

Figure S1. Representative flow cytometry plots of CEPs, hemangiocytes, and myeloid derived suppressor cells

Analysis of flow cytometry data obtained from peripheral blood of C57Bl/6 mice. CEPs are determined as positive for both VEGFR2 and CD117 and negative for CD45 surface marker. Myeloid derived suppressor cells (MDSCs) are determined as positive for both Gr-1 and CD11b. Hemangiocytes (Hemangio) are determined as positive for CD45, CXCR4 and VEGFR1 surface markers.

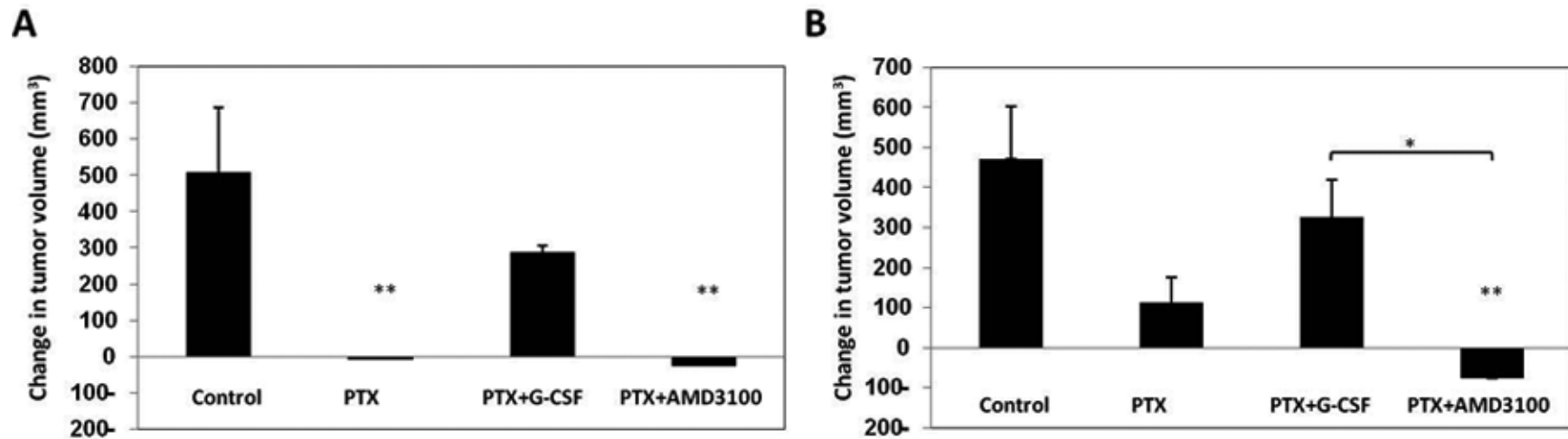


Figure S2. Changes in tumor volume of both LLC and EMT/6 tumors 3 days after treatment with PTX, or PTX in combination with either G-CSF or AMD3100

C57Bl/6 mice bearing 500mm³ LLC tumors (A) or BALB/c mice bearing 500mm³ EMT6 tumors (B) (n = 4–5 mice/group) were treated with PTX or PTX in combination with either G-CSF or AMD3100. Tumor volumes were assessed before and 3 days after treatment. Post treatment average change in tumor volume is calculated as the reduction from pretreatment volume. *, 0.05 > p > 0.01; **, 0.01 > p > 0.001.

