Supporting Information

Blackmore et al. 10.1073/pnas.1620415114

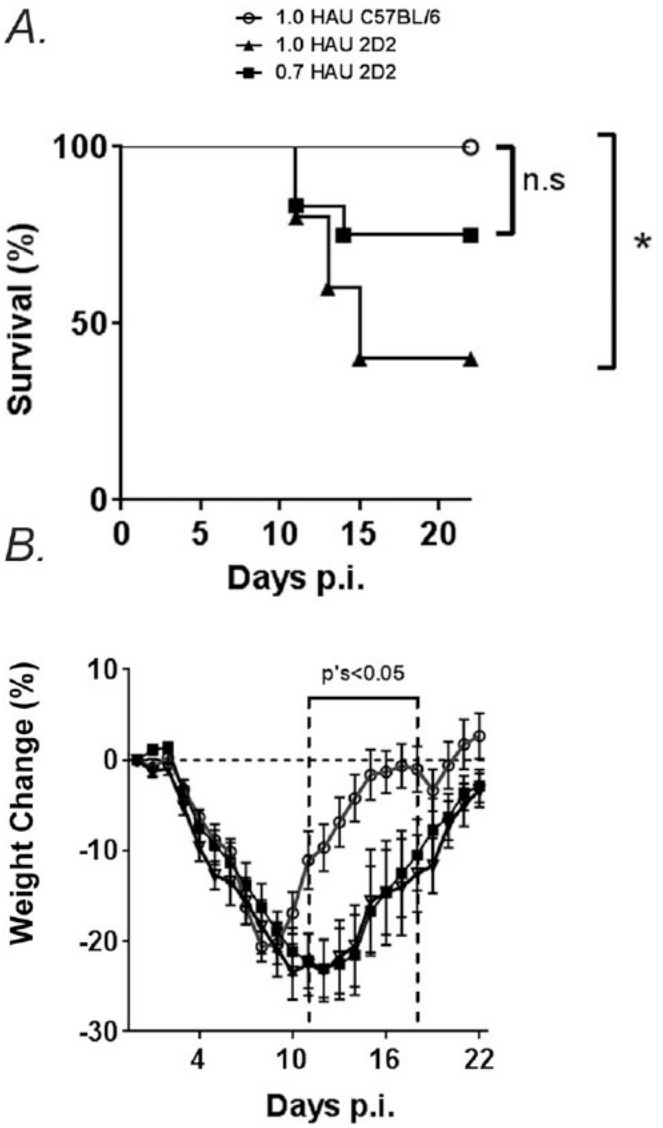

Fig. S1. Transgenic 2D2 mice survive infection with influenza. Control C57BL/6J and transgenic 2D2 mice were inoculated with influenza. (*A* and *B*) The effects of inoculation with 1.0 HAU on C57BL/6J (open circles) and transgenic 2D2 (closed squares) survival (*A*) and weight loss (*B*). Fewer 2D2 mice met the criteria for euthanasia following 0.7 HAU inoculation (*A*), although these mice exhibited prolonged sickness as determined by weight loss (*B*). Results in *A* are combined from four independent experiments. For C57BL/6 mice, $n = 5$; for 0.7 HAU 2D2 mice, $n = 24$; and for 1.0 HAU 2D2 mice, $n = 5$. Results in *B* are expressed as means ± SE ($n = 5$–7 per group). *Ps < 0.05.

