

Jag1 modulates an oscillatory Dll1-Notch-Hes1 signaling module to coordinate growth and fate of pancreatic progenitors

by

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1 **Summary**

2 Notch signaling controls proliferation of multipotent pancreatic progenitor cells
3 (MPCs) and their segregation into bipotent progenitors (BPs) and unipotent pro-acinar
4 cells (PACs). Here we uncover fast ultradian oscillations in the ligand Dll1, and the
5 transcriptional effector Hes1, which proved crucial for MPC expansion. Conversely
6 Jag1, a uniformly expressed ligand, curbed MPC growth, but as expression later
7 segregated to PACs it proved critical for specifying all but the most proximal 5% of BPs,
8 while BPs were entirely lost in *Jag1*, *Dll1* double mutants. Moreover, experimentally
9 induced changes in Hes1 oscillation parameters was associated with selective
10 adoption of BP or PAC fates. Anatomically, ductal morphogenesis and organ
11 architecture is minimally perturbed in *Jag1* mutants until later stages, when ductal
12 remodeling fails and signs of acinar-to-ductal metaplasia appear. Our study uncovers
13 oscillating Notch activity in the developing pancreas, which along with modulation by
14 Jag1 is required to coordinate MPC growth and fate.

15

16 **Keywords** Notch, oscillations, cis-inhibition, pancreas, development, fate, Dll1, Jag1,
17 Hes1

21 Introduction

22 Deciphering the mechanisms that control the differentiation of progenitor cells into
23 various mature cell types is crucial for understanding disease etiology and for using
24 pluripotent stem cells in cell replacement therapy applications. The principal cell
25 lineages of the mammalian pancreas, acinar, duct and endocrine, arise from
26 multipotent pancreatic progenitor cells (MPCs) through a series of binary cell fate
27 choices (Shih et al., 2013). MPCs are specified from the posterior foregut endoderm
28 as dorsal and ventral anlagen around embryonic day (E)8.5 (Wessells and Cohen,
29 1967), and are distinguished by their expression of several transcription factors
30 including Pdx1, Ptf1a, Hnf1 β , Sox9 and Nkx6-1. The multipotentiality of these cells has
31 been demonstrated at the population level by many lineage-tracing studies (Gu et al.,
32 2002; Kopp et al., 2011; Pan et al., 2013; Solar et al., 2009; Zhou et al., 2007) and, more
33 recently, at the clonal level (Larsen et al., 2017).

34 Dorsal MPCs give rise to an early wave of endocrine cells from ~E9.5 to ~E11 while
35 endocrinogenesis is delayed ~36 hours in the ventral anlage (Ahlgren et al., 1997;
36 Pictet et al., 1972; Spooner et al., 1970). The appearance of hormone-producing cells
37 is preceded by, and dependent on, expression of *Neurog3* (encoding Ngn3) in
38 endocrine precursors, which initiates at ~E8.5 in the dorsal bud and ~E10.0 in the
39 ventral bud (Gradwohl et al., 2000; Gu et al., 2002; Villasenor et al., 2008). A transient
40 decline in *Neurog3* expression from E11 to E12 (Villasenor et al., 2008) coincide with
41 peak segregation of Ptf1a⁺Nkx6-1⁺ MPCs into proximal, Ptf1a⁻Nkx6-1⁺ bipotent
42 progenitors (BPs) and distal, Ptf1a⁺Nkx6-1⁻ pro-acinar cells (PACs) (Hald et al., 2008;
43 Schaffer et al., 2010). This proximodistal (PD) patterning is regulated by Notch
44 signaling as forced expression of a Notch intracellular domain (NICD) prevents MPCs
45 from adopting a PAC fate (Hald et al., 2003; Murtaugh et al., 2003; Schaffer et al., 2010)
46 while dominant-negative Maml1 prevents a BP fate (Afelik et al., 2012; Horn et al.,
47 2012). Total inactivation of Notch signaling in the endoderm, as seen in *Foxa2*^{T2AiCre/+};
48 *Mib1*^{f/f} (*Mib1* ^{Δ Foxa2}) embryos, results in a complete shift from BP- to PAC fate (Horn et
49 al., 2012). Downstream of Notch, PD patterning depends on mutually antagonistic
50 interactions between Ptf1a and Nkx6-1/Nkx6-2 (Schaffer et al., 2010). Notch, and its
51 downstream target gene *Hes1*, also prevent precocious and excessive endocrine

52 differentiation (Apelqvist et al., 1999; Jensen et al., 2000b) and stimulate MPC
53 proliferation (Ahnfelt-Rønne et al., 2012). After PD patterning is complete around E14,
54 Notch signaling and Hes1 expression persist in BPs, maintaining Sox9 expression and
55 ductal fate, and inhibiting endocrine differentiation by repressing *Neurog3* (Bankaitis
56 et al., 2015; Klinck et al., 2011; Kopinke et al., 2011; Magenheim et al., 2011; Shih et
57 al., 2012). Less is known about which ligands regulate the adoption of distinct cell fates.
58 Dll1 regulates early endocrine differentiation (Ahnfelt-Rønne et al., 2012; Apelqvist et
59 al., 1999), yet PD patterning appears unaffected in *Dll1*^{ΔFoxa2} embryos and the
60 endocrinogenic phenotype of E10.5 *Dll1*^{ΔFoxa2} embryos is much weaker than that of
61 E10.5 *Mib1*^{ΔFoxa2} embryos (Horn et al., 2012; Jorgensen et al., 2018). These findings
62 show that additional Mib1 substrates, most likely other Notch ligands, are involved in
63 these cell fate decisions. In zebrafish, intrapancreatic duct cells are depleted in
64 *jag1b/jag2b* morphants (Lorent et al., 2004; Yee et al., 2005) and completely absent
65 from *jag1b*^{-/-}; *jag2b*^{-/-} embryos (Zhang et al., 2017) with the latter also showing a
66 reduction in endocrine cells while acinar cells were unaffected. However, in mice *Jag1*
67 is the only Jagged-type ligand expressed in the pancreatic endoderm (Lammert et al.,
68 2000), and *Jag1*^{ΔPdx1} animals displayed ductal malformation and paucity postnatally,
69 due to reduced duct cell proliferation (Golson et al., 2009b). Similarly, *Dll1; Jag1*^{ΔPtf1a}
70 embryos showed a modest phenotype with loss of only the terminal duct or
71 centroacinar cells (CACs) (Nakano et al., 2015). Thus, which ligands that regulate the
72 various cell fate decisions remains unclear as do dynamics and timing of Notch
73 signaling. In this study, we uncover a complex interaction between Dll1 and Jag1 that
74 regulates growth and differentiation of pancreatic progenitors. We demonstrate that
75 Dll1 and Hes1 expression oscillates and that oscillations are important for progenitor
76 expansion and fate choice. Conversely, we find uniformly expressed Jag1 to attenuate
77 Notch activity cell-autonomously. Yet, Jag1 can activate Notch in Jag1⁻ neighbors, in
78 partial redundancy with Dll1, and together the two ligands specify the entire BP
79 lineage. Anatomically, epithelial plexus formation and the gross architecture of the
80 organ are remarkably unperturbed in spite of the profound changes in cell fate.
81 However, at late stages larger ducts are missing or malformed, terminal ducts are
82 morphologically abnormal and signs of acinar-to-ductal metaplasia (ADM) appear in
83 *Jag1*^{ΔFoxa2} embryos.

84 **Results**

85 **Initially uniform Jag1 expression segregates to nascent PACs.** Since BPs are converted
86 to PACs in *Mib1*^{ΔF_{oxa2}} embryos, but not in *Dll1*^{ΔF_{oxa2}} embryos (Horn et al., 2012), we
87 reasoned that an additional Mib1 substrate, most likely Jag1 (Ahnfelt-Rønne et al.,
88 2012; Apelqvist et al., 1999; Golson et al., 2009a; Jensen et al., 2000a; Lammert et al.,
89 2000; Nakano et al., 2015), must act during PD patterning. Because the reported
90 expression patterns are not entirely consistent, we decided to re-analyze the
91 expression of these two ligands using fluorescent protein reporters targeted to the
92 *Dll1* and *Jag1* loci. The *Jag1*^{J1V_{mC}} and *Dll1*^{D1V_{mC}} alleles comprise a Venus-T2A-mCherry
93 cassette inserted in frame with the coding region of the last exons to generate Dll1-
94 and Jag1-Venus fusion proteins that serve as dynamic reporters of ligand protein
95 expression while mCherry acts as a more sensitive reporter owing to its longer half-
96 life after being cleaved from the fusion protein. Co-immunofluorescence (IF) analysis
97 with anti-GFP antibodies revealed uniform Jag1-Venus fusion protein expression in
98 E10.5 *Jag1*^{J1V_{mC}/+} Pdx1⁺Sox9⁺ MPCs in the dorsal pancreas epithelium and weaker
99 expression in the surrounding mesenchyme and a restriction to the distal epithelium
100 at E12.5, with most of the centrally located, emerging Sox9⁺ BPs being negative for
101 Jag1-Venus. At E15.5, Jag1-Venus expression was detected apically in acinar cells and
102 in non-parenchymal cells, including the vasculature, but expression was excluded from
103 Sox9⁺ BPs and Pdx1^{Hi} β-cells (Figure 1A). In *Dll1*^{D1V_{mC}} embryos we detected Dll1-Venus
104 fusion protein in scattered cells throughout the E10.5 and E12.5 dorsal pancreatic
105 epithelium, some of which were Sox9^{Lo/-}, and by E15.5, Dll1-Venus was found
106 intracellularly in the apical pole of acinar cells, and in dispersed Sox9^{Lo/-} cells in the
107 proximal epithelium that likely represent endocrine precursors (Figure 1A). Anti-RFP
108 antibodies confirmed these expression patterns and further revealed onset of Jag1
109 expression in MPCs between E9.5 and E10.5 (Figure S1A). Similarly, validated anti-Jag1
110 and anti-Dll1 antibodies (see Methods, Figure S7A-7L) reproduced the expression
111 patterns of the Jag1- and Dll1-reporters in wild type pancreas, and IF analysis of
112 lineage markers confirmed Jag1 expression in vascular cells and acinar cells at E15.5,
113 and its absence from E15.5 Sox9⁺ BPs and from the endocrine lineage at all stages
114 (Figure S1B). Equally, Dll1⁺ cells could be divided into subpopulations comprising
115 Dll1⁺Ptf1a⁺ MPCs or PACs present in E10.5, E12.5, and E15.5 pancreas epithelium as

116 well as Dll1⁺Ngn3⁺ endocrine precursors, evident at all stages, and Dll1⁺Ngn3⁻ cells in
117 E12.5 proximal epithelium (Figure S1C). Lastly, co-expression of Dll1 and Jag1 was
118 evident in some E10.5 MPCs and in a subset of distal cells at E12.5 (Figures 1B and 1C).
119 Co-IF for Jag1, Dll1, Ptf1a and Nkx6-1 revealed that these were Ptf1a⁺Nkx6-1^{Lo/-}
120 nascent PACs and MPCs and in the proximal region Dll1⁺ cells were Ptf1a⁻Jag1⁻ and
121 either Nkx6-1⁺ or Nkx6-1⁻ (Figure 1C).

122

123 **Notch activity is suppressed in nascent PACs.** We next examined the distribution of
124 Notch1 and Notch2 in emerging BPs and PACs of the E12.5 pancreatic epithelium.
125 While Notch1 was expressed in PACs and BPs, Notch2 was specifically enriched in BPs
126 (Figure 1D), as previously noted in E15.5 pancreas (Shih et al., 2012). To identify cells
127 in which Notch1 had been activated before and during PD patterning we first analyzed
128 embryonic pancreata from the Notch1 activity-trap mouse line N1IP::Cre^{HI}; Rosa26^{LSL-}
129 ^{Ai3} in which EYFP permanently labels the progeny of cells experiencing Notch1
130 activation (Liu et al., 2015). We detected YFP labeling in a few E10.5 Ptf1a⁺Sox9⁺ MPCs
131 and at E12.5 we found YFP expression in Ptf1a⁺ nascent PACs, Sox9⁺ nascent BPs and
132 in Ptf1a⁺Sox9⁺ MPCs (Figure 1E). Quantification revealed that YFP⁺ cell distribution
133 reflected no bias towards either nascent BPs or PACs at E12.5, consistent with the cells
134 experiencing Notch1 activation in MPCs, from which both lineages derive, prior to the
135 lineage segregation occurring by E12.5. We next analyzed Hes1 expression as an acute
136 readout of general, pan-Notch activation in relation to ligand protein expression in
137 E12.5 MPCs and emerging Nkx6-1⁺ BPs and Ptf1a⁺ PACs. Most Hes1^{Hi} cells were Nkx6-
138 1^{Hi}Ptf1a^{Lo/-}Jag1^{Lo/-} BPs and most Hes1^{Lo/-} cells were Nkx6-1^{Lo/-}Ptf1a^{Hi}Jag1^{Hi} PACs
139 except for a few Hes1^{Lo/-} cells that were Nkx6-1^{Hi}Ptf1a^{Hi}Jag1^{Hi} (Figure 1F). In relation
140 to Dll1, we found that Hes1^{Hi} cells typically were Nkx6-1^{Hi}Ptf1a^{Lo/-}Dll1^{Lo/-} BPs and,
141 when located in the periphery, often located next to Hes1^{Lo}Nkx6-1^{Lo/-}Ptf1a^{Hi}Dll1^{Hi} cells
142 (Figure 1G). Analyses of double reporter mice harboring either *Jag1*^{J1Vmc} or *Dll1*^{D1Vmc}
143 lines combined with a *Hes1*-EGFP reporter (Klinck et al., 2011) for EGFP and mCherry
144 expression confirmed the progressive confinement of Hes1 to BPs and Jag1 to PACs as
145 well as the heterogenous Dll1 expression at multiple stages, albeit the long half-life of
146 EGFP and mCherry likely overestimated the number of co-positive cells (Figure S1D).
147 A schematic representation of ligand and receptor expression patterns is shown in

148 Figure S1E. Overall, we observe an inverse correlation between Dll1 and Hes1
149 expression and the data suggest that BPs ($Jag1^{-}Dll1^{+/-}Notch1^{+}Notch2^{+}$) have receptors
150 in stoichiometric surplus, favoring signal reception, while ligands are in surplus in PACs
151 ($Jag1^{+}Dll1^{+/-}Notch1^{+}Notch2^{-}$), favoring signal sending. Together, the data show that
152 Notch receptor activation becomes suppressed in emerging $Jag1^{+}Dll1^{+/-}$ PACs, and
153 support the notion that these, together with $Ngn3^{+}Dll1^{Hi}$ endocrine precursors in the
154 central trunk epithelium, are activating Notch receptors in nascent, $Jag1^{Lo/-}Dll1^{Lo/-}$ BPs.

