Supplemental Methods

Cell culture conditions

Mouse pancreatic fibroblasts were established from adult pancreata. Tissues were minced and then incubated in 1 mg/mL collagenase (Sigma-Aldrich) in HBSS for 15 mins at 37°C before passing through a 40 μm cell strainer. Fibroblast cells were seeded to 80-90% confluency in IMDM with 10% FBS, then starved by replacing the media with IMDM with 0.5% of FBS. Sixteen hours later, cells were treated with conditioned medium from primary mouse pancreatic cancer cells (derived from KPC tumor). Cells were harvested for RNA at 6h, 12h and 24h after treatment.

N-Acetyl-L-Cysteine Treatment

iKras* or iKras*;IL6$^{-/-}$ mice were treated with N-Acetyl-L-Cysteine (NAC) (Sigma-Aldrich) at two weeks after pancreatitis induction and sustained for three weeks. NAC was administered in 200 μl PBS at a dose of 150 mg/kg body weight daily through intraperitoneal injection.

Histopathological analysis

The histopathological analysis was performed as previously described [1,2]. In brief, 5 randomly selected, non-overlapping high-power images (20X objective) were taken for each slide. A minimum of 50 total acinar or ductal clusters was counted from at least three independent animals for each group. Each cluster counted was classified as normal (nl), ADM, PanIN1A, 1B, 2 and 3 based on the classification consensus [3]. The data was expressed as percentage of total counted clusters. Error bars represent standard error.

Histological analysis

Pancreatic tissues from both experimental and control mice were fixed overnight in 10% neutral-buffered formalin, embedded in paraffin and sectioned. Embedding and sectioning were performed by the University of Michigan Cancer Center Histopathology Core. Sections were

subjected to Hematoxylin/eosin (H&E), periodic acid Schiff (PAS), Gomori's Trichrome, toluidine blue staining, immunohistochemical and immunofluorescence staining as previously described [4,5]. Antibodies used are listed in Table S1. For apoptosis detection, the ApopTag Red In Situ Apoptosis Detection Kit (S7165; Millipore) was used in accordance with the manufacturer's protocol. Images were taken with an Olympus BX-51 microscope, and Olympus DP71 digital camera, and DP Controller software. The immunofluorescent images were acquired using the Olympus IX-71 confocal microscope and FluoView FV500/IX software. Tissues for transmission electron microscope analysis were prepared by University of Michigan Microscopy & Image Analysis Laboratory and images were acquired using Philips CM-100 transmission electron microscope.

Proliferation analysis

Analysis of proliferating cells was performed using tissue stained for Ki67. Briefly, 3 randomly selected, non-overlapping high-power images (20X objective) were taken for each slide from at least 3 independent animals for each group. Nuclei positive for Ki67 were counted as actively proliferating cells. Epithelial and stromal compartments for each image were counted separately, and data was expressed as percentage of total counted nuclei for each compartment. Errors bars are expressed as standard error.

Western Blotting

Tissue was homogenized in RIPA buffer (Sigma R0278) and protease inhibitor (Sigma P8340). Equal amounts of proteins were electrophoresed in 12% SDS-PAGE gels, transferred to PVDF membrane (Bio-Rad). Membranes were blocked with 5% milk and incubated with primary antibody at 4°C overnight. HRP-conjugated secondary antibody was used at 1:5000 dilution and detected by ECL (Thermo Scientific).

Quantitative RT-PCR

Total RNA was prepared from pancreas tissue samples using RNeasy (Qiagen) according to the manufacturer's protocol. Reverse-transcription reactions were conducted using High Capacity cDNA Reverse Transcription kit (Applied Biosystems). Samples for QPCR were prepared with 1X SYBR Green PCR Master Mix (Applied Biosystems) and various primers (Supplemental Table 2). All primers were optimized for amplification under reaction conditions as follows: 95^{o}C 10mins, followed by 40 cycles of 95^{o}C 15 secs and 60^{o}C 1min. Melt curve analysis was performed for all samples after the completion of the amplification protocol. GAPDH was used as the housekeeping gene expression control for the in vitro experiments and Cyclophilin was used for in vivo experiments.

