Supplementary Materials and Methods

DT Treatment

To induce DTR expression globally in the pancreas, we crossed $R26^{DTR}$ or $R26^{DTR/lacZ}$ with PdxCre mice. Eight-week-old mice were injected intraperitoneally daily for 5 days with 0.5 ng/g body wt DT (Sigma-Aldrich, St Louis, MO) and killed at different time points up to 45 days after DT administration (Supplementary Figure 1*A*). Throughout this report, days 1 to 7, 8 to 25, and 26 to 45 after last DT injections are referred to as early, mid, and late stages, respectively, because of variable progression of the phenotype after ablation.

To allow DTR expression selectively in acinar cells, we crossed $R26^{DTR}$ or $R26^{DTR/lacZ}$ with ElaCreERT2 mice. Eight-week-old mice were first injected intraperitoneally with tamoxifen for 5 days to activate Cre. After a 7-day wait to allow for Cre-recombinase action, 0.5 ng/g body wt DT injections were performed for 5 consecutive days. Mice were killed 1, 3, 5, 7, or 8 days after DT administration (Supplementary Figure 1*B*).

Tamoxifen Treatment

Tamoxifen (Sigma-Aldrich) was dissolved in 100% ethanol at 100 mg/mL and subsequently resuspended in corn oil (Sigma-Aldrich) at a concentration of 10 mg/mL. Six-week-old mice were injected intraperitoneally with 2 mg tamoxifen for 5 consecutive days, for a total of 10 mg.

Immunofluorescence

For immunolabeling on cryopreserved sections, harvested pancreata were fixed overnight at 4°C in 4% paraformaldehyde, incubated in 30% sucrose solution overnight at 4°C, and subsequently embedded with OCT compound. Cryosections (5–6 μm) were collected serially so that each slide would contain semiadjacent sections across the entire tissue. Sections were permeabilized with 0.1% phosphate-buffered saline (PBS)/Triton X-100, washed in PBS, and blocked for 30 minutes in 10% normal donkey serum in 0.1% PBS/Tween. For BrdU staining, slides were pretreated with 2 mol/L HCl for 30 minutes at room temperature before permeabilization and blocking. For cytokeratin-19 staining, tissues were snap frozen in OCT and sections were subsequently fixed in 4% paraformaldehyde at room temperature for 10 minutes before permeabilization and blocking. Primary antibodies were incubated overnight at 4°C, whereas secondary antibodies were incubated for 1 hour at room temperature. Images were acquired on a Zeiss Imager Z1 microscope with a Zeiss AxioCam driven by Zeiss AxioVision Rel.4.7 software (Zeiss, Thornwood, NY).

For quantification analysis, marker$^+$ cells were counted using ImageJ software.

The sources of antibodies and dilutions used are summarized in Supplementary Table 1.

H&E Staining

For H&E staining, tissues were fixed in 4% paraformaldehyde, dehydrated in ethanol, and paraffin embedded. H&E staining was performed on 5-μm-thick sections according to standard protocol.

