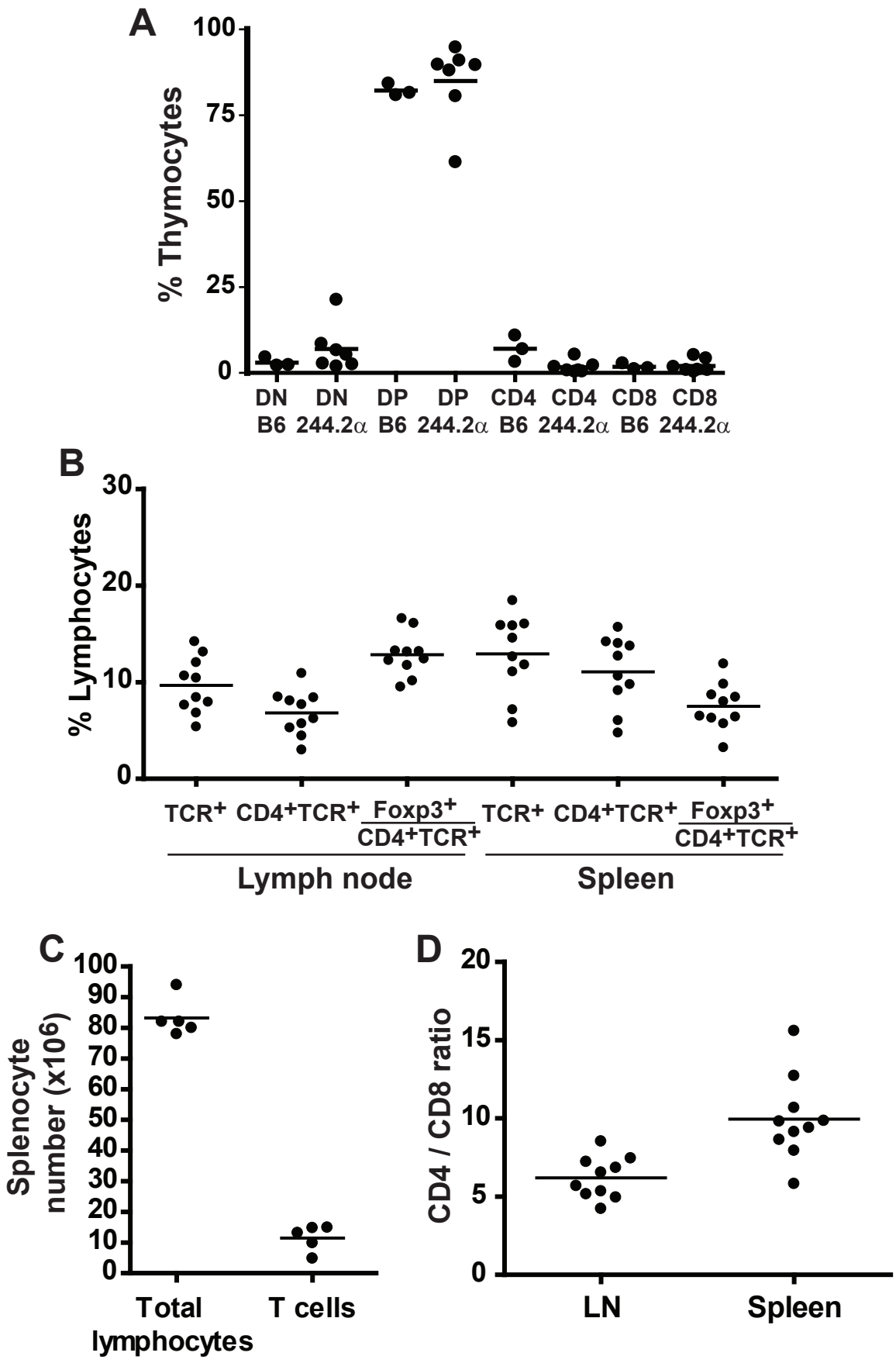
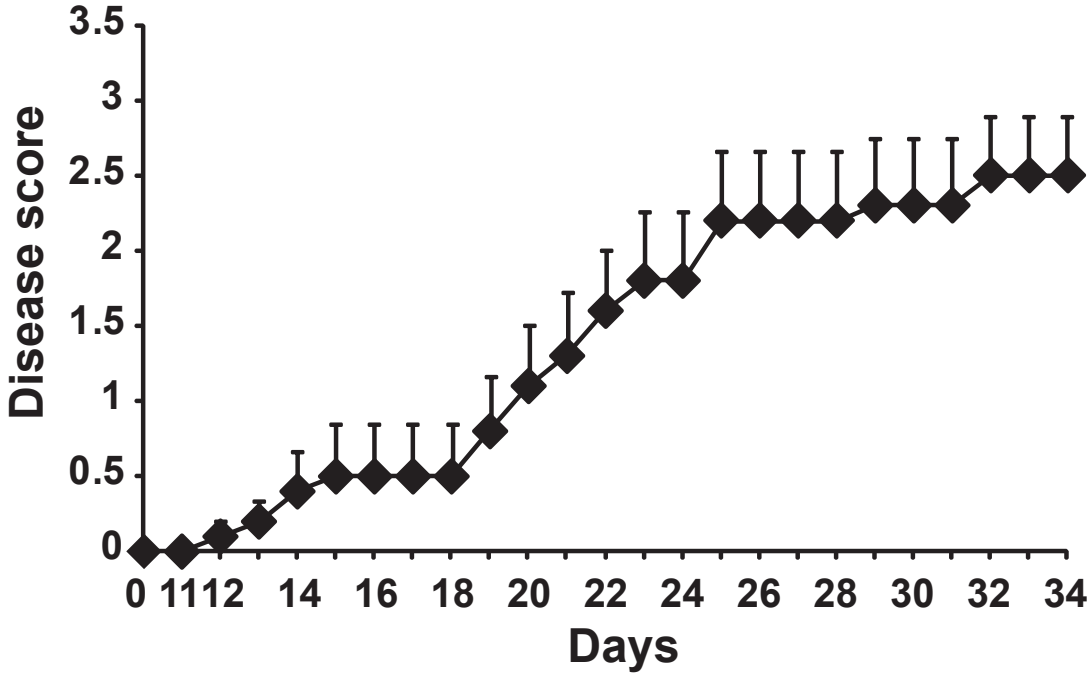
