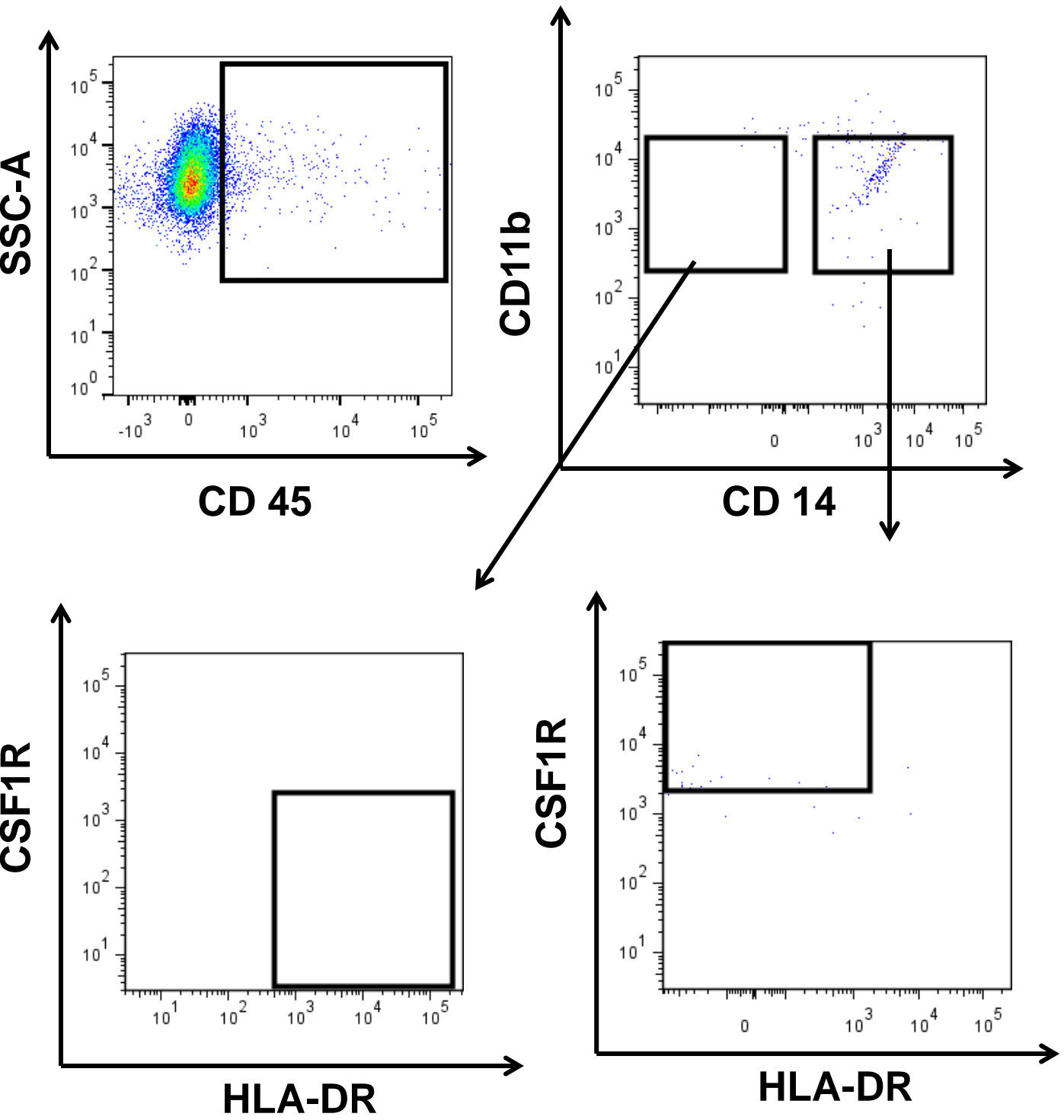


Normal Human Pancreas

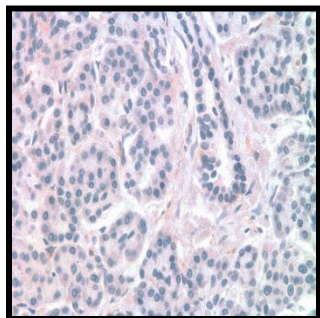


Supplementary figure 1: Flow cytometry performed on normal human pancreas samples (N=4). A representative flow plot shows 2.89% CD45⁺ cells infiltrating the normal human pancreas (gating is performed using isotype control).

