Supplementary Materials and Methods

Luciferase assays

HEK293T cells were seeded at 4 x 10^4 cells/well in DMEM supplemented with 10% Fetal Bovine Serum in 24-well tissue-culture plates (HEK293T cells were used as they do not endogenously express Pdx1). Each well was transfected with a total of 400 ng of plasmid DNA using Fugene6 (Promega) 24 hours after seeding. Cells were lysed in 200 µl /well of Passive Lysis Buffer (Promega) 24 hours after transfection and subjected to a single freeze–thaw cycle at -80°C (Anderson et al., 2009). Luciferase activity was measured for each well by reacting 20 ul of cell lysate with 50 µl of Luciferase Assay Buffer (Promega) in white 96-well plates, using a FLUOstar OPTIMA microplate reader (BMG Labtech). Individual wells were normalized to Beta-Galactosidase activity using the FluoReporter lacZ/Galactosidase Quantitation Kit (Life Technologies). Samples were performed in triplicate per experiment and each experiment was repeated for a total of 5 times.

Semiquantitative PCR

Individual pancreatic buds were isolated from E12.5 embryos. RNA from each bud was obtained using the Qiagen RNeasy Mini kit, following their protocol including incubation with DNase. After eluting the RNA from the column in 30 µl of water, 15 µl was used for cDNA synthesis with Promega iScript cDNA synthesis kit. One bud from each litter was retained as a no-reverse transcriptase control. After cDNA synthesis, samples were further diluted with water to 100 µl total volume. Semiquantitative PCRs were run using 1 µl of cDNA, 0.5 µL of each primer previously diluted to 20nM (sequences of primers used for each gene are listed in Supplementary table 3), and corresponding quantities of MMTaq and water per reaction to a total volume of 14 µls. Water blanks was prepared for each reaction using 1 µl of water instead of cDNA. Individual reactions were stopped at 30, 33 and 36 cycles. Water blanks were allowed to proceed to the 36^{th} cycle before stopping. 7 µl of each reaction was loaded onto a 2% agarose gel in TAE with ethidium bromide and visualized. Data for RT-PCR were collected from 3 individual pancreatic buds per genotype, in triplicate (6-12 repeats for each gene analyzed).

Real-time quantitative PCRs

Total RNA (250 ng) from mouse E11.5 pancreata was isolated using RNeasy Micro Kit (Qiagen) cDNA was using SuperScript II (Invitrogen). 1 µl cDNA in Power SybrGreen Master Mix (Applied Biosystems) was used for real-time quantitative reverse transcriptase polymerase chain reaction (qPCR) analysis (CFX96, Bio-Rad) of gene expression using primers described in supplementary table S3. Gene expression levels were determined by PCR reactions (95 °C, 30 s; 60 °C, 30 s; 72 °C, 30 s; 35 cycles); fluorescence was measured at 72 °C. Gene expression levels were calibrated based on the threshold cycle [C(t)] calibrated to a standard curve generated for each assay using a five-step 1:5 dilution curve of wildtype mouse E11.5 pancreas and lung cDNA. Gene expression levels were normalized to *Cyclophilin (Das et al., 2013).* Data were collected from 5 individual pancreatic buds of each genotype (n=5), all reactions were carried out in triplicates, (6-12 repeats for each gene analyzed).

Transfection for immunofluorescent staining

HEK293T cells were seeded at 1.6 x 10^5 cells/well in DMEM supplemented with 10% Fetal Bovine Serum onto coverslips coated with fibronectin in 6-well tissue-culture plates (HEK293T cells were used as they do not endogenously express Pdx1). Each well was transfected with a total of 1600 ng of plasmid DNA (either CS2GFP or CS2GFP-Pdx1 as described previously) using Fugene6 (Promega) 24 hours after seeding. After 24 hours, media was removed and coverslips were fixed in 4%PFA/PBS and then washed in 0.1% NP40/PBS (PBSN). The coverslips were blocked in 5% NDS for 30 minutes, followed by an hour long incubation in a mixture of primary antibodies (mouse anti-E-cadherin used at 1:100 and Chicken anti-GFP diluted to 1:500, further details for antibodies can be found on supplementary table 1). The cells were rinsed briefly several times in PBSN and then incubated in secondary antibodies (Invitrogen Alexa series Donkey anti-mouse-555 and Goat anti-chicken 488) for one hour. Coverslips were washed in PBSN and then mounted onto slides using Prolong Gold anti-fade media. Slides were visualized on a Zeiss LSM 710 microscope. E-cadherin levels were quantified for 150 of each type of transfected cells using the mean gray value function in ImageJ.

References

Anderson, D. M., Beres, B. J., Wilson-Rawls, J. and Rawls, A. (2009). The homeobox gene Mohawk represses transcription by recruiting the sin3A/HDAC co-repressor complex. *Developmental dynamics : an official publication of the American Association of Anatomists* **238**, 572-580.

Das, A., Tanigawa, S., Karner, C. M., Xin, M., Lum, L., Chen, C., Olson, E. N., Perantoni, A. O. and Carroll, T. J. (2013). Stromal-epithelial crosstalk regulates kidney progenitor cell differentiation. *Nature cell biology* **15**, 1035-1044.

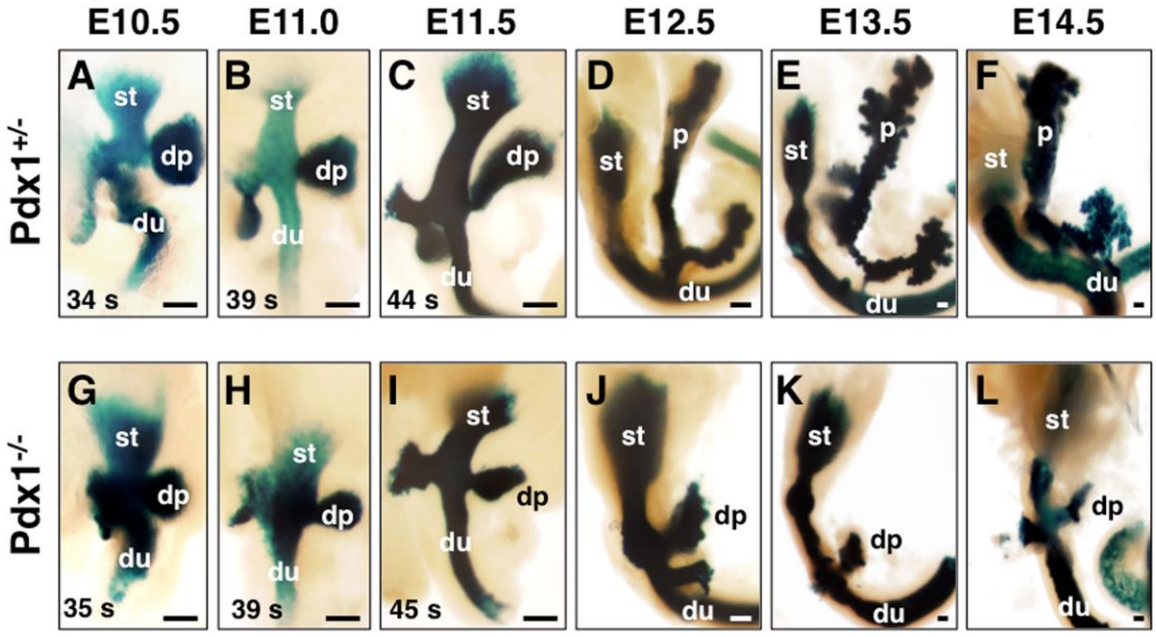


Fig. S1. The Pdx1$^{-/-}$ epithelium does not expand or branch after E10.5. Whole-mount beta-gal stains of Pdx1$^{LacZ/+}$ (Pdx1$^{+/-}$) and Pdx1$^{LacZ/LacZ}$ (Pdx1$^{-/-}$) show gross pancreatic morphology. Note Pdx1$^{-/-}$ epithelium does not expand or branch after E10.5, a time when the bud epithelium is most highly stratified. Dp, dorsal pancreatic bud; p, pancreas; st, stomach; du, duodenum. Numbers followed by (s) in E10.5, E11.0 and E11.5 stages indicate the number of somites in the embryo dissected. Anterior-posterior axis is oriented with anterior portion of gut located towards top of image. Scale bar, 100 μm.

