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Supplementary Information for

Neural progenitor cells pyroptosis contributes to Zika virus-induced brain atrophy and represents a therapeutic target

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Figures S1 to S5

Figure S1

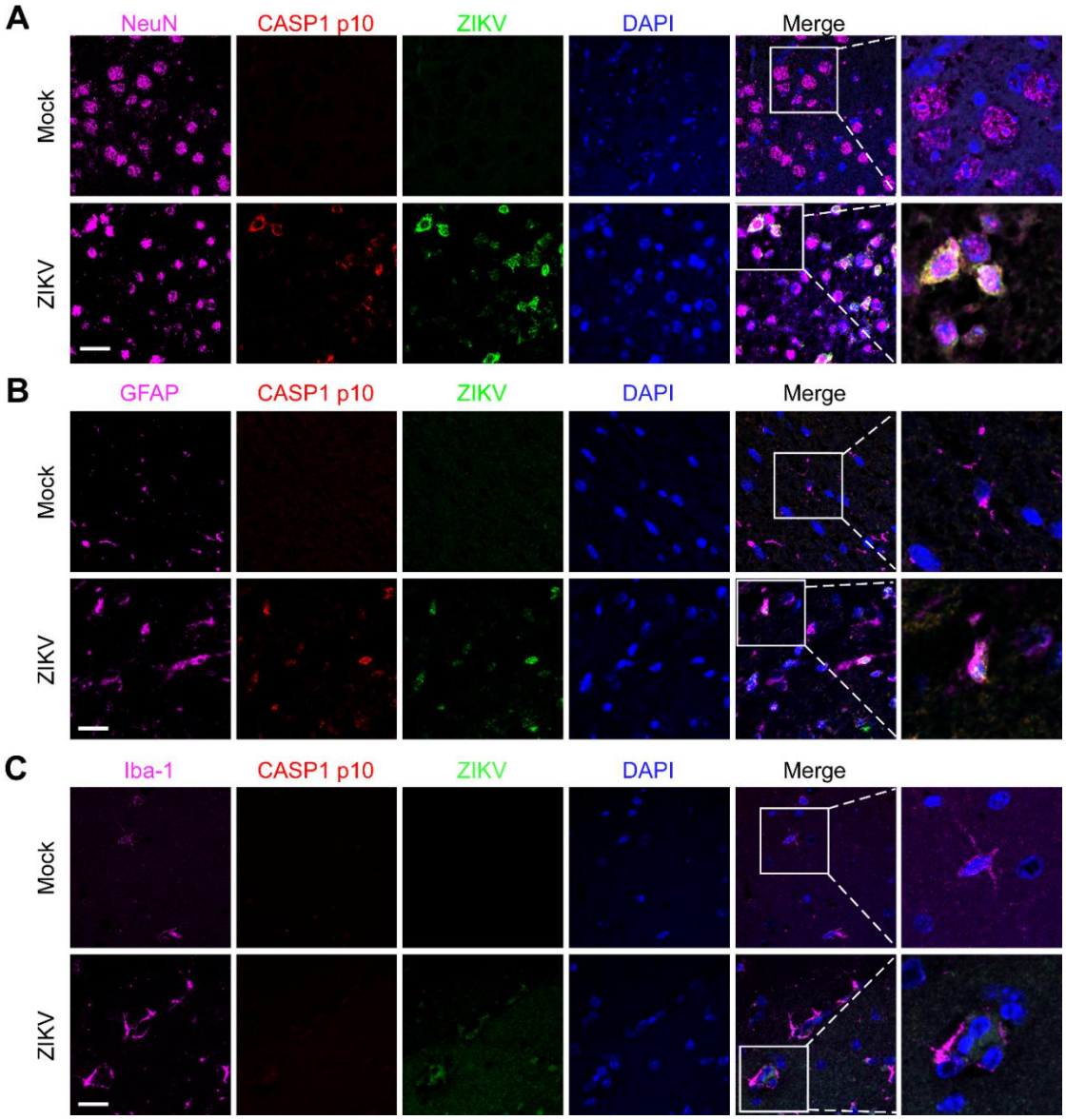

Fig. S1. ZIKV infects NPCs and causes pyroptosis *in vivo*.

