Supplementary Methods

Macrophage adoptive transfer experiments: CD11b+Gr1- cells were isolated from single cell suspensions of p53 tumors from donor mice by serial magnetic bead isolation. Purified cells were admixed 1:1 with p53 tumor cells and 5 x 10^5 total cells were injected into new host mice. Tumor dimensions were measured 3 times per week beginning on day 7.

Flow cytometry staining and analysis: Tumors were isolated, minced in a petri dish on ice and then enzymatically dissociated in Hanks Balanced Salt Solution containing 0.5 mg/ml Collagenase IV (Sigma), 0.1 mg/ml Hyaluronidase V (Sigma), 0.6 U/ml Dispase II (Roche) and 0.005 MU/ml DNAse I (Sigma) at 37°C for 15 min. Cell suspensions were filtered through a 70μm cell strainer. Single cell suspensions (10^6 cells in 100 μL total volume) were incubated with Aqua Live Dead fixable stain (Life Technologies, Carlsbad, CA), FcR-blocking reagent (BD Biosciences, San Jose, CA) and fluorescently labeled antibodies and incubated at 4°C for 1h. Primary antibodies to cell surface markers directed against CD11b (M1/70), Gr1 (RB6-8C5), CD3 (145-2C11), CD4 (GK1.5), CD8 (53-6.7), PD-1 (J43), PD-L1 (MIH5), CD45 (30-F11), CD25 (PC62.5) and FoxP3 (FJK-16s) were from eBioscience. Multicolor FACS Analysis was performed on a BD Canto RUO 11 Color Analyzer. All data analysis was performed using the flow cytometry analysis program FloJo (Treestar).

Magnetic bead purification of myeloid cells: Single cell preparations from tumors were incubated with FcR-blocking reagent (BD Biosciences) and then with 20μl magnetic microbeads conjugated to antibodies against CD11b and Gr1, (Miltenyi Biotech MACS Microbeads)/1xE7 cells for 20 min at 4°C. Cells bound to magnetic beads were then removed from the cell suspension according to manufacturers instructions.

Immunohistochemistry and immunofluorescence: Five μm thickness cryosections of murine tissues were prepared using a Leica cryostat CM1900. Slides were fixed in ice cold acetone or 3.7% paraformaldehyde for 5 min, permeabilized in 0.1% Triton X-100 in phosphate buffered saline (PBS), blocked in 8% normal donkey serum diluted in PBS for 1.5 h at RT, and incubated with rabbit anti-CD11b (M1/70, 1:200, BD Biosciences) or rabbit anti-F480 (BM8, 1:200 eBiosciences) diluted in 8% normal donkey serum overnight at 4ºC. After extensive washing, slides were incubated with 1-2 μg/ml cross-absorbed donkey anti-rabbit IgG (H+L) conjugated with Alexa Fluor 488 (Invitrogen). Slides were counterstained with DAPI (Invitrogen). Coverslips were mounted with Dako Cytomation fluorescent mounting medium. IHC was conducted with FFPE (5μm) tissue sections of human PDAC or murine PDAC that were subjected to heat-mediated antigen retrieval immersed in citrate buffer (pH 6.0) for 20 min. prior to blocking with 10% goat serum for 30 min. Primary tissues were then stained for 2h at RT

using mouse anti-human CD68 (PG-M1, 1:200, Dako) anti-human PI3Kγ monoclonal antibodies (kindly provided by E. Hirsch, University of Torino, Italy), rabbit anti-claudin-18 (Invitrogen 700178), rabbit anti-cytokeratin (Sigma Aldrich A8273), rabbit anti-Amylase (Abcam AB21156,), Following washing of antibody, primary antibodies were detected by incubation in goat anti-rabbit IgG-Alexa488 or with Goat anti-rabbit IgG/HRP. Masson's trichrome staining was performed according to routine protocol. Anti-CD68 stained slides were counterstained with eosin, and fluorescent antibody stained sections were counterstained with 4',6-diamidino-2-phenylindole (DAPI) to identify nuclei. Slides were washed and mounted in DAKO fluorescent mounting medium. Immunofluorescence images were collected on a Nikon microscope (Eclipse TE2000-U) and analyzed with MetaMorph Software. Pixels/field or cell number/field were quantified in five 100x fields from biological replicates.

