Supporting Information

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SI Text

Dissociation of Adult Mouse Pancreas. All animal studies were approved by the Animal Care and Use Committee at Johns Hopkins University. Whole adult mouse pancreas was harvested and digested in 1.4 mg/mL collagenase-P (Boehringer Mannheim) at 37 °C for 30 min. Peripheral acinar-ductal units, depleted of large ducts and endocrine islets, were prepared as described previously (1–3). Following multiple washes with HBSS supplemented with 5% FBS, collagenase-digested pancreatic tissue was filtered through 600 μm and 100 μm polypropylene mesh (Spectrum Laboratories), then spun through a 30% FBS cushion. Peripheral acinar-ductal units were either subjected to whole-mount immunofluorescent labeling or further dissociated for FACS analysis. For FACS, pelleted acinar-ductal units were resuspended in diluted trypsin (0.05%) (Invitrogen) and incubated at 37 °C for 15 min. Dispersed cells were then directly resuspended in Aldefluor buffer.

Immunofluorescent and Immunohistochemical Labeling. Dissected embryonic pancreas from E10.5–E18.5 embryos or adult mouse pancreas was fixed in 4% paraformaldehyde overnight at 4 °C, cryoprotected in 30% sucrose-PBS for 4–6 h at 4 °C, OCT embedded and cut into 3- to 4-μm sections. Sections were permeabilized for 15–30 min in 0.2% Triton X-100 in PBS, and blocking of nonspecific reactivity was performed for 1 h in 10% FBS/0.2% Triton X-100 in PBS at RT. Primary antibodies were incubated at the appropriate dilutions in 5% FBS/0.2% Triton X-100 in PBS overnight: rabbit anti-glucagon 1:400 (Novus Biologicals), rabbit anti-ALDH1 1:200 (Abcam), rabbit anti-ALDH1/2 1:200 (Santa Cruz), guinea pig anti-insulin 1:400 (Biomeda), rat anti-E-cadherin 1:400 (Zymed), rabbit anti-Sox9 1:1,000 (Chemicon), goat anti-insulin C-peptide 1:500 (Millipore), and rabbit anti-amylase 1:400 (Sigma). The next morning, slides were washed three times in 0.2% Triton X-100 in PBS, and sections were incubated with the appropriate Cy2- and/or Cy3- and/or Cy5-conjugated secondary IgG antibodies at 1:200 dilution for 1 h at RT in the dark. After three more washes in PBS, nuclei were labeled with DAPI (1:1,000) and slides were mounted in Vectashield mounting medium. Images were acquired using a Zeiss Axiovert imaging microscope. A similar protocol was used for whole-mount immunofluorescent labeling of collagenase-digested pancreas. For immunofluorescent labeling of FACS-sorted single cells, 7,000–8,000 sorted cells were pelleted at 1,200 rpm for 3 min onto coated slides using a Shandon Cytospin 4 (Thermo Electron) and dried at room temperature for 5 min before labeling. Immunohistochemical analysis of ALDH1 expression in normal and caerulein-treated adult mouse pancreas was performed as described (4).

Aldefluor Assay and Sorting of Aldefluor-Positive and -Negative Cells by FACS. The Aldefluor Kit (StemCell Technologies) was used to isolate population with high vs. low ALDH enzymatic activity (hereafter referred to as Aldefluor positive and Aldefluor negative). Dispersed cells resuspended in Aldefluor assay buffer containing ALDH substrate (BAAA, 0.6 ng/μL per $1{\cdot}10^7$ cells in 1 mL) were incubated for 50 min at 37 °C. As a negative control to confirm the specificity of Aldefluor labeling, an aliquot of $5{\cdot}10^6$ cells from each sample was treated with 1.6 mM diethylaminobenzaldehyde (DEAB), a specific ALDH inhibitor. The sorting gate of the Aldefluor-positive cells was established using DEAB-treated cells as a guide, so that the Aldefluor-positive population was defined by DEAB-sensitive Aldefluor activity. To exclude the possibility that the Aldefluor-positive population was contaminated with endocrine cells, pancreatic tissue was also harvested from *Tg(Ins1-DsRed*T4) 32Hara/J* mice (5) (obtained from the Jackson Laboratory) and subjected to FACS. Flow cytometry was performed using a FACSAria (Becton Dickinson) flow cytometer.

Labeling with additional antibodies was performed subsequent to Aldefluor staining and without permeabilization. Primary antibody incubations were done in the dark on ice in 10% FBS/PBS for 45 min. Following washes, incubation with the secondary antibodies was performed on ice in 5% FBS/PBS for 45 min. The following antibodies were used for flow cytometry: rat anti-E-cadherin (Zymed), rat anti-CD133 (eBioscience), rat anti-Sca-1 (eBioscience), rat anti-Pecam (BD Biosciences), and rat anti-CD45 (BD Biosciences).

