Supplemental methods

Cell lines and reagents

Human PDAC cell lines (PANC-1, Cat No. CRL-1469 and AsPC-1 Cat No. CRL-1682), THP-1 and RAW 264.7 (murine monocyte, macrophage Cat No. SC-6003) cells were purchased from ATCC (ATCC, Manassas, VA). ATCC authenticates these cell lines using short tandem repeat analysis. The cell lines were expanded and frozen immediately after receipt. The cumulative culture length of the cells was less than 6 months after resuscitation. Early passage cells were used for all experiments, and they were not reauthenticated. PANC-1 and RAW 264.7 cells were cultured in DMEM (Cat No. 11965092, Thermo Fisher Sci, Waltham, MA) and AsPC-1 were cultured in RPMI 1640 (Cat No. 11875093, Thermo Fisher Sci, Waltham, MA) supplemented with 10 % fetal bovine serum (FBS, Cat No. S11150, R&D Systems, Minneapolis, MN), 50-units/mL penicillin, and 50 µg/mL streptomycin (Cat No. P4458, Life Technologies Inc., Frederick, MD). Cells were incubated in a humidified 5 % CO_2 atmosphere at 37 °C. All the cell were frequently tested for mycoplasma contamination using a mycoplasma detection kit from Sigma (Cat No. SAB5600252, Sigma-Aldrich, St. Louis, MO).

Creation of mPDX KPC mouse cell lines

The ascites of two different tumor-bearing KPC mice were used to create mPDX KPC and mPDX KPC-2 cell lines (21). Ascites fluids were taken from the animals during necropsy and centrifuged to pellet tumor cells (21). The pellet was rinsed in PBS and centrifuged several times before being resuspended in RPMI1640 supplemented with 4% FBS and then placed into culture. This media was used to maintain mPDX KPC and mPDX KPC-2 cell lines. As with human

PDAC cell lines, cells were expanded, frozen and upon thawing cumulative length of time of cells in culture was less than 6 months.

Plasmid transfections

Plasmid transfection experiments used FuGene HD transfection reagent (Cat. No E2311, Promega, Madison, WI) using the manufacturer's protocol. Briefly, plasmids were complexed with FuGene HD reagent (1:3 ratio) in 500 µL of serum free medium and was added to 100-mm plates, which had 2.5 mL of serum-free DMEM F12 medium. After 6 hours transfection, complete medium was added, and cells were cultured further with indicated treatments (11, 21).

Reagents and antibodies

Antibodies specific for PARP (Cat No. 9542), pStat1 (Y701) (Cat. No 9167), Stat1(Cat. No 14994), pStat3 (Y705) (Cat. No 9145), Stat3 (Cat. No 30835), XIAP (Cat. No 14334), pAkt (Cat. No 4060), Bax (Cat. No 5023), CCL2 (Cat. No 2027), Survivin (Cat No. 2803), CD31 (Cat No. 3528) and Mcl-1 (Cat. No 94296) (Cell Signaling Technology Inc, Danvers, MA), and β-actin (Cat. No ab6276, Abcam, Cambridge, MA), were used in this study. The other reagents were Transcriptor First Strand cDNA Synthesis Kits (Cat No. 4368814 Applied biosystems, Thermo Fisher Sci, Waltham, MA), MTT cell growth assay kits (Cat No. CT02, Millipore Corporation, Billerica, MA), CCL2 antibody for animal studies was procured from BioXcell (Cat No. BE0185, BioXcell, Lebanon, NH), and MMP13 inhibitor (MMP13-I, Cat No. 444283) was purchased from Sigma (St. Louis, MO). TaqMan probes for Stat1, CCL2, MMP13, NLRP3, RETNLA, NOS2, CCL5, CXCL-13, IL-1Ra, TIMP-1, CXCL-10 and β-Actin (Applied Biosystems, Thermo Scientific, Walton, MA) were used in the study.

Sorting of monocytes using flow cytometry.

For sorting, after tumor dissociation, CD45+ cells were positively selected from the tumor lysate by magnetic-assisted cell sorting. Live CD45+ CD11b+ Ly6G− cells were sorted using a BD FACS Aria fusion cell sorter. Primary macrophages were derived from bone marrow cells of C57BL/6 mice. In brief, bone marrow cells were cultured in Dulbecco's modified essential media containing 10% FBS and 50 ng/ml M-CSF (R&D system) for 5 days to establish mouse bone marrow-derived macrophages (BMDMs). BMDMs were incubated in a humidified atmosphere 5% CO_2 at 37°C. BMDMs were stimulated with $p[IC]^{PEI}$ (0.5 μg/ml) for 24 hours, and whole cell lysates were generated. Cell lysates were resolved by SDS-PAGE and subjected to immunoblotting for pStat1 (Y701).

