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Supplemental Information

Activating Peripheral Innate Immunity Enables Safe and Effective Oncolytic Virotherapy in the Brain

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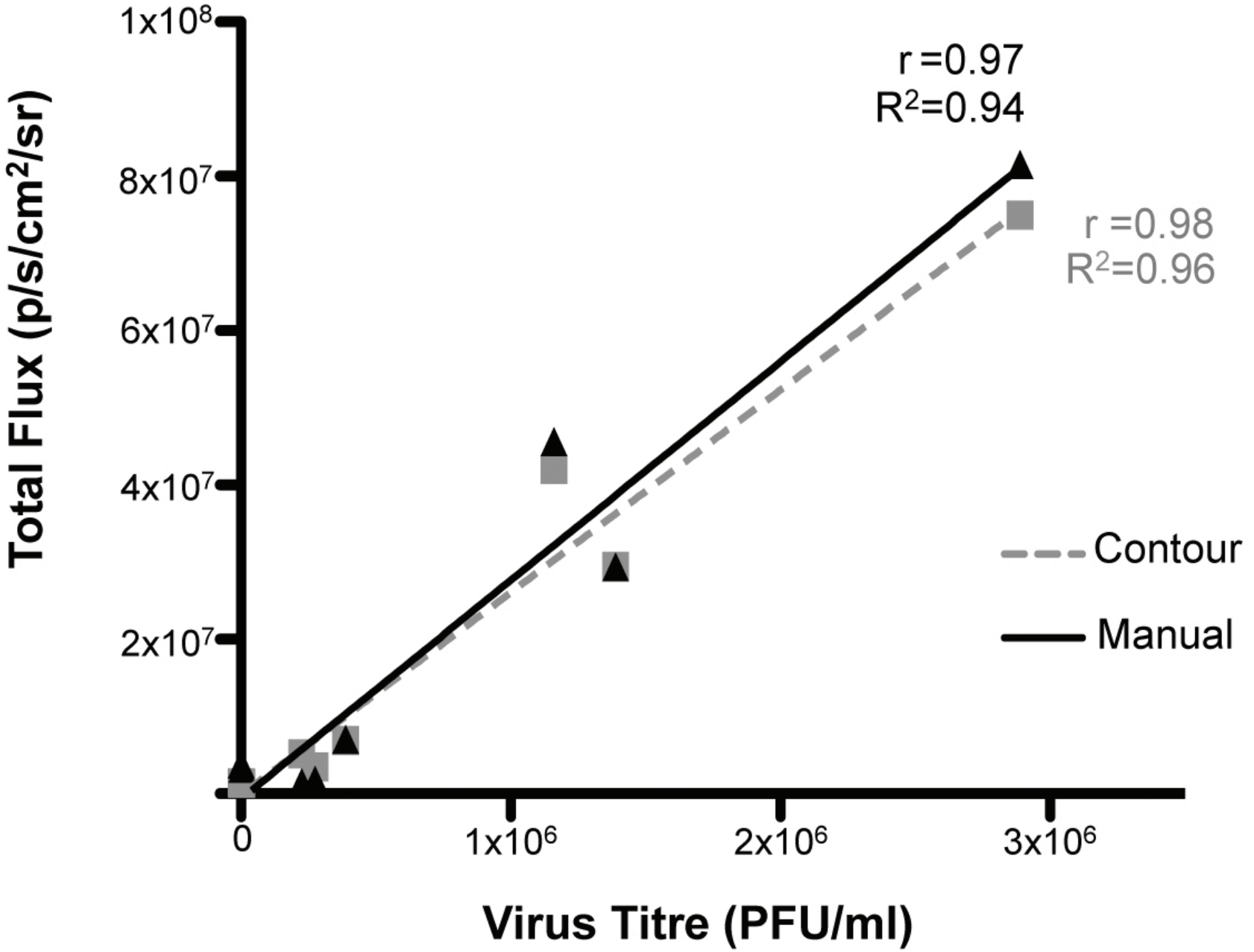
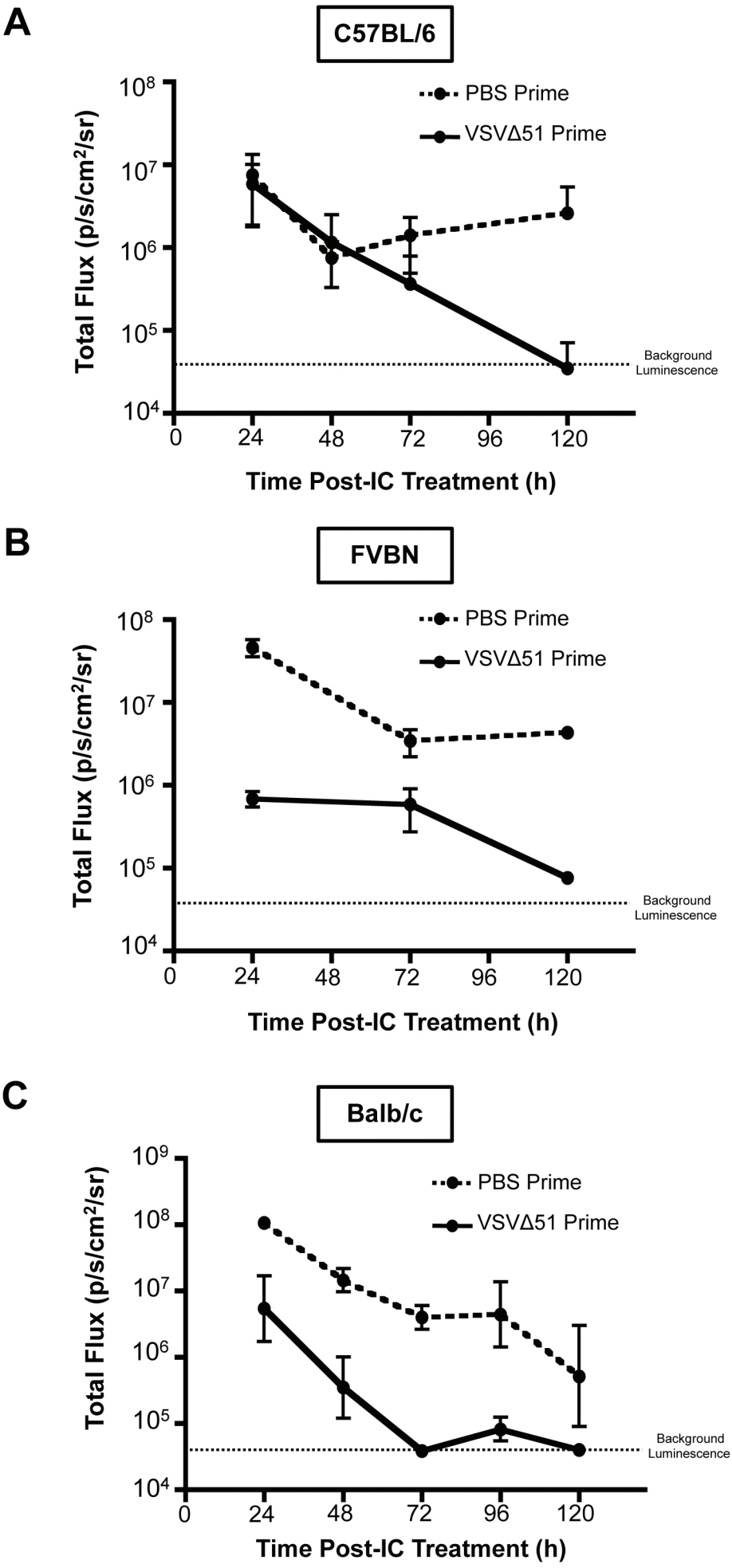
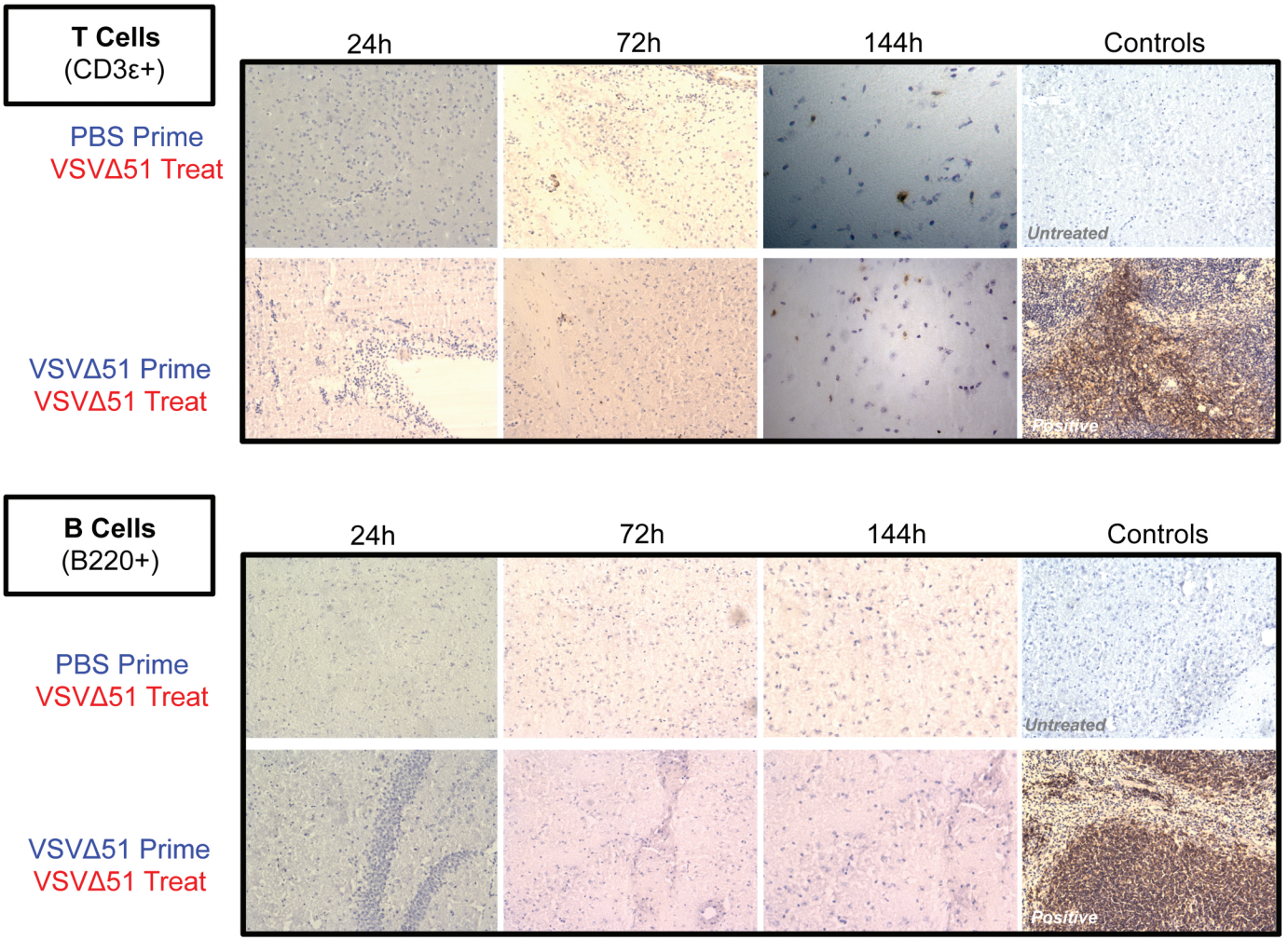


