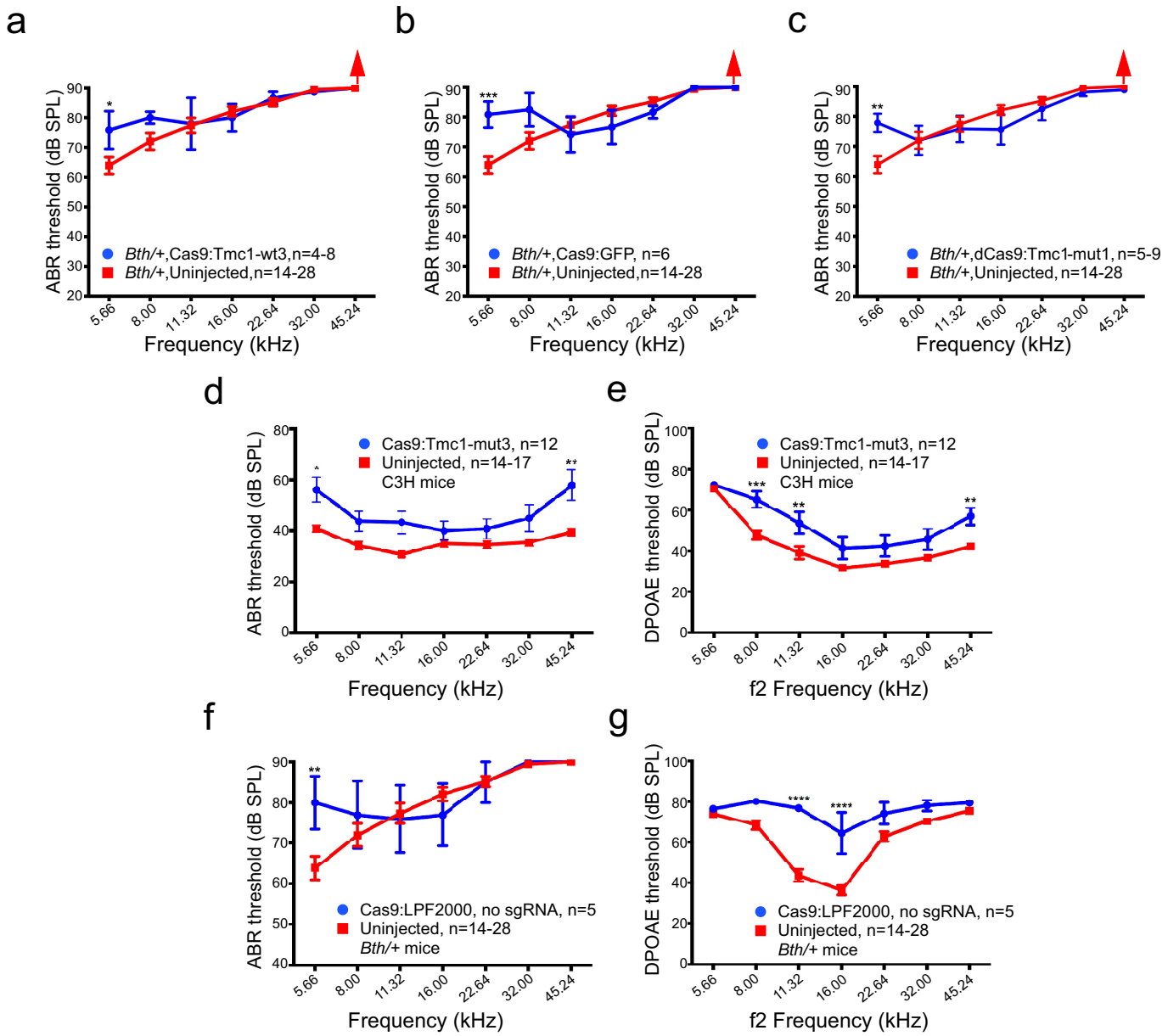

Extended Data Figure 6 | Hearing rescue is dependent on the *Tmc1*^{Bth} target specificity of the sgRNA, Cas9 nuclease activity, the presence of the *Tmc1*^{Bth} mutation, and the presence of the sgRNA. a, In *Tmc1*$^{Bth/+}$ ears injected with Cas9–Tmc1-wt3–lipid, which targets the wild-type *Tmc1* allele instead of the mutant *Tmc1*^{Bth} allele, ABR thresholds (blue) were comparable to or higher than those of uninjected controls (red) after four weeks. **b**, *Tmc1*$^{Bth/+}$ ears injected with Cas9–GFP sgRNA–lipid (blue) did not show improved ABR thresholds four weeks after treatment. **c**, *Tmc1*$^{Bth/+}$ ears injected with catalytically inactive dCas9–Tmc1-mut1–lipid did not show improved ABR thresholds four weeks after treatment. **d**, ABR thresholds of wild-type C3H mice injected with Cas9–Tmc1-mut3–lipid showed similar patterns to the uninjected control inner ears at four weeks, except at 5.66 and 45.24 kHz where ABR thresholds were elevated. **e**, Elevated DPOAE thresholds at three frequencies were observed after the treatment in **d**. **f**, Injection of Cas9–Lipofectamine 2000 (LPF2000) without sgRNA in *Tmc1*$^{Bth/+}$ mice did not improve ABR thresholds after four weeks. **g**, Elevated DPOAE thresholds at 11 and 16 kHz were observed after the treatment in **f**. Statistical analysis of ABR and DPOAE thresholds was performed by two-way ANOVA with Bonferroni correction for multiple comparisons: $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$. 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).