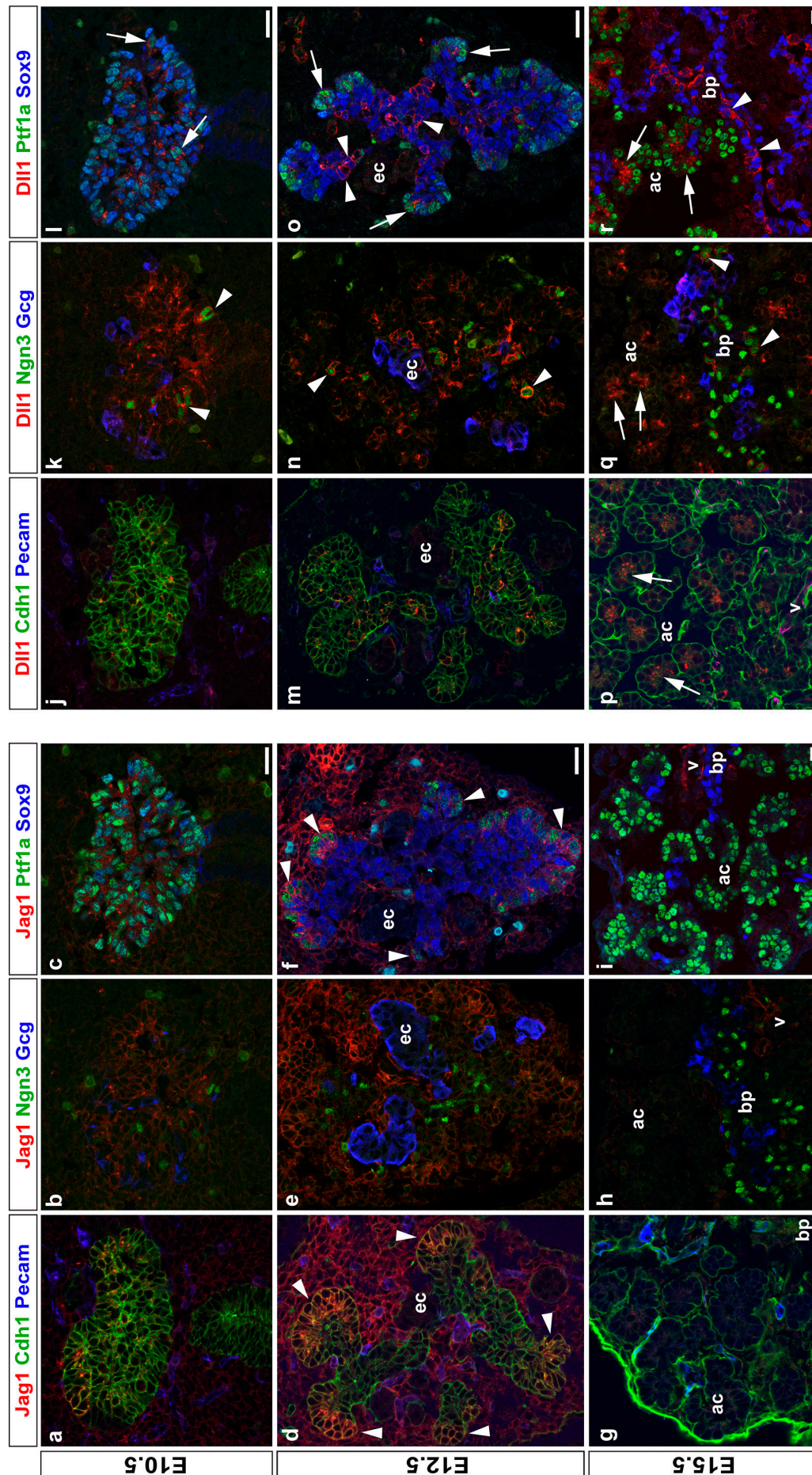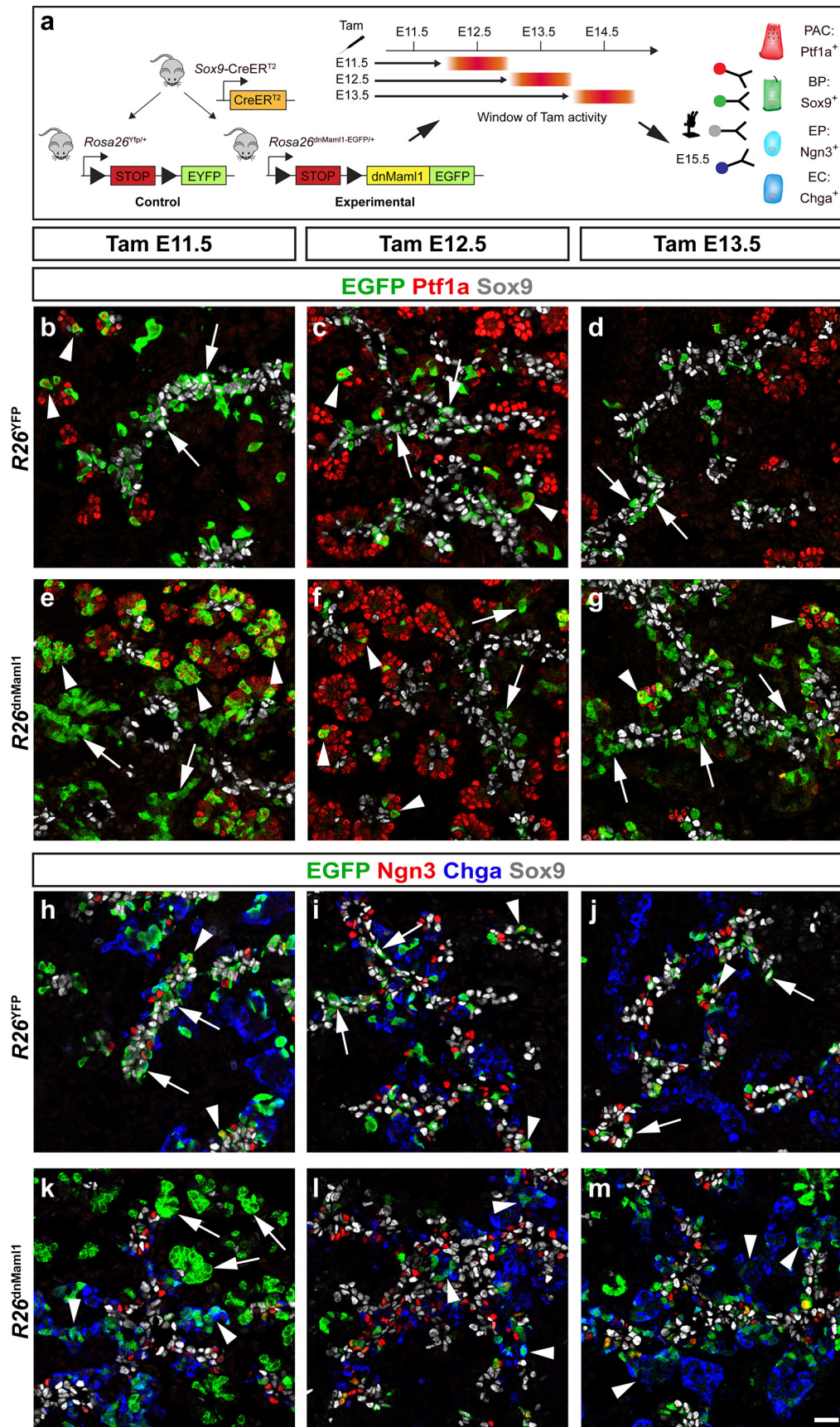**Supplementary Figure 1**

