Supplementary Information

Figure S1

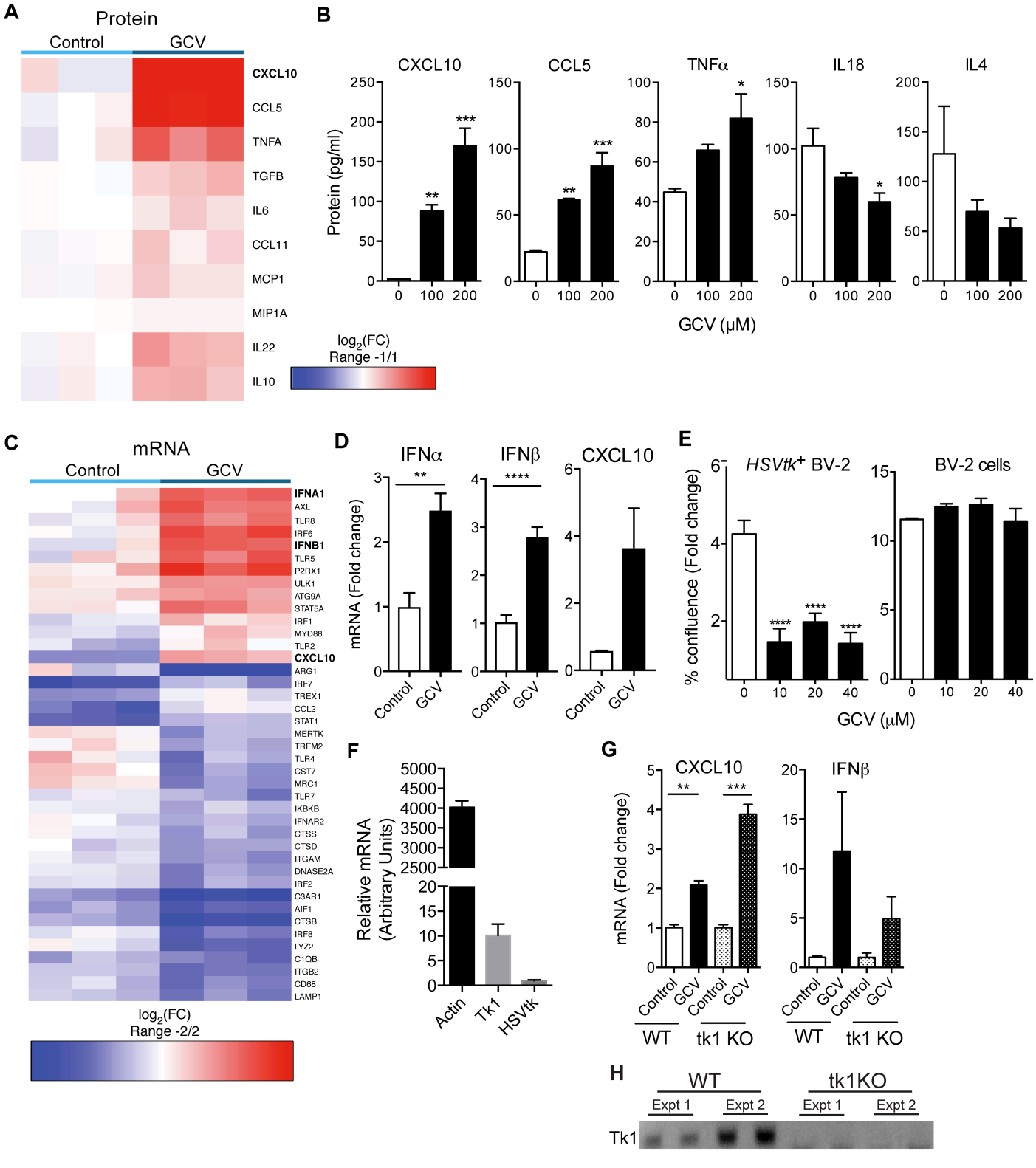

Figure S1. Ganciclovir induces interferon response in BV-2 microglia-like cells independent of thymidine kinase. Related to Figure 1. (A-B) Heatmap (A) and dose curves (B) of differentially secreted proteins in conditioned supernatants from BV-2 cells treated with GCV for 24h, analyzed by 38-plex Luminex-based array. (C-D) Heatmap (C) and quantification of type I interferon response genes (D) of microfluidic qRT-PCR array from BV-2 cells treated with GCV for 24h showing differentially expressed transcripts. (E) Cells stably expressing HSVtk and BV-2 cells used in this study were treated with indicated concentrations of GCV for 48h. Percent confluence measured using automated cell counting. Fold change is based on confluence at 0h. (F) qRT-PCR analysis of primary microglia used in this study showing no detection of HSVtk mRNA compared to Actin and Tk1. (G) Wild type (WT) and thymidine kinase 1 knockout (tk1 KO) primary microglia were treated with GCV for 6h and mRNA quantified. (H) RT-PCR followed by agarose gel electrophoresis shows Tk1 transcript in isolated wildtype (WT), but not tk1 knockout (tk1KO), microglia. Two replicates from each experiment are shown. Fold change is based on control treatment for each genotype. All GCV treatments were with 200μM unless otherwise noted. Bars represent mean + SEM from 2-3 independent biological repeats. Statistical test: one-way ANOVA followed by Dunnett's multiple comparison test (B, E), unpaired Student's t-test (D, G).

