SUPPLEMENTAL MATERIAL

Ma et al., http://www.jem.org/cgi/content/full/jem.20100269/DC1

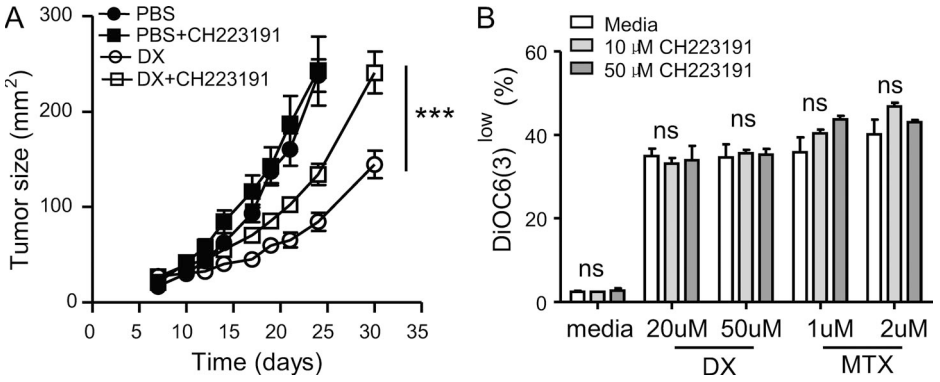

Figure S1. AhR antagonist partially impaired the efficacy of anthracyclines. (A) AhR antagonist CH223191 was dissolved with DMSO and diluted in olive oil. Mice treated with either PBS or DX received a daily systemic inoculation (i.p.) of CH223191 (2 mM, 100 µl) for 4 d from the day of PBS or DX treatment. Tumor size was measured at the indicated time points. One representative experiment out of three is shown. (B) Apoptosis of MCA205 cells treated with media, DX, or MTX with or without the indicated concentration of AhR inhibitor CH-223191. Apoptosis is indicated by a reduction in mitochondrial membrane potential detected by decreased DiOC6(3) fluorescence. This experiment was performed twice with similar results. ***, $P < 0.001$. ns, not significant.

