Supporting Information

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SI Methods

ELISA and Flow Cytometry. IL-10 and TGF-β (R&D Systems), HGF (B-Bridge), IL-4, IL-12p70, IFN-γ, and TNF-α (eBioscience) ELISAs were performed according to manufacturer's instructions. For flow cytometry, cells were incubated with antibodies directed against CD4, Vα3.2 (BD Pharmingen), CD8, CD11c, CD11b, CD25, CD40, CD80, CD86, MHC class II, CD44, CD62L, Vβ11, cMet (eBioscience), or isotype control. Expression of FoxP3 was detected in fixed/permeabilized T cells by labeling with anti-mouse FoxP3 mAb (eBioscience). Intracellular cytokine staining was performed with anti-IL-17A, anti-IFN-γ, and anti-IL-10 staining (eBioscience). Samples were run through a FACSCalibur flow cytometer (BD Biosciences) with standard equipment.

Histology. Spinal cords were removed from mice at peak disease, fixed, and paraffin embedded. Sections (5 μm) were stained with H&E to assess inflammatory infiltration, with luxol fast blue to assess the degree of demyelination, or with Bielschowsky's silver staining to assess the level of axonal damage. For immunostaining, frozen sections of spinal cord at peak disease were embedded in Tissue-Tek optimal cutting temperature compound (Miles Scientific), snap-frozen in liquid nitrogen, and then fixed with cold EtOH before staining with CD4, CD8, CD11b, and CD11c antibodies.

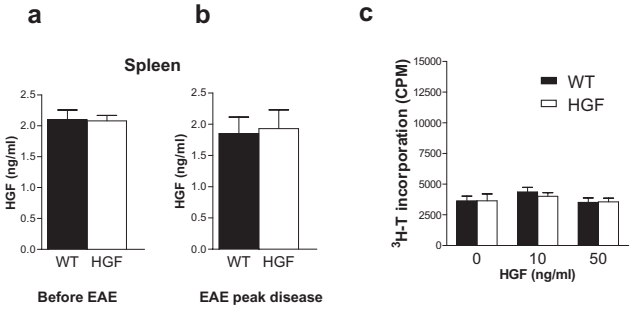

Fig. S1. Hepatocyte growth factor (HGF) in not increased in the spleen and does not induce proliferation of splenic T cells during experimental autoimmune encephalitis (EAE). HGF was measured by ELISA in spleen homogenates in WT mice and mice carrying an HGF transgene (HGF-Tg mice) before (*A*) and during (*B*) EAE peak disease. $n = 3$ per experiment. Results shown are mean ± standard error of the mean (SEM) of triplicate experiments. (*C*) Proliferation assay of splenocytes with recombinant mouse HGF (rHGF) (0–50 ng/mL) in HGF-Tg mice and WT littermates during EAE peak disease, day 25 postimmunization. $n = 3$ per experiment. Results shown are mean cpm ± SEM of triplicate experiments.

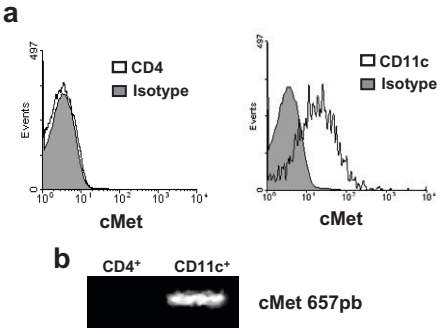

Fig. S2. The cMet (HGF) receptor is present on the surface of CD11c$^+$ cells but not on CD4$^+$ T cells. (*A*) cMet receptor staining on the surface of CD11c$^+$ and CD4$^+$ T cells from fresh C57BL/6 splenocytes. Results are representative of four independent experiments. (*B*) Detection of the cMet receptor by RT-PCR on CD11c$^+$ and CD4$^+$ T cells from fresh C57BL/6 splenocytes.

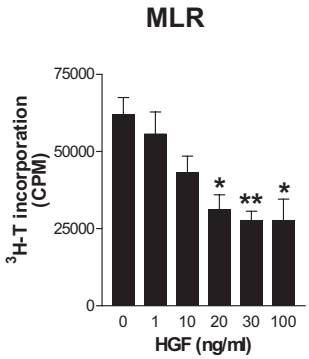

Fig. S3. Influence of HGF on dendritic cell (DC) function and T-cell proliferation assessed by mixed lymphocyte reaction (MLR). MLRs were performed through coincubation of purified CD11c$^+$ cells (DC) from BALB/c mice and purified CD4$^+$ T cells from C57BL/6 mice. T-cell proliferation was analyzed after CD11c$^+$ cells were preincubated with increasing concentrations of rHGF. Results shown are mean cpm ± SEM of triplicate wells. *, $P < 0.05$; **, $P < 0.01$.

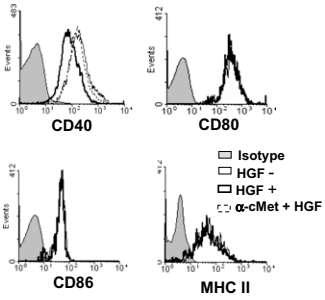

Fig. S4. HGF inhibits in vitro expression of CD40 in CD11c$^+$ DC. Antigen-specific response (ASR) staining for coreceptors of CD11c$^+$ cells including CD40, CD80, CD86, and MHC class II. CD40 expression was inhibited by rHGF at 30 ng/mL. Figures are representative of four independent experiments.

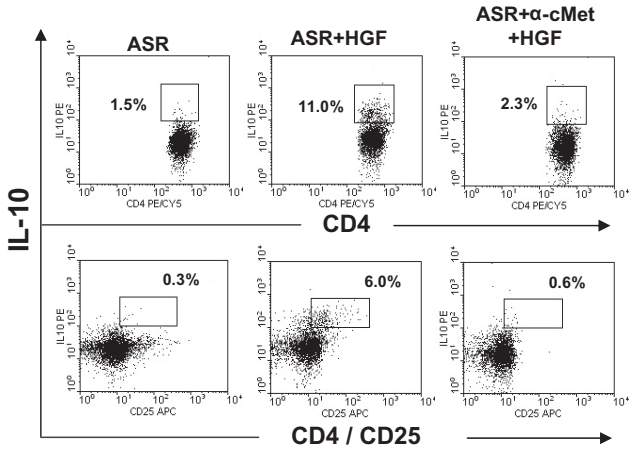

Fig. S5. HGF-treated DC induce IL-10 production in CD4$^+$ and CD4$^+$CD25$^+$ T cells. IL-10 intracellular staining of CD4$^+$ T cells and CD4$^+$CD25$^+$ T cells from ASR experiments shows IL-10 production was increased in both T-cell subtypes when T cells were cocultured with rHGF-treated CD11c$^+$ DC. The effect of HGF on CD11c$^+$ cells was inhibited by preincubation of CD11c$^+$ cells with α-cMet–blocking antibody. Figures are representative of four independent experiments.