Three-day-old mouse pups were subcutaneously infected with 5×10^5 PFUs of ZIKV or mock-infected. **(A)** Representative micrographs of the brain tissue co-stained for NeuN, cleaved-caspase-1 (p10) and ZIKV NS3 protein (21 dpi). **(B)** Representative micrographs of the brain tissue co-stained for GFAP, cleaved-caspase-1 (p10) and ZIKV NS3 protein (21 dpi). **(C)** Representative micrographs of the brain tissue co-stained for Iba-1, cleaved-caspase-1 (p10) and ZIKV NS3 protein (21 dpi). The nuclei were stained with DAPI. Scale bar, 25 μm.

Figure S2

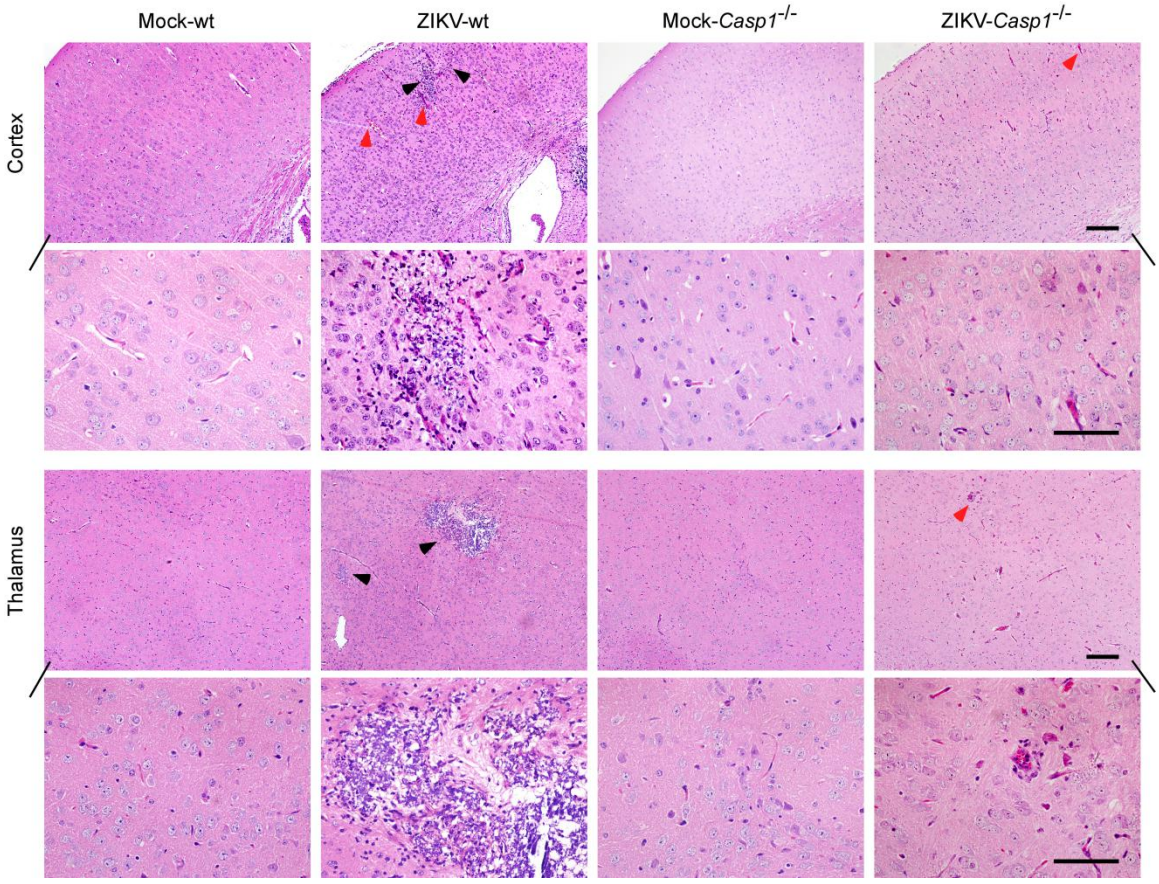

Fig. S2. *Caspase-1* KO restores neuropathological alterations induced by ZIKV in mice.

