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Supplemental Information

Rescue of auditory function by a single administration of AAV-*TMPRSS3* gene therapy in aged mice of human recessive deafness DFNB8

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Supplemental Figures

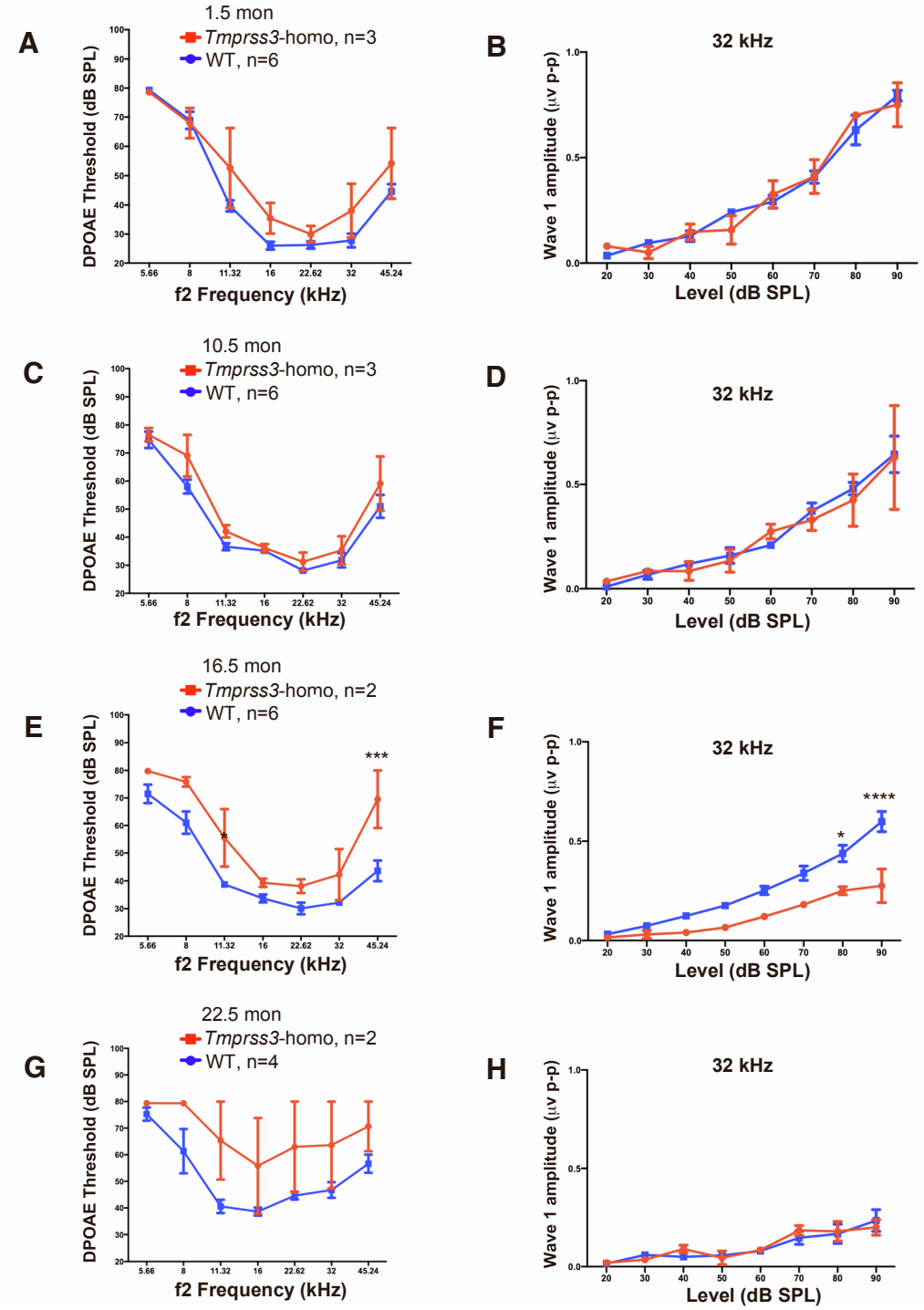

Figure S1. *Tmprss3*$^{A306T/A306T}$ KI mouse model exhibits late-onset progressive hearing loss. **(A-D)** Similar DPOAE thresholds and wave 1 amplitudes in *Tmprss3*$^{A306T/A306T}$ homozygous mice ears (red) and wild-type ears (blue) at 1.5 months **(A, B)** and 10.5 months **(C, D)**, respectively. **(E)** DPOAE thresholds were higher across frequencies in the *Tmprss3*$^{A306T/A306T}$ homozygous ears (red) compared to WT ears at the similar age (blue), an indication of reduced outer hair cell function. **(F)** The wave 1 amplitudes were