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(54) Title: GENE THERAPY TARGETING COCHLEAR CELLS

(57) Abstract: The present disclosure relates to methods of targeting specific cell types within the cochlea using optimized gene therapy vectors. In particular, the disclosure provides gene therapy vectors to specifically target cochlear cells and methods of treating hearing impairment and hearing-loss related disorders.



GENE THERAPY TARGETING COCHLEAR CELLS**INCORPORATION BY REFERENCE OF MATERIAL SUBMITTED
ELECTRONICALLY**

[0001] This application contains, as a separate part of the disclosure, a Sequence Listing in computer-readable form which is incorporated by reference in its entirety and identified as follows: 54882_Seqlisting.txt; Size: 81,037 bytes; Created: October 19, 2020.

Field

[0002] The present disclosure relates to methods of targeting specific cell types within the cochlea using optimized gene therapy vectors. In particular, the disclosure provides gene therapy vectors to specifically target cochlear cells and methods of treating hearing impairment and hearing-related disorders.

Background

[0003] Hearing loss is a common sensory disorder worldwide, and many of pre-lingual deafness is due to genetic causes. More than 300 genetic loci linked to hereditary hearing loss and >100 causative genes have also been identified. Therefore, gene therapy is an attractive method of therapy for hearing loss. However, sensory cells of the adult mammalian cochlea lack the capacity for self-renewal and invasive methods of administration may add to the damage to these cells.

[0004] The human inner ear is a small, three-dimensionally complex, fluid-filled structure encased in the densest bone in the body and located deep in the base of skull. Acoustic energy from sound is transmitted to the fluids of the cochlea via vibrations of the tympanic membrane and ossicular chain in the middle ear, producing a traveling wave along the basilar membrane. The length of the cochlea and stiffness of the basilar membrane enables the differentiation of sound frequencies. This in turn leads to activation of mechanotransduction by hair cells, specialized sensory cells located in the organ of Corti, which turn mechanical stimulation into electrical depolarization. The electrical signal initiated by the inner hair cells (IHCs) is then processed by spiral ganglion neurons (SGNs) that make up the auditory nerve and ultimately decoded in the auditory cortex of the temporal lobe¹.

[0005] The organ of Corti includes two classes of sensory hair cells: inner hair cells (IHCs), which convert mechanical information carried by sound into electrical signals transmitted to neuronal structures, and outer hair cells (OHCs), which amplify and tune the

cochlear response, a process required for complex hearing function. Other potential targets in the inner ear include spiral ganglion neurons, columnar cells of the spiral limbus, which are important for the maintenance of the adjacent tectorial membrane, and supporting cells, which have protective functions and can be triggered to transdifferentiate into hair cells up to an early neonatal stage.

[0006] The inner ear is a difficult to access fluid-filled space. There are significant physical and diffusion barriers to accessing the inner hair cells, including the blood-labyrinthine barrier, and therefore systemic delivery of therapeutic agents to the inner ear is limited. The blood-labyrinthine may slow distribution of large molecules, such as viral vectors and other gene therapy reagents. In addition, disruption of the barrier may lead to leakage of high potassium into the perilymphatic spaces that bathe the basolateral surface of hair cells and neurons can chronically depolarize these cells and lead to cell death. Further, breach of the tight junctions between endolymph and perilymph can lead to rundown of the endocochlear potential, which would reduce the driving force for sensory transduction in hair cells and therefore result in reduced cochlear sensitivity and elevated auditory thresholds. (See Ahmed et al., J Assoc Res Otolaryngol. 18(5): 649–670, 2017)

[0007] It has been hypothesized that direct access to hair cells for gene therapy may be achievable through vector injection into the cochlear duct. However, injections directly into the cochlear duct could alter the delicate high-potassium endolymph fluid in the duct and thereby disrupt the endocochlear potential, leading to damage of the sensory cells and irreversible hearing loss. The perilymph-filled spaces surrounding the cochlear duct, scala tympani and scala vestibuli can be accessed from the middle ear, either through the oval window membrane or the round window membrane (RWM). The RWM, which is the only non-bony opening into the inner ear, is relatively easily accessible in many animal models, and administration of viral vector using this route has been well tolerated (Askew et al., Sci Transl Med. 7(295):295ra108, 2015, Chien et al., Mol Ther. 24(1):17-25, 2016, Chien et al., Laryngoscope.; 125(11):2557, 201).

[0008] Viral gene therapy vectors such as adenovirus, AAV, lentivirus, herpes simplex virus I, vaccinia virus have been tested in the cochlea, and these vectors only resulted in transient or suboptimal gene transfer (Fukui & Raphael, Hear Res. 297:99-105, 2013). Only adenovirus to date has progressed to a clinical program (Luebke et al. Adv. Otorhinolaryngol: 87-98, 2009). Previous studies of AAV serotypes *in vivo* inner ear injection via different

routes of administration highlighted the difficulties in targeting outer hair cells (OHCs), particularly via RWM injection (Liu et al., Mol Ther. 12(4):725-33, 2005), and resulted in only partial rescue of hearing in mouse models of inherited deafness (Akil et al., Neuron. 75(2):283-93, 2012, Askew et al., Science Trans. Med. 7:295ra108, 2015; Chien et al. Mol Ther. 24(1):17-25, 2016). AAV vector Anc80L65 was shown to transduce OHC with high efficiency when administered via injection into the round window membrane (Landegger et al., Biotechnology 35(3): 280-284, 2017).

[0009] In particular, there are many difficulties for targeting gene therapy vectors to the inner hair cells within the cochlea. For example, there is a very low number of inner hair cells in the cochlea. In addition, hair cells do not proliferate and the final number of hair cells is reached early in development and the number will not increase later in life.

[0010] Currently there are no biological treatments for hearing loss. Current state-of-art treatments focus on sound amplification and implanted electrodes that stimulate the auditory nerve. These strategies offer partial recovery of function for a limited patient population but do not come close to restoring natural hearing. Thus, there is a need for development of methods of delivering gene therapy vectors to the relevant cell types in the organ of Corti in the cochlea that are not invasive or damaging to the cochlear cells.

Summary

[0011] The disclosure provides gene therapy vectors that target specific cell types in the cochlea. These gene therapy vectors are useful for delivering a transgene to cells within the cochlea. The disclosure provides for methods of treating hearing loss comprising administering the gene therapy vectors using systemic delivery such as intravenous delivery, intratympanic delivery or intrathecal delivery or any other delivery method used to apply the vector directly into the cerebrospinal fluid. Gene therapy methods that target specific cells types have advantages for treating hearing loss.

[0012] The term “hearing loss” is interchangeable with the term “hearing impairment” and these terms refer to hearing determined by audiometry to be below threshold levels for normal hearing.

[0013] The disclosure provides for methods of delivering a transgene to a cochlear cell in a subject comprising administering to the subject a gene therapy vector encoding the transgene, wherein the gene therapy vector is administered to the subject using systemic delivery, such as intravenous (IV) delivery, intratympanic delivery, intrathecal delivery or any other

delivery method used to apply the vector directly into the cerebrospinal fluid. For example, the disclosed methods result in delivering the transgene to cochlear cells such as inner hair cells, outer hair cells, ganglion cells, supporting cells, Deiters' cells, pillar cells and/or epithelium cells.

[0014] The disclosure also provides for methods of treating hearing-loss, such as hereditary hearing loss, age-related hearing loss, or hearing-loss related to injury or disease comprising administering to the subject a gene therapy vector encoding a transgene, wherein the gene therapy vector is administered using systemic delivery such as intravenous (IV) delivery, intratympanic delivery, intrathecal delivery or any other delivery method used to apply the vector directly into the cerebrospinal fluid. The provided methods treat hearing loss caused by genetic mutation or hearing loss caused by damage.

[0015] For example, the disclosure provides for method of treating a subject suffering from a hearing loss-related disorder such as Waardenburg syndrome (WS), Branchiootorenal spectrum disorders, Neurofibromatosis 2 (NF2), Stickler syndrome, Usher syndrome type I, Usher syndrome type II, Usher syndrome type III, Pendred syndrome, Jervell and Lange-Nielsen syndrome, Biotinidase deficiency Refsum disease, Alport syndrome, Deafness-dystonia-optic neuropathy syndrome, or Mohr-Tranebjaerg syndrome.

[0016] The disclosure also provides for compositions for delivering a transgene to a cochlear cell in a subject, wherein the composition comprises a gene therapy vector encoding the transgene, and wherein the composition is formulated for administration using systemic delivery, such as intravenous (IV) delivery, intratympanic delivery, intrathecal delivery or any other delivery method used to apply the vector directly into the cerebrospinal fluid. For example, the disclosed compositions deliver the transgene to cochlear cells such as inner hair cells, outer hair cells, ganglion cells, supporting cells, Deiters' cells, pillar cells and/or epithelium cells.

[0017] The disclosure also provides for composition for treating hearing-loss, such as hereditary hearing loss, age-related hearing loss, or hearing-loss related to injury or disease, wherein the composition comprises a gene therapy vector encoding a transgene, and wherein the composition is formulated for systemic delivery such as intravenous (IV) delivery, intratympanic delivery, intrathecal delivery or any other delivery method used to apply the vector directly into the cerebrospinal fluid. The provided compositions are useful for treating hearing loss caused by genetic mutation or hearing loss caused by damage.

[0018] For example, the disclosure provides for compositions for treating a subject suffering from a hearing loss-related disorder such as Waardenburg syndrome (WS), Branchiootorenal spectrum disorders, Neurofibromatosis 2 (NF2), Stickler syndrome, Usher syndrome type I, Usher syndrome type II, Usher syndrome type III, Pendred syndrome, Jervell and Lange-Nielsen syndrome, Biotinidase deficiency Refsum disease, Alport syndrome, Deafness-dystonia-optic neuropathy syndrome, or Mohr-Tranebjaerg syndrome.

[0019] The disclosure also provides for use of a gene therapy vector encoding a transgene for the preparation of a medicament for delivering a transgene to a cochlear cell in a subject, wherein the gene therapy encodes the transgene, and wherein the medicament is formulated for systemic delivery, such as intravenous (IV) delivery, intratympanic delivery, intrathecal delivery or any other delivery method used to apply the vector directly into the cerebrospinal fluid. For example, use of disclosed gene therapy vectors results in delivering the transgene to cochlear cells such as inner hair cells, outer hair cells, ganglion cells, supporting cells, Deiters' cells, pillar cells and epithelium cells.

[0020] The disclosure also provides use of a gene therapy vector for the preparation of a medicament for treating hearing-loss, such as hereditary hearing loss, age-related hearing loss, or hearing-loss related to injury or disease comprising administering to the subject a gene therapy vector encoding a transgene, wherein the medicament is formulated for systemic delivery such as intravenous (IV) delivery, intratympanic delivery, intrathecal delivery or any other delivery method used to apply the vector directly into the cerebrospinal fluid. For example, the disclosed gene therapy vectors may be used for preparation of a medicament for treating hearing loss caused by genetic mutation or hearing loss caused by damage.

[0021] For example, the disclosure provides for medicaments for treating a subject suffering from a hearing loss-related disorder such as Waardenburg syndrome (WS), Branchiootorenal spectrum disorders, Neurofibromatosis 2 (NF2), Stickler syndrome, Usher syndrome type I, Usher syndrome type II, Usher syndrome type III, Pendred syndrome, Jervell and Lange-Nielsen syndrome, Biotinidase deficiency Refsum disease, Alport syndrome, Deafness-dystonia-optic neuropathy syndrome, or Mohr-Tranebjaerg syndrome.

[0022] The disclosed methods, compositions and uses deliver any transgene of interest to a cochlear cell. The transgene is a polynucleotide sequence that encodes a polypeptide of interest or is a nucleic acid that inhibits, interferes or silences expression of a gene of interest,

such as a siRNA or miRNA. Exemplary transgenes are polynucleotides that encode human atonal transcription factor (ATOH1) (Genbank Accession no. NM_005172.2; SEQ ID NO: 2), otoferlin (SEQ ID NO: 4), gap junction protein beta 2 (Genbank Accession no. NM_004004.6; SEQ ID NO: 6), pendrin (SLC26A) (Genbank Accession No. XM_006716025.3; SEQ ID NO: 8), forkhead box 1 (FOXP1) (Genbank Accession No. NM_005249; SEQ ID NO: 10), activin A or Inhibin (Genbank Accession No. NM_002192; SEQ ID No: 12), follistatin (FST) (SEQ ID NO: 14), galectin-1 (Genbank Accession No. NM_002305.4; SEQ ID NO: 19) or galectin-3 (Genbank Accession No. AB006780.1; SEQ ID NO: 20). In addition, the transgenes are a wild type nucleotide sequence of a gene set out in Table 1, Table 2, Table 3, Table 4 or Table 5 herein.

[0023] In any of the disclosed methods, composition or uses, the gene therapy vector is AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAVRH10, AAVRH74, AAV11, AAV12, AAV13, AAVTT or Anc80, AAV7m8 and their derivatives.

[0024] In any of the disclosed methods, the gene therapy vector comprises the CB promoter (SEQ ID NO: 1), P546 promoter (SEQ ID NO: 16), CMV promoter (SEQ ID NO: 17 or the Myo7A promoter (SEQ ID NO: 18).

[0025] In addition, in any of the disclosed methods, compositions or uses, the gene therapy vector, composition or medicament is administered using intrathecal delivery, the subject is placed in the Trendelenburg position after administering of the gene therapy vector, composition or medicament.

Brief Description of the Drawings

[0026] **Figure 1** provides the schematic of the AAV vector tested in this disclosure.

[0027] **Figure 2** demonstrates delivery of the GFP transgene to the cochlear after intrathecal injection of the vector. The sagittal cryosection of a cochlea is stained with DAPI and GFP. The hematoxylin and eosin stain is provided for reference only.

[0028] **Figure 3** provides staining of DAPI and GFP in the cochlea from 4 different mice (shx2IT-LF, shx2IT-2F, shx2IT-LE and shx2IT-LFLR) after intrathecal injection of the AAV vector.

[0029] **Figure 4** provides staining of DAPI and GFP in the cochlea from a mouse after intrathecal injection of the AAV vector, and the different areas of the mouse cochlea are indicated.

[0030] **Figure 5** provides staining for DAPI, GFP and actin in the cochlea from a mouse after intrathecal injection of the AAV vector.

[0031] **Figure 6** provides staining for DAPI and GFP in the cochlea from a mouse after intrathecal injection for DAPI and GFP. The cut on this case is directed in a different orientation to allow the visualization of the targeting throughout the different areas of the cochlea.

[0032] **Figure 7** provides staining for DAPI and GFP in the cochlea from a mouse after intrathecal injection. The cut in this case is directed in a different orientation to allow the visualization of the targeting throughout the different areas of the cochlea.

[0033] **Figure 8** provides staining for DAPI, GFP and Actin in the vestibule of the cochlea from a mouse after intrathecal injection.

[0034] **Figure 9** provides staining for DAPI, GFP and Actin (phalloidin) in the cochlea from a mouse which was exposed to injury causing noise and the AAV was injected immediately after exposure to noise. The numbered sections are regions that are imaged at a higher magnification.

[0035] **Figure 10** provides staining for DAPI, GFP and Actin (phalloidin) in the cochlea from a mouse which was exposed to injury causing noise and the AAV was injected immediately after exposure to noise. The section labeled 1 is a region of the cochlea that is imaged at a higher magnification.

[0036] **Figure 11** provides staining for DAPI, GFP and Actin (phalloidin) in the cochlea from a mouse which was exposed to injury causing noise and the AAV was injected immediately after exposure to noise. The numbered sections are regions that are imaged at a higher magnification.

[0037] **Figure 12** provides staining for DAPI, GFP and Actin (phalloidin) in the cochlea from a mouse which was exposed to injury causing noise and the AAV was injected immediately after exposure to noise. The numbered sections are regions that are imaged at a higher magnification.

[0038] **Figure 13** provides staining for DAPI, GFP and myo7A in the cochlea from a mouse which was exposed to noise and the AAV was injected intrathecally 24 hours after exposure to noise.

[0039] **Figure 14** provides staining for DAPI, GFP and myo7A in the cochlea from a mouse which was exposed to noise and the AAV was injected intrathecally 24 hours after exposure to noise. The numbered sections are regions that are imaged at a higher magnification.

[0040] **Figure 156** provides staining for DAPI, GFP and myo7A in the cochlea from multiple mice which were exposed to noise and the AAV was injected intrathecally immediately after (0hpi) or 24 hours (24hpi) after exposure to noise.

[0041] **Figure 16** provides staining for DAPI and GFP in a cochlea from a mouse which was exposed to noise and the AAV was injected immediately after (0hpi) exposure to noise. This photo is imaged at the optimal exposure of the 24hpi IT injected mice. This photo demonstrates that GFP expression in the 24hpi intrathecal injected animals is lower than in 0hpi intrathecal injected animals, as imaging at the optimal exposure for 24hpi overexposes 0hpi.

[0042] **Figure 17** provides hematoxylin and eosin stain of a cochlear nerve. The abbreviations indicated the locations of structures in the Organ of Corti. SL=Spiral limbus; TM=Tectorial membrane; OHC=Outer hair cells; IHC=Inner hair cells; BM=Basilar membrane.

[0043] **Figure 18** provides staining for DAPI and GFP in the cochlea from a mouse after intravenous injection of AAV.

[0044] **Figure 19** provides staining for DAPI, GFP and MyoD in the cochlea from multiple mice after intravenous injection of AAV. Each panel is an image of the cochlea from a different mouse (top row is 10x and bottom row is 20x)

[0045] **Figure 20** provides staining for DAPI and GFP in the cochlea from a mouse after intravenous injection of AAV without noise damage.

[0046] **Figure 21** provides staining for DAPI and GFP in the cochlea from a mouse after intravenous injection of AAV without noise damage.

[0047] **Figure 22** provides staining for DAPI and GFP in the cochlea from a mouse after intravenous injection of AAV without noise damage.

[0048] **Figure 23** provides staining for DAPI and GFP in the cochlea from a that control mouse that was exposed to noise damage but did not receive an intravenous injection of AAV.

Detailed Description

[0049] The disclosure provides optimization of AAV gene therapy for targeting the cochlea to treat hearing loss disorders. The data provided herein focuses on administration of AAV9 vectors, but the disclosure contemplates using any gene therapy vector that comprises a promoter that specifically targets a cochlear cell, and these optimized vectors are administered using intravenous (IV) delivery or intrathecal delivery or any other delivery method that accesses the cerebrospinal fluid (CSF). For example, the data demonstrated that AAV9 injected directly into the cerebrospinal fluid via intrathecal injection was effective in targeting transgene expression in the inner hair cells of the cochlea. Thus, intrathecal injections can be used to deliver gene therapy vectors to the cochlea and specifically for delivering gene therapy vectors to inner hair cells.

Gene Therapy Vectors

[0050] Adeno-associated virus (AAV) is a replication-deficient parvovirus, the single-stranded DNA genome of which is about 4.7 kb in length including two 145 nucleotide inverted terminal repeats (ITRs) and may be used to refer to the virus itself or derivatives thereof. The term covers all subtypes and both naturally occurring and recombinant forms, except where specified otherwise. There are multiple serotypes of AAV. The serotypes of AAV are each associated with a specific clade, the members of which share serologic and functional similarities. Thus, AAVs may also be referred to by the clade. For example, AAV9 sequences are referred to as “clade F” sequences (Gao et al., J. Virol., 78: 6381-6388 (2004)). The present disclosure contemplates the use of any sequence within a specific clade, e.g., clade F. The nucleotide sequences of the genomes of the AAV serotypes are known. For example, the complete genome of AAV-1 is provided in GenBank Accession No. NC_002077; the complete genome of AAV-2 is provided in GenBank Accession No. NC_001401 and Srivastava et al., J. Virol., 45: 555-564 (1983); the complete genome of AAV-3 is provided in GenBank Accession No. NC_1829; the complete genome of AAV-4 is provided in GenBank Accession No. NC_001829; the AAV-5 genome is provided in GenBank Accession No. AF085716; the complete genome of AAV-6 is provided in GenBank Accession No. NC_001862; at least portions of AAV-7 and AAV-8 genomes are provided in GenBank Accession Nos. AX753246 and AX753249, respectively; the AAV-9 genome is provided in Gao et al., J. Virol., 78: 6381-6388 (2004); the AAV-10 genome is provided in *Mol. Ther.*, 13(1): 67-76 (2006); the AAV-11 genome is provided in *Virology*, 330(2): 375-

383 (2004); portions of the AAV-12 genome are provided in Genbank Accession No. DQ813647; portions of the AAV-13 genome are provided in Genbank Accession No. EU285562. The sequence of the AAV rh.74 genome is provided in see U.S. Patent 9,434,928, incorporated herein by reference. The sequence of the AAV-B1 genome is provided in Choudhury *et al.*, *Mol. Ther.*, 24(7): 1247-1257 (2016). Anc80 is an AAV vector that is of AAV1, AAV2, AAV8 and AAV9. The sequence of Anc80 is provided in Zinn *et al.*, *Cell Reports* 12: 1056-1068, 2015, Vandenberghe *et al.*, PCT/US2014/060163, both of which are incorporated by reference herein, in their entirety and GenBank Accession Nos. KT235804–KT235812.