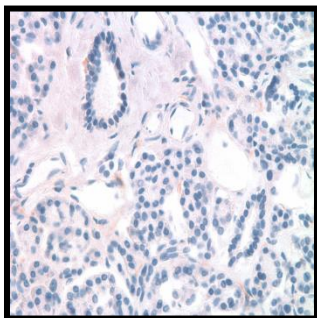
NORMAL HUMAN PANCREAS (40X)

BROWN=CD 14

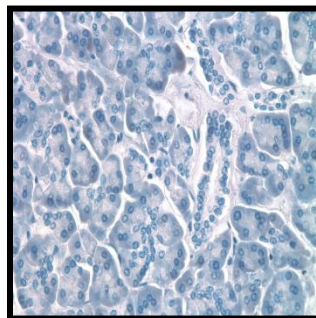
Normal # 1



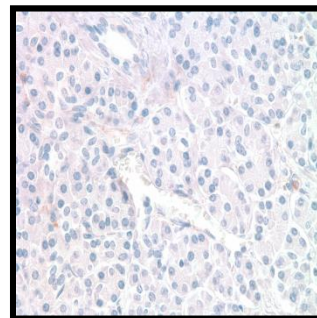
Normal # 2



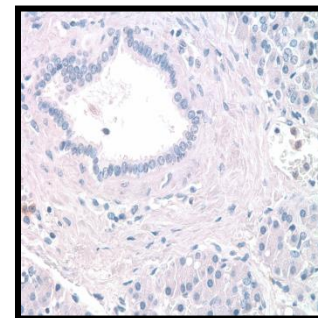
Normal # 3



Normal # 4

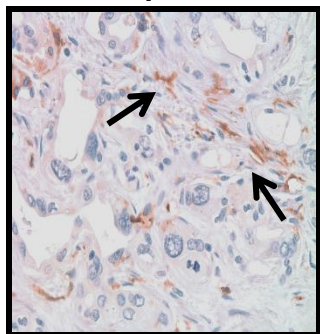


Normal # 5

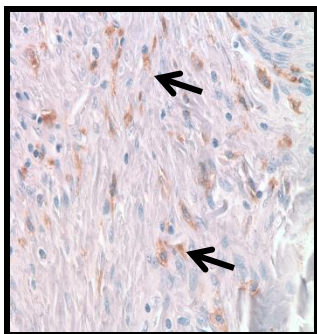


HUMAN PANCREATIC CANCER

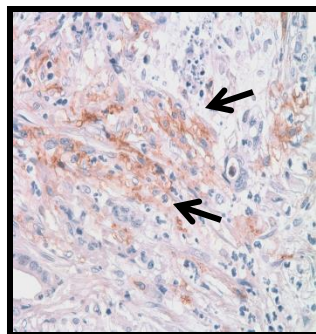
