Figure 1

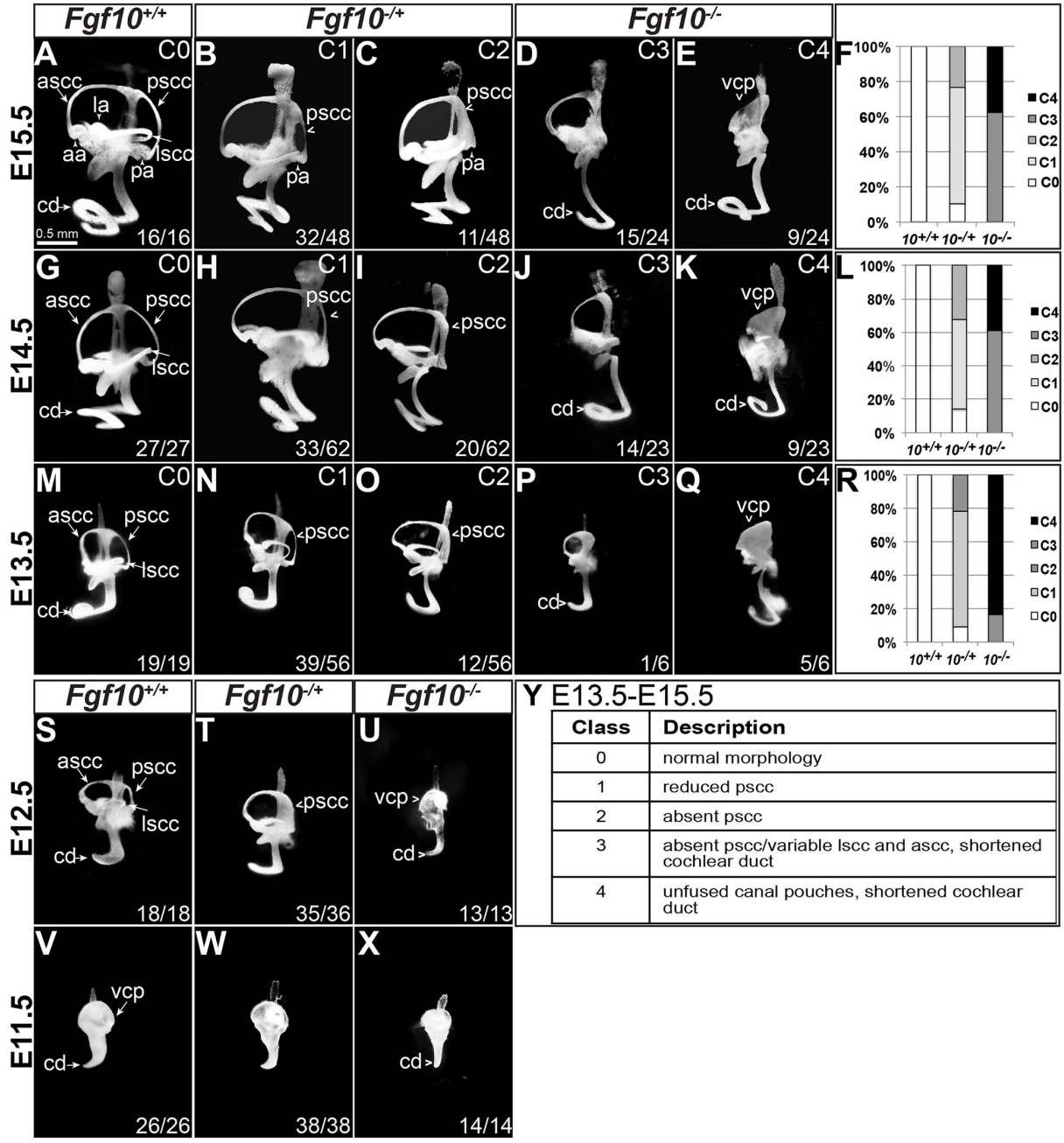

Figure 1. Vestibular morphogenesis is affected in both *Fgf10* heterozygous and *Fgf10* homozygous null mutants, whereas cochlear development is affected only in homozygotes. (A-E, G-K, M-Q, S-X) Inner ear epithelia were filled with paint at the stages indicated to the left of each row. Genotypes are indicated at the top of each column. The phenotypic class designations for E13.5-E15.5 samples as described in the main text (C0-C4) are summarized in panel Y and indicated in the upper right portion of each panel. The percentage of each E13.5-E15.5 genotype falling into each phenotypic class is shown (F, L, R). Abbreviations: aa, anterior ampula; ascc, anterior semicircular canal; cd, cochlear duct; la, lateral ampula; lscc, lateral semicircular canal; pa, posterior ampula; pscc, posterior semicircular canal; vcp, vertical canal plate.