[0051] *Cis*-acting sequences directing viral DNA replication (rep), encapsidation/packaging and host cell chromosome integration are contained within the ITRs. Three AAV promoters (named p5, p19, and p40 for their relative map locations) drive the expression of the two AAV internal open reading frames encoding rep and cap genes. The two rep promoters (p5 and p19), coupled with the differential splicing of the single AAV intron (at nucleotides 2107 and 2227), result in the production of four rep proteins (rep 78, rep 68, rep 52, and rep 40) from the rep gene. Rep proteins possess multiple enzymatic properties that are ultimately responsible for replicating the viral genome. The cap gene is expressed from the p40 promoter and it encodes the three capsid proteins VP1, VP2, and VP3. Alternative splicing and non-consensus translational start sites are responsible for the production of the three related capsid proteins. A single consensus polyadenylation site is located at map position 95 of the AAV genome. The life cycle and genetics of AAV are reviewed in Muzyczka, *Current Topics in Microbiology and Immunology*, 158: 97-129 (1992).

[0052] AAV possesses unique features that make it attractive as a vector for delivering foreign DNA to cells, for example, in gene therapy. AAV infection of cells in culture is noncytopathic, and natural infection of humans and other animals is silent and asymptomatic. Moreover, AAV infects many mammalian cells allowing the possibility of targeting many different tissues *in vivo*. Moreover, AAV transduces slowly dividing and non-dividing cells, and can persist essentially for the lifetime of those cells as a transcriptionally active nuclear episome (extrachromosomal element). The native AAV proviral genome is infectious as cloned DNA in plasmids which makes construction of recombinant genomes feasible. Furthermore, because the signals directing AAV replication, genome encapsidation and integration are contained within the ITRs of the AAV genome, some or all of the internal

approximately 4.3 kb of the genome (encoding replication and structural capsid proteins, rep-cap) may be replaced with foreign DNA such as a gene cassette containing a promoter, a DNA of interest and a polyadenylation signal. In some instances, the rep and cap proteins are provided *in trans*. Another significant feature of AAV is that it is an extremely stable and hearty virus. It easily withstands the conditions used to inactivate adenovirus (56° to 65°C for several hours), making cold preservation of AAV less critical. AAV may even be lyophilized. Finally, AAV-infected cells are not resistant to superinfection.

[0053] The term “AAV” as used herein refers to the wild type AAV virus or viral particles. The terms “AAV,” “AAV virus,” and “AAV viral particle” are used interchangeably herein. The term “rAAV” refers to a recombinant AAV virus or recombinant infectious, encapsulated viral particles. The terms “rAAV,” “rAAV virus,” and “rAAV viral particle” are used interchangeably herein.

[0054] The term “rAAV genome” refers to a polynucleotide sequence that is derived from a native AAV genome that has been modified. In some embodiments, the rAAV genome has been modified to remove the native cap and rep genes. In some embodiments, the rAAV genome comprises the endogenous 5’ and 3’ inverted terminal repeats (ITRs). In some embodiments, the rAAV genome comprises ITRs from an AAV serotype that is different from the AAV serotype from which the AAV genome was derived. In some embodiments, the rAAV genome comprises a transgene of interest flanked on the 5’ and 3’ ends by inverted terminal repeat (ITR). In some embodiments, the rAAV genome comprises a “gene cassette.”

[0055] The term “scAAV” refers to a rAAV virus or rAAV viral particle comprising a self-complementary genome. The term “ssAAV” refers to a rAAV virus or rAAV viral particle comprising a single-stranded genome.

[0056] The rAAV genomes provided herein, in some embodiments, comprise one or more AAV ITRs flanking the transgene polynucleotide sequence. The transgene polynucleotide sequence is operatively linked to transcriptional control elements (including, but not limited to, promoters, enhancers and/or polyadenylation signal sequences) that are functional in target cells to form a gene cassette. Examples of promoters are the CMV promoter, chicken β actin promoter (CB), the P546 promoter and the Myo7A promoter. Additional promoters are contemplated herein including, but not limited to the simian virus 40 (SV40) early promoter, mouse mammary tumor virus (MMTV), human immunodeficiency virus (HIV) long terminal

repeat (LTR) promoter, MoMuLV promoter, an avian leukemia virus promoter, an Epstein-Barr virus immediate early promoter, a Rous sarcoma virus promoter, as well as human gene promoters such as, but not limited to, the actin promoter, the myosin promoter, the elongation factor-1a promoter, the hemoglobin promoter, and the creatine kinase promoter.

[0057] Additionally provided herein are a CMV promoter sequence, a CB promoter sequence, a P546 promoter sequence, Myo7A promoter and promoter sequences at least: 65%, 70%, 75%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the nucleotide sequence of the CMV (SEQ ID NO: 17), CB (SEQ ID NO: 1) Myo7A (SEQ ID NO: 18) or P546 (SEQ ID NO: 16) sequence which exhibit transcription promoting activity.

[0058] Other examples of transcription control elements are tissue specific control elements, for example, promoters that allow expression specifically within neurons or specifically within astrocytes. Examples include neuron specific enolase and glial fibrillary acidic protein promoters. Inducible promoters are also contemplated. Non-limiting examples of inducible promoters include, but are not limited to a metallothionine promoter, a glucocorticoid promoter, a progesterone promoter, and a tetracycline-regulated promoter. The gene cassette may also include intron sequences to facilitate processing of a transgene RNA transcript when expressed in mammalian cells. One example of such an intron is the SV40 intron.

[0059] “Packaging” refers to a series of intracellular events that result in the assembly and encapsidation of an AAV particle. The term “production” refers to the process of producing the rAAV (the infectious, encapsulated rAAV particles) by the packing cells.

[0060] AAV “rep” and “cap” genes refer to polynucleotide sequences encoding replication and encapsidation proteins, respectively, of adeno-associated virus. AAV rep and cap are referred to herein as AAV “packaging genes.”

[0061] A “helper virus” for AAV refers to a virus that allows AAV (e.g. wild-type AAV) to be replicated and packaged by a mammalian cell. A variety of such helper viruses for AAV are known in the art, including adenoviruses, herpesviruses and poxviruses such as vaccinia. The adenoviruses may encompass a number of different subgroups, although Adenovirus type 5 of subgroup C is most commonly used. Numerous adenoviruses of human, non-human mammalian and avian origin are known and available from depositories such as the ATCC. Viruses of the herpes family include, for example, herpes simplex viruses (HSV) and

Epstein-Barr viruses (EBV), as well as cytomegaloviruses (CMV) and pseudorabies viruses (PRV); which are also available from depositories such as ATCC.

[0062] “Helper virus function(s)” refers to function(s) encoded in a helper virus genome which allow AAV replication and packaging (in conjunction with other requirements for replication and packaging described herein). As described herein, “helper virus function” may be provided in a number of ways, including by providing helper virus or providing, for example, polynucleotide sequences encoding the requisite function(s) to a producer cell in trans.

[0063] The rAAV genomes provided herein lack AAV rep and cap DNA. AAV DNA in the rAAV genomes (*e.g.*, ITRs) contemplated herein may be from any AAV serotype suitable for deriving a recombinant virus including, but not limited to, AAV serotypes Anc80, Anc80L65, AAV-1, AAV-2, AAV-3, AAV-4, AAV-5, AAV-6, AAV-7, AAV7mb, AAV-8, AAV-9, AAV-10, AAV-RH10, AAV-11, AAV-12, AAV-13, AAV rh.74 and AAV-B1 and their derivatives. As noted above, the nucleotide sequences of the genomes of various AAV serotypes are known in the art. rAAV with capsid mutations, are also contemplated. See, for example, Marsic *et al.*, *Molecular Therapy*, 22(11): 1900-1909 (2014). Modified capsids herein are also contemplated and include capsids having various post-translational modifications such as glycosylation and deamidation. Deamidation of asparagine or glutamine side chains resulting in conversion of asparagine residues to aspartic acid or isoaspartic acid residues, and conversion of glutamine to glutamic acid or isoglutamic acid is contemplated in rAAV capsids provided herein. See, for example, Giles *et al.*, *Molecular Therapy*, 26(12): 2848-2862 (2018). Modified capsids herein are also contemplated to comprise targeting sequences directing the rAAV to the affected tissues and organs requiring treatment.

[0064] DNA plasmids provided herein comprise rAAV genomes described herein. The DNA plasmids may be transferred to cells permissible for infection with a helper virus of AAV (*e.g.*, adenovirus, E1-deleted adenovirus or herpesvirus) for assembly of the rAAV genome into infectious viral particles with AAV9 capsid proteins. Techniques to produce rAAV, in which an rAAV genome to be packaged, rep and cap genes, and helper virus functions are provided to a cell are standard in the art. Production of rAAV particles requires that the following components are present within a single cell (denoted herein as a packaging cell): a rAAV genome, AAV rep and cap genes separate from (*i.e.*, not in) the rAAV genome,

and helper virus functions. The AAV rep and cap genes may be from any AAV serotype for which recombinant virus can be derived and may be from a different AAV serotype than the rAAV genome ITRs. Production of pseudotyped rAAV is disclosed in, for example, WO 01/83692 which is incorporated by reference herein in its entirety. In various embodiments, AAV capsid proteins may be modified to enhance delivery of the recombinant rAAV. Modifications to capsid proteins are generally known in the art. See, for example, US 2005/0053922 and US 2009/0202490, the disclosures of which are incorporated by reference herein in their entirety.

[0065] A method of generating a packaging cell is to create a cell line that stably expresses all the necessary components for rAAV production. For example, a plasmid (or multiple plasmids) comprising a rAAV genome lacking AAV rep and cap genes, AAV rep and cap genes separate from the rAAV genome, and a selectable marker, such as a neomycin resistance gene, may be integrated into the genome of a cell. rAAV genomes may be introduced into bacterial plasmids by procedures such as GC tailing (Samulski et al., 1982, Proc. Natl. Acad. Sci. USA, 79:2077-2081), addition of synthetic linkers containing restriction endonuclease cleavage sites (Laughlin et al., 1983, Gene, 23:65-73) or by direct, blunt-end ligation (Senapathy & Carter, 1984, J. Biol. Chem., 259:4661-4666). The packaging cell line may then be infected with a helper virus such as adenovirus. The advantages of this method are that the cells are selectable and are suitable for large-scale production of rAAV. Other non-limiting examples of suitable methods employ adenovirus or baculovirus rather than plasmids to introduce rAAV genomes and/or rep and cap genes into packaging cells.

[0066] General principles of rAAV particle production are reviewed in, for example, Carter, 1992, Current Opinions in Biotechnology, 1533-539; and Muzyczka, 1992, Curr. Topics in Microbial. and Immunol., 158:97-129). Various approaches are described in Ratschin et al., Mol. Cell. Biol. 4:2072 (1984); Hermonat et al., Proc. Natl. Acad. Sci. USA, 81:6466 (1984); Tratschin et al., Mol. Cell. Biol. 5:3251 (1985); McLaughlin et al., J. Virol., 62:1963 (1988); and Lebkowski et al., 1988 Mol. Cell. Biol., 7:349 (1988). Samulski et al. (1989, J. Virol., 63:3822-3828); U.S. Patent No. 5,173,414; WO 95/13365 and corresponding U.S. Patent No. 5,658,776 ; WO 95/13392; WO 96/17947; PCT/US98/18600; WO 97/09441 (PCT/US96/14423); WO 97/08298 (PCT/US96/13872); WO 97/21825 (PCT/US96/20777); WO 97/06243 (PCT/FR96/01064); WO 99/11764; Perrin et al. (1995) Vaccine 13:1244-1250; Paul et al. (1993) Human Gene Therapy 4:609-615; Clark et al. (1996) Gene Therapy

3:1124-1132; U.S. Patent. No. 5,786,211; U.S. Patent No. 5,871,982; and U.S. Patent. No. 6,258,595. The foregoing documents are hereby incorporated by reference in their entirety herein, with particular emphasis on those sections of the documents relating to rAAV particle production.

[0067] Further provided herein are packaging cells that produce infectious rAAV particles. In one embodiment packaging cells may be stably transformed cancer cells such as HeLa cells, 293 cells and PerC.6 cells (a cognate 293 line). In another embodiment, packaging cells may be cells that are not transformed cancer cells such as low passage 293 cells (human fetal kidney cells transformed with E1 of adenovirus), MRC-5 cells (human fetal fibroblasts), WI-38 cells (human fetal fibroblasts), Vero cells (monkey kidney cells) and FRhL-2 cells (rhesus fetal lung cells).

[0068] Also provided herein are rAAV (*e.g.*, infectious encapsidated rAAV particles) comprising a rAAV genome of the disclosure. The genomes of the rAAV lack AAV rep and cap DNA, that is, there is no AAV rep or cap DNA between the ITRs of the genomes of the rAAV. The rAAV genome can be a self-complementary (sc) genome. A rAAV with a sc genome is referred to herein as a scAAV. The rAAV genome can be a single-stranded (ss) genome. A rAAV with a single-stranded genome is referred to herein as an ssAAV.

[0069] The rAAV may be purified by methods standard in the art such as by column chromatography or cesium chloride gradients. Methods for purifying rAAV from helper virus are known in the art and may include methods disclosed in, for example, Clark *et al.*, *Hum. Gene Ther.*, 10(6): 1031-1039 (1999); Schenpp and Clark, *Methods Mol. Med.*, 69: 427-443 (2002); U.S. Patent No. 6,566,118 and WO 98/09657.

[0070] Compositions comprising rAAV are also provided. Compositions comprise a rAAV encoding a polypeptide of interest. Compositions may include two or more rAAV encoding different polypeptides of interest. In some embodiments, the rAAV is scAAV or ssAAV.

[0071] Compositions provided herein comprise rAAV and a pharmaceutically acceptable excipient or excipients. Acceptable excipients are nontoxic to recipients and are preferably inert at the dosages and concentrations employed, and include, but are not limited to, buffers such as phosphate *e.g.*, phosphate-buffered saline (PBS), citrate, or other organic acids; antioxidants such as ascorbic acid; low molecular weight polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as

polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, arginine or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrans; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; salt-forming counterions such as sodium; and/or nonionic surfactants such as Tween, copolymers such as poloxamer 188, pluronics (e.g., Pluronic F68) or polyethylene glycol (PEG).

Compositions provided herein can comprise a pharmaceutically acceptable aqueous excipient containing a non-ionic, low-osmolar compound or contrast agent such as iobitridol, iohexol, iomeprol, iopamidol, iopentol, iopromide, ioversol, or ioxilan, where the aqueous excipient containing the non-ionic, low-osmolar compound can have one or more of the following characteristics: about 180 mgI/mL, an osmolality by vapor-pressure osmometry of about 322mOsm/kg water, an osmolarity of about 273mOsm/L, an absolute viscosity of about 2.3cp at 20°C and about 1.5cp at 37°C, and a specific gravity of about 1.164 at 37°C. Exemplary compositions comprise about 20 to 40% non-ionic, low-osmolar compound or about 25% to about 35% non-ionic, low-osmolar compound. An exemplary composition comprises scAAV or rAAV viral particles formulated in 20mM Tris (pH8.0), 1mM MgCl₂, 200mM NaCl, 0.001% poloxamer 188 and about 25% to about 35% non-ionic, low-osmolar compound. Another exemplary composition comprises scAAV formulated in and 1X PBS and 0.001% Pluronic F68.

[0072] Dosages may be expressed in units of viral genomes (vg). Dosages contemplated herein include about 1×10^7 vg, about 1×10^8 vg, about 1×10^9 vg, about 5×10^9 vg, about 6×10^9 vg, about 7×10^9 vg, about 8×10^9 vg, about 9×10^9 vg, about 1×10^{10} vg, about 2×10^{10} vg, about 3×10^{10} vg, about 4×10^{10} vg, about 5×10^{10} vg, about 1×10^{11} vg, about 1.1×10^{11} vg, about 1.2×10^{11} vg, about 1.3×10^{11} vg, about 1.4×10^{11} vg, about 1.5×10^{11} vg, about 1.6×10^{11} vg, about 1.7×10^{11} vg, about 1.8×10^{11} vg, about 1.9×10^{11} vg, about 2×10^{11} vg, about 3×10^{11} vg, about 4×10^{11} vg, about 5×10^{11} vg, about 1×10^{12} vg, about 1×10^{13} vg, about 1.1×10^{13} vg, about 1.2×10^{13} vg, about 1.3×10^{13} vg, about 1.5×10^{13} vg, about 2×10^{13} vg, about 2.5×10^{13} vg, about 3×10^{13} vg, about 3.5×10^{13} vg, about 4×10^{13} vg, about 4.5×10^{13} vg, about 5×10^{13} vg, about 6×10^{13} vg, about 1×10^{14} vg, about 2×10^{14} vg, about 3×10^{14} vg, about 4×10^{14} vg, about 5×10^{14} vg, about 1×10^{15} vg, to about 1×10^{16} vg, or more total viral genomes. Dosages of about 1×10^9 vg to about 1×10^{10} vg, about 5×10^9 vg to about 5×10^{10} vg, about 1×10^{10} vg to about 1×10^{11} vg, about 1×10^{11} vg to about 1×10^{15} vg, about 1×10^{12} vg to about 1×10^{15} vg, about 1×10^{12} vg to about 1×10^{14} vg, about 1×10^{13} vg to about 6×10^{14} vg,

and about 6×10^{13} vg to about 1.0×10^{14} vg, 2.0×10^{14} vg, 3.0×10^{14} vg, 5.0×10^{14} are also contemplated. One dose exemplified herein is 1.65×10^{11} vg.

[0073] Methods of transducing target cochlear cells with rAAV are provided. The cochlear cells include inner hair cells, outer hair cells, ganglion cells, supporting cells, Deiters' cells, pillar cells and epithelium cells.

[0074] The term "transduction" is used to refer to the administration/delivery of the CLN6 polynucleotide to a target cell either *in vivo* or *in vitro*, via a replication-deficient rAAV of the disclosure resulting in expression of a functional polypeptide by the recipient cell. Transduction of cells with rAAV of the disclosure results in sustained expression of polypeptide or RNA encoded by the rAAV. The present disclosure thus provides methods of administering/delivering to a subject rAAV encoding a transgene encoded polypeptide by an intrathecal or IV delivery, or any combination thereof. Intrathecal delivery refers to delivery into the space under the arachnoid membrane of the brain or spinal cord. In some embodiments, intrathecal administration is via intracisternal administration.

Disorders Related to Hearing Loss

[0075] The disclosure provides methods of treating hearing loss. Conductive hearing loss results from abnormalities of the external ear and/or the ossicles of the middle ear.

Sensorineural hearing loss results from malfunction of inner ear structures (i.e., cochlea or auditory nerve). Mixed hearing loss is a combination of conductive and sensorineural hearing loss. Central auditory dysfunction results from damage or dysfunction at the level of the eighth cranial nerve, auditory brain stem, or cerebral cortex.

