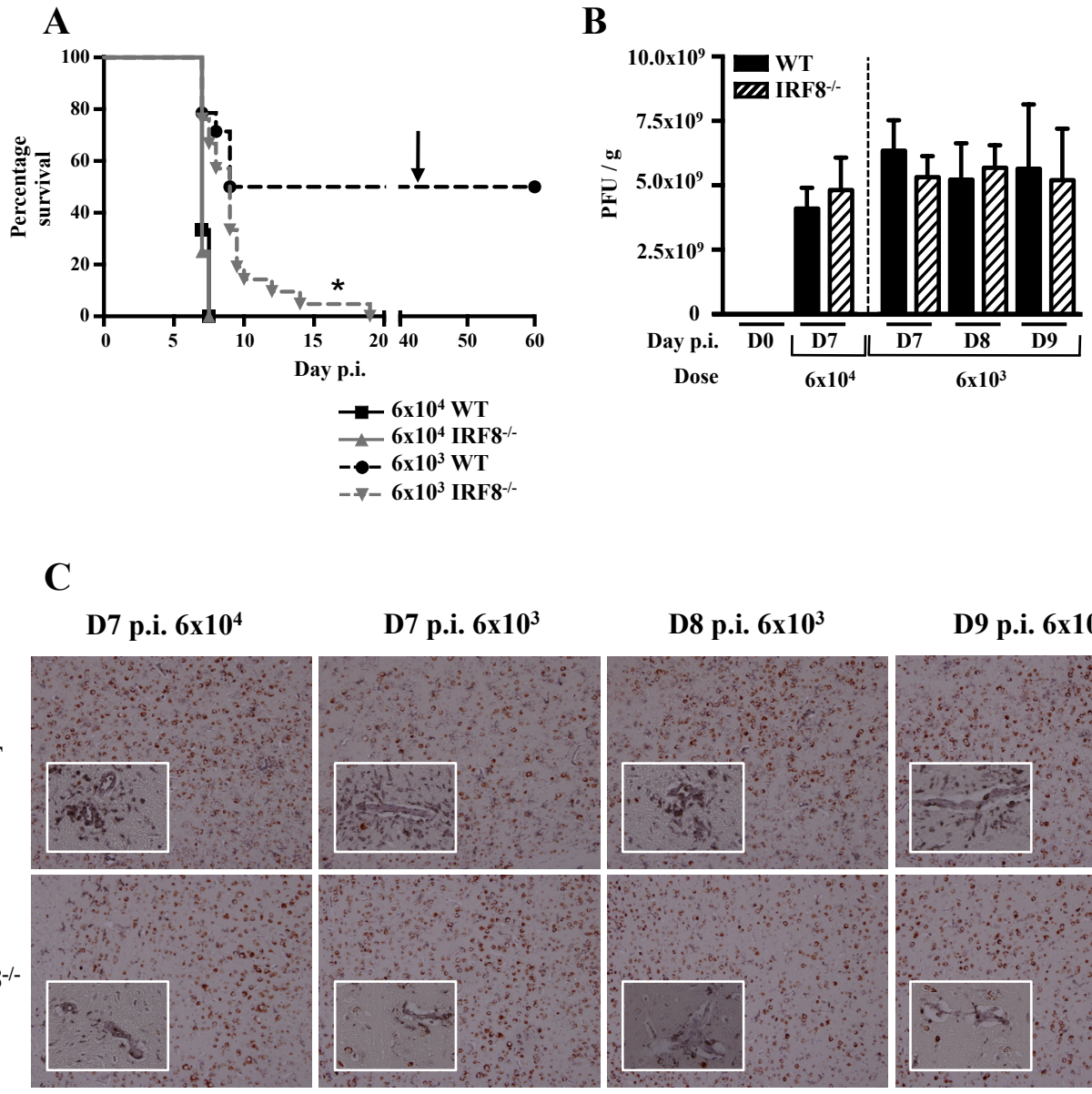
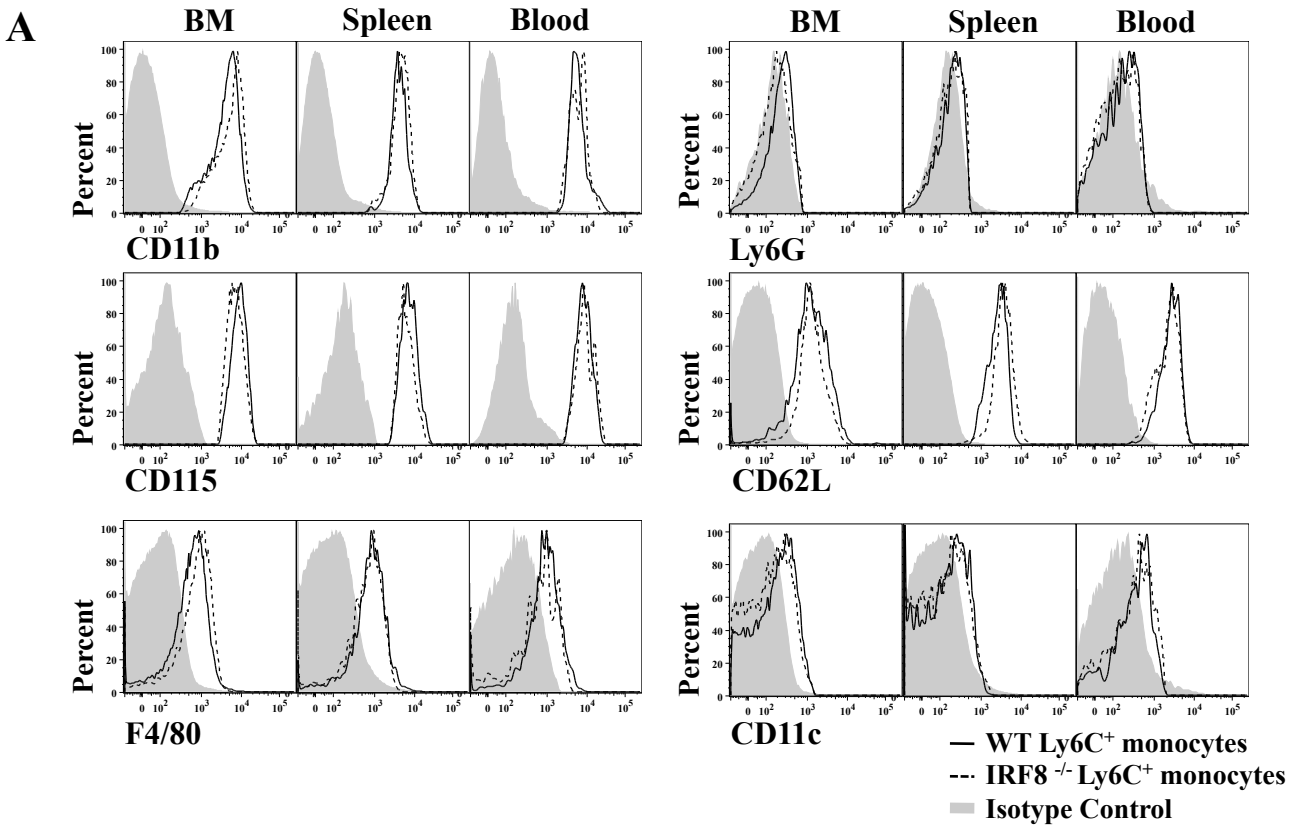
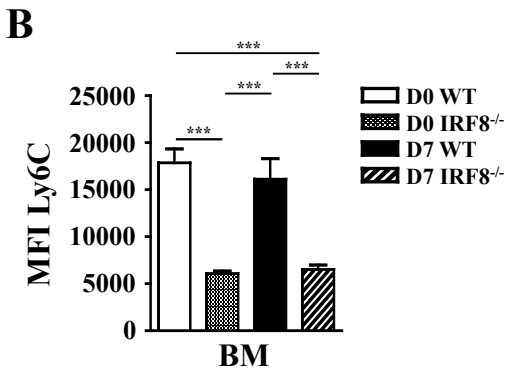
