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Supporting Information for:

Blockade of the Protease ADAM17 Ameliorates Experimental Pancreatitis

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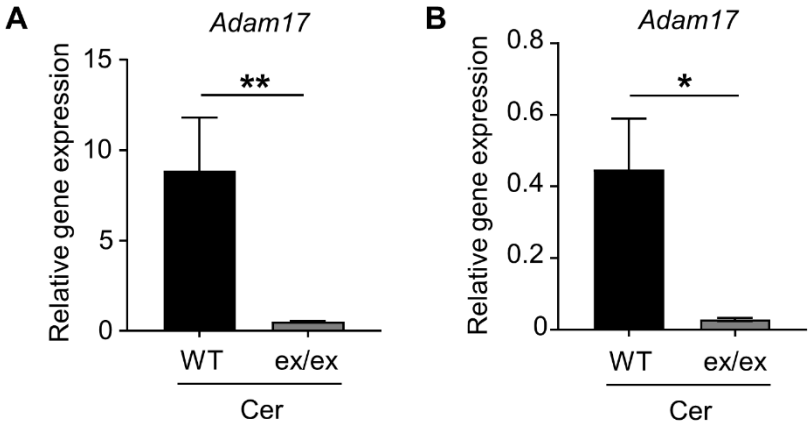
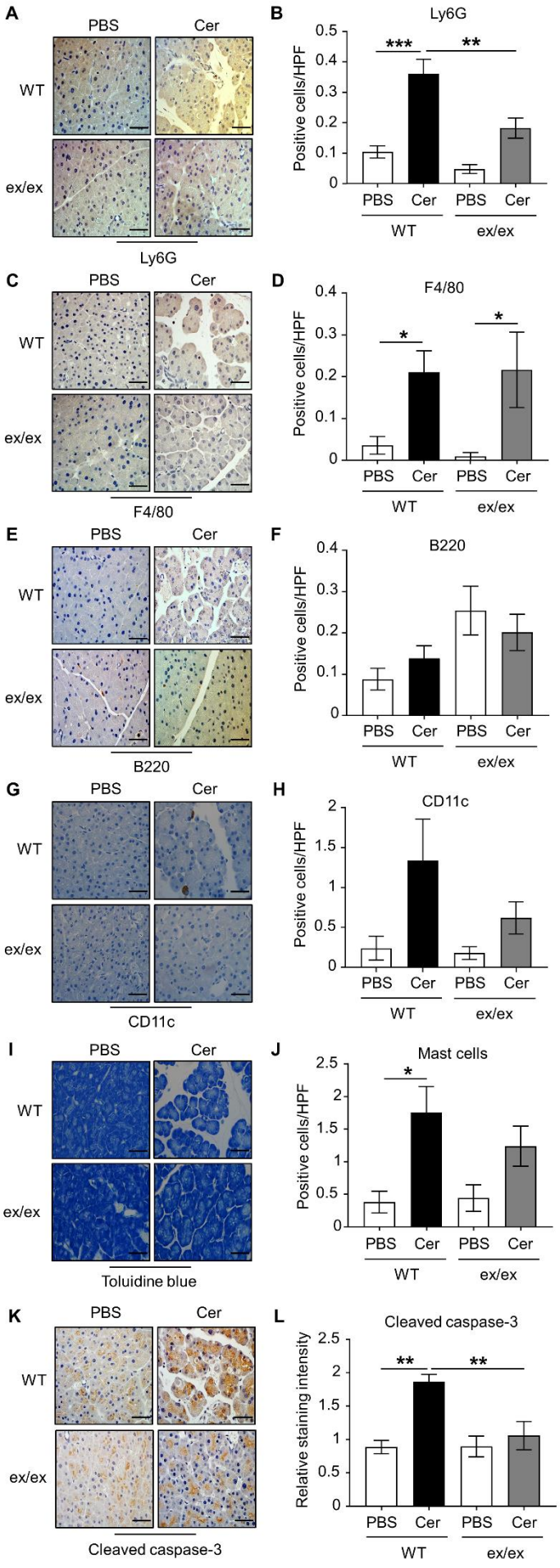
Supplementary Table 1: Characteristics of patients used in this study.

