Supporting Information

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SI Materials and Methods

Reagents. Cathepsin B (from bovine spleen) was purchased from Merck, and *N*-(p-Tosyl)-GPK-pNA (*p*-nitroanilide) and soy bean trypsin inhibitor (SBTI) were from Sigma.

Primers Used for Real-time PCR. Mouse β-actin primers (forward: 5′-TGGAATCCTGTGGCATCCATGAAAC-3′, reverse: 5′-TAAAACGCAGCTCAGTAACAGTCCG-3′), trypsinogen5 primers (forward: 5′-ATTGAAGTCACCTGCCATCC-3′, reverse: 5′-AGGACAGGAGCTTCCAGACA-3′), TLR3 primers (forward: 5′-TTGTCTTCTGCACGAACCTG-3′, reverse: 5′-CCCGTTCCCAACTTTGTAGA-3′), RIG-I primers (forward: 5′-TTGCTGAGTGCAATCTCGTC-3′, reverse: 5′-GTATGCGGTGAACCGTCTTT-3′), and MDA5 primers (forward: 5′-TGTCTTGGACACTTGCTTCG-3′, reverse: 5′-GGCCTCTGTCTCCAGACTTG-3′) were used for real-time PCR.

Primers Used for ChIP Assay. Mouse *trypsinogen5* promoter-specific primers (forward: 5′-CCGGGGGAAACAATGATGTGGAAAC-3′, reverse: 5′-AGCAGCTCCCAGAAAAGTGA-3′) and *Angiotensinogen* (*Agt*) exon2-specific primers (forward: 5′-CACCCCTGCTACAGTCCATT-3′, reverse: 5′-CAGACATGACTGGGGGAGGT-3′) were used for the ChIP assay.

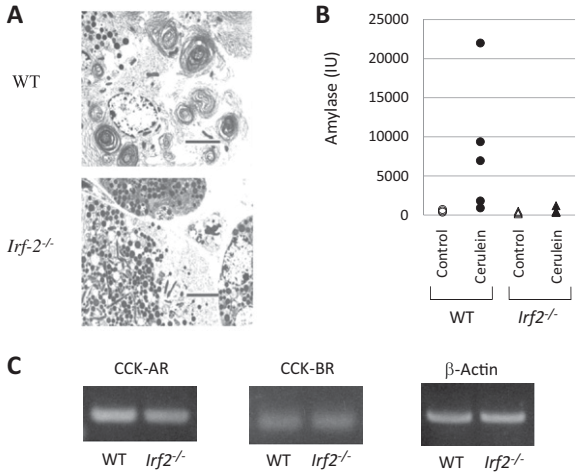

Fig. S1. Resistance of *Irf2*$^{-/-}$ mice to cerulein-induced pancreatitis. Wild-type or *Irf2*$^{-/-}$ mice were injected intraperitoneally with 50 μg/kg cerulein six times at hourly intervals. One hour after the last injection, the pancreas was subjected to electron microscopic analysis (*A*) and serum amylase levels were also measured (*B*). (Scale bars, 5 μm.) (*C*) The pancreatic mRNAs of WT and *Irf2*$^{-/-}$ mice were prepared by the acid guanidinium thiocyanate-phenol-chloroform extraction method after treatment with RNAlater Solution (Ambion) overnight at −80 °C, and the expression of two kinds of cholecystokinin receptors (CCK-AR and CCK-BR) was analyzed by Northern blotting with radiolabeled specific probes. β-Actin expression is shown as a control.

