

Figure S1. Supplemental data related to bead screen. (A) Description of methods used for screening candidate growth factors and quantifying changes in β cell position over time. Growth factor-soaked agarose beads (blue) were implanted into E13.5 fetal mouse pancreatic organ cultures. *Ins1*-GFP fluorescence was measured as a function of radius from the central bead; lower right fluorescence image indicates measurement of *Ins1*-GFP intensity along a line (red arrow) rotated 360° (white arrow) from the center of the bead (blue circle). Fluorescence intensity was measured along the circumference of each circle with radius from 0 to 325 μm and normalized for path length, with resulting chart indicated at right. (B) Examples of Shift calculations for three organs. Blue line indicates day 0 measurements; green line indicates day 5 measurements. The center of the bead is defined as radius = 0 μm . (C-F) Sema3a, Sema3b, and Sema3f are capable of inducing alpha cell swarming of beads in the distal dorsal pancreas. None induce expression of Ki67 in alpha cells. (G,H) No alteration of islet precursor marker Neurog3 expression with Sema3a bead. (I,J) Alpha cells responding to Sema3a beads invade into mesenchyme and lose E-Cadherin expression. White circles indicate bead placement. Scale bars: 100 μm (A, C-H) and 50 μm (I,J).

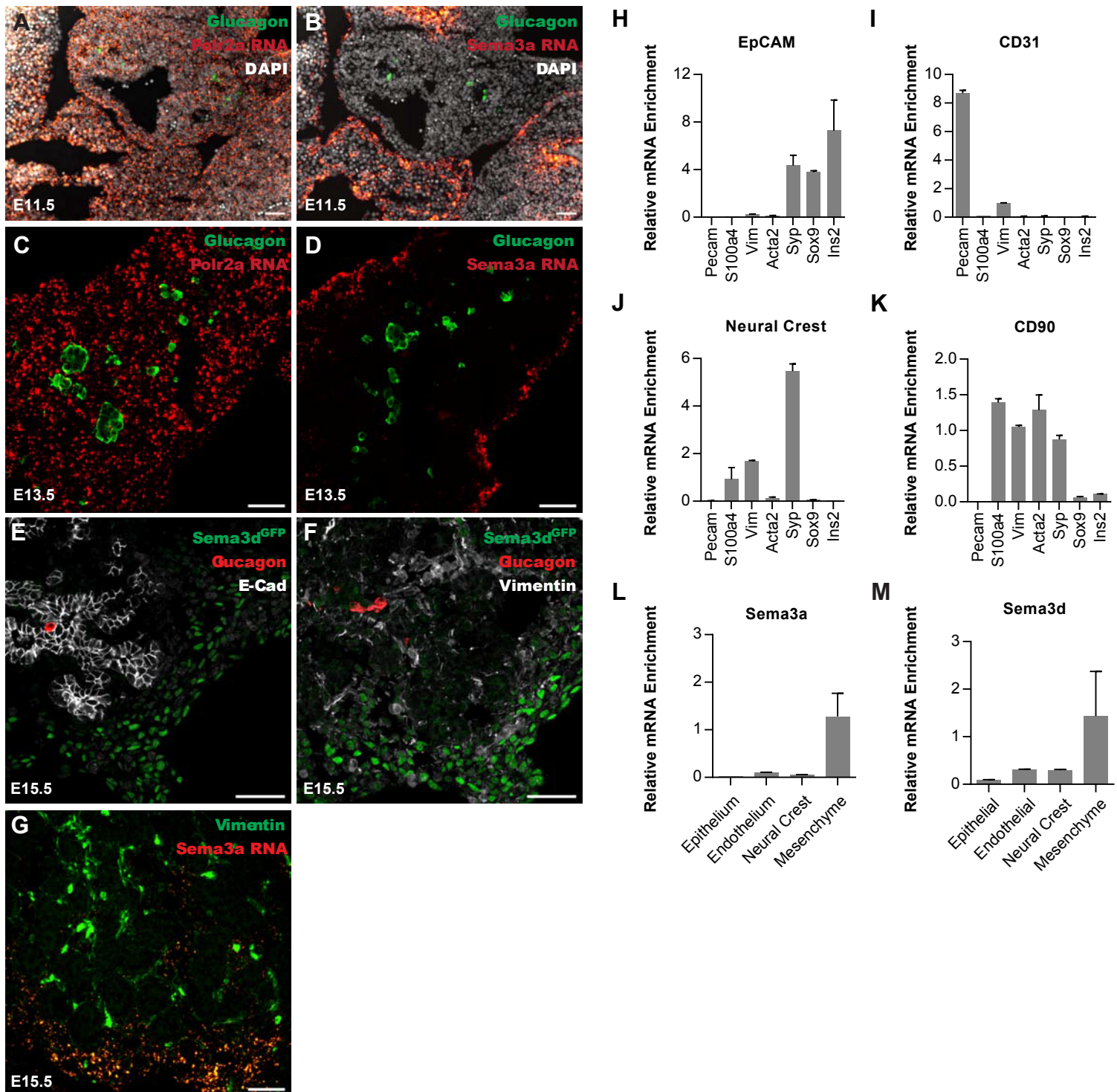


Figure S2. Supplemental data related to Semaphorin expression. (A-D) *Sema3a* mRNA is expressed along the pancreatic periphery at earlier developmental stages, including E11 and E13. (E,F) Expression of *Sema3d*^{GFP} transgene is restricted to peripheral pancreatic mesenchymal cells. (G) *Sema3a* mRNA expression overlaps with peripheral mesenchymal cells. (L-M) *Sema3a* and *Sema3d* mRNA are enriched in FACS-purified mesenchymal cells; validation of FACS purified cell populations are presented (H-K). Data in (H-M) presented as mean±SEM. Scale bars: 50 μm.

