SUPPLEMENTAL FIGURES AND LEGENDS

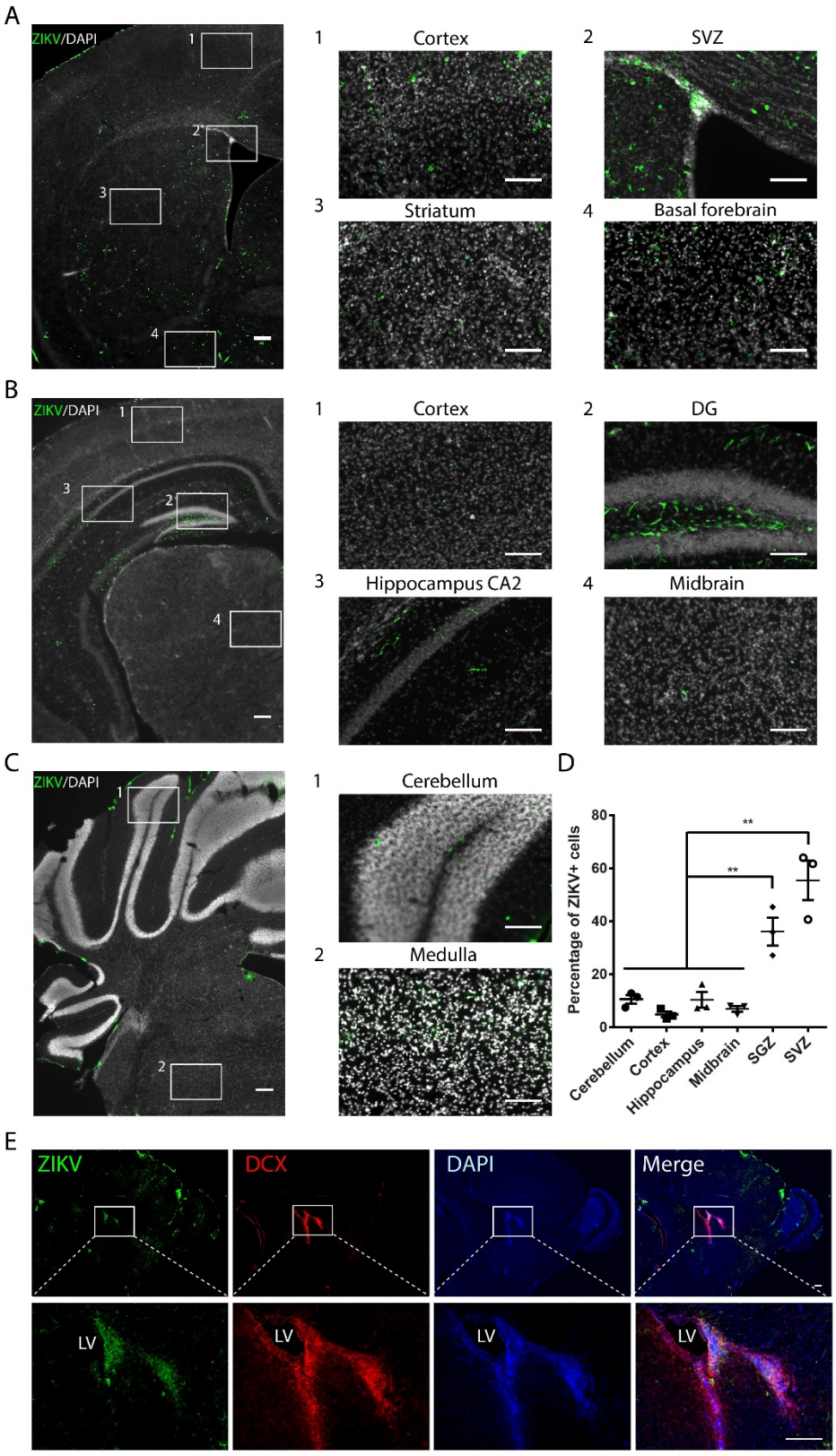

Figure S1. Distribution of ZIKV infection in the adult brain, related to Figure 1.

(A-C) Representative images of ZIKV infected cells across various brain regions. Numbered panels: high magnification of the areas outlined by a white box in images A to C. Scale bar, 100 μm.

