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## Supplemental Information

# Type I Interferon Receptor Signaling of Neurons and Astrocytes Regulates Microglia Activation during Viral Encephalitis

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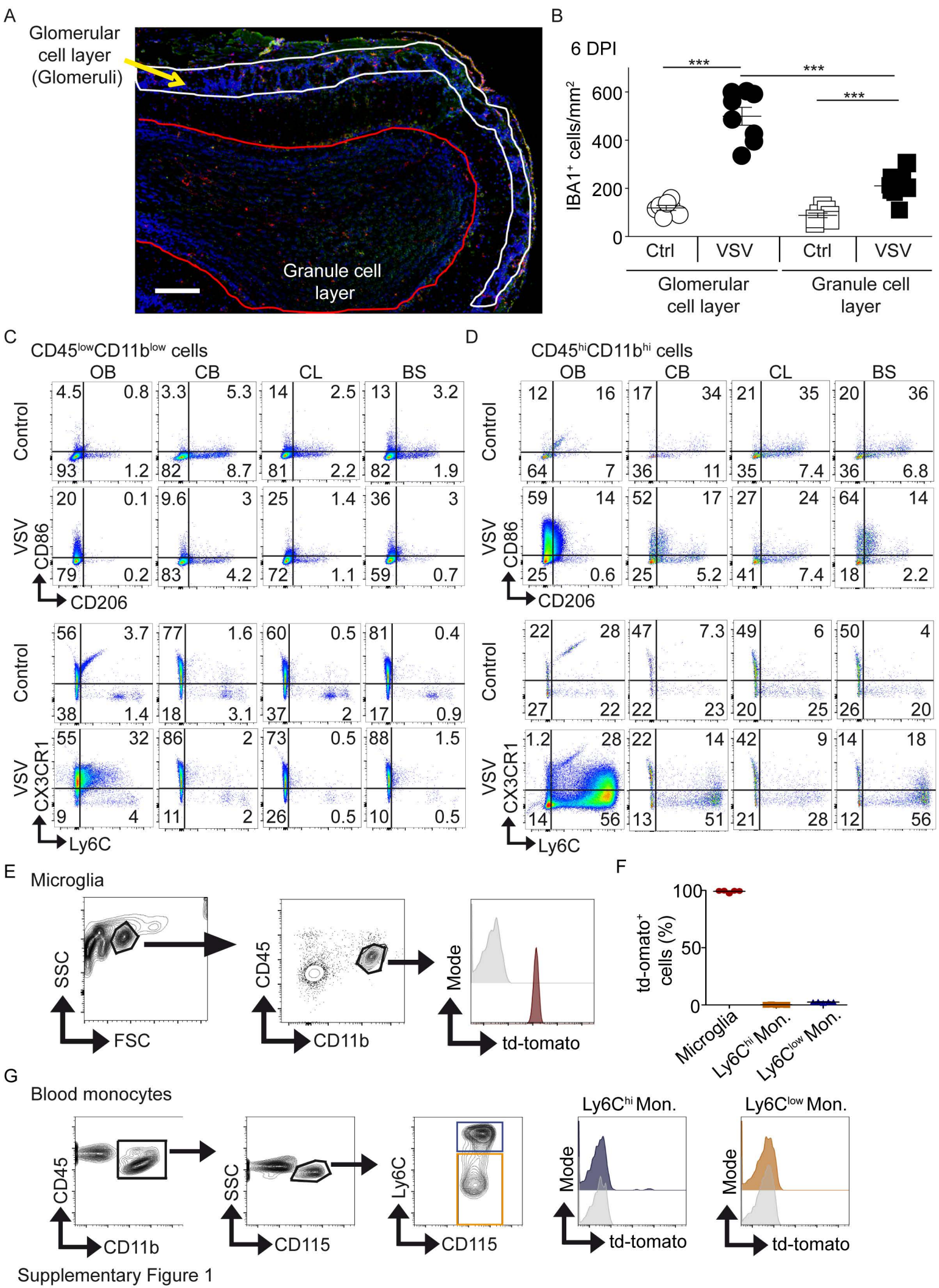
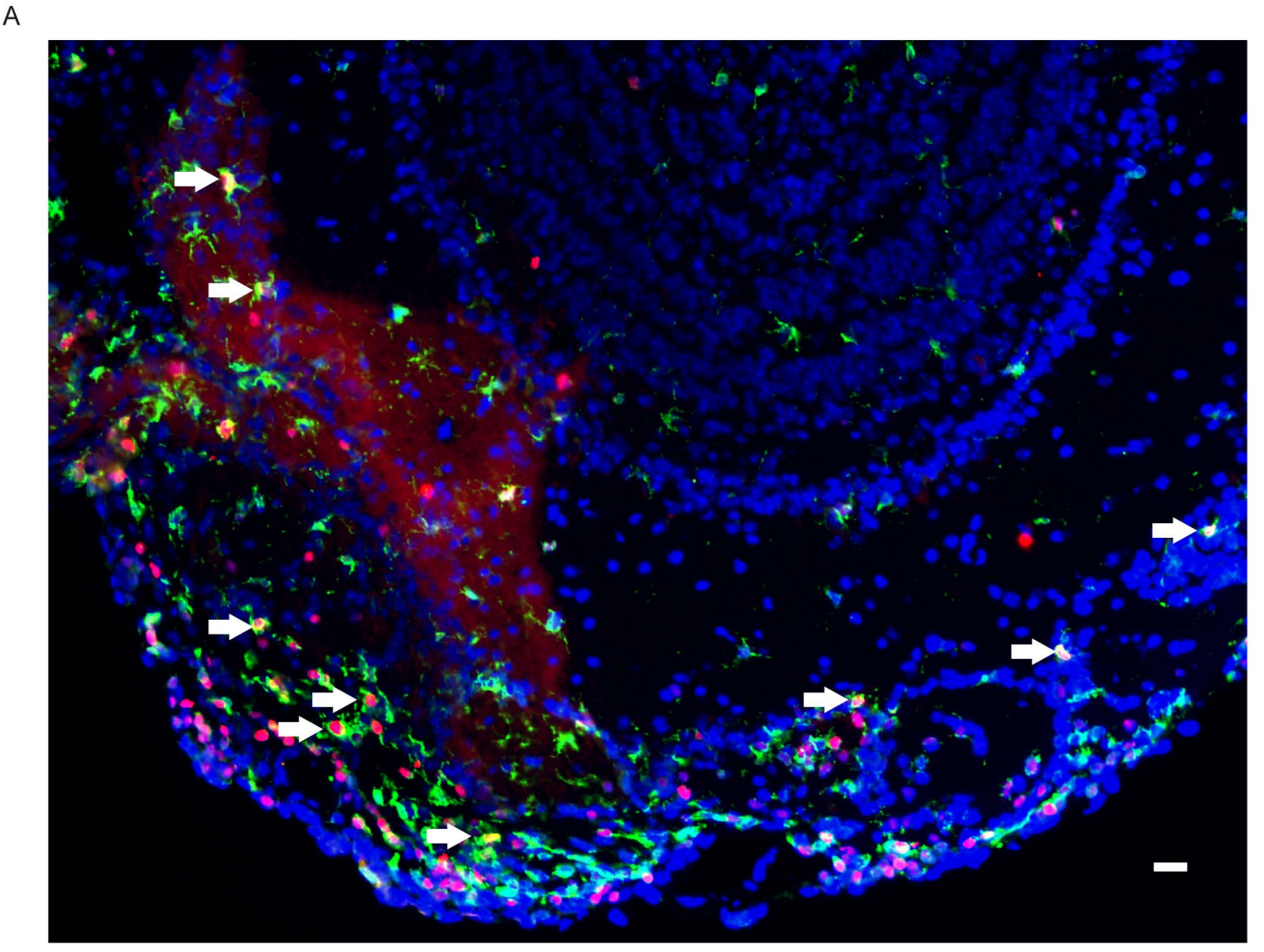
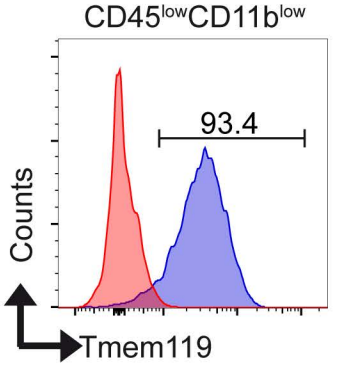
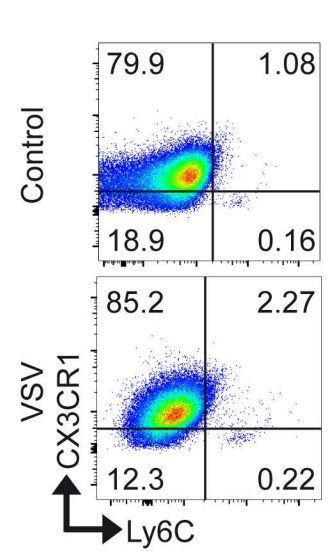