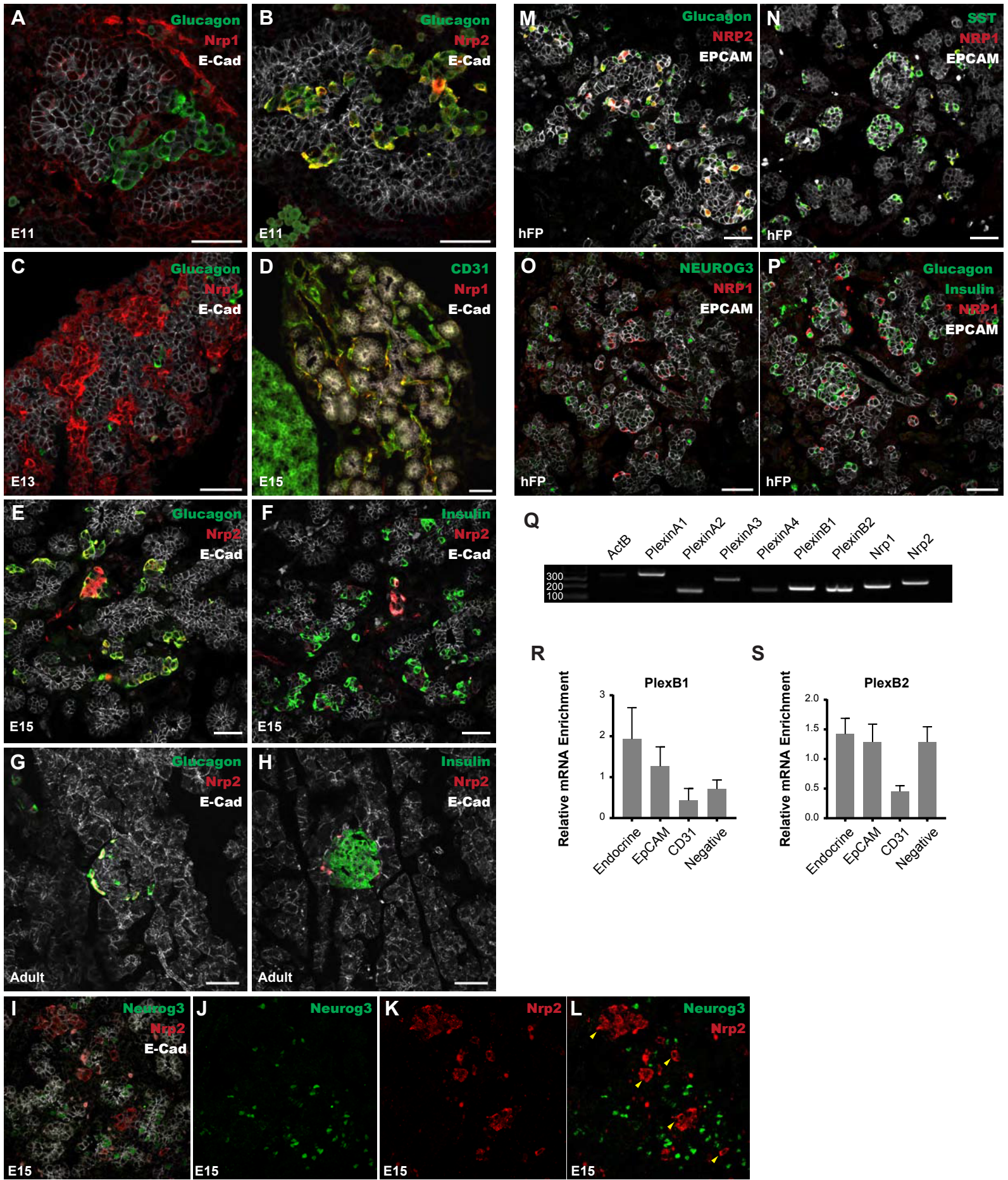


Figure S3. Expression of Semaphorin receptors in the pancreas. (A,B) At E11.5, Nrp1 is expressed within the pancreatic epithelium but not in α cells. Nrp2 is expressed in α cells. (C) Nrp1 is expressed primarily in the mesenchyme at E13.5, and in a subset of epithelial cells. (D) Expression of Nrp1 at E15.5 overlaps with CD31⁺ endothelial cells. (E-H) Nrp2 is expressed in α cells, but not β cells, at E15.5 and in adult mice. (I-L) Nrp2 is not expressed in islet progenitor cells, marked by Neurogenin 3⁺ signal, at e15. (M-P) In the human fetal pancreas, NRP2 is expressed in α cells, and NRP1 is expressed in δ cells. NRP1 is also expressed in a subset of EPCAM⁺ epithelial cells that does not coexpress NEUROG3, Insulin, or Glucagon. (Q) Expression of Plexin mRNA by RT-PCR in E15.5 pancreas. (R,S) Expression of *PlexinB* genes in FACS-purified cell populations shows a lack of enrichment in islet cells at E15.5 relative to EpCAM⁺ epithelial cells or whole pancreas. Data presented as mean \pm SEM. Scale bars: 50 μ m.

