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Supplemental Information

Expansion of Adult Human Pancreatic Tissue Yields Organoids Harboring Progenitor Cells with Endocrine Differentiation Potential

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Figure S1:

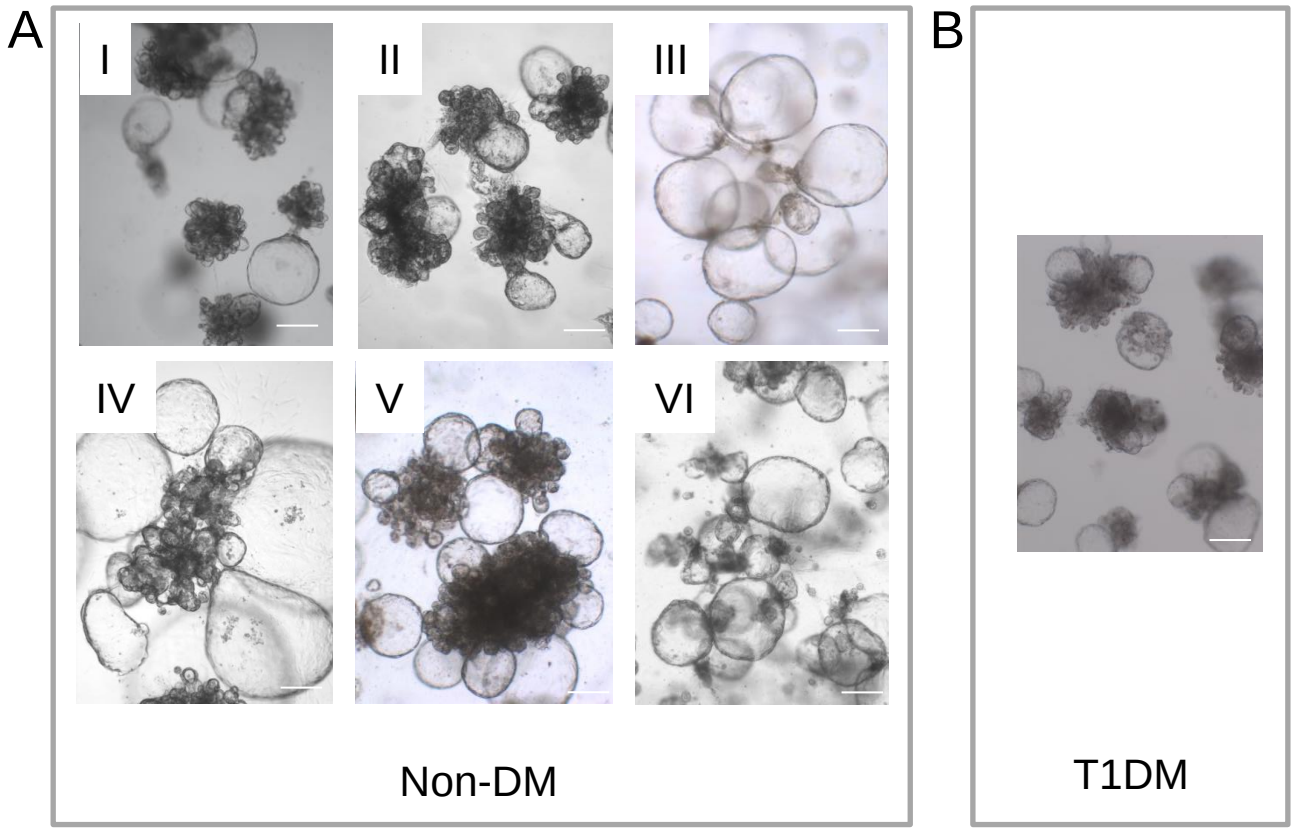
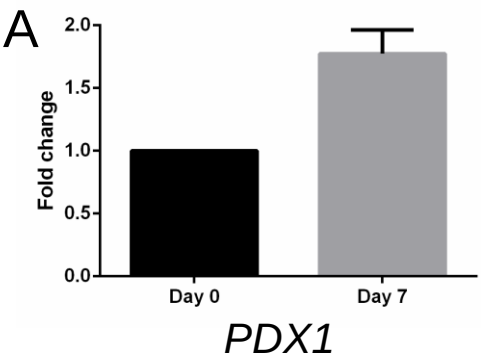
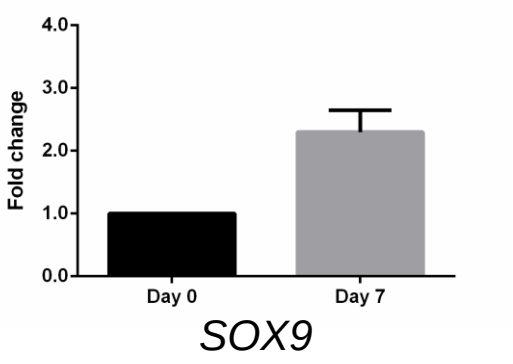
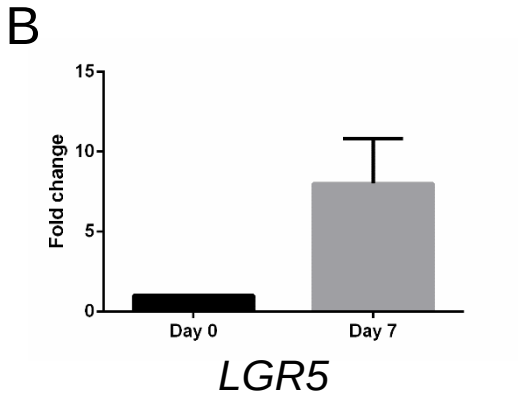
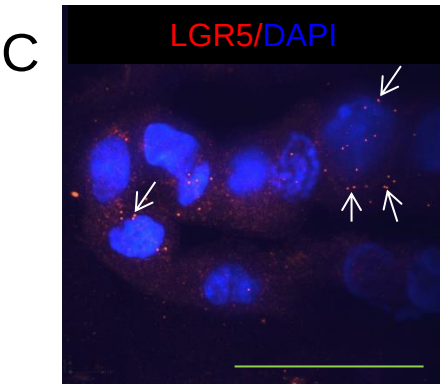