X-gal Staining

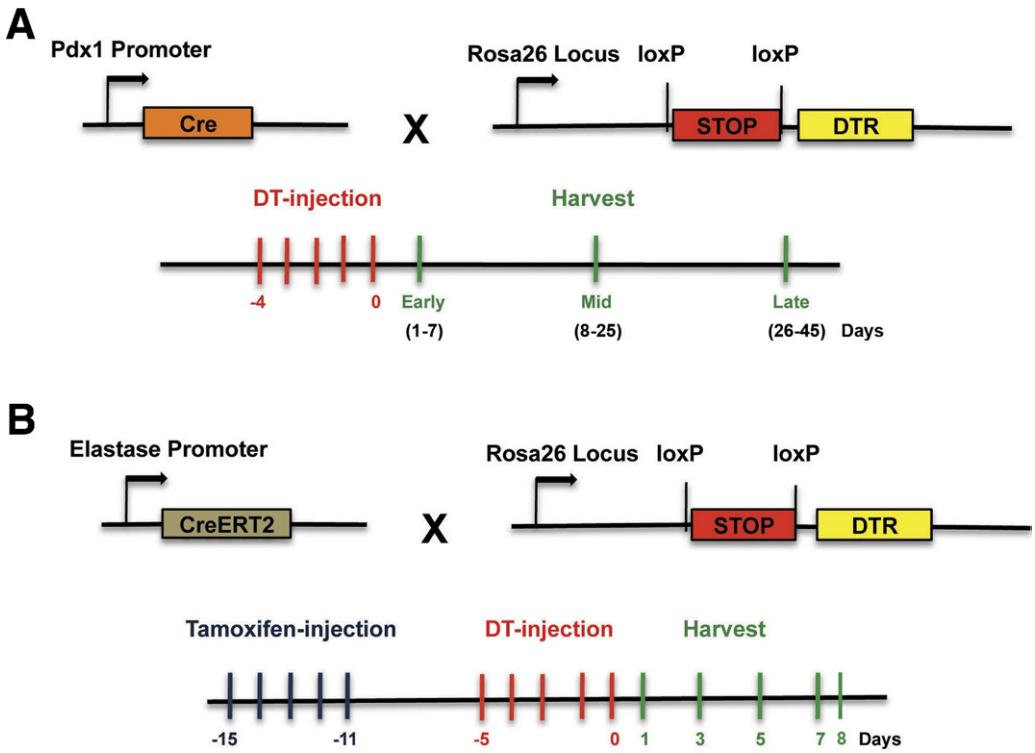
For β-galactosidase staining (X-gal staining), tissues were fixed at room temperature for 2 hours in 2% paraformaldehyde, washed in PBS and Rinse Buffer (2 mmol/L $MgCl_2$, 0.01% sodium deoxycholate, 0.02% Nonidet P-40 in PBS), and then incubated overnight at 37°C with X-gal solution (1 mg/mL X-gal [Research Products International Corp, Mount Prospect, IL], 5 mmol/L $K_4Fe(CN)_6$, 5 mmol/L $K_3Fe(CN)_6$ in Rinse Buffer). Following washes in PBS, tissues were postfixed in 4% paraformaldehyde at room temperature for 10 minutes, incubated in 30% sucrose solution overnight, and subsequently embedded with OCT compound. Cryosections were prepared as described for immunolabeling and counterstained with hematoxylin.

BrdU Labeling (Pulse-Chase) and Terminal Deoxynucleotidyl Transferase–Mediated Deoxyuridine Triphosphate Nick-End Labeling Assay

