



Extended Data Figure 7 | Hearing preservation following treatment with additional Tmc1-mut sgRNAs other than Tmc1-mut3. **a**, Mean ABR thresholds were significantly reduced at three frequencies in ears injected with Cas9–Tmc1-mut1–lipid compared to uninjected *Tmc1^{Bth/+}* ears after four weeks. **b**, DPOAE thresholds were elevated in the same group of inner ears after Cas9–Tmc1-mut1 injection as in **a** after four weeks. **c**, Mean ABR thresholds were significantly reduced at five frequencies in ears injected with Cas9–Tmc1-mut2–lipid compared to uninjected *Tmc1^{Bth/+}* ears after four weeks. **d**, DPOAE thresholds were elevated in the same group of inner ears after Cas9–Tmc1-mut2 injection as in **c** after four weeks. **e**, Mean ABR thresholds were significantly reduced at three frequencies in ears injected with Cas9–Tmc1-mut4–lipid compared to uninjected *Tmc1^{Bth/+}* ears after four weeks. **f**, DPOAE thresholds were elevated in the same group of inner ears after Cas9–Tmc1-mut4–lipid injection as in **e** after four weeks. **g**, Significantly stronger wave 1 amplitudes were detected in ears injected with each of the Cas9–Tmc1-mut–lipid complexes shown at 16 kHz (80 and 90 dB SPL). Individual

values ($n = 8, 13$, or 18) are shown; horizontal bars represent mean values. **h**, Eight weeks after Cas9–Tmc1-mut1–lipid injection into *Tmc1^{Bth/+}* ears, mean ABR thresholds were significantly reduced at five frequencies compared to uninjected *Tmc1^{Bth/+}* ears, which showed ABR thresholds >80 dB at all frequencies after eight weeks. Mean ABR thresholds of untreated wild-type (WT) C3H mice of eight weeks of age are shown in purple. Red arrows indicate no ABR response at the highest SPL level of 90 dB. **i**, DPOAE thresholds were significantly elevated at two frequencies (8 and 11 kHz) in the same group of inner ears after Cas9–Tmc1-mut1 injection as in **h** after eight weeks. Mean DPOAE thresholds of untreated wild-type C3H mice of eight weeks of age are shown in purple. Statistical analysis of ABR and DPOAE thresholds and wave 1 amplitudes was performed by two-way ANOVA with Bonferroni correction for multiple comparisons: $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, $****P < 0.0001$. Values and error bars reflect mean \pm s.e.m. Among the different frequencies assayed, the number of ears tested (n) varies within the range shown (Supplementary Table 2).