Supporting Information

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SI Materials and Methods

Ethics Statement. All experiments were performed in accordance with guidelines from the Guide for the Care and Use of Laboratory Animals of the NIH. Protocols were reviewed and approved by the Institutional Animal Care and Use Committee at Columbia University School of Medicine (assurance no. AC-AAAR5408).

Ex Utero Electroporation. Plasmids encoding GFP were transfected by intraventricular injection into the ventricle of dissected embryonic brains at the appropriate time. DNA was mixed with colored nontoxic dye and 1 μg of nucleic acid was injected into the ventricular space using a high gauge needle made from glass capillary tube. Post injection, five pulses of electrical current (50 V, 5 ms each, with 1-s intervals) were applied by directly placing electrodes on the outer surface of the brain, angled along the lateral aspect of the neocortex adjacent to the lateral ventricle targeted by injection. Brain slices were generated following transfection and cultures were maintained up to 8 d postelectroporation.

Organotypic Embryonic Mouse Brain Slice Cultures. Timed pregnant Swiss Webster mice (E13, E15, and E19) were purchased from Taconic Labs; E1 was defined as the day of confirmation of sperm-positive vaginal plug. Mice were killed and fetuses were harvested. Fetal brains were dissected into ice-cold artificial cerebrospinal fluid (ACSF) consisting of 125 mM NaCl, 5 mM KCl, 1.25 mM NaH_2PO_4, 1 mM $MgSO_4$, 2 mM $CaCl_2$, 25 mM $NaHCO_3$, and 20 mM glucose, pH 7.4, 310 $mOsm1^{-1}$. Brains were embedded in 4% low melting point agarose dissolved in ACSF and sliced into 300-μm coronal sections using a vibratome (Zeiss). Slices were maintained on 0.4-μm, 30-mm diameter Millicell-CM inserts (Millipore) in cortical culture medium (CCM) containing 25% HBSS, 47% basal MEM, 25% normal horse serum, 1× penicillin–streptomycin–glutamine, and 30% glucose. Cultures were maintained in a humidified incubator at 37 °C with constant 5% CO_2 supply.

Indirect Immunofluorescence Microscopy. Either 72 h, 96 h, or 8 d postinfection, the medium was removed from Zika virus-infected or -uninfected organotypic brain slice cultures and the cultures were placed overnight in 4% paraformaldehyde (PFA) fixative dissolved in 1× PBS at 4 °C. Following fixation, cultures were incubated in blocking solution of PBS, 0.3% Triton X-100, and 3% donkey serum. Cultures were incubated overnight at 4 °C in blocking solution containing appropriate primary antibodies. Sections were washed in 1× PBS and incubated in the presence of fluorophore-conjugated secondary in blocking solution. Sections were mounted on slides using Aqua-Poly/Mount (Polysciences, Inc) and imaged using a iZ80 laser scanning confocal microscope (Olympus FV100 spectral confocal system).

Plaque Assay. Vero cells were seeded on 60-mm plates for ∼70% confluence at the time of plaque assay. Next, 100λ portions of serial 10-fold virus dilutions were incubated with cells for 1 h at 37 °C. Two overlays were added to the infected cells. The first overlay consisted of 2 mL of 1 DMEM, 0.8% Noble agar, 0.1% BSA, 40 mM $MgCl_2$, and 10% bovine calf serum. After solidification, a second liquid overlay was added that was composed of 1 DMEM, 0.1% BSA, 40 mM $MgCl_2$, 0.2% glucose, 2 mM pyruvate, 4 mM glutamine, and 4 mM oxaloacetic acid. The cells were incubated at 37 °C for 6–8 d and developed by using 10% trichloroacetic acid and crystal violet.

Antibodies. Antibodies used in this study were chicken polyclonal against vimentin (AB5733, 1:1,000 dilution; Millipore), pan-flavivirus E glycoprotein (MAB 10216, Millipore), rabbit polyclonal against CDP (SC-1302, Santa Cruz), and cleaved caspase 3 (Cell Signaling). Donkey fluorophore-conjugated secondary antibodies (1:500 dilution; Jackson Labs) were used together with DAPI (4′,6-diamidino-2-phenylindole, 62248, 1:1,000 dilution; Thermo Fisher Scientific).

Data Analysis. GraphPad Prism software was used to analyze all data. Log10-transformed titers were used for graphing the results of plaque assays. Statistical analysis for Figs. 3*C* and 6*C* were performed by ANOVA test to determine significance among groups and then unpaired *t* tests between the mean values of three different experiments from embryos derived from three different mothers.