155

156 **Hes1 and Dll11 proteins display ultradian oscillations.** An inverse correlation
157 between heterogenous Dll1 and Hes1 expression is also observed in neural
158 progenitors, where it reflects oscillating protein levels (Imayoshi et al., 2013; Shimojo
159 et al., 2016; Shimojo et al., 2008), but it is unknown whether Notch components
160 oscillate in the fetal pancreas. To address this question, we examined the dynamics of
161 Hes1 and Dll1 protein expression in the developing pancreas using BAC-transgenic
162 Luc2-Hes1 fusion protein reporter mice (Imayoshi et al., 2013) and *Dll1*-Fluc knock-in
163 mice (Shimojo et al., 2016). E10.5 and E12.5 dorsal pancreata were explanted and
164 cultured with luciferin, and bioluminescence images were examined. Time-lapse
165 imaging analysis showed that Hes1 protein expression changed dynamically
166 throughout the explant and neighboring cells were frequently observed to oscillate in
167 anti-phase (Figure 2A, Movie S1-S3). Temporal analysis revealed well-defined
168 oscillations in Hes1 protein levels with an average period of ~90 min that remained
169 constant throughout a 6-day culture period (Figure 2A). Equally, time-lapse imaging
170 analysis showed that Dll1 protein expression also changed dynamically throughout the
171 explant (Figure 2B, Movie S4). Temporal analysis revealed that Dll1 protein levels also
172 oscillated with an average period of ~90 min (Figure 2B). As both *Hes1* and *Dll1* are
173 direct Hes1 target genes in pancreatic progenitors (de Lichtenberg et al., 2018a; de
174 Lichtenberg et al., 2018b), these data indicate that Hes1 and Dll1 participate in the
175 same oscillating gene regulatory network in the early pancreas.

176

177 **Dll1 oscillations augment and Jag1 attenuates MPC growth.** We have previously
178 shown that global mutations in either Hes1 or Dll1 reduces MPC proliferation and the
179 size of the pancreas anlage (Ahnfelt-Rønne et al., 2012). Given the different

180 spatiotemporal expression patterns of Dll1 and Jag1 in MPCs we first asked whether
181 Dll1 oscillations were important for Dll1 stimulated MPC expansion. To address this
182 question, we examined pancreatic bud size by whole-mount IF (WM-IF) in two
183 different *Dll1* mutant mouse lines (termed *Dll1 type1* and *Dll1 type2* mutants) that
184 display steady, intermediate level Dll1 expression and dampened Hes1 oscillations
185 (Shimojo et al., 2016) and compared to littermate wild-type controls. We found that
186 average dorsal pancreatic bud size was reduced by ~45% and ~25% in *Dll1*^{type1/type1} and
187 *Dll1*^{type2/type2} mutants, respectively, while ventral buds were reduced by ~55% and
188 ~65%, respectively (Figure 3A). The magnitude of this change is comparable to a ~65%
189 reduction in dorsal and ventral bud size observed in E10.5 *Dll1*^{ΔFoxa2} embryos (Figure
190 3A), and in *Dll1*^{-/-} mutants (Ahnfelt-Rønne et al., 2012). These results suggest that Dll1
191 oscillations are important for MPC expansion by ensuring adequate Notch activation,
192 perhaps by allowing MPCs to alternate between sending and receiving states multiple
193 times within a single cell cycle.

194 We then asked whether bud sizes were affected in E10.5 *Jag1*^{ΔFoxa2} embryos. Since
195 *Foxa2* is linked to *Jag1* on mouse chromosome 2 at a distance of ~5.7 cM we first
196 introduced a *Jag1* null allele (Xue et al., 1999) on the same chromosome as the
197 *Foxa2*^{iCre} allele via meiotic crossover. Animals carrying *Jag1*⁻; *Foxa2*^{iCre} chromosomes
198 were then backcrossed to homozygous *Foxa2*^{iCre/iCre} animals to secure this
199 chromosome from further crossover events. Timed matings of these mice with *Jag1*^{fl/fl};
200 *R26*^{YFP/YFP} animals generated *Jag1*^{ΔFoxa2/-} embryos (referred to as *Jag1*^{ΔFoxa2}) and
201 *Jag1*^{ΔFoxa2/+} heterozygote littermate controls. Remarkably, WM-IF analysis revealed
202 the E10.5 dorsal and ventral pancreata to be increased in size in *Jag1*^{ΔFoxa2} embryos
203 compared to controls (Figure 3A). Together, these data suggest that Notch activation
204 is mediated by oscillating Dll1 expression, which via induction of oscillating Hes1
205 expression stimulates MPC expansion. Conversely, Jag1 attenuates Dll1-mediated
206 Notch activation to limit MPC expansion, possibly by sequestering a fraction of the
207 available Notch receptors in *cis*.

208

209 **Suppressing Notch before ~E13 shunts progenitors to a PAC fate.** Before testing the
210 requirement for Jag1 and Dll1 in the segregation of MPCs into BP and PAC fates we

211 decided to define the temporal window through which Notch signaling affected this
212 fate choice. To address this question, we administered tamoxifen (Tam) to pregnant
213 dams carrying either *Hnf1b*-CreER^{T2}; *Rosa26*^{LSL-dnMaml1-eGFP/+} (hereafter referred to as
214 *R26*^{dnMaml1}) embryos or *Hnf1b*-CreER^{T2}; *Rosa26*^{LSL-YFP/+} (hereafter referred to as *R26*^{YFP})
215 control embryos at different timepoints and harvested embryos for analysis at E15.5
216 (Figure 4A). In agreement with a previous *Hnf1b*-CreER^{T2} lineage-tracing analysis (Solar
217 et al., 2009), IF examination of *R26*^{YFP} pancreata, treated with Tam between E11.5 –
218 E13.5, for Sox9 and Ptf1a revealed that while some *Hnf1b*-expressing cells retain their
219 multipotency at E13 and E14, they become progressively more biased towards the BP
220 lineage with time (Figures 4B-4D and 4N). In contrast, *R26*^{dnMaml1} pancreata treated
221 with Tam at E11.5 showed an ~11-fold reduction of EGFP⁺ cells adopting a BP fate,
222 while the fraction of EGFP⁺ cells allocated to a PAC fate had increased almost 3-fold.
223 The fraction of labeled cells expressing neither marker was unchanged (Figures 4E and
224 4N). Similarly, significantly fewer EGFP⁺ cells co-expressed Sox9 in *R26*^{dnMaml1}
225 pancreata injected with Tam at E12.5 or E13.5 compared to controls. However, in
226 sharp contrast to E11.5 Tam-induced pancreata, the proportion of EGFP⁺ cells
227 expressing Ptf1a was unchanged following Tam treatment at E12.5 and E13.5. Instead,
228 more EGFP⁺ cells were Sox9⁻Ptf1a⁻, compared to YFP⁺ cells in the controls (Figures 4F,
229 4G, 4N). Together, these data show that suppression of Notch signal reception in MPCs
230 and BPs before ~E13 shunts the cells to a PAC fate. However, if Notch signaling is
231 blocked in BPs after ~E13, they adopt an alternative fate.

232

233 **Suppressing Notch after ~E13 shunts progenitors to an endocrine fate.** We next
234 performed IF for EGFP/YFP, Sox9, Ngn3 and Chga to determine when progenitors
235 adopted an endocrine fate upon Notch blockade. As expected, the fraction of labelled
236 cells expressing Sox9 in E15.5 *R26*^{dnMaml1} pancreata was significantly lower than in
237 controls after Tam injection at E11.5 and consistent with the increase of labelled
238 Ptf1a⁺ cells mentioned above, significantly more labelled cells were triple-negative for
239 Sox9, Ngn3 and Chga in dnMaml1 embryos, compared to controls (Figures 4H, 4K, 4O).
240 Examining endocrine lineage markers, we found that the fraction of labelled cells
241 expressing either Ngn3 or Chga, was not significantly different between dnMaml1

242 embryos and controls (Figures 4H, 4K, 4O). However, fewer labelled cells co-expressed
243 Ngn3 in dnMaml1 embryos than in controls (Figure 4P). Conversely, when Tam was
244 injected at E12.5 and E13.5, the fraction of labelled cells expressing endocrine markers
245 was now markedly increased compared to controls, while the fraction of labelled cells
246 expressing Sox9 was reduced and labelled cells triple-negative for Sox9, Ngn3 and
247 Chga was unchanged between groups (Figures 4I, 4J, 4L, 4M). Again, fewer labelled
248 cells co-expressed Ngn3 in dnMaml1 embryos than in controls (Figure 4P). A
249 qualitative analysis of lineage-traced E15.5 *Sox9-CreER^{T2}; R26^{dnMaml1}* embryos
250 compared to stage-matched *Sox9-CreER^{T2}; R26^{YFP}* embryos revealed the same shift in
251 the fate of the labelled cells when comparing Tam injections at E11.5 to E12.5 and
252 E13.5 (Figure S2). Taken together, these results show that the time window through
253 which prevention of Notch activation can shunt cells to a PAC fate closes by ~E13 and
254 confirms that prevention of Notch activation in BPs after ~E13 induces endocrine
255 differentiation (Magenheim et al., 2011; Shih et al., 2012).

256

257 **Jag1 is required to exit multipotency.** Having established the temporal limit for BP
258 versus PAC specification we next sought to identify the ligands responsible for this fate
259 choice. IF analysis of E12.5 *Jag1^{ΔFoxa2}* mutant pancreas revealed that the fraction of
260 Ptf1a⁺Nkx6-1⁺ MPCs was significantly increased at the expense of Ptf1a⁻Nkx6-1⁺ BPs,
261 while the fraction of Ptf1a⁺Nkx6-1⁻ PACs was unchanged compared to controls
262 (Figures 3B-3C). While Ptf1a is essentially confined to the peripheral-most epithelial
263 cells in wild-type controls, Ptf1a expression was also seen in a few, more proximal cells
264 in *Jag1^{ΔFoxa2/+}* heterozygotes and prominently in many proximal cells in *Jag1^{ΔFoxa2/-}*
265 homozygote mutants (Figure 3B). We previously performed the same analysis on
266 E12.5 *Dll1^{ΔFoxa2}* mutant pancreas (Horn et al., 2012) so we calculated the percentage
267 of each cell type from those data and found that while the fraction of Ptf1a⁻Nkx6-1⁺
268 BPs was also reduced in these embryos, the fraction of Ptf1a⁺Nkx6-1⁺ MPCs was not
269 increased. Instead, the fraction of Ptf1a⁺Nkx6-1⁻ PACs was increased (Fig. 3C). Thus,
270 proper segregation of MPCs into PAC and BP domains at E12.5 requires Jag1, while
271 Dll1 is required for biasing MPCs towards a BP fate.

272 Analysis of later stages revealed that most Ptf1a⁺ cells remained co-positive for Nkx6-
273 1 and Sox9 at E13.5, thus maintaining an MPC marker profile. Not until E14.5 did we

274 observe a resolution into distinct Ptf1a⁺Nkx6-1⁻ PACs and Ptf1a⁻Nkx6-1⁺ BPs in the
275 *Jag1*^{ΔF_{oxa2}} pancreas (Figure S3). However, while BPs normally extend all the way into
276 the forming acini at this stage, the *Jag1*^{ΔF_{oxa2}} pancreas was nearly devoid of such cells
277 in the periphery (Figure S3). To begin to understand the mechanism causing MPCs to
278 maintain their multipotent state we assessed how loss of Jag1 impacts Notch activity
279 in *Jag1*^{ΔF_{oxa2}} pancreata by IF analysis of Hes1 expression. We found that Hes1
280 expression, and by inference active Notch signaling, was upregulated in the E12.5
281 *Jag1*^{ΔF_{oxa2}} pancreas compared to heterozygote littermates (Figure 3D-3E). This was
282 especially notable in the distal-most cells, in which average Hes1 levels were increased
283 ~2-fold (Figure 3E). This finding suggests that Jag1 is required cell-autonomously to
284 inhibit Notch activation in emerging PACs, and thus act as a symmetry breaker that
285 triggers exit from the multipotent state.

286

287 **The combined activities of *Jag1* and *Dll1* specify the entire BP population.** Since
288 E15.5 *Mib1*^{ΔF_{oxa2}} mutants are comprised exclusively of PACs cells (Horn et al., 2012)
289 we next examined the individual and combined requirement of Jag1 and Dll1 for BP
290 versus PAC fate at this stage. To address this question, we generated stage-matched
291 *Dll1*^{ΔF_{oxa2}} and *Jag1*^{ΔF_{oxa2}} mutants, compound *Jag1*; *Dll1*^{ΔF_{oxa2}} mutants, and control mice
292 without ligand deletions (*Foxa2*^{T2AiCre}; *R26*^{YFP}). Analysis of all four genotypes at E15.5
293 revealed no change in the numbers of PACs or BPs per unit area between single
294 *Dll1*^{ΔF_{oxa2}} mutant and control pancreata. However, the *Dll1*^{ΔF_{oxa2}} mutant pancreata
295 were clearly hypomorphic. In contrast, BPs were severely depleted in *Jag1*^{ΔF_{oxa2}}
296 mutants with a corresponding expansion of PACs, and in compound *Jag1*; *Dll1*^{ΔF_{oxa2}}
297 mutants this shift in fate was essentially all-encompassing (Figures 5A-5E). Notably,
298 *Jag1*^{ΔF_{oxa2}/+} heterozygotes showed an intermediate phenotype (Figures S4A-S4D). The
299 few remaining BPs found in *Jag1*^{ΔF_{oxa2}} mutants were confined to the central core of
300 the organ (Figure 5A-5B), which was also evident when plotting the fraction of Nkx6-
301 1⁺ and Ptf1a⁺ cells in the z-dimension (Figure 5C). IF staining for insulin revealed a ~50%
302 decrease in the number of insulin⁺ β-cells in both *Dll1*^{ΔF_{oxa2}} and *Jag1*^{ΔF_{oxa2}} pancreata
303 compared to controls, while compound *Jag1*; *Dll1*^{ΔF_{oxa2}} mutants exhibited a near
304 complete loss of β-cells (Figure 5D-5E). Together, these data show that Jag1 is required

305 for development of all but the central-most BPs and that the combined activities of
306 Jag1 and Dll1 specify the entire BP lineage and suggest that MPCs normally fated to
307 become BPs adopt a PAC fate in their absence.

