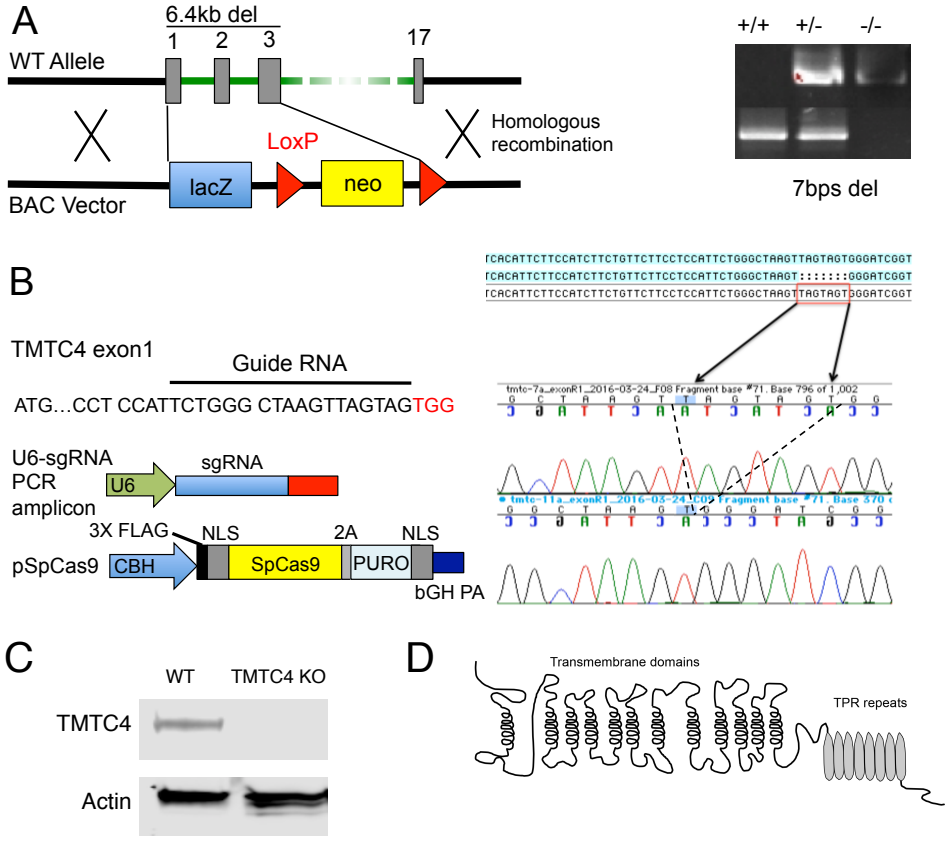
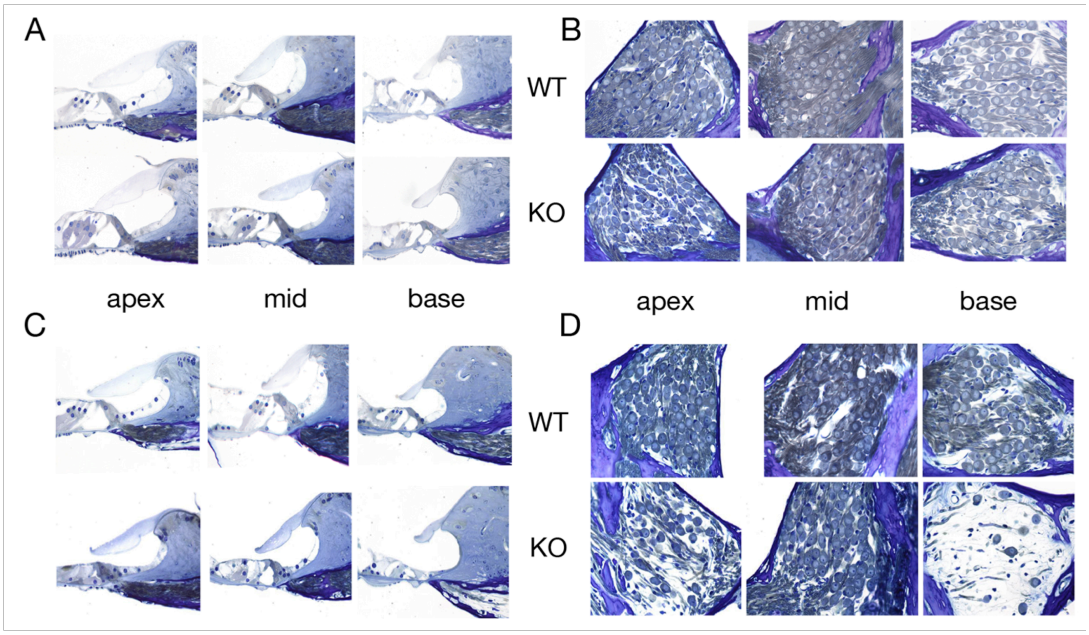
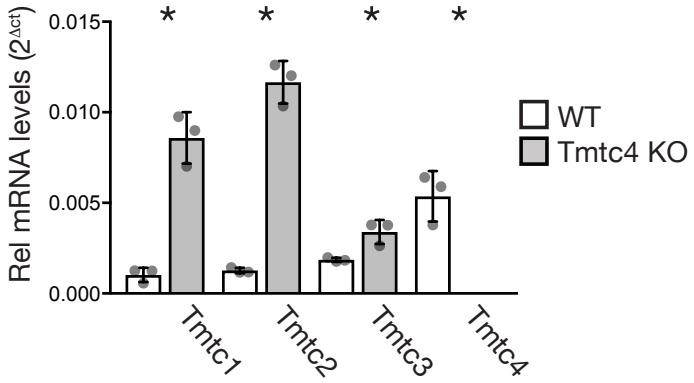


