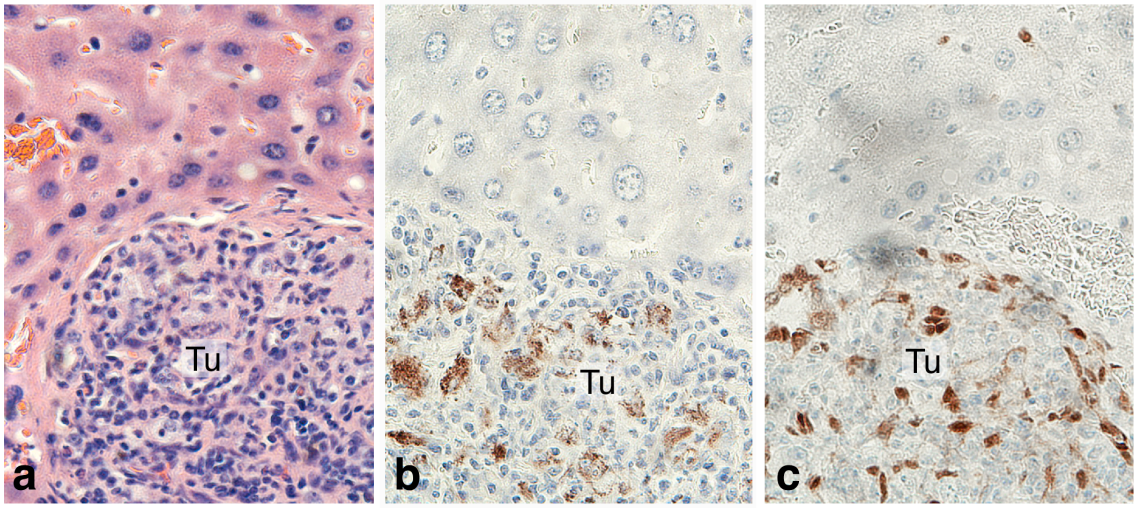
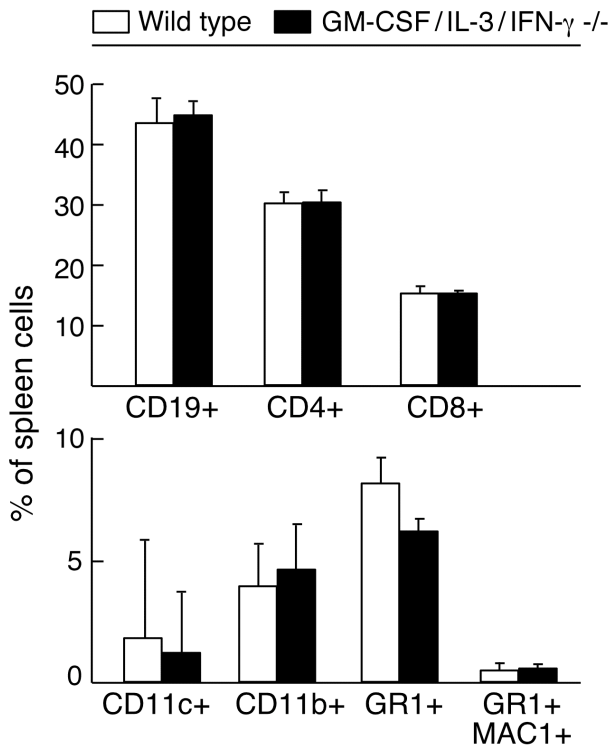
Supplemental Materials