Figure S2:


A
Fold change
2.0
1.5
1.0
0.5
0.0
Day 0
Day 7
PDX1


Fold change
4.0
3.0
2.0
1.0
0.0
Day 0
Day 7
SOX9


B
Fold change
15
10
5
0
Day 0
Day 7
LGR5


C
LGR5/DAPI

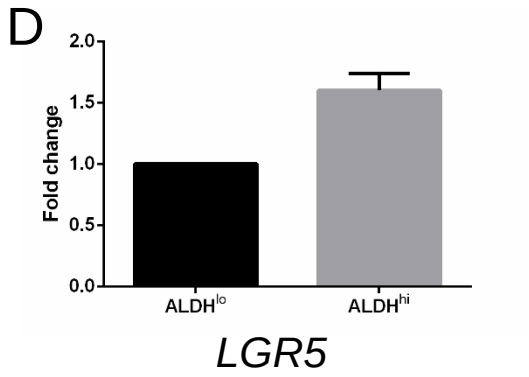

D
Fold change
2.0
1.5
1.0
0.5
0.0
ALDHlo
ALDHhi
LGR5

Figure S3:

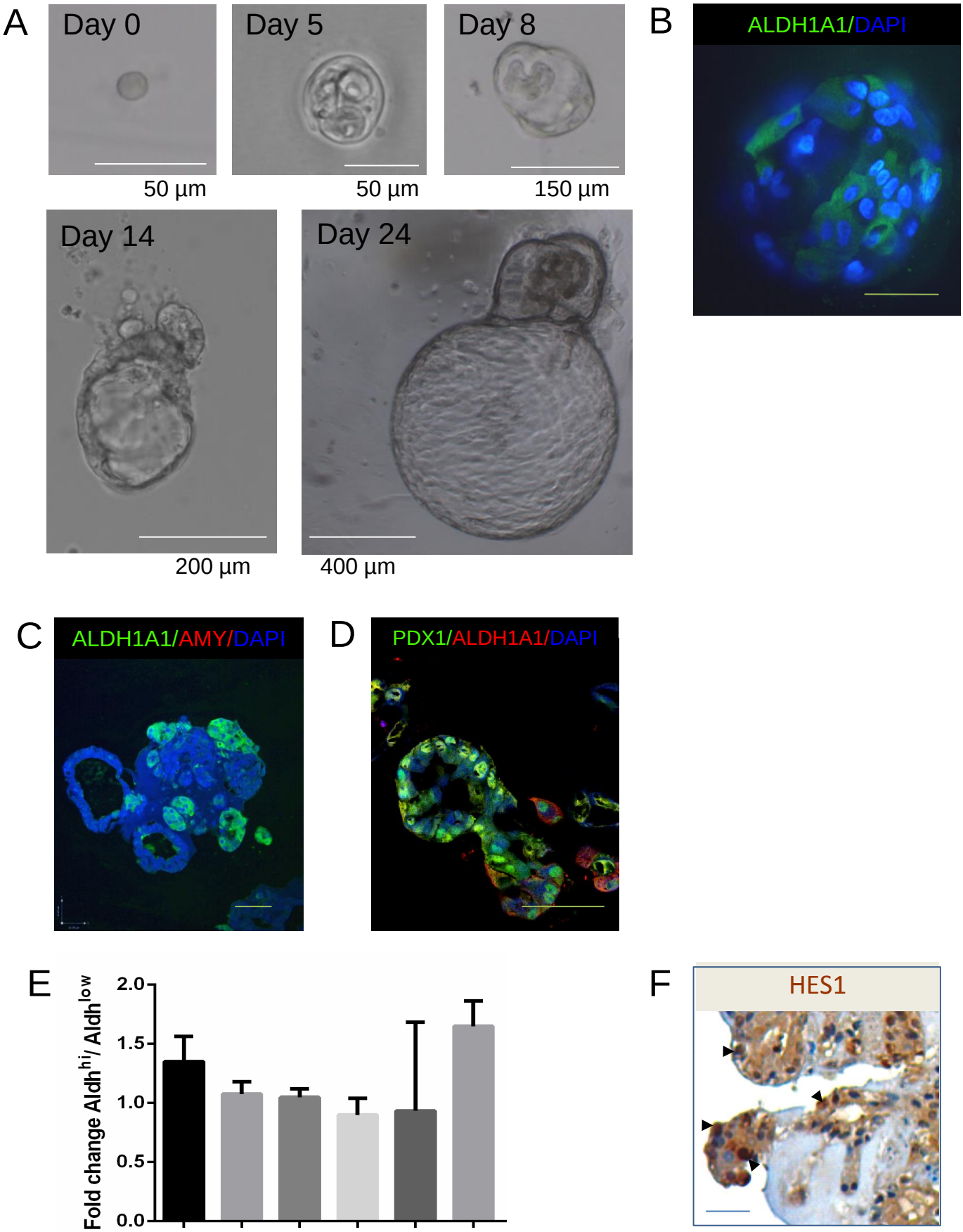

A
Day 0
Day 5
Day 8
50 µm
50 µm
150 µm
Day 14
Day 24
200 µm
400 µm
B
ALDH1A1/DAPI
C
ALDH1A1/AMY/DAPI
D
PDX1/ALDH1A1/DAPI
E
Fold change Aldh^hi/ Aldh^low
2.0
1.5
1.0
0.5
0.0
SOX9
SCA1
MET
NES
HEY1
HES1
F
HES1

