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Supplementary Materials for

Pancreatitis is an FGF21-deficient state that is corrected by replacement therapy

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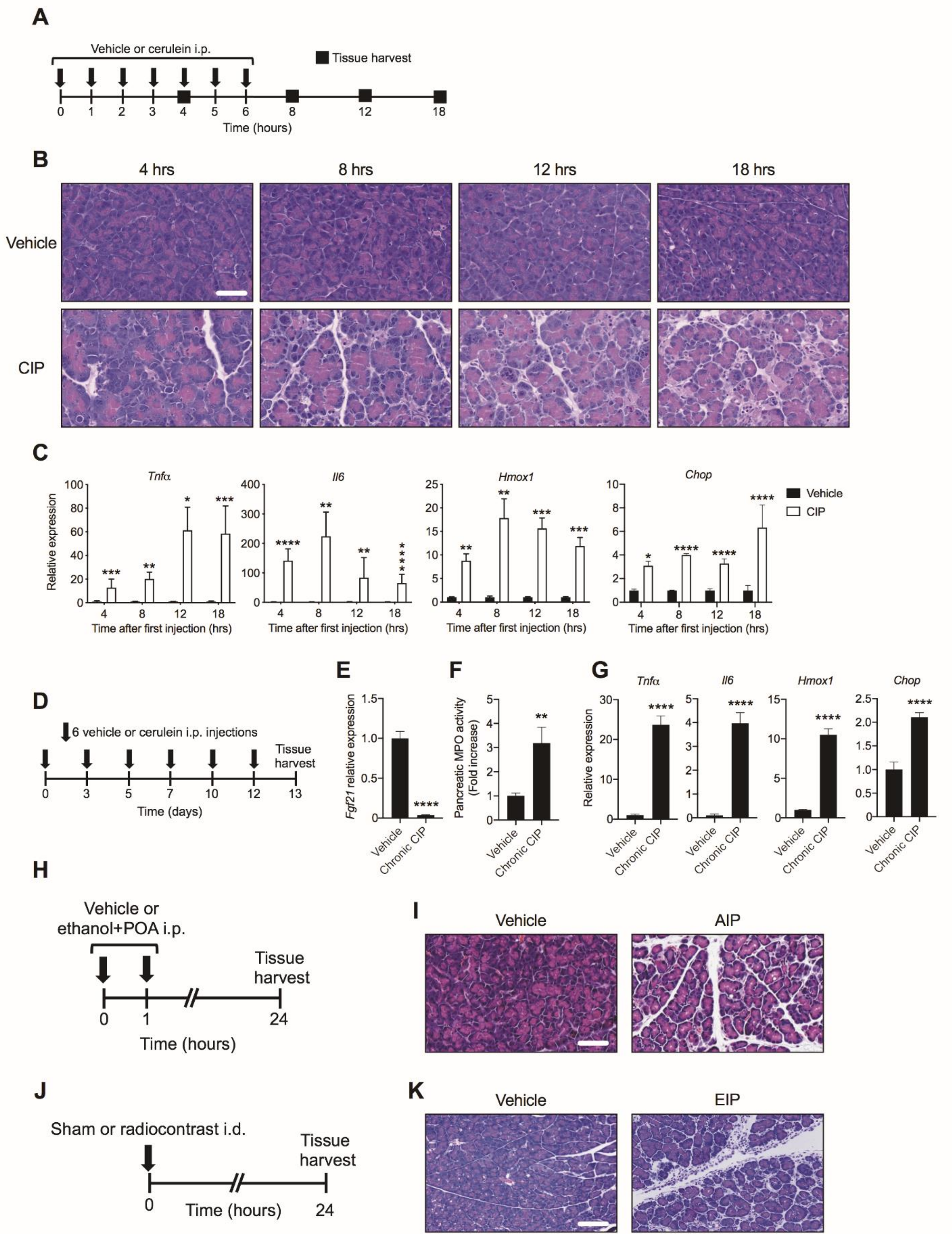
The PDF file includes:

Other Supplementary Material for this manuscript includes the following:

(available at stm.sciencemag.org/cgi/content/full/12/525/eaay5186/DC1)

Data file S1 (Microsoft Excel format). Human pancreas tissue donor information.
Data file S2 (Microsoft Excel format). Raw numbers for each data point in each graph from the study.


