List of Supplemental Material

Figures S1-S7

Table S1 (see Excel file)

Tables S2-S4

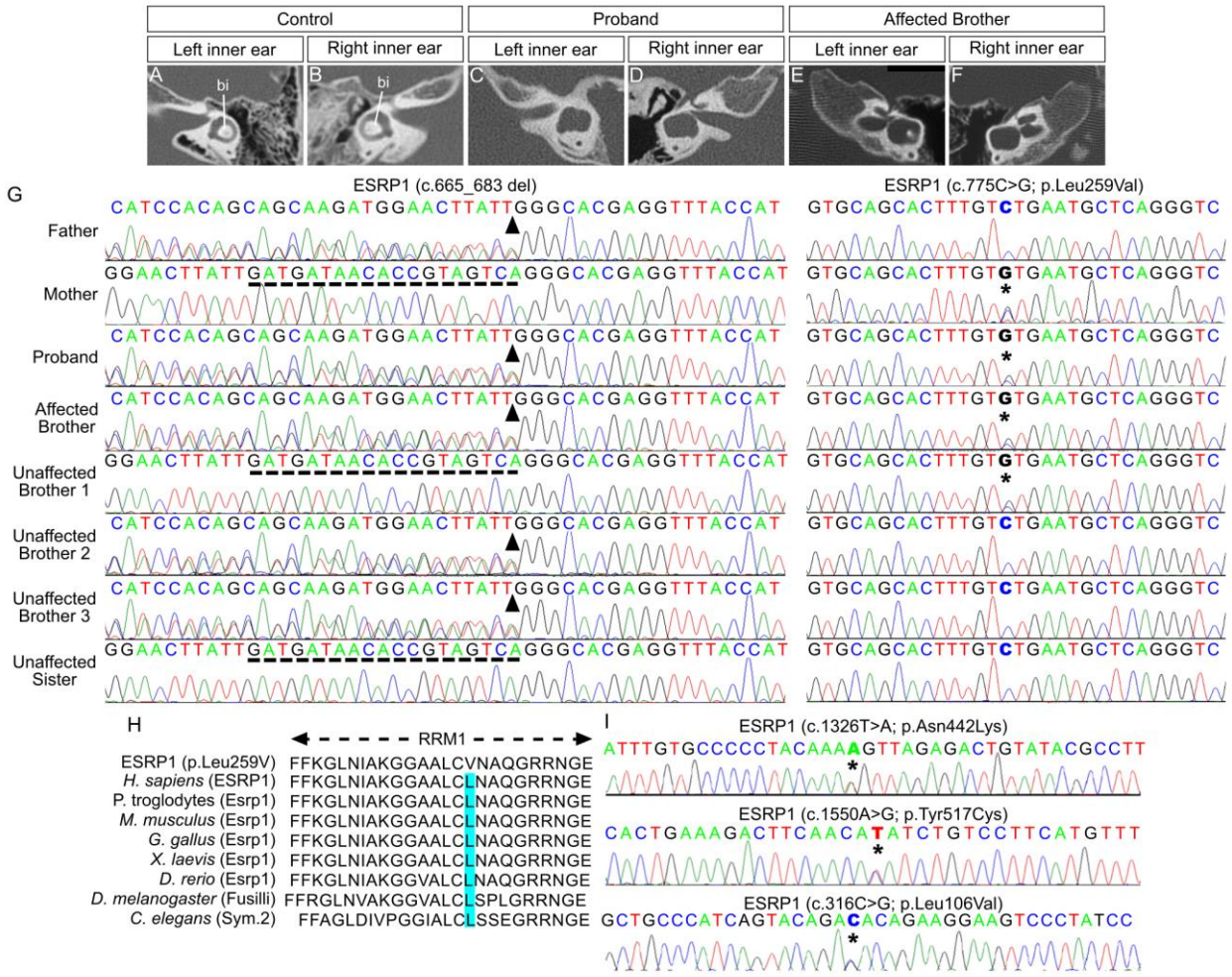

Figure S1. (Related to Figure 1) *ESRP1* mutations in individuals with SNHL. (A-F) Axial CT scans of the lateral vestibule from control and affected children in pedigree with SNHL showing absence of bony island (bi) in (C-F). (G) Sanger sequencing tracks of *ESRP1* from individuals in pedigree with SNHL showing position of paternally inherited 19 bp deletion (arrowhead) corresponding to underlined sequence, and maternally inherited point mutation (asterisk). (H) Cross-species comparison of the first RNA recognition motif (RRM) in ESRP1 showing amino acid conservation at site of ESRP1 mutation (Leu259Val). (I) Sanger sequencing tracks of *ESRP1* from three isolated cases of SNHL showing position of point mutations (asterisk).

