SUPPLEMENTAL MATERIAL

Wolf et al., https://doi.org/10.1084/jem.20160499

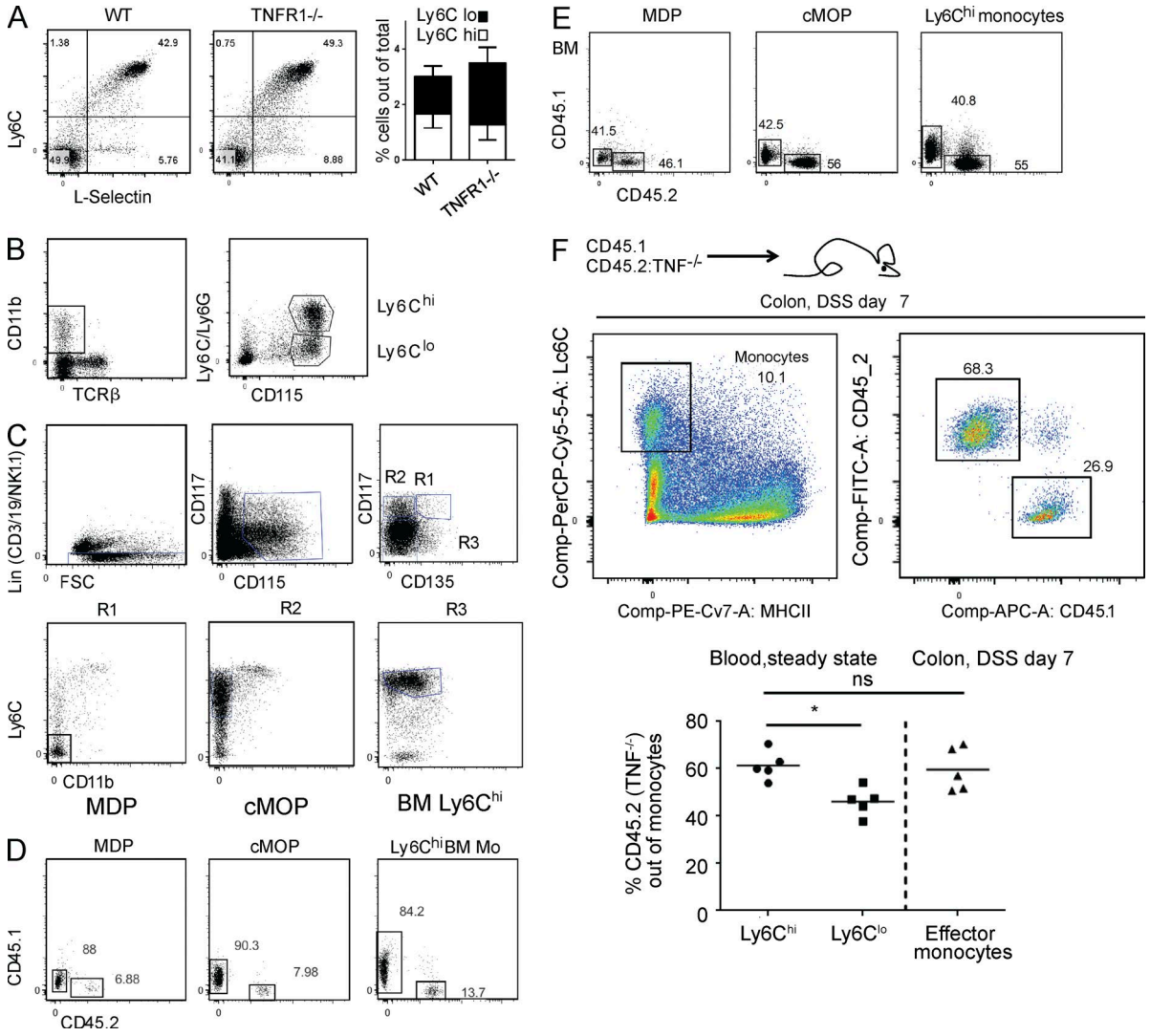

Figure S1. **Flow cytometric analysis of monocytes and monocyte precursors in the BM.** (A) FACS analysis of blood monocytes from nonchimeric WT and TNFR1$^{-/-}$ mice in steady state. $n = 8$. Results are expressed as means ± SEM. (B) FACS analysis illustrating definition of blood monocyte subsets. (C) FACS analysis illustrating definition of monocyte precursors. FSC, forward scatter. (D) FACS analysis of myeloid BM precursors: MDPs, cMOPs, and BM Ly6C^{hi} monocytes in [TNFR1$^{-/-}$/WT > WT] mixed BM chimeras. (E) FACS analysis of indicated myeloid BM precursors in [TNF/WT > WT] mixed BM chimeras as in F. Mo, monocyte. (F) FACS analysis of colons of [CD45.2 TNF$^{-/-}$/CD45.1 WT > WT] mice treated with 2% DSS for 7 d. Each mouse was assessed for frequency of CD45.2^{+} steady-state blood monocytes before DSS administration, as in Fig. 1 E. $n = 5$. *, $P < 0.05$; Mann-Whitney *U* test was used.

