Supplementary Information

Supplementary Figure Legends

Supplementary Figure 1 Morphological characterisation of metastatic PDAC lesions in human and mouse.

(a) Lower magnification of representative micrographs shown in Fig. 1a. Identification of pan-cytokeratin $(CK)^+$ metastatic pancreatic cancer cells, hematopoietic cells ($CD45^+$), macrophages ($CD68^+$) and myofibroblasts (αSMA^+) as predominant cell types at the hepatic metastatic microenvironment of pancreatic cancer by immunohistochemical analysis of human biopsies (data are from 5 PDAC patients and 5 healthy subjects; five fields assessed per sample). HL= healthy liver, LM = liver metastasis.

(b) Identification of cytokeratin (CK) 19^+ metastatic pancreatic cancer cells, tumour associated macrophages ($CD68^+$) and myofibroblasts ($PDGFR\alpha^+$) as predominant cell types at the hepatic metastatic microenvironment of pancreatic cancer by immunofluorescence analysis of human biopsies. Representative micrographs are shown and quantification of data (n= 5 PDAC, n = 5 healthy subjects; five fields assessed per sample; mean ± s.e.m; two-tailed unpaired t-test). HL= healthy liver, LM=liver metastasis.

(c) Representative Masson's trichrome staining and quantification of area occupied by fibrotic stroma in human biopsies (n= 5 PDAC patients, n = 5 healthy subjects; five fields assessed per sample; mean ± s.e.m; two-tailed unpaired t-test). HL= healthy liver, LM=liver metastasis.

(d) Experimental metastasis model by intrasplenic implantation of $1x10^6$ $Kras^{G12D}$;$Trp53^{R172H}$;Pdx1-Cre (KPC) mice derived pancreatic cancer cells expressing a luciferase/zsGreen lentiviral reporter plasmid ($KPC^{luc/zsGreen}$). Liver tissues were isolated at day 5 and day 12 post implantation. (Upper panel) Liver tissue sectioned stained by Hematoxylin and Eosin (HE) showing initial micrometastatic lesions of disseminated cancer cells at day 5 post implantation followed by the generation of an excessive stromal microenvironment surrounding disseminated cancer cells at day 12 post implantation. (Lower panel) Representative immunofluorescence staining of disseminated

KPC$^{luc/zsGreen}$ (zsGreen) cells. Data are from 6 mice per time point; four fields assessed per sample; data combine two independent experiments.

Scale bars = a, 200 μm; b, c, d, 100μm.

Supplementary Figure 2 FACS-based quantification of immune cell infiltrating the metastatic site in PDAC.

Experimental metastasis model by intrasplenic implantation of 1x10^6 KPC-derived pancreatic cancer cells. After 12 days, liver tissues were isolated, enzymatically digested and resulting single cell suspensions were stained for flow cytometry analysis. Naïve livers were used as controls (healthy).

(a) Representative flow cytometry dot plots showing gating strategy used to quantify intrametastatic B cells (CD45$^+$B220$^+$), T cell (CD45$^+$CD3$^+$), NK cells (CD45$^+$NK1.1$^+$CD3^{neg}B220^{neg}), Neutrophils (CD45$^+$CD11b$^+$F4/80^{neg}Ly6G$^+$Ly6C$^+$, inflammatory monocytes (CD45$^+$CD11b$^+$F4/80^{neg}Ly6G^{neg}Ly6C$^+$) and macrophages (CD45$^+$CD11b$^+$F4/80$^+$). Only viable cells (SYTOX^{neg}) were used (data are from 5 healthy livers or 8 liver metastasis; repeated two times with similar results).

(b) Quantification of CD45$^+$ hematopoietic cells detected in healthy control livers (Ctrl) and metastatic tumour bearing livers (LM) (n = 5 Ctrl mice; n = 8 LM mice; data combine two independent experiments; individual data points, horizontal lines represent mean ± s.e.m; two-tailed unpaired t-test).

