Supplemental Information

Western Zika Virus in Human Fetal Neural Progenitors
Persists Long Term with Partial Cytopathic
and Limited Immunogenic Effects

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Supplemental Experimental Procedures

Cells and viruses

Cells were maintained in the following media: human hepatoma Huh7.5 (DMEM), baby hamster kidney BHK-21J (MEM), African green monkey kidney Vero-E6 (MEM), THP-1 (RPMI), and STAT1-/- fibroblasts (RPMI) (Life Technologies). For all cells, media was supplemented with 10% FBS (Life Technologies) and 0.1 mM non-essential amino acids (Life Technologies). Human neural progenitor (hNP) cultures were obtained from Lonza or generated as previously described (Konopka et al., 2012). hNPs were maintained as previously described (Konopka et al., 2012). ZIKV PRVABC59 was obtained from the CDC (GenBank Accession #KU501215). The virus had been passaged three times in Vero cells prior to our acquisition. The virus was passaged a fourth time in Vero-E6 cells to establish a viral stock. Viral stocks were prepared by infecting Vero-E6 cells with 0.01-0.05 MOI of virus. Cell supernatant was collected 6 days later and purified by centrifugation at 1500 rpm to remove cellular debris. Viral stocks were titered on BHK-21J cells. YFV-17D and dengue virus (strain 16681) were generated from clones as previously described (Bredenbeek et al., 2003; Kinney et al., 1997).

Virus infections and titer determination

ZIKV stocks grown in Vero-E6 cells were titered by limiting dilution plaque assay on BHK-21J cells. Infections with YFV-17D or ZIKV were performed at 37°C for 1 h with occasional rocking before cells were overlaid with Avicel. After 4 days, plaques were visualized by crystal violet staining. Alternatively, viral titers were determined on Huh7.5 cells using a published FACS-based antibody protocol (Lambeth et al., 2005).
Indirect Immunofluorescence

Immortalized and hNP cells infected with ZIKV were fixed 72 h post-infection in 4% paraformaldehyde for at least 15 min. Cells were then permeabilized with 0.2% Triton for 5 min, washed with PBS+/+ and blocked in 10% BSA in PBS+/+ for 30 min. The cells were then washed with PBS+/+ and incubated for 2 h with primary antibody in 3% BSA in PBS+/+. The following primary antibodies were used: Anti-Flavivirus Group Antigen Antibody (D1-4G2-4-15), Nestin (rabbit, #ABD69 Millipore), cleaved caspase 3 (rabbit, #9664 Cell Signaling Technology). After 2 h, cells were washed three times with PBS+/+ and counterstained with AlexaFluor 488 goat anti-mouse or AlexaFluor 555 goat anti-mouse (Life Technologies #A21430). Cells were incubated at RT for 30 min, washed and stained with Hoechst dye (#33342 Thermo Scientific). Cells were observed by immunofluorescence using a Zeiss epifluorescent microscope equipped with an AxioCam MRm, or a Nikon epifluorescent microscope equipped with a Photometrics CoolSnap ES²camera. Images were obtained using camera specific software and processed in ImageJ or Adobe Photoshop.

Electron Microscopy

Control or infected Huh7.5 cells were fixed with 2.5% (v/v) glutaraldehyde in 0.1M sodium cacodylate buffer (pH7.4). After 10 min fixation, cells were released from the plastic dishes by gentle scraping, transferred to conical tube, kept in the same fixative for an additional hour at room temperature and pelleted. After three rinses with 0.1 M sodium cacodylate buffer, cell pellets were embedded in 3% agarose and sliced into small blocks (1mm3), rinsed with the same buffer three times and post-fixed with 1% osmium tetroxide and 0.8 % potassium ferricyanide in 0.1 M sodium cacodylate buffer for 1.5 h at room temperature. Cells were rinsed with water and en bloc stained with 4% uranyl acetate in 50% ethanol for two hours. Cells were dehydrated with increasing concentration of ethanol, transitioned into propylene oxide, infiltrated
with Embed-812 resin and polymerized in a 60°C oven overnight. Blocks were sectioned with a diamond knife (Diatome) on a Leica Ultracut 6 ultramicrotome (Leica Microsystems) and collected onto copper grids, post stained with 2% aqueous Uranyl acetate and lead citrate. Images were acquired on a Tecnai G² spirit transmission electron microscope (FEI) equipped with a LaB₆ source using a voltage of 120 kV.

Supplemental References

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Lambeth, C.R., White, L.J., Johnston, R.E., and de Silva, A.M. (2005). Flow cytometry-based assay for titrating dengue virus. Journal of clinical microbiology 43, 3267-3272.
Supplemental Figure 1. A. Quantitation of ZIKV and DENV genomes by RT-qPCR. Huh7.5 cells were infected with increasing doses of each virus. Total RNA from infected cells was assayed for either ZIKV or DENV genomes using virus-specific primers. B. Electron micrographs of Huh7.5 infected with DENV, ZIKV, and YFV. Images are representative of commonly visualized vesicle packets, large invaginated vesicles, convoluted membranes and viral particles.
Supplemental Figure 2. A. Antibody-based array to detect 102 cytokines in supernatants of hNP infected with ZIKV for 24 h. B, C. ELISA to detect CCL2 or CX3CL1 in hNP supernatants 24 h and 72 h after infection with 0.5 MOI ZIKV. Results are presented as mean ± S.D. n=3. Statistical analysis was performed by unpaired t-test. ns, not significant.