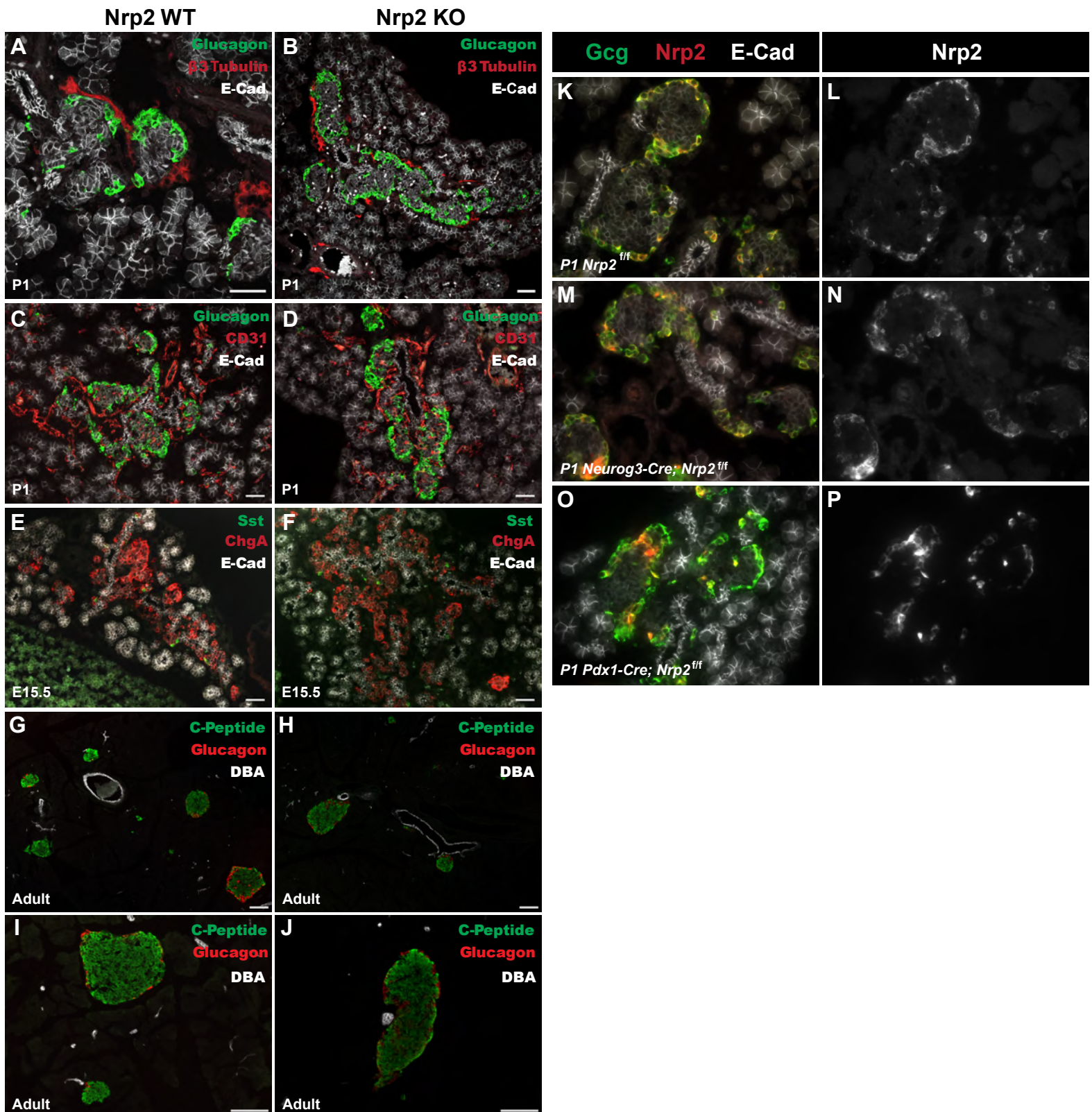


Figure S4. Supplemental data related to *Nrp2* knockout analysis. (A-D) No gross alterations in islet innervation or vascularization at P1. (E,F) No apparent changes in δ cell development at E15.5. (G-J) Islet histology in surviving adult *Nrp2* knockout mice demonstrates islet association with ducts. (K-P) The *Nrp2* floxed allele is not efficiently excised by pancreas specific Ngn3 cre and Pdx1 cre transgenes at P0. Scale bars: 50 μ m.

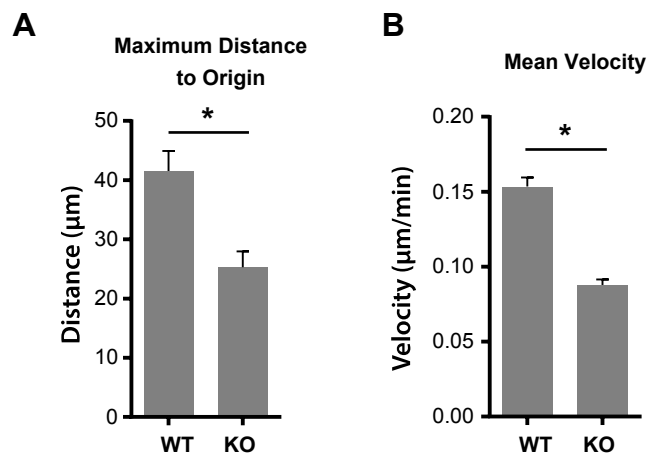
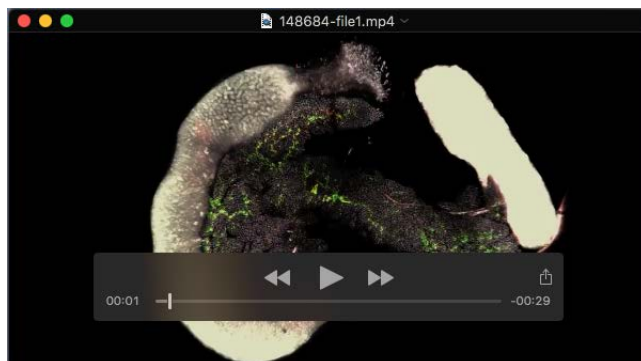