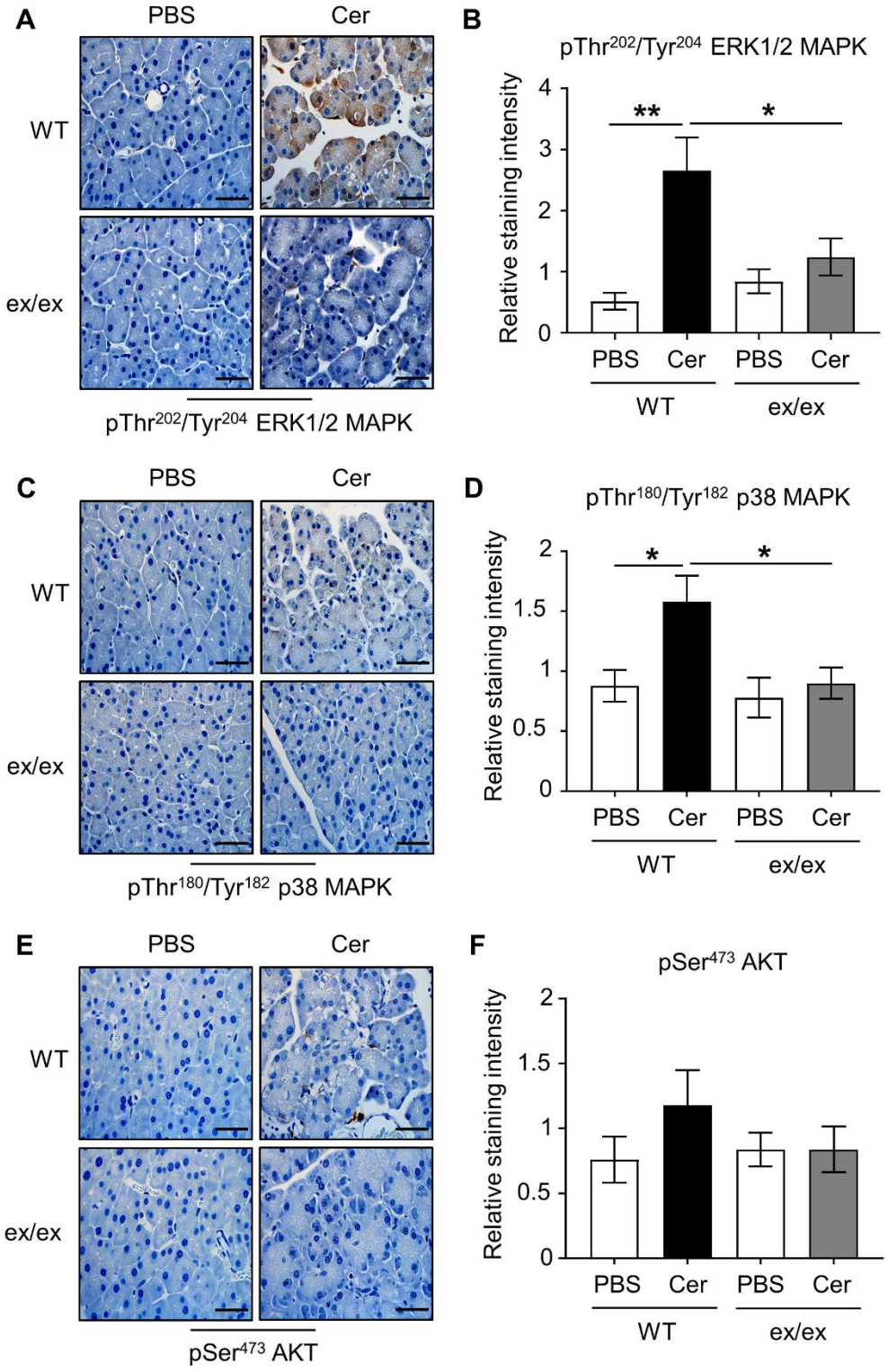
Supplementary Table 2: List of mouse primer sequences used in the study.



