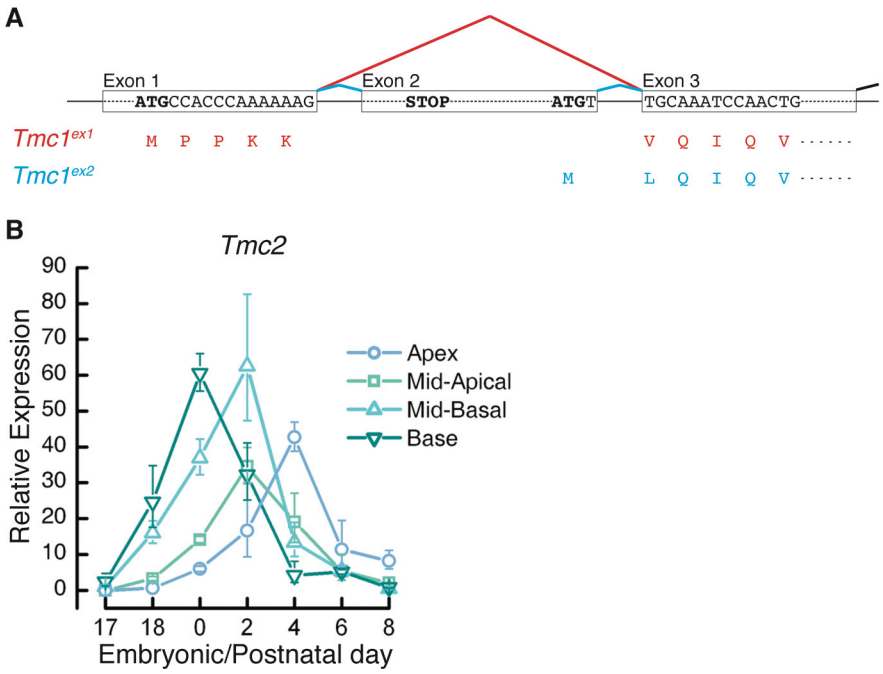
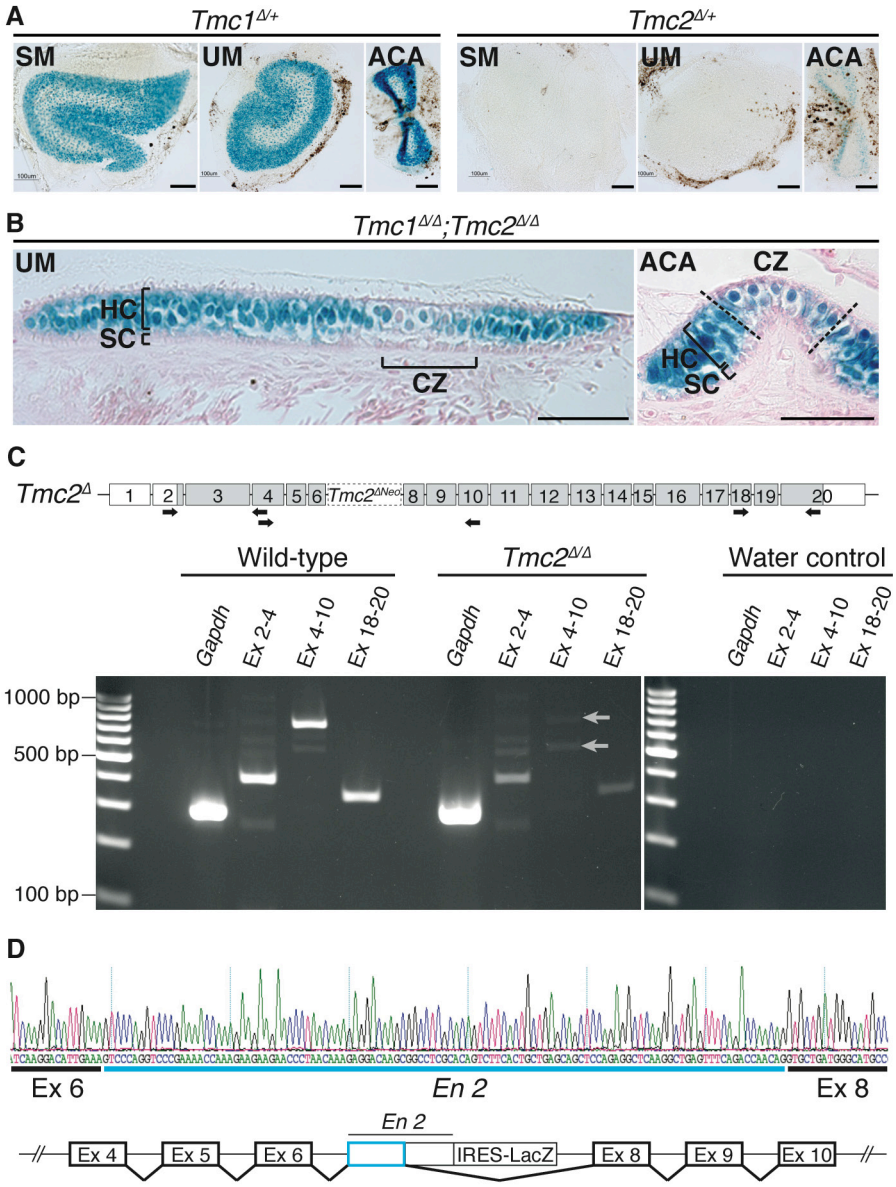
Supplemental Figure 1