Figure 2

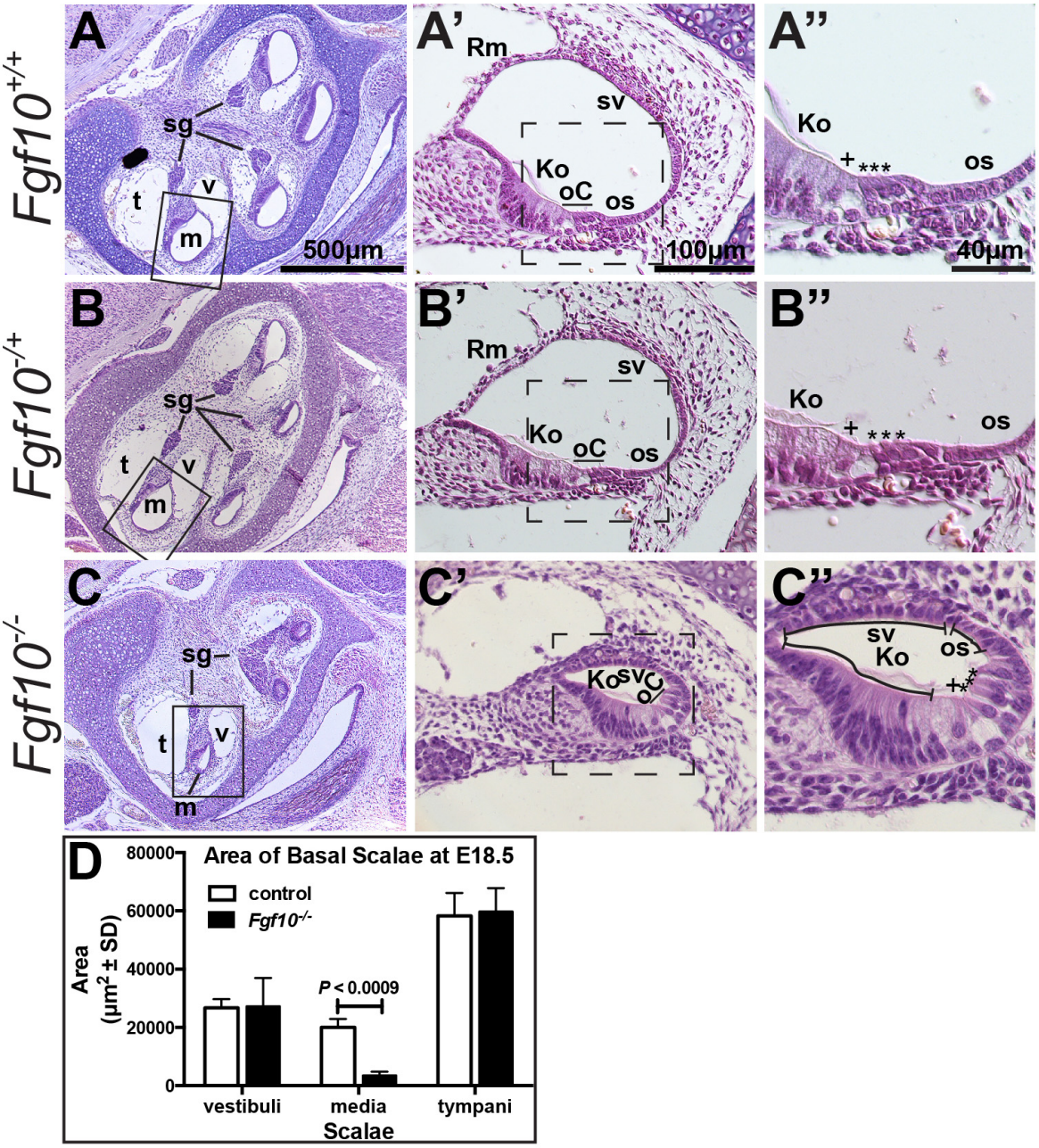

Figure 2. *Fgf10* null cochlear ducts lack non-sensory domains and have a reduced cross-sectional area. Hematoxylin and eosin-stained E18.5 cochlear duct cross sections at three magnifications. Boxes in A-C indicate the region magnified in A'-C'. Dashed boxes in A'-C'; indicate the region magnified in A"-C". C" Morphologic structures remaining in *Fgf10* mutants are indicated with lines. Genotypes are indicated to the left of each row. D. Graphical comparison of the cross sectional area of basal scalae (n = 3 controls and 3 mutants). Abbreviations: Ko, Kolliker's organ; m, scala media (cochlear duct), oC, organ of Corti; os, outer sulcus; Rm, Reissner's membrane; sg, spiral (cochlear) ganglion; sv, stria vascularis; t, scala tympani; v, scala vestibuli. Asterisks indicate outer hair cells, plus symbols indicate inner hair cells. Scale bars in A, A', A'' apply to all panels in the same column.