A
Vehicle or cerulein i.p.
Tissue harvest
0
1
2
3
4
5
6
8
12
18
Time (hours)
B
4 hrs
8 hrs
12 hrs
18 hrs
Vehicle
CIP
C
Tnfα
Il6
Hmox1
Chop
Relative expression
100
80
60
40
20
0
400
300
200
100
25
20
15
10
5
10
8
6
4
2
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****
Time after first injection (hrs)
Vehicle
CIP
D
6 vehicle or cerulein i.p. injections
Tissue harvest
0
3
5
7
10
12
13
Time (days)
E
Fgf21 relative expression
1.5
1.0
0.5
0
****
Vehicle
Chronic CIP
F
Pancreatic MPO activity (Fold increase)
5
4
3
2
1
0
**
G
Tnfα
Il6
Hmox1
Chop
Relative expression
30
20
10
5
4
3
2
1
15
2.5
2.0
1.5
1.0
0.5
****
****
****
****
H
Vehicle or ethanol+POA i.p.
Tissue harvest
0
1
24
Time (hours)
I
Vehicle
AIP
J
Sham or radiocontrast i.d.
Tissue harvest
0
Time (hours)
24
K
Vehicle
EIP

Fig. S1. Experimental design and characterization of acute and chronic pancreatitis mouse models. Data are related to Fig. 1A-D**. (A)** Schematic of CIP time-course experiment in mice where a black arrow represents a single 50 μg/kg cerulein i.p. injection and black boxes show tissue collection timepoints. **(B, C)** Representative H&E stained images of pancreata **(B)** and pancreatic mRNA markers of inflammation (*Tnfα, Il6*), oxidative stress (*Hmox1*), and ER stress (*Chop*) **(C)** from mice treated as described in (A). **(D)** Schematic of chronic cerulein-induced pancreatitis (chronic CIP), where each black arrow represents 6 hourly i.p. injections of 50 μg/kg cerulein. **(E-G)** Pancreatic *Fgf21* mRNA expression **(E)**, pancreatic MPO activity **(F)** and mRNA markers **(G)** of inflammation (*Tnfα, Il6*), oxidative stress (*Hmox1*), and ER stress (*Chop*) from mice treated as described in (D). **(H)** Schematic of alcohol-induced pancreatitis by 2 injections of 1.35 g/kg ethanol and 150 mg/kg palmitoleic acid (POA). **(I)** Representative H&E stained images of pancreata from mice treated as described in (H). **(J)** Schematic of the ERCP-induced pancreatitis (EIP) experimental model where the radiocontrast agent (iohexol, 300 mg/ml) was infused intraductally (i.d.) and tissue harvested at 24 h. **(K)** Representative H&E stained images of pancreata from mice treated as described in (J). Scale bar indicates 50 μm for all images. Results are means ± S.E.M. *n* = number of mice/group for all experiments. *n* = 3-4 in (A-C); *n* = 8 in (D, -G); *n* = 8 in (H, I); *n* = 5 in (J, K). * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$. Asterisks indicate statistically different values relative to vehicle.

