Modulation of the anti-tumor immune response by complement

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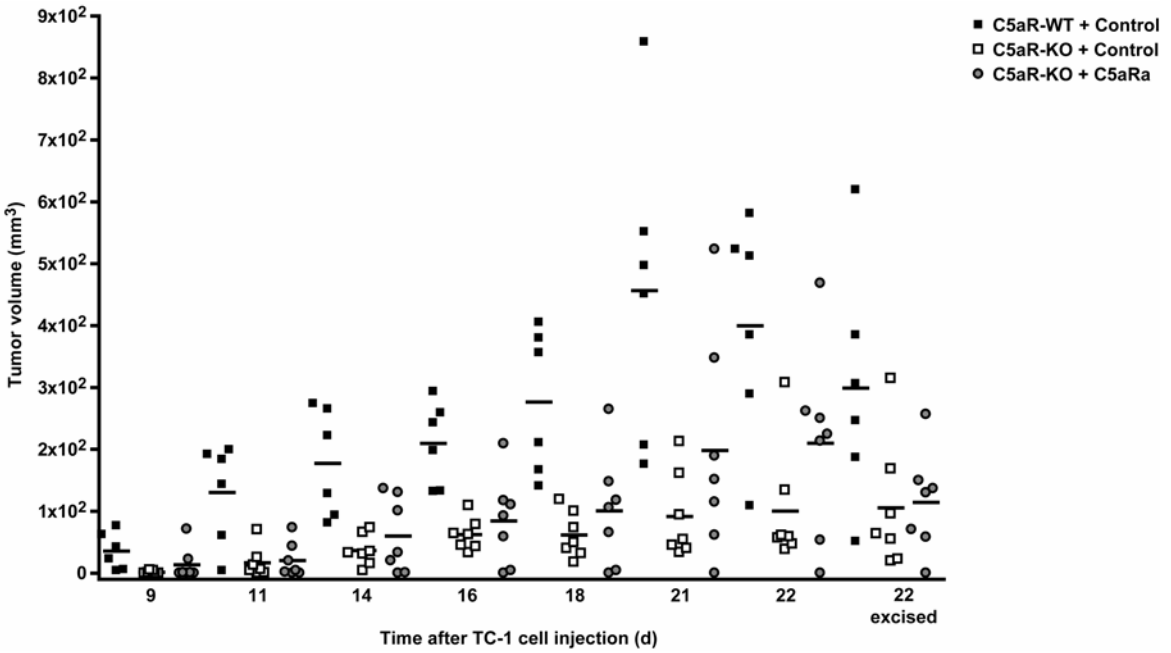
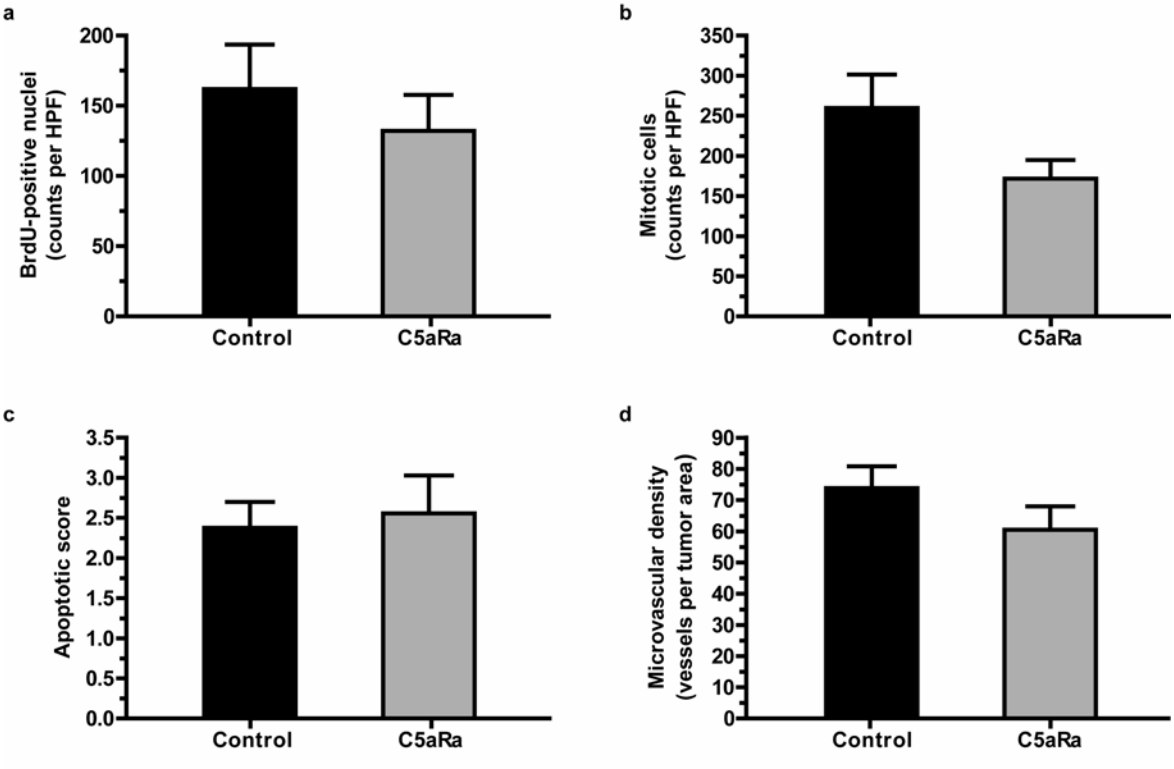
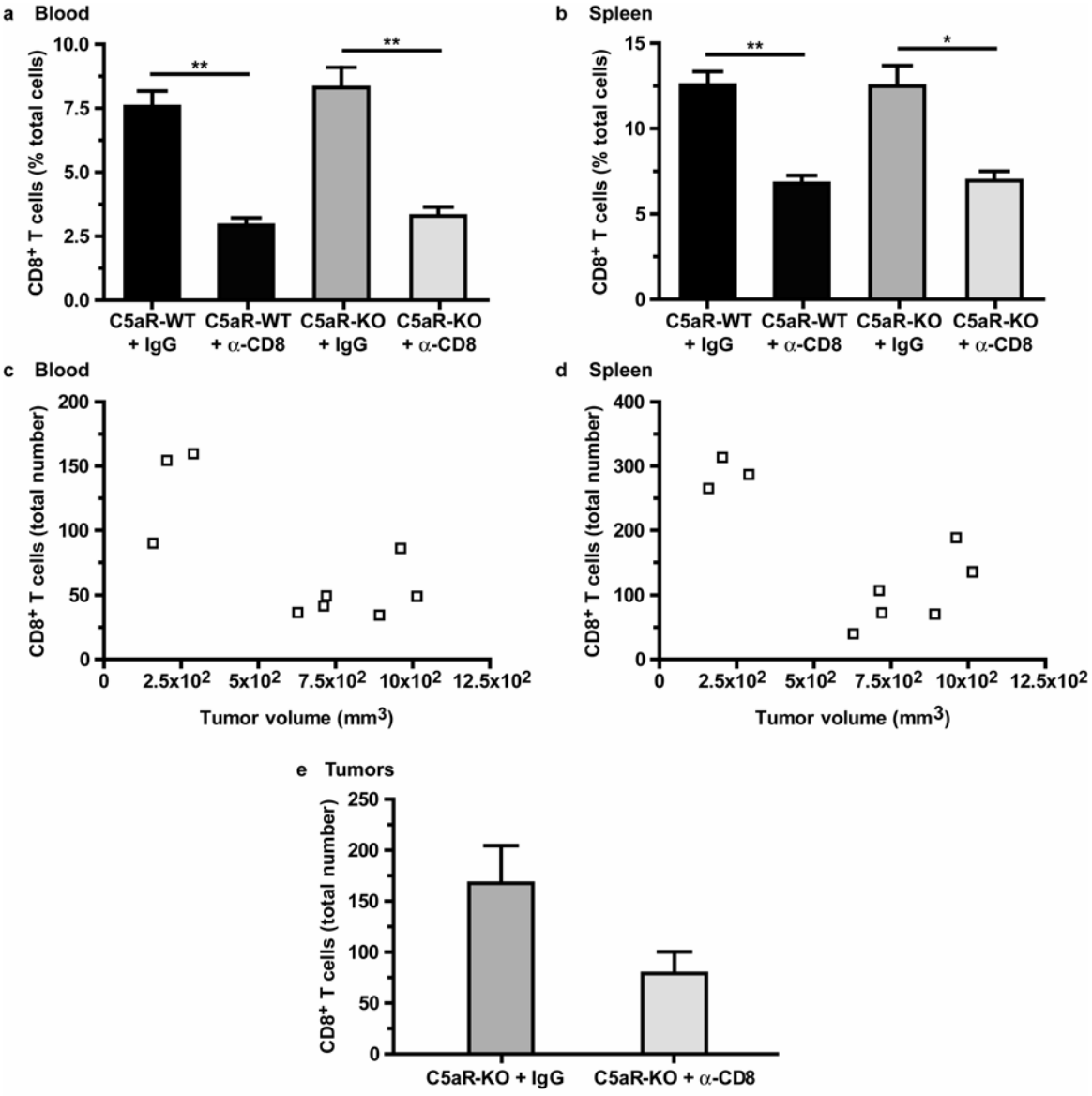


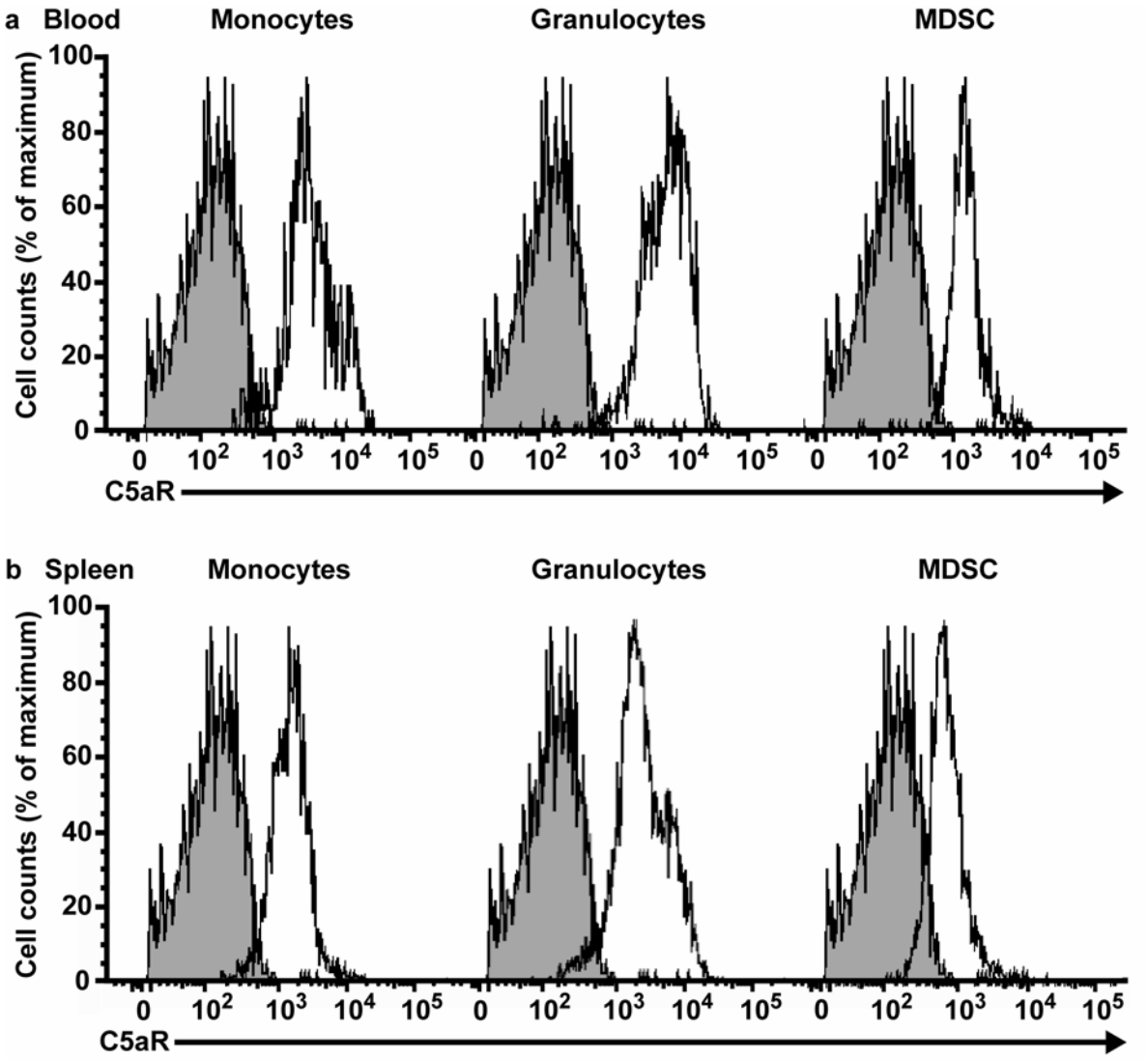
Supplementary Figure 1 C5aR antagonist does not exhibit cytotoxicity toward TC-1 cells. Tumor volumes for individual C5aR-deficient (C5aR-KO) mice and littermate wild-type controls (C5aR-WT) treated with either control peptide (control) or C5aR antagonist (C5aRa). The last panel (22 excised) indicates volumes based on measurements obtained after mice were sacrificed and the tumors removed. Horizontal lines among each group of data points represent the mean tumor volume