Supplemental Figures

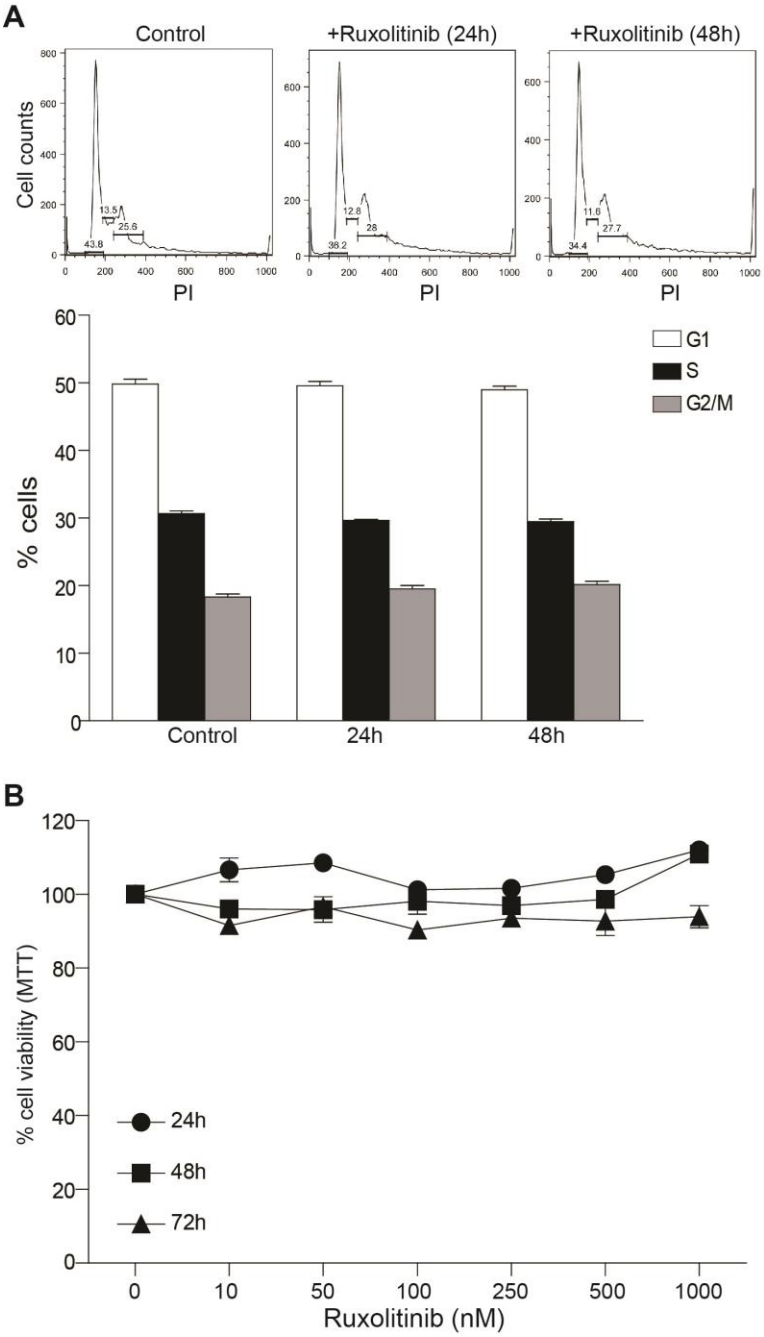

Figure S1. Ruxolitinib treatment does not alter cell cycle and proliferation of tumor cells. A. PANC02-H7 cells were cultured in the presence of ruxolitinib (250 nM). Cells were stained with PI and analyzed by flow cytometry at the indicated time points. The percentages of cells in the various cell cycle phases are indicated. Bottom panel: the percentage of cells in various stages of cell cycles as shown in the top panel is quantified. Column: mean; Bar: SD. B. PANC02-H7 cells were cultured in the presence of ruxolitinib at the indicated doses for the indicated time points and analyzed by MTT assays. The cell viability of untreated cells was set at 100%.

