



**Extended Data Figure 2 | Delivery of Cas9–Tmc1–mut3 sgRNA complexes into primary fibroblasts derived from wild-type or homozygous *Tmc1*<sup>Bth/Bth</sup> mice.** **a**, Using seven commercially available lipids: LPF2000 (Lipofectamine 2000); RNAiMAX (Lipofectamine RNAiMAX); LPF3000 (Lipofectamine 3000); CRISPRMAX (Lipofectamine CRISPRMAX); LTX (Lipofectamine LTX). **b**, Using ten biodegradable, bio-reducible lipids: Lipid 1 (75-O14B); Lipid 2 (76-O14B); Lipid 3 (80-O18B); Lipid 4 (87-O16B); Lipid 5 (113-O18B); Lipid 6 (306-O12B); Lipid 7 (306-O16B); Lipid 8 (306-O18B);

Lipid 9 (400-O12B); Lipid 10 (400-O16B). We delivered 100 nM purified Cas9–Tmc1–mut3 RNP using 3  $\mu\text{l}$  of the cationic lipid shown in DMEM-FBS. Fibroblast cells were collected 96 h after treatment, genomic DNA was extracted, and indels were detected by HTS. **c**, Synthetic route and chemical structure of lipids. **d**, Commercially available amine head groups used in lipid synthesis. Lipids were synthesized as previously described<sup>26</sup>. Individual values ( $n = 2-4$ ) are shown; horizontal lines and error bars represent mean  $\pm$  s.d. of three or more biological replicates.