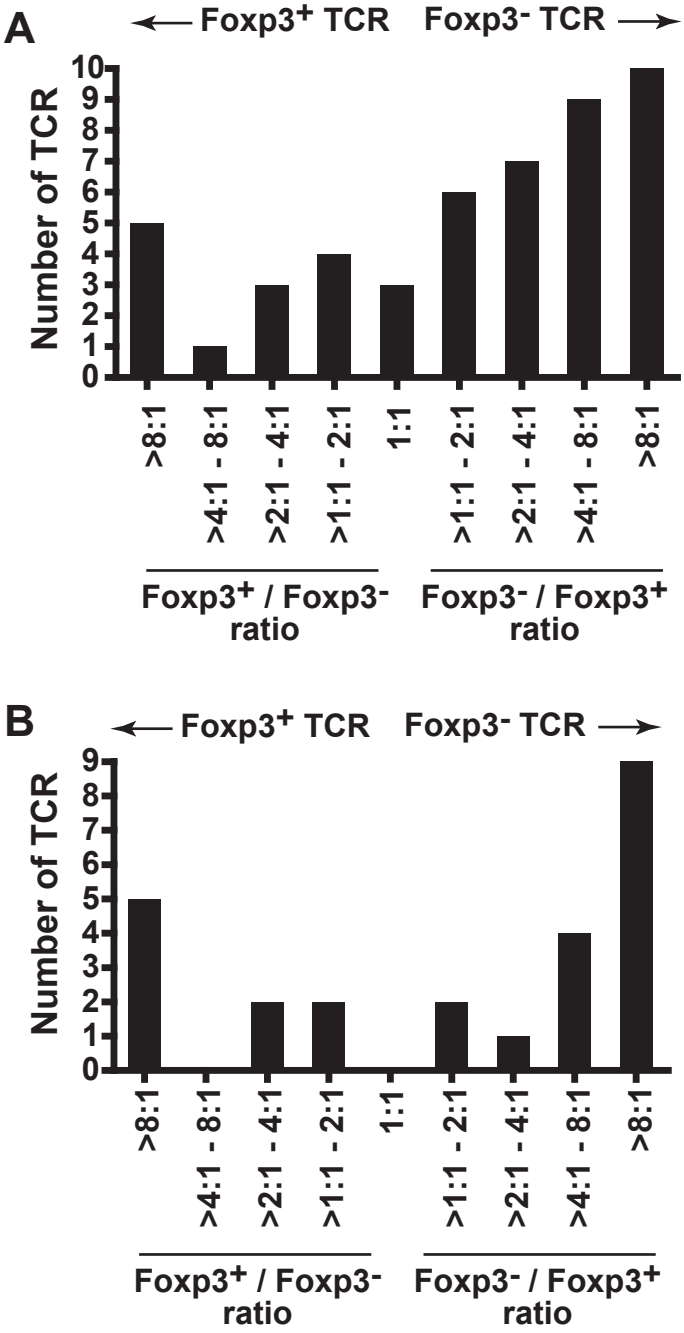


