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Supplemental Information

p120ctn-Mediated Organ Patterning Precedes and Determines Pancreatic Progenitor Fate

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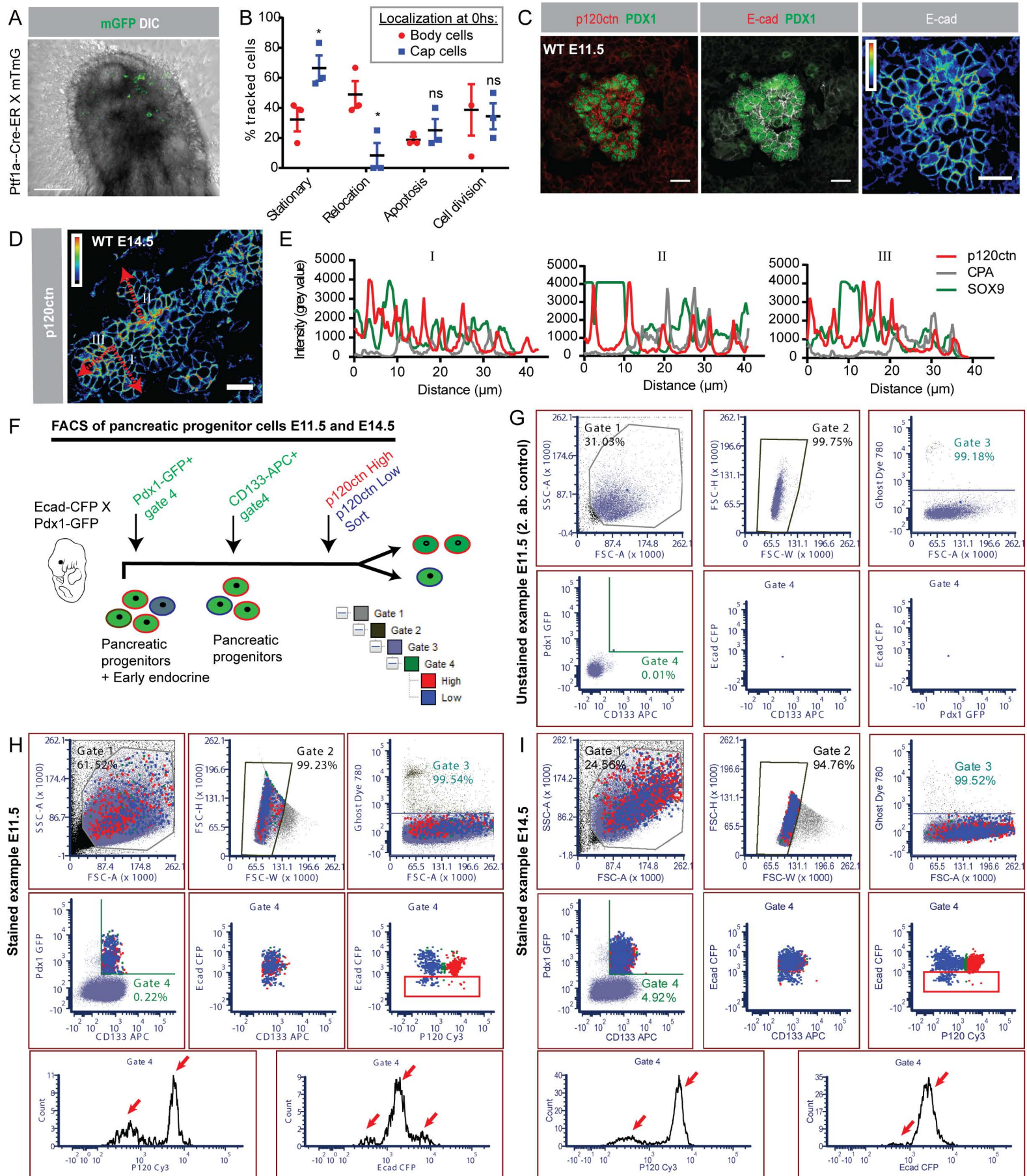
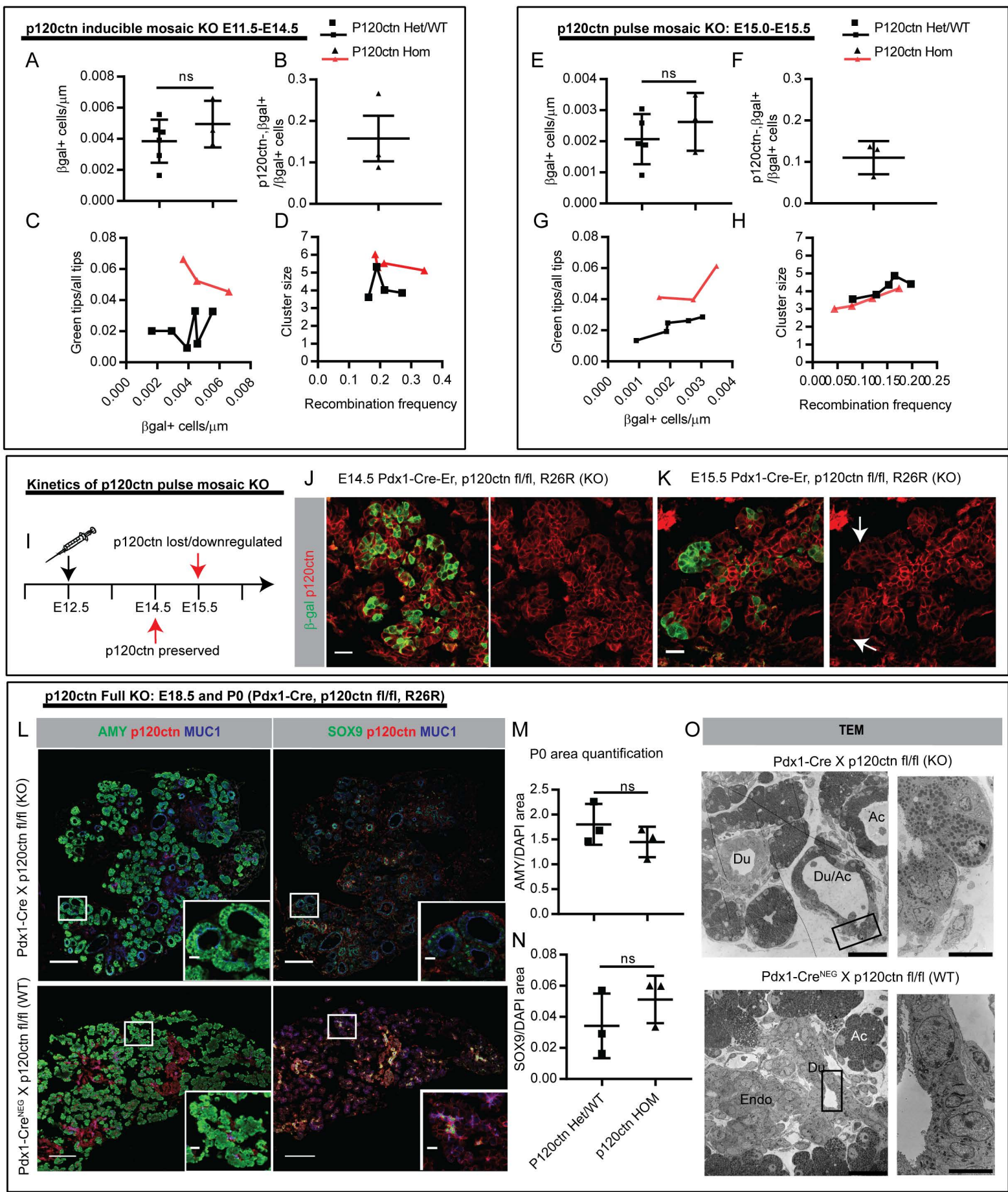


