



Figures and figure supplements

Differential regulation of hair cell actin cytoskeleton mediated by SRF and MRTFB

Ling-Yun Zhou et al.

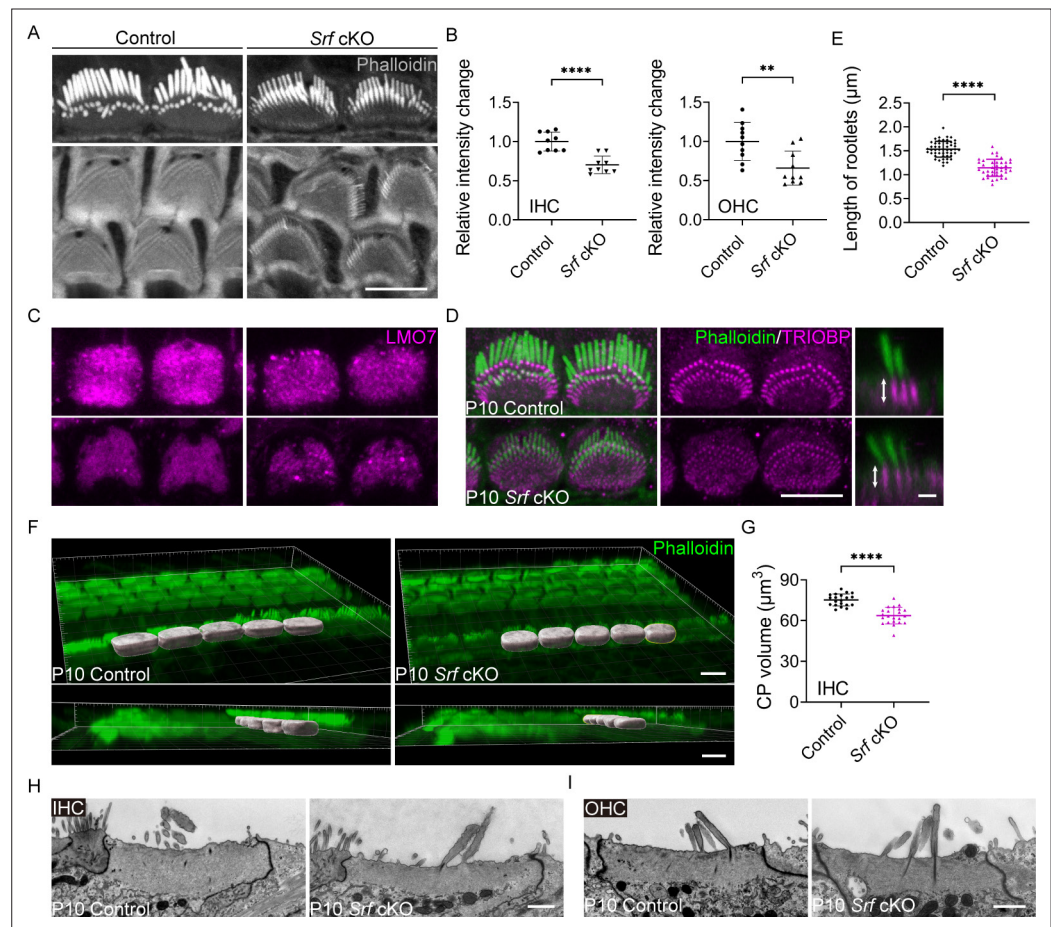


Figure 1. *Srf* cKO mice have defects in the cuticular plate. **(A)** Reduced phalloidin staining in the cuticular plates of *Srf* cKO hair cells (HCs) at P10. **(B)** Quantification of phalloidin reactivity in the cuticular plates of HC in control and *Srf* cKO mice at P10. The relative intensity changes are the ratio of phalloidin intensity of mutant and control cuticular plates. Analyzed numbers (cells, animals): inner hair cells (IHCs), control (68, 6), *Srf* cKO (67, 6). Outer hair cells (OHCs), control (240, 6), *Srf* cKO (269, 6). **(C)** LMO7 immunostaining in HC of control and *Srf* cKO at P10. **(D)** TRIOBP immunostaining in IHCs of control and *Srf* cKO at P10. Side views of TRIOBP labeling in IHCs are on the right. **(E)** Quantification of rootlet length of the longest row of IHC stereocilia (as shown by arrows in D) in control and *Srf* cKO at P10. Analyzed numbers (stereocilia, cells, animals): control (50, 14, 4), *Srf* cKO (41, 12, 4). **(F)** Imaris 3D reconstruction of phalloidin-labeled cuticular plates in control and *Srf* cKO mice at P10. The contour of the cuticular plate outlined by a yellow line in *Srf* cKO represents the area used to calculate the volume of the cuticular plate. **(G)** Quantification of cuticular plate volume of control and *Srf* cKO IHCs. Analyzed numbers (cells, animals): control (20, 5), *Srf* cKO (22, 5). **(H, I)** Transmission electron microscopy (TEM) analysis of cuticular plates of IHCs and OHCs in control and *Srf* cKO at P10. In D, scale bar for the side-view represents 1 μ m. In H and I, the scale bars represent 1 μ m. Scale bars in other panels represent 5 μ m. Error bars indicate standard deviation (SD), p values were derived from two-tailed unpaired Student's t-test, ****p-value <0.0001, **p-value <0.01.

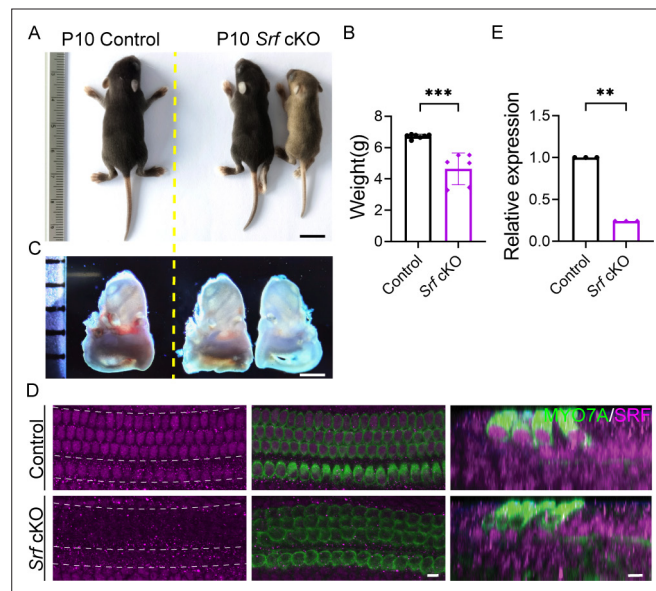


Figure 1—figure supplement 1. Conditional knockout of *Srf* in mice caused growth defects. **(A)** Representative examples of P10 control and *Srf* cKO pups. **(B)** Body mass measurements of control and *Srf* cKO mice at P10. Analyzed numbers (animals): control (7), *Srf* cKO (8). Error bars indicate SD, p values were derived from two-tailed unpaired Student's t-test, ***p value<0.001. **(C)** No significant size differences were found between control and *Srf* cKO inner ear at P10. **(D)** SRF immunoreactivity was obviously reduced in *Srf* cKO hair cells but not supporting cells. **(E)** Quantitative reverse transcription polymerase chain reaction (RT-qPCR) showed that compared to control, the expression level of *Srf* in *Srf* cKO hair cells reduced, normalized to GAPDH. In A, scale bar represents 1 cm. In C, scale bar presents 1 mm. In D, scale bars represent 5 μ m. Error bars indicate standard error of the mean (SEM), p values were derived from one-way analysis of variance (ANOVA) followed by Bonferroni post-test, **p value<0.01.