Table S1: Antibodies

Antibody	Supplier	Catalog Number	IHC dilution	IF Dilution	WB dilution
Primary Antibody					
Amylase	Sigma-Aldrich	A8273	-	1:100	-
Akt (pan)	Cell Signaling	4691	-	-	1:1000
Alpha-smooth muscle actin	Sigma-Aldrich	A2547	-	1:1000	-
CK19 (TromaⅢ)	Iowa Developmental Hybridoma Bank	-	1:100	1:100	-
Claudin-18	Invitrogen	700178	1:150	-	-
Cleaved Caspase-3	Cell Signaling	9661	1:100	1:100	-
ERK1/2 (p44/42)	Cell Signaling	4695	-	-	1:1000

GAPDH	abcam	ab9485	-	-	1:2500
IL6	abcam	ab6672	1:500	1:500	-
Mist1	-	-	1:500	-	-
Nrf2	Sigma-Aldrich	SAB4501984	1:70	-	1:800
Ki67	Vector Laboratories	VP-RM04	1:100	1:100	-
p-Akt	Cell Signaling	4060	1:100	-	1:2000
p-ERK1/2 (phospho-p44/42)	Cell Signaling	4370	1:100	1:100	1:1000
p-Stat3	Cell Signaling	9131	1:100	-	1:2000
Stat3	Cell Signaling	9139	-	-	1:1000
Secondary Antibody					
Alexa Fluor® 488 Donkey Anti-Mouse IgG	Invitrogen	A-21202	-	1:400	-
Alexa Fluor® 488 Donkey Anti-Rabbit IgG	Invitrogen	A-21026	-	1:400	-
Alexa Fluor® 555 Goat Anti-Rabbit IgG	Invitrogen	A-21428	-	1:400	-
Alexa Fluor® 555 Goat Anti-Rat IgG	Invitrogen	A-21434	-	1:400	-
Alexa Fluor® 594 Donkey Anti-Rabbit IgG	Invitrogen	A-21207	-	1:400	-

Alexa Fluor® 633 Goat Anti-Rat IgG	Invitrogen	A-21094	-	1:400	-
Alexa Fluor® 647 Donkey Anti-Rabbit IgG	Invitrogen	A-31573	-	1:400	-
Biotinylated Rabbit Anti-Goat IgG	Vector Laboratories	BA-5000	1:300	-	-
Biotinylated Goat Anti-Mouse IgG	Vector Laboratories	BA-9200	1:300	-	-
Biotinylated Goat Anti-Rabbit IgG	Vector Laboratories	BA-1000	1:300	-	-
Biotinylated Rabbit Anti-Rat IgG	Vector Laboratories	BA-4001	1:300	-	-
ECL Rabbit IgG, HRP-Linked Whole Ab	GE Healthcare	NA934	-	-	1:5000

Table S2: Primer sequences for quantitative RT-PCR

Gene	Forward Primer	Reverse Primer	Ref.
Human			
IL6	TCTCCACAAGCGCCTTCG	CTCAGGGCTGAGATGCCG	[6]
Mouse			
Il6	TTCCATCCAGTTGCCTTCTTGG	TTCTCATTTCCACGATTTCCCAG	

Transgenic Kras	CAAGGACAAGGTGTACAGTTATGTGACT	GCCTGCGACGGCGGCATCTGC	[7]
Il1β	GTGGCTGTGGAGAAGCTGTG	GAAGGTCCACGGGAAAGACAC	[8]
GM-CSF	ATGCCTGTCACGTTGAATGAAG	GCGGGTCTGCACACATGTTA	[9]
Slfn4	GCCCTCTGTTCAAGTCAAGTGTCC	CCCAGATGAAATCCTTTCCACGA	
MMP7	GGAGATGCTCACTTTGACAAGGA	ATTCATGGGTGGCAGCAAAC	[10]
MMP9	CGCCTTGGTGTAGCACAACA	ACAGGGTTTGCCTTCTCCGTT	
MT1MMP	ACCCTTTGATGGTGAAGGAGGGTT	TGGCGGAGGGATCGTTAGAATGTT	
TIMP1	TACACCCCAGTCATGGAAAGC	CGGCCCGTGATGAGAAACT	
Il2	CCTTCAAATTTTACTTGCCCA	TGAGTCAAATCCAGAACATGC	[11]
Il4	CCCCAGCTAGTTGTCATCCTG	CGCATCCGTGGATATGGCTC	
Il10	GCTATGCTGCCTGCTCTTACT	CCTGCTGATCCTCATGCCA	
Il11	CTGCACAGATGAGAGACAAATTCC	GAAGCTGCAAAGATCCCAATG	[8]
Il17	AAGGCAGCAGCGATCATCC	GGAACGGTTGAGGTAGTCTGAG	
COX2	TGAGCAACTATTCCAAACCAGC	GCACGTAGTCTTCGATCACTATC	
Tnfα	CATCTTCTCAAAATTCGAGTGACAA	TGGGAGTAGACAAGGTACAACCC	
Ifng	TCAAGTGGCATAGATGTGGAAGAA	TGGCTCTGCAGGATTTTCAGT	
Bak	TATTAACCGGCGCTACGACAC	CTTAAATAGGCTGGAGGCGATCTT	[12]
Bax	AAACTGGTGCTCAAGGCCCT	AGCAGCCGCTCACGGAG	[12]
Bcl-2	CCGGGAGAACAGGGTATGATAA	CCCACTCGTAGCCCCTCTG	[12]
Bcl-x	GGTCGCATCGTGGCCTTT	TCCGACTCACCAATACCTGCAT	[12]
Cyclophilin	TCACAGAATTATTCCAGGATTCATG	TGCCGCCAGTGCCATT	[10]
GAPDH	TTGATGGCAACAATCTCCAC	CGTCCCGTAGACAAAATGGT	[13]

