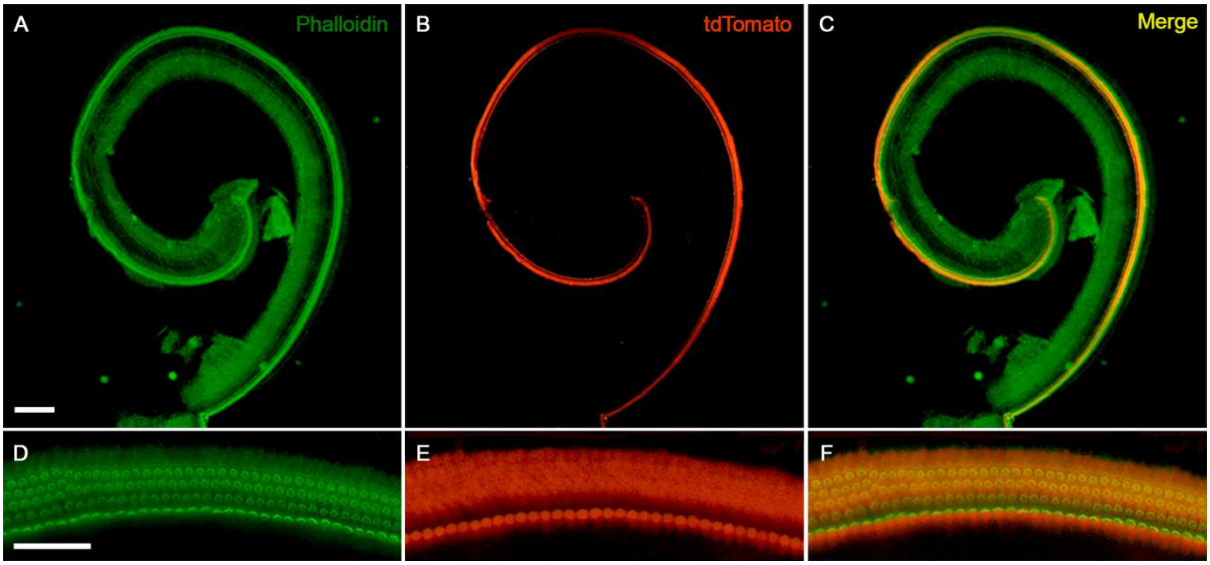
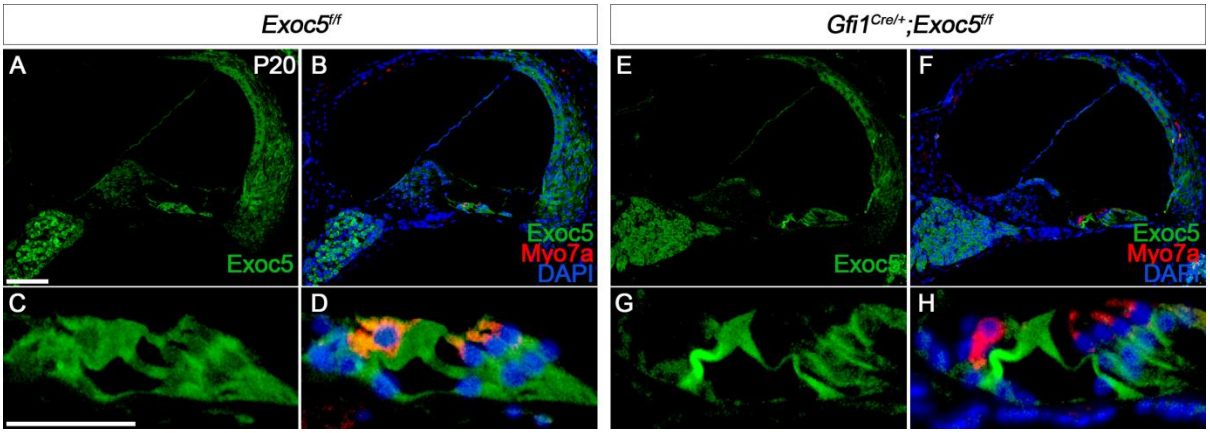
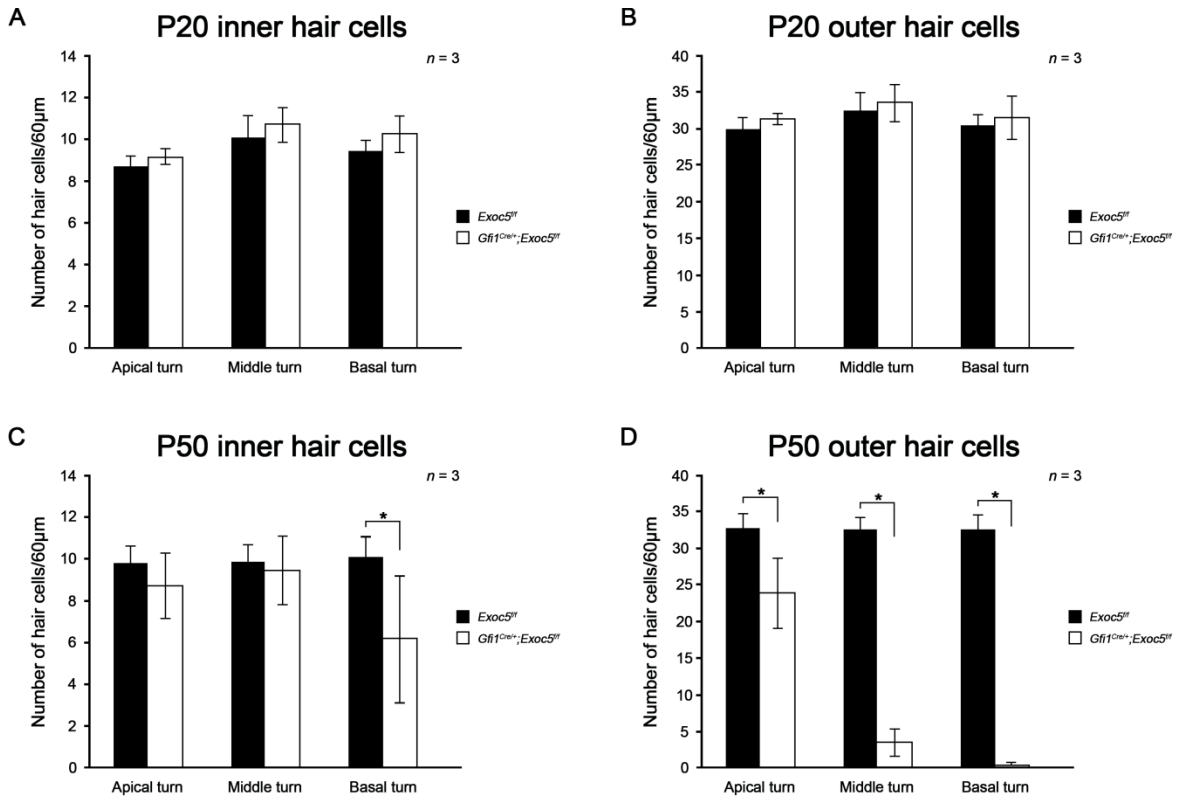


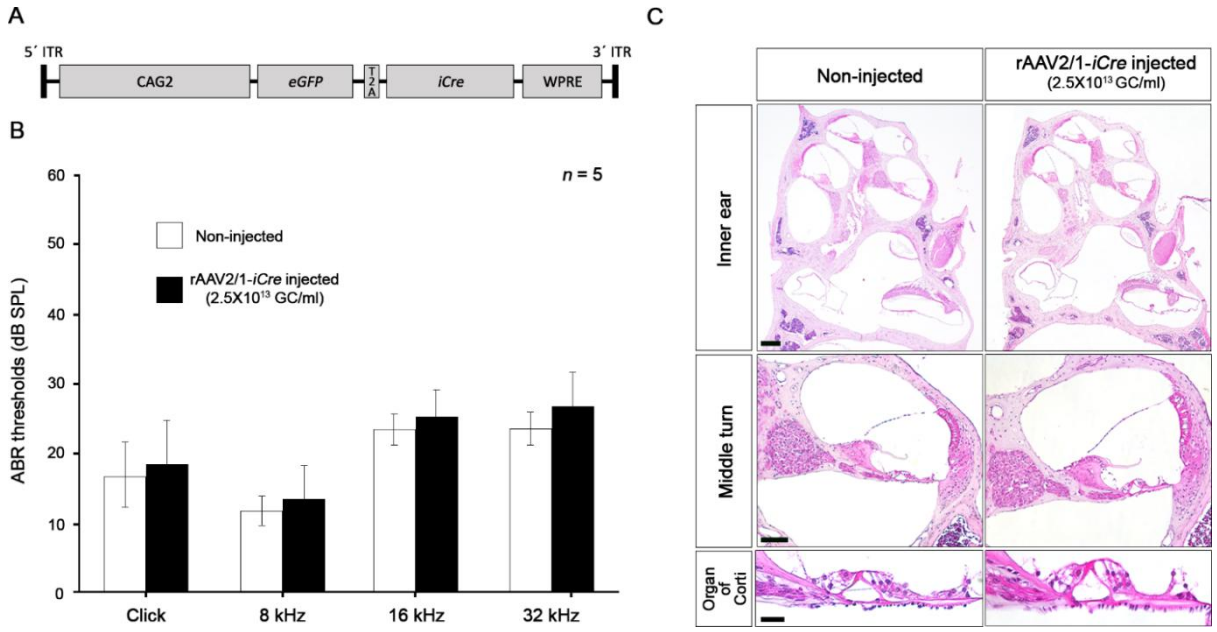
Supplementary Figure 1. *Gfi1*$^{Cre/+}$ expression in the inner ear. The phalloidin stained actin