SUPPLEMENTAL MATERIAL

Hou et al., http://www.jem.org/cgi/content/full/jem.20082030/DC1

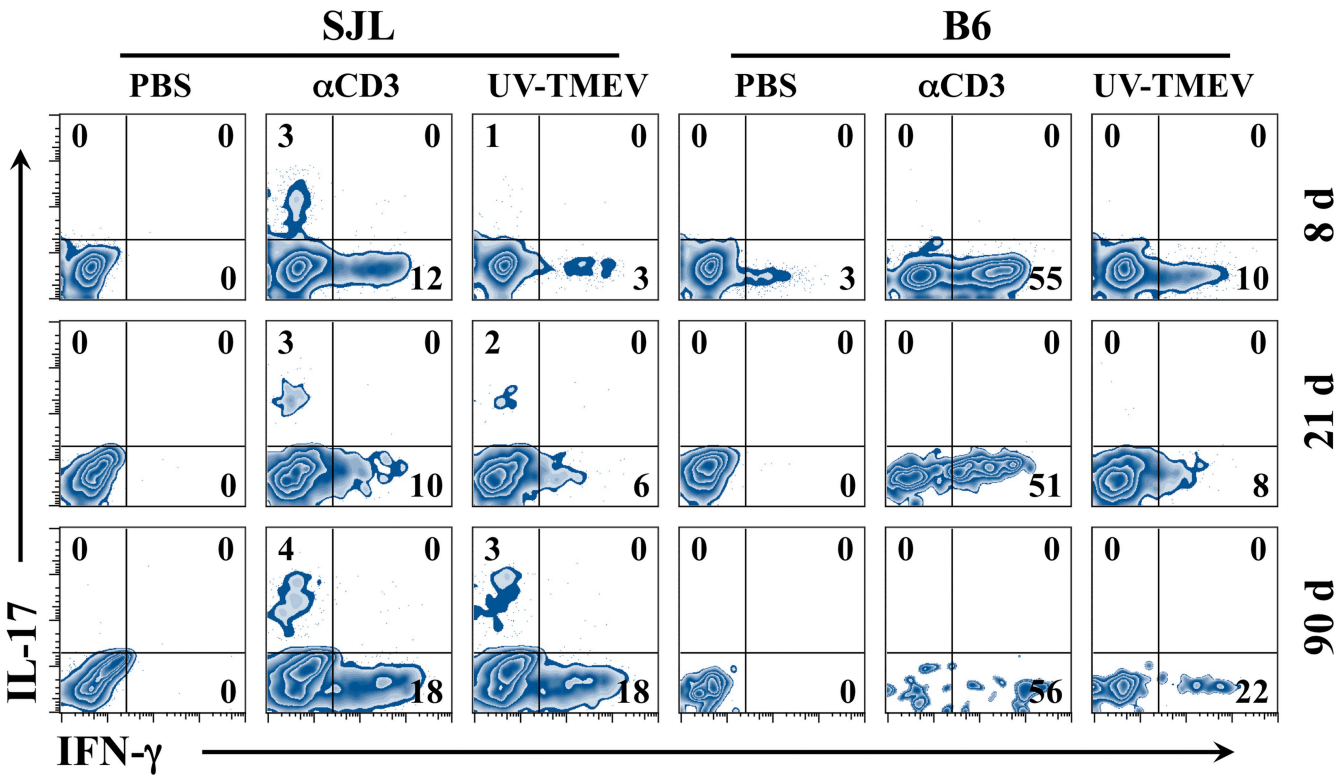

Figure S1. Enhanced Th17 development during persistent virus infection. Flow cytometry of CNS-infiltrating mononuclear cells collected from SJL and B6 mice at 8, 21, and 96 d after TMEV infection after restimulation with anti-CD3/anti-CD28 (αCD3) or 1 µg/ml UV-TMEV for 6 h. All flow cytometry plots were gated on $CD4^+$ T cells. A representative result with pooled CNS cells (two to four mice per group) from three separate experiments is shown. The numbers in each quadrant represent percentages.

