ranibizumab-PC particles (construct according to SEQ ID NO: 90). Cochlear and auditory function (DPOAEs, **FIG. 26A**, and ABR, **FIG. 26B**, respectively) demonstrated normal mean thresholds in mice administered with exemplary rAAVAnc80-ranibizumab-PC particles when compared with uninjected mice. The * indicates that one mouse administered rAAVAnc80-ranibizumab-PC particles died during the function tests, thus only DPOAEs and chirp-evoked ABRs were measured in this animal before that time; therefore, N=2 for 8, 16, and 32 kHz ABRs. Error bars represent standard deviation.

[0156] **FIGs. 27A-27B** provide aschematic description and graphical model depicting anti-VEGF protein concentration (modeled using measured concentrations from NHPs provided rAAVAnc80-ranibizumab particles (construct according to SEQ ID NO: 91) compared to distance to VS. **FIG. 27A** is a schematic of a computational modeling approach: depicting the three-dimensional diffusion of a constant source of anti-VEGF protein within a 90-µL sphere in relation to distance (in mm) from the surface of the sphere, *e.g.*, the border of scala tympani / fundus. Distance from fundus to medial and lateral borders of tumors (< 5 mm width) was estimated from data obtained from Koen 2020 which is incorporated herein in its entirety by reference (immediate **FIG. 2**). **FIG. 27B** represents a conservative modeling approach showing that perilymph anti-VEGF protein concentration decreases with diffusion distance, but remains within the reported biologically active range (area between 10-100% on the Y-axis and 0-11mm on the X-axis) within the vicinity of the tumor in the internal auditory canal. Estimated anti-VEGF protein concentration varies with choice of diffusion coefficient, represented as a range (shaded area with solid dots) based on the three reported diffusion coefficients. A color image of **FIG. 27B** with shading is

provided in **FIG. 27B** of U.S. Provisional patent application 63/152,832, the entire contents of which is incorporated herein by reference. The biologically active range, and predicted therapeutically-relevant range, was estimated as ~28 ng/mL; the concentration necessary to inhibit biological activity of VEGF-A by 50% in an *in-vitro* cellular proliferation assay is 11 to 27 ng/mL as described in Genentech 2017, incorporated herein in its entirety by reference).

[0157] **FIG. 28** depicts inner hair cell (IHC) transduction events for seven NHPs that underwent intracochlear (RWM) administration of AAVAnc80-eGFP with venting of the stapes footplate (6 unilateral, 1 bilateral), and two NHPs that underwent intracochlear (RWM) administration of AAVAnc80-eGFP without venting of the stapes footplate (bilateral). Cochleae were analyzed for eGFP expression in IHCs following a 3-week in-life duration. Transduction efficiency of ~80% to 100% can be achieved in macaque IHCs at higher doses (as seen with NHPs M6-M9), while at lower doses (as seen with NHPs M1-M3), an apex-to-base gradient in eGFP expression is observed. Only sporadic transduction at cochlear regions in the apical 75% of the cochlea were observed for animals that underwent surgery without venting of the stapes footplate.

[0158] **FIG. 29** illustrates a perspective of a device for delivering fluid to an inner ear, according to aspects of the present disclosure.

[0159] **FIG. 30** illustrates a sideview of a bent needle sub-assembly, according to aspects of the present disclosure.

[0160] **FIG. 31** illustrates a perspective view of a device for delivering fluid to an inner ear, according to aspects of the present disclosure.

[0161] **FIG. 32** 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.

DETAILED DESCRIPTION OF CERTAIN EMBODIMENTS

[0162] In certain embodiments, the present disclosure relates to an rAAV-antiVEGF particle intended for the treatment of subjects with otological diseases associated with neovascularization. In certain embodiments, such an otological disease associated with neovascularization is vestibular schwannoma (VS), or benign tumors that form in the cells around the vestibulocochlear nerve within the internal auditory canal. In certain embodiments, common symptoms associated with

early VS include hearing loss, tinnitus, and dizziness; as tumors continue to grow, they can compress the brainstem, representing a significant concern for more serious morbidity and, in rare cases, mortality.

[0163] In certain embodiments, the present disclosure relates to an rAAV-antiVEGF particle intended for the treatment of subjects with an inner ear disorder, e.g., as described herein. In some embodiments, an inner ear disorder comprises acoustic neuroma, vestibular schwannoma, or neurofibromatosis type II. In some embodiments, an inner ear disorder is or comprises acoustic neuroma. In some embodiments, an inner ear disorder is or comprises vestibular schwannoma. In some embodiments, an inner ear disorder is or comprises neurofibromatosis type II.

[0164] In some embodiments, the present disclosure relates to an rAAV-antiVEGF particle intended for the treatment of subjects with vestibular schwannoma.

[0165] In certain cases, therapy with inhibitors of vascular endothelial growth factor (VEGF) offers an opportunity to attenuate progressive VS tumors, rather than using invasive alternatives such as surgical resection and/or radiation therapy, the current standard of care. In certain embodiments, clinical data support the use of a systemically administered VEGF inhibitor in patients with VS tumors from an efficacy perspective; however, long-term systemic administration of VEGF inhibitors is associated with significant safety concerns. In certain embodiments, rAAV-antiVEGF is designed and intended to treat individuals with VS by gene transfer to the inner ear to promote localized expression and secretion of an anti-VEGF protein. In certain embodiments, an objective is to provide local exposure of the therapeutic VEGF inhibitor (e.g., an anti-VEGF protein, e.g., bevacizumab, ranibizumab, and/or aflibercept) at the VS site, thereby limiting systemic exposure and minimizing the potential for the adverse effects associated with systemic administration.

[0166] In certain embodiments, cochlear and vestibular cells of the inner ear are transduced by rAAV-antiVEGF, and secrete anti-VEGF protein into perilymph: a cochlear fluid that is in diffusional continuity with the interstitial and perineural spaces of the vestibulocochlear nerve where VS tumors are located. In certain embodiments, a lack of barriers to diffusion along the internal auditory canal provides a potential path for therapeutic anti-VEGF protein expressed in perilymph to reach the intended VS target in the nerve interstitium. In certain embodiments,

transduced cells of the cochlear modiolus are positioned to secrete the desired anti-VEGF protein directly into the interstitial fluid of the nerve.

[0167] In certain embodiments, escalating doses of compositions described herein (e.g., comprising an rAAV-antiVEGF) are administered via unilateral intracochlear injection to an individual (e.g., a mammal, e.g., a human patient in need thereof) with unilateral sporadic progressive VS. In certain embodiments, growth rates for these tumors are variable, and some VS tumors will not progress, in certain embodiments, an individual (e.g., a mammal, e.g., a human) may be limited to those individuals with tumors demonstrating clear evidence of progression, excluding those individuals with evidence of stable tumors on successive imaging evaluations. In certain embodiments, growth rates for these tumors are variable, and some VS tumors will not progress, in certain embodiments, an individual (e.g., a mammal, e.g., a human patient in need thereof) may be limited to those individuals with tumors demonstrating clear evidence of lack of progression, excluding those individuals with evidence of increasing tumor volume on successive imaging evaluations.

[0168] In certain embodiments, an individual (e.g., a mammal, e.g., a human patient in need thereof) with larger tumor(s) (e.g., tumors that have a greater potential to compress the brainstem) are excluded from treatment with compositions described herein, as in some embodiments, these individuals are at high risk for potentially life-threatening tumor-related sequelae that may potentially be avoided with the current standard of care of surgical resection and radiation therapy. In certain embodiments, individuals (e.g., a mammal, e.g., a human patient in need thereof) with larger tumors (e.g., tumors that have a greater potential to compress the brainstem) are expressly targeted for treatment with compositions described herein, as in some embodiments, these individuals are at high risk for potentially life-threatening tumor-related sequelae that may potentially be avoided using compositions as described herein in a less invasive manner and/or with greater or equal efficacy than the current standard of care of surgical resection and radiation therapy.

[0169] In certain embodiments, an individual (e.g., a mammal, e.g., a human) with growing tumors, where the tumor size is unlikely to impact brainstem, have the potential to derive greatest benefit from intervention with compositions as described herein (e.g., rAAV-antiVEGF therapy), while remaining candidates for future surgical resection and/or radiation as needed. In certain

embodiments, an individual (e.g., a mammal, e.g., a human) with growing tumors, where the tumor size may impact the brainstem, have the potential to derive greatest benefit from intervention with compositions as described herein (e.g., rAAV-antiVEGF therapy), while remaining candidates for future surgical resection and/or radiation as needed.

[0170] In certain embodiment, provided herein are methods comprising introducing into an inner ear of an individual, e.g., a mammal, e.g., a human, an effective amount, e.g., a therapeutically effective amount, of an rAAV particle comprising a construct nucleotide sequence encoding: (a) a polypeptide comprising an antibody heavy chain variable domain operably linked to a signal peptide and a polypeptide comprising an antibody light chain variable domain operably linked to a signal peptide; or (b) a polypeptide comprising an antigen-binding antibody fragment (e.g., a Fab or a scFv) operably linked to a signal peptide.

[0171] In certain embodiments, compositions as described herein (e.g., rAAV-antiVEGF) may be administered in the surgical suite under controlled aseptic conditions by an otologic surgeon.

[0172] In some embodiments, provided herein are methods for increasing the level of an antibody, in an inner ear and/or internal auditory canal of an individual, e.g., a mammal, e.g., a human in need thereof, comprising: introducing into the inner ear of the mammal an effective amount, e.g., a therapeutically effective amount of an rAAV particle comprising a nucleotide sequence encoding: (a) a polypeptide comprising an antibody heavy chain variable domain operably linked to a signal peptide and a polypeptide comprising an antibody light chain variable domain operably linked to a signal peptide; or (b) a polypeptide comprising an antigen-binding antibody fragment (e.g., a Fab or a scFv) operably linked to a signal peptide; wherein the introducing results in an increase in the level of the antibody or the antigen binding antibody fragment in the inner ear of the individual, e.g., mammal, e.g., human.

[0173] In some embodiment, the disclosure provides methods for treating an inner ear disorder in an individual, e.g., a mammal, e.g., a human in need thereof, comprising introducing into the inner ear of the mammal an effective amount, e.g., a therapeutically effective amount, of an rAAV particle comprising a nucleotide sequence encoding: (a) a polypeptide comprising an antibody heavy chain variable domain operably linked to a signal peptide and a polypeptide comprising an antibody light chain variable domain operably linked to a signal peptide; or (b) a polypeptide

comprising an antigen-binding antibody fragment linked to a signal peptide; where the introducing results in the treatment of the inner ear disorder in the mammal.

[0174] In some embodiments, provided herein are methods of reducing VEGF activity in an inner ear of an individual, e.g., a mammal, e.g., a human in need thereof, comprising introducing into the inner ear of the mammal an effective amount, e.g., a therapeutically effective amount, of an rAAV particle comprising a nucleotide sequence encoding (a) a polypeptide including an antibody heavy chain variable domain operably linked to a signal peptide and a polypeptide comprising an antibody light chain variable domain operably linked to a signal peptide; or (b) a polypeptide comprising an antigen-binding antibody fragment (e.g., a Fab or a scFv) operably linked to a signal peptide; wherein the polypeptide of (a) encodes an antibody that binds specifically to VEGF and reduces VEGF activity, the polypeptide of (b) encodes an antigen-binding antibody fragment that binds specifically to VEGF and reduces VEGF activity; and wherein the introducing results in a reduction in VEGF activity in the inner ear of the individual, e.g., mammal or human.

[0175] In some embodiments, provided herein are methods of treating an otological disease associated with neovascularization, e.g., acoustic neuroma, VS, or neurofibromatosis type II in an inner ear of an individual (e.g., a mammal, e.g., a human) comprising: introducing into the inner ear of the individual an effective amount (e.g., a therapeutically effective amount) of an rAAV particle comprising a nucleotide sequence encoding (a) a polypeptide comprising an antibody heavy chain variable domain operably linked to a signal peptide and a polypeptide comprising an antibody light chain variable domain operably linked to a signal peptide; or (b) a polypeptide comprising an antigen-binding antibody fragment (e.g., a Fab or a scFv) operably linked to a signal peptide; wherein the polypeptide of (a) encodes an antibody that binds specifically to VEGF and reduces VEGF activity, the polypeptide of (b) encodes an antigen-binding antibody fragment that binds specifically to VEGF and reduces VEGF activity; and wherein the introducing results in treatment of the otological disease associated with neovascularization, e.g., acoustic neuroma or VS in the inner ear of the individual.

[0176] In some embodiment, provided herein are methods comprising introducing into an inner ear of an individual (e.g., a mammal, e.g., a human) an effective amount (e.g., a

therapeutically effective amount) of an rAAV particle comprising a nucleotide sequence encoding a soluble VEGF receptor operably linked to a signal peptide.

[0177] In some embodiment, the disclosure provides methods for increasing the level of a soluble VEGF receptor in an inner ear of an individual (e.g., a mammal, e.g., a human) in need thereof, comprising introducing into the inner ear of the individual an effective amount (e.g., a therapeutically effective amount) of an rAAV particle comprising a nucleotide sequence encoding a soluble VEGF receptor operably linked to a signal peptide; wherein the introducing results in an increase in the level of the soluble VEGF receptor in the inner ear of the individual.

[0178] In some embodiment, provided herein are methods for treating an inner ear disorder in an individual (e.g., a mammal, e.g., a human) in need thereof comprising introducing into the inner ear of the individual an effective amount (e.g., a therapeutically effective amount) of an rAAV particle comprising a nucleotide sequence encoding at least a portion of a soluble VEGF receptor operably linked to a signal peptide; wherein the introducing results in the treatment of the inner ear disorder in the individual.

[0179] In some embodiment, provided herein are methods of reducing a VEGF activity in an inner ear of an individual (e.g., a mammal, e.g., a human) in need thereof comprising introducing into the inner ear of the individual an effective amount (e.g., a therapeutically effective amount) of an rAAV particle comprising a nucleotide sequence encoding at least a portion of a VEGF receptor operably linked to a signal peptide; wherein the introducing results in a reduction in the VEGF activity in the inner ear of the individual.

[0180] In some embodiment, provided herein are methods of treating an otological disease associated with neovascularization, acoustic neuroma, VS, or neurofibromatosis type 2 in an inner ear (including e.g., the internal auditory canal) of an individual (e.g., a mammal, e.g., a human) that include introducing into the inner ear of the individual an effective amount (e.g., a therapeutically effective amount) of an rAAV particle comprising a nucleotide sequence encoding at least a portion of a VEGF receptor operably linked to a signal peptide; wherein the introducing results in treatment of the otological disease associated with neovascularization, acoustic neuroma, VS or neurofibromatosis type II, respectively, in the inner ear of the individual.

[0181] In other embodiments, the disclosure also provides kits comprising any of the rAAV particles described herein.

[0182] Additional non-limiting aspects of the compositions, kits, and methods are described herein and can be used in any combination without limitation.

[0183] The present disclosure provides, *inter alia*, methods of gene therapy, e.g., using composition disclosed herein, to treat individuals with an otological disease associated with neovascularization, e.g., VS, by locally expressing secreted anti-VEGF protein in cells of the cochlea and vestibular system, in close proximity to and in diffusional continuity with the VS tumor environment in the internal auditory canal. In some embodiments, the method comprises gene transfer to the cochlea using an rAAV particle comprising a construct containing complimentary DNA (cDNA) encoding an anti-VEGF protein (rAAV-antiVEGF). Without wishing to be bound by theory, it is believed that in some embodiments, cochlear and vestibular cells of the inner ear transduced by an rAAV-antiVEGF (e.g., rAAVAnc80-antiVEGF) can secrete anti-VEGF protein into perilymph and the interstitial and perineural spaces of the vestibulocochlear nerve (comprised of the superior and inferior vestibular nerves and cochlear nerve). A majority of VS tumors originate in the lateral third, nearest the cochlea, of the internal auditory canal, which houses the vestibulocochlear nerve (**FIGS. 1-3**). The lack of barriers to diffusion along this canal results in the cochlear nerve being bathed in a continuum of fluid, with perilymph at its lateral end and CSF at its medial end; thus, diffusion from perilymph into the nerve interstitium provides a potential path for therapeutic anti-VEGF protein expressed in perilymph to reach the intended VS target. Although the precise mechanism by which VEGF inhibitors result in tumor control and regression is not fully understood, in some embodiments, mechanisms include decreasing vascular permeability and/or aberrant angiogenesis through inhibition of endothelial cell proliferation, as well as the normalization of tumor vasculature (Brastianos 2009, incorporated herein in its entirety by reference).

[0184] To date, there are no known gene transfer clinical trials comprising transfer to the inner ear using rAAV particles in humans; however, a clinical trial to evaluate an adenovirus particle comprising a construct encoding the complementary DNA (cDNA) for human Atonal transcription factor (Hath1) for the treatment of severe to profound hearing loss was initiated in 2014 and

completed in 2019 (Clinicaltrials.gov 2020a [NCT02132130] which is incorporated herein in its entirety by reference).

[0185] In some embodiments, the delivery approach disclosed herein comprises a synthetic AAV capsid (e.g., AAVAnc80) for transduction of inner ear cells, and/or a device for targeted delivery directly to the cochlea.

[0186] The current standard of care for patients with VS includes several approaches and several treatment objectives (Doherty 2006; Kaul 2018, each of which is incorporated herein in its entirety by reference). Treatments include imaging / observation, surgery, and radiation therapy. Treatment objectives can include preservation of hearing, but often patients present with complete or partial deafness, and the size and growth of tumors can dictate more aggressive interventions that accept hearing loss as an inevitable consequence of therapy. Both surgery and radiation carry with them adverse effects; importantly, neither is associated with improvement in quality of life metrics compared to observation alone (Carlson 2015, incorporated herein in its entirety by reference), so there is a clear need for less invasive treatments that can mitigate the impact of tumor growth.

[0187] The clinical efficacy of systemically administered bevacizumab for controlling tumor growth and associated hearing loss in NF2 patients is not without significant risk, as the continued intravenous infusions required to maintain therapeutic benefit also increase the risk for serious complications; in a meta-analysis of studies using bevacizumab for NF2, the pooled incidence of serious toxicity (Grade 3 or 4) was 17%, based on a meta-analysis of five clinical trial populations comprising 125 patients (Lu 2019, incorporated herein in its entirety by reference). Hypertension, proteinuria, elevated liver enzymes, arterial thromboembolic events (ATE), venous thromboembolic events, hemorrhage, and surgery and wound healing complications have all been associated with high doses of bevacizumab therapy (Chen 2009; Hanna 2019, each of which is incorporated herein in its entirety by reference). Disclosed herein, *inter alia*, is an alternative approach to treating VS that, e.g., does not require high levels of circulating anti-VEGF protein (e.g., bevacizumab), and in some embodiments, can present lower risk to patients with respect to events related to systemic exposure to the therapeutic molecule.

[0188] In some embodiments, use of an intracochlear route of administration to deliver rAAV-antiVEGF to the inner ear, transduction of inner ear cells, and subsequent expression and/or

secretion of an anti-VEGF protein (e.g., bevacizumab, ranibizumab, and/or aflibercept) can produce a sustained depot of the therapeutic drug in close proximity to the tumor. In some embodiments, cochlear and vestibular cells of the inner ear transduced by rAAV-antiVEGF can secrete anti-VEGF protein into nearby chambers (e.g., the perilymph, and cells of the cochlear modiolus, e.g., spiral ganglion neurons and satellite glial cells), and/or can secrete protein directly into the interstitial fluid of the cochlear nerve. In some embodiment, the lack of barriers to diffusion along the internal auditory canal results in the cochlear nerve being bathed in a continuum of fluid, with perilymph at its lateral end (nearest the cochlea, where the majority of VS tumors originate [**FIGS. 1-3**]) and CSF at its medial end. In some embodiments, diffusion from perilymph into the nerve interstitium provides a potential path for therapeutic anti-VEGF protein expressed in perilymph to reach the intended VS target.

[0189] In some embodiments, use of an intracochlear route of administration to deliver rAAV-antiVEGF to the inner ear, transduction of inner ear cells, and subsequent expression and/or secretion of an anti-VEGF protein (e.g., bevacizumab, ranibizumab, and/or aflibercept) results in non-therapeutically relevant or undetectable levels of anti-VEGF protein in non-cochlear tissue or fluid compartments. In some embodiments, an anti-VEGF protein is present at non-therapeutically relevant or undetectable levels in serum, CSF, liver, spleen, brainstem, auditory cortex, mandibular lymph nodes, or a combination thereof.

[0190] In some embodiments, local exposure to anti-VEGF proteins at the tumor surface can control tumor growth despite a different diffusion path to access and neutralize VEGF compared to extravasation of anti-VEGF proteins from the bloodstream. For example, Lichtenbeld et al.,1999 (incorporated herein in its entirety by reference) applied anti-VEGF proteins topically to tumors in mice and observed significantly reduced vascular permeability, notably at a 20-fold lower dose compared to a systemic dose that also achieved decreases in vascular permeability in mice to a similar degree (Yuan 1996, incorporated herein in its entirety by reference).

[0191] Without wishing to be bound by theory, it is believed in some embodiments that a low-level but sustained exposure to anti-VEGF protein in the fluid surrounding VS may stabilize and/or reduce tumor growth, through various mechanisms e.g., such as reducing permeability of tumor vessels and normalizing tumor vasculature. In some embodiments, by minimizing circulating levels of anti-VEGF proteins, local delivery of rAAV-antiVEGF to the ear and the resulting anti-

VEGF protein exposure in the tumor microenvironment can provide a durable therapeutic benefit while minimizing risk of adverse events associated with systemic anti-VEGF protein administration.

Vestibular Schwannoma (VS)

[0192] VS (also called acoustic neuroma) is a benign, usually slow-growing tumor (or tumors) resulting from neoplasia of Schwann cells that ensheathe the vestibulocochlear nerve (also referred to as cranial nerve VIII). VS often originate on the superior or inferior vestibular branches of the vestibulocochlear nerve –the nerve responsible for transmitting information about sound and equilibrium from the inner ear to the brain (see e.g., **FIGS. 1-3**). These tumors often arise within the internal auditory canal (e.g., immediately adjacent to the inner ear) and can extend into the cerebellopontine angle; they can occur as sporadic unilateral tumors or, less commonly, as bilateral tumors, which generally occurs in the setting of neurofibromatosis type 2 (NF2). A common area for VS occurrence is along the vestibulocochlear nerve (see, e.g., **FIGS. 1-3**). Small, intracanalicular tumors (less than 5 mm width) can arise, e.g., within the lateral third of the internal auditory canal, nearest to the cochlea (Koen 2020, incorporated herein in its entirety by reference).

[0193] Common symptoms associated with VS include hearing loss, tinnitus, and dizziness. As tumors continue to grow and expand outside the internal auditory canal and into the cranial space, they can compress the brainstem, representing a significant concern for more serious morbidity and a threat to survival. In the current standard of care, small or non-growing tumors may be followed by observation only, while surgical resection and/or radiation therapy are indicated for larger and/or progressive tumors.

[0194] In certain embodiments, compositions and methods described herein may reduce and/or ameliorate symptoms associated with VS and/or current standard of care methods for treating VS. Such symptoms may include but are not limited to: hearing loss, degeneration of hair cells, alteration of biochemical milieu of inner ear fluids, elevated intralabyrinthine protein, endolymphatic hydrops, cochlear aperture obstruction, intralabyrinthine hemorrhage, disruption of cochlear vascular supply, tinnitus, dizziness, intractable headache, facial neuropathy, trigeminal neuropathy, facial paralysis, facial paresthesia, hydrocephalus, cerebellar herniation, and/or death.

[0195] It is noteworthy that an increasing number of discovered VS tumors are asymptomatic and are identified in patients undergoing imaging for other indications (Reznitsky 2019,

incorporated herein in its entirety by reference). In some cases, symptoms of VSs can arise from compression of the cochlear nerve and invasion of the vestibular branches of the vestibulocochlear nerve (cranial nerve VIII). While the facial nerve is often stretched and splayed by the tumor, facial paralysis is generally uncommon. In some cases, compression of the nearby trigeminal nerve, which is responsible for transmitting facial sensory information to the brain, can result in facial paresthesia. Although histologically benign, in some cases, large tumors can compress the brainstem and result in hydrocephalus, cerebellar herniation, and, in rare cases, death.

[0196] Hearing loss induced by VS is thought to be e.g., produced by compression of the cochlear nerve and/or by cochlear dysfunction, which is supported by the presence of cochlear pathology in most cases. The mechanisms of VS-induced hearing loss are hypothesized to include, e.g., degeneration of hair cells, alteration of biochemical milieu of inner ear fluids (e.g., toxic cytokines from the tumor), elevated intralabyrinthine protein, endolymphatic hydrops, cochlear aperture obstruction, intralabyrinthine hemorrhage, and/or disruption of cochlear vascular supply (Roosli 2012; Dilwali 2015; Remenschneider 2017, each of which is incorporated herein in its entirety by reference).

[0197] While current treatments may reduce risks associated with tumor growth, they are not associated with stabilization of hearing loss or tinnitus, and they can often result in adverse effects including unilateral loss of residual hearing, intractable headache, and facial nerve defects (Pedrosa 1994; Sampath 1997; Sardhara 2020, each of which is incorporated herein in its entirety by reference). While rare, radiation therapy also carries the additional risk of secondary malignancy (Kapurch 2016, incorporated herein in its entirety by reference). Estimates of hearing preservation following current treatment regimens for VS vary, but in a large-scale retrospective comparison of surgical resection procedures, Ansari reported a range of average post-operative hearing loss from 40.6 to 82.7%, depending on the surgical approach used, as well as tumor size and location; this included surgical approaches such as middle cranial fossa and retrosigmoid that, depending on tumor size, can be favored over a translabyrinthine approach specifically to facilitate greater hearing preservation (Ansari 2012, incorporated herein in its entirety by reference). Guidelines from the Congress of Neurological surgeons recommend counseling patients considering radiosurgery, microsurgery, or observation to expect a 50 to 75% chance of hearing loss within 10 years, as they describe only a moderately low probability (defined as > 25 to 50%) of hearing preservation at 10 years with each of these treatment options (Carlson 2018,

incorporated herein in its entirety by reference). No currently available standard treatment for VS carries with it the potential for hearing improvement.

[0198] Decision-making about treatment for sporadic VSs is complex. The presence of a tumor is generally confirmed via magnetic resonance imaging (MRI) and initial standard of care is based on the severity of symptoms and tumor size. At the time of diagnosis, approximately 20 to 30% of cases are less than 1 cm, approximately 30% of cases are 1 to 2 cm (inclusive), and the remainder of cases (approximately 40 to 50%) are greater 2 cm (Peris-Celda 2019, incorporated herein in its entirety by reference). With the increase in availability and utilization of MRI scanners, in general, these tumors are being discovered earlier (smaller) and more often at an asymptomatic stage (Reznitsky 2019, incorporated herein in its entirety by reference). In certain embodiments, the present disclosure provides compositions and methods that may be particularly amenable to halting or slowing the growth of and/or shrinking tumors that are less than 0.5cm, less than 1 cm, less than 2 cm, less than 3 cm, less than 4 cm, or less than 5 cm.

[0199] In some cases, VS demonstrate variable and often unpredictable growth rates. This inherent behavior of the tumors is further complicated by variability in imaging modalities, tumor size estimates, and definitions of growth (Kondziolka 2012, incorporated herein in its entirety by reference). While overall tumor growth averages approximately 1 mm/year, between 30 and 60% of all tumors exhibit low or no apparent growth; for those that do grow, annual linear rates are between 2 and 3 mm/year (Paldor 2016; Lees 2018, each of which is incorporated herein in its entirety by reference). In some patients with small tumors, treatment comprises MRI scans alone, and additional treatment is considered only if the tumor displays measurable growth or if symptoms worsen (MacKeith 2013; Kirchmann 2017, each of which is incorporated in its entirety by reference herein). In some patients with tumors that continue to grow, and thus present a substantial increased risk for sequelae such as brainstem compression, current treatment options include surgical resection, radiation (Golfinos 2016, incorporated herein in its entirety by reference), or some combination of these approaches. While these treatments may reduce risks associated with tumor growth, they are not associated with long-term stabilization of hearing loss (Carlson 2018, incorporated herein in its entirety by reference) or improvement in tinnitus (Sardhara 2020, incorporated herein in its entirety by reference), and can often result in adverse effects including unilateral loss of residual hearing, intractable headache (Pedrosa 1994, incorporated herein in its entirety by reference), and facial nerve defects (Sampath 1997,

incorporated herein in its entirety by reference). Currently, about half of all VS cases will eventually require surgical resection and/or radiation. In some embodiments, the present disclosure provides compositions and methods that reduce the risks associated with tumor growth, such as hearing loss, loss of speech understanding, tinnitus, loss of quality of life, brainstem compression, and/or death. In some embodiments, the present disclosure provides compositions and methods that reduce the risks associated, such as tumor growth, hearing loss, degeneration of hair cells, alteration of biochemical milieu of inner ear fluids, elevated intralabyrinthine protein, endolymphatic hydrops, cochlear aperture obstruction, intralabyrinthine hemorrhage, disruption of cochlear vascular supply, tinnitus, dizziness, intractable headache, facial neuropathy, trigeminal neuropathy, facial paralysis, facial paresthesia, hydrocephalus, cerebellar herniation, and/or death.

[0200] Despite modern and recent improvements in both surgical and radiation therapies, in some embodiments, the more conservative approach of observation has gained favor; so long as the tumor is not growing, this represents the current best strategy to preserve remaining auditory function and minimize potential adverse impact of interventional treatments (MacKeith 2013; Kirchmann 2017; Torres Maldonado 2019, each of which is incorporated herein in its entirety by reference). In some embodiments, an improved treatment would be one that can promote VS stasis and/or regression, and thus circumvent the need for more invasive approaches including surgical resection or radiation therapy.

[0201] In the United States, the total incidence of VS is estimated to be 1.09 to 1.98 new cases per 100,000 population; thus, between 3300 and 6300 patients are diagnosed with VS in the US annually (Kshettry 2015; Ostrom 2018, each of which is incorporated herein in its entirety by reference). Increased reported incidence in recent years stems from the combination of an aging population and the continued enhancement of imaging technologies. The current standard of care for patients with VS includes several approaches and several treatment objectives (Doherty 2006; Kaul 2018, each of which is incorporated herein in its entirety by reference). Treatments include imaging / observation, surgery, and radiation therapy. Treatment objectives can include preservation of hearing, but often patients present with complete or partial deafness, and the size and growth of tumors can dictate more aggressive interventions that accept hearing loss as an inevitable consequence of therapy. Both surgery and radiation carry with them adverse effects; importantly, neither is associated with improvement in quality of life metrics compared to

observation alone (Carlson 2015, incorporated herein in its entirety by reference), so there is a clear need for less invasive treatments that can mitigate the impact of tumor growth.

[0202] For many of these patients with growing tumors, or tumors that are compressing or may eventually compress the brainstem, observation alone may not be acceptable and surgical resection or radiation may be required to prevent impact to neurological function. In some cases, it is not possible to remove the whole tumor without certain injury to the facial nerve. In these cases, subtotal resection is often done to preserve facial nerve function; however, this treatment approach leaves residual tumor that can continue to grow. In some embodiments, compositions and methods described herein may be utilized as a combination therapy in conjunction with surgical resection and/or radiation therapy. In some embodiments, such combination therapy approaches reduce the risk of facial nerve injury, or residual tumor cell growth.

[0203] In some embodiments, therapeutic approaches described herein utilizing compositions described herein, e.g., rAAV-antiVEGF, attenuate tumor growth pharmacologically, while avoiding and/or minimizing adverse effects associated with current standard of care treatments such as surgical resection and/or radiation.

[0204] The current standard of care for VS has evolved over the past 2 to 3 decades, as imaging techniques have evolved and the ability to identify and track the growth of tumors has improved. There has been a progressive trend toward a conservative, observational "wait and rescan" approach, with a growing awareness that many tumors exhibit slow or low growth and may not impact hearing so long as the low growth rate is maintained (MacKeith 2013; Reznitsky 2019, each of which is incorporated herein in its entirety by reference).

[0205] In certain embodiments, interventions, methods, and/or compositions described herein comprise benefits, including the opportunity to augment conservative treatment approaches by, e.g., halting tumor growth, stabilizing hearing, and/or obviating the need for more invasive treatment approaches such as surgical resection and/or radiation therapy. While halting VS growth is likely to provide a substantial clinical benefit, it is also possible that in some embodiments, methods and therapies utilizing compositions described herein (e.g., rAAV-antiVEGF) could go beyond tumor stasis and drive shrinkage of tumors, restoration of speech understanding, and reduction in perceived difficulty of speech understanding, as demonstrated in studies of bevacizumab-treated VS tumors in NF2 patients (Huang 2018; Plotkin 2019, each of which is

incorporated herein in its entirety by reference). In addition, studies with systemically administered VEGF inhibitor have shown improvements in NF2-related quality of life, including symptoms associated with VS in NF2 patients (Plotkin 2019, incorporated herein in its entirety by reference). However, these systemic administrations are often associated with negative side-effects. The results of these early studies may suggest that there is potential for anti-VEGF protein treatment to ameliorate and potentially combat the symptoms associated with VS. In certain embodiments, compositions described herein (e.g., rAAV-antiVEGF) may allow for less invasive treatment modalities, and sustained and localized expression of anti-VEGF protein in diffusional continuity with the tumor, potentially providing more concentrated and improved benefits of antiVEGF treatment without the systemic treatment associated side effects to patients with otological diseases associated with neovascularization (e.g., VS).

Vascular Endothelial Growth Factor (VEGF), and VEGF Inhibition in VS

[0206] New blood vessel development and vascularization have been found to be important factors in a number of tumor growth models, and may be important for VS growth. VEGF is one of the main regulators of angiogenesis. In certain cases, VEGF protein and its receptors are expressed in sporadic VS tumors (Cayé-Thomasen 2003; Plotkin 2009, each of which is incorporated herein in its entirety by reference), for instance all tumors examined in a study of 182 resected sporadic VS tumors expressed VEGF receptors (Koutsimpelas 2012, incorporated herein in its entirety by reference). In some cases, levels of VEGF protein and/or receptor expression in this type of schwannoma has been shown to correlate with tumor growth rates and/or growth indices determined by serial MRIs (Cayé-Thomasen 2005; Koutsimpelas 2007, each of which is incorporated herein in its entirety by reference) and with microvessel density (Koutsimpelas 2007, incorporated herein in its entirety by reference), suggesting a role for VEGF expression in VS growth. Additionally, systemic treatment with the VEGF inhibitor (bevacizumab) was effective in controlling growth and improving hearing in NF2 patients with VS. Without wishing to be bound by theory, it is believed that using VEGF inhibitors can result in the control of tumor growth, e.g., VS growth, by controlling vascular growth, e.g., tumor vascularization.

[0207] A VEGF gene encodes vascular endothelial growth factor (VEGF), formerly known as fms-like tyrosine kinase (Flt-1). A VEGF protein is a heparin-binding protein that induces

migration and proliferation of vascular endothelial cells. Non-limiting examples of protein and nucleotide sequences encoding a wildtype VEGF protein are described herein.

[0208] In some embodiments, local exposure to anti-VEGF protein at the tumor surface has the potential to control tumor growth despite a different diffusion path to access and neutralize VEGF compared to extravasation of anti-VEGF protein from the bloodstream. For example, Lichtenbeld et al., 1999 (incorporated herein in its entirety by reference), applied anti-VEGF protein topically to tumors in mice and observed significantly reduced vascular permeability, notably at a 20-fold lower dose compared to a systemic dose that also achieved decreases in vascular permeability in mice to a similar degree (Yuan 1996, incorporated herein in its entirety by reference). Accordingly, in some embodiments, compositions as described herein (e.g., comprising rAAV-antiVEGF) deliver low-level but sustained exposure to anti-VEGF protein in the fluid surrounding VS, thus having the potential to stabilize and/or reduce tumor growth. In certain embodiments, compositions as described herein (e.g., comprising rAAV-antiVEGF) stabilize and/or reduce tumor growth by reducing permeability of tumor vessels and/or normalizing tumor vasculature.

[0209] In certain embodiments, anti-VEGF protein therapy, e.g., as described herein, for VS attenuates growing tumors without the need for invasive alternatives such as surgical resection and/or radiation therapy, thereby, e.g., avoiding the complications of surgical resection and/or radiation therapy. It has been suggested that systemically administered VEGF inhibitor (bevacizumab) may demonstrate efficacy in stabilizing or reducing VS growth and hearing loss sequelae in patients with neurofibromatosis type 2 (NF2), where tumors resulting from germline mutations in NF2 also highly express VEGF and its receptors (Plotkin 2009; Plotkin 2012; Lu 2019, each of which is incorporated herein in its entirety by reference). However, systemic administration of VEGF inhibitors for controlling VS growth in NF2 patients may also be associated with adverse effects, with a pooled incidence of serious toxicity (Grade 3 or 4) of 17% in a meta-analysis of five groups of clinical trial participants, comprising 125 patients (Lu 2019, incorporated herein in its entirety by reference).

[0210] In certain embodiments, compositions as described herein (e.g., rAAV-antiVEGF) can be used in a method of treating an individual (e.g., a mammal, e.g., a human) with VS by gene transfer to the inner ear to, e.g., promote expression and secretion of anti-VEGF protein. In certain

embodiments, the compositions described herein, e.g., rAAV-antiVEGF, provide local exposure, e.g., in the inner ear, to the anti-VEGF protein. In certain embodiments, local exposure, e.g., in the inner ear, to the anti-VEGF protein at the VS site limits systemic exposure, and/or reduces, e.g., minimizes, potential adverse effects. In certain embodiments, compositions as described herein (e.g., rAAV-antiVEGF) comprise ranibizumab (48 kilodaltons [kDa]), a humanized monoclonal antibody fragment (Fab) derived from full-length murine anti-human VEGF monoclonal antibody. In certain embodiments, ranibizumab binds to VEGF and inhibits VEGF binding to its receptors VEGFR-1 and/or VEGFR-2, thereby reducing vascular leakage, aberrant angiogenesis, and/or tumor growth (Genentech 2017, incorporated herein in its entirety by reference).

[0211] In certain embodiments, cochlear and vestibular cells of the inner ear are transduced by compositions as described herein (e.g., comprising rAAV-antiVEGF). In certain embodiments, these cell types and/or others may secrete anti-VEGF protein into perilymph, which is an inner ear fluid that is in diffusional continuity with the interstitial and perineural spaces of the vestibulocochlear nerve, e.g., which is comprised of the superior and inferior vestibular nerves and the cochlear nerve, where the tumor is located. In some embodiments, a majority of VS tumors originate in the lateral third (nearest the cochlea) of the internal auditory canal, which houses the vestibulocochlear nerve. In some embodiments, lack of barriers to diffusion along this canal results in the cochlear nerve being bathed in a continuum of fluid, with perilymph at its lateral end and CSF at its medial end; thus, diffusion from perilymph into the nerve interstitium provides a potential path for therapeutic anti-VEGF protein expressed in perilymph to reach the intended VS target (Rask-Andersen 2006, incorporated herein in its entirety by reference). In some embodiments, spiral ganglion neurons and/or their satellite glial cells within the cochlear modiolus are transduced and/or transfected by compositions as described herein (e.g., comprising rAAV-antiVEGF), these cells are positioned to secrete protein directly into the interstitial fluid of the cochlear nerve.

[0212] In some embodiments, intracochlear administration of compositions described herein (e.g., rAAV-antiVEGF) has the potential to eliminate the need for future treatment and to preserve physiologic hearing in an individual (e.g., a mammal, e.g., a human) with an otological disease associated with neovascularization (e.g., VS), regardless of underlying etiology. In some embodiments, intracochlear administration of compositions described herein (e.g., rAAV-antiVEGF) has the potential to delay invasive treatment approaches, such as surgical resection

and/or radiation therapy, and associated loss of physiologic hearing. In some embodiments, intracochlear administration of compositions described herein (e.g., rAAV-antiVEGF) is followed by subsequent standard of care treatments. In some embodiments, intracochlear administration of compositions described herein (e.g., rAAV-antiVEGF) occurs before and/or after radiation therapy. In some embodiments, administration of compositions described herein (e.g., rAAV-antiVEGF) may improve an individual's (e.g., a mammal's, e.g., a human's) response to radiotherapy by sensitizing the tumor and allowing for lower radiation dosing (Koutsimpelas 2012; Gao 2015, each of which is incorporated herein in its entirety by reference).

[0213] As described above, new blood vessel development and vascularization have been found to be important factors in VS growth (Koutsimpelas 2007; Wong 2010, each of which is incorporated herein in its entirety by reference), and VEGF is one of the main regulators of angiogenesis. In addition to the angiogenic effect, VEGF also provides cellular protection and resistance to apoptosis induced by irradiation (Koutsimpelas 2012, incorporated herein in its entirety by reference). Over the past decade, clinical data have emerged demonstrating that VEGF inhibitors can halt or reverse the growth of VS. Bevacizumab (Avastin®) is currently the only pharmacologic agent for which preliminary evidence of effectiveness, in the setting of NF2, has been demonstrated in patients with VS.

[0214] The initial clinical evidence was published more than ten years ago in a seminal paper by Plotkin et al. (Plotkin 2009, incorporated herein in its entirety by reference). In this study, ten NF2 patients (six men and four women with a median age of 25 years) with baseline tumor size of 2.2 to 38.7cm^3 and baseline annual growth rates of 9 to 121%, and most presenting with hearing loss, were dosed with systemic bevacizumab 5 mg/kg every 2 weeks for an average of 12 months (3 to 19 months). After repeated bevacizumab dosing, tumors shrank in nine of ten patients, with six patients showing an MRI response of at least 20% reduction in tumor volume; responses were maintained in four of six patients during 11- to 16-month follow-up periods. The median best response to treatment for the nine of ten patients with smaller tumors was a volumetric reduction of 26%. Of the seven patients available for hearing testing, four demonstrated improvement, defined as a significant increase in word recognition score, two displayed stable hearing loss, and one demonstrated progressive hearing loss compared to baseline testing (Plotkin 2009, incorporated herein in its entirety by reference). Such results may suggest that this approach could potentially reverse progressive hearing loss observed in those with sustained tumor growth.

[0215] After this initial publication, a larger retrospective review of thirty-one NF2 patients treated systemically with intravenous bevacizumab(5 mg/kg every 2 weeks for 6 to 41 months; median duration 14 months) was reported (Plotkin 2012, incorporated herein in its entirety by reference). In this study of a similar patient population, 57% of evaluated patients demonstrated improvement in hearing, defined as increase in word recognition score from baseline, and 55% demonstrated a radiographic response, defined as at least a 20% decrease in tumor volume compared to baseline. When assessing long-term treatment response, 90% of patients had stable or improved hearing after one year and 61% of those patients continued that trend after three years. Additionally, 88% of patients had stable or decreased tumor size after one year of treatment and 54% of those patients remained stable at three years (Plotkin 2012, incorporated herein in its entirety by reference).

[0216] Most recently, Plotkin et al. published the results of a multicenter, prospective Phase 2 efficacy trial of systemic intravenous bevacizumab administration in NF2 patients with progressive VS (Plotkin 2019, incorporated herein in its entirety by reference). In this trial, patients (median age of 23 years) received bevacizumab systemically for six months at 10 mg/kg every two weeks, followed by eighteen months at 5 mg/kg every three weeks. Consistent with previous findings, interim trial results demonstrated that, six months into treatment, 41% of participants showed hearing improvement and 32% showed a radiographic response.

[0217] Preliminary efficacy and safety of systemically administered bevacizumab for NF2 patients with VS has also been reviewed in a meta-analysis of treatment outcomes from eight clinical trials conducted across the United States and Europe (Killeen 2019; Karajannis 2019; Lu 2019, each of which is incorporated herein in its entirety by reference). The treatment outcomes of 161 NF2 patients with 196 schwannomas across these eight studies, who received bevacizumab doses ranging from 5 to 10 mg/kg every 2 weeks (for an 11- to 22-month average range across the studies) were evaluated. Across these studies, the combined data demonstrate radiographic response (at least 20% volumetric reduction) in approximately 41% of schwannomas, tumor stability in approximately 47% of schwannomas, and tumor progression (at least 20% volumetric increase) in approximately 7% of schwannomas. In patients where audiometric data were available, these doses of bevacizumab were associated with improved hearing in approximately 20% of individuals, preserved hearing (stabilized hearing loss) in approximately 69% of individuals, and worsened hearing loss in approximately 6% of individuals. Subsequent surgical

intervention was required in 11% of patients during the reported follow-up time. In addition, side effects of bevacizumab such as serious toxicity (including hypertension, proteinuria, and amenorrhea) were assessed. The pooled incidence of serious toxicity (Grade 3 or 4) was 17%, based on a meta-analysis of five groups of clinical trial participants, comprising 125 patients (Lu 2019, which is incorporated herein in its entirety by reference). In certain embodiments, the present disclosure provides methods and compositions suitable for fulfilling a long-met need of efficacious treatment of otological diseases associated with neovascularization while potentially avoiding negative consequences associated with systemic delivery of therapeutic anti-VEGF proteins.

[0218] The above described body of clinical data (showing from ~30% to more than 60% response rates in tumor shrinkage) supports the systemic use of VEGF inhibitors to reduce VS growth and to improve associated symptoms of hearing loss despite the associated side-effects common with systemic use of VEGF inhibitors (Plotkin 2009; Plotkin 2012; Lu 2019; Plotkin 2019, each of which is incorporated herein in its entirety by reference). Without treatment, the mean growth rate of VS varies from 0.4 to 2.9 mm/year (Yoshimoto 2005, incorporated herein in its entirety by reference), with spontaneous tumor shrinkage reported to be from zero to 11% of tumors in studies of up to 212 patients (Tschudi 2000; Slattery 2004; Peyre 2013; Schnurman 2019, each of which is incorporated herein in its entirety by reference) or 3.8 % of tumors in a large study of 1261 patients (Huang 2013, incorporated herein in its entirety by reference). The limitation to systemic anti-VEGF protein therapies, to date, has been the inevitability of adverse systemic effects. In certain embodiments, the disclosure provides methods of administering an anti-VEGF protein. In certain embodiments, methods and compositions are provided that utilize an Adeno-Associated Virus (AAV) delivery mechanism. In certain embodiments, such an AAV comprises a recombinant construct, and is referred to as a recombinant AAV (rAAV). Embodiments of such constructs are described in further detail below. In certain embodiments, compositions are provided to deliver localized anti-VEGF proteins, e.g., rAAV-antiVEGF, into the cochlea, e.g., in close proximity to the location of a tumor, e.g., VS. In some embodiments, administration of a VEGF inhibitor, e.g., rAAV-antiVEGF, into the cochlea reduces side effects associated with systemic delivery of VEGF inhibitors.

[0219] Despite their inadequacy in evaluating a biologically active dose range for a gene therapy particle delivered via an intracochlear route of administration for the treatment of VS, several mouse models have been utilized to generate data supporting the biological plausibility of

VEGF inhibitors in reducing tumor vascular permeability. Using the cranial window model, Yuan et al. transplanted various human tumor cells lines and then administered an intravenous bolus of either neutralizing antibody against VEGF/VPF (vascular permeability factor) or phosphate-buffered saline control (Yuan 1996, incorporated herein in its entirety by reference). They showed that tumor vascular permeability to albumin in antibody-treated groups was lower than in the matched controls and that the tumor vessels became smaller in diameter and eventually disappeared after consecutive treatments. These data demonstrate that tumor vascular permeability can be reduced by neutralization of endogenous VEGF/VPF (Yuan 1996, incorporated herein in its entirety by reference). More recently, using both the sciatic nerve model and the intracranial window model, Gao et al. characterized the mechanism behind anti-VEGF protein treatment on bilateral VS (Gao 2015, incorporated herein in its entirety by reference). This group injected human HEI193 schwannoma cells into either the mouse sciatic nerve sheath or between the pia and arachnoid meninges of the right hemisphere of cranial window-implanted mice. After tumor size reached 4 mm in diameter, the VEGF inhibitor bevacizumab was administered 5mg/kg/week via an intraperitoneal (IP) route. The resulting data demonstrated that bevacizumab alleviated tumor edema, improved neurological function, and transiently normalized tumor vasculature in the mouse.

[0220] These studies provide proof-of-concept data supporting the scientific rationale for the use of VEGF inhibitors to slow progression of schwannomas in the mouse, which is consistent with the preliminary clinical data in NF2 patients treated systemically with Avastin® (Plotkin 2009; Plotkin 2012; Lu 2019; Plotkin 2019, each of which is incorporated herein in its entirety by reference). One limitation of these prior studies is that they do not reflect the enduring exposure levels in a limited target location.

[0221] In certain embodiments, methods and compositions disclosed herein (e.g., comprising rAAV-antiVEGF), result in sustained levels of a VEGF inhibitor in a limited target location, e.g., in the inner ear. Without wishing to be bound by theory, it is believed that compositions disclosed herein, e.g., VEGF inhibitors, could enter VS tumor cells through direct uptake from the surrounding fluid bath, which is in continuity with the perilymphatic compartment of the inner ear through the nerve interstitium from which an inner ear supply of anti-VEGF protein may diffuse. In tumor-bearing mice, anti-VEGF protein applied topically to the tumor tissue, rather than through

the bloodstream, resulted in beneficial effects such as reduced vascular permeability (Lichtenbeld 1999, incorporated herein in its entirety by reference).

[0222] In certain embodiments, the present disclosure provides methods and compositions suitable for delivery of VEGF inhibitors, e.g., rAAV-antiVEGF, locally to a tumor site. In some embodiments, these methods and compositions have the potential to maintain the benefit of anti-VEGF protein control of tumor growth while potentially minimizing the risk of serious toxicity that has been documented for systemic VEGF inhibitor administration. In certain embodiments, the present disclosure provides methods and compositions suitable for transduction of inner ear cells. In some embodiments, transduction of inner ear cells may enable long-lasting expression of anti-VEGF protein at and/or near the site of the tumor with minimal systemic exposure.

VEGF Polynucleotide Sequences

[0223] Among other things, the present disclosure provides polynucleotides, e.g., polynucleotides comprising a VEGF gene or characteristic portion thereof, as well as compositions including such polynucleotides and methods utilizing such polynucleotides and/or compositions.

[0224] In some embodiments, a polynucleotide comprising a VEGF gene or characteristic portion thereof can be DNA or RNA. In some embodiments, DNA can be genomic DNA or cDNA. In some embodiments, RNA can be an mRNA. In some embodiments, a polynucleotide comprises exons and/or introns of a VEGF gene.

[0225] In some embodiments, a gene product is expressed from a polynucleotide comprising a VEGF gene or characteristic portion thereof. In some embodiments, 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 embodiments, a polynucleotide provided herein can include one or more control elements.

[0226] In some embodiments, a VEGF gene is a mammalian VEGF gene. In some embodiments, a VEGF gene is a murine VEGF gene. In some embodiments, a VEGF gene is a primate VEGF gene. In some embodiments, a VEGF gene is a human VEGF gene. In some embodiments, a VEGF gene is a genomic DNA sequence. In some embodiments, a VEGF gene is an RNA sequence which encodes a protein product. In some embodiments, a VEGF gene is a

complementary DNA sequence which encodes the complement RNA sequence which encodes a protein product.

[0227] In some embodiments, an exemplary human VEGF gene is found at human Chromosomal location 6p21.1; location at NC_000006.12 (43770209..43786487) of the current 2020 assembly, and is known as VEGF-A with the NCBI Reference Sequence number: NG_008732.1. In some embodiments, an exemplary human VEGF gene is a cDNA sequence encompassed within the VEGF-A gene (e.g., VEGF-A transcript variant 1, transcript variant 2, transcript variant 3, etc.). In some embodiments, an exemplary human VEGF gene is one of the at least 9 known transcriptional isoforms of VEGF-A, one skilled in the art will understand these transcriptional isoforms may undergo alternative splicing to generate alternative translational isoforms. In some embodiments, an exemplary human VEGF-A gene is a cDNA sequence represented by transcript variant 1, encoding VEGF isoform A (NCBI Reference Sequence: NM_001025366.3). In some embodiments, an exemplary human VEGF-A gene is a cDNA sequence represented by transcript variant 2, encoding VEGF isoform B (NCBI Reference Sequence: NM_003376.6). In some embodiments, an exemplary human VEGF-A gene is a cDNA sequence represented by transcript variant 3, encoding VEGF isoform C (NCBI Reference Sequence: NM_001025367.3). In some embodiments, an exemplary human VEGF-A gene is a cDNA sequence represented by transcript variant 4, encoding VEGF isoform D (NCBI Reference Sequence: NM_001025368.3). In some embodiments, an exemplary human VEGF-A gene is a cDNA sequence represented by transcript variant 5, encoding VEGF isoform E (NCBI Reference Sequence: NM_001025369.3). In some embodiments, an exemplary human VEGF-A gene is a cDNA sequence represented by transcript variant 6, encoding VEGF isoform F (NCBI Reference Sequence: NM_001025370.3). In some embodiments, an exemplary human VEGF-A gene is a cDNA sequence represented by transcript variant 7, encoding VEGF isoform G (NCBI Reference Sequence: NM_001033756.3). In some embodiments, an exemplary human VEGF-A gene is a cDNA sequence represented by transcript variant 8, encoding VEGF isoform H (NCBI Reference Sequence: NM_001171622.2). In some embodiments, an exemplary human VEGF-A gene is a cDNA sequence represented by transcript variant , encoding VEGF isoform R (NCBI Reference Sequence: NM_001204385.2).

[0228] In some embodiments, an exemplary human VEGF gene is found at human Chromosomal location 11q13.1, at location NC_000011.10 (64234584..64239264), and is known

as VEGF-B (NCBI Reference Sequence: NG_029823.1). In some embodiments, an exemplary human VEGF gene is a cDNA sequence encompassed within the VEGF-B gene e.g., VEGF-B transcript variant 167 (NCBI Reference Sequence: NM_001243733.2), and/or transcript variant 186 (NCBI Reference Sequence: NM_003377.5).

[0229] In some embodiments, an exemplary human VEGF gene is found at human Chromosomal location 4q34.1-q34.3, at location NC_000004.12 (176683538..176792922, complement), and is known as VEGF-C (NCBI Reference Sequence: NG_034216.1). In some embodiments, an exemplary human VEGF gene is a cDNA sequence encompassed within the VEGF-C gene, .g., VEGF-C transcript variant 1(NCBI Reference Sequence: NM_005429.5).

[0230] In some embodiments, an exemplary human VEGF gene is found at human Chromosomal location Xp22.2, at location NC_000023.11 (15345596..15384413, complement), and is known as VEGF-D (NCBI Reference Sequence: NG_012509.1). In some embodiments, an exemplary human VEGF gene is a genomic sequence or a cDNA sequence encompassed within the VEGF-D gene, e.g., VEGF-D transcript variant 1(NCBI Reference Sequence: NM_004469.5).

VEGF Protein Sequences

[0231] In certain embodiments, proteins of interest are isoforms of the VEGF-A gene. This gene is a member of the PDGF (platelet-derived growth factor)/VEGF (vascular endothelial growth factor) family (PDGF/VEGF). It encodes a heparin-binding protein, which typically exists as a disulfide-linked homodimer. This growth factor induces proliferation and migration of vascular endothelial cells, and is essential for both physiological and pathological angiogenesis. Alternatively spliced transcript variants encoding different isoforms have been described. There is substantial evidence for alternative translation initiation from upstream non-AUG (CUG) codons resulting in additional isoforms. In some embodiments, proteins of interest are inhibitors of endogenous VEGF-A functions.

[0232] Exemplary Human VEGF-A isoform L-VEGF$_{206}$ precursor (also known as Isoform A) amino acid sequence (SEQ ID NO: 1)

MTDRQTDTAPSPSYHLLPGRRRTVDAAASRGQGPEPAPGGGVEGVGARGVALKLFVQL
LGCSRFGGAVVRAGEAEPSGAARSASSGREEPQPEEGEEEEEKEEERGPQWRLGARKPG
SWTGEAAVCADSAPAARAPQALARASGRGGRVARRGAEESGPPHSPSRRGSASRAGPG

RASETMNFLLSWVHWSLALLLYLHHAKWSQAAPMAEGGGQNHHEVVKFMDVYQRSY
CHPIETLVDIFQEYPDEIEYIFKPSCVPLMRCGGCCNDEGLECVPTEESNITMQIMRIKPHQ
GQHIGEMSFLQHNKCECRPKKDRARQEKKSVRGKGKGQKRKRKKSRYKSWSVYVGA
RCCLMPWSLPGPHPCGPCSERRKHLFVQDPQTCKCSCKNTDSRCKARQLELNERTCRC
DKPRR

[0233] Exemplary Human VEGF-A isoform L-VEGF$_{189}$ precursor (also known as Isoform B) amino acid sequence (SEQ ID NO: 2)

MTDRQTDTAPSPSYHLLPGRRRTVDAAASRGQGPEPAPGGGVEGVGARGVALKLFVQL
LGCSRFGGAVVRAGEAEPSGAARSASSGREEPQPEEGEEEEEKEEERGPQWRLGARKPG
SWTGEAAVCADSAPAARAPQALARASGRGGRVARRGAEESGPPHSPSRRGSASRAGPG
RASETMNFLLSWVHWSLALLLYLHHAKWSQAAPMAEGGGQNHHEVVKFMDVYQRSY
CHPIETLVDIFQEYPDEIEYIFKPSCVPLMRCGGCCNDEGLECVPTEESNITMQIMRIKPHQ
GQHIGEMSFLQHNKCECRPKKDRARQEKKSVRGKGKGQKRKRKKSRYKSWSVPCGPC
SERRKHLFVQDPQTCKCSCKNTDSRCKARQLELNERTCRCDKPRR

[0234] Exemplary Human VEGF-A isoform VEGF$_{111}$ precursor amino acid sequence (SEQ ID NO: 3)

MNFLLSWVHWSLALLLYLHHAKWSQAAPMAEGGGQNHHEVVKFMDVYQRSYCHPIE
TLVDIFQEYPDEIEYIFKPSCVPLMRCGGCCNDEGLECVPTEESNITMQIMRIKPHQGQHI
GEMSFLQHNKCECRCDKPRR

[0235] Exemplary Human VEGF-A isoform VEGF$_{121}$ precursor amino acid sequence (SEQ ID NO: 4)

MNFLLSWVHWSLALLLYLHHAKWSQAAPMAEGGGQNHHEVVKFMDVYQRSYCHPIE
TLVDIFQEYPDEIEYIFKPSCVPLMRCGGCCNDEGLECVPTEESNITMQIMRIKPHQGQHI
GEMSFLQHNKCECRPKKDRARQEKCDKPRR

[0236] Exemplary Human VEGF-A isoform VEGF$_{145}$ precursor amino acid sequence (SEQ ID NO: 5)

MNFLLSWVHWSLALLLYLHHAKWSQAAPMAEGGGQNHHEVVKFMDVYQRSYCHPIE
TLVDIFQEYPDEIEYIFKPSCVPLMRCGGCCNDEGLECVPTEESNITMQIMRIKPHQGQHI
GEMSFLQHNKCECRPKKDRARQEKKSVRGKGKGQKRKRKKSRYKSWSVCDKPRR

[0237] Exemplary Human VEGF-A isoform $VEGF_{165A}$ precursor amino acid sequence (SEQ ID NO: 6)

MNFLLSWVHWSLALLLYLHHAKWSQAAPMAEGGGQNHHEVVKFMDVYQRSYCHPIE
TLVDIFQEYPDEIEYIFKPSCVPLMRCGGCCNDEGLECVPTEESNITMQIMRIKPHQGQHI
GEMSFLQHNKCECRPKKDRARQENPCGPCSERRKHLFVQDPQTCKCSCKNTDSRCKAR
QLELNERTCRCDKPRR

[0238] Exemplary Human VEGF-A isoform $VEGF_{189}$ precursor amino acid sequence (SEQ ID NO: 7)

MNFLLSWVHWSLALLLYLHHAKWSQAAPMAEGGGQNHHEVVKFMDVYQRSYCHPIE
TLVDIFQEYPDEIEYIFKPSCVPLMRCGGCCNDEGLECVPTEESNITMQIMRIKPHQGQHI
GEMSFLQHNKCECRPKKDRARQEKKSVRGKGKGQKRKRKKSRYKSWSVPCGPCSERR
KHLFVQDPQTCKCSCKNTDSRCKARQLELNERTCRCDKPRR

[0239] Exemplary Human VEGF-A isoform $VEGF_{206}$ precursor amino acid sequence (SEQ ID NO: 8)

MNFLLSWVHWSLALLLYLHHAKWSQAAPMAEGGGQNHHEVVKFMDVYQRSYCHPIE
TLVDIFQEYPDEIEYIFKPSCVPLMRCGGCCNDEGLECVPTEESNITMQIMRIKPHQGQHI
GEMSFLQHNKCECRPKKDRARQEKKSVRGKGKGQKRKRKKSRYKSWSVYVGARCCL
MPWSLPGPHPCGPCSERRKHLFVQDPQTCKCSCKNTDSRCKARQLELNERTCRCDKPR
R

[0240] In certain embodiments, proteins of interest are isoforms of the VEGF-B gene. This gene encodes a member of the PDGF/VEGF. The VEGF family members regulate the formation of blood vessels and are involved in endothelial cell physiology. This member is a ligand for VEGFR-1 (vascular endothelial growth factor receptor 1) and NRP-1 (neuropilin-1). In some embodiments, proteins of interest are inhibitors of endogenous VEGF-B functions.

[0241] Exemplary Human VEGF-B isoform VEGFB-167 precursor amino acid sequence (SEQ ID NO: 9)

MSPLLRRLLLAALLQLAPAQAPVSQPDAPGHQRKVVSWIDVYTRATCQPREVVVPLTV
ELMGTVAKQLVPSCVTVQRCGGCCPDDGLECVPTGQHQVRMQILMIRYPSSQLGEMSL

EEHSQCECRPKKKDSAVKPDSPRPLCPRCTQHHQRPDPRTCRCRCRRRSFLRCQGRGLE
LNPDTCRCRKLRR

[0242] Exemplary Human VEGF-B isoform VEGFB-186 precursor amino acid sequence (SEQ ID NO: 10)

MSPLLRRLLLAALLQLAPAQAPVSQPDAPGHQRKVVSWIDVYTRATCQPREVVVPLTV
ELMGTVAKQLVPSCVTVQRCGGCCPDDGLECVPTGQHQVRMQILMIRYPSSQLGEMSL
EEHSQCECRPKKKDSAVKPDRAATPHHRPQPRSVPGWDSAPGAPSPADITHPTPAPGPS
AHAAPSTTSALTPGPAAAAADAAASSVAKGGA

[0243] In certain embodiments, proteins of interest are isoforms of the VEGF-C gene. The protein encoded by this gene is a member of the PDGF/VEGF family. The encoded protein promotes angiogenesis and endothelial cell growth, and can also affect the permeability of blood vessels. The precursor protein is further cleaved into a fully processed form that can bind and activate VEGFR-2 and VEGFR-3 receptors. In some embodiments, proteins of interest are inhibitors of endogenous VEGF-C functions.

[0244] Exemplary Human VEGF-C precursor amino acid sequence (SEQ ID NO: 11)

MHLLGFFSVACSLLAAALLPGPREAPAAAAAFESGLDLSDAEPDAGEATAYASKDLEEQ
LRSVSSVDELMTVLYPEYWKMYKCQLRKGGWQHNREQANLNSRTEETIKFAAAHYNT
EILKSIDNEWRKTQCMPREVCIDVGKEFGVATNTFFKPPCVSVYRCGGCCNSEGLQCMN
TSTSYLSKTLFEITVPLSQGPKPVTISFANHTSCRCMSKLDVYRQVHSIIRRSLPATLPQCQ
AANKTCPTNYMWNNHICRCLAQEDFMFSSDAGDDSTDGFHDICGPNKELDEETCQCVC
RAGLRPASCGPHKELDRNSCQCVCKNKLFPSQCGANREFDENTCQCVCKRTCPRNQPL
NPGKCACECTESPQKCLLKGKKFHHQTCSCYRRPCTNRQKACEPGFSYSEEVCRCVPSY
WKRPQMS

[0245] In certain embodiments, proteins of interest are isoforms of the VEGF-D gene. The protein encoded by this gene is a member of the PDGF/VEGF family and is active in angiogenesis, lymphangiogenesis, and endothelial cell growth. This secreted protein undergoes a complex proteolytic maturation, generating multiple processed forms which bind and activate VEGFR-2 and VEGFR-3 receptors. This protein is structurally and functionally similar to VEGF-C. In some embodiments, proteins of interest are inhibitors of endogenous VEGF-D functions.

[0246] Exemplary Human VEGF-D precursor amino acid sequence (SEQ ID NO: 12)

MYREWVVVNVFMMLYVQLVQGSSNEHGPVKRSSQSTLERSEQQIRAASSLEELLRITHS
EDWKLWRCRLRLKSFTSMDSRSASHRSTRFAATFYDIETLKVIDEEWQRTQCSPRETCV
EVASELGKSTNTFFKPPCVNVFRCGGCCNEESLICMNTSTSYISKQLFEISVPLTSVPELVP
VKVANHTGCKCLPTAPRHPYSIIRRSIQIPEEDRCSHSKKLCPIDMLWDSNKCKCVLQEE
NPLAGTEDHSHLQEPALCGPHMMFDEDRCECVCKTPCPKDLIQHPKNCSCFECKESLET
CCQKHKLFHPDTCSCEDRCPFHTRPCASGKTACAKHCRFPKEKRAAQGPHSRKNP

VEGF, VEGF-R, and VEGF Binding Proteins

[0247] In some embodiments, proteins of interest are ones that can bind VEGF. In some embodiments, VEGF binding proteins can be or comprise antibodies, and/or fragments thereof. In some embodiments, VEGF binding proteins can be or comprise vascular endothelial growth factor receptor (VEGFR) proteins, and/or fragments thereof.

[0248] As described above, data has been generated that may support the biological plausibility of VEGF inhibitors in reducing tumor vascular permeability. In some embodiments, such data was generated using mouse models, which have been utilized to generate data supporting the biological plausibility of VEGF inhibitors in reducing tumor vascular permeability. In some embodiments, such mouse models are not ideal for evaluating biologically active dose ranges for gene therapy particles which are delivered via an intracochlear route of administration, e.g., for the treatment of VS. Using the cranial window model, Yuan et al. transplanted various human tumor cells lines and then administered an intravenous bolus of either neutralizing antibody against VEGF/VPF (vascular permeability factor) or phosphate-buffered saline control (Yuan 1996, incorporated herein in its entirety by reference). The Authors showed that tumor vascular permeability to albumin in antibody-treated groups was lower than in the matched controls and that the tumor vessels became smaller in diameter and eventually disappeared after consecutive treatments. These data demonstrate that tumor vascular permeability can be reduced by neutralization of endogenous VEGF/VPF (Yuan 1996, incorporated herein in its entirety by reference). More recently, using both the sciatic nerve model and the intracranial window model, Gao et al. sought to characterize the mechanism behind anti-VEGF protein treatment on bilateral VS (Gao 2015, incorporated herein in its entirety by reference). This group injected human HEI193 schwannoma cells into either the mouse sciatic nerve sheath or between the pia and arachnoid

meninges of the right hemisphere of cranial window-implanted mice. After tumor size reached 4 mm in diameter, the VEGF inhibitor bevacizumab was administered 5mg/kg/week via an intraperitoneal (IP) route. The resulting data demonstrate that bevacizumab alleviated tumor edema, improved neurological function, and transiently normalized tumor vasculature in the mouse.

[0249] Taken together, these studies provide proof-of-concept data supporting the scientific rationale for the use of VEGF inhibitors to slow progression of schwannomas in the mouse, which is consistent with the preliminary clinical data in NF2 patients treated systemically with Avastin® (Plotkin 2009; Plotkin 2012; Lu 2019; Plotkin 2019, each of which is incorporated herein in its entirety by reference). One limitation of these prior studies is that they do not reflect the enduring exposure levels in a limited target location.

[0250] In some embodiments, methods and compositions disclosed herein result in sustained levels of a VEGF inhibitor, e.g., rAAV-antiVEGF, at a limited target location, e.g., in the inner ear of an individual, e.g., mammal, e.g., human. Without wishing to be bound by theory, it is believed that in some embodiments, a VEGF inhibitor could enter VS tumor cells through direct uptake from the surrounding fluid bath, which is in continuity with the perilymphatic compartment of the inner ear through the nerve interstitium from which an inner ear supply of anti-VEGF protein may diffuse. In tumor-bearing mice, anti-VEGF protein applied topically to the tumor tissue, rather than through the bloodstream, resulted in beneficial effects such as reduced vascular permeability (Lichtenbeld 1999, incorporated herein in its entirety by reference).

Anti-VEGF Antibodies

[0251] In some embodiments of any of the antibodies described herein, said antibodies can bind to a VEGF antigen (e.g., any of the exemplary VEGF proteins described herein, e.g., one or more of mature human VEGF-A, mature human VEGF-B, mature human VEGF-C, and mature human VEGF-D) (e.g., any of the binding affinities described herein).

[0252] In some embodiments described herein, an antibody can decrease an activity of a VEGF (e.g., one or more of any of the exemplary VEGF proteins described herein, e.g., one or more of mature human VEGF-A, mature human VEGF-B, mature human VEGF-C, and mature human VEGF-D). In some embodiments, an antibody can block a VEGF (e.g., one or more of any of the exemplary VEGF proteins described herein, e.g., one or more of mature human VEGF-A, mature

human VEGF-B, mature human VEGF-C, and mature human VEGF-D) from binding to one or more of its receptors (e.g., one or more VEGF receptors) See, e.g., WO 1998/045331, US 9,079,953, US 2015/0147317, US 2016/0289314, Plotkin et al., Otology & Neurotology 33: 1046-1052 (2012); and Ferrara et al. (2005) Biochem Biophys Res Commun 333(2): 328-335, each of which is hereby incorporated in their entirety by reference. In some embodiments, an antibody can decrease downstream signaling (e.g., signaling downstream of a VEGF receptor, e.g., one or more of any of the exemplary VEGF receptors described herein, e.g., one or more of human VEGFR-1, human VEGFR-2, and human VEGFR-3). In some embodiments, a decrease in an activity of a VEGF can be detected indirectly, e.g., through VS tumor size, and/or alteration of VS associated symptoms described herein, e.g., through an increase in hearing (e.g., a 1% to about 400% increase (or any of the subranges of this range described herein) in hearing) or a decrease (e.g., a 1% to 99%, a 1% to 95%, a 1% to 90%, a 1% to 85%, a 1% to 80%, a 1% to 75%, a 1% to 70%, a 1% to 65%, a 1% to 60%, a 1% to 55%, a 1% to 50%, a 1% to 45%, a 1% to 40%, a 1% to 35%, a 1% to 30%, a 1% to 25%, a 1% to 20%, a 1% to 15%, a 1% to 10%, a 1% to 5%, a 5% to 99%, a 5% to 95%, a 5% to 90%, a 5% to 85%, a 5% to 80%, a 5% to 75%, a 5% to 70%, a 5% to 65%, a 5% to 60%, a 5% to 55%, a 5% to 50%, a 5% to 45%, a 5% to 40%, a 5% to 35%, a 5% to 30%, a 5% to 25%, a 5% to 20%, a 5% to 15%, a 5% to 10%, a 10% to 99%, a 10% to 95%, a 10% to 90%, a 10% to 85%, a 10% to 80%, a 10% to 75%, a 10% to 70%, a 10% to 65%, a 10% to 60%, a 10% to 55%, a 10% to 50%, a 10% to 45%, a 10% to 40%, a 10% to 35%, a 10% to 30%, a 10% to 25%, a 10% to 20%, a 10% to 15%, a 15% to 99%, a 15% to 95%, a 15% to 90%, a 15% to 85%, a 15% to 80%, a 15% to 75%, a 15% to 70%, a 15% to 65%, a 15% to 60%, a 15% to 55%, a 15% to 50%, a 15% to 45%, a 15% to 40%, a 15% to 35%, a 15% to 30%, a 15% to 25%, a 15% to 20%, a 20% to 99%, a 20% to 95%, a 20% to 90%, a 20% to 85%, a 20% to 80%, a 20% to 75%, a 20% to 70%, a 20% to 65%, a 20% to 60%, a 20% to 55%, a 20% to 50%, a 20% to 45%, a 20% to 40%, a 20% to 35%, a 20% to 30%, a 20% to 25%, a 25% to 99%, a 25% to 95%, a 25% to 90%, a 25% to 85%, a 25% to 80%, a 25% to 75%, a 25% to 70%, a 25% to 65%, a 25% to 60%, a 25% to 55%, a 25% to 50%, a 25% to 45%, a 25% to 40%, a 25% to 35%, a 25% to 30%, a 30% to 99%, a 30% to 95%, a 30% to 90%, a 30% to 85%, a 30% to 80%, a 30% to 75%, a 30% to 70%, a 30% to 65%, a 30% to 60%, a 30% to 55%, a 30% to 50%, a 30% to 45%, a 30% to 40%, a 30% to 35%, a 35% to 99%, a 35% to 95%, a 35% to 90%, a 35% to 85%, a 35% to 80%, a 35% to 75%, a 35% to 70%, a 35% to 65%, a 35% to 60%, a 35% to 55%, a 35% to 50%,

a 35% to 45%, a 35% to 40%, a 40% to 99%, a 40% to 95%, a 40% to 90%, a 40% to 85%, a 40% to 80%, a 40% to 75%, a 40% to 70%, a 40% to 65%, a 40% to 60%, a 40% to 55%, a 40% to 50%, a 40% to 45%, a 45% to 99%, a 45% to 95%, a 45% to 90%, a 45% to 85%, a 45% to 80%, a 45% to 75%, a 45% to 70%, a 45% to 65%, a 45% to 60%, a 45% to 55%, a 45% to 50%, a 50% to 99%, a 50% to 95%, a 50% to 90%, a 50% to 85%, a 50% to 80%, a 50% to 75%, a 50% to 70%, a 50% to 65%, a 50% to 60%, a 50% to 55%, a 55% to 99%, a 55% to 95%, a 55% to 90%, a 55% to 85%, a 55% to 80%, a 55% to 75%, a 55% to 70%, a 55% to 65%, a 55% to 60%, a 60% to 99%, a 60% to 95%, a 60% to 90%, a 60% to 85%, a 60% to 80%, a 60% to 75%, a 60% to 70%, a 60% to 65%, a 65% to 99%, a 65% to 95%, a 65% to 90%, a 65% to 85%, a 65% to 80%, a 65% to 75%, a 65% to 70%, a 70% to 99%, a 70% to 95%, a 70% to 90%, a 70% to 85%, a 70% to 80%, a 70% to 75%, a 75% to 99%, a 75% to 95%, a 75% to 90%, a 75% to 85%, a 75% to 80%, a 80% to 99%, a 80% to 95%, a 80% to 90%, a 80% to 85%, a 85% to 99%, a 85% to 95%, a 85% to 90%, a 90% to 99%, a 90% to 95%, or a 95% to 99% decrease) in the size of a VS tumor and/or the severity of one or more symptoms of an acoustic neuroma, VS, or neurofibromatosis type II in a mammal as compared the severity of one or more symptoms of an acoustic neuroma (e.g., loss of hearing, tinnitus, vertigo, loss of quality of life etc.) and/or size of an acoustic neuroma, VS, or neurofibromatosis type II in the mammal, respectively, before administration of any rAAV particles described herein. In some embodiments, a decrease in a VEGF activity can be detected in an *in-vitro* assay.

[0253] In some embodiments, the antibody can be a humanized antibody, a chimeric antibody, or a multivalent antibody. In some embodiments, an antibody can be a scFv-Fc, a VHH domain, a VNAR domain, a (scFv)2, a minibody, or a BiTE. In some embodiments, an antibody can be a DVD-Ig, and a dual-affinity re-targeting antibody (DART), a triomab, kih IgG with a common LC, a crossmab, an ortho-Fab IgG, a 2-in-1-IgG, IgG-ScFv, scFv2-Fc, a bi-nanobody, tanden antibody, a DART-Fc, a scFv-HAS-scFv, DNL-Fab3, DAF (two-in-one or four-in-one), DutaMab, DT-IgG, knobs-in-holes common LC, knobs-in-holes assembly, charge pair antibody, Fab-arm exchange antibody, SEEDbody, Triomab, LUZ-Y, Fcab, kλ-body, orthogonal Fab, DVD-IgG, IgG(H)-scFv, scFv-(H)IgG, IgG(L)-scFv, scFv-(L)-IgG, IgG (L,H)-Fc, IgG(H)-V, V(H)-IgG, IgG(L)-V, V(L)-IgG, KIH IgG-scFab, 2scFv-IgG, IgG-2scFv, scFv4-Ig, Zybody, DVI-IgG, nanobody, nanobody-HSA, a diabody, a TandAb, scDiabody, scDiabody-CH3, Diabody-CH3, Triple Body, miniantibody, minibody, TriBi minibody, scFv-CH3 KIH, Fab-scFv, scFv-CH-CL-

scFv, F(ab')2-scFV2, scFv-KIH, Fab-scFv-Fc, tetravalent HCAb, scDiabody-Fc, diabody-Fc, tandem scFv-Fc, intrabody, dock and lock bispecific antibody, ImmTAC, HSAbody, scDiabody-HAS, tandem scFv, IgG-IgG, Cov-X-Body, and scFv1-PEG-scFv2.

[0254] Additional examples of an antibody include an Fv fragment, a Fab fragment, a F(ab')2 fragment, and a Fab' fragment. Additional examples of an antibody include an antigen-binding fragment of an IgG (e.g., an antigen-binding fragment of IgG1, IgG2, IgG3, or IgG4) (e.g., an antigen-binding fragment of a human or humanized IgG, e.g., human or humanized IgG1, IgG2, IgG3, or IgG4); an antigen-binding fragment of an IgA (e.g., an antigen-binding fragment of IgA1 or IgA2) (e.g., an antigen-binding fragment of a human or humanized IgA, e.g., a human or humanized IgA1 or IgA2); an antigen-binding fragment of an IgD (e.g., an antigen-binding fragment of a human or humanized IgD); an antigen-binding fragment of an IgE (e.g., an antigen-binding fragment of a human or humanized IgE); or an antigen-binding fragment of an IgM (e.g., an antigen-binding fragment of a human or humanized IgM).

[0255] In some embodiments, any of the antibodies described herein can bind specifically to VEGF. In some embodiments, any of the antibodies described herein can bind specifically to PDGF/VEGF.

[0256] A VHH domain is a single monomeric variable antibody domain that can be found in camelids. A VNAR domain is a single monomeric variable antibody domain that can be found in cartilaginous fish. Non-limiting aspects of VHH domains and VNAR domains are described in, e.g., Cromie et al., Curr. Top. Med. Chem. 15:2543-2557, 2016; De Genst et al., Dev. Comp. Immunol. 30:187-198, 2006; De Meyer et al., Trends Biotechnol. 32:263-270, 2014; Kijanka et al., Nanomedicine 10:161-174, 2015; Kovaleva et al., Expert. Opin. Biol. Ther. 14:1527-1539, 2014; Krah et al., Immunopharmacol. Immunotoxicol. 38:21-28, 2016; Mujic-Delic et al., Trends Pharmacol. Sci. 35:247-255, 2014; Muyldermans, J. Biotechnol. 74:277-302, 2001; Muyldermans et al., Trends Biochem. Sci. 26:230-235, 2001; Muyldermans, Ann. Rev. Biochem. 82:775-797, 2013; Rahbarizadeh et al., Immunol. Invest. 40:299-338, 2011; Van Audenhove et al., EBioMedicine 8:40-48, 2016; Van Bockstaele et al., Curr. Opin. Investig. Drugs 10:1212-1224, 2009; Vincke et al., Methods Mol. Biol. 911:15-26, 2012; and Wesolowski et al., Med. Microbiol. Immunol. 198:157-174, 2009, each of which is incorporated herein in its entirety by reference.

[0257] In some embodiments, a "Fv" fragment comprises a non-covalently-linked dimer of one heavy chain variable domain and one light chain variable domain.

[0258] In some embodiments, a "Fab" fragment comprises, the constant domain of the light chain and the first constant domain (CH1) of the heavy chain, in addition to the heavy and light chain variable domains of the Fv fragment.

[0259] In some embodiments, a "F(ab')2" fragment comprises two Fab fragments joined, near the hinge region, by disulfide bonds.

[0260] In some embodiments, a "dual variable domain immunoglobulin" or "DVD-Ig" refers to multivalent and multispecific binding proteins as described, e.g., in DiGiammarino et al., Methods Mol. Biol. 899:145-156, 2012; Jakob et al., MABs 5:358-363, 2013; and U.S. Patent Nos. 7,612,181; 8,258,268; 8,586,714; 8,716,450; 8,722,855; 8,735,546; and 8,822,645, each of which are incorporated in its entirety herein by reference.

[0261] In some embodiments, Drug Affinity Responsive Target Stability (DARTS) assays are described and performed as found in e.g., Garber, Nature Reviews Drug Discovery 13:799-801, 2014; which is incorporated in its entirety herein by reference.

[0262] In some embodiments, any of the antibodies described herein has a dissociation constant (KD) of less than 1 x 10-5 M (e.g., less than 0.5 x 10-5 M, less than 1 x 10-6 M, less than 0.5 x 10-6 M, less than 1 x 10-7 M, less than 0.5 x 10-7 M, less than 1 x 10-8 M, less than 0.5 x 10-8 M, less than 1 x 10-9 M, less than 0.5 x 10-9 M, less than 1 x 10-10 M, less than 0.5 x 10-10 M, less than 1 x 10-11 M, less than 0.5 x 10-11 M, or less than 1 x 10-12 M), e.g., as measured in phosphate buffered saline using surface plasmon resonance (SPR) for a VEGF protein (e.g., any of the VEGF proteins described herein, e.g., one or more of mature human VEGF-A, mature human VEGF-B, mature human VEGF-C, and mature human VEGF-D).

[0263] In some embodiments, any of the antibodies described herein has a KD of about 1 x 10-12 M to about 1 x 10-5 M, about 0.5 x 10-5 M, about 1 x 10-6 M, about 0.5 x 10-6 M, about 1 x 10-7 M, about 0.5 x 10-7 M, about 1 x 10-8 M, about 0.5 x 10-8 M, about 1 x 10-9 M, about 0.5 x 10-9 M, about 1 x 10-10 M, about 0.5 x 10-10 M, about 1 x 10-11 M, or about 0.5 x 10-11 M (inclusive); about 0.5 x 10-11 M to about 1 x 10-5 M, about 0.5 x 10-5 M, about 1 x 10-6 M, about 0.5 x 10-6 M, about 1 x 10-7 M, about 0.5 x 10-7 M, about 1 x 10-8 M, about 0.5 x 10-8 M, about

1 x 10-9 M, about 0.5 x 10-9 M, about 1 x 10-10 M, about 0.5 x 10-10 M, or about 1 x 10-11 M (inclusive); about 1 x 10-11 M to about 1 x 10-5 M, about 0.5 x 10-5 M, about 1 x 10-6 M, about 0.5 x 10-6 M, about 1 x 10-7 M, about 0.5 x 10-7 M, about 1 x 10-8 M, about 0.5 x 10-8 M, about 1 x 10-9 M, about 0.5 x 10-9 M, about 1 x 10-10 M, or about 0.5 x 10-10 M (inclusive); about 0.5 x 10-10 M to about 1 x 10-5 M, about 0.5 x 10-5 M, about 1 x 10-6 M, about 0.5 x 10-6 M, about 1 x 10-7 M, about 0.5 x 10-7 M, about 1 x 10-8 M, about 0.5 x 10-8 M, about 1 x 10-9 M, about 0.5 x 10-9 M, or about 1 x 10-10 M (inclusive); about 1 x 10-10 M to about 1 x 10-5 M, about 0.5 x 10-5 M, about 1 x 10-6 M, about 0.5 x 10-6 M, about 1 x 10-7 M, about 0.5 x 10-7 M, about 1 x 10-8 M, about 0.5 x 10-8 M, or about 1 x 10-9 M (inclusive); about 1 x 10-9 M to about 1 x 10-5 M, about 0.5 x 10-5 M, about 1 x 10-6 M, about 0.5 x 10-6 M, about 1 x 10-7 M, about 0.5 x 10-7 M, about 1 x 10-8 M, or about 0.5 x 10-8 M (inclusive); about 0.5 x 10-8 M to about 1 x 10-5 M, about 0.5 x 10-5 M, about 1 x 10-6 M, about 0.5 x 10-6 M, about 1 x 10-7 M, about 0.5 x 10-7 M, or about 1 x 10-8 M (inclusive); about 1 x 10-8 M to about 1 x 10-5 M, about 0.5 x 10-5 M, about 1 x 10-6 M, about 0.5 x 10-6 M, about 1 x 10-7 M, or about 0.5 x 10-7 M (inclusive); about 0.5 x 10-7 M to about 1 x 10-5 M, about 0.5 x 10-5 M, about 1 x 10-6 M, about 0.5 x 10-6 M, or about 1 x 10-7 M (inclusive); about 1 x 10-7 M to about 1 x 10-5 M, about 0.5 x 10-5 M, about 1 x 10-6 M, or about 0.5 x 10-6 M (inclusive); about 0.5 x 10-6 M to about 1 x 10-5 M, about 0.5 x 10-5 M, or about 1 x 10-6 M (inclusive); about 1 x 10-6 M to about 1 x 10-5 M or about 0.5 x 10-5 M (inclusive); or about 0.5 x 10-5 M to about 1 x 10-5 M (inclusive), e.g., as measured in phosphate buffered saline using surface plasmon resonance (SPR), for a VEGF protein (e.g., any of the VEGF proteins described herein, e.g., one or more of mature human VEGF-A, mature human VEGF-B, mature human VEGF-C, and mature human VEGF-D).

[0264] A variety of different methods known in the art can be used to determine the KD values of any of the antibodies described herein (e.g., an electrophoretic mobility shift assay, a filter binding assay, surface plasmon resonance, and a bimolecular binding kinetics assay, etc.).

[0265] In some embodiments of any of the antibodies described herein, the half-life of the antibody in a subject (e.g., a human) is decreased about 0.5-fold to about 4-fold (e.g., about 0.5-fold to about 3.5-fold, about 0.5-fold to about 3-fold, about 0.5-fold to about 2.5-fold, about 0.5-fold to about 2-fold, about 0.5-fold to about 1.5-fold, about 0.5-fold to about 1-fold, about 1-fold

to about 4-fold, about 1-fold to about 3.5-fold, about 1-fold to about 3-fold, about 1-fold to about 2.5-fold, about 1-fold to about 2-fold, about 1.5-fold to about 4-fold, about 1.5-fold to about 3.5-fold, about 1.5-fold to about 3-fold, about 1.5-fold to about 2.5-fold, about 1.5-fold to about 2-fold, about 2-fold to about 4-fold, about 2-fold to about 3.5-fold, about 2-fold to about 3-fold, about 2-fold to about 2.5-fold, about 2.5-fold to about 4-fold, about 2.5-fold to about 3.5-fold, about 2.5-fold to about 3-fold, about 3-fold to about 4-fold, about 3-fold to about 3.5-fold, or about 3.5-fold to about 4-fold) as compared to the half-life of a control antibody (e.g., any of the control antibodies or conditions described herein) in a similar subject. See, e.g., Leabman et al., MAbs. 5(6): 896-903, 2013, incorporated herein in its entirety by reference. In some embodiments, an antibody described herein has one or more amino acid substitutions in the Fc region that decrease its half-life in a mammal, and a control antibody lacks at least one (e.g., lacks all) of these one or more amino acid substitutions in the Fc region.

[0266] In some embodiments, the antibody that specifically binds to a VEGF is bevacizumab (Avastatin®). Bevacizumab (full size antibody ~150 kDa) inhibits all isoforms of VEGF-A. Bevacizumab received Food and Drug administration (FDA) approval in 2004 for colon cancer for intravenous (IV) dose of 4.0-7.5 mg/kg at 2-3 weeks (plasmatic half-life 21 days), for intravitreal (IVT) dose 1.25 mg in 0.05 mL (half-life 5.6 days). Bevacizumab has a K_D for VEGF 165 (VEGF-A) of 58 pM. *See,* e.g., WO 2017/050825; which is incorporated in its entirety herein by reference.

[0267] In some embodiments, the antibody that specifically binds to a VEGF is ranibizumab (Lucentis®). Ranibizumab (~50 kDa) inhibits all isoforms of VEGF-A. Ranibizumab received FDA approval in 2006 for ocular use for intravenous (IV) dose of 4.0-7.5 mg/kg at 2-3 weeks (plasma half-life of 0.5 days), for intravitreal (IVT) dose 0.5 mg in 0.05 mL (half-life of 3.2 days). Ranibizumab has a K_D for VEGF-A_{165} (VEGF-A Isoform 165, as represented by SEQ ID NO: 6) of 46 pM. See, e.g., WO 2014/178078; which is incorporated in its entirety herein by reference.

[0268] In some embodiments, the antibody that specifically binds to VEGF is sevacizumab (APX003/ SIM-BD0801), or a characteristic portion thereof.

[0269] In certain embodiments, an anti-VEGF protein coding sequence comprised within a composition as described herein (e.g., rAAV-antiVEGF) is selected from VEGF inhibitors approved for pathological vascularization in the retina, and in glioblastoma and other cancers,

including for example: bevacizumab (Avastin®); aflibercept (Eylea®); ziv-aflibercept (Zaltrap®); brolucizumab (Beovu®); and/or ranibizumab (Lucentis®). In addition, in some embodiments, biosimilars of many of these products in various stages of nonclinical and clinical development, and a ranibizumab biosimilar, Razumab®, may be utilized.

[0270] Ranibizumab, aflibercept, and brolucizumab are each approved for repeated intravitreal administration for wet age-related macular degeneration (AMD); ranibizumab and aflibercept are additionally approved for repeated intravitreal administration for retinal vein occlusion (RVO), diabetic macular edema (DME), and diabetic retinopathy (DR). It has been observed that targeted local delivery, and associated reduction in systemic exposure, may result in improved safety profiles (including lower rates of thromboembolic events) over intravenous treatment regimens of VEGF inhibitors. Gene therapy versions of these therapeutics are currently in clinical development for AMD and DME (Clinicaltrials.gov 2020b, 2020c, and 2020d [NCT03066258, NCT03748784, and NCT04418427], each of which is incorporated herein in its entirety by reference). Aflibercept is a recombinant fusion protein (97 kDa) consisting of portions of human VEGF receptors 1 and 2 extracellular domains fused to the Fc portion of human IgG1 that has been shown to be effective in wet AMD and DME clinical trials when delivered locally to the eye through repeated intravitreal administration. Like bevacizumab, ziv-aflibercept is also approved for intravenous infusion and carries similar risks and warnings regarding hemorrhage and wound healing (Sanofi-Aventis US 2020, which is incorporated herein in its entirety by reference).

[0271] In a study of patients with wet age-related macular degeneration (AMD), localized delivery of clinical doses of anti-VEGF proteins such as bevacizumab and ranibizumab administered via intravitreal injection were associated with significant improvements in visual acuity at 4 months; however, at this same time point VEGF plasma levels were significantly reduced only for the cohort injected with bevacizumab (Carneiro 2012, incorporated herein in its entirety by reference), In certain embodiments, methods and compositions described in the present disclosure provide solutions to the reduction of plasma VEGF levels associated with systemic delivery and/or potentially associated with acute localized delivery of bevacizumab. Certain studies have demonstrated results consistent with findings in pharmacokinetic studies, e.g., that in rabbits, bevacizumab, but not ranibizumab, is detected in the serum following intravitreal injection (Bakri 2007, incorporated herein in its entirety by reference). Review of clinical safety data from pivotal trials in AMD suggests that repeated intravitreal administration of ranibizumab for up to

two years is not associated with significant safety risks (Schmidt-Erfurth 2010, incorporated herein in its entirety by reference).

Ranibizumab

[0272] Ranibizumab is a humanized monoclonal antibody fragment (Fab) with similar clinical efficacy and an equivalent rate of thromboembolic events as aflibercept when delivered locally in the eye through intravitreal administration (Genentech 2017, incorporated herein in its entirety by reference). It is an IgG1 Fab that binds to and neutralizes VEGF. Ranibizumab is thought to bind to and inhibit biological activity all known isoforms of the human VEGF-A protein by preventing their interaction with the cognate receptors (VEGFR-1 and VEGFR-2). Ranibizumab has been marketed under the brand name Lucentis®. It is FDA approved for indications such as the treatment of macular edema after retinal vein occlusion, age-related macular degeneration (wet), and diabetic macular edema. Compared to bevacizumab, ranibizumab exhibits a more than 8-fold higher binding capacity and 66-fold higher binding affinity (Klettner 2008; Yang 2014, each of which incorporated herein in its entirety by reference). These differences yield an IC50 for VEGF-induced endothelial proliferation for ranibizumab that is 6-fold less than bevacizumab, with ranibizumab having a clinically relevant impact on VEGF activity down to 0.37 nanomolar (nM) (~17 nanograms per milliliter [ng/mL] ranibizumab; Yang 2014, incorporated herein in its entirety by reference). Importantly, ranibizumab also lacks an Fc region, allowing the molecule to avoid Fc recycling and making it significantly smaller (48 kDa) than the full-size antibody (149 kDa) (Meyer 2011, incorporated herein in its entirety by reference). In some embodiments, this smaller molecular size may be advantageous in improving diffusion to the target site, capacity to extravasate into the tumor interstitium, and/or efficiency to diffuse to target sites within the tumor (Xenaki 2017, incorporated herein in its entirety by reference). In some embodiments, compositions as described herein comprise a coding sequence of ranibizumab for development into an rAAV gene therapy product. In some embodiments, compositions as described herein (e.g., rAAV-antiVEGF) may be delivered into the cochlea, wherein intracochlear administration can result in low systemic exposure and thereby an improved safety profile compared to intravenous administration.

[0273] In certain embodiments, the present disclosure provides compositions comprising ranibizumab, an anti-VEGF protein that lacks the Fc region. An antibody Fc region as is found on

bevacizumab, is thought to allow for bevacizumab's distribution across biological barriers through Fc-receptor mediated transport, as well as bevacizumab's activation of an immune response (Kim 2009; Meyer 2011, each of which is incorporated herein in its entirety by reference). Certain studies have suggested that compared to bevacizumab, ranibizumab may exhibit a 17-fold higher binding capacity and 6-fold higher binding affinity when highly diluted (Ferrara 2006; Klettner 2008, each of which is incorporated herein in its entirety by reference), suggesting greater specific activity in lower concentrations. In some embodiments, the smaller molecular size of ranibizumab (48 kDa, compared to 149 kDa for bevacizumab) may also be advantageous in improving diffusion to the target site, capacity to extravasate into the tumor interstitium, and/or efficiency to diffuse to target sites within the tumor (Xenaki 2017, incorporated herein in its entirety by reference).

[0274] In some embodiments, an anti-VEGF protein as described herein that specifically binds to VEGF and/or antigen-presenting fragments thereof, is an antibody. In some embodiments, such an antibody comprises an immunoglobulin light chain variable domain that is or comprises a sequence that is at least 80% identical (e.g., at least 82%, at least 84%, at least 86%, at least 88%, at least 90%, at least 92%, at least 94%, at least 96%, at least 98%, or at least 99%) identical to an immunoglobulin light chain variable domain of ranibizumab, and/or comprises an immunoglobulin heavy chain variable domain that is or comprises a sequence that is at least 80% identical (e.g., at least 82%, at least 84%, at least 86%, at least 88%, at least 90%, at least 92%, at least 94%, at least 96%, at least 98%, or at least 99%) identical to an immunoglobulin heavy chain variable domain of ranibizumab.

[0275] In some embodiments, an anti-VEGF protein as described herein that specifically binds to VEGF and/or antigen-presenting fragments thereof, is an antibody. In some embodiments, such an antibody comprises, *inter alia*, an immunoglobulin light chain variable domain that is or comprises an immunoglobulin light chain variable domain of ranibizumab, and/or an immunoglobulin heavy chain variable domain that is or comprises an immunoglobulin heavy chain variable domain of ranibizumab. In some embodiments, an antibody comprises an immunoglobulin light chain variable domain that is or comprises a sequence of an immunoglobulin light chain variable domain of ranibizumab (as represented by SEQ ID NO: 20), except that it comprises one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, or fifteen amino acid substitutions, and/or comprises an immunoglobulin heavy chain variable domain that is or comprises a sequence of an immunoglobulin light chain variable domain of

ranibizumab (as represented by SEQ ID NO: 16, 17, or 18), except that it comprises one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, or fifteen amino acid substitutions. In some embodiments, an antigen-binding domain comprises three CDRs in an immunoglobulin light chain variable domain of ranibizumab, and/or three CDRs in an immunoglobulin heavy chain variable domain of ranibizumab.

[0276] Exemplary Ranibizumab Heavy Chain nucleotide sequence (SEQ ID NO: 13)

GAGGTGCAGCTGGTGGAATCTGGCGGCGGACTTGTTCAACCTGGCGGCTCTCTGAGA
CTGAGCTGTGCCGCTTCTGGCTACGACTTCACCCACTACGGCATGAACTGGGTCCGA
CAGGCCCCTGGCAAAGGCCTTGAATGGGTCGGATGGATCAACACCTACACCGGCGA
GCCAACATACGCCGCCGACTTCAAGCGGAGATTCACCTTCAGCCTGGACACCAGCA
AGAGCACCGCCTACCTGCAGATGAACAGCCTGAGAGCCGAGGACACCGCCGTGTAC
TACTGCGCCAAGTATCCCTACTACTACGGCACCAGCCACTGGTACTTTGACGTGTGG
GGACAGGGCACACTGGTCACAGTGTCTAGCGCCTCTACAAAGGGCCCCAGCGTTTTC
CCACTGGCTCCTAGCAGCAAGTCTACCAGCGGAGGAACAGCCGCTCTGGGCTGTCT
GGTCAAGGACTACTTTCCCGAGCCTGTGACCGTGTCCTGGAATTCTGGCGCTCTGAC
AAGCGGCGTGCACACCTTTCCAGCTGTGCTGCAAAGCAGCGGCCTGTACTCTCTGAG
CAGCGTCGTGACAGTGCCAAGCAGCTCTCTGGGCACCCAGACCTACATCTGCAATGT
GAACCACAAGCCTAGCAACACCAAGGTGGACAAGAAGGTGGAACCCAAGAGCTGC
GACAAGACCCACACCGGCAAG

[0277] Exemplary Ranibizumab Heavy Chain nucleotide sequence (SEQ ID NO: 14)

GAGGTGCAGCTGGTGGAATCTGGCGGCGGACTTGTTCAACCTGGCGGCTCTCTGAGA
CTGAGCTGTGCCGCTTCTGGCTACGACTTCACCCACTACGGCATGAACTGGGTCCGA
CAGGCCCCTGGCAAAGGCCTTGAATGGGTCGGATGGATCAACACCTACACCGGCGA
GCCAACATACGCCGCCGACTTCAAGCGGAGATTCACCTTCAGCCTGGACACCAGCA
AGAGCACCGCCTACCTGCAGATGAACAGCCTGAGAGCCGAGGACACCGCCGTGTAC
TACTGCGCCAAGTATCCCTACTACTACGGCACCAGCCACTGGTACTTTGACGTGTGG
GGACAGGGCACACTGGTCACAGTGTCTAGCGCCTCTACAAAGGGCCCCAGCGTTTTC
CCACTGGCTCCTAGCAGCAAGTCTACCAGCGGAGGAACAGCCGCTCTGGGCTGTCT
GGTCAAGGACTACTTTCCCGAGCCTGTGACCGTGTCCTGGAATTCTGGCGCTCTGAC
AAGCGGCGTGCACACCTTTCCAGCTGTGCTGCAAAGCAGCGGCCTGTACTCTCTGAG

CAGCGTCGTGACAGTGCCAAGCAGCTCTCTGGGCACCCAGACCTACATCTGCAATGT
GAACCACAAGCCTAGCAACACCAAGGTGGACAAGAAGGTGGAACCCAAGAGCTGC
GACAAGACCCACAAG

[0278] Exemplary Ranibizumab Heavy Chain nucleotide sequence (SEQ ID NO: 15)

GAGGTGCAGCTGGTGGAATCTGGCGGCGGACTTGTTCAACCTGGCGGCTCTCTGAGA
CTGAGCTGTGCCGCTTCTGGCTACGACTTCACCCACTACGGCATGAACTGGGTCCGA
CAGGCCCCTGGCAAAGGCCTTGAATGGGTCGGATGGATCAACACCTACACCGGCGA
GCCAACATACGCCGCCGACTTCAAGCGGAGATTCACCTTCAGCCTGGACACCAGCA
AGAGCACCGCCTACCTGCAGATGAACAGCCTGAGAGCCGAGGACACCGCCGTGTAC
TACTGCGCCAAGTATCCCTACTACTACGGCACCAGCCACTGGTACTTTGACGTGTGG
GGACAGGGCACACTGGTCACAGTGTCTAGCGCCTCTACAAAGGGCCCCAGCGTTTTC
CCACTGGCTCCTAGCAGCAAGTCTACCAGCGGAGGAACAGCCGCTCTGGGCTGTCT
GGTCAAGGACTACTTTCCCGAGCCTGTGACCGTGTCCTGGAATTCTGGCGCTCTGAC
AAGCGGCGTGCACACCTTTCCAGCTGTGCTGCAAAGCAGCGGCCTGTACTCTCTGAG
CAGCGTCGTGACAGTGCCAAGCAGCTCTCTGGGCACCCAGACCTACATCTGCAATGT
GAACCACAAGCCTAGCAACACCAAGGTGGACAAGAAGGTGGAACCCAAGAGC

[0279] Exemplary Ranibizumab Heavy Chain amino acid sequence (SEQ ID NO: 16)

EVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQAPGKGLEWVGWINTYTGE
PTYAADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPYYYGTSHWYFDVWG
QGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV
HTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTG
K

[0280] Exemplary Ranibizumab Heavy Chain amino acid sequence (SEQ ID NO: 17)

EVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQAPGKGLEWVGWINTYTGE
PTYAADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPYYYGTSHWYFDVWG
QGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV
HTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHL

[0281] Exemplary Ranibizumab Heavy Chain amino acid sequence (SEQ ID NO: 18)

EVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQAPGKGLEWVGWINTYTGE
PTYAADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPYYYGTSHWYFDVWG
QGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV
HTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKS

[0282] Exemplary Ranibizumab Light Chain nucleotide sequence (SEQ ID NO: 19)

GACATCCAGCTGACACAGAGCCCCAGCAGCCTGTCTGCCTCTGTGGGAGACAGAGT
GACCATCACCTGTAGCGCCAGCCAGGACATCTCCAACTACCTGAACTGGTATCAGCA
AAAGCCCGGCAAGGCCCCTAAGGTGCTGATCTACTTCACAAGCAGCCTGCACTCCG
GCGTGCCCAGCAGATTTTCTGGCTCTGGCAGCGGCACCGACTTCACCCTGACCATAT
CTAGCCTGCAGCCTGAGGACTTCGCCACCTACTACTGCCAGCAGTACAGCACCGTGC
CTTGGACATTTGGCCAGGGCACAAAGGTGGAAATCAAGCGGACTGTGGCCGCTCCT
AGCGTGTTCATCTTTCCACCTAGCGACGAGCAGCTGAAGTCTGGCACAGCCTCTGTC
GTGTGCCTGCTGAACAACTTCTACCCCAGAGAAGCCAAGGTGCAGTGGAAAGTGGA
CAATGCCCTGCAGAGCGGCAACAGCCAAGAGAGCGTGACAGAGCAGGACTCCAAG
GATAGCACCTATAGCCTGAGCAGCACCCTGACACTGAGCAAGGCCGACTACGAGAA
GCACAAAGTGTACGCCTGCGAAGTGACCCACCAGGGCCTTTCTAGCCCTGTGACCA
AGAGCTTCAACCGGGGCGAATGT

[0283] Exemplary Ranibizumab Light Chain amino acid sequence (SEQ ID NO: 20)

DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPSR
FSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTVAAPSVFIFPPSD
EQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTL
SKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

[0284] Exemplary Cleavable polypeptide comprising Heavy and Light Chain Ranibizumab, nucleotide sequence (SEQ ID NO: 103)

ATGTACCGGATGCAGCTGCTGAGCTGTATCGCCCTGTCTCTGGCCCTGGTCACCAAT
TCTGAGGTGCAGCTGGTGGAATCTGGCGGCGGACTTGTTCAACCTGGCGGCTCTCTG
AGACTGAGCTGTGCCGCTTCTGGCTACGACTTCACCCACTACGGCATGAACTGGGTC
CGACAGGCCCCTGGCAAAGGCCTTGAATGGGTCGGATGGATCAACACCTACACCGG
CGAGCCAACATACGCCGCCGACTTCAAGCGGAGATTCACCTTCAGCCTGGACACCA

GCAAGAGCACCGCCTACCTGCAGATGAACAGCCTGAGAGCCGAGGACACCGCCGTG
TACTACTGCGCCAAGTATCCCTACTACTACGGCACCAGCCACTGGTACTTTGACGTG
TGGGGACAGGGCACACTGGTCACAGTGTCTAGCGCCTCTACAAAGGGCCCCAGCGT
TTTCCCACTGGCTCCTAGCAGCAAGTCTACCAGCGGAGGAACAGCCGCTCTGGGCTG
TCTGGTCAAGGACTACTTTCCCGAGCCTGTGACCGTGTCCTGGAATTCTGGCGCTCT
GACAAGCGGCGTGCACACCTTTCCAGCTGTGCTGCAAAGCAGCGGCCTGTACTCTCT
GAGCAGCGTCGTGACAGTGCCAAGCAGCTCTCTGGGCACCCAGACCTACATCTGCA
ATGTGAACCACAAGCCTAGCAACACCAAGGTGGACAAGAAGGTGGAACCCAAGAG
CTGCGACAAGACCCACACCGGCAAGCGGAAGAGAAGAGGCTCTGGCGAAGGCAGA
GGCAGCCTGCTTACATGTGGCGACGTGGAAGAGAACCCCGGACCTATGTATAGAAT
GCAGCTCCTGTCCTGCATTGCCCTGAGCCTGGCTCTCGTGACCAACAGCGACATCCA
GCTGACACAGAGCCCCAGCAGCCTGTCTGCCTCTGTGGGAGACAGAGTGACCATCA
CCTGTAGCGCCAGCCAGGACATCTCCAACTACCTGAACTGGTATCAGCAAAAGCCC
GGCAAGGCCCCTAAGGTGCTGATCTACTTCACAAGCAGCCTGCACTCCGGCGTGCCC
AGCAGATTTTCTGGCTCTGGCAGCGGCACCGACTTCACCCTGACCATATCTAGCCTG
CAGCCTGAGGACTTCGCCACCTACTACTGCCAGCAGTACAGCACCGTGCCTTGGACA
TTTGGCCAGGGCACAAAGGTGGAAATCAAGCGGACTGTGGCCGCTCCTAGCGTGTT
CATCTTTCCACCTAGCGACGAGCAGCTGAAGTCTGGCACAGCCTCTGTCGTGTGCCT
GCTGAACAACTTCTACCCCAGAGAAGCCAAGGTGCAGTGGAAAGTGGACAATGCCC
TGCAGAGCGGCAACAGCCAAGAGAGCGTGACAGAGCAGGACTCCAAGGATAGCAC
CTATAGCCTGAGCAGCACCCTGACACTGAGCAAGGCCGACTACGAGAAGCACAAAG
TGTACGCCTGCGAAGTGACCCACCAGGGCCTTTCTAGCCCTGTGACCAAGAGCTTCA
ACCGGGGCGAATGT

[0285] Exemplary Cleavable polypeptide comprising Heavy and Light Chain Ranibizumab (SEQ ID NO: 21)

MYRMQLLSCIALSLALVTNSEVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWV
RQAPGKGLEWVGWINTYTGEPTYAADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYY
CAKYPYYYGTSHWYFDVWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVK
DYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPS
NTKVDKKVEPKSCDKTHTGKRKRRGSGEGRGSLLTCGDVEENPGPMYRMQLLSCIALS
LALVTNSDIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSL

HSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTVAAPS
VFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTY
SLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

Bevacizumab

[0286] Bevacizumab is a humanized monoclonal full-length antibody against VEGF that is approved for intravenous infusion for the treatment of glioblastoma, colorectal, lung, kidney, cervical, and ovarian cancers. However, the main drawbacks to bevacizumab therapy are the need for continued regular intravenous infusions and the side effects associated with high doses in systemic circulation, which include hypertension, proteinuria, elevated liver enzymes, arterial thromboembolic events (ATE), venous thromboembolic events, hemorrhage, and surgery and wound healing complications. Currently, bevacizumab is the only pharmacologic agent for which preliminary clinical evidence of effectiveness in VS patients has been demonstrated.

[0287] In some embodiments, an anti-VEGF protein as described herein that specifically binds to VEGF and/or antigen-presenting fragments thereof, is an antibody. In some embodiments, such an antibody comprises an immunoglobulin light chain variable domain that is or comprises a sequence that is at least 80% identical (e.g., at least 82%, at least 84%, at least 86%, at least 88%, at least 90%, at least 92%, at least 94%, at least 96%, at least 98%, or at least 99%) identical to an immunoglobulin light chain variable domain of Bevacizumab, and/or comprises an immunoglobulin heavy chain variable domain that is or comprises a sequence that is at least 80% identical (e.g., at least 82%, at least 84%, at least 86%, at least 88%, at least 90%, at least 92%, at least 94%, at least 96%, at least 98%, or at least 99%) identical to an immunoglobulin heavy chain variable domain of Bevacizumab.

[0288] In some embodiments, an anti-VEGF protein as described herein that specifically binds to VEGF and/or antigen-presenting fragments thereof, is an antibody. In some embodiments, such an antibody comprises an immunoglobulin light chain variable domain that is or comprises an immunoglobulin light chain variable domain of Bevacizumab, and/or an immunoglobulin heavy chain variable domain that is or comprises an immunoglobulin heavy chain variable domain of Bevacizumab. In some embodiments, an antibody comprises an immunoglobulin light chain variable domain that is or comprises the sequence of an immunoglobulin light chain variable domain of Bevacizumab, except that it comprises one, two, three, four, five, six, seven, eight, nine,

ten, eleven, twelve, thirteen, fourteen, or fifteen amino acid substitutions, and/or comprises an immunoglobulin heavy chain variable domain that is or comprises the sequence of an immunoglobulin heavy chain variable domain of Bevacizumab, except that it comprises one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, or fifteen amino acid substitutions.

[0289] In some embodiments, an anti-VEGF protein as described herein that specifically binds to VEGF and/or antigen-presenting fragments thereof, is an antibody. In some embodiments, such an antibody comprises an immunoglobulin light chain (e.g., comprising an immunoglobulin light chain variable domain and an immunoglobulin light chain constant domain) that is or comprises an immunoglobulin light chain constant domain of Bevacizumab, and/or an immunoglobulin heavy chain (e.g., comprising an immunoglobulin heavy chain variable domain and an immunoglobulin heavy chain constant domain) that is or comprises an immunoglobulin heavy chain constant domain of Bevacizumab. In some embodiments, an antibody comprises an immunoglobulin light chain constant domain that is or comprises a sequence of an immunoglobulin light chain constant domain of Bevacizumab, except that it comprises one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, or fifteen amino acid substitutions, and/or comprises an immunoglobulin heavy chain constant domain that is or comprises a sequence of an immunoglobulin heavy chain constant domain of Bevacizumab, except that it comprises one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, or fifteen amino acid substitutions.

[0290] In some embodiments a first antigen-binding domain comprises three CDRs in an immunoglobulin light chain variable domain of Bevacizumab, and/or three CDRs in an immunoglobulin heavy chain variable domain of Bevacizumab. In some embodiments a second antigen-binding domain comprises three CDRs in an immunoglobulin light chain variable domain of Bevacizumab, and/or three CDRs in an immunoglobulin heavy chain variable domain of Bevacizumab.

[0291] Exemplary Bevacizumab nucleotide sequence (SEQ ID NO: 22)

ATGTACCGGATGCAGCTGCTGAGCTGTATCGCCCTGTCTCTGGCCCTGGTCACCAAT
TCTGAGGTGCAGCTGGTGGAATCTGGCGGCGGACTTGTTCAACCTGGCGGCTCTCTG
AGACTGAGCTGTGCCGCTTCTGGCTACACCTTCACCAACTACGGCATGAACTGGGTC

CGACAGGCCCCTGGCAAAGGCCTTGAATGGGTCGGATGGATCAACACCTACACCGG
CGAGCCAACATACGCCGCCGACTTCAAGCGGAGATTCACCTTCAGCCTGGACACCA
GCAAGAGCACCGCCTACCTGCAGATGAACAGCCTGAGAGCCGAGGACACCGCCGTG
TACTACTGCGCCAAGTATCCCCACTACTACGGCAGCAGCCACTGGTACTTTGACGTG
TGGGGACAGGGCACACTGGTCACAGTGTCTAGCGCCTCTACAAAGGGCCCCAGCGT
TTTCCCACTGGCTCCTAGCAGCAAGTCTACCAGCGGAGGAACAGCCGCTCTGGGCTG
TCTGGTCAAGGACTACTTTCCCGAGCCTGTGACCGTGTCCTGGAATTCTGGCGCTCT
GACAAGCGGCGTGCACACCTTTCCAGCTGTGCTGCAAAGCAGCGGCCTGTACTCTCT
GAGCAGCGTCGTGACAGTGCCAAGCAGCTCTCTGGGCACCCAGACCTACATCTGCA
ATGTGAACCACAAGCCTAGCAACACCAAGGTGGACAAGAAGGTGGAACCCAAGAG
CTGCGACAAGACCCACACCTGTCCTCCATGTCCTGCTCCAGAACTGCTCGGCGGACC
TTCCGTGTTCCTGTTTCCTCCAAAGCCTAAGGACACCCTGATGATCAGCAGAACCCC
TGAAGTGACCTGCGTGGTGGTGGATGTGTCCCACGAGGATCCCGAAGTGAAGTTCA
ATTGGTACGTGGACGGCGTGGAAGTGCACAACGCCAAGACCAAGCCTAGAGAGGAA
CAGTACAACAGCACCTACAGAGTGGTGTCCGTGCTGACCGTGCTGCACCAGGATTG
GCTGAACGGCAAAGAGTACAAGTGCAAGGTGTCCAACAAGGCCCTGCCTGCTCCTA
TCGAGAAAACCATCAGCAAGGCCAAGGGCCAGCCTAGGGAACCCCAGGTTTACACA
CTGCCTCCAAGCCGGGAAGAGATGACCAAGAACCAGGTGTCCCTGACCTGCCTCGT
GAAGGGCTTCTACCCTTCCGATATCGCCGTGGAATGGGAGAGCAATGGCCAGCCAG
AGAACAACTACAAGACAACCCCTCCTGTGCTGGACAGCGACGGCTCATTCTTCCTGT
ACAGCAAGCTGACAGTGGACAAGTCCAGATGGCAGCAGGGCAACGTGTTCAGCTGC
AGCGTGATGCACGAGGCCCTGCACAACCACTACACCCAGAAGTCTCTGAGCCTGTCT
CCTGGCAAGCGGAAGAGAAGAGGCTCTGGCGAAGGCAGAGGCAGCCTGCTTACATG
TGGCGACGTGGAAGAGAACCCCGGACCTATGTATAGAATGCAGCTCCTGTCCTGCAT
TGCCCTGAGCCTGGCTCTCGTGACCAACAGCGACATCCAGATGACACAGAGCCCCA
GCAGCCTGTCTGCCTCTGTGGGAGACAGAGTGACCATCACCTGTAGCGCCAGCCAG
GACATCTCCAACTACCTGAACTGGTATCAGCAAAAGCCCGGCAAGGCCCCTAAGGT
GCTGATCTACTTCACAAGCAGCCTGCACTCCGGCGTGCCCAGCAGATTTTCTGGCTC
TGGCAGCGGCACCGACTTCACCCTGACCATATCTAGCCTGCAGCCTGAGGACTTCGC
CACCTACTACTGCCAGCAGTACAGCACCGTGCCTTGGACATTTGGCCAGGGCACAA
AGGTGGAAATCAAGCGGACTGTGGCCGCTCCTAGCGTGTTCATCTTTCCACCTAGCG

ACGAGCAGCTGAAGTCTGGCACAGCCTCTGTCGTGTGCCTGCTGAACAACTTCTACC
CCAGAGAAGCCAAGGTGCAGTGGAAAGTGGACAATGCCCTGCAGAGCGGCAACAG
CCAAGAGAGCGTGACAGAGCAGGACTCCAAGGATAGCACCTATAGCCTGAGCAGCA
CCCTGACACTGAGCAAGGCCGACTACGAGAAGCACAAAGTGTACGCCTGCGAAGTG
ACCCACCAGGGCCTTTCTAGCCCTGTGACCAAGAGCTTCAACCGGGGCGAATGT

[0292] Exemplary Cleavable Polypeptide Comprising Heavy and Light Chain Bevacizumab Amino Acid Sequence (SEQ ID NO: 23)

MYRMQLLSCIALSLALVTNSEVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVR
QAPGKGLEWVGWINTYTGEPTYAADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYC
AKYPHYYGSSHWYFDVWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKD
YFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSN
TKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSH
EDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVS
NKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESN
GQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLS
LSPGKRKRRGSGEGRGSLLTCGDVEENPGPMYRMQLLSCIALSLALVTNSDIQMTQSPSS
LSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPSRFSGSGSGTD
FTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTAS
VVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKH
KVYACEVTHQGLSSPVTKSFNRGEC

[0293] Exemplary Bevacizumab Heavy Chain Nucleotide Sequence (SEQ ID NO: 108)

ATGTACCGGATGCAGCTGCTGAGCTGTATCGCCCTGTCTCTGGCCCTGGTCACCAAT
TCTGAGGTGCAGCTGGTGGAATCTGGCGGCGGACTTGTTCAACCTGGCGGCTCTCTG
AGACTGAGCTGTGCCGCTTCTGGCTACACCTTCACCAACTACGGCATGAACTGGGTC
CGACAGGCCCCTGGCAAAGGCCTTGAATGGGTCGGATGGATCAACACCTACACCGG
CGAGCCAACATACGCCGCCGACTTCAAGCGGAGATTCACCTTCAGCCTGGACACCA
GCAAGAGCACCGCCTACCTGCAGATGAACAGCCTGAGAGCCGAGGACACCGCCGTG
TACTACTGCGCCAAGTATCCCCACTACTACGGCAGCAGCCACTGGTACTTTGACGTG
TGGGGACAGGGCACACTGGTCACAGTGTCTAGCGCCTCTACAAAGGGCCCCAGCGT
TTTCCCACTGGCTCCTAGCAGCAAGTCTACCAGCGGAGGAACAGCCGCTCTGGGCTG

TCTGGTCAAGGACTACTTTCCCGAGCCTGTGACCGTGTCCTGGAATTCTGGCGCTCT
GACAAGCGGCGTGCACACCTTTCCAGCTGTGCTGCAAAGCAGCGGCCTGTACTCTCT
GAGCAGCGTCGTGACAGTGCCAAGCAGCTCTCTGGGCACCCAGACCTACATCTGCA
ATGTGAACCACAAGCCTAGCAACACCAAGGTGGACAAGAAGGTGGAACCCAAGAG
CTGCGACAAGACCCACACCTGTCCTCCATGTCCTGCTCCAGAACTGCTCGGCGGACC
TTCCGTGTTCCTGTTTCCTCCAAAGCCTAAGGACACCCTGATGATCAGCAGAACCCC
TGAAGTGACCTGCGTGGTGGTGGATGTGTCCCACGAGGATCCCGAAGTGAAGTTCA
ATTGGTACGTGGACGGCGTGGAAGTGCACAACGCCAAGACCAAGCCTAGAGAGGAA
CAGTACAACAGCACCTACAGAGTGGTGTCCGTGCTGACCGTGCTGCACCAGGATTG
GCTGAACGGCAAAGAGTACAAGTGCAAGGTGTCCAACAAGGCCCTGCCTGCTCCTA
TCGAGAAAACCATCAGCAAGGCCAAGGGCCAGCCTAGGGAACCCCAGGTTTACACA
CTGCCTCCAAGCCGGGAAGAGATGACCAAGAACCAGGTGTCCCTGACCTGCCTCGT
GAAGGGCTTCTACCCTTCCGATATCGCCGTGGAATGGGAGAGCAATGGCCAGCCAG
AGAACAACTACAAGACAACCCCTCCTGTGCTGGACAGCGACGGCTCATTCTTCCTGT
ACAGCAAGCTGACAGTGGACAAGTCCAGATGGCAGCAGGGCAACGTGTTCAGCTGC
AGCGTGATGCACGAGGCCCTGCACAACCACTACACCCAGAAGTCTCTGAGCCTGTCT
CCTGGCAAG

[0294] Exemplary Bevacizumab Heavy Chain amino acid sequence (SEQ ID NO: 24)

EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYTGE
PTYAADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWYFDVWG
QGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV
HTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCP
PCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN
AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPR
EPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGS
FFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

[0295] Exemplary Bevacizumab Light Chain Nucleotide Sequence (SEQ ID NO: 109)

GACATCCAGATGACACAGAGCCCCAGCAGCCTGTCTGCCTCTGTGGGAGACAGAGT
GACCATCACCTGTAGCGCCAGCCAGGACATCTCCAACTACCTGAACTGGTATCAGCA
AAAGCCCGGCAAGGCCCCTAAGGTGCTGATCTACTTCACAAGCAGCCTGCACTCCG

GCGTGCCCAGCAGATTTTCTGGCTCTGGCAGCGGCACCGACTTCACCCTGACCATAT
CTAGCCTGCAGCCTGAGGACTTCGCCACCTACTACTGCCAGCAGTACAGCACCGTGC
CTTGGACATTTGGCCAGGGCACAAAGGTGGAAATCAAGCGGACTGTGGCCGCTCCT
AGCGTGTTCATCTTTCCACCTAGCGACGAGCAGCTGAAGTCTGGCACAGCCTCTGTC
GTGTGCCTGCTGAACAACTTCTACCCCAGAGAAGCCAAGGTGCAGTGGAAAGTGGA
CAATGCCCTGCAGAGCGGCAACAGCCAAGAGAGCGTGACAGAGCAGGACTCCAAG
GATAGCACCTATAGCCTGAGCAGCACCCTGACACTGAGCAAGGCCGACTACGAGAA
GCACAAAGTGTACGCCTGCGAAGTGACCCACCAGGGCCTTTCTAGCCCTGTGACCA
AGAGCTTCAACCGGGGCGAATGT

[0296] Exemplary Bevacizumab Light Chain amino acid sequence (SEQ ID NO: 25)

DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS
RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTVAAPSVFIFPPS
DEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTL
TLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

VEGF TRAP

[0297] A soluble VEGF receptor (also referred to herein as a VEGF TRAP) is a polypeptide that comprises a portion of an extracellular region of one or more (e.g., two or three) mammalian VEGF receptor(s) (e.g., one or more of VEGFR-1, VEGFR-2, and VEGFR-3) operably linked to a signal peptide (e.g., any of the exemplary signal peptides described herein), where the soluble VEGF receptor is capable of specifically binding to one or more mammalian VEGF protein(s) (e.g., one or more (e.g., two, three, or four) of VEGF-A, VEGF-B, VEGF-C, and VEGF-D, e.g., one or more (e.g., two, three, or four) of human wildtype VEGF-A, human wildtype VEGF-B, human wildtype VEGF-C, and human wildtype VEGF-D).

[0298] In some examples, a soluble VEGF receptor comprises a portion (e.g., about 10 amino acids to about 732 amino acids, about 10 amino acids to about 700 amino acids, about 10 amino acids to about 650 amino acids, about 10 amino acids to about 600 amino acids, about 10 amino acids to about 550 amino acids, about 10 amino acids to about 500 amino acids, about 10 amino acids to about 450 amino acids, about 10 amino acids to about 400 amino acids, about 10 amino acids to about 350 amino acids, about 10 amino acids to about 300 amino acids, about 10 amino acids to about 250 amino acids, about 10 amino acids to about 200 amino acids, about 10 amino

acids to about 150 amino acids, about 10 amino acids to about 100 amino acids, about 10 amino acids to about 50 amino acids, about 50 amino acids to about 732 amino acids, about 50 amino acids to about 700 amino acids, about 50 amino acids to about 650 amino acids, about 50 amino acids to about 600 amino acids, about 50 amino acids to about 550 amino acids, about 50 amino acids to about 500 amino acids, about 50 amino acids to about 450 amino acids, about 50 amino acids to about 400 amino acids, about 50 amino acids to about 350 amino acids, about 50 amino acids to about 300 amino acids, about 50 amino acids to about 250 amino acids, about 50 amino acids to about 200 amino acids, about 50 amino acids to about 150 amino acids, about 50 amino acids to about 100 amino acids, about 100 amino acids to about 732 amino acids, about 100 amino acids to about 700 amino acids, about 100 amino acids to about 650 amino acids, about 100 amino acids to about 600 amino acids, about 100 amino acids to about 550 amino acids, about 100 amino acids to about 500 amino acids, about 100 amino acids to about 450 amino acids, about 100 amino acids to about 400 amino acids, about 100 amino acids to about 350 amino acids, about 100 amino acids to about 300 amino acids, about 100 amino acids to about 250 amino acids, about 100 amino acids to about 200 amino acids, about 100 amino acids to about 150 amino acids, about 150 amino acids to about 732 amino acids, about 150 amino acids to about 700 amino acids, about 150 amino acids to about 650 amino acids, about 150 amino acids to about 600 amino acids, about 150 amino acids to about 550 amino acids, about 150 amino acids to about 500 amino acids, about 150 amino acids to about 450 amino acids, about 150 amino acids to about 400 amino acids, about 150 amino acids to about 350 amino acids, about 150 amino acids to about 300 amino acids, about 150 amino acids to about 250 amino acids, about 150 amino acids to about 200 amino acids, about 200 amino acids to about 732 amino acids, about 200 amino acids to about 700 amino acids, about 200 amino acids to about 650 amino acids, about 200 amino acids to about 600 amino acids, about 200 amino acids to about 550 amino acids, about 200 amino acids to about 500 amino acids, about 200 amino acids to about 450 amino acids, about 200 amino acids to about 400 amino acids, about 200 amino acids to about 350 amino acids, about 200 amino acids to about 300 amino acids, about 200 amino acids to about 250 amino acids, about 250 amino acids to about 732 amino acids, about 250 amino acids to about 700 amino acids, about 250 amino acids to about 650 amino acids, about 250 amino acids to about 600 amino acids, about 250 amino acids to about 550 amino acids, about 250 amino acids to about 500 amino acids, about 250 amino acids to about 450 amino acids, about 250 amino acids to about 400 amino acids, about 250 amino acids to about 350 amino acids, about 250 amino

acids to about 300 amino acids, about 300 amino acids to about 732 amino acids, about 300 amino acids to about 700 amino acids, about 300 amino acids to about 650 amino acids, about 300 amino acids to about 600 amino acids, about 300 amino acids to about 550 amino acids, about 300 amino acids to about 500 amino acids, about 300 amino acids to about 450 amino acids, about 300 amino acids to about 400 amino acids, about 300 amino acids to about 350 amino acids, about 350 amino acids to about 732 amino acids, about 350 amino acids to about 700 amino acids, about 350 amino acids to about 650 amino acids, about 350 amino acids to about 600 amino acids, about 350 amino acids to about 550 amino acids, about 350 amino acids to about 500 amino acids, about 350 amino acids to about 450 amino acids, about 350 amino acids to about 400 amino acids, about 400 amino acids to about 732 amino acids, about 400 amino acids to about 700 amino acids, about 400 amino acids to about 650 amino acids, about 400 amino acids to about 600 amino acids, about 400 amino acids to about 550 amino acids, about 400 amino acids to about 500 amino acids, about 450 amino acids to about 732 amino acids, about 450 amino acids to about 700 amino acids, about 450 amino acids to about 650 amino acids, about 450 amino acids to about 600 amino acids, about 450 amino acids to about 550 amino acids, about 450 amino acids to about 500 amino acids, about 500 amino acids to about 732 amino acids, about 500 amino acids to about 700 amino acids, about 500 amino acids to about 650 amino acids, about 500 amino acids to about 600 amino acids, about 500 amino acids to about 550 amino acids, about 550 amino acids to about 732 amino acids, about 550 amino acids to about 700 amino acids, about 550 amino acids to about 650 amino acids, about 550 amino acids to about 600 amino acids, about 600 amino acids to about 732 amino acids, about 600 amino acids to about 700 amino acids, about 600 amino acids to about 650 amino acids, about 650 amino acids to about 732 amino acids, about 650 amino acids to about 700 amino acids, or about 700 amino acids to about 732 amino acids) of an extracellular region of VEGFR-1 (e.g., a contiguous sequence from wildtype human VEGFR-1 (e.g., a contiguous sequence including one or more (e.g., one, two, three, four, five, six, or seven) immunoglobulin-like domains in the extracellular region from wildtype human VEGFR-1 (e.g., SEQ ID NO: 27, 29, 31, or 33) or a sequence that is at least 80% (e.g., at least 82%, at least 84%, at least 86%, at least 88%, at least 90%, at least 92%, at least 94%, at least 96%, at least 98%, or at least 99%) identical to a contiguous sequence from wildtype human VEGFR-1, e.g., a sequence that is at least 80% (e.g., least 82%, at least 84%, at least 86%, at least 88%, at least 90%, least

92%, at least 94%, at least 96%, at least 98%, or at least 99%) identical to a contiguous sequence in SEQ ID NO: 27, 29, 31, or 33.

[0299] In some examples, a soluble VEGF receptor comprises a portion (e.g., about 20 amino acids to about 745 amino acids, or any of the subranges of this range described herein) of an extracellular region of VEGFR-2 (e.g., a contiguous sequence from wildtype human VEGFR-2 (e.g., a contiguous sequence including one or more (e.g., one, two, three, four, five, six, or seven) immunoglobulin-like domains in the extracellular region from wildtype human VEGFR-2 (e.g., SEQ ID NO: 35) or a sequence that is at least 80% (e.g., at least 82%, at least 84%, at least 86%, at least 88%, at least 90%, at least 92%, at least 94%, at least 96%, at least 98%, or at least 99%) identical to a contiguous sequence from wildtype human VEGFR-2, e.g., a sequence that is at least 80% (e.g., at least 82%, at least 84%, at least 86%, at least 88%, at least 90%, at least 92%, at least 94%, at least 96%, at least 98%, or at least 99%) identical to a contiguous sequence in SEQ ID NO: 35).

[0300] In some examples, a soluble VEGF receptor comprises a portion of an extracellular region of VEGFR-1 (e.g., any of the portions of an extracellular region of VEGFR-1 described herein) and a portion of an extracellular region of VEGFR-2 (e.g., any of the portions of an extracellular region of VEGFR-2 described herein). For example, a soluble VEGF receptor can include one or more (e.g., two, three, four, five, six, or seven) immunoglobulin-like domains in the extracellular region from wildtype human VEGFR-1 and one or more (e.g., two, three, four, five, six, or seven) immunoglobulin-like domains in the extracellular region from wildtype human VEGFR-2 (e.g., aflibercept).

[0301] In some examples, a soluble VEGF receptor comprises a portion (e.g., about 20 amino acids to about 751 amino acids, or any of the subranges of this range described herein) of an extracellular region of VEGFR-3 (e.g., a contiguous sequence from wildtype human VEGFR-3 (e.g., a contiguous sequence including one or more (e.g., one, two, three, four, five, six, or seven) immunoglobulin-like domains in the extracellular region from wildtype human VEGFR-3 (e.g., SEQ ID NO: 37, 39, or 41) or a sequence that is at least 80% (e.g., at least 82%, at least 84%, at least 86%, at least 88%, at least 90%, at least 92%, at least 94%, at least 96%, at least 98%, or at least 99%) identical to a contiguous sequence from wildtype human VEGFR-3, e.g., a sequence that is at least 80% (e.g., at least 82%, at least 84%, at least 86%, at least 88%, at least 90%, at

least 92%, at least 94%, at least 96%, at least 98%, or at least 99%) identical to a contiguous sequence in SEQ ID NO: 37, 39, or 41).

