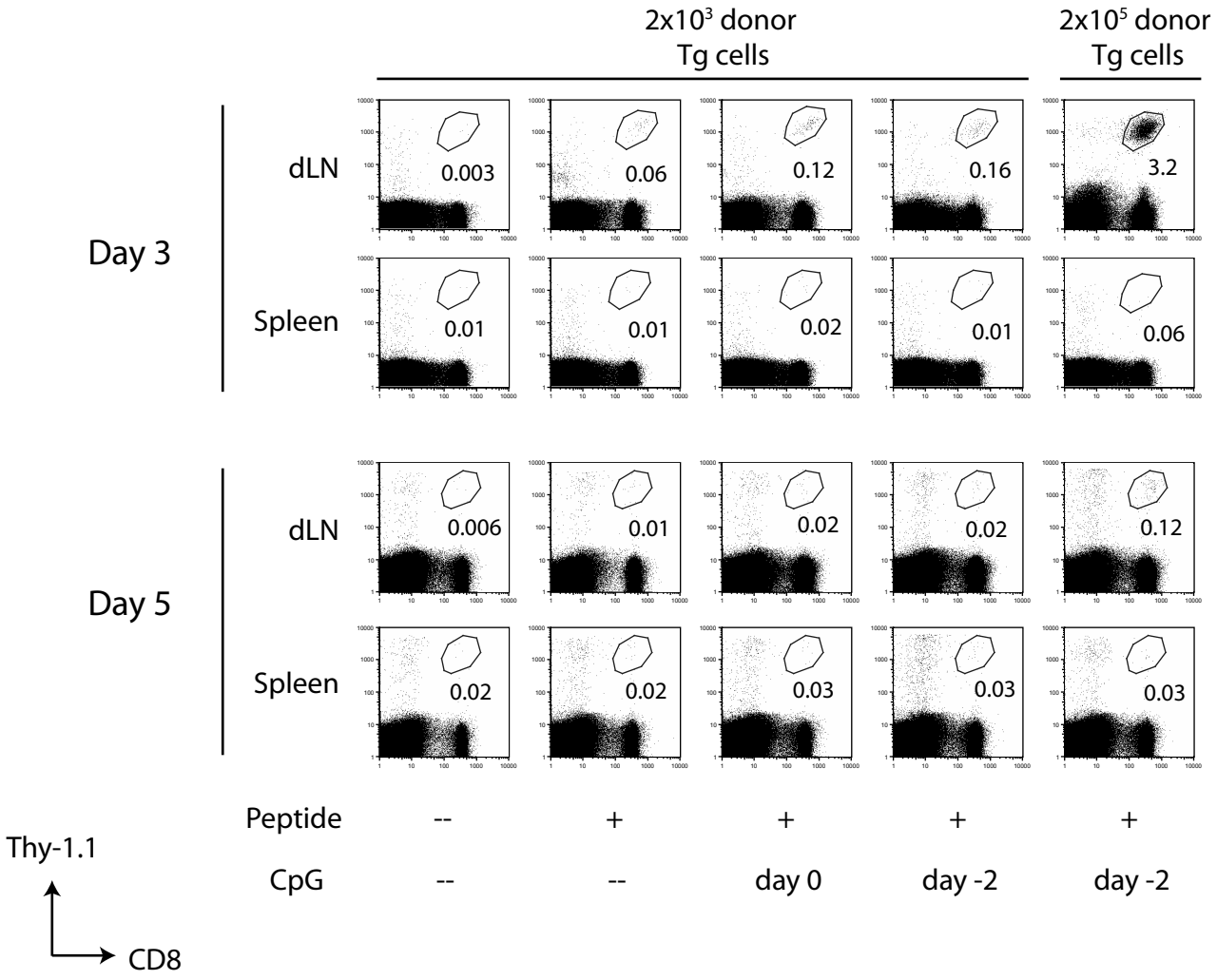
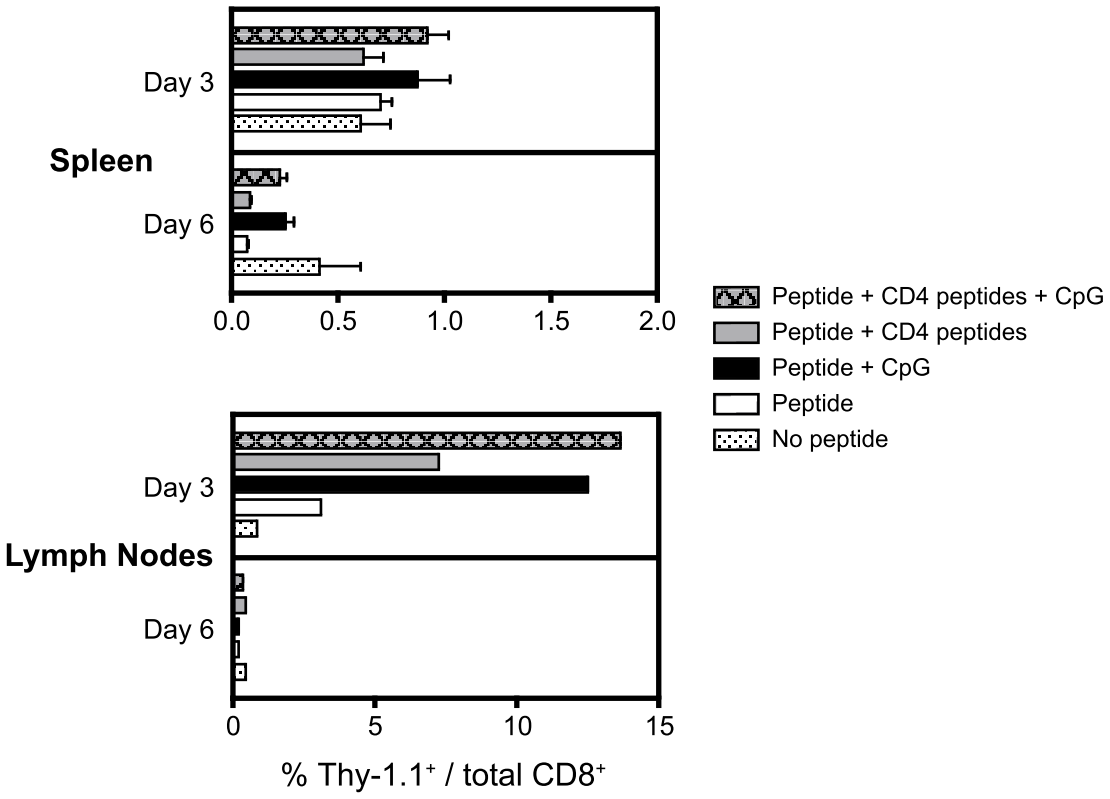
