Supplementary Methods

Animals and induction of pancreatitis. All experiments with mice were carried out as approved by the University Committee on Use and Care of Animals at the University of Michigan. FVB/N mice were obtained from Jackson Laboratory. Cerulein- or CDE diet-induced AP models were carried out using FVB/N mice as described[1, 2]. For the heparin experiment, 8-week old male mice were fasted for 14 h then injected intraperitonealy (I.P.) 7 times with saline or cerulein (50 μg/kg mouse weight, Sigma) hourly. In addition, 8 mg/kg low molecular weight heparin (Enoxaparin, Amphastar pharmaceuticals) was co-administered two times at the 5 h and 10 h post first injection of saline or cerulein. For L-Arg-induced AP model[3], FVB/N mice were injected (twice, at time 0 and one hour later) I.P. with 12% L-Arg (pH 7), at a dose of 4g/kg body weight. The ODD-luc mice were described previously[4]. *Hif1a*-floxed mice (C57BL/6 background) containing the loxP site[5] were crossed with *elastase-cre*^{ERT2} mice (C57BL/6 background) followed by Cre induction by daily administration of tamoxifen (3 mg/40 g of body weight) dissolved in corn oil for 3 days to generate acinar cell-specific HIF1α-null mice (*Hif1a*$^{AC-/-}$). After an additional three days from the last administration, mice were fasted overnight then given saline or cerulein intraperitonealy. There was no difference in the results between sexes (based on preliminary experiments we carried out, not shown; all mice used for the cerulein and L-arginine experiments were 8-10 weeks old) except for the CDE diet-induced AP model in that 4-week old female mice were used. Factor VIII-null mice (C57BL/6 background; 8 weeks old males) were kindly provided by Dr. Haig H Kazazian[6].

Acini isolation. Acini were isolated as described[7] with minor modifications. Briefly, eight weeks old *Hif1a*$^{F/F}$ and *Hif1a*$^{AC-/-}$ mice were sacrificed by CO_2 asphyxiation, and collected pancreata were washed and trimmed quickly. Each pancreas was injected with 5 ml of digestion solution

containing 0.025% bovine serum albumin (BSA), 0.001% trypsin inhibitor, and 0.001% collagenase (SERVA) in DMEM with a 27-gauge needle until well distended, then, incubated in the digestion solution with shaking (120 rpm, 37˚C, 10 min). The incubated solution was removed and the pancreas was incubated in 5 ml of fresh digestion solution for an additional 45 min under the same conditions. The digested pancreas-containing solution was resuspended ten times to disperse the acini, and the suspension was filtered through a 250-micron nylon mesh (Lab Pak). After pre-incubation at 37˚C for 30 min, the acini were incubated with cerulein (0, 3, 10, 30, 100, or 300 pM) for 30 min, then the culture media were subjected to measurement of amylase or to immune blotting.

Serum amylase, histologic analysis and human specimen. Amylase levels were measured using Phadebas reagent (Magle Life Sciences) as recommended by the supplier. For histological analysis, pancreata were fixed in 10% neutral buffered formalin (NBF) and the paraffin sections were stained with hematoxylin and eosin. Histological scoring was done using the following criteria: edema 1-3 scale (0, absent or rare; 1, involving interlobular space; 2, involving intralobular space; 3, involving the entire pancreatic acinus); necrosis [0, absent; 1, focal (<5%); 2, sublobular (<20%); 3, lobular (>20%)]; vacuoles, inflammation and hemorrhage (0, absent; 1, mild; 2, moderate; 3, severe). Scores were assigned in a blind fashion without knowledge of the genotype of the pancreata in the histology sections. For PicroSirius Red staining, deparaffinized pancreas sections were dipped in 0.1% PicroSirius Red solution (1 h, 22 ºC), followed by washes two times with 0.5% acetic acid. The stained area was quantified using ImageJ software[8]. Human pancreatic surgical specimens were obtained using a Human Subjects approved protocol that provides access to such samples without any patient identifiers. Nonidentifier patient information

is included in Supplemental Table 1. The normal pancreas specimen was obtained from the non-pancreatitis segment of the pancreas from patient 1.

Factor VIII activity, D-dimer assay and VEGF ELISA. Peripheral blood was collected from the retro-orbital venous plexus of mice and anticoagulated with 3.8% sodium citrate. Plasma was separated by centrifugation (2000 rpm, 20 min). Factor VIII activity was measured by a 2-stage chromogenic method using the COAMATIC assay kit (Chromogenix) according to the manufacturer's instructions. The calibration standard included with this kit is assayed according to the Fourth International WHO standard. The factor VIII plasma standard was normal pooled plasma from George King Biomedical. The ELISA for serum D-dimer and tissue VEGF were carried out using the colorimetric mouse D-dimer ELISA kit (NeoScientific) and mouse VEGF ELISA kit (R&D System), respectively, as recommended by the suppliers.

