Supplemental Methods

Animal experiments

All experimental use of animals abide by the Public Health Service Policy on Humane Care of Laboratory Animals, and was approved by the institutional animal care and use committee at Massachusetts General Hospital. All mice were bred and maintained in the Edwin L. Steele Laboratory gnotobiotic animal facility. Mice were maintained on a 12-h light-dark cycle in a temperature-controlled barrier facility, with ad libitum access to food and acidified water.

In this study, in addition to PDAC spontaneous models (described in the methods section of the main text), we used PAN02 and AK4.4 orthotopic tumor models. PAN02 tumor chunks and AK4.4 cells were authenticated by IDEXX Laboratories. (PAN02: IDEXX RADIL Case # 22366-2013. The sample was confirmed to be of mouse origin and no mammalian interspecies contamination was detected. A genetic profile was generated for the sample by using a panel of microsatellite markers for genotyping. Without a sample representing the original cell line, it is not possible to make any interpretations in terms of authentication of the cell line. However, the sample is consistent with originating from a C57BL/6 mouse strain from which this cell line originated. A comparison profile is provided and the reference profile for the C57BL/6NCr mouse strain is identical to the cell line except for an allele size difference at marker 39. The genetic profile generated for this sample can be used for comparison of samples in the future. AK4.4: IDEXX RADIL Case # 27818-2014. Without a sample representing the

original cell line, it is not possible to make any interpretations in terms of authentication of the cell line. However, the sample does have allele sizes consistent with the FVB mouse strain for 26 out of 27 markers tested (96%) and the cell line is consistent with originating from the FVB mouse strain. The genetic profile of the sample has an allele size difference at marker 5 compared to the FVB/NTac mouse strain.

For tumor implantation, mice were anesthetized for 30 minutes using intramuscular injections of ketamine/xylazine (90mg/9mg per kg BW). After surgery, Buprenorphine 0.1 mg/kg was administered every 12 hours for 72 hours. Criteria used for the decision to administer analgesics included ruffled fur, inability to self-ambulate, hyper- or hypo-activity and the appearance of dehydration. If the aforementioned symptoms persisted, despite the use of analgesics for three days, the animal harboring them was removed from the study and euthanized. For tumor-bearing animals, body weight was monitored at least twice a week to ensure that the mice did not experience greater than a 15% decrease in body weight. For tumor collection, mice were anesthetized for 30 minutes using intramuscular injections of ketamine/xylazine (90mg/9mg per kg BW). All animals were monitored by researchers and the Steele Lab Animal and Surgery Core personnel on a daily basis, including weekends and holidays following procedures.

Ultrasound measurement of tumor growth

Lean and obese mice were treated with losartan (90 mg/Kg i.p. every day) or an

equal volume of PBS intraperitoneally starting on day 5 after implantation for a period of 7 days. At appropriate time points (e.g., day 5 and 9 after implantation, an ultrasound device equipped with high-frequency ultrasound probes was used to longitudinally assess tumor growth noninvasively under isoflurane anesthesia. Final tumor volume was determined using a caliper.

Blood pressure measurements

Mice bearing orthotopic PAN02 pancreatic tumors were used for mean arterial blood pressure (MABP) measurements. Losartan treatment (90mg/kg) was initiated 5 days after tumor implantation, and MABP was measured 7 days after. Losartan was administered 2h before the measurement. Mean arterial pressure was measured by cannulation of the left carotid artery after a longitudinal skin incision above the trachea (1). After removal of the submandibular gland, the paratracheal muscles were split and the left carotid artery was isolated. The cranial end of the artery was ligated with a 6–0 silk suture and another suture was tied loosely around the central part of the artery. A metal clamp was then positioned caudally to stop blood flow during the cannulation. A polyethylene catheter (PE-10, Becton-Dickinson) filled with heparinized saline was then inserted through a hole cut proximally to the cranial ligature, and the other suture was tied tightly around the tubing and artery. The clamp was then removed and the end of the tubing was connected to a pressure transducer for the measurement of blood pressure.

Drug preparation

Angiotensin inhibitor losartan was obtained as pills, crushed and dissolved in PBS over 24 h. The solution was then sterile filtered for injection. 5-FU was obtained as a solution for injection. All drugs were purchased from the pharmacy at Massachusetts General Hospital.