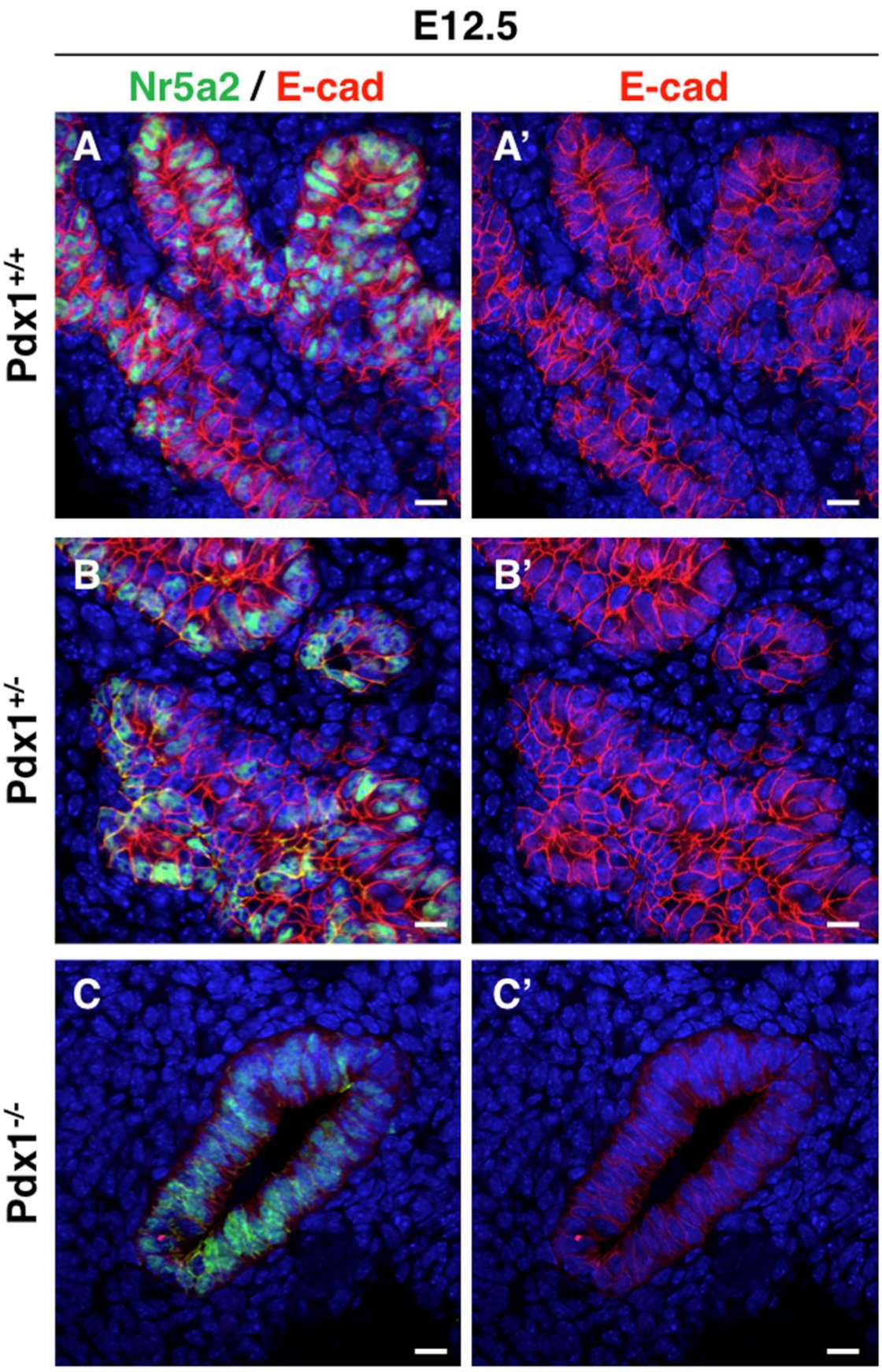


Fig. S2. Pdx1$^{-/-}$ epithelium fails to undergo morphogenesis and expand, while Pdx1$^{+/-}$ and Pdx1$^{+/+}$epithelium are unaffected. A-C) Immunofluorescent staining of E12.5 for the epithelial adhesion molecule E-cad (red) and the nuclear hormone receptor family member Nr5a2 show that the Pdx1$^{+/-}$ (B) continues to grow and expand in the same manner as the Pdx1$^{+/+}$ (A), making them equivalent for our analysis. However, the Pdx1$^{-/-}$ (C) bud is already abrogated in size and branching capability at this stage. A'-C') E-cad shown without Nr5a2 staining to better illustrate complexity of the Pdx1$^{+/+}$ (A') and Pdx1$^{+/-}$ (B') epithelia as they have continued to expand and differentiate, in contrast to the failure of Pdx1$^{-/-}$ bud growth. Scale bars, 25 μm.