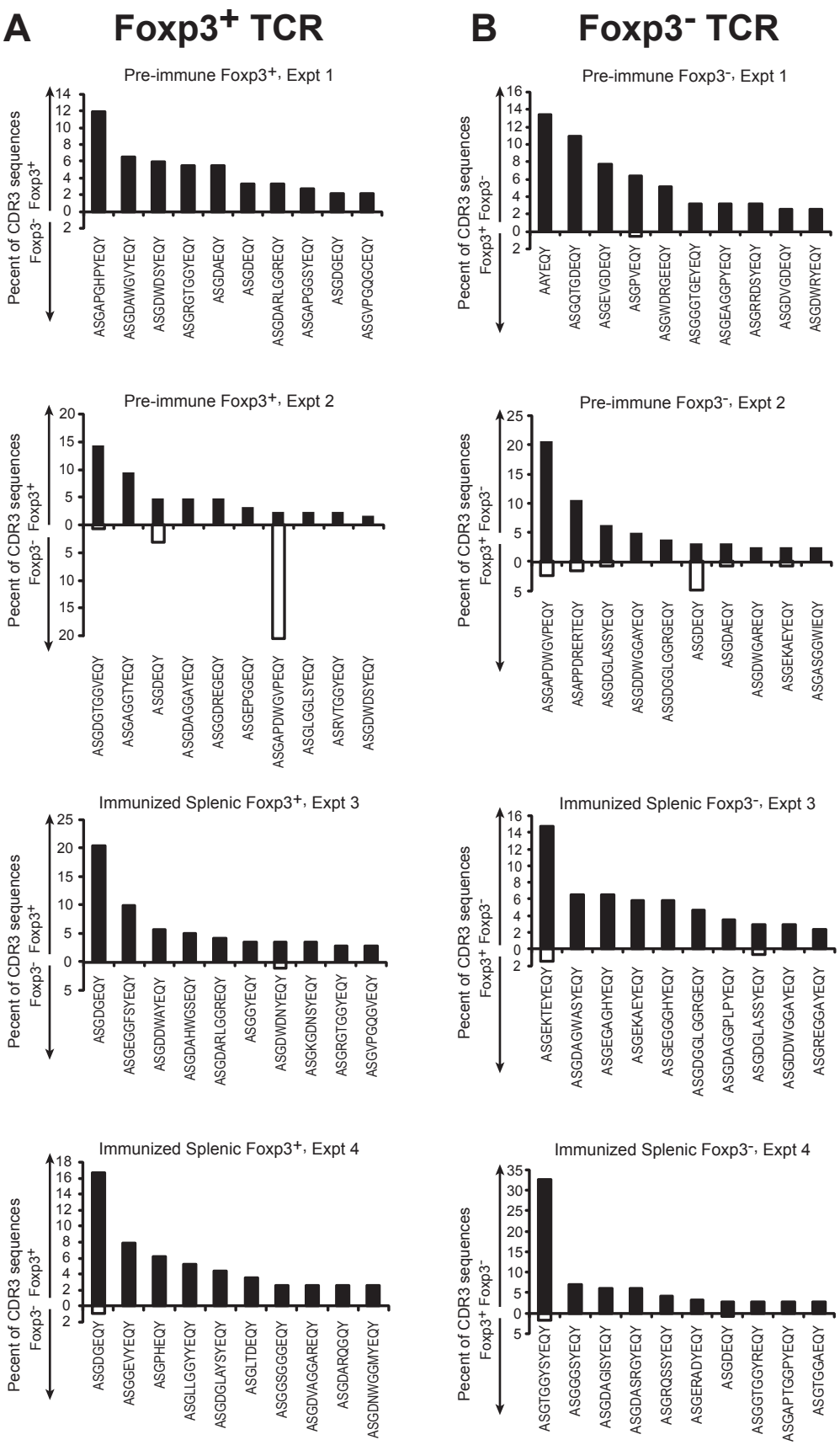
Liu, et. al. Supplemental Figure 1



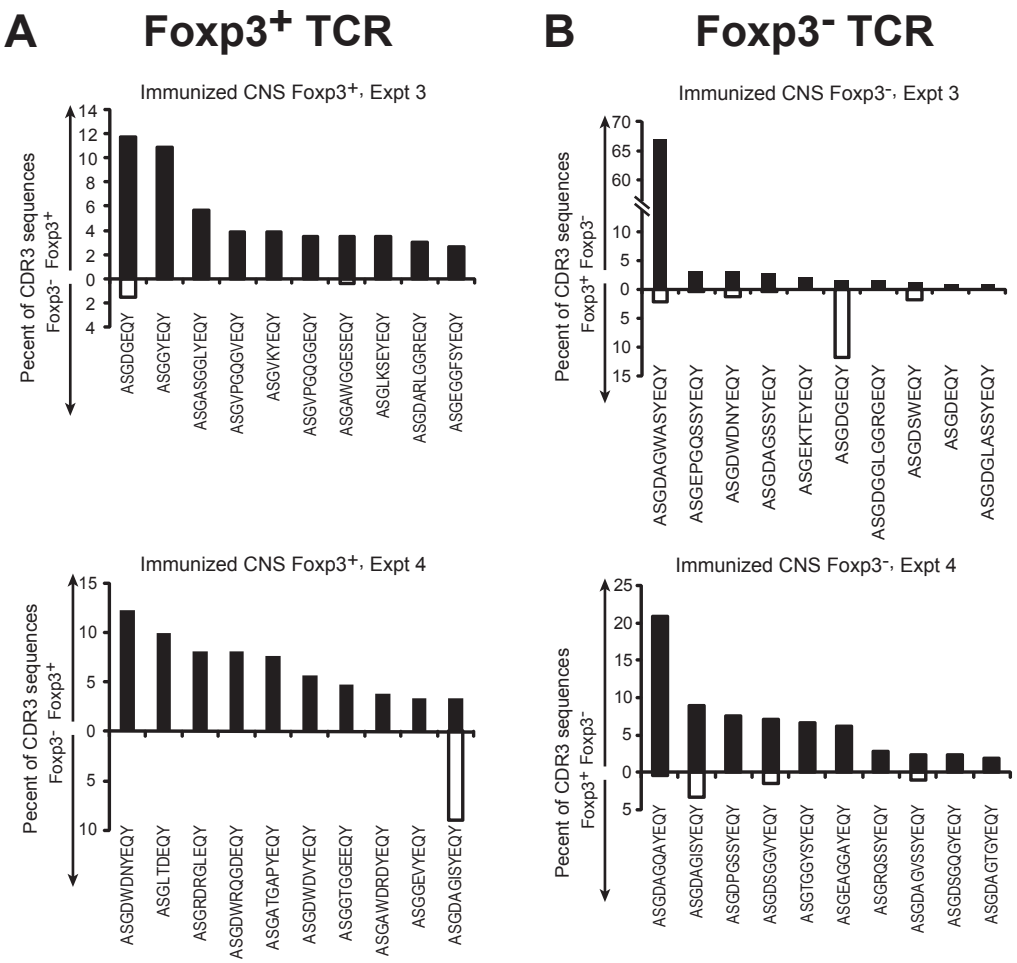
Liu, et. al. Supplemental Figure 2



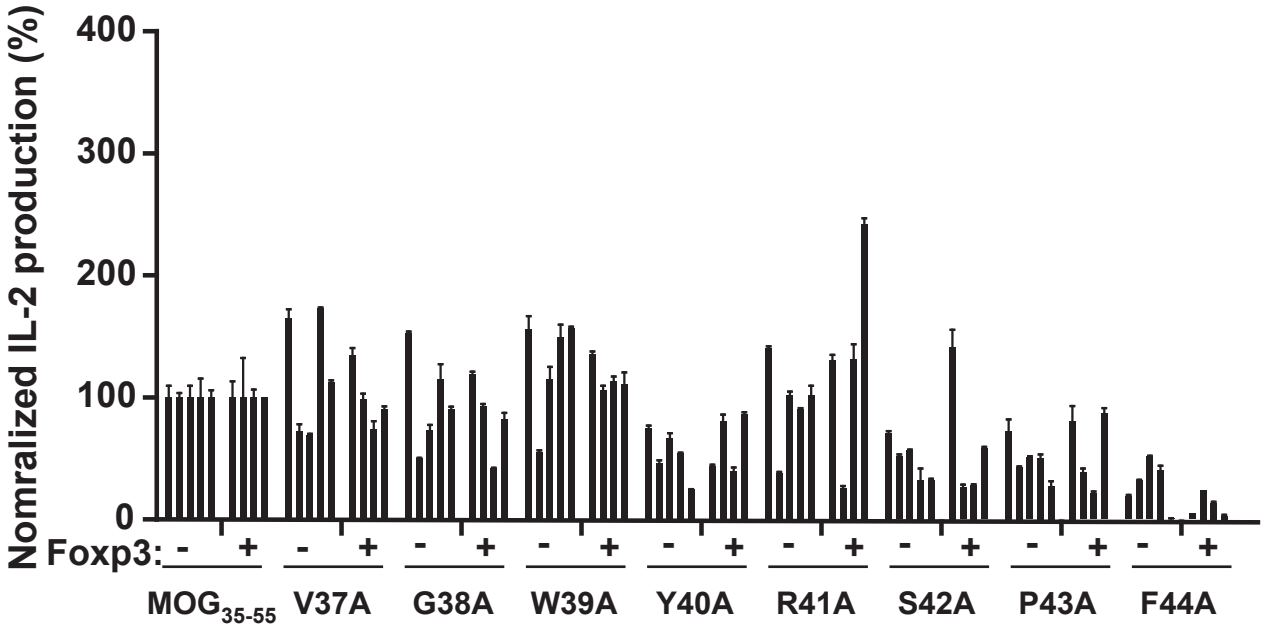
Liu, et. al. Supplemental Figure 3

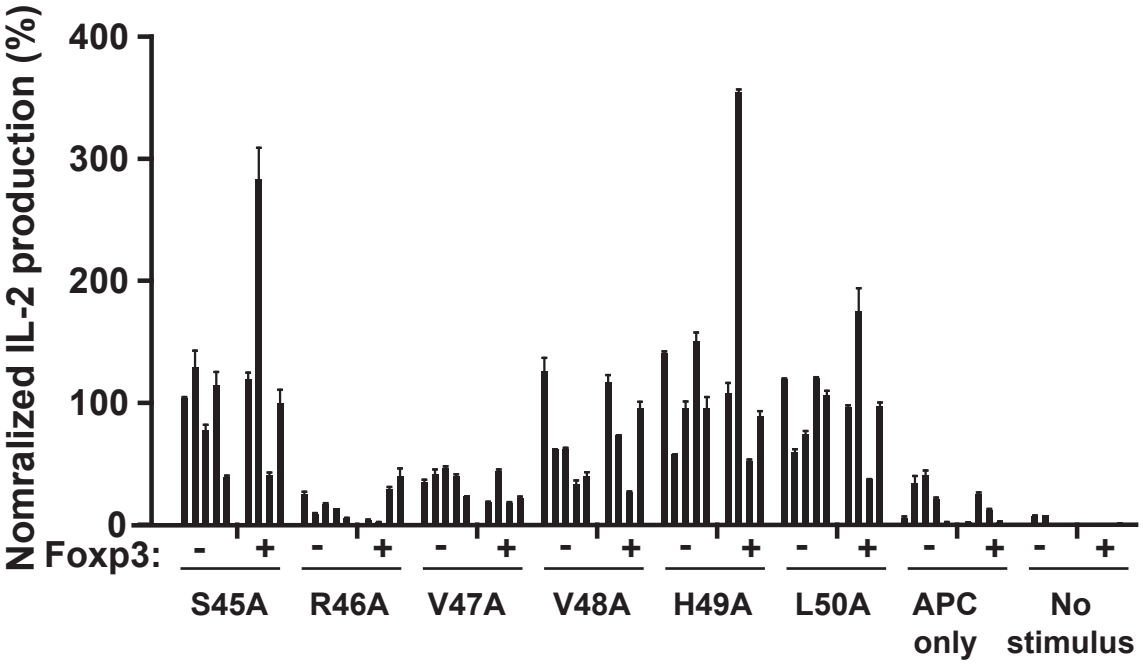


Liu, et. al. Supplemental Figure 4

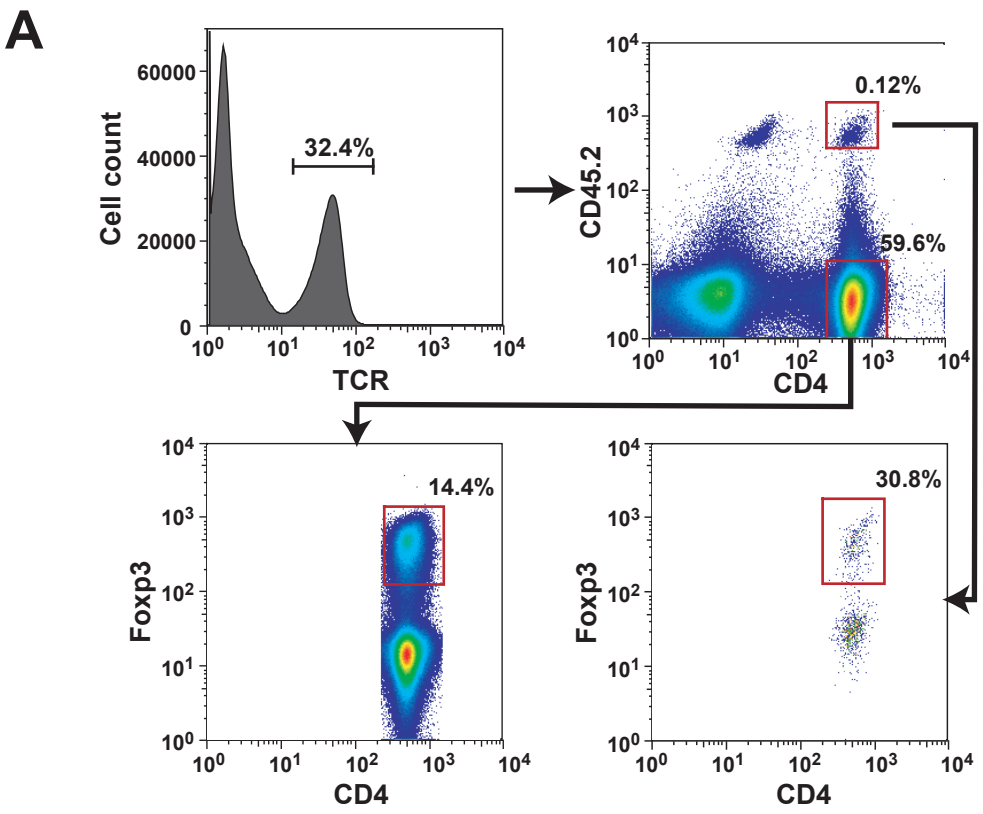


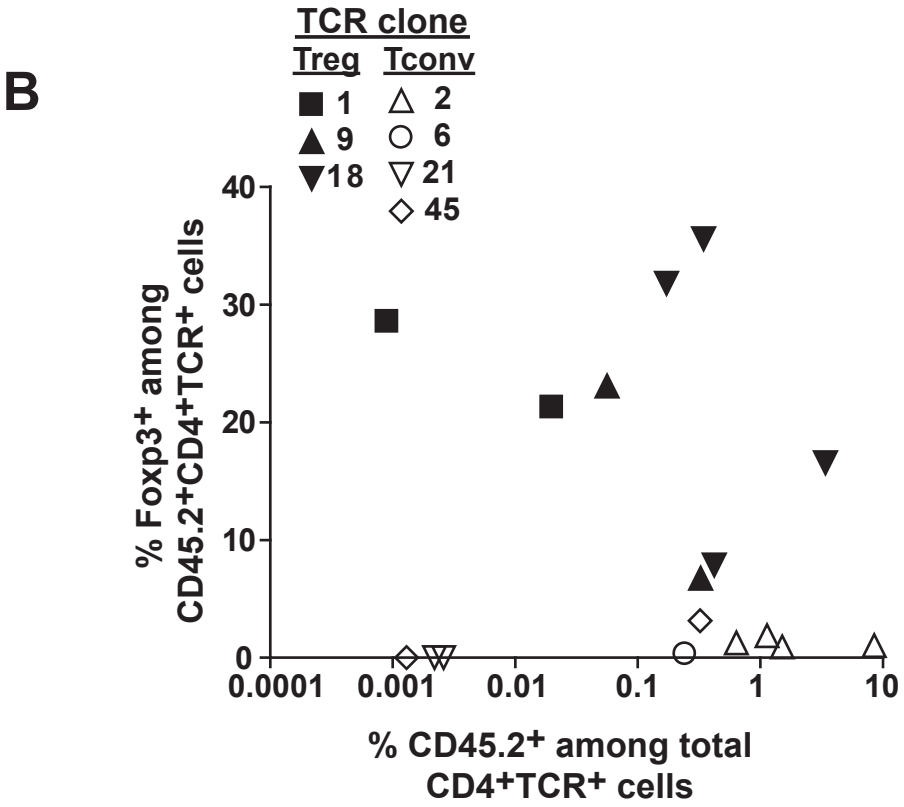
Liu, et. al. Supplemental Figure 5





Liu, et. al. Supplemental Figure 6