Figure S2

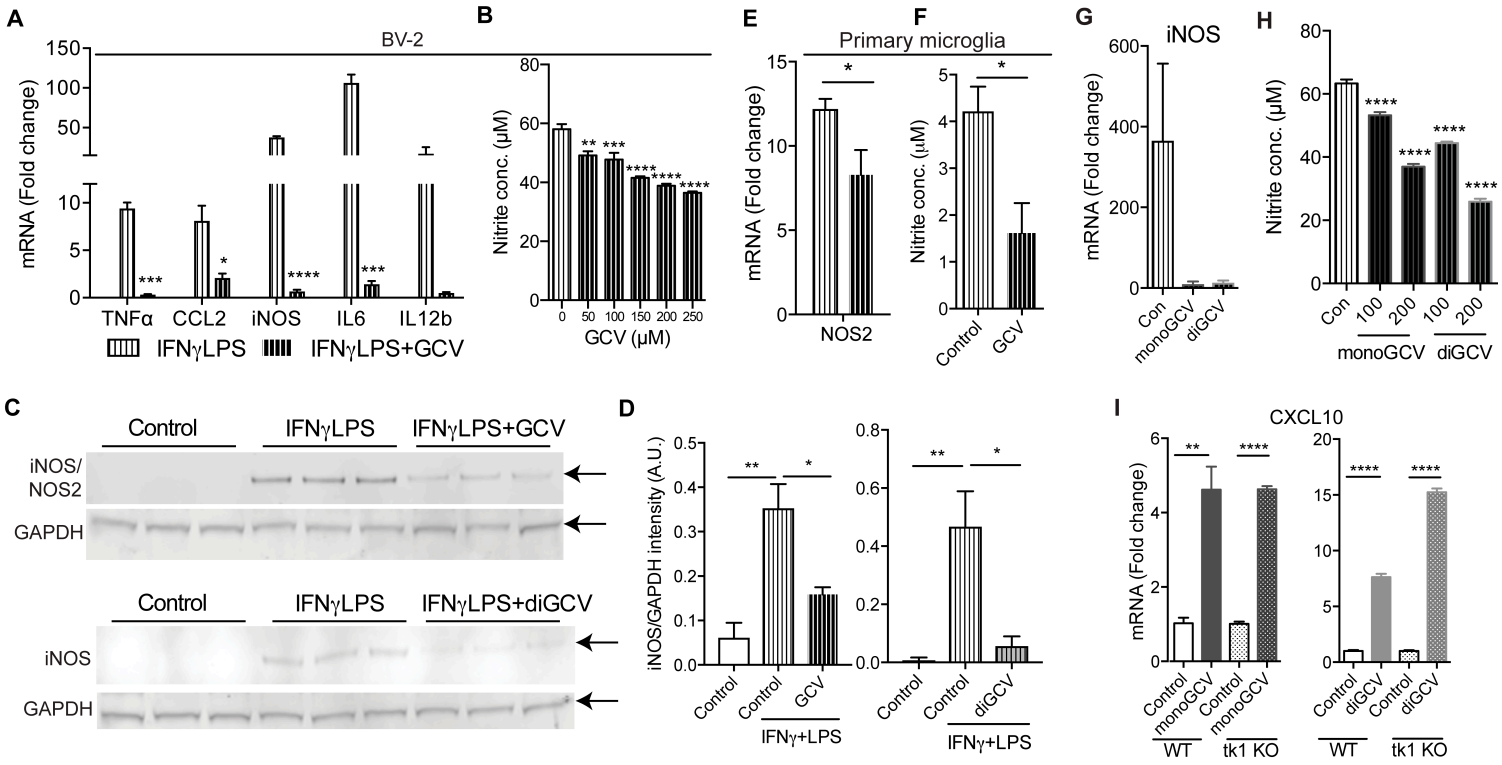

Figure S2. Ganciclovir and its derivatives reduce inflammation in microglia. Related to Figures 1 and 2. BV-2 cells stimulated with IFNγ/LPS for 24h showing differentially expressed transcripts (A), nitrite production (B) and; Western blot for iNOS/NOS2 (C) and quantification of protein (D). (E-F) Primary microglia were stimulated with IFNγ/LPS with or without GCV for 24h showing iNOS transcript (E) and nitrite production (F) after GCV treatment. (G-H) iNOS transcript (G) and nitrite production (H) in IFNγ/LPS treated BV2 cells with monoGCV or diGCV. (I) Wild type and thymidine kinase 1 knockout (tk1 KO) primary microglia were treated with indicated drugs for 6h and CXCL10 mRNA was quantified by qRT-PCR. Fold change is based on control treatment for each genotype. GCV, monoGCV and diGCV treatments were with 200μM unless otherwise noted. Statistical tests: one-way ANOVA followed by Dunnett's multiple comparison test (B, D, H), unpaired Student's t-test (A, E, G, H, I). mRNA fold change was determined by qRT-PCR. Bars represent mean + SEM from 3 independent biological repeats for BV-2 cells and 2 for primary microglia.