[0302] Non-limiting examples of extracellular regions of different mammalian VEGFR-1, different mammalian VEGFR-2, and different mammalian VEGFR-3 are described herein. Non-limiting examples of protein and nucleotide sequences encoding a wildtype VEGF receptor protein are shown below. As one skilled in the art can appreciate, a substitution in an amino acid that is conserved between species is more likely to result in a change in the function of a protein, while a substitution in an amino acid position that is not conserved between species is less likely to have an effect on the function of a protein.

[0303] The VEGFR-1 gene found at human chromosomal position 13q12.3 encodes a 33 exon containing member of the vascular endothelial growth factor receptor (VEGFR) family. VEGFR family members are receptor tyrosine kinases (RTKs) which contain an extracellular ligand-binding region with seven immunoglobulin (Ig)-like domains, a transmembrane segment, and a tyrosine kinase (TK) domain within the cytoplasmic domain. This protein binds to VEGF-A, VEGF-B and placental growth factor and plays an important role in angiogenesis and vasculogenesis. Expression of this receptor is found in vascular endothelial cells, placental trophoblast cells and peripheral blood monocytes. Multiple transcript variants encoding different isoforms have been found for this gene. Isoforms include a full-length transmembrane receptor isoform and shortened, soluble isoforms.

[0304] Exemplary Human VEGFR-1 isoform 1 cDNA sequence (SEQ ID NO: 26)

ATCGAGGTCCGCGGGAGGCTCGGAGCGCGCCAGGCGGACACTCCTCTCGGCTCCTC
CCCGGCAGCGGCGGCGGCTCGGAGCGGGCTCCGGGGCTCGGGTGCAGCGGCCAGCG
GGCGCCTGGCGGCGAGGATTACCCGGGGAAGTGGTTGTCTCCTGGCTGGAGCCGCG
AGACGGGCGCTCAGGGCGCGGGGCCGGCGGCGGCGAACGAGAGGACGGACTCTGG
CGGCCGGGTCGTTGGCCGCGGGGAGCGCGGGCACCGGGCGAGCAGGCCGCGTCGCG
CTCACCATGGTCAGCTACTGGGACACCGGGGTCCTGCTGTGCGCGCTGCTCAGCTGT
CTGCTTCTCACAGGATCTAGTTCAGGTTCAAAATTAAAAGATCCTGAACTGAGTTTA
AAAGGCACCCAGCACATCATGCAAGCAGGCCAGACACTGCATCTCCAATGCAGGGG
GGAAGCAGCCCATAAATGGTCTTTGCCTGAAATGGTGAGTAAGGAAAGCGAAAGGC
TGAGCATAACTAAATCTGCCTGTGGAAGAAATGGCAAACAATTCTGCAGTACTTTAA

CCTTGAACACAGCTCAAGCAAACCACACTGGCTTCTACAGCTGCAAATATCTAGCTG
TACCTACTTCAAAGAAGAAGGAAACAGAATCTGCAATCTATATATTTATTAGTGATA
CAGGTAGACCTTTCGTAGAGATGTACAGTGAAATCCCCGAAATTATACACATGACTG
AAGGAAGGGAGCTCGTCATTCCCTGCCGGGTTACGTCACCTAACATCACTGTTACTT
TAAAAAAGTTTCCACTTGACACTTTGATCCCTGATGGAAAACGCATAATCTGGGACA
GTAGAAAGGGCTTCATCATATCAAATGCAACGTACAAAGAAATAGGGCTTCTGACC
TGTGAAGCAACAGTCAATGGGCATTTGTATAAGACAAACTATCTCACACATCGACA
AACCAATACAATCATAGATGTCCAAATAAGCACACCACGCCCAGTCAAATTACTTA
GAGGCCATACTCTTGTCCTCAATTGTACTGCTACCACTCCCTTGAACACGAGAGTTC
AAATGACCTGGAGTTACCCTGATGAAAAAAATAAGAGAGCTTCCGTAAGGCGACGA
ATTGACCAAAGCAATTCCCATGCCAACATATTCTACAGTGTTCTTACTATTGACAAA
ATGCAGAACAAAGACAAAGGACTTTATACTTGTCGTGTAAGGAGTGGACCATCATT
CAAATCTGTTAACACCTCAGTGCATATATATGATAAAGCATTCATCACTGTGAAACA
TCGAAAACAGCAGGTGCTTGAAACCGTAGCTGGCAAGCGGTCTTACCGGCTCTCTAT
GAAAGTGAAGGCATTTCCCTCGCCGGAAGTTGTATGGTTAAAAGATGGGTTACCTGC
GACTGAGAAATCTGCTCGCTATTTGACTCGTGGCTACTCGTTAATTATCAAGGACGT
AACTGAAGAGGATGCAGGGAATTATACAATCTTGCTGAGCATAAAACAGTCAAATG
TGTTTAAAAACCTCACTGCCACTCTAATTGTCAATGTGAAACCCCAGATTTACGAAA
AGGCCGTGTCATCGTTTCCAGACCCGGCTCTCTACCCACTGGGCAGCAGACAAATCC
TGACTTGTACCGCATATGGTATCCCTCAACCTACAATCAAGTGGTTCTGGCACCCCT
GTAACCATAATCATTCCGAAGCAAGGTGTGACTTTTGTTCCAATAATGAAGAGTCCT
TTATCCTGGATGCTGACAGCAACATGGGAAACAGAATTGAGAGCATCACTCAGCGC
ATGGCAATAATAGAAGGAAAGAATAAGATGGCTAGCACCTTGGTTGTGGCTGACTC
TAGAATTTCTGGAATCTACATTTGCATAGCTTCCAATAAAGTTGGGACTGTGGGAAG
AAACATAAGCTTTTATATCACAGATGTGCCAAATGGGTTTCATGTTAACTTGGAAAA
AATGCCGACGGAAGGAGAGGACCTGAAACTGTCTTGCACAGTTAACAAGTTCTTAT
ACAGAGACGTTACTTGGATTTTACTGCGGACAGTTAATAACAGAACAATGCACTACA
GTATTAGCAAGCAAAAAATGGCCATCACTAAGGAGCACTCCATCACTCTTAATCTTA
CCATCATGAATGTTTCCCTGCAAGATTCAGGCACCTATGCCTGCAGAGCCAGGAATG
TATACACAGGGGAAGAAATCCTCCAGAAGAAAGAAATTACAATCAGAGATCAGGA
AGCACCATACCTCCTGCGAAACCTCAGTGATCACACAGTGGCCATCAGCAGTTCCAC

CACTTTAGACTGTCATGCTAATGGTGTCCCCGAGCCTCAGATCACTTGGTTTAAAAA
CAACCACAAAATACAACAAGAGCCTGGAATTATTTTAGGACCAGGAAGCAGCACGC
TGTTTATTGAAAGAGTCACAGAAGAGGATGAAGGTGTCTATCACTGCAAAGCCACC
AACCAGAAGGGCTCTGTGGAAAGTTCAGCATACCTCACTGTTCAAGGAACCTCGGA
CAAGTCTAATCTGGAGCTGATCACTCTAACATGCACCTGTGTGGCTGCGACTCTCTT
CTGGCTCCTATTAACCCTCTTTATCCGAAAAATGAAAAGGTCTTCTTCTGAAATAAA
GACTGACTACCTATCAATTATAATGGACCCAGATGAAGTTCCTTTGGATGAGCAGTG
TGAGCGGCTCCCTTATGATGCCAGCAAGTGGGAGTTTGCCCGGGAGAGACTTAAACT
GGGCAAATCACTTGGAAGAGGGGCTTTTGGAAAAGTGGTTCAAGCATCAGCATTTG
GCATTAAGAAATCACCTACGTGCCGGACTGTGGCTGTGAAAATGCTGAAAGAGGGG
GCCACGGCCAGCGAGTACAAAGCTCTGATGACTGAGCTAAAAATCTTGACCCACAT
TGGCCACCATCTGAACGTGGTTAACCTGCTGGGAGCCTGCACCAAGCAAGGAGGGC
CTCTGATGGTGATTGTTGAATACTGCAAATATGGAAATCTCTCCAACTACCTCAAGA
GCAAACGTGACTTATTTTTTCTCAACAAGGATGCAGCACTACACATGGAGCCTAAGA
AAGAAAAAATGGAGCCAGGCCTGGAACAAGGCAAGAAACCAAGACTAGATAGCGT
CACCAGCAGCGAAAGCTTTGCGAGCTCCGGCTTTCAGGAAGATAAAAGTCTGAGTG
ATGTTGAGGAAGAGGAGGATTCTGACGGTTTCTACAAGGAGCCCATCACTATGGAA
GATCTGATTTCTTACAGTTTTCAAGTGGCCAGAGGCATGGAGTTCCTGTCTTCCAGA
AAGTGCATTCATCGGGACCTGGCAGCGAGAAACATTCTTTTATCTGAGAACAACGTG
GTGAAGATTTGTGATTTTGGCCTTGCCCGGGATATTTATAAGAACCCCGATTATGTG
AGAAAAGGAGATACTCGACTTCCTCTGAAATGGATGGCTCCTGAATCTATCTTTGAC
AAAATCTACAGCACCAAGAGCGACGTGTGGTCTTACGGAGTATTGCTGTGGGAAAT
CTTCTCCTTAGGTGGGTCTCCATACCCAGGAGTACAAATGGATGAGGACTTTTGCAG
TCGCCTGAGGGAAGGCATGAGGATGAGAGCTCCTGAGTACTCTACTCCTGAAATCTA
TCAGATCATGCTGGACTGCTGGCACAGAGACCCAAAAGAAAGGCCAAGATTTGCAG
AACTTGTGGAAAAACTAGGTGATTTGCTTCAAGCAAATGTACAACAGGATGGTAAA
GACTACATCCCAATCAATGCCATACTGACAGGAAATAGTGGGTTTACATACTCAACT
CCTGCCTTCTCTGAGGACTTCTTCAAGGAAAGTATTTCAGCTCCGAAGTTTAATTCAG
GAAGCTCTGATGATGTCAGATACGTAAATGCTTTCAAGTTCATGAGCCTGGAAAGAA
TCAAAACCTTTGAAGAACTTTTACCGAATGCCACCTCCATGTTTGATGACTACCAGG
GCGACAGCAGCACTCTGTTGGCCTCTCCCATGCTGAAGCGCTTCACCTGGACTGACA

GCAAACCCAAGGCCTCGCTCAAGATTGACTTGAGAGTAACCAGTAAAAGTAAGGAG
TCGGGGCTGTCTGATGTCAGCAGGCCCAGTTTCTGCCATTCCAGCTGTGGGCACGTC
AGCGAAGGCAAGCGCAGGTTCACCTACGACCACGCTGAGCTGGAAAGGAAAATCGC
GTGCTGCTCCCCGCCCCCAGACTACAACTCGGTGGTCCTGTACTCCACCCCACCCAT
CTAGAGTTTGACACGAAGCCTTATTTCTAGAAGCACATGTGTATTTATACCCCCAGG
AAACTAGCTTTTGCCAGTATTATGCATATATAAGTTTACACCTTTATCTTTCCATGGG
AGCCAGCTGCTTTTTGTGATTTTTTTAATAGTGCTTTTTTTTTTTTTGACTAACAAGAAT
GTAACTCCAGATAGAGAAATAGTGACAAGTGAAGAACACTACTGCTAAATCCTCAT
GTTACTCAGTGTTAGAGAAATCCTTCCTAAACCCAATGACTTCCCTGCTCCAACCCC
CGCCACCTCAGGGCACGCAGGACCAGTTTGATTGAGGAGCTGCACTGATCACCCAA
TGCATCACGTACCCCACTGGGCCAGCCCTGCAGCCCAAAACCCAGGGCAACAAGCC
CGTTAGCCCCAGGGATCACTGGCTGGCCTGAGCAACATCTCGGGAGTCCTCTAGCAG
GCCTAAGACATGTGAGGAGGAAAAGGAAAAAAAGCAAAAAGCAAGGGAGAAAAG
AGAAACCGGGAGAAGGCATGAGAAAGAATTTGAGACGCACCATGTGGGCACGGAG
GGGGACGGGGCTCAGCAATGCCATTTCAGTGGCTTCCCAGCTCTGACCCTTCTACAT
TTGAGGGCCCAGCCAGGAGCAGATGGACAGCGATGAGGGGACATTTTCTGGATTCT
GGGAGGCAAGAAAAGGACAAATATCTTTTTTGGAACTAAAGCAAATTTTAGAACTT
TACCTATGGAAGTGGTTCTATGTCCATTCTCATTCGTGGCATGTTTTGATTTGTAGCA
CTGAGGGTGGCACTCAACTCTGAGCCCATACTTTTGGCTCCTCTAGTAAGATGCACT
GAAAACTTAGCCAGAGTTAGGTTGTCTCCAGGCCATGATGGCCTTACACTGAAAATG
TCACATTCTATTTTGGGTATTAATATATAGTCCAGACACTTAACTCAATTTCTTGGTA
TTATTCTGTTTTGCACAGTTAGTTGTGAAAGAAAGCTGAGAAGAATGAAAATGCAGT
CCTGAGGAGAGGAGTTTTCTCCATATCAAAACGAGGGCTGATGGAGGAAAAAGGTC
AATAAGGTCAAGGGAAAACCCCGTCTCTATACCAACCAAACCAATTCACCAACACA
GTTGGGACCCAAAACACAGGAAGTCAGTCACGTTTCCTTTTCATTTAATGGGGATTC
CACTATCTCACACTAATCTGAAAGGATGTGGAAGAGCATTAGCTGGCGCATATTAAG
CACTTTAAGCTCCTTGAGTAAAAAGGTGGTATGTAATTTATGCAAGGTATTTCTCCA
GTTGGGACTCAGGATATTAGTTAATGAGCCATCACTAGAAGAAAAGCCCATTTTCAA
CTGCTTTGAAACTTGCCTGGGGTCTGAGCATGATGGGAATAGGGAGACAGGGTAGG
AAAGGGCGCCTACTCTTCAGGGTCTAAAGATCAAGTGGGCCTTGGATCGCTAAGCTG
GCTCTGTTTGATGCTATTTATGCAAGTTAGGGTCTATGTATTTATGATGTCTGCACCT

TCTGCAGCCAGTCAGAAGCTGGAGAGGCAACAGTGGATTGCTGCTTCTTGGGGAGA
AGAGTATGCTTCCTTTTATCCATGTAATTTAACTGTAGAACCTGAGCTCTAAGTAACC
GAAGAATGTATGCCTCTGTTCTTATGTGCCACATCCTTGTTTAAAGGCTCTCTGTATG
AAGAGATGGGACCGTCATCAGCACATTCCCTAGTGAGCCTACTGGCTCCTGGCAGC
GGCTTTTGTGGAAGACTCACTAGCCAGAAGAGAGGAGTGGGACAGTCCTCTCCACC
AAGATCTAAATCCAAACAAAAGCAGGCTAGAGCCAGAAGAGAGGACAAATCTTTGT
TCTTCCTCTTCTTTACATACGCAAACCACCTGTGACAGCTGGCAATTTTATAAATCAG
GTAACTGGAAGGAGGTTAAACACAGAAAAAAGAAGACCTCAGTCAATTCTCTACTT
TTTTTTTTTTTTCCAAATCAGATAATAGCCCAGCAAATAGTGATAACAAATAAAACC
TTAGCTATTCATGTCTTGATTTCAATAATTAATTCTTAATCATTAAGAGACCATAATA
AATACTCCTTTTCAAGAGAAAAGCAAAACCATTAGAATTGTTACTCAGCTCCTTCAA
ACTCAGGTTTGTAGCATACATGAGTCCATCCATCAGTCAAAGAATGGTTCCATCTGG
AGTCTTAATGTAGAAAGAAAAATGGAGACTTGTAATAATGAGCTAGTTACAAAGTG
CTTGTTCATTAAAATAGCACTGAAAATTGAAACATGAATTAACTGATAATATTCCAA
TCATTTGCCATTTATGACAAAAATGGTTGGCACTAACAAAGAACGAGCACTTCCTTT
CAGAGTTTCTGAGATAATGTACGTGGAACAGTCTGGGTGGAATGGGGCTGAAACCA
TGTGCAAGTCTGTGTCTTGTCAGTCCAAGAAGTGACACCGAGATGTTAATTTTAGGG
ACCCGTGCCTTGTTTCCTAGCCCACAAGAATGCAAACATCAAACAGATACTCGCTAG
CCTCATTTAAATTGATTAAAGGAGGAGTGCATCTTTGGCCGACAGTGGTGTAACTGT
ATGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGGGTGTATGTGTGTTTTGTGCA
TAACTATTTAAGGAAACTGGAATTTTAAAGTTACTTTTATACAAACCAAGAATATAT
GCTACAGATATAAGACAGACATGGTTTGGTCCTATATTTCTAGTCATGATGAATGTA
TTTTGTATACCATCTTCATATAATAAACTTCCAAAAACACA

[0305] Exemplary Human VEGFR-1 isoform 1 precursor amino acid sequence (SEQ ID NO: 27)

MVSYWDTGVLLCALLSCLLLTGSSSGSKLKDPELSLKGTQHIMQAGQTLHLQCRGEAA
HKWSLPEMVSKESERLSITKSACGRNGKQFCSTLTLNTAQANHTGFYSCKYLAVPTSKK
KETESAIYIFISDTGRPFVEMYSEIPEIIHMTEGRELVIPCRVTSPNITVTLKKFPLDTLIPDG
KRIIWDSRKGFIISNATYKEIGLLTCEATVNGHLYKTNYLTHRQTNTIIDVQISTPRPVKLL
RGHTLVLNCTATTPLNTRVQMTWSYPDEKNKRASVRRRIDQSNSHANIFYSVLTIDKMQ
NKDKGLYTCRVRSGPSFKSVNTSVHIYDKAFITVKHRKQQVLETVAGKRSYRLSMKVK

AFPSPEVVWLKDGLPATEKSARYLTRGYSLIIKDVTEEDAGNYTILLSIKQSNVFKNLTA
TLIVNVKPQIYEKAVSSFPDPALYPLGSRQILTCTAYGIPQPTIKWFWHPCNHNHSEARC
DFCSNNEESFILDADSNMGNRIESITQRMAIIEGKNKMASTLVVADSRISGIYICIASNKVG
TVGRNISFYITDVPNGFHVNLEKMPTEGEDLKLSCTVNKFLYRDVTWILLRTVNNRTMH
YSISKQKMAITKEHSITLNLTIMNVSLQDSGTYACRARNVYTGEEILQKKEITIRDQEAPY
LLRNLSDHTVAISSSTTLDCHANGVPEPQITWFKNNHKIQQEPGIILGPGSSTLFIERVTEE
DEGVYHCKATNQKGSVESSAYLTVQGTSDKSNLELITLTCTCVAATLFWLLLTLFIRKM
KRSSSEIKTDYLSIIMDPDEVPLDEQCERLPYDASKWEFARERLKLGKSLGRGAFGKVVQ
ASAFGIKKSPTCRTVAVKMLKEGATASEYKALMTELKILTHIGHHLNVVNLLGACTKQG
GPLMVIVEYCKYGNLSNYLKSKRDLFFLNKDAALHMEPKKEKMEPGLEQGKKPRLDSV
TSSESFASSGFQEDKSLSDVEEEEDSDGFYKEPITMEDLISYSFQVARGMEFLSSRKCIHR
DLAARNILLSENNVVKICDFGLARDIYKNPDYVRKGDTRLPLKWMAPESIFDKIYSTKSD
VWSYGVLLWEIFSLGGSPYPGVQMDEDFCSRLREGMRMRAPEYSTPEIYQIMLDCWHR
DPKERPRFAELVEKLGDLLQANVQQDGKDYIPINAILTGNSGFTYSTPAFSEDFFKESISA
PKFNSGSSDDVRYVNAFKFMSLERIKTFEELLPNATSMFDDYQGDSSTLLASPMLKRFT
WTDSKPKASLKIDLRVTSKSKESGLSDVSRPSFCHSSCGHVSEGKRRFTYDHAELERKIA
CCSPPPDYNSVVLYSTPPI

[0306] This variant (2), also known as sFlt1 or sVEGFR-1, differs in the 3` coding region and 3` UTR, compared to variant 1. The encoded soluble protein (isoform 2) has a shorter, distinct C-terminus and lacks the transmembrane and cytoplasmic regions of isoform 1.

[0307] Exemplary Human VEGFR-1 isoform 2 (also known as sVEGFR-1) cDNA sequence (SEQ ID NO: 28)

ATCGAGGTCCGCGGGAGGCTCGGAGCGCGCCAGGCGGACACTCCTCTCGGCTCCTC
CCCGGCAGCGGCGGCGGCTCGGAGCGGGCTCCGGGGCTCGGGTGCAGCGGCCAGCG
GGCGCCTGGCGGCGAGGATTACCCGGGGAAGTGGTTGTCTCCTGGCTGGAGCCGCG
AGACGGGCGCTCAGGGCGCGGGGCCGGCGGCGGCGAACGAGAGGACGGACTCTGG
CGGCCGGGTCGTTGGCCGCGGGGAGCGCGGGCACCGGGCGAGCAGGCCGCGTCGCG
CTCACCATGGTCAGCTACTGGGACACCGGGGTCCTGCTGTGCGCGCTGCTCAGCTGT
CTGCTTCTCACAGGATCTAGTTCAGGTTCAAAATTAAAAGATCCTGAACTGAGTTTA
AAAGGCACCCAGCACATCATGCAAGCAGGCCAGACACTGCATCTCCAATGCAGGGG

GGAAGCAGCCCATAAATGGTCTTTGCCTGAAATGGTGAGTAAGGAAAGCGAAAGGC
TGAGCATAACTAAATCTGCCTGTGGAAGAAATGGCAAACAATTCTGCAGTACTTTAA
CCTTGAACACAGCTCAAGCAAACCACACTGGCTTCTACAGCTGCAAATATCTAGCTG
TACCTACTTCAAAGAAGAAGGAAACAGAATCTGCAATCTATATATTTATTAGTGATA
CAGGTAGACCTTTCGTAGAGATGTACAGTGAAATCCCCGAAATTATACACATGACTG
AAGGAAGGGAGCTCGTCATTCCCTGCCGGGTTACGTCACCTAACATCACTGTTACTT
TAAAAAAGTTTCCACTTGACACTTTGATCCCTGATGGAAAACGCATAATCTGGGACA
GTAGAAAGGGCTTCATCATATCAAATGCAACGTACAAAGAAATAGGGCTTCTGACC
TGTGAAGCAACAGTCAATGGGCATTTGTATAAGACAAACTATCTCACACATCGACA
AACCAATACAATCATAGATGTCCAAATAAGCACACCACGCCCAGTCAAATTACTTA
GAGGCCATACTCTTGTCCTCAATTGTACTGCTACCACTCCCTTGAACACGAGAGTTC
AAATGACCTGGAGTTACCCTGATGAAAAAAATAAGAGAGCTTCCGTAAGGCGACGA
ATTGACCAAAGCAATTCCCATGCCAACATATTCTACAGTGTTCTTACTATTGACAAA
ATGCAGAACAAAGACAAAGGACTTTATACTTGTCGTGTAAGGAGTGGACCATCATT
CAAATCTGTTAACACCTCAGTGCATATATATGATAAAGCATTCATCACTGTGAAACA
TCGAAAACAGCAGGTGCTTGAAACCGTAGCTGGCAAGCGGTCTTACCGGCTCTCTAT
GAAAGTGAAGGCATTTCCCTCGCCGGAAGTTGTATGGTTAAAAGATGGGTTACCTGC
GACTGAGAAATCTGCTCGCTATTTGACTCGTGGCTACTCGTTAATTATCAAGGACGT
AACTGAAGAGGATGCAGGGAATTATACAATCTTGCTGAGCATAAAACAGTCAAATG
TGTTTAAAAACCTCACTGCCACTCTAATTGTCAATGTGAAACCCCAGATTTACGAAA
AGGCCGTGTCATCGTTTCCAGACCCGGCTCTCTACCCACTGGGCAGCAGACAAATCC
TGACTTGTACCGCATATGGTATCCCTCAACCTACAATCAAGTGGTTCTGGCACCCCT
GTAACCATAATCATTCCGAAGCAAGGTGTGACTTTTGTTCCAATAATGAAGAGTCCT
TTATCCTGGATGCTGACAGCAACATGGGAAACAGAATTGAGAGCATCACTCAGCGC
ATGGCAATAATAGAAGGAAAGAATAAGATGGCTAGCACCTTGGTTGTGGCTGACTC
TAGAATTTCTGGAATCTACATTTGCATAGCTTCCAATAAAGTTGGGACTGTGGGAAG
AAACATAAGCTTTTATATCACAGATGTGCCAAATGGGTTTCATGTTAACTTGGAAAA
AATGCCGACGGAAGGAGAGGACCTGAAACTGTCTTGCACAGTTAACAAGTTCTTAT
ACAGAGACGTTACTTGGATTTTACTGCGGACAGTTAATAACAGAACAATGCACTACA
GTATTAGCAAGCAAAAAATGGCCATCACTAAGGAGCACTCCATCACTCTTAATCTTA
CCATCATGAATGTTTCCCTGCAAGATTCAGGCACCTATGCCTGCAGAGCCAGGAATG

TATACACAGGGGAAGAAATCCTCCAGAAGAAAGAAATTACAATCAGAGGTGAGCAC
TGCAACAAAAAGGCTGTTTTCTCTCGGATCTCCAAATTTAAAAGCACAAGGAATGAT
TGTACCACACAAAGTAATGTAAAACATTAAAGGACTCATTAAAAAGTAACAGTTGT
CTCATATCATCTTGATTTATTGTCACTGTTGCTAACTTTCAGGCTCGGAGGAGATGCT
CCTCCCAAAATGAGTTCGGAGATGATAGCAGTAATAATGAGACCCCCGGGCCCCAG
CTCTGGGCCCCCCCATTCAGGCCGAGGGGGCTGCTCCGGGGGGCCGACTTGGTGCAC
GTTTGGATTTGGAGGATCCCTGCACTGCCTTCTCTGTGTTTGTTGCTCTTGCTGTTTTC
TCCTGCCTGATAAACAACAACTTGGGATGATCCTTTCCTTCCATTTTGATGCCAACCT
CTTTTTATTTTTAAGTGTTGAAGCTGCACAAACTGAATAATTTAAACAAATGCTGGTT
TCTGCCAAAGATGGACACGAATAAGTTAATTTTCCAGCTCAGAATGAGTACAGTTGA
ATTTGAGACTCTGTCGGACTTCTGCCTGGTTTTATTTGGGACTATTTCATCTGCTCTT
GATTTGTAAATAGCACCTGGATAGCAAGTTATAATGCTTATTTATTTGAAAATGCTTT
TTTTTTTTTTACGTTAAGCACATTTATCTTGAACTGGAGCTTCTAAAATGGGCCCCAG
GGGTGCAAGATGTTGGTGTAATTCAGAGATAGTAAAGGTTTATCGCAGTGTGAATTA
TAAGAGTCCATCCAAATCAACGTCCCCTCCCTCCTCTCATGCGATCCAGGTAATTAT
GCAGTTAGTGCCACAGTAGACTAGCCTAGCAAAGGGTTTGCTCCTTGCTGTCTCTGA
CTGCACCACACAGCTATTGATGGCAGCTGAAAGAAAGTGGATCATGCCTTAATTTTA
AATATTCCTGTCCTCTGGTTATTATTTTAAGGAACTTCATCATGTTAAAATGACAGCA
TTCAAAGGTGTACCACAATCAATTTATCAAGGAAATAAAGGCTATTGTAACCAGAG
ATTTAATGCATTCTTCTAAATGTAAATTTAAAATTTGCCCTTTAAAAAAGTCCACTTT
CCCCATATGCAAATGTTAATAGGATTTTTATGGGGATTAAGAAGCGGCAAAACTACA
GAAGCAGAATTCAAAGTAATTTAAAAAATACACACCAGTTTTAAATCAAGAGAAGT
TGTAATCTCTTGTTTTAAGCTTGCGTTTGAGGGAAAATGACTTTTTCACCAATTTAAT
ATGCATTGTTCTGTTGTTTTTATTTATGATTGATCATTATATGTGACTTGCATAAACT
ATTTAAAAAAAAAAAACTATAATGACCAAAATAGCCATGGCTGAGAAACACAGTGGC
TGGGCAGTTCAATAGGAGGTGACAATATGACAACTTCTCAAGCTTGGGAACTCACC
AGACTGTTTCCTCCTTTAGGTAACAGATTCTGTCCCACGGCTAAACTTGTCTTTCACG
TGGGAATTGCTTTTGTCAAACGTGAAAGAGTAAACAATAGCATTTCCCCAGAATGCC
AGTTTTATGGAGCCCCAAATGCTCTGAAAACAATTAGTAACCTGGAAGTTGTCAGCC
CAAAGGAAAGAAAAATCAATTGTATCTTGAAATTTTACCTATGGCTCTTTGGCCTGG
CTTCTTTGTTCATTATAAGTTAGTGTGTTCCTTCAGGAAACAATGCCTTAATACCATA

GAACATGGGGGCCTTAATAGTTGCTAACATTAAAAAAGCAAACAGAATGATTGAGG
GATCCTTATGAAAACAAAATGGTGAATTGGACATGCAGAACCTACCATTTCCTTCCC
CTGTTTGCAATTTTTGTGGGGAGGGGAGGATGTTAGTATTTACAAAAGATGATTTTA
AGAACTTCCAAGAGATGAGTTTAAGAATTCCATAGAGTATTAGTTGTTCACTGTGTA
ATTAATCCTTCCGGAGAGTCTTTTTTTTTTTTTTTAAAGAAACTTTTGGGTGGGTTTTG
TTTTTTATTAGTTACCCTAGGGGTATGTTACCCTGGGGTATGAAGGGAGGTGAAGAT
AACGGAGGGGGGAGAAAAAAAAAAGGAGAAAAAAGGAGCCTAAAATGGGGAATA
ATTGAAATGGAACAGGGGGTGTGAGGCTGGTTCCTCAGTCCCCATTCCAAACGGAG
GATAGAAGCTGTGTATTTATGTGACCTGGCAGATCTCTGGGGCCATAACACTGAAAA
GTGAAAGAACCTGGTGGGCAGCTATCTTTGGCTACTGATAACCAGCAGAAATGTCTG
TTAATTCTGATTTTCTCAATTTGAAGGGATCAGCTACACTGTTAAATTTTGGAAAGCC
ACTACCTACTTCCATCAAGTAACTTAGGTTTCGAAATATGGGTTCAACGCACCTCCC
TTATTCAAAATGTCAAAATAGATTATTATAATGTATAAAGTAAGAATTGACAAAATA
TGATTCTTGGGTTGATTGGTCATTTAGAAACTAGCCAAAAGTGAGACTTTTAATGTA
GAACATTTTTCAGAAATGGGTACAAAGAAAAATGCATATTACTGTATATTTCAGAGT
GTTTATGTGAACCTTGTATTTAATTGAGAGTCCCATGTACGTTCTGCAGCCTTTTTGC
TGCTTCTATCATCTGAAGTTTGTGTAGTACAAATAAGGCCTTTGGGATTCTTAATGAC
ATTTATGTTAAAATGTTCTCTTCTCTTTAAACACCGTTTTCCAATCCACCTGTCAGGG
AGTCCAAATCGTGTCTGTGTTGATGATGCTATACTTTGTAGCTAGAAAAACAATTTT
AGTGTTGTGGGCTCTGTATTCAGACTTCCTTTTTACAAGACCGATGGGCAGTGATAG
ATTATTTTATCATATTTAATGCATGGGAAATAGTGTGCTGAGGAAGCTATTAAAAGT
ATAACTCAGTGAATTGGGTCTGAGTTTTAAATGAGATATTTCAAAATTGGCTTGCCA
CTGTAAAAGCGACTAAATAATAATATGATACTGTTCTTTATGATCTTGTCATGTTTCA
CTGATATGTTTGGGGTCTTCACTATGTAAAAAATGTCAAAATTGTAATGAGCAAGCA
TGTACAAGTAGTCGTAAATCAAAGGTTTTAAACAGGACTGCATTTTCAATTAGGAAA
AGCTGTTTGGCAGATAGCATCCAATGCAAAAACAGAAATATCGTAACGTTCTGCTTA
GTGGGCAAGATAAGATAGGAAAGACATGCTCAAAGAGGCAAAAGAATCATTGCTAT
CATTCATTCTACACTAGTTTGAAGAAGTTTTTGTACATCAGAGCACTTCCTTCAGCAC
ACTTTTTTGCCTTCAGATTTCATTTTTTATAAAATGAGAAGACTAATGATAAACTGTA
GAAATCAAAATTTATTGAGAAATCTGTTTCTCCTAACAGATAGTAACCCTGCCATGA
TATACTACTTCAACAATGTTATAAAATTTATGTGATAATATACATTTTAACCTGGGAT

TTCTAAATTGCTTTAACAAATGCTAATCCTGAGAGTTGCCCTGCAGGACTCAAAAGG
GAAAGGTTTTGGGACGTGGCAGAACCCTGCAGGGACATGGAATTAAGGCCATTGCA
ATGTATCATCTTTGTAGCATTGTCATCACTCCTAAGCTGCCTTCACAGTTTTAGTACA
CTAAGATGAGGAAATCGAAAATGGGCAGAGAAAGCTCATACTGTATAATTGAAGAC
AGTGACAGAGAACGTGTCAGTTATGCCAAAACTCTTTTGATTTCTGTTCCAGGATTT
CCAACAAGAGGGGAAAGGAATGACTTGGGAGGGTGGGAAAGACATTAGGAGTTGT
TTTTATTTTTTACCTTGGAAGCTTTAGCTACCAATCCAGTACCCTCCTAACTAGAATG
TATACACATCAGCAGGACTGACTGACTACTTCATTAGAGATATACTGTACTCATTGG
GGGCCTTGGGGGTACTGCTGTTCTTATGTGGGATTTTAATGTTGTAATGTATTGCATC
TTAATGTATTGAATTCATTTTGTTGTACTATATTGGTTGGCATTTTATTAAAATAAAT
TGTATTGTATCATATTTGTATGTTTTAAGAGAAAATAATATAAAATACAATATTTGTA
CTATTATATAGTGCAAAAACTACAAATCTGTGCCTCTGCCTCTTGAATTAATTCTTTG
GTTGCTTGCATTTGGGAAGGGAATGGAGAAAGGAAAGAACCAATAAAGCTTTCAAA
GTTCAAGAAA

[0308] Exemplary Human VEGFR-1 isoform 2 (also known as sVEGFR-1) precursor amino acid sequence (SEQ ID NO: 29)

MVSYWDTGVLLCALLSCLLLTGSSSGSKLKDPELSLKGTQHIMQAGQTLHLQCRGEAA
HKWSLPEMVSKESERLSITKSACGRNGKQFCSTLTLNTAQANHTGFYSCKYLAVPTSKK
KETESAIYIFISDTGRPFVEMYSEIPEIIHMTEGRELVIPCRVTSPNITVTLKKFPLDTLIPDG
KRIIWDSRKGFIISNATYKEIGLLTCEATVNGHLYKTNYLTHRQTNTIIDVQISTPRPVKLL
RGHTLVLNCTATTPLNTRVQMTWSYPDEKNKRASVRRRIDQSNSHANIFYSVLTIDKMQ
NKDKGLYTCRVRSGPSFKSVNTSVHIYDKAFITVKHRKQQVLETVAGKRSYRLSMKVK
AFPSPEVVWLKDGLPATEKSARYLTRGYSLIIKDVTEEDAGNYTILLSIKQSNVFKNLTA
TLIVNVKPQIYEKAVSSFPDPALYPLGSRQILTCTAYGIPQPTIKWFWHPCNHNHSEARC
DFCSNNEESFILDADSNMGNRIESITQRMAIIEGKNKMASTLVVADSRISGIYICIASNKVG
TVGRNISFYITDVPNGFHVNLEKMPTEGEDLKLSCTVNKFLYRDVTWILLRTVNNRTMH
YSISKQKMAITKEHSITLNLTIMNVSLQDSGTYACRARNVYTGEEILQKKEITIRGEHCNK
KAVFSRISKFKSTRNDCTTQSNVKH

[0309] This variant (3) differs in the 3` coding region and 3` UTR, compared to variant 1. The encoded soluble protein (isoform 3) has a shorter, distinct C-terminus and lacks the transmembrane and cytoplasmic regions of isoform 1.

[0310] Exemplary Human VEGFR-1 isoform 3 cDNA sequence (SEQ ID NO: 30)

ATCGAGGTCCGCGGGAGGCTCGGAGCGCGCCAGGCGGACACTCCTCTCGGCTCCTC
CCCGGCAGCGGCGGCGGCTCGGAGCGGGCTCCGGGGCTCGGGTGCAGCGGCCAGCG
GGCGCCTGGCGGCGAGGATTACCCGGGGAAGTGGTTGTCTCCTGGCTGGAGCCGCG
AGACGGGCGCTCAGGGCGCGGGGCCGGCGGCGGCGAACGAGAGGACGGACTCTGG
CGGCCGGGTCGTTGGCCGCGGGGAGCGCGGGCACCGGGCGAGCAGGCCGCGTCGCG
CTCACCATGGTCAGCTACTGGGACACCGGGGTCCTGCTGTGCGCGCTGCTCAGCTGT
CTGCTTCTCACAGGATCTAGTTCAGGTTCAAAATTAAAAGATCCTGAACTGAGTTTA
AAAGGCACCCAGCACATCATGCAAGCAGGCCAGACACTGCATCTCCAATGCAGGGG
GGAAGCAGCCCATAAATGGTCTTTGCCTGAAATGGTGAGTAAGGAAAGCGAAAGGC
TGAGCATAACTAAATCTGCCTGTGGAAGAAATGGCAAACAATTCTGCAGTACTTTAA
CCTTGAACACAGCTCAAGCAAACCACACTGGCTTCTACAGCTGCAAATATCTAGCTG
TACCTACTTCAAAGAAGAAGGAAACAGAATCTGCAATCTATATATTTATTAGTGATA
CAGGTAGACCTTTCGTAGAGATGTACAGTGAAATCCCCGAAATTATACACATGACTG
AAGGAAGGGAGCTCGTCATTCCCTGCCGGGTTACGTCACCTAACATCACTGTTACTT
TAAAAAAGTTTCCACTTGACACTTTGATCCCTGATGGAAAACGCATAATCTGGGACA
GTAGAAAGGGCTTCATCATATCAAATGCAACGTACAAAGAAATAGGGCTTCTGACC
TGTGAAGCAACAGTCAATGGGCATTTGTATAAGACAAACTATCTCACACATCGACA
AACCAATACAATCATAGATGTCCAAATAAGCACACCACGCCCAGTCAAATTACTTA
GAGGCCATACTCTTGTCCTCAATTGTACTGCTACCACTCCCTTGAACACGAGAGTTC
AAATGACCTGGAGTTACCCTGATGAAAAAAATAAGAGAGCTTCCGTAAGGCGACGA
ATTGACCAAAGCAATTCCCATGCCAACATATTCTACAGTGTTCTTACTATTGACAAA
ATGCAGAACAAAGACAAAGGACTTTATACTTGTCGTGTAAGGAGTGGACCATCATT
CAAATCTGTTAACACCTCAGTGCATATATATGATAAAGCATTCATCACTGTGAAACA
TCGAAAACAGCAGGTGCTTGAAACCGTAGCTGGCAAGCGGTCTTACCGGCTCTCTAT
GAAAGTGAAGGCATTTCCCTCGCCGGAAGTTGTATGGTTAAAAGATGGGTTACCTGC
GACTGAGAAATCTGCTCGCTATTTGACTCGTGGCTACTCGTTAATTATCAAGGACGT
AACTGAAGAGGATGCAGGGAATTATACAATCTTGCTGAGCATAAAACAGTCAAATG

TGTTTAAAAACCTCACTGCCACTCTAATTGTCAATGTGAAACCCCAGATTTACGAAA
AGGCCGTGTCATCGTTTCCAGACCCGGCTCTCTACCCACTGGGCAGCAGACAAATCC
TGACTTGTACCGCATATGGTATCCCTCAACCTACAATCAAGTGGTTCTGGCACCCCT
GTAACCATAATCATTCCGAAGCAAGGTGTGACTTTTGTTCCAATAATGAAGAGTCCT
TTATCCTGGATGCTGACAGCAACATGGGAAACAGAATTGAGAGCATCACTCAGCGC
ATGGCAATAATAGAAGGAAAGAATAAGATGGCTAGCACCTTGGTTGTGGCTGACTC
TAGAATTTCTGGAATCTACATTTGCATAGCTTCCAATAAAGTTGGGACTGTGGGAAG
AAACATAAGCTTTTATATCACAGATGTGCCAAATGGGTTTCATGTTAACTTGGAAAA
AATGCCGACGGAAGGAGAGGACCTGAAACTGTCTTGCACAGTTAACAAGTTCTTAT
ACAGAGACGTTACTTGGATTTTACTGCGGACAGTTAATAACAGAACAATGCACTACA
GTATTAGCAAGCAAAAAATGGCCATCACTAAGGAGCACTCCATCACTCTTAATCTTA
CCATCATGAATGTTTCCCTGCAAGATTCAGGCACCTATGCCTGCAGAGCCAGGAATG
TATACACAGGGGAAGAAATCCTCCAGAAGAAAGAAATTACAATCAGAGATCAGGA
AGCACCATACCTCCTGCGAAACCTCAGTGATCACACAGTGGCCATCAGCAGTTCCAC
CACTTTAGACTGTCATGCTAATGGTGTCCCCGAGCCTCAGATCACTTGGTTTAAAAA
CAACCACAAAATACAACAAGAGCCTGAACTGTATACATCAACGTCACCATCGTCAT
CGTCATCATCACCATTGTCATCATCATCATCATCGTCATCATCATCATCATCATAGCT
ATCATCATTATCATCATCATCATCATCATCATCATAGCTACCATTTATTGAAAACTAT
TATGTGTCAACTTCAAAGAACTTATCCTTTAGTTGGAGAGCCAAGACAATCATAACA
ATAACAAATGGCCGGGCATGGTGGCTCACGCCTGTAATCCCAGCACTTTGGGAGGC
CAAGGCAGGTGGATCATTTGAGGTCAGGAGTTCAAGACCAGCCTGACCAAGATGGT
GAAATGCTGTCTCTATTAAAAATACAAAATTAGCCAGGCATGGTGGCTCATGCCTGT
AATGCCAGCTACTCGGGAGGCTGAGACAGGAGAATCACTTGAACCCAGGAGGCAGA
GGTTGCAGGGAGCCGAGATCGTGTACTGCACTCCAGCCTGGGCAACAAGAGCGAAA
CTCCGTCTCAAAAAACAAATAAATAAATAAATAAATAAACAGACAAAATTCACTTT
TTATTCTATTAAACTTAACATACATGCATTAA

[0311] Exemplary Human VEGFR-1 isoform 3 cDNA sequence (SEQ ID NO: 31)

MVSYWDTGVLLCALLSCLLLTGSSSGSKLKDPELSLKGTQHIMQAGQTLHLQCRGEAA
HKWSLPEMVSKESERLSITKSACGRNGKQFCSTLTLNTAQANHTGFYSCKYLAVPTSKK
KETESAIYIFISDTGRPFVEMYSEIPEIIHMTEGRELVIPCRVTSPNITVTLKKFPLDTLIPDG
KRIIWDSRKGFIISNATYKEIGLLTCEATVNGHLYKTNYLTHRQTNTIIDVQISTPRPVKLL

RGHTLVLNCTATTPLNTRVQMTWSYPDEKNKRASVRRRIDQSNSHANIFYSVLTIDKMQ
NKDKGLYTCRVRSGPSFKSVNTSVHIYDKAFITVKHRKQQVLETVAGKRSYRLSMKVK
AFPSPEVVWLKDGLPATEKSARYLTRGYSLIIKDVTEEDAGNYTILLSIKQSNVFKNLTA
TLIVNVKPQIYEKAVSSFPDPALYPLGSRQILTCTAYGIPQPTIKWFWHPCNHNHSEARC
DFCSNNEESFILDADSNMGNRIESITQRMAIIEGKNKMASTLVVADSRISGIYICIASNKVG
TVGRNISFYITDVPNGFHVNLEKMPTEGEDLKLSCTVNKFLYRDVTWILLRTVNNRTMH
YSISKQKMAITKEHSITLNLTIMNVSLQDSGTYACRARNVYTGEEILQKKEITIRDQEAPY
LLRNLSDHTVAISSSTTLDCHANGVPEPQITWFKNNHKIQQEPELYTSTSPSSSSSSPLSSS
SSSSSSSSS

[0312] This variant (4) differs in the 3` coding region and 3` UTR, compared to variant 1. The encoded soluble protein (isoform 4) has a shorter, distinct C-terminus and lacks the transmembrane and cytoplasmic regions of isoform 1.

[0313] Exemplary Human VEGFR-1 isoform 4 cDNA sequence (SEQ ID NO: 32)

ATCGAGGTCCGCGGGAGGCTCGGAGCGCGCCAGGCGGACACTCCTCTCGGCTCCTC
CCCGGCAGCGGCGGCGGCTCGGAGCGGGCTCCGGGGCTCGGGTGCAGCGGCCAGCG
GGCGCCTGGCGGCGAGGATTACCCGGGGAAGTGGTTGTCTCCTGGCTGGAGCCGCG
AGACGGGCGCTCAGGGCGCGGGGCCGGCGGCGGCGAACGAGAGGACGGACTCTGG
CGGCCGGGTCGTTGGCCGCGGGGAGCGCGGGCACCGGGCGAGCAGGCCGCGTCGCG
CTCACCATGGTCAGCTACTGGGACACCGGGGTCCTGCTGTGCGCGCTGCTCAGCTGT
CTGCTTCTCACAGGATCTAGTTCAGGTTCAAAATTAAAAGATCCTGAACTGAGTTTA
AAAGGCACCCAGCACATCATGCAAGCAGGCCAGACACTGCATCTCCAATGCAGGGG
GGAAGCAGCCCATAAATGGTCTTTGCCTGAAATGGTGAGTAAGGAAAGCGAAAGGC
TGAGCATAACTAAATCTGCCTGTGGAAGAAATGGCAAACAATTCTGCAGTACTTTAA
CCTTGAACACAGCTCAAGCAAACCACACTGGCTTCTACAGCTGCAAATATCTAGCTG
TACCTACTTCAAAGAAGAAGGAAACAGAATCTGCAATCTATATATTTATTAGTGATA
CAGGTAGACCTTTCGTAGAGATGTACAGTGAAATCCCCGAAATTATACACATGACTG
AAGGAAGGGAGCTCGTCATTCCCTGCCGGGTTACGTCACCTAACATCACTGTTACTT
TAAAAAAGTTTCCACTTGACACTTTGATCCCTGATGGAAAACGCATAATCTGGGACA
GTAGAAAGGGCTTCATCATATCAAATGCAACGTACAAAGAAATAGGGCTTCTGACC
TGTGAAGCAACAGTCAATGGGCATTTGTATAAGACAAACTATCTCACACATCGACA

AACCAATACAATCATAGATGTCCAAATAAGCACACCACGCCCAGTCAAATTACTTA
GAGGCCATACTCTTGTCCTCAATTGTACTGCTACCACTCCCTTGAACACGAGAGTTC
AAATGACCTGGAGTTACCCTGATGAAAAAAATAAGAGAGCTTCCGTAAGGCGACGA
ATTGACCAAAGCAATTCCCATGCCAACATATTCTACAGTGTTCTTACTATTGACAAA
ATGCAGAACAAAGACAAAGGACTTTATACTTGTCGTGTAAGGAGTGGACCATCATT
CAAATCTGTTAACACCTCAGTGCATATATATGATAAAGCATTCATCACTGTGAAACA
TCGAAAACAGCAGGTGCTTGAAACCGTAGCTGGCAAGCGGTCTTACCGGCTCTCTAT
GAAAGTGAAGGCATTTCCCTCGCCGGAAGTTGTATGGTTAAAAGATGGGTTACCTGC
GACTGAGAAATCTGCTCGCTATTTGACTCGTGGCTACTCGTTAATTATCAAGGACGT
AACTGAAGAGGATGCAGGGAATTATACAATCTTGCTGAGCATAAAACAGTCAAATG
TGTTTAAAAACCTCACTGCCACTCTAATTGTCAATGTGAAACCCCAGATTTACGAAA
AGGCCGTGTCATCGTTTCCAGACCCGGCTCTCTACCCACTGGGCAGCAGACAAATCC
TGACTTGTACCGCATATGGTATCCCTCAACCTACAATCAAGTGGTTCTGGCACCCCT
GTAACCATAATCATTCCGAAGCAAGGTGTGACTTTTGTTCCAATAATGAAGAGTCCT
TTATCCTGGATGCTGACAGCAACATGGGAAACAGAATTGAGAGCATCACTCAGCGC
ATGGCAATAATAGAAGGAAAGAATAAGCTTCCACCAGCTAACAGTTCTTTCATGTTG
CCACCTACAAGCTTCTCTTCCAACTACTTCCATTTCCTTCCGTGA

[0314] Exemplary Human VEGFR-1 isoform 4 cDNA sequence (SEQ ID NO: 33)

MVSYWDTGVLLCALLSCLLLTGSSSGSKLKDPELSLKGTQHIMQAGQTLHLQCRGEAA
HKWSLPEMVSKESERLSITKSACGRNGKQFCSTLTLNTAQANHTGFYSCKYLAVPTSKK
KETESAIYIFISDTGRPFVEMYSEIPEIIHMTEGRELVIPCRVTSPNITVTLKKFPLDTLIPDG
KRIIWDSRKGFIISNATYKEIGLLTCEATVNGHLYKTNYLTHRQTNTIIDVQISTPRPVKLL
RGHTLVLNCTATTPLNTRVQMTWSYPDEKNKRASVRRRIDQSNSHANIFYSVLTIDKMQ
NKDKGLYTCRVRSGPSFKSVNTSVHIYDKAFITVKHRKQQVLETVAGKRSYRLSMKVK
AFPSPEVVWLKDGLPATEKSARYLTRGYSLIIKDVTEEDAGNYTILLSIKQSNVFKNLTA
TLIVNVKPQIYEKAVSSFPDPALYPLGSRQILTCTAYGIPQPTIKWFWHPCNHNHSEARC
DFCSNNEESFILDADSNMGNRIESITQRMAIIEGKNKLPPANSSFMLPPTSFSSNYFHFLP

[0315] The VEGFR-2 gene found at human chromosomal position 4q12 encodes a 30 exon containing member of the vascular endothelial growth factor receptor family (VEGFR) and is one of two genes encoding receptors for VEGF-A. This receptor, known as kinase insert domain

receptor, is a type III receptor tyrosine kinase. It functions as the main mediator of VEGF-A induced endothelial proliferation, survival, migration, tubular morphogenesis and sprouting. The signaling and trafficking of this receptor are regulated by multiple factors, including Rab GTPase, P2Y purine nucleotide receptor, integrin alphaVbeta3, T-cell protein tyrosine phosphatase, etc.

[0316] Exemplary Human VEGFR-2 cDNA sequence (SEQ ID NO: 34)

ACTGAGTCCCGGGACCCCGGGAGAGCGGTCAATGTGTGGTCGCTGCGTTTCCTCTGC
CTGCGCCGGGCATCACTTGCGCGCCGCAGAAAGTCCGTCTGGCAGCCTGGATATCCT
CTCCTACCGGCACCCGCAGACGCCCCTGCAGCCGCGGTCGGCGCCCGGGCTCCCTAG
CCCTGTGCGCTCAACTGTCCTGCGCTGCGGGGTGCCGCGAGTTCCACCTCCGCGCCT
CCTTCTCTAGACAGGCGCTGGGAGAAAGAACCGGCTCCCGAGTTCTGGGCATTTCGC
CCGGCTCGAGGTGCAGGATGCAGAGCAAGGTGCTGCTGGCCGTCGCCCTGTGGCTC
TGCGTGGAGACCCGGGCCGCCTCTGTGGGTTTGCCTAGTGTTTCTCTTGATCTGCCCA
GGCTCAGCATACAAAAAGACATACTTACAATTAAGGCTAATACAACTCTTCAAATTA
CTTGCAGGGGACAGAGGGACTTGGACTGGCTTTGGCCCAATAATCAGAGTGGCAGT
GAGCAAAGGGTGGAGGTGACTGAGTGCAGCGATGGCCTCTTCTGTAAGACACTCAC
AATTCCAAAAGTGATCGGAAATGACACTGGAGCCTACAAGTGCTTCTACCGGGAAA
CTGACTTGGCCTCGGTCATTTATGTCTATGTTCAAGATTACAGATCTCCATTTATTGC
TTCTGTTAGTGACCAACATGGAGTCGTGTACATTACTGAGAACAAAAACAAAACTGT
GGTGATTCCATGTCTCGGGTCCATTTCAAATCTCAACGTGTCACTTTGTGCAAGATAC
CCAGAAAAGAGATTTGTTCCTGATGGTAACAGAATTTCCTGGGACAGCAAGAAGGG
CTTTACTATTCCCAGCTACATGATCAGCTATGCTGGCATGGTCTTCTGTGAAGCAAA
AATTAATGATGAAAGTTACCAGTCTATTATGTACATAGTTGTCGTTGTAGGGTATAG
GATTTATGATGTGGTTCTGAGTCCGTCTCATGGAATTGAACTATCTGTTGGAGAAAA
GCTTGTCTTAAATTGTACAGCAAGAACTGAACTAAATGTGGGGATTGACTTCAACTG
GGAATACCCTTCTTCGAAGCATCAGCATAAGAAACTTGTAAACCGAGACCTAAAAA
CCCAGTCTGGGAGTGAGATGAAGAAATTTTTGAGCACCTTAACTATAGATGGTGTAA
CCCGGAGTGACCAAGGATTGTACACCTGTGCAGCATCCAGTGGGCTGATGACCAAG
AAGAACAGCACATTTGTCAGGGTCCATGAAAAACCTTTTGTTGCTTTTGGAAGTGGC
ATGGAATCTCTGGTGGAAGCCACGGTGGGGGAGCGTGTCAGAATCCCTGCGAAGTA
CCTTGGTTACCCACCCCCAGAAATAAAATGGTATAAAAATGGAATACCCCTTGAGTC
CAATCACACAATTAAAGCGGGGCATGTACTGACGATTATGGAAGTGAGTGAAAGAG

ACACAGGAAATTACACTGTCATCCTTACCAATCCCATTTCAAAGGAGAAGCAGAGC
CATGTGGTCTCTCTGGTTGTGTATGTCCCACCCCAGATTGGTGAGAAATCTCTAATCT
CTCCTGTGGATTCCTACCAGTACGGCACCACTCAAACGCTGACATGTACGGTCTATG
CCATTCCTCCCCCGCATCACATCCACTGGTATTGGCAGTTGGAGGAAGAGTGCGCCA
ACGAGCCCAGCCAAGCTGTCTCAGTGACAAACCCATACCCTTGTGAAGAATGGAGA
AGTGTGGAGGACTTCCAGGGAGGAAATAAAATTGAAGTTAATAAAAATCAATTTGC
TCTAATTGAAGGAAAAAACAAAACTGTAAGTACCCTTGTTATCCAAGCGGCAAATG
TGTCAGCTTTGTACAAATGTGAAGCGGTCAACAAAGTCGGGAGAGGAGAGAGGGTG
ATCTCCTTCCACGTGACCAGGGGTCCTGAAATTACTTTGCAACCTGACATGCAGCCC
ACTGAGCAGGAGAGCGTGTCTTTGTGGTGCACTGCAGACAGATCTACGTTTGAGAAC
CTCACATGGTACAAGCTTGGCCCACAGCCTCTGCCAATCCATGTGGGAGAGTTGCCC
ACACCTGTTTGCAAGAACTTGGATACTCTTTGGAAATTGAATGCCACCATGTTCTCT
AATAGCACAAATGACATTTTGATCATGGAGCTTAAGAATGCATCCTTGCAGGACCAA
GGAGACTATGTCTGCCTTGCTCAAGACAGGAAGACCAAGAAAAGACATTGCGTGGT
CAGGCAGCTCACAGTCCTAGAGCGTGTGGCACCCACGATCACAGGAAACCTGGAGA
ATCAGACGACAAGTATTGGGGAAAGCATCGAAGTCTCATGCACGGCATCTGGGAAT
CCCCCTCCACAGATCATGTGGTTTAAAGATAATGAGACCCTTGTAGAAGACTCAGGC
ATTGTATTGAAGGATGGGAACCGGAACCTCACTATCCGCAGAGTGAGGAAGGAGGA
CGAAGGCCTCTACACCTGCCAGGCATGCAGTGTTCTTGGCTGTGCAAAAGTGGAGGC
ATTTTTCATAATAGAAGGTGCCCAGGAAAAGACGAACTTGGAAATCATTATTCTAGT
AGGCACGGCGGTGATTGCCATGTTCTTCTGGCTACTTCTTGTCATCATCCTACGGACC
GTTAAGCGGGCCAATGGAGGGGAACTGAAGACAGGCTACTTGTCCATCGTCATGGA
TCCAGATGAACTCCCATTGGATGAACATTGTGAACGACTGCCTTATGATGCCAGCAA
ATGGGAATTCCCCAGAGACCGGCTGAAGCTAGGTAAGCCTCTTGGCCGTGGTGCCTT
TGGCCAAGTGATTGAAGCAGATGCCTTTGGAATTGACAAGACAGCAACTTGCAGGA
CAGTAGCAGTCAAAATGTTGAAAGAAGGAGCAACACACAGTGAGCATCGAGCTCTC
ATGTCTGAACTCAAGATCCTCATTCATATTGGTCACCATCTCAATGTGGTCAACCTTC
TAGGTGCCTGTACCAAGCCAGGAGGGCCACTCATGGTGATTGTGGAATTCTGCAAAT
TTGGAAACCTGTCCACTTACCTGAGGAGCAAGAGAAATGAATTTGTCCCCTACAAGA
CCAAAGGGGCACGATTCCGTCAAGGGAAAGACTACGTTGGAGCAATCCCTGTGGAT
CTGAAACGGCGCTTGGACAGCATCACCAGTAGCCAGAGCTCAGCCAGCTCTGGATT

TGTGGAGGAGAAGTCCCTCAGTGATGTAGAAGAAGAGGAAGCTCCTGAAGATCTGT
ATAAGGACTTCCTGACCTTGGAGCATCTCATCTGTTACAGCTTCCAAGTGGCTAAGG
GCATGGAGTTCTTGGCATCGCGAAAGTGTATCCACAGGGACCTGGCGGCACGAAAT
ATCCTCTTATCGGAGAAGAACGTGGTTAAAATCTGTGACTTTGGCTTGGCCCGGGAT
ATTTATAAAGATCCAGATTATGTCAGAAAAGGAGATGCTCGCCTCCCTTTGAAATGG
ATGGCCCCAGAAACAATTTTTGACAGAGTGTACACAATCCAGAGTGACGTCTGGTCT
TTTGGTGTTTTGCTGTGGGAAATATTTTCCTTAGGTGCTTCTCCATATCCTGGGGTAA
AGATTGATGAAGAATTTTGTAGGCGATTGAAAGAAGGAACTAGAATGAGGGCCCCT
GATTATACTACACCAGAAATGTACCAGACCATGCTGGACTGCTGGCACGGGGAGCC
CAGTCAGAGACCCACGTTTTCAGAGTTGGTGGAACATTTGGGAAATCTCTTGCAAGC
TAATGCTCAGCAGGATGGCAAAGACTACATTGTTCTTCCGATATCAGAGACTTTGAG
CATGGAAGAGGATTCTGGACTCTCTCTGCCTACCTCACCTGTTTCCTGTATGGAGGA
GGAGGAAGTATGTGACCCCAAATTCCATTATGACAACACAGCAGGAATCAGTCAGT
ATCTGCAGAACAGTAAGCGAAAGAGCCGGCCTGTGAGTGTAAAAACATTTGAAGAT
ATCCCGTTAGAAGAACCAGAAGTAAAAGTAATCCCAGATGACAACCAGACGGACAG
TGGTATGGTTCTTGCCTCAGAAGAGCTGAAAACTTTGGAAGACAGAACCAAATTATC
TCCATCTTTTGGTGGAATGGTGCCCAGCAAAAGCAGGGAGTCTGTGGCATCTGAAGG
CTCAAACCAGACAAGCGGCTACCAGTCCGGATATCACTCCGATGACACAGACACCA
CCGTGTACTCCAGTGAGGAAGCAGAACTTTTAAAGCTGATAGAGATTGGAGTGCAA
ACCGGTAGCACAGCCCAGATTCTCCAGCCTGACTCGGGGACCACACTGAGCTCTCCT
CCTGTTTAAAAGGAAGCATCCACACCCCCAACTCCTGGACATCACATGAGAGGTGCT
GCTCAGATTTTCAAGTGTTGTTCTTTCCACCAGCAGGAAGTAGCCGCATTTGATTTTC
ATTTCGACAACAGAAAAAGGACCTCGGACTGCAGGGAGCCAGTCTTCTAGGCATAT
CCTGGAAGAGGCTTGTGACCCAAGAATGTGTCTGTGTCTTCTCCCAGTGTTGACCTG
ATCCTCTTTTTCATTCATTTAAAAAGCATTTATCATGCCCCCTGCTGCGGGTCTCACC
ATGGGTTTAGAACAAAGACGTTCAAGAAATGGCCCCATCCTCAAAGAAGTAGCAGT
ACCTGGGGAGCTGACACTTCTGTAAAACTAGAAGATAAACCAGGCAATGTAAGTGT
TCGAGGTGTTGAAGATGGGAAGGATTTGCAGGGCTGAGTCTATCCAAGAGGCTTTGT
TTAGGACGTGGGTCCCAAGCCAAGCCTTAAGTGTGGAATTCGGATTGATAGAAAGG
AAGACTAACGTTACCTTGCTTTGGAGAGTACTGGAGCCTGCAAATGCATTGTGTTTG
CTCTGGTGGAGGTGGGCATGGGGTCTGTTCTGAAATGTAAAGGGTTCAGACGGGGTT

TCTGGTTTTAGAAGGTTGCGTGTTCTTCGAGTTGGGCTAAAGTAGAGTTCGTTGTGCT
GTTTCTGACTCCTAATGAGAGTTCCTTCCAGACCGTTACGTGTCTCCTGGCCAAGCCC
CAGGAAGGAAATGATGCAGCTCTGGCTCCTTGTCTCCCAGGCTGATCCTTTATTCAG
AATACCACAAAGAAAGGACATTCAGCTCAAGGCTCCCTGCCGTGTTGAAGAGTTCT
GACTGCACAAACCAGCTTCTGGTTTCTTCTGGAATGAATACCCTCATATCTGTCCTGA
TGTGATATGTCTGAGACTGAATGCGGGAGGTTCAATGTGAAGCTGTGTGTGGTGTCA
AAGTTTCAGGAAGGATTTTACCCTTTTGTTCTTCCCCCTGTCCCCAACCCACTCTCAC
CCCGCAACCCATCAGTATTTTAGTTATTTGGCCTCTACTCCAGTAAACCTGATTGGGT
TTGTTCACTCTCTGAATGATTATTAGCCAGACTTCAAAATTATTTTATAGCCCAAATT
ATAACATCTATTGTATTATTTAGACTTTTAACATATAGAGCTATTTCTACTGATTTTT
GCCCTTGTTCTGTCCTTTTTTTCAAAAAAGAAAATGTGTTTTTTGTTTGGTACCATAG
TGTGAAATGCTGGGAACAATGACTATAAGACATGCTATGGCACATATATTTATAGTC
TGTTTATGTAGAAACAAATGTAATATATTAAAGCCTTATATATAATGAACTTTGTAC
TATTCACATTTTGTATCAGTATTATGTAGCATAACAAAGGTCATAATGCTTTCAGCA
ATTGATGTCATTTTATTAAAGAACATTGAAAAACTTGAAAAAAAAAAAAAAAAAAA

[0317] Exemplary Human VEGFR-2 precursor amino acid sequence (SEQ ID NO: 35)

MQSKVLLAVALWLCVETRAASVGLPSVSLDLPRLSIQKDILTIKANTTLQITCRGQRDLD
WLWPNNQSGSEQRVEVTECSDGLFCKTLTIPKVIGNDTGAYKCFYRETDLASVIYVYVQ
DYRSPFIASVSDQHGVVYITENKNKTVVIPCLGSISNLNVSLCARYPEKRFVPDGNRISW
DSKKGFTIPSYMISYAGMVFCEAKINDESYQSIMYIVVVVGYRIYDVVLSPSHGIELSVGE
KLVLNCTARTELNVGIDFNWEYPSSKHQHKKLVNRDLKTQSGSEMKKFLSTLTIDGVTR
SDQGLYTCAASSGLMTKKNSTFVRVHEKPFVAFGSGMESLVEATVGERVRIPAKYLGY
PPPEIKWYKNGIPLESNHTIKAGHVLTIMEVSERDTGNYTVILTNPISKEKQSHVVSLVVY
VPPQIGEKSLISPVDSYQYGTTQTLTCTVYAIPPPHHIHWYWQLEEECANEPSQAVSVTN
PYPCEEWRSVEDFQGGNKIEVNKNQFALIEGKNKTVSTLVIQAANVSALYKCEAVNKV
GRGERVISFHVTRGPEITLQPDMQPTEQESVSLWCTADRSTFENLTWYKLGPQPLPIHVG
ELPTPVCKNLDTLWKLNATMFSNSTNDILIMELKNASLQDQGDYVCLAQDRKTKKRHC
VVRQLTVLERVAPTITGNLENQTTSIGESIEVSCTASGNPPPQIMWFKDNETLVEDSGIVL
KDGNRNLTIRRVRKEDEGLYTCQACSVLGCAKVEAFFIIEGAQEKTNLEIIILVGTAVIAM
FFWLLLVIILRTVKRANGGELKTGYLSIVMDPDELPLDEHCERLPYDASKWEFPRDRLKL
GKPLGRGAFGQVIEADAFGIDKTATCRTVAVKMLKEGATHSEHRALMSELKILIHIGHH

LNVVNLLGACTKPGGPLMVIVEFCKFGNLSTYLRSKRNEFVPYKTKGARFRQGKDYVG
AIPVDLKRRLDSITSSQSSASSGFVEEKSLSDVEEEEAPEDLYKDFLTLEHLICYSFQVAK
GMEFLASRKCIHRDLAARNILLSEKNVVKICDFGLARDIYKDPDYVRKGDARLPLKWM
APETIFDRVYTIQSDVWSFGVLLWEIFSLGASPYPGVKIDEEFCRRLKEGTRMRAPDYTT
PEMYQTMLDCWHGEPSQRPTFSELVEHLGNLLQANAQQDGKDYIVLPISETLSMEEDSG
LSLPTSPVSCMEEEEVCDPKFHYDNTAGISQYLQNSKRKSRPVSVKTFEDIPLEEPEVKVI
PDDNQTDSGMVLASEELKTLEDRTKLSPSFGGMVPSKSRESVASEGSNQTSGYQSGYHS
DDTDTTVYSSEEAELLKLIEIGVQTGSTAQILQPDSGTTLSSPPV

[0318] The VEGFR-3 gene found at human chromosomal position 5q35.3 encodes a 35 exon containing member of the vascular endothelial growth factor receptor family (VEGFR) This gene encodes a tyrosine kinase receptor for vascular endothelial growth factors C and D. The protein is thought to be involved in lymphangiogenesis and maintenance of the lymphatic endothelium.

[0319] Exemplary Human VEGFR-3 Isoform 1 cDNA sequence (SEQ ID NO: 36)

ACTTTCAGCCCCGAGCCGCGGCCGCTCGGGTCGGACCCACGCGCAGCGGCCGGAGA
TGCAGCGGGGCGCCGCGCTGTGCCTGCGACTGTGGCTCTGCCTGGGACTCCTGGACG
GCCTGGTGAGTGGCTACTCCATGACCCCCCCGACCTTGAACATCACGGAGGAGTCAC
ACGTCATCGACACCGGTGACAGCCTGTCCATCTCCTGCAGGGGACAGCACCCCCTCG
AGTGGGCTTGGCCAGGAGCTCAGGAGGCGCCAGCCACCGGAGACAAGGACAGCGA
GGACACGGGGGTGGTGCGAGACTGCGAGGGCACAGACGCCAGGCCCTACTGCAAG
GTGTTGCTGCTGCACGAGGTACATGCCAACGACACAGGCAGCTACGTCTGCTACTAC
AAGTACATCAAGGCACGCATCGAGGGCACCACGGCCGCCAGCTCCTACGTGTTCGT
GAGAGACTTTGAGCAGCCATTCATCAACAAGCCTGACACGCTCTTGGTCAACAGGA
AGGACGCCATGTGGGTGCCCTGTCTGGTGTCCATCCCCGGCCTCAATGTCACGCTGC
GCTCGCAAAGCTCGGTGCTGTGGCCAGACGGGCAGGAGGTGGTGTGGGATGACCGG
CGGGGCATGCTCGTGTCCACGCCACTGCTGCACGATGCCCTGTACCTGCAGTGCGAG
ACCACCTGGGGAGACCAGGACTTCCTTTCCAACCCCTTCCTGGTGCACATCACAGGC
AACGAGCTCTATGACATCCAGCTGTTGCCCAGGAAGTCGCTGGAGCTGCTGGTAGG
GGAGAAGCTGGTCCTGAACTGCACCGTGTGGGCTGAGTTTAACTCAGGTGTCACCTT
TGACTGGGACTACCCAGGGAAGCAGGCAGAGCGGGGTAAGTGGGTGCCCGAGCGA
CGCTCCCAGCAGACCCACACAGAACTCTCCAGCATCCTGACCATCCACAACGTCAGC

CAGCACGACCTGGGCTCGTATGTGTGCAAGGCCAACAACGGCATCCAGCGATTTCG

GGAGAGCACCGAGGTCATTGTGCATGAAAATCCCTTCATCAGCGTCGAGTGGCTCA

AAGGACCCATCCTGGAGGCCACGGCAGGAGACGAGCTGGTGAAGCTGCCCGTGAAG

CTGGCAGCGTACCCCCCGCCCGAGTTCCAGTGGTACAAGGATGGAAAGGCACTGTC

CGGGCGCCACAGTCCACATGCCCTGGTGCTCAAGGAGGTGACAGAGGCCAGCACAG

GCACCTACACCCTCGCCCTGTGGAACTCCGCTGCTGGCCTGAGGCGCAACATCAGCC

TGGAGCTGGTGGTGAATGTGCCCCCCCAGATACATGAGAAGGAGGCCTCCTCCCCC

AGCATCTACTCGCGTCACAGCCGCCAGGCCCTCACCTGCACGGCCTACGGGGTGCCC

CTGCCTCTCAGCATCCAGTGGCACTGGCGGCCCTGGACACCCTGCAAGATGTTTGCC

CAGCGTAGTCTCCGGCGGCGGCAGCAGCAAGACCTCATGCCACAGTGCCGTGACTG

GAGGGCGGTGACCACGCAGGATGCCGTGAACCCCATCGAGAGCCTGGACACCTGGA

CCGAGTTTGTGGAGGGAAAGAATAAGACTGTGAGCAAGCTGGTGATCCAGAATGCC

AACGTGTCTGCCATGTACAAGTGTGTGGTCTCCAACAAGGTGGGCCAGGATGAGCG

GCTCATCTACTTCTATGTGACCACCATCCCCGACGGCTTCACCATCGAATCCAAGCC

ATCCGAGGAGCTACTAGAGGGCCAGCCGGTGCTCCTGAGCTGCCAAGCCGACAGCT

ACAAGTACGAGCATCTGCGCTGGTACCGCCTCAACCTGTCCACGCTGCACGATGCGC

ACGGGAACCCGCTTCTGCTCGACTGCAAGAACGTGCATCTGTTCGCCACCCCTCTGG

CCGCCAGCCTGGAGGAGGTGGCACCTGGGGCGCGCCACGCCACGCTCAGCCTGAGT

ATCCCCCGCGTCGCGCCCGAGCACGAGGGCCACTATGTGTGCGAAGTGCAAGACCG

GCGCAGCCATGACAAGCACTGCCACAAGAAGTACCTGTCGGTGCAGGCCCTGGAAG

CCCCTCGGCTCACGCAGAACTTGACCGACCTCCTGGTGAACGTGAGCGACTCGCTGG

AGATGCAGTGCTTGGTGGCCGGAGCGCACGCGCCCAGCATCGTGTGGTACAAAGAC

GAGAGGCTGCTGGAGGAAAAGTCTGGAGTCGACTTGGCGGACTCCAACCAGAAGCT

GAGCATCCAGCGCGTGCGCGAGGAGGATGCGGGACGCTATCTGTGCAGCGTGTGCA

ACGCCAAGGGCTGCGTCAACTCCTCCGCCAGCGTGGCCGTGGAAGGCTCCGAGGAT

AAGGGCAGCATGGAGATCGTGATCCTTGTCGGTACCGGCGTCATCGCTGTCTTCTTC

TGGGTCCTCCTCCTCCTCATCTTCTGTAACATGAGGAGGCCGGCCCACGCAGACATC

AAGACGGGCTACCTGTCCATCATCATGGACCCCGGGGAGGTGCCTCTGGAGGAGCA

ATGCGAATACCTGTCCTACGATGCCAGCCAGTGGGAATTCCCCCGAGAGCGGCTGC

ACCTGGGGAGAGTGCTCGGCTACGGCGCCTTCGGGAAGGTGGTGGAAGCCTCCGCT

TTCGGCATCCACAAGGGCAGCAGCTGTGACACCGTGGCCGTGAAAATGCTGAAAGA

GGGCGCCACGGCCAGCGAGCACCGCGCGCTGATGTCGGAGCTCAAGATCCTCATTC
ACATCGGCAACCACCTCAACGTGGTCAACCTCCTCGGGGCGTGCACCAAGCCGCAG
GGCCCCCTCATGGTGATCGTGGAGTTCTGCAAGTACGGCAACCTCTCCAACTTCCTG
CGCGCCAAGCGGGACGCCTTCAGCCCCTGCGCGGAGAAGTCTCCCGAGCAGCGCGG
ACGCTTCCGCGCCATGGTGGAGCTCGCCAGGCTGGATCGGAGGCGGCCGGGGAGCA
GCGACAGGGTCCTCTTCGCGCGGTTCTCGAAGACCGAGGGCGGAGCGAGGCGGGCT
TCTCCAGACCAAGAAGCTGAGGACCTGTGGCTGAGCCCGCTGACCATGGAAGATCT
TGTCTGCTACAGCTTCCAGGTGGCCAGAGGGATGGAGTTCCTGGCTTCCCGAAAGTG
CATCCACAGAGACCTGGCTGCTCGGAACATTCTGCTGTCGGAAAGCGACGTGGTGA
AGATCTGTGACTTTGGCCTTGCCCGGGACATCTACAAAGACCCCGACTACGTCCGCA
AGGGCAGTGCCCGGCTGCCCCTGAAGTGGATGGCCCCTGAAAGCATCTTCGACAAG
GTGTACACCACGCAGAGTGACGTGTGGTCCTTTGGGGTGCTTCTCTGGGAGATCTTC
TCTCTGGGGGCCTCCCCGTACCCTGGGGTGCAGATCAATGAGGAGTTCTGCCAGCGG
CTGAGAGACGGCACAAGGATGAGGGCCCCGGAGCTGGCCACTCCCGCCATACGCCG
CATCATGCTGAACTGCTGGTCCGGAGACCCCAAGGCGAGACCTGCATTCTCGGAGCT
GGTGGAGATCCTGGGGGACCTGCTCCAGGGCAGGGGCCTGCAAGAGGAAGAGGAG
GTCTGCATGGCCCCGCGCAGCTCTCAGAGCTCAGAAGAGGGCAGCTTCTCGCAGGT
GTCCACCATGGCCCTACACATCGCCCAGGCTGACGCTGAGGACAGCCCGCCAAGCC
TGCAGCGCCACAGCCTGGCCGCCAGGTATTACAACTGGGTGTCCTTTCCCGGGTGCC
TGGCCAGAGGGGCTGAGACCCGTGGTTCCTCCAGGATGAAGACATTTGAGGAATTC
CCCATGACCCCAACGACCTACAAAGGCTCTGTGGACAACCAGACAGACAGTGGGAT
GGTGCTGGCCTCGGAGGAGTTTGAGCAGATAGAGAGCAGGCATAGACAAGAAAGC
GGCTTCAGCTGTAAAGGACCTGGCCAGAATGTGGCTGTGACCAGGGCACACCCTGA
CTCCCAAGGGAGGCGGCGGCGGCCTGAGCGGGGGGCCCGAGGAGGCCAGGTGTTTT
ACAACAGCGAGTATGGGGAGCTGTCGGAGCCAAGCGAGGAGGACCACTGCTCCCCG
TCTGCCCGCGTGACTTTCTTCACAGACAACAGCTACTAAGCAGCATCGGACAAGACC
CCCAGCACTTGGGGGTTCAGGCCCGGCAGGGCGGGCAGAGGGCTGGAGGCCCAGGC
TGGGAACTCATCTGGTTGAACTCTGGTGGCACAGGAGTGTCCTCTTCCCTCTCTGCA
GACTTCCCAGCTAGGAAGAGCAGGACTCCAGGCCCAAGGCTCCCGGAATTCCGTCA
CCACGACTGGCCAGGGCCACGCTCCAGCTGCCCCGGCCCCTCCCCCTGAGATTCAGA
TGTCATTTAGTTCAGCATCCGCAGGTGCTGGTCCCGGGGCCAGCACTTCCATGGGAA

TGTCTCTTTGGCGACCTCCTTTCATCACACTGGGTGGTGGCCTGGTCCCTGTTTTCCC
ACGAGGAATCTGTGGGTCTGGGAGTCACACAGTGTTGGAGGTTAAGGCATACGAGA
GCAGAGGTCTCCCAAACGCCCTTTCCTCCTCAGGCACACAGCTACTCTCCCCACGAG
GGCTGGCTGGCCTCACCCACCCCTGCACAGTTGAAGGGAGGGGCTGTGTTTCCATCT
CAAAGAAGGCATTTGCAGGGTCCTCTTCTGGGCCTGACCAAACAGCCAACTAGCCC
CTGGGGTGGCCACCAGTATGACAGTATTATACGCTGGCAACACAGAGGCAGCCCGC
ACACCTGCGCCTGGGTGTTGAGAGCCATCCTGCAAGTCTTTTTCAACAGAACTTCAC
AGACTGTTAGAGCTGCTGAGAAGAATTTGCTTTCCGAATTCAGCCTGGAAGGCGCCC
AGGGACAGCTGTACTGAGTCTAGATGACTCTGACCCCCGCCCCAGGTCAAGGCCAG
CAGAGCAGTCAGTGCCTCTGGAGAAGGCCCTTGCTCTCCCACCTGGCCCAGACTCCG
AGGAGCCTGGGTCTGGAGCTGCCGGTCTGGTTCTTCCCTTTAGAGCCCGGATCTGCC
ACCTGCGGCCCCTCCCAAGCCGTGAACCAGCTCATGAGAGATGAACACTGTGGGAT
CCACTCAGGAAGGCTCGGGGCTGGCACAAAGGACCACCCAGCATTGCCCTGTGCCA
CCCAGCACTCAGTGGACATTCTGGGGACCTGCCTTCAGCCTTTTCCTGCCCTGTGCCT
GACATCAGCACCCTGGCTGGTCAGAATGCCGCCCTCCCAGAGGAGCAGCCGAGAGA
TCCCCTGAAGGCTGGAGGCATTCTGCTCAGGACCCCTATCCCAGCTCACAGTGCCCA
ACCATCTCACCAGGAGAAAGAGCCACATCCCCACGTTAGGACCACGGAGACTGACC
ACCACCCTGACCCCCCAAACCCACGCACCAGACGCTTGCAGGACAGGCGCCGCGCA
GCGGGCAGGGGCTTGCCCGGCCGACCCTCCCCTCCCCACCTCCCCCACTGCGCGTTA
CTCCAGGATATGCCGAGTGCACGTATAAGGTCATCTTCGTCGTCCCCGTGGACCTCC
CCCTTCCTCTGCACGTCGTCCAACGTGGGACTGGCGTGTCAGGCTTCCCTGGGAGGA
TCTGGAGGTTGTTCTCTGCAGAGAACCAGCCTGGCTCCTGGCGCGCACCTCTGCTCC
CTTCTCCTCACTACCCACCCACGCATGTACCGGGAAAAAAACTACTATGCCCTTCTA
GACCATGTTCTGAGAAAAGATCGAAAATATTTAACAAGAGATAATAATAAATCTGA
TGCCGGTCTTTGTGTGTGTTGCGGA

[0320] Exemplary Human VEGFR-3 Isoform 1 precursor amino acid sequence (SEQ ID NO: 37)

MQRGAALCLRLWLCLGLLDGLVSGYSMTPPTLNITEESHVIDTGDSLSISCRGQHPLEW
AWPGAQEAPATGDKDSEDTGVVRDCEGTDARPYCKVLLLHEVHANDTGSYVCYYKYI
KARIEGTTAASSYVFVRDFEQPFINKPDTLLVNRKDAMWVPCLVSIPGLNVTLRSQSSVL
WPDGQEVVWDDRRGMLVSTPLLHDALYLQCETTWGDQDFLSNPFLVHITGNELYDIQL

LPRKSLELLVGEKLVLNCTVWAEFNSGVTFDWDYPGKQAERGKWVPERRSQQTHTELS
SILTIHNVSQHDLGSYVCKANNGIQRFRESTEVIVHENPFISVEWLKGPILEATAGDELVK
LPVKLAAYPPPEFQWYKDGKALSGRHSPHALVLKEVTEASTGTYTLALWNSAAGLRRN
ISLELVVNVPPQIHEKEASSPSIYSRHSRQALTCTAYGVPLPLSIQWHWRPWTPCKMFAQ
RSLRRRQQQDLMPQCRDWRAVTTQDAVNPIESLDTWTEFVEGKNKTVSKLVIQNANVS
AMYKCVVSNKVGQDERLIYFYVTTIPDGFTIESKPSEELLEGQPVLLSCQADSYKYEHLR
WYRLNLSTLHDAHGNPLLLDCKNVHLFATPLAASLEEVAPGARHATLSLSIPRVAPEHE
GHYVCEVQDRRSHDKHCHKKYLSVQALEAPRLTQNLTDLLVNVSDSLEMQCLVAGAH
APSIVWYKDERLLEEKSGVDLADSNQKLSIQRVREEDAGRYLCSVCNAKGCVNSSASV
AVEGSEDKGSMEIVILVGTGVIAVFFWVLLLLIFCNMRRPAHADIKTGYLSIIMDPGEVPL
EEQCEYLSYDASQWEFPRERLHLGRVLGYGAFGKVVEASAFGIHKGSSCDTVAVKMLK
EGATASEHRALMSELKILIHIGNHLNVVNLLGACTKPQGPLMVIVEFCKYGNLSNFLRA
KRDAFSPCAEKSPEQRGRFRAMVELARLDRRRPGSSDRVLFARFSKTEGGARRASPDQE
AEDLWLSPLTMEDLVCYSFQVARGMEFLASRKCIHRDLAARNILLSESDVVKICDFGLA
RDIYKDPDYVRKGSARLPLKWMAPESIFDKVYTTQSDVWSFGVLLWEIFSLGASPYPGV
QINEEFCQRLRDGTRMRAPELATPAIRRIMLNCWSGDPKARPAFSELVEILGDLLQGRGL
QEEEEVCMAPRSSQSSEEGSFSQVSTMALHIAQADAEDSPPSLQRHSLAARYYNWVSFP
GCLARGAETRGSSRMKTFEEFPMTPTTYKGSVDNQTDSGMVLASEEFEQIESRHRQESG
FSCKGPGQNVAVTRAHPDSQGRRRRPERGARGGQVFYNSEYGELSEPSEEDHCSPSARV
TFFTDNSY

[0321] This variant (2) contains an alternate 3` terminal exon compared to variant 1. This results in an isoform (2) with a shorter C-terminus compared to isoform 1.

[0322] Exemplary Human VEGFR-3 Isoform 2 cDNA sequence (SEQ ID NO: 38)

ACTTTCAGCCCCGAGCCGCGGCCGCTCGGGTCGGACCCACGCGCAGCGGCCGGAGA
TGCAGCGGGGCGCCGCGCTGTGCCTGCGACTGTGGCTCTGCCTGGGACTCCTGGACG
GCCTGGTGAGTGGCTACTCCATGACCCCCCCGACCTTGAACATCACGGAGGAGTCAC
ACGTCATCGACACCGGTGACAGCCTGTCCATCTCCTGCAGGGGACAGCACCCCCTCG
AGTGGGCTTGGCCAGGAGCTCAGGAGGCGCCAGCCACCGGAGACAAGGACAGCGA
GGACACGGGGGTGGTGCGAGACTGCGAGGGCACAGACGCCAGGCCCTACTGCAAG
GTGTTGCTGCTGCACGAGGTACATGCCAACGACACAGGCAGCTACGTCTGCTACTAC

AAGTACATCAAGGCACGCATCGAGGGCACCACGGCCGCCAGCTCCTACGTGTTCGT
GAGAGACTTTGAGCAGCCATTCATCAACAAGCCTGACACGCTCTTGGTCAACAGGA
AGGACGCCATGTGGGTGCCCTGTCTGGTGTCCATCCCCGGCCTCAATGTCACGCTGC
GCTCGCAAAGCTCGGTGCTGTGGCCAGACGGGCAGGAGGTGGTGTGGGATGACCGG
CGGGGCATGCTCGTGTCCACGCCACTGCTGCACGATGCCCTGTACCTGCAGTGCGAG
ACCACCTGGGGAGACCAGGACTTCCTTTCCAACCCCTTCCTGGTGCACATCACAGGC
AACGAGCTCTATGACATCCAGCTGTTGCCCAGGAAGTCGCTGGAGCTGCTGGTAGG
GGAGAAGCTGGTCCTGAACTGCACCGTGTGGGCTGAGTTTAACTCAGGTGTCACCTT
TGACTGGGACTACCCAGGGAAGCAGGCAGAGCGGGGTAAGTGGGTGCCCGAGCGA
CGCTCCCAGCAGACCCACACAGAACTCTCCAGCATCCTGACCATCCACAACGTCAGC
CAGCACGACCTGGGCTCGTATGTGTGCAAGGCCAACAACGGCATCCAGCGATTTCG
GGAGAGCACCGAGGTCATTGTGCATGAAAATCCCTTCATCAGCGTCGAGTGGCTCA
AAGGACCCATCCTGGAGGCCACGGCAGGAGACGAGCTGGTGAAGCTGCCCGTGAAG
CTGGCAGCGTACCCCCCGCCCGAGTTCCAGTGGTACAAGGATGGAAAGGCACTGTC
CGGGCGCCACAGTCCACATGCCCTGGTGCTCAAGGAGGTGACAGAGGCCAGCACAG
GCACCTACACCCTCGCCCTGTGGAACTCCGCTGCTGGCCTGAGGCGCAACATCAGCC
TGGAGCTGGTGGTGAATGTGCCCCCCCAGATACATGAGAAGGAGGCCTCCTCCCCC
AGCATCTACTCGCGTCACAGCCGCCAGGCCCTCACCTGCACGGCCTACGGGGTGCCC
CTGCCTCTCAGCATCCAGTGGCACTGGCGGCCCTGGACACCCTGCAAGATGTTTGCC
CAGCGTAGTCTCCGGCGGCGGCAGCAGCAAGACCTCATGCCACAGTGCCGTGACTG
GAGGGCGGTGACCACGCAGGATGCCGTGAACCCCATCGAGAGCCTGGACACCTGGA
CCGAGTTTGTGGAGGGAAAGAATAAGACTGTGAGCAAGCTGGTGATCCAGAATGCC
AACGTGTCTGCCATGTACAAGTGTGTGGTCTCCAACAAGGTGGGCCAGGATGAGCG
GCTCATCTACTTCTATGTGACCACCATCCCCGACGGCTTCACCATCGAATCCAAGCC
ATCCGAGGAGCTACTAGAGGGCCAGCCGGTGCTCCTGAGCTGCCAAGCCGACAGCT
ACAAGTACGAGCATCTGCGCTGGTACCGCCTCAACCTGTCCACGCTGCACGATGCGC
ACGGGAACCCGCTTCTGCTCGACTGCAAGAACGTGCATCTGTTCGCCACCCCTCTGG
CCGCCAGCCTGGAGGAGGTGGCACCTGGGGCGCGCCACGCCACGCTCAGCCTGAGT
ATCCCCCGCGTCGCGCCCGAGCACGAGGGCCACTATGTGTGCGAAGTGCAAGACCG
GCGCAGCCATGACAAGCACTGCCACAAGAAGTACCTGTCGGTGCAGGCCCTGGAAG
CCCCTCGGCTCACGCAGAACTTGACCGACCTCCTGGTGAACGTGAGCGACTCGCTGG

AGATGCAGTGCTTGGTGGCCGGAGCGCACGCGCCCAGCATCGTGTGGTACAAAGAC

GAGAGGCTGCTGGAGGAAAAGTCTGGAGTCGACTTGGCGGACTCCAACCAGAAGCT

GAGCATCCAGCGCGTGCGCGAGGAGGATGCGGGACGCTATCTGTGCAGCGTGTGCA

ACGCCAAGGGCTGCGTCAACTCCTCCGCCAGCGTGGCCGTGGAAGGCTCCGAGGAT

AAGGGCAGCATGGAGATCGTGATCCTTGTCGGTACCGGCGTCATCGCTGTCTTCTTC

TGGGTCCTCCTCCTCCTCATCTTCTGTAACATGAGGAGGCCGGCCCACGCAGACATC

AAGACGGGCTACCTGTCCATCATCATGGACCCCGGGGAGGTGCCTCTGGAGGAGCA

ATGCGAATACCTGTCCTACGATGCCAGCCAGTGGGAATTCCCCCGAGAGCGGCTGC

ACCTGGGGAGAGTGCTCGGCTACGGCGCCTTCGGGAAGGTGGTGGAAGCCTCCGCT

TTCGGCATCCACAAGGGCAGCAGCTGTGACACCGTGGCCGTGAAAATGCTGAAAGA

GGGCGCCACGGCCAGCGAGCACCGCGCGCTGATGTCGGAGCTCAAGATCCTCATTC

ACATCGGCAACCACCTCAACGTGGTCAACCTCCTCGGGGCGTGCACCAAGCCGCAG

GGCCCCCTCATGGTGATCGTGGAGTTCTGCAAGTACGGCAACCTCTCCAACTTCCTG

CGCGCCAAGCGGGACGCCTTCAGCCCCTGCGCGGAGAAGTCTCCCGAGCAGCGCGG

ACGCTTCCGCGCCATGGTGGAGCTCGCCAGGCTGGATCGGAGGCGGCCGGGGAGCA

GCGACAGGGTCCTCTTCGCGCGGTTCTCGAAGACCGAGGGCGGAGCGAGGCGGGCT

TCTCCAGACCAAGAAGCTGAGGACCTGTGGCTGAGCCCGCTGACCATGGAAGATCT

TGTCTGCTACAGCTTCCAGGTGGCCAGAGGGATGGAGTTCCTGGCTTCCCGAAAGTG

CATCCACAGAGACCTGGCTGCTCGGAACATTCTGCTGTCGGAAAGCGACGTGGTGA

AGATCTGTGACTTTGGCCTTGCCCGGGACATCTACAAAGACCCCGACTACGTCCGCA

AGGGCAGTGCCCGGCTGCCCCTGAAGTGGATGGCCCCTGAAAGCATCTTCGACAAG

GTGTACACCACGCAGAGTGACGTGTGGTCCTTTGGGGTGCTTCTCTGGGAGATCTTC

TCTCTGGGGGCCTCCCCGTACCCTGGGGTGCAGATCAATGAGGAGTTCTGCCAGCGG

CTGAGAGACGGCACAAGGATGAGGGCCCCGGAGCTGGCCACTCCCGCCATACGCCG

CATCATGCTGAACTGCTGGTCCGGAGACCCCAAGGCGAGACCTGCATTCTCGGAGCT

GGTGGAGATCCTGGGGGACCTGCTCCAGGGCAGGGGCCTGCAAGAGGAAGAGGAG

GTCTGCATGGCCCCGCGCAGCTCTCAGAGCTCAGAAGAGGGCAGCTTCTCGCAGGT

GTCCACCATGGCCCTACACATCGCCCAGGCTGACGCTGAGGACAGCCCGCCAAGCC

TGCAGCGCCACAGCCTGGCCGCCAGGTATTACAACTGGGTGTCCTTTCCCGGGTGCC

TGGCCAGAGGGGCTGAGACCCGTGGTTCCTCCAGGATGAAGACATTTGAGGAATTC

CCCATGACCCCAACGACCTACAAAGGCTCTGTGGACAACCAGACAGACAGTGGGAT

GGTGCTGGCCTCGGAGGAGTTTGAGCAGATAGAGAGCAGGCATAGACAAGAAAGC
GGCTTCAGGTAGCTGAAGCAGAGAGAGAAGGCAGCATACGTCAGCATTTTCTTC
TCTGCACTTATAAGAAAGATCAAAGACTTTAAGACTTTCGCTATTTCTTCTACTGCTA
TCTACTACAAACTTCAAAGAGGAACCAGGAGGACAAGAGGAGCATGAAAGTGGAC
AAGGAGTGTGACCACTGAAGCACCACAGGGAGGGGTTAGGCCTCCGGATGACTGCG
GGCAGGCCTGGATAATATCCAGCCTCCCACAAGAAGCTGGTGGAGCAGAGTGTTCC
CTGACTCCTCCAAGGAAAGGGAGACGCCCTTTCATGGTCTGCTGAGTAACAGGTGCC
TTCCCAGACACTGGCGTTACTGCTTGACCAAAGAGCCCTCAAGCGGCCCTTATGCCA
GCGTGACAGAGGGCTCACCTCTTGCCTTCTAGGTCACTTCTCACAATGTCCCTTCAG
CACCTGACCCTGTGCCCACCAGTTATTCCTTGGTAATATGAGTAATACATCAAAGAG
TAGTATTAAAAGCTAATTAATCATGTTTATACTAA

[0323] Exemplary Human VEGFR-3 Isoform 1 precursor amino acid sequence (SEQ ID NO: 39)

MQRGAALCLRLWLCLGLLDGLVSGYSMTPPTLNITEESHVIDTGDSLSISCRGQHPLEW
AWPGAQEAPATGDKDSEDTGVVRDCEGTDARPYCKVLLLHEVHANDTGSYVCYYKYI
KARIEGTTAASSYVFVRDFEQPFINKPDTLLVNRKDAMWVPCLVSIPGLNVTLRSQSSVL
WPDGQEVVWDDRRGMLVSTPLLHDALYLQCETTWGDQDFLSNPFLVHITGNELYDIQL
LPRKSLELLVGEKLVLNCTVWAEFNSGVTFDWDYPGKQAERGKWVPERRSQQTHTELS
SILTIHNVSQHDLGSYVCKANNGIQRFRESTEVIVHENPFISVEWLKGPILEATAGDELVK
LPVKLAAYPPPEFQWYKDGKALSGRHSPHALVLKEVTEASTGTYTLALWNSAAGLRRN
ISLELVVNVPPQIHEKEASSPSIYSRHSRQALTCTAYGVPLPLSIQWHWRPWTPCKMFAQ
RSLRRRQQQDLMPQCRDWRAVTTQDAVNPIESLDTWTEFVEGKNKTVSKLVIQNANVS
AMYKCVVSNKVGQDERLIYFYVTTIPDGFTIESKPSEELLEGQPVLLSCQADSYKYEHLR
WYRLNLSTLHDAHGNPLLLDCKNVHLFATPLAASLEEVAPGARHATLSLSIPRVAPEHE
GHYVCEVQDRRSHDKHCHKKYLSVQALEAPRLTQNLTDLLVNVSDSLEMQCLVAGAH
APSIVWYKDERLLEEKSGVDLADSNQKLSIQRVREEDAGRYLCSVCNAKGCVNSSASV
AVEGSEDKGSMEIVILVGTGVIAVFFWVLLLLIFCNMRRPAHADIKTGYLSIIMDPGEVPL
EEQCEYLSYDASQWEFPRERLHLGRVLGYGAFGKVVEASAFGIHKGSSCDTVAVKMLK
EGATASEHRALMSELKILIHIGNHLNVVNLLGACTKPQGPLMVIVEFCKYGNLSNFLRA
KRDAFSPCAEKSPEQRGRFRAMVELARLDRRRPGSSDRVLFARFSKTEGGARRASPDQE
AEDLWLSPLTMEDLVCYSFQVARGMEFLASRKCIHRDLAARNILLSESDVVKICDFGLA

RDIYKDPDYVRKGSARLPLKWMAPESIFDKVYTTQSDVWSFGVLLWEIFSLGASPYPGV
QINEEFCQRLRDGTRMRAPELATPAIRRIMLNCWSGDPKARPAFSELVEILGDLLQGRGL
QEEEEVCMAPRSSQSSEEGSFSQVSTMALHIAQADAEDSPPSLQRHSLAARYYNWVSFP
GCLARGAETRGSSRMKTFEEFPMTPTTYKGSVDNQTDSGMVLASEEFEQIESRHRQESG
FR

[0324] This variant (3) contains an alternate 3` terminal exon compared to variant 1. This results in an isoform (3) with a shorter C-terminus compared to isoform 1.

[0325] Exemplary Human VEGFR-3 Isoform 2 cDNA sequence (SEQ ID NO: 40)

ACTTTCAGCCCCGAGCCGCGGCCGCTCGGGTCGGACCCACGCGCAGCGGCCGGAGA
TGCAGCGGGGCGCCGCGCTGTGCCTGCGACTGTGGCTCTGCCTGGGACTCCTGGACG
GCCTGGTGAGTGGCTACTCCATGACCCCCCCGACCTTGAACATCACGGAGGAGTCAC
ACGTCATCGACACCGGTGACAGCCTGTCCATCTCCTGCAGGGGACAGCACCCCCTCG
AGTGGGCTTGGCCAGGAGCTCAGGAGGCGCCAGCCACCGGAGACAAGGACAGCGA
GGACACGGGGGTGGTGCGAGACTGCGAGGGCACAGACGCCAGGCCCTACTGCAAG
GTGTTGCTGCTGCACGAGGTACATGCCAACGACACAGGCAGCTACGTCTGCTACTAC
AAGTACATCAAGGCACGCATCGAGGGCACCACGGCCGCCAGCTCCTACGTGTTCGT
GAGAGACTTTGAGCAGCCATTCATCAACAAGCCTGACACGCTCTTGGTCAACAGGA
AGGACGCCATGTGGGTGCCCTGTCTGGTGTCCATCCCCGGCCTCAATGTCACGCTGC
GCTCGCAAAGCTCGGTGCTGTGGCCAGACGGGCAGGAGGTGGTGTGGGATGACCGG
CGGGGCATGCTCGTGTCCACGCCACTGCTGCACGATGCCCTGTACCTGCAGTGCGAG
ACCACCTGGGGAGACCAGGACTTCCTTTCCAACCCCTTCCTGGTGCACATCACAGGC
AACGAGCTCTATGACATCCAGCTGTTGCCCAGGAAGTCGCTGGAGCTGCTGGTAGG
GGAGAAGCTGGTCCTGAACTGCACCGTGTGGGCTGAGTTTAACTCAGGTGTCACCTT
TGACTGGGACTACCCAGGGAAGCAGGCAGAGCGGGGTAAGTGGGTGCCCGAGCGA
CGCTCCCAGCAGACCCACACAGAACTCTCCAGCATCCTGACCATCCACAACGTCAGC
CAGCACGACCTGGGCTCGTATGTGTGCAAGGCCAACAACGGCATCCAGCGATTTCG
GGAGAGCACCGAGGTCATTGTGCATGAAAATCCCTTCATCAGCGTCGAGTGGCTCA
AAGGACCCATCCTGGAGGCCACGGCAGGAGACGAGCTGGTGAAGCTGCCCGTGAAG
CTGGCAGCGTACCCCCCGCCCGAGTTCCAGTGGTACAAGGATGGAAAGGCACTGTC
CGGGCGCCACAGTCCACATGCCCTGGTGCTCAAGGAGGTGACAGAGGCCAGCACAG

GCACCTACACCCTCGCCCTGTGGAACTCCGCTGCTGGCCTGAGGCGCAACATCAGCC
TGGAGCTGGTGGTGAATGTGCCCCCCCCAGATACATGAGAAGGAGGCCTCCTCCCCC
AGCATCTACTCGCGTCACAGCCGCCAGGCCCTCACCTGCACGGCCTACGGGGTGCCC
CTGCCTCTCAGCATCCAGTGGCACTGGCGGCCCTGGACACCCTGCAAGATGTTTGCC
CAGCGTAGTCTCCGGCGGCGGCAGCAGCAAGACCTCATGCCACAGTGCCGTGACTG
GAGGGCGGTGACCACGCAGGATGCCGTGAACCCCATCGAGAGCCTGGACACCTGGA
CCGAGTTTGTGGAGGGAAAGAATAAGACTGTGAGCAAGCTGGTGATCCAGAATGCC
AACGTGTCTGCCATGTACAAGTGTGTGGTCTCCAACAAGGTGGGCCAGGATGAGCG
GCTCATCTACTTCTATGTGACCACCATCCCCGACGGCTTCACCATCGAATCCAAGCC
ATCCGAGGAGCTACTAGAGGGCCAGCCGGTGCTCCTGAGCTGCCAAGCCGACAGCT
ACAAGTACGAGCATCTGCGCTGGTACCGCCTCAACCTGTCCACGCTGCACGATGCGC
ACGGGAACCCGCTTCTGCTCGACTGCAAGAACGTGCATCTGTTCGCCACCCCTCTGG
CCGCCAGCCTGGAGGAGGTGGCACCTGGGGCGCGCCACGCCACGCTCAGCCTGAGT
ATCCCCCGCGTCGCGCCCGAGCACGAGGGCCACTATGTGTGCGAAGTGCAAGACCG
GCGCAGCCATGACAAGCACTGCCACAAGAAGTACCTGTCGGTGCAGGCCCTGGAAG
CCCCTCGGCTCACGCAGAACTTGACCGACCTCCTGGTGAACGTGAGCGACTCGCTGG
AGATGCAGTGCTTGGTGGCCGGAGCGCACGCGCCCAGCATCGTGTGGTACAAAGAC
GAGAGGCTGCTGGAGGAAAAGTCTGGAGTCGACTTGGCGGACTCCAACCAGAAGCT
GAGCATCCAGCGCGTGCGCGAGGAGGATGCGGGACGCTATCTGTGCAGCGTGTGCA
ACGCCAAGGGCTGCGTCAACTCCTCCGCCAGCGTGGCCGTGGAAGGCTCCGAGGAT
AAGGGCAGCATGGAGATCGTGATCCTTGTCGGTACCGGCGTCATCGCTGTCTTCTTC
TGGGTCCTCCTCCTCCTCATCTTCTGTAACATGAGGAGGCCGGCCCACGCAGACATC
AAGACGGGCTACCTGTCCATCATCATGGACCCCGGGGAGGTGCCTCTGGAGGAGCA
ATGCGAATACCTGTCCTACGATGCCAGCCAGTGGGAATTCCCCCGAGAGCGGCTGC
ACCTGGGGAGAGTGCTCGGCTACGGCGCCTTCGGGAAGGTGGTGGAAGCCTCCGCT
TTCGGCATCCACAAGGGCAGCAGCTGTGACACCGTGGCCGTGAAAATGCTGAAAGA
GGGCGCCACGGCCAGCGAGCACCGCGCGCTGATGTCGGAGCTCAAGATCCTCATTC
ACATCGGCAACCACCTCAACGTGGTCAACCTCCTCGGGGCGTGCACCAAGCCGCAG
GGCCCCCTCATGGTGATCGTGGAGTTCTGCAAGTACGGCAACCTCTCCAACTTCCTG
CGCGCCAAGCGGGACGCCTTCAGCCCCTGCGCGGAGAAGTCTCCCGAGCAGCGCGG
ACGCTTCCGCGCCATGGTGGAGCTCGCCAGGCTGGATCGGAGGCGGCCGGGGAGCA

GCGACAGGGTCCTCTTCGCGCGGTTCTCGAAGACCGAGGGCGGAGCGAGGCGGGCT
TCTCCAGACCAAGAAGCTGAGGACCTGTGGCTGAGCCCGCTGACCATGGAAGATCT
TGTCTGCTACAGCTTCCAGGTGGCCAGAGGGATGGAGTTCCTGGCTTCCCGAAAGTG
CATCCACAGAGACCTGGCTGCTCGGAACATTCTGCTGTCGGAAAGCGACGTGGTGA
AGATCTGTGACTTTGGCCTTGCCCGGGACATCTACAAAGACCCCGACTACGTCCGCA
AGGGCAGTGCCCGGCTGCCCCTGAAGTGGATGGCCCCTGAAAGCATCTTCGACAAG
GTGTACACCACGCAGAGTGACGTGTGGTCCTTTGGGGTGCTTCTCTGGGAGATCTTC
TCTCTGGGGGCCTCCCCGTACCCTGGGGTGCAGATCAATGAGGAGTTCTGCCAGCGG
CTGAGAGACGGCACAAGGATGAGGGCCCCGGAGCTGGCCACTCCCGCCATACGCCG
CATCATGCTGAACTGCTGGTCCGGAGACCCCAAGGCGAGACCTGCATTCTCGGAGCT
GGTGGAGATCCTGGGGGACCTGCTCCAGGGCAGGGGCCTGCAAGAGGAAGAGGAG
GTCTGCATGGCCCCGCGCAGCTCTCAGAGCTCAGAAGAGGGCAGCTTCTCGCAGGT
GTCCACCATGGCCCTACACATCGCCCAGGCTGACGCTGAGGACAGCCCGCCAAGCC
TGCAGCGCCACAGCCTGGCCGCCAGGTATTACAACTGGGTGTCCTTTCCCGGGTGCC
TGGCCAGAGGGGCTGAGACCCGTGGTTCCTCCAGGATGAAGACATTTGAGGAATTC
CCCATGACCCCAACGACCTACAAAGGCTCTGTGGACAACCAGACAGACAGTGGGAT
GGTGCTGGCCTCGGAGGAGTTTGAGCAGATAGAGAGCAGGCATAGACAAGAAAGC
GGCTTCAGAGGAACCAGGAGGACAAGAGGAGCATGAAAGTGGACAAGGAGTGTGA
CCACTGAAGCACCACAGGGAGGGGTTAGGCCTCCGGATGACTGCGGGCAGGCCTGG
ATAATATCCAGCCTCCCACAAGAAGCTGGTGGAGCAGAGTGTTCCCTGACTCCTCCA
AGGAAAGGGAGACGCCCTTTCATGGTCTGCTGAGTAACAGGTGCCTTCCCAGACACT
GGCGTTACTGCTTGACCAAAGAGCCCTCAAGCGGCCCTTATGCCAGCGTGACAGAG
GGCTCACCTCTTGCCTTCTAGGTCACTTCTCACAATGTCCCTTCAGCACCTGACCCTG
TGCCCACCAGTTATTCCTTGGTAATATGAGTAATACATCAAAGAGTAGTATTAAAAG
CTAATTAATCATGTTTATACTAA

[0326] Exemplary Human VEGFR-3 Isoform 1 precursor amino acid sequence (SEQ ID NO: 41)

MQRGAALCLRLWLCLGLLDGLVSGYSMTPPTLNITEESHVIDTGDSLSISCRGQHPLEW
AWPGAQEAPATGDKDSEDTGVVRDCEGTDARPYCKVLLLHEVHANDTGSYVCYYKYI
KARIEGTTAASSYVFVRDFEQPFINKPDTLLVNRKDAMWVPCLVSIPGLNVTLRSQSSVL
WPDGQEVVWDDRRGMLVSTPLLHDALYLQCETTWGDQDFLSNPFLVHITGNELYDIQL

LPRKSLELLVGEKLVLNCTVWAEFNSGVTFDWDYPGKQAERGKWVPERRSQQTHTELS
SILTIHNVSQHDLGSYVCKANNGIQRFRESTEVIVHENPFISVEWLKGPILEATAGDELVK
LPVKLAAYPPPEFQWYKDGKALSGRHSPHALVLKEVTEASTGTYTLALWNSAAGLRRN
ISLELVVNVPPQIHEKEASSPSIYSRHSRQALTCTAYGVPLPLSIQWHWRPWTPCKMFAQ
RSLRRRQQQDLMPQCRDWRAVTTQDAVNPIESLDTWTEFVEGKNKTVSKLVIQNANVS
AMYKCVVSNKVGQDERLIYFYVTTIPDGFTIESKPSEELLEGQPVLLSCQADSYKYEHLR
WYRLNLSTLHDAHGNPLLLDCKNVHLFATPLAASLEEVAPGARHATLSLSIPRVAPEHE
GHYVCEVQDRRSHDKHCHKKYLSVQALEAPRLTQNLTDLLVNVSDSLEMQCLVAGAH
APSIVWYKDERLLEEKSGVDLADSNQKLSIQRVREEDAGRYLCSVCNAKGCVNSSASV
AVEGSEDKGSMEIVILVGTGVIAVFFWVLLLLIFCNMRRPAHADIKTGYLSIIMDPGEVPL
EEQCEYLSYDASQWEFPRERLHLGRVLGYGAFGKVVEASAFGIHKGSSCDTVAVKMLK
EGATASEHRALMSELKILIHIGNHLNVVNLLGACTKPQGPLMVIVEFCKYGNLSNFLRA
KRDAFSPCAEKSPEQRGRFRAMVELARLDRRRPGSSDRVLFARFSKTEGGARRASPDQE
AEDLWLSPLTMEDLVCYSFQVARGMEFLASRKCIHRDLAARNILLSESDVVKICDFGLA
RDIYKDPDYVRKGSARLPLKWMAPESIFDKVYTTQSDVWSFGVLLWEIFSLGASPYPGV
QINEEFCQRLRDGTRMRAPELATPAIRRIMLNCWSGDPKARPAFSELVEILGDLLQGRGL
QEEEEVCMAPRSSQSSEEGSFSQVSTMALHIAQADAEDSPPSLQRHSLAARYYNWVSFP
GCLARGAETRGSSRMKTFEEFPMTPTTYKGSVDNQTDSGMVLASEEFEQIESRHRQESG
FRGTRRTRGA

Fusion Proteins

[0327] Fusion proteins or chimeric proteins are proteins created through the joining of two or more genes that originally coded for separate proteins. Translation of a fusion gene results in creation of a single or multiple polypeptides with functional properties derived from each of the original proteins. Recombinant fusion proteins are created artificially, and resultant proteins may have underlying polypeptides with different functions and/or physico-chemical patterns. In some embodiments, anti-VEGF protein compositions as described herein comprise fusions of one or more VEGF-R proteins or characteristic portions thereof as described herein.

[0328] For example, antibody fusion proteins may combine an antibody that targets a specific antigen, with a protein that is able to modify an immune response or induce direct damage to a cancer cell. For example, cytolitic fusion proteins increase the potency of antibodies to eliminate

cancer cells, by attaching them to a toxin. These so called "immunotoxins" derive their potency from the toxin and their specificity from the antibody or antibody fragment to which they are attached. In certain embodiments, such fusion proteins may comprise a polypeptide (e.g., antibody and/or characteristic portion thereof) fused to a cytokine, to create an "immunocytokines". Such a fusion protein may combine a tumor-targeting antibody (e.g., an anti-VEGF antibody) with a cytokine (an innate immune response mediator). In some such embodiments, this increases local activation of an immune system in a tumor microenvironment, potentially supporting elimination of a cancerous cell and/or tumor (e.g., VS).

[0329] In some embodiments, any of the anti-VEGF proteins described herein (e.g., an antibody, soluble VEGF receptor, and/or characteristic VEGF binding portion thereof) can include one or more (e.g., two, three, four, five, six, seven, eight, nine, or ten) amino acid substitutions in an Fc region that decrease the half-life of the antibody, and/or soluble VEGF receptor in a mammal, e.g., as compared to the half-life of an otherwise identical antibody, and/or soluble VEGF receptor not including at least one of the one or more amino acid substitutions. Methods for determining the half-life of an antibody, and/or soluble VEGF receptor in a mammal are well-known in the art. Non-limiting examples of point mutations in a Fc mutation that can decrease the half-life of an antibody, and/or soluble VEGF receptor are described in Leabman et al., *MAbs* 5(6):896-903, 2013; which is incorporated in its entirety herein by reference.

Aflibercept

[0330] In certain embodiments, an anti-VEGF protein as described herein is Aflibercept. Aflibercept is a recombinant fusion protein consisting of portions of human VEGF receptors 1 and 2 extracellular domains fused to the Fc portion of human IgG1. Aflibercept is a dimeric glycoprotein with a protein molecular weight of approximately 97 kilodaltons (kDa) and may contain post-translational glycosylation, which may constitute an additional 15% of the total molecular mass, resulting in a total molecular weight of 115 kDa. Aflibercept is FDA approved, and has been indicated for the treatment of Neovascular (Wet) Age-Related Macular Degeneration, Macular Edema Following Retinal Vein Occlusion, Diabetic Macular Edema, Metastatic Colorectal Cancer, and Diabetic Retinopathy.

[0331] In certain embodiments, Aflibercept functions as a VEGF-TRAP. In contrast to certain exemplary antibody-based VEGF binding strategies described herein, e.g., utilizing ranibizumab

and/or bevacizumab, in some embodiments, the VEGF-TRAP incorporates the second binding domain of the VEGFR-1 receptor and the third domain of the VEGFR-2 receptor. By fusing these extracellular protein sequences to the Fc segment of a human IgG backbone a chimeric protein is produced with a very high VEGF binding affinity (Kd≈1 pM). Like ranibizumab and bevacizumab, a VEGF-TRAP such as aflibercept binds all isomers of the VEGF-A family, and also binds VEGF-B and placental growth factor. In certain embodiments, an Aflibercept protein is conjugated to a suitable secretion signal sequence, such as an IL2SS (described below). In certain embodiments, a VEGF-TRAP protein product is represented by SEQ ID NO: 43 or 44.

[0332] Exemplary Aflibercept nucleotide coding sequence (SEQ ID NO: 42)

ATGTACCGGATGCAGCTGCTGAGCTGTATCGCCCTGTCTCTGGCCCTGGTCACCAAT
TCTAGCGATACCGGCAGACCCTTCGTGGAAATGTACAGCGAGATCCCCGAGATCAT
CCACATGACCGAGGGCAGAGAGCTGGTCATCCCCTGCAGAGTGACAAGCCCCAACA
TCACCGTGACTCTGAAGAAGTTCCCTCTGGACACACTGATCCCCGACGGCAAGAGA
ATCATCTGGGACAGCCGGAAGGGCTTCATCATCAGCAACGCCACCTACAAAGAGAT
CGGCCTGCTGACCTGTGAAGCCACCGTGAATGGCCACCTGTACAAGACCAACTACCT
GACACACAGACAGACCAACACCATCATCGACGTGGTGCTGAGCCCTAGCCACGGCA
TTGAACTGTCTGTGGGCGAGAAGCTGGTGCTGAACTGTACCGCCAGAACCGAGCTG
AACGTGGGCATCGACTTCAACTGGGAGTACCCCAGCAGCAAGCACCAGCACAAGAA
ACTGGTCAACCGGGACCTGAAAACCCAGAGCGGCAGCGAGATGAAGAAATTCCTGA
GCACCCTGACCATCGACGGCGTGACCAGATCTGACCAGGGCCTGTACACATGTGCC
GCCAGCTCTGGCCTGATGACCAAGAAAAACAGCACCTTCGTGCGGGTGCACGAGAA
GGACAAGACCCACACCTGTCCTCCATGTCCTGCTCCAGAACTGCTCGGCGGACCTTC
CGTGTTCCTGTTTCCTCCAAAGCCTAAGGACACCCTGATGATCAGCAGAACCCCTGA
AGTGACCTGCGTGGTGGTGGATGTGTCCCACGAGGATCCCGAAGTGAAGTTCAATTG
GTACGTGGACGGCGTGGAAGTGCACAACGCCAAGACCAAGCCTAGAGAGGAACAG
TACAATAGCACCTACAGAGTGGTGTCCGTGCTGACCGTGCTGCACCAGGATTGGCTG
AACGGCAAAGAGTACAAGTGCAAGGTGTCCAACAAGGCCCTGCCTGCTCCTATCGA
GAAAACCATCTCCAAGGCCAAGGGCCAGCCTAGGGAACCCCAGGTTTACACACTGC
CTCCAAGCAGGGACGAGCTGACAAAGAACCAGGTGTCCCTGACCTGCCTGGTCAAG
GGCTTCTACCCTTCCGATATCGCCGTGGAATGGGAGAGCAATGGCCAGCCTGAGAA
CAACTACAAGACAACCCCTCCTGTGCTGGACAGCGACGGCTCATTCTTCCTGTACAG

CAAGCTGACAGTGGACAAGAGCAGATGGCAGCAGGGCAACGTGTTCAGCTGCAGCG
TGATGCACGAGGCCCTGCACAACCACTACACCCAGAAGTCCCTGAGCCTGTCTCCTG
GA

[0333] Exemplary Aflibercept amino acid sequence (SEQ ID NO: 43)

MYRMQLLSCIALSLALVTNSSDTGRPFVEMYSEIPEIIHMTEGRELVIPCRVTSPNITVTLK
KFPLDTLIPDGKRIIWDSRKGFIISNATYKEIGLLTCEATVNGHLYKTNYLTHRQTNTIIDV
VLSPSHGIELSVGEKLVLNCTARTELNVGIDFNWEYPSSKHQHKKLVNRDLKTQSGSEM
KKFLSTLTIDGVTRSDQGLYTCAASSGLMTKKNSTFVRVHEKDKTHTCPPCPAPELLGG
PSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY
NSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSR
DELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDK
SRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG

[0334] Exemplary Aflibercept Extracellular (EC) Domain amino acid sequence (SEQ ID NO: 112)

MYRMQLLSCIALSLALVTNSSDTGRPFVEMYSEIPEIIHMTEGRELVIPCRVTSPNITVTLK
KFPLDTLIPDGKRIIWDSRKGFIISNATYKEIGLLTCEATVNGHLYKTNYLTHRQTNTIIDV
VLSPSHGIELSVGEKLVLNCTARTELNVGIDFNWEYPSSKHQHKKLVNRDLKTQSGSEM
KKFLSTLTIDGVTRSDQGLYTCAASSGLMTKKNSTFVRVHEK

[0335] Exemplary Aflibercept amino acid sequence (SEQ ID NO: 44)

SDTGRPFVEMYSEIPEIIHMTEGRELVIPCRVTSPNITVTLKKFPLDTLIPDGKRIIWDSRKG
FIISNATYKEIGLLTCEATVNGHLYKTNYLTHRQTNTIIDVVLSPSHGIELSVGEKLVLNC
TARTELNVGIDFNWEYPSSKHQHKKLVNRDLKTQSGSEMKKFLSTLTIDGVTRSDQGLY
TCAASSGLMTKKNSTFVRVHEKDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPE
VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWL
NGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYP
SDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEAL
HNHYTQKSLSLSPG

[0336] Exemplary Fragment, Crystallizable (Fc) Nucleotide Sequence (SEQ ID NO: 110)

GACAAGACCCACACCTGTCCTCCATGTCCTGCTCCAGAACTGCTCGGCGGACCTTCC
GTGTTCCTGTTTCCTCCAAAGCCTAAGGACACCCTGATGATCAGCAGAACCCCTGAA
GTGACCTGCGTGGTGGTGGATGTGTCCCACGAGGATCCCGAAGTGAAGTTCAATTGG
TACGTGGACGGCGTGGAAGTGCACAACGCCAAGACCAAGCCTAGAGAGGAACAGT
ACAACAGCACCTACAGAGTGGTGTCCGTGCTGACCGTGCTGCACCAGGATTGGCTG
AACGGCAAAGAGTACAAGTGCAAGGTGTCCAACAAGGCCCTGCCTGCTCCTATCGA
GAAAACCATCAGCAAGGCCAAGGGCCAGCCTAGGGAACCCCAGGTTTACACACTGC
CTCCAAGCCGGGAAGAGATGACCAAGAACCAGGTGTCCCTGACCTGCCTCGTGAAG
GGCTTCTACCCTTCCGATATCGCCGTGGAATGGGAGAGCAATGGCCAGCCAGAGAA
CAACTACAAGACAACCCCTCCTGTGCTGGACAGCGACGGCTCATTCTTCCTGTACAG
CAAGCTGACAGTGGACAAGTCCAGATGGCAGCAGGGCAACGTGTTCAGCTGCAGCG
TGATGCACGAGGCCCTGCACAACCACTACACCCAGAAGTCTCTGAGCCTGTCTCCTG
GC

[0337] Exemplary Fragment, Crystallizable (Fc) Amino Acid Sequence (SEQ ID NO: 111)

DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV
DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTIS
KAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPP
VLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG

Constructs

[0338] 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 an anti-VEGF protein, e.g., an anti-VEGF antibody, and/or a soluble VEGF Receptor (e.g., an anti-VEGF-TRAP protein (e.g., a fusion protein such as aflibercept)). 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 embodiments, a construct is a plasmid (e.g., a circular DNA molecule that can autonomously

replicate inside a cell). In some embodiments, a construct can be a cosmid (e.g., pWE or sCos series).

[0339] In some embodiments, a construct is a viral construct. In some embodiments, a viral construct is a lentivirus, retrovirus, adenovirus, or adeno-associated virus construct. In some embodiments, 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). A simplified example of a WT AAV genome is represented in **FIG. 5, Panel (A)**, while a simplified example of a recombinant AAV (rAAV) construct is represented in **FIG. 5, Panel (B)**. In some embodiments, a viral construct is an adenovirus construct. In some embodiments, 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 in its entirety by reference.

[0340] Constructs provided herein can be of different sizes. In some embodiments, 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 8kb, up to about 9 kb, up to about 10 kb, up to about 11 kb, up to about 12 kb, up to about 13 kb, up to about 14 kb, or up to about 15 kb. In some embodiments, 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.

[0341] In some embodiments, a construct is a viral construct and can have a total number of nucleotides of up to 10 kb. In some embodiments, 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.

[0342] In some embodiments, 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 8kb, about 6 kb to about 7 kb, or about 7 kb to about 8 kb

[0343] In some embodiments, a construct is an adenovirus construct and can have a total number of nucleotides of up to 8 kb. In some embodiments, 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.

[0344] 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 embodiments, 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.

AAV Particles

[0345] Among other things, the present disclosure provides AAV particles that comprise an AAV capsid described herein containing a recombinant AAV (rAAV) construct encoding an anti-VEGF protein, e.g., an anti-VEGF antibody and/or an anti-VEGF-TRAP protein. In some embodiments, AAV particles can be described as having a serotype, which is a description of the construct strain and the capsid strain. For example, in some embodiments 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 embodiments, 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. This same nomenclature can also be used for rAAV capsids, constructs, and/or particles.

[0346] In certain embodiments, gene therapy using rAAV particles is a promising therapeutic modality for inner ear disorders for several reasons: (1) an inner ear, which contains the auditory and vestibular sensory epithelia, has modified immune surveillance, similar to that in the central

nervous system (Fujioka 2014, incorporated herein in its entirety by reference); (2) sensory and supporting cells of the cochlear organ of Corti are post-mitotic, allowing for the possibility of long-term expression following a single administration of rAAV; and (3) aggregate clinical experience with rAAV delivery in both adults and children, via multiple routes of administration, suggests a strong safety profile for rAAV as a delivery vehicle, particularly in localized delivery and/or at low to moderate doses.

[0347] Clinical trials utilizing rAAV particles began more than two decades ago, and rAAV particles have been administered to hundreds of participants in dozens of clinical trials at doses of up to approximately 1E15 vg or more for systemic administration (Flotte 1996; Flotte 2013; Parente 2018; Wang 2019, each of which is incorporated herein in its entirety by reference). The number of trials in which rAAV particles have been used for *in-vivo* gene transfer has steadily increased. The safety profile, together with the high efficiency of transduction of a broad range of target tissues, has established rAAV particles as a platform of choice for *in-vivo* gene therapy (Wang 2019, incorporated herein in its entirety by reference). Successful application of rAAV technology has been achieved in the clinic for a variety of conditions, including coagulation disorders, inherited blindness, and neurodegenerative diseases (Colella 2018; Wang 2019, each of which is incorporated herein in its entirety by reference).

[0348] In certain embodiments, compositions comprising an rAAV particle comprising constructs encoding an anti-VEGF protein are referred to as rAAV-antiVEGF. In certain compositions, wherein Anc80 capsid is utilized, such a composition may be referred to as rAAVAnc80-antiVEGF. In some embodiments, these compositions may also be referred to as AK-antiVEGF. In some embodiments, an rAAVAnc80-antiVEGF particle comprises a specific rAAV construct, for example it may comprise a construct as represented by **FIG. 6, Panel (A)**. Such a construct is referred to herein as a V_H/V_L construct, and/or rAAVAnc80-V_H/V_L; an exemplary rAAVAnc80-V_H/V_L construct encodes ranibizumab. In some embodiments, an rAAVAnc80-antiVEGF particle comprises a construct as represented by **FIG. 6, Panel (B)**, wherein the construct may be referred to as an antibody GFP construct (ABGFP), and/or rAAVAnc80-ABGFP; an exemplary rAAVAnc80-ABGFP construct encodes ranibizumab or bevacizumab transcriptionally linked to GFP. In some embodiments, an rAAVAnc80-antiVEGF construct comprises a construct as represented by **FIG. 6 Panel (C)**, wherein the construct may be referred to as an antibody (AB) construct, and/or rAAVAnc80-AB; an exemplary rAAVAnc80-AB

construct encodes bevacizumab. In some embodiments, an rAAVAnc80-antiVEGF construct comprises a construct as represented by **FIG. 6, Panel (D)**, wherein the construct may be referred to as anti-VEGF-TRAP (TRAP), and/or rAAVAnc80-TRAP; an exemplary rAAVAnc80-TRAP construct encodes aflibercept. In some embodiments, a compositions disclosed herein, e.g., rAAV-antiVEGF, is a gene therapy composition and is intended for the treatment of patients with VS. In some embodiments, a composition disclosed herein comprises AAVAnc80, a putative common ancestor of adeno-associated viral (AAV) particle serotypes 1, 2, 8, and 9 (Zinn 2015, incorporated herein in its entirety by reference), as a delivery vehicle for a transgene encoding the sequence for ranibizumab, an antibody fragment (Fab) previously optimized for inhibition of VEGF following intraocular administration (Genentech 2017, incorporated herein in its entirety by reference). In some embodiments, a composition disclosed herein is a non-enveloped icosahedral virion of approximately 26 nanometer (nm) in diameter. In some embodiments, adeno-associated viruses, on which a composition disclosed herein, e.g., synthetic AAVAnc80 capsid sequence, is based, are non-pathogenic, single-stranded DNA genome-containing, helper virus-dependent members of the Parvoviridae family.

[0349] Recombinant AAV (rAAV) particles, while retaining the same overall capsid structure as found in wild-type adeno-associated viruses, encapsidate genomes that are devoid of any viral protein-coding sequences and have therapeutic gene expression cassettes inserted in their place. The only sequences of viral origin are the inverted terminal repeat (ITR) sequences, which are necessary to guide genome replication and packaging during particle production. The removal of viral coding sequences maximizes the packaging capacity of rAAV particles and contributes to their low immunogenicity and cytotoxicity when delivered *in-vivo* (Wang 2019, incorporated herein in its entirety by reference).

[0350] AAVAnc80 is a rationally designed AAV capsid whose sequence was inferred by ancestral sequence reconstruction (Zinn 2015, incorporated herein in its entirety by reference). The first reported uses of AAVAnc80 in the mammalian inner ear revealed a high transduction efficiency in cochlear and vestibular hair cells and evidence for recovery of auditory, cochlear, and vestibular function in a knock-in mouse model (Landegger 2017; Pan 2017, each of which is incorporated herein in its entirety by reference). Subsequent studies have confirmed that cochlear- and vestibular cell transduction efficiency of AAVAnc80 is significantly higher than other AAV serotypes in mice (Landegger 2017; Tao 2018; Yoshimura 2018; Omichi 2020, each of which is

incorporated herein in its entirety by reference) and in non-human primates (Andres-Mateos 2019, incorporated herein in its entirety by reference).

[0351] In some embodiments, a composition disclosed herein, e.g., rAAV-antiVEGF, comprises two components: AAVAnc80 (also referred to as Anc80L65; Zinn 2015, incorporated herein in its entirety by reference) capsid and a single-stranded rAAV DNA genome encoding an anti-VEGF protein, such as an rAAV-V_H/V_L (e.g., ranibizumab), rAAV-AB (e.g., bevacizumab), rAAV-ABGFP (e.g., an antibody tagged with GFP, e.g., ranibizumab or bevacizumab tagged with GFP), and/or rAAV-TRAP (e.g., aflibercept) encapsidated by said AAVAnc80 capsid. In some embodiments, a composition disclosed herein, e.g., rAAV-antiVEGF, comprises two components: AAVAnc80 (also referred to as Anc80L65; Zinn 2015, incorporated herein in its entirety by reference) capsid and a single-stranded DNA genome of 3000-5000 nucleotides, exclusive of ITR sequences (e.g., as shown by **FIG. 6, Panels (A)-(D)**), encapsidated by said AAVAnc80 capsid.

[0352] In some embodiments, a composition disclosed herein, e.g., a recombinant construct DNA genome is flanked by AAV2 ITR sequences and comprises a eukaryotic expression cassette encoding the following promoter and regulatory sequences: the cytomegalovirus (CMV) early enhancer element; the chicken beta actin (*CβA*) gene sequence located between the 5` flanking region and the proximal region of the second exon; and the 3` splice acceptor sequence derived from the rabbit beta globin (*RβG*) gene, commonly referred to as the CAG promoter (Miyazaki 1989; Niwa 1991; Orbán 2009, each of which is incorporated herein in its entirety by reference). In some embodiments, following this hybrid regulatory element is a bicistronic cassette encoding ranibizumab heavy and light chains, separated by furin and thosea asigna virus-derived protease 2A (T2A) cleavage sites. In some embodiments, to facilitate protein secretion, a 20 amino-acid human interleukin-2 (IL-2) leader sequence is situated upstream of each Fab chain. In some embodiments of a composition disclosed herein, downstream of an anti-VEGF protein coding sequence is a bovine growth hormone polyadenylation site.

[0353] The current standard of care for VS has evolved over the past 2 to 3 decades, as imaging techniques have evolved and the ability to identify and track the growth of tumors has improved. There has been a progressive trend toward a conservative, observational "wait and rescan" approach, with a growing awareness that many tumors exhibit slow or low growth and may not impact hearing so long as the low growth rate is maintained (MacKeith 2013; Reznitsky 2019,

each of which is incorporated herein in its entirety by reference). In some embodiments, a key potential benefit of a composition disclosed herein, e.g., rAAV-antiVEGF, is the opportunity, e.g., to augment this conservative treatment approach by halting tumor growth, stabilizing hearing, and/or obviating the need for more invasive treatment approaches, such as surgical resection and/or radiation therapy. Without wishing to be bound by theory, it is believed that in some embodiments, while halting VS growth is likely to provide a substantial clinical benefit, it is also possible that a composition disclosed herein, e.g., rAAV-antiVEGF therapy could go beyond tumor stasis and drive shrinkage of tumors, restoration of speech understanding, and/or reduction in perceived difficulty of speech understanding, as demonstrated in studies of bevacizumab-treated VS tumors in NF2 patients (Huang 2018; Plotkin 2019, each of which is incorporated herein in its entirety by reference). In addition, studies with systemically administered VEGF inhibitor have shown improvements in NF2-related quality of life, including symptoms associated with VS in NF2 patients (Plotkin 2019, incorporated herein in its entirety by reference). In some embodiments, these clinical data suggest that rAAV-antiVEGF administration, and sustained and localized expression of anti-VEGF protein in diffusional continuity with the tumor, has the potential to provide these additional benefits to patients with VS.

