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Supplemental Information

Gene Therapy Restores Balance and Auditory Functions in a Mouse Model of Usher Syndrome

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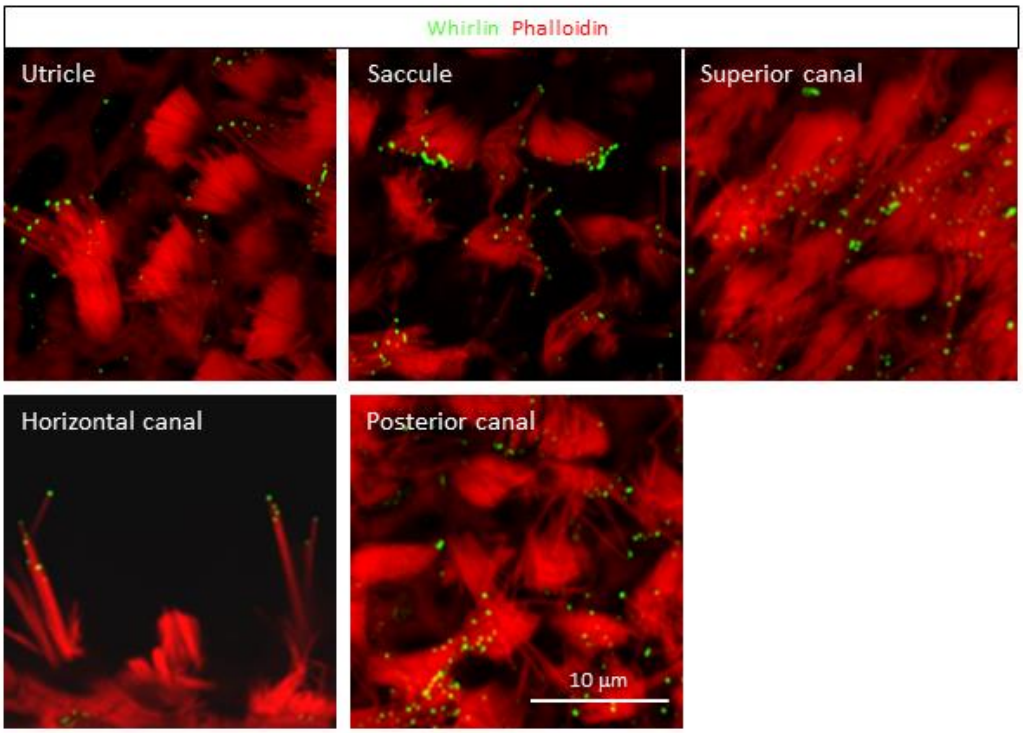
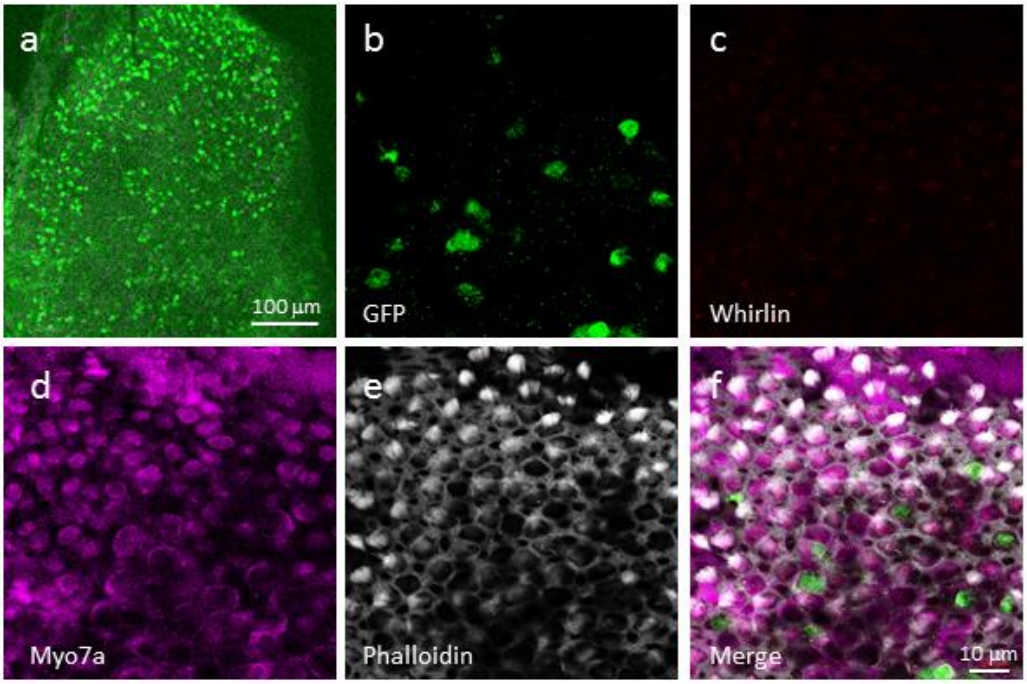
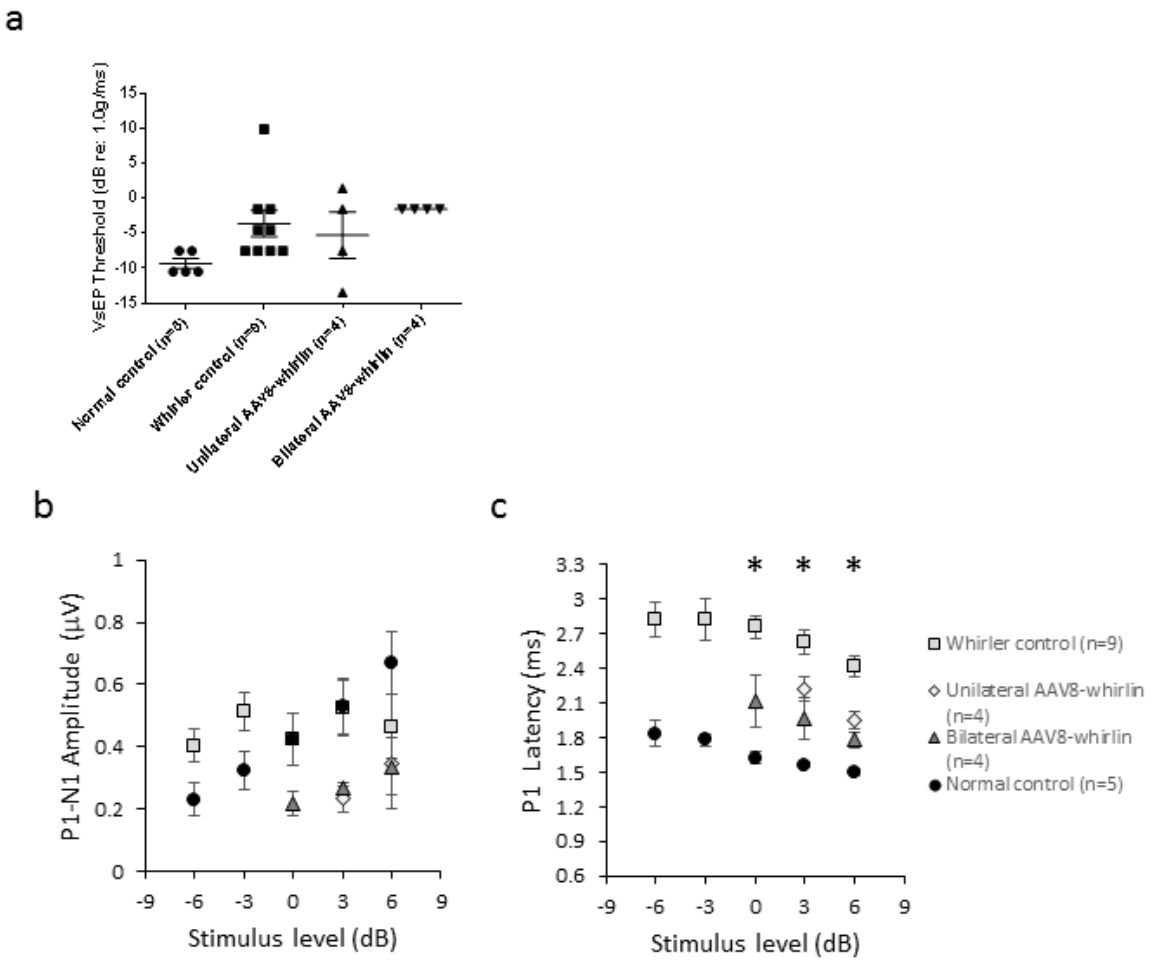