significantly higher at 32 kHz at 80 and 90 dB in WT ears compared to KI ears at 16.5 months. **(G)** At 22.5 months of age, DPOAE thresholds were higher across frequencies in the *Tmprss3*$^{A306T/A306T}$ homozygous ears (red) compared to WT ears at the similar age (blue), although there was a big variation among the KI ears. **(H)** At 22.5 months, the wave1 amplitudes in WT and the KI ears were similar, which were lower than at 16.5 months, an indication that the age-related reduction in neural activities of WT ears that matched the reduction in the KI ears. Values and error bars reflect mean ± SEM.

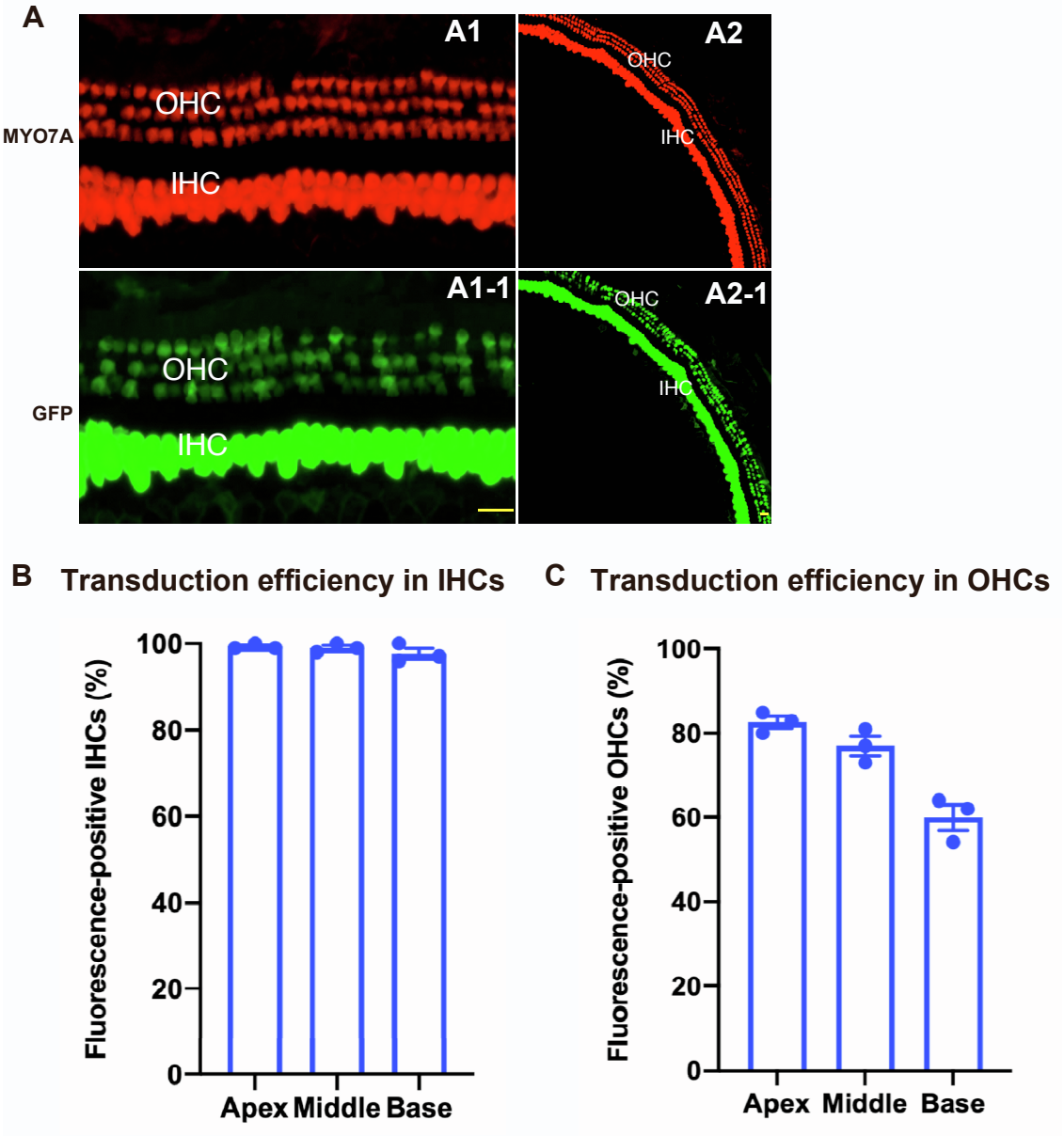
A
A1
A2
OHC
IHC
MYO7A
A1-1
A2-1
GFP
B Transdution efficiency in IHCs
Fluorescence-positive IHCs (%)
100
80
60
40
20
0
Apex Middle Base
C Transdution efficiency in OHCs
Fluorescence-positive OHCs (%)
100
80
60
40
20
0
Apex Middle Base