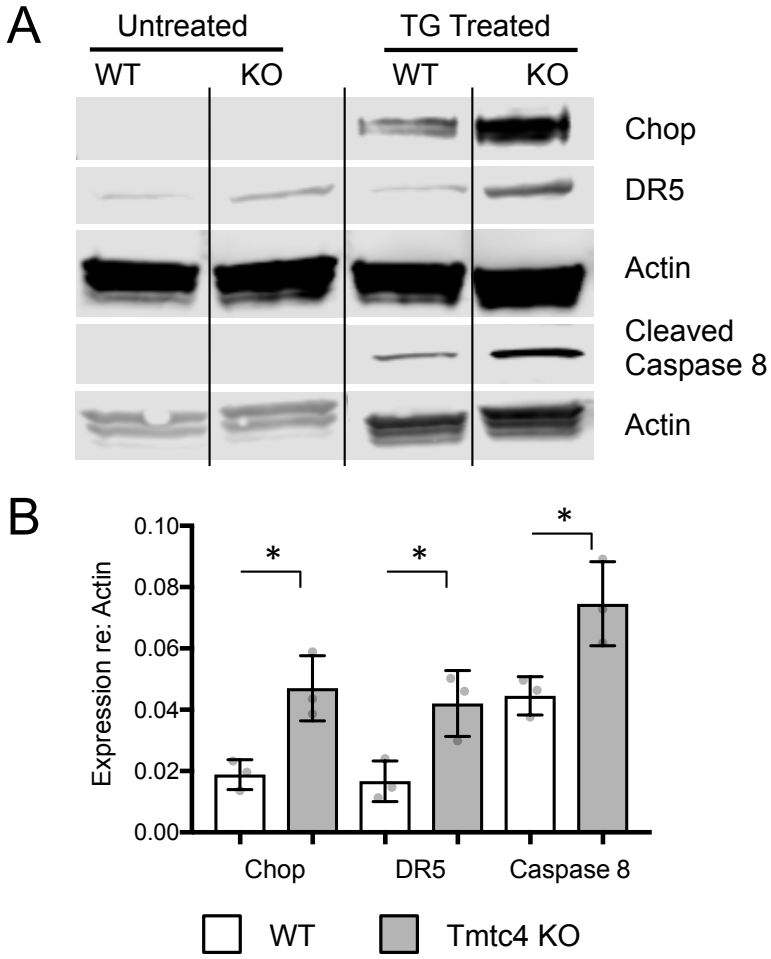

**Supplementary Figure 1.** Constructs and strategies for genetic manipulation of Tmtc4. **A.** Strategy for homologous recombination at the mouse Tmtc4 allele, removing exons 1-3, replaced with the gene for beta-galactosidase and the neomycin gene flanked by loxP sites. The gel displays the WT and KO alleles (in WT/Het/KO mice) as identified by qPCR. **B.** The constructs and approach using CRISPR to generate a bi-allelic deletion of a seven-base-pair region in exon 1 of Tmtc4 in HEK cells. **C.** Western blot of ER fractions from WT and CRISPR-deleted *Tmtc4* KO HEK cells demonstrates effective disruption of endogenous *Tmtc4* expression. Image is representative of 3 experiments. **D.** Predicted topology of TMTC4 demonstrates 10 putative transmembrane domains and eight TPR (tetratricopeptide repeat) domains.



