Supplemental Materials and Methods

Generation of PACE-trypsinogen transgenic mice. To generate PACE-trypsinogen, a fragment containing loxP-GFP-stop-loxP (LGL) followed by mutant PACE-trypsinogen (Gene ID 25052) [1] was cloned into the pCAGGS vector (obtained from BCCM/LMBP Plasmid Collection, Belgium) [2], which contains a chimeric human CMV-IE enhancer and chicken β-actin promoter (CAG). The construct was digested with *Spe*I and *Hin*dIII to remove vector sequences and subsequently purified. Microinjection of this fragment into fertilized pronuclei of C57BL/6 mice was performed at the institutional Genetically Engineered Mouse Facility following standard procedures. Founder mice were identified using standard PCR genotyping with crude tail DNA and primers spanning the LGL cassette and PACE-trypsinogen (forward, TGGAGTTCGTGACCGCCGCCG; reverse, CTGCTCATTGCCCTCAAGGAC). Similar protein expression levels were found in each of the founder lines.

Conditional transgenic expression of PACE-trypsinogen in pancreatic acinar cells. To target conditional expression of the transgene specifically to pancreatic acinar cells, PACE-trypsinogen transgenic mice were bred with full length elastase promoter regulated CreErT transgenic mice [3]. While the Cre- animals used in this study have previously been found to possess significant tamoxifen-independent Cre-activity [3], we did not observe any obvious morphological changes in LGL-PACE-trypsinogen X CreErT mice without tamoxifen induction (data not shown). To initiate expression of PACE-trypsinogen, Cre recombination was induced with TM as previously described [4].

Briefly, TM was dissolved in a 20% ethanol and 80% corn oil solution (100mg/ml, Sigma) and adult animals (35-50d of age) were given daily TM doses of 5 mg orally for five consecutive days (TM^{max}, Supplemental Figure 1A) or in gradual repetitive fashion once every 5^{th} day for a period of 40d (TM^{grad}, Supplemental Figure 1B). For experimental analyses sex and age matched animals were killed at specified times after the initial TM treatment. Single transgenic littermates received the same treatment and served as controls.

Preparation of tissue samples and histopathological evaluation. Mice were killed and their pancreata were carefully removed, rinsed with phosphate-buffered saline, and either fixed in 10% buffered formalin overnight or embedded in OCT medium and frozen. Serum and snap-frozen pancreatic tissue samples were also prepared following standard procedures. Formalin-fixed samples were then embedded in paraffin, serially sectioned (5 μm thick) and stained with standard H&E. Histopathological examination was performed by a pathologist familiar with typical findings of experimental as well as human pancreatitis. Evaluation was performed in a blinded manner. Prevalence of pancreatic edema, inflammatory infiltration (including neutrophilic leucocytes and including monocytes and macrophages), stellate cell activation, presence of autophagic vacuoles and fat as wells acinar cell mitosis, apoptosis and necrosis were assessed. The extent of affected acinar cells was also estimated. To build a histology score the sum of all features present (present = 1 point) and the extent of affected acinar cells (1-4 points) were used. The resulting range of this score from 0 (normal) to 13 (complete destruction of the pancreas) allowed

quantification of the occurring histopathological changes. To assess the extent of lung injury a similar strategy was applied. After H&E staining lung injury was defined as alveolar membrane thickening or disruption and inflammatory infiltration. Degree of lung injury was given a score dependant on the average extent of 10 different vision fields (200x) as follows (score range 0-3): intact lung alveoli, no apparent inflammation and presence of only minor irregularities of alveolar membrane (score 0); apparent alveolar membrane thickening in most areas, possible inflammation (score 1); extensive alveolar membrane thickening, reducing the original lumen by >40%, obvious inflammatory infiltration (score 2). Alveolar lumen is nearly total occluded by swollen alveolar membranes and infiltrating inflammatory cells (score 3). Scores from 3 independent researchers were averaged and used for semiquantitative group comparison.

Western blot analysis. Western blotting was performed using standard procedures. Briefly, snap frozen pancreatic samples were homogenized in lysis buffer (50mM HEPES, pH7.5, 150mM NaCl, 2.5mM EDTA, 0.1% Tween-20, 1mM dithiothreitol, 1mM NaF, 0.1mM sodium orthovanidate, 0.1mM phenyl-methylsulfonyl fluoride, protease inhibitor cocktail: complete mini, Roche Diagnostics), sonicated twice and centrifuged to remove cellular debris. Samples of 50 μg protein were denatured with Laemmli buffer and subjected to electrophoresis on sodium dodecyl polyacrylamide gels and transferred by electroblotting to nitrocellulose membranes. Primary antibodies for immunodetection were anti-hemagglutinin (anti-HA, 1:4000, Rockland) and anti-RPS6 (1:1000, Santa Cruz). Appropriate fluorescent dye-labeled secondary

antibodies were used to allow detection with the Odyssey Infrared Imaging System (LI-COR Biosciences).

