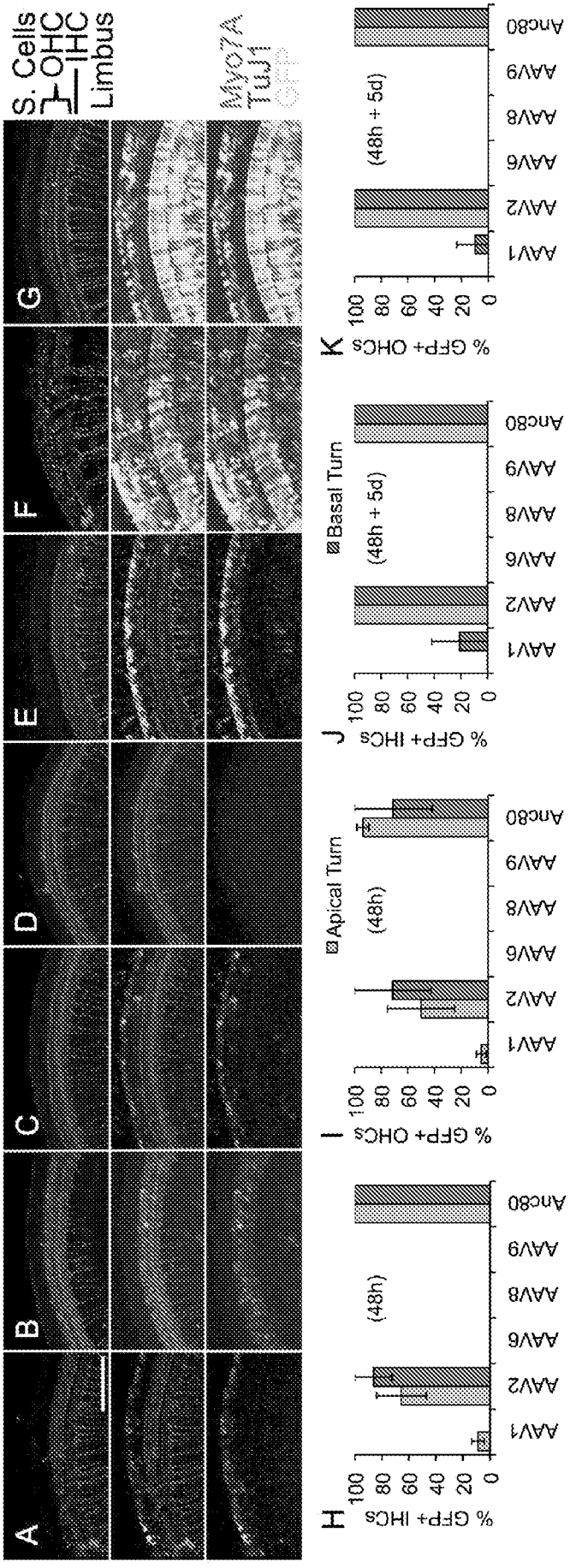

AAV1
AAV2
AAV6
AAV8
AAV9
Anc80 apex
Anc80 base
S. Cells
OHC
IHC
Limbus
Myo7A
Tuj1
A
B
C
D
E
F
G
H
I
J
K
% GFP+ IHCs
% GFP+ OHCs
Apical Turn
Basal Turn
(48h)
(48h + 5d)
AAV1
AAV2
AAV6
AAV8
AAV9
Anc80

FIG. 1

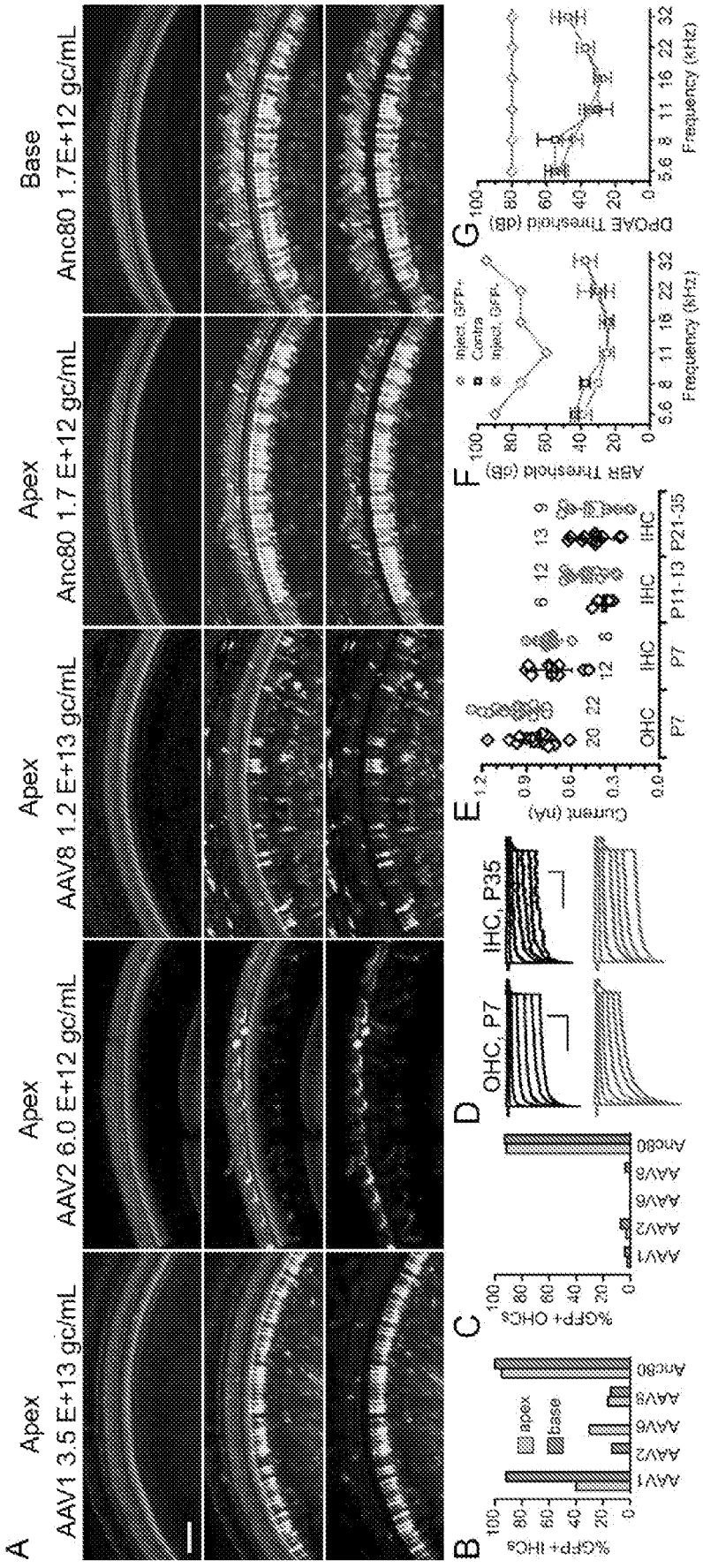

A
Apex
AAV1 3.5 E+13 gc/mL
Apex
AAV2 6.0 E+12 gc/mL
Apex
AAV8 1.2 E+13 gc/mL
Apex
Anc80 1.7 E+12 gc/mL
Base
Anc80 1.7E+12 gc/mL
B
%GFP+ IHCs
apex
base
AAV1
AAV2
AAV6
AAV8
Anc80
C
%GFP+ OHCs
D
OHC, P7
IHC, P35
E
Current (nA)
OHC
IHC
P7
P7
P11-13
P21-35
F
ABR Threshold (dB)
inject. GFP+
Contra
inject. GFP-
Frequency (kHz)
G
DPOAE Threshold (dB)
Frequency (kHz)

FIG. 2

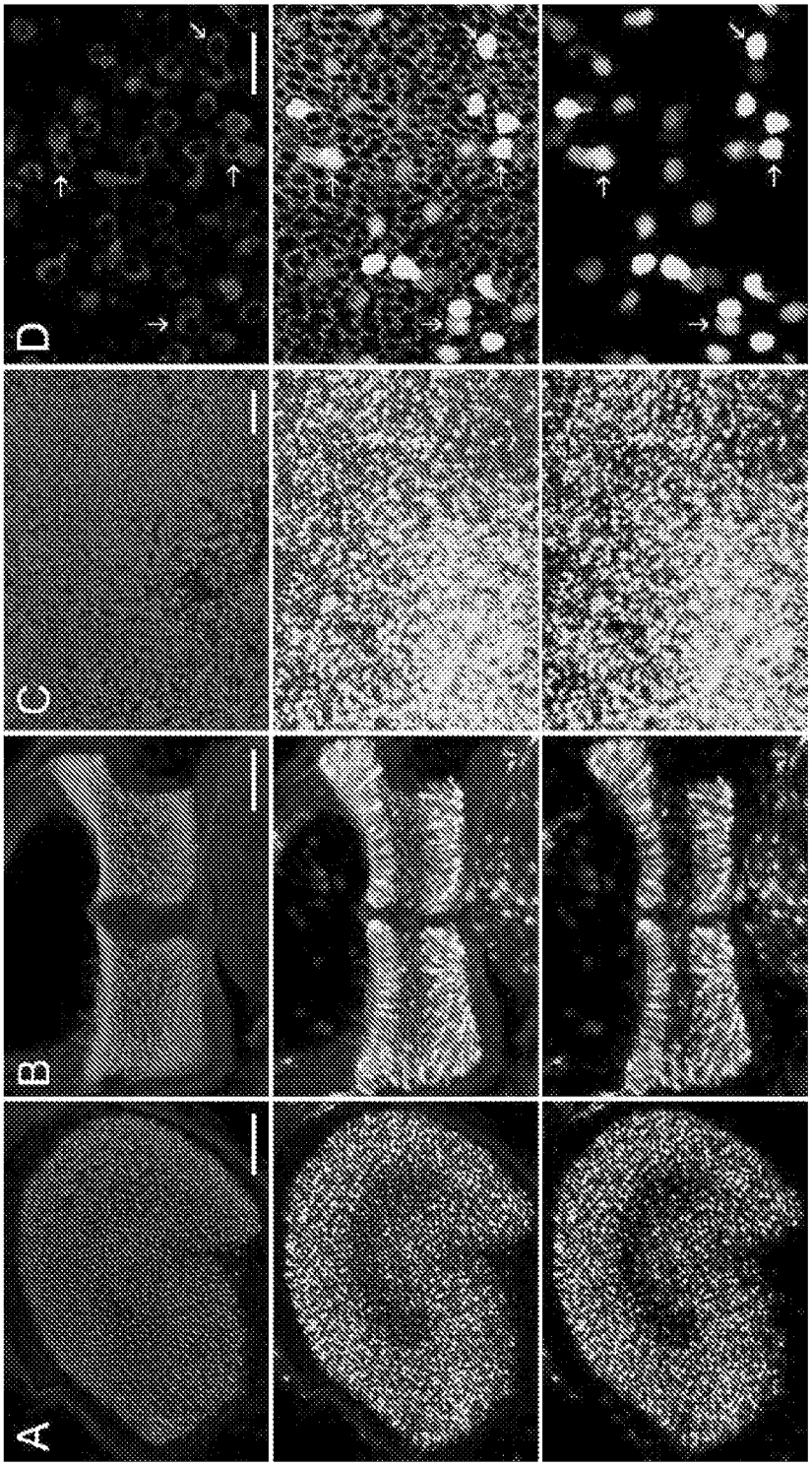


FIG. 3

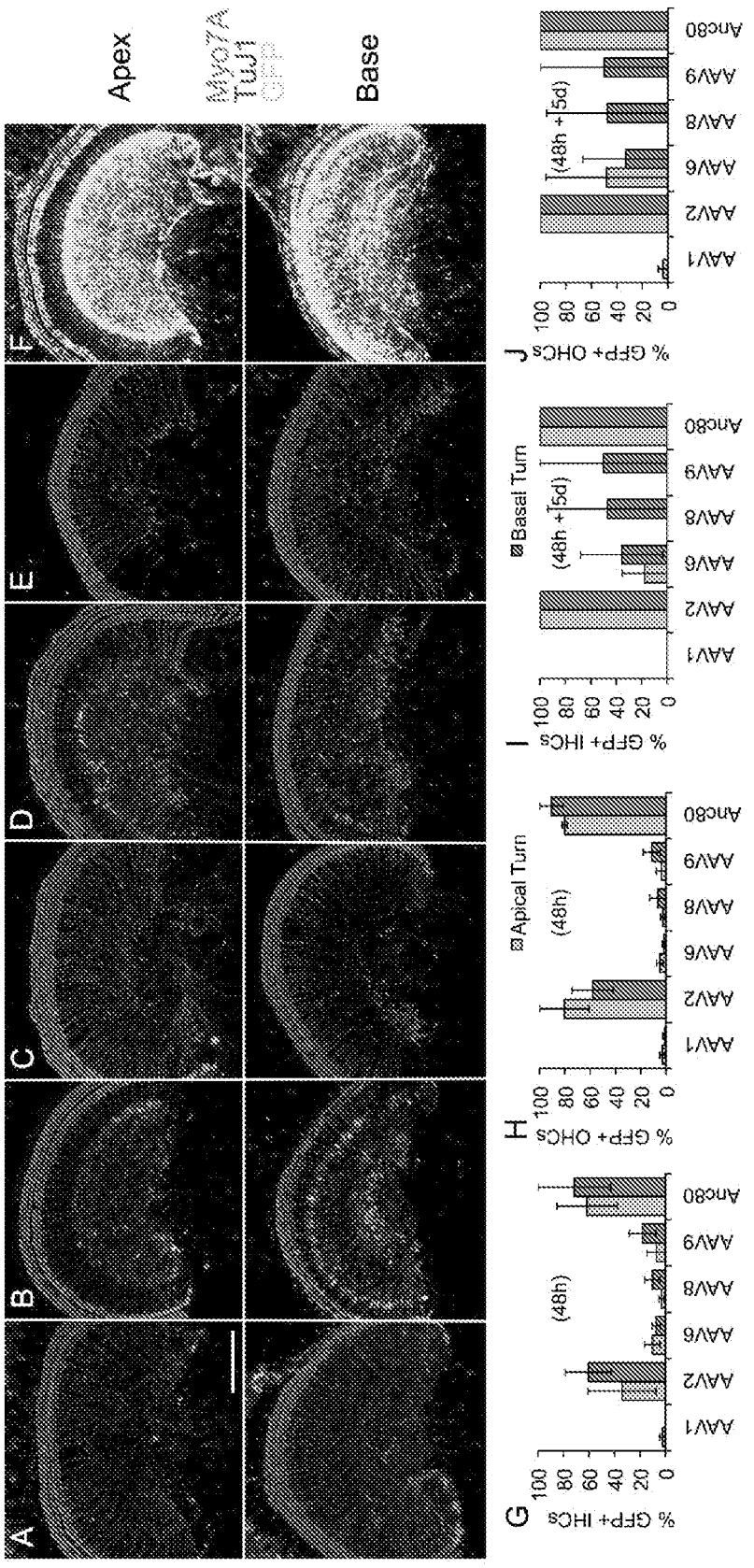

AAV1
AAV2
AAV6
AAV8
AAV9
Anc80
Apex
Base
MyoVA
TuJ1
GFP
A
B
C
D
E
F
G
H
I
J
% GFP+ IHCs
% GFP+ OHCs
(48h)
(48h +5d)
Apical Turn
Basal Turn
AAV1
AAV2
AAV6
AAV8
AAV9
Anc80