Figure S4:

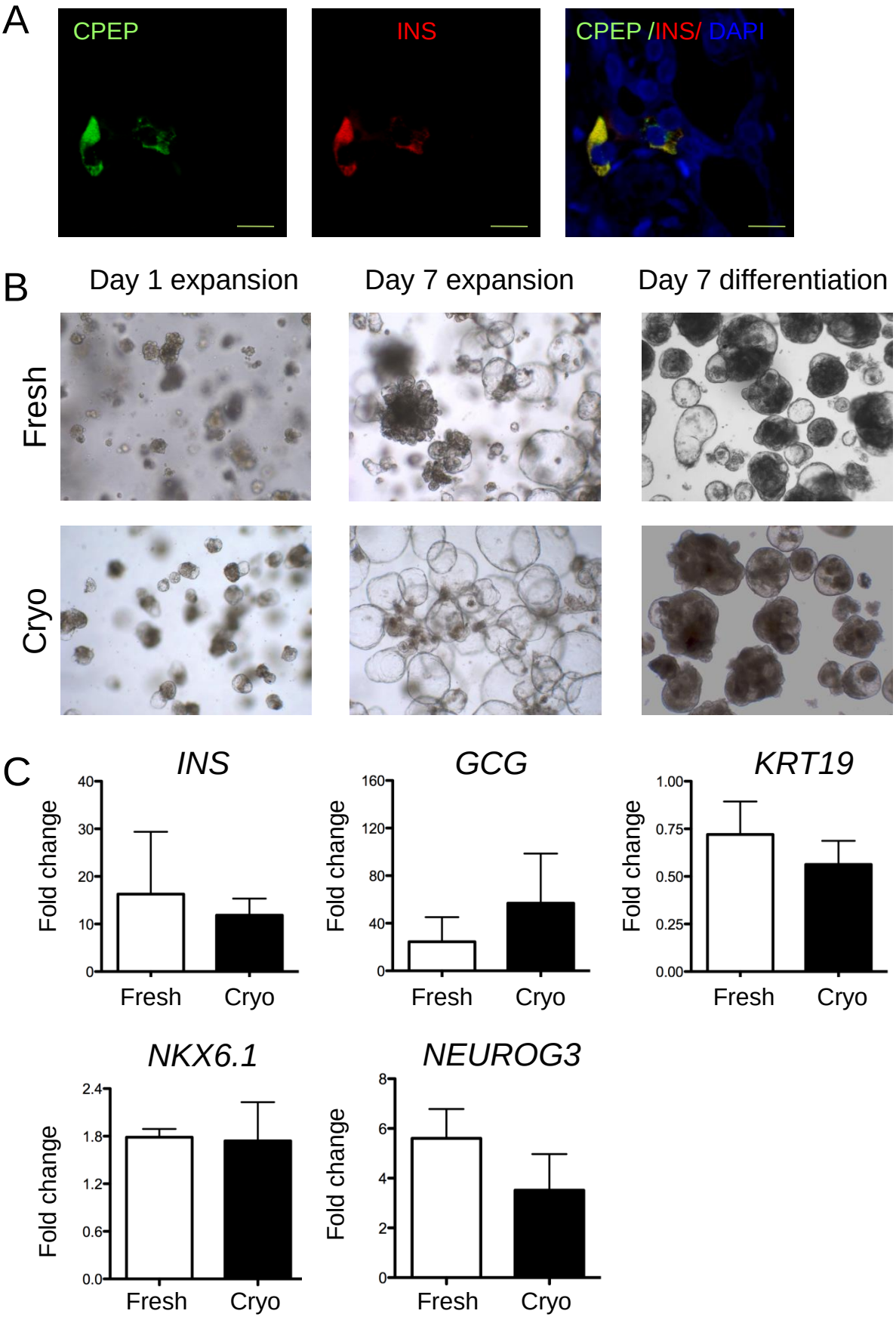

A
CPEP
INS
CPEP /INS/ DAPI
B
Day 1 expansion
Day 7 expansion
Day 7 differentiation
Fresh
Cryo
C
INS
GCG
KRT19
NKX6.1
NEUROG3
Fold change
Fresh
Cryo

Figure S5:

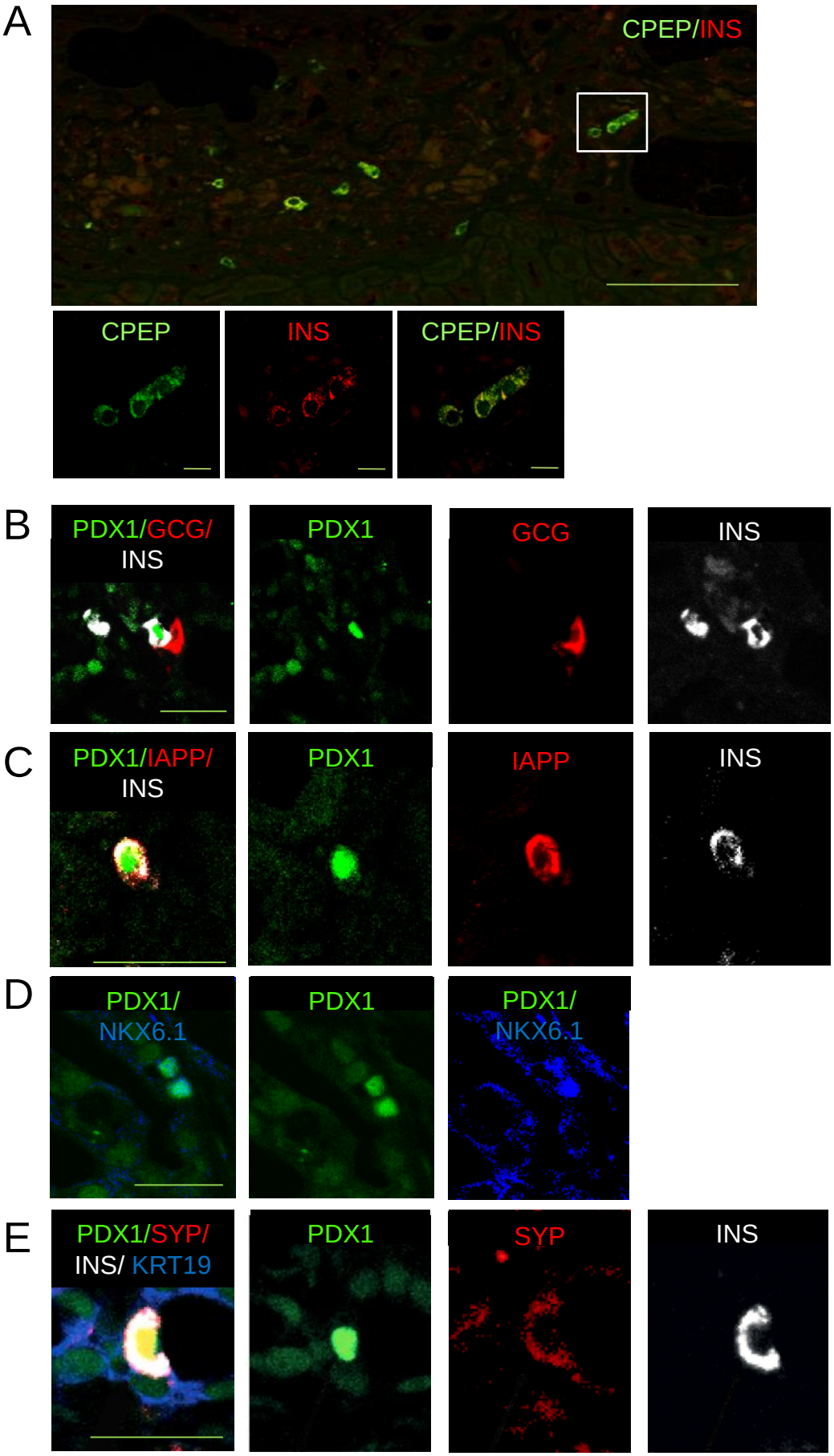

A
CPEP/INS
CPEP
INS
CPEP/INS
B
PDX1/GCG/
INS
PDX1
GCG
INS
C
PDX1/IAPP/
INS
PDX1
IAPP
INS
D
PDX1/
NKX6.1
PDX1
PDX1/
NKX6.1
E
PDX1/SYP/
INS/ KRT19
PDX1
SYP
INS

Table S1: Antibody list

Use	Antibody	Source	Supplier	Dilution	Amplification	Antigen retrieval	Cat. number
Primary	ALDH1A1	goat	R and D systems	1:100	biotin	none	AF5669
Primary	ALDH1A1	rabbit	Abcam	1:100	no	Citrate PH=6.0	ab52492
Primary	AMY2B	mouse	Santa Cruz	1:100	no	Citrate PH=6.0	sc-46657
Primary	C-PEP	mouse	Millipore	1:1000	no	not necessary	05-1109
Primary	CPA1	rabbit	Sigma-Aldrich	1:1000	no	none	HPA021836
Primary	GCG	rabbit	Vector labs	1:200	no	none	VP-G806
Primary	GCG	mouse	Sigma-Aldrich	1:100	no	none	G2654
Primary	HES1	rabbit	Santa Cruz	1:200	no	Citrate PH=6.0	Sc-25392
Primary	IAPP	Mouse	Anne Clark, OCDEM, Oxford	1:1000	no	Citrate PH=6.0	n/a
Primary	INS	Guinea pig	Millipore	1:200	no	none	4011-01F
Primary	Ki67	mouse	Invitrogen	1:200	Biotin	Citrate PH=6.0	18-0192Z
Primary	KRT19	mouse	Dako	1:20	Biotin	Proteinase K	M 0888
Primary	MUCIN1	Armenian hamster	Thermo Scientific	1:200	Biotin	Citrate PH=6.0	HM-1630-R7
Primary	NEUROG3	sheep	R and D systems	1:500	no	Citrate PH=6.0	AF3444
Primary	NKX6.1	rabbit	Sigma Prestige Antibodies	1:500	no	Citrate PH=6.0	HPA036774
Primary	PDX1-Biotinylated	Goat	R and D systems	1:5	Biotin	Citrate PH=6.0	BAF2419
Primary	SOX9	rabbit	Millipore	1:400	no	Citrate PH=6.0	AB5535
Primary	SYP	mouse	Millipore		no	Citrate PH=6.0	MAB5258
Primary	VIM	mouse	Dako	1:100	Biotin	Citrate PH=6.0	M0725
biotinylation	Biotin-anti mouse IgG (H+L)	donkey	Jackson Immuno research labs	1:200	no	n/a	715-065-151
biotinylation	Biotin-anti goat IgG (H+L)	rabbit	Dako	1:200	no	n/a	E 0466
biotinylation	Biotin-anti armenian hamster IgG (H+L)	goat	Affymetrix eBioscience	1:200	n/a	n/a	13-4113
Secondary	Anti rabbit IgG (H+L) Alexafluor 488/568	donkey	Molecular probes	1:200	n/a	n/a	A21206 (488) A10042 (568)
Secondary	Anti goat IgG (H+L) Alexafluor 488	donkey	Molecular probes	1:200	n/a	n/a	A11055
Secondary	Anti mouse IgG (H+L) Alexafluor 488/568	donkey	Molecular probes	1:200	n/a	n/a	A21202 (488) A11061 (568)
Secondary	Tritc-anti guinea pig	donkey	Jackson Immuno research labs	1:400	n/a	n/a	706-025-148
Secondary	Anti sheep IgG (H+L) Alexafluor 568	donkey	Molecular probes	1:200	n/a	n/a	A21099
Secondary	Streptavidin-Alexafluor 488/568	n/a	Molecular probes	1:200	n/a	n/a	S11223 (488) S11226 (568)
Secondary	Anti sheep-HRP	Rabbit	Dako	1:200	n/a	n/a	
Secondary	TSA Plus Cyanine 3/5 System	n/a	Perkin Elmer	1:50	n/a	n/a	NEL744001KT (568) NEL745001KT (647)
F-actin staining	Alexafluor 488 Phalloidin	n/a	Life Technologies	1:1000	n/a	not necessary	A12379
Nuclear staining	DAPI	n/a	Molecular probes	1:10000	n/a	not necessary	D1306

