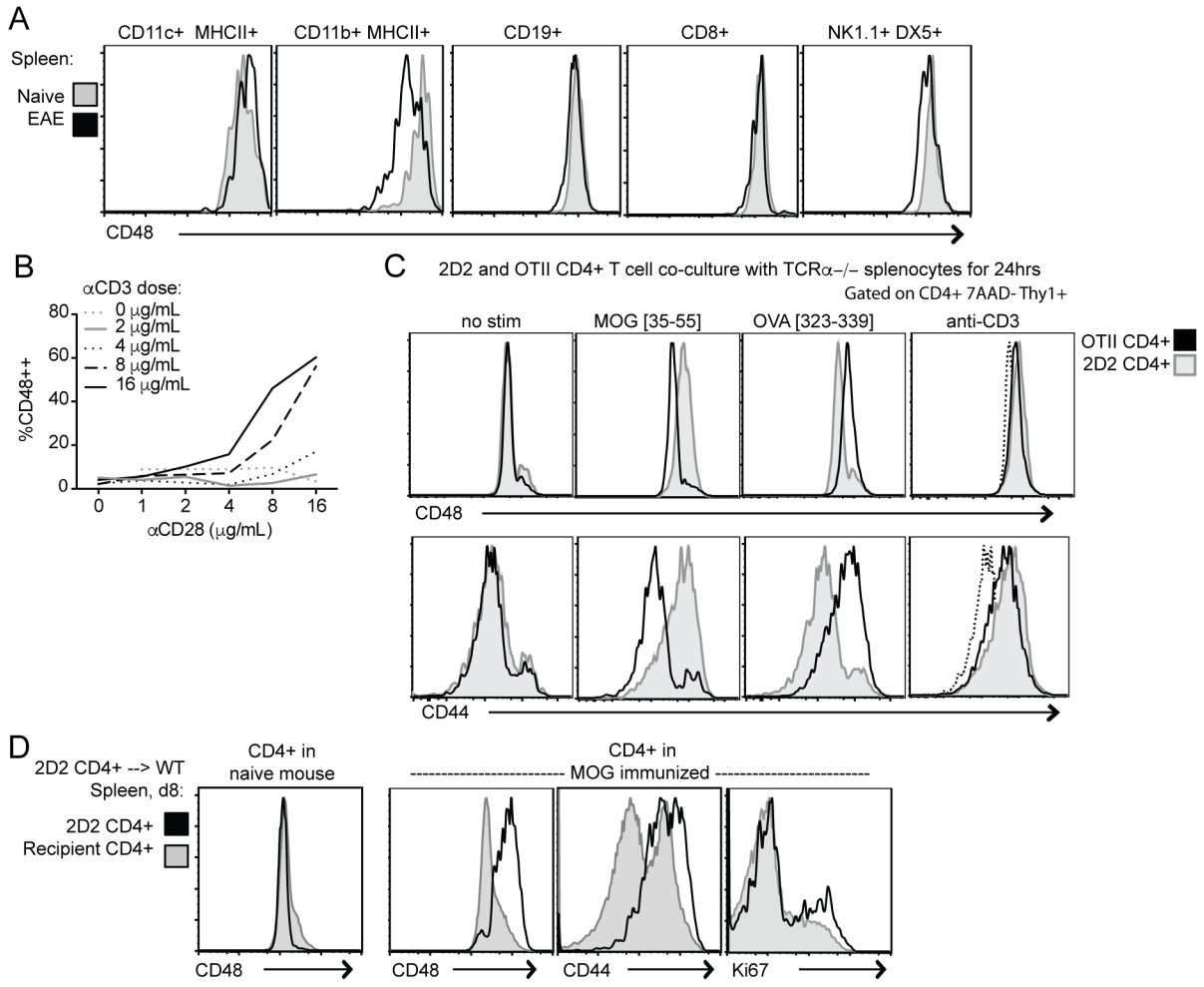
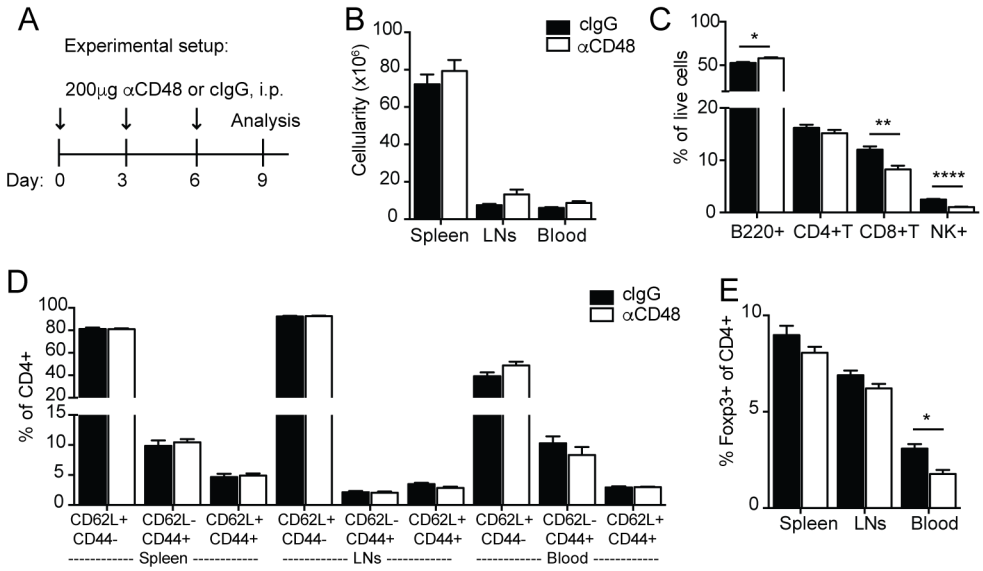
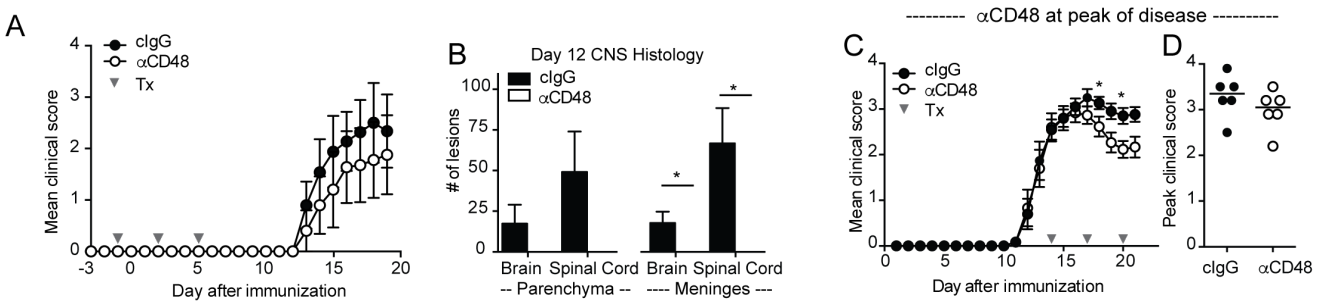


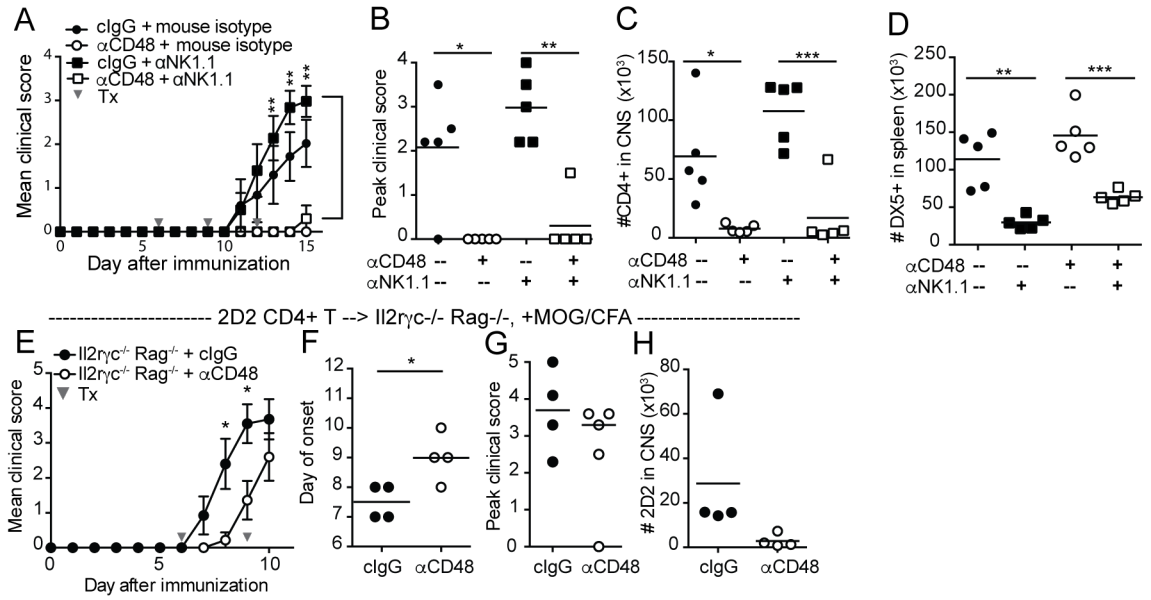
Supplemental Figure 1. CD48 upregulation on CD4+ T cells is dose-dependent and antigen-specific. A. Representative CD48 expression in the spleen of a naïve mouse and a mouse at the peak of EAE. **B.** Naïve CD62L+CD44- CD4+ T cells were sorted from a WT mouse, and stimulated in vitro on anti-CD3/anti-CD28-coated plates for 48hrs. Level of CD48 expression was evaluated by flow cytometry. **C**. MACS-purified CD4+ T cells from 2D2 Thy1.1 and OTII Thy1.1/1.2 TCR Tg mice were cultured with TCRα-/- splenocytes and without peptide, indicated peptide, or anti-CD3. CD48 and CD44 expression were assessed 24 hours later. Dotted black line in far right panels indicates no stimulation condition. **D.** 2D2 CD4+ T cells (Thy1.1) were transferred to WT Thy1.2 recipients, followed by immunization with MOG_{35-55}/CFA. Left, representative CD48 staining on recipient (Thy1.1-) and 2D2 (Thy1.1+) CD4+ T cells from an unimmunized mouse. Right, representative CD48, CD44 and Ki67 expression on recipient and 2D2 CD4+ T cells from spleens on day 8 after immunization. Representative of 3 independent experiments with 4-6 mice per group (A), 2 independent experiments (B,C), and 5 independent experiments with 3-7 mice (D).