308

309 **Impeding ligand trans-activation in late development only affects CACs.** The strong
310 phenotype we observe in *Jag1; Dll1^{ΔFoxa2}* mutant embryos contrasts with the rather
311 modest phenotype seen in *Jag1; Dll1^{ΔPtf1a}* mutant embryos, which only lack CACs
312 (Nakano et al., 2015). This suggests that the timing of Cre-mediated recombination,
313 which is mosaic and occurs considerably later with *Ptf1a^{Cre}* compared with *Foxa2^{iCre}*
314 ^{44,48}, is critical for deciding the outcome. Considering that Mib1 is required for all Notch
315 ligand trans-activation (Itoh et al., 2003; Koo et al., 2005; Koo et al., 2007) and that its
316 elimination in *Mib1^{ΔFoxa2}* pancreata phenocopies the cell fate changes in *Jag1;*
317 *Dll1^{ΔFoxa2}* embryos seen here (Horn et al., 2012), we therefore asked whether
318 *Mib1^{ΔPtf1a}* pancreata would phenocopy *Jag1; Dll1^{ΔPtf1a}* pancreata. *Mib1*-depleted cells
319 and their progeny were identified by *R26^{YFP}* recombination. Examination of *Mib1^{ΔPtf1a}*
320 pancreata prior to E13.5 failed to reveal any obvious defects. In contrast, analysis at
321 E15.5 revealed the ductal tree to be truncated distally. The distal-most, Sox9⁺ cells
322 (prospective CACs), which normally protrude into the nascent Ptf1a⁺ acini are
323 specifically depleted in *Mib1^{ΔPtf1a}* embryos (Figure S5). Notably, this phenotype closely
324 resembles the reported loss of CACs following *Ptf1a^{Cre}*-driven compound deletion of
325 *Dll1* and *Jag1* (Nakano et al., 2015). Taken together, these results suggest that the
326 trans-activation of Notch receptors by Jag1 and Dll1 expressed on PACs and emerging
327 acinar cells is required to specify and/or maintain adjacent CAC precursors.

328

329 **Coupling of Hes1 oscillation parameters to cell fate.** Similar to *Jag1; Dll1^{ΔFoxa2}* mutants,
330 *Hes1^{ΔFoxa2}* embryos also show severely reduced numbers of BPs and a corresponding
331 increase in PACs (Horn et al., 2012). We therefore asked whether perturbing the
332 period or amplitude of Hes1 oscillations would similarly affect cell fate. To address this
333 question, we exploited the ability of NICD levels to modulate oscillation parameters
334 (Wiedermann et al., 2015). We explanted E10.5 dorsal buds from *Hes1*-Luc2 embryos
335 and perturbed NICD levels with small molecule inhibitors. We monitored the period
336 and amplitude of Hes1 oscillation by bioluminescence imaging and conducted end-

337 point IF analysis of cell fate allocation. Western blot analysis showed that treatment
338 with a low dose of the γ -secretase inhibitor DAPT reduced average N1ICD levels by
339 ~50% compared to vehicle controls (Figure 6A) and bioluminescence imaging revealed
340 a ~70% decrease in the amplitude of Hes1 oscillations, while the period was
341 unaffected (Figure 6B). This was associated with an increase of Ptf1a⁺ PACs and
342 Ptf1a⁺Sox9⁺ MPCs at the expense of Sox9⁺ BPs at the end of the 4-day culture period
343 (Figure 6C-6D). In contrast, exposure to MLN4924, a Nedd8 activating enzyme
344 inhibitor, increased N1ICD levels by ~60% compared to vehicle controls (Figure 6A)
345 and prolonged the mean oscillation period from ~90 to ~120 minutes while only
346 marginally affecting the amplitude (Figure 6B). IF analysis showed that Ptf1a⁺ cells
347 (PACs and MPCs) were strongly reduced by MLN4924 treatment and the explants
348 were composed almost entirely of Sox9⁺ cells (BPs and/or duct cells) (Figure 6C-6D).
349 Together these data suggest that the both the amplitude and period of Hes1
350 oscillations are important for MPC fate choice.

351

352 **Early *Jag1* ^{Δ Foxa2} mutants have normal plexus formation and organ architecture.** We
353 next analyzed how the BP-to-PAC fate switch affects ductal morphogenesis and overall
354 organ development in *Jag1* ^{Δ Foxa2} mutants. In spite of the delayed PD patterning, we
355 found that formation of the epithelial plexus occurred normally and overall organ size
356 and morphology was comparable between *Jag1* ^{Δ Foxa2} mutants and heterozygote
357 littermate controls (Figure 7A). Remarkably, even at E15.5 the overall organ
358 architecture is essentially unaffected. The dorsal pancreas had a well-formed, anvil-
359 shaped head and the body gradually tapered into the narrow connection with a
360 normal-sized ventral pancreas located in the duodenal loop (Figure 7B). In contrast,
361 the *Dll1* ^{Δ Foxa2} dorsal pancreas was hypoplastic with the head being malformed and the
362 body of the pancreas being greatly truncated (Figure 7B). However, closer inspection
363 of *Jag1* ^{Δ Foxa2} embryos revealed that although the ductal plexus appeared to have
364 remodeled into a hierarchical tree-like structure, the smooth walls of the intercalated
365 ducts seen in controls and *Dll1* ^{Δ Foxa2} mutants, showed a more serrated appearance in
366 *Jag1* ^{Δ Foxa2} mutants (Figure 7B). Nevertheless, acinar structure appears normal with
367 apical localization of Muc1, ZO-1 and PKC ζ , indicating that acinar cytoarchitecture is

368 maintained in the E15.5 *Jag1*^{ΔF_{oxa2}} pancreas (Figure 7H-7I). In the domain usually
369 occupied by Sox9⁺ prospective ducts, we were able to identify elongated tubular
370 structures expressing Ptf1a instead of Sox9 (Figure S4E). Similar to normal PACs these
371 mis-specified PACs also expressed Bhlha15/Mist1 (Figure S4F). Taken together, these
372 findings suggest that the overall organ architecture and remodeling of the ductal
373 plexus is regulated independently from the differentiation programs that allocate
374 MPCs to endocrine, duct and acinar lineages. In contrast, finer morphological features
375 of the ductal tree are clearly perturbed by the BP-to-PAC fate switch.

376

377 **Late *Jag1*^{ΔF_{oxa2}} embryos show signs of acute pancreatitis and ADM.** E18.5 *Jag1*^{ΔF_{oxa2}}
378 pancreata remained equivalent to controls in size and overall organ morphology. The
379 dorsal and ventral pancreas appeared fused normally, the gastric lobe was present,
380 and the normal “anvil” shape of the dorsal pancreas (Villasenor et al., 2010) was
381 evident (Figure 7C). However, closer inspection revealed that the larger ducts were
382 not formed properly. The main duct was disrupted, interlobular as well as larger
383 intralobular ducts were largely absent and many of the terminal ducts appeared
384 serrated, occasionally connected by ducts of relatively normal morphology despite
385 being composed of Ptf1a⁺ cells (Figure 7D). As expected, we saw a prominent loss of
386 endocrine cells in E18.5 *Jag1*^{ΔF_{oxa2}} pancreata, with scattered α-cells and a central
387 cluster of β-cells. The late-arising, somatostatin⁺ δ-cells were nearly absent, even in
388 the central cluster (Figure 7E). Examination of Muc1 and Krt19 expression and DBA
389 lectin binding revealed an obvious paucity of the ductal tree and throughout the
390 epithelium we observed numerous “ring-like” structures with perturbed apicobasal
391 polarity and co-expression of amylase, Cpa1 and Krt19 (Figure 7F-7I), reminiscent of
392 the acinar-to-ductal metaplasia (ADM) associated with Cerulein-induced acute
393 pancreatitis (Nishikawa et al., 2019).

394

395 Discussion

396 Regulation of MPC proliferation and fate choice by Notch has been comprehensively
397 documented by previous work (Afelik et al., 2012; Ahnfelt-Rønne et al., 2012;
398 Apelqvist et al., 1999; Fujikura et al., 2006; Fujikura et al., 2007; Hald et al., 2003;
399 Jensen et al., 2000a; Jensen et al., 2000b; Murtaugh et al., 2003; Schaffer et al., 2010;

400 Shih et al., 2012). In this study we show that the Notch pathway components Dll1 and
401 Hes1 display ultradian oscillations in the developing mouse pancreas and that Jag1,
402 which is uniformly expressed in MPCs, dampens Dll1-mediated Notch activation to
403 restrain MPC growth, secure timely exit from the multipotent state, and coordinate
404 MPC fate choice (Figure S6).

405 We found the period of pancreatic Dll1 and Hes1 oscillations to be in the range of 1-2
406 hours with an average of ~90 minutes. This is noticeably shorter than the period
407 observed in neural progenitors, which is in the range of 2-3 hours with an average of
408 ~150 minutes (Imayoshi et al., 2013; Shimojo et al., 2016). At first glance this may
409 appear surprising given that the regulatory network between and within cells has a
410 similar architecture for neural and pancreatic progenitors (this work and (Kageyama
411 et al., 2008; Tiedemann et al., 2017)). Moreover, the oscillations in neighboring
412 pancreatic and neural progenitors are typically not in phase, while groups of oscillating
413 cells in the pre-somitic mesoderm *are* in phase. Indeed, we often observed Hes1
414 oscillations in neighboring pancreatic cells to be in anti-phase, as is also observed in
415 neural progenitors (Imayoshi et al., 2013). So how can the different periods be
416 explained? The oscillatory period in pre-somitic mesoderm is sensitive to Notch
417 activity and modeling suggests that this may be a general property of Notch
418 oscillations (Kim et al., 2011; Wiedermann et al., 2015). We therefore speculate that
419 the high expression levels of Notch pathway components in neural progenitors
420 compared to MPCs may cause the former to attain a longer period. This notion is
421 supported by our observation of an extended period in pancreatic progenitors treated
422 with MLN4924 but raises the question of why the period is not shortened by DAPT
423 treatment. However, a minimal period is imposed on the system, partly by the sum of
424 the transcriptional and translational delays in the delayed negative feedback
425 mechanism of Hes1 and partly by the parameters governing the dynamics of
426 Dll1/Notch-mediated intercellular signaling (Lewis, 2003), and it is possible that a
427 minimal period is reached in the pancreas.

428 We have previously shown that E10.5 *Dll1*^{-/-} pancreata are hypoplastic due to reduced
429 proliferation (Ahnfelt-Rønne et al., 2012) and now we show that E10.5 *Dll1*^{ΔFoxa2}
430 mutants and *Dll1* Type 1 and Type 2 oscillation mutants also present with pancreatic
431 hypoplasia. Both Type 1 and Type 2 mutants encode the wildtype protein, but due to

432 accelerated or delayed protein synthesis, respectively, a steady intermediate level of
433 Dll1 expression is achieved as oscillations of both Dll1 and Hes1 are dampened
434 (Shimojo and Kageyama, 2016). The hypoplasia seen in these mutants suggests that
435 oscillatory expression of Dll1 and/or Hes1 is important for stimulating MPC growth.
436 This can be explained if ultradian Dll1 oscillations enables a temporal symmetry where
437 MPCs alternate between sending and receiving input via Notch. Without oscillations
438 it would prove difficult for the cells to reach a ligand^{Lo}, receiving state as they would
439 be subject to *cis*-inhibition and fail to receive input via Notch receptors that ultimately
440 couples to the mitotic machinery (Figure S6A).

441 Notably, we found that E10.5 Jag1-deficient pancreata were hyperplastic, showing
442 that Jag1 limits MPC growth. Together, these results suggest that Jag1 antagonizes
443 Dll1 function at this stage, possibly by a *cis*-inhibitory interaction that sequesters a
444 fraction of the available Notch receptors (Figure S6A). It is possible that Jag1-mediated
445 dampening of Notch activation is part of the mechanism that sets the oscillatory
446 frequency of Hes1 and Dll1 expression.

447

448 In pancreata undergoing PD patterning, we found that emerging PACs (Ptf1a⁺)
449 expressed high levels of ligands and little to no Hes1, indicating a state of low Notch
450 activity. Conversely, adjacent MPCs (Ptf1a⁺Nkx6-1⁺) or BPs (Nkx6-1⁺) were generally
451 expressing no or low levels of ligand and high levels of Hes1, indicating Notch
452 activation. This suggests that mutually inactivating *cis*-interactions between ligands
453 and receptors (Sprinzak et al., 2010) are crucial for exiting the multipotent stage and
454 for the cells to adopt either a PAC or BP fate. Such a notion is supported by the
455 downregulation of Hes1 and absence of Notch2 expression in PACs and thus overall
456 lower levels of Notch receptor expression than seen in emerging Notch1⁺Notch2⁺ BPs.
457 Conversely, Notch1/2 co-expression, and the absence of Jag1 expression in nascent
458 BPs, would render these more sensitive to signal reception and less prone to signal
459 emission due to *cis* interactions (Figure S6B).

460 To test these ideas and to investigate the role of individual ligands in PD patterning
461 we performed single and double Dll1/Jag1 loss-of-function experiments. Our marker
462 analyses showed that most epithelial cells in the E12.5 Jag1^{ΔFoxa2} embryos maintained
463 a Ptf1a⁺Nkx6-1⁺Sox9⁺ marker profile suggesting that they failed to exit the MPC stage.