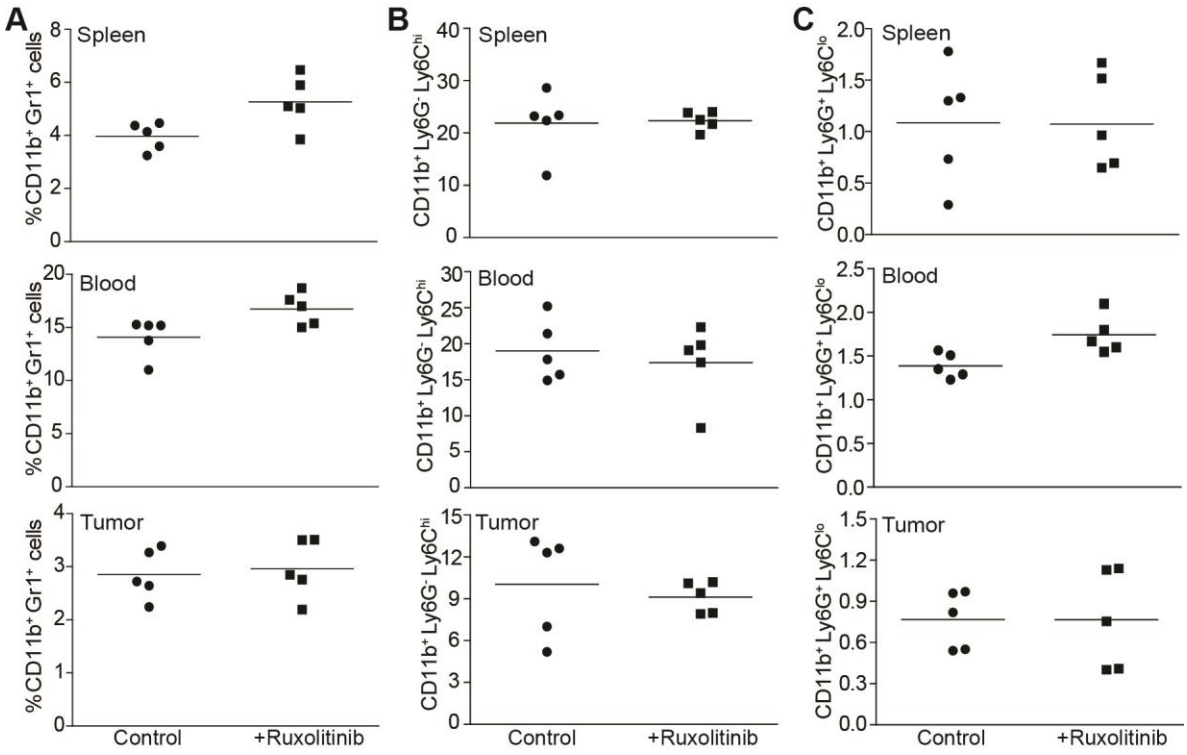

Figure S2. MDSC level in tumor-bearing mice. PANC02-H7 cells were orthotopicaally transplanted to mice. The tumor-bearing mice were treated daily with solvent (n=5) or ruxolitinib (n=5, 50 mg/kg body weight) for 10 days. Spleen, blood and tumors were collected to make single cells. The cell mixtures were stained with CD11b- and Gr1-specific mAbs (A), CD11b-Ly6G- and Ly6C-specific mAbs (B & C) and analyzed by flow cytometry. A: Quantification of $CD11b^{+}Gr1^{+}$ general MDSCs. B: $CD11b^{+}Ly6G^{-}Ly6Chi$ M-MDSCs. C: Quantification of $CD11b^{+}Ly6G^{+}Ly6C^{lo}$ PMN-MDSCs. Each dot represents % MDSCs of one mouse.

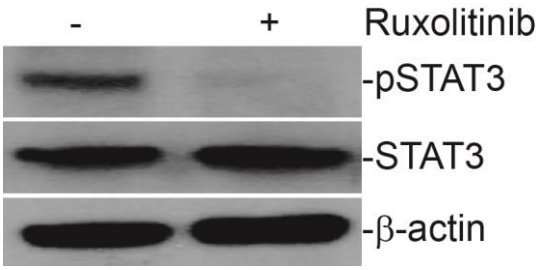

Figure S3. Ruxolitinib inhibits STAT3 activation in tumor-infiltrating CTLs. $CD8^+$ T cells were purified from tumor tissues from control (n=5) and Ruxolitinib-treated (n=5) mice. The purified cells from the five control and five Ruxolitinib-treated mice were pooled and lysed for total protein, and analyzed by Western blotting using the indicated antibodies. β-actin is used as normalization control.