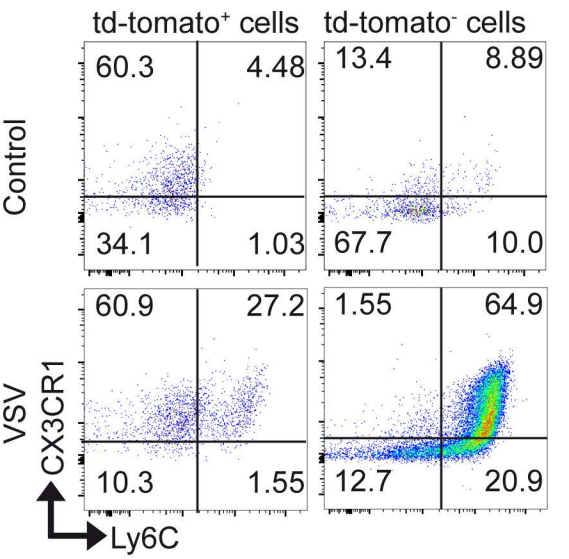

Supplementary Figure 1

**Supplementary Figure 1. Activated myeloid cells accumulate in the glomerular layer of the olfactory bulb (OB) upon VSV infection via intranasal route (Data related to Figure 1).** (A) Anatomy of the murine OB. The outer region of the OB marked by the white line is the glomerular cell layer containing glomerular structures, which are also called glomeruli. The central region marked by the red line is called granule cell layer. (B) Quantitation of Iba-$1^{+}$ cells in the OB glomeruli and the granule cell layer in Figure 1A on 6 dpi (n= 7-8, N= 2, combined data). (C) Representative data plots for expression of CD86 vs CD206 and CX3CR1 vs Ly6C on the $CD45^{low}CD11b^{low}$ cells (black marked population in Figure 1E) in different brain regions. (D) Representative data plots for expression of CD86 vs CD206 and CX3CR1 vs Ly6C on the $CD45^{hi}CD11b^{hi}$ cells (red marked population in Figure 1E) in different brain regions. (E) Analysis of td-tomato reporter protein expression in blood monocytes. *CX3CR1-cre-*$^{ER+/-}$*td-tomato*$^{St/Wt}$ mice were tamoxifen treated after weaning and 8 weeks later the blood was collected and then mice were perfused and brain was prepared. Representative flow cytometry data for analysis of td-tomato expression on $CD45^{low}CD11b^{low}$ cells from the brain (gray plot: untreated control. Red plot: tamoxifen treated sample). (F) Percentage of td-$tomato^{+}$ cells in brain (from E) and blood (from G) (n= 5, N= 2, combined data). (G) Representative flow cytometry data for analysis of td-tomato expression on $Ly6C^{hi}$ and $Ly6C^{low}$ blood monocytes in singlet population based on FSC and SCC (Grey plot: untreated control. dark grey plot and brown plot: tamoxifen treated sample). Data in B are shown as mean ± s.e.m. Scale bar in A is 200 µm.



