Microglia have a protective role in a mouse model of viral encephalitis-induced seizure development and hippocampal damage

Inken Waltl[1,2], Christopher Käufer[1], Ingo Gerhauser[3], Chintan Chhatbar[4], Luca Ghita[4], Ulrich Kalinke[4], and Wolfgang Löscher[1,2]

Supplemental Data

Fig. S1

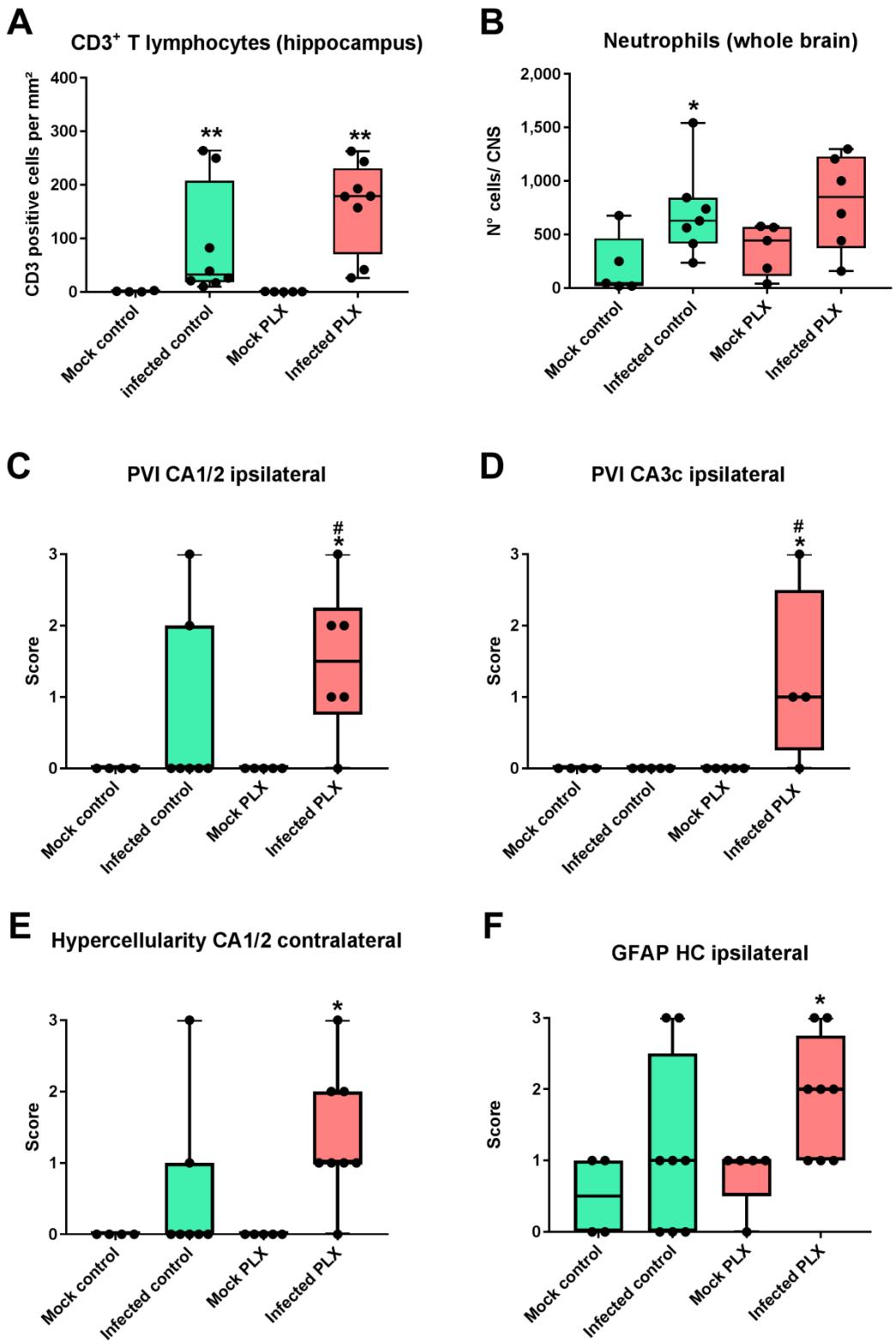

Fig. S1

Neuroinflammation as indicated by CD3$^+$ T cell (A) and neutrophil invasion (B),

perivascular infiltrates (PVI; perivascular cuffing; C,D), hypercellularity (E), and astrogliosis (F). Brain infiltration by $CD3^+$ cells and neutrophils was determined in the whole brain by flow cytometry, while PVI, hypercellularity, and GFAP were semiquantitatively analyzed by (immuno)histology in the hippocampus. Data are shown as boxplots with whiskers from minimum to maximal values; the horizontal line in the boxes represents the median value. In addition, individual data are shown. Sample size: 4 mock-infected controls; 5 mock-infected mice with PLX5622; 7-8 infected controls; 6-8 infected mice with PLX5622. Significant differences to mock-infected mice are indicated by asterisks (*P<0.05; **P<0.01) while significant differences between infected mice treated with PLX5622 and infected controls are indicated by the hash sign ($^{\#}$P<0.05).