Mouse *Tmc1* isoforms and cochlear expression of *Tmc2*. (**A**) *Tmc1*^{ex1} encodes a transcript lacking exon 2 and its translation initiation codon is predicted to be in exon 1. *Tmc1*^{ex2} encodes a transcript including exon 2 and the translation initiation codon is predicted to be in exon 2. (**B**) Quantitative RT-PCR analysis of mouse cochlear RNA shows transient expression of *Tmc2* between embryonic day 17 (E17) and postnatal day 8 (P8) . RNA was harvested from cochleae divided into four equal quarters. Error bars indicate ± s.d.



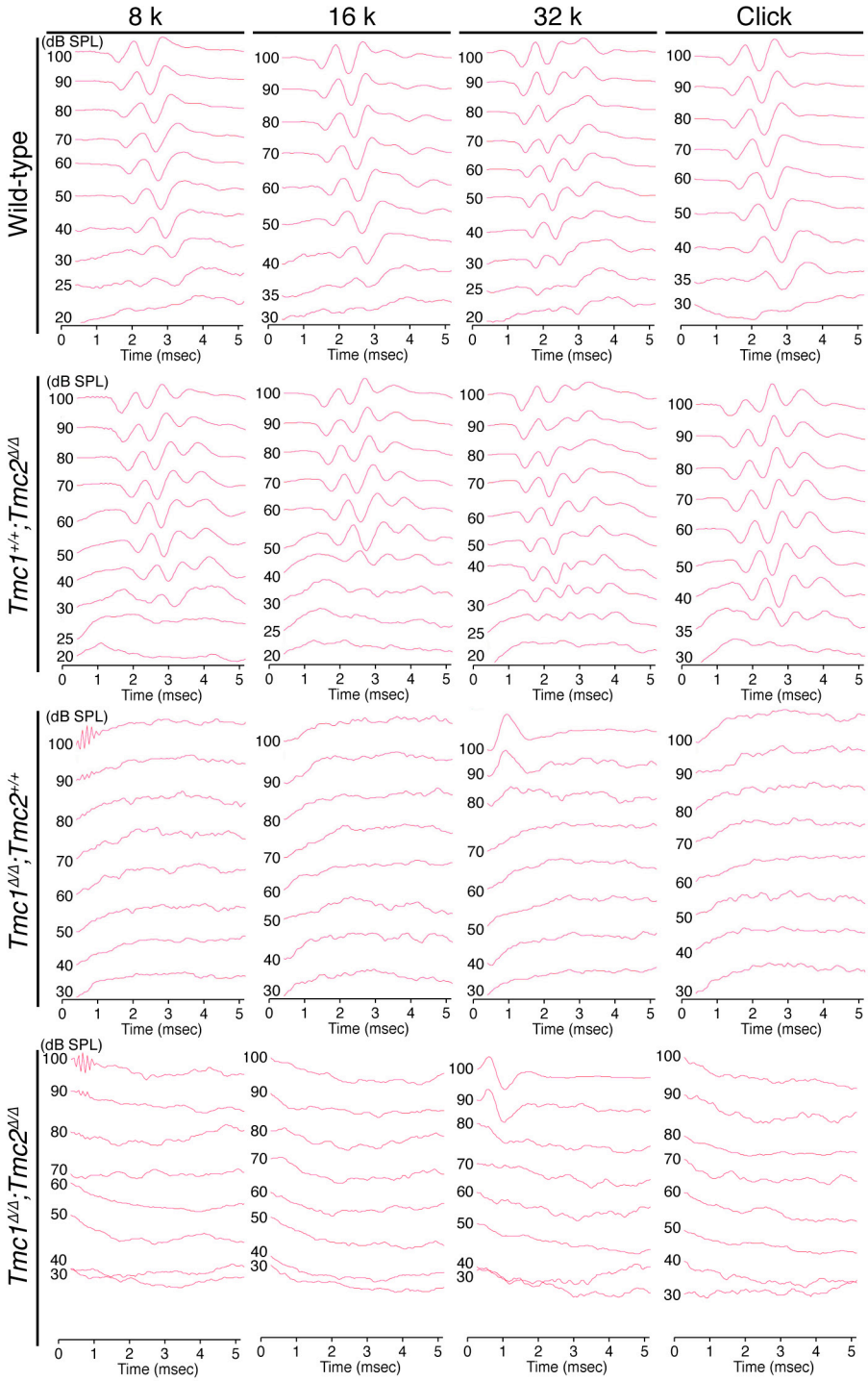
Supplemental Figure 2

Tmc1$^{\Delta}$ and *Tmc2*$^{\Delta}$ lacZ reporter expression and *Tmc2*$^{\Delta}$ transcript. (**A**) X-gal detection of *Tmc1*$^{\Delta}$ and *Tmc2*$^{\Delta}$ lacZ reporter expression. The saccular macula (SM), utricular macula (UM) and anterior crista ampullaris (ACA) are shown for P28 *Tmc1*$^{\Delta/+}$*;Tmc2*$^{+/+}$ and *Tmc1*$^{+/+}$*;Tmc2*$^{\Delta/+}$ mice. No X-gal staining was observed in corresponding samples from C57BL/6J wild-type mice (data not shown). Scale bars: 100 µm. (**B**) P28 *Tmc1*$^{\Delta/\Delta}$*;Tmc2*$^{\Delta/\Delta}$ inner ears were embedded in paraffin, sectioned at 8-µm thickness and stained with eosin and with X-gal. This experiment visualizes collective expression from the lacZ reporter genes integrated at both *Tmc1*$^{\Delta}$ and *Tmc2*$^{\Delta}$. β-galactosidase activity is restricted to hair cells. Subcellular concentration of stain in nuclei is due to a nuclear localization signal in the *lacZ* reporter gene. In the vestibular end organs, X-gal staining intensity in nuclei of hair cells is low in the central zone (CZ; demarcated by dashed lines) compared with the peripheral zone. Nuclei in supporting cell layers are not stained. UM, utricle macula; SM, saccular macula; ACA, anterior crista ampullaris; HC, hair cells; SC, supporting cells. Scale bars: 50 µm. (**C**) RT-PCR analysis confirms the existence of residual *Tmc2*$^{\Delta}$ transcripts. The RT-PCR substrate was 200 ng of total RNA extracted from P3 C57BL/6J wild-type or *Tmc1*$^{+/+}$*;Tmc2*$^{\Delta/\Delta}$ inner ears. One µl of 84 µl of total RT-PCR products were PCR-amplified and separated by agarose gel electrophoresis. Primer information is shown in Supplemental Table 1. (**D**) Alternative (leaky) splicing of the gene trap construct in *Tmc2*$^{\Delta}$. RT-PCR products indicated by arrows in (C) were subcloned for nucleotide sequence analysis. The higher molecular-weight band encodes an alternative transcript with a portion of the mouse *En2* sequence (indicated by the blue line and box) and a frameshift of the downstream *Tmc2* coding sequence in exon 8. The lower molecular-weight band is also detected from wild-type RNA and is an amplification product of the unrelated G protein pathway suppressor 1 gene.


A
Tmc1Δ/+
SM
UM
ACA
Tmc2Δ/+
SM
UM
ACA
B
Tmc1Δ/Δ;Tmc2Δ/Δ
UM
HC
SC
CZ
ACA
CZ
HC
SC
C
Tmc2Δ
1
2
3
4
5
6
Tmc2ΔNeo
8
9
10
11
12
13
14
15
16
17
18
19
20
Wild-type
Tmc2Δ/Δ
Water control
Gapdh
Ex 2-4
Ex 4-10
Ex 18-20
1000 bp
500 bp
100 bp
D
Ex 6
En 2
Ex 8
En 2
Ex 4
Ex 5
Ex 6
IRES-LacZ
Ex 8
Ex 9
Ex 10

