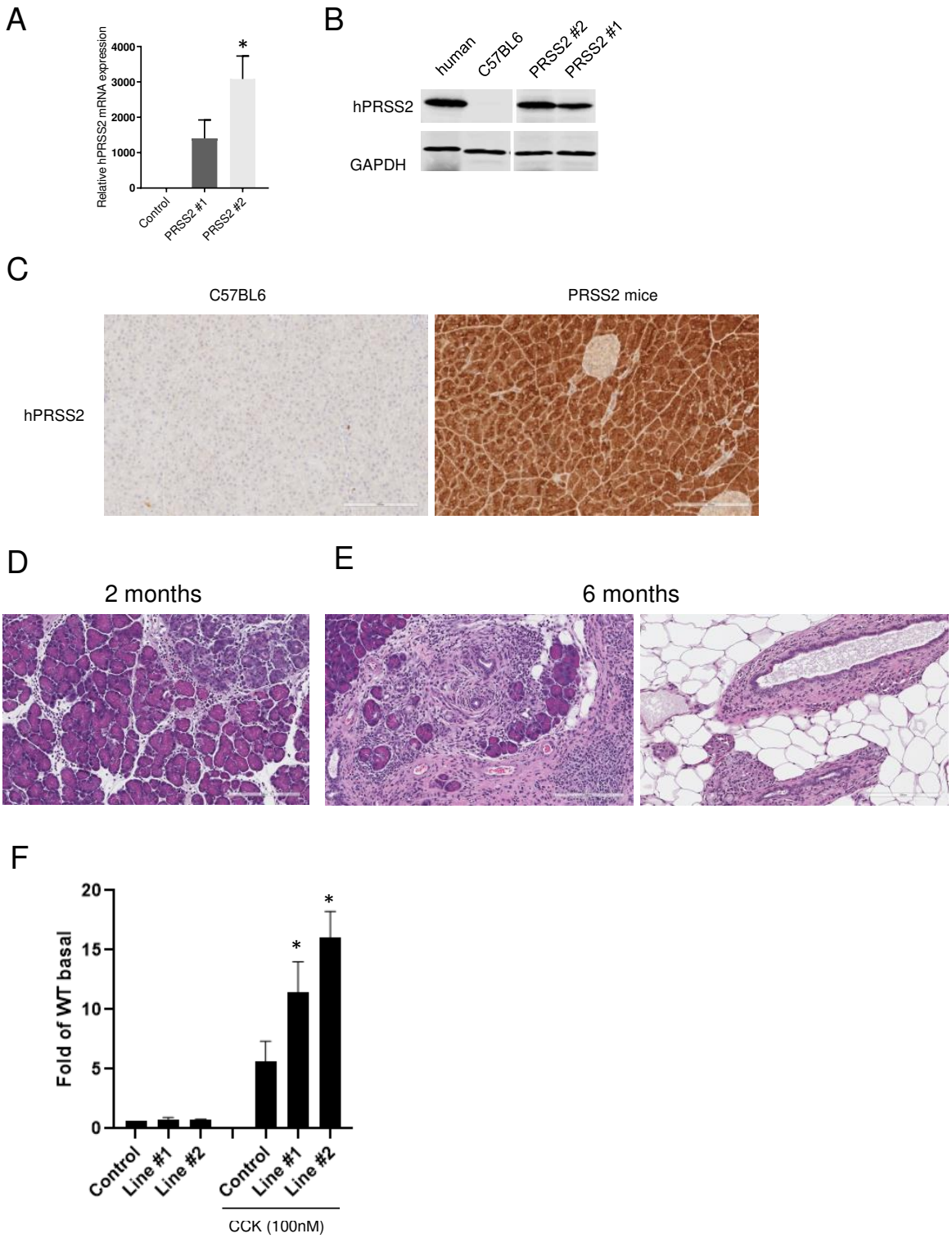
Supplemental materials

Supplemental Results:

