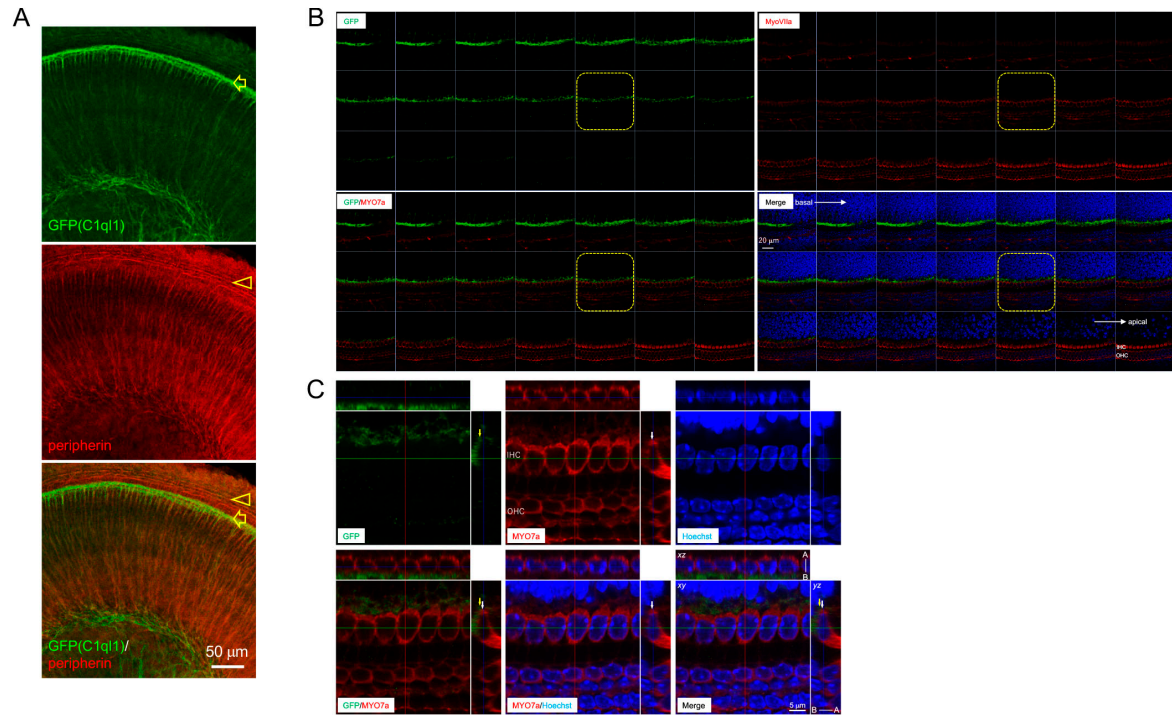
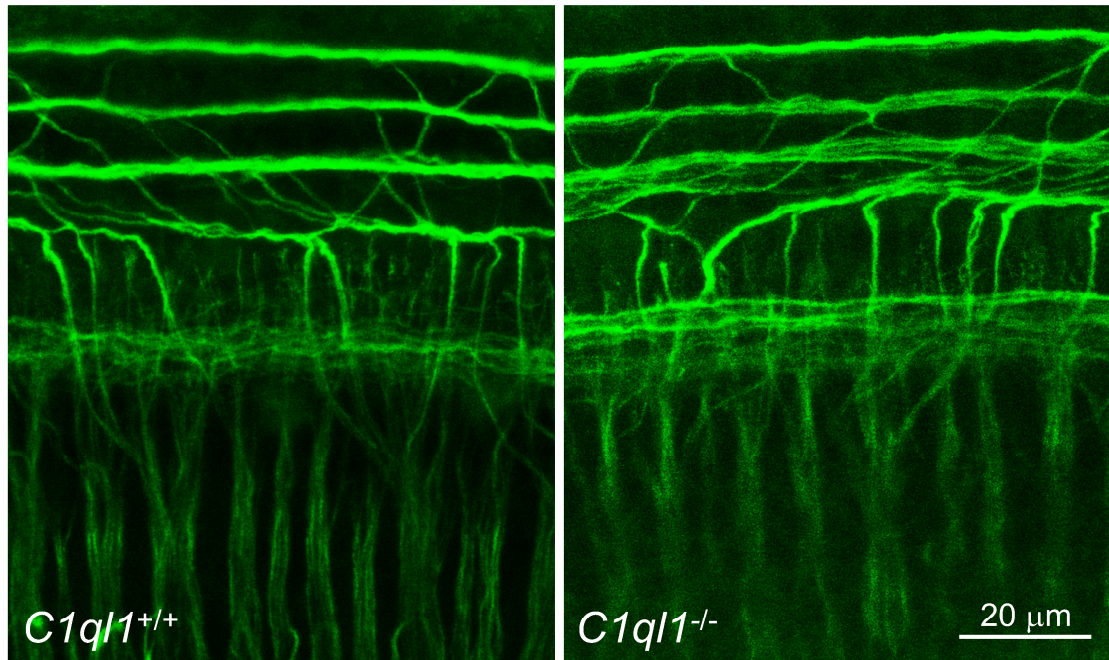


