**Supplementary Methods**

**Cells and viruses**

Low passage Asian lineage ZIKV strain (PLCal ZV) isolated from a Canadian traveler in 2013 [1], the prototype Asian ZIKV strain isolated in Puerto Rico (PRVABC-59) in 2015 [2] and dengue virus-2 (DENV-2, New Guinea C) were kindly provided by the National Microbiology Lab of Canada (Winnipeg, MB). The African virus strain (MR766) generated from an infectious clone of the 1947 Uganda ZIKV was obtained from Dr. Matthew J. Evans laboratory at the Icahn School of Medicine at Mount Sinai, New York [3] Viruses were propagated in *Aedes albopictus* C6/36 cells grown in Minimum Essential Medium (MEM) supplemented with 10% fetal bovine serum (Gibco), L-glutamine, Penicillin-Streptomycin and MEM non-essential amino acids at 32°C.

ZIKV and DENV-2 stocks were prepared after inoculating C6/36 cells with the multiplicity of infection (MOI) of 0.2 and harvesting supernatants at 48 and 96 hr post-infection. Virus-containing media were clarified by centrifugation at 3200 x g for 10 minutes. HFAs [4] were prepared as previously described. Briefly, human fetal brain tissues were obtained from 15 to 19-week aborted fetuses. After isolation of astrocytes, cells were split and all experiments were conducted with cells from the fifth to the seventh passage. HFAs (three donors) were grown in MEM (1g/L Glucose, 15 mM HEPES, Gibco) supplemented with 10% fetal bovine serum (Gibco), L-glutamine, MEM non-essential amino acids, sodium pyruvate, and 1g/mL glucose. Vero (ATCC, CCL-81) cells were maintained in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum, 15 mM HEPES (Gibco), L-glutamine and Penicillin-Streptomycin.

**Viral infections**

For infection, cells were seeded in 6-well plates at 4 -5 x 105 cells per well (Greiner) or 96-wells plates (Greiner or CELLSTAR) at 1 x 104 cells per well. Cells were rinsed once with PBS and ZIKV or DENV-2 at an MOI of 0.3 or 3.0 were added to the cells. Cells were then incubated for 2 hr at 37°C using fresh media supplemented with 3% fetal bovine serum (Gibco). Next, the inoculum was removed and the cells were washed twice with PBS. Complete culture medium was added to each well, and cells were incubated at 37°C and 5% CO2. Mock cells were incubated with the culture supernatant from uninfected C6/36 cells.

**Viral plaque assay**

ZIKV wasserially diluted (10-fold dilutions) and infected monolayers of Vero cells at 37 °C for 2 hr. The monolayer was overlaid with a mixture of MEM (Gibco) and 1.5% carboxymethylcellulose (Sigma-Aldrich) following the infection. The cells were maintained at 37°C for 4 days for plaque development. For the plaque assay of DENV-2, cells were cultured for 7 days. Before plaque counting, cells were fixed with 10% formaldehyde and stained with 1% crystal violet in 20% ethanol.

**Tissue and cellular RNA purification, cDNA synthesis, and qRT-PCR**

Total RNA was extracted from cultured HFAs or brain tissue using RNeasy mini kits (QIAGEN). Samples were then treated with RNase-free DNase (QIAGEN) before a portion (0.5-1 µg total RNA) was subjected to reverse transcription using ImProm-II Reverse Transcriptase (Promega). Cellular transcripts and viral RNA were quantitated by qRT-PCR using PerfeCTa SYBR Green SuperMix (Quanta BioSciences) in an Mx3000P instrument (Agilent Technologies) under the following cycling conditions: 45 cycles of 94°C for 30 s, 55°C for 60 s, and 68°C for 20 s. Gene expression (fold change) was calculated using the 2(-ΔΔCT) method with human β-actin mRNA transcript as the internal control.