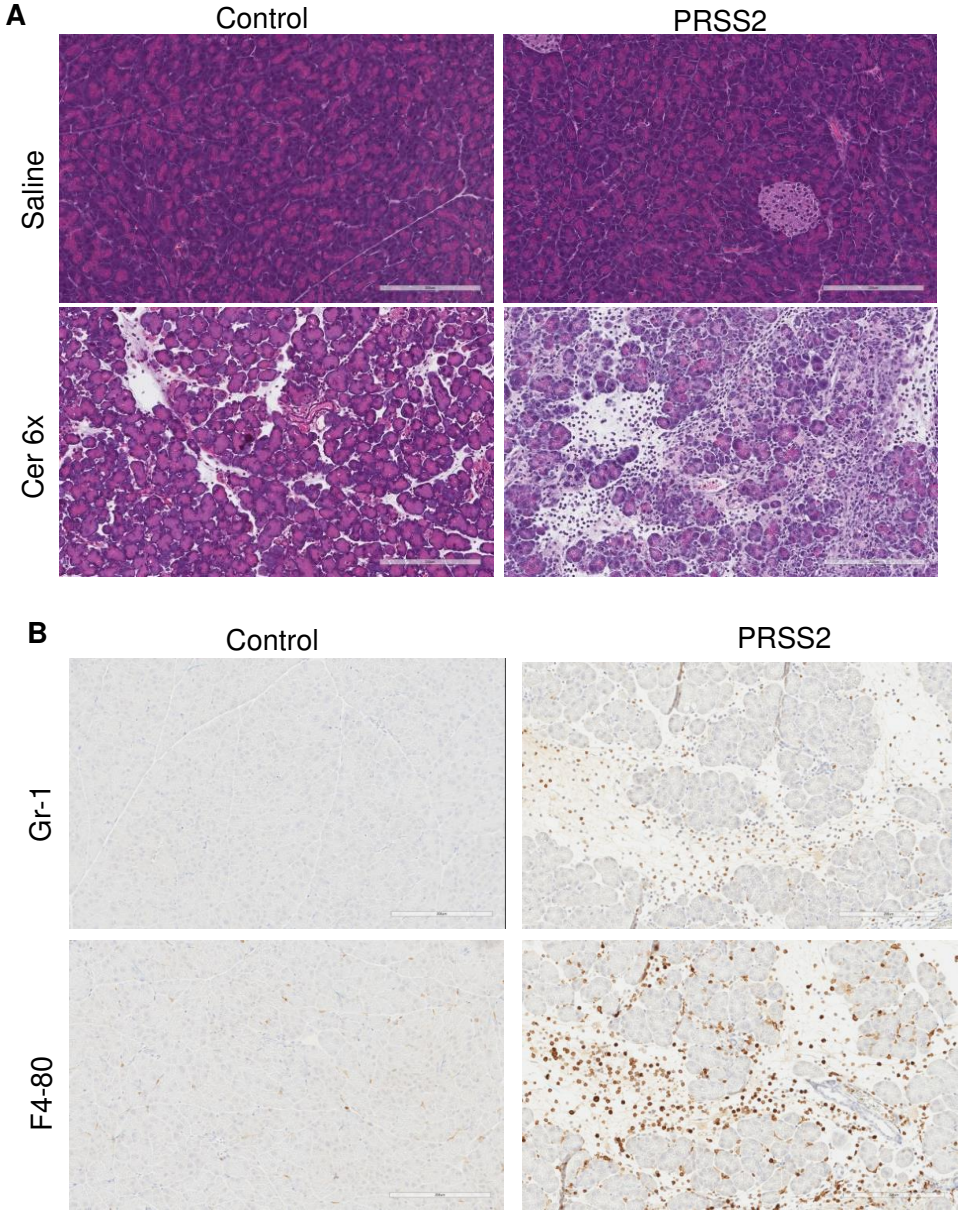
Two lines of PRSS2 mice were successfully validated for PRSS2 expression by PRSS2-specific RT-PCR **(Supplemental Figure 1A),** Western blot and immunohistochemical staining using a human PRSS2-specific antibody **(Supplemental Figure 1B and 1C**). PRSS2 expression levels were higher in line #2 than line #1. Spontaneous pancreatitis was not observed in PRSS2 line #1 mice until 1 year old (**Data not shown**). However, in PRSS2 line #2 focal pancreatitis (area <10%) was evident at the age of 2 months old (**Supplemental Figure 1D**). As the mice aged, spontaneous focal chronic inflammatory lesions were observed in approximately 1/3 of the mice at six months old (**Supplemental Figure 1E**). These observations suggest that upregulation of human PRSS2 dose-dependently increased sensitivity to pancreatitis development, even under normal physiological conditions. In consistent with these findings, cholecystokinin (CCK100nM) induced more trypsin activity in pancreatic acinar cells isolated from PRSS2 transgenic mice than those from control mice **(Supplemental Figure 1F),** in contrast to the observation that increasing PRSS2 yields less active trypsin in test tube assays.[1]

