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Supplemental information

Dual-AAV vector-mediated expression of *MYO7A* improves vestibular function in a mouse model of Usher syndrome 1B

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Supplemental Figures

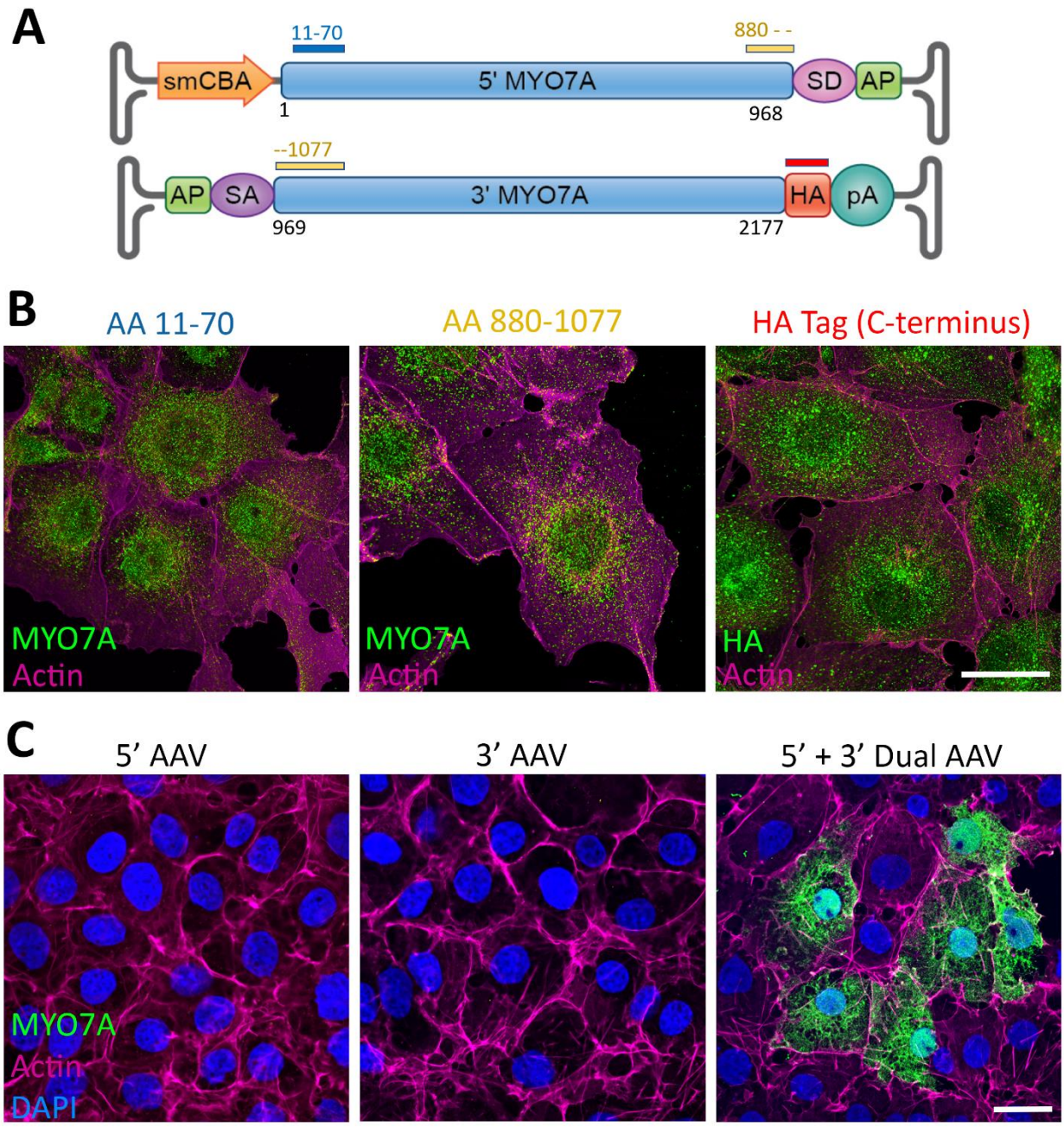

Figure S1. Dual-AAV8(Y733F)-*MYO7A* vectors expressed full-length MYO7A in COS-7 cells. (A) Schematics of the dual-AAV8(Y733F)-MYO7A vectors used in this study. The

