Supplemental information

Title:

The dengue virus conceals double-stranded RNA in the intracellular membrane to escape from an interferon response.

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Supplemental Table S1 | Sequence of primers for RT-qPCR.

Gene	Primer orientation	Primer sequences (5' to 3')
Flavi NS5[a]	Forward	AACATGATGGGAAAAAGAGAGAAGAAGC
	Reverse	CGTGCTCCAAGCCACATG
Human IFN-β[b]	Forward	TGCCTCAAGGACAGGATGAAC
	Reverse	GCGTCCTCCTTCTGGAACTG
Human GAPDH[b]	Forward	AAATCAAGTGGGGCGATGCTG
	Reverse	CAAATGAGCCCCAGCCTTCTC

[a] Source, Kinney *et al*., [38]. [b] Source, Espada-Murao, L. A. and K. Morita, [17].

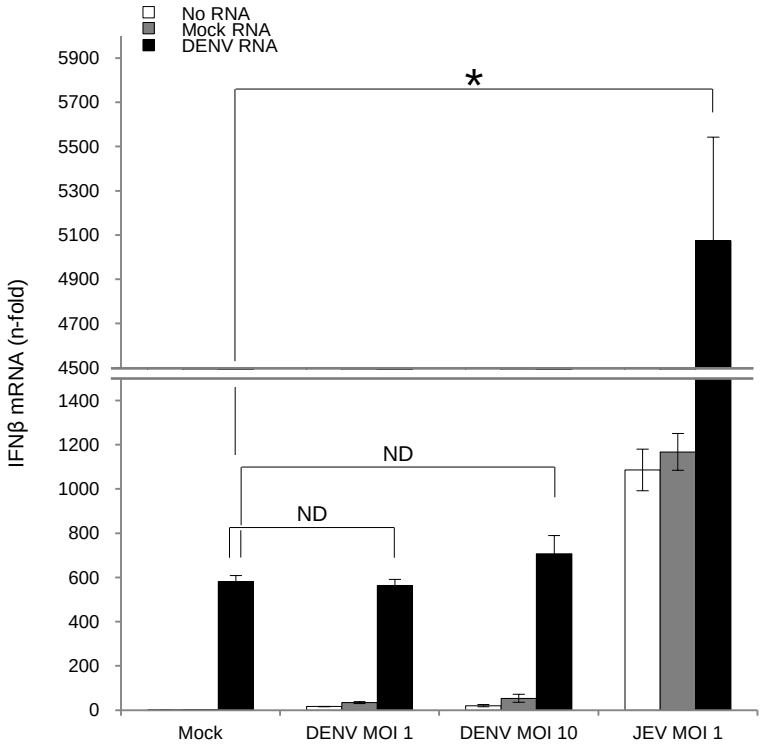
[17] Espada-Murao, L. A. & Morita, K. Delayed cytosolic exposure of Japanese encephalitis virus double-stranded RNA impedes interferon activation and enhances viral dissemination in porcine cells. *J. Virol.* **85,** 6736-6749 (2011).

[38] Kinney, R. M. *et al*. Construction of infectious cDNA clones for dengue 2 virus: strain 16681 and its attenuated vaccine derivative, strain PDK-53. *Virology* **230,** 300-308 (1997).