[0076] Genetic hearing loss is subcategorized into Mendelian inheritance including both syndromic and non-syndromic cases, or complex inheritance which includes both genetic and environmental factors. A summary of the genetic causes of syndromic hearing loss are provided in Table 1. A summary of the genetic causes of nonsyndromic hearing loss are provided in Table 2 and Table 3. A summary of the X-linked nonsyndromic hearing Loss is provided in Table 4 (See Shearer AE, Hildebrand MS, Smith RJH. Hereditary Hearing Loss and Deafness Overview. 1999 Feb 14 [Updated 2017 Jul 27]. In: Adam MP, Ardinger HH, Pagon RA, et al., editors. GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2019.)

Table 1

Syndrome	Mode of Inheritance	Genes	Hearing Impairment		
			Type	Onset	Severity
Waardenburg syndrome (WS)	Autosomal Dominant	<i>PAX3</i> <i>MITF</i> <i>EDNRB</i> <i>EDN3</i> <i>SOX10</i>	WS1: sensorineural	Congenital	Variable
Branchiootorenal spectrum disorders	Autosomal Dominant	<i>EYA1</i> <i>SIX1</i> <i>SIX5</i>	Conductive; sensorineural; mixed	Variable	Variable
Neurofibromatosis 2 (NF2)	Autosomal Dominant	<i>NF2</i>	sensorineural	~3rd decade	Generally unilateral & gradual; can be bilateral & sudden
Stickler syndrome	Autosomal Dominant	<i>COL2A1</i> <i>COL11A1</i> <i>COL11A2</i> <i>COL9A1</i> <i>COL9A2</i> <i>COL9A3</i>	Conductive, sensorineural	Variable	Variable
Usher syndrome type I	Autosomal Recessive	<i>MYO7A</i> <i>USH1C</i> <i>CDH23</i> <i>PCDH15</i> <i>USH1G</i> <i>CIB2</i>	sensorineural	Congenital	Severe to profound
Usher syndrome type II	Autosomal Recessive	<i>ADGRV1</i> <i>WHRN</i> <i>USH2A</i>	sensorineural	Congenital	Mild to severe
Usher syndrome type III	Autosomal Recessive	<i>CLRN1</i> <i>HARS1</i>	sensorineural	Congenital	Progressive
Pendred syndrome	Autosomal Recessive	<i>SLC26A4</i>	sensorineural	Congenital	Usually (but not invariably) severe to profound
Jervell and Lange-Nielsen syndrome	Autosomal Recessive	<i>KCNQ1</i> <i>KCNE1</i>	sensorineural	Congenital	Profound Severe; progressive
Biotinidase deficiency	Autosomal Recessive	<i>BTD</i>	sensorineural	Variable	Variable
Refsum disease	Autosomal Recessive	<i>PHYH</i> <i>PEX7</i>	sensorineural	Variable	severity; progressive
Alport syndrome	X-linked (85%)	<i>COL4A5</i> <i>COL4A3</i> ⁷	sensorineural	After age 10	Varying severity;

	Autosomal Recessive (15%)	<i>COL4A4</i>			progressive
Deafness-dystonia-optic neuropathy syndrome (Mohr-Tranebjaerg syndrome)	X-linked	<i>TIMM8A</i>	sensorineural	Early childhood	Progressive; pre- or postlingual

Table 2: Autosomal Dominant Nonsyndromic Hearing Loss:

Gene	Locus	Onset/Decade	Audioprofile
<i>ACTG1</i>	DFNA20/26	Postlingual	High frequency; progressive
<i>CCDC50</i>	DFNA440	Postlingual	Low to mild frequencies; progressive
<i>CD164</i>	DFNA66	Postlingual	Flat or mid-frequency; progressive
<i>CEACAM16</i>	DFNA4B	Postlingual	Flat; progressive
<i>COCH</i>	DFNA9	Postlingual/2nd	High frequency; progressive
<i>COL11A2</i>	DFNA13	Postlingual/2nd	Mid-frequency loss
<i>GSDME</i>	DFNA5	Postlingual/1st	High frequency; progressive
<i>DIAPH1</i>	DFNA1	Postlingual/1st	Low frequency; progressive
<i>DMXL2</i>	-	Postlingual/2nd	Flat; progressive
<i>DSPP</i>	DFNA39	Postlingual	High frequency; progressive
<i>EYA4</i>	DFNA10	Postlingual/3rd, 4th	Flat/gently downsloping
<i>GJB2</i> ¹	DFNA3	Prelingual	High frequency; progressive
<i>GJB3</i>	DFNA2B	Postlingual/4th	High frequency; progressive
<i>GJB6</i> ¹	DFNA3	Prelingual	High frequency; progressive
<i>GRHL2</i>	DFNA28	Postlingual	Flat/gently downsloping
<i>HOMER2</i>	DFNA68	Postlingual/1st	High frequency; progressive
<i>KCNO4</i>	DFNA2	Postlingual/2nd	High frequency; progressive
<i>MIR96</i>	DFNA50	Postlingual/2nd	Flat; progressive
<i>MCM2</i>	DFNA70	Postlingual	High frequency; progressive
<i>MYH14</i>	DFNA4	Postlingual	Flat/gently downsloping
<i>MYH9</i>	DFNA17	Postlingual	High frequency; progressive
<i>MYO1A</i>	DFNA48	Postlingual	Progressive
<i>MYO6</i>	DFNA22	Postlingual	High frequency; progressive
<i>MYO7A</i> ²	DFNA11	Postlingual/1st	Flat/gently downsloping
<i>OSBPL2</i>	DFNA67	Postlingual	High frequency; progressive
<i>P2RX2</i>	DFNA41	Postlingual	Flat; progressive
<i>POU4F3</i>	DFNA15	Postlingual	High frequency; progressive
<i>SIX1</i>	DFNA23	Prelingual	Downsloping
<i>SLC17A8</i>	DFNA25	Postlingual/2nd-6th	High frequency; progressive
<i>TBC1D24</i>	DFNA65	Postlingual	High frequency; progressive

<i>TECTA</i> ^{3,4}	DFNA8/12	Prelingual	Mid-frequency loss
<i>TJP2</i> & <i>FAM189A2</i>	DFNA51	Postlingual/4th	High frequency; progressive
<i>TMC1</i>	DFNA36	Postlingual	Flat/gently downsloping
<i>WFS1</i> ⁵	DFNA6/14/38	Prelingual	Low frequency; progressive

Table 3: Autosomal Recessive Nonsyndromic Hearing Loss

Gene	Locus	Onset	Type
<i>ADCY1</i>	DFNB44	Prelingual	Mild to moderate; stable
<i>BDP1</i>	DFNB49	Postlingual	High frequency; stable
<i>BSND</i>	DFNB73	Prelingual	Severe to profound; stable
<i>CABP2</i>	DFNB93	Prelingual	Moderate to severe; stable
<i>CDC14A</i>	DFNB105	Prelingual	Severe to profound
<i>CDH23</i> ¹	DFNB12	Prelingual	Severe to profound; stable
<i>CIB2</i>	DFNB48	Prelingual	Severe to profound
<i>CLDN14</i>	DFNB29	Prelingual	Severe to profound; stable
<i>CLIC5</i>	DFNB103	Prelingual	High frequency; progressive
<i>COL11A2</i>	DFNB53	Prelingual	Severe to profound; stable
<i>DCDC2</i>	DFNB66	Prelingual	Severe to profound
<i>PJVK</i>	DFNB59	Prelingual	Severe to profound; stable
<i>ELMOD3</i>	DFNB88	Prelingual	Severe to profound; mixed
<i>EPS8</i>	DFNB102	Prelingual	Severe to profound
<i>EPS8L2</i>	-	Postlingual	High frequency; progressive
<i>ESPN</i>	DFNB36	Prelingual	—
<i>ESRRB</i>	DFNB35	Unknown	Severe to profound
<i>GIPC3</i> ²	DFNB15/72/95	Prelingual	Severe to profound
<i>GJB2</i> ³	DFNB1	Prelingual ⁴	Usually stable
<i>GJB6</i> ³	DFNB1	Prelingual ⁴	Usually stable
<i>GPSM2</i>	DFNB32/82	Prelingual	Severe to profound; stable
<i>GRXCR1</i>	DFNB25	Prelingual	Moderate to profound; progressive
<i>GRXCR2</i>	DFNB101	Prelingual	High frequency; progressive
<i>HGF</i>	DFNB39	Prelingual	Severe to profound; downsloping
<i>ILDR1</i>	DFNB42	Prelingual	Moderate to severe
<i>KARS1</i>	DFNB89	Prelingual	Moderate to severe; stable
<i>LHFPL5</i>	DFNB67	Prelingual	Severe to profound; stable
<i>LOXHD1</i>	DFNB77	Postlingual	Moderate to profound; progressive
<i>LRTOMT</i>	DFNB63	Prelingual	Severe to profound; stable
<i>MARVELD2</i>	DFNB49	Prelingual	Moderate to profound; stable
<i>MET</i>	DFNB97	Prelingual	Severe to profound
<i>MSRB3</i>	DFNB74	Prelingual	Severe to profound
<i>MYO15A</i>	DFNB3	Prelingual	Severe to profound; stable
<i>MYO3A</i>	DFNB30	Prelingual	Severe to profound; stable
<i>MYO6</i>	DFNB37	Prelingual	—
<i>MYO7A</i> ⁵	DFNB2	Prelingual, postlingual	Unspecified

<i>NARS2</i> ⁶	DFNB94	Prelingual	Severe to profound; stable
<i>OTOG</i>	DFNB18B	Prelingual	Mild to moderate; stable
<i>OTOGL</i>	DFNB84	Prelingual	High frequency; stable
<i>OTOA</i>	DFNB22	Prelingual	Severe to profound; stable
<i>OTOF</i>	DFNB9	Prelingual	Usually severe to profound; stable
<i>PCDH15</i>	DFNB23	Prelingual	Severe to profound; stable
<i>PNPT1</i>	DFNB70	Prelingual	Severe to profound; stable
<i>PTPRQ</i>	DFNB84	Prelingual	Moderate to profound; progressive
<i>RDX</i>	DFNB24	Prelingual	Severe to profound; stable
<i>RIPOR2</i>	DFNB104	Prelingual	Severe to profound
<i>ROR1</i> ⁷	-	Prelingual	Severe to profound
<i>S1PR2</i>	DFNB68	Prelingual	Severe to profound
<i>SERPINB6</i>	DFNB91	Prelingual	Moderate to severe
<i>SLC22A4</i>	DFNB60	Prelingual	Severe to profound
<i>SLC26A4</i> ⁸	DFNB4	Prelingual, postlingual	Stable; progressive
<i>SLC26A5</i>	DFNB61	Prelingual	Severe to profound; stable
<i>STRC</i>	DFNB16	Prelingual	Severe to profound; stable
<i>SYNE4</i>	DFNB76	Prelingual	High frequency; progressive
<i>TECTA</i> ^{9, 10}	DFNB21	Prelingual	Severe to profound; stable
<i>TBC1D24</i>	DFNB86	Prelingual	Severe to profound
<i>TMC1</i>	DFNB7/11	Prelingual	Severe to profound; stable
<i>TMEM132E</i>	DFNB99	Prelingual	Severe to profound
<i>TMIE</i>	DFNB6	Prelingual	Severe to profound; stable
<i>TMPRSS3</i>	DFNB8/10	Postlingual ¹¹ , prelingual	Progressive; stable
<i>TPRN</i>	DFNB79	Prelingual	Severe to profound; stable
<i>TRIOBP</i>	DFNB28	Prelingual	Severe to profound; stable
<i>TSPEAR</i>	DFNB98	Prelingual	Severe to profound
<i>USH1C</i> ¹²	DFNB18	Prelingual	Severe to profound; stable
<i>WBP2</i>	-	Prelingual	High frequency; progressive
<i>WHRN</i>	DFNB31	Prelingual	—

Table 4: X-linked Nonsyndromic Hearing Loss:

Gene	Locus	Onset	Type / Degree	Frequencies
<i>PRPS1</i>	DFNX1	Postlingual	Progressive sensorineural / severe to profound	All
<i>POU3F4</i>	DFNX2	Prelingual	Progressive, mixed / variable, but progresses to profound	All
<i>SMPX</i>	DFNX4	Postlingual	Progressive sensorineural / mild to profound	All

<i>AIFM1</i>	DFNX5	Prelingual	Progressive auditory neuropathy	Low frequency
<i>COL4A6</i>	DFNX6	Prelingual	Progressive sensorineural / severe to profound	All

[0077] Mitochondrial DNA pathogenic variants have been implicated in a variety of diseases with hearing loss ranging from rare neuromuscular syndromes such as Kearns-Sayre syndrome, mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes (MELAS), myoclonic epilepsy with ragged-red fibers (MERRF), and neurogenic weakness with ataxia and retinitis pigmentosa (NARP), to common conditions such as diabetes mellitus, Parkinson disease, and Alzheimer disease. For examples. An exemplary pathogenic variant associated with hearing loss in diabetes mellitus patients is the 3243 A-to-G transition in *MTTL1* and MELAS. A summary of mitochondrial DNA mutations related to hearing loss is provided in Table 5. (See Shearer AE, Hildebrand MS, Smith RJH. Hereditary Hearing Loss and Deafness Overview. 1999 Feb 14 [Updated 2017 Jul 27]. In: Adam MP, Ardinger HH, Pagon RA, et al., editors. GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2019.)

Table 5:

Gene	Pathogenic Variant	Severity	Penetrance
<i>MT-RNR1</i>	961 different variants	Variable	Highly variable, aminoglycoside induced
	1494C>T	Variable	
	1555A>G	Variable	
<i>MT-TS1</i>	7445A>G	Variable	Highly variable
	7472insC	Variable	
	7510T>C	Variable	
	7511T	Variable	

<i>MT-COI</i>	7444G>A	Severe to profound	Complete, aminoglycoside associated; associated with <i>MT-RNR1</i> 1555A>G
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[0078] In any of the disclosed methods, the step of treating hearing-loss in a subject in need results in improved hearing in the subject or an improvement or reduction in hearing impairment in the subject. Tests for determining whether a method of treatment described herein improves or reduces hearing loss or hearing impairment include physiological tests which objectively determine the functional status of the auditory system; and audiometry which subjectively determines how the individual processes auditory information. Physiological tests include the auditory brain stem response testing (ABR, also known as BAER, BSER), which uses a stimulus (e.g. clicks) to evoke electrophysiologic responses, which originate in the eighth cranial nerve and auditory brain stem and are recorded with surface electrodes. ABR “wave V detection threshold” correlates best with hearing sensitivity in the 1500- to 4000-Hz region in neurologically normal individuals; ABR does not assess low frequency (<1500 Hz) sensitivity.

[0079] Auditory steady-state response testing (ASSR) uses an objective, statistics-based mathematical detection algorithm to detect and define hearing thresholds. ASSR can be obtained using broadband or frequency-specific stimuli and can offer hearing threshold differentiation in the severe-to-profound range. The ASSR test is frequently used to give frequency-specific information that ABR does not give. Test frequencies of 500, 1000, 2000, and 4000 Hz are commonly used.

[0080] Evoked otoacoustic emissions (EOAEs) are sounds originating within the cochlea that are measured in the external auditory canal using a probe with a microphone and transducer. EOAEs reflect primarily the activity of the outer hair cells of the cochlea across a broad frequency range and are present in ears with hearing sensitivity better than 40-50 dB HL. Immittance testing (tympanometry, acoustic reflex thresholds, acoustic reflex decay) assesses the peripheral auditory system, including middle ear pressure, tympanic membrane mobility, Eustachian tube function, and mobility of the middle ear ossicles.

[0081] Audiometry includes behavioral tests such as behavioral observation audiometry (BOA) and visual reinforcement audiometry (VRA). Pure-tone audiometry (air and bone conduction) involves determination of the lowest intensity at which an individual “hears” a pure tone, as a function of frequency (or pitch). Octave frequencies from 250 (close to middle

C) to 8000 Hz are tested using earphones. Intensity or loudness is measured in decibels (dB), defined as the ratio between two sound pressures. 0 dB HL is the average threshold for a normal hearing adult; 120 dB HL is so loud as to cause pain. Speech reception thresholds (SRTs) and speech discrimination are assessed. For air conduction audiometry sounds are presented through earphones; thresholds depend on the condition of the external ear canal, middle ear, and inner ear. For bone conduction audiometry sounds are presented through a vibrator placed on the mastoid bone or forehead, thus bypassing the external and middle ears; thresholds depend on the condition of the inner ear. Additional tests include conditioned play audiometry which develops a complete frequency-specific audiogram for each ear, and conventional audiometry which indicates when an individual hears a sound.

[0082] An audioprofile refers to the recording of several audiograms on a single graph. These audiograms may be from one individual at different times, but more frequently they are from different members of the same family segregating deafness usually in an autosomal dominant fashion. By plotting numerous audiograms with age on the same graph, the age-related progression of hearing loss can be appreciated within these families.

Transgenes

[0083] The disclosed methods comprising delivering any transgene of interest to a cochlear cell. The transgene is a polynucleotide sequence that encodes a polypeptide of interest or is a nucleic acid that inhibits, interferes or silences expression of a gene of interest, such as a siRNA or miRNA. Exemplary transgenes are the human atonal transcription factor (ATOH1) cDNA (SEQ ID NO: 2), otoferlin cDNA (SEQ ID NO: 4), gap junction protein beta 2 (GJB2) cDNA (SEQ ID NO: 6), SLC264 cDNA (SEQ ID NO: 8), Forkhead Box O3 (FOXO3) cDNA (SEQ ID NO: 10), Activin A cDNA (SEQ ID NO: 12), follistatin cDNA (SEQ ID NO: 14).

[0084] Exemplary transgenes are polynucleotides that encode human atonal transcription factor (SEQ ID NO: 3), otoferlin (SEQ ID NO: 5), gap junction protein beta 2 (SEQ ID NO: 7), pendrin (SEQ ID NO: 9), forkhead box 1 (SEQ ID NO: 11), activin A or inhibin (SEQ ID NO: 13), follistatin (SEQ ID NO: 15) galectin-1 (SEQ ID NO: 20) and galectin-3 (SEQ ID NO: 22) proteins.

[0085] miRNA shRNA and miRNA, that are expressed in the cochlea, are contemplated as transgenes to include in the disclosed optimized gene therapy vectors.

[0086] rAAV genomes provided herein may comprise a wild type nucleic acid sequence of any of the genes provided in Table 1, Table 2, Table 3, Table 4 or Table 5 herein. In addition, rAAV genomes provided herein may comprise a cDNA sequence of one of the following human atonal transcription factor (ATOH1) cDNA (SEQ ID NO: 2), otoferlin cDNA (SEQ ID NO: 4), gap junction protein beta 2 (GJB2) cDNA (SEQ ID NO: 6), SLC26A4 cDNA (SEQ ID NO: 8), forkhead Box 1 (FOXO3) cDNA (SEQ ID NO: 10), activin A cDNA (SEQ ID NO: 12), follistatin (SEQ ID NO: 14), galectin-1 (SEQ ID NO: 19) or galectin-3 (SEQ ID NO: 21) cDNA. For example, the polypeptide encoded by the transgene include polypeptides comprising an amino acid sequence that is at least: 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence encoded by the transgene sequence, and retains the desired activity.

[0087] For example, rAAV genomes provided herein comprise a polynucleotide encoding a human atonal transcription factor (SEQ ID NO: 3), otoferlin (SEQ ID NO: 5), gap junction protein beta 2 (SEQ ID NO: 7), pendrin (SEQ ID NO: 9), forkhead box 1 (SEQ ID NO: 11), activin A or inhibin (SEQ ID NO: 13), follistatin (SEQ ID NO: 15), galectin-1 (SEQ ID NO: 20) or galectin-3 (SEQ ID NO: 22). The polypeptide include polypeptides comprising an amino acid sequence that is at least: 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to an amino acid sequence encoded by the transgene sequence and retains the desired activity.