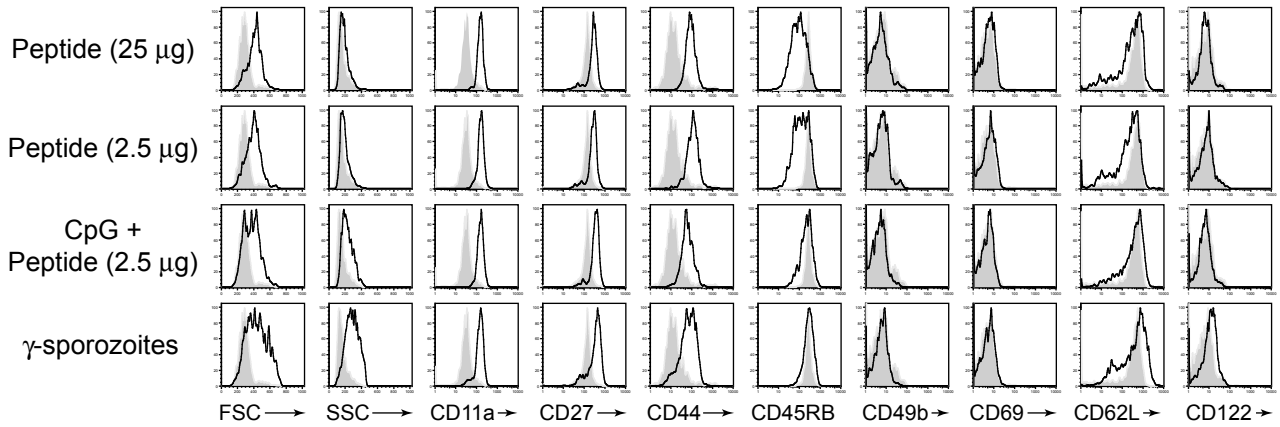


