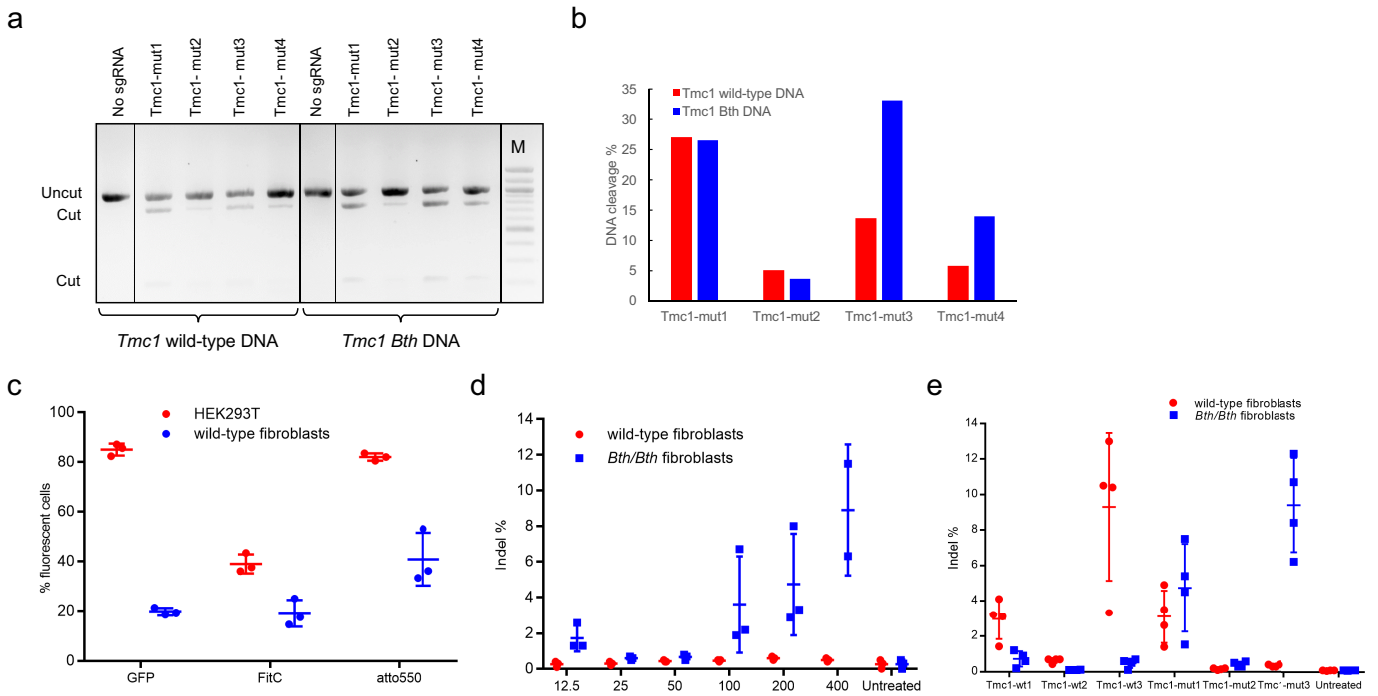

Extended Data Figure 1 | Allele-selective editing of wild-type or *Bth* mutant *Tmc1* in cleavage assays *in vitro* and by lipid-mediated delivery into primary fibroblasts. a, *In vitro* Cas9–sgRNA-mediated *Tmc1* DNA cleavage. We incubated 100 nM of a 995-bp DNA fragment containing wild-type *Tmc1* (lanes 1–5) or $Tmc1^{Bth}$ (lanes 6–10) with 300 nM of each of the four Cas9–sgRNAs shown for 15 min at 37 °C. Expected cleavage products are 774–778 bp and 217–221 bp. M, 100-bp ladder; the lower two heavy bands are 500 and 1,000 bp. **b**, Quantification of DNA cleavage in **a** by densitometry using imageJ. **c**, Comparison of transfection efficiency in HEK293T cells and wild-type primary fibroblasts. Fifty nanograms GFP plasmid, 10 nM Cas9–FitC–Tmc1-mut3 sgRNA RNP, or 10 nM Cas9–CrRNA–Tmc1-mut3–atto-550-TracrRNA RNP were delivered into HEK293T cells or wild-type primary fibroblasts using 3 μl Lipofectamine 2000. For samples with GFP plasmid, the fraction of GFP-positive cells was measured by flow cytometry 24 h after delivery. For samples with Cas9–FitC–Tmc1-mut3 RNP or Cas9–CrRNA–Tmc1-mut3–atto-550-TracrRNA RNP, medium was removed 6 h after delivery. The cells were trypsinized, washed three times with 500 μl PBS containing 20 U ml^{-1} heparin, and subjected to flow cytometry. **d**, Wild-type or *Bth* mutant *Tmc1* allele editing in primary fibroblasts derived from wild-type or $Tmc1^{Bth/Bth}$ mice as a function of the dose of Cas9–Tmc1-mut3–lipid complex. Cas9–Tmc1-mut3 (12.5, 25, 50, 100, 200, or 400 nM) was delivered into the primary fibroblasts using Lipofectamine 2000 in DMEM–FBS. **e**, Lipid-mediated delivery of Cas9–sgRNA complexes into primary fibroblasts derived from wild-type or $Tmc1^{Bth/Bth}$ mice. We delivered 100 nM of purified Cas9 protein and each wild-type *Tmc1*-targeting sgRNA (Tmc1-wt1, Tmc1-wt2, or Tmc1-wt3) or $Tmc1^{Bth}$ mutant-targeting sgRNA (Tmc1-mut1, Tmc1-mut2, or Tmc1-mut3) into wild-type fibroblasts (red) and $Tmc1^{Bth/Bth}$ fibroblasts (blue) using Lipofectamine 2000 in DMEM–FBS. Primary fibroblast cells were harvested 96 h after treatment. Genomic DNA was extracted and indels were detected by HTS. Individual values ($n = 3$–4) are shown; horizontal lines and error bars represent mean ± s.d. of biological replicates.