Figure S1

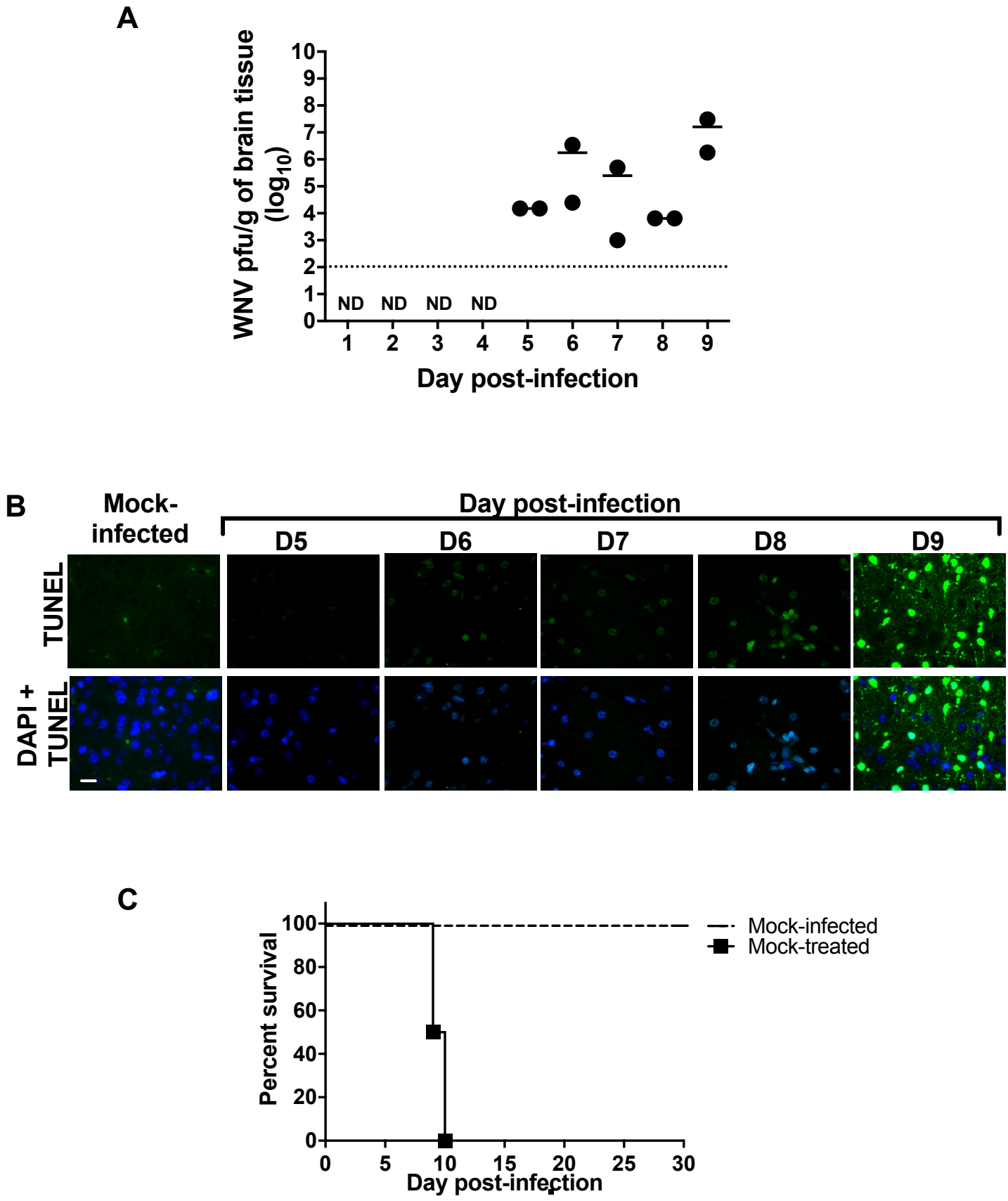

A
WNV pfu/g of brain tissue (log10)
10
9
8
7
6
5
4
3
2
1
0
ND ND ND ND
1 2 3 4 5 6 7 8 9
Day post-infection
B
Mock-infected
Day post-infection
D5
D6
D7
D8
D9
TUNEL
DAPI + TUNEL
C
Percent survival
100
80
60
40
20
0
0 5 10 15 20 25 30
Day post-infection
Mock-infected
Mock-treated