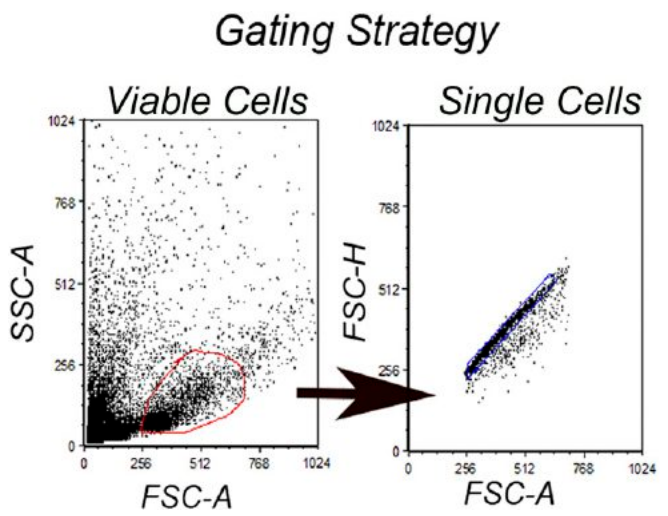

Fig. S2. Gating strategy for comparing percentages of leukocytes isolated from the brain of 2D2 mice. The gating strategy used to determine viable and single cells obtained from the brains of saline- and influenza-inoculated 2D2 mice. This gating strategy was used to generate data shown in Fig. 1.

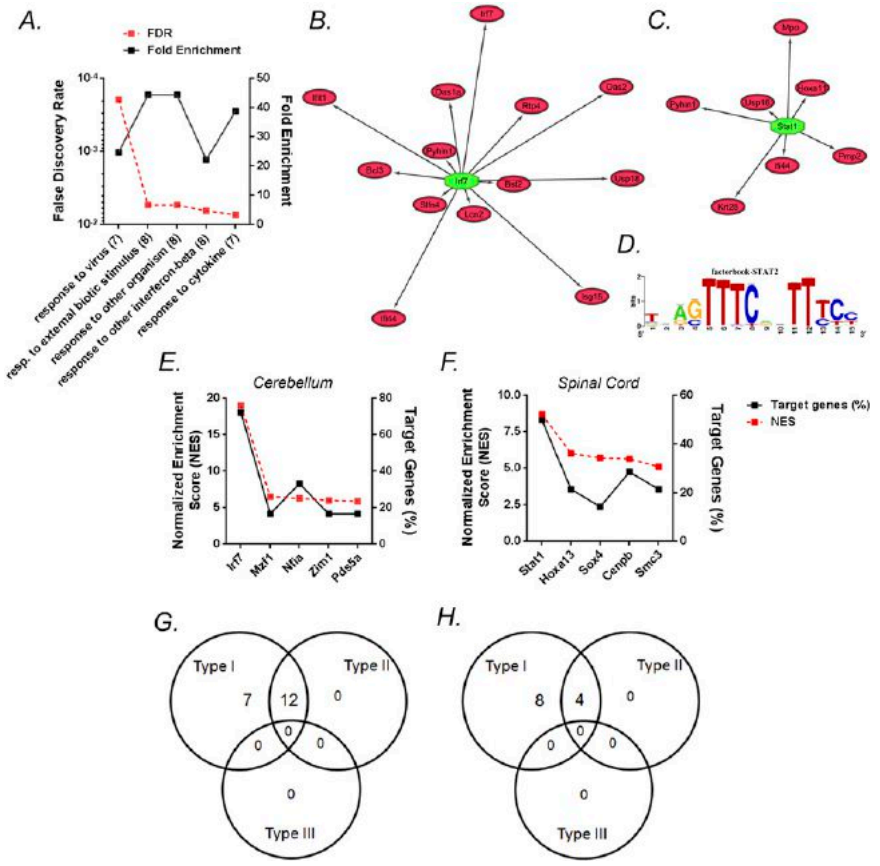

Fig. S3. IFN genes are up-regulated in the cerebellum and spinal cord at day 4 p.i. (*A*) Gene ontology analysis of the cerebellum at day 4 p.i. The numbers of genes associated with each GO term are shown in parentheses. (*B* and *C*) Top putative regulon controlling expression of up-regulated genes in the spinal cord (*Left*) and cerebellum (*Right*) as well as the top transcription factor-binding motif (*C*) as determined by iRegulon analysis. Analysis were performed on 20 kb flanking the transcriptional start site of each target gene. (*D* and *E*) Top five putative regulons, the percentage of target genes, and normalized enrichment scores identified from cerebellar and spinal cord up-regulated genes. (*F* and *G*) Interferome analysis conducted on up-regulated genes in the cerebellum (*Left*) and spinal cord (*Right*). For all up-regulated genes, fold change was >1.5 and FDR was <0.05.