(c) Percentage of intrametastatic immune cells among total viable cells gated according to (a) in healthy control livers (Ctrl) and metastatic tumour bearing livers (LM) (individual data points, horizontal lines represent mean ± s.e.m; two-tailed unpaired t-test). Data shown combine two independent experiments; total mice: n = 5 Ctrl mice, n = 6 LM mice (B220, NK1.1, CD3); n = 5 Ctrl mice; n = 9 LM mice (Ly6G, Ly6C, F4/80).

(d) Representative flow cytometry dot plots showing gating strategy to analyse CCR2 (CD192) expression levels. Intrametastatic inflammatory monocytes (CD45$^+$CD11b$^+$F4/80^{neg}Ly6G^{neg}Ly6C$^+$) and macrophages (CD45$^+$CD11b$^+$F4/80$^+$) express CCR2 (data are from 5 Ctrl mice or 8 LM mice; repeated two times with similar results).

ns, not significant.

Supplementary Figure 3: Liver PDAC metastasis induces the recruitment of monocyte-derived macrophages, followed by the activation of resident hStCs.

(a, b) Experimental metastasis model by intrasplenic implantation of $1x10^6$ KPC cells. (a) Intrametastatic $CD11b^+$ cells showing a marked expansion of the macrophage population ($F4/80^+$, blue) in established macro-metastatic lesions (day 12, D12), while inflammatory monocytes ($Ly6C^+Ly6G^{neg}$, red) predominantly increased during early micro-metastatic spreading (day 5, D5). Ctrl= 5 mice; Day 5 = 2 mice, Day 12 = 5 mice; data combine two independent experiments.

(b) Representative immunofluorescence staining showing increased $CD11b^+$ cells (upper) in micro-metastatic livers, followed by excessive accumulation of αSMA^+ myofibroblasts in livers with macro-metastatic lesions. Macrophages (lower) are equally distributed in healthy livers (Kupffer cells) and show increase clustering in tumour bearing livers. Data are from 6 mice per time point; two independent experiments.

(c) Representative Masson's trichrome staining (MTS) of healthy control liver indicating absence of fibrotic stroma (lack of blue colour). Data are from 6 mice; two independent experiments.

(d) Statistical comparison showing a positive correlation (Pearson) between increased numbers of αSMA^+ myofibroblasts and area occupied by metastatic cancer cells in tumour bearing murine livers. solid line = best fit, dashed lines, 95% confidence intervals. Total n = 20 mice; four independent experiments.

(e - g) Primary tumours and spontaneous metastatic hepatic tumours derived KPC mice.

(e) Representative HE images showing the presence of an excessive stromal compartment at both sites (data are from 5 mice per condition, four fields assessed per sample).

(f) Representative images of liver tumour sections stained for MAMs ($F4/80^+$), pancreatic cancer cells ($CK19^+$), or myofibroblasts (αSMA^+) showing the presence of an excessive metastatic

microenvironment mainly consisting of MAMs and myofibroblasts surrounding the tumour cells (data are from 5 mice per condition, four fields assessed per sample).

(g) Flow cytometry analysis showing a marked expansion of the macrophage population ($F4/80^+$) in metastatic livers (ML) compared to healthy livers (HL) (n = 5 mice per condition, data combine five independent experiments; mean ± s.e.m.; two-tailed unpaired t-test).

(h - j) Chimeric WT + tdTomatoRed BM (WT+tdTR BM) mice. (h) Successful BM reconstitution was confirmed by flow cytometry analysing total circulating $CD11b^+Gr1^+$ cells. (i, j) Representative images showing co-localization of tdTomatoRed signal with F4/80+ MAMs (i), but not with αSMA+ myofibroblasts in the metastatic lesion (below white line). Data are from 6 mice per condition; one experiment.

Scale bars, b, c, f, 100μm; e, 50μm; i, j, 25μm

Supplementary Figure 4 The recruitment of monocyte derived macrophages is necessary for efficient pancreatic cancer metastasis.

$1x10^6$ KPC-derived cells (a) or $1x10^6$ Panc02 cells (b - e) were intrasplenically injected into age and sex matched WT and PI3K$\gamma^{-/-}$ (-/-) mice. After 12 days, total livers were harvested and analysed by flow cytometry, immunohistochemistry, and immune fluorescence based methods.