Supplementary Fig. 1. Expression of Jag1 and Dll1 in the developing pancreas. **a**, **g** Schematics showing the structure of the $Jag1^{J1VmC}$ **a** and $Dll1^{D1VmC}$ **g** reporters. **b-f** Sections of E9.5 **b**, E10.5 **c**, E11.5 **d**, E12.5 **e** and E15.5 **f** $Jag1^{J1VmC}$ embryos stained for Jag1-mCherry, Pdx1 and Sox9 as indicated. Higher magnifications of boxed areas are shown in insets. **h-l** Sections of E9.5 **h**, E10.5 **i**, E11.5 **j**, E12.5 **k** and E15.5 **l** $Dll1^{D1VmC}$ embryos stained for Dll1-mCherry, Pdx1 and Sox9 as indicated. Higher magnifications of boxed areas are shown in insets. dp: dorsal pancreas; vp: ventral pancreas; bp: bi-potent progenitor domain; ac: emerging acini. Scale bar 20 µm.
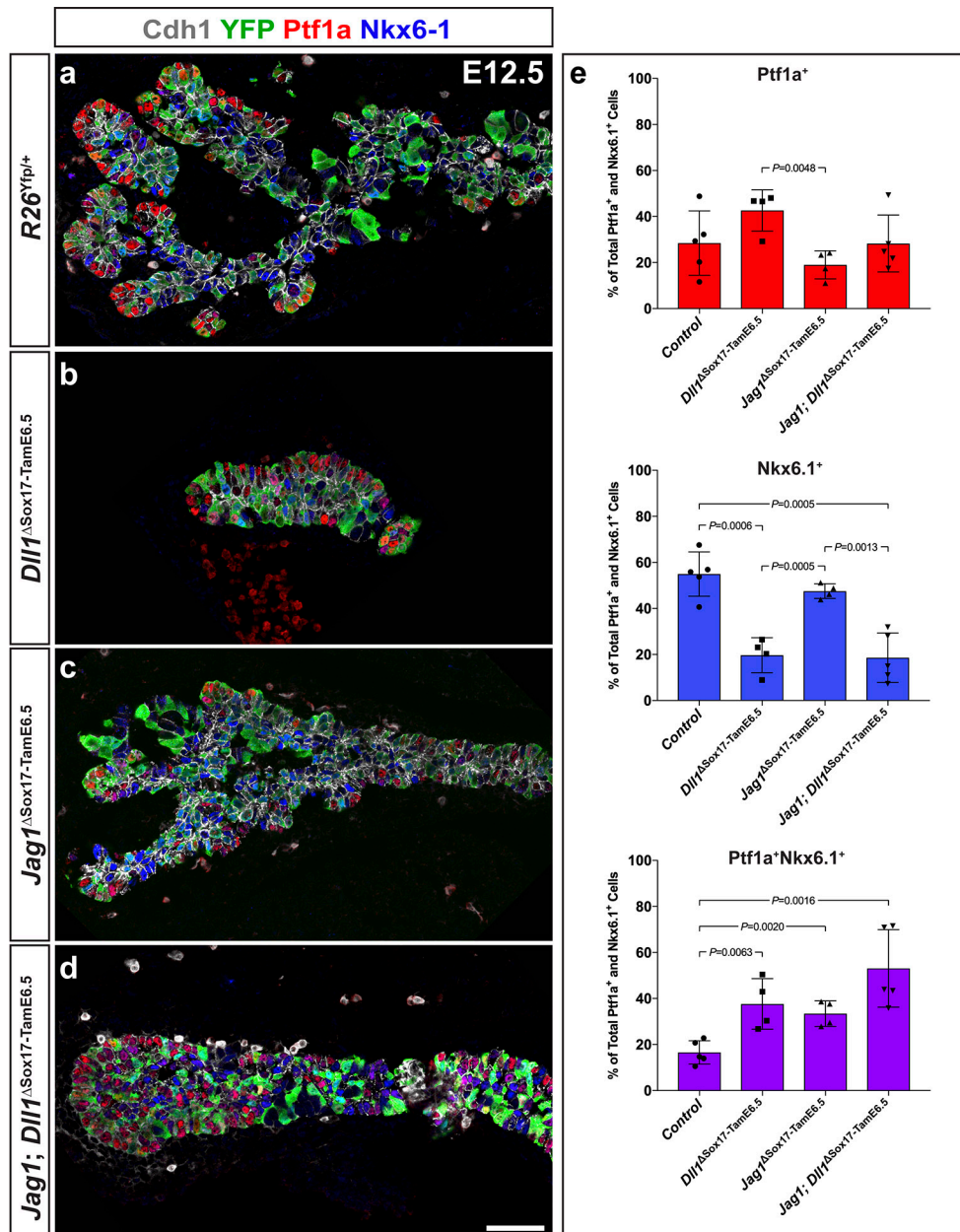
Supplementary Figure 2