Figure S3

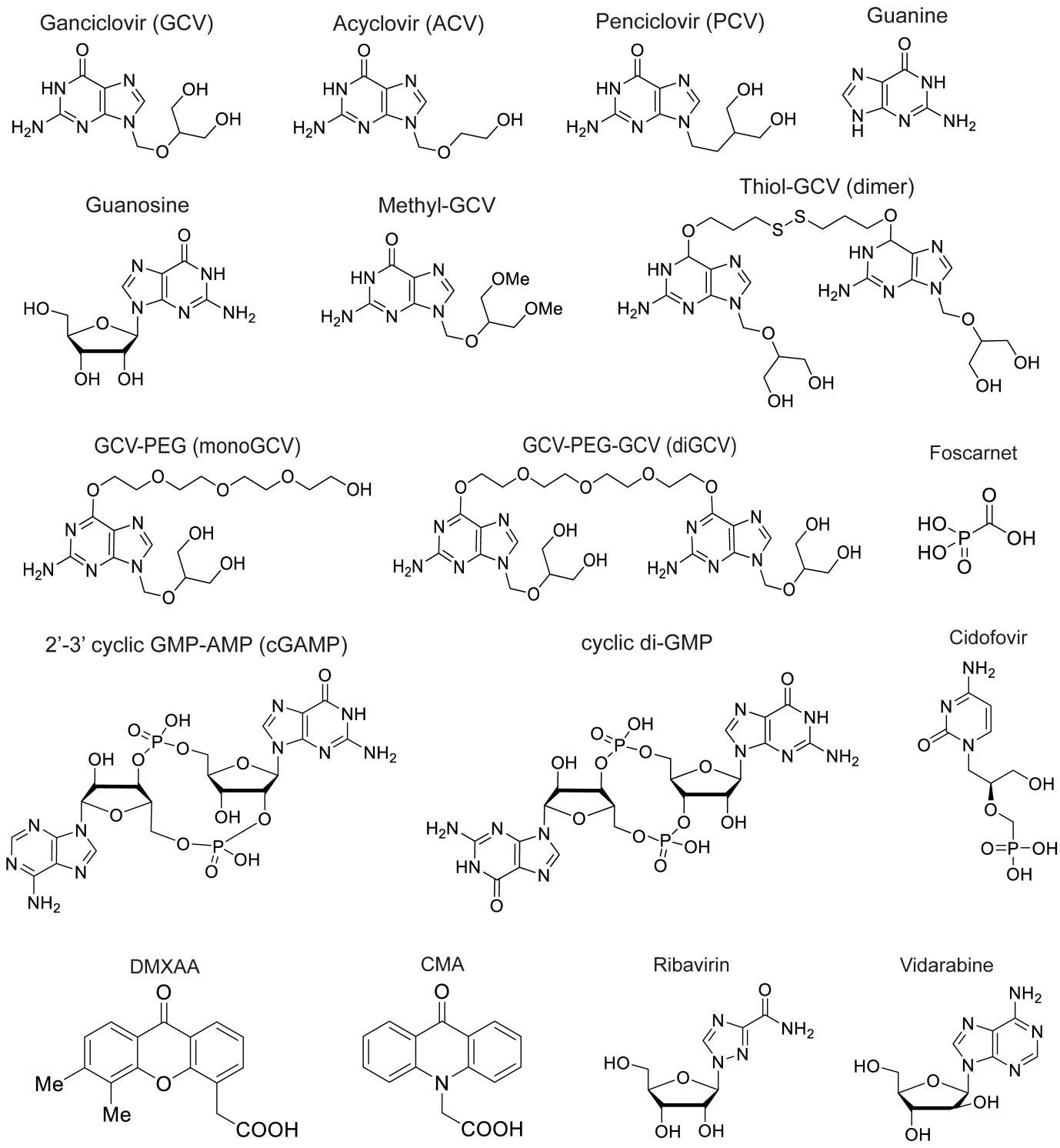

Figure S3. Related to Figures 2, 3 and 4. Structures of compounds used in this study.

Figure S4

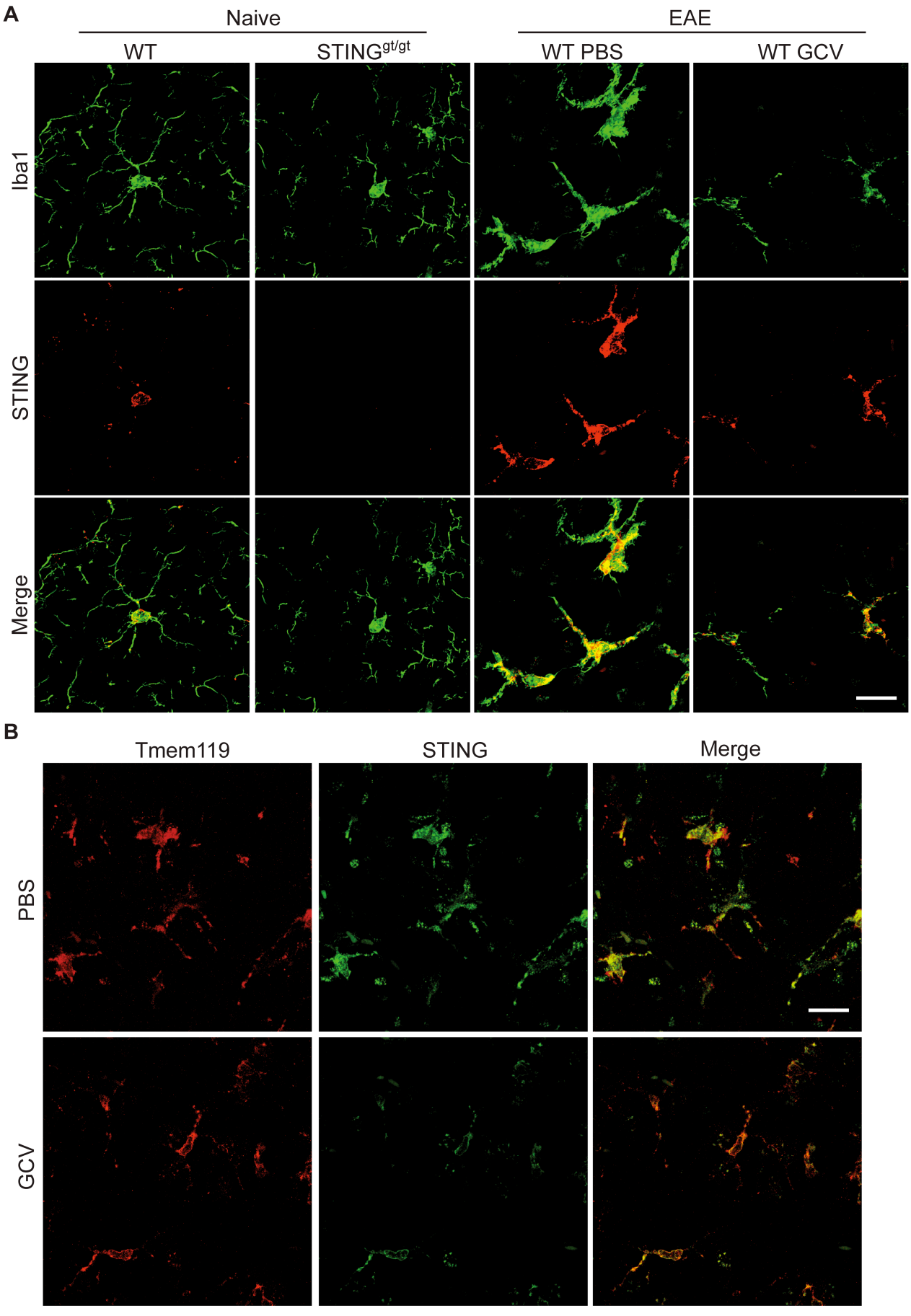
A
Naive
EAE
WT
STING$^{gt/gt}$
WT PBS
WT GCV
Iba1
STING
Merge
B
Tmem119
STING
Merge
PBS
GCV