PC pt # 1



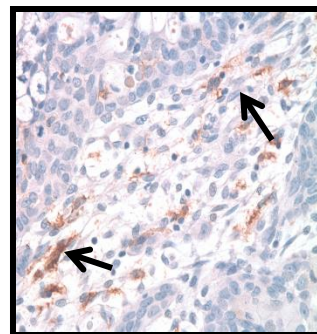
PC pt # 2



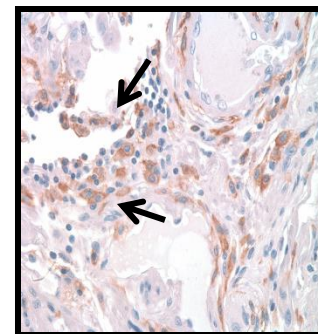
PC pt # 3



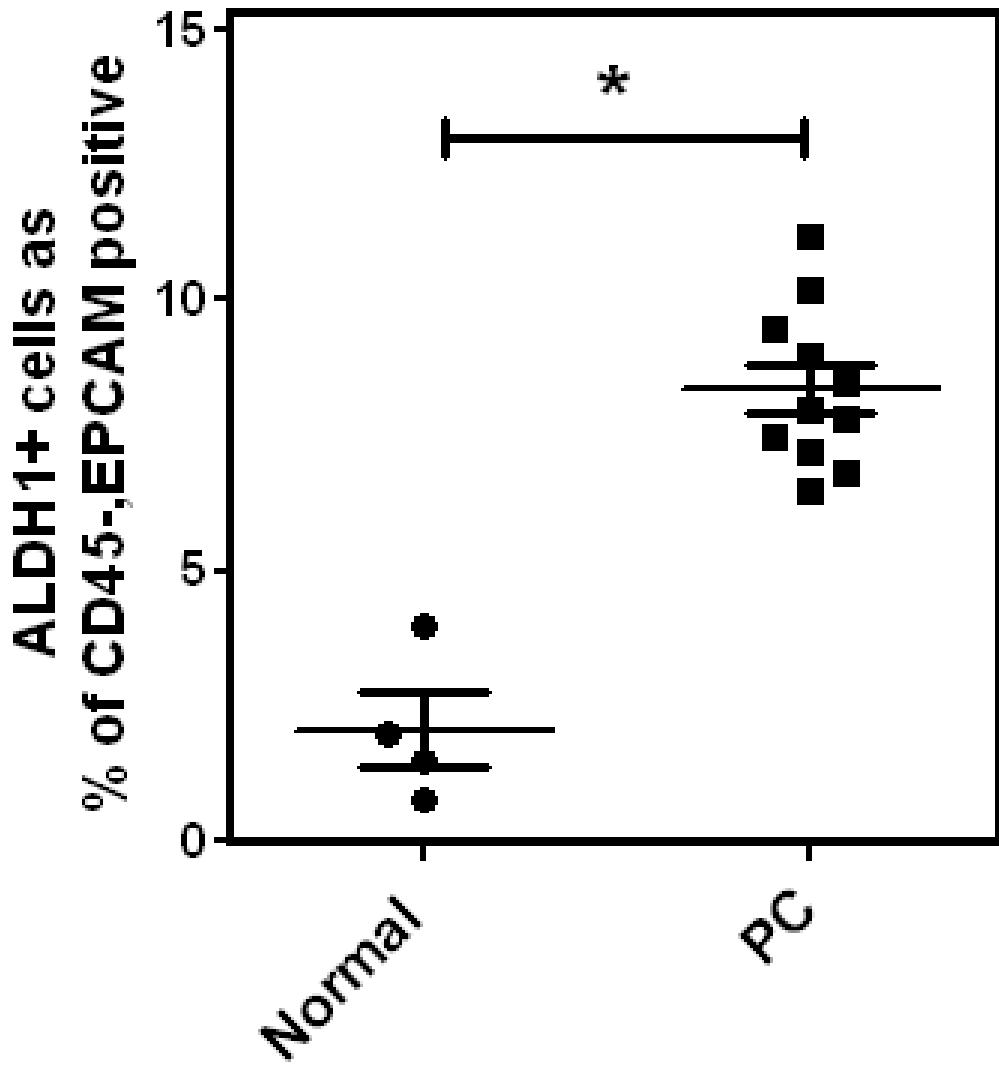
PC pt # 4



PC pt # 5



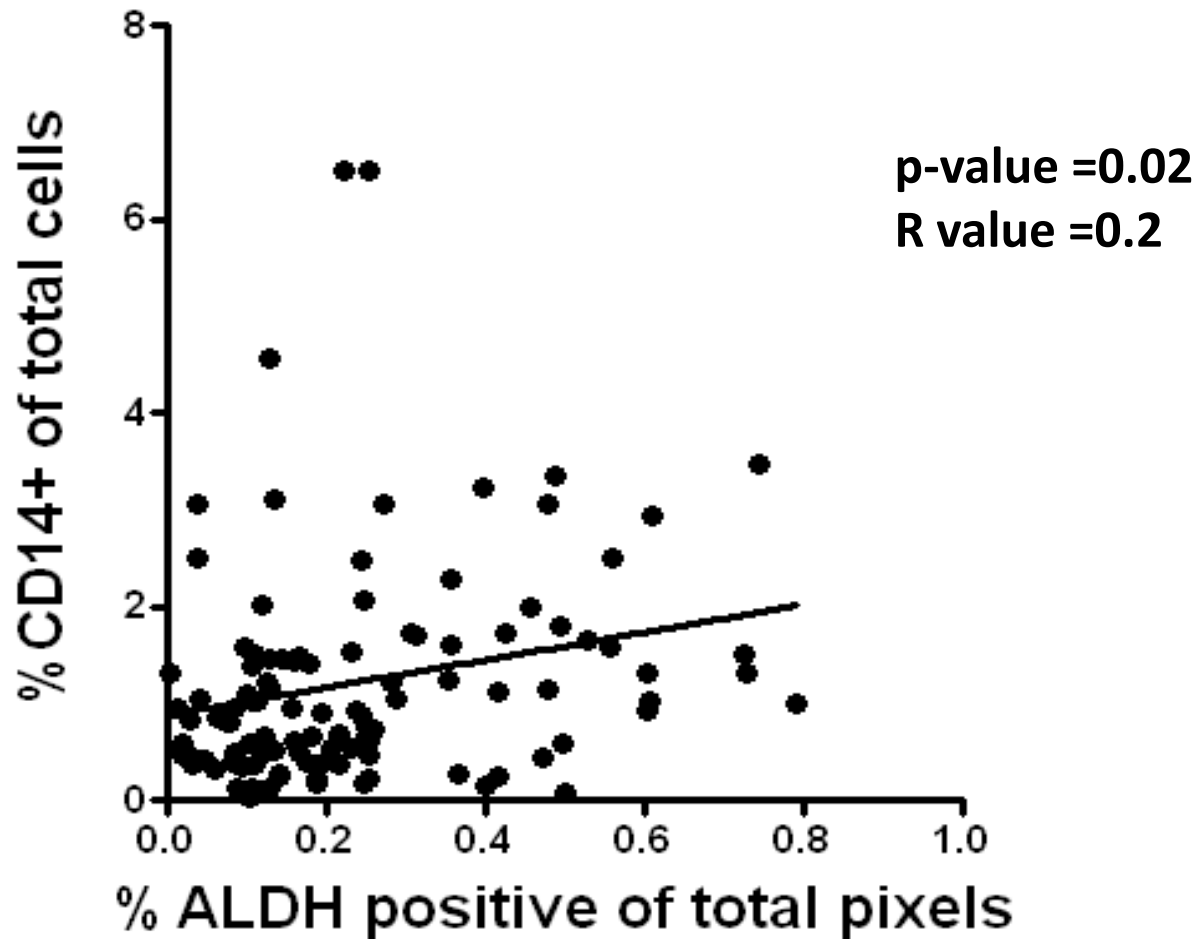
Supplementary figure 2: Immunohistochemistry images (40X) of PC tumor specimens (N=5) and normal human pancreas (N=5) stained for CD14⁺ cells (Brown). Arrows point towards CD14⁺ cells in the tumor.