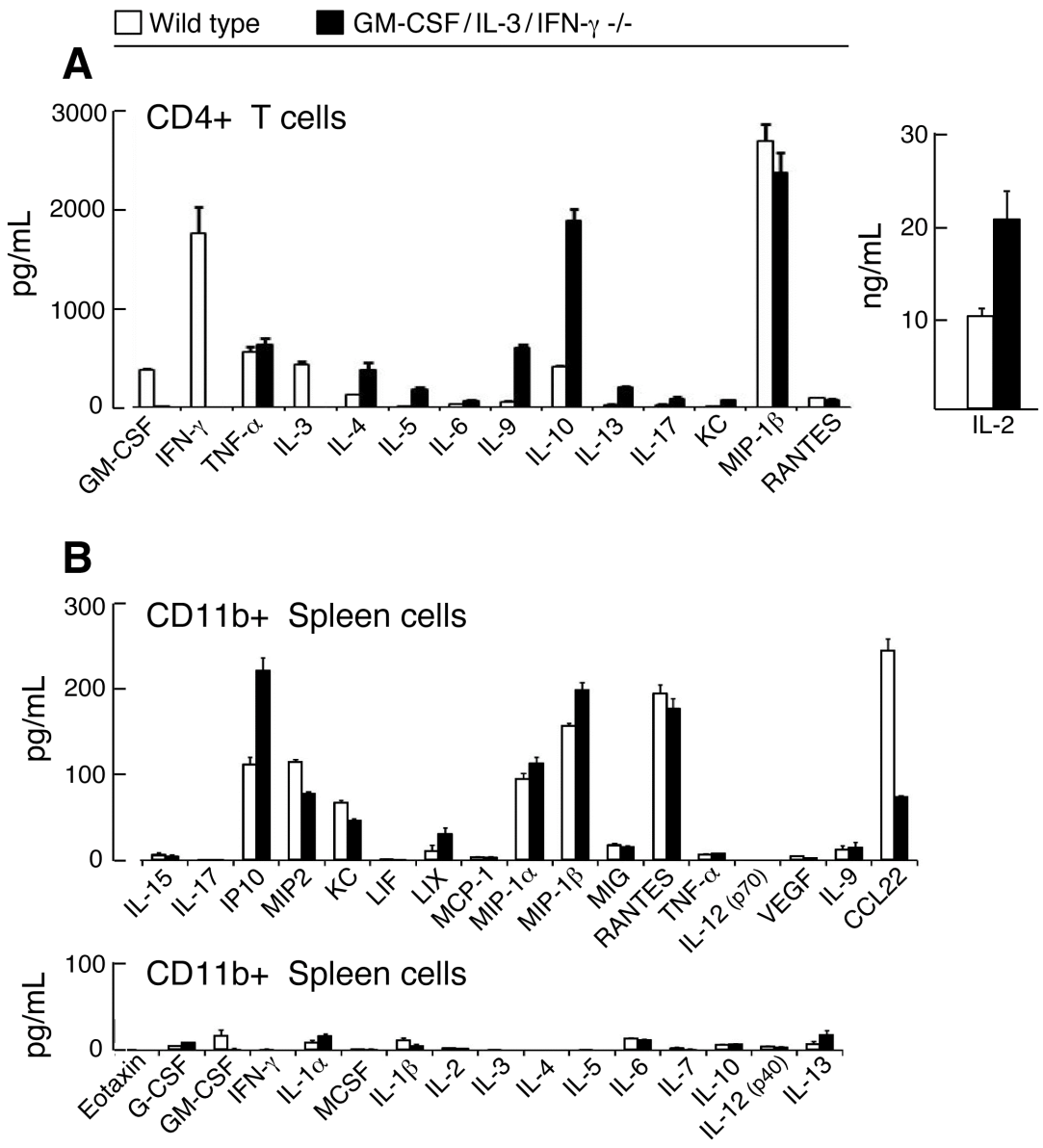
Supplemental Figure 1: SPC expression and ERK activation are maintained in BALB/c TKO tumor metastases

(A) – (C) Liver metastases from a 14 month old BALB/c TKO mouse. 400X magnification. (A) H&E. (B) SPC. (C) pERK.



Supplemental Figure 2: Lymphoid and myeloid composition of BALB/c WT and TKO spleens