Supplementary Fig. 2. Cell-specific expression of Jag1 and Dll1 in the developing pancreas. Sections of E10.5 **a**-**c**, **j**-**l**; E12.5 **d**-**f**, **m**-**o**; and E15.5 **g**-**i**, **p**-**r** dorsal pancreata stained for Jag1, Pecam and Cdh1 **a**, **d**, **g**; Jag1, Ngn3 and Gcg **b**, **e**, **h**; Jag1, Ptf1a, and Sox9 **c**, **f**, **l**; Dll1, Pecam and Cdh1 **j**, **m**, **p**; Dll1, Ngn3 and Gcg **k**, **n**, **q** or Dll1, Ptf1a, and Sox9 **l**, **o**, **r** in the indicated colors. bp: bi-potent progenitor domain; ec: endocrine cells; ac: emerging acini; v: vessel. Scale bars, 25 μm. Note the initial uniform epithelial expression of Jag1 at E10.5 **a**-**c**, which becomes confined to Ptf1a^{+} PACs in the distal epithelium at E12.5 (arrowheads in **d** and **f**). Note Dll1 expression in Ngn3^{+} cells (arrows in **k**, **n**, **q**), in Ptf1a^{+} cells (arrows in **l**, **o**-**r**) at all stages and in Sox9^{+} BPs at E12.5 and E15.5 (arrowheads in **o** and **r**).
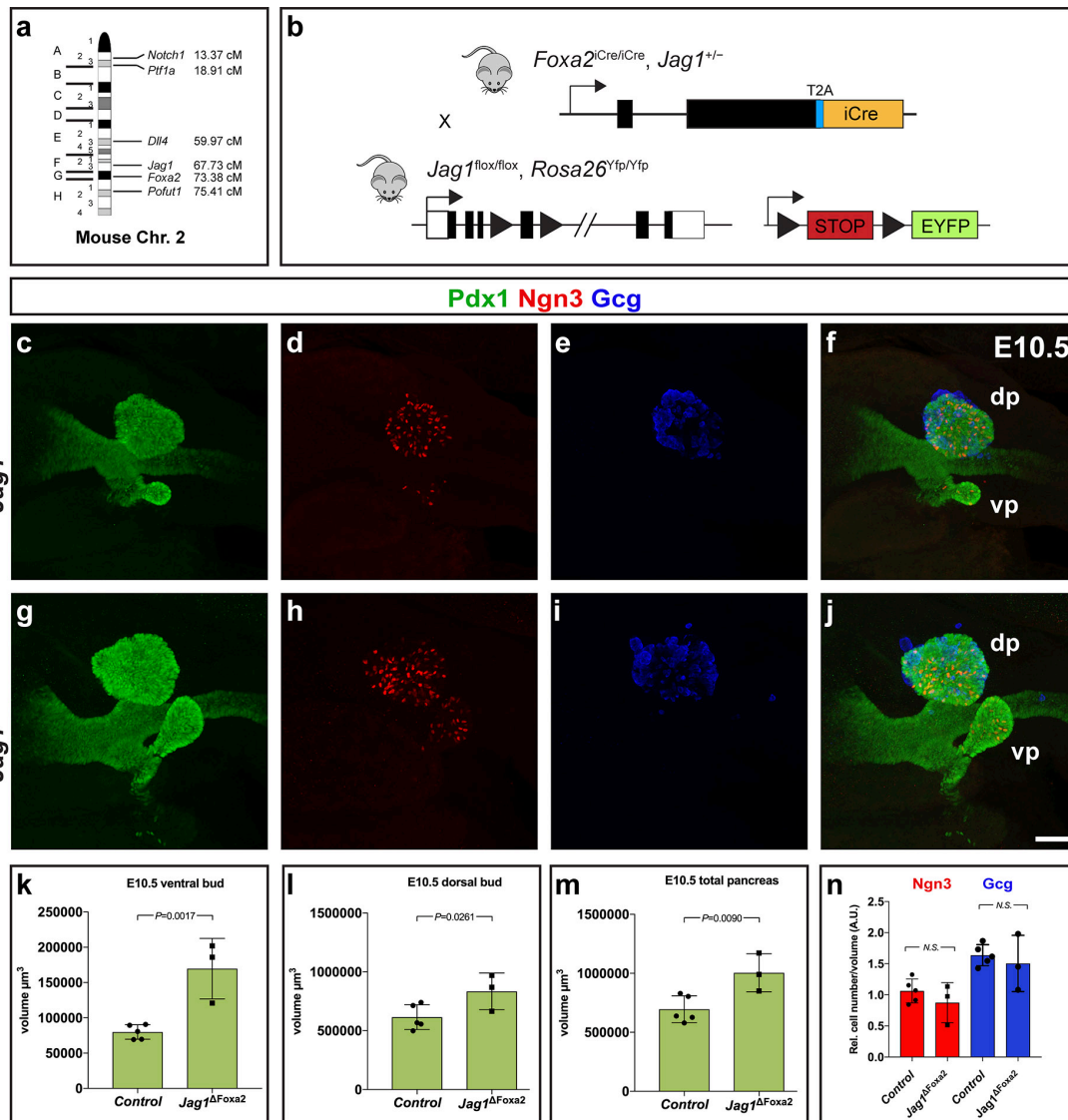
Supplementary Figure 3

Supplementary Fig. 3. Stage-dependent allocation of progenitor fate induced by Notch suppression. **a** Schematic overview of strategy applied to identify fates of progeny from pancreatic progenitors in which Notch-mediated transcriptional activation is prevented by *Sox9-CreER^{T2}*-mediated expression of a dnMaml1-GFP fusion protein from the *Rosa26* locus at different developmental stages. Approximate temporal windows of Tamoxifen (Tam) activity resulting from single intraperitoneal injections at E11.5, E12.5 or E13.5 are indicated. **b-m** Sections of E15.5 *Sox9-CreER^{T2}*; *R26*^YFP control **b-d**, **h-j** or *Sox9-CreER^{T2}*; *R26*^dnMaml1 Notch-blocked **e-g**, **k-m** pancreata stained for EYFP/EGFP and Sox9 combined with either Ptf1a **b-g** or Ngn3 and Chga **h-m** in the indicated colors. Note lineage-labeled Sox9⁺ BPs (arrows in **b-d**) and Ptf1a⁺ PACs (arrowheads in **b** and **c**) in control panels (*R26*^YFP) and lineage-labeled Ptf1a⁺ PACs (arrowheads in **e-g**) and Ptf1a/Sox9 double negative cells (arrows in **e-g**) in experimental panels (*R26*^dnMaml1). **h-m** Note lineage-labelled Sox9⁺ BPs (arrows in **h-j**) and Ngn3⁺ endocrine precursors (arrowheads in **h-j**) in control panels (*R26*^YFP) and lineage-labeled Ngn3/Chga/Sox9 triple negative cells (arrows in **k**) and Chga⁺ endocrine cells (arrowheads in **k-m**) in experimental panels (*R26*^dnMaml1). Scale bar, 50 µm.

