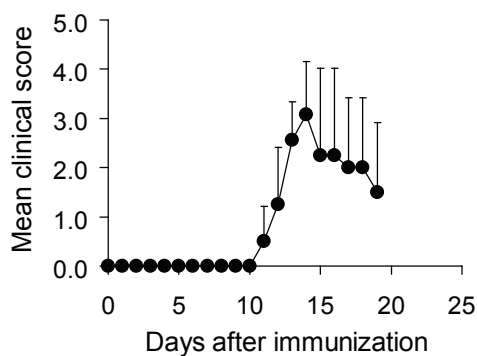
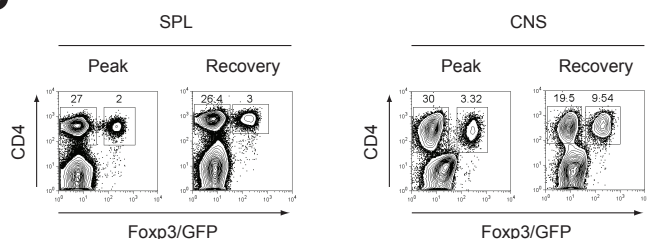


Supplementary Figure 4

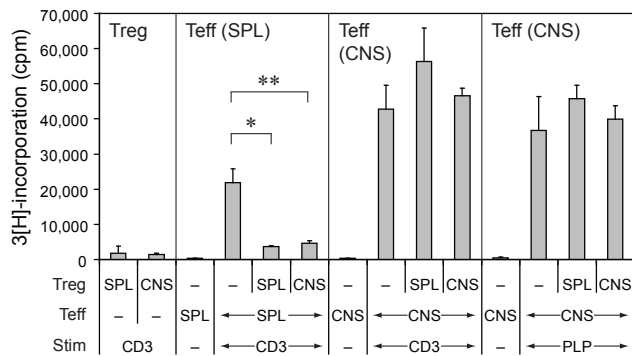
a



b

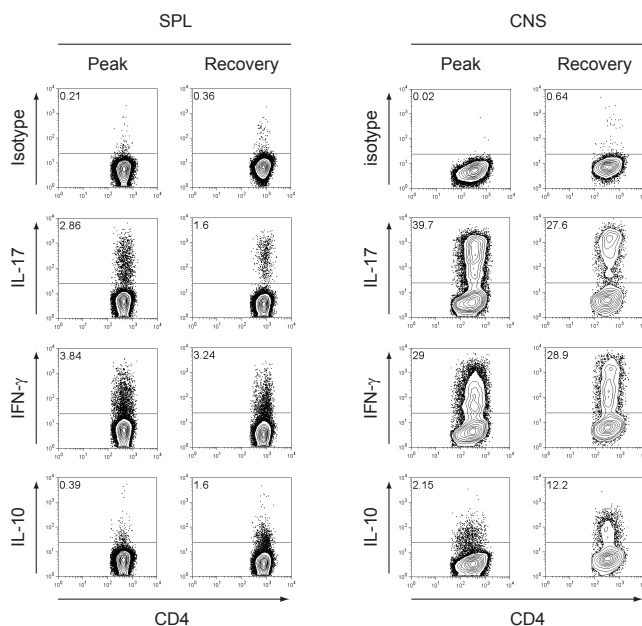


e



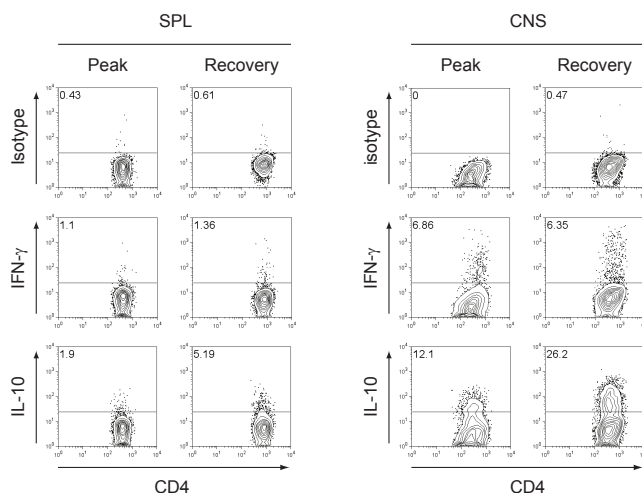
c

T-eff (CD4⁺Fopx3/GFP⁻) cytokines:



d

T-reg (CD4⁺Fopx3/GFP⁺) cytokines:



Supplementary Figure 4. T-eff and T-reg dynamics and function during the first disease episode in PLP₁₃₉₋₁₅₁ induced EAE. **(a)** SJL mice were crossed with *Foxp3gfp.KI*-mice and back-crossed onto the SJL genetic background for at least four generations (SJL *Foxp3gfp.KI*). In these mice, the expression of Foxp3 was linked to the expression of GFP as described for the original *Foxp3gfp.KI*-mice. EAE was induced by immunization with PLP₁₃₉₋₁₅₁/CFA and the disease course was followed for 20 days ($n=9$, +SD). **(b)** Splenocytes and CNS mononuclear cells were isolated from female SJL *Foxp3gfp.KI*-mice at the peak of disease (d14) and during recovery (d19) and stained for CD4. The percentages of CD4⁺Fopx3/GFP⁻ T-cells (T-eff) and CD4⁺Fopx3/GFP⁺ T-cells (T-reg) within the mononuclear cell populations of the spleen and the CNS are shown. **(c, d)** Mononuclear cells were prepared from SJL *Foxp3gfp.KI*-mice at the peak of disease and during recovery, stimulated *ex vivo* with PMA/ionomycin and stained for CD4 and intracellular cytokines. **(c)** Cytokine expression (percentages) in the T-eff gate (CD4⁺Fopx3/GFP⁻). **(d)** Cytokine expression (percentages) in the T-reg gate (CD4⁺Fopx3/GFP⁺). **(e)** T-eff (CD4⁺Fopx3/GFP⁻) and T-reg (CD4⁺Fopx3/GFP⁺) were isolated by FACS-sorting from the spleen and the CNS of SJL *Foxp3gfp.KI*-mice at the peak of disease (d14). Splenic T-eff and CNS-T-eff were compared for their susceptibility to suppression by spleen-derived or CNS-derived T-reg in anti-CD3-driven and PLP₁₃₉₋₁₅₁-specific proliferation assays. Mean 3[H]-thymidine-incorporation of triplicate cultures (+SD). * $P<5 \times 10^{-5}$, ** $P<3 \times 10^{-5}$, t-test.