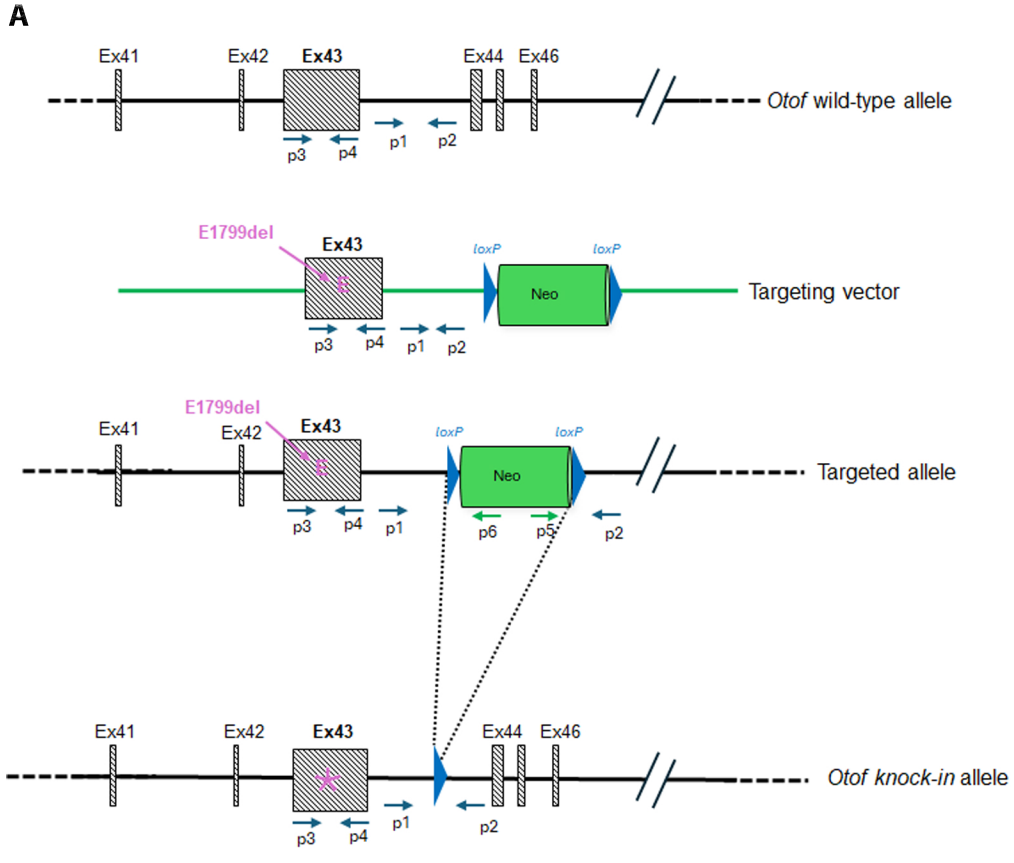
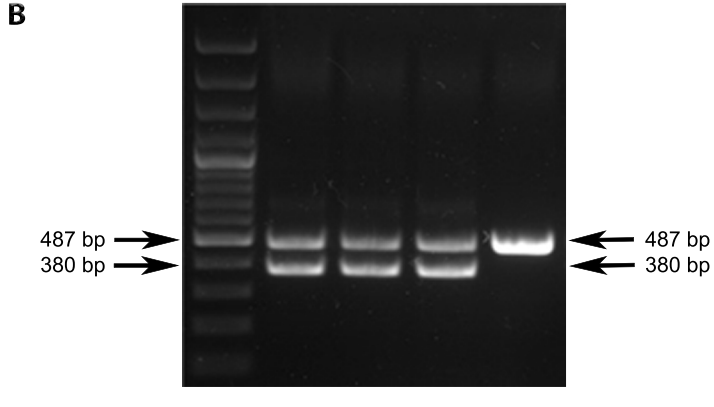
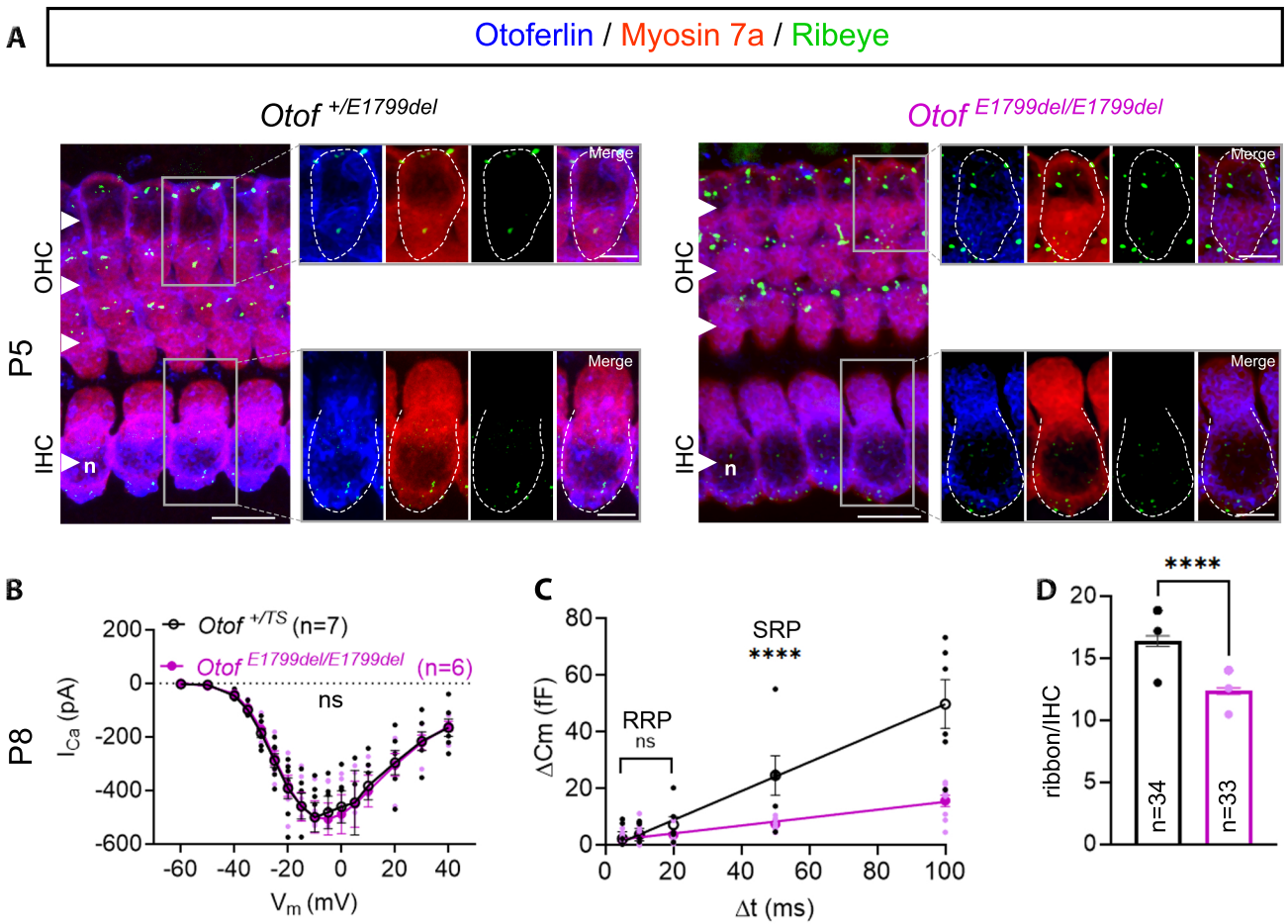
Supplementary Figure1