Table S2: Primer sequences

Gene name	Forward (5' →3')	Reverse (5' →3')
INS	GCAGCCTTTGTCAACCAACA	TTCCCCGCACACTAGGTAGAGA
GCG	CAAGGCAGCTGGCAACGT	CTGGTGAATGTGCCCTGTGA
SST	CCCAGACTCCGTCAGTTTCT	ATCATTCTCCGTCTGGTTGG
AMY	GATGGGTTGATATTGCTCTTG	GTATCTTTCCCACCAAGGTC
KRT19	CTACAGCCACTACTACACGAC	CAGAGCCTGTTCCGTCTCAAA
NEUROG3	ACCCTCACTCAAGTCTGTCTG	ATTGGGAGTAGAGCAGATCG
PDX1	CCATGGATGAAGTCTACCAAAGCT	CGTGAGATGTACTTGTTGAATAGGAACT
SOX9	GCCGAGCTCAGCAAGAC	TACTTGTAATCCGGGTGGTC
NKX6.1	CTGGCCTGTACCCCTCATCA	CTTCCCGTCTTTGTCCAACAA
CPA1	TGGTGTTGAGTGTCCTGTTG	GCTCCTTCACCTTCTGTACC
PTF1A	AGGCCCAGAAGGTCATC	AGGGAGGCCATAATCAGG
MYC	TCTCTGAAAGGCTCTCCTTG	CCTGTTGGTGAAGCTAACG
CCD1	GTCCTACTACCGCCTCACAC	AGCAGGGCTTCGATCTG
B2M	GCGCTACTCTCTCTTTCTGG	GCTGGATGACGTGAGTAAAC
GAPDH	GGAAGCTTGTCATCAATGG	TGATGATCTTGAGGCTGTTG

Figure S1. Donor variation in growth of human pancreatic organoids. Related to Figure 1.

A) Brightfield images of pancreatic organoids derived from 6 non-diabetic (non-DM) donors (I –VI) show variability in morphology observed after expansion for 7 days. No clear donor age or BMI dependent differences were observed that relate to variable morphology. Scale bars 50 µm.

B) Brightfield image of pancreatic organoids derived from a donor with Type 1 diabetes mellitus (T1DM), expanded for 7 days. Scale bar 50 µm.

Figure S2. Progenitor markers during expansion and *LGR5* enrichment in ALDH^{hi} cells. Related to Figure 2A-C.

A) Gene expression of *SOX9* and *PDX1* in expansion organoids (d7) compared to islet-depleted starting material (d0). Mean±SEM (n=3 donors).

B) *LGR5* gene expression in expansion organoids (d7) compared to islet-depleted starting material (d0). mean±SEM (n=3 donors).

C) Single molecule fluorescent *in situ* hybridisation (smFISH) of *LGR5* transcripts (red, see arrows) in an organoid budding structure at day 7 of expansion. Scale bar 50µm.

D) *LGR5* gene expression in sorted ALDH^{hi} compared to ALDH^{lo} cells at day 7 of expansion. mean±SEM (n=2 donors).

Figure S3. Characterization of organoids from ALDH^{hi} cells and islet-depleted pancreatic tissue. Related to Figure 2D-H.

A-B) Organoid-forming capacity of sorted ALDH^{hi} cells. A) Representative example of a sorted single ALDH^{hi} cell during expansion. Days of expansion are depicted above the images, the length of scale bars are in the bottom of the pictures. B) Confocal image of a wholemount-stained organoid formed after 2 weeks' expansion culture from a sorted ALDH^{hi} cell. Organoids consists of ALDH1A1^{+} cells (green) as well as ALDH1A1^{-} cells. Scale bar 50µm.

C-F) Gene and protein expression of exocrine and progenitor markers. C) Confocal image of a pancreatic organoid expanded for 7 days. ALDH1A1^{+} cells (green) were located at the tips of budding structures. Immunostaining for amylase was not observed. Scale bar 50µm. D) Confocal image of a

pancreatic organoid expanded for 7 days and immunostained for PDX1 (green) and ALDH1A1 (red). Scale bar 50µm. E) Gene expression for markers, known to be upregulated in mouse centroacinar cells, in sorted $ALDH^{lo}$ and $ALDH^{hi}$ cells derived from organoids expanded for 7 days. The graph shows the gene expression ratio in $ALDH^{hi}$ to $ALDH^{lo}$ cells for the different markers. Mean±SEM (n=2). F) Chromogenic immunostaining of HES1 (brown) in organoids on d7 of expansion. Arrows indicate examples of $HES1^{+}$ cells. Scale bar 50 µM.

Figure S4. Characterization of in vitro differentiated organoids from fresh and cryopreserved pancreatic tissue. Related to Figure 4.