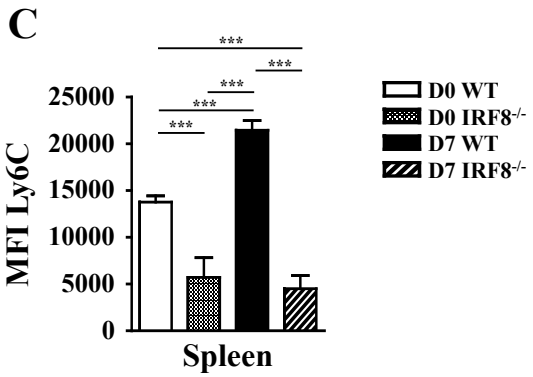
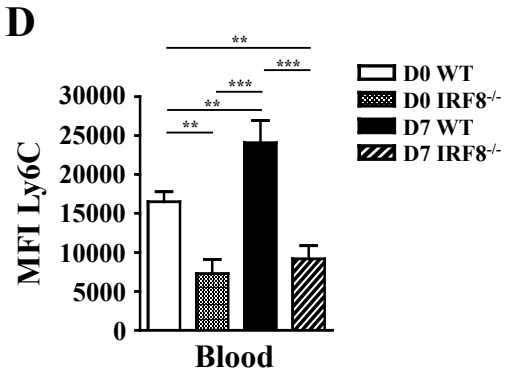


