

Figure S1. Gating strategies used. Seven days after infection, brain cells were isolated from untreated mice, and stained with antibodies to cell markers. Data were acquired on a FACS-Verse and analyzed using the gating strategies shown in the figure. (A) Gating of microglia, macrophages, and lymphocytes. (B) Gating strategy for virus-specific CD4 T cells. CD8 T cells were gated in the same manner.

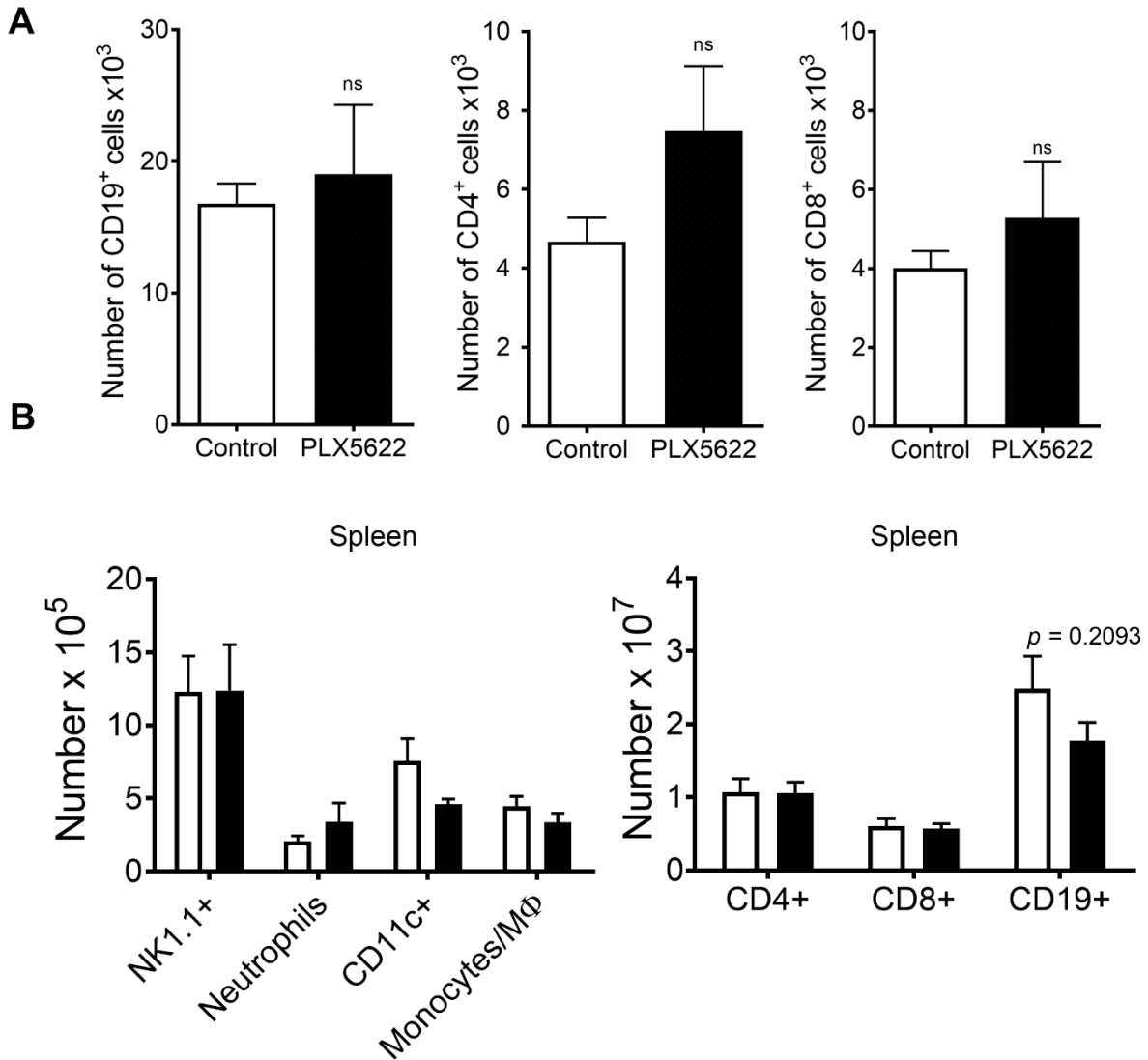


Figure S2. Mice were treated with PLX5622 or control feed for 7 days. (A) CD19⁺ B cells, CD4 and CD8 T cell numbers in the brain. (B) Total numbers of indicated cell types isolated from spleens after treatment with PLX5622. Data are representative of 2 independent experiments with 5 mice per group. Statistical significance determined by Mann Whitney U tests.