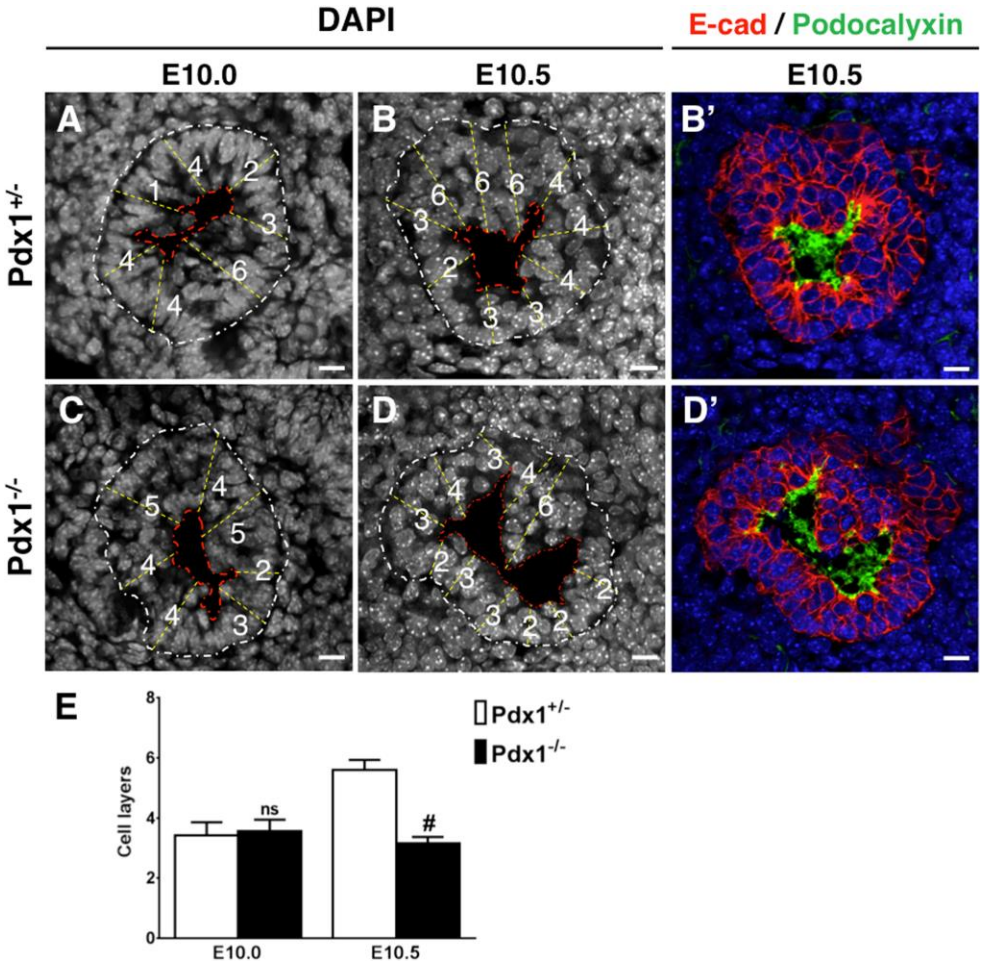


Fig. S3. Methodology for quantifying cell layers in pancreatic bud stratified epithelium. A, C) The $Pdx1^{+/-}$ and $Pdx1^{-/-}$ pancreata display similar levels of stratification at E10.0. B, D) While the $Pdx1^{-/-}$ bud is stratified at E10.5, it shows fewer layers overall in the epithelium than that of a $Pdx1^{+/-}$ littermate. Outer white line indicates the basal surface of the pancreatic bud, red dotted line outlines the primary central lumen and yellow dotted lines indicated perpendicular trajectories analyzed. Numbers indicate how many nuclei were encountered in a straight line extending from the apical central lumen surface to the basal outer surface of the bud (yellow dotted lines). B', D') Representative staining with E-cad (in red) and the sialomucin Podocalyxin (in green) were used to label the epithelium and primary central lumen respectively, in order to confirm number of cell layers through the buds in section stained with DAPI (in blue). Scale bars in all panels, 20 μm. E) Quantification of average cell layers at E10.0 and E10.5. ns = not statistically significant, #= $p<0.0001$.

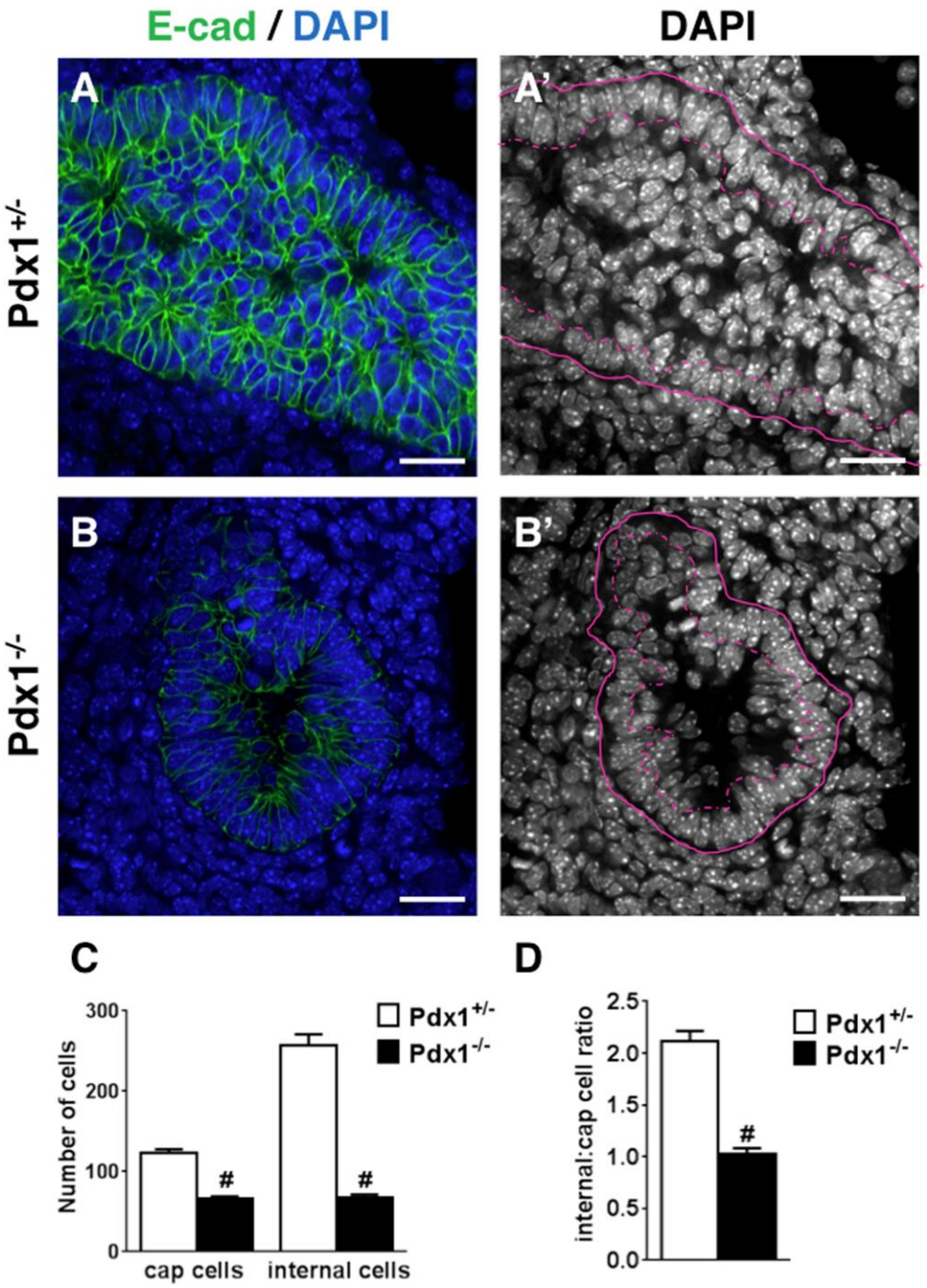


Fig. S4. The E11.5 Pdx1$^{-/-}$ bud displays fewer internal cells than its Pdx1$^{+/-}$ littermate. A-B) 10 μm sections through E11.5 pancreatic buds stained for E-cad and DAPI demonstrate both smaller size and reduced internal cell number in the Pdx1$^{-/-}$ bud. A'-B') DAPI staining shown in grayscale to more clearly show nuclei. Solid pink line denotes border of pancreas next to surrounding mesenchyme, dotted yellow line separates cap cells from internal (body) cells. A-B' Scale bars, 20 μm C) Comparison of the number of cap cells and internal cells in the Pdx1$^{+/-}$ and Pdx1$^{-/-}$. D) Comparison of the ratio of internal cells to cap cells at E11.5. #= $p<0.0001$ (Student t-test).