for that group ($n \geq 6$ mice per cohort). $P < 0.0001$ for C5aR-WT + Control vs. each C5aR-KO group, regardless of treatment (two-way ANOVA).



Supplementary Figure 2 Blockade of C5aR by antagonist does not significantly affect cell proliferation, apoptosis, or angiogenesis in tumors. Tumor sections from C5aRa-treated or PBS-treated mice (Control) ($n \geq 9$ mice per cohort) were analyzed for **(a)** The number of BrdU-positive nuclei per high-power field (HPF; magnification, 400x; $P = 0.4770$), **(b)** The number of mitotic figures per HPF ($P = 0.0927$), **(c)** The amount of apoptosis ($P = 0.7580$), and **(d)** Microvascular density ($P = 0.2137$). *P* values were determined by *t*-test.



Supplementary Figure 3 Treatment of C5aR-deficient mice with CD8 antibody depletes $CD8^+$ T cells and accelerates tumor growth. **(a)** Percentage of $CD3^+CD8^+$ T cells, determined by flow cytometry, in the blood of tumor-bearing C5aR-deficient (C5aR-KO) mice and littermate wild-type controls (C5aR-WT) treated with either IgG or CD8 antibody (α-CD8). Cells were isolated from mice represented in Fig. 4e. (b) Same analysis as in (a) but for spleens. For (a) and (b), $n \geq 9$ mice per cohort; *, $P = 0.0005$; **, $P < 0.0001$ (*t*-test). **(c)** Number of $CD3^+CD8^+$ T cells infiltrating tumors in relation to tumor volumes for C5aR-KO mice treated with α-CD8, based on studies shown in Fig. 4e ($n = 9$ mice, $P = 0.0323$, $r = -0.7094$, Pearson correlation). **(d)** Same analysis as in (c) but for spleens ($n = 9$ mice, $P = 0.0302$, $r = -0.7154$, Pearson correlation). **(e)** Number of $CD8^+$ T cells, determined by immunofluorescence, in tumors from C5aR-KO mice treated with IgG or α-CD8 (from studies shown in Fig. 4e; $P = 0.0705$, *t*-test).



Supplementary Figure 4 C5a receptor is expressed on myeloid-derived cells. **(a)** Expression of C5aR (white areas) versus isotype controls (grey areas) in monocytes, granulocytes, and myeloid-derived suppressor cells (MDSC) in the blood of wild-type mice without tumors (*n* = 3). Viable cells were gated based on their scatter properties and CD45 expression. MDSCs were identified as cells co-expressing CD11b and Gr-1. **(b)** Same analysis as in (a) but for spleens.