Supplemental Figure 4

Supplementary Fig. 4. Jag1 and Dll1 are both involved in PD patterning. **a-d** IF on Sections of E12.5 embryos of the indicated genotypes stained for Cdh1, YFP, Ptf1a, and Nkx-1 as indicated. **e** Combined bar graphs/scatter plots showing quantifications of the distribution of Ptf1a$^+$ (Ptf1a$^+$Nkx6-1$^-$) PACs (upper panel), Nkx6.1$^+$ (Ptf1a$^-$Nkx6-1$^+$) BPs (middle panel) and Ptf1a$^+$Nkx6-1$^+$ cells (lower panel) relative to the number of progenitor cells (the total number of Ptf1a$^+$ and/or Nkx6-1$^+$ cells) in dorsal pancreata of the different genotypes. Data are presented as mean ± S.D. Significant *P*-values are

indicated on the plots. N=4-5 as indicated by the number of data points in the scatter plots.

Supplementary Figure 5

Supplementary Fig. 5. The pancreas primordia are enlarged in E10.5 $Jag1^{\Delta Foxa2/-}$ mutants. **a** Schematic showing linkage of relevant Cre-driver genes and key Notch pathway genes on mouse chromosome 2. Note the need for meiotic recombination and carefully designed and monitored genotyping in order to obtain e.g. $Jag1^{\Delta Foxa2}$ or $Notch1^{\Delta Ptf1a}$ mutants. **b** Schematic showing mating strategy to obtain mutant embryos once the proper parental meiotic recombinants are identified. **c-j** 3D maximum intensity projections of E10.5 $Jag1^{\Delta Foxa2/+}$ **c-f** and $Jag1^{\Delta Foxa2/-}$ **g-j** pancreata stained for Pdx1, Ngn3 and Gcg as indicated. dp: dorsal pancreas; vp: ventral pancreas. **k-m** Combined bar graphs/scatter plots showing quantifications of volume of Pdx1^{Hi} cells in ventral **k**, dorsal **l** and total **m** pancreas primordia. **n** Combined bar graphs/scatter plots showing quantifications of Ngn3$^+$ and Gcg$^+$ cells relative to progenitor volume.

