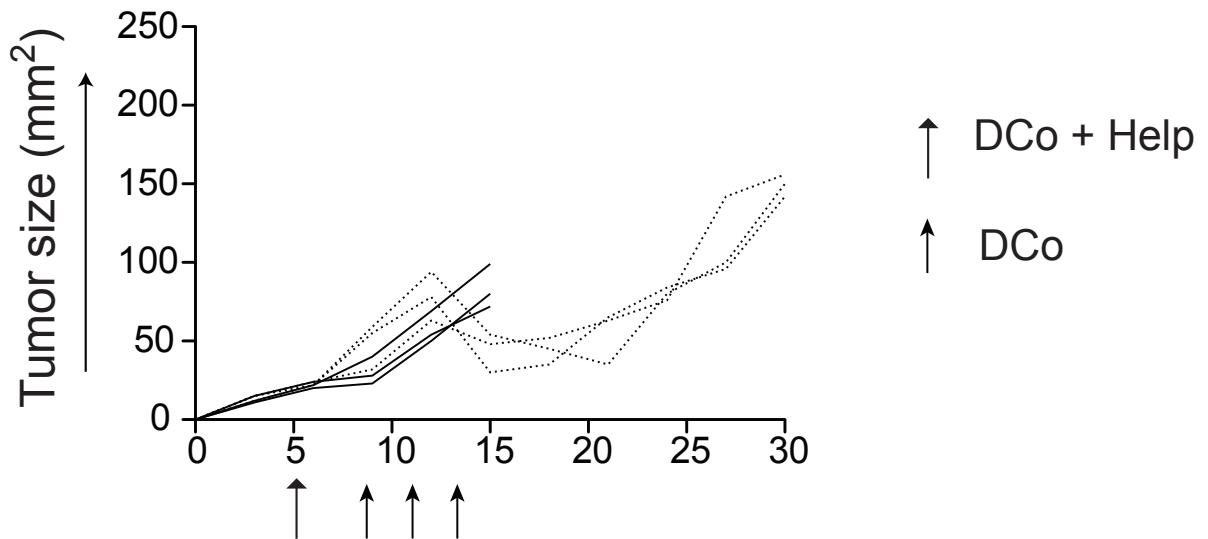
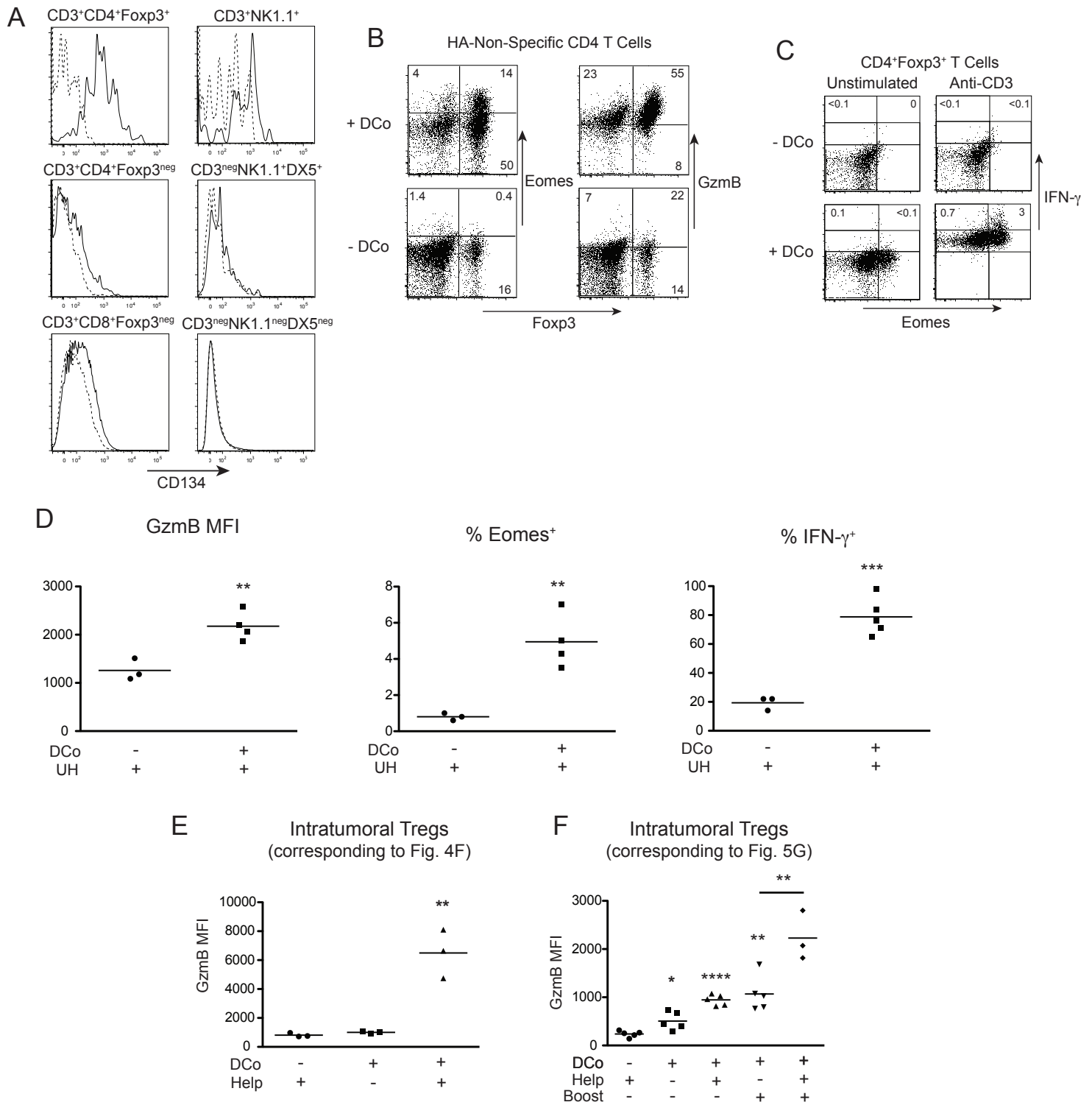