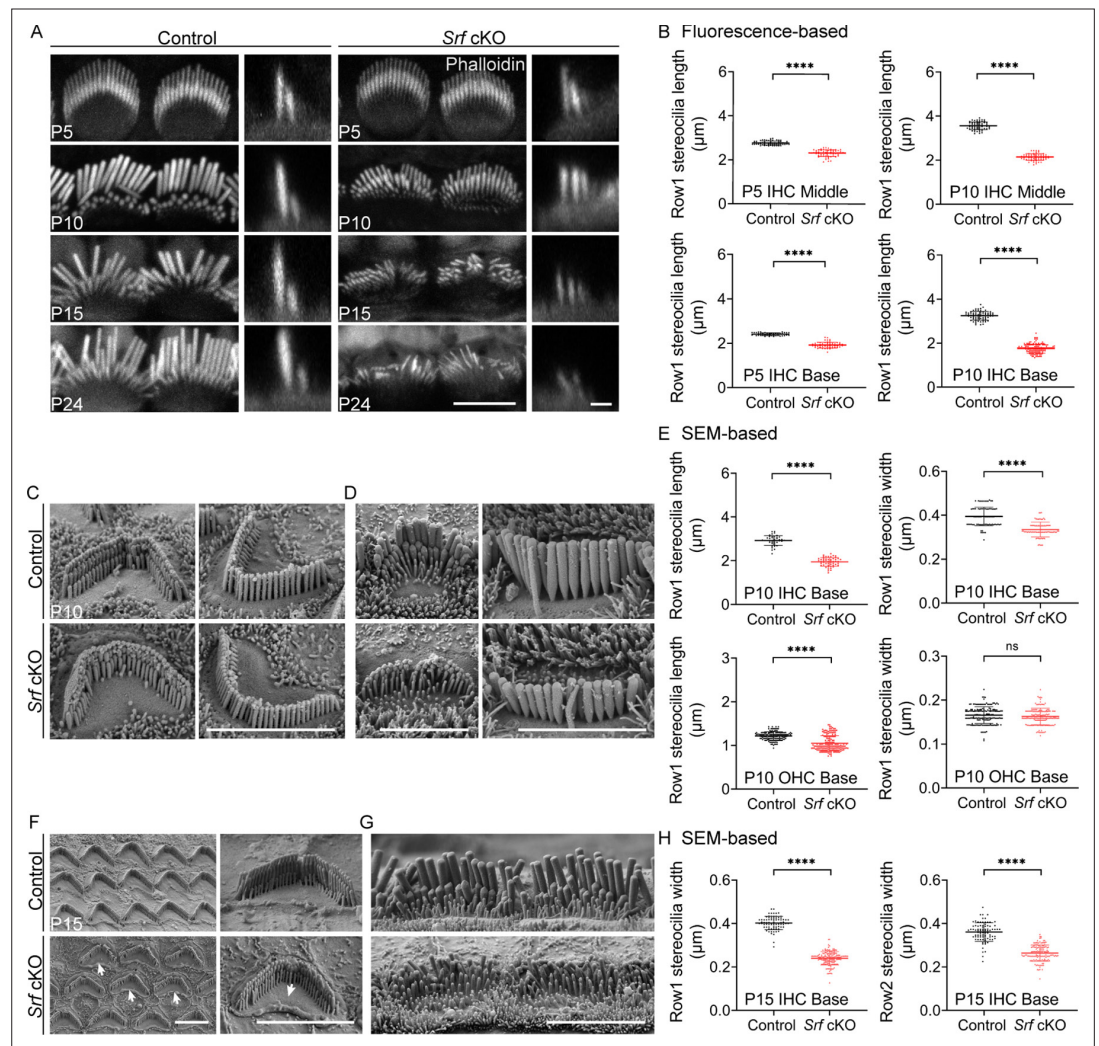


Figure 2. The defects of stereocilia dimensions in *Srf* cKO mice. **(A)** Phalloidin-stained inner hair cell (IHC) bundles of control and *Srf* cKO at different ages. En face views (scale bar, 5 μ m) are on the left, and side views (scale bar, 1 μ m) are on the right. **(B)** Fluorescence-based quantification of the length of row 1 IHC stereocilia at different cochlear positions, at P5 and P10. Analyzed numbers (stereocilia, cells, animals): middle IHCs, P5 control (60, 30, 5), P5 *Srf* cKO (62, 30, 5), P10 control (61, 25, 6), P10 *Srf* cKO (62, 26, 6). Basal IHCs, P5 control (41, 21, 5), P5 *Srf* cKO (53, 24, 5), P10 control (74, 28, 6), P10 *Srf* cKO (153, 53, 6). **(C, D)** Scanning electron microscopy (SEM) of outer hair cells (OHCs) and IHCs in control and *Srf* cKO mice at P10. **(E)** SEM-based quantification of row 1 stereocilia length and width in basal HCs at P10. Analyzed numbers: lengths: IHCs, control (42, 20, 5), *Srf* cKO (52, 18, 5). OHCs, control (149, 43, 5), *Srf* cKO (165, 42, 5). Widths: IHCs, control (88, 22, 5), *Srf* cKO (81, 20, 5). OHCs, control (164, 45, 5), *Srf* cKO (147, 42, 5). **(F, G)** SEM of OHCs and IHCs in control and *Srf* cKO mice at P15. **(H)** SEM-based quantification of rows 1 and 2 stereocilia width in basal IHCs at P15. Analyzed numbers: row 1, control (76, 11, 3), *Srf* cKO (150, 15, 3). Row 2, control (85, 11, 3), *Srf* cKO (134, 14, 3). Scale bars in C, D, F and G represent 5 μ m. Error bars indicate standard deviation (SD), p values were derived from two-tailed unpaired Student's t-test, ****p value<0.0001.

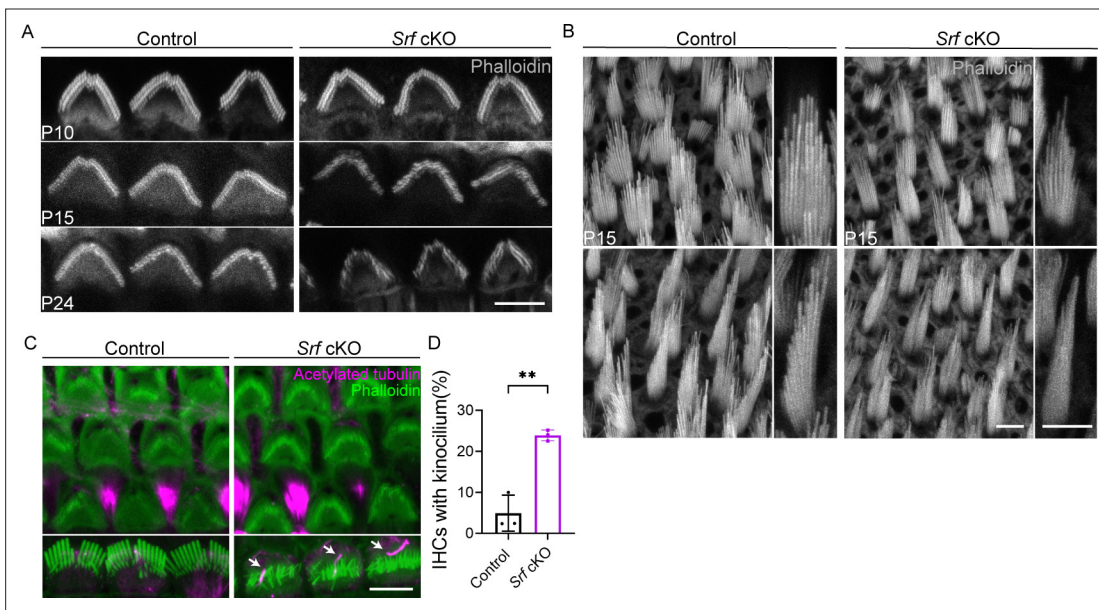
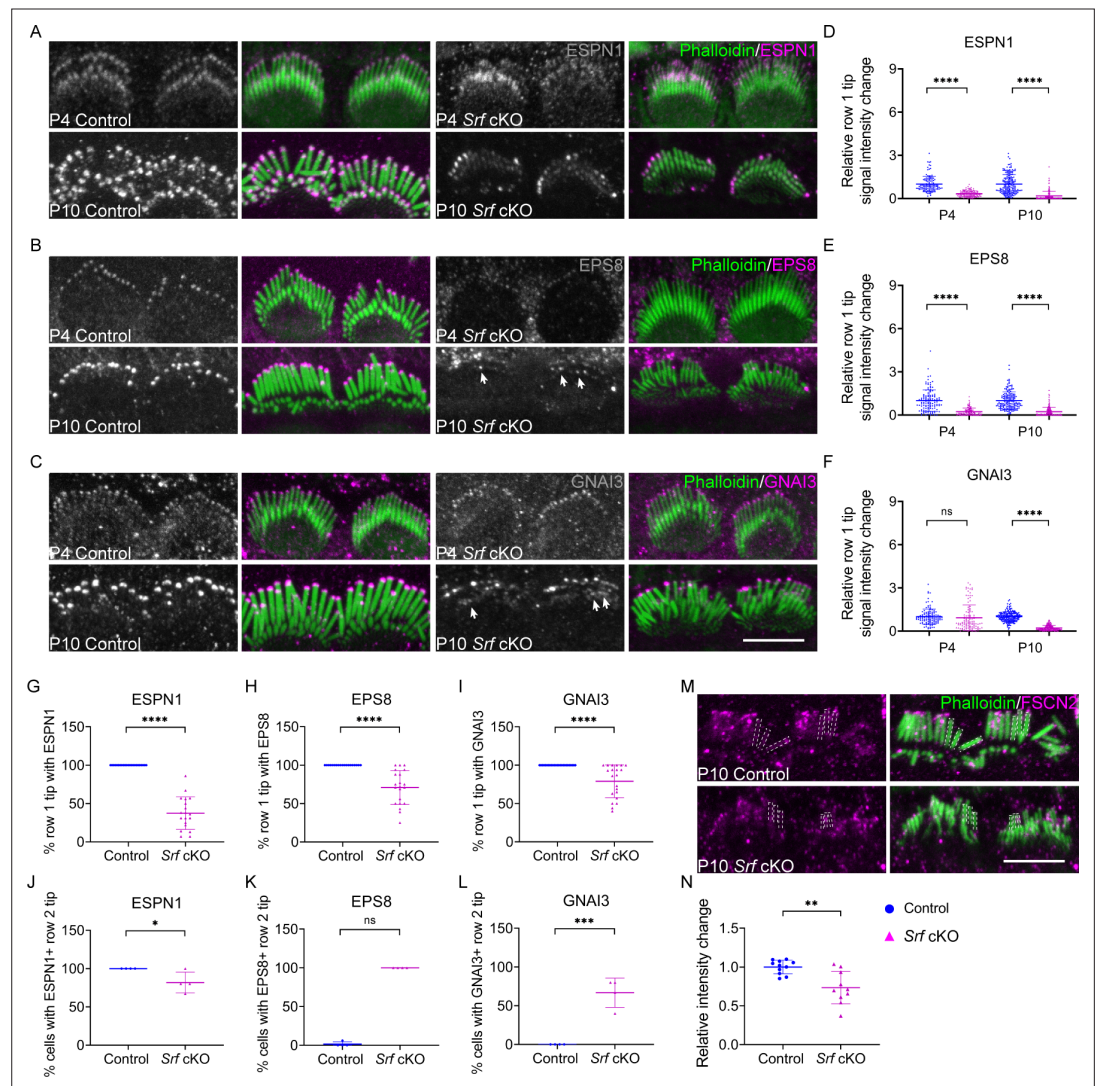
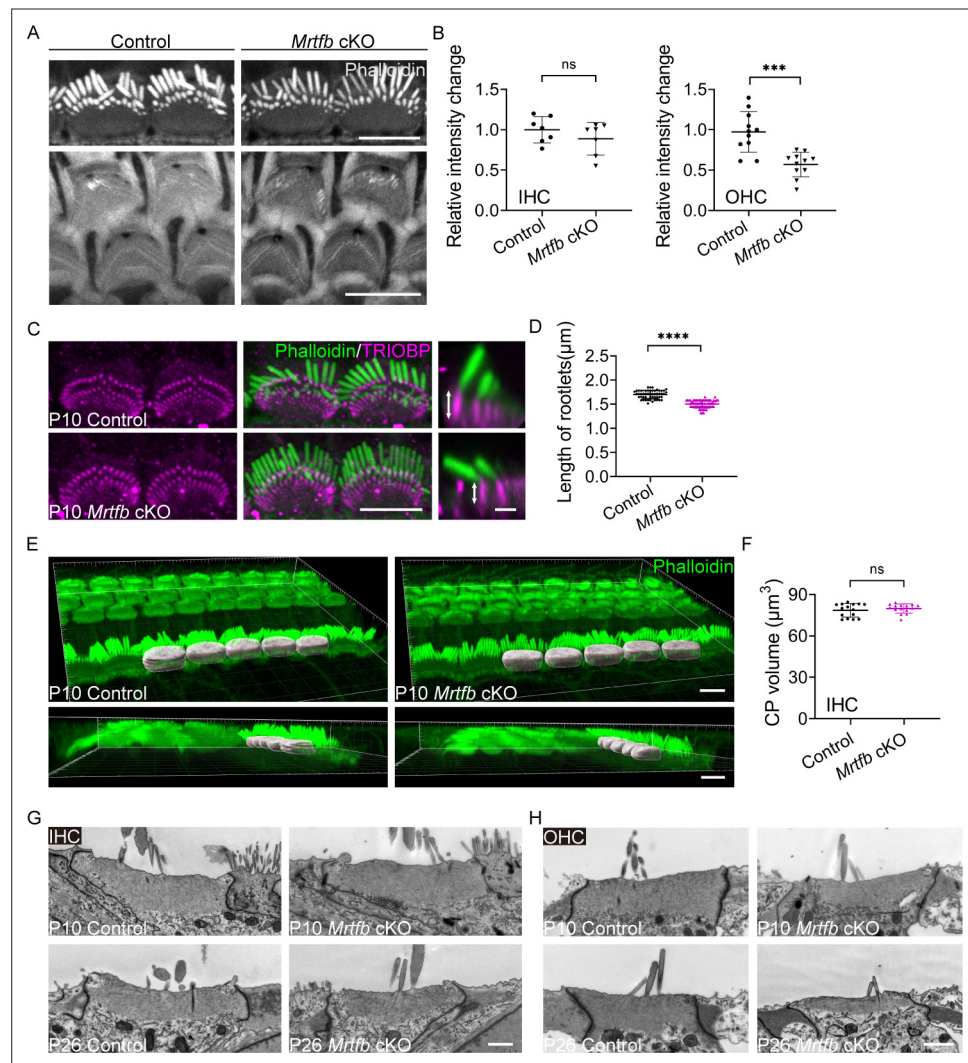


