Alpha-mangostin inhibits viral replication and suppresses nuclear factor kappa B (NF-κB)-mediated inflammation in dengue virus infection

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Supplementary Information

Supplementary Table 1. Fold changes of antiviral gene expression in DENV-infected HepG2 cells in the presence or absence of 20 μM of α-MG

Gene abbreviation	Formal gene name	Fold changes	
		DENV-2 + EtOH	DENV-2 + α-MG
CCL5	Chemokine (C–C motif) ligand 5	1,509.65	265.03
IFNB1	Interferon beta, beta 1, fibroblast	362.04	34.06
CXCL10	Chemokine (C–X–C motif) ligand 10	349.71	25.99
TNF	Tumor necrosis factor	171.25	3.25
OAS2	2'-5'-Oligoadenylate synthetase 2	114.56	15.78
CXCL11	Chemokine (C–X–C motif) ligand 11	30.48	4.53
CCL3	Chemokine (C–C motif) ligand 3	29.04	1.31
CXCL8	Chemokine (C–X–C motif) ligand 8	27.28	6.87
MX1	Myxovirus (influenza virus) resistance 1, interferon-inducible protein p78 (mouse)	12.38	2.85
FOS	FBJ murine osteosarcoma viral oncogene homolog	7.36	2.75
ISG15	ISG15 ubiquitin-like modifier	6.54	2.10
IFIH1	Interferon induced with helicase C domain 1	6.15	1.85
NFKBIA	Nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha	5.10	1.14
DHX58	DEXH (Asp-Glu-X-His) box polypeptide 58	3.66	2.25
DDX58	DEAD (Asp-Glu-Ala-Asp) box polypeptide 58	2.97	1.95
IL6	Interleukin 6	2.89	1.17
NOD2	Nucleotide-binding oligomerization domain-containing protein 2	2.85	0.69
TLR-3	Toll-like receptor 3	2.81	0.41
IL15	Interleukin 15	2.75	0.67
STAT1	Signal transducer and activator of transcription 1	2.38	1.37
CYLD	Cylindromatosis (turban tumor syndrome)	2.35	1.11
JUN	Jun proto-oncogene	2.25	1.21
IL18	Interleukin 18	2.20	2.79
MEFV	Mediterranean fever	2.20	0.72
CXCL9	Chemokine (C–X–C motif) ligand 9	2.13	1.61

Values represent mRNA fold changes of antiviral genes in DENV-infected HepG2 cells in the presence or absence of 20 μM of α-MG relative to mock-infected cells

Abbreviations: DENV, dengue virus; HepG2 cells, human hepatocellular carcinoma cells; α-MG, alpha-mangostin

Supplementary Table 2. Fold changes of cytokine and chemokine gene expression in DENV-infected HepG2 cells in the presence or absence of 20 μM of α-MG

Gene abbreviation	Formal gene name	Fold changes	
		DENV-2 + EtOH	DENV-2 + α-MG
CCL5	Chemokine (C–C motif) ligand 5	1,039.73	191.34
CXCL10	Chemokine (C–X–C motif) ligand 10	270.97	13.09
TNF	Tumor necrosis factor	117.95	6.87
CXCL2	Chemokine (C–X–C motif) ligand 2	45.00	3.27
LTB	Lymphotoxin beta (TNF superfamily, member 3)	28.48	1.75
CXCL8	C-X-C motif chemokine ligand 8 (Interleukin 8)	26.95	5.70
CXCL11	Chemokine (C–X–C motif) ligand 11	20.14	2.33
CCL3	Chemokine (C–C motif) ligand 3	15.05	1.35
CXCL1	Chemokine (C–X–C motif) ligand 1	13.76	2.11
CSF3	Colony stimulating factor 3	10.00	2.00
IL23A	Interleukin 23, alpha subunit p19	6.11	1.97
CCL20	Chemokine (C–C motif) ligand 20	5.66	1.46
IL11	Interleukin 11	4.67	3.20
OSM	Oncostatin M	3.08	1.38
BMP6	Bone morphogenetic protein 6	3.04	5.17
LIF	Leukemia inhibitory factor	2.64	1.68
TGFB2	Transforming growth factor, beta 2	2.59	0.88
IL6	Interleukin 6	2.54	0.85
CXCL5	Chemokine (C–X–C motif) ligand 5	2.32	0.75
IL15	Interleukin 15	2.30	0.74
LTA	Lymphotoxin alpha (TNF superfamily, member 1)	2.02	1.17

Values represent mRNA fold changes of cytokine and chemokine genes in DENV-infected HepG2 cells in the presence or absence of 20 μM of α-MG relative to mock-infected cells