Drug delivery

Mice bearing orthotopic PAN02 were injected with 30 mg/kg of 5-FU or Doxorubicin 3 weeks after tumor implantation, administered retro-orbitally 30 min prior to tumor removal. The tissue was dabbed off excess blood and then snap-frozen in liquid nitrogen for analysis. 5-FU was isolated from the tissues and measured using liquid–liquid extraction followed by reverse-phase HPLC with tandem mass spectrometry.

Analysis of desmoplasia

To assess obesity-induced extracellular matrix (ECM) remodeling and fibrosis we quantified fibrillar collagen accumulation using second harmonic generation imaging, collagen-I and hyaluronan content, number of activated pancreatic stellate cells (PSCs) using αSMA activated fibroblast marker, MMPs levels and fibrosis-related signaling pathways (e.g. AT1, TGFβ1, SMAD2 and AKT/ERK/P38JNK signaling) were measured by PCR Array, Western blot, ELISA, and immunostaining. Immune cell profile was assessed by flow cytometry. Standard protocols were previously described (2-6).

Gene Expression

Immediately after excision, tumor tissue was snap-frozen and stored in liquid nitrogen. Total RNA was extracted, and relative gene expression was determined using RT2 Profiler PCR Arrays system (Qiagen) on a Stratagene Mx3000P QPCR System. The pre-made pathway-focused arrays used (mouse genes) was "Fibrosis" (Cat. Number: PAMM011Z), "Inflammatory Cytokines and Receptors" (Cat. Number: PAMM011Z), and "Common Cytokines" (Cat. Number: PAMM021Z).

Protein Expression

- Western blot analysis

Each tumor sample was homogenized directly in lysis buffer for protein extraction. 30ug of denatured protein per sample was loaded on 7%, 10% and 12% SDS-polyacrylamide gels. Membranes were blotted with antibodies against: phospho-AKT^{Ser473} and AKT; phospho-p38 MAPKT$^{180/Y182}$ and p38; phospho-pS6$^{Ser235/236}$; phospho-ERK(p44/42 MAPK)$^{T202/Y204}$ and ERK; phospho-4EBP1$^{Thr37/46}$ and 4EBP1; phospho-JNK (SAPK/JNK)$^{Thr183/Tyr185}$; phospho-NF-κB p65 ^{Ser536}; TGF-ß; smad-2; E-cadherin; vimentin; LDHa and snail. All Antibodies listed above were obtained from Cell Signaling Technology (Beverly, MA), and diluted 1:1000 with the exception of phospho-JNK (SAPK/JNK)$^{Thr183/Tyr185}$ (1:500) and Phospho-NF-κB p65 ^{Ser536} (1:500). Other antibodies used were: for αSMA (1:1000, Abcam, MA); Collagen 1 [(1:1000, LF-68 antibody provided by Dr Larry

Fisher (NIDCR)]; MMP-9 (1:500, EMD Millipore-Billerica, MA), AT1 (1:1000, LifeSpan BioSciences Inc, WA), ZEB1 (1:1000, Novus Biologicals, CO), CA-IX (1:1000, Abcam, MA), Hif-1α (1:1000, Abcam, MA), GAPDH (1:2000, Ambion, NY), ß-actin (1:5000, Sigma, MO), and α-tubilin (1:5000, Sigma, MO). Quantification of protein expression relative to total receptor or ß-actin was obtained using Image J software.

- ELISA/Multiplex array

Each tumor sample was homogenized directly in lysis buffer for protein extraction. 2ug/ul of cell/tumor sample was used. Data are expressed as pg/mg of tissue protein. A pre-made inflammatory multiple cytokines protein array was used (V-PLEX Proinflammatory Panel1 mouse kit, cat. number K15048D). For detection of hyaluronan, an ELISA kit was used (Quantikine Kit, R&D, cat. number DHYAL0).