Figure S2

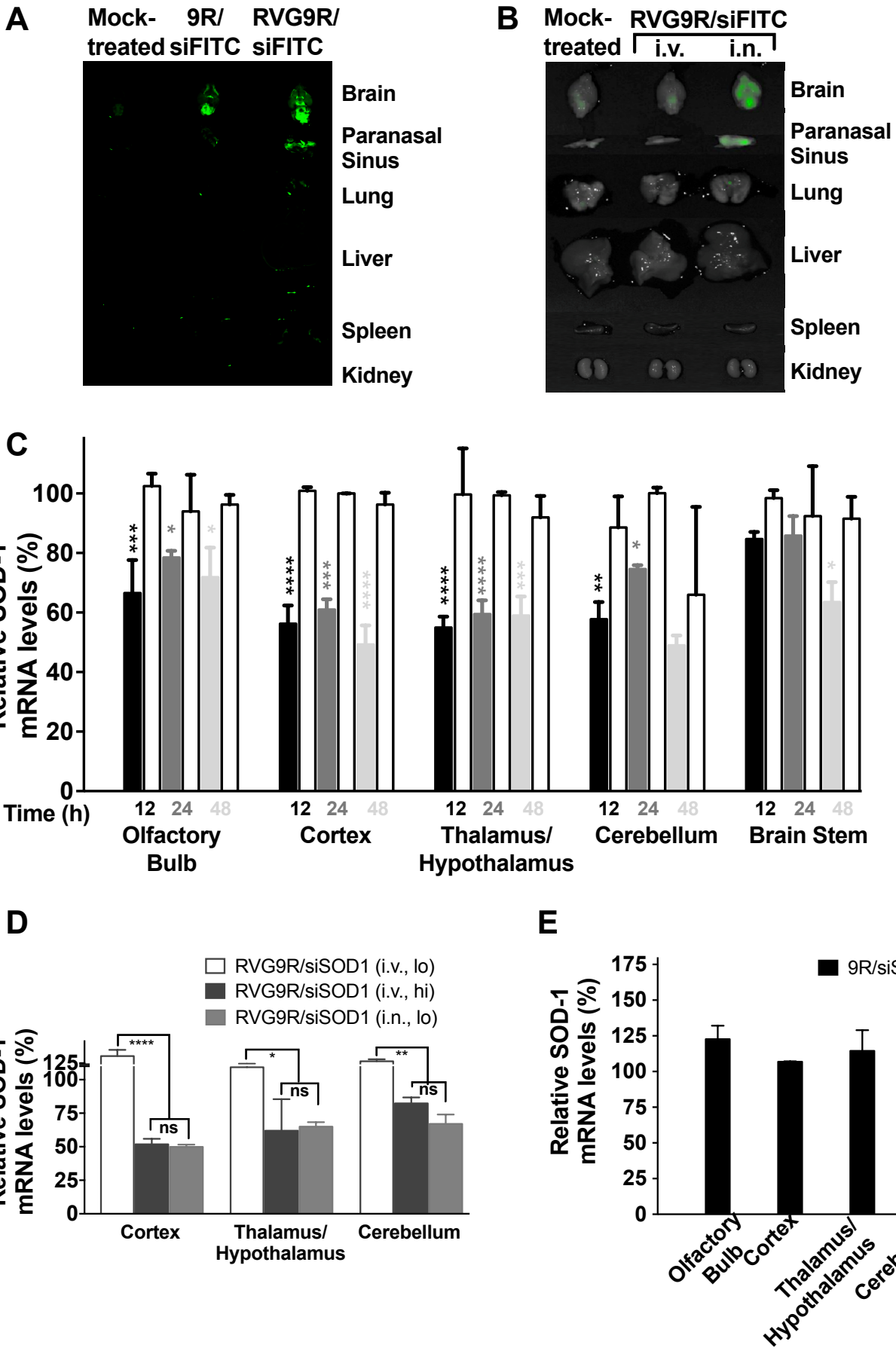