[0088] rAAV genomes provided herein, in some cases, comprise a polynucleotide encoding a polypeptide or a polynucleotide at least: 65%, 70%, 75%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the nucleotide sequence that encodes a polypeptide with the desired activity.

[0089] rAAV genomes provided herein, in some embodiments, comprise a transgene comprising a polynucleotide sequence that encodes a polypeptide with a desired activity and that hybridizes under stringent conditions to any one of nucleic acid sequence of a known transgene of interest, or the complement thereof.

[0090] The term “stringent” is used to refer to conditions that are commonly understood in the art as stringent. Hybridization stringency is principally determined by temperature, ionic strength, and the concentration of denaturing agents such as formamide. Examples of stringent conditions for hybridization and washing include but are not limited to 0.015 M sodium chloride, 0.0015 M sodium citrate at 65-68°C or 0.015 M sodium chloride, 0.0015M

sodium citrate, and 50% formamide at 42°C. See, for example, Sambrook et al., *Molecular Cloning: A Laboratory Manual*, 2nd Ed., Cold Spring Harbor Laboratory, (Cold Spring Harbor, N.Y. 1989).

Methods of Administration

[0091] Intrathecal administration is exemplified herein. These methods include transducing target cells with one or more rAAV described herein. In some embodiments, the rAAV viral particle comprising a transgene is administered or delivered to the ear, brain and/or spinal cord of a patient. In some embodiments, the polynucleotide is delivered to the brain. Areas of the brain contemplated for delivery include, but are not limited to, the motor cortex, visual cortex, cerebellum and the brain stem. In some embodiments, the polynucleotide is delivered to the spinal cord. In some embodiments, the polynucleotide is delivered to a lower motor neuron. The polynucleotide may be delivered to a cochlear cell such as inner hair cells, outer hair cells, ganglion cells, supporting cells, Deiters' cells, pillar cells and epithelium cells.

[0092] In some embodiments of methods provided herein, the patient is held in the Trendelenberg position (head down position) after administration of the rAAV (e.g., for about 5, about 10, about 15 or about 20 minutes). For example, the patient may be tilted in the head down position at about 1 degree to about 30 degrees, about 15 to about 30 degrees, about 30 to about 60 degrees, about 60 to about 90 degrees, or about 90 to about 180 degrees).

[0093] For intracerebroventricular injections, a needle is inserted into the skull and the liquid is injected into the ventricles containing cerebrospinal fluid. For example, intracerebroventricular injections are carried out by a clinically trained surgeon using methods known in the art.

[0094] The methods provided herein comprise the step of administering an effective dose, or effective multiple doses, of a composition comprising a rAAV provided herein to a subject (e.g., an animal including, but not limited to, a human patient) in need thereof. If the dose is administered prior to development of the symptoms of the hearing loss-related disorder, the administration is prophylactic. If the dose is administered after the development of symptoms of the hearing loss-related disorder, the administration is therapeutic. An effective dose is a dose that alleviates (eliminates or reduces) at least one symptom associated with the hearing loss-related disorder, that slows or prevents progression of the disorder, that

diminishes the extent of disorder, that results in remission (partial or total) of disorder, and/or that prolongs hearing and/or survival . In comparison to the subject before treatment or in comparison to an untreated subject, methods provided herein result in stabilization, reduced progression of hearing loss, or improvement in hearing.

Examples

[0095] While the following examples describe specific embodiments, it is understood that variations and modifications will occur to those skilled in the art. Accordingly, only such limitations as appear in the claims should be placed on the invention.

Example 1

Production of scAAV9.GFP

[0096] A human GFP cDNA clone was obtained from Origene, Rockville, MD. GFP cDNA alone or GFP cDNA and shSOD-1 cDNA was further subcloned into a self-complementary AAV9 genome under the hybrid chicken β -Actin promoter (CB). The plasmid construct also included one or more of the CB promoter (SEQ ID NO: 1), an intron such as the simianvirus 40 (SV40) chimeric intron and a Bovine Growth Hormone (BGH) polyadenylation signal (BGH PolyA). A schematic of the plasmid constructs showing the GFP cDNA inserted between AAV2 ITRs is provided in **Fig. 1** referred to as AAV9.CB.GFP. The AAV9.CB.shSOD1.GFP plasmid has a human SOD-1 cDNA inserted between the SV40 intron and the GFP cDNA. The constructs were packaged into either AAV9 genome.

Example 2

Intrathecal AAV9 Delivery and Cochlear Cell Targeting

[0097] Mice received one intrathecal (IT) injection of either 1.65×10^{11} vg or 3.3×10^{11} vg of scAAV9.shSOD1.CB.GFP or scAAV9.CB.GFP. The scAAV9.shSOD1.CB.GFP or scAAV9.CB.GFP was formulated in 1x PBS and 0.001% Pluronic F68 (denoted as PBS/F68). The mice were euthanized 3 weeks after injection. The cochlea of the treated mice were cryosectioned and stained for GFP. The section was also stained with DAPI to show the location of the cells.

[0098] Figures 2- 5 provide data for the mice injected with scAAV9.shSOD1.CB.GFP. Figure 2 is a staining for GFP in a sagittal cryosection of a cochlea from a IT treated. The hematoxylin and eosin staining is provided as a reference and is not a photo of a cochlea from an injected mouse (shx2IT-LFLR). This data demonstrates the strong GFP staining throughout the mouse cochlea after IT injection. Figure 3 provides staining of 4 different

mice (shx2IT-LF, shx2IT-2F, shx2IT-LE and shx2IT-LFLR). This figure shows widespread GFP expression and homogenous pattern of GFP expression in all injected mice. The cells in the organ of corti express high levels of GFP. Figure 4 highlights the different areas of the mouse cochlea which had GFP expression after IT injection. In Figure 5, the sagittal cryosection of the cochlea from a IT injected mouse (shx2IT-LE) was stained for actin, in addition to GFP and DAPI. This figure shows successful targeting of inner hair cells (IHCs) as they express the GFP transgene in several organ of corti. The figure shows a zoom in to larger magnification to clearly see the IHC cells.

[0099] Figures 6-8 provide data for the mice injected with scAAV9.CB.GFP. The cochlea of the IT injected mice were also sectioned in coronal orientation with a vibratome for a different view of the GFP expression pattern. Figures 6 and 7 demonstrates that the inner hair cells within the cochlea stain strongly for GFP in an injected mouse (mouse #2 and mouse #0). These figures also demonstrate that IT injection results in broad localization of GFP expression. Figure 8 provides staining of the vestibule of the IT injected mice with DAPI, GFP and actin. These figures show targeting of the inner hair cells throughout the entire cochlea throughout the different turns where different wave lengths are processed.

Example 3

Transduction of Mouse Cochleae After Intrathecal Delivery and Exposure to Noise

[00100] Stress to the hair cells in the inner ear of mice was induced by exposing the mouse to 100 db sound pressure level noise for 2 hours. scAAV9.CB.GFP was administered to mice via one intrathecal injection (IT) after exposure to the noise, either immediately after exposure (0 h.p.i.) or 24 hours after exposure (24 h.p.i.). The mice were injected with 1.65×10^{11} vg of scAAV9.CB.GFP. The scAAV9.CB.GFP was formulated in 1x PBS and 0.001% Pluronic F68 (denoted as PBS/F68). The mice were euthanized 3 weeks after injection.

[00101] To examine the cochlear expression of the transgene, an immunohistochemistry analysis was performed to visualize GFP protein. Cross sections of the organ tissue was stained for Dapi, GFP and phalloidin, which stains actin filaments or myo7A which stains myosin-VIIa. Staining for myo7a was a specific method for labeling hair cells. As demonstrated in Figures 9-12, the inner hair cells expressed transgene GFP after IT injection of scAAV9.CB.GFP immediately after exposure to noise.

[00102] Figures 13-16 provide staining of GFP and myo7A, and showed GFP expression was detected when IT injection occurred 24 hours after exposure to noise, but expression

level was overall lower in inner hair cells. GFP expression was lower when the IT injection was delayed 24 hours after exposure to noise when compared to GFP expression when the IT injection occurred immediately after exposure to noise. Figure 16 and 17 demonstrate that administering scAAV9.CB.GFP 24 hours after injury resulted in a weaker GFP signal but targeting of the inner hair cells still occurs with the delayed administration. Figure 16 is imaged at the optimal exposure of the 24hpi IT injected mice. This photo demonstrates that GFP expression in the 24hpi intrathecal injected animals is lower than in 0hpi intrathecal injected animals, as imaging at the optimal exposure for 24hpi overexposes 0hpi. Hematoxylin and Eosin staining shown in Figure 17 demonstrates that the inner hair cells are not damaged by the noise injury protocol used in this study.

Example 4

Transduction of Mouse Cochlear After Intravenous Injection

[00103] scAAV9.CB.GFP was also administered to normal mice via one tail vein injection. The mice were injected with 1.65×10^{12} vg/kg of scAAV9.CB.GFP. The scAAV9.CB.GFP was formulated in 1x PBS and 0.001% Pluronic F68 (denoted as PBS/F68). The mice were euthanized 3 weeks after injection. As shown in Figure 18, the preliminary data provided herein show that scAAV9.CB.GFP did deliver GFP to the cochlea but did not transduce the inner hair cells or the spinal ganglion neurons when administered using tail vein injection. Instead, it seems the transduction remained around blood vessels.

[00104] A subsequent study was carried out in which 1.65×10^{12} of scAAV9.CB.GFP formulated in PBS/F68 was administered to normal mice via one tail vein injection, and the mice were euthanized 3 weeks after injection. The immunohistochemistry data provided in Figures 19-22, intravenous injection did not result in transduction of inner hair cells in healthy cochlea. Figure 23 provides data from an uninjected control mouse that was exposed to noise injury.

Claims

What is claimed:

1. A method of delivering a transgene to a cochlear cell in a subject comprising administering a gene therapy vector encoding the transgene, wherein the gene therapy vector is administered to the subject using intravenous delivery, intrathecal delivery or any method of delivery that accesses the cerebrospinal fluid.
2. The method of claim 1 wherein the cochlear cell is an inner hair cell.
3. A method of treating hearing-loss or a hearing loss-related disorder in a subject comprising administering a gene therapy vector encoding a transgene to the subject, wherein the gene therapy vector is administered using intravenous delivery, intrathecal delivery or any method of delivery that accesses the cerebrospinal fluid.
4. The method of claim 3, wherein the hearing loss-related disorder is Waardenburg syndrome (WS), Branchiootorenal spectrum disorders, Neurofibromatosis 2 (NF2), Stickler syndrome, Usher syndrome type I, Usher syndrome type II, Usher syndrome type III, Pendred syndrome, Jervell and Lange-Nielsen syndrome, Biotinidase deficiency Refsum disease, Alport syndrome, Deafness-dystonia-optic neuropathy syndrome, or Mohr-Tranebjaerg syndrome.
5. The method of claims 1 or 2, wherein the subject is suffering from hearing-loss or a hearing loss-related disorder.
6. The method of claim 5, wherein the hearing loss-related disorder is Waardenburg syndrome (WS), Branchiootorenal spectrum disorders, Neurofibromatosis 2 (NF2), Stickler syndrome, Usher syndrome type I, Usher syndrome type II, Usher syndrome type III, Pendred syndrome, Jervell and Lange-Nielsen syndrome, Biotinidase deficiency Refsum disease, Alport syndrome, Deafness-dystonia-optic neuropathy syndrome, or Mohr-Tranebjaerg syndrome.
7. The method of any one of claims 1-6 wherein the transgene encodes human atonal transcription factor (ATOH1) (SEQ ID NO: 2), otoferlin (SEQ ID NO: 4), gap junction

protein beta 2 (SEQ ID NO:6), pendrin (SLC26A) (SEQ ID NO: 8), forkhead box 1 (FOXG1) (SEQ ID NO: 10), activin A or Inhibin (SEQ ID No: 12), follistatin (FST) (SEQ ID NO: 14), galectin-1 (SEQ ID NO: 19) or galectin-3 (SEQ ID NO: 21).

8. The method of any one of claims 1-6 wherein the transgene is a miRNA or, siRNA against human atonal transcription factor (ATOH1) (SEQ ID NO: 2), otoferlin (SEQ ID NO: 4), gap junction protein beta 2 (SEQ ID NO:6), pendrin (SLC26A) (SEQ ID NO: 8), forkhead box 1 (FOXG1) (SEQ ID NO: 10), activin A or Inhibin (SEQ ID No: 12), follistatin (FST) (SEQ ID NO: 14), galectin-1 (SEQ ID NO: 19) or galectin-3 (SEQ ID NO: 21).

9. The method of any one of claims 1-8 wherein the gene therapy vector is AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAVRH10, AAVRH74, AAV11, AAV12, AAV13, AAVTT or Anc80, AAV7m8 or their derivatives.

10. The method of any one of claims 1-9, wherein the gene therapy vector is administered using intrathecal delivery, and the method further comprises placing the subject in the Trendelenberg position after administering of the gene therapy vector.

11. The method of any one of claims 1-9, wherein the gene therapy vector is administered using a delivery method which directly applies the vector into the cerebrospinal fluid.

12. A composition for delivering a transgene to a cochlear cell in a subject, wherein the composition comprises a gene therapy vector encoding the transgene, and wherein composition is formulated for intravenous delivery, intrathecal delivery or any method of delivery that accesses the cerebrospinal fluid.

13. The composition of claim 1 wherein the cochlear cell is an inner hair cell.

14. A composition for treating hearing-loss or a hearing loss-related disorder in a subject, the composition comprises a gene therapy vector encoding a transgene, and wherein the composition is formulated for intravenous delivery, intrathecal delivery or any method of delivery that accesses the cerebrospinal fluid.

15. A composition of claim 14, wherein the hearing loss-related disorder is Waardenburg syndrome (WS), Branchiootorenal spectrum disorders, Neurofibromatosis 2 (NF2), Stickler syndrome, Usher syndrome type I, Usher syndrome type II, Usher syndrome type III,

Pendred syndrome, Jervell and Lange-Nielsen syndrome, Biotinidase deficiency Refsum disease, Alport syndrome, Deafness-dystonia-optic neuropathy syndrome, or Mohr-Tranebjaerg syndrome.

16. The composition of claims 12 or 13, wherein the subject is suffering from hearing-loss or a hearing loss-related disorder.

17. The composition of claim 16, wherein the hearing loss-related disorder is Waardenburg syndrome (WS), Branchiootorenal spectrum disorders, Neurofibromatosis 2 (NF2), Stickler syndrome, Usher syndrome type I, Usher syndrome type II, Usher syndrome type III, Pendred syndrome, Jervell and Lange-Nielsen syndrome, Biotinidase deficiency Refsum disease, Alport syndrome, Deafness-dystonia-optic neuropathy syndrome, or Mohr-Tranebjaerg syndrome.

18. The composition of any one of claim 12-17 wherein the transgene encodes human atonal transcription factor (ATOH1) (SEQ ID NO: 2), otoferlin (SEQ ID NO: 4), gap junction protein beta 2 (SEQ ID NO: 6), pendrin (SLC26A) (SEQ ID NO: 8), forkhead box 1 (FOXP1) (SEQ ID NO: 10), activin A or Inhibin (SEQ ID No: 12), follistatin (FST) (SEQ ID NO: 14), galectin-1 (SEQ ID NO: 19) or galectin-3 (SEQ ID NO: 21).

19. The composition of any one of claims 12-17 wherein the transgene is a miRNA or, siRNA against human atonal transcription factor (ATOH1) (SEQ ID NO: 2), otoferlin (SEQ ID NO: 4), gap junction protein beta 2 (SEQ ID NO: 6), pendrin (SLC26A) (SEQ ID NO: 8), forkhead box 1 (FOXP1) (SEQ ID NO: 10), activin A or Inhibin (SEQ ID No: 12), follistatin (FST) (SEQ ID NO: 14), galectin-1 (SEQ ID NO: 19) or galectin-3 (SEQ ID NO: 21).

20. The composition of any one of claims 12-19 wherein the gene therapy vector is AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAVRH10, AAVRH74, AAV11, AAV12, AAV13, AAVTT or Anc80, AAV7m8 or their derivatives.

21. The composition of any one of claims 12-20, wherein the composition is formulated for intrathecal delivery to a subject, wherein the subject is placed in the Trendelenburg position after administering of the composition.

22. The composition of any one of claims 12-20, wherein the is formulated for administration using a delivery method which directly applies the vector into the cerebrospinal fluid.
23. Use of a gene therapy vector for the preparation of a medicament for delivering a transgene to a cochlear cell in a subject, wherein the gene therapy vector encodes the transgene, and there wherein the medicament is formulated for administration to the subject using intravenous delivery, intrathecal delivery or any method of delivery that accesses the cerebrospinal fluid.
24. The use of claim 23 wherein the cochlear cell is an inner hair cell.
25. Use of a gene therapy vector for the preparation of a medicament for treating hearing-loss or a hearing loss-related disorder in a subject, wherein the gene therapy vector encodes a transgene, and wherein the medicament is formulated for intravenous delivery, intrathecal delivery or any method of delivery that accesses the cerebrospinal fluid.
26. The use of claim 25, wherein the hearing loss-related disorder is Waardenburg syndrome (WS), Branchiootorenal spectrum disorders, Neurofibromatosis 2 (NF2), Stickler syndrome, Usher syndrome type I, Usher syndrome type II, Usher syndrome type III, Pendred syndrome, Jervell and Lange-Nielsen syndrome, Biotinidase deficiency Refsum disease, Alport syndrome, Deafness-dystonia-optic neuropathy syndrome, or Mohr-Tranebjaerg syndrome.
27. The use of claims 23 or 24, wherein the subject is suffering from hearing-loss or a hearing loss-related disorder.
28. The use of claim 27, wherein the hearing loss-related disorder is Waardenburg syndrome (WS), Branchiootorenal spectrum disorders, Neurofibromatosis 2 (NF2), Stickler syndrome, Usher syndrome type I, Usher syndrome type II, Usher syndrome type III, Pendred syndrome, Jervell and Lange-Nielsen syndrome, Biotinidase deficiency Refsum disease, Alport syndrome, Deafness-dystonia-optic neuropathy syndrome, or Mohr-Tranebjaerg syndrome.
29. The use of any one of claims 23-28 wherein the transgene encodes human atonal transcription factor (ATOH1) (SEQ ID NO: 2), otoferlin (SEQ ID NO: 4), gap junction

protein beta 2 (SEQ ID NO:6), pendrin (SLC26A) (SEQ ID NO: 8), forkhead box 1 (FOXG1) (SEQ ID NO: 10), activin A or Inhibin (SEQ ID No: 12), follistatin (FST) (SEQ ID NO: 14), galectin-1 (SEQ ID NO: 19) or galectin-3 (SEQ ID NO: 21).

30. The use of any one of claims 23-28 wherein the transgene is a miRNA or, siRNA against human atonal transcription factor (ATOH1) (SEQ ID NO: 2), otoferlin (SEQ ID NO: 4), gap junction protein beta 2 (SEQ ID NO:6), pendrin (SLC26A) (SEQ ID NO: 8), forkhead box 1 (FOXG1) (SEQ ID NO: 10), activin A or Inhibin (SEQ ID No: 12), follistatin (FST) (SEQ ID NO: 14), .

31. The use of any one of claims 23-30, wherein the gene therapy vector is AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAVRH10, AAVRH74, AAV11, AAV12, AAV13, AAVTT or Anc80, AAV7m8 or their derivatives.

32. The use of any one of claims 23-31, wherein the medicament is formulated for intrathecal delivery, and the subject is placed in the Trendelenberg position after administering of the medicament.

33. The use of any one of claims 23-31, wherein the medicament is formulated for a delivery method which directly applies the vector into the cerebrospinal fluid.