Figure 2—figure supplement 1. *Srf* cKO mice have defects in hair bundles. **(A)** Phalloidin staining of control and *Srf* cKO outer hair cells (OHCs) hair bundles at various ages. **(B)** Phalloidin staining of control and *Srf* cKO hair bundles in P15 utricle. Upper, bundles of striolar HCs. Bottom, bundles of extrastriolar HCs. Higher magnification of hair bundles were shown on the right side of the panel. **(C)** Acetylated-tubulin immunostaining at P10 showed delayed kinocilia degeneration in *Srf* cKO inner hair cells (IHCs) compared to control. **(D)** Quantification of kinocilium-bearing control and *Srf* cKO IHCs at P10. Analyzed numbers (cells, animals): Apex, control (300, 3), *Srf* cKO (300, 3). Middle, control (300, 3), *Srf* cKO (307, 3). Base, control (390, 3), *Srf* cKO (400, 3). At least 100 cells were counted in each cochlear position for each mouse. Scale bars in all panels represent 5 μ m. Error bars indicate standard deviation (SD), p values were derived from two-tailed unpaired Student's *t*-test, **p value < 0.01.





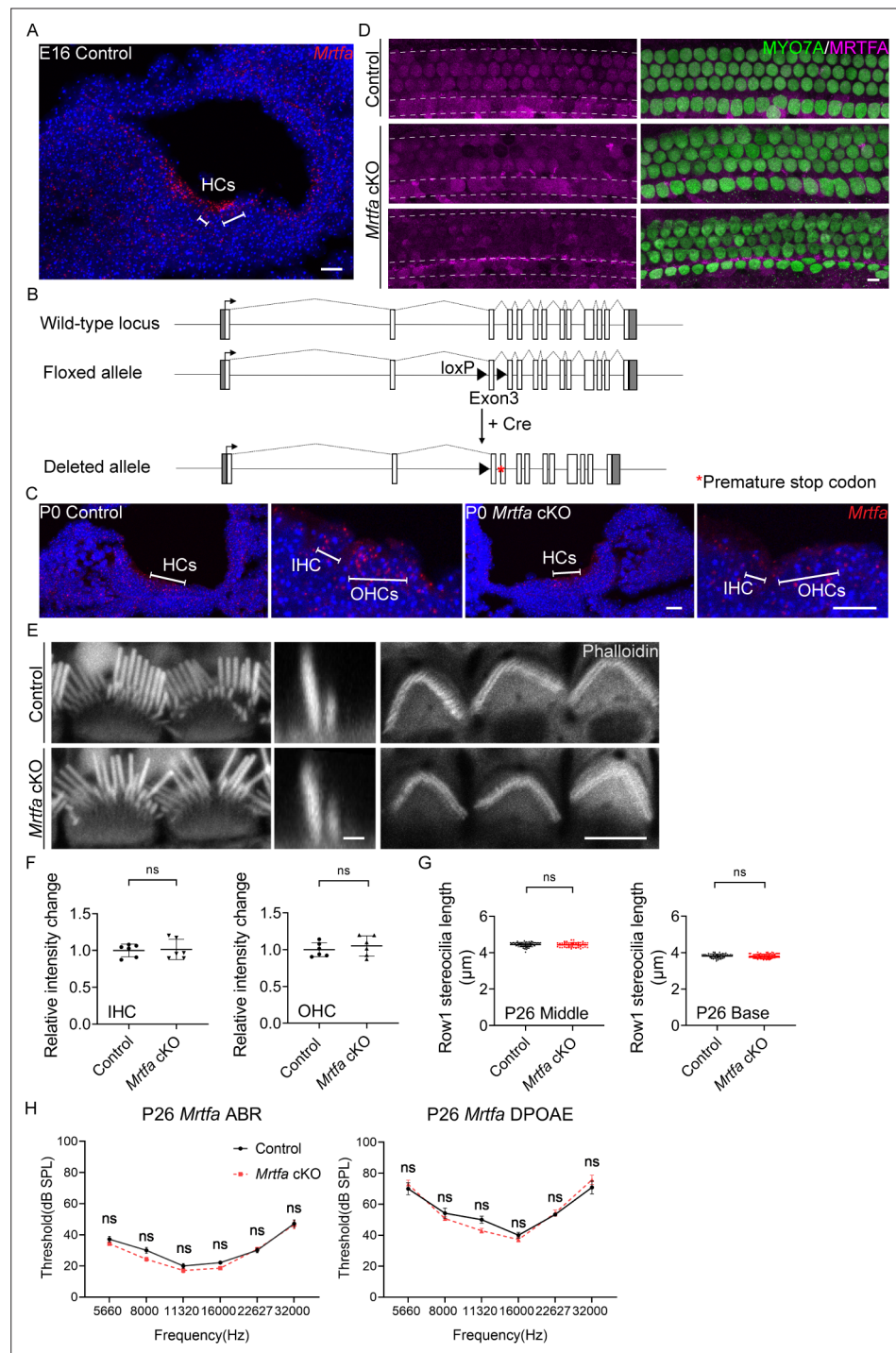


Figure 4—figure supplement 1. No obvious defects in HCs and hearing function of *Mrtfa* cKO mice. **(A)** RNAscope in situ hybridization of *Mrtfa* in E16 control cochleae. **(B)** Genomic structures of mouse *Mrtfa* gene, CRISPR-mediated targeting allele, and mutated allele after Cre-mediated recombination. Exons are indicated by open boxes; untranslated regions are indicated by gray boxes. The loxP sites and premature stop codon are shown with black triangles and red asterisk. **(C)** RNAscope in situ hybridization of *Mrtfa* in P0 cochleae of control and *Mrtfa* cKO mice. **(D)** MRTFA immunostaining in P0 control and *Mrtfa* cKO cochleae. MRTFA immunoreactivity was significantly reduced in mutants. **(E)** Phalloidin staining of hair bundles in control and *Mrtfa* cKO at P26. Left and right panels are en face views of inner hair cell (IHC) and outer hair cell (OHC) bundles, middle panels are side views of IHC bundles. **(F)** Quantification of phalloidin reactivity in the cuticular plates of control and *Mrtfa* cKO HCs at P26. Analyzed numbers (cells, animals): IHCs, control (48, 4), *Mrtfa* cKO (48, 4). OHCs, control (144, 4), *Mrtfa* cKO (144, 4). **(G)** Quantification of stereocilia length in P26 Middle and P26 Base. **(H)** ABR and DPOAE thresholds for Control and *Mrtfa* cKO mice. *ns*, not significant.