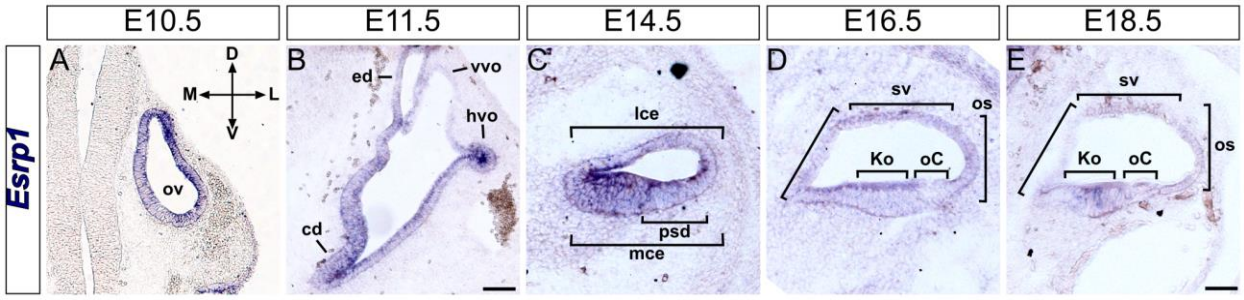

Figure S2. (Related to Figures 1 and 2) *Esrp1* expression in the developing mouse cochlea. (A-E) Transverse sections through the otic vesicle (A,B) and cochlear duct (C-E) of wild type embryos over several developmental stages showing broad *Esrp1* expression in the otic epithelium. Scale bar = 100μm (A,B) and 50μm (C-E). Abbreviations: cochlear duct (cd), dorsal (D), endolymphatic duct (ed), horizontal vestibular outpouch (hvo), Kolliker's organ (Ko), lateral (L), lateral cochlear epithelium (lce), medial (M), medial cochlear epithelium (mce), neural tube (nt), periotic mesenchyme (pom), prosensory domain (psd), organ of Corti (oC), otic vesicle (ov), outer sulcus (os), Reissner's membrane (Rm), stria vascularis (sv), ventral (V), vertical vestibular outpouch (vvo).