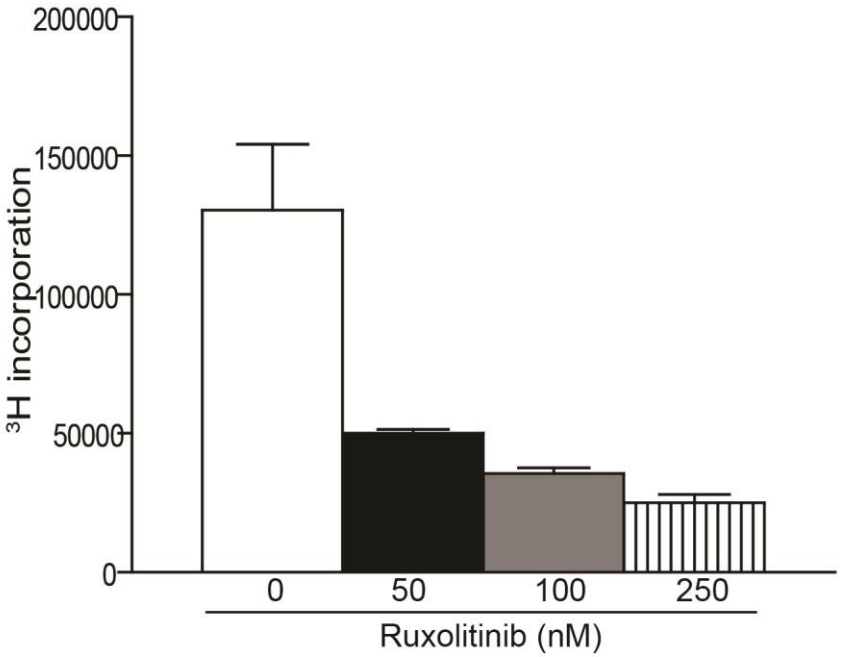

Figure S4. Ruxolitinib inhibits T cell proliferation in vitro. Purified $CD3^+$ T cells were cultured in anti-CD3/CD28-coated plates in the presence of ruxolitinib for 3 days. T cell proliferation was measured by 3H-thymidine incorporation.