Figure 4—figure supplement 1 continued on next page

Figure 4—figure supplement 1 continued

4), *Mrtfa* cKO (134, 4). **(G)** Quantification of row 1 stereocilia length in P26 control and *Mrtfa* cKO IHCs at different cochlear positions. Analyzed numbers (stereocilia, cells, animals): middle IHCs, control (62, 30, 4), *Mrtfa* cKO (47, 25, 4). Basal IHCs, control (55, 27, 4), *Mrtfa* cKO (71, 32, 4). **(H)** No significant difference was observed in auditory brainstem response (ABR) and distortion product otoacoustic emission (DPOAE) analysis between control and *Mrtfa* cKO mice at P26. Analyzed numbers (animals): control (7), *Mrtfa* cKO (7). In A and C, scale bars present 20 μm . In E, scale bar for the side-view represents 1 μm . Scale bars in other panels represent 5 μm . Error bars of F and G indicate standard deviation (SD), p values were derived from two-tailed unpaired Student's *t*-test. Error bars of H indicate standard error of the mean (SEM), p values were derived from two-way analysis of variance (ANOVA) followed by Bonferroni post-test.

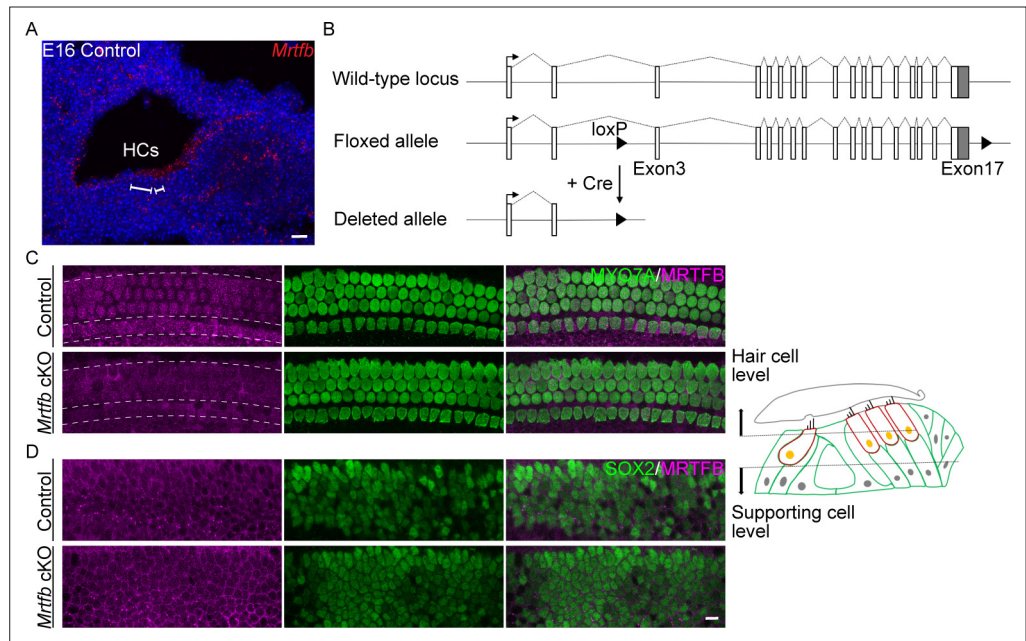


Figure 4—figure supplement 2. Conditional knockout of *Mrtfb* in mice. **(A)** RNAscope in situ hybridization of *Mrtfb* in E16 control cochleae. **(B)** Genomic structures of mouse *Mrtfb* gene, CRISPR-mediated targeting allele, and mutated allele after Cre-mediated recombination. Exons are indicated by open boxes; untranslated regions are indicated by gray boxes. The loxP sites are shown with black triangles. **(C, D)** MRTFB immunoreactivity was reduced in P1 *Mrtfb* cKO cochlear hair cells, but not supporting cells. In A, scale bar represents 20 μ m. In D, scale bar represents 5 μ m.

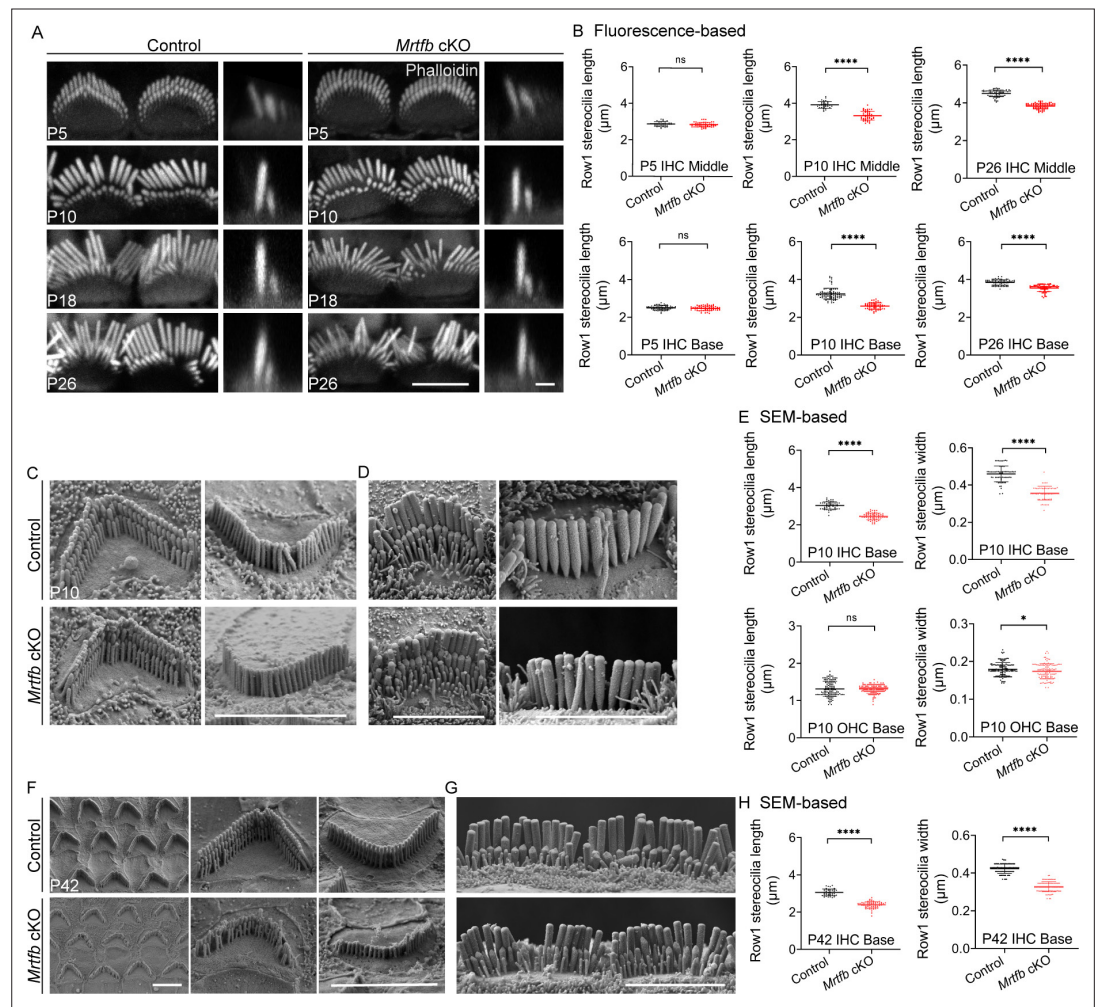


Figure 5. The defects of stereocilia dimensions in *Mrtfb* cKO mice. **(A)** Phalloidin staining of inner hair cell (IHC) bundles at different ages. En face views (scale bar, 5 μ m) are on the left, and side views (scale bar, 1 μ m) are on the right. **(B)** Fluorescence-based quantification of row 1 IHC stereocilia length at different cochlear positions and ages. Analyzed numbers: middle IHCs, P5 control (41, 20, 6), P5 *Mrtfb* cKO (53, 23, 6), P10 control (80, 35, 6), P10 *Mrtfb* cKO (36, 18, 6), P26 control (73, 23, 5), P26 *Mrtfb* cKO (71, 24, 5). Basal IHCs, P5 control (65, 28, 6), P5 *Mrtfb* cKO (60, 25, 6), P10 control (83, 39, 6), P10 *Mrtfb* cKO (48, 22, 6), P26 control (74, 25, 5), P26 *Mrtfb* cKO (73, 24, 5). **(C, D)** Scanning electron microscopy (SEM) of outer hair cells (OHCs) and IHCs at P10. **(E)** SEM-based quantification of row 1 stereocilia length and width in basal HCs of control and *Mrtfb* cKO at P10. Analyzed numbers: lengths: IHCs, control (50, 20, 6), *Mrtfb* cKO (59, 20, 6). OHCs, control (127, 33, 6), *Mrtfb* cKO (132, 39, 6). Widths: IHCs, control (65, 22, 5), *Mrtfb* cKO (58, 22, 5). OHCs, control (135, 35, 5), *Mrtfb* cKO (118, 39, 5). **(F, G)** SEM of OHCs and IHCs at P42. **(H)** SEM-based quantification of row 1 stereocilia length and width in basal IHCs of control and *Mrtfb* cKO at P42. Analyzed numbers: lengths, control (42, 22, 6), *Mrtfb* cKO (63, 24, 6). Widths, control (86, 22, 6), *Mrtfb* cKO (92, 24, 6). Scale bars in C, D, F and G represent 5 μ m. Error bars indicate standard deviation (SD), p values were derived from two-tailed unpaired Student's t-test, ****p value<0.0001, and *p value<0.05.