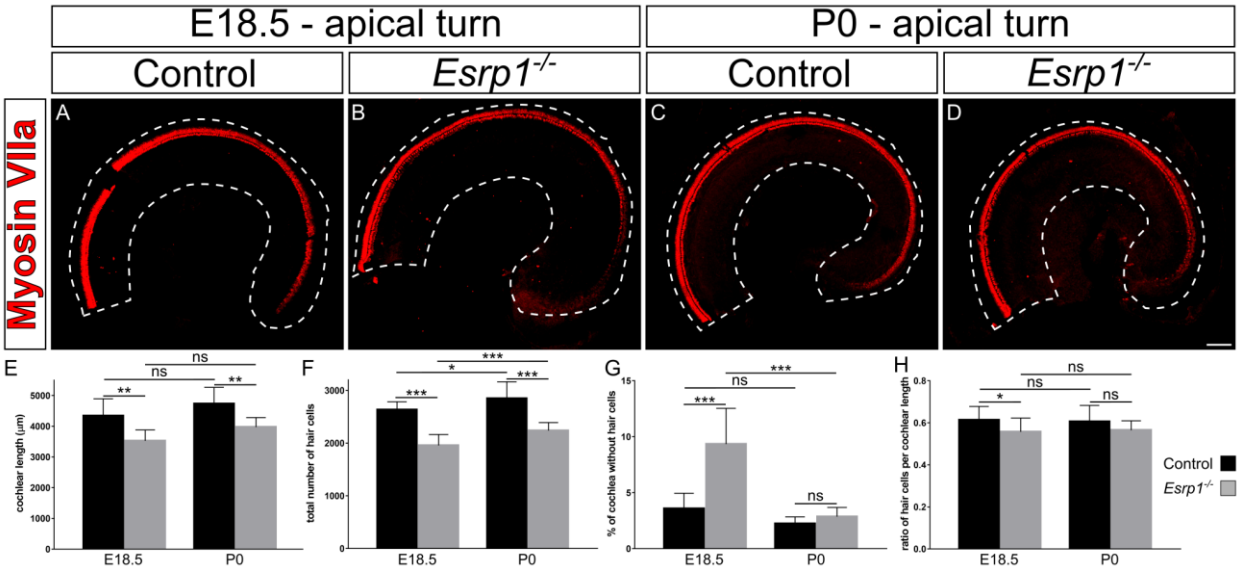

Figure S3. (Related to Figure 3) Hair cell differentiation is delayed at the apex of the cochlear duct in *Esrp1*$^{-/-}$ mutants. (A-D) Whole mount cochlear preparations from control and *Esrp1*$^{-/-}$ embryos immunostained for Myosin VIIa at E18.5 (A-B) and P0 (C-D). Scale bar = 100µm. (E-F) Quantification of cochlear length (E), total number of hair cells (F), percent of cochlea without hair cells (G), and ratio of hair cells per cochlear length (H) presented as mean ± SD (*P<0.05; ***P<0.0001, one-way ANOVA with Tukey's test, n=10).