Figure S1. Related to Figure 1 and Video S1. Pancreatic epithelial progenitor migration drives patterning.

A: MIP overlay of green fluorescent channel and DIC image of one Ptf1a-Cre-ER X mTmG explant in culture prior to imaging, showing scattered green cells. **B:** Graph detailing the quantification of Ptf1a-Cre-ER tracking. Data are represented as mean ± SEM. *p<0.05 by t-test. **C:** Confocal images from E11.5 WT pancreatic tissue stained for p120ctn, E-cad and PDX1. Bar=20μm. **D:** Confocal image from E14.5 WT pancreatic tissue stained for p120ctn. Arrows show the location of intensity profile measurements. Bar=20μm. **E:** Intensity profile measurements. **F:** FACS strategy. **G:** Example of E11.5 unstained control FACS analysis. **H:** Example of E11.5 stained sample FACS analysis. **I:** Example of E14.5 stained sample FACS analysis. Red box: E-cad low cells. Red arrows: Intensity peaks.



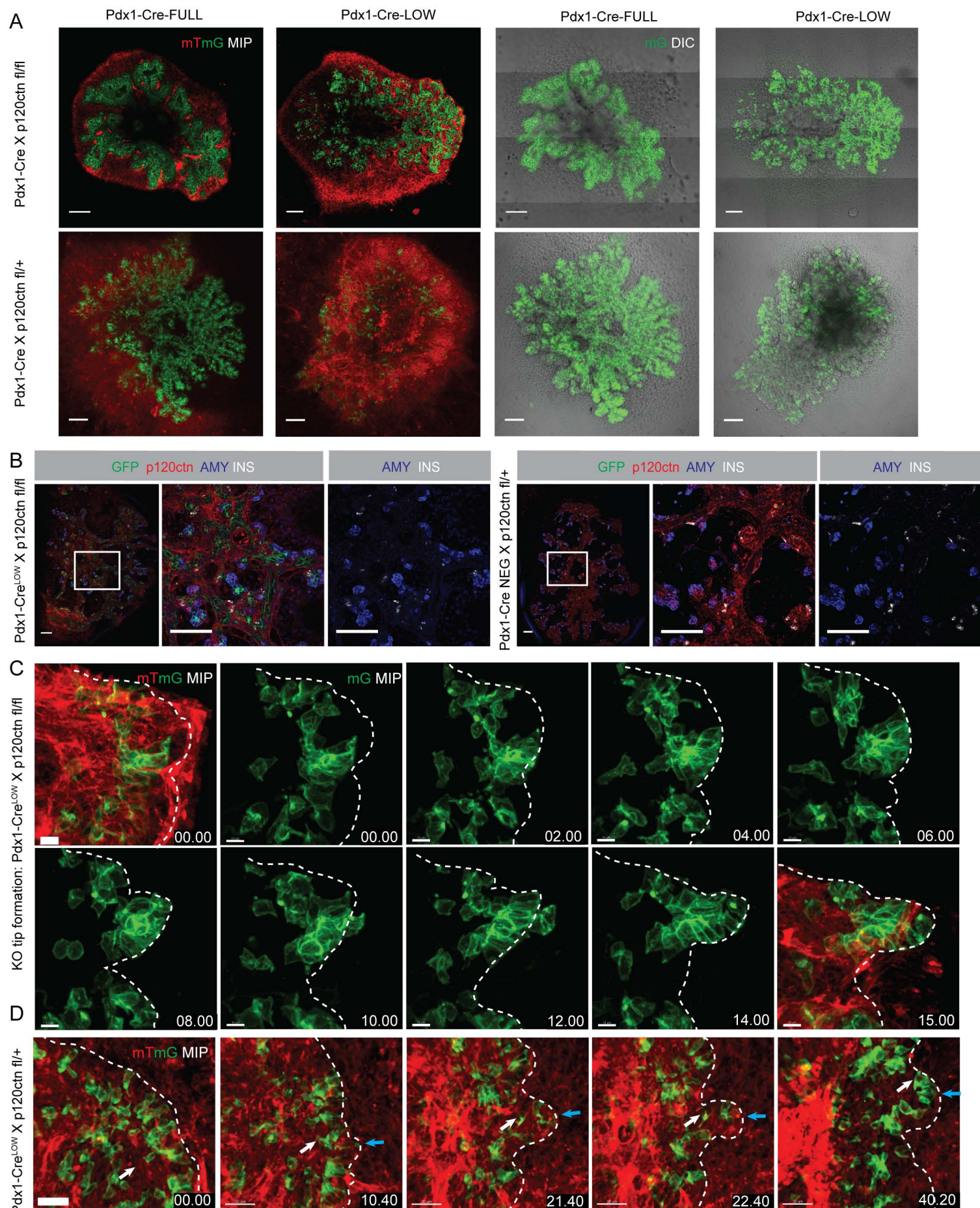


Figure S3. Related to Figure 3 and Video S5-6. Differential expression of P120ctn triggers tip formation by collective cell migration.

A: Examples of phenotypes of explanted dorsal pancreata post imaging from Pdx1-Cre-FULL and Pdx1-Cre-LOW crossed with either p120ctn fl/fl (HOM KO) or p120ctn fl/+ (HET) and the mTmG reporter. Maximum intensity projections (MIPs) for Tomato/GFP (left) or overlay of differential interference contrast (DIC) and GFP (right) confocal tiled images. Bar=100µm. **B:** Confocal images of Pdx1-Cre-LOW, p120ctn fl/fl, mTmG (HOM KO) or Pdx1-Cre NEG, p120ctn fl/+, mTmG (WT) explants stained for GFP, p120ctn, Amylase and Insulin post live-imaging. Bar=100µm. **C, D:** Time lapse imaging stills showing tip formation in Pdx1-Cre-ER, p120ctn fl/fl, mTmG HOM KO at E12.5 + 1 days culture (**C**) and HET at E11.5 + 1 days culture (**D**). Examples of MIPs of Tomato and GFP signal is shown in red and green respectively. Time is specified in hours and minutes. White stippled line: boundary between epithelium and mesenchyme based on DIC images (not shown). White arrow: migrating green cell. Blue arrow: forming tip. The images were normalized by median filtering and corrected in x-y for drift and organ growth. Bar=10µm in C and 30µm in D

