Supplemental Figure 1. Substitution of *Hnf1β-CreER^{T2}* in Pulse-Chase Analyses with *Sox9-CreER^{T2}* Confirms that Notch-Dependent TrPC Allocation Closes by E13. (**A,B**) Tamoxifen (Tam) administration to experimental mice induces *Sox9-CreER^{T2}*-mediated expression of a dnMaml1-GFP fusion protein from the *Rosa26* locus, rendering Sox9$^+$ TrPC-biased MPCs and their progeny permanently Notch-insensitive and GFP-labeled (**A**) while in control embryos, the same cell population is YFP-labeled but remains Notch signaling-intact following tamoxifen-induced *Sox9-CreER^{T2}*-mediated recombination of the *Rosa26^{YFP}* reporter (**B**). (**C**) Approximate temporal windows of Tam activity resulting from single intraperitoneal (i.p.) injections at E11.5, E12.5 or E13.5: pancreata were analysed at E15.5 whereupon cell fate of labeled TrPCs and their progeny was assessed on the basis of marker expression (**D**). *Sox9-CreER^{T2}*-mediated recombination efficiency for both *Rosa26$^{dnMaml1\text{-}eGFP}$* and *Rosa26^{YFP}* alleles is of an order of magnitude greater than that achieved with *Hnf1β-CreER^{T2}* (**E-P**). Qualitatively, cell fate distributions of control YFP$^+$ cells in E15.5 *Sox9-CreER^{T2}; Rosa26^{YFP}* and Notch-insensitive dnMaml1-GFP$^+$ cells in *Sox9-CreER^{T2}; Rosa26$^{dnMaml1\text{-}eGFP}$* pancreata following E11.5, E12.5 or E13.5 Tam i.p. mirror those seen using *Hnf1β-CreER^{T2}*. While after an E11.5 Tam i.p., YFP$^+$ cells in control *Sox9-CreER^{T2}; Rosa26^{YFP}* pancreata can be found to express markers of all three compartments (**E,G**), by an E13.5 Tam i.p., YFP$^+$ cells are predominantly Sox9$^+$ or endocrine (Ngn3$^+$ or ChrA$^+$) (**I,K,M,O**). Following Tam i.p. at E11.5, dnMaml1-GFP$^+$ cells in *Sox9-CreER^{T2}; Rosa26$^{dnMaml1\text{-}GFP}$* pancreata are predominantly Sox9$^-$ Ptf1a$^+$ with a proportion expressing endocrine markers (Ngn3, Chr-A) (**F,H**). Tam i.p at E12.5 or E13.5 similarly results in the vast majority of dnMaml1-GFP$^+$ cells being Sox9$^-$ although in contrast to the principally acinar labeling following E11.5 Tam i.p., dnMaml1-GFP$^+$ cells following E12.5 or E13.5 Tam i.p. are predominantly endocrine (Chr-A$^+$) with a smaller proportion expressing Ptf1a (**J,L,N,P**). Scale bar = 50 μm.

Supplemental Figure 2. Validation of Specificity of Anti-Dll1, -Jag1 and -Hes1 Antisera. Rabbit monoclonal anti-Jag1 antibody (Cell Signaling Technology: 2620) gives membraneous signal on cells in the neural tube and weaker but specific membraneous staining throughout both pancreatic buds in E10.5 control mice (**A,A''**) but not *Jag1$^{-/-}$* littermates (**B,B''**). Similarly, goat anti-Jag1 antibody (Santa Cruz Biotechnology: sc-6011) gives membraneous signal on cells in the neural tube and weaker but specific membraneous staining throughout both pancreatic buds in E10.5 control