Liu, et. al. Supplemental Figure 7

Supplemental Figure Legends

Supplemental figure 1. Lymphoid reconstitution of retrogenic mice. 1MOG244.2α TCR retrogenic mice were sacrificed ~8 weeks after HPC transfer, and thymus, spleen and LN cells isolated and quantitatively analyzed for the indicated cell subsets. (A) Percent of DN, DP, or $CD4^+$ or $CD8^+$ SP T cells among 1MOG244.2α or simultaneously analyzed C57BL/6 thymocytes is shown. (B) Percent splenic and LN reconstitution with total T lymphocytes (TCR+) and CD4 T Lymphocytes ($CD4^+TCR^+$), and fractional engraftment with $CD4^+$ TCR^+ GFP-$Foxp3^+$ T lymphocytes ($Foxp3^+/CD4^+TCR^+$) are plotted for individual mice. Means are indicated by the horizontal bar. The remaining lymphocytes were composed predominantly of B cells. (C) Absolute number of total splenic lymphocytes and TCR^+ lymphocytes is plotted. (D) The ratio of $CD4^+$ / $CD8^+$ T lymphocytes is plotted.

Supplemental figure 2. EAE induction in 1MOG244.2α TCR retrogenic mice. 1MOG244.2α mice (n=10) were immunized to induce EAE and clinically monitored. Ten of 10 mice developed disease symptoms with a mean±1 s.d. maximal score of 2.6±1.2 (range 1-4). Plot shows mean clinical score + 1 s.e.m.

Supplemental figure 3. Distribution of shared CDR3 sequences. Numbers of CDR3 amino acid sequences identified in both $Foxp3^+$ and $Foxp3^-$ populations at the indicated ratio is shown. Left sides of plots indicate sequences that were primarily identified among $Foxp3^+$ cells, and right sides indicate sequences primarily identified among $Foxp3^-$ cells. A 1:1 ratio indicates equal numbers of sequences were identified among $Foxp3^+$ and

Foxp3$^-$ populations. (A) Data is plotted for all sequences identified (see Table I). (B) Data is plotted only for those sequences identified 10 or more times.