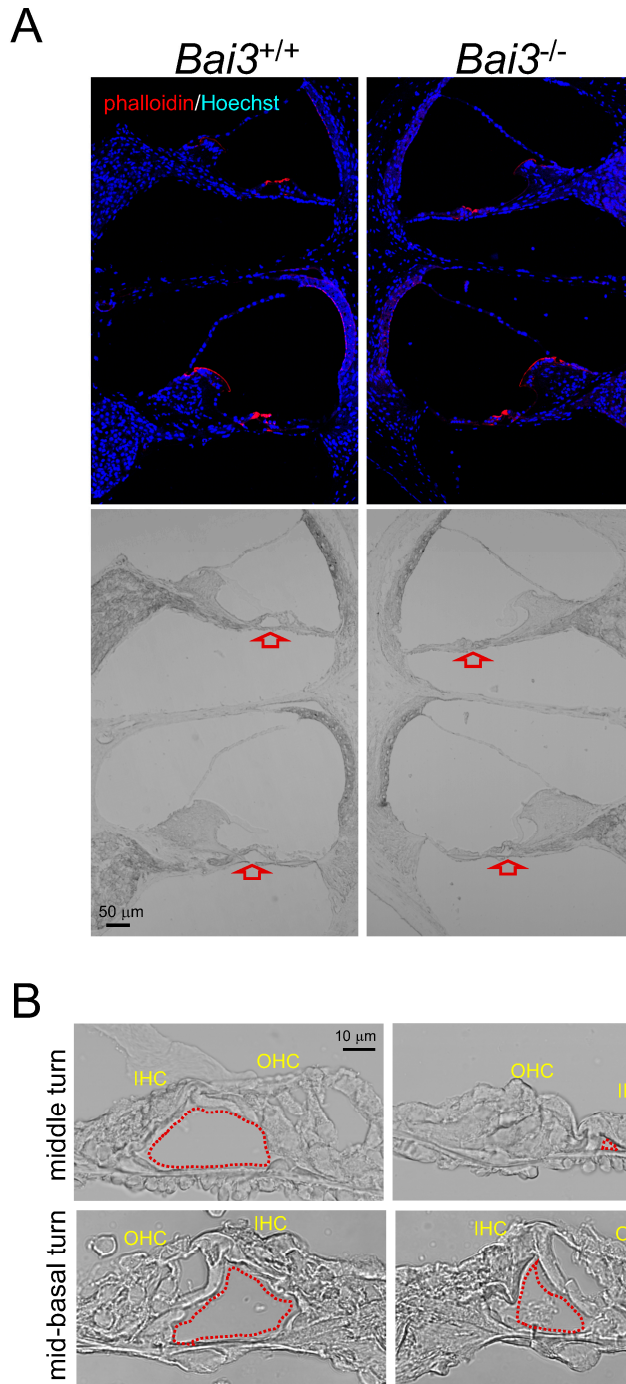
**Figure S1:** Immunofluorescence of *Bai3* (green) is observed in hair cells of *Bai3*<sup>+/+</sup> mice at E18.5 (A) and P0 (B). Tissues are co-immunostained with MYO7a (red), SOX2 (white). Nuclei were stained with Hoechst 33258 (blue). Yellow hollow arrows indicate organ of Corti. In adult *Bai3*<sup>+/+</sup> mice, the expression pattern of *Bai3* changes from the neonatal stage during which *Bai3* is expressed in hair cells, resulting in a pillar cell-specific pattern as shown in Figure 1B. An antibody against the N-terminal extracellular domain of *Bai3* was generated in a previous study [7], and its specificity was confirmed by staining the cochlear section of *Bai3*<sup>-/-</sup> mice. Immunofluorescence of *Bai3* in pillar cells were abolished in adult *Bai3*<sup>-/-</sup> mice (C). Tissues were co-immunostained with MYO7a (white) and phalloidin (red). Nuclei were stained with Hoechst 33258 (blue).