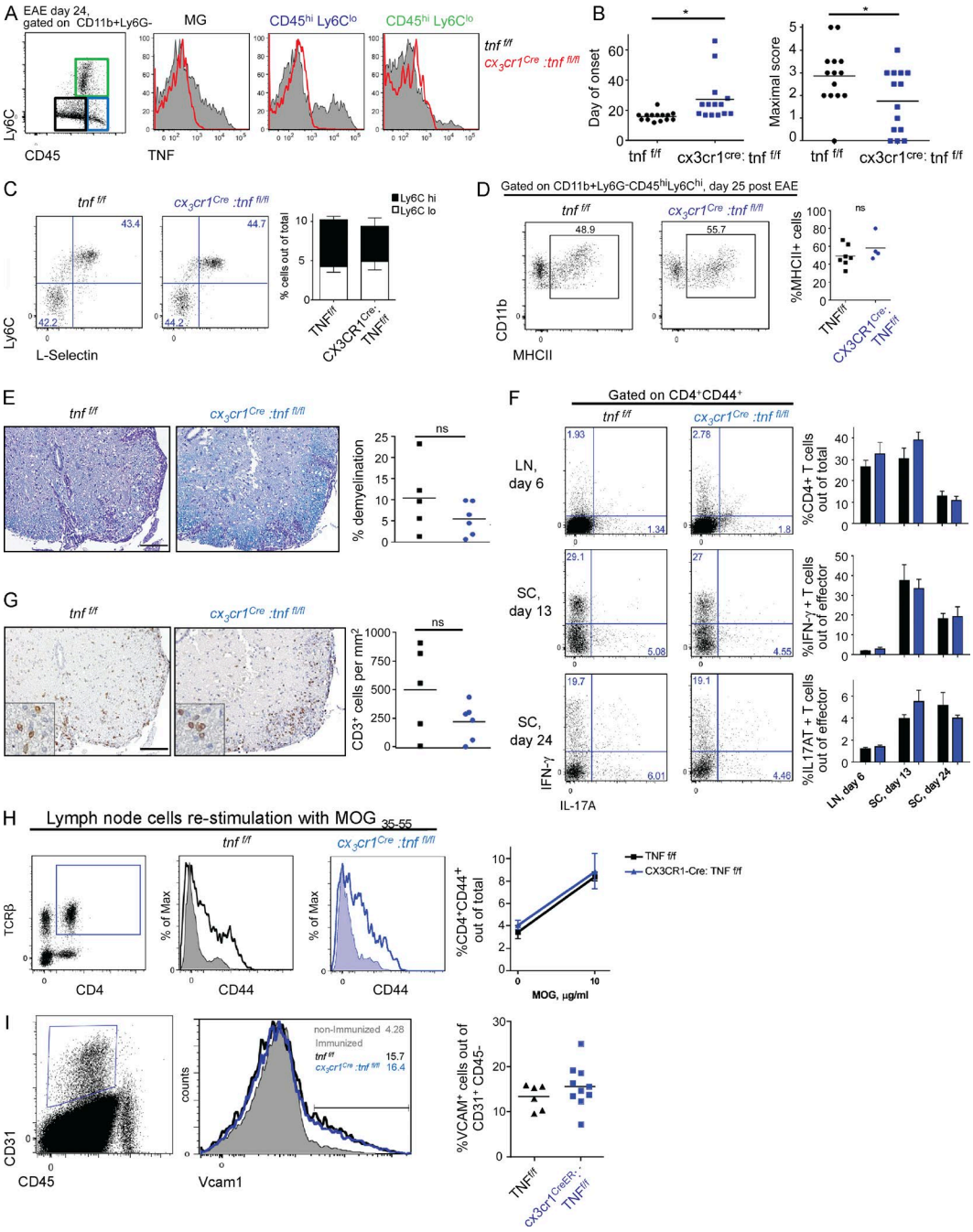

Figure S2. **Additional characterization of *cx3cr1*^{Cre}*:tnf*$^{f/f}$ mice.** (A) FACS analysis of intracellular TNF expression in different myeloid populations in the inflamed spinal cord of *tnf*$^{f/f}$ and *cx3cr1*^{cre}*:tnf*$^{f/f}$ mice. MG, microglia. (B) Pooled day-of-onset (left) and maximal-score (right) data of MOG_{35-55}-immunized *tnf*$^{f/f}$ and *cx3cr1*^{cre}*:tnf*$^{f/f}$ mice from three independent experiments. $n = 14$. *, $P < 0.05$. Student's *t* test was used. (C) FACS analysis of blood monocytes from *tnf*$^{f/f}$ and *cx3cr1*^{cre}*:tnf*$^{f/f}$ mice in steady state. $n = 8–7$. (D) FACS analysis of CD45^{hi}Ly6C^{hi}MHCII^{+} effector monocytes (gated as in Fig. 3 A) from spinal cords of MOG_{35-55}-immunized *tnf*$^{f/f}$ and *cx3cr1*^{cre}*:tnf*$^{f/f}$ mice 24 d after immunization (the same cells are shown also in Fig. 4 C). $n = 4–7$. (E) Histological analysis of demyelination by luxol-fast blue stain in spinal cords of in *tnf*$^{fl/fl}$ and *cx*$_3$*cr1*^{Cre}*:tnf*$^{fl/fl}$ mice at day 24 after immunization. $n = 5–6$. (F) FACS analysis of Th1 and Th17 cells in lymph nodes (LN) at day 6 after immunization or in spinal cord (SC) at days 13 and 24 in *tnf*$^{fl/fl}$ and *cx*$_3$*cr1*^{Cre}*:tnf*$^{fl/fl}$. $n = 4–6$. (G) CD3 histological stain in spinal cords of *tnf*$^{fl/fl}$ and *cx*$_3$*cr1*^{Cre}*:tnf*$^{fl/fl}$ mice at day 24 after immunization. $n = 5–6$. (E and G) Bars, 100 µm. (H) FACS analysis of lymph node T cells of *tnf*$^{fl/fl}$ and *cx*$_3$*cr1*^{Cre}*:tnf*$^{fl/fl}$ mice immunized with MOG_{35-55} in vivo and restimulated ex vivo with 10 µg/ml MOG_{35-55}. Shaded histograms represent nonstimulated cells. Unshaded histograms represent stimulated cells. $n = 3$ biological repeats. (I) FACS analysis for the activation marker VCAM1 on endothelial cells (CD31^{+}CD45) isolated from spinal cord of *tnf*$^{fl/fl}$ and *cx*$_3$*cr1*^{Cre}*:tnf*$^{fl/fl}$ mice 9 d after MOG_{35-55} immunization. $n = 6–10$. Results are expressed as means ± SEM.