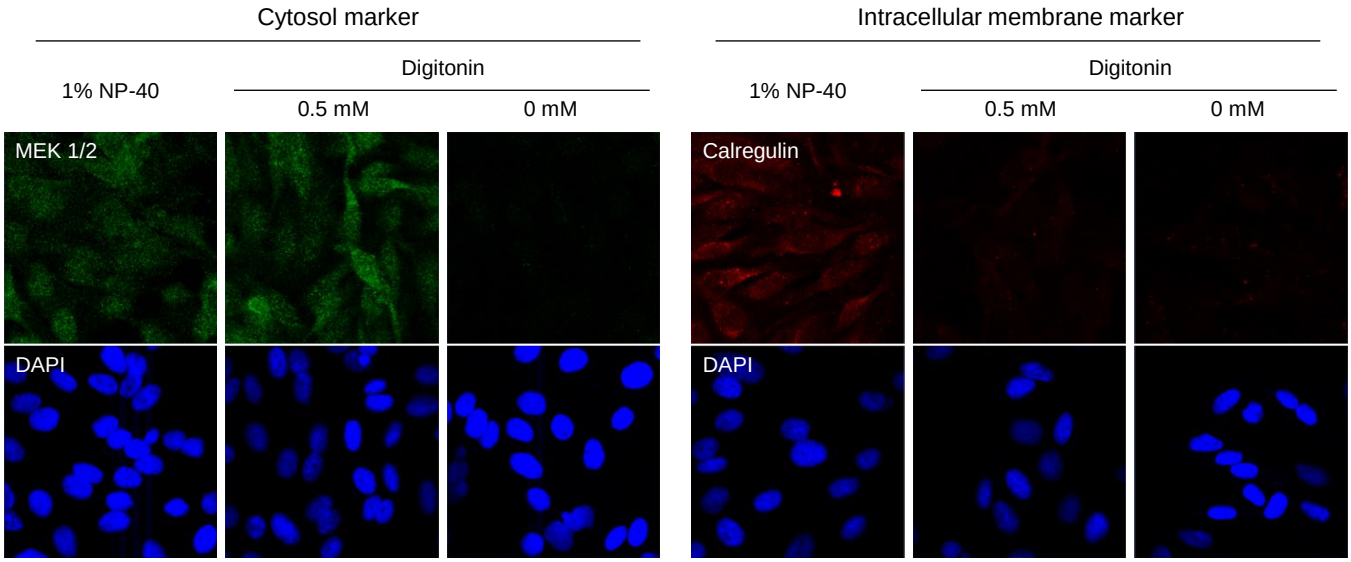
Supplemental Table S2 | vRNA and IFN-β mRNA levels in DENV- and JEV-infected cells.

	DENV MOI 1		
	12 h	24 h	48 h
vRNA copies (log 10)	5.52 ± 0.59	6.50 ± 0.45	7.29 ± 0.50
IFNβ mRNA (n-fold)	0.61 ± 0.08	0.98 ± 0.14	1.93 ± 0.17
	DENV MOI 10		
	12 h	24 h	48 h
vRNA copies (log 10)	6.44 ± 0.55	7.61 ± 0.42	8.22 ± 0.50
IFNβ mRNA (n-fold)	0.69 ± 0.06	2.55 ± 0.38	15.74 ± 2.90
	JEV MOI 1		
	12 h	24 h	48 h
vRNA copies (log 10)	7.76 ± 0.49	8.70 ± 0.47	8.97 ± 0.48
IFNβ mRNA (n-fold)	1.91 ± 0.32	496.00 ± 132.00	678.85 ± 161.53

