Supporting Information:

Supplementary Figures:

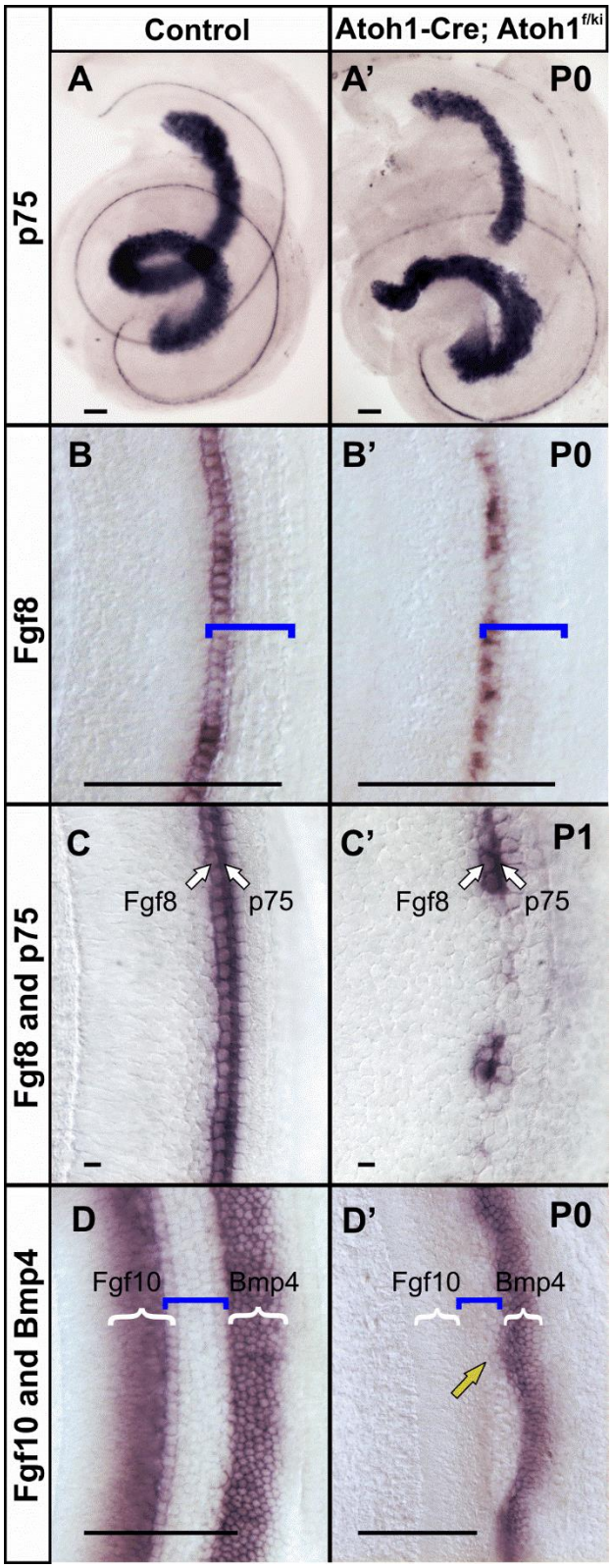

Figure S1. ***Fgf8* expression loss correlates with the *p75* expression loss and *Fgf10* and *Bmp4* are changed.** ISH of p75 reveals expression in the IP cells with a gradient from apex to base of the cochlea in the P0 *Atoh1-Cre; Atoh1*$^{f/kiNeurog1}$ mice (A, A'). *Fgf8* expression is also reduced in the IHC in the P0 *Atoh1-Cre; Atoh1*$^{f/kiNeurog1}$ mice (B, B'). Double ISH of *p75* and *Fgf8* in the P1 cochlea reveal that the loss of *Fgf8* expressing IHC correlates with the loss of *p75* positive IP cells (C,C'). Double ISH of *Fgf10* and *Bmp4* shows that *Fgf10* is nearly eliminated and *Bmp4* flanking the lateral/abneural boundary of the OC shows medial expansion in the *Atoh1-Cre; Atoh1*$^{f/kiNeurog1}$ mice (D, yellow arrow in D') like self-terminating mice (Pan et al., 2012a). Bar indicates 100 μm in all except 10 μm in C,C'.

