

Figure S1. Differential expression profiling identifies Shh repressed genes in the inner ear. (A) Top 50 Shh repressed genes that show significant upregulation in *Smo^{ecko}* (red) and downregulation in *Shh-P1* (blue) inner ears (\log_2 fold change). (B) DAVID Gene Ontology (Biological Processes) term enrichment for Shh repressed genes.

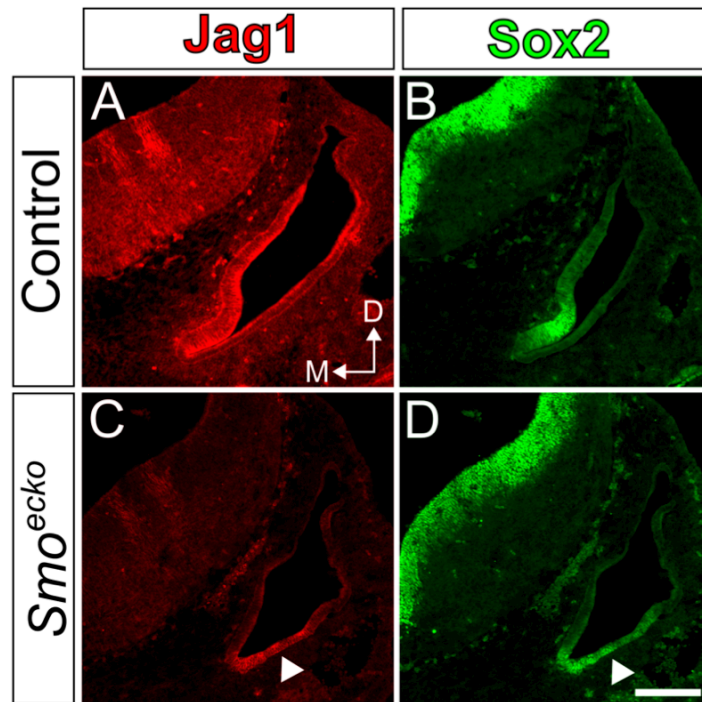


Figure S2. Misexpression of prosensory markers, Jag1 and Sox2, in *Smo^{ecko}* inner ears. Transverse sections through the otic vesicle of control and *Smo^{ecko}* embryos at E11.5 (n=4) immunostained for Jag1 and Sox2. *Smo^{ecko}* embryos display a loss of medial and gain of lateral (white arrowhead) otic expression of Jag1 and Sox2.