Figure S4. Related to Figure 5. Representative immunohistological images. (A) Iba1 and STING double stain in the hippocampus (Naïve) and cerebella (EAE) of indicated mice. (B) Tmem119 and STING double stain in the cerebella of mice with EAE. Scale bar = 20μm.

Figure S5

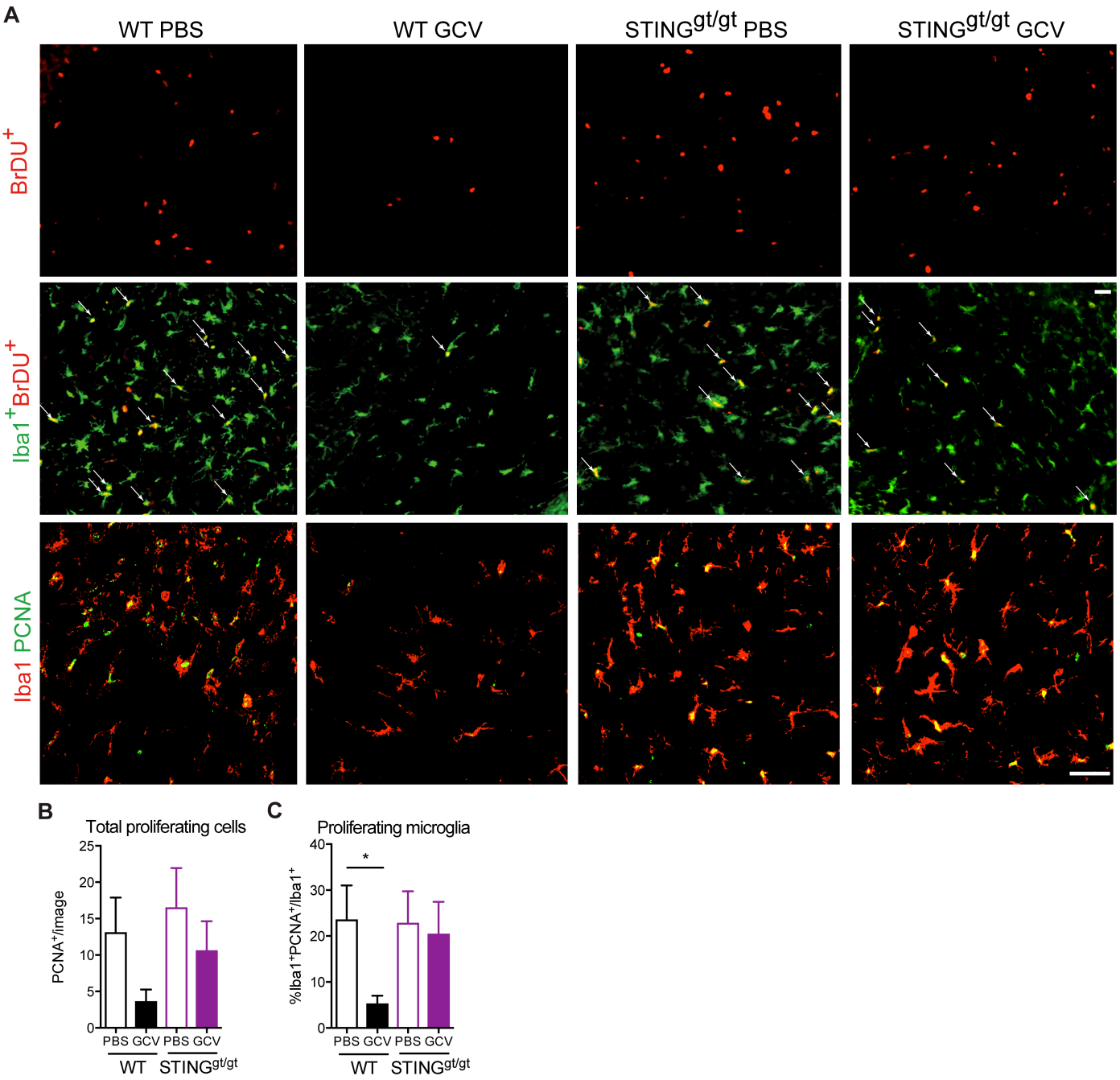

Figure S5. Related to Figure 5. Ganciclovir inhibits proliferation of myeloid cells in mice with EAE. (A) Representative images from immunohistological analysis of cerebella of mice with EAE. Arrows point to Iba1^{+}BrDU^{+} cells. (B) Percent cell counts of Iba1 and PCNA from immunohistological staining of cerebella of mice with EAE. Scale bar = 20μm. n= 6 mice/group. Bars