The following forward and reverse primer pairs were used for PCR: β-actin: 5’-GGATCAGCAAGCAGGAGTATG-3’ and 5’-GCATTTGCGGTGGACGAT-3’, ZIKV NS5 region [5]: 5’-CCTTGGATTCTTGAACGAGGA-3’ and 5’-AGAGCTTCATTCTCCAGATCAA-3’, MX2: 5’-CAGCCACCACCAGGAAACA-3’ and 5’-TTCTGCTCGTACTGGCTGTACAG-3’, IFN-β: 5’-GACGCCGCATTGACCATCTA-3’ and 5’-TTGGCCTTCAGGTAATGCAGAA-3’, RSAD2 (viperin): 5’-CTTTTGCTGGGAAGCTCTTG-3’ and 5’-CAGCTGCTGCTTTCTCCTCT-3’, OAS-1: 5’- TTCTTAAAGCATGGGTAATTC-3’, 5’- GAAGGCAGCTCACGAAAC-3’, DENV: 5’-TTGAGTAAACTGTGCAGCCTGTAGCTC-3’ and 5’-GGGTCTCCTCTAACCTCTAGTCCT-3’.

**Cytotoxicity assays**

The CellTiter-Glo Luminescent Cell Viability Assay (Promega) was used for quantitation of ATP in cultured cells. Cells lysates were assayed after mixing 100 µl of complete media with 100 µl of reconstituted CellTiter-Glo Reagent (buffer plus substrate) following the manufacturer’s instructions. Samples were mixed by shaking the plates after which luminescence was recorded with a GloMax Explorer Model GM3510 (Promega) 10 min after adding the reagent.

CytoTox 96 Non-Radioactive Cytotoxicity Assay (Promega) was used to measure the release of LDH from lysed cells to the supernatants of explants. Fifty µl of supernatants were mixed with 50 µl of Substrate Mix. After 30 minutes of incubation at room temperature, the reactions were stopped with 50 µl of Stop Solution. Absorbance at 490 nm was measured using a GloMax Explorer Model GM3510 (Promega) over the next 60 minutes.

**Immunofluorescence staining and cell imaging**

Cells on coverslips or 96-well plates (CELLSTAR) were processed by fixing for 15 min at room temperature with 4% paraformaldehyde in PBS. Cells were washed three times in PBS and then permeabilized/blocked with 0.2% Triton-X100 and 3% BSA in PBS for 1 hr at room temperature followed by washing with PBS containing 0.3% BSA. Incubations with primary antibodies diluted 1:500 (mouse anti-Flavivirus Group Antigen 4G2, Millipore) in blocking buffer (3% BSA and PBS) were carried out at room temperature for 1.5 hr followed by three washes in 0.02% Triton-X100 with 0.3% BSA and PBS. Samples were then incubated with secondary antibodies (1:1000) in blocking buffer containing 1 µg/mL of DAPI for 1 hr at room temperature followed by three washes in 0.3% BSA in PBS. Secondary antibodies (Invitrogen) were Alexa Fluor 488 donkey anti-mouse. Confocal images of cells on coverslips were acquired using an Olympus 1x81 spinning disk confocal microscope and images were analyzed using Volocity 6.2.1 software. Images of cells on 96-well plates were acquired using the Operetta High Content Imaging System (Perkin Elmer) with 20 X objective lens and analyzed using Harmony 3.5 software. Total and antigen-positive cells were counted in 10 image fields per well, and the percentage of infected cells was obtained.

**Fetal brain explant cultures**

Human brain tissue from 15 to 19-week aborted fetuses was obtained (after written consent) under protocol 1420 of the University of Alberta Human Research Ethics Board. After delivery to the laboratory in ice-cold PBS, the fresh tissue was placed in a 100 mM Petri dish and then dissected with sterile scalpel and forceps into approximately 5 mm x 5 cm x 1 mm blocks [6]. The small tissue blocks were immediately immersed in 24-well dishes containing the same media used to culture HFAs (see above). The explant cultures were maintained in a humidified 37°C incubator containing 5% CO2 for up to 5 days under different experimental treatments.

Where indicated, brain explant tissue was treated with the FGF receptor inhibitor BGJ398 (10 nM) or DMSO for 48 hr before infection overnight with 106 PFU/mL of PRVABC-59 ZIKV strain. The next day, explants were washed once with PBS and then fresh media containing inhibitor or DMSO was added. Media samples were collected for viral titration and LDH measurement at 24 and 48 hr time points. Explant tissue was subjected to total RNA isolation at 48 hr and then qRT-PCR analyses or fixed with 10% buffered formalin for processing at the HistoCore facility of the University of Alberta.