Figure 1

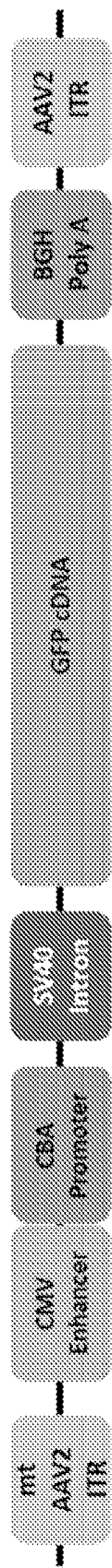
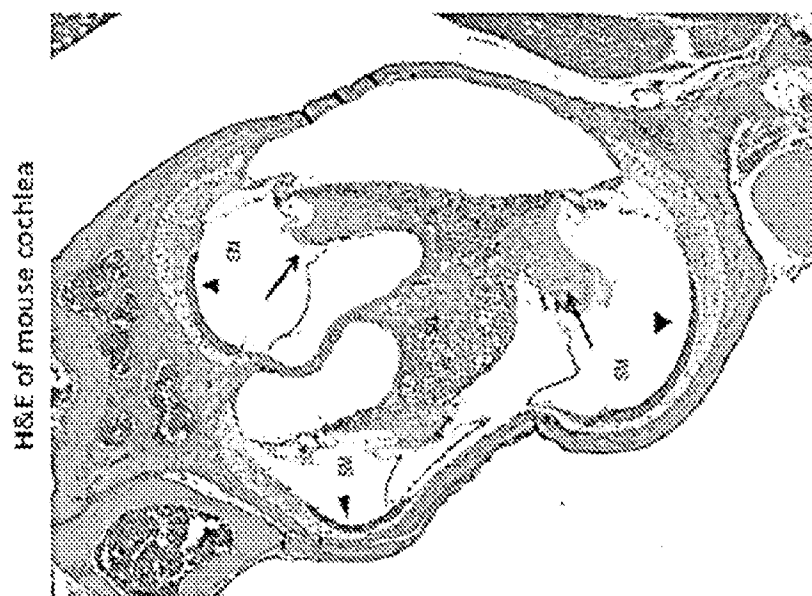


Figure 2



shx2IT-1FLR cochlea

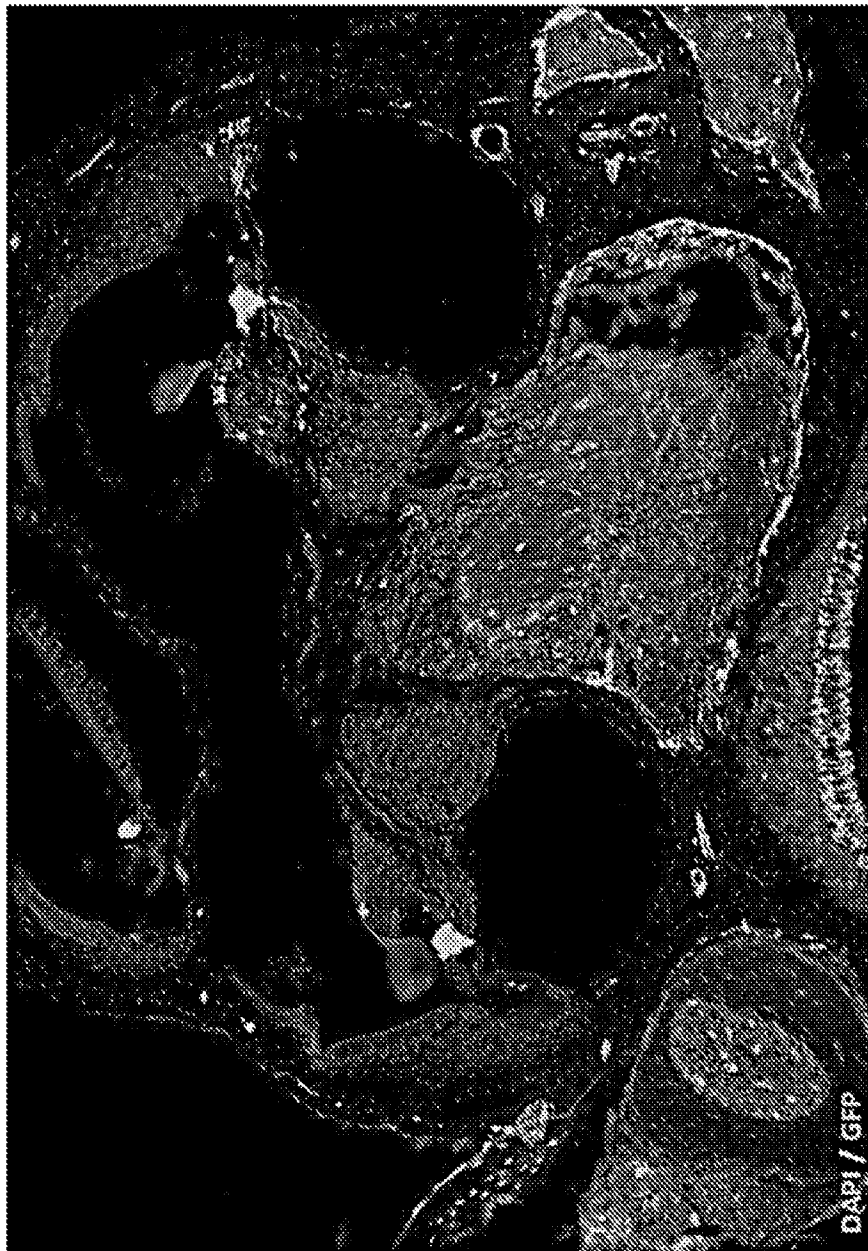
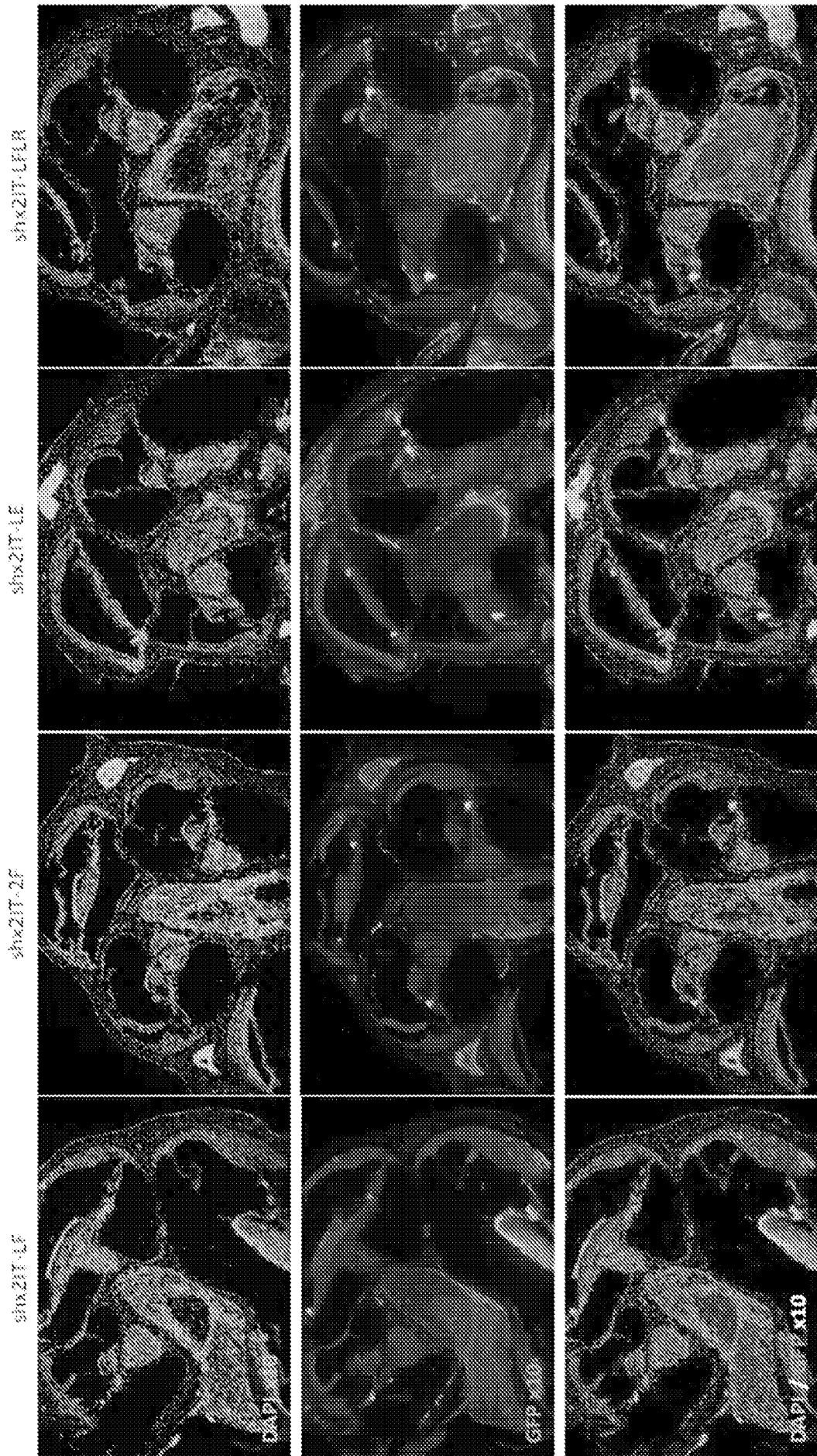


Figure 3



- 1 -- Organ of Corti
- 2 -- Organ of Corti (Focus 1)
- 3 -- Spiral Limbus
- 4 -- Organ of Corti
- 5 -- Spiral ganglion
- 6 -- Vestibular epithelium

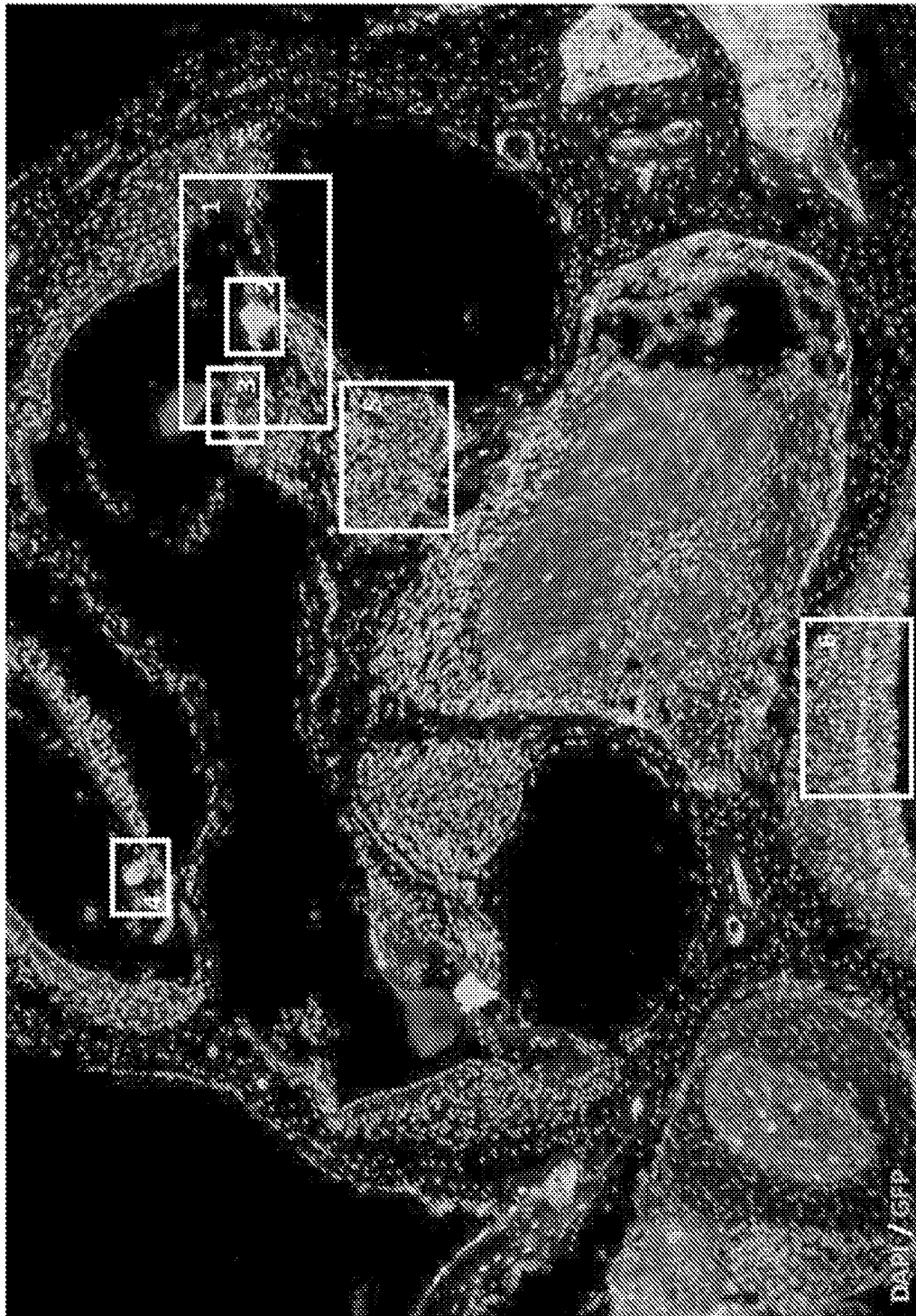


Figure 4

Figure 5

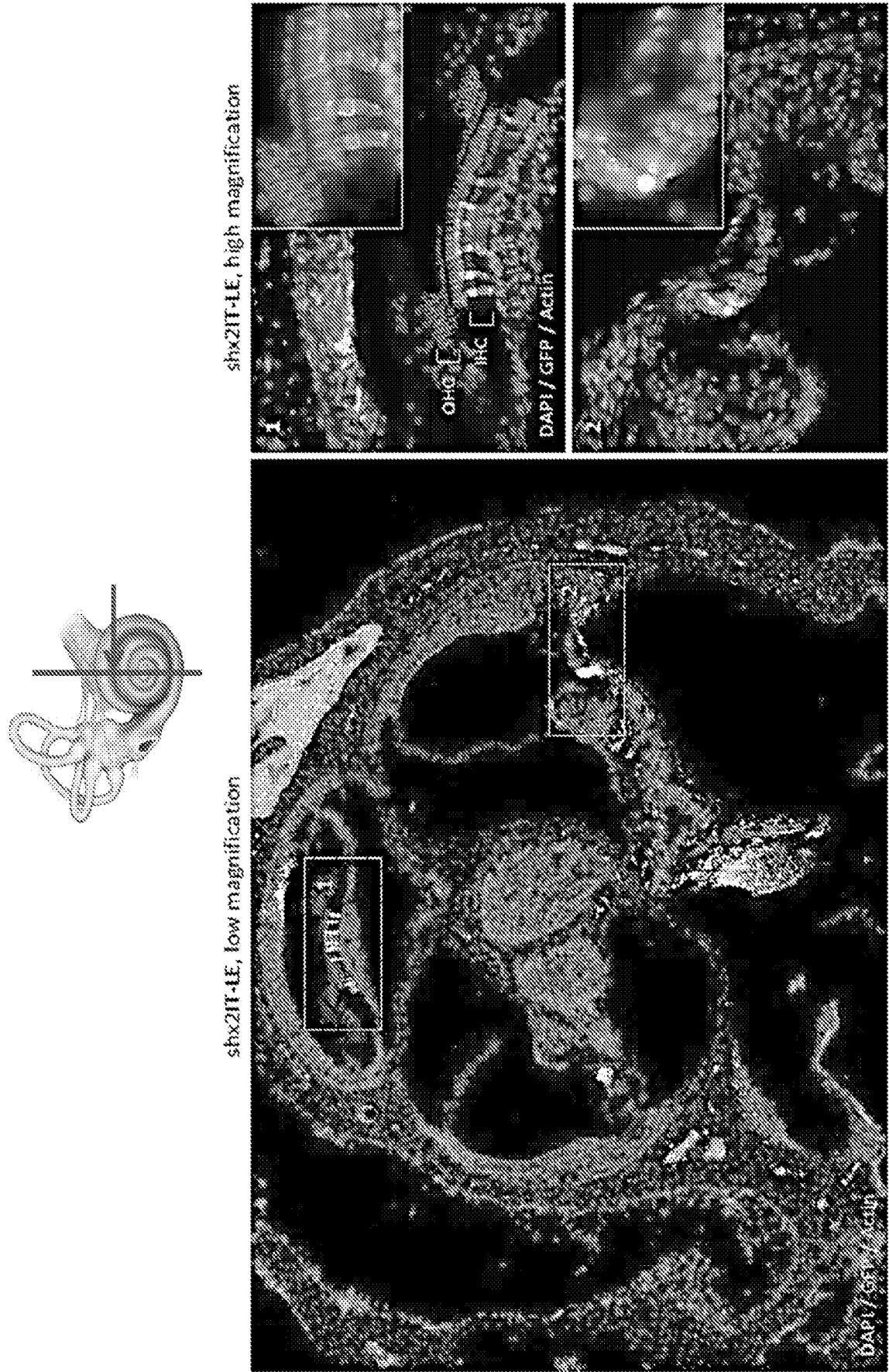
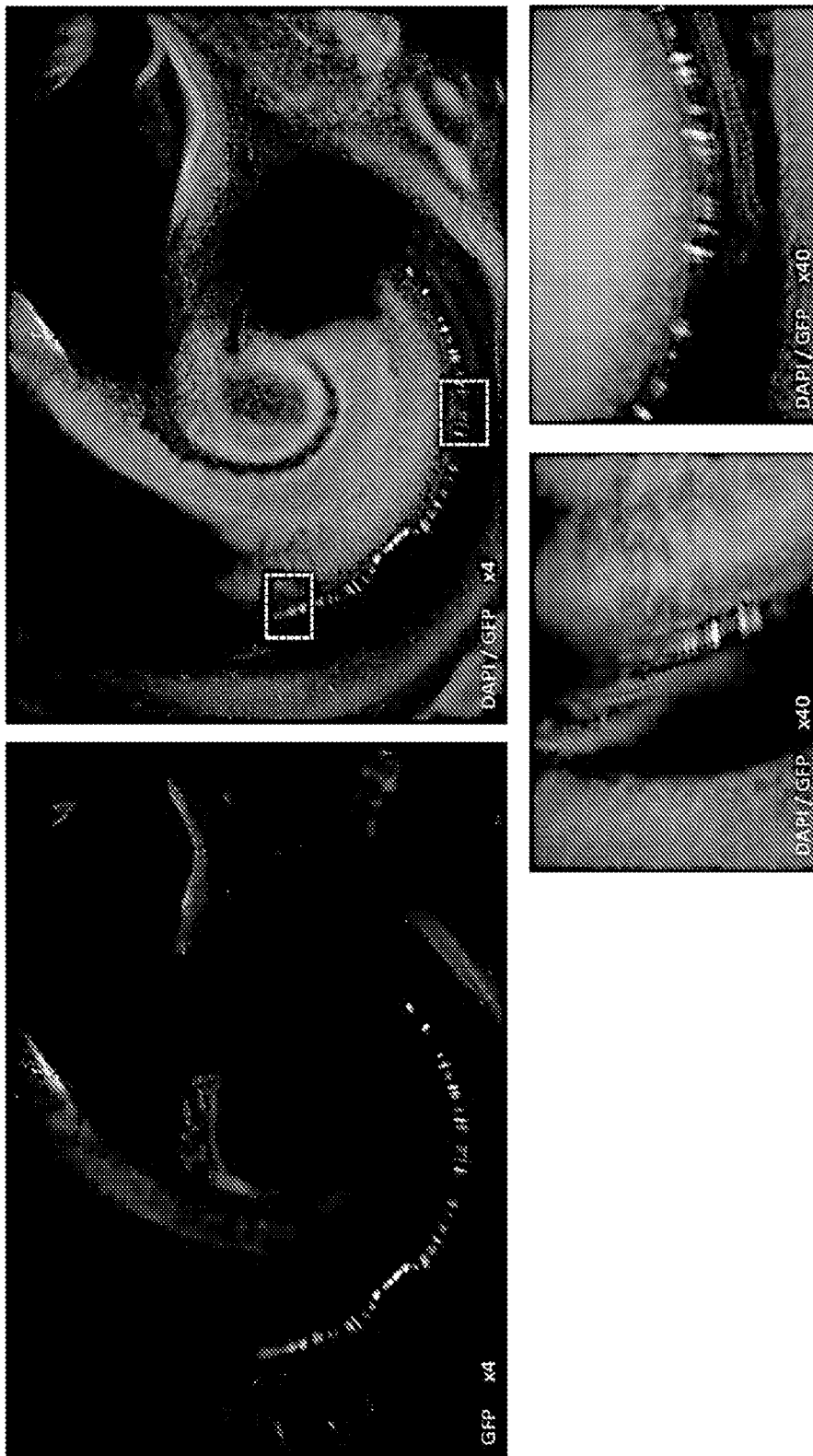