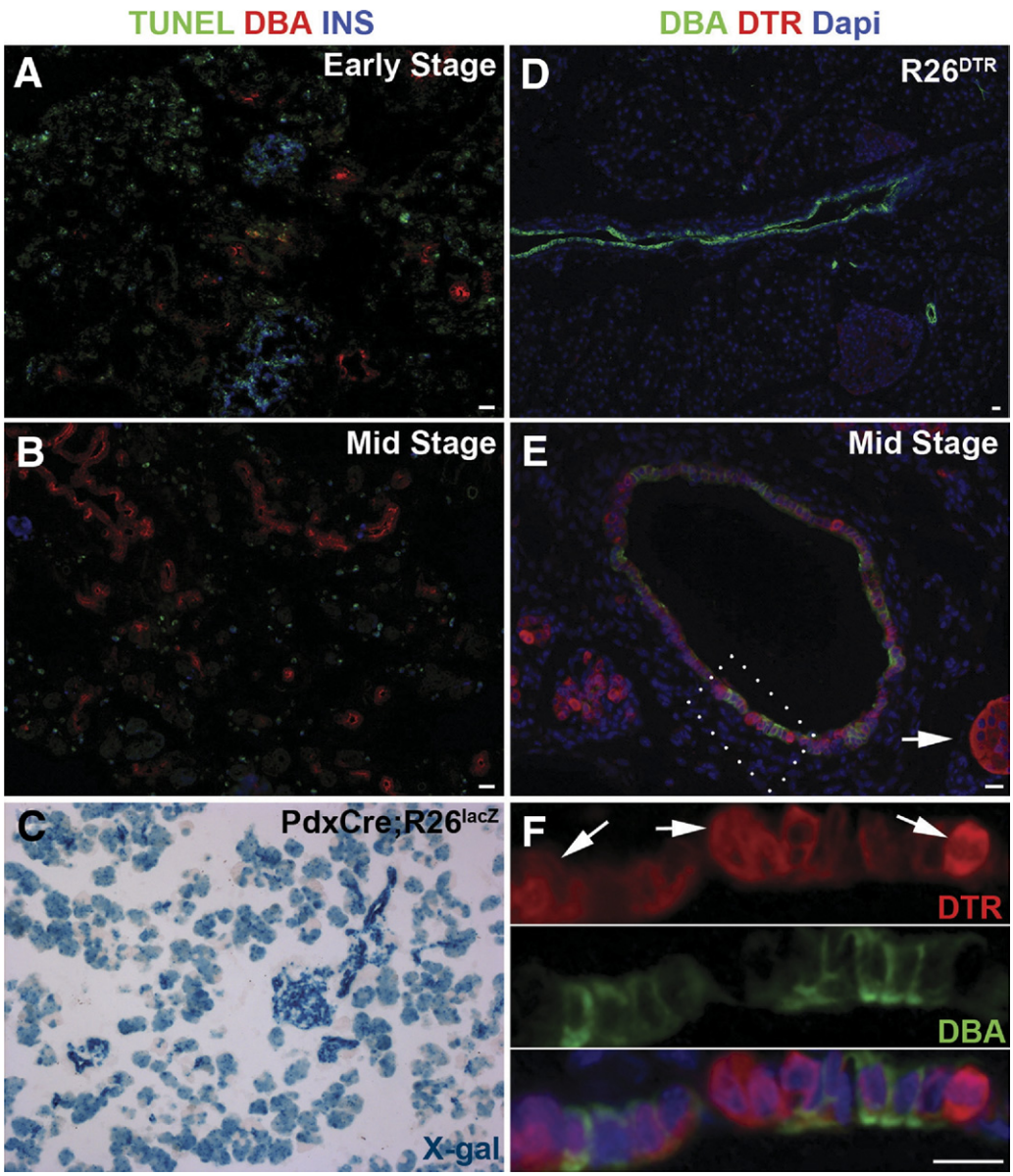
BrdU labeling was performed by injecting BrdU intraperitoneally (0.2 mg/g body wt) (Sigma) daily for 2 consecutive days before the mice were killed. Alternatively, BrdU (0.8 g/L) was provided in drinking water for 2 days, with water changed daily. Apoptotic cells were recognized by TUNEL assay with a TUNEL Apoptosis Detection Kit (Millipore, Billerica, MA), following the manufacturer's instructions.