*Sample processing and immunohistochemistry***.** Mice pancreata were either frozen in optimal cutting temperature compound (OCT, Tissue-Tek) or fixed in 10% formalin (24-48h) and embedded in paraffin. Immunohistochemical (IHC) staining for cleaved caspase-3 (1:200, Cell Signaling), HMGB1 (1:1000, Abcam), p65 (1:50, Cell Signaling) and Ki67 (1:200, Lab Vision) was performed in pancreatic paraffin sections. Briefly, after deparaffinization and antigen retrieval sections unspecific binding was blocked and primary antibodies were applied (4°C, over night). Then after washing incubation with the appropriate HRP-labeled polymers (BioCare) was performed. Positive labeling was detected by exposing the sample to stable 3,3‘-diaminobenzidine (Phoenix BioTechnologies). Samples were counterstained with Gill no. 3 hematoxylin solution. IHC for CD 45 (1:50, BD Pharmingen), Gr1 (1:20, BD Pharmingen), F4/80 (1:20, eBioscience) were performed in frozen pancreatic sections. Briefly, frozen sections were fixed in pure acetone at -20°C for 10 min and blocked with 10% fetal bovine serum/2% normal horse serum for 1 h at room temperature. Primary antibodies were applied for 1 h at room temperature. After brief washing, incubation with biotinylated secondary antibodies and streptavidin-labeled horseradish peroxidase was performed. Finally, positive reactions in the sections were detected using the NovaRED substrate kit for peroxidase (Vector Laboratories). Counterstaining was performed with Gill no. 3 hematoxylin.

Immunofluorescence. The same sections were sequentially labeled with anti-amylase and anti-HA antibodies. Briefly, Frozen sections (10 µm) were placed on Superfrost glass slides, fixed for 10 min with acetone at −20 °C, air-dried, and then blocked with 4% Fish Gelatin in phosphate-buffered saline (PBS) containing 0.02% Triton X-100 for 1h at room temperature. For labeling of amylase, sections were incubated overnight with rabbit anti-α-Amylase antibody (1:1000, SIGMA ALDRICH INC, Cat: A8273) and then Cy3 labeled AffiniPure F(ab')2 Fragment Goat Anti-Rabbit IgG for 1h (1:1000, Jackson ImmunoResearch Laboratories, Inc; Code 111-166-047). To prepare labeling with a second rabbit anti-HA antibody, the sections were blocked at room temperature with Normal Rabbit Serum (1:3000) in 4% Fish Gelatin PBS for 30 minutes followed by incubation with unlabelled AffiniPure F(ab')2 Fragment Goat Anti-Rabbit IgG in 4% Fish Gelatin PBS (1:10, Jackson ImmunoResearch Laboratories, Inc; Code 111-006-047) for 2h and normal goat serum 5% for 1h. After incubating with 4% Fish Gelatin in PBS for 5 minutes, sections were covered overnight with Anti-HA EPITOPE TAG (RABBIT) Antibody (1:500, ROCKLAND IMMUNOCHEMICALS INC, Gilbertsville, PA; Cat: 600-401-384) followed by incubation with Cy5-AffiniPure F(ab')2 Fragment Goat Anti-Rabbit IgG (1:1000, Jackson ImmunoResearch Laboratories, Inc; Code 111-006-046) for 1h. The nuclei were counterstained with SYTOX® Green Nucleic Acid Stain (1:50000, INVITROGEN CORP, PALATINE, IL; Cat: S7020). Fluorescent imaging was performed on a Zeiss LSM510 confocal scanning microscope (Zeiss, Thornwood, NY) and pseudo colors were set green for Cy3, red for Cy5

and blue for SYTOX® Green Nucleic Acid. Overlay of green and red was shown as yellow.

TUNEL staining. Paraffin-embedded sections were deparaffinized, treated with proteinase K (20 mg/ml, 1:1000, 10 min, room temperature) and incubated with a reaction buffer mix containing a biotin-labeled nucleotide mix and terminal deoxynucleotidyl transferase enzyme. After incubation with horseradish peroxidase-conjugated streptavidin (60 min, 37°C, 1:400, Dako) positive reaction was detected by exposing the sample to stable 3,3‘-diaminobenzidine (Phoenix BioTechnologies). Samples were counterstained with Gill no. 3 hematoxylin solution. Quantification of cell death was performed by determining the average number of TUNEL-positive acinar cells of four different vision fields per sample (200x magnification). Quantification of apoptotic cell death was performed by determining the average number of cleaved Caspase-3/TUNEL positive acinar cells of four different vision fields (200x magnification) per sample.