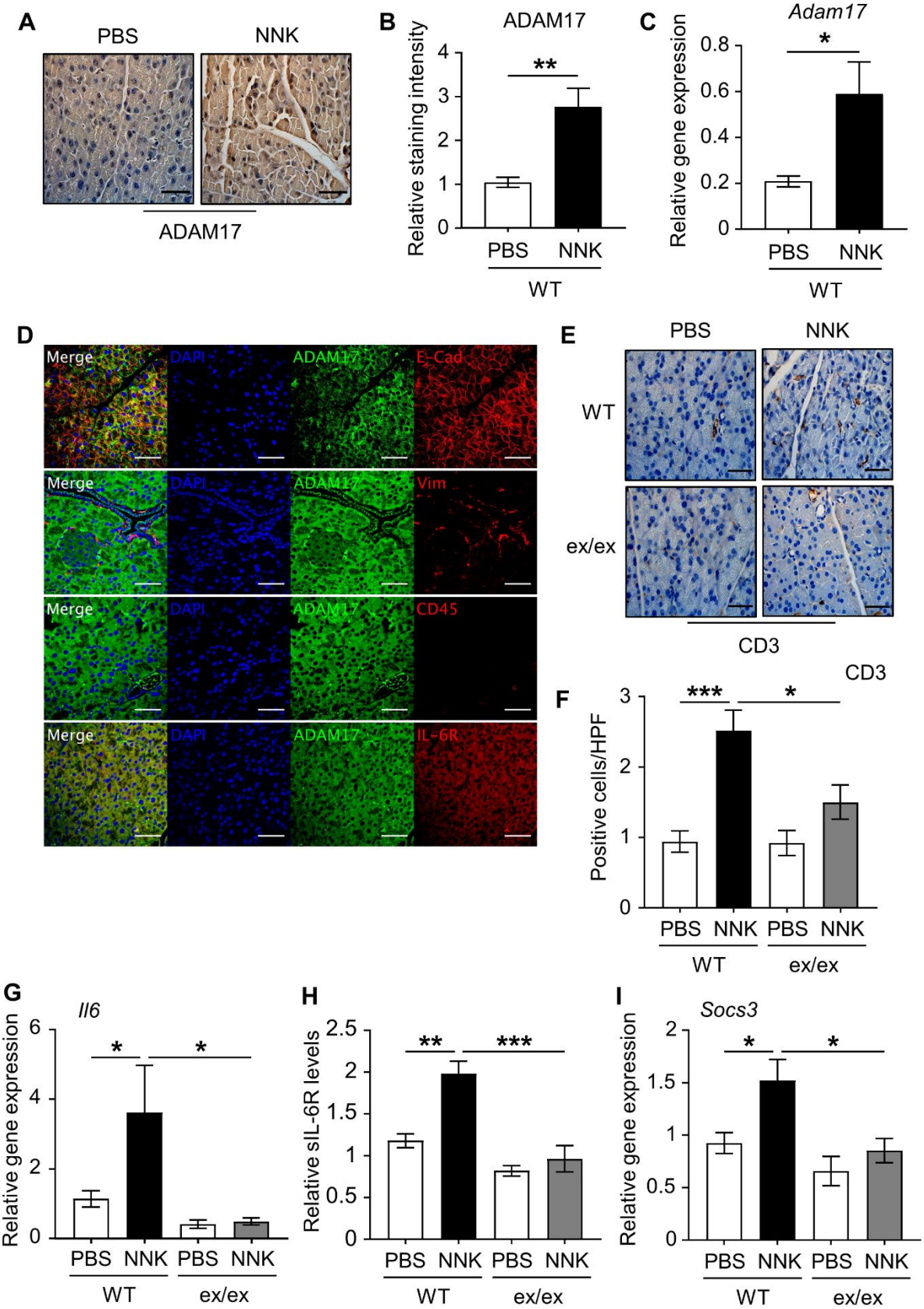
Supplementary Figure 1: **Reduced expression of ADAM17 in the pancreas of** *Adam17*$^{ex/ex}$ mice **during cerulein-mediated pancreatitis.** (**A**) qPCR expression analysis of *Adam17* gene (normalized against *18S rRNA*) in pancreatic tissues from wild-type (WT) and *Adam17*$^{ex/ex}$ (ex/ex) mice treated with cerulein (Cer) (n = 5/group). $^{**}P < 0.01$, Student's *t*-test. (**B**) qPCR expression analysis of the *Adam17* gene (normalized against *18SrRNA*) in pancreatic acinar cell cultures from WT and *Adam17*$^{ex/ex}$ (ex/ex) mice treated with cerulein (Cer) (n = 3-5/group). $^{*}P < 0.05$, Student's *t*-test.