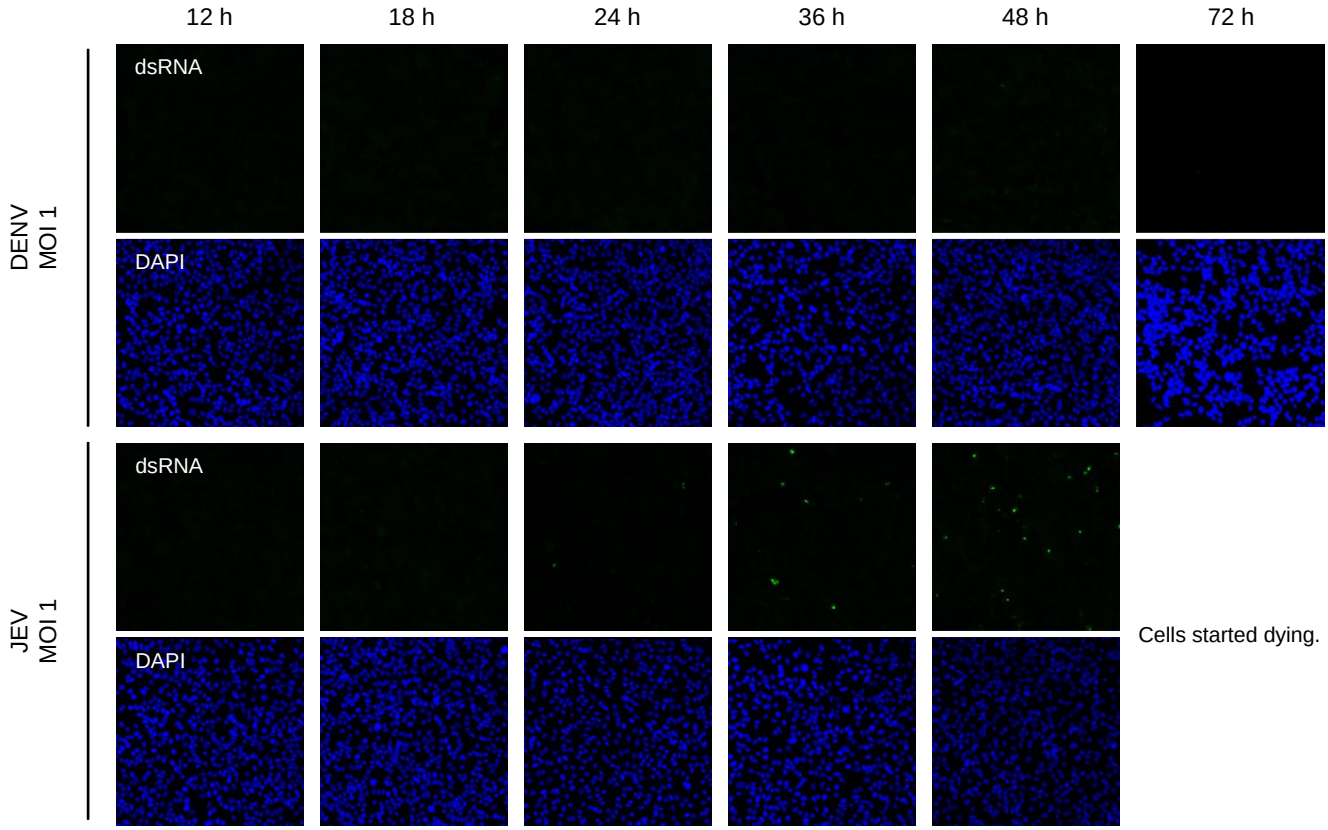
(Mean ± SE)



