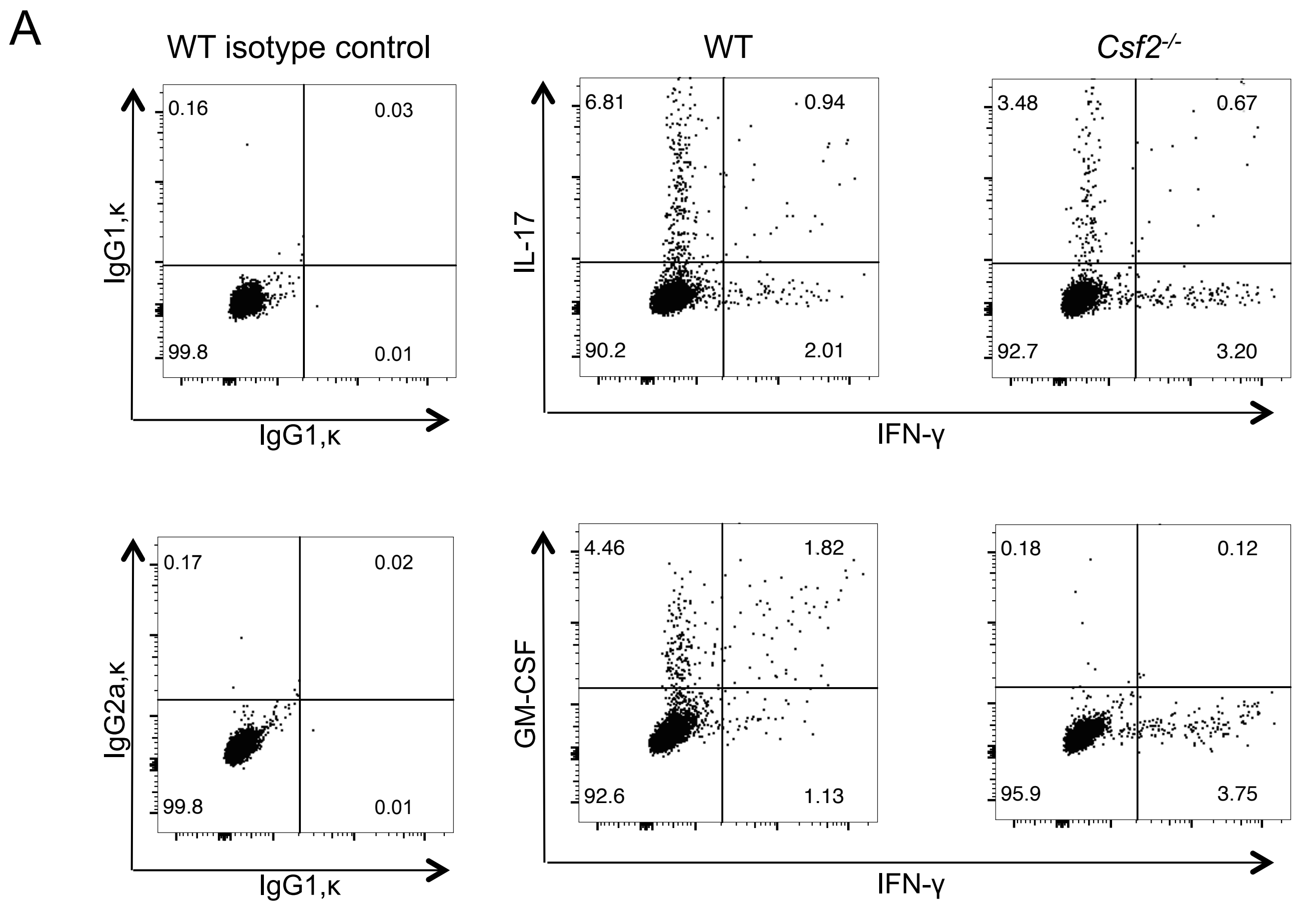
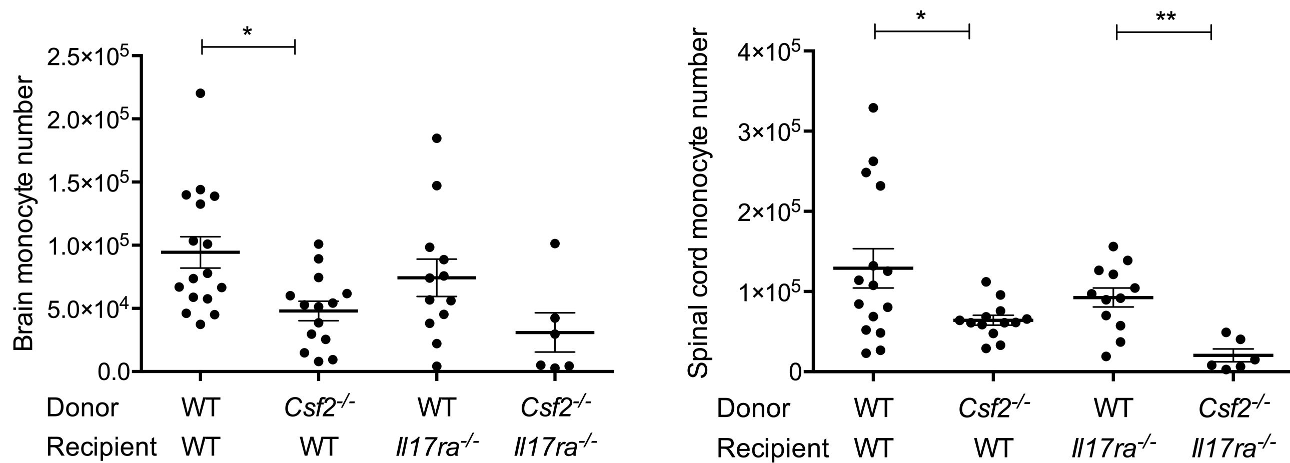