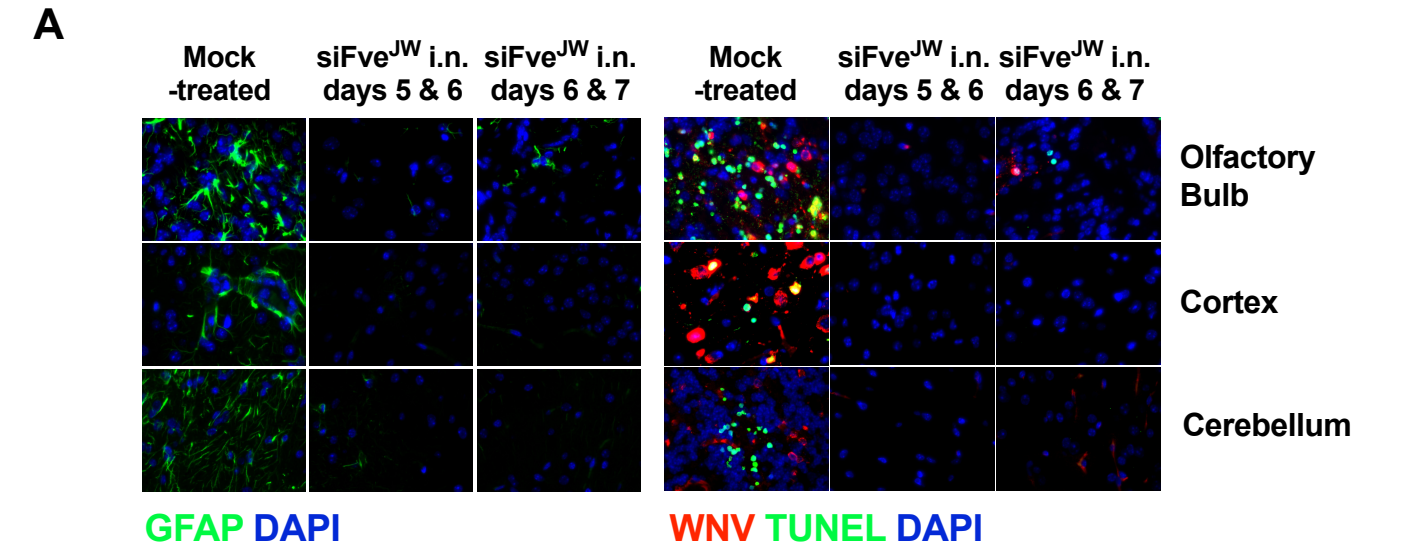
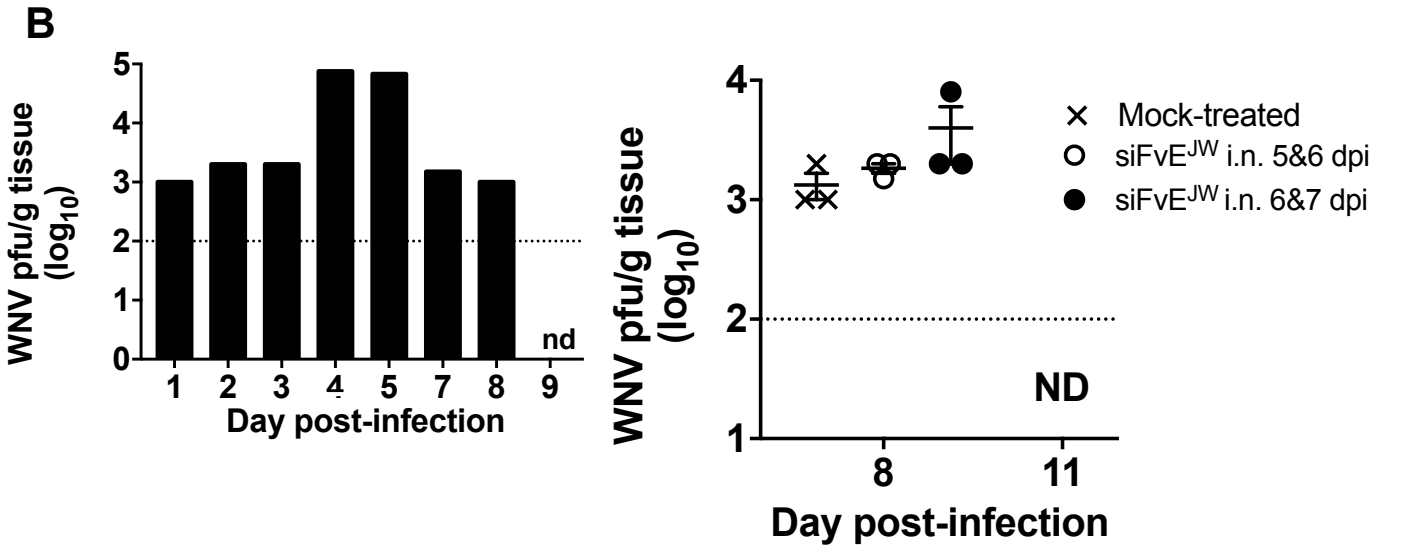