Figure 3

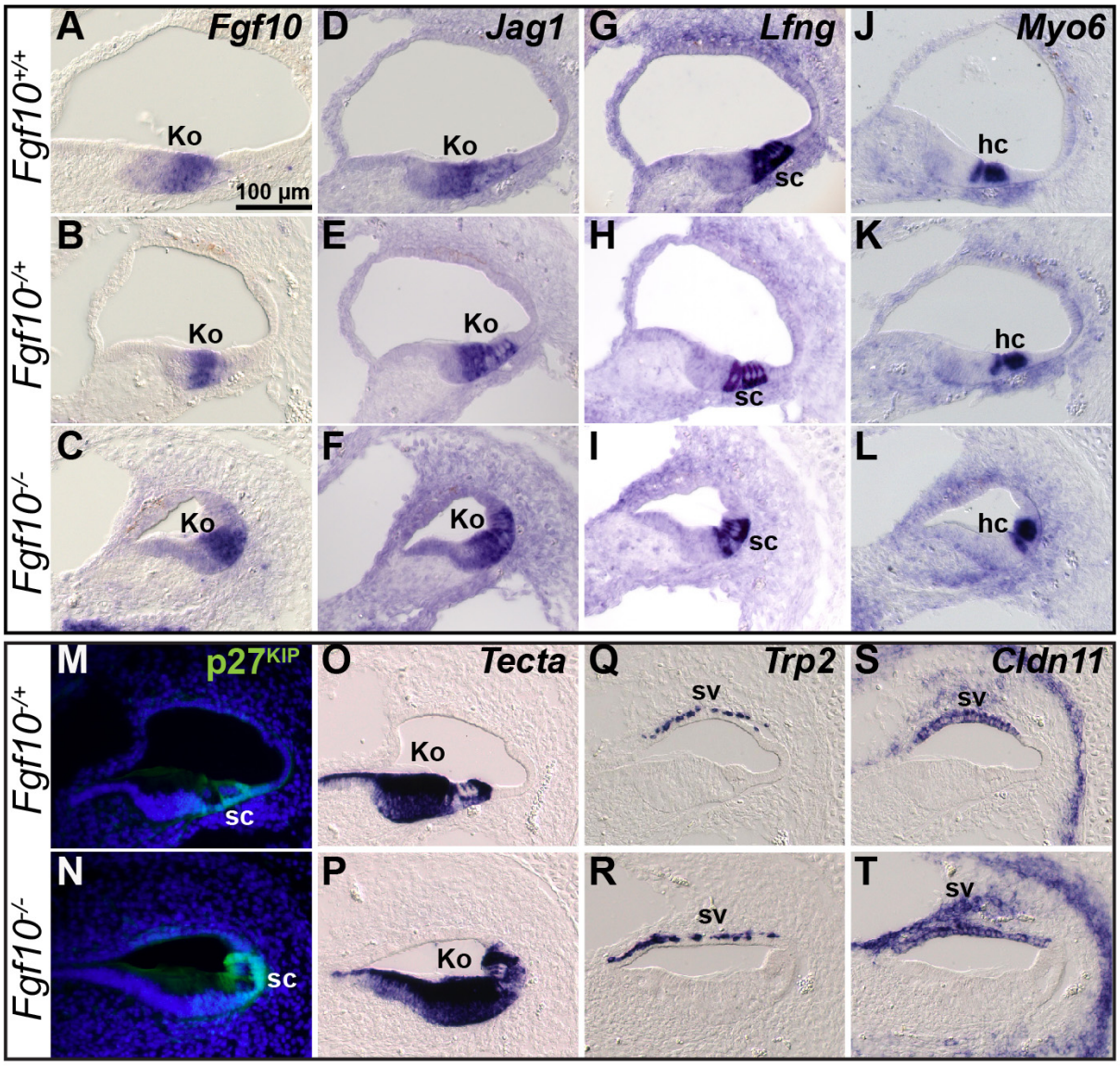

Figure 3. Markers of Kolliker's organ, supporting cells, hair cells and the stria vascularis are unchanged in E18.5 *Fgf10* null mutants. In situ hybridization (A-L, O-T) or immunostaining analysis (M, N) of cochlear cross sections. Genotypes are indicated to the left of each row and probes are indicated at the upper right of the top panel in each series. Abbreviations: hc, hair cells; Ko, Kolliker's organ; sc, supporting cells; sv, stria vascularis. Scale bar in A applies to all panels.

Figure 4

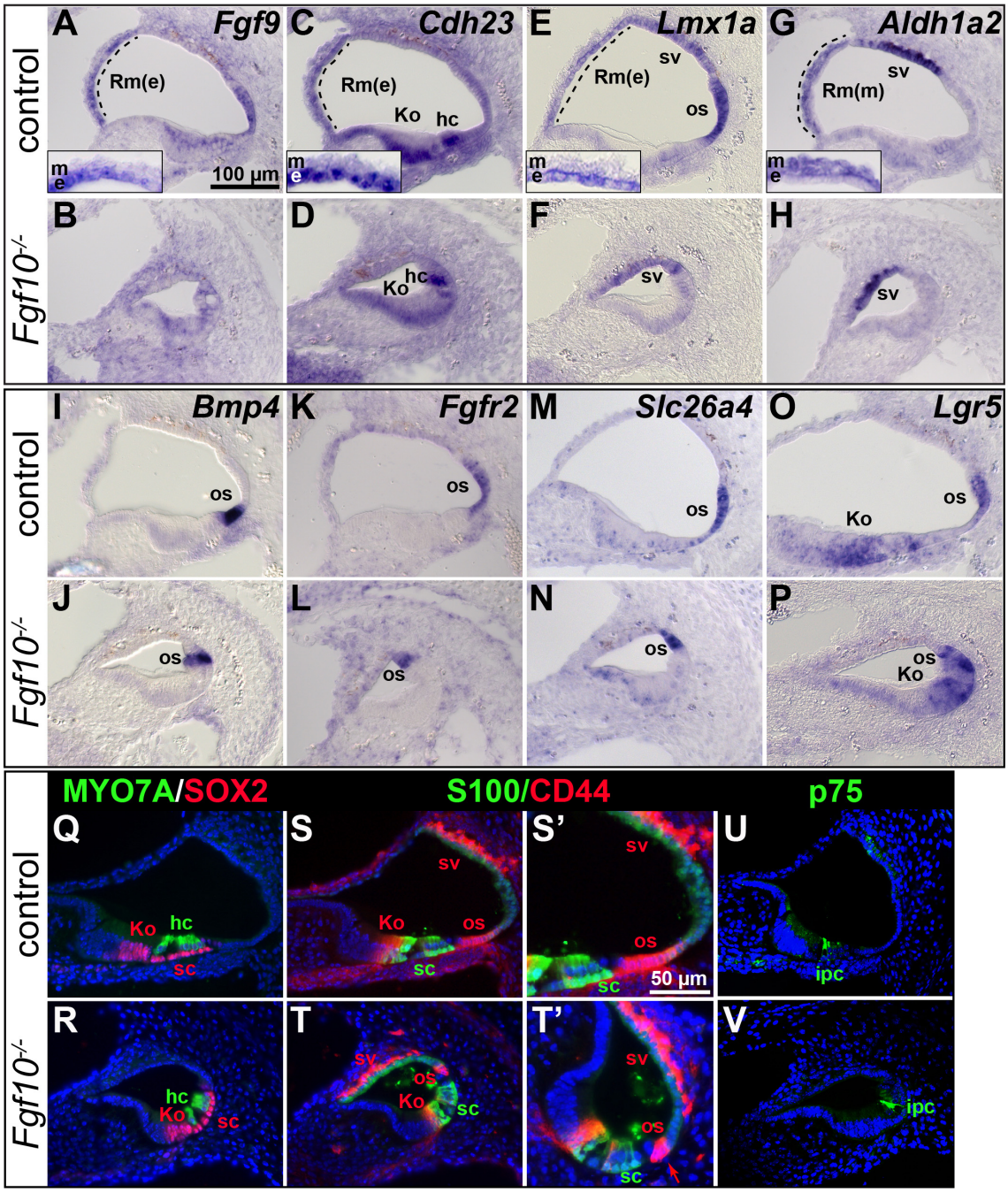

Figure 4. Markers of Reissner's membrane are absent and outer sulcus markers show a reduced domain in E18.5 *Fgf10* null mutant cochleae. In situ hybridization (A-P) and immunostaining (Q-V) analyses of basal cochlear duct cross sections. Genotypes are indicated to the left of each row and probes are indicated to the upper right of each pair of panels. Insets in A, C, E, G show magnifications of Reissner's membrane. S'-T' provide enlargements of S-T, with the arrow in T' indicating the remnant Claudius cells in the mutant os. Abbreviations: Rm(e), Reissner's membrane-epithelial layer; Rm(m), Reissner's membrane-mesenchymal/mesothelial domain; os, outer sulcus. See Figure 2 or 3 legend for others. Scale bar in A applies to all panels except S'-T'. Scale bar in S' applies to T'.