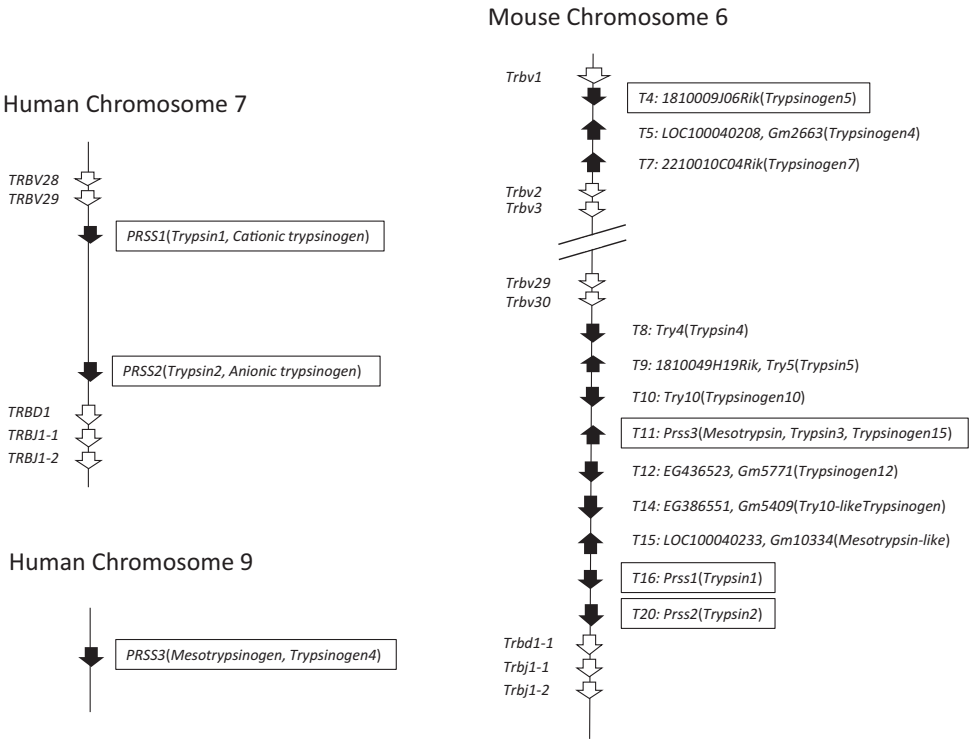

Fig. S2. Schematic structures of human and mouse trypsinogen genes in the *T-cell receptor β-chain* (*TRB*) locus. Three *trypsinogen* genes (shown as filled thick arrows) are found in humans, although *PRSS3* encodes three transcriptional trypsinogen isoforms. Twenty *trypsinogen* genes (*T1-T20*) are found in mouse, but only 12 of them are thought to be expressed, as indicated by the filled arrows. The human *TRB V, D*, and *J* and mouse *Trb v, d*, and *j* genes are shown as open arrows.