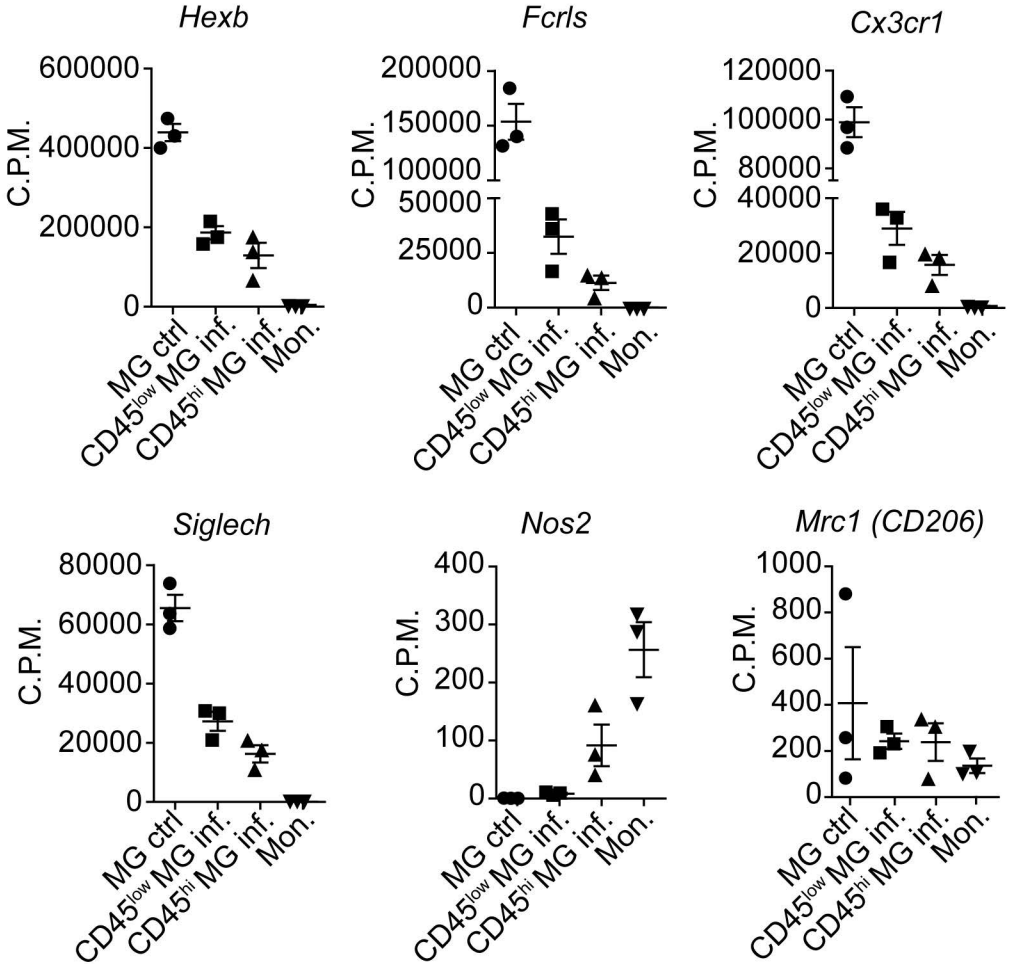



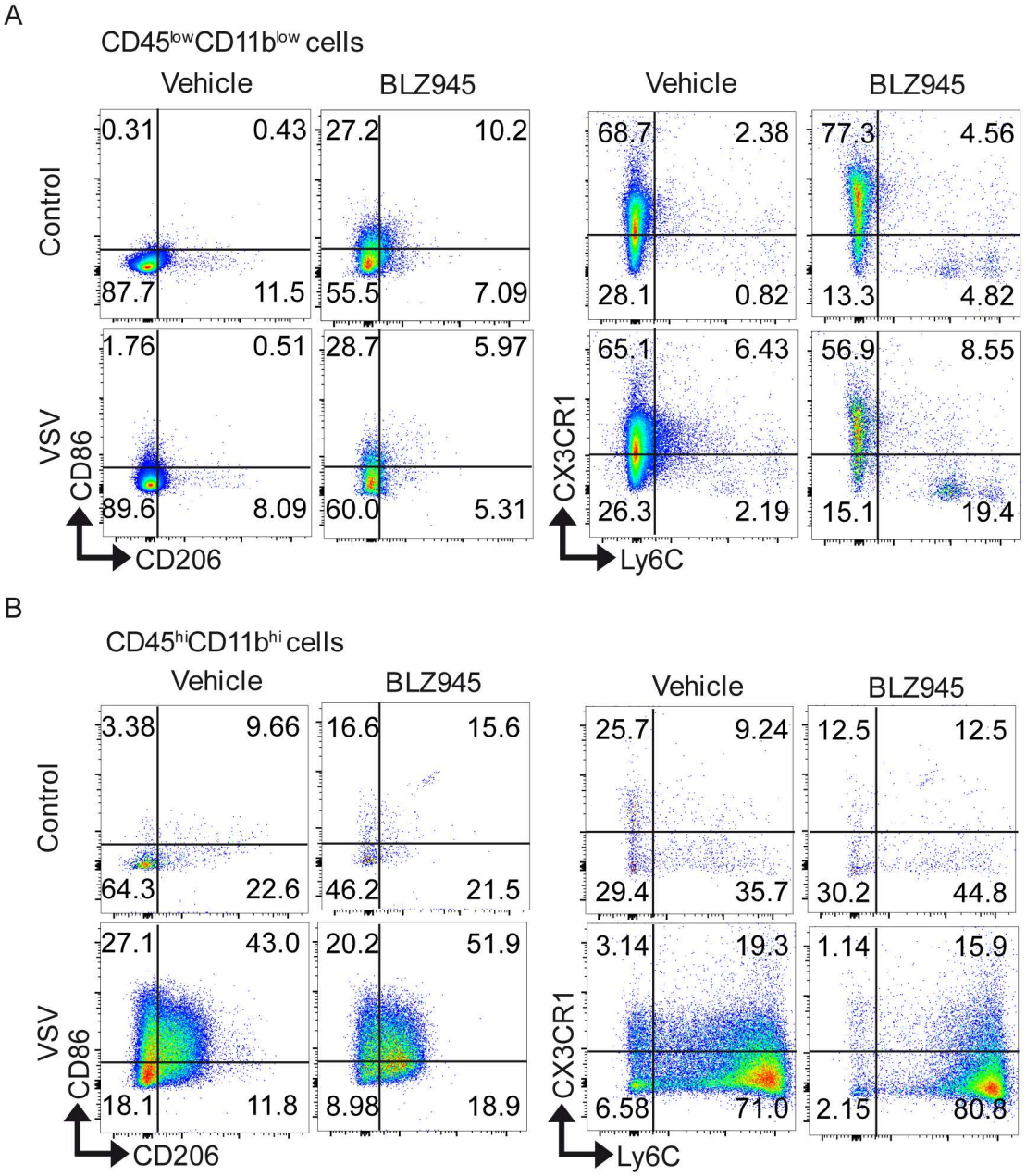

Supplementary Figure 2

**Supplementary Figure 2. Activated myeloid cells proliferate within the OB of VSV infected mice and in tamoxifen treated *CX3CR1-cre-$^{ER+/-}$td-tomato$^{St/Wt}$* mice CD45$^{low}$CD11b$^{low}$ cells express microglia marker Tmem119 (Data related to Figure 2).** (A) Representative enlarged image of the Iba-1$^{+}$Ki67$^{+}$ immunofluorescence staining of OB from VSV infected animal shown in Figure 2A. White arrows highlight some of the cells which are Iba-1$^{+}$Ki67$^{+}$. (B) Representative flow cytometry data for the expression of Tmem119 in CD45$^{low}$CD11b$^{low}$ cells from the CNS of tamoxifen treated *CX3CR1-cre-$^{ER+/-}$td-tomato$^{St/Wt}$* animals (Red plot: secondary control antibody only; blue plot: Tmem119 staining) (n= 5 total, N= 2). Representative data plots for the expression of CX3CR1 and Ly6C in (C) CD45$^{low}$CD11b$^{low}$ population from CNS in Figure 2C and (D) CD45$^{hi}$CD11b$^{hi}$td-tomato$^{+}$ and CD45$^{hi}$CD11b$^{hi}$td-tomato$^{-}$ population from Figure 2E. Scale bar in A is 10 µm.