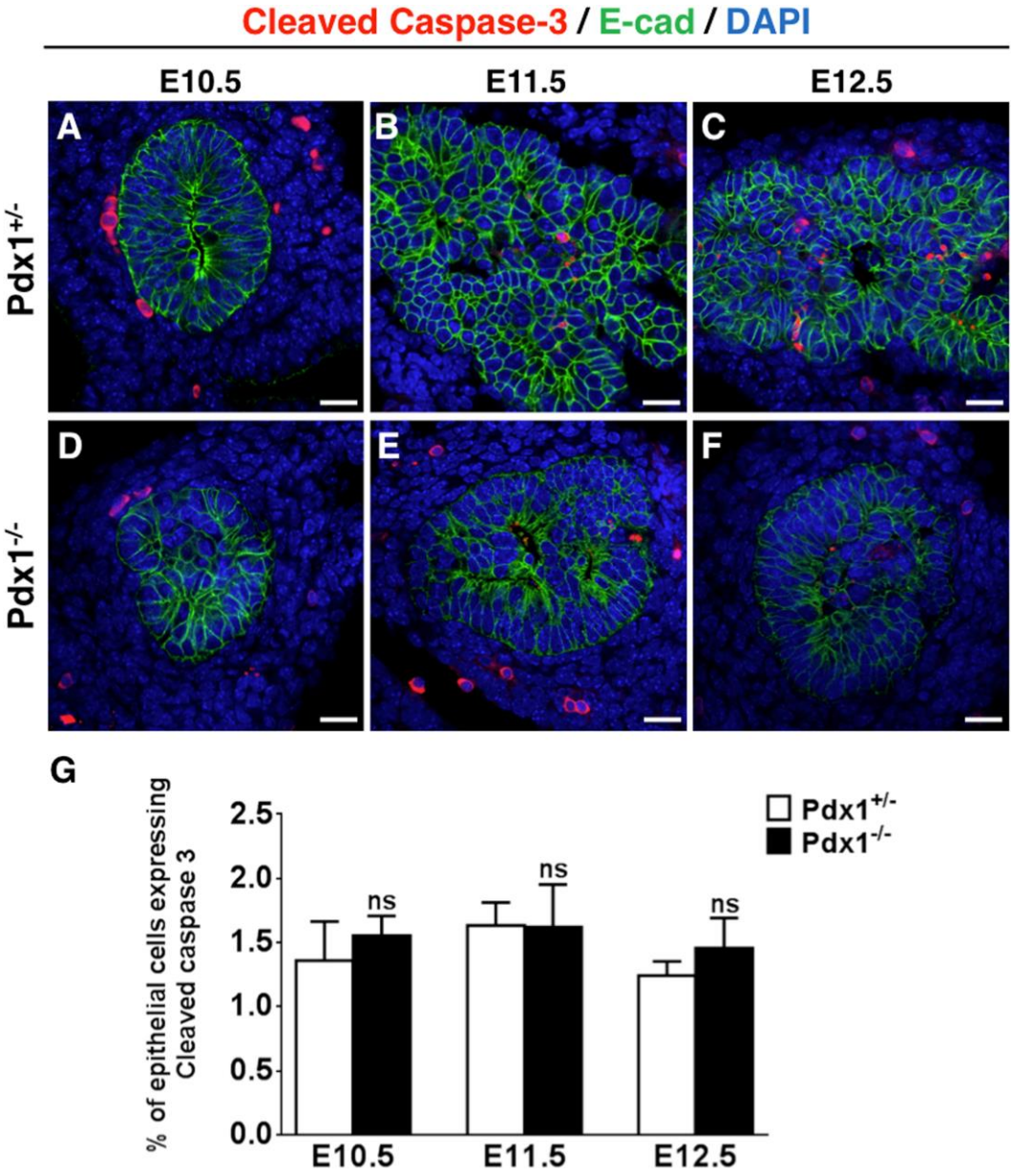


Fig. S5. The Pdx1$^{-/-}$ pancreatic bud does not exhibit increased cell death. A-F) Cell death was assayed using cleaved Caspase3 (in red), E-cad to delineate the epithelium (in green) and DAPI (in blue) staining. Scale bars = 10 μm. G) Quantification at E10.5-12.5 shows that there is not a significant increase in cell death. ns = not statistically significant (Student t-test).

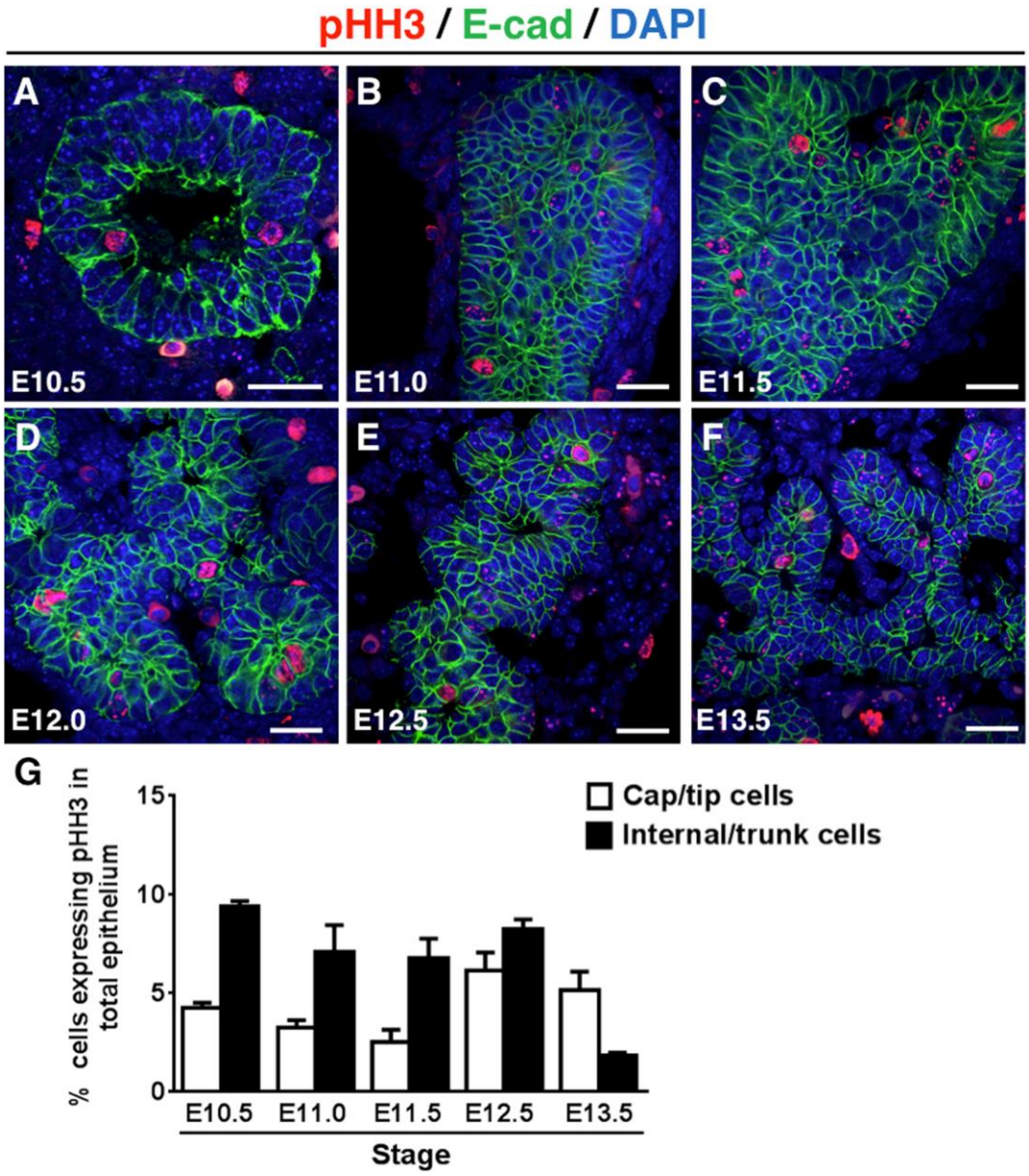


Fig. S6. Increased proliferation in internal cells compared to cap/tip cells of the WT developing pancreas until E13.5. A-F) Wild type pancreas sections were stained with pHH3 (in red) and DAPI (in blue). White outline denotes outer boundary of pancreatic epithelium. Scale bars, 20 μm. G) Quantification of the percentage of $pHH3^{+}$ epithelial cells in either the cap/tip or internal/trunk domains per pancreatic section at different developmental time points. Prior to E13.5, proliferation of internal cells occurs at higher frequency than peripheral (cap/tip).

