# Supporting Information

## Qin et al. 10.1073/pnas.1117218109

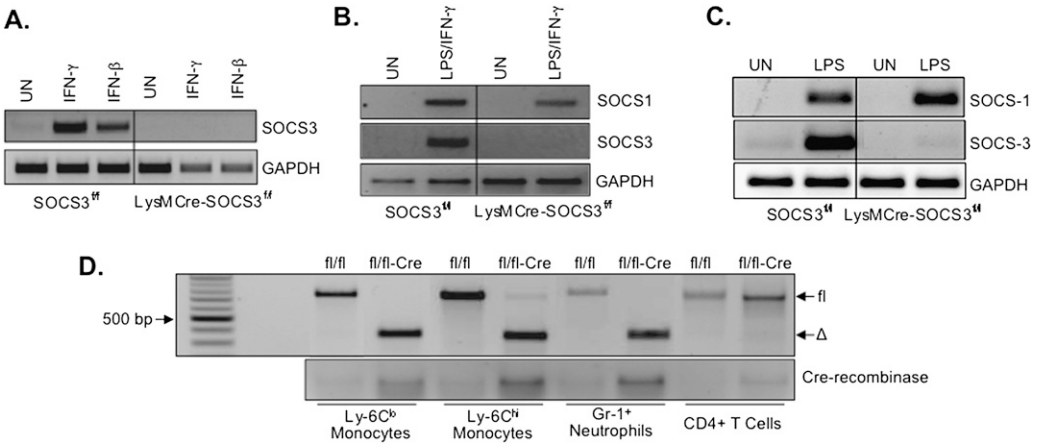



**Fig. S1.** Suppressor of cytokine signaling 3 (SOCS3) deletion in LysMCre-SOCS3$^{fl/fl}$ mice. (*A*) Primary microglia from SOCS3$^{fl/fl}$ and LysMCre-SOCS3$^{fl/fl}$ mice were treated with medium (UN), IFN-γ (10 ng/mL) or IFN-β (100 units/mL) for 4 h, and mRNA was analyzed by RT-PCR for SOCS3 and GAPDH expression. (*B*) Peritoneal macrophages from SOCS3$^{fl/fl}$ and LysMCre-SOCS3$^{fl/fl}$ mice were treated with medium (UN) or LPS (10 ng/mL) plus IFN-γ (10 ng/mL) for 4 h, and mRNA was analyzed by RT-PCR for SOCS1, SOCS3, and GAPDH expression. (*C*) Bone marrow derived dendritic cells (BMDCs) from SOCS3$^{fl/fl}$ and LysMCre-SOCS3$^{fl/fl}$ mice were treated with medium (UN) or LPS (10 ng/mL) for 4 h, and mRNA was analyzed by RT-PCR for SOCS1, SOCS3, and GAPDH expression. (*D*) Ly-6C$^{lo}$ monocytes, Ly-6C$^{hi}$ monocytes, and CD4$^{+}$ T cells were isolated from spleen and Gr-1$^{+}$ neutrophils were isolated from peripheral blood. Genomic DNA was distinguished as floxP (fl) and cre-excised alleles (Δ), and Cre-recombinase mRNA expression was confirmed by PCR. Three independent experiments are represented.

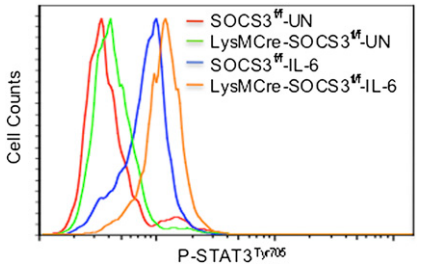



**Fig. S2.** Phosphorylation of STAT3 in macrophages from SOCS3$^{fl/fl}$ and LysMCre-SOCS3$^{fl/fl}$ mice. BMDMs were incubated with medium (UN) or IL-6/sIL-6R for 1 h, then STAT3 phosphorylation (Tyr-705) was determined by intracellular staining. Three independent experiments are represented.

