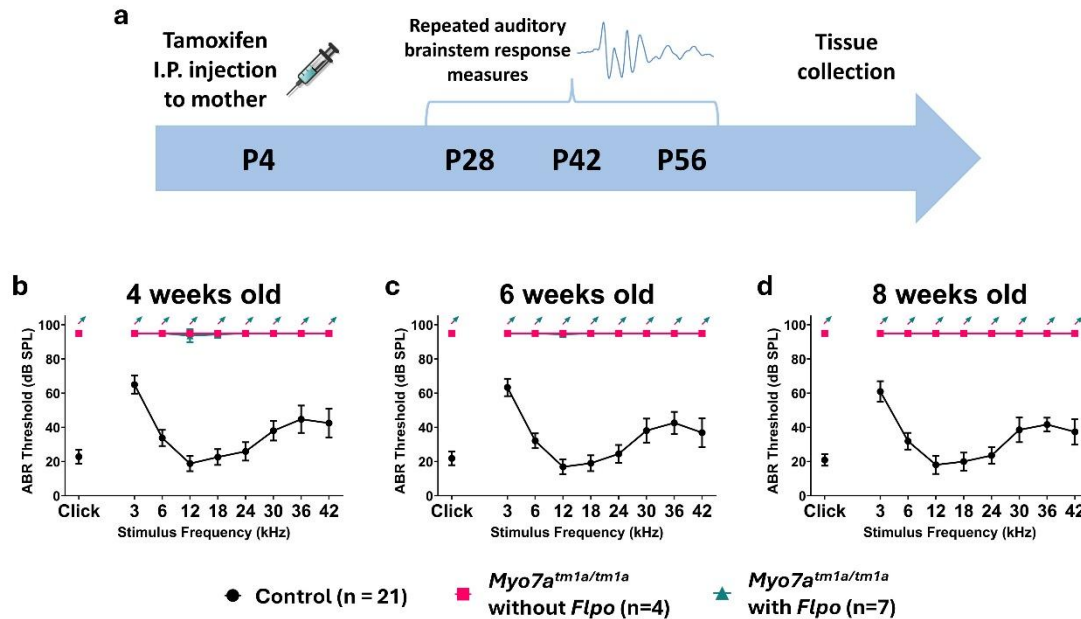


Limited potential to reverse deafness caused by mutation of *Myo7a*

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Supplementary Figure 1



Supplementary Fig. 1 Tamoxifen administration at P4 does not recover hearing loss of

Myo7a^{tm1a/tm1a} mice (a) Schematic showing the experimental timeline for the P4 cohort; pups

received tamoxifen from P4 via their mother's milk, ABRs were performed at four, six and

eight weeks old prior to inner ear collection. (B) ABR thresholds of control (black),

Myo7a^{tm1a/tm1a} without *Flpo* (pink), and *Myo7a^{tm1a/tm1a}* with *Flpo* (teal) mice at four, six and

eight weeks old between 3-42kHz. Data points indicate the lowest sound level (mean ± 1

SD) at which features of the ABR waveform start to appear. Data points at 95dB SPL