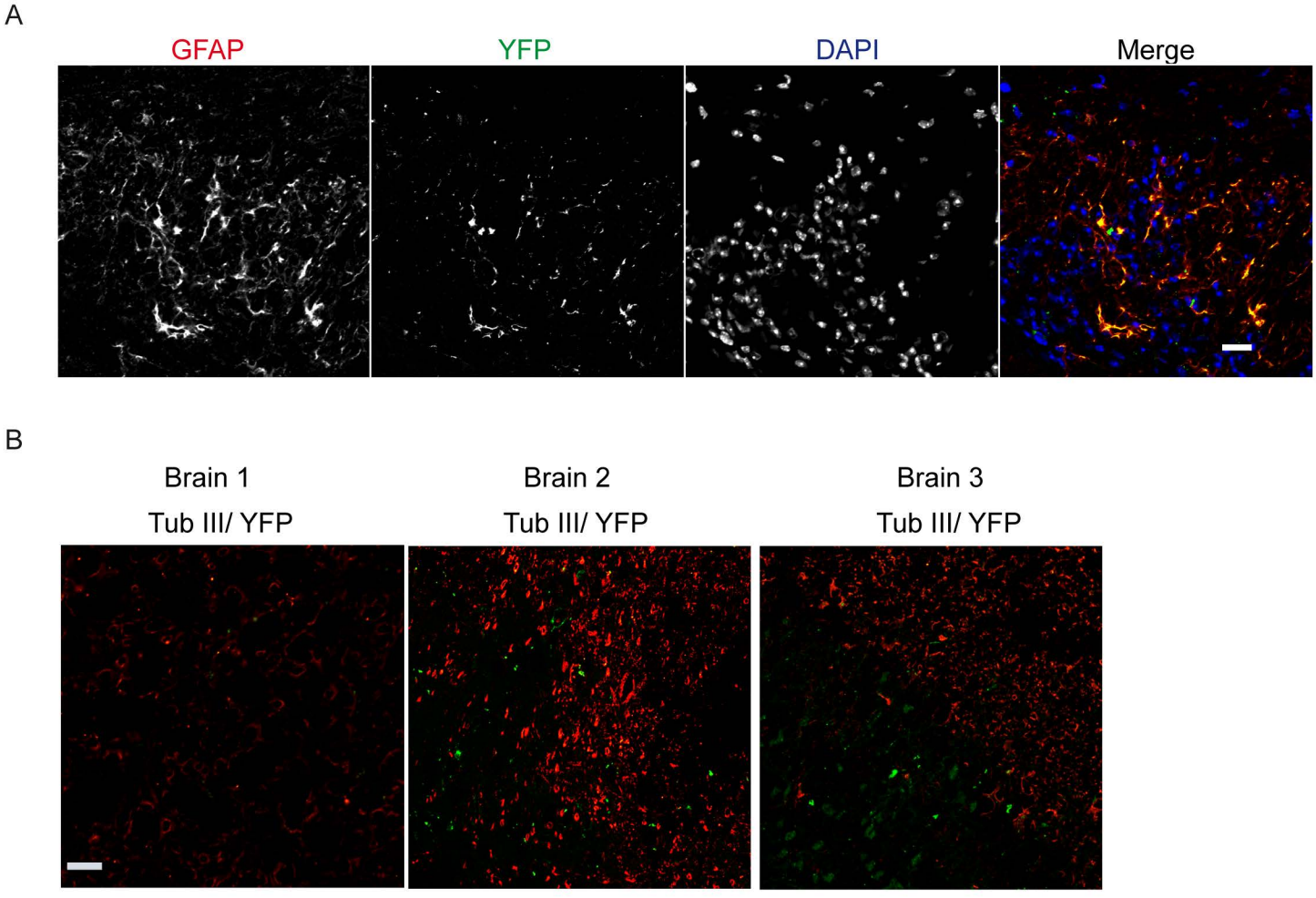

Supplementary Figure 3

**Supplementary Figure 3. mRNA expression levels of different CNS resident myeloid cell subsets (Data related to Figure 3).** mRNA expression levels of the indicated genes are shown for the indicated myeloid cell populations from Figure 3. Data are shown as mean ± s.e.m.