Suppl. Figure 1



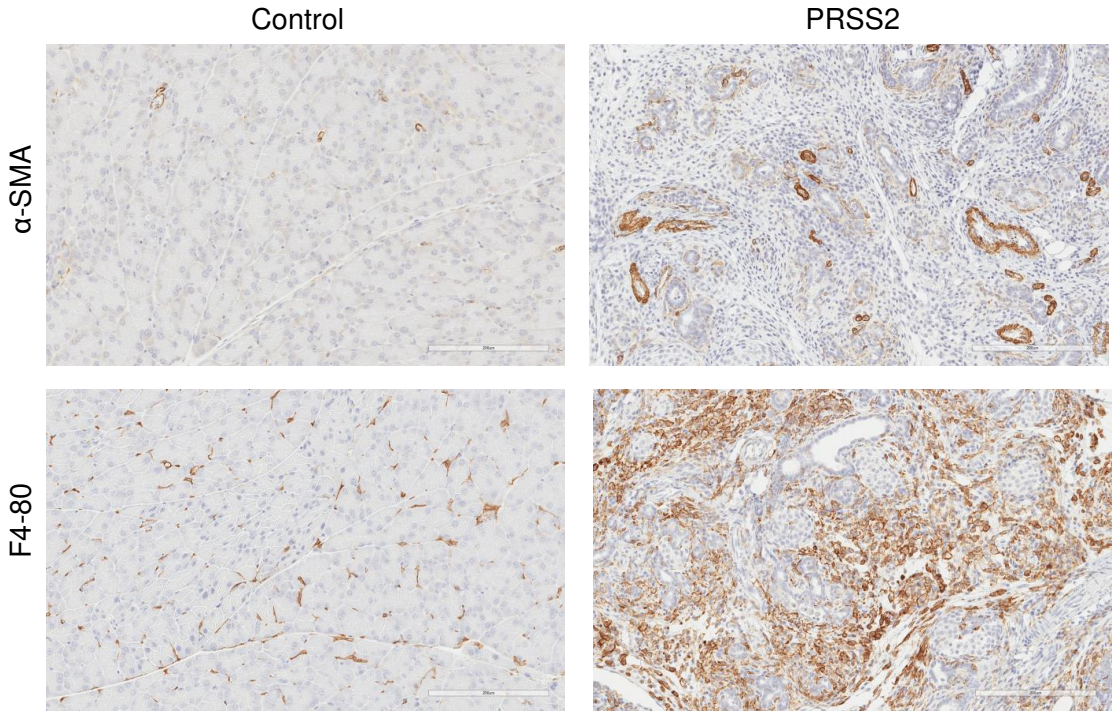
Supplemental Figure 1: Transgenic expression of human PRSS2 caused focal chronic pancreatitis and increased trypsin activity. A. Human PRSS2 mRNA was not present in WT control mice but highly expressed in transgenic mice as detected by real-time RT-PCR (n=3). **B.** Human PRSS2 protein expression levels were detected by Western Blot (the blot was run on the same gel. Unrelated sample bands were cropped). **C.** Immunohistochemical staining for human PRSS2 showed its expression in nearly 100% pancreatic acinar cells of PRSS2 line #1 mice. **D.** Spontaneous pancreatitis in PRSS2 line #2 (2 month old). **E.** Spontaneous pancreatitis in PRSS2 line #2 (6 month old). **F.** Pancreatic trypsin activity was measured 30 min after CCK (100nM) stimulation in isolated primary pancreatic acinar cells (n=3). * $p<0.05$.

