Supplemental Figure Legend

Figure S1. Influence of anthracycline-based chemotherapy on myeloid cells in distinct lymphoid organs. (A-G) Wildtype (WT) C57BL/6 mice bearing MCA205 fibrosarcomas were treated with doxorubicin (DX) i.t. or an equivalent volume of PBS, as a single injection. Neoplastic lesions, tumor-draining lymph nodes (DLNs), spleens and the bone marrow (BM) were harvested 38 hours later, dissociated and processed for the quantification of tumor-infiltrating leukocytes (TILs) of the indicated phenotype by cytofluorometry (A-F). Representative dot plots for CD11c+MHCII+ TILs are illustrated in A (numbers refer to the percentage of cells found in the corresponding gate), while the quantitative data relative to tumors and DLNs are depicted in B and C, respectively. Panels D, E and F report quantitative data on CD11b+, CD11b+Ly6Chigh and CD11b+Ly6G+ TILs, respectively. Alternatively, CD11b+Ly6Chigh cells from neoplastic lesions, spleens and BM were monitored for the expression of the indicated phenotypic markers (G). (Iso BM = BM cells stained with an isotype-matched control antibody). Quantitative results are reported as means ± SEM (n = 4-5 mice/group). *p<0.05, **p<0.01, ***p<0.001,ns = non-significant (unpaired, two-tailed Student's t test), as compared to the same organ from PBS-receiving animals.

Figure S2. Impact of chemotherapy on the expression of M1 and M2 macrophage-related genes by Ly6Chigh cells. Wild type C57BL/6 mice bearing MCA205 fibrosarcomas were treated with doxorubicin (DX) i.t. or an equivalent volume of PBS, as a single injection. Thirty-eight hours later, CD11b+Ly6Chigh leukocytes were isolated from neoplastic lesions by cytofluorometry and processed for the RT-PCR-assisted quantification of transcripts coding for M1 and M2 macrophage related factors. Results are representative of 3 independent experiments and shown as means ± SEM (n = 5 mice/group). *p<0.05, **p<0.01 (unpaired, two-tailed Student's t test), as compared to CD11b+Ly6Chigh cells isolated from PBS-treated fibrosarcomas.

Figure S3. Anthracycline-based chemotherapy activates antigen-specific T cells within neoplastic lesions. (A) Wild-type C57BL/6 mice were inoculated with ovalbumin (OVA)-expressing MCA205 cells s.c. (on day -9), and then (on day -5) received CFSE-labeled OT1 cells i.v.. On day 0, mice were treated with doxorubicin (DX) i.t. or an equivalent volume of PBS, as a single injection, followed (on day 1) by lymphadenectomy or sham surgery (as a control condition). On day 3, tumors were harvested and dissociated to determine the proliferation (CFSE dilution) of OT1 cells. Typical dot plots of DX treated tumor are shown to illustrate the gating strategy employed to analyze the proliferation of adoptively infused, CFSE stained

CD3+CD8+TCRV□5+ cells in situ. (B) Alternatively, naïve CD45.1 mice were adoptively transferred with CD45.2+ OT1 cells (on day -8), inoculated with OVA-expressing MCA205 tumor cells (on day -7) and - on day 0 - treated with DX or PBS i.t.. Sixty hrs later, neoplastic lesions, tumor-draining lymph nodes (DLNs), contralateral lymph nodes (CLNs) and spleens were harvested and processed for the quantification of CD69+ (activated) OT1 cells. Data are shown as means ± SEM (n = 5 mice/group). *p<0.05, (unpaired, two-tailed Student's t test), as compared to the same organ of PBS-receiving mice.

Figure S4. Anthracycline-based chemotherapy fails to affect the proliferation of antigen-specific T cells within tumor draining lymph nodes. Wild-type C57BL/6 mice were inoculated with ovalbumin (OVA)-expressing MCA205 cells s.c. (on day -9), and then (on day -5) received CFSE-labeled OT1 cells i.v.. On day 0, mice were treated with doxorubicin (DX) i.t. or an equivalent volume of PBS, as a single injection. On day 3, tumor-draining lymph nodes (DLNs) were harvested and dissociated to determine the proliferation (CFSE dilution) of OT1 cells. Typical dot plots and histograms are shown to illustrate the gating strategy employed as well as the proliferative response of adoptively infused, CFSE-stained CD3+CD8+TCRV□5+ cells within DLNs.

Figure S5. Anthracycline-based chemotherapy induces fluctuations in the circulating levels of various myeloid cell populations. The peripheral blood of wild-type C57BL/6 mice bearing established MCA205 fibrosarcomas was harvested atindicated time points after a single intra tumoral injection of doxorubicin (DX) or PBS. Upon red blood cell lysis, samples were characterized by cytofluorometry for the abundance of myeloid cells of the indicated phenotype. Naïve C57BL/6 mice were employed as a reference for the baseline levels of circulating myeloid cells. Results are reported as means ± SEM (n = 5 mice/group). *p<0.05, **p<0.01 (unpaired, two-tailed Student's t test), as compared to PBS-treated, MCA205 fibrosarcoma-bearing mice.

Figure S6. Chemotherapy regulates the expression of chemokine-encoding genes by tumor-infiltrating leukocytes. Wild type (WT) C57BL/6 mice bearing WT or CD39-expressing (CD39) MCA205 fibrosarcomas were treated with doxorubicin (DX) i.t. or an equivalent volume of PBS, as a single injection. Twenty hrs later, tumor-infiltrating leukocytes of the indicated phenotype were isolated by cytofluorometry and processed for the RT-PCR-assisted quantification of Cxcl1, Cxcl2, Cxcl9, Cxcl10, Cxcl11, Cx3cl1, Ccl3, Ccl4 and Ccl5 expression levels. Results (means ± SEM, n = 5 mice/group) are expressed in arbitrary units (AUs) upon normalization to the expression levels of Ppia. *p<0.05, **p<0.01 (unpaired, two tailed Student's t test), as compared to cells of

the same phenotype isolated from PBS-treated mice bearing the same type of tumor (unless otherwise indicated).

Figure S7. Expression of Ccr2 on malignant cells and tumor-infiltrating CD11b+ cells. Wild-type (WT) C57BL/6 mice bearing WT MCA205 fibrosarcomas were treated with doxorubicin (DX) i.t. or an equivalent volume of PBS, as a single injection. Forty-eight hrs later, neoplastic lesions were harvested and processed for the cytofluorometric assessment of Ccr2 expression on neoplastic (CD45-) and tumor-infiltrating myeloid (CD45+CD11b+) cells. Representative expression profiles are shown.