Supplementary Figure S1. Loss of Ccr7 does not impact leukocyte accumulation or T cell priming in the spleen during WNV infection. (A-F) Following FSC/SSC and UV<sup>-</sup> gating for live cells, total cell numbers of mDC (MHCII<sup>hi</sup>CD11c<sup>+</sup>), CD8<sup>+</sup> T cells (CD3<sup>+</sup>CD8<sup>+</sup>), CD4<sup>+</sup> T cells (CD3<sup>+</sup>CD4<sup>+</sup>), activated CD8<sup>+</sup> T cells (CD3<sup>+</sup>CD8<sup>+</sup>CD44<sup>+</sup>), and activated CD4<sup>+</sup> T cells (CD3<sup>+</sup>CD4<sup>+</sup>CD44<sup>+</sup>) were assessed by flow cytometry on days 0, 1, 3, and 5 p.i. from the spleen of WT and  $Ccr7^{/-}$  mice. (G) Viral load was assessed from the spleen of WT and  $Ccr7^{/-}$  mice by an FFU assay. (H) The percentage of  $CD3^+CD4^+$  T cells expressing IL-17, after stimulation ex vivo for 8 hours with an immunodominant Db-restricted WNV NS4B peptide and gating on FSC/SSC and UV<sup>-</sup> for live cells, from the spleen of day 8 WNV-infected WT and  $Ccr7^{-1}$  mice was assessed by flow cytometry. (K) The total number of Treg cells from the spleen of day 8 WNV-infected WT and Ccr7<sup>-/-</sup> mice is shown and were gated on FSC/SSC, UV<sup>-</sup> for live cells, CD3<sup>+</sup>CD4<sup>+</sup>, and FoxP3<sup>+</sup>CD25<sup>+</sup> by flow cytometry analysis. (**I-J and L**) Splenocytes from day 8 WNV-infected WT and  $Ccr7^{-}$  mice were stimulated ex vivo for 8 hours with an immunodominant D<sup>b</sup>-restricted WNV NS4B peptide and intracellular cytokine expression of IFN- $\gamma$  and/or TNF- $\alpha$  was assessed by flow cytometry analysis with the dot plots, percentages, and total numbers shown. Gating strategy: FSC/SSC UV  $CD3^+CD8^+$  followed by IFN- $\gamma$  and/or TNF- $\alpha$  expression. Data are shown as mean ±SD for n=3-9 mice per genotype and time point from two independent experiments.



Supplementary Figure S2. Cytokines are elevated in the CNS of Ccr7-deficient mice. (A-H) Protein levels of IL-1 $\beta$ , IL-12, IL-22, Ccl3, Ccl4, Ccl5, Ccl2, and Ccl7 in the CNS of WT and  $Ccr7^{-/-}$  mice at days 0, 8, 10, and 12 p.i. were measured by multiplex ELISA. Data are shown as mean ±SD for n=3-9 mice per genotype and time point from two independent experiments. \*, P < 0.05 and \*\*\*, P < 0.001.