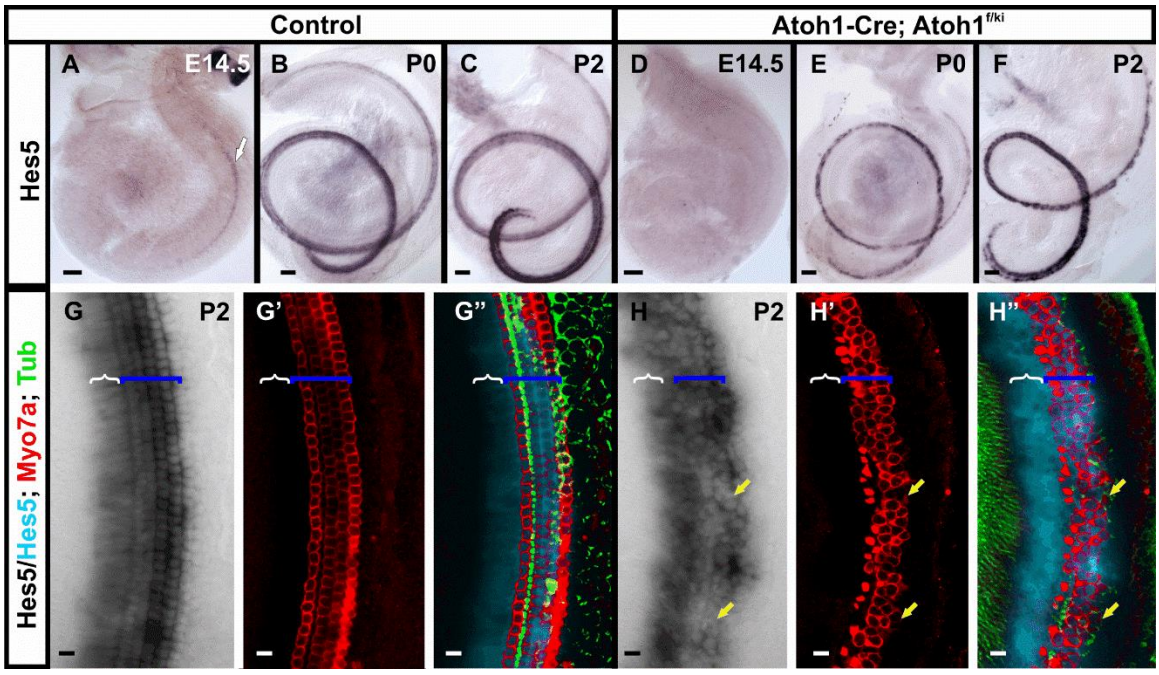

Figure S2. **Aberration of *Hes5* expression in the *Atoh1-Cre; Atoh1*$^{f/kiNeurog1}$ mice.** *Hes5* is upregulated in the mid-base of the control cochlea at E14.5 (A). *Hes5* is delayed in the littermate *Atoh1-Cre; Atoh1*$^{f/kiNeurog1}$ cochlea (D) that is upregulated later at P0 and P2 (E, F). *Hes5*, is dominantly expressed in the lateral SCs in the P2 control mice (G). In the *Atoh1-Cre; Atoh1*$^{f/kiNeurog1}$ mice, *Hes5* is differentially downregulated, particularly toward the base. Myo7a and Tubulin immunohistochemistry in the *Hes5* ISH reacted cochlea reveals that patchy downregulation of *Hes5* in *Atoh1-Cre; Atoh1*$^{f/kiNeurog1}$ mice is not associated with the quantitative changes of HCs (arrows in H-H") as reported in *Hes5* deletion mutants (Zine et al., 2001; Zine and de Ribaupierre, 2002). We use a false cyan color to show the ISH stain while combined with the immunohistochemistry. '{'demarcates the expression of *Hes5* in the GER and '[' in the OC. Bar indicates 100 µm in A-F and 10 µm in G-H".