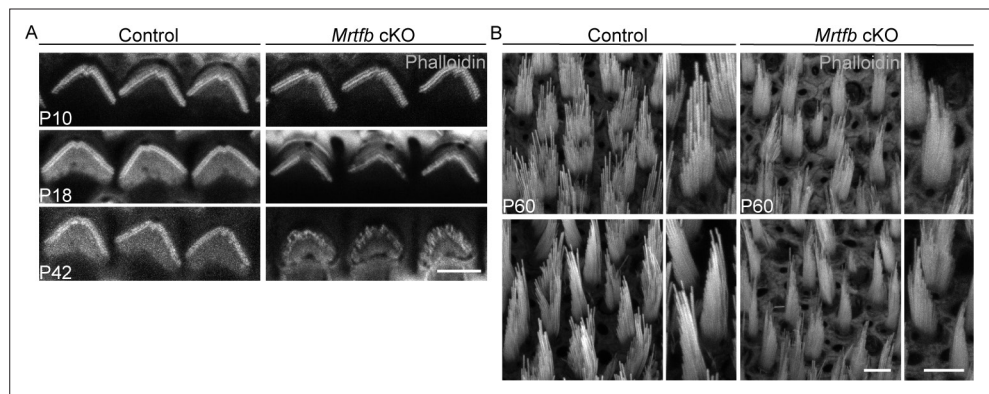


Figure 5—figure supplement 1. *Mrtfb* cKO mice have stereocilia impairments. **(A)** Phalloidin staining of outer hair cell (OHC) bundles in control and *Mrtfb* cKO at different ages. **(B)** Phalloidin staining of hair bundles in P60 control and *Mrtfb* cKO utricle. Upper, bundles of striolar HCs. Bottom, bundles of extrastriolar HCs. Higher magnification of hair bundles were shown on the right side of the panel. Scale bars in all panels represent 5 μm.

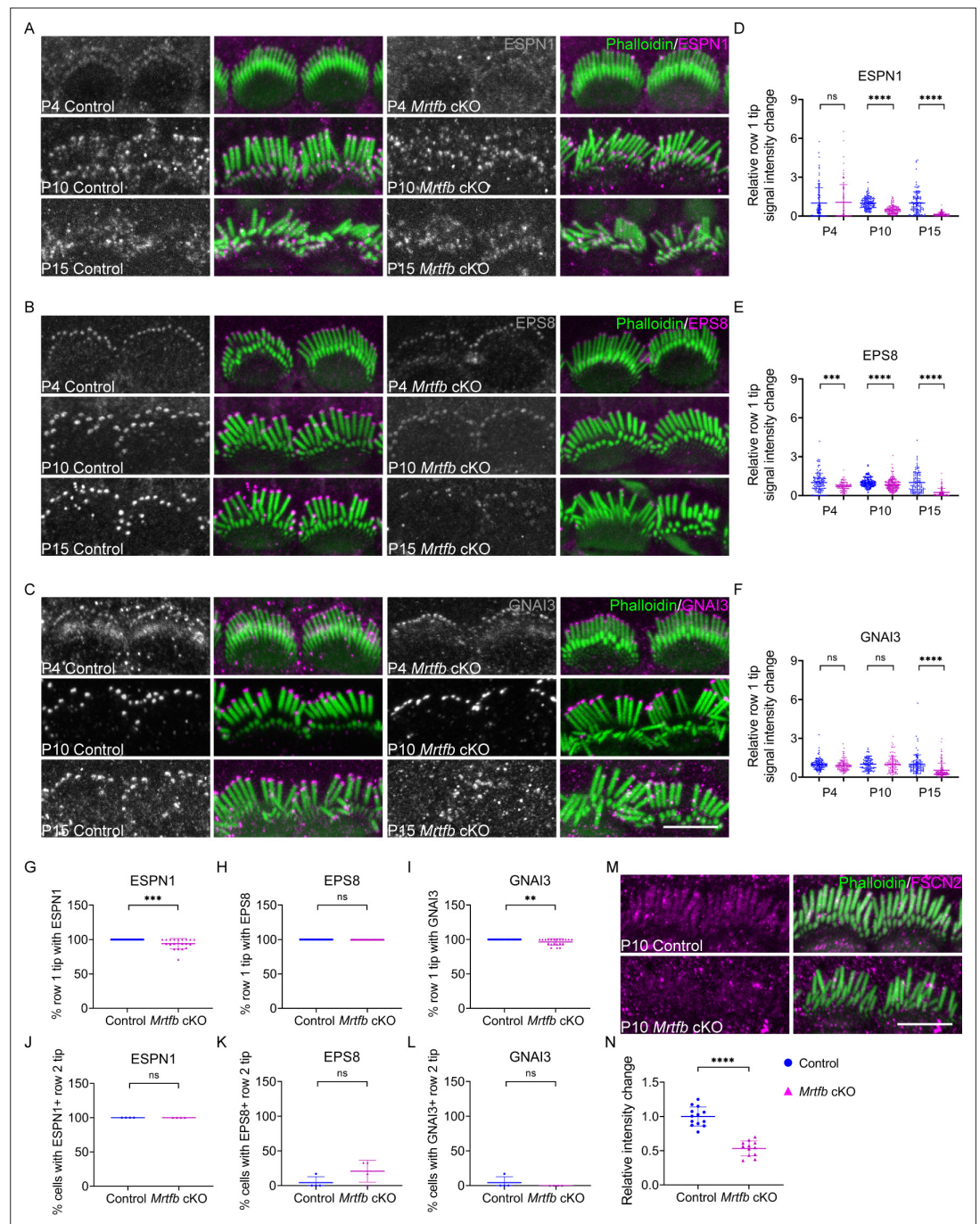


Figure 6. Altered distribution of tip proteins and actin crosslinker in *Mrtfb* cKO mice. **(A–C)** Immunofluorescence localization of ESPN1, EPS8, and GNAI3 in inner hair cells (IHCs) of control and *Mrtfb* cKO mice at different ages. Left panels are proteins staining separately from the phalloidin (in grayscale). **(D–F)** Quantitation of immunoreactivity of ESPN1, EPS8, and GNAI3 in IHCs of control and *Mrtfb* cKO at different ages. Analyzed numbers (stereocilia, cells, animals): ESPN1, P4 control (110, 11, 4), P4 *Mrtfb* cKO (110, 11, 4), P10 control (130, 13, 5), P10 *Mrtfb* cKO (140, 14, 5), P15 control (110, 11, 4), P15 *Mrtfb* cKO (120, 12, 4). EPS8, P4 control (120, 12, 5), P4 *Mrtfb* cKO (100, 10, 5), P10 control (190, 19, 5), P10 *Mrtfb* cKO (200, 20, 5), P15 control (110, 11, 5), P15 *Mrtfb* cKO (110, 11, 5). GNAI3, P4 control (120, 12, 5), P4 *Mrtfb* cKO (110, 11, 5) P10 control (100, 10, 4), P10 *Mrtfb* cKO (100, 10, 4), P15 control (100, 11, 4), P15 *Mrtfb* cKO (120, 11, 4). **(G–I)** Percentage of row 1 tip with ESPN1, EPS8, or GNAI3 staining to all row 1 stereocilia in IHCs of control and *Mrtfb* cKO at P10. **(J–L)** Percentage of IHCs with ESPN1, EPS8, or GNAI3 staining on the row 2 tips of control and *Mrtfb* cKO at P10. Analyzed numbers in G–L (cells, animals): ESPN1, control (24, 5), *Mrtfb* cKO (20, 5). EPS8, control (24, 5), *Mrtfb* cKO (24, 5). GNAI3, control

Figure 6 continued on next page

Figure 6 continued

(24, 4), *Mrtfb* cKO (21, 4). **(M)** FSCN2 immunostaining in control and *Mrtfb* cKO IHCs. **(N)** Quantification of FSCN2 reactivity in IHC stereocilium shafts at P10. Analyzed numbers (stereocilia, cells, animals): control (130, 13, 6), *Mrtfb* cKO (120, 12, 6). Scale bars, 5 μ m. Error bars indicate standard deviation (SD), p values were derived from two-tailed unpaired Student's t-test, ****p value<0.0001, ***p value<0.001, and **p value<0.01.