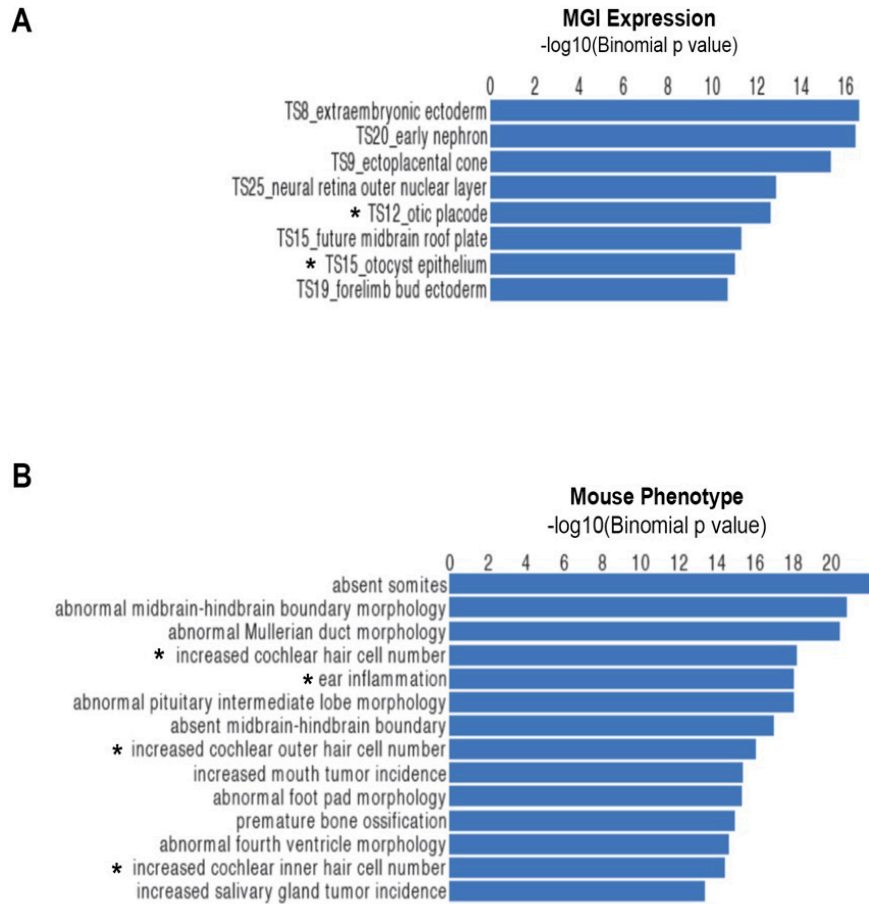


Figure S3. GREAT based computational analysis of inner ear ATAC-seq sites. (A, B) Enrichment terms for genes in the vicinity of ATAC-seq peaks based on mouse genome informatics (MGI) expression (A) and phenotypes (B). Significant inner ear enrichment terms are indicated (*).

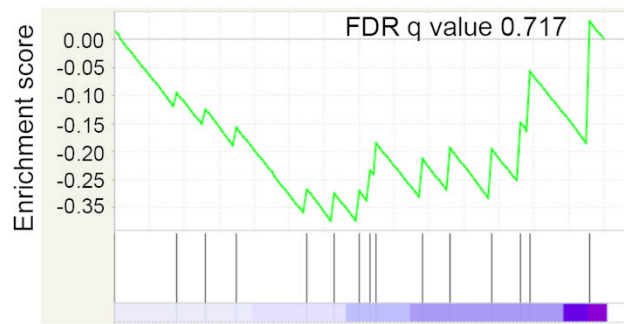


Figure S4. GSEA analysis of intersected ATAC-seq, Gli2 ChIP-seq and H3K27ac ChIP-seq peaks. No significant enrichment is detected for intersected peaks in the vicinity of Shh responsive genes (FDR q value =0.717).

Table S1

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Table S2

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Table S3

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Table S4

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Table S5. Primer sequences used in Gli2 ChIP-qPCR and Six1 ChIP-qPCR assays**Gli2 ChIP-qPCR**

Target sites	Primer Sequence
<i>Frem1</i>	FWD: TGTGCCATGGAAGTCACAAG; REV: TCTTGTCTGAATCTGATGTAGCTC
<i>Ecel1</i>	FWD: CACGTGCGTGTAAGTGATCATA; REV: TCTTGATGGGTGGACGAAAC
<i>Fam107a</i>	FWD: GCAAGACAGCTTCCCTTTGA; REV: TCAGCAGACAGGGTCCTAAA
<i>Hey1</i>	FWD: CTGCCTGCCTCTGTCTC; REV: GAAACAGCTCCTCCTTCACA
<i>Cldn22</i>	FWD: CCGAGAGAGAGCTCGGA; REV: CCAGACCCAGGCAAGTT
<i>Jag1</i>	FWD: TCTGGGTGGCCAGAGTATAA; REV: CCACACATGGACTCTCTGAAC
<i>Pls1</i>	FWD: CTAAGGCTACACCGTGAACAA; REV: TCATGCCCAGACTTCAACAA
<i>Brip1</i>	FWD: ACACCACAATACTGGAGATCAA; REV: CTCTCGGGTTGCCAGTAAC
<i>Ptch1</i>	FWD: CGCCGTAGTACATTACTGAAG; REV: AATGGAAGGGCCGGAAC

Six1 ChIP-qPCR

Target sites	Primer Sequence
<i>Frem1</i>	FWD: CATGGAGTCACTCGAATGGAAG; REV: GGTGGTCTGAACAGGGCTT
<i>Ecel1</i>	FWD: TACCAGCCGGGTACACAT; REV: TGTTAGAGTTACCGCGAACAAG
<i>Fam107a</i>	FWD: GAAAGTCCCAGGCCTTGTT; REV: TAACAGGGAGTCTACCACAGT
<i>Hey1</i>	FWD: TGTGCTGCCCACTTACA; REV: GGAGCACTGGAGATGAGG
<i>Cldn22</i>	FWD: CCAACTTGCCTGGGTCTG; REV: CAGAGCAGCTTCAGCCTATC