464 This correlated with increased Hes1 expression, suggesting that Notch activity is
465 increased and that Jag1 normally acts cell-autonomously to inhibit Notch activation in
466 emerging PACs. We suggest that upregulation of Jag1 *cis*-inhibits Notch receptors and
467 thus acts as a symmetry breaker that terminates oscillatory Hes1 expression in
468 nascent PACs (Figure S6B). Loss of Notch activity may additionally downregulate Nkx6-
469 1 (Afelik et al., 2012) and/or liberate Rbpj from N1ICD, which would then be free to
470 complex with Ptf1a (Cras-Meneur et al., 2009). Both of these mechanisms would favor
471 a PAC fate (Cras-Meneur et al., 2009; Schaffer et al., 2010). Concurrently, free ligand
472 molecules would be able to convey *trans*-activation of receptors in neighboring cells
473 if these are in a responsive, ligand^{Lo}/receptor^{Hi} state, and instruct these to adopt a BP
474 fate (Figure S6C).

475 In spite of increased Notch activity at early stages, *Jag1*^{ΔFoxa2} MPCs eventually adopt a
476 PAC fate, which depends on the cells attaining a state of low Notch activation (Afelik
477 et al., 2012; Horn et al., 2012; Schaffer et al., 2010). However, it remains to be
478 determined what triggers a reduction in Notch activation, but it is noteworthy that the
479 timing coincides with the onset of *Lfng* (*Lunatic fringe*) expression at E14.5 in PACs
480 (Svensson et al., 2009). Lunatic fringe has been shown to inhibit Notch activity in
481 presomitic mesoderm (Dale et al., 2003) and could potentially strengthen *cis*-
482 inhibitory activity of Dll1 (LeBon et al., 2014) in E14.5 *Jag1*^{ΔFoxa2} progenitors. This
483 would attenuate expression of the BP-promoting Notch target genes Nkx6-1 (Afelik et
484 al., 2012) and Sox9 (Shih et al., 2012) in Dll1^{Hi} cells allowing these to exit from the MPC
485 state and ultimately adopt a PAC fate. Testing this hypothesis and identifying the
486 precise role of different Notch receptors awaits future experiments.

487 The strong effect on PD patterning we observe is surprising since previous analyses of
488 pancreas-specific Jag1 or Jag1/Dll1 deletions did not uncover a prominent expansion
489 of the PAC domain at the expense of BPs (Golson et al., 2009a; Golson et al., 2009b;
490 Nakano et al., 2015) . We suspect that this can be attributed to the different timing of
491 efficient, non-mosaic recombination between different Cre lines. Conditional Jag1
492 deletion with *Pdx1*-Cre or *Foxa3*-Cre driver lines occurs much later than with our
493 targeted *Foxa2*^{Cre} driver, which recombined with >99% efficacy prior to pancreas
494 specification (Horn et al., 2012). More recently, compound *Dll1*; *Jag1*^{ΔPtf1a} mutants
495 were found to have a loss of CACs, the terminal-most cell type in the ductal tree, while

496 single mutant littermates did not show any phenotype (Nakano et al., 2015). As our
497 *Mib1*^{ΔPtf1a} mutants phenocopy the loss of CACs reported in *Dll1*; *Jag1*^{ΔPtf1a} mutants,
498 this suggests that the *Ptf1a*^{Cre}-driver only becomes non-mosaic in PACs and their
499 progeny and that CACs are specified by PACs late in pancreatic development.

500 In spite of the prolonged MPC state, the ductal plexus forms normally. However, at
501 later stages the consequence of the BP-to-PAC switch becomes evident as normal
502 remodeling of the ductal plexus into a well-structured ductal tree is disrupted. The
503 tubular network comprising the intercalated ducts seems to form but the normal
504 smooth morphology of the ductal lumen is perturbed by E15.5. We also noted a
505 complete absence of larger intralobular ducts and interlobular ducts at E18.5 and
506 ductal structures in the area of the main duct appear interrupted as also previously
507 noted in *Jag1*^{ΔPdx1} animals (Golson et al., 2009b). These perturbations are not
508 surprising given that acinar cells have not evolved to form cuboidal or columnar
509 epithelia, but rather to adopt a pyramidal shape fitting for cells forming an acinus.
510 However, in spite of these disturbances the overall organ morphology is surprisingly
511 well preserved. We conclude that the regulatory principles governing the overall
512 shape of the pancreas are highly resilient to cell fate changes, at least as long as these
513 occur in the internal part of the organ.

514

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521

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523 and wrote the manuscript. M.C.J. performed all staining, imaging and quantitative
524 analysis of whole-mount specimens. K.H.L. contributed to phenotype analysis. H.S., I.I.
525 and R.Ka. contributed to bioluminescence imaging and generated the *Jag1*^{J1V^{mC}} and
526 *Dll1*^{D1V^{mC}} mouse lines. R.Ko. generated the N1IP::Cre^{HI}; *Rosa26*^{LSL-Ai3} embryos. P.S.

527 conceived the study, designed and interpreted experiments and wrote the manuscript.

528 All authors revised and approved the manuscript.

529

530 **Declaration of Interests.** The authors declare no competing or financial interests.

531

532 **Figure legends**

533 **Figure 1.** Differential expression of Notch components in MPCs and their progeny.

534 (A) Sections of E10.5, E12.5 and E15.5 *Jag1*^{J1V^{mC}} and *Dll1*^{D1V^{mC}} dorsal pancreata stained
535 for Venus, Pdx1 and Sox9 as indicated. Arrowheads in E12.5 panel indicate emerging
536 PACs. Arrows in E15.5 panels indicate endocrine precursors and arrowheads indicate
537 forming acini. v: vessel. Lower panels show schematic representations of the *Jag1*^{J1V^{mC}}
538 and *Dll1*^{D1V^{mC}} fusion protein reporters.

539 (B) Sections of E10.5 *Jag1*^{J1V^{mC}} and *Dll1*^{D1V^{mC}} dorsal buds stained for Venus, Sox9 and
540 Dll1 or Jag1 as indicated. Arrows indicate Dll1⁺Jag1⁻ cells and arrowheads indicate
541 Sox9⁺ MPCs co-expressing Jag1 and Dll1.

542 (C) Section of E12.5 dorsal pancreas stained for Ptf1a, Nkx6-1, Dll1 and Jag1 as
543 indicated. Arrows indicate Nkx6-1⁺ BPs expressing Dll1 but not Jag1 and arrowheads
544 indicate Ptf1a⁺ PACs co-expressing Jag1 and Dll1.

545 (D) Serial sections of E12.5 dorsal pancreas stained for Ptf1a, Nkx6-1, and Notch1 or
546 Notch2 as indicated. Arrows and arrowheads in Notch1 panels indicate Notch1⁺Nkx6-
547 1⁺ and Notch1⁺Ptf1a⁺ cells, respectively. Arrows and arrowheads in Notch2 panels
548 indicate Notch2⁻Ptf1a⁺ and Notch2⁺Nkx6-1⁺ cells, respectively.

549 (E) Upper panel shows a schematic of the lineage-tracing strategy using N1IP::Cre to
550 label cells with a history of Notch1 activation. Lower panels show sections of E10.5
551 and E12.5 dorsal pancreas from an N1IP::Cre; Rosa26LSL-Ai3 embryo stained for YFP,
552 Ptf1a and Sox9 as indicated. Insets show individual channels for clarity. Arrow: YFP-
553 labeled, Ptf1a⁺ PAC. Arrowheads: YFP-labeled, Sox9⁺ BPs. Asterisks: YFP-labeled
554 endothelial cells. The bar graph shows quantification of YFP-labeled versus unlabeled
555 epithelial cells expressing Sox9, Ptf1a, or both. Mean ± S.D., N = 3 embryos.

556 (F) Section of E12.5 dorsal pancreas stained for Ptf1a, Nkx6-1, Hes1 and Jag1 as
557 indicated. Arrows: Ptf1a⁺Jag1⁺Nkx6-1^{Lo/-} cells. Arrowheads: Nkx6-1⁺ cells; either Hes1⁺
558 (white arrowheads) or Hes1⁻ (yellow arrowheads).

559 (G) Section of E12.5 dorsal pancreas stained for Ptf1a, Nkx6-1, Hes1 and Dll1 as
560 indicated. Arrows: Nkx6-1^{Hi}Hes1^{Hi} cells in distal epithelium adjacent to Ptf1a⁺Dll1^{Hi}
561 cells (arrowheads). Scale bars are 20 μ m in all panels. See also Figures S1 and S7.

562

563 **Figure 2.** Hes1 and Dll1 protein levels display ultradian oscillations.

564 (A) Bioluminescence images and quantification of Luc2-Hes1 reporter expression in
565 E10.5 pancreatic explants cultured for 1, 4 or 6 days prior to time-lapse imaging.

566 (B) Bioluminescence images and quantification of Dll1-Fluc reporter expression in
567 E12.5 pancreatic explants cultured for 4 hours prior to time-lapse imaging.

568 (A, B) Grayscale and false color montages are shown for the same cell and each frame
569 is 10 minutes (10'). Representative tracks from two separate cells are shown for each
570 condition and the scatter plots shows the distribution and average of the oscillation
571 periods (Mean \pm S.D., N > 50 cells for each Hes1-Luc2 time point and N = 18 for Dll1-
572 Fluc).

573

574 **Figure 3.** Dll1 and Jag1 differentially affects MPC expansion and differentiation.

575 (A) 3D maximum intensity projections and bud volume quantifications of E10.5
576 *Dll1*^{T1/T1}, *Dll1*^{T2/T2}, *Dll1* ^{Δ Foxa2}, *Jag1* ^{Δ Foxa2/-} mutants and their wildtype or heterozygote
577 littermate controls stained by whole-mount IF for Pdx1, Ngn3, and Gcg as indicated.
578 dp: dorsal pancreas; vp: ventral pancreas. Scale bar is 50 μ m.

579 (B) Sections of E12.5 wildtype *R26*^{Yfp/Yfp}, *Jag1* ^{Δ Foxa2/+} and *Jag1* ^{Δ Foxa2/-} mutants stained
580 by IF for Ptf1a and Nkx6-1 as indicated. Insets shows higher magnification views of the
581 boxed areas in the main panels. Scale bars, 25 μ m (main panels) and 10 μ m (insets).

582 (C) Quantification of Ptf1a⁺, Nkx6-1⁺ and Ptf1a⁺Nkx6-1⁺ co-expressing cells in E12.5
583 wildtype *R26*^{Yfp/Yfp}, *Jag1* ^{Δ Foxa2/+} and *Jag1* ^{Δ Foxa2/-} mutants as well as in *Dll1*^{f/f} wildtypes
584 and *Dll1* ^{Δ Foxa2} mutants. Mean \pm S.D., N = 3-5 as indicated by individual data points.

585 (D) Sections of E12.5 *R26*^{Yfp/Yfp}, *Jag1* ^{Δ Foxa2/+} and *Jag1* ^{Δ Foxa2/-} pancreata stained for Ptf1a
586 and Hes1 as indicated. Scale bars are 25 μ m (main panels) and 10 μ m (insets).

587 (E) Quantification of Hes1 fluorescence intensity in distal Ptf1a⁺ cells in *Jag1* ^{Δ Foxa2/-}
588 mutants and *Jag1* ^{Δ Foxa2/+} littermate controls. Scatter plots show intensity values for
589 individual cells in three mutant-control pairs in three, separate IF experiments, mean

590 ± S.D., N ≥ 192 cells/embryo. Changes in mean Hes1 levels for each pair of embryos
591 are shown in the right-hand panel, color-coded for embryo ID (a.u., arbitrary units).
592

593 **Figure 4.** Stage-dependent allocation of progenitor fate by Notch suppression.

594 (A) Overview of strategy applied to identify fates of progeny from pancreatic
595 progenitors. Approximate temporal windows of Tamoxifen (Tam) activity resulting
596 from single intraperitoneal injections at E11.5, E12.5 or E13.5 and cell fate-specific
597 markers are indicated. PAC: pre-acinar cell; BP: bipotent progenitor; EP: endocrine
598 precursor; EC: endocrine cell.

599 (B-M) Sections of E15.5 *Hnf1b-CreER^{T2}; R26^{YFP}* control (B-D, H-J) or *Hnf1b-CreER^{T2};*
600 *R26^{dnMaml1}* Notch-blocked (E-G, K-M) pancreata stained for EYFP/EGFP and Sox9
601 combined with either Ptf1a (B-G) or Ngn3 and Chga (H-M), as indicated. bp: bi-potent
602 progenitor domain; ac: emerging acini; ec: endocrine cells. Scale bar 50 μm. Arrows in
603 (B-D, H-J) lineage-labeled Sox9⁺ BPs. Arrowheads in (B, E): lineage-labeled Ptf1a⁺ PACs.
604 Arrowheads in (F, G): Ptf1a/Sox9 double negative cells. Arrowheads in (I-J, L-M) Chga⁺
605 endocrine cells. Red arrowhead in (J): Ngn3⁺ endocrine precursor. Arrowheads in (K):
606 lineage-labeled Ngn3/Chga/Sox9 triple negative cells. Scale bar is 50 μm.

607 (N-O) Quantification of *R26^{YFP}*- (EYFP) versus *R26^{dnMaml1}* (EGFP)-labeled cell
608 distribution at E15.5 following Tam administration at E11.5, E12.5 or E13.5 as
609 indicated. Pie charts show fractions of EYFP/EGFP-labeled cells expressing Ptf1a, Sox9
610 or neither marker (D-neg) or Sox9, endocrine markers (Endo, Ngn3+Chga) or none of
611 the three (T-neg). (P) Pie chart showing the proportions of labeled cells in the
612 endocrine lineage expressing either Ngn3 or Chga. P-values for significant changes in
613 allocation to distinct cell fates between *R26^{dnMaml1}* and controls are indicated by font
614 color. N = 3 embryos per genotype for each Tam injection time-point. See also Figure
615 S2.

616

617 **Figure 5.** Progenitors adopt a PAC fate in *Jag1-Dll1* double mutant mice.

618 (A) IF for Ptf1a and Nkx6-1 as indicated on sections of E15.5 *R26^{Yfp/+}, Dll1^{ΔFoxa2},*
619 *Jag1^{ΔFoxa2},* and *Jag1; Dll1^{ΔFoxa2}* dorsal pancreata. Di: distal; Pr: proximal. Scale bars are
620 100 μm (main panels) and 25 μm (insets).