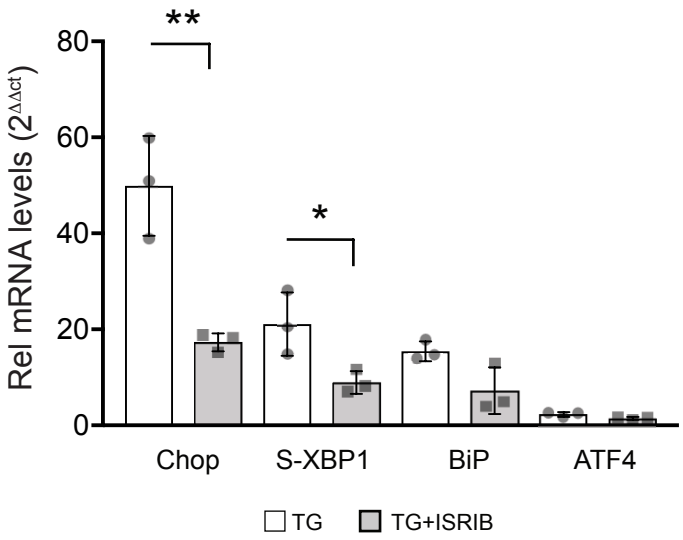

**Supplementary Figure 2**. Progressive degeneration in the cochlea and spiral ganglion of Tmtc4 KO mice. Histologic sections of cochleae (**A**) and spiral ganglia (**B**) from P30 WT (top) and Tmtc4 KO (bottom) mice demonstrate degeneration of the organ of Corti in all cochlear turns with preservation of the spiral ganglion. Middle panels from **A** are identical to those shown as Figure 2C (top row) and 2F (bottom row). When examined again at 4 months of age, cochlear degeneration is more severe (**C**), and spiral ganglion neuron degeneration is observed (**D**). Images are representative of data from 3 WT and 3 Tmtc4 KO mice experiments.




**Supplementary Figure 3.** Expression of mRNA for Tmtc1, Tmtc2, Tmtc3, and Tmtc4 (relative to GAPDH by the $2^{\Delta ct}$ method) was measured in Tmtc4 KO and WT mouse cochleae. Tmtc4 KO mice exhibited significant upregulation of all 3 Tmtc4 isoforms (N=3 mice of each genotype (WT and KO)). Data represent means and SE. * $p < 0.01$ by one-way ANOVA followed by Tukey multiple comparisons test.




**Supplementary Figure 4.** Increased susceptibility of Tmtc4 KO cells to UPR activation and downstream apoptosis. **A.** WT and Tmtc4 KO cultured fibroblasts were treated with 1 $\mu$ M thapsigargin, or untreated, for 24 hours before harvesting. Whole cell lysates were stained with antibodies against Chop, DR5, cleaved Caspase 8, and actin. Actin control was shared between Chop and DR5, and separately run for Caspase 8. Images are representative of 3 experiments. **B.** The levels of TG-induced Chop, DR5, and cleaved Caspase 8 expression were normalized to actin and compared between WT and Tmtc4 KO fibroblasts (N=3 for each condition). Data represent means and SD. * $p < 0.05$ by one-way ANOVA followed by Tukey multiple comparisons test.




**Supplementary Figure 5**. Attenuation of ER stress with the eIF2B activator (and indirect ATF4 inhibitor) ISRIB **A.** Wild-type fibroblasts were treated with 1 μM thapsigargin (TG), alone or with 0.2 μM ISRIB. mRNA expression of Chop, S-XBP1, BiP, and ATF4 upon TG-induced ER stress was measured relative to expression of GAPDH and uninduced fibroblasts by the $2^{\Delta\Delta ct}$ method (N = 3 for each condition). Data represent means and SD. * $p < 0.05$; ** $p < 0.001$ by one-way ANOVA followed by Tukey multiple comparisons test.