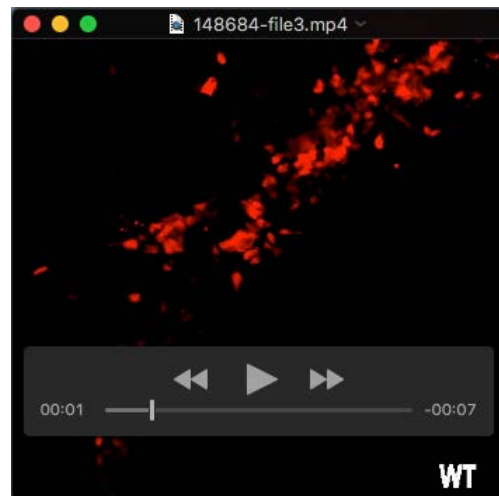
Figure S5. Live imaging analysis supplement. (A) Quantification of maximum distance from cell position at $t=0$ hrs in live imaging of *Nrp2* wild-type and knockout cells ($P=0.001$, $n = 38$ WT and 25 KO). (B) Quantification of mean cell velocity in live imaging ($P=2 \times 10^{-11}$, $n = 38$ WT and 25 KO). Both presented as mean \pm SEM, P values from two-tailed t-test.



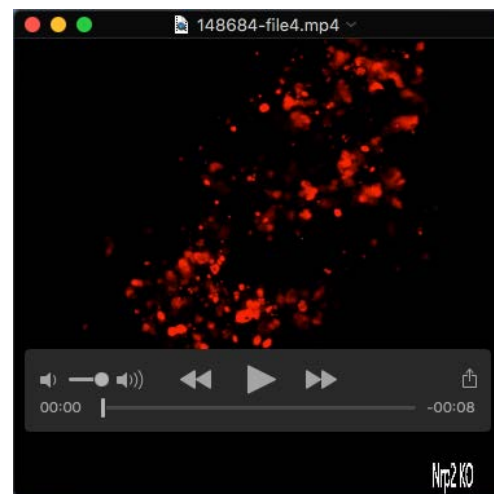
Movie 1. CLARITY 3D reconstruction of *Nrp2* wild type whole pancreas at P1. Islets (glucagon, red; c-peptide, green) in *Nrp2* wild type pancreas form distinct small islet clusters both close and distant to ductal structures (mucin, white). The 3D reconstruction movies were created in Arivis Vision4D software using data from Figure 4.



Movie 2. CLARITY 3D reconstruction of *Nrp2* knockout type whole pancreas at P1. Islets (glucagon, red; c-peptide, green) in *Nrp2* knockout type pancreas form distinct small islets clusters, but also form large islet aggregates that are in close proximity to ductal structures (mucin, white). The 3D reconstruction movies were created in Arivis Vision4D software using data from Figure 4.



Movie 3. 24 hour time-lapse video of endocrine cell migration and deformation in *Nrp2* wild type embryonic pancreas explant. Endocrine lineage cells (red) in *Nrp2* wild type pancreas (*Nrp2*^{+/+}; Neurog3-tdTomato) readily display cell migration and cell deformation, such as filopodia extensions. Time-lapse video is created from data in Figure 5.



Movie 4. 24 hour time-lapse video of endocrine cell migration and deformation in *Nrp2* knockout embryonic pancreas explant. Endocrine lineage cells (red) in *Nrp2* knockout pancreas (*Nrp2*^{-/-}; Neurog3-tdTomato) show limited migration and cell deformation compared to *Nrp2* wild type control (Movie S3). Time-lapse video is created from data in Figure 5.