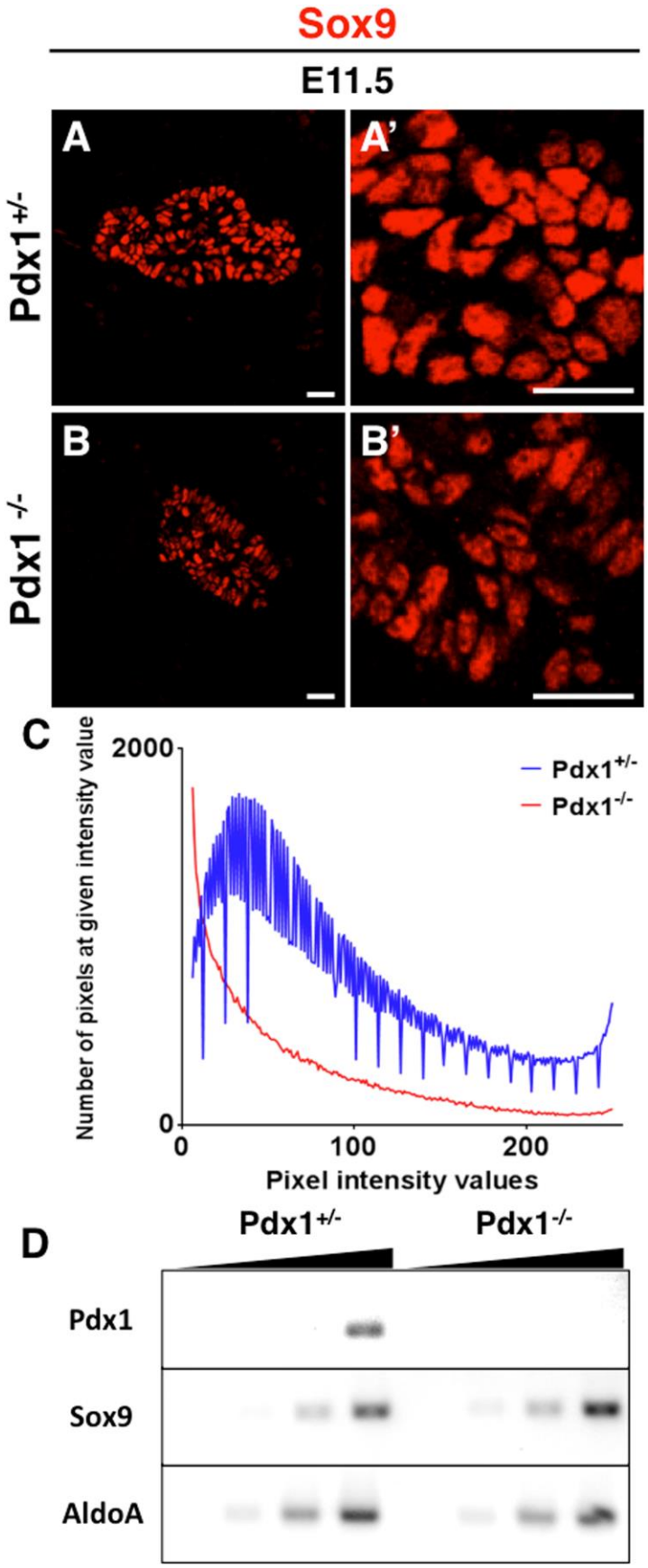


Fig. S7. Sox9 is expressed at lower levels in the Pdx1$^{-/-}$ pancreatic epithelium at E11.5. A-B') Staining for the bipotential (ductal and endocrine) progenitor marker Sox9 (expressed at high levels, red) indicates that this cell population is reduced in the Pdx1$^{-/-}$ pancreas. Sox9 staining (in red) in the Pdx1$^{+/-}$ and Pdx1$^{-/-}$ epithelium shows that fewer cells express high levels of Sox9 (Sox9^{hi}) associated with bipotential progenitors. (A'-B') High magnification images. C) Quantification of pixel intensity distribution derived from Sox9 stained images from 5 sections through each of 3 buds per genotype (Pdx1$^{+/-}$ and Pdx1$^{-/-}$). Measurements show that Pdx1$^{-/-}$ epithelium expressed lower levels of Sox9 (error bars omitted to allow for graph clarity, as have pixel intensity values from 0-10 to eliminate any background signal and those from 245-255 to account for saturation). D) Expression of the progenitor marker gene Sox9 is not significantly decreased at the mRNA level, as per semi-quantitative PCR.

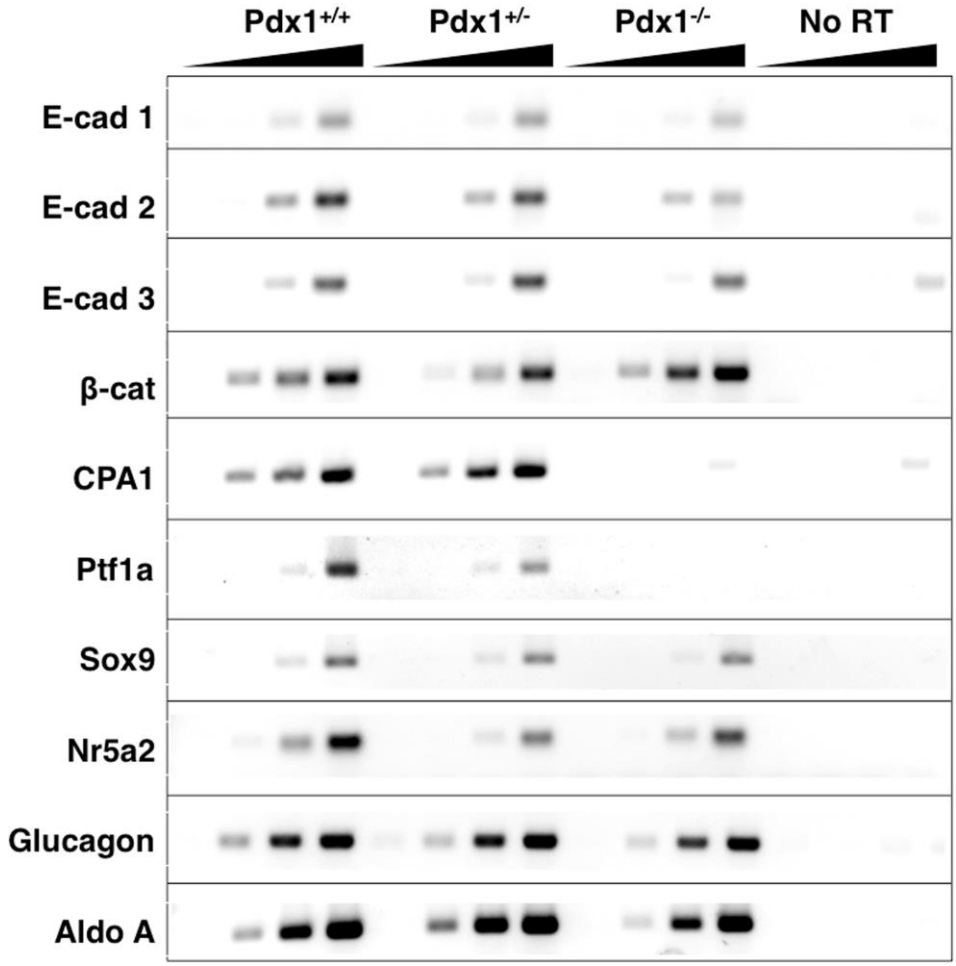


Fig. S8. Semi-quantitative RT-PCRs on E12.5 buds confirm decrease in E-cadherin and MPC markers CPA1 and Ptf1a. cDNA isolated from individual buds was used for semi-quantitative PCRs. 3 different primer sets were used to test for E-cad, and transcript levels were observed to be decreased in the $Pdx1^{-/-}$ for all 3 primer sets used. β-cat transcript levels are unaffected however, indicating that Pdx1 does not affect transcription of this gene. MPC genes CPA1 and Ptf1a are almost completely ablated in the Pdx1 null bud, but Sox9 and Nr5a2 transcripts are at similar levels as those seen in the WT and het buds. Glucagon and Aldolase A were used as loading controls, as glucagon arising from the primary transition endocrine cells had previously been reported to be unaffected. cDNA analyzed was collected from 3 individual pancreatic buds per genotype, reactions were carried out in triplicate.

