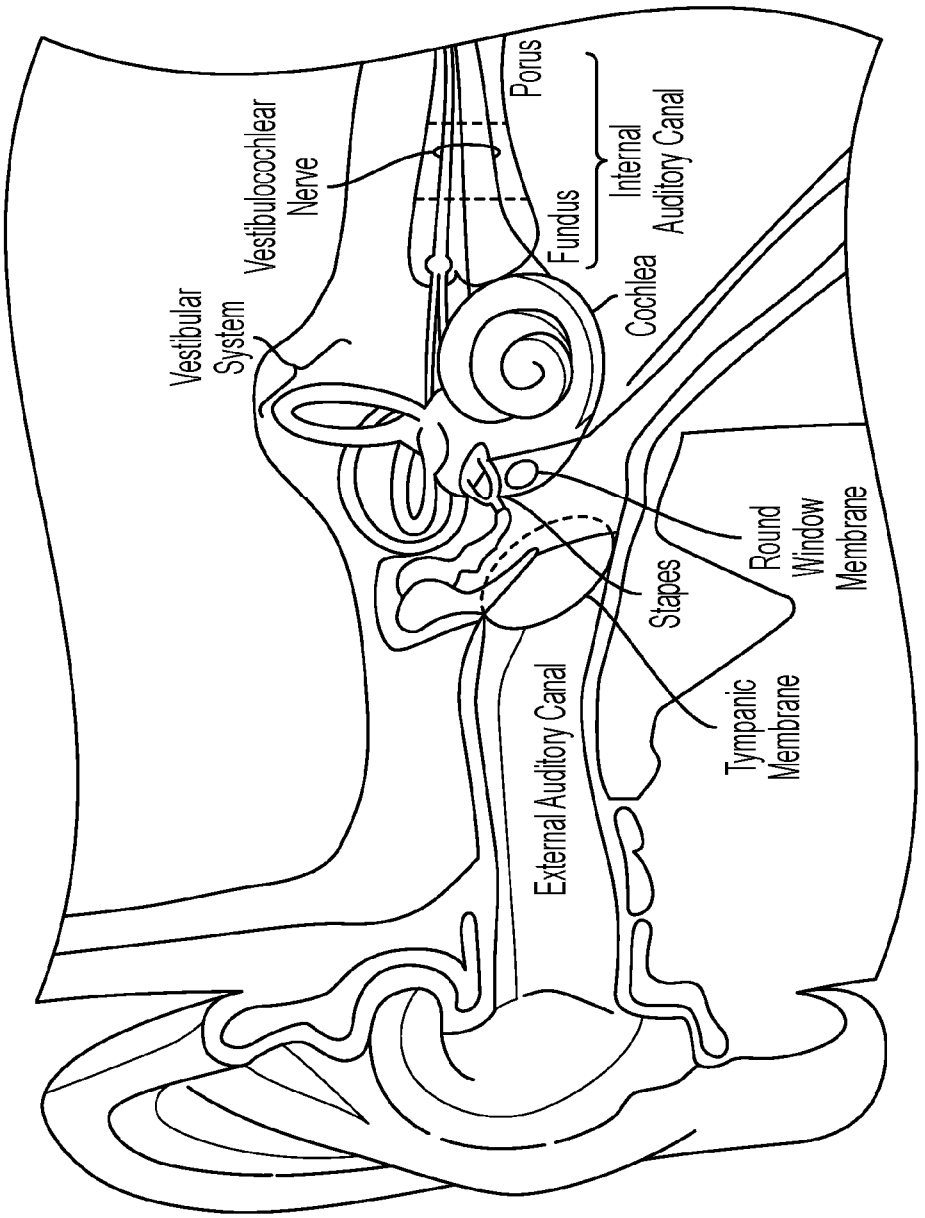

Porus
Internal Auditory Canal
Fundus
Vestibulocochlear Nerve
Vestibular System
Cochlea
Round Window Membrane
Stapes
Tympanic Membrane
External Auditory Canal

FIG. 1

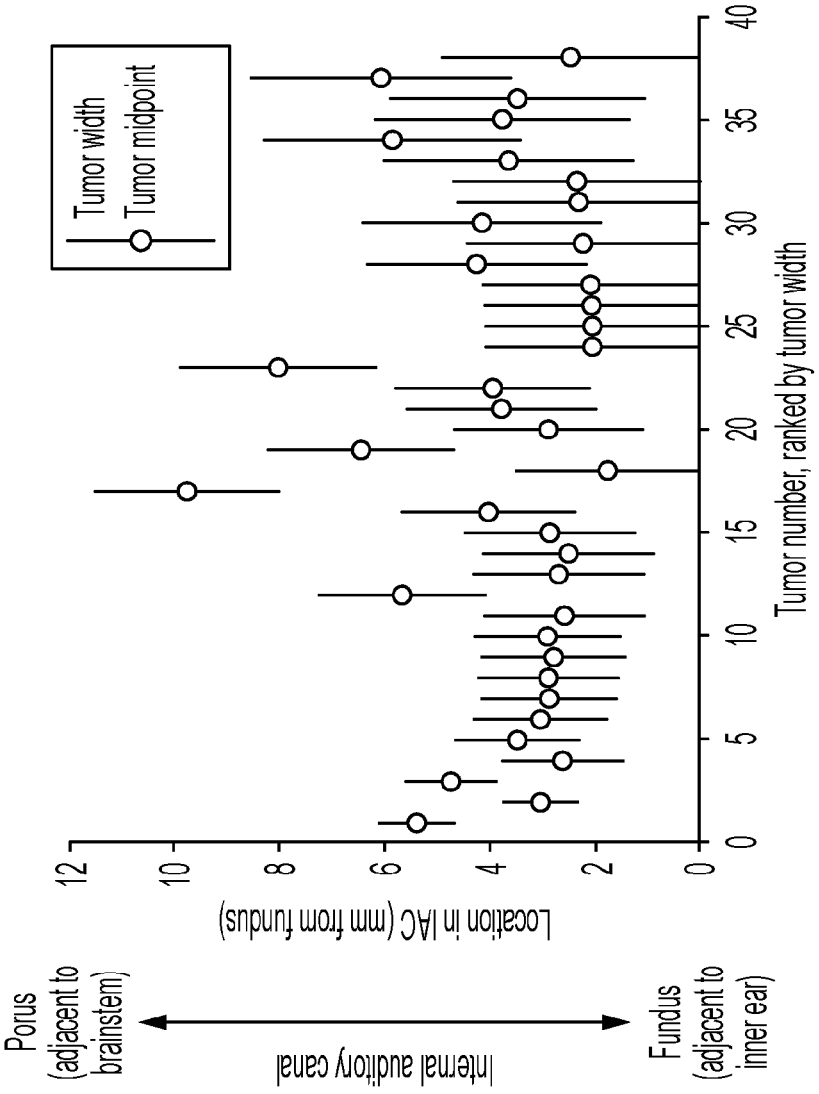

Tumor width
Tumor midpoint
Location in IAC (mm from fundus)
0
2
4
6
8
10
12
Tumor number, ranked by tumor width
0
5
10
15
20
25
30
35
40
Porus
(adjacent to
brainstem)
Internal auditory canal
Fundus
(adjacent to
inner ear)

FIG. 2

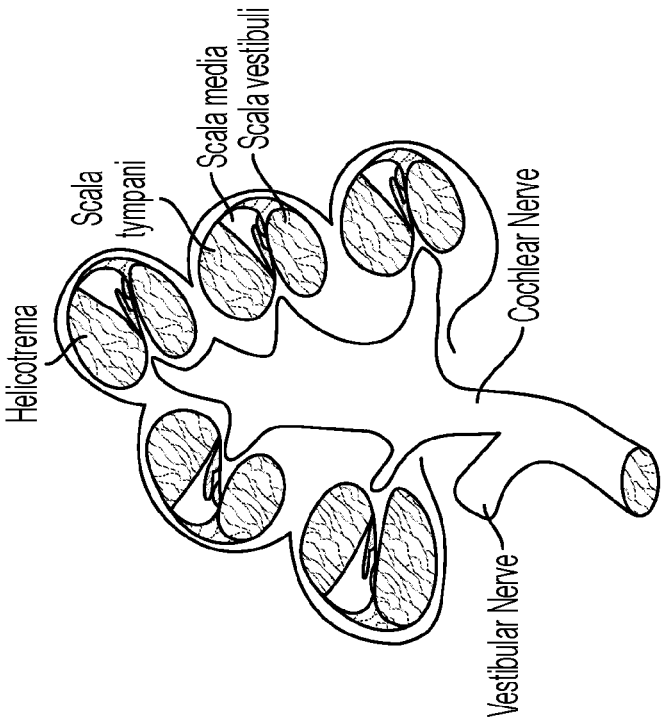

Helicotrema
Scala tympani
Scala media
Scala vestibuli
Cochlear Nerve
Vestibular Nerve

FIG. 3B

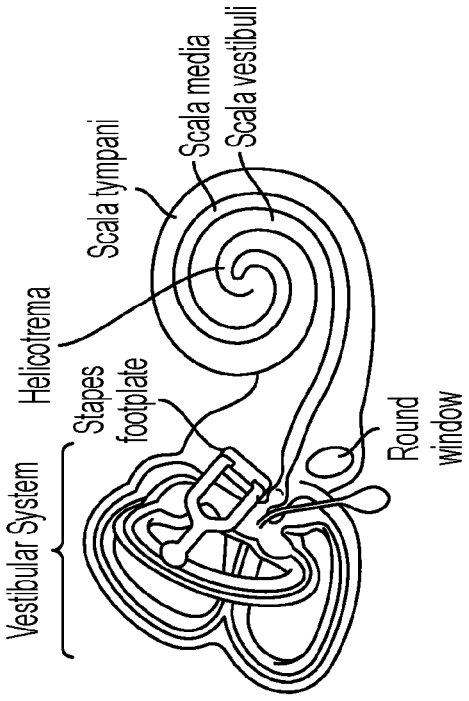

Vestibular System
Helicotrema
Stapes footplate
Scala tympani
Scala media
Scala vestibuli
Round window

FIG. 3A

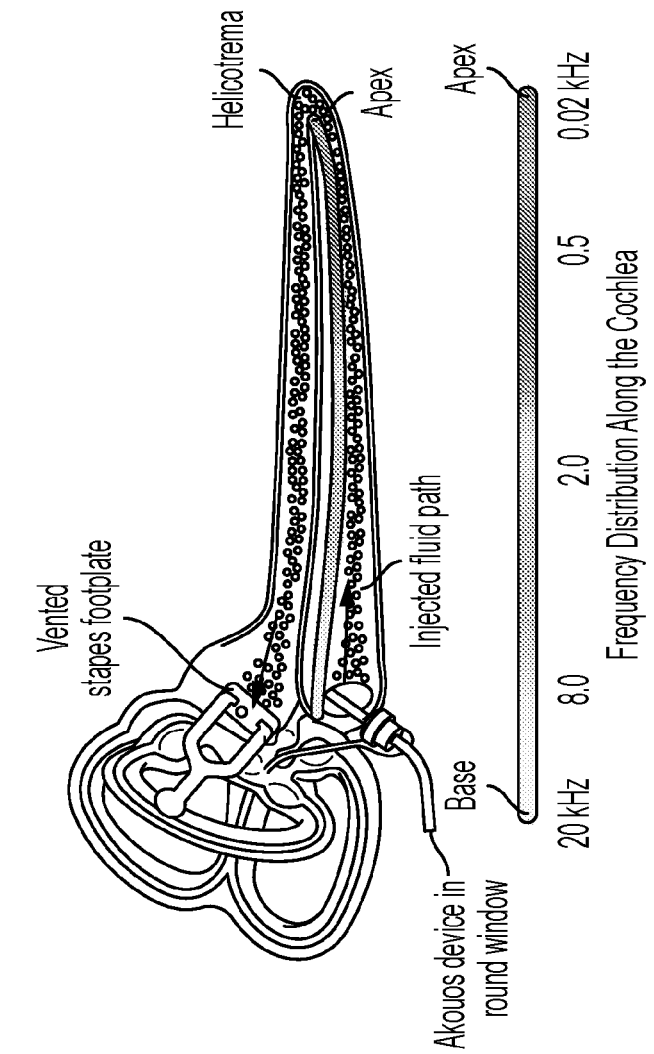

Helicotrema
Apex
Apex
0.02 kHz
0.5
2.0
8.0
20 kHz
Base
Frequency Distribution Along the Cochlea
Injected fluid path
Vented
stapes footplate
Akouos device in
round window

FIG. 4B

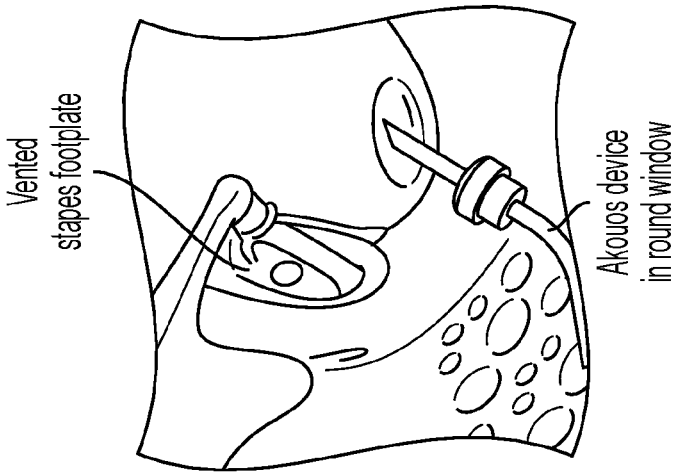

Vented
stapes footplate
Akouos device
in round window

FIG. 4A

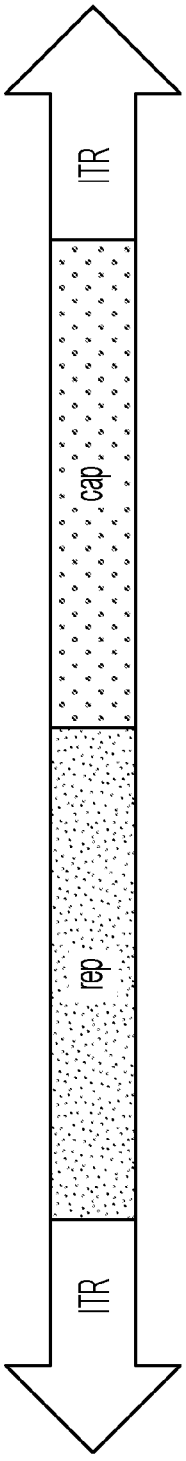

ITR
cap
rep
ITR

FIG. 5A

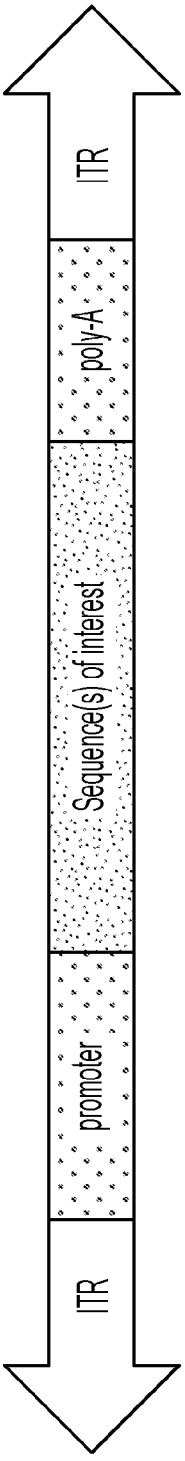

ITR
poly-A
Sequence(s) of interest
promoter
ITR

FIG. 5B

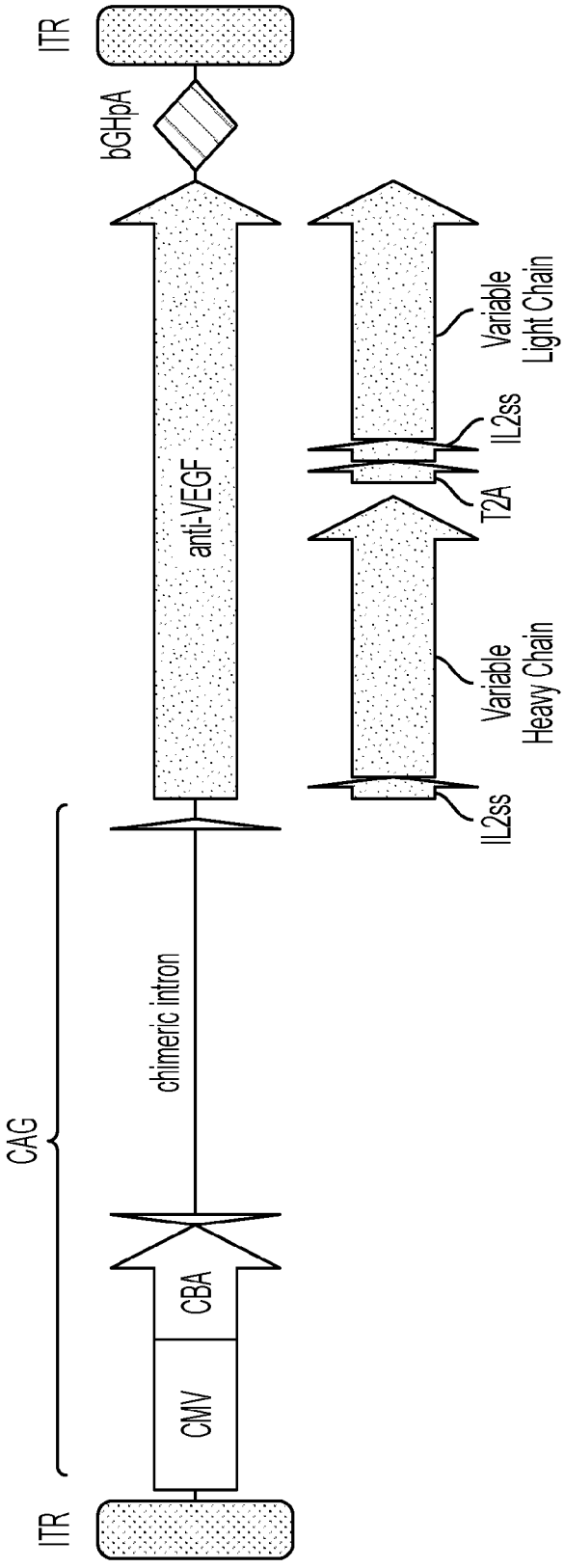

ITR
bGHpA
anti-VEGF
IL2ss
Variable Heavy Chain
T2A
IL2ss
Variable Light Chain
chimeric intron
CAG
CBA
CMV
ITR