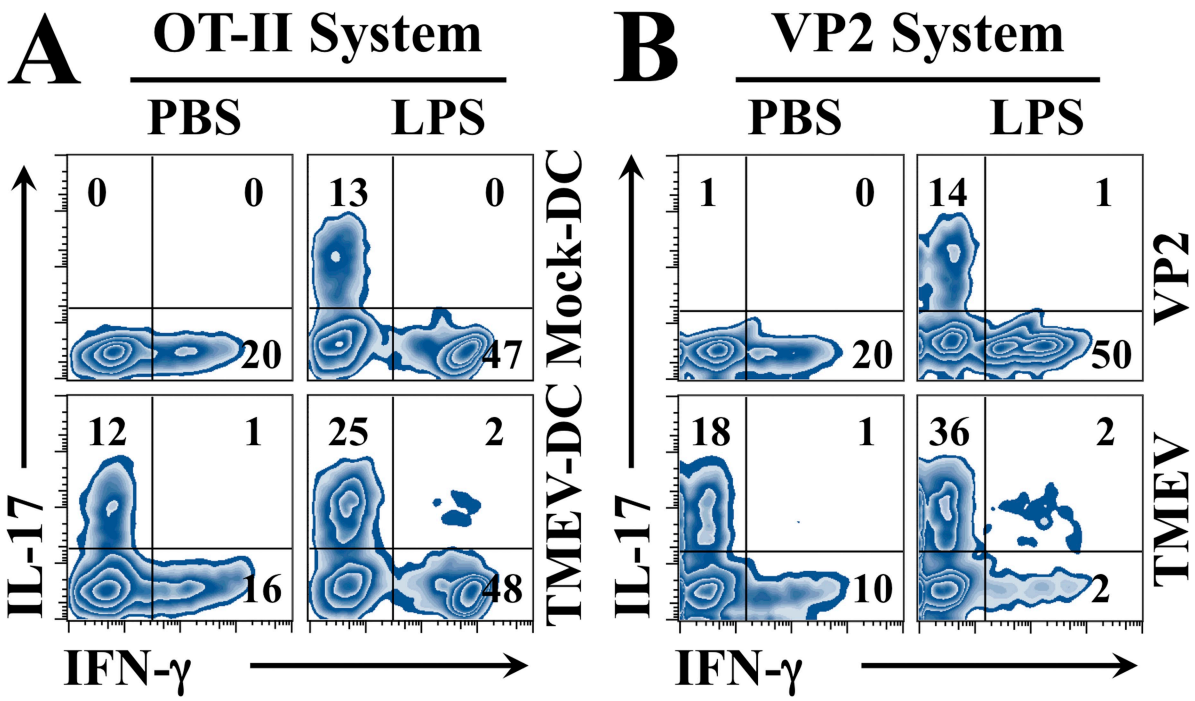

Figure S2. LPS treatment in vitro enhances the differentiation of Th17 cells induced by virus-infected DCs. Flow cytometry of (A) OT-II or (B) VP2 transgenic CD4+ T cells were cultured for 4 d with mock-infected BMDCs or BMDCs infected with TMEV (MOI of 10) in the presence of LPS. For OT-II T cells, the cognate peptide $OVA_{323-339}$ was added. Viral epitope VP2–pulsed BMDCs were added to VP2 transgenic CD4+ T cells as a positive control (B). Levels of intracellular cytokines were determined after restimulation with PMA plus ionomycin for 6 h. A representative result from three independent experiments is shown. The numbers in each quadrant represent percentages.