Real-Time Quantitative Polymerase Chain Reaction

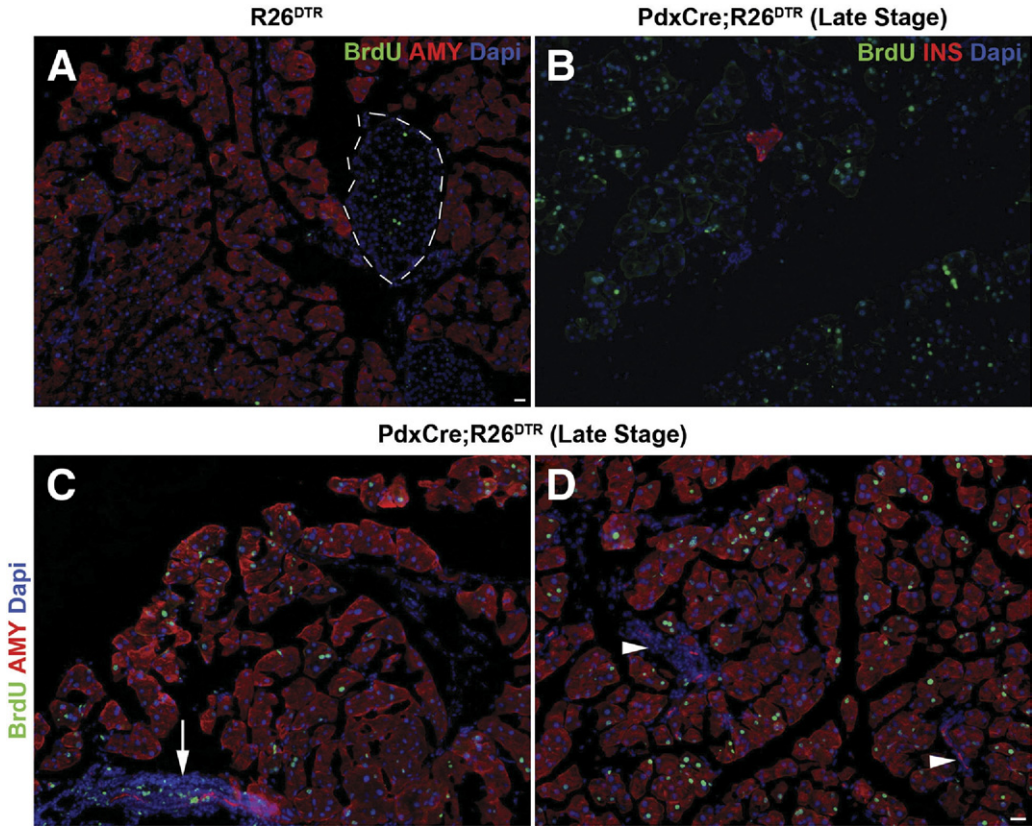
After harvesting, tissues were preserved in RNAlater (Ambion, Austin, TX) and stored until use. Messenger RNA isolation and subsequent complementary DNA synthesis were performed using μMACS One-step cDNA Kit (Miltenyi Biotec, Auburn, CA) according to the manufacturer's instructions. Primers were purchased from Qiagen (QuantiTect Primer Assays; Valencia, CA) and are listed in Supplementary Table 2. Reactions were performed with QuantiTect SYBR Green PCR Kit (Qiagen) using a LightCycler 1.5 Instrument (Roche, Branchburg, NJ). Reactions were performed at least in triplicate. Specificity of the amplified products was determined by melting peak analysis. Quantification for each gene of interest was performed with the $2^{-\Delta\Delta Ct}$ method. Quantified values were normalized against the housekeeping gene GAPDH, which proved to be stable across the samples.



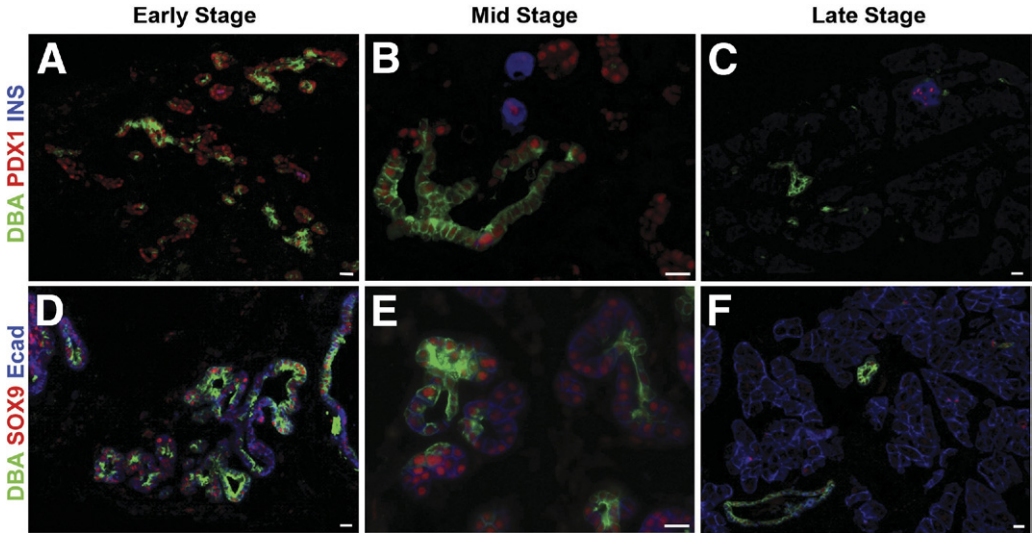
Supplementary Figure 1. Experimental design of the (*A*) PdxCre;R26^{DTR} and (*B*) ElaCreERT;R26^{DTR} transgenic mouse model.