FIG. 6A

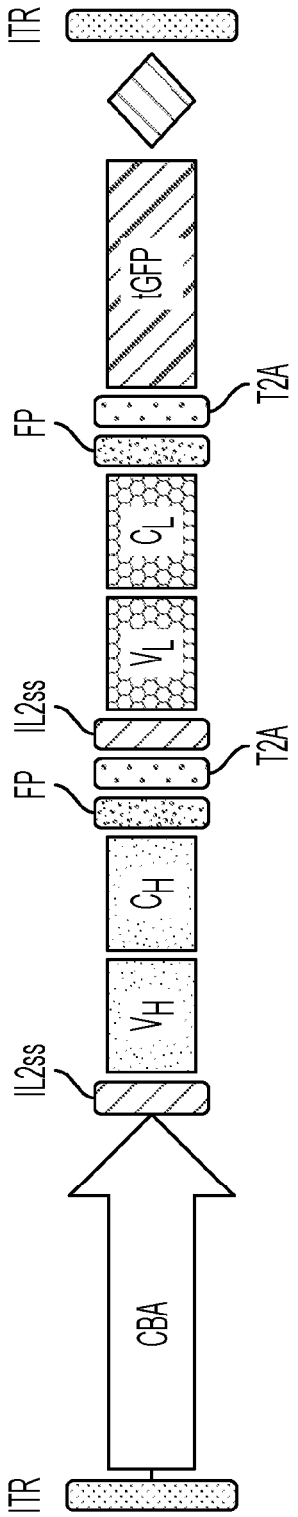


FIG. 6B

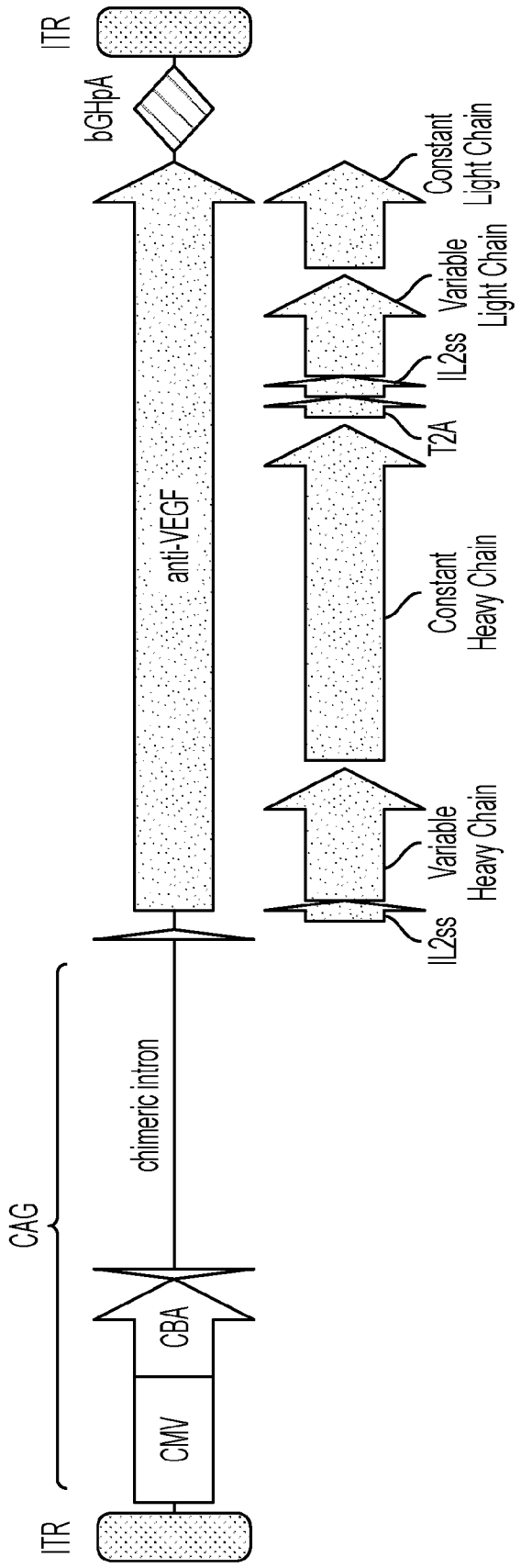

ITR
bGHpA
anti-VEGF
Constant Light Chain
Variable Light Chain
IL2ss
T2A
Constant Heavy Chain
Variable Heavy Chain
IL2ss
CAG
chimeric intron
CBA
CMV
ITR

FIG. 6C

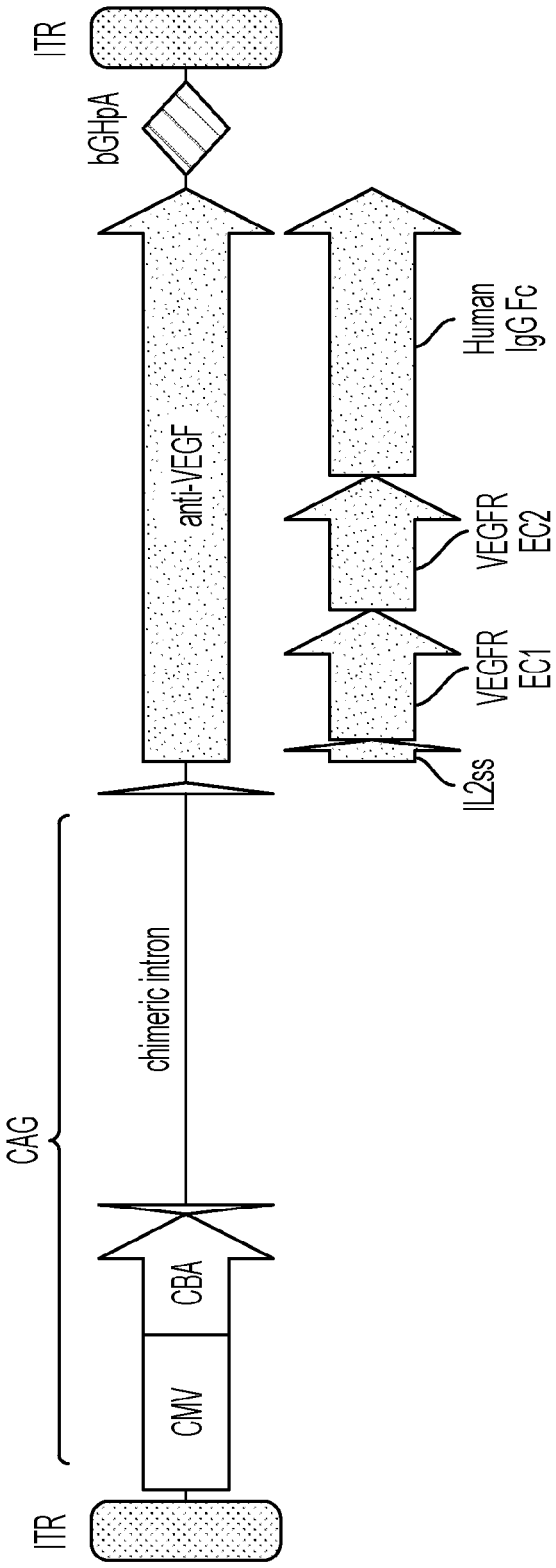

ITR
bGHpA
anti-VEGF
Human IgG Fc
VEGFR EC2
VEGFR EC1
IL2ss
chimeric intron
CAG
CBA
CMV
ITR

FIG. 6D

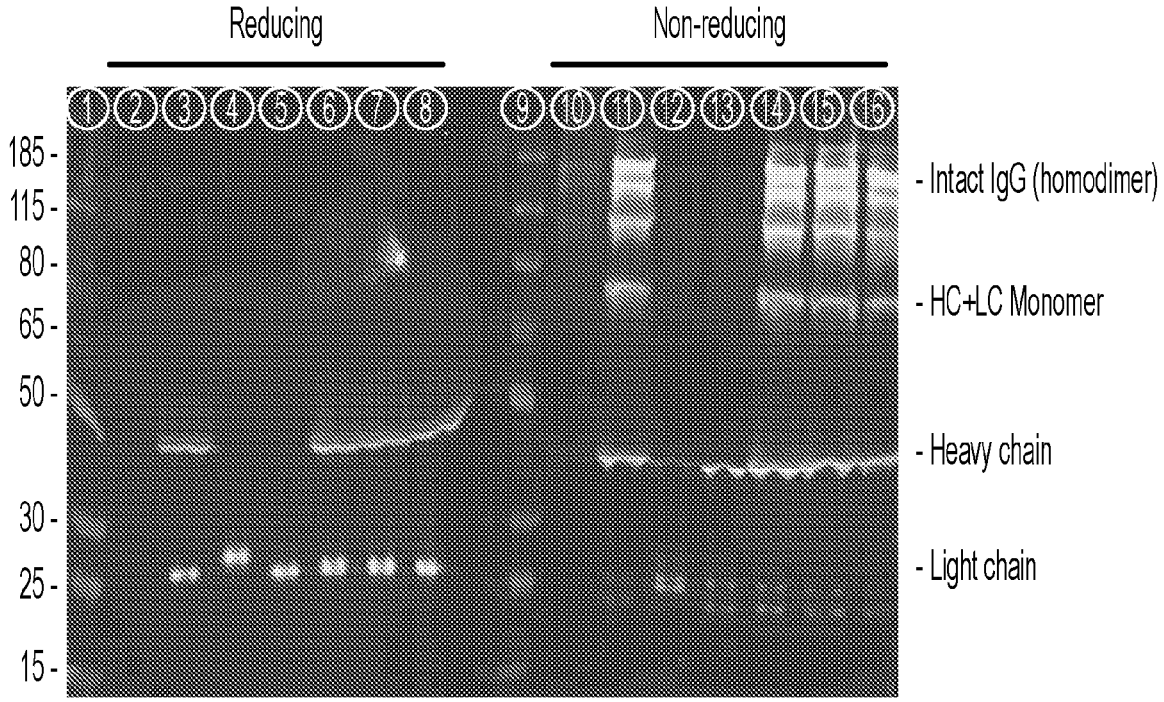

Reducing
Non-reducing
① ② ③ ④ ⑤ ⑥ ⑦ ⑧ ⑨ ⑩ ⑪ ⑫ ⑬ ⑭ ⑮ ⑯
185 -
115 -
80 -
65 -
50 -
30 -
25 -
15 -
- Intact IgG (homodimer)
- HC+LC Monomer
- Heavy chain
- Light chain

FIG. 7

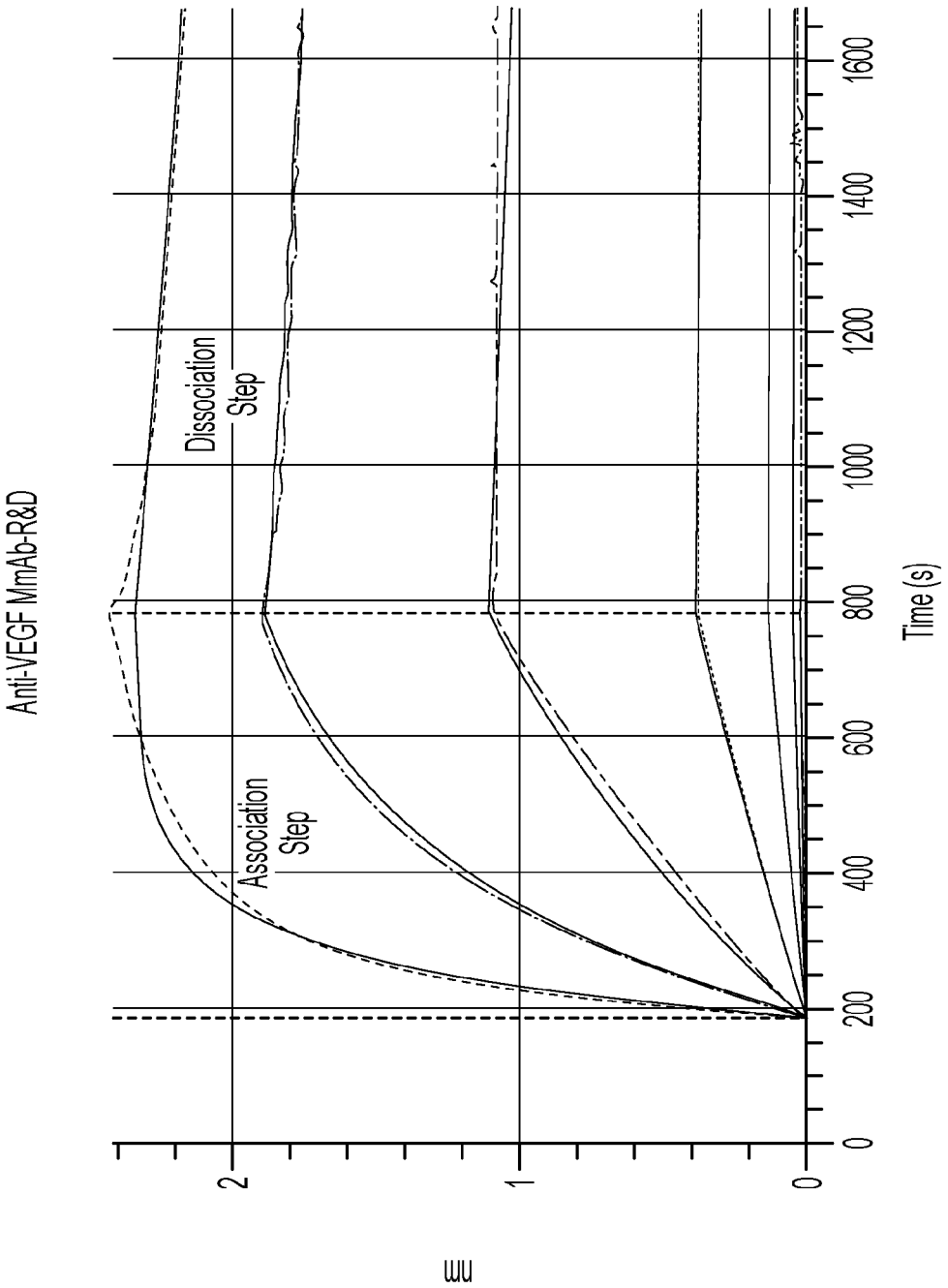

Anti-VEGF MmAb-R&D
Dissociation Step
Association Step
nm
2
1
0
0
200
400
600
800
1000
1200
1400
1600
Time (s)

FIG. 8A

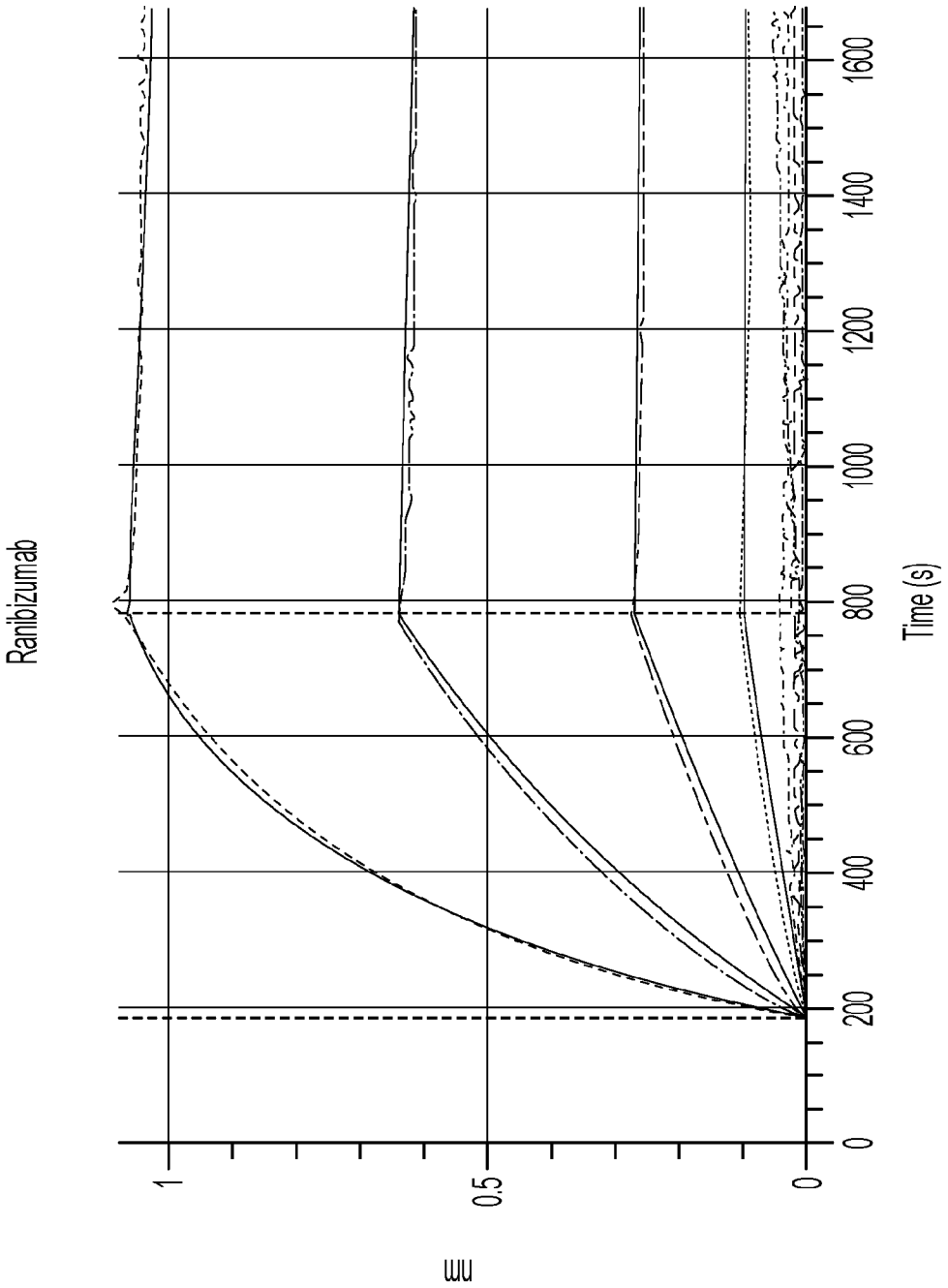

Ranibizumab
mu
1
0.5
0
0
200
400
600
800
1000
1200
1400
1600
Time (s)

FIG. 8B

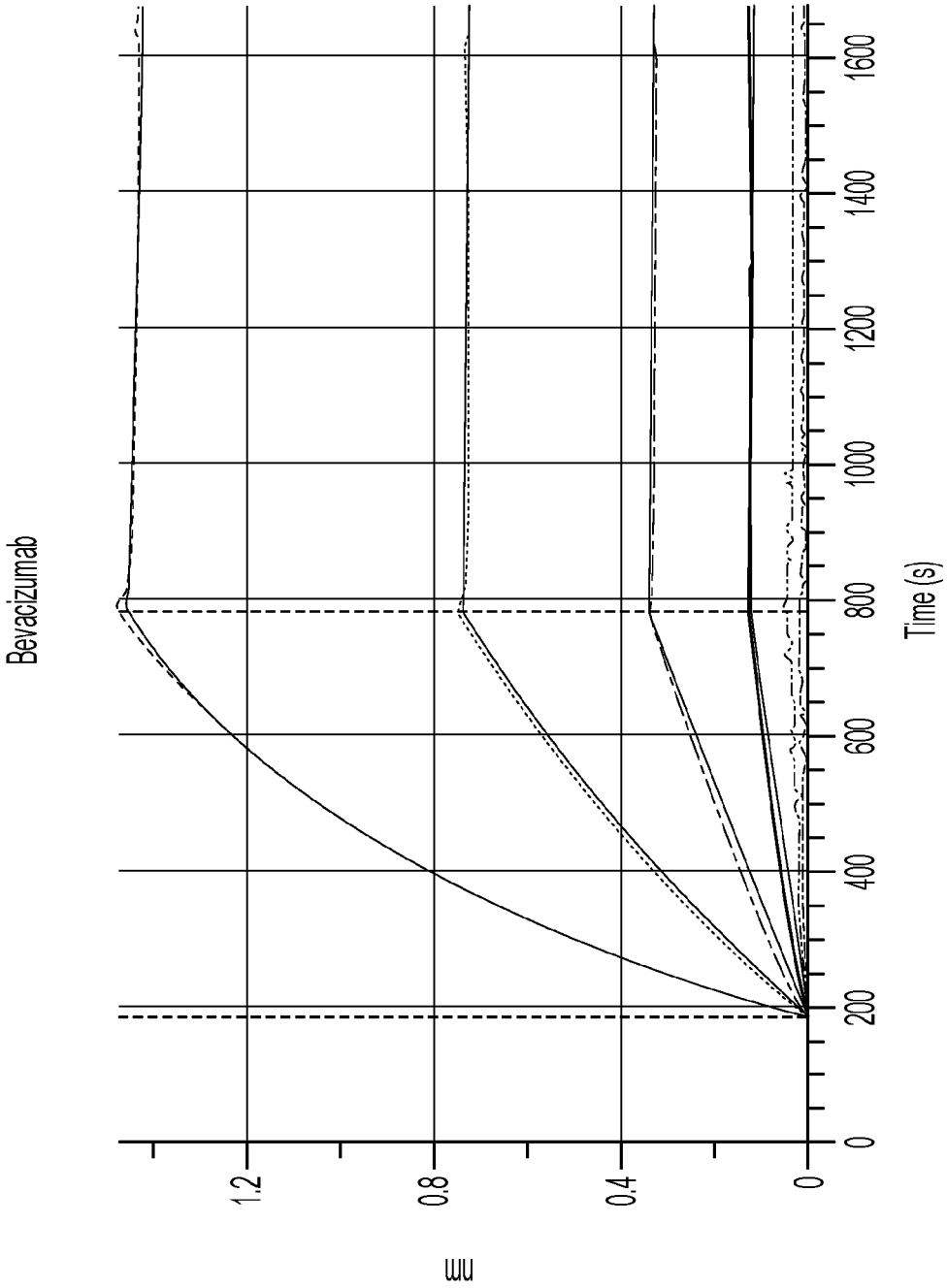

Bevacizumab
nm
1.2
0.8
0.4
0
0
200
400
600
800
1000
1200
1400
1600
Time (s)