Three-day-old mouse pups of WT or *Casp1*$^{-/-}$ were subcutaneously infected with 5×10^5 PFUs of ZIKV or mock-infected. Representative micrographs of the cortex and thalamus of WT or *Casp1*$^{-/-}$ mice with or without ZIKV infection (21 dpi). Black arrow: necrotic loci; Red arrowhead: perivascular cuffing. Scale bars, 100 μm (upper panels), 50 μm (lower panels).

Figure S3

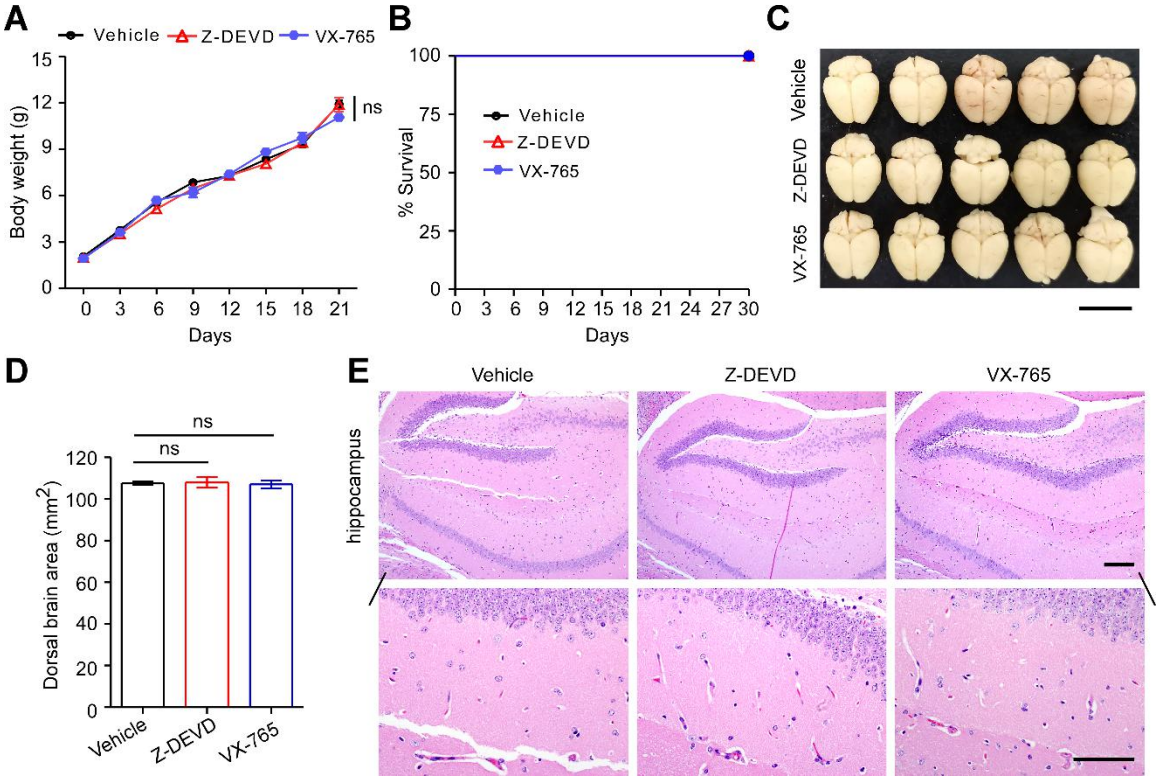

Fig. S3. VX-765 or Z-DEVD-FMK treatment does not induce neuroinflammation and brain atrophy in mock-infected mice.