Supplementary figure 3:

A scatter plot comparing ALDH1^{Bright} cells as percent of EpCAM positive cells in PC and normal human pancreas by flow cytometry. The ALDH1^{Bright} population (as percent of EpCAM positive cells) is significantly higher in PC compared to Normal human pancreas ($p < 0.05$ by Mann Whitney test).

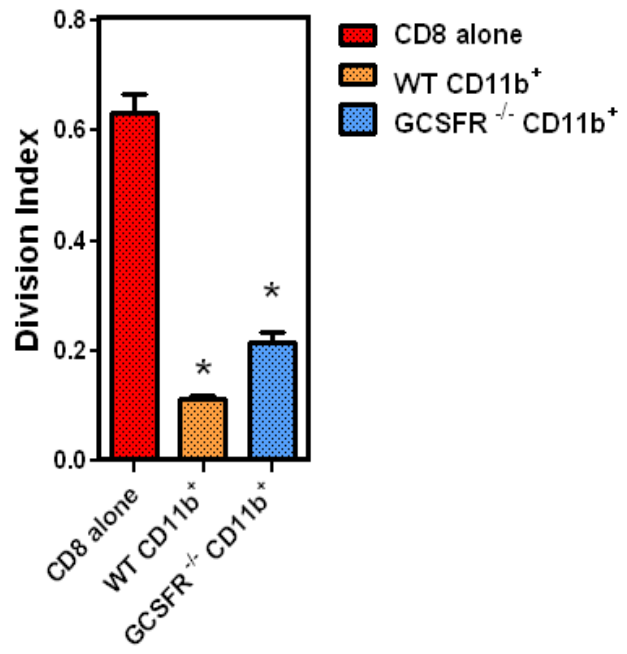
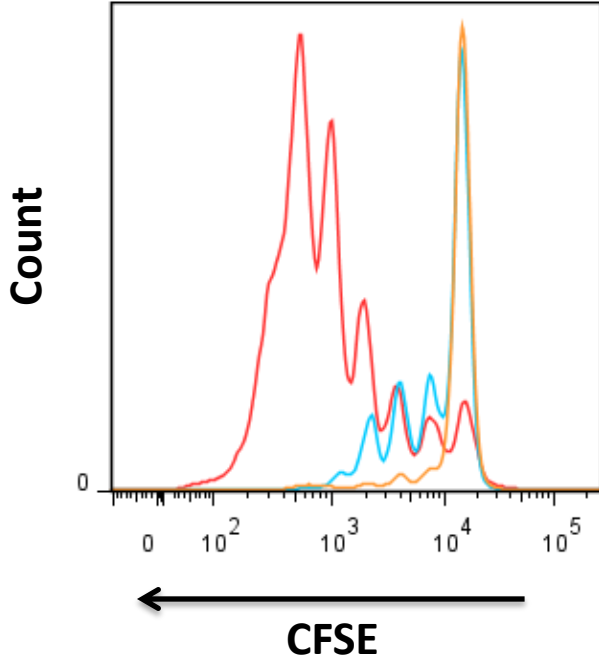
Correlation between CD14+ infiltrate and ALDH1 expression



Supplementary Figure 4: Pearson correlation analysis shows a significant positive correlation of percent CD14⁺ cells versus ALDH1^{Bright} 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⁺



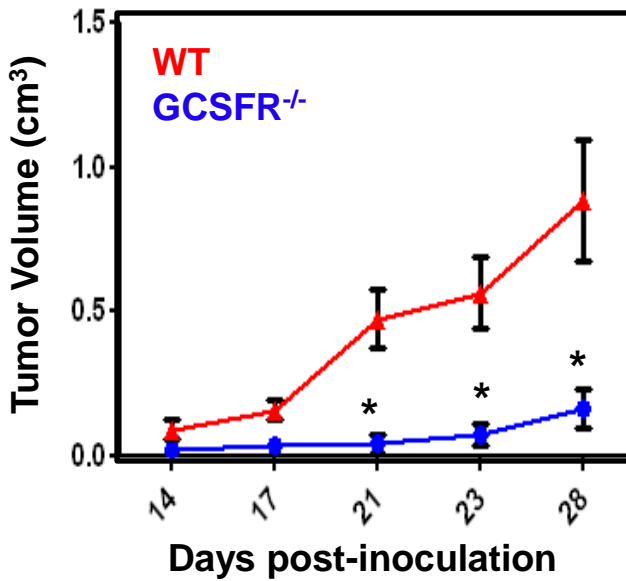
Supplementary Figure 5:

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

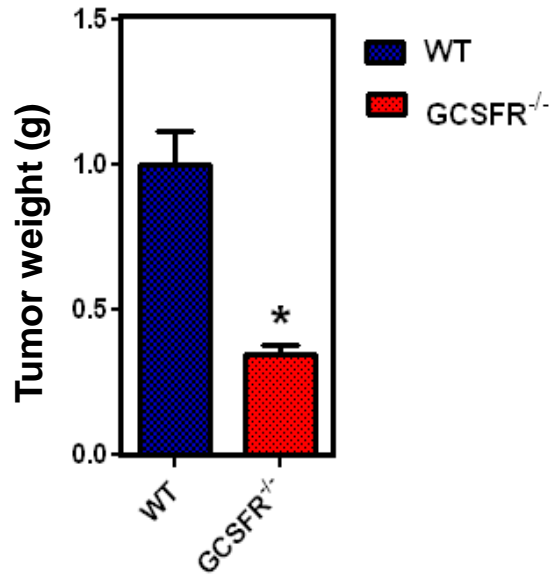
Graphs depict means \pm SEM with asterisk (*) denoting statistically significant differences between groups defined as $p < 0.05$ by Mann-Whitney test.