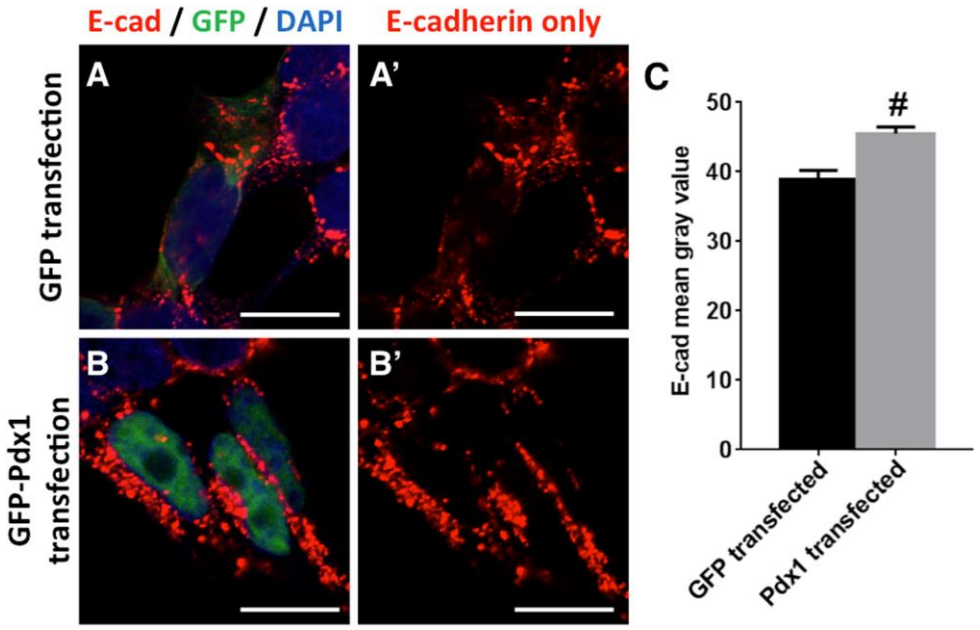


Fig. S9. Addition of Pdx1 *in vitro* causes an increase of E-cadherin at cellular junctions. A-B) Cells transfected with either control GFP constructs (CS2GFP) or CS2GFP-Pdx1 were stained for E-cad (in red), GFP (in green) and DAPI (in blue). A'-B') E-cad staining shows an increase of E-cad levels in the GFP-Pdx1 transfected cells (B') compared to the GFP transfected cells (A). C) E-cad intensity was increased in GFP-Pdx1 transfected cells, as assessed through the mean gray value function on ImageJ. 150 individual transfected cells were analyzed, from 3 separate transfections and staining assays, with 3 replicate wells each. #= $p<0.0001$.

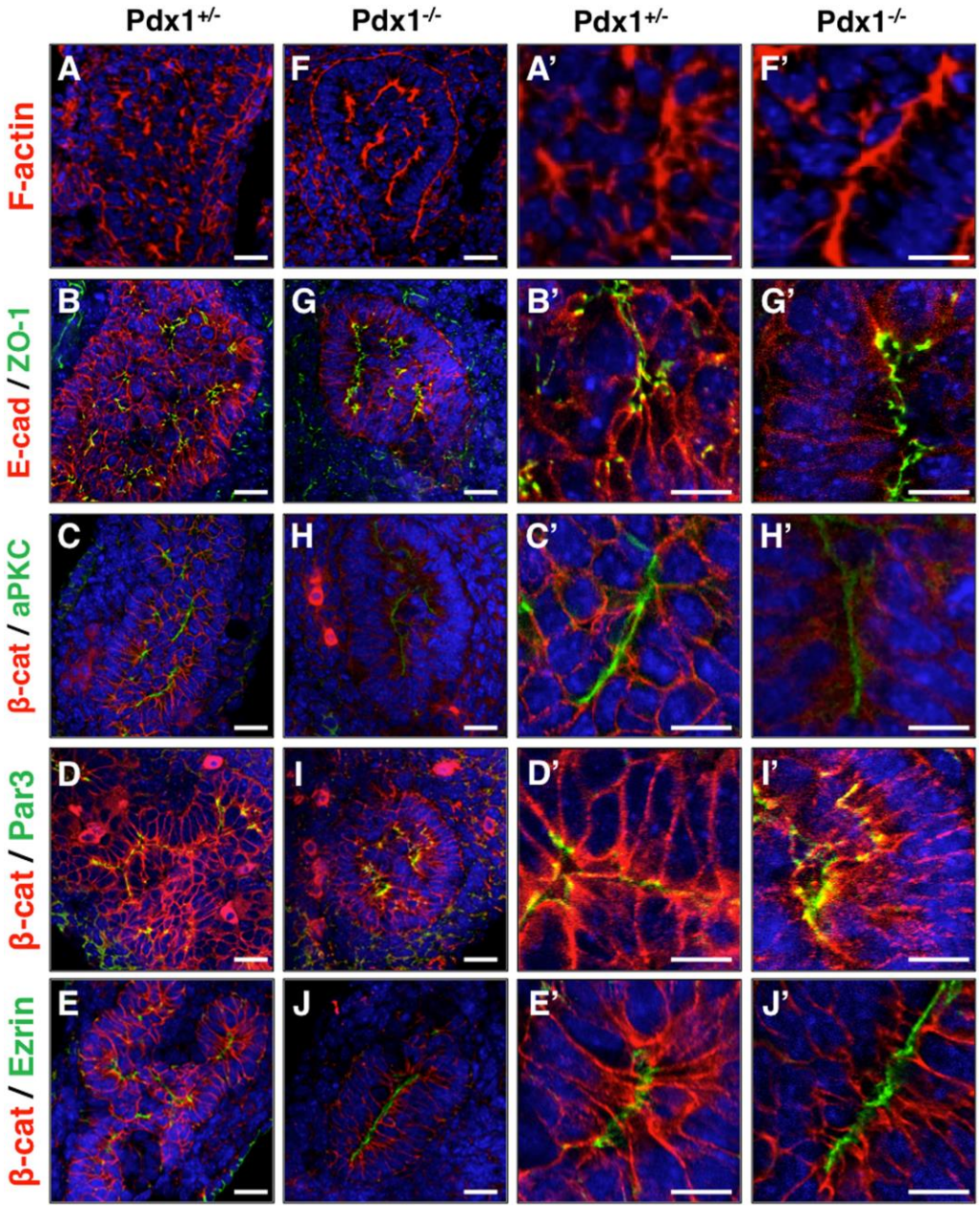


Fig. S10. Apical polarity determinants are correctly organized in Pdx1$^{-/-}$ buds at E11.5. While apical polarity molecules (all in green, except for F-actin) are located towards the apical surface in the Pdx1$^{-/-}$ bud, the organization of the epithelium exhibits disruptions compared to Pdx1$^{+/-}$. Apical markers localize primarily to regions adjacent to the primary central lumen. DAPI (in blue) was used to stain nuclei. Scale bars in A-J, 20 μm, panels A'-J', 10 μm.