Flow Cytometric Analysis

mPDX KPC tumors were harvested in cold RPMI. They were cut into pieces using surgical scalpels and further enzymatically dissociated through the addition of 200 μg/ml DNase I (Cat No. 10104159001), 1 mg/ml collagenase (Cat No. C0130, Roche, Sigma Aldrich, St Louis, MO), and 1 M HEPES (Cat No. 15630080, Thermo Fisher Sci, Waltham, MA) for 30–45 min at 37°C with stirring. After 20 min of incubation, 0.1 M EDTA (Cat No. AM9260G, Thermo Fisher Sci, Waltham, MA) was added to avoid clumping. The samples were incubated for an additional 10 min on ice to allow isolation of macrophages and dendritic cells. Thereafter, the preparations were passed through a 100-μm filter strainer and washed thoroughly with Hank's balanced salt solution (HBSS) buffer supplemented with 2% FCS, 20 mM HEPES, and 5 mM EDTA. Finally,

the cells were resuspended in PBS and stained for flow cytometry. Non-specific labeling was blocked with anti-CD16/32 (Cat No. 156603, Fc Block; Bio Legend) before specific labeling. LIVE/DEAD Aqua staining was used to remove dead cells. The following mAbs were used for cell surface marker staining: anti-CD45 FITC (Cat No. NB11081719); anti-CD11b BUV395 (Cat No. BDB563553); anti-F4/80 PE (Cat No. PIMA516631) anti-CD11c AF647 (Cat No. NBP250451AH); anti-Ly6G AF700 (Cat No. NBP200441Z) (Fisher Sci, Waltham, MA); anti-Ly6C PerCP-Cy5.5 (Cat No. 128011); anti-MHCII PE/Cy7 (Cat No. 25-5321-82, Thermo Fisher Sci, Waltham, MA**)**. The samples were analyzed using a BD LSR Fortessa X-20 cytometer and analyzed with FlowJo software (BD Biosciences, Ashland, OR).

Western blotting

Western blotting analysis was performed as described [18]. PDAC cells were plated for 24 hours and treated with either $[pIC]^{PEI}$ (0.5 μg/ml) or Gem (5 μM) alone or in combination for 48 hours and cells were collected and lysed, or tumors were collected from $[pIC]^{PEI}$-treated animals and single cell suspensions were made and then lysed. Equal amounts of proteins were resolved on SDS PAGE, and Western blotting analysis was performed as described (11, 21).

Cell proliferation assays

Cell growth was determined using a modified 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay as described (11). Cells were treated with either $[pIC]^{PEI}$ (0.5 μg/ml) or gemcitabine (Gem) (5 μM) alone or in combination and incubated at 37 °C for 48 hours. MTT reagent was added to the cells, and they were incubated for 4 hours at 37 °C. The absorbance of formazan was measured with a microplate reader at A560.

Terminal Deoxy Nucleotidyl Transferase-Mediated Nick-End Labeling (TUNEL) Assay

Apoptosis induction in tumor sections of mock-treated or $[pIC]^{PEI}$ (1 mg/kg, total 8 doses) treated animals was assessed with TUNEL (Cat No. 11684795910, TUNEL Kit, Sigma-Aldrich, St. Louis, MO) assay as described earlier (11) following the manufacturer's instructions. Briefly, TUNEL reaction mixture was added to permeabilized samples and incubated for 1 hour in the dark in a humidified atmosphere at 37 °C. These slides were counterstained with DAPI, and images were captured with an Olympus research fluorescence microscope and a representative image is shown.

Immunohistochemical Analysis

IHC analysis was done as described previously. Briefly, the FFPE sections were deparaffinized, rehydrated, and were permeabilized and then blocked with 2% goat serum and incubated with indicated antibodies anti-Survivin (1:100), XIAP (1:100), and anti-CD31 (1:100), overnight at 4°C. These slides were then washed with PBS and incubated with suitable secondary antibodies for an additional one hour. These sections were then developed using 3,3′-diaminobenzidine

solution as chromogen and nuclei were counterstained with hematoxylin and dehydrated and mounted as per standard protocol (11, 21).