Figure 5

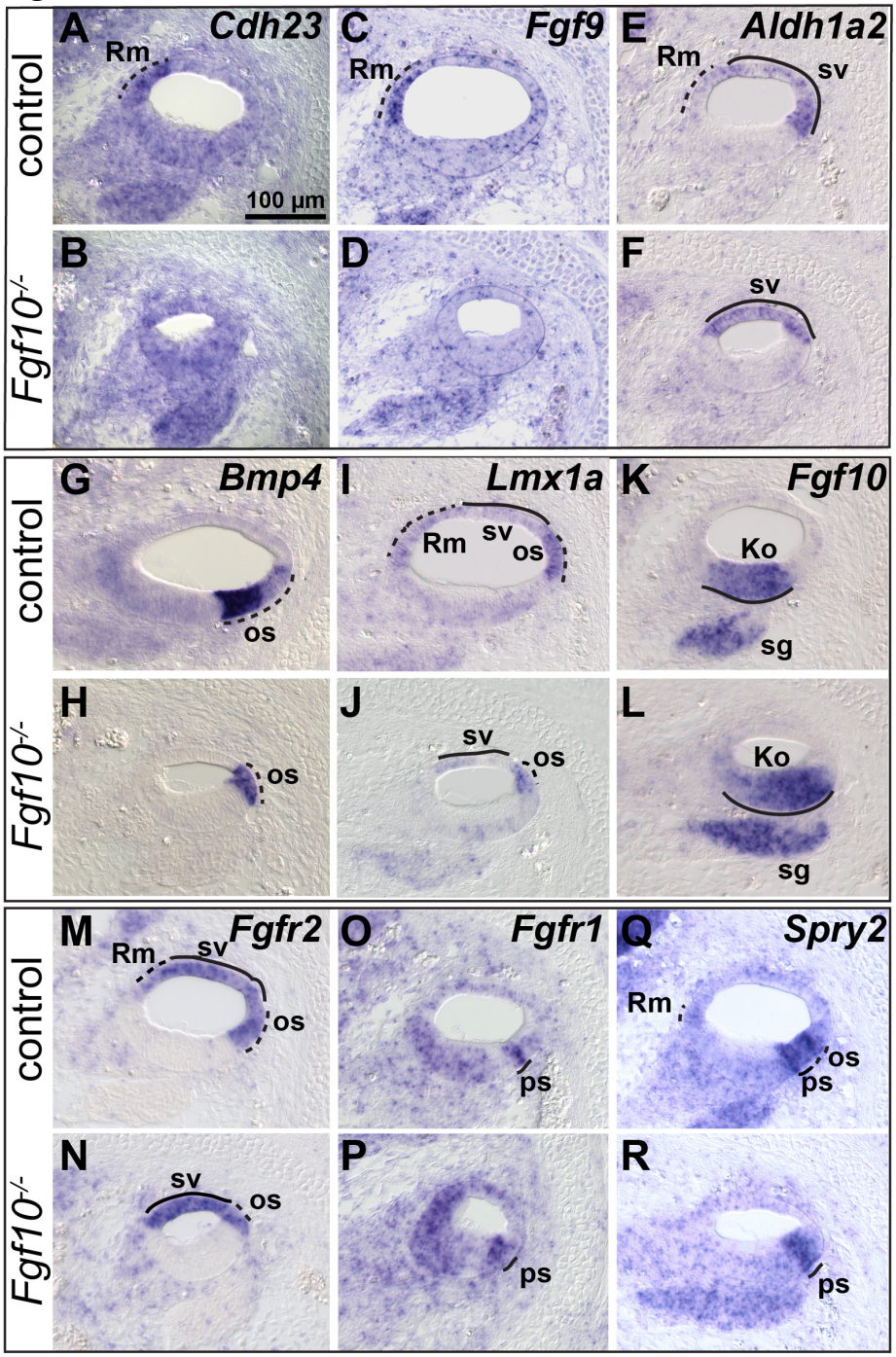

Figure 5. Presumptive Reissner's membrane markers are absent and presumptive outer sulcus markers show a reduced domain in E15.5 *Fgf10* null mutant cochleae. In situ hybridization analyses of basal cochlear duct cross sections are shown. Genotypes are indicated to the left of each row and probes are indicated to the upper right of each pair of panels. Dashed and solid lines indicate expression that is altered or unchanged, respectively, in mutants. Abbreviations: Ko, presumptive Kolliker's organ; os, presumptive outer sulcus; ps, presumptive prosensory domain; Rm, presumptive Reissner's membrane; sg, spiral ganglion; sv, stria vascularis. Scale bar in A applies to all panels.