Engineering of a mouse line with the E1799del mutation of the *Otof* gene





Supplementary Figure 2



Supplementary Figure 1. Engineering of *Otof*$^{E1799del/E1799del}$ mice

(**A**) A targeting vector was designed to carry the *Otof* E1799del mutation in exon 43, incorporating a neomycin cassette (Neo) flanked by loxP sites (blue) as a selectable marker. PCR primers (p1 and p2) were designed to distinguish between the wild-type and *knock-in* alleles. (**B**) Amplicons obtained by RT-PCR from *Otof* $^{+/E1799del}$ and *Otof* $^{E1799del/E1799del}$ mice with the p1 and p2 primers used to detect the wild-type allele (380 bp amplicon) and the *Otof knock-in* allele (487 bp amplicon).

Supplementary Figure 2. Otoferlin expression, ribbon numbers and IHC exocytosis in neonatal *Otof*$^{E1799del/E1799del}$ mice

(**A**) Confocal microscopy images of IHCs and OHCs from whole-mount preparations of the organ of Corti from P5 *Otof* $^{+/E1799del}$ (left) and P5 *Otof* $^{E1799del/E1799del}$ (right) mice immunolabeled for otoferlin (blue), myosin 7a (red) and ribeye (green). Insets show higher magnifications of the boxed OHCs and IHCs highlighting otoferlin expression in both cell types. Scale bar: 10 µm. Inset scale bar: 5 µm. (B) Mean Ca^{2+} current amplitudes (ICa^{2+}) of P8 *Otof* $^{+/E1799del}$ (black, *n* = 7 IHCs from 4 mice, 2 females and 2 males) and *Otof* $^{E1799del/E1799del}$ (purple, *n* = 6 IHCs from 3 mice, 2 females and 1 males) IHCs obtained from patch-clamp recordings after 20 ms depolarizations to potentials between -60 mV and +40 mV. (C) Mean corresponding capacitance changes (ΔC_m) evoked by voltage-steps from -80 to -10 mV with an increasing duration of depolarization from 5 to 100 ms, for P8 *Otof* $^{+/E1799del}$ (black, *n* = 6 IHCs from 4 mice, 2 females and 2 males) and *Otof* $^{E1799del/E1799del}$ (purple, *n* = 9 IHCs from 3 mice, 2 females and 1 males) IHCs. The fusion of the readily releasable pool (RRP) and the sustained release pool (SRP) vesicles was evaluated for durations of 5 to 20 ms and 30 to 100 ms, respectively. In (C-D), data are presented as the mean ± SEM. **** $p<0.0001$, ns,

not significant (two-way ANOVA test followed by a Bonferroni's multiple comparison post hoc test). (**D**) Histogram depicting IHC ribbon quantification in the apical cochlear turns of P8 *Otof* $^{+/E1799del}$ where the capacitance measurements were performed (black, n = 34 IHCs from 3 mice, 2 females and 1 male) and *Otof* $^{E1799del/E1799del}$ (purple, n = 33 IHCs from 3 mice, 2 females and 1 male) mice. Data are presented as the mean ± SEM. **** p<0.0001 (unpaired t-test).