Histochemical staining of collagen. Sirius red staining of collagen in paraffin-embedded pancreatic tissue sections was performed to detect collagen deposits. Briefly, sections were deparaffinized and stained with Weigert’s hematoxylin for 8 min at room temperature. After extensive washing, sections were incubated with picro-Sirius red for 1 h followed by double washing with acidified water. Samples were then briefly dried and dehydrated.

Measurement of edema, serum amylase and trypsin activity. To determine the level of edema, tissue water content was measured. Briefly, a portion of the pancreas was weighed (wet weight), dried (>20 h, 60°C), and weighed again (dry

weight). Pancreatic tissue water content was assessed using the formula wet weight - dry weight / wet weight x 100 = pancreatic tissue water content (%). Serum amylase activity was measured using the Phadebas test as recommended (Pharmacia Diagnostics). Results were expressed as fold increase compared to control. Trypsin enzymatic activity was measured in homogenates of the pancreas fluorometrically previously described [5]. Results were normalized to protein content and expressed as fold increase compared to control.

Electron microscopy. After one week of induction of Cre recombination pancreatic samples were removed and fixed with a solution containing 3% glutaraldehyde plus 2% paraformaldehyde in 0.1 M cacodylate buffer, pH 7.3, for 1 h. After fixation, the samples were washed and treated with 0.1% Millipore-filtered cacodylate-buffered tannic acid, postfixed with 1% buffered osmium tetroxide for 30 min, and stained *en bloc* with 1% Millipore-filtered uranyl acetate. The samples were then dehydrated in ethanol at increasing concentrations, infiltrated and embedded in LX-112 medium. The samples were polymerized in a 70°C oven for 2 days. Ultrathin sections of the samples were cut using a Leica Ultracut microtome, stained with uranyl acetate and lead citrate using a Leica EM stainer and examined under a JEM 1010 transmission electron microscope (JEOL Ltd.) at an accelerating voltage of 80 kV. Digital images pancreatic samples were obtained using an AMT imaging system (Advanced Microscopy Techniques).

Statistical analysis. Results are displayed with mean ± SE. To determine whether results were significantly different from controls, data were analyzed using the two-sided unpaired *t* -test. For the survival analysis a Kaplan-Meier survival curve was created. To determine whether the difference between the survival curves was significant the Gehan-Breslow-Wilcoxon test was performed using GraphPad Prism version 5.00 for Windows.

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Supplemental Figure 1. Cre-recombination induction schemes. A, TM^{max} induction scheme: For maximum-rapid induction TM was given once a day for five consecutive days (TM^{max}) and animals were examined as indicated following initiation of treatment (5d, 7d or later). This treatment allowed maximum recombination efficiency simultaneously throughout the entire pancreas. **B,** TM^{grad} induction scheme: For gradual induction TM was given once every 5^{th} day for a period of 40 days and animals were examined 5d and 30d following the last dose of TM (TM^{grad}). This treatment strategy allowed gradual Cre-recombination of a fraction of the exocrine pancreas at a given time. **C,** Conditional expression of DTR was achieved by using the TM^{max} induction strategy. After an interval of 14d following maximum induction DT (100ng i.p) was administered twice daily for 3 consecutive days. These DT treated DTR^{on} mice were then examined at different time points as indicated.

Supplemental Figure 2. Langerhans' islets were not damaged in homozygous PACE-tryp^{on} mice. A-B, Despite the widespread destruction of pancreatic tissue and the acute inflammation in homozygous PACE-$tryp^{on}$ mice Langerhans' islets remained intact as observed at 7d after TM initiation (A, 100x; B, 400x). **C,** Measurement of blood glucose levels in non-fastened mice early (7d) and and late (>300d) after TM^{max} treatment demonstrated that basal glucose levels were not altered.

Supplemental Figure 3. Limited acinar cell regeneration occurred in homozygous PACE-tryp^{on} mice. A-C, Controls showed few stained cells when IHC for a proliferation marker, Ki67 was performed after TM^{max} (10d) (**A**). In homozygous PACE-tryp^{on} mice staining was sparse in areas with near total depletion of acinar cells (**B**) but abundant where injury was less extensive (**C**). This staining pattern was similar with both induction schemes, TM^{max} and TM^{grad} (all panels 100x).

Supplemental Figure 4. Lack of immunostaining in the absence of primary antibodies denoted the specificity of these antibodies in immunohistochemistry. Extra frozen (A) or paraffin (B) sections were incubated with HRP labeled secondary antibody in the absence of primary antibodies.