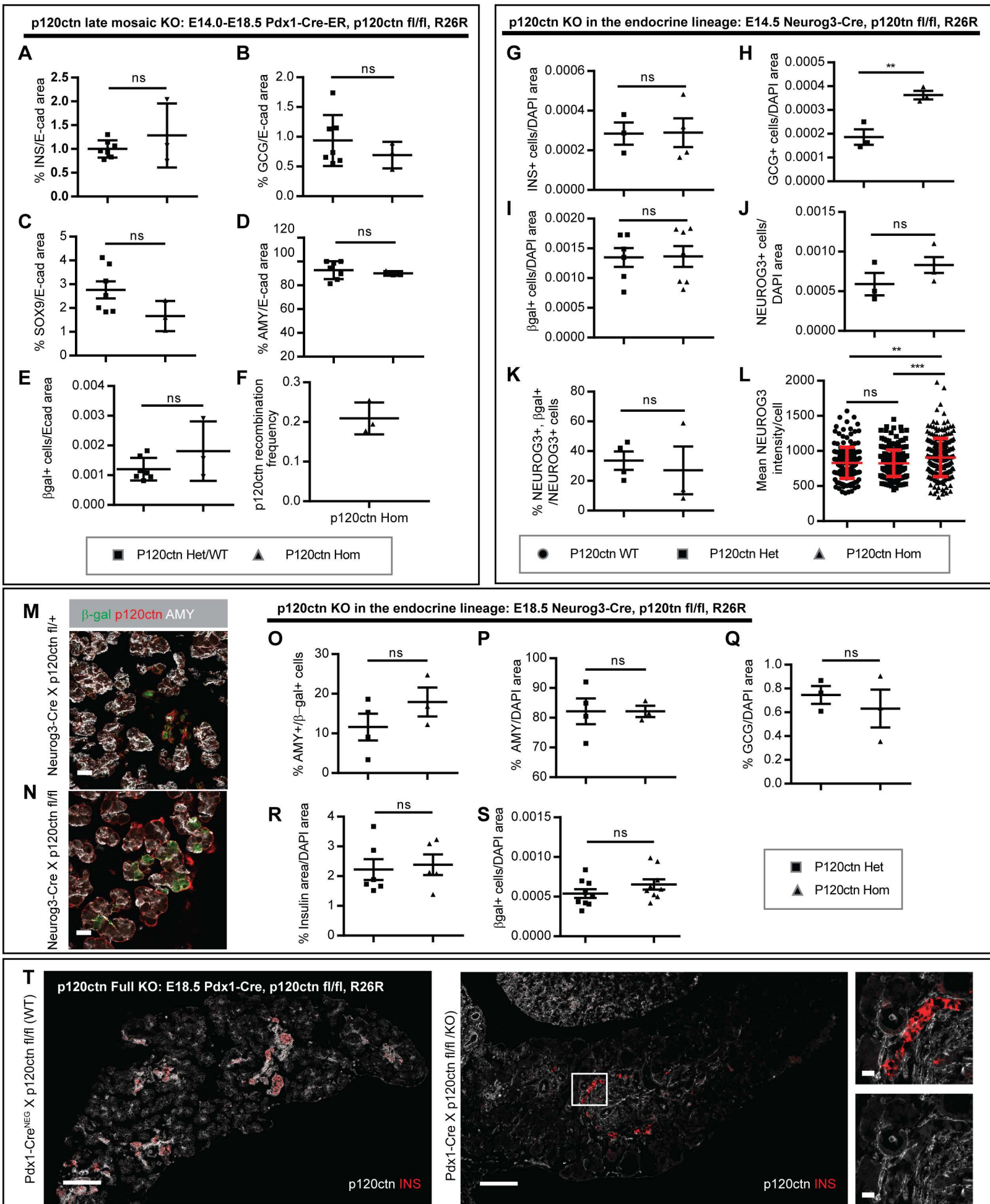


Figure S4. Related to Figure 4 and 5. P120ctn-deficient cells ablated after tip-trunk patterning or in the endocrine lineage
A-F: Quantification relating to Pdx1-Cre-ER, p120ctn fl/fl, R26R inducible mosaic KO from E14.0 -E18.5. **G-L:** Quantification relating to Neurog3-Cre, p120ctn fl/fl, R26R p120ctn endocrine lineage KO analysed at E14.5. Data in A-L is represented as mean \pm SEM. ns=not significant. ** $p < 0.01$, *** $p < 0.001$ by two-tailed t-test. **M, N:** Confocal images from E18.5 HET (M) and HOM KO (N) stained for β -gal, p120ctn and Amylase. Bar=20 μ m. **O-S:** Quantification relating to p120ctn endocrine lineage KO analysed at E18.5. Data is represented as mean \pm SEM. ns=not significant by two-tailed t-test. **T:** Confocal images from Pdx1-Cre, p120ctn fl/fl, R26R E18.5 WT and HOM FULL KO stained for p120ctn and Insulin. Bar=200 μ m and 20 μ m (magnification from boxed area).

Characterization of endocrine progenitors and insulin+ cells (WT)