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Supplementary Figure 6

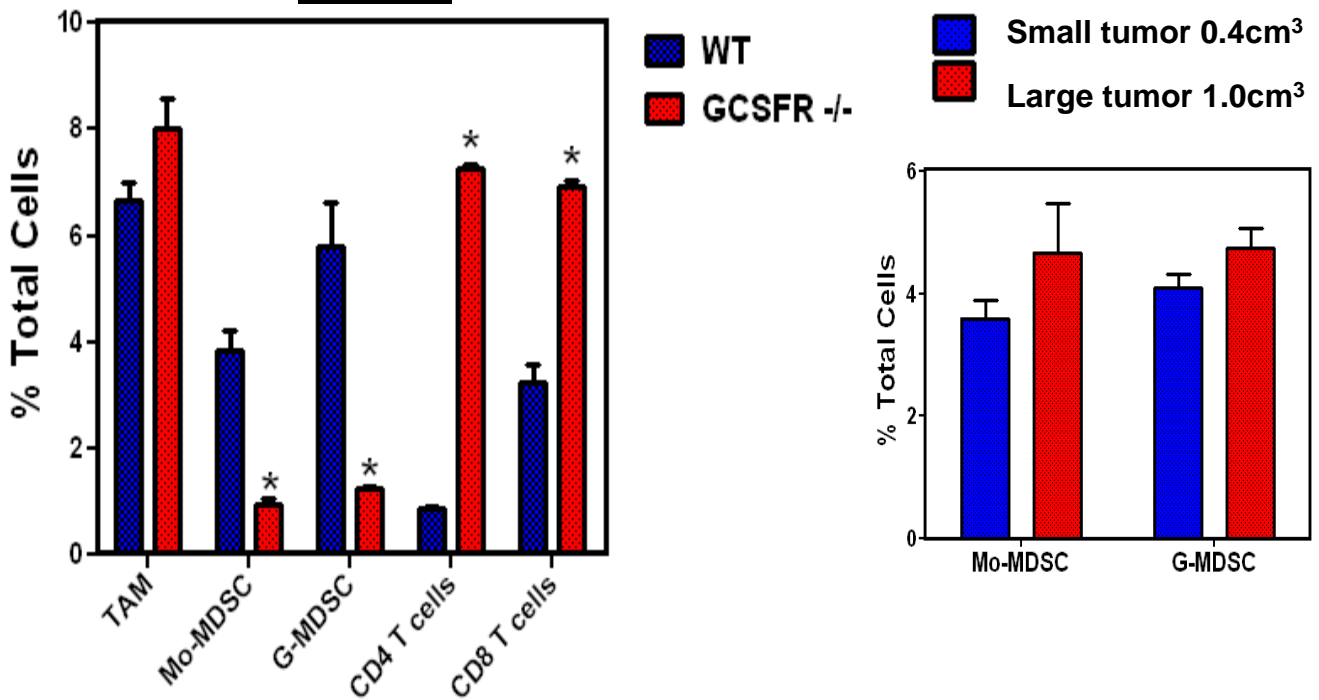
A. TUMOR GROWTH CURVE
(Pan02)



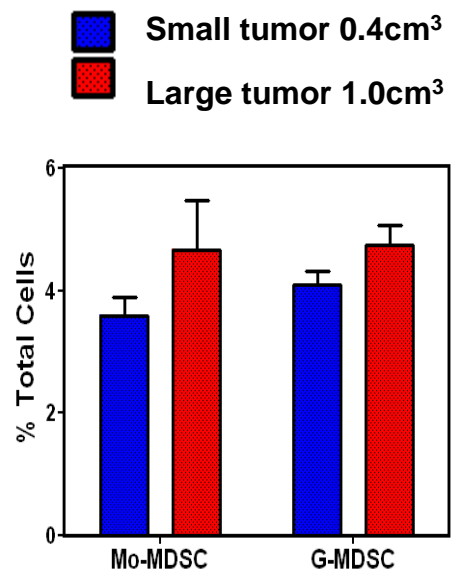
B. TUMOR WEIGHT
(Pan02)



C. TUMOR ASSOCIATED MYELOID
AND LYMPHOID INFILTRATE
(Pan02)



D. MYELOID INFILTRATE IN KCM
TUMORS



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.