human *MYO7A* cDNA was separated into two halves (labeled “5’ *MYO7A*” and “3’ *MYO7A*”), and its expression was driven by the CMV/chicken β-actin promoter (labeled “smCBA”), which was only present in the 5’ vector. The 3’ vector had a polyadenylation signal (pA). The highly recombinogenic coding sequence for alkaline phosphatase (AP), splice donor sites (SD), and splice acceptor sites (SA) were used to facilitate the recombination of the 5’ and 3’ cDNAs. The location of the antigens targeted by both anti-MYO7A antibodies as well as the C-terminal HA tag are marked with color coded rectangles. (B) COS-7 cells were infected with the dual-AAV8(Y733F)-MYO7A vectors which resulted in the expression of full-length human MYO7A. Specimens were imaged using super-resolution AiryScan mode. MYO7A was detected using the anti-MYO7A antibodies directed against the N-terminus, middle, and C-terminus (anti-HA), with very similar cellular distribution across the three specimens. HA refers to the hemagglutinin tag inserted at the C-terminus which facilitates its detection. Scale bar represents 20 μm. (C) COS-7 cells infected with either the 5’ or 3’ vector alone showed no MYO7A expression when the anti-MYO7A antibody targeting the middle portion of the protein was used (AA880-1077). Specimens were imaged using confocal mode. MYO7A expression was detected when both the 5’ and 3’ vectors were simultaneously used. Scale bar represents 20 μm.