621 (B) Quantitative analyses of the ratio of Ptf1a⁺, Nkx6-1⁺ or Ptf1a⁺Nkx6-1⁺ (Co⁺) cells in
622 E15.5 mutant and control pancreata.

623 (C) Distribution of Ptf1a⁺, Nkx6-1⁺ or Ptf1a⁺Nkx6-1⁺ cells on every 10th section in the z-
624 dimension is shown for a representative embryo from each genotype.

625 (D) IF for Sox9, Ngn3 and insulin as indicated on sections of E15.5 *R26^{Yfp/+}*, *Dll1^{ΔFoxa2}*,
626 *Jag1^{ΔFoxa2}*, and *Jag1; Dll1^{ΔFoxa2}* dorsal pancreata. Di: distal; Pr: proximal. Scale bars are
627 100 μm (main panels) and 25 μm (insets).

628 (E) Quantitative analysis of Sox9⁺ and insulin⁺ β-cells in E15.5 mutant and control
629 pancreata. Graphs show mean ± S.D., N = 3 except for *Jag1; Dll1^{ΔFoxa2}* where N = 2. See
630 also Figures S3, S4 and S5.

631

632 **Figure 6.** Modulation of NICD levels affect Hes1 oscillation parameters and cell fate.

633 (A) Western blots showing reduced or elevated N1ICD levels in response to DAPT (2.5
634 μM) or MLN4924 (5 μM) treatment, respectively. Bar graph shows quantification of
635 band intensities from individual experiments (mean ± S.D., N = 4).

636 (B) Distributions and averages of the of amplitudes and periods for Hes1 oscillations
637 in E10.5 pancreas explants treated with DAPT or MLN4924. (Mean ± S.D., N > 50 cells
638 for each condition.

639 (C) 3D maximum intensity projections of E10.5 pancreas explants cultured for 5 days
640 in DAPT or MLN4924 and stained by whole-mount IF for Sox9 and Ptf1a. Scale bar is
641 100 μm.

642 (D) Quantitative analyses of the ratio of Ptf1a⁺, Sox9⁺ or Ptf1a⁺Sox9⁺ cells from E10.5
643 pancreas explants cultured as in (C). Median and interquartile ranges as well as
644 minimum and maximum values are indicated, N = 13 for DMSO- and MLN4924-treated
645 samples and N = 7 for DAPT treated samples. See also Figure S6.

646

647 **Figure 7.** Ductal remodeling is defective in *Jag1^{ΔFoxa2}* mutants.

648 (A) 3D maximum intensity projections of the midgut region of E12.5 *Jag1^{ΔFoxa2/+}* and
649 *Jag1^{ΔFoxa2/-}* littermates stained for Cdh1, Muc1 and Sox9 as indicated by whole-mount
650 IF. White brackets indicate the Muc1 staining in the body region of the dorsal
651 pancreata to emphasize the luminal plexus regions shown in insets below the main

652 panels. dp: dorsal pancreas, vp: ventral pancreas. Scale bars are 50 μm (main panels)
653 and 25 μm (insets).

654 (B) Upper panels: 3D maximum intensity projections of whole-mount, Muc1 labeled
655 E15.5 *Jag1* ^{Δ Foxa2/+}, *Jag1* ^{Δ Foxa2/-}, *Dll1* ^{Δ Foxa2/+}, and *Dll1* ^{Δ Foxa2/-} pancreata. Scale bar is 300
656 μm . Middle panels: Higher power images of maximum intensity projections from sub-
657 stacks acquired from the boxed areas indicated in upper panels showing Muc1 and
658 Sox9 distribution. Scale bar is 50 μm . Lower panels: Higher power images of boxed
659 areas indicated in middle panels showing Muc1 alone to emphasize the structure of
660 the terminal ducts. Arrowheads: Terminal duct lumens appear serrated in *Jag1* ^{Δ Foxa2/-}
661 mutants while heterozygote littermate controls and *Dll1* ^{Δ Foxa2/-} mutants show smooth
662 lumens. dp: dorsal pancreas, vp: ventral pancreas. Scale bar is 10 μm .

663 (C) 3D maximum intensity projections of E18.5 *Jag1* ^{Δ Foxa2/+} and *Jag1* ^{Δ Foxa2/-} whole-
664 mount pancreata stained for Muc1 and YFP as indicated. dp: dorsal pancreas, gl:
665 gastric lobe, vp: ventral pancreas, du:duodenum. Scale bar is 700 μm .

666 (D) 3D maximum intensity projections of the same pancreata shown in (C). Note
667 absence of both interlobular and large intralobular ducts in *Jag1* ^{Δ Foxa2/-} mutants
668 compared to controls. Boxed areas indicate regions shown at higher magnifications in
669 lower panels. Scale bars are 100 μm (upper panels) and 25 μm (lower panels).

670 (E-G) Sections of E18.5 *Jag1* ^{Δ Foxa2/+} and *Jag1* ^{Δ Foxa2/-} pancreata stained for insulin (Ins),
671 glucagon (Gcg) and somatostatin (Sst) (E), Muc1, Krt19 and DBA (F), or Cpa1, amylase
672 (Amy) and Ptf1a as indicated (G). md: main duct, (disrupted in mutants), icd:
673 intercalated ducts (abnormal morphology in mutants), "ADM": ADM-like structures.
674 Scale bars are 25 μm .

675 (H-I) Sections of E15.5 and E18.5 *Jag1* ^{Δ Foxa2/+} and *Jag1* ^{Δ Foxa2/-} pancreata stained for ZO-
676 1, Ptf1a, Sox9 and YFP or ZO-1, Ptf1a, Cpa1 and YFP as indicated (H) and PKC ζ , Ptf1a,
677 Pdx1 and YFP or PKC ζ , Ptf1a, Cpa1 and YFP as indicated (I). Arrows: Ptf1a⁺ duct-like
678 structures in E15.5 *Jag1* ^{Δ Foxa2/-} mutants and apical ZO-1 and PKC ζ in E18.5 duct cells in
679 control mice. Arrowheads: apical ZO-1 in the E15-5 Ptf1a⁺ PAC domain and in E18.5
680 Ptf1a⁺Cpa1⁺ acinar cells. Asterisks: Absence of apical ZO-1 and PKC ζ in
681 YFP⁺Ptf1a⁺Cpa1⁺ ADM-like structures ("ADM") in E18.5 *Jag1* ^{Δ Foxa2/-} pancreas. Scale
682 bars, 25 μm . ac: acini, bp: bi-potent progenitor domain, dt: ducts.

683

684 **Methods**

685 **Animals.** Published mouse strains were genotyped according to the original work:
686 *R26^{LSL-dnMam1-EGFP}* (Horn et al., 2012), *Gt(ROSA)26Sortm1(EYFP)Cos* (*R26^{LSL-YFP}* reporter
687 (Srinivas et al., 2001)), *Gt(ROSA)26Sortm3(CAG-EYFP)Hze* (*R26^{LSL-Ai3}* reporter (Madisen
688 et al., 2010)), *Mib1^{tm2Kong}* (floxed *Mib1* (Koo et al., 2007)), *Dll1^{tm1Gos}* (*Dll1^{LacZ}* null allele
689 (Hrabe de Angelis et al., 1997)), *Dll1^{tm1.1Hri}* (floxed *Dll1* (Horn et al., 2012)), *Jag1^{tm1Grid}*
690 (*Jag1* null allele (Xue et al., 1999)), *Jag1^{tm2Grid}* (floxed *Jag1* (Kiernan et al., 2006)),
691 *Tg(Hes1-EGFP)^{1Hri}* (BAC transgenic *Hes1-EGFP* reporter (Klinck et al., 2011)), *Hes1^{tm1Fgu}*
692 (*Hes1* null allele (Ishibashi et al., 1995)), *Foxa2^{T2AiCre}* (Cre add-on allele (Horn et al.,
693 2012)), *Hnf1b-CreER^{T2}* (Solar et al., 2009), *Sox9-CreER^{T2}* (Kopp et al., 2011), *Ptf1a^{Cre}*
694 (Kawaguchi et al., 2002) and *Notch1^{tm4(cre)Rko}* (N1IP::Cre^{HI} (Liu et al., 2015)), Luc2-Hes1
695 (BAC transgenic Luciferase-Hes1 fusion protein reporter (Imayoshi et al., 2013)), and
696 lastly *Dll1^{tm1.1Kag}* (*Dll1-Fluc*), *Dll1^{tm4.1(Dll1)Kag}* (*Dll1* Type 1 mutant), and *Dll1^{tm5.1(Dll1)Kag}*
697 (*Dll1* Type 2 mutant) all from (Shimojo and Kageyama, 2016). Additional genotyping
698 primers are given in Supplementary Table 1. Homozygous *Dll1^{D1Vmc}* and *Jag1^{J1Vmc}* mice
699 are viable and fertile, but were maintained and analyzed as heterozygotes due to
700 *Dll1^{D1Vmc}* being a weak hypomorph evident by short, kinky tails in homozygote animals.
701 Generation of Jag1 C-terminal Venus-T2A-mCherry fusion reporter knock-in construct
702 was conducted using a BAC clone (RP23-173O12) from the BACPAC Resources Center
703 at Children's Hospital Oakland Research Institute. An frt-PGK-EM7-Neo-frt cassette
704 was inserted downstream of a Venus-T2A-mCherry reporter in pBluescript II SK+,
705 flanked by 300-500-bp homology arms from the *Jag1* gene with the *Jag1* stop codon
706 removed. BAC targeting cassettes were excised and electroporated into competent
707 SW105 cells containing the BAC clone of interest. Correctly targeted BAC clones were
708 identified by a panel of PCR primers and restriction digestions. The knock-in cassette
709 fragment was retrieved and cloned into pMCS-DTA (a kind gift from Dr. Kosuke Yusa,
710 Osaka University, Japan). The 5'- and 3'-homology arms in the retrieval vector were
711 designed such that 2.5- and 7.5-kb DNA segments flanked the Venus-T2A-mCherry
712 reporter-frt-PGK-EM7-Neo-frt cassette in the BAC clone, which was then subcloned
713 into pMCS-DTA. The shorter homology arm was used to design PCR-based screening
714 for targeted ES cells (TT2). Chimeric mice were produced from successfully targeted

715 ES cell clones by aggregation with ICR embryos. Germ line transmission of the targeted
716 allele was assessed by PCR of tail DNA. pCAG-FLPe mice (Kanki et al., 2006) were used
717 to remove the frt-PGK-EM7-Neo-frt cassette. The Dll1-Venus-T2A-mCherry knock-in
718 mice were generated by a similar strategy using a *Dll1* containing BAC clone (RP23-
719 306J23).

720 *Jag1*^{ΔFoxa2} and *Dll1*; *Jag1*^{ΔFoxa2}: We first introduced a *Jag1* null allele (Xue et al., 1999)
721 on the chromosome carrying the *Foxa2*^{iCre} allele via meiotic crossover. Animals
722 carrying *Jag1*⁻; *Foxa2*^{iCre} chromosomes were then backcrossed to homozygous
723 *Foxa2*^{iCre/iCre} animals to secure this chromosome from further crossover events. *Jag1*^{+/-};
724 *Foxa2*^{iCre/iCre} animals were next crossed with *Jag1*^{fl/fl}*R26*^{YFP/YFP} animals to generate
725 *Jag1*^{ΔFoxa2} embryos and to *Dll1*^{fl/+}; *Foxa2*^{T2AiCre/T2AiCre} animals to generate *Jag1*^{+/-}; *Dll1*^{fl/+};
726 *Foxa2*^{T2AiCre/T2AiCre} mice. The latter was then crossed with *Jag1*^{fl/fl}; *Dll1*^{fl/fl}; *R26*^{YFP/YFP}
727 animals to generate *Jag1*; *Dll1*^{ΔFoxa2} embryos. *Dll1*^{ΔFoxa2} embryos and *R26*^{YFP} controls
728 were made as previously described (Horn et al., 2012). Noon on the day of vaginal plug
729 appearance was considered E0.5. All animal experiments described herein were
730 conducted in accordance with local legislation and authorized by the local regulatory
731 authorities.

732

733 **Tamoxifen administration.** Tamoxifen (Sigma-Aldrich, St. Louis, MO) was dissolved at
734 10 mg/ml in corn oil (Sigma) and a single dose of 75 μg/g (for *Hnf1b*- and *Sox9*-CreERT²-
735 mediated *R26*^{YFP/dnMaml1-eGFP} induction) or 40 μg/g (for *Sox17*^{CreERT2}-mediated ligand
736 deletion) body-weight administered by intraperitoneal injection at noon ± 1 hour.

737

738 **Explant culture.** Dorsal pancreata from luciferase reporter embryos were isolated at
739 E10.5 or E12.5 and cultured on 35 mm glass-bottom μ-dishes (Ibidi) coated with 0.1
740 mg/mL Fibronectin (Sigma-Aldrich, St. Louis, MO) in explant culture medium: M199
741 (Gibco?) supplemented with 10% FCS, 1% Pen/strep, 1% Fungizone, 25 ng/ml EGF, 25
742 ng/ml FGF2 and 100 ng/ml FGF10 (R&D Systems). The explants were incubated at 37°C,
743 20% O₂ and 5% CO₂. The explants dissected at E10.5 were cultured for various
744 durations as indicated in the main text. The Dll1-Luc2 explants dissected at E12.5 were
745 cultured for 4 hours before over-night (20 hours) imaging was initiated.

746

747 **Western blots.** 8-10 dorsal pancreata from E10.5 embryos were pooled and cultured
748 for three hours in hanging drops of explant culture medium supplemented with either
749 2.5 μ M DAPT (Sigma-Aldrich, St. Louis, MO), 5 μ M MLN4924 (Cayman Chemical, Ann
750 Arbor, MI) or DMSO. Explants were lysed in lysis buffer (Cell Signaling Technology,
751 Leiden, NL). Samples were sonicated and spun at 15.000 rpm for 20 min at 4°C, then
752 reduced at 95°C for 10 min and loaded on a Bolt™ 4-12% gel Bis-Tris Plus acrylamide
753 gel (Thermo Fischer Scientific, Slangerup, DK). Protein was transferred using a Novex™
754 Semi-Dry Blotter (InVitrogen, Slangerup, DK) according to the manufacturers
755 instructions and blocked in blocking buffer (5% milk in PBST (PBS w. 0.1% Tween20)).
756 Membranes were incubated overnight at 4°C in primary antibodies diluted in blocking
757 buffer and after washing in PBST the blots were developed with the SuperSignal West
758 Dura ECL kit (InVitrogen, Slangerup, DK). Primary antibodies were rabbit monoclonal
759 anti-cleaved Notch1 (Val1744) (Cell Signaling Technology, Leiden, NL) at 1:1000 and
760 rat monoclonal anti-tubulin at 1:5000 (Abcam, Cambridge, UK).