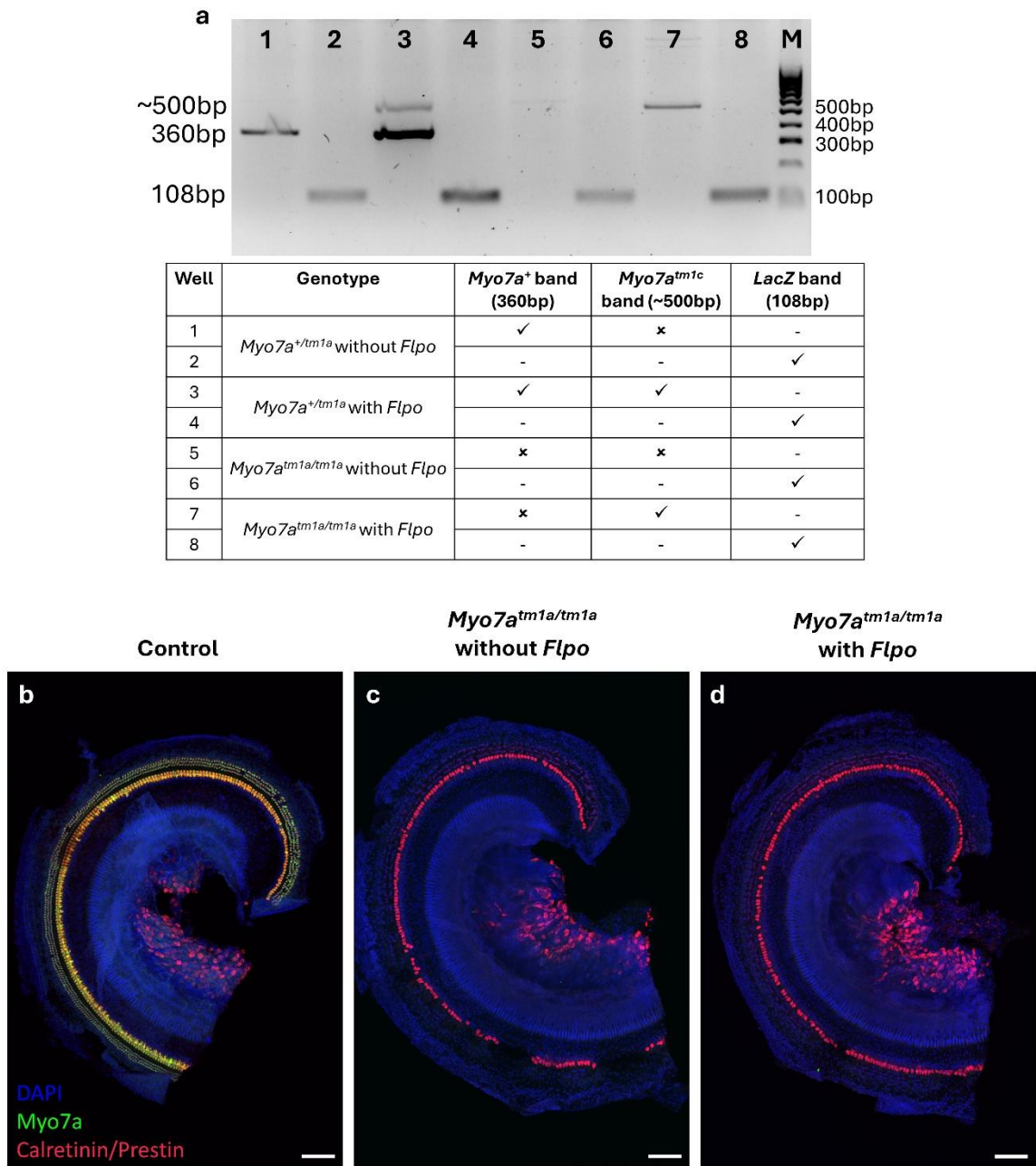
indicate no response up to the highest sound level used. Responses of both *Myo7a^{tm1a/tm1a}*

with and without *Flpo* mice were significantly raised compared to control mice ($p < 0.01$,

Kruskal-Wallis test). There was no significant difference between responses of *Myo7a^{tm1a/tm1a}*

with and without *Flpo* mice.

Supplementary Figure 2



Supplementary Fig. 2 Tamoxifen administration via mother’s milk to pups at P4 does not result in *Myo7a* protein expression in *Myo7a*^{tm1a/tm1a} with *Flpo* mice (a) Agarose gel showing the genomic PCR product using pinna tissue, showing the excision of the *tm1a* cassette: *Myo7a* F and *Myo7a* R primers produced either a 360bp product from the wildtype *Myo7a*⁺ allele or a ~500bp product from the *Myo7a*^{tm1c} allele if the *tm1a* cassette has been

excised. Thus, genomic template from *Myo7a*^{tm1a/tm1a} with *Flpo* mice produced a single ~500bp band, and template from *Myo7a*^{tm1a/tm1a} without *Flpo* mice did not produce a band. If a control *Myo7a*^{+/tm1a} mouse carried *Flpo*, 2 bands of 360bp and ~500bp were produced. If any unexcised tm1a cassette remains, the *LacZ* F and *LacZ* R primers produced a 108bp product from the *Myo7a*^{tm1a} allele. (b-d) Confocal images of apical pieces of organ of Corti whole mounts from control, *Myo7a*^{tm1a/tm1a} without *Flpo*, and *Myo7a*^{tm1a/tm1a} with *Flpo* mice at nine weeks old. Tamoxifen was administered to all mice via mother's milk from P4 regardless of genotype. IHCs and OHCs labelled with anti-myosin VIIA (both - green), anti-calretinin (IHCs - red), anti-prestin (OHCs - red), nuclei labelled with DAPI (blue). Scale bars = 100µm.