A) Co-localisation of C-peptide with insulin in organoids in vitro. Confocal image of organoids expanded (7 days) and differentiated (7days) *in vitro*, immunostained for human C-peptide (CPEP; green) and insulin (INS; red). All INS^{+} cells in the organoids are also $CPEP^{+}$. DAPI (blue) was used as counterstain. Scale bars 10 µm.

B-C) Cryopreservation of human pancreatic organoids. B) Brightfield images of organoids grown from either freshly retrieved pancreatic tissue (Fresh) (upper panel) or cryopreserved pancreatic tissue (lower panel). Same donors were used to test characteristics (n=4). Representative pictures are shown from day 1 and day 7 expansion and from differentiation cultures (day 7). C) Gene expression profiles of organoids expanded from cryopreserved and fresh pancreatic tissue. Fold change in gene expression between differentiation and expansion conditions was calculated for organoids expanded from fresh and cryopreserved material (mean±SEM, n=4). No significant difference in gene expression was detected between fresh and cryopreserved organoids.

Figure S5. Endocrine markers in grafts of human pancreatic organoids. Related to Figure 5.

A) Immunostaining for C-peptide (CPEP; green) and insulin (INS; red) in grafts of pancreatic organoids, shows that all INS^{+} cells are also $CPEP^{+}$. Scale bar 100 µm. The bottom pictures show confocal images of the insert with co-localisation of C-peptide and insulin. DAPI (blue) was used as counterstain. Scale bars 10 µm.

(B-E) Confocal images from a graft of human pancreatic organoids. (B) PDX1 (green) co-localised with insulin (white), whereas these markers were not detected in GCG^+ cells (red); (C-E) PDX1 (green) also co-localised with IAPP (red), NKX6.1 (blue), and SYP (red). Scale bars 50 μM.

Supplemental movie S1. Formation of budding structures from human islet-depleted adult pancreatic tissue

Time-lapse imaging shows the formation of budding structures from human islet-depleted pancreatic tissue. Imaging was started at day 1 and images were taken for 2.5 days at a rate of 1 image per hour.

Supplemental Table S1: Antibodies used in this study. Related to Figure 1,2,4,5.

Supplemental Table S2: Primer sets used in this study. Related to Figure 2 and 4.

Human adult pancreatic tissue

Human islets were isolated according to a modified Ricordi method by the human islet isolation unit at the Leiden University Medical Center (LUMC). The islet-depleted tissue remaining after islet isolation was used in the studies within one day of islet isolation and the human islets were used within several days of islet isolation (after at least one day culture). CMRL-1066 medium (Cellgro) supplemented with 10% human serum (Blood bank LUMC, Leiden) and 1% penicillin/streptomycin (Invitrogen) was used to culture islet-depleted tissue and human islets. Pancreases from cadaveric organ donors with diabetes mellitus were excluded from the studies apart from one organ donor with a history of type 1 diabetes.

Expansion of human adult and fetal pancreatic organoids

Human adult pancreatic organoids

Small clumps of adult human pancreatic exocrine tissue were obtained by mechanical dissociation of the islet-depleted tissue with a glass pipette. These clumps were plated in 24-well plates (Costar) at a density of ~15-20 clumps in a 30-40 µl drop of Matrigel (Basement membrane Growth Factor Reduced; BD Biosciences) as previously described (Sato et al., 2011). We next added 'Expansion Medium' (450µl/well), consisting of Advanced DMEM/F12 (Invitrogen) supplemented with 10 mM HEPES (Invitrogen), 1x Glutamax (Invitrogen), 1% penicillin/streptomycin (Sigma): B27 supplement (Invitrogen), N-2 supplement (Invitrogen), 1,25 µM N-acetylcysteine (Sigma), 50ng/ml EGF (Peprotech), 10 nM Gastrin (Tocris), 100 ng/ml FGF10 (Peprotech), 10% R-spondin1 conditioned medium (prepared in house), 100 ng/ml Noggin (Peprotech) or 10% Noggin conditioned medium (prepared in house), 500nM TGFβ inhibitor (A83-01; Stemgent). This Epidermal Growth Factor/Noggin/R-spondin (ENR)-based medium was adapted from Sato et al. (Sato et al., 2009). Passages were performed each 7 to 14 days. During passaging the organoids were

retrieved from the Matrigel with ice-cold Advanced DMEM/F12, mechanically dissociated into small pieces and transferred to fresh Matrigel in a 1:4 split ratio. The expansion culture medium was refreshed every 2-3 days. Cryopreservation of adult pancreatic tissue was done directly in CryoStem™ Freezing Medium (Stemgent). CoolCell^{R} freezing containers (Biocision) were used to allow for controlled freezing of the tissue to -80ºC. Cells were stored in liquid nitrogen.

Human fetal pancreatic organoids

Fetal pancreas for organoid culture was either plated directly or cryopreserved at -80C in freezing medium (50% FCS, 50% DMSO). To prepare samples for seeding, cryopreserved fetal pancreas was first transferred from -80C to 3ml Advanced DMEM/F12 (Invitrogen) at 37C, and incubated for 10 min in an incubator, before repeating this twice in fresh medium. Defrosted cryopreserved samples or fresh fetal pancreas were then minced with a sterile razorblade to obtain small clumps of pancreatic tissue. Lastly, minced tissue was plated in Matrigel and cultured under similar conditions as adult pancreatic organoids. Samples for RNA-extraction were isolated, and stored at -80C for later analysis.

Growth curve of expansion organoids

Organoids were dissociated by trypsinisation with Tryple Express (Gibco) for 5 minutes to retrieve single cells. Cell numbers were counted with a Burker-Turk haemocytometer at the end of each passage. 5 wells were counted per time point and the mean number of cells per well was determined.