761

762 **Immunostaining.** All primary antibodies are listed with dilution in Supplementary
763 Table 2. Dissected whole embryos (E10.5-E12.5) and foregut preparations (E13.5-
764 E18.5) were fixed in 4% paraformaldehyde in PBS, embedded in Tissue-Tek O.C.T.
765 (Sakura Finetek) and cryosectioned at 10 μ m. For immunofluorescence analysis,
766 antigen retrieval was conducted in pH6.0 citrate buffer, followed by permeabilization
767 in 0.15% Triton X-100 in PBS. After blocking in 1% normal donkey serum in PBS with
768 0.1% Tween-20, sections were incubated overnight at 4°C with primary antibodies
769 diluted in the same buffer. Primary antibodies were detected with anti-rabbit, guinea
770 pig, mouse, rat, goat, sheep or chicken donkey-raised secondary antibodies
771 conjugated to either Cy5 (1:500), Cy3 (1:1,000), Alexa Fluor 488 (1:1,000) or DyLight
772 405 (1:200) (all Jackson ImmunoResearch Europe, Ely, UK). Slides were mounted in
773 Vectashield (Vector Laboratories, Burlingame, CA) with or without DAPI for
774 counterstaining nuclei. Whole-mount IF of E10.5 whole embryos and E12.5, E15.5 and
775 E18.5 foregut preparations was performed as previously described (Ahnfelt-Ronne et
776 al., 2007). Specimens were cleared with BABB (benzyl alcohol:benzyl benzoate 1:2)

777 then scanned confocally for z-stack image acquisition. Images were captured on a
778 Leica SP8 or Zeiss LSM780 confocal microscope and figures prepared using Adobe
779 Photoshop/Illustrator CS6 (Adobe Systems, San Jose, CA, USA). Cultured explants were
780 fixed in 4% PFA for 40 min at room temperature followed by antigen retrieval for 1
781 hour at 37°C in citrate buffer (pH 6.0, 0.1 M 9.5% citric acid, 0.1 M 41.5% sodium
782 citrate and 49% ddH₂O). 3x 10 min wash in PBS at room temperature. Permeabilization
783 in 1% Triton-X-100 diluted in PBS for 40 min. Block in 1% Normal Donkey Serum (#017-
784 00-121) (Jackson ImmunoResearch Europe, Ely, UK) diluted in PBST. Explant were
785 incubated in primary antibodies diluted in blocking buffer at 4°C overnight. The
786 following day, explants were washed 3x 10 min in PSB followed by 2-hour incubation
787 at room temperature with secondary antibodies diluted in blocking buffer.

788

789 **Antibody validation.** Antisera against Dll1, Jag1, and Hes1 were validated by IF
790 analysis of E10.5 neural tube from embryos that were either wildtype or null for the
791 relevant gene (Figure S7). Two characteristic stripes of Jag1 in the neural tube
792 (Johnston et al., 1997) and uniform but weak Jag1 in the E10.5 pancreas was detected
793 by both anti-Jag1 antisera in wild type tissue, but not in equivalent *Jag1*-null tissue.
794 The anti-Dll1 antibody detected Dll1 in the expected reciprocal pattern (compared to
795 Jag1) in the neural tube and scattered cells in the pancreas of wild type embryos, but
796 not *Dll1*^{-/-} embryos. The rabbit monoclonal anti-Hes1 antibody detected Hes1 in the
797 expected patterns in both wild type tissues (i.e. prominently in floor plate and dorsal
798 neural tube as well as pancreatic epithelium and weakly in the surrounding
799 mesenchyme), but not in *Hes1*^{-/-} tissues.

800

801 **Bioluminescence imaging.** Explants were imaged on a heated stage of an inverted
802 microscope (Olympus IX83) and maintained at 37°C in 5% CO₂. The bioluminescence
803 signal was collected with an Olympus 40x UPLFN Universal Plan Fluorite oil immersion
804 objective was transmitted directly to either a cooled Andor iXon Ultra 888 EMCCD
805 camera or a cooled Andor iKon-M934 CCD camera (Oxford Instruments, Oxford, UK).
806 The exposure time for Luc2-Hes1 was 5-10 min with no binning and for Dll1-Fluc it was
807 10 minutes with 4x4 binning.

808

809 **Bioluminescence quantification.** Image sequences were analysed in Fiji (ImageJ
810 version 2.0.0, NIH). Noise from cosmic rays was removed using the SpikeNoise Filter,
811 while single-cell tracking was optimized using the Savitzky-Golay Temporal Filter in Fiji.
812 To track single cells the path was defined with the ROI tool and supported by a
813 Maximum Projection image. False colour (Fire) was used to illustrate the signal
814 intensity in each cell. Z-axis Profiler Plus was used to extract the bioluminescence
815 signal per cell over time for peak-to-peak quantification in Microsoft Excel. Mean
816 amplitude for each oscillating cell was calculated as the difference in relative
817 luciferase activity (a.u.) between individual peaks (P) and troughs (T) using the
818 equation: Mean amplitude = $(P_1 - T_1) + (P_2 - T_2) + \dots + (P_n - T_n)/n$. The local background
819 arising from light scattering in the explants was measured in nine ROIs adjacent to
820 oscillatory cells for each time-lapse movie and the average local background was
821 subtracted from the raw bioluminescence signals before plotting tracks. Background
822 from noise in the EMCCD camera was negligible.

823

824 **Cell quantification.** ~400 cells YFP⁺ lineage-traced cells were counted on 9-11 evenly
825 spaced optical sections through the pancreas from each of three E12.5 N1IP::Cre^{H1};
826 *Rosa26*^{LSL-Ai3} embryos and scored for co-expression of Sox9 and Ptf1a. YFP⁺ and GFP⁺
827 lineage-traced cells from *Hnf1b*-CreERT²; *R26*^{YFP} and *R26*^{dnMam1-GFP} embryos,
828 respectively, were counted on every fifth section throughout the pancreas, for a total
829 of >200 cells/embryo, for each marker combination. Ptf1a⁺Nkx6.1⁻, Ptf1a⁻Nkx6.1⁺ and
830 Ptf1a⁺Nkx6.1⁺ cells were quantified on every fifth section throughout dorsal pancreas
831 from E12.5 controls (*R26*^{Yfp/+}; *Sox17*^{CreERT/+}), *Dll1*^{ΔSox17Tam}, *Jag1*^{ΔSox17Tam},
832 *Dll1/Jag1*^{ΔSox17Tam} using Imaris™ (Oxford Instruments, Oxford, UK). E12.5 *Jag1*^{ΔFoxa2}
833 embryos were quantified the same way but with their own controls (see below). For
834 quantification of E12.5 *Jag1*^{ΔFoxa2} Hes1 IF signal intensity in distal-most Ptf1a⁺ cells,
835 corrected total cell fluorescence (CTCF) was determined using FIJI (Burgess et al., 2010;
836 McCloy et al., 2014). Numbers of Ptf1a⁺Nkx6.1⁻, Ptf1a⁻Nkx6.1⁺ and Ptf1a⁺Nkx6.1⁺ cells
837 as well as Sox9⁺ and insulin⁺ cells were manually scored from every tenth section of
838 dorsal pancreata from E15.5 controls (*R26*^{Yfp/+}; *Foxa2*^{iCre/+}; *Dll1*^{+/+}; *Jag1*^{+/+}), *Jag1*^{ΔFoxa2},
839 *Dll1*^{ΔFoxa2} and *Jag1/Dll1*^{ΔFoxa2} embryos and expressed relative to the area (in mm²) of
840 the YFP⁺ dorsal pancreatic epithelium using Fiji.

841

842 **Statistical analyses.** An F test was used to compare variances. Data sets with two
 843 groups having equal variances were analyzed by a two-tailed (unless otherwise
 844 indicated) Student's t tests. For data with unequal variances we used two-tailed
 845 Welch's t tests. Data sets with multiple groups were analyzed by one-way ANOVA,
 846 followed by Tukey's post hoc test for multiple comparisons using the Prism statistical
 847 program (GraphPad, San Diego, CA). Results were expressed as mean \pm S.D. and
 848 sample numbers are indicated in the figures. A minimum of three embryos per
 849 genotype were examined, except for *Jag1*; *Dll1* ^{Δ Foxa2} for which three years of breeding
 850 have so far only yielded two embryos.

851

852 **Supplemental information**

853 **Supplementary Table 1**

854 Genotyping primers.

855 D1VmC genotyping primers: Fwd: 5'-CTTCAAAGGACACCAAGTACCAGTCG-3', WT-Rev:

856 5'-CTGTCCATAGTGCAATGGGAACAACC 3', Venus-Rev: 5'

857 CTTGCTCACCATAAAGATGCGACCTCC 3'

858

859 J1VmC genotyping primers: Fwd: 5' CAACACGGTCCCCATTAAGGATTACGAG 3', WT-

860 Rev: 5' CTGTCCATAGTGCAATGGGAACAACC 3', Venus-Rev: as above.

861

862 **Supplemental Table 2. Antibodies employed in immunofluorescence analyses**

863

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
PKC ζ	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

864

865 ¹ Detected with (1:500) Cy3-conjugated F(ab')₂ fragment donkey anti-rabbit IgG (Jackson

866 ImmunoResearch Europe; 711-166-152)

867 ² Blocking and antibody incubations performed using M.O.M. Basic Kit (Vector Labs; BMK-

868 2202)

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

870 ⁴ Concentrate

871

872

873 **Movie S1.** Time-lapse movie of explanted E10.5 Luc2-Hes1 dorsal pancreatic bud
874 cultured for 24 hours before imaging commenced. Individual Luc2-Hes1 expressing
875 nuclei are displaying ultradian oscillations. The movie shows 20 hours of development
876 and each frame is 10 minutes.

877

878 **Movie S2.** Time-lapse movie of explanted E10.5 Luc2-Hes1 dorsal pancreatic bud
879 cultured for 96 hours before imaging commenced. Individual Luc2-Hes1 expressing
880 nuclei are displaying ultradian oscillations. The movie shows 20 hours of development
881 and each frame is 10 minutes.

882

883 **Movie S3.** Time-lapse movie of explanted E10.5 Luc2-Hes1 dorsal pancreatic bud
884 cultured for 144 hours before imaging commenced. Individual Luc2-Hes1 expressing
885 nuclei are displaying ultradian oscillations. The movie shows 20 hours of development
886 and each frame is 10 minutes.

887

888 **Movie S4.** Time-lapse movie of explanted E12.5 Dll1-Fluc dorsal pancreatic bud
889 cultured for 4 hours before imaging commenced. Individual Luc2-Hes1 expressing
890 nuclei are displaying ultradian oscillations. The movie shows 20 hours of development
891 and each frame is 10 minutes.

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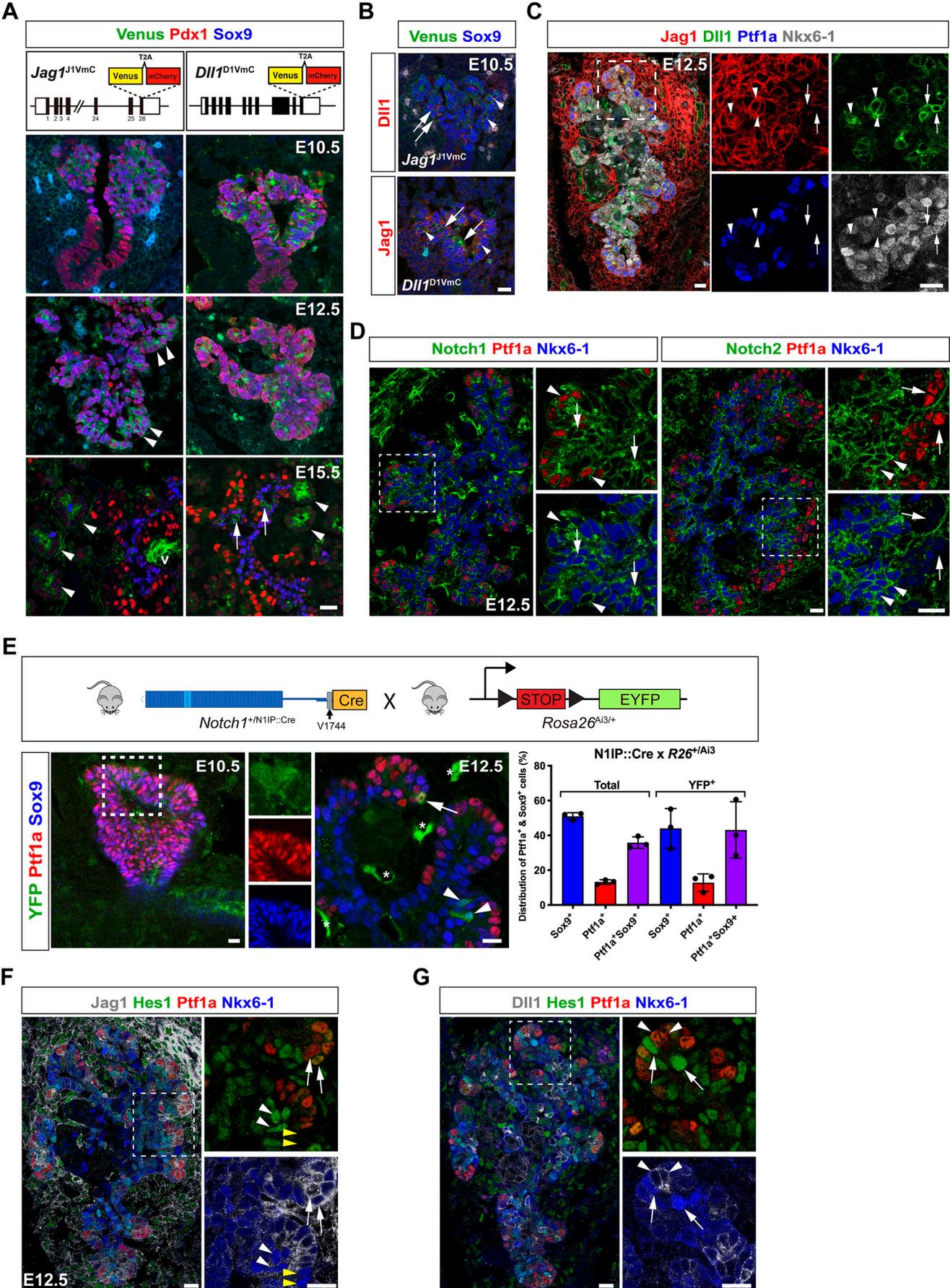


