Supplemental Figure 1

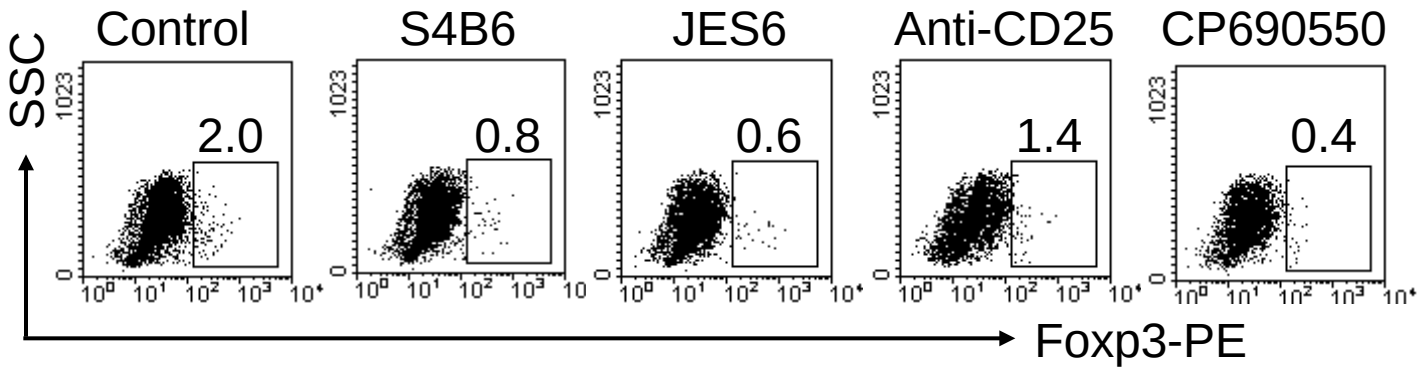

Figure S1. Foxp3 was not expressed after the conditioning step. Purified $CD4^+CD25^-$ T cells were activated by anti-CD3/anti-CD28 mAbs in the presence of anti-IL-2 mAb (clone S4B6 or JES6, respectively), anti-CD25 mAb, or CP690550, as indicated. At 3 days after cell culture, cells were intracellularly stained with anti-Foxp3-PE. The representative dot plots show the frequencies of Foxp3-expressing cells.