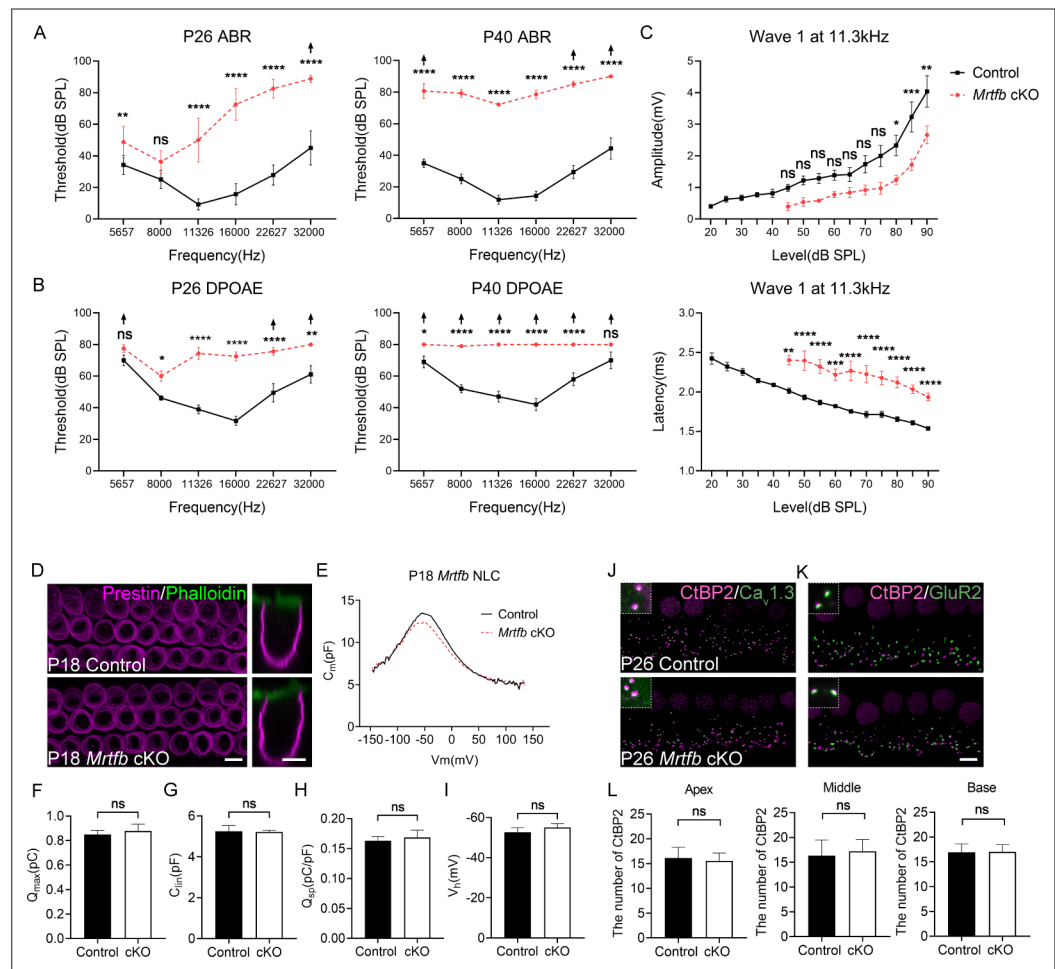


Figure 7. Early-onset and progressive hearing loss in *Mrtfb* cKO mice. **(A)** Auditory brainstem response (ABR) analysis demonstrates *Mrtfb* cKO mice exhibited early-onset and progressive hearing loss. **(B)** Distortion product otoacoustic emission (DPOAE) thresholds in *Mrtfb* cKO mice were significantly elevated at P26 and at P42 compared to control. **(C)** ABR wave1 amplitude (upper panel) and latency (lower panel) at 11.3 kHz in control and *Mrtfb* cKO mice at P26. Analyzed numbers (animals): P26, control (7), *Mrtfb* cKO (8). P42, control (8), *Mrtfb* cKO (7). Arrows indicate that at fixed frequency, no response was identified at the maximum output. Error bars indicate standard error of the mean (SEM), p values were derived from two-way analysis of variance (ANOVA) followed by Bonferroni post-test. **(D)** Prestin immunostaining in outer hair cells (OHCs) at P18, side views of OHCs are on the right. **(E)** Nonlinear capacitance (NLC) recordings of OHCs in control and *Mrtfb* cKO mice at P18. **(F–I)** Quantitative analysis of Q_{max} , C_{in} , Q_{sp} , and V_h showed no significant difference between control and *Mrtfb* cKO OHCs. Analyzed numbers (cells, animals): control (5, 4), *Mrtfb* cKO (5, 4). **(J, K)** CtBP2-Ca_v1.3 and CtBP2-GluR2 co-immunostaining in inner hair cells (IHCs). Higher magnification images are in the upper dotted box. **(L)** Comparison of IHC ribbon synapse numbers between control and *Mrtfb* cKO mice at different cochlear positions at P26. Analyzed numbers (cells, animals): apical turns, control (28, 5), *Mrtfb* cKO (28, 5). Middle turns, control (20, 6), *Mrtfb* cKO (26, 6). Basal turns, control (34, 6), *Mrtfb* cKO (32, 6). Scale bars in all panels represent 5 μ m. Error bars indicate standard deviation (SD), p values were derived from two-tailed unpaired Student's *t*-test. ****p-value <0.0001, ***p-value <0.001, **p-value <0.01, and *p-value <0.05.

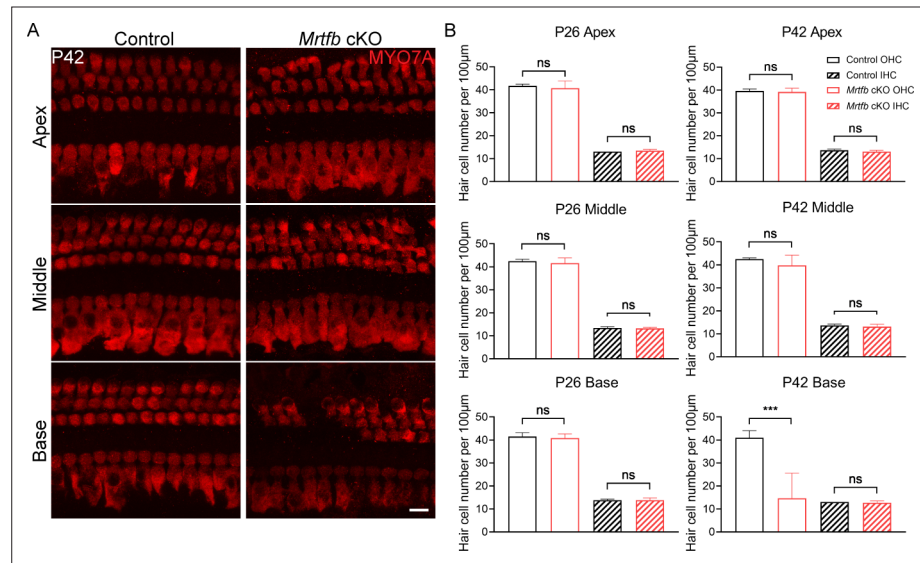


Figure 7—figure supplement 1. Hair cell numbers were not affected in *Mrtfb* cKO mice. **(A, B)** The hair cell numbers were not affected in *Mrtfb* cKO mice at P26. Analyzed numbers (animals): P26, control (4), *Mrtfb* cKO (4). P42, control (3), *Mrtfb* cKO (3). Scale bar, 5 µm. Error bars indicate standard deviation (SD), p values were derived from two-tailed unpaired Student's t-test, ***p value<0.001.

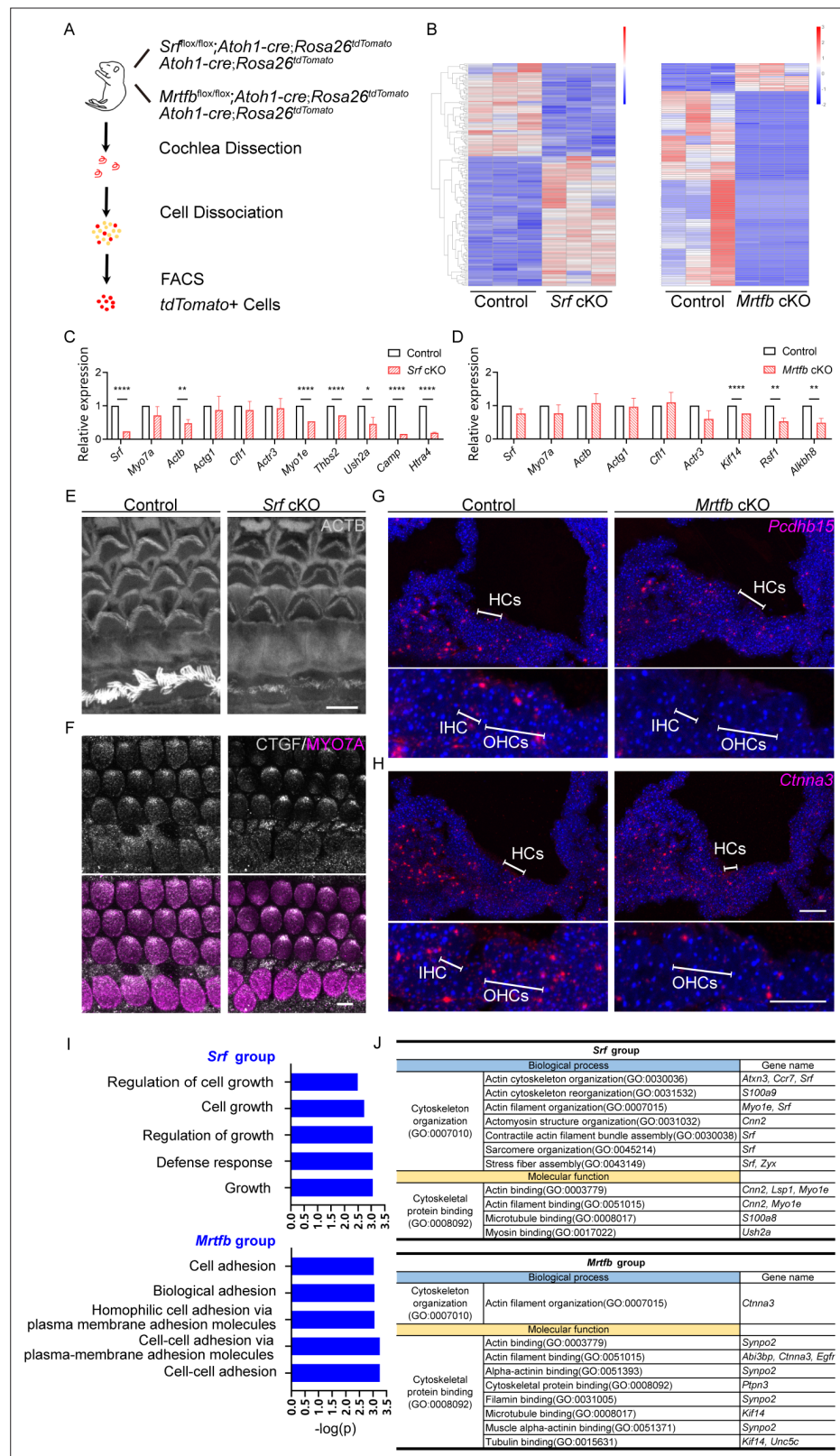


Figure 8. *Srf* cKO and *Mrtfb* cKO hair cell transcriptome analysis. **(A)** Diagram of fluorescence-activated cell sorting (FACS) purification strategy to isolate hair cells. The genotypes of mice used for cell sorting are on the right of the mouse cartoon. **(B)** The heatmaps of all the differentially expressed genes (DEGs) in *Srf* cKO and *Mrtfb* cKO ($p_{adj} < 0.05$ and $|\log_2(\text{fold change})| > 1$). Color-coded key bar indicates normalized expression values, Figure 8 continued on next page

Figure 8 continued

relatively high expression levels are indicated by red colors, whereas blue colors represent lower expression levels. **(C, D)** RT-qPCR validation of the expression levels of genes identified in control, *Srf* cKO and *Mrtfb* cKO RNA-Seq, normalized to GAPDH. Error bars indicate standard error of the mean (SEM), p values were derived from one-way analysis of variance (ANOVA) followed by Bonferroni post-test, ****p value<0.0001, **p value<0.01, and *p value<0.05. **(E, F)** Reduced ACTB and CTGF immunostaining in *Srf* cKO HCs. Scale bars, 5 μ m. **(G, H)** RNAscope in situ hybridizations analysis of *Pcdhb15* and *Ctnna3* expression in P2 frozen sections of cochleae from control and *Mrtfb* mutants. Nuclei were labeled with 4'-6-diamidino-2-phenylindole (DAPI). Scale bar, 20 μ m. **(I)** Gene Ontology (GO) enrichment analysis (Biological Process) of down-regulated DEGs in *Srf* cKO and *Mrtfb* cKO. **(J)** Summary of functional clustering of down-regulated DEGs by *Srf* or *Mrtfb* deficiency, according to gene's GO annotations in Gene Ontology Resource.