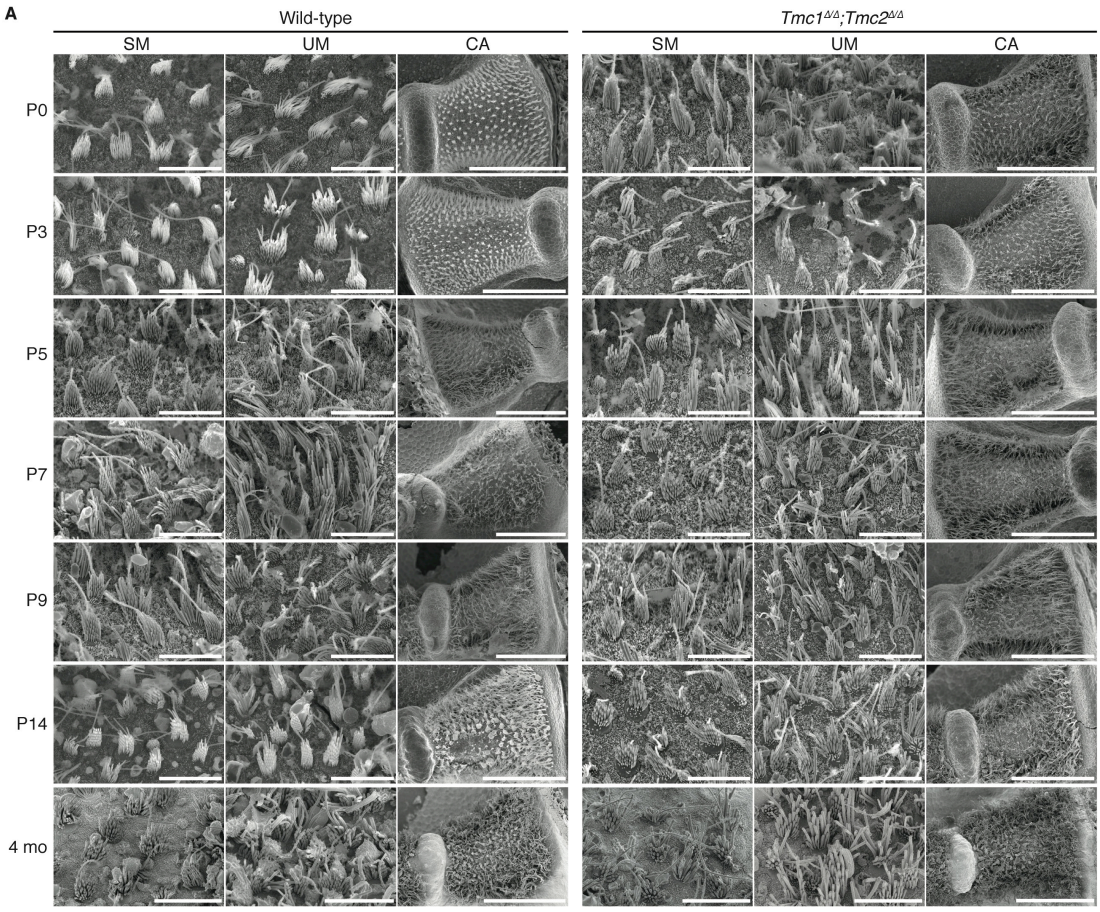
Supplemental Figure 3

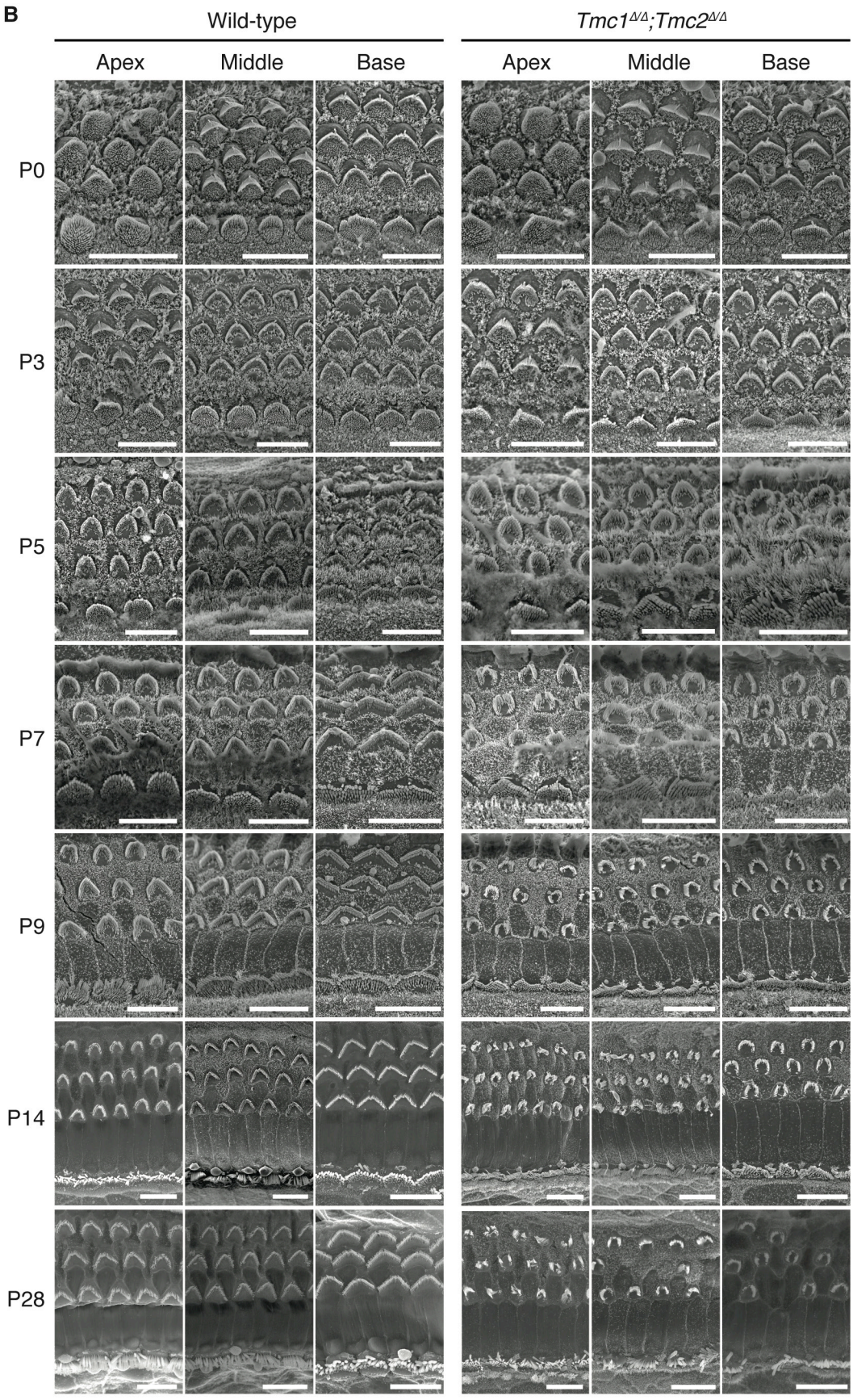
Representative auditory brainstem response waveforms at 5 weeks of age. *Tmc1*$^{\Delta/\Delta}$ mice show profound hearing loss, with no effect of *Tmc2* genotype at any tested frequencies. There is an artifactual waveform within 1.5 msec after high-intensity 8-kHz or 32-kHz tone-burst stimuli.