Supplemental Tables

Table S1. Growth factors used in bead screen			
Growth factor	Company	Catalog Number	n (organs in screen)
PDGF-BB (human recombinant)	R&D Systems	220-BB-010	3
BMP4 (human recombinant)	R&D Systems	314-BP-010	6
Noggin (human recombinant)	R&D Systems	6057-NG-025	5
HGF (human recombinant)	R&D Systems	294-HG-005	5
Activin A (human recombinant)	R&D Systems	338-AC-010	5
GDF3 (human recombinant)	R&D Systems	5754-G3-010	7
IGF1 (human recombinant)	R&D Systems	291-G1-200	3
TGF β 1 (human recombinant)	R&D Systems	240-B-002	5
Sema3a (human recombinant, Fc fusion)	R&D Systems	1250-S3-025	6
TGF β 3 (human recombinant)	R&D Systems	243-B3-002	6
VEGF (human recombinant)	Peprtech	AF-100-20A	7
Wnt3a (mouse recombinant)	R&D Systems	1324-WN-002	4
EGF (human recombinant)	Sigma	E9644	4
RSPO1 (human recombinant)	Peprtech	120-38	5
Shh (human recombinant)	Peprtech	315-22	3
Cxcl13 (mouse recombinant)	Peprtech	250-24	4
Cxcl12 (mouse recombinant)	R&D System	460-SD-010	6
Fgf2 (human recombinant)	Peprtech	100-18B	5
Exendin4	Tocris	1933	3
Ang1 (human recombinant)	Peprtech	130-06	3
sFRP1 (human recombinant)	Peprtech	120-29	3
Sema3a (mouse recombinant, Fc fusion)	R&D Systems	5926-S3-025	N/A
Sema3b (mouse recombinant, Fc fusion)	R&D Systems	5440-S3-025	N/A
Sema3f (mouse recombinant, Fc fusion)	R&D Systems	3237-S3-025	N/A

Inhibitor	Concentration	Company	Catalog Number
CHIR99021 (GSK3 inhibitor)	1 μ M	Selleck Chemicals	S2924
LIMKi3 (LIM Kinase inhibitor)	10 μ M	EMD Millipore	435930
IPA3 (PAK inhibitor)	10 μ M	EMD Millipore	506106
Y-27632 (ROCK inhibitor)	10 μ M	EMD Millipore	688000
Roscovitine (CDK5 inhibitor)	10 μ M	EMD Millipore	557360

Antigen	Antibody type	Source	Catalog number	Dilution
Insulin	Guinea pig polyclonal	Dako	A0564	500
c-Peptide	Rabbit polyclonal	Cell Signaling Technologies	4593	500
Glucagon	Guinea pig polyclonal	Clontech	M182	5,000
Chromogranin A	Rabbit polyclonal	Immunostar	20085	500
E-Cadherin	Rat monoclonal	Life Technologies	13-1900	500
Mucin	Hamster monoclonal	Thermo Scientific	HM-1630	500
Nrp1	Rabbit monoclonal (EPR3113)	Abcam	ab81321	250
Nrp2	Rabbit monoclonal (D39A5)	Cell Signaling Technologies	3366	250
Vimentin	Rabbit monoclonal (D21H3)	Cell Signaling Technologies	5741	200
PDGFRA	Rabbit monoclonal (D1E1E)	Cell Signaling Technologies	3174	400
CD90 (AF488)	Rat monoclonal (30-H12)	BioLegend	105315	250
EpCAM (Biotin)	Mouse monoclonal (G8.8)	BioLegend	118204	250
CD31 (BV421)	Mouse monoclonal (390)	BioLegend	102423	250
EPCAM	Mouse monoclonal (1B7)	eBioscience	13-9326	250
DBA	(<i>Dolichos biflorus agglutinin</i> lectin, biotinylated)	Vector Laboratories	B-1035	1000

*All antibodies were validated using positive and negative control tissue samples.

Gene	Forward Primer Sequence	Reverse Primer Sequence	Product Size
ActB	CATAGCACAGCTTCTCTTTGATGTC	CTAAGGCCAACCGTGAAAAGATG	321 bp
PlexinA1	TCAATGACAAAGTTGCCATTCCAC	AAATACCGCCATGTTTGCACC	350 bp
PlexinA2	TGAGATGGTCTCTGTGTTCAAAGAT	GATCCCCTGAGCTTAGACACTCT	167 bp
PlexinA3	CTGTTGGATACTGTGTACAAGGGTA	GCTATCCGGGCTGCTGG	301 bp
PlexinA4	CACAATGGGGGTCACCTGAG	AAATGCCCTCCAATATGAGACTGT	188 bp
PlexinB1	ATGCTGGGGAGTATGATGTCTCTA	GAGCTTAGAACCATGCAGGGTAA	214 bp
PlexinB2	GCAGTCATAGAAGGGATACTGGTG	GACTGAGGACGATGAGTTACTGTG	206 bp
Nrp1	GGCCCTTCTCTTCATCAAATTTGT	CTCCAGGTCAAACCTTTCAAACCTCC	214 bp
Nrp2	TACATCAAGTTCACCTCAGACTACG	CAGCCAGTCATATTTACAGTCTCCT	254 bp