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Supplemental Information

Viral Infection or IFN-α Alters Mitotic Spindle Orientation by Modulating Pericentrin Levels

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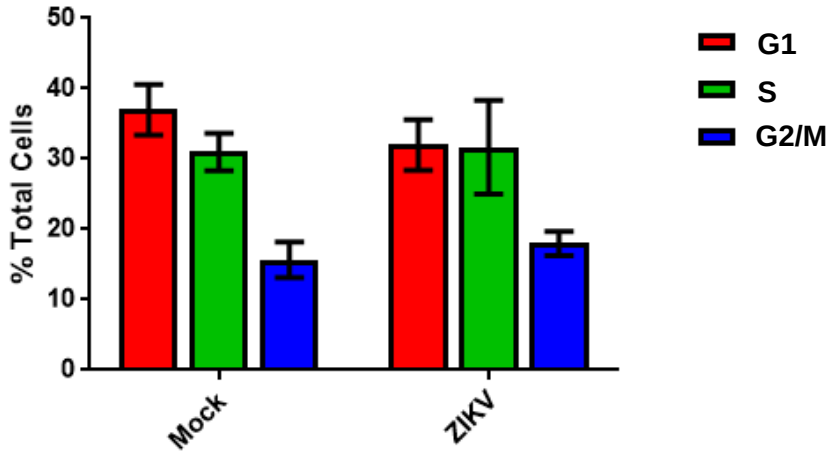
A



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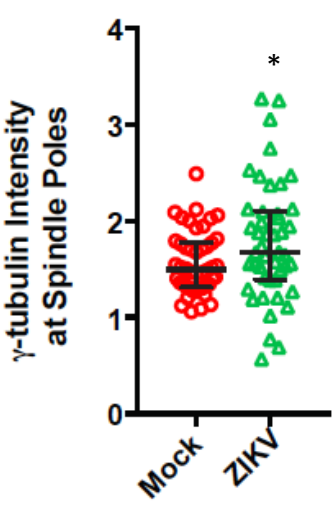

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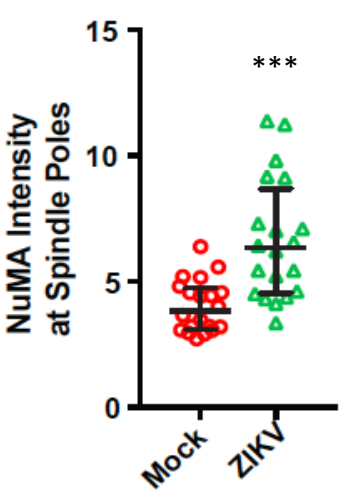

Sup Figure 1

Supplemental Figure 1. Related to Figure 1. ZIKV infection does not alter cell cycle progression, and increases γ-tubulin or NuMA levels at the spindle poles.

(A) Cell-cycle analysis of G0-synchronized A549 cells after ZIKV infection. A549 cells were serum starved for 48 hr, then infected with ZIKV (PRVABC59) at an MOI of 5 for 48 hr. After infection cells were stained with propidium iodine and analyzed for DNA content by flow cytometry. The mean percent cells in G1 (red), S (Green), or G2/M (Blue) phase are shown for either mock infected (mock) or ZIKV-infected samples.

(B) Quantification of γ-Tubulin intensity in the spindle poles of mitotic cells for mock (red, n = 49), or 24 hr (green, n = 48) ZIKV infection.

(C) Quantification of NuMA intensity in the spindle poles of mitotic cells for mock (red, 21), or 24 hr (green, 20) ZIKV infection. Statistical significance (p-value) of the angle changed or integrated intensity between mock and a condition in all quantified data is represented using asterisks above the data, where p-values < 0.001 are "***", and p-values < 0.5 are "*". All data represent the mean +/- SD from three independent experiments.

Transparent Methods

Cell Culture

A549 (ATCC CCL-185), and U2OS (ATCC HTB-96) cells were grown in complete DMEM (Dulbecco's Modified Eagle Media (Sigma, D5671) supplemented with 10% FBS (GIBCO, 10437), 2 mM L-glutamine (GIBCO, 25030), 100 U/mL Pen-Strep (GIBCO, 15140), and Plasmocin (Invivogen, ant-mpt-1). HepG2 (ATCC HB-8065) were transduced with NTCP cDNA using the retroviral transfer vector pQXCIH (Clontech, 631516) to create HepG2-NTCP cells and were grown in complete DMEM. H9 neural progenitors were obtained from Fisher Scientific (N7800100) and grown in KnockOut DMEM/F-12 (GIBCO, 12660) supplemented with 2 mM Glutamax (GIBCO, 35050), 20 ng/mL bFGF (GIBCO, PHG0024), 20 ng/mL EGF (GIBCO, PHG0314) and 2% StemPro Neural Supplement (GIBCO, A10508), and cultured according to manufacturer's instructions.