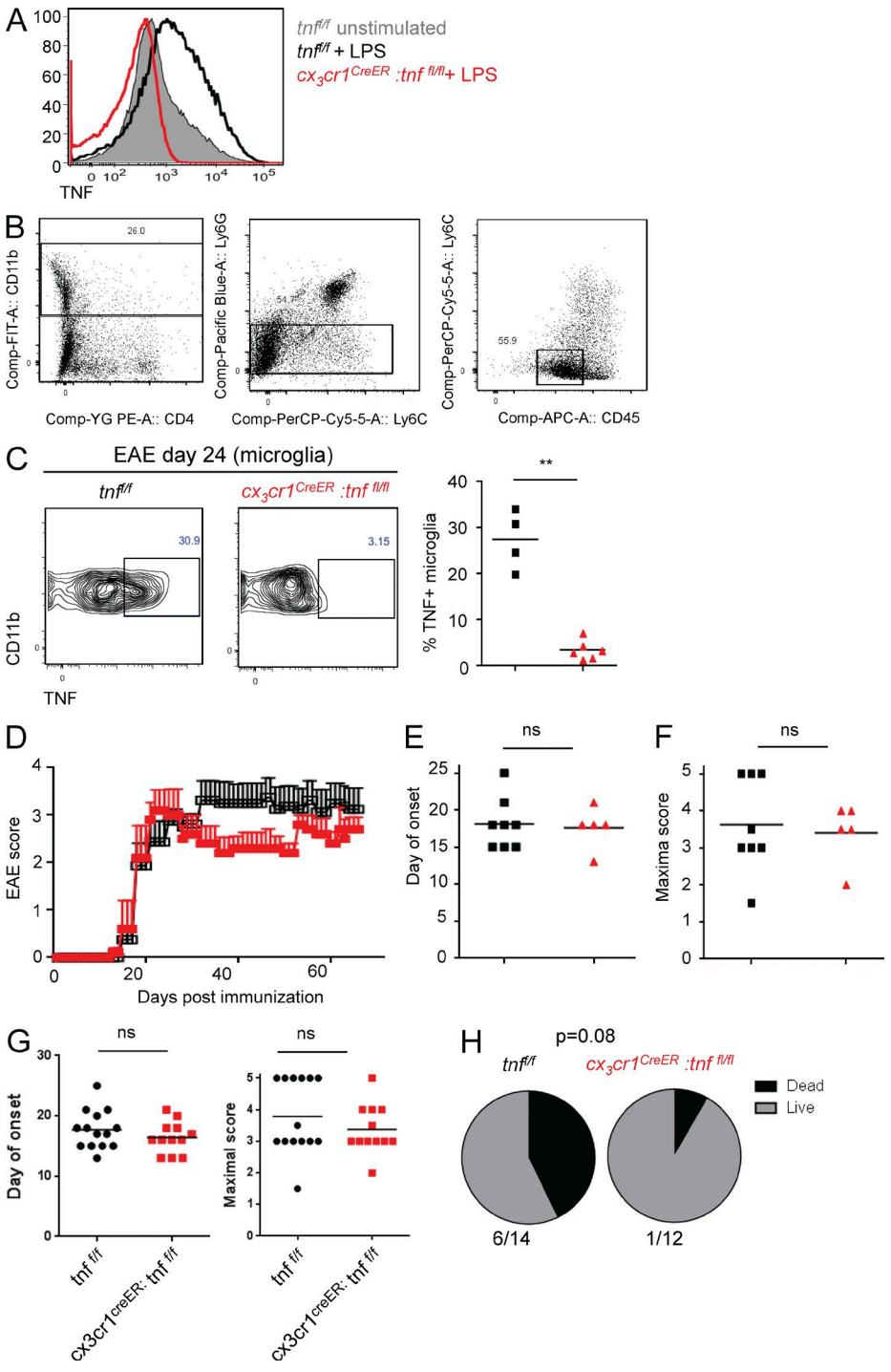

Figure S3. **Microglia-restricted TNF deletion does not affect EAE onset.** (A) Intracellular FACS staining for TNF expression of microglia isolated from *tnf*$^{fl/fl}$ and *cx*$_3$*cr1*^{CreER}*:tnf*$^{fl/fl}$ animals 6 wk after TAM treatment. Microglia were treated ex vivo with 10 µg/ml LPS for 4 h or left unstimulated. Data are representative of three biological repeats. (B) Gating strategy for microglia in the inflamed spinal cord. (C) TNF production by microglia (CD11b^{+}CD45^{int}Ly6C^{-}Ly6G^{-}) in spinal cords of TAM-treated *tnf*$^{fl/fl}$ and *cx*$_3$*cr1*^{CreER}*:tnf*$^{fl/fl}$ mice 24 d after MOG$_{35-55}$ immunization, analyzed by FACS. n = 4–6. **, P < 0.01. Mann-Whitney U test was used. (D–F) EAE disease course, individual day of onset, and individual maximal score of TAM-treated *tnf*$^{fl/fl}$ and *cx*$_3$*cr1*^{CreER}*:tnf*$^{fl/fl}$ mice immunized with MOG$_{35-55}$. n = 5–8. Data are representative of three independent experiments. Results are expressed as means ± SEM. (G) Pooled day of onset (left) and maximal score (right) of MOG$_{35-55}$-immunized *tnf*$^{f/f}$ and *cx3cr1*^{creER}*:tnf*$^{f/f}$ mice from three independent experiments. n = 12–14. (H) Mortality rate of pooled *tnf*$^{f/f}$ and *cx3cr1*^{creER}*:tnf*$^{f/f}$ mice shown in G. Numbers indicate the death incidence out of total mice in each group. χ test was performed to assess the significance.

Tables S1 and S2 are included as Excel files. Table S1 lists genes up-regulated in spinal cord effector monocytes at EAE day 9 compared with Ly6C^{hi} monocytes. Table S2 lists genes down-regulated in spinal cord effector monocytes at EAE day 9 compared with Ly6C^{hi} monocytes.