A
Mock-treated
9R/siFITC
RVG9R/siFITC
Brain
Paranasal Sinus
Lung
Liver
Spleen
Kidney
B
Mock-treated
RVG9R/siFITC
i.v.
i.n.
Brain
Paranasal Sinus
Lung
Liver
Spleen
Kidney
C
Relative SOD-1 mRNA levels (%)
Time (h)
12
24
48
Olfactory Bulb
Cortex
Thalamus/ Hypothalamus
Cerebellum
Brain Stem
D
RVG9R/siSOD1 (i.v., lo)
RVG9R/siSOD1 (i.v., hi)
RVG9R/siSOD1 (i.n., lo)
Relative SOD-1 mRNA levels (%)
ns
Cortex
Thalamus/ Hypothalamus
Cerebellum
E
9R/siSOD-1
Relative SOD-1 mRNA levels (%)
Olfactory Bulb
Cortex
Thalamus/ Hypothalamus
Cerebellum
Brain Stem

Figure S3


A
Mock -treated
siFve^JW i.n. days 5 & 6
siFve^JW i.n. days 6 & 7
Mock -treated
siFve^JW i.n. days 5 & 6
siFve^JW i.n. days 6 & 7
Olfactory Bulb
Cortex
Cerebellum
GFAP DAPI
WNV TUNEL DAPI


B
WNV pfu/g tissue (log10)
Day post-infection
nd
WNV pfu/g tissue (log10)
Mock-treated
siFvE^JW i.n. 5&6 dpi
siFvE^JW i.n. 6&7 dpi
ND
Day post-infection