Figure S2. AAV2 transduction in adult mice cochleae. **(A)** Representative confocal images of apex-middle region of AAV2 transduction in adult mouse cochlea (**A1**, high magnification; **A2**, low magnification). Cochlea was stained with MYO7A (red, **A1** and **A2**), anti-GFP (green, **A1-1** and **A2-1**). Scale bar, 20μm. **(B, C)** Quantification of IHCs **(B)** and OHCs **(C)** AAV2-GFP transduction across different regions of the cochleae (apex, middle, and base). Time of injection: four months of age. Time of imaging: five months of age. Values and error bars reflect mean ± SEM, n=3.

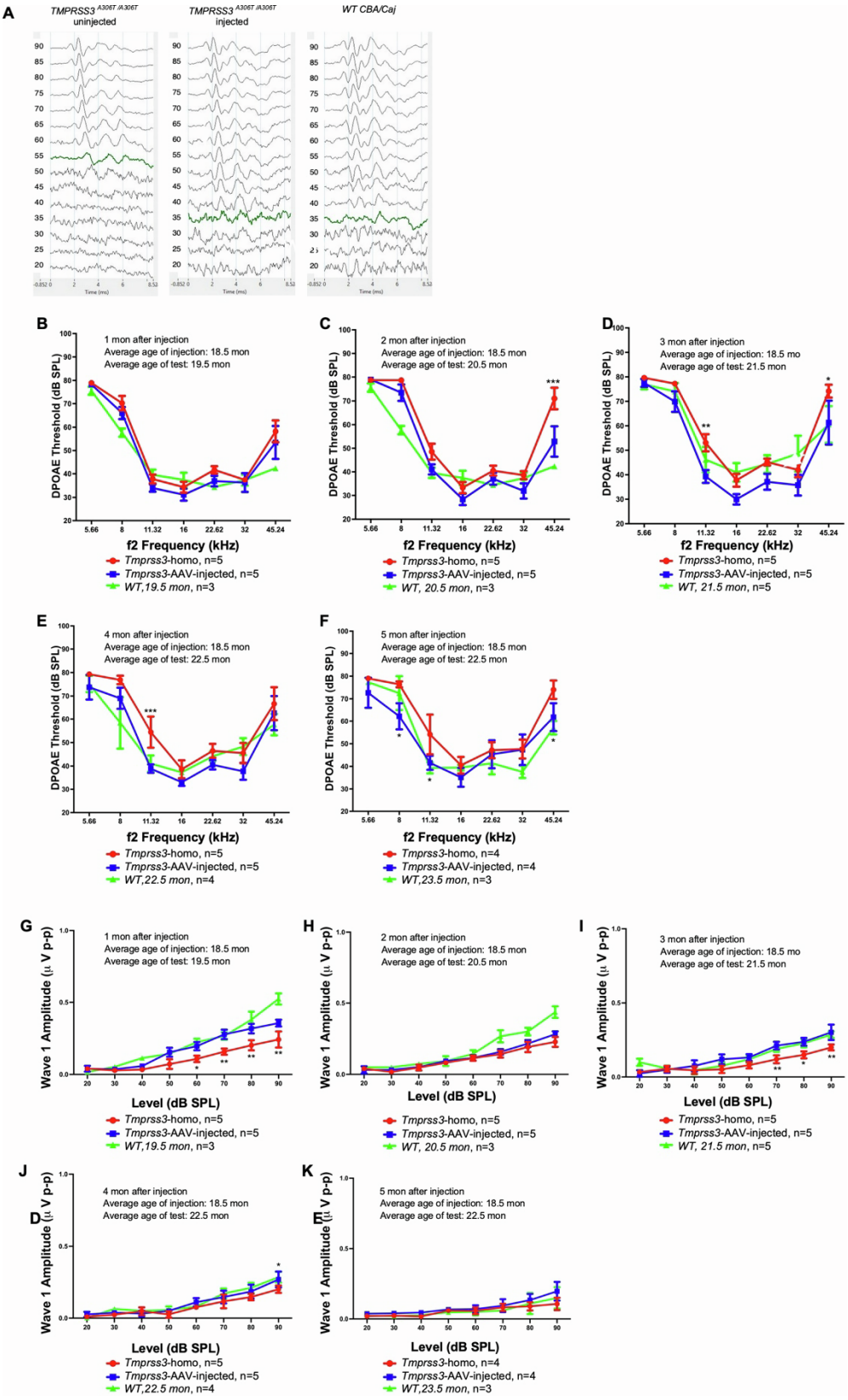
A
TMPRSS3 A306T /A306T uninjected
TMPRSS3 A306T /A306T injected
WT CBA/Caj
B
1 mon after injection
Average age of injection: 18.5 mon
Average age of test: 19.5 mon
C
2 mon after injection
Average age of injection: 18.5 mon
Average age of test: 20.5 mon
D
3 mon after injection
Average age of injection: 18.5 mo
Average age of test: 21.5 mon
DPOAE Threshold (dB SPL)
f2 Frequency (kHz)
Tmprss3-homo, n=5
Tmprss3-AAV-injected, n=5
WT,19.5 mon, n=3
WT, 20.5 mon, n=3
WT, 21.5 mon, n=5
E
4 mon after injection
Average age of injection: 18.5 mon
Average age of test: 22.5 mon
F
5 mon after injection
Average age of injection: 18.5 mon
Average age of test: 22.5 mon
Tmprss3-homo, n=4
Tmprss3-AAV-injected, n=4
WT,22.5 mon, n=4
WT,23.5 mon, n=3
G
1 mon after injection
Average age of injection: 18.5 mon
Average age of test: 19.5 mon
H
2 mon after injection
Average age of injection: 18.5 mon
Average age of test: 20.5 mon
I
3 mon after injection
Average age of injection: 18.5 mo
Average age of test: 21.5 mon
Wave 1 Amplitude (μ V p-p)
Level (dB SPL)
Tmprss3-homo, n=5
Tmprss3-AAV-injected, n=5
WT,19.5 mon, n=3
WT, 20.5 mon, n=3
WT, 21.5 mon, n=5
J
4 mon after injection
Average age of injection: 18.5 mon
Average age of test: 22.5 mon
K
5 mon after injection
Average age of injection: 18.5 mon
Average age of test: 22.5 mon
Tmprss3-homo, n=4
Tmprss3-AAV-injected, n=4
WT,22.5 mon, n=4
WT,23.5 mon, n=3