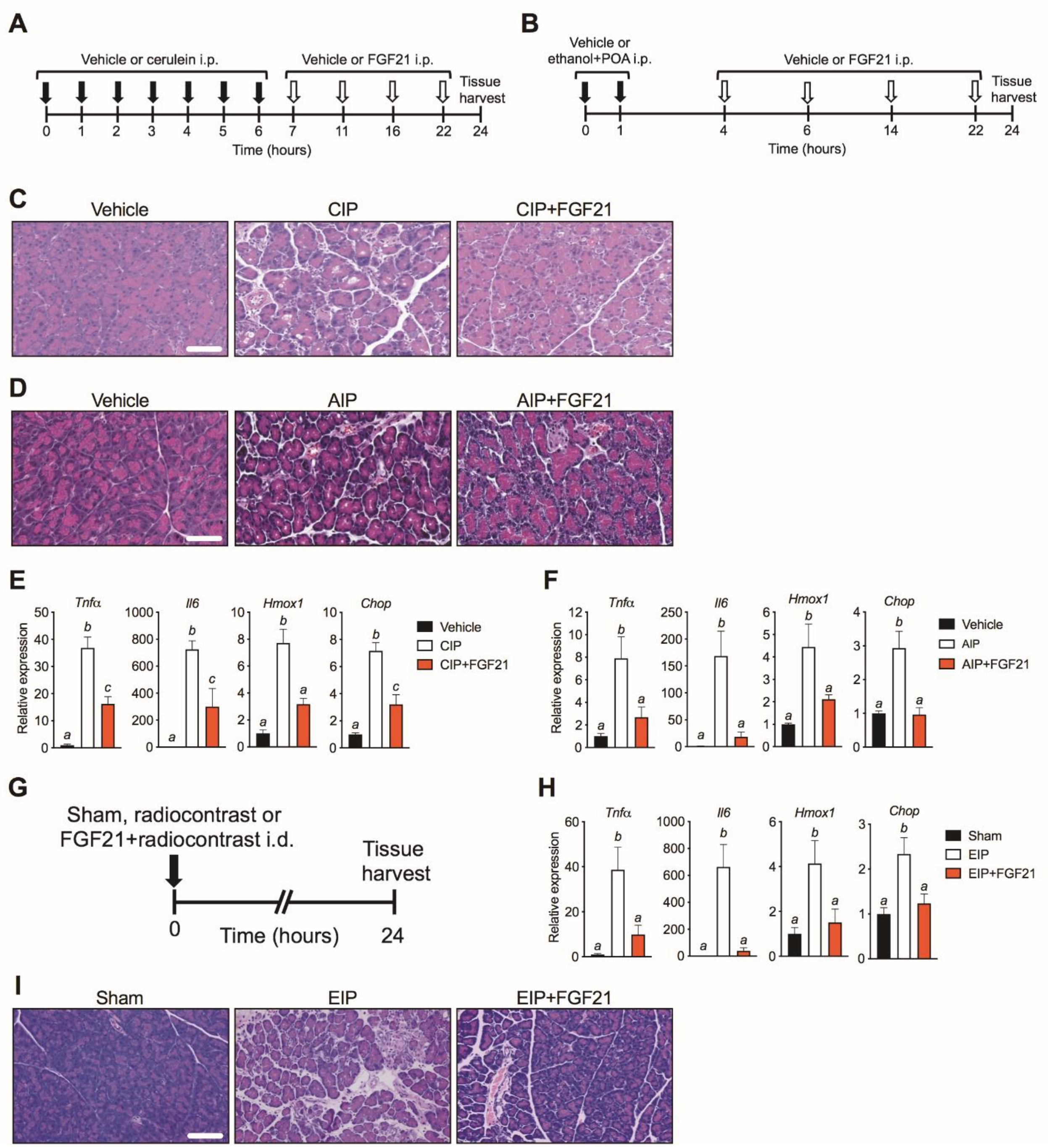

Fig. S2. Experimental design, histology, and gene expression data for FGF21 therapy experiments in three different models of pancreatitis. Data are related to Fig. 1E-N. **(A)** Schematic of CIP in mice with 50 μg/kg cerulein (black arrow) and 1 mg/kg FGF21 (white arrow) i.p. injections. **(B)** Schematic of AIP in mice with 1.35 g/kg ethanol plus 150 mg/kg palmitoleic acid (POA) (black arrow) and 1 mg/kg FGF21 (white arrow) i.p. injections. **(C, D)** Representative H&E images of stained pancreata from mice with CIP **(C)** or AIP **(D)** and treated as described in (A) and (B) respectively**. (E, F)** Pancreatic mRNA markers of inflammation (*Tnfα, Il6*), oxidative stress (*Hmox1*), and ER stress (*Chop*) from mice in Fig. 1E **(E)** and Fig. 1F **(F)**. **(G)** Schematic of the EIP experimental model with FGF21 treatment where the radiocontrast agent (iohexol, 300 mg/ml) was infused i.d. with 1 μg/ml FGF21. **(H)** Pancreatic mRNA markers of inflammation (*Tnfa, Il6*), oxidative stress (*Hmox1*) and ER stress (*Chop*) from mice in Fig. 1M. **(I)** Representative H&E images of stained pancreata from mice treated as described in (G). Scale bar indicates 50 μm for all images. Results are means ± S.E.M. *n* = number of mice/group for all experiments. *n* = 6 in (A, C and E); *n* = 8 in (B, D, and F); *n* = 5 in (G-H). Statistical differences ($p > 0.05$) are indicated between all values that have different letters.