represent mean + SEM. Statistical tests: Two- way ANOVA followed by Sidak's multiple comparisons test between indicated groups.

Figure S6

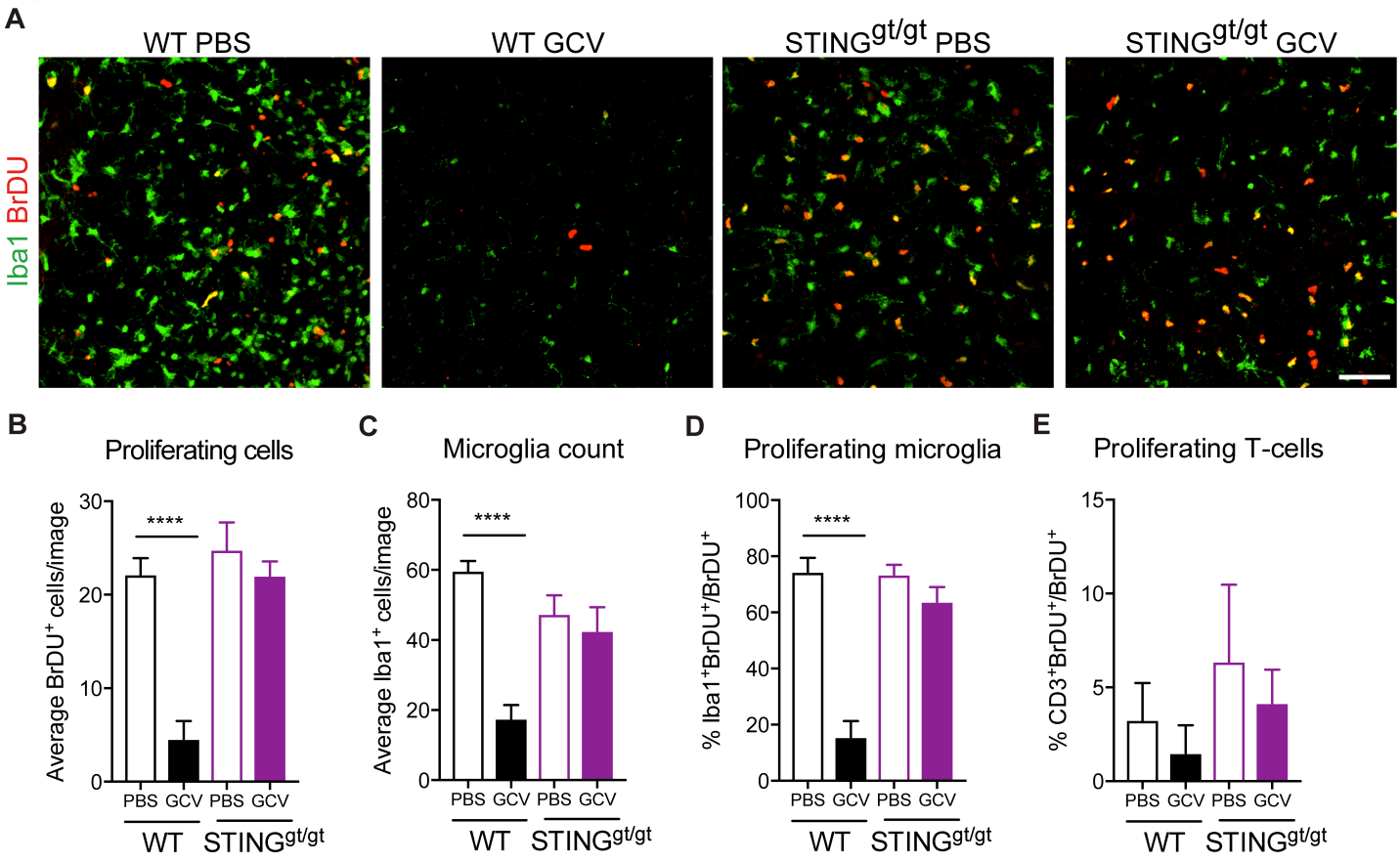

Figure S6. Ganciclovir inhibits proliferation of myeloid cells in the spinal cords of mice with EAE. Related to Figure 5. Representative images (A) and quantification of average number of $BrDU^+$ proliferating cells (B), $Iba1^+$ myeloid cells (C), percent $Iba1^+BrDU^+$ proliferating myeloid cells (D) and $CD3^+BrDU^+$ proliferating T-cells (E). Scale bar = 20μm. n= 6 mice/group. Bars represent mean + SEM. Statistical tests: Two- way ANOVA followed by Sidak's multiple comparisons test between indicated groups.