FIG. 4

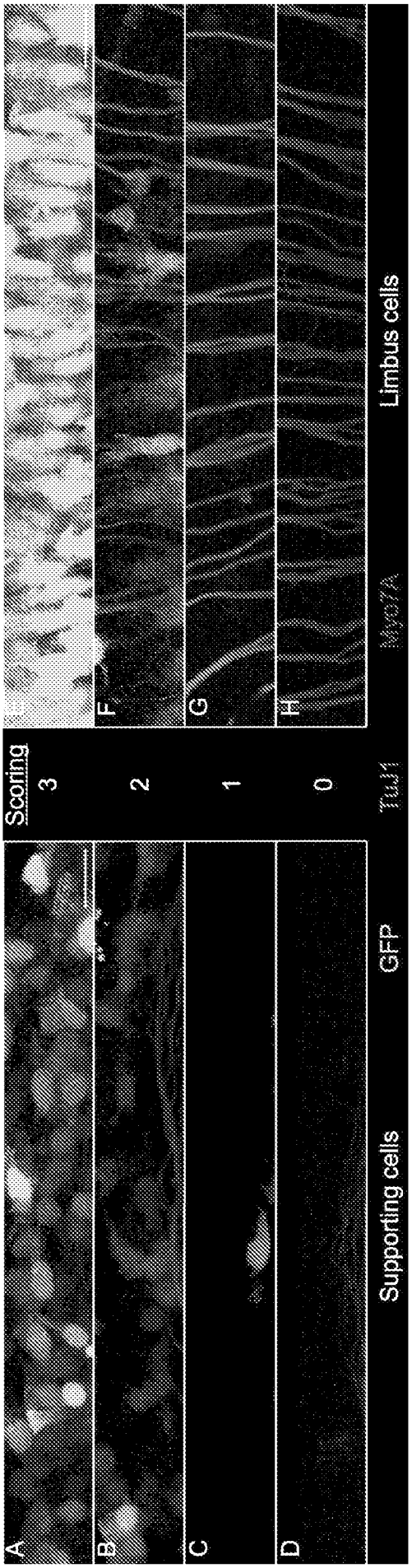

A
B
C
D
Supporting cells
GFP
Scoring
3
2
1
0
Tuj1
E
F
G
H
MyO7A
Limbus cells

FIG. 5

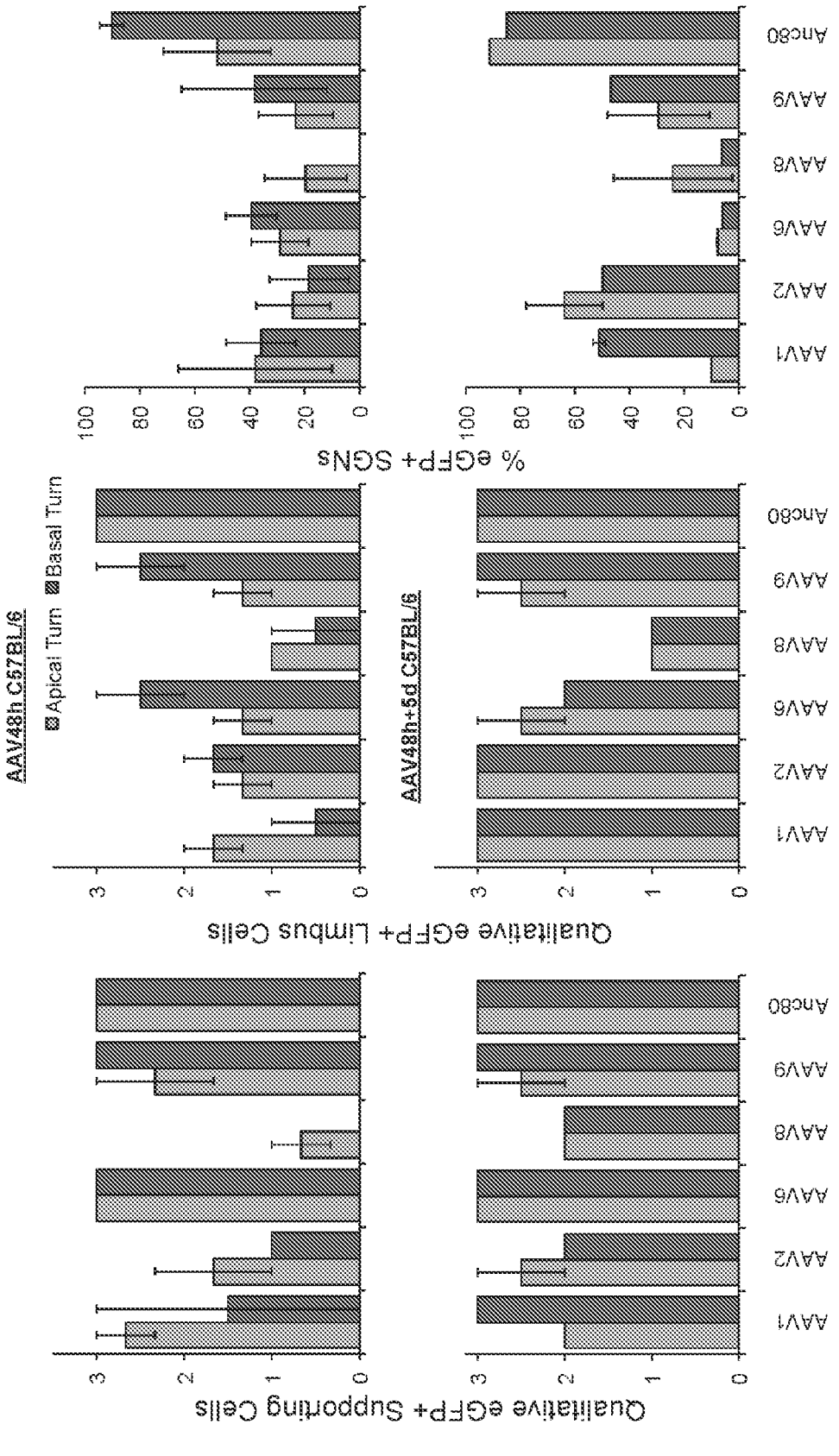

Qualitative eGFP+ Supporting Cells
Qualitative eGFP+ Limbus Cells
% eGFP+ SGNs
AAV48h C57BL/6
AAV48h+5d C57BL/6
Apical Turn
Basal Turn
AAV1
AAV2
AAV6
AAV8
AAV9
Anc80

FIG. 6

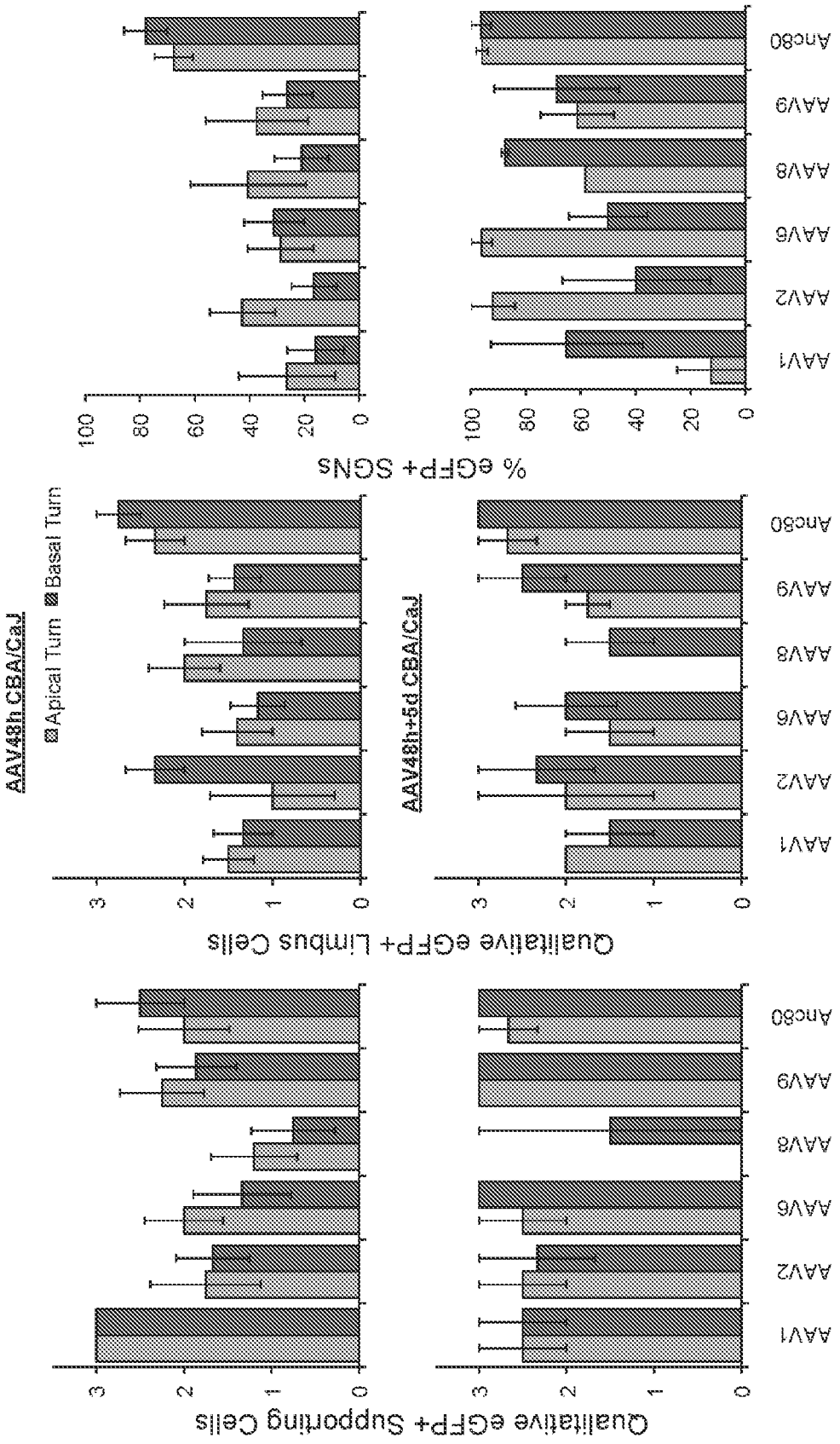

% eGFP+ SGNs
Qualitative eGFP+ Limbus Cells
Qualitative eGFP+ Supporting Cells
AAV48h CBA/CaJ
AAV48h+5d CBA/CaJ
Apical Turn
Basal Turn
AAV1
AAV2
AAV6
AAV8
AAV9
Anc80

FIG. 7

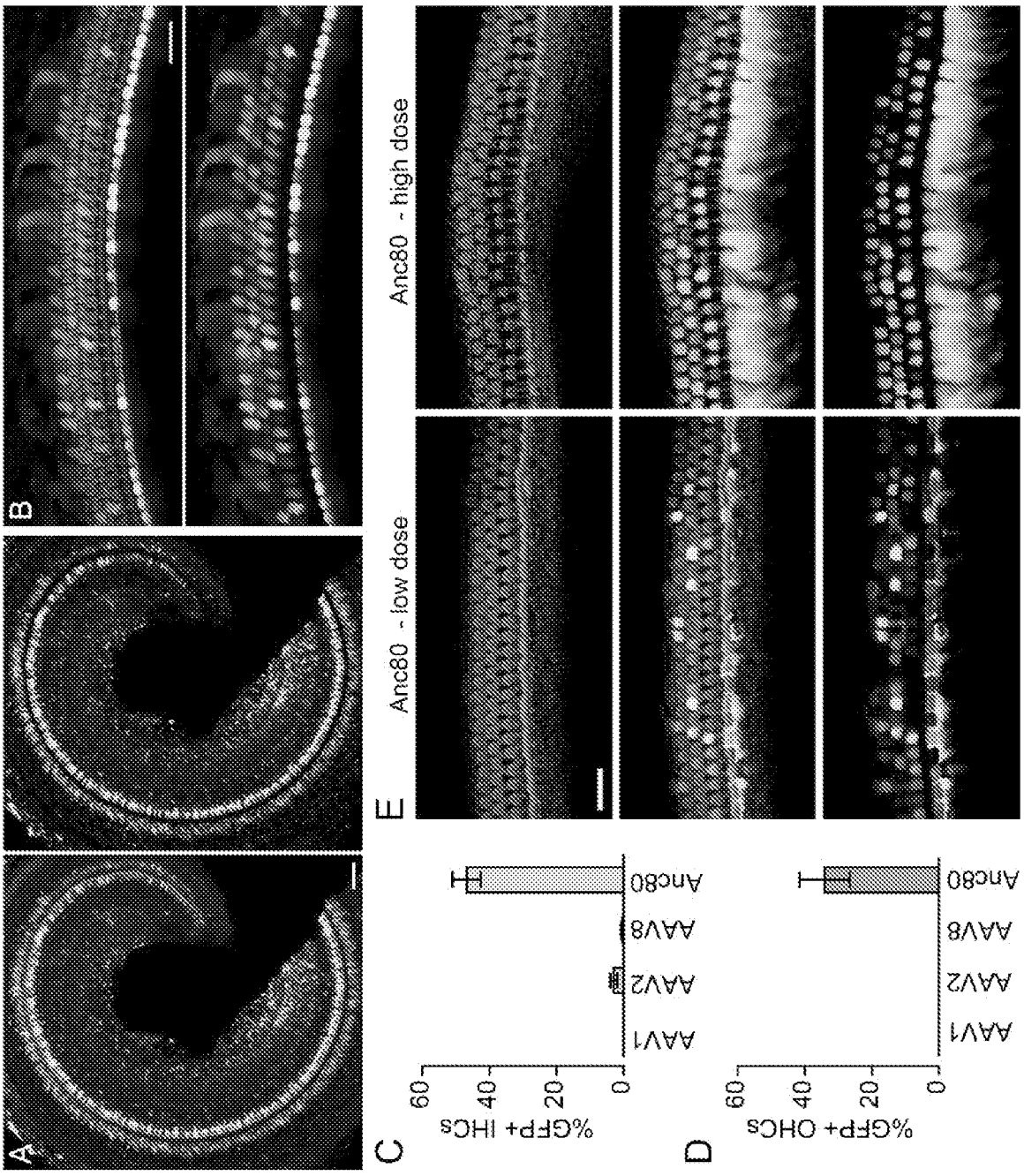

A
B
C
D
E
Anc80 - low dose
Anc80 - high dose
%GFP+ IHCs
%GFP+ OHCs
0
20
40
60
AAV1
AAV2
AAV8
Anc80