Figure 6



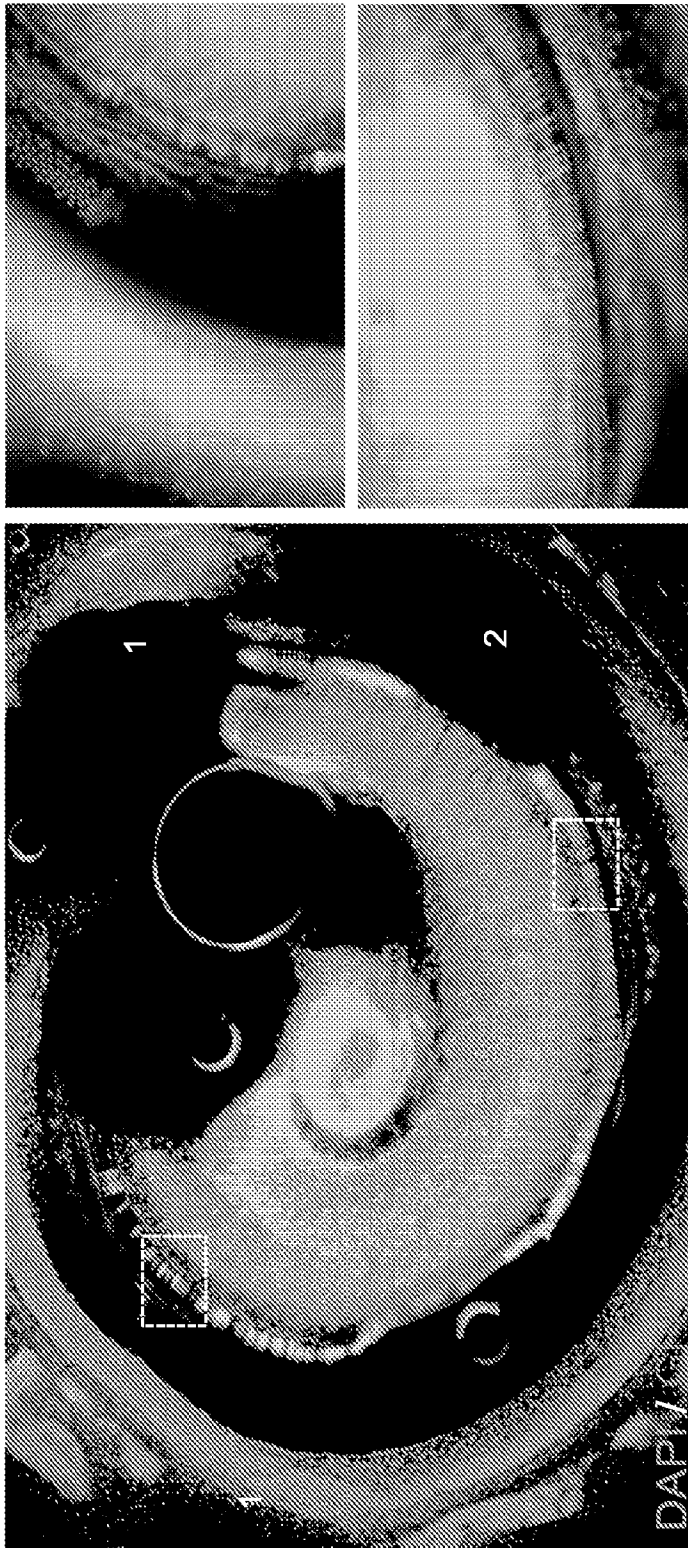


Figure 7

Figure 8

Vestibule

Cochlea Mouse #1

Cochlea Mouse #2

Cochlea Mouse #30

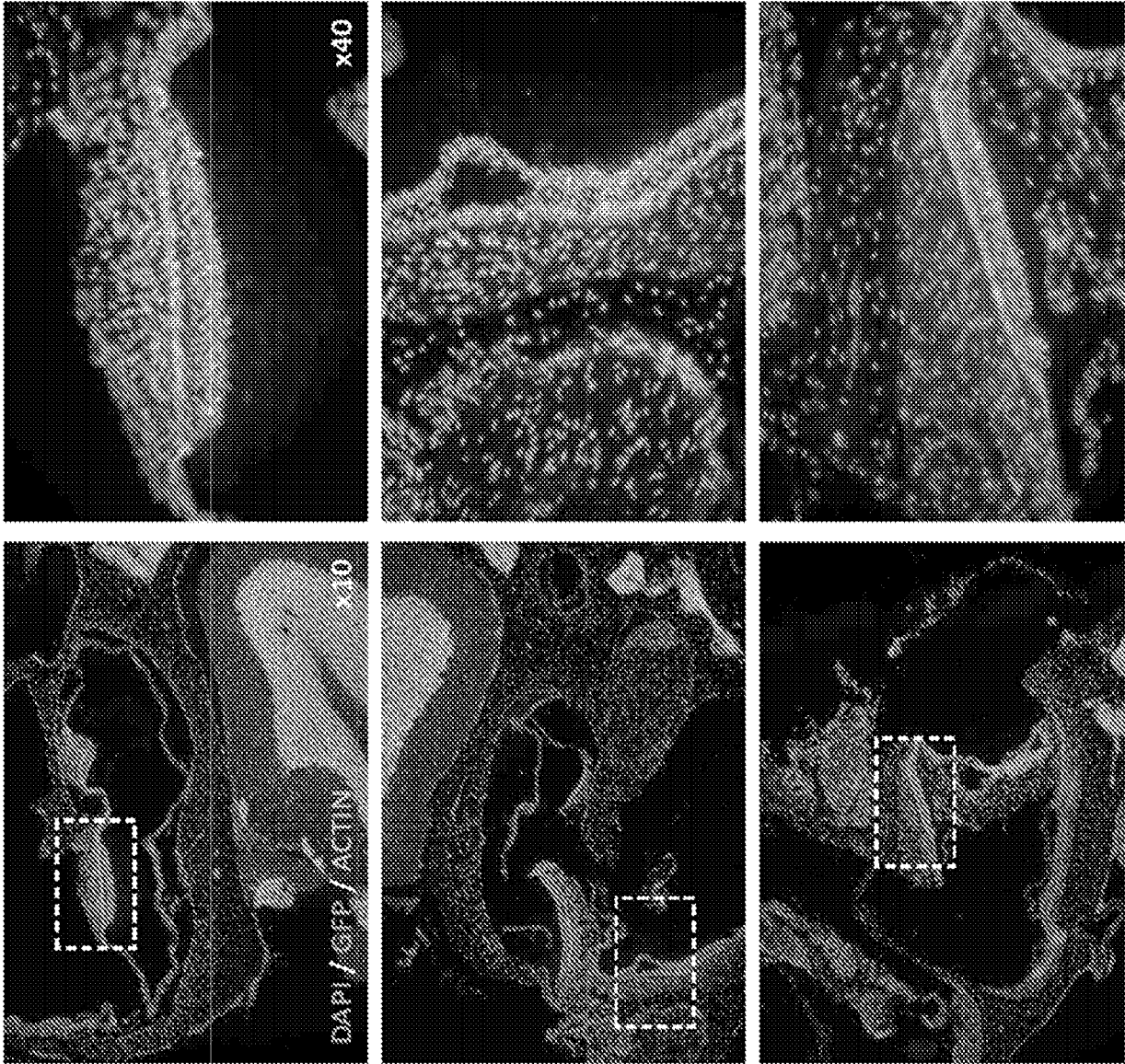


Figure 9

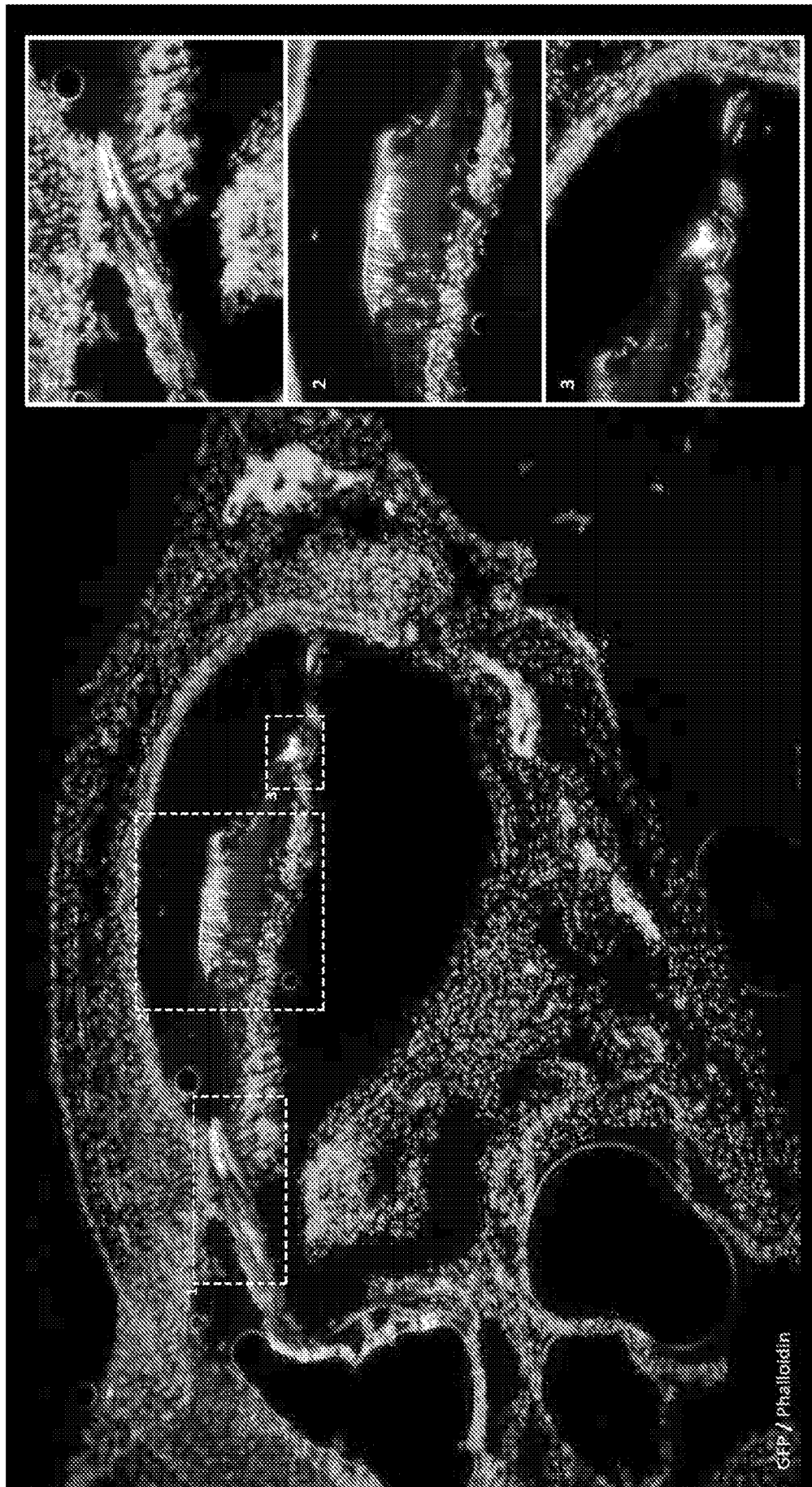


Figure 10

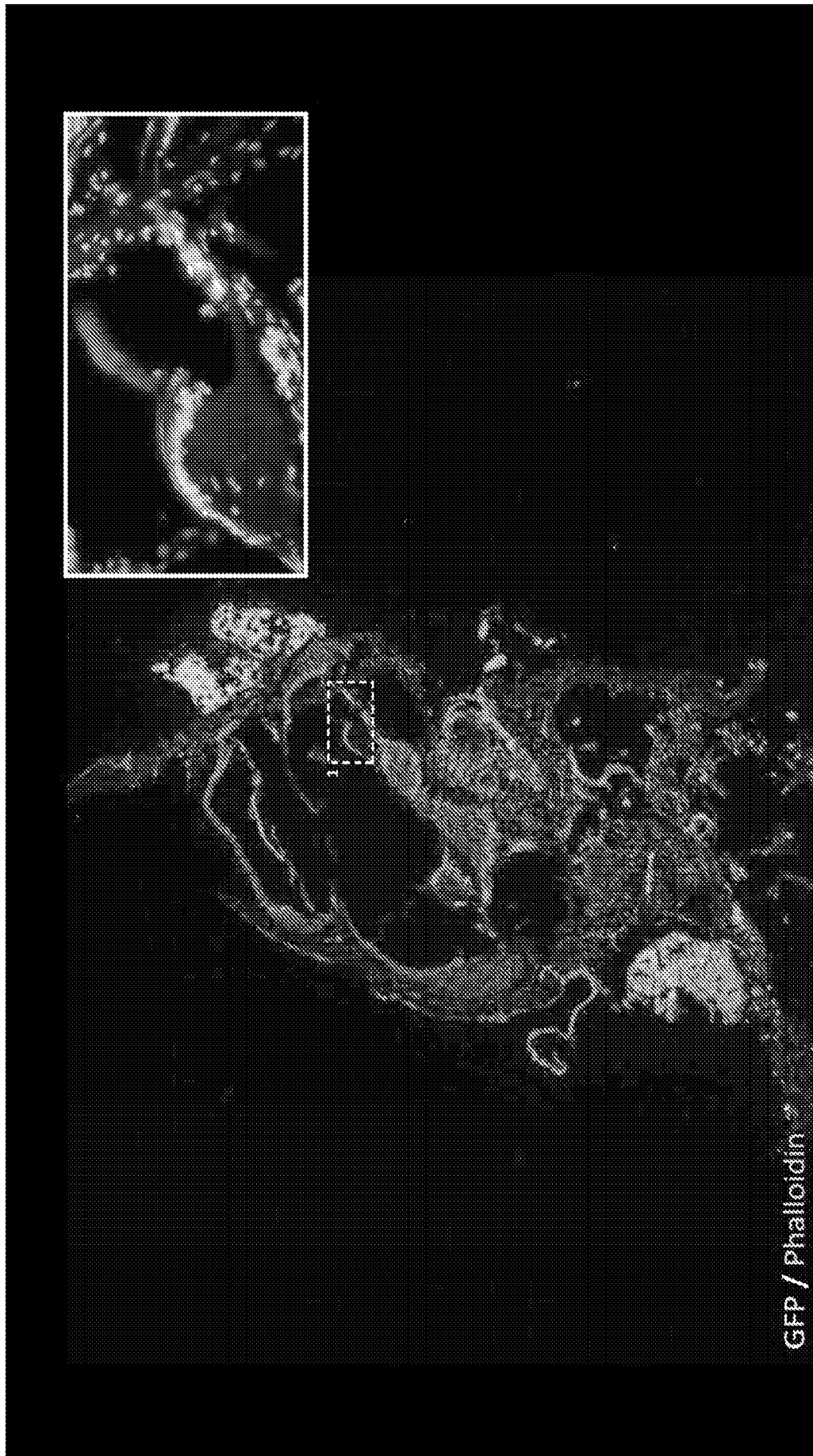
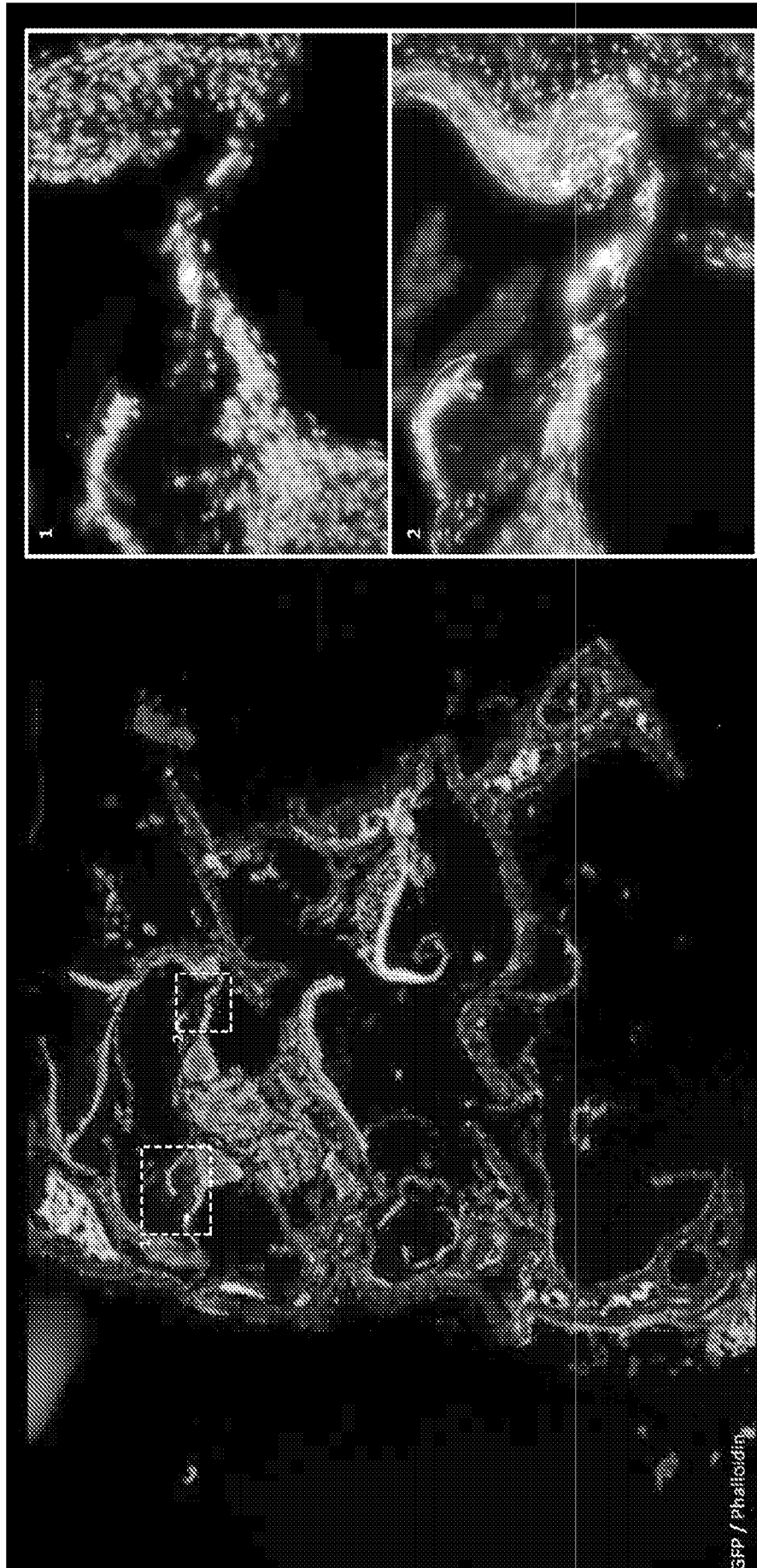