FIG. 8C

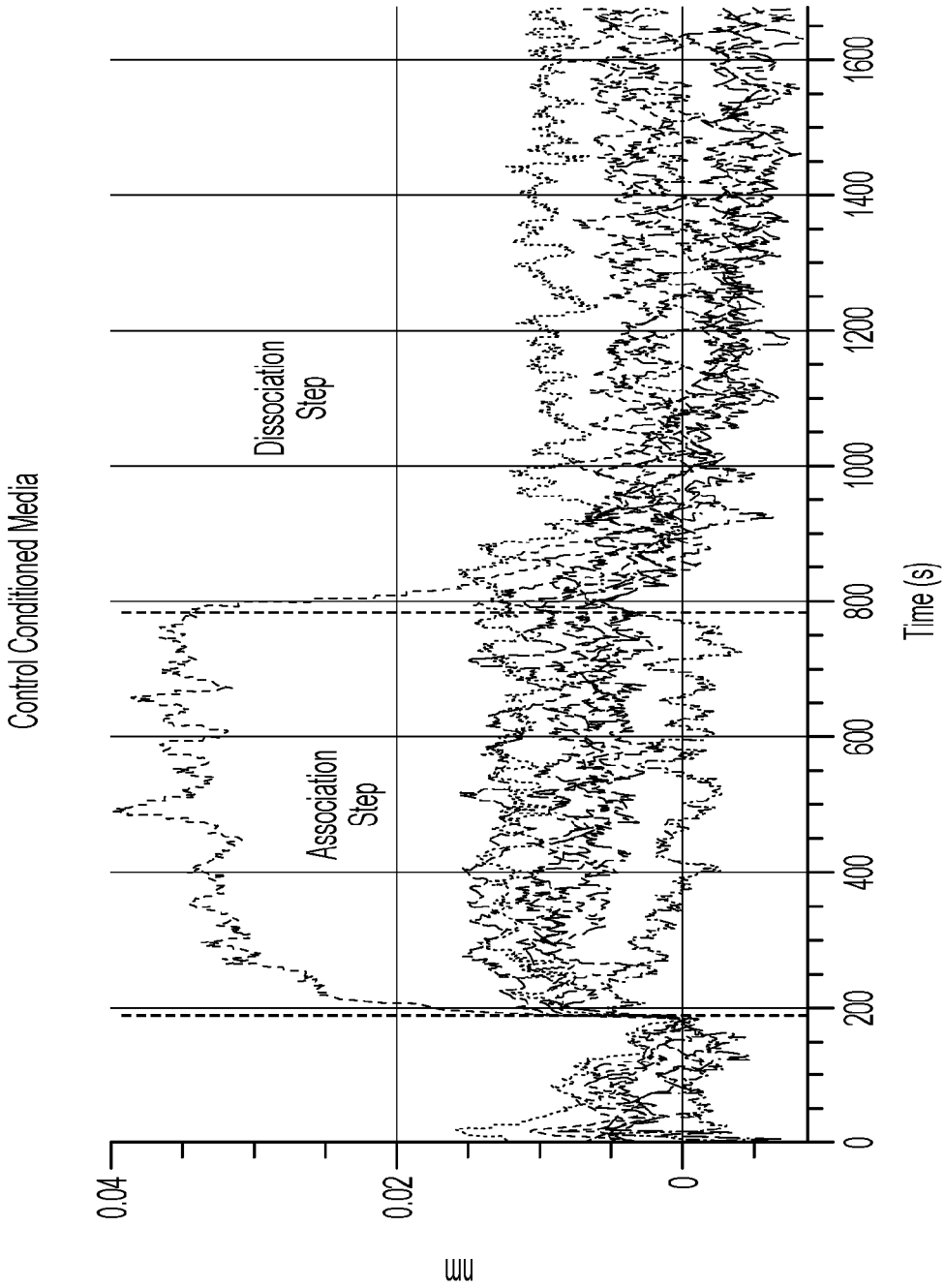

Control Conditioned Media
Association Step
Dissociation Step
nm
0.04
0.02
0
0
200
400
600
800
1000
1200
1400
1600
Time (s)

FIG. 8D

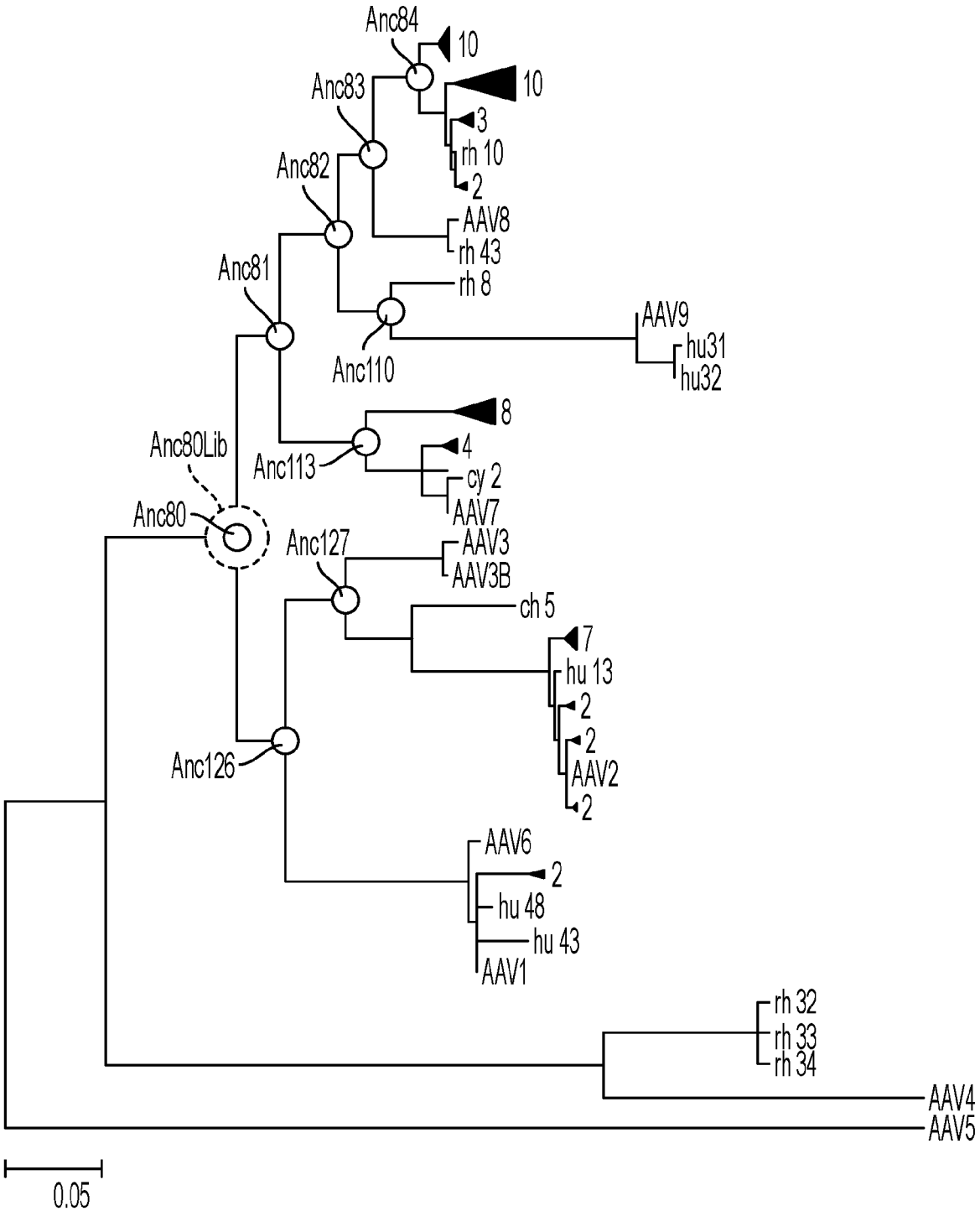

Anc84
10
10
Anc83
3
rh 10
2
Anc82
AAV8
rh 43
Anc81
rh 8
AAV9
hu31
hu32
Anc110
8
Anc80Lib
4
Anc113
cy 2
AAV7
Anc80
Anc127
AAV3
AAV3B
ch 5
7
hu 13
2
2
AAV2
2
Anc126
AAV6
2
hu 48
hu 43
AAV1
rh 32
rh 33
rh 34
AAV4
AAV5
0.05

FIG. 9

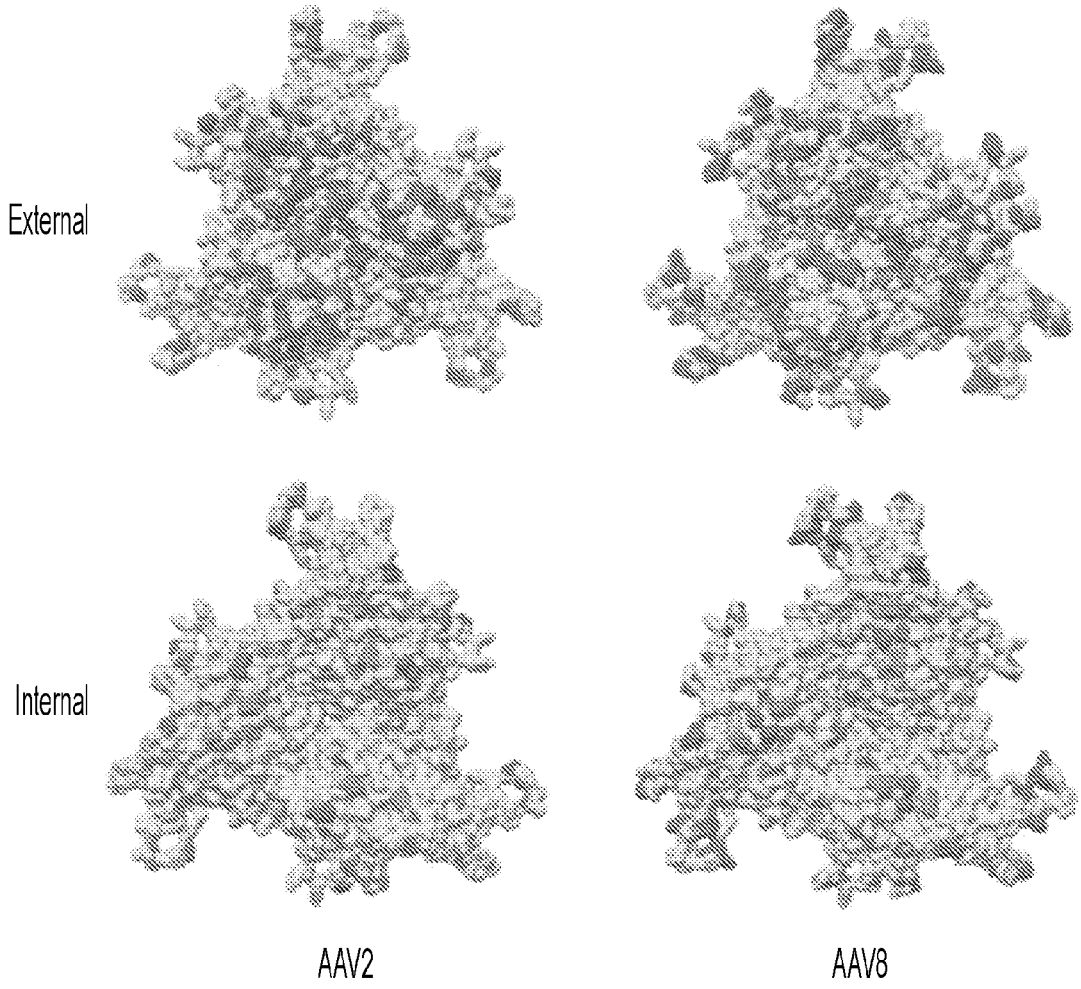


FIG. 10

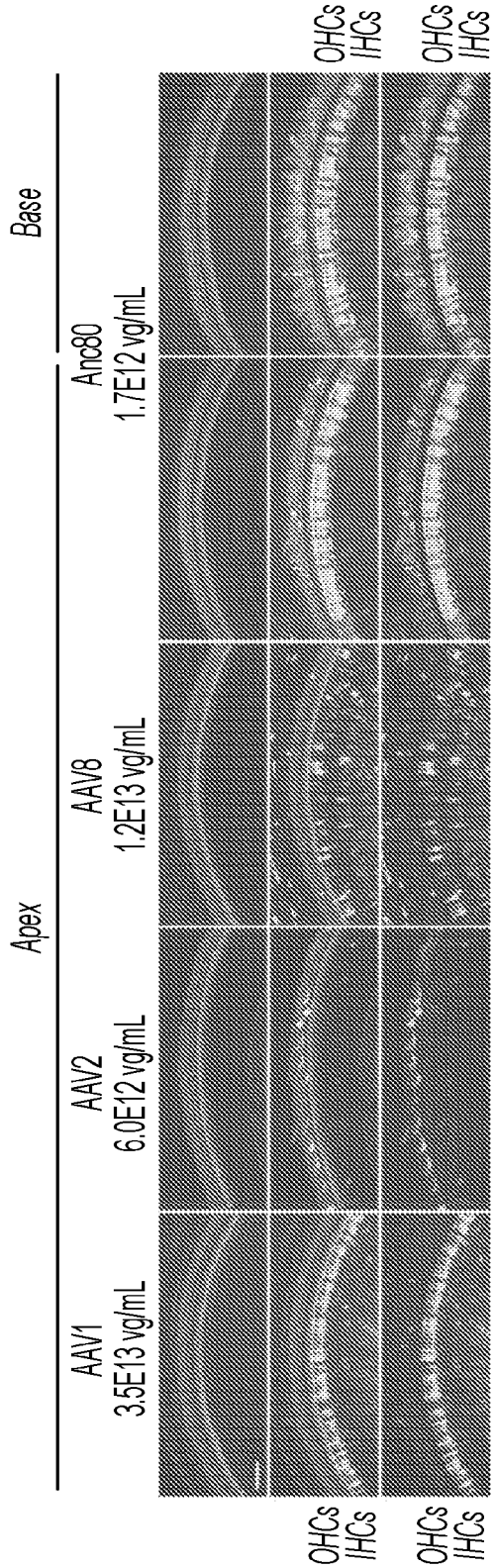

Apex
Base
AAV1
3.5E13 vg/mL
AAV2
6.0E12 vg/mL
AAV8
1.2E13 vg/mL
Anc80
1.7E12 vg/mL
OHCs
IHCs
OHCs
IHCs
OHCs
IHCs
OHCs
IHCs

FIG. 11

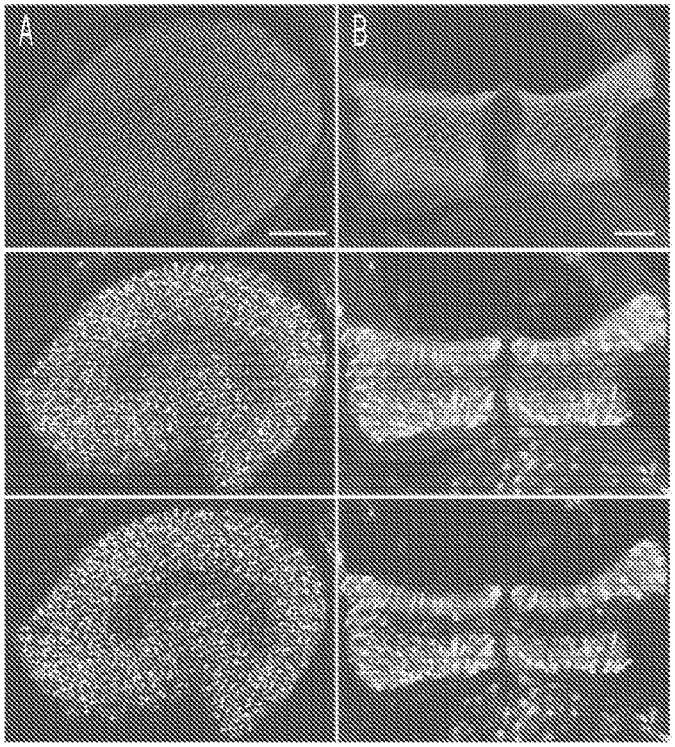


FIG. 12

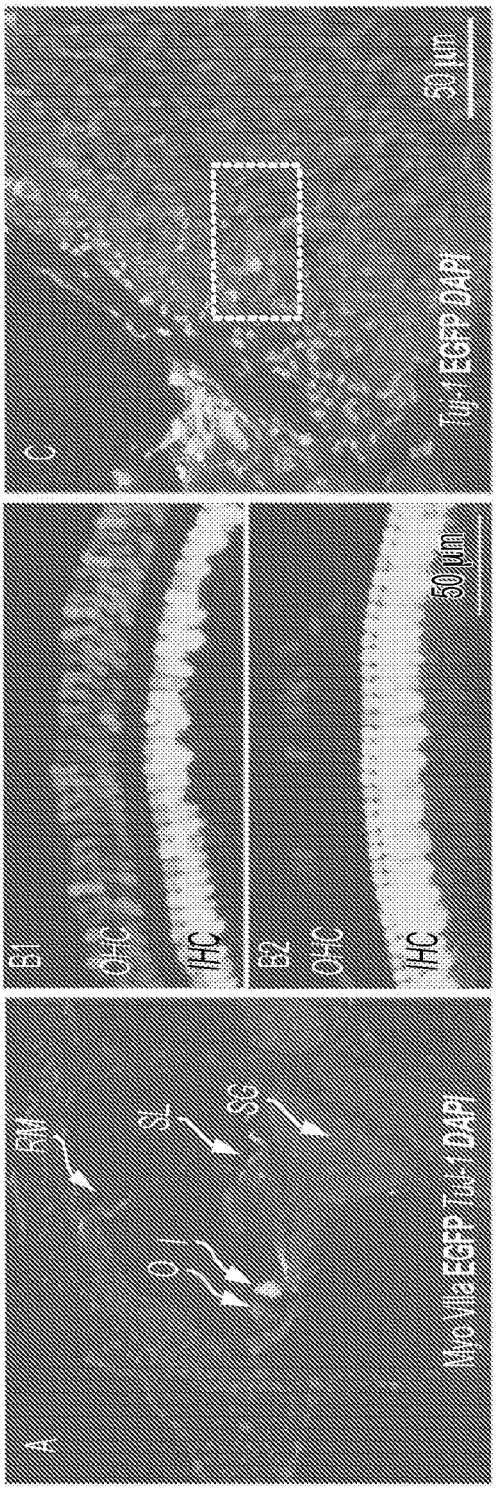

A
Myo7a EGFP Tom1 DAPI
BM
SL
SG
OHC
IHC
B1
OHC
IHC
B2
OHC
IHC
50 µm
C
Tyr-1 EGFP DAPI
50 µm

FIG. 13

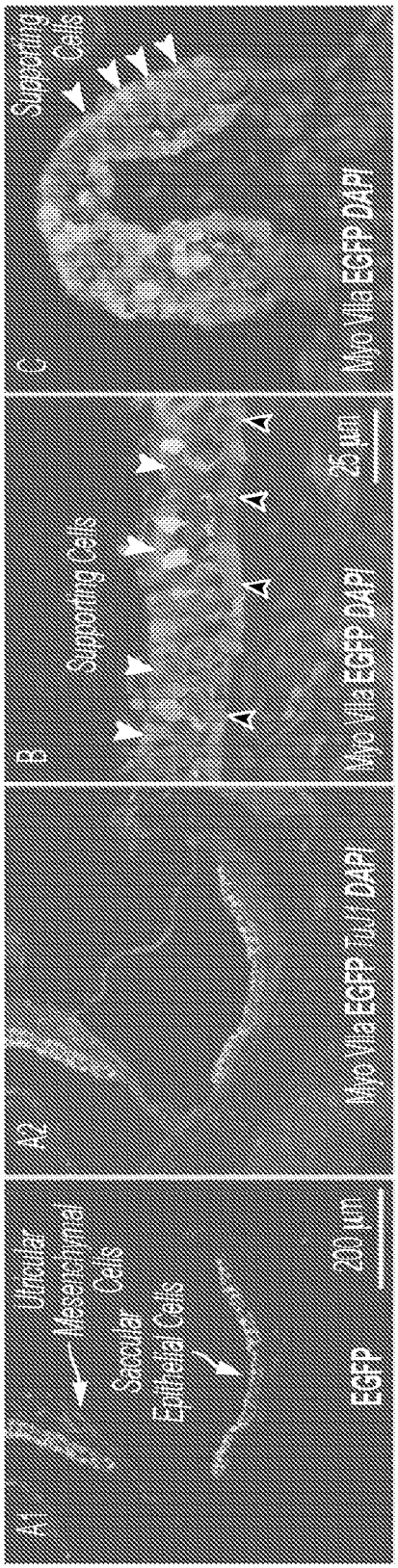

A1
Ureteric Mesenchyme
Vascular Cells
Epithelial Cells
EGFP
200 µm
A2
Myo Vila EGFP Tuj1 DAPI
B
Supporting Cells
Myo Vila EGFP DAPI
25 µm
C
Supporting Cells
Myo Vila EGFP DAPI