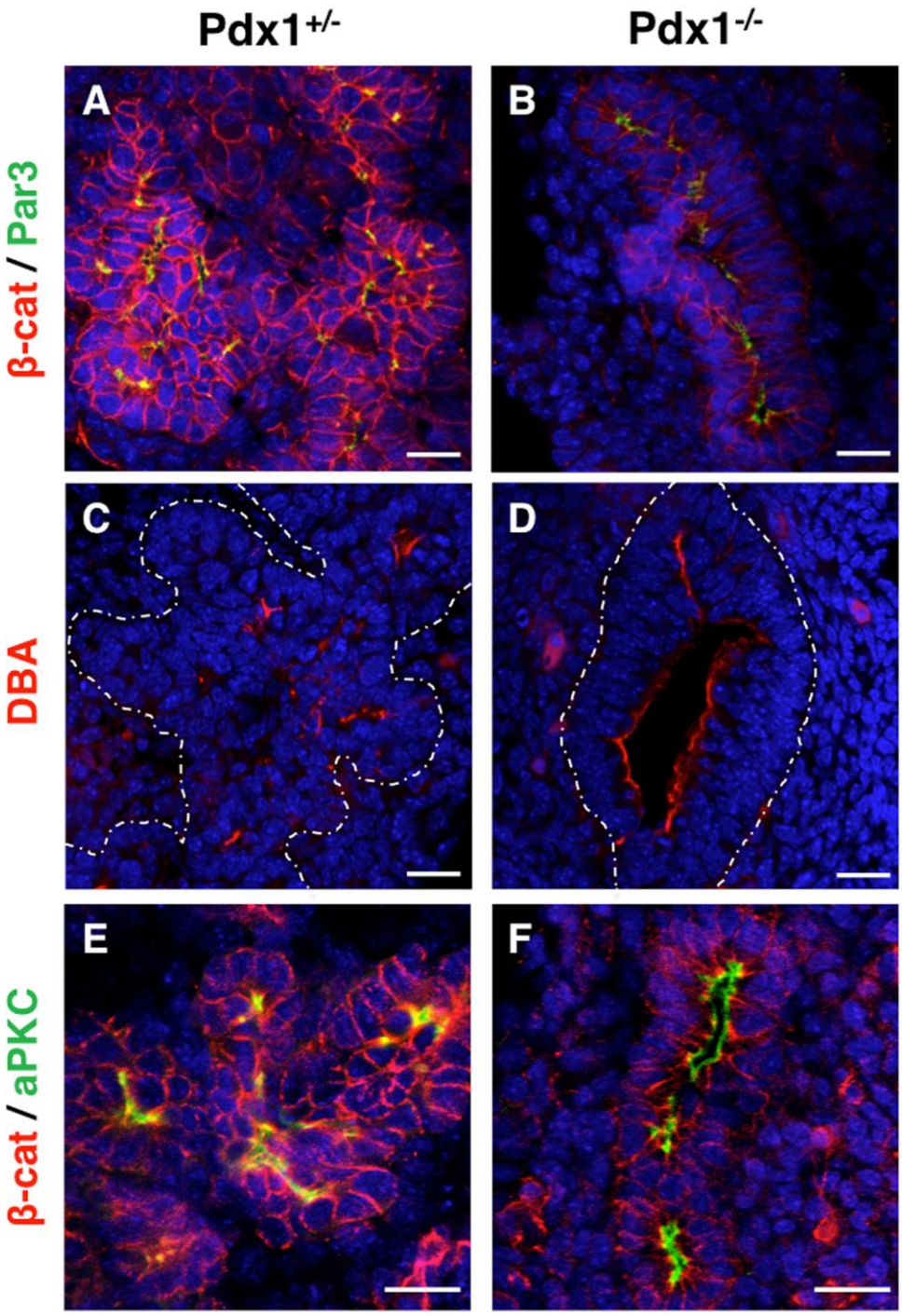


Fig. S11. Apical polarity determinants remain unaffected in Pdx1$^{-/-}$ buds at E12.5. Apical polarity cues Par3 (green, A-B), DBA (red, C-D) and aPKC (green, E-F) were assessed in Pdx1$^{+/-}$ and Pdx1$^{-/-}$ pancreatic buds. We find that apical markers are properly oriented towards the luminal surface in the Pdx1$^{-/-}$ epithelium, in the same pattern observed in the Pdx1$^{+/-}$ bud, at this same stage. Beta-catenin was used as a counterstain in A-B and E-F (in red). DAPI (in blue) was used to stain nuclei. Scale bars, 20 μm.

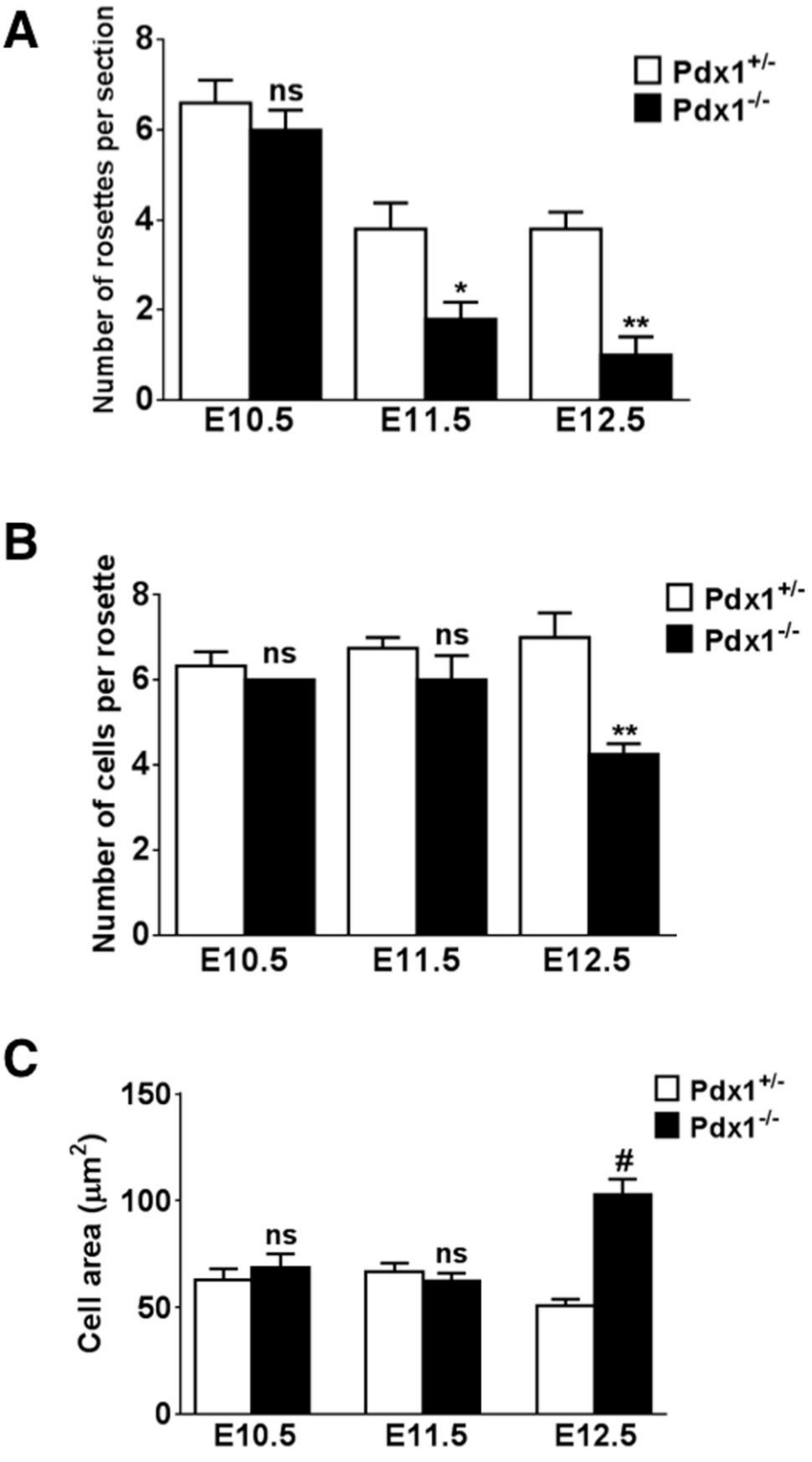
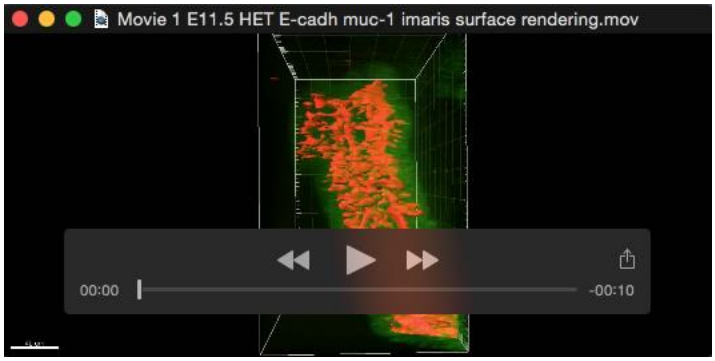
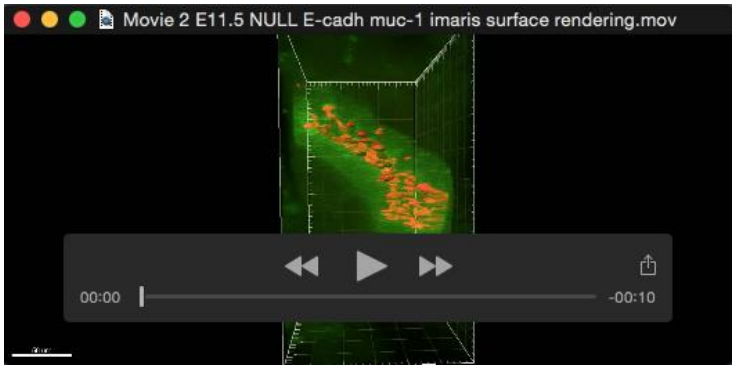