**Histology**

Formalin fixed tissues were embedded in paraffin for standard hematoxylin and eosin (H&E) staining [7]. H&E-stained sections were viewed by bright field microscopy.

For immunohistochemical staining, fetal brain tissue sections were deparaffinized using xylene and then rehydrated with a graded series of ethanol. Antigen retrieval was carried out by boiling slides (microwave 80% power) for 15 minutes in 10 mM sodium citrate, pH 6.0. Next, samples were blocked with 20% normal goat serum in PBS for 1 hr followed by overnight incubation at 4oC with mouse anti-Flavivirus Group Antigen 4G2 antibody (Millipore) diluted 1:50 in 5% normal goat serum in PBS. The samples were then incubated with goat anti-mouse Alexa Flour-488 (Invitrogen) diluted 1:200 in 5% normal goat serum in PBS for 1 hr at room temperature. Wash steps after antibody incubations were performed with 5% normal goat serum in PBS. Coverslips were mounted onto slides with ProLong Gold Antifade with DAPI (Thermo Fisher Scientific).

Where indicated, apoptotic cells in the formalin-fixed, paraffin-embedded tissue of fetal brain explants were detected by TUNEL staining using the in situ bromodeoxyuridine-red DNA fragmentation kit (Abcam) according to the manufacturer's protocol. Confocal images were acquired using an Olympus 1x81 spinning disk confocal microscope equipped with Volocity 6.2.1 software.

**RNAseq libraries**

RNAseq libraries were constructed using the TruSeq RNA Sample Prep V2 Kit (Illumina) as per manufacturer instructions. In brief, 1 μg of total RNA was diluted in 50 μL of nuclease-free H2O and mixed with one volume of messenger RNA purification beads containing oligos dT conjugated to paramagnetic beads and the suspension was heated to 65°C for 5 min, cooled down to 4°C and then incubated at room temperature for 5 min. The suspension was then incubated on a magnetic stand for 5 min at room temperature, the supernatant removed, and the beads washed with 200 μL of beads washing solution. Finally, the RNA was eluted from the beads in 50 μL of elution buffer at 85°C, for 2 min and then cooled down to 25°C. RNA was re-bound to magnetic beads to increase specificity adding 50 μL of Bead Binding Buffer and beads were washed as described above. On-beads RNA was supplemented with 19.5 μL Elute, Prime Fragment mix containing random hexamers for cDNA synthesis. The solution was heated at 94°C for 8 min and then cooled down to 4°C to allow for annealing of random hexamers. cDNA synthesis was conducted in 8 μL First Strand Master Mix containing SuperScript II reverse transcriptase at a 1:9 ratio using the following thermo cycling program 25°C for 10 min, 42°C for 50 min, 70°C for 15 min, hold at 4°C. Second strand cDNA synthesis was conducted in the presence of 25 μL of Second Strand Master Mix at 16°C for 1 hr. cDNA reaction was supplemented with 90 μL of AMPure XP beads, incubated for 15 min at room temperature, and then incubated for 5 min on a magnetic stand. Beads were washed twice with 200 μL of 80% ethanol, air-dried, and finally the cDNA was eluted in 50 μL Resuspension Buffer. End repair was conducted in 40 μL of End Repair Mix for 30 min at 30°C. End-repaired cDNA was supplemented with 160 μL of AMPure XP beads, which were washed and end-repaired cDNA eluted in 15 μL as described above. End-repaired cDNA was 3’ adenylated in 12.5 μL of A-tailing mix with the following thermo cycler program: 37°C for 30 min, 70°C for 5 min, 4°C hold. Adapters were ligated by adding 2.5 μL of Ligation Mix, incubated at 30°C for 10 min; reaction was stopped with 5 μL of Stop Ligation Buffer. Reaction was cleaned up twice, in the first round with 42 μL of AMPure XP Beads and eluted in 50 μL of Resuspension Buffer, and in the second round with 50 μL AMPure XP Beads and eluted in 20 μL, all as described above. cDNA was enriched by adding 25 μL of PCR Master Mix using the following PCR program: 98°C for 30 sec; [15X] 98°C for 10 sec, 60°C for 30 sec, 72°C for 30 sec, 72°C for 5 min, 4°C hold. PCR reaction was cleaned up with 50 μL of AMPure XP Beads and recovered in 30 μL of Resuspension Buffer, as described above. Libraries were sequenced in a NextSeq instrument, using a 75 cycles paired end V3 sequencing kit (Thermo Fisher).