Murine macrophage polarization: Bone marrow derived cells (BMDC) were aseptically harvested from 6-8 week-old female mice by flushing leg bones of euthanized mice with phosphate buffered saline (PBS), 0.5% BSA, 2mM EDTA, incubating in red cell lysis buffer (155 mM NH_4Cl, 10 mM $NaHCO_3$ and 0.1 mM EDTA) and centrifuging over Histopaque 1083 to purify the mononuclear cells. Approximately $5X10^7$ BMDC were purified by gradient centrifugation from the femurs and tibias of a single mouse. Purified mononuclear cells were cultured in RPMI + 20% serum + 50ng/ml M-CSF (PeproTech). Macrophages were polarized with either interferon gamma (20 ng/ml, Peprotech) plus lipopolysaccharide (100 ng/ml, Sigma) for 24h or IL-4 (20 ng/ml, Peprotech), for 48h. Total RNA was harvested from macrophages using the RNeasy Mini Kit (Qiagen) according to the manufacture's instructions.

***In vitro* fibroblast stimulation:** Primary murine embryonic fibroblasts (Lonza) were serum starved for 6h and incubated with serum and stimuli free conditioned media from polarized macrophages. In some studies, 1 μg/ml neutralizing anti-TGFβ (R&D Systems, AB-100-NA) or anti-PDFG-BB (R&D Systems, AB-23-NA) was added to the stimulation media. Collagen mRNA expression was determined by RT-PCR after 24h of stimulation.

RT-PCR: cDNA was prepared using 1 μg RNA with the qScript cDNA Synthesis Kit (Quanta Biosciences). Sybr green-based qPCR was performed using human and murine primers to *Gapdh, Pdgfb,* (Qiagen QuantiTect Primer Assay). mRNA levels were normalized to *Gapdh* (dCt = Ct gene of interest – Ct *Gapdh*) and reported as either relative mRNA expression ($ddCt = 2^{\wedge-(dCt\ sample - dCt\ control)}$) or Log base 2 fold change (fold change = Log_2 (dCt test / dCt control).

ELISA assays: Whole tumors or CD11b+Gr1- cells isolated from tumors were lysed in RIPA buffer and total protein concentration was determined using a BCA Protein Assay (Pierce). Macrophage supernatants (100 μl) or 500 μg of total protein lysate from tumors were used in ELISAs to detect PDGF-BB (R&D

Biosystems). Protein expression was normalized to total volume (supernatants) or 1 mg total protein (tumor lysates).

Immunoblotting: Macrophage cultures or tumor cells were solubilized in RIPA buffer containing protease and phosphatase inhibitors. Thirty μg protein was electrophoresed on Biorad precast gradient gels and electrophoretically transferred onto PVDF membranes. Proteins were detected by incubation with 1:1000 dilutions of primary antibodies, washed and detected with Goat anti-rabbit-HRP antibodies and detected after incubation with a chemiluminescent substrate. Primary antibodies directed against p110γ (#4252) and p110α (#4255) were from Cell Signaling Technology, and anti-β-actin was from Sigma.

Tumor cell migration assay: LMP or p53 2.1.1 PDAC cells were plated on the upper surface of collagen coated 8 μm pore transwell membrane inserts and incubated in the presence of basal media (RPMI + 1% pen/strep). Serum free conditioned media collected from in vitro polarized macrophages was added to the lower chambers of transwell plates, and plates were incubated at 37°C, 5%CO_2 overnight. The upper chambers of transwell inserts were washed and wiped free of cells and then inserts were incubated in DAPI. Tumor cells/field were then quantified by fluorescence microscopy. In some studies, Imatinib (Cayman Chemical) was added to both the cell suspension and the WT macrophage conditioned media in lower chambers. For other studies, macrophage conditioned media in the lower chamber was supplemented with recombinant PDGF-AA or PDGF-BB (Peprotech) at a final concentration of 100 ng/ml.

Supplementary Figure Legends

Supplementary Figure 1: Time course of PDAC tumor inflammation

A. Size of orthotopic LMP PDAC tumors (n=5) over time. **B**. Quantification of CD11bGr1+ (n=3) and CD11b+F480+ (n=3) myeloid cell populations in LMP PDAC tumors over time. **C**. Quantification of CD4+ (n=3) and CD8+ T (n=3) cell populations in LMP PDAC tumors over time. **D**. Quantification of B cell population of LMP PDAC tumors (n=3) over time. Significance testing was performed by parametric t test.

Supplementary Figure 2: Effect of PI3Kγ inhibition on immune cell populations

A. Western blotting to detect PI3Kγ and β-actin in WT and p110γ-/- macrophages and in PDAC cell lines DT6606, DT4313 and K8484. **B**. Representative FACS plots from Control and TG100-115 treated tumors and quantification of CD11b+Gr1- (macrophages) and CD11b+Gr1+ (monocytes and neutrophils) cells

from Control (n=3) and TG100-115 (n=3) treated mice. **C-D.** Quantification of CD4 and CD8 T cell populations in spleen, lung, and liver from (**C**) normal and (**D**) PDAC-bearing WT and p110γ-/- animals. Significance testing was performed by parametric *t* test.