Viral Culture

ZIKV (Puerto Rico 2015, PRVABC59, ATCC, VR-1843) and DENV (New Guinea C 1944, ATCC VR-1584) were propagated in C6/36 cells grown in RPMI-1640 (Sigma, R0883). After infection for 72 hours supernatants were collected and tittered by infecting A549 cells. HCMV TB40E-GFP virus was generated from electroporation of bacterial artificial chromosome into HEL fibroblasts, and tittered using a standard plaque assay (Paredes and Yu, 2012). HBV (subtype-ayw) was grown in HepAD38 cells cultured in DMEM/F12 (Sigma, D6421) supplemented with 2 mM L-glutamine, 100 U/mL Pen-Strep, and Plasmocin (Ladner et al., 1997). Following weekly collection of media for 4 weeks, viral supernatants are precipitated with 8% PEG8000 (Sigma, 89510) followed by centrifugation at 3500 x g to collect viral particles. HBV titers were determined by infection of HepG2 cells and immunostaining using the HB25B10 hybridoma

supernatants. IAV-A/WSN33/H1N1 was grown by infecting MDCK (ATCC CCL-34) cells grown in complete DMEM. After 72 hrs. of infection supernatants were collected and tittered by infecting A549 cells and immunostaining.

Viral Infections

For ZIKV (PRVABC59), DENV (New Guinea C), and IAV-A/WSN33/ (H1N1) infections, A549 cells were grown to 70% confluence and were infected at an MOI of 5 for ZIKV and DENV, and a MOI of 0.5 for IAV-WSN33 for the indicated time points. H-9 neural progenitor cells were grown to 60% confluence and infected with ZIKV (PRVABC59 MOI 5) for 24 hrs. or were infected with HCMV (TB40E-GFP MOI 3) for 48 hrs. For HBV (serotype-ayw) infections HepG2-NTCP cells were grown to 70% confluence and infected with an MOI of 0.4-0.7 for 24 hr. After 24hr of HBV infection media was replaced with D10G containing 2.5% DMSO (Sigma, D2660) and allowed to incubate for 5 days. After all infections, cells were fixed using 4% Formalin and processed as described below.

Immunostaining and immunofluorescence

Coverslips with the indicated adherent cells were first fixed in 4% formaldehyde (Sigma F8775) in D-PBS (Invitrogen, D8537) for 7 min followed by the addition of ice cold methanol and stored at -20 °C. Cells were then permeabilized in 0.1% Triton X-100 (Fisher, BP151) and 0.1% Tween 20 (Sigma, P7949) in D-PBS for 20 min followed by 3 washes in D-PBS. Blocking was performed for 30 min in 1% BSA (BioPharm, 71-010) in D-PBS containing 0.3 M glycine (Sigma, G7126). Cells were next incubated in primary antibodies (please see antibodies methods section) diluted in 1% BSA in D- PBS for 1 hr, followed by 3 washes with D-PBS. Cells were then incubated in secondary antibodies (see antibodies) diluted 1:1000 in D-PBS containing 1% BSA. Cells were washed 3 times with D-PBS before mounting to slides using Vectashield with DAPI (4', 6'-diamidino-2-phenylindole, Vector Laboratories, H-1200). Samples were then imaged using a Nikon A1 inverted confocal microscope. All microscope settings were kept constant throughout entirety of a given experiment. All other image analyses and all quantifications were done using the FIJI software.

Imaging and analysis for spindle pole angles

Mitotic cells in metaphase were identified by metaphase plate of DAPI staining and PCNT-labeled spindle poles. Z-stacks of mitotic cells from 5 μm-7 μm were acquired using a Nikon A1 inverted confocal microscope (pinhole 0.9 AU) in 0.2 μm steps to capture the entire height of the cell of interest. Spindle angles were calculated by first measuring the linear and vertical distance between PCNT foci followed by calculating the angle between the axis of the spindle and that of the coverslip using the inverse tangent function (arctan(x)), so that 2 PCNT foci focused in the same z-plane have a calculated spindle orientation of 0°.