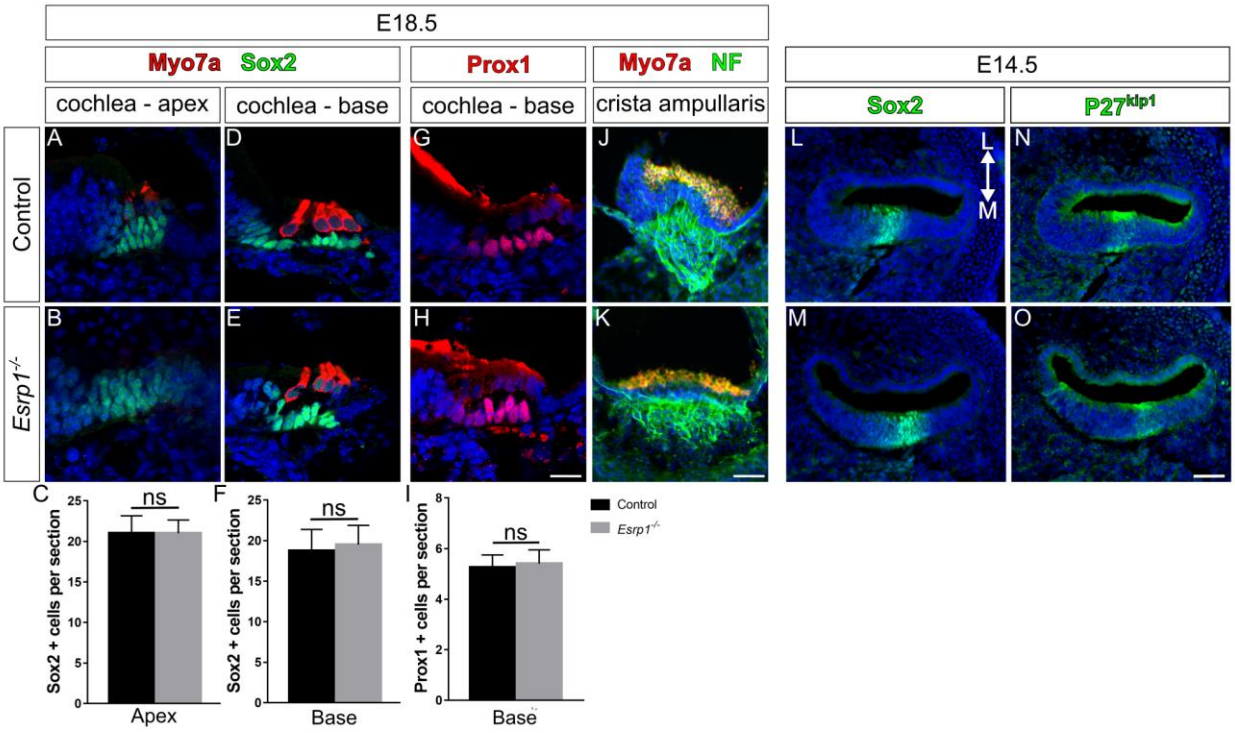

Figure S4. (Related to Figures 2 and 4) Specification of prosensory progenitors, support cells, vestibular hair cells and neurons is not compromised in *Esrp1*$^{-/-}$ embryos. (A-C) Sox2 staining reveals a normal pool of sensory progenitors at the apex of the cochlear duct of *Esrp1* mutants. (D-F) Sox2 and (G-I) Prox1 positive support cells are present in similar number and organization at the base of *Esrp1* mutants. Myo7a labels hair cells. All quantifications are presented as mean ± SD (ns, unpaired t-test). (J,K) Vestibular hair cells and neurons labeled with Myo7a and neurofilament (NF), respectively, are unaffected in *Esrp1* mutants. (L-O) The expression of Sox2 and P27^{kip1} in prosensory progenitors is not compromised in *Esrp1*$^{-/-}$ embryos. Scale bar = 10µm (A,B,D,E,G,H) and 50µm (J-O). n=5 for all panels. Abbreviations: L (lateral), M (medial).