Figure 11



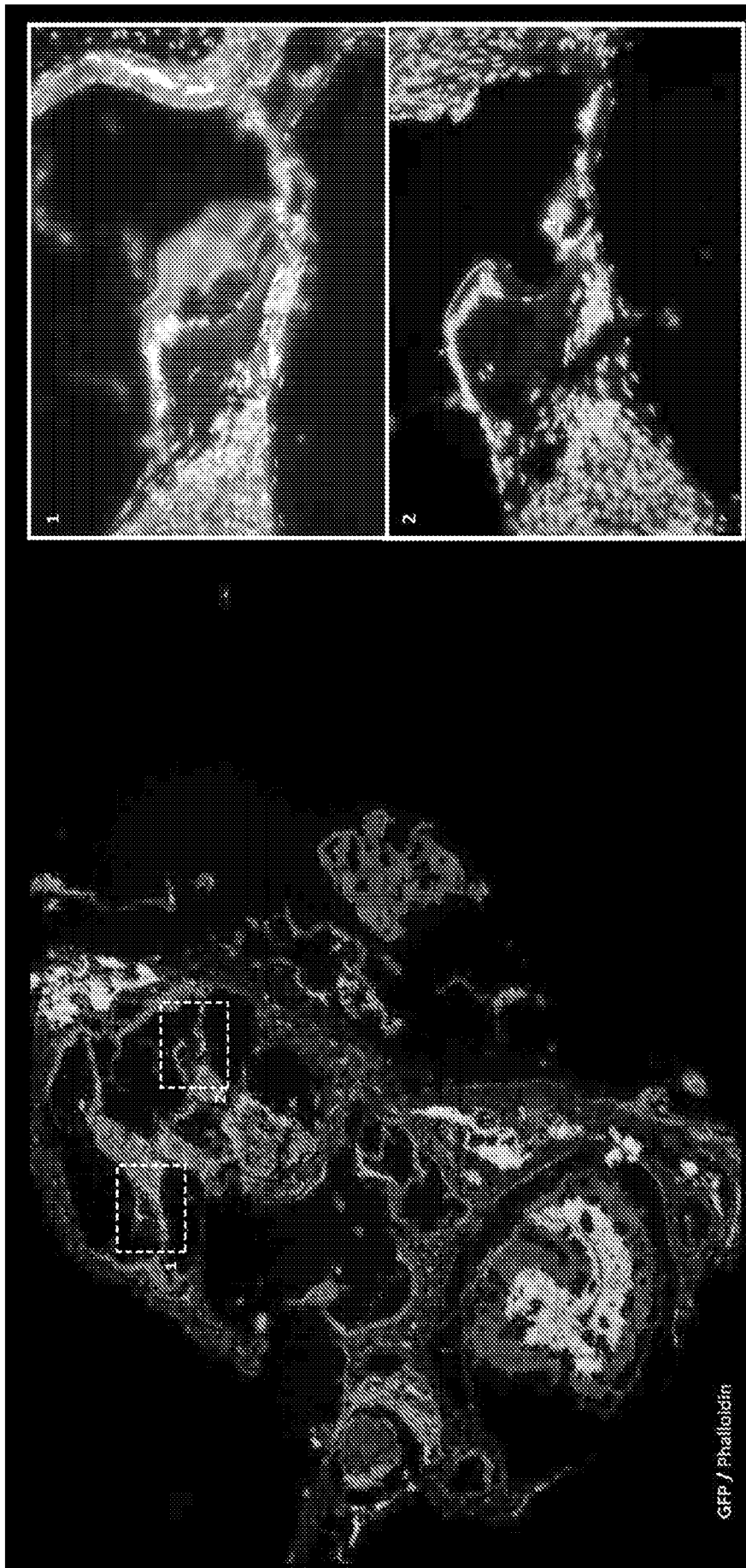


Figure 12

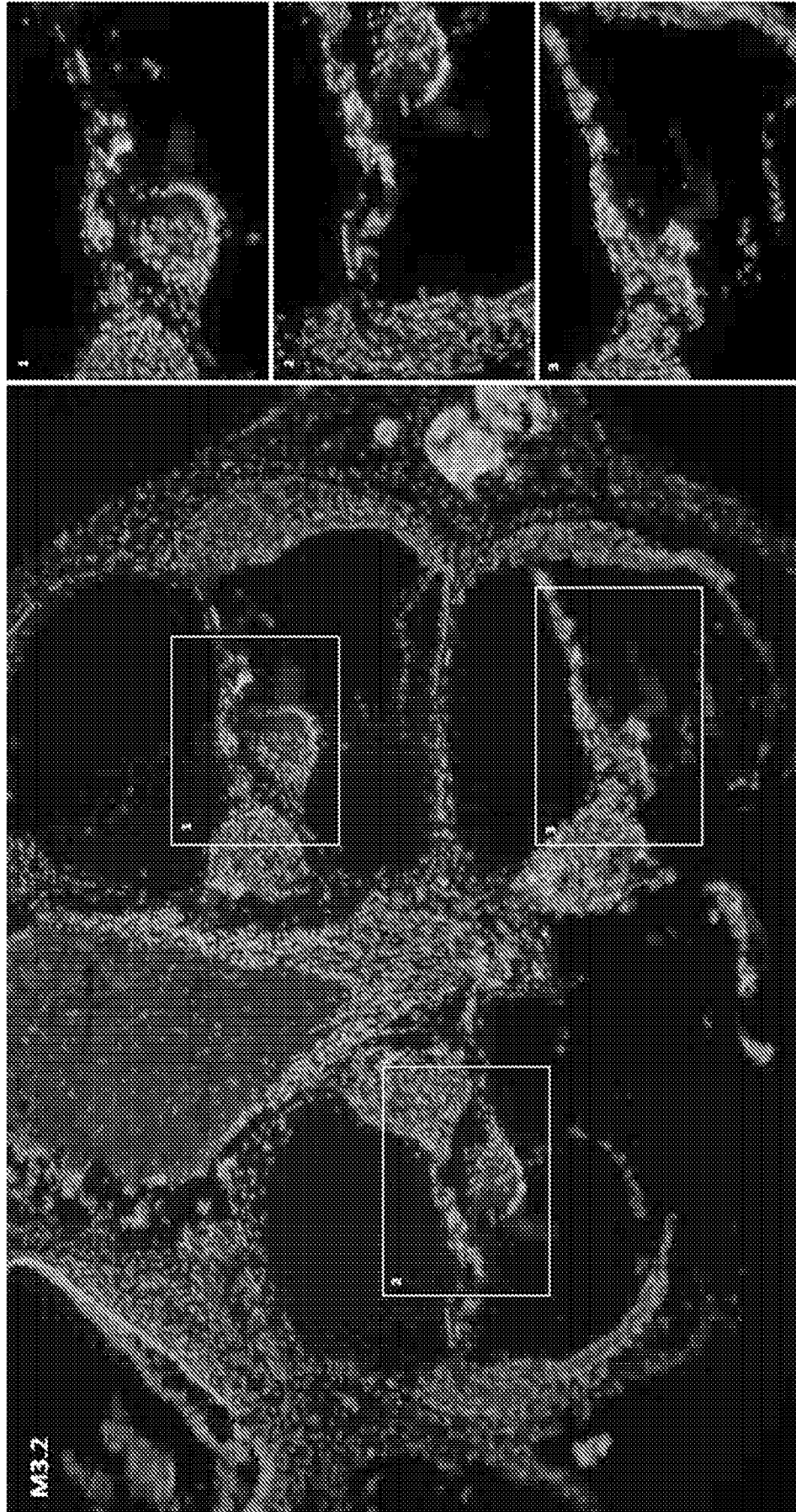


Figure 13

Figure 14

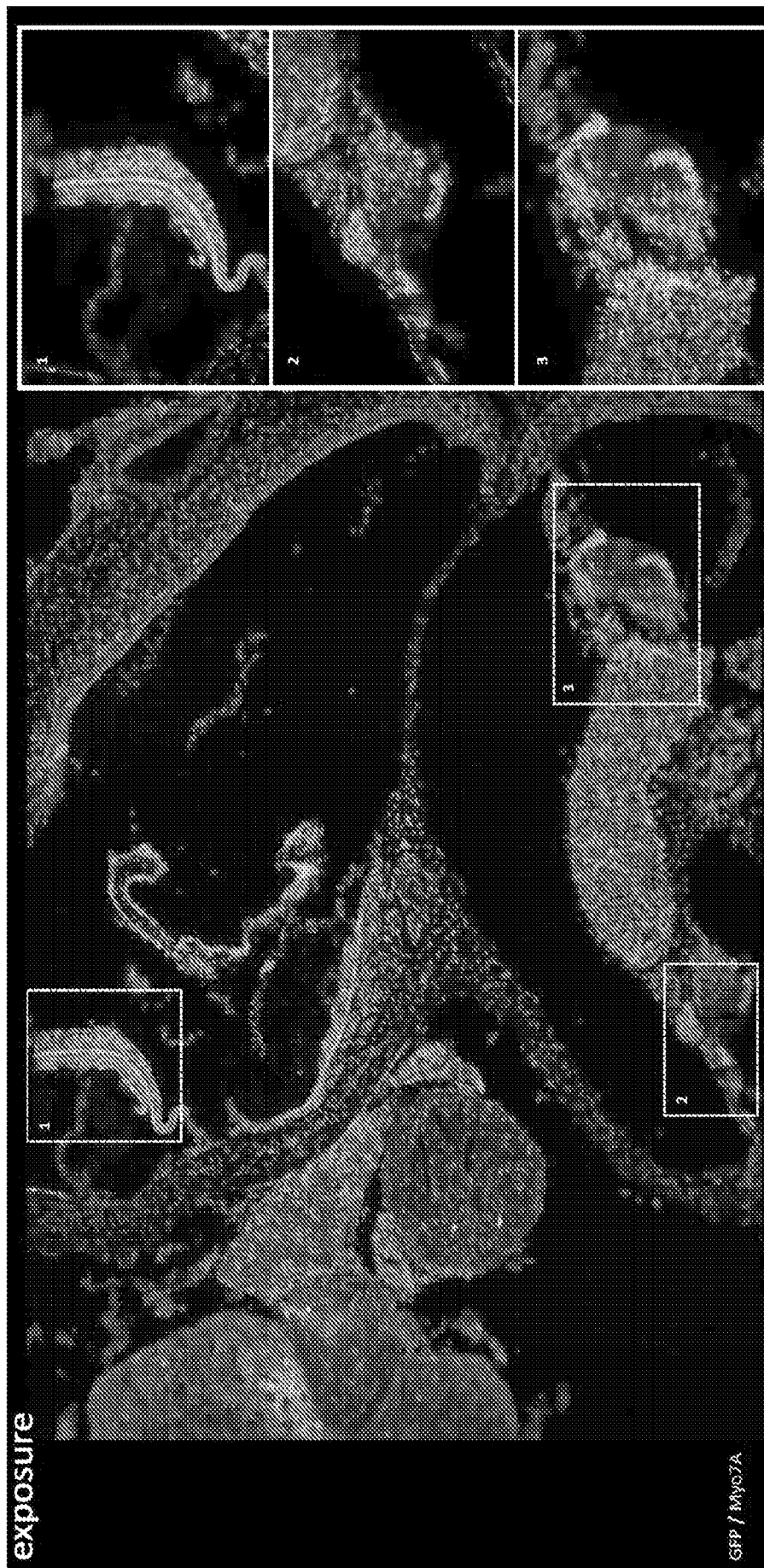


Figure 15

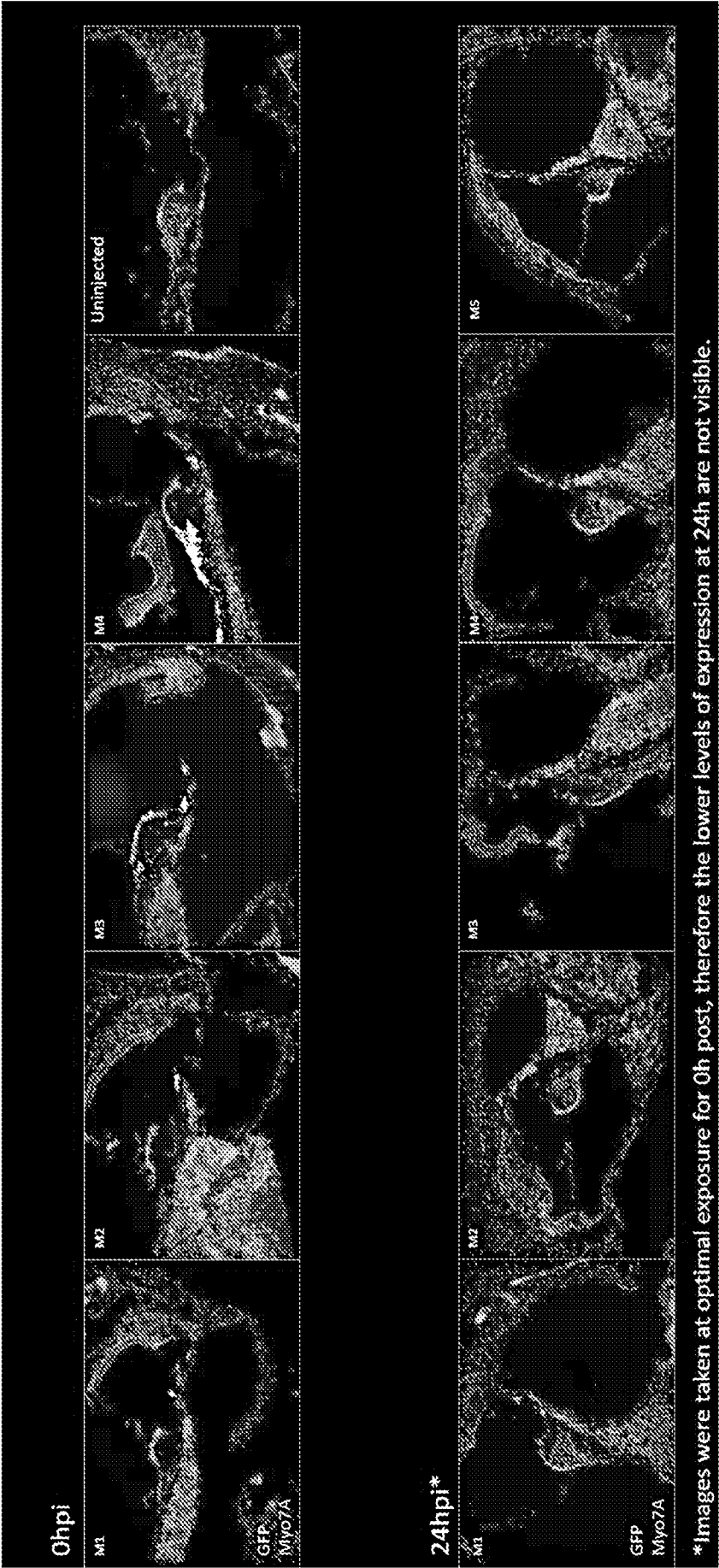
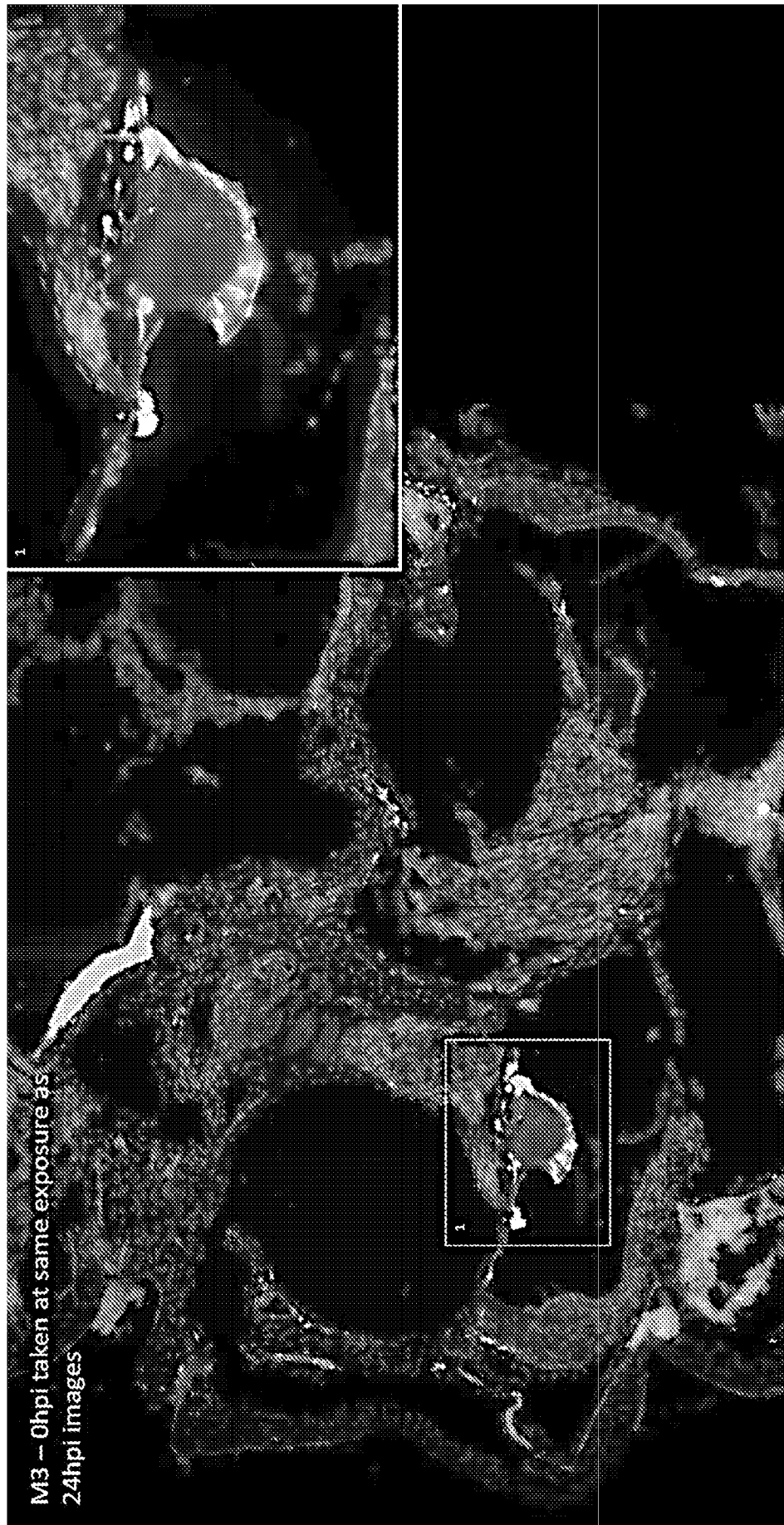


