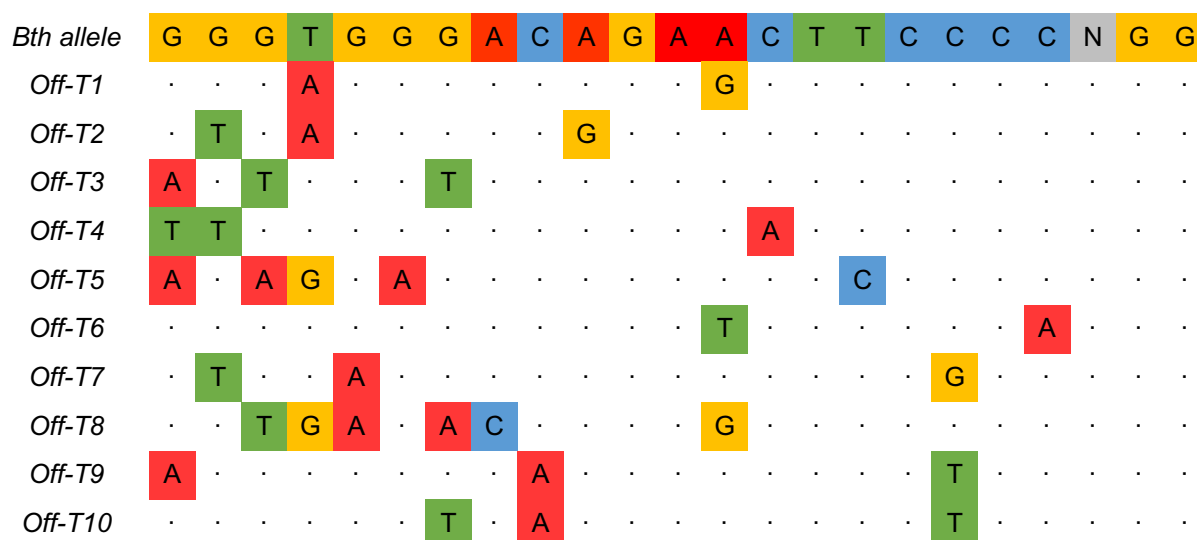
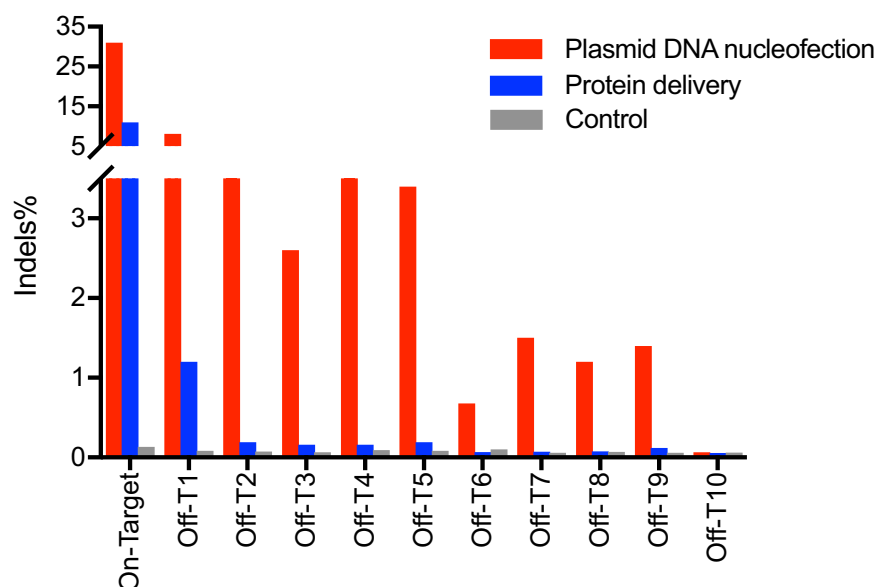


a



b



Extended Data Figure 3 | Off-target sites identified by GUIDE-seq after nucleofection of DNA plasmids encoding Cas9 and Tmc1-mut3 sgRNA into primary fibroblasts from *Tmc1^{Bth/+}* mice. a, One thousand nanograms of Cas9 plasmid, 300 ng Tmc1-mut3 sgRNA plasmid, 400 ng pmaxGFP plasmid, and 50 pmol double-stranded oligodeoxynucleotides (dsODN) were nucleofected into *Tmc1^{Bth/+}* fibroblasts using a LONZA 4D-Nucleofector. Genomic DNA was extracted 96 h after nucleofection and subjected to GUIDE-seq as previously described²³. *Off-T1* to *Off-T10* are ten off-target sites detected by GUIDE-seq. Mismatches compared to the on-target site are shown and highlighted in colour. The *Tmc1^{Bth}*

allele targeted by sgRNA Tmc1-mut3 is shown in the top row. **b**, Indel frequency at the *Tmc1* locus and at each of the off-target loci in Cas9–Tmc1-mut3-treated *Tmc1^{Bth/Bth}* primary fibroblasts following plasmid DNA nucleofection or following RNP delivery. For RNP delivery, 100 nM Cas9–Tmc1-mut3 RNP was delivered to the *Tmc1^{Bth/Bth}* fibroblasts using 3 µl Lipofectamine 2000. Indels were detected by HTS at the *Tmc1* on-target site and at each off-target site. Red, samples nucleofected with DNA plasmids encoding Cas9 and Tmc1-mut3 sgRNA; blue, samples treated with Cas9–Tmc1-mut3 RNPs; grey, control samples nucleofected with unrelated dsDNA only.