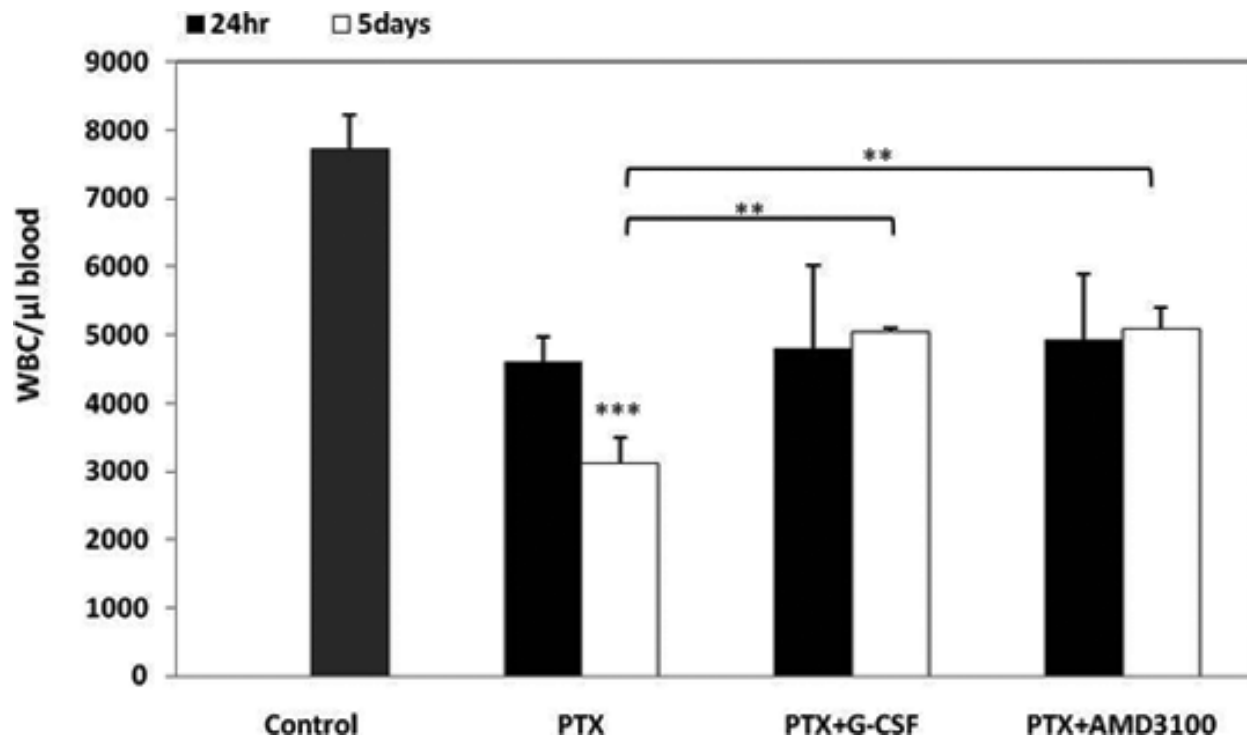


Figure S3. Evaluation of white blood cell (WBC) count in mice treated with either PTX or PTX in combination with either G-CSF or AMD3100

Non-tumor bearing C57Bl/6 mice (n=4–5 mice/group) were treated with PTX, PTX+G-CSF, or PTX+AMD3100. Twenty-four hours and 5 days later, mice were bled via retro-orbital sinus for the evaluation of white blood cell counts (WBC). **, 0.01>p>0.001; ***, p<0.001, from control group, unless indicated otherwise.

