Supplemental Experimental Procedures

Plasmids and antibodies

cDNAs corresponding to the NS5 sequence for YOKV, IGUV, SPOV and ZIKV French Polynesia strain (GenBank accession numbers AB114858.1, AY632538.4, DQ859064.1) were synthesized (GenScript) and subcloned into pCAGGS-COOH-HA expression vector. For cloning NS5 of Uganda ZIKV, Vero cells were infected with MR766 ZIKV strain (ATCC VR-84), total RNA was isolated using trizol and cDNA was RT-PCR amplified using gene specific primers and cloned into pCAGGS-COOH-HA expression vector. Upon sequencing it corresponded to Genbank: HQ234498. USUV NS5 was cloned from viral RNA (SAAR-1776 strain, GenBank accession number AY453412.1). Gene specific primers were used to amplify the USUV NS5 gene and the resulting cDNA was subcloned into pCAGGS-COOH-HA expression vector. All primer sequences are available upon request. The N-terminal proteolytically cleaved version of the DENV2 NS5-HA plasmid used in this study was previously described (Ashour et al., 2009). GFP and mTRIM61-HA expression plasmids were used as negative controls. The NiV V-HA expression plasmid was kindly provided by Megan Shaw. The following antibodies were used in this study: anti-UBR4 (Abcam; ab86738), anti-STAT2 (Santa Cruz; sc-476), mouse anti-HA (Sigma;H9658), rabbit anti-HA (Cell Signaling; 3724), anti-FLAG (Sigma; F1804), anti-pSTAT2 (Millipore; 07-224), anti-STAT1 (BD Biosciences; 610120), anti-tubulin (Cell Signaling; 2146s), anti-pSTAT1 (Cell Signaling; 9167S), anti-4G2 (EMD Millipore; MAB10216); anti-actin (Sigma; A5441); the anti-ZIKV NS5 antibody was raised in hens to the peptide sequence DEE KYM DYL STQ VRY LGE E (NS5 residues #878-896).

Cells

293T (ATCC), Vero cells (ATCC), primary human fibroblasts (ATCC), and MEFs were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum and 5% Penicillin Streptomycin. UBR4 CRISPR/Cas9 KO 293T cells have been described previously (Tripathi et al., 2015).

MEFs were derived from C57Bl/6 (WT) and the IFNAR1-/- cells were derived as previously described (Guo et al., 2008).

Immunoprecipitation assays

293T cells were transfected with the indicated plasmid and 24 hours post transfection cells were treated with universal type I IFN (PBL) for 45 minutes, or mock treated (or left untreated for the UBR4 and mouse STAT2 coIPs). Cells were then lysed in non-denaturing co-immunoprecipitation buffer. Lysates were collected (sonication was performed on UBR4 IP lysates due to the membrane bound nature of UBR4) and centrifuged to obtain whole cell extract and the remaining lysate was incubated with anti-HA beads (Sigma). HA beads were washed and bound protein was eluted by boiling the beads in SDS buffer. SDS-PAGE followed by immunoblot analysis was performed on the IP and whole cell extracts with the indicated antibodies.

Immunofluorescence assays

Vero cells were transfected or co-transfected with the indicated proteins and 24 hours post transfection cells were treated with universal IFN (PBL) for 30 min. Cells were fixed with 4% PFA, permeabilized with 0.1% NP40 and incubated with the indicated antibodies. For viral infection, Vero cells were infected at the indicated MOI, treated with universal IFN (PBL) for 30 min 24hpi and fixed with 4% PFA, permeablized with 0.1% NP40 and incubated with the indicated antibodies. Images were taken with the Zeiss AxioImager, ISMMS Microscopy Core facility.

Flow cytometry

293T cells were transfected with equal amounts of the indicated HA-tagged protein and GFP expression plasmid. Twenty-four hours posttransfection, cells were mock or IFN treated (1000 U universal IFN, PBL)

h for 45 min. Cells were then fixed in 4% PFA and sorted on same GFP signal intensity using the BD CSM5L cell sorter, ISMMS FACS SRF. Recovered cells were then lysed and SDS-PAGE was performed followed by immunoblot analysis with the indicated antibodies.

Virus infections

Cells were either mock infected or infected with DENV2, ZIKV Uganda, Puerto Rico (Lanciotti et al., 2016) or Fortaleza (isolated from a human microcephaly case in Brazil, 2015 and kindly provided by Steve Whitehead, NIAID) at the indicated MOI. Virus was allowed to adsorb at room temperature for 1 hour before incubation at 37 degrees C for the indicated time. Immunofluorescence assays and SDS-PAGE followed by immunoblot analysis was performed in order to detect STAT2 and viral NS5 protein levels. Plaque assays were performed to determine viral titer. For the proteasome inhibitor experiment, cells were treated with 20uM of MG132 (Sigma) or lactacystin (Sigma) 12 hpi. For the ISG induction experiment measured via qPCR, Vero cells were treated with 50 U of universal IFN (PBL) 24 hpi and processed for RNA extraction 14 hours post IFN treatment.

References:

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