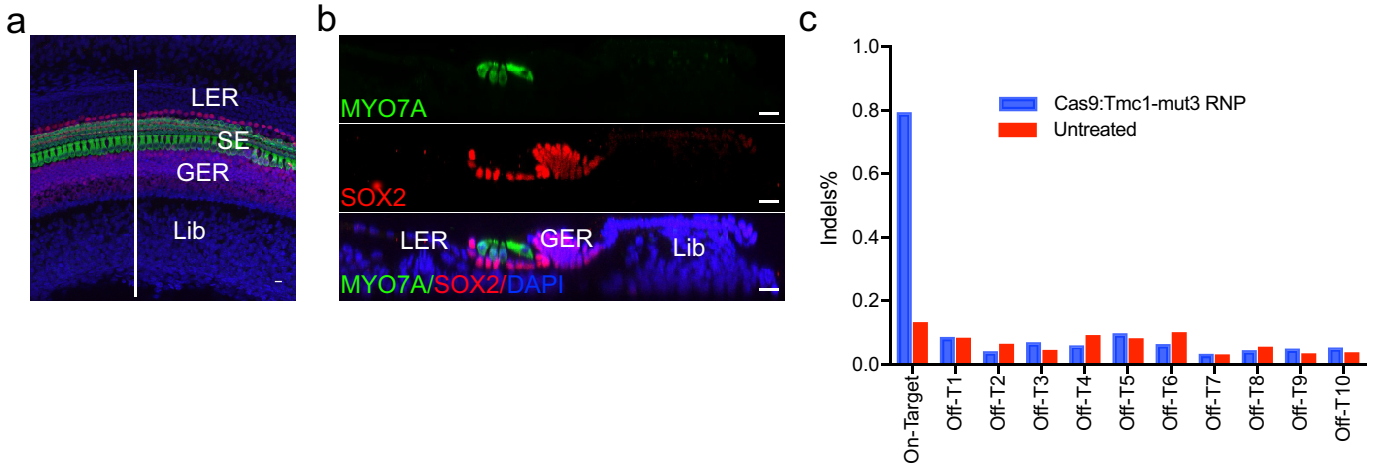

Extended Data Figure 9 | *In vivo* editing of the *Tmc1* locus from *Tmc1*$^{Bth/+}$ ears injected with Cas9–Tmc1-mut3 sgRNA. A representation of the organ of Corti removed at P5 for high-throughput DNA sequencing. **a**, A confocal *z*-stack image showing the surface view of a dissected and labelled organ of Corti used for HTS. **b**, A cross-sectional view of the organ of Corti (along the white line in **a**) showing the positions of hair cells (MYO7A), supporting cells (SOX2) and the cells from other cochlear regions that were used for quantification. LER, lesser epithelial ridge; GER, greater epithelial ridge; SE, sensory epithelium; Lib, limbus region. DAPI-labelled nuclei are shown in blue. Quantification showed that hair cells represented 1.45% ± 0.05% (mean ± s.e.m., $n = 4$) of all cells in the dissected cochlea. Scale bars, 10 μm. **c**, On-target and off-target *in vivo* editing of the *Tmc1* locus in organ of Corti samples. No indels were observed at frequencies substantially above that of an untreated control sample at any of the ten off-target sites identified by GUIDE-seq (*Off-T1* to *Off-T10*). Indels were detected by HTS at the *Tmc1* on-target site and each off-target site from *in vivo* tissue samples dissected from the inner ear of neonatal mice 4 days after Cas9–Tmc1-mut3 RNP injection (blue), or from untreated control samples (red).