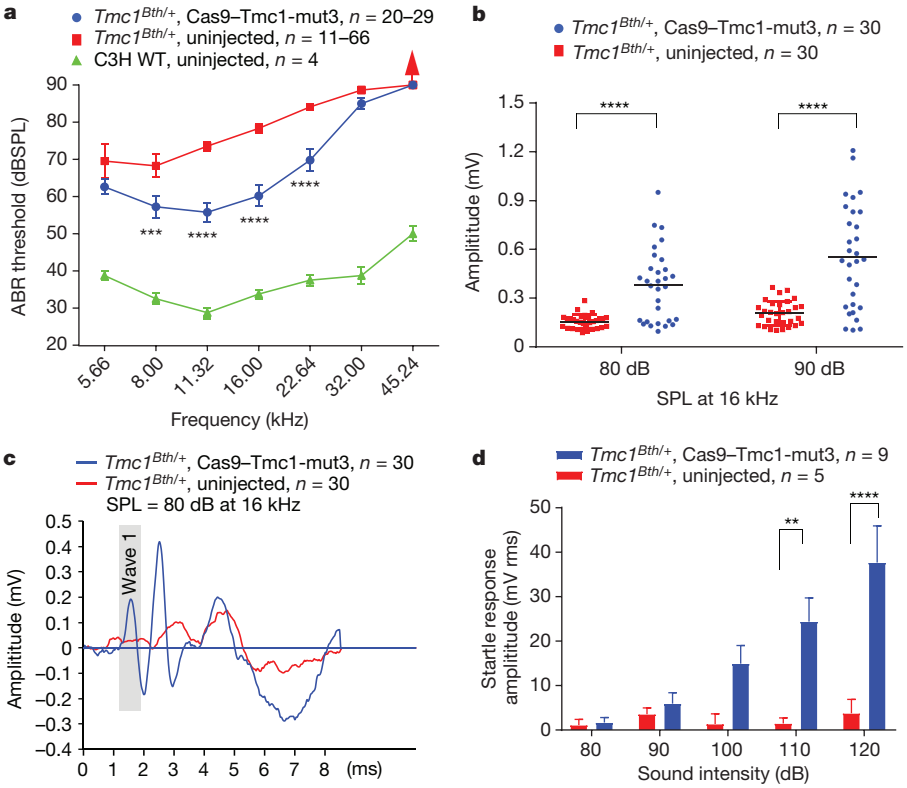

Figure 3 | Cas9–Tmc1-mut3–lipid injections reduce hearing loss in *Tmc1*$^{Bth/+}$ mice. a, ABR thresholds in *Tmc1*$^{Bth/+}$ ears injected with Cas9–Tmc1-mut3–lipid (blue), uninjected *Tmc1*$^{Bth/+}$ ears (red), and wild-type C3H ears (green) after four weeks. **b**, Peak amplitudes of ABR wave 1 at 16 kHz in Cas9–Tmc1-mut3–lipid-injected ears (blue) compared with uninjected ears (red) after four weeks. Horizontal bars are mean values. **c**, Mean ABR waveforms in Cas9–Tmc1-mut3–lipid-injected ears (blue) and uninjected ears (red). **d**, Startle responses in Cas9–Tmc1-mut3–lipid-injected mice (blue) and in uninjected mice (red) eight weeks after treatment. Red arrow in **a** indicates no ABR response at the highest stimulus level tested (90 dB). Statistical tests were two-way ANOVA with Bonferroni correction for multiple comparisons: $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$. Values and error bars reflect mean ± s.e.m. Among the different frequencies assayed, the number of ears tested (*n*) varies within the range shown (Supplementary Table 2).

mice by canalostomy. Two weeks after injection, loss of GFP fluorescence in the apical turn suggested target gene disruption with 25 ± 2.1% efficiency (Extended Data Fig. 8), comparable to previous observations of 20% GFP editing in neonatal hair cells[15]. These results suggest that this approach may be applicable to dominant genetic deafness that manifests with late-onset hearing loss.