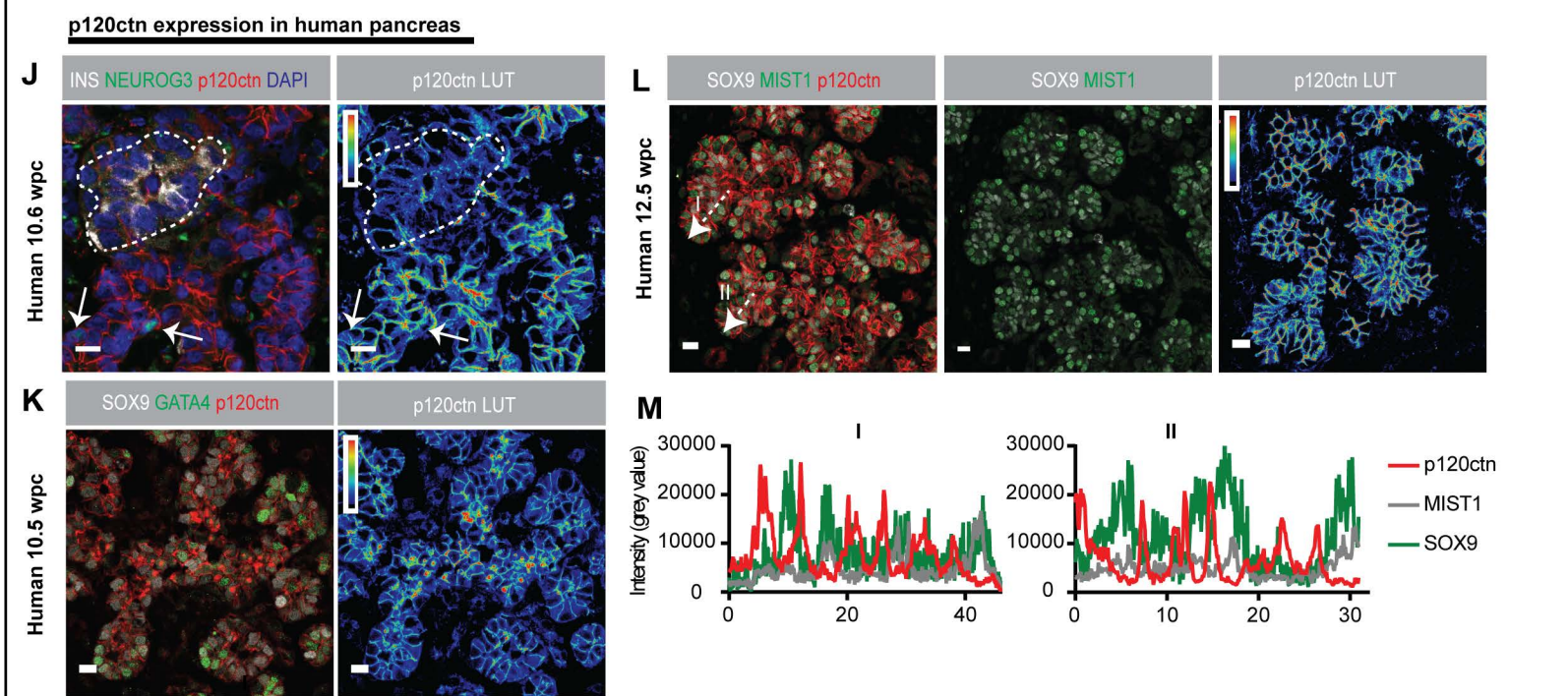
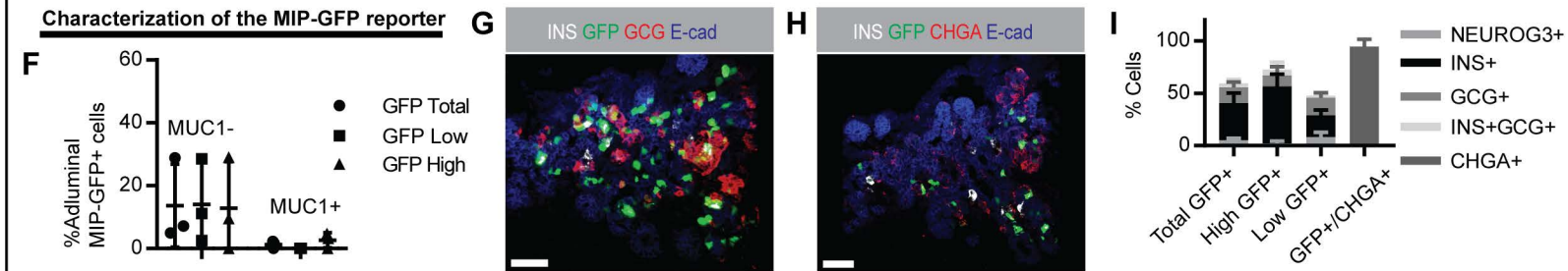
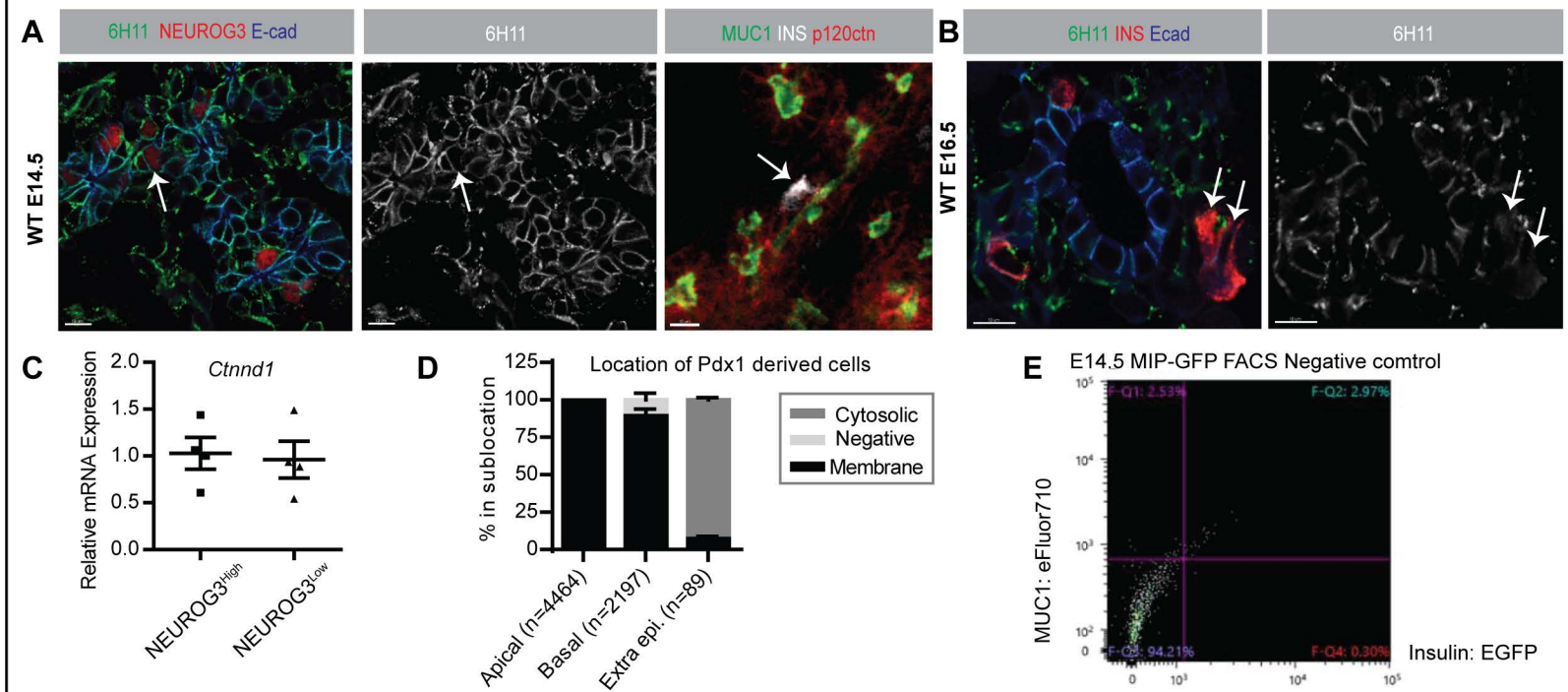