RT-qPCR. Total RNA was prepared using the RNeasy Micro Kit (QIAGEN). RT-qPCR was performed using a C1000 Thermal Cycler Thermo (BioRad) and the IQ SYBR Green SuperMix (BioRad). The PCR volume was 20 μL containing 1.5 μL of diluted cDNA and 250 nM of each primer. Thermocycling conditions included an initial polymerase activation step for 3 min at 95 °C, followed by 40 cycles of 30 s at 95 °C for template denaturalization, 30 s at 58 °C for annealing, and 30 s at 72 °C for extension and fluorescence measure. Afterward, a dissociation protocol with a gradient from 65 °C to 95 °C was used for each primer pair to verify the specificity of the RT-qPCR reaction and the absence of primer dimers. In addition, each PCR included a reverse-transcription negative control to check for potential genomic DNA contamination. Reagent contamination was also detected by a reaction mix without template. All samples were amplified in duplicate and normalized against GAPDH as an internal control. The relative quantification of mRNA was performed with the CFX96 Real-Time PCR Detection System.

Pancreatosphere Formation Assay. Pancreatosphere formation assays on sorted Aldefluor-positive and -negative cells were performed by plating cells in 24-well ultra-low attachment plates (Corning) at a density of 6 cells/μL. Cells were grown for 5–7 days in DMEM/F-12 (GIBCO, Invitrogen), 1× N2 Supplement (StemCell Technologies), 20 ng/mL EGF (Peprotech), 20 ng/mL FGF2 (Invitrogen), 1× B27 (StemCell Technologies), 100 μM β-mercaptoethanol (Sigma), 1× nonessential amino acid (Sigma), 1× [enicillin/streptomycin (Cellgro), 10 ng/mL LIF (Sigma), and 3% FBS (GIBCO, Invitrogen). For quantitative assays of pancreatosphere formation, cells were sorted directly into 96-well ultra-low attachment plates (Corning) at a density of 1, 10, or 100 cells per well (0.01 cell/μL; 0.1 cell/μL; 1 cell/μL). Serial passages were performed by dissociating spheres using the NeuroCult Chemical Dissociation Kit (StemCell Technologies), selecting viable cells based on trypan blue exclusion, and replating at a density of 6 cells/μL.

Insulin (C-peptide) Secretion Assays. Assays of pancreatosphere insulin secretion were performed after 7 days in culture. For comparison, parallel assays were performed on Ins-1 cells (clone 832/13). Pancreatospheres were washed with PBS to eliminate any remaining FBS and then incubated in D-glucose-free RPMI supplemented with 0.25% BSA at three different glucose concentrations (0 mM, 5 mM, and 11 mM). After 12 h, we removed media, lysed pancreatospheres in 1 M glacial acetic acid, and determined insulin C-peptide levels by ELISA (Alpco). Insulin C-peptide secretion was expressed as a fraction of total cellular C-peptide content.

Injection of Aldefluor-Positive/mCherry Cells in E12.5 Embryonic Pancreas. Dissected embryonic pancreas from E12.5 embryos were isolated and cultured in RPMI1640 + 10% FCS, and

microinjected (Eppendorf TransferMan NK) with 1,000 Aldefluor-positive or -negative cells isolated from adult (4- to 8-week-old) *pCAG:mCherry* transgenic mouse pancreas. *pCAG:mCherry* mice were kindly provided by Dr. Michael Wolfgang, Johns Hopkins University. The embryonic buds were kept in culture for 7 days, changing the medium every other day (1).

1. Esni F, Miyamoto Y, Leach SD, Ghosh B (2005) Primary explant cultures of adult and embryonic pancreas. *Methods Mol Med* 103:259–271.
2. Miyamoto Y, et al. (2003) Notch mediates TGF alpha-induced changes in epithelial differentiation during pancreatic tumorigenesis. *Cancer Cell* 3:565–576.
3. Means AL, et al. (2005) Pancreatic epithelial plasticity mediated by acinar cell transdifferentiation and generation of nestin-positive intermediates. *Development* 132:3767–3776.
4. Strobel O, et al. (2007) In vivo lineage tracing defines the role of acinar-to-ductal transdifferentiation in inflammatory ductal metaplasia. *Gastroenterology* 133: 1999–2009.
5. Hara M, et al. (2006) Imaging pancreatic beta-cells in the intact pancreas. *Am J Physiol Endocrinol Metab* 290:E1041–E1047.

