Supporting Information

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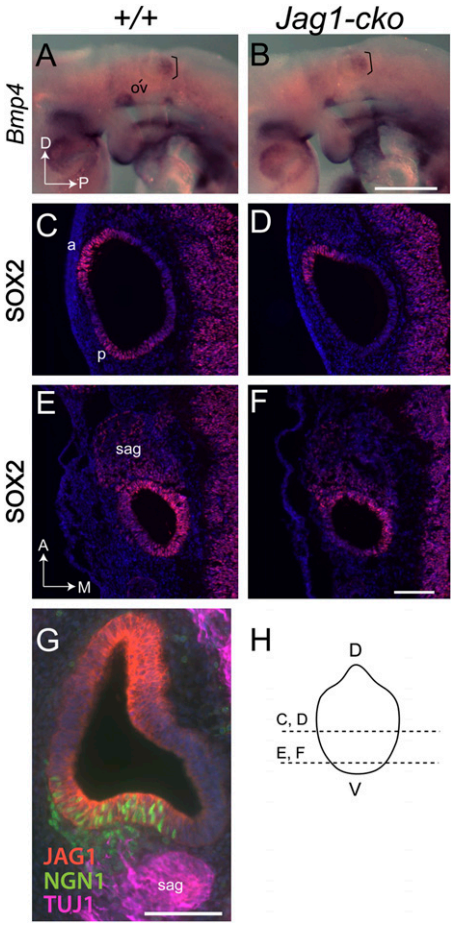

Fig. S1. Marker expression in WT and *Jag1-cko* early otocysts. (*A* and *B*) Whole-mount in situ hybridization in E9.5 embryos demonstrating *Bmp4* expression in the posterior region of the otocyst/otic cup (brackets). (*C–F*) SOX2 expression in different regions of the E10.25 otocyst demonstrating the missing posterior and smaller/weaker anterior domain in the anterior region. (*G*) NGN1 is expressed within the anterior JAG1 expression domain, which implicates JAG1 in neuronal formation. (*H*) Schematic E10.25 otocyst demonstrating the relative positions of the sections shown in *C–F*. a, anterior; D, dorsal; M, medial; ov, otic vesicle; p, posterior; sag, statoacoustic ganglion; V, ventral. (Scale bars: 500 um for *A* and *B*; 100 μm for *C–G*.) In *G*, posterior is up and ventral is to the right.

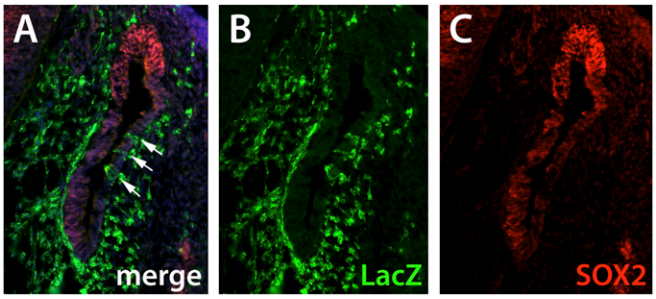

Fig. S2. The *Col2a1-Cre* drives LacZ expression in small clusters of cells in the nonsensory regions of the otocyst. E11.5 inner ear derived from a cross between the *Col2a1-Cre* and a *ROSA-LacZ* reporter demonstrating small clusters of β-galactosidase–positive cells in the nonsensory (SOX-negative) region of the cochlea (arrows in *A*). β-galactosidase is also detected in the mesenchyme surrounding the otocyst (*A* and *B*). (*C*) SOX2 expression indicates the sensory domain in the ear. Dorsal is up and lateral is to the right.