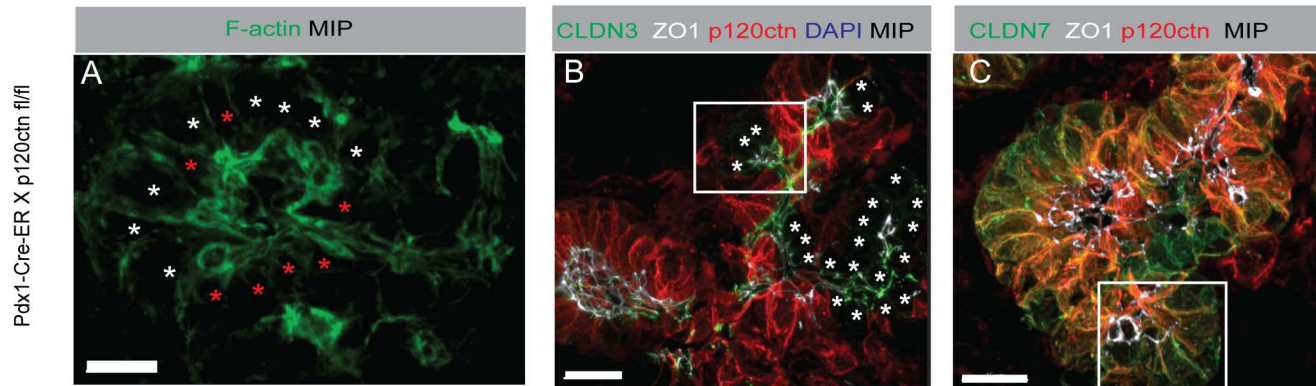
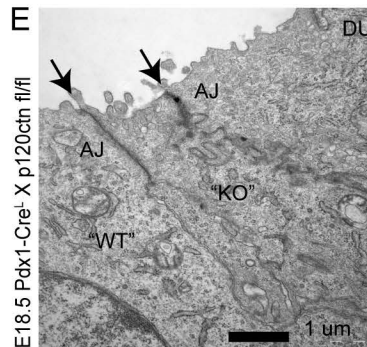
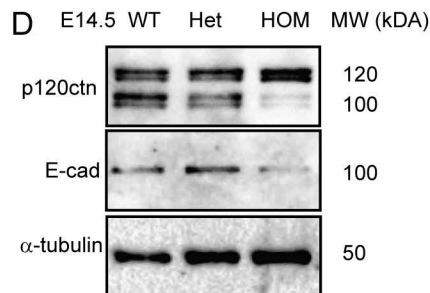