FIG. 14

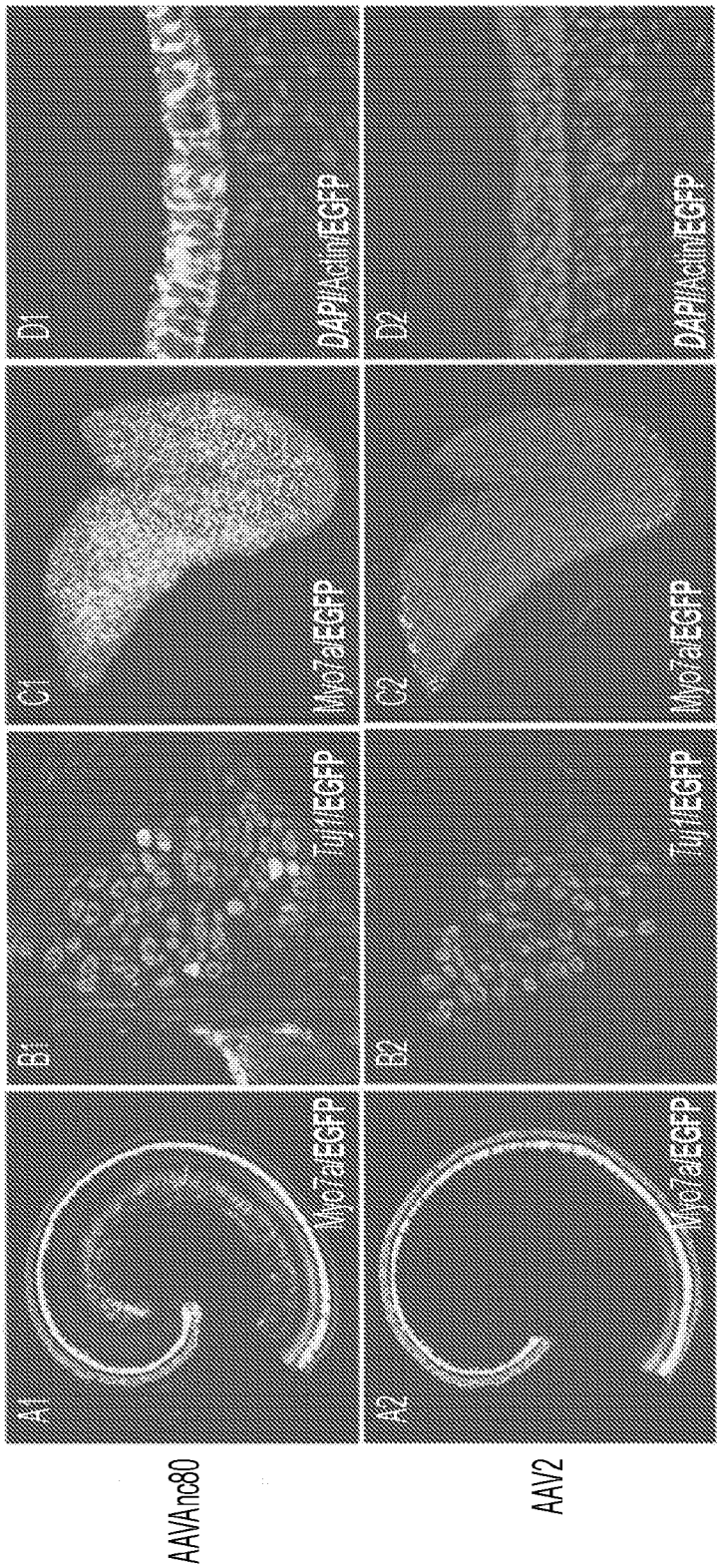

A1
B1
C1
D1
A2
B2
C2
D2
MyoTaIEGFP
Tuj1EGFP
DAPI/Actin/EGFP
AAVAnc80
AAV2

FIG. 15

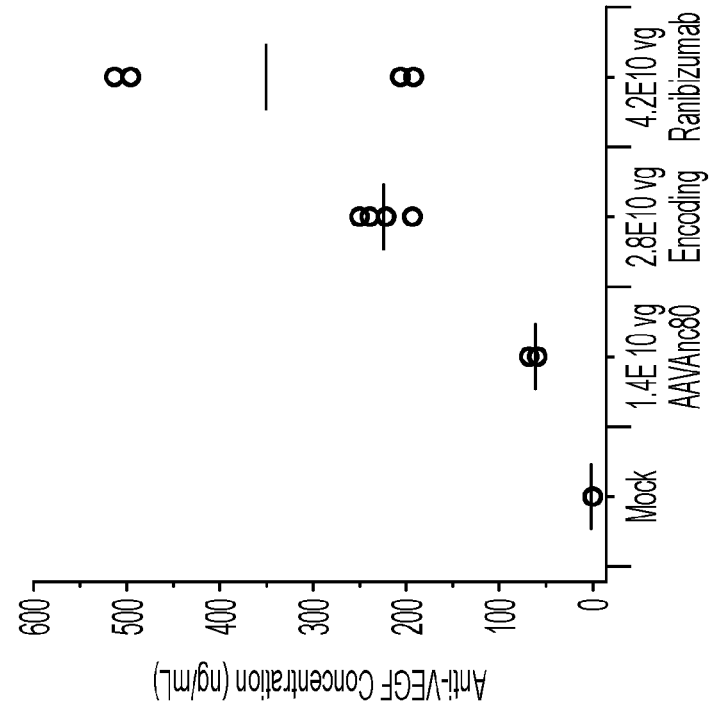


FIG. 16B

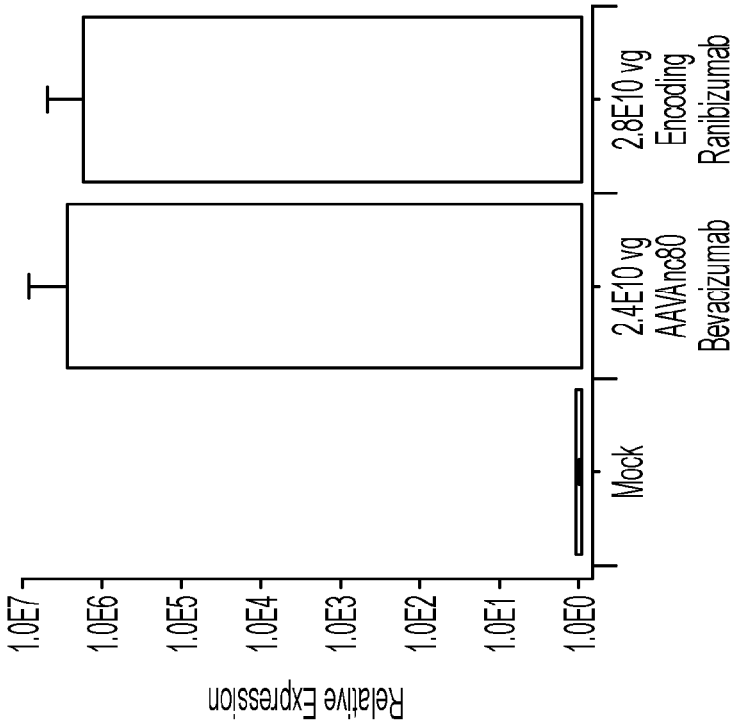


FIG. 16A

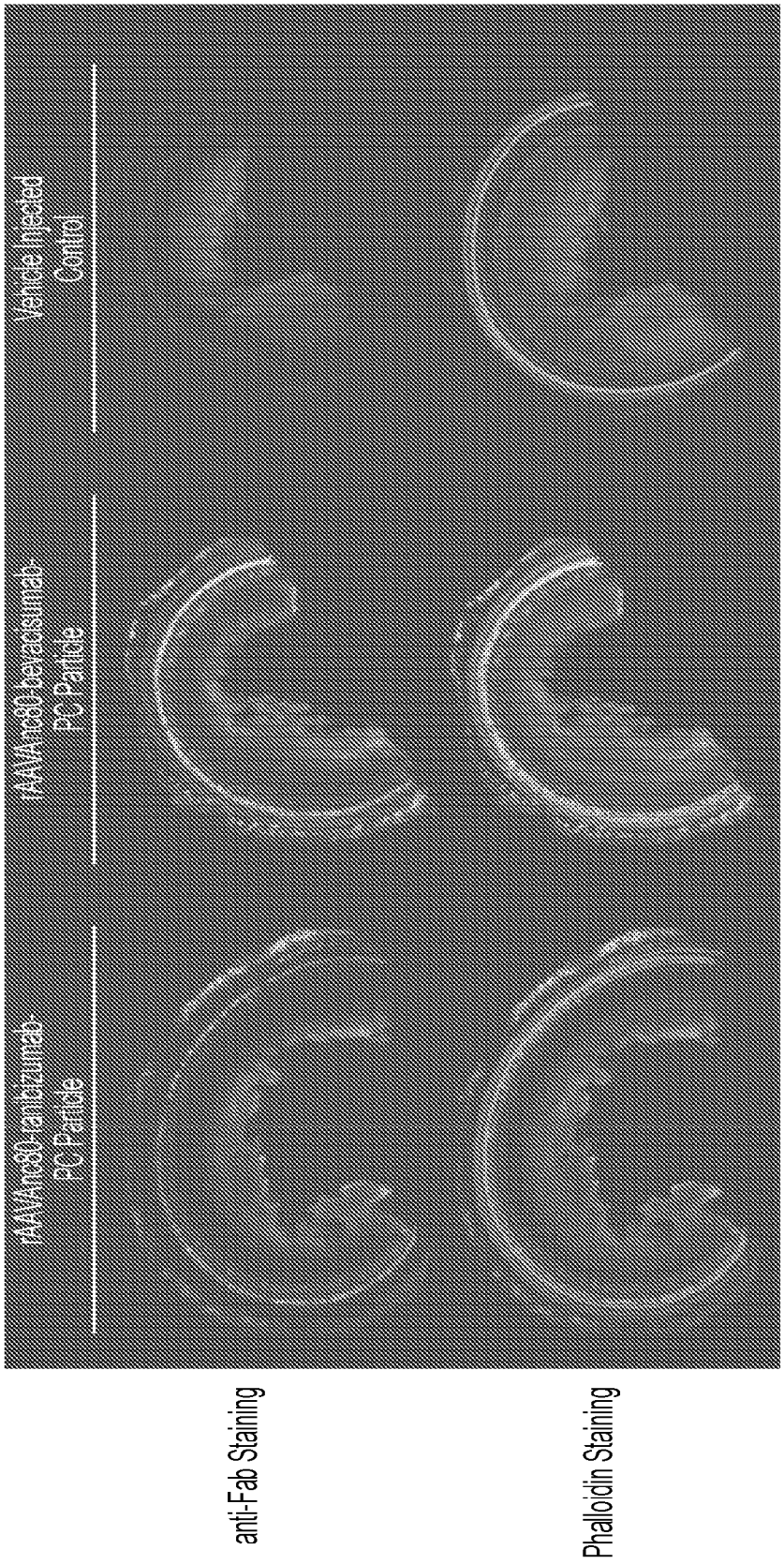

rAAVAnc80 ranibizumab: PO Particle
rAAVAnc80 bevacizumab: PO Particle
Vehicle Injected Control
anti-Fab Staining
Phalloidin Staining

FIG. 17

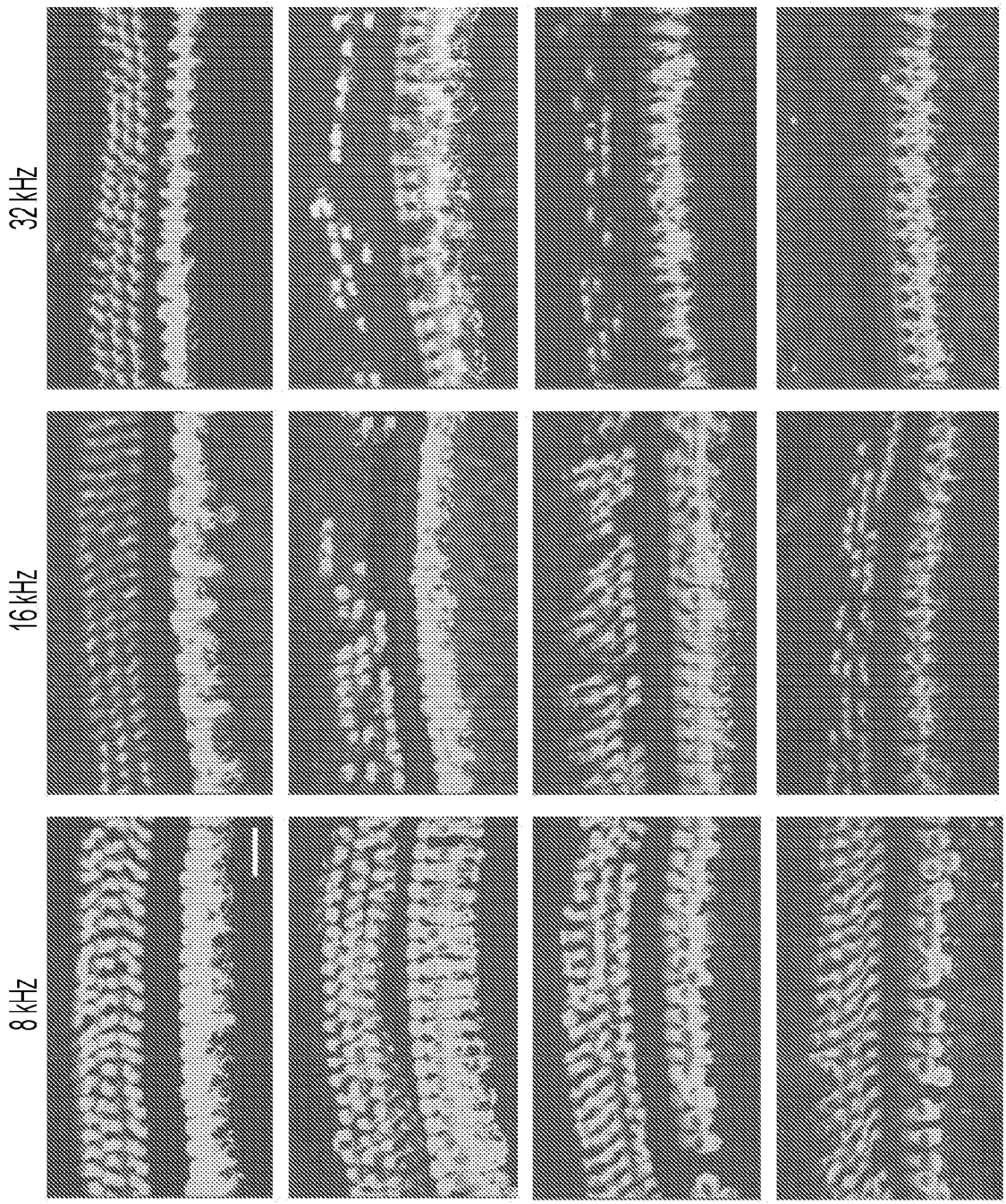

8 kHz
16 kHz
32 kHz
control non-injected ear
Vehicle injected ear
rAAVAnc80-ranibizumab-PC at 1.4E10 vg/cochlea
rAAVAnc80-bevacizumab-PC at 1.2E10 vg/cochlea