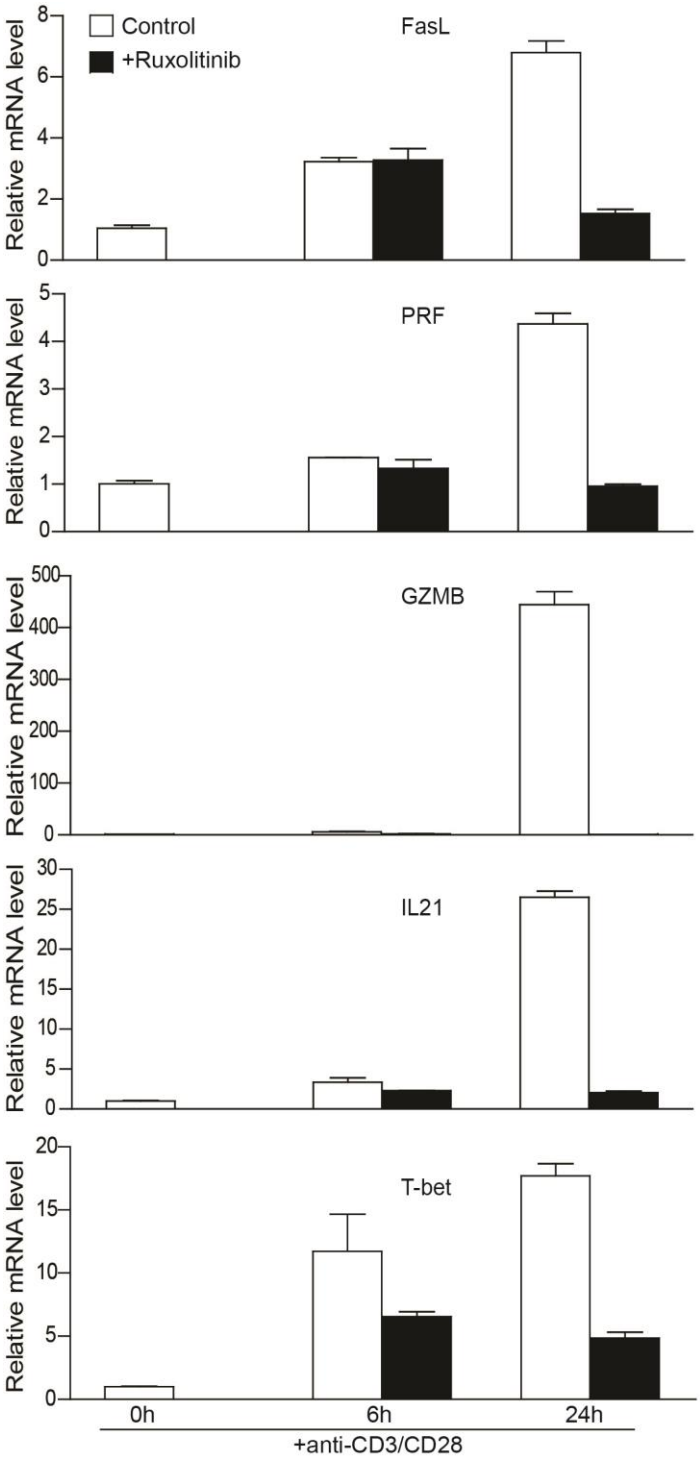

Figure S5. Ruxolitinib inhibits T cell effector expression. $CD3^+$ T cells were purified from spleen of C57BL/6 mice and cultured in anti-CD3/CD28-coated plates in the absence or presence of ruxolitinib. RNA was isolated at the indicated time points and analyzed for expression levels of the indicated genes by real-time PCR.

Table S1. Reagents

Name	Company	Cat log#	Application
Pstat (Y701)	BD transduction laboratory	612133	WB
STST1 (C-terminus)	BD transduction laboratory	51-90002093	WB
pSTAT2 (Y690)	Abcam	ab53132	WB
STAT2 (H190)	Santa cruz	sc22816	WB
pSTAT3 (Y705)	Upstate	05-485	WB
STAT3	BD transduction laboratory	610190	WB
pSTAT4 (Y693)	Santa cruz	sc101804	WB
STAT4 (H119)	Santa cruz	sc7959	WB
pSTAT5 (Y641)	BD Bioscience Pharmigen	558242	WB
STAT 5	BD transduction laboratory	51-9002096	WB
pSTAT6 (Y641)	Santa cruz	sc11762	WB
STAT6	BD transduction laboratory	51-9002196	WB
β-actin	Sigma	A5441	WB
APC anti-mouse PD-L1	Biolegend	124312	Flow
FITC anti-mouse CD11b	Biolegend	101206	Flow
PE anti-mouse Gr1	Biolegend	108408	Flow
PE anti-mouse Ly6G	BD Bioscience	551461	Flow
APC anti-mouse Ly6C	Biolegend	128016	Flow
APC anti-human PD-L1	Biolegend	329708	Flow
CD8	Dako	M103	IHC
PD-L1	Abcam	ab205921	IHC
Hamest anti-mouse CD3e	BD Pharmigen	553057	Coating
Hamest anti-mouse CD28	BD Pharmigen	553294	Coating
Recombinant mouse IL21	Biolegend	574502	Coating
IgG	BioXCell	BP0091	In-vivo treatment
anti-IFNγ	BioXCell	BP0055	In-vivo treatment
Fludarabine	Santa cruz	sc204755	In-vitro treatment