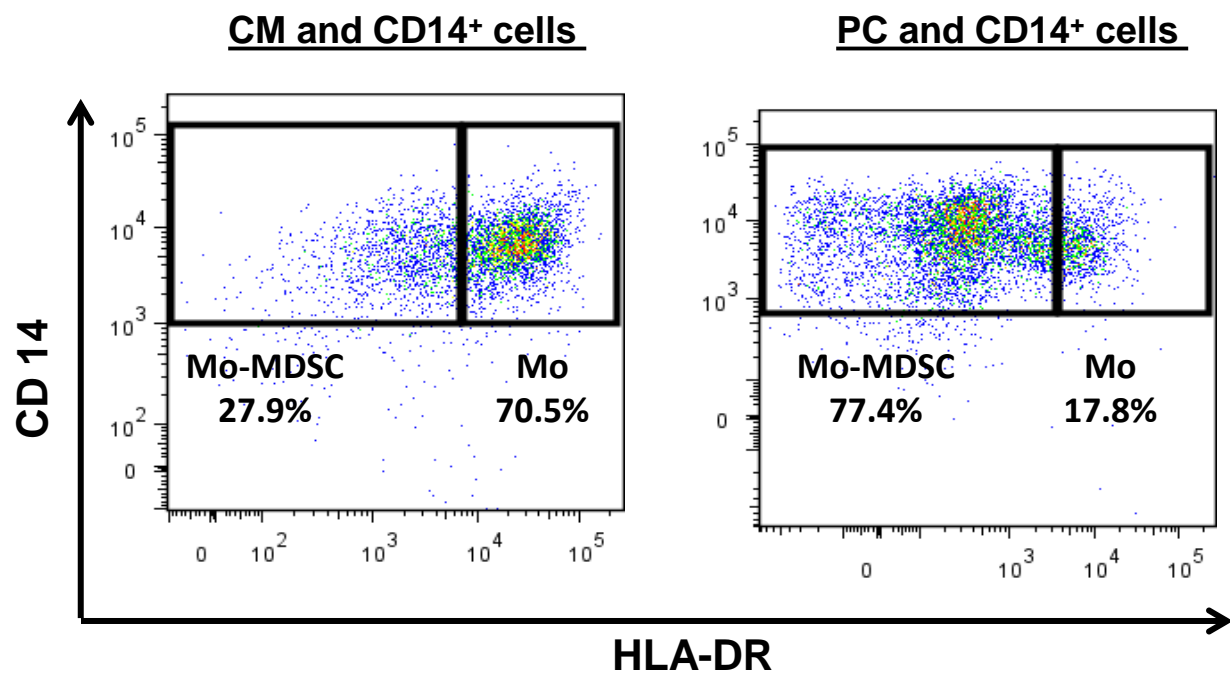
B. Bar graph compares tumor weights of orthotopically implanted Pan02 in WT and GCSFR^{-/-} mice 28 days post injection.

C. Analysis compares tumor myeloid and lymphoid infiltrate by flow cytometry in WT and GCSFR^{-/-}, Pan02 tumor-bearing mice. Mo-MDSC = CD11b⁺/Ly6C^{hi}/Gr1⁺/F4/80^{mid}, G-MDSC = CD11b⁺/Gr-1⁺/Ly6G^{hi}/Ly6C^{mid}, T cells = CD45⁺/CD3⁺; TAM = CD45⁺/CD11b⁺/F4/80^{hi}/Ly6C^{low}/MHCII⁺.

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).

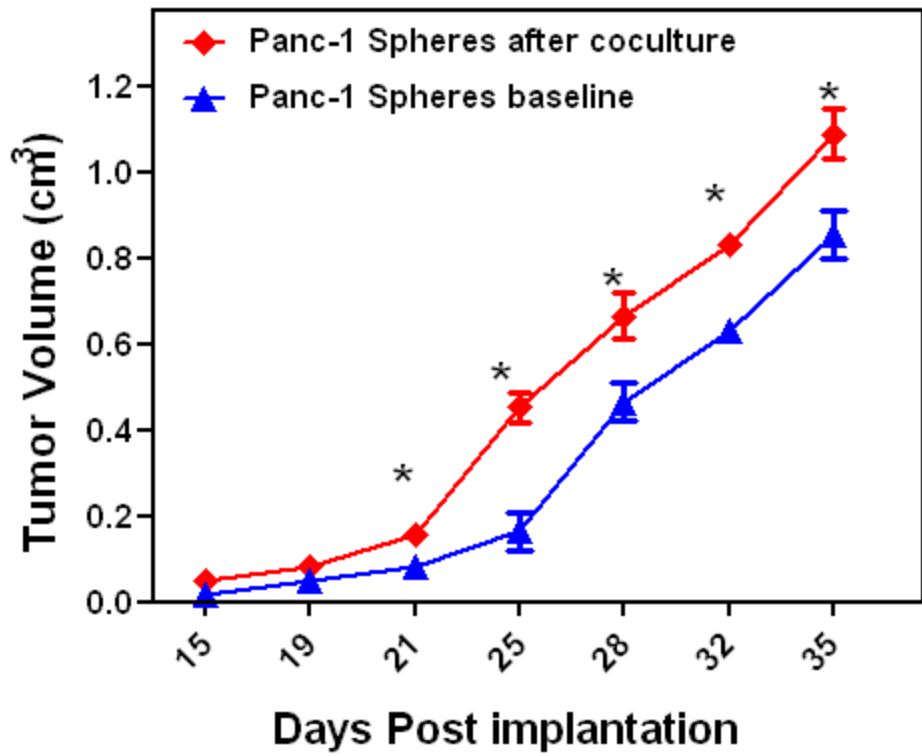
Graphs depict means ± SEM with asterisk (*) denoting statistically significant differences between groups defined as p < 0.05 by Mann-Whitney test.