FIG. 18

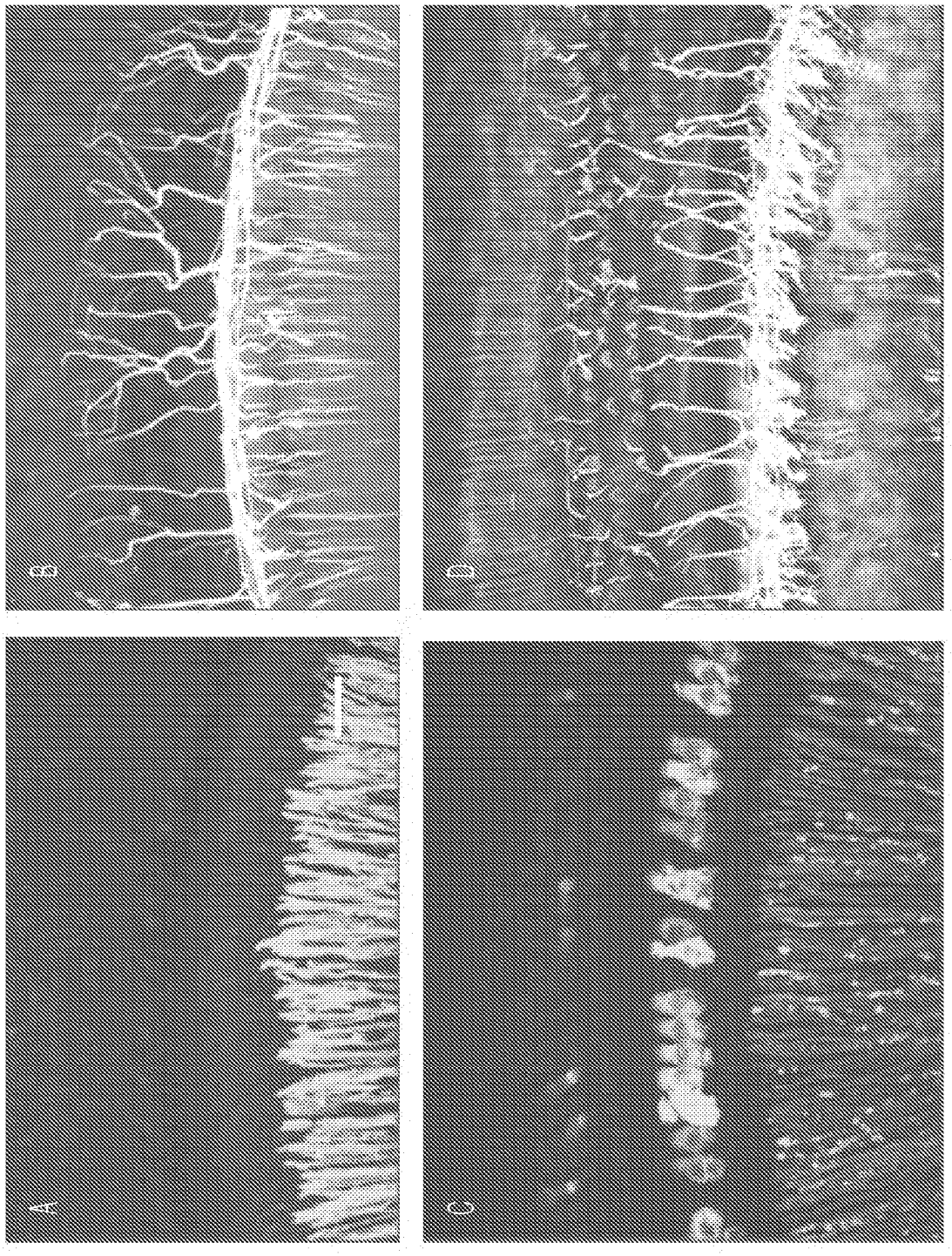

A
B
C
D
Vehicle injected control
rAAVAnc80-ranibizumab-PC at 1.4E10 vg/cochlea

FIG. 19

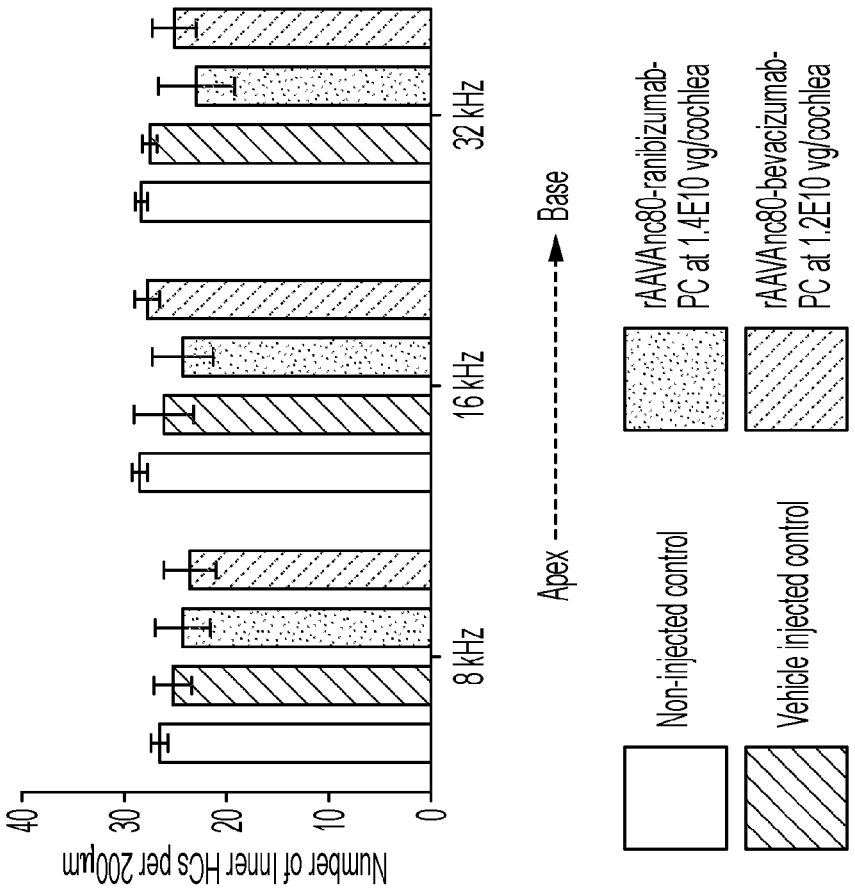


FIG. 20B

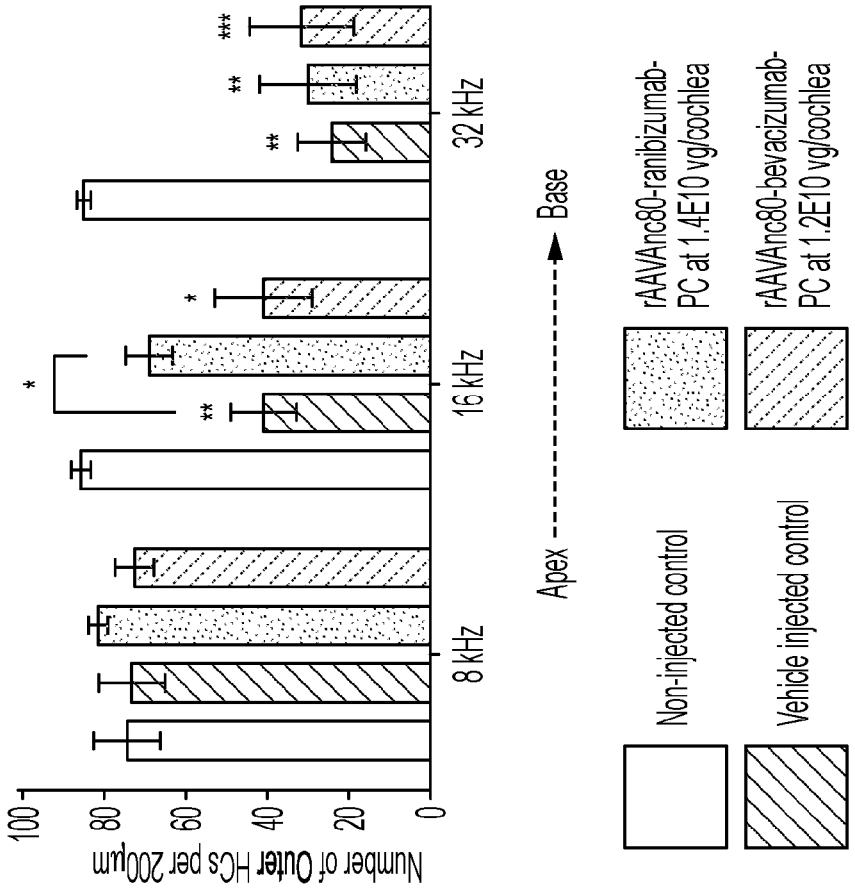


FIG. 20A

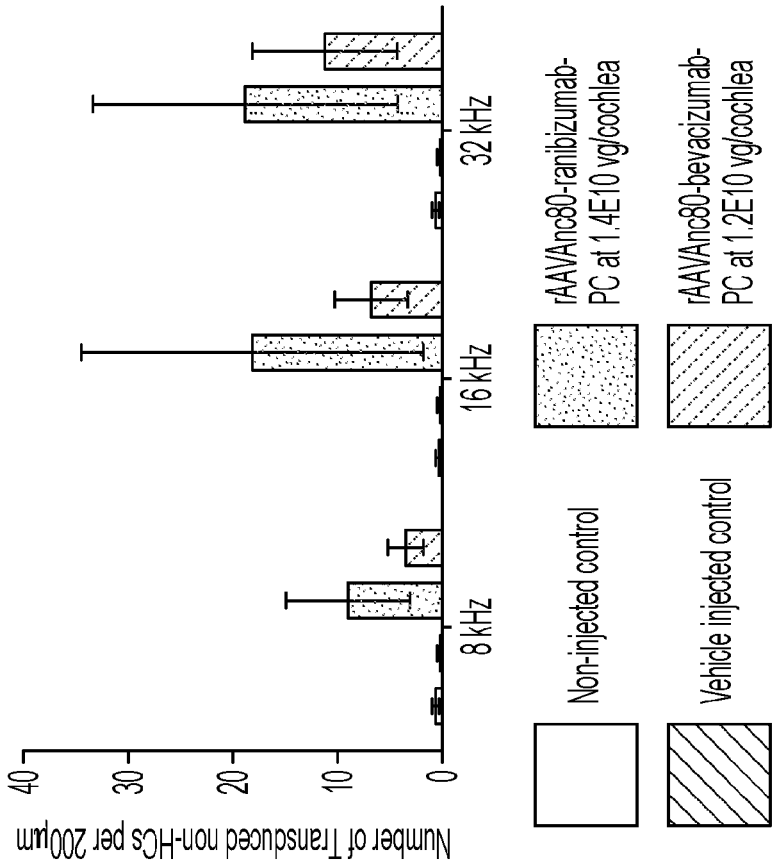


FIG. 21B

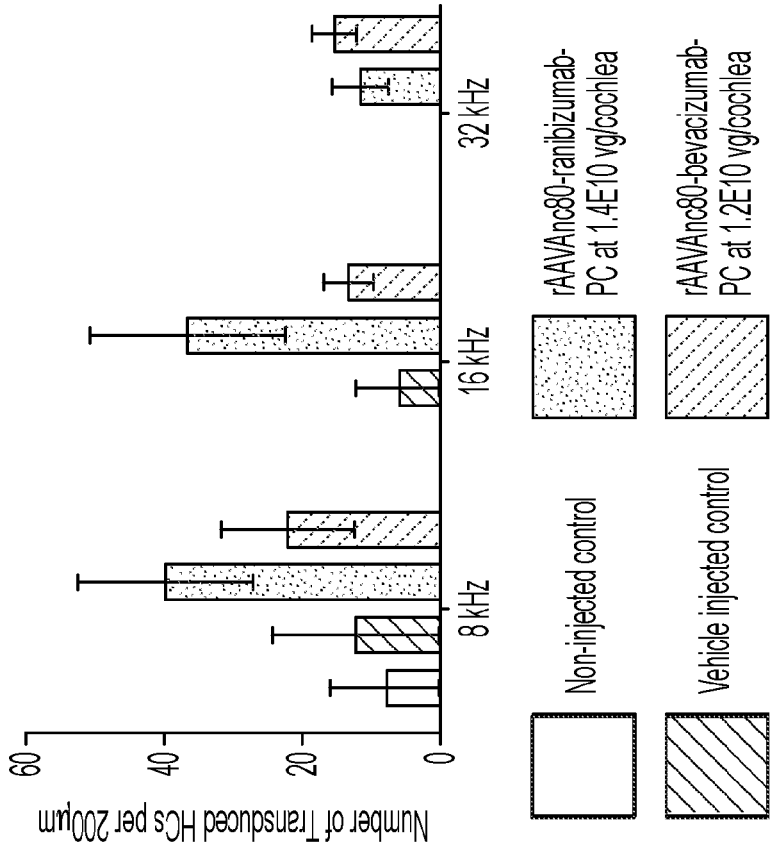


FIG. 21A

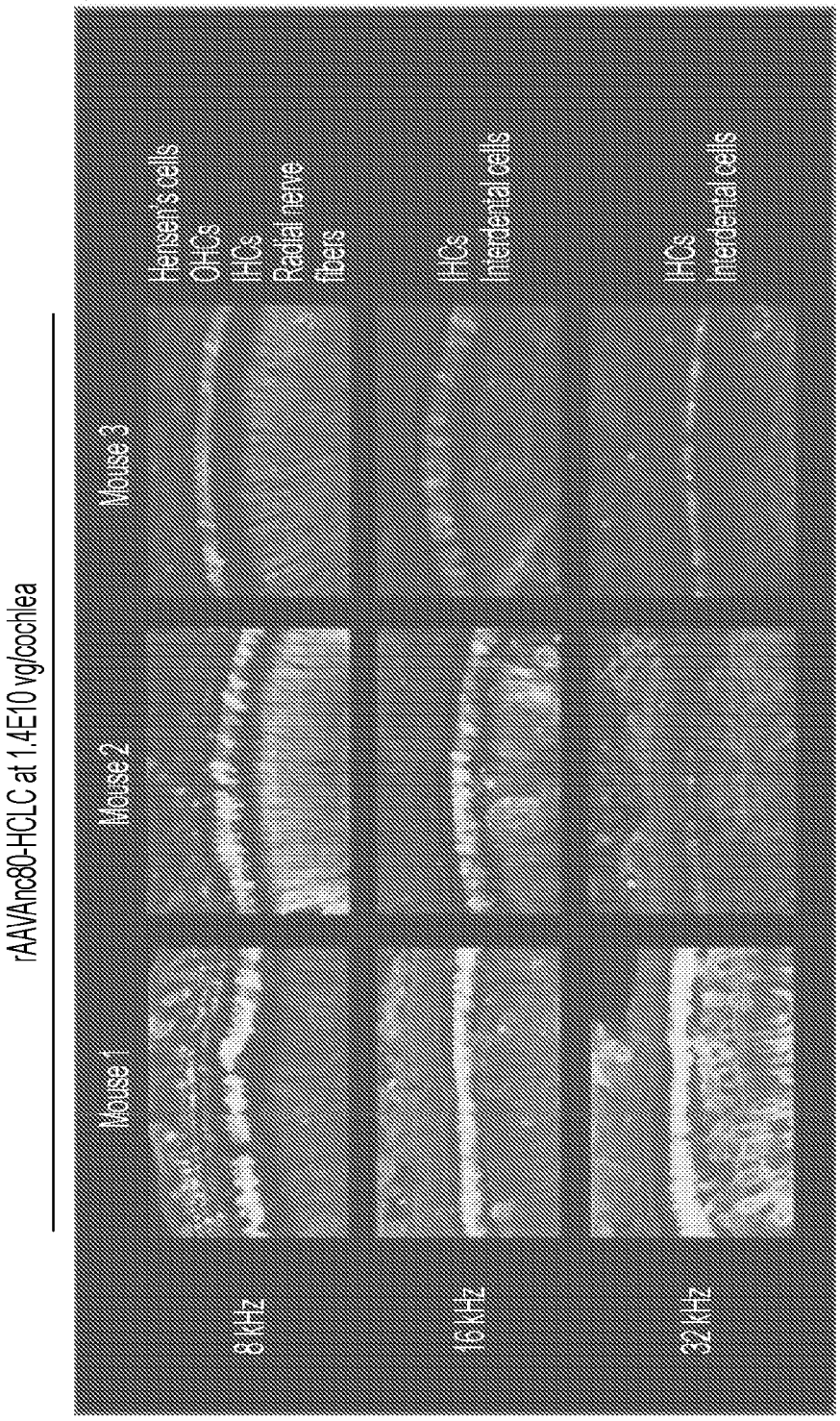

rAAVAnc80-HCLC at 1.4E10 vg/cochlea
Mouse 1
Mouse 2
Mouse 3
Hensen's cells
OHCs
IHCs
Radial nerve fibers
IHCs
Interdental cells
IHCs
Interdental cells
8 kHz
16 kHz
32 kHz

FIG. 22

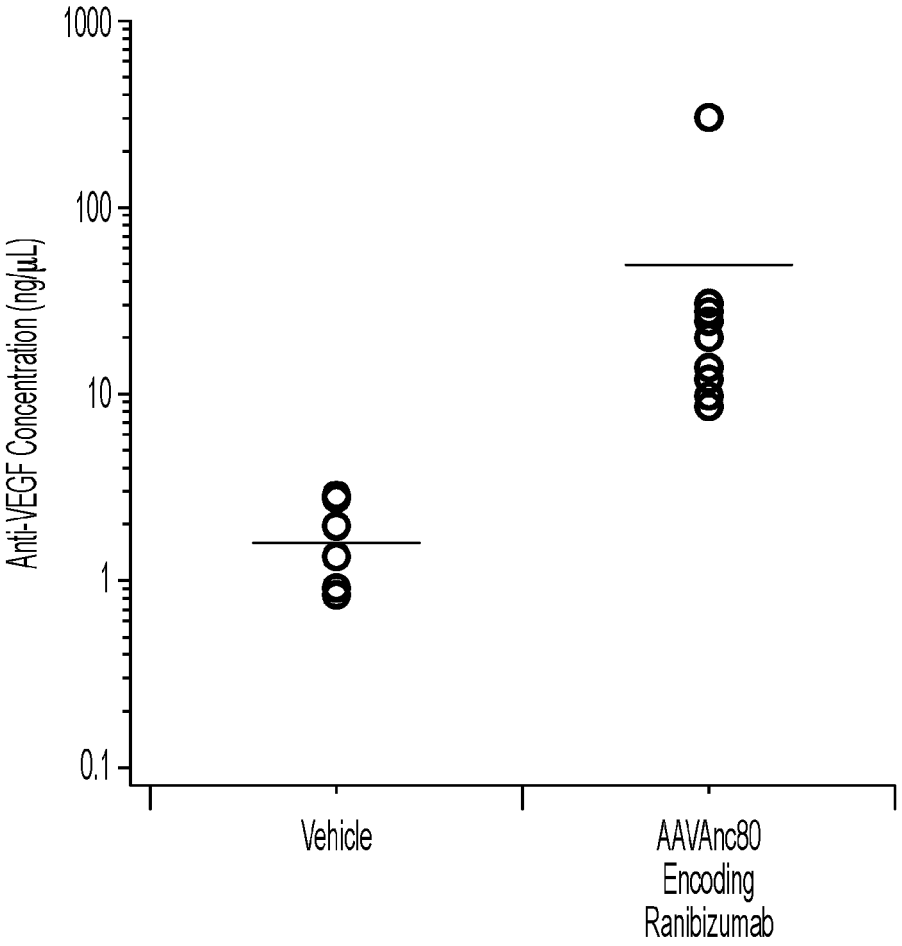

1000
100
10
1
0.1
Anti-VEGF Concentration (ng/µL)
Vehicle
AAVAnc80
Encoding
Ranibizumab

FIG. 23

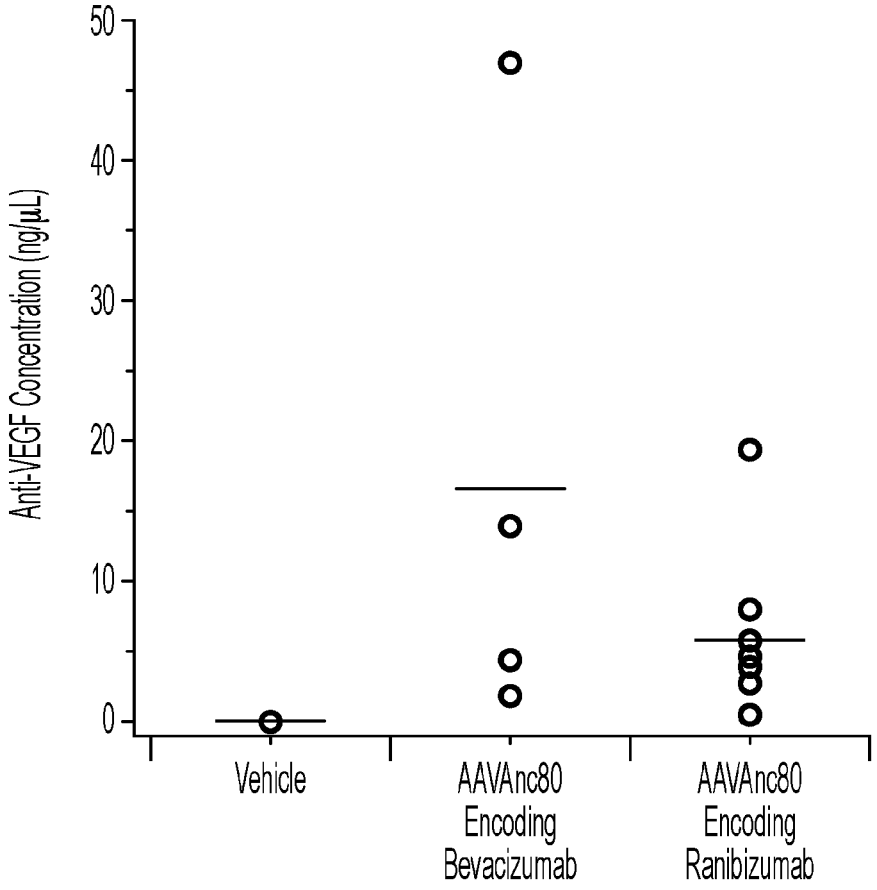

50
40
30
20
10
0
Anti-VEGF Concentration (ng/µL)
Vehicle
AAVAnc80 Encoding Bevacizumab
AAVAnc80 Encoding Ranibizumab

