Supplementary figure: 1

Normal Human Pancreas

**CD45**

**CD14**

**CSF1R**

**HLA-DR**

**HLA-DR**

**CD11b**

**SSC-A**
Supplementary figure 1: Flow cytometry performed on normal human pancreas samples (N=4). A representative flow plot shows 2.89% CD45+ cells infiltrating the normal human pancreas (gating is performed using isotype control).
NORMAL HUMAN PANCREAS (40X)
BROWN=CD 14

Normal # 1  Normal # 2  Normal # 3  Normal # 4  Normal # 5

HUMAN PANCREATIC CANCER

PC pt # 1  PC pt # 2  PC pt # 3  PC pt # 4  PC pt # 5

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Supplementary figure: 2
Supplementary figure 2: Immunohistochemistry images (40X) of PC tumor specimens (N=5) and normal human pancreas (N=5) stained for CD14$^+$ cells (Brown). Arrows point towards CD14$^+$ cells in the tumor.
Supplementary figure: 3

ALDH1+ cells as % of CD45+EPCAM positive

Normal | PC

*
Supplementary figure 3:

A scatter plot comparing ALDH1\textsuperscript{Bright} cells as percent of EpCAM positive cells in PC and normal human pancreas by flow cytometry. The ALDH1\textsuperscript{Bright} population (as percent of EpCAM positive cells) is significantly higher in PC compared to Normal human pancreas ($p$\textless{}0.05 by Mann Whitney test).
Correlation between CD14+ infiltrate and ALDH1 expression

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Supplementary figure: 4

p-value = 0.02
R value = 0.2
Supplementary Figure 4: Pearson correlation analysis shows a significant positive correlation of percent CD14⁺ cells versus ALDH₁Bright cells (Spearman r=0.2, p=0.02).
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Supplementary figure: 5

**RED:** Splenocytes alone
**BLUE:** Splenocytes & TCM (GCSFR\(^{-/-}\))CD11b\(^{+}\)
**ORANGE:** Splenocytes & TCM (WT)CD11b\(^{+}\)

![Flow cytometry histogram and bar graph showing cell counts and division index for different conditions.](image-url)
**Supplementary Figure 5:**

Representative CFSE dilution FACS analysis of splenocytes stimulated with a-CD3 and cultured alone or in the presence of tumor conditioned medium (TCM) and CD11b^+ cells from either GCSFR^−/− or WT mice.

Graphs depict means ± SEM with asterisk (*) denoting statistically significant differences between groups defined as p<0.05 by Mann-Whitney test.
A. TUMOR GROWTH CURVE (Pan02)

B. TUMOR WEIGHT (Pan02)

C. TUMOR ASSOCIATED MYELOID AND LYMPHOID INFILTRATE (Pan02)

D. MYELOID INFILTRATE IN KCM TUMORS

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Supplementary Figure 6
Supplementary Figure 6:

A. Tumor growth curves comparing subcutaneous tumor growth, Pan02 by caliper measurements in WT mice and GCSFR⁻/⁻ mice. Points on curve represent mean values± SEM at indicated time points.

B. Bar graph compares tumor weights of orthotopically implanted Pan02 in WT and GCSFR⁻/⁻ mice 28 days post injection.


D. Analysis compares tumor myeloid infiltrate by flow cytometry in WT tumors. Blue bars show G-MDSC and Mo-MDSC in a small sized KCM tumor (volume= 0.4cm³) and red bars show myeloid cells in large tumor (volume= 1.0cm³).

Graphs depict means ± SEM with asterisk (*) denoting statistically significant differences between groups defined as p<0.05 by Mann-Whitney test.
CM and CD14+ cells

Mo-MDSC 27.9%
Mo 70.5%

PC and CD14+ cells

Mo-MDSC 77.4%
Mo 17.8%
Supplementary figure 7: CD14^+ cells were isolated from normal human PBMC by magnetic bead isolation and were co-cultured with human PC cell line BxPC3 for 72 hours.