**Bioinformatics**

Libraries were demultiplexed using the appropriate workflow in the NextSeq instrument. After quality control, libraries were aligned to the GRCh38.81 version of the human genome with RSEM [8], TopHat2 [9] or Kallisto [10]. Data was parsed with in-house Python scripts. Differential expression analysis was conducted with the programming language R, using the packages EdgeR [11], DESeq2 [12] or Sleuth [13]. Plots were generated with R scripts. We evaluated the performance of three of the most popular bioinformatics pipelines for the analysis of RNAseq data. Namely, for quantification/counting and differential expression analyses, the following pairs of packages were used: RSEM/ EdgeR [8, 11], TopHat2/DESqeq2 [9, 12] and Kallisto/Sleuth [10, 13]. In all cases, a general linear model that accounted for variability between HFAs donors was used. Respectively, 1273 transcripts, 3498 genes, and 2641 transcripts were identified as differentially expressed (p < 0.05; FDR < 0.05; Fold Change > 2) with each approach. We select to interpret the Kallisto/Sleuth results because such pipeline had intermediate astringency and also because it was found to outperform the other two methods on simulated data, likely because Sleuth uses bootstrapping and response error linear modeling to partition the total variance into experimental and biological components [13].

**Supplementary References**

1. Fonseca K, Meatherall B, Zarra D, et al. First case of Zika virus infection in a returning Canadian traveler. Am J Trop Med Hyg **2014**; 91:1035-8.

2. Lanciotti RS, Lambert AJ, Holodniy M, Saavedra S, Signor Ldel C. Phylogeny of Zika Virus in Western Hemisphere, 2015. Emerg Infect Dis **2016**; 22:933-5.

3. Schwarz MC, Sourisseau M, Espino MM, et al. Rescue of the 1947 Zika Virus Prototype Strain with a Cytomegalovirus Promoter-Driven cDNA Clone. mSphere **2016**; 1.

4. Vivithanaporn P, Maingat F, Lin LT, et al. Hepatitis C virus core protein induces neuroimmune activation and potentiates Human Immunodeficiency Virus-1 neurotoxicity. PLoS One **2010**; 5:e12856.

5. Balm MN, Lee CK, Lee HK, Chiu L, Koay ES, Tang JW. A diagnostic polymerase chain reaction assay for Zika virus. J Med Virol **2012**; 84:1501-5.

6. Grivel JC, Margolis L. Use of human tissue explants to study human infectious agents. Nat Protoc **2009**; 4:256-69.

7. Cardiff RD, Miller CH, Munn RJ. Manual hematoxylin and eosin staining of mouse tissue sections. Cold Spring Harb Protoc **2014**; 2014:655-8.

8. Li B, Dewey C. RSEM: accurate transcript quantification from RNA-Seq data with or without a reference genome. BMC Bioinformatics **2011**; 12:1-16.

9. Kim D, Pertea G, Trapnell C, Pimentel H, Kelley R, Salzberg SL. TopHat2: accurate alignment of transcriptomes in the presence of insertions, deletions and gene fusions. Genome Biol **2013**; 14:R36.

10. Bray NL, Pimentel H, Melsted P, Pachter L. Near-optimal probabilistic RNA-seq quantification. Nat Biotechnol **2016**; 34:525-7.

11. Robinson MD, McCarthy DJ, Smyth GK. edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. Bioinformatics **2010**; 26:139-40.

12. Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol **2014**; 15:550.

13. Pimentel H, Bray N, Puente S, Melsted P, Pachter L. Differential analysis of RNA-Seq incorporating quantification uncertainty. biorxiv **2016**.