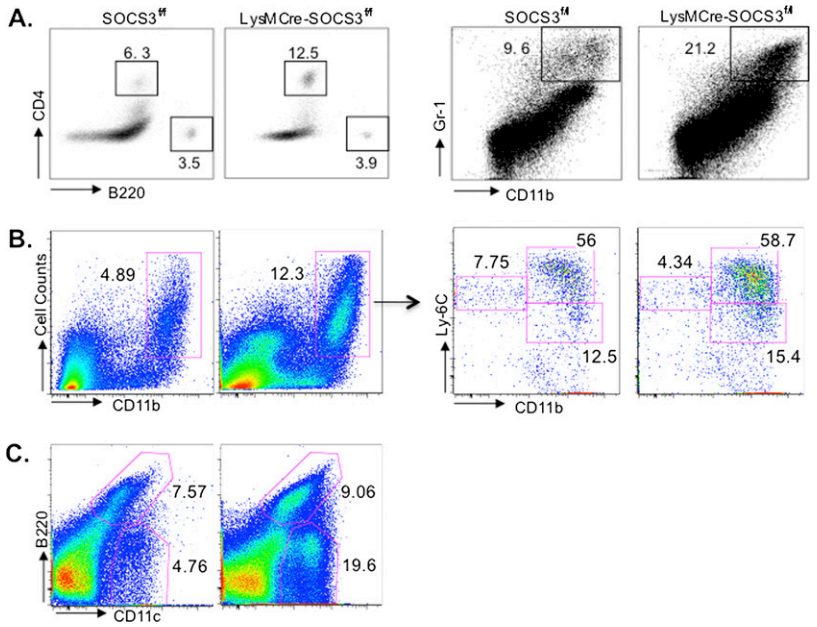



**Fig. S3.** CNS-infiltrating mononuclear cells from SOCS3$^{fl/fl}$ and LysMCre-SOCS3$^{fl/fl}$ mice. (*A*) CNS-infiltrating mononuclear cells were isolated from brain at day 12 after myelin oligodendrocyte glycoprotein (MOG) immunization. Cells were stained with antibodies to CD4, B220, CD11b, and Gr-1. The percentages of CD4$^+$ T cells, B220$^+$ B cells, and CD11b$^+$/Gr-1$^+$ neutrophils were gated. (*B*) CNS-infiltrating mononuclear cells were obtained from SOCS3$^{fl/fl}$ and LysMCre-SOCS3$^{fl/fl}$ mice and stained with antibodies to CD11b and Ly-6C, and the percentages of CD11b$^+$/Ly-6C$^{hi}$ monocytes and CD11b$^+$/Ly-6C$^{lo}$ monocytes were gated. (*C*) Cells were stained with antibodies to B220 and CD11c, and the percentages of B220$^+$ B cells and CD11c$^+$ dendritic cells were gated. Three independent experiments are represented.

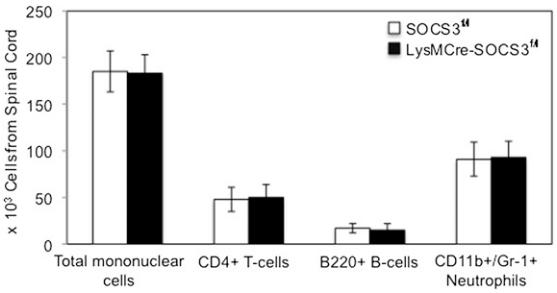



**Fig. S4.** Total cells, CD4$^+$ T cells, B cells, and neutrophils in spinal cord-infiltrating mononuclear cells from SOCS3$^{fl/fl}$ and LysMCre-SOCS3$^{fl/fl}$ mice. CNS-infiltrating mononuclear cells were isolated from spinal cord at day 12 after MOG immunization. Cells were counted and stained with antibodies to CD4, B220, CD11b, and Gr-1, and the percentage of CD4$^+$ T cells, B220$^+$ B cells, and CD11b$^+$/Gr-1$^+$ neutrophils were gated. Three independent experiments are represented.