Immunofluorescence/Immunohistochemistry

For analysis of tumor tissues on frozen sections, tumors were excised and frozen in optimal cutting temperature compound (Tissue-Tek). Transverse tumor sections, 10 mm thick, were immunostained with specific antibodies. To obtain mosaic images of tumors, an Olympus FV1000 confocal laser-scanning microscope was used. A 10x air objective acquired 1260-µm square tiles, and an automated stage scanned throughout the entire cross-section of tumor tissue. The imaged tiles were stitched into a final mosaic image using Olympus

software. Antigen expression was quantified by measuring the area occupied by the stain of interest normalized by the area of DAPI-stained nuclei (i.e., unitless), and analyzed using a custom algorithm in MATLAB (The MathWorks). Identical analysis settings and thresholds were used for all tumors. For vascular analysis, vessels were skeletonized and segmented using a custom, semi-automated tracing program developed in MATLAB allowing the removal of structures under 30 pixels and regions of autofluorescence. To obtain the perfusion fraction, the number of vessels counted by this program with colocalization of lectin and CD31 staining was divided by the total viable tumor area and by the number of vessels counted with CD31 staining. Antibodies used for immunofluorescence were the following: Collagen-I (LF-68 antibody, 1:50 dilution); Hyaluronan (biotinylated hyaluronan proteoglycan fragment, 385911, Calbiochem); αSMA (C6198 antibody, Sigma, 1:500 dilution); AT1 (18801 antibody, Abcam, 1:100 dilution); CD31 (1398Z, Millipore, 1:200 dilution), Ly6G (551459, BD Pharmigen, 1:200, IL-1β (9722, Abcam, 1:200). To detect lectin in tumors, on last day of treatment, mice were slowly injected with 100 µl of 1 mg/ml biotinylated lectin (Vector Labs), administered via the retro-orbital sinus 5 min before tumor removal. Cy3-, Cy5- or FITC-conjugated secondary antibodies were used for the detection of signals by confocal microscopy. Slides were counterstained with DAPI for nuclear staining.

For analysis of tissues on paraffin sections, freshly excised adipose tissues and tumors were fixed in 4% (vol/vol) paraformaldehyde overnight. 10µm sections were stained with Masson’s Trichrome and counterstained for hematoxylin. Images were taken using a brightfield microscope with a camera attached. To

quantify adipocyte number and diameter, pictures were taken at 10X magnification. No less than eight regions of interest (ROI) were used, and cross-sectional diameter and mean adipocyte number per tumor were obtained using ImageJ image software. For the detection of collagen-I and biotinylated hyaluronan proteoglycan fragment and quantification of adipocyte diameter in human pancreatic ductal adenocarcinoma, the paraffin-embedded sections were treated with a pH-9.0 antigen retrieval solution and counterstained with hematoxylin. Slides were scanned, and images were obtained using Aperio ImageScope. After tumors had been individually annotated, they were analyzed using imageJ software.

Flow Cytometry

Tumor-bearing mice were perfused through intracardiac injection of PBS and sacrificed. Pancreatic tumor tissues were harvested, minced, and digested at 37 °C for 1 h with DMEM containing collagenase type 1A (1.5 mg/mL), hyaluronidase (1.5 mg/mL), and DNase (2 mg/mL). The digestion mixtures were filtered through 70-μm cell strainers. Single-cell suspensions were incubated with rat anti-mouse CD16/CD32 mAb for blocking and stained with fluorochrome-conjugated antibodies in cold buffer cold buffer (1% BSA, 0.1% NaN3 in PBS). 7-amino-actinomycin D (7AAD) reagent (eBioscience) was added to the stained tubes per manufacturer's instruction immediately before running the flow analysis. Flow cytometry data were acquired on an LSRII flow cytometer (Becton Dickinson) and were analyzed with FACSDiva software. FSC-A vs. FSC-W and

SSC-A vs. SSC-W were applied to discriminate the doublet/aggregated events.

The following monoclonal anti-mouse antibodies were used: CD45-PE, CD45-PE-Cy7, CD45-FITC, GR1-APC, CD11b-APC-Cy7, CD11b-APC, F4/80-APC, Ly6G-FITC, CD4-FITC, CD4-PE-Cy7, CD8-FITC, CD8a-PE, CD25-APC-Cy7 (BD Biosciences) and F4/80-FITC and F4/80-PE (eBioscience).

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