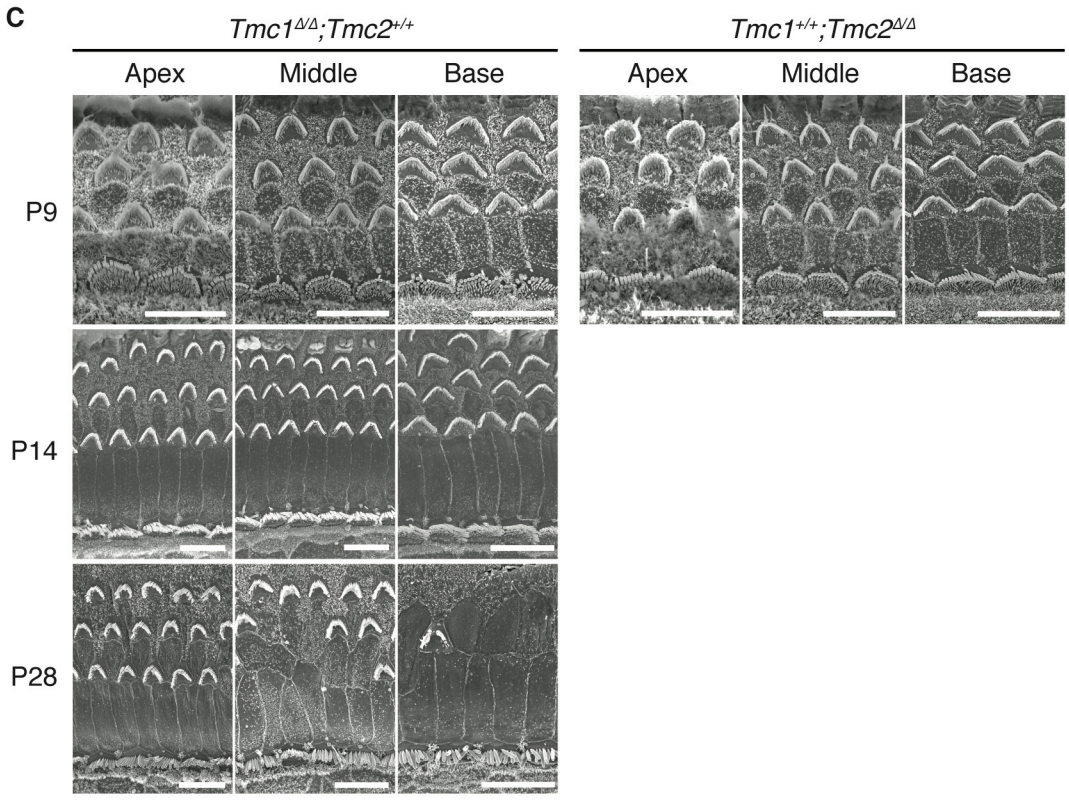


Supplemental Figure 4

Developmental hair bundle phenotypes. (**A**) Scanning electron micrographs of *Tmc1*$^{\Delta/\Delta}$*;Tmc2*$^{\Delta/\Delta}$ hair bundles of saccular macula (SM), utricular macula (UM) and crista ampullaris (CA) appear normal through four months of age (mo). Scale bars: 10 μm (SM, UM); 100 μm (CA). (**B**) Degeneration of *Tmc1*$^{\Delta/\Delta}$*;Tmc2*$^{\Delta/\Delta}$ outer hair cell bundles is first apparent at P5 and P7 in the basal and apical cochlear turns, respectively. (**C**) *Tmc1*$^{\Delta/\Delta}$;*Tmc2*$^{+/+}$ outer hair cell bundles appear normal until approximately P14 and then begin to degenerate. *Tmc1*$^{+/+}$*;Tmc2*$^{\Delta/\Delta}$ outer hair cell bundles appear normal at P9 and were not evaluated at later time points since adult mice with this genotype have hearing that is indistinguishable from that of wild-type controls. Scale bars: 10 μm.