FIG. 8

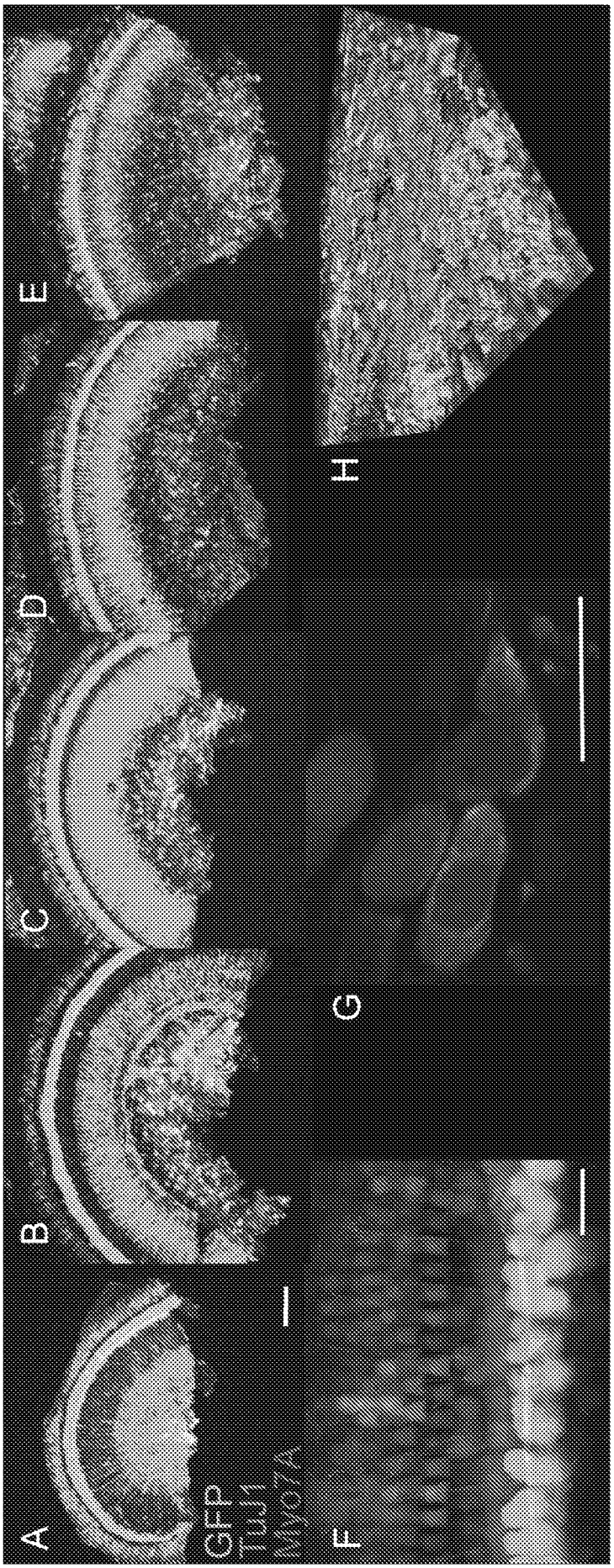


FIG. 9

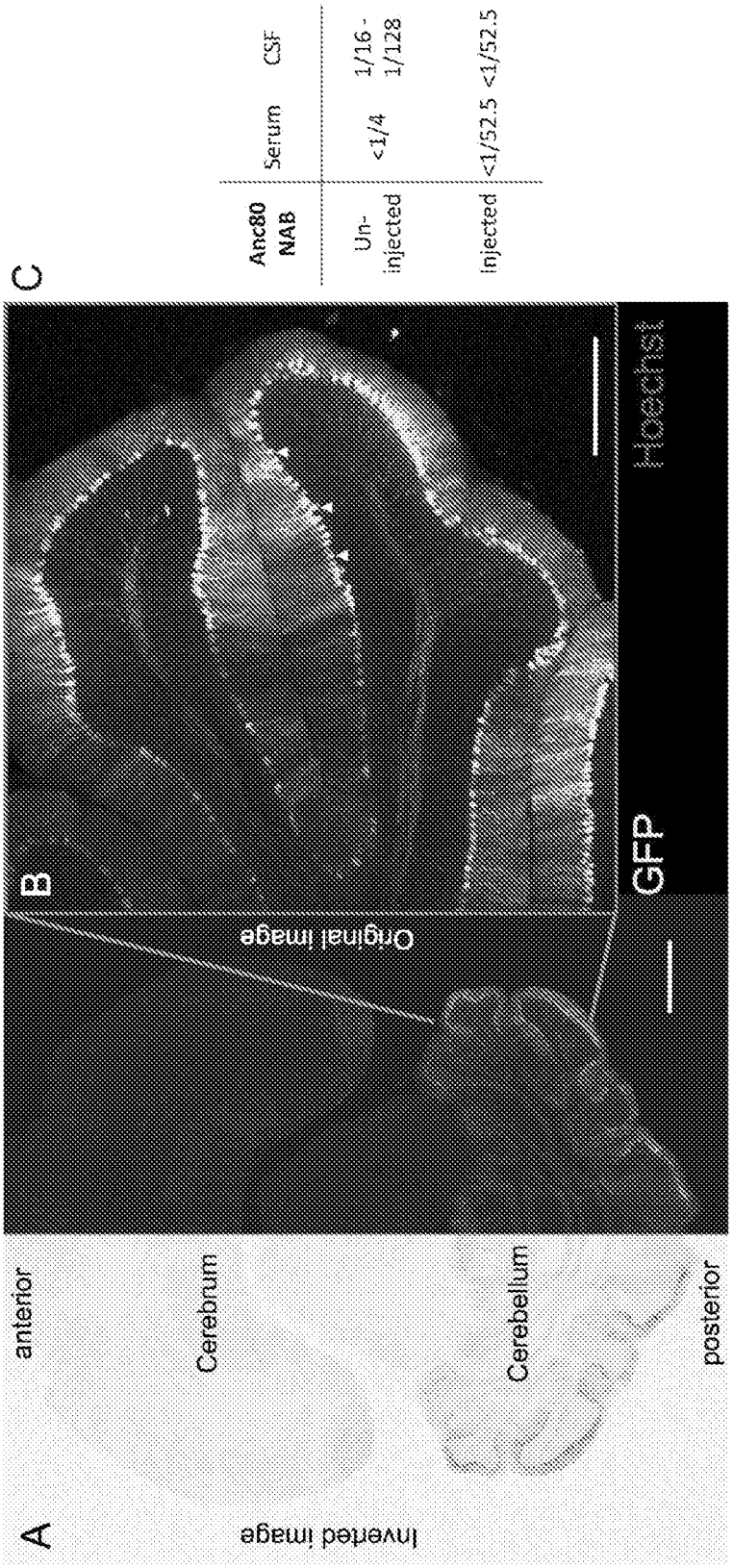

A
B
C
anterior
posterior
Cerebrum
Cerebellum
Inverted image
Original image
GFP
Hoechst
Anc80 NAB
Serum
CSF
Un-injected
<1/4
1/16 - 1/128
Injected
<1/52.5
<1/52.5