Figure 6

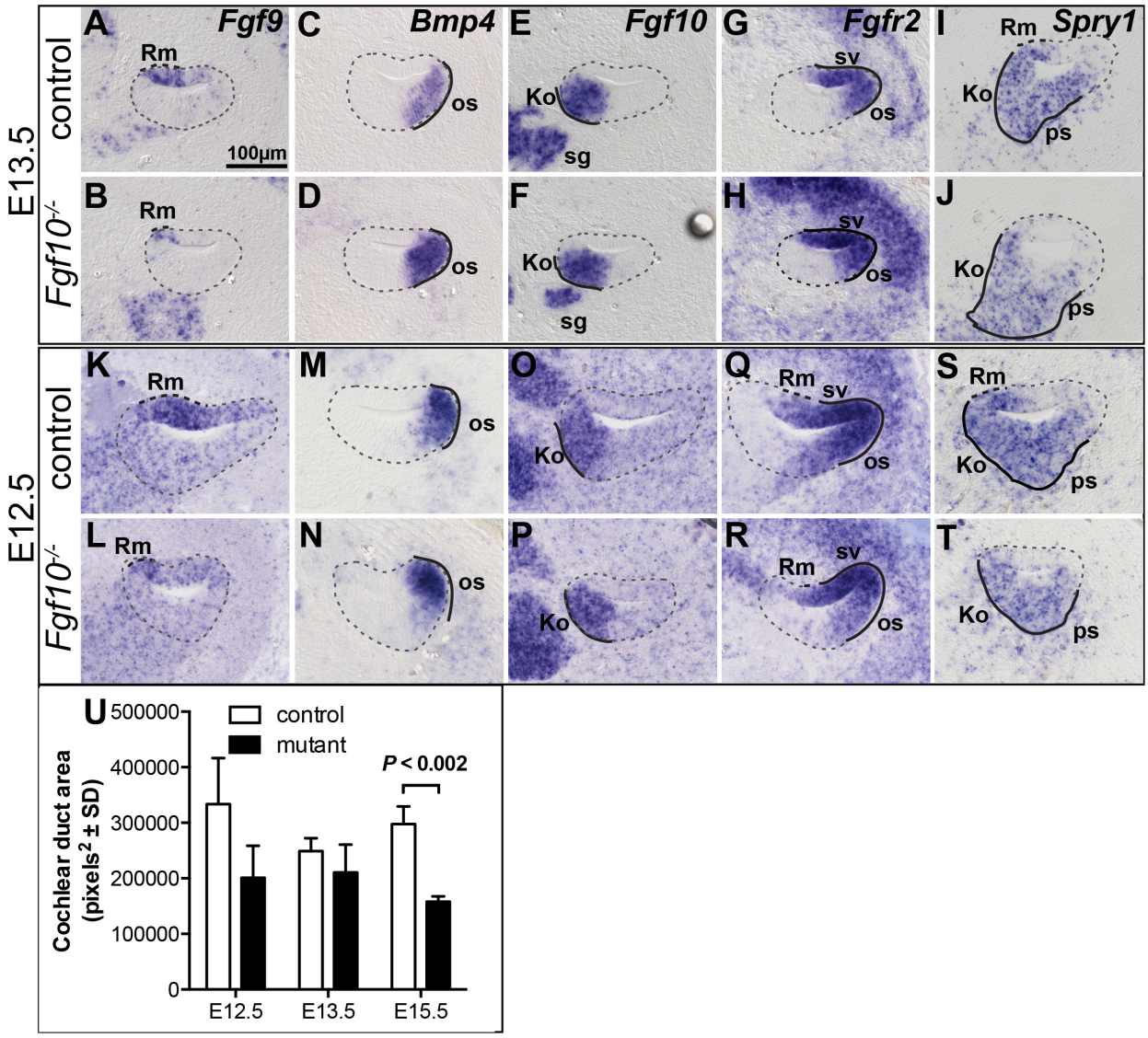

Figure 6. The effects of FGF10 absence on Reissner's membrane development precede those on outer sulcus development. In situ hybridization analyses of basal cochlear duct cross sections at E13.5 (A-J) and E12.5 (K-T). Genotypes are indicated to the left of each row and probes are indicated to the upper right of the top panels. Dashed and solid black lines indicate expression that is altered or unchanged, respectively, in mutants. The cochlear duct is outlined with a dashed grey line. U. Graphical comparison of the cochlear duct area of the basal scala media at the developmental ages shown (n = 3 controls and 3 mutants). Abbreviations: Ko, Kolliker's organ; Rm, presumptive Reissner's membrane; os, outer sulcus; ps, prosensory region; sg, spiral ganglion; sv, stria vascularis. Scale bar in A applies to all panels.