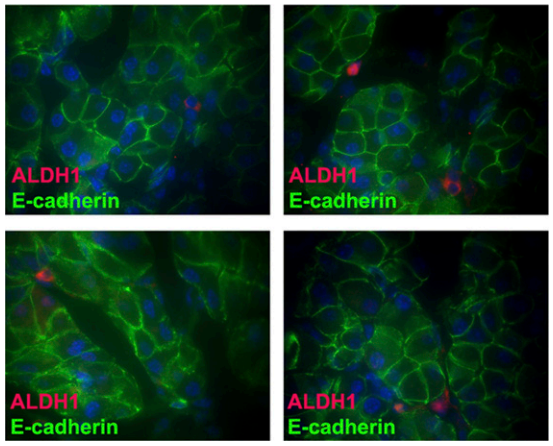

Fig. S1. Additional presence of ALDH1-positive, E-cadherin-negative mesenchymal cells in adult mouse pancreas.

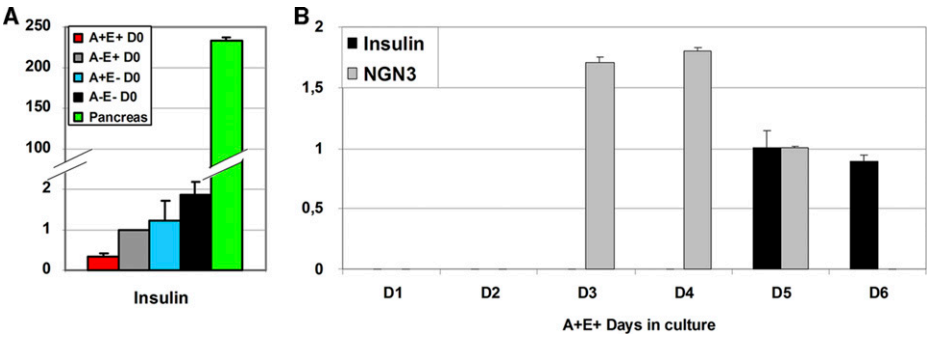

Fig. S2. Detection of transcripts for insulin (*A* and *B*) and Ngn3 (*B*) by qRT-PCR. (*A*) quantification of insulin transcripts in freshly sorted Aldefluor-positive, E-cadherin-positive (A+E+; red), Aldefluor-negative, E-cadherin-positive (A−E+; gray), Aldefluor-positive, E-cadherin-negative (A+E−; blue), Aldefluor-negative, E-cadherin-negative (A−E−; black), and total pancreas (green). Note marked depletion of insulin expression in all four sorted cell fractions, confirming marked depletion of islets in preparations of peripheral acinar-ductal units used for cell sorting. (*B*) Sequential activation of Ngn3 and insulin expression in pancreatospheres formed from A+E+ cells.