Significant *P*-values are indicated on the plots. N=3-5 as indicated by the number of data points in the scatter plots.
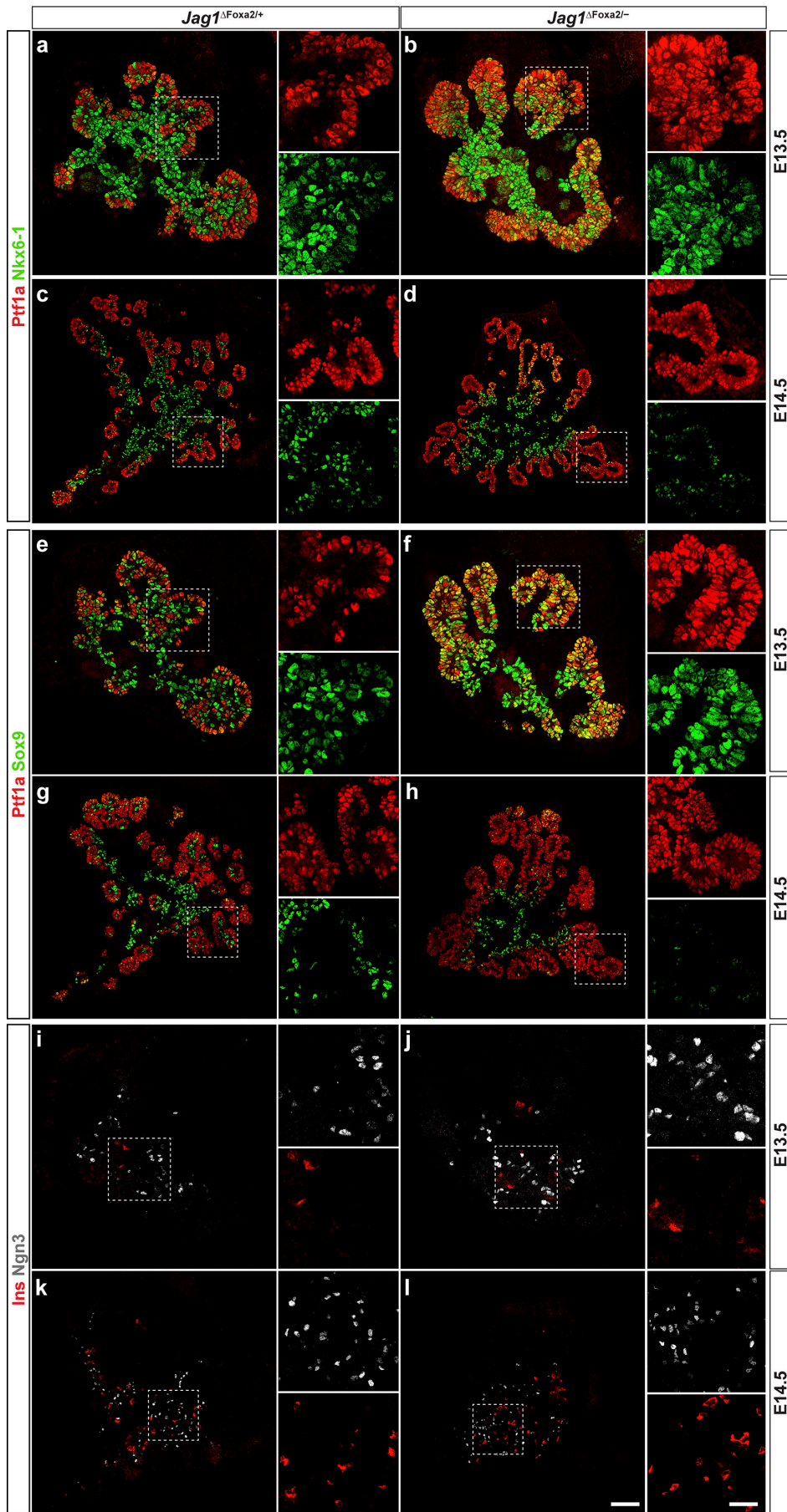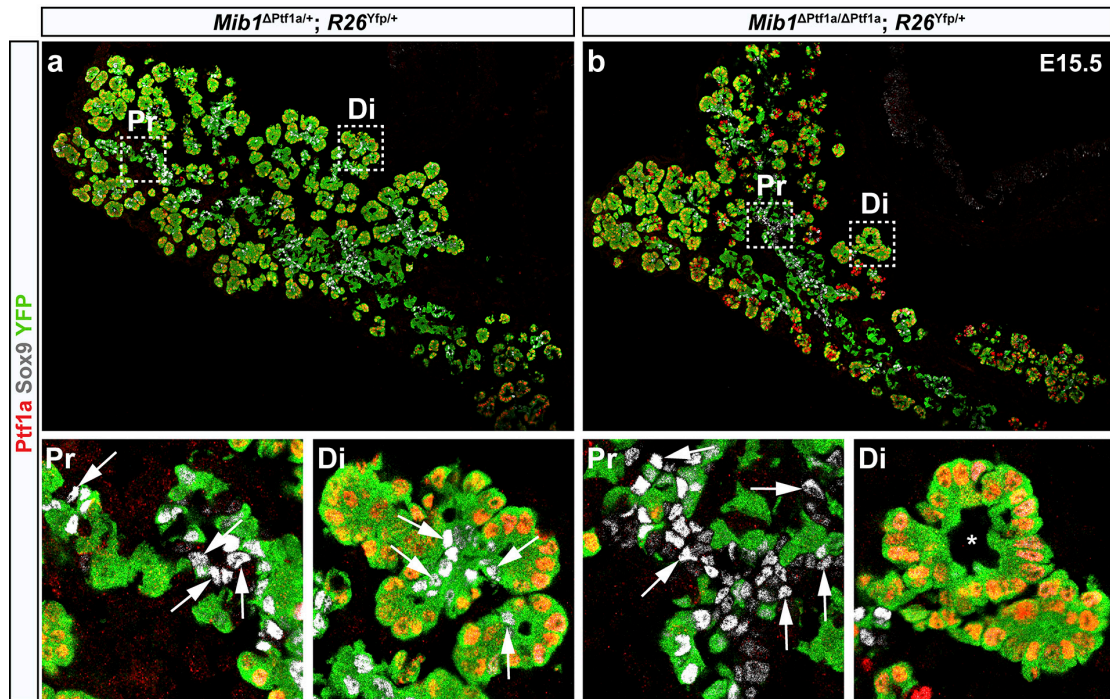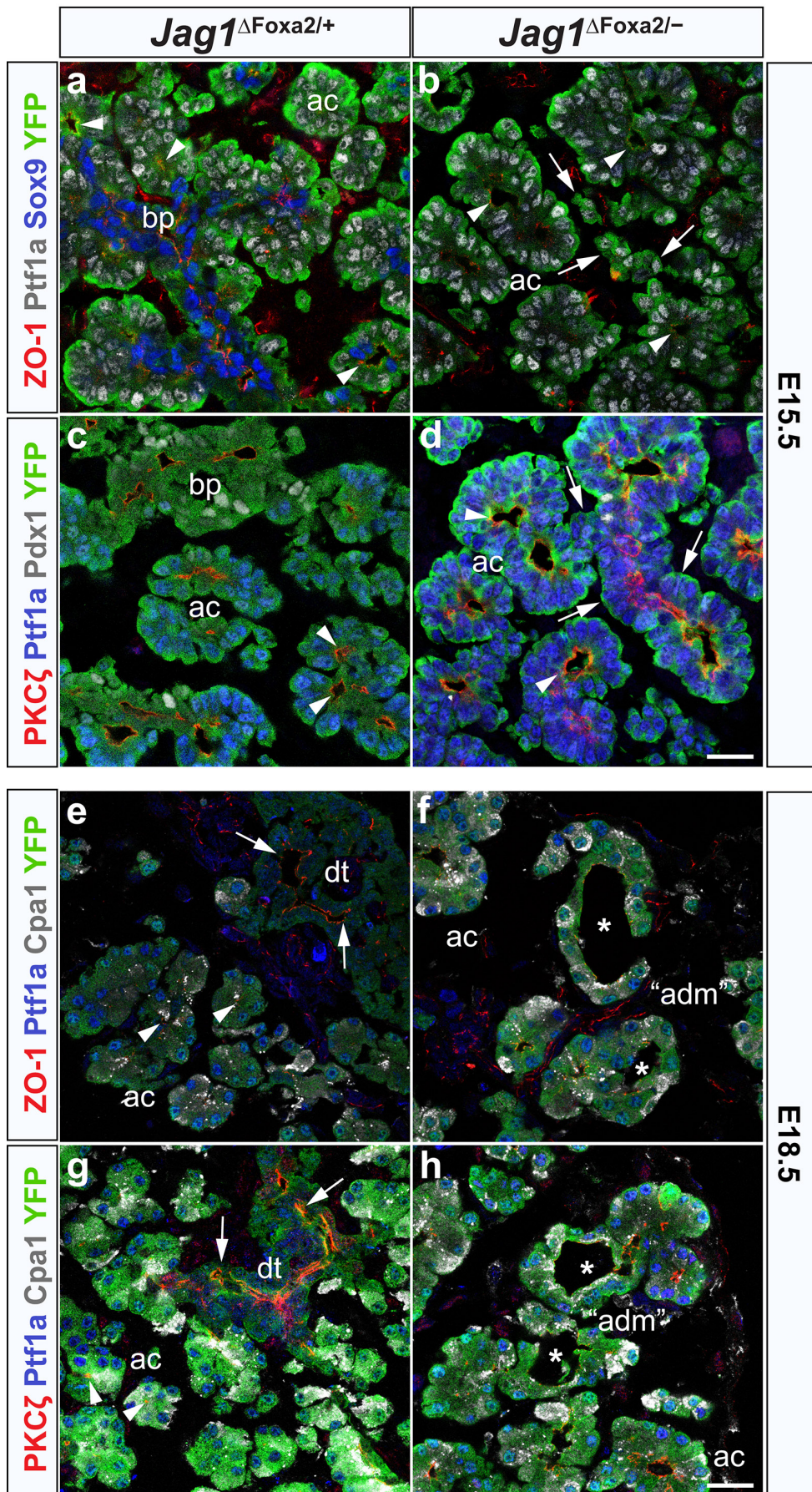
Supplemental Figure 6

Supplementary Fig. 6. Delayed resolution of PD patterning in the $Jag1^{\Delta Foxa2}$ pancreas.