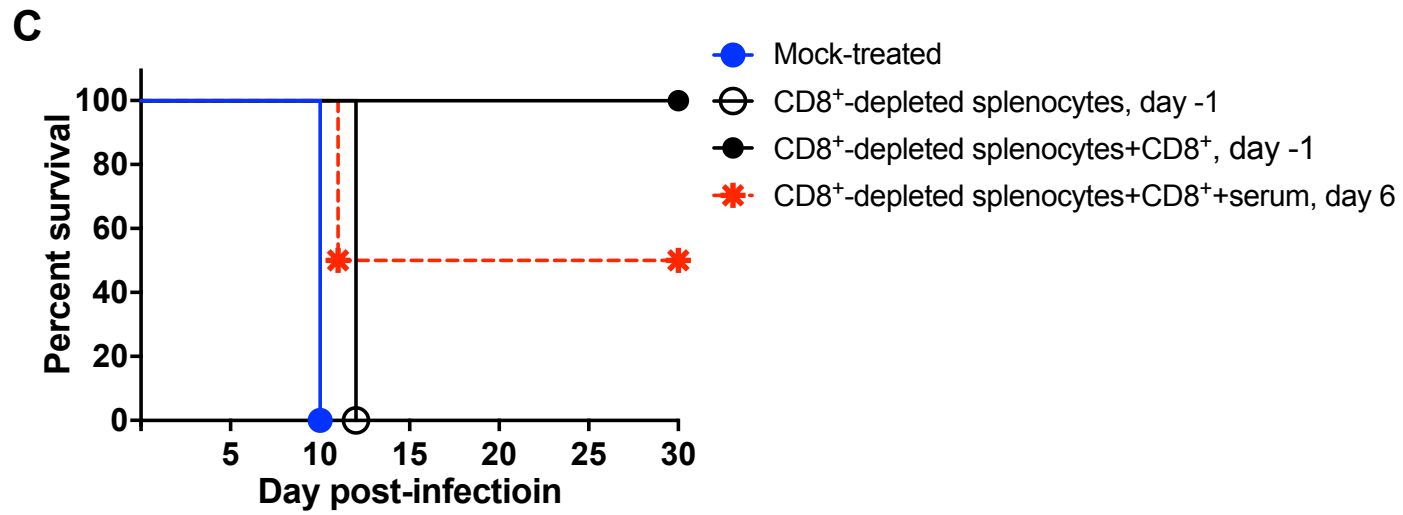
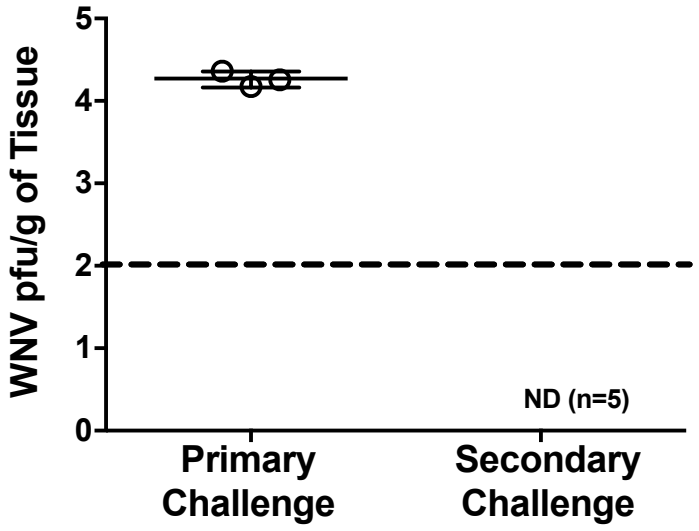
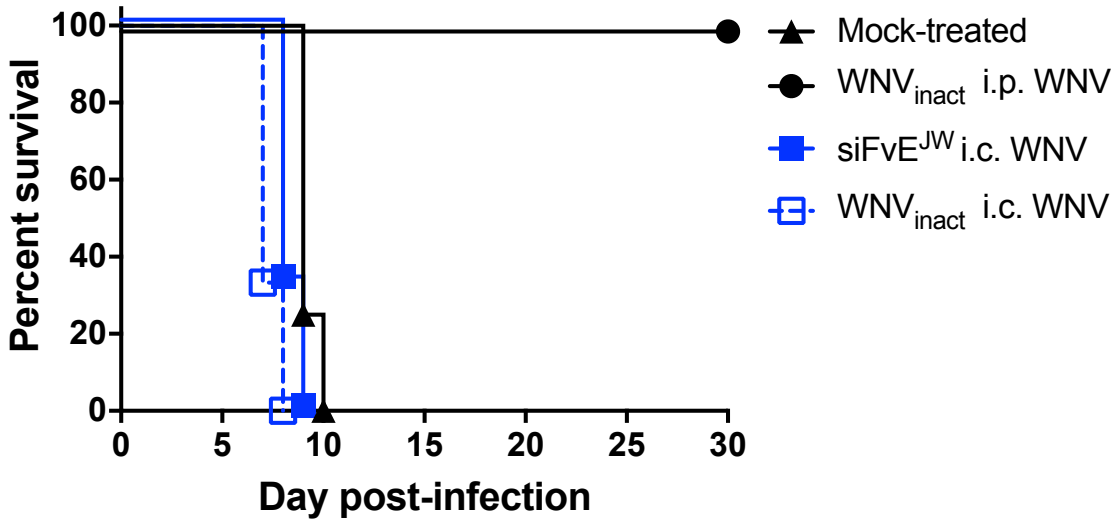