Figure S3. AAV2-h*TMPRSS3* transduction rescues auditory function in *Tmprss3*$^{A306T/A306T}$ mice. **(A)** Representative ABR waveform recorded from an untreated *Tmprss3*$^{A306T/A306T}$ mouse ear (left), a *Tmprss3*$^{A306T/A306T}$ mouse ear treated with AAV2-h*TMPRSS3* (middle), and a WT ear (right) at 20.5 months using 11.32 kHz tone bursts, respectively. Threshold was determined by the detection of peak 1 and is indicated by green color trace. **(B-F)** DPOAE thresholds in *Tmprss3*$^{A306T/A306T}$ mice treated with AAV2-*hTMPRSS3* ears (blue), untreated *Tmprss3*$^{A306T/A306T}$ ears (red), and WT ears (green) at one month **(B)**, two months **(C)**, three months **(D)**, four months **(E)**, and five months after injection **(F)**, respectively. Generally lower DPOAE thresholds were seen in injected compared to uninjected *Tmprss3*$^{A306T/A306T}$ ears. **(G-K)** Amplitudes of ABR wave 1 at 32kHz in *Tmprss3*$^{A306T/A306T}$ mice treated with AAV2-h*TMPRSS3* ears (blue), untreated *Tmprss3*$^{A306T/A306T}$ ears (red), and WT ears (green) at one month **(G)**, two months **(H)**, three months **(I)**, four months **(J)**, and five months after injection **(K)**, respectively. Wave 1 amplitudes were generally higher in the injected compared to uninjected *Tmprss3*$^{A306T/A306T}$ ears up to four months after injection. By five months after injection (at the age of 23.5 months), wave 1 amplitudes in WT, injected and uninjected KI ears were indistinguishable. Values and error bars reflect mean ± SEM.