## Both + Boost



**SUPPLEMENTAL FIGURE 1.** Extended tumor growth data (until day 30) for the Both + Boost treatment group shown in Figure 5A. The three mice harboring growing tumors (solid lines) were euthanized on day 15 and their TIL analyzed (shown in Figure 5C-G). The three mice whose tumors regressed between days 12-15 (dotted lines) were monitored until day 30.



**SUPPLEMENTAL FIGURE 2.** Characterization of CD4<sup>+</sup>Foxp3<sup>+</sup> Tregs in mice treated with DCo plus unrelated help. (A) CD134 surface expression on the indicated populations of naive spleen cells. Overlaid histograms are shown for WT (solid lines) and CD134<sup>-/-</sup> cells (dashed lines, used as a background control). (B) Using the paradigm described in Reference #34, Thy1.1<sup>+</sup> TCR transgenic HA-specific CD4 T cells were adoptively transferred into Thy1.2<sup>+</sup> self-HA recipients and treated +/- DCo. Spleens were analyzed on day 5 for ex vivo staining of Foxp3 versus Eomes and GzmB on CD4<sup>+</sup>Thy1.1<sup>neg</sup> cells. (C) Eomes versus IFN-γ staining in CD4<sup>+</sup>Foxp3<sup>+</sup> cells following 4-hour stimulation +/- anti-CD3 mAb. Plots in B and C are representative of 3-4 replicates per group. (D) Using the adoptive transfer paradigm described in Figures 2A and 7, CD4<sup>+</sup>Foxp3<sup>+</sup> Tregs from spleens of mice treated 5 days earlier with DCo + unrelated help (UH) or unrelated help only (control) were stained ex vivo for Eomes and GzmB or IFN-γ following 5 hr stimulation with anti-CD3 mAb. (E & F) Ex vivo GzmB expression in intratumoral Thy1.1<sup>neg</sup>CD4<sup>+</sup>Foxp3<sup>+</sup> cells analyzed from the samples described in Figures 4F (E) and 5G (F).