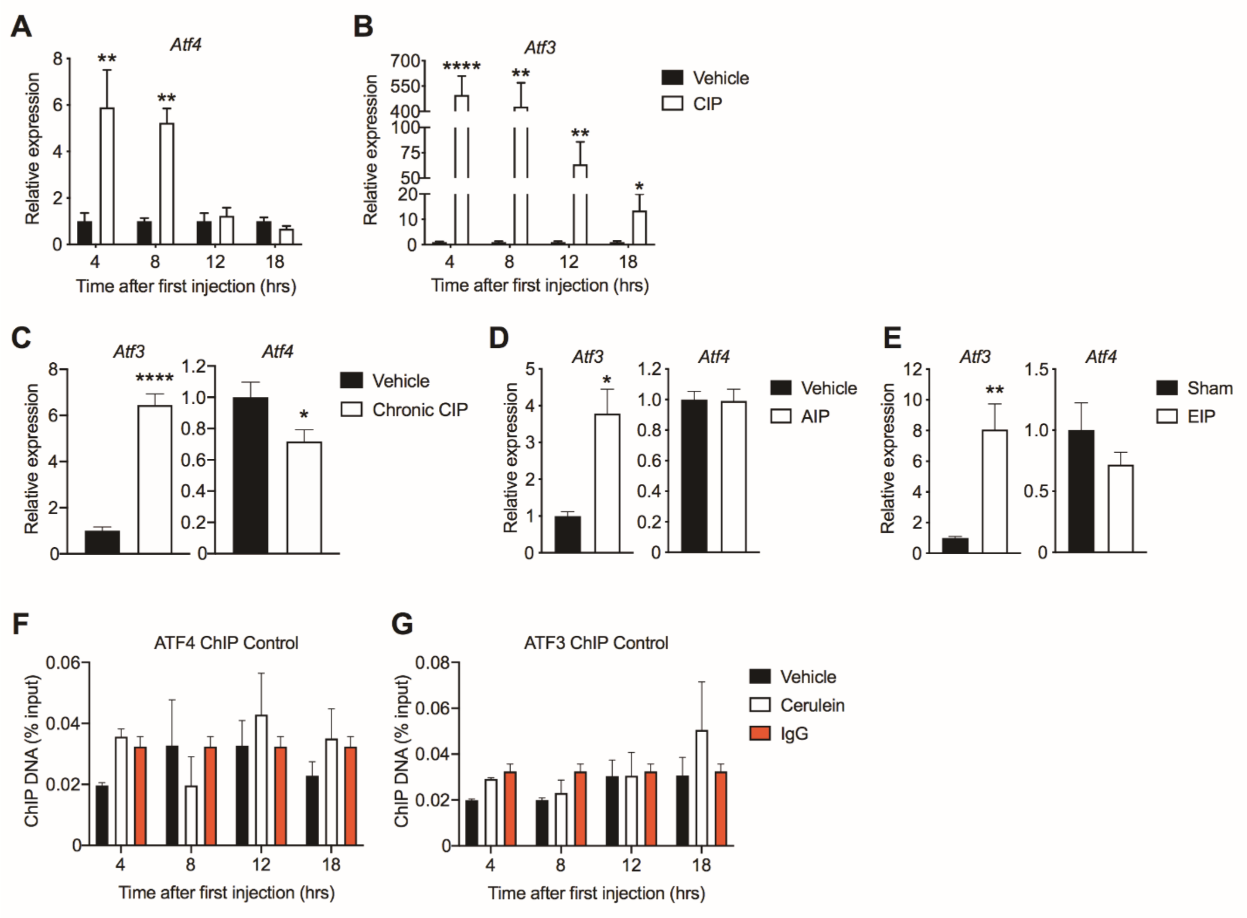

Fig. S3. *Atf3* and *Atf4* expression in pancreatitis mouse models and ATF3 and ATF4 ChIP experimental controls. Data are related to Fig. 3. **(A, B)** Pancreatic *Atf4* **(A)** and *Atf3* **(B)** mRNA expression in the time course of CIP from Fig. 3A. **(C-E)** Pancreatic *Atf3* and *Atf4* mRNA in CIP, **(C)** AIP, **(D)** and EIP **(E)**. **(F, G)** ATF4 **(F)** and ATF3 **(G)** ChIP-qPCR controls targeting a genomic region located at -6614/-6551 from the *Fgf21* transcription start site for the experiments in Fig. 3E-H. Results are expressed as means ± S.E.M.; *n* = number of mice/group for all experiments. *n* = 3-4 in (A, B, F, and G); *n* = 8 in (C, D); *n* = 5 in (E). * p < 0.05; ** p < 0.01; **** p < 0.0001. Asterisks indicate statistically different values relative to vehicle.