(D) Quantification of ZIKV infected cells as percentage of DAPI cells in selective brain regions. All data represent means ± SEM. n=3 animals for each group $^{**}P < 0.01$. One-way ANOVA with Tukey's multiple comparisons test.

(E) Representative images of sagittal sections showing ZIKV infected DCX+ cells along the rostral migratory stream. Lower images are high magnification of the areas outlined by a white box in top images. Scale bar, 100 μm.

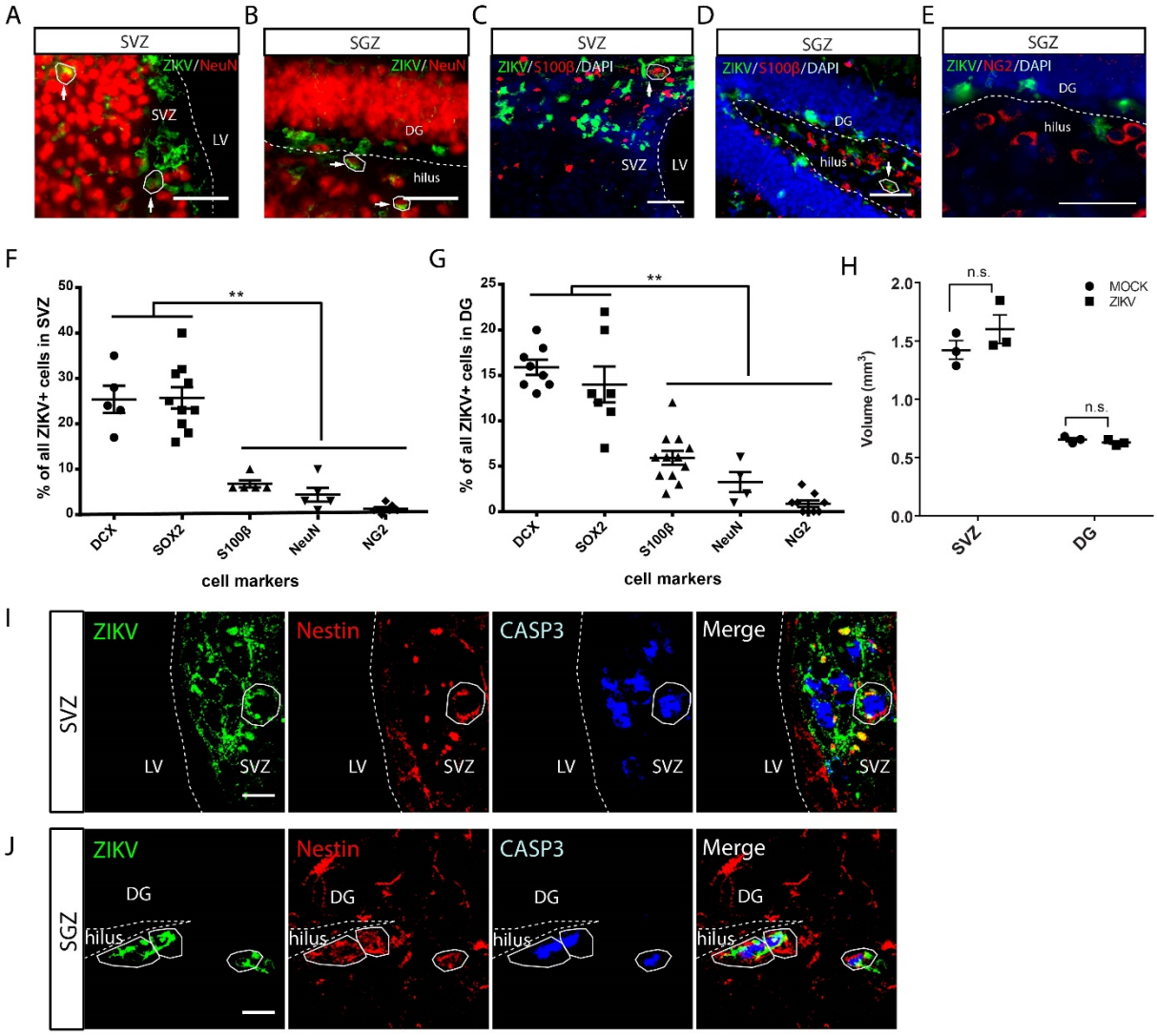
A
SVZ
ZIKV/NeuN
SVZ
LV
B
SGZ
ZIKV/NeuN
DG
hilus
C
SVZ
ZIKV/S100β/DAPI
SVZ
LV
D
SGZ
ZIKV/S100β/DAPI
DG
hilus
E
SGZ
ZIKV/NG2/DAPI
DG
hilus
F
% of all ZIKV+ cells in SVZ
**
DCX
SOX2
S100β
NeuN
NG2
cell markers
G
% of all ZIKV+ cells in DG
**
DCX
SOX2
S100β
NeuN
NG2
cell markers
H
Volume (mm3)
n.s.
n.s.
MOCK
ZIKV
SVZ
DG
I
SVZ
ZIKV
Nestin
CASP3
Merge
LV
SVZ
J
SGZ
ZIKV
Nestin
CASP3
Merge
DG
hilus