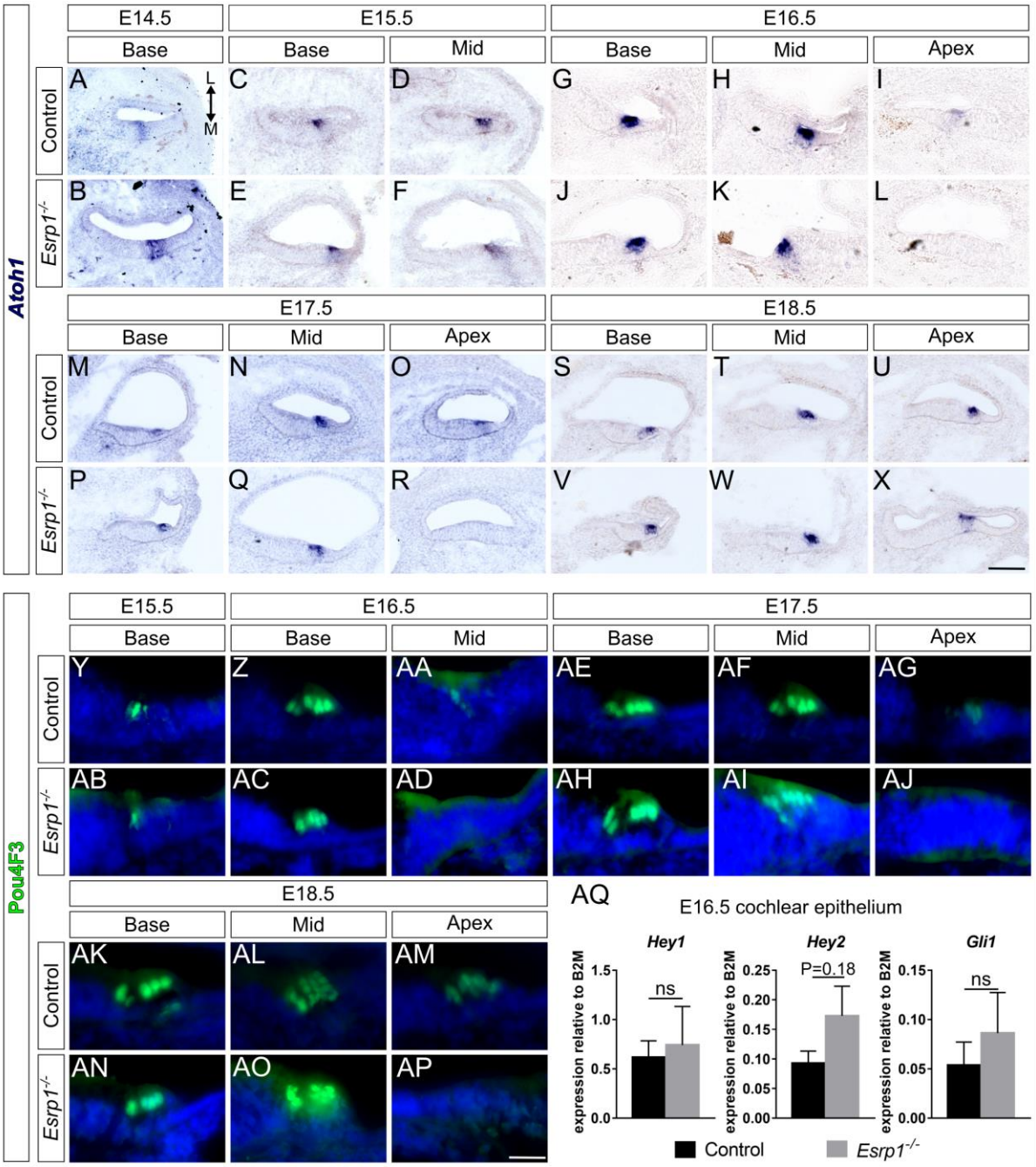

Figure S5. (Related to Figure 4) Hair cell differentiation and maturation is delayed in *Esrp1*$^{-/-}$ embryos. (A-X) Transverse sections through the cochlear duct of control and *Esrp1*$^{-/-}$ embryos stained for *Atoh1* mRNA at progressively later stages of hair cell development. (Y-AP) Transverse sections through the cochlear duct of control and *Esrp1*$^{-/-}$ embryos immunostained for Pou4F3 at progressively later stages of hair cell development. Scale bar = 100μm (A-X) and 10μm (Y-AP). (AQ) qRT-PCR of sensory markers on E16.5 cochlear epithelium represented as mean ± SD (ns, unpaired t-test). (A-AP) n=5, (AQ) n=6. Abbreviations: L (lateral), M (medial).