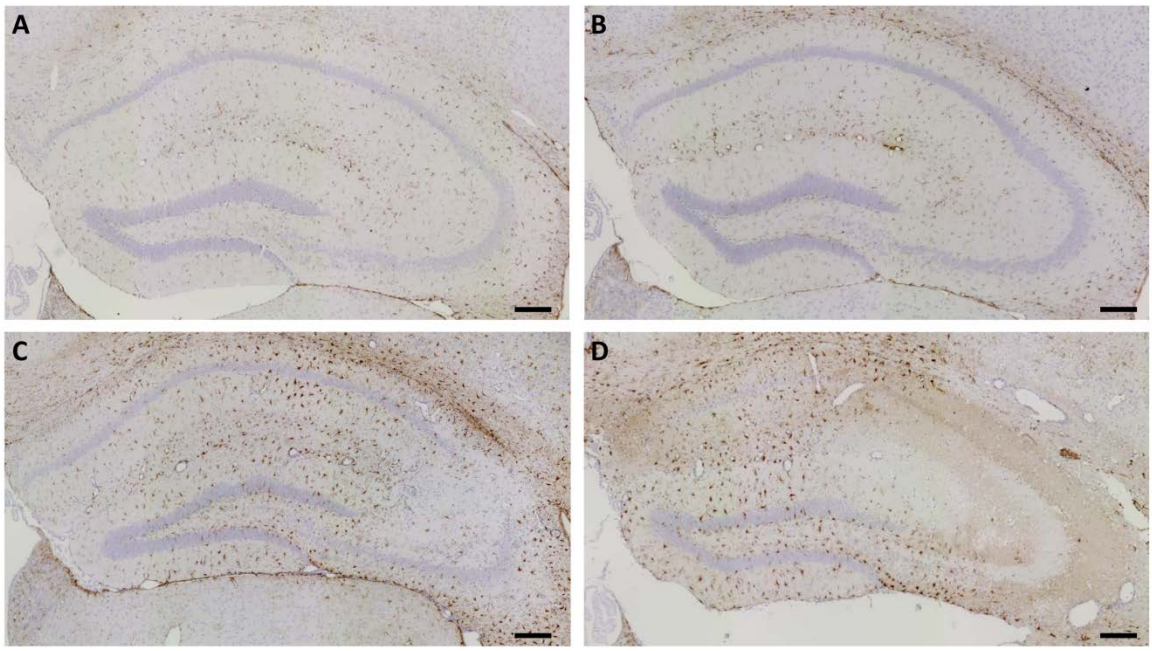

Fig. S2

GFAP-labeled astrocytes in the ipsilateral hippocampal area. Representative photomicrographs are shown for a mock-infected control (A), a mock-infected PLX5622-treated mouse (B), an infected control mouse (C), and an infected PLX5622-treated mouse (D). Note the increased GFAP labeling in the infected mice (C,D). See Fig. 8D for semiquantitative analysis of labeling. Scale bar in D = 200 µm.

