Extended Data Figure 4 | Cas9–Tmc1-mut3–lipid injection reduces hearing loss, improves acoustic startle response, and preserves stereocilia in *Tmc1*^{Bth/+} mice. a, Phalloidin labelling showed the preservation of stereocilia of IHCs in an ear eight weeks after injection with Cas9–Tmc1-mut3 sgRNA at three frequency locations indicated, whereas the uninjected contralateral inner ear of the same mouse showed severe degeneration of stereocilia at locations corresponding to 16 and 32 kHz. The boxes indicate the stereocilia, which are shown at the bottom of each image at higher magnification. Scale bars, 10 μm. Similar results were observed in other injected ears that were immunolabelled (*n* = 5). **b**, Representative ABR waveforms showing reduced threshold (red traces) at 16 kHz in a Cas9–Tmc1-mut3–lipid-injected *Tmc1*^{Bth/+} ear (left) compared to the uninjected contralateral ear (right) of the same mouse after four weeks. **c**, Eight weeks after Cas9–Tmc1-mut3 injection into *Tmc1*^{Bth/+} ears (blue), mean ABR thresholds were significantly reduced at

three frequencies. Uninjected *Tmc1*^{Bth/+} ears (red) showed ABR thresholds >85 dB at all frequencies after eight weeks. ABR thresholds from wild-type C3H mice are shown in green. **d**, ABR wave 1 amplitudes following 90 dB SPL stimulation at 16 kHz were greater in injected *Tmc1*^{Bth/+} ears than in uninjected ears eight weeks after treatment. Individual values (*n* = 15 or 20 for uninjected, and 24 for injected) are shown; horizontal bars represent mean values. **e**, Startle responses at 16 kHz in individual Cas9–Tmc1-mut3 sgRNA-injected mice (blue) were significantly stronger (*P* < 0.001) than in uninjected mice (red) eight weeks after treatment. Among the different frequencies assayed, the number of ears tested (*n*) varies within the range shown (Supplementary Table 2). Statistical analyses of ABR thresholds, amplitudes, and startle responses were performed by two-way ANOVA with Bonferroni correction for multiple comparisons: *P < 0.05, **P < 0.01, ****P < 0.0001. Values and error bars reflect mean ± s.e.m.