C
Percent survival
Day post-infectioin
Mock-treated
CD8+-depleted splenocytes, day -1
CD8+-depleted splenocytes+CD8+, day -1
CD8+-depleted splenocytes+CD8++serum, day 6

Figure S4

A


WNV pfu/g of Tissue
5
4
3
2
1
0
ND (n=5)
Primary Challenge
Secondary Challenge

B


Percent survival
100
80
60
40
20
0
0
5
10
15
20
25
30
Day post-infection
Mock-treated
WNVinact i.p. WNV
siFvEJW i.c. WNV
WNVinact i.c. WNV

Supplemental Information

Figure S1, Related to STAR Methods. Kinetics of WNV infection in C3H mice

(A) Brain viral loads (days 1 through 9 p.i.) in C3H mice challenged intraperitoneally (i.p.) with $100LD_{50}$ WNV. Data are mean WNV titers per gram of tissue and each dot represents one mouse. The dotted line represents the limit of detection for the assay. ND-not detectable. (B) Fluorescence microscopy images of brain tissue sections from control mice (Mock-infected) or mice infected with $100LD_{50}$ of WNV days 5 through 9 p.i. depicting TUNEL-positive cells. Merged images depict DAPI nuclear stain (blue) overlaid with TUNEL stain (green). Scale bar=30 μm (C) Kaplan-Meier survival curves of C3H mice after i.p. challenge with $100LD_{50}$ WNV. Data are from multiple experiments representing a total of 22 mice in each group. Mock-infected mice- No WNV and Mock-treated mice- mice infected i.p. with $100LD_{50}$ of WNV.

Figure S2, Related to Figure 1. Analysis of i.n. siRNA delivery to the brain

(A) Fluorescent images of the organs in Figure 1A. Mock- Mice administered i.n. PBS. (B) Representative images of organs isolated at 24 h after i.n. administration of PBS (mock), and RVG9R-complexed siFITC (5 μg) administered i.v. or i.n. Flourescent images are overlaid on their bright-field counterparts. (C) qPCR for SOD1 mRNA in isolated mouse brain regions at 12, 24 and 48 h following i.n. treatment with RVG9R-siSOD1 (solid bars) and RVG9R-siNT (open bars). NT- non-targeting siRNA, siCD4. A single dose of RVG9R-siRNA (13.5 μg) was administered. Data are percent relative gene expression (± SD) relative to GAPDH after normalizing with corresponding data from the mock (PBS)-treated cohort. Data are combined from 2 experiments with individual mouse cohorts of n = 3 per treatment at each time point. (C) qPCR for SOD1 mRNA in mouse brain regions at 48 h after the fourth treatment with 13.5 μg (lo) or 70 μg (hi) of RVG9R-siSOD1 aministered i.v. or i.n. Data are percent relative gene expression (± SD) relative to GAPDH after normalizing with corresponding data from the mock (PBS)-treated cohort. Data are with individual mouse cohorts of n = 3 per treatment. (E) qPCR for SOD1 gene expression in brain regions at 24 h following i.n. treatment with siSOD1 complexed to 9R. Data are mean percent gene expression (± SE) relative to GAPDH after normalizing with corresponding data in a mock-treated cohort (n=3). *$P \leq 0.05$; ***$P \leq 0.001$; ****$P \leq 0.0001$; ns- not-significant.