Figure S5. Related to Figure 1, 5 and 6. Endocrine delamination is associated with decreased expression of p120ctn in mouse and human pancreas
A, B: Confocal images from E14.5 (A) and E16.5 (B) WT pancreas stained for the p120ctn isoform1 specific antibody 6H11, NEUROG3 and E-cad or MUC1, INS and p120ctn. Bars=10µm. Arrows:NEUROG3+ or INS+ cells **C:** QPCR quantification of *Ctnnd1* expression in FACS sorted NEUROG3-high and NEUROG3-low populations from n=4 E15.5 Neurog3-YFP embryos. Data is represented as mean ± SEM. **D:** Quantification of p120ctn expression pattern relative to cell position in all β-gal+ cells in Pdx1-Cre, R26R embryos at E14.5. Based on 3D IHC staining of MUC1, p120ctn, and β-gal. Data are represented as mean ±SEM of n=3 WT embryos. **E:** FACS plot of the gating strategy in negative control (unstained, GFP-negative). E14.5 WT pancreata. **F:** Quantification of mean % of adluminal GFP+ cells +/- SEM in E14.5 MIP-GFP embryos (n=3). **G-H:** Maximum intensity projection of IF staining of E14.5 MIP-GFP embryo. **G:** INS, GFP, GCG and E-cad. **H:** INS, GFP, Chromogranin A (CHGA) and E-cad. Bars=50µm **I:** Histogram of mean % GFP+ cells +/- SEM with expression of NEUROG3, INS and/or GCG. n=4 E14.5 MIP-GFP mice. **J-L:** Confocal images from human fetal 10.6 (J) wpc pancreas stained for INS, NEUROG3, p120ctn and DAPI (left), 10.5 (K) and 12.5 wpc (L) pancreas stained for SOX9, MIST1, and p120ctn (K) or SOX9, GATA4, and p120ctn (L). LUT of p120ctn intensities (right). Bars=10µm. Solid arrows in J: NEUROG3+ cells. Outline: INS+ cells. Stippled arrow in L: Location of intensity profile measurements. **M:** p120ctn intensity profile measurements.