embryos (**C,C''**) but not *Jag1*$^{-/-}$ littermates (**D,D''**). Note that adjacent sections are shown in **A-D''**: Jag1 is detected in the same cell populations using both antisera although the goat antibody yields stronger signal. Sheep anti-Dll1 antibody (R&D Systems: AF3970) yields heterogeneous membraneous signal on cells in the neural tube (nt), and weaker but specific signal in dorsal (dp) and ventral (vp) pancreas in E10.5 control embryos (**E,E''**), but not nullizygous *Dll1*$^{lacZ/lacZ}$ littermates (**F,F''**). Rabbit monoclonal anti-Hes1 antibody (Cell Signaling Technology: 11988) yields strong nuclear Hes1 signal in the neural tube and floorplate (fp) and throughout both pancreatic buds in E10.25 control embryos (**G,G''**) but not *Hes1*$^{-/-}$ littermates (**H,H''**). Sox9 signal is shown for reference. Pancreatic buds are demarcated by white dashed lines in **A'',B'',C'',D'',E'',F'',G'',H''**. Images of Sox9, ligand or Hes1 signal within each antibody combination were captured at the same exposure and processed equivalently except for inset panels for Dll1 (**E'',F''**) or Jag1 (**A'',B''**) in which weaker signal in pancreatic buds compared to neural tube is enhanced to show pancreas-specific signal (parallel enhancement performed for control and nullizygous images). duo, duodenum; cbd, common bile duct. Scale bar = 50 μm.

Supplemental Figure 3. Cell-Specific Expression of Jag1 and Dll1 in the Developing Pancreas. Sections of an E15.5 *Jag1*^{J1VmC} embryo stained for Venus (Jag1-Venus) and Sox9 (**A**) or mCherry (Jag1-mCherry), GFP (from Hes1-eGFP) and Sox9 (**C**) and sections of an E15.5 (or E12.5, **E**) *Dll1*^{D1VmC} embryo stained for Venus (Dll1-Venus) and Sox9 (**B**) or mCherry (Dll1-mCherry) and Sox9 with either GFP (Hes1-eGFP) (**D**) or Jag1 (**E,F**). Sections of E12.5 (**G-I**) or E15.5 (**J-L**) pancreata stained for Jag1, Pecam and E-Cadherin (**G,J**), Jag1, Ngn3 and glucagon with insulin (**H,K**) or Jag1, Ptf1a, and Sox9 (**I,L**). Sections of E12.5 (**M-O**) or E15.5 (**P-R**) pancreata stained for Dll1, Pecam and E-Cadherin (**M,P**), Dll1, Ngn3 and glucagon with insulin (**N,Q**) or Dll1, Ptf1a, and Sox9 (**O,R**).

Supplemental Figure 4. PD Patterning is Perturbed Following Mosaic Endodermal Deletion of Jag1 and Dll1. (**A-D'**) IF on sections of E12.5 embryos of the indicated genotypes for Ptf1a, Nkx6.1, YFP and E-Cadherin. (**E-G**) Scatter plots showing quantifications of the number of Ptf1a$^+$ (Ptf1a$^+$ Nkx6.1$^-$) TiPCs (**E**), Nkx6.1$^+$ (Ptf1a$^-$ Nkx6.1$^+$) TrPCs (**F**) and Ptf1a$^+$ Nkx6.1$^+$ cells (**G**) relative to the number of progenitor cells (the total number of Ptf1a$^+$ and/or Nkx6.1$^+$ cells) in dorsal pancreata. Data are

presented as mean ± S.E.M. *P*-values are indicated on the plots. Genotypes are abbreviated in scatter plots by the first letter (D = Dll or J = Jag) of the ligand gene(s) deleted by *Sox17*^{CreERT2}.