Figure 1

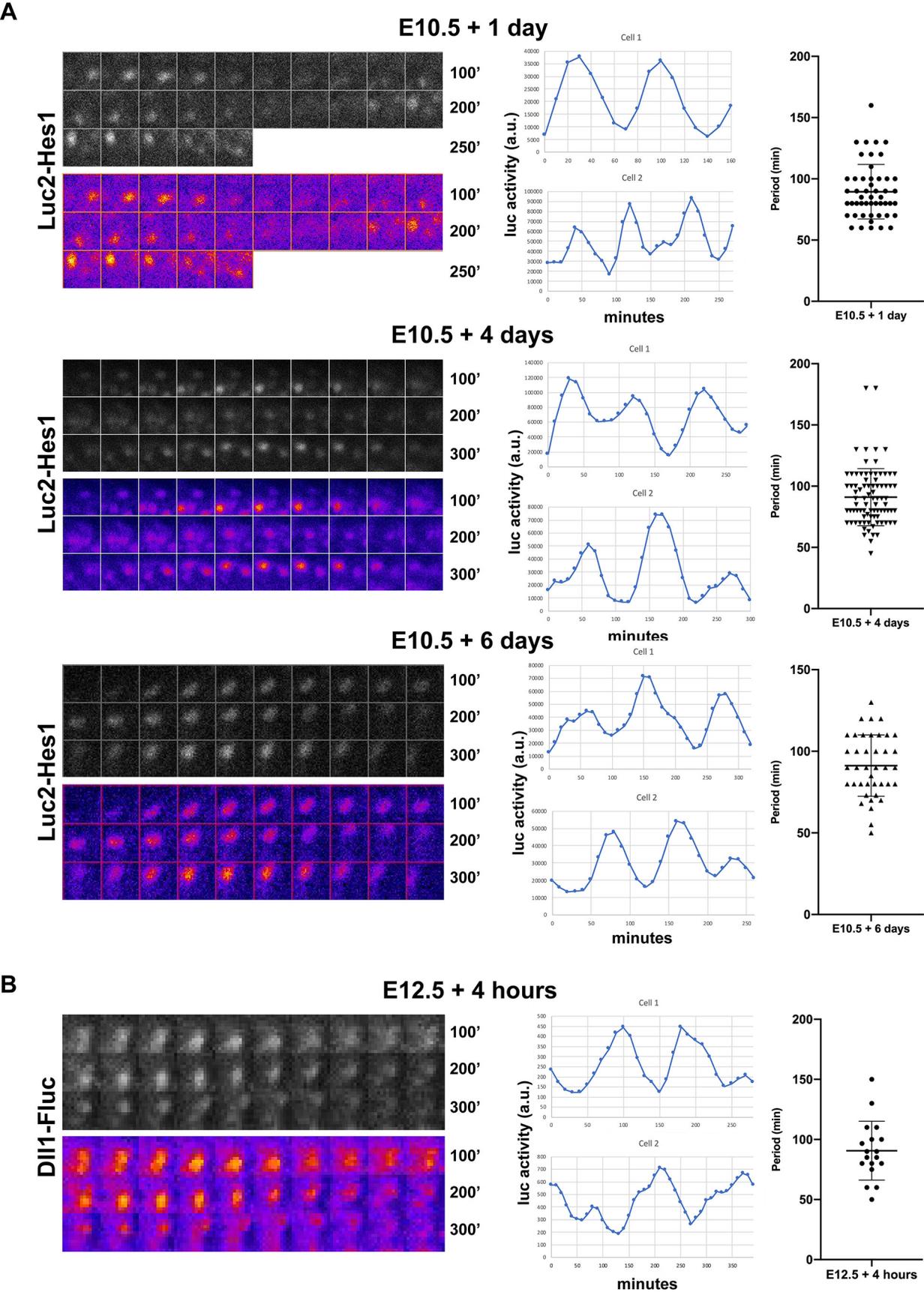
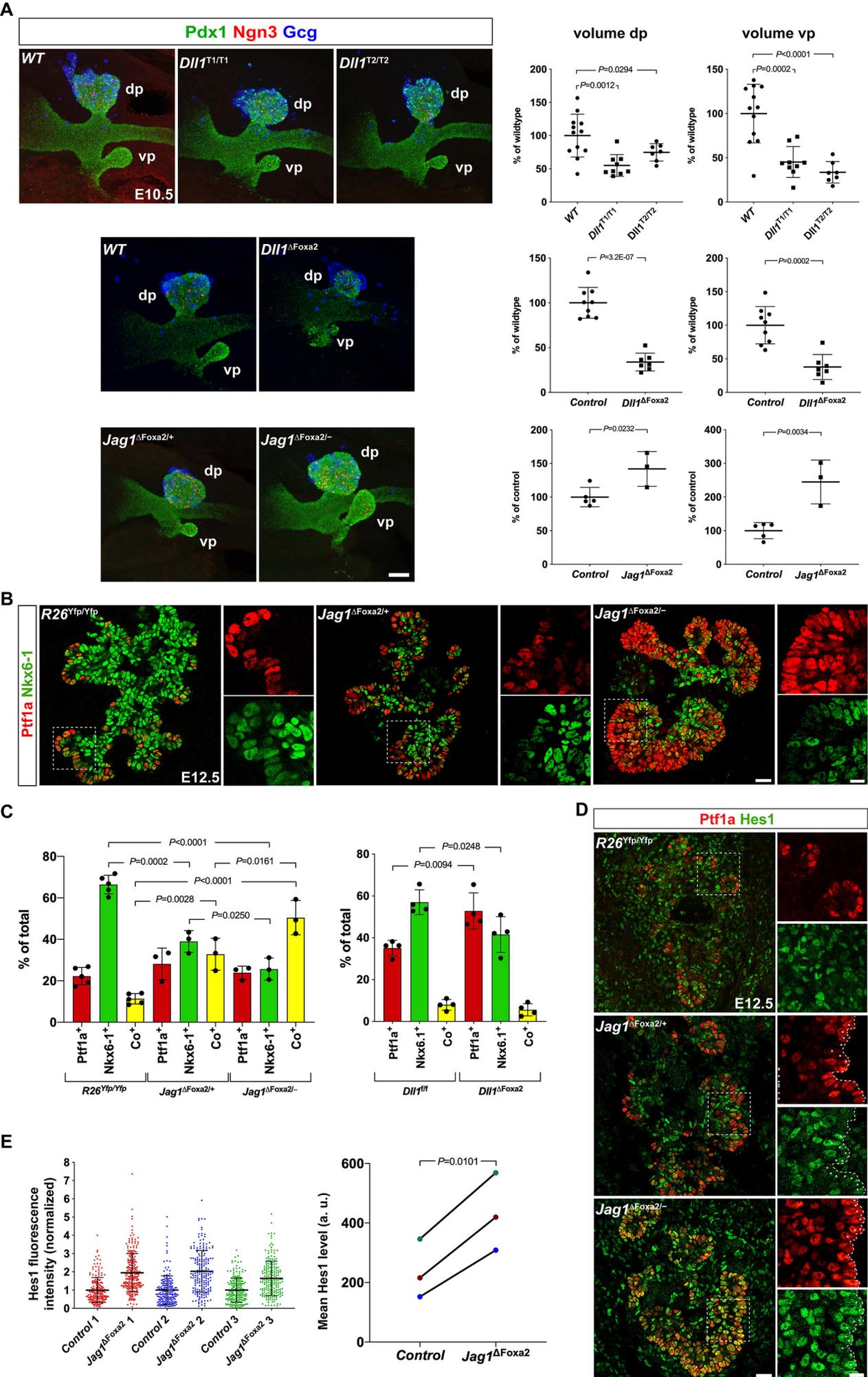
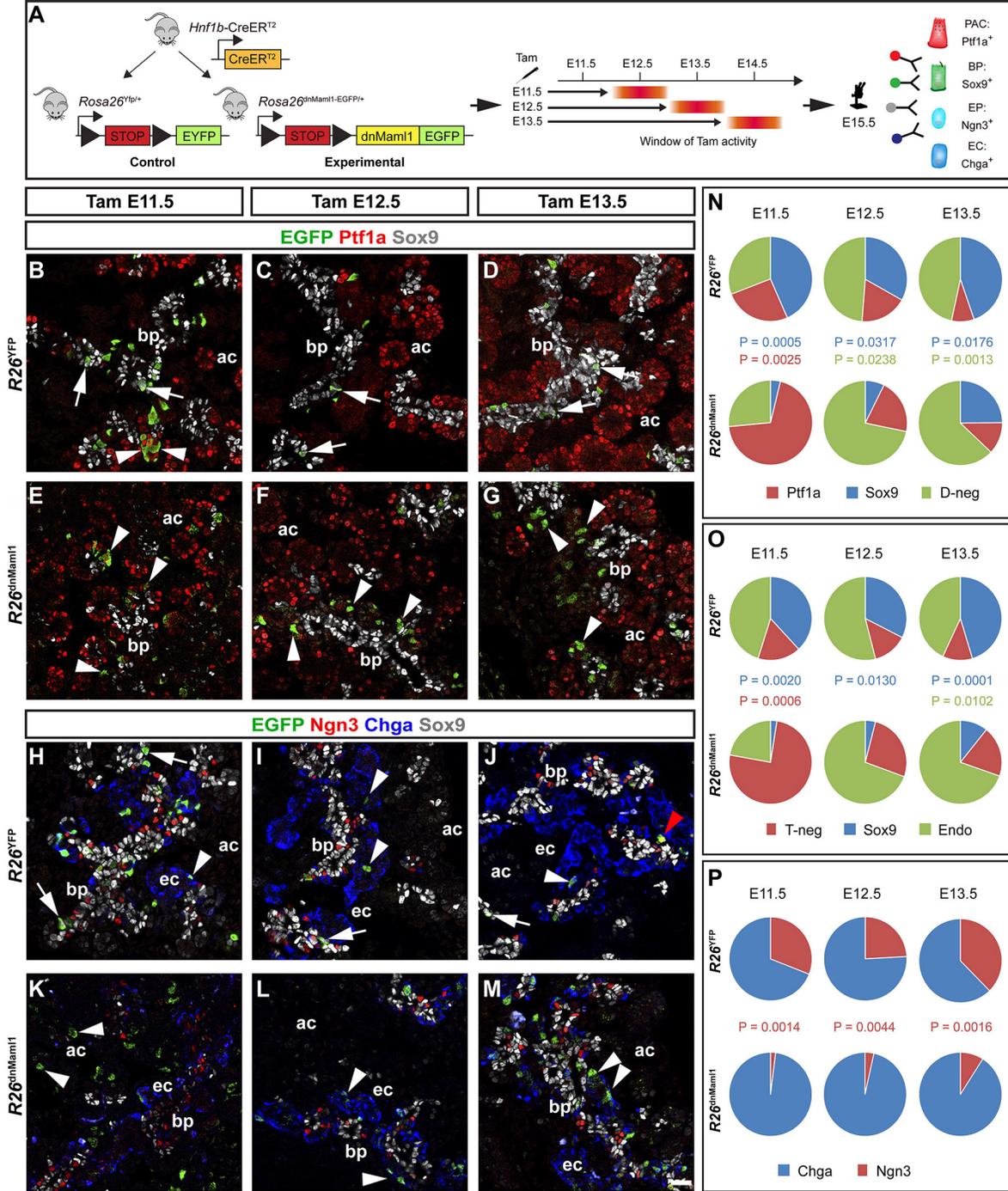