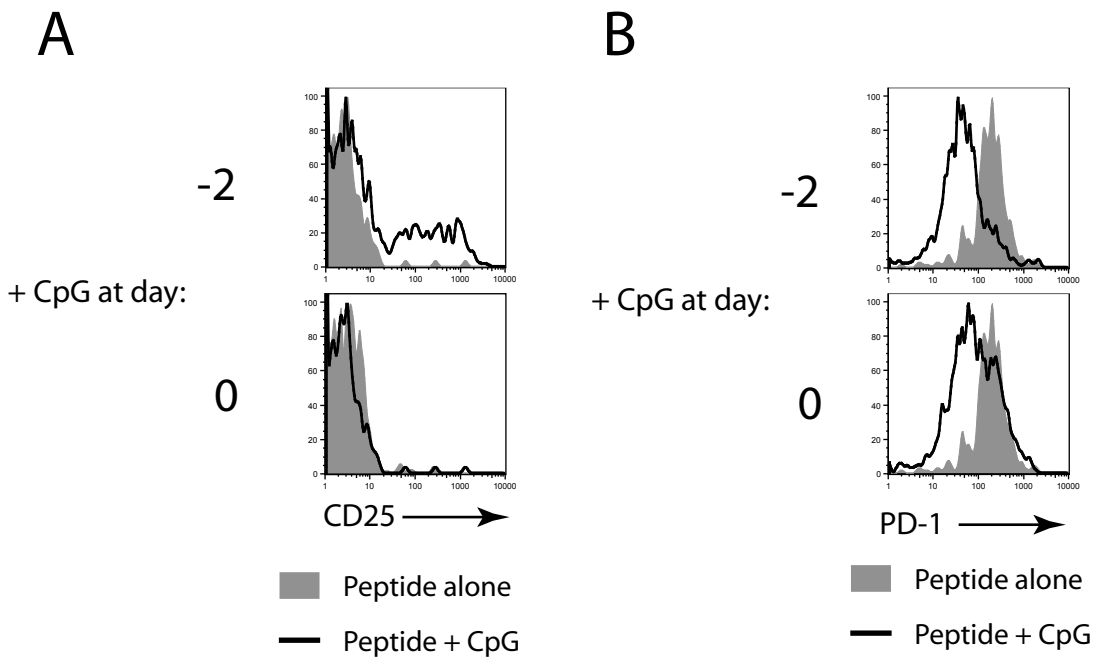
Supplementary Figure 1. Large numbers of TCR-Tg cells are required to visualize early events of T cell priming with peptide. Naïve BALB/c mice received indicated numbers of Thy-1.1$^+$ TCR-Tg cells (2×10^3 or 2×10^5) and were immunized with 2.5 µg SYVPSAEQI peptide with or without CpG, as indicated. Three days later, the frequencies of CD8$^+$Thy-1.1$^+$ cells in the lymph nodes and spleen was evaluated by FACS and are expressed as a percentage of total CD8$^+$ cells. Data plots are from a representative individual spleen sample and pooled lymph node samples of two-three mice per group per time point.