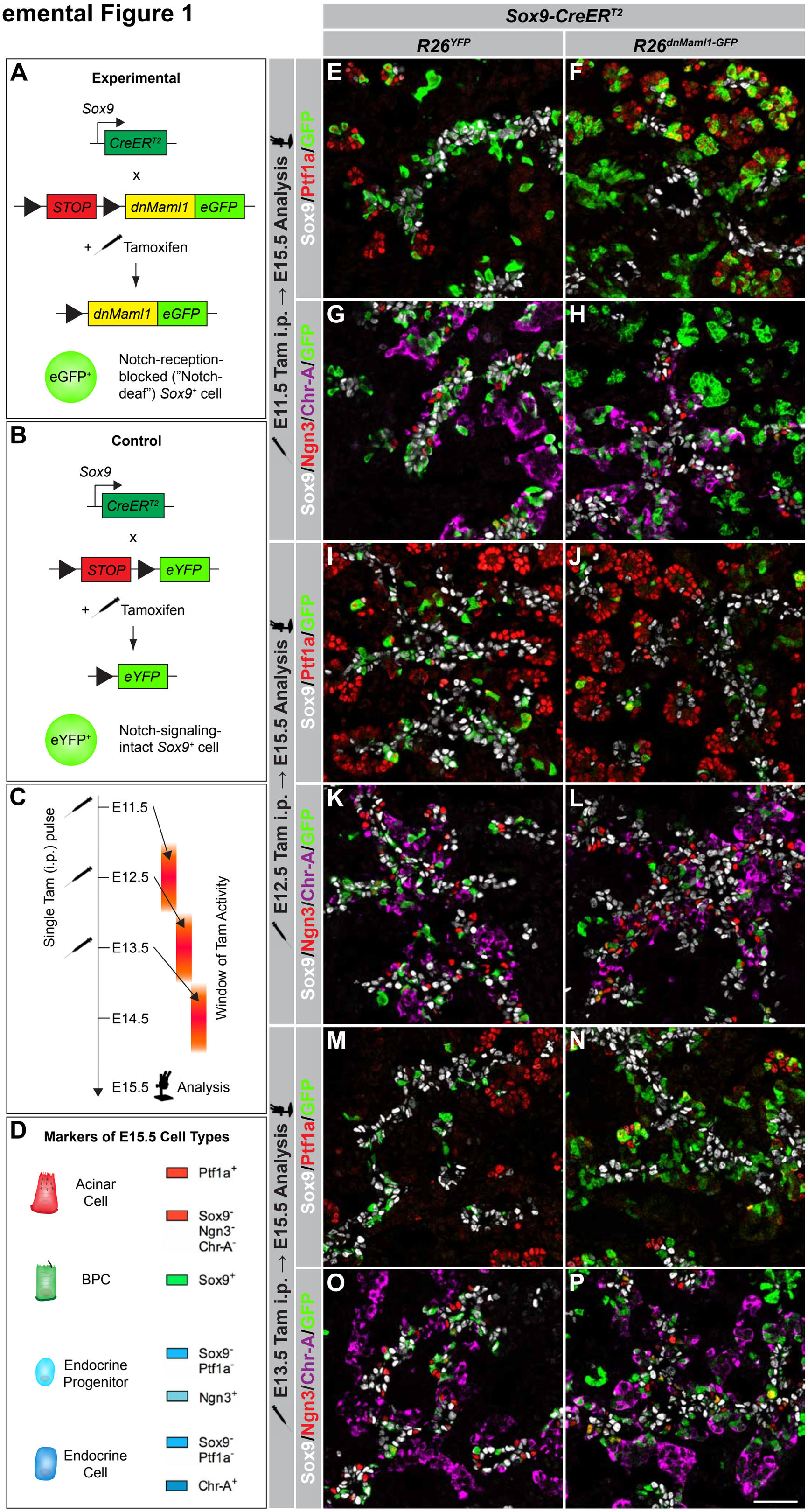
Supplemental Figure 5. Resolution of PD Patterning is Delayed by Two Days in the *Jag1*$^{\Delta Foxa2}$ Pancreas. IF for Ptf1a and either Nkx6.1 (**A,B,G,H**) or Sox9 (**C,D,I,J**) shows that while Ptf1a expression is maintained proximally in the *Jag1*$^{\Delta Foxa2}$ pancreas at E13.5 (**B,D**) compared to controls (**A,C**), Ptf1a and Nkx6.1 expression domains have resolved by E14.5 in the mutant with loss of the distal-most Nkx6.1^{+} (**H**, inset) and Sox9^{+} (**J**, inset) TrPC-derived duct cells. Presence of insulin^{+} β-cells and Ngn3^{+} endocrine precursors is ostensibly unaffected in E13.5 (**F**) or E14.5 (**L**) *Jag1*$^{\Delta Foxa2}$ pancreata relative to control E13.5 (**E**) or E14.5 (**K**) littermates.