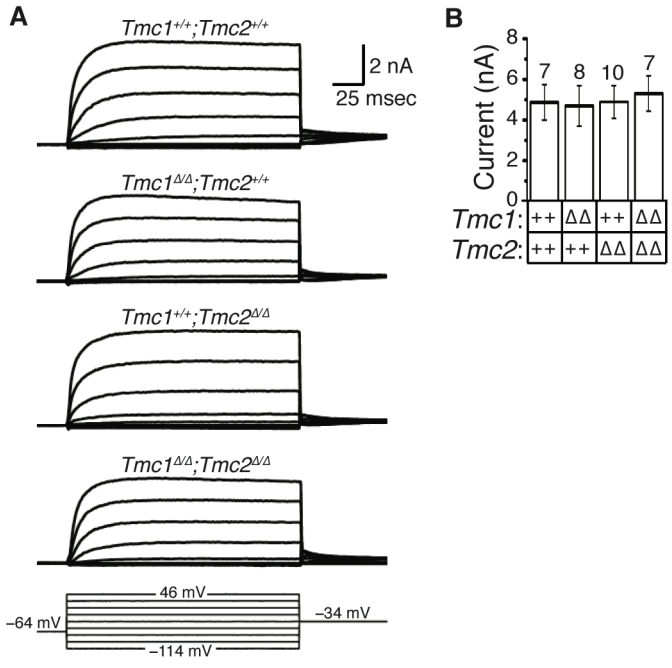



B
Wild-type
Tmc1Δ/Δ;Tmc2Δ/Δ
Apex
Middle
Base
Apex
Middle
Base
P0
P3
P5
P7
P9
P14
P28


C
Tmc1Δ/Δ;Tmc2+/+
Tmc1+/+;Tmc2Δ/Δ
Apex
Middle
Base
Apex
Middle
Base
P9
P14
P28

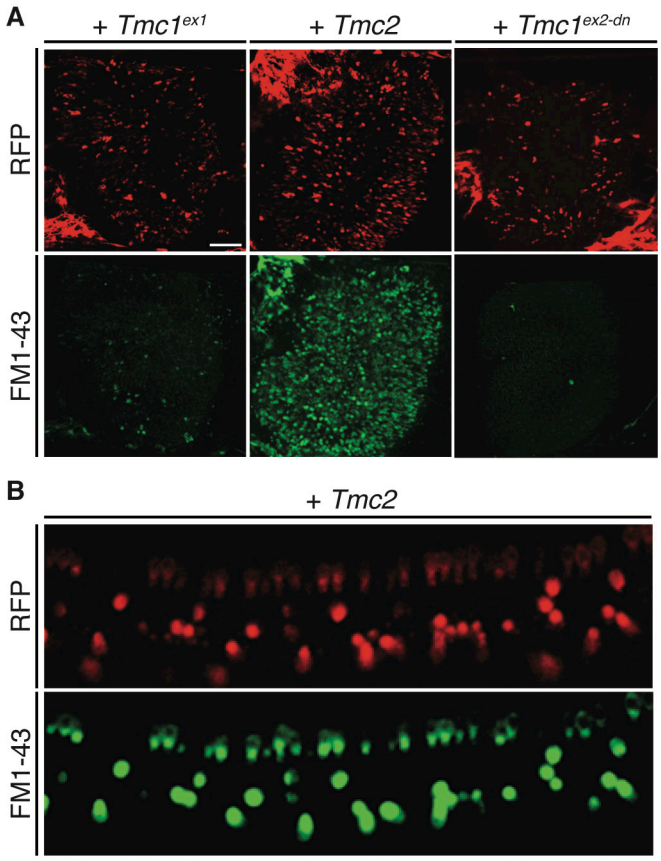
Supplemental Figure 5

Voltage-dependent currents measured from type II hair cells. (**A**) Utricles were harvested from wild-type and mutant mice at P5-P7. Representative families of currents evoked using the voltage-protocol shown at the bottom. There were no significant differences in kinetics, voltage-dependence or amplitude in the outward currents for any of the genotypes examined. (**B**) The bar graph shows the mean maximal current amplitudes evoked by the step to 46 mV. Error bars indicate ± s.d. The number indicates the total number of hair cells examined for each genotype.