[0354] In some embodiments, intracochlear administration of a composition disclosed herein, e.g., rAAV-antiVEGF, has the potential to eliminate the need for future treatment and to preserve physiologic hearing in those with VS, regardless of underlying etiology. In some embodiments, a composition disclosed herein can result in a delay of invasive treatment approaches, such as surgical resection and/or radiation therapy, and associated loss of physiologic hearing. In some embodiments of any of the methods disclosed herein, the method further comprises administration of standard of care treatments. In some embodiments, there may also be potential benefit to subjects who subsequently undergo radiation therapy, as anti-VEGF protein treatment may improve a subject's response to radiotherapy by sensitizing the tumor and allowing for lower radiation dosing (Koutsimpelas 2012; Gao 2015, each of which is incorporated herein in its entirety by reference).

AAV Constructs

[0355] The present disclosure provides polynucleotide constructs that comprise an anti-VEGF antibody and/or a soluble VEGF receptor (e.g., an anti-VEGF TRAP protein, such as the fusion

protein aflibercept). In some embodiments described herein, a polynucleotide comprising an anti-VEGF antibody and/or a soluble VEGF receptor is referred to as a construct, and can be encompassed within an AAV capsid, to create an rAAV particle. In certain embodiments, an rAAV construct may be referred to as a vector, and may be quantified as vector genomes (vg); for example, as vg per milliliter (mL). A simplified schematic representation of a WT AAV genome is depicted in **FIG. 5, Panel (A)**, while a simplified schematic representation of an rAAV construct is depicted in **FIG. 5, Panel (B)**.

[0356] In some embodiments, a polynucleotide construct comprises one or more components derived from or modified from a naturally occurring AAV genomic construct. In some embodiments, 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, or AAVAnc80 construct. 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 herein in its entirety by reference.

[0357] In some embodiments, provided constructs comprise coding sequence, e.g., an anti-VEGF gene or a characteristic portion thereof, one or more regulatory and/or control sequences, and optionally 5` and 3` AAV derived inverted terminal repeats (ITRs). In some embodiments 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 embodiments, provided rAAV constructs are packaged into an AAV capsid to form an rAAV particle.

[0358] In some embodiments, 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 in its entirety by reference). Typical AAV2-derived ITR sequences are about 145 nucleotides in length. In some embodiments, at least 80% of a typical ITR sequence (e.g., 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 herein in its entirety by reference). In some embodiments, any of the coding sequences and/or constructs described herein are flanked by 5` and 3` AAV ITR sequences. An AAV ITR sequences may be obtained from any known AAV, including presently identified AAV types.

[0359] In some embodiments, 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 in its entirety by reference) 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 embodiments, provided constructs can be packaged into a capsid to create an rAAV particle. An rAAV particle may be delivered to a selected target cell. In some embodiments, 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 embodiments, 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.

[0360] As shown in **FIG. 5, Panel (A)**, an unmodified AAV endogenous genome comprises two open reading frames, "cap" and "rep," which are flanked by ITRs. As shown in **FIG. 5, Panel (B)**, exemplary rAAV constructs similarly include ITRs flanking a coding region, e.g., a coding sequence (e.g., an antiVEGF gene). In some embodiments, 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 embodiments, a rAAV construct optionally comprises a promoter (shown in **FIG. 5, 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. 5, Panel (B)**), or any combination thereof. Such additional elements are described further herein.

[0361] In some embodiments, a construct is a rAAV construct. In some embodiments, a 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 embodiments, an rAAV 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 embodiments, an rAAV 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 5kb, about 3 kb to about 4 kb, about 3 kb to about 5 kb, or about 4 kb to about 5 kb.

[0362] 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 embodiments, 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.

Exemplary Construct Components

Inverted Terminal Repeat Sequences (ITRs)

[0363] 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 ITRs ability to self-prime, allowing primase-independent synthesis of a second DNA strand. ITRs can also aid in efficient encapsidation of an rAAV construct in an rAAV particle.

[0364] In some embodiments, a rAAV particle (e.g., an AAV2/Anc80 particle) of the present disclosure can comprise a rAAV construct comprising a coding sequence (e.g., an anti-VEGF gene) and associated elements flanked by a 5` and a 3` AAV ITR sequences. In some embodiments, an ITR is or comprises about 145 nucleic acids. In some embodiments, 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 embodiments an ITR is an AAV2 ITR.

[0365] 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 embodiments, 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 embodiments, 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.

[0366] For example, an ITR (e.g., a 5` ITR) can have a sequence according to SEQ ID NO: 45. In some embodiments, an ITR (e.g., a 3` ITR) can have a sequence according to SEQ ID NO: 46. In some embodiments, an ITR comprises one or more modifications, e.g., truncations, deletions, substitutions or insertions, as is known in the art. In some embodiments, an ITR comprises fewer than 145 nucleotides, e.g., 127, 130, 134 or 141 nucleotides. For example, in some embodiments, 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 embodiments, an ITR (e.g., a 5` ITR) can have a sequence according to SEQ ID NO: 47. In some embodiments, an ITR (e.g., a 3` ITR) can have a sequence according to SEQ ID NO: 48.

[0367] A non-limiting example of a 5` AAV ITR sequence is SEQ ID NO: 45. A non-limiting example of a 3` AAV ITR sequence is SEQ ID NO: 46. A non-limiting example of a 5` AAV ITR sequence is SEQ ID NO: 47. A non-limiting example of a 3` AAV ITR sequence is SEQ ID NO: 48. In some embodiments, rAAV constructs of the present disclosure comprise a 5` AAV ITR

and/or a 3` AAV ITR. In some embodiments, a 5` AAV ITR sequence is SEQ ID NO: 45. In some embodiments, a 3` AAV ITR sequence is SEQ ID NO: 46. In some embodiments, the 5` and a 3` AAV ITRs (e.g., SEQ ID NOs: 45 and 46, or 47 and 48) flank a portion of a coding sequence, e.g., all or a portion of an anti-VEGF gene (e.g., SEQ ID NOs: 13, 14, 15, 19, 22, 42 and/or 103). 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 embodiments, a 5` ITR sequence is at least 85%, 90%, 95%, 98% or 99% identical to a 5` ITR sequence represented by SEQ ID NOs: 45 or 47. In some embodiments, a 3` ITR sequence is at least 85%, 90%, 95%, 98% or 99% identical to a 3` ITR sequence represented by SEQ ID NOs: 46 or 48.

[0368] Exemplary 5` AAV ITR (SEQ ID NO: 45)

TTGGCCACTCCCTCTCTGCGCGCTCGCTCGCTCACTGAGGCCGCCCGGGCAAAGCCC
GGGCGTCGGGCGACCTTTGGTCGCCCGGCCTCAGTGAGCGAGCGAGCGCGCAGAGA
GGGAGTGGCCAACTCCATCACTAGGGGTTCCT

[0369] Exemplary 3` AAV ITR (SEQ ID NO: 46)

AGGAACCCCTAGTGATGGAGTTGGCCACTCCCTCTCTGCGCGCTCGCTCGCTCACTG
AGGCCGGGCGACCAAAGGTCGCCCGACGCCCGGGCTTTGCCCGGGCGGCCTCAGTG
AGCGAGCGAGCGCGCAGAGAGGGAGTGGCCAA

[0370] Exemplary 5` AAV ITR (SEQ ID NO: 47)

CTGCGCGCTCGCTCGCTCACTGAGGCCGCCCGGGCGTCGGGCGACCTTTGGTCGCCC
GGCCTCAGTGAGCGAGCGAGCGCGCAGAGAGGGAGTGGCCAACTCCATCACTAGGG
GTTCCT

[0371] Exemplary 3` AAV ITR (SEQ ID NO: 48)

AGGAACCCCTAGTGATGGAGTTGGCCACTCCCTCTCTGCGCGCTCGCTCGCTCACTG
AGGCCGGGCGACCAAAGGTCGCCCGACGCCCGGGCTTTGCCCGGGCGGCCTCAGTG
AGCGAGCGAGCGCGCAG

Promoters

[0372] In some embodiments, 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., an anti-VEGF gene). 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 embodiments, a construct (e.g., an rAAV construct) comprises a promoter operably linked to one of the non-limiting example promoters described herein.

[0373] In some embodiments, 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, or any other type of promoter known in the art. In some embodiments, a promoter is a RNA polymerase II promoter, such as a mammalian RNA polymerase II promoter. In some embodiments, 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 embodiments, a promoter is a cochlea-specific promoter or a cochlea-oriented promoter. In some embodiments, a promoter is a hair cell specific promoter, or a supporting cell specific promoter.

[0374] A variety of promoters are known in the art, which can be used herein. Non-limiting examples of promoters that can be used herein include: human EFlα, human cytomegalovirus (CMV) (US Patent No. 5,168,062, which is incorporated in its entirety herein by reference), human ubiquitin C (UBC), mouse phosphoglycerate kinase 1, polyoma adenovirus, simian virus 40 (SV40), β-globin, β-actin, α-fetoprotein, γ-globin, β-interferon, γ-glutamyl transferase, mouse mammary tumor virus (MMTV), Rous sarcoma virus, rat insulin, glyceraldehyde-3-phosphate dehydrogenase, metallothionein II (MT II), amylase, cathepsin, MI muscarinic receptor, retroviral LTR (e.g., human T-cell leukemia virus HTLV), AAV ITR, interleukin-2, collagenase, platelet-derived growth factor, adenovirus 5 E2, stromelysin, murine MX gene, glucose regulated proteins (GRP78 and GRP94), α-2-macroglobulin, vimentin, MHC class I gene H-2$_K$ b, HSP70, proliferin, tumor necrosis factor, thyroid stimulating hormone a gene, immunoglobulin light chain, T-cell receptor, HLA DQa and DQ , interleukin-2 receptor, MHC class II, MHC class II HLA-DRa, muscle creatine kinase, prealbumin (transthyretin), elastase I, albumin gene, c-fos, c-HA-ras, neural cell adhesion molecule (NCAM), H2B (TH2B) histone, rat growth hormone, human serum amyloid (SAA), troponin I (TN I), duchenne muscular dystrophy, human immunodeficiency virus,

and Gibbon Ape Leukemia Virus (GALV) promoters. Additional examples of promoters are known in the art. See, e.g., Lodish, Molecular Cell Biology, Freeman and Company, New York 2007, each of which is incorporated in its entirety herein by reference. In some embodiments, a promoter is the CMV immediate early promoter. In some embodiments, the promoter is a CAG promoter or a CAG/CBA promoter. In some embodiments, the promoter comprises or consists of SEQ ID NO: 49. In some embodiments, a promoter comprises or consists of SEQ ID NO: 50. In certain embodiments, a promoter comprises a CMV/CBA enhancer/promoter construct exemplified in SEQ ID NO: 51. In certain embodiments, a promoter comprises a CMV/CBA enhancer/promoter construct exemplified in SEQ ID NO: 52. In certain embodiments, a promoter comprises a CAG promoter or CMV/CBA/SV-40 enhancer/promoter construct exemplified in SEQ ID NO: 53. In certain embodiments, a promoter comprises a CAG promoter or CMV/CBA/SV-40 enhancer/promoter construct exemplified in SEQ ID NO: 54. In some embodiments, a promoter sequence is at least 85%, 90%, 95%, 98% or 99% identical to the promoter sequences represented by SEQ ID NOs: 49 or 50. In some embodiments, an enhancer-promoter sequence is at least 85%, 90%, 95%, 98% or 99% identical to enhancer-promoter sequence represented by SEQ ID NOs: 51, 52, 53, or 54.

[0375] The term "constitutive" promoter refers to a nucleotide sequence that, when operably linked with a nucleic acid encoding a protein (e.g., an anti-VEGF protein), causes RNA to be transcribed from the nucleic acid in a cell under most or all physiological conditions.

[0376] 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).

[0377] Inducible promoters allow regulation of gene expression and can be regulated by exogenously supplied compounds, environmental factors such as temperature, or the presence of a specific physiological state, e.g., acute phase, a particular differentiation state of the cell, or in replicating cells only. Inducible promoters and inducible systems are available from a variety of commercial sources, including, without limitation, Invitrogen, Clontech, and Ariad. Additional examples of inducible promoters are known in the art.

[0378] Examples of inducible promoters regulated by exogenously supplied compounds include the zinc-inducible sheep metallothionein (MT) promoter, the dexamethasone (Dex)-inducible mouse mammary tumor virus (MMTV) promoter, the T7 polymerase promoter system (WO 98/10088, which is incorporated in its entirety herein by reference); the ecdysone insect promoter (No et al, Proc. Natl. Acad Sci. US.A. 93:3346-3351, 1996, which is incorporated in its entirety herein by reference), the tetracycline-repressible system (Gossen et al, Proc. Natl. Acad Sci. US.A. 89:5547-5551, 1992, which is incorporated in its entirety herein by reference), the tetracycline-inducible system (Gossen et al, Science 268:1766-1769, 1995, see also Harvey et al, Curr. Opin. Chem. Biol. 2:512-518, 1998, each of which is incorporated in their entirety herein by reference), the RU486-inducible system (Wang et al, Nat. Biotech. 15:239- 243, 1997, and Wang et al, Gene Ther. 4:432-441, 1997, each of which is incorporated in their entirety herein by reference), and the rapamycin-inducible system (Magari et al. J Clin. Invest. 100:2865-2872, 1997, which is incorporated in its entirety herein by reference).

[0379] The term "tissue-specific" promoter refers to a promoter that is active only in certain specific cell types and/or tissues (e.g., transcription of a specific gene occurs only within cells expressing transcription regulatory and/or control proteins that bind to the tissue-specific promoter). In some embodiments, regulatory and/or control sequences impart tissue-specific gene expression capabilities. In some cases, tissue-specific regulatory and/or control sequences bind tissue-specific transcription factors that induce transcription in a tissue-specific manner.

[0380] In some embodiments, a tissue-specific promoter is a cochlea-specific promoter. In some embodiments, a tissue-specific promoter is a cochlear hair cell-specific promoter. Non-limiting examples of cochlear hair cell-specific promoters include but are not limited to: a ATOH1 promoter, a POU4F3 promoter, a LHX3 promoter, a MYO7A promoter, a MYO6 promoter, a α9ACHR promoter, and a αl0ACHR promoter. In some embodiments, a promoter is a cochlear hair cell-specific promoter such as a PRESTIN promoter or an ONCOMOD promoter. See, e.g., Zheng et al., Nature 405:149-155, 2000; Tian et al. Dev. Dyn. 23 l: 199-203, 2004; and Ryan et al., Adv. Otorhinolaryngol. 66: 99-115, 2009, each of which is incorporated in their entirety herein by reference.

[0381] In some embodiments, a tissue-specific promoter is an ear cell specific promoter. In some embodiments, a tissue-specific promoter is an inner ear cell specific promoter. Non-limiting

examples of inner ear non-sensory cell-specific promoters include but are not limited to: GJB2, GJB6, SLC26A4, TECTA, DFNA5, COCH, NDP, SYN1, GFAP, PLP, TAK1, or SOX21. In some embodiments, a cochlear non-sensory cell specific promoter may be an inner ear supporting cell specific promoter. Non-limiting examples of inner ear supporting cell specific promoters include but are not limited to: SOX2, FGFR3, PROX1, GLAST1, LGR5, HES1, HES5, NOTCH1, JAG1, CDKN1A, CDKN1B, SOX10, P75, CD44, HEY2, LFNG, or S100b.

[0382] In some embodiments, provided rAAV constructs comprise a promoter sequence selected from a CAG, a CBA, a CMV, or a CB7 promoter. In some embodiments of any of the therapeutic compositions described herein, the first and/or sole rAAV construct further comprises at least one promoter sequence selected from Cochlea and/or inner ear specific promoters.

[0383] Exemplary CBA promoter (SEQ ID NO: 49)

GTCGAGGTGAGCCCCACGTTCTGCTTCACTCTCCCCATCTCCCCCCCCCTCCCCACCCC
CAATTTTGTATTTATTTATTTTTTAATTATTTTGTGCAGCGATGGGGGCGGGGGGGGGG
GGGGGGGCGCGCGCCAGGCGGGGCGGGGCGGGGCGAGGGGCGGGGCGGGGCGAG
GCGGAGAGGTGCGGCGGCAGCCAATCAGAGCGGCGCGCTCCGAAAGTTTCCTTTTA
TGGCGAGGCGGCGGCGGCGGCGGCCCTATAAAAAGCGAAGCGCGCGGCGGGCG

[0384] Exemplary CBA promoter (SEQ ID NO: 50)

GTCGAGGTGAGCCCCACGTTCTGCTTCACTCTCCCCATCTCCCCCCCCCTCCCCACCCC
CAATTTTGTATTTATTTATTTTTTAATTATTTTGTGCAGCGATGGGGGCGGGGGGGGGG
GGGGGCGCGCGCCAGGCGGGGCGGGGCGGGGCGAGGGGCGGGGCGGGGCGAGGC
GGAGAGGTGCGGCGGCAGCCAATCAGAGCGGCGCGCTCCGAAAGTTTCCTTTTATG
GCGAGGCGGCGGCGGCGGCGGCCCTATAAAAAGCGAAGCGCGCGGCGGGCG

[0385] Exemplary CMV/CBA enhancer/promoter (SEQ ID NO: 51)

GACATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATA
GCCCATATATGGAGTTCCGCGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGAC
CGCCCAACGACCCCCGCCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGC
CAATAGGGACTTTCCATTGACGTCAATGGGTGGACTATTTACGGTAAACTGCCCACT
TGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACG
GTAAATGGCCCGCCTGGCATTATGCCCAGTACATGACCTTATGGGACTTTCCTACTT

GGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGGTCGAGGTGAGCCCCACG
TTCTGCTTCACTCTCCCCATCTCCCCCCCCCTCCCCACCCCCCAATTTTGTATTTATTTAT
TTTTTAATTATTTTGTGCAGCGATGGGGGCGGGGGGGGGGGGGGGCGCGCGCCAG
GCGGGGCGGGGCGGGGCGAGGGGCGGGGCGGGGCGAGGCGGAGAGGTGCGGCGG
CAGCCAATCAGAGCGGCGCGCTCCGAAAGTTTCCTTTTATGGCGAGGCGGCGGCGG
CGGCGGCCCTATAAAAAGCGAAGCGCGCGGCGGGCG

[0386] Exemplary CMV/CBA enhancer/promoter (SEQ ID NO: 52)

GACATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATA
GCCCATATATGGAGTTCCGCGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGAC
CGCCCAACGACCCCCGCCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGC
CAATAGGGACTTTCCATTGACGTCAATGGGTGGACTATTTACGGTAAACTGCCCACT
TGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACG
GTAAATGGCCCGCCTGGCATTATGCCCAGTACATGACCTTATGGGACTTTCCTACTT
GGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGGTCGAGGTGAGCCCCACG
TTCTGCTTCACTCTCCCCATCTCCCCCCCCCTCCCCACCCCCCAATTTTGTATTTATTTAT
TTTTTAATTATTTTGTGCAGCGATGGGGGCGGGGGGGGGGGGGGGCGCGCGCCAGGC
GGGGCGGGGCGGGGCGAGGGGCGGGGCGGGGCGAGGCGGAGAGGTGCGGCGGCA
GCCAATCAGAGCGGCGCGCTCCGAAAGTTTCCTTTTATGGCGAGGCGGCGGCGGCG
GCGGCCCTATAAAAAGCGAAGCGCGCGGCGGGCG

[0387] Exemplary CAG enhancer/promoter (SEQ ID NO: 53)

GACATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATA
GCCCATATATGGAGTTCCGCGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGAC
CGCCCAACGACCCCCGCCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGC
CAATAGGGACTTTCCATTGACGTCAATGGGTGGACTATTTACGGTAAACTGCCCACT
TGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACG
GTAAATGGCCCGCCTGGCATTATGCCCAGTACATGACCTTATGGGACTTTCCTACTT
GGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGGTCGAGGTGAGCCCCACG
TTCTGCTTCACTCTCCCCATCTCCCCCCCCCTCCCCACCCCCCAATTTTGTATTTATTTAT
TTTTTAATTATTTTGTGCAGCGATGGGGGCGGGGGGGGGGGGGGGCGCGCGCCAG
GCGGGGCGGGGCGGGGCGAGGGGCGGGGCGGGGCGAGGCGGAGAGGTGCGGCGG

CAGCCAATCAGAGCGGCGCGCTCCGAAAGTTTCCTTTTATGGCGAGGCGGCGGCGG
CGGCGGCCCTATAAAAAGCGAAGCGCGCGGCGGGCGGGAGTCGCTGCGTTGCCTTC
GCCCCGTGCCCCGCTCCGCGCCGCCTCGCGCCGCCCGCCCCGGCTCTGACTGACCGC
GTTACTCCCACAGGTGAGCGGGCGGGACGGCCCTTCTCCTCCGGGCTGTAATTAGCG
CTTGGTTTAATGACGGCTCGTTTCTTTTCTGTGGCTGCGTGAAAGCCTTAAAGGGCTC
CGGGAGGGCCCTTTGTGCGGGGGGGAGCGGCTCGGGGGGTGCGTGCGTGTGTGTGT
GCGTGGGGAGCGCCGCGTGCGGCCCGCGCTGCCCGGCGGCTGTGAGCGCTGCGGGC
GCGGCGCGGGGCTTTGTGCGCTCCGCGTGTGCGCGAGGGGAGCGCGGCCGGGGGCG
GTGCCCCGCGGTGCGGGGGGGCTGCGAGGGGAACAAAGGCTGCGTGCGGGGTGTGT
GCGTGGGGGGGTGAGCAGGGGGTGTGGGCGCGGCGGTCGGGCTGTAACCCCCCCCT
GCACCCCCCTCCCCGAGTTGCTGAGCACGGCCCGGCTTCGGGTGCGGGGCTCCGTGC
GGGGCGTGGCGCGGGGCTCGCCGTGCCGGGCGGGGGGTGGCGGCAGGTGGGGGTG
CCGGGCGGGGCGGGGCCGCCTCGGGCCGGGGAGGGCTCGGGGGAGGGGCGCGGCG
GCCCCCGGAGCGCCGGCGGCTGTCGAGGCGCGGCGAGCCGCAGCCATTGCCTTTTA
TGGTAATCGTGCGAGAGGGCGCAGGGACTTCCTTTGTCCCAAATCTGTGCGGAGCCG
AAATCTGGGAGGCGCCGCCGCACCCCCTCTAGCGGGCGCGGGGCGAAGCGGTGCGG
CGCCGGCAGGAAGGAAATGGGCGGGGAGGGCCTTCGTGCGTCGCCGCGCCGCCGTC
CCCTTCTCCCTCTCCAGCCTCGGGGCTGTCCGCGGGGGGGACGGCTGCCTTCGGGGGG
GACGGGGCAGGGCGGGGTTCGGCTTCTGGCGTGTGACCGGCGGCTCTAGAGCCTCT
GCTAACCATGTTCATGCCTTCTTCTTTTTCCTACAG

[0388] Exemplary CAG enhancer/promoter (SEQ ID NO: 54)

GACATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATA
GCCCATATATGGAGTTCCGCGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGAC
CGCCCAACGACCCCCGCCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGC
CAATAGGGACTTTCCATTGACGTCAATGGGTGGACTATTTACGGTAAACTGCCCACT
TGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACG
GTAAATGGCCCGCCTGGCATTATGCCCAGTACATGACCTTATGGGACTTTCCTACTT
GGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGGTCGAGGTGAGCCCCACG
TTCTGCTTCACTCTCCCCATCTCCCCCCCCCTCCCCACCCCCAATTTTGTATTTATTTAT
TTTTTAATTATTTTGTGCAGCGATGGGGGCGGGGGGGGGGGGGGCGCGCGCCAGGC
GGGGCGGGGCGGGGCGAGGGGCGGGGCGGGGCGAGGCGGAGAGGTGCGGCGGCA

GCCAATCAGAGCGGCGCGCTCCGAAAGTTTCCTTTTATGGCGAGGCGGCGGCGGCG
GCGGCCCTATAAAAAGCGAAGCGCGCGGCGGGCGGGAGTCGCTGCGTTGCCTTCGC
CCCGTGCCCCGCTCCGCGCCGCCTCGCGCCGCCCGCCCCGGCTCTGACTGACCGCGT
TACTCCCACAGGTGAGCGGGCGGGACGGCCCTTCTCCTCCGGGCTGTAATTAGCGCT
TGGTTTAATGACGGCTCGTTTCTTTTCTGTGGCTGCGTGAAAGCCTTAAAGGGCTCCG
GGAGGGCCCTTTGTGCGGGGGGGAGCGGCTCGGGGGGTGCGTGCGTGTGTGTGTGC
GTGGGGAGCGCCGCGTGCGGCCCGCGCTGCCCGGCGGCTGTGAGCGCTGCGGGCGC
GGCGCGGGGCTTTGTGCGCTCCGCGTGTGCGCGAGGGGAGCGCGGCCGGGGGCGGT
GCCCCGCGGTGCGGGGGGGGCTGCGAGGGGAACAAAGGCTGCGTGCGGGGTGTGTGC
GTGGGGGGGTGAGCAGGGGGTGTGGGCGCGGCGGTCGGGCTGTAACCCCCCCCTGC
ACCCCCCTCCCCGAGTTGCTGAGCACGGCCCGGCTTCGGGTGCGGGGCTCCGTGCGG
GGCGTGGCGCGGGGCTCGCCGTGCCGGGCGGGGGGGTGGCGGCAGGTGGGGGTGCC
GGGCGGGGCGGGGCCGCCTCGGGCCGGGGAGGGCTCGGGGGAGGGGCGCGGCGGC
CCCCGGAGCGCCGGCGGCTGTCGAGGCGCGGCGAGCCGCAGCCATTGCCTTTTATG
GTAATCGTGCGAGAGGGCGCAGGGACTTCCTTTGTCCCAAATCTGTGCGGAGCCGA
AATCTGGGAGGCGCCGCCGCACCCCCTCTAGCGGGCGCGGGGCGAAGCGGTGCGGC
GCCGGCAGGAAGGAAATGGGCGGGGAGGGCCTTCGTGCGTCGCCGCGCCGCCGTCC
CCTTCTCCCTCTCCAGCCTCGGGGCTGTCCGCGGGGGGGACGGCTGCCTTCGGGGGGG
ACGGGGCAGGGCGGGGTTCGGCTTCTGGCGTGTGACCGGCGGCTCTAGAGCCTCTG
CTAACCATGTTCATGCCTTCTTCTTTTTCCTACAG

[0389] In certain embodiments, a promoter is an endogenous human ATOH1 enhancer-promoter as set forth in SEQ ID NO: 55. In some embodiments, an enhancer-promoter sequence is at least 85%, 90%, 95%, 98% or 99% identical to enhancer-promoter sequence represented by SEQ ID NO: 55.

[0390] **Exemplary Human ATOH1 enhancer-promoter (SEQ ID NO: 55)**

CTATGGAGTTTGCATAACAAACGTTTGGCAGCTCGCTCTCTTACACTCCATTAACAA
GCTGTAACATATAGCTGCAGGTTGCTATAATCTCATTAATATTTTGGAAACTTGAAT
ATTGAGTATTTCTGAGTGCTCATTCCCCATATGCCAGCCACTTCTGCCATGCTGACTG
GTTCCTTTCTCTCCATTATTAGCAATTAGCTTCTTACCTTCCAAAGTCAGATCCAAGG
TATCCAAGATACTAGCAAAGGAATCAACTATGTGTGCAAGTTAAGCATGCTTAATAT

CACCCAAACAAACAAAGAGGCAGCATTTCTTAAAGTAATGAAGATAGATAAATCGG
GTTAGTCCTTTGCGACACTGCTGGTGCTTTCTAGAGTTTTATATATTTTAAGCAGCTT
GCTTTATATTCTGTCTTTGCCTCCCACCCCACCAGCACTTTTATTTGTGGAGGGTTTT
GGCTCGCCACACTTTGGGAAACTTATTTGATTTCACGGAGAGCTGAAGGAAGATCAT
TTTTGGCAACAGACAAGTTTAAACACGATTTCTATGGGACATTGCTAACTGGGGCCC
CTAAGGAGAAAGGGGAAACTGAGCGGAGAATGGGTTAAATCCTTGGAAGCAGGGG
AGAGGCAGGGGAGGAGAAGTCGGAGGAGTATAAAGAAAAGGACAGGAACCAA
GAAGCGTGGGGGTGGTTTGCCGTAATGTGAGTGTTTCTTAATTAGAGAACGGTTGAC
AATAGAGGGTCTGGCAGAGGCTCCTGGCCGCGGTGCGGAGCGTCTGGAGCGGAGCA
CGCGCTGTCAGCTGGTGAGCGCACTCTCCTTTCAGGCAGCTCCCCGGGGAGCTGTGC
GGCCACATTTAACACCATCATCACCCCTCCCCGGCCTCCTCAACCTCGGCCTCCTCCT
CGTCGACAGCCTTCCTTGGCCCCCACCAGCAGAGCTCACAGTAGCGAGCGTCTCTCG
CCGTCTCCCGCACTCGGCCGGGGCCTCTCTCCTCCCCCAGCTGCGCAGCGGGAGCCG
CCACTGCCCACTGCACCTCCCAGCAACCAGCCCAGCACGCAAAGAAGCTGCGCAAA
GTTAAAGCCAAGCAATGCCAAGGGGAGGGGAAGCTGGAGGCGGGCTTTGAGTGGCT
TCTGGGCGCCTGGCGGGTCCAGAATCGCCCAGAGCCGCCCGCGGTCGTGCACATCT
GACCCGAGTCAGCTTGGGCACCAGCCGAGAGCCGGCTCCGCACCGCTCCCGCACCC
CAGCCGCCGGGGTGGTGACACACACCGGAGTCGAATTACAGCCCTGCAATTAACAT
ATGAATCTGACGAATTTAAAAGAAGGAAAAAAAAAAAAAAAACCTGAGCAGGCTTG
GGAGTCCTCTGCACACAAGAACTTTTCTCGGGGTGTAAAAACTCTTTGATTGGCTGC
TCGCACGCGCCTGCCCGCGCCCTCCATTGGCTGAGAAGACACGCGACCGGCGCGAG
GAGGGGGTTGGGAGAGGAGCGGGGGGAGACTGAGTGGCGCGTGCCGCTTTTTAAAG
GGGCGCAGCGCCTTCAGCAACCGGAGAAGCATAGTTGCACGCGACCTGGTGTGTGA
TCTCCGAGTGGGTGGGGGAGGGTCGAGGAGGGAAAAAAAAATAAGACGTTGCAGA
AGAGACCCGGAAAGGGCCTTTTTTTTGGTTGAGCTGGTGTCCCAGTGCTGCCTCCGA
TCCTGAGCCTCCGAGCCTTTGCAGTGCAA

[0391] In certain embodiments, a promoter is an endogenous human SLC26A4 immediate promoter as set forth in SEQ ID NO: 56 or 57. In certain embodiments, a promoter is an endogenous human SLC26A4 enhancer-promoter as set forth in SEQ ID NO: 58, 59, or 60. In some embodiments, an enhancer-promoter sequence is at least 85%, 90%, 95%, 98% or 99% identical to a promoter or enhancer-promoter sequence represented by SEQ ID NO: 56, 57, 58, 59,

or 60. In certain embodiments, a promoter is a human SLC26A4 endogenous enhancer-promoter sequence comprised within SEQ ID NO: 58, 59, or 60.

[0392] Exemplary Human SLC26A4 immediate promoter (SEQ ID NO: 56)

CTGCCTTCTGAGAGCGCTATAAAGGCAGCGGAAGGGTAGTCCGCGGGGCATTCCGG
GCGG

[0393] Exemplary Human SLC26A4 immediate promoter (SEQ ID NO: 57)

CTCTAGGCGGGCTCTGCTCTTCTTTAAGGAGTCCCACAGGGCCTGGCCCGCCCCTGA
CCT

[0394] Exemplary Human SLC26A4 enhancer-promoter (SEQ ID NO: 58)

TAAAGAGTTGTGAGTTGTGTAGGTGAGTTGCCATGGAGCTACAAATATGAGTTGATA
TTCTGAAATCCTAGACAGCCATCTCCAAGGTTAAGAAAAATCCTTATGCACTCACTT
GCAAAGATATCCACAGCATGCTCTTAATGGAGAAAAACAAAGCCTTAGATCAAATA
TGTAAAGTAATTTTTAGTTTTTTGAAAAGGTATGTTTGGGCTATAGATAAATCTGTTC
AAAAAACATGAGAGAAGATAATAATGGTTGAAAGGAGACACAGTGCTTGCCCTCAA
GAAGTTTTTGTCTAGTGAGGGAGAGAGAACTTGTATGTAAATAAAATTGTGTTACTA
AGGTAGATAGTGAGAAGTAACTTAAGAGAGGATCAGATAAGGTATTAAGAGAATAC
AGAAAAGGGTCTGGATTAATTCTGAACAGCATCAAAGAATGTTCTTGCAAGAGATA
GTGTTTTCACCAGATCTTGAAGGTATGGATGAGGGTATACAGAGTGAGTATATTCAG
ATTCTACTTTAAAACAAATACTTTCCTCTGTTGTAGTGGAGTTGAGCTATACATCCAA
CAATAATGAAAAAATACACGCATATATACATATATGGAGAGAGATACATATTTTAG
TACATGTAGCAATTGATTAATAAATGTACAGTTTAAGTCGCATGCAAAACCTTGGAG
TGATAGCAAACTTCATTGTAGGATGTTTAGCAGCATCTCTGGTCTCTACTCACTAGAT
CCCAATAGCATCTCCCTAGGTGTGACAACCAAAAATGTCTCCAGGCATTGACCTCTG
GAGGCAAAAAAAGCCCTTTATTAAGAACCAGTGGTATACATAAGTAAAACATACAC
AAGAGATTCCTCCCCTCTTCTCTGTATGTGAATAAAAATTGCAAAGTTCATGACCTG
GATTTTCCTTTTAGGTTTCTTCTTTAGTGGTTCTTAACTTCATTGGGTGAAGTAAGCCT
TTGAAGATCTGTTGAAAGCTGTTGACTCATTCACTTCTCAGGAAAACGCACATGCTG
ACTACCATTTCAGAGAATTTGCATCAGGGTTCTCTGGGGAGGAGTTCTGAGTTCTGT
TTCCAGGAGCTCGTAGAATTGTCATGGTCTGCATATGCAAGGCAGGTGGATTACGGA

AGGTTGATGTACAGAGGTCTGTATTTTGGAGCCTCTTCTGTATTTACTTCAGAACACT
AACAATCAGGCGAGAATGTTCTGGTTTATCAAACCCTTCCTTCTGCCTTTCATCTTAA
CCATGCATTAGTTTTAACAAAGTTCATCCCAACAGAAGACAAAACACTGATGAGGT
AGGATAGCTCCAGCTCCTCCTCCCTCTCTTCTAGTCTTGATTTCCATGTAGTCCAGTT
TATTCCTTCCCTGATTGTCCAGGAGAATGAGAAAAAGAAAAAACAGAGTCTAGTGG
GTAAGAAAGGGCCACCTGGACGGCTTGATTTGGATTGTGAAATAAAACACACACAC
ATGCACACGTAGAATAAGTGGCTAAAATCTGAGTAAATCGTGAACTCTCTGTATCCT
CCACCCATTGAATACTCCTAAAAGACTTTCTAGAAATTCAAGGACTTATTAATATAG
AAACCTGGCCATTGTTCCTCTTCTCCTCCCCATGTGGTATGAGAGCACCTGTGGCAG
GCTCCCAGAGACCACGGACCTCTTCCTCTAGGCGGGCTCTGCTCTTCTTTAAGGAGT
CCCACAGGGCCTGGCCCGCCCCTGACCTCGCAACCCTTGAGATTAGTAACGGGATG
AGTGAGGATCCGGGTGGCCCCTGCGTGGCAGCCAGTAAGAGTCTCAGCCTTCCCGG
TTCGGGAAAGGGGAAGAATGCAGGAGGGGTAGGATTTCTTTCCTGATAGGATCGGT
TGGGAAAGACCGCAGCCTGTGTGTGTCTTTCCCTTCGACCAAGGTGTCTGTTGCTCC
GTAAATAAAACGTCCCACTGCCTTCTGAGAGCGCTATAAAGGCAGCGGAAGGGTAG
TCCGCGGGGC

[0395] Exemplary Human SLC26A4 enhancer-promoter (SEQ ID NO: 59)

GGCTGCTCGGAAAACAGGACGAGGGGAGAGACTTGCTCAATAAGCTGAAAGTTCTG
CCCCCGAGAGGGCTGCGACAGCTGCTGGAATGTGCCTGCAGCGTCCGCCTCTTGGG
GACCCGCGGAGCGCGCCCTGACGGTTCCACGCCTGGCCCGGGGGTCTGCACCTCTCC
TCCAGTGCGCACCTGGAGCTGCGTCCCGGGTCAGGTGCGGGGAGGGAGGGAATCTC
AGTGTCCCCTTCCAGCCTTGCAAGCGCCTTTGGCCCCTGCCCCAGCCCCTCGGTTTGG
GGGAGATTTCAGAACGCGGACAGCGCCCTGGCTGCGGGCCATAGGGGACTGGGTGG
AACTCGGGAAGCCCCCAGAGCAGGGGCTTACTCGCTTCAAGTTTGGGGAACCCCGG
GCAGCGGGTGCAGGCCACGAGACCCGAAGGTTCTCAGGTGCCCCCCTGCAGGCTGG
CCGTGCGCGCCGTGGGGCGCTTGTCGCGAGCGCCGAGGGCTGCAGGACGCGGACCA
GACTCGCGGTGCAGGGGGGCCTGGCTGCAGCTAACAGGTGATCCCGTTCTTTCTGTT
CCTCGCTCTTCCCCTCCGATCGTCCTCGCTTACCGCGTGTCCTCCCTCCTCGCTGTCCT
CTGGCTCGCAGGTCATGGCAGCGCCAGGCGGCAGGTCGGAGCCGCCGCAGCTCCCC
GAGTACAGCTGCAGCTACATGGTGTCGCGGCCGGTCTACAGCGAGCTCGCTTTCCAG
CAACAGCACGAGCGGCGCCTGCAGGAGCGCAAGACGCTGCGGGAGAGCCTGGCCA

AGTGCTGCAGGTAGCGGCCGCGCGGGCCTGCGTAGAGAGAAGCGGAGCGGGGCGT
CCACGCCTTGGGGAGGGAAGGGCGTCCCCAGCGGGCGAGAGTGGGGTGCGGGCGG
CGGAGCCCCTGGGCGCCAGCTGCTTCTCCCAGAGGCCCGACTTTCGGTCTCCGGTCC
TCCACGCCGCCCTTCTGGTGGGAGGGTGGCTCCATCAGTCTCGGGCCCGAAATGAAC
TTACCTGGGAAACTCGCCTTTGGGGAGAGTGGGTTCTAGGAGCCCCGTCTCTCTTTTT
CCTCTCTGAAGGAAACTTGGAGTGCCTCTTGGGGTACAGTGGGTCCCTGTTGCCTTC
TTGGGAGCTTGTTTAAATGAAATGAATAGGGAAACCCAGCTCTTGACCAGGAGGAG
TCCTTGAAACACTCAAGCTAAGTAGGCGGGCTACCATTCAGTTAGAGACCAGGATG
CAAGCTAGAACCCAGGGGAGCGCGGGGTGTGCCAAGTACTTCATCAGCAGGCTGTG
GGACCCCTGGGGAAAGCCACCCTCAGTCTCTAAACCCAAACATGCCGTAACTAGAT
GTCACAAACATAAAGAAATTAGAGTTTCTAAAACCTTTCATTATAG

[0396] Exemplary Human SLC26A4 enhancer-promoter (SEQ ID NO: 60)

CGGAAGGTTGATGTACAGAGGTCTGTATTTTGGAGCCTCTTCTGTATTTACTTCAGA
ACACTAACAATCAGGCGAGAATGTTCTGGTTTATCAAACCCTTCCTTCTGCCTTTCAT
CTTAACCATGCATTAGTTTTAACAAAGTTCATCCCAACAGAAGACAAAACACTGATG
AGGTAGGATAGCTCCAGCTCCTCCTCCCTCTCTTCTAGTCTTGATTTCCATGTAGTCC
AGTTTATTCCTTCCCTGATTGTCCAGGAGAATGAGAAAAAGAAAAAACAGAGTCTA
GTGGGTAAGAAAGGGCCACCTGGACGGCTTGATTTGGATTGTGAAATAAAACACAC
ACACATGCACACGTAGAATAAGTGGCTAAAATCTGAGTAAATCGTGAACTCTCTGTA
TCCTCCACCCATTGAATACTCCTAAAAGACTTTCTAGAAATTCAAGGACTTATTAAT
ATAGAAACCTGGCCATTGTTCCTCTTCTCCTCCCCATGTGGTATGAGAGCACCTGTG
GCAGGCTCCCAGAGACCACGGACCTCTTCCTCTAGGCGGGCTCTGCTCTTCTTTAAG
GAGTCCCACAGGGCCTGGCCCGCCCCTGACCTCGCAACCCTTGAGATTAGTAACGG
GATGAGTGAGGATCCGGGTGGCCCCTGCGTGGCAGCCAGTAAGAGTCTCAGCCTTC
CCGGTTCGGGAAAGGGGAAGAATGCAGGAGGGGTAGGATTTCTTTCCTGATAGGAT
CGGTTGGGAAAGACCGCAGCCTGTGTGTGTCTTTCCCTTCGACCAAGGTGTCTGTTG
CTCCGTAAATAAAACGTCCCACTGCCTTCTGAGAGCGCTATAAAGGCAGCGGAAGG
GTAGTCCGCGGGGCATTCCGGGCGGGGCGCGAGCAGAGACAGGTGAGTT

[0397] In certain embodiments, a promoter is a human LGR5 enhancer-promoter as set forth in SEQ ID NO: 61. In some embodiments, an enhancer-promoter sequence is at least 85%, 90%,

95%, 98% or 99% identical to enhancer-promoter sequence represented by SEQ ID NO: 61. In some embodiments, a promoter is a human LGR5 endogenous enhancer-promoter sequence comprised within SEQ ID NO: 61.

[0398] Exemplary Human LGR5 enhancer-promoter (SEQ ID NO: 61)

AGGGCTATTTGTACCTCAACGAGGGCTTCTCTCCAAGAAAGCCCTGAATCCTTTTCC
TCCTTTTTCCTGCAGATTCACTATAGGACACTTTTTGAAGCAAGAGCATGCATTTTCC
CCCTGGCGCTCTGCAGCGGTTCTCAGAGCCCAGTGTCACTCACATAGGTGGGACTGC
TCTCAGTTCAGAGAGCGCTGGGACACTTAAGATGAAAAGTCCCTGGAAGTTAGCAA
ACAGCCATCTGTCACTCTGGCATCGATTTACTAAAAGTGACTTCTAGGGTATTCTAA
ACCACTTTTAAAAAACAAATGAGTCACTTCGACTTCCTCACCCCGCAAGAGATAGGA
AGGCAGCAGTGGAGTGCTCGCTCAGGAGCTGTATTTGTTTAGCGATTAGCCTAGAGC
TTTGATTTTAGGGCAAAAGCGAGCCAGACAGTGCGGCAGACGTAAGGATCAAAAAG
GCCACCTATCATTCGCCGGGGACGCCTGCCTCCTTACCCTGATAACGTAACTATTTCT
CTGCATAGGATTTTAGTTTTTGTGTTTTTGTTTTGTTTTATTCTGTTTAATCACTTCAA
GTATCTCATCCATTATTTGAAGCGGGCTCGGAGGAAACGTGCCGCATCCTCCAGTCC
TTGTGCGTCTGTTTAGGTCTCTCCGAAGCAGGTCCCTCTCGACTCTTAGATCTGGGTC
TCCAGCACGCATGAAGGGGTAAGGGTGGGGGGGTCCCCTATTCCGGCGCGCGGCGT
TGAGCACTGAATCTTCCAGGCGGAGGCTCAGTGGGAGCGCCGAGAACTCGCCAGTA
CCGCGCGCTGCCTGCTGCCTGCTGCCTCCCAGCCCAGGACTTGGGAAAGGAGGGAG
GGGACAAGTGGAGGGAAAGTGGGGCCGGGCGGGGGGTGCCTGGGAAGCCAGGCTG
CGCTGACGTCACTGGGCGCGCAATTCGGGCTGGAGCGCTTTAAAAAACGAGCGTGC
AAGCAGAGATGCTGCTCCACACCGCTCAGGCCGCGAGCAGCAGCAAGGCGCACCGC
CACTGTCGCCGCTGCAGCCAGGGCTGCTCCGAAGGCCGGCGTGGCGGCAACCGGCA
CCTCTGTCCCCGCCGCGCTTCTCCTCGCCGCCCACGCCGTGGGGTCAGGAACGCGGC
GTCTGGCGCTGCAGACGCCCGCTGAGTTGCAGAAGCCCACGGAGCGGCGCCCGGCG
CGCCACGGCCCGTAGCAGTCCGGTGCTGCTCTCCGCCCGCGTCCGGCTCGTGGCCCC
CTACTTCGGGCACCGACCGGT

[0399] In certain embodiments, a promoter is a human SYN1 enhancer-promoter as set forth in SEQ ID NO: 62. In some embodiments, an enhancer-promoter sequence is at least 85%, 90%, 95%, 98% or 99% identical to enhancer-promoter sequence represented by SEQ ID NO: 62. In

some embodiments, a promoter is a human SYN1 endogenous enhancer-promoter sequence comprised within SEQ ID NO: 62.

[0400] Exemplary Human SYN1 enhancer-promoter (SEQ ID NO: 62)

TGCGTATGAGTGCAAGTGGGTTTTAGGACCAGGATGAGGCGGGGTGGGGGTGCCTA
CCTGACGACCGACCCCGACCCACTGGACAAGCACCCAACCCCCATTCCCCAAATTGC
GCATCCCCTATCAGAGAGGGGGAGGGGAAACAGGATGCGGCGAGGCGCGTGCGCA
CTGCCAGCTTCAGCACCGCGGACAGTGCCTTCGCCCCCGCCTGGCGGCGCGCGCCAC
CGCCGCCTCAGCACTGAAGGCGCGCTGACGTCACTCGCCGGTCCCCCGCAAACTCCC
CTTCCCGGCCACCTTGGTCGCGTCCGCGCCGCCGCCGGCCCAGCCGGACCGCACCAC
GCGAGGCGCGAGATAGGGGGGCACGGGCGCGACCATCTGCGCTGCGGCGCCGGCG
ACTCAGCGCTGCCTCAGTCTGCGGTGGGCAGCGGAGGAGTCGTGTCGTGCCTGAGA
GCGCAGTCGAGAA

[0401] In certain embodiments, a promoter is a human GFAP enhancer-promoter as set forth in SEQ ID NO: 63. In some embodiments, an enhancer-promoter sequence is at least 85%, 90%, 95%, 98% or 99% identical to enhancer-promoter sequence represented by SEQ ID NO: 63. In some embodiments, a promoter is a human GFAP endogenous enhancer-promoter sequence comprised within SEQ ID NO: 63.

[0402] Exemplary Human GFAP enhancer-promoter (SEQ ID NO: 63)

CCCACCTCCCTCTCTGTGCTGGGACTCACAGAGGGAGACCTCAGGAGGCAGTCTGTC
CATCACATGTCCAAATGCAGAGCATACCCTGGGCTGGGCGCAGTGGCGCACAACTG
TAATTCCAGCACTTTGGGAGGCTGATGTGGAAGGATCACTTGAGCCCAGAAGTTCTA
GACCAGCCTGGGCAACATGGCAAGACCCTATCTCTACAAAAAAAGTTAAAAAATCA
GCCACGTGTGGTGACACACACCTGTAGTCCCAGCTATTCAGGAGGCTGAGGTGAGG
GGATCACTTAAGGCTGGGAGGTTGAGGCTGCAGTGAGTCGTGGTTGCGCCACTGCA
CTCCAGCCTGGGCAACAGTGAGACCCTGTCTCAAAAGACAAAAAAAAAAAAAAAAA
AAAAAAAGAACATATCCTGGTGTGGAGTAGGGGACGCTGCTCTGACAGAGGCTCGG
GGGCCTGAGCTGGCTCTGTGAGCTGGGGAGGAGGCAGACAGCCAGGCCTTGTCTGC
AAGCAGACCTGGCAGCATTGGGCTGGCCGCCCCCCAGGGCCTCCTCTTCATGCCCAG
TGAATGACTCACCTTGGCACAGACACAATGTTCGGGGTGGGCACAGTGCCTGCTTCC
CGCCGCACCCCAGCCCCCCTCAAATGCCTTCCGAGAAGCCCATTGAGCAGGGGGCTT

GCATTGCACCCCAGCCTGACAGCCTGGCATCTTGGGATAAAAGCAGCACAGCCCCC
TAGGGGCTGCCCTTGCTGTGTGGCGCCACCGGCGGTGGAGAACAAGGCTCTATTCAG
CCTGTGCCCAGGAAAGGGGATCAGGGGATGCCCAGGCATGGACAGTGGGTGGCAGG
GGGGGAGAGGAGGGCTGTCTGCTTCCCAGAAGTCCAAGGACACAAATGGGTGAGGG
GACTGGGCAGGGTTCTGACCCTGTGGGACCAGAGTGGAGGGCGTAGATGGACCTGA
AGTCTCCAGGGACAACAGGGCCCAGGTCTCAGGCTCCTAGTTGGGCCCAGTGGCTC
CAGCGTTTCCAAACCCATCCATCCCCAGAGGTTCTTCCCATCTCTCCAGGCTGATGT
GTGGGAACTCGAGGAAATAAATCTCCAGTGGGAGACGGAGGGGTGGCCAGGGAAA
CGGGGCGCTGCAGGAATAAAGACGAGCCAGCACAGCCAGCTCATGTGTAACGGCTT
TGTGGAGCTGTCAAGGCCTGGTCTCTGGGAGAGAGGCACAGGGAGGCCAGACAAGG
AAGGGGTGACCTGGAGGGACAGATCCAGGGGCTAAAGTCCTGATAAGGCAAGAGA
GTGCCGGCCCCCTCTTGCCCTATCAGGACCTCCACTGCCACATAGAGGCCATGATTG
ACCCTTAGACAAAGGGCTGGTGTCCAATCCCAGCCCCCAGCCCCAGAACTCCAGGG
AATGAATGGGCAGAGAGCAGGAATGTGGGACATCTGTGTTCAAGGGAAGGACTCCA
GGAGTCTGCTGGGAATGAGGCCTAGTAGGAAATGAGGTGGCCCTTGAGGGTACAGA
ACAGGTTCATTCTTCGCCAAATTCCCAGCACCTTGCAGGCACTTACAGCTGAGTGAG
ATAATGCCTGGGTTATGAAATCAAAAAGTTGGAAAGCAGGTCAGAGGTCATCTGGT
ACAGCCCTTCCTTCCCTTTTTTTTTTTTTTTTTTGTGAGACAAGGTCTCTCTCTGTTGC
CCAGGCTGGAGTGGCGCAAACACAGCTCACTGCAGCCTCAACCTACTGGGCTCAAG
CAATCCTCCAGCCTCAGCCTCCCAAAGTGCTGGGATTACAAGCATGAGCCACCCCAC
TCAGCCCTTTCCTTCCTTTTTAATTGATGCATAATAATTGTAAGTATTCATCATGGTC
CAACCAACCCTTTCTTGACCCACCTTCCTAGAGAGAGGGTCCTCTTGCTTCAGCGGT
CAGGGCCCCAGACCCATGGTCTGGCTCCAGGTACCACCTGCCTCATGCAGGAGTTGG
CGTGCCCAGGAAGCTCTGCCTCTGGGCACAGTGACCTCAGTGGGGTGAGGGGAGCT
CTCCCCATAGCTGGGCTGCGGCCCAACCCCACCCCCTCAGGCTATGCCAGGGGGTGT
TGCCAGGGGCACCCGGGCATCGCCAGTCTAGCCCACTCCTTCATAAAGCCCTCGCAT
CCCAGGAGCGAGCAGAGCCAGAGCAGGTTGGAGAGGAGACGCATCACCTCCGCTGC
TCGC

Enhancers

[0403] 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., an anti-VEGF protein). 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 embodiments, an enhancer sequence is found within an intronic sequence. Unlike promoter sequences, enhancer sequences can act at much larger distance away from the transcription start site (e.g., as compared to a promoter). Non-limiting examples of enhancers include a RSV enhancer, a CMV enhancer, and/or a SV40 enhancer. In some embodiments, a construct comprises a CMV enhancer exemplified by SEQ ID NO: 64. In some embodiments, an enhancer sequence is at least 85%, 90%, 95%, 98% or 99% identical to the enhancer sequence represented by SEQ ID NO: 64. In some embodiments, an SV-40 derived enhancer is the SV-40 T intron sequence, which is exemplified by SEQ ID NO: 65. In some embodiments, a an enhancer sequence is at least 85%, 90%, 95%, 98% or 99% identical to the enhancer sequence represented by SEQ ID NO: 65.

[0404] Exemplary CMV enhancer (SEQ ID NO: 64)

GACATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATA
GCCCATATATGGAGTTCCGCGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGAC
CGCCCAACGACCCCCGCCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGC
CAATAGGGACTTTCCATTGACGTCAATGGGTGGACTATTTACGGTAAACTGCCCACT
TGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACG
GTAAATGGCCCGCCTGGCATTATGCCCAGTACATGACCTTATGGGACTTTCCTACTT
GGCAGTACATCTACGTATTAGTCATCGCTATTACCATGG

[0405] Exemplary SV-40 synthetic intron (SEQ ID NO: 65)

GGAGTCGCTGCGTTGCCTTCGCCCCGTGCCCCGCTCCGCGCCGCCTCGCGCCGCCCG
CCCCGGCTCTGACTGACCGCGTTACTCCCACAGGTGAGCGGGCGGGACGGCCCTTCT
CCTCCGGGCTGTAATTAGCGCTTGGTTTAATGACGGCTCGTTTCTTTTCTGTGGCTGC
GTGAAAGCCTTAAAGGGCTCCGGGAGGGCCCTTTGTGCGGGGGGGAGCGGCTCGGG
GGGTGCGTGCGTGTGTGTGTGCGTGGGGAGCGCCGCGTGCGGCCCGCGCTGCCCGG
CGGCTGTGAGCGCTGCGGGCGCGGCGCGGGGCTTTGTGCGCTCCGCGTGTGCGCGA
GGGGAGCGCGGCCGGGGGCGGTGCCCCGCGGTGCGGGGGGGCTGCGAGGGGAACA
AAGGCTGCGTGCGGGGTGTGTGCGTGGGGGGGTGAGCAGGGGGTGTGGGCGCGGCG
GTCGGGCTGTAACCCCCCCCTGCACCCCCCTCCCCGAGTTGCTGAGCACGGCCCGGC

TTCGGGTGCGGGGCTCCGTGCGGGGCGTGGCGCGGGGCTCGCCGTGCCGGGCGGGG
GGTGGCGGCAGGTGGGGGTGCCGGGCGGGGCGGGGCCGCCTCGGGCCGGGGAGGG
CTCGGGGGGAGGGGCGCGGCGGCCCCCGGAGCGCCGGCGGCTGTCGAGGCGCGGCG
AGCCGCAGCCATTGCCTTTTATGGTAATCGTGCGAGAGGGCGCAGGGACTTCCTTTG
TCCCAAATCTGTGCGGAGCCGAAATCTGGGAGGCGCCGCCGCACCCCCTCTAGCGG
GCGCGGGGCGAAGCGGTGCGGCGCCGGCAGGAAGGAAATGGGCGGGGAGGGCCTT
CGTGCGTCGCCGCGCCGCCGTCCCCTTCTCCCTCTCCAGCCTCGGGGCTGTCCGCGG
GGGGACGGCTGCCTTCGGGGGGGGACGGGGCAGGGCGGGGTTCGGCTTCTGGCGTGT
GACCGGCGGCTCTAGAGCCTCTGCTAACCATGTTCATGCCTTCTTCTTTTTCCTACAG

Untranslated Regions: 5` UTRs and 3` UTRs

[0406] In some embodiments, 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 an anti-VEGF protein.

[0407] Natural 5` UTRs include a sequence that plays a role in translation initiation. in some embodiments, 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.

[0408] In some embodiments, 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.

[0409] In some embodiments, 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 embodiments, a 5` UTR is derived from an endogenous gene loci and may include all or part of an endogenous sequence.

[0410] 3` UTRs are found immediately 3` to the stop codon of the gene of interest. In some embodiments, 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 embodiments, a 3` UTR is derived from an endogenous gene loci and may include all or part of an endogenous sequence.

[0411] 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., Mal. Cell. Biol. 15:5777-5788, 1995; Chen et al., Mal. Cell Biol. 15:2010-2018, 1995, each of which is incorporated herein in its entirety by reference): 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.

[0412] 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*.

[0413] In some embodiments, the introduction, removal, or modification of 3` UTR AREs can be used to modulate the stability of an mRNA encoding an anti-VEGF protein. In other embodiments, AREs can be removed or mutated to increase the intracellular stability and thus increase translation and production of an anti-VEGF protein.

[0414] In other embodiments, non-ARE sequences may be incorporated into the 5` or 3` UTRs. In some embodiments, introns or portions of intron sequences may be incorporated into the flanking regions of the polynucleotides in any of the constructs, compositions, kits, and methods

provided herein. Incorporation of intronic sequences may increase protein production as well as mRNA levels.

Internal Ribosome Entry Sites (IRES)

[0415] In some embodiments, a construct encoding an anti-VEGF protein 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, Mal. Cell. Biol. 8(3):1103-1112, 1988, incorporated herein in its entirety by reference).

[0416] 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.

[0417] In some embodiments, the IRES sequence that is incorporated into a construct that encodes an anti-VEGF protein, or a portion of an anti-VEGF protein 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 I I: 1921-1931, 2000; and Klump et al., Gene Therapy 8:811-817, 2001, each of which is incorporated in its entirety herein by reference).

[0418] An IRES can be utilized in an rAAV construct. In some embodiments, a construct encoding a C-terminal portion of an anti-VEGF protein can include a polynucleotide internal ribosome entry site (IRES). In some embodiments, an IRES can be part of a composition

comprising more than one construct. In some embodiments, an IRES is used to produce more than one polypeptide from a single gene transcript.

Secretion and Cleavage Signals

[0419] In some embodiments, any of the constructs provided herein can include secretion signals, cleavage sites, and/or linker sequences. In some embodiments, these sites are functional in a translated protein, and result in post-translational modifications and/or processing events. In some embodiments, constructs as described herein are translated into a relatively long precursor polypeptide, such a precursor polypeptide may then undergo post translational modifications and/or processing, which may involve endogenous cellular enzymatic. Such a processing step may produce multiple peptides, the biological function of such peptides may be accomplished either solely by one peptide, or by the function of multiple peptides acting in concert.

[0420] In some embodiments, constructs provided herein include a signal peptide. In some embodiments, a signal peptide may be a signal sequence, targeting signal, localization signal, localization sequence, transit peptide, leader sequence or leader peptide. In some embodiments, such a sequence is generally short (e.g., approximately 16-30 amino acids in length). In some embodiments, such a signal peptide is present at the N-terminus of a peptide of interest. In some embodiments, more than one signal peptide may exist in a translational product. In some embodiments, an exemplary signal peptide comprises a secretion signal from interleukin-2 (IL2SS). In some embodiments, an exemplary signal peptide is encoded by a nucleotide sequence represented by SEQ ID NO: 66 or 67. In some embodiments, such an amino acid sequence is represented by SEQ ID NO: 68, and can be 95%, 90%, 85%, 80%, or 75% identical to such a sequence. One skilled in the art will recognize that alternative secretion signal sequences exist, and may be incorporated into constructs as described herein.

[0421] In some embodiments, constructs provided herein include a linker peptide. In some embodiments, a linker peptide is utilized to join two or more functional peptides in a translational product. In some embodiments, such a linker peptide may include additional functional sequences, such as recognition sequences for endogenous peptidases. In some embodiments, such a linker sequence comprises a furin cleavage signal. In some embodiments, an exemplary linker sequence comprising a furin cleavage signal is encoded by a nucleotide sequence represented by SEQ ID NO: 71. In some embodiments, such a linker sequence comprises a furin cleavage signal and can

be represented by SEQ ID NO: 72, or can be approximately 90%, 80%, 70%, or 60% similar to such a sequence. In some embodiments, a linker peptide sequence may be one amino acid in length, two amino acids in length, three amino acids in length, four amino acids in length, five amino acids in length, six amino acids in length, seven amino acids in length, eight amino acids in length, nine amino acids in length, ten amino acids in length, eleven amino acids in length, twelve amino acids in length, thirteen amino acids in length, fourteen amino acids in length, fifteen amino acids in length, sixteen amino acids in length, seventeen amino acids in length, eighteen amino acids in length, nineteen amino acids in length, or twenty amino acids in length. In some embodiments, a linker peptide sequence may be up to fifty amino acids in length. One skilled in the art will recognize that alternative linker sequences exist (functional or not), and may be incorporated into constructs as described herein.

[0422] In some embodiments, constructs provided herein include a peptide sequence that induces polypeptide cleavage and/or failure to form a peptide linkage during translation. In some embodiments, constructs as described herein may include a self-cleaving peptide, that in some embodiments may be a 2A self-cleaving peptide. In some embodiments, such a peptide is approximately 18 to 22 amino acids in length, e.g., 18 amino acids in length, 19 amino acids in length, 20 amino acids in length, 21 amino acids in length, or 22 amino acids in length. In some embodiments, such a peptide may induce ribosomal skipping during translation of a protein. In some embodiments, a 2A self-cleaving peptide is represented by a core sequence motif of DxExNPGP (SEQ ID NO: 115), and are found endogenously in a range of viral families. In some embodiments, a self-cleaving peptide generates polyproteins from a single transcript by causing the ribosome to fail at making a peptide bond. In some embodiments, a self-cleaving and/or cleavage signal is encoded by a nucleotide sequence represented by SEQ ID NO: 69. In some embodiments, a self-cleaving and/or cleavage signal is represented by SEQ ID NO: 70, or a sequence sharing approximately 95%, 90%, 80%, 75%, 70%, 65%, 60%, 55%, or 50% identity. One skilled in the art will recognize that alternative peptide cleavage sequences exist (self-cleaving or requiring the aid of endogenous cellular machinery), and may be incorporated into constructs as described herein.

[0423] Exemplary Signal Secretion nucleotide sequence (SEQ ID NO: 66)

ATGTACCGGATGCAGCTGCTGAGCTGTATCGCCCTGTCTCTGGCCCTGGTCACCAAT
TCT

[0424] Exemplary Signal Secretion nucleotide sequence (SEQ ID NO: 67)

ATGTATAGAATGCAGCTCCTGTCCTGCATTGCCCTGAGCCTGGCTCTCGTGACCAAC
AGC

[0425] Exemplary Signal Secretion amino acid sequence (SEQ ID NO: 68)

MYRMQLLSCIALSLALVTNS

[0426] Exemplary Cleavage signal nucleotide sequence (SEQ ID NO: 69)

GGCTCTGGCGAAGGCAGAGGCAGCCTGCTTACATGTGGCGACGTGGAAGAGAACCC
CGGACCT

[0427] Exemplary Cleavage signal amino acid sequence (SEQ ID NO: 70)

GSGEGRGSLLTCGDVEENPGP

[0428] Exemplary Linker/Cleavage signal nucleotide sequence (SEQ ID NO: 71)

GACAAGACCCACACCGGCAAGCGGAAGAGAAGA

[0429] Exemplary Linker/Cleavage signal amino acid sequence (SEQ ID NO: 72)

DKTHTGKRKRR

Splice Sites

[0430] In some embodiments, 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 embodiments, splice sites are involved in trans-splicing.

[0431] Exemplary splice donor intron (SEQ ID NO: 73)

GTAAGTATCAAGGTTACAAGACAGGTTTAAGGAGACCAATAGAAACTGGGCTTGTC
GAGACAGAGAAGACTCTTGCGTTTCT

[0432] Exemplary splice acceptor intron (SEQ ID NO: 74)

GATAGGCACCTATTGGTCTTACTGACATCCACTTTGCCTTTCTCTCCACAG

Polyadenylation Sequences

[0433] In some embodiments, 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 comprises 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 in its entirety by reference). 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 in its entirety by reference). In some embodiments, a poly(A) signal sequence is positioned 3` to the coding sequence.

[0434] 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. 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)(SEQ ID NO: 117) added to the pre-mRNA through the action of an enzyme, polyadenylate polymerase. In some embodiments, a poly(A) tail is added onto transcripts that contain a specific sequence, e.g., a 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.

[0435] 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.

[0436] 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. US.A. 81(13):3944-3948, 1984; U.S. Patent No. 5,122,458, each of which is incorporated herein in its entirety by reference), 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 in its entirety by reference), human collagen, polyoma virus (Batt et al., Mal. Cell Biol. 15(9):4783-4790, 1995, which is incorporated herein in its entirety by reference), the Herpes simplex virus thymidine kinase gene (HSV TK), IgG heavy-chain gene polyadenylation signal (US 2006/0040354, which is incorporated herein in its entirety by reference), human growth hormone (hGH) (Szymanski et al., Mal. Therapy 15(7):1340-1347, 2007, which is incorporated herein in its entirety by reference), the group consisting of SV40 poly(A) site, such as the SV40 late and early poly(A) site (Schek et al., Mal. Cell Biol. 12(12):5386-5393, 1992, which is incorporated herein in its entirety by reference).

[0437] 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 in its entirety by reference).

[0438] In some embodiments, a poly(A) signal sequence can be a synthetic polyadenylation site (see, e.g., the pCl-neo expression construct of Promega that is based on Levitt el al, Genes Dev. 3(7):1019-1025, 1989, which is incorporated herein in its entirety by reference). In some embodiments, a poly(A) signal sequence is the polyadenylation signal of soluble neuropilin-1 (sNRP) (AAATAAAATACGAAATG) (SEQ ID NO: 116) (see, e.g., WO 05/073384, which is incorporated herein in its entirety by reference). In some embodiments, a poly(A) signal sequence comprises or consists of the SV40 poly(A) site. In some embodiments, a poly(A) signal comprises or consists of SEQ ID NO: 76. In some embodiments, a poly(A) signal sequence comprises or consists of bGHpA. In some embodiments, a poly(A) signal comprises or consists of SEQ ID NO: 75. Additional examples of poly(A) signal sequences are known in the art. In some embodiments, a poly(A) sequence is at least 85%, 90%, 95%, 98% or 99% identical to the poly(A) sequence represented by SEQ ID NOs: 75 or 76.

[0439] Exemplary bGH poly(A) signal sequence (SEQ ID NO: 75)

CTGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGCCCCTCCCCCGTGCCTTCCTTGAC
CCTGGAAGGTGCCACTCCCACTGTCCTTTCCTAATAAAATGAGGAAATTGCATCGCA

TTGTCTGAGTAGGTGTCATTCTATTCTGGGGGGTGGGGTGGGGCAGGACAGCAAGG
GGGAGGATTGGGAAGACAATAGCAGGCATGCTGGGGATGCGGTGGGCTCTATGG

[0440] Exemplary SV40 poly(A) signal sequence (SEQ ID NO: 76)

AACTTGTTTATTGCAGCTTATAATGGTTACAAATAAAGCAATAGCATCACAAATTTC
ACAAATAAAGCATTTTTTTCACTGCATTCTAGTTGTGGTTTGTCCAAACTCATCAATG
TATCTTA

Additional Sequences

[0441] In some embodiments, constructs of the present disclosure may comprise one or more cloning sites. In some such embodiments, cloning sites may not be fully removed prior to manufacturing for administration to a subject. In some embodiments, cloning sites may have functional roles including as linker sequences, as nucleotide and/or peptide cleavage signals, and/or as portions of a Kozak site. 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 embodiments, constructs may contain any combination of cloning sites, exemplary cloning sites are represented by SEQ ID NO: 77-82.

[0442] Exemplary cloning site A (SEQ ID NO: 77)

TTGTCGACGCGGCCGCACGCGT

[0443] Exemplary cloning site B (SEQ ID NO: 78)

CTCCTGGGCAACGTGCTGGTTATTGTGACCGGTGCCACC

[0444] Exemplary cloning site C (SEQ ID NO: 79)

TAAGAGCTCGCTGATCAGCCTCGA

[0445] Exemplary cloning site D (SEQ ID NO: 80)

AAGCTTGAATTCAGCTGACGTGCCTCGGACCGCCTAGG

[0446] Exemplary cloning site E (SEQ ID NO: 81)

GCGGCCGCACGCGT

[0447] Exemplary cloning site F (SEQ ID NO: 82)

AAGCTTGAATTCAGCTGACGTGCCTCGGACCGCT

Destabilization Domains

[0448] In some embodiments, any of the constructs provided herein can optionally include a sequence encoding a destabilizing domain ("a destabilizing sequence") for temporal control of protein expression. Non-limiting examples of destabilizing sequences include sequences encoding a FK506 sequence, a dihydrofolate reductase (DHFR) sequence, or other exemplary destabilizing sequences.

[0449] In the absence of a stabilizing ligand, a protein sequence operatively linked to a destabilizing sequence is degraded by ubiquitination. In contrast, in the presence of a stabilizing ligand, protein degradation is inhibited, thereby allowing the protein sequence operatively linked to the destabilizing sequence to be actively expressed. As a positive control for stabilization of protein expression, protein expression can be detected 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).

[0450] Additional examples of destabilizing sequences are known in the art. In some embodiments, the destabilizing sequence is a FK506- and rapamycin-binding protein (FKBP12) sequence, and the stabilizing ligand is Shield-1 (Shld1) (Banaszynski et al. (2012) Cell 126(5): 995-1004, which is incorporated in its entirety herein by reference). In some embodiments, a destabilizing sequence is a DHFR sequence, and a stabilizing ligand is trimethoprim (TMP) (Iwamoto et al. (2010) Chem Biol 17:981-988, which is incorporated in its entirety herein by reference).

[0451] In some embodiments, a destabilizing sequence is a FKBP12 sequence, and a presence of an rAAV construct carrying the FKBP12 gene in a subject cell (e.g., a supporting cochlear outer hair cell) is detected by western blotting. In some embodiments, a destabilizing sequence can be used to verify the temporally-specific activity of any of the rAAV constructs described herein.

[0452] Exemplary DHFR destabilizing amino acid sequence (SEQ ID NO: 83)

MISLIAALAVDYVIGMENAMPWNLPADLAWFKRNTLNKPVIMGRHTWESIGRPLPGRK
NIILSSQPSTDDRVTWVKSVDEAIAACGDVPEIMVIGGGRVIEQFLPKAQKLYLTHIDAEV
EGDTHFPDYEPDDWESVFSEFHDADAQNSHSYCFEILERR

[0453] Exemplary DHFR destabilizing nucleotide sequence (SEQ ID NO: 84)

GGTACCATCAGTCTGATTGCGGCGTTAGCGGTAGATTACGTTATCGGCATGGAAAAC
GCCATGCCGTGGAACCTGCCTGCCGATCTCGCCTGGTTTAAACGCAACACCTTAAAT
AAACCCGTGATTATGGGCCGCCATACCTGGGAATCAATCGGTCGTCCGTTGCCAGGA
CGCAAAAATATTATCCTCAGCAGTCAACCGAGTACGGACGATCGCGTAACGTGGGT
GAAGTCGGTGGATGAAGCCATCGCGGCGTGTGGTGACGTACCAGAAATCATGGTGA
TTGGCGGCGGTCGCGTTATTGAACAGTTCTTGCCAAAAGCGCAAAAACTGTATCTGA
CGCATATCGACGCAGAAGTGGAAGGCGACACCCATTTCCCGGATTACGAGCCGGAT
GACTGGGAATCGGTATTCAGCGAATTCCACGATGCTGATGCGCAGAACTCTCACAGC
TATTGCTTTGAGATTCTGGAGCGGCGATAA

[0454] Exemplary destabilizing domain (SEQ ID NO: 85)

ATCAGTCTGATTGCGGCGTTAGCGGTAGATTACGTTATCGGCATGGAAAACGCCATG
CCGTGGAACCTGCCTGCCGATCTCGCCTGGTTTAAACGCAACACCTTAAATAAACCC
GTGATTATGGGCCGCCATACCTGGGAATCAATCGGTCGTCCGTTGCCAGGACGCAAA
AATATTATCCTCAGCAGTCAACCGAGTACGGACGATCGCGTAACGTGGGTGAAGTC
GGTGGATGAAGCCATCGCGGCGTGTGGTGACGTACCAGAAATCATGGTGATTGGCG
GCGGTCGCGTTATTGAACAGTTCTTGCCAAAAGCGCAAAAACTGTATCTGACGCATA
TCGACGCAGAAGTGGAAGGCGACACCCATTTCCCGGATTACGAGCCGGATGACTGG
GAATCGGTATTCAGCGAATTCCACGATGCTGATGCGCAGAACTCTCACAGCTATTGC
TTTGAGATTCTGGAGCGGCGA

[0455] Exemplary FKBP12 destabilizing peptide amino acid sequence (SEQ ID NO: 86)

MGVEKQVIRPGNGPKPAPGQTVTVHCTGFGKDGDLSQKFWSTKDEGQKPFSFQIGKGA
VIKGWDEGVIGMQIGEVARLRCSSDYAYGAGGFPAWGIQPNSVLDFEIEVLSVQ

Reporter Sequences or Elements

[0456] In some embodiments, 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), and a luciferase. Additional examples of reporter sequences are known in the art. 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).

[0457] In some embodiments, 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 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 embodiments, 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.

[0458] In some embodiments, 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: 87.

[0459] In some embodiments, a reporter sequence is an Enhanced Green Fluorescent Protein (eGFP) tag (e.g., an eGFP tag), and the presence of a construct carrying an eGFP tag in a mammalian cell (e.g., an inner ear cell, e.g., a cochlear hair or supporting cell) is detected by fluorescence, protein binding, or detection assays (e.g., Western blots, immunohistochemistry, radioimmunoassay (RIA), mass spectrometry). Exemplary eGFP tag sequences are provided as SEQ ID NO: 104 and 105.

[0460] Exemplary 3xFLAG tag sequence (SEQ ID NO: 87)

GGATCCCGGGCTGACTACAAAGACCATGACGGTGATTATAAAGATCATGACATCGA
CTACAAGGATGACGATGACAAG

[0461] Exemplary Enhanced Green Fluorescent Protein (eGFP) nucleotide sequence (SEQ ID NO: 104)

MVSKGEELFTGVVPILVELDGDVNGHKFSVSGEGEGDATYGKLTLKFICTTGKLPVPWP
TLVTTLTYGVQCFSRYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEG
DTLVNRIELKGIDFKEDGNILGHKLEYNYNSHNVYIMADKQKNGIKVNFKIRHNIEDGSV
QLADHYQQNTPIGDGPVLLPDNHYLSTQSALSKDPNEKRDHMVLLEFVTAAGITLGMD
ELYK

[0462] Exemplary Enhanced Green Fluorescent Protein (eGFP) Amino Acid sequence (SEQ ID NO: 105)

ATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCGAGCT
GGACGGCGACGTAAACGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGAT
GCCACCTACGGCAAGCTGACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCCGTG
CCCTGGCCCACCCTCGTGACCACCCTGACCTACGGCGTGCAGTGCTTCAGCCGCTAC
CCCGACCACATGAAGCAGCACGACTTCTTCAAGTCCGCCATGCCCGAAGGCTACGTC
CAGGAGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGACCCGCGCCGAGGT
GAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCA
AGGAGGACGGCAACATCCTGGGGCACAAGCTGGAGTACAACTACAACAGCCACAA
CGTCTATATCATGGCCGACAAGCAGAAGAACGGCATCAAGGTGAACTTCAAGATCC
GCCACAACATCGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAGCAGAACACC
CCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTACCTGAGCACCCAGTCC
GCCCTGAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTTCGT
GACCGCCGCCGGGATCACTCTCGGCATGGACGAGCTGTACAAG

AAV Capsids

[0463] The present disclosure provides one or more polynucleotide constructs packaged into an AAV capsid. In some embodiments, 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 or Anc80 serotype, or one or more hybrids thereof. In some embodiments, an AAV capsid is from an AAV ancestral serotype. In some embodiments, 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 embodiments, an AAV capsid is an Anc80 capsid (e.g., an Anc80L65 capsid). In some embodiments, an AAV capsid is created using a template nucleotide coding sequence comprising SEQ ID NO: 88. In some embodiments, the capsid comprises a polypeptide represented by SEQ ID NO: 89. In some embodiments, the capsid comprises a polypeptide with at least 85%, 90%, 95%, 98% or 99% sequence identity to the polypeptide represented by SEQ ID NO: 89. In some embodiments, the capsid comprises a polypeptide represented by SEQ ID NO: 113. In some embodiments, the capsid comprises a polypeptide with at least 85%, 90%, 95%, 98% or 99% sequence identity to the polypeptide represented by SEQ ID NO: 113. In some embodiments, the capsid comprises a polypeptide represented by SEQ ID NO: 114. In some embodiments, the capsid comprises a polypeptide with at least 85%, 90%, 95%, 98% or 99% sequence identity to the polypeptide represented by SEQ ID NO: 114.

[0464] As provided herein, any combination of AAV capsids and rAAV 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, wild type or variant AAV2 ITRs and AAV6 capsid, etc. In some embodiments of the present disclosure, an AAV particle is comprised of AAV2 components (e.g., capsid and ITRs are AAV2 serotype). In some embodiments, 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 embodiments 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: 89, SEQ ID NO: 113 or SEQ ID NO: 114) that encapsidates an AAV construct with AAV2 ITRs (e.g., SEQ ID NOs: 45 and 46, or 47 and 48) flanking a portion of a coding sequence, for example, a sequence encoding an anti-VEGF protein (e.g., SEQ ID NO: 13, 14, 15, 19, 22, 42, and/or 103). 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 herein in its entirety by reference. In some embodiments, a capsid sequence is at least 85%, 90%, 95%, 98% or 99% identical to a capsid nucleotide or amino acid sequence represented by SEQ ID NO: 88 or 89, respectively.

[0465] In some embodiments, a platform delivery approach disclosed herein combines a library of synthetic AAV capsids, known as ancestral AAV (AAVAnc) capsids that recreate the evolutionary lineage of current naturally occurring viruses. In some embodiments, these AAV

capsids are coupled with a novel, minimally invasive administration procedure to deliver product candidates directly to the cochlea. In some embodiment, a delivery approach utilizes an AAVAnc80 capsid variant from this library (also known as Anc80L65). In some embodiments, such a capsid is utilized to create an rAAV particle, wherein such a particle is created through the addition of a construct as described herein, e.g., a construct comprising an anti-VEGF protein as described herein, to create an rAAVAnc80-antiVEGF. In some embodiments, such a particle can deliver cDNA for an inhibitor of VEGF, a protein that can cause abnormal blood vessel growth. In some embodiments, such a treatment is for an otological disorder characterized by neovascularization, e.g., for the treatment of VS, a tumor of the vestibulocochlear nerve, also referred to as the cranial nerve VIII.

[0466] In some embodiments, a composition disclosed herein comprises an AAVAnc80 capsid, which is a rationally designed, synthetic AAV capsid whose sequence was inferred by ancestral sequence reconstruction. Ancestral sequence reconstruction uses available sequence information from naturally occurring adeno-associated viruses and, as a result of phylogenetic and statistical prediction, identifies the ancestral state of a sequence at various intermediary evolutionary nodes (**FIG. 9**). During the creation of AAVAnc80, nine nodes were reconstructed, and *in silico* derived sequences across the AAV lineage were synthesized *de novo* and characterized. This led to the identification of the Anc80 Library node (Anc80Lib), the putative ancestor of the widely studied AAV serotypes 1, 2, 8, and 9. Anc80Lib protein sequences were subsequently reverse-translated and generated by gene synthesis, and individual clones were evaluated in isolation for packaging, infectivity, and biological properties. In some embodiments, and based on these results, AAVAnc80, the 65th Anc80Lib clone (Anc80L65), was selected for further characterization. An AAVAnc80 capsid variant has a distinctive composition; although the sequences of AAV8 and AAV2 differ by only approximately 9% and 12%, respectively, from AAVAnc80, structural modeling of AAVAnc80 has shown that around 20% of its particle external surface is divergent from known circulating AAVs (**FIG. 9** and **FIG. 10**) in a manner that is distributed across the capsid surface (Zinn 2015, incorporated herein in its entirety by reference).

[0467] In some embodiments, AAVAnc80's performance as a gene therapy particle *in-vivo* has been characterized and rAAV particles comprising AAVAnc80 have demonstrated a potential to act as a broadly applicable gene therapy particle. In some embodiments, studies conducted in mice and non-human primates (NHPs) have shown that AAVAnc80 has a similar transduction

efficiency to AAV8 when targeting the liver after intravenous injection, without obvious signs of systemic toxicity (Zinn 2015; Murillo 2019, each of which is incorporated herein in its entirety by reference). In addition, in some embodiments, AAVAnc80 has shown tropism for and efficient transduction of the mouse anterior segment of the eye (Wang 2017, incorporated herein in its entirety by reference), mouse and NHP retina (Zinn 2015; Carvalho 2018, each of which is incorporated herein in its entirety by reference), mouse skeletal muscle (Zinn 2015, incorporated herein in its entirety by reference), mouse central nervous system (CNS) by systemic and intraparenchymal delivery (Hudry 2018, incorporated herein in its entirety by reference), and murine kidney (Ikeda 2018, incorporated herein in its entirety by reference).

[0468] In some embodiments, compositions as described herein (e.g., comprising rAAV-antiVEGF) comprise an AAVAnc80 capsid. In some embodiments, AAVAnc80 capsid demonstrates high transduction efficiency for cochlear and vestibular cells. AAVAnc80 is a rationally designed AAV capsid whose sequence was inferred by ancestral sequence reconstruction (Zinn 2015, incorporated herein in its entirety by reference). Ancestral sequence reconstruction uses available sequence information from naturally occurring AAVs and, as a result of phylogenetic and statistical prediction, identifies the ancestral state of a sequence at various intermediary evolutionary nodes. As described in the literature, *de novo* synthesis and characterization of *in silico* derived sequences across the AAV lineage led to identification of the Anc80 Library (Anc80Lib) node, the putative ancestor of the widely studied AAV serotypes 1, 2, 8, and 9. Subsequent evaluation of Anc80Lib sequences led to the further characterization of AAVAnc80, the 65th Anc80Lib clone (Anc80L65). These studies indicated that the AAVAnc80 capsid variant has a distinctive composition with a divergent external surface particle distribution which yields a stable and functional AAV variant with a similar transduction efficiency to AAV8 (Zinn 2015, incorporated herein in its entirety by reference). The first reported use of AAVAnc80 in the mammalian inner ear revealed a high transduction efficiency in cochlear and vestibular hair cells (Landegger 2017, incorporated herein in its entirety by reference). Multiple subsequent, independent investigations have confirmed the increased cochlear and vestibular cell transduction efficiency of AAVAnc80 relative to other AAV serotypes; in mice of various ages (Landegger 2017; Tao 2018; Yoshimura 2018; Omichi 2020, each of which is incorporated herein in its entirety by reference) and in non-human primates (Andres-Mateos 2019, incorporated herein in its entirety

by reference), AAVAnc80 has a higher transduction efficiency and broader tropism compared to a number of other AAV capsids.

[0469] Gene therapy using AAV particles is a promising therapeutic modality for inner ear disorders for several reasons, such as: (1) the inner ear, which contains the auditory and vestibular sensory epithelia, has modified immune surveillance, similar to that in the central nervous system (Fujioka 2014, incorporated herein in its entirety by reference); (2) the sensory and supporting cells of the cochlear organ of Corti are post-mitotic, allowing for the possibility of long-term expression following a single administration of AAV; and (3) the aggregate clinical experience with rAAV delivery in both adults and children, via multiple routes of administration, suggests a strong safety profile for AAV as a delivery vehicle, particularly in localized delivery and/or at low to moderate doses.

[0470] Beginning with initial clinical trials more than two decades ago, rAAV particles have been administered to hundreds of participants in dozens of clinical trials at doses of up to approximately 1E15 vg or more for systemic administration (Flotte 1996; Flotte 2013; Parente 2018; Wang 2019, each of which is incorporated herein in its entirety by reference). The number of trials in which AAV particles have been used for *in-vivo* gene transfer has steadily increased. The safety profile, together with the high efficiency of transduction of a broad range of target tissues, has established rAAV particles as the platform of choice for *in-vivo* gene therapy (Wang 2019, incorporated herein in its entirety by reference). Successful application of the rAAV technology has been achieved in the clinic for a variety of conditions, including coagulation disorders, inherited blindness, and neurodegenerative diseases (Colella 2018; Wang 2019, each of which is incorporated herein in its entirety by reference).

[0471] An rAAV particle product (alipogene tiparvovec; Glybera®) was first approved by the European Medicines Agency (EMA) for treatment of lipoprotein lipase deficiency in 2012. Subsequently, two rAAV products, voretigene neparvovec-rzyl (Luxturna®) for the treatment of confirmed biallelic *RPE65* mutation-associated retinal dystrophy and onasemnogene abeparvovec-xioi (Zolgensma®) for the treatment of spinal muscular atrophy (SMA) with biallelic mutations in the *SMN1* gene, were approved by the U.S. Food and Drug Administration (FDA) in 2017 and 2019, respectively; voretigene neparvovec-rzyl (Luxturna®) was also approved by the EMA for

the treatment of loss of vision due to inherited retinal dystrophy, when the disease is caused by mutations in the gene *RPE65*.

[0472] In some embodiments, drugs and biologics, including rAAV particles, can reach many target cells in the inner ear by delivering them into the perilymph. Perilymph is a fluid very similar in composition to (Lysaght 2011, incorporated herein in its entirety by reference), and in diffusional continuity with, cerebrospinal fluid (CSF). Perilymph bathes most of the sensory, neural, and supporting cells of the cochlea and of the vestibular system, housed in the bony labyrinth of the inner ear (**FIGS. 1** and **3**). In some embodiments, perilymphatic space of cochlea, to which a composition disclosed herein, e.g., rAAV-antiVEGF, is delivered, comprises two scalae, or passages: scala tympani and scala vestibuli, which are continuous with one another at the apex of the cochlear spiral via the helicotrema. Many cells of the inner ear are in fluid continuity with perilymph through interstitial spaces in the tissue.

[0473] In some embodiments, also disclosed herein is a sterile, one-time use delivery device for intracochlear administration, to deliver a composition disclosed herein, e.g., rAAV-antiVEGF to perilymph fluid of inner ear through a round window membrane with a vent located in a stapes footplate. In some embodiments, in this intracochlear administration approach, a composition disclosed herein, e.g., rAAV-antiVEGF, will be administered into the scala tympani through the round window membrane, with a vent in a stapes footplate within the oval window, such that composition is perfused through scala tympani, then through scala vestibuli via connection at the helicotrema, and follows the fluid path to a vent in a stapes footplate (**FIG. 4**). In some embodiments, presence of a vent distinct from the injection port allows for more even distribution of drug along the length of the cochlea and prevents the deleterious build-up of additional fluid pressure within the inner ear. In some embodiments, as evidenced by transduction of vestibular cells using this dual-fenestrae, injection plus venting technique, this delivery approach also permits diffusion of a composition disclosed herein, e.g., injectate, to a vestibular system. In some embodiments, the entire process can be accomplished in a subject with a relatively nontraumatic approach through an external auditory canal; see **FIG. 1**, and the Examples for additional information regarding the surgical administration procedure.

[0474] A number of studies on AAVAnc80 transduction in mice have been published. Different types of viral vectors (e.g., adenoviral vector, herpes simplex viral vectors) have been

considered for gene delivery to the inner ear in animal models (Chen 2001; Wenzel 2007; Husseman 2009, each of which is incorporated herein in its entirety by reference); however, rAAV particles appear to be a promising tool for gene delivery directly to the cochlea given the acceptable safety profile and the long-lasting transgene expression, including recovery of auditory, cochlear, and vestibular function in knock-out and knock-in mouse models (Akil 2012; Kim 2016; Pan 2017; Akil 2019; Al-Moyed 2019; György 2019, each of which is incorporated herein in its entirety by reference). Several AAV serotypes have been delivered into the inner ear, using different surgical approaches and doses, in both neonatal and adult mice (Akil 2012; Askew 2015; Chien 2016; Landegger 2017; Suzuki 2017; Tao 2018; Yoshimura 2018; Akil 2019; Al-Moyed 2019; György 2019; Kim 2019; Omichi 2020, each of which is incorporated herein in its entirety by reference). Transduction efficiency, as assessed by GFP expression in different cell types of the cochlear and vestibular organs, differs depending on the mouse postnatal age, method use to deliver the particle, and serotype or capsid variant evaluated.

[0475] In some embodiments, AAVAnc80 variant has shown high efficiency targeting of cochlear and vestibular sensory cells (hair cells) and accessory cells of cochlear and vestibular organs in neonatal and adult mice compared with other AAV capsids (Landegger 2017; Suzuki 2017; Omichi 2020, each of which is incorporated herein in its entirety by reference). Anc80 neonatal tropism and gene transfer efficiency was evaluated *in-vivo* in C57BL/6 mice injected at postnatal day 1 (P1) by round-window administration (Landegger 2017, incorporated herein in its entirety by reference). Consistent with prior studies, AAV2, AAV6, and AAV8 targeted a low percentage of IHCs, and AAV1 was able to transduce IHCs with higher efficiency, but OHC transduction was minimal; in contrast, AAVAnc80 (1.7E9 vg/cochlea) was able to transduce around 100% of IHCs and ~90% of OHCs (**FIG. 11**) without any deleterious effects to cochlear or auditory function (Landegger 2017, incorporated herein in its entirety by reference). In some embodiments, in the vestibular system, AAVAnc80 transduced type I and type II hair cells of the utricle as well as cells of the semicircular canal cristae (**FIG. 12**), without impacting vestibular function (Landegger 2017, incorporated herein in its entirety by reference).

[0476] Using a different route of administration via the posterior semicircular canal, AAVAnc80 tropism and gene transfer efficiency was evaluated in adult (7 wks) CBA/CaJ mice (Suzuki 2017, incorporated herein in its entirety by reference). AAVAnc80 (9.6E8 vg/cochlea) targeted sensory and accessory cells of the cochlea, including approximately 100% of IHCs

throughout the cochlear length as well as a significant fraction of OHCs, cells of the spiral limbus and Reissner's membrane, and cells of the cochlear modiolus (e.g., spiral ganglion neurons and satellite glial cells) (**FIG. 13)** while maintaining normal cochlear and auditory function (Suzuki 2017, incorporated herein in its entirety by reference). Multiple cell types of the vestibular system were also transduced, including a subset of hair cells and virtually 100% of supporting cells in the utricle and semicircular canal cristae, as well as the saccule (**FIG. 14**), all without deleterious effects on vestibular function (Suzuki 2017, incorporated herein in its entirety by reference).

[0477] More recently, AAVAnc80 *in-vivo* transduction in adult (4 wks) C3H/FeJ mice was evaluated using a route of delivery utilized herein (via round window membrane delivery with posterior semicircular canal fenestration) and directly compared to transduction by naturally occurring serotypes AAV1, AAV2, AAV8, and AAV9 (Omichi 2020, incorporated herein in its entirety by reference). All particles produced some degree of transduction without deleterious effects to auditory function, as demonstrated by control-like (uninjected) ABR thresholds. AAVAnc80 (5.5E9 vg/cochlea) transduced virtually 100% of IHCs along the cochlear length, and approximately 27 to 66% of OHCs depending on cochlear location (**FIG. 15**). Despite a slightly higher transduction efficiency for OHCs by AAV2 (3.68E9 vg/cochlea) compared to AAVAnc80, AAVAnc80 maintained a significantly broader tropism than AAV2 as evidenced by eGFP-positive hair cells of the saccule and spiral ganglion neurons in the cochlea; in these same cell types, AAV2 produced little-to-no transduction (**FIG. 15**) (Omichi 2020, incorporated herein in its entirety by reference).

[0478] In some embodiments, the ability of AAVAnc80 to target a wide range of inner ear cell types, including cochlear IHCs and OHCs, supporting cells, cells of the cochlear spiral ganglion, vestibular hair cells of utricle, saccule, and crista ampularis, and cochlear and vestibular supporting/accessory cells, in neonatal to adult mice, suggests, e.g., that AAVAnc80 could facilitate development of gene therapy approaches for disorders of the inner ear.

[0479] In certain embodiments, platform and supportive studies are conducted in non-human primates (NHPs) using reporter constructs to evaluate AAVAnc80 transduction in an inner ear. In some embodiments, exploratory platform and other supportive studies are performed in NHPs to evaluate AAVAnc80 tropism and dose-dependent effects of delivery of AAVAnc80 particles. In some embodiments, evaluation of AAVAnc80 capsid tropism for NHP inner ear cell types is

facilitated using rAAVAnc80 particles comprising constructs encoding an enhanced GFP (eGFP) reporter transgene and delivered via intracochlear injection through the round window membrane. In some embodiments, such particles may also be useful for development of novel devices for efficient delivery directly to the cochlea as described herein.

[0480] In some embodiments, NHP ears were histologically evaluated for transduction efficiency of cochlear hair cells and dose-dependency of hair cell transduction. In some embodiments, such ears were also analyzed for breadth of cell-type transduction in cochlear and/or vestibular organs. In some embodiments, it is likely that increasing the populations of inner ear cells that are transduced with rAAV particles as described herein (e.g., comprising rAAVAnc80-antiVEGF) supports the likelihood that, in the case of rAAVAnc80-antiVEGF, the inner ear can maintain a sustained depot of secreted anti-VEGF protein; in turn, this protein has the potential to control VS growth via proximity and diffusional continuity with the site of the tumor in the adjacent internal auditory canal. In some embodiments, tolerability of a procedure on hair cell survival, transduction in the presence of pre-existing neutralizing antibodies (NAbs), and any potential transduction of the contralateral (uninjected) ear were also evaluated in NHP. Results are summarized in the Examples.

[0481] In some embodiments, the rationally designed, synthetic AAVAnc80L65 capsid variant (AAVAnc80) is highly efficient at transducing primate IHCs, in a dose-dependent manner, using a delivery device designed for round window membrane administration. In some embodiments, approximately 75 to 100% transduction of IHCs in an injected ear is achieved, using an AAVAnc80 particle comprising a construct encoding a transgene, at a dose of about 6.0×10^{10} vg/cochlea or higher.

[0482] In some embodiments, pre-existing neutralizing antibodies against AAVAnc80, at least at moderate levels in serum, do not inhibit IHC or supporting cell transduction when particles are delivered via an intracochlear route of administration.

[0483] In some embodiments, qualitative assessments of injected ears demonstrates transduction across multiple cochlear and vestibular cell types, all of which are positioned to secrete anti-VEGF protein either into perilymph or directly into interstitial fluid of the cochlear nerve following transduction by rAAVAnc80-antiVEGF and thereby potentially control growth of VSs through diffusion in the vestibulocochlear nerve interstitium.

[0484] Exemplary AAV Anc80 Capsid DNA Sequence (SEQ ID NO: 88)

ATGGCTGCCGATGGTTATCTTCCAGATTGGCTCGAGGACAACCTCTCTGAGGGCATT
CGCGAGTGGTGGGACTTGAAACCTGGAGCCCCGAAACCCAAAGCCAACCAGCAAAA
GCAGGACGACGGCCGGGGTCTGGTGCTTCCTGGCTACAAGTACCTCGGACCCTTCAA
CGGACTCGACAAGGGGGAGCCCGTCAACGCGGCGGACGCAGCGGCCCTCGAGCAC
GACAAGGCCTACGACCAGCAGCTCAAAGCGGGTGACAATCCGTACCTGCGGTATAA
CCACGCCGACGCCGAGTTTCAGGAGCGTCTGCAAGAAGATACGTCTTTTGGGGGCA
ACCTCGGGCGAGCAGTCTTCCAGGCCAAGAAGCGGGTTCTCGAACCTCTCGGTCTGG
TTGAGGAAGGCGCTAAGACGGCTCCTGGAAAGAAGAGACCGGTAGAGCAATCACCC
CAGGAACCAGACTCCTCTTCGGGCATCGGCAAGAAAGGCCAGCAGCCCGCGAAGAA
GAGACTCAACTTTGGGCAGACAGGCGACTCAGAGTCAGTGCCCGACCCTCAACCAC
TCGGAGAACCCCCCGCAGCCCCCTCTGGTGTGGGATCTAATACAATGGCAGCAGGC
GGTGGCGCTCCAATGGCAGACAATAACGAAGGCGCCGACGGAGTGGGTAACGCCTC
AGGAAATTGGCATTGCGATTCCACATGGCTGGGCGACAGAGTCATCACCACCAGCA
CCCGAACCTGGGCCCTCCCCACCTACAACAACCACCTCTACAAGCAAATCTCCAGCC
AATCGGGAGCAAGCACCAACGACAACACCTACTTCGGCTACAGCACCCCCTGGGGG
TATTTTGACTTTAACAGATTCCACTGCCACTTCTCACCACGTGACTGGCAGCGACTCA
TCAACAACAACTGGGGATTCCGGCCCAAGAGACTCAACTTCAAGCTCTTCAACATCC
AGGTCAAGGAGGTCACGACGAATGATGGCACCACGACCATCGCCAATAACCTTACC
AGCACGGTTCAGGTCTTTACGGACTCGGAATACCAGCTCCCGTACGTCCTCGGCTCT
GCGCACCAGGGCTGCCTGCCTCCGTTCCCGGCGGACGTCTTCATGATTCCTCAGTAC
GGGTACCTGACTCTGAACAATGGCAGTCAGGCCGTGGGCCGTTCCTCCTTCTACTGC
CTGGAGTACTTTCCTTCTCAAATGCTGAGAACGGGCAACAACTTTGAGTTCAGCTAC
ACGTTTGAGGACGTGCCTTTTCACAGCAGCTACGCGCACAGCCAAAGCCTGGACCG
GCTGATGAACCCCCTCATCGACCAGTACCTGTACTACCTGTCTCGGACTCAGACCAC
GAGTGGTACCGCAGGAAATCGGACGTTGCAATTTTCTCAGGCCGGGCCTAGTAGCAT
GGCGAATCAGGCCAAAAACTGGCTACCCGGGCCCTGCTACCGGCAGCAACGCGTCT
CCAAGACAGCGAATCAAAATAACAACAGCAACTTTGCCTGGACCGGTGCCACCAAG
TATCATCTGAATGGCAGAGACTCTCTGGTAAATCCCGGTCCCGCTATGGCAACCCAC
AAGGACGACGAAGACAAATTTTTTCCGATGAGCGGAGTCTTAATATTTGGGAAACA
GGGAGCTGGAAATAGCAACGTGGACCTTGACAACGTTATGATAACCAGTGAGGAAG

AAATTAAAACCACCAACCCAGTGGCCACAGAACAGTACGGCACGGTGGCCACTAAC
CTGCAATCGTCAAACACCGCTCCTGCTACAGGGACCGTCAACAGTCAAGGAGCCTT
ACCTGGCATGGTCTGGCAGAACCGGGACGTGTACCTGCAGGGTCCTATCTGGGCCA
AGATTCCTCACACGGACGGACACTTTCATCCCTCGCCGCTGATGGGAGGCTTTGGAC
TGAAACACCCGCCTCCTCAGATCCTGATTAAGAATACACCTGTTCCCGCGAATCCTC
CAACTACCTTCAGTCCAGCTAAGTTTGCGTCGTTCATCACGCAGTACAGCACCGGAC
AGGTCAGCGTGGAAATTGAATGGGAGCTGCAGAAAGAAAACAGCAAACGCTGGAA
CCCAGAGATTCAATACACTTCCAACTACAACAAATCTACAAATGTGGACTTTGCTGT
TGACACAAATGGCGTTTATTCTGAGCCTCGCCCCATCGGCACCCGTTACCTCACCCG
TAATCTG

[0485] Exemplary AAV Anc80 Capsid Amino Acid Sequence (SEQ ID NO: 89)

MAADGYLPDWLEDNLSEGIREWWDLKPGAPKPKANQQKQDDGRGLVLPGYKYLGPF
NGLDKGEPVNAADAAALEHDKAYDQQLKAGDNPYLRYNHADAEFQERLQEDTSFGGN
LGRAVFQAKKRVLEPLGLVEEGAKTAPGKKRPVEQSPQEPDSSSGIGKKGQQPAKKRLN
FGQTGDSESVPDPQPLGEPPAAPSGVGSNTMAAGGGAPMADNNEGADGVGNASGNWH
CDSTWLGDRVITTSTRTWALPTYNNHLYKQISSQSGASTNDNTYFGYSTPWGYFDFNRF
HCHFSPRDWQRLINNNWGFRPKRLNFKLFNIQVKEVTTNDGTTTIANNLTSTVQVFTDS
EYQLPYVLGSAHQGCLPPFPADVFMIPQYGYLTLNNGSQAVGRSSFYCLEYFPSQMLRT
GNNFEFSYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYLSRTQTTSGTAGNRTLQFSQA
GPSSMANQAKNWLPGPCYRQQRVSKTANQNNNSNFAWTGATKYHLNGRDSLVNPGP
AMATHKDDEDKFFPMSGVLIFGKQGAGNSNVDLDNVMITSEEEIKTTNPVATEQYGTV
ATNLQSSNTAPATGTVNSQGALPGMVWQNRDVYLQGPIWAKIPHTDGHFHPSPLMGGF
GLKHPPPQILIKNTPVPANPPTTFSPAKFASFITQYSTGQVSVEIEWELQKENSKRWNPEIQ
YTSNYNKSTNVDFAVDTNGVYSEPRPIGTRYLTRNL

[0486] Exemplary AAV Anc80 Capsid Amino Acid Sequence (SEQ ID NO: 113)

MAADGYLPDWLEDNLSEGIREWWDLKPGAPKPKANQQKQDDGRGLVLPGYKYL
GPFNGLDKGEPVNAADAAALEHDKAYDQQLKAGDNPYLRYNHADAEFQERLQED
TSFGGNLGRAVFQAKKRVLEPLGLVEEGAKTAPGKKRPVEQSPQEPDSSSGIGKKG
QQPAX$_1$KRLNFGQTGDSESVPDPQPLGEPPAAPSGVGSNTMX$_2$AGGGAPMADNNEG
ADGVGNASGNWHCDSTWLGDRVITTSTRTWALPTYNNHLYKQISSQSGX$_3$STNDN
TYFGYSTPWGYFDFNRFHCHFSPRDWQRLINNNWGFRPKX$_4$LNFKLFNIQVKEVTT
NDGTTTIANNLTSTVQVFTDSEYQLPYVLGSAHQGCLPPFPADVFMIPQYGYLTLNN

GSQAVGRSSFYCLEYFPSQMLRTGNNFX$_5$FSYTFEDVPFHSSYAHSQSLDRLMNPLI
DQYLYYLSRTQTTSGTAGNRX$_6$LQFSQAGPSSMANQAKNWLPGPCYRQQRVSKTX$_7$
NQNNNSNFAWTGATKYHLNGRDSLVNPGPAMATHKDDEDKFFPMSGVLIFGKQG
AGNSNVDLDNVMITX$_8$EEEIKTTNPVATEX$_9$YGTVATNLQSX$_{10}$NTAPATGTVNSQG
ALPGMVWQX$_{11}$RDVYLQGPIWAKIPHTDGHFHPSPLMGGFGLKHPPPQILIKNTPVP
ANPPTTFSPAKFASFITQYSTGQVSVEIEWELQKENSKRWNPEIQYTSNYNKSTNVD
FAVDTNGVYSEPRPIGTRYLTRNL

Symbol in SEQ ID NO: 113	Corresponding Amino Acid
X_1	K or R
X_2	A or S
X_3	A or G
X_4	R or k
X_5	E or Q
X_6	T or E
X_7	A or T
X_8	S or N
X_9	Q or E
X_{10}	S or A
X_{11}	N or D

[0487] Exemplary AAV Anc80 Capsid Amino Acid Sequence (SEQ ID NO: 114)

MAADGYLPDWLEDNLSEGIREWWDLKPGAPKPKANQQKQDDGRGLVLPGYKYLGPF
NGLDKGEPVNAADAAALEHDKAYDQQLKAGDNPYLRYNHADAEFQERLQEDTSFGGN
LGRAVFQAKKRVLEPLGLVEEGAKTAPGKKRPVEQSPQEPDSSSGIGKKGQQPARKRLN
FGQTGDSESVPDPQPLGEPPAAPSGVGSNTMAAGGGAPMADNNEGADGVGNASGNWH
CDSTWLGDRVITTSTRTWALPTYNNHLYKQISSQSGGSTNDNTYFGYSTPWGYFDFNRF
HCHFSPRDWQRLINNNWGFRPKKLNFKLFNIQVKEVTTNDGTTTIANNLTSTVQVFTDS
EYQLPYVLGSAHQGCLPPFPADVFMIPQYGYLTLNNGSQAVGRSSFYCLEYFPSQMLRT
GNNFQFSYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYLSRTQTTSGTAGNRTLQFSQ
AGPSSMANQAKNWLPGPCYRQQRVSKTTNQNNNSNFAWTGATKYHLNGRDSLVNPGP
AMATHKDDEDKFFPMSGVLIFGKQGAGNSNVDLDNVMITNEEEIKTTNPVATEEYGTV
ATNLQSANTAPATGTVNSQGALPGMVWQDRDVYLQGPIWAKIPHTDGHFHPSPLMGG
FGLKHPPPQILIKNTPVPANPPTTFSPAKFASFITQYSTGQVSVEIEWELQKENSKRWNPEI
QYTSNYNKSTNVDFAVDTNGVYSEPRPIGTRYLTRNL

Exemplary AAV Particles

[0488] In certain embodiments, the present disclosure relates to an rAAV-antiVEGF composition comprising a particle comprising rAAV2/Anc80L65-CAG.ranibizumab.bGH. In certain embodiments, an rAAV-antiVEGF composition comprises: a) Anc80L65, a rationally

designed, synthetic AAV capsid; b) Inverted terminal repeats derived from AAV2 (upstream and downstream); c) Cytomegalovirus (CMV) early enhancer element, the chicken beta actin (*CβA*) gene sequence located between the 5` flanking region and the proximal region of the second exon, and the 3` splice sequence derived from the rabbit beta globin (*RβG*) gene (Miyazaki 1989; Niwa 1991; and Orbán 2009, each of which is incorporated herein in its entirety by reference), which together are commonly referred to as the CAG promoter (upstream); d) Ranibizumab (antibody fragment [Fab]) coding sequence comprising, i) Bicistronic cassette encoding ranibizumab heavy and light chain variable regions, separated by furin and thosea asigna virus-derived protease 2A (T2A) cleavage sites, and ii) A 20 amino-acid human interleukin-2 (IL-2) leader sequence cloned upstream of each Fab chain to facilitate protein secretion; and e) Bovine growth hormone (bGH) polyadenylation (pA) signal (downstream).

[0489] In certain embodiments, the present disclosure relates to an rAAVAnc80-antiVEGF composition comprising a particle comprising rAAV2/Anc80L65-CAG.bevacizumab.bGH. In certain embodiments, an rAAV-antiVEGF composition comprises: a) Anc80L65, a rationally designed, synthetic AAV capsid; b) Inverted terminal repeats derived from AAV2 (upstream and downstream); c) Cytomegalovirus (CMV) early enhancer element, the chicken beta actin (*CβA*) gene sequence located between the 5` flanking region and the proximal region of the second exon, and the 3` splice sequence derived from the rabbit beta globin (*RβG*) gene (Miyazaki 1989; Niwa 1991; Orbán 2009, each of which are incorporated herein in their entirety), which together are commonly referred to as the CAG promoter (upstream); d) bevacizumab coding sequence (antibody comprising Fab and Fc regions) comprising, i) Bicistronic cassette encoding heavy and light chain variable and constant regions, separated by furin and thosea asigna virus-derived protease 2A (T2A) cleavage sites, and ii) A 20 amino-acid human interleukin-2 (IL-2) leader sequence cloned upstream of each Fab chain to facilitate protein secretion; and e) Bovine growth hormone (bGH) polyadenylation (pA) signal (downstream).

[0490] In certain embodiments, the present disclosure relates to an rAAVAnc80-antiVEGF composition comprising a particle comprising rAAV2/Anc80L65-CAG. aflibercept.bGH. In certain embodiments, an rAAV-antiVEGF composition comprises: a) Anc80L65, a rationally designed, synthetic AAV capsid; b) Inverted terminal repeats derived from AAV2 (upstream and downstream); c) Cytomegalovirus (CMV) early enhancer element, the chicken beta actin (*CβA*) gene sequence located between the 5` flanking region and the proximal region of the second exon,

and the 3` splice sequence derived from the rabbit beta globin (*RβG*) gene (Miyazaki 1989; Niwa 1991; Orbán 2009, each of which are incorporated herein in their entirety), which together are commonly referred to as the CAG promoter (upstream); d) aflibercept coding sequence comprising, i) an optional 20 amino-acid human interleukin-2 (IL-2) leader sequence, and ii) a recombinant fusion protein consisting of portions of human VEGF receptors 1 and 2 extracellular domains fused to the Fc portion of human IgG1; and e) Bovine growth hormone (bGH) polyadenylation (pA) signal (downstream).

Compositions

[0491] Among other things, the present disclosure provides compositions. In some embodiments, a composition comprises a construct as described herein. In some embodiments, a composition comprises one or more constructs as described herein. In some embodiments, a composition comprises a plurality of constructs as described herein. In some embodiments, when more than one construct is included in the composition, the constructs are each different.

[0492] In some embodiments, a composition comprises an rAAV particle as described herein. In some embodiments, a composition comprises one or more rAAV particles as described herein. In some embodiments, a composition comprises a plurality of rAAV particles. In come embodiments, when more than one rAAV particle is included in the composition, the rAAV particles are each different.

[0493] In some embodiments, a composition comprises an anti-VEGF protein. In some embodiments, a composition comprises a cell. In some embodiments, a composition is or comprises a pharmaceutical composition.

[0494] In some embodiments, an rAAVAnc80-antiVEGF particle is comprised of an AAVAnc80 capsid and a single-stranded DNA genome encoding ranibizumab, a 48 kilodalton (kDa) humanized monoclonal antibody fragment (Fab), which is used clinically to inhibit VEGF. In certain embodiments, rAAVAnc80-antiVEGF will be manufactured at a CRO or at an in-house operational GMP facility.

[0495] In some embodiments, rAAVAnc80-antiVEGF is produced through transient transfection of human embryonic kidney (HEK) 293 epithelial cells. In some embodiments, a clarified cell harvest is purified by affinity chromatography, followed by further purification and

enrichment for full rAAVAnc80 particles by cesium chloride isopycnic gradient separation. In some embodiments, a formulation buffer is comprised of sterile water containing mono-potassium phosphate at a range of 0.1-5 mM (e.g., at 1.5 mM mono-potassium phosphate), sodium phosphate dibasic at a range of 1-20mM (e.g., at 8.1 mM sodium phosphate dibasic), potassium chloride at a range of 0.1-10mM (e.g., 2.7 mM potassium chloride), sodium chloride at a range of 100-1000mM (e.g., at 172 mM sodium chloride), and Poloxamer at a range of 0.0001-0.001% (e.g., 0.001% Poloxamer 188). In some embodiments, once purified and formulated, an rAAVAnc80 particle comprising a construct is referred to as a drug substance. In some embodiments, a drug substance is subsequently sterile filtered (e.g., using a 0.2 micron [μm] filter) and aseptically filled for single use into sterile vials and stoppered. In some embodiments, these vialed aliquots constitute drug product and are stored at an appropriate temperature until the day of administration (e.g., from 4°C to -100°C, e.g., at ≤ -65 °C).

[0496] In some embodiments, a separate Diluent drug product can be created (e.g., an AAVAnc80 diluent), and can at or near equivalence in composition to the formulation buffer. In some embodiments, such a diluent can be sterile filtered (0.2 μm filter), aseptically filled for single use into sterile vials, and stored a at an appropriate temperature until the day of administration (e.g., from 4°C to -100°C, e.g., at ≤ -65 °C). In some embodiments, a Diluent drug product is utilized to prepare the concentration necessary for doses of drug substance described herein.

Dosing and Volume of Administration

[0497] In some embodiments, 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.

[0498] In some embodiments, a composition disclosed herein is administered as a single dose. In some embodiments, a composition disclosed herein is administered as a plurality of doses, e.g., 2, 3, 4, 5, 6, 7, 8, 9 or 10 doses.

[0499] In some embodiments, 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 embodiments, a composition disclosed herein is administered at a

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

[0500] In some embodiments, 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 embodiments 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.

[0501] In some embodiments, 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 mL to about 0.30 mL, about 0.01 mL to about 0.25 mL, about 0.01 mL to about 0.20 mL, about 0.01 mL to about 0.15 mL, about 0.01 mL to about 0.14 mL, about 0.01 mL to about 0.13 mL, about 0.01 mL to about 0.12 mL, about 0.01 mL to about 0.11 mL, about 0.01 mL to about 0.10 mL, about 0.01 mL to about 0.09 mL, about 0.01 mL to about 0.08 mL, about 0.01 mL to about 0.07 mL, about 0.01 mL to about 0.06 mL, about 0.01 mL to about 0.05 mL, about 0.01 mL to about 0.04 mL, about 0.01 mL to about 0.03 mL, or about 0.01 mL to about 0.02 mL.

[0502] In some embodiments, 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 0.02 mL to about 0.30 mL, 0.03 mL to about 0.30 mL, 0.04 mL to about 0.30 mL, 0.05 mL to about 0.30 mL, 0.06 mL to about 0.30 mL, 0.07 mL to about 0.30 mL, 0.08 mL to about 0.30 mL, 0.09 mL to about 0.30 mL, 0.10 mL to about 0.30 mL, 0.11 mL to about 0.30 mL, 0.12 mL to about 0.30 mL, 0.13 mL to about 0.30 mL, 0.14 mL to about 0.30 mL, 0.15 mL to about 0.30 mL, 0.16 mL to about 0.30 mL, 0.17 mL to about 0.30 mL, 0.18 mL to about 0.30 mL, 0.19 mL to about 0.30 mL, 0.20 mL to about 0.30 mL, or 0.25 mL to about 0.30 mL.

[0503] In some embodiments, 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 mL to about 0.03 mL, about 0.02 mL to about 0.25 mL, about 0.03 mL to about 0.20 mL, about 0.04 mL to about 0.18 mL, about 0.05 mL to about 0.16 mL, about 0.06 mL to about 0.14 mL, about 0.07 mL to about 0.12 mL, or about 0.08 mL to about 0.1 mL.

[0504] In some embodiments, a composition disclosed herein (e.g., a composition comprising one or a plurality of rAAV constructs disclosed herein) is administered at an amount of about 1 x 10^{11} vg/mL to about 1 x 10^{15} vg/mL, about 1 x 10^{11} vg/mL to about 9 x 10^{14} vg/mL, about 1 x 10^{11} vg/mL to about 8 x 10^{14} vg/mL, about 1 x 10^{11} vg/mL to about 7 x 10^{14} vg/mL, about 1 x 10^{11} vg/mL to about 6 x 10^{14} vg/mL, about 1 x 10^{11} vg/mL to about 5 x 10^{14} vg/mL, about 1 x 10^{11} vg/mL to about 4 x 10^{14} vg/mL, about 1 x 10^{11} vg/mL to about 3 x 10^{14} vg/mL, about 1 x 10^{11} vg/mL to about 2 x 10^{14} vg/mL, about 1 x 10^{11} vg/mL to about 1 x 10^{14} vg/mL, about 1 x 10^{11}

vg/mL to about 9 x 10^{13} vg/mL, about 1 x 10^{11} vg/mL to about 8 x 10^{13} vg/mL, about 1 x 10^{11} vg/mL to about 7 x 10^{13} vg/mL, about 1 x 10^{11} vg/mL to about 6 x 10^{13} vg/mL, about 1 x 10^{11} vg/mL to about 5 x 10^{13} vg/mL, about 1 x 10^{11} vg/mL to about 4 x 10^{13} vg/mL, about 1 x 10^{11} vg/mL to about 3 x 10^{13} vg/mL, about 1 x 10^{11} vg/mL to about 2 x 10^{13} vg/mL, about 1 x 10^{11} vg/mL to about 1 x 10^{13} vg/mL, about 1 x 10^{11} vg/mL to about 9 x 10^{12} vg/mL, about 1 x 10^{11} vg/mL to about 8 x 10^{12} vg/mL, about 1 x 10^{11} vg/mL to about 7 x 10^{12} vg/mL, about 1 x 10^{11} vg/mL to about 6 x 10^{12} vg/mL, about 1 x 10^{11} vg/mL to about 5 x 10^{12} vg/mL, about 1 x 10^{11} vg/mL to about 4 x 10^{12} vg/mL, about 1 x 10^{11} vg/mL to about 3 x 10^{12} vg/mL, about 1 x 10^{11} vg/mL to about 2 x 10^{12} vg/mL, about 1 x 10^{11} vg/mL to about 1 x 10^{12} vg/mL, about 1 x 10^{11} vg/mL to about 9.5 x 10^{11} vg/ mL, about 1 x 10^{11} vg/mL to about 9 x 10^{11} vg/ mL, about 1 x 10^{11} vg/mL to about 8.5 x 10^{11} vg/ mL, about 1 x 10^{11} vg/mL to about 8 x 10^{11} vg/ mL, about 1 x 10^{11} vg/mL to about 7.5 x 10^{11} vg/ mL, about 1 x 10^{11} vg/mL to about 7 x 10^{11} vg/ mL, about 1 x 10^{11} vg/mL to about 6.5 x 10^{11} vg/ mL, about 1 x 10^{11} vg/mL to about 6 x 10^{11} vg/ mL, about 1 x 10^{11} vg/mL to about 5.5 x 10^{11} vg/ mL, about 1 x 10^{11} vg/mL to about 5 x 10^{11} vg/ mL, about 1 x 10^{11} vg/mL to about 4.5 x 10^{11} vg/ mL, about 1 x 10^{11} vg/mL to about 4 x 10^{11} vg/ mL, about 1 x 10^{11} vg/mL to about 3.5 x 10^{11} vg/ mL, about 1 x 10^{11} vg/mL to about 3 x 10^{11} vg/ mL, about 1 x 10^{11} vg/mL to about 2.5 x 10^{11} vg/ mL, about 1 x 10^{11} vg/mL to about 2 x 10^{11} vg/ mL, about 1 x 10^{11} vg/mL to about 1.5 x 10^{11} vg/ mL.

[0505] In some embodiments, a composition disclosed herein (e.g., a composition comprising one or a plurality of rAAV constructs disclosed herein) is administered at an amount of about 1 x 10^{11} vg/mL to about 1 x 10^{15} vg/mL, about 2 x 10^{11} vg/mL to about 1 x 10^{15} vg/mL, about 3 x 10^{11} vg/mL to about 1 x 10^{15} vg/mL, about 4 x 10^{11} vg/mL to about 1 x 10^{15} vg/mL, about 5 x 10^{11} vg/mL to about 1 x 10^{15} vg/mL, about 6 x 10^{11} vg/mL to about 1 x 10^{15} vg/mL, about 7 x 10^{11} vg/mL to about 1 x 10^{15} vg/mL, about 8 x 10^{11} vg/mL to about 1 x 10^{15} vg/mL, about 9 x 10^{11} vg/mL to about 1 x 10^{15} vg/mL, about 1 x 10^{12} vg/mL to about 1 x 10^{15} vg/mL, about 2 x 10^{12} vg/mL to about 1 x 10^{15} vg/mL, about 3 x 10^{12} vg/mL to about 1 x 10^{15} vg/mL, about 4 x 10^{12} vg/mL to about 1 x 10^{15} vg/mL, about 5 x 10^{12} vg/mL to about 1 x 10^{15} vg/mL, about 6 x 10^{12} vg/mL to about 1 x 10^{15} vg/mL, about 7 x 10^{12} vg/mL to about 1 x 10^{15} vg/mL, about 8 x 10^{12} vg/mL to about 1 x 10^{15} vg/mL, about 9 x 10^{12} vg/mL to about 1 x 10^{15} vg/mL, about 1 x 10^{13} vg/mL to about 1 x 10^{15} vg/mL, about 2 x 10^{13} vg/mL to about 1 x 10^{15} vg/mL, about 3 x 10^{13} vg/mL to about 1 x 10^{15} vg/mL, about 4 x 10^{13} vg/mL to about 1 x 10^{15} vg/mL, about 5 x 10^{13}

vg/mL to about 1 x 10^{15} vg/mL, about 6 x 10^{13} vg/mL to about 1 x 10^{15} vg/mL, about 7 x 10^{13} vg/mL to about 1 x 10^{15} vg/mL, about 8 x 10^{13} vg/mL to about 1 x 10^{15} vg/mL, about 9 x 10^{13} vg/mL to about 1 x 10^{15} vg/mL, about 1 x 10^{14} vg/mL to about 1 x 10^{15} vg/mL, about 2 x 10^{14} vg/mL to about 1 x 10^{15} vg/mL, about 3 x 10^{14} vg/mL to about 1 x 10^{15} vg/mL, about 4 x 10^{14} vg/mL to about 1 x 10^{15} vg/mL, about 5 x 10^{14} vg/mL to about 1 x 10^{15} vg/mL, about 6 x 10^{14} vg/mL to about 1 x 10^{15} vg/mL, about 7 x 10^{14} vg/mL to about 1 x 10^{15} vg/mL, about 8 x 10^{14} vg/mL to about 1 x 10^{15} vg/mL, or about 9 x 10^{14} vg/mL to about 1 x 10^{15} vg/mL.

[0506] In some embodiments, a composition disclosed herein (e.g., a composition comprising one or a plurality of rAAV constructs disclosed herein) is administered at an amount of at least 1 x 10^{11}, at least 5 x 10^{11}, at least 1 x 10^{12}, at least 1 x 10^{12}, at least 2 x 10^{12}, at least 3 x 10^{12}, at least 4 x 10^{12}, at least 5 x 10^{12}, at least 6 x 10^{12}, at least 7 x 10^{12}, at least 8 x 10^{12}, at least 9 x 10^{12}, at least 1 x 10^{13}, at least 2 x 10^{13}, at least 3 x 10^{13}, at least 4 x 10^{13}, at least 5 x 10^{13}, at least 6 x 10^{13}, at least 7 x 10^{13}, at least 8 x 10^{13}, at least 9 x 10^{13}, or at least 1 x 10^{14} vector genomes (vg) per milliliter (mL) (vg/mL).

[0507] In some embodiments, a composition disclosed herein (e.g., a composition comprising one or a plurality of rAAV constructs disclosed herein) is administered at an amount of at most 1 x 10^{15}, at most 5 x 10^{14}, at most 1 x 10^{14}, at most 5 x 10^{13}, at most 1 x 10^{13}, at most 9 x 10^{12}, at most 8 x 10^{12}, at most 7 x 10^{12}, at most 6 x 10^{12}, at most 5 x 10^{12}, at most 4 x 10^{12}, at most 3 x 10^{12}, at most 2 x 10^{12}, or at most 1 x 10^{12} vector genomes (vg) per milliliter (mL).

[0508] In some embodiments, a composition disclosed herein (e.g., a composition comprising one or a plurality of rAAV constructs disclosed herein) is administered at an amount of about 1 x 10^{12} vg/mL, about 1.1 x 10^{12} vg/mL, 1.2 x 10^{12} vg/mL, about 1.3 x 10^{12} vg/mL, about 1.4 x 10^{12} vg/mL, about 1.5 x 10^{12} vg/mL, about 1.6 x 10^{12} vg/mL, about 1.7 x 10^{12} vg/mL, about 1.8 x 10^{12} vg/mL, about 1.9 x 10^{12} vg/mL, about 2.0 x 10^{12} vg/mL, about 2.1 x 10^{12} vg/mL, about 2.2 x 10^{12} vg/mL, about 2.3 x 10^{12} vg/mL, about 2.4 x 10^{12} vg/mL, about 2.5 x 10^{12} vg/mL, about 2.6 x 10^{12} vg/mL, about 2.7 x 10^{12} vg/mL, about 2.8 x 10^{12} vg/mL, about 2.9 x 10^{12} vg/mL, about 3.0 x 10^{12} vg/mL, about 3.1 x 10^{12} vg/mL, about 3.2 x 10^{12} vg/mL, about 3.3 x 10^{12} vg/mL, about 3.4 x 10^{12} vg/mL, about 3.5 x 10^{12} vg/mL, about 3.6 x 10^{12} vg/mL, about 3.7 x 10^{12} vg/mL, about 3.8 x 10^{12} vg/mL, about 3.9 x 10^{12} vg/mL, about 4.0 x 10^{12} vg/mL, about 4.1 x 10^{12} vg/mL, about 4.2 x 10^{12} vg/mL, about 4.3 x 10^{12} vg/mL, about 4.4 x 10^{12} vg/mL, about 4.5 x 10^{12} vg/mL, about 4.6 x 10^{12}

vg/mL, about 4.7 x 10^{12} vg/mL, about 4.8 x 10^{12} vg/mL, about 4.9 x 10^{12} vg/mL, about 5.0 x 10^{12} vg/mL, about 5.1 x 10^{12} vg/mL, about 5.2 x 10^{12} vg/mL, about 5.3 x 10^{12} vg/mL, about 5.4 x 10^{12} vg/mL, about5.5 x 10^{12} vg/mL, about 5.6 x 10^{12} vg/mL, about 5.7 x 10^{12} vg/mL, about 5.8 x 10^{12} vg/mL, about 5.9 x 10^{12} vg/mL, about 6.0 x 10^{12} vg/mL, about 7.0 x 10^{12} vg/mL, about 8.0 x 10^{12} vg/mL, about 9.0 x 10^{12} vg/mL, about 9.1 x 10^{12} vg/mL, about 9.2 x 10^{12} vg/mL, about 9.3 x 10^{12} vg/mL, about 9.4 x 10^{12} vg/mL, about 9.5 x 10^{12} vg/mL, about 9.6 x 10^{12} vg/mL, about 9.7 x 10^{12} vg/mL, about 9.8 x 10^{12} vg/mL, about 9.9 x 10^{12} vg/mL, about 1 x 10^{13} vg/mL, 1.1 x 10^{13} vg/mL, 1.2 x 10^{13} vg/mL, 1.3 x 10^{13} vg/mL, 1.4 x 10^{13} vg/mL, 1.5 x 10^{13} vg/mL, 1.6 x 10^{13} vg/mL, 1.7 x 10^{13} vg/mL, 1.8 x 10^{13} vg/mL, 1.9 x 10^{13} vg/mL, 2 x 10^{13} vg/mL, 5 x 10^{13} vg/mL, or 1 x 10^{14} vg/mL.

[0509] In some embodiments, a composition disclosed herein (e.g., a composition comprising one or a plurality of rAAV constructs disclosed herein) is administered at an amount of 2.5 x 10^{12} vg/mL +/- 10%. In some embodiments, a composition disclosed herein (e.g., a composition comprising one or a plurality of rAAV constructs disclosed herein) is administered at an amount of 2.5 x 10^{12} vg/mL.

[0510] In some embodiments, a composition disclosed herein (e.g., a composition comprising one or a plurality of rAAV constructs disclosed herein) is administered at an amount of 5 x 10^{12} vg/mL +/- 10%. In some embodiments, a composition disclosed herein (e.g., a composition comprising one or a plurality of rAAV constructs disclosed herein) is administered at an amount of 5 x 10^{12} vg/mL.

[0511] In some embodiments, a composition disclosed herein (e.g., a composition comprising one or a plurality of rAAV constructs disclosed herein) is administered at an amount of 1 x 10^{13} vg/mL +/- 10%. In some embodiments, a composition disclosed herein (e.g., a composition comprising one or a plurality of rAAV constructs disclosed herein) is administered at an amount of 1 x 10^{13} vg/mL.

[0512] In some embodiments, a composition disclosed herein (e.g., a composition comprising one or a plurality of rAAV constructs disclosed herein) is administered at an amount of about 1 x 10^{10} to about 1 x 10^{13} vg/cochlea, about 2 x 10^{10} to about 1 x 10^{13} vg/cochlea, about 3 x 10^{10} to about 1 x 10^{13} vg/cochlea, about 4 x 10^{10} to about 1 x 10^{13} vg/cochlea, about 5 x 10^{10} to about 1 x 10^{13} vg/cochlea, about 6 x 10^{10} to about 1 x 10^{13} vg/cochlea, about 7 x 10^{10} to about 1 x 10^{13}

vg/cochlea, about 8 x 10^{10} to about 1 x 10^{13} vg/cochlea, about 9 x 10^{10} to about 1 x 10^{13} vg/cochlea, about 1 x 10^{11} to about 1 x 10^{13} vg/cochlea, about 2 x 10^{11} to about 1 x 10^{13} vg/cochlea, about 3 x 10^{11} to about 1 x 10^{13} vg/cochlea, about 4 x 10^{11} to about 1 x 10^{13} vg/cochlea, about 5 x 10^{11} to about 1 x 10^{13} vg/cochlea, about 6 x 10^{11} to about 1 x 10^{13} vg/cochlea, about 7 x 10^{11} to about 1 x 10^{13} vg/cochlea, about 8 x 10^{11} to about 1 x 10^{13} vg/cochlea, about 9 x 10^{11} to about 1 x 10^{13} vg/cochlea, about 1 x 10^{12} to about 1 x 10^{13} vg/cochlea, about 2 x 10^{12} to about 1 x 10^{13} vg/cochlea, about 3 x 10^{12} to about 1 x 10^{13} vg/cochlea, about 4 x 10^{12} to about 1 x 10^{13} vg/cochlea, about 5 x 10^{12} to about 1 x 10^{13} vg/cochlea, about 6 x 10^{12} to about 1 x 10^{13} vg/cochlea, about7 x 10^{12} to about 1 x 10^{13} vg/cochlea, about 8 x 10^{12} to about 1 x 10^{13} vg/cochlea, or about 9 x 10^{12} to about 1 x 10^{13} vg/cochlea.

[0513] In some embodiments, a composition disclosed herein (e.g., a composition comprising one or a plurality of rAAV constructs disclosed herein) is administered at an amount of about 1 x 10^{10} to about 1 x 10^{13} vg/cochlea, about 1 x 10^{10} to about 9 x 10^{12} vg/cochlea, about 1 x 10^{10} to about 8 x 10^{12} vg/cochlea, about 1 x 10^{10} to about 7 x 10^{12} vg/cochlea, about 1 x 10^{10} to about 6 x 10^{12} vg/cochlea, about 1 x 10^{10} to about 5 x 10^{12} vg/cochlea, about 1 x 10^{10} to about 4 x 10^{12} vg/cochlea, about 1 x 10^{10} to about 3 x 10^{12} vg/cochlea, about 1 x 10^{10} to about 2 x 10^{12} vg/cochlea, about 1 x 10^{10} to about 1 x 10^{12} vg/cochlea, about 1 x 10^{10} to about 9 x 10^{11} vg/cochlea, about 1 x 10^{10} to about 8 x 10^{11} vg/cochlea, about 1 x 10^{10} to about 7 x 10^{11} vg/cochlea, about 1 x 10^{10} to about 6 x 10^{11} vg/cochlea, about 1 x 10^{10} to about 5 x 10^{11} vg/cochlea, about 1 x 10^{10} to about 4 x 10^{11} vg/cochlea, about 1 x 10^{10} to about 3 x 10^{11} vg/cochlea, about 1 x 10^{10} to about 2 x 10^{11} vg/cochlea, about 1 x 10^{10} to about 1 x 10^{11} vg/cochlea, about 1 x 10^{10} to about 9 x 10^{10} vg/cochlea, about 1 x 10^{10} to about 8 x 10^{10} vg/cochlea, about 1 x 10^{10} to about 7 x 10^{10} vg/cochlea, about 1 x 10^{10} to about 6 x 10^{10} vg/cochlea, about 1 x 10^{10} to about 5 x 10^{10} vg/cochlea, about 1 x 10^{10} to about 4 x 10^{10} vg/cochlea, about 1 x 10^{10} to about 3 x 10^{10} vg/cochlea, to about 1 x 10^{10} to about 2 x 10^{10} vg/cochlea.