Supplemental Figure 6. Apicobasal Polarity is Retained in E15.5 *Jag1*$^{\Delta Foxa2}$ Acini But Lost Prior to Birth. IF for ZO-1 shows it to be maintained apically on Ptf1a^{+} acinar and Sox9^{+} duct cells in the E15.5 *Jag1*$^{\Delta Foxa2}$ (**B-C'**) as control (**A,A'**) pancreas. Similarly, IF for PKCζ demonstrates it to be maintained apically on Ptf1a^{+} acinar and Pdx1^{Lo} duct cells in the E15.5 *Jag1*$^{\Delta Foxa2}$ (**E-F'**) as control (**D,D'**) pancreas. At E18.5 however, while apical ZO-1 is still expressed on Ptf1a^{+} CPA1^{+} acinar cells (as well as duct cells) in control mice (**G-G'**), it is downregulated in epithelial ((*R26*)YFP^{+}) Ptf1a^{+} CPA1^{+} "ring"-like structures (asterisks) in the *Jag1*$^{\Delta Foxa2}$ pancreas (**H-I'**). Likewise, PKCζ, expressed apically on Ptf1a^{+} CPA1^{+} acinar cells (and duct cells) in the E18.5 control pancreas (**J,J'**) is depleted in Ptf1a^{+} CPA1^{+} ring-like structures (asterisks) in the *Jag1*$^{\Delta Foxa2}$ pancreas (**K-L'**).

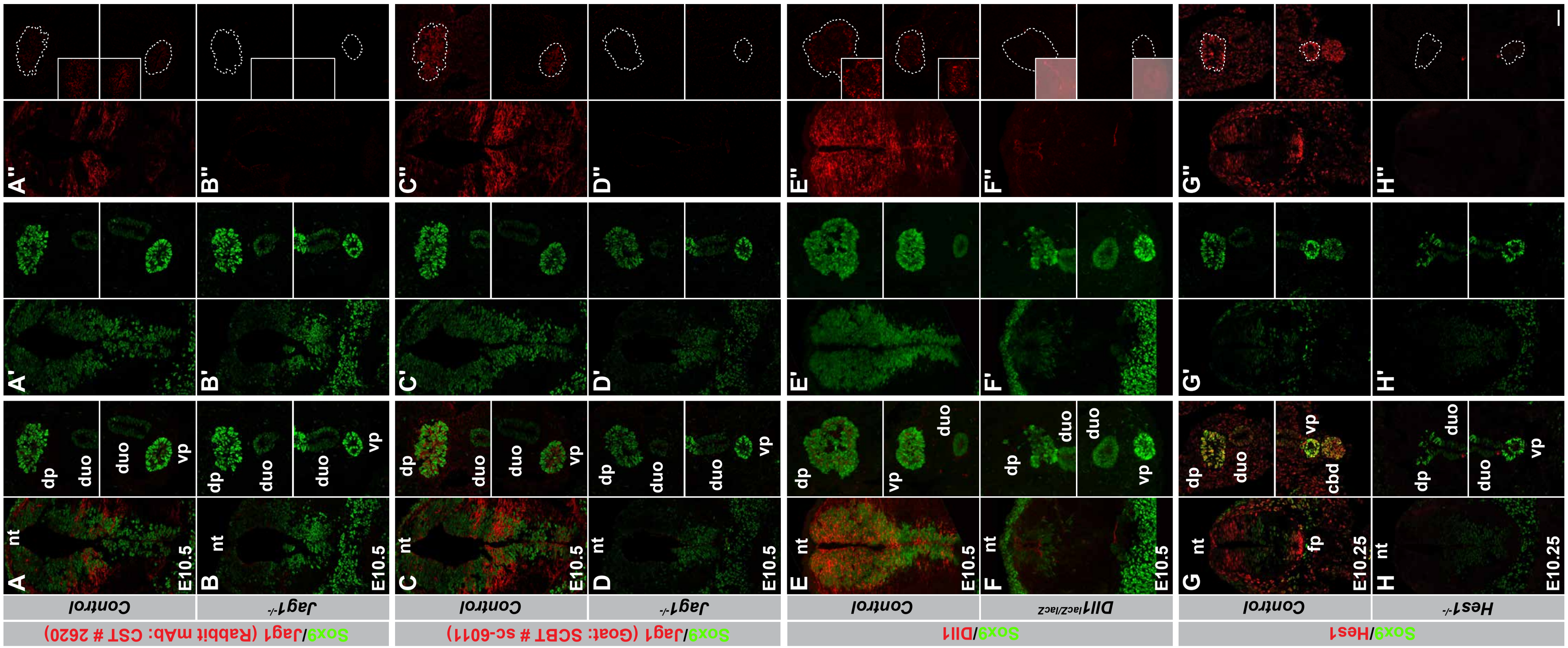
Supplemental Figure 7. Endocrine Paucity and Acinar Dysmorphogenesis are Exacerbated with Development. IF for insulin, glucagon and somatostatin shows a greater extent of loss of the later-arising somatostatin^{+} δ-cells than of either insulin^{+} β-cells or glucagon^{+} α-cells in the E18.5 *Jag1*$^{\Delta Foxa2}$ pancreas (**B**) compared with control littermates (**A**). Expression of the acinar markers amylase and CPA1 is retained in acinar cells of E18.5 *Jag1*$^{\Delta Foxa2}$ (**D**) as in control (**C**) pancreas, including dilated "ring"-like structures although their expression is aberrantly basal (**D**, inset). IF for Muc1 and the ductal markers Cytokeratin 19 (CK19) and DBA reveals discontinuous, dispersed ductal structures in the E18.5 *Jag1*$^{\Delta Foxa2}$ pancreas (**F**) in contrast to the extensive, continuous ductal tree of control littermates (**E**). The amylase^{+} CPA1^{+} ring-like structures also express CK19 (ring-like structure in