Figure S2. Distribution of ZIKV infection in different cell types in the adult brain and evidence for activation of apoptotic pathway in ZIKV-infected NPCs, related to Figure 2.

(A, B) SVZ (A) and SGZ (B) regions co-stained for NeuN and ZIKV. Arrows and white circle outlines: infected neurons evidenced by NeuN co-staining. Dense cell zone in F: hippocampal dentate granule layer. LV: Lateral ventricle. SVZ: subventricular zone. DG: dentate gyrus. Most ZIKV signal is in neurogenic non-NeuN expressing regions. Scale bar, 50μm.

(C, D) SVZ (C) and SGZ (D) regions co-stained for S100β and ZIKV envelope protein. Arrows and white outlines: infected astrocytes evidenced by S100β co-staining. LV: Lateral ventricle. SVZ: subventricular zone. DG: dentate gyrus. Scale bar, 50μm.

(E) SGZ region co-stained for NG2 and ZIKV envelope protein. NG2 marks oligodendrocytes, which were negative for ZIKV. DG: dentate gyrus. Scale bar, 50μm.

(F, G) Quantification of infected DCX+, SOX2+, S100β+, NeuN+ and NG2+ cells in SVZ (F) and DG (G). n=4-12 sections in 3 ZIKV infected animals. $^{**}P < 0.01$. One-way ANOVA with Tukey's multiple comparisons test.

(H) Volume of SVZ and DG in MOCK and ZIKV infected animals. n=3 animals for each group. Student's t test.

(I, J) Representative confocal images of infected Nestin+ neural progenitors shows CASP3+ reactivity in SVZ (I) and SGZ (J). White circle outlines: infected apoptotic cells. LV: Lateral ventricle. DG: dentate gyrus. Scale bar, 10μm.

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

ZIKV Selection of strain and Preparation

Three lineages of ZIKV have been identified: East African, West African, and Asian {Lanciotti, 2016 #12}. ZIKV strain MR-766 of the East African lineage was first isolated in the 1940s, whereas both West African and Asian strains were first isolated in the 1960s. Recent studies have incorporated the use of all of the publicly available strains as well as recent isolates from infected individuals. ZIKV strains of interest among the Asian lineage include FSS13025 which was isolated from Cambodia in 2010 {Haddow, 2012 #13;Heang, 2012 #14} and shares >99% identity at the nucleotide level with strain SPH2015, isolated from the current outbreak in Brazil {Lanciotti, 2016 #12;Cunha, 2016 #16}. Differences in tropism or cytopathology is not well understood, although some strains produce more pathogenicity than the original MR-766 strain {Lazear, 2016 #17}.

The Asian lineage ZIKV strain FSS13025 (Cambodia, 2010), obtained from the World Reference Center for Emerging Viruses and Arboviruses (WRCEVA), was used in this study. ZIKV was cultured using the C6/36 Aedes albopictus mosquito cells grown in Leitbovitz L-15 medium with 5% Fetal Bovine Serum (FBS) at 28°C in absence of CO2. Supernatant was harvested after 7-10 days of culture, followed by clarification by centrifugation and concentrated via ultracentrifugation as previously described (Prestwood et al., 2008). Virus was titrated via a standard Focus Forming Assay (FFA) using Baby Hamster Kidney (BHK)-21 cells and the pan Flavivirus anti-envelope (E) antibody 4G2.