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Supplementary figure: 7



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.

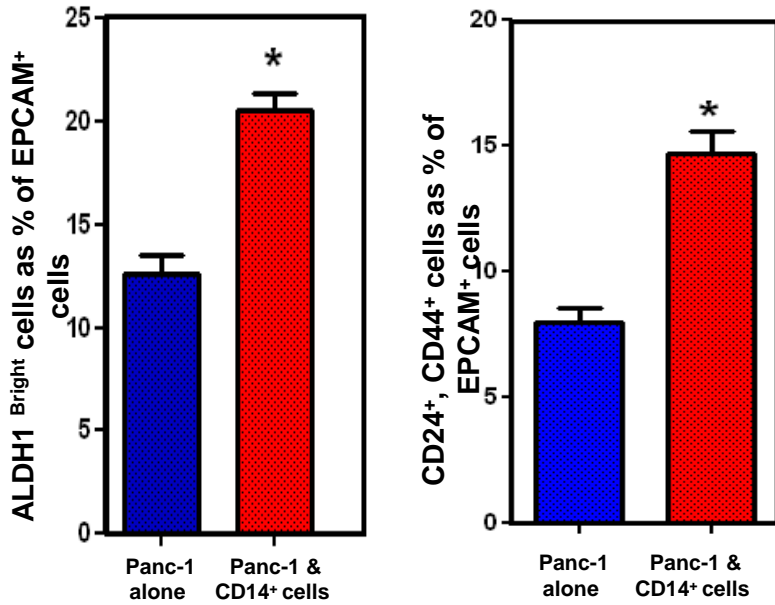
SUBCUTANEOUS TUMOR GROWTH CURVE



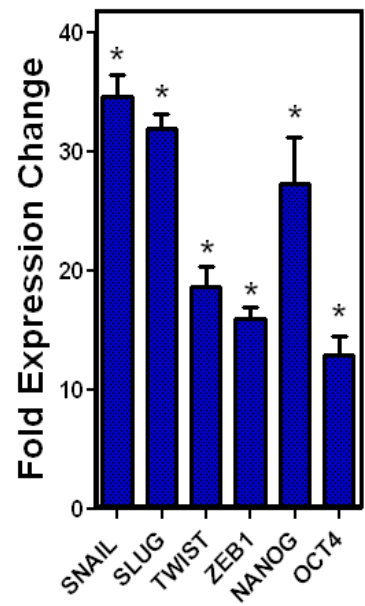
Supplementary Figure 8: Tumor growth curves comparing subcutaneous tumor growth, Panc-1 spheres (with baseline ALDH1 activity) and Panc-1 spheres after co-culture (increased ALDH1 activity) by caliper measurements in NU/J mice. Points on curve represent mean values \pm SEM are indicated time points.

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Supplementary Figure 9

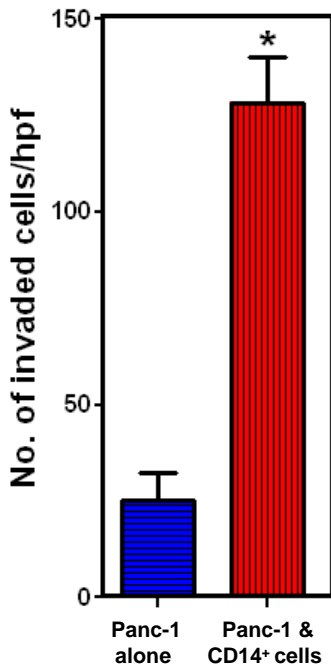
A.



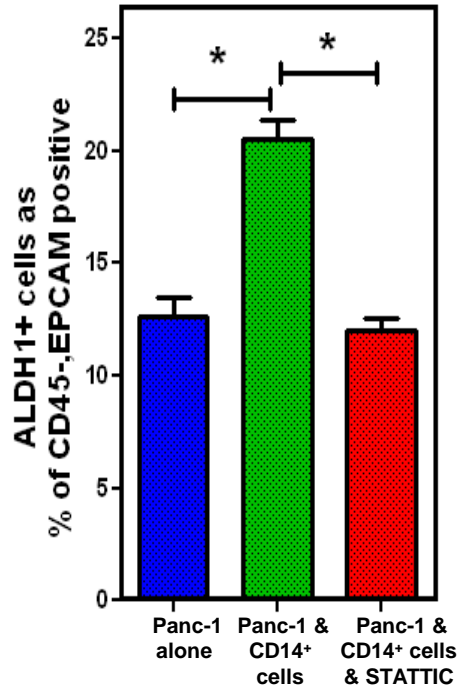
B.