insets on **D** and **F** from the same region of adjacent sections). Insets are displayed with and without the YFP channel to demarcate epithelium for clarity.

Supplemental Figure 1



Supplemental Figure 2



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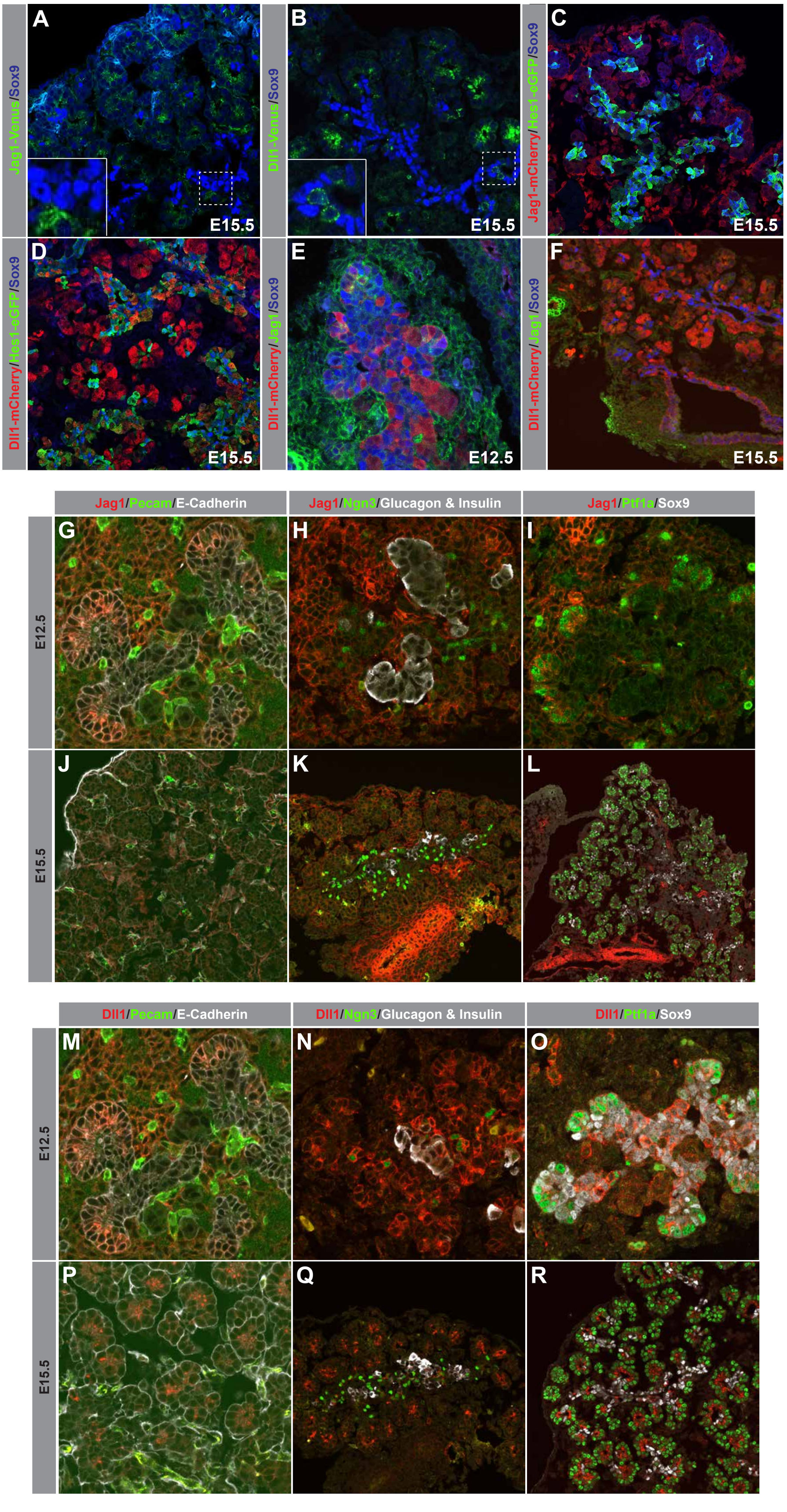