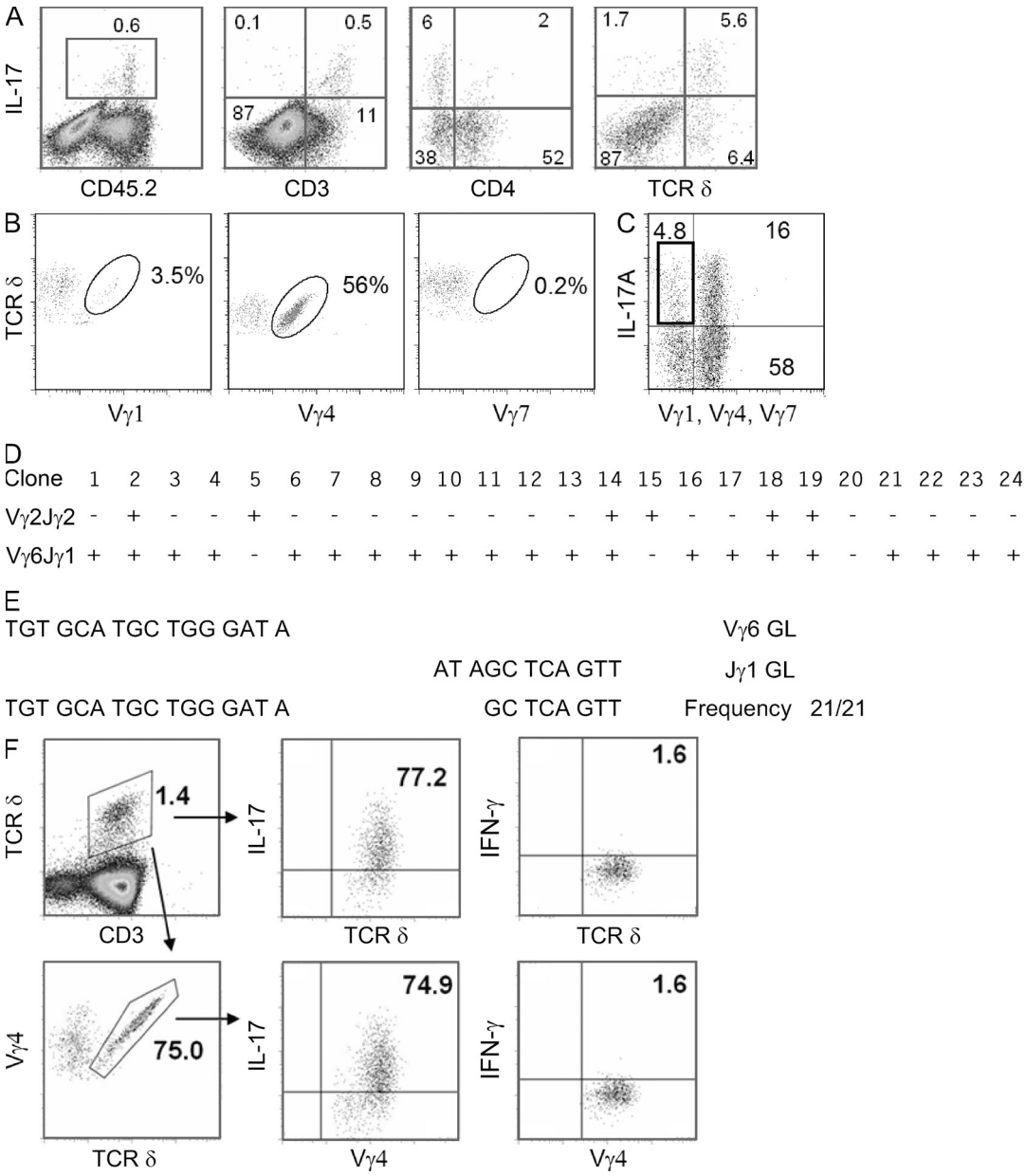

Figure S2. Vγ chain usage by γδ T17 cells in tumor bed and skin draining LNs of naive mice. (A) CD45, CD3, CD4, and TCR δ expression by IL-17A–producing cells from tumor beds of mice 8 d after chemotherapy. (B) Vγ usage in live CD45$^+$ CD3$^+$ TCR δ$^+$ IL-17$^+$ cells in tumor beds after DX. Numbers represent the percentage of Vγ1$^+$, Vγ4$^+$, or Vγ7$^+$ cells among TCR δ$^+$ IL-17$^+$ cells as indicated. One experiment representative of three is shown. (C–E) Individual Vγ1$^-$Vγ4$^-$Vγ7$^-$ γδ T17 TILs as gated in (C) were sorted in PCR plates, and DNA was amplified with primers specific for Vγ2-Jγ2 or Vγ6-Jγ1 rearrangements. (D) Presence (+) or absence (−) of specific amplification bands in 24 clones analyzed. (E) Junctional sequences of the Vγ6-Jγ1 amplifications present in 21 clones in D. GL denotes the germline sequences of the Vγ6 and Jγ1 ends as indicated. (F) LN cells from naive mice were stimulated with PMA/ionomycin, and total γδ T cells (top) or Vγ4$^+$ cells (bottom) were gated and analyzed for intracellular IL-17 (middle) or IFN-γ (right) by FACS. Numbers indicate the percentage of cells inside the gates. One experiment representative of four is shown.

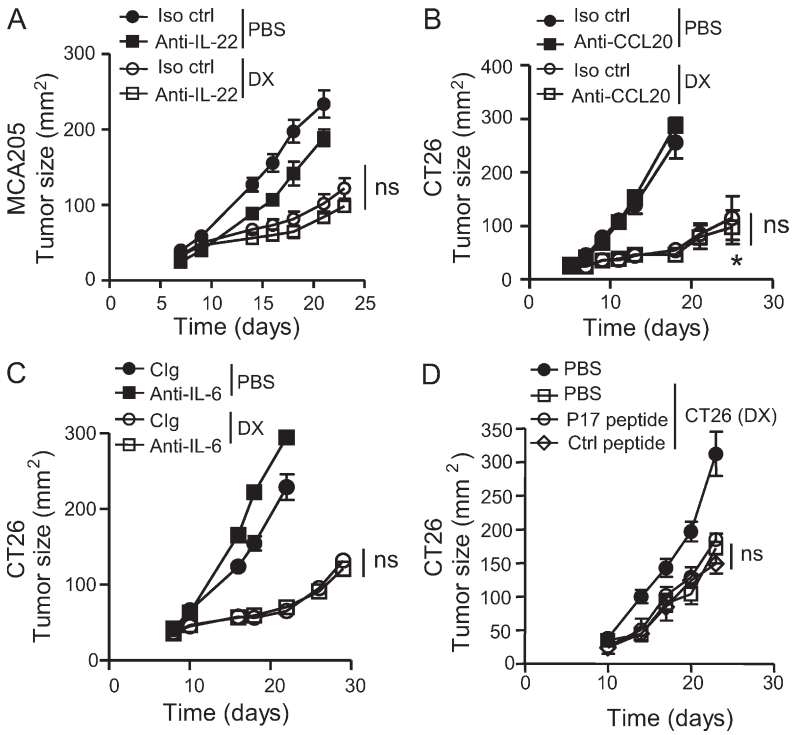

Figure S3. Dispensable roles of IL-22, CCL20, IL-6, and TGF-β for the efficacy of chemotherapy. (A and B) Neutralizing antibodies against IL-22 (50 µg/mouse; A), CCL20 (200 µg/mouse; B), or Clg were administered i.p. every other day for 1 wk starting at the day of chemotherapy in MCA205- or CT26-bearing WT mice. Tumor growth was measured at the indicated time points. One representative experiment out of two is shown. (C) Subcutaneous CT26 colon cancers were treated with DX in the presence of systemic administration of neutralizing antibody against IL-6 (300 µg/mouse) or Clg. (D) Mice were immunized with DX-treated CT26 (injected s.c. into the right flank) and concomitantly challenged with live CT26 tumor cells (injected into the opposite flank at day 0). In parallel, anti–TGF-β or a control peptide (100 µg/mouse) was administered locally (on the site of the vaccination) daily from day 0 to 10. Tumor size was measured at the indicated time points (n = 5 mice/group). The experiment was performed twice with similar results. ns, not significant.