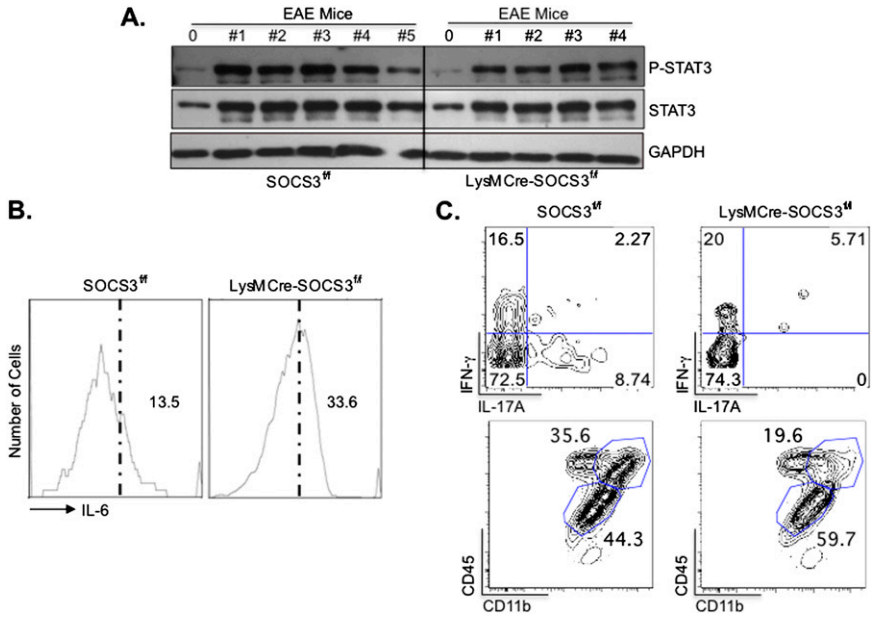



**Fig. S5.** STAT3 activation, IL-6 expression, and mononuclear cell infiltration in spinal cord from SOCS3$^{fl/fl}$ and LysMCre-SOCS3$^{fl/fl}$ mice. (*A*) Protein extracts from spinal cords of unimmunized (day 0) or MOG35-55–immunized (day 13) SOCS3$^{fl/fl}$ ($n$ = 5) and LysMCre-SOCS3$^{fl/fl}$ mice ($n$ = 4) were immunoblotted with indicated antibodies. (*B*) CNS-infiltrating mononuclear cells were isolated from brain at day 12 after immunization and stained for the surface marker CD11b and by intracellular flow for IL-6. (*C*) CNS-infiltrating mononuclear cells from spinal cord at the peak of atypical disease (MOG35-55–immunized for 13 d) from SOCS3$^{fl/fl}$ and LysMCre-SOCS3$^{fl/fl}$ mice. Cells were stained for the surface marker CD4 and intracellular flow for IFN-γ and IL-17A and the surface markers CD11b and CD45. Five independent experiments are represented.

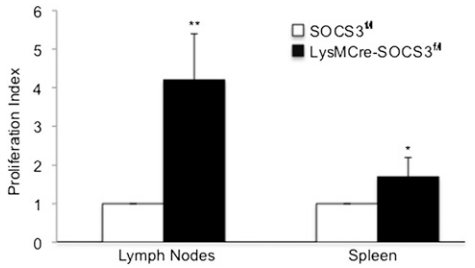



**Fig. S6.** Proliferation of CD4$^+$ T cells from lymph nodes and spleen of SOCS3$^{fl/fl}$ and LysMCre-SOCS3$^{fl/fl}$ mice. CD4$^+$ T cells were isolated from the lymph nodes or spleen of MOG-injected SOCS3$^{fl/fl}$ and LysMCre-SOCS3$^{fl/fl}$ mice after 7 d. CD4$^+$ T cells and GM-CSF matured dendritic cells (DCs) were cultured at a 5:1 ratio for T-cell proliferation with MOG (5 μg/mL) stimulation for 80 h, and cell proliferation was analyzed with the Click-iT EdU Alexa Fluor 488 assay kit. *$P < 0.05$, **$P < 0.001$. Three independent experiments are represented.