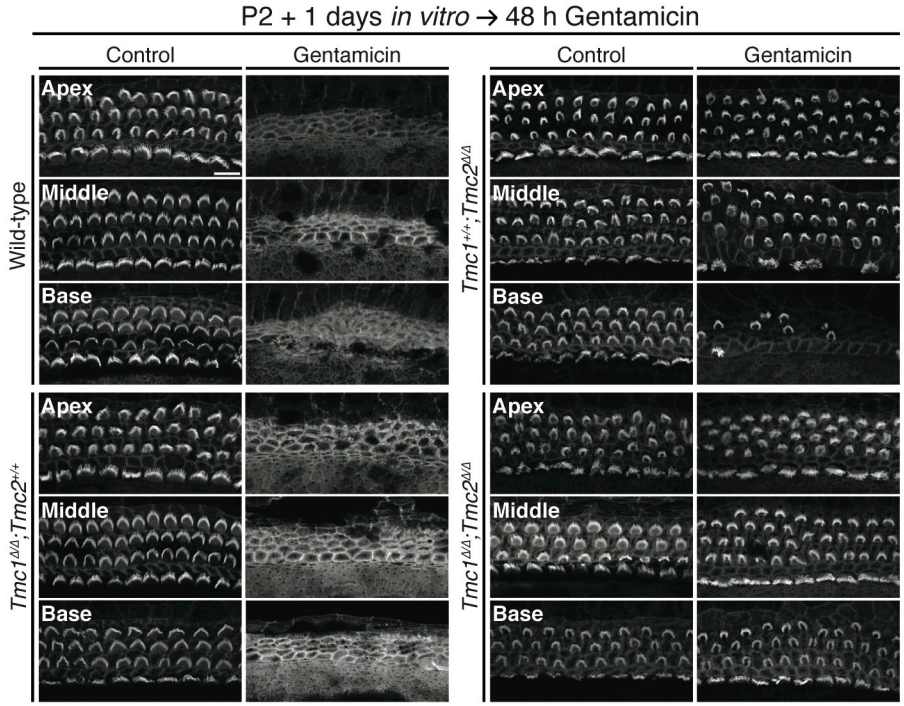
Supplemental Figure 6

Rescue of FM1-43 uptake with *Tmc1* and *Tmc2*. (**A**) Organotypic cultures were generated from utricles excised from $Tmc1^{\Delta/\Delta};Tmc2^{\Delta/\Delta}$ mice at P0 to P2, exposed to adenoviral expression vectors encoding $Tmc1^{ex1}$ (n=2 utricles), *Tmc2* (n=4 utricles), or $Tmc1^{ex2\text{-}dn}$ (n=2 utricles), and maintained in culture for 2-4 days. Red fluorescence from the RFP transfection marker was imaged prior to exposure to FM1-43 (green) to avoid red fluorescence from FM1-43. Some non-hair cells transfected with $Tmc1^{ex1}$ or *Tmc2* do not take up FM1-43. Conversely, there is FM1-43 uptake in a few cells of cultures transfected with $Tmc1^{ex1}$ or *Tmc2* and low or undetectable levels of RFP. No FM1-43 uptake was detected in utricles transfected with $Tmc1^{ex2\text{-}dn}$. Scale bars: 100 μm. (**B**) Same experiments were done on cochlear cultures (n=4 cochleae). $Tmc1^{\Delta/\Delta};Tmc2^{\Delta/\Delta}$ cochlea hair cells that were RFP-positive, indicating transfection with *Tmc2*, had robust uptake of FM1-43.