(a) Representative HE staining of liver tissue sections showing a marked decreased size of metastatic tumours in livers of PI3Kγ deficient (-/-) mice (data are from 7 WT and 9 PI3K$\gamma^{-/-}$ mice; data combine two independent experiments).

(b) Percentage of intrametastatic macrophages among $CD45^+$ cells quantified by flow cytometry. PI3Kγ deficiency (-/-) results in a marked reduction of MAMs compared to WT (n = 6 mice per condition; one experiment; individual data points, horizontal lines represent mean ± s.e.m; two-tailed unpaired t-test).

(c, d) Quantification of metastatic frequency (c) and average metastatic lesion size (d) in WT and PI3Kγ knockout mice (-/-) by hematoxylin and eosin (HE) stained liver sections (n = 6 mice per

condition; all metastatic nodules assessed from one section per sample; one experiment; c, individual data points, horizontal lines represent mean ± s.e.m; d, mean ± s.e.m; two-tailed unpaired t-test).

(e) Representative immunofluorescence staining and quantification of myofibroblasts (αSMA^+) cell frequency in livers in WT and PI3Kγ knockout mice (-/-). Nuclei were counterstained with DAPI (n = 6 mice per condition; two fields assessed per sample; one experiment; mean ± s.e.m; two-tailed unpaired t-test).

Supplementary Figure 5 Disseminated PDAC cells depend on MAMs to sustain growth after colonization of the metastatic site.

(a, b) *In vivo* bioluminescence imaging to monitor metastatic colonization of the liver (a) Representative image showing radiance of a mouse two days post intrasplenically implantation of 1x 10^6 PDAC cancer cells ($KPC^{luc/zsGreen}$) and tumour free control mouse. Main signal detected originates from the liver area, while some residual signal is measured from the injection site, the spleen. (b) Quantification of radiance (total flux) measure two days post implantation confirming colonization of the liver by implanted cancer cells has occurred (data are from 2 control or 10 $KPC^{luc/zsGreen}$ mice; one experiment, individual data points, horizontal lines represent mean).

(c) Representative flow cytometry dot plots and quantification showing a marked reduction of macrophages ($F4/80^+$) and myofibroblasts ($PDGFR\alpha^+$) in KPC cell induced tumour bearing livers of mice treated with Clodronate Liposomes (CL) compared to control mice treated with PBS Liposomes (PL) (n = 3 mice per condition; one experiment; individual data points, horizontal lines represent mean ± s.e.m; two-tailed unpaired t-test).

(d) Percentage of macrophages ($F4/80^+$) among viable cells in Panc02 cell induced tumour bearing livers of mice treated with Clodronate Liposomes (CL, data from 2 mice) compared to control mice treated with PBS Liposomes (PL, data from 2 mice) (one experiment; individual data points, horizontal lines represent mean).

(e) Representative immunofluorescence staining and quantification of MAMs ($F4/80^+$) and myofibroblasts (αSMA^+) cell frequency in Panc02 cell induced liver tumours of mice treated with

liposomes containing control PBS (PL) or clodronate (CL). Nuclei were counterstained with DAPI (n = 4 mice per condition; five fields assessed per sample; one experiment; mean ± s.e.m; two-tailed unpaired t-test).

(f, g) Evaluation of metastatic frequency (f) and lesion area covered by metastatic cells (g) in Panc02 tumour bearding livers of mice treated with liposomes containing control PBS (PL) or clodronate (CL). Nuclei were counterstained with DAPI (n = 5 mice per condition; all metastatic nodules assessed from one section per sample; one experiment; individual data points, horizontal lines represent mean ± s.e.m; two-tailed unpaired t-test).

Scale bars, 100μm.

Supplementary Figure 6 High levels of granulin are specifically expressed by tumour educated macrophages in vitro and in vivo.

(a) Quantification of *aSMA (Acta2*) and *collagen 1a (Col1a*) mRNA levels in primary murine dermal fibroblasts stimulated with isogenic macrophage conditioned media (CM) as determined by qPCR. Bar graph show fold up regulation compared to unstimulated and are displayed as mean (data are from a single experiment, repeated four times with similar results; Supplementary Table 6).