Figure S7

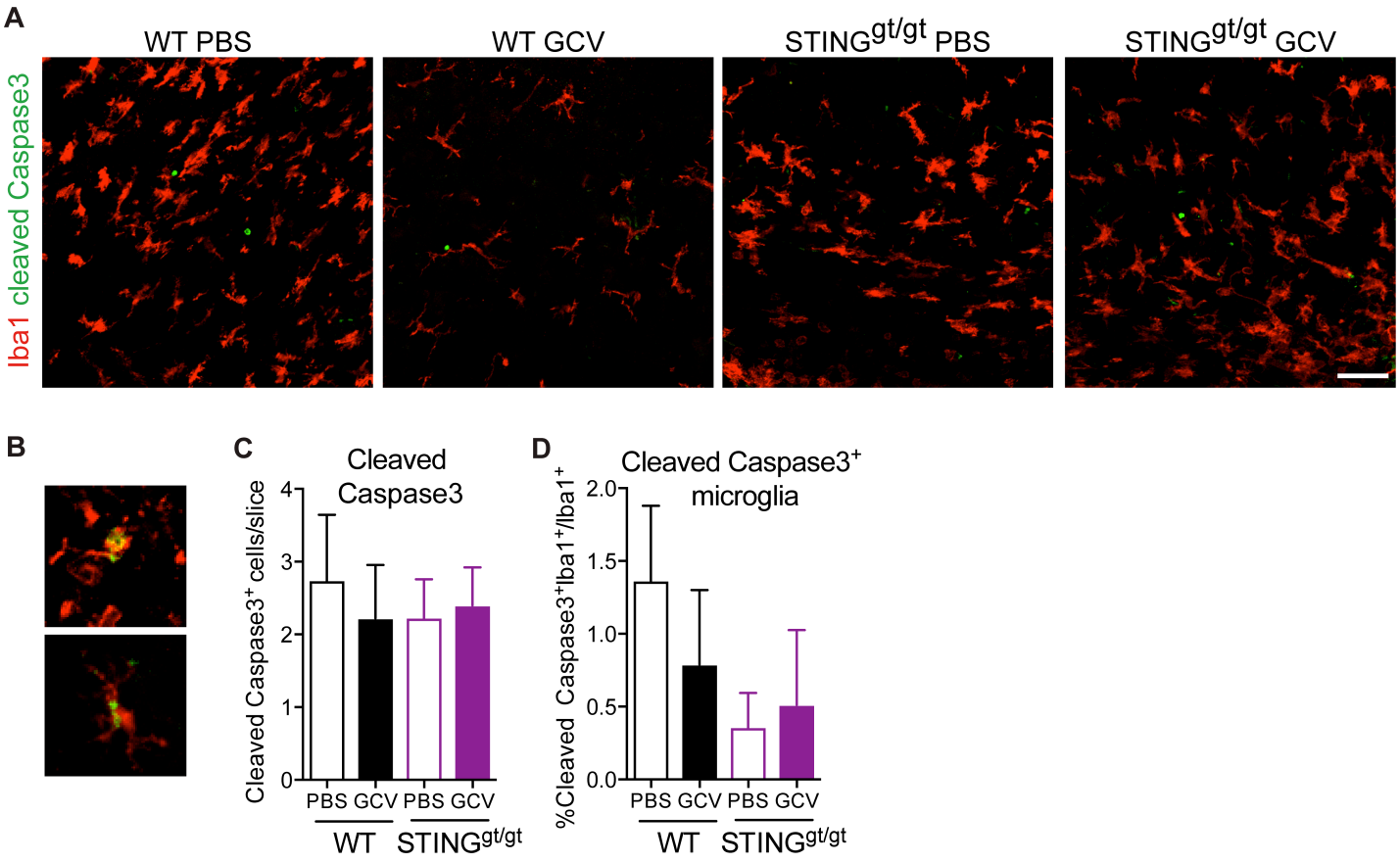

Figure S7. Related to Figure 5. Ganciclovir does not induce apoptosis in myeloid cells of mice with EAE. Representative images (A-B) and percent cell counts of cleaved Caspase 3^{+} (C) and cleaved Caspase3^{+} Iba1^{+} (D) cells in the cerebella of mice with EAE. (B) shows rare Iba1^{+} cells that stain for cleaved Caspase3 as well. Scale bar = 20μm. n= 6 mice/group. Bars represent mean + SEM. Statistical tests: Two- way ANOVA followed by Sidak's multiple comparisons test between indicated groups.