Figure 7

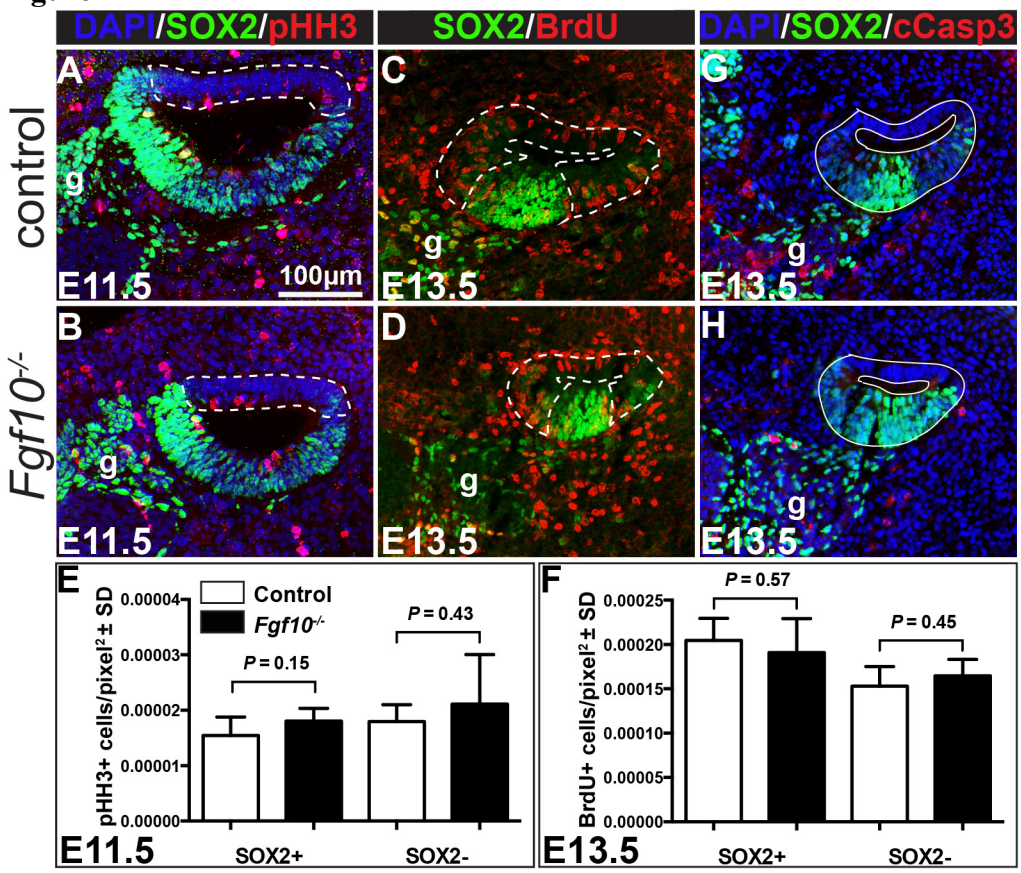

Figure 7. Neither cell proliferation nor cell survival is altered in *Fgf10* null cochlear ducts. Double labeling of control (A) and *Fgf10* null (B) E11.5 cochlear duct cross sections with antibodies directed against phospho-Histone H3 (red) and SOX2 (green). Nuclei are counterstained with DAPI (blue). Double labeling of control (C) and *Fgf10* null (D) E13.5 cochlear duct cross sections with antibodies directed against BrdU (red) and SOX2 (green). Dotted lines delineate the regions considered non-sensory (SOX2-). The remaining SOX2+ areas were considered prosensory. Graphical comparisons of the mean number of proliferating cells per pixel^2 in SOX2+ (E) and SOX2- (F) regions of control (white bars) and *Fgf10* null mutants (black bars). Error bars indicate standard deviation (SD). Double labeling of control (G) and *Fgf10* null (H) E13.5 cochlear duct cross sections with antibodies directed against cleaved Caspase 3 (cCasp3; red) and SOX2 (green). Nuclei are counterstained with DAPI (blue). Abbreviation: g, ganglion. Scale bar in panel A applies to all images.

Figure 8

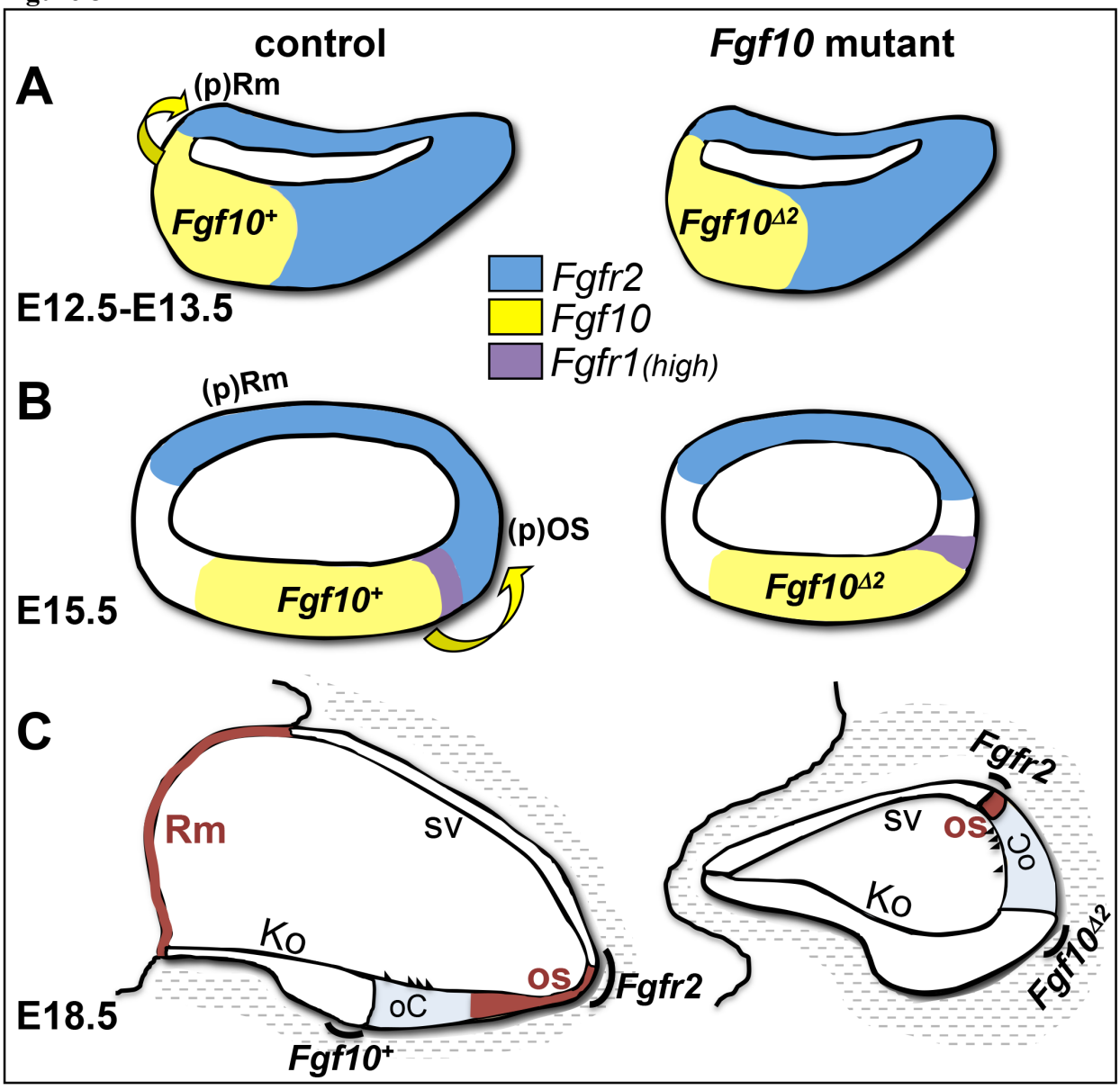

Figure 8. Model depicting the effects of FGF10 absence on development of non-sensory cochlear domains. (A) At E12.5-E13.5 FGF10 induces Reissner's membrane development at the medial boundary of *Fgf10/Fgfr2* expression. (B) By E15.5, FGF10 induces outer sulcus development at the lateral boundary of *Fgf10/Fgfr2* expression. Expression domains of *Fgf10* and receptor genes are color coded in A and B. (C) Failure of FGF10 signaling leads by E18.5 to a total loss of Reissner's membrane and a significant reduction in the outer sulcus. Both affected domains are colored brown. Other morphologic domains are delineated and the now quite separated expression domains for *Fgf10* and *Fgfr2* are indicated with black arcs. There is low-level diffuse expression of *Fgfr1* throughout much of the cochlear duct at all stages (not shown). *Fgf10*$^{\Delta 2}$ refers to the stable exon 2-deleted transcript that does not encode functional FGF10, but perdures in the mutant. All other abbreviations have been defined previously.