filaments (green) and hair cell-specific tdTomato signals (red) are shown in the cochlea of *Gfi1*$^{Cre/+}$*;tdTomato* mice at the P3. (A-C) Whole cochlea. (D-F) Middle turn of cochlea. Scale bars, 200 μm in (A-C) and 25 μm in (D-F).



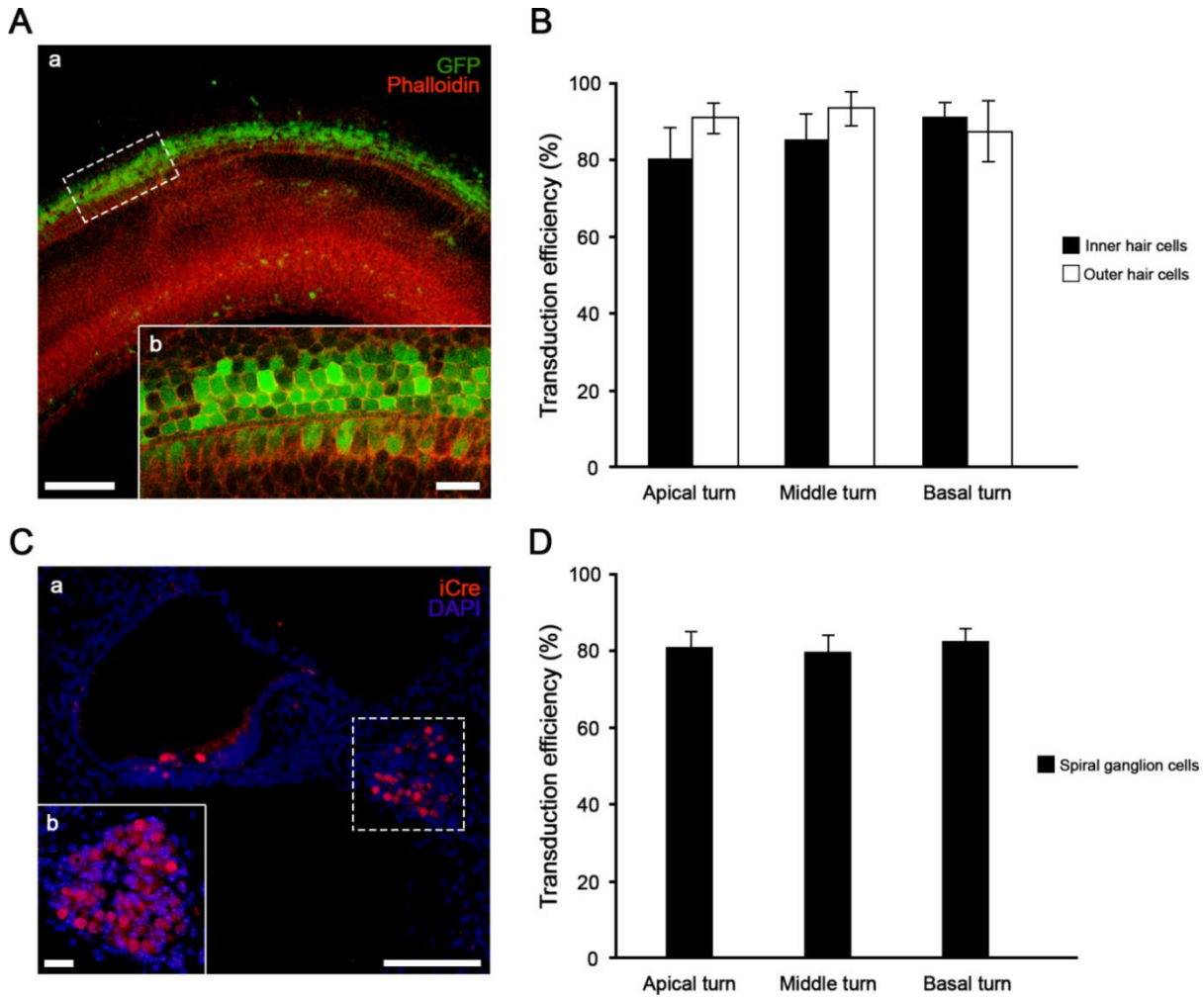
Supplementary Figure 2. Exoc5 antibody validation by immunofluorescence in Exoc5f/f and *Gfi1*$^{Cre/+}$*;Exoc5*$^{f/f}$ inner ear at P20. Sensory and non-sensory epithelial expression of EXOC5 was confirmed using rabbit anti-Exoc5 antibody purchased from Abcam Inc. (A-D). In hair-cell-specific *Exoc5* knockout cochlea (*Gfi1*$^{Cre/+}$*;Exoc5*$^{f/f}$, negative control), EXOC5 expression was negative in both inner and outer hair cell specifically (E-H). Scale bars, 50 μm in (A, B, E, F) and 25 μm in (C, D, G, H).