A
PBS
Cer
WT
ex/ex
Ly6G
B
Ly6G
Positive cells/HPF
***
**
PBS
Cer
PBS
Cer
WT
ex/ex
C
F4/80
D
F4/80
*
*
E
B220
F
B220
G
CD11c
H
CD11c
I
Toluidine blue
J
Mast cells
K
Cleaved caspase-3
L
Cleaved caspase-3
Relative staining intensity
**
**

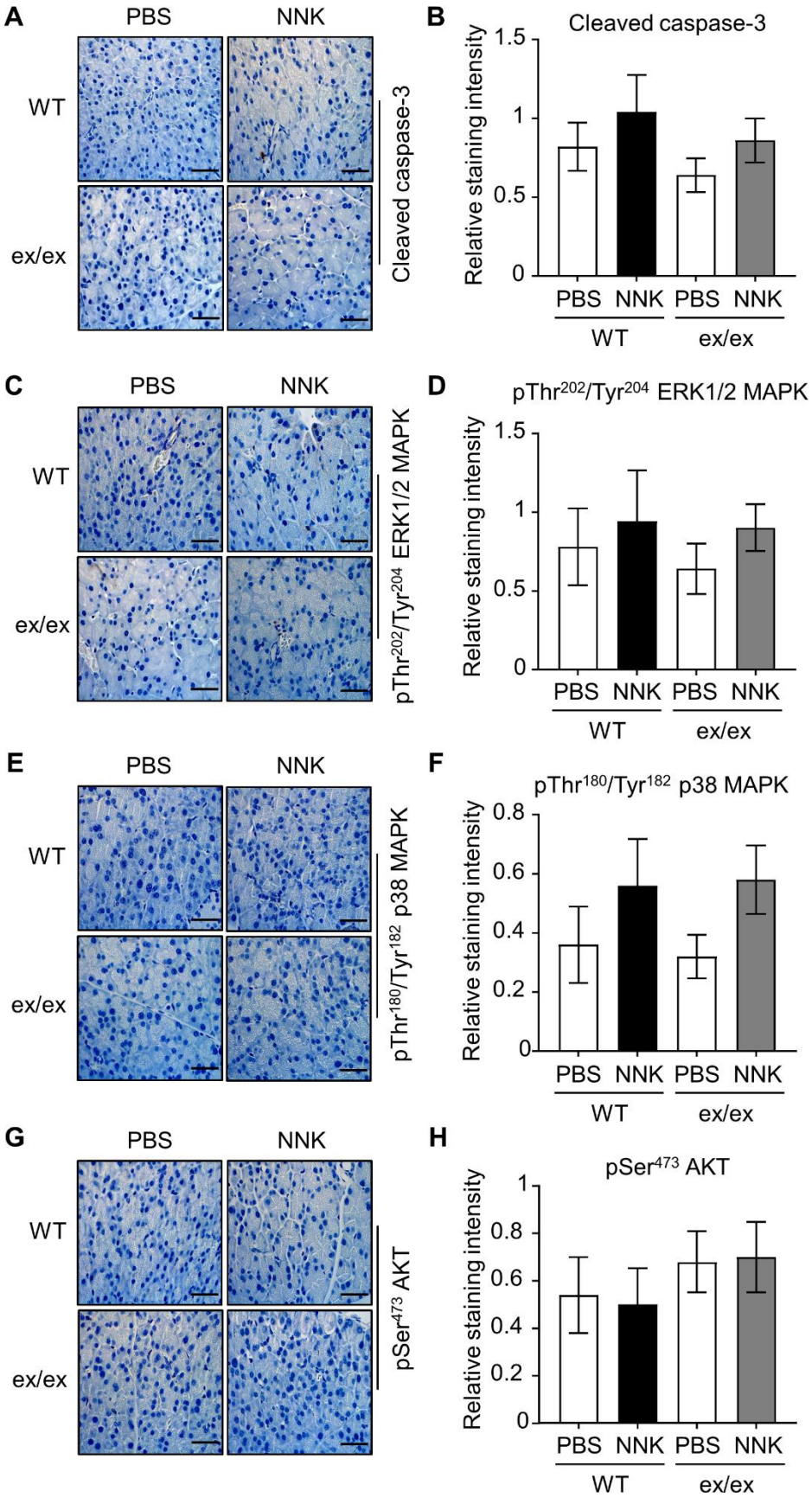
Supplementary Figure 2: **Immunohistochemical analysis of immune cell subsets and acinar cell death in pancreatic tissues following the induction of cerulein-mediated pancreatitis.** (**A**, **C**, **E**, **G**, **I** and **K**) Representative images of Ly6G, F4/80, B220, CD11c, Toluidine blue and cleaved caspase-3-stained pancreatic sections from WT mice and *Adam17*$^{ex/ex}$ mice (ex/ex) treated with either PBS (as a control) or cerulein (Cer), respectively. Scale bar, 100μm. (**B**, **D**, **F**, **H** and **J**) Quantification of Ly6G, F4/80, B220, CD11c and Toluidine blue-positive cells per high-power field (HPF) in pancreatic tissues of the indicated groups, respectively (n = 5-8/group). (**L**) Quantification of cleaved caspase-3-staining intensity in pancreatic tissues of the indicated groups (n = 6-8/group). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, one-way ANOVA.