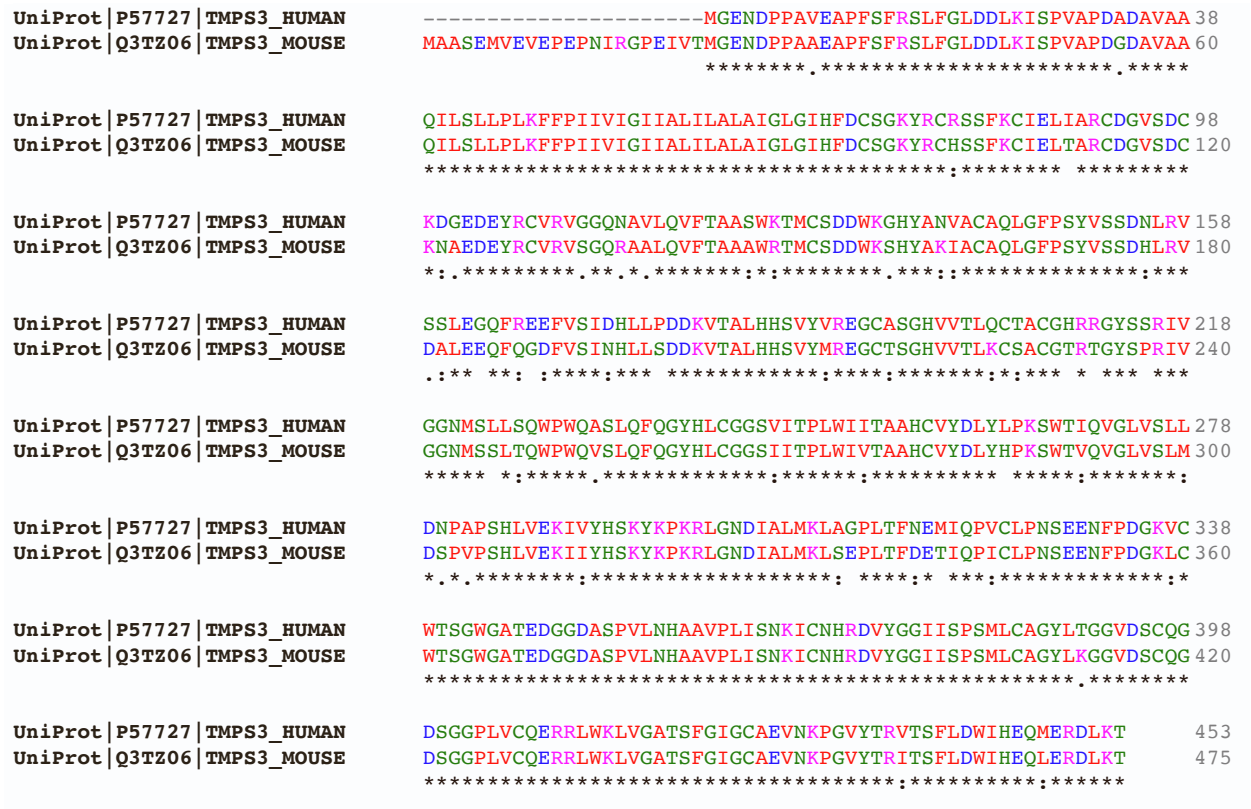
UniProt|P57727|TMPS3_HUMAN ----------MGENDPPAVEAPFSFRSLFGLDDLKISPVAPDADAVAA 38
UniProt|Q3TZ06|TMPS3_MOUSE MAASEMVEVEPEPNIRGPEIVTMGENDPPAAEAPFSFRSLFGLDDLKISPVAPDGDAVAA 60
UniProt|P57727|TMPS3_HUMAN QILSLLPLKFFPIIVIGIIALILALAIGLGIHFDCSGKYRCRSSFKCIELIARCDGVSDC 98
UniProt|Q3TZ06|TMPS3_MOUSE QILSLLPLKFFPIIVIGIIALILALAIGLGIHFDCSGKYRCHSSFKCIELTARCDGVSDC 120
UniProt|P57727|TMPS3_HUMAN KDGEDEYRCVRVGGQNAVLQVFTAASWKTMCSDDWKGHYANVACAQLGFPSYVSSDNLRV 158
UniProt|Q3TZ06|TMPS3_MOUSE KNAEDEYRCVRVSGQRAALQVFTAAAWRTMCSDDWKSHYAKIACAQLGFPSYVSSDHLRV 180
UniProt|P57727|TMPS3_HUMAN SSLEGQFREEFVSIDHLLPDDKVTALHHSVYVREGCASGHVVTLQCTACGHRRGYSSRIV 218
UniProt|Q3TZ06|TMPS3_MOUSE DALEEQFQGDFVSINHLLSDDKVTALHHSVYMREGCTSGHVVTLKCSACGTRTGYSPRIV 240
UniProt|P57727|TMPS3_HUMAN GGNMSLLSQWPWQASLQFQGYHLCGGSVITPLWIITAAHCVYDLYLPKSWTIQVGLVSLL 278
UniProt|Q3TZ06|TMPS3_MOUSE GGNMSSLTQWPWQVSLQFQGYHLCGGSIITPLWIVTAAHCVYDLYHPKSWTVQVGLVSLM 300
UniProt|P57727|TMPS3_HUMAN DNPAPSHLVEKIVYHSKYKPKRLGNDIALMKLAGPLTFNEMIQPVCLPNSEENFPDGKVC 338