[0514] In some embodiments, a composition disclosed herein (e.g., a composition comprising one or a plurality of rAAV constructs disclosed herein) is administered at an amount of about 1 x 10^{10} to about 1 x 10^{13} vg/cochlea, about 5 x 10^{10} to about 5 x 10^{12} vg/cochlea, about 8 x 10^{10} to about 1 x 10^{12} vg/cochlea, about 1 x 10^{11} to about 9 x 10^{11} vg/cochlea, about 2 x 10^{11} to about 8 x 10^{11} vg/cochlea, about 2.5 x 10^{11} to about 5 x 10^{11} vg/cochlea, about 3 x 10^{11} to about 4.5 x 10^{11} vg.cochlea.

[0515] In some embodiments, a composition disclosed herein (e.g., a composition comprising one or a plurality of rAAV constructs disclosed herein) is administered at an amount of about 1 x 10^{10} vg/cochlea, about 5 x 10^{10} vg/cochlea, about 1 x 10^{11} vg/cochlea, about 1.5 x 10^{11} vg/cochlea, about 2 x 10^{11} vg/cochlea, about 2.1 x 10^{11} vg/cochlea, 2.2 x 10^{11} vg/cochlea, 2.3 x 10^{11} vg/cochlea, 2.4 x 10^{11} vg/cochlea, about 2.5 x 10^{11} vg/cochlea, 2.6 x 10^{11} vg/cochlea, 2.7 x 10^{11} vg/cochlea, 2.8 x 10^{11} vg/cochlea, 2.9 x 10^{11} vg/cochlea, about 3 x 10^{11} vg/cochlea, 3.1 x 10^{11} vg/cochlea, 3.2 x 10^{11} vg/cochlea, 3.3 x 10^{11} vg/cochlea, 3.4 x 10^{11} vg/cochlea, about 3.5 x 10^{11} vg/cochlea, 3.6 x 10^{11} vg/cochlea, 3.7 x 10^{11} vg/cochlea, 3.8 x 10^{11} vg/cochlea, 3.9 x 10^{11} vg/cochlea, about 4 x 10^{11} vg/cochlea, 4.1 x 10^{11} vg/cochlea, 4.2 x 10^{11} vg/cochlea, 4.3 x 10^{11} vg/cochlea, 4.4 x 10^{11} vg/cochlea, about 4.5 x 10^{11} vg/cochlea, 4.6 x 10^{11} vg/cochlea, 4.7 x 10^{11} vg/cochlea, 4.8 x 10^{11} vg/cochlea, 4.9 x 10^{11} vg/cochlea, about 5 x 10^{11} vg/cochlea, about 5.5 x 10^{11} vg/cochlea, about 6 x 10^{11} vg/cochlea, about 6.5 x 10^{11} vg/cochlea, about 7 x 10^{11} vg/cochlea, about 7.5 x 10^{11} vg/cochlea, about 8 x 10^{11} vg/cochlea, about 8.5 x 10^{11} vg/cochlea, about 9 x 10^{11} vg/cochlea, about 9.5 x 10^{11} vg/cochlea, about 1 x 10^{12} vg/cochlea.

[0516] In some embodiments, a composition disclosed herein (e.g., a composition comprising one or a plurality of rAAV constructs disclosed herein) is administered at an amount of 2.3 x 10^{11} vg/cochlea, +/- 10%. In some embodiments, a composition disclosed herein (e.g., a composition comprising one or a plurality of rAAV constructs disclosed herein) is administered at an amount of 2.3 x 10^{11} vg/cochlea.

[0517] In some embodiments, a composition disclosed herein (e.g., a composition comprising one or a plurality of rAAV constructs disclosed herein) is administered at an amount of 4.5 x 10^{11} vg/cochlea, +/- 10%. In some embodiments, a composition disclosed herein (e.g., a composition comprising one or a plurality of rAAV constructs disclosed herein) is administered at an amount of 4.5 x 10^{11} vg/cochlea.

[0518] In some embodiments, a composition disclosed herein (e.g., a composition comprising one or a plurality of rAAV constructs disclosed herein) is administered at an amount of 9 x 10^{11} vg/cochlea, +/- 10%. In some embodiments, a composition disclosed herein (e.g., a composition comprising one or a plurality of rAAV constructs disclosed herein) is administered at an amount of 9 x 10^{11} vg/cochlea.

Single AAV Construct Compositions

[0519] In some embodiments, the present disclosure provides compositions or systems comprising rAAV particles comprised of a single construct. In some such embodiments, a single construct may deliver a polynucleotide that encodes a functional (e.g., as previously described and/or otherwise functional, e.g., codon optimized) copy of an anti-VEGF gene. In some embodiments, a construct is or comprises a rAAV construct. In some embodiments described herein, a single rAAV construct is capable of expressing a full-length anti-VEGF messenger RNA (mRNA) or a characteristic protein thereof in a target cell (e.g., an inner ear cell). In some embodiments, a single construct (e.g., any of the constructs described herein) can include a sequence encoding a functional anti-VEGF protein (e.g., any construct that generates functional anti-VEGF protein). In some embodiments, a single construct (e.g., any of the constructs described herein) can include a sequence encoding a functional anti-VEGF protein (e.g., any construct that generates functional antiVEGF protein) and optionally additional polypeptide sequences (e.g., regulatory sequences, and/or reporter sequences).

[0520] In some embodiments, a single construct composition or system may comprise any or all of the exemplary construct components described herein. In some embodiments, an exemplary single construct is represented by SEQ ID NO: 90. In some embodiments, an exemplary single construct is represented by SEQ ID NO: 91. In some embodiments, an exemplary single construct is represented by SEQ ID NO: 92. In some embodiments, an exemplary single construct is represented by SEQ ID NO: 93. In some embodiments, an exemplary single construct is represented by SEQ ID NO: 94. In some embodiments, an exemplary single construct is represented by SEQ ID NO: 95. In some embodiments, an exemplary single construct is represented by SEQ ID NO: 96. In some embodiments, an exemplary single construct is represented by SEQ ID NO: 106. In some embodiments, an exemplary single construct is represented by SEQ ID NO: 107. In some embodiments, an exemplary single construct is at least 85%, 90%, 95%, 98% or 99% identical to the sequence represented by SEQ ID NO: 90, 91, 92, 93, 94, 95, 96, 106, or 107. 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.

[0521] In some embodiments, a construct has a sequence according to SEQ ID NO: 90, this construct may be referred to as an rAAV-ranibizumab-PC construct, is an exemplary embodiment

of the schematic shown in **FIG. 6, Panel (A),** and encodes for the anti-VEGF protein ranibizumab. In some embodiments, an exemplary construct comprises: a 5` ITR exemplified by SEQ ID NO: 47, optionally a cloning site exemplified by SEQ ID NO: 81, a CMV enhancer exemplified by SEQ ID NO: 64, a CBA promoter exemplified by SEQ ID NO: 50, a chimeric intron exemplified by SEQ ID NO: 65, optionally a cloning site exemplified by SEQ ID NO: 78, an anti-VEGF coding region exemplified by SEQ ID NO: 103, optionally a cloning site exemplified by SEQ ID NO: 79, a poly(A) site exemplified by SEQ ID NO: 75, optionally a cloning site exemplified by SEQ ID NO: 82, and a 3` ITR exemplified by SEQ ID NO: 48.

[0522] In some embodiments, a construct has a sequence according to SEQ ID NO: 91, this construct may be referred to as an rAAV-ranibizumab construct, is an exemplary embodiment of the schematic shown in **FIG. 6, Panel (A),** and encodes for the anti-VEGF protein ranibizumab. In some embodiments, an exemplary construct comprises: a 5` ITR exemplified by SEQ ID NO: 45, optionally a cloning site exemplified by SEQ ID NO: 77, a CMV enhancer exemplified by SEQ ID NO: 64, a CBA promoter exemplified by SEQ ID NO: 49, a chimeric intron exemplified by SEQ ID NO: 65, optionally a cloning site exemplified by SEQ ID NO: 78, an anti-VEGF coding region exemplified by SEQ ID NO: 103, optionally a cloning site exemplified by SEQ ID NO: 79, a poly(A) site exemplified by SEQ ID NO: 75, optionally a cloning site exemplified by SEQ ID NO: 80, and a 3` ITR exemplified by SEQ ID NO: 46.

[0523] In some embodiments, a construct has a sequence according to SEQ ID NO: 92, this construct may be referred to as an rAAV-ranibizumab.2 construct, is an exemplary embodiment of the schematic shown in **FIG. 6, Panel (A),** and encodes for the anti-VEGF protein ranibizumab. In some embodiments, an exemplary construct comprises: a 5` ITR exemplified by SEQ ID NO: 45, optionally a cloning site exemplified by SEQ ID NO: 77, a CMV enhancer exemplified by SEQ ID NO: 64, a CBA promoter exemplified by SEQ ID NO: 50, a chimeric intron exemplified by SEQ ID NO: 65, optionally a cloning site exemplified by SEQ ID NO: 78, an anti-VEGF coding region exemplified by SEQ ID NO: 103, optionally a cloning site exemplified by SEQ ID NO: 79, a poly(A) site exemplified by SEQ ID NO: 75, optionally a cloning site exemplified by SEQ ID NO: 80, and a 3` ITR exemplified by SEQ ID NO: 46.

[0524] In some embodiments, a construct has a sequence according to SEQ ID NO: 106, this construct may be referred to as an rAAV-ranibizumab-GFP construct, is an exemplary

embodiment of the schematic shown in **FIG. 6, Panel (B),** and encodes for the anti-VEGF protein ranibizumab. In some embodiments, an exemplary construct comprises: a 5` ITR exemplified by SEQ ID NO: 47, optionally a cloning site exemplified by SEQ ID NO: 81, a CMV enhancer exemplified by SEQ ID NO: 64, a CBA promoter exemplified by SEQ ID NO: 50, a chimeric intron exemplified by SEQ ID NO: 65, optionally a cloning site exemplified by SEQ ID NO: 78, an anti-VEGF coding region exemplified by SEQ ID NO: 103, optionally a reporter sequence exemplified by SEQ ID NO: 104, optionally a cloning site exemplified by SEQ ID NO: 79, a poly(A) site exemplified by SEQ ID NO: 75, optionally a cloning site exemplified by SEQ ID NO: 82, and a 3` ITR exemplified by SEQ ID NO: 48.

[0525] In some embodiments, a construct has a sequence according to SEQ ID NO: 93, this construct may be referred to as an rAAV-bevacizumab-PC construct, is an exemplary embodiment of the schematic shown in **FIG. 6, Panel (C),** and encodes for the anti-VEGF protein bevacizumab. In some embodiments, an exemplary construct comprises: a 5` ITR exemplified by SEQ ID NO: 47, optionally a cloning site exemplified by SEQ ID NO: 81, a CMV enhancer exemplified by SEQ ID NO: 64, a CBA promoter exemplified by SEQ ID NO: 50, a chimeric intron exemplified by SEQ ID NO: 65, optionally a cloning site exemplified by SEQ ID NO: 78, an anti-VEGF coding region exemplified by SEQ ID NO: 22, optionally a cloning site exemplified by SEQ ID NO: 79, a poly(A) site exemplified by SEQ ID NO: 75, optionally a cloning site exemplified by SEQ ID NO: 82, and a 3` ITR exemplified by SEQ ID NO: 48.

[0526] In some embodiments, a construct has a sequence according to SEQ ID NO: 94, this construct may be referred to as an rAAV-bevacizumab construct, is an exemplary embodiment of the schematic shown in **FIG. 6, Panel (C),** and encodes for the anti-VEGF protein bevacizumab. In some embodiments, an exemplary construct comprises: a 5` ITR exemplified by SEQ ID NO: 45, optionally a cloning site exemplified by SEQ ID NO: 77, a CMV enhancer exemplified by SEQ ID NO: 64, a CBA promoter exemplified by SEQ ID NO: 49, a chimeric intron exemplified by SEQ ID NO: 65, optionally a cloning site exemplified by SEQ ID NO: 78, an anti-VEGF coding region exemplified by SEQ ID NO: 22, optionally a cloning site exemplified by SEQ ID NO: 79, a poly(A) site exemplified by SEQ ID NO: 75, optionally a cloning site exemplified by SEQ ID NO: 80, and a 3` ITR exemplified by SEQ ID NO: 46.

[0527] In some embodiments, a construct has a sequence according to SEQ ID NO: 107, this construct may be referred to as an rAAV-bevacizumab-GFP construct, is an exemplary embodiment of the schematic shown in **FIG. 6, Panel (B),** and encodes for the anti-VEGF protein bevacizumab. In some embodiments, an exemplary construct comprises: a 5` ITR exemplified by SEQ ID NO: 47, optionally a cloning site exemplified by SEQ ID NO: 81, a CMV enhancer exemplified by SEQ ID NO: 64, a CBA promoter exemplified by SEQ ID NO: 50, a chimeric intron exemplified by SEQ ID NO: 65, optionally a cloning site exemplified by SEQ ID NO: 78, an anti-VEGF coding region exemplified by SEQ ID NO: 22, optionally a reporter sequence exemplified by SEQ ID NO: 104, optionally a cloning site exemplified by SEQ ID NO: 79, a poly(A) site exemplified by SEQ ID NO: 75, optionally a cloning site exemplified by SEQ ID NO: 82, and a 3` ITR exemplified by SEQ ID NO: 48.

[0528] In some embodiments, a construct has a sequence according to SEQ ID NO: 95, this construct may be referred to as an rAAV-aflibercept-PC construct, is an exemplary embodiment of the schematic shown in **FIG. 6, Panel (D),** and encodes for the anti-VEGF protein aflibercept. In some embodiments, an exemplary construct comprises: a 5` ITR exemplified by SEQ ID NO: 47, optionally a cloning site exemplified by SEQ ID NO: 81, a CMV enhancer exemplified by SEQ ID NO: 64, a CBA promoter exemplified by SEQ ID NO: 50, a chimeric intron exemplified by SEQ ID NO: 65, optionally a cloning site exemplified by SEQ ID NO: 78, an anti-VEGF coding region exemplified by SEQ ID NO: 42, optionally a cloning site exemplified by SEQ ID NO: 79, a poly(A) site exemplified by SEQ ID NO: 75, optionally a cloning site exemplified by SEQ ID NO: 82, and a 3` ITR exemplified by SEQ ID NO: 48.

[0529] In some embodiments, a construct has a sequence according to SEQ ID NO: 96, this construct may be referred to as an rAAV-aflibercept construct, is an exemplary embodiment of the schematic shown in **FIG. 6, Panel (D),** and encodes for the anti-VEGF protein aflibercept. In some embodiments, an exemplary construct comprises: a 5` ITR exemplified by SEQ ID NO: 45, optionally a cloning site exemplified by SEQ ID NO: 77, a CMV enhancer exemplified by SEQ ID NO: 64, a CBA promoter exemplified by SEQ ID NO: 49, a chimeric intron exemplified by SEQ ID NO: 65, optionally a cloning site exemplified by SEQ ID NO: 78, an anti-VEGF coding region exemplified by SEQ ID NO: 42, optionally a cloning site exemplified by SEQ ID NO: 79, a poly(A) site exemplified by SEQ ID NO: 75, optionally a cloning site exemplified by SEQ ID NO: 80, and a 3` ITR exemplified by SEQ ID NO: 46.

[0530] In certain embodiments, rAAVAnc80 particles comprising constructs encoding anti-VEGF proteins were produced and used to evaluate biological activity and inner ear tolerability of said construct, in order to support the biological plausibility and reasonable safety of local anti-VEGF therapy for VS. In some embodiments, such evaluations were made through a series of studies using the following approaches/models: transduction and/or transfection of a cell line, transduction and/or transfection of cochlear explants; intracochlear administration and transduction of wildtype mice cells; and/or intracochlear administration and transduction of NHP cells. Summaries of certain anti-VEGF particles are shown in **Table 1**.

[0531] Table 1 - Summary of Particles Used or Planned for Use in Certain Studies

Capsid	Promoter	VEGF inhibitor coding sequence	pA Signal
AAVAnc80	CAG	Bevacizumab	bGH
AAVAnc80	CAG	Ranibizumab	bGH
AAVAnc80	CAG	Aflibercept	bGH

Abbreviations: bGH = bovine growth hormone; CAG = cytomegalovirus (CMV) early enhancer element, the chicken beta actin (*CβA*) gene sequence located between the 5` flanking region and the proximal region of the second exon, and the 3` splice sequence derived from the rabbit beta globin (*RβG*) gene (Miyazaki 1989; Niwa 1991; Orbán 2009, each of which is incorporated herein in its entirety by reference), which together are commonly referred to as the CAG promoter; GLP = Good Laboratory Practice; pA = polyadenylation.

[0532] Exemplary rAAV-Ranibizumab-PC Construct Sequence (SEQ ID NO: 90)

CTGCGCGCTCGCTCGCTCACTGAGGCCGCCCGGGCGTCGGGCGACCTTTGGTCGCCC
GGCCTCAGTGAGCGAGCGAGCGCGCAGAGAGGGAGTGGCCAACTCCATCACTAGGG
GTTCCTGCGGCCGCACGCGTGACATTGATTATTGACTAGTTATTAATAGTAATCAAT
TACGGGGTCATTAGTTCATAGCCCATATATGGAGTTCCGCGTTACATAACTTACGGT
AAATGGCCCGCCTGGCTGACCGCCCAACGACCCCCGCCCATTGACGTCAATAATGA
CGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGACT
ATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGC
CCCCTATTGACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCCAGTACATGA
CCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCAT
GGGTCGAGGTGAGCCCCACGTTCTGCTTCACTCTCCCCATCTCCCCCCCCTCCCCACC
CCCAATTTTGTATTTATTTATTTTTTAATTATTTTGTGCAGCGATGGGGGCGGGGGGG
GGGGGGGGCGCGCGCCAGGCGGGGCGGGGCGGGGCGAGGGGCGGGGCGGGGCGAG

GCGGAGAGGTGCGGCGGCAGCCAATCAGAGCGGCGCGCTCCGAAAGTTTCCTTTTA
TGGCGAGGCGGCGGCGGCGGCGGCCCTATAAAAAGCGAAGCGCGCGGCGGGCGGG
AGTCGCTGCGTTGCCTTCGCCCCGTGCCCCGCTCCGCGCCGCCTCGCGCCGCCCGCC
CCGGCTCTGACTGACCGCGTTACTCCCACAGGTGAGCGGGCGGGACGGCCCTTCTCC
TCCGGGCTGTAATTAGCGCTTGGTTTAATGACGGCTCGTTTCTTTTCTGTGGCTGCGT
GAAAGCCTTAAAGGGCTCCGGGAGGGCCCTTTGTGCGGGGGGGAGCGGCTCGGGGG
GTGCGTGCGTGTGTGTGTGCGTGGGGAGCGCCGCGTGCGGCCCGCGCTGCCCGGCG
GCTGTGAGCGCTGCGGGCGCGGCGCGGGGCTTTGTGCGCTCCGCGTGTGCGCGAGG
GGAGCGCGGCCGGGGGCGGTGCCCCGCGGTGCGGGGGGGGCTGCGAGGGGAACAAA
GGCTGCGTGCGGGGTGTGTGCGTGGGGGGGTGAGCAGGGGGTGTGGGCGCGGCGGT
CGGGCTGTAACCCCCCCCTGCACCCCCCTCCCCGAGTTGCTGAGCACGGCCCGGCTT
CGGGTGCGGGGCTCCGTGCGGGGCGTGGCGCGGGGCTCGCCGTGCCGGGCGGGGGG
TGGCGGCAGGTGGGGGTGCCGGGCGGGGCGGGGCCGCCTCGGGCCGGGGAGGGCT
CGGGGGAGGGGCGCGGCGGCCCCCGGAGCGCCGGCGGCTGTCGAGGCGCGGCGAG
CCGCAGCCATTGCCTTTTATGGTAATCGTGCGAGAGGGCGCAGGGACTTCCTTTGTC
CCAAATCTGTGCGGAGCCGAAATCTGGGAGGCGCCGCCGCACCCCCTCTAGCGGGC
GCGGGGCGAAGCGGTGCGGCGCCGGCAGGAAGGAAATGGGCGGGGAGGGCCTTCG
TGCGTCGCCGCGCCGCCGTCCCCTTCTCCCTCTCCAGCCTCGGGGCTGTCCGCGGGG
GGACGGCTGCCTTCGGGGGGGGACGGGGCAGGGCGGGGTTCGGCTTCTGGCGTGTGA
CCGGCGGCTCTAGAGCCTCTGCTAACCATGTTCATGCCTTCTTCTTTTTCCTACAGCT
CCTGGGCAACGTGCTGGTTATTGTGACCGGTGCCACCATGTACCGGATGCAGCTGCT
GAGCTGTATCGCCCTGTCTCTGGCCCTGGTCACCAATTCTGAGGTGCAGCTGGTGGA
ATCTGGCGGCGGACTTGTTCAACCTGGCGGCTCTCTGAGACTGAGCTGTGCCGCTTC
TGGCTACGACTTCACCCACTACGGCATGAACTGGGTCCGACAGGCCCCTGGCAAAG
GCCTTGAATGGGTCGGATGGATCAACACCTACACCGGCGAGCCAACATACGCCGCC
GACTTCAAGCGGAGATTCACCTTCAGCCTGGACACCAGCAAGAGCACCGCCTACCT
GCAGATGAACAGCCTGAGAGCCGAGGACACCGCCGTGTACTACTGCGCCAAGTATC
CCTACTACTACGGCACCAGCCACTGGTACTTTGACGTGTGGGGACAGGGCACACTG
GTCACAGTGTCTAGCGCCTCTACAAAGGGCCCCAGCGTTTTCCCACTGGCTCCTAGC
AGCAAGTCTACCAGCGGAGGAACAGCCGCTCTGGGCTGTCTGGTCAAGGACTACTTT
CCCGAGCCTGTGACCGTGTCCTGGAATTCTGGCGCTCTGACAAGCGGCGTGCACACC

TTTCCAGCTGTGCTGCAAAGCAGCGGCCTGTACTCTCTGAGCAGCGTCGTGACAGTG
CCAAGCAGCTCTCTGGGCACCCAGACCTACATCTGCAATGTGAACCACAAGCCTAG
CAACACCAAGGTGGACAAGAAGGTGGAACCCAAGAGCTGCGACAAGACCCACACC
GGCAAGCGGAAGAGAAGAGGCTCTGGCGAAGGCAGAGGCAGCCTGCTTACATGTG
GCGACGTGGAAGAGAACCCCGGACCTATGTATAGAATGCAGCTCCTGTCCTGCATTG
CCCTGAGCCTGGCTCTCGTGACCAACAGCGACATCCAGCTGACACAGAGCCCCAGC
AGCCTGTCTGCCTCTGTGGGAGACAGAGTGACCATCACCTGTAGCGCCAGCCAGGA
CATCTCCAACTACCTGAACTGGTATCAGCAAAAGCCCGGCAAGGCCCCTAAGGTGC
TGATCTACTTCACAAGCAGCCTGCACTCCGGCGTGCCCAGCAGATTTTCTGGCTCTG
GCAGCGGCACCGACTTCACCCTGACCATATCTAGCCTGCAGCCTGAGGACTTCGCCA
CCTACTACTGCCAGCAGTACAGCACCGTGCCTTGGACATTTGGCCAGGGCACAAAG
GTGGAAATCAAGCGGACTGTGGCCGCTCCTAGCGTGTTCATCTTTCCACCTAGCGAC
GAGCAGCTGAAGTCTGGCACAGCCTCTGTCGTGTGCCTGCTGAACAACTTCTACCCC
AGAGAAGCCAAGGTGCAGTGGAAAGTGGACAATGCCCTGCAGAGCGGCAACAGCC
AAGAGAGCGTGACAGAGCAGGACTCCAAGGATAGCACCTATAGCCTGAGCAGCACC
CTGACACTGAGCAAGGCCGACTACGAGAAGCACAAAGTGTACGCCTGCGAAGTGAC
CCACCAGGGCCTTTCTAGCCCTGTGACCAAGAGCTTCAACCGGGGCGAATGTTAAG
AGCTCGCTGATCAGCCTCGACTGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGCCCC
TCCCCCGTGCCTTCCTTGACCCTGGAAGGTGCCACTCCCACTGTCCTTTCCTAATAAA
ATGAGGAAATTGCATCGCATTGTCTGAGTAGGTGTCATTCTATTCTGGGGGGTGGGG
TGGGGCAGGACAGCAAGGGGGAGGATTGGGAAGACAATAGCAGGCATGCTGGGGA
TGCGGTGGGCTCTATGGAAGCTTGAATTCAGCTGACGTGCCTCGGACCGCTAGGAAC
CCCTAGTGATGGAGTTGGCCACTCCCTCTCTGCGCGCTCGCTCGCTCACTGAGGCCG
GGCGACCAAAGGTCGCCCGACGCCCGGGCTTTGCCCGGGCGGCCTCAGTGAGCGAG
CGAGCGCGCAG

[0533] Exemplary rAAV-Ranibizumab Construct Sequence (SEQ ID NO: 91)

TTGGCCACTCCCTCTCTGCGCGCTCGCTCGCTCACTGAGGCCGCCCGGGCAAAGCCC
GGGCGTCGGGCGACCTTTGGTCGCCCGGCCTCAGTGAGCGAGCGAGCGCGCAGAGA
GGGAGTGGCCAACTCCATCACTAGGGGTTCCTTTGTCGACGCGGCCGCACGCGTGAC
ATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCC
ATATATGGAGTTCCGCGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCC

CAACGACCCCCGCCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAAT
AGGGACTTTCCATTGACGTCAATGGGTGGACTATTTACGGTAAACTGCCCACTTGGC
AGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAA
ATGGCCCGCCTGGCATTATGCCCAGTACATGACCTTATGGGACTTTCCTACTTGGCA
GTACATCTACGTATTAGTCATCGCTATTACCATGGGTCGAGGTGAGCCCCACGTTCT
GCTTCACTCTCCCCATCTCCCCCCCCTCCCCACCCCCAATTTTGTATTTATTTATTTTT
TAATTATTTTGTGCAGCGATGGGGGCGGGGGGGGGGGGGGGCGCGCGCCAGGCGG
GGCGGGGCGGGGCGAGGGGCGGGGCGGGGCGAGGCGGAGAGGTGCGGCGGCAGCC
AATCAGAGCGGCGCGCTCCGAAAGTTTCCTTTTATGGCGAGGCGGCGGCGGCGGCG
GCCCTATAAAAAGCGAAGCGCGCGGCGGGCGGGAGTCGCTGCGTTGCCTTCGCCCC
GTGCCCCGCTCCGCGCCGCCTCGCGCCGCCCGCCCCGGCTCTGACTGACCGCGTTAC
TCCCACAGGTGAGCGGGCGGGACGGCCCTTCTCCTCCGGGCTGTAATTAGCGCTTGG
TTTAATGACGGCTCGTTTCTTTTCTGTGGCTGCGTGAAAGCCTTAAAGGGCTCCGGG
AGGGCCCTTTGTGCGGGGGGGAGCGGCTCGGGGGGTGCGTGCGTGTGTGTGTGCGT
GGGGAGCGCCGCGTGCGGCCCGCGCTGCCCGGCGGCTGTGAGCGCTGCGGGCGCGG
CGCGGGGCTTTGTGCGCTCCGCGTGTGCGCGAGGGGAGCGCGGCCGGGGGCGGTGC
CCCGCGGTGCGGGGGGGCTGCGAGGGGAACAAAGGCTGCGTGCGGGGTGTGTGCGT
GGGGGGGTGAGCAGGGGGTGTGGGCGCGGCGGTCGGGCTGTAACCCCCCCCTGCAC
CCCCCTCCCCGAGTTGCTGAGCACGGCCCGGCTTCGGGTGCGGGGCTCCGTGCGGGG
CGTGGCGCGGGGCTCGCCGTGCCGGGCGGGGGGTGGCGGCAGGTGGGGGTGCCGG
GCGGGGCGGGGCCGCCTCGGGCCGGGGAGGGCTCGGGGGAGGGGCGCGGCGGCCC
CCGGAGCGCCGGCGGCTGTCGAGGCGCGGCGAGCCGCAGCCATTGCCTTTTATGGT
AATCGTGCGAGAGGGCGCAGGGACTTCCTTTGTCCCAAATCTGTGCGGAGCCGAAA
TCTGGGAGGCGCCGCCGCACCCCCTCTAGCGGGCGCGGGGCGAAGCGGTGCGGCGC
CGGCAGGAAGGAAATGGGCGGGGAGGGCCTTCGTGCGTCGCCGCGCCGCCGTCCCC
TTCTCCCTCTCCAGCCTCGGGGCTGTCCGCGGGGGGGACGGCTGCCTTCGGGGGGGAC
GGGGCAGGGCGGGGTTCGGCTTCTGGCGTGTGACCGGCGGCTCTAGAGCCTCTGCTA
ACCATGTTCATGCCTTCTTCTTTTTCCTACAGCTCCTGGGCAACGTGCTGGTTATTGT
GACCGGTGCCACCATGTACCGGATGCAGCTGCTGAGCTGTATCGCCCTGTCTCTGGC
CCTGGTCACCAATTCTGAGGTGCAGCTGGTGGAATCTGGCGGCGGACTTGTTCAACC
TGGCGGCTCTCTGAGACTGAGCTGTGCCGCTTCTGGCTACGACTTCACCCACTACGG

CATGAACTGGGTCCGACAGGCCCCTGGCAAAGGCCTTGAATGGGTCGGATGGATCA
ACACCTACACCGGCGAGCCAACATACGCCGCCGACTTCAAGCGGAGATTCACCTTC
AGCCTGGACACCAGCAAGAGCACCGCCTACCTGCAGATGAACAGCCTGAGAGCCGA
GGACACCGCCGTGTACTACTGCGCCAAGTATCCCTACTACTACGGCACCAGCCACTG
GTACTTTGACGTGTGGGGACAGGGCACACTGGTCACAGTGTCTAGCGCCTCTACAAA
GGGCCCCAGCGTTTTCCCACTGGCTCCTAGCAGCAAGTCTACCAGCGGAGGAACAG
CCGCTCTGGGCTGTCTGGTCAAGGACTACTTTCCCGAGCCTGTGACCGTGTCCTGGA
ATTCTGGCGCTCTGACAAGCGGCGTGCACACCTTTCCAGCTGTGCTGCAAAGCAGCG
GCCTGTACTCTCTGAGCAGCGTCGTGACAGTGCCAAGCAGCTCTCTGGGCACCCAGA
CCTACATCTGCAATGTGAACCACAAGCCTAGCAACACCAAGGTGGACAAGAAGGTG
GAACCCAAGAGCTGCGACAAGACCCACACCGGCAAGCGGAAGAGAAGAGGCTCTG
GCGAAGGCAGAGGCAGCCTGCTTACATGTGGCGACGTGGAAGAGAACCCCGGACCT
ATGTATAGAATGCAGCTCCTGTCCTGCATTGCCCTGAGCCTGGCTCTCGTGACCAAC
AGCGACATCCAGCTGACACAGAGCCCCAGCAGCCTGTCTGCCTCTGTGGGAGACAG
AGTGACCATCACCTGTAGCGCCAGCCAGGACATCTCCAACTACCTGAACTGGTATCA
GCAAAAGCCCGGCAAGGCCCCTAAGGTGCTGATCTACTTCACAAGCAGCCTGCACT
CCGGCGTGCCCAGCAGATTTTCTGGCTCTGGCAGCGGCACCGACTTCACCCTGACCA
TATCTAGCCTGCAGCCTGAGGACTTCGCCACCTACTACTGCCAGCAGTACAGCACCG
TGCCTTGGACATTTGGCCAGGGCACAAAGGTGGAAATCAAGCGGACTGTGGCCGCT
CCTAGCGTGTTCATCTTTCCACCTAGCGACGAGCAGCTGAAGTCTGGCACAGCCTCT
GTCGTGTGCCTGCTGAACAACTTCTACCCCAGAGAAGCCAAGGTGCAGTGGAAAGT
GGACAATGCCCTGCAGAGCGGCAACAGCCAAGAGAGCGTGACAGAGCAGGACTCC
AAGGATAGCACCTATAGCCTGAGCAGCACCCTGACACTGAGCAAGGCCGACTACGA
GAAGCACAAAGTGTACGCCTGCGAAGTGACCCACCAGGGCCTTTCTAGCCCTGTGA
CCAAGAGCTTCAACCGGGGCGAATGTTAAGAGCTCGCTGATCAGCCTCGACTGTGC
CTTCTAGTTGCCAGCCATCTGTTGTTTGCCCCTCCCCCGTGCCTTCCTTGACCCTGGA
AGGTGCCACTCCCACTGTCCTTTCCTAATAAAATGAGGAAATTGCATCGCATTGTCT
GAGTAGGTGTCATTCTATTCTGGGGGGGTGGGGTGGGGCAGGACAGCAAGGGGGAGG
ATTGGGAAGACAATAGCAGGCATGCTGGGGATGCGGTGGGCTCTATGGAAGCTTGA
ATTCAGCTGACGTGCCTCGGACCGCCTAGGAGGAACCCCTAGTGATGGAGTTGGCC
ACTCCCTCTCTGCGCGCTCGCTCGCTCACTGAGGCCGGGCGACCAAAGGTCGCCCGA

CGCCCGGGCTTTGCCCGGGCGGCCTCAGTGAGCGAGCGAGCGCGCAGAGAGGGAGT
GGCCAA

[0534] Exemplary rAAV-Ranibizumab.2 Construct Sequence (SEQ ID NO: 92)

TTGGCCACTCCCTCTCTGCGCGCTCGCTCGCTCACTGAGGCCGCCCGGGCAAAGCCC
GGGCGTCGGGCGACCTTTGGTCGCCCGGCCTCAGTGAGCGAGCGAGCGCGCAGAGA
GGGAGTGGCCAACTCCATCACTAGGGGTTCCTTTGTCGACGCGGCCGCACGCGTGAC
ATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCC
ATATATGGAGTTCCGCGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCC
CAACGACCCCCGCCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAAT
AGGGACTTTCCATTGACGTCAATGGGTGGACTATTTACGGTAAACTGCCCACTTGGC
AGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAA
ATGGCCCGCCTGGCATTATGCCCAGTACATGACCTTATGGGACTTTCCTACTTGGCA
GTACATCTACGTATTAGTCATCGCTATTACCATGGGTCGAGGTGAGCCCCACGTTCT
GCTTCACTCTCCCCATCTCCCCCCCCTCCCCACCCCCAATTTTGTATTTATTTATTTTT
TAATTATTTTGTGCAGCGATGGGGGCGGGGGGGGGGGGGCGCGCGCCAGGCGGGG
CGGGGCGGGGCGAGGGGCGGGGCGGGGCGAGGCGGAGAGGTGCGGCGGCAGCCAA
TCAGAGCGGCGCGCTCCGAAAGTTTCCTTTTATGGCGAGGCGGCGGCGGCGGCGGC
CCTATAAAAAGCGAAGCGCGCGGCGGGCGGGAGTCGCTGCGTTGCCTTCGCCCCGT
GCCCCGCTCCGCGCCGCCTCGCGCCGCCCGCCCCGGCTCTGACTGACCGCGTTACTC
CCACAGGTGAGCGGGCGGGACGGCCCTTCTCCTCCGGGCTGTAATTAGCGCTTGGTT
TAATGACGGCTCGTTTCTTTTCTGTGGCTGCGTGAAAGCCTTAAAGGGCTCCGGGAG
GGCCCTTTGTGCGGGGGGGAGCGGCTCGGGGGGTGCGTGCGTGTGTGTGTGCGTGG
GGAGCGCCGCGTGCGGCCCGCGCTGCCCGGCGGCTGTGAGCGCTGCGGGCGCGGCG
CGGGGCTTTGTGCGCTCCGCGTGTGCGCGAGGGGAGCGCGGCCGGGGGCGGTGCCC
CGCGGTGCGGGGGGGCTGCGAGGGGAACAAAGGCTGCGTGCGGGGTGTGTGCGTGG
GGGGGTGAGCAGGGGGGTGTGGGCGCGGCGGTCGGGCTGTAACCCCCCCCCTGCACCC
CCCTCCCCGAGTTGCTGAGCACGGCCCGGCTTCGGGTGCGGGGCTCCGTGCGGGGC
GTGGCGCGGGGCTCGCCGTGCCGGGCGGGGGGTGGCGGCAGGTGGGGGTGCCGGG
CGGGGCGGGGCCGCCTCGGGCCGGGGAGGGCTCGGGGGAGGGGCGCGGCGGCCCC
CGGAGCGCCGGCGGCTGTCGAGGCGCGGCGAGCCGCAGCCATTGCCTTTTATGGTA
ATCGTGCGAGAGGGCGCAGGGACTTCCTTTGTCCCAAATCTGTGCGGAGCCGAAATC

TGGGAGGCGCCGCCGCACCCCCTCTAGCGGGCGCGGGGCGAAGCGGTGCGGCGCCG
GCAGGAAGGAAATGGGCGGGGAGGGCCTTCGTGCGTCGCCGCGCCGCCGTCCCCTT
CTCCCTCTCCAGCCTCGGGGCTGTCCGCGGGGGGGACGGCTGCCTTCGGGGGGGACG
GGGCAGGGCGGGGTTCGGCTTCTGGCGTGTGACCGGCGGCTCTAGAGCCTCTGCTAA
CCATGTTCATGCCTTCTTCTTTTTCCTACAGCTCCTGGGCAACGTGCTGGTTATTGTG
ACCGGTGCCACCATGTACCGGATGCAGCTGCTGAGCTGTATCGCCCTGTCTCTGGCC
CTGGTCACCAATTCTGAGGTGCAGCTGGTGGAATCTGGCGGCGGACTTGTTCAACCT
GGCGGCTCTCTGAGACTGAGCTGTGCCGCTTCTGGCTACGACTTCACCCACTACGGC
ATGAACTGGGTCCGACAGGCCCCTGGCAAAGGCCTTGAATGGGTCGGATGGATCAA
CACCTACACCGGCGAGCCAACATACGCCGCCGACTTCAAGCGGAGATTCACCTTCA
GCCTGGACACCAGCAAGAGCACCGCCTACCTGCAGATGAACAGCCTGAGAGCCGAG
GACACCGCCGTGTACTACTGCGCCAAGTATCCCTACTACTACGGCACCAGCCACTGG
TACTTTGACGTGTGGGGACAGGGCACACTGGTCACAGTGTCTAGCGCCTCTACAAAG
GGCCCCAGCGTTTTCCCACTGGCTCCTAGCAGCAAGTCTACCAGCGGAGGAACAGC
CGCTCTGGGCTGTCTGGTCAAGGACTACTTTCCCGAGCCTGTGACCGTGTCCTGGAA
TTCTGGCGCTCTGACAAGCGGCGTGCACACCTTTCCAGCTGTGCTGCAAAGCAGCGG
CCTGTACTCTCTGAGCAGCGTCGTGACAGTGCCAAGCAGCTCTCTGGGCACCCAGAC
CTACATCTGCAATGTGAACCACAAGCCTAGCAACACCAAGGTGGACAAGAAGGTGG
AACCCAAGAGCTGCGACAAGACCCACACCGGCAAGCGGAAGAGAAGAGGCTCTGG
CGAAGGCAGAGGCAGCCTGCTTACATGTGGCGACGTGGAAGAGAACCCCGGACCTA
TGTATAGAATGCAGCTCCTGTCCTGCATTGCCCTGAGCCTGGCTCTCGTGACCAACA
GCGACATCCAGCTGACACAGAGCCCCAGCAGCCTGTCTGCCTCTGTGGGAGACAGA
GTGACCATCACCTGTAGCGCCAGCCAGGACATCTCCAACTACCTGAACTGGTATCAG
CAAAAGCCCGGCAAGGCCCCTAAGGTGCTGATCTACTTCACAAGCAGCCTGCACTC
CGGCGTGCCCAGCAGATTTTCTGGCTCTGGCAGCGGCACCGACTTCACCCTGACCAT
ATCTAGCCTGCAGCCTGAGGACTTCGCCACCTACTACTGCCAGCAGTACAGCACCGT
GCCTTGGACATTTGGCCAGGGCACAAAGGTGGAAATCAAGCGGACTGTGGCCGCTC
CTAGCGTGTTCATCTTTCCACCTAGCGACGAGCAGCTGAAGTCTGGCACAGCCTCTG
TCGTGTGCCTGCTGAACAACTTCTACCCCAGAGAAGCCAAGGTGCAGTGGAAAGTG
GACAATGCCCTGCAGAGCGGCAACAGCCAAGAGAGCGTGACAGAGCAGGACTCCA
AGGATAGCACCTATAGCCTGAGCAGCACCCTGACACTGAGCAAGGCCGACTACGAG

AAGCACAAAGTGTACGCCTGCGAAGTGACCCACCAGGGCCTTTCTAGCCCTGTGAC
CAAGAGCTTCAACCGGGGCGAATGTTAAGAGCTCGCTGATCAGCCTCGACTGTGCCT
TCTAGTTGCCAGCCATCTGTTGTTTGCCCCTCCCCCGTGCCTTCCTTGACCCTGGAAG
GTGCCACTCCCACTGTCCTTTCCTAATAAAATGAGGAAATTGCATCGCATTGTCTGA
GTAGGTGTCATTCTATTCTGGGGGGTGGGGTGGGGCAGGACAGCAAGGGGGAGGAT
TGGGAAGACAATAGCAGGCATGCTGGGGATGCGGTGGGCTCTATGGAAGCTTGAAT
TCAGCTGACGTGCCTCGGACCGCCTAGGAGGAACCCCTAGTGATGGAGTTGGCCAC
TCCCTCTCTGCGCGCTCGCTCGCTCACTGAGGCCGGGCGACCAAAGGTCGCCCGACG
CCCGGGCTTTGCCCGGGCGGCCTCAGTGAGCGAGCGAGCGCGCAGAGAGGGAGTGG
CCAA

[0535] Exemplary rAAV-Ranibizumab-GFP Construct Sequence (SEQ ID NO: 106)

CTGCGCGCTCGCTCGCTCACTGAGGCCGCCCGGGCGTCGGGCGACCTTTGGTCGCCC
GGCCTCAGTGAGCGAGCGAGCGCGCAGAGAGGGAGTGGCCAACTCCATCACTAGGG
GTTCCTGCGGCCGCACGCGTGACATTGATTATTGACTAGTTATTAATAGTAATCAAT
TACGGGGTCATTAGTTCATAGCCCATATATGGAGTTCCGCGTTACATAACTTACGGT
AAATGGCCCGCCTGGCTGACCGCCCAACGACCCCCGCCCATTGACGTCAATAATGA
CGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGACT
ATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGC
CCCCTATTGACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCCAGTACATGA
CCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCAT
GGGTCGAGGTGAGCCCCACGTTCTGCTTCACTCTCCCCATCTCCCCCCCCTCCCCACC
CCCAATTTTGTATTTATTTATTTTTTAATTATTTTGTGCAGCGATGGGGGCGGGGGGG
GGGGGGGCGCGCGCCAGGCGGGGCGGGGCGGGGCGAGGGGCGGGGCGGGGCGAG
GCGGAGAGGTGCGGCGGCAGCCAATCAGAGCGGCGCGCTCCGAAAGTTTCCTTTTA
TGGCGAGGCGGCGGCGGCGGCGGCCCTATAAAAAGCGAAGCGCGCGGCGGGCGGG
AGTCGCTGCGTTGCCTTCGCCCCGTGCCCCGCTCCGCGCCGCCTCGCGCCGCCCGCC
CCGGCTCTGACTGACCGCGTTACTCCCACAGGTGAGCGGGCGGGACGGCCCTTCTCC
TCCGGGCTGTAATTAGCGCTTGGTTTAATGACGGCTCGTTTCTTTTCTGTGGCTGCGT
GAAAGCCTTAAAGGGCTCCGGGAGGGCCCTTTGTGCGGGGGGGAGCGGCTCGGGGG
GTGCGTGCGTGTGTGTGTGCGTGGGGAGCGCCGCGTGCGGCCCGCGCTGCCCGGCG
GCTGTGAGCGCTGCGGGCGCGGCGCGGGGCTTTGTGCGCTCCGCGTGTGCGCGAGG

GGAGCGCGGCCGGGGGCGGTGCCCCGCGGTGCGGGGGGGCTGCGAGGGGAACAAA
GGCTGCGTGCGGGGTGTGTGCGTGGGGGGGTGAGCAGGGGGTGTGGGCGCGGCGGT
CGGGCTGTAACCCCCCCCTGCACCCCCCTCCCCGAGTTGCTGAGCACGGCCCGGCTT
CGGGTGCGGGGCTCCGTGCGGGGCGTGGCGCGGGGCTCGCCGTGCCGGGCGGGGGG
TGGCGGCAGGTGGGGGTGCCGGGCGGGGCGGGGCCGCCTCGGGCCGGGGAGGGCT
CGGGGGAGGGGCGCGGCGGCCCCCGGAGCGCCGGCGGCTGTCGAGGCGCGGCGAG
CCGCAGCCATTGCCTTTTATGGTAATCGTGCGAGAGGGCGCAGGGACTTCCTTTGTC
CCAAATCTGTGCGGAGCCGAAATCTGGGAGGCGCCGCCGCACCCCCTCTAGCGGGC
GCGGGGCGAAGCGGTGCGGCGCCGGCAGGAAGGAAATGGGCGGGGAGGGCCTTCG
TGCGTCGCCGCGCCGCCGTCCCCTTCTCCCTCTCCAGCCTCGGGGCTGTCCGCGGGG
GGACGGCTGCCTTCGGGGGGGACGGGGCAGGGCGGGGTTCGGCTTCTGGCGTGTGA
CCGGCGGCTCTAGAGCCTCTGCTAACCATGTTCATGCCTTCTTCTTTTTCCTACAGCT
CCTGGGCAACGTGCTGGTTATTGTGACCGGTGCCACCATGTACCGGATGCAGCTGCT
GAGCTGTATCGCCCTGTCTCTGGCCCTGGTCACCAATTCTGAGGTGCAGCTGGTGGA
ATCTGGCGGCGGACTTGTTCAACCTGGCGGCTCTCTGAGACTGAGCTGTGCCGCTTC
TGGCTACGACTTCACCCACTACGGCATGAACTGGGTCCGACAGGCCCCTGGCAAAG
GCCTTGAATGGGTCGGATGGATCAACACCTACACCGGCGAGCCAACATACGCCGCC
GACTTCAAGCGGAGATTCACCTTCAGCCTGGACACCAGCAAGAGCACCGCCTACCT
GCAGATGAACAGCCTGAGAGCCGAGGACACCGCCGTGTACTACTGCGCCAAGTATC
CCTACTACTACGGCACCAGCCACTGGTACTTTGACGTGTGGGGACAGGGCACACTG
GTCACAGTGTCTAGCGCCTCTACAAAGGGCCCCAGCGTTTTCCCACTGGCTCCTAGC
AGCAAGTCTACCAGCGGAGGAACAGCCGCTCTGGGCTGTCTGGTCAAGGACTACTTT
CCCGAGCCTGTGACCGTGTCCTGGAATTCTGGCGCTCTGACAAGCGGCGTGCACACC
TTTCCAGCTGTGCTGCAAAGCAGCGGCCTGTACTCTCTGAGCAGCGTCGTGACAGTG
CCAAGCAGCTCTCTGGGCACCCAGACCTACATCTGCAATGTGAACCACAAGCCTAG
CAACACCAAGGTGGACAAGAAGGTGGAACCCAAGAGCTGCGACAAGACCCACACC
GGCAAGCGGAAGAGAAGAGGCTCTGGCGAAGGCAGAGGCAGCCTGCTTACATGTG
GCGACGTGGAAGAGAACCCCGGACCTATGTATAGAATGCAGCTCCTGTCCTGCATTG
CCCTGAGCCTGGCTCTCGTGACCAACAGCGACATCCAGCTGACACAGAGCCCCAGC
AGCCTGTCTGCCTCTGTGGGAGACAGAGTGACCATCACCTGTAGCGCCAGCCAGGA
CATCTCCAACTACCTGAACTGGTATCAGCAAAAGCCCGGCAAGGCCCCTAAGGTGC

TGATCTACTTCACAAGCAGCCTGCACTCCGGCGTGCCCAGCAGATTTTCTGGCTCTG
GCAGCGGCACCGACTTCACCCTGACCATATCTAGCCTGCAGCCTGAGGACTTCGCCA
CCTACTACTGCCAGCAGTACAGCACCGTGCCTTGGACATTTGGCCAGGGCACAAAG
GTGGAAATCAAGCGGACTGTGGCCGCTCCTAGCGTGTTCATCTTTCCACCTAGCGAC
GAGCAGCTGAAGTCTGGCACAGCCTCTGTCGTGTGCCTGCTGAACAACTTCTACCCC
AGAGAAGCCAAGGTGCAGTGGAAAGTGGACAATGCCCTGCAGAGCGGCAACAGCC
AAGAGAGCGTGACAGAGCAGGACTCCAAGGATAGCACCTATAGCCTGAGCAGCACC
CTGACACTGAGCAAGGCCGACTACGAGAAGCACAAAGTGTACGCCTGCGAAGTGAC
CCACCAGGGCCTTTCTAGCCCTGTGACCAAGAGCTTCAACCGGGGCGAATGTATGGT
GAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCGAGCTGGACG
GCGACGTAAACGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACC
TACGGCAAGCTGACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCCGTGCCCTGG
CCCACCCTCGTGACCACCCTGACCTACGGCGTGCAGTGCTTCAGCCGCTACCCCGAC
CACATGAAGCAGCACGACTTCTTCAAGTCCGCCATGCCCGAAGGCTACGTCCAGGA
GCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGACCCGCGCCGAGGTGAAGT
TCGAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAGGAG
GACGGCAACATCCTGGGGCACAAGCTGGAGTACAACTACAACAGCCACAACGTCTA
TATCATGGCCGACAAGCAGAAGAACGGCATCAAGGTGAACTTCAAGATCCGCCACA
ACATCGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAGCAGAACACCCCCATC
GGCGACGGCCCCGTGCTGCTGCCCGACAACCACTACCTGAGCACCCAGTCCGCCCTG
AGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTTCGTGACCGC
CGCCGGGATCACTCTCGGCATGGACGAGCTGTACAAGTAAGAGCTCGCTGATCAGC
CTCGACTGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGCCCCTCCCCCGTGCCTTCC
TTGACCCTGGAAGGTGCCACTCCCACTGTCCTTTCCTAATAAAATGAGGAAATTGCA
TCGCATTGTCTGAGTAGGTGTCATTCTATTCTGGGGGGTGGGGTGGGGCAGGACAGC
AAGGGGGAGGATTGGGAAGACAATAGCAGGCATGCTGGGGATGCGGTGGGCTCTAT
GGAAGCTTGAATTCAGCTGACGTGCCTCGGACCGCTAGGAACCCCTAGTGATGGAG
TTGGCCACTCCCTCTCTGCGCGCTCGCTCGCTCACTGAGGCCGGGCGACCAAAGGTC
GCCCGACGCCCGGGCTTTGCCCGGGCGGCCTCAGTGAGCGAGCGAGCGCGCAG

[0536] Exemplary rAAV-Bevacizumab-PC Construct Sequence (SEQ ID NO: 93)

CTGCGCGCTCGCTCGCTCACTGAGGCCGCCCGGGCGTCGGGCGACCTTTGGTCGCCC
GGCCTCAGTGAGCGAGCGAGCGCGCAGAGAGGGAGTGGCCAACTCCATCACTAGGG
GTTCCTGCGGCCGCACGCGTGACATTGATTATTGACTAGTTATTAATAGTAATCAAT
TACGGGGTCATTAGTTCATAGCCCATATATGGAGTTCCGCGTTACATAACTTACGGT
AAATGGCCCGCCTGGCTGACCGCCCAACGACCCCCGCCCATTGACGTCAATAATGA
CGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGACT
ATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGC
CCCCTATTGACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCCAGTACATGA
CCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCAT
GGGTCGAGGTGAGCCCCACGTTCTGCTTCACTCTCCCCATCTCCCCCCCCTCCCCACC
CCCAATTTTGTATTTATTTATTTTTTAATTATTTTGTGCAGCGATGGGGGCGGGGGGG
GGGGGGGCGCGCGCCAGGCGGGGCGGGGCGGGGCGAGGGGCGGGGCGGGGCGAG
GCGGAGAGGTGCGGCGGCAGCCAATCAGAGCGGCGCGCTCCGAAAGTTTCCTTTTA
TGGCGAGGCGGCGGCGGCGGCGGCCCTATAAAAAGCGAAGCGCGCGGCGGGCGGG
AGTCGCTGCGTTGCCTTCGCCCCGTGCCCCGCTCCGCGCCGCCTCGCGCCGCCCGCC
CCGGCTCTGACTGACCGCGTTACTCCCACAGGTGAGCGGGCGGGACGGCCCTTCTCC
TCCGGGCTGTAATTAGCGCTTGGTTTAATGACGGCTCGTTTCTTTTCTGTGGCTGCGT
GAAAGCCTTAAAGGGCTCCGGGAGGGCCCTTTGTGCGGGGGGGAGCGGCTCGGGGG
GTGCGTGCGTGTGTGTGTGCGTGGGGAGCGCCGCGTGCGGCCCGCGCTGCCCGGCG
GCTGTGAGCGCTGCGGGCGCGGCGCGGGGCTTTGTGCGCTCCGCGTGTGCGCGAGG
GGAGCGCGGCCGGGGGCGGTGCCCCGCGGTGCGGGGGGGCTGCGAGGGGAACAAA
GGCTGCGTGCGGGGTGTGTGCGTGGGGGGGTGAGCAGGGGGTGTGGGCGCGGCGGT
CGGGCTGTAACCCCCCCCTGCACCCCCCTCCCCGAGTTGCTGAGCACGGCCCGGCTT
CGGGTGCGGGGCTCCGTGCGGGGCGTGGCGCGGGGCTCGCCGTGCCGGGCGGGGGG
TGGCGGCAGGTGGGGGTGCCGGGCGGGGCGGGGCCGCCTCGGGCCGGGGAGGGCT
CGGGGGAGGGGCGCGGCGGCCCCCGGAGCGCCGGCGGCTGTCGAGGCGCGGCGAG
CCGCAGCCATTGCCTTTTATGGTAATCGTGCGAGAGGGCGCAGGGACTTCCTTTGTC
CCAAATCTGTGCGGAGCCGAAATCTGGGAGGCGCCGCCGCACCCCCTCTAGCGGGC
GCGGGGCGAAGCGGTGCGGCGCCGGCAGGAAGGAAATGGGCGGGGAGGGCCTTCG
TGCGTCGCCGCGCCGCCGTCCCCTTCTCCCTCTCCAGCCTCGGGGCTGTCCGCGGGG
GGACGGCTGCCTTCGGGGGGGGACGGGGCAGGGCGGGGTTCGGCTTCTGGCGTGTGA

CCGGCGGCTCTAGAGCCTCTGCTAACCATGTTCATGCCTTCTTCTTTTTCCTACAGCT
CCTGGGCAACGTGCTGGTTATTGTGACCGGTGCCACCATGTACCGGATGCAGCTGCT
GAGCTGTATCGCCCTGTCTCTGGCCCTGGTCACCAATTCTGAGGTGCAGCTGGTGGA
ATCTGGCGGCGGACTTGTTCAACCTGGCGGCTCTCTGAGACTGAGCTGTGCCGCTTC
TGGCTACACCTTCACCAACTACGGCATGAACTGGGTCCGACAGGCCCCTGGCAAAG
GCCTTGAATGGGTCGGATGGATCAACACCTACACCGGCGAGCCAACATACGCCGCC
GACTTCAAGCGGAGATTCACCTTCAGCCTGGACACCAGCAAGAGCACCGCCTACCT
GCAGATGAACAGCCTGAGAGCCGAGGACACCGCCGTGTACTACTGCGCCAAGTATC
CCCACTACTACGGCAGCAGCCACTGGTACTTTGACGTGTGGGGACAGGGCACACTG
GTCACAGTGTCTAGCGCCTCTACAAAGGGCCCCAGCGTTTTCCCACTGGCTCCTAGC
AGCAAGTCTACCAGCGGAGGAACAGCCGCTCTGGGCTGTCTGGTCAAGGACTACTTT
CCCGAGCCTGTGACCGTGTCCTGGAATTCTGGCGCTCTGACAAGCGGCGTGCACACC
TTTCCAGCTGTGCTGCAAAGCAGCGGCCTGTACTCTCTGAGCAGCGTCGTGACAGTG
CCAAGCAGCTCTCTGGGCACCCAGACCTACATCTGCAATGTGAACCACAAGCCTAG
CAACACCAAGGTGGACAAGAAGGTGGAACCCAAGAGCTGCGACAAGACCCACACC
TGTCCTCCATGTCCTGCTCCAGAACTGCTCGGCGGACCTTCCGTGTTCCTGTTTCCTC
CAAAGCCTAAGGACACCCTGATGATCAGCAGAACCCCTGAAGTGACCTGCGTGGTG
GTGGATGTGTCCCACGAGGATCCCGAAGTGAAGTTCAATTGGTACGTGGACGGCGT
GGAAGTGCACAACGCCAAGACCAAGCCTAGAGAGGAACAGTACAACAGCACCTAC
AGAGTGGTGTCCGTGCTGACCGTGCTGCACCAGGATTGGCTGAACGGCAAAGAGTA
CAAGTGCAAGGTGTCCAACAAGGCCCTGCCTGCTCCTATCGAGAAAACCATCAGCA
AGGCCAAGGGCCAGCCTAGGGAACCCCAGGTTTACACACTGCCTCCAAGCCGGGAA
GAGATGACCAAGAACCAGGTGTCCCTGACCTGCCTCGTGAAGGGCTTCTACCCTTCC
GATATCGCCGTGGAATGGGAGAGCAATGGCCAGCCAGAGAACAACTACAAGACAA
CCCCTCCTGTGCTGGACAGCGACGGCTCATTCTTCCTGTACAGCAAGCTGACAGTGG
ACAAGTCCAGATGGCAGCAGGGCAACGTGTTCAGCTGCAGCGTGATGCACGAGGCC
CTGCACAACCACTACACCCAGAAGTCTCTGAGCCTGTCTCCTGGCAAGCGGAAGAG
AAGAGGCTCTGGCGAAGGCAGAGGCAGCCTGCTTACATGTGGCGACGTGGAAGAGA
ACCCCGGACCTATGTATAGAATGCAGCTCCTGTCCTGCATTGCCCTGAGCCTGGCTC
TCGTGACCAACAGCGACATCCAGATGACACAGAGCCCCAGCAGCCTGTCTGCCTCT
GTGGGAGACAGAGTGACCATCACCTGTAGCGCCAGCCAGGACATCTCCAACTACCT

GAACTGGTATCAGCAAAAGCCCGGCAAGGCCCCTAAGGTGCTGATCTACTTCACAA
GCAGCCTGCACTCCGGCGTGCCCAGCAGATTTTCTGGCTCTGGCAGCGGCACCGACT
TCACCCTGACCATATCTAGCCTGCAGCCTGAGGACTTCGCCACCTACTACTGCCAGC
AGTACAGCACCGTGCCTTGGACATTTGGCCAGGGCACAAAGGTGGAAATCAAGCGG
ACTGTGGCCGCTCCTAGCGTGTTCATCTTTCCACCTAGCGACGAGCAGCTGAAGTCT
GGCACAGCCTCTGTCGTGTGCCTGCTGAACAACTTCTACCCCAGAGAAGCCAAGGTG
CAGTGGAAAGTGGACAATGCCCTGCAGAGCGGCAACAGCCAAGAGAGCGTGACAG
AGCAGGACTCCAAGGATAGCACCTATAGCCTGAGCAGCACCCTGACACTGAGCAAG
GCCGACTACGAGAAGCACAAAGTGTACGCCTGCGAAGTGACCCACCAGGGCCTTTC
TAGCCCTGTGACCAAGAGCTTCAACCGGGGCGAATGTTAAGAGCTCGCTGATCAGC
CTCGACTGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGCCCCTCCCCCGTGCCTTCC
TTGACCCTGGAAGGTGCCACTCCCACTGTCCTTTCCTAATAAAATGAGGAAATTGCA
TCGCATTGTCTGAGTAGGTGTCATTCTATTCTGGGGGGTGGGGTGGGGCAGGACAGC
AAGGGGGAGGATTGGGAAGACAATAGCAGGCATGCTGGGGATGCGGTGGGCTCTAT
GGAAGCTTGAATTCAGCTGACGTGCCTCGGACCGCTAGGAACCCCTAGTGATGGAG
TTGGCCACTCCCTCTCTGCGCGCTCGCTCGCTCACTGAGGCCGGGCGACCAAAGGTC
GCCCGACGCCCGGGCTTTGCCCGGGCGGCCTCAGTGAGCGAGCGAGCGCGCAG

[0537] Exemplary rAAV-Bevacizumab Construct Sequence (SEQ ID NO: 94)

TTGGCCACTCCCTCTCTGCGCGCTCGCTCGCTCACTGAGGCCGCCCGGGCAAAGCCC
GGGCGTCGGGCGACCTTTGGTCGCCCGGCCTCAGTGAGCGAGCGAGCGCGCAGAGA
GGGAGTGGCCAACTCCATCACTAGGGGTTCCTTTGTCGACGCGGCCGCACGCGTGAC
ATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCC
ATATATGGAGTTCCGCGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCC
CAACGACCCCCGCCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAAT
AGGGACTTTCCATTGACGTCAATGGGTGGACTATTTACGGTAAACTGCCCACTTGGC
AGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAA
ATGGCCCGCCTGGCATTATGCCCAGTACATGACCTTATGGGACTTTCCTACTTGGCA
GTACATCTACGTATTAGTCATCGCTATTACCATGGGTCGAGGTGAGCCCCACGTTCT
GCTTCACTCTCCCCATCTCCCCCCCCTCCCCACCCCCAATTTTGTATTTATTTATTTTT
TAATTATTTTGTGCAGCGATGGGGGCGGGGGGGGGGGGGGGCGCGCGCCAGGCGG
GGCGGGGCGGGGCGAGGGGCGGGGCGGGGCGAGGCGGAGAGGTGCGGCGGCAGCC

AATCAGAGCGGCGCGCTCCGAAAGTTTCCTTTTATGGCGAGGCGGCGGCGGCGGCG
GCCCTATAAAAAGCGAAGCGCGCGGCGGGCGGGAGTCGCTGCGTTGCCTTCGCCCC
GTGCCCCGCTCCGCGCCGCCTCGCGCCGCCCGCCCCGGCTCTGACTGACCGCGTTAC
TCCCACAGGTGAGCGGGCGGGACGGCCCTTCTCCTCCGGGCTGTAATTAGCGCTTGG
TTTAATGACGGCTCGTTTCTTTTCTGTGGCTGCGTGAAAGCCTTAAAGGGCTCCGGG
AGGGCCCTTTGTGCGGGGGGGAGCGGCTCGGGGGGTGCGTGCGTGTGTGTGTGCGT
GGGGAGCGCCGCGTGCGGCCCGCGCTGCCCGGCGGCTGTGAGCGCTGCGGGCGCGG
CGCGGGGCTTTGTGCGCTCCGCGTGTGCGCGAGGGGAGCGCGGCCGGGGGCGGTGC
CCCGCGGTGCGGGGGGGGCTGCGAGGGGAACAAAGGCTGCGTGCGGGGTGTGTGCGT
GGGGGGGTGAGCAGGGGGTGTGGGCGCGGCGGTCGGGCTGTAACCCCCCCCTGCAC
CCCCCTCCCCGAGTTGCTGAGCACGGCCCGGCTTCGGGTGCGGGGCTCCGTGCGGGG
CGTGGCGCGGGGCTCGCCGTGCCGGGCGGGGGGTGGCGGCAGGTGGGGGTGCCGG
GCGGGGCGGGGCCGCCTCGGGCCGGGGAGGGCTCGGGGGAGGGGCGCGGCGGCCC
CCGGAGCGCCGGCGGCTGTCGAGGCGCGGCGAGCCGCAGCCATTGCCTTTTATGGT
AATCGTGCGAGAGGGCGCAGGGACTTCCTTTGTCCCAAATCTGTGCGGAGCCGAAA
TCTGGGAGGCGCCGCCGCACCCCCTCTAGCGGGCGCGGGGCGAAGCGGTGCGGCGC
CGGCAGGAAGGAAATGGGCGGGGAGGGCCTTCGTGCGTCGCCGCGCCGCCGTCCCC
TTCTCCCTCTCCAGCCTCGGGGCTGTCCGCGGGGGGGACGGCTGCCTTCGGGGGGGAC
GGGGCAGGGCGGGGTTCGGCTTCTGGCGTGTGACCGGCGGCTCTAGAGCCTCTGCTA
ACCATGTTCATGCCTTCTTCTTTTTCCTACAGCTCCTGGGCAACGTGCTGGTTATTGT
GACCGGTGCCACCATGTACCGGATGCAGCTGCTGAGCTGTATCGCCCTGTCTCTGGC
CCTGGTCACCAATTCTGAGGTGCAGCTGGTGGAATCTGGCGGCGGACTTGTTCAACC
TGGCGGCTCTCTGAGACTGAGCTGTGCCGCTTCTGGCTACACCTTCACCAACTACGG
CATGAACTGGGTCCGACAGGCCCCTGGCAAAGGCCTTGAATGGGTCGGATGGATCA
ACACCTACACCGGCGAGCCAACATACGCCGCCGACTTCAAGCGGAGATTCACCTTC
AGCCTGGACACCAGCAAGAGCACCGCCTACCTGCAGATGAACAGCCTGAGAGCCGA
GGACACCGCCGTGTACTACTGCGCCAAGTATCCCCACTACTACGGCAGCAGCCACTG
GTACTTTGACGTGTGGGGACAGGGCACACTGGTCACAGTGTCTAGCGCCTCTACAAA
GGGCCCCAGCGTTTTCCCACTGGCTCCTAGCAGCAAGTCTACCAGCGGAGGAACAG
CCGCTCTGGGCTGTCTGGTCAAGGACTACTTTCCCGAGCCTGTGACCGTGTCCTGGA
ATTCTGGCGCTCTGACAAGCGGCGTGCACACCTTTCCAGCTGTGCTGCAAAGCAGCG

GCCTGTACTCTCTGAGCAGCGTCGTGACAGTGCCAAGCAGCTCTCTGGGCACCCAGA
CCTACATCTGCAATGTGAACCACAAGCCTAGCAACACCAAGGTGGACAAGAAGGTG
GAACCCAAGAGCTGCGACAAGACCCACACCTGTCCTCCATGTCCTGCTCCAGAACTG
CTCGGCGGACCTTCCGTGTTCCTGTTTCCTCCAAAGCCTAAGGACACCCTGATGATC
AGCAGAACCCCTGAAGTGACCTGCGTGGTGGTGGATGTGTCCCACGAGGATCCCGA
AGTGAAGTTCAATTGGTACGTGGACGGCGTGGAAGTGCACAACGCCAAGACCAAGC
CTAGAGAGGAACAGTACAACAGCACCTACAGAGTGGTGTCCGTGCTGACCGTGCTG
CACCAGGATTGGCTGAACGGCAAAGAGTACAAGTGCAAGGTGTCCAACAAGGCCCT
GCCTGCTCCTATCGAGAAAACCATCAGCAAGGCCAAGGGCCAGCCTAGGGAACCCC
AGGTTTACACACTGCCTCCAAGCCGGGAAGAGATGACCAAGAACCAGGTGTCCCTG
ACCTGCCTCGTGAAGGGCTTCTACCCTTCCGATATCGCCGTGGAATGGGAGAGCAAT
GGCCAGCCAGAGAACAACTACAAGACAACCCCTCCTGTGCTGGACAGCGACGGCTC
ATTCTTCCTGTACAGCAAGCTGACAGTGGACAAGTCCAGATGGCAGCAGGGCAACG
TGTTCAGCTGCAGCGTGATGCACGAGGCCCTGCACAACCACTACACCCAGAAGTCTC
TGAGCCTGTCTCCTGGCAAGCGGAAGAGAAGAGGCTCTGGCGAAGGCAGAGGCAGC
CTGCTTACATGTGGCGACGTGGAAGAGAACCCCGGACCTATGTATAGAATGCAGCT
CCTGTCCTGCATTGCCCTGAGCCTGGCTCTCGTGACCAACAGCGACATCCAGATGAC
ACAGAGCCCCAGCAGCCTGTCTGCCTCTGTGGGAGACAGAGTGACCATCACCTGTA
GCGCCAGCCAGGACATCTCCAACTACCTGAACTGGTATCAGCAAAAGCCCGGCAAG
GCCCCTAAGGTGCTGATCTACTTCACAAGCAGCCTGCACTCCGGCGTGCCCAGCAGA
TTTTCTGGCTCTGGCAGCGGCACCGACTTCACCCTGACCATATCTAGCCTGCAGCCT
GAGGACTTCGCCACCTACTACTGCCAGCAGTACAGCACCGTGCCTTGGACATTTGGC
CAGGGCACAAAGGTGGAAATCAAGCGGACTGTGGCCGCTCCTAGCGTGTTCATCTTT
CCACCTAGCGACGAGCAGCTGAAGTCTGGCACAGCCTCTGTCGTGTGCCTGCTGAAC
AACTTCTACCCCAGAGAAGCCAAGGTGCAGTGGAAAGTGGACAATGCCCTGCAGAG
CGGCAACAGCCAAGAGAGCGTGACAGAGCAGGACTCCAAGGATAGCACCTATAGC
CTGAGCAGCACCCTGACACTGAGCAAGGCCGACTACGAGAAGCACAAAGTGTACGC
CTGCGAAGTGACCCACCAGGGCCTTTCTAGCCCTGTGACCAAGAGCTTCAACCGGG
GCGAATGTTAAGAGCTCGCTGATCAGCCTCGACTGTGCCTTCTAGTTGCCAGCCATC
TGTTGTTTGCCCCTCCCCCGTGCCTTCCTTGACCCTGGAAGGTGCCACTCCCACTGTC
CTTTCCTAATAAAATGAGGAAATTGCATCGCATTGTCTGAGTAGGTGTCATTCTATTC

TGGGGGGTGGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAGACAATAGCAG
GCATGCTGGGGATGCGGTGGGCTCTATGGAAGCTTGAATTCAGCTGACGTGCCTCGG
ACCGCCTAGGAGGAACCCCTAGTGATGGAGTTGGCCACTCCCTCTCTGCGCGCTCGC
TCGCTCACTGAGGCCGGGCGACCAAAGGTCGCCCGACGCCCGGGCTTTGCCCGGGC
GGCCTCAGTGAGCGAGCGAGCGCGCAGAGAGGGAGTGGCCAA

[0538] Exemplary rAAV-Bevacizumab-GFP Construct Sequence (SEQ ID NO: 107)

CTGCGCGCTCGCTCGCTCACTGAGGCCGCCCGGGCGTCGGGCGACCTTTGGTCGCCC
GGCCTCAGTGAGCGAGCGAGCGCGCAGAGAGGGAGTGGCCAACTCCATCACTAGGG
GTTCCTGCGGCCGCACGCGTGACATTGATTATTGACTAGTTATTAATAGTAATCAAT
TACGGGGTCATTAGTTCATAGCCCATATATGGAGTTCCGCGTTACATAACTTACGGT
AAATGGCCCGCCTGGCTGACCGCCCAACGACCCCCGCCCATTGACGTCAATAATGA
CGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGACT
ATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGC
CCCCTATTGACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCCAGTACATGA
CCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCAT
GGGTCGAGGTGAGCCCCACGTTCTGCTTCACTCTCCCCATCTCCCCCCCCTCCCCACC
CCCAATTTTGTATTTATTTATTTTTTAATTATTTTGTGCAGCGATGGGGGCGGGGGGG
GGGGGGGCGCGCGCCAGGCGGGGCGGGGCGGGGCGAGGGGCGGGGCGGGGCGAG
GCGGAGAGGTGCGGCGGCAGCCAATCAGAGCGGCGCGCTCCGAAAGTTTCCTTTTA
TGGCGAGGCGGCGGCGGCGGCGGCCCTATAAAAAGCGAAGCGCGCGGCGGGCGGG
AGTCGCTGCGTTGCCTTCGCCCCGTGCCCCGCTCCGCGCCGCCTCGCGCCGCCCGCC
CCGGCTCTGACTGACCGCGTTACTCCCACAGGTGAGCGGGCGGGACGGCCCTTCTCC
TCCGGGCTGTAATTAGCGCTTGGTTTAATGACGGCTCGTTTCTTTTCTGTGGCTGCGT
GAAAGCCTTAAAGGGCTCCGGGAGGGCCCTTTGTGCGGGGGGGAGCGGCTCGGGGG
GTGCGTGCGTGTGTGTGTGCGTGGGGAGCGCCGCGTGCGGCCCGCGCTGCCCGGCG
GCTGTGAGCGCTGCGGGCGCGGCGCGGGGCTTTGTGCGCTCCGCGTGTGCGCGAGG
GGAGCGCGGCCGGGGGCGGTGCCCCGCGGTGCGGGGGGGCTGCGAGGGGAACAAA
GGCTGCGTGCGGGGTGTGTGCGTGGGGGGGTGAGCAGGGGGTGTGGGCGCGGCGGT
CGGGCTGTAACCCCCCCCTGCACCCCCCTCCCCGAGTTGCTGAGCACGGCCCGGCTT
CGGGTGCGGGGCTCCGTGCGGGGCGTGGCGCGGGGCTCGCCGTGCCGGGCGGGGGG
TGGCGGCAGGTGGGGGTGCCGGGCGGGGCGGGGCCGCCTCGGGCCGGGGAGGGCT

CGGGGGAGGGGCGCGGCGGCCCCCGGAGCGCCGGCGGCTGTCGAGGCGCGGCGAG
CCGCAGCCATTGCCTTTTATGGTAATCGTGCGAGAGGGCGCAGGGACTTCCTTTGTC
CCAAATCTGTGCGGAGCCGAAATCTGGGAGGCGCCGCCGCACCCCCTCTAGCGGGC
GCGGGGCGAAGCGGTGCGGCGCCGGCAGGAAGGAAATGGGCGGGGAGGGCCTTCG
TGCGTCGCCGCGCCGCCGTCCCCTTCTCCCTCTCCAGCCTCGGGGCTGTCCGCGGGG
GGACGGCTGCCTTCGGGGGGGGACGGGGCAGGGCGGGGTTCGGCTTCTGGCGTGTGA
CCGGCGGCTCTAGAGCCTCTGCTAACCATGTTCATGCCTTCTTCTTTTTCCTACAGCT
CCTGGGCAACGTGCTGGTTATTGTGACCGGTGCCACCATGTACCGGATGCAGCTGCT
GAGCTGTATCGCCCTGTCTCTGGCCCTGGTCACCAATTCTGAGGTGCAGCTGGTGGA
ATCTGGCGGCGGACTTGTTCAACCTGGCGGCTCTCTGAGACTGAGCTGTGCCGCTTC
TGGCTACACCTTCACCAACTACGGCATGAACTGGGTCCGACAGGCCCCTGGCAAAG
GCCTTGAATGGGTCGGATGGATCAACACCTACACCGGCGAGCCAACATACGCCGCC
GACTTCAAGCGGAGATTCACCTTCAGCCTGGACACCAGCAAGAGCACCGCCTACCT
GCAGATGAACAGCCTGAGAGCCGAGGACACCGCCGTGTACTACTGCGCCAAGTATC
CCCACTACTACGGCAGCAGCCACTGGTACTTTGACGTGTGGGGACAGGGCACACTG
GTCACAGTGTCTAGCGCCTCTACAAAGGGCCCCAGCGTTTTCCCACTGGCTCCTAGC
AGCAAGTCTACCAGCGGAGGAACAGCCGCTCTGGGCTGTCTGGTCAAGGACTACTTT
CCCGAGCCTGTGACCGTGTCCTGGAATTCTGGCGCTCTGACAAGCGGCGTGCACACC
TTTCCAGCTGTGCTGCAAAGCAGCGGCCTGTACTCTCTGAGCAGCGTCGTGACAGTG
CCAAGCAGCTCTCTGGGCACCCAGACCTACATCTGCAATGTGAACCACAAGCCTAG
CAACACCAAGGTGGACAAGAAGGTGGAACCCAAGAGCTGCGACAAGACCCACACC
TGTCCTCCATGTCCTGCTCCAGAACTGCTCGGCGGACCTTCCGTGTTCCTGTTTCCTC
CAAAGCCTAAGGACACCCTGATGATCAGCAGAACCCCTGAAGTGACCTGCGTGGTG
GTGGATGTGTCCCACGAGGATCCCGAAGTGAAGTTCAATTGGTACGTGGACGGCGT
GGAAGTGCACAACGCCAAGACCAAGCCTAGAGAGGAACAGTACAACAGCACCTAC
AGAGTGGTGTCCGTGCTGACCGTGCTGCACCAGGATTGGCTGAACGGCAAAGAGTA
CAAGTGCAAGGTGTCCAACAAGGCCCTGCCTGCTCCTATCGAGAAAACCATCAGCA
AGGCCAAGGGCCAGCCTAGGGAACCCCAGGTTTACACACTGCCTCCAAGCCGGGAA
GAGATGACCAAGAACCAGGTGTCCCTGACCTGCCTCGTGAAGGGCTTCTACCCTTCC
GATATCGCCGTGGAATGGGAGAGCAATGGCCAGCCAGAGAACAACTACAAGACAA
CCCCTCCTGTGCTGGACAGCGACGGCTCATTCTTCCTGTACAGCAAGCTGACAGTGG

ACAAGTCCAGATGGCAGCAGGGCAACGTGTTCAGCTGCAGCGTGATGCACGAGGCC
CTGCACAACCACTACACCCAGAAGTCTCTGAGCCTGTCTCCTGGCAAGCGGAAGAG
AAGAGGCTCTGGCGAAGGCAGAGGCAGCCTGCTTACATGTGGCGACGTGGAAGAGA
ACCCCGGACCTATGTATAGAATGCAGCTCCTGTCCTGCATTGCCCTGAGCCTGGCTC
TCGTGACCAACAGCGACATCCAGATGACACAGAGCCCCAGCAGCCTGTCTGCCTCT
GTGGGAGACAGAGTGACCATCACCTGTAGCGCCAGCCAGGACATCTCCAACTACCT
GAACTGGTATCAGCAAAAGCCCGGCAAGGCCCCTAAGGTGCTGATCTACTTCACAA
GCAGCCTGCACTCCGGCGTGCCCAGCAGATTTTCTGGCTCTGGCAGCGGCACCGACT
TCACCCTGACCATATCTAGCCTGCAGCCTGAGGACTTCGCCACCTACTACTGCCAGC
AGTACAGCACCGTGCCTTGGACATTTGGCCAGGGCACAAAGGTGGAAATCAAGCGG
ACTGTGGCCGCTCCTAGCGTGTTCATCTTTCCACCTAGCGACGAGCAGCTGAAGTCT
GGCACAGCCTCTGTCGTGTGCCTGCTGAACAACTTCTACCCCAGAGAAGCCAAGGTG
CAGTGGAAAGTGGACAATGCCCTGCAGAGCGGCAACAGCCAAGAGAGCGTGACAG
AGCAGGACTCCAAGGATAGCACCTATAGCCTGAGCAGCACCCTGACACTGAGCAAG
GCCGACTACGAGAAGCACAAAGTGTACGCCTGCGAAGTGACCCACCAGGGCCTTTC
TAGCCCTGTGACCAAGAGCTTCAACCGGGGCGAATGTATGGTGAGCAAGGGCGAGG
AGCTGTTCACCGGGGTGGTGCCCATCCTGGTCGAGCTGGACGGCGACGTAAACGGC
CACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGAC
CCTGAAGTTCATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCCCACCCTCGTGAC
CACCCTGACCTACGGCGTGCAGTGCTTCAGCCGCTACCCCGACCACATGAAGCAGC
ACGACTTCTTCAAGTCCGCCATGCCCGAAGGCTACGTCCAGGAGCGCACCATCTTCT
TCAAGGACGACGGCAACTACAAGACCCGCGCCGAGGTGAAGTTCGAGGGCGACACC
CTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAGGAGGACGGCAACATCCT
GGGGCACAAGCTGGAGTACAACTACAACAGCCACAACGTCTATATCATGGCCGACA
AGCAGAAGAACGGCATCAAGGTGAACTTCAAGATCCGCCACAACATCGAGGACGGC
AGCGTGCAGCTCGCCGACCACTACCAGCAGAACACCCCCATCGGCGACGGCCCCGT
GCTGCTGCCCGACAACCACTACCTGAGCACCCAGTCCGCCCTGAGCAAAGACCCCA
ACGAGAAGCGCGATCACATGGTCCTGCTGGAGTTCGTGACCGCCGCCGGGATCACT
CTCGGCATGGACGAGCTGTACAAGTAAGAGCTCGCTGATCAGCCTCGACTGTGCCTT
CTAGTTGCCAGCCATCTGTTGTTTGCCCCTCCCCCGTGCCTTCCTTGACCCTGGAAGG
TGCCACTCCCACTGTCCTTTCCTAATAAAATGAGGAAATTGCATCGCATTGTCTGAG

TAGGTGTCATTCTATTCTGGGGGGTGGGGTGGGGCAGGACAGCAAGGGGGAGGATT
GGGAAGACAATAGCAGGCATGCTGGGGATGCGGTGGGCTCTATGGAAGCTTGAATT
CAGCTGACGTGCCTCGGACCGCTAGGAACCCCTAGTGATGGAGTTGGCCACTCCCTC
TCTGCGCGCTCGCTCGCTCACTGAGGCCGGGCGACCAAAGGTCGCCCGACGCCCGG
GCTTTGCCCGGGCGGCCTCAGTGAGCGAGCGAGCGCGCAG

[0539] Exemplary rAAV-aflibercept-PC Construct Sequence (SEQ ID NO: 95)

CTGCGCGCTCGCTCGCTCACTGAGGCCGCCCGGGCGTCGGGCGACCTTTGGTCGCCC
GGCCTCAGTGAGCGAGCGAGCGCGCAGAGAGGGAGTGGCCAACTCCATCACTAGGG
GTTCCTGCGGCCGCACGCGTGACATTGATTATTGACTAGTTATTAATAGTAATCAAT
TACGGGGTCATTAGTTCATAGCCCATATATGGAGTTCCGCGTTACATAACTTACGGT
AAATGGCCCGCCTGGCTGACCGCCCAACGACCCCCGCCCATTGACGTCAATAATGA
CGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGACT
ATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGC
CCCCTATTGACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCCAGTACATGA
CCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCAT
GGGTCGAGGTGAGCCCCACGTTCTGCTTCACTCTCCCCATCTCCCCCCCCTCCCCACC
CCCAATTTTGTATTTATTTATTTTTTAATTATTTTGTGCAGCGATGGGGGCGGGGGGG
GGGGGGGCGCGCGCCAGGCGGGGCGGGGCGGGGCGAGGGGCGGGGCGGGGCGAG
GCGGAGAGGTGCGGCGGCAGCCAATCAGAGCGGCGCGCTCCGAAAGTTTCCTTTTA
TGGCGAGGCGGCGGCGGCGGCGGCCCTATAAAAAGCGAAGCGCGCGGCGGGCGGG
AGTCGCTGCGTTGCCTTCGCCCCGTGCCCCGCTCCGCGCCGCCTCGCGCCGCCCGCC
CCGGCTCTGACTGACCGCGTTACTCCCACAGGTGAGCGGGCGGGACGGCCCTTCTCC
TCCGGGCTGTAATTAGCGCTTGGTTTAATGACGGCTCGTTTCTTTTCTGTGGCTGCGT
GAAAGCCTTAAAGGGCTCCGGGAGGGCCCTTTGTGCGGGGGGGAGCGGCTCGGGGG
GTGCGTGCGTGTGTGTGTGCGTGGGGAGCGCCGCGTGCGGCCCGCGCTGCCCGGCG
GCTGTGAGCGCTGCGGGCGCGGCGCGGGGCTTTGTGCGCTCCGCGTGTGCGCGAGG
GGAGCGCGGCCGGGGGCGGTGCCCCGCGGTGCGGGGGGGGCTGCGAGGGGAACAAA
GGCTGCGTGCGGGGTGTGTGCGTGGGGGGGTGAGCAGGGGGTGTGGGCGCGGCGGT
CGGGCTGTAACCCCCCCCTGCACCCCCCTCCCCGAGTTGCTGAGCACGGCCCGGCTT
CGGGTGCGGGGCTCCGTGCGGGGCGTGGCGCGGGGCTCGCCGTGCCGGGCGGGGGG
TGGCGGCAGGTGGGGGTGCCGGGCGGGGCGGGGCCGCCTCGGGCCGGGGAGGGCT

CGGGGGAGGGGCGCGGCGGCCCCCGGAGCGCCGGCGGCTGTCGAGGCGCGGCGAG
CCGCAGCCATTGCCTTTTATGGTAATCGTGCGAGAGGGCGCAGGGACTTCCTTTGTC
CCAAATCTGTGCGGAGCCGAAATCTGGGAGGCGCCGCCGCACCCCCTCTAGCGGGC
GCGGGGCGAAGCGGTGCGGCGCCGGCAGGAAGGAAATGGGCGGGGAGGGCCTTCG
TGCGTCGCCGCGCCGCCGTCCCCTTCTCCCTCTCCAGCCTCGGGGCTGTCCGCGGGG
GGACGGCTGCCTTCGGGGGGGGACGGGGCAGGGCGGGGTTCGGCTTCTGGCGTGTGA
CCGGCGGCTCTAGAGCCTCTGCTAACCATGTTCATGCCTTCTTCTTTTTCCTACAGCT
CCTGGGCAACGTGCTGGTTATTGTGACCGGTGCCACCATGTACCGGATGCAGCTGCT
GAGCTGTATCGCCCTGTCTCTGGCCCTGGTCACCAATTCTAGCGATACCGGCAGACC
CTTCGTGGAAATGTACAGCGAGATCCCCGAGATCATCCACATGACCGAGGGCAGAG
AGCTGGTCATCCCCTGCAGAGTGACAAGCCCCAACATCACCGTGACTCTGAAGAAG
TTCCCTCTGGACACACTGATCCCCGACGGCAAGAGAATCATCTGGGACAGCCGGAA
GGGCTTCATCATCAGCAACGCCACCTACAAAGAGATCGGCCTGCTGACCTGTGAAG
CCACCGTGAATGGCCACCTGTACAAGACCAACTACCTGACACACAGACAGACCAAC
ACCATCATCGACGTGGTGCTGAGCCCTAGCCACGGCATTGAACTGTCTGTGGGCGAG
AAGCTGGTGCTGAACTGTACCGCCAGAACCGAGCTGAACGTGGGCATCGACTTCAA
CTGGGAGTACCCCAGCAGCAAGCACCAGCACAAGAAACTGGTCAACCGGGACCTGA
AAACCCAGAGCGGCAGCGAGATGAAGAAATTCCTGAGCACCCTGACCATCGACGGC
GTGACCAGATCTGACCAGGGCCTGTACACATGTGCCGCCAGCTCTGGCCTGATGACC
AAGAAAAACAGCACCTTCGTGCGGGTGCACGAGAAGGACAAGACCCACACCTGTCC
TCCATGTCCTGCTCCAGAACTGCTCGGCGGACCTTCCGTGTTCCTGTTTCCTCCAAAG
CCTAAGGACACCCTGATGATCAGCAGAACCCCTGAAGTGACCTGCGTGGTGGTGGA
TGTGTCCCACGAGGATCCCGAAGTGAAGTTCAATTGGTACGTGGACGGCGTGGAAG
TGCACAACGCCAAGACCAAGCCTAGAGAGGAACAGTACAATAGCACCTACAGAGTG
GTGTCCGTGCTGACCGTGCTGCACCAGGATTGGCTGAACGGCAAAGAGTACAAGTG
CAAGGTGTCCAACAAGGCCCTGCCTGCTCCTATCGAGAAAACCATCTCCAAGGCCA
AGGGCCAGCCTAGGGAACCCCAGGTTTACACACTGCCTCCAAGCAGGGACGAGCTG
ACAAAGAACCAGGTGTCCCTGACCTGCCTGGTCAAGGGCTTCTACCCTTCCGATATC
GCCGTGGAATGGGAGAGCAATGGCCAGCCTGAGAACAACTACAAGACAACCCCTCC
TGTGCTGGACAGCGACGGCTCATTCTTCCTGTACAGCAAGCTGACAGTGGACAAGA
GCAGATGGCAGCAGGGCAACGTGTTCAGCTGCAGCGTGATGCACGAGGCCCTGCAC

AACCACTACACCCAGAAGTCCCTGAGCCTGTCTCCTGGATAAGAGCTCGCTGATCAG
CCTCGACTGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGCCCCTCCCCCGTGCCTTC
CTTGACCCTGGAAGGTGCCACTCCCACTGTCCTTTCCTAATAAAATGAGGAAATTGC
ATCGCATTGTCTGAGTAGGTGTCATTCTATTCTGGGGGGTGGGGTGGGGCAGGACAG
CAAGGGGGAGGATTGGGAAGACAATAGCAGGCATGCTGGGGATGCGGTGGGCTCTA
TGGAAGCTTGAATTCAGCTGACGTGCCTCGGACCGCTAGGAACCCCTAGTGATGGA
GTTGGCCACTCCCTCTCTGCGCGCTCGCTCGCTCACTGAGGCCGGGCGACCAAAGGT
CGCCCGACGCCCGGGCTTTGCCCGGGCGGCCTCAGTGAGCGAGCGAGCGCGCAG

[0540] Exemplary rAAV-Aflibercept Construct Sequence (SEQ ID NO: 96)

TTGGCCACTCCCTCTCTGCGCGCTCGCTCGCTCACTGAGGCCGCCCGGGCAAAGCCC
GGGCGTCGGGCGACCTTTGGTCGCCCGGCCTCAGTGAGCGAGCGAGCGCGCAGAGA
GGGAGTGGCCAACTCCATCACTAGGGGTTCCTTTGTCGACGCGGCCGCACGCGTGAC
ATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCC
ATATATGGAGTTCCGCGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCC
CAACGACCCCCGCCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAAT
AGGGACTTTCCATTGACGTCAATGGGTGGACTATTTACGGTAAACTGCCCACTTGGC
AGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAA
ATGGCCCGCCTGGCATTATGCCCAGTACATGACCTTATGGGACTTTCCTACTTGGCA
GTACATCTACGTATTAGTCATCGCTATTACCATGGGTCGAGGTGAGCCCCACGTTCT
GCTTCACTCTCCCCATCTCCCCCCCCTCCCCACCCCCAATTTTGTATTTATTTATTTTT
TAATTATTTTGTGCAGCGATGGGGGCGGGGGGGGGGGGGGGCGCGCGCCAGGCGG
GGCGGGGCGGGGCGAGGGGCGGGGCGGGGCGAGGCGGAGAGGTGCGGCGGCAGCC
AATCAGAGCGGCGCGCTCCGAAAGTTTCCTTTTATGGCGAGGCGGCGGCGGCGGCG
GCCCTATAAAAAGCGAAGCGCGCGGCGGGCGGGAGTCGCTGCGTTGCCTTCGCCCC
GTGCCCCGCTCCGCGCCGCCTCGCGCCGCCCGCCCCGGCTCTGACTGACCGCGTTAC
TCCCACAGGTGAGCGGGCGGGACGGCCCTTCTCCTCCGGGCTGTAATTAGCGCTTGG
TTTAATGACGGCTCGTTTCTTTTCTGTGGCTGCGTGAAAGCCTTAAAGGGCTCCGGG
AGGGCCCTTTGTGCGGGGGGGAGCGGCTCGGGGGGTGCGTGCGTGTGTGTGTGCGT
GGGGAGCGCCGCGTGCGGCCCGCGCTGCCCGGCGGCTGTGAGCGCTGCGGGCGCGG
CGCGGGGCTTTGTGCGCTCCGCGTGTGCGCGAGGGGAGCGCGGCCGGGGGCGGTGC
CCCGCGGTGCGGGGGGGGCTGCGAGGGGAACAAAGGCTGCGTGCGGGGTGTGTGCGT

GGGGGGGTGAGCAGGGGGTGTGGGCGCGGCGGTCGGGCTGTAACCCCCCCCTGCAC

CCCCCTCCCCGAGTTGCTGAGCACGGCCCGGCTTCGGGTGCGGGGCTCCGTGCGGGG

CGTGGCGCGGGGCTCGCCGTGCCGGGCGGGGGGTGGCGGCAGGTGGGGGTGCCGG

GCGGGGCGGGGCCGCCTCGGGCCGGGGAGGGCTCGGGGGAGGGGCGCGGCGGCCC

CCGGAGCGCCGGCGGCTGTCGAGGCGCGGCGAGCCGCAGCCATTGCCTTTTATGGT

AATCGTGCGAGAGGGCGCAGGGACTTCCTTTGTCCCAAATCTGTGCGGAGCCGAAA

TCTGGGAGGCGCCGCCGCACCCCCTCTAGCGGGCGCGGGGCGAAGCGGTGCGGCGC

CGGCAGGAAGGAAATGGGCGGGGAGGGCCTTCGTGCGTCGCCGCGCCGCCGTCCCC

TTCTCCCTCTCCAGCCTCGGGGCTGTCCGCGGGGGGGACGGCTGCCTTCGGGGGGGAC

GGGGCAGGGCGGGGTTCGGCTTCTGGCGTGTGACCGGCGGCTCTAGAGCCTCTGCTA

ACCATGTTCATGCCTTCTTCTTTTTCCTACAGCTCCTGGGCAACGTGCTGGTTATTGT

GACCGGTGCCACCATGTACCGGATGCAGCTGCTGAGCTGTATCGCCCTGTCTCTGGC

CCTGGTCACCAATTCTAGCGATACCGGCAGACCCTTCGTGGAAATGTACAGCGAGAT

CCCCGAGATCATCCACATGACCGAGGGCAGAGAGCTGGTCATCCCCTGCAGAGTGA

CAAGCCCCAACATCACCGTGACTCTGAAGAAGTTCCCTCTGGACACACTGATCCCCG

ACGGCAAGAGAATCATCTGGGACAGCCGGAAGGGCTTCATCATCAGCAACGCCACC

TACAAAGAGATCGGCCTGCTGACCTGTGAAGCCACCGTGAATGGCCACCTGTACAA

GACCAACTACCTGACACACAGACAGACCAACACCATCATCGACGTGGTGCTGAGCC

CTAGCCACGGCATTGAACTGTCTGTGGGCGAGAAGCTGGTGCTGAACTGTACCGCCA

GAACCGAGCTGAACGTGGGCATCGACTTCAACTGGGAGTACCCCAGCAGCAAGCAC

CAGCACAAGAAACTGGTCAACCGGGACCTGAAAACCCAGAGCGGCAGCGAGATGA

AGAAATTCCTGAGCACCCTGACCATCGACGGCGTGACCAGATCTGACCAGGGCCTG

TACACATGTGCCGCCAGCTCTGGCCTGATGACCAAGAAAAACAGCACCTTCGTGCG

GGTGCACGAGAAGGACAAGACCCACACCTGTCCTCCATGTCCTGCTCCAGAACTGCT

CGGCGGACCTTCCGTGTTCCTGTTTCCTCCAAAGCCTAAGGACACCCTGATGATCAG

CAGAACCCCTGAAGTGACCTGCGTGGTGGTGGATGTGTCCCACGAGGATCCCGAAG

TGAAGTTCAATTGGTACGTGGACGGCGTGGAAGTGCACAACGCCAAGACCAAGCCT

AGAGAGGAACAGTACAATAGCACCTACAGAGTGGTGTCCGTGCTGACCGTGCTGCA

CCAGGATTGGCTGAACGGCAAAGAGTACAAGTGCAAGGTGTCCAACAAGGCCCTGC

CTGCTCCTATCGAGAAAACCATCTCCAAGGCCAAGGGCCAGCCTAGGGAACCCCAG

GTTTACACACTGCCTCCAAGCAGGGACGAGCTGACAAAGAACCAGGTGTCCCTGAC

CTGCCTGGTCAAGGGCTTCTACCCTTCCGATATCGCCGTGGAATGGGAGAGCAATGG
CCAGCCTGAGAACAACTACAAGACAACCCCTCCTGTGCTGGACAGCGACGGCTCAT
TCTTCCTGTACAGCAAGCTGACAGTGGACAAGAGCAGATGGCAGCAGGGCAACGTG
TTCAGCTGCAGCGTGATGCACGAGGCCCTGCACAACCACTACACCCAGAAGTCCCT
GAGCCTGTCTCCTGGATAAGAGCTCGCTGATCAGCCTCGACTGTGCCTTCTAGTTGC
CAGCCATCTGTTGTTTGCCCCTCCCCCGTGCCTTCCTTGACCCTGGAAGGTGCCACTC
CCACTGTCCTTTCCTAATAAAATGAGGAAATTGCATCGCATTGTCTGAGTAGGTGTC
ATTCTATTCTGGGGGGTGGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAGAC
AATAGCAGGCATGCTGGGGATGCGGTGGGCTCTATGGAAGCTTGAATTCAGCTGAC
GTGCCTCGGACCGCCTAGGAGGAACCCCTAGTGATGGAGTTGGCCACTCCCTCTCTG
CGCGCTCGCTCGCTCACTGAGGCCGGGCGACCAAAGGTCGCCCGACGCCCGGGCTT
TGCCCGGGCGGCCTCAGTGAGCGAGCGAGCGCGCAGAGAGGGAGTGGCCAA

Multiple AAV Construct Compositions

[0541] The present disclosure recognizes that some coding sequences encoding a protein (e.g., an anti-VEGF protein) may be delivered by dividing the coding sequence into multiple portions, which are each included in a different construct. In some embodiments, provided herein are compositions or systems comprising at least two different constructs (e.g., two, three, four, five, or six). In some embodiments, each of the at least two different constructs comprises a coding sequence that encodes a different portion of a coding region (e.g., encoding a target protein, e.g., an inner ear target protein, e.g., an anti-VEGF protein), each of the encoded portions being at least 10 amino acids (e.g., at least about 10 amino acids, at least about 20 amino acids, at least about 30 amino acids, at least about 60 amino acids, at least about 70 amino acids, at least about 80 amino acids, at least about 90 amino acids, at least about 100 amino acids, at least about 110 amino acids, at least about 120 amino acids, at least about 130 amino acids, at least about 140 amino acids, at least about 150 amino acids, at least about 160 amino acids, at least about 170 amino acids, at least about 180 amino acids, at least about 190 amino acids, at least about 200 amino acids, at least about 210 amino acids, at least about 220 amino acids, at least about 230 amino acids, at least about 240 amino acids, at least about 250 amino acids, at least about 260 amino acids, at least about 270 amino acids, at least about 280 amino acids, at least about 290 amino acids, at least about 300 amino acids, at least about 310 amino acids, at least about 320 amino acids, at least about 330 amino acids, at least about 340 amino acids, at least about 350 amino acids, at least

about 360 amino acids, at least about 370 amino acids, at least about 380 amino acids, at least about 390 amino acids, at least about 400 amino acids, at least about 410 amino acids, at least about 420 amino acids, at least about 430 amino acids, at least about 440 amino acids, at least about 450 amino acids, at least about 460 amino acids, at least about 470 amino acids, at least about 480 amino acids, at least about 490 amino acids, at least about 500 amino acids, at least about 510 amino acids, at least about 520 amino acids, at least about 530 amino acids, at least about 540 amino acids, at least about 550 amino acids, at least about 560 amino acids, at least about 570 amino acids, at least about 580 amino acids, at least about 590 amino acids, at least about 600 amino acids, at least about 610 amino acids, at least about 620 amino acids, at least about 630 amino acids, at least about 640 amino acids, at least about 650 amino acids, at least about 660 amino acids, at least about 670 amino acids, at least about 680 amino acids, at least about 690 amino acids, at least about 700 amino acids, at least about 710 amino acids, at least about 720 amino acids, at least about 730 amino acids, at least about 740 amino acids, at least about 750 amino acids, at least about 760 amino acids, at least about 770 amino acids, at least about 780 amino acids, at least about 790 amino acids, at least about 800 amino acids, at least about 810 amino acids, or at least about 820 amino acids) where the amino acid sequence of each of the encoded portions may optionally partially overlap with the amino acid sequence of a different one of the encoded portions; no single construct of the at least two different constructs encodes the active target protein; and, when introduced into a subject cell (e.g., an animal cell, e.g., a primate cell, e.g., a human cell), the at least two different may undergo homologous recombination with each other, where the recombined nucleic acid encodes an active target protein (e.g., an anti-VEGF protein). In some embodiments, one of the nucleic acid constructs can include a coding sequence that encodes a portion of a target protein (e.g., an anti-VEGF protein), where the encoded portion is at most about 820 amino acids (e.g., at most about 10 amino acids, at most about 20 amino acids, at most about 30 amino acids, at most about 60 amino acids, at most about 70 amino acids, at most about 80 amino acids, at most about 90 amino acids, at most about 100 amino acids, at most about 110 amino acids, at most about 120 amino acids, at most about 130 amino acids, at most about 140 amino acids, at most about 150 amino acids, at most about 160 amino acids, at most about 170 amino acids, at most about 180 amino acids, at most about 190 amino acids, at most about 200 amino acids, at most about 210 amino acids, at most about 220 amino acids, at most about 230 amino acids, at most about 240 amino acids, at most about 250

amino acids, at most about 260 amino acids, at most about 270 amino acids, at most about 280 amino acids, at most about 290 amino acids, at most about 300 amino acids, at most about 310 amino acids, at most about 320 amino acids, at most about 330 amino acids, at most about 340 amino acids, at most about 350 amino acids, at most about 360 amino acids, at most about 370 amino acids, at most about 380 amino acids, at most about 390 amino acids, at most about 400 amino acids, at most about 410 amino acids, at most about 420 amino acids, at most about 430 amino acids, at most about 440 amino acids, at most about 450 amino acids, at most about 460 amino acids, at most about 470 amino acids, at most about 480 amino acids, at most about 490 amino acids, at most about 500 amino acids, at most about 510 amino acids, at most about 520 amino acids, at most about 530 amino acids, at most about 540 amino acids, at most about 550 amino acids, at most about 560 amino acids, at most about 570 amino acids, at most about 580 amino acids, at most about 590 amino acids, at most about 600 amino acids, at most about 610 amino acids, at most about 620 amino acids, at most about 630 amino acids, at most about 640 amino acids, at most about 650 amino acids, at most about 660 amino acids, at most about 670 amino acids, at most about 680 amino acids, at most about 690 amino acids, at most about 700 amino acids, at most about 710 amino acids, at most about 720 amino acids, at most about 730 amino acids, at most about 740 amino acids, at most about 750 amino acids, at most about 760 amino acids, at most about 770 amino acids, at most about 780 amino acids, at most about 790 amino acids, at most about 800 amino acids, at most about 810 amino acids, or at most about 820 amino acids).

[0542] In some embodiments, an amino acid sequence of an encoded portion of each of the constructs does not overlap, even in part, with an amino acid sequence of a different one of the encoded portions. In some embodiments, an amino acid sequence of an encoded portion of a construct partially overlaps with an amino acid sequence of an encoded portion of a different construct. In some embodiments, an amino acid sequence of an encoded portion of each construct partially overlaps with an amino acid sequence of an encoded portion of at least one different construct. In some embodiments, an overlapping amino acid sequence is between about 10 amino acid residues to about 820 amino acids, or any of the subranges of this range (e.g., about 10 amino acids, about 20 amino acids, about 30 amino acids, about 60 amino acids, about 70 amino acids, about 80 amino acids, about 90 amino acids, about 100 amino acids, about 110 amino acids, about 120 amino acids, about 130 amino acids, about 140 amino acids, about 150 amino acids, about 160

amino acids, about 170 amino acids, about 180 amino acids, about 190 amino acids, about 200 amino acids, about 210 amino acids, about 220 amino acids, about 230 amino acids, about 240 amino acids, about 250 amino acids, about 260 amino acids, about 270 amino acids, about 280 amino acids, about 290 amino acids, about 300 amino acids, about 310 amino acids, about 320 amino acids, about 330 amino acids, about 340 amino acids, about 350 amino acids, about 360 amino acids, about 370 amino acids, about 380 amino acids, about 390 amino acids, about 400 amino acids, about 410 amino acids, about 420 amino acids, about 430 amino acids, about 440 amino acids, about 450 amino acids, about 460 amino acids, about 470 amino acids, about 480 amino acids, about 490 amino acids, about 500 amino acids, about 510 amino acids, about 520 amino acids, about 530 amino acids, about 540 amino acids, about 550 amino acids, about 560 amino acids, about 570 amino acids, about 580 amino acids, about 590 amino acids, about 600 amino acids, about 610 amino acids, about 620 amino acids, about 630 amino acids, about 640 amino acids, about 650 amino acids, about 660 amino acids, about 670 amino acids, about 680 amino acids, about 690 amino acids, about 700 amino acids, about 710 amino acids, about 720 amino acids, about 730 amino acids, about 740 amino acids, about 750 amino acids, about 760 amino acids, about 770 amino acids, about 780 amino acids, about 790 amino acids, about 800 amino acids, about 810 amino acids, or about 820 amino acids in length).

[0543] In some examples, a desired gene product (e.g., a therapeutic gene product) is encoded by at least two different constructs. In some embodiments, each of at least two different constructs comprises a different segment of a potentially coding region, wherein the coding region may or may not comprise non-coding sequences such as introns and/or regulatory regions. In some embodiments, the at least two different constructs transduce the same cell. In some embodiments, the transduced cell transcribes and translates the two distinct constructs. In some embodiments, the translated polypeptides may then combine at the tertiary and/or quaternary structure level to create a functional anti-VEGF protein. For example, in some embodiments, a ranibizumab light chain, and a ranibizumab heavy chain, are encoded on at least two different constructs, and following translation combine to form a ranibizumab protein in the manner described herein and known in the art.

[0544] In some embodiments, compositions described herein comprising an anti-VEGF gene include naturally occurring and/or synthetic intron sequences. An intron may include a nucleotide sequence of an intron that is present in an endogenous genomic DNA sequence (e.g., an inner ear

cell target genomic DNA (e.g., a VEGF and/or VEGF-R genomic DNA sequence). In some embodiments, different intron segments overlap. In some embodiments, different intron segments overlap in sequence by at most about 12,000 nucleotides (e.g., at most about 100 nucleotides, at most about 200 nucleotides, at most about 300 nucleotides, at most about 600 nucleotides, at most about 700 nucleotides, at most about 800 nucleotides, at most about 900 nucleotides, at most about 1,000 nucleotides, at most about 1,100 nucleotides, at most about 1,200 nucleotides, at most about 1,300 nucleotides, at most about 1,400 nucleotides, at most about 1,500 nucleotides, at most about 1,600 nucleotides, at most about 1,700 nucleotides, at most about 1,800 nucleotides, at most about 1,900 nucleotides, at most about 2,000 nucleotides, at most about 2,100 nucleotides, at most about 2,200 nucleotides, at most about 2,300 nucleotides, at most about 2,400 nucleotides, at most about 2,500 nucleotides, at most about 2,600 nucleotides, at most about 2,700 nucleotides, at most about 2,800 nucleotides, at most about 2,900 nucleotides, at most about 3,000 nucleotides, at most about 3,100 nucleotides, at most about 3,200 nucleotides, at most about 3,300 nucleotides, at most about 3,400 nucleotides, at most about 3,500 nucleotides, at most about 3,600 nucleotides, at most about 3,700 nucleotides, at most about 3,800 nucleotides, at most about 3,900 nucleotides, at most about 4,000 nucleotides, at most about 4,100 nucleotides, at most about 4,200 nucleotides, at most about 4,300 nucleotides, at most about 4,400 nucleotides, at most about 4,500 nucleotides, at most about 4,600 nucleotides, at most about 4,700 nucleotides, at most about 4,800 nucleotides, at most about 4,900 nucleotides, at most about 5,000 nucleotides, at most about 5,100 nucleotides, at most about 5,200 nucleotides, at most about 5,300 nucleotides, at most about 5,400 nucleotides, at most about 5,500 nucleotides, at most about 5,600 nucleotides, at most about 5,700 nucleotides, at most about 5,800 nucleotides, at most about 5,900 nucleotides, at most about 6,000 nucleotides, at most about 6,100 nucleotides, at most about 6,200 nucleotides, at most about 6,300 nucleotides, at most about 6,400 nucleotides, at most about 6,500 nucleotides, at most about 6,600 nucleotides, at most about 6,700 nucleotides, at most about 6,800 nucleotides, at most about 6,900 nucleotides, at most about 7,000 nucleotides, at most about 7,100 nucleotides, at most about 7,200 nucleotides, at most about 7,300 nucleotides, at most about 7,400 nucleotides, at most about 7,500 nucleotides, at most about 7,600 nucleotides, at most about 7,700 nucleotides, at most about 7,800 nucleotides, at most about 7,900 nucleotides, at most about 8,000 nucleotides, at most about 8,100 nucleotides, at most about 8,200 nucleotides, at most about 8,300 nucleotides, at most about 8,400 nucleotides, at most about 8,500 nucleotides, at most about 8,600 nucleotides, at most about 8,700 nucleotides, at most about

8,800 nucleotides, at most about 8,900 nucleotides, at most about 9,000 nucleotides, at most about 9,100 nucleotides, at most about 9,200 nucleotides, at most about 9,300 nucleotides, at most about 9,400 nucleotides, at most about 9,500 nucleotides, at most about 9,600 nucleotides, at most about 9,700 nucleotides, at most about 9,800 nucleotides, at most about 9,900 nucleotides, at most about 10,000 nucleotides, at most about 10,100 nucleotides, at most about 10,200 nucleotides, at most about 10,300 nucleotides, at most about 10,400 nucleotides, at most about 10,500 nucleotides, at most about 10,600 nucleotides, at most about 10,700 nucleotides, at most about 10,800 nucleotides, at most about 10,900 nucleotides, at most about 11,000 nucleotides, at most about 11,100 nucleotides, at most about 11,200 nucleotides, at most about 11,300 nucleotides, at most about 11,400 nucleotides, at most about 11,500 nucleotides, at most about 11,600 nucleotides, at most about 11,700 nucleotides, at most about 11,800 nucleotides, at most about 11,900 nucleotides, or at most about 12,000 nucleotides) in length. In some embodiments, the overlapping nucleotide sequence in any two of the different constructs can include part or all of one or more exons of a target gene (e.g., anti-VEGF gene).

[0545] In some embodiments, a composition or system is or comprises two, three, four, or five different constructs. In compositions where the number of different constructs in the composition is two, the first of the two different constructs can include a coding sequence that encodes an N-terminal portion of a protein (e.g., an anti-VEGF protein), which may be referred to as a lead portion, a first construct, or a 5` portion (e.g., one of at least two antibody chains, e.g., a sequence encoding a heavy chain variable region and/or a light chain variable region). In some examples, an N-terminal portion of the target gene is at least about 10 amino acids (e.g., at least about 10 amino acids, at least about 20 amino acids, at least about 30 amino acids, at least about 60 amino acids, at least about 70 amino acids, at least about 80 amino acids, at least about 90 amino acids, at least about 100 amino acids, at least about 110 amino acids, at least about 120 amino acids, at least about 130 amino acids, at least about 140 amino acids, at least about 150 amino acids, at least about 160 amino acids, at least about 170 amino acids, at least about 180 amino acids, at least about 190 amino acids, at least about 200 amino acids, at least about 210 amino acids, at least about 220 amino acids, at least about 230 amino acids, at least about 240 amino acids, at least about 250 amino acids, at least about 260 amino acids, at least about 270 amino acids, at least about 280 amino acids, at least about 290 amino acids, at least about 300 amino acids, at least about 310 amino acids, at least about 320 amino acids, at least about 330 amino acids, at least

about 340 amino acids, at least about 350 amino acids, at least about 360 amino acids, at least about 370 amino acids, at least about 380 amino acids, at least about 390 amino acids, at least about 400 amino acids, at least about 410 amino acids, at least about 420 amino acids, at least about 430 amino acids, at least about 440 amino acids, at least about 450 amino acids, at least about 460 amino acids, at least about 470 amino acids, at least about 480 amino acids, at least about 490 amino acids, at least about 500 amino acids, at least about 510 amino acids, at least about 520 amino acids, at least about 530 amino acids, at least about 540 amino acids, at least about 550 amino acids, at least about 560 amino acids, at least about 570 amino acids, at least about 580 amino acids, at least about 590 amino acids, at least about 600 amino acids, at least about 610 amino acids, at least about 620 amino acids, at least about 630 amino acids, at least about 640 amino acids, at least about 650 amino acids, at least about 660 amino acids, at least about 670 amino acids, at least about 680 amino acids, at least about 690 amino acids, at least about 700 amino acids, at least about 710 amino acids, at least about 720 amino acids, at least about 730 amino acids, at least about 740 amino acids, at least about 750 amino acids, at least about 760 amino acids, at least about 770 amino acids, at least about 780 amino acids, at least about 790 amino acids, at least about 800 amino acids, at least about 810 amino acids, or at least about 820 amino acids) in length. In some examples, a first construct comprises one or both of a promoter (e.g., any of the promoters described herein or known in the art) and a Kozak sequence (e.g., any of the exemplary Kozak sequences described herein or known in the art). In some examples, a first construct comprises a promoter that is an inducible promoter, a constitutive promoter, or a tissue-specific promoter. In some examples, a second of the two different constructs comprises a coding sequence that encodes a C-terminal portion of the protein, which may be referred to as a terminal portion, a second construct, or a 3` portion (e.g., one of at least two antibody chains, e.g., a sequence encoding a heavy chain variable region and/or a light chain variable region). In some examples, a C-terminal portion of the target protein is at least about 10 amino acids (e.g., at least about 10 amino acids, at least about 20 amino acids, at least about 30 amino acids, at least about 60 amino acids, at least about 70 amino acids, at least about 80 amino acids, at least about 90 amino acids, at least about 100 amino acids, at least about 110 amino acids, at least about 120 amino acids, at least about 130 amino acids, at least about 140 amino acids, at least about 150 amino acids, at least about 160 amino acids, at least about 170 amino acids, at least about 180 amino acids, at least about 190 amino acids, at least about 200 amino acids, at least

about 210 amino acids, at least about 220 amino acids, at least about 230 amino acids, at least about 240 amino acids, at least about 250 amino acids, at least about 260 amino acids, at least about 270 amino acids, at least about 280 amino acids, at least about 290 amino acids, at least about 300 amino acids, at least about 310 amino acids, at least about 320 amino acids, at least about 330 amino acids, at least about 340 amino acids, at least about 350 amino acids, at least about 360 amino acids, at least about 370 amino acids, at least about 380 amino acids, at least about 390 amino acids, at least about 400 amino acids, at least about 410 amino acids, at least about 420 amino acids, at least about 430 amino acids, at least about 440 amino acids, at least about 450 amino acids, at least about 460 amino acids, at least about 470 amino acids, at least about 480 amino acids, at least about 490 amino acids, at least about 500 amino acids, at least about 510 amino acids, at least about 520 amino acids, at least about 530 amino acids, at least about 540 amino acids, at least about 550 amino acids, at least about 560 amino acids, at least about 570 amino acids, at least about 580 amino acids, at least about 590 amino acids, at least about 600 amino acids, at least about 610 amino acids, at least about 620 amino acids, at least about 630 amino acids, at least about 640 amino acids, at least about 650 amino acids, at least about 660 amino acids, at least about 670 amino acids, at least about 680 amino acids, at least about 690 amino acids, at least about 700 amino acids, at least about 710 amino acids, at least about 720 amino acids, at least about 730 amino acids, at least about 740 amino acids, at least about 750 amino acids, at least about 760 amino acids, at least about 770 amino acids, at least about 780 amino acids, at least about 790 amino acids, at least about 800 amino acids, at least about 810 amino acids, or at least about 820 amino acids) in length. In some examples, a second construct further comprises a poly(A) sequence.

[0546] In some examples where the number of different constructs in the composition is two, an N-terminal portion encoded by one of the two constructs can include a portion including amino acid position 1 to about amino acid position 820, or any subrange of this range (e.g., amino acid 1 to at least about amino acid 10, amino acid 1 to at least about amino acid 20, amino acid 1 to at least about amino acid 30, amino acid 1 to at least about amino acid 60, amino acid 1 to at least about amino acid 70, amino acid 1 to at least about amino acid 80, amino acid 1 to at least about amino acid 90, amino acid 1 to at least about amino acid 100, amino acid 1 to at least about amino acid 110, amino acid 1 to at least about amino acid 120, amino acid 1 to at least about amino acid 130, amino acid 1 to at least about amino acid 140, amino acid 1 to at least about amino acid 150,

amino acid 1 to at least about amino acid 160, amino acid 1 to at least about amino acid 170, amino acid 1 to at least about amino acid 180, amino acid 1 to at least about amino acid 190, amino acid 1 to at least about amino acid 200, amino acid 1 to at least about amino acid 210, amino acid 1 to at least about amino acid 220, amino acid 1 to at least about amino acid 230, amino acid 1 to at least about amino acid 240, amino acid 1 to at least about amino acid 250, amino acid 1 to at least about amino acid 260, amino acid 1 to at least about amino acid 270, amino acid 1 to at least about amino acid 280, amino acid 1 to at least about amino acid 290, amino acid 1 to at least about amino acid 300, amino acid 1 to at least about amino acid 310, amino acid 1 to at least about amino acid 320, amino acid 1 to at least about amino acid 330, amino acid 1 to at least about amino acid 340, amino acid 1 to at least about amino acid 350, amino acid 1 to at least about amino acid 360, amino acid 1 to at least about amino acid 370, amino acid 1 to at least about amino acid 380, amino acid 1 to at least about amino acid 390, amino acid 1 to at least about amino acid 400, amino acid 1 to at least about amino acid 410, amino acid 1 to at least about amino acid 420, amino acid 1 to at least about amino acid 430, amino acid 1 to at least about amino acid 440, amino acid 1 to at least about amino acid 450, amino acid 1 to at least about amino acid 460, amino acid 1 to at least about amino acid 470, amino acid 1 to at least about amino acid 480, amino acid 1 to at least about amino acid 490, amino acid 1 to at least about amino acid 500, amino acid 1 to at least about amino acid 510, amino acid 1 to at least about amino acid 520, amino acid 1 to at least about amino acid 530, amino acid 1 to at least about amino acid 540, amino acid 1 to at least about amino acid 550, amino acid 1 to at least about amino acid 560, amino acid 1 to at least about amino acid 570, amino acid 1 to at least about amino acid 580, amino acid 1 to at least about amino acid 590, amino acid 1 to at least about amino acid 600, amino acid 1 to at least about amino acid 610, amino acid 1 to at least about amino acid 620, amino acid 1 to at least about amino acid 630, amino acid 1 to at least about amino acid 640, amino acid 1 to at least about amino acid 650, amino acid 1 to at least about amino acid 660, amino acid 1 to at least about amino acid 670, amino acid 1 to at least about amino acid 680, amino acid 1 to at least about amino acid 690, amino acid 1 to at least about amino acid 700, amino acid 1 to at least about amino acid 710, amino acid 1 to at least about amino acid 720, amino acid 1 to at least about amino acid 730, amino acid 1 to at least about amino acid 740, amino acid 1 to at least about amino acid 750, amino acid 1 to at least about amino acid 760, amino acid 1 to at least about amino acid 770, amino acid 1 to at least about amino acid 780, amino acid 1 to at least about amino acid 790, amino acid 1 to at least about amino acid 800, amino acid 1 to at

least about amino acid 810, or amino acid 1 to at least about amino acid 820) of an anti-VEGF protein (e.g., SEQ ID NOs: 16, 17, 18, 20, 21, 23, and/or 43). In some examples where the number of different constructs in the composition is two, an N-terminal portion of the precursor anti-VEGF protein can include a portion including at most amino acid position 1 to amino acid position 820 or any subrange of this range (e.g., amino acid 1 to at most about amino acid 10, amino acid 1 to at most about amino acid 20, amino acid 1 to at most about amino acid 30, amino acid 1 to at most about amino acid 60, amino acid 1 to at most about amino acid 70, amino acid 1 to at most about amino acid 80, amino acid 1 to at most about amino acid 90, amino acid 1 to at most about amino acid 100, amino acid 1 to at most about amino acid 110, amino acid 1 to at most about amino acid 120, amino acid 1 to at most about amino acid 130, amino acid 1 to at most about amino acid 140, amino acid 1 to at most about amino acid 150, amino acid 1 to at most about amino acid 160, amino acid 1 to at most about amino acid 170, amino acid 1 to at most about amino acid 180, amino acid 1 to at most about amino acid 190, amino acid 1 to at most about amino acid 200, amino acid 1 to at most about amino acid 210, amino acid 1 to at most about amino acid 220, amino acid 1 to at most about amino acid 230, amino acid 1 to at most about amino acid 240, amino acid 1 to at most about amino acid 250, amino acid 1 to at most about amino acid 260, amino acid 1 to at most about amino acid 270, amino acid 1 to at most about amino acid 280, amino acid 1 to at most about amino acid 290, amino acid 1 to at most about amino acid 300, amino acid 1 to at most about amino acid 310, amino acid 1 to at most about amino acid 320, amino acid 1 to at most about amino acid 330, amino acid 1 to at most about amino acid 340, amino acid 1 to at most about amino acid 350, amino acid 1 to at most about amino acid 360, amino acid 1 to at most about amino acid 370, amino acid 1 to at most about amino acid 380, amino acid 1 to at most about amino acid 390, amino acid 1 to at most about amino acid 400, amino acid 1 to at most about amino acid 410, amino acid 1 to at most about amino acid 420, amino acid 1 to at most about amino acid 430, amino acid 1 to at most about amino acid 440, amino acid 1 to at most about amino acid 450, amino acid 1 to at most about amino acid 460, amino acid 1 to at most about amino acid 470, amino acid 1 to at most about amino acid 480, amino acid 1 to at most about amino acid 490, amino acid 1 to at most about amino acid 500, amino acid 1 to at most about amino acid 510, amino acid 1 to at most about amino acid 520, amino acid 1 to at most about amino acid 530, amino acid 1 to at most about amino acid 540, amino acid 1 to at most about amino acid 550, amino acid 1 to at most about amino acid 560,

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[0547] In some examples where the number of different constructs in the composition is two, a C-terminal portion encoded by one of the two constructs can include a portion including the final amino acid (e.g., about amino acid position 820) to about amino acid position 1, or any subrange of this range (e.g., amino acid 820 to at least about amino acid 10, amino acid 820 to at least about amino acid 20, amino acid 820 to at least about amino acid 30, amino acid 820 to at least about amino acid 60, amino acid 820 to at least about amino acid 70, amino acid 820 to at least about amino acid 80, amino acid 820 to at least about amino acid 90, amino acid 820 to at least about amino acid 100, amino acid 820 to at least about amino acid 110, amino acid 820 to at least about amino acid 120, amino acid 820 to at least about amino acid 130, amino acid 820 to at least about amino acid 140, amino acid 820 to at least about amino acid 150, amino acid 820 to at least about amino acid 160, amino acid 820 to at least about amino acid 170, amino acid 820 to at least about amino acid 180, amino acid 820 to at least about amino acid 190, amino acid 820 to at least about amino acid 200, amino acid 820 to at least about amino acid 210, amino acid 820 to at least about amino acid 220, amino acid 820 to at least about amino acid 230, amino acid 820 to at least about amino acid 240, amino acid 820 to at least about amino acid 250, amino acid 820 to at least about amino acid 260, amino acid 820 to at least about amino acid 270, amino acid 820 to at least about amino acid 280, amino acid 820 to at least about amino acid 290, amino acid 820 to at least about

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amino acid 20, amino acid 820 to at most about amino acid 30, amino acid 820 to at most about amino acid 60, amino acid 820 to at most about amino acid 70, amino acid 820 to at most about amino acid 80, amino acid 820 to at most about amino acid 90, amino acid 820 to at most about amino acid 100, amino acid 820 to at most about amino acid 110, amino acid 820 to at most about amino acid 120, amino acid 820 to at most about amino acid 130, amino acid 820 to at most about amino acid 140, amino acid 820 to at most about amino acid 150, amino acid 820 to at most about amino acid 160, amino acid 820 to at most about amino acid 170, amino acid 820 to at most about amino acid 180, amino acid 820 to at most about amino acid 190, amino acid 820 to at most about amino acid 200, amino acid 820 to at most about amino acid 210, amino acid 820 to at most about amino acid 220, amino acid 820 to at most about amino acid 230, amino acid 820 to at most about amino acid 240, amino acid 820 to at most about amino acid 250, amino acid 820 to at most about amino acid 260, amino acid 820 to at most about amino acid 270, amino acid 820 to at most about amino acid 280, amino acid 820 to at most about amino acid 290, amino acid 820 to at most about amino acid 300, amino acid 820 to at most about amino acid 310, amino acid 820 to at most about amino acid 320, amino acid 820 to at most about amino acid 330, amino acid 820 to at most about amino acid 340, amino acid 820 to at most about amino acid 350, amino acid 820 to at most about amino acid 360, amino acid 820 to at most about amino acid 370, amino acid 820 to at most about amino acid 380, amino acid 820 to at most about amino acid 390, amino acid 820 to at most about amino acid 400, amino acid 820 to at most about amino acid 410, amino acid 820 to at most about amino acid 420, amino acid 820 to at most about amino acid 430, amino acid 820 to at most about amino acid 440, amino acid 820 to at most about amino acid 450, amino acid 820 to at most about amino acid 460, amino acid 820 to at most about amino acid 470, amino acid 820 to at most about amino acid 480, amino acid 820 to at most about amino acid 490, amino acid 820 to at most about amino acid 500, amino acid 820 to at most about amino acid 510, amino acid 820 to at most about amino acid 520, amino acid 820 to at most about amino acid 530, amino acid 820 to at most about amino acid 540, amino acid 820 to at most about amino acid 550, amino acid 820 to at most about amino acid 560, amino acid 820 to at most about amino acid 570, amino acid 820 to at most about amino acid 580, amino acid 820 to at most about amino acid 590, amino acid 820 to at most about amino acid 600, amino acid 820 to at most about amino acid 610, amino acid 820 to at most about amino acid 620, amino acid 820 to at most about amino acid 630, amino acid 820 to at most about amino acid 640, amino acid 820 to at most about amino acid 650, amino acid 820 to at most about

amino acid 660, amino acid 820 to at most about amino acid 670, amino acid 820 to at most about amino acid 680, amino acid 820 to at most about amino acid 690, amino acid 820 to at most about amino acid 700, amino acid 820 to at most about amino acid 710, amino acid 820 to at most about amino acid 720, amino acid 820 to at most about amino acid 730, amino acid 820 to at most about amino acid 740, amino acid 820 to at most about amino acid 750, amino acid 820 to at most about amino acid 760, amino acid 820 to at most about amino acid 770, amino acid 820 to at most about amino acid 780, amino acid 820 to at most about amino acid 790, amino acid 820 to at most about amino acid 800, amino acid 820 to at most about amino acid 810, or amino acid 820 to at most about amino acid 820, or any length sequence there between of an anti-VEGF protein (e.g., SEQ ID NO: 16, 17, 18, 20, 21, 23, and/or 43).

[0548] In some embodiments, splice sites are involved in trans-splicing. In some embodiments, a splice donor site (Trapani et al. EMBO Mol. Med. 6(2):194-211, 2014, which is incorporated herein in its entirety by reference) follows the coding sequence in the N-terminal construct. In the C-terminal construct, a splice acceptor site may be subcloned just before the coding sequence for a second portion of an anti-VEGF gene. In some embodiments, within the coding sequence, a silent mutation can be introduced, generating an additional site for restriction digestion.

[0549] In some embodiments, any of the constructs provided herein can be included in a composition suitable for administration to an animal for the amelioration of symptoms associated with an otological disease characterized by neovascularization (e.g., VS).

Pharmaceutical Compositions

[0550] In certain embodiments, a composition of the present disclosure relates to a separate diluent drug product (AAV diluent drug product), at or near equivalence in composition to an rAAV-antiVEGF formulation buffer. In certain embodiments, a diluent drug product will be manufactured, sterile filtered (0.2 μm filter), and aseptically filled into single-use vials. In certain embodiments, the formulation buffer comprises sterile water containing 1.5 mM mono-potassium phosphate, 8.1 mM sodium phosphate dibasic, 2.7 mM potassium chloride, 172 mM sodium chloride, and 0.001% Poloxamer 188. In certain embodiments, diluent drug product will be utilized to prepare the concentrations necessary for the doses described for use in a mammal of interest (e.g., a human in need thereof).

[0551] Among other things, the present disclosure provides pharmaceutical compositions. In some embodiments compositions provided herein are suitable for administration to an animal for the amelioration of symptoms associated with an otological disease characterized by neovascularization (e.g., VS).

[0552] In some embodiments, pharmaceutical compositions of the present disclosure may comprise, e.g., a polynucleotide, e.g., one or more constructs, as described herein. In some embodiments, 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.

[0553] In some embodiments, a pharmaceutical composition comprises one or more pharmaceutically or physiologically acceptable carriers, diluents or excipients. As used herein, the term "pharmaceutically acceptable carrier" comprises 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 embodiments, formulations are in a dosage forms, such as injectable solutions, injectable gels, drug-release capsules, and the like.

[0554] In some embodiments, compositions of the present disclosure are formulated for intravenous administration. In some embodiments compositions of the present disclosure are formulated for intra-cochlear administration. In some embodiments, a therapeutic composition is formulated to comprise a lipid nanoparticle, a polymeric nanoparticle, a mini-circle DNA and/or a CELiD DNA.

[0555] In some embodiments, a therapeutic composition is formulated to comprise a synthetic perilymph solution. For example, in some embodiments, a synthetic perilymph solution comprises 20-200mM NaCl; 1-5 mM KCl; 0.1-10mM $CaCl_2$; 1-10mM glucose; and 2-50 mM HEPES, with a pH between about 6 and about 9. In some embodiments, a therapeutic composition is formulated at appropriate dilutions in a physiologically acceptable solution (*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). In some embodiments, a therapeutic composition is formulated to comprise a physiologically suitable solution. For example, in some embodiments, 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 embodiments, alternative pluronic acids are utilized. In some embodiments, alternative ion concentrations are utilized.

[0556] In some embodiments, 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 embodiments, 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^{TM} (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).

[0557] In some embodiments, a composition comprises a pharmaceutically acceptable carrier (e.g., phosphate buffered saline, saline, or bacteriostatic water). Upon formulation, in some embodiments, solutions can be administered in a manner compatible with a dosage formulation

and in such amount as is therapeutically effective. In some embodiments, formulations are administered in a variety of dosage forms such as injectable solutions, injectable gels, drug-release capsules, and the like.

[0558] In some embodiments, a composition provided herein can be 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).