Mouse

IRF-TKO mice were bred at La Jolla Institute for Allergy & Immunology Animal Facility (La Jolla, CA). This mouse strain was derived from mice provided by Dr. Tadatsugu Taniguchi (U. Tokyo)

and obtained from Dr. Michael Diamond (Washington U., St. Louis, MO). Three pairs of mice between 5-6 weeks of age were infected retro-orbitally (Yardeni et al., 2011) with 1 x 10^3 Focus Forming Units (FFU) of virus or mock treatment, then sacrificed 6 days later. This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health, the US Public Health Service Policy on Humane Care and Use of Laboratory Animals, and the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC). All experimental procedures were approved and performed according to the guidelines set by the Animal Care and Use Committee (protocol number AP-28SS1-0809).

EdU Labeling

Mice infected with ZIKV 6d prior received intraperitoneal injections with 10 mg/kg of EdU in PBS and brains were harvested after 2h according to established protocols (Chehrehasa et al., 2009). EdU staining was performed on 20 uM sections using Click-iT™ EdU protocol (Invitrogen, Carlsbad, CA). Briefly, sections were incubated with a Click-iT™ reaction cocktail containing Click-iT™ reaction buffer, CuSO4, Alexa Fluor® 594 Azide, and reaction buffer additive for 30 min, then rinsed, DAPI counterstained and mounted.

Immunohistochemistry and Antibodies

Mice were sacrificed then perfused with normal saline solution followed by fresh 4% paraformaldehyde (PFA) in PBS. Brains were isolated then post-fixed in 4% PFA/PBS overnight, cryoprotected in 30% sucrose, then embedded in TissueTek. Serial 40 µm coronal sections were prepared on sliding microtome across the entire brain. Sections were permeabilized with 1% Triton X-100 in PBS (PBT), blocked with Donkey Serum (Millipore) and immunostained with antibodies.

We selected antibodies to label specific developmental timepoints of NSCs. Cells in the SVZ migrate along the rostral migratory stream and contribute to the olfactory granule neurons. Cells in the SGZ migrate and contribute to the dentate granule neuron population. Neural stem cells (NSCs) express markers such as GFAP, SOX2 and Nestin; Intermediate progenitor cells (IPCs) lose GFAP expression and start to express doublecortin (DCX); the migratory immature neurons express markers such as DCX, the post-migratory mature neurons express markers such as NeuN in a clear temporal fashion. In addition to antibody agains the Flavivirus envelope protein (MAB10216, 4G2 clone 1, EMD Millipore 1:500), we also used GFAP (G9269, Sigma Aldrich 1:400), Nestin (Avēs 1:2000), SOX2 (AB5603, Millipore 1:500), S100β (ab868, Sigma Aldrich 1:300), Caspase 3 active (cleaved) form (AB3623, Millipore 1:100), Doublecortin (sc-8067, Santa Cruz 1:200) and NeuN (ab177487, Abcam 1:300). Ki67 (RM-9106-S1, ThermoFisher, 1:200), and pH3-Ser10 (06-570, Millipore, 1:500). Alexa Fluor® secondary antibodies (Invitrogen) were used for detection.

Imaging and Quantification

Images were acquired on an Olympus IX51with QImaing Retiga-2000R camera or Zeiss Axio Observer Z1 confocal microscope. Images were analyzed with ImageJ (NIH) and Stereo Investigator (MBF Bioscience). In the quantification analysis, we estimated SGZ and SVZ area as SOX2+ regions in dentate gyrus and lateral ventricle. Stereological counting of the total EdU, Ki67, and pH3 labeled cells was performed by counting all positive cells using every 6th serial sections throughout the hippocampus and SVZ. The two-tailed unpaired Student's *t*-test was used to calculate *P* values for comparisons. Multiple comparisons in the same data set were analyzed by one-way ANOVA followed by Tukey's multiple comparisons test. $P < 0.05$ was considered to be statistically significant. Statistical processing was performed using Microsoft Excel and GraphPad Prism Software.