Expansion of organoids derived from dispersed single cells.

Human adult pancreatic organoids expanded for 7 days were dispersed by trypsinisation with Tryple Express (Gibco) for 5 minutes. Single cells were obtained by filtering the cell

suspension over a 40 µm cell strainer (Fisher scientific) and plated (1000 cells/well) in a drop of 50 µl Matrigel/well of a 24-well plate, and cultured with Expansion Medium (see above) supplemented with 10nM Rock inhibitor Y27632 (BioVision). Rock inhibitor enhances survival of dissociated cells in our organoid culture system, in line with published results for dissociated hESCs (Watanabe et al., 2007).

Aldefluor™ labelling and cell sorting

Labelling of progenitor cells in the organoids using the Aldefluor™ fluorescent reagent system (Stemcell) was performed according to the manufacturer's protocol. Organoids were exposed to the fluorescent reagent for 45-60 minutes. They were imaged (Leica AF7000) immediately in the presence of the Aldefluor assay buffer, which contains ABC transport inhibitors that prevent active efflux of the Aldefluor product. For FACS experiments, single cells were isolated as described above, labelled for 45 minutes with the fluorescent reagent, washed several times and collected in Aldefluor™ assay buffer. ABD FACSAria II was used for sorting $ALDH^{lo}$ and $ALDH^{hi}$ cell populations. 4-Diethylaminobenzaldehyde (DEAB; an ALDH enzyme inhibitor) was used to set $ALDH^{hi}$ and $ALDH^{lo}$ gates, according to the manufacturer's protocol. Standard sorting protocols were followed and first a cell size gate was set on FSC/SCC plots to remove debris, then FSC-W and SSC-W were plotted to enable doublet discrimination and third, a live cell gate was made using the live/dead marker (7-AAD).

Colony-forming unit assay

After labelling with Aldelfuor and FACS (as described above), single $ALDH^{lo}$ and $ALDH^{hi}$ cells were plated in a dilution series of 1000 cells/well to 1 cell/well in 5µL Matrigel (8 replica per dilution; n=4). Cells were then provided with Expansion Medium supplemented

with 10nM Rock inhibitor Y27632 (BioVision), and colony (organoid)-forming units were counted on day 7 of expansion.

In vitro differentiation of human pancreatic organoids

The differentiation culture medium consisted of serum-free Dulbecco's modified Eagle medium/F12 (Invitrogen) supplemented with 2g/l human albumin (CeAlb, Sanquin), Insulin-transferrin-selenite-X (100X) supplement (Gibco), used at 1:1000, 10 mM nicotinamide (Pharmacy Leiden University Medical Center) and 1% penicillin/streptomycin (Sigma) (Yatoh et al., 2007).

In vivo differentiation: transplantation under the kidney capsule

To assess further *in vivo* differentiation of human pancreatic organoids, transplantation experiments were performed in 8–12 week old male NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wjl}/SzJ (NOD scid gamma, NSG) immunodeficient mice (Jax labs). Mice were cared for according to institutional guidelines of the Leiden University Medical Center or Hubrecht Institute. Transplantation of organoids was performed in normoglycemic or hyperglycemic mice under isofluorane anaesthesia. Hyperglycemia was induced less than 6 days before transplantation with a single dose of 130 mg/kg streptozotocin (Sigma). Blood glucose levels were measured three times a week using an Accu-Check Comfort Glucometer (Roche), and animals with blood glucose concentration >18 mM were considered hyperglycemic. Randomization for blood glucose levels was done just before transplantation, assuring similar levels for each donor (n=8) in both groups. Organoids were retrieved from the differentiation medium and washed with PBS several times before approximately 2.5 – 3.0 mm^3 of tissue (2.4-4.5x10^5 cells) was packed in a PE50 transplantation tube. The tissue was then carefully placed under the kidney capsule using a Hamilton syringe with a threaded plunger. Blood glucose levels of

hyperglycemic animals were measured 3 times a week. Grafted tissue and blood plasma samples were obtained 1 day or 1 month after transplantation. INS^+ cells within or closely associated with the $KRT19^+$ ductal structures were counted in the grafts. Animal experiments were approved by the institutional animal welfare committee.

Imaging and immunohistochemical staining

For immunohistochemical analysis, organoids were fixed in 4% paraformaldehyde (PFA) and embedded in paraffin. Tissue grafts under the kidney capsule were also fixed in 4% PFA overnight. Tissue sections (4 μm) were cut and incubated with primary antibodies at 4ºC overnight and with secondary antibodies at room temperature for at least 2 hours. Primary antibodies, their dilution, source and amplification strategies are listed in Supplemental Table S1.

Fluorescent images were captured with a Leica SP5 confocal/multiphoton microscope or with a PerkinElmer ultraview vox spinning disk microscope. Images were further processed with either LAS AF lite or Volocity software, respectively. Quantitative analyses were performed with image J (NIH, Bethesda). The proportion of immunostained cells out of DAPI-positive cells is presented. Brightfield pictures were captured with a Leica DMIL microscope and processed with Leica LAS AF lite software.

Quantitative-PCR (qPCR) and analysis

For qPCR gene expression analysis, total RNA was extracted using the RNeasy Mini Kit (Qiagen) or with Trizol extraction (Invitrogen). Complementary DNA (cDNA) was prepared by reverse transcription of 1 μg total RNA using 25 ng Oligo dT-primers (Invitrogen), 0.5 mM dNTP (Fermentas), M-MLV RT 5x buffer (Promega), 10mM dithiothreitol (Promega), 40 Units RNasin (Promega) and 200 Units M-MLV reverse transcriptase (Invitrogen). cDNA

was used for gene profiling.To determine quantitative gene expression levels, real time PCR was performed in the Icycler iQ Real-Time PCR Detection System (Bio-Rad). PCR reactions were set up with 40ng cDNA and ready-to-use reaction master mix (iQ SYBR green supermix, Biorad). Primers used are specified in Supplemental Table S2. Gene expression levels were determined as the average of three replicate wells, and levels were normalised to each of two internal controls GAPDH and B2M. B2M-normalised data are shown, and all statistically significant changes were confirmed with GAPDH normalisation.

