

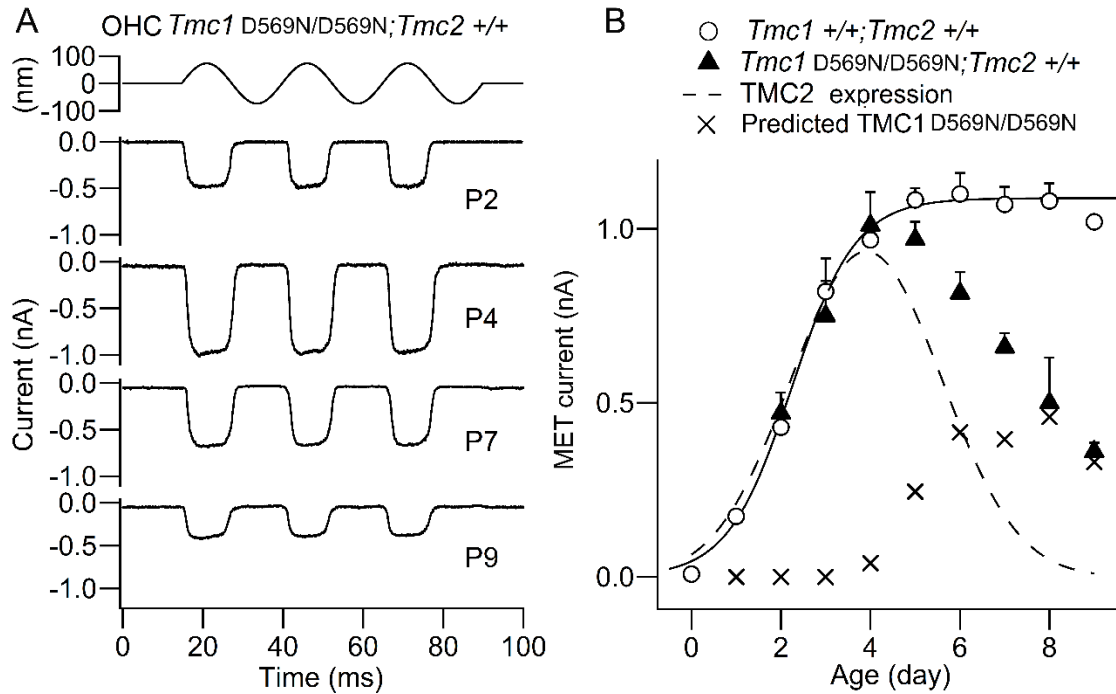
**Supplementary Fig. S1**

GACAAACCACTTGTTTGGGTGCATTAGTTCGAGATGCTAACCCAGTGTATGTATCTT  
CTCTAGCCTTCAT**ACACAGAGTTCAATATCAG**TGGCAACGTCCTCGCTCTGATCTT  
CAACCAAGGCATGATCTGGTATGTCAGCTGTTGGATACTTGAGCATTATGGAG

Single-stranded oligodeoxynucleotide donor sequence to generate *Tmc1 D569N*.

The guide RNA recognition site is shown in bold, and the desired mutation is underlined.

**Supplementary Fig. S2**



**A.** Examples of peak MET currents in response to sinusoidal deflection of the hair bundle at postnatal days P2, P4, P7 and P9 in *Tmc1* D569N/D569N; *Tmc2*+/+ mice. **B.** Plots of the developmental time course of the OHC mean MET current ( $\pm$  SD) in wild type control (open circles), and D569N mutant (filled triangles). The expected developmental expression of TMC2 (dashed line) was calculated as the mean of the two values in Fig. 1e of reference (13); it was subtracted from the *Tmc1* D569N/D569N; *Tmc2*+/+ mutant (filled triangles) to yield the expression of the TMC1 D569N mutant channels alone (crosses). Three to nine recordings were used to generate each experimental point. Currents measured at -84 mV holding potential.