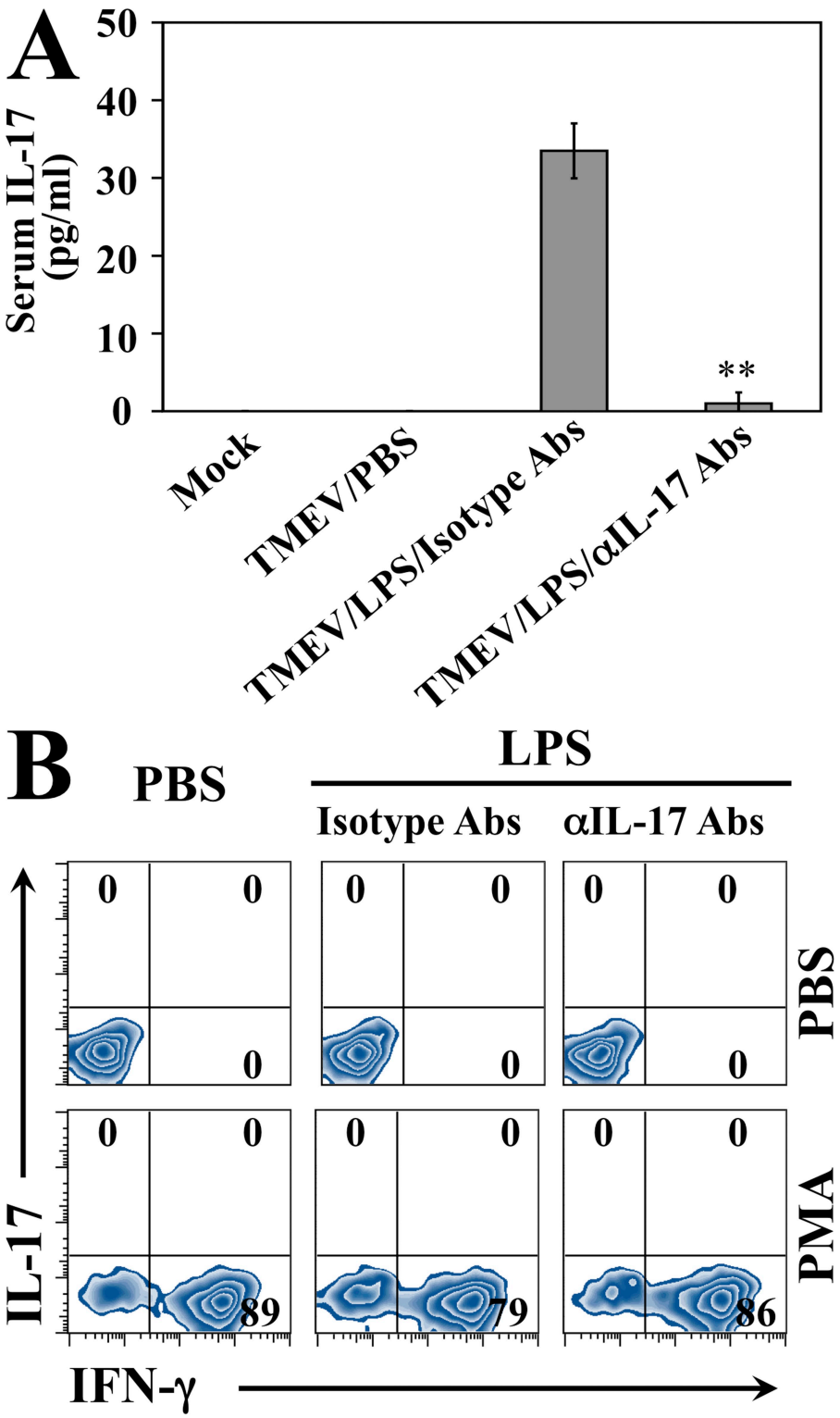

Figure S3. Serum IL-17 levels and CNS-infiltrating CD8⁺ T cell responses in B6 mice treated with anti-IL-17 antibodies during TMEV infection. (A) ELISA analysis of serum IL-17 levels at 8 d after mock or TMEV infection in B6 mice treated with PBS (TMEV/PBS) or LPS and isotype control (TMEV/LPS/Isotype Abs), or anti-IL-17 (TMEV/LPS/αIL-17 Abs) antibodies. Means ± SD are shown. **, $P < 0.01$ between the anti-IL-17 and isotype control antibody-treated groups. (B) Flow cytometry of cytokine expression of CNS-infiltrating cells obtained at 8 d after TMEV infection in B6 mice treated with PBS or LPS and isotype control or anti-IL-17 antibodies followed by restimulation with PMA plus ionomycin. Flow cytometry plots were gated on CD8⁺ T cells. Results represent values with CNS cells pooled from two mice per group. The numbers in each quadrant represent percentages. Data in A and B are representatives of three separate experiments.