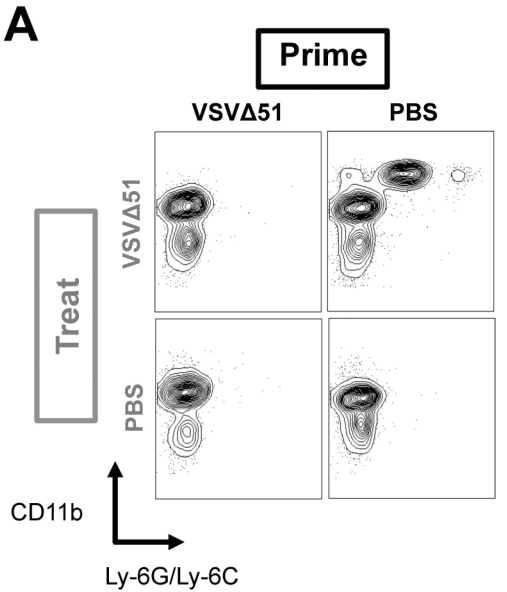
Supplemental Figure 1

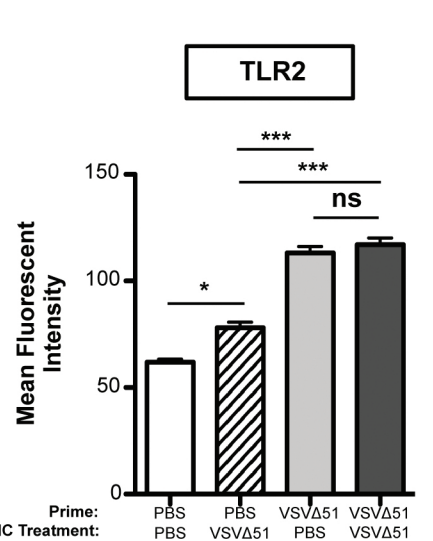


Supplemental Figure 2

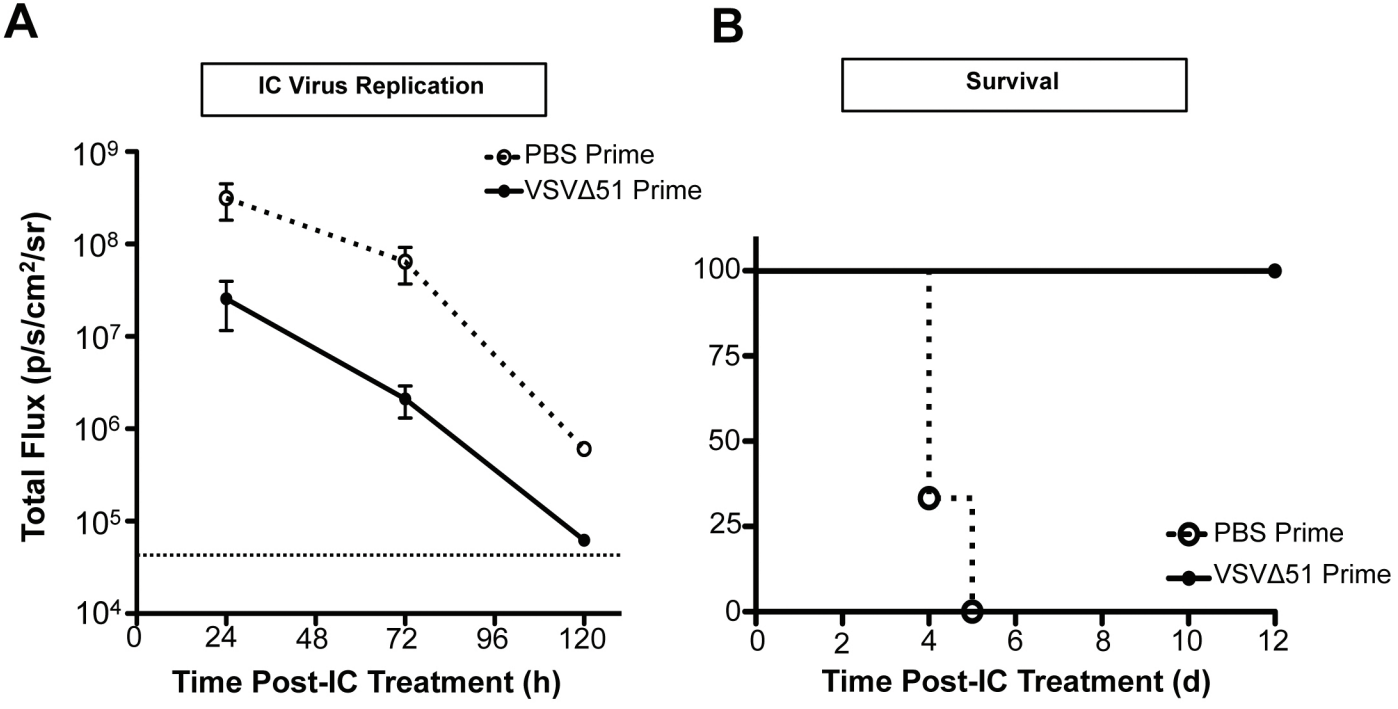


Supplemental Figure 3

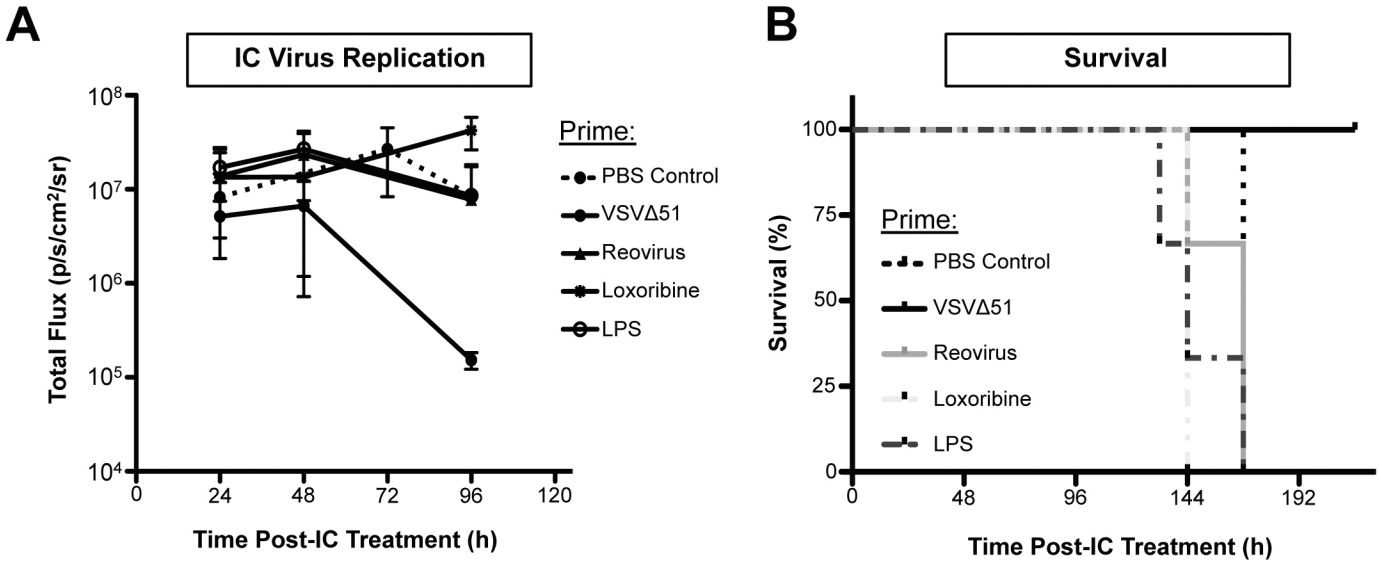




Supplemental Figure 4



Supplemental Figure 5



Supplemental Figure 6

SUPPLEMENTAL FIGURE LEGENDS

Supplemental Figure 1. Viral luciferase activity correlates with viral titres in the brain. Intracranial virus luciferase activity was measured by total photon flux via IVIS imaging. Intracranial viral titres were analyzed by plaque assay. Non-linear regression analysis was used to assess the correlation between flux and titres, and generate a correlation co-efficient (Pearson's r score) and co-efficient of determination (R2) value for both manual and contour profiles (R2=0.94 (manual), R2=0.96 (contour); r=0.97 (manual), r=0.98 (contour)).

Supplemental Figure 2. Protection induced by peripheral VSVΔ51 priming is not mouse strain-specific. Replication of IC virus was measured via IVIS imaging following a 24h prime with PBS or VSVΔ51-GFP in a) C57BL/6, b) FVBN and c) Balb/c mice, based on 3 mice per group. Dotted lines represent background luminescence.

Supplemental Figure 3. Adaptive cellular populations are not present in the brains of mice when the protective effect is established. Immunohistochemistry was performed to detect T cells or B cells in the brains of primed and IC-treated mice. Brains were analyzed at 24h, 72h and 144h post-IC treatment. The brain from a naive animal was included as an "untreated" control, and the spleen from a naive animal was included as a positive control.