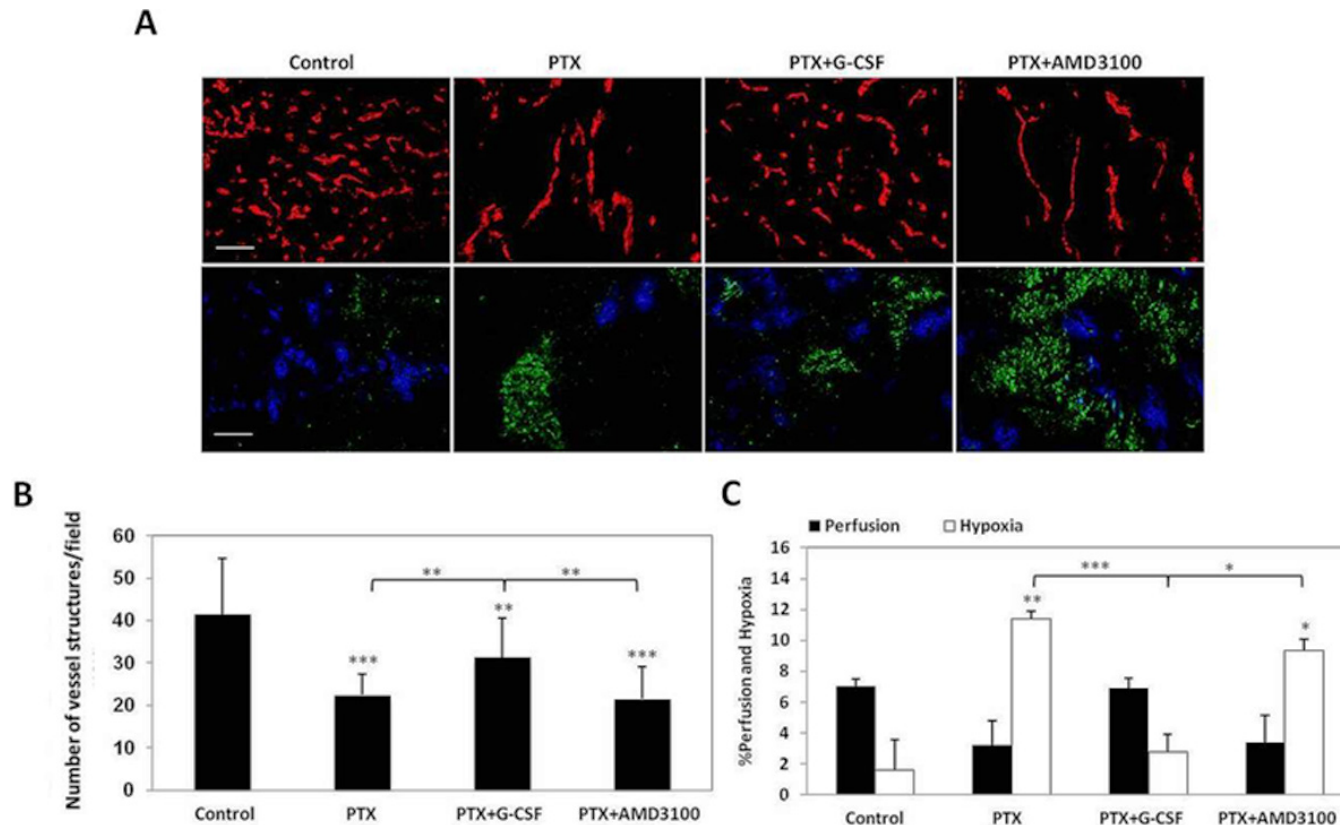


Figure S4. Evaluation of microvessel density, perfusion and hypoxia in EMT/6 tumors grown in BALB/c mice after treatment with PTX or PTX in combination with either G-CSF or AMD3100

500mm³ EMT/6 tumors were removed 3 days after treatment with PTX, PTX+G-CSF or PTX+AMD3100. Tumors were evaluated for (A) microvessel density using CD31 immunostaining as a marker for endothelial cells (red) (Scale bar = 100 μm); and hypoxia (green) and vessel perfusion (blue) using hypoxic probe and Hoechst as described in Materials and Methods (Scale bars = 200 μm). Quantification of (B) microvessel density, and (C) perfusion and hypoxia is provided. *, 0.05>p>0.01; **, 0.01>p>0.001; ***, p<0.001, from control group, unless indicated otherwise.