Supplementary Table 1

cDNA clones used to prepare digoxigenin-labeled cRNA antisense transcripts for in situ hybridization

Gene	Insert size	Enzyme	Polymerase	Source	Reference
Bmp4	~900 bp	Acc1	T7	Anne Boulet	Jones et al., 1991
Cldn11	~1.8 kb	NcoI	T3	Andreas Kispert	Trowe et al., 2011
Fgf9	~860 bp	HindIII	SP6	David Ornitz via Sabine Fuhrmann	Colvin et al., 1999
Fgf10	~550 bp	EcoR1	SP6	David Ornitz	Xu et al., 1998
Fgfr1	459 bp	EcoRI	T7	GenBank BC010200	Li et al., 2007
Fgfr2	~1.8 kb	EcoRI	SP6	Olivia Bermingham-McDonogh	Hayashi et al., 2010
Lfng	~1 kb	HindIII	T3	Doris Wu	Morsli et al., 1998
Myo6	834 bp	NotI	T7	Karen Avraham	Avraham et al., 1995
Spry1	~1.5 kb	EcoRI	T7	Kathy Shim	Minowada et al., 1999
Spry2	~1.5 kb	XbaI	T3	Kathy Shim	Minowada et al., 1999
Tecta	~4.5 kb	EcoRI	T7	Guy Richardson	Rau et al., 1999
Trp2	~1kb	HindIII	T7	Doris Wu	Morsli et al., 1999

Purified DNA was digested with the indicated restriction enzyme and then transcribed with the indicated RNA polymerase to produce antisense probes for in situ hybridization.

Reference List for Probes:

Avraham, K. B., Hasson, T., Steel, K. P., Kingsley, D. M., Russell, L. B., Mooseker, M. S., Copeland, N. G., Jenkins, N. A., 1995. The mouse Snell's waltzer deafness gene encodes an unconventional myosin required for structural integrity of inner ear hair cells. Nat Genet. 11**,** 369-375.

Colvin, J. S., Feldman, B., Nadeau, J. H., Goldfarb, M., Ornitz, D. M., 1999. Genomic organization and embryonic expression of the mouse fibroblast growth factor 9 gene. Dev Dyn. 216**,** 72-88.

Hayashi, T., Ray, C. A., Younkins, C., Bermingham-McDonogh, O., 2010. Expression patterns of FGF receptors in the developing mammalian cochlea. Dev Dyn. 239**,** 1019-1026.

Jones, C. M., Lyons, K. M., Hogan, B. L., 1991. Involvement of Bone Morphogenetic Protein-4 (BMP-4) and Vgr-1 in morphogenesis and neurogenesis in the mouse. Development. 111**,** 531-542.

Li, C., Scott, D. A., Hatch, E., Tian, X., Mansour, S. L., 2007. Dusp6 (Mkp3) is a negative feedback regulator of FGF-stimulated ERK signaling during mouse development. Development. 134**,** 167-176.

Minowada, G., Jarvis, L. A., Chi, C. L., Neubuser, A., Sun, X., Hacohen, N., Krasnow, M. A., Martin, G. R., 1999. Vertebrate Sprouty genes are induced by FGF signaling and can cause chondrodysplasia when overexpressed. Development. 126**,** 4465-4475.

Morsli, H., Choo, D., Ryan, A., Johnson, R., Wu, D. K., 1998. Development of the mouse inner ear and origin of its sensory organs. J Neurosci. 18**,** 3327-3335.

Morsli, H., Tuorto, F., Choo, D., Postiglione, M. P., Simeone, A., Wu, D. K., 1999. Otx1 and Otx2 activities are required for the normal development of the mouse inner ear. Development. 126**,** 2335-2343.

Rau, A., Legan, P. K., Richardson, G. P., 1999. Tectorin mRNA expression is spatially and temporally restricted during mouse inner ear development. J Comp Neurol. 405**,** 271-280.

Trowe, M. O., Maier, H., Petry, M., Schweizer, M., Schuster-Gossler, K., Kispert, A., 2011. Impaired stria vascularis integrity upon loss of E-cadherin in basal cells. Dev Biol. 359**,** 95-107.

Xu, X., Weinstein, M., Li, C., Naski, M., Cohen, R. I., Ornitz, D. M., Leder, P., Deng, C., 1998. Fibroblast growth factor receptor 2 (FGFR2)-mediated reciprocal regulation loop between FGF8 and FGF10 is essential for limb induction. Development. 125**,** 753-765.

Supplementary Table 2

Primers used to generate DNA fragments for cRNA probe production

Gene	Primers	Primer sequences (5'-3')	Product size (bp)
Cdh23	F-816	CAAGATGGAATCCACATGGTTCATGG	651
	R-817	ATGCTTTGTTCCCTCATCCACTGG	
Fgf16	F-832	TGTGACCCATGTACTCTGTGACC	457
	R-833	ACGAATGAGAGATCTGCAGAGCC	
Jag1	F-812	TGCTGAGCTCTGTCTTAACAGTGG	605
	R-813	ACAGAAACTACCAGTGCCAGTGG	
Lgr5	F-826	CCTATTTGGTAGCTGGCTGATCC	657
	R-827	AGCAACAGAGCAATGTGCTTCACC	
Lmx1a	F-828	TCAGTAACCTGGGAGACTGCTTCC	836
	R-829	GCCATCTGGAGTTGTATTCTAGACC	
Aldh1a2	F-810	CTCACAACAAGTGAGCTTCAGCC	407
	R-811	ACTGTAGGAGGAACAGAGAGCC	
Slc26a4	F-822	GTAGACTTGCTTCCTGAGAGAGG	448
	R-823	CACATGGATTTCAGAGTCAGTCAGG	

Forward (F) and reverse (R) primers used to PCR-amplify 3'UTR regions of each indicated gene from mouse genomic DNA. All reverse primers include the T7 promoter sequence (GGATCCTAATACGACTCACTATAGGGAG) at the 5' end. The antisense-RNA strand was produced by transcription of the PCR product using T7 RNA polymerase.