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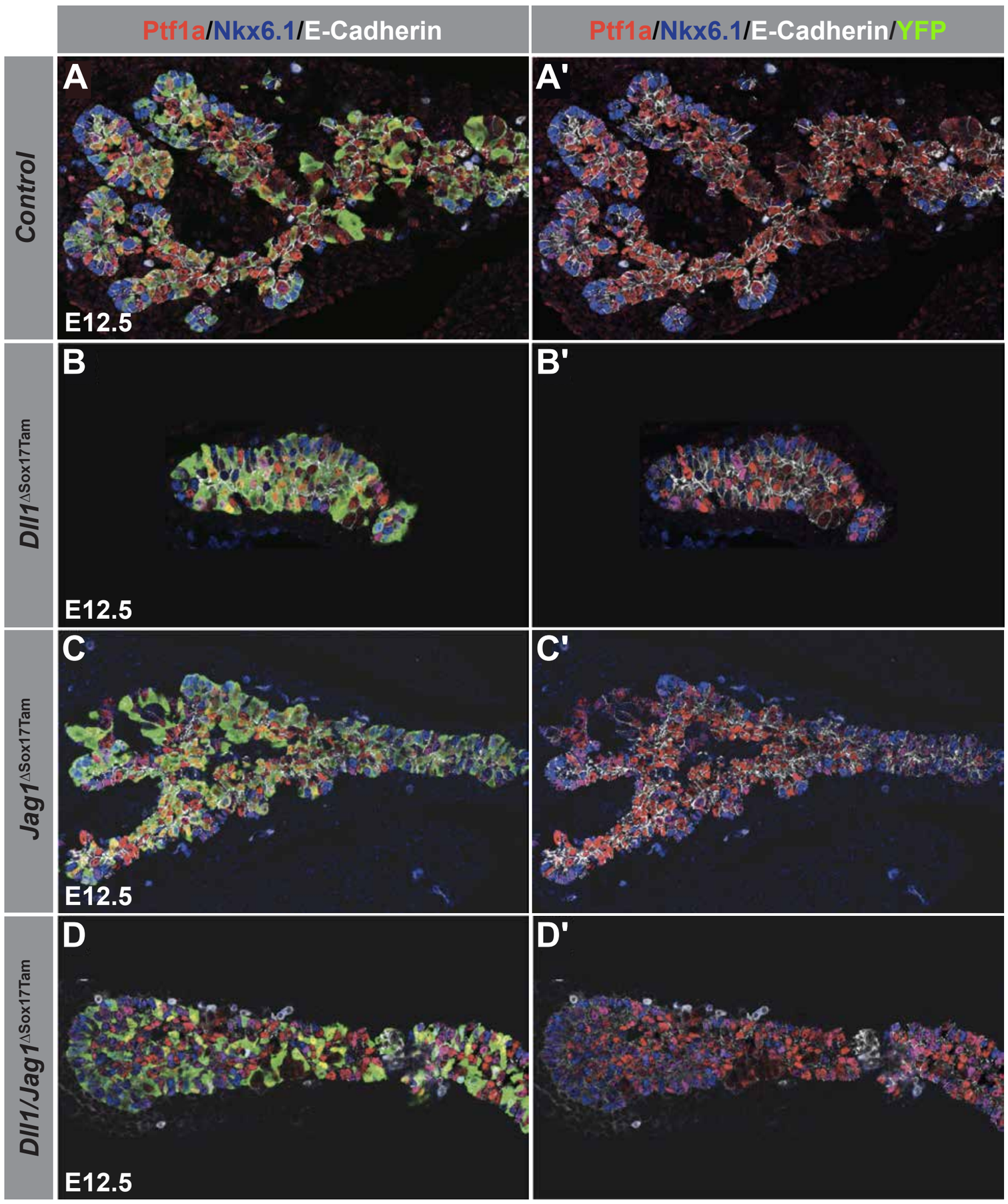

