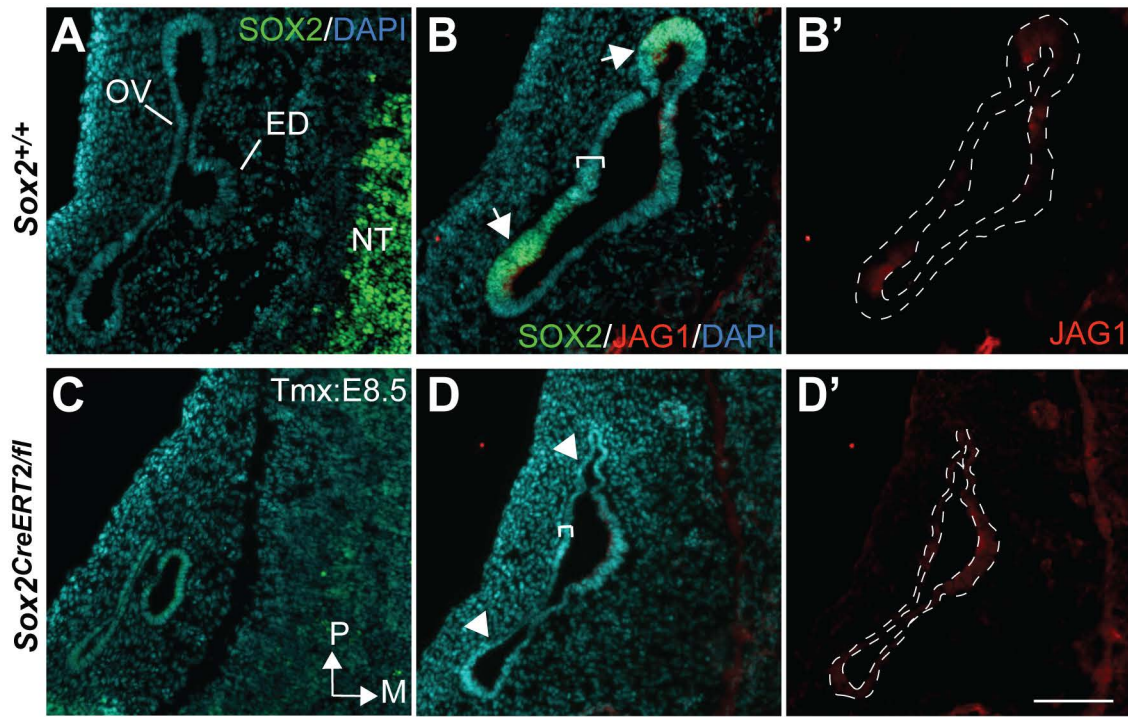
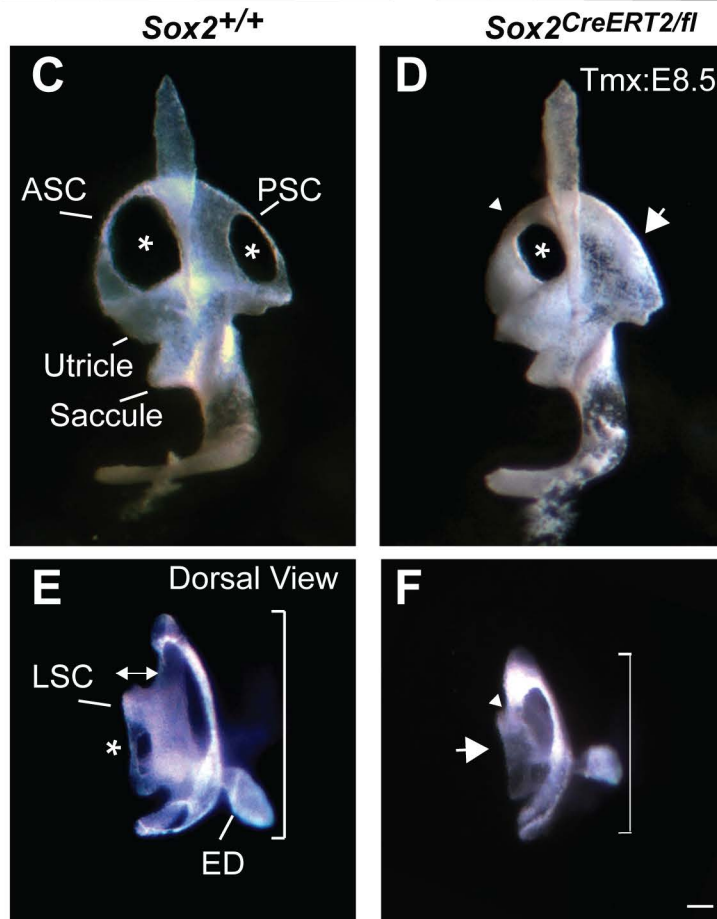


