

Supplementary Figure Legends

Supplementary Figure 1: (A-C) Immunostaining of sections obtained from NP (A), PP1 (B) or PP21 (C) $Gcg^{iCre};R26^{Tom}$ dams for detection of glucagon (blue) along with tomato-red, showing expansion of tomato⁺/glucagon⁻ cells in the PP21 islets. (D) Quantification of the number of tomato⁺/glucagon⁺ cells in the indicated time points. Values are mean \pm SD of at least four biological replicates. **p<0.001 by one-way ANOVA test. Tom: tomato-red, GCG: glucagon. NP: non-pregnant, PP1: post-partum day 1, PP21: post-partum day 21, GD17.5: gestational day 17.5. Scale bars 20 μ m.

Supplementary Figure 2: Pregnancy does not promote higher proliferation rate among α -cells.

BrdU was given via drinking water between GD10.5 and PP1 at which the mice were euthanized. NP $Gcg^{iCre};R26^{Tom}$ females that were given BrdU for the same window of time served as control for baseline BrdU incorporation. (A, B) Detection of tomato-red in conjunction with BrdU (cyan) on sections obtained from NP (A) or PP1 (B) $Gcg^{iCre};R26^{Tom}$ dams. (C) Quantification of percentage of proliferating tomato-labeled cells among total tomato-positive cells. Values are mean \pm SD of five biological replicates. *p<0.0001 by unpaired t-test. NP: non-pregnant, PP21: post-partum day 21. Scale bars 20 μ m.

Supplementary Figure 3: Lgr5-expressing cells are not detected prior to gestational day 15.

(A) NP or $Lgr5^{CreERT};R26^{Tomato}$ dams that were treated with tamoxifen prior to mating were allowed to deliver and were euthanized at the time for weaning on PP21. No tomato-red labeled cells could be detected. (B) $Lgr5^{CreERT};R26^{Tomato}$ dams that were treated with tamoxifen on GD12 were euthanized on GD15. No tomato-red labeled cells could be detected. (C) $Lgr5^{CreERT};R26^{Tomato}$ dams that were treated with tamoxifen on GD12 were allowed to deliver and were euthanized at the time for weaning on PP21. No tomato-red labeled cells could be detected.

Supplementary Figure 4: Identification of Lgr5-progenies in the adult mouse pancreas.

(A, B) Detection of tomato-red, GFP (green), E-cadherin (blue) and Dapi (grey) in the intestine (A) or the pancreas of *Lgr5^{CreERT};R26^{Tomato}* dams treated with tamoxifen on PP1 and euthanized on PP14. Scale bars 20 μ m.

(C-E) Detection of tomato-red along with somatostatin (green) and Dapi (blue) (C), pancreatic polypeptide (green) an Dapi (blue) (D), or insulin (green), glucagon (blue) and Dapi (grey) in pancreatic tissues of *Lgr5^{CreERT};R26^{Tomato}* dams treated with tamoxifen on PP1 and euthanized on PP21. Ecad: E-cadherin, Tom: tomato-red, SOM: somatostatin, PP: pancreatic polypeptide, INS: insulin, GCG: glucagon, PP1: post-partum day 1, PP14: post-partum day 14, PP21: post-partum day 21. Scale bars 20 μ m.

Research Methods

Supplementary Table 1.

Antigen	Species	Company	Cat. No.	Dilution
Amylase	rabbit	Sigma	A8273	1:300
BrdU	rat	Abcam	Ab6326	1:100
Cleaved Caspase-3	mouse	Abcam	Ab208161	1:200
CD90.1-VioBlue	mouse	Miltenyi	130-102-637	1:10 (Flow)
Cytokeratin	rabbit	DAKO	Z0622	1:100
DBA	biotin	Vector Lab	B1035	1:100 (Flow)
E-cadherin	goat	R&D	AF748	1:200 (IF) 1:100 (Flow)
F4/80	rat	Invitrogen	MF48000	1:100
GFP	rabbit	Life tech.	A6455	1:500
GFP	goat	Abcam	ab5450	1:500
Glucagon	guinea pig	Linco/Millipore	4031-01F	1:1000
Glucagon	rabbit	Linco/Millipore	4030-01F	1:1000
Insulin	guinea pig	Linco/Millipore	4011-01	1:1000
PP	guinea pig	DAKO	4041-01	1:200
Pdx1	goat	Abcam	ab47383	1:2000
Somatostatin	goat	Santa Cruz	sc-7819	1:100
Urocortin-3	rabbit	P. Sawchenko, Salk Institute	N/A	1:1500

All of the secondary antibodies used for Immunostaining were purchased from Jackson ImmunoResearch Laboratories: biotin-conjugated anti-rabbit (1:500), biotin-conjugated anti-rat (1:500), biotin conjugated anti-guinea pig (1:500), biotin-conjugated anti-goat (1:250); Cy2-conjugated streptavidin (1:500); Cy3-conjugated streptavidin (1:500); Cy5-conjugated streptavidin (1:100); and Cy2- and Cy3- anti-guinea pig, anti-rabbit, anti-rat, anti-goat (all 1:300). For flow cytometry, secondary antibodies used were Cy5 anti-goat, anti-rat, anti-rabbit

(1:100), Cy5-conjugated streptavidin (1:100) and Donkey anti-Goat IgG (H+L) Alexa Fluor® 350 conjugate (Thermo fisher).