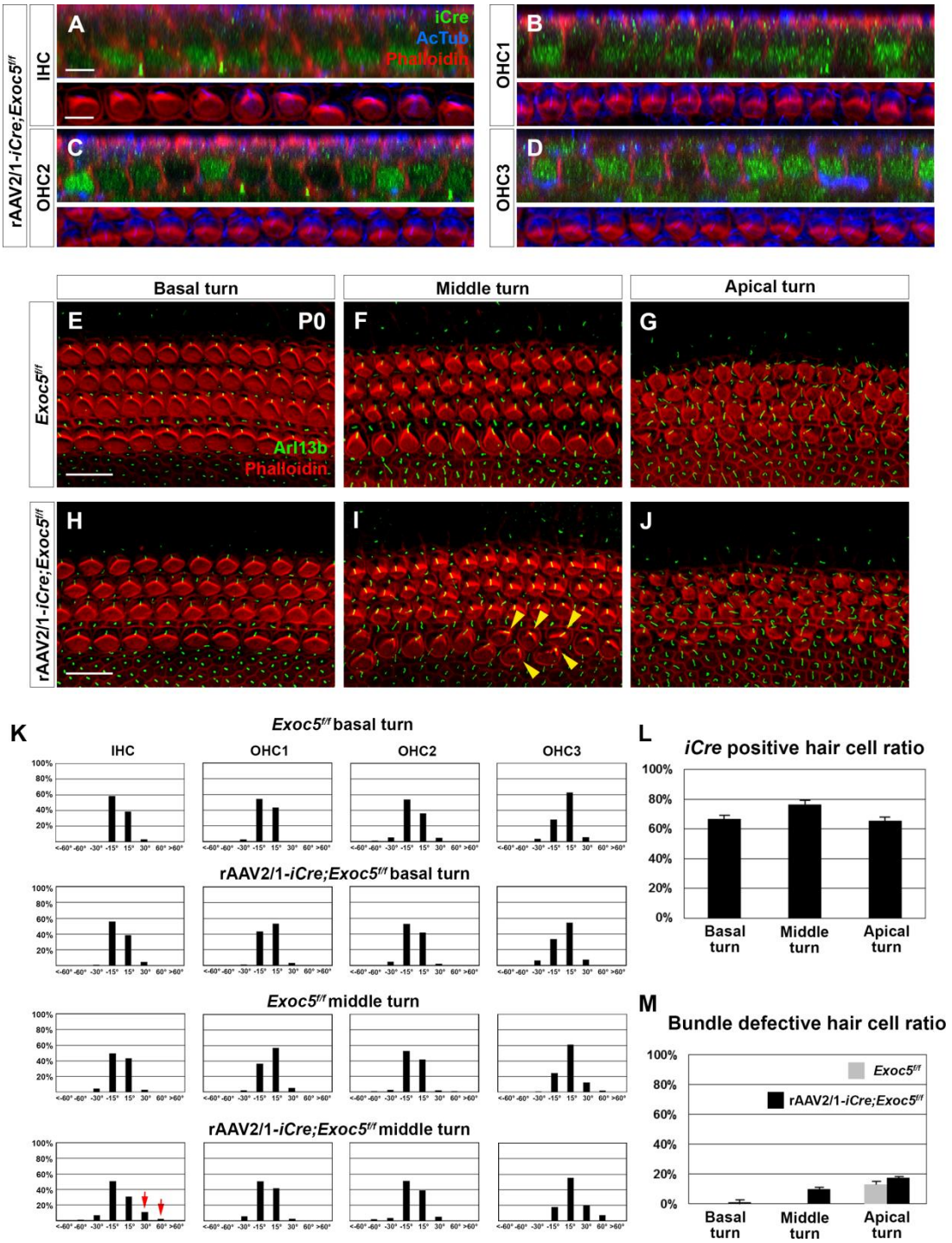
Supplementary Figure 3. Quantitation analysis of remaining hair cells in *Gfi1*$^{Cre/+}$*;Exoc5*$^{f/f}$. The average number of inner and outer hair cells per 60 μm length in the organ of Corti was counted at each turn in *Exoc5*$^{f/f}$ (black) and *Gfi1-Cre;Exoc5*$^{f/f}$ (white). (A) Inner hair cells at P20. (B) Outer hair cells at P20. (C) Inner hair cells at P50. (D) Outer hair cells at P50 (* $P < 0.01$). The data are shown as the mean±SD.



Supplementary Figure 4. Safety validation of rAAV2/1-*iCre* injection. (A) Construction of rAAV2/1-*iCre*. (B) Click and tone-burst (8, 16, and 32 kHz) ABR thresholds in the rAAV2/1-*iCre* non-injected inner ear (white rectangle) and 2.5×10^{13}GC/ml of rAAV2/1-*iCre* injected inner ear (black rectangle) of wild-type mice at P20. No statistically significant difference between the rAAV2/1-*iCre* non-injected group and injected group was observed. *(*n = 5, P > 0.05). (C) H&E staining is performed in the non-injected inner ear and the rAAV2/1-*iCre* injected inner ear of wild type mice at P20. The histology of whole inner ear (upper panels), middle turn of cochlea (middle panels) and organ of Corti (lower panels) was shown. Scale bars, 200 μm in upper panels, 100 μm in middle panels, and 25 μm in lower panels.