RNA-sequencing and analysis

Samples for RNA extraction were re-suspended in RLT buffer (Qiagen), and extractions were carried out using the RNeasy Mini or Micro RNA Extraction Kit (Qiagen). For mRNA sequencing, 10ng of RNA per sample was processed using the CEL-Seq protocol (Hashimshony et al., 2012), and 75 bp paired end sequencing was carried out on an Illumina Nextseq. Next, Read 1 was aligned with the hg19 RefSeq human transcriptome (available from UCSC genome browser (Kent et al., 2002)) using Burrows-Wheeler Alignment (Li et al., 2010) with default parameters. Read 2 contained a barcode that identified the sample-of-origin for that read. The CEL-Seq method sequences only a short stretch of the 3′ end of a transcript, and yields one read per transcript. Reads-per-million (RPM) normalization was used per sample and only genes with more than 10 RPM in 4 or more samples were accepted for subsequent analysis. The total transcript count in each sample was first normalized to the median number of transcripts per sample. After normalisation of the samples, R was used to generate a correlation heatmap based on spearman correlations, and a principle component analysis was performed. The accession number for the RNA sequencing data reported in this paper is GEO: GSE108854.

smFISH

Organoids for single molecule FISH (smFISH) assessment were harvested and freed from Matrigel using Cell Recovery Solution (BD Biosciences), according to the manufacturer protocol, and subsequently washed and fixed in 4% formaldehyde, 30% sucrose PBS solution overnight. Next, fixed samples were transferred to optimal-cutting-temperature compound in a mold and frozen. Frozen samples were then sectioned, and processed for smFISH as previously described (Itzkovitz et al., 2011). Individual transcripts were imaged using previously described methods (Raj et al., 2008).

Human C-peptide measurements in mice

To assess insulin secretory function after organoid transplantation in immunodeficient mice, analysis of human C-peptide, which is secreted in a 1:1 molecular ratio with insulin from insulin producing cells, was performed. Blood was drawn from tail cuts of normoglycemic or hyperglycemic mice. In normoglycemic animals, an intraperitoneal glucose challenge (2 g/kg) was performed 45 minutes before blood withdrawal in order to stimulate insulin-producing cells. The blood was centrifuged and plasma kept at –20 ºC. Analysis of human C-peptide was performed using the human-specific ultra-sensitive C-peptide ELISA kit (Mercodia) according to the manufacturer's instructions.

Statistical Analysis

Values are shown as mean ± standard error of the mean (SEM). The Mann-Whitney U test was used as a non-parametric test. *P* values <0.05 were considered significant. Statistical software comprised SPSS and GraphPad Prism. Power calculations for animal studies were based on the expected variations from the experiments in NSG mice and based on previous

experience with human pancreatic tissue grafts. All animals that survived and where grafts were found under the kidney capsule (92%) were included in the study. No blinding of investigators was done with animal studies and their analyses.

Reference List

Hashimshony, T., Wagner, F., Sher, N., and Yanai, I. (2012). CEL-Seq: single-cell RNA-Seq by multiplexed linear amplification. Cell Rep. *2*, 666-673.

Itzkovitz, S., Lyubimova, A., Blat, I.C., Maynard, M., van, E.J., Lees, J., Jacks, T., Clevers, H., and van, O.A. (2011). Single-molecule transcript counting of stem-cell markers in the mouse intestine. Nat. Cell Biol. *14*, 106-114.

Kent, W.J., Sugnet, C.W., Furey, T.S., Roskin, K.M., Pringle, T.H., Zahler, A.M., and Haussler, D. (2002). The human genome browser at UCSC. Genome Res. *12*, 996-1006.

Li, H. and Durbin, R. (2010). Fast and accurate long-read alignment with Burrows-Wheeler transform. Bioinformatics. *26*, 589-595.

Raj, A., van den Bogaard, P., Rifkin, S.A., van, O.A., and Tyagi, S. (2008). Imaging individual mRNA molecules using multiple singly labeled probes. Nat. Methods *5*, 877-879.

Sato, T., Stange, D.E., Ferrante, M., Vries, R.G., van Es, J.H., van den Brink, S., Van Houdt, W.J., Pronk, A., Van, G.J., Siersema, P.D. et al. (2011). Long-term expansion of epithelial organoids from human colon, adenoma, adenocarcinoma, and Barrett's epithelium. Gastroenterology *141*, 1762-1772.

Sato, T., Vries, R.G., Snippert, H.J., van de Wetering, M., Barker, N., Stange, D.E., van Es, J.H., Abo, A., Kujala, P., Peters, P.J. et al. (2009). Single Lgr5 stem cells build crypt-villus structures in vitro without a mesenchymal niche. Nature *459*, 262-265.

Watanabe, K., Ueno, M., Kamiya, D., Nishiyama, A., Matsumura, M., Wataya, T., Takahashi, J.B., Nishikawa, S., Nishikawa, S., Muguruma, K. et al. (2007). A ROCK inhibitor permits survival of dissociated human embryonic stem cells. Nat. Biotechnol. *25*, 681-686.

Yatoh, S., Dodge, R., Akashi, T., Omer, A., Sharma, A., Weir, G.C., and Bonner-Weir, S. (2007). Differentiation of affinity-purified human pancreatic duct cells to beta-cells. Diabetes *56*, 1802-1809.