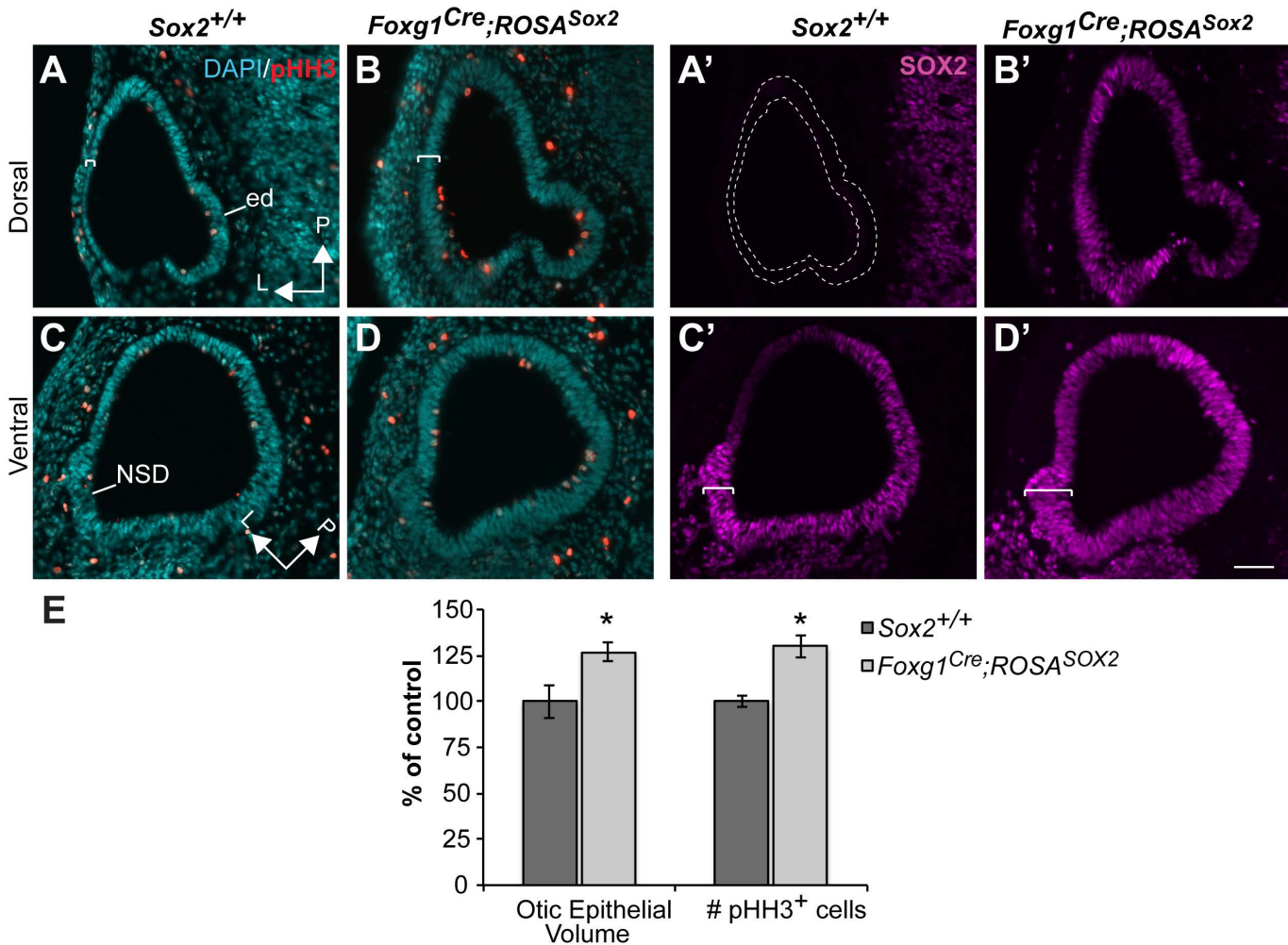
### E11.5 Dorsal Otocyst



### E12.5 Inner Ears



**Figure S1. Early (E8.5) SOX2 deletion impairs canal formation.** Cryosections of *Sox2* control (**A**) and *Sox2*<sup>CreERT2/fl</sup> mutant (**B**) show that E8.5 SOX2-depletion reduces the dorsal otic epithelial thickness compared to control at E11.5 (**A-D; brackets, B and D**). Weak or absent dorsal JAG1 expression in the anterior and posterior regions, which marks the presumed cristae, is observed in mutants compared to controls (**B' and D'**). E12.5 paint-filled inner ears show canal formation but smaller canal regions as well as fusion defects in the posterior and lateral canal (**C-F**). **C** and **D** are medial views showing a smaller but fused anterior semicircular canal and a small unfused posterior semicircular canal (**D; asterisk, arrow**). **E** and **F** are dorsal views showing that E8.5 SOX2-deficient inner ears have a smaller and unfused lateral semicircular canal primordium (**F; arrow, small arrowhead**). Double-headed arrows (**E**) indicate the size of the outpocketing of the control lateral semicircular canal and the small arrowhead indicates the smaller size in the mutant. The reduced anterior and posterior canal size in the SOX2-deleted inner ears compared to control are indicated by brackets (**E-F**). Sites of epithelial fusion are marked by an asterisk (**C-E**). Site of non-fusion are marked by an arrow (**D, F**). Scale bars: 100  $\mu$ m. ASC, anterior semicircular canal; ED, endolymphatic duct; LSC, lateral semicircular canal; PSC, posterior semicircular canal.



**Figure S2. Overexpression of SOX2 expands the number of otic progenitors.** Dorsal and ventral E10.5 sections from control and SOX2-overexpressed otocysts. Dorsal sections of *Sox2*<sup>+/+</sup> (A) and *Foxg1*<sup>Cre</sup>;*ROSA*<sup>SOX2</sup> (B) showing that overexpression of SOX2 increases epithelial thickness, notably in the typically thinner lateral wall (brackets). Ventral sections (C-D') showing a similar pattern. (A'-D') Confirmation of SOX2 overexpression showing SOX2 upregulation even in the dorsal otocyst that normally expresses very little SOX2 (A' and B'). (E) Quantification normalized to the control of epithelial volume \*P = .03 and pHH3-positive cells \*P = .02. Significance determined by a Student's *t*-test. Bars represent the SEM. Scale bar: 50  $\mu$ m