Figure S1

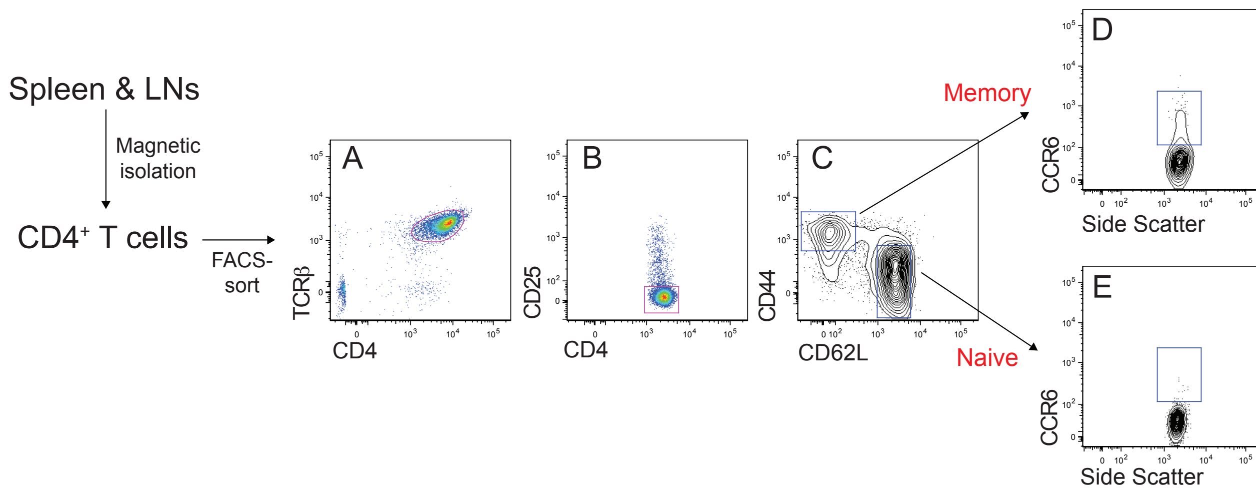

Figure S1. Sorting of endogenous CD4^{+} T cell subsets by FACS. Total CD4^{+} T cells magnetically isolated from pooled spleen/peripheral lymph node mononuclear cells of mice were FACS-sorted. Briefly, TCRβ^{+}CD4^{+} T cells were gated **(A)**, followed by gating out CD25^{hi} regulatory T cells **(B)**. Naïve (CD44^{lo}CD62L^{hi}) or memory (CD44^{hi}CD62L^{lo}) T cells were then gated as shown in **(C)**, and both subsets were analyzed for/sorted on CCR6 expression **(D, E)**.