A
PBS
Cer
WT
ex/ex
pThr202/Tyr204 ERK1/2 MAPK
B
pThr202/Tyr204 ERK1/2 MAPK
Relative staining intensity
0
1
2
3
4
**
*
PBS
Cer
PBS
Cer
WT
ex/ex
C
PBS
Cer
WT
ex/ex
pThr180/Tyr182 p38 MAPK
D
pThr180/Tyr182 p38 MAPK
Relative staining intensity
0
0.5
1
1.5
2
*
*
PBS
Cer
PBS
Cer
WT
ex/ex
E
PBS
Cer
WT
ex/ex
pSer473 AKT
F
pSer473 AKT
Relative staining intensity
0
0.5
1
1.5
2
PBS
Cer
PBS
Cer
WT
ex/ex

Supplementary Figure 3: **Immunohistochemical analysis of major IL-6 trans-signalling pathways in pancreatic tissues following the induction of cerulein-mediated pancreatitis**. (**A**, **C** and **E**) Representative images of pThr^{202}/Tyr^{204} ERK1/2 MAPK, pThr^{180}/Tyr^{182} p38 MAPK and pSer^{473} AKT-stained pancreatic sections from WT mice and *Adam17*$^{ex/ex}$ mice (ex/ex) treated with either PBS (as a control) or cerulein (Cer), respectively. Scale bar, 100µm. (**B**, **D** and **F**) Quantification of pThr^{202}/Tyr^{204} ERK1/2 MAPK, pThr^{180}/Tyr^{182} p38 MAPK and pSer^{473} AKT staining intensity in pancreatic tissues of the indicated groups, respectively (n = 5/group). *P < 0.05, **P < 0.01, one-way ANOVA.


A
PBS
NNK
ADAM17
B
ADAM17
Relative staining intensity
**
PBS
NNK
WT
C
Adam17
Relative gene expression
*
PBS
NNK
WT
D
Merge
DAPI
ADAM17
E-Cad
Vim
CD45
IL-6R
E
PBS
NNK
WT
ex/ex
CD3
F
CD3
Positive cells/HPF
***
*
PBS
NNK
WT
ex/ex
G
Il6
Relative gene expression
H
Relative sIL-6R levels
**
***
I
Socs3
Relative gene expression