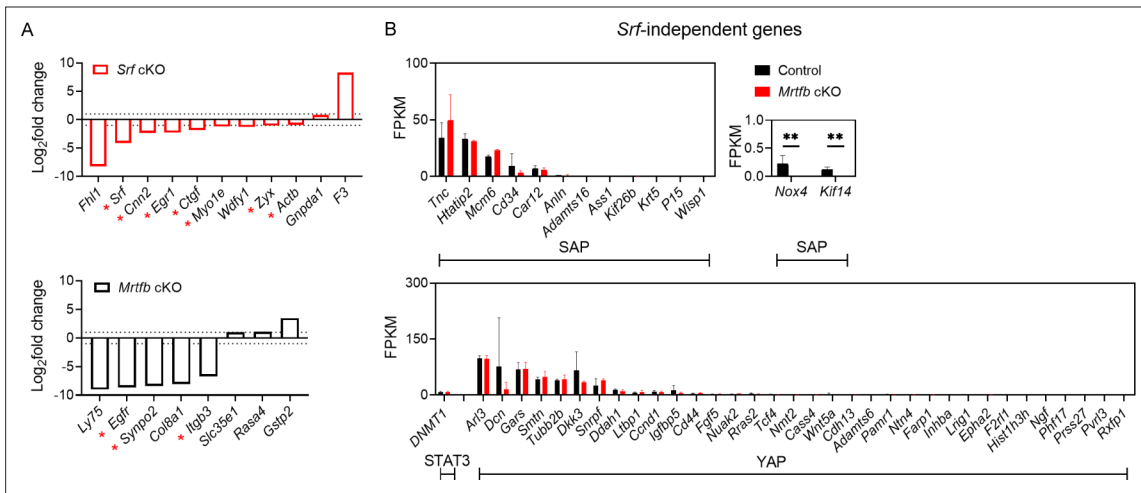


Figure 8—figure supplement 1. Validation of RNA-sequencing results. **(A)** Comparison of *Srf* cKO or *Mrtfb* cKO's regulated genes which are known downstream of MRTF–SRF pathway. Dotted lines on the plot represent the cutoff to annotate the differentially expressed genes (DEGs). Asterisks indicate the genes involved in the regulation of cytoskeleton. **(B)** The expression level of the reported *Srf*-independent genes sets in control and *Mrtfb* cKO, indicated by FPKM. Error bars indicate standard deviation (SD), **p-value <0.01.

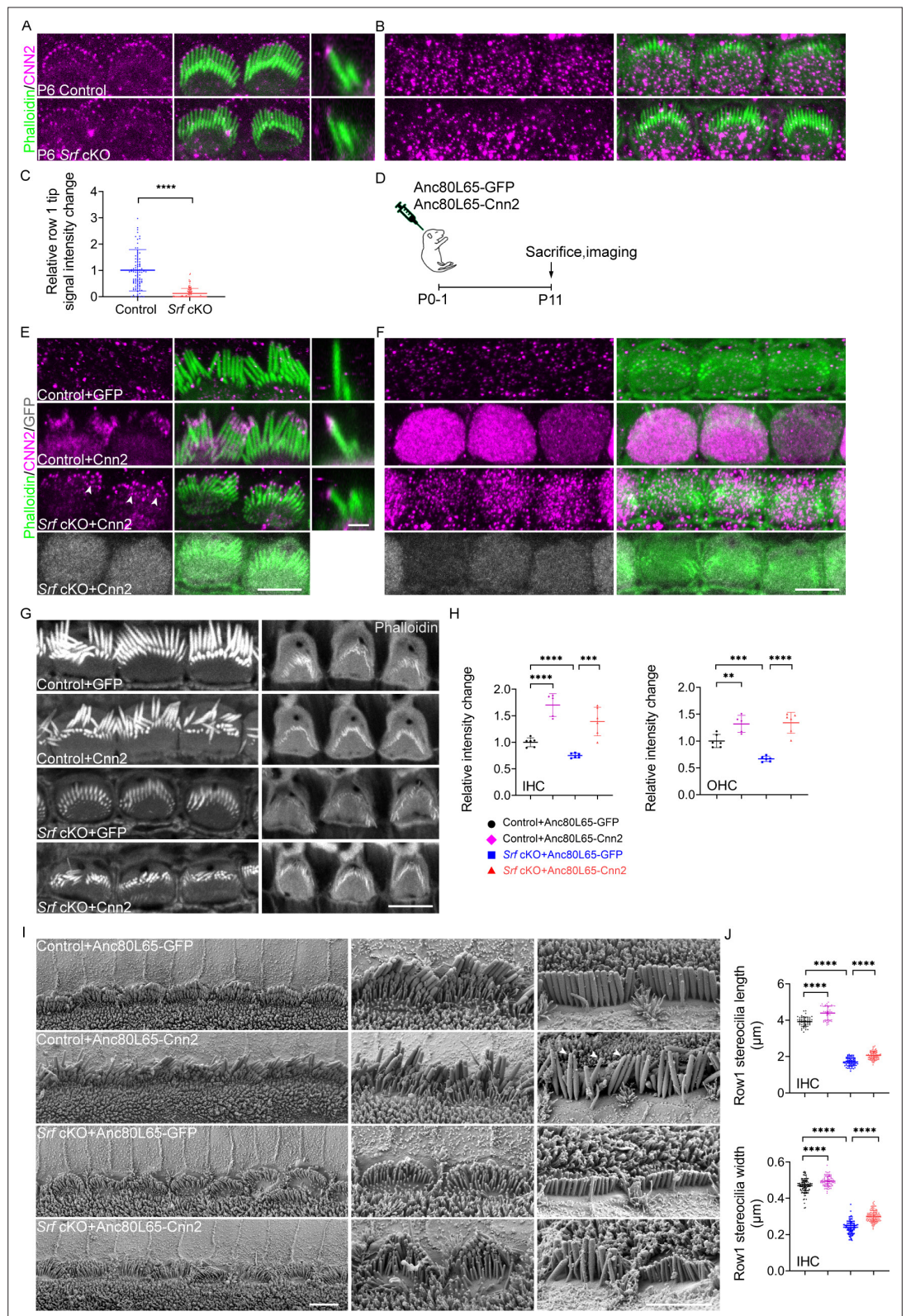


Figure 9. The injection of Anc80L65-Cnn2 partially restores the cuticular plate integrity and stereocilia morphology in *Srf* cKO mice. **(A, B)** CNN2 immunostaining in the stereocilia and cuticular plates of P6 inner hair cells (IHCs). **(C)** Quantitation of CNN2 reactivity in row 1 IHC stereocilia. Analyzed numbers (stereocilia, cells, animals): control (100, 10, 4), *Srf* cKO (110, 11, 4). **(D)** Depiction of the experimental paradigm for in vivo virus injection. **(E, F)** CNN2 Figure 9 continued on next page

Figure 9 continued

immunostainings in the stereocilia and cuticular plates of P11 control mice injected with Anc80L65-GFP or Anc80L65-Cnn2, and *Srf* cKO injected with Anc80L65-Cnn2. The bottom panels in E and all panels in F were taken at the level of the cuticular plate. The GFP expression in IHCs of *Srf* cKO injected with Anc80L65-Cnn2 indicate the efficiency of *Cnn2* delivery. Arrowheads indicate CNN2 at row 2 stereocilia tips. **(G)** Phalloidin staining in the cuticular plates of Anc80L65-GFP-injected control, Anc80L65-Cnn2-injected control, Anc80L65-GFP-injected *Srf* cKO, and Anc80L65-Cnn2-injected *Srf* cKO hair cells at P11. **(H)** Quantification of phalloidin reactivity in the cuticular plates of P12 IHCs and outer hair cells (OHCs) from Anc80L65-GFP-injected control, Anc80L65-Cnn2-injected control, Anc80L65-GFP-injected *Srf* cKO, and Anc80L65-Cnn2-injected *Srf* cKO mice. Analyzed numbers (cells, animals): IHCs, Anc80L65-GFP-injected control (52, 5), Anc80L65-Cnn2-injected control (47, 5), Anc80L65-GFP-injected *Srf* cKO (49, 5), Anc80L65-Cnn2-injected *Srf* cKO (61, 6). OHCs, Anc80L65-GFP-injected control (194, 5), Anc80L65-Cnn2-injected control (147, 5), Anc80L65-GFP-injected *Srf* cKO (150, 5), Anc80L65-Cnn2-injected *Srf* cKO (204, 6). **(I)** Representative scanning electron microscopy (SEM) images of P11 apical IHCs of Anc80L65-GFP-injected control, Anc80L65-Cnn2-injected control, Anc80L65-GFP-injected *Srf* cKO, and Anc80L65-Cnn2-injected *Srf* cKO. Arrowheads indicate abnormal thin stereocilia tips. **(J)** SEM-based quantification of row 1 stereocilia length and width of P11 apical IHCs. Analyzed numbers (stereocilia, cells, animals): lengths, Anc80L65-GFP-injected control (52, 16, 4), Anc80L65-Cnn2-injected control (44, 13, 5), Anc80L65-GFP-injected *Srf* cKO (70, 20, 5), Anc80L65-Cnn2-injected *Srf* cKO (99, 30, 6). Widths, Anc80L65-GFP-injected control (107, 20, 4), Anc80L65-Cnn2-injected control (75, 20, 5), Anc80L65-GFP-injected *Srf* cKO (121, 25, 5), Anc80L65-Cnn2-injected *Srf* cKO (130, 30, 6). In E, the scale bar for side-view represents 1 μm and scale bars in other panels represent 5 μm . Error bars indicate standard deviation (SD), p values were derived from two-tailed unpaired Student's t-test, ****p value < 0.0001, ***p value < 0.001, and **p value < 0.01.