Three-day-old mouse pups were subsequently treated with VX-765 (50 mg/kg, i.p.), or Z-DEVD-FMK (50 mg/kg, i.p.), or with vehicle (PBS/DMSO) every two days. **(A)** Body weight curves of mock-infected mice treated with VX-765, Z-DEVD-FMK or control vehicle. **(B)** Survival curves of mock-infected mice treated with VX-765, Z-DEVD-FMK or control vehicle. **(C)** Representative images of brains from mock-infected mice treated with VX-765, Z-DEVD-FMK or vehicle (30 days). Scale bars, 1 cm. **(D)** Dorsal brain area of mice was measured and shown in the histogram. **(E)** Representative micrographs of histopathological analysis with H&E staining of the hippocampus of mock-infected mice treated with VX-765, Z-DEVD-FMK or vehicle. Scale bars, 100 μm (upper panels), 50 μm (lower panels).

Figure S4

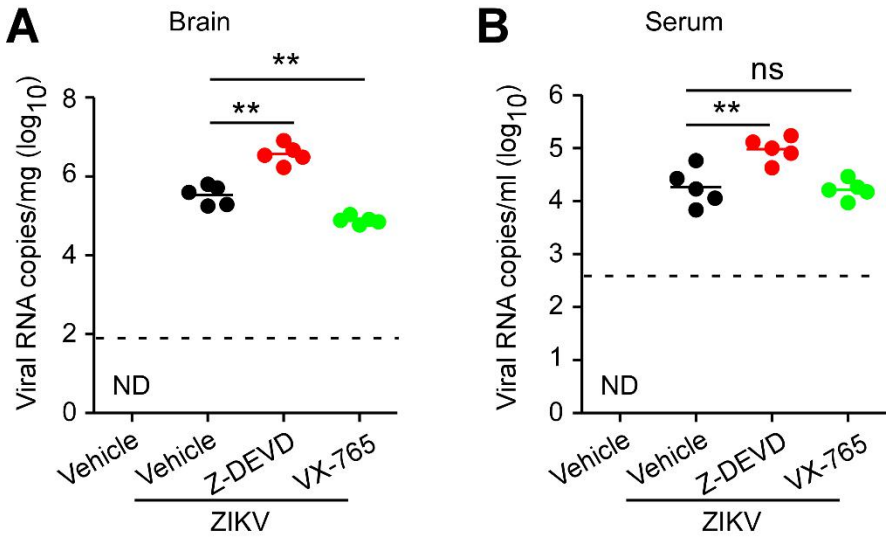

Fig. S4. Quantification of ZIKV RNA copies in mice brain and serum.

Three-day-old mouse pups were subcutaneously infected with 1×10^6 PFUs of ZIKV or mock-infected, and subsequently treated with VX-765 (50 mg/kg, i.p.), or Z-DEVD-FMK (50 mg/kg, i.p.), or with vehicle (PBS/DMSO) every two days. Brain (**A**) and serum (**B**) specimens of mice were collected and subjected to ZIKV RNA examination using qRT-PCR method (n = 5). Dotted line depicts the limit of detection for the assays. Data are presented as mean ± SD, Student's t-test. ns, not significant. ND, not detectable.