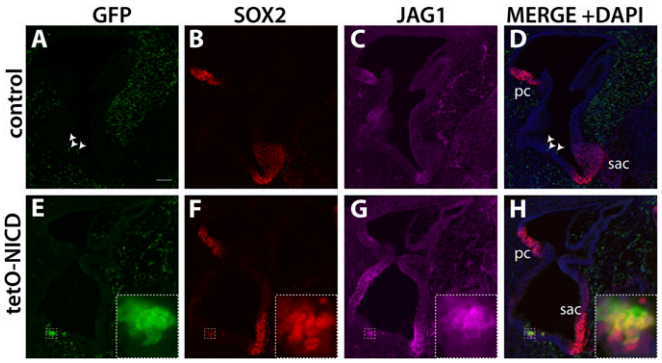

Fig. S3. Ectopic Notch signaling in the vestibular region induces the expression of JAG1 and SOX2 (a marker of sensory progenitors) at E12.5. (*A–D*) In the control *Col2a1-Cre/rtTA* bigenic inner ear EGFP$^+$ cells (arrowheads) are found in the nonsensory regions between the saccule (sac) and posterior cristae (pc), but these cells do not express SOX2 and JAG1. (*E–H*) SOX2 and JAG1 are up-regulated in the EGFP$^+$ cells in the *Col2a1-Cre/rtTA/tetO-NICD* trigenic inner ear. A high-power view of the boxed area is shown in the upper right corner. Note that the expression of SOX2 is slightly wider than the EGFP expression. (Scale bar: 50 μm.)

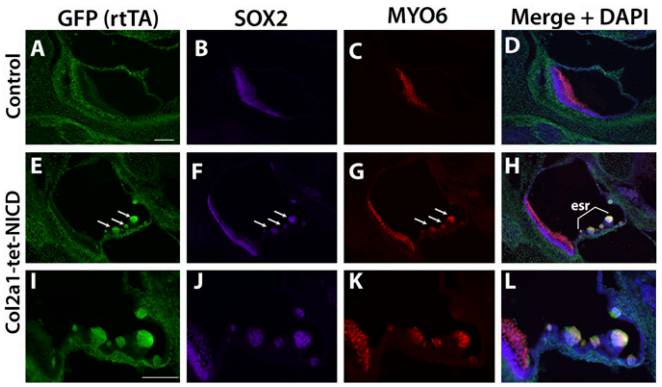

Fig. S4. Ectopic Notch activation induces the generation of hair cells and supporting cells in the vestibule at E18.5. SOX2 is a marker for supporting cells, Myosin VI (MYO6) is a marker for hair cells, and EGFP reflects expression of rtTA. (*A–D*) Control inner ear of *Col2a1-Cre/rtTA* bigenic mouse. (*E–H*) EGFP$^+$ clusters of cells (arrows) in the nonsensory regions of the *Col2a1-Cre/rtTA/tetO-NICD* saccule colocalize with SOX2 and MYO6. (*I–L*) High-power view of the ESRs shown in *E–H*. Bracket indicates the ESRs in *H*. (Scale bar: 100 μm.)

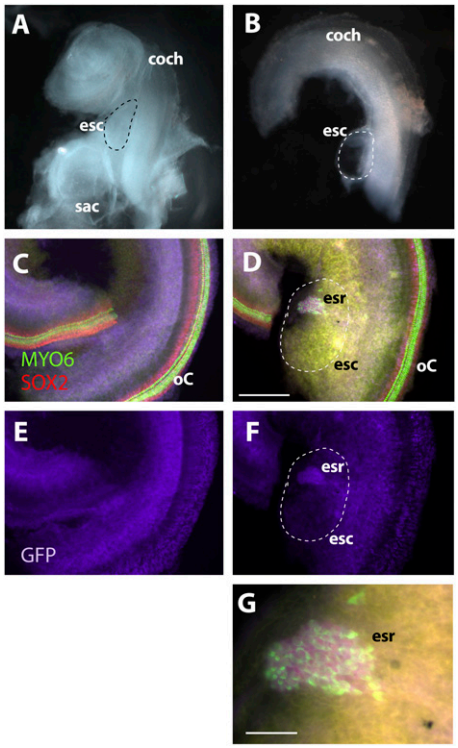

Fig. S5. Ectopic Notch activation induces a separate sensory compartment. (*A*) Dissected whole-mount *Col2a1-Cre/rtTA/tetO-NICD* inner ear showing the ectopic sensory compartment (esc) located at the basal cochlea (dotted area). (*B*) A further dissected cochlea with the ectopic sensory compartment attached at the base. (*C–G*) SOX2 is a marker for supporting cells, Myosin VI (MYO6) is a marker for hair cells, and EGFP labels rtTA-expressing cells. (*C* and *E*) Cochlea of a *Col2a1-Cre/rtTA* bigenic control. (*D* and *F*) An EGFP-positive sensory region is present within the ectopic compartment in the *Col2a1-Cre/rtTA/tetO-NICD* triple transgenic basal cochlea. Dotted lines in *B*, *D*, and *F* indicate the entire ectopic compartment. (*G*) High-power view of the ESR shown in *D*. coch, cochlea; oC, organ of Corti; sac, saccule. (Scale bar: 100 μm for *C–F*; 50 μm for *G*.)