[0559] In some embodiments, a provided composition comprises one nucleic acid construct. In some embodiments, a provided composition comprises two or more different constructs. In some embodiments, a composition that include a single nucleic acid construct comprising a coding sequence that encodes an anti-VEGF protein and/or a functional characteristic portion thereof. In some embodiments, compositions comprise a single nucleic acid construct comprising a coding sequence that encodes an anti-VEGF protein 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. In some embodiments, a composition comprising at least two different constructs, e.g., constructs comprise coding sequences that encode a different portion of an anti-VEGF protein, the constructs can be combined to generate a sequence encoding an active anti-VEGF protein (e.g., a full-length ranibizumab, bevacizumab, or aflibercept protein) in a mammalian cell, and thereby treat associated VS and/or other otology associated diseases characterized by neovascularization and amenable to anti-VEGF protein treatment as described herein.

[0560] Also provided are kits including any of the compositions described herein. In some embodiments, a kit can include a solid composition (e.g., a lyophilized composition including the at least two different constructs described herein) and a liquid for solubilizing the lyophilized composition. In some embodiments, a kit can include a pre-loaded syringe including any of the compositions described herein.

[0561] In some embodiments, a kit comprises a vial comprising any of the compositions described herein (e.g., formulated as an aqueous composition, e.g., an aqueous pharmaceutical composition). In some embodiments, a kit can include instructions for performing any of the methods described herein.

Genetically Modified Cells

[0562] 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 comprises any of the nucleic acids, constructs or compositions described herein. In some embodiments, an animal cell is a human cell (e.g., a human supporting cell or a human hair cell). In other embodiments, 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.

[0563] In some embodiments, 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.

[0564] In some embodiments, an animal cell is a specialized cell of the cochlea. In some embodiments, an animal cell is a hair cell. In some embodiments, an animal cell is a cochlear inner hair cell or a cochlear outer hair cell. In some embodiments, an animal cell is a cochlear inner hair cell. In some embodiments, an animal cell is a cochlear outer hair cell.

[0565] In some embodiments, an animal cell is *in-vitro*. In some embodiments, 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 embodiments, an animal cell is an autologous cell obtained from an animal and cultured *ex-vivo*.

Methods

[0566] Among other things, the present disclosure provides methods for treating a subject having an otological disease associated with vascularization, e.g., VS. In some embodiments, a method comprises introducing a composition as described herein into an inner ear (e.g., a cochlea) of a subject. For example, provided herein are methods that in some embodiments 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) an effective amount, e.g., a therapeutically effective amount, of any

composition described herein. In some embodiments of any of the methods disclosed herein, a 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 expression and/or activity of a supporting and/or hearing cell target protein encoded by the gene, e.g., a mutation in NF2). Some embodiments of any of the methods disclosed herein further include, prior to the introducing or administering step, determining that a subject has a defective inner ear cell target gene. Some embodiments of any of the methods disclosed herein can further include detecting a mutation in an inner ear cell target gene in a subject. Some embodiments of any of the methods disclosed herein can further include identifying or diagnosing a subject as having nonsyndromic or syndromic sensorineural hearing loss.

[0567] In some embodiments, a method disclosed herein comprises administering a composition disclosed herein, e.g., rAAV-antiVEGF, for the treatment of a subject, e.g., mammal, e.g., human, e.g., patient, with VS. In some embodiments, a composition disclosed herein is delivered via surgical delivery, e.g., to a cochlea.

[0568] In some embodiments, a subject has a progressive tumor, e.g., a tumor that has a growth rate of at least 5%, at least 10%, at least 20%, at least 30%, at least 40%, or at least 50% in successive scans, e.g., a tumor that has evidence of progression. In some embodiments, a subject has a progressive tumor, e.g., a tumor that has a growth rate of at least 5%, at least 10%, at least 20%, at least 30%, at least 40%, or at least 50% in consecutive scans, e.g., a tumor that has evidence of progression.

[0569] In some embodiments, a subject has a stable tumor, e.g., a tumor that has a growth rate that is less than 5%, less than 10%, less than 20%, or less than 30% in successive scans, e.g., a tumor that lacks evidence of substantive progression. In some embodiments, a subject has a stable tumor, e.g., a tumor that has a growth rate that is less than 5%, less than 10%, less than 20%, or less than 30% in consecutive scans, e.g., a tumor that lacks evidence of substantive progression.

[0570] In some embodiments, a subject does not have a tumor that is 2 cm or more maximum dimension (e.g., a tumor that is likely to invade a cerebellopontine angle and potentially compress the brainstem). In some embodiments, a subject is not at high risk for potentially life-threatening tumor-related sequelae that may be avoided with the current standard of care of surgical resection

and radiation therapy. In some embodiments, a method disclosed herein further comprises administering surgical resection or radiation.

[0571] In some embodiments, a subject has a tumor that is 2 cm or more maximum dimension (e.g., a tumor that is likely to invade a cerebellopontine angle and potentially compress a brainstem). In some embodiments, a subject is at high risk for potentially life-threatening tumor-related sequelae that may be avoided through administration of methods and compositions as described herein. In some embodiments, methods and compositions as described herein are utilized as a combination therapy with current standard of care methods, e.g., in some embodiments a method disclosed herein further comprises administering surgical resection or radiation.

[0572] In some embodiments, a subject does not have profound deafness in the contralateral ear. In some embodiment, a subject has auditory function in the contralateral ear. In some embodiments, a subject can maintain access to important auditory domains such as speech reception and environmental awareness. In some embodiments, a subject having NF2 is not administered a composition disclosed herein. In some embodiments, safety and tolerability of a composition disclosed herein can be assessed in unilateral sporadic progressive VS. In some embodiments of any of the methods disclosed herein, a subject has progressive growth of small (less than 2 cm maximum dimension), and/or unilateral sporadic tumors. In some embodiments, safety and tolerability of a composition disclosed herein, e.g., rAAV-antiVEGF, can be assessed in a subject disclosed herein.

[0573] In some embodiments, a composition disclosed herein, e.g., rAAV-antiVEGF, is formulated as a sterile suspension for intracochlear administration. In some embodiments, a composition comprises constructs in an amount of at least 1×10^{11}, at least 5×10^{11}, at least 1×10^{12}, at least 1×10^{12}, at least 2×10^{12}, at least 3×10^{12}, at least 4×10^{12}, at least 5×10^{12}, at least 6×10^{12}, at least 7×10^{12}, at least 8×10^{12}, at least 9×10^{12}, at least 1×10^{13}, at least 2×10^{13}, at least 3×10^{13}, at least 4×10^{13}, at least 5×10^{13}, at least 6×10^{13}, at least 7×10^{13}, at least 8×10^{13}, at least 9×10^{13}, or at least 1×10^{14} vector genomes (vg) per milliliter (mL) (vg/mL). In some embodiments, a composition comprises constructs in an amount of at most 1×10^{15}, at most 5×10^{14}, at most 1×10^{14}, at most 5×10^{13}, at most 1 x 10(13), at most 9×10^{12}, at most 8×10^{12}, at most 7×10^{12}, at most 6×10^{12}, at most 5×10^{12}, at most 4 x 10(12), at most 3×10^{12}, at most 2×10^{12}, or at most 1×10^{12} vector genomes (vg) per milliliter (mL). In some embodiments, a

composition comprises constructs in an amount of about 1 x 10^{12} vg/mL to 1 x 10^{13} vg/mL, about 5 x 10(12) vg/mL to 5 x 10^{13} vg/mL, or about 1 x 10^{13} vg/mL to 2 x 10^{13} vg/mL.

[0574] In some embodiments, a composition comprises constructs in an amount of about 1 x 10^{12} vg/mL, about 1.1 x 10^{12} vg/mL, 1.2 x 10^{12} vg/mL, about 1.3 x 10^{12} vg/mL, about 1.4 x 10^{12} vg/mL, about 1.5 x 10^{12} vg/mL, about 1.6 x 10^{12} vg/mL, about 1.7 x 10^{12} vg/mL, about 1.8 x 10^{12} vg/mL, about 1.9 x 10^{12} vg/mL, about 2.0 x 10^{12} vg/mL, about 2.1 x 10^{12} vg/mL, about 2.2 x 10^{12} vg/mL, about 2.3 x 10^{12} vg/mL, about 2.4 x 10^{12} vg/mL, about 2.5 x 10^{12} vg/mL, about 2.6 x 10^{12} vg/mL, about 2.7 x 10^{12} vg/mL, about 2.8 x 10^{12} vg/mL, about 2.9 x 10^{12} vg/mL, about 3.0 x 10^{12} vg/mL, about 3.1 x 10^{12} vg/mL, about 3.2 x 10^{12} vg/mL, about 3.3 x 10^{12} vg/mL, about 3.4 x 10^{12} vg/mL, about 3.5 x 10^{12} vg/mL, about 3.6 x 10^{12} vg/mL, about 3.7 x 10^{12} vg/mL, about 3.8 x 10^{12} vg/mL, about 3.9 x 10^{12} vg/mL, about 4.0 x 10^{12} vg/mL, about 4.1 x 10^{12} vg/mL, about 4.2 x 10^{12} vg/mL, about 4.3 x 10^{12} vg/mL, about 4.4 x 10^{12} vg/mL, about 4.5 x 10^{12} vg/mL, about 4.6 x 10^{12} vg/mL, about 4.7 x 10^{12} vg/mL, about 4.8 x 10^{12} vg/mL, about 4.9 x 10^{12} vg/mL, about 5.0 x 10^{12} vg/mL, about 5.1 x 10^{12} vg/mL, about 5.2 x 10^{12} vg/mL, about 5.3 x 10^{12} vg/mL, about 5.4 x 10^{12} vg/mL, about5.5 x 10^{12} vg/mL, about 5.6 x 10^{12} vg/mL, about 5.7 x 10^{12} vg/mL, about 5.8 x 10^{12} vg/mL, about 5.9 x 10^{12} vg/mL, about 6.0 x 10^{12} vg/mL, about 7.0 x 10^{12} vg/mL, about 8.0 x 10^{12} vg/mL, about 9.0 x 10^{12} vg/mL, about 9.1 x 10^{12} vg/mL, about 9.2 x 10^{12} vg/mL, about 9.3 x 10^{12} vg/mL, about 9.4 x 10^{12} vg/mL, about 9.5 x 10^{12} vg/mL, about 9.6 x 10^{12} vg/mL, about 9.7 x 10^{12} vg/mL, about 9.8 x 10^{12} vg/mL, about 9.9 x 10^{12} vg/mL, about 1 x 10^{13} vg/mL, 1.1 x 10^{13} vg/mL, 1.2 x 10^{13} vg/mL, 1.3 x 10^{13} vg/mL, 1.4 x 10^{13} vg/mL, 1.5 x 10^{13} vg/mL, 1.6 x 10^{13} vg/mL, 1.7 x 10^{13} vg/mL, 1.8 x 10^{13} vg/mL, 1.9 x 10^{13} vg/mL, 2 x 10^{13} vg/mL, 5 x 10^{13} vg/mL, or 1 x 10^{14} vg/mL.

[0575] In some embodiments, a composition comprises constructs in an amount of 2.5 x 10^{12} vg/mL +/- 10%.

[0576] In some embodiments, a composition comprises constructs in an amount of 5 x 10^{12} vg/mL +/- 10%.

[0577] In some embodiments, a composition comprises constructs in an amount of 1 x 10^{13} vg/mL +/- 10%.

[0578] In some embodiments, a composition disclosed herein, e.g., rAAV-antiVEGF, is administered in the surgical suite under controlled aseptic conditions by a board-certified surgeon

experienced in performing otologic surgery. In some embodiments, an administration procedure is a microscope- or endoscope-assisted transcanal exploratory tympanotomy and laser-assisted microstapedotomy, followed by a round window injection to deliver, e.g., about 0.09 mL, of a solution containing a composition disclosed herein, e.g., rAAV-antiVEGF particles. Transcanal exploratory tympanotomy is a common procedure used to expose the structures of the middle ear; transcanal exploratory tympanotomy is often accompanied by laser-assisted stapedectomy (removal of the stapes footplate) or stapedotomy (creating a hole in the stapes footplate), e.g., for patients with otosclerosis. In some embodiments, the approximately 0.25 mm vent hole in the stapes footplate (made using an otologic laser) serves to prevent a potential deleterious rise in intralabyrinthine pressure.

[0579] In some embodiments, disclosed herein is a sterile, one-time use delivery device to administer a composition disclosed herein to the perilymph fluid of an inner ear through a round window membrane with a vent located in a stapes footplate. In some embodiments, this custom device affords advantages over available materials, e.g., both with respect to the potential for safety and efficacy of a therapeutic agent, as it was specifically designed for an intracochlear route of administration. In some embodiments, design elements of a delivery device 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 flow rate (coupled with the use of a standard pump); allowance for visualization of round window membrane and delivery through the external auditory canal by the surgeon; minimization of damage to the round window membrane, or to cochlear structures beyond the round window membrane; and/or minimization of efflux back out through round window membrane.

[0580] In some embodiments, any of the methods disclosed herein comprise a dose-escalation study to assess safety and tolerability in subjects, e.g., mammals, e.g., humans, e.g., patients, e.g., with unilateral progressive sporadic VS. In some embodiments, a composition disclosed herein, e.g., rAAV-antiVEGF, is administered at a dosing regimen disclosed herein. In some embodiments, the dosing regimen comprises either unilateral or bilateral intracochlear administrations of a dose, e.g., as described herein, of a composition disclosed herein, e.g,. rAAV-antiVEGF. In some embodiments, 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 embodiments, 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 embodiments, a 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, e.g., depending on the population.

[0581] In some embodiments, a dosing regimen comprises delivery in a volume of about 0.01 mL to about 0.30 mL, about 0.01 mL to about 0.25 mL, about 0.01 mL to about 0.20 mL, about 0.01 mL to about 0.15 mL, about 0.01 mL to about 0.14 mL, about 0.01 mL to about 0.13 mL, about 0.01 mL to about 0.12 mL, about 0.01 mL to about 0.11 mL, about 0.01 mL to about 0.10 mL, about 0.01 mL to about 0.09 mL, about 0.01 mL to about 0.08 mL, about 0.01 mL to about 0.07 mL, about 0.01 mL to about 0.06 mL, about 0.01 mL to about 0.05 mL, about 0.01 mL to about 0.04 mL, about 0.01 mL to about 0.03 mL, or about 0.01 mL to about 0.02 Ml.

[0582] In some embodiments, a dosing regimen comprises delivery in a volume of about 0.02 mL to about 0.30 mL, 0.03 mL to about 0.30 mL, 0.04 mL to about 0.30 mL, 0.05 mL to about 0.30 mL, 0.06 mL to about 0.30 mL, 0.07 mL to about 0.30 mL, 0.08 mL to about 0.30 mL, 0.09 mL to about 0.30 mL, 0.10 mL to about 0.30 mL, 0.11 mL to about 0.30 mL, 0.12 mL to about 0.30 mL, 0.13 mL to about 0.30 mL, 0.14 mL to about 0.30 mL, 0.15 mL to about 0.30 mL, 0.16 mL to about 0.30 mL, 0.17 mL to about 0.30 mL, 0.18 mL to about 0.30 mL, 0.19 mL to about 0.30 mL, 0.20 mL to about 0.30 mL, or 0.25 mL to about 0.30 mL.

[0583] In some embodiments, a dosing regimen comprises delivery in a volume of about 0.01 mL to about 0.03 mL, about 0.02 mL to about 0.25 mL, about 0.03 mL to about 0.20 mL, about 0.04 mL to about 0.18 mL, about 0.05 mL to about 0.16 mL, about 0.06 mL to about 0.14 mL, about 0.07 mL to about 0.12 mL, or about 0.08 mL to about 0.1 mL.

[0584] In some embodiments, a subject having sporadic progressive VS typically presents unilaterally. In some embodiments, a subject having unilateral sporadic progressive VS is administered unilateral administration of a composition disclosed herein. In some embodiments, a

subject having NF2 typically experiences bilateral VSs. In some embodiments, a subject having NF2 is administered either unilateral or bilateral administration of a composition disclosed herein.

[0585] In some embodiments, a method disclosed herein evaluates the safety and tolerability of escalating doses of a composition disclosed herein, e.g., rAAV-antiVEGF, administered via unilateral intracochlear injection to a subject, e.g.,18 to 80 years of age, with unilateral sporadic progressive VS. In some embodiments, a composition disclosed herein, e.g., rAAV-antiVEGF, inhibits growth of a tumor.

[0586] In some embodiments, any of the methods disclosed herein comprise an evaluation of the safety and tolerability of a composition disclosed herein, e.g., rAAV-antiVEGF. In some embodiments, evaluation of the efficacy of a composition disclosed herein, e.g., rAAV-antiVEGF to treat sporadic progressive VS, is performed in a randomized, controlled setting (using a concurrent, non-intervention observation arm).

[0587] In some embodiments, any of the methods disclosed herein comprise a determination of an optimal dose of a composition disclosed herein, e.g., rAAV anti-VEGF. In some embodiments, any of the methods disclosed herein comprise evaluation of efficacy of a composition disclosed herein, e.g., rAAV-antiVEGF in a broader VS population. In some embodiments, any of the methods disclosed herein comprise determination of an optimal dosing regimen, e.g., including for bilateral VS tumors (in the setting of NF2).

[0588] In some embodiments of any of the methods disclosed herein, the method further comprises administration of the current standard of care, e.g., surgical resection of the tumor and/or radiation therapy.

[0589] In some embodiments, of any of the methods disclosed herein, pre-existing neutralizing antibodies (NAb), as detected in serum, do not substantially impact transduction of rAAV particles delivered by an intracochlear route of administration. In some embodiments, a subject administered a composition disclosed herein has pre-existing neutralizing antibodies (NAb), e.g., as described herein. In some embodiments, a subject administered a composition disclosed herein does not have pre-existing neutralizing antibodies (NAb), e.g., as described herein.

[0590] In some embodiments, provided herein are methods of ameliorating and/or treating a disease characterized by neovascularization (e.g., VS) by expressing an anti-VEGF protein (e.g.,

a ranibizumab, bevacizumab, and/or aflibercept protein) 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 embodiments, methods include administering to the inner ear of a subject an effective amount, e.g., a therapeutically effective amount of any of the compositions described herein, wherein the administering ameliorates and/or treats a disease characterized by neovascularization by expression an anti-VEGF protein. In some embodiments, the inner ear target cell that produces an anti-VEGF protein 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.

[0591] Also provided herein are methods of increasing the expression level of an anti-VEGF 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 an effective amount, e.g., a therapeutically effective amount of any of the compositions described herein, wherein the administering results in an increase in the expression level of an anti-VEGF protein (e.g., ranibizumab, bevacizumab, and/or aflibercept) in any cell subset of the inner ear of a subject. In some embodiments, 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.

[0592] Also provided herein are methods of treating VS associated symptoms (e.g., hearing loss, tinnitus, vertigo, dizziness, a feeling of fullness in the ear, facial numbness, facial paresthesia, headaches, clumsy gait, and/or mental confusion etc.) in a subject (e.g., an animal, e.g., a mammal, e.g., a primate, e.g., a human) identified as having an otology related disease characterized by neovascularization, comprising: administering to the inner ear of the subject an effective amount, e.g., a therapeutically effective amount of any of the compositions described herein. Also provided herein are methods that include administering to an inner ear of a subject an effective amount, e.g., a therapeutically effective amount of any of the compositions described herein.

[0593] Also provided herein are surgical methods for treatment of VS. In some embodiments, the methods include the steps of: introducing into a cochlea of a subject an incision, e.g., a first incision, at a first incision point; and administering intra-cochlearly an effective amount, e.g., a therapeutically effective amount of any of the compositions provided herein. In some embodiments, the composition is administered to the subject at the incision point, e.g., the first

incision point. In some embodiments, the composition is administered to the subject into or through the first incision.

[0594] In some embodiments 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 embodiments of any of the methods described herein, any of the compositions described herein is administered to the subject into or through the cochlea round window membrane. In some embodiments of any of the methods described herein, the composition is administered using a medical device capable of creating a plurality of incisions in the round window membrane. In some embodiments, the medical device comprises a plurality of micro-needles. In some embodiments, the medical device comprises a plurality of micro-needles including a generally circular first aspect, where each micro-needle has a diameter of at least about 10 microns. In some embodiments, the medical device comprises a base and/or a reservoir capable of holding the composition. In some embodiments, the medical device comprises a plurality of hollow micro-needles individually including a lumen capable of transferring the composition. In some embodiments, the medical device comprises a means for generating at least a partial vacuum.

[0595] In some embodiments, technologies of the present disclosure are used to treat subjects with or at risk of hearing loss. In some embodiments, technologies of the present disclosure are used to treat subjects with or at risk of tinnitus. In some embodiments, technologies of the present disclosure are used to treat subjects with or at risk of vertigo. In some embodiments, technologies of the present disclosure are used to treat subjects with or at risk of VS tumor growth. In some embodiments, technologies of the present disclosure are used to treat subjects with or at risk of myriad symptoms associated with VS and/or standard of care VS treatment options. For example, in some embodiments, a subject has symptoms attributed to at least one pathogenic variant of NF2. It will be understood by those in the art that many different mutations in NF2 can result in a pathogenic variant. In some such embodiments, a pathogenic variant causes or is at risk of causing hearing loss, tinnitus, vertigo, tumor growth etc. by increasing the likelihood of disease characterized by neovascularization.

[0596] In some embodiments, a subject experiencing VS associated symptoms (e.g., hearing loss, tinnitus, vertigo, dizziness, a feeling of fullness in the ear, facial numbness, facial paralysis, headaches, clumsy gait, and/or mental confusion etc.) will be evaluated to determine if and where

one or more mutations may exist that may be causing said VS associated symptoms. In some such embodiments, the status of certain gene products or function (e.g., via protein or sequencing analyses) will be evaluated. In some embodiments of any of the methods described herein, the subject or animal is a mammal, in some embodiments the mammal is a domestic animal, a farm animal, a zoo animal, a non-human primate, or a human. In some embodiments 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 embodiments 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 embodiments of any of the methods described herein, the subject or mammal is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or 11 months of age.

[0597] In some embodiments, any of the methods and/or compositions described herein, may result in improvement(s) in VS associated symptoms (e.g., hearing loss, tinnitus, vertigo, dizziness, a feeling of fullness in the ear, facial numbness, facial paresthesia, headaches, clumsy gait, and/or mental confusion etc.). In some embodiments, such a symptom is measured and determined (e.g., using any of the metrics for determining improvement in such a symptom as described herein or known in the art) 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.

[0598] In some embodiments a subject (e.g., an animal, e.g., a mammal, e.g., a human) has or is at risk of developing VS associated symptoms (e.g., hearing loss, tinnitus, vertigo, dizziness, a feeling of fullness in the ear, facial numbness, facial paresthesia, headaches, clumsy gait, and/or mental confusion etc.). In some embodiments a subject (e.g., an animal, e.g., a mammal, e.g., a human) has been previously identified as having a mutation in an NF2 gene. In some embodiments a subject (e.g., an animal, e.g., a mammal, e.g., a human) has any of the mutations in an NF2 gene

that are known in the art to be associated with a disease characterized by neovascularization, e.g., VS. In some such embodiments, 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 associated with VS (e.g., via genetic testing) that has not previously been identified. In some such embodiments, identified mutations may be novel (e.g., not previously described in the literature), and methods of treatment for a subject suffering from or susceptible to VS will be personalized to the mutation(s) of the particular patient.

[0599] In some embodiments, 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 an NF2 gene (e.g., via genetic testing). In some embodiments, a subject (e.g., an animal, e.g., a mammal, e.g., a human) has been identified as having a mutation in an NF2 gene and has been diagnosed with VS associated symptom(s) (e.g., hearing loss, tinnitus, vertigo, dizziness, a feeling of fullness in the ear, facial numbness, facial paresthesia, headaches, clumsy gait, and/or mental confusion etc.). In some embodiments, a subject (e.g., an animal, e.g., a mammal, e.g., a human) has been identified as having symptoms associated with a disease characterized by neovascularization.

[0600] In some embodiments, successful treatment of VS and VS associated symptom(s) (e.g., hearing loss, tinnitus, vertigo, dizziness, a feeling of fullness in the ear, facial numbness, facial paresthesia, headaches, clumsy gait, and/or mental confusion etc.) can be determined in a subject using any of the conventional methods known in the art, including but not limited to: hearing tests, tinnitus tests, gait tests, cognitive tests, imaging techniques, and/or diagnostic biomarker sampling. For example, 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).

[0601] In some embodiments 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 embodiments 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).

[0602] In some embodiments of any method provided herein, the composition can be formulated for intra-cochlear administration. In some embodiments of any of the methods described herein, the compositions described herein can be administered via intra-cochlear administration or local administration. In some embodiments of any of the methods described herein, the compositions are administered through the use of a medical device (e.g., any of the exemplary medical devices described herein).

[0603] In some embodiments, intra-cochlear administration can be performed using any of the methods described herein or known in the art. For example, in some embodiments, 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.

[0604] In some embodiments of any method provided herein, a subject has or is at risk of developing VS and/or VS associated symptoms. In some embodiments of any method provided herein, a subject has been previously identified as having a mutation in a gene implicated in VS development, such a gene may be expressed in supporting cells and/or hair cells.

[0605] In some embodiments, a subject cell is *in-vitro*. In some embodiments, a subject cell is originally obtained from a subject and is cultured *ex-vivo*. In some embodiments, a subject cell is considered otherwise healthy, and is cultured and expanded *ex-vivo*. In some embodiments, a subject cell has previously been determined to have a defective inner ear cell target gene. In some embodiments, a subject cell has previously been determined to have a defective hair cell target

gene. In some embodiments, a subject cell has previously been determined to have a defective supporting cell target gene.

Devices, Administration, and Surgical Methods

[0606] Provided herein are therapeutic delivery systems for treating hearing loss (e.g., nonsyndromic sensorineural hearing loss or syndromic sensorineural hearing loss). In some embodiments, 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 embodiments, a medical device includes a plurality of micro-needles.

[0607] In some embodiments of any of the methods disclosed herein, a subject will receive a treatment comprising dosing with a composition disclosed herein, e.g., rAAV-antiVEGF. In some embodiments, a subject may receive at least one additional treatment comprising dosing with a composition disclosed herein. In some embodiments, a subject will be monitored for at least 30 days between each test article composition (e.g., solution comprising rAAV particles) administration.

[0608] In certain embodiments, methods and compositions as described herein are utilized as a combination therapy with certain current treatment options, such as continual observation by MRI with no additional active intervention, stereotactic radiosurgery (SRS), fractionated radiotherapy (FRT), and/or Microsurgery (MS).

[0609] In some embodiments, methods and compositions as described herein are utilized as a combination therapy with continual observation using MRI. In certain embodiments, MRI measurements may identify tumor growth, tumor stasis, or tumor regression. In certain embodiments, methods as described herein coupled with continual observation without additional intervention increases hearing preservation rate when compared to methods utilizing continual MRI based observation alone.

[0610] In some embodiments, methods and compositions as described herein are utilized as a combination therapy with radiosurgery (e.g., gamma knife surgery) as is known in the art. In some embodiments, a single effective dose of radiation is delivered to the tumor (e.g., from 5 to 10 gy of radiation, from 7 to 12 gy of radiation, from 9 to 14 gy of radiation, from 11 to 16 gy of radiation,

from 13 to 18 gy of radiation, or from 12 to 17gy of radiation) before, or after treatment with compositions and methods as described herein. In some embodiments, combination therapy utilizing methods and compositions as described herein reduces necessary radiation dose profiles, reducing the chance that radiation profiles may overlap structures adjacent to a tumor. In some embodiments, this combination therapy approach reduces the potential of radiosurgery induced dysfunction of the cranial nerve or brainstem structures. In some embodiments, combination therapy approaches reduce post-operative hearing loss associated with increased radiation dose to the cochlea or associated brainstem nuclei. In some embodiments, combination therapy approaches reduce the possibility of post-operative hydrocephalus. In some embodiments, combination therapy approaches reduce the possibility of post-operative secondary malignancy. In some embodiments, combination therapy approaches slow tumor growth, arrest tumor growth, and/or shrink tumor size. In some embodiments, combination therapy approaches increase the efficacy of radiosurgery, for instance, increasing the chances of tumor growth arrest, reducing the requirements for additional treatments, increasing the odds of hearing preservation, reducing the odds of permanent facial neuropathy, reducing the odds of trigeminal neuropathy, and/or reducing the odds of hydrocephalus.

[0611] In some embodiments, methods and compositions as described herein are utilized as a combination therapy with fractionated radiotherapy as is known in the art. In some embodiments, an effective dose of radiation is delivered to the tumor through multiple micro doses (e.g., ~5 micro doses, ~10 micro doses, ~15 micro doses, ~20 micro doses, ~25 micro doses, or ~30 micro doses) over a period of weeks (e.g., 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 7 weeks, 8 weeks, 9 weeks, 10 weeks, 11 weeks, 12 weeks, or greater than 12 weeks) which together culminate in one large dose of radiation to the tumor (e.g., ~30 gy, ~40 gy, ~50gy ~60 gy, or ~70 gy of radiation), that is administered before, during, or after treatment with compositions and methods as described herein. In some embodiments, combination therapy approaches as described herein result in continual damage the tumor, with the inhibition of neovascularization, while allowing the surrounding tissues to heal between doses. In some embodiments, combination therapy approaches as described herein increase the efficacy of fractionated radiotherapy, for instance, increasing the mean rate of tumor arrest, increasing the 5-year actuarial rate, reducing the chances for additional treatments being required, increasing hearing preservation, decreasing the

odds of developing facial neuropathy, decreasing the odds of developing trigeminal neuropathy, and/or decreasing the odds of hydrocephalus.

[0612] In some embodiments, methods and compositions as described herein are utilized as a combination therapy with surgical interventions as are known in the art. In some embodiments, such a combination therapy approach increases the efficacy of surgical intervention while reducing the chances of additional surgical complications such as damage to cranial nerves, cerebrospinal fluid (CSF) leaks, and/or post-operative infection. In some embodiments, such a combination approach reduces the odds of additional treatments being required, increases the odds of hearing preservation, reduces the odds of permanent facial neuropathy, reduces the odds of acute morbidity associated with CSF patients, and/or reduces the odds of mortality.

[0613] Also provided herein are surgical methods for treatment of hearing loss (e.g., nonsyndromic sensorineural hearing loss or syndromic sensorineural hearing loss). In some embodiments, a method includes 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 embodiments, a composition is administered to a subject at the first incision point. In some embodiments, a composition is administered to a subject into or through the first incision.

[0614] In some embodiments, systemic administration of VEGF inhibitors has been evaluated for the treatment of VS in NF2 patients and the results of these studies provide preliminary clinical evidence for the use of VEGF inhibitors to treat VS. In some embodiments, NF2 patients are at heightened risk for administration of a composition disclosed herein, as they generally present with bilateral schwannomas resulting in compromised hearing in both ears. In some embodiments, a subject having NF2 is not administered a composition disclosed herein. In some embodiments, a subject having NF2 is administered a composition disclosed herein, e.g., based on safety and efficacy of a composition disclosed herein, e.g., rAAV-antiVEGF, delivered via unilateral or bilateral intracochlear administration.

[0615] In some embodiments, sporadic VS tumors express VEGF (Saito 2003; Cayé-Thomasen 2003, each of which is incorporated herein in its entirety by reference), and the level of VEGF expression, e.g., correlates with the growth rate of the tumor (Cayé-Thomasen 2003; Cayé-Thomasen 2005, each of which is incorporated herein in its entirety by reference). In some

embodiments of any of the methods disclosed herein, a subject has progressive sporadic VS (e.g., defined as a single tumor with a growth rate of at least 20% over successive scans), but whose tumors are still small enough where an additional observation period of one year would not put them at high risk for further tumor-related sequelae (apart from hearing loss). In some embodiments, the subject is an adult who is receiving or will be receiving invasive surgical resection and/or radiation therapy. In some embodiments of any of the methods disclosed herein, the method comprises evaluating a safe dose, e.g., a starting dose (8-fold below the anticipated NOAEL). In some embodiments, the starting dose results in VEGF inhibitor exposure levels in tissues and fluids in close proximity to the tumor location.

[0616] In some embodiments, compositions as described herein (e.g., rAAV-antiVEGF) may reach many target cells in the inner ear through delivery into the perilymph. Perilymph is a fluid very similar in composition to, and in diffusional continuity with, cerebrospinal fluid (CSF) (Lysaght 2011, incorporated herein in its entirety by reference). Perilymph bathes most of the sensory, neural, and supporting cells of the cochlea and of the vestibular system, housed in the bony labyrinth of the inner ear. The perilymphatic space of the cochlea, to which in some embodiments, compositions as described herein (e.g., comprising rAAV-antiVEGF) will be delivered, comprises two scalae, or passages: the scala tympani and the scala vestibuli, which are continuous with one another at the apex of the cochlear spiral via the helicotrema. Many cells of the inner ear are in fluid continuity with perilymph through interstitial spaces in the tissue.

[0617] Intracochlear administration of an rAAV-antiVEGF composition with a delivery device as described herein typically will occur through the round window membrane (*see* **FIG. 1**). In some embodiments, compositions as described herein are delivered to an individual (e.g., a mammal, e.g., a human) using a sterile, one-time use delivery device for intracochlear administration. In certain embodiments, compositions as described herein are delivered to the perilymph fluid of the inner ear through the round window membrane with a vent located in the stapes footplate. In certain embodiments, an intracochlear administration approach comprises administration of compositions as described herein into the scala tympani through the round window membrane, with a vent in the stapes footplate within the oval window, such that rAAV particles are perfused through scala tympani, then through scala vestibuli via connection at the helicotrema, and follows the fluid path to the vent in the stapes footplate. In some embodiments, presence of a vent distinct from the injection port allows for a more even and/or controlled

distribution of drug along the length of the cochlea and/or prevents the potentially deleterious build-up of additional fluid pressure within the inner ear. In some embodiments, this delivery approach also permits diffusion of the injectate to the vestibular system. In some embodiments, a process such as that described herein can be accomplished in patients with a relatively nontraumatic approach through the external auditory canal.

[0618] In some embodiments 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 embodiments 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 embodiments 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 embodiments, a medical device includes a plurality of micro-needles. In some embodiments, 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 embodiments, a medical device includes a base and/or a reservoir capable of holding a composition. In some embodiments, a medical device includes a plurality of hollow micro-needles individually including a lumen capable of transferring a composition. In some embodiments, a medical device includes a means for generating at least a partial vacuum.

[0619] In some embodiments, disclosed herein is a sterile, one-time use delivery device to administer a composition disclosed herein to the perilymph fluid of the inner ear through the round window membrane with a vent located in the stapes footplate. In some embodiments, this custom device affords advantages over available materials and general-purpose surgical instruments, both with respect to safety and the potential for efficacy of a therapeutic agent, as it was specifically designed for the intracochlear route of administration. Important design elements of the delivery device 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 flow rate (coupled with the use of a standard pump); allowance for visualization of round window membrane and delivery through the external auditory canal by the surgeon; minimization of damage to the round window membrane, or to cochlear structures beyond the round window membrane; and minimization of efflux back out through round window membrane. In some embodiments, a custom, dedicated

device will be removed from the ear following delivery of about 0.09 mL of a composition disclosed herein, e.g., rAAV-antiVEGF into the perilymph of the cochlea.

[0620] Delivery to the inner ear is challenging as a result of the frailty and inaccessibility of the sensory cells and non-sensory supporting cells/structures, limiting the type of surgical approaches without unintended damage. In some embodiments, appropriate venting of the fluid-filled cochlear space prevents damage in the inner ear due to the increased fluid volume.

[0621] The structure and function of the mammalian inner ear is highly conserved across mammals (Manley 2017, incorporated herein in its entirety by reference), albeit with differences in timing of cochlear development relative to gestation and birth between primates and other mammals. Cochlear development in the mouse is still ongoing at the time of birth, with hearing onset at around postnatal day 12 (P12; Bulankina 2012, incorporated herein in its entirety by reference). Humans, in contrast, undergo complete cochlear development in utero, and experience hearing onset by gestational week 25 to 28 (Litovsky 2015, incorporated herein in its entirety by reference). Two significant structural aspects of the mature inner ear that must be taken into consideration in the development of potential therapies and their delivery in the ear are: 1) size differences of the inner ear among species; and 2) patency of the cochlear aqueduct that runs between the cochlear perilymph and the cerebrospinal fluid (CSF). In some embodiments of any of the methods disclosed herein, cochlear volume is used for dose extrapolation, e.g., from animals to humans.

[0622] In some embodiments, when the subject is a rodent, e.g., a mouse, a surgical approach described in Yoshimura et al., 2018, which comprises delivery through the round window membrane with fenestration of the posterior semicircular canal, which has demonstrated robust and reliable transduction, independent of the age of the animal at the time of injection is used (Yoshimura 2018, incorporated herein in its entirety by reference). A postauricular incision is made to access the temporal bone. A portion of the sternocleidomastoid muscle is divided to expose the otic bulla. A 0.5 to 0.6 mm diameter otologic drill is used to make a small hole in the bulla; the hole is then widened to visualize the stapedial artery and the round window membrane. Fenestration of the posterior semicircular canal is performed with the otologic drill (0.5 to 0.6 mm diameter) to serve as a vent the inner ear during cochlear administration. The round window membrane is penetrated with the mouse delivery device, which consists of a borosilicate capillary

pipette and a 10 uL Hamilton syringe, and 1 μL of solution comprising viral particles (approximately 40 to 50% of the total inner ear volume) is delivered through the round window membrane, into the scala tympani, at rate of 300 nL/min.

[0623] In some embodiments, when the subject is a NHP, a postauricular incision is made and dissection of the soft tissue is performed down to the level of the periosteum. In some embodiments, the periosteum is incised and elevated to expose the mastoid bone. A cortical mastoidectomy is performed with a combination of high-speed cutting and diamond drill burs. The facial recess is then opened, allowing for adequate round window and oval window (OW) visualization. Fenestration of the stapes footplate in the OW is performed using a Rosen needle. As with other models, the fenestration allows for injection of a larger volume without damage to the inner ear; additionally, venting allows solution comprising rAAV particles to flow toward the apex of the cochlea. Thirty μL of solution comprising rAAV particles (approximately 40 to 50% of the total inner ear volume) is delivered through the round window membrane at rate 30 μL/min.

[0624] In some embodiments, when the subject is a mammal, e.g., a human, a less invasive approach through the external auditory canal is used, e.g., since the relevant structures are relatively large, even at birth. In some embodiments, the clinical administration procedure is a transcanal exploratory tympanotomy and laser-assisted microstapedotomy (using a potassium titanyl phosphate [KTP] or CO2 otologic laser to place a small vent hole [approximately 0.25 mm] in the stapes footplate), followed by a round window injection to deliver about 0.09 mL (or 90 μL, approximately 40 to 50% of the total inner ear volume) of solution comprising a composition disclosed herein, e.g., rAAV-antiVEGF particles, through the round window membrane within a three-minute period. In some embodiments, venting serves to prevent a potential deleterious rise in intralabyrinthine pressure. Transcanal exploratory tympanotomy is a common procedure used to expose the structures of the middle ear; transcanal exploratory tympanotomy is often accompanied by laser-assisted stapedectomy (removal of the stapes footplate) or stapedotomy (creating a hole in the stapes footplate), e.g., for patients with otosclerosis.

[0625] In some embodiments, 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. 33-36 (or microcatheter) to deliver a composition disclosed herein at a controlled flow rate and in a fixed volume, via the round window membrane.

[0626] In some embodiments, 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 embodiments, 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 embodiments, venting facilitates transduction rates of about 75-100% of IHCs throughout the cochlea. In some embodiments, 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 (see **FIG. 28**). In some embodiments, 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 (see **FIG. 28**).

[0627] 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 embodiments, the flow rate (or infusion rate) may include a rate of about 30 μL/min, or from about 25 μL/min to about 35 μL/min, or from about 20 μL/min to about 40 μL/min, or from about 20 μL/min to about 70 μL/min, or from about 20 μL/min to about 90 μL/min, or from about 20 μL/min to about 100 μL/min. In some embodiments, the flow rate is about 20 μL/min, about 30 μL/min, about 40 μL/min, about 50 μL/min, about 60 μL/min, about 70 μL/min, about 80 μL/min, about 90 μL/min or about100 μL/min. In some embodiments, 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 embodiments, 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 embodiments, the total volume of a composition disclosed herein equates to from about 40% to about 50% of the volume of the inner ear.

[0628] Once the delivery has been completed, the device may be removed. In some embodiments, a device described herein, may be configured as a single-use disposable product. In other embodiments, 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.

[0629] In some embodiments, a composition disclosed herein comprises one or a plurality of rAAV constructs. In some embodiments, when more than one rAAV construct is included in the composition, the rAAV constructs are each different. In some embodiments, an rAAV construct comprises an anti-VEGF coding region, e.g., as described herein. In some embodiments, a composition comprises an rAAV particle comprising an AAV construct described herein. In some embodiments, the rAAV particle is encapsidated by an Anc80 capsid. In some embodiment, an Anc80 capsid comprises a polypeptide of SEQ ID NO: 89. In some embodiment, an Anc80 capsid comprises a polypeptide of SEQ ID NO: 113. In some embodiment, an Anc80 capsid comprises a polypeptide of SEQ ID NO: 114.

[0630] In some embodiments, a composition disclosed herein can be administered to a subject with a surgical procedure. In some embodiments, 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 embodiments, 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.

[0631] In some embodiments, 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.

[0632] In some embodiments, 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.

[0633] In some embodiments, performing a laser-assisted micro-stapedotomy includes using a KTP otologic laser and/or a CO2 otologic laser.

[0634] As another example, a composition disclosed herein is administered using a device and/or system specifically designed for intracochlear route of administration. In some embodiments, 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.

[0635] 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.

[0636] An exemplary device for use in any of the methods disclosed herein is described in **FIGs. 29-32**. **Fig. 29** 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. 30**) with stopper 28 (shown in **Fig. 30**) 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. 30**) disposed therewithin.

[0637] Referring still to **Fig. 29**, 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 embodiments, may be coupled to a control system and/or power supply (not shown)). In some embodiments, the bent needle 38 (shown in **Fig. 30**) 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 embodiments, 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 embodiments 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)).

[0638] **Fig. 30** illustrates a sideview of the bent needle sub-assembly 26, according to aspects of the present disclosed embodiments. 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. 33**) 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.

[0639] **Fig. 31** 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. 31**) 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. 31**), 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. 31**), 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. 31**) 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 form 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.

[0640] **Fig. 32** 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 embodiments. As shown in **Fig. 32**, bent needle sub-assembly 26 may include a needle 38 coupled to a bent portion 32. In other embodiments, 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. 32**, bent needle sub-assembly 26 may also include a stopper 28 disposed around bent portion 32. **Fig. 32** 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 embodiments, 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. 32** 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.

Methods of Treating a Subject

[0641] The present disclosure provides, among other things, that technologies described herein may be used to treat an underlying disease and/or symptoms in a subject suffering from or at risk of an otological disease characterized by neovascularization (e.g., acoustic neuromas, e.g., VS).

[0642] In some embodiments, a method comprises administering a construct (e.g., an rAAV construct) described herein, a particle (e.g., an rAAV particle), or a composition described herein to a subject. In some embodiments, a method is a method of treatment. In some embodiments, a subject is a subject suffering from or at risk of an otological disease characterized by neovascularization (e.g., acoustic neuromas, e.g., VS).

[0643] In some embodiments, administering a construct (e.g., an rAAV construct) described herein, a particle (e.g., an rAAV particle), or a composition described herein to a subject may alleviate and/or ameliorate one or more symptoms associated with an otological disease characterized by neovascularization (e.g., acoustic neuromas, e.g., VS). Symptoms can include, for example, hearing loss, degeneration of hair cells, alteration of biochemical milieu of inner ear fluids, elevated intralabyrinthine protein, endolymphatic hydrops, cochlear aperture obstruction, intralabyrinthine hemorrhage, disruption of cochlear vascular supply, tinnitus, dizziness, intractable headache, facial neuropathy, trigeminal neuropathy, facial paralysis, facial paresthesia, hydrocephalus, cerebellar herniation, and/or death.

[0644] In some embodiments, an otological disease characterized by neovascularization (e.g., acoustic neuromas, e.g., VS) is associated with a gene mutation (e.g., a deletion mutation, a frameshift mutation, a nonsense mutation, a hypomorphic mutation, a hypermorphic mutation, a neomorphic mutation, aberrant over expression, aberrant under expression, etc.). In some embodiments, a subject suffering from or at risk of an otological disease characterized by neovascularization (e.g., acoustic neuromas, e.g., VS) may have a mutation in a gene related to otological tumor progression, which may be characterized as described herein.

[0645] In some embodiments, a subject is genetically and/or symptomatically characterized prior to, during, and/or after treatment with technologies described herein (e.g. real-time PCR, quantitative real-time PCR, Western blotting, immunoprecipitation, immunohistochemistry, mass spectrometry, or immunofluorescence, indirect phenotypic determination of expression of a gene and/or protein (e.g., through functional hearing tests, ABRs, DPOAEs, etc.), etc.). In some embodiments, a subject suffering from or at risk of an otological disease characterized by neovascularization (e.g., acoustic neuromas, e.g., VS) may have their associated disease state characterized through tissue sampling (e.g., comprising one or more inner ear cells, e.g., comprising one or more hair cells and/or one or more supporting cells). In some embodiments, tissues are evaluated via morphological analysis to determine morphology of hair cells and/or support cells before, during, and/or after administration of any technologies (e.g., methodologies, e.g., compositions, e.g., compositions comprising constructs, and/or particles, etc.) as described herein. In some such embodiments, standard immunohistochemical or histological analyses may be performed. In some embodiments, if cells are used *in-vitro* or *ex-vivo*, additional immunocytochemical or immunohistochemical analyses may be performed. In some

embodiments, one or more assays of one or more proteins or transcripts (e.g., western blot, ELISA, polymerase chain reactions) may be performed on one or more samples from a subject or *in-vitro* cell populations.

[0646] In some embodiments, administering a construct (e.g., an rAAV construct) described herein, a particle (e.g., an rAAV particle), or a composition described herein to a subject improves a subject's immunohistochemical evaluation (e.g., tests as described above) when compared to immunohistochemical tests performed prior to treatment with technologies described herein or when compared to a control population.

Treating a Subject to Improve Symptoms of Hearing Loss, Tinnitus, Dizziness, and/or Speech

[0647] In some embodiments, a subject suffering from or at risk of an otological disease characterized by neovascularization (e.g., acoustic neuromas, e.g., VS) may receive a treatment regimen that is characterized by hearing function. In some embodiments, functionality of a treatment regimen is characterized through hearing function, wherein such hearing function is determined in an individual using auditory brainstem response measurements (ABR) before, after, and/or during treatment with compositions and methods described herein. In some embodiments, functionality of a treatment regimen is characterized through hearing function, wherein such hearing function is determined in an individual by measuring distortion product optoacoustic emissions (DPOAEs) before, after, and/or during treatment with compositions and methods described herein. In some such embodiments, hearing measurements are taken from one or both ears of a subject. In some such embodiments, 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 embodiments, a subject has ABR and/or DPOAE measurements recorded prior to receiving any treatment. In some embodiments, 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 embodiments, ABR and/or DPOAE measurements are taken after treatment is administered and at regular follow-up intervals post-treatment. In some embodiments, treatment with technologies described herein improve a subject's test evaluation (e.g., tests as described above) when compared to tests performed prior to treatment with technologies described herein or when compared to a control population.

[0648] In some embodiments, a subject suffering from or at risk of an otological disease characterized by neovascularization (e.g., acoustic neuromas, e.g., VS) may have a treatment regimen that is characterized by hearing function as a function of speech, speech understanding, and/or tone recognition. In some embodiments, functionality of a treatment regimen is characterized through determination of speech pattern recognition and/or is determined by a speech therapist. In some embodiments, functionality of a therapeutic treatment regimen with technologies described herein is determined by pure tone testing. In some embodiments, functionality of a therapeutic treatment regimen with technologies described herein is determined by bone conduction testing. In some embodiments, functionality of a therapeutic treatment regimen with technologies described herein is determined by acoustic reflex testing. In some embodiments, functionality of a therapeutic treatment regimen with technologies described herein is determined by tympanometry. In some embodiments, functionality of a therapeutic treatment regimen with technologies described herein is determined by any combination of hearing analysis known in the art. In some embodiments, functionality of a therapeutic treatment regimen with technologies described herein is measured holistically, and/or from one or both ears of a subject. In some embodiments, functionality of a therapeutic treatment regimen with technologies described herein utilizes recordings and/or professional analysis taken post-regime 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 embodiments, a treatment 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 embodiments 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 embodiments, 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. In some embodiments, treatment with technologies described herein improve a subject's test evaluation (e.g., tests as described above) when compared to tests performed prior to treatment with technologies described herein or when compared to a control population.

[0649] In some embodiments, a subject suffering from or at risk of an otological disease characterized by neovascularization (e.g., acoustic neuromas, e.g., VS) may undergo treatment functionality evaluation through a method comprising behavioral audiometry evaluation. In some embodiments, behavioral audiometry evaluation comprises pure-tone audiometry with air and bone curves with appropriate masking, Speech audiometry, Words in quiet, or words in noise analysis. In some embodiments, behavioral audiometry evaluation comprises electrophysiologic audiometry by auditory brainstem response testing. In some embodiments, behavioral audiometry evaluation comprises standardized questionnaires: HHIA: Hearing Handicap Inventory for Adults, DHI: Dizziness Handicap Inventory, THI: Tinnitus Handicap Inventory, PANQOL: Penn Acoustic Neuroma Quality of Life (QoL). In some embodiments, treatment with technologies described herein improve a subject's test evaluation (e.g., tests as described above) when compared to tests performed prior to treatment with technologies described herein or when compared to a control population.

[0650] In some embodiments, a subject suffering from or at risk of an otological disease characterized by neovascularization (e.g., acoustic neuromas, e.g., VS) may be monitored by evaluation of otologic, vestibular, and/or systemic adverse events, as well as hematology, clinical chemistry, and/or urinalysis parameters. In some embodiments, a subject undergoing treatment with technologies described herein may be assessed for anti-VEGF protein levels in blood and construct DNA in ear swabs, nasal swabs, saliva, and blood. In some embodiments, a subject undergoing treatment with technologies described herein may have blood collected for evaluation of potential humoral immune responses to the capsid and/or secreted transgene product.

[0651] In some embodiments, a subject suffering from or at risk of an otological disease characterized by neovascularization (e.g., acoustic neuromas, e.g., VS) may have their treatment functionality analyzed through ABR tests. In some embodiments, an ABR test measures whether the subject's cochlea, cochlear nerve, and brainstem responds to each sound stimulus, such tests are often used as a measure of the health of the ear. In some embodiments, in addition to measuring the lowest intensity of each stimulus frequency that the subject's brainstem could reliably process (threshold), the amplitude of Wave I in response to suprathreshold stimuli can be measured in order to assess the integrity of the afferent flow of information from the cochlear hair cells to the auditory nerve. In some embodiments, ABR thresholds can provide important information about the lowest level of sound that a subject's ear passes along to and is processed by the brainstem, the

suprathreshold responses in the amplitude of the ABR Wave I has increasingly been used as a proxy for the integrity of the ribbon synapse connection between the base of the inner hair cells and the auditory nerve dendrites. In some embodiments, treatment with technologies described herein improve a subject's ABR evaluation when compared to tests performed prior to treatment with technologies described herein or when compared to a control population.

[0652] In some embodiments, a subject suffering from or at risk of an otological disease characterized by neovascularization (e.g., acoustic neuromas, e.g., VS) may have their treatment functionality analyzed through DPOAE based tests. In some embodiments, DPOAEs are sounds created by movement of the cochlear outer hair cells and are non-invasively measured in the ear canal with a transducer & microphone combination. In some embodiments, a size of evoked DPOAEs is a useful measure of outer hair cell function. In some embodiments, two primary tones (f1 and f2) are presented to an ear producing mechanical vibrations that causes pressure changes in cochlear fluids at stimulus and distortion frequencies. In some embodiments, these pressure changes drive the ear in reverse, activating the middle ear and then eardrum to produce sound in the ear canal. In some embodiments, DPOAEs are collected at the same test frequencies used in ABRs (8, 16, 32 kHz) and may be performed while a subject is under anesthesia for ABRs. In some embodiments, an f2 is centered at 8, 16, and 32 kHz while an f1=f2*0.8 + 10 dB. In some embodiments, at each frequency, tones are presented from 10-80 dB SPL in 5-dB ascending increments. In some embodiments, DPOAEs assess outer hair cell function and are therefore typically used as a suprathreshold assessment of the strength of the response, measured as an amplitude of the distortion product emission response. In some embodiments, all three test frequencies are used. In some embodiments, reliable distortion product responses may not be obtainable for each frequency tested, in such cases, analyses may be done as appropriate. In some embodiments, treatment with technologies described herein improve a subject's BPOAE evaluation when compared to tests performed prior to treatment with technologies described herein or when compared to a control population.

Treating a Subject to Reduce Tumor Size and/or Vasculature

[0653] In some embodiments, a subject suffering from or at risk of an otological disease characterized by neovascularization (e.g., acoustic neuromas, e.g., VS) may have their treatment functionality analyzed through imaging based techniques. In some embodiments, efficacy of

treatment of an individual with compositions and methods described herein may be measured using radiographic methods. In some embodiments, such radiographic methods may comprise but are not limited to computed tomography (CT) (also known as a computerized axial tomography (CAT) scan), X-ray, magnetic resonance imaging (MRI), three-dimensional fluid-attenuated inversion recovery (3D-FLAIR) MRI, positron emission tomography (PET), and/or PET-CT scans. Additional radiographic techniques and methods that may be suitable are known in the art. In some embodiments, treatment with technologies described herein improve a subject's imaging based evaluation when compared to tests performed prior to treatment with technologies described herein or when compared to a control population.

[0654] In some embodiments, treatment(s) of a subject with technologies described herein are associated with a decrease in VS size as measured by a radiographic technique. In some embodiments, treatment(s) of a subject with technologies described herein are associated with a stasis of VS size as measured by a radiographic technique. In some embodiments, treatment(s) of a subject with technologies described herein are associated with a slowing of VS size growth as measured by a radiographic technique.

[0655] In some embodiments, treatment(s) of a subject with technologies described herein are associated with a decreased signal intensity of the fluid on three-dimensional fluid-attenuated inversion recovery (3D-FLAIR) MRI. In certain embodiments, there is a correlation between a higher cochlear signal on 3D FLAIR images and hearing loss in patients with VS. In certain embodiments, treatment(s) of a subject with technologies described herein reduce concentration of proteins in the perilymphatic space associated with enhanced cochlear signal on FLAIR images in subjects with VS.

Treating a Subject to Improve Neurological Function

[0656] In some embodiments, treatment(s) of a subject with technologies described herein are associated with an improvement in neurological function. In some embodiments, treatment(s) of a subject with technologies described herein alleviates tumor edema. In some embodiments, treatment(s) of a subject with technologies described herein transiently and/or stably normalizes tumor vasculature in an individual.

[0657] In some embodiments, treatment(s) of a subject with technologies described herein are associated with an improvement and/or restoration of speech understanding. In some

embodiments, treatment(s) of a subject with technologies described herein are associated with a patient perceived reduction in the difficulty of speech understanding. In some embodiments, treatment(s) of a subject with technologies described herein are associated with an improvement and/or restoration of an individual's reported quality of life.

Methods of Characterizing a Disease State

[0658] Among other things, the present disclosure provides methods of characterizing a disease state of a subject, e.g., a subject suffering from or at risk of VS. In some embodiments, a disease state can be characterized by determining the presence (or absence) of a mutation in a gene. The term "mutation in a gene" refers to a modification in a known consensus functional gene that results in the production of a protein having one or more of: a deletion in one or more amino acids, one or more amino acid substitutions, and one or more amino acid insertions as compared to the consensus functional protein, and/or results in a decrease in the expressed level of the encoded protein in a mammalian cell as compared to the expressed level of the encoded protein in a mammalian cell not having a mutation. In some embodiments, a mutation can result in the production of a protein having a deletion in one or more amino acids (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 16, 17, 18, 19, 20, or more amino acids). In some embodiments, the mutation can result in a frameshift in the gene. The term "frameshift" is known in the art to encompass any mutation in a coding sequence that results in a shift in the reading frame of the coding sequence. In some embodiments, a frameshift can result in a nonfunctional protein. In some embodiments, a point mutation can be a nonsense mutation (e.g., result in a premature stop codon in an exon of the gene). A nonsense mutation can result in the production of a truncated protein (as compared to a corresponding consensus functional protein) that may or may not be functional. In some embodiments, the mutation can result in the loss (or a decrease in the level) of expression of mRNA or protein or both the mRNA and protein. In some embodiments, the mutation can result in the production of an altered protein having a loss or decrease in one or more biological activities (functions) as compared to a consensus functional protein.

[0659] In some embodiments, the mutation is an insertion of one or more nucleotides into a gene. In some embodiments, the mutation is in a regulatory and/or control sequence of the gene, e.g., a portion of the gene that is not coding sequence. In some embodiments, a mutation in a regulatory and/or control sequence may be in a promoter or enhancer region and prevent or reduce

the proper transcription of the gene. In some embodiments, a mutation is in a known heterologous gene known to interact with a protein.

[0660] Methods of genotyping and/or detecting expression or activity of a gene mRNA and/or protein are known in the art (see e.g., Ito et al., World J Otorhinolaryngol. 2013 May 28; 3(2): 26–34, and Roesch et al., Int J Mol Sci. 2018 Jan; 19(1): 209, each of which is incorporated in its entirety herein by reference). In some embodiments, level of expression of mRNA or protein may be detected directly (e.g., detecting a protein, detecting an mRNA etc.). Non-limiting examples of techniques that can be used to detect expression and/or activity of a gene directly include, e.g., real-time PCR, quantitative real-time PCR, Western blotting, immunoprecipitation, immunohistochemistry, mass spectrometry, or immunofluorescence. In some embodiments, expression of a gene and/or protein can be detected indirectly (e.g., through functional hearing tests, ABRs, DPOAEs, etc.).

[0661] In some embodiments, tissue samples (e.g., comprising one or more inner ear cells, e.g., comprising one or more hair cells and/or one or more supporting cells) may be evaluated via morphological analysis to determine morphology of hair cells and/or support cells before and after administration of any agents (e.g., compositions, e.g., compositions comprising constructs, and/or particles, etc.) as described herein. In some such embodiments, standard immunohistochemical or histological analyses may be performed. In some embodiments, if cells are used *in-vitro* or *ex-vivo*, additional immunocytochemical or immunohistochemical analyses may be performed. In some embodiments, one or more assays of one or more proteins or transcripts (e.g., western blot, ELISA, polymerase chain reactions) may be performed on one or more samples from a subject or *in-vitro* cell populations.

Evaluating Hearing Loss, Tinnitus, Dizziness, and Symptom Recovery

[0662] In some embodiments, hearing function is determined in an individual using auditory brainstem response measurements (ABR) before, after, and/or during treatment with compositions and methods described herein. In some embodiments, hearing function is determined in an individual by measuring distortion product optoacoustic emissions (DPOAEs) before, after, and/or during treatment with compositions and methods described herein. In some such embodiments, measurements are taken from one or both ears of a subject. In some such embodiments, 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 embodiments, a subject has ABR and/or DPOAE measurements recorded prior to receiving any treatment. In some embodiments, 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 embodiments, ABR and/or DPOAE measurements are taken after treatment is administered and at regular follow-up intervals post-treatment.

[0663] In some embodiments, hearing function is determined using speech pattern recognition or is determined by a speech therapist. In some embodiments, hearing function is determined by pure tone testing. In some embodiments, hearing function is determined by bone conduction testing. In some embodiments, hearing function is determined by acoustic reflex testing. In some embodiments hearing function is determined by tympanometry. In some embodiments, hearing function is determined by any combination of hearing analysis known in the art. In some such embodiments, measurements are taken holistically, and/or from one or both ears of a subject. In some such embodiments, 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 embodiments, 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 embodiments 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 embodiments, 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.

[0664] In some embodiments, any of the methods disclosed herein comprise behavioral audiometry evaluation. In some embodiments, behavioral audiometry evaluation comprises pure-tone audiometry with air and bone curves with appropriate masking, Speech audiometry, Words in quiet, or words in noise. In some embodiments, behavioral audiometry evaluation comprises electrophysiologic audiometry by auditory brainstem response testing. In some embodiments,

behavioral audiometry evaluation comprises standardized questionnaires: HHIA: Hearing Handicap Inventory for Adults, DHI: Dizziness Handicap Inventory, THI: Tinnitus Handicap Inventory, PANQOL: Penn Acoustic Neuroma Quality of Life (QoL).

[0665] In some embodiments of any of the methods disclosed herein, safety and efficacy may be monitored by evaluation of otologic, vestibular, and systemic adverse events, as well as hematology, clinical chemistry, and/or urinalysis parameters. In some embodiments of any of the methods disclosed herein, additional parameters to be assessed will include anti-VEGF protein levels in blood and construct DNA in ear swabs, nasal swabs, saliva, and blood. In some embodiments of any of the methods disclosed herein, blood may also be collected for evaluation of potential humoral immune responses to the capsid and secreted transgene product.

[0666] The ABR test measures whether the animal's cochlea, cochlear nerve, and brainstem responds to each sound stimulus and is often used as a measure of the health of the ear. This same basic test is commonly used to test hearing of newborn humans in hospitals and it is a standard hearing test used in lab animals. In some embodiments, an ABR test involves injecting mice with an IP dose of ketamine/xylazine anesthetic to minimize movement and muscle artifacts and to aid in the placement of measurement electrodes. In some embodiments, an ABR test (when done with the DPOAE at the same time, and in both ears) takes approximately 45 minutes to complete, so a booster dose of anesthetic is sometimes required. In some embodiments, an initial dose of anesthetic consists of ketamine (100 mg/kg) and xylazine (10 mg/kg) IP and if needed, a ketamine only booster which consists of ¼-½ of the original ketamine dose. In some embodiments, no redosing of xylazine is performed based on veterinarian recommendations. In some embodiments, transdermal recording electrodes through the skin surface are placed at three standard locations (at vertex of the skull equidistant between the ears, over the mastoid behind the pinna on the test-ear, and over the mastoid behind the pinna for the opposite ear for the grounding electrode). In some embodiments, stimuli consists of low (8 kHz), middle (16 kHz), and high (32 kHz) frequency pure tone pip stimuli (0.1 ms rise fall, 1.5 ms duration) from 10 - 80 dB SPL in ascending 5-dB steps. In some embodiments, stimuli will be presented at a rate of 30/sec, and 512 artifact-free averages are acquired at each stimulus level. In some embodiments, ABRs were collected at 2-3 separate time points, depending upon the group; baseline and at terminal week 4-6 with a subset of animals also tested at 3 weeks post-surgery. In some embodiments, in addition to measuring the lowest intensity of each stimulus frequency that the animal's brainstem could reliably process (threshold),

the amplitude of Wave I in response to suprathreshold stimuli can be measured in order to assess the integrity of the afferent flow of information from the cochlear hair cells to the auditory nerve. In some embodiments, ABR thresholds can provide important information about the lowest level of sound that an animal's ear passes along to and is processed by the brainstem, the suprathreshold responses in the amplitude of the ABR Wave I has increasingly been used as a proxy for the integrity of the ribbon synapse connection between the base of the inner hair cells and the auditory nerve dendrites.

[0667] DPOAEs are sounds created by movement of the cochlear outer hair cells and are non-invasively measured in the ear canal with a transducer & microphone combination. In some embodiments, a size of evoked DPOAEs is a useful measure of outer hair cell function. This same basic test is also commonly used to test hearing of newborn humans in hospitals and it is a standard hearing test used in lab animals. In some embodiments, two primary tones (f1 and f2) are presented to an ear producing mechanical vibrations that causes pressure changes in cochlear fluids at stimulus and distortion frequencies. In some embodiments, these pressure changes drive the ear in reverse, activating the middle ear and then eardrum to produce sound in the ear canal. In some embodiments, DPOAEs are collected at the same test frequencies used in ABRs (8, 16, 32 kHz) and while under anesthesia for ABRs. In some embodiments, an f2 is centered at 8, 16, and 32 kHz while an f1=f2*0.8 + 10 dB. In some embodiments, at each frequency, tones are presented from 10-80 dB SPL in 5-dB ascending increments. In some embodiments, DPOAEs assess outer hair cell function and are therefore typically used as a suprathreshold assessment of the strength of the response, measured as an amplitude of the distortion product emission response. In some embodiments, all three test frequencies are used. In some embodiments, reliable distortion product responses may not be obtainable for each frequency tested, in such cases, analyses may be done as appropriate.

Evaluating Tumor Size and Vasculature

[0668] In some embodiments, efficacy of treatment of an individual with compositions and methods described herein may be measured using radiographic methods. In some embodiments, such radiographic methods may comprise computed tomography (CT) (also known as a computerized axial tomography (CAT) scan), X-ray, magnetic resonance imaging (MRI), three-dimensional fluid-attenuated inversion recovery (3D-FLAIR) MRI, positron emission tomography

(PET), and/or PET-CT scans. Additional radiographic techniques and methods that may be suitable are known in the art.

[0669] In some embodiments, treatment of an individual with methods and/or compositions described herein are associated with a decrease in VS size as measured by a radiographic technique. In some embodiments, treatment of an individual with methods and/or compositions described herein are associated with a stasis of VS size as measured by a radiographic technique. In some embodiments, treatment of an individual with methods and/or compositions described herein are associated with a slowing of VS size growth as measured by a radiographic technique.

[0670] In some embodiments, treatment of an individual with methods and/or compositions described herein are associated with a decreased signal intensity of the fluid on three-dimensional fluid-attenuated inversion recovery (3D-FLAIR) MRI. In certain embodiments, there is a correlation between a higher cochlear signal on 3D FLAIR images and hearing loss in patients with VS. In certain embodiments, methods and/or compositions as described herein reduce concentration of proteins in the perilymphatic space associated with enhanced cochlear signal on FLAIR images in subjects with VS.

Evaluating Neurological Function

[0671] In some embodiments, treatment of an individual with methods and/or compositions described herein are associated with an improvement in neurological function. In some embodiments, treatment of an individual with methods and/or compositions alleviates tumor edema. In some embodiments, treatment of an individual with methods and/or compositions transiently and/or stably normalizes tumor vasculature in an individual.

[0672] In some embodiments, treatment of an individual with methods and/or compositions described herein are associated with an improvement and/or restoration of speech understanding. In some embodiments, treatment of an individual with methods and/or compositions described herein are associated with a patient perceived reduction in the difficulty of speech understanding. In some embodiments, treatment of an individual with methods and/or compositions described herein are associated with an improvement and/or restoration of an individual's reported quality of life.

Evaluating anti-VEGF Protein Concentration in Biological Samples

[0673] In some embodiments, methods described herein include evaluating anti-VEGF protein concentrations in one or more biological samples form an individual before, during, and/or after treatment with compositions described herein.

[0674] In some embodiments 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 anti-VEGF protein. In some embodiments, an increase in expression of an active anti-VEGF protein as described herein when compared relative to a control level, e.g., as compared to the level of expression of an anti-VEGF protein prior to introduction of the compositions comprising any construct(s) as described herein.

[0675] Methods of detecting expression and/or activity of a target RNA and/or protein are known in the art. In some embodiments, 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 directly include: real-time PCR, Western blotting, immunoprecipitation, immunohistochemistry, mass spectrometry, or immunofluorescence. In some embodiments, expression of an inner ear cell target protein can be detected indirectly (e.g., through functional hearing tests).

[0676] In some embodiments, biodistribution and/or shedding analysis of rAAV particles is performed. In some embodiments, disclosed herein is a composition comprising an rAAV-antiVEGF replication-defective, rAAV particle for delivering a cDNA, e.g., to express an anti-VEGF protein. In some embodiments, an rAAV particle as described herein is used to treat a subject, e.g., a human, e.g., a patient, with VS or at risk of developing VS. In some embodiments, a composition disclosed herein is administered via an intracochlear route. In some embodiments, a composition disclosed herein is administered at a dose of rAAV particles described herein (e.g., as measured by vector genome qPCR analysis). In some embodiments, a composition disclosed herein is administered to a localized area of the body. In some embodiments, a composition disclosed herein results in detectable expression of construct sequences at a localized area of the body, e.g., a cochlea and/or perilymph. In some embodiments, a composition disclosed herein, is expected to result in limited vascular spread and systemic exposure. In some embodiments, a composition disclosed herein results in higher levels of construct sequences in the cochlea, but not

in non-cochlear tissue or fluid compartments, e.g., liver, spleen, lymph nodes, brainstem, auditory cortext, serum and/or CSF. In some embodiments, a composition disclosed herein results in lower but detectable levels of construct sequences in non-cochlear tissues and fluids collected. In some embodiments, expression of construct sequences in non-cochlear tissue is at a level that is not therapeutically relevant. In some embodiments, levels of construct sequences were generally decreased overall by six months. In some embodiments, levels of construct sequences decrease over time in blood samples, e.g., one month following intracochlear administration of a composition disclosed herein.

[0677] In some embodiments of any of the methods disclosed herein, relevant fluids for biodistribution and shedding (e.g., blood, serum, urine, saliva, nasal and ear swabs, and CSF fluid) are collected and/or evaluated. In some embodiments of any of the methods disclosed herein, non-target tissues are collected and/or evaluated. In some embodiments, capsid variant is expected to determine tropism. In some embodiments, a composition disclosed herein comprises a capsid variant, e.g., AAVAnc80 capsid variant. In some embodiments, delivery of a composition disclosed herein comprising a capsid variant, e.g., via the same route of administration, in the same particle formulation, and/or at equivalent or lower particle doses, is not expected to result in differences in biodistribution and shedding patterns.

[0678] In some embodiments, a composition disclosed herein, e.g., rAAV-antiVEGF, is dosed at a level less than about 5 x 10(12) total vg/cochlea. In some embodiments, a composition disclosed herein, e.g., rAAV-antiVEGF, is dosed at a level less than about 5×10^{13} vg/mL. In some embodiments, a dose of a composition disclosed herein is a function of, e.g., limitations of volume and rAAV particle concentration.

[0679] In contrast, clinical trials using rAAV particles have delivered more than 1×10^{14} vg/kg via systemic routes of administration, in some cases to participants younger than 6 months of age (AveXis 2019, incorporated herein in its entirety by reference).

[0680] Other localized deliveries with relatively low doses of rAAV particles have not reported extensive biodistribution beyond the target area, e.g., throughout the body, or extensive rAAV particle shedding (excretion/secretion; generally measuring underlying construct DNA concentration through methods such as qPCR). For example, low levels of distribution beyond the ocular target area (specifically in the optic nerve of the particle-injected eye, optic chiasm, spleen

and liver, and sporadically in the lymph nodes of study animals) have been reported for Luxturna® delivered bilaterally at a dose of 7.5x 10^{11} vg/eye. Similarly, in the Phase 3 clinical trial, rAAV particle was shed transiently and at low levels in tears of 45% of the participants; it was detected, also at low levels, in serum (but not whole blood) samples of 10% of the participants in the days immediately following subretinal administration of Luxturna® delivered bilaterally at a dose of 1.5 x 10^{11} vg/eye (Russell 2017; Spark Therapeutics 2017, each of which is incorporated herein in its entirety by reference).

[0681] In some embodiments, any of the methods disclosed herein comprise evaluating a distribution of rAAV particle sequences in blood (e.g., serum and whole blood) over a time course following unilateral administration to the cochlea using, e.g., a validated qPCR method. In some embodiments, additional specimens (e.g., external auditory canal swabs, nasal swabs, saliva, and urine) will be collected for evaluation of shedding from the subject. In some embodiments, a specimen is collected from a subject until at least three consecutive negative samples are obtained.

[0682] In some embodiments, proteins correlating with VS associated hearing loss are measured before, during, and/or after treatment with compositions and/or methods described herein. In certain embodiments, such VS associated hearing loss associated proteins include: μ-Crystallin (CRYM), low density lipoprotein receptor-related protein 2 (LRP2), immunoglobulin (Ig) γ-4 chain C region, Ig κ-chain C region, complement C3, immunoglobulin heavy constant γ 3, and/or chemokine receptor-4 (CXCR4).

[0683] In some embodiments, immunogenicity to AAV capsids and/or particles are measured. Immunogenicity to AAV capsids and/or particles delivered to localized areas, and in relatively low doses compared to systemic applications, have generally not yielded specific patterns of immune responses; importantly, responses observed through both humoral and cell-mediated immunological monitoring (e.g., through enzyme-linked immunosorbent assay [ELISA]/neutralizing antibody [NAb] and enzyme-linked immunosorbent spot [ELISPOT] assays, respectively) have predominantly been without clinical correlate for route(s) of administration (ROA) that afford some immunological protection (e.g., direct administration to the brain).

[0684] In some embodiments of any of the methods disclosed herein, an intracochlear ROA, e.g., in species with a non-patent cochlear aqueduct (e.g., NHPs and humans), is expected to provide a similar level of protection. In some embodiments of any of the methods disclosed herein,

a subject will receive a short, peri-operative course of an immunomodulatory regimen, e.g., systemic oral corticosteroids, for approximately 17 days, beginning 3 days before administration of a compositions disclosed herein, e.g., rAAV-antiVEGF. In some embodiments, an immunomodulatory regimen reduces inflammation related to the surgical administration procedure. In some embodiments, an immunomodulatory regimen can also further reduce the potential for an immune reaction to either a capsid (e.g., AAVAnc80) or the underlying construct (e.g., a transgene product, e.g., an anti-VEGF protein).

[0685] In some embodiments, any method disclosed herein further comprise evaluating humoral immunity (e.g., antibody responses) in response to administration of a composition disclosed herein. In some embodiments, effect of pre-existing immunity, measured e.g., by serum NAb levels, on the transduction of a compositions disclosed herein when delivered via the intracochlear ROA is evaluated. In some embodiments, pre-existing NAb levels do not inhibit transduction of AAV particles delivered by an intracochlear route of administration. In some embodiments, any method disclosed herein further comprise evaluating serum for potential systemic humoral responses to both the AAV capsid and/or the transgene product (e.g., a secreted protein). In some embodiments, responsive to the evaluation of systemic humoral responses, a treatment interval for bilateral intracochlear administration of a composition disclosed herein, e.g., rAAV-antiVEGF can be developed.

[0686] In some embodiments, any method disclosed herein does not result in cytotoxic T cell responses, e.g., to either an AAV particle, capsid, and/or construct (e.g. underlying transgene) product from rAAV particles delivered via a localized route of administration (ROA), such as intracochlear administration. For example, the labeling for Luxturna® notes that no subject had a clinically significant, cytotoxic T cell response (Spark Therapeutics 2017, incorporated herein in its entirety by reference); isolated positive interferon-gamma (IFN-gamma) ELISPOT assay results were obtained during the clinical development program (Bennett 2012, incorporated herein in its entirety by reference), but the significance of these isolated results is unknown, as no clinical inflammatory response was observed and no dose limiting toxicity was seen in the clinical program.

[0687] In certain embodiments, pharmacokinetics of any of the compositions or products of compositions described herein are measured and collected. In certain embodiments, anti-VEGF

protein is secreted; prior art has described high doses of systemic anti-VEGF protein as associated with hypertension, proteinuria, elevated liver enzymes, arterial thromboembolic events, venous thromboembolic events, hemorrhage, and surgery and wound healing complications when delivered systemically at high doses over time (5 to 15 mg/kg every 2 to 3 weeks). In some embodiments of any of the methods disclosed herein, a composition disclosed herein is administered locally. In some embodiments, local delivery of a composition disclosed herein results in a decrease in likelihood of any one or more detrimental off-target effects. In some embodiments, local delivery of a composition disclosed herein does not result in any detrimental off-target effects. In some embodiments of any of the methods disclosed herein, a subject is followed-up by monitoring anti-VEGF protein in serum (e.g., using an electrochemiluminescence assay), vital signs, urinalysis, and/or clinical chemistry. In some embodiments, monitoring of a subject administered a composition disclosed herein allows for early intervention and/or minimization of any off-target effects.

[0688] In some embodiments, following administration of a composition as described herein, serum can be collected and analyzed for anti-VEGF protein measurement. In some embodiments, such measurements can take place prior to composition administration (Baseline), at week 2 following administration, and monthly for an appropriate duration (e.g., 3 months, 6 months, 1 year, 2 years, 3 years, 4 years, or greater than 5 years). In some embodiments, anti-VEGF protein will not be detected in an individual's serum at baseline, or at any timepoint post-administration in individuals that received intracochlear delivery of either vehicle or a dose of rAAV-antiVEGF particles described herein.

[0689] In some embodiments of any method disclosed herein, a method comprises collection and/or evaluation of serum for presence of an anti-VEGF protein using, (e.g., an electrochemiluminescence assay as described herein).

Production Methods

[0690] AAV systems are generally well known in the art (see, e.g., Kelleher and Vos, Biotechniques, 17(6):1110-17 (1994); Cotten 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.

[0691] 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.

[0692] In some embodiments, 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 embodiments, such a stable host cell contains such component(s) under the control of an inducible promoter. In some embodiments, such component(s) may be under the control of a constitutive promoter. In some embodiments, 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.

[0693] 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).

[0694] In some embodiments, 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 embodiments, 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 (e.g., rep and cap), which function in trans for productive AAV replication and encapsidation. In some embodiments, the AAV helper function construct supports efficient AAV construct production without generating any detectable wild type AAV particles (e.g., 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 (e.g., "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.

[0695] 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 AAV--the helper functions (e.g., 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.

[0696] In some embodiments, viral construct titers post-purification are determined. In some embodiments, titers are determined using quantitative PCR. In certain embodiments, a TaqMan probe specific to a construct is utilized to determine construct levels. In certain embodiments, the TaqMan probe is represented by SEQ ID NO: 97, while forward and reverse amplifying primers are exemplified by SEQ ID NO: 98 and 99, respectively.

[0697] **Exemplary TaqMan probe for quantification of constructs (SEQ ID NO: 97)**

/56-FAM/TAATTCCAA/ZEN/CCAGCAGAGTCAGGGC/3IABkFQ/

[0698] **Exemplary forward qPCR primer for quantification of constructs (SEQ ID NO: 98)**

GATACAGCTAGAGTCCTGATTGC

[0699] **Exemplary reverse qPCR primer for quantification of constructs (SEQ ID NO: 99)**

GATCTGCCAAGTACCTCACTATG

[0700] As described herein, in some embodiments, 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 embodiments, an AAV particle is an AAV2/6, AAV2/8, AAV2/9, or AAV2/Anc80 particle (e.g., with AAV6, AAV8, AAV9 or Anc80 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 particle 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 embodiments, an AAV construct is a self-complementary AAV construct.

[0701] The present disclosure provides, among other things, methods of making AAV-based constructs. In some embodiments, such methods include use of host cells. In some embodiments, 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.

[0702] 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 (e.g., 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 cells with constructs that encode helper functions, or cells can be engineered to stably contain genes encoding helper functions, expression of which can be controlled at the transcriptional or posttranscriptional level.

[0703] 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.

[0704] In some embodiments of any of the methods disclosed herein, a focused set of analytical testing and a shorter stability testing protocol is used for a composition disclosed herein, e.g., rAAV-antiVEGF, e.g., AAV-anti VEGF drug substance (DS). In some embodiments, the DS and the drug product (DP) for a composition disclosed herein, e.g., rAAV-antiVEGF, are quite similar, with, e.g., only an additional filtration step and vialing constituting the DP manufacture from the DS. In some embodiments, DP undergoes a more comprehensive analytical testing and a longer stability testing protocol. In some embodiments, DS is not stored long-term, e.g., DS is moved promptly from DS to DP manufacture. In some embodiments, DS is stored, e.g., held at ≤ -65 °C prior to the fill/finish process.

[0705] In some embodiments of any of the methods disclosed herein, stability of a composition disclosed herein, e.g., rAAV-antiVEGF DS and DP, is evaluated using assays that are, e.g., indicative of stability of the product including Appearance, Vector Genome Titer (ddPCR), Potency, and/or Bioburden (DS) or Sterility (DP). In some embodiments, a potency assay comprises a specification once sufficient data have been generated to support the establishment of a specification. In some embodiments, DS and DP will be placed on 2-8 °C stability for about 6 weeks. In some embodiments, DS is not stored at 2-8 °C for extended periods of time. In some embodiments, DP is stored for 60 months at the target storage temperature (≤ -65 °C). In some embodiments, DS is stored, e.g., placed on stability, for about 12 months.

[0706] In some embodiments, potency and strength of a composition disclosed herein, e.g., rAAV-antiVEGF is evaluated using an assay, e.g., a matrix of assays as described herein. In some embodiments, the assays comprise evaluating multiple product characteristics, and will be used to assess product functional activity, consistency, stability, and/or to provide evidence of comparability after any changes are introduced to the manufacturing process. In some embodiments, ddPCR titer assay are qualified for suitability prior to administering a composition disclosed herein to a subject. In some embodiments, expression of anti-VEGF protein is assessed in a mammalian cell line *in-vitro*. In some embodiments, protein expression is assessed across

several multiplicities of infection (MOIs) and compared relative to a reference standard. In some embodiments, a functional bioassay is used in a method disclosed herein, , e.g., including commercially available kits and published methods for determining anti-VEGF activity. In some embodiments, a functional bioassay that assesses potency is used, e.g., a sensitive and quantitative measure of the bioactivity of expressed anti-VEGF protein.

[0707] In some embodiments, a manufacturing method as disclosed herein is utilized. In some embodiments a manufacturing method is not expected to impact quality attributes of an rAAV particle, or to affect the interpretation of the associated studies.

[0708] In some embodiments, a composition disclosed herein comprises a recombinant particle, e.g., rAAV-antiVEGF, which comprises two components, e.g., an AAVAnc80 capsid (Zinn 2015, which is incorporated herein in its entirety by reference) and single-stranded DNA genome of 3555 nucleotides, exclusive of inverted terminal repeat (ITR) sequences (an example of a construct represented by **FIG. 6, Panel (A)**, and represented by SEQ ID NO: 92), encapsidated by the AAVAnc80 capsid. In some embodiments, a composition disclosed herein, e.g., an rAAV-antiVEGF particle, expresses ranibizumab, a 48 kDa humanized monoclonal Fab (derived from full-length murine anti-human VEGF monoclonal antibody A.4.6.1) which is used, e.g., clinically to inhibit VEGF.

EXAMPLES

[0709] 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.

[0710] It is believed that one or ordinary skill in the art can, using the preceding description and following Examples, as well as what is known in the art, to make and utilize technologies of the present disclosure.

Example 1: Construction of Viral Constructs

[0711] This example provides a description of generating exemplary viral constructs encoding an anti-VEGF protein, as described herein. 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 derived anti-VEGF proteins under single, dual, or multi construct strategies can be generated. AAV serotypes 1, 2, 3, 4, 5, 6, 7, 8, 9, rh8, rhl0, rh39, rh43, and Anc80 can each be prepared to encapsulate anti-VEGF protein constructs, and form rAAV particles. Among other things, the rAAV particles can be utilized 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/or (iv) a single construct strategy.

Example 1.1: Construction of rAAV-Ranibizumab, rAAV-Ranibizumab-PC, and rAAV-Bevacizumab-PC Constructs

[0712] This example provides a description of generating rAAV-ranibizumab, rAAV-ranibizumab-GFP, and rAAV-bevacizumab-PC constructs, as described herein. A first recombinantly generated construct had a sequence according to SEQ ID NO: 90. This construct is referred to throughout the Examples as an "rAAV-ranibizumab-PC construct." An rAAV-ranibizumab-PC construct is an exemplary embodiment of an rAAV-V_H/V_L construct represented by the schematic shown in **FIG. 6, Panel (A)**, and comprises a sequence that encodes for ranibizumab. A second recombinantly generated construct had a sequence according to SEQ ID NO: 91. This construct is referred to throughout the Examples as an "rAAV-ranibizumab construct." An rAAV-ranibizumab construct is an exemplary embodiment of an rAAV-V_H/V_L construct represented by the schematic shown in **FIG. 6, Panel (A)**, and comprises a sequence that encodes for ranibizumab. A third recombinantly generated construct had a sequence according to SEQ ID NO: 106. This construct is referred to throughout the Examples as an "rAAV-ranibizumab-GFP construct." An rAAV-ranibizumab-GFP construct is an exemplary embodiment of an rAAV-ABGFP construct represented by the schematic shown in **FIG. 6, Panel (B)**, and comprises a sequence that encodes for ranibizumab. A fourth recombinantly generated construct had a sequence according to SEQ ID NO: 93. This construct is referred to throughout the Examples as an "rAAV-bevacizumab-PC construct." An rAAV-bevacizumab-PC construct is an exemplary

embodiment of an rAAV-AB construct represented by the schematic shown in **FIG. 6, Panel (C)**, and comprises a sequence that encodes for the anti-VEGF protein bevacizumab.

[0713] Certain constructs as described above, a 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. An AAVAnc80 capsid was then generated with an rAAV construct above to form either rAAVAnc80-ranibizumab, rAAVAnc80-ranibizumab-PC, and rAAVAnc80-bevacizumab-PC particles.

Example 1.2: Construction of rAAV-Bevacizumab, rAAV-Bevacizumab-GFP, rAAV-Aflibercept-PC, and rAAV-Aflibercept Constructs

[0714] This example provides a description of generating rAAV-bevacizumab, rAAV-bevacizumab-GFP, rAAV-aflibercept-PC, and rAAV-aflibercept constructs, as described herein. One construct has a sequence according to SEQ ID NO: 94. This construct is referred to throughout the Examples as an "rAAV-bevacizumab construct." An rAAV-bevacizumab construct is an exemplary embodiment of an rAAV-AB construct represented by the schematic shown in **FIG. 6, Panel (C)**, and comprises a sequence that encodes for the anti-VEGF protein bevacizumab. Another construct has a sequence according to SEQ ID NO: 107. This construct is referred to throughout the Examples as an "rAAV-bevacizumab-GFP construct." An rAAV-bevacizumab-GFP construct is an exemplary embodiment of an rAAV-ABGFP construct represented by the schematic shown in **FIG. 6, Panel (B)**, and comprises a sequence that encodes for the anti-VEGF protein bevacizumab. Another construct has a sequence according to SEQ ID NO: 95. This construct is referred to throughout the examples as an "rAAV-aflibercept-PC construct." An rAAV-aflibercept-PC construct is an exemplary embodiment of an rAAV-TRAP construct represented by the schematic shown in **FIG. 6, Panel (D)**, and comprises a sequence that encodes for the anti-VEGF protein aflibercept. Another construct has a sequence according to SEQ ID NO: 96. This construct is referred to throughout the examples as an "rAAV-aflibercept construct." An rAAV-aflibercept construct is an exemplary embodiment of an rAAV-TRAP construct represented by the schematic shown in **FIG. 6, Panel (D)**, and comprises a sequence that encodes for the anti-VEGF protein aflibercept. rAAV particles are generated by transfection with an adenovirus-free method as described by Xiao et al. J Virol. 73(5):3994-4003, 1999, which is incorporated in its entirety herein by reference. An individual rAAV-bevacizumab-PC, rAAV-bevacizumab-GFP, rAAV-aflibercept-PC, or rAAV-aflibercept construct as described above, a trans plasmid with

AAV Rep and Cap genes, and a helper plasmid with an essential region from an adenovirus genome are co-transfected in HEK293 cells. An AAVAnc80 capsid is then generated with an rAAV construct described above to form either an rAAVAnc80-bevacizumab, rAAVAnc80-bevacizumab-PC, rAAV-Anc80-ranibizumab-GFP, rAAVAnc80-aflibercept-PC, and rAAVAnc80-aflibercept particles.

Example 2: Purifying Viral Particles

[0715] This example provides a description of methods that can be used to purify constructs encoding anti-VEGF proteins, as described herein.

Example 2.1: Purifying rAAVAnc80-Ranibizumab, rAAVAnc80-Ranibizumab-PC, and rAAVAnc80-Bevacizumab-PC Particles

[0716] This example provides a description of methods that can be used to purify rAAV particles encoding anti-VEGF proteins, e.g., those formed in Example 1. rAAVAnc80-ranibizumab, rAAVAnc80-ranibizumab, and rAAVAnc80-bevacizumab-PC particles produced in Example 1.1 were each independently 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. Following the second CsCl density gradient centrifugation step, 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 particles 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 probes corresponding to the ITR region of the AAV construct genome (see, e.g., Bartoli et al. Gene. Ther. 13:20-28, 2006, which is incorporated in its entirety herein by reference).

Example 2.2: Purifying rAAVAnc80-Ranibizumab-GFP, rAAVAnc80-Bevacizumab, rAAVAnc80-Bevacizumab-GFP, rAAVAnc80-Aflibercept, and rAAVAnc80-Aflibercept-PC Particles

[0717] This example also provides a description of methods that can be used to purify rAAV particles encoding anti-VEGF proteins, e.g., those formed in Example 1. rAAVAnc80-ranibizumab particles produced in Example 1.1, and rAAVAnc80-bevacizumab, rAAVAnc80-bevacizumab-GFP, rAAVAnc80-aflibercept-PC, and rAAVAnc80-aflibercept particles produced in Example 1.2 are each independently 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. Following the second CsCl density gradient centrifugation step, 11 fractions of 500 μL are recovered from the CsCl density gradient tube and purified through dialysis in 1x PBS. The fractions are analyzed by dot blot to determine those containing rAAV genomes. The viral genome number (vg) of each preparation is determined by a quantitative real-time PCR-based titration method using primers and probes corresponding to the ITR region of the AAV construct genome (see, e.g., Bartoli et al. Gene. Ther. 13:20-28, 2006, which is incorporated in its entirety herein by reference).

Example 2.3: Purifying rAAVAnc80 Particles

[0718] This example also provides a description of additional methods that can be used to purify rAAV particles encoding anti-VEGF proteins, e.g., those formed in Example 1. rAAV-antiVEGF particles are purified in the following manner 72 hours post transfection. 10% Triton is added to the solution (for a final concentration of 2%) and the cell-culture is incubated for 1 hour at 37°C on a cell-shaker. Subsequently, 100U/mL benzonase and 2uM MgCl2 are added, and the cells are incubated for 2 hours at 37°C on a cell-shaker. Next, NaCl (150uM) is added and the cells are incubated for 20 minutes at 37°C on a cell-shaker. The samples are centrifuged at 2000xg for 10 minutes. The supernatant is transferred to a clean container and stored at -80°C, or immediately subjected to Tangential Flow Filtration (TFF). The filtered supernatant is then loaded into AVB columns to undergo AVB column chromatography and rAAV filtration. Filtered eluate is then subjected to CsCl density gradient centrifugation (RI=1.3905 – 1.3915, spin speed = 65,000 RPM for ≥ 16 h at 15 °C). The purified full-particle sample is then subjected to a final round of TFF and formulations are vialed for later use.

Example 3: Formulation of rAAVAnc80-antiVEGF Particles

[0719] This example relates to the preparation of compositions comprising viral particles including constructs encoding an anti-VEGF protein, as described herein.

Example 3.1: Formulation of rAAVAnc80-Ranibizumab, rAAVAnc80-Ranibizumab-PC, and rAAVAnc80-Bevacizumab-PC Particles

[0720] rAAVAnc80 particles including constructs rAAV-ranibizumab, rAAV-ranibizumab-PC, or rAAV-bevacizumab-PC, each encoding an anti-VEGF protein, as described herein were

produced and purified, as set forth in the Examples above, to an appropriate titer as determined by quantitative digital droplet (dd) PCR (*e.g.*, of approximately 1.39^{13} vg/mL). Purified particles were then prepared at known dilutions (*e.g.*, at $6x10^4$, $1.3x10^5$, $1.8x10^5$, $4.5x10^9$, and $1.3x10^{10}$ vg/mL) in physiologically acceptable solution. The physiologically acceptable solution used included a 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). A titer quantification of rAAVAnc80-ranibizumab particles, rAAVAnc80-ranibizumab-PC particles, or rAAVAnc80-bevacizumab-PC particles, was performed using a forward primer sequence: CCGATTTCGGCCTATTGGTTA (SEQ ID NO: 100), a reverse primer sequence CTGTGGAGAGAAAGGCAAAGT (SEQ ID NO: 101), and a probe sequence GGCACCTATTGGTCTTACTGACATCC (SEQ ID NO: 102) with an appropriate fluorophore (FAM).

Example 3.2: Formulation of rAAVAnc80-Ranibizumab, rAAVAnc80-Ranibizumab-PC, and rAAVAnc80-Bevacizumab-PC Particles

[0721] rAAVAnc80 particles including constructs rAAV-ranibizumab-GFP, rAAV-bevacizumab, rAAV-bevacizumab-GFP, rAAV-aflibercept, or rAAV-aflibercept-PC, each encoding an anti-VEGF protein, as described herein are produced and purified, as set forth in the Examples above, to an appropriate titer as determined by quantitative digital droplet (dd) PCR (*e.g.*, of approximately 1.39^{13} vg/mL). Purified particles are then prepared at known dilutions (*e.g.*, at $6x10^4$, $1.3x10^5$, $1.8x10^5$, $4.5x10^9$, and $1.3x10^{10}$ vg/mL) in physiologically acceptable solution. The physiologically acceptable solution can include a commercially available 1xPBS with pluronic acid F68, and can be 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). A titer quantification of rAAVAnc80-ranibizumab-GFP particles, rAAVAnc80-bevacizumab particles, rAAVAnc80-bevacizumab-GFP particles, rAAVAnc80-aflibercept particles, or rAAVAnc80-aflibercept-PC particles, is performed using a forward primer sequence: CCGATTTCGGCCTATTGGTTA (SEQ ID NO: 100), a reverse primer sequence CTGTGGAGAGAAAGGCAAAGT (SEQ ID NO: 101), and a probe sequence GGCACCTATTGGTCTTACTGACATCC (SEQ ID NO: 102) with an appropriate fluorophore (e.g., FAM).

Example 4: Device Description

[0722] This example relates to a device suitable for the delivery of rAAV particles, including the rAAV particles comprising constructs encoding anti-VEGF proteins described herein, to the inner ear. A specialized microcatheter designed for consistent and safe penetration of the round window membrane can be used to deliver a composition as provided herein to the cochlea of a subject (e.g., as depicted in **Figure 4**). The microcatheter is shaped such that a 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 round window membrane. 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 round window membrane that are sufficient to allow rAAV particles comprising constructs as described herein (*e.g.*, comprising a coding sequence for an anti-VEGF protein) 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 µL/min to approximately 90 µL/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 $1x10^{12}$ to $5x10^{13}$ 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: Expression and Secretion of anti-VEGF Proteins Produced From Cell Lines

[0723] This example relates to the transduction and/or transfection of exemplary constructs, and expression and secretion of exemplary proteins described herein.

Example 5.1: Expression and Secretion of Proteins from rAAV-Ranibizumab-PC, rAAV-Ranibizumab-GFP, and rAAV-Bevacizumab-PC Constructs

[0724] Cell lines (*e.g.*, HEK239FT) were transfected with exemplary rAAV-antiVEGF constructs or transduced with exemplary rAAVAnc80-antiVEGF particles as described herein. For transfection events, HEK293FT cells were seeded overnight at $7x10^4$ cells/well in a 24-well plate format with a culture volume of 400 µL. Approximately 800 ng of anti-VEGF constructs rAAV-ranibizumab-PC, rAAV-bevacizumab-PC, or rAAV-ranibizumab-GFP (as described in Example 1) were transfected into cells using jetprime transfection reagent (Polyplus-transfection® SA). For transduction events, HEK293FT cells were seeded for 6 hours at $4x10^4$ cells/well in a 96-well plate

format with a culture volume of 50 µL in the presence of 2 µM etoposide (Fisher Scientific 34120525MG), exemplary rAAVAnc80-bevacizumab-PC particles (as described in Example 1) were added into the media at an MOI of $7.5x10^4$, $2.2x10^5$, and $5.5x10^5$ respectively. For cells either transfected or transduced, supernatant was harvested at 72 hours post treatment for each sample. For protein expression analysis, 30 µL of samples were loaded into individual wells in a 4-12% Bis-Tris protein gel and standard western blotting procedures as known in the art were conducted. Banding patterns were determined using a fluorescent reader, with test anti-ranibizumab antibody (targeted at the Fab epitope; supplied by Abcam, ab168684-25UG) as the primary detection probe, and anti-human IgG as the secondary detection probe (see **FIG. 7**).

Example 5.2: Expression and Secretion of Proteins from rAAV-Ranibizumab, and rAAV-Bevacizumab, rAAV-Bevacizumab-GFP, rAAV-Aflibercept, and rAAV-Aflibercept-PC Constructs

[0725] Cell lines (*e.g.,* HEK239FT) are transfected with exemplary anti-VEGF constructs or transduced with exemplary rAAVAnc80-antiVEGF particles as described herein. For transfection events, HEK293FT cells are seeded overnight at approximately 7x104 cells/well in a 24-well plate format with a culture volume of 400 µL. Approximately 800 ng of rAAV-Ranibizumab, rAAV-Bevacizumab, rAAV-Bevacizumab-GFP, rAAV-Aflibercept-PC, or rAAV-Aflibercept constructs(*e.g.,* as described in Example 1) are transfected into cells using jetprime transfection reagent (Polyplus-transfection® SA). For transduction events, HEK293FT cells are seeded for 6 hours at $4x10^4$ cells/well in a 96-well plate format with a culture volume of 50 µL in the presence of 2 µM etoposide (Fisher Scientific 34120525MG). rAAVAnc80-ranibizumab, rAAVAnc80-ranibizumab-PC, rAAVAnc80-ranibizumab-GFP, rAAVAnc80-bevacizumab, rAAVAnc80-bevacizumab-GFP, rAAVAnc80-aflibercept-PC, or rAAVAnc80-aflibercept (*e.g.,* as described in Example 1) are added to the cell media at an MOI of approximately $7.5x10^4$, $2.2x10^5$, and/or $5.5x10^5$. For cells either transfected or transduced, supernatant is harvested at approximately 72 hours post treatment for each sample. For protein expression analysis, 30 µL of samples are loaded into individual wells in a 4-12% Bis-Tris protein gel and standard western blotting procedures as known in the art are conducted. Banding patterns are determined using a fluorescent reader, with test anti-ranibizumab (targeted at the Fab epitope; supplied by Abcam, ab168684-25UG) and test anti-aflibercept antibodies as the primary detection probe, and anti-human IgG as the secondary detection probe.

Example 6: Binding Activity of Anti-VEGF Proteins Produced From Cell Lines

[0726] This example describes how to determine a binding activity of exemplary anti-VEGF proteins produced as described in Example 5 above.

Example 6.1: Binding Activity of Anti-VEGF Proteins Ranibizumab and Bevacizumab

[0727] Binding activity of exemplary anti-VEGF proteins ranibizumab or bevacizumab produced through transfection as described in Example 5 above were analyzed using Octet® HTX biosensor instruments. A first set of control experiments were performed to calibrate the plasmon surface resonance instrumentation, *e.g.*, using a mouse anti-human VEGF monoclonal antibody (anti-hVEGF MmAb; R&D Systems, MAB293-100) added into control cell conditioned medium using recombinant human VEGF as the binding agent (**FIG. 8, Panel (A)**). A second set of experiments were performed to determine the VEGF-binding activity of particles comprised in conditioned medium from HEK293TF cells following transfection of exemplary constructs rAAV-ranibizumab-PC, or rAAV-bevacizumab-PC as described in Example 1, or control non-transfected cell conditioned medium. Graphical results for test materials are presented in **FIG. 8, Panel (B)** or **Panel (C)**, with control media represented in **FIG. 8, Panel (D)**.

[0728] Test samples included soluble ranibizumab and bevacizumab secreted and present in medium from HEK293TF cells transfected with constructs rAAV-ranibizumab-PC, or rAAV-bevacizumab-PC as described in Example 1. Samples were prepared by diluting cell conditioned media 1:10 in 1x kinetics buffer (Fortebio, 18-1105) and aliquoted into a 384-well sample plate. Anti-hVEGF MmAb (R&D Systems, MAB293-100) was diluted at a concentration of 10 μg/mL as a positive control. The capture agent, recombinant human VEGF (R&D Systems, 293-VE-010) was diluted in a series of 1:2 dilution ratio from 200 nM to 3.125 nM.

[0729] The binding affinities of the conditioned medium samples and mouse anti-human VEGF antibody (R&D Systems) samples were measured in 1x kinetics buffer in Octet® HTX biosensor instrument. The binding features and values were generated by the Octet® analysis software, Data Analysis HT10.0. Data collected is represented in **Table 2**, where the respective K_D, equilibrium association constant (k_a), and the dissociation (k_{dis}), are listed.

[0730] The anti-hVEGF mouse antibody (R&D Systems) showed high binding affinity. Similarly, rAAV construct encoded ranibizumab or bevacizumab showed high binding affinity to human VEGF. No K_D value could be extrapolated from the binding data of control conditioned medium sample. Together, these data show that the constructs as provided herein can result in expression and secretion of anti-VEGF proteins ranibizumab or bevacizumab, and that constructs as provided herein may be used to express anti-VEGF proteins in the inner ear of a mammal.

[0731] **Table 2: Binding kinetics of recombinant anti-VEGF proteins**

Sample ID	KD (M)*	ka (1/Ms)	kdis (1/s)	Full R^2
Bevacizumab	3.30E-10	8.49E+04	2.80E-05	0.9998
Ranibizumab	1.68E-10	2.45E+05	4.11E-05	0.9991
MmAb-R&D	2.29E-10	3.40E+05	7.77E-05	0.9993

[0732] Secreted ranibizumab was capable of binding biotinylated recombinant human VEGF (Acro Biosystems, VE5-H8210) with a KD of 168 pM and secreted bevacizumab was capable of binding with a KD of 330 pM (KD = 229 pM for positive control mouse anti-human VEGF recombinant antibody [R&D Systems, MAB293-100]). Compared to the binding affinity for ranibizumab and bevacizumab to VEGF-A165, 20.6 and 35.1 pM, respectively (Papadopoulos 2012, incorporated herein in its entirety by reference), these data suggest that rAAV constructs in plasmid form comprising an anti-VEGF protein coding sequence are capable of producing a protein *in-vitro* with an acceptable binding affinity.

Example 6.2: Binding Activity of Anti-VEGF Proteins Ranibizumab, Bevacizumab, and Aflibercept

[0733] Binding activity of exemplary anti-VEGF binding agents as described herein are analyzed using Octet® HTX biosensor instruments. anti-VEGF proteins are produced in cell lines (*e.g.*, HEK293FT cells) following transfection of exemplary constructs rAAV-ranibizumab, rAAV-bevacizumab, rAAV-ranibizumab-GFP, rAAV-bevacizumab-GFP, rAAV-aflibercept-PC, or rAAV-aflibercept as described in Example 1, or transduction with the same constructs comprised within an AAV Anc80 capsid to form an rAAV particle, e.g., rAAVAnc80-ranibizumab particle, rAAVAnc80-bevacizumab particle, rAAVAnc80-ranibizumab-GFP particle, rAAVAnc80-bevacizumab-GFP particle, rAAVAnc80-aflibercept-PC particle, or rAAVAnc80-aflibercept particle. A first set of control experiments are performed to calibrate the plasmon surface resonance instrumentation, *e.g.*, using a mouse anti-human VEGF monoclonal antibody

(anti-hVEGF MmAb; R&D Systems, MAB293-100) in control conditioned medium using recombinant human VEGF as the binding agent. A second set of experiments are performed to determine the human VEGF-binding activity of conditioned medium from HEK293TF cells following transfection or transduction of exemplary constructs or rAAV particles as described herein, or control conditioned medium. Graphical results depict exemplary binding activity of transgenic anti-VEGF proteins.

[0734] Test samples include soluble ranibizumab, bevacizumab, and/or aflibercept secreted and present in medium from HEK293TF cells transduced or transfected with constructs as described herein (*e.g.*, as described in Example 1). Samples are prepared by diluting cell conditioned media 1:10 in 1x kinetics buffer (Fortebio, 18-1105) and aliquoting into a 384-well sample plate. Anti-hVEGF MmAb (R&D Systems, MAB293-100) is diluted at a concentration of 10 μg/mL as a positive control. The capture agent, recombinant human VEGF (R&D Systems, 293-VE-010) is diluted in a series of 1:2 dilution ratio from 200 nM to 3.125 nM.

[0735] The binding affinities of the conditioned medium samples and mouse anti-human VEGF antibody (R&D Systems) samples are measured in 1x kinetics buffer in Octet® HTX biosensor instrument. The binding features and values are generated by the Octet® analysis software, Data Analysis HT10.0. Data collected is represented as K_D, equilibrium association constant (k_a), and the dissociation rate (k_{dis}).

Example 7: *Ex-vivo* demonstration of anti-VEGF protein production

[0736] This example relates to the introduction, and expression analysis of rAAV constructs expressing an anti-VEGF protein in mammalian cochlear explants grown *in-vitro* or *ex-vivo*. Cochlear explant culture models can provide a reliable experimental system to mimic the morphology and molecular characteristics of sensory hair cells and non-sensory supporting cells of the cochlea to study transduction and expression of rAAV particles within the intrinsic cellular environment found *in-vivo*.

Example 7.1: Ex-Vivo Production of Anti-VEGF Proteins Ranibizumab and Bevacizumab

[0737] Described herein are *ex-vivo* evaluations of anti-VEGF protein expression and secretion from newborn (P2) wild type (WT) mice cochlear explants transduced by rAAV (*e.g.*,

rAAVAnc80) particles comprising constructs rAAV-V_H/V_L or rAAV-AB, exemplified by rAAV-ranibizumab-PC or rAAV-bevacizumab-PC (as described in Example 1). In these experiments, an organ of Corti was dissected and mounted on coverslips, followed by incubation for three to four days with either vehicle or a range of doses of rAAV particles - rAAVAnc80-ranibizumab-PC particles or rAAVAnc80-bevacizumab-PC particles. An rAAVAnc80-bevacizumab-PC particle was transduced at 1.2E10, 2.4E10, and 3.5E10 vg/explant, while an rAAVAnc80-ranibizumab-PC particle was transduced at 1.4E10, 2.8E10, and 4.2E10 vg/explant.

[0738] The media surrounding the explants was harvested and analyzed using an MSD immunoassay, a platform employing an electrochemiluminescent detection technology to quantify analytes with higher sensitivity and larger dynamic range than traditional ELISA analysis. Streptavidin-coated MSD assay plates were blocked and coated with biotinylated VEGF ligand before incubation with the sample. Anti-VEGF proteins bound to the assay plate were then detected with a tagged goat polyclonal anti-κ light-chain (pAb) antibody (**FIG. 16, Panel (B)**). The explants were lysed for ribonucleic acid (RNA) expression analysis using quantitative real-time polymerase chain reaction (qRT-PCR) with Taqman primer-probes for GAPDH (housekeeping control) and for anti-VEGF protein encoding nucleotides (mRNA products encoding bevacizumab or ranibizumab) (**FIG. 16, Panel (A)**).

[0739] Anti-VEGF RNA was detected in the explants receiving rAAVAnc80-antiVEGF particles, but not those receiving vehicle (**FIG. 16, Panel (A)**), and secreted anti-VEGF protein was detected in the media of the explants receiving rAAVAnc80-antiVEGF particles, but not those receiving vehicle (**FIG. 16, Panel (B)**). These data suggest that administration of rAAVAnc80-antiVEGF particles comprising constructs encoding anti-VEGF proteins to WT mouse cochlea *ex-vivo* yields expression and secretion of the target protein from transduced cells. Cochlear explant results support *in-vivo* testing of intracochlear injection of WT mice with rAAVAnc80-antiVEGF particles to determine at least tolerability *in-vivo*, as well as to assess *in-vivo* secreted levels of anti-VEGF protein.

Example 7.2: Ex-Vivo Production of Anti-VEGF Proteins Ranibizumab, Bevacizumab, and Aflibercept

[0740] Mock rAAV particles or rAAV particles rAAVAnc80-ranibizumab, rAAVAnc80-ranibizumab-GFP, rAAVAnc80-bevacizumab, rAAVAnc80-bevacizumab-GFP, rAAVAnc80-aflibercept-PC, or rAAVAnc80-aflibercept (*e.g.,* as described in Example 1) are prepared and

transduced into newborn (P2) wild type (WT) mice cochlear explants at a suitable MOI (e.g., at 1.2E10, 1.4E10, 2.4E10, 2.8E10, 3.5E10, and/or 4.2E10 vg/explant, etc.) In these experiments, an organ of Corti is dissected and mounted on coverslips, followed by incubation for three to four days with either vehicle or a range of doses of rAAVAnc80-ranibizumab particles, rAAVAnc80-ranibizumab-GFP particles, rAAVAnc80-bevacizumab particles, rAAVAnc80-bevacizumab-GFP particles, rAAVAnc80-aflibercept-PC particles, or rAAVAnc80-aflibercept particles, as described in Example 1. Media surrounding the explants is harvested and analyzed using an MSD immunoassay. Streptavidin-coated MSD assay plates are blocked and coated with biotinylated VEGF ligand before incubation with the sample. Anti-VEGF proteins (e.g., ranibizumab, bevacizumab, and/or aflibercept) bound to the assay plate are then detected with a tagged goat polyclonal anti-κ light-chain (pAb) antibody.

[0741] Optionally, experiments are conducted to determine the mRNA expression level from rAAV-antiVEGF constructs transduced into wild type explants (*ex-vivo*). Mock rAAV particles or rAAV particles comprising rAAV constructs (*e.g.*, as described in Example 1) encapsidated by Anc80 capsids are prepared and transduced into explants at a suitable MOI (*e.g.*, at those described above, and/or at an MOI of approximately $4.5x10^9$ vg/cochlea or $1.5x10^{10}$ vg/cochlea). Supernatant is collected, and cells are harvested 72 hours post transduction using 350µL RLT Plus RNA lysis buffer (Qiagen), and RNA samples are prepared using the RNeasy Micro Kit (Qiagen). Relative mRNA expression levels are determined using quantitative real-time PCR with anti-VEGF protein encoding nucleotide specific amplifying primers (*e.g.*, primers specific for bevacizumab, ranibizumab, and/or aflibercept encoding nucleotides) and a TaqMan probe and are compared relative to an appropriate control (e.g., mouse GAPDH probe as control (Life Technologies)). Robust and dose dependent anti-VEGF protein encoding mRNA production is observed. Soluble and secreted anti-VEGF protein levels are determined as described above.

Example 8: Surgical Methods and rAAV Particle Analysis in Model Animals

[0742] The current example relates to the introduction of technologies (e.g., constructs, particles, and/or compositions) described herein to the inner ear of aged mice. rAAV particles comprising a construct encoding an anti-VEGF protein 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, doi·10 1089/hum.2016 053, June 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 (approximately 5-10 mg/kg) and ketamine (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. 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 µL of construct containing buffer (*e.g.*, rAAV constructs described herein at approximately $4.5x10^9$ to $5x10^{10}$ vg/per cochlea in artificial perilymph or 1xPBS with pluronic acid F68) to the scala tympani at a rate of 2 nL/second. The glass micropipette is left in place for 5 minutes post-injection. Following cochleostomy and injection, the opening in the tympanic bulla and the posterior semicircular canal 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.

[0743] Studies such as those described in the present example are conducted in preparation for first-in-human safety and dose escalation trials in adult participants with unilateral progressive sporadic VS. Intracochlear delivery of rAAV (*e.g.*, AAVAnc80, *e.g.*, AAVAnc80-antiVEGF) can be used to transduce cochlear and vestibular cells of the inner ear, which are then expected to secrete anti-VEGF protein into perilymph or directly into the interstitial fluid of the cochlear nerve. The lack of barriers to diffusion along the internal auditory canal results in the cochlear nerve being bathed in a continuum of fluid, with perilymph at its lateral end (nearest the cochlea, where the majority of VS tumors originate (**FIGS. 1 and 3**) and CSF at its medial end; thus, diffusion from perilymph into the nerve interstitium provides a path for therapeutic anti-VEGF protein expressed in perilymph to reach the intended VS target.

[0744] In mice, a surgical approach, e.g., that described in Yoshimura et al., 2018, can be utilized for the delivery of rAAV particles as described herein (Yoshimura 2018, incorporated herein in its entirety by reference). Specifically, delivery through the round window membrane

with fenestration of the posterior semicircular canal, which has demonstrated robust and reliable transduction, independent of the age of the animal at the time of injection (Yoshimura 2018, incorporated herein in its entirety by reference). A postauricular incision is made to access the temporal bone. A portion of the sternocleidomastoid muscle is divided to expose the otic bulla. A 0.5 to 0.6 mm diameter otologic drill is used to make a small hole in the bulla; the hole is then widened to visualize the stapedial artery and the round window membrane. Fenestration of the posterior semicircular canal is performed with the otologic drill (0.5 to 0.6 mm diameter) to serve as a vent the inner ear during cochlear administration. The round window membrane is penetrated with the mouse delivery device, which comprises a borosilicate capillary pipette and a 10 uL Hamilton syringe, and 1 μL of solution comprising viral particles (approximately 40 to 50% of the total inner ear volume) is delivered through the round window membrane, into the scala tympani, at rate of 300 nL/min. Studies of AAVAnc80 particles comprising constructs encoding an anti-VEGF protein are performed in mice with mature cochleae.

Example 9: Transgenic Expression, Analysis, and Imaging of Anti-VEGF Proteins in Mice

[0745] This example relates to the transgenic expression of anti-VEGF proteins in mice, and the subsequent imaging and phenotypic analysis of said animals.

Example 9.1: In-Vivo Expression, and Imaging of Anti-VEGF Proteins Ranibizumab and Bevacizumab in Mice

[0746] Biological tolerability and/or exposure to compositions comprising anti-VEGF proteins described herein were evaluated, including measuring anti-VEGF levels in CSF and serum, after intracochlear delivery of rAAV particles as described herein comprising constructs encoding antiVEGF. rAAVAnc80-ranibizumab-PC particles or rAAVAnc80-bevacizumab-PC particles (as described in Example 1) were injected into P30 WT CBA/CaJ mice). An outline of Study 1 groups can be found in Table 3. An additional study (referred to herein as “Study 2”) described herein was also performed, results of which are described below, an outline of the study groups can be found in Table 4. These studies captured data related to surgical introduction, rAAV particle transduction, and subsequent expression of- and exposure to- secreted anti-VEGF proteins. These results are summarized below and their details can be found in **FIGS. 17-26**. In these studies, rAAVAnc80-ranibizumab-PC particles or rAAVAnc80-bevacizumab-PC particles (as described in Example 1)were administered via unilateral intracochlear injection through the round window

membrane with posterior semicircular canal fenestration (as described herein). Vehicle-control animals were injected with formulation buffer.

[0747] CAB/CaJ wild type mice were anesthetized to prepare for introduction of compositions described herein. Vehicle controls, rAAVAnc80-ranibizumab-PC particles, or rAAVAnc80-bevacizumab-PC particles (as described in Example 1) were prepared and introduced to the mouse inner ear through the round window membrane. Introduction of rAAV particles was performed through the following steps: A) preauricular incision to expose the cochlear bulla, B) glass micropipettes (cat # 4878 - WPI) pulled with a micropipette puller (cat # P87 - Sutter instruments) to a final OD of about 10 μm were used to manually deliver (micropipettes held by a Nanoliter 2000 micromanipulator – WPI) compositions containing rAAV particles into the scala tympanic, which allows access to inner ear cells, C) 1 μL of a composition described herein at a suitable concentration in suitable buffer (described in Example 1, rAAVAnc80-ranibizumab-PC particles at approximately $1.4x10^{10}$ vg/per cochlea, or rAAVAnc80-bevacizumab-PC particles at approximately $1.2x10^{10}$ vg/per cochlea; each diluted in in 1xPBS with pluronic acid F68) was injected into each tested cochlea at a release rate of 0.3 μL/min (controlled by MICRO4 microinjection controller - WPI). Sham surgeries were performed as above with vehicle as a negative control. Mice were allowed to recover from surgery without additional intervention. At 5 weeks post-injection, animals were euthanized, cerebrospinal fluid (CSF) samples, serum samples, and inner ear sections for immunofluorescence analysis were collected.

Table 3: Study 1 Group Assignments

Group	Number of Animals*	Test Article	Concentration (vector genome [vg]/mL)	Dose (vg/cochlea)	Survival Period
1	10	rAAVAnc80-ranibizumab-PC particles	1.4E13	1.4E10	4 to 5 weeks
2	10	rAAVAnc80-bevacizumab-PC particles	1.2E13	1.2E10	4 to 5 weeks
3**	10	Vehicle	n/a	n/a	4 to 5 weeks

*Combined males and females (4 to 6 males per group). **Uninjected, contralateral ears from Vehicle-control group used as histological controls. Abbreviations: n/a = not applicable.

[0748] To evaluate anti-VEGF protein exposure as a result of inner ear transduction by rAAV particles as described herein (rAAVAnc80-antiVEGF particles), blood and CSF were collected upon termination at 4 to 5 weeks following intracochlear delivery, and anti-VEGF protein levels in CSF and serum were measured using an MSD immunoassay (e.g., as described in Example 7). Levels of soluble anti-VEGF protein (ranibizumab or bevacizumab) within the CSF was measured and ranged from 2.71-19.29 ng/mL. Following collection of these fluids, mice were transcardially perfused and cochleae were harvested and submersion-fixed for histological assessment. Cochlear samples were decalcified and microdissected. Dissected organ of Corti whole-mounts and cross-sections were stained with anti-ranibizumab (Abcam, ab168684-25UG, mAb) determine transgenic expression of constructs as described herein, and anti-Phalloidin (Thermo Fisher A12381) staining was used to characterize different inner ear cell types (**FIG. 17**) and anti-Myosin VIIa staining was used to label hair cells (depicted in **FIG. 18** and quantified in **FIG. 20**). Half of the samples were also stained with anti-Sox2 to label supporting cells and the other half with anti-neurofilament 200 to label neuronal projections (**FIG. 19**). Confocal analysis of immunostained tissue was used to identify transduction efficiency/anti-VEGF expression, and cell survival (**FIGS. 20 and 21**).

[0749] Anti-VEGF protein expression was evaluated based on the appearance of a signal and not the intensity. Without wishing to be bound to any particular theory, one reason for this could be that the polyclonal secondary antibody could potentially bind a larger number of epitopes on the humanized mouse antibody bevacizumab when conjugated to the mouse monoclonal primary anti-ranibizumab antibody (MmAb described above) as compared to the corresponding smaller sized humanized mouse antibody ranibizumab when conjugated to the monoclonal primary anti-ranibizumab antibody. Inner hair cells robustly expressed a transgene in a majority of cochleae (17 of 19 ears) receiving rAAVAnc80-ranibizumab-PC particles or rAAVAnc80-bevacizumab-PC particles (as described in Example 1, results depicted in **FIG. 17** and quantified in **FIG. 21**). Of these 17 cochleae, 14 also showed expression in a subset of OHCs and/or non-hair cells. No substantial difference was detected in the cell identity and expression pattern between ears receiving rAAVAnc80-ranibizumab-PC particles compared to those receiving rAAVAnc80-bevacizumab-PC particles.

[0750] Confirmation that this expression in cochlear tissues led to production and secretion of anti-VEGF protein (ranibizumab or bevacizumab) was determined. CSF (sampled from cisterna

magna) and serum samples were collected and analyzed from animals (**FIGS. 23 and 24**). Anti-VEGF protein concentrations in the perilymph can also be measured to better understand the ratio of anti-VEGF protein exposure between perilymph and CSF. Perilymph sample collection in mice can be technically challenging. In the present example, one perilymph sample was collected. Anti-VEGF protein (ranibizumab or bevacizumab) was detected in CSF of rAAV particle-injected animals compared to no detection in vehicle-injected controls (**FIG. 24**). Detection of secreted anti-VEGF protein by an MSD assay was limited to qualitative comparisons between ranibizumab and bevacizumab, owing to use of a similar polyclonal secondary as used in detecting expression in cochlear tissues (as described above), where a stronger signal is observed for samples from ears receiving rAAVAnc80-bevacizumab-PC particles due to greater availability of epitopes. Without wishing to be bound to any particular theory, clear detection of anti-VEGF protein in the CSF may be due to communication between perilymph and CSF in this species via the patent cochlear aqueduct.

[0751] Mouse serum was assessed for anti-VEGF protein (ranibizumab) via an MSD assay with an anti-κ light-chain (pAb) antibody that detects ranibizumab (described above and in Example 7). Compared to vehicle-injected animals, mice that received intracochlear delivery of rAAV-ranibizumab-PC particles demonstrated higher levels of anti-VEGF protein (ranibizumab) in serum at 4 to 5 weeks following delivery (**FIG. 23**).

[0752] Serum from mice that received intracochlear delivery of rAAV-ranibizumab, rAAV-ranibizumab-GFP, rAAV-bevacizumab-PC, rAAV-bevacizumab, rAAV-bevacizumab-GFP, rAAV-aflibercept-PC, or rAAV-aflibercept particles (e.g., comprising ranibizumab, bevacizumab, or aflibercept) can be evaluated.

[0753] Study 2 was also performed using the same parameters: e.g., mouse strain, age, rAAV capsid, ranibizumab encoding construct, and intracochlear administration procedure to address emerging data indicating possible surgical challenges. The study utilized rAAVAnc80-ranibizumab-PC particles (as described in Example 1), as in certain embodiments, ranibizumab may have different features when compared to bevacizumab, e.g., a higher binding capacity and higher binding affinity, no Fc domain, and a smaller molecular mass. rAAVAnc80-ranibizumab-PC particles (as described in Example 1) were administered at 1.4E10 vg/cochlea via unilateral intracochlear injection through the round window membrane with posterior semicircular canal

fenestration (as described herein). Uninjected ears of vehicle-injected mice served as controls. Upon study termination, animals were transcardially perfused and cochleae were harvested, decalcified, and microdissected for confocal microscopy imaging using the following labels: anti-ranibizumab (mAb) to detect transgene expression; anti-Myosin VIIa to label hair cells; and anti-Tuj1 to label projections of cochlear nerve fibers (*e.g.*, radial nerve fibers).

Table 4: Study 2 Group Assignments

Group	Number of Animals*	Test Article	Concentration (vector genome [vg]/mL)	Dose (vg/cochlea)	Survival Period
1	3	rAAV-ranibizumab-PC particle	1.4E13	1.4E10	4 weeks
2	2	Uninjected	n/a	n/a	4 weeks*

* From place-holder surgery date based on age of mice (no sham surgery). Abbreviations: n/a = not applicable.

[0754] Cochlear histology of the sensory epithelium in this study depicted broad distribution of anti-VEGF protein (ranibizumab) expression across the length of the cochlea (**FIG. 22**) in multiple cell types, including inner and outer hair cells, lateral supporting cells, and interdental cells of the spiral limbus. In general, hair cells were present in the analyzed regions of the cochleae, with the exception that some IHC loss was observed in the apical-most region of the cochlea of Mouse 1.

Example 9.2: Phenotypic Analysis of In-Vivo Ranibizumab and Bevacizumab Delivery and Production in Mice

[0755] rAAV-antiVEGF particles as described herein were evaluated for cochlear tolerability in mice. As part of a study conducted to evaluate transduction and subsequent protein secretion in mice following intracochlear delivery of exemplary rAAVAnc80-ranibizumab-PC particles, or rAAVAnc80-bevacizumab-PC particles (as described in Example 1), overall tolerability, auditory function, and cochlear hair cell survival were also assessed to evaluate cochlear tolerability of the surgical administration and transgene expression. No deleterious change in body weight or body condition score was detected throughout the 4 to 5 weeks post-intracochlear delivery, with the exception of one animal that died 1 week post-delivery for an unidentified reason (no surgical complications or aberrant findings on health observations were noted). No indication of damage to the vestibular system, as evidenced by circling or head-bobbing, was detected.

[0756] Assessment of auditory function via ABRs was performed prior to surgery and 4 to 5 weeks post-surgery (immediately prior to euthanasia). Threshold shifts were observed in groups that received rAAVAnc80-ranibizumab-PC particles, or rAAVAnc80-bevacizumab-PC particles, as well as in the group that received vehicle. Threshold shifts were of similar magnitude across groups, suggesting potential damage to the cochlea due to the surgical procedure and not due to transgene expression (**FIG. 25**).

[0757] Consistent with the functional assessments (ABRs; **FIG. 25**), hair cell loss was detected in groups that received rAAVAnc80-ranibizumab-PC particles, or rAAVAnc80-bevacizumab-PC particles, as well as the group that received vehicle, but not in the uninjected (contralateral) control ears. Damage to the basilar membrane at the base of the cochlea was detected in 16 of 19 ears that received rAAVAnc80-antiVEGF particles and in 9 of 10 ears that received vehicle, and a subsequent loss of outer hair cells in these damaged regions was prominent at 16 and 32 kHz (**FIG. 18**). Overall, IHC loss was not significant; sporadic IHC loss was only detected in the 8 kHz frequency region of 2 of 19 ears that received rAAVAnc80-antiVEGF particles (**FIG. 18**). While the route of administration resulted in hair cell damage in this particular study (referred to herein as "Study 1"), consistency between functional assessments and cell survival across ears, whether receiving rAAVAnc80-antiVEGF particles or vehicle, suggests that cell loss may have been due to the surgical procedure rather than from rAAV capsid toxicity, transgene expression, or subsequent protein secretion.

[0758] In light of the findings that surgical procedure may result in cell loss, Study 2 was conducted. This study showed no systematic damage to the basilar membrane and subsequent loss of OHC, and only sporadic loss at the extreme apex and base, at 4 weeks following intracochlear delivery of rAAVAnc80-ranibizumab-PC particles (as described in Example 1) (**FIG. 22**). Consistent with the qualitative assessment of overall hair cell survival in the corresponding regions, assessments of cochlear function (DPOAEs) and auditory function (ABRs) revealed normal thresholds at 4 weeks post-administration as compared to uninjected controls (**FIG. 26, Panels (A)** and **(B)**).

[0759] The combined data reveal delivery of rAAVAnc80-antiVEGF particles (such as rAAVAnc80-ranibizumab-PC particles, or rAAVAnc80-bevacizumab-PC particles (as described in Example 1), when introduced to mice, resulted in transgene expression in multiple cell types,

including both hair cells and various supporting cells, as well as detection of the secreted anti-VEGF protein (ranibizumab or bevacizumab) in CSF. In mouse, anti-VEGF protein was present in the CSF, without wishing to be bound to any particular theory, this may potentially occur through active fluidic exchange of perilymph and CSF via the patent cochlear aqueduct in this species. These results depict 1) robust anti-VEGF protein expression in cochlear cells and 2) detection of secreted anti-VEGF protein in CSF, supporting advancement of programs to more anatomically relevant and translatable species such as non-human primates.

Example 10: Phenotypic analysis of transgenic expression of anti-VEGF protein encoding mRNA and protein expression in murine models of Vestibular Schwannoma (VS)

[0760] The present example pertains to an analysis of phenotypes related to VS in murine models (*e.g.*, models as described in Chen et al., Nature Protocols 2019, which is incorporated herein in its entirety by reference). Model mice are prepared as described in Chen et al., mice are allowed to recover from surgery for an appropriate period of time (*e.g.*, approximately 1 week), and then are anesthetized to prepare for introduction of compositions described herein. Mock rAAV particles or rAAV constructs (e.g., such as those described in Example 1) encapsidated by Anc80 capsids are prepared and introduced to the mouse inner ear through the round window membrane. Introduction of rAAV-antiVEGF particles is performed through the following steps: A) preauricular incision to expose the cochlear bulla, B) glass micropipettes (e.g., cat # 4878 - WPI) pulled with a micropipette puller (e.g., cat # P87 - Sutter instruments) to a final OD of ~10 μm are used to manually deliver (e.g., micropipettes held by a Nanoliter 2000 micromanipulator – WPI) compositions containing rAAV particles into the scala tympanic, which allows access to inner ear cells, C) 1 μL of a composition described herein (*e.g.*, rAAV constructs described herein at approximately $4.5x10^9$ to $5x10^{10}$ vg/per cochlea in 1xPBS with pluronic acid F68) is injected into each tested cochlea at a release rate of 0.3μl /min (e.g., controlled by MICRO4 microinjection controller - WPI). Sham surgeries are performed as above with vehicle as a negative control. Mice are allowed to recover from surgery without additional intervention.

[0761] At appropriate intervals post-surgery (*e.g.*, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 7 weeks, and 8 weeks), VS murine models which have undergone unilateral or bilateral composition or sham injections are anesthetized with sodium pentobarbital (e.g., 35 mg/kg) delivered intraperitoneally. Mice are then placed and maintained in a head-holder within a

grounded and acoustically and electrically insulated test room. An evoked potential detection system (e.g., Smart EP 3.90, Intelligent Hearing Systems, Miami, FL, USA) is used to measure the thresholds of the auditory brainstem response (ABR) in mice. Click sounds as well as 8, 16, and 32 kHz tone bursts at varying intensity (e.g., from 10 to 130 dB SPL) are used to evoke ABRs in test mice. The response signals are recorded with a subcutaneous needle electrode inserted ventrolaterally into the ears of the mice. This example confirms that the introduction of exemplary constructs as described herein (*e.g.*, as described in Example 1) can reduce and/or prevent VS induced symptoms, further analysis may assist in determining the exact administration timing window and how anti-VEGF protein expression acts to inhibit VS induced symptoms. Results are depicted as ABR traces, and test animals and/or ears are compared to controls, which may include non-injected animals or non-injected ears. Improvements in ABR performance are observed in test ears when compared to control ears, or in test animals when compared to control animals. Improvements in response to stimuli may also be observed in non-injected ears due to crossover from the injected ear to the non-injected ear.

[0762] At appropriate intervals post-surgery (*e.g.*, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 7 weeks, and 8 weeks), VS murine models which have undergone unilateral or bilateral composition or sham injections are anesthetized (*e.g.*, with sodium pentobarbital (35 mg/kg) delivered intraperitoneally). Mice are subsequently euthanized, and VS tumors are harvested. Tumors volume, transcriptional pattern, protein composition, and/or histology are compared. Test mice injected with exemplary compositions as described herein display reduced tumor volume, altered transcriptional patterns, altered protein composition, and/or altered histology when compared to control non-injected VS model mice.

[0763] To evaluate the potential for inner ear toxicity, following gross examination, a cochleae can be processed, dissected, and stained, and a cytocochleogram analysis can be performed to evaluate loss of IHCs and/or OHCs. The opposite cochlea/ear can be processed for middle- and inner-ear histopathology and microscopic evaluation (*e.g.*, by a board-certified pathologist) to evaluate any gross and microscopic lesions.

Example 11: Analysis of Transgenic Expression of Anti-VEGF mRNA and Protein Expression in Non-Human Primate Animal Models

[0764] The present example provides *in-vivo* studies of rAAVAnc80-antiVEGF particles in non-human primates (NHP). These studies can determine among other things, the tolerability, toxicology, and phenotypic response following administration of methods and/or compositions as described herein. Appropriate NHP models (*e.g.,* Cynomolgus macque aka Macaca fascicularis) are utilized for this purpose as the inner-ear physiological similarities between NHP and humans are more pronounced than those found in murine models. The NHP animal model macaque has an inner-ear morphology that is largely representative of primate inner-ear morphology, where the lack of patent cochlear aqueduct facilitates the predictable and quantitative assessment of injectate volume dosed into the perilymph. Because of the relatively narrow diameter of the external auditory canal in NHPs compared with humans, the required surgical approach for this animal model is more invasive (entry through the mastoid/facial recess rather than through the external auditory canal). Without wishing to be bound to any particular theory, for the foregoing reasons, this approach may result in an overestimate of expected conductive loss, surgical trauma, or both. Despite having about one third the total inner ear volume of humans, the macaque cochlea is anatomically similar and the proposed drug delivery process (e.g., round window membrane injection) itself is conducted in the manner previously outlined. For analyzing and investigating the intracochlear administration of rAAV-antiVEGF particles as described herein, the macaque represents the most practical anatomically correct animal model to evaluate both safety and tolerability as well as the dose of rAAV particles needed to result in effective anti-VEGF protein exposure levels in close proximity to VS and/or related tumors.