Imaging and analysis for fluorescence intensities of PCNT, PLK1, NuMA and γ- Tubulin

Imaging was performed as described above and PCNT and PLK1 intensities were quantified by using the 2D sum projection of the z-stack followed by measurement of the integrated

fluorescence density of the PCNT and PLK1 foci, and subtracting the background intensity. Intensities were then normalized to mock conditions.

Images for NuMA and γ-tubulin were acquired with a Zeiss Axiovert 200M, a Perkin Elmer Ultraview spinning disc microscope and Hamamatsu ORCA-ER camera (100x NA1.4 or 40x NA 1.3 Plan-Apochromat Oil objective). Z stacks are shown as 2D maximum projections (MetaMorph, Molecular Devices). Fluorescence range intensity was adjusted identically for each series of panels. Intensity profiles and fluorescence intensity quantification were obtained from sum projections of Z stacks using MetaMorph software. For fluorescence intensity quantification, computer-generated concentric circles of 60 (inner area) or 80 (outer area) pixels in diameter were used to measure centrosome (inner area) and calculate local background (difference between the outer and inner area) fluorescence intensity.

Immunoblotting

Lysates of ZIKV infected A549 or U2OS cells were created by lysing 1x106 cells in Laemmli Sample Buffer (62.5 mM Tris pH 6.8, 10% Glycerol, 2% SDS, 5% β- mercaptoethanol, 0.004% Bromophenol Blue) then separated on a 4-20% Tris-Glycine SDS-PAGE gel. After transfer to PVDF membrane (Thermo, 88518), membranes were blocked with 5% milk then immunoblotted using the antibodies described below.

Cell cycle analysis

0.2 x10^5 A549 cells were plated into 6-well dishes and allowed to attach for 12hrs. Cells were synchronized in G0 by serum starvation after replacing complete media with DMEM supplemented with 2 mM L-glutamine for 48hrs. Following serum starvation, serum free media was replaced with ZIKV containing media or complete DMEM for 48 hrs. After infection, cells were stained with propidium iodine (Abcam, ab139418) and analyzed for DNA content using a MACSQuant flow cytometer and data was analyzed using a univariate model of DNA content in FlowJo™ where 2N counts were assigned G1- phase, 4N/2N were assigned to S-phase, and 4N were assigned G2/M-phase.

siRNA transfections and IFN-α treatment

A549 cells were transfected with siRNAs (non-targeting NT Dharmacon D-001206-14,or IFNAR Dharmacon, L-020209-00) at a final concentration of 50 nM using Oligofectamine (Invitrogen, 1225201) for 48 hr as previously described. After 48 hr, the media was replaced with fresh complete media containing 100 IU/mL IFN-α (Invitrogen, PCH4014); 24 hr later the cells were fixed and processed as described above.

Antibodies

Primary antibodies used in these studies are D1-4G2-4-15 hybridoma (ATCC, HB-112) used to detect ZIKV and DENV, H18-S210 hybridoma (Coriell, WC00029) used to detect IAV-WSN33, H25B10 Hybridoma (ATCC, CRL-8017) used to detect HBV, Pericentrin (Rabbit pAb Abcam, ab4448, 1:300 dilution), α-tubulin (Rat mAb Abcam, ab6161, 1:50 dilution), α-tubulin-FITC conjugated (Mouse mAb Abcam, ab195887 1:100), GFP antibody (mouse mAb, Santa Cruz, sc-

9996 1:500 dilution), PLK1 (Rabbit pAb Novus Biologics, NB100-547 1:1000 dilution), ZIKV (Rabbit pAb Genetex, GTX133314 1:1000 dilution), NuMA (Rabbit pAb Abcam, ab36999), γ-tubulin (Mouse mAb Sigma, GTU-88).

Secondary antibodies for immunofluorescence were all from Thermo Fisher and used at a 1:1000 dilution: AlexaFluor 488 goat anti-mouse IgG (A11001), AlexaFluor 568 goat anti-rabbit IgG (A11011), Alexa Fluor 647 goat anti-rat IgG (A21247). Secondary antibodies for western blot were purchased from Jackson ImmunoResearch and used at a 1:10,000 dilution: goat anti-mouse IgG-HRP (115-035-003), goat anti-rabbit IgG-HRP (111-035-003), goat anti-rat IgG-HRP (112-035-003).

Statistical analysis

All p-values were calculated using a student's t-test with Welche's correction in GraphPad Prism.

References

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