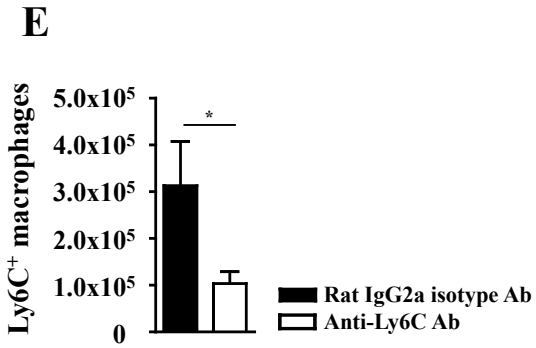
Supplementary Fig. 1.



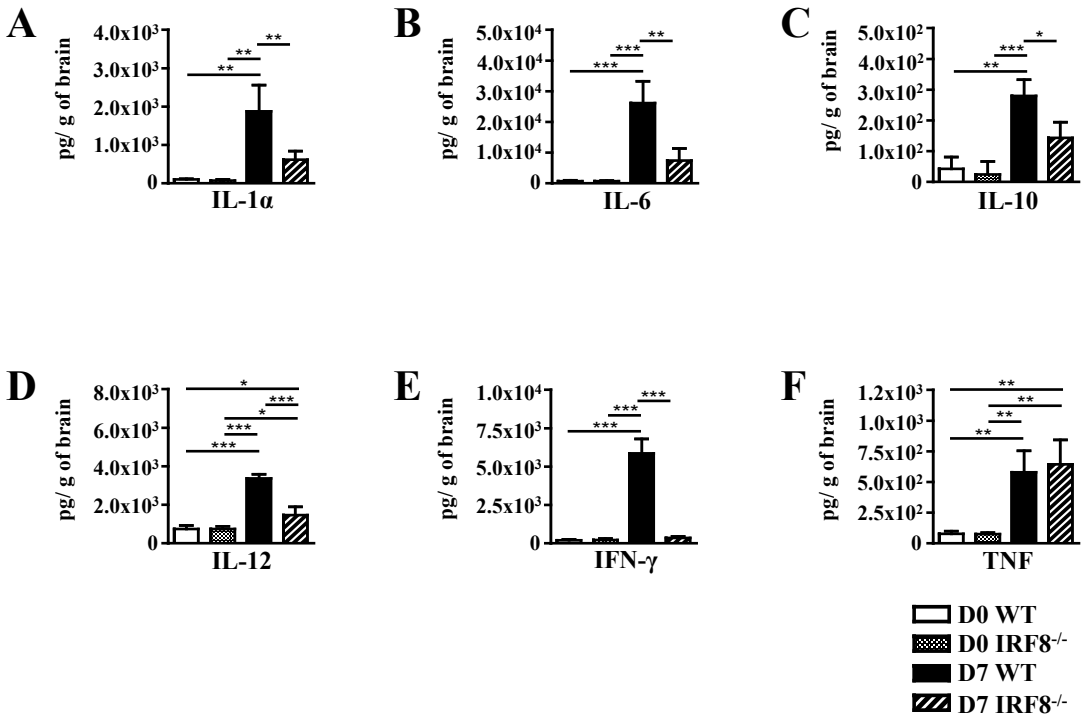