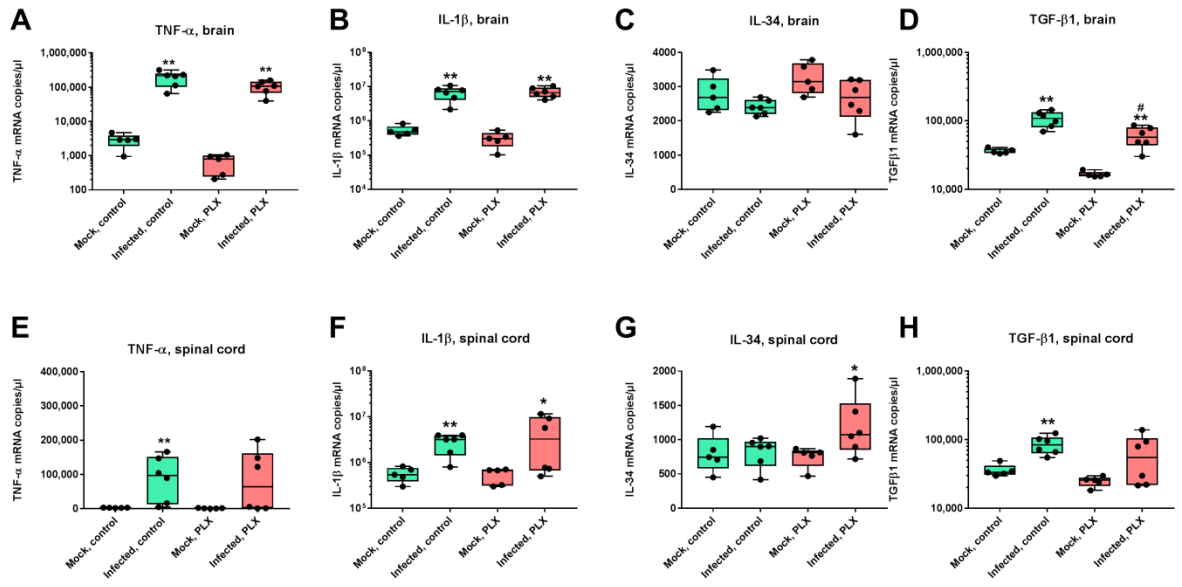

Fig. S3

TNF-α, IL-1ß, IL-34, and TGF-β1 mRNA expression in brain and spinal cord of infected mice. Data are shown as boxplots with whiskers from minimum to maximal values; the horizontal line in the boxes represents the median value. Note that y-axis has a logarithmic scale. In addition, individual data are shown. Sample size is 5 for all noninfected mice and 6 for all infected mice. Significant differences to mock-infected mice are indicated by asterisks (*P<0.05; **P<0.01) while significant differences between infected mice treated with PLX5622 and infected controls are indicated by the hash sign ($^{\#}$P<0.05; $^{\#\#}$P<0.01). (A) TNF-α mRNA expression in brain. (B) IL-1β mRNA expression in brain. (C) IL-34 mRNA expression in brain. (D) TGF-β1 mRNA expression in brain. (E) TNF-α mRNA expression in spinal cord. (F) Il-1ß mRNA expression in spinal cord, (G) IL-34 mRNA expression in spinal cord , (H) TGF-β1 mRNA expression in spinal cord.