FIG. 10

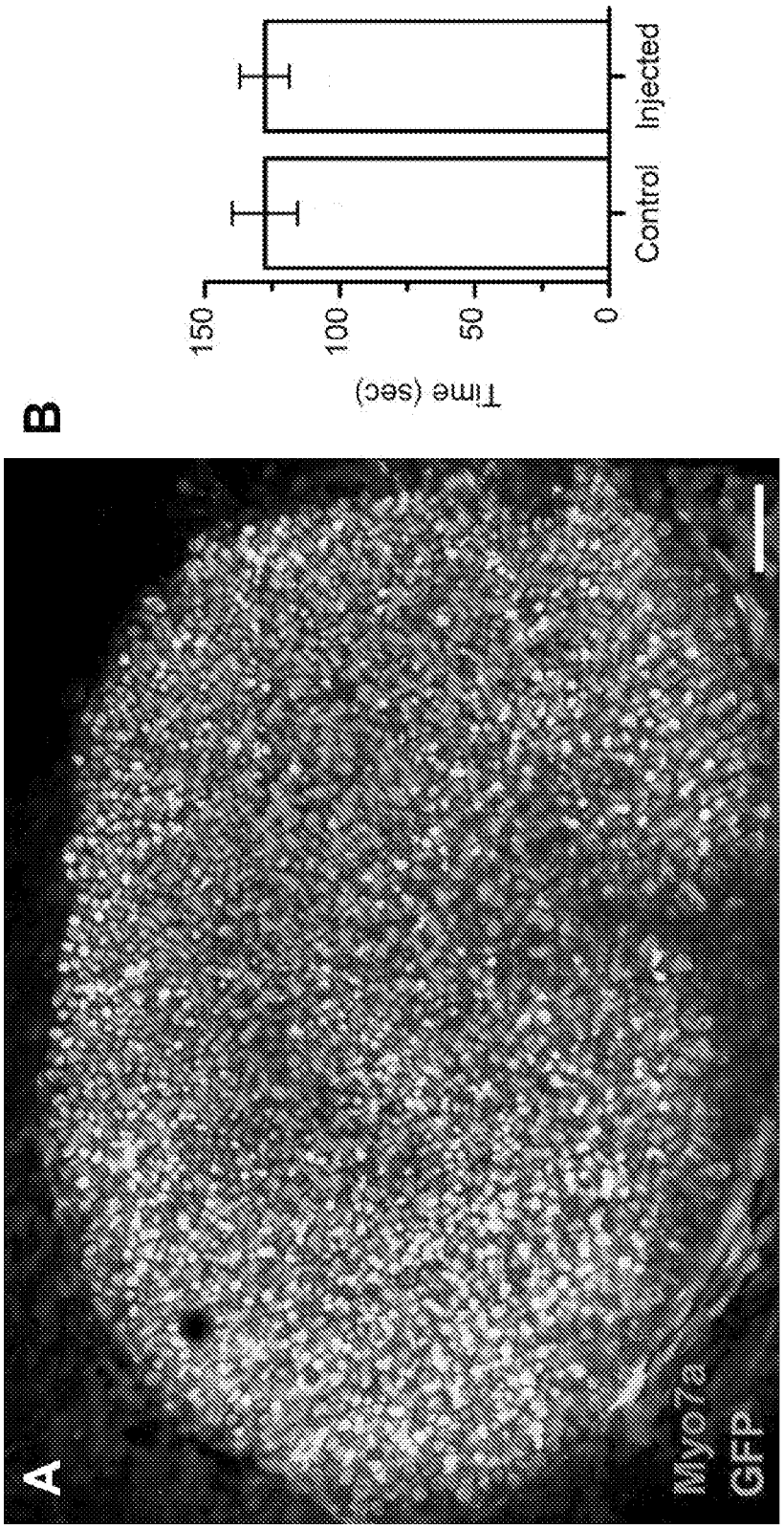


FIG. 11

Fig. 11

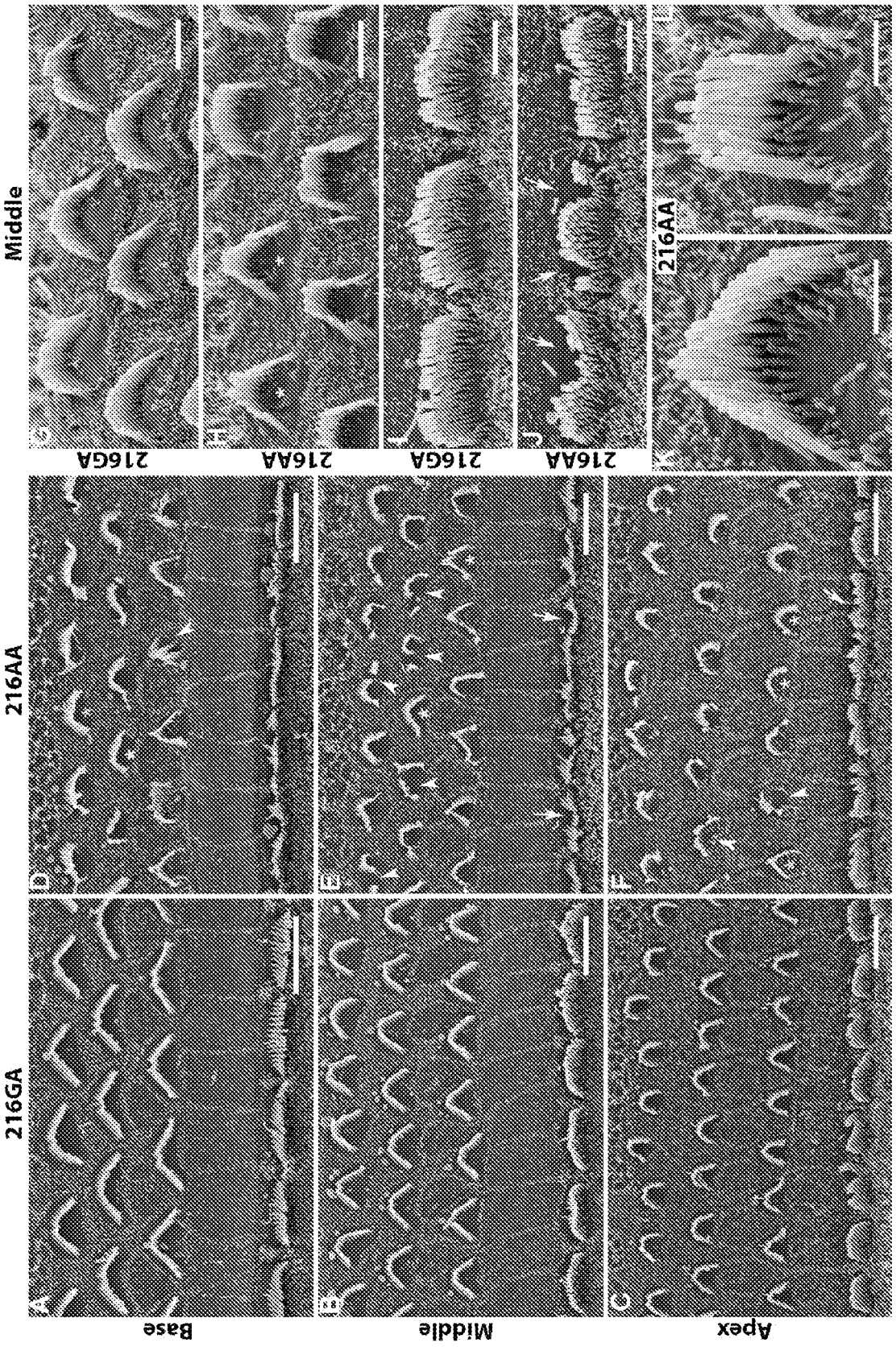

216GA
216AA
Base
Middle
Apex
216GA
216AA
216GA
216AA
Middle
216AA

FIG. 12

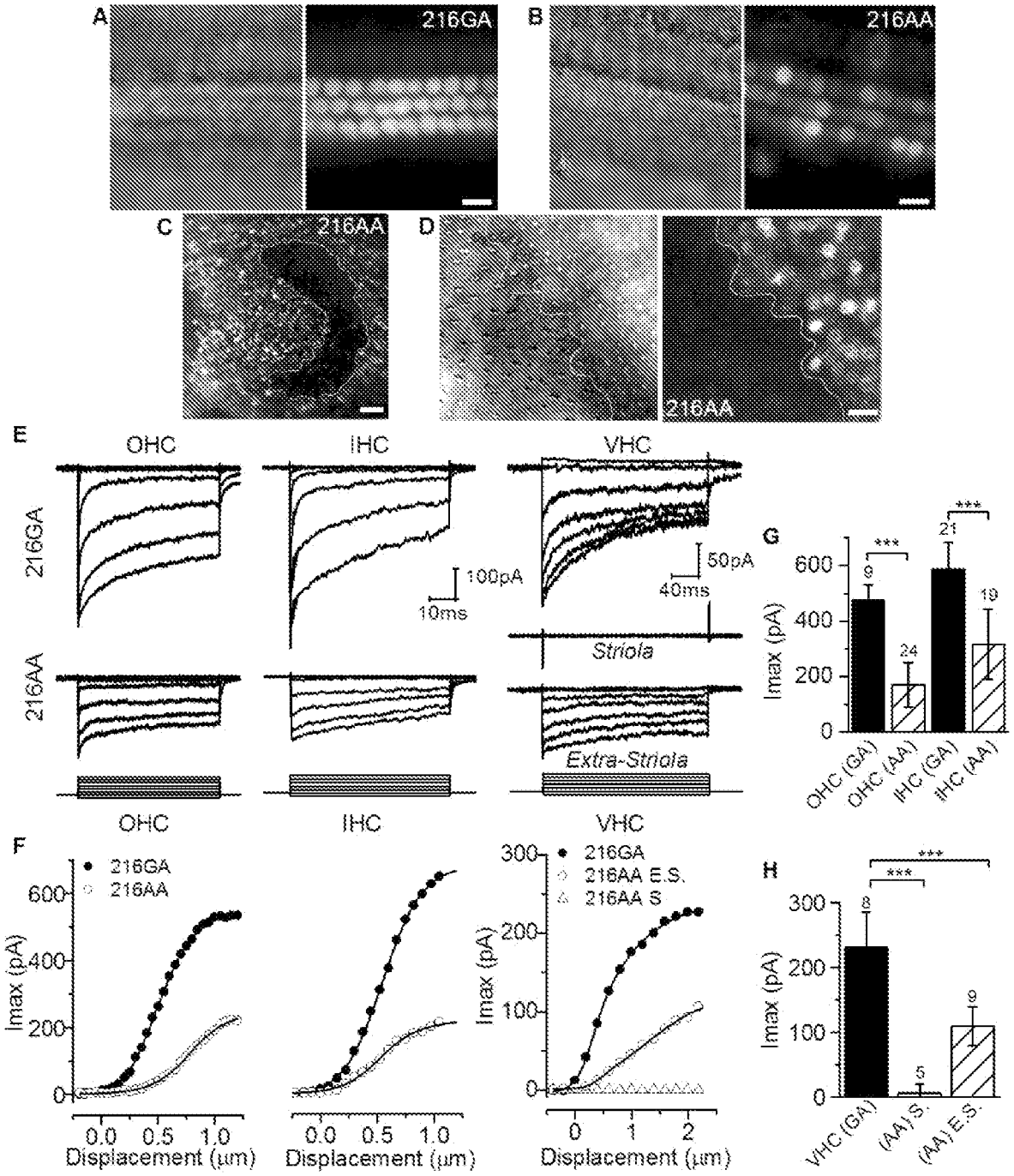

A
216GA
B
216AA
C
216AA
D
216AA
E
OHC
IHC
VHC
216GA
216AA
100pA
10ms
50pA
40ms
Striola
Extra-Striola
G
Imax (pA)
600
400
200
0
***
***
9
24
21
19
OHC (GA)
OHC (AA)
IHC (GA)
IHC (AA)
F
OHC
IHC
VHC
216GA
216AA
216AA E.S.
216AA S
Imax (pA)
600
400
200
0
300
200
100
0.0 0.5 1.0
0 1 2
Displacement (µm)
H
***
***
8
5
9
300
200
100
0
VHC (GA)
(AA) S.
(AA) E.S.

FIG. 13

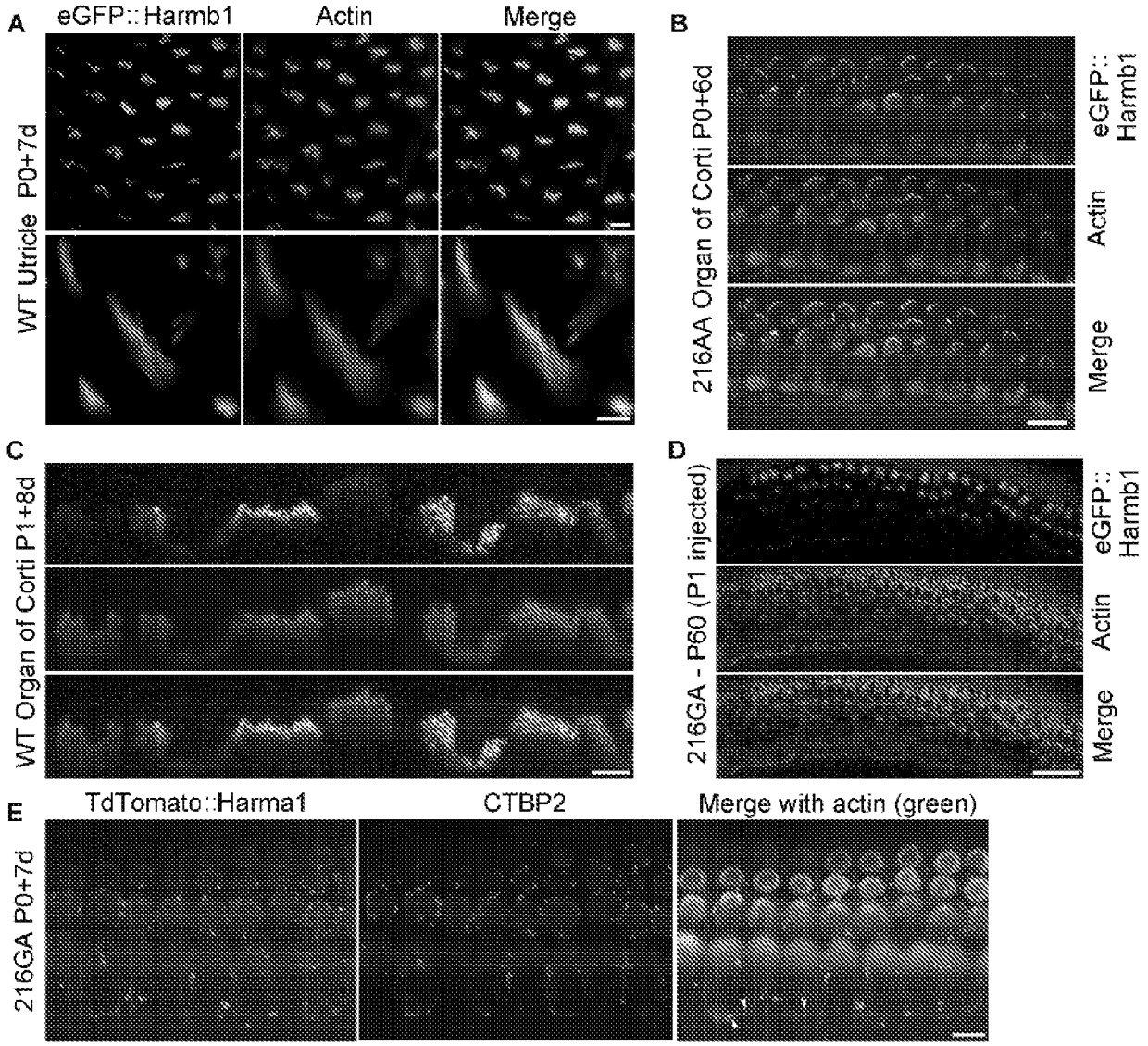

A
eGFP::Harmb1
Actin
Merge
WT Utricle P0+7d
B
216AA Organ of Corti P0+6d
eGFP::Harmb1
Actin
Merge
C
WT Organ of Corti P1+8d
D
216GA - P60 (P1 injected)
eGFP::Harmb1
Actin
Merge
E
TdTomato::Harma1
CTBP2
Merge with actin (green)
216GA P0+7d

FIG. 14

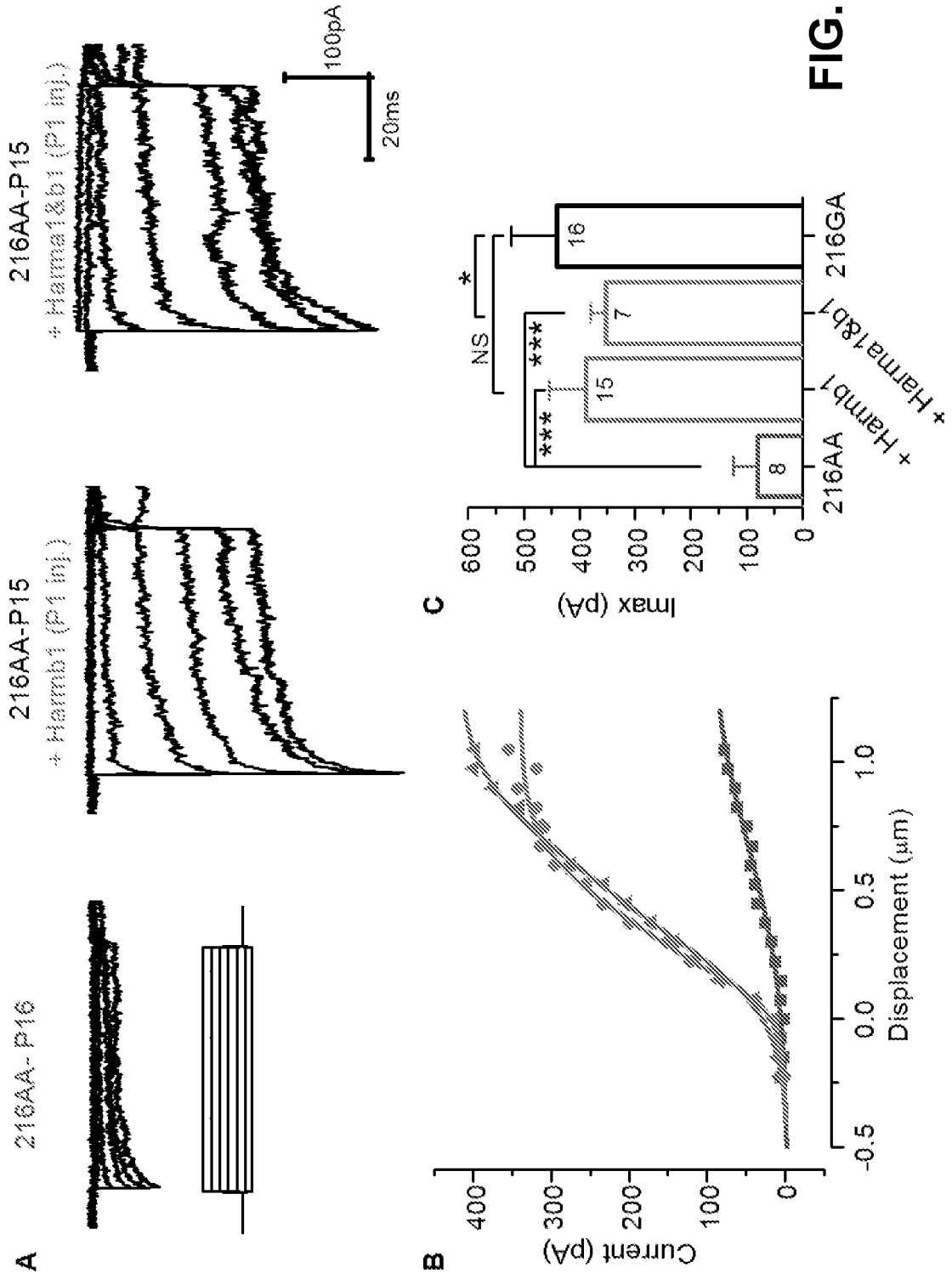

A
216AA- P16
216AA-P15
+ Hamb1 (P1 inj.)
216AA-P15
+ Hama1&b1 (P1 inj.)
100pA
20ms
B
Current (pA)
Displacement (μm)
C
Imax (pA)
216AA
+ Hamb1
+ Hama1&b1
216GA
NS
*
***
***

FIG. 15

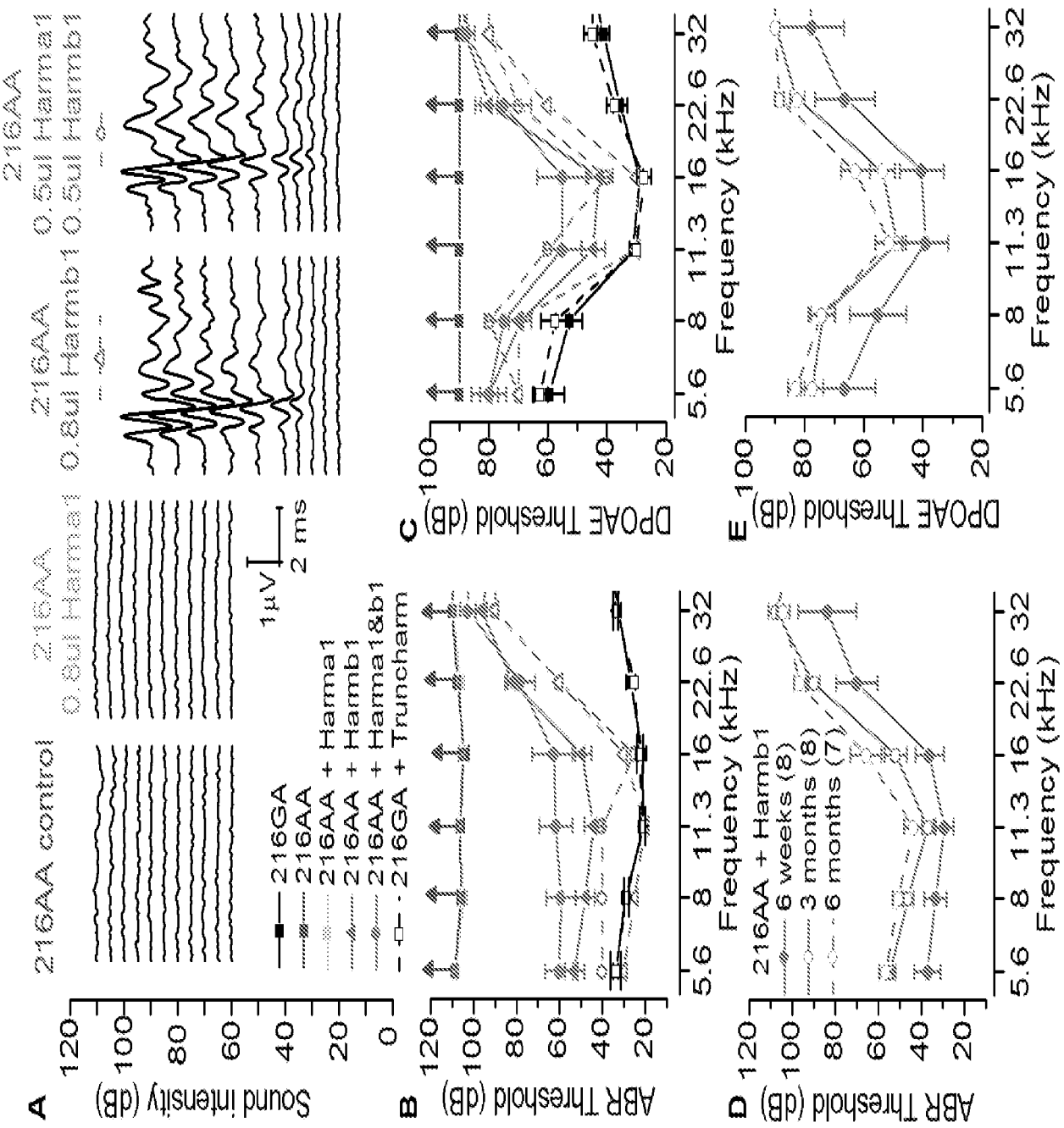

A
216AA control
216AA
0.8ul Harma1
216AA
0.8ul Harmb1
216AA
0.5ul Harma1
0.5ul Harmb1
Sound intensity (dB)
120
100
80
60
40
20
0
216GA
216AA
216AA + Harma1
216AA + Harmb1
216AA + Harma1&b1
216GA + Truncharm
1μV
2 ms
B
ABR Threshold (dB)
C
DPOAE Threshold (dB)
D
ABR Threshold (dB)
E
DPOAE Threshold (dB)
5.6
8
11.3
16
22.6
32
Frequency (kHz)
216AA + Harmb1
6 weeks (8)
3 months (8)
6 months (7)