Supplemental Figure 2

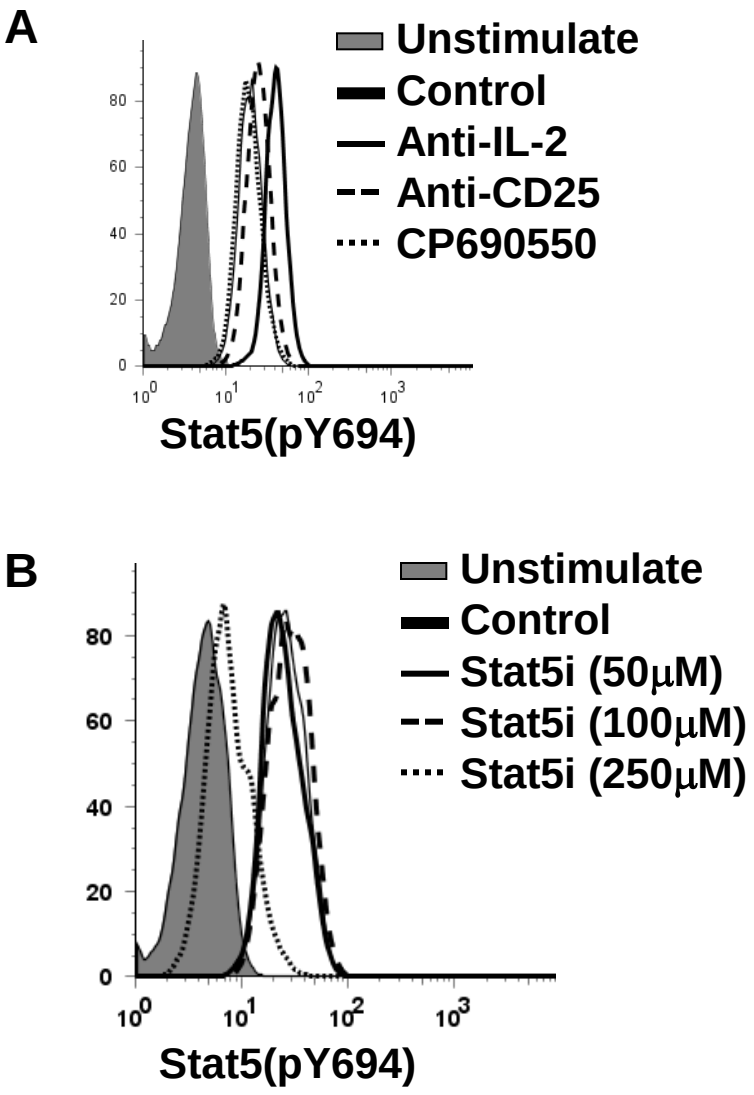

Figure S2. Stat5 phosphorylation during the two-step generation of iTreg cells. Purified $CD4^+CD25^-Foxp3/GFP^-$ T cells were used in the two-step model of iTreg cell generation. **(A)** During the conditioning step, cells were activated by anti-CD3/anti-CD28 mAbs for 3 days, and were either untreated (control group) or treated with 10 μg/ml anti-IL-2 mAb, 10 μg/ml anti-CD25 mAb, or 50 nM CP690550. Overlay histogram shows the phospho-Stat5 expression in the day-3 cultured cells. **(B)** During the conditioning step, cells were activated by anti-CD3/anti-CD28 mAbs for 3 days, and were either untreated (control group) or treated with the indicated concentrations of Stat5 inhibitor. Overlay histogram shows the phospho-Stat5 expression in the day-3 cultured cells.