Supplementary Figure 3: Intravital imaging of orthotopic PDAC growth and spread
Brightfield, mCherry, and merged intravital images of mice orthotopically implanted with mCherry labeled LMP PDAC cells and treated with either TG100-115 or inert control.

Supplementary Figure 4: Effect of PI3Kγ inhibitors on PDAC growth and survival
A. Schematic of late stage TG100-115 treatment of orthotopic p53 2.1.1 PDAC tumors. **B.** Weights of TG100-115 and control treated p53 2.1.1 tumors that were treated beginning two weeks after tumor cell inoculation, as shown in **A**. **C**. Incidence of metastases in animals from **A.** Significance testing for **C** was performed by Fisher's exact test. Significance testing for **B** was performed by unpaired *t* test for two groups. **D**. Effect TG100-115 on in vitro cell proliferation of DT4313, DT6606 and K8484 PDAC cells. **E**. Effect of TG100-115 on in vitro cell proliferation of LMP and p53 2.1.1 PDAC cells (n=3-4). Significance testing was performed by parametric *t* test.

Supplementary Figure 5: Immune expression profile of macrophages isolated from PDAC tumors
A. mRNA expression levels of immune response factors in CD11b+ and CD11b- cells isolated from PDAC tumors. **B**. Relative mRNA expression of immune response genes in orthotopic TG100-115 and control treated Panc02 tumors. **C**. Relative mRNA expression of immune response genes in tumor associated macrophages (TAMs) isolated from control and TG100-115 treated orthotopic Panc02 tumors. **D-E.** mRNA expression levels of immune response factors in CD11b+ cells isolated from spleen of liver of PDAC-tumor bearing WT and p110γ□/- animals. **F.** CD8+ T cell content in WT and p110γ-/- tumors with adoptively transferred macrophages from Figure 5D (n=5-6). Significance testing was performed by parametric *t* test.

Supplementary Figure 6: Immune checkpoint characterization of PDAC tumors A. Representative plots of PD-1 expression on CD4+ and CD8+ T cells from WT and p110γ-/- p53 2.1.1 tumors and quantification of positive cells (Percent PD-1+ cells) and mean fluorescence intensity of PD-L1 expression (MFI) via flow cytometry (n=3). **B.** Representative plots of PD-L1 expression on CD45- tumor cells, CD11b+Gr1- macrophages and CD11b+Gr1+ myeloid cells in WT and p110γ-/- p53 2.1.1 tumors and quantification of positive cells (Percent PD-L1+ cells) as well as mean fluorescence intensity of PD-L1 (MFI) (n=3). **C.** Representative FACS plots of FoxP3 and CD25 expression on CD4+ T cells from WT and p110γ-/- p53 2.1.1 tumors and quantification of CD4+CD25+ FoxP3+ T cells in WT and p110γ-/- tumors (n=3). **D.** PDAC growth in WT and p110$\gamma^{-/-}$ animals treated with anti-PD1 or isotype

control (n=8) according to the dosing scheme shown. **E**. PDAC growth in animals treated with vehicle and TG100-115, with anti-PD1 or isotype control (n=8) according to the depicted dosing schemes.. Significance testing was performed by one-way Anova with Tukey's posthoc testing for multiple pairwise testing or by parametric or nonparametric Student's *t* test as appropriate.

Supplementary Figure 7: Effect of macrophage secrete factors on PDAC cell migration in vitro

A. Chemotaxis of p53 2.1.1 PDAC cells towards stimulus free conditioned medium from IL-4 stimulated WT or p110γ-/- macrophages. **B**. Effect of stimulus free conditioned medium from IL4-stimulated WT or p110γ-/-macrophages on the proliferation of LMP and p53 2.1.1 PDAC cells in vitro. **C**. Chemotaxis of p53 2.1.1 PDAC cells towards stimulus free conditioned medium from IL-4 stimulated WT macrophages in the presence or absence of dilutions of the PDGFR inhibitor Imatinib. **D**. Chemotaxis of LMP PDAC cells towards stimulus free conditioned medium from IL-4 stimulated WT macrophages in the presence or absence of Lapatinib or Erlotinib. **E**. Chemotaxis of p53 2.1.1 PDAC cells towards medium (RPMI) or stimulus free conditioned medium from IL-4 stimulated WT or p110γ -/- macrophages in the presence or absence of 100 ng/ml SCF, CCL2, PDGF-AA or PDGF-BB. **F**. Chemotaxis of PANC-1 human PDAC cells toward basal medium or basal medium containing PDGF-BB. Significance testing was performed by one-way Anova with Tukey's posthoc testing for multiple pairwise testing or by parametric or nonparametric Student's *t* test as appropriate.