B

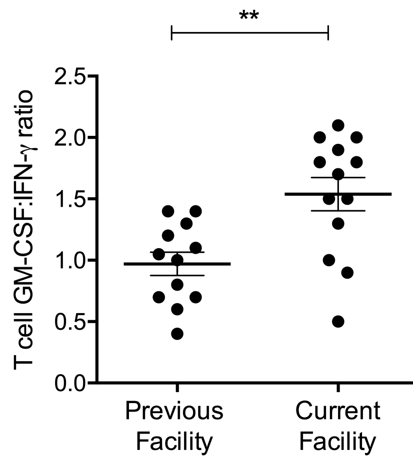
Donor cells	WT	*Csf2*$^{-/-}$
IL-17:IFN-γ ratio	2.5	1.0
GM-CSF:IFN-γ ratio	2.1	0
Percent atypical in WT recipients	100	80
Percent atypical in IL-17R KO recipients	80	0

Supplemental Figure 1. Representative example of cytokine production by WT and *Csf2*$^{-/-}$ donor T cells, cytokine ratios, and resulting incidence of atypical EAE.

(A) Intracellular cytokine staining of $CD4^+$ T cells from WT or *Csf2*$^{-/-}$ donor mice for IL-17, GM-CSF, and IFN-γ or isotype controls. Cells were isolated from spleens and lymph nodes of MOG-immunized mice on d7, cultured with $MOG_{97\text{-}114}$ peptide and IL-23 for three days, and stimulated in vitro with $MOG_{97\text{-}114}$ for four hours prior to intracellular staining and flow cytometric analysis. (B) IL-17:IFN-γ and GM-CSF:IFN-γ ratios were calculated from the cytokine data in (A) for the WT and *Csf2*$^{-/-}$ donor T cells, and the incidence of atypical EAE in WT and *Il17ra*$^{-/-}$ recipients of those same donor T cells in the representative experiment is shown. Data is from one of 16 experiments. EAE, experimental autoimmune encephalomyelitis; MOG, myelin oligodendrocyte glycoprotein.



Supplemental Figure 2. Fewer monocytes accumulate in the brain and spinal cord in the absence of T cell-derived GM-CSF.
Numbers of brain- and spinal cord-infiltrating monocytes ($CD11b^+$ $CD45^+$ $Ly6G^-$ $Ly6C^{hi}$) were determined by flow cytometric analysis of cells isolated from the brains (left) and spinal cords (right) of WT and *Il17ra*$^{-/-}$ recipients of WT or *Csf2*$^{-/-}$ $CD4^+$ T cells at peak EAE. Data were pooled from 4 experiments. Error bars represent SEM. *$P<0.05$, **$P<0.01$, One-way ANOVA with Tukey's post test. EAE, experimental autoimmune encephalomyelitis.



Supplemental Figure 3. Differences in T cell production of GM-CSF relative to IFN-γ seen in two animal facilities.
Ratios of GM-CSF-producing to IFN-γ-producing $CD4^+$ T cells were determined from experiments performed in two separate animal facilities at the University of Washington. Experiments reported in Simmons et al. were performed in the "previous" animal facility. All experiments reported here were performed in the "current" animal facility. Data from the "current facility" is also depicted in Figure 3. Animals studied in each facility were derived from the same breeding stocks. Intracellular cytokine staining of $CD4^+$ T cells from MOG-immunized mice was performed after three days of culture with MOG_{97-114} and IL-23 as described in Methods. Error bars represent SEM. *P*=0.004, Student's *t* test. MOG, myelin oligodendrocyte glycoprotein.