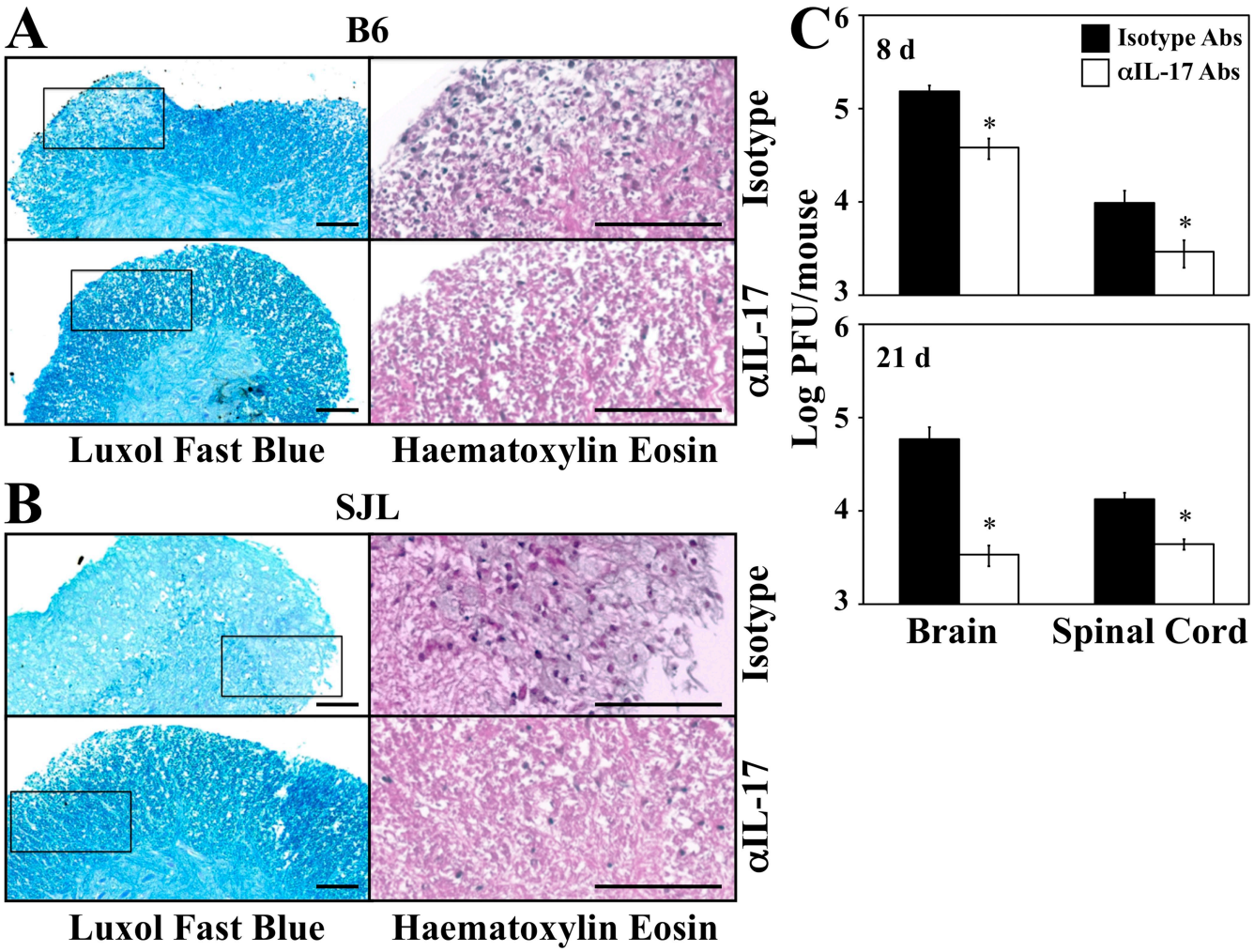

Figure S4. Histopathology and viral persistence in mice treated with anti–IL-17 antibody. LPS-injected (A) B6 and (B) SJL mice were treated with isotype control or anti–IL-17 antibodies, and the spinal cords of these mice were stained with Luxol fast blue and hematoxylin and eosin at 50 d after infection. (right) Enlarged images of the left micrographs. Bars, 100 µm. (C) Viral persistence in the CNS was determined by plaque assay 8 and 21 d after infection. Representative results of three separate experiments derived from pooled brains and spinal cords from two mice per each group are presented (means ± SD are shown). *, $P < 0.05$ between the anti–IL-17 and isotype control antibody–treated groups.