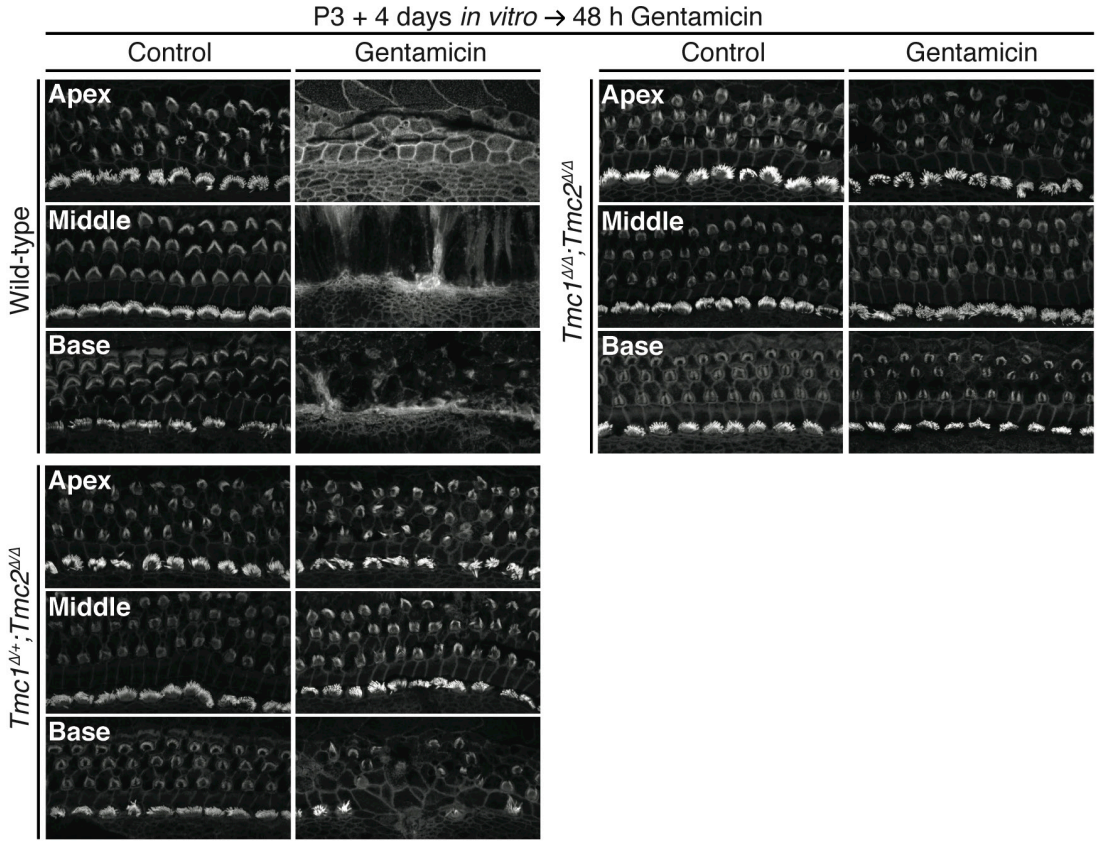
Supplemental Figure 7

Tmc1$^{\Delta}$ and *Tmc2*$^{\Delta}$ cochlear hair cell uptake of gentamicin. Cochlear explant cultures from mice at P2 were incubated overnight followed by a 48-hour incubation in culture medium with 1 mM gentamicin. Wild-type cochlear hair bundles were completely eliminated, while *Tmc1*$^{\Delta/\Delta}$;*Tmc2*$^{\Delta/\Delta}$ hair cells retained intact hair bundles. *Tmc1*$^{+/+}$;*Tmc2*$^{\Delta/\Delta}$ hair cells show a loss of most hair bundles in the base and a small proportion of hair bundles in the middle turn, while apical hair bundles remained intact. Scale bar: 10 µm.



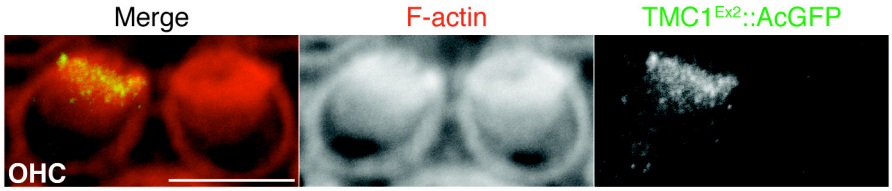
Supplemental Figure 8

Tmc1$^{\Delta}$ and *Tmc2*$^{\Delta}$ hair cell uptake of gentamicin at P3 + 4 days in vitro. Cochlear cultures were explanted at P3, incubated in culture medium for four days, followed by 48 h in 1 mM gentamicin. Wild-type hair bundles are completely eliminated, whereas *Tmc1*$^{\Delta/\Delta}$*;Tmc2*$^{\Delta/\Delta}$ hair bundles remain intact. *Tmc1*$^{\Delta/+}$*;Tmc2*$^{\Delta/\Delta}$ hair bundles show intermediate susceptibility to gentamicin.



Supplemental Figure 9

Localization of exogenously expressed TMC1^{Ex2}::AcGFP. Mouse outer hair cells were transfected with *CMV-Tmc1^{ex2}::GFP* at E16.5 + 1 day in vitro and incubated for 48h. Gamma settings for red and green channels are adjusted equally throughout entire images using Adobe Photoshop CS5.