Fig. S12. Rosette number and morphology is altered in the Pdx1$^{-/-}$ pancreatic bud. A) Average number of rosettes was assessed across 45 individual 10μm sections per genotype at E10.5, E11.5 and E12.5. We found that while there is an overall decrease in rosette number over time during development in the Pdx1$^{+/-}$, rosette number is decreased in the Pdx1$^{-/-}$ by E11.5 and even more dramatically by E12.5. B) Number of cells per rosette is reduced in the Pdx1 null bud by E12.5. C) Cell size (assessed by measuring the area of cells in a rosette on 10μm sections) is increased in the Pdx1$^{-/-}$ by E12.5. ns = not statistically significant, * = $p<0.05$, ** = $p<0.01$, # = $p<0.001$.



Movie 1. Microlumens begin to connect and form a ductal plexus in the Pdx1$^{+/-}$ pancreas bud by E11.5. Imaris surface reconstruction of lumens stained with Muc1 (in red) show that the E11.5 Pdx1$^{+/-}$ has thin lumens that are starting to interconnect and form the net-like ductal plexus. E-cad (in green) was used as a counterstain to mark the entire pancreatic epithelium.



Movie 2. Lumen size is altered in the Pdx1$^{-/-}$ epithelium by E11.5. Imaris surface reconstruction of the Pdx1$^{-/-}$ lumens, stained with Muc1 (in red), at the same stage shows an altered lumen morphology, where lumen size has expanded and overall plexus morphology has simplified. E-cad (in green) was used as a counterstain to mark the entire pancreatic epithelium.

Table S1. Antibodies used in this paper

Antibody	Host animal	Dilution	Source company / Cat No.
aPKC	Rabbit	1:100	Santa Cruz / sc-216
Alpha-catenin	Rabbit	1:1000	Sigma / C8114
Beta-catenin	Goat	1:100	Santa Cruz / sc-1496
Caspase3 (cleaved)	Rabbit	1:400	Cell signaling technologies / 9661
C-Myc	Rabbit	1:50 with TSA	Cell signaling technologies/ 9402
CPA	Goat	1:300	R&D systems / AF2765
DBA (biotinylated)	-	1:1000	Vector Labs / B-1035
E-cadherin	Mouse	1:100	BD Transduction / 610182
Ezrin	Rabbit	1:100	Millipore / 07-130
Fibronectin	Mouse	1:100	Santa Cruz / SC-71113
Glucagon	Rabbit	1:2000	Millipore / 4030-01F
GM-130	Mouse	1:100	BD Biosciences / 610822
Phospho-histone H3	Rabbit	1:200	Millipore / 06-570
Insulin	Rabbit	1:100	Cell signaling technologies / 4590
Laminin	Rabbit	1:200	Sigma / L9393
MafA	Rabbit		Bethyl laboratories / A300-611A
Muc-1	Armenian Hamster	1:200	ThermoScientific / HM-1630
Ngn3	Goat	1:100	Gu Lab
Par3	Rabbit	1:100	Millipore / 07-330
Phalloidin (F-actin)		1:100	Invitrogen / A34055
pMLC	Mouse	1:100 with TSA	Cell signaling technologies / 3675S
Podocalyxin	Goat	1:100	R&D systems / AF1556
Ptf1a	Guinea Pig	1:1000	BCBC (Jane Johnson and Ray MacDonald)
Sox9	Rabbit	1:1000	Millipore / AB5535
Synaptophysin	Rabbit	1:600	DAKO / A0010
Yap	Rabbit	1:100 with TSA	Cell signaling / 4912
ZO-1	Rabbit	1:100	Invitrogen / 40-2200
ZO-1	Mouse	1:100	Invitrogen / 33-9100

Table S2. Primers used for cloning

Primer Name	Sequence
E-cadherin 5'-primer (for both 300 bp and 3 Kb fragments)	5'-taaactgaggaaggtcactactgc-3'
E-cadherin 3'-primer for 3 Kb fragment	5'-cctgtctgtagttggtggca-3'
E-cadherin 3'-primer for 300 bp fragment	5'-ttatatcatggctgggtgcagg-3'
E-cadh Mut-site 1 5' primer	5'-gaagggtgTGCCGAacctgacc-3'
E-cadh Mut-site 1 3' primer	5'-caggtTCGGCAcacccttcag-3'
E-cadh Mut-site 2 5' primer	5'-gaattatcactgtACGGCAgaaggg-3'
E-cadh Mut-site 2 3' primer	5'-cattgacccttcTGCCGTacagtg-3'
β-catenin 5'-primer for 2.3 Kb fragment	5'-gtatggctctgctgggaaag-3'
β-catenin 3'-primer for 2.3 Kb fragment	5'-ccgctccattggaaactaaa-3'
Pdx1 5'-primer to insert into CS2 vector (containing XhoI site)	5'-tatcgctcgagatgaacagtgaggagcagtactacgc-3'
Pdx1 3'-primer to insert into CS2 vector (containing XbaI site)	5'-gatactctagactaccggggttcctgcggtc-3'

*Capital letters in Mut primers indicate mutated Pdx1 consensus sequence

Table S3. Primers used for semi-quantitative PCR

Gene	Primer sequences
Pdx1	For 5'-AAAACCGTCGCATGAAGTG-3' Rev 5'-TAAGGCCCGAAGGCAGTAG-3'
E-cad 1 (Harvard PrimerBank ID 118129809c3)	For 5'-CTCCAGTCATAGGGAGCTGTC-3' Rev 5'-TCTTCTGAGACCTGGGTACAC-3'
E-cad 2	For 5'-GAAGACGCTGAGCATGTGAA-3' Rev 5'-TGGATCCAAGATGGTGATGA-3'
E-cad 3	For 5'-ACCGGAAGTGACTCGAAATG-3' Rev 5'-GTCCTGATCCGACTCAGAGG-3'
β-catenin	For 5'-CTGCACAACCTTTCTCACCA-3' Rev 5'-CAACCATTTTCTGCAGTCCA-3'
Sox9	For 5'-CCACGGAACAGACTCACATC-3' Rev 5'-CCCTCTCGCTTCAGATCAAC-3'
Nr5a2	For 5'-CTGCTGGAGTGAGCTCTTGA-3' Rev 5'-ATACAAACTCCCGCTGATCG-3'
CPA1	For 5'-ACACGGGACCAAGTTCAAGT-3' Rev 5'-GGTCCATGATGGTCAAAAGG-3'
Ptf1a	For 5'-GCACCTCGGAGAGGACAGT-3' Rev 5'-CCTCTGGGGTCCACACTTTA-3'
Glucagon	For 5'-TGAATTTGAGAGGCATGCTG-3' Rev 5'-GAATGGTGCTCATCTCGTCA-3'
Aldolase A	For 5'-CTGAGCGACCACCATGTCTA-3' Rev 5'-TTGATGGATGCCTCTTCCTC-3'
Cyclophilin	For 5'-GGAGATGGCACAGGAGGAA-3' Rev 5'-GCCCGTAGTGCTTCAGCTT-3'