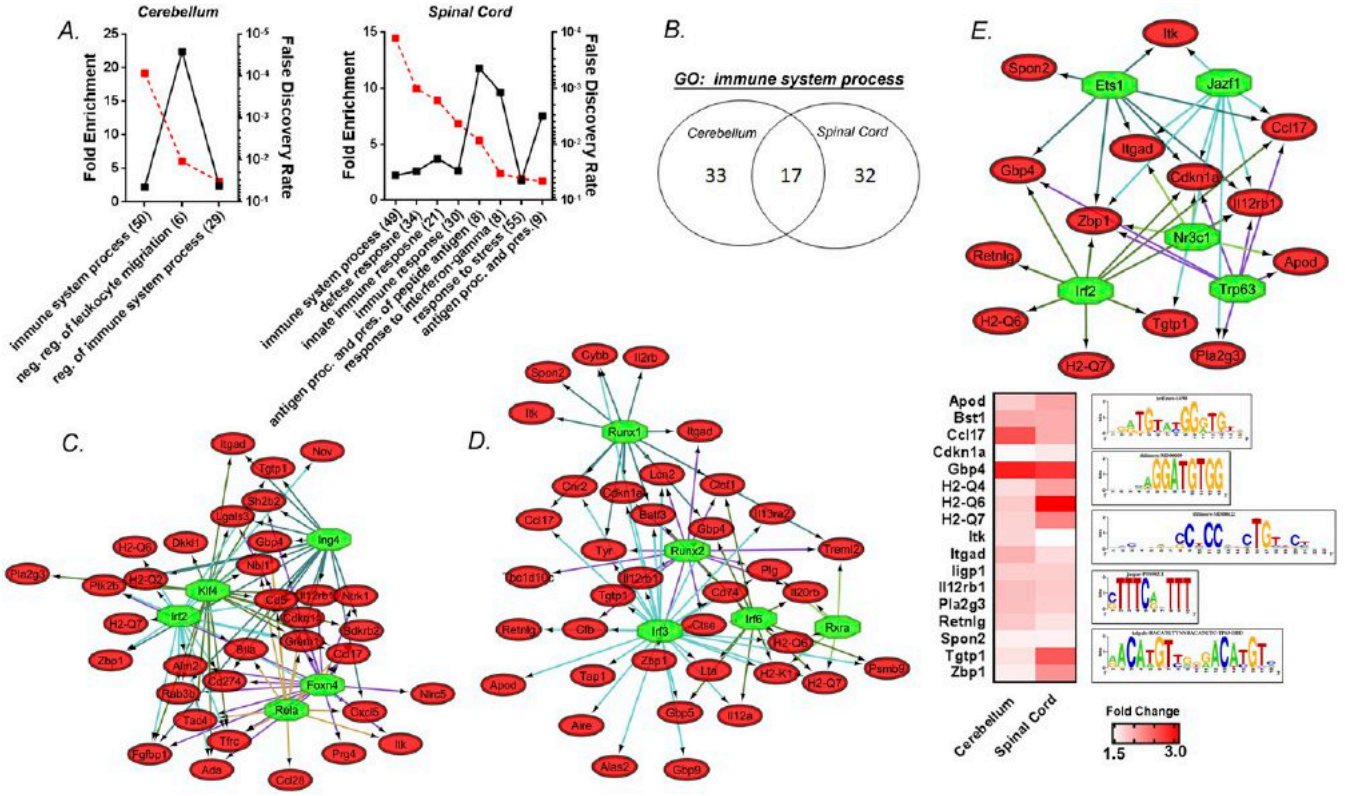

Fig. S4. Bioinformatic analysis of up-regulated genes at day 8 p.i. (*A*) Significant gene ontology terms of up-regulated genes showing >1.4-fold change in expression and an FDR <0.05 in the cerebellum (*Left*) and spinal cord (*Right*). The numbers of genes associated with a GO term are shown in parentheses. The numbers of genes comprising the GO term immune system process that are distinct and shared between each tissue (*B*). (*C* and *D*) Gene regulatory network analysis showing the top five transcription factors and their putative targets for cerebellar (*C*) and spinal cord (*D*) genes. (*E*) Putative transcriptional regulators of shared genes (*Top*), their expression levels compared with control samples (*Bottom, Left*), and the top transcription factor-binding motifs (*Bottom, Right*). Scale bar for shared up-regulated genes is shown.