Supplemental References

1. Collins MA, Bednar F, Zhang Y, Brisset JC, Galban S, et al. (2012) Oncogenic Kras is required for both the initiation and maintenance of pancreatic cancer in mice. J Clin Invest 122: 639-653.
2. Kojima K, Vickers SM, Adsay NV, Jhala NC, Kim HG, et al. (2007) Inactivation of Smad4 accelerates Kras(G12D)-mediated pancreatic neoplasia. Cancer Res 67: 8121-8130.
3. Hruban RH, Adsay NV, Albores-Saavedra J, Anver MR, Biankin AV, et al. (2006) Pathology of genetically engineered mouse models of pancreatic exocrine cancer: consensus report and recommendations. Cancer Res 66: 95-106.
4. Pasca di Magliano M, Sekine S, Ermilov A, Ferris J, Dlugosz AA, et al. (2006) Hedgehog/Ras interactions regulate early stages of pancreatic cancer. Genes Dev 20: 3161-3173.
5. Zare-Mirzaie A, Lotfi M, Sadeghipour A, Haghi-Ashtiani MT (2012) Analysis of colonic mucosa mast cell count in patients with chronic diarrhea. Saudi J Gastroenterol 18: 322-326.
6. Good M, Siggers RH, Sodhi CP, Afrazi A, Alkhudari F, et al. (2012) Amniotic fluid inhibits Toll-like receptor 4 signaling in the fetal and neonatal intestinal epithelium. Proc Natl Acad Sci U S A 109: 11330-11335.
7. Fisher GH, Wellen SL, Klimstra D, Lenczowski JM, Tichelaar JW, et al. (2001) Induction and apoptotic regression of lung adenocarcinomas by regulation of a K-Ras transgene in the presence and absence of tumor suppressor genes. Genes Dev 15: 3249-3262.
8. Bollrath J, Phesse TJ, von Burstin VA, Putoczki T, Bennecke M, et al. (2009) gp130-mediated Stat3 activation in enterocytes regulates cell survival and cell-cycle progression during colitis-associated tumorigenesis. Cancer Cell 15: 91-102.
9. Mirabella F, Baxter EW, Boissinot M, James SR, Cockerill PN (2010) The human IL-3/granulocyte-macrophage colony-stimulating factor locus is epigenetically silent in immature thymocytes and is progressively activated during T cell development. J Immunol 184: 3043-3054.

10. Pasca di Magliano M, Biankin AV, Heiser PW, Cano DA, Gutierrez PJ, et al. (2007) Common activation of canonical Wnt signaling in pancreatic adenocarcinoma. PLoS ONE 2: e1155.

11. Zhao F, Zhang Y, Wang H, Jin M, He S, et al. (2011) Blockade of osteopontin reduces alloreactive CD8+ T cell-mediated graft-versus-host disease. Blood 117: 1723-1733.

12. Greten FR, Weber CK, Greten TF, Schneider G, Wagner M, et al. (2002) Stat3 and NF-kappaB activation prevents apoptosis in pancreatic carcinogenesis. Gastroenterology 123: 2052-2063.

13. Grivennikov S, Karin E, Terzic J, Mucida D, Yu GY, et al. (2009) IL-6 and Stat3 are required for survival of intestinal epithelial cells and development of colitis-associated cancer. Cancer Cell 15: 103-113.