Figure S8

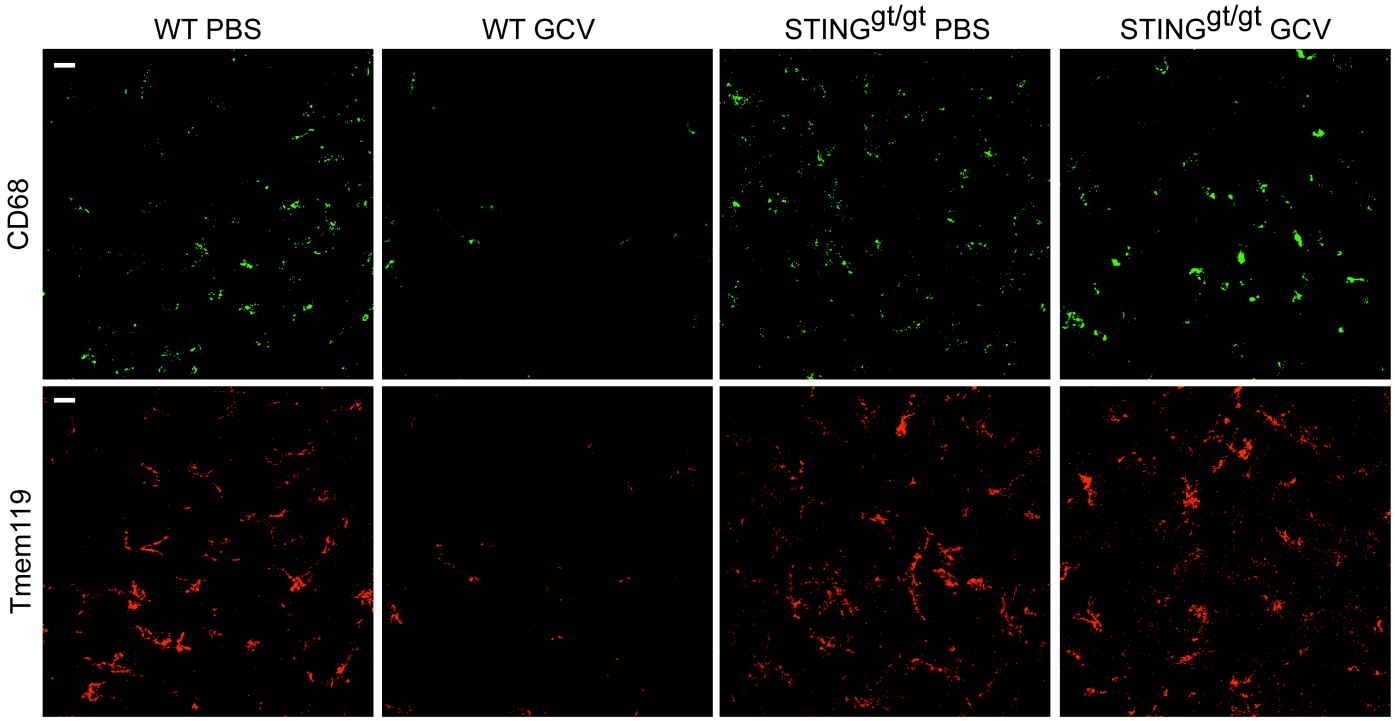

Figure S8. Related to Figure 5. Representative images from immunohistological analysis of CD68 and Tmem119 in the cerebella of mice with EAE. Scale bar = 20μm.

Figure S9

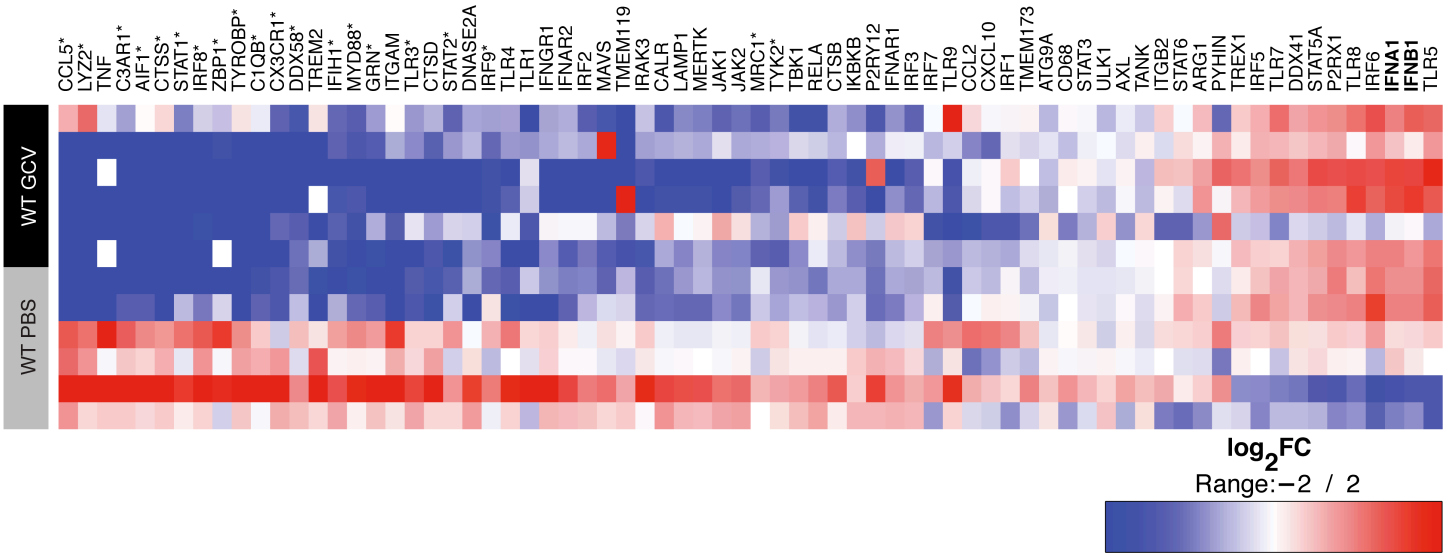

Figure S9. Related to Figure 7. Ganciclovir increases interferon production and decreases inflammatory factors in brains of mice with EAE. Heatmap from microfluidic qRT-PCR array on RNA from cerebella of mice with EAE. *indicates significance ($p < 0.05$ unpaired Student's t-test). n= 6 mice/group.