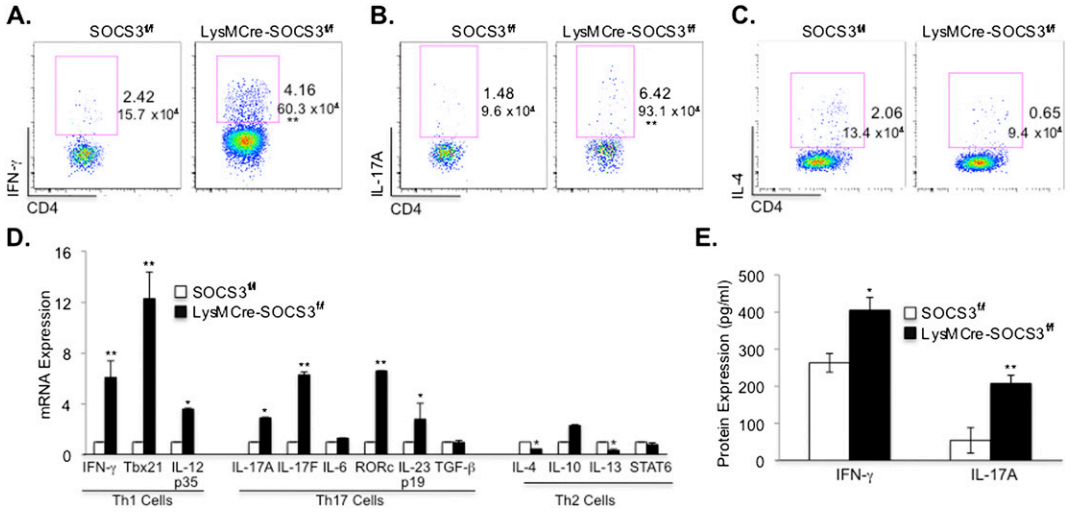



**Fig. S7.** MOG-induced Th1, Th17, and Th2 differentiation in the lymph nodes of MOG-injected SOCS3$^{fl/fl}$ and LysMCre-SOCS3$^{fl/fl}$ mice. CD4$^+$ T cells were isolated from the lymph nodes of 7-d MOG-injected SOCS3$^{fl/fl}$ and LysMCre-SOCS3$^{fl/fl}$ mice. CD4$^+$ T cells and BMDCs were cultured at a 5:1 ratio for Th1, Th17, and Th2 cell differentiation. Th1 cells were differentiated with MOG peptide (5 μg/mL), IL-12 (10 ng/mL) plus anti–IL-4 (10 μg/mL) for 3 d. Th17 cells were differentiated with MOG peptide (5 μg/mL), TGF-β (5 ng/mL), IL-6 (20 ng/mL), IL-23 (10 ng/mL) plus anti–IFN-γ (10 μg/mL) and anti–IL-4 (10 μg/mL) for 3 d. Th2 cells were differentiated with MOG peptide (5 μg/mL), IL-4 (10 ng/mL) plus anti–IFN-γ (10 μg/mL) for 3 d. At day 3, cells were stimulated with PMA/ionomycin (25 ng/mL and 1 μg/mL) plus GolgiStop (BD Pharmingen) for 4 h and were analyzed for intracellular production of IFN-γ (*A*), IL-17A (*B*), and IL-4 (*C*); total cell numbers were calculated with total CD4 T cells. Th1, Th2, and Th17 factor mRNA expression was analyzed by qRT-PCR (*D*). IFN-γ and IL-17A protein expression in the supernatants was examined by ELISA (*E*). *$P < 0.05$, **$P < 0.001$. Three independent experiments are represented.

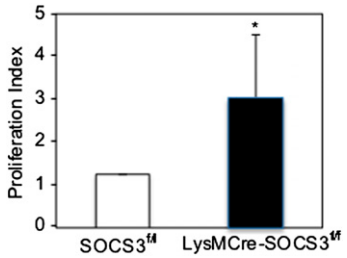



**Fig. S8.** Myeloid cell SOCS3 expression influences CD4$^+$ T-cell proliferation. Naive CD4$^+$ T cells were isolated from the spleen of MOG-TCR transgenic 2D2 mice. Macrophages from SOCS3$^{fl/fl}$ and LysMCre-SOCS3$^{fl/fl}$ mice were polarized to the M1 phenotype with LPS plus IFN-γ for 48 h and then used as antigen-presenting cells. M1 macrophages and CD4$^+$ T cells were cultured at a 1:5 ratio for T-cell proliferation with MOG (2 μg/mL) stimulation for 80 h, and cell proliferation was analyzed with the Click-iT EdU Alexa Fluor 488 assay kit. *$P < 0.05$. Three independent experiments are represented.