FIG. 24

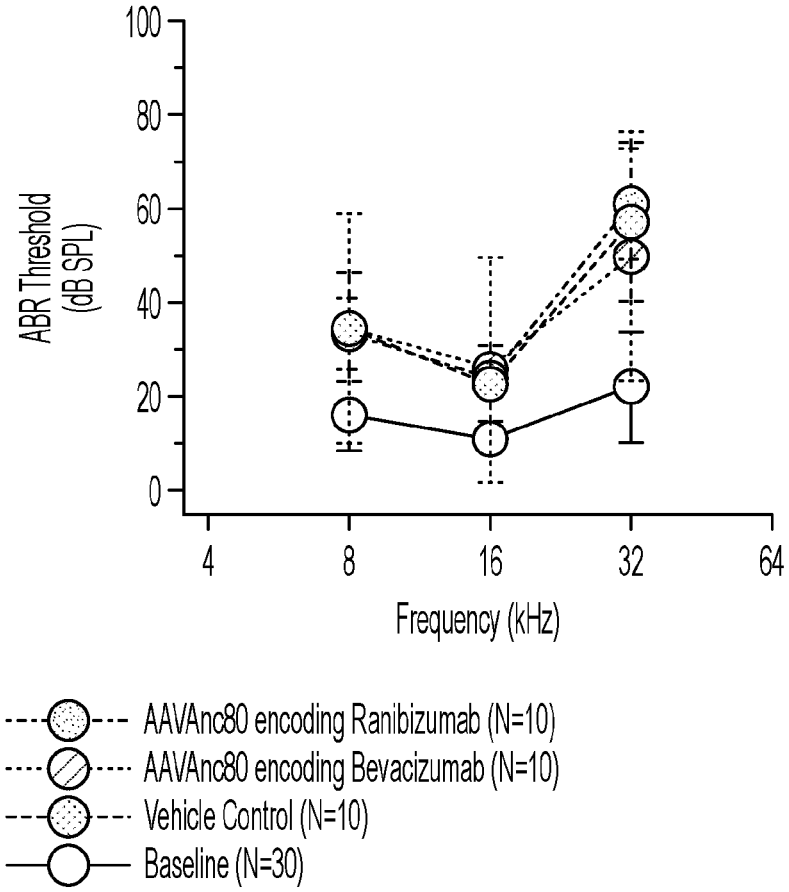

100
80
60
40
20
0
ABR Threshold
(dB SPL)
4
8
16
32
64
Frequency (kHz)
AAVAnc80 encoding Ranibizumab (N=10)
AAVAnc80 encoding Bevacizumab (N=10)
Vehicle Control (N=10)
Baseline (N=30)

FIG. 25

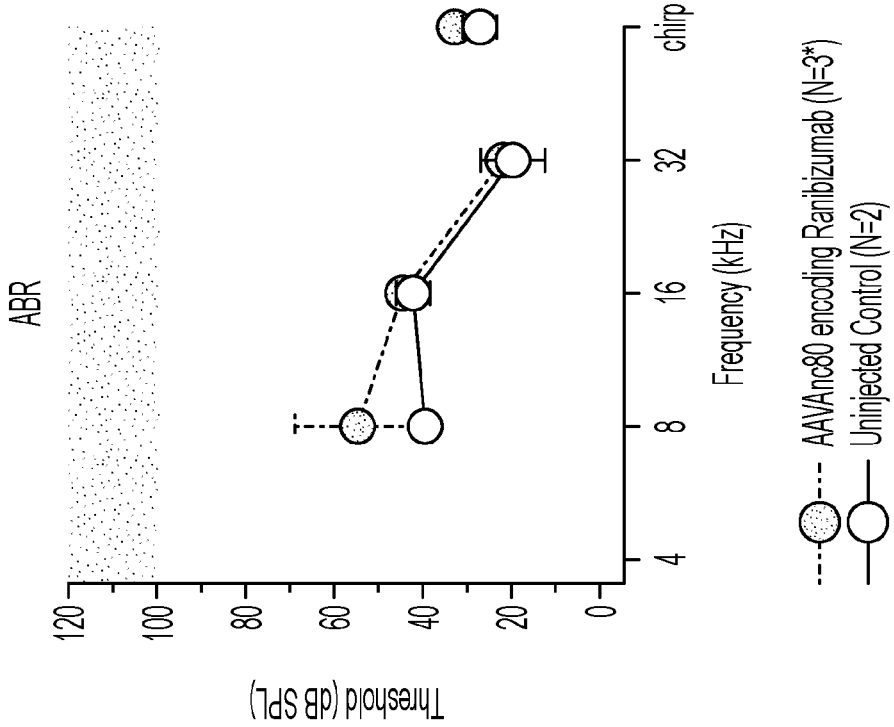


FIG. 26B

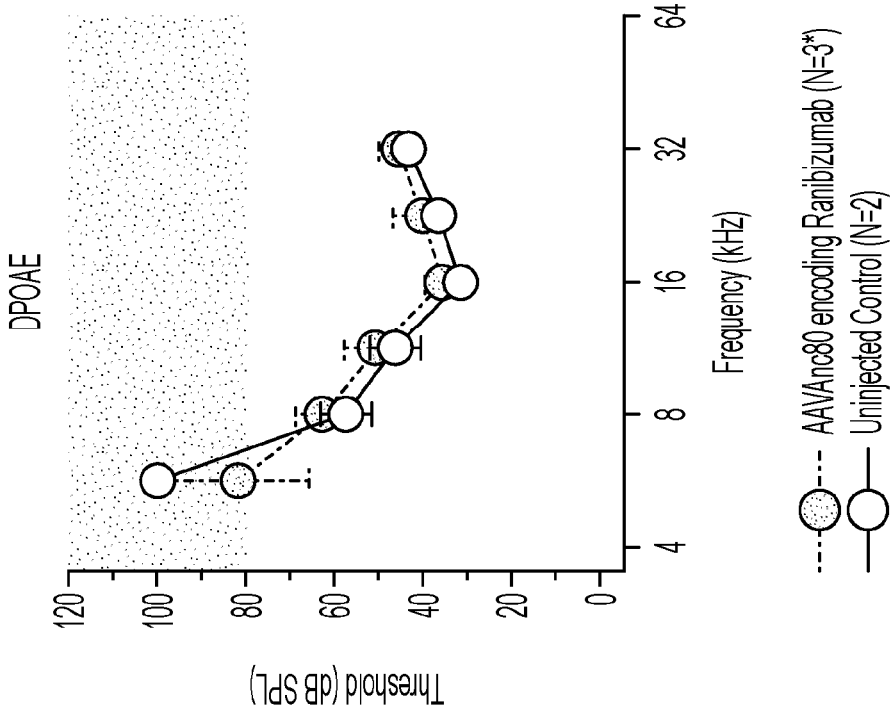


FIG. 26A

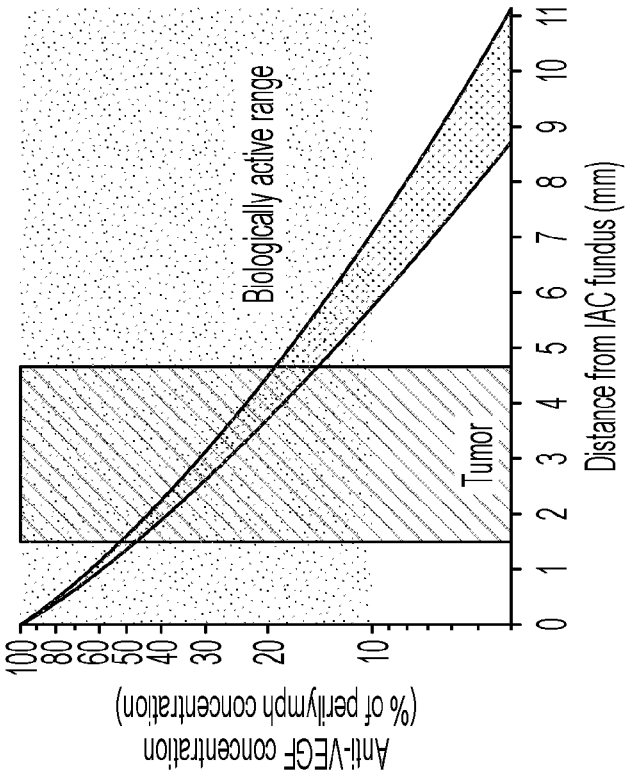


FIG. 27B

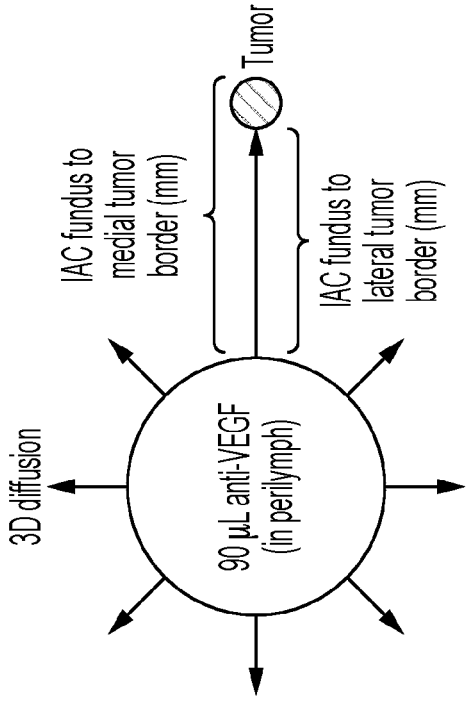


FIG. 27A

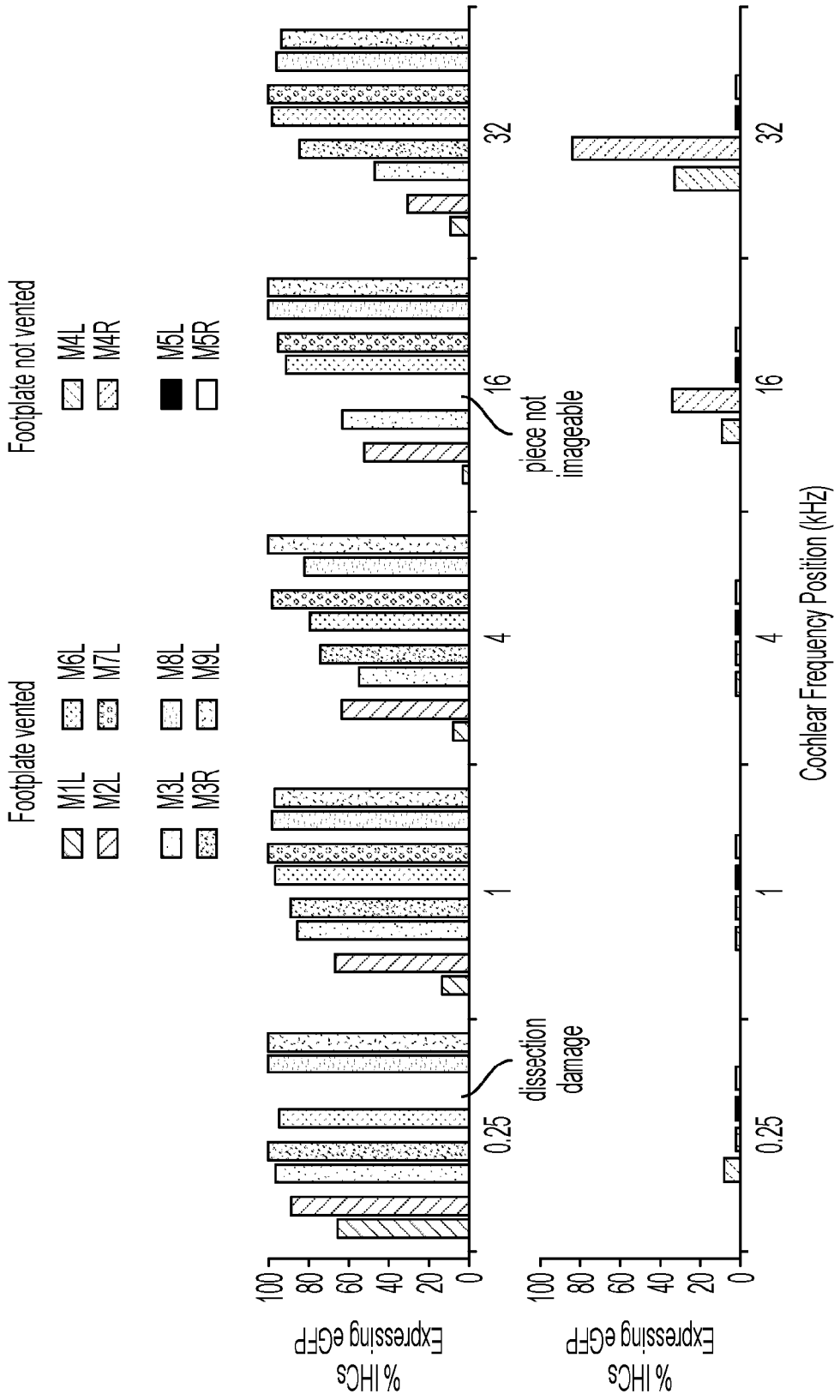

Footplate vented
M1L
M2L
M6L
M7L
M3L
M3R
M8L
M9L
Footplate not vented
M4L
M4R
M5L
M5R
% IHCs Expressing eGFP
100
80
60
40
20
0
0.25
1
4
16
32
dissection damage
piece not imageable
Cochlear Frequency Position (kHz)