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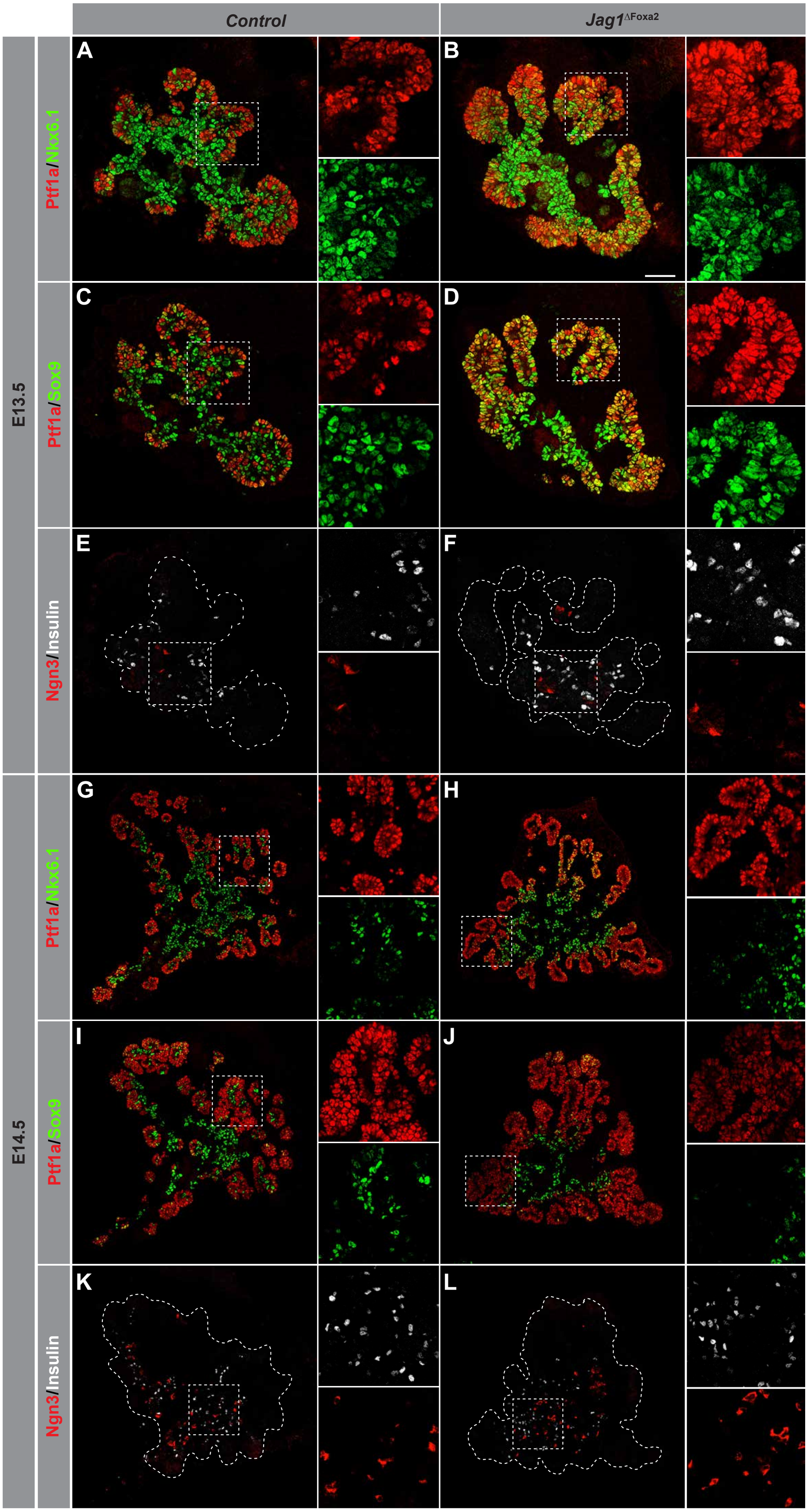