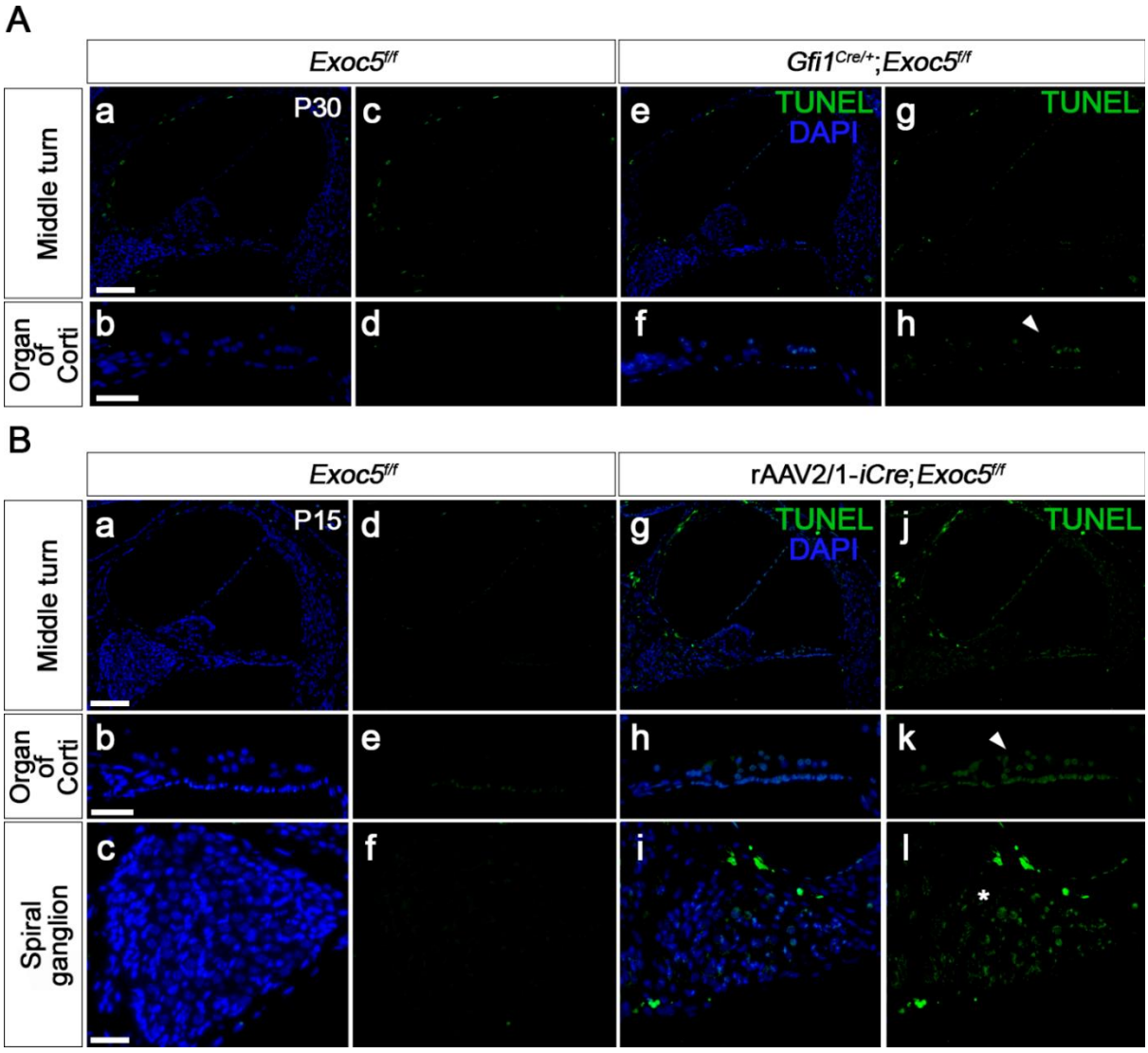


Supplementary Figure 5. Analysis of rAAV2/1-*iCre* transduction efficiency in the inner ear. iCre transduction was confirmed by immunostaining for iCre (red) or GFP (green) (A, C), and transduction efficiency was quantified (B, D) in rAAV2/1-*iCre* injected *Exoc5*$^{f/f}$ mice at P0. (A) GFP expression detected in iCre-transduced hair cells and phalloidin-stained actin filaments (red). (B) The percentage (%) of iCre-transduced inner and outer hair cells at each turn. (C) Expression of iCre (red) in sensory epithelium (a) and spiral ganglions (b). (D) The percentage (%) of iCre-positive cells in spiral ganglions. White dotted rectangles highlight regions of higher magnification (b, d). The data are shown as the mean±SD. Scale bars, 100 μm in (A-a and C-a), 20 μm in (A-b, C-b).



Supplementary Figure 6. Whole mount immunostaining of hair cells and kinocilia in the cochlea of rAAV2/1-*iCre;Exoc5*$^{f/f}$ mice. Primary cilia (kinocilia) and stereocilia were stained in P0 *Exoc5*$^{f/f}$ and rAAV2/1-*iCre;Exoc5*$^{f/f}$ cochlea with AcTub antibody (blue), iCre antibody (green) and phalloidin (red) (A-D) or Arl13b antibody (green) and phalloidin (red) (E-J), respectively. Kinocilia are

present in all hair cells in both *Exoc5*$^{f/f}$ and rAAV2/1-*iCre;Exoc5*$^{f/f}$ cochlea. A few extra hair cells were present in the middle and apical turns of rAAV2/1-*iCre;Exoc5*$^{f/f}$ cochlea (I, arrowheads). (K) The locations of kinocilia in the hair cells were not significantly altered in