Supplementary Methods

Histological analysis

Tumor tissues from both experimental and control mice were fixed overnight in 10% neutral-buffered formalin, embedded in paraffin and sectioned. Hematoxylin/eosin staining (H&E), immunohistochemistry and immunofluorescent stainings were performed as previously described.[1] For CD8 immunohistochemistry staining, tissues were fixed in 4% PFA and embedded for frozen sections. Antibodies used are listed in Table S1. For immunofluorescence, Alexa Fluor (Invitrogen) secondary antibodies were used. Cell nuclei were counterstained with DAPI (Invitrogen). ApopTag Red In Situ Apoptosis Detection Kit (S7165; Millipore) was used for apoptosis detection. Images were taken using Olympus BX-51 microscope with an 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. Image-Pro Plus 4.5 was used to measure the percentage of positive area of immuhistochemistry staining and to count the number of immunofluorescent staining positive cells per high power field image. At least three images per sample were analyzed for immuhistochemistry staining or immunofluorescent staining. Periodic acid–Schiff (PAS) staining for intracellular mucins, to identify low-grade PanINs, was performed following the manufacturer's instructions (Alcian Blue/PAS Special Stain Kit, Thermo Scientific).

Flow cytometric analysis and sorting

Single-cell suspensions of fresh spleen, pancreas or tumor tissue were prepared as previously described[2] and incubated with fluorescently conjugated antibodies listed in Table S1. Flow cytometric analysis was performed on a Cyan^{TM} ADP analyzer (Beckman Coulter) and data were analyzed with Summit 4.3 software. Cell sorting was performed using a MoFlo Astrio (Beckman Coulter).

Western blotting

Western blotting was conducted as previously described.[3] Detailed antibody information is included in Table S1.

Quantitative RT-PCR

Total RNA was isolated using RNeasy (Qiagen) and reverse-transcription reactions were conducted using High Capacity cDNA Reverse Transcription kit (Applied Biosystems) according to the manufacturer's protocol. Samples for qRT-PCR were prepared with 1X SYBR Green PCR Master Mix (Applied Biosystems) and various primers (Table S2). RT-PCR was performed as follows: 95^{o}C 10mins, followed by 40 cycles of 95^{o}C 15 secs and 60^{o}C 1min. Cyclophilin A expression was used for normalization.

Table S1: Antibodies

Antibody	Supplier	Catalog Number	IHC dilution	IF Dilution	Western-blot Dilution
α-Amylase	Sigma-Aldrich	A8273	-	1:100	-
Alpha-smooth muscle actin	Sigma-Aldrich	A2547	1:1000	1:1000	-
α-Tubulin	Cell Signaling	3973	-	-	1:1000
AKT	Cell Signaling	4691	-	-	1:1000
B-Raf	Cell Signaling	9433	-	-	1:1000
β-Actin	Santa Cruz	sc-69879	-	-	1:1000
c-Raf	Cell Signaling	9422	-	-	1:1000
CD3	Abcam	Ab5690	-	1:75	-
CD8	eBioscience	14-0083	-	1:75	-
CK19 (TromaIII)	Iowa Developmental Hybridoma Bank	-	1:100	1:100	-
Cleaved Caspase-3	Cell Signaling	9661	1:400	-	-
EGF receptor	Cell Signaling	4267	-	-	1:1000

ERK1/2 (p44/42)	Cell Signaling	4695	-	-	1:1000
E-cadherin	BD Pharmingen	610181	-	1:100	-
F4/80	BMA Biomedicals	T-2006	1:100	-	-
GAPDH	abcam	Ab9485	-	-	1:2500
Ki67	Vector Laboratories	VP-RM04	1:100	-	-
MEK	Cell Signaling	4694	-	-	1:1000
PD-L1	eBioscience	14-5982	1:100	-	1:1000
PD-L1	Cell Signaling	13684	-	-	1:1000
p-AKT	Cell Signaling	4060	-	-	1:2000
p-B-Raf (Ser445)	Cell Signaling	2696	-	-	1:1000
p-c-Raf (Ser338)	Cell Signaling	9427	-	-	1:1000
p-EGFR (Tyr1068)	Cell Signaling	3777	-	-	1:1000
p-ERK1/2 (phospho-p44/42)	Cell Signaling	4370	1:100	-	1:1000
p-MEK	Cell Signaling	2338	-	-	1:2000
p-Stat3	Cell Signaling	9131	1:100	-	1:1000
Stat3	Cell Signaling	9139	-	-	1:1000
Flow Cytometry Antibody	**Supplier**	**Clone**	**Dilution**		
CD3	BD Pharmingen	17A2	1:50		
CD4	BD Pharmingen	RM4-5	1:50		
CD8α	BD Pharmingen	53-6.7	1:50		
CD11b	BD Pharmingen	M1/70	1:50		
CD25	BD Pharmingen	PC61	1:50		
CD45	Invitrogen	30-F11	1:50		
CD64	BD Pharmingen	X54-5/7.1.1	1:50		
CD274 (PD-L1)	BD Pharmingen	MIH5	1:50		
CD326 (EpCAM)	eBioscience	G8.8	1:50		
F4/80	BD Pharmingen	BM8	1:50		
Foxp3	BD Pharmingen	FJK-16s	1:50		

Gr-1 (Ly-6G and ly-6C)	BD Pharmingen	RB6-8C5	1:50		

Table S2: Primer sequences for quantitative RT-PCR

Gene	Forward Primer	Reverse Primer
Mouse		
Arg1	CTCCAAGCCAAAGTCCTTAGAG	AGGAGCTGTCATTAGGGACATC
Ctla-4	TTTTGTAGCCCTGCTCACTCT	CTGAAGGTTGGGTCACCTGTA
Cyclophilin A	TCACAGAATTATTCCAGGATTCATG	TGCCGCCAGTGCCATT
F4/80	CCCAGCTTCTGCCACCTGCA	GGAGCCATTCAAGACAAAGCC
Gzmb	CCACTCTCGACCCTACATGG	GGCCCCCAAAGTGACATTTATT
Ifnβ1	GTGGCTGTGGAGAAGCTGTG	GAAGGTCCACGGGAAAGACAC
Ifnγ	TCAAGTGGCATAGATGTGGAAGAA	TGGCTCTGCAGGATTTTCAGT
iNos2	CCAAGCCCTCACCTACTTCC	CTCTGAGGGCTGACACAAGG
Mrc1	CTCTGTTCAGCTATTGGACGC	CGGAATTTCTGGGATTCAGCTTC
Msr1	GCACAATCTGTGATGATCGCT	CCCAGCATCTTCTGAATGTGAA
Pdcd1	ACCCTGGTCATTCACTTGGG	CATTTGCTCCCTCTGACACTG
Pdcd1lg1	GCTGAAGTCAATGCCCCATA	TCCACGGAAATTCTCTGGTTG
Pdcd1lg2	TTGTCGGTGTGATTGGCTTC	AAAAGGCAGCACACAGTTGC
Prf-1	AGCACAAGTTCGTGCCAGG	GCGTCTCTCATTAGGGAGTTTTT
Human		
Cyclophilin A	CCCACCGTGTTCTTCGACATT	GGACCCGTATGCTTTAGGATGA
Il6	TGCGTCCGTAGTTTCCTTCT	GCCTCAGACATCTCCAGTCC
PDCDLG1	TGGCATTTGCTGAACGCATTT	TGCAGCCAGGTCTAATTGTTTT

PDCDLG2	ATTGCAGCTTCACCAGATAGC	AAAGTTGCATTCCAGGGTCAC

References

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