Table S2.PCR Primers

mCTLA4-F	CCCTGCTCACTCTTCTTTTCATCC
mCTLA4-B	TTTGGTCATTTGTCTGCCGC
mIL21-F	AAGAGGCAAGGGTGTAGTAAGAAGC
mIL21-B	GGAAAGGATGTGGGAGAGGAGAC
mRORC-F	AACTTGGGGAACCAGAACAGGG
mRORC-B	GCTTGGCAAACTCCACCACATAC
mIL10-F	GCTGGACAACATACTGCTAACCGAC
mIL10-B	CTTGCTCTTATTTTCACAGGGGAG
mTGFβ-F	TTGAGTCCCTCGCATCCCAG
mTGFβ-B	TCCCAAGGAAAGGTAGGTGATAGTC
mCD8-F	ACCTGGACATCAGAGCCCCTTG
mCD8-B	AATCCTACGCTTTGCCCACC
mCD4-F	CCTCAAGATACCCCAGGTCTCG
mCD4-B	CAAGGAAACCCAGAAAGCCG
mIFNγ-F	CCATCAGCAACAACATAAGCGTC
mIFNγ-B	TCTCTTCCCCACCCCGAATCAGCAG
mPRF1-F	CCTATGGCACGCACTTTATCACG
mPRF1-B	TTCACTGGAGACGCTGGCTTGG
mGZMB-F	GCCCACAACATCAAAGAACAGG
mGZMB-B	CCAACCAGCCACATAGCACAC
mIL17A-F	CCCTCAGACTACCTCAACCGTTC
mIL17A-B	TCTCAGGCTCCCTCTTCAGGAC
mIL6-F	TCTGGGAAATCGTGGAAATGAG
mIL6-B	TCTCTGAAGGACTCTGGCTTTGTC
mTbx21-F	TGTTCCCATTCCTGTCCTTCAC
mTbx21-B	TGCTGCCTTCTGCCTTTCC
mIL23α-F	ATAATGTGCCCCGTATCCAGTG
mIL23α-B	GCTCCCCTTTGAAGATGTCAGAG
mPD-L1-F	ATTGCTCCTTGACTGCTGGCTG
mPD-L1-B	TTCTGGGTTCCTCCTCCTTTCC
mPD-1-F	CCGCCTTCTGTAATGGTTTGAG
mPD-1-B	CGATTTTTGCCTTGGGGTGC
mFasL-F	CTTGGGCTCCTCCAGGGTCAGT
mFasL-B	TCTCCTCCATTAGCACCAGATCC
mIL24-F	AATGAATGCTGACTGAGCCTGCCC
mIL24-B	CCAAATCGGAACTCTTGACCCTC
mIL27-F	TGCTTCCTCGCTACCACACTTC
mIL27-B	TCCTCTTCCTCTTCCTCCTTGTCC
mCSF1-F	CAACACCCCCAATGCTAACG
mCSF1-B	CCCTCTGTCCTCTGAGAATCATCC
mGM-CSF-F	TCCTGGGCATTGTGGTCTACAG
mGM-CSF-B	TGGGGGGCAGTATGTCTGG
mCXCL9-F1	TCATTGCTACACTGAAGAACGGAG
mCXCL9-B1	ACGACGACGACTTTGGGGTG
mCXCL10-F1	TCTCTCCATCACTCCCCTTTACC
mCXCL10-B1	CTTGCTTCGGCAGTTACTTTTGTC
mIL9-F1	GCTTGTGTCTCTCCGTCCCAAC
mIL9-B1	CACCCGATGGAAAACAGGC
mGATA3-F1	CCTCTACGCTCCTTGCTACTCAGG
mGATA3-B1	CCCCCCCCAAAAAAAAAGC
mIFNβ-F1	CTGCGTTCCTGCTGTGCTTC
mIFNβ-B1	TCTTCTCCGTCATCTCCATAGGG
mIFNα1-F1	CTGAAGGACAGGAAGGACTTTGG
mIFNα1-B1	CTGCTGGTGGAGGTCATTGC
mβ-Actin-F	CTGGCACCACACCTTCTACAATG
mβ-Actin-B	GGGTCATCTTTTCACGGTTGG

Table S3. Pancreatic cancer patient information

Patient*	Gender	Ethnicity	Age	Cancer Stage	Primary or Metastatic	Before or After Treatment
1	Male	Black	47	pT3N1M0 (Stage IIB)	Primary (G2 histology)	Patient with prior HNSCC treated with cisplatin and radiation. Last treatment 7 months prior to surgery to remove pancreatic tumor
2	Male	Black	62	pT3N1M0 (Stage IIB)	Primary (G2 histology)	none
3	Female	White	53	pT2N0M0 (Stage IB)	Primary (G2 histology)	none
4	Male	Black	67	pT4N0M0 (Stage III)	Primary (G2 histology)	none
5	Female	White	58	pT3N0M0 (Stage IIA)	Primary (G2 histology)	none

*Patient number as shown in Figure 7.