Suppl. Figure 2



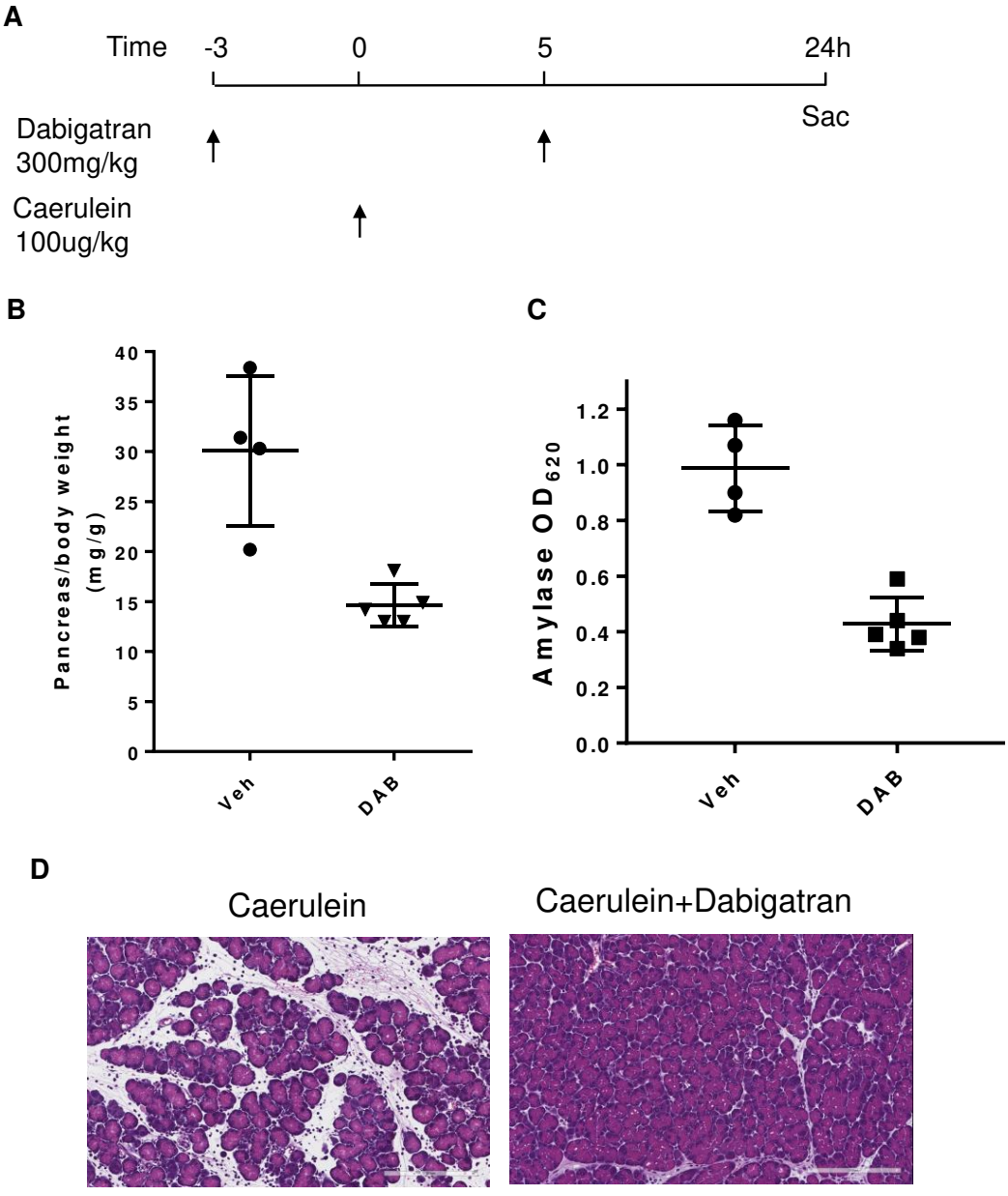
Supplemental Figure 2: Pancreatitis evaluation in PRSS2 transgenic mice. **A.** Pancreatitis was induced by six dose caerulein (100μg/kg/h) in control and PRSS2 line #1 mice. At 24 hours after initial injection, pancreas histology (H &E) was examined. **B.** Immunohistochemical staining was performed for Gr-1 (neutrophil) and F4/80 (macrophage) in the pancreata of control and transgenic PRSS2 line #1mice.

Suppl. Figure 3



Supplemental Figure 3: At 21 days after a single injection of caerulein, immunohistochemical staining was performed for alpha-smooth muscle actin (α-SMA, a maker for stellate activation) and F4/80 (macrophage) in the pancreata of control and transgenic PRSS2 line #1 mice.