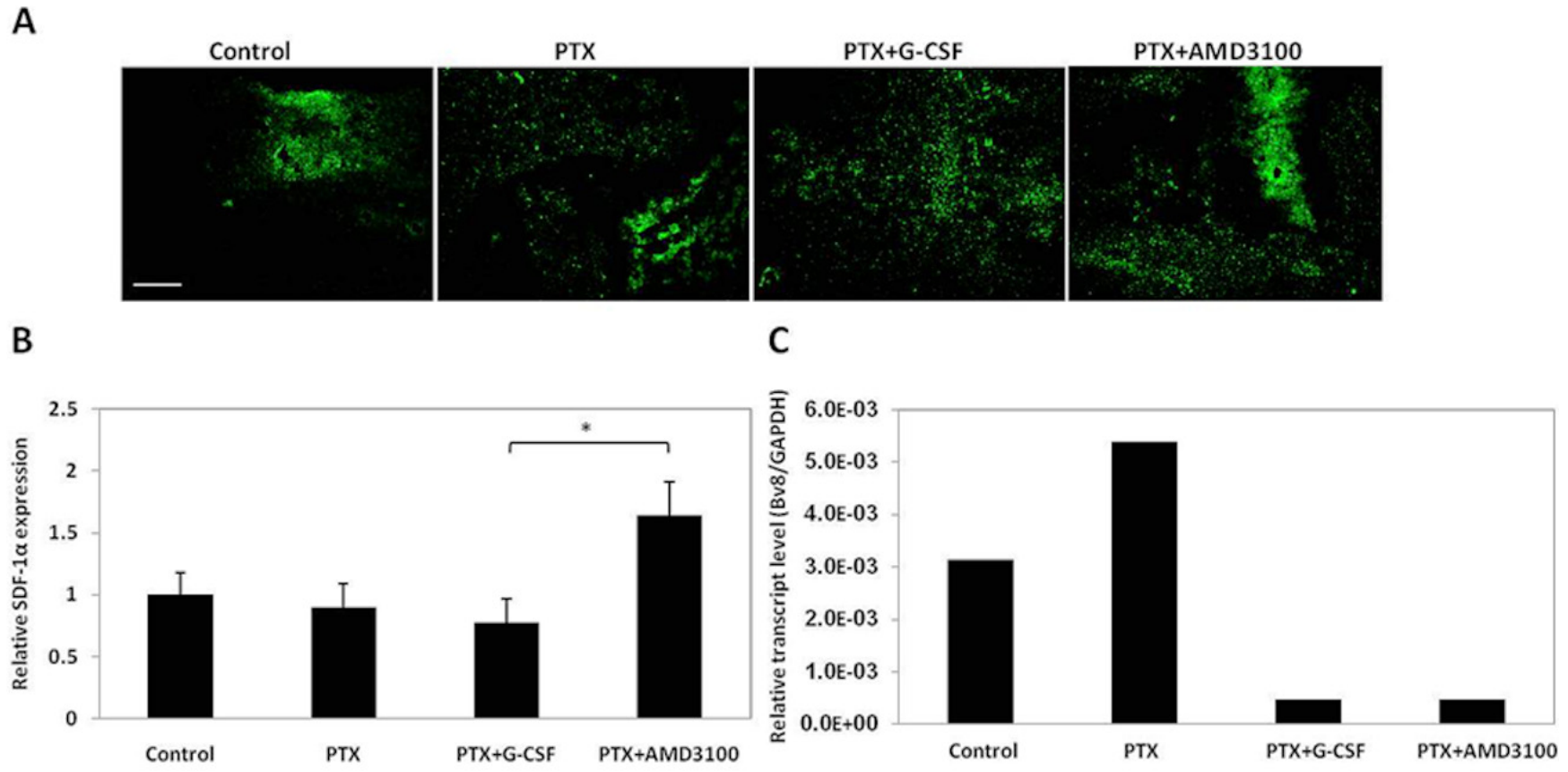


Figure S5. Evaluation of SDF-1 α expression and Bv8 mRNA levels in EMT/6 tumors following treatment with PTX or PTX in combination with either G-CSF or AMD3100

BALB/c mice bearing 500mm³ EMT/6 tumors (n = 4–5 mice/group) were treated with PTX or PTX in combination with either G-CSF or AMD3100. Three days later, tumors were removed and sections were evaluated for SDF-1 α expression (green) (Scale bar = 200 μ m). (B) Quantification of relative SDF-1 α expression is provided. (C) In parallel, mRNA was extracted from the same tumors, and Bv8 mRNA levels were measured by qRT-PCR. *: p<0.05, from control group, unless indicated otherwise.