FIG. 16

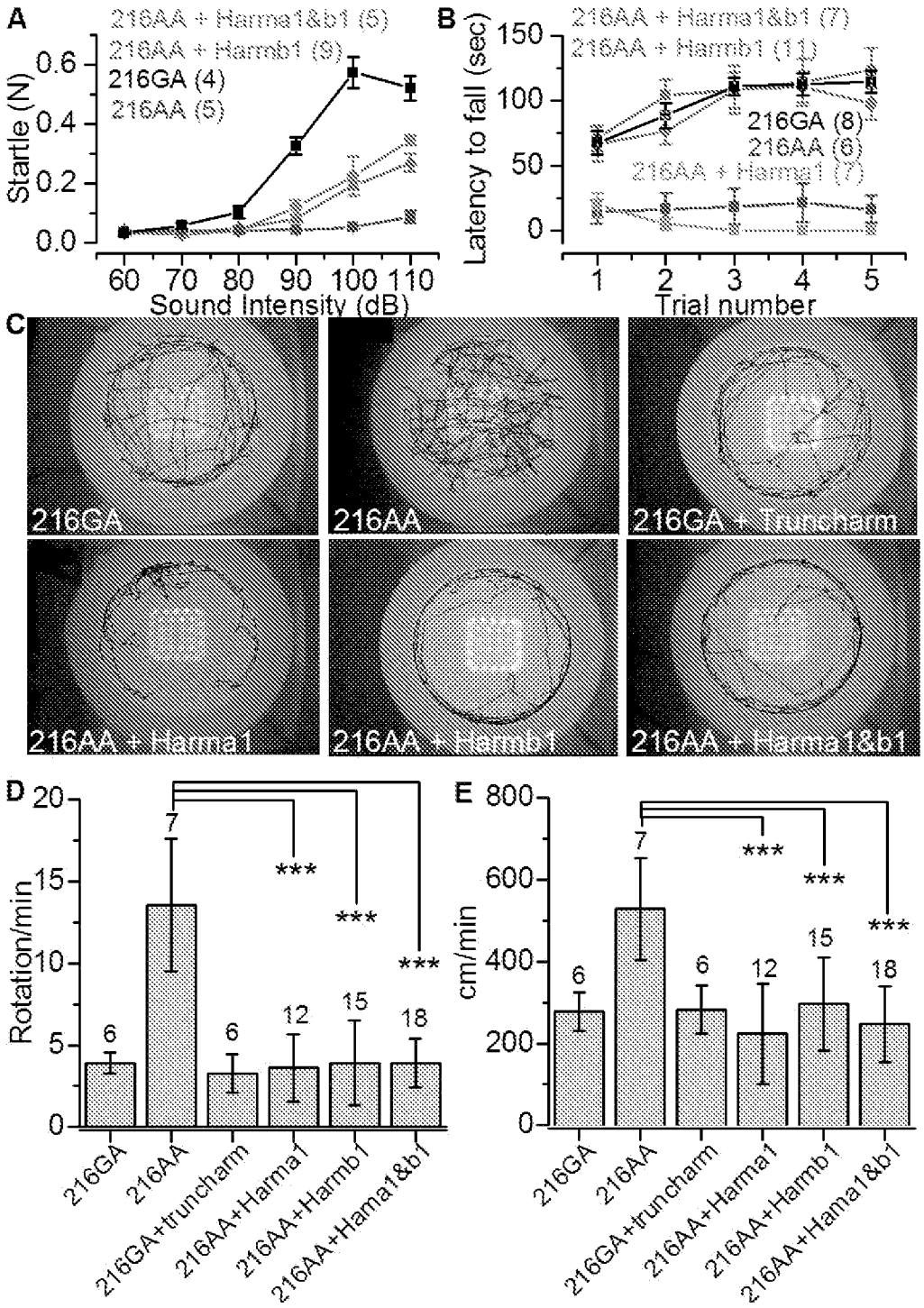

A
216AA + Harma1&b1 (5)
216AA + Hamb1 (9)
216GA (4)
216AA (5)
Startle (N)
0.0
0.2
0.4
0.6
60 70 80 90 100 110
Sound Intensity (dB)
B
216AA + Harma1&b1 (7)
216AA + Hamb1 (11)
216GA (8)
216AA (6)
216AA + Harma1 (7)
Latency to fall (sec)
0
50
100
150
1 2 3 4 5
Trial number
C
216GA
216AA
216GA + Truncharm
216AA + Harma1
216AA + Hamb1
216AA + Harma1&b1
D
Rotation/min
0
5
10
15
20
6
7
6
12
15
18
***
***
***
216GA
216AA
216GA+truncharm
216AA+Harma1
216AA+Hamb1
216AA+Hama1&b1
E
cm/min
0
200
400
600
800
6
7
6
12
15
18
***
***
***
216GA
216AA
216GA+truncharm
216AA+Harma1
216AA+Hamb1
216AA+Hama1&b1

FIG. 17

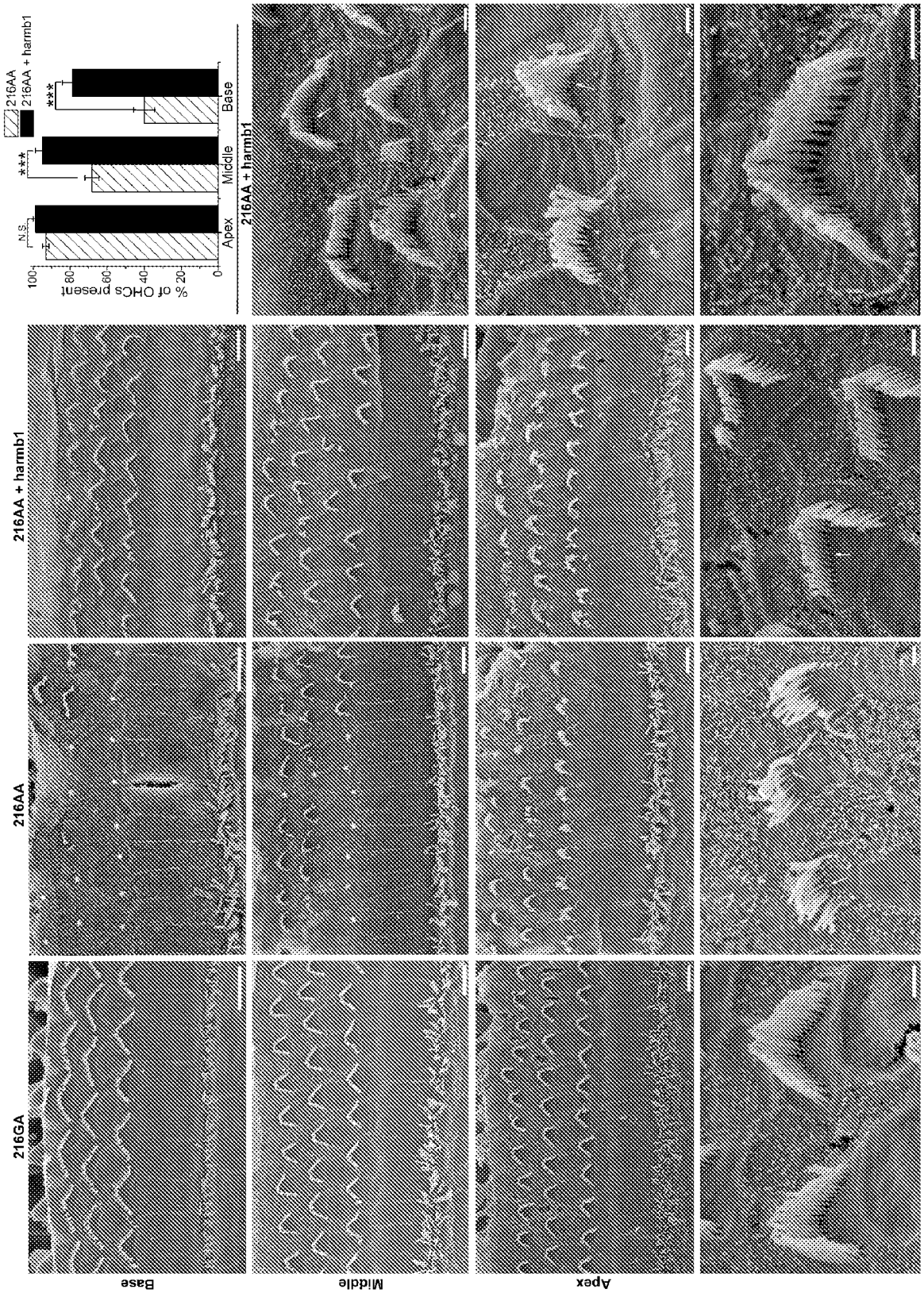

216GA
216AA
216AA + harmb1
Base
Middle
Apex
% of OHCs present
100
80
60
40
20
0
N.S.
***
***
Apex
Middle
Base
216AA
216AA + harmb1