Supplemental Figure 4. Peripheral priming modulates the recruitment and phenotype of immune cells in the brain. a) Influx of granulocytic/monocytic populations in the brain following peripheral priming. Mice were primed for 24h followed by IC treatment. At 12h post-intracranial dose, cells were isolated from brain homogenates, stained, and analyzed by flow cytometry. Plots were first gated on CD45+ cells (not shown), followed by CD11b and Ly-6G/Ly-6C. b) TLR2 expression in microglial populations in the brain following peripheral priming. Mice were primed for 24h, followed by intracranial treatment. After 12h, brains were homogenized, stained and analyzed by flow cytometry. Microglial cells were defined as $CD45^{lo}$CD11b+Gr-1-. P-values were calculated by one-way ANOVA. * = $P<0.05$, *** = $P<0.001$.

Supplemental Figure 5. IFNγ is not essential for VSVΔ51 priming-induced CNS protection. IFNγ-/- mice were primed and intracranially treated, and the replication kinetics of the IC virus dose were monitored by IVIS imaging. Dotted line represents background luminescence. Survival was monitored over time. Based on n=3 per group.

Supplemental Figure 6. Heterologous viruses or virus-like signals don't recapitulate the protective benefits of a VSVΔ51 prime. Balb/c mice were primed for 24h with Reovirus, loxoribine or LPS (or PBS or $1x10^8$ PFU VSVΔ51-GFP controls). Following IC treatment, virus replication was monitored by IVIS imaging to capture luminescence (a), and survival was assessed (b).