Induced mosaic p120ctn KO: E14.5 Pdx1-Cre-ER, p120ctn fl/fl, R26R



Full p120ctn KO: Pdx1-Cre, p120ctn fl/fl, R26R



Inducible mosaic E-cad KO: E14.5 Pdx1-Cre-ER, Ecad fl/fl, R26R

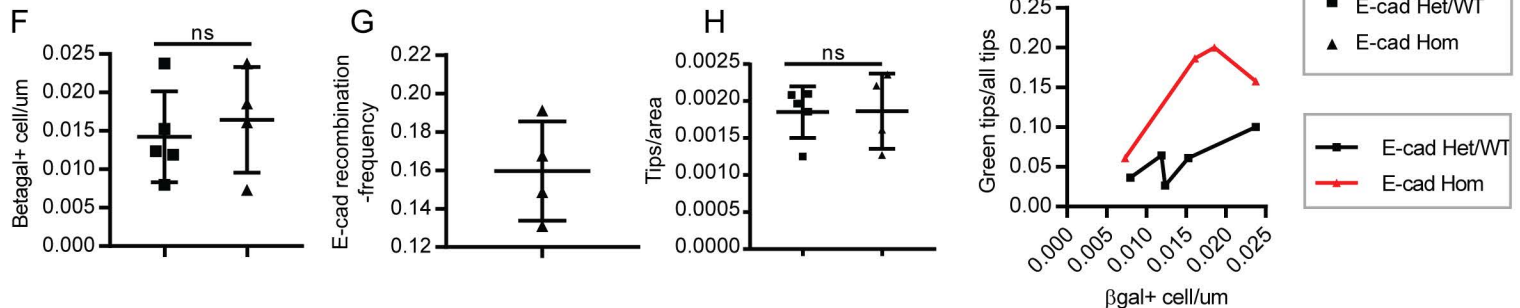


Figure S6. Related to Figure 7. E-cadherin acts downstream of p120ctn

A-C: Maximum intensity projections (MIP) from E14.5 Pdx1-Cre-ER, p120ctn fl/fl, R26R HOM KO pancreas induced at E8.5 and stained for F-actin (**A**), Claudin3, ZO1, p120ctn and DAPI (**B**) or Claudin7, ZO1, p120ctn and DAPI (**C**). Boxed area is magnified in main Fig 7. White* KO cell, Red* WT cell. Bar=10μm in A and 15 μm in B,C. **D:** Example of WB analysis of E14.5 pancreas from Pdx1-Cre, p120ctn fl/fl, R26R WT, HET and HOM FULL p120ctn KO probed with p120ctn, E-cad and α-tubulin antibodies. E-cad and p120ctn are from separate blots. **E:** TEM micrograph from FULL pancreatic HOM KO at E18.5. Arrows: apical cell-cell junctions. DU: ductal. Bar=1μm. **F-I:** Quantification relating to Pdx1-Cre-ER, Ecad fl/fl, R26R E-cad mosaic KO induced at E8.5 and analyzed at E14.5. Data are represented as mean ± SEM of n=5 E14.5 WT/HET and n=4 HOM KO embryos. ns=not significant by two-tailed t-test.