FIG. 18

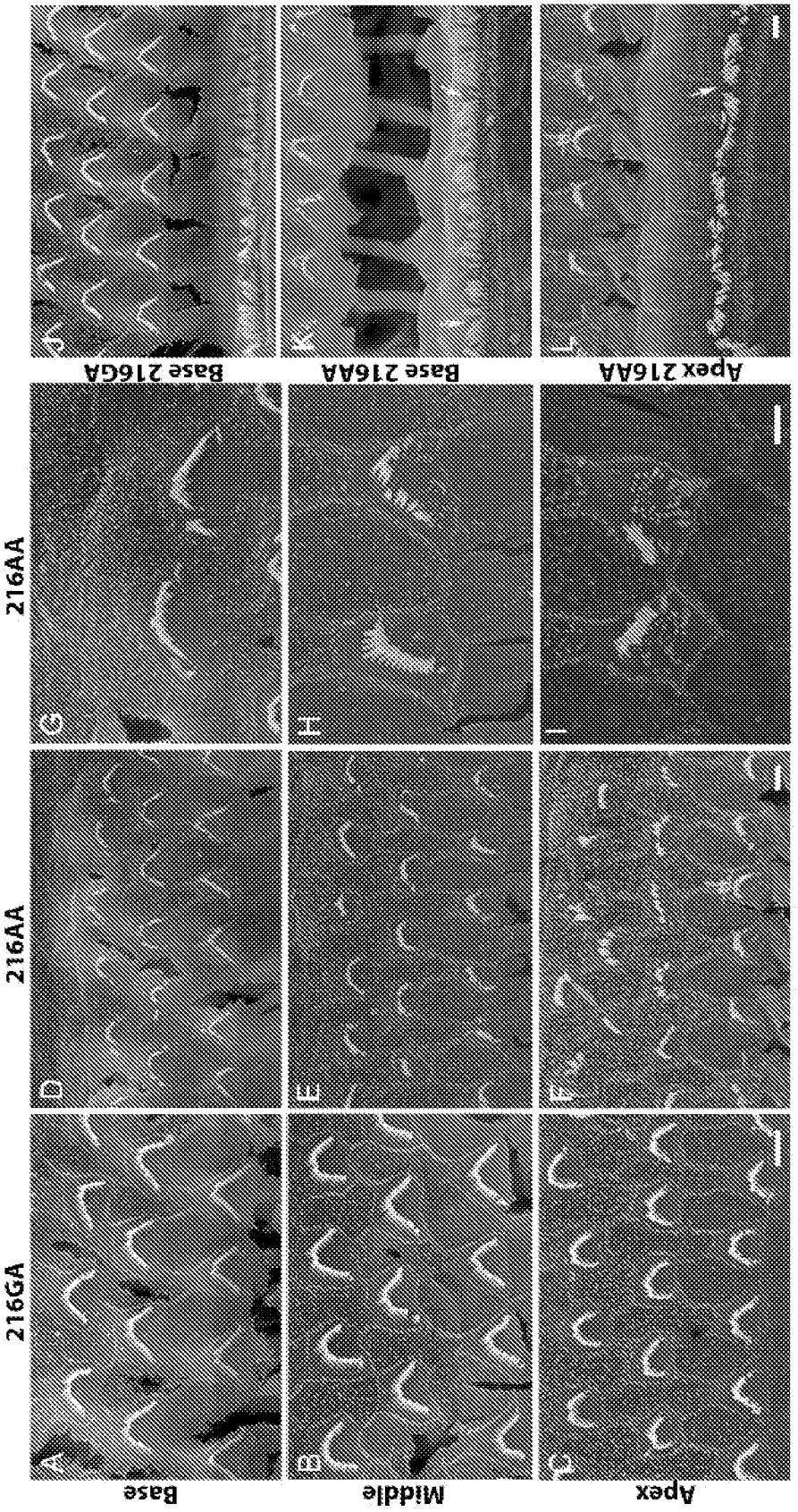

216GA
216AA
216AA
Base
Middle
Apex
Base 216GA
Base 216AA
Apex 216AA

FIG. 19

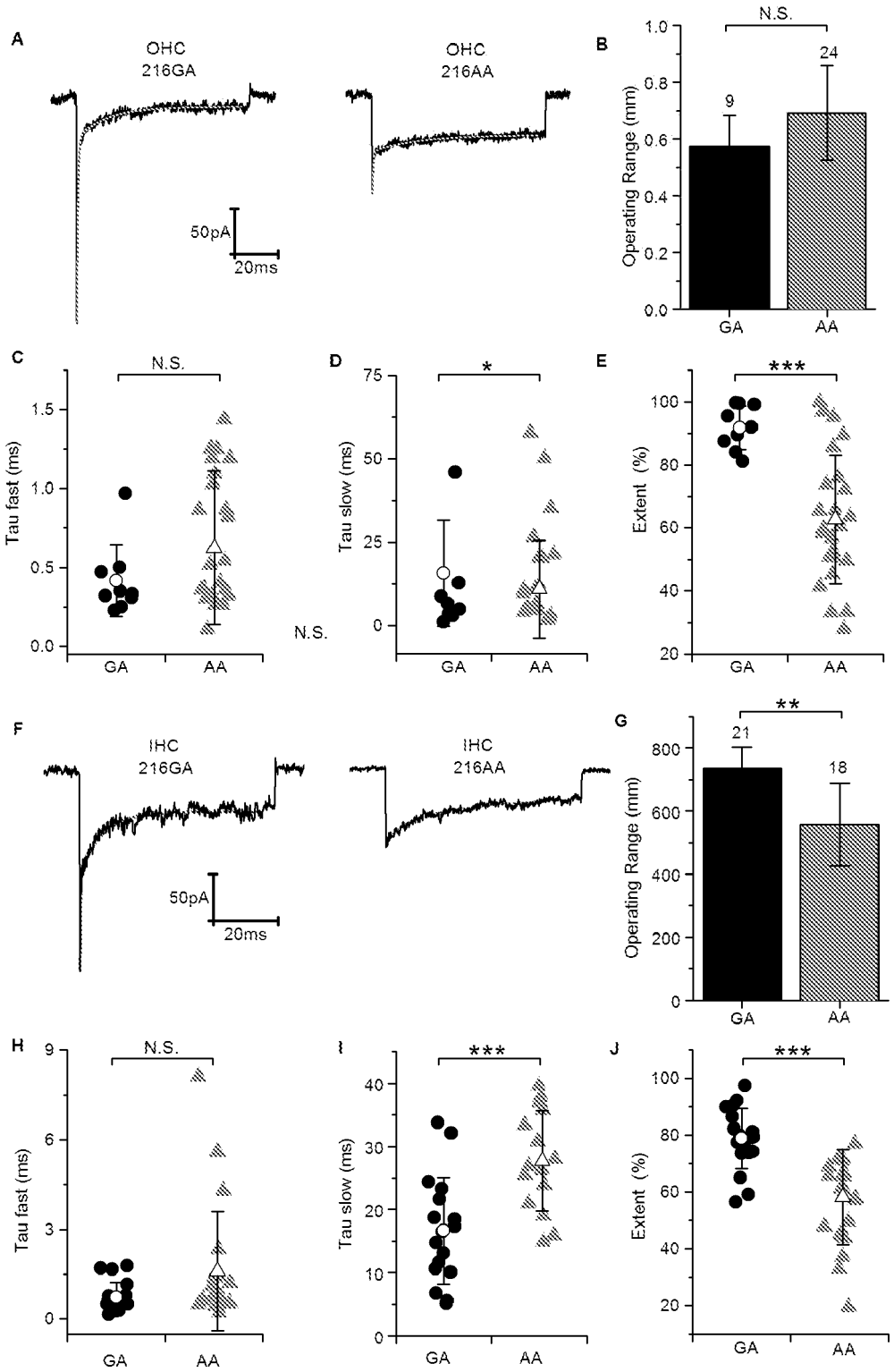

A
OHC
216GA
50pA
20ms
OHC
216AA
B
N.S.
9
24
Operating Range (mm)
GA
AA
C
N.S.
Tau fast (ms)
GA
AA
D
*
Tau slow (ms)
N.S.
GA
AA
E
***
Extent (%)
GA
AA
F
IHC
216GA
50pA
20ms
IHC
216AA
G
**
21
18
Operating Range (mm)
GA
AA
H
N.S.
Tau fast (ms)
GA
AA
I
***
Tau slow (ms)
GA
AA
J
***
Extent (%)
GA
AA

FIG. 20

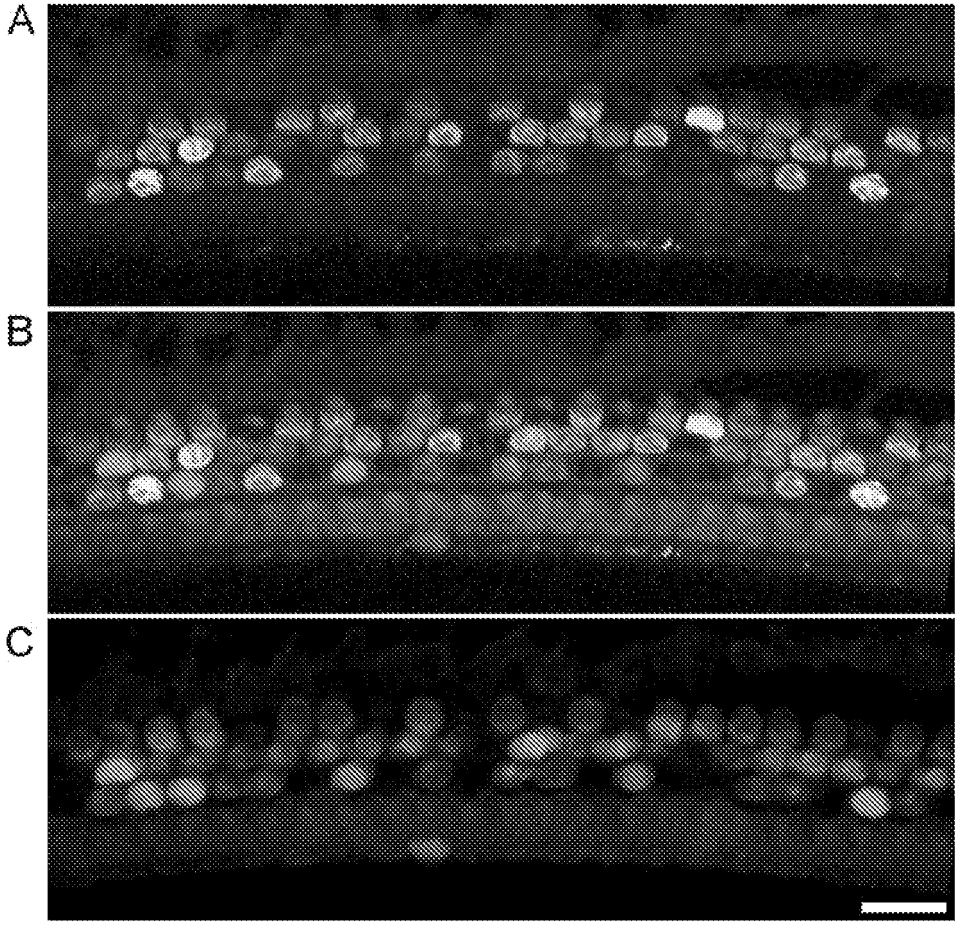


FIG. 21

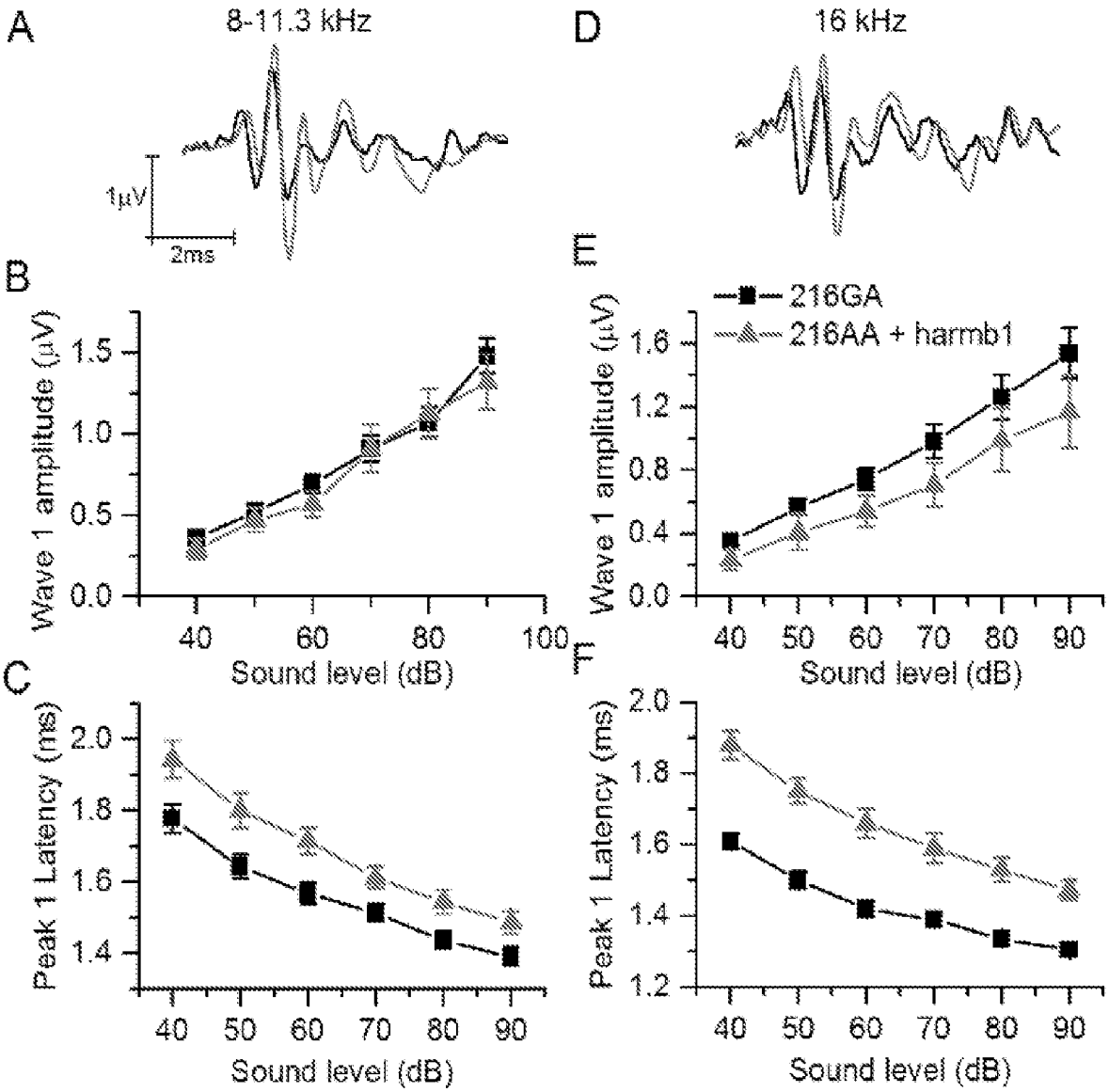

A
8-11.3 kHz
1μV
2ms
D
16 kHz
B
Wave 1 amplitude (μV)
Sound level (dB)
C
Peak 1 Latency (ms)
Sound level (dB)
E
216GA
216AA + harmb1
Wave 1 amplitude (μV)
Sound level (dB)
F
Peak 1 Latency (ms)
Sound level (dB)

FIG. 22

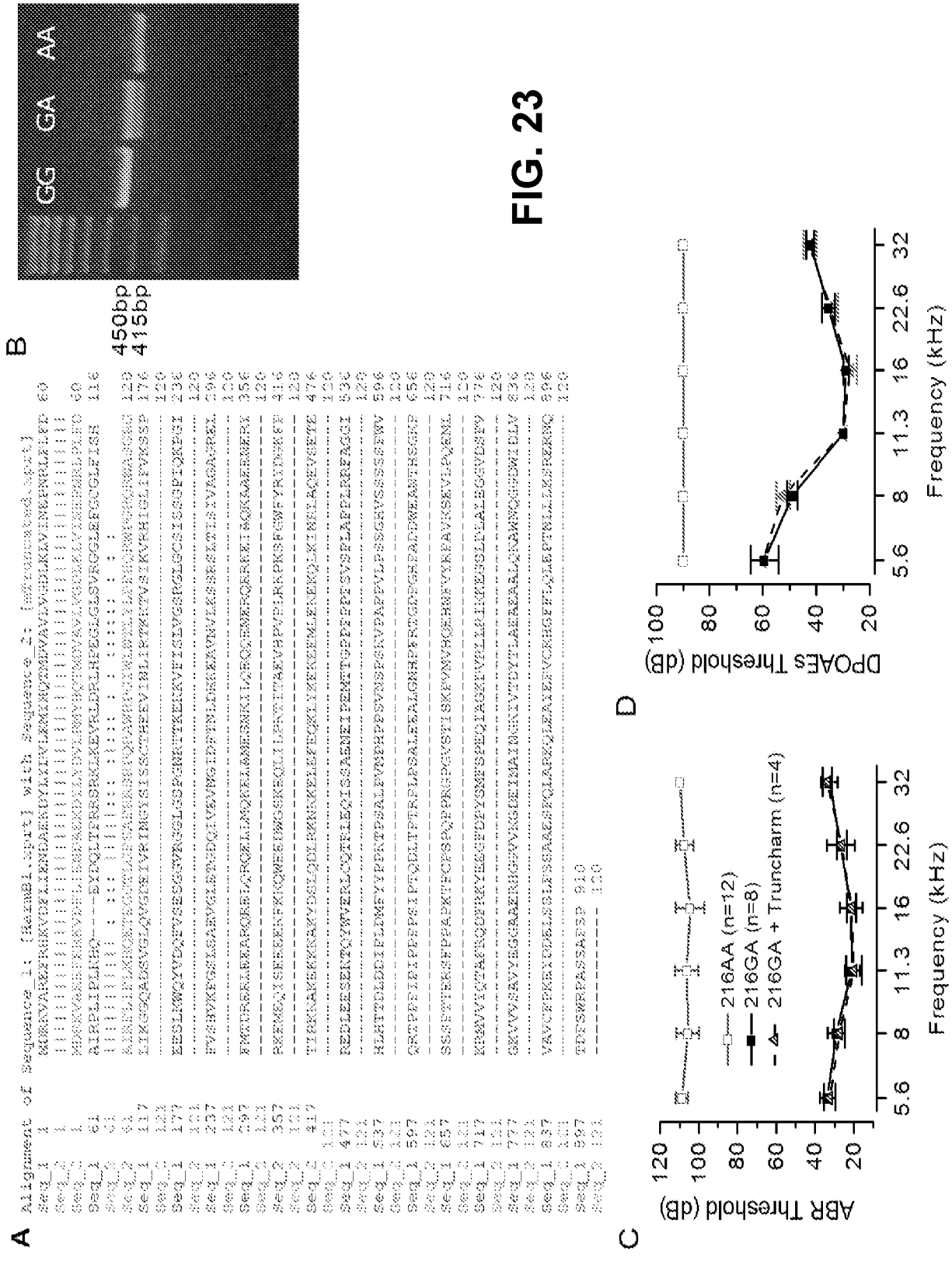
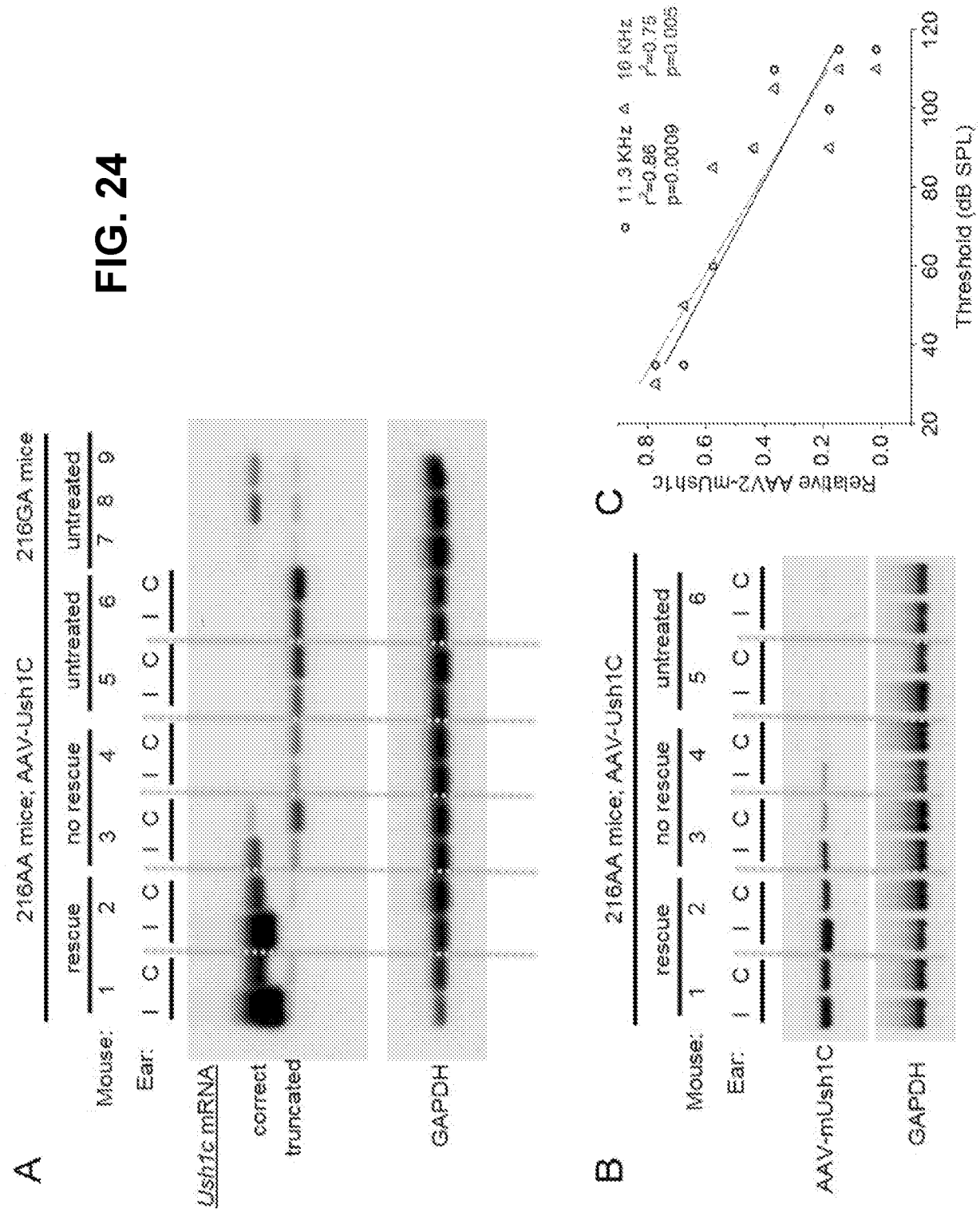