Supplementary Tables:

Table S1. Total length measurement of the cochlea at P7. The total lengths of the cochleae at P7 display no substantial difference in both *Atoh1-Cre; Atoh1$^{f/kiNeurog1}$* and *Atoh1-Cre; Atoh1$^{f/f}$* mice compared to control littermates.

Total Length in µm	**Control**	***Atoh1-Cre; Atoh1$^{f/ki}$***	***Atoh1-Cre; Atoh1$^{f/f}$***
Mean (N=3)	6561±497	6523±187	6459±288

Table S2. The quantification of HCs and IP cells in the equivalent segments of the control, *Atoh1-Cre; Atoh1$^{f/kiNeurog1}$* and *Atoh1-Cre; Atoh1$^{f/f}$* cochlea at P7.

Mean of IHCs count (N=6)	**Control**	***Atoh1-Cre; Atoh1$^{f/ki}$***	***Atoh1-Cre; Atoh1$^{f/f}$***
Apex	37±1	33±8	10±1
Middle	40±1	42±5	18±4
Base	33±3	33±4	20±5
Mean of OHCs count	Control	*Atoh1-Cre; Atoh1$^{f/ki}$*	*Atoh1-Cre; Atoh1$^{f/f}$*
Apex	127±3	96±25	63±10
Middle	129±4	71±17	41±8
Base	115±6	64±16	28±13
Mean of IP cells count	Control	*Atoh1-Cre; Atoh1$^{f/ki}$*	*Atoh1-Cre; Atoh1$^{f/f}$*
Apex	53±6	45±4	34±6
Middle	59±3	42±3	28±5
Base	51±8	43±7	21±6

Table S3. Primer sequences used for the RT-qPCR are shown here.

Name	**Accession number**	**Primer Sequence**	**In silico Tm**	**% GC**	**Amplicon Length (bp)**
Neurog1	NM_010896.2	**Forward**: ggcctttgtaaggcaacatc **Reverse:** cagccagttccccatctatt	59/59	50/50	73
Pou4f3	NM_138945.2	**Forward**: ccccgtactgcaagaacc **Reverse:** catcaaagcttccaaatatattaccc	59/60	61/35	113
Barhl1	NM_019446.4	**Forward**: ggtaccagaaccgcagga **Reverse:** tggagcgccgagtaattg	59/60	61/56	88
Actb	NM_007393.3	**Forward**: ctaaggccaaccgtgaaaag **Reverse:** accagaggcatacagggaca	59/60	50/55	104

Supplementary Materials and Methods:

Genotyping

EconoTaq plus green 2X master mix (Lucigen, 30033) and a three primer sets were used for the genotyping of tail DNA. All resultant products were electrophoresed and visualized on a 2% agarose gel. The different primer sets were used to detect *Atoh1* floxed allele, *Atoh1*^{kiNeurog1} allele and *Cre*-specific primers to detect *Atoh1-Cre* transgene as described (Jahan et al., 2012; Pan et al., 2012a).

In situ hybridization

For *in situ* hybridization, the plasmids containing the cDNAs were used to generate the RNA probe by *in vitro* transcription. After being anesthetized with 2,2,2 tribromoethanol (Avertin), mice were perfused in 4% paraformaldehyde (PFA) and fixed overnight in 4% PFA. The ears were dissected in 0.4% PFA and dehydrated and rehydrated in graded methanol series and then digested briefly with 20 µg/ml of Proteinase K (Ambion, Austin, TX, USA) for 15-20 minutes. The samples were then hybridized overnight at 60°C to the riboprobe in hybridization solution. The samples were incubated overnight with an anti-digoxigenin antibody after washing off the unbound probe (Roche Diagnostics GmbH, Mannheim, Germany). After a series of washes, the samples were reacted with nitroblue phosphate/ 5-bromo, 4-chloro, 3-indolil phosphate (BM purple substrate, Roche Diagnostics, Germany) which is enzymatically converted to a purple colored product. The ears were mounted flat in glycerol and viewed in a Nikon Eclipse 800 microscope using differential interference contrast microscopy and images were captured with Metamorph software. The ears of the littermate of different genotype for the same gene expression were performed in the same reaction tubes to maintain the reaction accuracy.