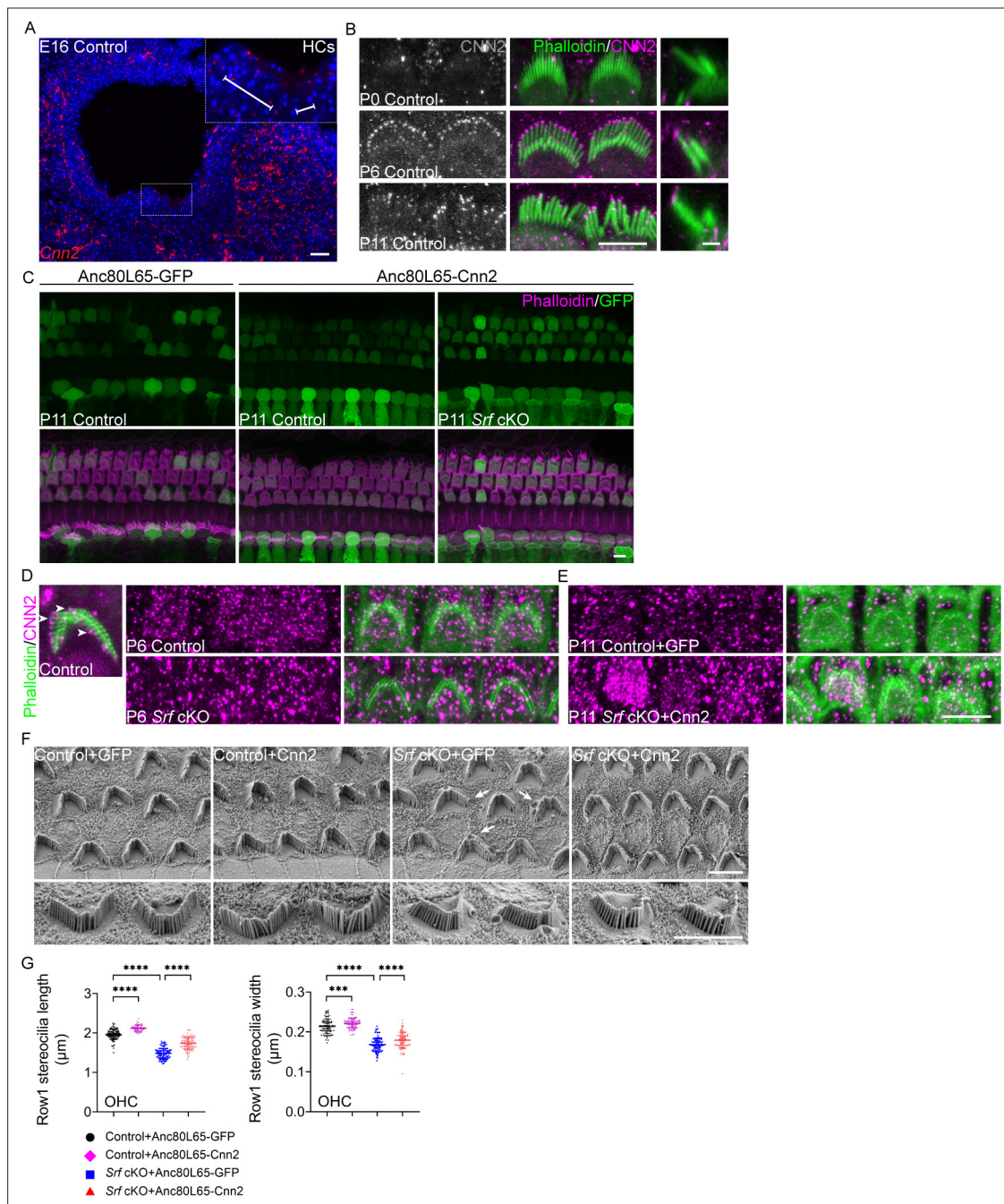


Figure 9—figure supplement 1. The injection of AAV-Anc80L65-Cnn2 partially restores outer hair cell (OHC) stereocilia morphology in *Srf* cKO mice. **(A)** RNAscope in situ hybridization of *Cnn2* in E16 control cochlea. Inset indicates hair cells. **(B)** Immunohistochemical analysis of CNN2 expression in control inner hair cells (IHCs) at different ages. Right panels are side views. **(C)** The infection patterns of Anc80L65-GFP and Anc80L65-Cnn2 in hair cells of control and *Srf* cKO. **(D)** CNN2 immunoreactivity in the stereocilia and cuticular plates of OHCs. Left: CNN2 staining in the stereocilia of control OHCs at P6. Arrowheads indicate CNN2 at row 1 stereocilia tips. Right: CNN2 staining in the cuticular plates of control and *Srf* cKO OHCs at P6. **(E)** CNN2 immunostainings in the cuticular plates of Anc80L65-GFP-injected control and Anc80L65-Cnn2-injected *Srf* cKO OHCs at P11. **(F)** Representative scanning electron microscopy (SEM) images of P11 apical OHCs of Anc80L65-GFP-injected control, Anc80L65-Cnn2-injected control, Anc80L65-GFP-injected *Srf* cKO, and Anc80L65-Cnn2-injected *Srf* cKO. Arrows indicate the raised apical plasma membrane of cuticular plate in *Srf* cKO hair cells. **(G)** SEM-based quantification of row 1 stereocilia length and width in P11 apical OHCs of Anc80L65-GFP-injected control, Anc80L65-GFP-injected *Srf* cKO, and Anc80L65-Cnn2-injected *Srf* cKO. Analyzed numbers (stereocilia, cells, animals): lengths: Anc80L65-GFP-injected control (117, 35, 4), Anc80L65-Cnn2-injected control (66, 25, 5), Anc80L65-GFP-injected *Srf* cKO (100, 32, 5), Anc80L65-Cnn2-injected *Srf* cKO (101, 30, 6). Widths: Anc80L65-GFP-injected control (90, 30, 4), Anc80L65-Cnn2-injected control (94, 30, 5), Anc80L65-GFP-injected *Srf* cKO (105, 35, 5), Anc80L65-Cnn2-injected *Srf* cKO (111, 32, 6). In A, scale bar represents 20 µm. In B, scale bar for the side-view represents 1 µm. Scale bars in others panels represent 5 µm. Error bars indicate standard deviation (SD), p values were derived from two-tailed unpaired Student's t-test, ****p value<0.0001, and ***p value<0.001.

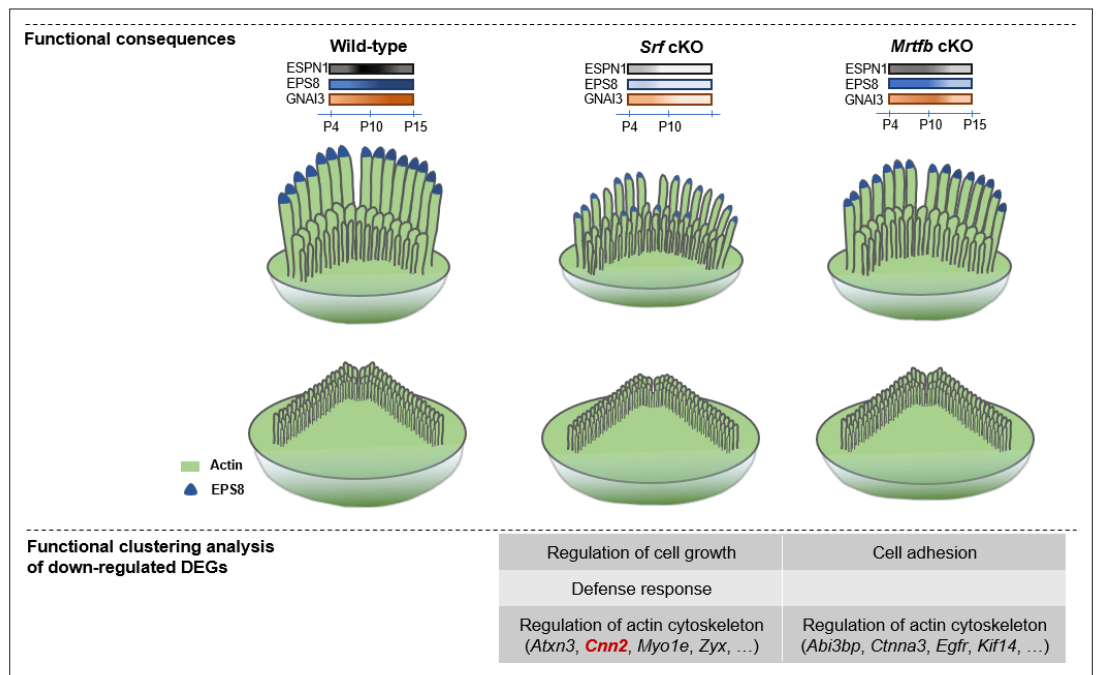


Figure 10. An illustration model of differential regulation of actin cytoskeleton mediated by SRF and MRTFB in hair cells.