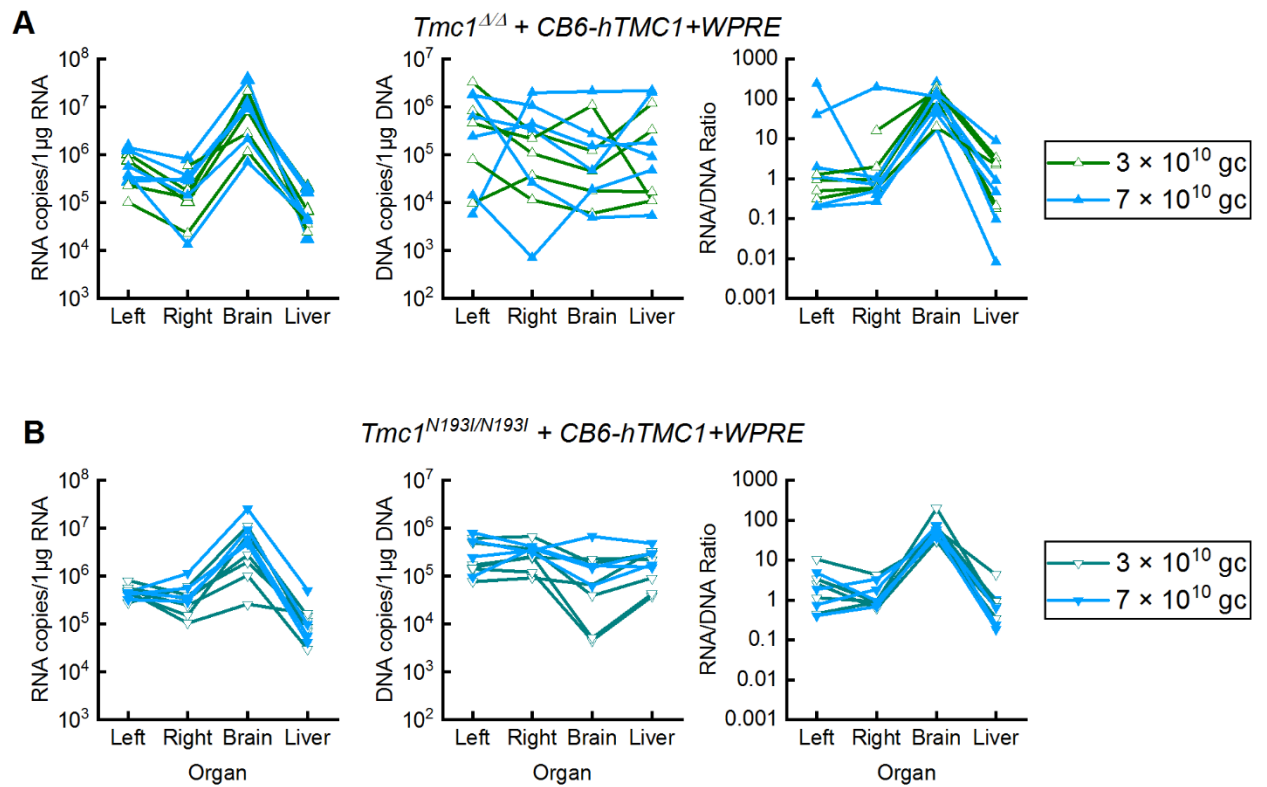


**Figure S1.** Wild-type mice injected with AAV2/9-PHP.B-*CB6-hTMC1-WPRE* have normal hearing. (A) ABRs (above) and DPOAEs (below) as a function of frequency of *Tmc1<sup>+/+</sup>* mice injected with the *CB6-hTMC1-WPRE* construct ( $3 \times 10^{10}$  gc) at 4 (left), 8 (center) and 12 weeks (right) of age. Mean  $\pm$  S.E.M. thresholds are shown in bold traces and individual recordings are shown as lighter traces. Dashed lines indicate mean ABR or DPOAE thresholds for uninjected *Tmc1<sup>+/+</sup>* mice. (B) Mean  $\pm$  S.E.M. ABR (above) or DPOAE (below) thresholds at each individual timepoint are shown in a single plot. (C) Representative confocal images from middlecochlear sections of *Tmc1<sup>+/+</sup>* mice injected with *CMV-hTMC1* ( $3 \times 10^{10}$  gc) from 12-week-old mice immunostained against myosin 7a. Scale bars: upper panels (10 $\times$ ) 200  $\mu$ m; lower panels (63 $\times$ ) 15  $\mu$ m. (D) Mean  $\pm$  S.E.M hair cell counts per 100  $\mu$ m sections for IHCs (left) and OHCs (right) from untreated (white circles) and injected *Tmc1<sup>+/+</sup>* (purple squares) mice. Individual samples are included in the scatterplots.



**Figure S2.** Viral nucleic acid biodistribution profile in target and off-target mouse tissue. Viral RNA (left) or DNA (center) concentration (copies per 1 μg of DNA/RNA) in (A) *Tmc1<sup>Δ/Δ</sup>* or (B) *Tmc1<sup>N193I/N193I</sup>* mice injected with AAV9-PHP.B-CB6-hTMC1+WPPE in the left (injected) or right (contralateral) inner ears, the brain and liver. RNA to DNA ratio (RNA/DNA) is shown (right). Mice injected with the  $3 \times 10^{10}$  gc dose are shown using green triangles and those injected with  $7 \times 10^{10}$  gc dose are shown using light blue filled triangles. Measurements from the same animal are linked by connecting lines.

**Tables S1-S6.** Values and statistics for data presented in Figures 1-5 and Supp. Figure S1, respectively.