Supplementary Figure 4: **Expression of distinct cell-type markers and IL-6 trans-signalling components in pancreatic tissues of the cigarette smoke carcinogen (NNK)-induced pancreatitis model**. (**A**) Representative images of ADAM17-stained pancreatic sections from PBS or NNK-treated wild-type (WT) pseudo-A/J mice. Scale bar, 100µm. (**B**) Quantification of ADAM17 staining intensity in pancreatic tissues of the indicated groups (n = 5/group). $^{**}P < 0.01$, Student's *t*-test. (**C**) qPCR expression analysis of *Adam17* gene (normalized against *18SrRNA*) in pancreatic acinar cell cultures from WT pseudo-A/J mice treated with either PBS (as a control) or NNK (n = 3-5/group). $^{*}P < 0.05$, Student's *t*-test. (**D**) Representative immunofluorescence images of pancreatic sections from NNK-treated pseudo-A/J mice co-stained for ADAM17 and markers for epithelial cells (E-cadherin; E-Cad), endothelial cells (Vimentin; Vim), total immune cells (CD45) and IL-6R. DAPI nuclear staining is blue. Scale bars, 100µm. (**E**) Representative images of CD3-stained pancreatic sections from WT or *Adam17*$^{ex/ex}$ (ex/ex) pseudo-A/J mice treated with either PBS (as a control) or NNK. (**F**) Quantification of CD3-positive cells per high-power field (HPF) in pancreatic tissues of the indicated groups (n = 5/group). (**G**) qPCR expression analysis of *Il6* gene (normalized against *18SrRNA*) in pancreatic acinar cell cultures from WT or *Adam17*$^{ex/ex}$ (ex/ex) pseudo-A/J mice treated with either PBS (as a control) or NNK (n = 3-5/group). (**H**) Relative soluble IL-6R levels (sIL-6R) in pancreatic acinar cell culture supernatants from the indicated groups (n = 3-5/group). (**I**) qPCR expression analysis of *Socs3* gene (normalized against *18SrRNA*) in pancreatic tissues of the indicated groups (n = 5/group). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, one-way ANOVA.


A
PBS
NNK
WT
ex/ex
Cleaved caspase-3
B
Cleaved caspase-3
Relative staining intensity
1.5
1
0.5
0
PBS NNK PBS NNK
WT
ex/ex
C
PBS
NNK
WT
ex/ex
pThr202/Tyr204 ERK1/2 MAPK
D
pThr202/Tyr204 ERK1/2 MAPK
Relative staining intensity
1.5
1
0.5
0
PBS NNK PBS NNK
WT
ex/ex
E
PBS
NNK
WT
ex/ex
pThr180/Tyr182 p38 MAPK
F
pThr180/Tyr182 p38 MAPK
Relative staining intensity
0.8
0.6
0.4
0.2
0
PBS NNK PBS NNK
WT
ex/ex
G
PBS
NNK
WT
ex/ex
pSer473 AKT
H
pSer473 AKT
Relative staining intensity
1
0.8
0.6
0.4
0.2
0
PBS NNK PBS NNK
WT
ex/ex

Supplementary Figure 5: **Immunohistochemical analysis of major IL-6-trans signalling pathways in pancreatic tissues of the cigarette smoke carcinogen (NNK)-induced pancreatitis model**. (**A**, **C**, **E** and **G**) Representative images of cleaved caspase-3, pThr^{202}/Tyr^{204} ERK1/2 MAPK, pThr^{180}/Tyr^{182} p38 MAPK and pSer^{473} AKT-stained pancreatic sections from PBS (as a control) or NNK-treated wild-type (WT) and *Adam17*$^{ex/ex}$ mice (ex/ex), respectively. Scale bar, 100µm. (**B**, **D**, **F** and **H**) Quantification of cleaved caspase-3, pThr^{202}/Tyr^{204} ERK1/2 MAPK, pThr^{180}/Tyr^{182} p38 MAPK and pSer^{473} AKT staining intensity in pancreatic tissues of the indicated groups, respectively (n = 5/group).

Supplementary Table 1: Characteristics of the patients used in this study.

	Normal	Pancreatitis
Mean age		
Years (range)	36.8 (23-57)	62.3 (45-78)
Sex [number (%)]		
Male	9 (60)	6 (40)
Female	6 (40)	9 (60)

Supplementary Table 2: List of mouse primer sequences used in the study.

Gene	Forward	Reverse
Cxcl1	CCTTGACCCTGAAGCTCCCT	CGGGTGCCATCAGAGCAGTCT
Cxcl2	AACATCCAGAGCTTGAGTGTGA	TTCAGGGTCAAGGCAAACTT
Ccl2	AGGTGTCCCAAAGAAGCTGTA	ATGTCTGGACCCATTCCTTCT
Il6	ATGGATGCTACCAAACTGGAT	TGAAGGACTCTGGCTTTGTCT
Socs3	GCGGGCACCTTTCTTATCC	TCCCCGACTGGGTCTTGAC