Supplemental Figure 2. Anti-CD48 treatment alters lymphocyte distribution in naïve mice. WT mice were treated with anti-CD48 or control IgG (cIgG) for 9 days. **A.** Experimental schematic. **B.** Numbers of live cells in spleen, LNs, and blood. **C.** Percentages of B220+, CD3+CD4+, CD3+CD8+ and NK1.1+ cells in the spleen. **D**. Proportions of naïve (CD62L+CD44-), activated (CD62L-CD44+) and memory (CD62L+CD44+) cells among CD4+CD3+ T cells. **E.** Percentages of Foxp3+ among CD4+CD3+ T cells. Representative of 4 independent experiments with 3-5 mice per group.



Supplemental Figure 3. The effects of anti-CD48 mAb depend on timing of administration during EAE. Mice were immunized with MOG_{35-55}/CFA to induce EAE. **A**. Anti-CD48 or cIgG was given 1 day before, and 2 and 5 days after immunization. Mean clinical scores, ±SEM. **B**. Anti-CD48 or cIgG was given on days 6 and 9 after immunization, and brain and spinal cord were collected on day 12 for histological analysis as in Figure 2. 4/5 of cIgG mice showed clinical disease, median score of 0.5. 0/5 of anti-CD48 mice showed clinical disease. Data are mean ±SEM. **C-D**. Anti-CD48 or cIgG was given on days 14, 17 and 20 after immunization, when all mice showed clinical disease. Treatment groups were assigned on day 14, such that the average score in each group was equivalent. **C**. Mean clinical scores, ±SEM. **D**. Peak clinical scores. Representative of 2 independent experiments with 5-6 mice per group (A, C, D), and 5 mice per group (B). Gray arrows indicate antibody treatment (Tx). Dots in D represent individual mice.



Supplemental Figure 4. NK cells are not required for anti-CD48 mediated attenuation of EAE. A-D WT mice were immunized with MOG_{35-55}/CFA to induce EAE. Anti-NK1.1 mAb or mouse isotype control was given one day before immunization and every 5 days thereafter to deplete NK cells. Anti-CD48 or cIgG was given on days 6, 9 and 12 after immunization. Spleen and CNS were collected on day 15 for analysis by flow cytometry **A**. Mean clinical scores, ±SEM. Asterisks indicate statistical significance in comparison of anti-NK1.1 + cIg vs. anti-NK1.1 + anti-CD48 by Mann-Whitney test. **B**. Peak clinical scores; bar represents median. **C**. Number of CD4+ T cells in the CNS. **D**. Number of DX5+ NK cells in the spleen. **E-H.** 2D2 CD4+ T cells were transferred into Il2rγc-/-Rag2-/- mice, recipients were rested for 2 weeks then immunized with MOG_{35-55}/CFA. Pertussis toxin was given on d0 only. Anti-CD48 or cIgG was given on days 6 and 9 after immunization. **E**. Mean clinical scores, ±SEM. **F**. Day of EAE onset, for mice that developed EAE. **G**. Peak clinical scores for all mice; bar represents median. **H**. Number of CD4+ T cells recovered from the CNS of mice on day 10 after immunization. Representative of 4 independent experiments with 5 mice per group (A-D), and 3 independent experiments with 4-5 mice per group (E-H). Error bars represent SEM. Gray arrows indicate anti-CD48 treatments (Tx). Dots in B, C, D, F, G and H represent individual mice.