UniProt|Q3TZ06|TMPS3_MOUSE DSPVPSHLVEKIIYHSKYKPKRLGNDIALMKLSEPLTFDETIQPICLPNSEENFPDGKLC 360
UniProt|P57727|TMPS3_HUMAN WTSGWGATEDGGDASPVLNHAAVPLISNKICNHRDVYGGIISPSMLCAGYLTGGVDSCQG 398
UniProt|Q3TZ06|TMPS3_MOUSE WTSGWGATEDGGDASPVLNHAAVPLISNKICNHRDVYGGIISPSMLCAGYLKGGVDSCQG 420
UniProt|P57727|TMPS3_HUMAN DSGGPLVCQERRLWKLVGATSFGIGCAEVNKPGVYTRVTSFLDWIHEQMERDLKT 453
UniProt|Q3TZ06|TMPS3_MOUSE DSGGPLVCQERRLWKLVGATSFGIGCAEVNKPGVYTRITSFLDWIHEQLERDLKT 475

Figure S4. Alignment of TMPRSS3 proteins from human and mouse. Clustal Omega (v 1.2.4) alignment of TMPRSS3 proteins from human and mouse listed by their names and UniProt IDs showed that TMPRSS3 sequences are 88% identical between humans and mice. An asterisk (*) denotes identical residues; double dots (:) represent a conserved residue substitution; a single dot (.) shows partial conservation of the residue. Red, small and hydrophobic; blue, acidic; magenta, basic; green, hydroxyl or sulfhydryl or amine or G.

Table S1. The information on the animals studied including the age of injection, the age of hearing tests, and the age of inner ear harvest.