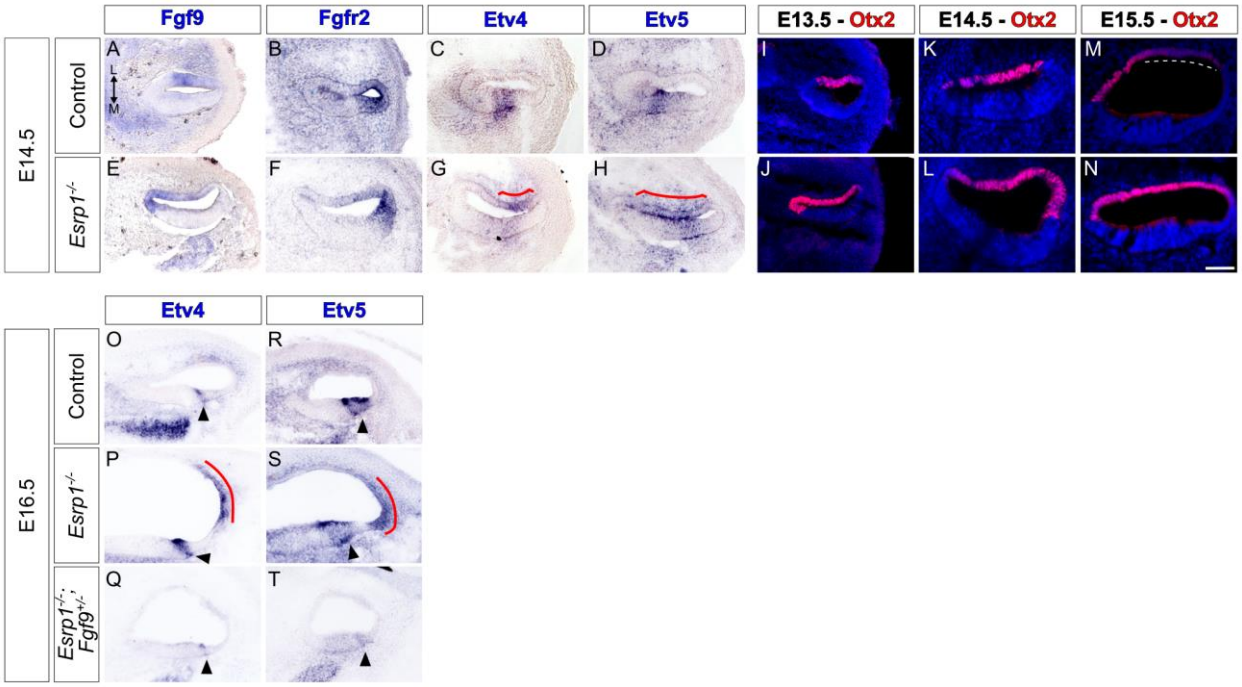

Figure S6. (Related to Figures 5 and 7) Fgf signaling is ectopically activated in the lateral cochlear epithelium of *Esrp1* mutants. (A-H) Transverse sections through the cochlear duct of control and *Esrp1*$^{-/-}$ mutants at E14.5 stained with markers of Fgf signaling. Ectopic expression of *Fgf9*, *Etv4* and *Etv5* along the lateral cochlear epithelium is highlighted (red-bracket). (I-N) Otx2 is progressively restricted to Reissner's membrane in control embryos but remains broadly expressed throughout the lateral cochlear epithelium in *Esrp1* mutants. Otx2 repression from the stria vascularis is indicated (dashed line). (O-T) Transverse sections through the cochlear duct of control, *Esrp1*$^{-/-}$, and *Esrp1*$^{-/-}$;*Fgf9*$^{+/-}$ embryos at E16.5 stained for *Etv4* and *Etv5*. Ectopic expression of *Etv4* and *Etv5* along the lateral cochlear epithelium (red-bracket) and normal sensory expression (arrowhead) are highlighted. Scale bar = 50μm. n=4 for all panels. Abbreviations: L (lateral), M (medial).

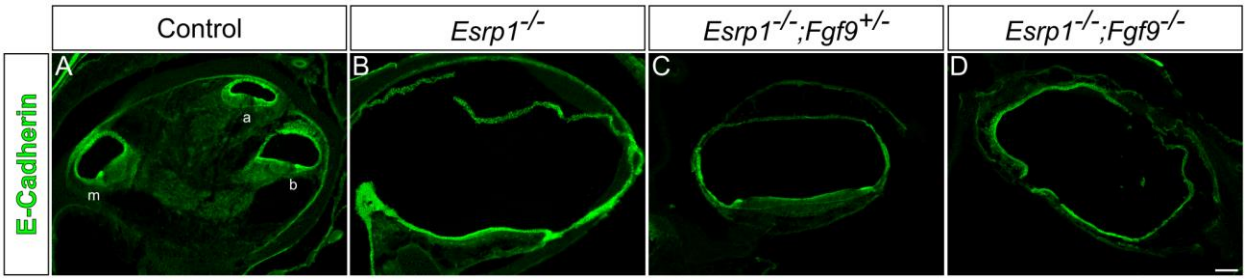

Figure S7. (Related to Figures 2 and 7) The cystic otic phenotype in *Esrp1*$^{-/-}$ embryos is exacerbated by the dose dependent loss of Fgf9. (A-D) Transverse sections through the cochlear ducts of control (n=6), *Esrp1*$^{-/-}$ (n=4), *Esrp1*$^{-/-}$*; Fgf9*$^{+/-}$ (n=4) and *Esrp1*$^{-/-}$*; Fgf9*$^{-/-}$ (n=3) embryos at E18.5 immunostained for E-cadherin. Scale bar=100µm. Abbreviations: apical turn (a), basal turn (b), medial turn (m).

Table S2. (Related to Figures 4, 5, S5, and S6) Expression values of selected genes from RNA-seq on E16.5 control and *Esrp1*$^{-/-}$ cochlear epithelium.

Gene	Control FPKM	*Esrp1*$^{-/-}$ FPKM	Fold Change	P-Value
Atoh1	3.46 ± 0.59	2.43 ± 0.38	-0.596	0.040
Atp1a1	96.99 ± 19.03	81.56 ± 4.37	-0.334	0.089
Bsnd	0.53 ± 0.14	0.01 ± 0.01	-5.029	8.513E-16
Cd44	1.92 ± 0.18	1.37 ± 0.10	-0.570	0.014
Cldn11	13.24 ± 4.30	13.12 ± 2.44	-0.161	0.546
Esrrb	10.61 ± 2.38	0.72 ± 0.33	-4.012	3.959E-40
Etv4	6.32 ± 1.14	9.17 ± 1.78	0.403	0.047
Etv5	11.50 ± 1.01	11.81 ± 0.33	-0.048	0.758
Fgfr1	17.18 ± 0.52	18.69 ± 0.65	0.029	0.954
Fgfr2	15.10 ± 2.02	12.43 ± 1.30	-0.343	0.076
Fgfr3	6.55 ± 1.22	3.91 ± .054	-0.796	2.055E-04
Gjb2	55.99 ± 9.04	39.31 ± 7.70	-0.547	0.012
Gli1	1.92 ± 0.17	1.58 ± 0.24	-0.341	0.188
Hey1	15.66 ± 1.85	13.05 ± 1.59	-0.318	0.096
Hey2	7.27 ± 0.41	8.51 ± 1.14	0.176	0.501
Kcne1	1.52 ± 0.3	0.06 ± 0.03	-4.503	9.817E-24
Kcnq1	1.72 ± 0.3	0.97 ± 0.38	-0.989	0.004
Myo7a	2.29 ± 0.43	1.89 ± 0.76	-0.441	0.093
Otx2	8.75 ± 2.75	18.29 ± 1.12	0.995	9.071E-05
Pou4f3	5.89 ± 0.69	3.49 ± 1.11	-0.914	0.004
Prox1	3.67 ± 0.18	2.02 ± 0.57	-0.876	0.001
Ptch1	6.54 ± 0.68	6.18 ± 1.30	-0.128	0.492
Sox2	19.14 ± 0.28	18.54 ± 2.95	-0.110	0.501