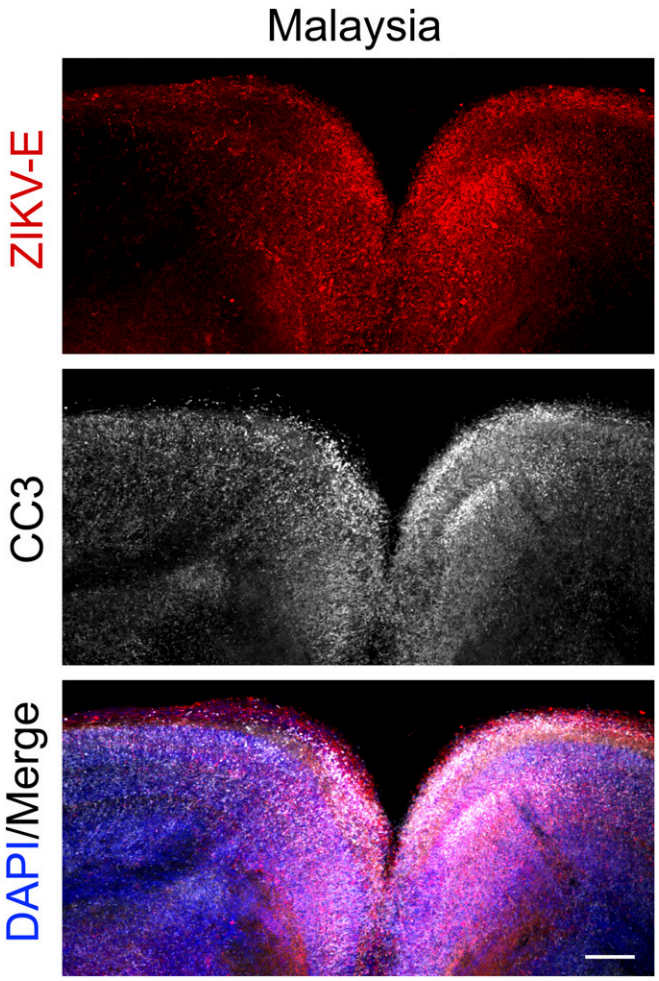

Fig. S1. Tissue distribution and replication of Malaysian ZIKV isolate in E15 organotypic mouse brain slices. Brain slice cultures from E15 embryos were infected with 10^5 pfu of the Malaysian ZIKV isolate and at 4 dpi, were fixed and stained with pan-flavivirus antibody against the E glycoprotein (ZIKV-E), cleaved caspase 3 (CC3), and DAPI. Notably the regions of apoptosis overlapped with regions of ZIKV infection, as determined by ZIKV-E staining. (Scale bar: 250 μm.)

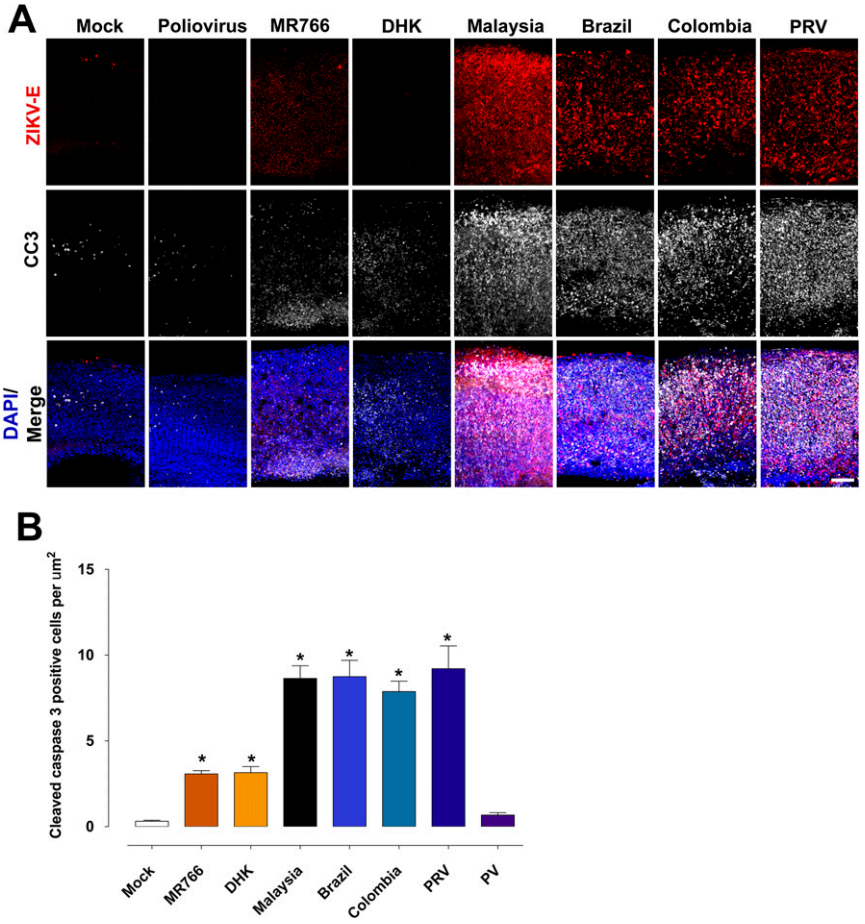

Fig. S2. ZIKV infection of organotypic mouse brain slices leads to increased apoptosis. (*A*) Brain slice cultures from E15 embryos were infected with 10^5 pfu of the indicated ZIKV isolates or poliovirus and at 8 dpi, were fixed and stained with pan-flavivirus antibody against the E glycoprotein (ZIKV-E), antibody to cleaved caspase 3 (CC3), and DAPI. All isolates of ZIKV tested led to infection of organotypic slices and induced apoptosis. (Scale bar: 100 μm.) (*B*) Infection with all isolates of ZIKV led to significant increases in apoptosis compared with control, and infection with Asian ZIKV isolates led to increase in apoptosis compared with infection with African ZIKV isolates (ANOVA, $P < 0.01$ across all groups; unpaired *t* test, $^*P < 0.05$, $n = 3$ different experiments with embryos from different mothers). Apoptotic index, as defined by number of cleaved CC3 positive cells per square micrometer, in E15 brain slice cultures infected with the indicated ZIKV isolates. PV, poliovirus. Data represented as mean ± SEM for each condition.