FIG. 28

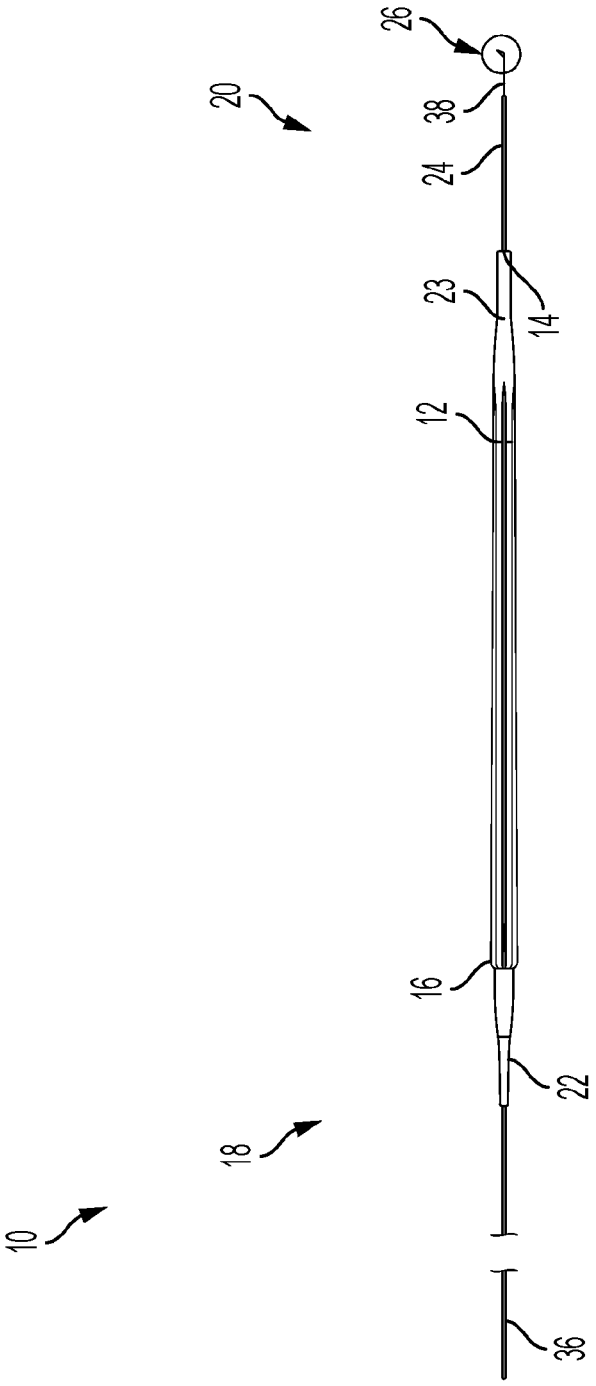


FIG. 29

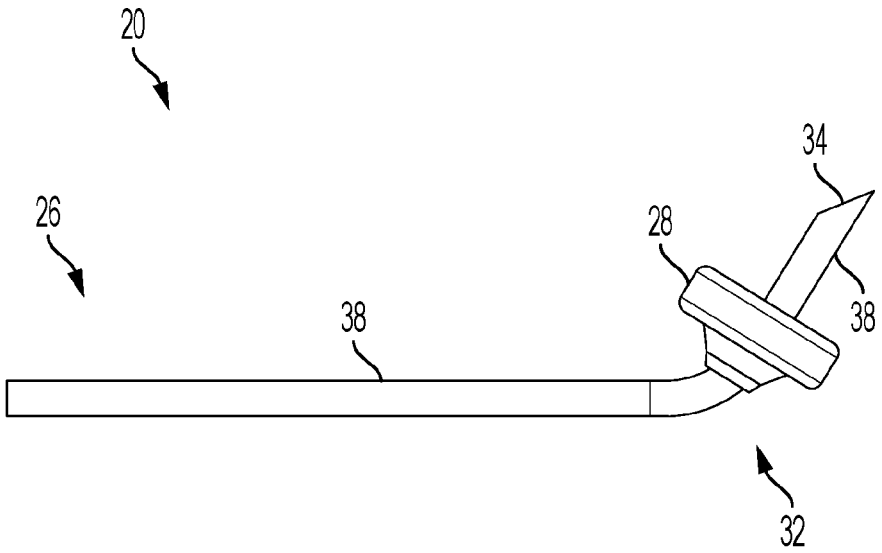


FIG. 30

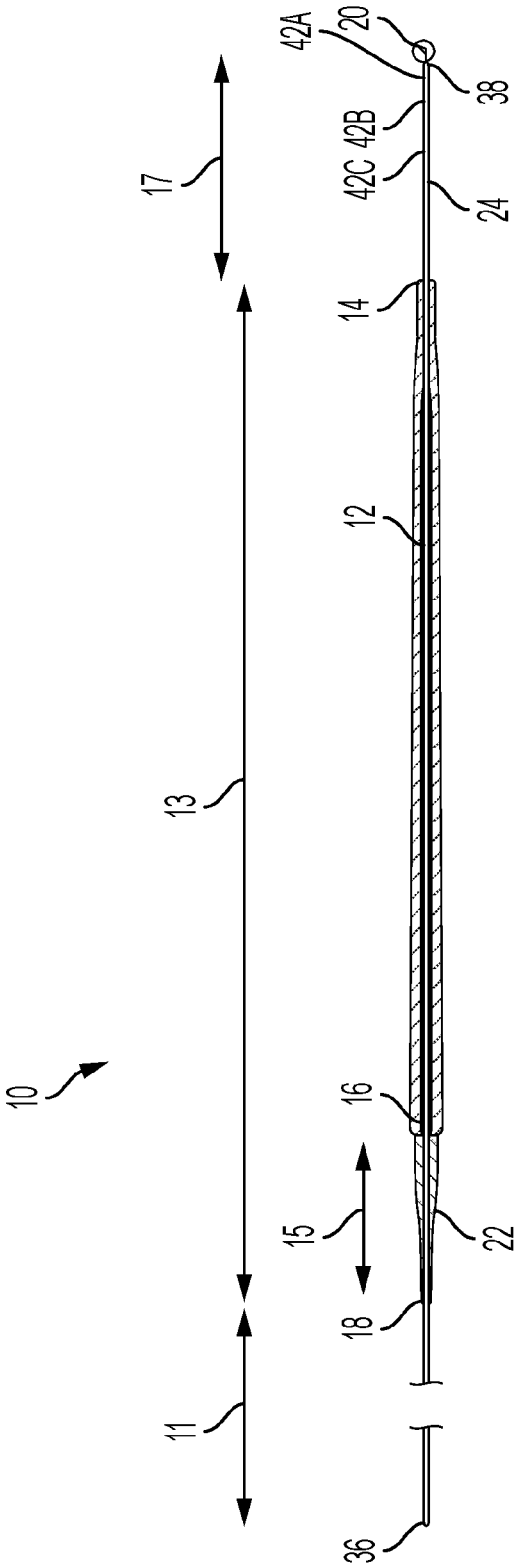


FIG. 31

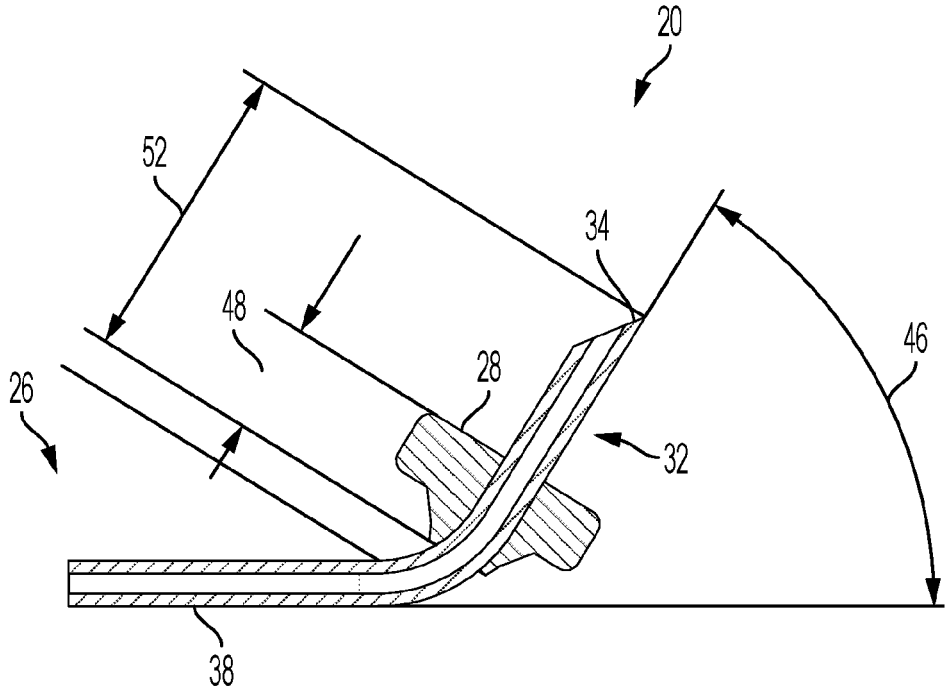


FIG. 32

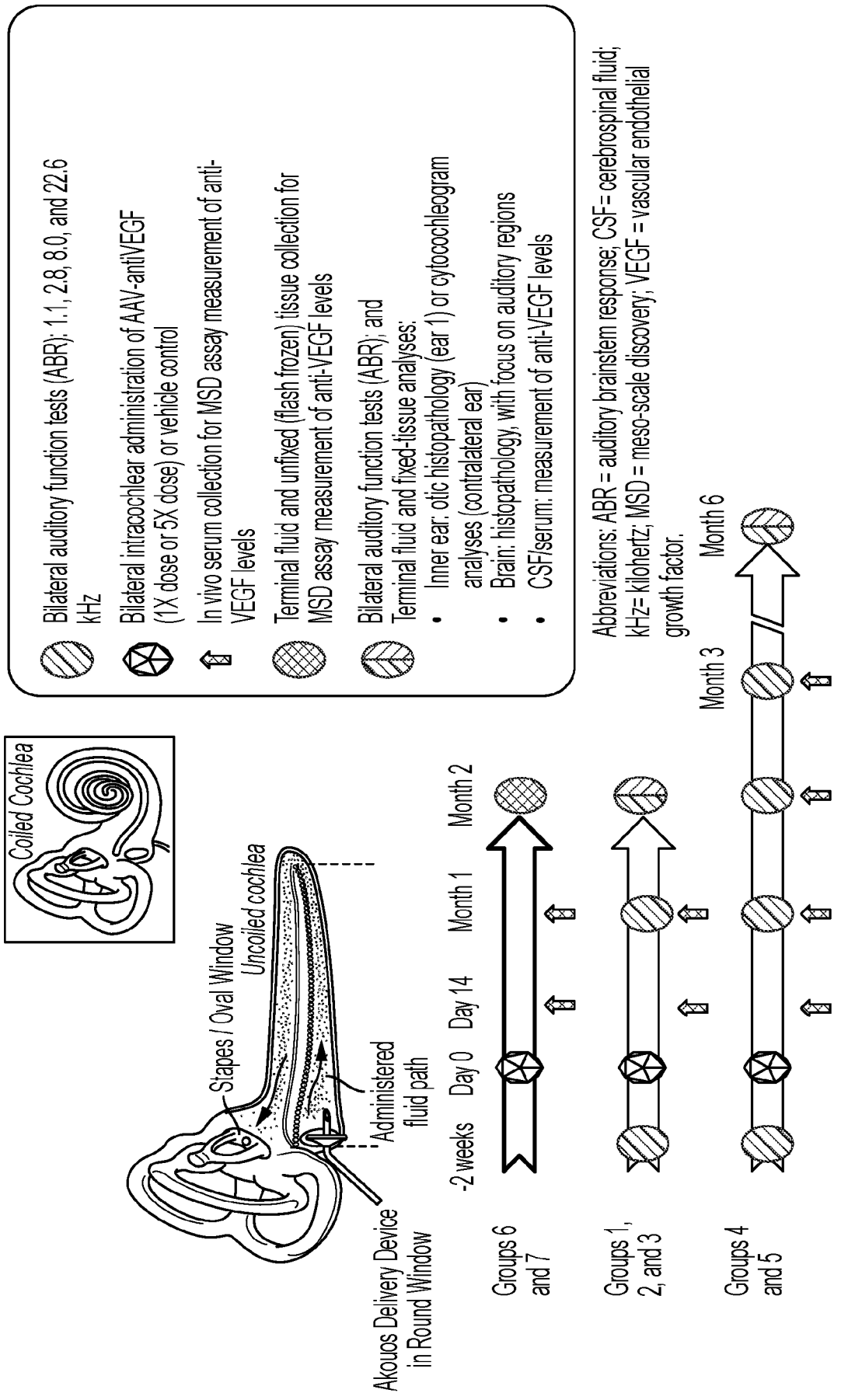

Bilateral auditory function tests (ABR): 1.1, 2.8, 8.0, and 22.6 kHz
Bilateral intracochlear administration of AAV-antiVEGF (1X dose or 5X dose) or vehicle control
In vivo serum collection for MSD assay measurement of anti-VEGF levels
Terminal fluid and unfixed (flash frozen) tissue collection for MSD assay measurement of anti-VEGF levels
Bilateral auditory function tests (ABR); and
Terminal fluid and fixed-tissue analyses:
Inner ear: otic histopathology (ear 1) or cytocochleogram analyses (contralateral ear)
Brain: histopathology, with focus on auditory regions
CSF/serum: measurement of anti-VEGF levels
Abbreviations: ABR = auditory brainstem response; CSF= cerebrospinal fluid; kHz= kilohertz; MSD = meso-scale discovery; VEGF = vascular endothelial growth factor.
Coiled Cochlea
Uncoiled cochlea
Stapes / Oval Window
Administered fluid path
Akouos Delivery Device in Round Window
-2 weeks
Day 0
Day 14
Month 1
Month 2
Month 3
Month 6
Groups 6 and 7
Groups 1, 2, and 3
Groups 4 and 5

FIG. 33

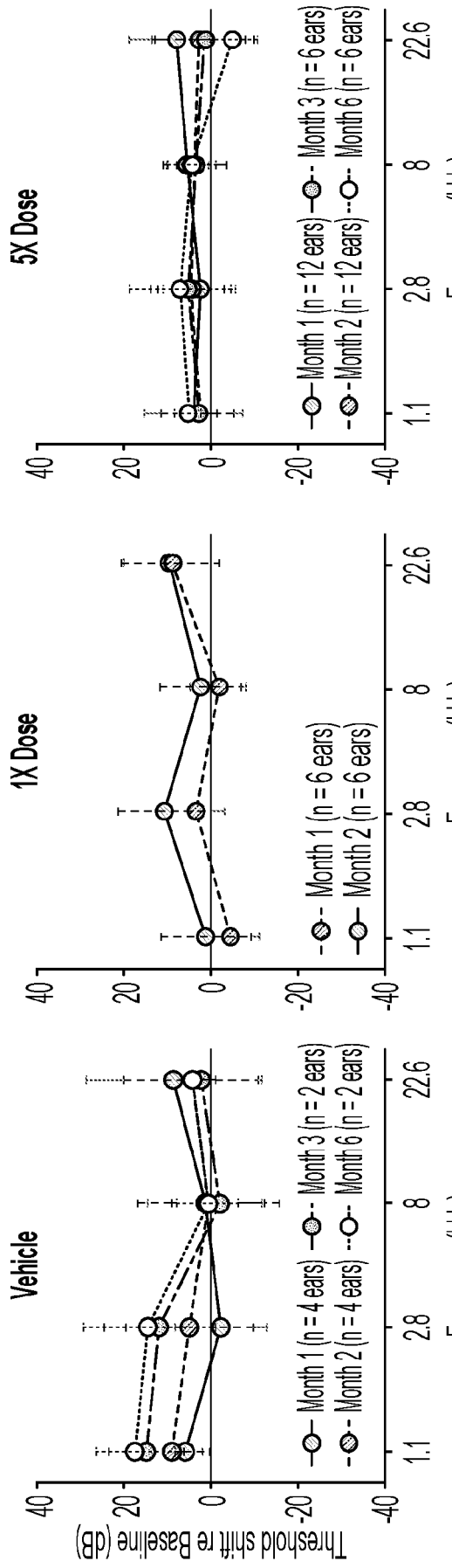


FIG. 34A

FIG. 34B

FIG. 34C

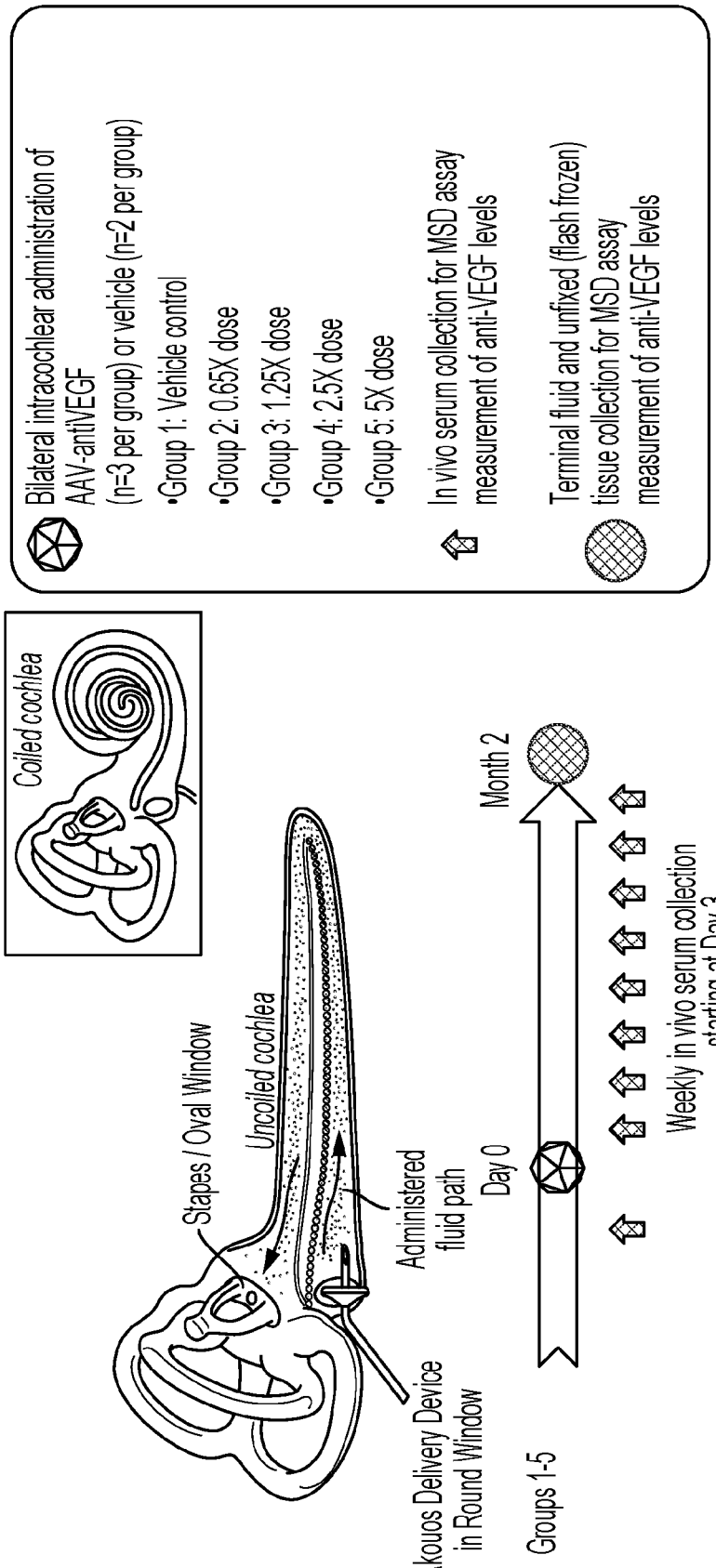


FIG. 35

Abbreviations: CSF= cerebrospinal fluid; NHP = non-human primate(s); MSD = meso-scale discovery; VEGF = vascular endothelial growth factor.

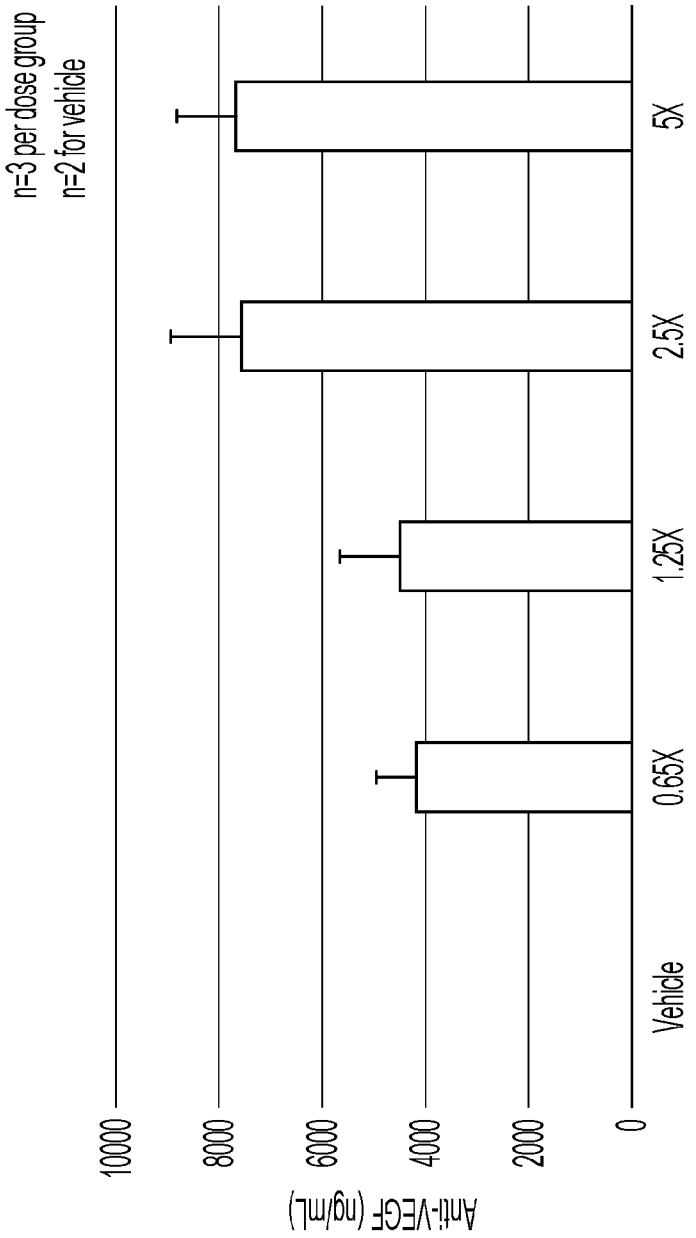

n=3 per dose group
n=2 for vehicle
Anti-VEGF (ng/mL)
10000
8000
6000
4000
2000
0
Vehicle
0.65X
1.25X
2.5X
5X
Dose Groups

