**Supplemental Experimental Methods**

**Cell culture**

All cells were cultured in IMDM supplemented with 10% FBS and 1% penicillin/streptomycin (Gibco). Primary mouse pancreatic fibroblasts were derived from control or KC pancreata. Pancreas were minced with sterile scissors and subsequently digested in 1mg/mL collagenase. MSCs were sorted from cultures using Fluorescence Activated Cell Sorting. For bone marrow MSC extraction, the tibia and femur was flushed with culture media. Collected marrow was plated after being extruded through a 20 G syringe to disassociate tissue. After 2 days, cultures were washed twice with PBS and adherent cells cultured for an additional week prior to FACS. Primary MSCs were verified using both flow cytometry for defined markers as well as functional testing of differentiation capacity into bone and fat. MSC differentiation was verified up to passage 10, and all experiments were performed on MSCs below this passage number. For MSC differentiation experiments, cells were plated following instructions on commercially available differentiation kits for osteogenesis, chondrogenesis, and adipogenesis (all Gibco). Bone-marrow derived macrophages were derived using an established protocol ([1](#_ENREF_1)). Briefly, extruded bone marrow precursors were cultured in one week in growth media supplemented with 20% L929 conditioned media, 10% FBS, and 1% pen/strep. For RAW264.7 polarization experiments, neutralizing antibodies in PBS were used at indicated concentrations to block IL10 (R&D Systems #AF519) and IL6 (R&D Systems #MAB406).

**Mice**

Mice were housed in specific pathogen-free facilities of the University of Michigan Comprehensive Cancer Center. This study was approved by the University of Michigan University Committee on Use and Care of Animals (UCUCA) guidelines. Ptf1a-Cre;LSL-KrasG12D (KC) animals were generated by crossing Ptf1a-Cre mice ([2](#_ENREF_2)) with LSL-KrasG12D ([3](#_ENREF_3)). Acute pancreatitis was induced as previously described ([4](#_ENREF_4)) by two 8-hourly series of intraperitoneal injections with caerulein (Sigma) at a concentration of 75ug/kg over a 48-hour period.

**Flow Cytometry**

Single cell suspensions were prepared from the pancreas as follows: tissues were minced with sterile scalpels prior to digestion in 1mg/mL collagenase (Sigma-Aldrich) at 37°C for 15min. Digested samples were then filtered through a 40μm strainer. Single cell suspensions were prepared in HBSS with 2% FBS for Fluorescence activated cell sorting (FACS). Antibodies used for MSCs were CD44-FITC (1:50, BD Pharm), CD73-PeCy7 (1:50, ebioscience), CD49a-PE (1:50, BD Pharm), and CD90-APC (1:50, BD Pharm). Antibodies used for immune cells were CD45-Pacific Orange (1:50, BD Pharm), CD11b-APCCy7 (1:50, BD Pharm), CD3-PE (1:50, BD Pharm), F4/80-PECy5 (1:50, ebioscience), CD64-PE (1:50, BD Pharm). FACS was performed on a MoFlo Astrios (Beckman Coulter) and data analyzed using Summit 6.1 Software.

**Immunohistochemistry and Immunofluorescence**

Primary antibodies used were CK19 (1:100, Iowa Developmental Hybridoma Bank), F4/80 (1:100, BMA Biomedicals), Ki67 (1:100, Vector Laboratories), Cleaved Capsase 3 (1:300, Cell Signaling). Images were taken with an Olympus BX-51 microscope, Olympus DP71 digital camera, and CellSens standard v1.6 software. For immunofluorescence, Alexa Fluor-conjugated (Invitrogen) secondary antibodies were used. Cell nuclei were counterstained with Prolong Gold-DAPI (Invitrogen). The images were acquired using an Olympus IX-71 confocal microscope and FluoView FV500/IX software.

**Reverse Transcription Real-Time Quantitative PCR (RT-qPCR)**

Cells for RNA extraction were collected in lysis buffer (Ambion). RNA was isolated using a PureLink RNA Mini Kit (Ambion) as per manufacturer instructions. Reverse transcription was conducted with a High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems). RT-qPCR reactions were prepared with 1x SYBR Green PCR Master Mix (Applied Biosystems) and primers were optimized for amplification under the following reaction conditions: 95°C 10 minutes, followed by 40 cycles of 95°C 15 seconds and 60°C 1 minute.