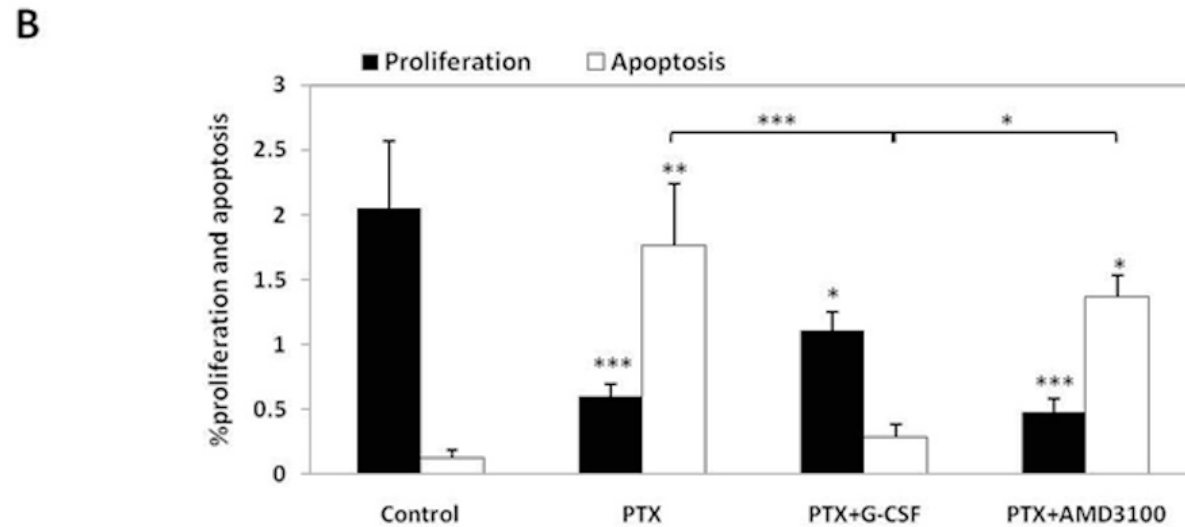
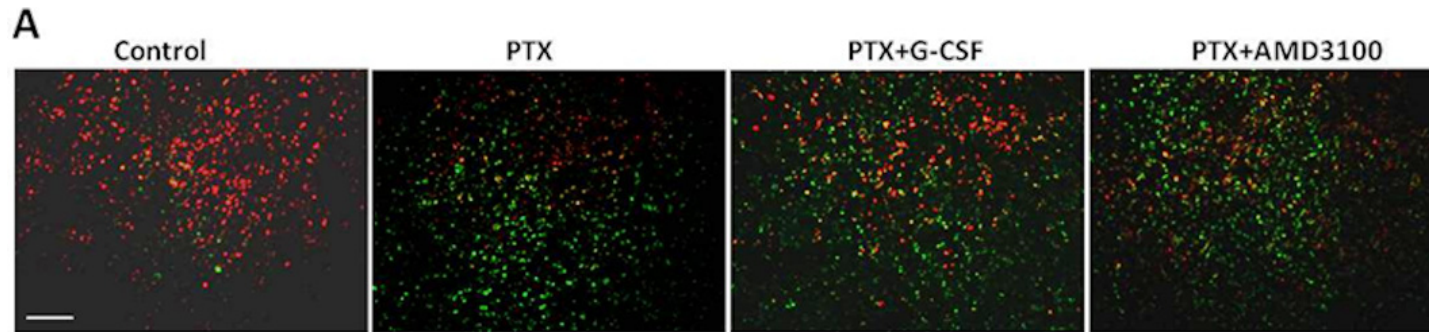


Figure S6. Evaluation of tumor cell proliferation and apoptosis in EMT/6 tumors following treatment with PTX or PTX in combination with either G-CSF or AMD3100

(A) BALB/c mice bearing 500 mm³ EMT/6 tumors (n = 4–5 mice/group) were treated with PTX or PTX in combination with either G-CSF or AMD3100. Three days later, tumors were removed and evaluated for proliferation (red) and apoptosis (green) (Scale bar= 200 μm). (B) Quantification of proliferation and apoptosis is expressed as a percentage. *, 0.05>p>0.01; **, 0.01>p>0.001; ***, p<0.001, from control group, unless indicated otherwise.