To confirm that *in vivo* treatment of *Tmc1*$^{Bth/+}$ mice with Cas9–Tmc1-mut3 sgRNA disrupted the *Tmc1*^{Bth} allele, we sequenced DNA from cochlea tissue collected from injected *Tmc1*$^{Bth/+}$ and untreated *Tmc1*$^{Bth/+}$ mice. After injection on P1, tissues were removed on P5 and separated into organ of Corti (containing hair cells), spiral ganglion, and spiral ligament samples (Extended Data Fig. 9a, b). We estimated the fraction of hair cells in dissected cochlear tissue to be only about 1.5% of the total cells used for DNA sequencing (Extended Data Fig. 9a, b). Nevertheless, we observed unambiguous indels at the *Tmc1*^{Bth} locus in cochlear tissue from treated mice (Fig. 4a). The organ of Corti samples contained, on average, *Tmc1* editing of 0.92% of total sequenced DNA, which corresponds to about 1.8% *Tmc1*^{Bth} allele disruption in the heterozygous mice (Fig. 4a). We also isolated samples of much smaller numbers of cells (up to a few dozen, mostly hair cells) from

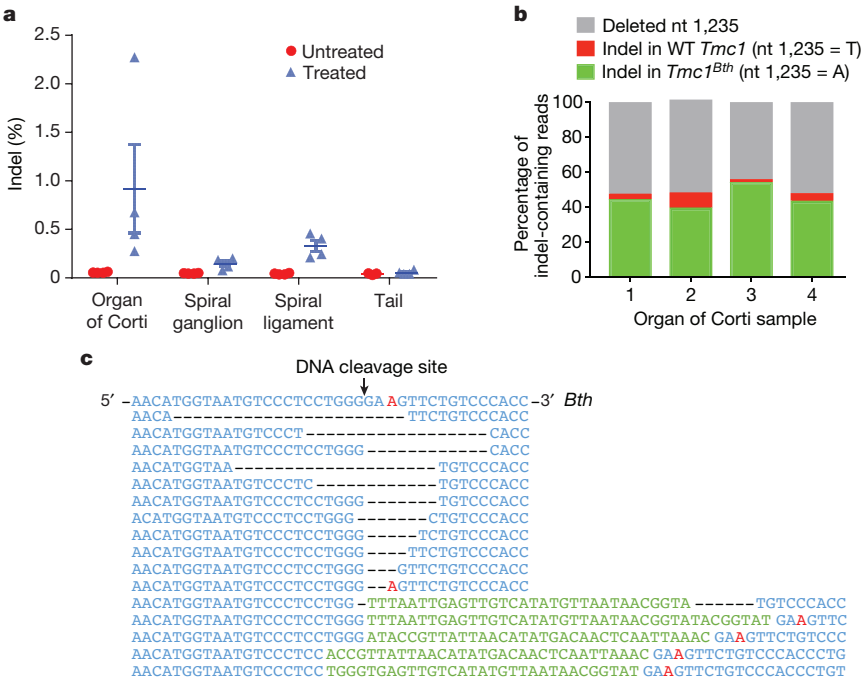

Figure 4 | Genome modification at *Tmc1* induced by lipid-mediated delivery of Cas9–Tmc1-mut3 RNP into *Tmc1*$^{Bth/+}$ mice. a, *Tmc1* indel frequencies from tissue samples four days after injection of Cas9–Tmc1-mut3–lipid (blue) or from uninjected mice (red). Individual values ($n = 4$) are shown; horizontal lines and error bars reflect mean ± s.e.m. Note that *Tmc1*^{Bth} allele editing frequencies in these heterozygous mice are approximately double the observed indel frequencies. **b**, Analysis of indel-containing *Tmc1* sequencing reads from four injected organ of Corti samples in **a**. **c**, The most abundant 16 *Tmc1* sequences, grouped by similarity, from organ of Corti samples in **b**. The T1235A *Tmc1*^{Bth} mutation is shown in red.