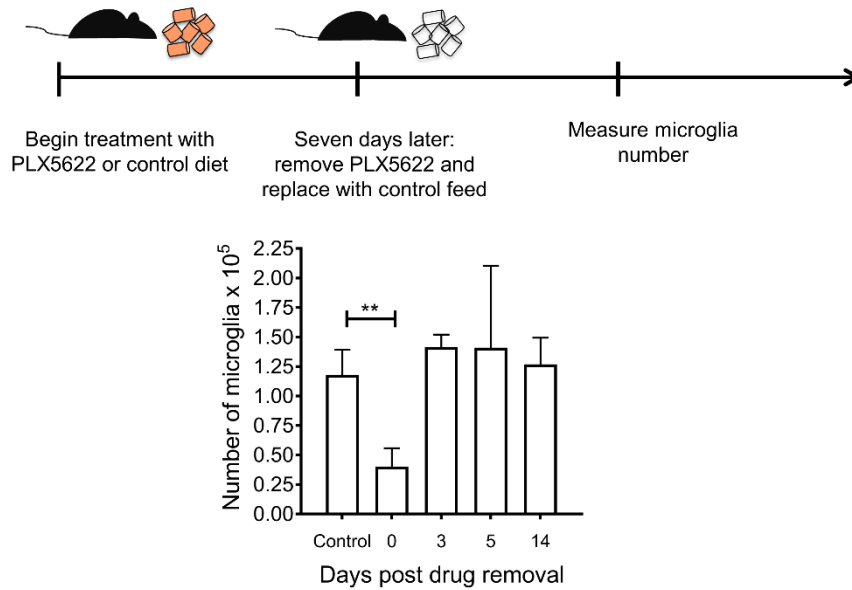


Figure S3. Microglia recovery after PLX5622 removal. Mice were treated with PLX5622 for 7 days and then PLX5622-containing food was replaced with control feed. Microglia numbers were measured by flow cytometry at day 0, 3, 5, and 14 post-removal of PLX5622. Data are from 3 mice per group. *** $p < 0.001$ by Mann-Whitney U.

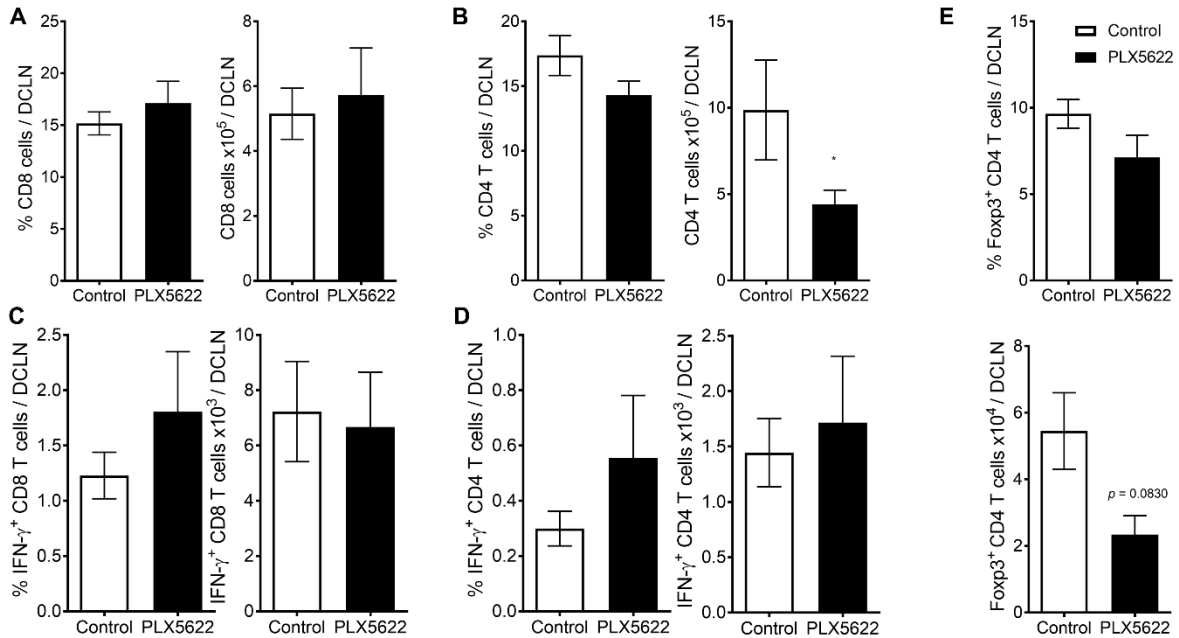


Figure S4. Altered CD4 T cell response in the DCLN after microglia depletion. Mice were treated with PLX5622 for 7 days prior to intracranial MHV infection and immune cells in the DCLNs were assessed by flow cytometry at day 7 p.i. Frequency and number of CD8 T cells (A), CD4 T cells (B) and Tregs (E) in the DCLN. Virus-specific CD8 and CD4 T cell frequency and number determined by IFN- γ ICS after stimulation with S510 peptide (C) or M133 peptide (D), respectively. Data represent combined data from 3 independent experiments with a total of seven-eight mice per group. * $p < 0.05$, as assessed by Mann-Whitney U test.

Table S1. List of primers

Gene		Primers
mHPRT	Fwd	5'-gcgtcgtgattagcgatgatg
	Rev	5'-ctcgagcaagtctttcagtc
gRNA	Fwd	5'-agggagtttgaccttggtcag
	Rev	5'-ataatgcacctgtcatcctcg
IFN α 4	Fwd	5'-tccatcagcagctcaatgac
	Rev	5'-aggaagagagggctctccag
IFN β	Fwd	5'-tcagaatgagtggtggttc
	Rev	5'-gaccttcaaatgcagtagattca
IL-6	Fwd	5'-gctaccaaactggatataatcagga
	Rev	5'-ccaggtagctatggtactccagaa