a-l Sections of E13.5 **a, b, e, f, i, j** and E14.5 **c, d, g, h, k, l** pancreata from $Jag1^{\Delta Foxa2/+}$ **a, c, e, g, i, k** and $Jag1^{\Delta Foxa2/-}$ **b, d, f, h, j, l** embryos stained for Ptf1a and Nkx6-1 **a-d**, Ptf1a and Sox9 **e-h**, or insulin (Ins) and Ngn3 as indicated **i-l**. Insets show higher magnifications of individual channels. Note that while extensive co-expression of Ptf1a with Nkx6-1 and Sox9 is maintained in the E13.5 $Jag1^{\Delta Foxa2/-}$ pancreas **b, f** compared to $Jag1^{\Delta Foxa2/+}$ littermates **a, e**, the Ptf1a and Nkx6-1 expression domains have largely resolved by E14.5 in the mutant **d, h**. However, distal Nkx6-1$^+$Sox9$^+$ BPs are essentially absent from $Jag1^{\Delta Foxa2/-}$ mutants (compare insets in **c** and **g** to **d** and **h**). Presence of Ins$^+$ β-cells and Ngn3$^+$ endocrine precursors appear unaffected in E13.5 and E14.5 $Jag1^{\Delta Foxa2/-}$ pancreata relative to $Jag1^{\Delta Foxa2/+}$ littermates (compare **i** and **k** to **j** and **l**). Scala bars, 50 μm (main panels) and 25 μm (insets).
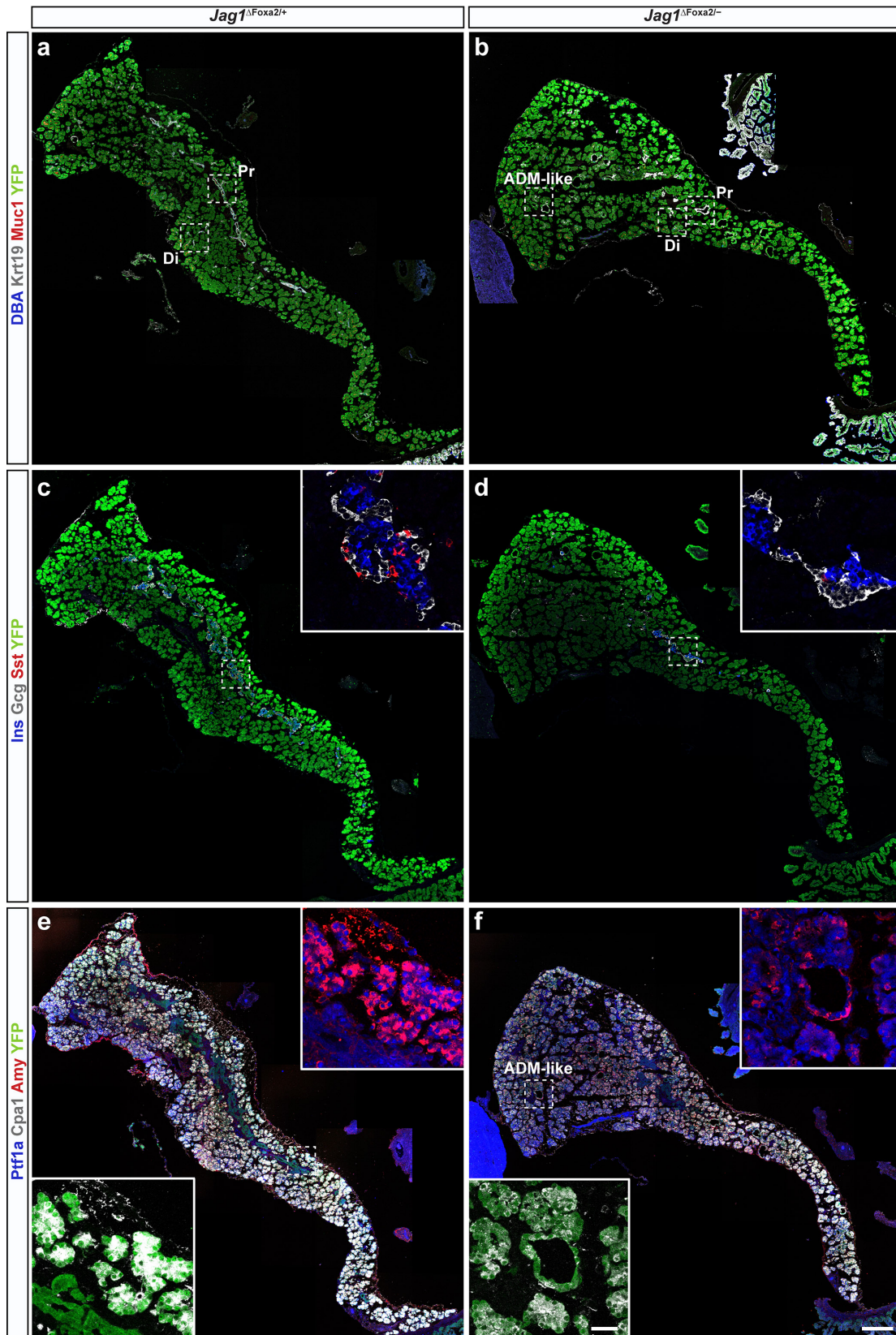
Supplementary Figure 7