Abbreviations: DENV, dengue virus; HepG2 cells, human hepatocellular carcinoma cells; α-MG, alpha-mangostin

Supplementary Table 3. Primers used for DENV-2-RdRp construction

Primer	**Orientation**	**Sequence (5'-3')**
DENV-2 RdRp_F	Forward	GGGTCGCGGATCCACTTACGAGCCAGATGTAGACCTCGG
DENV-2 RdRp_R	Reverse	TCGAGTGCGGCCGCGCCCTGAAAATACAGGTTTTCTGCCTCTTCCTCTTCTCTTCTGAATCTTTTCATG

Original immunoblot for Fig.1

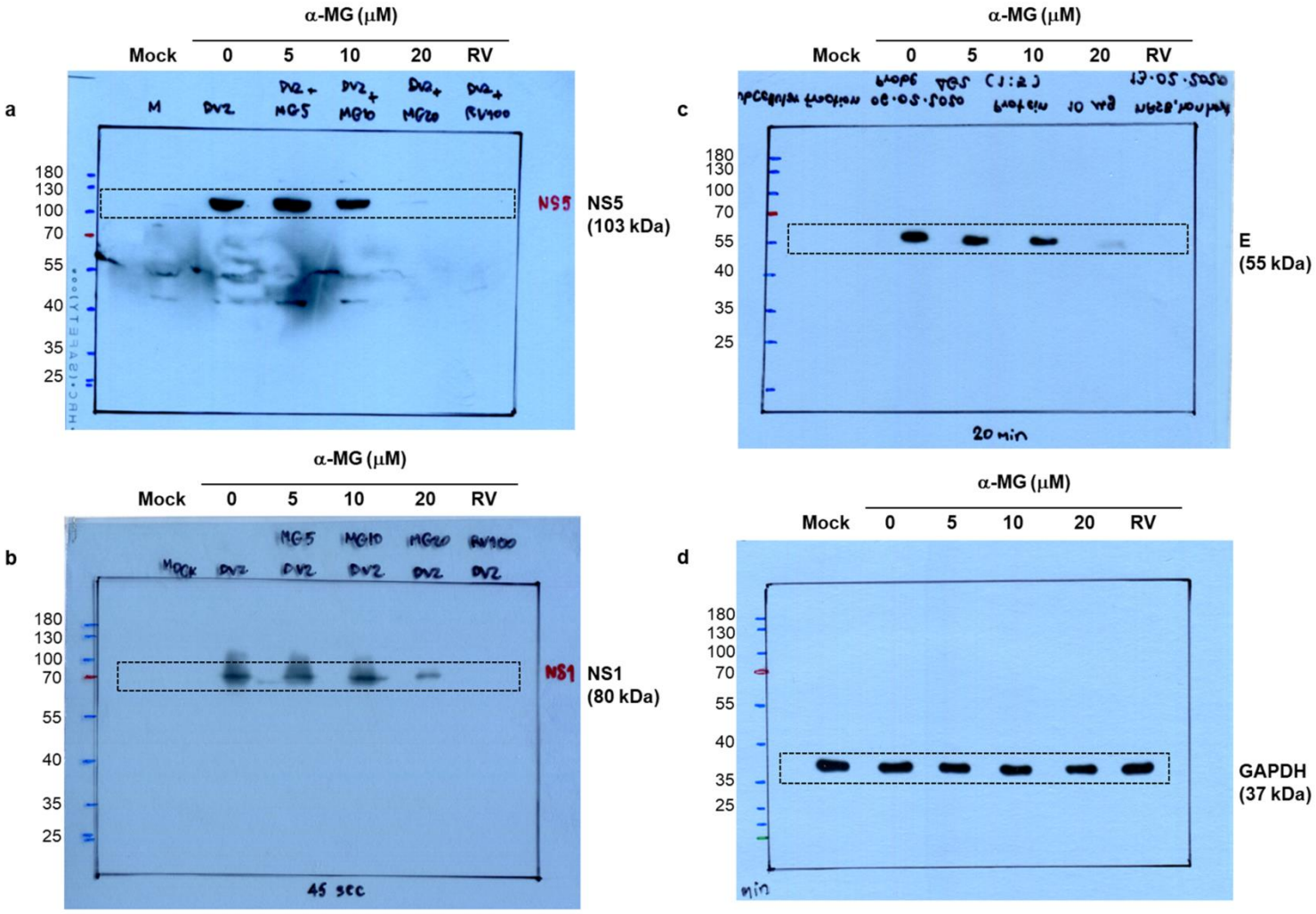

Fig. S1 Immunoblot images with cropped areas indicated by dashed lines. The results are the representatives from three independent experiments. The samples were obtained from the same experiment and the blots were processed in parallel. Each membrane was separately incubated with specific antibody to detect either (a-c) DENV NS5, NS1, or E proteins. (d) The same membrane was incubated with anti-GAPDH antibody.