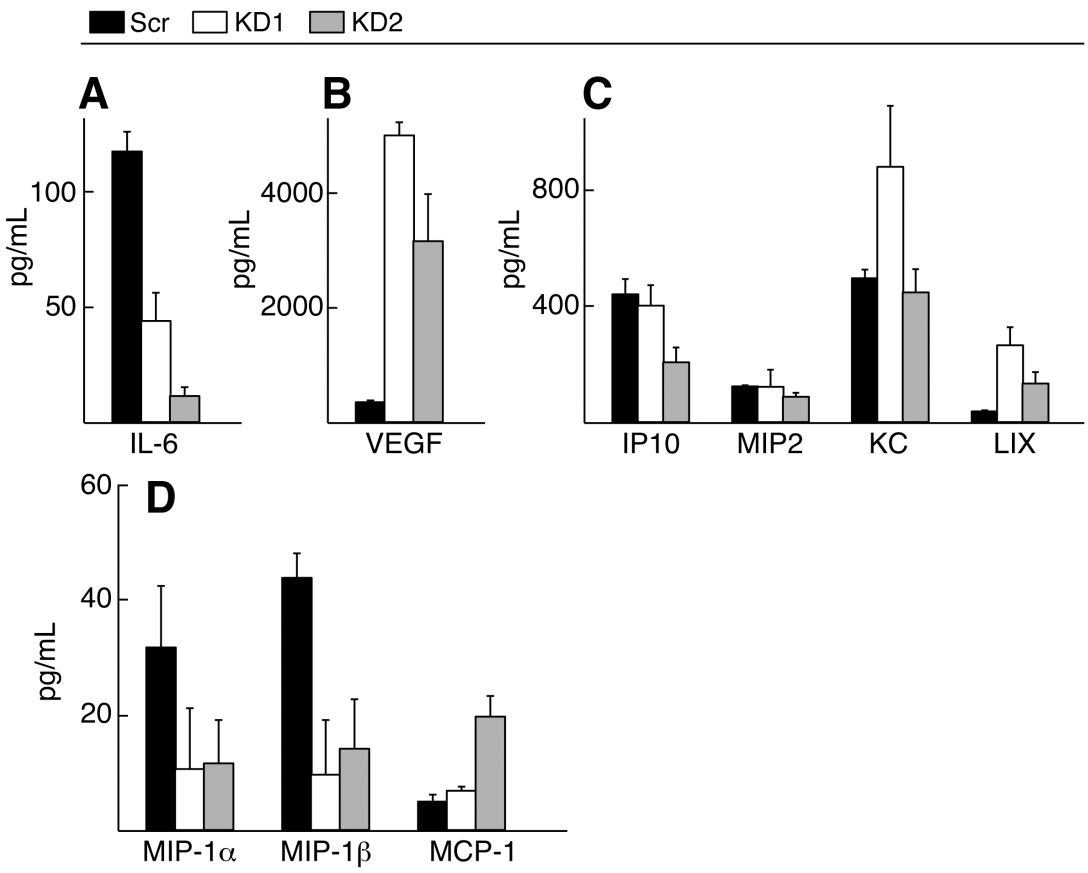
Analysis of lymphoid (top panel) and myeloid (bottom panel) spleen cells in 3 month old BALB/c WT and TKO mice. Cell types were identified by flow cytometry using the indicated antibodies. Data show the combined results from three independent experiments including a total of six animals in each group. Error bars represent SEM.



Supplemental Figure 3: Skewed cytokine and chemokine production from BALB/c TKO T cells and macrophages

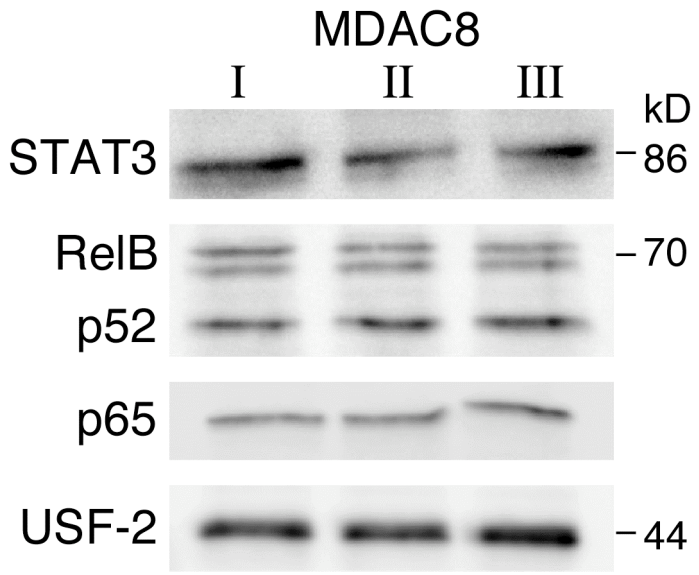
Cytokine and chemokine production from CD4+ T cells (top panel) and CD11b+ myeloid cells (bottom panel). Samples are identical to those depicted in figure 3F (CD4+) and figure 3G (CD11b+). Cytokines and chemokines were measured using anti-cytokine fluorescent beads. Error bars represent SEM. (top panel) IL-2 is presented on a separate