FIG. 36

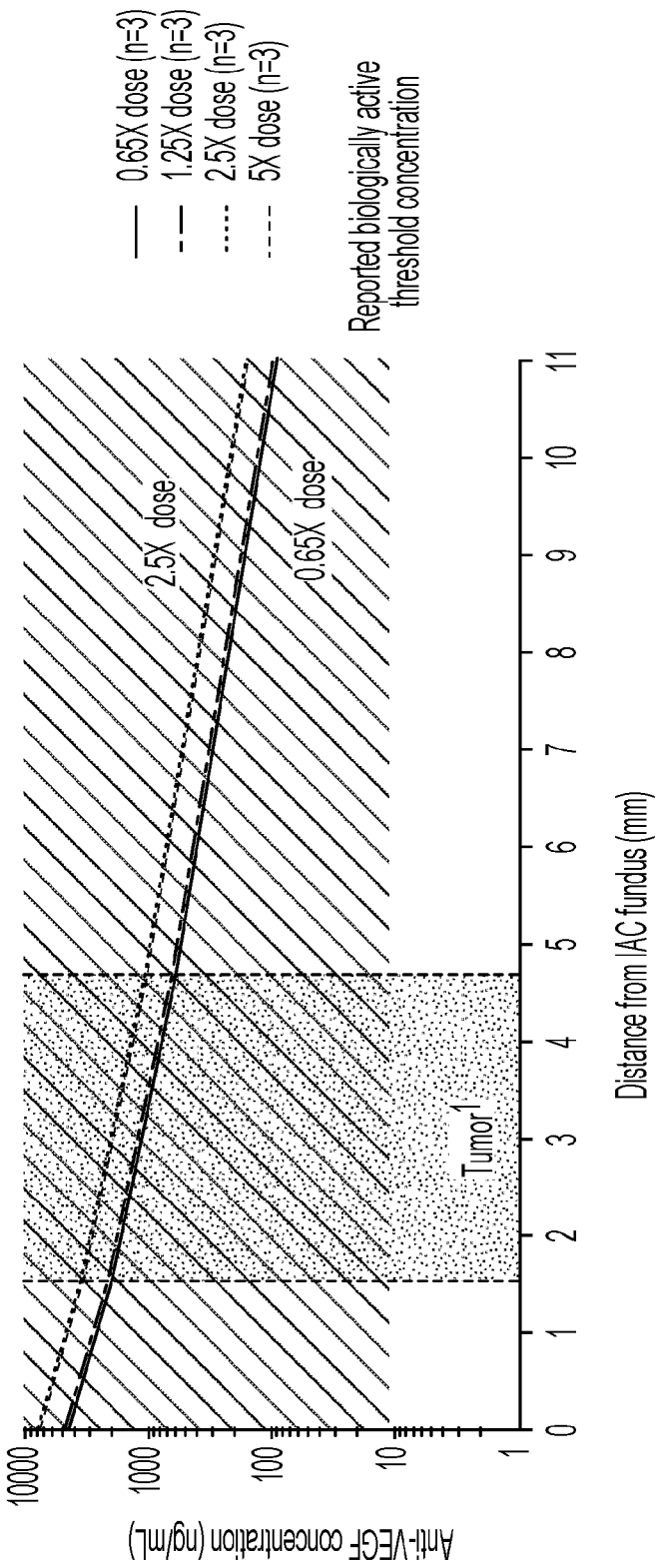

0.65X dose (n=3)
1.25X dose (n=3)
2.5X dose (n=3)
5X dose (n=3)
Reported biologically active threshold concentration
2.5X dose
0.65X dose
Tumor
Anti-VEGF concentration (ng/mL)
10000
1000
100
10
1
0
1
2
3
4
5
6
7
8
9
10
11
Distance from IAC fundus (mm)

FIG. 37

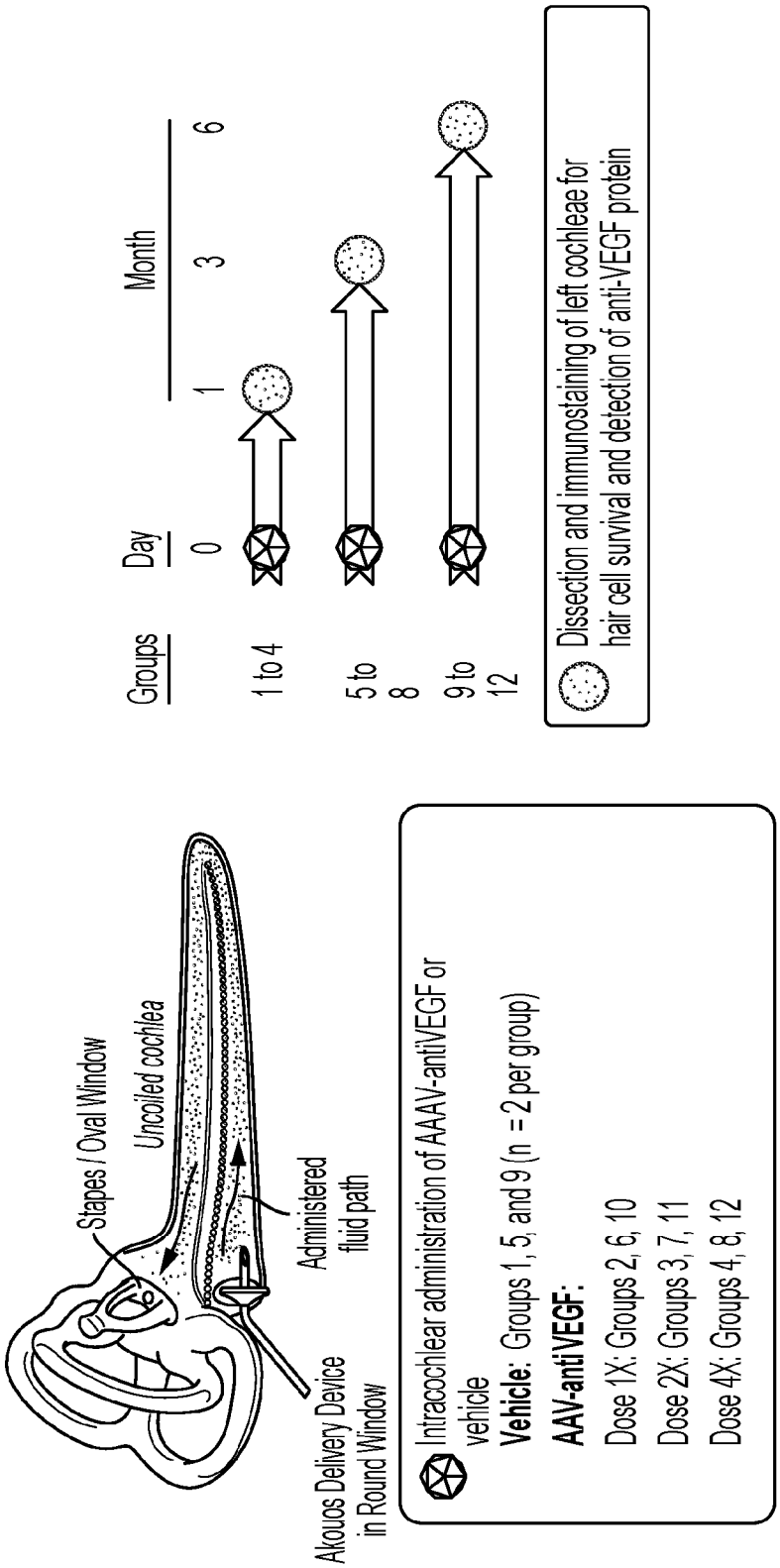
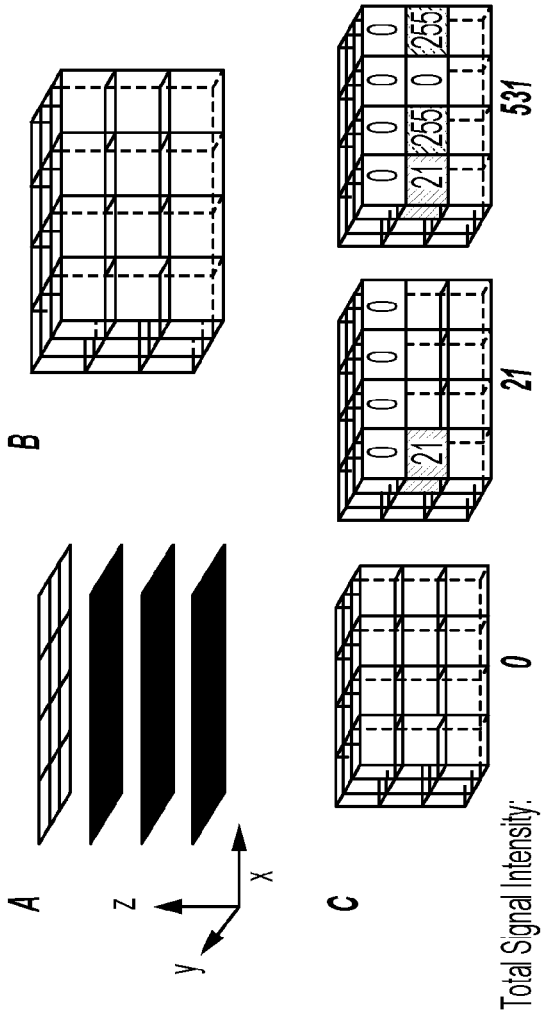
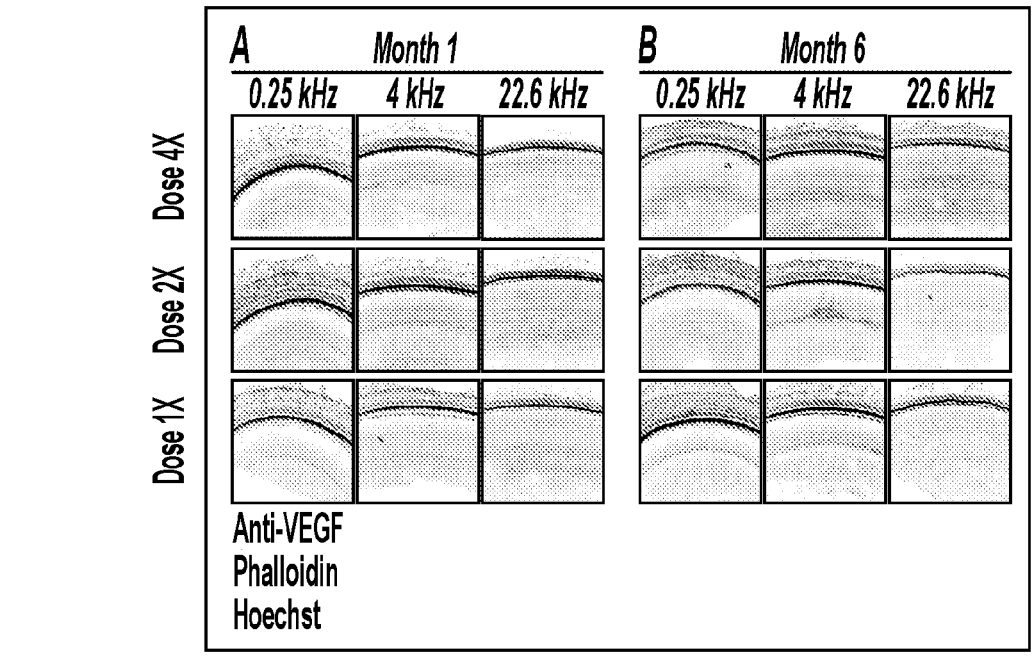
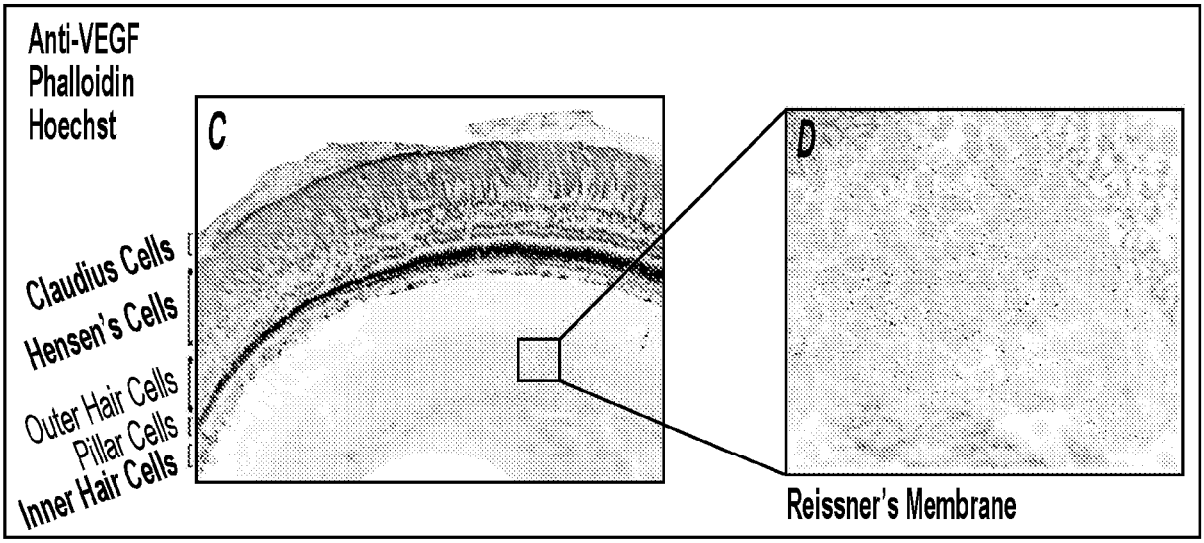

Stapes / Oval Window
Uncoiled cochlea
Administered fluid path
Akoustos Delivery Device in Round Window
Intracochlear administration of AAAV-antiVEGF or vehicle
Vehicle: Groups 1, 5, and 9 (n = 2 per group)
AAV-antiVEGF:
Dose 1X: Groups 2, 6, 10
Dose 2X: Groups 3, 7, 11
Dose 4X: Groups 4, 8, 12
Groups
Day
Month
0
1
3
6
1 to 4
5 to 8
9 to 12
Dissection and immunostaining of left cochleae for hair cell survival and detection of anti-VEGF protein

FIG. 38



FIGs. 39A-39C


A
Month 1
0.25 kHz
4 kHz
22.6 kHz
B
Month 6
0.25 kHz
4 kHz
22.6 kHz
Dose 4X
Dose 2X
Dose 1X
Anti-VEGF
Phalloidin
Hoechst


Anti-VEGF
Phalloidin
Hoechst
C
D
Claudius Cells
Hensen's Cells
Outer Hair Cells
Pillar Cells
Inner Hair Cells
Reissner's Membrane

FIGs. 40A-40D

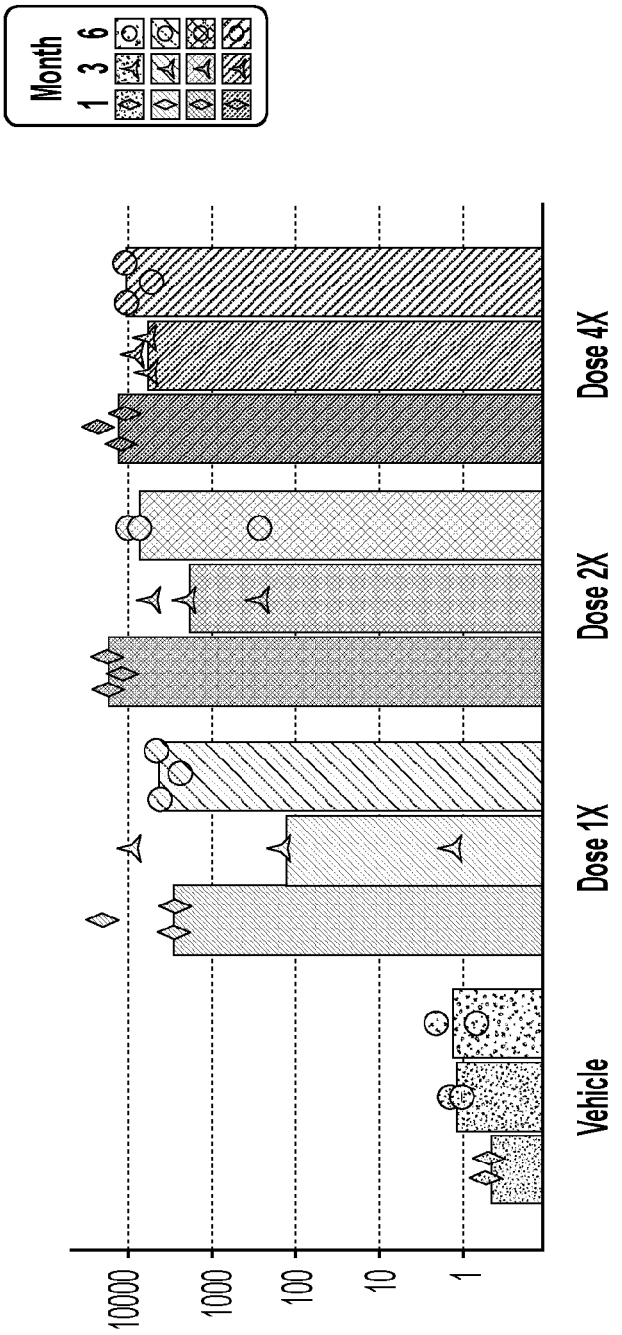
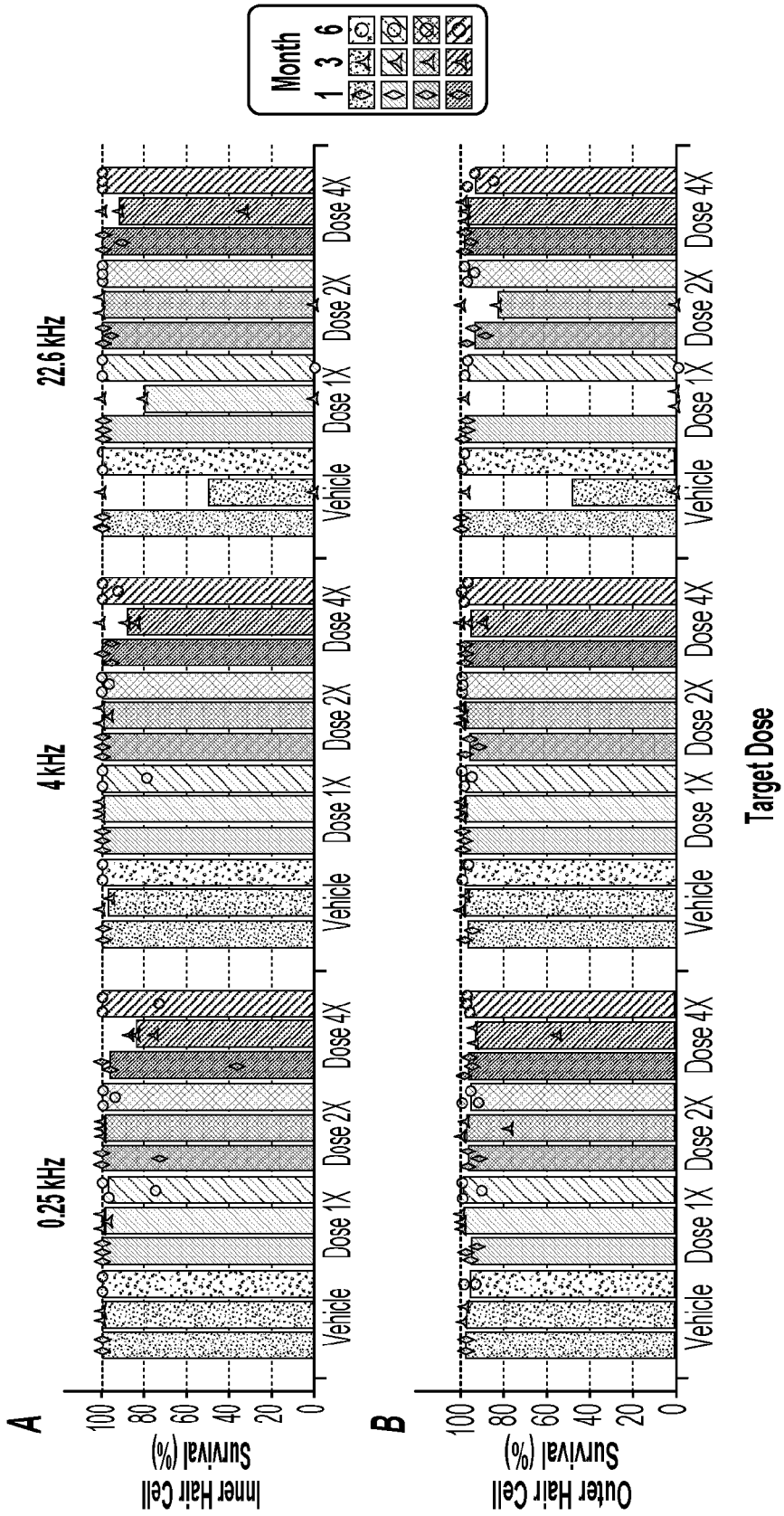


FIG. 41


A
B
0.25 kHz
4 kHz
22.6 kHz
Month
1 3 6
Inner Hair Cell
Survival (%)
Outer Hair Cell
Survival (%)
0
20
40
60
80
100
Vehicle
Dose 1X
Dose 2X
Dose 4X
Target Dose

FIGs. 42A-42B