Figure S2

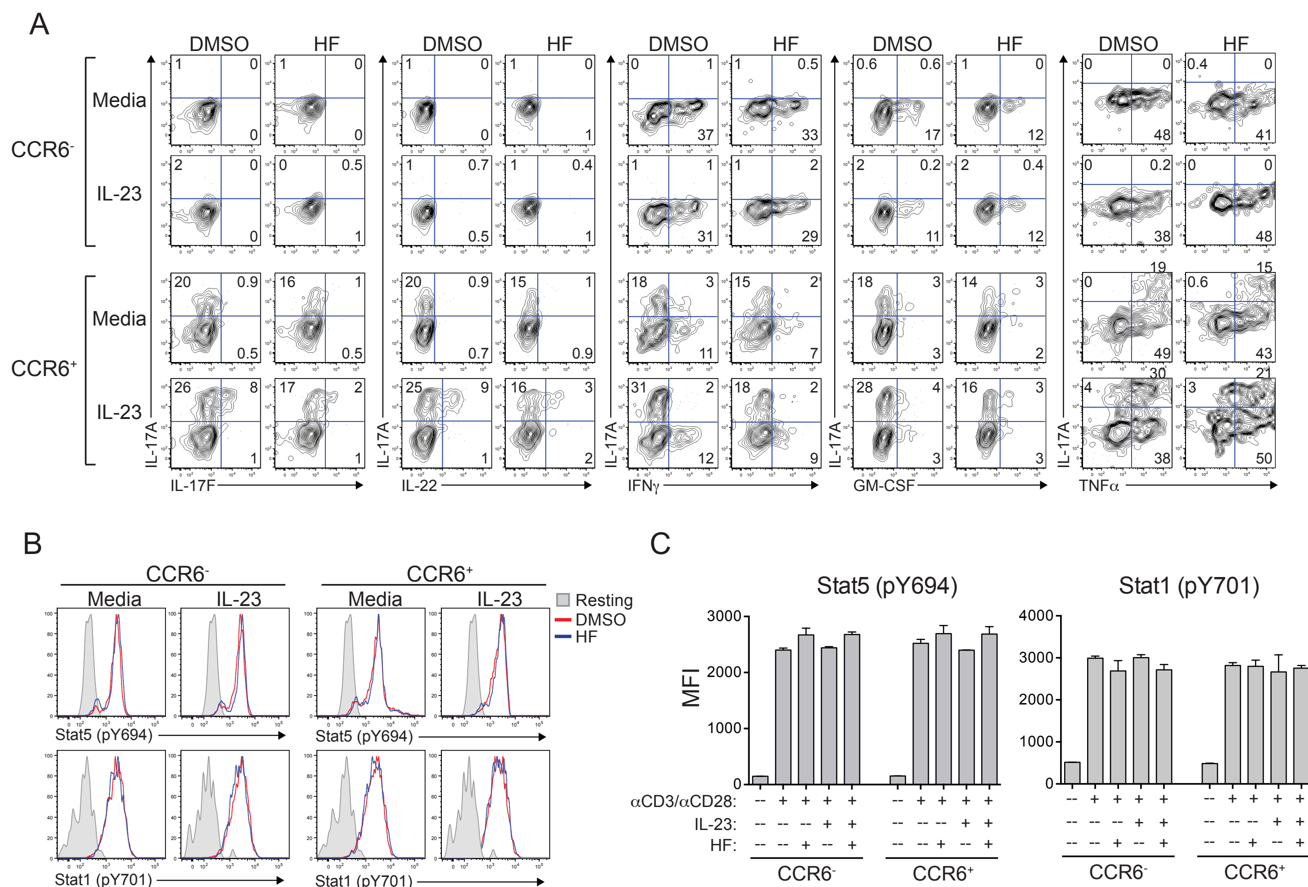

Figure S2. HF selectively regulates IL-23-dependent cytokine expression in endogenous memory T cells. (A) Cytokine expression in FACS-sorted and CD3/CD28-stimulated $CCR6^-$ or $CCR6^+$ memory T cells cultured for 48 hours +/- IL-23 in the presence of DMSO or HF (20 nM). Cells were washed and restimulated with PMA and ionomycin prior to intracellular staining. Data represent 5 independent experiments, each using cells pooled and sorted from 10-20 mice. TNFα data represent 3 experiments. (B) Endogenous $CCR6^-$ or $CCR6^+$ $CD4^+CD25^-CD44^{hi}CD62L^{lo}$ effector/memory T cells were FACS-sorted, left unstimulated or stimulated with anti-CD3/anti-CD28, and stimulated cells were cultured for 48 hours +/- IL-23. These cultures were further treated with vehicle (DMSO) or 10 nM HF, and phosphorylation of Stat5 (Y694) and Stat1 (Y701) was determined by phospho-specific intracellular staining and FACS analysis. The overlayed histograms are representative of 3 independent experiments. (C) Combined analysis of Stat5 (*Left*) and Stat1 (*Right*) phosphorylation in endogenous $CCR6^-$ or $CCR6^+$ memory T cells determined as in (B). Data are presented as mean Stat5 or Stat1 mean fluorescence intensity (MFI) $\pm$ SD from 3 experiments.