Example 11.1: In-Vivo Expression, and Analysis of anti-VEGF Protein Ranibizumab in NHPs

[0765] In NHPs, rAAV-antiVEGF particles as described herein (e.g., rAAVAnc80-antiVEGF) were delivered as follows: a postauricular incision was made and dissection of the soft tissue was performed down to the level of the periosteum. The periosteum was incised and elevated to expose the mastoid bone. A cortical mastoidectomy was performed with a combination of high-speed cutting and diamond drill burs. The facial recess was then opened, allowing for adequate round window and oval window (OW) visualization. Fenestration of the stapes footplate in the OW was performed using a Rosen needle. As with other models, the fenestration allows for injection of a larger volume without damage to the inner ear; additionally, venting allows solution comprising rAAV-antiVEGF particles to flow toward the apex of the cochlea. Thirty μL of solution

comprising viral particles (approximately 40 to 50% of the total inner ear volume) was delivered through the round window membrane at a rate of 30 μL/min.

[0766] Compositions and methods described herein were evaluated for local and systemic tolerability using simultaneous bilateral intracochlear administration of rAAVAnc80-ranibizumab particles (as described in Example 1) in NHPs (cynomolgus macaques) followed for 2-month or 6-month recovery periods. Among other things, this evaluation: 1) demonstrated local and systemic tolerability and 2) permitted evaluation of expression levels of anti-VEGF protein in perilymph, CSF, serum, and other tissues after intracochlear administration of an rAAVAnc80-antiVEGF particle. Both low- and high-dose groups were used. The low- and high-dose groups were based on previous platform and supportive studies in NHPs in which doses around the corresponding concentration of the higher dose (1.0E13 vg/mL corresponding to 3.0E11 vg/cochlea; with a human equivalent of 9.0E11 vg/cochlea) were not associated with pathology in NHP hair cells, whereas a higher concentration (3.7E13 vg/mL corresponding to 1.1E12 vg/cochlea; human equivalent 3.3E12 vg/cochlea) was associated with potentially capsid-related pathology in hair cells of some animals. Studies conducted herein included an additional high-dose group and vehicle group with the primary objective of collecting perilymph to ensure sample availability in which to measure anti-VEGF protein levels (e.g., by MSD); these separate groups were needed, as perilymph collection almost invariably damages the surrounding cochlea.

[0767] The results obtained from these experiments show that anti-VEGF protein (Ranibizumab) was detected in the perilymph of NHPs. No anti-VEGF protein was detected in the perilymph of the vehicle-injected control NHP, and no anti-VEGF protein was detected in terminal CSF of any NHP, regardless of post-administration duration (2 or 6 months).

[0768] **FIG. 27** demonstrates a computational model which incorporates perilymph exposure levels to estimate diffusion properties from the inner ear to the typical VS site. Computational modeling (as depicted in **FIG. 27**) of diffusion of anti-VEGF protein through the internal auditory canal predicted that anti-VEGF levels in the perilymph exceeded the biologically active threshold concentration (IC 50 of Ranibizumab) within the reported range of early VS locations. The concentration necessary to inhibit biological activity of VEGF-A by 50% in an *in-vitro* cellular proliferation assay is 11 to 27 ng/mL as described in Genentech 2017, incorporated herein in its entirety by reference.

[0769] Serum anti-VEGF protein levels were evaluated in NHPs and the level of anti-VEGF protein detected was minimal to undetectable in all NHPs. Anti-VEGF protein was also not detected in any non-cochlear tissue lysate of any NHP including liver, spleen, brainstem, left and right auditory cortex, and left and right mandibular lymph node.

[0770] Taken together with clinical data demonstrating efficacy of systemic anti-VEGF protein in controlling VS tumor growth and alleviating VS tumor symptoms, these experiments highlighted the feasibility of producing an inner-ear depot of anti-VEGF protein for the purposes of potentially shrinking tumor size, and/or controlling tumor growth and its sequelae through local exposure to anti-VEGF proteins, thereby limiting adverse effects related to repeated systemic anti-VEGF protein administration.

Example 12: Phenotypic Analysis of NHPs Delivered rAAVAnc80-Ranibizumab

[0771] As described above, an exposure and tolerability study in NHPs (cynomolgus macaques) using rAAVAnc80-ranibizumab particles (as described in Example 1) was performed, where particles were delivered bilaterally into the cochlea, to assess local and systemic effects of rAAVAnc80-antiVEGF particles in NHPs. Among other things, this study provided data to inform cochlear tolerability (serial ABR measurements up to 6 months post- administration; cytocochleograms at 2- and 6-months post-administration; and otic histopathology at 2- and 6-months post-administration).

[0772] Bilateral intracochlear administrations of rAAVAnc80-ranibizumab particles (as described in Example 1) with two exemplary doses (Dose 1 and Dose 2) or vehicle were performed using a transmastoid/facial recess surgical approach to access the round and oval windows as described above. Dose 1 was lower than Dose 2.

[0773] Following administration, there were no documented significant clinical observations related to intracochlear administration and/or the test rAAVAnc80-antiVEGF particle. Minor neurological signs were considered to be related to surgical maneuvers that potentially affect the vestibular system, and the NHPs recovered without medical intervention. Overall, clinical observations for all NHPs, with 2- and 6-month recoveries, were mainly expected temporary findings following inner ear surgery.

Example 13: Human Administration

[0774] This example discloses treatment of a subject having hearing loss and/or additional symptoms associated with VS arising either incidentally or as associated with a syndrome (*e.g.*, Neurofibromatosis-2).

[0775] In humans, a less invasive approach for delivery of agents as described herein can be utilized when compared to that utilized in model organisms as described above, *e.g.,* through the external auditory canal; as in some embodiments, it is more practical since relevant structures are relatively large in humans, even at birth. In brief, a clinical administration method described herein can comprise a transcanal exploratory tympanotomy and laser-assisted microstapedotomy (using a potassium titanyl phosphate [KTP] or CO2 otologic laser to place a small vent hole [approximately 0.25 mm] in the stapes footplate), followed by a round window injection to deliver about 0.09 mL (or about 90 μL, approximately 40 to 50% of the total inner ear volume) of solution containing rAAV-antiVEGF particles as described herein through the round window membrane within a three-minute period. As with nonclinical and cadaveric models, venting serves to prevent a potential deleterious rise in intralabyrinthine pressure. Additional information regarding the clinical administration procedure and a delivery device can be found throughout the present disclosure.

[0776] A patient can be diagnosed as having Unilateral or Bilateral VS (*e.g.*, as determined by MRI). A clinical administration procedure can be a transcanal exploratory tympanotomy and laser-assisted microstapedotomy, followed by a round window injection to deliver an appropriate volume of solution containing rAAV-antiVEGF particles as described herein at an appropriate titer through the round window over a short period of time. Prior to surgery, an ear to be operated on can be identified and a mark placed above an ear with an indelible marker for confirmation in keeping with institutional requirements. Availability of an rAAV particle as described herein can be confirmed prior to anesthesia being administered. After induction with general anesthesia, subjects are positioned in the supine position with the head turned to the side and the operative ear facing up. An ear can be prepped with betadine and draped in the usual fashion. With use of an operating microscope or endoscope, a four-quadrant block of the external auditory canal meatus is performed with anesthesia (e.g., 1% lidocaine with epinephrine). A posterior tympanomeatal flap

is developed and the middle ear entered to expose both the oval and the round window. This may require the removal of a small amount of bone at the junction of the bony canal and tympanic membrane using a micro-curette or drill. Using an appropriate laser (e.g., a KTP or CO2 otologic laser), a small hole (e.g., approximately 0.25 mm) is made in the stapes footplate. Such a hole can serve as a vent during injection to prevent a potential deleterious rise in intralabyrinthine pressure. An injection catheter can then be passed through the round window membrane to an appropriate depth (e.g., not to exceed 1 mm), as determined by a stopper near the tip of the injection device. Exposure of a round window may require removal of a pseudomembrane or ledge of overhanging bone. A delivery device can be held in place while an appropriate volume of solution is instilled. Upon removal of a catheter, a blood/tissue patch and/or drop of sealant (e.g., sodium hyaluronate, e.g., Healon®) is placed over the round window membrane and stapes footplate to create a functional seal of both fenestrae while biological healing occurs over the next 24 to 48 hours. A tympanomeatal flap is then returned to its anatomic position and held in place with antibiotic-soaked absorbable gelatin sponge.

[0777] At appropriate intervals, a subject may undergo follow-up imaging analysis (e.g., MRI analysis) to measure tumor volume and/or tumor growth rate. Subjects that are treated with exemplary methods and/or compositions as described herein may exhibit decreased tumor volume and/or a reduced tumor growth rate when compared to subjects that are not treated with exemplary methods and/or compositions as described herein.

[0778] A sterile, one-time use delivery device is utilized for delivery. Such a device is designed to allow for safe and effective delivery of product candidate rAAV-antiVEGF particles described herein, through the round window membrane into the cochlea. A delivery device is intended for use in delivery of gene therapy product candidates described herein, often in conjunction with an intracochlear route of administration. A one-time use device can be developed to deliver test articles to perilymph fluid of the inner ear through the round window membrane with a vent located in the stapes footplate; a form factor of a device optimizes the approach to the round window membrane through the external auditory canal. A custom device affords advantages over commercially available materials, both with respect to safety and the potential for efficacy of a therapeutic agent, as such a device is specifically designed for this intracochlear route of administration (**FIG. 4**). Certain design elements of a device include but are not limited to: maintenance of sterility of injected fluid; minimization of air bubbles introduced to an inner ear;

ability to precisely deliver small volumes at a controlled flow rate (coupled with the use of a standard pump); allowance for visualization of round window membrane and delivery through an external auditory canal by a surgeon; minimization of damage to a round window membrane, or to cochlear structures beyond a round window membrane; and/or minimization of efflux back out through round window membrane.

EXEMPLARY EMBODIMENTS

[0779] Embodiment 1. A construct comprising a coding sequence operably linked to a promoter, wherein the coding sequence encodes a vascular endothelial growth factor (VEGF) binding agent or a portion thereof.

[0780] Embodiment 2. The construct of embodiment 1, wherein the promoter is an inducible promoter, a constitutive promoter, or a tissue-specific promoter.

[0781] Embodiment 3. The construct of embodiment 1 or 2, wherein the promoter is a CAG promoter, a CBA promoter, a CMV promoter, or a CB7 promoter.

[0782] Embodiment 4. The construct of any one of embodiments 1-3, wherein the promoter comprises a nucleic acid sequence according to SEQ ID NO: 64, SEQ ID NO: 49, and/or SEQ ID NO: 65.

[0783] Embodiment 5. The construct of any one of embodiments 1-4, wherein the coding sequence is or comprises a primate coding sequence.

[0784] Embodiment 6. The construct of any one of embodiments 1-5, wherein the VEGF binding agent or portion thereof is a primate VEGF binding agent.

[0785] Embodiment 7. The construct of any one of embodiments 1-4, wherein the coding sequence is or comprises a human coding sequence.

[0786] Embodiment 8. The construct of any one of embodiments 1-4 or 6, wherein the VEGF binding agent is or comprises a human VEGF binding agent.

[0787] Embodiment 9. The construct of any one of embodiments 1-4, wherein the coding sequence is or comprises an engineered coding sequence.

[0788] Embodiment 10. The construct of any one of embodiments 1-4 or 9, wherein the VEGF binding agent is or comprises a humanized VEGF binding agent.

[0789] Embodiment 11. The construct of any one of embodiments 1-10, wherein the VEGF binding agent is capable of binding to at least one VEGF protein or a fragment thereof.

[0790] Embodiment 12. The construct of embodiment 11, wherein the at least one VEGF protein is VEGF-A, VEGF-B, VEGF-C, VEGF-D, or a combination thereof.

[0791] Embodiment 13. The construct of embodiment 11, wherein the at least one VEGF protein is or comprises VEGF-A.

[0792] Embodiment 14. The construct of any one of embodiments 1-13, wherein the VEGF binding agent comprises at least one polypeptide.

[0793] Embodiment 15. The construct of any one of embodiments 1-14, wherein the VEGF binding agent is or comprises an antibody or a fragment thereof.

[0794] Embodiment 16. The construct of embodiment 15, wherein the antibody fragment is a Fab fragment, a Fab' fragment, a F(ab')2 fragment, a Fd fragment, a Fd' fragment, a complementarity determining region (CDR), a single chain Fv, or an Fc domain.

[0795] Embodiment 17. The construct of any one of embodiments 1-15, wherein the VEGF binding agent is or comprises an immunoglobulin heavy chain, an immunoglobulin light chain, or a combination thereof.

[0796] Embodiment 18. The construct of any one of embodiments 1-15 or 17, wherein the VEGF binding agent comprises:

(i) a polypeptide that comprises an amino sequence according to SEQ ID NO: 16,

(ii) a polypeptide that comprises an amino sequence according to SEQ ID NO: 20, or

(iii) a combination thereof.

[0797] Embodiment 19. The construct of embodiment 18, wherein the VEGF binding agent is or comprises ranibizumab.

[0798] Embodiment 20. The construct of any one of embodiments 1-15 or 17-19, wherein the coding sequence comprises:

(i) a nucleic acid sequence comprising SEQ ID NO: 13,

(ii) a nucleic acid sequence comprising SEQ ID NO: 19, or

(iii) a combination thereof.

[0799] Embodiment 21. The construct of any one of embodiments 1-20, wherein the coding sequence comprises one or more nucleic acid sequences that each encode a signal peptide.

[0800] Embodiment 22. The construct of embodiment 21, wherein at least one nucleic acid sequence encodes an interleukin 2 (IL2) signal peptide.

[0801] Embodiment 23. The construct of any one of embodiments 1-22, wherein the coding sequence comprises one or more sequences encoding a self-cleaving peptide.

[0802] Embodiment 24. The construct of embodiment 23, wherein the self-cleaving peptide is a thosea asigna virus 2A (T2A) peptide.

[0803] Embodiment 25. The construct of any one of embodiments 1-15 or 17-24, wherein the coding sequence is or comprises a nucleic acid sequence according to SEQ ID NO: 21.

[0804] Embodiment 26. The construct of any one of embodiments 1-15 or 17, wherein the VEGF binding agent comprises:

(i) a polypeptide that comprises an amino sequence according to SEQ ID NO: 24,

(ii) a polypeptide that comprises an amino sequence according to SEQ ID NO: 25, or

(iii) a combination thereof.

[0805] Embodiment 27. The construct of embodiment 26, wherein the VEGF binding agent is or comprises bevacizumab.

[0806] Embodiment 28. The construct of any one of embodiments 1-15, 17, 26, or 27, wherein the coding sequence comprises:

(i) a nucleic acid sequence comprising SEQ ID NO: 108,

(ii) a nucleic acid sequence comprising SEQ ID NO: 109, or

(iii) a combination thereof.

[0807] Embodiment 29. The construct of any one of embodiments 26-28, wherein the coding sequence comprises one or more sequences that each encode a signal peptide.

[0808] Embodiment 30. The construct of embodiment 29, wherein at least one nucleic acid sequence encodes an IL2 signal peptide.

[0809] Embodiment 31. The construct of any one of embodiments 26-30, wherein the coding sequence comprises one or more sequences encoding a self-cleaving peptide.

[0810] Embodiment 32. The construct of embodiment 31, wherein the self-cleaving peptide is a T2A peptide.

[0811] Embodiment 33. The construct of any one of embodiments 1-15, 17, or 26-32 wherein the coding sequence is or comprises the nucleic acid sequence according to SEQ ID NO: 22.

[0812] Embodiment 34. The construct of any one of embodiments 1-15, wherein the VEGF binding agent comprises an Fc domain.

[0813] Embodiment 35. The construct of any one of embodiments 1-15, or 34, wherein the Fc domain comprises the amino acid sequence according to SEQ ID NO: 111.

[0814] Embodiment 36. The construct of any one of embodiments 1-15, 34, or 35, wherein the coding sequence comprises the nucleic acid sequence according to SEQ ID NO: 110.

[0815] Embodiment 37. The construct of any one of embodiments 1-15, or 34-36, wherein the VEGF binding agent comprises one or more extracellular domains of a VEGF receptor.

[0816] Embodiment 38. The construct of embodiment 37, wherein the one or more extracellular domains of the VEGF receptor comprises the amino sequence according to SEQ ID NO: 112.

[0817] Embodiment 39. The construct of any one of embodiments 1-15, or 34-38, wherein the VEGF binding agent comprises two extracellular domains of a VEGF receptor.

[0818] Embodiment 40. The construct of any one of embodiments 34-39, wherein the coding sequence comprises one or more nucleic acid sequences each encoding a signal peptide.

[0819] Embodiment 41. The construct of embodiment 40, wherein at least one nucleic acid sequence encodes an IL2 signal peptide.

[0820] Embodiment 42. The construct of any one of embodiments 1-41, further comprising two AAV inverted terminal repeats (ITRs), wherein the two AAV ITRs flank the coding sequence and promoter.

[0821] Embodiment 43. The construct of embodiment 42, wherein the two AAV ITRs are or are derived from AAV2 ITRs.

[0822] Embodiment 44. The construct of embodiment 42 or 43, wherein the two AAV ITRs comprise a 5` ITR comprising a nucleic acid sequence according to SEQ ID NO: 45 or 47, and a 3` ITR comprising a nucleic acid sequence according to SEQ ID NO: 46 or 48.

[0823] Embodiment 45. The construct of embodiment 1, wherein the construct comprises a nucleic acid sequence according to any of SEQ ID NOs: 90, 91, 92, 93, 94, 106, or 107.

[0824] Embodiment 46. The construct of embodiment 1, wherein the construct comprises a nucleic acid sequence according to any of SEQ ID NOs: 95, or 96.

[0825] Embodiment 47. An AAV particle comprising the construct of any one of embodiments 1-46.

[0826] Embodiment 48. The AAV particle of embodiment 47, 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.

[0827] Embodiment 49. The AAV particle of embodiment 48, wherein the AAV capsid is an AAV Anc80 capsid.

[0828] Embodiment 50. A composition comprising the construct of any one of embodiments 1-46.

[0829] Embodiment 51. A composition comprising the AAV particle of any one of embodiments 47-50.

[0830] Embodiment 52. The composition of embodiment 51, wherein the 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 capsid.

[0831] Embodiment 53. The composition of embodiment 52, wherein the AAV capsid of the AAV particle is an AAV Anc80 capsid.

[0832] Embodiment 54. The composition of embodiment 53, wherein the AAV Anc80 capsid is an AAV Anc80L65 capsid.

[0833] Embodiment 55. The composition of any one of embodiments 50-54, wherein the composition is a pharmaceutical composition.

[0834] Embodiment 56. The composition of embodiment 55, further comprising a pharmaceutically acceptable carrier.

[0835] Embodiment 57. The composition of embodiment 55 or 56, which is formulated at a dose of about 1×10^{11} vg/mL to about 1×10^{15} vg/mL.

[0836] Embodiment 58. The composition of any one of embodiments 55-57, which is formulated at a dose of about 1×10^{10} to about 1×10^{13} vg/cochlea.

[0837] Embodiment 59. The composition of any one of embodiments 55-58, which is administered at a volume of about 0.01mL to 0.1 mL.

[0838] Embodiment 60. A cell comprising the composition of any one of embodiments 50-59.

[0839] Embodiment 61. The cell of embodiment 60, wherein the cell is in vivo, ex vivo, or in vitro.

[0840] Embodiment 62. The cell of embodiment 60 or 61, wherein the cell is a mammalian cell.

[0841] Embodiment 63. The cell of embodiment 62, wherein the mammalian cell is a human cell.

[0842] Embodiment 64. The cell of embodiment 60, wherein the cell is immortalized to generate a stable cell line.

[0843] Embodiment 65. The cell of embodiment 63, wherein the human cell is in the ear of a subject.

[0844] Embodiment 66. The cell of embodiment 63, wherein the human cell is an inner ear cell.

[0845] Embodiment 67. The cell of embodiment 66, wherein the inner ear cell is an inner hair cell, an outer hair cell, or both.

[0846] Embodiment 68. A system comprising the composition of any one of embodiments 50-59.

[0847] Embodiment 69. A method comprising contacting an inner ear cell with the construct of any one of embodiments 1-46.

[0848] Embodiment 70. A method comprising contacting an inner ear cell with the AAV particle of any one of embodiments 47-49.

[0849] Embodiment 71. A method comprising contacting an inner ear cell with the composition of any one of embodiments 50-59.

[0850] Embodiment 72. The method of any one of embodiments 69-71, where the inner ear cell is an outer hair cell.

[0851] Embodiment 73. The method of any one of embodiments 69-71, where the inner ear cell is an inner hair cell.

[0852] Embodiment 74. The method of any one of embodiments 69-73, wherein the inner ear cell is in the ear of a subject.

[0853] Embodiment 75. The method of any one of embodiments 69-73, wherein the inner ear cell is in vitro or ex vivo.

[0854] Embodiment 76. A method comprising, contacting a cell with:

(i) the construct of any one of embodiments 1-46; and

(ii) one or more constructs comprising an AAV Rep gene, AAV Cap gene, AAV VA gene, AAV E2a gene, and AAV E4 gene.

[0855] Embodiment 77. The method of embodiment 76, where the cell is an inner ear cell.

[0856] Embodiment 78. The method of embodiment 77, wherein the inner ear cell is an outer hair cell.

[0857] Embodiment 79. The method of embodiment 77, wherein the inner ear cell is an inner hair cell.

[0858] Embodiment 80. The method of any one of embodiments 77-79, wherein the inner ear cell is in vitro or ex vivo.

[0859] Embodiment 81. A method comprising introducing the construct of any one of embodiments 1-46 into the inner ear of a subject.

[0860] Embodiment 82. A method comprising introducing the AAV particle of any one of embodiments 47-49 into the inner ear of a subject.

[0861] Embodiment 83. A method comprising introducing the composition of any one of embodiments 50-59 into the inner ear of a subject.

[0862] Embodiment 84. The method of any one of embodiments 81-83, wherein the construct, AAV particle, or composition is introduced into the cochlea of the subject.

[0863] Embodiment 85. The method of any one of embodiments 81-84, wherein the construct, AAV particle, or composition is introduced via a round window membrane injection.

[0864] Embodiment 86. The method of any one of embodiments 69-75 and 81-85, further comprising measuring a hearing level of the subject.

[0865] Embodiment 87. The method of embodiment 86, a hearing level is measured by performing an auditory brainstem response (ABR) test.

[0866] Embodiment 88. The method of embodiment 86 or 87, further comprising comparing the hearing level of the subject to a reference hearing level.

[0867] Embodiment 89. The method of embodiment 88, wherein the reference hearing level is a published or historical reference hearing level.

[0868] Embodiment 90. The method of embodiment 89, wherein the hearing level of the subject is measured after the construct of any one of embodiments 1-46, the AAV particle of any one of embodiments 47-49, or the composition of any one of embodiments 50-59 is introduced, and the reference hearing level is a hearing level of the subject that was measured before the construct of any one of embodiments 1-46, the AAV particle of any one of embodiments 47-49, or the composition of any one of embodiments 50-59 was introduced.

[0869] Embodiment 91. The method of any one of embodiments 69-75 and 81-90, further comprising measuring a level of a vascular endothelial growth factor (VEGF) binding agent or a portion thereof in a subject.

[0870] Embodiment 92. The method of embodiment 91, wherein the level of the vascular endothelial growth factor (VEGF) binding agent or portion thereof is measured in the inner ear of the subject.

[0871] Embodiment 93. The method of embodiment 91 or 92, wherein the level of the vascular endothelial growth factor (VEGF) binding agent or portion thereof is measured in the cochlea of the subject.

[0872] Embodiment 94. The method of any one of embodiments 69-75 and 81-93, further comprising comparing the level of the vascular endothelial growth factor (VEGF) binding agent or a portion thereof in the subject to a reference level of vascular endothelial growth factor (VEGF) binding agent or a portion thereof.

[0873] Embodiment 95. The method of embodiment 94, wherein the reference level of vascular endothelial growth factor (VEGF) binding agent or a portion thereof is a published or historical reference level of vascular endothelial growth factor (VEGF) binding agent or a portion thereof.

[0874] Embodiment 96. The method of any one of embodiments 91-95, wherein the level of the vascular endothelial growth factor (VEGF) binding agent or a portion thereof in the subject is measured after the construct of any one of embodiments 1-46, the AAV particle of any one of embodiments 47-49, or the composition of any one of embodiments 50-59 is introduced, and the reference level of vascular endothelial growth factor (VEGF) binding agent or a portion thereof is the level the vascular endothelial growth factor (VEGF) binding agent or a portion thereof in the subject that was measured before the construct of any one of embodiments 1-46, the AAV particle of any one of embodiments 47-49, or the composition of any one of embodiments 50-59 was introduced.

[0875] Embodiment 97. The method of any one of embodiments 69-75 and 81-96, further comprising measuring a volume of a tumor in a subject.

[0876] Embodiment 98. The method of embodiments 97, further comprising comparing a volume of a tumor in the subject to a reference tumor volume.

[0877] Embodiment 99. The method of embodiment 98, wherein the reference tumor volume is a published or historical reference tumor volume.

[0878] Embodiment 100. The method of any one of embodiments 97-99, wherein the volume of a tumor in the subject is measured after the construct of any one of embodiments 1-46, the AAV particle of any one of embodiments 47-49, or the composition of any one of embodiments 50-59 is introduced, and the reference tumor volume is the volume of the tumor in the subject that was measured before the construct of any one of embodiments 1-46, the AAV particle of any one of embodiments 47-49, or the composition of any one of embodiments 50-59 was introduced.

[0879] Embodiment 101. A method of treating hearing loss or treating an inner ear disorder, comprising administering the construct of any one of embodiments 1-46, the AAV particle of any one of embodiments 47-49, or the composition of any one of embodiments 50-59 to a subject in need thereof.

[0880] Embodiment 102. The method of any one of embodiments 74 and 81-101, wherein the subject is suffering from or is at risk of an otological disease characterized by neovascularization.

[0881] Embodiment 103. The method of embodiment 102, wherein the otological disease is or comprises an acoustic neuroma.

[0882] Embodiment 104. The method of embodiment 102, wherein the otological disease is or comprises a vestibular schwannoma.

[0883] Embodiment 105. The method of embodiment 104, wherein the subject having vestibular schwannoma has neurofibromatosis type 2 (NF2),

[0884] Embodiment 106. The method of any one of embodiments 102-105, wherein one or more symptoms associated with the otological disease is alleviated or ameliorated.

[0885] Embodiment 107. The method of embodiment 106, wherein the one or more symptoms comprises hearing loss, degeneration of hair cells, alteration of biochemical milieu of inner ear fluids, elevated intralabyrinthine protein, endolymphatic hydrops, cochlear aperture obstruction, intralabyrinthine hemorrhage, disruption of cochlear vascular supply, tinnitus, dizziness,

intractable headache, facial neuropathy, trigeminal neuropathy, facial paralysis, facial paresthesia, hydrocephalus, cerebellar herniation, death, or a combination thereof.

[0886] Embodiment 108. The method of any one of embodiments 69-75, 81-102, and 104-107, wherein the method is a method of treating vestibular schwannoma.

[0887] Embodiment 109. The method of any one of embodiments 69-75 and 81-107, wherein the method is a method of modulating the level of VEGF.

[0888] Embodiment 110. The method of any one of embodiments 69-75 and 81-107, wherein the method is a method of modulating the level of active VEGF.

[0889] Embodiment 111. The method of any one of embodiments 69-75 and 81-107, wherein the method is a method of decreasing the activity of VEGF.

[0890] Embodiment 112. A construct of any one of embodiments 1-46 for use in the treatment of an otological disease characterized by neovascularization and/or one or more symptoms associated with the otological disease.

[0891] Embodiment 113. An AAV particle of any one of embodiments 47-49 for use in the treatment of an otological disease characterized by neovascularization and/or one or more symptoms associated with the otological disease.

[0892] Embodiment 114. A composition of any one of embodiments 50-59 for use in the treatment of an otological disease characterized by neovascularization and/or one or more symptoms associated with the otological disease.

[0893] Embodiment 115. The construct of embodiment 112, the AAV particle of embodiment 113, or the composition of embodiment 114, wherein the one or more symptoms associated with the otological disease comprise hearing loss, degeneration of hair cells, alteration of biochemical milieu of inner ear fluids, elevated intralabyrinthine protein, endolymphatic hydrops, cochlear aperture obstruction, intralabyrinthine hemorrhage, disruption of cochlear vascular supply, tinnitus, dizziness, intractable headache, facial neuropathy, trigeminal neuropathy, facial paralysis, facial paresthesia, hydrocephalus, cerebellar herniation, death, or a combination thereof.

[0894] Embodiment 116. Use of a construct of any one of embodiments 1-46 for the manufacture of a medicament to treat an otological disease characterized by neovascularization and/or one or more symptoms associated with the otological disease.

[0895] Embodiment 117. Use of a particle of any one of embodiments 47-49 for the manufacture of a medicament to treat an otological disease characterized by neovascularization and/or one or more symptoms associated with the otological disease.

[0896] Embodiment 118. Use of a composition of any one of embodiments 50-59 for the manufacture of a medicament to treat an otological disease characterized by neovascularization and/or one or more symptoms associated with the otological disease.

[0897] Embodiment 119. The use of any one of embodiments 116-118, wherein the one or more symptoms associated with the otological disease comprise hearing loss, degeneration of hair cells, alteration of biochemical milieu of inner ear fluids, elevated intralabyrinthine protein, endolymphatic hydrops, cochlear aperture obstruction, intralabyrinthine hemorrhage, disruption of cochlear vascular supply, tinnitus, dizziness, intractable headache, facial neuropathy, trigeminal neuropathy, facial paralysis, facial paresthesia, hydrocephalus, cerebellar herniation, death, or a combination thereof.

[0898] Embodiment 120. A population of cells comprising one or more cells according to any one of embodiments 60-67, wherein the population is or comprises a stable cell line.

[0899] Embodiment 121. A kit comprising a composition of any one of embodiments 50-59.

[0900] Embodiment 122. The kit of embodiment 121, wherein the composition is pre-loaded in a device.

[0901] Embodiment 123. The kit of embodiment 122, wherein the device is a microcatheter.

[0902] Embodiment 124. The kit of embodiment 123, 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.

[0903] Embodiment 125. The kit of embodiment 123 or 124, wherein a distal end of the microcatheter is comprised of at least one microneedle with a diameter of between 10 and 1,000 microns.

[0904] Embodiment 126. The kit of any one of embodiments 121-125, further comprising a device.

[0905] Embodiment 127. The kit of embodiment 126, wherein the device is a device described in any one of FIGS. 29-32.

[0906] Embodiment 128. The kit of any one of embodiments 122-127, wherein the device comprises a needle comprising a bent portion and an angled tip.

CLAIMS

1. A construct comprising a coding sequence operably linked to a promoter, wherein the coding sequence encodes a vascular endothelial growth factor (VEGF) binding agent or a portion thereof.

2. The construct of claim 1, wherein the promoter is a CAG promoter, a CBA promoter, a CMV promoter, or a CB7 promoter.

3. The construct of claim 1 or 2, wherein the VEGF binding agent is or comprises an antibody or a fragment thereof.

4. The construct of claim 3, wherein the antibody fragment is a Fab fragment, a Fab' fragment, a F(ab')2 fragment, a Fd fragment, a Fd' fragment, a complementarity determining region (CDR), a single chain Fv, or an Fc domain.

5. The construct of any one of claims 1-4, wherein the VEGF binding agent is or comprises an immunoglobulin heavy chain, an immunoglobulin light chain, or a combination thereof.

6. The construct of any one of claims 1-5, wherein the VEGF binding agent comprises:
(i) a polypeptide that comprises an amino sequence according to SEQ ID NO: 16,
(ii) a polypeptide that comprises an amino sequence according to SEQ ID NO: 20, or
(iii) a combination thereof.

7. The construct of claim 6, wherein the VEGF binding agent is or comprises ranibizumab.

8. The construct of any one of claims 1-3 or 5-6, wherein the coding sequence comprises:
(i) a nucleic acid sequence comprising SEQ ID NO: 13,
(ii) a nucleic acid sequence comprising SEQ ID NO: 19, or
(iii) a combination thereof.

9. The construct of any one of claims 1-8, wherein the coding sequence comprises one or more nucleic acid sequences that each encode a signal peptide.

10. The construct of claim 9, wherein at least one nucleic acid sequence encodes an interleukin 2 (IL2) signal peptide.

11. The construct of any one of claims 1-10, wherein the coding sequence comprises one or more sequences encoding a self-cleaving peptide.

12. The construct of claim 11, wherein the self-cleaving peptide is a thosea asigna virus 2A (T2A) peptide.

13. The construct of any one of claims 1-3 or 5-12, wherein the coding sequence is or comprises a nucleic acid sequence according to SEQ ID NO: 21.

14. The construct of any one of claims 1-13, further comprising two AAV inverted terminal repeats (ITRs), wherein the two AAV ITRs flank the coding sequence and promoter.

15. The construct of claim 14, wherein the two AAV ITRs are or are derived from AAV2 ITRs.

16. The construct of claim 14 or 15, wherein the two AAV ITRs comprise a 5` ITR comprising a nucleic acid sequence according to SEQ ID NO: 45 or 47, and a 3` ITR comprising a nucleic acid sequence according to SEQ ID NO: 46 or 48.

17. The construct of claim 1, wherein the construct comprises a nucleic acid sequence according to any of SEQ ID NOs: 90, 91, 92, or 106.

18. An AAV particle comprising the construct of any one of claims 1-17.

19. The AAV particle of claim 18, 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.

20. The AAV particle of claim 19, wherein the AAV capsid is an AAV Anc80 capsid, optionally an AAV Anc80L65 capsid.

21 The AAV particle of claim 19 or 20, wherein the AAV capsid comprises a polypeptide according to SEQ ID NO: 113 or SEQ ID NO: 114.

22. A composition comprising the construct of any one of claims 1-17 or the AAV particle of any one of claims 18-21.

23. The composition of claim 22, wherein the composition is a pharmaceutical composition further comprising a pharmaceutically acceptable carrier.

24. The composition of claim 22 or 23, which is formulated at a dose of about 1 x 10^{11} vg/mL to about 1 x 10^{15} vg/mL.

25. The composition of any one of claims 22-24, which is formulated at a dose of about 1 x 10^{10} to about 1 x 10^{13} vg/cochlea.

26. The composition of any one of claims 22-25, which is administered at a volume of about 0.01mL to 0.1 mL.

27. A method comprising contacting an inner ear cell with the construct of any one of claims 1-17, the AAV particle of any one of claims 18-21, or the composition of any one of claims 22-26.

28. A method comprising, contacting a cell with:

(i) the construct of any one of claims 1-17; and

(ii) one or more constructs comprising an AAV Rep gene, AAV Cap gene, AAV VA gene, AAV E2a gene, and AAV E4 gene.

29. A method comprising introducing the construct of any one of claims 1-17, the AAV particle of any one of claims 18-21, or the composition of any one of claims 22-26 into the inner ear of a subject.

30. The method of claim 29, further comprising measuring a hearing level of the subject.

31. The method of claim 30, further comprising comparing the hearing level of the subject to a reference hearing level.

32. The method of claim 31, wherein the hearing level of the subject is measured after the construct of any one of claims 1-17, the AAV particle of any one of claims 18-21, or the composition of any one of claims 22-26 is introduced, and the reference hearing level is a hearing level of the subject that was measured before the construct of any one of claims 1-17, the AAV particle of any one of claims 18-21, or the composition of any one of claims 22-26 was introduced.

33. The method of any one of claims 29-32, further comprising measuring a level of a vascular endothelial growth factor (VEGF) binding agent or a portion thereof in a subject.

34. The method of claim 33, further comprising comparing the level of the vascular endothelial growth factor (VEGF) binding agent or a portion thereof in the subject to a reference level of vascular endothelial growth factor (VEGF) binding agent or a portion thereof.

35. The method of any one of claims 29-34, further comprising measuring a volume of a tumor in a subject.

36. The method of claim 35, further comprising comparing a volume of a tumor in the subject to a reference tumor volume.

37. A method of treating hearing loss comprising administering the construct of any one of claims 1-17, the AAV particle of any one of claims 18-21, or the composition of any one of claims 22-26 to a subject in need thereof.

38. A method of treating an inner ear disorder in a mammal comprising administering the construct of any one of claims 1-17, the AAV particle of any one of claims 18-21, or the composition of any one of claims 22-26 to a subject in need thereof.

39. The method of claim 38, wherein the inner ear disorder is acoustic neuroma, vestibular schwannoma, or neurofibromatosis type II.

40. A method of treating vestibular schwannoma in a mammal comprising administering the construct of any one of claims 1-17, the AAV particle of any one of claims 18-21, or the composition of any one of claims 22-26 to a subject in need thereof.

41. A construct of any one of claims 1-17, an AAV particle of any one of claims 18-21, or a composition of any one of claims 22-26 for use in the treatment of an otological disease characterized by neovascularization and/or one or more symptoms associated with the otological disease.

42. A construct of any one of claims 1-17, an AAV particle of any one of claims 18-21, or a composition of any one of claims 22-26 for use in the treatment of an inner ear disorder.

43. The construct for use of claim 42, wherein the inner ear disorder is acoustic neuroma, vestibular schwannoma, or neurofibromatosis type II.

44. A construct of any one of claims 1-17, an AAV particle of any one of claims 18-21, or a composition of any one of claims 22-26 for use in the treatment of vestibular schwannoma.

45. Use of a construct of any one of claims 1-17, a particle of any one of claims 18-21, or a composition of any one of claims 22-26 for the manufacture of a medicament to treat an otological disease characterized by neovascularization and/or one or more symptoms associated with the otological disease.

46. Use of a construct of any one of claims 1-17, a particle of any one of claims 18-21, or a composition of any one of claims 22-26 for the manufacture of a medicament to treat an inner ear disorder.

47. The use of claim 46, wherein the inner ear disorder is acoustic neuroma, vestibular schwannoma, or neurofibromatosis type II.

48. Use of a construct of any one of claims 1-17, a particle of any one of claims 18-21, or a composition of any one of claims 22-26 for the manufacture of a medicament to treat a vestibular schwannoma.

49. The method of any one of claims 37-40, the AAV construct for use of any one of claims 41-44, or the use of any one of claims 45-48, wherein the mammal is a human.

50. A kit comprising a construct of any one of claims 1-17, a particle of any one of claims 18-21, or a composition of any one of claims 22-26.

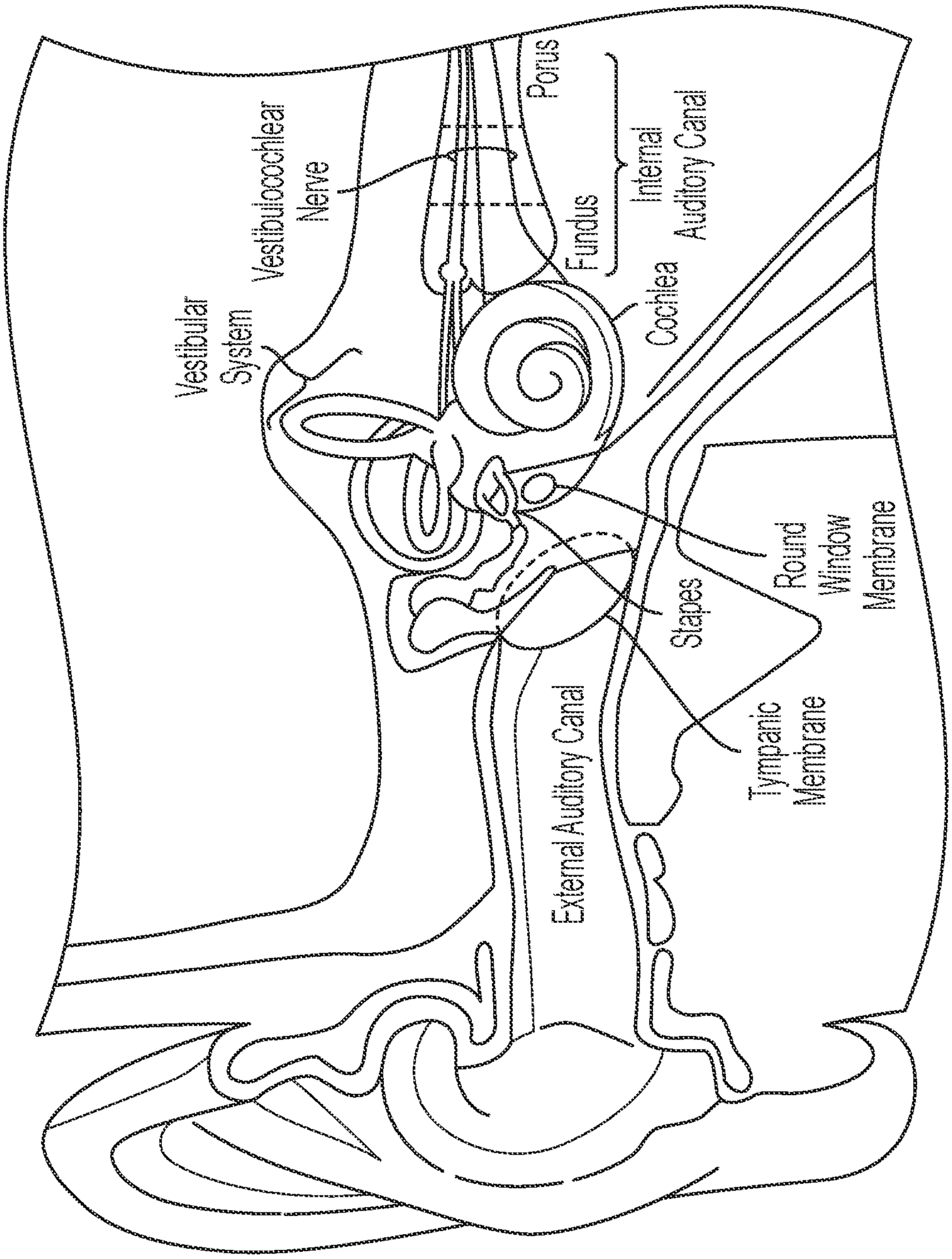

Vestibular System
Vestibulocochlear Nerve
Porus
Fundus
Internal Auditory Canal
Cochlea
Stapes
Round Window Membrane
External Auditory Canal
Tympanic Membrane

FIG. 1

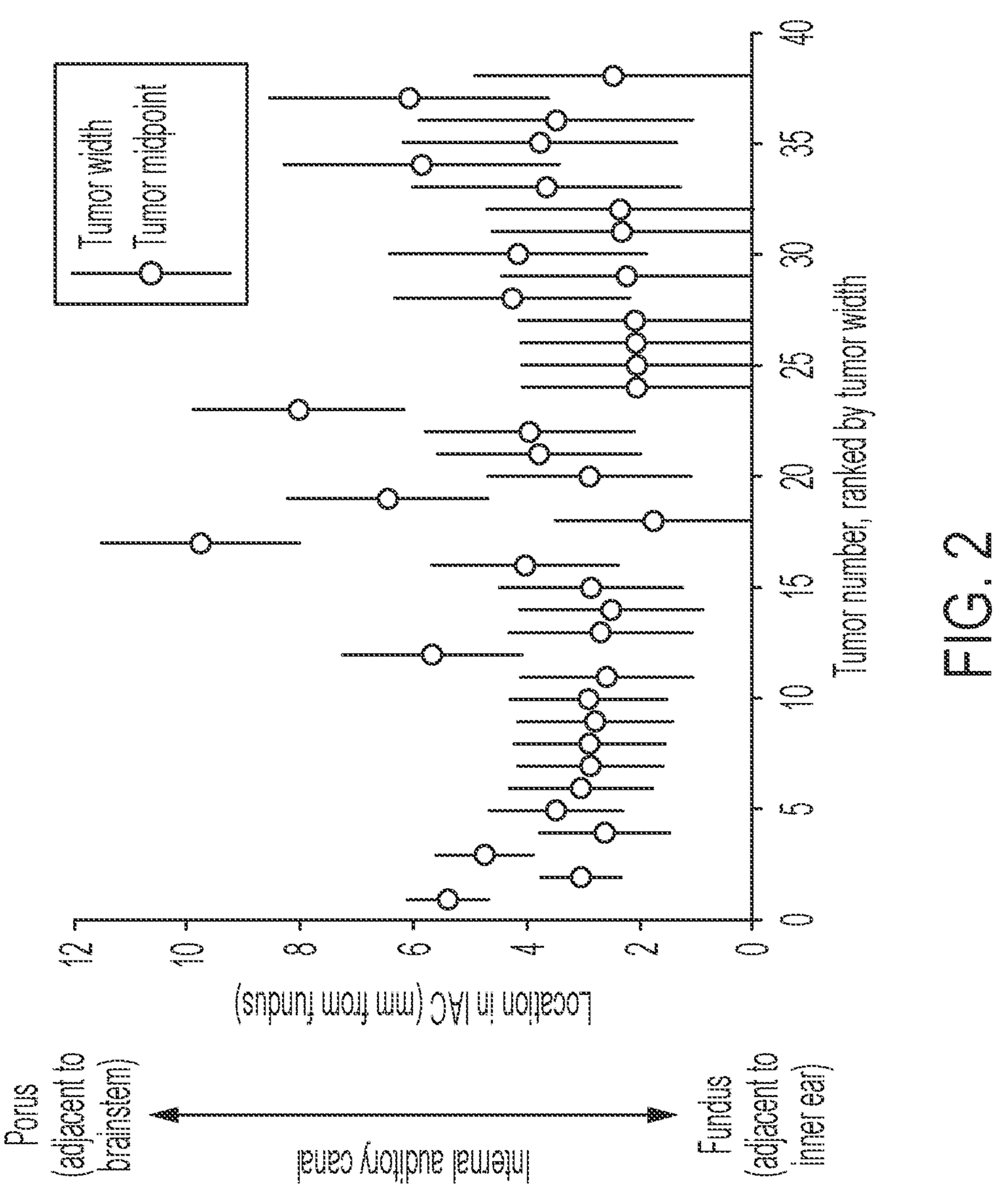

Tumor width
Tumor midpoint
Tumor number, ranked by tumor width
0
5
10
15
20
25
30
35
40
Location in IAC (mm from fundus)
0
2
4
6
8
10
12
Porus
(adjacent to
brainstem)
Internal auditory canal
Fundus
(adjacent to
inner ear)

FIG. 2

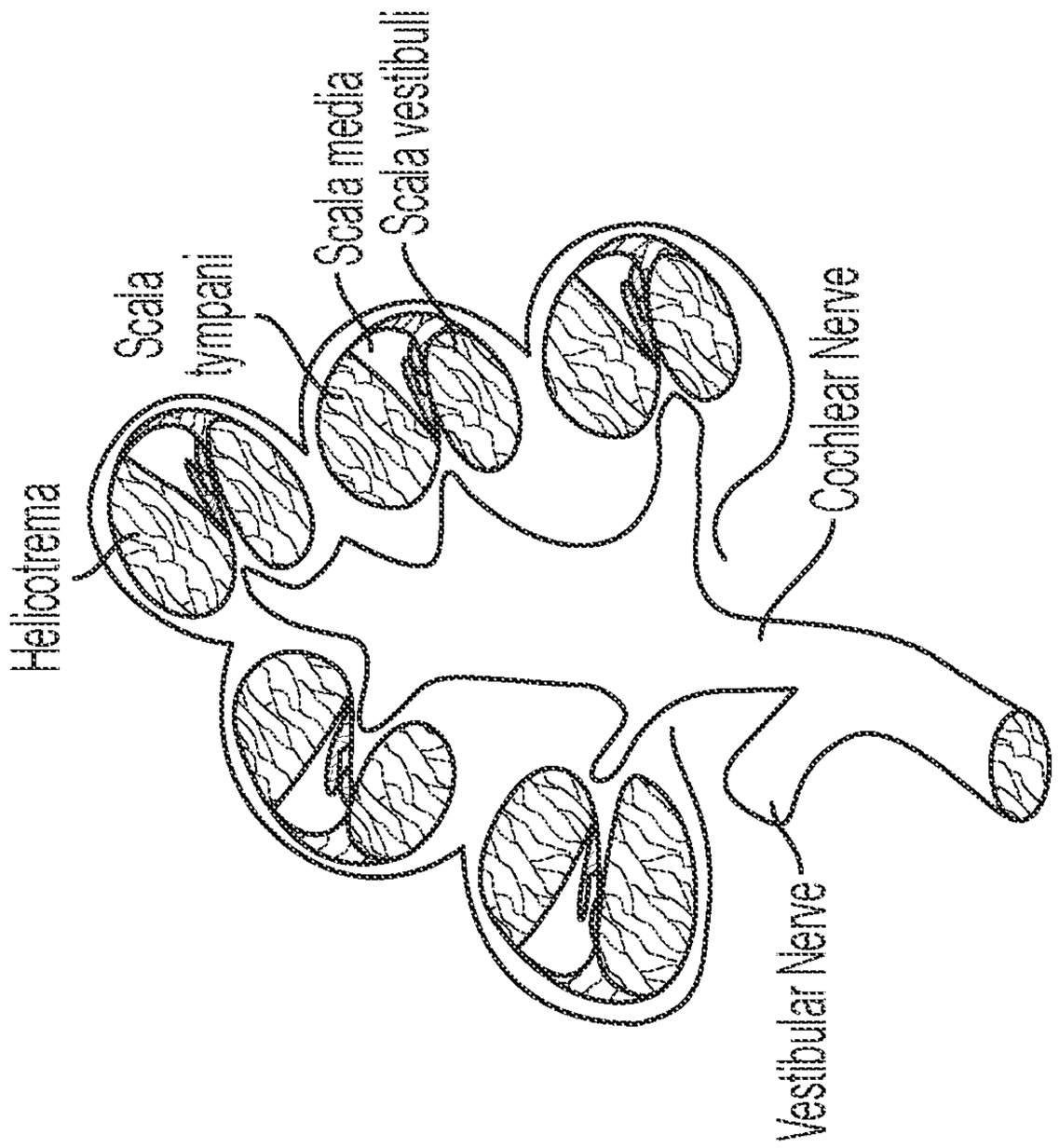

Helicotrema
Scala tympani
Scala media
Scala vestibuli
Cochlear Nerve
Vestibular Nerve

FIG. 3B

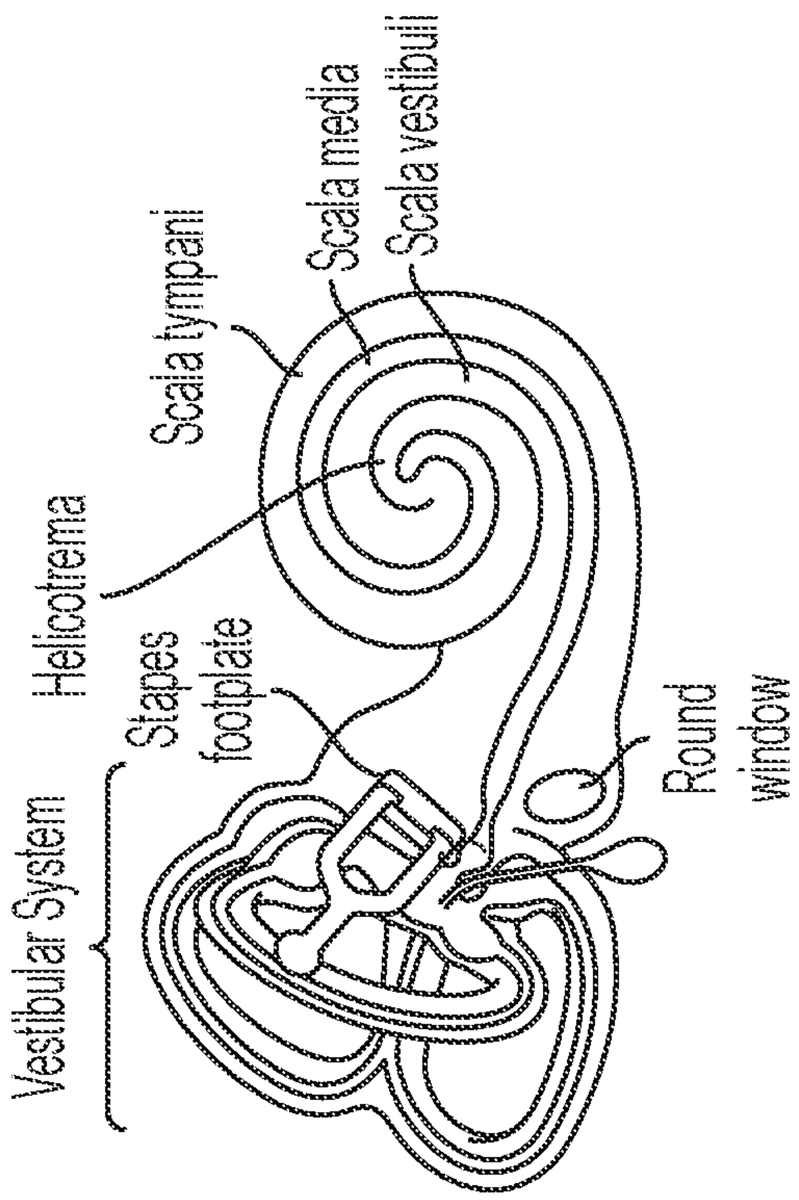

Helicotrema
Scala tympani
Scala media
Scala vestibuli
Stapes footplate
Round window
Vestibular System

FIG. 3A

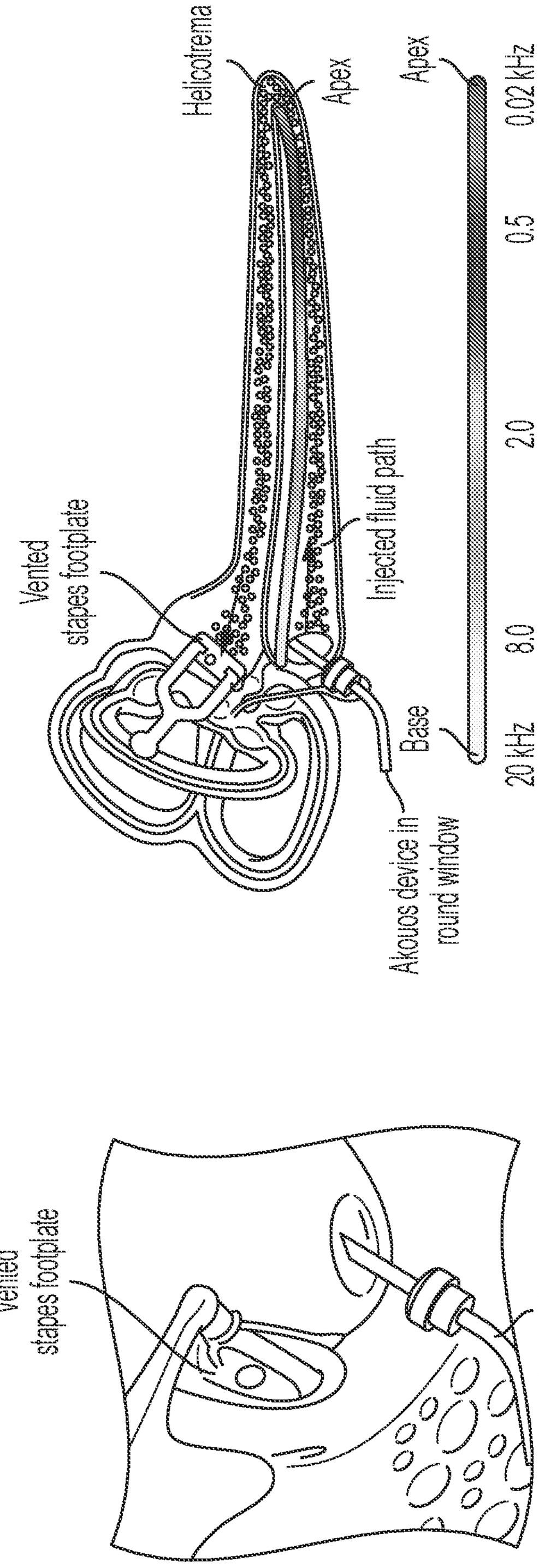

Helicotrema
Apex
Apex
0.02 kHz
0.5
2.0
8.0
20 kHz
Frequency Distribution Along the Cochlea
Vented
stapes footplate
Injected fluid path
Base
Akouos device in
round window

FIG. 4B

Vented
stapes footplate
Akouos device
in round window

FIG. 4A

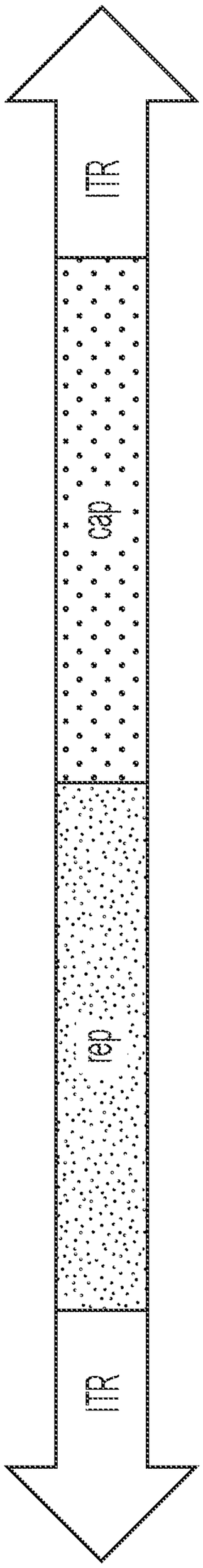

ITR
cap
rep
ITR

FIG. 5A

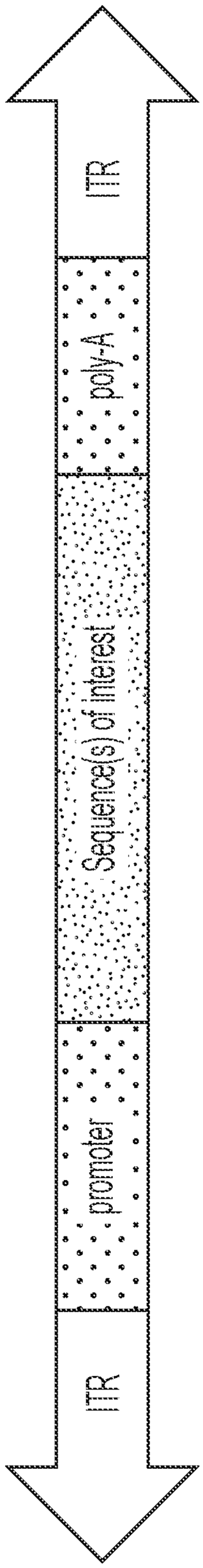

ITR
poly-A
Sequence(s) of interest
promoter
ITR

FIG. 5B

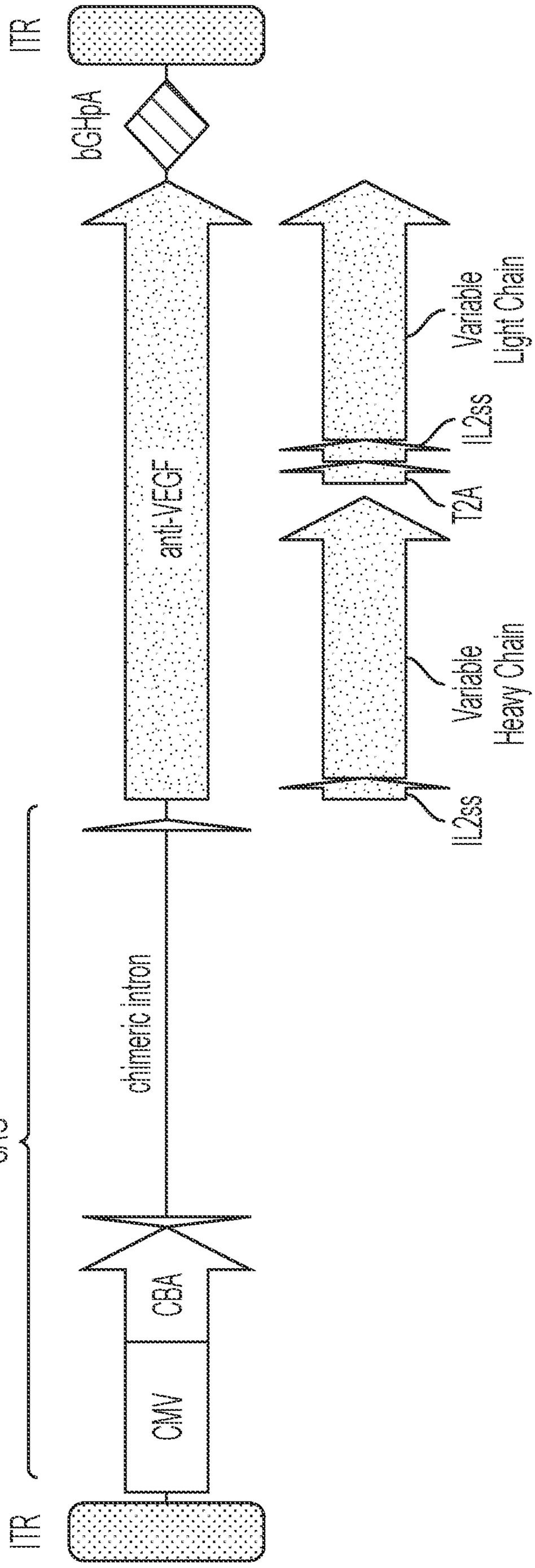

ITR
bGHpA
anti-VEGF
IL2ss
Variable
Heavy Chain
T2A
IL2ss
Variable
Light Chain
CAG
chimeric intron
CBA
CMV
ITR

FIG. 6A

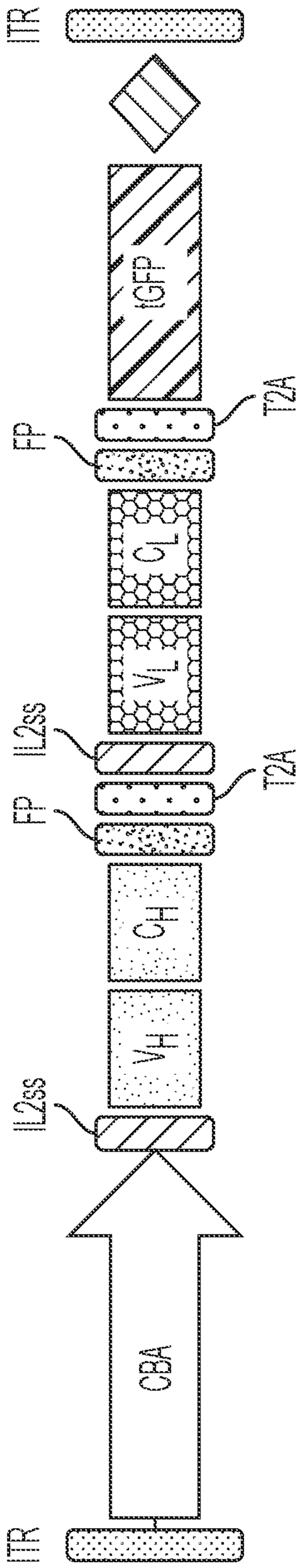


FIG. 6B

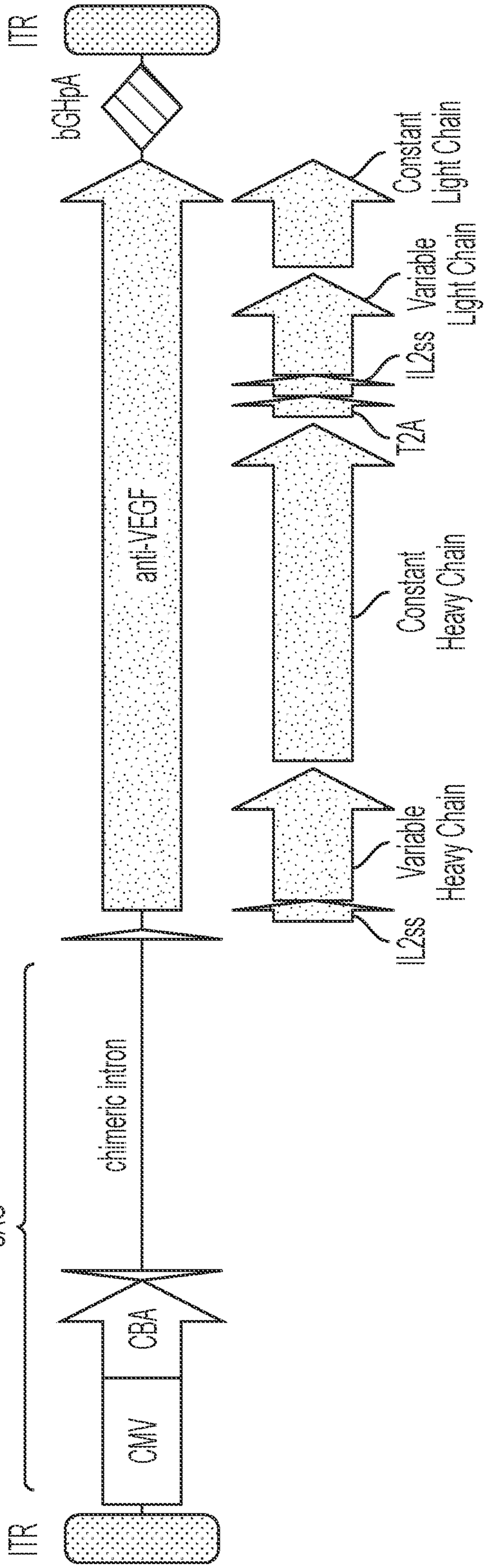

ITR
CBA
CMV
chimeric intron
CAG
IL2ss
Variable Heavy Chain
T2A
IL2ss
Variable Light Chain
Constant Heavy Chain
anti-VEGF
bGHpA
ITR
Constant Light Chain

FIG. 6C

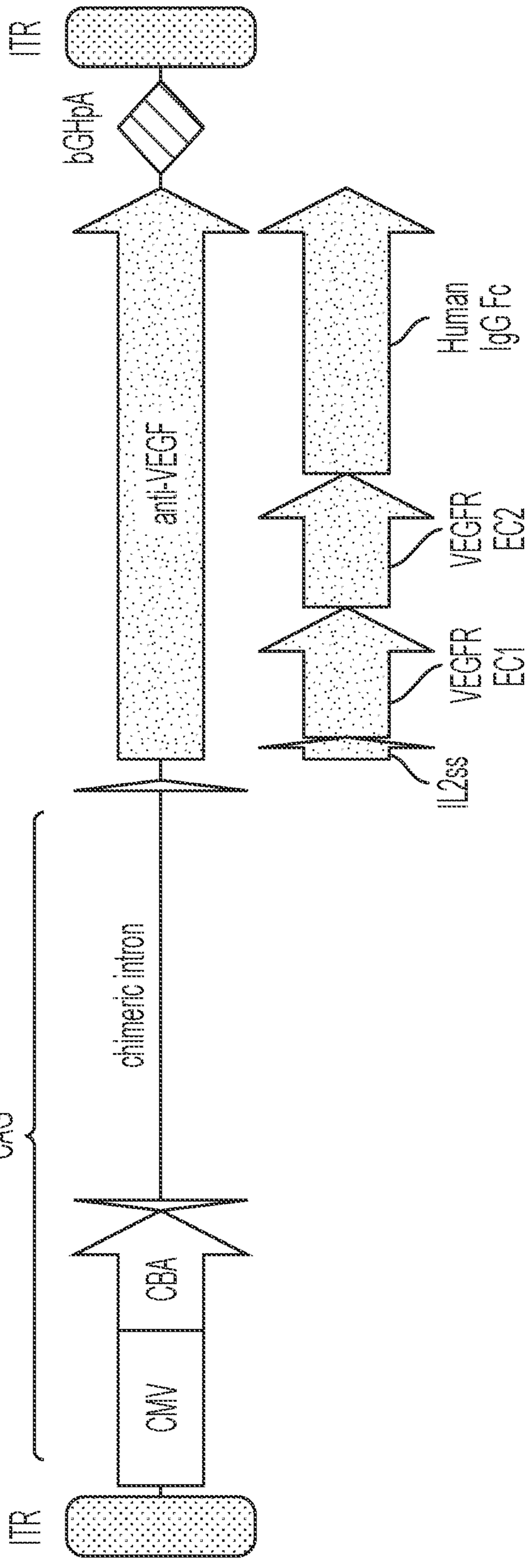

ITR
bGHpA
anti-VEGF
Human IgG Fc
VEGFR EC2
VEGFR EC1
IL2SS
CAG
chimeric intron
CBA
CMV
ITR

FIG. 6D

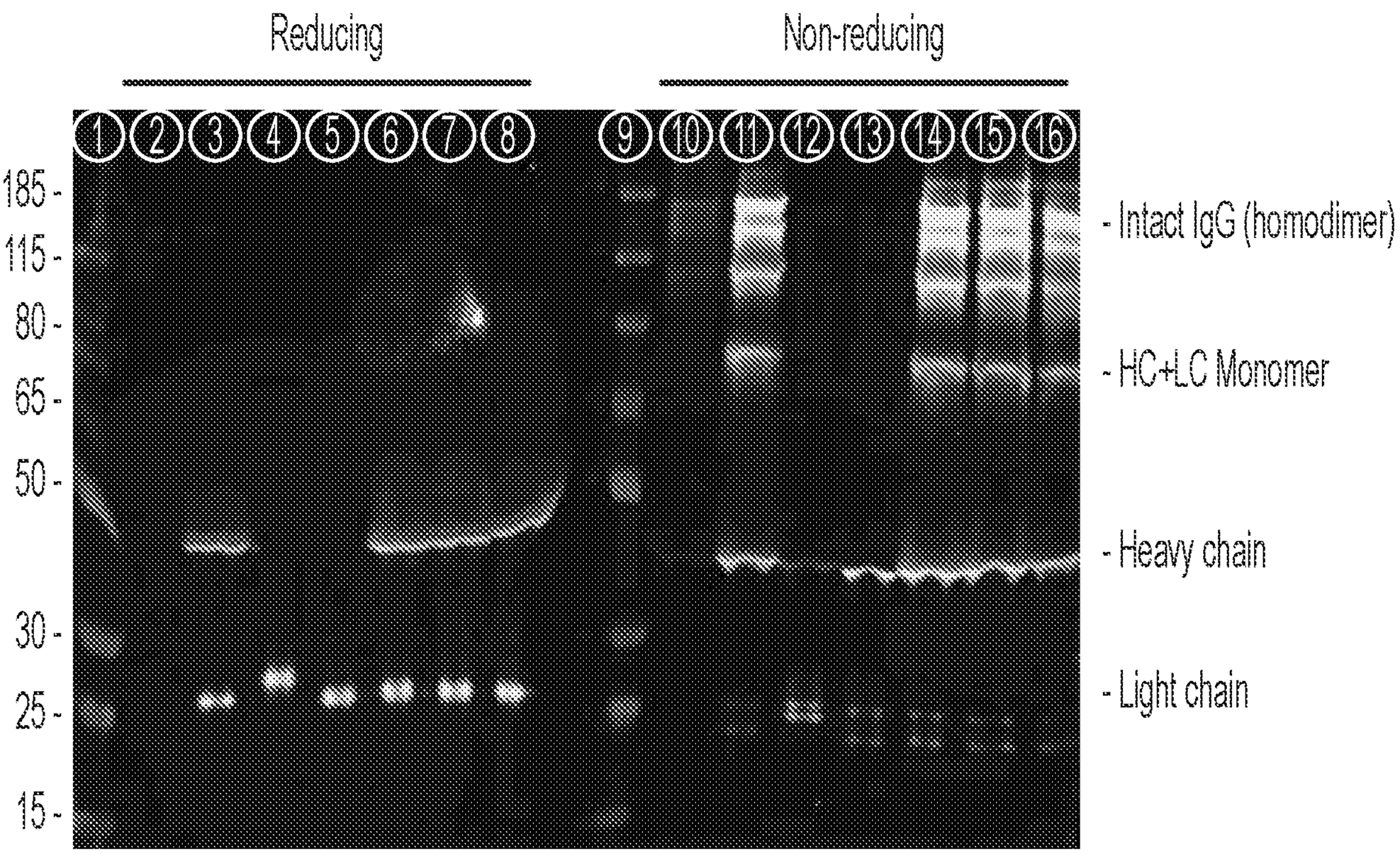

Reducing
Non-reducing
1 2 3 4 5 6 7 8
9 10 11 12 13 14 15 16
185 -
115 -
80 -
65 -
50 -
30 -
25 -
15 -
- Intact IgG (homodimer)
- HC+LC Monomer
- Heavy chain
- Light chain

FIG. 7

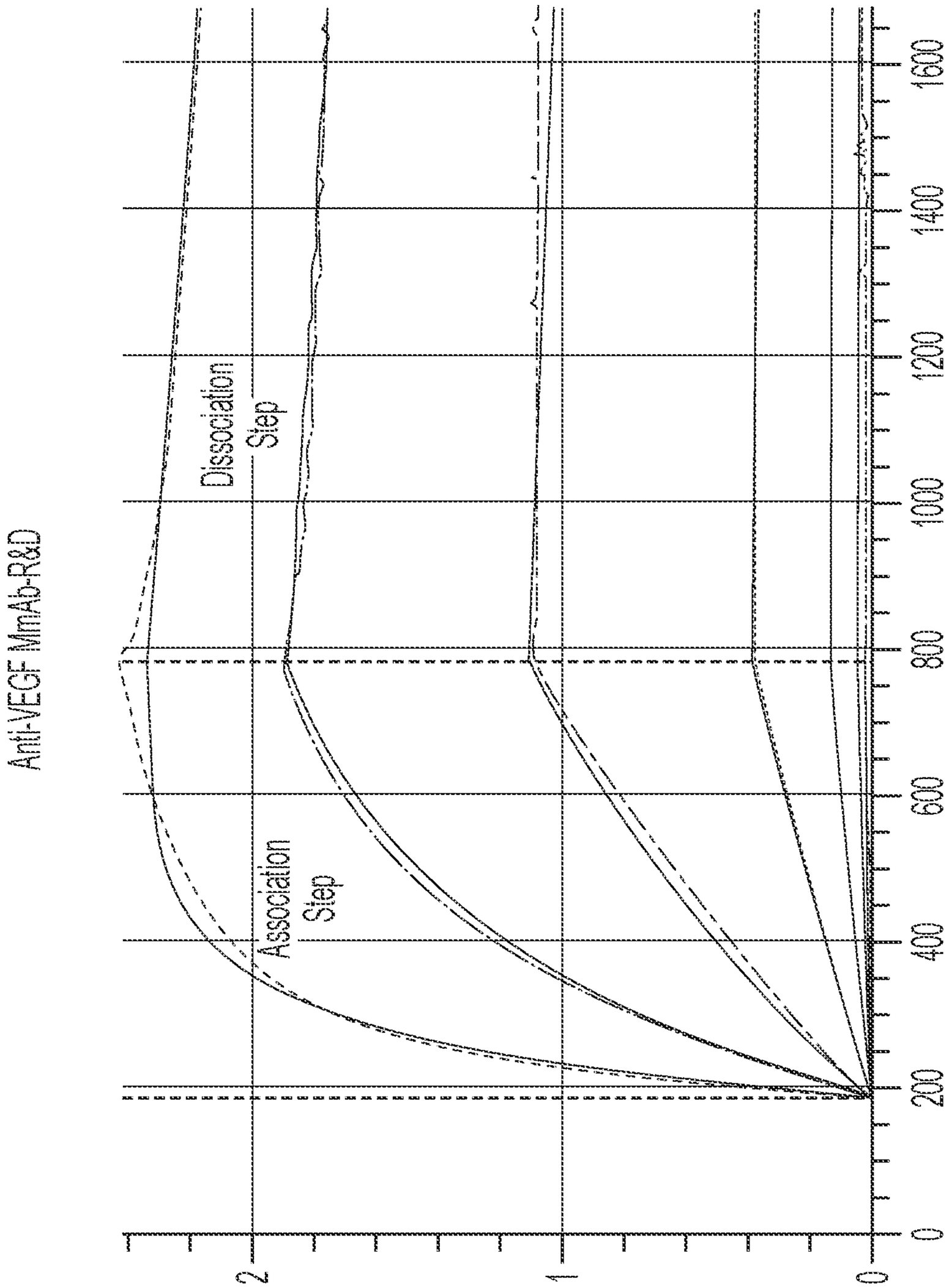

Anti-VEGF MmAb-R&D
Association Step
Dissociation Step
0
200
400
600
800
1000
1200
1400
1600
Time (s)
nm
0
1
2

FIG. 8A

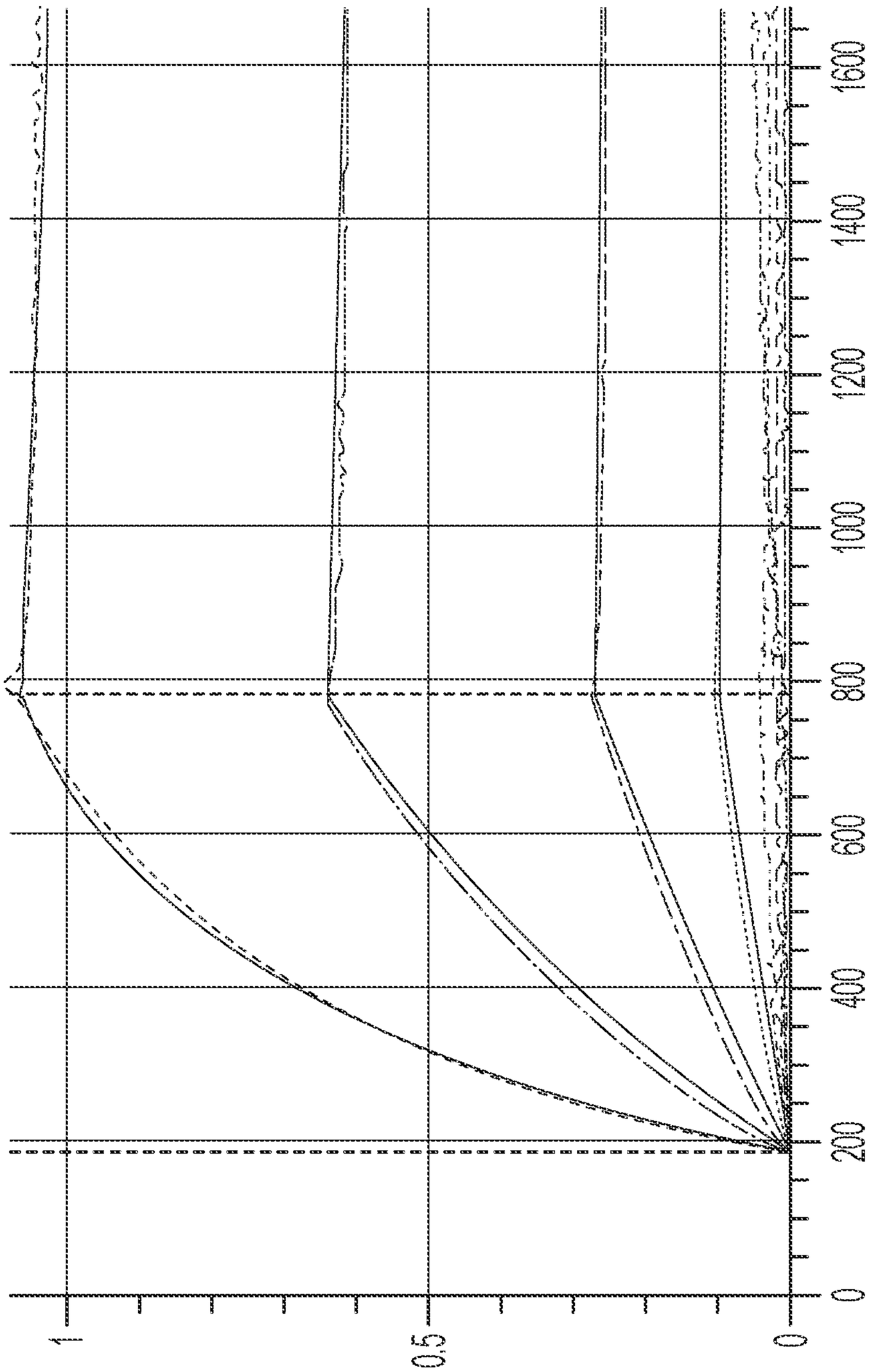


FIG. 8B

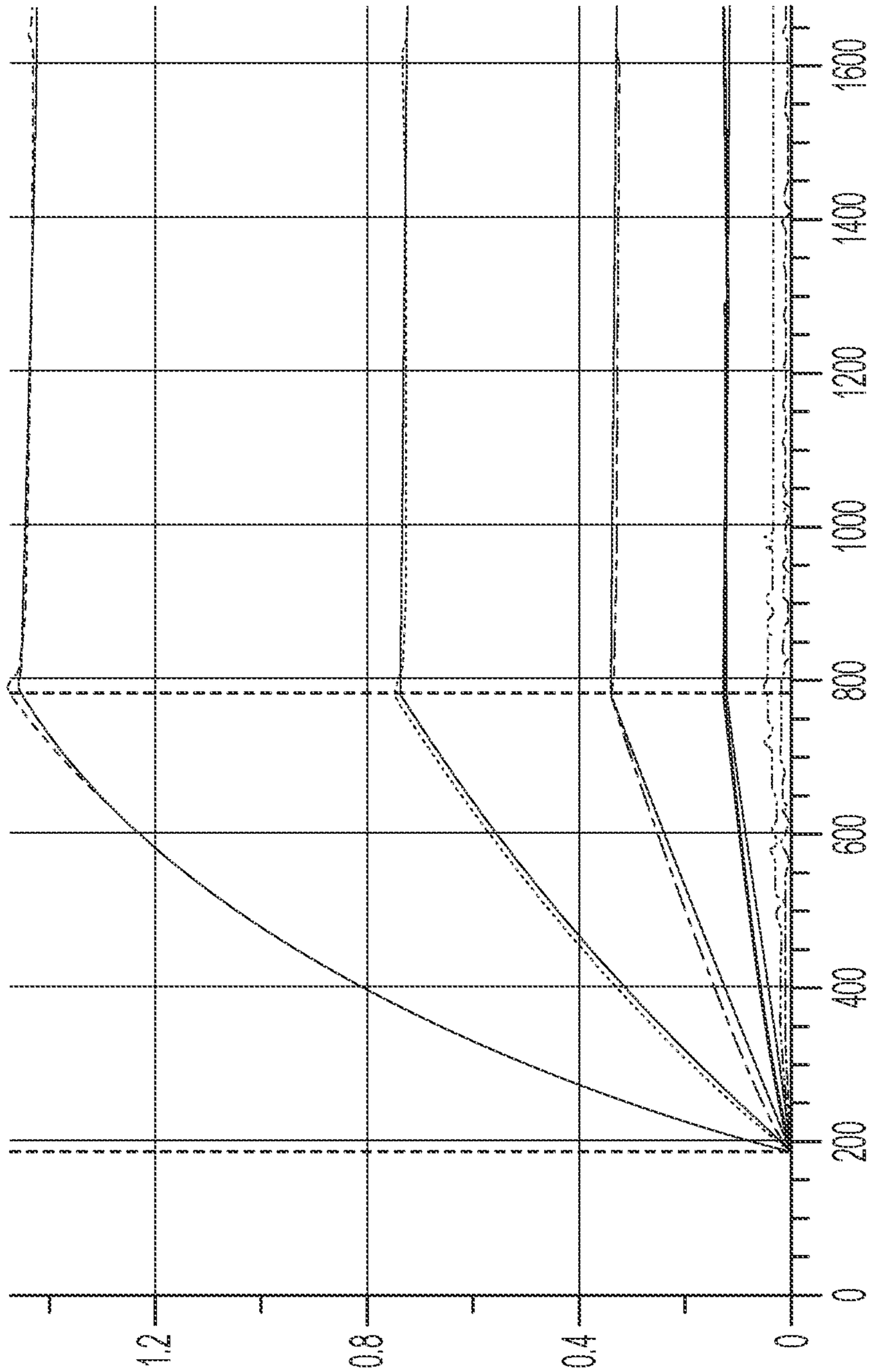

Bevacizumab
Time (s)
0
200
400
600
800
1000
1200
1400
1600
0
0,4
0,8
1,2
nm

FIG. 8C

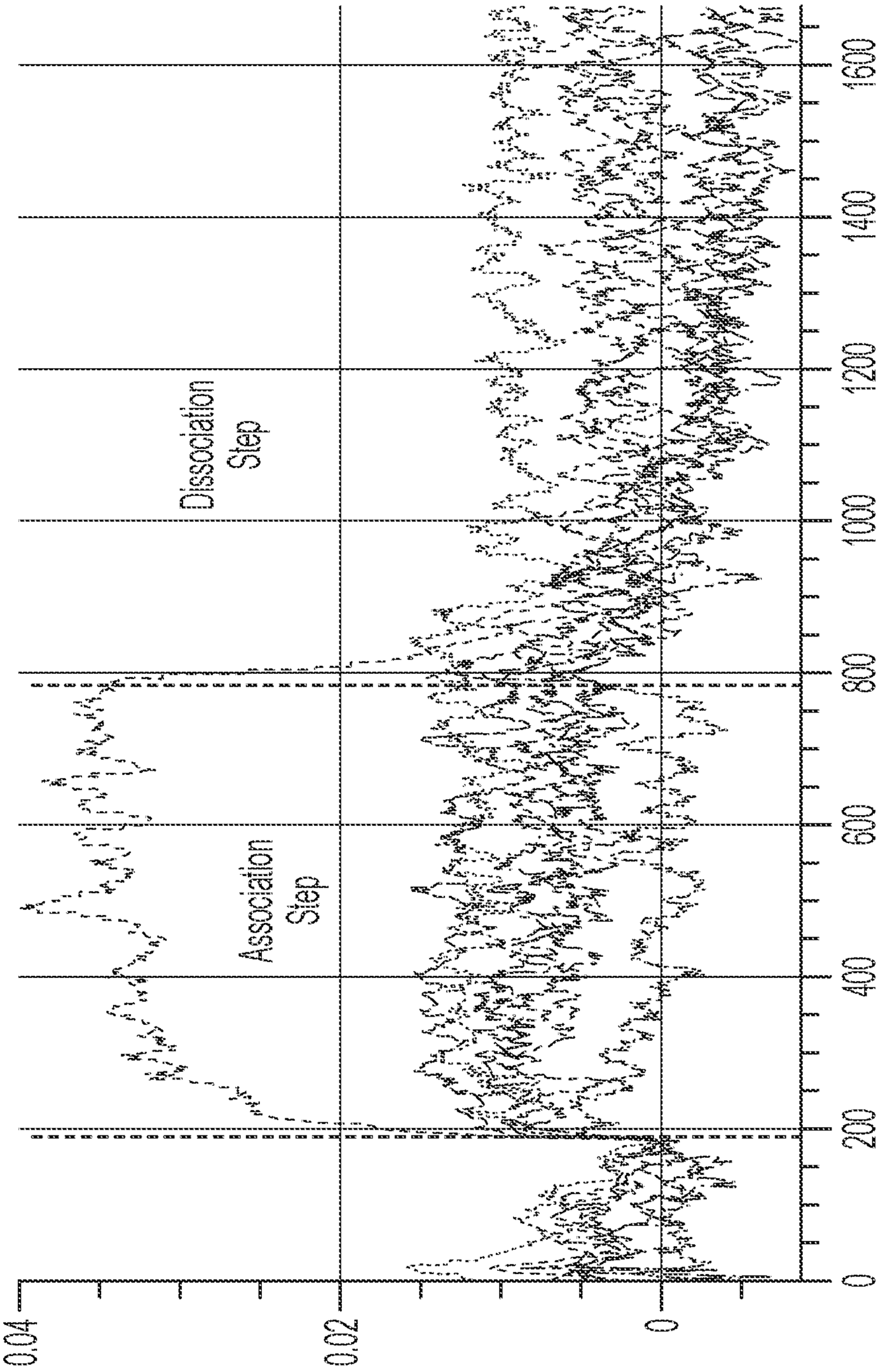

Control Conditioned Media
Association Step
Dissociation Step
0.04
0.02
0
nm
0
200
400
600
800
1000
1200
1400
1600
Time (s)

FIG. 8D

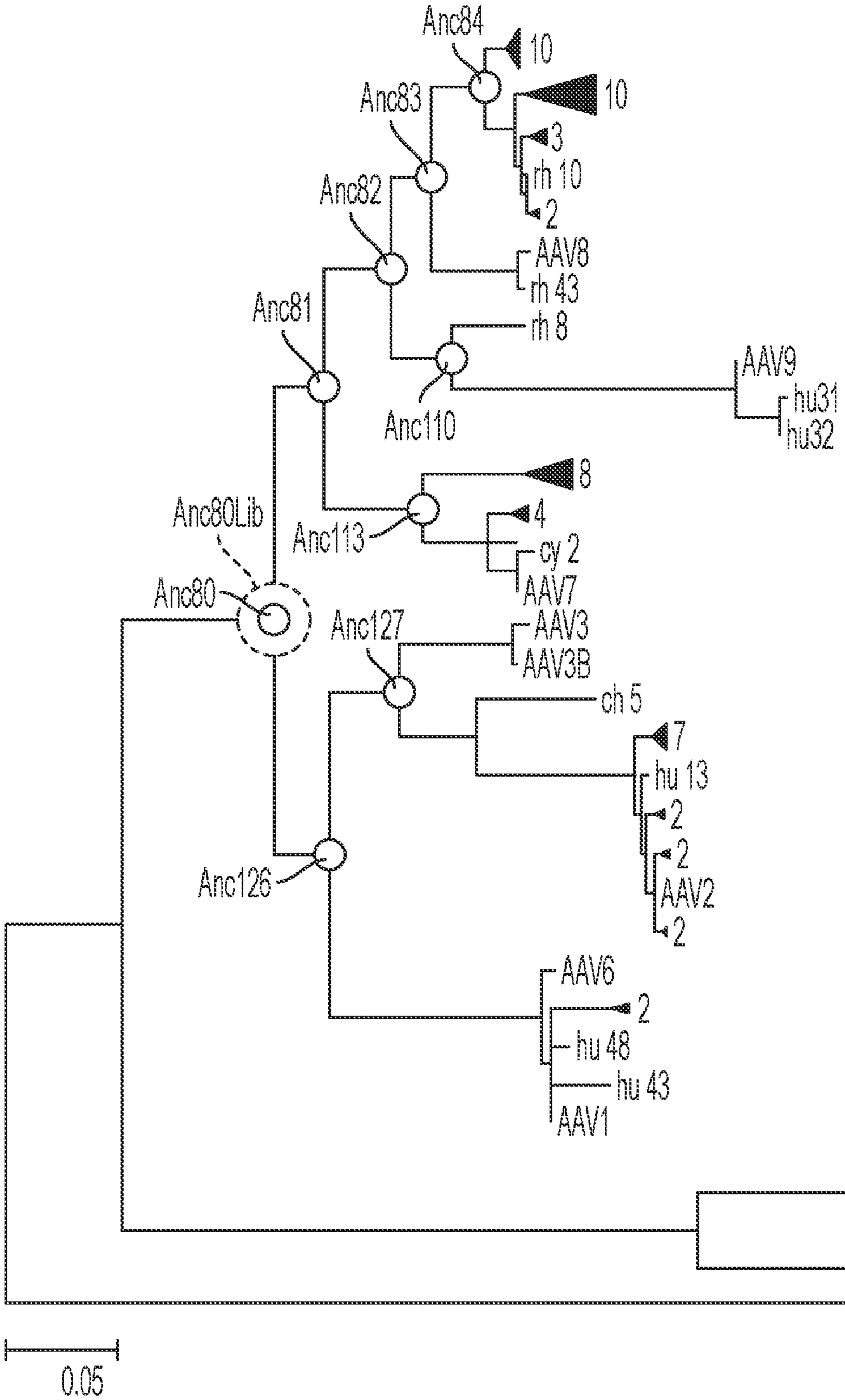

Anc84
10
Anc83
10
3
rh 10
2
Anc82
AAV8
rh 43
Anc81
rh 8
AAV9
hu31
hu32
Anc110
8
Anc80Lib
4
Anc113
cy 2
AAV7
Anc80
Anc127
AAV3
AAV3B
ch 5
7
hu 13
2
2
AAV2
2
Anc126
AAV6
2
hu 48
hu 43
AAV1
rh 32
rh 33
rh 34
AAV4
AAV5
0.05

FIG. 9

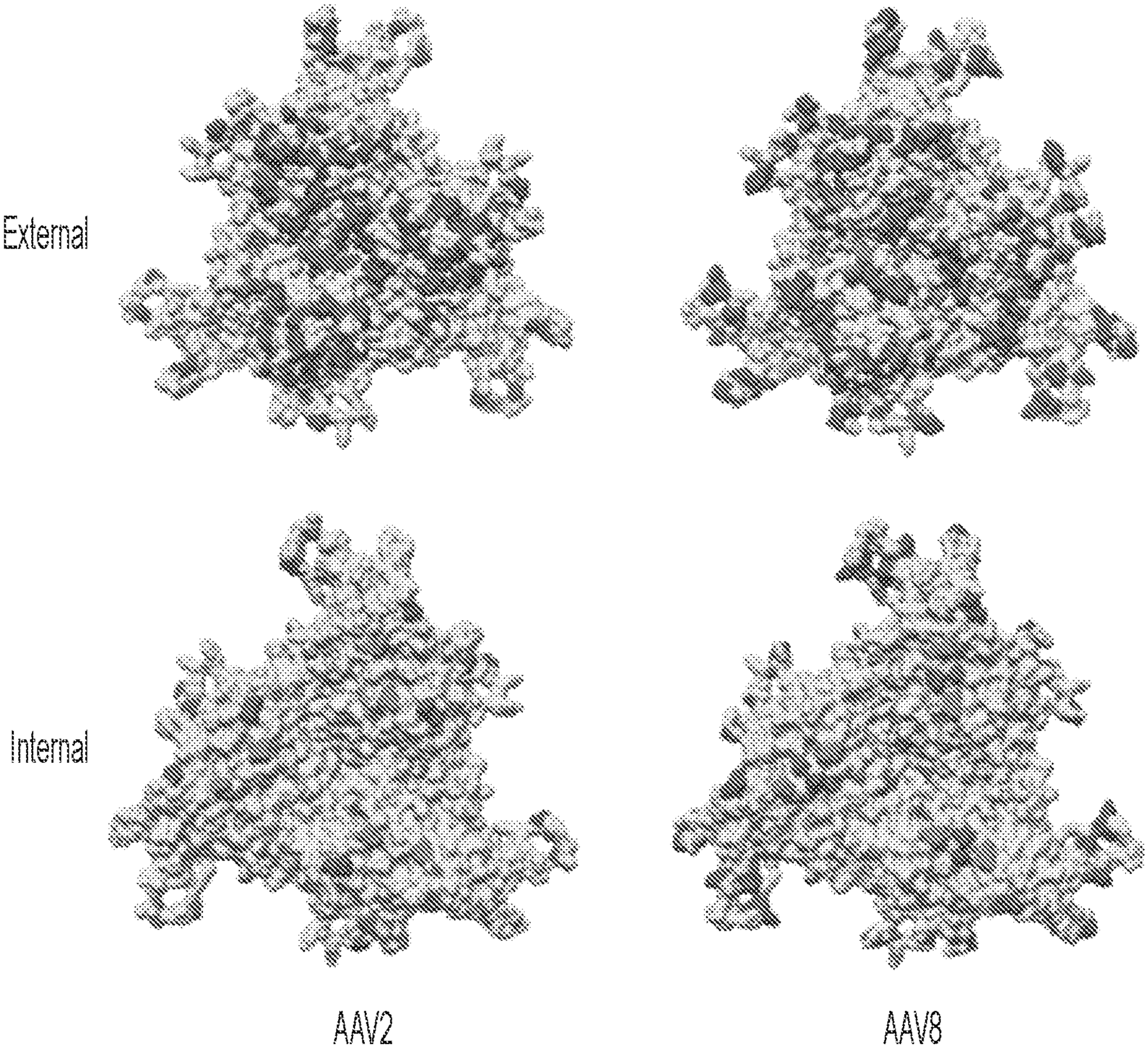


FIG. 10

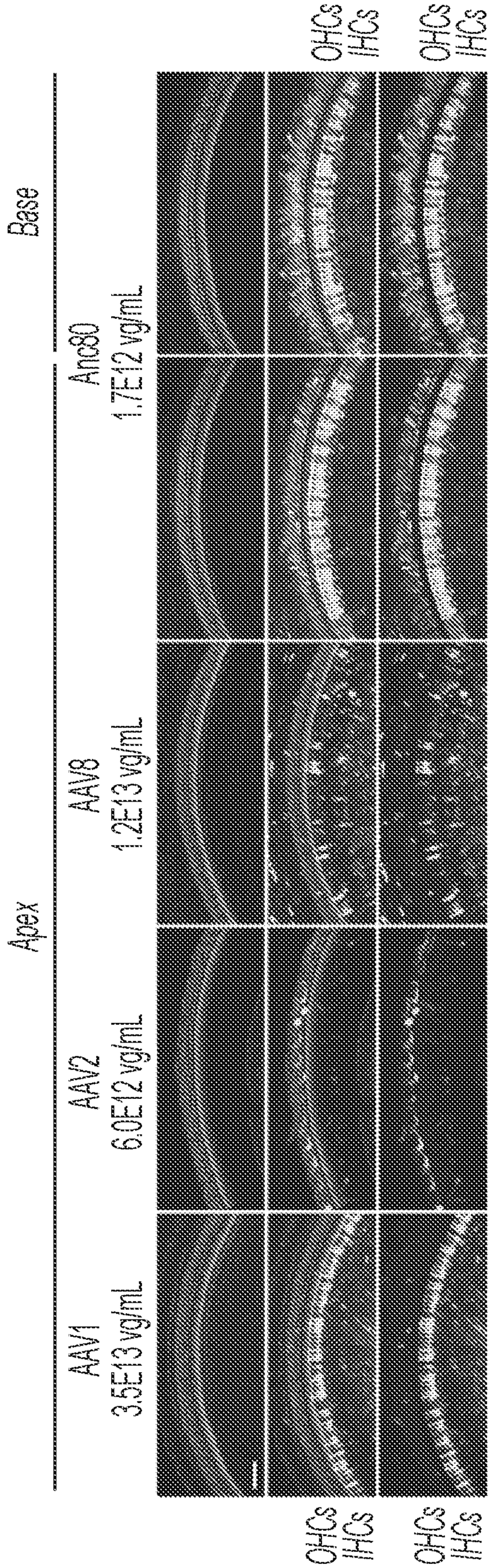

Base
Apex
AAV2
6.0E12 vg/mL
AAV1
3.5E13 vg/mL
Anc80
1.7E12 vg/mL
AAV8
1.2E13 vg/mL
OHCs
IHCs
OHCs
IHCs
OHCs
IHCs
OHCs
IHCs

FIG. 11

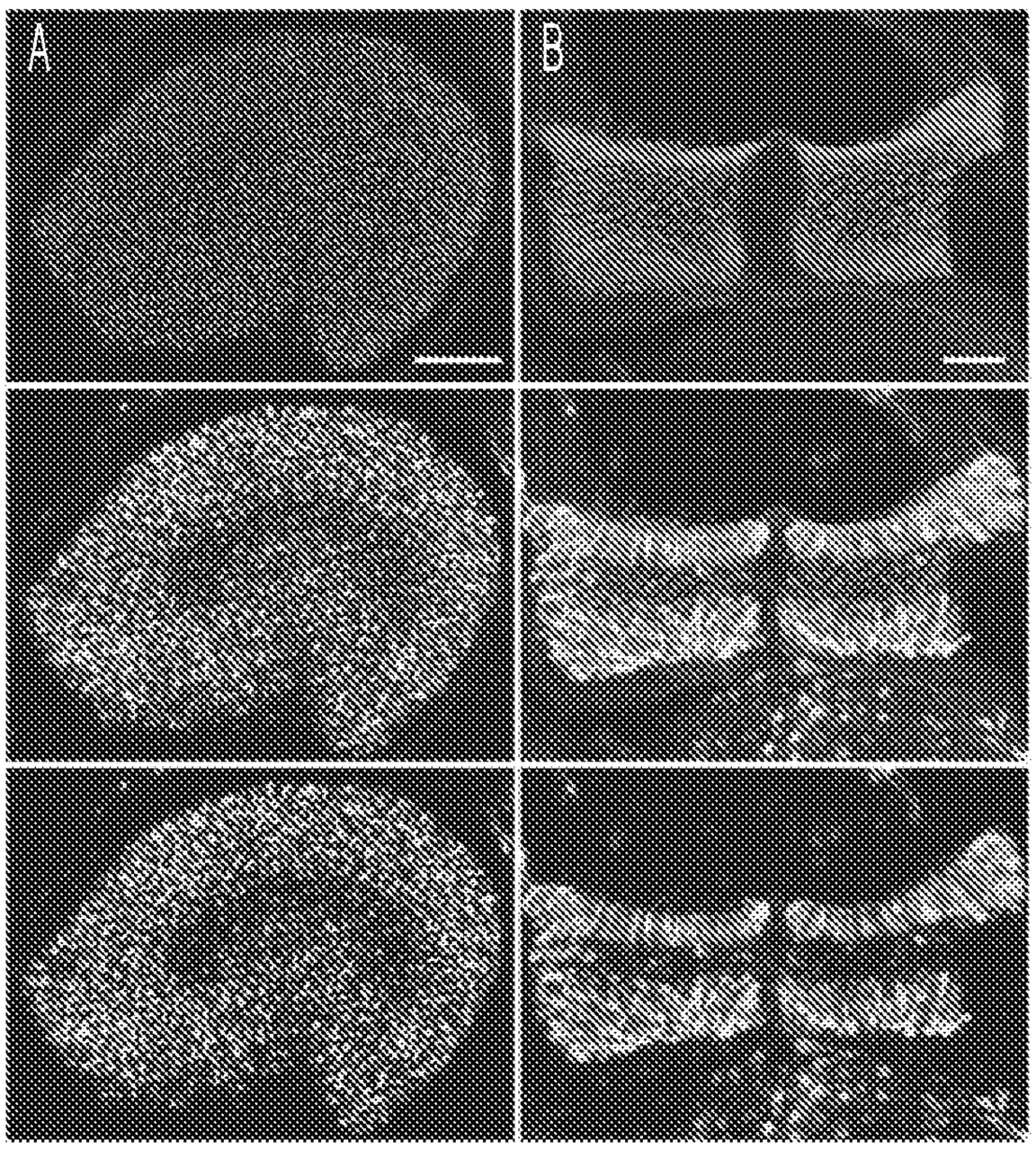


FIG. 12

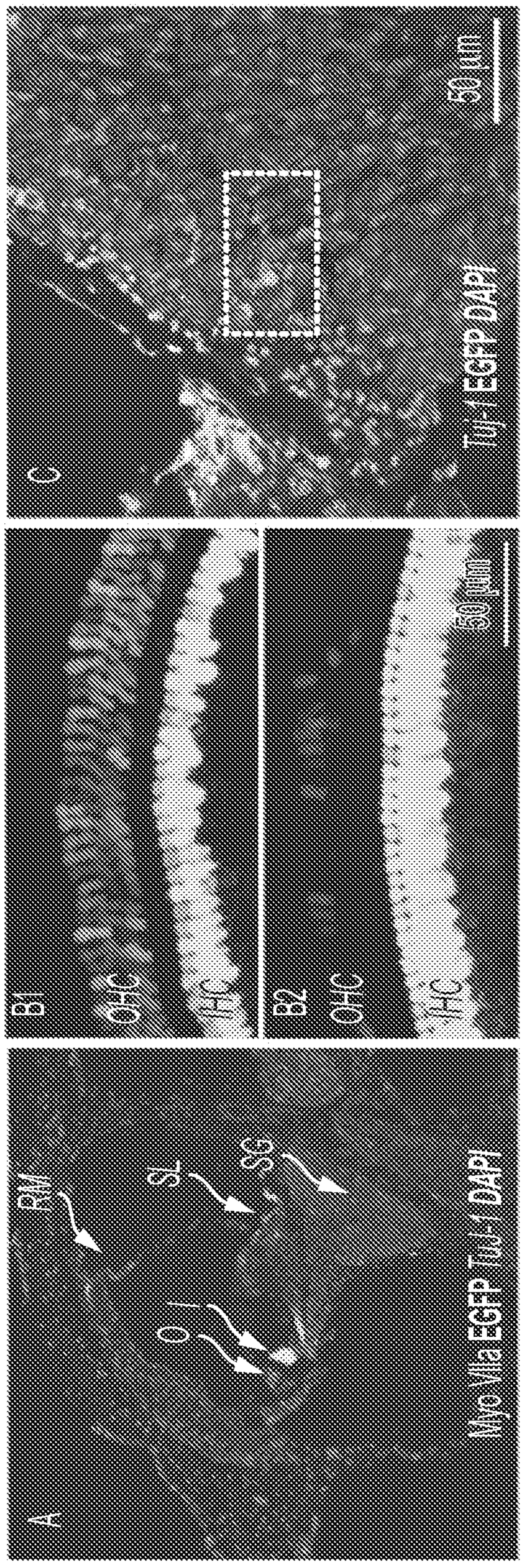

A
My VIIa EGFP Tuj-1 DAPI
RM
SL
SG
O
B1
OHC
IHC
B2
OHC
IHC
50 µm
C
Tuj-1 EGFP DAPI
50 µm

FIG. 13

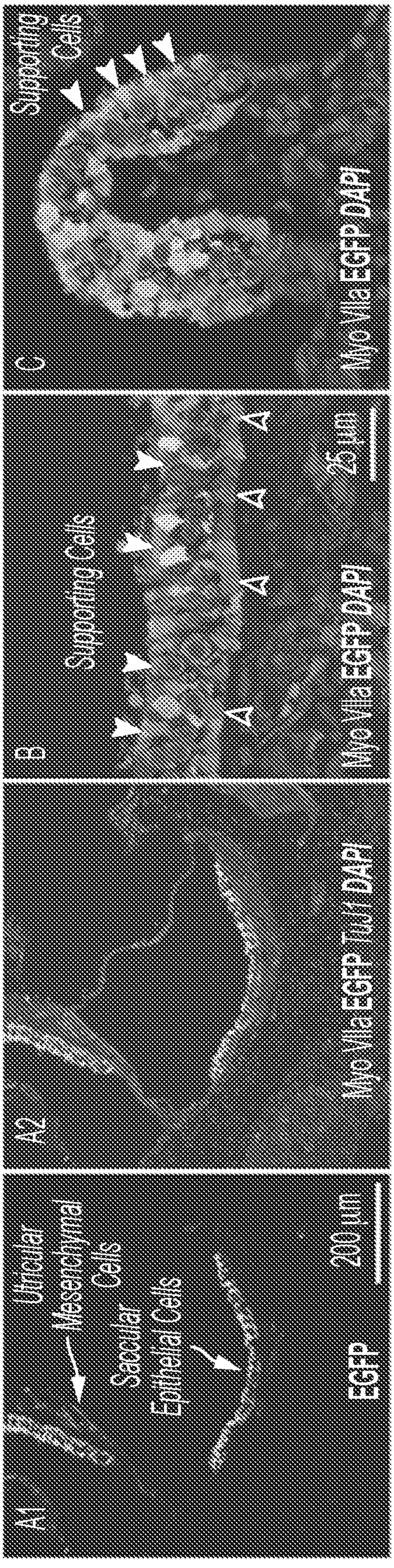

A1
Unicular Mesenchymal Cells
Saccular Epithelial Cells
EGFP DAPI
200 µm
A2
Myo Vila EGFP TdT DAPI
B
Supporting Cells
Myo Vila EGFP DAPI
25 µm
C
Supporting Cells
MyoVila EGFP DAPI

FIG. 14

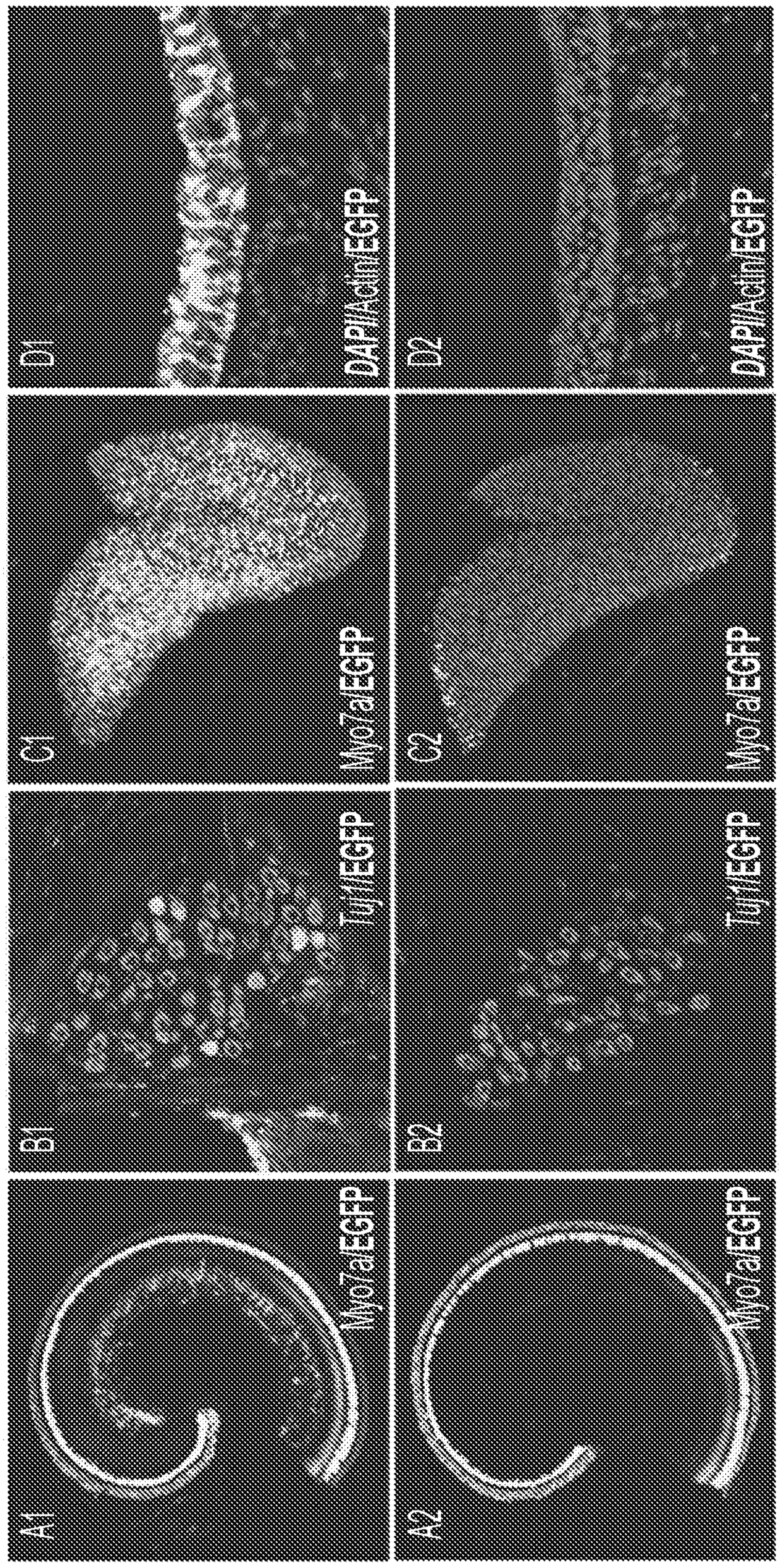

AAVAnc80
AAV2
A1
A2
B1
B2
C1
C2
D1
D2
MyO7aEGFP
Tuj1EGFP
DAPI/ActinEGFP

FIG. 15

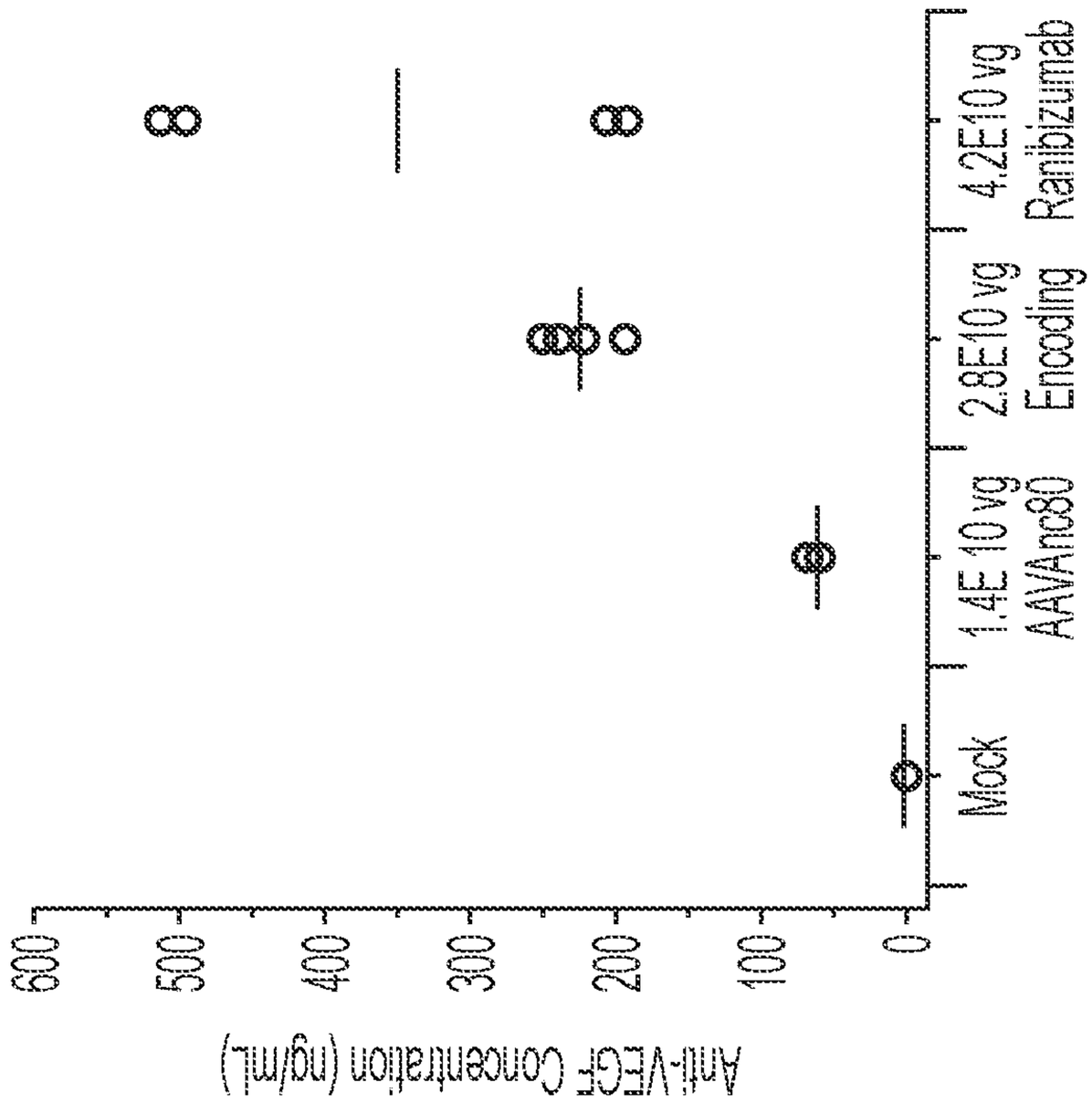


FIG. 16B

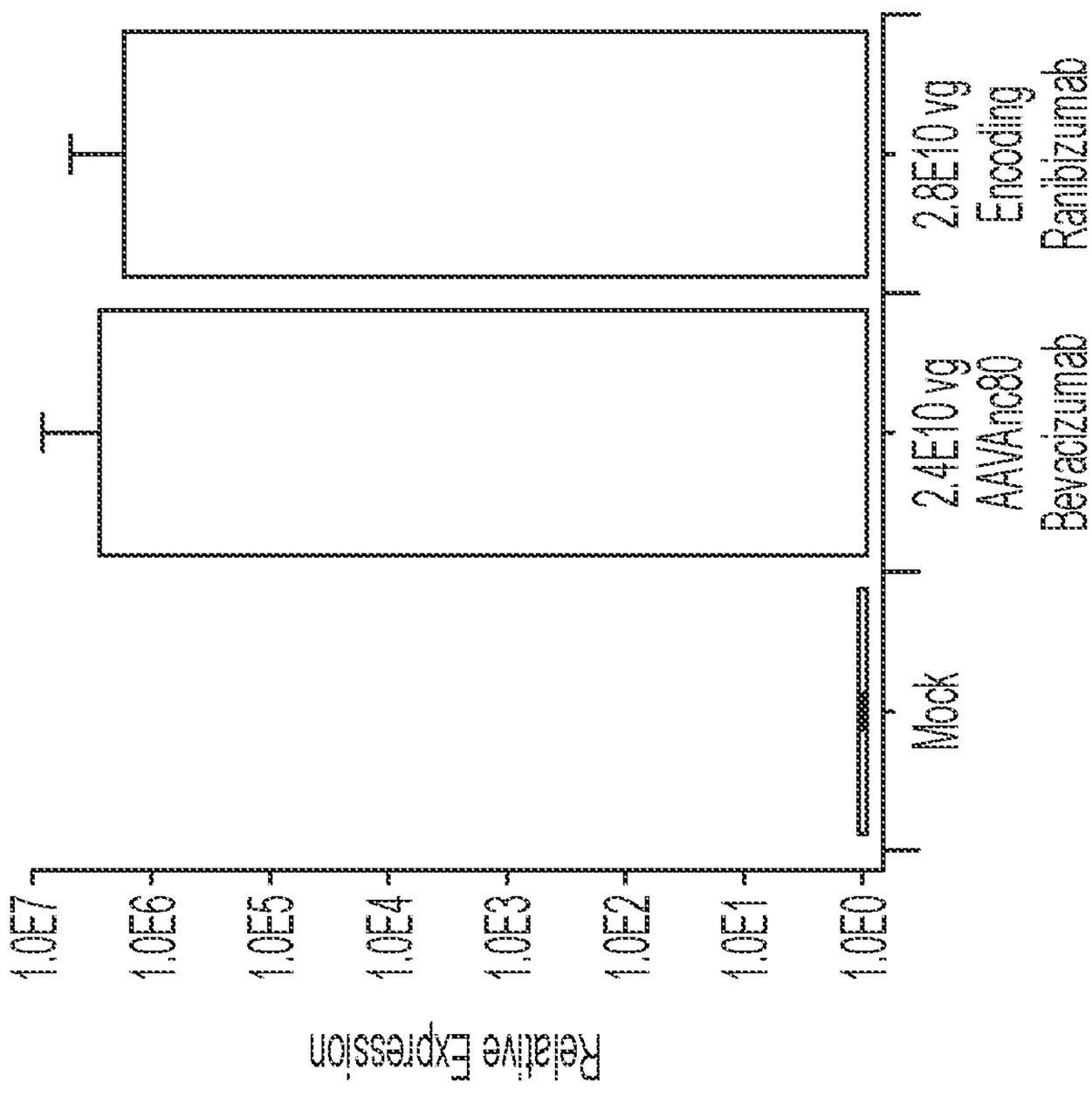


FIG. 16A

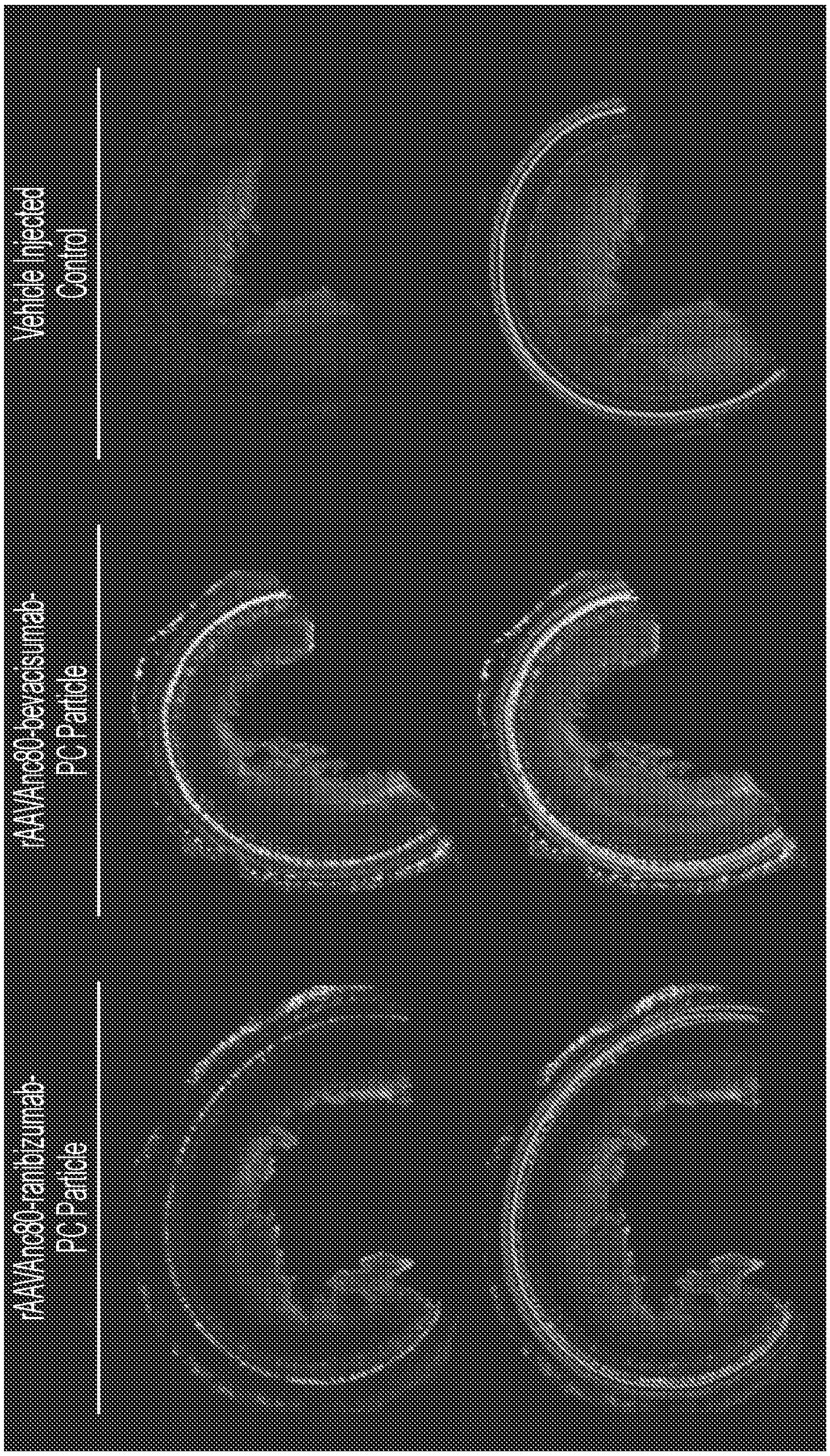

rAAVAnc80-bevacizumab-PC Particle
Vehicle Injected Control
rAAVAnc80-ranibizumab-PC Particle
anti-Fab Staining
Phalloidin Staining

FIG. 17

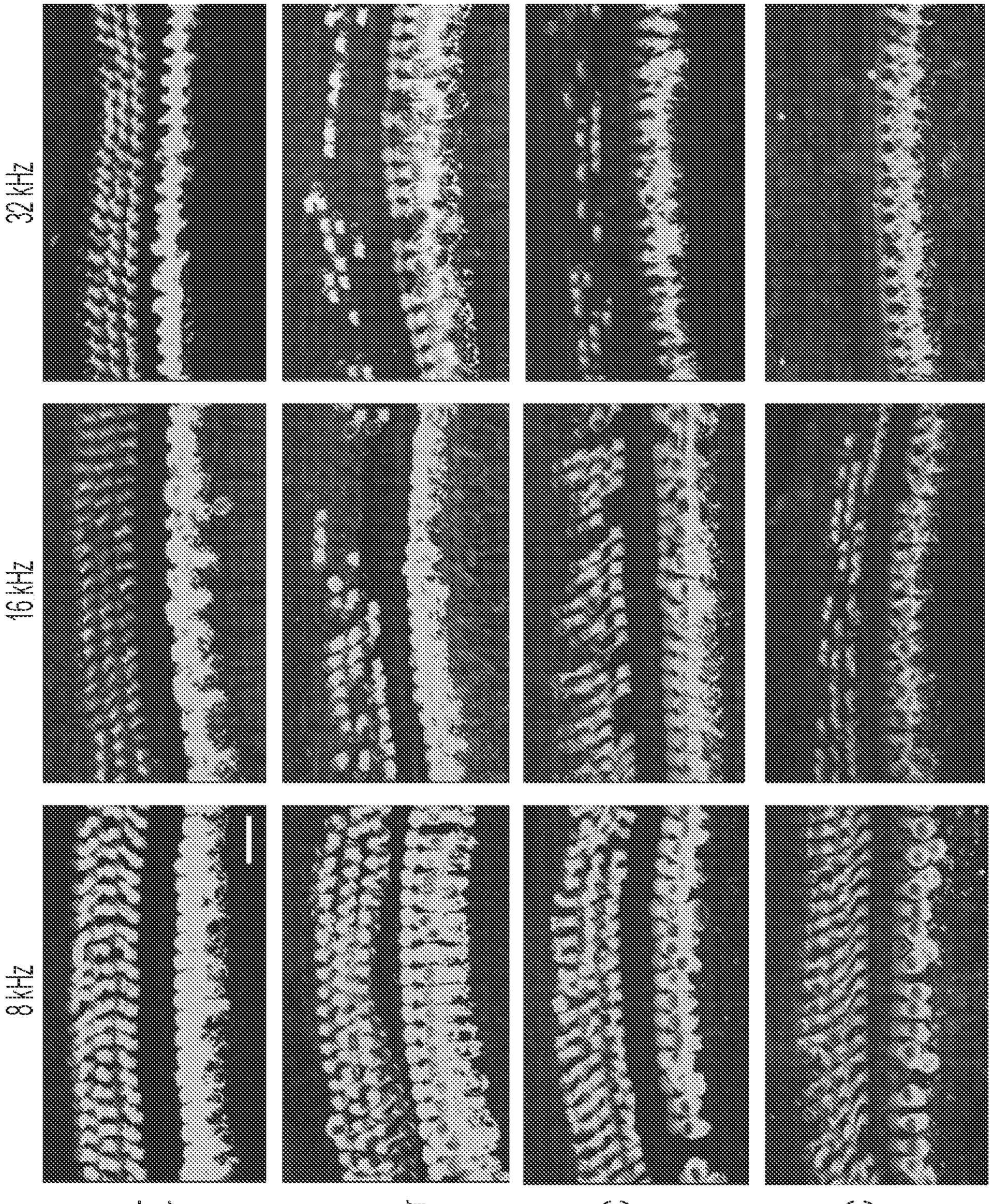

32 kHz
16 kHz
8 kHz
control non-injected ear
Vehicle injected ear
rAAVAnc80-ranibizumab-PC at 1.4E10 vg/cochlea
rAAVAnc80-bevacizumab-PC at 1.2E10 vg/cochlea