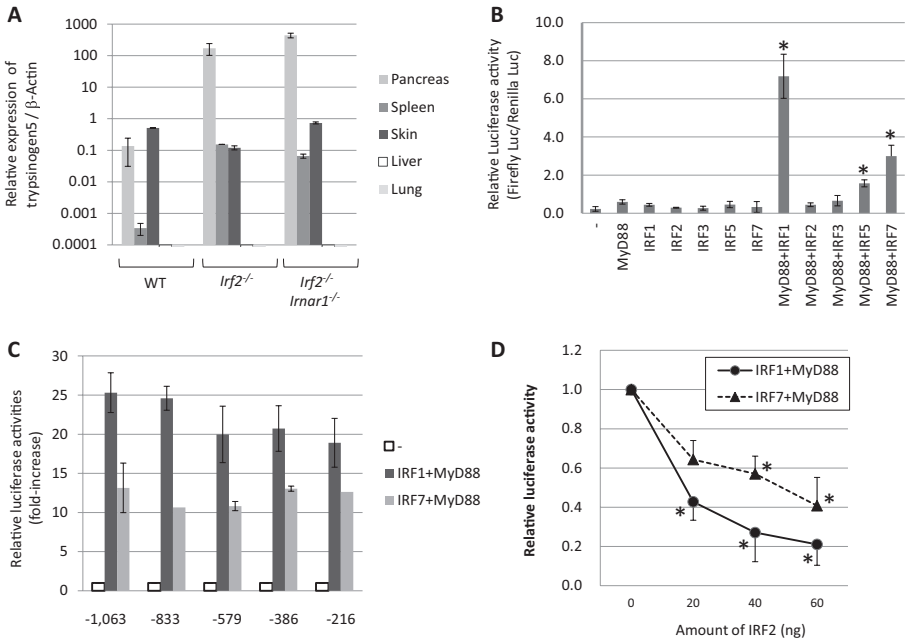

Fig. S3. (*A*) Trypsinogen5 mRNA expression levels in the indicated tissues were normalized to β-actin expression and are shown on a log scale. It was expressed at low but accurately measurable levels in wild-type pancreas and skin, compared with spleen, liver, and lung. Trypsinogen5 mRNA expression in pancreas and spleen was up-regulated about 1,000-fold in $Irf2^{-/-}$ mice compared with wild type. However, the up-regulation was not affected by further abolishing *IFNα/β receptor 1* (*Ifnar1*). (*B*) The promoter activity of *trypsinogen5* was examined by placing the 1.1-kb *trypsinogen5* promoter region before the firefly luciferase gene as reporter in 293T cells. The expression vectors for IRF1, -2, -3, -5, and -7 were cotransfected with the reporter in the presence or absence of MyD88. Luciferase activity was determined with a dual luciferase assay system. IRF1, -5, and -7 significantly enhanced (*$P < 0.02$) the *trypsinogen5* promoter in the presence of MyD88. (*C*) The promoter activities of deletion mutants of *trypsinogen5* promoter were measured in the absence or presence of both MyD88 and IRF1 or IRF7 in 293T cells. (*D*) Dose-dependent effects of IRF2 expression on IRF1- and IRF7-dependent *trypsinogen5* promoter activation in the presence of MyD88. *$P < 0.02$ versus the absence of IRF2.