(b) Quantification of human (green) and mouse (red) myofibroblast that migrated towards human and mouse macrophages, respectively, in a matrigel-coated transwell assay (n = 4 independent experiments; mean ± s.e.m.; two-tailed unpaired t-test)

(c) Quantification of human myofibroblast proliferation in the presence of control media, THP-1 derived macrophage CM, or primary macrophage CM (n = 4 independent experiments; mean ± s.e.m.; two-tailed unpaired t-test).

(d) Quantification of *granulin* mRNA expression levels by qPCR in human primary unstimulated macrophages (M0) or primary macrophages stimulated with either interleukin (IL) -4 (M2-like phenotype), interferon (IFN) γ / LPS (M1-like phenotype) or educated with tumour conditioned media generated from human Panc1 cells (n = 3 independent experiments mean ± s.e.m.; two-tailed unpaired t-test).

(e) Quantification of *granulin* mRNA expression levels by qPCR in murine primary macrophages stimulated with either interleukin (IL) -4 (M2-like phenotype), interferon (IFN) γ / LPS (M1-like phenotype) or educated with tumour conditioned media generated from murine KPC and Panc02 PDAC cancer cells (n = 3 independent experiments; mean ± s.e.m; two-tailed unpaired t-test).

(f) Metastatic hepatic tumours derived from the spontaneous mouse pancreatic cancer model Pdx1Cre-ERT;$Kras^{G12D}$;$Trp53^{R172H}$; (PdxCre-ERT KP mice) were isolated and morphometrically analysed. Representative images of immunohistochemistry staining for MAMs (CD68+) and granulin expression on serial tissue sections from metastatic lesions and healthy liver and quantification of the data (n = 1 mouse per condition; eight fields assessed per sample; bars represent means).

ns, not significant.

Supplementary Figure 7 Depletion of granulin does not affect the recruitment of macrophage to the metastatic site, their activation, or intrametastatic effector T cell numbers, but markedly reduces stromal expansion.

$5x10^5$ KPC-derived cells were intrasplenically injected into WT and $Grn^{-/-}$ mice (a), and chimeric WT+ WT BM and WT + $Grn^{-/-}$ BM mice (b). After 12 days, total livers were harvested and analysed my flow cytometry.

(a) Quantification of intrametastatic total MAMs ($F4/80^+$), $CD206^+$ MAMs, and $CD8^+$ effector T cells by flow cytometry isolated from WT and $Grn^{-/-}$ mice (n = 6 mice per condition; one experiment; individual data points, horizontal lines represent mean ± s.e.m; two-tailed unpaired t-test).

(b) Quantification of intrametastatic total MAMs ($F4/80^+$), $CD206^+$ MAMs, and $CD8^+$ effector T cells by flow cytometry isolated from WT + WT BM and WT + $Grn^{-/-}$ BM mice mice (n = 6 mice per condition; one experiment; individual data points, horizontal lines represent mean ± s.e.m; two-tailed unpaired t-test).

(c) Quantification of murine KPC cancer cell proliferation in the presence or absence of Mf CM and periostin neutralising antibody (anti-Periostin) (n = 4 independent experiments; mean +/- SEM; two-tailed unpaired t-test)

(d) Quantification of periostin expression by qPCR in primary activated murine myofibroblasts and KPC cells. Periostin expression was undetectable in KPC cells while myofibroblasts expressed high levels of periostin (n = 3 independent experiments; mean ± s.e.m).

(e) Immunohistochemical analysis of periostin in healthy liver (HL) and spontaneous metastatic livers (ML) collected from Pdx1-CreERT KP mice, respectively. Representative micrographs and quantification of the data are shown (n = 1 mouse per condition; eight fields assessed per sample; bars represent means).

(f, g) Representative images of the evaluation of periostin deposition and fibrotic stroma formation (MTS) in metastatic livers of control WT, WT mice treated with clodronate liposomes, PI3K$\gamma^{-/-}$, Grn$^{-/-}$, and WT + Grn$^{-/-}$ BM mice 12 days after intra-splenic implantation of KPC cells (data are from 4 mice per condition, four fields assessed per sample; data combine five independent experiments).