Table S3. (Related to STAR Methods) PCR primers used to amplify human *ESRP1* and *ESRP2* exons.

ESRP1 Primers			
Exon	**Forward primer (5'-3')**	**Reverse Primer (5'-3')**	**Size (bp)**
Exon 1	TAGCAGTAGCAAGGAAGGGG	GGGTCCAGACTCACAAGTGG	396
Exon 2	GAGAGCTTTGATTCTGCGTC	ATGAAGAGAAGCAGCGACC	445
Exon 3	AAGGGGAGCTACTTTGCAG	AGAACAGAGAAACTTGAATAAACC	279
Exon 4	TAGGTTCCTAATTGTGAGAGAAAC	ATTTTGGATAGGCCAATTTTATC	450
Exon 5-6	GTTGAATGACTGACTGATGGG	ATTAGCCAGGTATGGTAGCG	662
Exon 7-9	AACTAGGCAGTTGTCTTGCAG	AAAACCATTCATGACCCTATTC	686
Exon 10	CATTGGCAGGACTTTTATCAC	TGAGCAATGTAGGACTATTTTCAG	469
Exon 11	GTATCATGCTTTCCCTGCTG	CTTAGTGTGTAACTGAGGATTACG	675
Exon 12	TTGTCACTTTGCATGTTTTG	AGGGGCACAGAAAGTTACG	369
Exon 13	TGTGTAGATGGAACAGTGACTATG	GAGCTCTTGCACTGGCTATC	334
Exon 14	GCTCTGAGAAGTATAAGAAAGGTTG	ATCTGTTTCTTTCCTGGCTG	360
Exon 15	TCTAAATGTCCTATACTTTTGTTGG	TCTAAATGCCTTCAGAATCTTG	286
ESRP2 Primers			
Exon	**Forward primer (5'-3')**	**Reverse Primer (5'-3')**	**Size (bp)**
Exon 1-2	GTAACTCGCCTGGAGAGGG	ACAAGAGCCCAGTCCTGC	844
Exon 3-4	TTCCTCCTACCCTCACTTCC	ATACTCAGACACATGTACCCACAG	475
Exon 5	GGAGTAAACAGGATTAGTGCTTATG	GAGCAATAGGCATTTGTCATC	217
Exon 5	GTGTCTATGGAGGGGAGTTG	TAGAGAAGGTCACAGTGGGG	265
Exon 6-8	GCAGAACTTGGTGGGGAC	TATACACCTGTGGGTACAGAGAGC	599
Exon 6-8	TTTTGCAGAACTTGGTGG	CACCTGTGGGTACAGAGAGC	599
Exon 9-10	GGCCAATCTGAGTCAGGC	GTAGGGCAGGGACACAGAG	612
Exon 11	AGATGTACTGTTACTCCAGGGC	TACCCCTAACACACACCCC	353
Exon 12	GTGTGTGTTAGGGGTAAGGC	TAGAAGGTAGGGGCTTACAACAG	331
Exon 13	ATAGCGGGGACTCCTTTC	TTCCCCAGATTGGGACAG	323
Exon14-15	GGTCTCGGTCTAGGCCAC	GTTGAGGGTGCTGGTCTTC	469

Table S4. (Related to STAR Methods) Primers used to quantify changes in alternative splicing and gene expression by RT-PCR.