Supplementary Fig. 7. E15.5 *Mib1*$^{\Delta Ptf1a}$ pancreas lacks CAC precursors. **a**, **b** Sections of E15.5 *Mib1*$^{\Delta Ptf1a/+}$; *R26*$^{YFP/+}$ **a** and *Mib1*$^{\Delta Ptf1a/\Delta Ptf1a}$; *R26*$^{YFP/+}$ **b** pancreata stained for Ptf1a, Sox9 and YFP as indicated. Insets show higher magnification views of boxed areas; Pr (proximal) and Di (distal). Note that recombination is mosaic in the Sox9$^+$ domain (arrows in Pr insets) but uniformly high in the Ptf1a$^+$ domain. Also note that terminal most Sox9$^+$ cells located within forming acini of the *Mib1*$^{\Delta Ptf1a/+}$ pancreas (arrows in Di inset) are missing from the *Mib1*$^{\Delta Ptf1a/\Delta Ptf1a}$ pancreas (asterisk). Scale bars, 100 μm (main panels) and 20 μm (insets).
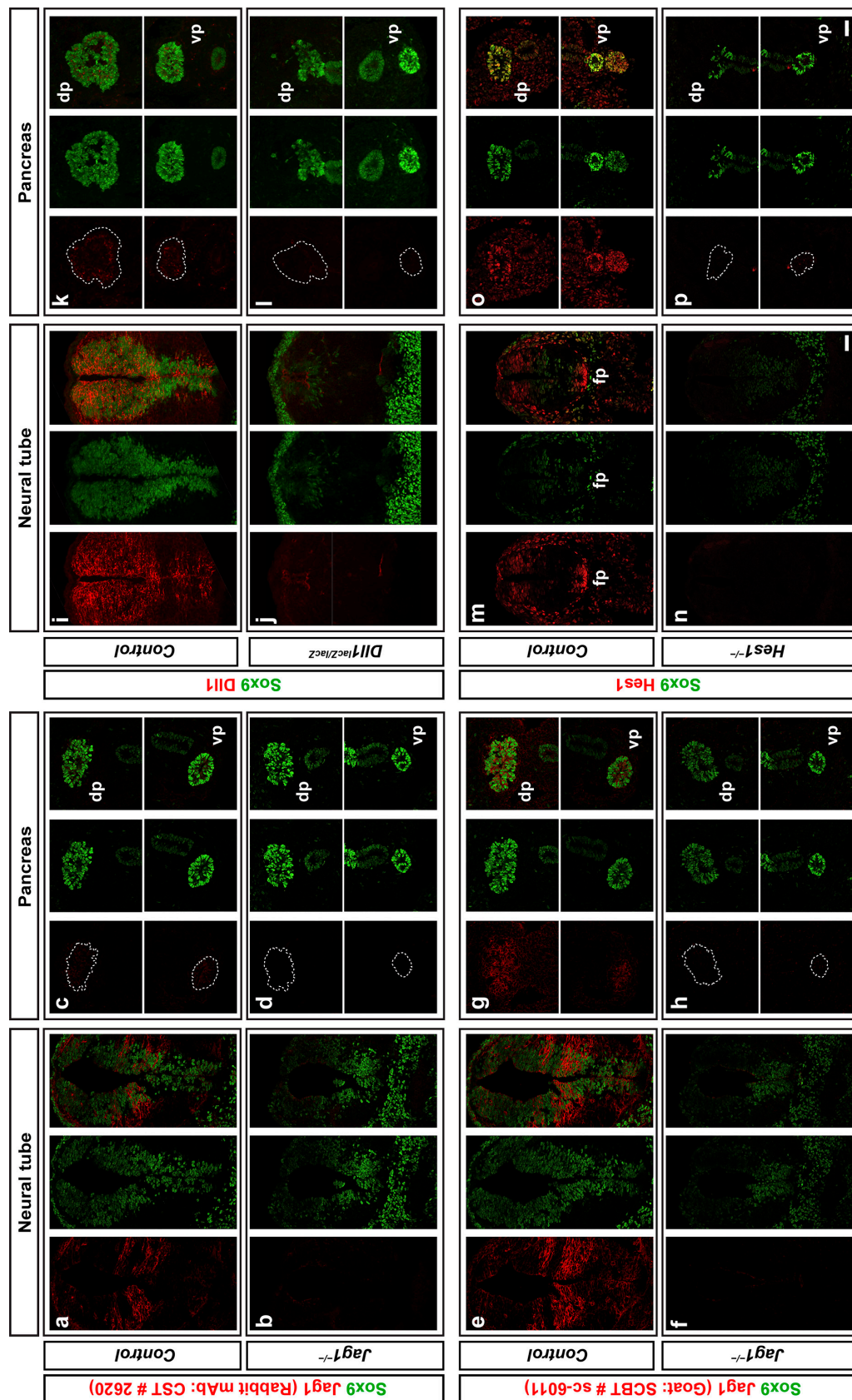
Supplementary Figure 8

Supplementary Fig. 8. Apicobasal polarity is retained in E15.5 *Jag1*$^{\Delta Foxa2}$ acini but lost prior to birth. **a-h** Sections of E15.5 **a-d** and E18.5 **e-h** *Jag1*$^{\Delta Foxa2/+}$ **a**, **c**, **e**, **g** and *Jag1*$^{\Delta Foxa2/-}$ **b**, **d**, **f**, **h** pancreata stained for ZO-1, Ptf1a, Sox9 and YFP **a**, **b**, **e**, **f** and PKCζ, Ptf1a, Cpa1 and YFP **c**, **d**, **g**, **h** as indicated. Note that ZO-1 and PKCζ are found apically in the Ptf1a$^+$ domain of both controls and at E15.5 (arrowheads in **a-d**). Also note Ptf1a$^+$ duct-like structures in E15.5 *Jag1*$^{\Delta Foxa2/-}$ mutants (arrows in **b** and **d**). At E18.5 however, apical ZO-1 and PKCζ are downregulated in YFP$^+$Ptf1a$^+$Cpa1$^+$ ADM-like structures ("adm", asterisks in **f** and **h**) in the *Jag1*$^{\Delta Foxa2/-}$ pancreas, while still expressed on Ptf1a$^+$Cpa1$^+$ acinar cells (arrowheads in **e** and **g**) and in duct cells (arrows in **e** and **g**) in control mice. Scale bars, 25 µm. ac: acini, bp: bi-potent progenitor domain, dt: ducts, "adm": ADM-like structures.

Supplementary Figure 9

Supplementary Fig. 9. Ductal malformations, endocrine hypoplasia and ADM-like lesions in late development. **a-f** Composite low magnification panels of ~10 separate

images stitched together to cover the entire dorsal pancreas of E18.5 $Jag1^{\Delta Foxa2/+}$ **a**, **c**, **e** and $Jag1^{\Delta Foxa2/-}$ **b**, **d**, **f** embryos. **a**, **b** IF for Muc1, Cytokeratin 19 (Krt19), and DBA as indicated reveals discontinuous, dispersed ductal structures in the E18.5 $Jag1^{\Delta Foxa2}$ pancreas in contrast to the extensive, continuous ductal tree of control littermates. Boxed areas are shown in high magnification in Fig. 7b-k. **c**, **d** IF for insulin (Ins), glucagon (Gcg) and somatostatin (Sst) as indicated shows a greater extent of loss of the late-arising Sst$^+$ δ-cells than of either Ins$^+$ β-cells or Gcg$^+$ α-cells in the E18.5 $Jag1^{\Delta Foxa2/-}$ pancreas compared with control littermates. **e**, **f** Expression of the acinar markers amylase (Amy) and Cpa1 is retained in acinar cells of E18.5 $Jag1^{\Delta Foxa2/-}$ as well as in control littermates, including in the dilated ADM-like structures although aberrantly located basally (inset in **f**). Note that the Amy$^+$Cpa1$^+$ ADM-like structures also express Krt19 (the insets labeled ADM-like in **b** and **f**) are from the same region of adjacent sections). Some insets are displayed without the YFP channel for the sake of clarity. Scale bars, 250 μm (main panels) and 25 μm (insets).