rAAV2/1-*iCre;Exoc5*$^{f/f}$ cochlea when compared to *Exoc5*$^{f/f}$ except for a few inner hair cells in the middle turn (arrows). (L) The graph showing that at least 60% or more of hair cells are iCre-positive in each of the three turns. (M) The ratio of bundle defective hair cells were less than 20% of total iCre-positive hair cells. Scale bars, 10 μm.



Supplementary Figure 7. Apoptotic cell death in the sensory epithelial cells of the *Gfi1*$^{Cre/+}$*;Exoc5*$^{f/f}$ mice and rAAV2/1-*iCre;Exoc5*$^{f/f}$ mice by TUNEL assay. Apoptotic cell death was analyzed using TUNEL assay in the both of two different lines of Exoc5 deficiency mice. (A) The green

fluorescence labeled apoptotic death cells represented in the middle turn of cochlea (a, c, e, and g) and in the magnified image of organ of Corti (b, d, f, and h) from *Exoc5*$^{f/f}$ and *Gfi1*$^{Cre/+}$*;Exoc5*$^{f/f}$ mice at P30. (B) The green fluorescence labeled apoptotic death cells represented in the middle turn of cochlea (a, d, g, and j), magnified image of organ of Corti (b, e, h, and k), and magnified image of organ of Corti (c, f, i, and l) from the contralateral ear (*Exoc5*$^{f/f}$) and the rAAV2/1-*iCre* injected ear (rAAV2/1-*iCre;Exoc5*$^{f/f}$) at P15. Arrow heads are indicated in which represented TUNEL signal in hair cells and asterisks are indicated in which represented TUNEL signal in spiral ganglions The nuclei were counterstained with DAPI (blue). Scale bars, 100 μm in (Middle turn panels) and 25 μm in (Organ of Corti and spiral ganglion panels).