Alternative Splicing Primers		
Gene	Forward-Primer	Reverse-primer
Mouse		
Arhgef11	TCAAGCTCAGAACCAGCAGGAAGT	TGCTCGATGGTGTGGAAGATCACA
Col11a2	GGACGGCCCTCAGACTCAGAAG	GCCAGGGGGTCCAGCTAATCCAG
Col11a2	GCCGCCCTGTCCGCTTTCTC	GGCCCTTGTCACCCCCACTACTG
Csnk1d	CTCACGGGCCGACAAGATACCT	GAGCTCCCGGCGTTAACATTTCAG
Ctnnd1	AACCTCGCTGGATTTGTCTTTC	TGATCCTGGGGTCCGTTGAGTTTC
Dgkd	CTAAACGCAGCCGTAGTGGTAAA	TTTGTTAGAGACAGGAAGCCCAAT
Enah	GCTGGGCGTGGGAATGGACCTCTT	TTGCCAGCTCTTTTCTCATCTCAT
Epb4.1	TTAACATCAACGGGCAAGTC	ATATCGGCATCTCCTGTGA
Eya4	GAGGTGCTTTCCCCATTG	ATTTCATCCGTTAAGACCATT
Eya4	TCCCCATTGAGAACATTTACAG	AGAGGTCCGAGTGGCTTGATA
Fgfr1	CCTGGGCAGCAATGTGGAGTT	AAGGCCCCGGTGCAGTAGATA
Fgfr2	CCCGGGTCTAGATTTATAGTGATGCCCAGCC	CCCGGGGAATTCACCACCATGCAGGCGATTAA
Fgfr3	TGCCGGCCAACCAGACAGC	GCGCAGGCGGCAGAGTATCACA
Flnb	GGCGAGGAGGTGGGCTTTGTAG	CCTGACGGCAAATGGAATCACCAA
Ganab	GCCCGGCTTTCTGTCTCTGGTC	CATCCCTGGGTGTTTCCTCA
Limch1	AGAGCCTGAAGCAACCCTGACG	TCGCCGGCGCTCCTCTTCT
Lsm14b	CCTCGGCGACACAGCTCAATGGT	ATCACAGCTGGGTCCTTCTCTTCC
Map3k7	TTCCTGCCACAAACGACAC	ATTCTGACACTAGGGCTGGATGAC
Nf2	TGACTTCAAGGATACGGACAT	CAAACAAGCCAGCCCTCTACT
Ocrl	TGAACTCCGGTGAAGATAAGATTG	GAAGGGGTCGCTCACTGGTA
Phactr4	TGGAAGCAGGGGACACAACAC	GGGCTAGAGGACCTGGCACTG
Ralgps2	ACCTGCTGGATGATAGTGTC	CTTCAGAGATTTGGCGGCATAGTA
Scrib	ATCCGCAAAGACACGCCCCACTAC	AGCCTTTTCCCCCTGCGATACTGA
Sec22c	CAGCCCTGGGTGTCCTCTC	CCAGCATCAGCAGCACCTTCA

Human		
ARHGEF11	GAAATTCTGGGATATGGGAGTC	GCCTGTTGAGCTTGAGAGTGA
ENAH	TGCTGGCCAGGAGGAGAAGA	ACTGGGCTGTGATAAGGGTGT
NF2	TGGCAGCAGCAAGCACA	CCCGGTAGCAGGAGAAGT
RALGPS	AGACCTCATGGCCTGCTTTTG	TGTAGGCTTTTTGCCTTCTTT
SYBR Green Primers		
hβACTIN	AGGCACCAGGGCGTGAT	GCCCACATAGGAATCCTTCTGAC
hESRP1	TGATCTTCGAAAAGAATTCAAGAAA	CGAGAGACTGAACTACTCTTCTCAAAA
hESRP2	CCCTACATGCTCTGCACTGA	GGAATTCTCTTCGGAGGTCA
mB2M	TCTCTCTTTCTGGCCTGGAG	AATGTCGGATGGATGAAACC
mAtoh1	ATGCACGGGCTGAACCA	TCGTTGTTGAAGGACGGGATA
mGli1	GGAAGTCCTATTCACGCCTTGA	CAACCTTCTTGCTCACACATGTAA
mHey1	CACTGCAGGAGGGAAAGGTTA	CCCCAAACTCCGATAGTCCAT
mHey2	AAGCGCCCTTGTGAGGAAA	TCGCTCCCCACGTCGAT
mPtch1	CTGGCTCTGATGACCGTTGA	GCACTCAGCTTGATCCCAATG