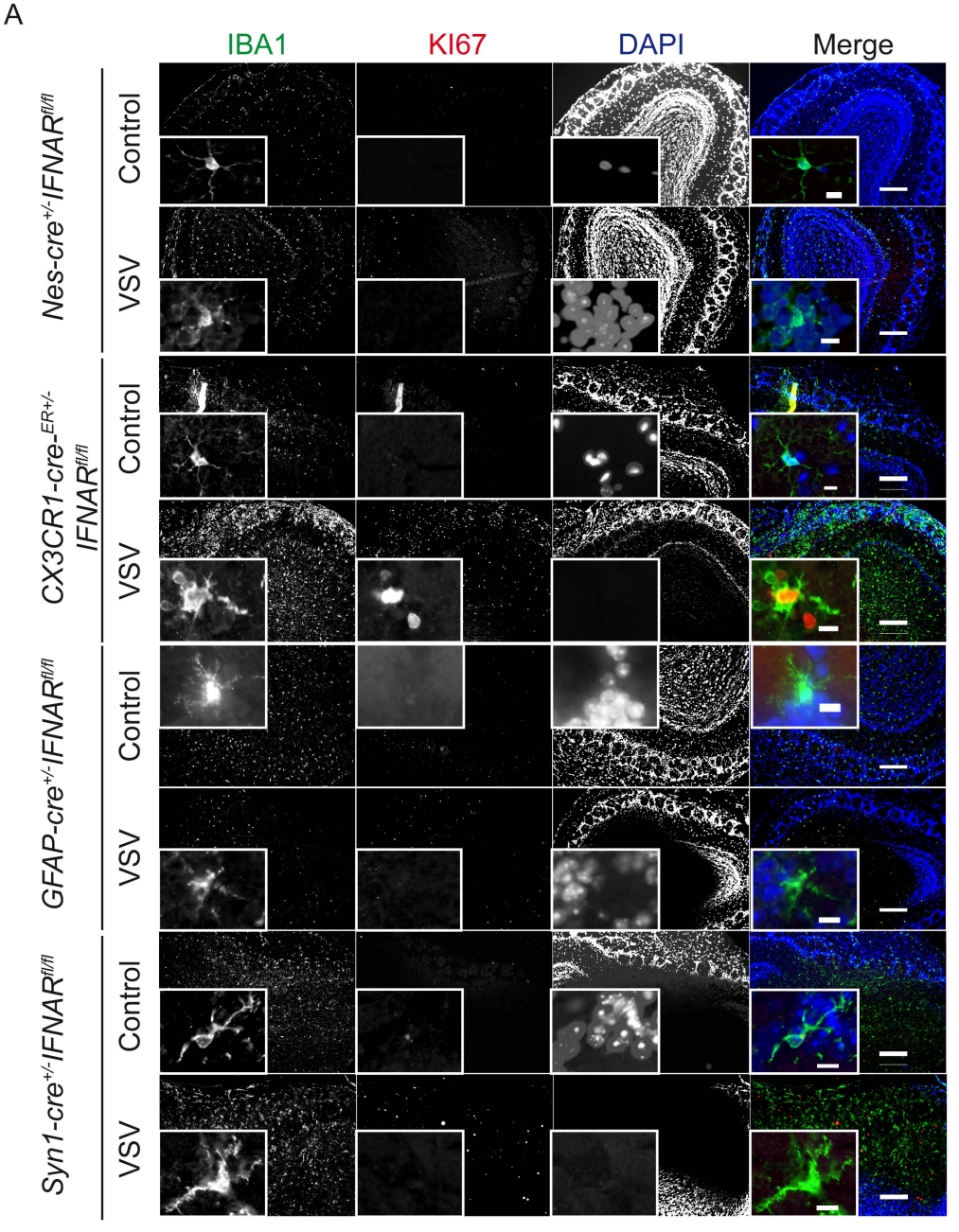

Supplementary Figure 4

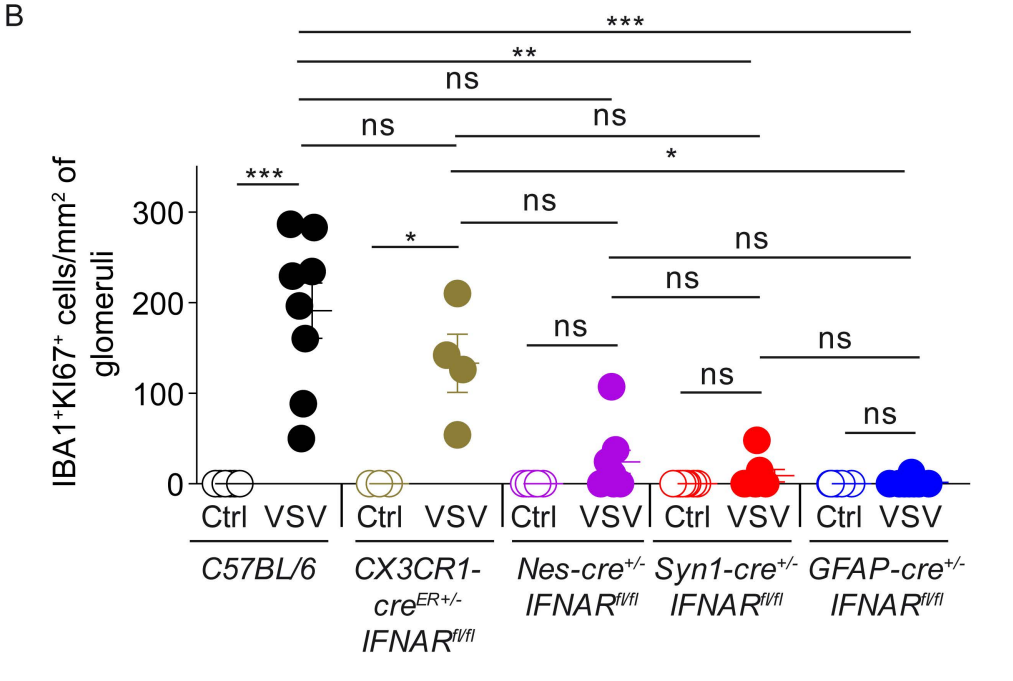
**Supplementary Figure 4. BLZ945 treatment does not induce general immune suppression (Data related to Figure 4).** Representative data plots for expression of CD86, CD206, CX3CR1 and Ly6C in (A) $CD45^{low}CD11b^{low}$ population from the CNS in Figure 4D and (B) $CD45^{hi}CD11b^{hi}$ population from Figure 4D. (C) Virus titers in the liver, lung and spleen of the animals in Figure 4F (n= 10-11, N= 2, combined data). Data in C are shown as mean ± s.e.m.