A
B
CG
GA
AA
450bp
415bp
C
ABR Threshold (dB)
216AA (n=12)
216GA (n=8)
216GA + Truncharm (n=4)
Frequency (kHz)
D
DPOAEs Threshold (dB)
Frequency (kHz)

FIG. 23

FIG. 24


A
216AA mice; AAV-Ush1C
216GA mice
rescue
no rescue
untreated
untreated
Mouse:
Ear:
Ush1c mRNA
correct
truncated
GAPDH
B
216AA mice; AAV-Ush1C
AAV-mUsh1C
GAPDH
C
11.3 KHz
16 KHz
Relative AAV2-mUsh1c
Threshold (dB SPL)

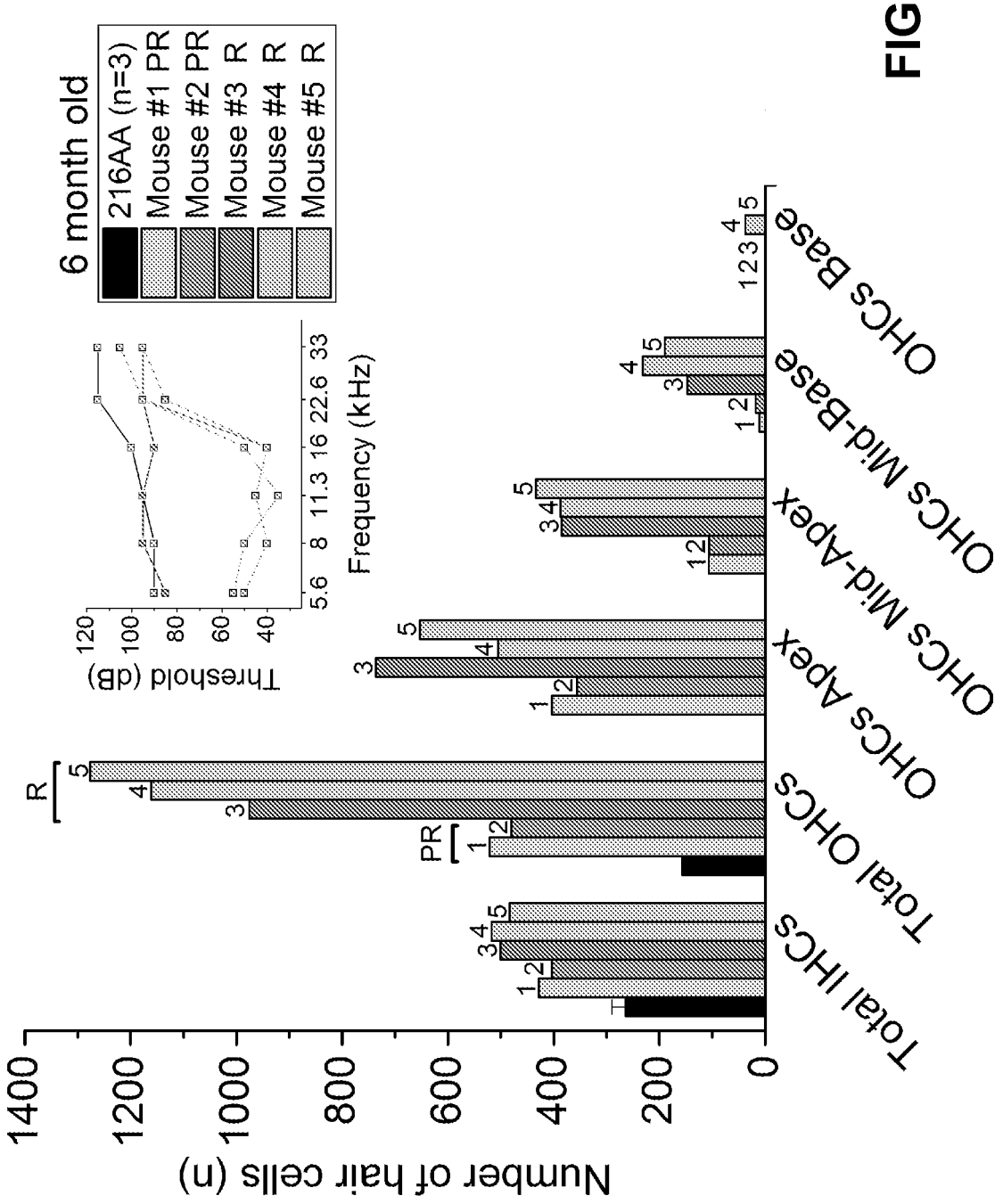

6 month old
216AA (n=3)
Mouse #1 PR
Mouse #2 PR
Mouse #3 R
Mouse #4 R
Mouse #5 R
Threshold (dB)
Frequency (kHz)
Number of hair cells (n)
Total IHCs
Total OHCs
OHCs Apex
OHCs Mid-Apex
OHCs Mid-Base
OHCs Base
R
PR

FIG. 25

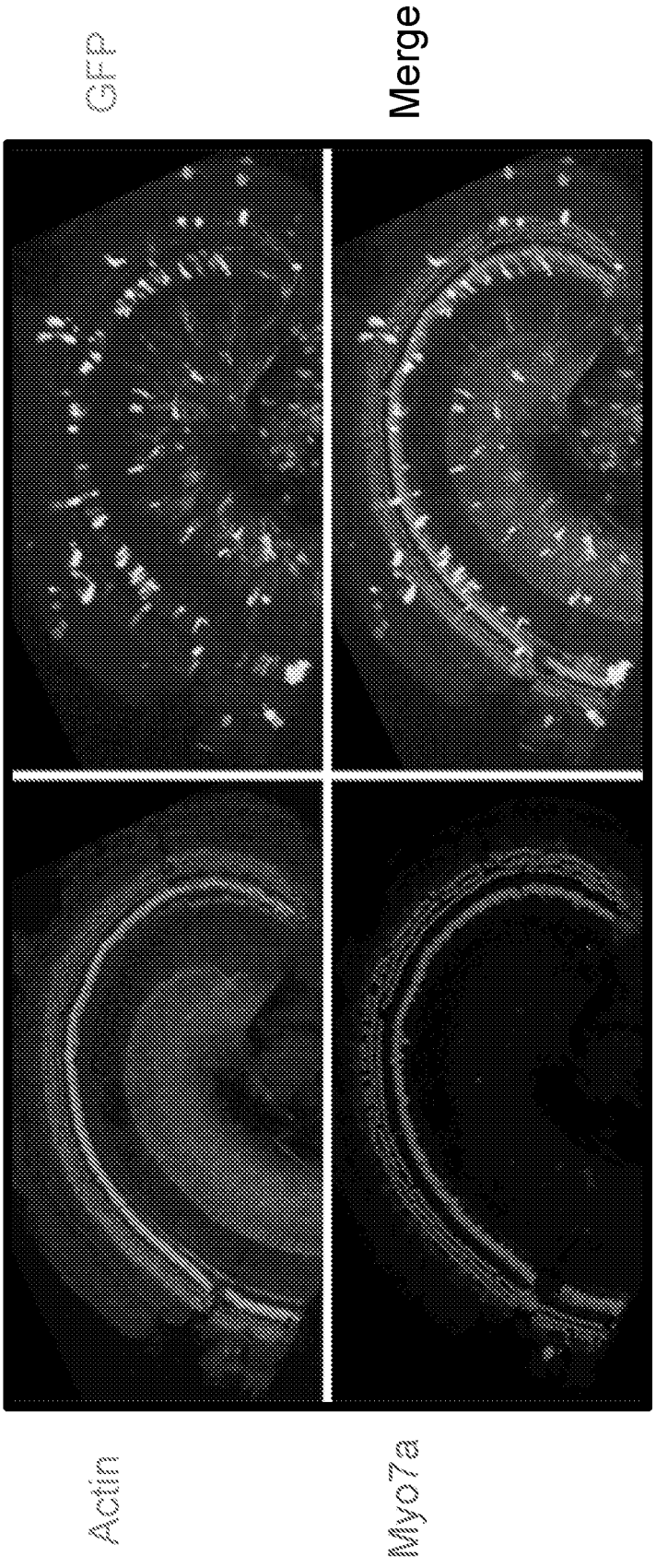


FIG. 26

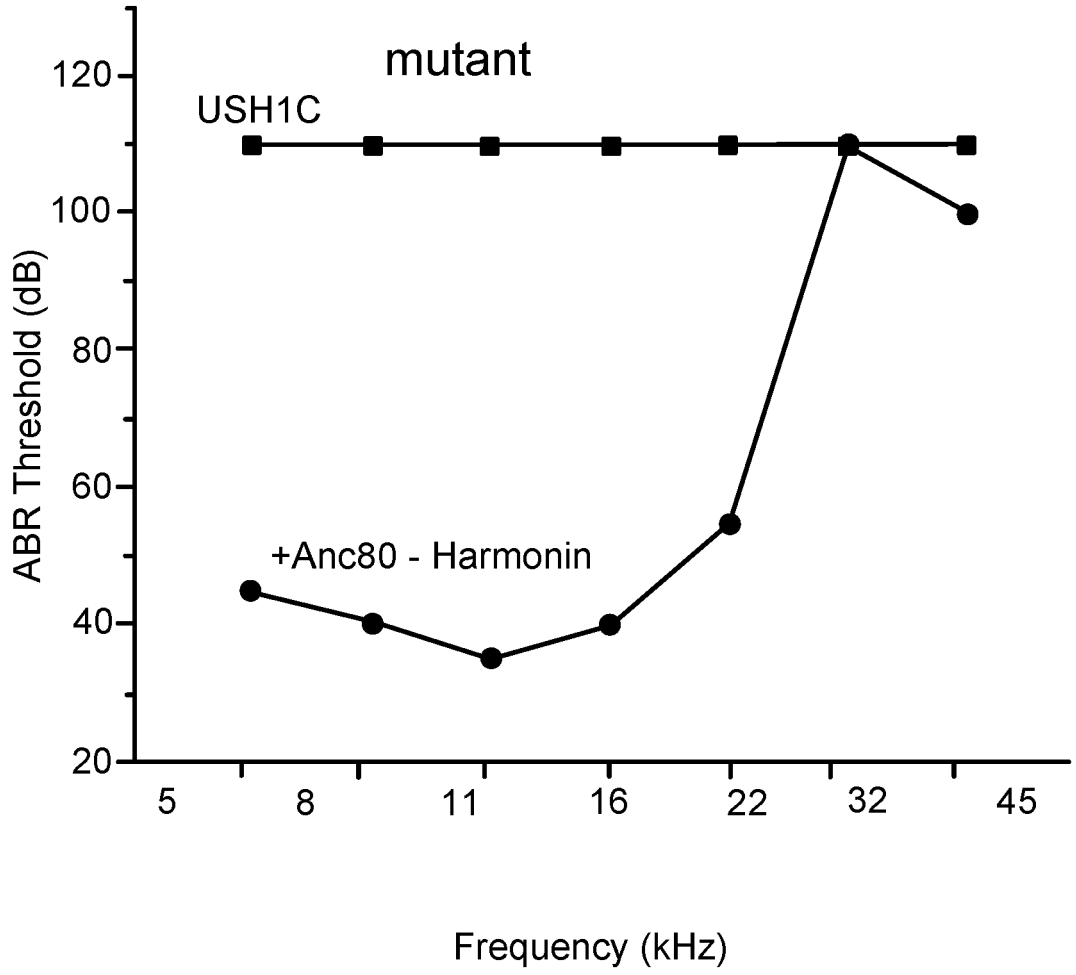

mutant
USH1C
+Anc80 - Harmonin
ABR Threshold (dB)
120
100
80
60
40
20
5
8
11
16
22
32
45
Frequency (kHz)