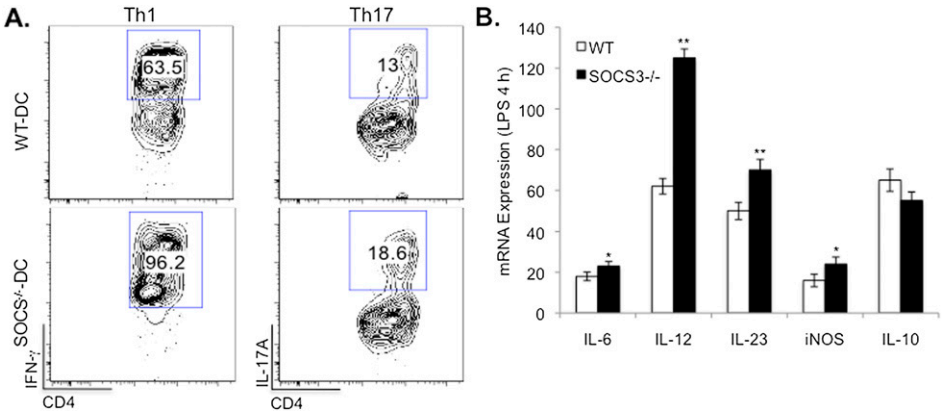



**Fig. S9.** SOCS3 deletion in BMDCs leads to enhanced Th1/Th17 cell differentiation and M1 gene expression. (*A*) Mature BMDCs from SOCS3$^{fl/fl}$ and LysMCre-SOCS3$^{fl/fl}$ mice [cultured with GM-CSF (10 ng/mL) for 7 d] were used as antigen-presenting cells and cultured with naive CD4$^+$ T cells isolated from the spleen of SOCS3$^{fl/fl}$ mice at a 1:5 ratio for Th1 and Th17 cell differentiation. At day 4, cells were stimulated with PMA/ionomycin plus GolgiStop for 4 h, stained for the surface marker CD4 and by intracellular flow for IFN-γ and IL-17A protein expression. (*B*) BMDCs from SOCS3$^{fl/fl}$ and LysMCre-SOCS3$^{fl/fl}$ mice were differentiated in GM-CSF (10 ng/mL) medium for 7 d and then treated with LPS for 4 h. mRNA was analyzed by qRT-PCR for IL-6, IL-12p40, IL-23p19, iNOS, and IL-10. *$P < 0.05$, **$P < 0.001$. Three independent experiments are represented.

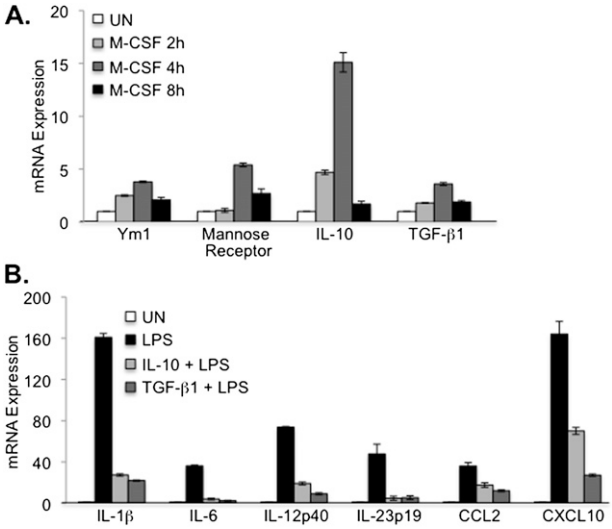
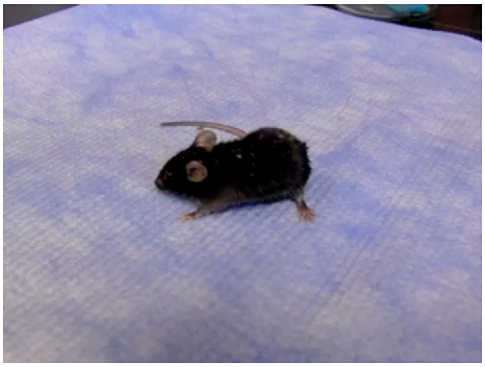



**Fig. S10.** IL-10 and TGF-β inhibit M1 macrophage polarization. (*A*) BMDMs from SOCS3$^{fl/fl}$ mice were treated with medium (UN) or M-CSF (10 ng/mL) for up to 8 h, then mRNA was analyzed by qRT-PCR. (*B*) BMDMs from SOCS3$^{fl/fl}$ mice were pretreated with IL-10 (10 ng/mL) or TGF-β1 (10 ng/mL) for 2 h, and then cells were treated with LPS for 4 h. IL-1β, IL-6, IL-12p40, IL-23p19, CCL2, and CXCL10 mRNA expression was analyzed by qRT-PCR. Three independent experiments are represented.



**Movie S1.** SOCS3$^{fl/fl}$ mouse at day 13 after MOG immunization.

Movie S1

**Movie S2.** LysMCre-SOCS3$^{fl/fl}$ mouse at day 13 after MOG immunization.

Movie S2