Figure S3

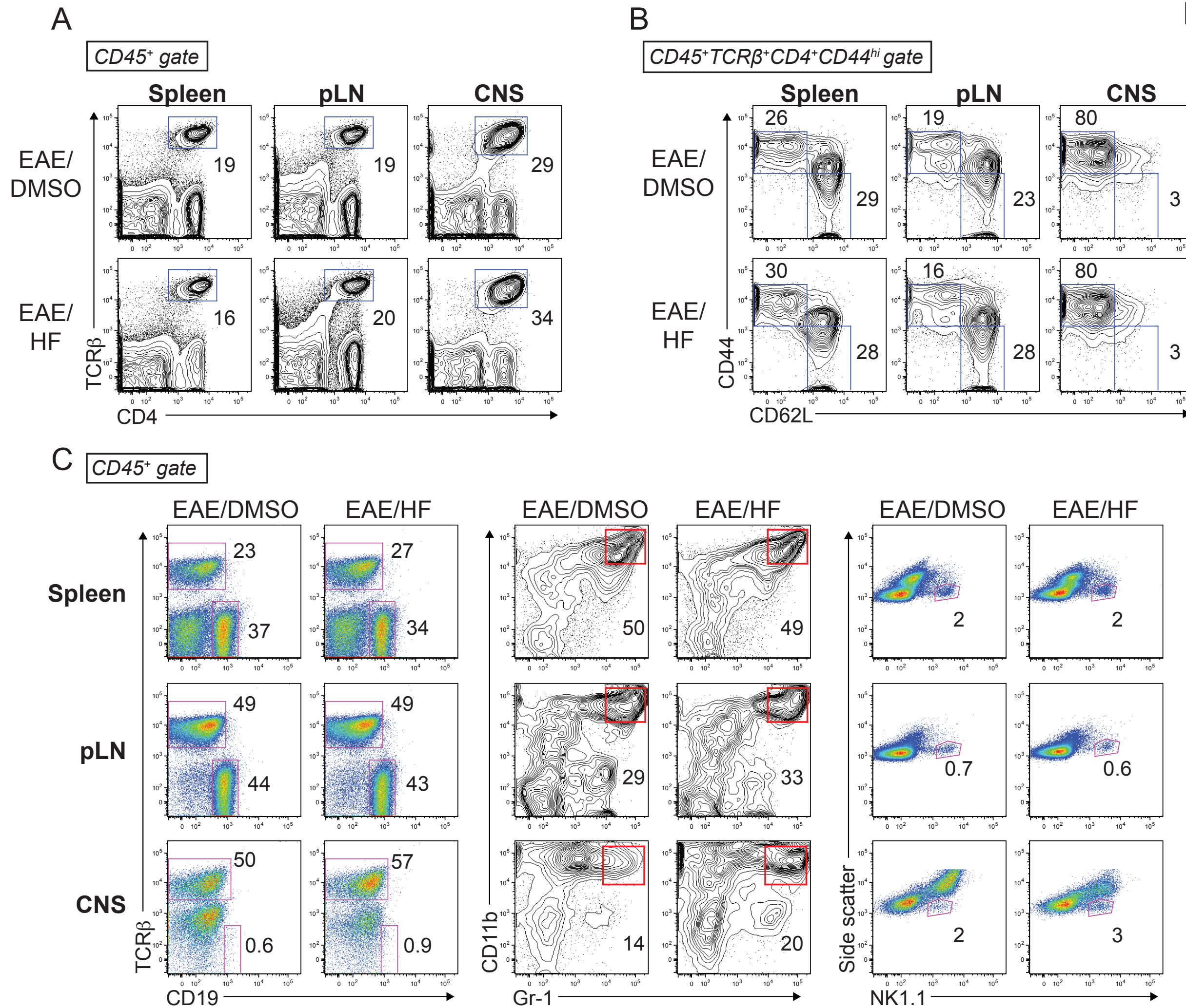

Figure S3. Representative analyses of EAE mice dosed therapeutically with HF. (A) Expression of TCRβ and CD4 within CD45^{+} mononuclear cells from the spleen, peripheral lymph nodes (pLNs), or brain and spinal cord (CNS) of DMSO- or HF-treated mice with active EAE. **(B)** Expression of CD44 and CCR6 in CD45^{+}TCRβ^{+}CD4^{+} T cells isolated from DMSO- or HF-treated mice (as in [A]). **(C)** Immunophenotypic analyses of CD45^{+} mononuclear cells isolated from the spleen, draining lymph nodes, or CNS of EAE mice treated with DMSO or HF. All data represent analysis of 6-8 mice per group.