FIG. 27

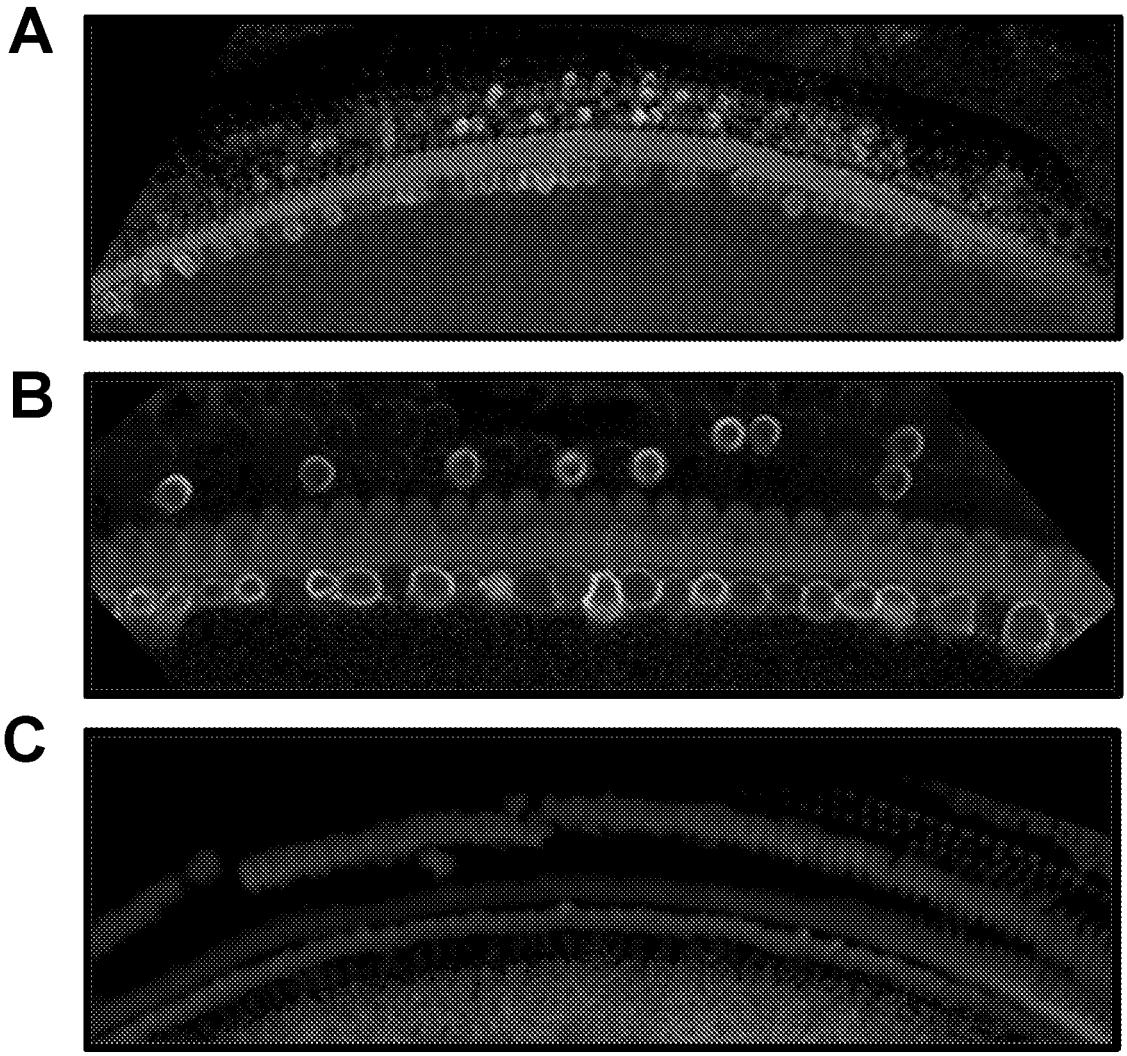


FIG. 28

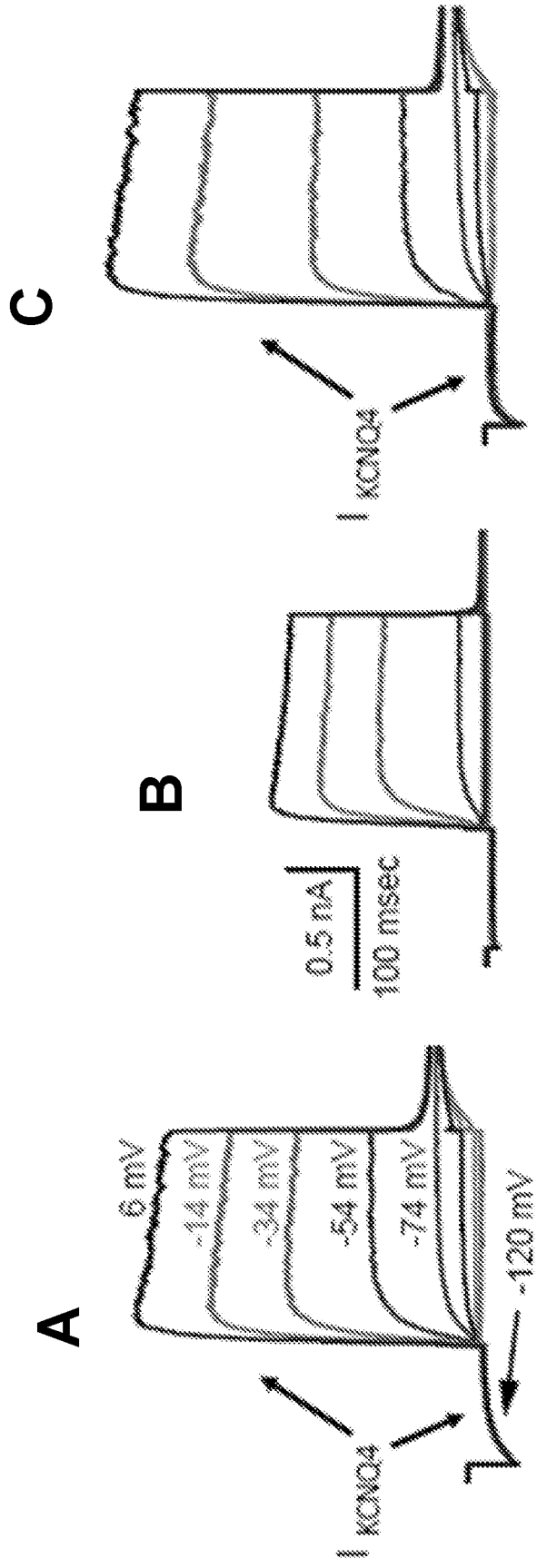

A
6 mV
-14 mV
-34 mV
-54 mV
-74 mV
-120 mV
I KCNQ4
B
0.5 nA
100 msec
C
I KCNQ4

FIG. 29

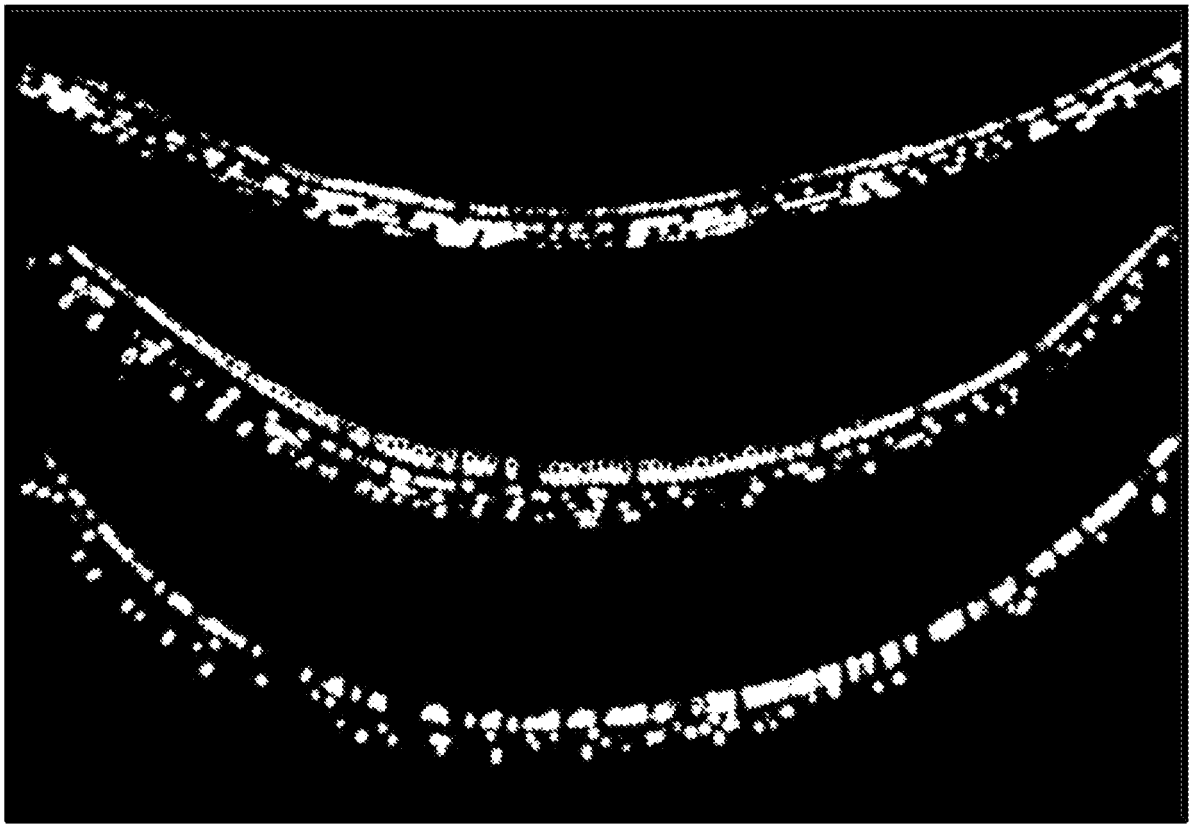


FIG. 30

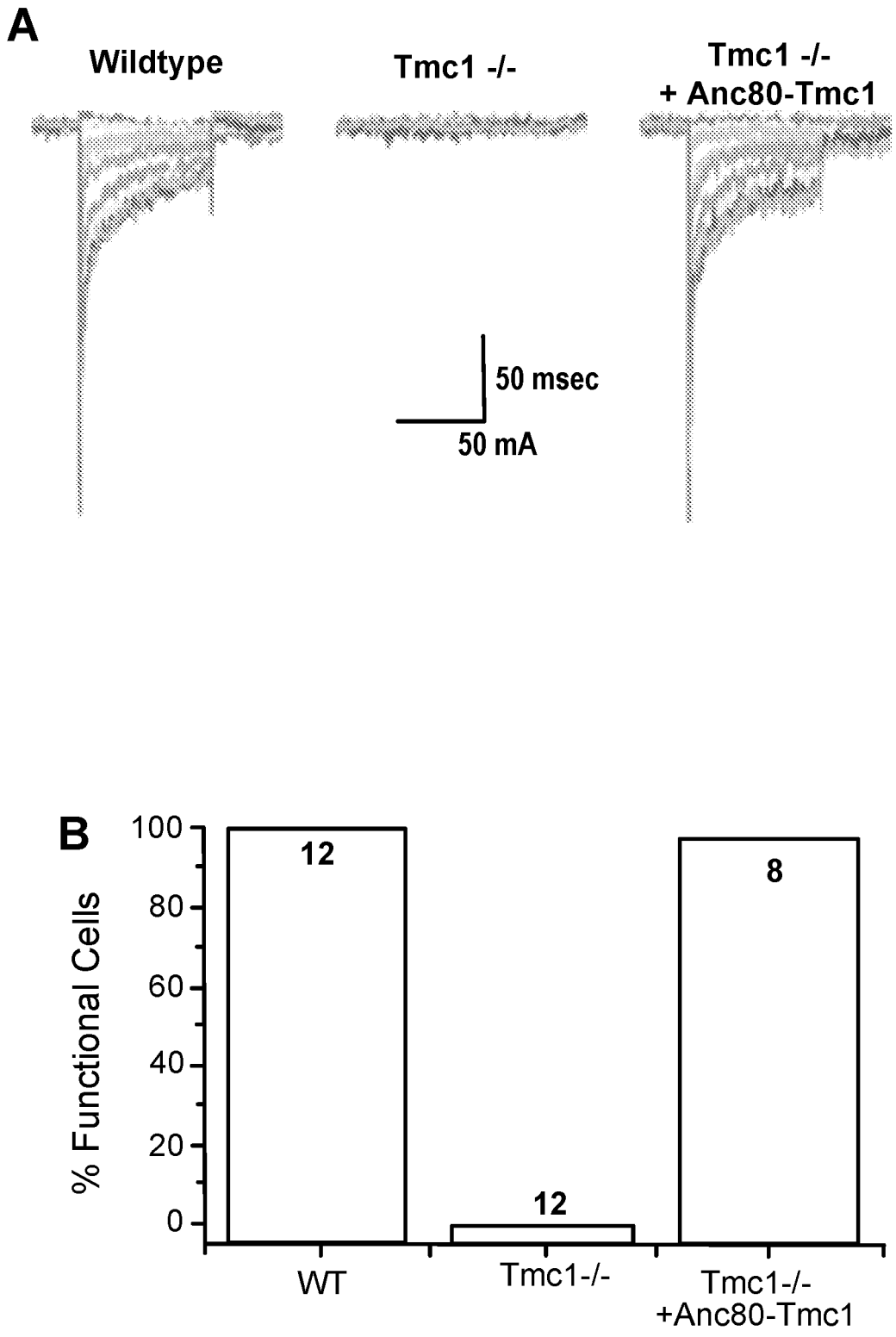

A
Wildtype
Tmc1 -/-
Tmc1 -/-
+ Anc80-Tmc1
50 msec
50 mA
B
% Functional Cells
100
80
60
40
20
0
12
12
8
WT
Tmc1-/-
Tmc1-/-
+Anc80-Tmc1

FIG. 31

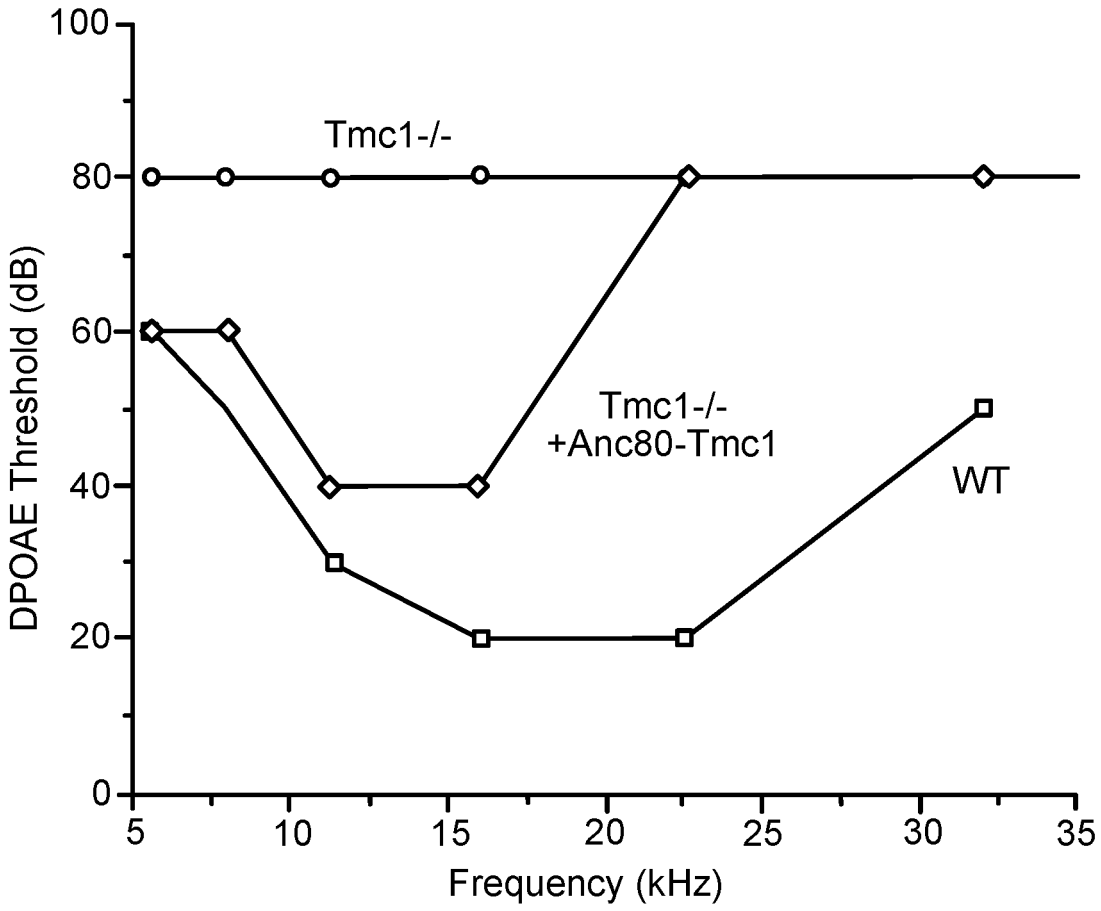

100
80
60
40
20
0
DPOAE Threshold (dB)
Tmc1-/-
Tmc1-/-
+Anc80-Tmc1
WT
5
10
15
20
25
30
35
Frequency (kHz)

FIG. 32