FIG. 18

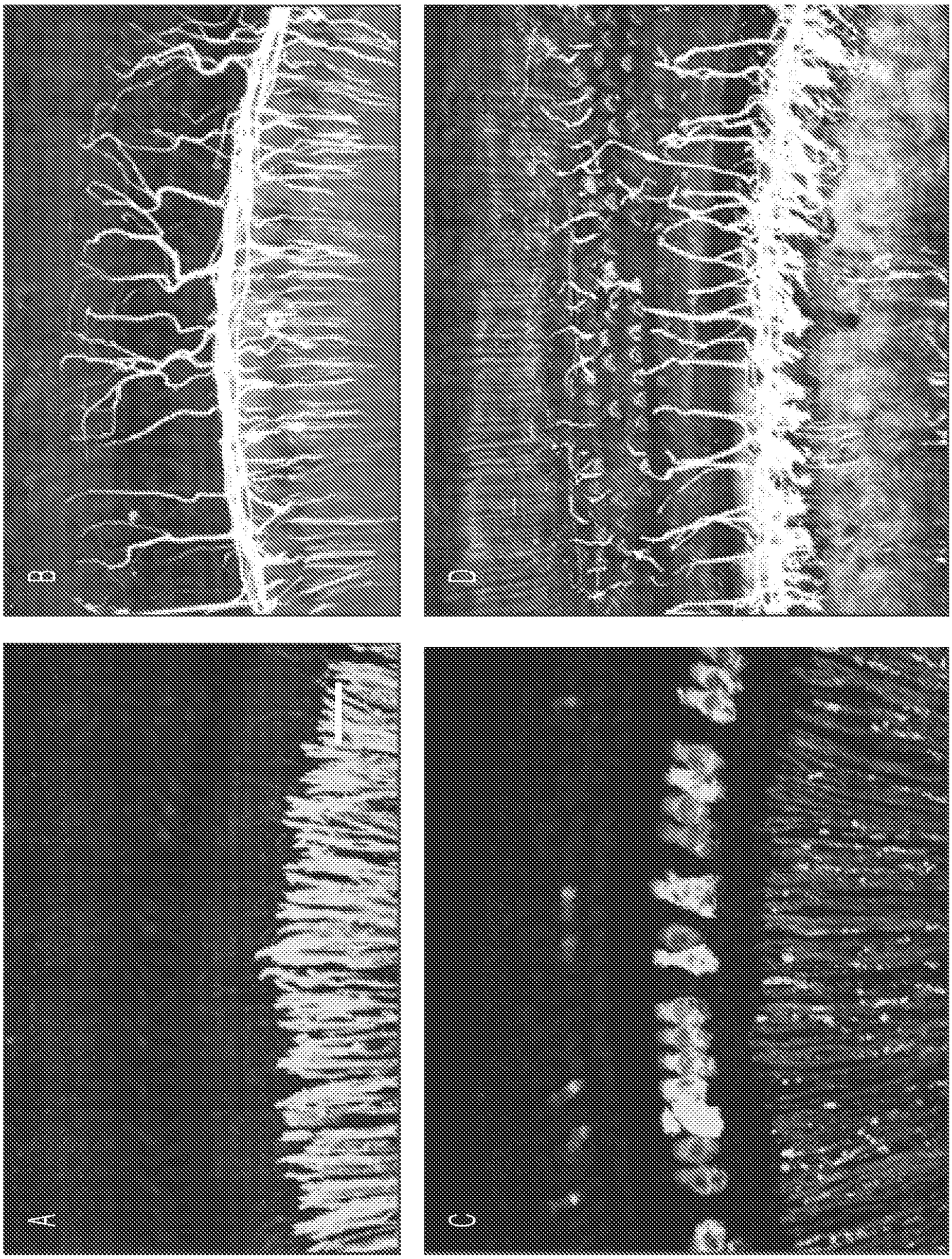

A
Vehicle injected control
B
C
rAAVAnc80-ranibizumab-PC at 1.4E10 vg/cochlea
D

FIG. 19

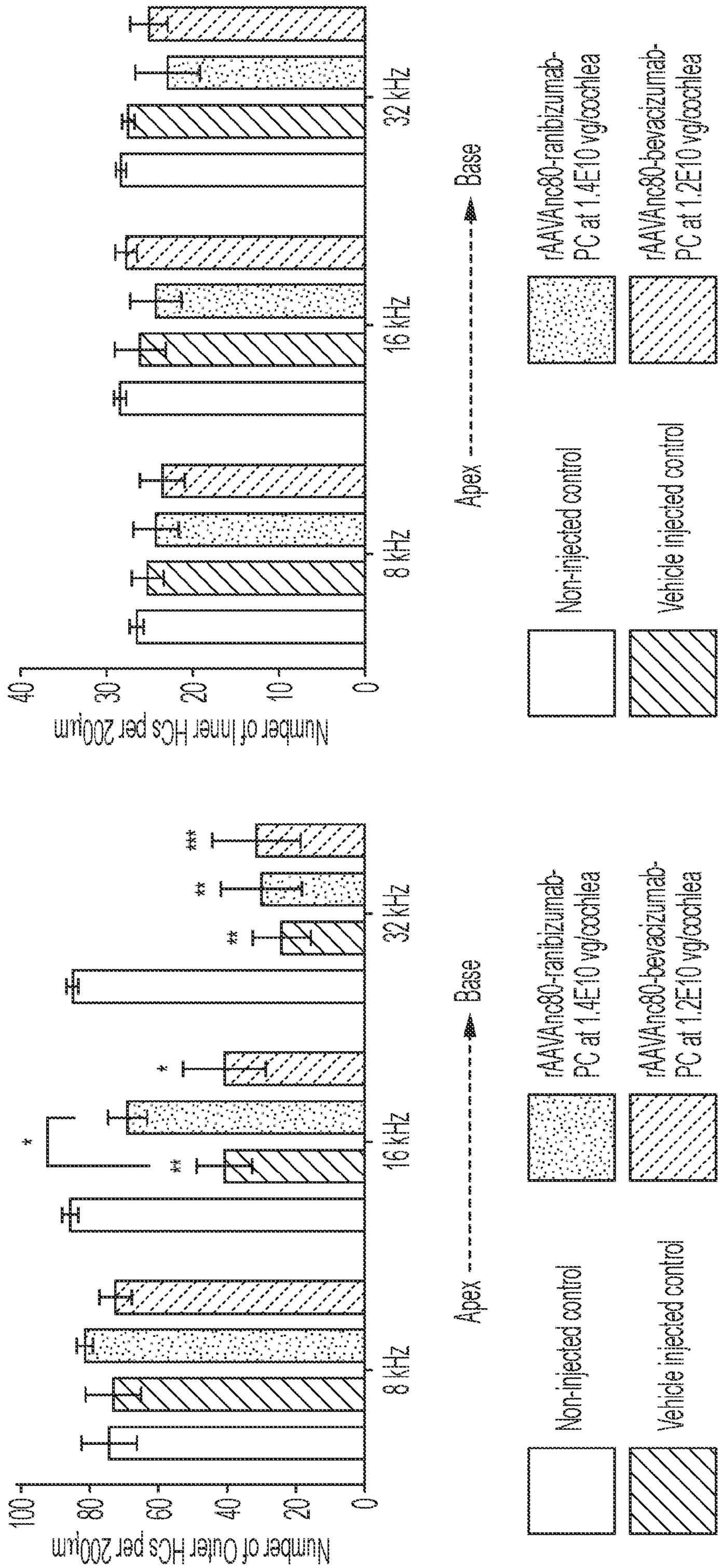


FIG. 20A

FIG. 20B

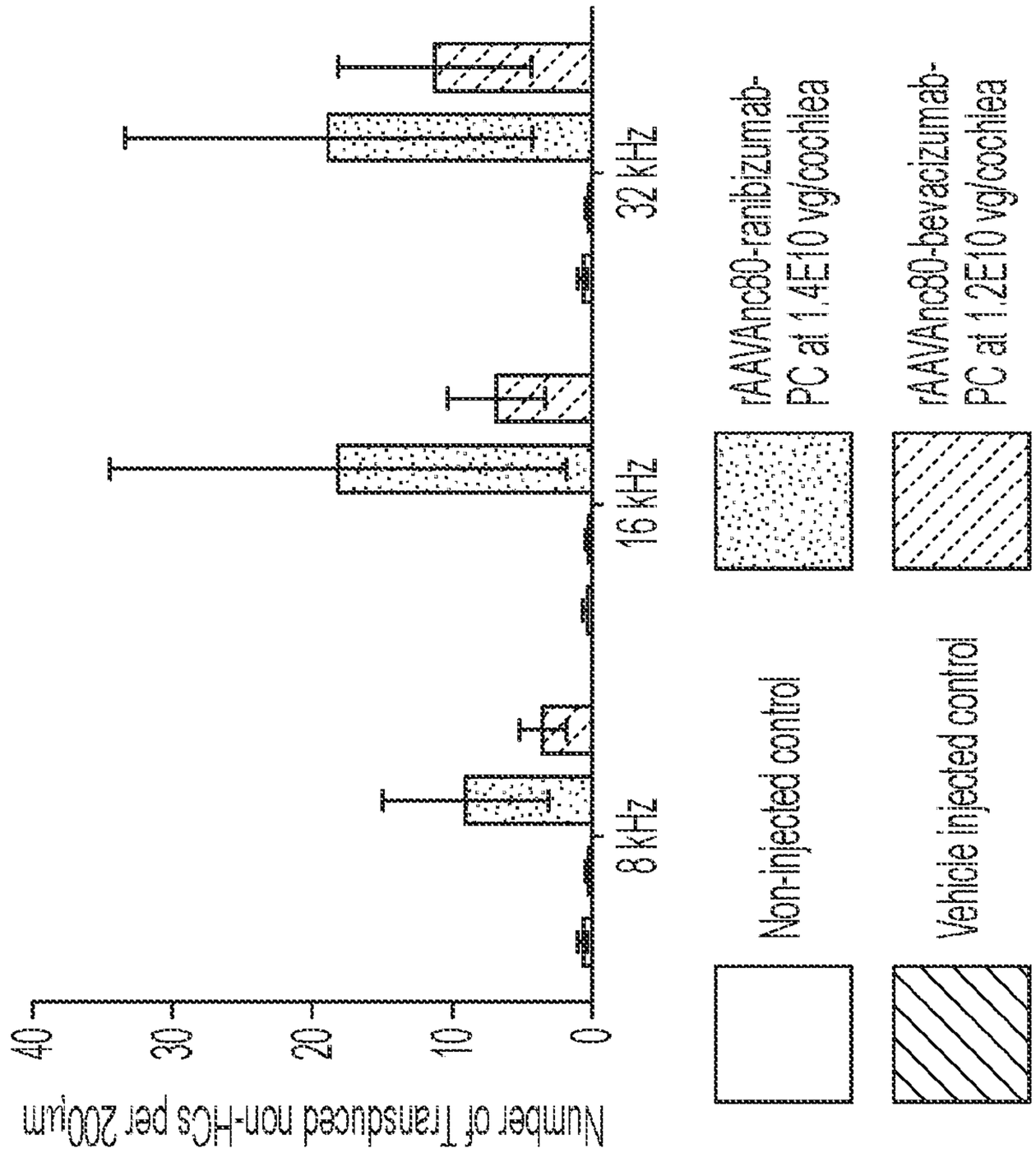


FIG. 21B

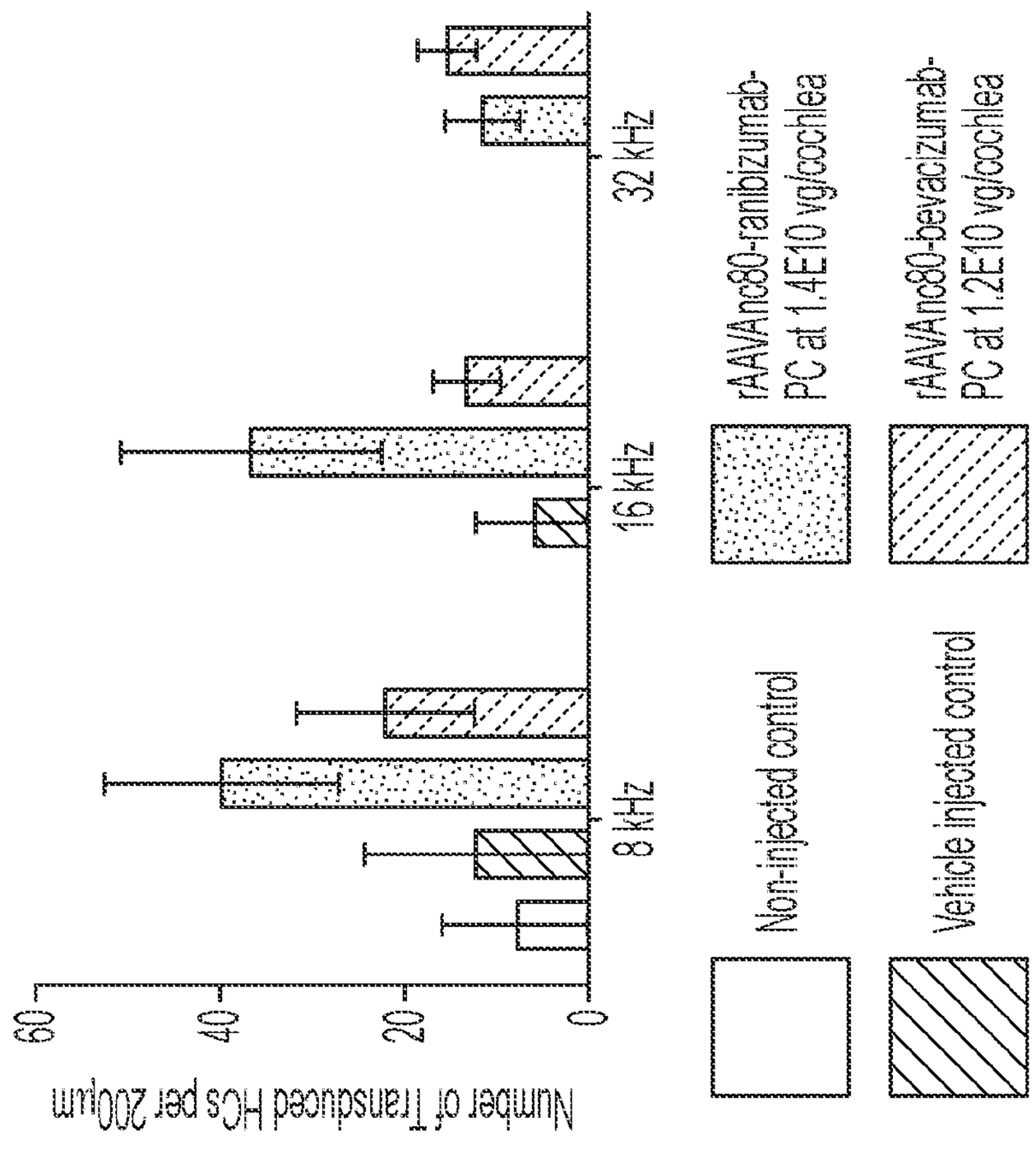


FIG. 21A


rAAVAnc80-HCLC at 1.4E10 vg/cochlea
Mouse 1
Mouse 2
Mouse 3
Hensen's cells
OHCs
IHCs
Radial nerve fibers
IHCs
Interdental cells
IHCs
Interdental cells
8 kHz
16 kHz
32 kHz

FIG. 22

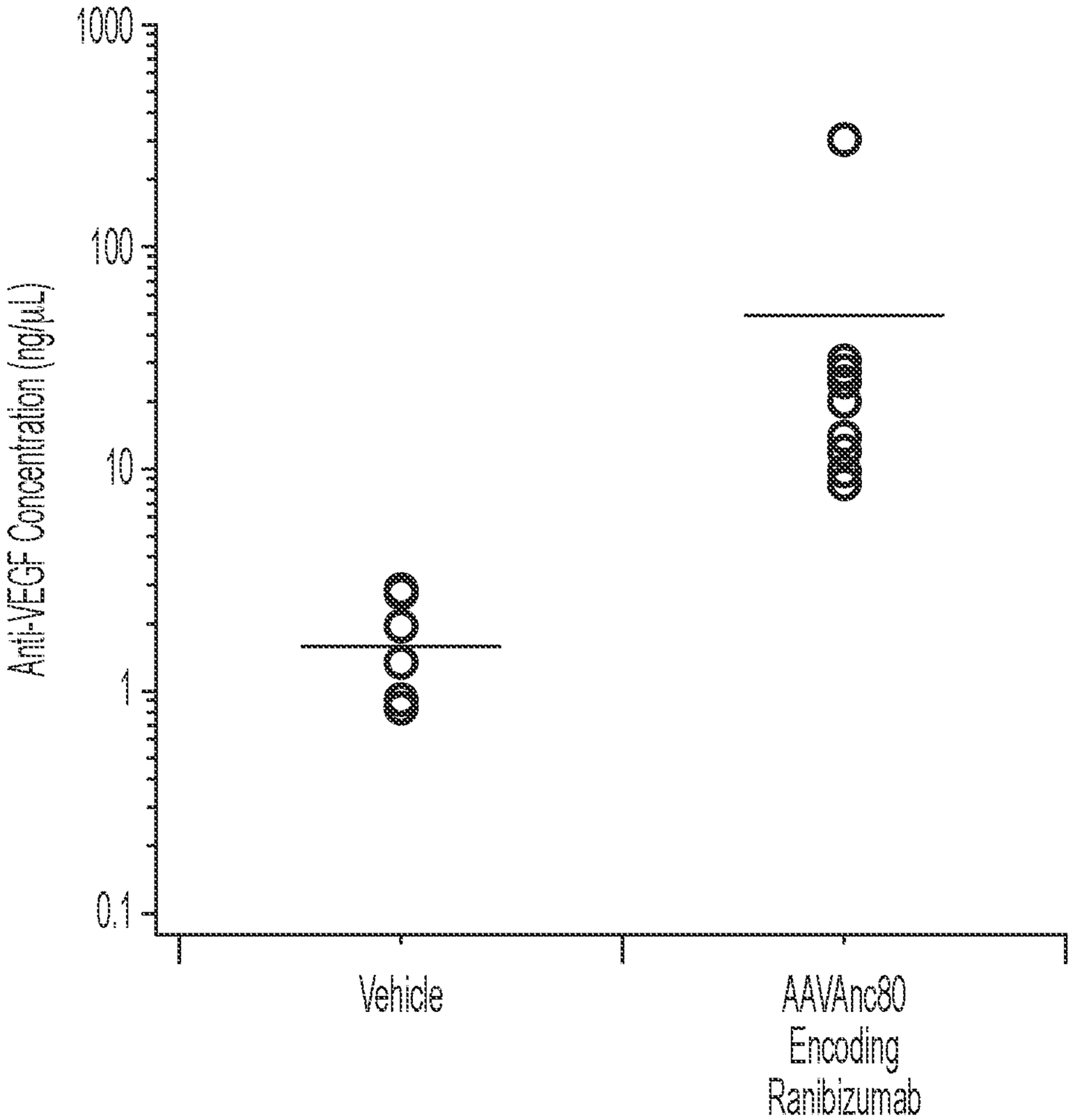

Anti-VEGF Concentration (ng/μL)
1000
100
10
1
0.1
Vehicle
AAVAnc80
Encoding
Ranibizumab

FIG. 23

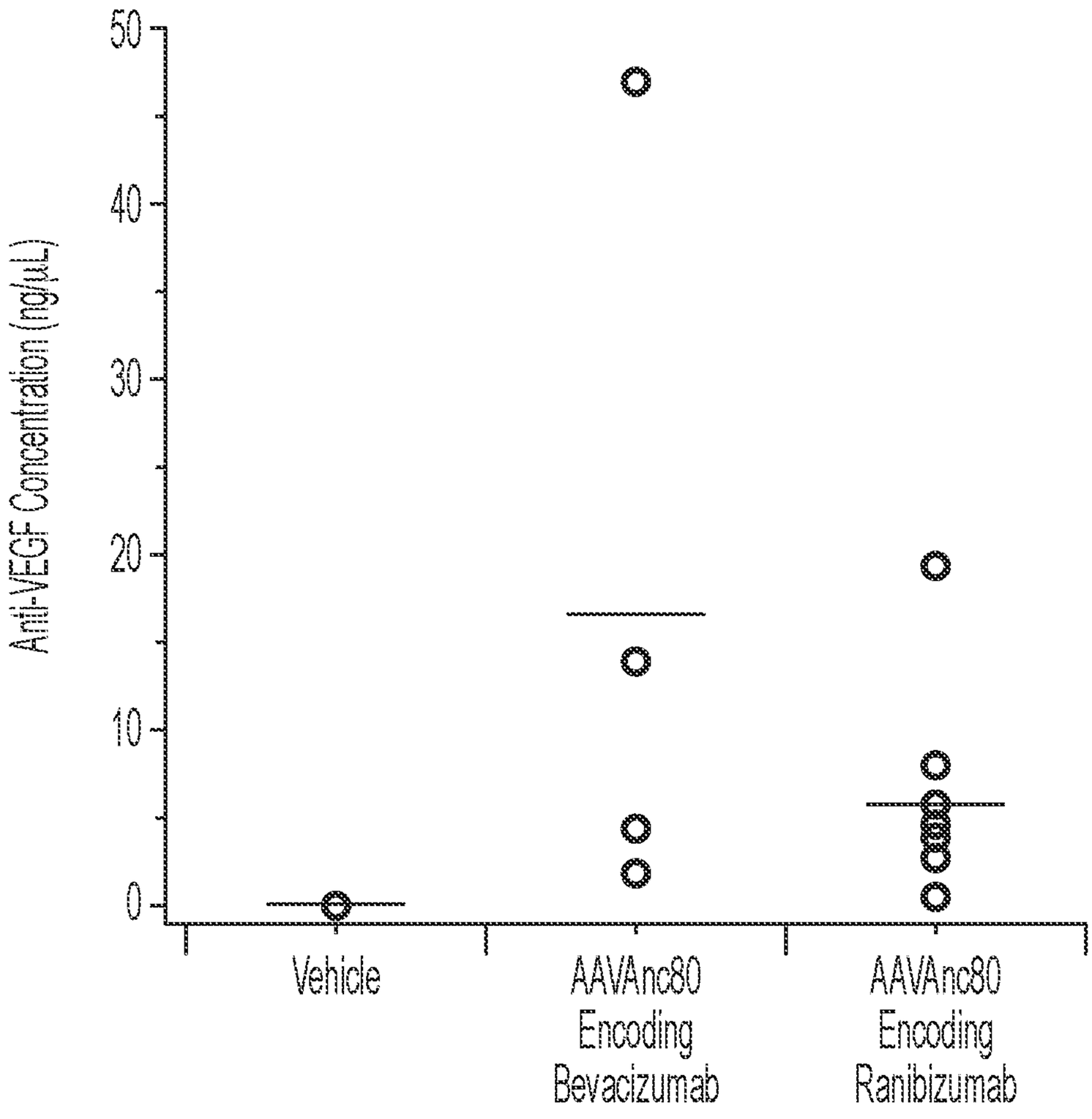

Anti-VEGF Concentration (ng/µL)
50
40
30
20
10
0
Vehicle
AAVAnc80 Encoding Bevacizumab
AAVAnc80 Encoding Ranibizumab

FIG. 24

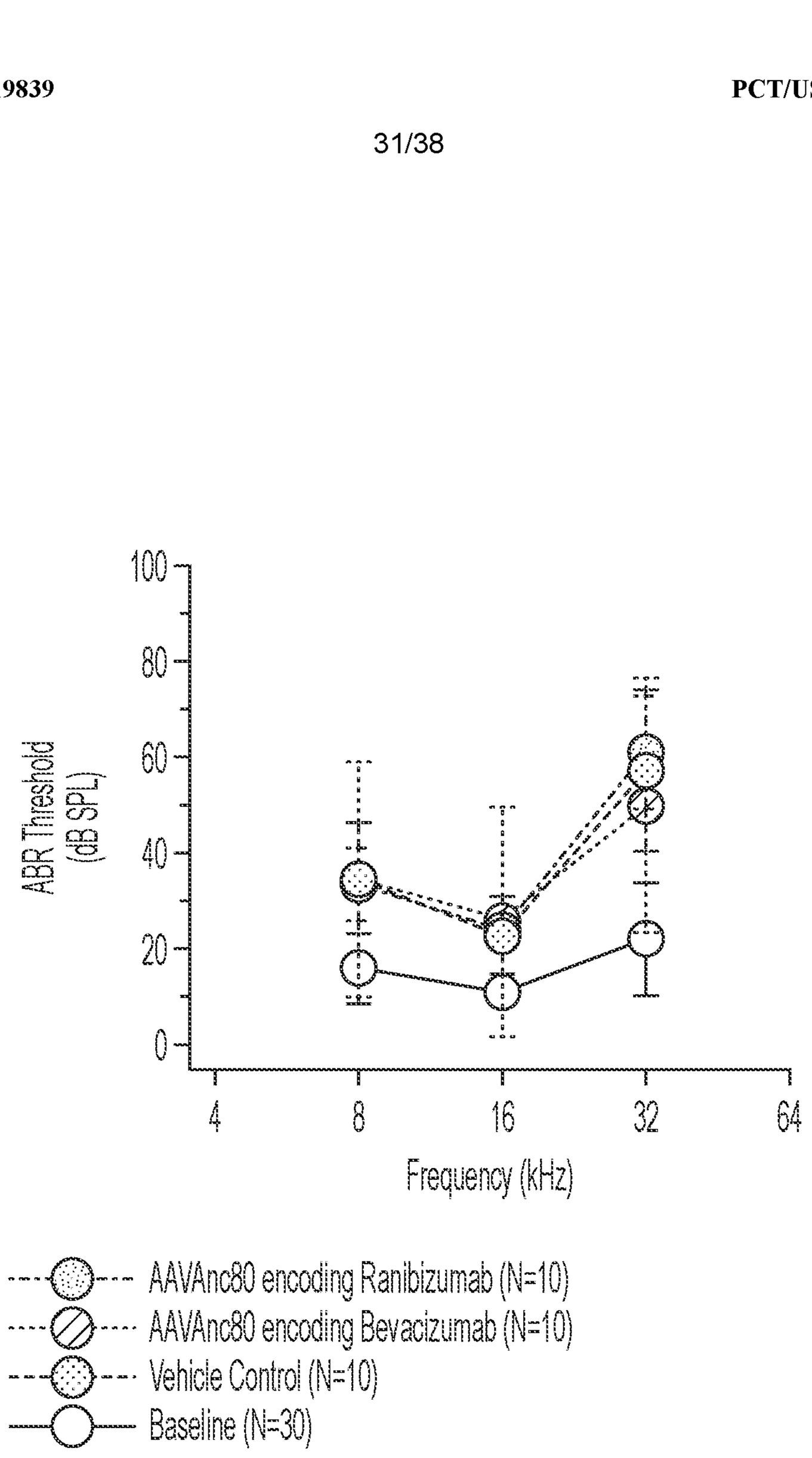

ABR Threshold
(dB SPL)
0
20
40
60
80
100
4
8
16
32
64
Frequency (kHz)
AAVAnc80 encoding Ranibizumab (N=10)
AAVAnc80 encoding Bevacizumab (N=10)
Vehicle Control (N=10)
Baseline (N=30)

FIG. 25

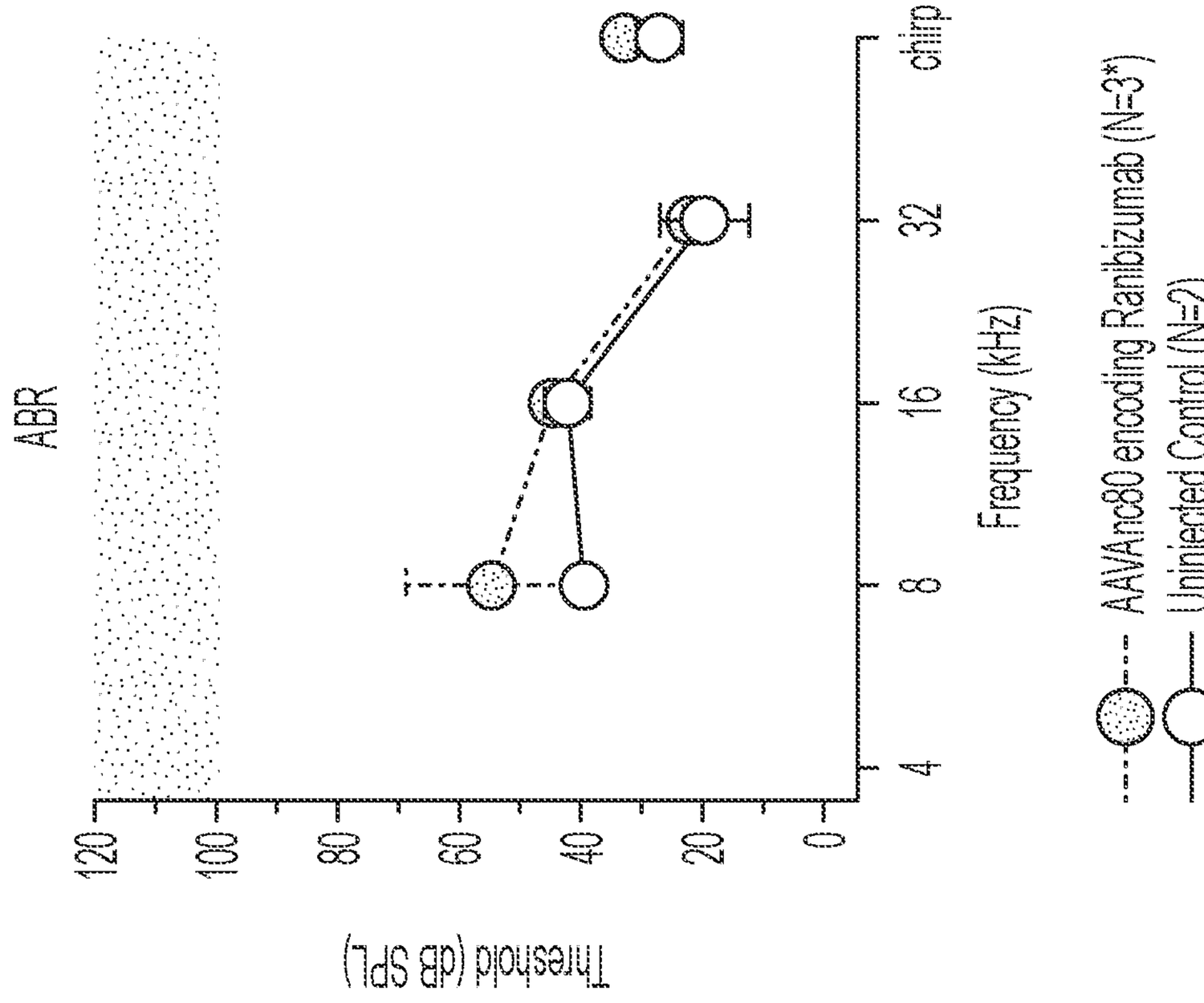


FIG. 26B

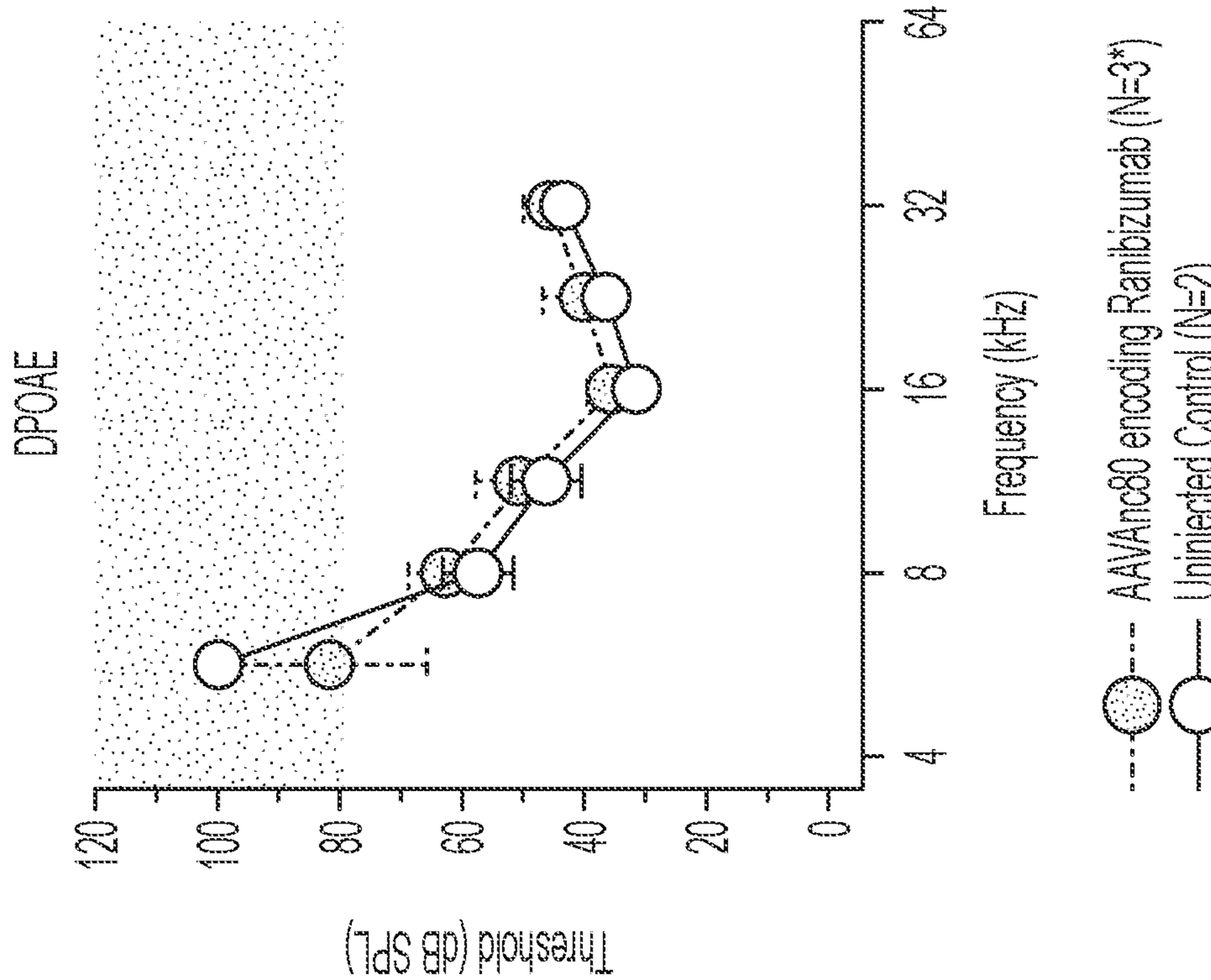


FIG. 26A

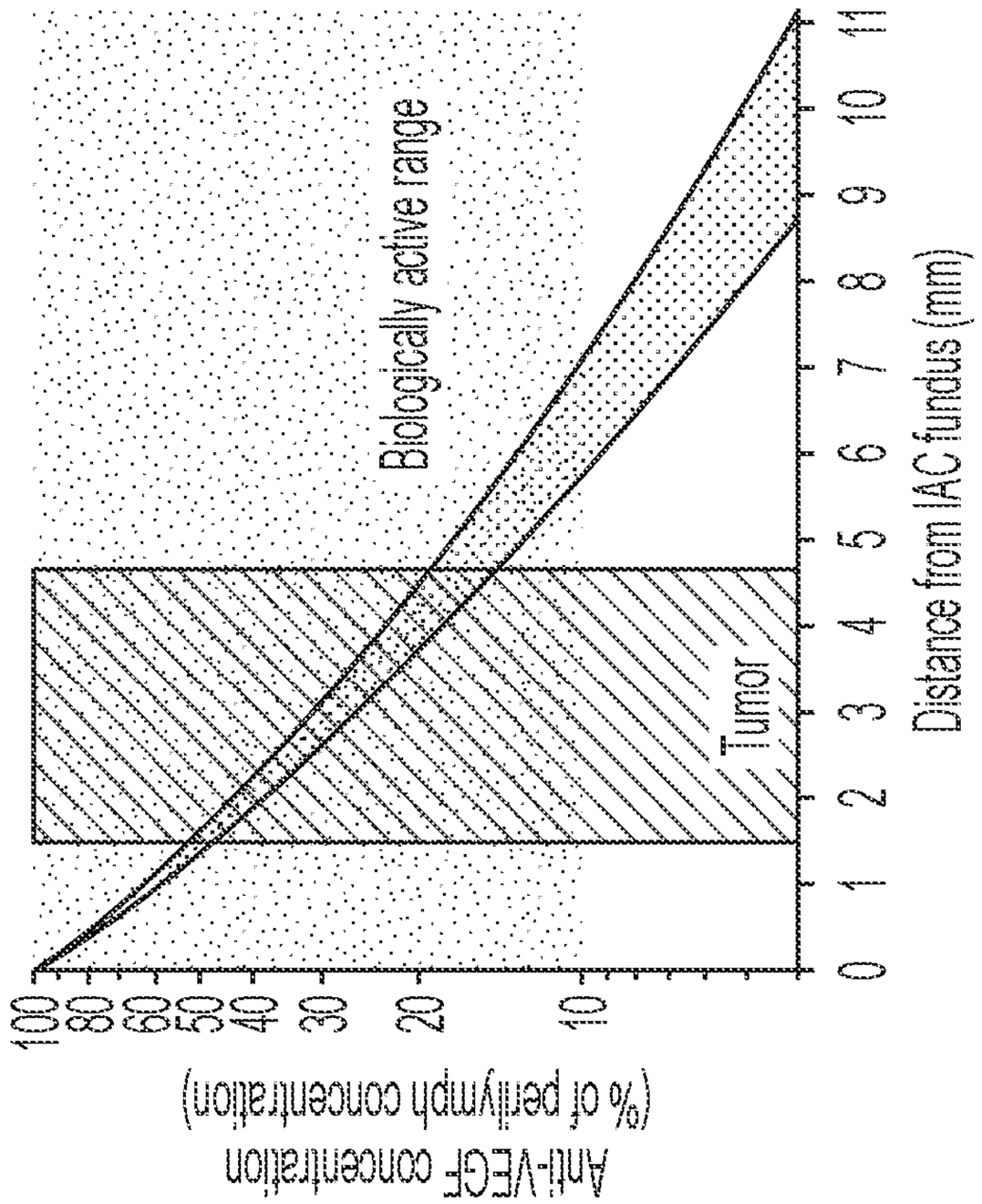


FIG. 27B

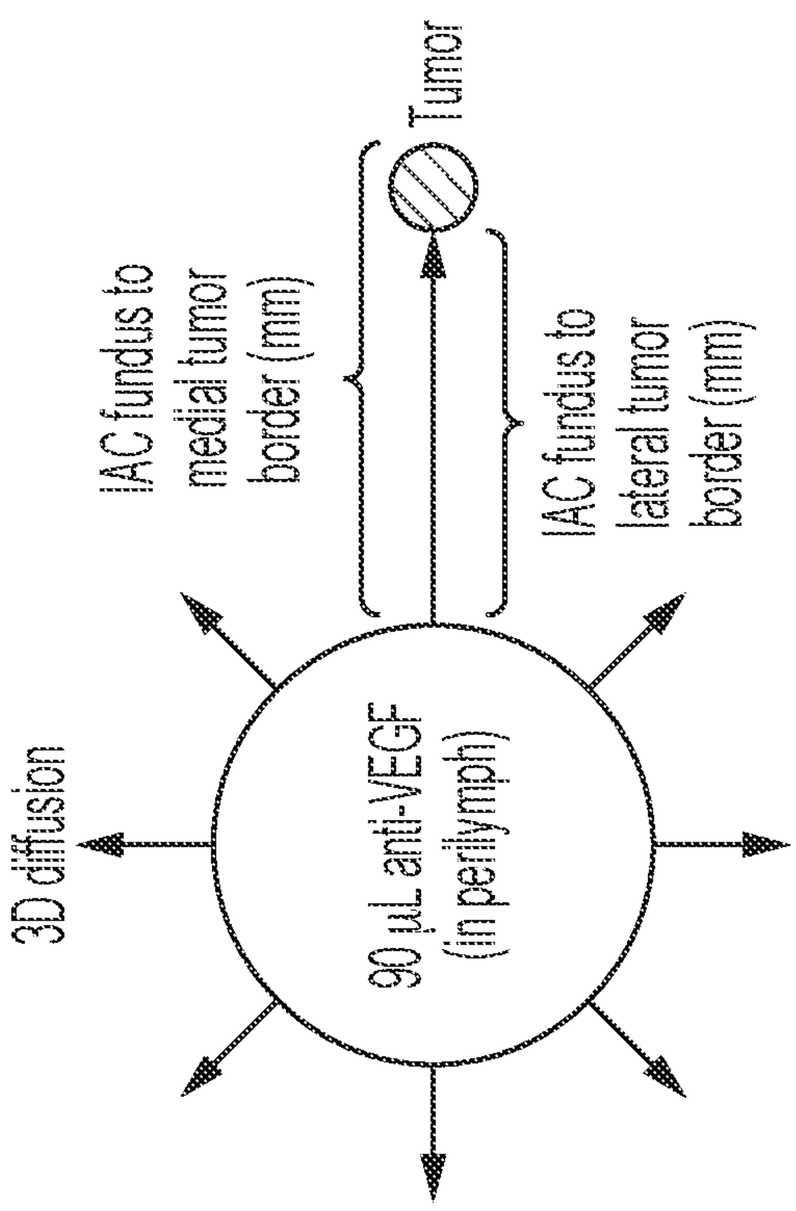


FIG. 27A

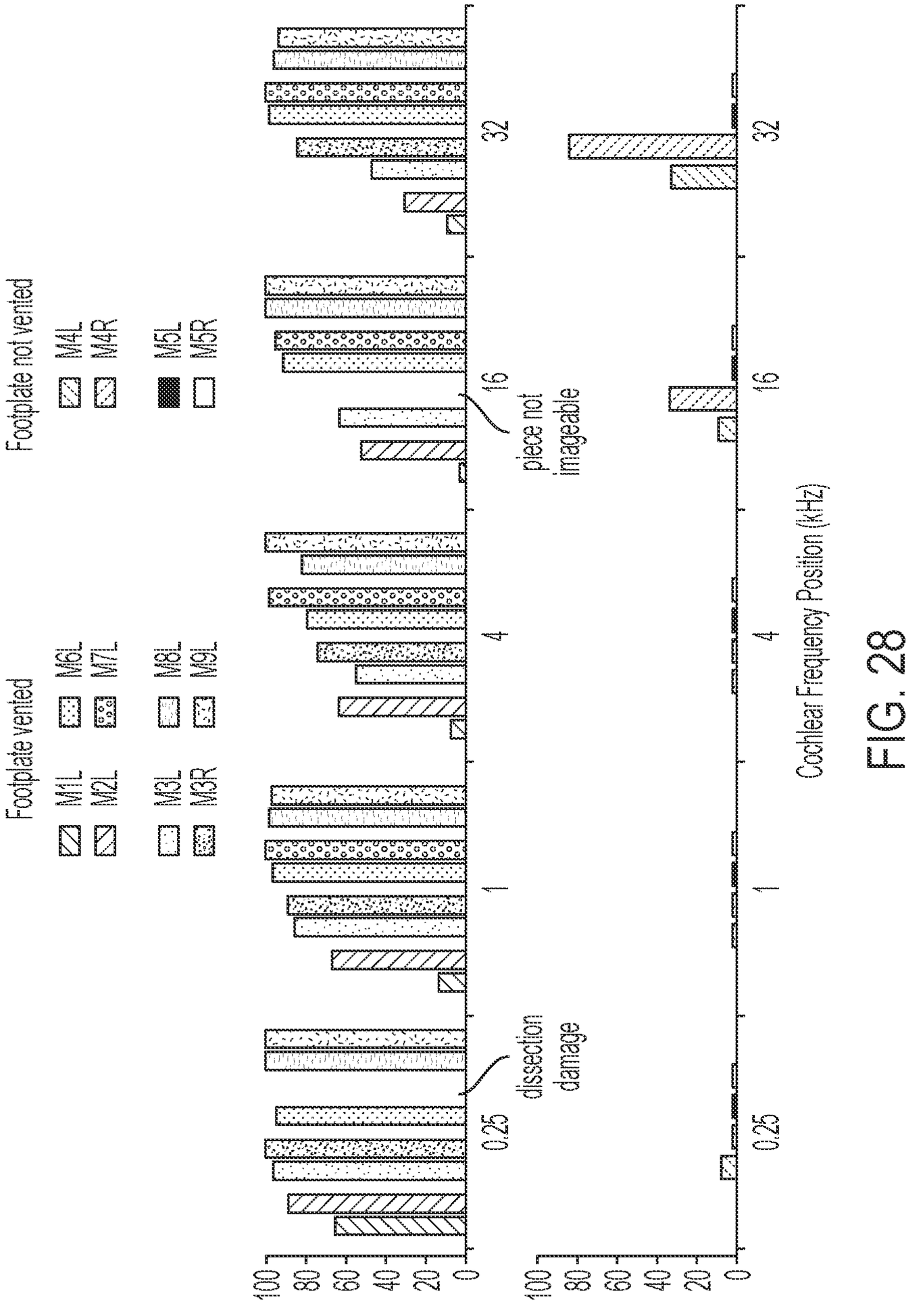

Footplate not vented
M4L
M4R
M5L
M5R
Footplate vented
M1L
M2L
M3L
M3R
M6L
M7L
M8L
M9L
32
16
piece not imageable
4
1
dissection damage
0.25
100
80
60
40
20
0
% IHCs Expressing eGFP
Cochlear Frequency Position (kHz)

FIG. 28

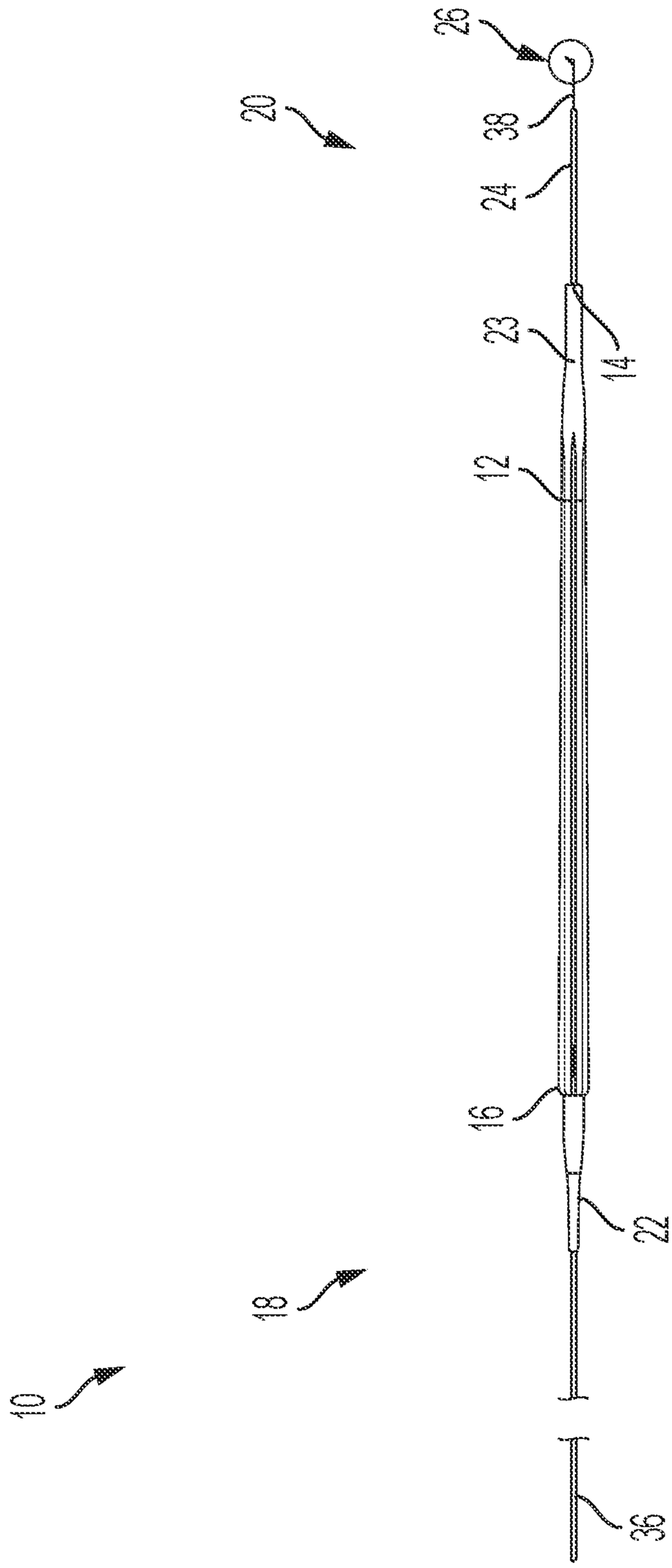


FIG. 29

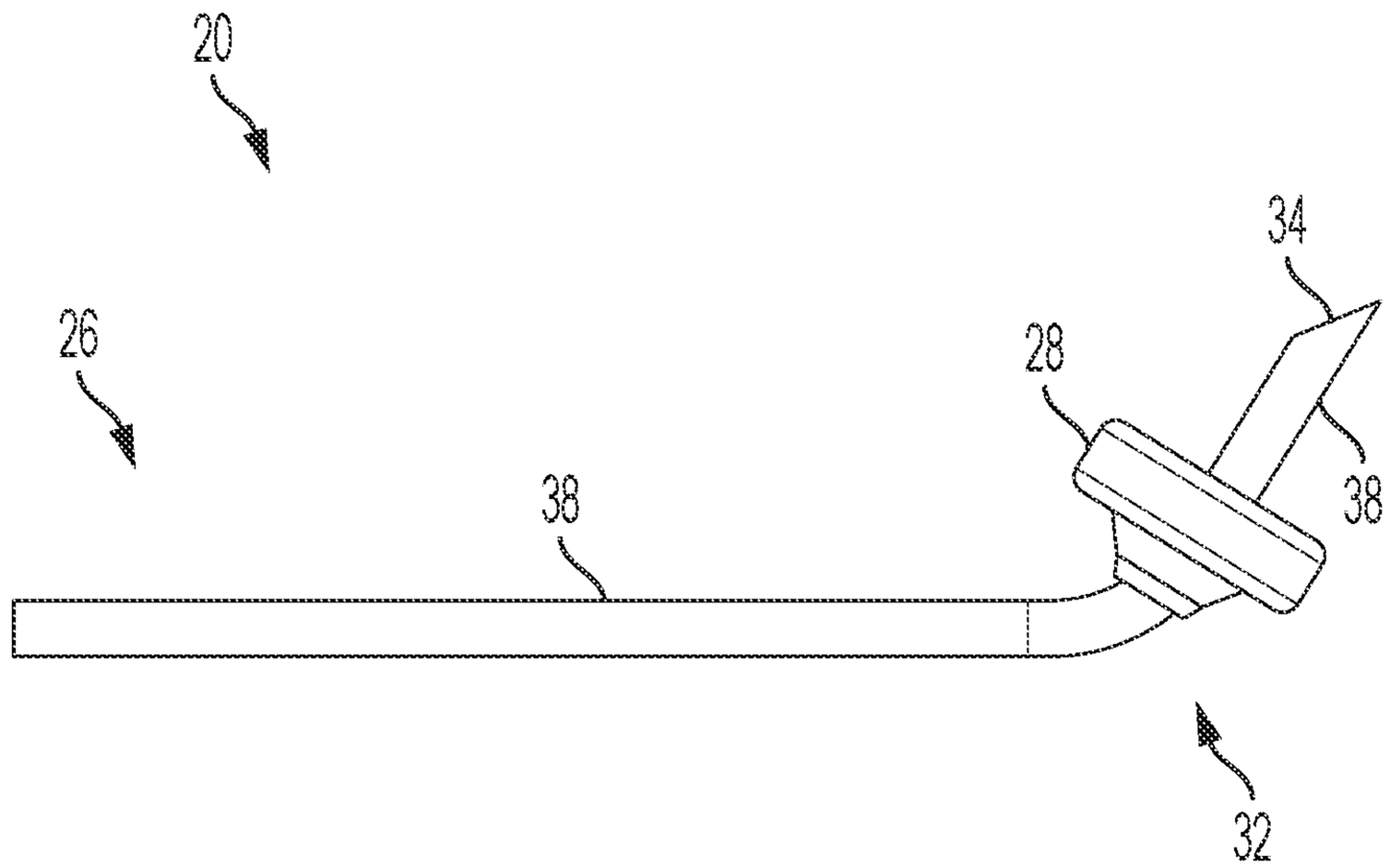


FIG. 30

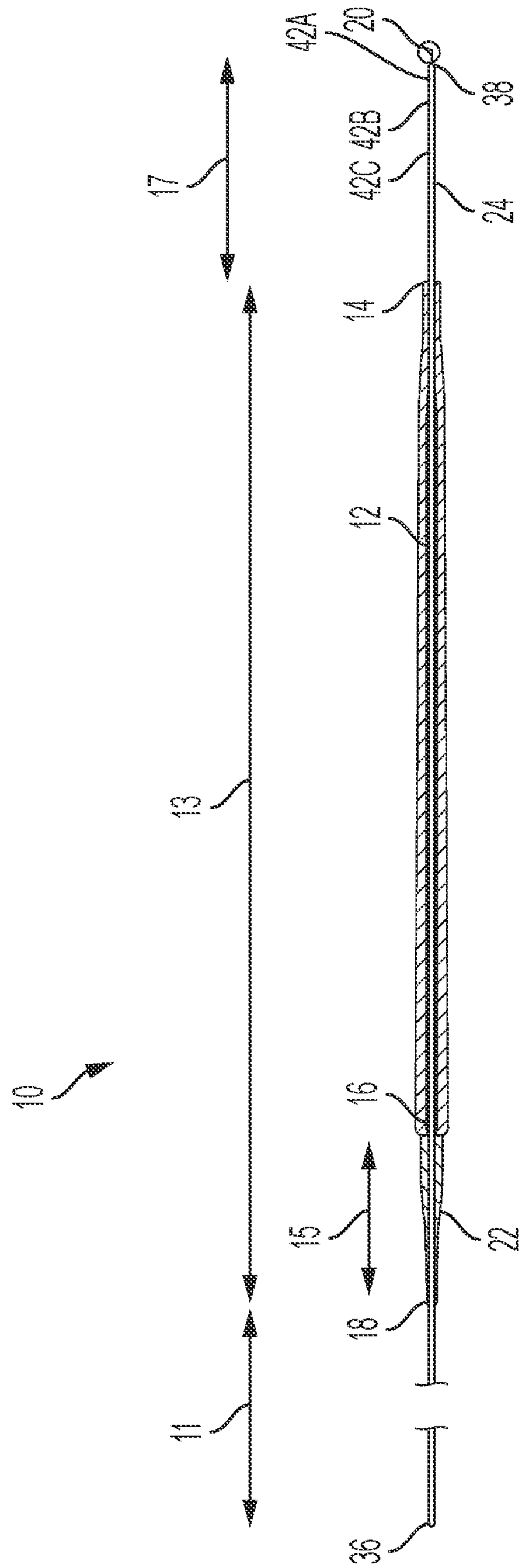


FIG. 31

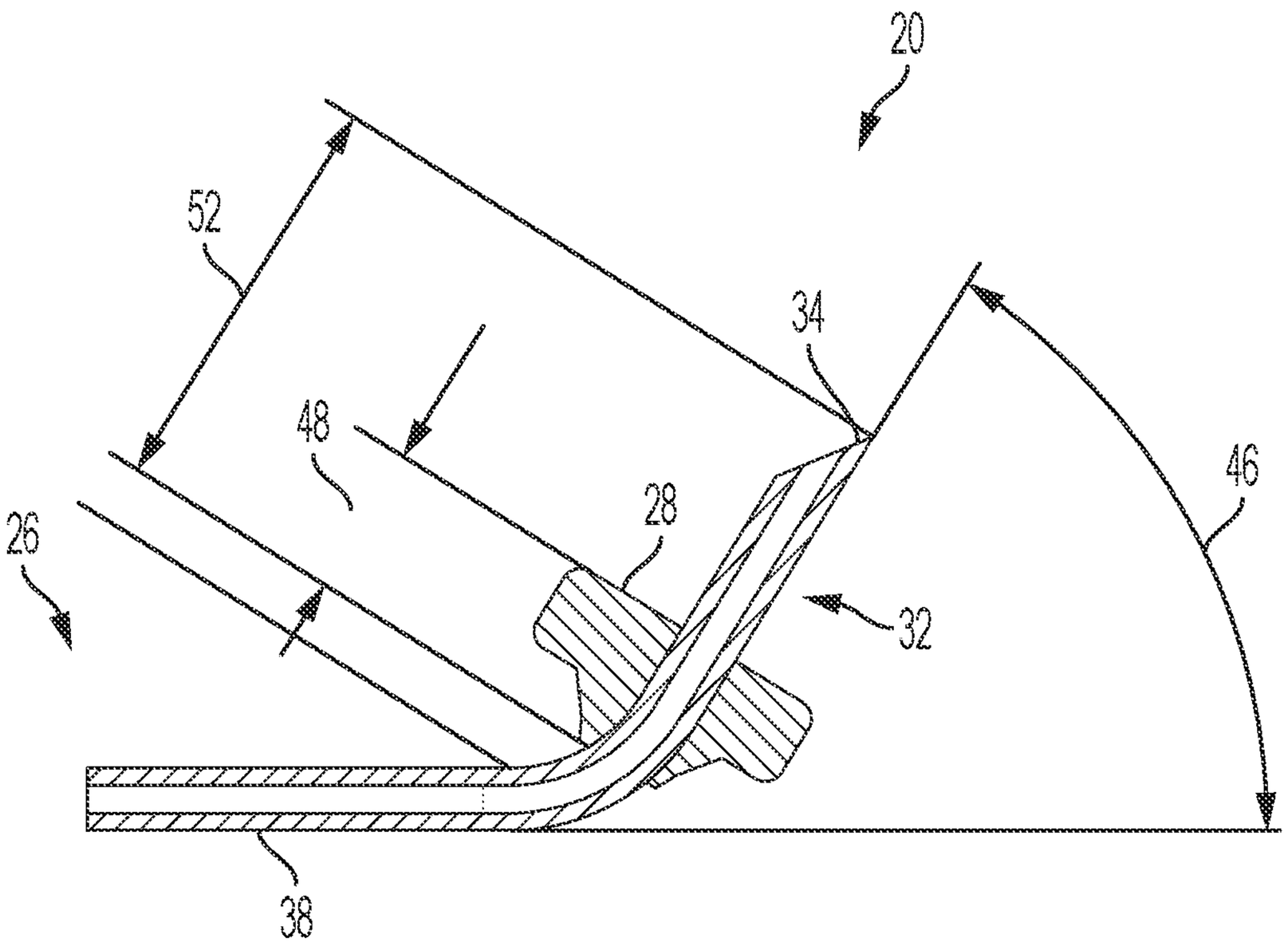


FIG. 32

SEQUENCE LISTING

<110> AKOUOS, INC.

<120> ANTI-VEGF ANTIBODY CONSTRUCTS AND RELATED METHODS FOR TREATING VESTIBULAR SCHWANNOMA ASSOCIATED SYMPTOMS

<130> 2013615-0503 (AK020.WO)

<140>
<141>

<150> US 63/152,832
<151> 2021-02-23

<150> US 63/120,189
<151> 2020-12-01

<160> 117

<170> PatentIn version 3.5

<210> 1
<211> 412
<212> PRT
<213> Homo sapiens

<400> 1

```
Met Thr Asp Arg Gln Thr Asp Thr Ala Pro Ser Pro Ser Tyr His Leu
1               5                  10                  15


Leu Pro Gly Arg Arg Arg Thr Val Asp Ala Ala Ala Ser Arg Gly Gln
            20                  25                  30


Gly Pro Glu Pro Ala Pro Gly Gly Gly Val Glu Gly Val Gly Ala Arg
        35                  40                  45


Gly Val Ala Leu Lys Leu Phe Val Gln Leu Leu Gly Cys Ser Arg Phe
    50                  55                  60


Gly Gly Ala Val Val Arg Ala Gly Glu Ala Glu Pro Ser Gly Ala Ala
65                  70                  75                  80


Arg Ser Ala Ser Ser Gly Arg Glu Glu Pro Gln Pro Glu Glu Gly Glu
                85                  90                  95
```

```
Glu Glu Glu Glu Lys Glu Glu Glu Arg Gly Pro Gln Trp Arg Leu Gly
            100                 105                 110

Ala Arg Lys Pro Gly Ser Trp Thr Gly Glu Ala Ala Val Cys Ala Asp
        115                 120                 125

Ser Ala Pro Ala Ala Arg Ala Pro Gln Ala Leu Ala Arg Ala Ser Gly
    130                 135                 140

Arg Gly Gly Arg Val Ala Arg Arg Gly Ala Glu Glu Ser Gly Pro Pro
145                 150                 155                 160

His Ser Pro Ser Arg Arg Gly Ser Ala Ser Arg Ala Gly Pro Gly Arg
                165                 170                 175

Ala Ser Glu Thr Met Asn Phe Leu Leu Ser Trp Val His Trp Ser Leu
            180                 185                 190

Ala Leu Leu Leu Tyr Leu His His Ala Lys Trp Ser Gln Ala Ala Pro
        195                 200                 205

Met Ala Glu Gly Gly Gly Gln Asn His His Glu Val Val Lys Phe Met
    210                 215                 220

Asp Val Tyr Gln Arg Ser Tyr Cys His Pro Ile Glu Thr Leu Val Asp
225                 230                 235                 240

Ile Phe Gln Glu Tyr Pro Asp Glu Ile Glu Tyr Ile Phe Lys Pro Ser
                245                 250                 255

Cys Val Pro Leu Met Arg Cys Gly Gly Cys Cys Asn Asp Glu Gly Leu
            260                 265                 270

Glu Cys Val Pro Thr Glu Glu Ser Asn Ile Thr Met Gln Ile Met Arg
        275                 280                 285
```

```
Ile Lys Pro His Gln Gly Gln His Ile Gly Glu Met Ser Phe Leu Gln
    290                 295                 300

His Asn Lys Cys Glu Cys Arg Pro Lys Lys Asp Arg Ala Arg Gln Glu
305                 310                 315                 320

Lys Lys Ser Val Arg Gly Lys Gly Lys Gly Gln Lys Arg Lys Arg Lys
                325                 330                 335

Lys Ser Arg Tyr Lys Ser Trp Ser Val Tyr Val Gly Ala Arg Cys Cys
            340                 345                 350

Leu Met Pro Trp Ser Leu Pro Gly Pro His Pro Cys Gly Pro Cys Ser
        355                 360                 365

Glu Arg Arg Lys His Leu Phe Val Gln Asp Pro Gln Thr Cys Lys Cys
    370                 375                 380

Ser Cys Lys Asn Thr Asp Ser Arg Cys Lys Ala Arg Gln Leu Glu Leu
385                 390                 395                 400

Asn Glu Arg Thr Cys Arg Cys Asp Lys Pro Arg Arg
                405                 410
```

<210> 2
<211> 395
<212> PRT
<213> Homo sapiens

<400> 2

```
Met Thr Asp Arg Gln Thr Asp Thr Ala Pro Ser Pro Ser Tyr His Leu
1               5                   10                  15

Leu Pro Gly Arg Arg Arg Thr Val Asp Ala Ala Ala Ser Arg Gly Gln
            20                  25                  30

Gly Pro Glu Pro Ala Pro Gly Gly Gly Val Glu Gly Val Gly Ala Arg
        35                  40                  45
```

```
Gly Val Ala Leu Lys Leu Phe Val Gln Leu Leu Gly Cys Ser Arg Phe
    50                  55                  60

Gly Gly Ala Val Val Arg Ala Gly Glu Ala Glu Pro Ser Gly Ala Ala
65                  70                  75                  80

Arg Ser Ala Ser Ser Gly Arg Glu Glu Pro Gln Pro Glu Glu Gly Glu
                85                  90                  95

Glu Glu Glu Glu Lys Glu Glu Glu Arg Gly Pro Gln Trp Arg Leu Gly
            100                 105                 110

Ala Arg Lys Pro Gly Ser Trp Thr Gly Glu Ala Ala Val Cys Ala Asp
        115                 120                 125

Ser Ala Pro Ala Ala Arg Ala Pro Gln Ala Leu Ala Arg Ala Ser Gly
    130                 135                 140

Arg Gly Gly Arg Val Ala Arg Arg Gly Ala Glu Glu Ser Gly Pro Pro
145                 150                 155                 160

His Ser Pro Ser Arg Arg Gly Ser Ala Ser Arg Ala Gly Pro Gly Arg
                165                 170                 175

Ala Ser Glu Thr Met Asn Phe Leu Leu Ser Trp Val His Trp Ser Leu
            180                 185                 190

Ala Leu Leu Leu Tyr Leu His His Ala Lys Trp Ser Gln Ala Ala Pro
        195                 200                 205

Met Ala Glu Gly Gly Gly Gln Asn His His Glu Val Val Lys Phe Met
    210                 215                 220

Asp Val Tyr Gln Arg Ser Tyr Cys His Pro Ile Glu Thr Leu Val Asp
225                 230                 235                 240

Ile Phe Gln Glu Tyr Pro Asp Glu Ile Glu Tyr Ile Phe Lys Pro Ser
                245                 250                 255
```

```
Cys Val Pro Leu Met Arg Cys Gly Gly Cys Cys Asn Asp Glu Gly Leu
            260                 265                 270

Glu Cys Val Pro Thr Glu Glu Ser Asn Ile Thr Met Gln Ile Met Arg
        275                 280                 285

Ile Lys Pro His Gln Gly Gln His Ile Gly Glu Met Ser Phe Leu Gln
    290                 295                 300

His Asn Lys Cys Glu Cys Arg Pro Lys Lys Asp Arg Ala Arg Gln Glu
305                 310                 315                 320

Lys Lys Ser Val Arg Gly Lys Gly Lys Gly Gln Lys Arg Lys Arg Lys
                325                 330                 335

Lys Ser Arg Tyr Lys Ser Trp Ser Val Pro Cys Gly Pro Cys Ser Glu
            340                 345                 350

Arg Arg Lys His Leu Phe Val Gln Asp Pro Gln Thr Cys Lys Cys Ser
        355                 360                 365

Cys Lys Asn Thr Asp Ser Arg Cys Lys Ala Arg Gln Leu Glu Leu Asn
    370                 375                 380

Glu Arg Thr Cys Arg Cys Asp Lys Pro Arg Arg
385                 390                 395


<210> 3
<211> 137
<212> PRT
<213> Homo sapiens

<400> 3
Met Asn Phe Leu Leu Ser Trp Val His Trp Ser Leu Ala Leu Leu Leu
1               5                   10                  15

Tyr Leu His His Ala Lys Trp Ser Gln Ala Ala Pro Met Ala Glu Gly
            20                  25                  30
```

```
Gly Gly Gln Asn His His Glu Val Val Lys Phe Met Asp Val Tyr Gln
        35                  40                  45

Arg Ser Tyr Cys His Pro Ile Glu Thr Leu Val Asp Ile Phe Gln Glu
    50                  55                  60

Tyr Pro Asp Glu Ile Glu Tyr Ile Phe Lys Pro Ser Cys Val Pro Leu
65                  70                  75                  80

Met Arg Cys Gly Gly Cys Cys Asn Asp Glu Gly Leu Glu Cys Val Pro
                85                  90                  95

Thr Glu Glu Ser Asn Ile Thr Met Gln Ile Met Arg Ile Lys Pro His
            100                 105                 110

Gln Gly Gln His Ile Gly Glu Met Ser Phe Leu Gln His Asn Lys Cys
        115                 120                 125

Glu Cys Arg Cys Asp Lys Pro Arg Arg
    130                 135
```

<210> 4
<211> 147
<212> PRT
<213> Homo sapiens

<400> 4

```
Met Asn Phe Leu Leu Ser Trp Val His Trp Ser Leu Ala Leu Leu Leu
1               5                   10                  15

Tyr Leu His His Ala Lys Trp Ser Gln Ala Ala Pro Met Ala Glu Gly
            20                  25                  30

Gly Gly Gln Asn His His Glu Val Val Lys Phe Met Asp Val Tyr Gln
        35                  40                  45

Arg Ser Tyr Cys His Pro Ile Glu Thr Leu Val Asp Ile Phe Gln Glu
    50                  55                  60
```

Tyr Pro Asp Glu Ile Glu Tyr Ile Phe Lys Pro Ser Cys Val Pro Leu
65 70 75 80

Met Arg Cys Gly Gly Cys Cys Asn Asp Glu Gly Leu Glu Cys Val Pro
85 90 95

Thr Glu Glu Ser Asn Ile Thr Met Gln Ile Met Arg Ile Lys Pro His
100 105 110

Gln Gly Gln His Ile Gly Glu Met Ser Phe Leu Gln His Asn Lys Cys
115 120 125

Glu Cys Arg Pro Lys Lys Asp Arg Ala Arg Gln Glu Lys Cys Asp Lys
130 135 140

Pro Arg Arg
145

<210> 5
<211> 171
<212> PRT
<213> Homo sapiens

<400> 5
Met Asn Phe Leu Leu Ser Trp Val His Trp Ser Leu Ala Leu Leu Leu
1 5 10 15

Tyr Leu His His Ala Lys Trp Ser Gln Ala Ala Pro Met Ala Glu Gly
20 25 30

Gly Gly Gln Asn His His Glu Val Val Lys Phe Met Asp Val Tyr Gln
35 40 45

Arg Ser Tyr Cys His Pro Ile Glu Thr Leu Val Asp Ile Phe Gln Glu
50 55 60

Tyr Pro Asp Glu Ile Glu Tyr Ile Phe Lys Pro Ser Cys Val Pro Leu
65 70 75 80

```
Met Arg Cys Gly Gly Cys Cys Asn Asp Glu Gly Leu Glu Cys Val Pro
                85                  90                  95

Thr Glu Glu Ser Asn Ile Thr Met Gln Ile Met Arg Ile Lys Pro His
            100                 105                 110

Gln Gly Gln His Ile Gly Glu Met Ser Phe Leu Gln His Asn Lys Cys
        115                 120                 125

Glu Cys Arg Pro Lys Lys Asp Arg Ala Arg Gln Glu Lys Lys Ser Val
    130                 135                 140

Arg Gly Lys Gly Lys Gly Gln Lys Arg Lys Arg Lys Lys Ser Arg Tyr
145                 150                 155                 160

Lys Ser Trp Ser Val Cys Asp Lys Pro Arg Arg
                165                 170
```

<210> 6
<211> 191
<212> PRT
<213> Homo sapiens

<400> 6

```
Met Asn Phe Leu Leu Ser Trp Val His Trp Ser Leu Ala Leu Leu Leu
1               5                   10                  15

Tyr Leu His His Ala Lys Trp Ser Gln Ala Ala Pro Met Ala Glu Gly
            20                  25                  30

Gly Gly Gln Asn His His Glu Val Val Lys Phe Met Asp Val Tyr Gln
        35                  40                  45

Arg Ser Tyr Cys His Pro Ile Glu Thr Leu Val Asp Ile Phe Gln Glu
    50                  55                  60

Tyr Pro Asp Glu Ile Glu Tyr Ile Phe Lys Pro Ser Cys Val Pro Leu
65                  70                  75                  80
```

```
Met Arg Cys Gly Gly Cys Cys Asn Asp Glu Gly Leu Glu Cys Val Pro
                85                  90                  95

Thr Glu Glu Ser Asn Ile Thr Met Gln Ile Met Arg Ile Lys Pro His
            100                 105                 110

Gln Gly Gln His Ile Gly Glu Met Ser Phe Leu Gln His Asn Lys Cys
        115                 120                 125

Glu Cys Arg Pro Lys Lys Asp Arg Ala Arg Gln Glu Asn Pro Cys Gly
    130                 135                 140

Pro Cys Ser Glu Arg Arg Lys His Leu Phe Val Gln Asp Pro Gln Thr
145                 150                 155                 160

Cys Lys Cys Ser Cys Lys Asn Thr Asp Ser Arg Cys Lys Ala Arg Gln
                165                 170                 175

Leu Glu Leu Asn Glu Arg Thr Cys Arg Cys Asp Lys Pro Arg Arg
            180                 185                 190
```

<210> 7
<211> 215
<212> PRT
<213> Homo sapiens

<400> 7

```
Met Asn Phe Leu Leu Ser Trp Val His Trp Ser Leu Ala Leu Leu Leu
1               5                   10                  15

Tyr Leu His His Ala Lys Trp Ser Gln Ala Ala Pro Met Ala Glu Gly
            20                  25                  30

Gly Gly Gln Asn His His Glu Val Val Lys Phe Met Asp Val Tyr Gln
        35                  40                  45

Arg Ser Tyr Cys His Pro Ile Glu Thr Leu Val Asp Ile Phe Gln Glu
    50                  55                  60
```

```
Tyr Pro Asp Glu Ile Glu Tyr Ile Phe Lys Pro Ser Cys Val Pro Leu
65                  70                  75                  80

Met Arg Cys Gly Gly Cys Cys Asn Asp Glu Gly Leu Glu Cys Val Pro
                85                  90                  95

Thr Glu Glu Ser Asn Ile Thr Met Gln Ile Met Arg Ile Lys Pro His
            100                 105                 110

Gln Gly Gln His Ile Gly Glu Met Ser Phe Leu Gln His Asn Lys Cys
        115                 120                 125

Glu Cys Arg Pro Lys Lys Asp Arg Ala Arg Gln Glu Lys Lys Ser Val
    130                 135                 140

Arg Gly Lys Gly Lys Gly Gln Lys Arg Lys Arg Lys Lys Ser Arg Tyr
145                 150                 155                 160

Lys Ser Trp Ser Val Pro Cys Gly Pro Cys Ser Glu Arg Arg Lys His
                165                 170                 175

Leu Phe Val Gln Asp Pro Gln Thr Cys Lys Cys Ser Cys Lys Asn Thr
            180                 185                 190

Asp Ser Arg Cys Lys Ala Arg Gln Leu Glu Leu Asn Glu Arg Thr Cys
        195                 200                 205

Arg Cys Asp Lys Pro Arg Arg
    210                 215
```

<210> 8
<211> 232
<212> PRT
<213> Homo sapiens

<400> 8

```
Met Asn Phe Leu Leu Ser Trp Val His Trp Ser Leu Ala Leu Leu Leu
1               5                   10                  15
```

```
Tyr Leu His His Ala Lys Trp Ser Gln Ala Ala Pro Met Ala Glu Gly
            20                  25                  30

Gly Gly Gln Asn His His Glu Val Val Lys Phe Met Asp Val Tyr Gln
        35                  40                  45

Arg Ser Tyr Cys His Pro Ile Glu Thr Leu Val Asp Ile Phe Gln Glu
    50                  55                  60

Tyr Pro Asp Glu Ile Glu Tyr Ile Phe Lys Pro Ser Cys Val Pro Leu
65                  70                  75                  80

Met Arg Cys Gly Gly Cys Cys Asn Asp Glu Gly Leu Glu Cys Val Pro
                85                  90                  95

Thr Glu Glu Ser Asn Ile Thr Met Gln Ile Met Arg Ile Lys Pro His
            100                 105                 110

Gln Gly Gln His Ile Gly Glu Met Ser Phe Leu Gln His Asn Lys Cys
        115                 120                 125

Glu Cys Arg Pro Lys Lys Asp Arg Ala Arg Gln Glu Lys Lys Ser Val
    130                 135                 140

Arg Gly Lys Gly Lys Gly Gln Lys Arg Lys Arg Lys Lys Ser Arg Tyr
145                 150                 155                 160

Lys Ser Trp Ser Val Tyr Val Gly Ala Arg Cys Cys Leu Met Pro Trp
                165                 170                 175

Ser Leu Pro Gly Pro His Pro Cys Gly Pro Cys Ser Glu Arg Arg Lys
            180                 185                 190

His Leu Phe Val Gln Asp Pro Gln Thr Cys Lys Cys Ser Cys Lys Asn
        195                 200                 205
```

```
Thr Asp Ser Arg Cys Lys Ala Arg Gln Leu Glu Leu Asn Glu Arg Thr
    210                 215                 220


Cys Arg Cys Asp Lys Pro Arg Arg
225                 230


<210> 9
<211> 188
<212> PRT
<213> Homo sapiens

<400> 9
Met Ser Pro Leu Leu Arg Arg Leu Leu Leu Ala Ala Leu Leu Gln Leu
1               5                   10                  15


Ala Pro Ala Gln Ala Pro Val Ser Gln Pro Asp Ala Pro Gly His Gln
            20                  25                  30


Arg Lys Val Val Ser Trp Ile Asp Val Tyr Thr Arg Ala Thr Cys Gln
        35                  40                  45


Pro Arg Glu Val Val Val Pro Leu Thr Val Glu Leu Met Gly Thr Val
    50                  55                  60


Ala Lys Gln Leu Val Pro Ser Cys Val Thr Val Gln Arg Cys Gly Gly
65                  70                  75                  80


Cys Cys Pro Asp Asp Gly Leu Glu Cys Val Pro Thr Gly Gln His Gln
                85                  90                  95


Val Arg Met Gln Ile Leu Met Ile Arg Tyr Pro Ser Ser Gln Leu Gly
            100                 105                 110


Glu Met Ser Leu Glu Glu His Ser Gln Cys Glu Cys Arg Pro Lys Lys
        115                 120                 125


Lys Asp Ser Ala Val Lys Pro Asp Ser Pro Arg Pro Leu Cys Pro Arg
    130                 135                 140
```

```
Cys Thr Gln His His Gln Arg Pro Asp Pro Arg Thr Cys Arg Cys Arg
145                 150                 155                 160


Cys Arg Arg Arg Ser Phe Leu Arg Cys Gln Gly Arg Gly Leu Glu Leu
                165                 170                 175


Asn Pro Asp Thr Cys Arg Cys Arg Lys Leu Arg Arg
            180                 185


<210> 10
<211> 207
<212> PRT
<213> Homo sapiens

<400> 10
Met Ser Pro Leu Leu Arg Arg Leu Leu Leu Ala Ala Leu Leu Gln Leu
1               5                   10                  15


Ala Pro Ala Gln Ala Pro Val Ser Gln Pro Asp Ala Pro Gly His Gln
            20                  25                  30


Arg Lys Val Val Ser Trp Ile Asp Val Tyr Thr Arg Ala Thr Cys Gln
        35                  40                  45


Pro Arg Glu Val Val Val Pro Leu Thr Val Glu Leu Met Gly Thr Val
    50                  55                  60


Ala Lys Gln Leu Val Pro Ser Cys Val Thr Val Gln Arg Cys Gly Gly
65                  70                  75                  80


Cys Cys Pro Asp Asp Gly Leu Glu Cys Val Pro Thr Gly Gln His Gln
                85                  90                  95


Val Arg Met Gln Ile Leu Met Ile Arg Tyr Pro Ser Ser Gln Leu Gly
            100                 105                 110


Glu Met Ser Leu Glu Glu His Ser Gln Cys Glu Cys Arg Pro Lys Lys
        115                 120                 125
```

```
Lys Asp Ser Ala Val Lys Pro Asp Arg Ala Ala Thr Pro His His Arg
    130                 135                 140

Pro Gln Pro Arg Ser Val Pro Gly Trp Asp Ser Ala Pro Gly Ala Pro
145                 150                 155                 160

Ser Pro Ala Asp Ile Thr His Pro Thr Pro Ala Pro Gly Pro Ser Ala
                165                 170                 175

His Ala Ala Pro Ser Thr Thr Ser Ala Leu Thr Pro Gly Pro Ala Ala
            180                 185                 190

Ala Ala Ala Asp Ala Ala Ala Ser Ser Val Ala Lys Gly Gly Ala
        195                 200                 205
```

<210> 11
<211> 419
<212> PRT
<213> Homo sapiens

<400> 11

```
Met His Leu Leu Gly Phe Phe Ser Val Ala Cys Ser Leu Leu Ala Ala
1               5                   10                  15

Ala Leu Leu Pro Gly Pro Arg Glu Ala Pro Ala Ala Ala Ala Ala Phe
            20                  25                  30

Glu Ser Gly Leu Asp Leu Ser Asp Ala Glu Pro Asp Ala Gly Glu Ala
        35                  40                  45

Thr Ala Tyr Ala Ser Lys Asp Leu Glu Glu Gln Leu Arg Ser Val Ser
    50                  55                  60

Ser Val Asp Glu Leu Met Thr Val Leu Tyr Pro Glu Tyr Trp Lys Met
65                  70                  75                  80

Tyr Lys Cys Gln Leu Arg Lys Gly Gly Trp Gln His Asn Arg Glu Gln
                85                  90                  95
```

```
Ala Asn Leu Asn Ser Arg Thr Glu Glu Thr Ile Lys Phe Ala Ala Ala
            100                 105                 110

His Tyr Asn Thr Glu Ile Leu Lys Ser Ile Asp Asn Glu Trp Arg Lys
        115                 120                 125

Thr Gln Cys Met Pro Arg Glu Val Cys Ile Asp Val Gly Lys Glu Phe
    130                 135                 140

Gly Val Ala Thr Asn Thr Phe Phe Lys Pro Pro Cys Val Ser Val Tyr
145                 150                 155                 160

Arg Cys Gly Gly Cys Cys Asn Ser Glu Gly Leu Gln Cys Met Asn Thr
                165                 170                 175

Ser Thr Ser Tyr Leu Ser Lys Thr Leu Phe Glu Ile Thr Val Pro Leu
            180                 185                 190

Ser Gln Gly Pro Lys Pro Val Thr Ile Ser Phe Ala Asn His Thr Ser
        195                 200                 205

Cys Arg Cys Met Ser Lys Leu Asp Val Tyr Arg Gln Val His Ser Ile
    210                 215                 220

Ile Arg Arg Ser Leu Pro Ala Thr Leu Pro Gln Cys Gln Ala Ala Asn
225                 230                 235                 240

Lys Thr Cys Pro Thr Asn Tyr Met Trp Asn Asn His Ile Cys Arg Cys
                245                 250                 255

Leu Ala Gln Glu Asp Phe Met Phe Ser Ser Asp Ala Gly Asp Asp Ser
            260                 265                 270

Thr Asp Gly Phe His Asp Ile Cys Gly Pro Asn Lys Glu Leu Asp Glu
        275                 280                 285

Glu Thr Cys Gln Cys Val Cys Arg Ala Gly Leu Arg Pro Ala Ser Cys
    290                 295                 300
```

```
Gly Pro His Lys Glu Leu Asp Arg Asn Ser Cys Gln Cys Val Cys Lys
305                 310                 315                 320


Asn Lys Leu Phe Pro Ser Gln Cys Gly Ala Asn Arg Glu Phe Asp Glu
                325                 330                 335


Asn Thr Cys Gln Cys Val Cys Lys Arg Thr Cys Pro Arg Asn Gln Pro
            340                 345                 350


Leu Asn Pro Gly Lys Cys Ala Cys Glu Cys Thr Glu Ser Pro Gln Lys
        355                 360                 365


Cys Leu Leu Lys Gly Lys Lys Phe His His Gln Thr Cys Ser Cys Tyr
    370                 375                 380


Arg Arg Pro Cys Thr Asn Arg Gln Lys Ala Cys Glu Pro Gly Phe Ser
385                 390                 395                 400


Tyr Ser Glu Glu Val Cys Arg Cys Val Pro Ser Tyr Trp Lys Arg Pro
                405                 410                 415


Gln Met Ser
```

<210> 12
<211> 354
<212> PRT
<213> Homo sapiens

<400> 12

```
Met Tyr Arg Glu Trp Val Val Val Asn Val Phe Met Met Leu Tyr Val
1               5                   10                  15


Gln Leu Val Gln Gly Ser Ser Asn Glu His Gly Pro Val Lys Arg Ser
            20                  25                  30


Ser Gln Ser Thr Leu Glu Arg Ser Glu Gln Gln Ile Arg Ala Ala Ser
        35                  40                  45
```

```
Ser Leu Glu Glu Leu Leu Arg Ile Thr His Ser Glu Asp Trp Lys Leu
    50                  55                  60

Trp Arg Cys Arg Leu Arg Leu Lys Ser Phe Thr Ser Met Asp Ser Arg
65                  70                  75                  80

Ser Ala Ser His Arg Ser Thr Arg Phe Ala Ala Thr Phe Tyr Asp Ile
                85                  90                  95

Glu Thr Leu Lys Val Ile Asp Glu Glu Trp Gln Arg Thr Gln Cys Ser
            100                 105                 110

Pro Arg Glu Thr Cys Val Glu Val Ala Ser Glu Leu Gly Lys Ser Thr
        115                 120                 125

Asn Thr Phe Phe Lys Pro Pro Cys Val Asn Val Phe Arg Cys Gly Gly
    130                 135                 140

Cys Cys Asn Glu Glu Ser Leu Ile Cys Met Asn Thr Ser Thr Ser Tyr
145                 150                 155                 160

Ile Ser Lys Gln Leu Phe Glu Ile Ser Val Pro Leu Thr Ser Val Pro
                165                 170                 175

Glu Leu Val Pro Val Lys Val Ala Asn His Thr Gly Cys Lys Cys Leu
            180                 185                 190

Pro Thr Ala Pro Arg His Pro Tyr Ser Ile Ile Arg Arg Ser Ile Gln
        195                 200                 205

Ile Pro Glu Glu Asp Arg Cys Ser His Ser Lys Lys Leu Cys Pro Ile
    210                 215                 220

Asp Met Leu Trp Asp Ser Asn Lys Cys Lys Cys Val Leu Gln Glu Glu
225                 230                 235                 240
```

```
Asn Pro Leu Ala Gly Thr Glu Asp His Ser His Leu Gln Glu Pro Ala
                245                 250                 255

Leu Cys Gly Pro His Met Met Phe Asp Glu Asp Arg Cys Glu Cys Val
            260                 265                 270

Cys Lys Thr Pro Cys Pro Lys Asp Leu Ile Gln His Pro Lys Asn Cys
        275                 280                 285

Ser Cys Phe Glu Cys Lys Glu Ser Leu Glu Thr Cys Cys Gln Lys His
    290                 295                 300

Lys Leu Phe His Pro Asp Thr Cys Ser Cys Glu Asp Arg Cys Pro Phe
305                 310                 315                 320

His Thr Arg Pro Cys Ala Ser Gly Lys Thr Ala Cys Ala Lys His Cys
                325                 330                 335

Arg Phe Pro Lys Glu Lys Arg Ala Ala Gln Gly Pro His Ser Arg Lys
            340                 345                 350

Asn Pro
```

<210> 13
<211> 699
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
polynucleotide"

<400> 13
gaggtgcagc tggtggaatc tggcggcgga cttgttcaac ctggcggctc tctgagactg 60

agctgtgccg cttctggcta cgacttcacc cactacggca tgaactgggt ccgacaggcc 120

cctggcaaag gccttgaatg ggtcggatgg atcaacacct acaccggcga gccaacatac 180

gccgccgact tcaagcggag attcaccttc agcctggaca ccagcaagag caccgcctac 240

ctgcagatga acagcctgag agccgaggac accgccgtgt actactgcgc caagtatccc 300

tactactacg gcaccagcca ctggtacttt gacgtgtggg gacagggcac actggtcaca 360

gtgtctagcg cctctacaaa gggccccagc gttttcccac tggctcctag cagcaagtct 420

accagcggag gaacagccgc tctgggctgt ctggtcaagg actactttcc cgagcctgtg 480

accgtgtcct ggaattctgg cgctctgaca agcggcgtgc acacctttcc agctgtgctg 540

caaagcagcg gcctgtactc tctgagcagc gtcgtgacag tgccaagcag ctctctgggc 600

acccagacct acatctgcaa tgtgaaccac aagcctagca acaccaaggt ggacaagaag 660

gtggaaccca agagctgcga caagacccac accggcaag 699

<210> 14
<211> 693
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
polynucleotide"

<400> 14
gaggtgcagc tggtggaatc tggcggcgga cttgttcaac ctggcggctc tctgagactg 60

agctgtgccg cttctggcta cgacttcacc cactacggca tgaactgggt ccgacaggcc 120

cctggcaaag gccttgaatg ggtcggatgg atcaacacct acaccggcga gccaacatac 180

gccgccgact tcaagcggag attcaccttc agcctggaca ccagcaagag caccgcctac 240

ctgcagatga acagcctgag agccgaggac accgccgtgt actactgcgc caagtatccc 300

tactactacg gcaccagcca ctggtacttt gacgtgtggg gacagggcac actggtcaca 360

gtgtctagcg cctctacaaa gggccccagc gttttcccac tggctcctag cagcaagtct 420

accagcggag gaacagccgc tctgggctgt ctggtcaagg actactttcc cgagcctgtg 480

accgtgtcct ggaattctgg cgctctgaca agcggcgtgc acacctttcc agctgtgctg 540

caaagcagcg gcctgtactc tctgagcagc gtcgtgacag tgccaagcag ctctctgggc 600

acccagacct acatctgcaa tgtgaaccac aagcctagca acaccaaggt ggacaagaag 660

gtggaaccca agagctgcga caagacccac aag 693

<210> 15
<211> 675
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
polynucleotide"

<400> 15
gaggtgcagc tggtggaatc tggcggcgga cttgttcaac ctggcggctc tctgagactg 60

agctgtgccg cttctggcta cgacttcacc cactacggca tgaactgggt ccgacaggcc 120

cctggcaaag gccttgaatg ggtcggatgg atcaacacct acaccggcga gccaacatac 180

gccgccgact tcaagcggag attcaccttc agcctggaca ccagcaagag caccgcctac 240

ctgcagatga acagcctgag agccgaggac accgccgtgt actactgcgc caagtatccc 300

tactactacg gcaccagcca ctggtacttt gacgtgtggg gacagggcac actggtcaca 360

gtgtctagcg cctctacaaa gggccccagc gttttcccac tggctcctag cagcaagtct 420

accagcggag gaacagccgc tctgggctgt ctggtcaagg actactttcc cgagcctgtg 480

accgtgtcct ggaattctgg cgctctgaca agcggcgtgc acacctttcc agctgtgctg 540

caaagcagcg gcctgtactc tctgagcagc gtcgtgacag tgccaagcag ctctctgggc 600

acccagacct acatctgcaa tgtgaaccac aagcctagca acaccaaggt ggacaagaag 660

gtggaaccca agagc 675

<210> 16
<211> 233
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
polypeptide"

<400> 16
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1   5   10   15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Tyr Asp Phe Thr His Tyr
            20   25   30

Gly Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35   40   45

Gly Trp Ile Asn Thr Tyr Thr Gly Glu Pro Thr Tyr Ala Ala Asp Phe
    50   55   60

Lys Arg Arg Phe Thr Phe Ser Leu Asp Thr Ser Lys Ser Thr Ala Tyr
65   70   75   80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85   90   95

Ala Lys Tyr Pro Tyr Tyr Tyr Gly Thr Ser His Trp Tyr Phe Asp Val
            100   105   110

Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly
        115   120   125

Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly
    130   135   140

Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val
145   150   155   160

Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe
                165   170   175

Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val
            180   185   190

Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val

195 200 205

Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys
210 215 220

Ser Cys Asp Lys Thr His Thr Gly Lys
225 230

<210> 17
<211> 231
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic polypeptide"

<400> 17
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Tyr Asp Phe Thr His Tyr
20 25 30

Gly Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Gly Trp Ile Asn Thr Tyr Thr Gly Glu Pro Thr Tyr Ala Ala Asp Phe
50 55 60

Lys Arg Arg Phe Thr Phe Ser Leu Asp Thr Ser Lys Ser Thr Ala Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Lys Tyr Pro Tyr Tyr Tyr Gly Thr Ser His Trp Tyr Phe Asp Val
100 105 110

```
Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly
        115                 120                 125


Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly
    130                 135                 140


Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val
145                 150                 155                 160


Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe
                165                 170                 175


Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val
            180                 185                 190


Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val
        195                 200                 205


Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys
    210                 215                 220


Ser Cys Asp Lys Thr His Leu
225                 230


<210> 18
<211> 225
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      polypeptide"

<400> 18
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1               5                   10                  15


Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Tyr Asp Phe Thr His Tyr
            20                  25                  30
```

```
Gly Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45

Gly Trp Ile Asn Thr Tyr Thr Gly Glu Pro Thr Tyr Ala Ala Asp Phe
    50                  55                  60

Lys Arg Arg Phe Thr Phe Ser Leu Asp Thr Ser Lys Ser Thr Ala Tyr
65                  70                  75                  80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Lys Tyr Pro Tyr Tyr Tyr Gly Thr Ser His Trp Tyr Phe Asp Val
            100                 105                 110

Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly
        115                 120                 125

Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly
    130                 135                 140

Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val
145                 150                 155                 160

Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe
                165                 170                 175

Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val
            180                 185                 190

Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val
        195                 200                 205

Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys
    210                 215                 220

Ser
```

225

<210> 19
<211> 642
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
polynucleotide"

<400> 19
gacatccagc tgacacagag ccccagcagc ctgtctgcct ctgtgggaga cagagtgacc 60

atcacctgta gcgccagcca ggacatctcc aactacctga actggtatca gcaaaagccc 120

ggcaaggccc ctaaggtgct gatctacttc acaagcagcc tgcactccgg cgtgcccagc 180

agattttctg gctctggcag cggcaccgac ttcaccctga ccatatctag cctgcagcct 240

gaggacttcg ccacctacta ctgccagcag tacagcaccg tgccttggac atttggccag 300

ggcacaaagg tggaaatcaa gcggactgtg gccgctccta gcgtgttcat ctttccacct 360

agcgacgagc agctgaagtc tggcacagcc tctgtcgtgt gcctgctgaa caacttctac 420

cccagagaag ccaaggtgca gtggaaagtg gacaatgccc tgcagagcgg caacagccaa 480

gagagcgtga cagagcagga ctccaaggat agcacctata gcctgagcag caccctgaca 540

ctgagcaagg ccgactacga gaagcacaaa gtgtacgcct gcgaagtgac ccaccagggc 600

ctttctagcc ctgtgaccaa gagcttcaac cggggcgaat gt 642

<210> 20
<211> 214
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
polypeptide"

<400> 20
Asp Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

```
Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Gln Asp Ile Ser Asn Tyr
            20                  25                  30

Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Val Leu Ile
        35                  40                  45

Tyr Phe Thr Ser Ser Leu His Ser Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65                  70                  75                  80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Ser Thr Val Pro Trp
                85                  90                  95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala
            100                 105                 110

Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
        115                 120                 125

Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
    130                 135                 140

Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
145                 150                 155                 160

Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
                165                 170                 175

Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
            180                 185                 190

Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
        195                 200                 205
```

Phe Asn Arg Gly Glu Cys
    210

<210> 21
<211> 512
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      polypeptide"

<400> 21
Met Tyr Arg Met Gln Leu Leu Ser Cys Ile Ala Leu Ser Leu Ala Leu
1   5   10   15

Val Thr Asn Ser Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val
            20   25   30

Gln Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Tyr Asp
        35   40   45

Phe Thr His Tyr Gly Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly
    50   55   60

Leu Glu Trp Val Gly Trp Ile Asn Thr Tyr Thr Gly Glu Pro Thr Tyr
65   70   75   80

Ala Ala Asp Phe Lys Arg Arg Phe Thr Phe Ser Leu Asp Thr Ser Lys
                85   90   95

Ser Thr Ala Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala
            100   105   110

Val Tyr Tyr Cys Ala Lys Tyr Pro Tyr Tyr Tyr Gly Thr Ser His Trp
        115   120   125

Tyr Phe Asp Val Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala
    130   135   140

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Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser
145                 150                 155                 160

Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe
                165                 170                 175

Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly
            180                 185                 190

Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu
        195                 200                 205

Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr
    210                 215                 220

Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys
225                 230                 235                 240

Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr Gly Lys Arg Lys Arg
                245                 250                 255

Arg Gly Ser Gly Glu Gly Arg Gly Ser Leu Leu Thr Cys Gly Asp Val
            260                 265                 270

Glu Glu Asn Pro Gly Pro Met Tyr Arg Met Gln Leu Leu Ser Cys Ile
        275                 280                 285

Ala Leu Ser Leu Ala Leu Val Thr Asn Ser Asp Ile Gln Leu Thr Gln
    290                 295                 300

Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr
305                 310                 315                 320

Cys Ser Ala Ser Gln Asp Ile Ser Asn Tyr Leu Asn Trp Tyr Gln Gln
                325                 330                 335

Lys Pro Gly Lys Ala Pro Lys Val Leu Ile Tyr Phe Thr Ser Ser Leu
```

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            340                 345                 350

His Ser Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp
        355                 360                 365

Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr
    370                 375                 380

Tyr Cys Gln Gln Tyr Ser Thr Val Pro Trp Thr Phe Gly Gln Gly Thr
385                 390                 395                 400

Lys Val Glu Ile Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe
                405                 410                 415

Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys
            420                 425                 430

Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val
        435                 440                 445

Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln
    450                 455                 460

Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser
465                 470                 475                 480

Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His
                485                 490                 495

Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
            500                 505                 510


<210> 22
<211> 2196
<212> DNA
<213> Artificial Sequence

<220>
<221> source
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<223> /note="Description of Artificial Sequence: Synthetic
polynucleotide"

<400> 22
atgtaccgga tgcagctgct gagctgtatc gccctgtctc tggccctggt caccaattct 60

gaggtgcagc tggtggaatc tggcggcgga cttgttcaac ctggcggctc tctgagactg 120

agctgtgccg cttctggcta caccttcacc aactacggca tgaactgggt ccgacaggcc 180

cctggcaaag gccttgaatg ggtcggatgg atcaacacct acaccggcga gccaacatac 240

gccgccgact tcaagcggag attcaccttc agcctggaca ccagcaagag caccgcctac 300

ctgcagatga acagcctgag agccgaggac accgccgtgt actactgcgc caagtatccc 360

cactactacg gcagcagcca ctggtacttt gacgtgtggg gacagggcac actggtcaca 420

gtgtctagcg cctctacaaa gggccccagc gttttcccac tggctcctag cagcaagtct 480

accagcggag gaacagccgc tctgggctgt ctggtcaagg actactttcc cgagcctgtg 540

accgtgtcct ggaattctgg cgctctgaca agcggcgtgc acacctttcc agctgtgctg 600

caaagcagcg gcctgtactc tctgagcagc gtcgtgacag tgccaagcag ctctctgggc 660

acccagacct acatctgcaa tgtgaaccac aagcctagca acaccaaggt ggacaagaag 720

gtggaaccca agagctgcga caagacccac acctgtcctc catgtcctgc tccagaactg 780

ctcggcggac cttccgtgtt cctgtttcct ccaaagccta aggacaccct gatgatcagc 840

agaacccctg aagtgacctg cgtggtggtg gatgtgtccc acgaggatcc cgaagtgaag 900

ttcaattggt acgtggacgg cgtggaagtg cacaacgcca agaccaagcc tagagaggaa 960

cagtacaaca gcacctacag agtggtgtcc gtgctgaccg tgctgcacca ggattggctg 1020

aacggcaaag agtacaagtg caaggtgtcc aacaaggccc tgcctgctcc tatcgagaaa 1080

accatcagca aggccaaggg ccagcctagg gaaccccagg tttacacact gcctccaagc 1140

cgggaagaga tgaccaagaa ccaggtgtcc ctgacctgcc tcgtgaaggg cttctaccct 1200

tccgatatcg ccgtggaatg ggagagcaat ggccagccag agaacaacta caagacaacc 1260

cctcctgtgc tggacagcga cggctcattc ttcctgtaca gcaagctgac agtggacaag 1320

tccagatggc agcagggcaa cgtgttcagc tgcagcgtga tgcacgaggc cctgcacaac 1380

cactacaccc agaagtctct gagcctgtct cctggcaagc ggaagagaag aggctctggc 1440

gaaggcagag gcagcctgct tacatgtggc gacgtggaag agaaccccgg acctatgtat 1500

agaatgcagc tcctgtcctg cattgccctg agcctggctc tcgtgaccaa cagcgacatc 1560

cagatgacac agagccccag cagcctgtct gcctctgtgg gagacagagt gaccatcacc 1620

tgtagcgcca gccaggacat ctccaactac ctgaactggt atcagcaaaa gcccggcaag 1680

gcccctaagg tgctgatcta cttcacaagc agcctgcact ccggcgtgcc cagcagattt 1740

tctggctctg gcagcggcac cgacttcacc ctgaccatat ctagcctgca gcctgaggac 1800

ttcgccacct actactgcca gcagtacagc accgtgcctt ggacatttgg ccagggcaca 1860

aaggtggaaa tcaagcggac tgtggccgct cctagcgtgt tcatctttcc acctagcgac 1920

gagcagctga agtctggcac agcctctgtc gtgtgcctgc tgaacaactt ctaccccaga 1980

gaagccaagg tgcagtggaa agtggacaat gccctgcaga gcggcaacag ccaagagagc 2040

gtgacagagc aggactccaa ggatagcacc tatagcctga gcagcaccct gacactgagc 2100

aaggccgact acgagaagca caaagtgtac gcctgcgaag tgacccacca gggcctttct 2160

agccctgtga ccaagagctt caaccggggc gaatgt 2196

<210> 23
<211> 732
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
polypeptide"

<400> 23
Met Tyr Arg Met Gln Leu Leu Ser Cys Ile Ala Leu Ser Leu Ala Leu
1 5 10 15

Val Thr Asn Ser Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val
20 25 30

Gln Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Tyr Thr
35 40 45

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Phe Thr Asn Tyr Gly Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly
    50                  55                  60

Leu Glu Trp Val Gly Trp Ile Asn Thr Tyr Thr Gly Glu Pro Thr Tyr
65                  70                  75                  80

Ala Ala Asp Phe Lys Arg Arg Phe Thr Phe Ser Leu Asp Thr Ser Lys
                85                  90                  95

Ser Thr Ala Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala
            100                 105                 110

Val Tyr Tyr Cys Ala Lys Tyr Pro His Tyr Tyr Gly Ser Ser His Trp
        115                 120                 125

Tyr Phe Asp Val Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala
    130                 135                 140

Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser
145                 150                 155                 160

Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe
                165                 170                 175

Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly
            180                 185                 190

Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu
        195                 200                 205

Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr
    210                 215                 220

Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys
225                 230                 235                 240
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Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro
                245                 250                 255

Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys
            260                 265                 270

Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val
        275                 280                 285

Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr
    290                 295                 300

Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu
305                 310                 315                 320

Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His
                325                 330                 335

Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys
            340                 345                 350

Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln
        355                 360                 365

Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met
    370                 375                 380

Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro
385                 390                 395                 400

Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn
                405                 410                 415

Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu
            420                 425                 430

Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val
        435                 440                 445
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Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln
    450                 455                 460

Lys Ser Leu Ser Leu Ser Pro Gly Lys Arg Lys Arg Arg Gly Ser Gly
465                 470                 475                 480

Glu Gly Arg Gly Ser Leu Leu Thr Cys Gly Asp Val Glu Glu Asn Pro
                485                 490                 495

Gly Pro Met Tyr Arg Met Gln Leu Leu Ser Cys Ile Ala Leu Ser Leu
            500                 505                 510

Ala Leu Val Thr Asn Ser Asp Ile Gln Met Thr Gln Ser Pro Ser Ser
        515                 520                 525

Leu Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys Ser Ala Ser
    530                 535                 540

Gln Asp Ile Ser Asn Tyr Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys
545                 550                 555                 560

Ala Pro Lys Val Leu Ile Tyr Phe Thr Ser Ser Leu His Ser Gly Val
                565                 570                 575

Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr
            580                 585                 590

Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln
        595                 600                 605

Tyr Ser Thr Val Pro Trp Thr Phe Gly Gln Gly Thr Lys Val Glu Ile
    610                 615                 620

Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp
625                 630                 635                 640
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Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn
645 650 655

Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu
660 665 670

Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp
675 680 685

Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr
690 695 700

Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser
705 710 715 720

Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
725 730

<210> 24
<211> 453
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
polypeptide"

<400> 24
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Tyr Thr Phe Thr Asn Tyr
20 25 30

Gly Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Gly Trp Ile Asn Thr Tyr Thr Gly Glu Pro Thr Tyr Ala Ala Asp Phe
50 55 60

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Lys Arg Arg Phe Thr Phe Ser Leu Asp Thr Ser Lys Ser Thr Ala Tyr
65                  70                  75                  80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Lys Tyr Pro His Tyr Tyr Gly Ser Ser His Trp Tyr Phe Asp Val
            100                 105                 110

Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly
        115                 120                 125

Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly
    130                 135                 140

Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val
145                 150                 155                 160

Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe
                165                 170                 175

Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val
            180                 185                 190

Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val
        195                 200                 205

Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys
    210                 215                 220

Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu
225                 230                 235                 240

Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr
                245                 250                 255

Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val
```

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            260                 265                 270

Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val
        275                 280                 285

Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser
    290                 295                 300

Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu
305                 310                 315                 320

Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala
                325                 330                 335

Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro
            340                 345                 350

Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln
        355                 360                 365

Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala
    370                 375                 380

Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr
385                 390                 395                 400

Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu
                405                 410                 415

Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser
            420                 425                 430

Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser
        435                 440                 445

Leu Ser Pro Gly Lys
    450
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<210> 25
<211> 214
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
polypeptide"

<400> 25
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Gln Asp Ile Ser Asn Tyr
20 25 30

Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Val Leu Ile
35 40 45

Tyr Phe Thr Ser Ser Leu His Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Ser Thr Val Pro Trp
85 90 95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala
100 105 110

Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
115 120 125

Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
130 135 140

Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
145 150 155 160

Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
165 170 175

Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
180 185 190

Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
195 200 205

Phe Asn Arg Gly Glu Cys
210

<210> 26
<211> 7123
<212> DNA
<213> Homo sapiens

<400> 26
atcgaggtcc gcgggaggct cggagcgcgc caggcggaca ctcctctcgg ctcctccccg 60

gcagcggcgg cggctcggag cgggctccgg ggctcgggtg cagcggccag cgggcgcctg 120

gcggcgagga ttacccgggg aagtggttgt ctcctggctg gagccgcgag acgggcgctc 180

agggcgcggg gccggcggcg gcgaacgaga ggacggactc tggcggccgg gtcgttggcc 240

gcggggagcg cgggcaccgg gcgagcaggc cgcgtcgcgc tcaccatggt cagctactgg 300

gacaccgggg tcctgctgtg cgcgctgctc agctgtctgc ttctcacagg atctagttca 360

ggttcaaaat taaaagatcc tgaactgagt ttaaaaggca cccagcacat catgcaagca 420

ggccagacac tgcatctcca atgcaggggg gaagcagccc ataaatggtc tttgcctgaa 480

atggtgagta aggaaagcga aaggctgagc ataactaaat ctgcctgtgg aagaaatggc 540

aaacaattct gcagtacttt aaccttgaac acagctcaag caaaccacac tggcttctac 600

agctgcaaat atctagctgt acctacttca aagaagaagg aaacagaatc tgcaatctat 660

atatttatta gtgatacagg tagacctttc gtagagatgt acagtgaaat ccccgaaatt 720

atacacatga ctgaaggaag ggagctcgtc attccctgcc gggttacgtc acctaacatc 780

actgttactt taaaaaagtt tccacttgac actttgatcc ctgatggaaa acgcataatc 840

tgggacagta gaaagggctt catcatatca aatgcaacgt acaaagaaat agggcttctg 900

acctgtgaag caacagtcaa tgggcatttg tataagacaa actatctcac acatcgacaa 960

accaatacaa tcatagatgt ccaaataagc acaccacgcc cagtcaaatt acttagaggc 1020

catactcttg tcctcaattg tactgctacc actcccttga acacgagagt tcaaatgacc 1080

tggagttacc ctgatgaaaa aaataagaga gcttccgtaa ggcgacgaat tgaccaaagc 1140

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aaaggacttt atacttgtcg tgtaaggagt ggaccatcat tcaaatctgt taacacctca 1260

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accgtagctg gcaagcggtc ttaccggctc tctatgaaag tgaaggcatt tccctcgccg 1380

gaagttgtat ggttaaaaga tgggttacct gcgactgaga aatctgctcg ctatttgact 1440

cgtggctact cgttaattat caaggacgta actgaagagg atgcagggaa ttatacaatc 1500

ttgctgagca taaaacagtc aaatgtgttt aaaaacctca ctgccactct aattgtcaat 1560

gtgaaacccc agatttacga aaaggccgtg tcatcgtttc cagacccggc tctctaccca 1620

ctgggcagca gacaaatcct gacttgtacc gcatatggta tccctcaacc tacaatcaag 1680

tggttctggc acccctgtaa ccataatcat tccgaagcaa ggtgtgactt ttgttccaat 1740

aatgaagagt cctttatcct ggatgctgac agcaacatgg gaaacagaat tgagagcatc 1800

actcagcgca tggcaataat agaaggaaag aataagatgg ctagcacctt ggttgtggct 1860

gactctagaa tttctggaat ctacatttgc atagcttcca ataaagttgg gactgtggga 1920

agaaacataa gcttttatat cacagatgtg ccaaatgggt ttcatgttaa cttggaaaaa 1980

atgccgacgg aaggagagga cctgaaactg tcttgcacag ttaacaagtt cttatacaga 2040

gacgttactt ggattttact gcggacagtt aataacagaa caatgcacta cagtattagc 2100

aagcaaaaaa tggccatcac taaggagcac tccatcactc ttaatcttac catcatgaat 2160

gtttccctgc aagattcagg cacctatgcc tgcagagcca ggaatgtata cacaggggaa 2220

gaaatcctcc agaagaaaga aattacaatc agagatcagg aagcaccata cctcctgcga 2280

aacctcagtg atcacacagt ggccatcagc agttccacca ctttagactg tcatgctaat 2340

ggtgtccccg agcctcagat cacttggttt aaaaacaacc acaaaataca acaagagcct 2400

ggaattattt taggaccagg aagcagcacg ctgtttattg aaagagtcac agaagaggat 2460

gaaggtgtct atcactgcaa agccaccaac cagaagggct ctgtggaaag ttcagcatac 2520

ctcactgttc aaggaacctc ggacaagtct aatctggagc tgatcactct aacatgcacc 2580

tgtgtggctg cgactctctt ctggctccta ttaaccctct ttatccgaaa aatgaaaagg 2640

tcttcttctg aaataaagac tgactaccta tcaattataa tggacccaga tgaagttcct 2700

ttggatgagc agtgtgagcg gctcccttat gatgccagca agtgggagtt tgcccgggag 2760

agacttaaac tgggcaaatc acttggaaga ggggcttttg gaaaagtggt tcaagcatca 2820

gcatttggca ttaagaaatc acctacgtgc cggactgtgg ctgtgaaaat gctgaaagag 2880

ggggccacgg ccagcgagta caaagctctg atgactgagc taaaaatctt gacccacatt 2940

ggccaccatc tgaacgtggt taacctgctg ggagcctgca ccaagcaagg agggcctctg 3000

atggtgattg ttgaatactg caaatatgga aatctctcca actacctcaa gagcaaacgt 3060

gacttatttt ttctcaacaa ggatgcagca ctacacatgg agcctaagaa agaaaaaatg 3120

gagccaggcc tggaacaagg caagaaacca agactagata gcgtcaccag cagcgaaagc 3180

tttgcgagct ccggctttca ggaagataaa agtctgagtg atgttgagga agaggaggat 3240

tctgacggtt tctacaagga gcccatcact atggaagatc tgatttctta cagttttcaa 3300

gtggccagag gcatggagtt cctgtcttcc agaaagtgca ttcatcggga cctggcagcg 3360

agaaacattc ttttatctga gaacaacgtg gtgaagattt gtgattttgg ccttgcccgg 3420

gatatttata agaaccccga ttatgtgaga aaaggagata ctcgacttcc tctgaaatgg 3480

atggctcctg aatctatctt tgacaaaatc tacagcacca agagcgacgt gtggtcttac 3540

ggagtattgc tgtgggaaat cttctcctta ggtgggtctc catacccagg agtacaaatg 3600

gatgaggact tttgcagtcg cctgagggaa ggcatgagga tgagagctcc tgagtactct 3660

actcctgaaa tctatcagat catgctggac tgctggcaca gagacccaaa agaaaggcca 3720

agatttgcag aacttgtgga aaaactaggt gatttgcttc aagcaaatgt acaacaggat 3780

ggtaaagact acatcccaat caatgccata ctgacaggaa atagtgggtt tacatactca 3840

actcctgcct tctctgagga cttcttcaag gaaagtattt cagctccgaa gtttaattca 3900

ggaagctctg atgatgtcag atacgtaaat gctttcaagt tcatgagcct ggaaagaatc 3960

aaaacctttg aagaactttt accgaatgcc acctccatgt ttgatgacta ccagggcgac 4020

agcagcactc tgttggcctc tcccatgctg aagcgcttca cctggactga cagcaaaccc 4080

aaggcctcgc tcaagattga cttgagagta accagtaaaa gtaaggagtc ggggctgtct 4140

gatgtcagca ggcccagttt ctgccattcc agctgtgggc acgtcagcga aggcaagcgc 4200

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ggagctgcac tgatcaccca atgcatcacg taccccactg ggccagccct gcagcccaaa 4680

acccagggca acaagcccgt tagccccagg gatcactggc tggcctgagc aacatctcgg 4740

gagtcctcta gcaggcctaa gacatgtgag gaggaaaagg aaaaaaagca aaaagcaagg 4800

gagaaaagag aaaccgggag aaggcatgag aaagaatttg agacgcacca tgtgggcacg 4860

gagggggacg gggctcagca atgccatttc agtggcttcc cagctctgac ccttctacat 4920

ttgagggccc agccaggagc agatggacag cgatgagggg acattttctg gattctggga 4980

ggcaagaaaa ggacaaatat ctttttgga actaaagcaa attttagaac tttacctatg 5040

gaagtggttc tatgtccatt ctcattcgtg gcatgttttg atttgtagca ctgagggtgg 5100

cactcaactc tgagcccata cttttggctc ctctagtaag atgcactgaa aacttagcca 5160

gagttaggtt gtctccaggc catgatggcc ttacactgaa aatgtcacat tctattttgg 5220

gtattaatat atagtccaga cacttaactc aatttcttgg tattattctg ttttgcacag 5280

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Leu His Leu Gln Cys Arg Gly Glu Ala Ala His Lys Trp Ser Leu Pro
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Glu Met Val Ser Lys Glu Ser Glu Arg Leu Ser Ile Thr Lys Ser Ala
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Cys Gly Arg Asn Gly Lys Gln Phe Cys Ser Thr Leu Thr Leu Asn Thr
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Ala Gln Ala Asn His Thr Gly Phe Tyr Ser Cys Lys Tyr Leu Ala Val
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Pro Thr Ser Lys Lys Lys Glu Thr Glu Ser Ala Ile Tyr Ile Phe Ile
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Thr Ser Pro Asn Ile Thr Val Thr Leu Lys Lys Phe Pro Leu Asp Thr
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Leu Ile Pro Asp Gly Lys Arg Ile Ile Trp Asp Ser Arg Lys Gly Phe
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Ile Ile Ser Asn Ala Thr Tyr Lys Glu Ile Gly Leu Leu Thr Cys Glu
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Ala Thr Val Asn Gly His Leu Tyr Lys Thr Asn Tyr Leu Thr His Arg
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Gln Thr Asn Thr Ile Ile Asp Val Gln Ile Ser Thr Pro Arg Pro Val
225                 230                 235                 240

Lys Leu Leu Arg Gly His Thr Leu Val Leu Asn Cys Thr Ala Thr Thr
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Pro Leu Asn Thr Arg Val Gln Met Thr Trp Ser Tyr Pro Asp Glu Lys
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Asn Lys Arg Ala Ser Val Arg Arg Arg Ile Asp Gln Ser Asn Ser His
        275                 280                 285

Ala Asn Ile Phe Tyr Ser Val Leu Thr Ile Asp Lys Met Gln Asn Lys
    290                 295                 300

Asp Lys Gly Leu Tyr Thr Cys Arg Val Arg Ser Gly Pro Ser Phe Lys
305                 310                 315                 320

Ser Val Asn Thr Ser Val His Ile Tyr Asp Lys Ala Phe Ile Thr Val
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Lys His Arg Lys Gln Gln Val Leu Glu Thr Val Ala Gly Lys Arg Ser
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Tyr Arg Leu Ser Met Lys Val Lys Ala Phe Pro Ser Pro Glu Val Val
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Trp Leu Lys Asp Gly Leu Pro Ala Thr Glu Lys Ser Ala Arg Tyr Leu
    370                 375                 380

Thr Arg Gly Tyr Ser Leu Ile Ile Lys Asp Val Thr Glu Glu Asp Ala
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Gly Asn Tyr Thr Ile Leu Leu Ser Ile Lys Gln Ser Asn Val Phe Lys
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Asn Leu Thr Ala Thr Leu Ile Val Asn Val Lys Pro Gln Ile Tyr Glu
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Lys Ala Val Ser Ser Phe Pro Asp Pro Ala Leu Tyr Pro Leu Gly Ser
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Arg Gln Ile Leu Thr Cys Thr Ala Tyr Gly Ile Pro Gln Pro Thr Ile
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Lys Trp Phe Trp His Pro Cys Asn His Asn His Ser Glu Ala Arg Cys
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Asp Phe Cys Ser Asn Asn Glu Glu Ser Phe Ile Leu Asp Ala Asp Ser
                485                 490                 495

Asn Met Gly Asn Arg Ile Glu Ser Ile Thr Gln Arg Met Ala Ile Ile
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Glu Gly Lys Asn Lys Met Ala Ser Thr Leu Val Val Ala Asp Ser Arg
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Gly Arg Asn Ile Ser Phe Tyr Ile Thr Asp Val Pro Asn Gly Phe His
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Val Asn Leu Glu Lys Met Pro Thr Glu Gly Glu Asp Leu Lys Leu Ser
                565                 570                 575

Cys Thr Val Asn Lys Phe Leu Tyr Arg Asp Val Thr Trp Ile Leu Leu
            580                 585                 590

Arg Thr Val Asn Asn Arg Thr Met His Tyr Ser Ile Ser Lys Gln Lys
        595                 600                 605

Met Ala Ile Thr Lys Glu His Ser Ile Thr Leu Asn Leu Thr Ile Met
    610                 615                 620

Asn Val Ser Leu Gln Asp Ser Gly Thr Tyr Ala Cys Arg Ala Arg Asn
625                 630                 635                 640

Val Tyr Thr Gly Glu Glu Ile Leu Gln Lys Lys Glu Ile Thr Ile Arg
                645                 650                 655

Asp Gln Glu Ala Pro Tyr Leu Leu Arg Asn Leu Ser Asp His Thr Val
            660                 665                 670

Ala Ile Ser Ser Ser Thr Thr Leu Asp Cys His Ala Asn Gly Val Pro
        675                 680                 685

Glu Pro Gln Ile Thr Trp Phe Lys Asn Asn His Lys Ile Gln Gln Glu
    690                 695                 700

Pro Gly Ile Ile Leu Gly Pro Gly Ser Ser Thr Leu Phe Ile Glu Arg
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Val Thr Glu Glu Asp Glu Gly Val Tyr His Cys Lys Ala Thr Asn Gln
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Lys Gly Ser Val Glu Ser Ser Ala Tyr Leu Thr Val Gln Gly Thr Ser
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Asp Lys Ser Asn Leu Glu Leu Ile Thr Leu Thr Cys Thr Cys Val Ala
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Ala Thr Leu Phe Trp Leu Leu Leu Thr Leu Phe Ile Arg Lys Met Lys
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Arg Ser Ser Ser Glu Ile Lys Thr Asp Tyr Leu Ser Ile Ile Met Asp
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Pro Asp Glu Val Pro Leu Asp Glu Gln Cys Glu Arg Leu Pro Tyr Asp
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Ala Ser Lys Trp Glu Phe Ala Arg Glu Arg Leu Lys Leu Gly Lys Ser
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Leu Gly Arg Gly Ala Phe Gly Lys Val Val Gln Ala Ser Ala Phe Gly
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Ile Lys Lys Ser Pro Thr Cys Arg Thr Val Ala Val Lys Met Leu Lys
    850                 855                 860

Glu Gly Ala Thr Ala Ser Glu Tyr Lys Ala Leu Met Thr Glu Leu Lys
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Ile Leu Thr His Ile Gly His His Leu Asn Val Val Asn Leu Leu Gly
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Ala Cys Thr Lys Gln Gly Gly Pro Leu Met Val Ile Val Glu Tyr Cys
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Lys Tyr Gly Asn Leu Ser Asn Tyr Leu Lys Ser Lys Arg Asp Leu Phe
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Phe Leu Asn Lys Asp Ala Ala Leu His Met Glu Pro Lys Lys Glu Lys
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Thr Ser Ser Glu Ser Phe Ala Ser Ser Gly Phe Gln Glu Asp Lys Ser
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Leu Ser Asp Val Glu Glu Glu Glu Asp Ser Asp Gly Phe Tyr Lys Glu
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Pro Ile Thr Met Glu Asp Leu Ile  Ser Tyr Ser Phe Gln  Val Ala Arg
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Gly Met  Glu Phe Leu Ser Ser  Arg Lys Cys Ile His  Arg Asp Leu
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Ala Ala  Arg Asn Ile Leu Leu  Ser Glu Asn Asn Val  Val Lys Ile
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Cys Asp  Phe Gly Leu Ala Arg  Asp Ile Tyr Lys Asn  Pro Asp Tyr
    1040                1045                1050

Val Arg  Lys Gly Asp Thr Arg  Leu Pro Leu Lys Trp  Met Ala Pro
    1055                1060                1065

Glu Ser  Ile Phe Asp Lys Ile  Tyr Ser Thr Lys Ser  Asp Val Trp
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Ser Tyr  Gly Val Leu Leu Trp  Glu Ile Phe Ser Leu  Gly Gly Ser
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Pro Tyr  Pro Gly Val Gln Met  Asp Glu Asp Phe Cys  Ser Arg Leu
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Arg Glu  Gly Met Arg Met Arg  Ala Pro Glu Tyr Ser  Thr Pro Glu
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Arg Pro  Arg Phe Ala Glu Leu  Val Glu Lys Leu Gly  Asp Leu Leu
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Gln Ala  Asn Val Gln Gln Asp  Gly Lys Asp Tyr Ile  Pro Ile Asn
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Ala Ile  Leu Thr Gly Asn Ser  Gly Phe Thr Tyr Ser  Thr Pro Ala
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Phe Ser  Glu Asp Phe Phe Lys  Glu Ser Ile Ser Ala  Pro Lys Phe
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Asn Ser  Gly Ser Ser Asp Asp  Val Arg Tyr Val Asn  Ala Phe Lys
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Phe Met  Ser Leu Glu Arg Ile  Lys Thr Phe Glu Glu  Leu Leu Pro
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Asn Ala  Thr Ser Met Phe Asp  Asp Tyr Gln Gly Asp  Ser Ser Thr
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Leu Leu  Ala Ser Pro Met Leu  Lys Arg Phe Thr Trp  Thr Asp Ser
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Lys Pro  Lys Ala Ser Leu Lys  Ile Asp Leu Arg Val  Thr Ser Lys
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Ser Lys  Glu Ser Gly Leu Ser  Asp Val Ser Arg Pro  Ser Phe Cys
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His Ser  Ser Cys Gly His Val  Ser Glu Gly Lys Arg  Arg Phe Thr
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35 40 45

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Glu Met Val Ser Lys Glu Ser Glu Arg Leu Ser Ile Thr Lys Ser Ala
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Cys Gly Arg Asn Gly Lys Gln Phe Cys Ser Thr Leu Thr Leu Asn Thr
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Pro Thr Ser Lys Lys Lys Glu Thr Glu Ser Ala Ile Tyr Ile Phe Ile
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Ile Ile Ser Asn Ala Thr Tyr Lys Glu Ile Gly Leu Leu Thr Cys Glu
        195                 200                 205

Ala Thr Val Asn Gly His Leu Tyr Lys Thr Asn Tyr Leu Thr His Arg
    210                 215                 220

Gln Thr Asn Thr Ile Ile Asp Val Gln Ile Ser Thr Pro Arg Pro Val
225                 230                 235                 240
```

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Lys Leu Leu Arg Gly His Thr Leu Val Leu Asn Cys Thr Ala Thr Thr
                245                 250                 255

Pro Leu Asn Thr Arg Val Gln Met Thr Trp Ser Tyr Pro Asp Glu Lys
            260                 265                 270

Asn Lys Arg Ala Ser Val Arg Arg Arg Ile Asp Gln Ser Asn Ser His
        275                 280                 285

Ala Asn Ile Phe Tyr Ser Val Leu Thr Ile Asp Lys Met Gln Asn Lys
    290                 295                 300

Asp Lys Gly Leu Tyr Thr Cys Arg Val Arg Ser Gly Pro Ser Phe Lys
305                 310                 315                 320

Ser Val Asn Thr Ser Val His Ile Tyr Asp Lys Ala Phe Ile Thr Val
                325                 330                 335

Lys His Arg Lys Gln Gln Val Leu Glu Thr Val Ala Gly Lys Arg Ser
            340                 345                 350

Tyr Arg Leu Ser Met Lys Val Lys Ala Phe Pro Ser Pro Glu Val Val
        355                 360                 365

Trp Leu Lys Asp Gly Leu Pro Ala Thr Glu Lys Ser Ala Arg Tyr Leu
    370                 375                 380

Thr Arg Gly Tyr Ser Leu Ile Ile Lys Asp Val Thr Glu Glu Asp Ala
385                 390                 395                 400

Gly Asn Tyr Thr Ile Leu Leu Ser Ile Lys Gln Ser Asn Val Phe Lys
                405                 410                 415

Asn Leu Thr Ala Thr Leu Ile Val Asn Val Lys Pro Gln Ile Tyr Glu
            420                 425                 430

Lys Ala Val Ser Ser Phe Pro Asp Pro Ala Leu Tyr Pro Leu Gly Ser
        435                 440                 445
```

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Arg Gln Ile Leu Thr Cys Thr Ala Tyr Gly Ile Pro Gln Pro Thr Ile
    450                 455                 460

Lys Trp Phe Trp His Pro Cys Asn His Asn His Ser Glu Ala Arg Cys
465                 470                 475                 480

Asp Phe Cys Ser Asn Asn Glu Glu Ser Phe Ile Leu Asp Ala Asp Ser
                485                 490                 495

Asn Met Gly Asn Arg Ile Glu Ser Ile Thr Gln Arg Met Ala Ile Ile
            500                 505                 510

Glu Gly Lys Asn Lys Met Ala Ser Thr Leu Val Val Ala Asp Ser Arg
        515                 520                 525

Ile Ser Gly Ile Tyr Ile Cys Ile Ala Ser Asn Lys Val Gly Thr Val
    530                 535                 540

Gly Arg Asn Ile Ser Phe Tyr Ile Thr Asp Val Pro Asn Gly Phe His
545                 550                 555                 560

Val Asn Leu Glu Lys Met Pro Thr Glu Gly Glu Asp Leu Lys Leu Ser
                565                 570                 575

Cys Thr Val Asn Lys Phe Leu Tyr Arg Asp Val Thr Trp Ile Leu Leu
            580                 585                 590

Arg Thr Val Asn Asn Arg Thr Met His Tyr Ser Ile Ser Lys Gln Lys
        595                 600                 605

Met Ala Ile Thr Lys Glu His Ser Ile Thr Leu Asn Leu Thr Ile Met
    610                 615                 620

Asn Val Ser Leu Gln Asp Ser Gly Thr Tyr Ala Cys Arg Ala Arg Asn
625                 630                 635                 640
```

Val Tyr Thr Gly Glu Glu Ile Leu Gln Lys Lys Glu Ile Thr Ile Arg
645 650 655

Gly Glu His Cys Asn Lys Lys Ala Val Phe Ser Arg Ile Ser Lys Phe
660 665 670

Lys Ser Thr Arg Asn Asp Cys Thr Thr Gln Ser Asn Val Lys His
675 680 685

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<212> DNA
<213> Homo sapiens

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gcggcgagga ttacccgggg aagtggttgt ctcctggctg gagccgcgag acgggcgctc 180

agggcgcggg gccggcggcg gcgaacgaga ggacggactc tggcggccgg gtcgttggcc 240

gcggggagcg cgggcaccgg gcgagcaggc cgcgtcgcgc tcaccatggt cagctactgg 300

gacaccgggg tcctgctgtg cgcgctgctc agctgtctgc ttctcacagg atctagttca 360

ggttcaaaat taaaagatcc tgaactgagt ttaaaaggca cccagcacat catgcaagca 420

ggccagacac tgcatctcca atgcaggggg gaagcagccc ataaatggtc tttgcctgaa 480

atggtgagta aggaaagcga aaggctgagc ataactaaat ctgcctgtgg aagaaatggc 540

aaacaattct gcagtacttt aaccttgaac acagctcaag caaaccacac tggcttctac 600

agctgcaaat atctagctgt acctacttca aagaagaagg aaacagaatc tgcaatctat 660

atatttatta gtgatacagg tagacctttc gtagagatgt acagtgaaat ccccgaaatt 720

atacacatga ctgaaggaag ggagctcgtc attccctgcc gggttacgtc acctaacatc 780

actgttactt taaaaaagtt tccacttgac actttgatcc ctgatggaaa acgcataatc 840

tgggacagta gaaagggctt catcatatca aatgcaacgt acaaagaaat agggcttctg 900

acctgtgaag caacagtcaa tgggcatttg tataagacaa actatctcac acatcgacaa 960

accaatacaa tcatagatgt ccaaataagc acaccacgcc cagtcaaatt acttagaggc 1020

catactcttg tcctcaattg tactgctacc actcccttga acacgagagt tcaaatgacc 1080

tggagttacc ctgatgaaaa aaataagaga gcttccgtaa ggcgacgaat tgaccaaagc 1140

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cgtggctact cgttaattat caaggacgta actgaagagg atgcagggaa ttatacaatc 1500

ttgctgagca taaaacagtc aaatgtgttt aaaaacctca ctgccactct aattgtcaat 1560

gtgaaacccc agatttacga aaaggccgtg tcatcgtttc cagacccggc tctctaccca 1620

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gaactgtata catcaacgtc accatcgtca tcgtcatcat caccattgtc atcatcatca 2460

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<212> PRT
<213> Homo sapiens

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20 25 30

Glu Leu Ser Leu Lys Gly Thr Gln His Ile Met Gln Ala Gly Gln Thr
35 40 45

Leu His Leu Gln Cys Arg Gly Glu Ala Ala His Lys Trp Ser Leu Pro
50 55 60

Glu Met Val Ser Lys Glu Ser Glu Arg Leu Ser Ile Thr Lys Ser Ala
65 70 75 80

Cys Gly Arg Asn Gly Lys Gln Phe Cys Ser Thr Leu Thr Leu Asn Thr
85 90 95

Ala Gln Ala Asn His Thr Gly Phe Tyr Ser Cys Lys Tyr Leu Ala Val

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            100                 105                 110

Pro Thr Ser Lys Lys Lys Glu Thr Glu Ser Ala Ile Tyr Ile Phe Ile
        115                 120                 125

Ser Asp Thr Gly Arg Pro Phe Val Glu Met Tyr Ser Glu Ile Pro Glu
    130                 135                 140

Ile Ile His Met Thr Glu Gly Arg Glu Leu Val Ile Pro Cys Arg Val
145                 150                 155                 160

Thr Ser Pro Asn Ile Thr Val Thr Leu Lys Lys Phe Pro Leu Asp Thr
                165                 170                 175

Leu Ile Pro Asp Gly Lys Arg Ile Ile Trp Asp Ser Arg Lys Gly Phe
            180                 185                 190

Ile Ile Ser Asn Ala Thr Tyr Lys Glu Ile Gly Leu Leu Thr Cys Glu
        195                 200                 205

Ala Thr Val Asn Gly His Leu Tyr Lys Thr Asn Tyr Leu Thr His Arg
    210                 215                 220

Gln Thr Asn Thr Ile Ile Asp Val Gln Ile Ser Thr Pro Arg Pro Val
225                 230                 235                 240

Lys Leu Leu Arg Gly His Thr Leu Val Leu Asn Cys Thr Ala Thr Thr
                245                 250                 255

Pro Leu Asn Thr Arg Val Gln Met Thr Trp Ser Tyr Pro Asp Glu Lys
            260                 265                 270

Asn Lys Arg Ala Ser Val Arg Arg Arg Ile Asp Gln Ser Asn Ser His
        275                 280                 285

Ala Asn Ile Phe Tyr Ser Val Leu Thr Ile Asp Lys Met Gln Asn Lys
    290                 295                 300
```

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Asp Lys Gly Leu Tyr Thr Cys Arg Val Arg Ser Gly Pro Ser Phe Lys
305                 310                 315                 320

Ser Val Asn Thr Ser Val His Ile Tyr Asp Lys Ala Phe Ile Thr Val
                325                 330                 335

Lys His Arg Lys Gln Gln Val Leu Glu Thr Val Ala Gly Lys Arg Ser
            340                 345                 350

Tyr Arg Leu Ser Met Lys Val Lys Ala Phe Pro Ser Pro Glu Val Val
        355                 360                 365

Trp Leu Lys Asp Gly Leu Pro Ala Thr Glu Lys Ser Ala Arg Tyr Leu
    370                 375                 380

Thr Arg Gly Tyr Ser Leu Ile Ile Lys Asp Val Thr Glu Glu Asp Ala
385                 390                 395                 400

Gly Asn Tyr Thr Ile Leu Leu Ser Ile Lys Gln Ser Asn Val Phe Lys
                405                 410                 415

Asn Leu Thr Ala Thr Leu Ile Val Asn Val Lys Pro Gln Ile Tyr Glu
            420                 425                 430

Lys Ala Val Ser Ser Phe Pro Asp Pro Ala Leu Tyr Pro Leu Gly Ser
        435                 440                 445

Arg Gln Ile Leu Thr Cys Thr Ala Tyr Gly Ile Pro Gln Pro Thr Ile
    450                 455                 460

Lys Trp Phe Trp His Pro Cys Asn His Asn His Ser Glu Ala Arg Cys
465                 470                 475                 480

Asp Phe Cys Ser Asn Asn Glu Glu Ser Phe Ile Leu Asp Ala Asp Ser
                485                 490                 495

Asn Met Gly Asn Arg Ile Glu Ser Ile Thr Gln Arg Met Ala Ile Ile
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            500                 505                 510

Glu Gly Lys Asn Lys Met Ala Ser Thr Leu Val Val Ala Asp Ser Arg
        515                 520                 525

Ile Ser Gly Ile Tyr Ile Cys Ile Ala Ser Asn Lys Val Gly Thr Val
    530                 535                 540

Gly Arg Asn Ile Ser Phe Tyr Ile Thr Asp Val Pro Asn Gly Phe His
545                 550                 555                 560

Val Asn Leu Glu Lys Met Pro Thr Glu Gly Glu Asp Leu Lys Leu Ser
                565                 570                 575

Cys Thr Val Asn Lys Phe Leu Tyr Arg Asp Val Thr Trp Ile Leu Leu
            580                 585                 590

Arg Thr Val Asn Asn Arg Thr Met His Tyr Ser Ile Ser Lys Gln Lys
        595                 600                 605

Met Ala Ile Thr Lys Glu His Ser Ile Thr Leu Asn Leu Thr Ile Met
    610                 615                 620

Asn Val Ser Leu Gln Asp Ser Gly Thr Tyr Ala Cys Arg Ala Arg Asn
625                 630                 635                 640

Val Tyr Thr Gly Glu Glu Ile Leu Gln Lys Lys Glu Ile Thr Ile Arg
                645                 650                 655

Asp Gln Glu Ala Pro Tyr Leu Leu Arg Asn Leu Ser Asp His Thr Val
            660                 665                 670

Ala Ile Ser Ser Ser Thr Thr Leu Asp Cys His Ala Asn Gly Val Pro
        675                 680                 685

Glu Pro Gln Ile Thr Trp Phe Lys Asn Asn His Lys Ile Gln Gln Glu
    690                 695                 700
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Pro Glu Leu Tyr Thr Ser Thr Ser Pro Ser Ser Ser Ser Ser Ser Pro
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Leu Ser Ser Ser Ser Ser Ser Ser Ser Ser Ser Ser Ser Ser
725 730

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<212> DNA
<213> Homo sapiens

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gcggcgagga ttacccgggg aagtggttgt ctcctggctg gagccgcgag acgggcgctc 180

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gacaccgggg tcctgctgtg cgcgctgctc agctgtctgc ttctcacagg atctagttca 360

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ggccagacac tgcatctcca atgcaggggg gaagcagccc ataaatggtc tttgcctgaa 480

atggtgagta aggaaagcga aaggctgagc ataactaaat ctgcctgtgg aagaaatggc 540

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atatttatta gtgatacagg tagacctttc gtagagatgt acagtgaaat ccccgaaatt 720

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actgttactt taaaaaagtt tccacttgac actttgatcc ctgatggaaa acgcataatc 840