Supplementary Figure 1: AAV8-whirlin gene therapy delivered through the posterior semicircular canal successfully perfused all vestibular end-organs. Whirlin expression (green) is seen at the stereocilia tips of hair cells from all five vestibular end-organs (utricle, saccule, superior/horizontal/posterior semicircular canals). All images were taken at P120 from the same whirler mouse that received AAV8-whirlin gene therapy.

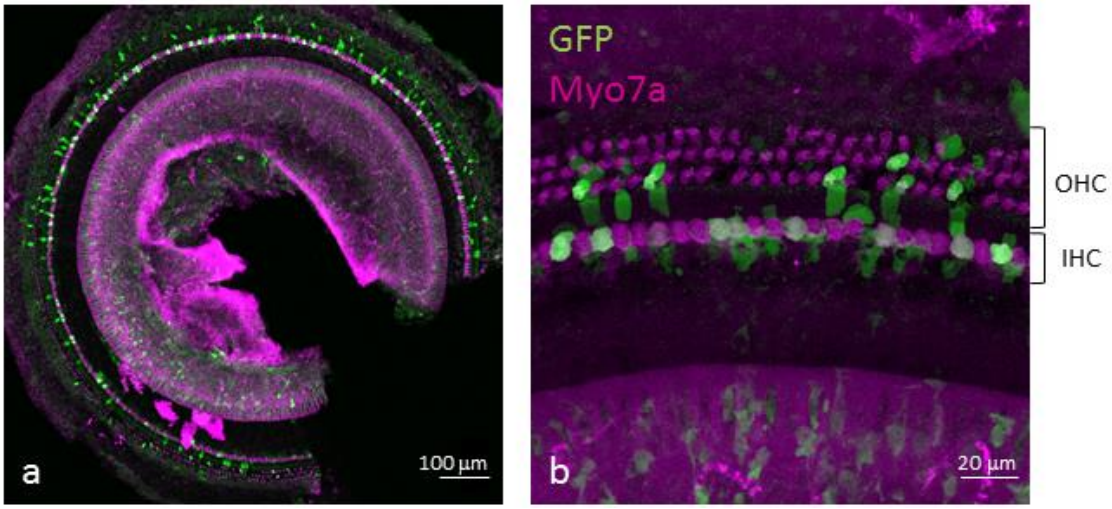


Supplementary Figure 2: AAV8-GFP injection into the whirler posterior semicircular canal had no effect on stereocilia length. When the negative control virus AAV8-GFP was injected into the whirler posterior semicircular canal, GFP expression was seen in both infected hair cells and supporting cells throughout the utricle (**a**). However, stereocilia bundles (white) remained abnormally short (**b-f**).