Figure S3, Related to Figures 2 and 3. Analysis of RVG9R-siFvE^{JW}-treated WNV-infected mice

(A) Representative images of WNV-infected mouse brain sections from the olfactory bulb, cortex and cerebellum analyzed by fluorescent immunohistochemistry at day 9 p.i. for GFAP expression (left) and TUNEL-positive cells and WNV antigen (right). Nuclei were stained with DAPI (blue). Mock-treated- Mice administered i.n. PBS. Scale bar= 30 μm. (B) Spleen viral burden measured by IPMA in the spleens of wild-type C3H mice at the indicated days (left) and RVG9R-siFvE^{JW} treated C3H mice on day 8 (right) after infection with WNV. Each data point represents one mouse. Data are mean WNV titers per gram of tissue and the dashed line represents limit of detection for the assay. nd-not done; ND- not detected. Mock-treated- Mice administered i.n. PBS. (C) Kaplan-Meier survival curves of C3H recipients that received splenocyte populations (w/w/o immune serum) from donor RVG9R-siFvE^{JW} treated WNV-infected mice that survived challenge. C3H recipients underwent adoptive transfer either a day before or on day 6 p.i. with WNV. n = 2-3 mice per group.

Figure S4, Related to Figure 4. Analysis of viral titers and survival kinetics of RVG9R-siFvE^{JW}-treated mice after secondary challenge with WNV.

(A) Infectious viral loads in the spleens of RVG9R-siFvE^{JW} treated C3H mice challenged for a second time with 100LD$_{50}$ WNV on day 4 after second challenge. Data are mean WNV titers per gram of tissue and the dashed line represents limit of detection for the assay. Each data point represents one mouse. ND- not detectable. (B) Kaplan-Meier survival curves of mice challenged intraperitoneally (i.p. 100LD$_{50}$) or intracranially (i.c. 20LD$_{50}$) with WNV. For mice treated with i.n. siRNA, this represented a second exposure to the virus. WNV$_{inact}$ refers to mice immunized with H_2O_2-inactivated WNV.

Oligonucleotides	**Sequence 5'-3'**	**Reference**
WNV primers	F: TCACGCATCTCTCCACCAAAG3 R: GGGTCAGCACGTTTGTCATTG3	(Papin et al., 2004)
SOD-1 primers	F: CCAGTGCAGGACCTCATTTT3 R: CACCTTTGCCCAAGTCATCT3	This paper
GAPDH primers	F: AACTTTGGCATTGTGGAAGG R: GGAGACAACCTGGTCCTCAG	This paper
siSOD1	Sense strand: GGUGGAAAUGAAGAAAGUA	(Kumar et al., 2007)
Human siCD4	Sense strand: GAUCAAGAGACUCCUCAGU3	(Kumar et al., 2007)
siLuc (siNT)	Sense strand: GGACAUUACUAGUGACUCA	(Kumar et al., 2006)
$siFvE^{JW}$	Sense strand: GGGAGCAUUGACACAUGUGCA	(Kumar et al., 2006)
siScr	Sense strand: GTGAACCTATCGACGGAGTGA	This paper

Table 1. Oligonuleotide sequences. Related to STAR methods.