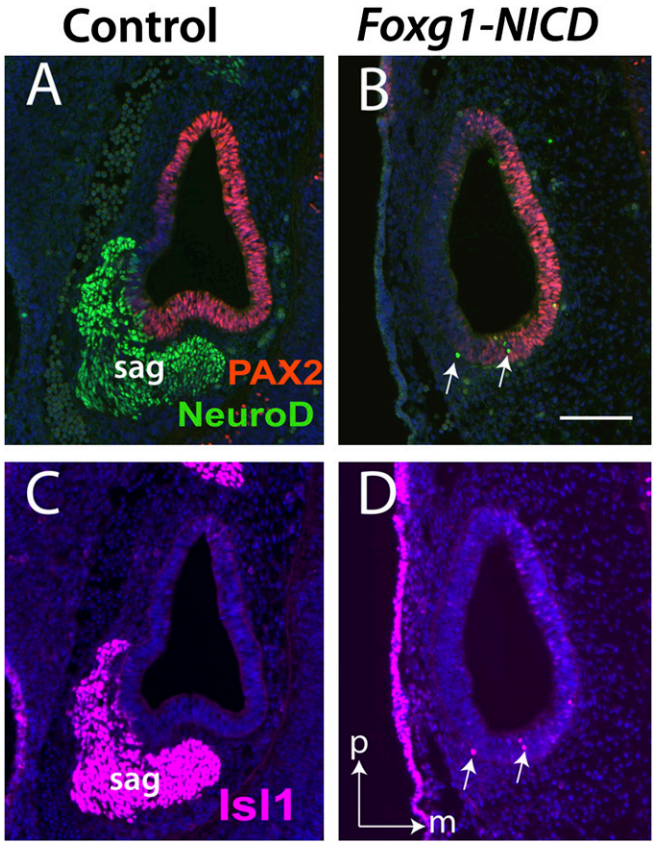

Fig. S6. *Foxg1-NICD* inner ears do not form statoacoustic ganglion but are not delayed in development. (*A* and *B*) PAX2 expression pattern in *Fox-NICD* transgenic otocyst is similar to that of control at E10.5, indicating the mutant otocysts are not delayed in their development. Notch activation also inhibits formation of the SAG, which is marked by NeuroD (*A* and *B*) and Isl1 (*C* and *D*). In *B* and *D*, arrows point to a few cells that appear to still express NeuroD and Isl1 in the *Foxg1-NICD* transgenic inner ear. P, posterior; m, medial. (Scale bar: 100 μm.)

Table S1. Summary of ESRs in *Col2a1-Cre/rtTA/tetO-NICD* ears

Cochlea only	Saccule only	Cochlea and saccule	Separate compartment
11 (f/w)	135 (I)	29 (f/w)	58
81 (I)	357 (f/w)	58 (f/w)*	206
226 (I)		69 (f/w)	233
234 (I)		84 (f/w)	366
285 (I)		133 (I)	430
366 (f/w)*		206 (I)*	580 (f/w)
430 (f/w)*		225 (I)	
		233 (I)*	
		274 (I)	
		403 (f/w)	

Numbers refer to individual ear samples. The mode of administration (f/w, food and water; I, injection) is indicated.

*Samples that had an ESR in the cochlea and/or saccule that also had an ESR in a separate compartment.

Table S2. Summary of antibodies

	Dilution	Source
Primary antibody		
Activated caspase-3	1:2,000	R&D Systems
β-galactosidase	1:500	Abcam
GFP	1:1,000	Abcam
JAG1	1:100	Santa Cruz Biotechnology
Myosin VI	1:1,000	Proteus Biosciences
Neurogenin 1	1:100	Santa Cruz Biotechnology
NeuroD	1:500	Santa Cruz Biotechnology
pHistone H3 (Ser-10)	1:400	Santa Cruz Biotechnology
pHistone H3 (Ser-10)	1:100	Cell Signaling Technology
SOX2	1:500	Santa Cruz Biotechnology
TUJ1	1:1,000	Covance
Secondary antibody		
Alexa Fluor 647 donkey anti-mouse	1:1,000	Molecular Probes
Alexa Fluor 488 donkey anti-rabbit	1:1,000	Molecular Probes
Alexa Fluor 546 donkey anti-goat	1:1,000	Molecular Probes
Cy3-conjugated donkey anti-chicken	1:500	Jackson ImmunoResearch