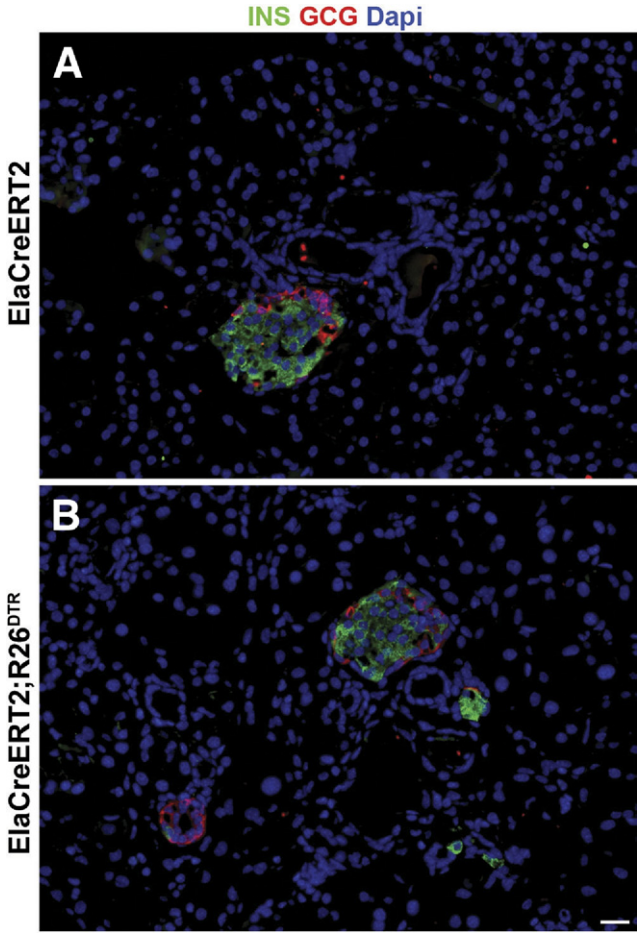
Supplementary Figure 2. (*A*) TUNEL assay in PdxCre;R26^{DTR} mice. DBA/TUNEL/insulin triple staining showed a high level of apoptosis among non-DBA^{+} epithelial cells (*A*) early after insult but (*B*) not during mid stage regeneration. (*C*) X-gal staining of PdxCre;R26^{lacZ} pancreas revealed high penetrance of Cre-transgene in all pancreatic epithelial cell lineages in the PdxCre strain. Immunofluorescent analyses of sections obtained from (*D*) R26;^{DTR} control and (*E* and *F*) mid stage regenerating PdxCre;R26^{DTR} pancreas for detection of DBA and DTR revealed expression of DTR in DBA^{+} cells. (*F*) Higher magnification of *E*. *Arrow* in *E* highlights a regenerated acinus. *Arrows* in *F* mark cytoplasmic and membrane localization of DTR in DBA^{-} cells within the duct-like structures. *Scale bars* = 20 μm.