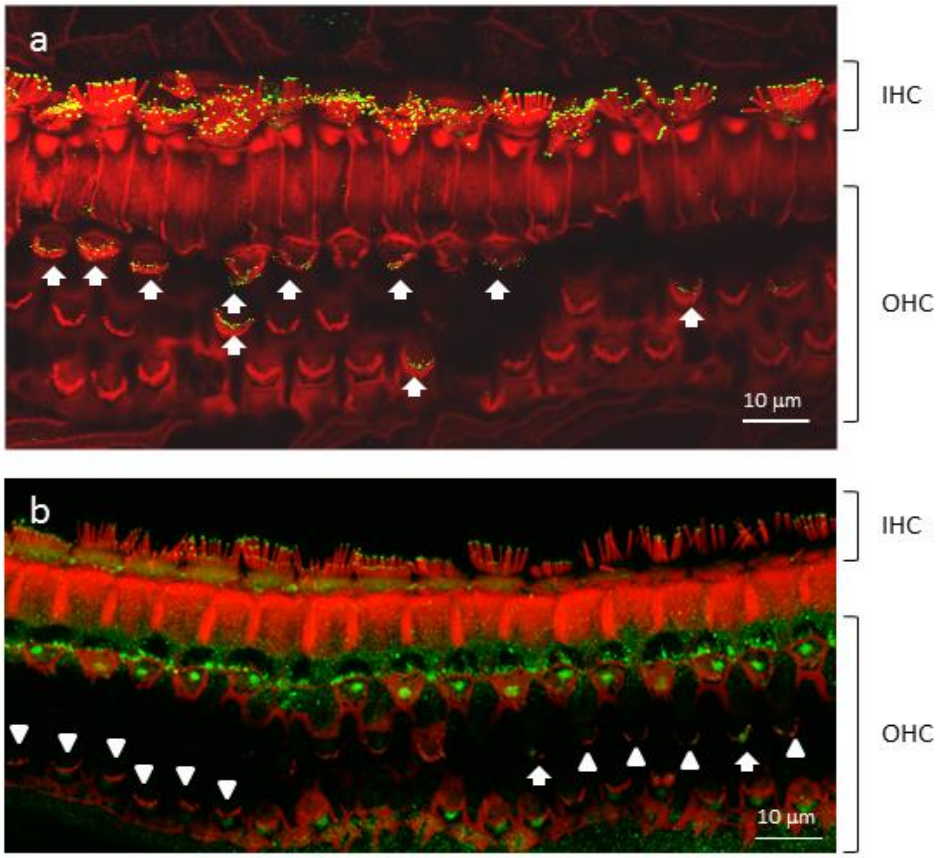


Supplementary Figure 3: VsEP measurements in whirlers that underwent AAV8-whirlin injections. (a) All whirler mice that received unilateral and bilateral AAV8-whirlin gene therapy had measurable VsEP thresholds. One of 9 whirler controls that did not receive AAV8-whirlin gene therapy had absent VsEP threshold (a threshold of 10 dB indicates a lack of measurable threshold). (**b**) Normal control littermates showed a steady increase in P1-N1 amplitude with increasing stimulus levels. This stimulus-level-dependent P1-N1 amplitude was also seen in whirlers that received bilateral AAV8-whirlin gene therapy, and was absent in whirler controls. (**c**) Whirlers that

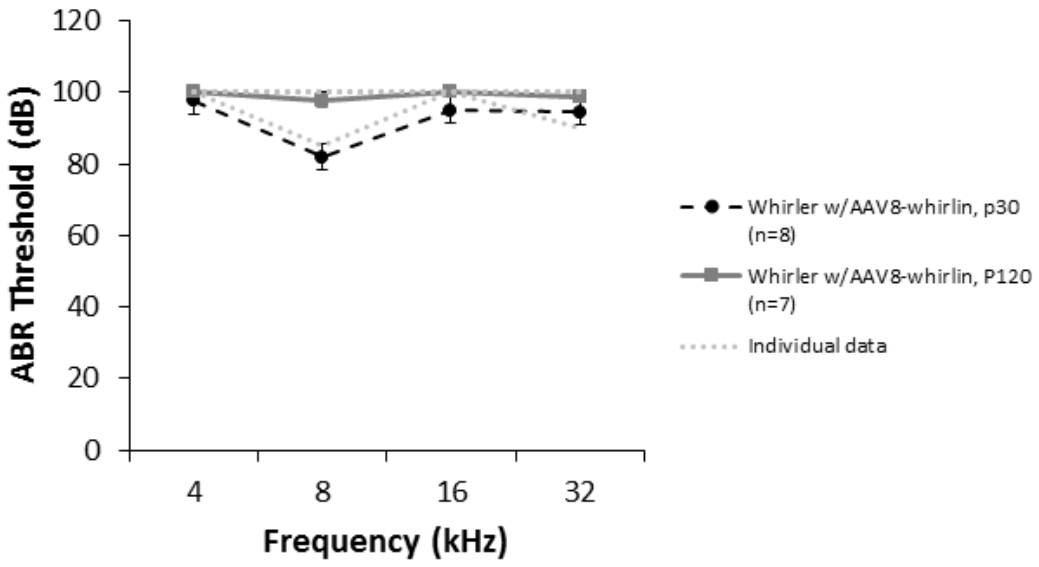
received bilateral AAV8-whirlin gene therapy had significant improvement in VsEP P1 latency compared to untreated whirler controls.



Supplementary Figure 4: AAV8-GFP injected through the posterior semicircular canal infected both IHCs and OHCs in the cochlea. Low (a) and high (b) magnification images of the cochlear middle turn from a neonatal whirler mouse that underwent AAV8-GFP injections into the posterior semicircular canal. AAV8-GFP efficiently infected both inner and outer hair cells.



Supplementary Figure 5: AAV8-whirlin gene therapy infected both IHCs and OHCs in the cochlea. (**a**) Middle turn of the cochlea of a P30 whirler mouse that received AAV8-whirlin gene therapy. There is robust AAV8-whirlin infection (green) in all IHCs, as well as some OHCs (white arrows). (**b**) Apical turn of the cochlea of a P120 whirler mouse that received AAV8-whirlin gene therapy. While some outer hair cells were infected and expressed whirlin at the stereocilia tips (white arrows), most outer hair cells were not infected (white arrowheads).



Supplementary Figure 6: Comparison of ABR thresholds at P30 and P120 in whirler mice that received AAV8-whirlin gene therapy. Two out of seven mice had measurable ABR thresholds at P120 (at 8 and 32 kHz). The average ABR threshold data from P30 are shown for comparison (black dashed line).