|  |  |  |
| --- | --- | --- |
| **Primer** | **Forward (5’-3’)** | **Reverse (5’-3’)** |
| *Arg1* | ctccaagccaaagtccttagag | aggagctgtcattagggacatc |
| *BMP2* | gggacccgctgtcttctagt | tcaactcaaattcgctgaggac |
| *BMP4* | ttcctggtaaccgaatgctga | cctgaatctcggcgacttttt |
| *CD206* | ggcaggatcttggcaacctagta | gtttggatcggcacacaaagtc |
| *Cox-2* | tgagcaactattccaaaccagc | gcacgtagtcttcgatcactatc |
| *G-CSF* | atggctcaactttctgcccag | ctgacagtgaccaggggaac |
| *GM-CSF* | atgcctgtcacgttgaatgaag | gcgggtctgcacacatgtta |
| *IL6* | ttccatccagttgccttcttgg | ttctcatttccacgatttcccag |
| *IL10* | gctcttactgactggcatgag | cgcagctctaggagcatgtg |
| *IL12p35* | cctcagtttggccagggtc | caggtttcgggactggctaag |
| *iNOS* | ccaagccctcacctacttcc | ctctgagggctgacacaagg |
| *M-CSF* | gacttcatgccagattgcc | ggtggctttagggtacagg |
| *Mcp-1* | ttaaaaacctggatcggaaccaa | gcattagcttcagatttacgggt |
| *TGFβ1* | tgacgtcactggagttgtacgg | ggttcatgtcatggatggtgc |

Melt curve analysis was included in the amplification protocol for all samples. *Cyclophilin A* and *gapdh* were used as control housekeeping genes.

**Western Blot**

Protein was isolated from Raw264.7 cells in lysis buffer (50mM Tris pH 8.0, 1% Triton X-100, 130mM NaCL, 1mM Na3VO4\*, 10mM Na4P2O7, 10mM NaF, 1mM EDTA). Equal amounts of protein were electrophoresed in SDS-PAGE gels and transferred to PVDF membranes (Bio-Rad). The following primary antibodies from Cell Signaling Technologies were used: AKT (1:1000, Cat# 9272), pAKT (Ser473) (1:1000, Cat# 9271), ERK (1:1000, Cat# 9102), pERK (Thr202/Tyr204) (1:1000, Cat# 9101), RalA (1:2500, Cat# 3526), STAT3 (1:1000, Cat# 9139), pSTAT3 (Tyr705) (1:1000, Cat# 9131). The following horseradish peroxidase-conjugated secondary antibodies from Bio-Rad were used (1:5000): Goat Anti-Mouse (Cat# 1721011) and Goat Anti-Rabbit (Cat# 1706515). Individual protein bands were visualized using Western Lightning Plus Enhanced Chemiluminescence (PerkinElmer, Cat# NEL103001EA) and film.

**Supplemental Figure Legends**

**Figure S1: CA-MSCs have a different expression profile than P-MSCs.** (A) *Bmp2* and *Bmp4* expression in freshly sorted MSCs. (B) Expression of *Il6, Cox2, Tgfβ1, M-csf, Gm-csf,* and *G-csf* in MSCs cultured *in vitro*. (C) Expression of *Il6, Cox2, Tgfβ1,* and *Il10* in bone marrow and pancreatic MSCs.

**Figure S2: CA-MSCs promote tumor growth.** (A) Experimental design. (B) Final tumor mass. (C) Histopathological analysis of tumors from co-injection of 13442 KPC tumor cells with MSCs, scale bar: 50μm. Inset: antibody detection of F4/80. Scale bar: 20μm. (D) Antibody detection of Ki67 (green) and CK19 (red). Nuclei marked by DAPI (blue), scale bar: 20μm. (E) Quantitiation of Ki67 staining. (F) Flow cytometry analysis of tumor CD11b+;CD64+;F4/80+ macrophages. (G) RT-qPCR analysis of *Mcp-1.* (H) RT-qPCR analysis of *M-csf.* (I) Final tumor mass of iKras\*p53\*#3 tumor cells co-injected with pancreatic and bone marrow MSCs. (J) Final tumor mass of 13442 KPC tumor cells co-injected with pancreatic and bone marrow MSCs. (K) Final tumor mass for 13442 KPC tumor cells co-injected with MSCs at 2:1 and 1:1 ratios.

**Figure S3: CA-MSCs require myeloid cells to promote tumor growth.** (A) Antibody detection of CK19, scale bar: 20μm. (B) Experimental design. (C) Percent change in tumor mass after CD11b cell depletion from co-injections of tumor cells with MSCs and non-MSCs.

**Figure S4: CA-MSCs polarize macrophages to an M2-like phenotype.** (A) RT-qPCR analysis of tumor tissue from co-injections of iKras\*p53\*#3 tumor cells with MSCs for *iNos, Arg1, Cd206, Il-10.* (B) RT-qPCR analysis of tumor tissue from co-injections of 13442 KPC tumor cells with MSCs for *iNos, Arg1, Cd206, Il-10. (C)* RT-qPCR analysis of RAW264.7 macrophages treated with conditioned media from MSCs and non-MSCs (CAFs).(D) RT-qPCR analysis of RAW264.7 macrophages for *iNos*. (E) RT-qPCR analysis of RAW264.7 macrophages for *IL12p35*. # indicate significant differences from CA-MSC stimulated cells.

**Supplemental References**

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