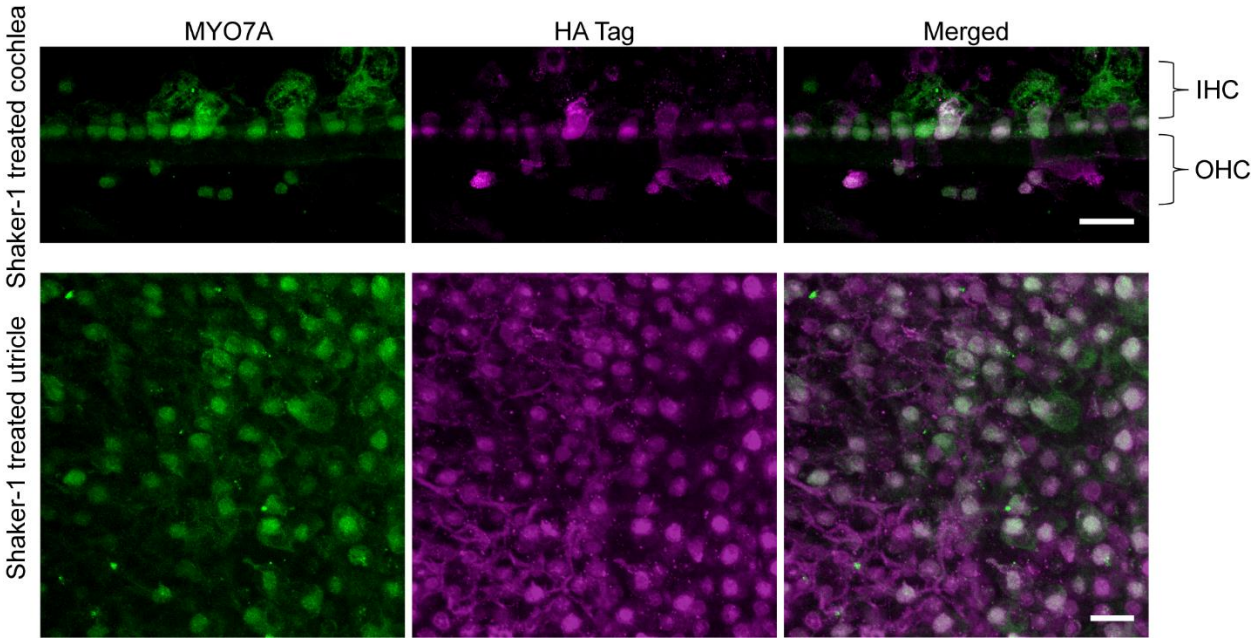

Figure S2. MYO7A co-localized with anti-HA antibodies in treated shaker-1 cochlear and vestibular hair cells. Representative confocal images of the cochlea (top row) and the utricle (bottom row) from a shaker-1 mouse treated with dual-AAV8(Y733F)-*MYO7A* vectors. MYO7A expression in the cochlear and vestibular hair cells of the treated shaker-1 mice co-localized with HA expression. This demonstrates that MYO7A expression in treated shaker-1 mice is mediated by the dual-AAV vectors. The top scale bar from the cochlea represents 20 µm. The bottom scale bar from the utricle represents 10 µm.

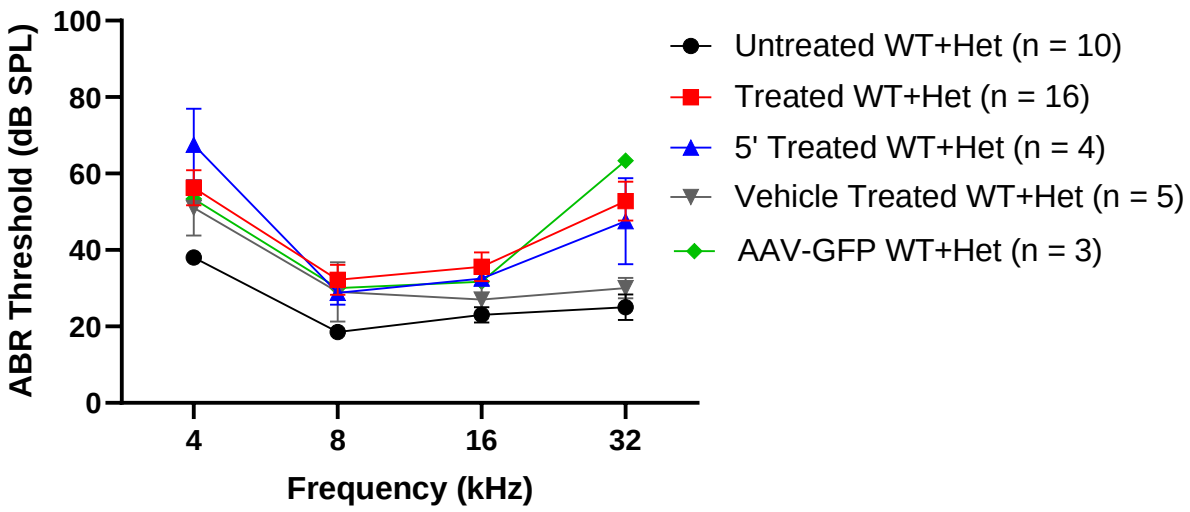

Figure S3. Dual-AAV8(Y733F)-*MYO7A* vectors cause an ABR threshold elevation in wild-type and heterozygous littermates. Auditory brainstem response (ABR) thresholds at the four measured frequencies (4, 8, 16, 32 kHz) are shown. ABR testing was done at ~P30. Injection of dual-AAV8(Y733F)-*MYO7A* vectors (labeled “Treated WT+Het”), 5’ vector alone (labeled “5’ Treated WT+Het”), and AAV8(Y733F)-GFP (labeled “AAV-GFP WT+Het”) caused a 10-30 dB ABR threshold elevation in the wild-type (WT) and heterozygous littermates. In contrast, wild-type and heterozygous littermates that were injected with vehicle (PBS with 5% glycerol, labeled “Vehicle Treated WT+Het”) did not show significant ABR threshold elevation compared to untreated wild-type and heterozygous mice (labeled “Untreated WT+Het”). Error bars represent standard errors.