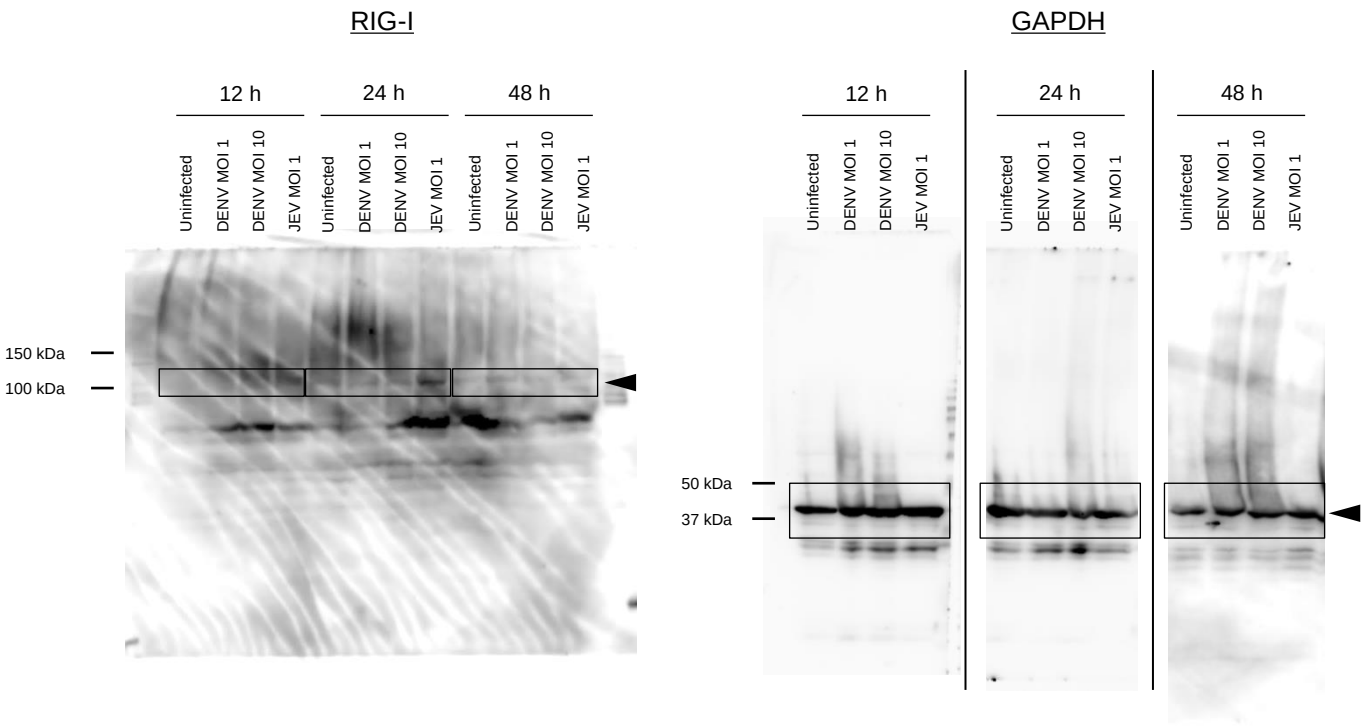
Supplemental Figure S1 | Effect of JEV-derived dsRNA on the IFN activation pathway.
Cells were infected with DENV2 strain 16681 at MOI 1, 10 or JEV strain JaOArS982 at MOI 1 prior to IFN-β stimulating assay. After 48 h p.i., 1 μg/well of the mock RNA or the JEV-derived dsRNA was transfected into the cells with the uninfected control. After 6 h from the RNA transfection, the cells were harvested and the IFN-β and the GAPDH mRNA level were quantified by RT-qPCR. The relative-fold increase of IFN-β mRNA levels was normalized with the GAPDH mRNA levels. The mean ± SE of the IFN-β mRNA levels was obtained from two independent duplicate experiments. The values between the two groups were tested by the Welch test analysis. The asterisks indicate a statistical significance at $p<0.01$, and ND indicates no significant difference.



Supplemental Figure S2 | Evaluation of digitonin permeabilization method using organelle markers.
The localization of MEK1/2 (cytosol marker) and calregulin (intracellular membrane marker) was observed using two different permeabilization methods. Uninfected cells were permeabilized using 1% NP-40 or 0 to 0.5 mM digitonin and the organelle markers were visualized by the immunofluorescence assay.



Supplemental Figure S3 | Detection of cytosolic dsRNA in the DENV- and JEV- infected cells.
HeLa cells were infected with DENV2 strain 16681 or JEV strain JaOArS982 at MOI 1. At the indicated times p.i., the infected cells were fixed and permeabilized by 0.5 mM of digitonin and the viral dsRNA was visualized by the immunofluorescence assay. The nuclei were stained with DAPI.



Supplemental Figure S4 | Representative blots of RIG-I and GAPDH in DENV- and JEV-infected cells. HeLa cells were infected with DENV2 strain 16681 at MOI 1 or 10 or JEV strain JaOArS982 at MOI 1. At the indicated time points, the cells were harvested and the target proteins were detected by the immunoblotting assay. GAPDH was used as an internal control. The black bars beside the images indicate the position of molecular size markers. The black allow heads indicate the position of the target proteins. The images inside the boxes are shown in Fig. 3C in the manuscript. All the samples were derived from the same experiment and blotting was processed in parallel.