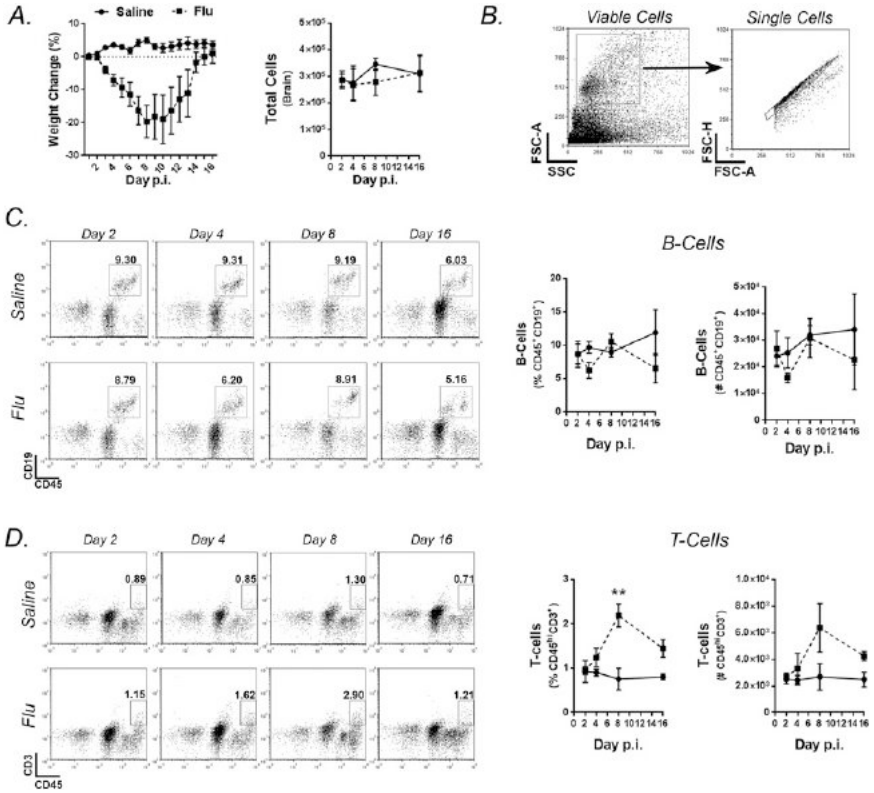

Fig. S5. Influenza inoculation increased T-cell surveillance of the brain. (*A*–*E*) Mice were inoculated with saline ($n = 12$) or influenza ($n = 20$). On days 2, 4, 8, and 16 p.i., CNS-IL were isolated and stained with antibodies specific for hematogenous cells ($CD45^+$), T-cells ($CD3^+$), and B-cells ($CD19^+$). (*A*) Effect of infection on weights (*Left*) and total number of cells isolated from brain (*Right*). (*B*) Gating strategy for viable and single cells. (*C* and *D*) Representative flow cytometry plots, cell percentage, and numbers of $CD45^+$ $CD3^+$ T-cells (*C*) and $CD45^+$ $CD19^+$ B-cells (*D*) in saline-inoculated mice ($n = 3$) and influenza-inoculated mice ($n = 5$) at days 2, 4, 8, and 16 in the brain. Significance was determined by two-way ANOVA. Results are means ± SE; $^{**}P < 0.01$.