**Figure S2:** (A) Cochleae of *C1ql1*<sup>+/GFP</sup> mice at P3 were immunostained using anti-green fluorescent protein (GFP) antibody (green) and anti-peripherin antibody (red). Peripherin-positive neurons innervate the outer hair cells (arrowhead), whereas GFP-positive neurons innervate the inner hair cells (arrows). Each image represents the maximum projection of a confocal z-stack. (B) Images of the sensory epithelium were serially taken along the z-axis from the basal (top left) to apical (bottom right) regions using confocal microscopy. The images marked with yellow dotted lines are enlarged in (C). GFP-positive neurons (green, indicated by yellow arrows) innervate MYO7a-positive inner hair cells (red, indicated by white arrows). A and B in the images denote apical and basal regions of hair cells, respectively. Nuclei were stained with Hoechst 33258 (blue).



**Figure S3:** The cochleae of *C1ql1*<sup>+/+</sup> and homozygous null mutant *C1ql1*<sup>-/-</sup> mice at P3 were immunostained using the pan-neuronal marker, NF-H antibody (green). The innervation pattern of NF-H-positive neurons in hair cells was not significantly different between *C1ql1*<sup>+/+</sup> and *C1ql1*<sup>-/-</sup> mice at P3. Each image represents the maximum projection of a confocal z-stack (4.32 μm x 5 sections).



**Figure S4:** *Bai3*<sup>+/+</sup> and *Bai3*<sup>-/-</sup> cochlea were stained with phalloidin (red in A) and Hoechst (blue in A). Arrows in bright field photo (A, bottom) indicate the location of organ of Corti. (B) shows the organ of Corti of *Bai3*<sup>+/+</sup> (left) and *Bai3*<sup>-/-</sup> (right) mice. Histological analysis revealed that tunnel of Corti, marked by dotted red line, are collapsed in *Bai3*<sup>-/-</sup> mice compared to *Bai3*<sup>+/+</sup> mice. Scale bar, 50  $\mu$ m in (A) and 10  $\mu$ m in (B).



**Table S1:** Experimental conditions for immunostaining.

## Supplemental Table 1\_Methods for Immunohistochemistry

tissue (mouse age)	antigen retrieval	permeabilization	blocking
E18.5, P0	80°C, 1h	0.3% Triton, 10min	10% NDS/PBST
P3	60°C, 1h	0.3% Triton, 20min	10% NDS/PBS
11 weeks (frozen section)	60°C, 1h	-	10% NDS/PBS
11 weeks (paraffin section)	Microwave 5 min x 3	-	10% NDS/PBST
17 months	-	0.3% Triton, 10min	10% NDS/PBST