Scale bars = 100μm. NS, not significant.

Supplementary Figure 8 Depletion of granulin in the hematopoietic compartment reduces pulmonary metastasis and myofibroblast activation in the lung.

(a - e) 5x10^5 KPC-derived cells were intravenously injected into age and sex matched chimeric WT + WT BM and WT + Grn$^{-/-}$ BM mice. After 12 days, total lungs were harvested and analysed.

(a) Representative images of immunohistochemistry staining for MAMs (CD68+) and granulin expression on serial tissue sections from lung metastatic lesions and quantification of the data (data are from 5 WT + WT BM mice or 4 WT + Grn$^{-/-}$ BM mice; four fields assessed per sample; one experiment).

(b) Representative images and quantification of WT BM and Grn$^{-/-}$ BM derived lung tissue stained with HE showing a marked reduction of area covered by metastatic cancer cells in Grn$^{-/-}$ BM mice compared to WT BM mice (mean ± s.e.m.; two-tailed unpaired t-test), while frequency of metastatic lesions (metastatic foci) remained unchanged (n = 5 WT + WT BM mice, n = 4 WT + Grn$^{-/-}$ BM mice; all metastatic nodules assessed from one section per sample; one experiment; individual data points, horizontal line represents mean ± s.e.m.; two-tailed unpaired t-test).

(c) Representative immunofluorescence staining and quantification of MAMs ($F4/80^+$) and myofibroblasts (αSMA^+) cell frequency in tumour bearing lungs of WT + WT BM and WT + $Grn^{-/-}$ BM mice. Nuclei were counterstained with DAPI (n = 5 WT + WT BM mice, n = 4 WT + $Grn^{-/-}$ BM mice; five fields assessed per sample; one experiment; mean ± s.e.m.; two-tailed unpaired t-test).

(d) Representative immunofluorescence staining and quantification of periostin expression in tumour bearing lungs of WT + WT BM and WT + $Grn^{-/-}$ BM mice. Nuclei were counterstained with DAPI (n = 5 WT + WT BM mice, n = 4 WT + $Grn^{-/-}$ BM mice; five fields assessed per sample; one experiment; mean ± s.e.m.; two-tailed unpaired t-test).

(e) Representative Masson's trichrome staining and quantification of area occupied by fibrotic stroma in tumour bearing lungs of WT + WT BM and WT + $Grn^{-/-}$ BM mice (n = 5 WT + WT BM mice, n = 4 WT + $Grn^{-/-}$ BM mice; five fields assessed per sample; one experiment; mean ± s.e.m.; two-tailed unpaired t-test).

(f) Immunohistochemical analysis of periostin and granulin expression in human primary PDAC tumours. Representative micrographs are shown (data are from 4 different patients per condition).

(g) IM from blood samples from healthy subjects and metastatic PDAC patients expressed high levels of the chemokine receptor CCR2 (CD192). Representative histogram shown from 6 different samples per condition.

Scale bars a, b, c, e, f, 100 μm; d, e, 200 μm; inset, 20 μm

Supplementary Table 1: Identified proteins in tumour educated macrophage secretome.

Top 20 identified proteins secreted by tumour educated macrophages are listed according to their abundance.

Supplementary Table 2*. *Secretome macrophages.

Complete list of secreted proteins associated with GO term “Extracellular Vesicular Exosome” identified by mass spectrometry in human macrophages educated with Panc1 conditioned media.

Supplementary Table 3. Secretome myofibroblasts.

Complete list of secreted proteins associated with GO term “Extracellular Matrix Organistion” identified by mass spectrometry as differentially expressed in human fibroblasts educated with primary macrophage conditioned media compared to unstimulated fibroblasts.

Supplementary Table 4: Primers.

List of primers used for qPCR analysis in this study.

Supplementary Table 5: Antibodies.

List of antibodies used in this study.

Supplementary Table 6: Statistics source data.

Activation assays primary dermal fibroblasts.