Suppl. Figure 4



Supplemental Figure 4: Trypsin inhibition ameliorated the severity of acute pancreatitis in PRSS2 mice. A. PRSS2 line #2 mice were induced with a single injection of caerulein (100ug/kg). One group of mice were pretreated with a trypsin inhibitor Dabigatran (300mg/kg) by oral gavage (n=4-5). B. As compared with vehicle (Veh) group, trypsin inhibition with Dabigatran (DAB) reduced pancreas edema. C. DAB reduced the level of serum amylase. D. Histology examination showed DAB improved pancreatitis.

Supplemental Methods:

Study approval and induction of pancreatitis: All animal experiments were approved by the Institutional Animal Care and Use Committee (IACUC) at Mayo Clinic. Pancreatitis was induced by caerulein (100μg/kg, Cat: CCKS-001; CPC Scientific, CA, USA).

Genetically engineered mice:

A human bacterial artificial chromosome (BAC) (clone RP11-701D14) harboring the full-length human *PRSS2* gene was obtained from BACPAC Resources (UCSF Benioff Children's Hospital, Oakland, CA). *PRSS1* gene in this construct was inactivated by an S200T point mutation.[2] All animal experiments were approved by the Institutional Animal Care and Use Committee (IACUC) at Mayo Clinic. Mice were maintained in 12-hour light/12-hour dark cycles with free access to food and water. Sex- and age-matched mice were used in control and experimental groups. All mice in C57BL/6J background, ages 2-8 months old, were used in the experiments. No phenotypic differences between sex and ages were noticed in the experiments.

Histologic analysis

Pancreatic tissue was collected at 24h after initiating acute pancreatitis, and 1 or 3 weeks after chronic pancreatitis. Fresh pancreatic tissue was fixed in 10% formalin for 24 hours and then dehydrated and paraffin-embedded. Cut into 4 μm thick slides for H&E staining. Methods of pancreatic pathology score was evaluated as previously described. [2]

Immunohistochemistry

The paraffin-embedded pancreatic samples were sliced into 4μm thick sections. Immunohistochemical staining was performed on the Dako Autostainer System (Agilent) according to the manufacturer's instructions. The anti-Human Trypsin 2 (1:200) (Cat: MAB3586) antibodies was purchased from R&D Systems. The anti-CD11b (Cat: ab133357) antibody was purchased from Abcam (MA, USA). The anti-F4/80 (Cat: sc-52664) antibody was obtained from Santa Cruz (Santa Cruz, CA, USA). The

anti-Gr-1 (Cat: 14-5931) antibody was purchased from Affymetrix (Santa Clara, CA, USA).

Western blot

Fresh pancreatic tissue was added to the protein lysis buffer containing SDS for ice lysis for 30 min, and the supernatant was centrifuged at 4℃. SDS-PAGE was used to separate proteins of different molecular weights, and then transferred to the nitrocellulose membrane. After blocking with skim milk, the primary antibody was incubated overnight. The anti-Human Trypsin 2/PRSS2 (1:1000) (Cat: MAB3586) was purchased from R&D Systems. The anti-GAPDH antibody (Cat: G8795) was obtained from Sigma (MO, USA).

Real-time qPCR

Total mRNA was extracted from pancreatic tissue by using the RNeasy Plus Mini Kit (Qiagen, CA, USA). The cDNA was synthesized by using the Go Script RT kit (Cat: A5003; Promega, WI, USA). Quantitative real-time polymerase chain reaction (PCR) was performed by using PowerUp SYBR Green Master Mix (Cat:A25742; Applied Biosystems, TX, USA). Forward accccaaatacaacagccgg and Reverse agccggagatgagggactcg primers were used for the detection of human PRSS2 mRNA expression.

Serum amylase activity and trypsin activity assays

Serum amylase, pancreatic acinar isolation and stimulation, trypsin activity assay were performed as previously described.[2]

Statistical analysis

All data are expressed as the mean±SEM. Two-tailed Student's t-test was performed using the GraphPad Prism 5.0 (GraphPad, La Jolla, CA). p values<0.05 was considered statistically significant.

References:

1 Kukor Z, Toth M, Sahin-Toth M. Human anionic trypsinogen: properties of autocatalytic activation and degradation and implications in pancreatic diseases. Eur J Biochem 2003;**270**:2047-58.

2 Gui F, Zhang Y, Wan J, Zhan X, Yao Y, Li Y*, et al.* Trypsin activity governs increased susceptibility to pancreatitis in mice expressing human PRSS1R122H. J Clin Invest 2019.