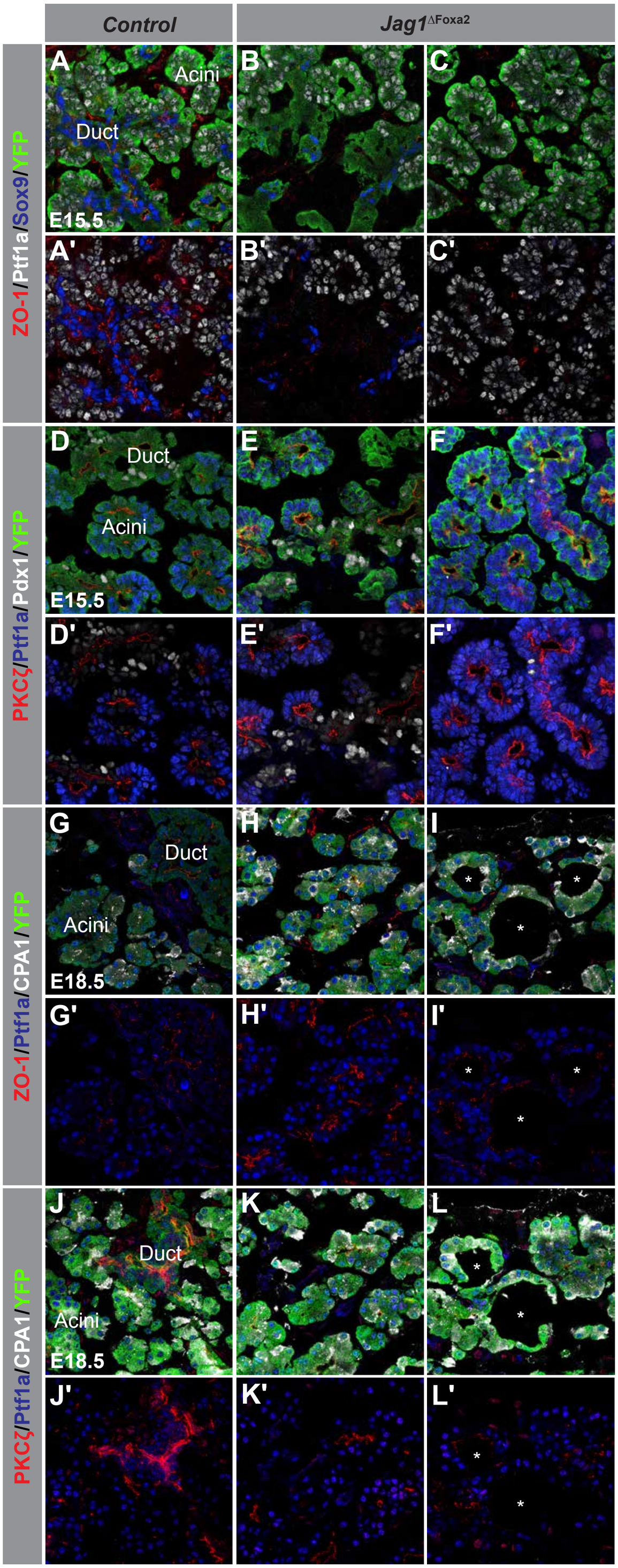


Supplemental Figure 6



Supplemental Figure 7