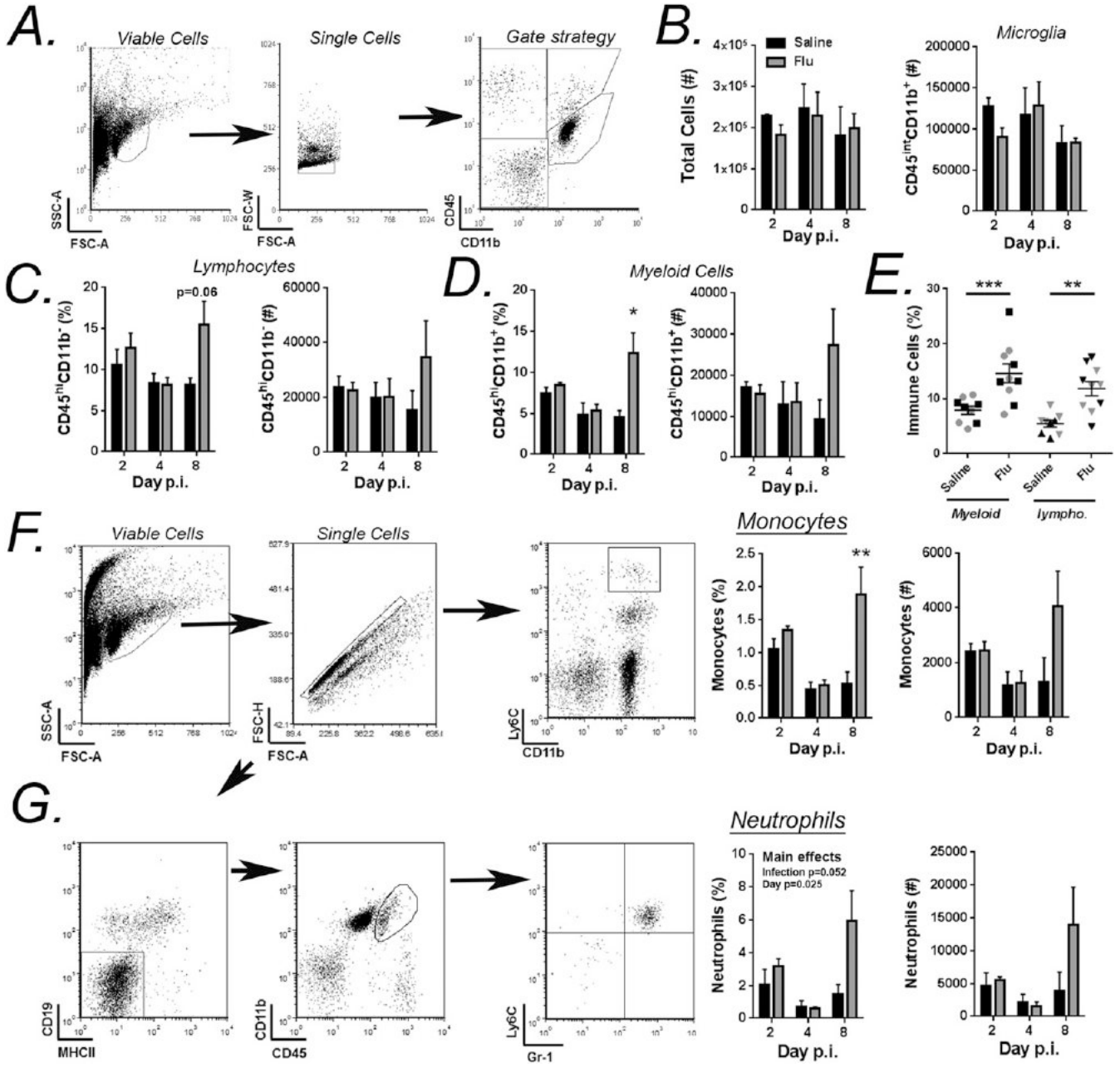

Fig. S6. The effect of infection on monocyte and neutrophil brain infiltration. (*A*–*E*) C57BL/6 mice were inoculated with saline or influenza (1.0 HAU), and brain-infiltrating lymphocytes were isolated by percoll gradient centrifugation at days 2, 4, and 8 p.i. (*A*) Gating strategy used to determine viable (*Left*) and single (*Middle*) cells. Antibodies specific for CD45 and CD11b were used to determine microglia ($CD45^{int}CD11b^{+}$), lymphocytes ($CD45^{+}CD11b^{-}$), and myeloid cells ($CD45^{hi}CD11b^{+}$) (*Right*). (*B*) The total number of recovered cells (*Left*) and microglia (*Right*) are shown. (*C* and *D*) The effect of infection on the percentages and numbers of lymphocytes (*C*) and myeloid cells (*D*) is shown. Results are expressed as means ± SE from two independent experiments ($n = 3$–5 per group). (*E*) The combined effect of infection on the percentages of myeloid cells (*Left*) and lymphocytes (*Right*) in the