scale due to the magnitude of cytokine secretion. IL-1α, IL-1β, IL-12p40, IL-12p70, VEGF, and MCP-1 were also assessed and were below the detection limit of the assay for both groups. For GM-CSF, IL-3, IL-5, IL-9, IL-10, IL-13, and KC $p < 0.001$; for IFN-γ $p = 0.003$; for IL-6 $p = 0.004$; for IL-2, IL-4, and IL-17 $p = 0.04$. TNF-α, MIP-1β, and RANTES were not significantly different. (bottom panel) For IP-10, MIP-2, KC, MIP-1β, and CCL22 $p = 0.002$. For all other cytokines, differences did not reach statistical significance. P values were not adjusted for multiple comparisons in either experiment.



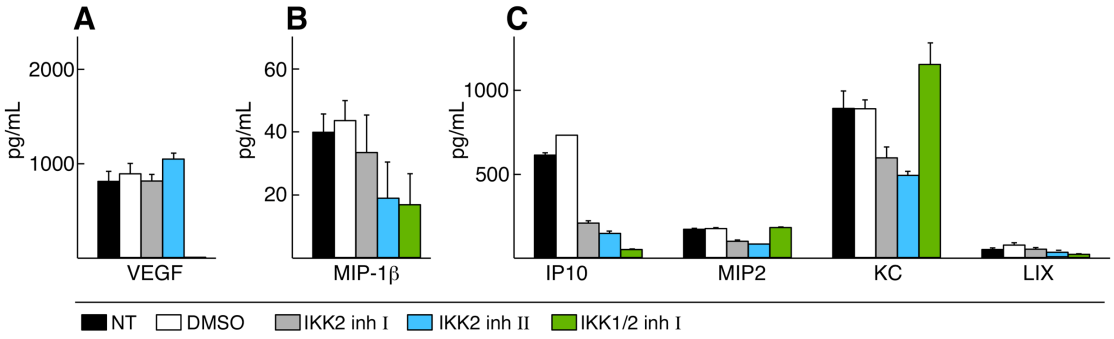
Supplemental Figure 4: IL-6 KD is associated with altered cytokine and chemokine expression in a BALB/c TKO tumor cell line

$3x10^5$ MDAC8 cells stably expressing shRNA directed against IL-6 (KD1, KD2) or a non-targeting construct (scr) as in figure 5 were cultured for 72 hours. Cytokines and chemokines were measured using anti-cytokine fluorescent beads except for MCP-1 which was measured by ELISA. Results represent the combination of two independent experiments containing either six samples (Scr, KD2) or four samples (KD1) per construct. Error bars represent SEM. KD1 was not included in the statistical analysis. For IL-6 and VEGF $p = 0.002$; for IP-10 and MIP-1β $p = 0.02$; for MCP-1 $p = 0.03$; for LIX $p = 0.05$. For MIP-2, KC, and MIP-1α differences did not reach statistical significance. P values were not adjusted for multiple comparisons.



Supplemental Figure 5: The BALB/c TKO tumor cell line MDAC8 shows nuclear localization of NF-κB and STAT3

Western blot using the indicated antibodies on nuclear lystates from cultured MDAC8 cells. Samples were gathered from 3 independent cultures (I, II, and III).



Supplemental Figure 6: Inhibition of IKK alters chemokine secretion by MDAC8 cells

MDAC8 cells were treated with IKK inhibitors; samples are identical to those presented in figure 6E. Supernatants were analyzed using anti-cytokine fluorescent beads. Samples treated with IKK1/2 inhibitor II were not presented in the analysis due to lack of viability of the cells within several hours of exposure to inhibitors. Error bars represent SEM. For statistical analysis, vehicle treated cultures were compared to IKK1/2 inhibitor I. For IP-10 $p = 0.0009$, for LIX $p = 0.02$, for VEGF $p = 0.002$. Differences in all other cytokines did not reach statistical significance.