Downregulation of HLA-DR expression after tumor exposure; flow-cytometry was performed on CD14^+ cells (from blood) after 72 hours of co-culture with tumor cells. Representative plots show that approximately 70.5% CD14^+ cells had high HLA-DR expression when these cells are incubated in complete medium (CM). After tumor exposure, these cells downregulate HLA-DR expression and 77.4% CD14^+ cells have low HLA-DR expression.
SUBCUTANEOUS TUMOR GROWTH CURVE

Tumor Volume (cm³)

Days Post implantation

Panc-1 Spheres after coculture

Panc-1 Spheres baseline
Supplementary Figure 8: Tumor growth curves comparing subcutaneous tumor growth, Panc-1 spheres (with baseline ALDH1 activity) and Panc-1 spheres after co-culture (increased ALDH1 activity) by caliper measurements in NU/J mice. Points on curve represent mean values ± SEM are indicated time points.
Supplementary Figure 9

A. ALDH1+ cells as % of EPCAM+ cells

B. CD24+, CD44+ cells as % of EPCAM+ cells

C. Panni et al. Supplementary Figure 9

D. Fold Expression Change

E. No. of invaded cells/hpf

F. No. of ALDH1+ cells as % of CD45+ cells

G. No. of spheres

H. Panni et al. Supplementary Figure 9
Supplementary figure 9:

A. CD14+/HLA-DR_{low/-} cells were co-cultured with Panc-1 for 72 hours and ALDH1, CD24 and CD44 staining was performed. Graph shows ALDH1^{Bright} CSCs and CD24^+, CD44^+ cells as a percentage of CD45^−, EpCAM^+, PI^− cells.

B. Tumor EMT Markers: RT-PCR shows that markers of cell pluripotency and EMT were upregulated in Panc-1 tumor cells after co-culture with CD14+/HLA-DR_{low/-} cells.

C. Graph for invasion assays showing that Panc-1 tumor cells have increased invasion through matrigel matrix membrane in the presence of CD14^+ cells relative to tumor cells alone. Cells per high power field were quantified. Graph depicts the number of invaded cells per high powered field (mean ± S.E.M of three independent experiments).

D. Inhibition of STAT3 signaling in CD14+/HLA-DR_{low/-} cells prevents the increase in ALDH1^{Bright} CSCs in Panc-1 from baseline. Representative flow-cytometry plots show ALDH1^{Bright} CSC population (gated on CD45^−, EpCAM^+, PI^− cells) which is approximately 13.5% in Panc-1 alone, 20.8% when Panc-1 was co-cultured with CD14^+/HLA-DR_{low/-} cells and 12.02% when Panc-1 was co-cultured with STATTC treated CD14^+/HLA-DR_{low/-} cells. Graph shows that inhibition of STAT3 signaling by STATTC (20µM) blocks the increase in frequency of ALDH1^{Bright} cells from baseline.

E. Bar graph shows tumor spheroid formation in Panc-1 cells with and without CD14^+/HLA-DR_{low/-} cells in the co-culture. The mean number of tumor spheroids formatted after 10 days is depicted.
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Supplementary Figure 10

pSTAT3 IN CD11b+ CELLS AFTER CO-CULTURE WITH TUMOR CELLS (KCM)

<table>
<thead>
<tr>
<th>Baseline</th>
<th>After co-culture</th>
</tr>
</thead>
<tbody>
<tr>
<td>pSTAT3</td>
<td></td>
</tr>
<tr>
<td>ACTIN 47kDa</td>
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</table>
Supplementary Figure 10:

Western blot analysis shows increased phosphorylated STAT3 in CD11b⁺ cells after co-culture with murine tumor cells (KCM).
IL-6 LEVELS IN CD14$^+$ CELLS WITH STAT3 INHIBITION

![Bar graph showing relative mRNA expression levels for CD14$^+$ alone, CD14$^+$ & Tumor, and CD14$^+$ & Tumor & STAT3 inhibition. The graph compares the expression levels and indicates significant differences with asterisks (*) to denote statistical significance.]
Supplementary Figure 11:

Inhibition of STAT3 signaling by STATTIC (20uM) downregulates the expression of IL-6 in tumor conditioned CD14+/HLA-DR<sub>low</sub>- cells by RT-PCR.

Bar graph depicts mean± SEM and asterisk (*) denotes statistically significant difference between the two groups p<0.05 by Mann-Whitney test.