brain from experiments described above (gray) and those represented in Fig. 5 (black). Individual mice are shown. Results are expressed as means ± SE ($n = 9$–10 per group). (*F* and *G*) Gating strategy used to determine the effect of infection on percentageand number of brain-infiltrating inflammatory monocytes (*F*) and neutrophils (*G*). Statistical analyses were done using two-way ANOVAs. Main effects are shown in *G*. Results are from two independent experiments ($n = 3$–5 per group) and are expressed as means ± SE; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

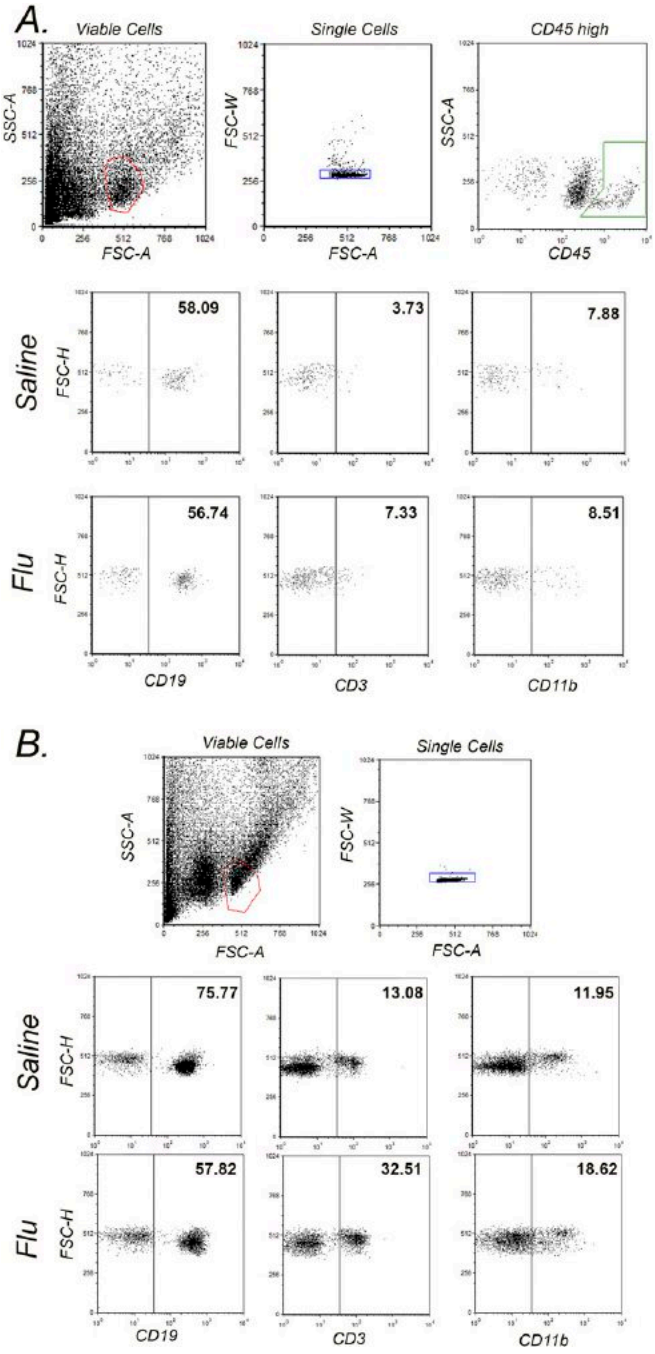

Fig. S7. Gating strategy for comparing percentages of leukocytes isolated from brain and blood. (*A* and *B*) The gating strategy used to determine the effect of infection on percentages of B-cells ($CD45^{hi}CD19^{+}$), T-cells ($CD45^{hi}CD3^{+}$), and monocyte/neutrophils ($CD45^{hi}CD11b^{+}$) isolated from the brain (*A*) and peripheral blood (*B*) is shown.