Supplementary Figure 2. MHC II-restricted peptides do not enhance the CD8$^+$ T cell response to peptide. Naïve BALB/c mice received TCR-Tg cells (5×10^5) and were immunized with 2.5 µg SYVPSAEQI peptide in combination with MHC II-restricted peptides and CpG, as indicated. For the helper peptides, 50 µg each of three unique previously-characterized peptides were added to the inoculum (P. yoelii $CS_{291\text{-}310}$ (As44), P. yoelii $CS_{59\text{-}79}$ (Py-1), and $OVA_{323\text{-}339}$). For CpG treatment, 30 µg of CpG 1826 was added. Final volumes of all immunizations were 100 µL PBS. Mice were sacrificed three or six days after immunization the frequencies of CD8$^+$Thy-1.1$^+$ cells in the lymph nodes and spleen was evaluated by FACS and are expressed as a percentage of total CD8$^+$ cells. Data are from individual spleen samples and pooled lymph node samples. Bars represent mean ± SD of three mice. Data are from one of two experiments with similar results.

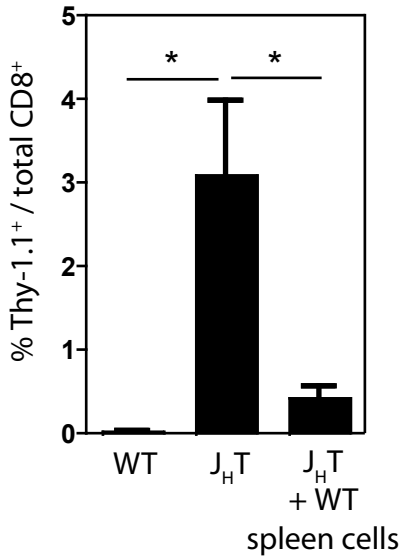


Supplementary Figure 3. CpG given two days before peptide modifies surface marker expression. Naïve BALB/c mice received TCR-Tg cells (2×10^4) and were immunized with the indicated amounts of peptide or γ-irradiated sporozoites three days later. One group of mice also received CpG two days before peptide, as indicated. Mice were sacrificed three days after peptide immunization and surface marker expression on $CD8^+Thy\text{-}1.1^+$ cells in the lymph nodes and spleen (not shown) was evaluated by FACS from peptide-immunized (solid line) and control non-immunized (shaded) mice. Data are from one of three experiments with similar results.



Supplementary Figure 4. CpG given simultaneously with peptide does not induce CD25 expression onT cells.

Naïve BALB/c mice received TCR-Tg cells (2×10^4) and were immunized with 2.5 µg of peptide three days later. Mice received CpG either two days before peptide ("-2"), the same time as peptide ("0"), or not at all ("peptide alone"), as indicated. (A) Mice were sacrificed three days after peptide immunization and surface expression of CD25 on $CD8^+Thy\text{-}1.1^+$ cells in the lymph nodes and spleen (not shown) was evaluated by FACS from peptide-immunized (shaded histogram) and CpG-treated mice (solid line). (B) Mice were sacrificed six days after peptide and expression of PD-1 was evaluated as in (B). Data are from one of two experiments with similar results.



Supplementary Figure 5. Unfractionated spleen cells from WT mice inhibit T cell responses in J_HT mice. Naïve BALB/c (WT) and two groups of B cell-deficient (J_HT) mice received Thy-1.1$^+$ TCR-Tg cells (2×10^3) and were immunized with CpG the following day and peptide two days after CpG. One group of J_HT mice also received whole WT spleen cells containing 3×10^6 B cells prior to CpG treatment. All mice were sacrificed ten days after peptide immunization and the frequency of TCR-Tg cells in the spleen was determined by FACS. Bars represent mean ± SD of three mice per group from one experiment. Student T-test: *, $p < 0.05$.