Figure S5

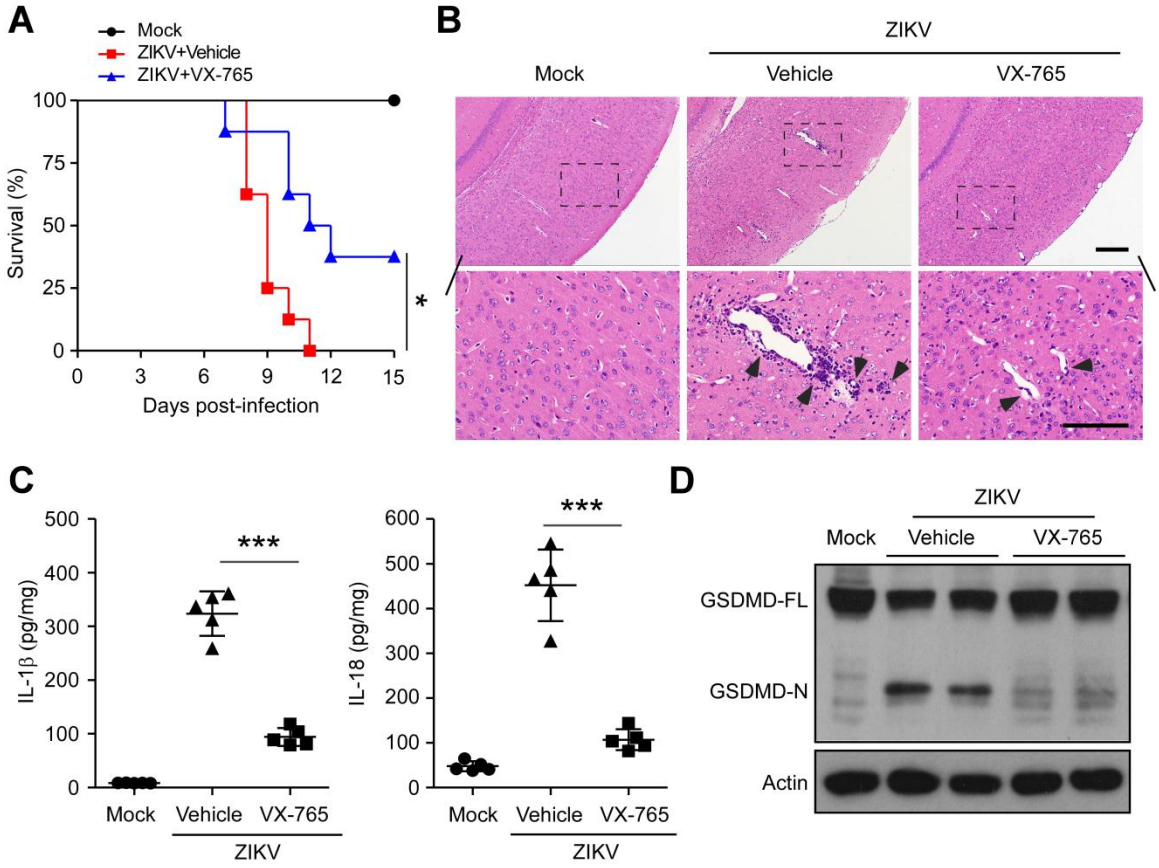

Fig. S5. VX-765 treatment improves survival of ZIKV-infected mice and reduces neuroinflammation and pyroptosis.

Five-week-old *Ifnar1*$^{-/-}$ mice were infected with ZIKV (1×10^5 PFU) or mock-infected, and subsequently treated with VX-765 (50 mg/kg, i.p.) or with vehicle (PBS/DMSO) daily. **(A)** Mice were monitored for survival over 15 days (n = 8). *P* value is based on a log-rank test. **P* < 0.05. **(B)** Representative micrographs of histopathological changes in brain sections of the cortex. Brain tissue sections obtained from ZIKV-infected mice showed inflammation characterized by infiltration of immune cells in the perivascular cuffing (black arrow). Scale bars, 100 μm (upper panels), 50 μm (lower panels). **(C)** IL-1β and IL-18 were measured by ELISA in brain specimens of ZIKV- and mock-infected mice treated with VX-765 or control vehicle. All data are presented as mean ± SD, Student's t-test, ****P* < 0.001. **(D)** Proteolytic cleavage of GSDMD in the brain

specimens of indicated mice was determined by immunoblotting analysis. GSDMD-FL, full-length GSDMD; GSDMD-N, the N- terminal cleavage product of GSDMD.