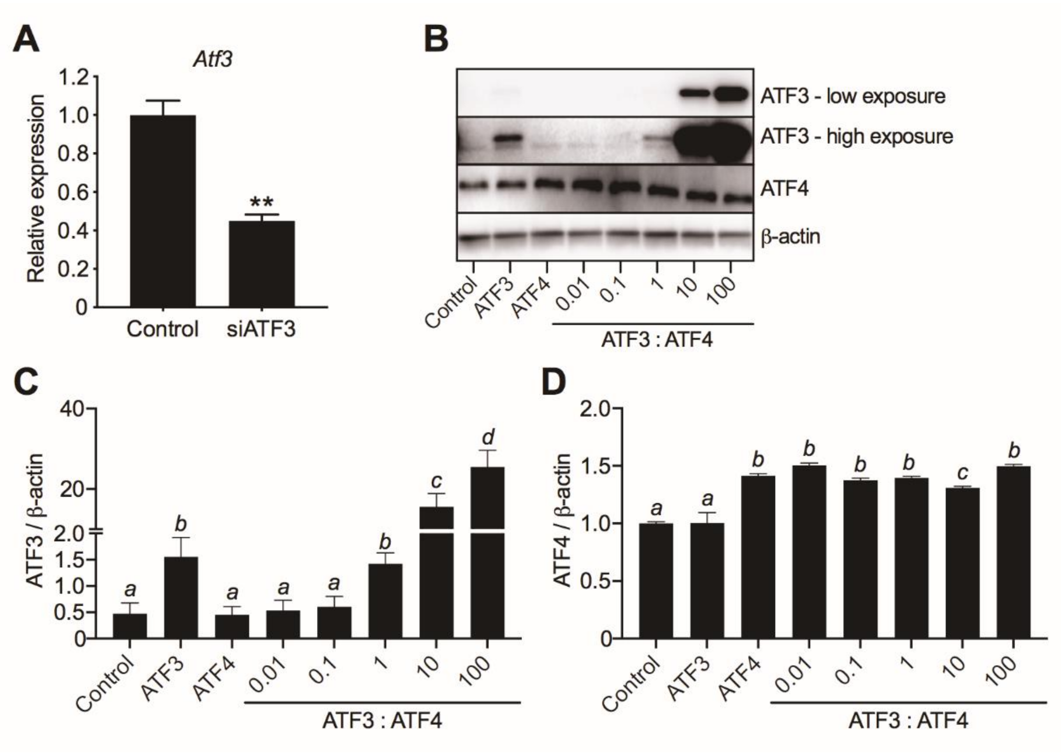

Fig. S4. ATF3 and ATF4 expression in 266-6 cells. Data are related to Fig. 4. **(A)** *Atf3* mRNA expression in control or *Atf3*-siRNA knockdown 266-6 cells. **(B)** ATF3 and ATF4 immunoblot with β-actin as loading control in 266-6 cells transfected with 0.01-100 ng of *Atf3* and 1 ng of *Atf4* plasmid. High and low exposure times are shown for ATF3. **(C, D)** Quantification of ATF3 **(C)** and ATF4 **(D)** in (B). Results are expressed as means ± S.E.M.; n = 3 for all experiments. * $p < 0.05$ relative to control. Statistical differences ($p > 0.05$) are indicated between all values that have different letters.