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acctgtgaag caacagtcaa tgggcatttg tataagacaa actatctcac acatcgacaa 960

accaatacaa tcatagatgt ccaaataagc acaccacgcc cagtcaaatt acttagaggc 1020

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tggagttacc ctgatgaaaa aaataagaga gcttccgtaa ggcgacgaat tgaccaaagc 1140

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ttgctgagca taaaacagtc aaatgtgttt aaaaacctca ctgccactct aattgtcaat 1560

gtgaaacccc agatttacga aaaggccgtg tcatcgtttc cagacccggc tctctaccca 1620

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actcagcgca tggcaataat agaaggaaag aataagcttc caccagctaa cagttctttc 1860

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<211> 541
<212> PRT
<213> Homo sapiens

<400> 33
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Cys Leu Leu Leu Thr Gly Ser Ser Ser Gly Ser Lys Leu Lys Asp Pro
20 25 30

Glu Leu Ser Leu Lys Gly Thr Gln His Ile Met Gln Ala Gly Gln Thr
35 40 45

Leu His Leu Gln Cys Arg Gly Glu Ala Ala His Lys Trp Ser Leu Pro
50 55 60

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Glu Met Val Ser Lys Glu Ser Glu Arg Leu Ser Ile Thr Lys Ser Ala
65                  70                  75                  80

Cys Gly Arg Asn Gly Lys Gln Phe Cys Ser Thr Leu Thr Leu Asn Thr
                85                  90                  95

Ala Gln Ala Asn His Thr Gly Phe Tyr Ser Cys Lys Tyr Leu Ala Val
            100                 105                 110

Pro Thr Ser Lys Lys Lys Glu Thr Glu Ser Ala Ile Tyr Ile Phe Ile
        115                 120                 125

Ser Asp Thr Gly Arg Pro Phe Val Glu Met Tyr Ser Glu Ile Pro Glu
    130                 135                 140

Ile Ile His Met Thr Glu Gly Arg Glu Leu Val Ile Pro Cys Arg Val
145                 150                 155                 160

Thr Ser Pro Asn Ile Thr Val Thr Leu Lys Lys Phe Pro Leu Asp Thr
                165                 170                 175

Leu Ile Pro Asp Gly Lys Arg Ile Ile Trp Asp Ser Arg Lys Gly Phe
            180                 185                 190

Ile Ile Ser Asn Ala Thr Tyr Lys Glu Ile Gly Leu Leu Thr Cys Glu
        195                 200                 205

Ala Thr Val Asn Gly His Leu Tyr Lys Thr Asn Tyr Leu Thr His Arg
    210                 215                 220

Gln Thr Asn Thr Ile Ile Asp Val Gln Ile Ser Thr Pro Arg Pro Val
225                 230                 235                 240

Lys Leu Leu Arg Gly His Thr Leu Val Leu Asn Cys Thr Ala Thr Thr
                245                 250                 255
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Pro Leu Asn Thr Arg Val Gln Met Thr Trp Ser Tyr Pro Asp Glu Lys
            260                 265                 270

Asn Lys Arg Ala Ser Val Arg Arg Arg Ile Asp Gln Ser Asn Ser His
        275                 280                 285

Ala Asn Ile Phe Tyr Ser Val Leu Thr Ile Asp Lys Met Gln Asn Lys
    290                 295                 300

Asp Lys Gly Leu Tyr Thr Cys Arg Val Arg Ser Gly Pro Ser Phe Lys
305                 310                 315                 320

Ser Val Asn Thr Ser Val His Ile Tyr Asp Lys Ala Phe Ile Thr Val
                325                 330                 335

Lys His Arg Lys Gln Gln Val Leu Glu Thr Val Ala Gly Lys Arg Ser
            340                 345                 350

Tyr Arg Leu Ser Met Lys Val Lys Ala Phe Pro Ser Pro Glu Val Val
        355                 360                 365

Trp Leu Lys Asp Gly Leu Pro Ala Thr Glu Lys Ser Ala Arg Tyr Leu
    370                 375                 380

Thr Arg Gly Tyr Ser Leu Ile Ile Lys Asp Val Thr Glu Glu Asp Ala
385                 390                 395                 400

Gly Asn Tyr Thr Ile Leu Leu Ser Ile Lys Gln Ser Asn Val Phe Lys
                405                 410                 415

Asn Leu Thr Ala Thr Leu Ile Val Asn Val Lys Pro Gln Ile Tyr Glu
            420                 425                 430

Lys Ala Val Ser Ser Phe Pro Asp Pro Ala Leu Tyr Pro Leu Gly Ser
        435                 440                 445

Arg Gln Ile Leu Thr Cys Thr Ala Tyr Gly Ile Pro Gln Pro Thr Ile
    450                 455                 460
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Lys Trp Phe Trp His Pro Cys Asn His Asn His Ser Glu Ala Arg Cys
465                 470                 475                 480


Asp Phe Cys Ser Asn Asn Glu Glu Ser Phe Ile Leu Asp Ala Asp Ser
                485                 490                 495


Asn Met Gly Asn Arg Ile Glu Ser Ile Thr Gln Arg Met Ala Ile Ile
            500                 505                 510


Glu Gly Lys Asn Lys Leu Pro Pro Ala Asn Ser Ser Phe Met Leu Pro
        515                 520                 525


Pro Thr Ser Phe Ser Ser Asn Tyr Phe His Phe Leu Pro
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<211> 5849
<212> DNA
<213> Homo sapiens

<400> 34

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ccggcacccg cagacgcccc tgcagccgcg gtcggcgccc gggctcccta gccctgtgcg      180

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cctacaagtg cttctaccgg gaaactgact tggcctcggt catttatgtc tatgttcaag      660
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35 40 45

Leu Gln Ile Thr Cys Arg Gly Gln Arg Asp Leu Asp Trp Leu Trp Pro
50 55 60

Asn Asn Gln Ser Gly Ser Glu Gln Arg Val Glu Val Thr Glu Cys Ser
65 70 75 80

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Asp Gly Leu Phe Cys Lys Thr Leu Thr Ile Pro Lys Val Ile Gly Asn
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Asp Thr Gly Ala Tyr Lys Cys Phe Tyr Arg Glu Thr Asp Leu Ala Ser
            100                 105                 110

Val Ile Tyr Val Tyr Val Gln Asp Tyr Arg Ser Pro Phe Ile Ala Ser
        115                 120                 125

Val Ser Asp Gln His Gly Val Val Tyr Ile Thr Glu Asn Lys Asn Lys
    130                 135                 140

Thr Val Val Ile Pro Cys Leu Gly Ser Ile Ser Asn Leu Asn Val Ser
145                 150                 155                 160

Leu Cys Ala Arg Tyr Pro Glu Lys Arg Phe Val Pro Asp Gly Asn Arg
                165                 170                 175

Ile Ser Trp Asp Ser Lys Lys Gly Phe Thr Ile Pro Ser Tyr Met Ile
            180                 185                 190

Ser Tyr Ala Gly Met Val Phe Cys Glu Ala Lys Ile Asn Asp Glu Ser
        195                 200                 205

Tyr Gln Ser Ile Met Tyr Ile Val Val Val Val Gly Tyr Arg Ile Tyr
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Asp Val Val Leu Ser Pro Ser His Gly Ile Glu Leu Ser Val Gly Glu
225                 230                 235                 240

Lys Leu Val Leu Asn Cys Thr Ala Arg Thr Glu Leu Asn Val Gly Ile
                245                 250                 255

Asp Phe Asn Trp Glu Tyr Pro Ser Ser Lys His Gln His Lys Lys Leu
            260                 265                 270

Val Asn Arg Asp Leu Lys Thr Gln Ser Gly Ser Glu Met Lys Lys Phe
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Leu Ser Thr Leu Thr Ile Asp Gly Val Thr Arg Ser Asp Gln Gly Leu
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Tyr Thr Cys Ala Ala Ser Ser Gly Leu Met Thr Lys Lys Asn Ser Thr
305                 310                 315                 320

Phe Val Arg Val His Glu Lys Pro Phe Val Ala Phe Gly Ser Gly Met
                325                 330                 335

Glu Ser Leu Val Glu Ala Thr Val Gly Glu Arg Val Arg Ile Pro Ala
            340                 345                 350

Lys Tyr Leu Gly Tyr Pro Pro Pro Glu Ile Lys Trp Tyr Lys Asn Gly
        355                 360                 365

Ile Pro Leu Glu Ser Asn His Thr Ile Lys Ala Gly His Val Leu Thr
    370                 375                 380

Ile Met Glu Val Ser Glu Arg Asp Thr Gly Asn Tyr Thr Val Ile Leu
385                 390                 395                 400

Thr Asn Pro Ile Ser Lys Glu Lys Gln Ser His Val Val Ser Leu Val
                405                 410                 415

Val Tyr Val Pro Pro Gln Ile Gly Glu Lys Ser Leu Ile Ser Pro Val
            420                 425                 430

Asp Ser Tyr Gln Tyr Gly Thr Thr Gln Thr Leu Thr Cys Thr Val Tyr
        435                 440                 445

Ala Ile Pro Pro Pro His His Ile His Trp Tyr Trp Gln Leu Glu Glu
    450                 455                 460

Glu Cys Ala Asn Glu Pro Ser Gln Ala Val Ser Val Thr Asn Pro Tyr
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Ile Glu Val Asn Lys Asn Gln Phe Ala Leu Ile Glu Gly Lys Asn Lys
            500                 505                 510

Thr Val Ser Thr Leu Val Ile Gln Ala Ala Asn Val Ser Ala Leu Tyr
        515                 520                 525

Lys Cys Glu Ala Val Asn Lys Val Gly Arg Gly Glu Arg Val Ile Ser
    530                 535                 540

Phe His Val Thr Arg Gly Pro Glu Ile Thr Leu Gln Pro Asp Met Gln
545                 550                 555                 560

Pro Thr Glu Gln Glu Ser Val Ser Leu Trp Cys Thr Ala Asp Arg Ser
                565                 570                 575

Thr Phe Glu Asn Leu Thr Trp Tyr Lys Leu Gly Pro Gln Pro Leu Pro
            580                 585                 590

Ile His Val Gly Glu Leu Pro Thr Pro Val Cys Lys Asn Leu Asp Thr
        595                 600                 605

Leu Trp Lys Leu Asn Ala Thr Met Phe Ser Asn Ser Thr Asn Asp Ile
    610                 615                 620

Leu Ile Met Glu Leu Lys Asn Ala Ser Leu Gln Asp Gln Gly Asp Tyr
625                 630                 635                 640

Val Cys Leu Ala Gln Asp Arg Lys Thr Lys Lys Arg His Cys Val Val
                645                 650                 655

Arg Gln Leu Thr Val Leu Glu Arg Val Ala Pro Thr Ile Thr Gly Asn
            660                 665                 670

Leu Glu Asn Gln Thr Thr Ser Ile Gly Glu Ser Ile Glu Val Ser Cys
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Thr Ala Ser Gly Asn Pro Pro Pro Gln Ile Met Trp Phe Lys Asp Asn
    690                 695                 700

Glu Thr Leu Val Glu Asp Ser Gly Ile Val Leu Lys Asp Gly Asn Arg
705                 710                 715                 720

Asn Leu Thr Ile Arg Arg Val Arg Lys Glu Asp Glu Gly Leu Tyr Thr
                725                 730                 735

Cys Gln Ala Cys Ser Val Leu Gly Cys Ala Lys Val Glu Ala Phe Phe
            740                 745                 750

Ile Ile Glu Gly Ala Gln Glu Lys Thr Asn Leu Glu Ile Ile Ile Leu
        755                 760                 765

Val Gly Thr Ala Val Ile Ala Met Phe Phe Trp Leu Leu Leu Val Ile
    770                 775                 780

Ile Leu Arg Thr Val Lys Arg Ala Asn Gly Gly Glu Leu Lys Thr Gly
785                 790                 795                 800

Tyr Leu Ser Ile Val Met Asp Pro Asp Glu Leu Pro Leu Asp Glu His
                805                 810                 815

Cys Glu Arg Leu Pro Tyr Asp Ala Ser Lys Trp Glu Phe Pro Arg Asp
            820                 825                 830

Arg Leu Lys Leu Gly Lys Pro Leu Gly Arg Gly Ala Phe Gly Gln Val
        835                 840                 845

Ile Glu Ala Asp Ala Phe Gly Ile Asp Lys Thr Ala Thr Cys Arg Thr
    850                 855                 860

Val Ala Val Lys Met Leu Lys Glu Gly Ala Thr His Ser Glu His Arg
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Ala Leu Met Ser Glu Leu Lys Ile Leu Ile His Ile Gly His His Leu
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Asn Val Val Asn Leu Leu Gly Ala Cys Thr Lys Pro Gly Gly Pro Leu
            900                 905                 910


Met Val Ile Val Glu Phe Cys Lys Phe Gly Asn Leu Ser Thr Tyr Leu
        915                 920                 925


Arg Ser Lys Arg Asn Glu Phe Val Pro Tyr Lys Thr Lys Gly Ala Arg
    930                 935                 940


Phe Arg Gln Gly Lys Asp Tyr Val Gly Ala Ile Pro Val Asp Leu Lys
945                 950                 955                 960


Arg Arg Leu Asp Ser Ile Thr Ser Ser Gln Ser Ser Ala Ser Ser Gly
               965                 970                 975


Phe Val Glu Glu Lys Ser Leu Ser Asp Val Glu Glu Glu Glu Ala Pro
            980                 985                 990


Glu Asp Leu Tyr Lys Asp Phe Leu  Thr Leu Glu His Leu  Ile Cys Tyr
        995                 1000                1005


Ser Phe  Gln Val Ala Lys Gly  Met Glu Phe Leu Ala  Ser Arg Lys
    1010                1015                1020


Cys Ile  His Arg Asp Leu Ala  Ala Arg Asn Ile Leu  Leu Ser Glu
    1025                1030                1035


Lys Asn  Val Val Lys Ile Cys  Asp Phe Gly Leu Ala  Arg Asp Ile
    1040                1045                1050


Tyr Lys  Asp Pro Asp Tyr Val  Arg Lys Gly Asp Ala  Arg Leu Pro
    1055                1060                1065


Leu Lys  Trp Met Ala Pro Glu  Thr Ile Phe Asp Arg  Val Tyr Thr
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Ile Gln  Ser Asp Val Trp Ser  Phe Gly Val Leu Leu  Trp Glu Ile
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Phe Ser  Leu Gly Ala Ser Pro  Tyr Pro Gly Val Lys  Ile Asp Glu
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Glu Phe  Cys Arg Arg Leu Lys  Glu Gly Thr Arg Met  Arg Ala Pro
    1115                1120                1125

Asp Tyr  Thr Thr Pro Glu Met  Tyr Gln Thr Met Leu  Asp Cys Trp
    1130                1135                1140

His Gly  Glu Pro Ser Gln Arg  Pro Thr Phe Ser Glu  Leu Val Glu
    1145                1150                1155

His Leu  Gly Asn Leu Leu Gln  Ala Asn Ala Gln Gln  Asp Gly Lys
    1160                1165                1170

Asp Tyr  Ile Val Leu Pro Ile  Ser Glu Thr Leu Ser  Met Glu Glu
    1175                1180                1185

Asp Ser  Gly Leu Ser Leu Pro  Thr Ser Pro Val Ser  Cys Met Glu
    1190                1195                1200

Glu Glu  Glu Val Cys Asp Pro  Lys Phe His Tyr Asp  Asn Thr Ala
    1205                1210                1215

Gly Ile  Ser Gln Tyr Leu Gln  Asn Ser Lys Arg Lys  Ser Arg Pro
    1220                1225                1230

Val Ser  Val Lys Thr Phe Glu  Asp Ile Pro Leu Glu  Glu Pro Glu
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Val Lys  Val Ile Pro Asp Asp  Asn Gln Thr Asp Ser  Gly Met Val
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Leu Ala  Ser Glu Glu Leu Lys  Thr Leu Glu Asp Arg  Thr Lys Leu
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Ser Pro  Ser Phe Gly Gly Met  Val Pro Ser Lys Ser  Arg Glu Ser
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Val Ala  Ser Glu Gly Ser Asn  Gln Thr Ser Gly Tyr  Gln Ser Gly
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Tyr His  Ser Asp Asp Thr Asp  Thr Thr Val Tyr Ser  Ser Glu Glu
    1310                 1315                 1320

Ala Glu  Leu Leu Lys Leu Ile  Glu Ile Gly Val Gln  Thr Gly Ser
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Thr Ala  Gln Ile Leu Gln Pro  Asp Ser Gly Thr Thr  Leu Ser Ser
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Pro Pro  Val
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cctcactacc cacccacgca tgtaccggga aaaaaactac tatgcccttc tagaccatgt 5760

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tgtgtgttgc gga 5833

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<212> PRT
<213> Homo sapiens

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Leu Leu Asp Gly Leu Val Ser Gly Tyr Ser Met Thr Pro Pro Thr Leu
20 25 30

Asn Ile Thr Glu Glu Ser His Val Ile Asp Thr Gly Asp Ser Leu Ser
35 40 45

```
Ile Ser Cys Arg Gly Gln His Pro Leu Glu Trp Ala Trp Pro Gly Ala
    50                  55                  60

Gln Glu Ala Pro Ala Thr Gly Asp Lys Asp Ser Glu Asp Thr Gly Val
65                  70                  75                  80

Val Arg Asp Cys Glu Gly Thr Asp Ala Arg Pro Tyr Cys Lys Val Leu
                85                  90                  95

Leu Leu His Glu Val His Ala Asn Asp Thr Gly Ser Tyr Val Cys Tyr
            100                 105                 110

Tyr Lys Tyr Ile Lys Ala Arg Ile Glu Gly Thr Thr Ala Ala Ser Ser
        115                 120                 125

Tyr Val Phe Val Arg Asp Phe Glu Gln Pro Phe Ile Asn Lys Pro Asp
    130                 135                 140

Thr Leu Leu Val Asn Arg Lys Asp Ala Met Trp Val Pro Cys Leu Val
145                 150                 155                 160

Ser Ile Pro Gly Leu Asn Val Thr Leu Arg Ser Gln Ser Ser Val Leu
                165                 170                 175

Trp Pro Asp Gly Gln Glu Val Val Trp Asp Asp Arg Arg Gly Met Leu
            180                 185                 190

Val Ser Thr Pro Leu Leu His Asp Ala Leu Tyr Leu Gln Cys Glu Thr
        195                 200                 205

Thr Trp Gly Asp Gln Asp Phe Leu Ser Asn Pro Phe Leu Val His Ile
    210                 215                 220

Thr Gly Asn Glu Leu Tyr Asp Ile Gln Leu Leu Pro Arg Lys Ser Leu
225                 230                 235                 240

Glu Leu Leu Val Gly Glu Lys Leu Val Leu Asn Cys Thr Val Trp Ala
                245                 250                 255
```

```
Glu Phe Asn Ser Gly Val Thr Phe Asp Trp Asp Tyr Pro Gly Lys Gln
            260                 265                 270

Ala Glu Arg Gly Lys Trp Val Pro Glu Arg Arg Ser Gln Gln Thr His
        275                 280                 285

Thr Glu Leu Ser Ser Ile Leu Thr Ile His Asn Val Ser Gln His Asp
    290                 295                 300

Leu Gly Ser Tyr Val Cys Lys Ala Asn Asn Gly Ile Gln Arg Phe Arg
305                 310                 315                 320

Glu Ser Thr Glu Val Ile Val His Glu Asn Pro Phe Ile Ser Val Glu
                325                 330                 335

Trp Leu Lys Gly Pro Ile Leu Glu Ala Thr Ala Gly Asp Glu Leu Val
            340                 345                 350

Lys Leu Pro Val Lys Leu Ala Ala Tyr Pro Pro Pro Glu Phe Gln Trp
        355                 360                 365

Tyr Lys Asp Gly Lys Ala Leu Ser Gly Arg His Ser Pro His Ala Leu
    370                 375                 380

Val Leu Lys Glu Val Thr Glu Ala Ser Thr Gly Thr Tyr Thr Leu Ala
385                 390                 395                 400

Leu Trp Asn Ser Ala Ala Gly Leu Arg Arg Asn Ile Ser Leu Glu Leu
                405                 410                 415

Val Val Asn Val Pro Pro Gln Ile His Glu Lys Glu Ala Ser Ser Pro
            420                 425                 430

Ser Ile Tyr Ser Arg His Ser Arg Gln Ala Leu Thr Cys Thr Ala Tyr
        435                 440                 445
```

```
Gly Val Pro Leu Pro Leu Ser Ile Gln Trp His Trp Arg Pro Trp Thr
    450                 455                 460

Pro Cys Lys Met Phe Ala Gln Arg Ser Leu Arg Arg Arg Gln Gln Gln
465                 470                 475                 480

Asp Leu Met Pro Gln Cys Arg Asp Trp Arg Ala Val Thr Thr Gln Asp
                485                 490                 495

Ala Val Asn Pro Ile Glu Ser Leu Asp Thr Trp Thr Glu Phe Val Glu
            500                 505                 510

Gly Lys Asn Lys Thr Val Ser Lys Leu Val Ile Gln Asn Ala Asn Val
        515                 520                 525

Ser Ala Met Tyr Lys Cys Val Val Ser Asn Lys Val Gly Gln Asp Glu
    530                 535                 540

Arg Leu Ile Tyr Phe Tyr Val Thr Thr Ile Pro Asp Gly Phe Thr Ile
545                 550                 555                 560

Glu Ser Lys Pro Ser Glu Glu Leu Leu Glu Gly Gln Pro Val Leu Leu
                565                 570                 575

Ser Cys Gln Ala Asp Ser Tyr Lys Tyr Glu His Leu Arg Trp Tyr Arg
            580                 585                 590

Leu Asn Leu Ser Thr Leu His Asp Ala His Gly Asn Pro Leu Leu Leu
        595                 600                 605

Asp Cys Lys Asn Val His Leu Phe Ala Thr Pro Leu Ala Ala Ser Leu
    610                 615                 620

Glu Glu Val Ala Pro Gly Ala Arg His Ala Thr Leu Ser Leu Ser Ile
625                 630                 635                 640

Pro Arg Val Ala Pro Glu His Glu Gly His Tyr Val Cys Glu Val Gln
                645                 650                 655
```

```
Asp Arg Arg Ser His Asp Lys His Cys His Lys Lys Tyr Leu Ser Val
            660                 665                 670

Gln Ala Leu Glu Ala Pro Arg Leu Thr Gln Asn Leu Thr Asp Leu Leu
        675                 680                 685

Val Asn Val Ser Asp Ser Leu Glu Met Gln Cys Leu Val Ala Gly Ala
    690                 695                 700

His Ala Pro Ser Ile Val Trp Tyr Lys Asp Glu Arg Leu Leu Glu Glu
705                 710                 715                 720

Lys Ser Gly Val Asp Leu Ala Asp Ser Asn Gln Lys Leu Ser Ile Gln
                725                 730                 735

Arg Val Arg Glu Glu Asp Ala Gly Arg Tyr Leu Cys Ser Val Cys Asn
            740                 745                 750

Ala Lys Gly Cys Val Asn Ser Ser Ala Ser Val Ala Val Glu Gly Ser
        755                 760                 765

Glu Asp Lys Gly Ser Met Glu Ile Val Ile Leu Val Gly Thr Gly Val
    770                 775                 780

Ile Ala Val Phe Phe Trp Val Leu Leu Leu Leu Ile Phe Cys Asn Met
785                 790                 795                 800

Arg Arg Pro Ala His Ala Asp Ile Lys Thr Gly Tyr Leu Ser Ile Ile
                805                 810                 815

Met Asp Pro Gly Glu Val Pro Leu Glu Glu Gln Cys Glu Tyr Leu Ser
            820                 825                 830

Tyr Asp Ala Ser Gln Trp Glu Phe Pro Arg Glu Arg Leu His Leu Gly
        835                 840                 845
```

```
Arg Val Leu Gly Tyr Gly Ala Phe Gly Lys Val Val Glu Ala Ser Ala
    850                 855                 860

Phe Gly Ile His Lys Gly Ser Ser Cys Asp Thr Val Ala Val Lys Met
865                 870                 875                 880

Leu Lys Glu Gly Ala Thr Ala Ser Glu His Arg Ala Leu Met Ser Glu
                885                 890                 895

Leu Lys Ile Leu Ile His Ile Gly Asn His Leu Asn Val Val Asn Leu
            900                 905                 910

Leu Gly Ala Cys Thr Lys Pro Gln Gly Pro Leu Met Val Ile Val Glu
        915                 920                 925

Phe Cys Lys Tyr Gly Asn Leu Ser Asn Phe Leu Arg Ala Lys Arg Asp
    930                 935                 940

Ala Phe Ser Pro Cys Ala Glu Lys Ser Pro Glu Gln Arg Gly Arg Phe
945                 950                 955                 960

Arg Ala Met Val Glu Leu Ala Arg Leu Asp Arg Arg Arg Pro Gly Ser
                965                 970                 975

Ser Asp Arg Val Leu Phe Ala Arg Phe Ser Lys Thr Glu Gly Gly Ala
            980                 985                 990

Arg Arg Ala Ser Pro Asp Gln Glu  Ala Glu Asp Leu Trp  Leu Ser Pro
        995                 1000                1005

Leu Thr  Met Glu Asp Leu Val  Cys Tyr Ser Phe Gln  Val Ala Arg
    1010                1015                1020

Gly Met  Glu Phe Leu Ala Ser  Arg Lys Cys Ile His  Arg Asp Leu
    1025                1030                1035

Ala Ala  Arg Asn Ile Leu Leu  Ser Glu Ser Asp Val  Val Lys Ile
    1040                1045                1050
```

```
Cys Asp  Phe Gly Leu Ala Arg  Asp Ile Tyr Lys Asp  Pro Asp Tyr
    1055                1060                1065

Val Arg  Lys Gly Ser Ala Arg  Leu Pro Leu Lys Trp  Met Ala Pro
    1070                1075                1080

Glu Ser  Ile Phe Asp Lys Val  Tyr Thr Thr Gln Ser  Asp Val Trp
    1085                1090                1095

Ser Phe  Gly Val Leu Leu Trp  Glu Ile Phe Ser Leu  Gly Ala Ser
    1100                1105                1110

Pro Tyr  Pro Gly Val Gln Ile  Asn Glu Glu Phe Cys  Gln Arg Leu
    1115                1120                1125

Arg Asp  Gly Thr Arg Met Arg  Ala Pro Glu Leu Ala  Thr Pro Ala
    1130                1135                1140

Ile Arg  Arg Ile Met Leu Asn  Cys Trp Ser Gly Asp  Pro Lys Ala
    1145                1150                1155

Arg Pro  Ala Phe Ser Glu Leu  Val Glu Ile Leu Gly  Asp Leu Leu
    1160                1165                1170

Gln Gly  Arg Gly Leu Gln Glu  Glu Glu Glu Val Cys  Met Ala Pro
    1175                1180                1185

Arg Ser  Ser Gln Ser Ser Glu  Glu Gly Ser Phe Ser  Gln Val Ser
    1190                1195                1200

Thr Met  Ala Leu His Ile Ala  Gln Ala Asp Ala Glu  Asp Ser Pro
    1205                1210                1215

Pro Ser  Leu Gln Arg His Ser  Leu Ala Ala Arg Tyr  Tyr Asn Trp
    1220                1225                1230
```

```
Val Ser  Phe Pro Gly Cys Leu  Ala Arg Gly Ala Glu  Thr Arg Gly
    1235                1240                1245

Ser Ser  Arg Met Lys Thr Phe  Glu Glu Phe Pro Met  Thr Pro Thr
    1250                1255                1260

Thr Tyr  Lys Gly Ser Val Asp  Asn Gln Thr Asp Ser  Gly Met Val
    1265                1270                1275

Leu Ala  Ser Glu Glu Phe Glu  Gln Ile Glu Ser Arg  His Arg Gln
    1280                1285                1290

Glu Ser  Gly Phe Ser Cys Lys  Gly Pro Gly Gln Asn  Val Ala Val
    1295                1300                1305

Thr Arg  Ala His Pro Asp Ser  Gln Gly Arg Arg Arg  Arg Pro Glu
    1310                1315                1320

Arg Gly  Ala Arg Gly Gly Gln  Val Phe Tyr Asn Ser  Glu Tyr Gly
    1325                1330                1335

Glu Leu  Ser Glu Pro Ser Glu  Glu Asp His Cys Ser  Pro Ser Ala
    1340                1345                1350

Arg Val  Thr Phe Phe Thr Asp  Asn Ser Tyr
    1355                1360
```

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<212> DNA
<213> Homo sapiens

<400> 38
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gagtggctac tccatgaccc ccccgacctt gaacatcacg gaggagtcac acgtcatcga 180

caccggtgac agcctgtcca tctcctgcag gggacagcac cccctcgagt gggcttggcc 240

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<210> 39
<211> 1298
<212> PRT
<213> Homo sapiens

<400> 39
Met Gln Arg Gly Ala Ala Leu Cys Leu Arg Leu Trp Leu Cys Leu Gly

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Leu Leu Asp Gly Leu Val Ser Gly Tyr Ser Met Thr Pro Pro Thr Leu
            20                  25                  30

Asn Ile Thr Glu Glu Ser His Val Ile Asp Thr Gly Asp Ser Leu Ser
        35                  40                  45

Ile Ser Cys Arg Gly Gln His Pro Leu Glu Trp Ala Trp Pro Gly Ala
    50                  55                  60

Gln Glu Ala Pro Ala Thr Gly Asp Lys Asp Ser Glu Asp Thr Gly Val
65                  70                  75                  80

Val Arg Asp Cys Glu Gly Thr Asp Ala Arg Pro Tyr Cys Lys Val Leu
                85                  90                  95

Leu Leu His Glu Val His Ala Asn Asp Thr Gly Ser Tyr Val Cys Tyr
            100                 105                 110

Tyr Lys Tyr Ile Lys Ala Arg Ile Glu Gly Thr Thr Ala Ala Ser Ser
        115                 120                 125

Tyr Val Phe Val Arg Asp Phe Glu Gln Pro Phe Ile Asn Lys Pro Asp
    130                 135                 140

Thr Leu Leu Val Asn Arg Lys Asp Ala Met Trp Val Pro Cys Leu Val
145                 150                 155                 160

Ser Ile Pro Gly Leu Asn Val Thr Leu Arg Ser Gln Ser Ser Val Leu
                165                 170                 175

Trp Pro Asp Gly Gln Glu Val Val Trp Asp Asp Arg Arg Gly Met Leu
            180                 185                 190

Val Ser Thr Pro Leu Leu His Asp Ala Leu Tyr Leu Gln Cys Glu Thr
        195                 200                 205
```

```
Thr Trp Gly Asp Gln Asp Phe Leu Ser Asn Pro Phe Leu Val His Ile
    210                 215                 220

Thr Gly Asn Glu Leu Tyr Asp Ile Gln Leu Leu Pro Arg Lys Ser Leu
225                 230                 235                 240

Glu Leu Leu Val Gly Glu Lys Leu Val Leu Asn Cys Thr Val Trp Ala
                245                 250                 255

Glu Phe Asn Ser Gly Val Thr Phe Asp Trp Asp Tyr Pro Gly Lys Gln
            260                 265                 270

Ala Glu Arg Gly Lys Trp Val Pro Glu Arg Arg Ser Gln Gln Thr His
        275                 280                 285

Thr Glu Leu Ser Ser Ile Leu Thr Ile His Asn Val Ser Gln His Asp
    290                 295                 300

Leu Gly Ser Tyr Val Cys Lys Ala Asn Asn Gly Ile Gln Arg Phe Arg
305                 310                 315                 320

Glu Ser Thr Glu Val Ile Val His Glu Asn Pro Phe Ile Ser Val Glu
                325                 330                 335

Trp Leu Lys Gly Pro Ile Leu Glu Ala Thr Ala Gly Asp Glu Leu Val
            340                 345                 350

Lys Leu Pro Val Lys Leu Ala Ala Tyr Pro Pro Pro Glu Phe Gln Trp
        355                 360                 365

Tyr Lys Asp Gly Lys Ala Leu Ser Gly Arg His Ser Pro His Ala Leu
    370                 375                 380

Val Leu Lys Glu Val Thr Glu Ala Ser Thr Gly Thr Tyr Thr Leu Ala
385                 390                 395                 400

Leu Trp Asn Ser Ala Ala Gly Leu Arg Arg Asn Ile Ser Leu Glu Leu
```

```
                405                 410                 415

Val Val Asn Val Pro Pro Gln Ile His Glu Lys Glu Ala Ser Ser Pro
            420                 425                 430

Ser Ile Tyr Ser Arg His Ser Arg Gln Ala Leu Thr Cys Thr Ala Tyr
        435                 440                 445

Gly Val Pro Leu Pro Leu Ser Ile Gln Trp His Trp Arg Pro Trp Thr
    450                 455                 460

Pro Cys Lys Met Phe Ala Gln Arg Ser Leu Arg Arg Arg Gln Gln Gln
465                 470                 475                 480

Asp Leu Met Pro Gln Cys Arg Asp Trp Arg Ala Val Thr Thr Gln Asp
                485                 490                 495

Ala Val Asn Pro Ile Glu Ser Leu Asp Thr Trp Thr Glu Phe Val Glu
            500                 505                 510

Gly Lys Asn Lys Thr Val Ser Lys Leu Val Ile Gln Asn Ala Asn Val
        515                 520                 525

Ser Ala Met Tyr Lys Cys Val Val Ser Asn Lys Val Gly Gln Asp Glu
    530                 535                 540

Arg Leu Ile Tyr Phe Tyr Val Thr Thr Ile Pro Asp Gly Phe Thr Ile
545                 550                 555                 560

Glu Ser Lys Pro Ser Glu Glu Leu Leu Glu Gly Gln Pro Val Leu Leu
                565                 570                 575

Ser Cys Gln Ala Asp Ser Tyr Lys Tyr Glu His Leu Arg Trp Tyr Arg
            580                 585                 590

Leu Asn Leu Ser Thr Leu His Asp Ala His Gly Asn Pro Leu Leu Leu
        595                 600                 605
```

```
Asp Cys Lys Asn Val His Leu Phe Ala Thr Pro Leu Ala Ala Ser Leu
    610                 615                 620

Glu Glu Val Ala Pro Gly Ala Arg His Ala Thr Leu Ser Leu Ser Ile
625                 630                 635                 640

Pro Arg Val Ala Pro Glu His Glu Gly His Tyr Val Cys Glu Val Gln
                645                 650                 655

Asp Arg Arg Ser His Asp Lys His Cys His Lys Lys Tyr Leu Ser Val
            660                 665                 670

Gln Ala Leu Glu Ala Pro Arg Leu Thr Gln Asn Leu Thr Asp Leu Leu
        675                 680                 685

Val Asn Val Ser Asp Ser Leu Glu Met Gln Cys Leu Val Ala Gly Ala
    690                 695                 700

His Ala Pro Ser Ile Val Trp Tyr Lys Asp Glu Arg Leu Leu Glu Glu
705                 710                 715                 720

Lys Ser Gly Val Asp Leu Ala Asp Ser Asn Gln Lys Leu Ser Ile Gln
                725                 730                 735

Arg Val Arg Glu Glu Asp Ala Gly Arg Tyr Leu Cys Ser Val Cys Asn
            740                 745                 750

Ala Lys Gly Cys Val Asn Ser Ser Ala Ser Val Ala Val Glu Gly Ser
        755                 760                 765

Glu Asp Lys Gly Ser Met Glu Ile Val Ile Leu Val Gly Thr Gly Val
    770                 775                 780

Ile Ala Val Phe Phe Trp Val Leu Leu Leu Leu Ile Phe Cys Asn Met
785                 790                 795                 800

Arg Arg Pro Ala His Ala Asp Ile Lys Thr Gly Tyr Leu Ser Ile Ile
```

```
                805                 810                 815

Met Asp Pro Gly Glu Val Pro Leu Glu Glu Gln Cys Glu Tyr Leu Ser
            820                 825                 830

Tyr Asp Ala Ser Gln Trp Glu Phe Pro Arg Glu Arg Leu His Leu Gly
        835                 840                 845

Arg Val Leu Gly Tyr Gly Ala Phe Gly Lys Val Val Glu Ala Ser Ala
    850                 855                 860

Phe Gly Ile His Lys Gly Ser Ser Cys Asp Thr Val Ala Val Lys Met
865                 870                 875                 880

Leu Lys Glu Gly Ala Thr Ala Ser Glu His Arg Ala Leu Met Ser Glu
                885                 890                 895

Leu Lys Ile Leu Ile His Ile Gly Asn His Leu Asn Val Val Asn Leu
            900                 905                 910

Leu Gly Ala Cys Thr Lys Pro Gln Gly Pro Leu Met Val Ile Val Glu
        915                 920                 925

Phe Cys Lys Tyr Gly Asn Leu Ser Asn Phe Leu Arg Ala Lys Arg Asp
    930                 935                 940

Ala Phe Ser Pro Cys Ala Glu Lys Ser Pro Glu Gln Arg Gly Arg Phe
945                 950                 955                 960

Arg Ala Met Val Glu Leu Ala Arg Leu Asp Arg Arg Arg Pro Gly Ser
                965                 970                 975

Ser Asp Arg Val Leu Phe Ala Arg Phe Ser Lys Thr Glu Gly Gly Ala
            980                 985                 990

Arg Arg Ala Ser Pro Asp Gln Glu  Ala Glu Asp Leu Trp  Leu Ser Pro
        995                 1000                1005
```

```
Leu Thr  Met Glu Asp Leu Val  Cys Tyr Ser Phe Gln  Val Ala Arg
    1010                1015                1020

Gly Met  Glu Phe Leu Ala Ser  Arg Lys Cys Ile His  Arg Asp Leu
    1025                1030                1035

Ala Ala  Arg Asn Ile Leu Leu  Ser Glu Ser Asp Val  Val Lys Ile
    1040                1045                1050

Cys Asp  Phe Gly Leu Ala Arg  Asp Ile Tyr Lys Asp  Pro Asp Tyr
    1055                1060                1065

Val Arg  Lys Gly Ser Ala Arg  Leu Pro Leu Lys Trp  Met Ala Pro
    1070                1075                1080

Glu Ser  Ile Phe Asp Lys Val  Tyr Thr Thr Gln Ser  Asp Val Trp
    1085                1090                1095

Ser Phe  Gly Val Leu Leu Trp  Glu Ile Phe Ser Leu  Gly Ala Ser
    1100                1105                1110

Pro Tyr  Pro Gly Val Gln Ile  Asn Glu Glu Phe Cys  Gln Arg Leu
    1115                1120                1125

Arg Asp  Gly Thr Arg Met Arg  Ala Pro Glu Leu Ala  Thr Pro Ala
    1130                1135                1140

Ile Arg  Arg Ile Met Leu Asn  Cys Trp Ser Gly Asp  Pro Lys Ala
    1145                1150                1155

Arg Pro  Ala Phe Ser Glu Leu  Val Glu Ile Leu Gly  Asp Leu Leu
    1160                1165                1170

Gln Gly  Arg Gly Leu Gln Glu  Glu Glu Glu Val Cys  Met Ala Pro
    1175                1180                1185

Arg Ser  Ser Gln Ser Ser Glu  Glu Gly Ser Phe Ser  Gln Val Ser
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    1190                1195                1200

Thr Met  Ala Leu His Ile Ala  Gln Ala Asp Ala Glu  Asp Ser Pro
    1205                1210                1215

Pro Ser  Leu Gln Arg His Ser  Leu Ala Ala Arg Tyr  Tyr Asn Trp
    1220                1225                1230

Val Ser  Phe Pro Gly Cys Leu  Ala Arg Gly Ala Glu  Thr Arg Gly
    1235                1240                1245

Ser Ser  Arg Met Lys Thr Phe  Glu Glu Phe Pro Met  Thr Pro Thr
    1250                1255                1260

Thr Tyr  Lys Gly Ser Val Asp  Asn Gln Thr Asp Ser  Gly Met Val
    1265                1270                1275

Leu Ala  Ser Glu Glu Phe Glu  Gln Ile Glu Ser Arg  His Arg Gln
    1280                1285                1290

Glu Ser  Gly Phe Arg
    1295
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<210> 40
<211> 4359
<212> DNA
<213> Homo sapiens

<400> 40

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gcggggcgcc gcgctgtgcc tgcgactgtg gctctgcctg ggactcctgg acggcctggt      120

gagtggctac tccatgaccc ccccgacctt gaacatcacg gaggagtcac acgtcatcga      180

caccggtgac agcctgtcca tctcctgcag gggacagcac cccctcgagt gggcttggcc      240

aggagctcag gaggcgccag ccaccggaga caaggacagc gaggacacgg gggtggtgcg      300

agactgcgag ggcacagacg ccaggcccta ctgcaaggtg ttgctgctgc acgaggtaca      360

tgccaacgac acaggcagct acgtctgcta ctacaagtac atcaaggcac gcatcgaggg      420
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caccacggcc gccagctcct acgtgttcgt gagagacttt gagcagccat tcatcaacaa 480

gcctgacacg ctcttggtca acaggaagga cgccatgtgg gtgccctgtc tggtgtccat 540

ccccggcctc aatgtcacgc tgcgctcgca aagctcggtg ctgtggccag acgggcagga 600

ggtggtgtgg gatgaccggc ggggcatgct cgtgtccacg ccactgctgc acgatgccct 660

gtacctgcag tgcgagacca cctggggaga ccaggacttc ctttccaacc ccttcctggt 720

gcacatcaca ggcaacgagc tctatgacat ccagctgttg cccaggaagt cgctggagct 780

gctggtaggg gagaagctgg tcctgaactg caccgtgtgg gctgagttta actcaggtgt 840

caccttttgac tgggactacc cagggaagca ggcagagcgg ggtaagtggg tgcccgagcg 900

acgctcccag cagacccaca cagaactctc cagcatcctg accatccaca acgtcagcca 960

gcacgacctg ggctcgtatg tgtgcaaggc caacaacggc atccagcgat ttcgggagag 1020

caccgaggtc attgtgcatg aaaatccctt catcagcgtc gagtggctca aaggacccat 1080

cctggaggcc acggcaggag acgagctggt gaagctgccc gtgaagctgg cagcgtaccc 1140

cccgcccgag ttccagtggt acaaggatgg aaaggcactg tccgggcgcc acagtccaca 1200

tgccctggtg ctcaaggagg tgacagaggc cagcacaggc acctacaccc tcgccctgtg 1260

gaactccgct gctggcctga ggcgcaacat cagcctggag ctggtggtga atgtgccccc 1320

ccagatacat gagaaggagg cctcctcccc cagcatctac tcgcgtcaca gccgccaggc 1380

cctcacctgc acggcctacg gggtgcccct gcctctcagc atccagtggc actggcggcc 1440

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catgccacag tgccgtgact ggagggcggt gaccacgcag gatgccgtga accccatcga 1560

gagcctggac acctggaccg agtttgtgga gggaaagaat aagactgtga gcaagctggt 1620

gatccagaat gccaacgtgt ctgccatgta caagtgtgtg gtctccaaca aggtgggcca 1680

ggatgagcgg ctcatctact tctatgtgac caccatcccc gacggcttca ccatcgaatc 1740

caagccatcc gaggagctac tagagggcca gccggtgctc ctgagctgcc aagccgacag 1800

ctacaagtac gagcatctgc gctggtaccg cctcaacctg tccacgctgc acgatgcgca 1860

cgggaacccg cttctgctcg actgcaagaa cgtgcatctg ttcgccaccc ctctggccgc 1920

cagcctggag gaggtggcac ctggggcgcg ccacgccacg ctcagcctga gtatcccccg 1980

cgtcgcgccc gagcacgagg gccactatgt gtgcgaagtg caagaccggc gcagccatga 2040

caagcactgc cacaagaagt acctgtcggt gcaggccctg gaagcccctc ggctcacgca 2100

gaacttgacc gacctcctgg tgaacgtgag cgactcgctg gagatgcagt gcttggtggc 2160

cggagcgcac gcgcccagca tcgtgtggta caaagacgag aggctgctgg aggaaaagtc 2220

tggagtcgac ttggcggact ccaaccagaa gctgagcatc cagcgcgtgc gcgaggagga 2280

tgcgggacgc tatctgtgca gcgtgtgcaa cgccaagggc tgcgtcaact cctccgccag 2340

cgtggccgtg gaaggctccg aggataaggg cagcatggag atcgtgatcc ttgtcggtac 2400

cggcgtcatc gctgtcttct tctgggtcct cctcctcctc atcttctgta acatgaggag 2460

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agagcggctg cacctgggga gagtgctcgg ctacggcgcc ttcgggaagg tggtggaagc 2640

ctccgctttc ggcatccaca agggcagcag ctgtgacacc gtggccgtga aaatgctgaa 2700

agagggcgcc acggccagcg agcaccgcgc gctgatgtcg gagctcaaga tcctcattca 2760

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catggtggag ctcgccaggc tggatcggag gcggccgggg agcagcgaca gggtcctctt 3000

cgcgcggttc tcgaagaccg agggcggagc gaggcgggct tctccagacc aagaagctga 3060

ggacctgtgg ctgagcccgc tgaccatgga agatcttgtc tgctacagct tccaggtggc 3120

cagagggatg gagttcctgg cttcccgaaa gtgcatccac agagacctgg ctgctcggaa 3180

cattctgctg tcggaaagcg acgtggtgaa gatctgtgac tttggccttg cccgggacat 3240

ctacaaagac cccgactacg tccgcaaggg cagtgcccgg ctgcccctga agtggatggc 3300

ccctgaaagc atcttcgaca aggtgtacac cacgcagagt gacgtgtggt cctttggggt 3360

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ggagttctgc cagcggctga gagacggcac aaggatgagg gccccggagc tggccactcc 3480

cgccatacgc cgcatcatgc tgaactgctg gtccggagac cccaaggcga gacctgcatt 3540

ctcggagctg gtggagatcc tgggggacct gctccagggc aggggcctgc aagaggaaga 3600

ggaggtctgc atggccccgc gcagctctca gagctcagaa gagggcagct tctcgcaggt 3660

gtccaccatg gccctacaca tcgcccaggc tgacgctgag gacagcccgc caagcctgca 3720

gcgccacagc ctggccgcca ggtattacaa ctgggtgtcc tttcccgggt gcctggccag 3780

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aacgacctac aaaggctctg tggacaacca gacagacagt gggatggtgc tggcctcgga 3900

ggagtttgag cagatagaga gcaggcatag acaagaaagc ggcttcagag gaaccaggag 3960

gacaagagga gcatgaaagt ggacaaggag tgtgaccact gaagcaccac agggaggggt 4020

taggcctccg gatgactgcg ggcaggcctg gataatatcc agcctcccac aagaagctgg 4080

tggagcagag tgttccctga ctcctccaag gaaagggaga cgccctttca tggtctgctg 4140

agtaacaggt gccttcccag acactggcgt tactgcttga ccaaagagcc ctcaagcggc 4200

ccttatgcca gcgtgacaga gggctcacct cttgccttct aggtcacttc tcacaatgtc 4260

ccttcagcac ctgaccctgt gcccaccagt tattccttgg taatatgagt aatacatcaa 4320

agagtagtat taaaagctaa ttaatcatgt ttatactaa 4359

<210> 41
<211> 1306
<212> PRT
<213> Homo sapiens

<400> 41
Met Gln Arg Gly Ala Ala Leu Cys Leu Arg Leu Trp Leu Cys Leu Gly
1 5 10 15

Leu Leu Asp Gly Leu Val Ser Gly Tyr Ser Met Thr Pro Pro Thr Leu
20 25 30

Asn Ile Thr Glu Glu Ser His Val Ile Asp Thr Gly Asp Ser Leu Ser
35 40 45

```
Ile Ser Cys Arg Gly Gln His Pro Leu Glu Trp Ala Trp Pro Gly Ala
    50                  55                  60

Gln Glu Ala Pro Ala Thr Gly Asp Lys Asp Ser Glu Asp Thr Gly Val
65                  70                  75                  80

Val Arg Asp Cys Glu Gly Thr Asp Ala Arg Pro Tyr Cys Lys Val Leu
                85                  90                  95

Leu Leu His Glu Val His Ala Asn Asp Thr Gly Ser Tyr Val Cys Tyr
            100                 105                 110

Tyr Lys Tyr Ile Lys Ala Arg Ile Glu Gly Thr Thr Ala Ala Ser Ser
        115                 120                 125

Tyr Val Phe Val Arg Asp Phe Glu Gln Pro Phe Ile Asn Lys Pro Asp
    130                 135                 140

Thr Leu Leu Val Asn Arg Lys Asp Ala Met Trp Val Pro Cys Leu Val
145                 150                 155                 160

Ser Ile Pro Gly Leu Asn Val Thr Leu Arg Ser Gln Ser Ser Val Leu
                165                 170                 175

Trp Pro Asp Gly Gln Glu Val Val Trp Asp Asp Arg Arg Gly Met Leu
            180                 185                 190

Val Ser Thr Pro Leu Leu His Asp Ala Leu Tyr Leu Gln Cys Glu Thr
        195                 200                 205

Thr Trp Gly Asp Gln Asp Phe Leu Ser Asn Pro Phe Leu Val His Ile
    210                 215                 220

Thr Gly Asn Glu Leu Tyr Asp Ile Gln Leu Leu Pro Arg Lys Ser Leu
225                 230                 235                 240
```

```
Glu Leu Leu Val Gly Glu Lys Leu Val Leu Asn Cys Thr Val Trp Ala
                245                 250                 255

Glu Phe Asn Ser Gly Val Thr Phe Asp Trp Asp Tyr Pro Gly Lys Gln
            260                 265                 270

Ala Glu Arg Gly Lys Trp Val Pro Glu Arg Arg Ser Gln Gln Thr His
        275                 280                 285

Thr Glu Leu Ser Ser Ile Leu Thr Ile His Asn Val Ser Gln His Asp
    290                 295                 300

Leu Gly Ser Tyr Val Cys Lys Ala Asn Asn Gly Ile Gln Arg Phe Arg
305                 310                 315                 320

Glu Ser Thr Glu Val Ile Val His Glu Asn Pro Phe Ile Ser Val Glu
                325                 330                 335

Trp Leu Lys Gly Pro Ile Leu Glu Ala Thr Ala Gly Asp Glu Leu Val
            340                 345                 350

Lys Leu Pro Val Lys Leu Ala Ala Tyr Pro Pro Pro Glu Phe Gln Trp
        355                 360                 365

Tyr Lys Asp Gly Lys Ala Leu Ser Gly Arg His Ser Pro His Ala Leu
    370                 375                 380

Val Leu Lys Glu Val Thr Glu Ala Ser Thr Gly Thr Tyr Thr Leu Ala
385                 390                 395                 400

Leu Trp Asn Ser Ala Ala Gly Leu Arg Arg Asn Ile Ser Leu Glu Leu
                405                 410                 415

Val Val Asn Val Pro Pro Gln Ile His Glu Lys Glu Ala Ser Ser Pro
            420                 425                 430

Ser Ile Tyr Ser Arg His Ser Arg Gln Ala Leu Thr Cys Thr Ala Tyr
        435                 440                 445
```

```
Gly Val Pro Leu Pro Leu Ser Ile Gln Trp His Trp Arg Pro Trp Thr
    450                 455                 460

Pro Cys Lys Met Phe Ala Gln Arg Ser Leu Arg Arg Arg Gln Gln Gln
465                 470                 475                 480

Asp Leu Met Pro Gln Cys Arg Asp Trp Arg Ala Val Thr Thr Gln Asp
                485                 490                 495

Ala Val Asn Pro Ile Glu Ser Leu Asp Thr Trp Thr Glu Phe Val Glu
            500                 505                 510

Gly Lys Asn Lys Thr Val Ser Lys Leu Val Ile Gln Asn Ala Asn Val
        515                 520                 525

Ser Ala Met Tyr Lys Cys Val Val Ser Asn Lys Val Gly Gln Asp Glu
    530                 535                 540

Arg Leu Ile Tyr Phe Tyr Val Thr Thr Ile Pro Asp Gly Phe Thr Ile
545                 550                 555                 560

Glu Ser Lys Pro Ser Glu Glu Leu Leu Glu Gly Gln Pro Val Leu Leu
                565                 570                 575

Ser Cys Gln Ala Asp Ser Tyr Lys Tyr Glu His Leu Arg Trp Tyr Arg
            580                 585                 590

Leu Asn Leu Ser Thr Leu His Asp Ala His Gly Asn Pro Leu Leu Leu
        595                 600                 605

Asp Cys Lys Asn Val His Leu Phe Ala Thr Pro Leu Ala Ala Ser Leu
    610                 615                 620

Glu Glu Val Ala Pro Gly Ala Arg His Ala Thr Leu Ser Leu Ser Ile
625                 630                 635                 640
```

```
Pro Arg Val Ala Pro Glu His Glu Gly His Tyr Val Cys Glu Val Gln
                645                 650                 655

Asp Arg Arg Ser His Asp Lys His Cys His Lys Lys Tyr Leu Ser Val
            660                 665                 670

Gln Ala Leu Glu Ala Pro Arg Leu Thr Gln Asn Leu Thr Asp Leu Leu
        675                 680                 685

Val Asn Val Ser Asp Ser Leu Glu Met Gln Cys Leu Val Ala Gly Ala
    690                 695                 700

His Ala Pro Ser Ile Val Trp Tyr Lys Asp Glu Arg Leu Leu Glu Glu
705                 710                 715                 720

Lys Ser Gly Val Asp Leu Ala Asp Ser Asn Gln Lys Leu Ser Ile Gln
                725                 730                 735

Arg Val Arg Glu Glu Asp Ala Gly Arg Tyr Leu Cys Ser Val Cys Asn
            740                 745                 750

Ala Lys Gly Cys Val Asn Ser Ser Ala Ser Val Ala Val Glu Gly Ser
        755                 760                 765

Glu Asp Lys Gly Ser Met Glu Ile Val Ile Leu Val Gly Thr Gly Val
    770                 775                 780

Ile Ala Val Phe Phe Trp Val Leu Leu Leu Leu Ile Phe Cys Asn Met
785                 790                 795                 800

Arg Arg Pro Ala His Ala Asp Ile Lys Thr Gly Tyr Leu Ser Ile Ile
                805                 810                 815

Met Asp Pro Gly Glu Val Pro Leu Glu Glu Gln Cys Glu Tyr Leu Ser
            820                 825                 830

Tyr Asp Ala Ser Gln Trp Glu Phe Pro Arg Glu Arg Leu His Leu Gly
        835                 840                 845
```

```
Arg Val Leu Gly Tyr Gly Ala Phe Gly Lys Val Val Glu Ala Ser Ala
    850                 855                 860

Phe Gly Ile His Lys Gly Ser Ser Cys Asp Thr Val Ala Val Lys Met
865                 870                 875                 880

Leu Lys Glu Gly Ala Thr Ala Ser Glu His Arg Ala Leu Met Ser Glu
                885                 890                 895

Leu Lys Ile Leu Ile His Ile Gly Asn His Leu Asn Val Val Asn Leu
            900                 905                 910

Leu Gly Ala Cys Thr Lys Pro Gln Gly Pro Leu Met Val Ile Val Glu
        915                 920                 925

Phe Cys Lys Tyr Gly Asn Leu Ser Asn Phe Leu Arg Ala Lys Arg Asp
    930                 935                 940

Ala Phe Ser Pro Cys Ala Glu Lys Ser Pro Glu Gln Arg Gly Arg Phe
945                 950                 955                 960

Arg Ala Met Val Glu Leu Ala Arg Leu Asp Arg Arg Arg Pro Gly Ser
                965                 970                 975

Ser Asp Arg Val Leu Phe Ala Arg Phe Ser Lys Thr Glu Gly Gly Ala
            980                 985                 990

Arg Arg Ala Ser Pro Asp Gln Glu  Ala Glu Asp Leu Trp  Leu Ser Pro
        995                 1000                1005

Leu Thr  Met Glu Asp Leu Val  Cys Tyr Ser Phe Gln  Val Ala Arg
    1010                1015                1020

Gly Met  Glu Phe Leu Ala Ser  Arg Lys Cys Ile His  Arg Asp Leu
    1025                1030                1035
```

```
Ala Ala  Arg Asn Ile Leu Leu  Ser Glu Ser Asp Val  Val Lys Ile
    1040                1045                1050

Cys Asp  Phe Gly Leu Ala Arg  Asp Ile Tyr Lys Asp  Pro Asp Tyr
    1055                1060                1065

Val Arg  Lys Gly Ser Ala Arg  Leu Pro Leu Lys Trp  Met Ala Pro
    1070                1075                1080

Glu Ser  Ile Phe Asp Lys Val  Tyr Thr Thr Gln Ser  Asp Val Trp
    1085                1090                1095

Ser Phe  Gly Val Leu Leu Trp  Glu Ile Phe Ser Leu  Gly Ala Ser
    1100                1105                1110

Pro Tyr  Pro Gly Val Gln Ile  Asn Glu Glu Phe Cys  Gln Arg Leu
    1115                1120                1125

Arg Asp  Gly Thr Arg Met Arg  Ala Pro Glu Leu Ala  Thr Pro Ala
    1130                1135                1140

Ile Arg  Arg Ile Met Leu Asn  Cys Trp Ser Gly Asp  Pro Lys Ala
    1145                1150                1155

Arg Pro  Ala Phe Ser Glu Leu  Val Glu Ile Leu Gly  Asp Leu Leu
    1160                1165                1170

Gln Gly  Arg Gly Leu Gln Glu  Glu Glu Glu Val Cys  Met Ala Pro
    1175                1180                1185

Arg Ser  Ser Gln Ser Ser Glu  Glu Gly Ser Phe Ser  Gln Val Ser
    1190                1195                1200

Thr Met  Ala Leu His Ile Ala  Gln Ala Asp Ala Glu  Asp Ser Pro
    1205                1210                1215

Pro Ser  Leu Gln Arg His Ser  Leu Ala Ala Arg Tyr  Tyr Asn Trp
    1220                1225                1230
```

Val Ser Phe Pro Gly Cys Leu Ala Arg Gly Ala Glu Thr Arg Gly
    1235   1240   1245

Ser Ser Arg Met Lys Thr Phe Glu Glu Phe Pro Met Thr Pro Thr
    1250   1255   1260

Thr Tyr Lys Gly Ser Val Asp Asn Gln Thr Asp Ser Gly Met Val
    1265   1270   1275

Leu Ala Ser Glu Glu Phe Glu Gln Ile Glu Ser Arg His Arg Gln
    1280   1285   1290

Glu Ser Gly Phe Arg Gly Thr Arg Arg Thr Arg Gly Ala
    1295   1300   1305

<210> 42
<211> 1353
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      polynucleotide"

<400> 42
atgtaccgga tgcagctgct gagctgtatc gccctgtctc tggccctggt caccaattct 60

agcgataccg gcagaccctt cgtggaaatg tacagcgaga tccccgagat catccacatg 120

accgagggca gagagctggt catcccctgc agagtgacaa gccccaacat caccgtgact 180

ctgaagaagt tccctctgga cacactgatc cccgacggca agagaatcat ctgggacagc 240

cggaagggct tcatcatcag caacgccacc tacaaagaga tcggcctgct gacctgtgaa 300

gccaccgtga atggccacct gtacaagacc aactacctga cacacagaca gaccaacacc 360

atcatcgacg tggtgctgag ccctagccac ggcattgaac tgtctgtggg cgagaagctg 420

gtgctgaact gtaccgccag aaccgagctg aacgtgggca tcgacttcaa ctgggagtac 480

cccagcagca agcaccagca caagaaactg gtcaaccggg acctgaaaac ccagagcggc 540

agcgagatga agaaattcct gagcaccctg accatcgacg gcgtgaccag atctgaccag 600

ggcctgtaca catgtgccgc cagctctggc ctgatgacca agaaaaacag caccttcgtg 660

cgggtgcacg agaaggacaa gacccacacc tgtcctccat gtcctgctcc agaactgctc 720

ggcggacctt ccgtgttcct gtttcctcca aagcctaagg acaccctgat gatcagcaga 780

acccctgaag tgacctgcgt ggtggtggat gtgtcccacg aggatcccga agtgaagttc 840

aattggtacg tggacggcgt ggaagtgcac aacgccaaga ccaagcctag agaggaacag 900

tacaatagca cctacagagt ggtgtccgtg ctgaccgtgc tgcaccagga ttggctgaac 960

ggcaaagagt acaagtgcaa ggtgtccaac aaggccctgc ctgctcctat cgagaaaacc 1020

atctccaagg ccaagggcca gcctagggaa ccccaggttt acacactgcc tccaagcagg 1080

gacgagctga caaagaacca ggtgtccctg acctgcctgg tcaagggctt ctacccttcc 1140

gatatcgccg tggaatggga gagcaatggc cagcctgaga acaactacaa gacaacccct 1200

cctgtgctgg acagcgacgg ctcattcttc ctgtacagca agctgacagt ggacaagagc 1260

agatggcagc agggcaacgt gttcagctgc agcgtgatgc acgaggccct gcacaaccac 1320

tacacccaga agtccctgag cctgtctcct gga 1353

<210> 43
<211> 451
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
polypeptide"

<400> 43
Met Tyr Arg Met Gln Leu Leu Ser Cys Ile Ala Leu Ser Leu Ala Leu
1 5 10 15

Val Thr Asn Ser Ser Asp Thr Gly Arg Pro Phe Val Glu Met Tyr Ser
20 25 30

Glu Ile Pro Glu Ile Ile His Met Thr Glu Gly Arg Glu Leu Val Ile

```
        35                  40                  45

Pro Cys Arg Val Thr Ser Pro Asn Ile Thr Val Thr Leu Lys Lys Phe
    50                  55                  60

Pro Leu Asp Thr Leu Ile Pro Asp Gly Lys Arg Ile Ile Trp Asp Ser
65                  70                  75                  80

Arg Lys Gly Phe Ile Ile Ser Asn Ala Thr Tyr Lys Glu Ile Gly Leu
                85                  90                  95

Leu Thr Cys Glu Ala Thr Val Asn Gly His Leu Tyr Lys Thr Asn Tyr
            100                 105                 110

Leu Thr His Arg Gln Thr Asn Thr Ile Ile Asp Val Val Leu Ser Pro
        115                 120                 125

Ser His Gly Ile Glu Leu Ser Val Gly Glu Lys Leu Val Leu Asn Cys
    130                 135                 140

Thr Ala Arg Thr Glu Leu Asn Val Gly Ile Asp Phe Asn Trp Glu Tyr
145                 150                 155                 160

Pro Ser Ser Lys His Gln His Lys Lys Leu Val Asn Arg Asp Leu Lys
                165                 170                 175

Thr Gln Ser Gly Ser Glu Met Lys Lys Phe Leu Ser Thr Leu Thr Ile
            180                 185                 190

Asp Gly Val Thr Arg Ser Asp Gln Gly Leu Tyr Thr Cys Ala Ala Ser
        195                 200                 205

Ser Gly Leu Met Thr Lys Lys Asn Ser Thr Phe Val Arg Val His Glu
    210                 215                 220

Lys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
225                 230                 235                 240
```

```
Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
                245                 250                 255

Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
            260                 265                 270

His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
        275                 280                 285

Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
    290                 295                 300

Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
305                 310                 315                 320

Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
                325                 330                 335

Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
            340                 345                 350

Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val
        355                 360                 365

Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
    370                 375                 380

Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
385                 390                 395                 400

Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
                405                 410                 415

Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
            420                 425                 430

Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
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435 440 445

Ser Pro Gly
450

<210> 44
<211> 431
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic polypeptide"

<400> 44
Ser Asp Thr Gly Arg Pro Phe Val Glu Met Tyr Ser Glu Ile Pro Glu
1 5 10 15

Ile Ile His Met Thr Glu Gly Arg Glu Leu Val Ile Pro Cys Arg Val
20 25 30

Thr Ser Pro Asn Ile Thr Val Thr Leu Lys Lys Phe Pro Leu Asp Thr
35 40 45

Leu Ile Pro Asp Gly Lys Arg Ile Ile Trp Asp Ser Arg Lys Gly Phe
50 55 60

Ile Ile Ser Asn Ala Thr Tyr Lys Glu Ile Gly Leu Leu Thr Cys Glu
65 70 75 80

Ala Thr Val Asn Gly His Leu Tyr Lys Thr Asn Tyr Leu Thr His Arg
85 90 95

Gln Thr Asn Thr Ile Ile Asp Val Val Leu Ser Pro Ser His Gly Ile
100 105 110

Glu Leu Ser Val Gly Glu Lys Leu Val Leu Asn Cys Thr Ala Arg Thr
115 120 125

```
Glu Leu Asn Val Gly Ile Asp Phe Asn Trp Glu Tyr Pro Ser Ser Lys
    130                 135                 140

His Gln His Lys Lys Leu Val Asn Arg Asp Leu Lys Thr Gln Ser Gly
145                 150                 155                 160

Ser Glu Met Lys Lys Phe Leu Ser Thr Leu Thr Ile Asp Gly Val Thr
                165                 170                 175

Arg Ser Asp Gln Gly Leu Tyr Thr Cys Ala Ala Ser Ser Gly Leu Met
            180                 185                 190

Thr Lys Lys Asn Ser Thr Phe Val Arg Val His Glu Lys Asp Lys Thr
        195                 200                 205

His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser
    210                 215                 220

Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg
225                 230                 235                 240

Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro
                245                 250                 255

Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala
            260                 265                 270

Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val
        275                 280                 285

Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr
    290                 295                 300

Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr
305                 310                 315                 320

Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu
                325                 330                 335
```

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Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys
            340                 345                 350

Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser
        355                 360                 365

Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp
    370                 375                 380

Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser
385                 390                 395                 400

Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala
                405                 410                 415

Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
            420                 425                 430
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<210> 45
<211> 145
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic polynucleotide"

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cgtcgggcga cctttggtcg cccggcctca gtgagcgagc gagcgcgcag agagggagtg 120

gccaactcca tcactagggg ttcct 145

<210> 46
<211> 145
<212> DNA
<213> Artificial Sequence

<220>

<221> source
<223> /note="Description of Artificial Sequence: Synthetic
polynucleotide"

<400> 46
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ccgggcgacc aaaggtcgcc cgacgcccgg gctttgcccg ggcggcctca gtgagcgagc 120

gagcgcgcag agagggagtg gccaa 145

<210> 47
<211> 119
<212> DNA
<213> Artificial Sequence

<220>
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<223> /note="Description of Artificial Sequence: Synthetic
polynucleotide"

<400> 47
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ctcagtgagc gagcgagcgc gcagagaggg agtggccaac tccatcacta ggggttcct 119

<210> 48
<211> 130
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
polynucleotide"

<400> 48
aggaacccct agtgatggag ttggccactc cctctctgcg cgctcgctcg ctcactgagg 60

ccgggcgacc aaaggtcgcc cgacgcccgg gctttgcccg ggcggcctca gtgagcgagc 120

gagcgcgcag 130

<210> 49
<211> 279
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
polynucleotide"

<400> 49
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gggcgcgcgc caggcggggc ggggcggggc gaggggcggg gcggggcgag gcggagaggt 180

gcggcggcag ccaatcagag cggcgcgctc cgaaagtttc cttttatggc gaggcggcgg 240

cggcggcggc cctataaaaa gcgaagcgcg cggcgggcg 279

<210> 50
<211> 277
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
polynucleotide"

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gcgcgcgcca ggcggggcgg ggcggggcga ggggcggggc ggggcgaggc ggagaggtgc 180

ggcggcagcc aatcagagcg gcgcgctccg aaagtttcct tttatggcga ggcggcggcg 240

gcggcggccc tataaaaagc gaagcgcgcg gcgggcg 277

<210> 51
<211> 660
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
polynucleotide"

<400> 51
gacattgatt attgactagt tattaatagt aatcaattac ggggtcatta gttcatagcc 60

catatatgga gttccgcgtt acataactta cggtaaatgg cccgcctggc tgaccgccca 120

acgacccccg cccattgacg tcaataatga cgtatgttcc catagtaacg ccaatagga 180

ctttccattg acgtcaatgg gtggactatt tacggtaaac tgcccacttg gcagtacatc 240

aagtgtatca tatgccaagt acgccccta ttgacgtcaa tgacggtaaa tggcccgcct 300

ggcattatgc ccagtacatg accttatggg actttcctac ttggcagtac atctacgtat 360

tagtcatcgc tattaccatg ggtcgaggtg agccccacgt tctgcttcac tctccccatc 420

tcccccccct ccccaccccc aattttgtat ttatttattt tttaattatt ttgtgcagcg 480

atgggggcgg gggggggggg ggggcgcgcg ccaggcgggg cggggcgggg cgaggggcgg 540

ggcggggcga ggcggagagg tgcggcggca gccaatcaga gcggcgcgct ccgaaagttt 600

ccttttatgg cgaggcggcg gcggcggcgg ccctataaaa agcgaagcgc gcggcgggcg 660

<210> 52
<211> 658
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
polynucleotide"

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catatatgga gttccgcgtt acataactta cggtaaatgg cccgcctggc tgaccgccca 120

acgacccccg cccattgacg tcaataatga cgtatgttcc catagtaacg ccaatagga 180

ctttccattg acgtcaatgg gtggactatt tacggtaaac tgcccacttg gcagtacatc 240

aagtgtatca tatgccaagt acgccccta ttgacgtcaa tgacggtaaa tggcccgcct 300

ggcattatgc ccagtacatg accttatggg actttcctac ttggcagtac atctacgtat 360

tagtcatcgc tattaccatg ggtcgaggtg agccccacgt tctgcttcac tctccccatc 420

tcccccccct ccccaccccc aattttgtat ttatttattt tttaattatt ttgtgcagcg 480

atgggggcgg gggggggggg ggcgcgcgcc aggcggggcg gggcggggcg aggggcgggg 540

cggggcgagg cggagaggtg cggcggcagc caatcagagc ggcgcgctcc gaaagtttcc 600

ttttatggcg aggcggcggc ggcggcggcc ctataaaaag cgaagcgcgc ggcgggcg 658

<210> 53
<211> 1673
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
polynucleotide"

<400> 53
gacattgatt attgactagt tattaatagt aatcaattac ggggtcatta gttcatagcc 60

catatatgga gttccgcgtt acataactta cggtaaatgg cccgcctggc tgaccgccca 120

acgacccccg cccattgacg tcaataatga cgtatgttcc catagtaacg ccaatagggа 180

ctttccattg acgtcaatgg gtggactatt tacggtaaac tgcccacttg gcagtacatc 240

aagtgtatca tatgccaagt acgccccctа ttgacgtcaa tgacggtaaa tggcccgcct 300

ggcattatgc ccagtacatg accttatggg actttcctac ttggcagtac atctacgtat 360

tagtcatcgc tattaccatg ggtcgaggtg agccccacgt tctgcttcac tctccccatc 420

tccccccccc ccccacccсc aattttgtat ttatttattt tttaattatt ttgtgcagcg 480

atgggggcgg gggggggggg ggggcgcgcg ccaggcgggg cggggcgggg cgaggggcgg 540

ggcggggcga ggcggagagg tgcggcggca gccaatcaga gcggcgcgct ccgaaagttt 600

ccttttatgg cgaggcggcg gcggcggcgg ccctataaaa agcgaagcgc gcggcgggcg 660

ggagtcgctg cgttgccttc gccccgtgcc ccgctccgcg ccgcctcgcg ccgcccgccc 720

cggctctgac tgaccgcgtt actcccacag gtgagcgggc gggacggccc ttctcctccg 780

ggctgtaatt agcgcttggt ttaatgacgg ctcgtttctt ttctgtggct gcgtgaaagc 840

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gtgtgtgtgc gtggggagcg ccgcgtgcgg cccgcgctgc ccggcggctg tgagcgctgc 960

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cggggctcgc cgtgccgggc gggggtggc ggcaggtggg ggtgccgggc ggggcggggc 1260

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ttcgtgcgtc gccgcgccgc cgtccccttc tccctctcca gcctcggggc tgtccgcggg 1560

gggacggctg ccttcggggg ggacggggca gggcggggtt cggcttctgg cgtgtgaccg 1620

gcggctctag agcctctgct aaccatgttc atgccttctt ctttttccta cag 1673

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<211> 1671
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<213> Artificial Sequence

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<221> source
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catatatgga gttccgcgtt acataactta cggtaaatgg cccgcctggc tgaccgccca 120

acgacccccg cccattgacg tcaataatga cgtatgttcc catagtaacg ccaataggga 180

ctttccattg acgtcaatgg gtggactatt tacggtaaac tgcccacttg gcagtacatc 240

aagtgtatca tatgccaagt acgccccta ttgacgtcaa tgacggtaaa tggcccgcct 300

ggcattatgc ccagtacatg accttatggg actttcctac ttggcagtac atctacgtat 360

tagtcatcgc tattaccatg ggtcgaggtg agccccacgt tctgcttcac tctccccatc 420

tccccccccc ccccaccccc aattttgtat ttatttattt tttaattatt ttgtgcagcg 480

atgggggcgg gggggggggg ggcgcgcgcc aggcggggcg gggcggggcg aggggcgggg 540

cggggcgagg cggagaggtg cggcggcagc caatcagagc ggcgcgctcc gaaagtttcc 600

ttttatggcg aggcggcggc ggcggcggcc ctataaaaag cgaagcgcgc ggcgggcggg 660

agtcgctgcg ttgccttcgc cccgtgcccc gctccgcgcc gcctcgcgcc gcccgccccg 720

gctctgactg accgcgttac tcccacaggt gagcgggcgg gacggccctt ctcctccggg 780

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gacggctgcc ttcgggggggg acggggcagg gcggggttcg gcttctggcg tgtgaccggc 1620

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<210> 55
<211> 1725
<212> DNA
<213> Homo sapiens

<400> 55

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tatttctgag tgctcattcc ccatatgcca gccacttctg ccatgctgac tggttccttt 180

ctctccatta ttagcaatta gcttcttacc ttccaaagtc agatccaagg tatccaagat 240

actagcaaag gaatcaacta tgtgtgcaag ttaagcatgc ttaatatcac ccaaacaaac 300

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cctcccaccc caccagcact tttatttgtg gagggttttg gctcgccaca ctttgggaaa 480

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acgatttcta tgggacattg ctaactgggg cccctaagga gaaaggggaa actgagcgga 600

gaatgggtta aatccttgga agcaggggag aggcagggga ggagagaagt cggaggagta 660

taaagaaaag gacaggaacc aagaagcgtg ggggtggttt gccgtaatgt gagtgtttct 720

taattagaga acggttgaca atagagggtc tggcagaggc tcctggccgc ggtgcggagc 780

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gccgccactg cccactgcac ctcccagcaa ccagcccagc acgcaaagaa gctgcgcaaa 1080

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cattggctga gaagacacgc gaccggcgcg aggagggggt tgggagagga gcgggggggag 1500

actgagtggc gcgtgccgct ttttaaaggg gcgcagcgcc ttcagcaacc ggagaagcat 1560

agttgcacgc gacctggtgt gtgatctccg agtgggtggg ggagggtcga ggagggaaaa 1620

aaaaataaga cgttgcagaa gagacccgga aagggccttt tttttggttg agctggtgtc 1680

ccagtgctgc ctccgatcct gagcctccga gcctttgcag tgcaa 1725

<210> 56
<211> 60
<212> DNA
<213> Homo sapiens

<400> 56
ctgccttctg agagcgctat aaaggcagcg gaagggtagt ccgcggggca ttccgggcgg 60

<210> 57
<211> 60
<212> DNA
<213> Homo sapiens

<400> 57
ctctaggcgg gctctgctct tctttaagga gtcccacagg gcctggcccg cccctgacct 60

<210> 58
<211> 2000
<212> DNA
<213> Homo sapiens

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atatccacag catgctctta atggagaaaa acaaagcctt agatcaaata tgtaaagtaa 180

tttttagttt tttgaaaagg tatgtttggg ctatagataa atctgttcaa aaaacatgag 240

agaagataat aatggttgaa aggagacaca gtgcttgccc tcaagaagtt tttgtctagt 300

gagggagaga gaacttgtat gtaaataaaa ttgtgttact aaggtagata gtgagaagta 360

acttaagaga ggatcagata aggtattaag agaatacaga aaagggtctg gattaattct 420

gaacagcatc aaagaatgtt cttgcaagag atagtgtttt caccagatct tgaaggtatg 480

gatgagggta tacagagtga gtatattcag attctacttt aaaacaaata ctttcctctg 540

ttgtagtgga gttgagctat acatccaaca ataatgaaaa aatacacgca tatatacata 600

tatggagaga gatacatatt ttagtacatg tagcaattga ttaataaatg tacagtttaa 660

gtcgcatgca aaaccttgga gtgatagcaa acttcattgt aggatgttta gcagcatctc 720

tggtctctac tcactagatc ccaatagcat ctccctaggt gtgacaacca aaaatgtctc 780

caggcattga cctctggagg caaaaaaagc cctttattaa gaaccagtgg tatacataag 840

taaaacatac acaagagatt cctcccctct tctctgtatg tgaataaaaa ttgcaaagtt 900

catgacctgg attttccttt taggtttctt ctttagtggt tcttaacttc attgggtgaa 960

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<211> 1400
<212> DNA
<213> Homo sapiens

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gagcgcgccc tgacggttcc acgcctggcc cgggggtctg cacctctcct ccagtgcgca 180

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tctaaaacct ttcattatag 1400

<210> 60
<211> 900
<212> DNA
<213> Homo sapiens

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ctaacaatca ggcgagaatg ttctggttta tcaaaccctt ccttctgcct ttcatcttaa 120

ccatgcatta gttttaacaa agttcatccc aacagaagac aaaacactga tgaggtagga 180

tagctccagc tcctcctccc tctcttctag tcttgatttc catgtagtcc agtttattcc 240

ttccctgatt gtccaggaga atgagaaaaa gaaaaaacag agtctagtgg gtaagaaagg 300

gccacctgga cggcttgatt tggattgtga aataaaacac acacacatgc acacgtagaa 360

taagtggcta aaatctgagt aaatcgtgaa ctctctgtat cctccaccca ttgaatactc 420

ctaaaagact ttctagaaat tcaaggactt attaatatag aaacctggcc attgttcctc 480

ttctcctccc catgtggtat gagagcacct gtggcaggct cccagagacc acggacctct 540

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gcagcggaag ggtagtccgc ggggcattcc gggcggggcg cgagcagaga caggtgagtt 900

<210> 61
<211> 1269
<212> DNA
<213> Homo sapiens

<400> 61

agggctattt gtacctcaac gagggcttct ctccaagaaa gccctgaatc cttttcctcc 60

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gcgctctgca gcggttctca gagcccagtg tcactcacat aggtgggact gctctcagtt 180

cagagagcgc tgggacactt aagatgaaaa gtccctggaa gttagcaaac agccatctgt 240

cactctggca tcgatttact aaaagtgact tctagggtat tctaaaccac ttttaaaaaa 300

caaatgagtc acttcgactt cctcaccccg caagagatag gaaggcagca gtggagtgct 360

cgctcaggag ctgtatttgt ttagcgatta gcctagagct ttgattttag ggcaaaagcg 420

agccagacag tgcggcagac gtaaggatca aaaaggccac ctatcattcg ccggggacgc 480

ctgcctcctt accctgataa cgtaactatt tctctgcata ggattttagt ttttgtgttt 540

ttgttttgtt ttattctgtt taatcacttc aagtatctca tccattattt gaagcgggct 600

cggaggaaac gtgccgcatc ctccagtcct tgtgcgtctg tttaggtctc tccgaagcag 660

gtccctctcg actcttagat ctgggtctcc agcacgcatg aaggggtaag ggtgggggggg 720

tcccctattc cggcgcgcgg cgttgagcac tgaatcttcc aggcggaggc tcagtgggag 780

cgccgagaac tcgccagtac cgcgcgctgc ctgctgcctg ctgcctccca gcccaggact 840

tgggaaagga gggaggggac aagtggaggg aaagtggggc cgggcggggg gtgcctggga 900

agccaggctg cgctgacgtc actgggcgcg caattcgggc tggagcgctt taaaaaacga 960

gcgtgcaagc agagatgctg ctccacaccg ctcaggccgc gagcagcagc aaggcgcacc 1020

gccactgtcg ccgctgcagc cagggctgct ccgaaggccg gcgtggcggc aaccggcacc 1080

tctgtccccg ccgcgcttct cctcgccgcc cacgccgtgg ggtcaggaac gcggcgtctg 1140

gcgctgcaga cgcccgctga gttgcagaag cccacggagc ggcgcccggc gcgccacggc 1200

ccgtagcagt ccggtgctgc tctccgcccg cgtccggctc gtggcccccc acttcgggca 1260

ccgaccggt 1269

<210> 62
<211> 463
<212> DNA
<213> Homo sapiens

<400> 62
tgcgtatgag tgcaagtggg ttttaggacc aggatgaggc ggggtggggg tgcctacctg 60

acgaccgacc ccgacccact ggacaagcac ccaaccccca ttccccaaat tgcgcatccc 120

ctatcagaga gggggagggg aaacaggatg cggcgaggcg cgtgcgcact gccagcttca 180

gcaccgcgga cagtgccttc gcccccgcct ggcggcgcgc gccaccgccg cctcagcact 240

gaaggcgcgc tgacgtcact cgccggtccc ccgcaaactc cccttcccgg ccaccttggt 300

cgcgtccgcg ccgccgccgg cccagccgga ccgcaccacg cgaggcgcga gatagggggg 360

cacgggcgcg accatctgcg ctgcggcgcc ggcgactcag cgctgcctca gtctgcggtg 420

ggcagcggag gagtcgtgtc gtgcctgaga gcgcagtcga gaa 463

<210> 63
<211> 2204
<212> DNA
<213> Homo sapiens

<400> 63
cccacctccc tctctgtgct gggactcaca gagggagacc tcaggaggca gtctgtccat 60

cacatgtcca aatgcagagc ataccctggg ctgggcgcag tggcgcacaa ctgtaattcc 120

agcactttgg gaggctgatg tggaaggatc acttgagccc agaagttcta gaccagcctg 180

ggcaacatgg caagacccta tctctacaaa aaaagttaaa aaatcagcca cgtgtggtga 240

cacacacctg tagtcccagc tattcaggag gctgaggtga ggggatcact taaggctggg 300

aggttgaggc tgcagtgagt cgtggttgcg ccactgcact ccagcctggg caacagtgag 360

accctgtctc aaaagacaaa aaaaaaaaaa aaaaaaaaaa gaacatatcc tggtgtggag 420

taggggacgc tgctctgaca gaggctcggg ggcctgagct ggctctgtga gctggggagg 480

aggcagacag ccaggccttg tctgcaagca gacctggcag cattgggctg gccgcccccc 540

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attgagcagg gggcttgcat tgcaccccag cctgacagcc tggcatcttg ggataaaagc 720

agcacagccc cctaggggct gcccttgctg tgtggcgcca ccggcggtgg agaacaaggc 780

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gcaggggggg agaggagggc tgtctgcttc ccagaagtcc aaggacacaa atgggtgagg 900

ggactgggca gggttctgac cctgtgggac cagagtggag ggcgtagatg gacctgaagt 960

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gaggaaataa atctccagtg ggagacggag gggtggccag ggaaacgggg cgctgcagga 1140

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gtgttcaagg gaaggactcc aggagtctgc tgggaatgag gcctagtagg aaatgaggtg 1500

gcccttgagg gtacagaaca ggttcattct tcgccaaatt cccagcacct tgcaggcact 1560

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tcaagcaatc ctccagcctc agcctcccaa agtgctggga ttacaagcat gagccacccc 1800

actcagccct ttccttcctt tttaattgat gcataataat tgtaagtatt catcatggtc 1860

caaccaaccc tttcttgacc caccttccta gagagagggt cctcttgctt cagcggtcag 1920

ggccccagac ccatggtctg gctccaggta ccacctgcct catgcaggag ttggcgtgcc 1980

caggaagctc tgcctctggg cacagtgacc tcagtggggt gaggggagct ctccccatag 2040

ctgggctgcg gcccaacccc acccccctcag gctatgccag ggggtgttgc caggggcacc 2100

cgggcatcgc cagtctagcc cactccttca taaagccctc gcatcccagg agcgagcaga 2160

gccagagcag gttggagagg agacgcatca cctccgctgc tcgc 2204

<210> 64

<211> 381
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
polynucleotide"

<400> 64
gacattgatt attgactagt tattaatagt aatcaattac ggggtcatta gttcatagcc 60

catatatgga gttccgcgtt acataactta cggtaaatgg cccgcctggc tgaccgccca 120

acgacccccg cccattgacg tcaataatga cgtatgttcc catagtaacg ccaatagggа 180

ctttccattg acgtcaatgg gtggactatt tacggtaaac tgcccacttg gcagtacatc 240

aagtgtatca tatgccaagt acgccccctа ttgacgtcaa tgacggtaaa tggcccgcct 300

ggcattatgc ccagtacatg accttatggg actttcctac ttggcagtac atctacgtat 360

tagtcatcgc tattaccatg g 381

<210> 65
<211> 1013
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
polynucleotide"

<400> 65
ggagtcgctg cgttgccttc gccccgtgcc ccgctccgcg ccgcctcgcg ccgcccgccc 60

cggctctgac tgaccgcgtt actcccacag gtgagcgggc gggacggccc ttctcctccg 120

ggctgtaatt agcgcttggt ttaatgacgg ctcgtttctt ttctgtggct gcgtgaaagc 180

cttaaagggc tccgggaggg ccctttgtgc gggggggagc ggctcggggg gtgcgtgcgt 240

gtgtgtgtgc gtggggagcg ccgcgtgcgg cccgcgctgc ccggcggctg tgagcgctgc 300

gggcgcggcg cggggctttg tgcgctccgc gtgtgcgcga ggggagcgcg gccgggggcg 360

gtgccccgcg gtgcgggggg gctgcgaggg gaacaaaggc tgcgtgcggg gtgtgtgcgt 420

ggggggtga gcaggggtg tgggcgcggc ggtcgggctg taacccccc ctgcaccccc 480

ctccccgagt tgctgagcac ggcccggctt cgggtgcggg gctccgtgcg gggcgtggcg 540

cggggctcgc cgtgccgggc gggggtggc ggcaggtggg ggtgccgggc ggggcggggc 600

cgcctcgggc cggggagggc tcgggggagg ggcgcggcgg cccccggagc gccggcggct 660

gtcgaggcgc ggcgagccgc agccattgcc ttttatggta atcgtgcgag agggcgcagg 720

gacttccttt gtcccaaatc tgtgcggagc cgaaatctgg gaggcgccgc cgcacccccc 780

ctagcgggcg cggggcgaag cggtgcggcg ccggcaggaa ggaaatgggc ggggagggcc 840

ttcgtgcgtc gccgcgccgc cgtccccttc tccctctcca gcctcggggc tgtccgcggg 900

gggacggctg ccttcggggg ggacggggca gggcggggtt cggcttctgg cgtgtgaccg 960

gcggctctag agcctctgct aaccatgttc atgccttctt ctttttccta cag 1013

<210> 66
<211> 60
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 66
atgtaccgga tgcagctgct gagctgtatc gccctgtctc tggccctggt caccaattct 60

<210> 67
<211> 60
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 67
atgtatagaa tgcagctcct gtcctgcatt gccctgagcc tggctctcgt gaccaacagc 60

<210> 68

<211> 20
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
peptide"

<400> 68
Met Tyr Arg Met Gln Leu Leu Ser Cys Ile Ala Leu Ser Leu Ala Leu
1 5 10 15

Val Thr Asn Ser
20

<210> 69
<211> 63
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 69
ggctctggcg aaggcagagg cagcctgctt acatgtggcg acgtggaaga gaaccccgga 60

cct 63

<210> 70
<211> 21
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
peptide"

<400> 70
Gly Ser Gly Glu Gly Arg Gly Ser Leu Leu Thr Cys Gly Asp Val Glu
1 5 10 15

Glu Asn Pro Gly Pro

20

<210> 71
<211> 33
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 71
gacaagaccc acaccggcaa gcggaagaga aga 33

<210> 72
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
peptide"

<400> 72
Asp Lys Thr His Thr Gly Lys Arg Lys Arg Arg
1 5 10

<210> 73
<211> 82
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 73
gtaagtatca aggttacaag acaggtttaa ggagaccaat agaaactggg cttgtcgaga 60

cagagaagac tcttgcgttt ct 82

<210> 74
<211> 51

<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 74
gataggcacc tattggtctt actgacatcc actttgcctt tctctccaca g 51

<210> 75
<211> 225
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
polynucleotide"

<400> 75
ctgtgccttc tagttgccag ccatctgttg tttgcccctc ccccgtgcct tccttgaccc 60

tggaaggtgc cactcccact gtcctttcct aataaaatga ggaaattgca tcgcattgtc 120

tgagtaggtg tcattctatt ctgggggggtg gggtggggca ggacagcaag ggggaggatt 180

gggaagacaa tagcaggcat gctggggatg cggtgggctc tatgg 225

<210> 76
<211> 122
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
polynucleotide"

<400> 76
aacttgttta ttgcagctta taatggttac aaataaagca atagcatcac aaatttcaca 60

aataaagcat ttttttcact gcattctagt tgtggtttgt ccaaactcat caatgtatct 120

ta 122

<210> 77
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 77
ttgtcgacgc ggccgcacgc gt 22

<210> 78
<211> 39
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 78
ctcctgggca acgtgctggt tattgtgacc ggtgccacc 39

<210> 79
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 79
taagagctcg ctgatcagcc tcga 24

<210> 80
<211> 38
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic

oligonucleotide"

<400> 80
aagcttgaat tcagctgacg tgcctcggac cgcctagg 38

<210> 81
<211> 14
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 81
gcggccgcac gcgt 14

<210> 82
<211> 34
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 82
aagcttgaat tcagctgacg tgcctcggac cgct 34

<210> 83
<211> 159
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic polypeptide"

<400> 83
Met Ile Ser Leu Ile Ala Ala Leu Ala Val Asp Tyr Val Ile Gly Met
1 5 10 15

Glu Asn Ala Met Pro Trp Asn Leu Pro Ala Asp Leu Ala Trp Phe Lys

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            20                  25                  30

Arg Asn Thr Leu Asn Lys Pro Val Ile Met Gly Arg His Thr Trp Glu
        35                  40                  45

Ser Ile Gly Arg Pro Leu Pro Gly Arg Lys Asn Ile Ile Leu Ser Ser
    50                  55                  60

Gln Pro Ser Thr Asp Asp Arg Val Thr Trp Val Lys Ser Val Asp Glu
65                  70                  75                  80

Ala Ile Ala Ala Cys Gly Asp Val Pro Glu Ile Met Val Ile Gly Gly
                85                  90                  95

Gly Arg Val Ile Glu Gln Phe Leu Pro Lys Ala Gln Lys Leu Tyr Leu
            100                 105                 110

Thr His Ile Asp Ala Glu Val Glu Gly Asp Thr His Phe Pro Asp Tyr
        115                 120                 125

Glu Pro Asp Asp Trp Glu Ser Val Phe Ser Glu Phe His Asp Ala Asp
    130                 135                 140

Ala Gln Asn Ser His Ser Tyr Cys Phe Glu Ile Leu Glu Arg Arg
145                 150                 155


<210> 84
<211> 483
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      polynucleotide"

<400> 84
ggtaccatca gtctgattgc ggcgttagcg gtagattacg ttatcggcat ggaaaacgcc       60

atgccgtgga acctgcctgc cgatctcgcc tggtttaaac gcaacacctt aaataaaccc      120
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gtgattatgg gccgccatac ctgggaatca atcggtcgtc cgttgccagg acgcaaaaat 180

attatcctca gcagtcaacc gagtacggac gatcgcgtaa cgtgggtgaa gtcggtggat 240

gaagccatcg cggcgtgtgg tgacgtacca gaaatcatgg tgattggcgg cggtcgcgtt 300

attgaacagt tcttgccaaa agcgcaaaaa ctgtatctga cgcatatcga cgcagaagtg 360

gaaggcgaca cccatttccc ggattacgag ccggatgact gggaatcggt attcagcgaa 420

ttccacgatg ctgatgcgca gaactctcac agctattgct ttgagattct ggagcggcga 480

taa 483

<210> 85
<211> 474
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
polynucleotide"

<400> 85
atcagtctga ttgcggcgtt agcggtagat tacgttatcg gcatggaaaa cgccatgccg 60

tggaacctgc ctgccgatct cgcctggttt aaacgcaaca ccttaaataa acccgtgatt 120

atgggccgcc atacctggga atcaatcggt cgtccgttgc caggacgcaa aaatattatc 180

ctcagcagtc aaccgagtac ggacgatcgc gtaacgtggg tgaagtcggt ggatgaagcc 240

atcgcggcgt gtggtgacgt accagaaatc atggtgattg gcggcggtcg cgttattgaa 300

cagttcttgc caaaagcgca aaaactgtat ctgacgcata tcgacgcaga agtggaaggc 360

gacacccatt tcccggatta cgagccggat gactgggaat cggtattcag cgaattccac 420

gatgctgatg cgcagaactc tcacagctat tgctttgaga ttctggagcg gcga 474

<210> 86
<211> 112
<212> PRT
<213> Artificial Sequence

<220>
<221> source

<223> /note="Description of Artificial Sequence: Synthetic
polypeptide"

<400> 86
Met Gly Val Glu Lys Gln Val Ile Arg Pro Gly Asn Gly Pro Lys Pro
1 5 10 15

Ala Pro Gly Gln Thr Val Thr Val His Cys Thr Gly Phe Gly Lys Asp
20 25 30

Gly Asp Leu Ser Gln Lys Phe Trp Ser Thr Lys Asp Glu Gly Gln Lys
35 40 45

Pro Phe Ser Phe Gln Ile Gly Lys Gly Ala Val Ile Lys Gly Trp Asp
50 55 60

Glu Gly Val Ile Gly Met Gln Ile Gly Glu Val Ala Arg Leu Arg Cys
65 70 75 80

Ser Ser Asp Tyr Ala Tyr Gly Ala Gly Gly Phe Pro Ala Trp Gly Ile
85 90 95

Gln Pro Asn Ser Val Leu Asp Phe Glu Ile Glu Val Leu Ser Val Gln
100 105 110

<210> 87
<211> 78
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 87
ggatcccggg ctgactacaa agaccatgac ggtgattata aagatcatga catcgactac 60

aaggatgacg atgacaag 78

<210> 88
<211> 2208

<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
polynucleotide"

<400> 88
atggctgccg atggttatct tccagattgg ctcgaggaca acctctctga gggcattcgc 60

gagtggtggg acttgaaacc tggagccccg aaacccaaag ccaaccagca aaagcaggac 120

gacggccggg gtctggtgct tcctggctac aagtacctcg gacccttcaa cggactcgac 180

aaggggggagc ccgtcaacgc ggcggacgca gcggccctcg agcacgacaa ggcctacgac 240

cagcagctca aagcgggtga caatccgtac ctgcggtata accacgccga cgccgagttt 300

caggagcgtc tgcaagaaga tacgtctttt gggggcaacc tcgggcgagc agtcttccag 360

gccaagaagc gggttctcga acctctcggt ctggttgagg aaggcgctaa gacggctcct 420

ggaaagaaga gaccggtaga gcaatcaccc caggaaccag actcctcttc gggcatcggc 480

aagaaaggcc agcagcccgc gaagaagaga ctcaactttg ggcagacagg cgactcagag 540

tcagtgcccg accctcaacc actcggagaa ccccccgcag ccccctctgg tgtgggatct 600

aatacaatgg cagcaggcgg tggcgctcca atggcagaca ataacgaagg cgccgacgga 660

gtgggtaacg cctcaggaaa ttggcattgc gattccacat ggctgggcga cagagtcatc 720

accaccagca cccgaacctg ggccctcccc acctacaaca accacctcta caagcaaatc 780

tccagccaat cgggagcaag caccaacgac aacacctact tcggctacag caccccctgg 840

gggtattttg actttaacag attccactgc cacttctcac cacgtgactg gcagcgactc 900

atcaacaaca actggggatt ccggcccaag agactcaact tcaagctctt caacatccag 960

gtcaaggagg tcacgacgaa tgatggcacc acgaccatcg ccaataacct taccagcacg 1020

gttcaggtct ttacggactc ggaataccag ctcccgtacg tcctcggctc tgcgcaccag 1080

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tctcaaatgc tgagaacggg caacaacttt gagttcagct acacgtttga ggacgtgcct 1260

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cagtacctgt actacctgtc tcggactcag accacgagtg gtaccgcagg aaatcggacg 1380

ttgcaatttt ctcaggccgg gcctagtagc atggcgaatc aggccaaaaa ctggctaccc 1440

gggccctgct accggcagca acgcgtctcc aagacagcga atcaaaataa caacagcaac 1500

tttgcctgga ccggtgccac caagtatcat ctgaatggca gagactctct ggtaaatccc 1560

ggtcccgcta tggcaaccca caaggacgac gaagacaaat tttttccgat gagcggagtc 1620

ttaatatttg ggaaacaggg agctggaaat agcaacgtgg accttgacaa cgttatgata 1680

accagtgagg aagaaattaa aaccaccaac ccagtggcca cagaacagta cggcacggtg 1740

gccactaacc tgcaatcgtc aaacaccgct cctgctacag ggaccgtcaa cagtcaagga 1800

gccttacctg gcatggtctg gcagaaccgg gacgtgtacc tgcagggtcc tatctgggcc 1860

aagattcctc acacggacgg acactttcat ccctcgccgc tgatgggagg ctttggactg 1920

aaacacccgc ctcctcagat cctgattaag aatacacctg ttcccgcgaa tcctccaact 1980

accttcagtc cagctaagtt tgcgtcgttc atcacgcagt acagcaccgg acaggtcagc 2040

gtggaaattg aatgggagct gcagaaagaa aacagcaaac gctggaaccc agagattcaa 2100

tacacttcca actacaacaa atctacaaat gtggactttg ctgttgacac aaatggcgtt 2160

tattctgagc ctcgccccat cggcacccgt tacctcaccc gtaatctg 2208

<210> 89
<211> 736
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
polypeptide"

<400> 89
Met Ala Ala Asp Gly Tyr Leu Pro Asp Trp Leu Glu Asp Asn Leu Ser
1 5 10 15

Glu Gly Ile Arg Glu Trp Trp Asp Leu Lys Pro Gly Ala Pro Lys Pro

```
            20                  25                  30

Lys Ala Asn Gln Gln Lys Gln Asp Asp Gly Arg Gly Leu Val Leu Pro
        35                  40                  45

Gly Tyr Lys Tyr Leu Gly Pro Phe Asn Gly Leu Asp Lys Gly Glu Pro
    50                  55                  60

Val Asn Ala Ala Asp Ala Ala Ala Leu Glu His Asp Lys Ala Tyr Asp
65                  70                  75                  80

Gln Gln Leu Lys Ala Gly Asp Asn Pro Tyr Leu Arg Tyr Asn His Ala
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Asp Ala Glu Phe Gln Glu Arg Leu Gln Glu Asp Thr Ser Phe Gly Gly
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Asn Leu Gly Arg Ala Val Phe Gln Ala Lys Lys Arg Val Leu Glu Pro
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Leu Gly Leu Val Glu Glu Gly Ala Lys Thr Ala Pro Gly Lys Lys Arg
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Pro Val Glu Gln Ser Pro Gln Glu Pro Asp Ser Ser Ser Gly Ile Gly
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Lys Lys Gly Gln Gln Pro Ala Lys Lys Arg Leu Asn Phe Gly Gln Thr
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Gly Asp Ser Glu Ser Val Pro Asp Pro Gln Pro Leu Gly Glu Pro Pro
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Thr Thr Ser Thr Arg Thr Trp Ala Leu Pro Thr Tyr Asn Asn His Leu
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Tyr Lys Gln Ile Ser Ser Gln Ser Gly Ala Ser Thr Asn Asp Asn Thr
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Tyr Phe Gly Tyr Ser Thr Pro Trp Gly Tyr Phe Asp Phe Asn Arg Phe
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His Cys His Phe Ser Pro Arg Asp Trp Gln Arg Leu Ile Asn Asn Asn
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Trp Gly Phe Arg Pro Lys Arg Leu Asn Phe Lys Leu Phe Asn Ile Gln
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Val Lys Glu Val Thr Thr Asn Asp Gly Thr Thr Thr Ile Ala Asn Asn
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Leu Thr Ser Thr Val Gln Val Phe Thr Asp Ser Glu Tyr Gln Leu Pro
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Tyr Val Leu Gly Ser Ala His Gln Gly Cys Leu Pro Pro Phe Pro Ala
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Asp Val Phe Met Ile Pro Gln Tyr Gly Tyr Leu Thr Leu Asn Asn Gly
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Ser Gln Ala Val Gly Arg Ser Ser Phe Tyr Cys Leu Glu Tyr Phe Pro
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Gly Pro Cys Tyr Arg Gln Gln Arg Val Ser Lys Thr Ala Asn Gln Asn
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Asn Asn Ser Asn Phe Ala Trp Thr Gly Ala Thr Lys Tyr His Leu Asn
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Thr Ser Glu Glu Glu Ile Lys Thr Thr Asn Pro Val Ala Thr Glu Gln
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Tyr Gly Thr Val Ala Thr Asn Leu Gln Ser Ser Asn Thr Ala Pro Ala
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Thr Gly Thr Val Asn Ser Gln Gly Ala Leu Pro Gly Met Val Trp Gln
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Asn Arg Asp Val Tyr Leu Gln Gly Pro Ile Trp Ala Lys Ile Pro His
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Asn Pro Pro Thr Thr Phe Ser Pro Ala Lys Phe Ala Ser Phe Ile Thr
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Gln Tyr Ser Thr Gly Gln Val Ser Val Glu Ile Glu Trp Glu Leu Gln
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<211> 3664
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
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ccaa 3664

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<211> 16
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
probe"

<400> 97
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<210> 98
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<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
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<400> 98
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<210> 99
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
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<223> /note="Description of Artificial Sequence: Synthetic
primer"

<400> 99
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<210> 100
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<220>
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<223> /note="Description of Artificial Sequence: Synthetic
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<210> 101
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<213> Artificial Sequence

<220>
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<223> /note="Description of Artificial Sequence: Synthetic primer"

<400> 101
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<210> 102
<211> 26
<212> DNA
<213> Artificial Sequence

<220>
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<400> 102
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<210> 103
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<212> DNA
<213> Artificial Sequence

<220>
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<223> /note="Description of Artificial Sequence: Synthetic polynucleotide"

<400> 103
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accgtgtcct ggaattctgg cgctctgaca agcggcgtgc acacctttcc agctgtgctg 600

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gtggaaccca agagctgcga caagacccac accggcaagc ggaagagaag aggctctggc 780

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<210> 104
<211> 239
<212> PRT

<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
polypeptide"

<400> 104

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Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile
        35                  40                  45


Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr
    50                  55                  60


Leu Thr Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys
65                  70                  75                  80


Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu
                85                  90                  95


Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu
            100                 105                 110


Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly
        115                 120                 125


Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr
    130                 135                 140


Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn
145                 150                 155                 160


Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser
                165                 170                 175
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Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly
180 185 190

Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu
195 200 205

Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe
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<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic polynucleotide"

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ggcatcaagg tgaacttcaa gatccgccac aacatcgagg acggcagcgt gcagctcgcc 540

gaccactacc agcagaacac ccccatcggc gacggccccg tgctgctgcc cgacaaccac 600

tacctgagca cccagtccgc cctgagcaaa gaccccaacg agaagcgcga tcacatggtc 660

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<210> 106
<211> 4509
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
polynucleotide"

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<210> 107
<211> 5169
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic polynucleotide"

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gcgaggggcg gggcggggcg aggcggagag gtgcggcggc agccaatcag agcggcgcgc 720

tccgaaagtt tccttttatg gcgaggcggc ggcggcggcg gccctataaa aagcgaagcg 780

cgcggcgggc gggagtcgct gcgttgcctt cgccccgtgc cccgctccgc gccgcctcgc 840

gccgcccgcc ccggctctga ctgaccgcgt tactcccaca ggtgagcggg cgggacggcc 900

cttctcctcc gggctgtaat tagcgcttgg tttaatgacg gctcgtttct tttctgtggc 960

tgcgtgaaag ccttaaaggg ctccgggagg gcccttttgtg cgggggggag cggctcgggg 1020

ggtgcgtgcg tgtgtgtgtg cgtggggagc gccgcgtgcg gcccgcgctg cccggcggct 1080

gtgagcgctg cgggcgcggc gcggggcttt gtgcgctccg cgtgtgcgcg aggggagcgc 1140

ggccgggggc ggtgccccgc ggtgcggggg ggctgcgagg ggaacaaagg ctgcgtgcgg 1200

ggtgtgtgcg tgggggggtg agcagggggt gtgggcgcgg cggtcgggct gtaacccccc 1260

cctgcacccc cctccccgag ttgctgagca cggcccggct tcgggtgcgg ggctccgtgc 1320

ggggcgtggc gcggggctcg ccgtgccggg cgggggggtgg cggcaggtgg gggtgccggg 1380

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cgccggcggc tgtcgaggcg cggcgagccg cagccattgc cttttatggt aatcgtgcga 1500

gagggcgcag ggacttcctt tgtcccaaat ctgtgcggag ccgaaatctg ggaggcgccg 1560

ccgcaccccc tctagcgggc gcggggcgaa gcggtgcggc gccggcagga aggaaatggg 1620

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acagctcctg ggcaacgtgc tggttattgt gaccggtgcc accatgtacc ggatgcagct 1860

gctgagctgt atcgccctgt ctctggccct ggtcaccaat tctgaggtgc agctggtgga 1920

atctggcggc ggacttgttc aacctggcgg ctctctgaga ctgagctgtg ccgcttctgg 1980

ctacaccttc accaactacg gcatgaactg ggtccgacag gcccctggca aaggccttga 2040

atgggtcgga tggatcaaca cctacaccgg cgagccaaca tacgccgccg acttcaagcg 2100

gagattcacc ttcagcctgg acaccagcaa gagcaccgcc tacctgcaga tgaacagcct 2160

gagagccgag gacaccgccg tgtactactg cgccaagtat ccccactact acggcagcag 2220

ccactggtac tttgacgtgt ggggacaggg cacactggtc acagtgtcta gcgcctctac 2280

aaagggcccc agcgttttcc cactggctcc tagcagcaag tctaccagcg gaggaacagc 2340

cgctctgggc tgtctggtca aggactactt tcccgagcct gtgaccgtgt cctggaattc 2400

tggcgctctg acaagcggcg tgcacacctt tccagctgtg ctgcaaagca gcggcctgta 2460

ctctctgagc agcgtcgtga cagtgccaag cagctctctg ggcacccaga cctacatctg 2520

caatgtgaac cacaagccta gcaacaccaa ggtggacaag aaggtggaac ccaagagctg 2580

cgacaagacc cacacctgtc ctccatgtcc tgctccagaa ctgctcggcg gaccttccgt 2640

gttcctgttt cctccaaagc ctaaggacac cctgatgatc agcagaaccc ctgaagtgac 2700

ctgcgtggtg gtggatgtgt cccacgagga tcccgaagtg aagttcaatt ggtacgtgga 2760

cggcgtggaa gtgcacaacg ccaagaccaa gcctagagag gaacagtaca acagcaccta 2820

cagagtggtg tccgtgctga ccgtgctgca ccaggattgg ctgaacggca aagagtacaa 2880

gtgcaaggtg tccaacaagg ccctgcctgc tcctatcgag aaaaccatca gcaaggccaa 2940

gggccagcct agggaacccc aggtttacac actgcctcca agccgggaag agatgaccaa 3000

gaaccaggtg tccctgacct gcctcgtgaa gggcttctac ccttccgata tcgccgtgga 3060

atgggagagc aatggccagc cagagaacaa ctacaagaca acccctcctg tgctggacag 3120

cgacggctca ttcttcctgt acagcaagct gacagtggac aagtccagat ggcagcaggg 3180

caacgtgttc agctgcagcg tgatgcacga ggccctgcac aaccactaca cccagaagtc 3240

tctgagcctg tctcctggca agcggaagag aagaggctct ggcgaaggca gaggcagcct 3300

gcttacatgt ggcgacgtgg aagagaaccc cggacctatg tatagaatgc agctcctgtc 3360

ctgcattgcc ctgagcctgg ctctcgtgac caacagcgac atccagatga cacagagccc 3420

cagcagcctg tctgcctctg tgggagacag agtgaccatc acctgtagcg ccagccagga 3480

catctccaac tacctgaact ggtatcagca aaagcccggc aaggccccta aggtgctgat 3540

ctacttcaca agcagcctgc actccggcgt gcccagcaga ttttctggct ctggcagcgg 3600

caccgacttc accctgacca tatctagcct gcagcctgag gacttcgcca cctactactg 3660

ccagcagtac agcaccgtgc cttggacatt tggccagggc acaaaggtgg aaatcaagcg 3720

gactgtggcc gctcctagcg tgttcatctt tccacctagc gacgagcagc tgaagtctgg 3780

cacagcctct gtcgtgtgcc tgctgaacaa cttctacccc agagaagcca aggtgcagtg 3840

gaaagtggac aatgccctgc agagcggcaa cagccaagag agcgtgacag agcaggactc 3900

caaggatagc acctatagcc tgagcagcac cctgacactg agcaaggccg actacgagaa 3960

gcacaaagtg tacgcctgcg aagtgaccca ccagggcctt tctagccctg tgaccaagag 4020

cttcaaccgg ggcgaatgta tggtgagcaa gggcgaggag ctgttcaccg gggtggtgcc 4080

catcctggtc gagctggacg gcgacgtaaa cggccacaag ttcagcgtgt ccggcgaggg 4140

cgagggcgat gccacctacg gcaagctgac cctgaagttc atctgcacca ccggcaagct 4200

gcccgtgccc tggcccaccc tcgtgaccac cctgacctac ggcgtgcagt gcttcagccg 4260

ctaccccgac cacatgaagc agcacgactt cttcaagtcc gccatgcccg aaggctacgt 4320

ccaggagcgc accatcttct tcaaggacga cggcaactac aagacccgcg ccgaggtgaa 4380

gttcgagggc gacaccctgg tgaaccgcat cgagctgaag ggcatcgact tcaaggagga 4440

cggcaacatc ctggggcaca agctggagta caactacaac agccacaacg tctatatcat 4500

ggccgacaag cagaagaacg gcatcaaggt gaacttcaag atccgccaca acatcgagga 4560

cggcagcgtg cagctcgccg accactacca gcagaacacc cccatcggcg acggccccgt 4620

gctgctgccc gacaaccact acctgagcac ccagtccgcc ctgagcaaag accccaacga 4680

gaagcgcgat cacatggtcc tgctggagtt cgtgaccgcc gccgggatca ctctcggcat 4740

ggacgagctg tacaagtaag agctcgctga tcagcctcga ctgtgccttc tagttgccag 4800

ccatctgttg tttgcccctc ccccgtgcct tccttgaccc tggaaggtgc cactcccact 4860

gtcctttcct aataaaatga ggaaattgca tcgcattgtc tgagtaggtg tcattctatt 4920

ctgggggggtg gggtggggca ggacagcaag ggggaggatt gggaagacaa tagcaggcat 4980

gctggggatg cggtgggctc tatggaagct tgaattcagc tgacgtgcct cggaccgcta 5040

ggaaccccta gtgatggagt tggccactcc ctctctgcgc gctcgctcgc tcactgaggc 5100

cgggcgacca aaggtcgccc gacgcccggg ctttgcccgg gcggcctcag tgagcgagcg 5160

agcgcgcag 5169

<210> 108
<211> 1419
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
polynucleotide"

<400> 108
atgtaccgga tgcagctgct gagctgtatc gccctgtctc tggccctggt caccaattct 60

gaggtgcagc tggtggaatc tggcggcgga cttgttcaac ctggcggctc tctgagactg 120

agctgtgccg cttctggcta caccttcacc aactacggca tgaactgggt ccgacaggcc 180

cctggcaaag gccttgaatg ggtcggatgg atcaacacct acaccggcga gccaacatac 240

gccgccgact tcaagcggag attcaccttc agcctggaca ccagcaagag caccgcctac 300

ctgcagatga acagcctgag agccgaggac accgccgtgt actactgcgc caagtatccc 360

cactactacg gcagcagcca ctggtacttt gacgtgtggg gacagggcac actggtcaca 420

gtgtctagcg cctctacaaa gggccccagc gttttcccac tggctcctag cagcaagtct 480

accagcggag gaacagccgc tctgggctgt ctggtcaagg actactttcc cgagcctgtg 540

accgtgtcct ggaattctgg cgctctgaca agcggcgtgc acacctttcc agctgtgctg 600

caaagcagcg gcctgtactc tctgagcagc gtcgtgacag tgccaagcag ctctctgggc 660

acccagacct acatctgcaa tgtgaaccac aagcctagca acaccaaggt ggacaagaag 720

gtggaaccca agagctgcga caagacccac acctgtcctc catgtcctgc tccagaactg 780

ctcggcggac cttccgtgtt cctgtttcct ccaaagccta aggacaccct gatgatcagc 840

agaacccctg aagtgacctg cgtggtggtg gatgtgtccc acgaggatcc cgaagtgaag 900

ttcaattggt acgtggacgg cgtggaagtg cacaacgcca agaccaagcc tagagaggaa 960

cagtacaaca gcacctacag agtggtgtcc gtgctgaccg tgctgcacca ggattggctg 1020

aacggcaaag agtacaagtg caaggtgtcc aacaaggccc tgcctgctcc tatcgagaaa 1080

accatcagca aggccaaggg ccagcctagg gaaccccagg tttacacact gcctccaagc 1140

cgggaagaga tgaccaagaa ccaggtgtcc ctgacctgcc tcgtgaaggg cttctaccct 1200

tccgatatcg ccgtggaatg ggagagcaat ggccagccag agaacaacta caagacaacc 1260

cctcctgtgc tggacagcga cggctcattc ttcctgtaca gcaagctgac agtggacaag 1320

tccagatggc agcagggcaa cgtgttcagc tgcagcgtga tgcacgaggc cctgcacaac 1380

cactacaccc agaagtctct gagcctgtct cctggcaag 1419

<210> 109
<211> 642
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
polynucleotide"

<400> 109
gacatccaga tgacacagag ccccagcagc ctgtctgcct ctgtgggaga cagagtgacc 60

atcacctgta gcgccagcca ggacatctcc aactacctga actggtatca gcaaaagccc 120

ggcaaggccc ctaaggtgct gatctacttc acaagcagcc tgcactccgg cgtgcccagc 180

agattttctg gctctggcag cggcaccgac ttcaccctga ccatatctag cctgcagcct 240

gaggacttcg ccacctacta ctgccagcag tacagcaccg tgccttggac atttggccag 300

ggcacaaagg tggaaatcaa gcggactgtg gccgctccta gcgtgttcat ctttccacct 360

agcgacgagc agctgaagtc tggcacagcc tctgtcgtgt gcctgctgaa caacttctac 420

cccagagaag ccaaggtgca gtggaaagtg gacaatgccc tgcagagcgg caacagccaa 480

gagagcgtga cagagcagga ctccaaggat agcacctata gcctgagcag caccctgaca 540

ctgagcaagg ccgactacga gaagcacaaa gtgtacgcct gcgaagtgac ccaccagggc 600

ctttctagcc ctgtgaccaa gagcttcaac cggggcgaat gt 642

<210> 110
<211> 678

<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
polynucleotide"

<400> 110
gacaagaccc acacctgtcc tccatgtcct gctccagaac tgctcggcgg accttccgtg 60

ttcctgtttc ctccaaagcc taaggacacc ctgatgatca gcagaacccc tgaagtgacc 120

tgcgtggtgg tggatgtgtc ccacgaggat cccgaagtga agttcaattg gtacgtggac 180

ggcgtggaag tgcacaacgc caagaccaag cctagagagg aacagtacaa cagcacctac 240

agagtggtgt ccgtgctgac cgtgctgcac caggattggc tgaacggcaa agagtacaag 300

tgcaaggtgt ccaacaaggc cctgcctgct cctatcgaga aaaccatcag caaggccaag 360

ggccagccta gggaacccca ggtttacaca ctgcctccaa gccgggaaga gatgaccaag 420

aaccaggtgt ccctgacctg cctcgtgaag ggcttctacc cttccgatat cgccgtggaa 480

tgggagagca atggccagcc agagaacaac tacaagacaa cccctcctgt gctggacagc 540

gacggctcat tcttcctgta cagcaagctg acagtggaca agtccagatg gcagcagggc 600

aacgtgttca gctgcagcgt gatgcacgag gccctgcaca accactacac ccagaagtct 660

ctgagcctgt ctcctggc 678

<210> 111
<211> 226
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
polypeptide"

<400> 111
Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly
1 5 10 15

Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met

```
            20                  25                  30

Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His
        35                  40                  45

Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val
    50                  55                  60

His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr
65                  70                  75                  80

Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly
                85                  90                  95

Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile
            100                 105                 110

Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val
        115                 120                 125

Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser
    130                 135                 140

Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
145                 150                 155                 160

Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro
                165                 170                 175

Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val
            180                 185                 190

Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met
        195                 200                 205

His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser
    210                 215                 220
```

Pro Gly
225

<210> 112
<211> 225
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
polypeptide"

<400> 112
Met Tyr Arg Met Gln Leu Leu Ser Cys Ile Ala Leu Ser Leu Ala Leu
1 5 10 15

Val Thr Asn Ser Ser Asp Thr Gly Arg Pro Phe Val Glu Met Tyr Ser
20 25 30

Glu Ile Pro Glu Ile Ile His Met Thr Glu Gly Arg Glu Leu Val Ile
35 40 45

Pro Cys Arg Val Thr Ser Pro Asn Ile Thr Val Thr Leu Lys Lys Phe
50 55 60

Pro Leu Asp Thr Leu Ile Pro Asp Gly Lys Arg Ile Ile Trp Asp Ser
65 70 75 80

Arg Lys Gly Phe Ile Ile Ser Asn Ala Thr Tyr Lys Glu Ile Gly Leu
85 90 95

Leu Thr Cys Glu Ala Thr Val Asn Gly His Leu Tyr Lys Thr Asn Tyr
100 105 110

Leu Thr His Arg Gln Thr Asn Thr Ile Ile Asp Val Val Leu Ser Pro
115 120 125

Ser His Gly Ile Glu Leu Ser Val Gly Glu Lys Leu Val Leu Asn Cys
130 135 140

```
Thr Ala Arg Thr Glu Leu Asn Val Gly Ile Asp Phe Asn Trp Glu Tyr
145                 150                 155                 160


Pro Ser Ser Lys His Gln His Lys Lys Leu Val Asn Arg Asp Leu Lys
                165                 170                 175


Thr Gln Ser Gly Ser Glu Met Lys Lys Phe Leu Ser Thr Leu Thr Ile
            180                 185                 190


Asp Gly Val Thr Arg Ser Asp Gln Gly Leu Tyr Thr Cys Ala Ala Ser
        195                 200                 205


Ser Gly Leu Met Thr Lys Lys Asn Ser Thr Phe Val Arg Val His Glu
    210                 215                 220


Lys
225


<210> 113
<211> 736
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      polypeptide"


<220>
<221> VARIANT
<222> (168)..(168)
<223> /replace="R"

<220>
<221> VARIANT
<222> (204)..(204)
<223> /replace="S"

<220>
<221> VARIANT
<222> (266)..(266)
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<223> /replace="G"

<220>
<221> VARIANT
<222> (311)..(311)
<223> /replace="K"

<220>
<221> VARIANT
<222> (411)..(411)
<223> /replace="Q"

<220>
<221> VARIANT
<222> (460)..(460)
<223> /replace="E"

<220>
<221> VARIANT
<222> (493)..(493)
<223> /replace="T"

<220>
<221> VARIANT
<222> (562)..(562)
<223> /replace="N"

<220>
<221> VARIANT
<222> (576)..(576)
<223> /replace="E"

<220>
<221> VARIANT
<222> (587)..(587)
<223> /replace="A"

<220>
<221> VARIANT
<222> (609)..(609)
<223> /replace="D"

<220>
<221> SITE
<222> (1)..(736)
<223> /note="Variant residues given in the sequence have no
preference with respect to those in the annotations
for variant positions"

<400> 113

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Met Ala Ala Asp Gly Tyr Leu Pro Asp Trp Leu Glu Asp Asn Leu Ser
1               5                  10                  15

Glu Gly Ile Arg Glu Trp Trp Asp Leu Lys Pro Gly Ala Pro Lys Pro
            20                  25                  30

Lys Ala Asn Gln Gln Lys Gln Asp Asp Gly Arg Gly Leu Val Leu Pro
        35                  40                  45

Gly Tyr Lys Tyr Leu Gly Pro Phe Asn Gly Leu Asp Lys Gly Glu Pro
    50                  55                  60

Val Asn Ala Ala Asp Ala Ala Ala Leu Glu His Asp Lys Ala Tyr Asp
65                  70                  75                  80

Gln Gln Leu Lys Ala Gly Asp Asn Pro Tyr Leu Arg Tyr Asn His Ala
                85                  90                  95

Asp Ala Glu Phe Gln Glu Arg Leu Gln Glu Asp Thr Ser Phe Gly Gly
            100                 105                 110

Asn Leu Gly Arg Ala Val Phe Gln Ala Lys Lys Arg Val Leu Glu Pro
        115                 120                 125

Leu Gly Leu Val Glu Glu Gly Ala Lys Thr Ala Pro Gly Lys Lys Arg
    130                 135                 140

Pro Val Glu Gln Ser Pro Gln Glu Pro Asp Ser Ser Ser Gly Ile Gly
145                 150                 155                 160

Lys Lys Gly Gln Gln Pro Ala Lys Lys Arg Leu Asn Phe Gly Gln Thr
                165                 170                 175

Gly Asp Ser Glu Ser Val Pro Asp Pro Gln Pro Leu Gly Glu Pro Pro
            180                 185                 190

Ala Ala Pro Ser Gly Val Gly Ser Asn Thr Met Ala Ala Gly Gly Gly
        195                 200                 205
```

```
Ala Pro Met Ala Asp Asn Asn Glu Gly Ala Asp Gly Val Gly Asn Ala
    210                 215                 220

Ser Gly Asn Trp His Cys Asp Ser Thr Trp Leu Gly Asp Arg Val Ile
225                 230                 235                 240

Thr Thr Ser Thr Arg Thr Trp Ala Leu Pro Thr Tyr Asn Asn His Leu
                245                 250                 255

Tyr Lys Gln Ile Ser Ser Gln Ser Gly Ala Ser Thr Asn Asp Asn Thr
            260                 265                 270

Tyr Phe Gly Tyr Ser Thr Pro Trp Gly Tyr Phe Asp Phe Asn Arg Phe
        275                 280                 285

His Cys His Phe Ser Pro Arg Asp Trp Gln Arg Leu Ile Asn Asn Asn
    290                 295                 300

Trp Gly Phe Arg Pro Lys Arg Leu Asn Phe Lys Leu Phe Asn Ile Gln
305                 310                 315                 320

Val Lys Glu Val Thr Thr Asn Asp Gly Thr Thr Thr Ile Ala Asn Asn
                325                 330                 335

Leu Thr Ser Thr Val Gln Val Phe Thr Asp Ser Glu Tyr Gln Leu Pro
            340                 345                 350

Tyr Val Leu Gly Ser Ala His Gln Gly Cys Leu Pro Pro Phe Pro Ala
        355                 360                 365

Asp Val Phe Met Ile Pro Gln Tyr Gly Tyr Leu Thr Leu Asn Asn Gly
    370                 375                 380

Ser Gln Ala Val Gly Arg Ser Ser Phe Tyr Cys Leu Glu Tyr Phe Pro
385                 390                 395                 400
```

```
Ser Gln Met Leu Arg Thr Gly Asn Asn Phe Glu Phe Ser Tyr Thr Phe
                405                 410                 415

Glu Asp Val Pro Phe His Ser Ser Tyr Ala His Ser Gln Ser Leu Asp
            420                 425                 430

Arg Leu Met Asn Pro Leu Ile Asp Gln Tyr Leu Tyr Tyr Leu Ser Arg
        435                 440                 445

Thr Gln Thr Thr Ser Gly Thr Ala Gly Asn Arg Thr Leu Gln Phe Ser
    450                 455                 460

Gln Ala Gly Pro Ser Ser Met Ala Asn Gln Ala Lys Asn Trp Leu Pro
465                 470                 475                 480

Gly Pro Cys Tyr Arg Gln Gln Arg Val Ser Lys Thr Ala Asn Gln Asn
                485                 490                 495

Asn Asn Ser Asn Phe Ala Trp Thr Gly Ala Thr Lys Tyr His Leu Asn
            500                 505                 510

Gly Arg Asp Ser Leu Val Asn Pro Gly Pro Ala Met Ala Thr His Lys
        515                 520                 525

Asp Asp Glu Asp Lys Phe Phe Pro Met Ser Gly Val Leu Ile Phe Gly
    530                 535                 540

Lys Gln Gly Ala Gly Asn Ser Asn Val Asp Leu Asp Asn Val Met Ile
545                 550                 555                 560

Thr Ser Glu Glu Glu Ile Lys Thr Thr Asn Pro Val Ala Thr Glu Gln
                565                 570                 575

Tyr Gly Thr Val Ala Thr Asn Leu Gln Ser Ser Asn Thr Ala Pro Ala
            580                 585                 590

Thr Gly Thr Val Asn Ser Gln Gly Ala Leu Pro Gly Met Val Trp Gln
        595                 600                 605
```

Asn Arg Asp Val Tyr Leu Gln Gly Pro Ile Trp Ala Lys Ile Pro His
    610   615   620

Thr Asp Gly His Phe His Pro Ser Pro Leu Met Gly Gly Phe Gly Leu
625   630   635   640

Lys His Pro Pro Pro Gln Ile Leu Ile Lys Asn Thr Pro Val Pro Ala
                645   650   655

Asn Pro Pro Thr Thr Phe Ser Pro Ala Lys Phe Ala Ser Phe Ile Thr
            660   665   670

Gln Tyr Ser Thr Gly Gln Val Ser Val Glu Ile Glu Trp Glu Leu Gln
        675   680   685

Lys Glu Asn Ser Lys Arg Trp Asn Pro Glu Ile Gln Tyr Thr Ser Asn
    690   695   700

Tyr Asn Lys Ser Thr Asn Val Asp Phe Ala Val Asp Thr Asn Gly Val
705   710   715   720

Tyr Ser Glu Pro Arg Pro Ile Gly Thr Arg Tyr Leu Thr Arg Asn Leu
                725   730   735

<210> 114
<211> 736
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      polypeptide"

<400> 114
Met Ala Ala Asp Gly Tyr Leu Pro Asp Trp Leu Glu Asp Asn Leu Ser
1   5   10   15

Glu Gly Ile Arg Glu Trp Trp Asp Leu Lys Pro Gly Ala Pro Lys Pro

```
            20                  25                  30

Lys Ala Asn Gln Gln Lys Gln Asp Asp Gly Arg Gly Leu Val Leu Pro
        35                  40                  45

Gly Tyr Lys Tyr Leu Gly Pro Phe Asn Gly Leu Asp Lys Gly Glu Pro
    50                  55                  60

Val Asn Ala Ala Asp Ala Ala Ala Leu Glu His Asp Lys Ala Tyr Asp
65                  70                  75                  80

Gln Gln Leu Lys Ala Gly Asp Asn Pro Tyr Leu Arg Tyr Asn His Ala
                85                  90                  95

Asp Ala Glu Phe Gln Glu Arg Leu Gln Glu Asp Thr Ser Phe Gly Gly
            100                 105                 110

Asn Leu Gly Arg Ala Val Phe Gln Ala Lys Lys Arg Val Leu Glu Pro
        115                 120                 125

Leu Gly Leu Val Glu Glu Gly Ala Lys Thr Ala Pro Gly Lys Lys Arg
    130                 135                 140

Pro Val Glu Gln Ser Pro Gln Glu Pro Asp Ser Ser Ser Gly Ile Gly
145                 150                 155                 160

Lys Lys Gly Gln Gln Pro Ala Arg Lys Arg Leu Asn Phe Gly Gln Thr
                165                 170                 175

Gly Asp Ser Glu Ser Val Pro Asp Pro Gln Pro Leu Gly Glu Pro Pro
            180                 185                 190

Ala Ala Pro Ser Gly Val Gly Ser Asn Thr Met Ala Ala Gly Gly Gly
        195                 200                 205

Ala Pro Met Ala Asp Asn Asn Glu Gly Ala Asp Gly Val Gly Asn Ala
    210                 215                 220
```

```
Ser Gly Asn Trp His Cys Asp Ser Thr Trp Leu Gly Asp Arg Val Ile
225                 230                 235                 240

Thr Thr Ser Thr Arg Thr Trp Ala Leu Pro Thr Tyr Asn Asn His Leu
                245                 250                 255

Tyr Lys Gln Ile Ser Ser Gln Ser Gly Gly Ser Thr Asn Asp Asn Thr
            260                 265                 270

Tyr Phe Gly Tyr Ser Thr Pro Trp Gly Tyr Phe Asp Phe Asn Arg Phe
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His Cys His Phe Ser Pro Arg Asp Trp Gln Arg Leu Ile Asn Asn Asn
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Trp Gly Phe Arg Pro Lys Lys Leu Asn Phe Lys Leu Phe Asn Ile Gln
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Val Lys Glu Val Thr Thr Asn Asp Gly Thr Thr Thr Ile Ala Asn Asn
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Leu Thr Ser Thr Val Gln Val Phe Thr Asp Ser Glu Tyr Gln Leu Pro
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Tyr Val Leu Gly Ser Ala His Gln Gly Cys Leu Pro Pro Phe Pro Ala
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Asp Val Phe Met Ile Pro Gln Tyr Gly Tyr Leu Thr Leu Asn Asn Gly
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Ser Gln Ala Val Gly Arg Ser Ser Phe Tyr Cys Leu Glu Tyr Phe Pro
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Ser Gln Met Leu Arg Thr Gly Asn Asn Phe Gln Phe Ser Tyr Thr Phe
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Glu Asp Val Pro Phe His Ser Ser Tyr Ala His Ser Gln Ser Leu Asp
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Arg Leu Met Asn Pro Leu Ile Asp Gln Tyr Leu Tyr Tyr Leu Ser Arg
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Gln Ala Gly Pro Ser Ser Met Ala Asn Gln Ala Lys Asn Trp Leu Pro
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Gly Pro Cys Tyr Arg Gln Gln Arg Val Ser Lys Thr Thr Asn Gln Asn
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Asn Asn Ser Asn Phe Ala Trp Thr Gly Ala Thr Lys Tyr His Leu Asn
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Gly Arg Asp Ser Leu Val Asn Pro Gly Pro Ala Met Ala Thr His Lys
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Asp Asp Glu Asp Lys Phe Phe Pro Met Ser Gly Val Leu Ile Phe Gly
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Lys Gln Gly Ala Gly Asn Ser Asn Val Asp Leu Asp Asn Val Met Ile
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Thr Asn Glu Glu Glu Ile Lys Thr Thr Asn Pro Val Ala Thr Glu Glu
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Tyr Gly Thr Val Ala Thr Asn Leu Gln Ser Ala Asn Thr Ala Pro Ala
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Thr Gly Thr Val Asn Ser Gln Gly Ala Leu Pro Gly Met Val Trp Gln
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Asp Arg Asp Val Tyr Leu Gln Gly Pro Ile Trp Ala Lys Ile Pro His
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Thr Asp Gly His Phe His Pro Ser Pro Leu Met Gly Gly Phe Gly Leu
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Lys His Pro Pro Pro Gln Ile Leu Ile Lys Asn Thr Pro Val Pro Ala
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Asn Pro Pro Thr Thr Phe Ser Pro Ala Lys Phe Ala Ser Phe Ile Thr
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Gln Tyr Ser Thr Gly Gln Val Ser Val Glu Ile Glu Trp Glu Leu Gln
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Lys Glu Asn Ser Lys Arg Trp Asn Pro Glu Ile Gln Tyr Thr Ser Asn
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