Supplementary Figure 5

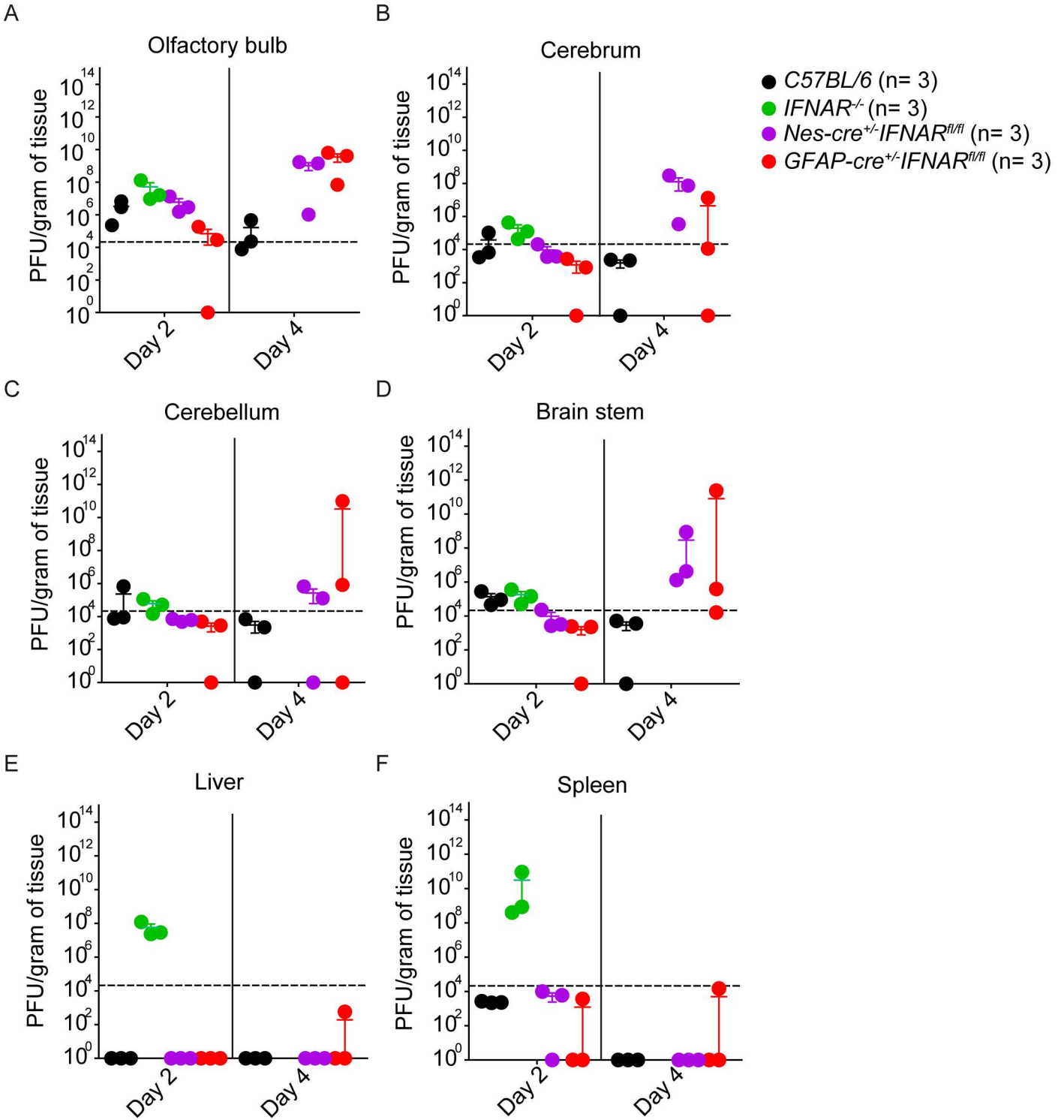
**Supplementary Figure 5. GFAP-cre targets astrocytes but not neurons (Data related to Figure 5).** Untreated *GFAP-cre*$^{+/-}$*Rosa26eYFP*$^{St/Wt}$ mice aged between 10-12 weeks were sacrificed and brains were prepared for cryostaining. Brain slices were immunolabelled with anti-GFAP antibody or Anti-ß-Tubulin III antibody, counter stained with DAPI and confocal microscopy was performed. Representative image showing expression of (A) GFAP, YFP and a combined image showing co-localization (n= 3) (B) ß-Tubulin III (in red) and YFP (in green) showing mutually exclusive expression (n= 3). Scale bar is 20 µm.




Supplementary Figure 6

**Supplementary Figure 6. IFNAR signaling of neurons and astrocytes, but not of microglia, is essential for microglia proliferation (Data related to Figure 5).** Animals were treated and brains were prepared as described in Figure 1. (A) OB slices were immunolabelled for Iba-1 and Ki-67, counter stained with DAPI and immunofluorescence microscopy was performed. (B) Quantitation of Iba-$1^{+}$Ki-$67^{+}$ cells in the OB glomeruli in A (n= 4-8, N= 2, combined data). WT data are from Figure 2. Data in B are shown as mean ± s.e.m. *P < 0.05, **P <0.01, ***P < 0.001, ns= not significant. Scale bar in A is 200 µm for main panels and 10 µm for insets.




Supplementary Figure 7

**Supplementary Figure 7. IFNAR signaling of neurons and astrocytes is essential for restriction of VSV spreads in the CNS after intranasal infection (Data related to Figure 7).** Viral titers of (A) olfactory bulb, (B) cerebrum, (C) cerebellum, (D) brain stem, (E) liver, and (F) spleen at 2 and 4 dpi after intranasal VSV infection in animals (n= 3 per genotype). Data are shown as mean ± s.e.m.