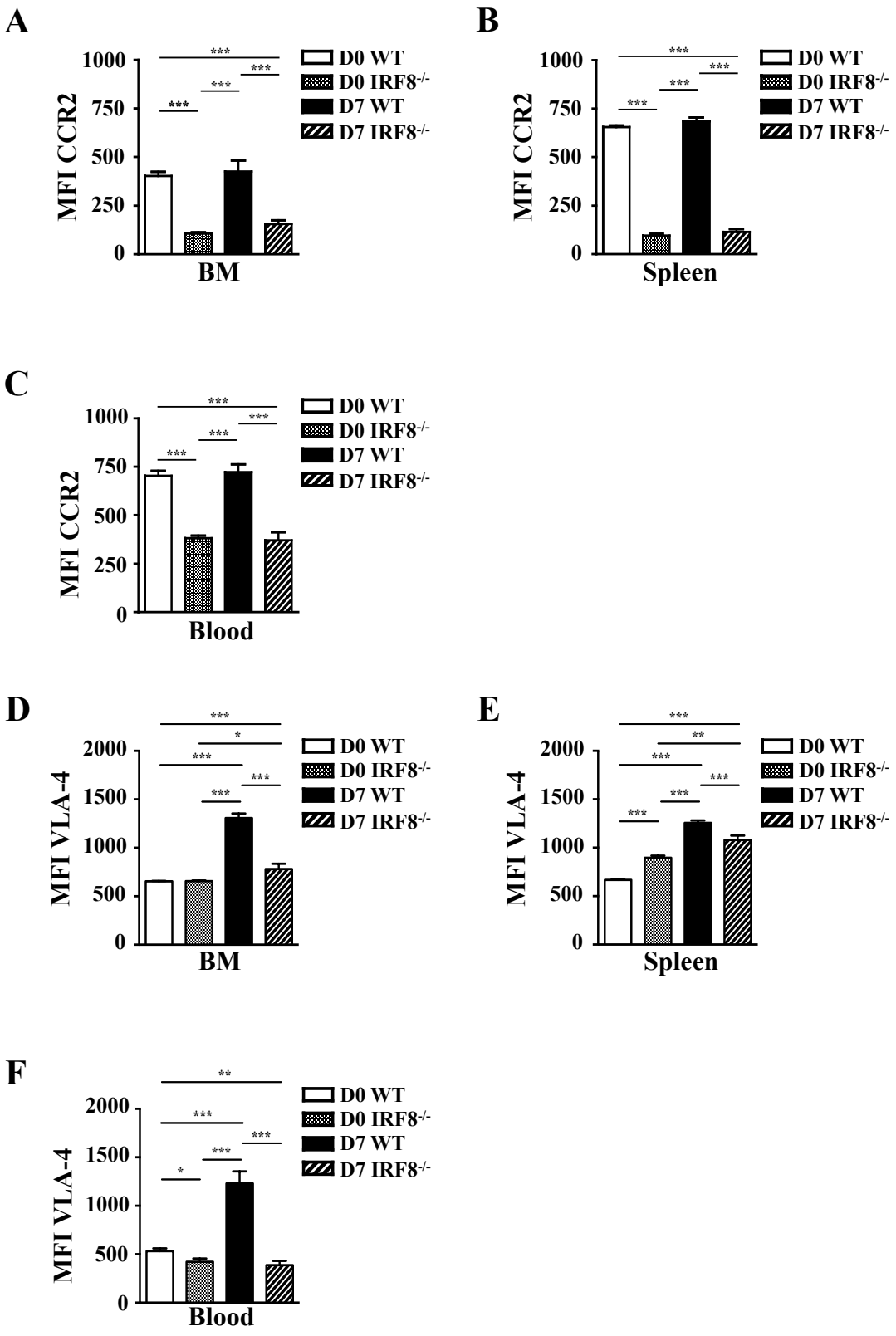




Supplementary Fig. 2.



Supplementary Fig. 3.



Supplementary Fig. 4.