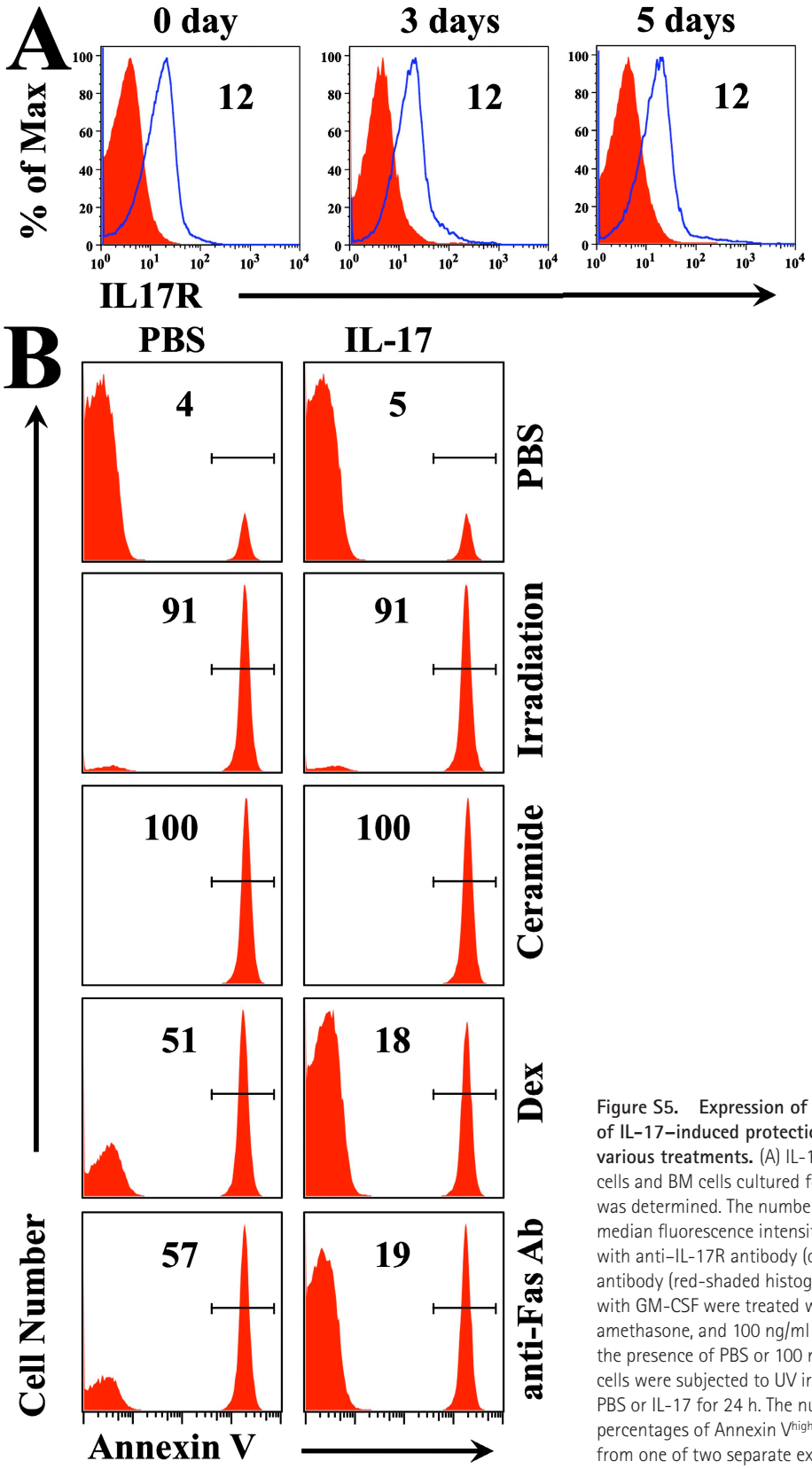

Figure S5. Expression of IL-17R on BM cells, and the range of IL-17–induced protection against apoptosis triggered by various treatments. (A) IL-17R expression on freshly isolated BM cells and BM cells cultured for 3 and 5 d in the presence of GM-CSF was determined. The number in each histogram represents relative median fluorescence intensity differences between cells stained with anti–IL-17R antibody (open histograms) and isotype control antibody (red-shaded histograms). (B) BM cells cultured for 2 d with GM-CSF were treated with 100 µM C2-ceramide, 5 µM dexamethasone, and 100 ng/ml anti-Fas mAb (clone Jo2) for 24 h in the presence of PBS or 100 ng/ml IL-17. In parallel experiments, BM cells were subjected to UV irradiation for 2 h and incubated with PBS or IL-17 for 24 h. The numbers in each histogram show the percentages of Annexin V^{high} cells. Data in A and B represent results from one of two separate experiments.