Immunohistochemistry

For immunohistochemistry, decalcification was performed by incubating the postnatal ears in EDTA in 0.4% PFA before the microdissection. Then the ears were dehydrated in 100% ethanol and rehydrated in graded ethanol series and then washed in PBS and blocked with 2.5% normal goat serum in PBS containing 0.5% Triton-X-100 for 1 hour. Then the ears were incubated in

primary antibodies for Myo7a (Myosin 7a, Proteus Biosciences), tubulin (Sigma) and p75 (Sigma) in dilutions of 1:200, 1:800 and 1:1000 respectively for 24-48 hours at 4˚C. After several washes with PBS, corresponding secondary antibodies (1:500) (Alexa fluor molecular probe 647 or 532 or 488; Invitrogen) were added and incubated overnight at 4°C. Hoechst nuclear stain (Polysciences; 10mg/ml) was used at a dilution of 1:1000 at room temperature for 1 hour. The ears were washed with PBS and mounted in glycerol and images were taken with a Leica TCS SP5 confocal microscope.

Cell Counts

IHCs, OHCs and IP cells were counted in the comparable regions of P7 control, *Atoh1-Cre; Atoh1*$^{f/kiNeurog1}$ and *Atoh1-Cre; Atoh1*$^{f/f}$ cochleae after performing the immunohistochemistry of Myo7a and tubulin. Each cochlea was divided into 3 equal segments as apex, middle and base and the overview images (100x magnification) were taken to select the area for quantification. 10% distant from the apical tip was chosen for 'apex', 50% for 'Mid' and 90% for the 'base' quantification. Counting was performed on enlarged images at the 400x magnification in SP5 confocal microscope using the LIF software in the 300 µm stretch of apex, middle and base of the cochleae. LIF software allows computerized number markings after each count to facilitate accurate quantification. Tubulin positive IP cells were used to demarcate IHCs as medial to IP cells and OHCs as lateral to the IP cells.

Auditory brainstem response (ABR) recording

2,2,2 tribromoethanol (0.025 ml/g of body weight) was injected in one month old control and *Atoh1-Cre; Atoh1*$^{f/kiNeurog1}$ littermate mice and absence of ocular and pedal reflexes were assessed for surgical level of anesthesia. Needle electrodes were then inserted subcutaneously in the vertex, slightly posterior to the pinna and in the contralateral hind limb. A loudspeaker was placed 10 cm away from the pinna of the test ear and computer-generated clicks were given in an open field environment in a soundproof chamber. Click responses were averaged across 512 presentations using Tucker-Davis Technologies System hardware running BioSig® Software. Recorded signals were bandpass filtered (300 Hz–5 kHz) and 60Hz notch filter. The sound level was decreased in 10-dB steps from a 96-dB sound pressure level until there was no noticeable response.

Scanning electron microscopy (SEM)

The mice for SEM were perfused and fixed in 2.5% gluteraldehyde in 1% PFA after sedating with 2,2,2 tribromoethanol. Ears of postnatal mice were decalcified with EDTA. Following osmication in 2% osmium tetroxide in 0.1 M phosphate buffer (pH 7.4) for up to 1 hour, the ears were microdissected including removal of the Reissners membrane and the tectorial membrane. The samples were then washed several times with distilled water to remove ions, dehydrated in a graded ethanol series, critical point dried, mounted on stubs and coated with gold/palladium. Stubs were viewed with a Hitachi S-4800 Scanning Electron Microscope with 3MeV acceleration.