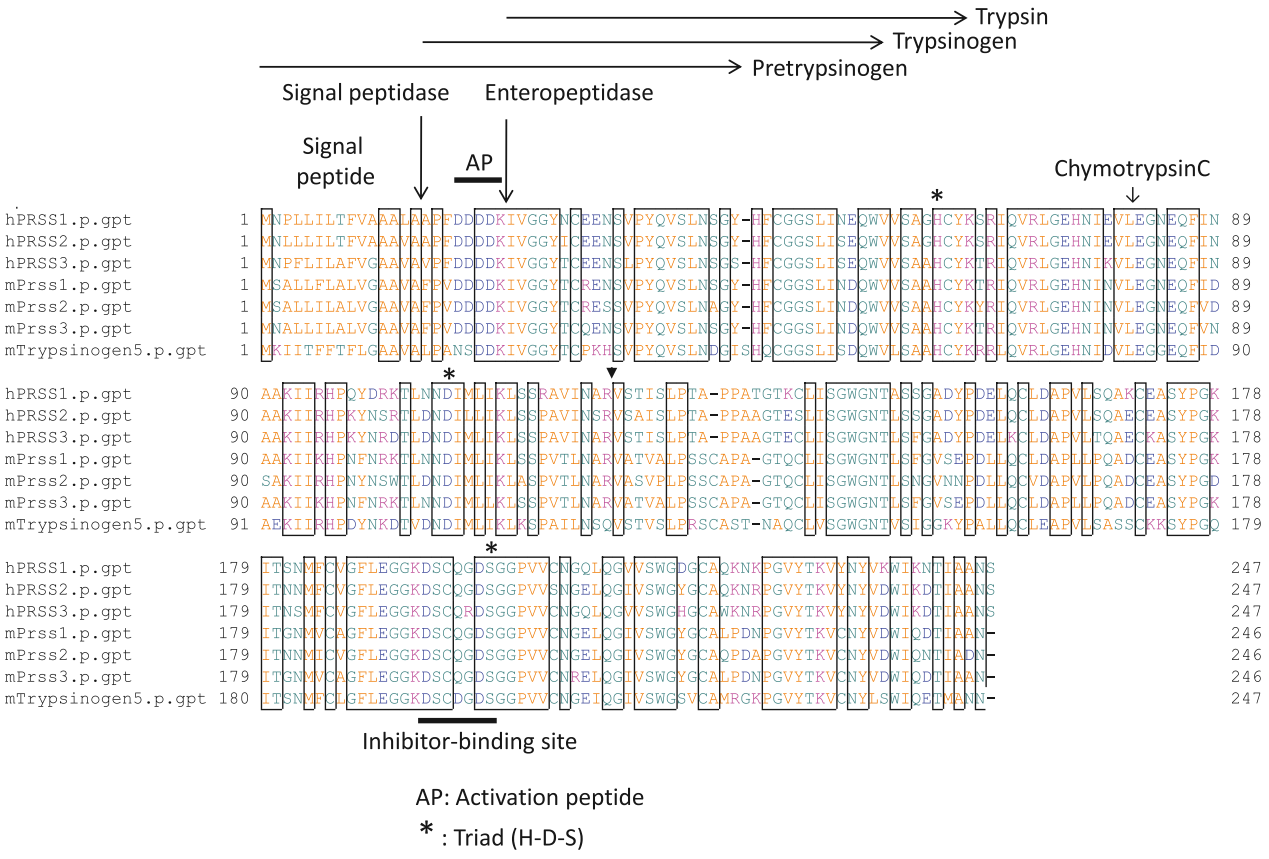

Fig. S4. The amino acid sequences of major human trypsinogens (PRSS1, -2, and -3) and mouse Prss1, -2, and -3 and trypsinogen5 are aligned. The signal peptidase, enteropeptidase, and chymotrypsin C cleavage sites are indicated with arrows, and the trypsin autolytic cleavage site is indicated with an arrowhead. Active trypsin is generated from pretrypsinogen by cleavage at the signal peptide in the endoplasmic reticulum and at the activation peptide in the duodenum. Because mouse trypsinogen5 contains the triad (H-D-S) of amino acids essential for trypsin activity (each amino acid is indicated with an asterisk), a signal peptide, and an activation peptide in the N-terminal region, this trypsinogen5 is predicted to have enzymatic activity. The inhibitor binding site D^{198} is unique in trypsinogen5, and may confer resistance to trypsin inhibitors, similar to the human PRSS3, R^{198}. Conserved amino acids are boxed.

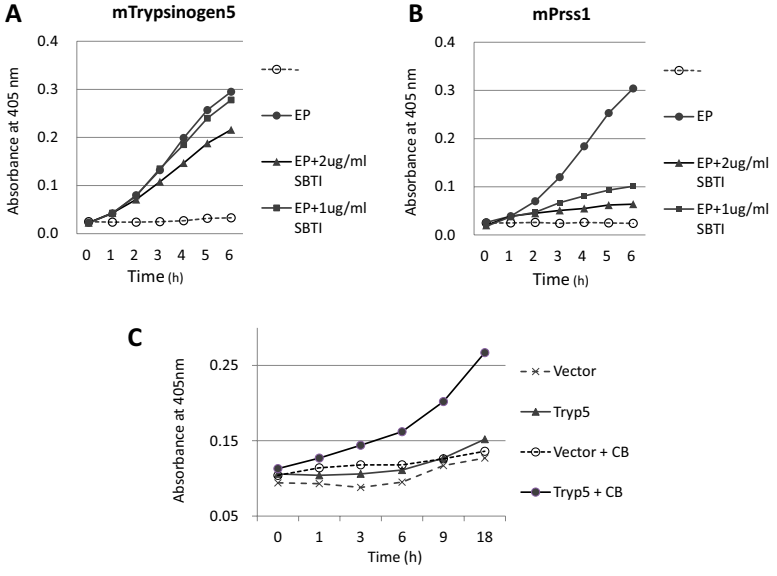

Fig. S5. Trypsin activity was monitored by the amount of released pNA from a specific substrate, measuring spectrophotometric units at 405 nm (Trypsin Activity Assay Kit; BioVision). The activities were dependent on the addition of enteropeptidase (EP), and increased in proportion to the amounts of lysates added to the reaction. Addition of 1 and 2 μg/mL SBTI had no effect on the mouse trypsinogen5 activities observed (*A*). In contrast, mouse Prss1 activities were inhibited with SBTI in a dose-dependent fashion (*B*). (*C*) Activation of trypsinogen5 with cathepsin B. The same amounts of 293FT cell lysates transfected with mouse trypsinogen5 (Tryp5) or vector only (Vector) were pretreated with 0.008 U of cathepsin B (CB) in 50 mM acetic acid buffer (pH 5.0) for 2 h at 37 °C, and then subjected to trypsin assay using *N*-(p-Tosyl)-GPK-pNA as substrate in 100 mM Tris buffer (pH 8.0) at 30 °C for the indicated periods. The amount of pNA released from the specific substrate was measured as trypsin activity.