Bioluminescence imaging. ODD-luc reporter mice were administered cerulein to induce AP. Mice were then injected with 50 mg/kg luciferin and imaged to assess HIF1α activity. For the fasting/re-feeding experiments, liquid diet (Dyets, Inc.) was administered by oral gavage of mice fasted for 14 h. After 10 minutes, mice were anesthetized using I.P. injection of pentobarbital or by inhalation of isoflurane, then imaged after another 5 minutes using the Xenogen IVIS 200 bioluminescence system.

Protein isolation and immunoblotting. Total pancreatic lysates were prepared by homogenizing the pancreata in SDS-containing sample buffer. The preparation of insoluble proteins was described previously[9]. Lysates were subjected to SDS-PAGE and transferred onto a polyvinylidene difluoride membrane. The blots were incubated with primary antibodies (information on antibodies is included in Supplemental Table 2) and processed using standard protocols.

Quantitative RT-PCR. Total RNA was extracted using RNAeasy kit (Qiagen), and 1 μg of RNA was reverse transcribed to cDNA using TaqMan reverse transcriptase kit (Applied Biosystems). Quantitative PCR was done using Brilliant SYBR Green Master Mix (Bio-Rad) and Eppendorf MasterCycler RealPlex (Thermo Fisher Scientific). Primer information is included in Supplemental Table 3.

Immunofluorescence staining, transmission electron microscopy and toluidine blue staining. Pancreata were embedded in optimal cutting temperature compound (Thermo Fisher Scientific), and cut into 5 μm sections. For crosslinked fibrin staining, sections were fixed with 2% acetic acid in 10% neutral buffered formalin (NBF) (30 min) then rinsed with PBS and blocked with 10% goat serum in PBS. Sections were incubated with antibodies to: fibrinogen-γ (1:100, Abcam), CD31 (1:100, BD Biosciences), HIF1 (1:100, Santa Cruz Biotechnology), CD45 (1:200, BD Biosciences), α-SMA (1:50, Sigma-Aldrich) (4℃, overnight). Sections were mounted in ProLong Gold antifade reagent that included 4',6-diamidino-2-phenylindole (DAPI, Thermo Fisher Scientific) to visualize the nuclei. For amylase staining, sections were fixed with 10% NBF (10 min) and amylase antibodies (1:100, Santa Cruz Biotechnology) were used. Samples were analyzed by Axio Imager.M2 Microscope (Carl Zeiss).

Ultrastructural analysis of mouse pancreata was carried out similar to what has been described[10]. In brief, tissues were excised, minced into small pieces and fixed in a solution of 2% paraformaldehyde & 2% glutaraldehyde in PBS for two hours at room temperature. After washing in PBS the tissue samples were post fixed in osmium tetroxide for 45 min at room temperature. Dehydration of the samples was accomplished by transferring the samples through a series of graded ethanol and then 100% propylene oxide. The tissue was then infiltrated by transferring the samples into increasing concentrations of Epon to propylene oxide solutions; 1:3,

1:1, 3:1 then 100% Epon and finally embedded. Sections were cut with a Leica EM UC7 ultramicrotome (Leica), stained for 15 min with 7% (saturated) aqueous uranyl acetate, washed, stained with lead citrate, and examined with a JEOL JEM 1400 plus transmission electron microscope (JEOL USA). Epon sections were also subjected to toluidine blue staining (stained for 15 seconds with 0.1% solution), then analyzed by Axio Imager.M2 Microscope (Carl Zeiss).

Measurement of MPO activity. Myeloperoxidase activity was assay as previously described. To measure myeloperoxidase activity of pancreas or lung, small pieces of tissues were homogenized in 20 mM sodium phosphate buffer (pH 7.4) and centrifuged at 13,000 x g for 10 min at 4˚C. The pellet was resuspended in 0.5% cetrimonium bromide in phosphate buffer (50 mM, pH 6.0), then frozen and thawed four times followed by centrifugation (10 min, 13,000 x g, 4˚C). The supernatant was mixed with the same volume of 3,3´,5,5´-tetramethylbenzidine using a 96-well plate and incubated at 37˚C for 2 min. The reaction was stopped by addition of 2N H_2SO_4, then measurement of the absorbance (405 nm) and normalization of the values to tissue DNA content.

Statistical analysis. Statistical analysis was performed using analysis of variance or *t* test and the GraphPad Prism 6 statistical software. A p-value < 0.05 was considered statistically significant.

References for Supplementary Methods

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