Figure 2





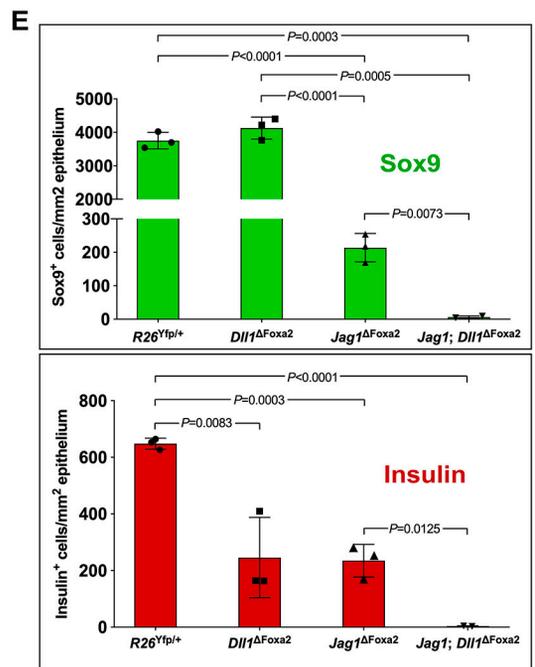
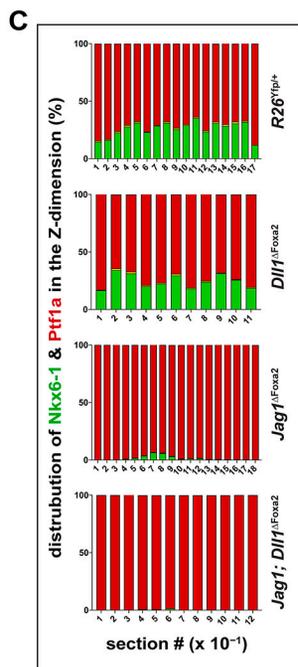
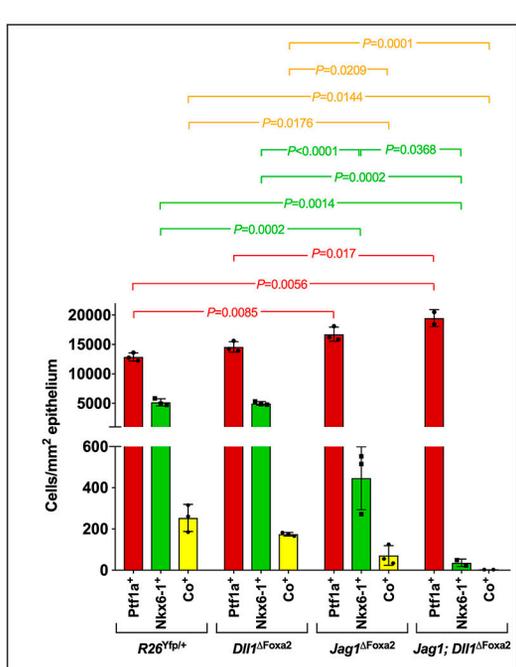
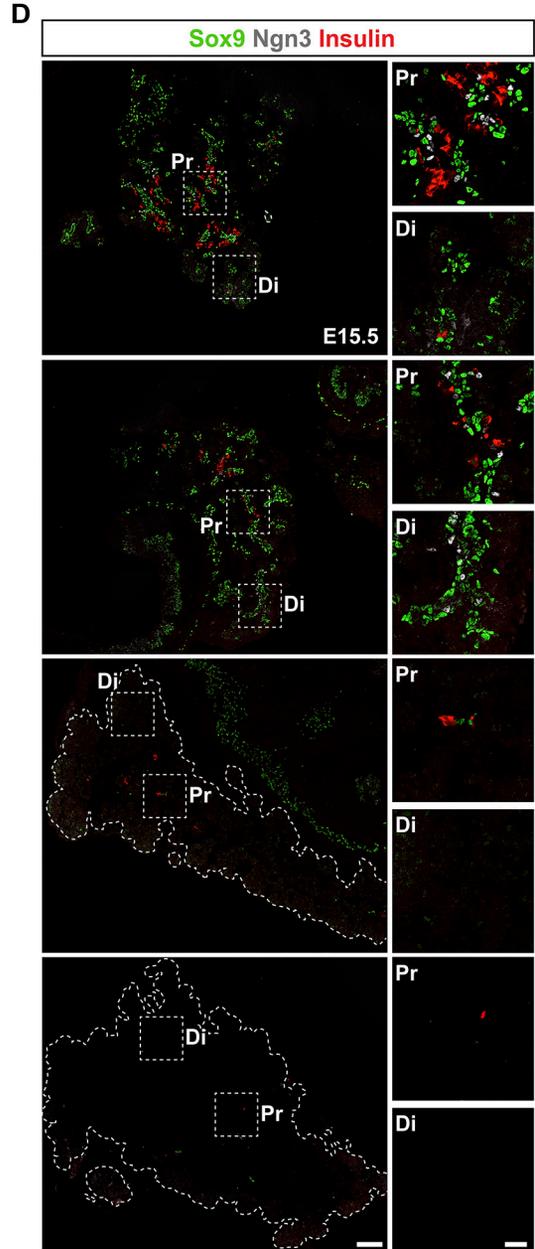
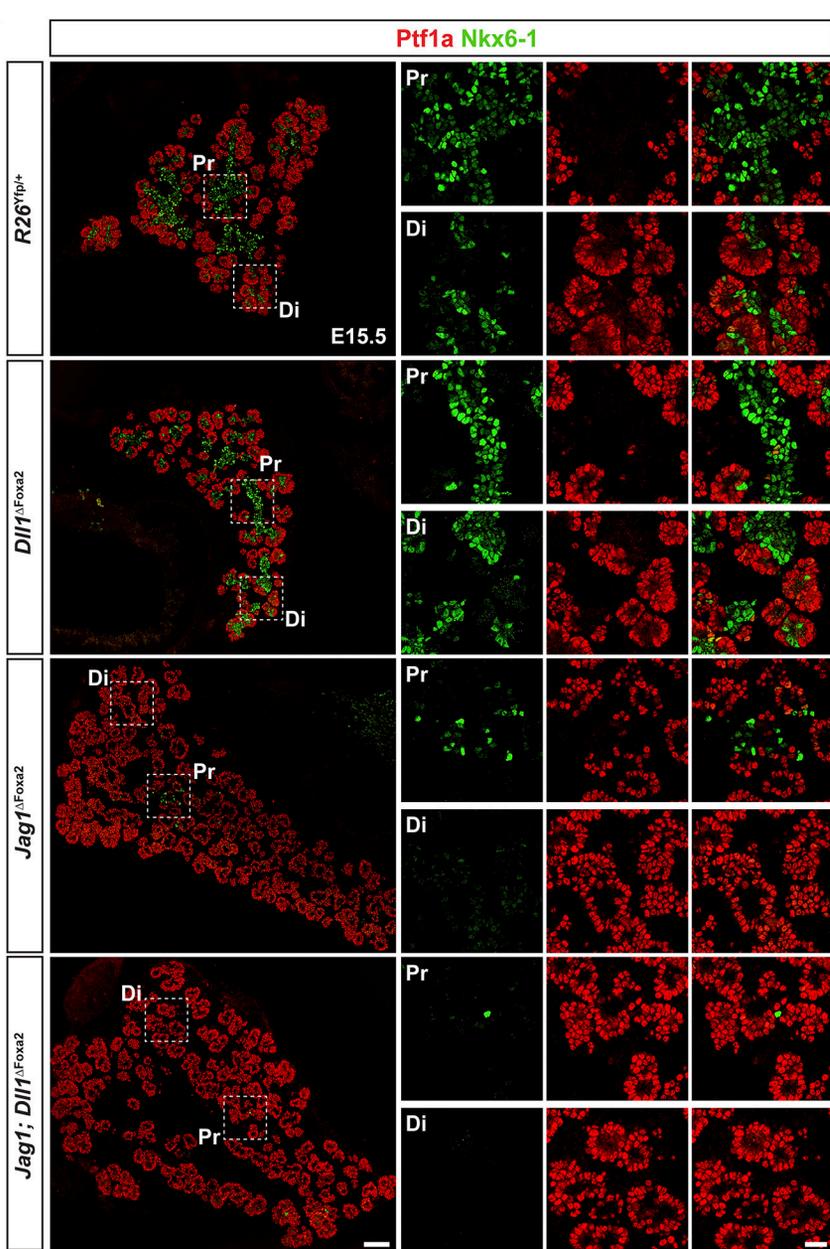


Figure 5

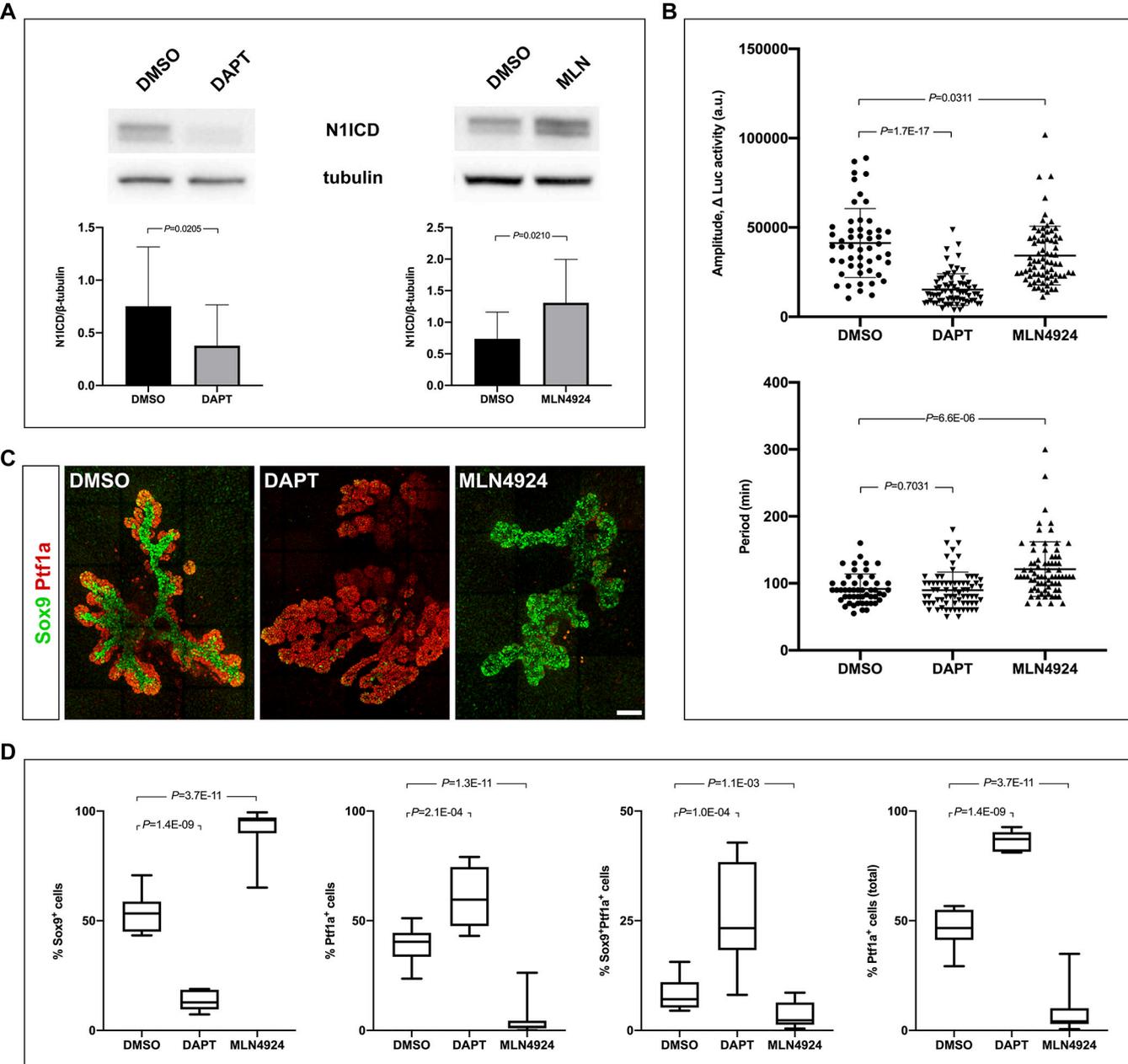


Figure 6

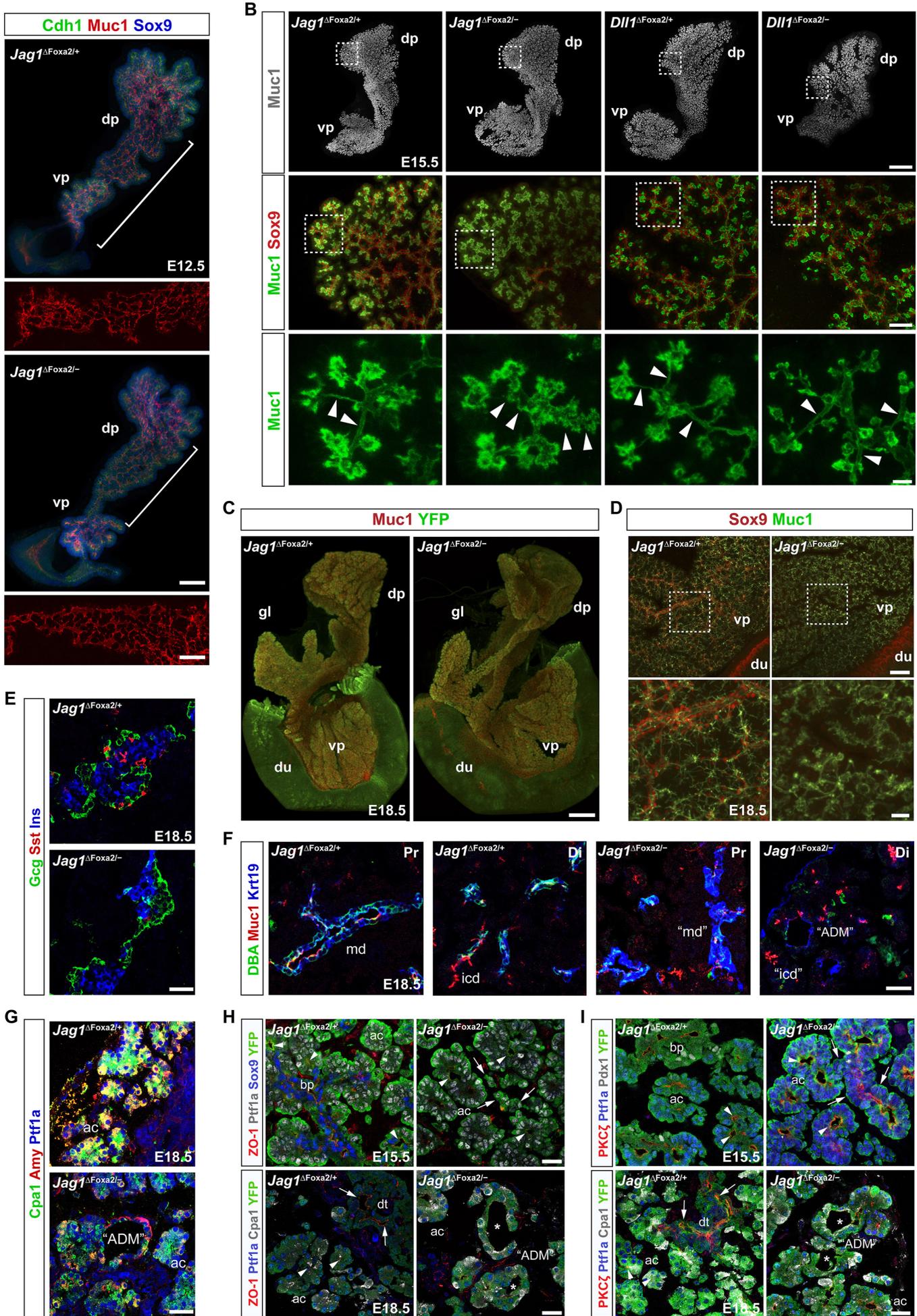


Figure 7