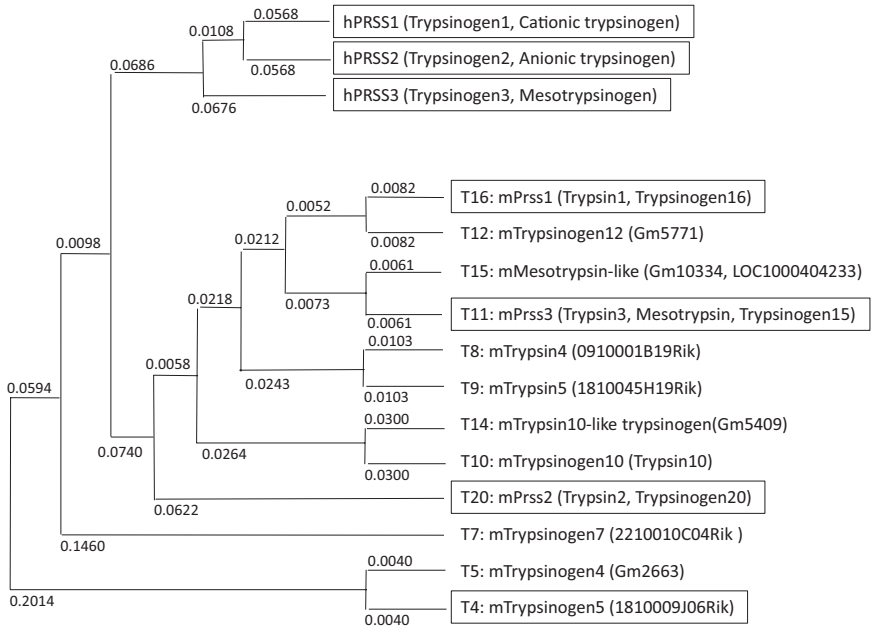

Fig. S6. The evolutionary pedigree of 3 human trypsinogens and 12 mouse trypsinogens. Human PRSS1, -2, and -3, mouse Prss1, -2, and 3, and trypsinogen5 are boxed. The pedigree was made using the unweighted pair group with arithmetic mean method. The associated number calculated by this method indicates the evolutionary distance such that smaller values are more homologous and evolutionarily closer to each other whereas larger values are less homologous and evolutionarily more distant.

Table S1. Significantly up- and down-regulated genes in *Irf2*$^{-/-}$ mice

Gene transcripts	WT (–)	WT (pIC)	*Irf2*$^{-/-}$ (–)	*Irf2*$^{-/-}$ (pIC)
Trypsinogen5 (1810009J06Rik)	80	69	14,345	15,470
Fetuin β (Fetub)	14	15	926	1,623
3-Hydroxy-3-methylglutaryl-CoA synthase 2 (Hmgcs2, HMG-CoA synthase)	23	240	1,375	777
Annexin A10 (Anxa10)	11	11	647	427
Ep1 (epithelial progenitor 1)	10	12	303	111
Lectin, galactose-binding, soluble 9 (Lgals9)	90	625	1,369	1,629
α-2-HS-glycoprotein (Ahsg, Fetuin-A)	10	12	157	591
Transmembrane 4 superfamily member 4 (Tm4sf4)	58	64	783	349
Guanylate-binding protein 2 (Gbp2)	22	1,064	258	1,230
Regenerating islet-derived 3γ (Reg3g)	268	4,511	2,756	2,758
Macrophage activation 2-like (Gbp6, guanylate-binding protein 6)	17	177	174	349
Lipocalin 2 (Lcn2)	28	146	283	217
Group-specific component (Gc, Vitamin D-binding protein)	30	21	186	577
S100 calcium-binding protein G (S100-G)	58	19	114	252
Ig κ chain variable 8 (Igk-V8)	25	455	24	7
Carbonic anhydrase 3 (Car3)	152	1,301	103	49
Complement factor D (adipsin)	157	707	56	38
Ig joining chain (Igj)	30	168	25	11
Galanin (Gal)	879	1,057	213	71
Cxcl13 [chemokine (C-X-C motif) ligand 13]	82	806	29	60
Ig λ variable 1 (Iklv1)	18	147	25	14
Solute carrier family 38, member 5 (Slc38a5)	1,533	1,683	143	196