Figure 16



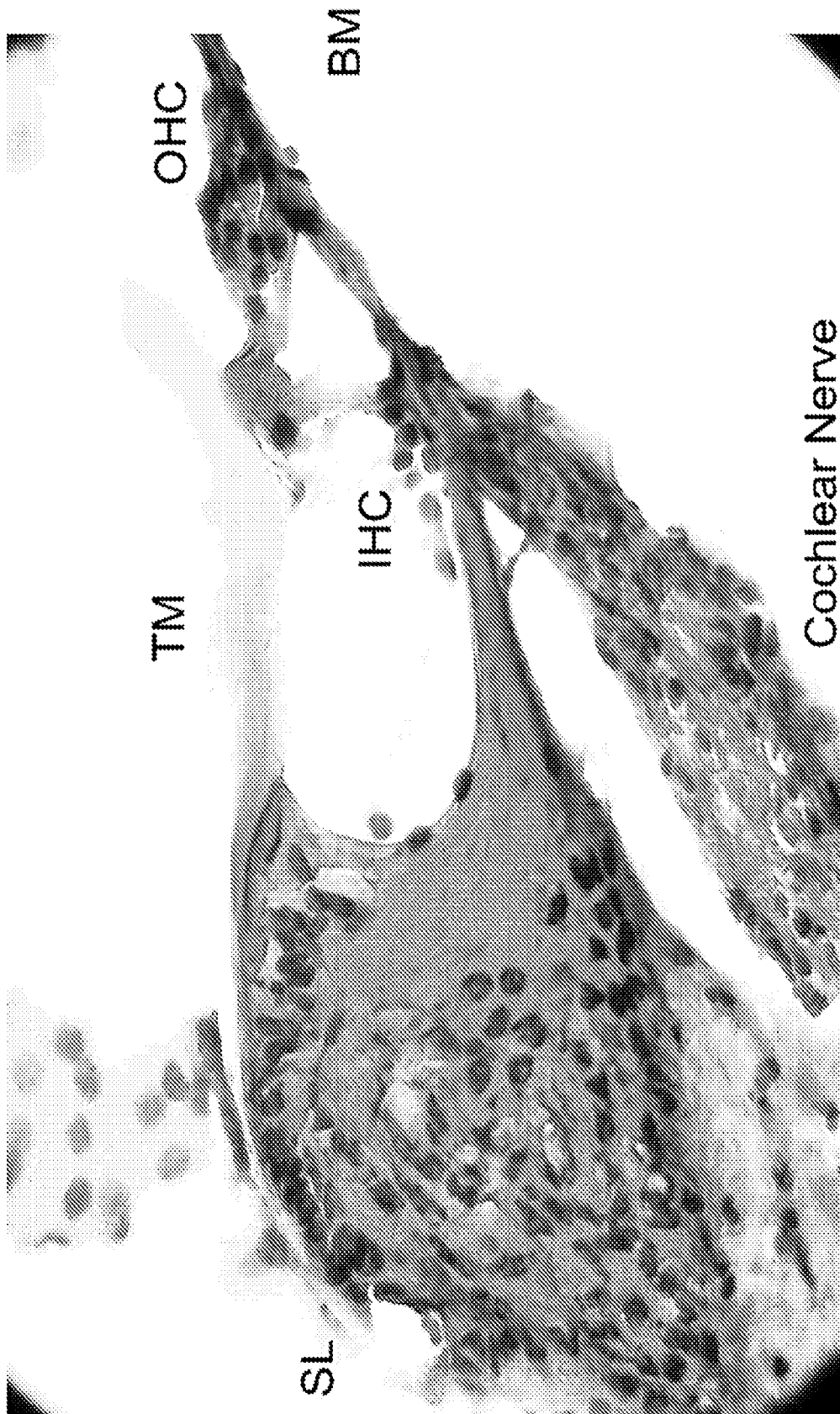


Figure 17

Figure 18

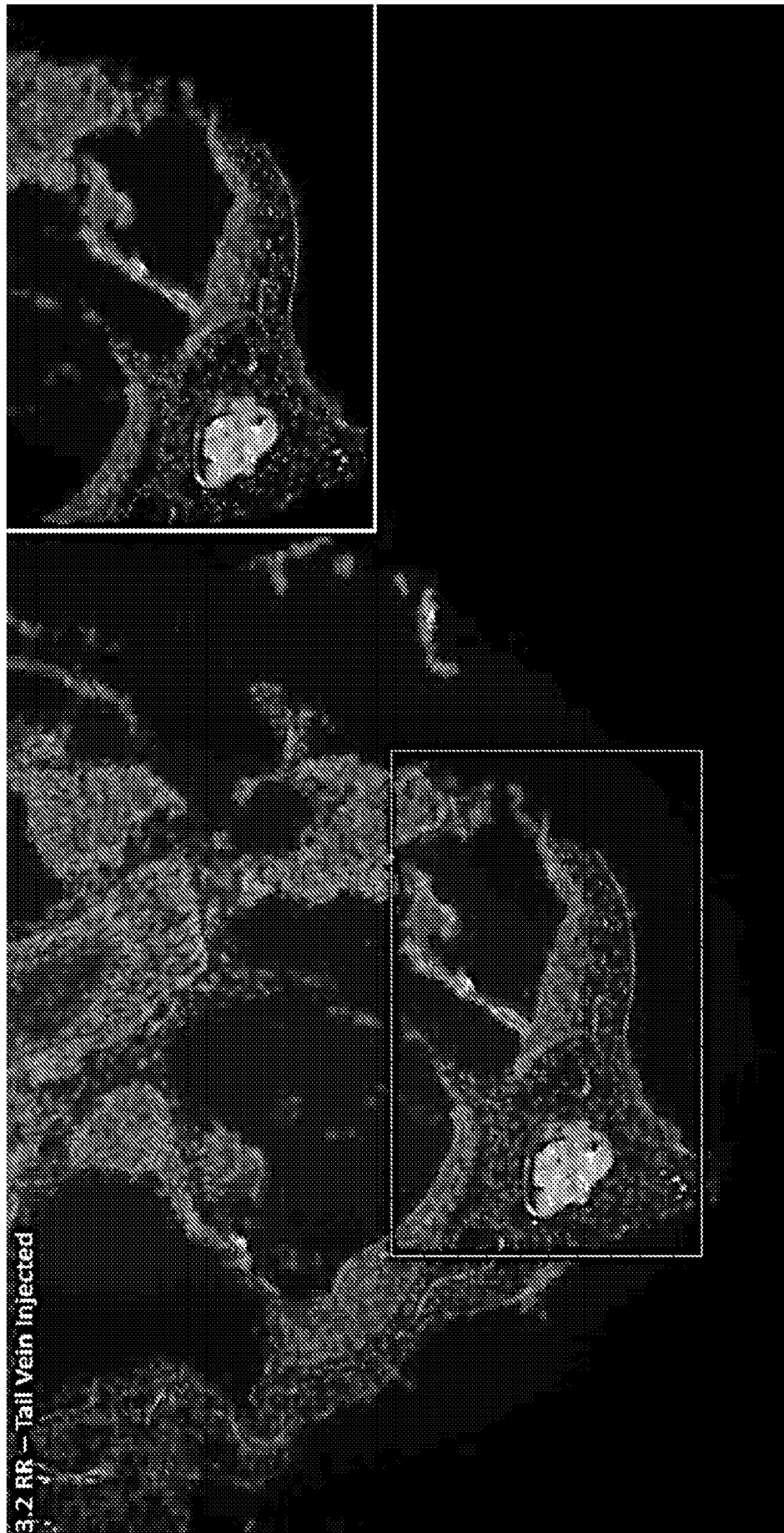


Figure 19

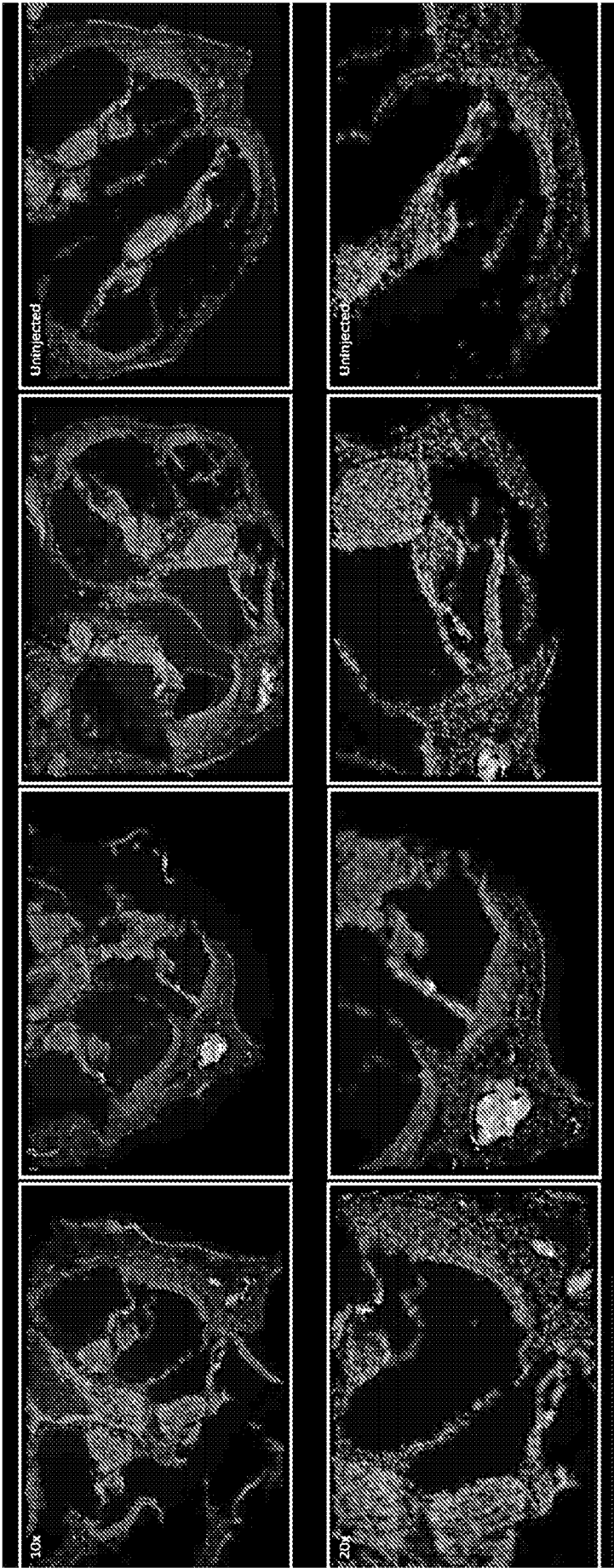


Figure 20

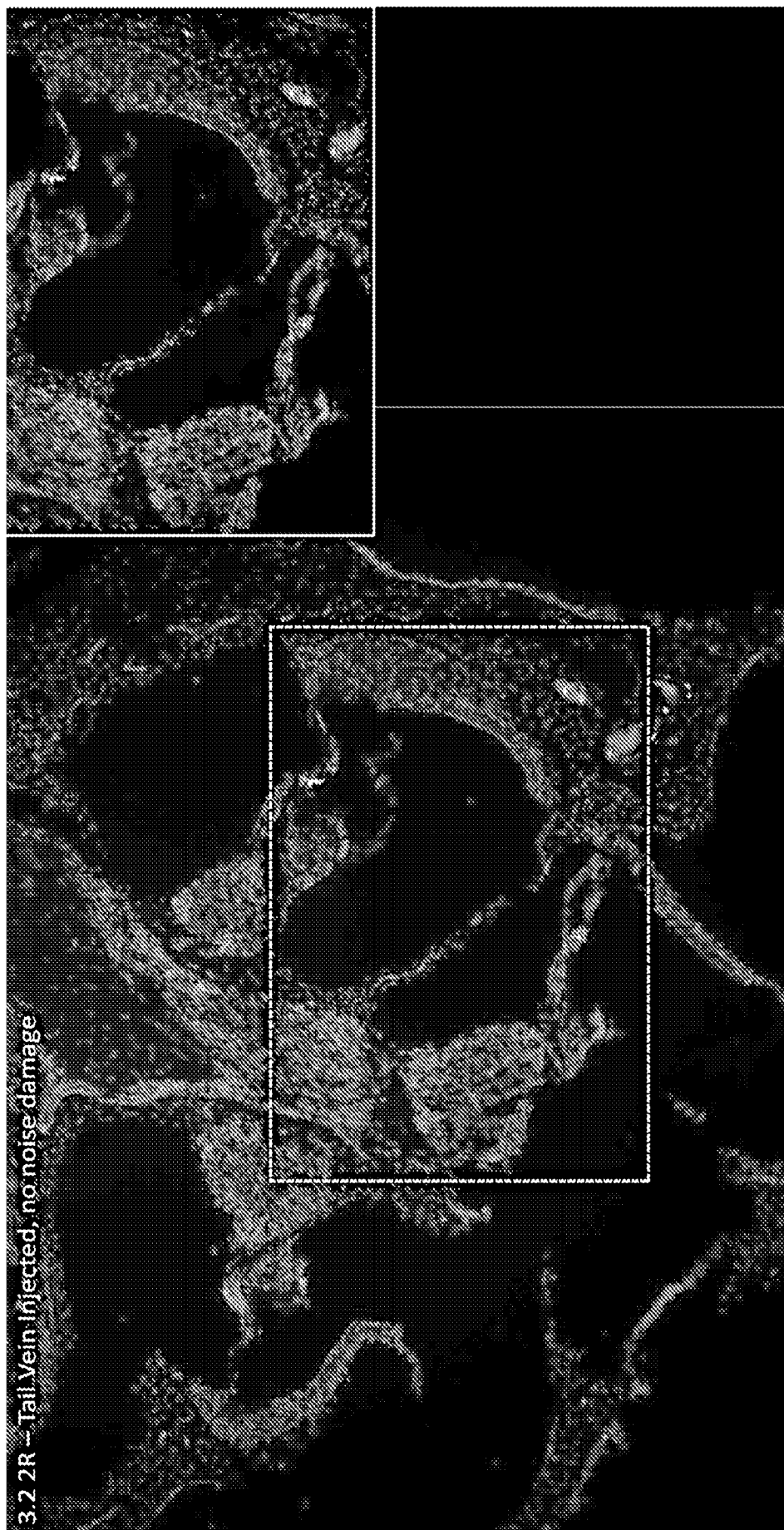


Figure 21

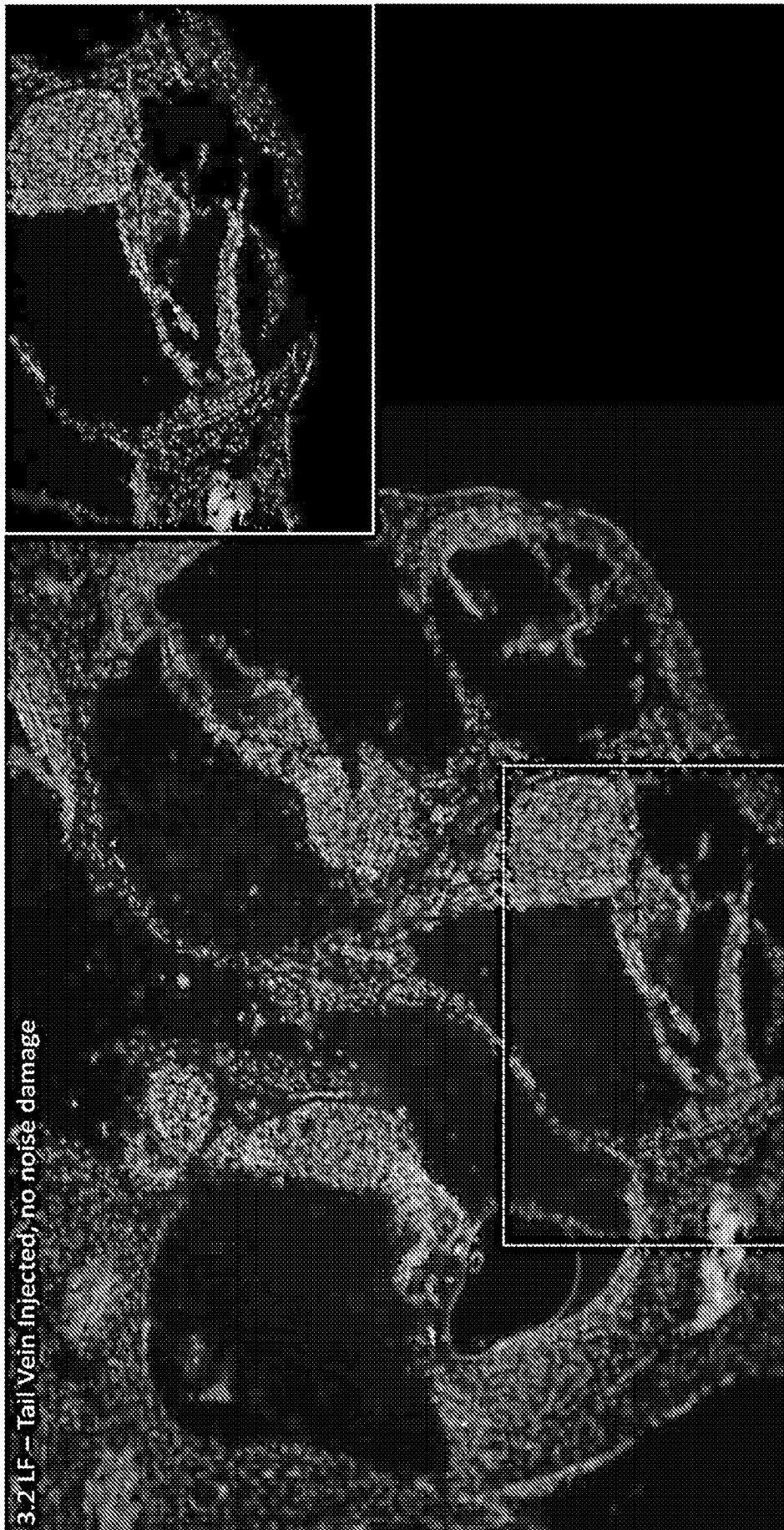


Figure 22

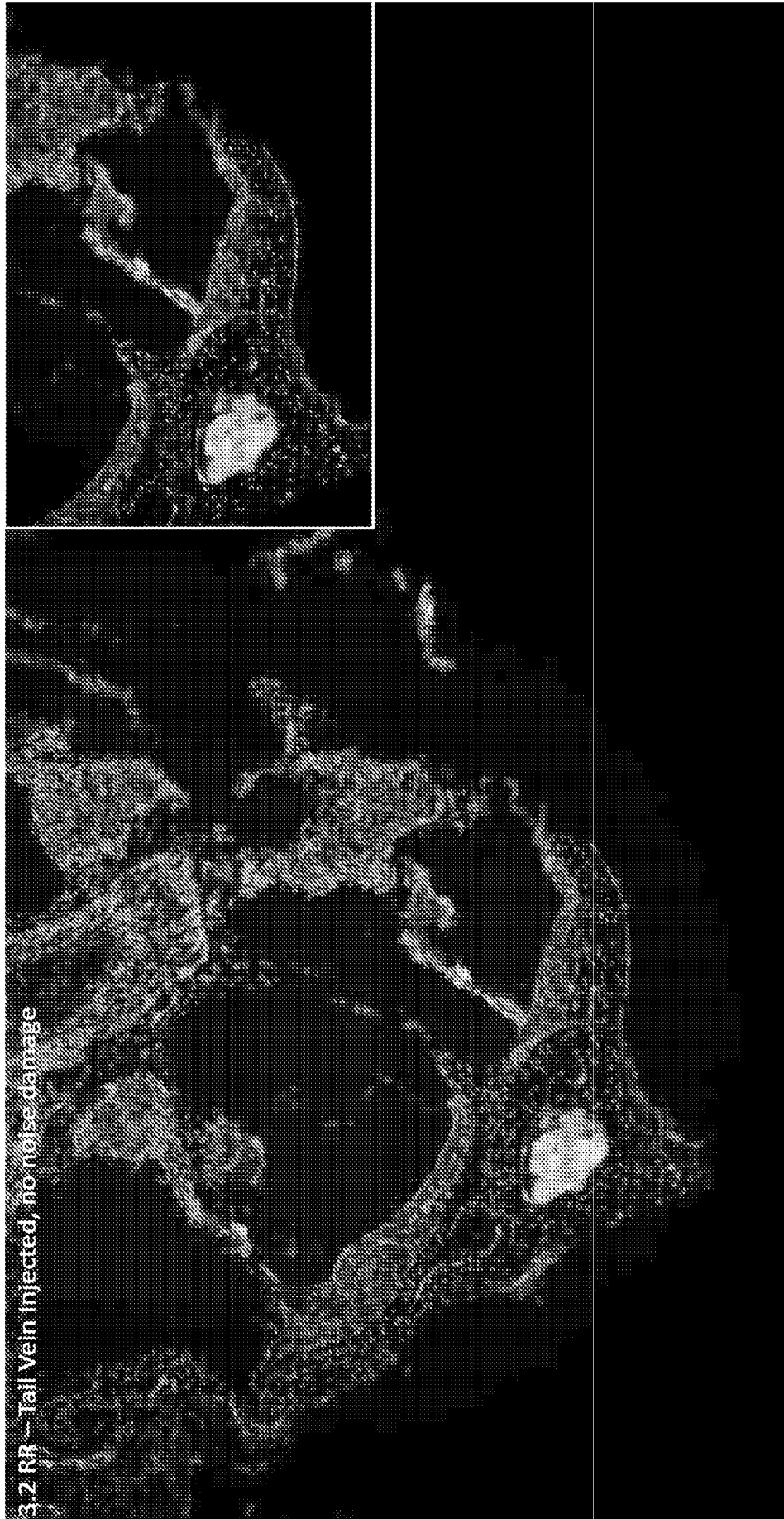


Figure 23