Supplementary Figure 1

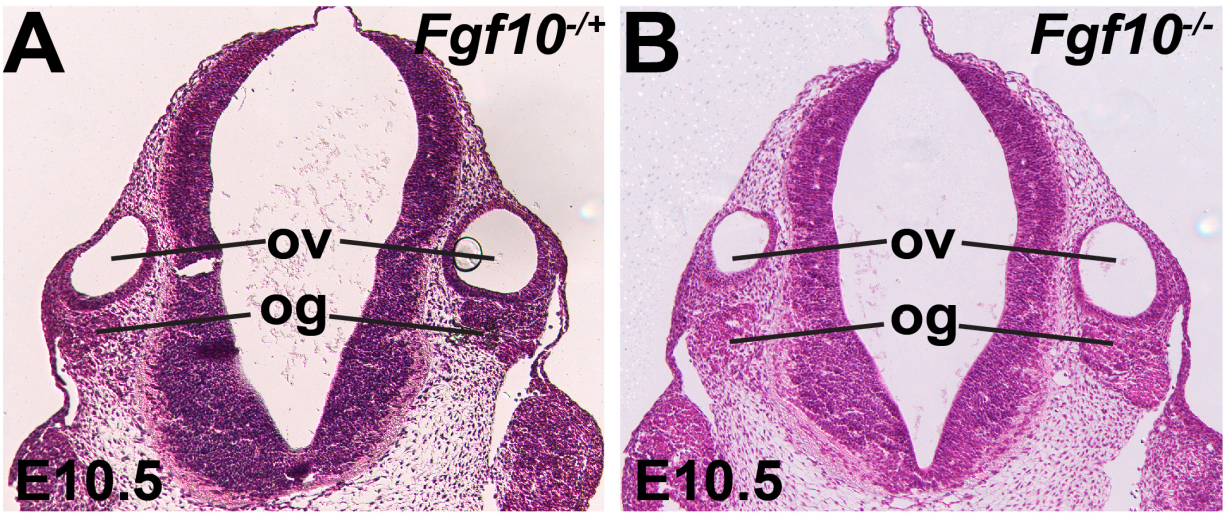

Figure S1. Otic development is normal in E10.5 *Fgf10* null mutants. Hematoxylin and eosin stained transverse sections of (A) heterozygous and (B) homozygous null *Fgf10* mutants show similar development of the otic vesicle (ov) and otic ganglion (og).

Supplementary Figure 2

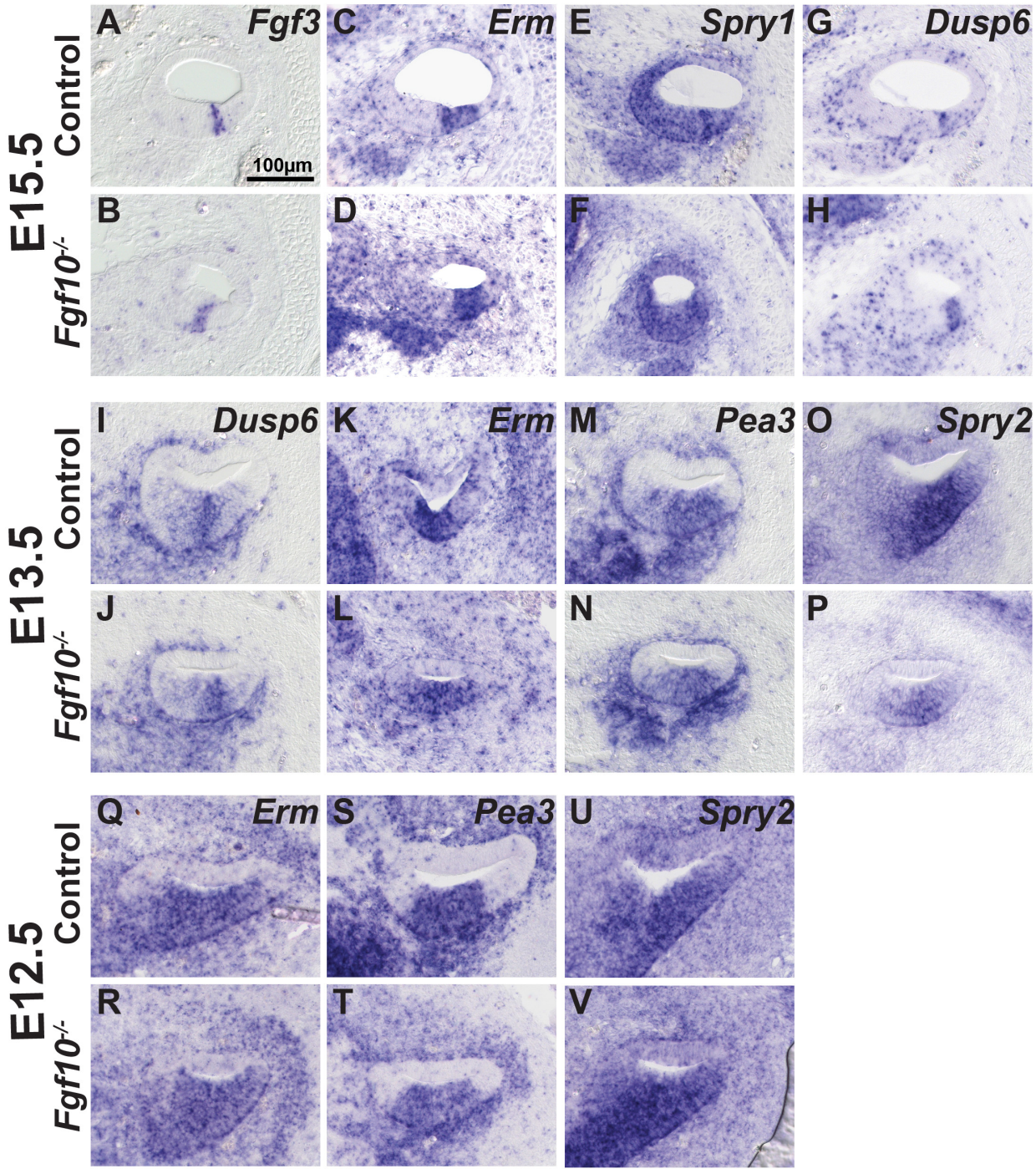

Figure S2. *Fgf3* and several FGF signaling indicators are expressed similarly in control and *Fgf10*$^{-/-}$ embryos. Cochlear duct cross sections at the stages indicated to the left were hybridized with the indicated probes. The scale bar in A applies to all panels.