Supplementary Fig. 10. Validation of anti-Dll1, -Jag1 and -Hes1 antisera. **a-d** Rabbit monoclonal anti-Jag1 antibody (Cell Signaling Technology #2620) gives a membranous signal on cells in the neural tube **a**, **b** and a weaker but specific membranous staining

throughout both pancreatic buds **c**, **d** in E10.5 control mice **a**, **c** but not *Jag1*$^{-/-}$ littermates **b**, **d**. **e-h** Similarly, goat anti-Jag1 antibody (Santa Cruz Biotechnology: #sc-6011) gives membranous signal on cells in the neural tube **e**, **f** and weaker but specific membranous staining throughout both pancreatic buds **g**, **h** in E10.5 control embryos **e**, **g** but not *Jag1*$^{-/-}$ littermates **f**, **h**. **i-l** Sheep anti-Dll1 antibody (R&D Systems: #AF3970) yields heterogeneous membranous signal on cells in the neural tube **i**, **j** and weaker but specific signal in dorsal (dp) and ventral (vp) pancreas **k**, **l** in E10.5 control embryos **i**, **k**, but not nullizygous *Dll1*$^{\text{lacZ/lacZ}}$ littermates **j**, **l**. **m-p** Rabbit monoclonal anti-Hes1 antibody (Cell Signaling Technology: #11988) yields strong nuclear Hes1 signal in the dorsal neural tube and floorplate (fp) **m**, **n** and throughout both pancreatic buds **o**, **p** in E10.25 control embryos **m**, **o** but not *Hes1*$^{-/-}$ littermates **n**, **p**. Scale bars, 50 μm. Sox9 signal is shown for reference. Weak signals in pancreatic buds compared to neural tube are visualized by enhancing the brightness equally for control and nullizygous images in Adobe Photoshop.

Supplementary Table 1

Genotyping primers.

D1VmC genotyping primers: Fwd: 5'-CTTCAAAGGACACCAAGTACCAGTCG-3', WT-Rev: 5'-CTGTCCATAGTGCAATGGGAACAACC 3', Venus-Rev: 5' CTTGCTCACCATAAAGATGCGACCTCC 3'

J1VmC genotyping primers: Fwd: 5' CAACACGGTCCCCATTAAGGATTACGAG 3', WT-Rev: 5' CTGTCCATAGTGCAATGGGAACAACC 3', Venus-Rev: as above.

*Sox17*CreERT2: Genotyping primers: Fwd: 5'-TGCCACGACCAAGTGACAGC-3', Rev: 5'-CCAGGTTACGGATATAGTTCATG-3'

Supplemental Table 2. Antibodies employed in immunofluorescence analyses

Primary Antibodies				
Antigen	**Species**	**Source**	**Catalogue #**	**Dilution**
Pdx1	Goat	NIH Beta Cell Biology Consortium (BCBC)	AB2027	1:10,000
Sox9	Guinea Pig	Gift from Ole Madsen/Novo Nordisk	N/A	1:2,000
Sox9	Rabbit	EMD Millipore (Merck)	AB5535	1:1,000
Ptf1a	Rabbit	NIH BCBC	AB2153	1:3,000
Ptf1a	Guinea Pig	Gift from Jane E. Johnson, UT Southwestern Medical Center	N/A	1:5,000
CPA1	Goat	R&D Systems	AF2765	1:200
Amylase	Rabbit	Sigma-Aldrich (Merck)	A8273	1:500[1]
Mist1	Rabbit mAb	Cell Signaling Technology	14896	1:500[1]
Nkx6.1	Mouse			1:500[2]
Nkx6.1	Rabbit	NIH BCBC	AB1069	1:2,000[1]
Ngn3	Rabbit	NIH BCBC	AB2011	1:4,000
Ngn3	Chicken	NIH BCBC/Chris V. E. Wright	AB3854	1:1,000
Chromogranin-A	Goat	Santa Cruz	sc-1488	1:200
Insulin	Guinea Pig	Abcam	ab7842	1:200
Insulin	Guinea Pig	Dako (Agilent)	A0564	1:800
Glucagon	Mouse	Sigma-Aldrich (Merck)	G2654	1:800
Glucagon	Guinea Pig	Millipore	4031-01F	1:4,000
Somatostatin	Rabbit	Dako (Agilent)	A0566	1:2,000[1]
DBA	N/A	Vector Labs	B-1035	1:500[3]
Krt19 (CK19, TROMA-III)	Rat	Developmental Studies Hybridoma Bank	N/A	1:100[4]
Muc1	Armenian Hamster mAb	Thermo Fisher (Invitrogen)	MA5-11202	1:200
ZO-1 (R26.4C)	Rat	Developmental Studies Hybridoma Bank	N/A	1:1,000[4]
PKCζ	Rabbit	Santa Cruz	sc-216	1:800
E-Cadherin	Mouse	BD Biosciences	610181	1:2,000
E-Cadherin	Rat	Novo Nordisk	N/A	1:1,000
Pecam1/CD31	Rat	BD Biosciences	550274	1:50
Dll1	Sheep	R&D Systems	AF3970	1:200
Jag1	Goat	Santa Cruz Biotechnology	sc-6011	1:200
Jag1	Rabbit mAb	Cell Signaling Technology	2620	1:50
Notch1	Sheep	R&D Systems	AF5267	1:200
Notch2	Goat	R&D Systems	AF1190	1:200
Hes1	Rabbit mAb	Cell Signaling Technology	11988	1:200
GFP	Chicken	Abcam	ab13970	1:1,000
GFP	Rabbit	Clontech	632460	1:1,000
RFP	Rabbit	Rockland	600-401-379	1:2,000

[1] Detected with (1:500) Cy3-conjugated F(ab')$_2$ fragment donkey anti-rabbit IgG (Jackson ImmunoResearch Europe; 711-166-152)

[2] Blocking and antibody incubations performed using M.O.M. Basic Kit (Vector Labs; BMK-2202)

[3] Biotinylated DBA detected with (30 μg/ml) AMCA Avidin D (Vector Labs; A-2008)

[4] Concentrate