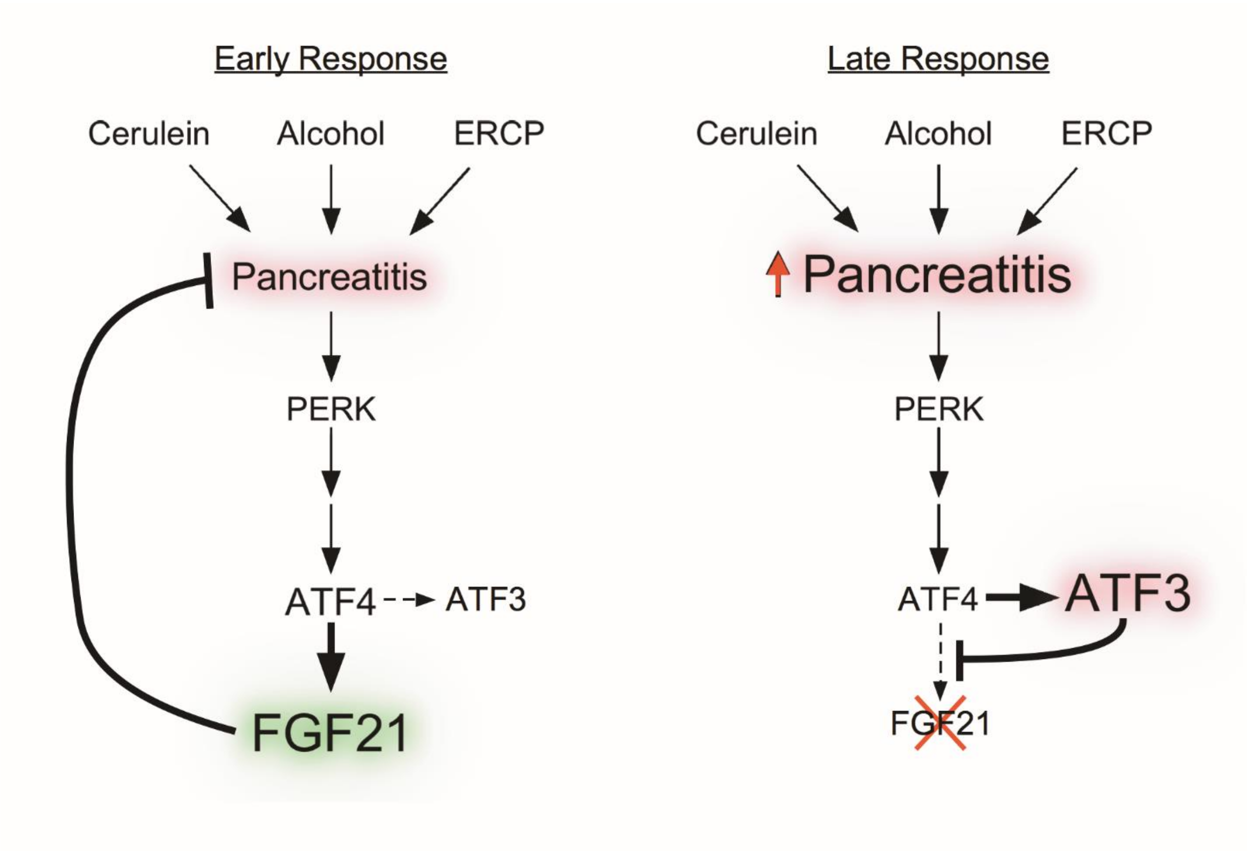

Fig. S5. Model of FGF21's protective role in pancreatitis.

Supplementary Tables

Table S1. qPCR primer sequences.

Gene name	**Accession No.**	**Forward primer sequence (5'→3')**	**Reverse primer sequence (5'→3')**
Atf3	NM_007498.2	tggagatgtcagtcaccaagtct	gcagcagcaattttatttctttct
Atf4	NM_009716.2	actctaatccctccatgtgtaaagg	caggtaggactctgggctcat
Chop	NM_007837.3	ccagaaggaagtgcatcttca	actgcacgtggaccaggtt
Fgf21	NM_020013	cctctaggtttctttgccaacag	aagctgcaggcctcaggat
Hmox1	NM_010442.2	caggtgtccagagaaggcttt	tcttccagggccgtgtagat
Il6	NM_031168	tcgtggaaatgagaaaagagttg	agtgcatcatcgttgttcataca
Tnfa	NM_13049	ctgaggtcaatctgcccaagtac	cttcacagagcaatgactccaaag
*U36B4**	NM_007475	cgtcctcgttggagtgaca	cggtgcgtcagggattg

* *U36B4* was used as the housekeeping gene for gene expression normalization.

Table S2. Primary antibody list.

Target protein	Company	Application
ATF3	Abcam	IF
ATF3	CST	IB and ChIP
ATF4	CST	IB, IF and ChIP
FGF21	Abcam	IF
Normal Rabbit IgG	CST	ChIP
β-actin	Abcam	IB

IB, immunoblot; IF, immunofluorescence; ChIP, chromatin immunoprecipitation, CST, Cell Signaling Technology.

Table S3. ChIP-qPCR primer sequences.

Primer set name	Position relative to *Fgf21* TSS*	Forward primer sequence (5’→3’)	Reverse primer sequence (5’→3’)
Control	-6614/-6551	tcagcatgcctccaaagc	tcagccttgaggaagagtagaca
AARE1+3	-1060/-981	gcaggacgctgtctggtg	gcttagcattcgggccttg
AARE2	-168/-81	ttcagacccctgttggaaag	cacacttggcaggaacctgaat

*TSS, transcription start site.