Figure S10

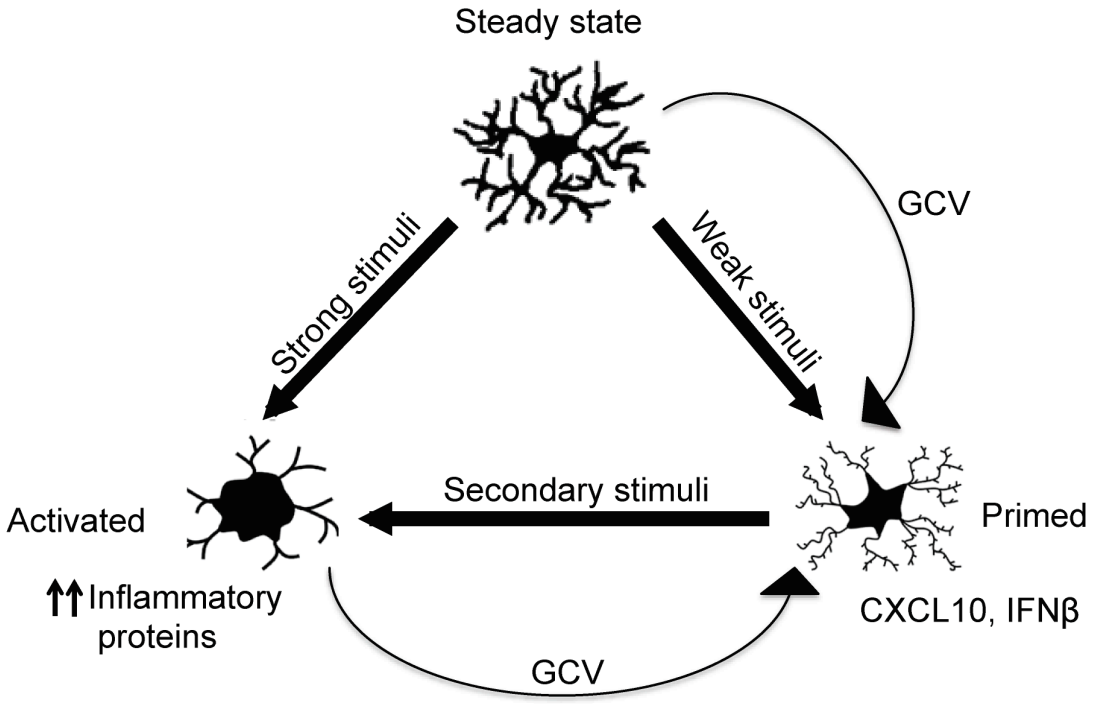

Figure S10. Cartoon showing Ganciclovir activity. We hypothesize that GCV shifts microglia to a primed state.

Table S1. Related to Figure 1. Microfluidic panel gene list with 86 microglia related genes and 10 house-keeping genes. Log_2Fold change (GCV/Control) for each gene in primary microglia is listed.

Table S2. Related to Figure 7. RNA-seq analysis showing log_2fold change, p-value and false discovery rate (FDR or q-value) for three different comparisons: WT GCV vs WT PBS (Sheet 1), $STING^{gt/gt}$ PBS vs WT PBS (Sheet 2), $STING^{gt/gt}$ GCV vs $STING^{gt/gt}$ PBS (Sheet 3).

Table S3. Related to STAR Methods. RT-PCR primers used in this study. All sequences were obtained from Harvard Primer Bank.

Gene	Fwd Primer	Reverse Primer
CXCL10	CCAAGTGCTGCCGTCATTTTC	GGCTCGCAGGGATGATTTCAA
IFNB1	CAGCTCCAAGAAAGGACGAAC	GGCAGTGTAACTCTTCTGCAT
STAT1	TCACAGTGGTTCGAGCTTCAG	GCAAACGAGACATCATAGGCA
IRF3	GGCAGTGTAACTCTTCTGCAT	CTTCCAGGTTGACACGTCCG
cGAS	CATCTTCCCAGCCTGACATT	CACGCTTCCTGCTATGATGA
Tk1	AAGTGCCTGGTCATCAAGTATG	GCTGCCACAATTACTGTCTTGC
HSVtk	GCTTCGTACCCCTGCCATCAAC	GCCCCCAGCACCCGCCAGTAAG
TLR3	GTGAGATACAACGTAGCTGACTG	TCCTGCATCCAAGATAGCAAGT
JAK1	TCCTGCATCCAAGATAGCAAGT	TTGGTAAAGTAGAACCTCATGCG
TNFa	TGGAACTGGCAGAAGAG	CCATAGAACTGATGAGAGG
iNOS	GTTCTCAGCCCAACAATACAAGA	GTGGACGGGTCGATGTCAC
CCL2	TTAAAAACCTGGATCGGAACCAA	GCATTAGCTTCAGATTTACGGGT
IL6	TAGTCCTTCCTACCCCAATTTCC	TTGGTCCTTAGCCACTCCTTC
IL12b	TGGTTTGCCATCGTTTTGCTG	ACAGGTGAGGTTCACTGTTTCT
ACTB	GGCTGTATTCCCCTCCATCG	CCAGTTGGTAACAATGCCATGT
human ACTB	CATGTACGTTGCTATCCAGGC	CTCCTTAATGTCACGCACGAT
human CXCL10	GTGGCATTCAAGGAGTACCTC	TGATGGCCTTCGATTCTGGATT
human IFNB1	GCTTGGATTCCTACAAAGAAGCA	ATAGATGGTCAATGCGGCGTC