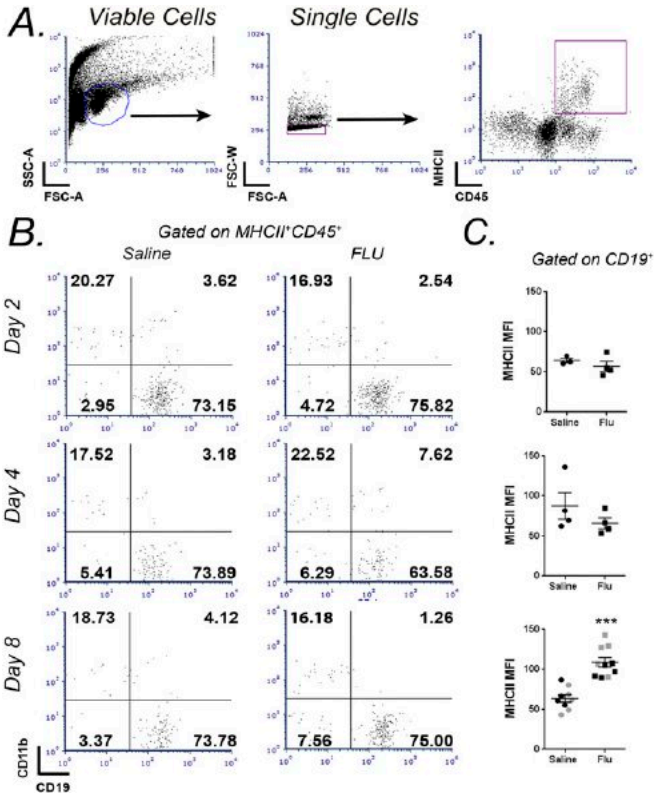

Fig. S8. Infection increases the percentage of MHC class II-expressing B-cells in the CNS. (*A–C*) C57BL/6 mice were inoculated with sterile saline or influenza (1.0 HAU) i.n., and MHC class II expression on myeloid/microglia ($CD11b^+$) and B-cells ($CD19^+$) was examined by flow cytometry during the course of infection. (*A* and *B*) Gating strategy for identifying MHC class II-expressing cells in the CNS of naive mice. (*C*) The effect of infection on MHC class II mean fluorescent intensity of $CD11b^-CD19^+$ B-cells during the course of infection is shown. Results are expressed as means ± SE. Individual mice are shown (n = 4–10 per group). Results at day 8 p.i. are from two cohorts of mice that are distinguishable by color. ***$P < 0.001$.

Table S1. Patient demographics for CSF samples in relapsing-remitting multiple sclerosis patients in relapse or remission

Sex	Age (y)*	Disease status	Therapy
M	42	Relapse	IFN-β
M	29	Relapse	IFN-β
M	31	Relapse	NA
M	42	Relapse	NA
M	47	Relapse	NA
M	53	Relapse	NA
F	37	Relapse	NA
M	58	Relapse	NA
M	49	Relapse	NA
M	47	Relapse	NA
M	35	Remission	Copolymer-1
M	32	Remission	Copolymer-1
M	32	Remission	NA
M	39	Remission	NA
F	51	Remission	Chlorambucil
F	44	Remission	Chlorambucil
F	51	Remission	Chlorambucil
M	40	Remission	Chlorambucil
M	48	Remission	Chlorambucil
M	25	Remission	Chlorambucil

Information not available (NA).
*Age did not differ between groups.