The levels of gene expression in the pancreas are shown in Affymetrix units. The upper 14 annotated genes were up-regulated more than 10-fold in *Irf2*$^{-/-}$ mice. The up-regulation of trypsinogen5 with another probe (Point 2 in Fig. 2) by >100-fold by inactivating *Irf2* gene was noteworthy, because trypsinogens are reported to play important roles in developing pancreatitis. Anxa10, Ahsg, and S100-G are Ca^{2+}-binding proteins. The lower eight genes were down-regulated >10-fold in *Irf2*$^{-/-}$ mice. Galanin is reported to mediate cerulein-induced pancreatitis (1). pIC, poly(I:C).

1. Bhandari M, et al. (2010) Galanin mediates the pathogenesis of cerulein-induced acute pancreatitis in the mouse. *Pancreas* 39:182–187.

Table S2. Expression of IFN-signaling molecules

Gene transcripts	WT (–)	WT (pIC)	*Irf2*$^{-/-}$ (–)	*Irf2*$^{-/-}$ (pIC)
irf1	35	286 ↑	44	361 ↑
irf3	97	113	136	114
irf4	19	16	10	15
irf5	34	47	36	46
irf6	114	148	197	171
irf7	26	375 ↑	107	224 ↑
irf8	32	81	43	54
irf9	44	191	88	237
IPS-1	109	113	121	123
trif	53	55	51	53
Myd88	31	311 ↑	77	297 ↑
tirap	23	46	36	47
mda5	35	921 ↑	76	733 ↑
rig-I	47	538 ↑	93	458 ↑
tlr1	9	9	10	6
tlr2	55	151	55	133
tlr3	13	98 ↑	24	118 ↑
tlr4	20	26	26	27
tlr6	15	18	14	12
tlr7	6	21	16	12
tlr8	8	9	10	9
tlr9	42	80	40	36

The levels of gene expression in the pancreas are shown in Affymetrix units. The DNA microarray analysis indicated that IRF1, IRF7, MyD88, MDA5, RIG-I, and TLR3 were significantly up-regulated with poly(I:C) in the pancreas of WT and *Irf2*$^{-/-}$ mice.

Table S3. Relationship among relevant gene expression, cell death, and pancreatitis

	WT		*Irf2*$^{-/-}$		*Irf2*$^{-/-}$*Ifnar1*$^{-/-}$		*Irf2*$^{-/-}$*Trif*$^{-/-}$	
	–	Poly(I:C)	–	Poly(I:C)	–	Poly(I:C)	–	Poly(I:C)
Granule retention	—	—	+++	+++	+++	+++	+++	+++
Trypsinogen5	—	—	+++	+++	+++	+++	+++	+++
TLR3/TRIF	+	+++	+	+++	+	+	—	—
MDA5/RIG-I/IPS-1	+	+++	+	+++	+	+	+	+++
Cell death	—	+	—	+	—	—	—	+
"Enhancing loop"	—	—	—	+++	—	—	—	+
Pancreatitis	—	—	—	+++	—	—	—	+
Serum amylase	—	—	—	+++	—	—	—	+

IRF2$^{-/-}$ caused abnormal granule retention (+++) and conspicuous expression of trypsinogen5 (+++), and the characteristics were not rescued by further destroying IFNAR1 or TRIF. There were at least two cell death pathways: TLR3/TRIF and MDA5/RIG-I/IPS-1, and high expression of TLR3/TRIF or MDA5/RIG-I/IPS-1 (+++) caused cell death. In addition, high expression of spink3-resistant trypsinogen5 (+++) required for the "enhancing loop" of cell death led to pancreatitis with elevated serum amylase (+++). –, none; +, low; +++, high.