Supplemental Table 1

Supplemental Table 1

Primer(s) for	Name	Sequence		Size (bp)
		Forward	Reverse	
Tmc1 WT	*Tmc1* exon 8	5'- GATTACTTTGGAGGATCACTAAGAGAA -3'	5'- TGAAGGTTAAGCTTGTTGTAAATCCTA -3'	360
Tmc1 WT	*Tmc1* exon 9	5'- GATGAACATTTTGGTACCCTTCTACTA -3'	5'- CACACTTTGACACGTACAGTCTTTTAT -3'	557
Tmc1$^{\Delta}$	*Tmc1* KO5'	5'- TCTGAGCTTCTTAATCTCTGGTAGAAC -3'	5'- ATACAGTCCTCTTCACATCCATGCT -3'	408
Tmc2 WT	*Tmc2* exon 7	5'- CGGTTCTTCTGTGGCATCTTACTT -3'	5'- ACCAGGCAATTGACATGAATA -3'	401
Tmc2$^{\Delta}$	*Tmc2* KO5'	5'- CTGCCTTCTGGTTAGATCACTTCA -3'	5'- GTGTTTTAAGTGTACCCACGGTCA -3'	625
Tmc2$^{\Delta}$	*Tmc2* KO3'	5'- ATTACCAGTTGGTCTGGTGTCAAAAATAAT -3'	5'- AGAGTATAACCAATCAGATGTGAGACATGC -3'	939
Tmc2	*Tmc2* exon 2-4	5'- GTGAGGGCCTTGAAACTCTG -3'	5'- AGACACTGGAGCCCAAGGA -3'	400
Tmc2	*Tmc2* exon 18-20	5'- GCTGCGAAAGAAGATCCAAG -3'	5'- TGGGTTCTCTTTCCAGAAGGT -3'	361
Tmc2	*Tmc2* exon 4-10	5'- TGGCTCAGATCCTGGAACA -3'	5'- AGCTGGTGAACATCTTGAAGC -3'	699
Tmc2	*Tmc2* exon 5-9	5'- GCAAGGGCAAACACCTCTAC -3'	5'- TCAAGCTGTAGCCAAACACG -3'	459
Tmc2	*Tmc2* exon 6-8	5'- CCCTGGGAAATGAAGATCAA -3'	5'- CCTCAAAATCCCAAAGGACA -3'	238

PCR condition; 94°C, 2 min, 32 cycles of 94°C, 20 sec, 57°C, 20 sec, 72°C, 45 sec, followed by 72°C, 3 min. WT, wild-type