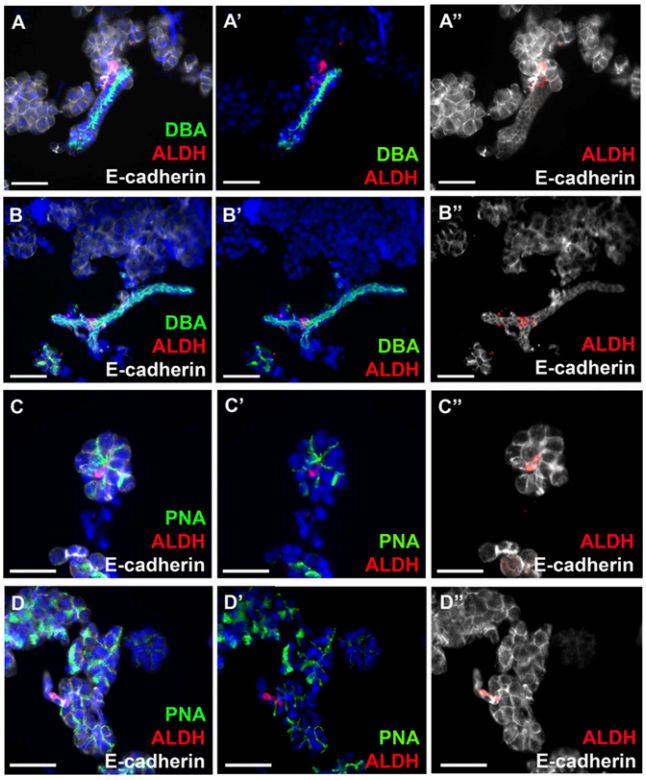

Fig. S3. Adult ALDH1-expressing cells are localized at the junction of terminal ductal epithelium and exocrine acini. Following collagenase digestion and isolation of terminal acinar-ductal units, whole-mount immunofluorescent labeling was performed for ALDH1 protein (red) in combination with E-cadherin (white) and FITC-conjugated DBA to mark terminal ductal epithelium (*A*, *A′*, *A′′*, *B*, *B′*, *B′′*) or FITC-conjugated PNA to mark the apical membrane of acinar cells (*C*, *C′*, *C′′*, *D*, *D′*, *D′′*). Note that E-cadherin-positive ALDH1-expressing cells are located in centroacinar and terminal ductal positions. (Scale bars: 50 μM.)

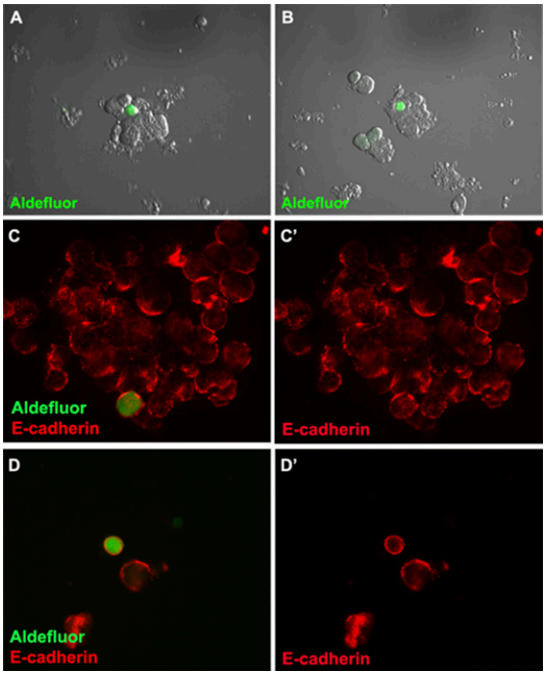

Fig. S4. Images of living ALDH1-expressing cells within peripheral acinar-ductal units isolated from collagenase-digested mouse pancreas. ALDH1 enzymatic activity is revealed by labeling with the Aldefluor reagent (green). Note terminal ductal/centroacinar position (*A* and *B*), as well as positive membrane labeling for E-cadherin (*C* and *D*). Images in *C′* and *D′* correspond to images in *C* and *D*, with ALDH1 labeling removed.

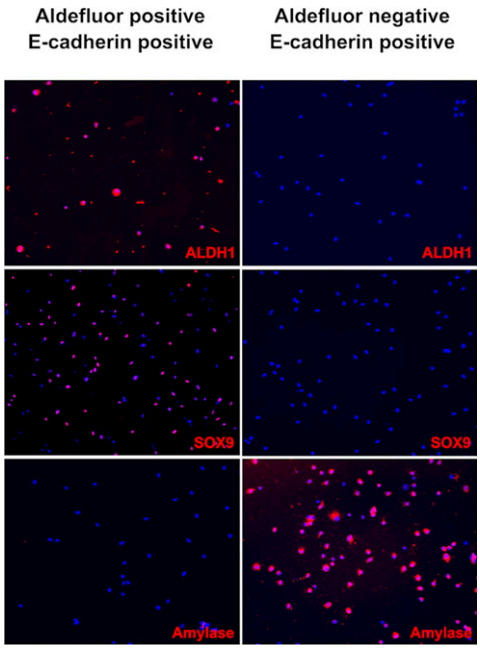

Fig. S5. FACS-sorted Aldefluor (+), E-cadherin (+) and Aldefluor (–), E-cadherin (+) epithelial cells display differential expression of ALDH1, Sox9, and amylase protein as assessed by immunofluorescent labeling of cytospin preparations.

Table S1. Rates of pancreatosphere formation 7 days following plating of FACS-sorted Aldefluor-positive, E-cadherin-positive (A+E+), Aldefluor-negative, E-cadherin-positive (A−E+), Aldefluor-positive, E-cadherin-negative (A+E−), and Aldefluor-negative, E-cadherin-negative (A−E−) cells

Cell type	No. of cells plated per well	Total wells	Wells with sphere(s) at day 7	% of wells with spheres
A+E+	1	19	3	15.8
A+E+	10	20	7	35.0
A+E+	100	12	7	58.3
A−E+	1	21	0	0.0
A−E+	100	24	1	4.2
A+E−	1	17	0	0.0
A+E−	10	19	0	0.0
A+E−	100	12	1	8.3
A−E−	1	17	0	0.0
A−E−	100	24	0	0.0