Figure S4

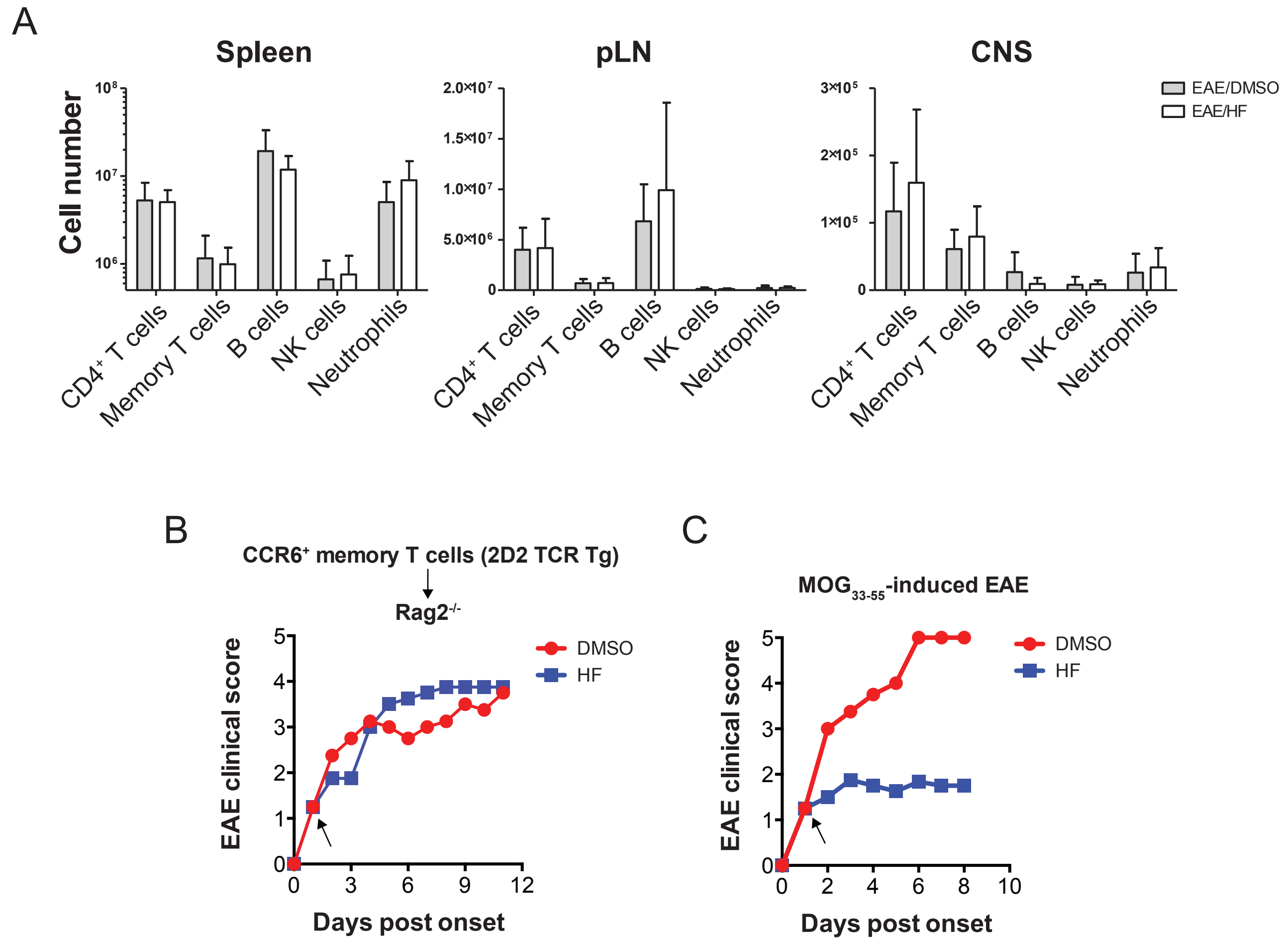

Figure S4. Effects of therapeutic HF dosing in EAE. (A) Mean mononuclear cell numbers + SD from 6-8 mice per group quantified by cell counting and FACS analysis (as in Figure S3C) of spleen (*Left*), peripheral lymph nodes (pLN – *Middle*), or brain and spinal cords (CNS – Right) of mice treated for 10 days with DMSO or HF following onset of EAE. No P values are significant (< .05) by paired student's *t* test. Data incorporate 2 independent experiments analyzing 3-4 mice per treatment group. (B) Endogenous $CCR6^+$ effector/memory T cells were FACS-sorted from spleens and peripheral lymph nodes of 2D2 TCR transgenic mice. Purified cells were expanded *in vitro* for 5 days with anti-CD3/anti-CD28 beads + IL-2 and 10^5 cells were injected into *Rag1*$^{-/-}$ mice. Recipients were treated daily with DMSO or 0.3 mg/kg HF (*n* = 4 per group) beginning at onset of clinical disease; symptoms were followed over 10 days. (C) The same batch of HF used in (B) was used to treat wild-type C57BL/6 mice immunized with MOG_{33-55}/CFA beginning at disease onset (as in Figure 4). Recipients were treated daily with DMSO or 0.3 mg/kg HF (*n* = 4 per group). Data are presented as mean EAE clinical score. Arrows indicate start of treatment.