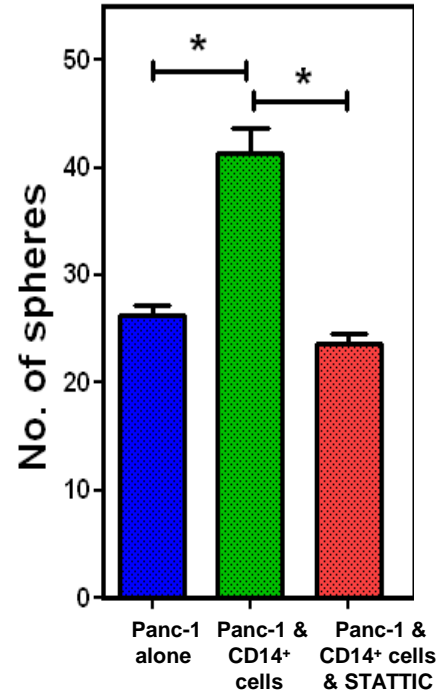
C.



D.



E.



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).

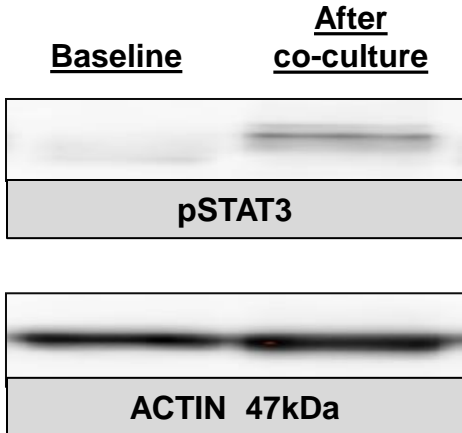
D. Inhibition of STAT3 signaling in CD14⁺/HLA-DR^{low/-} cells prevents the increase in ALDH1^{Bright} CSCs in Panc-1 from baseline. Representative flow-cytometry plots show ALDH1^{Bright} CSC population (gated on CD45⁻, EpCAM⁺, PI⁻ cells) which is approximately 13.5% in Panc-1 alone, 20.8% when Panc-1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

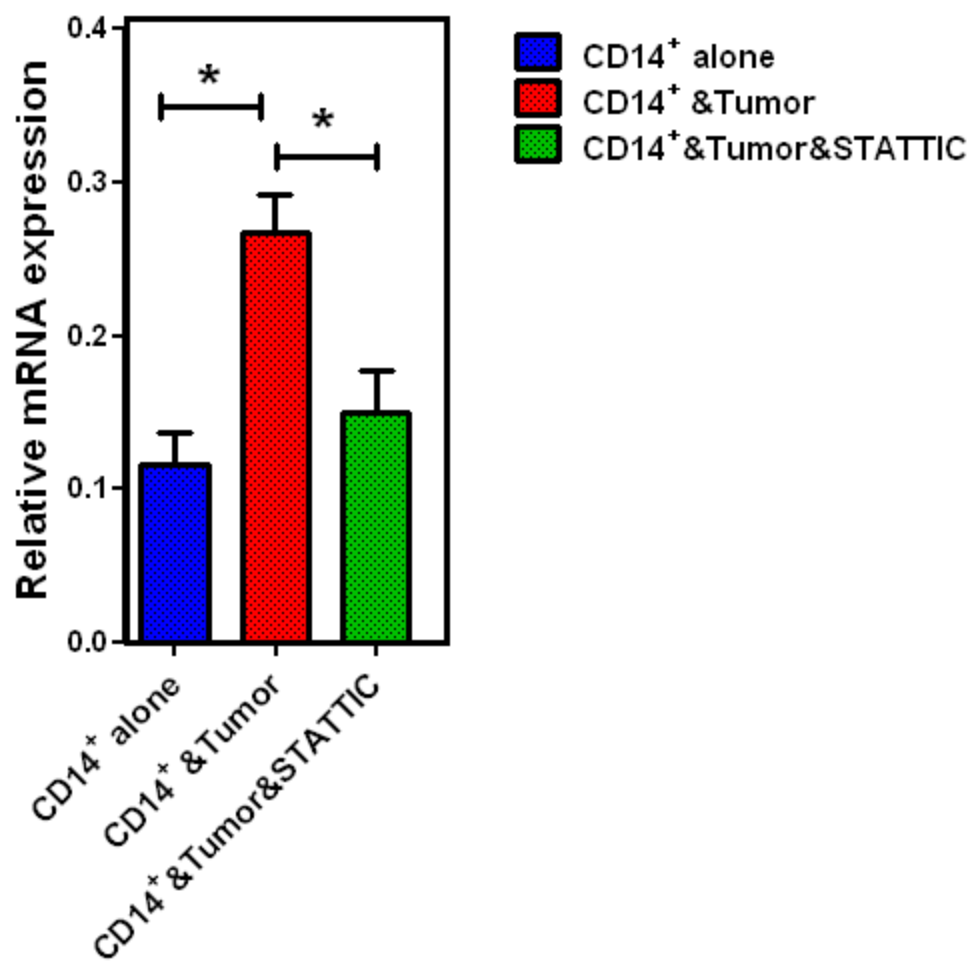
pSTAT3 IN CD11b+ CELLS AFTER CO-CULTURE WITH TUMOR CELLS (KCM)



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



Supplementary Figure 11:

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

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