Supplementary Figure 3. Pulse-chase BrdU experiments. Immunofluorescent analyses of (*A*) R26^{DTR} control or (*B–D*) day 30 after DT treatment regenerating PdxCre;R26^{DTR} pancreas using antibodies against (*A*, *C*, and *D*) BrdU/amylase or (*B*) BrdU/insulin. BrdU was administrated for 2 days on the day of last DT injection and the following day via the drinking water, and the pancreas was harvested on day 30 after BrdU withdrawal. *Arrow* in *C* marks a large duct with retained BrdU. *Arrowheads* in *D* highlight smaller ducts with no retained BrdU. *Dotted lines* highlight an islet. *Scale bars* = 20 μm.



Supplementary Figure 4. Immunofluorescent analyses of sections obtained from (*A* and *D*) early-stage, (*B* and *E*) mid stage, or (*C* and *F*) late-stage regenerating DT-treated PdxCre;R26^{DTR} pancreas for detection of (*A–C*) DBA/PDX1/insulin or (*D–F*) DBA/SOX9/E-cadherin. (*A* and *B*) In DT-treated PdxCre;R26^{DTR} pancreata, surviving ductal cells re-expressed PDX1 in early and mid stages. (*C*) Notably, once regeneration was completed, Pdx1 expression was once again restricted to β-cells. *Scale bars* = 20 μm.



Supplementary Figure 5. Acinar-specific cell ablation in tamoxifen-induced DT-treated ElaCreERT2;R26^{DTR} pancreas. Double immunostaining of tissues obtained from (*A*) control ElaCreERT2 or (*B*) regenerating ElaCreERT2;R26^{DTR} pancreas using antibodies recognizing insulin or glucagon showed the survival of nonacinar cells. *Scale bar* = 20 μm.

Supplementary Table 1. List of Antibodies Used for Immunofluorescence Analysis

Antigen	Species	Company	Catalog no.	Dilution
E-cadherin	Goat	R&D	AF748	1:200
E-cadherin	Rat	Invitrogen	13–1900	1:200
panCK	Rabbit	DAKO	Z0622	1:100
CK19	Rat	DSHB	TROMA III	1:100
CD31 (PECAM)	Rat	BD Pharmingen	BD550274	1:50
Insulin	Guinea pig	Linco/Millipore	4011–01	1:1000
Insulin	Mouse	Abcam	Ab8305–100	1:500
Glucagon	Guinea pig	Linco/Millipore	4031–01F	1:1000
Glucagon	Rabbit	Linco/Millipore	4030–01F	1:2000
Pdx-1	Rabbit	Abcam	AB47267	1:2000
Pdx-1	Goat	Abcam	AB47383	1:10,000
Sox9	Rabbit	Millipore	AB5535	1:1000
Ngn3	Guinea pig	Dr Maike Sander		1:500
Amylase	Rabbit	Sigma	A8273	1:300
Amylase	Goat	Santa Cruz Biotech	Sc-12821	1:250
FITC-DBA		Vector Laboratories	FL1031-2	1:100
BrdU	Rat	Abcam	AB6326	1:100
β-gal	Chicken	Abcam	9361	1:1000
HB-EGF	Goat	R&D Systems	AF-259-NA	1:500

NOTE. All secondary antibodies 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-conjugated donkey anti-guinea pig, anti-rabbit, anti-rat, anti-mouse, anti-goat (all 1:300).

Supplementary Table 2. List of Primers Used for qRT-PCR

Gene	Qiagen QuantiTect Primer Assay	Catalog no.
Notch1	Mm_Notch1_1_SG	QT00156982
Hes1	Mm_Hes1_1_SG	QT00313537
Mist1	Mm_Bhlha15_1_SG	QT00315182
Ptf1a	Mm_Ptf1a_1_SG	QT00124187
PDX1	Mm_Pdx1_1_SG	QT00102235
NGN3	Mm_Neurog3_1_SG	QT00262850
NeuroD	Mm_Neurod2_1_SG	QT00248892
MAFA	Mm_Mafa_2_SG	QT01037638
INS2	Mm_Ins2_1_SG	QT00114289
GCK	Mm_Gck_1_SG	QT00140007
GLUT2	Mm_Slc2a2_1_SG	QT00103537