Supplemental Figure 3

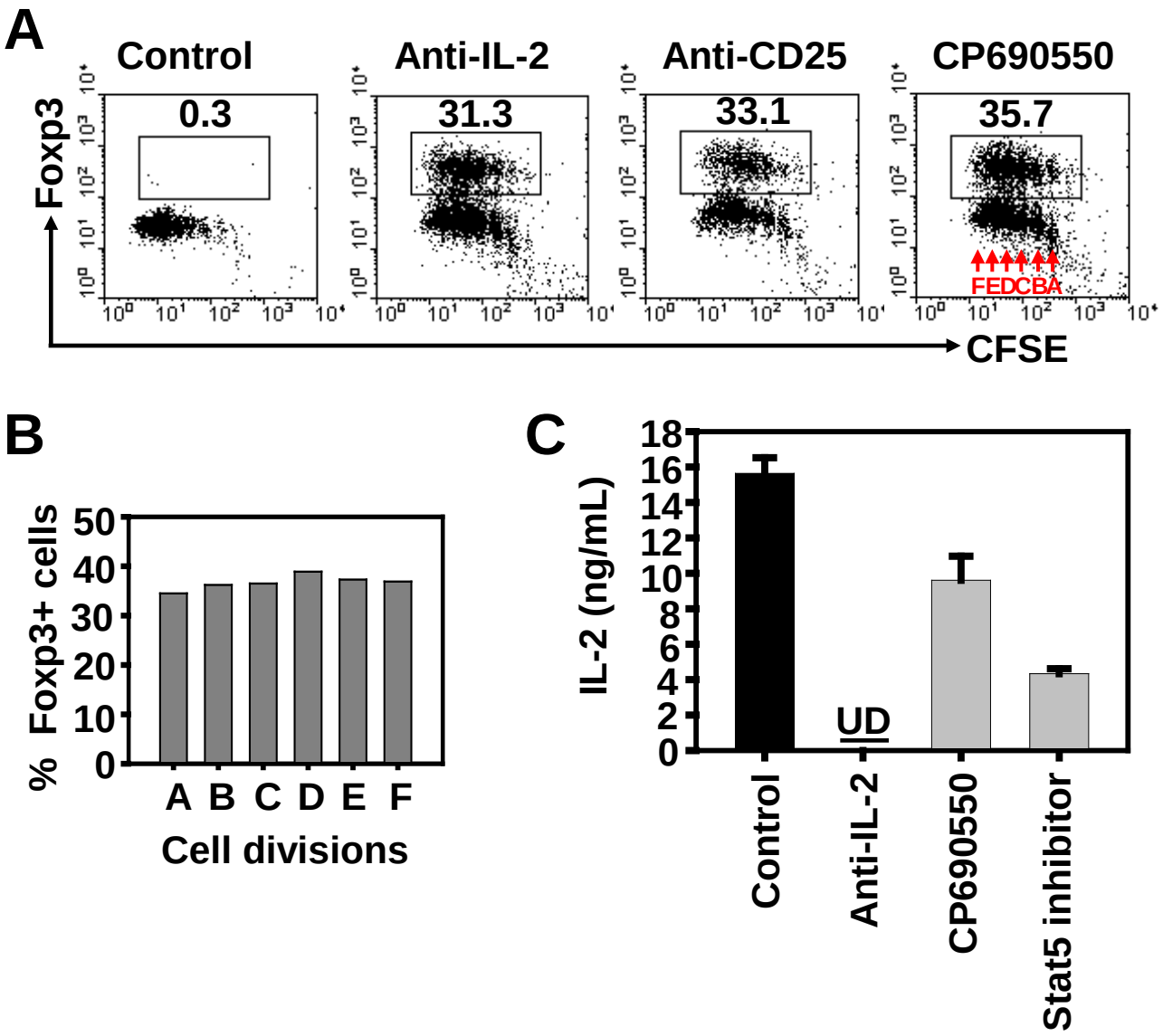

Figure S3. (A and B) Proliferation and Foxp3 expression during the two-step generation of iTreg cells. $CD4^+CD25^-$ T cells were stimulated with anti-CD3/anti-CD28 mAbs for 3 days in the absence (control group) or presence of anti-IL-2 mAb, anti-CD25 mAb, or CP690550 for 3 days, followed by exposure to IL-2 for 3 days. Cells were analyzed on day 6 after culture. In A, $CD4^+CD25^-$ T cells were CFSE labeled prior to cell culture. On day 6, cells were intracellularly stained with anti-Foxp3-PE. The frequencies of Foxp3-expressing cells are shown. In B, the bar graph shows the % Foxp3-expressing cells in each cell division, which is indicated by the red arrows in the right panel of A. ***(C) IL-2 levels during the conditioning step of iTreg cell generation.*** Purified $CD4^+CD25^-$ cells were activated by anti-CD3/anti-CD28 mAbs in the absence (control) or presence of anti-IL-2 mAb, CP690550, or Stat5 inhibitor as indicated. At 3 days after cell culture, IL-2 levels in the supernatants of cultures were detected by ELISA.

Supplemental Figure 4

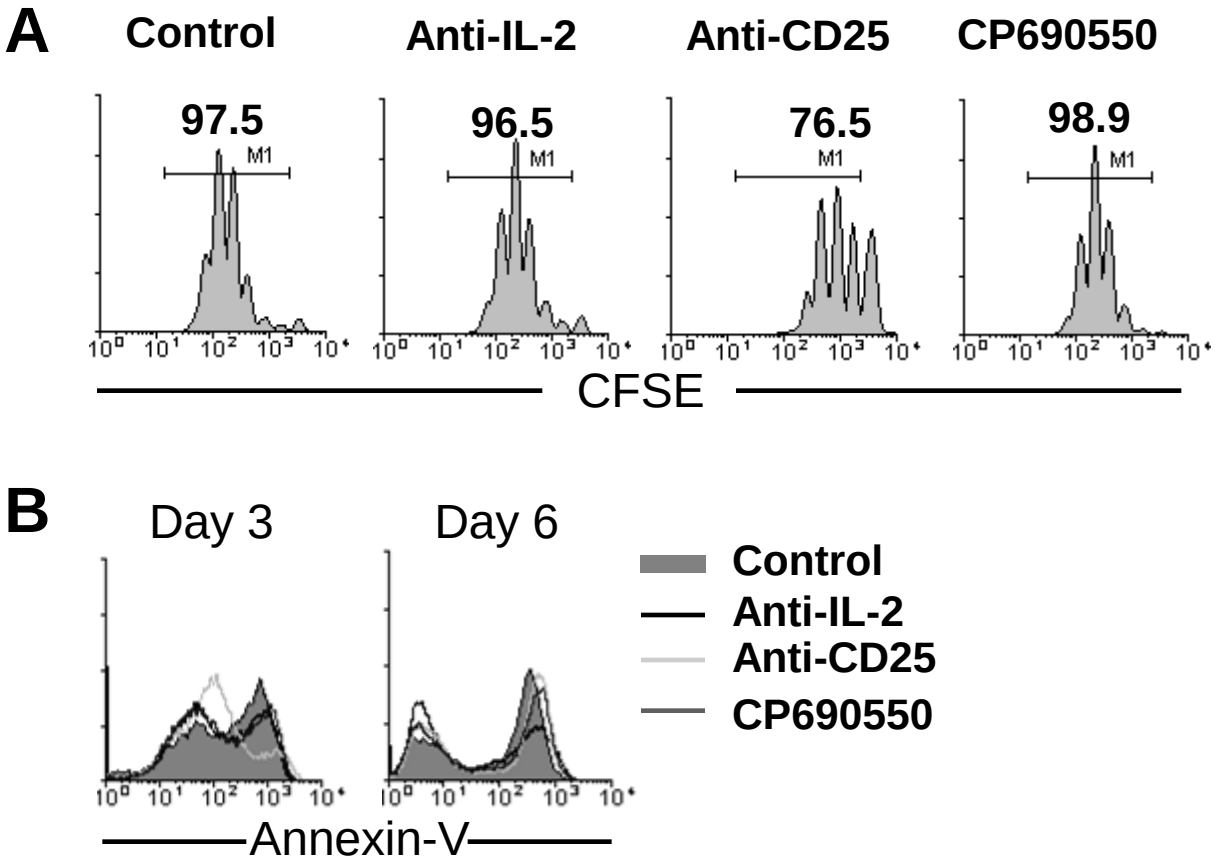

Figure S4. Charactering proliferation and survival of T cells during iTreg cell differentiation. **(A)** CFSE-labeled $CD4^+CD25^-$ T cells were activated by anti-CD3/anti-CD28 mAbs in the absence (control) or presence of 10 μg/ml anti-IL-2 mAb, 10 μg/ml anti-CD25 mAb, and 50 nM CP690550 as indicated. Histograms show the proliferation of CFSE-labeled cells in the day 3 cultures. **(B)** Purified $CD4^+CD25^-$ T cells were activated by anti-CD3/anti-CD28 mAbs in the presence of anti-IL-2 mAb, anti-CD25 mAb, and CP690550 for 3 days (the conditioning step), followed by exposure to IL-2 for additional 3 days (the Foxp3-induction step). Overlay histograms show the expression of Annexin V in cells from day 3 or day 6 cultures.