Supplementary Figure Legends

Supplementary Fig. 1. IRF8$^{-/-}$ mice have higher mortality following low dose WNV infection. WT and IRF8$^{-/-}$ mice were infected intranasally with 6x10^4 or 6x10^3 PFU WNV. Surviving mice were re-inoculated with 6x10^4 PFU on D42 p.i. to confirm protective immunity (indicated by arrow on graph). Mortality was recorded (**A**). BHK plaque assays were performed on brains from WT and IRF8$^{-/-}$ mice succumbing to disease on D7-D9 p.i. following infection with 6x10^4 or 6x10^3 PFU WNV (**B**). Immunohistology for WNV NS-1 (brown) and lectin (black; myeloid cells and endothelial cells) was performed on brain sections taken from WT and IRF8$^{-/-}$ mice succumbing to disease on D7-D9 p.i. following infection with 6x10^4 or 6x10^3 PFU WNV (**C**). Disease courses were performed 3 times with a minimum of 6 mice/group. Statistical analysis was conducted using the Log-rank (Mantel-Cox) test and $P \leq 0.05$ (*) in comparing IRF8$^{-/-}$ mice infected with 6x10^3 PFU to WT animals infected at this dose. BHK plaque assay data shown are means ± SD represent three separate experiments with a minimum of 4 mice/group. Immunohistology was performed on 4 sagittal brain sections from a minimum of 4 mice/group and was repeated twice.

Supplementary Fig. 2. IRF8$^{-/-}$ Ly6C$^+$ inflammatory monocytes express lower levels of Ly6C than WT cells. CD45$^+$ CD115$^+$ CD11b$^+$ Ly6G$^-$ Ly6C$^+$ monocytes were gated in the BM, spleen and blood and the expression of CD11b, CD115, F4/80, Ly6G, CD62L and CD11c were compared (**A**). Ly6C$^+$ monocytes were gated in the BM (**B**), spleen (**C**) and blood (**D**) and the MFI of Ly6C expression was compared. Following WNV infection, WT mice were injected i.v. with 100µg anti-Ly6C mAb or rat Ig2a isotype control on D5 and D6 p.i. Brains were processed on D7 p.i. and CD45$^+$ CD115$^+$ CD11b$^+$ Ly6G$^-$ Ly6C$^+$ macrophages were gated. Total numbers of macrophages were calculated using flow cytometry percentages and absolute live cells counts for each brain (**E**). Flow cytometry data shown are means ± SD and represent three separate experiments with 4 mice/group. Statistical analysis was conducted using one-way ANOVA and Tukey-Kramer post-test. $P \leq 0.05$ (*), $P \leq 0.01$ (**), $P \leq 0.001$ (***).

Supplementary Fig. 3. Significantly lower levels of proinflammatory cytokines are detected in the IRF8$^{-/-}$ WNV-infected brain. Brains from mock-infected and WNV-infected WT and IRF8$^{-/-}$ mice were collected on D7 p.i. and processed for multiplex ELISA. Levels of IL-1α (**A**), IL-6 (**B**), IL-10 (**C**), IL-12 (**D**), IFN-γ (**E**) and TNF (**F**) were compared. Multiplex ELISA data shown are means ± SD and was performed twice with 4 mice/group. Statistical analysis was conducted using one-way ANOVA and Tukey-Kramer post-test. $P \leq 0.05$ (*), $P \leq 0.01$ (**), $P \leq 0.001$ (***).

Supplementary Fig. 4. IRF8$^{-/-}$ monocytes express low levels of CCR2 and VLA-4. The BM (**A, D**), spleen (**B, E**) and blood (**C,F**) from mock-infected and WNV-infected WT and IRF8$^{-/-}$ mice were processed for flow cytometric analysis on D7 p.i. CD45$^+$ Ly6G$^-$ CD11b$^+$ CD115$^+$ Ly6C$^+$ monocytes were gated and the MFI of CCR2 (**A-C**) and VLA-4 (**D-F**) expression was compared. Flow cytometry data shown are means ± SD and represent three separate experiments with 4 mice/group. Statistical

analysis was conducted using one-way ANOVA and Tukey-Kramer post-test. $P \leq 0.05$ (*), $P \leq 0.01$ (**), $P \leq 0.001$ (***).