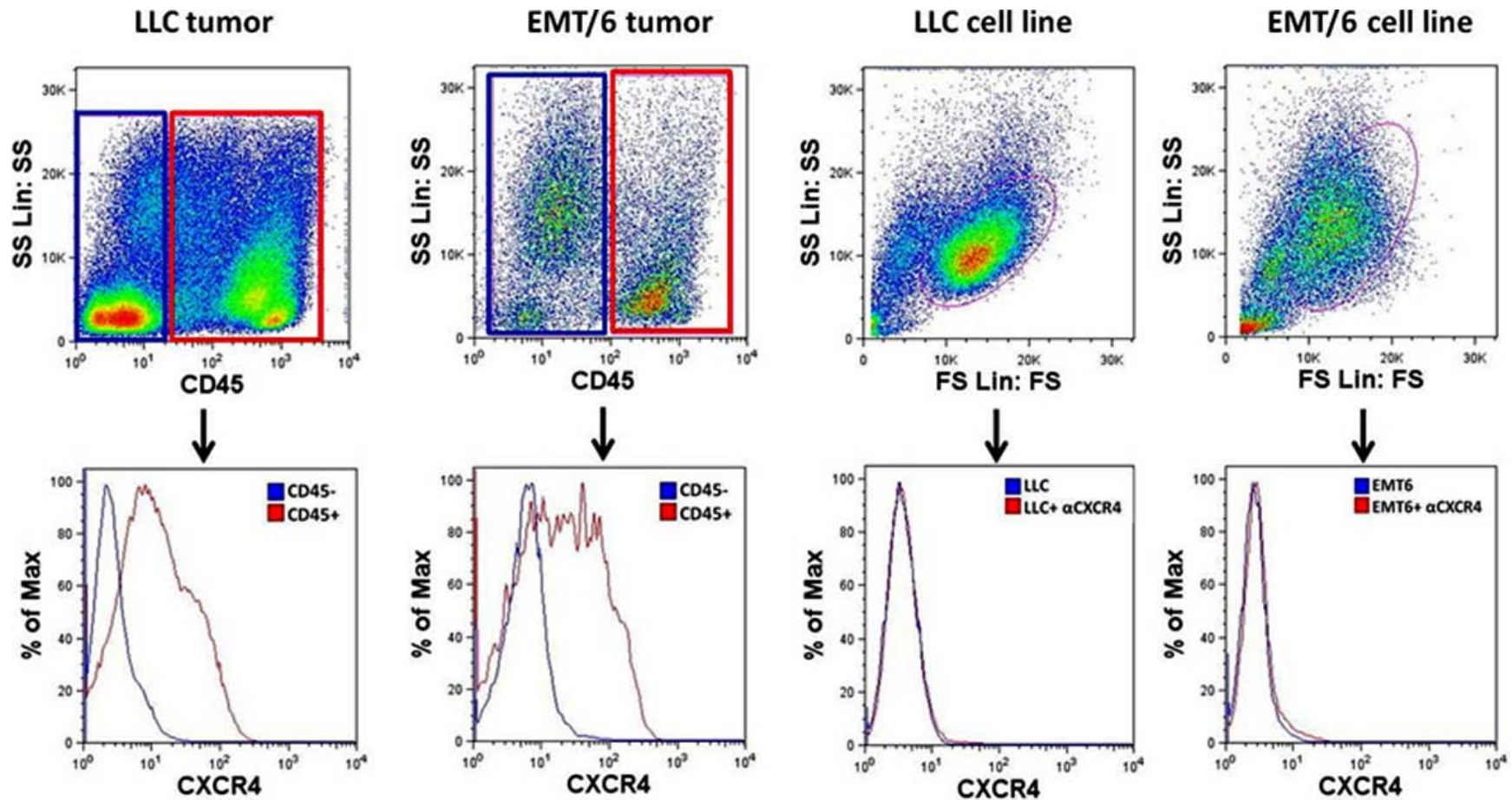


Figure S7. Evaluation of CXCR4 surface marker in LLC and EMT/6 tumors or cultured cells

500mm³ LLC and EMT/6 tumors grown in C57Bl/6 and BALB/c mice, respectively, were removed. Tumors were prepared as single cell suspension and subsequently immunostained with CD45 (to exclude hematopoietic cells infiltrating tumors) and CXCR4 in order to evaluate its expression by the tumor cells. Representative flow cytometry plots are presented. In addition, LLC and EMT/6 cultured cells were removed from the culture dish by trypsin, and subsequently washed twice with PBS. Then, cells were immunostained with CXCR4, and analyzed by flow cytometry. Representative flow cytometry plots are presented.