treated mice. Decreasing the number of cells entering the genomic DNA amplification and sequencing process increased the observed editing percentage to as high as 10% *Tmc1*^{Bth} allele disruption, but also elevated background *Tmc1* indel rates of untreated mice to an average of $0.82 \pm 0.57\%$ and a maximum of 1.6%, probably reflecting increased noise from processing of minute quantities of genomic DNA. No indel frequencies above that of untreated controls at any of the above-identified off-target sites were observed in Cas9–Tmc1-mut3–lipid-treated tissues (Extended Data Fig. 9c). Together, these observations confirm that Cas9–Tmc1-mut3–lipid treatment *in vivo* edits the *Tmc1* locus with no detected editing at GUIDE-seq-identified off-target loci.

An analysis of indel-containing *Tmc1* sequencing reads from treated *Tmc1*$^{Bth/+}$ mice allowed us to directly assess the allele specificity of Cas9–Tmc1-mut3 *in vivo*. Of 11,694 sequencing reads containing indels from four treated organ of Corti samples, 6,118 (52%) contained an intact nucleotide at *Tmc1* position 1,235. Of these, 5,736 (94%) contained modification of the mutant *Tmc1*^{Bth} allele, whereas only 382 (6%) contained modification of the wild-type *Tmc1* allele (Fig. 4b). Therefore, samples after treatment on average contained 15-fold higher modification of the *Tmc1*^{Bth} allele over the wild-type allele (Fig. 4b, c). These results demonstrate selective disruption of the *Tmc1*^{Bth} allele in *Tmc1*$^{Bth/+}$ mice, consistent with observed hearing phenotypes, even though the *Tmc1*^{Bth} and wild-type *Tmc1* alleles differ only at a single base pair.

This work shows that cationic lipid-mediated Cas9–sgRNA complex delivery *in vivo* can achieve allele-specific gene disruption in a mouse model of a human genetic disease, resulting in amelioration of the disease phenotype. Our results suggest that this approach has potential for the treatment of autosomal-dominant hearing loss related to hair cell dysfunction, and provide a complementary strategy to other approaches that use antisense oligos (ASOs) or RNA interference[6,25]. The genome editing strategy developed here may inform the future development of a DNA-free, virus-free, one-time treatment for certain genetic hearing loss disorders.

Online Content Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to these sections appear only in the online paper.

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