Fig. S4

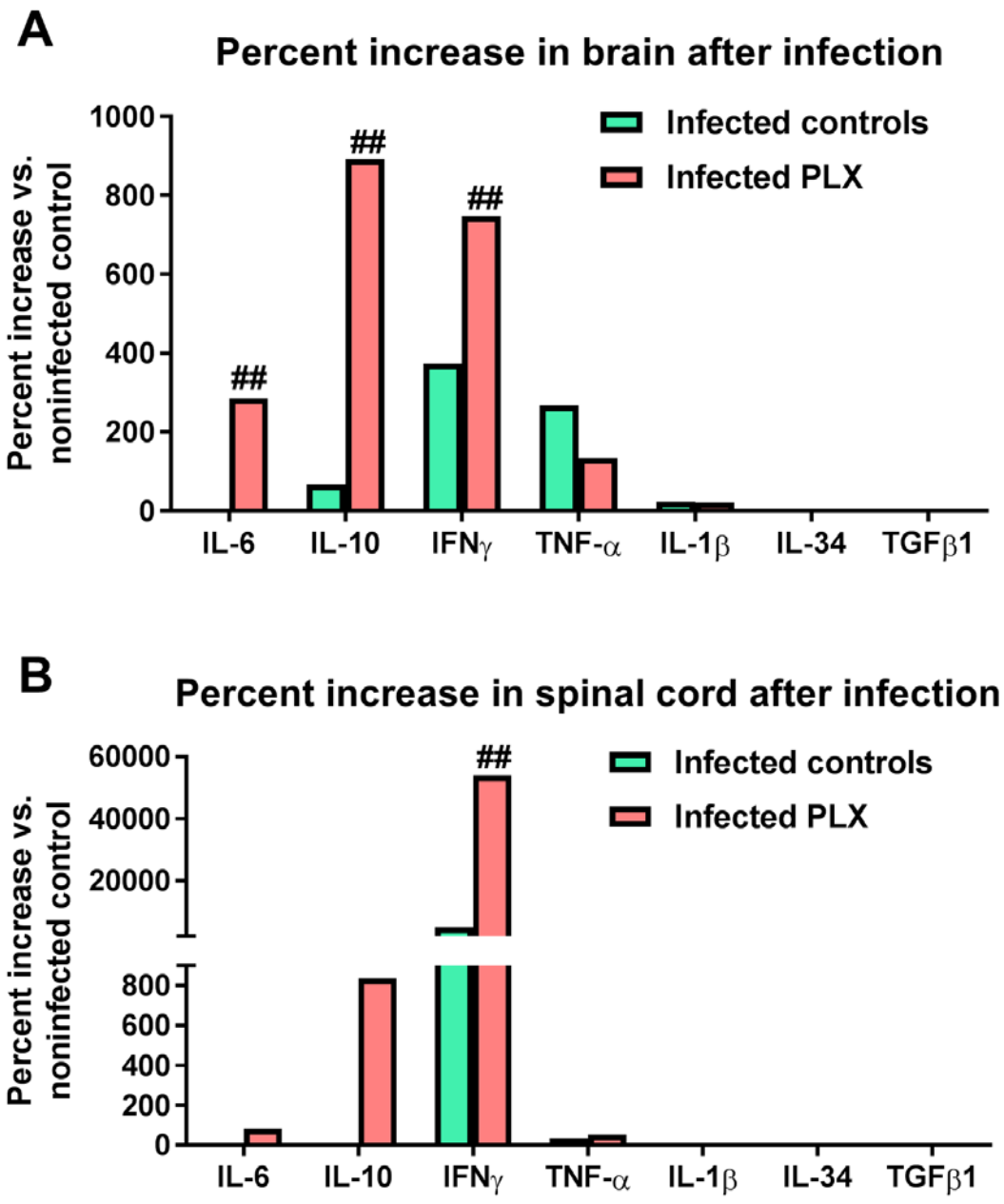

Fig. S4

Comparison of percent increase of cytokines in infected B6 controls versus infected mice treated with PLX5622 in brain (A) and spinal cord (B). Significant differences between infected mice treated with PLX5622 and infected controls are indicated by the hash sign ($^{\#\#}P<0.01$). For details see Fig. 12 and Suppl. Fig. S3.

Supplementary Table S1. Overview of differences between infected controls and PLX5622-treated infected mice.

Variable	**TMEV-infected C57BL/6 mice (7 days after infection)**	
	Controls	**PLX5622-treated**
Clinical symptoms		
Seizures	Yes	Yes (increased vs. infected controls)
Hindlimb paralysis	No	Yes
Mortality	No	Yes
Brain alterations		
TMEV antigen (IHC)	Present in CA1, CA2 and, less intense, cerebral cortex	Present in all hippocampal subregions plus cerebral cortex, thalamus, hypothalamus, striatum and other regions
Microglia (FACS)	No difference to mock	Depletion
Monocytes (FACS)	Increase	Increase
$CD8^+$ T cells (FACS)	Increase	Increase
$CD4^+$ T cells (FACS)	Increase	Increase, but less than in infected controls
$CD3^+$ T cells (IHC)	Increase in hippocampus	Increase in hippocampus
$Foxp3^+$ T cells (IHC)	No significant increase	Increase in hippocampus
Neutrophils (FACS)	Increase	No significant increase
Microglia (TMEM119 IHC)	Increase	Depletion
Mac-3^+ cells (IHC)	No significant increase in hippocampus, but cerebral cortex, thalamus and hypothalamus	Significant increase in hippocampus, cerebral cortex, thalamus and hypothalamus
$Iba1^+$ cells (IHC)	Increase in CA1/2; both ramified and round shaped cells	No increase in CA1/2, but increase in CA3c; only round shaped cells
Iba1/Mac-3 double labeled cells (IHC)	Increase in Ca1/2 and CA3c	Increase in Ca1/2 and CA3c
Perivascular infiltrates (H&E)	No significant increase in CA1/2; increase in cortex, hypothalamus, thalamus, and corpus striatum	Increase in Ca1/2 and CA3c; increase in hypothalamus, thalamus, corpus striatum, vessel layer
Hypercellularity (H&E)	No significant increase in CA1/2; increase in corpus striatum	Increase in Ca1/2 and CA3c; increase in vessel layer, corpus striatum, cortex, hypothalamus, thalamus
$GFAP^+$ cells (IHC)	No increase in hippocampus	Increase in hippocampus
NeuN (IHC)	Neurodegeneration in CA1 and CA2	Neurodegeneration in CA1, CA2, CA3a, CA3c, dentate gyrus
FluoroJade C	Labeled cells in CA1/2 and, less frequently, CA3a	Labelled cells in all hippocampal regions
Cytokines	Increase in TNFα, IL-6, IL-10, IFNγ, IL-1β, TGFβ1	More marked increase in IL-6, IL-10 and IFNγ
Spinal cord alterations		
TMEV antigen (IHC)	No	Yes
$CD3^+$ T cells (IHC)	Increase	More marked increase
$Foxp3^+$ T cells (IHC)	No significant increase	Increase
Neuronal necrosis	No	Yes
Mac-3^+ cells (IHC)	Yes	Yes (more marked vs. infected controls)
Perivascular infiltrates	Not significant	Significant
Cytokines	Increase in TNFα, IL-6, IL-10, IFNγ, IL-1β, TGFβ1	Much more marked increase in IFNγ
Alterations in periphery		
Monocytes in blood (FACS)	Increase	Increase, but less than in infected controls
Macrophages in spleen (FACS)	Increase	Increase