Supplemental figure 4. Frequency representation of the most common splenic Vβ8.2$^+$Jβ2.7$^+$ CDR3 isolates. Frequencies of the 10 most commonly isolated splenic CDR3 sequences from GFP-Foxp3$^+$ (A) or GFP-Foxp3$^-$ (B) T cells in each of the 4 experiments is plotted (filled bars). CDR3 frequencies are shown as percent values and were defined as 100*(Foxp3$^+$ or Foxp3$^-$ CDR3 isolates with the indicated sequence) / (total number of Foxp3$^+$ or Foxp3$^-$ splenic CDR3 sequences for the indicated experiment). Experiments 1 and 2 assayed pre-immune mice, and 3 and 4 studied mice with EAE. For sequences shared among Foxp3$^+$ and Foxp3$^-$ populations, below each filled bar the representation of the identical sequence in the alternative population is plotted, Foxp3$^-$ cells in (A) and Foxp3$^+$ cells in (B) (unfilled bars).

Supplemental figure 5. Frequency representation of the most common CNS-derived Vβ8.2$^+$Jβ2.7$^+$ CDR3 isolates. Analyses were performed as in Supp. fig. 4, but for CNS-derived sequences from experiments 3 and 4.

Supplemental figure 6. Fine specificity of Foxp3$^+$ and Foxp3$^-$ TCR. 4G4.CD4 cell lines expressing Foxp3$^-$ or Foxp3$^+$ T cell derived TCRs were stimulated with irradiated TCR C$\alpha^{-/-}$ APCs in the absence of antigen or in the presence of 100 μg/ml MOG_{35-55} peptide or the indicated alanine-substituted mutants of this peptide. IL-2 was measured in cell free

supernatant at 24 h as an indicator of stimulation. Percent IL-2 production compared with MOG_{35-55} stimulation is plotted. Bars indicate from left to right IL-2 production by clones 6, 21, 29, 45, 244.2, 1, 16, 18, and 35 after stimulation with the indicated peptide.

Supplemental figure 7. Foxp3 expression among $CD4^+$ T cells in TCR-retrogenic mixed chimeras. To evaluate the lineage preference of MOG-specific T_{reg} or T_{conv} TCR identified from 1MOG244.2α mice in mice with a wild type thymus and few transgenic precursor cells, we produced mixed chimeras using select cloned TCR. Retroviral constructs incorporating the TCR$\alpha\beta$ from MOG-specific T_{reg}-derived TCR clones 1, 9, and 18, and T_{conv} derived clones 2, 6, 21, and 45 were transduced into B6.129S7-*Rag1*^{tm1Mom}/J HPC. The Rag1 deficiency ensures that all T cells developing from the transduced population express the TCR transgene. An ~1-5% transduction efficiency was obtained. The HPC were mixed at a 1:1 or 1:10 ratio with untransduced, wild type, T-cell depleted CD45.1 congenic B6.SJL-*Ptprc*^{a} *Pep3*^{b}/BoyJ-derived bone marrow, and transplanted into B6.SJL-*Ptprc*^{a} *Pep3*^{b}/BoyJ mice. At 6-8 weeks after transplant mice were sacrificed, and splenocytes stained with CD45.2, TCR, CD4, and intracytoplasmic Foxp3 antibodies. (A) Gating strategy to identify cell populations is shown. Forward and side scatter gated lymphocytes were gated based on TCR positivity. $CD4^+CD45.2^+$ (transduced) or $CD4^+CD45.2^-$ (wild type) T cell populations were then gated from this population and the percent of $Foxp3^+$ cells determined based on intracytoplasmic staining. (B) Engraftment and percent Foxp3 positivity among TCR-transduced $CD45.2^+$ T cells is indicated. Percent engraftment of $CD4^+CD45.2^+$ T cells was calculated as 100 x (number of $CD45.2^+CD4^+TCR^+$

cells identified) / (total number of $CD4^+TCR^+$ cells). Percent $Foxp3^+$ T cells is calculated as 100 x (number of $CD45.2^+CD4^+TCR^+Foxp3^+$ cells) / (total number of $CD45.2^+CD4^+TCR^+$ cells).