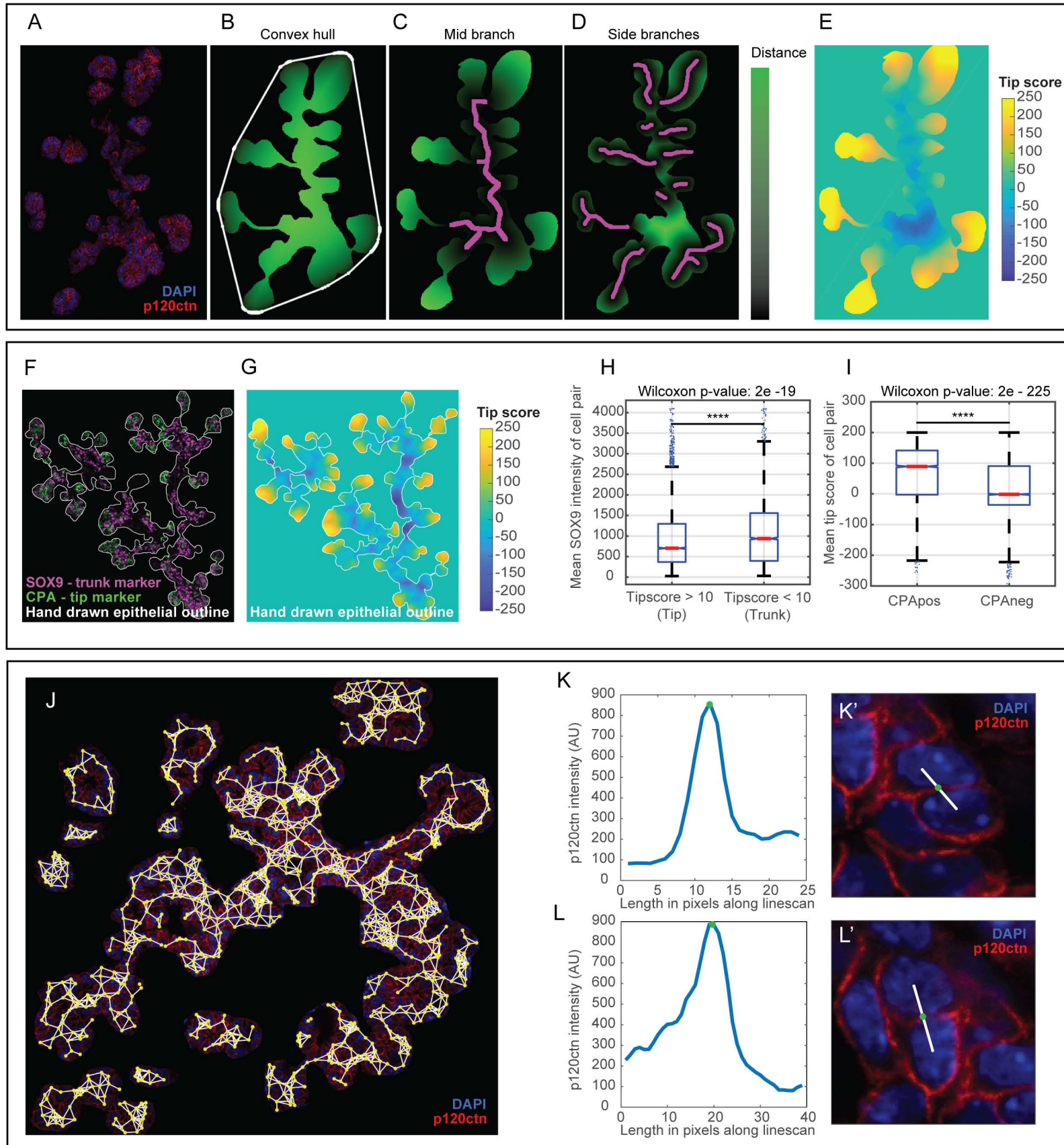


Figure S7. Related to Figure 1. Computational image analysis methods

A: Confocal image of E14.5 pancreas stained for p120ctn and DAPI. **B-D**: Black-green color map shows local distance to Convex hull/mid branch/side branches respectively. **B**: White line: Convex hull of epithelial outline. **C**: Magenta line: Mid branch of skeletonization of epithelial outline. **D**: Magenta lines: Side branches of skeletonization of epithelial outline. **E**: Color map shows tip score value in different locations for image shown in A. **F**: Confocal image of E14.5 pancreas stained for CPA, (tip marker) and SOX9 (trunk marker). White line: hand drawn epithelial outline. **G**: Color map showing tip score value in different locations for image shown in F. White line: hand drawn epithelial outline. **H**: Mean SOX9 intensity for cell pairs with mean tip score <10 (n=9398 cell pairs) and >10 (n=7506 cell pairs) respectively. Red lines mark median values. $p=2e-19$ (Wilcoxon signed-rank test). Difference between groups is also statistically significant on a per embryo basis, (data not shown). **I**: Mean tip score for CPA positive cell pairs (n=4514 cell pairs) and CPA negative cell pairs (n=8051 cell pairs) respectively. Red lines mark median values. $p=2e-225$ (Wilcoxon signed-rank test). Difference between groups is also statistically significant on a per embryo basis, (data not shown). **H-I**: Data comes from n=4 WT embryos (3 or 4 sections per embryo). **J**: Confocal image of E14.5 pancreas stained for p120ctn and DAPI. Yellow dots: cell positions. White lines: linescans between neighboring cells. **K-L**: Examples of p120ctn linescans extracted between neighboring cells show in image K' and L' respectively. Green dots show maximum value along curves in K and L. As seen in image K' and L', the position of the max. coincides with the position of the double membrane between the cells.