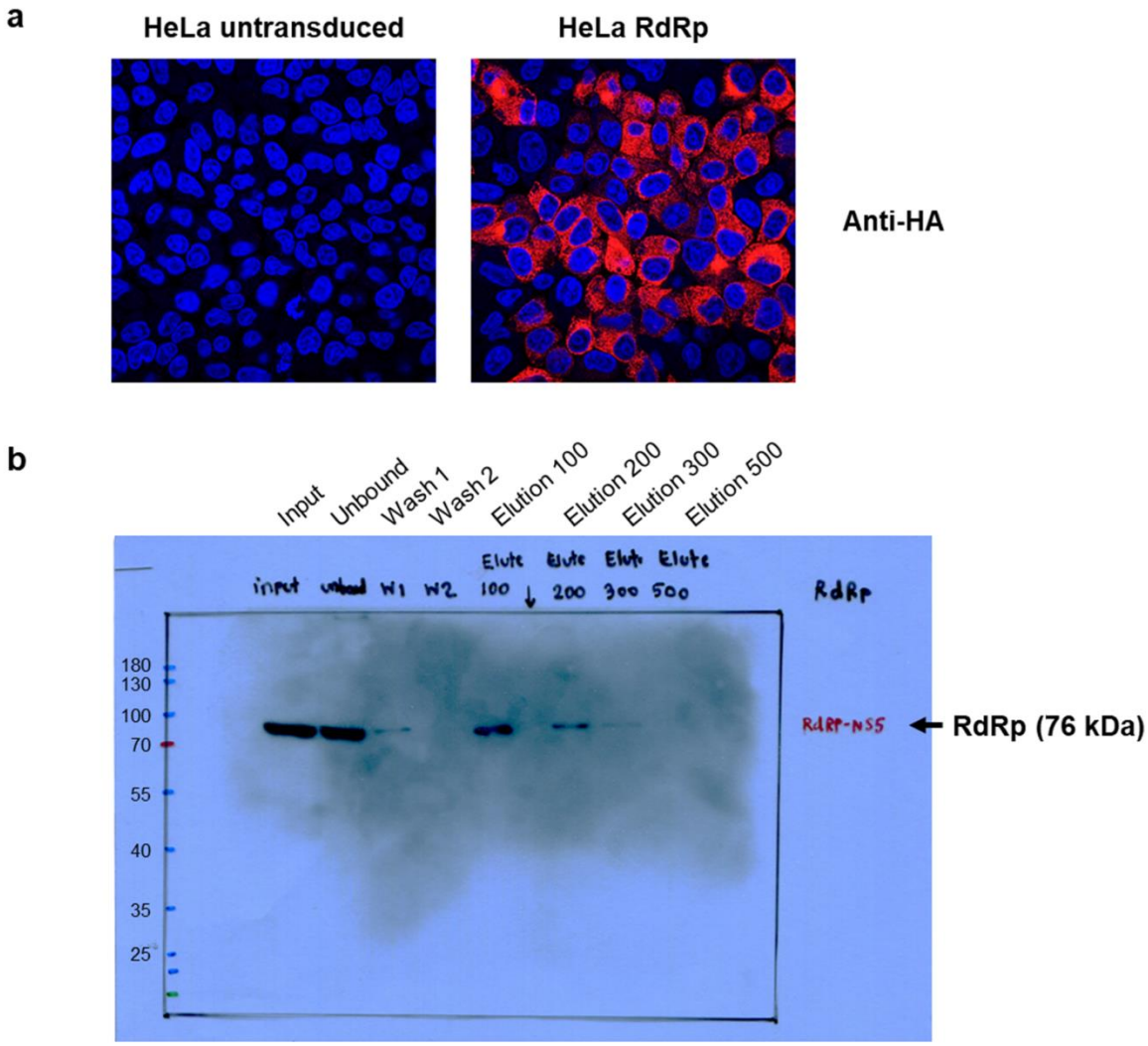

Fig. S2 Expression and purification of DENV-2 RdRp. (a) HeLa cells expressing stable DENV-2 RdRp are shown by indirect immunofluorescence assay using anti-HA antibody and Cy3-conjugated goat anti-mouse IgG antibody (red). The HeLa untransduced cells were used as control. (b) Immunoblot analysis for determining the purified recombinant DENV-2 RdRp protein. The protein fractions were subjected to electrophoresis and the purified DENV-2 RdRp protein was detected using anti-HA antibody and HRP-conjugated rabbit anti-mouse IgG antibody. The histidine-tagged DENV-2 RdRp protein was eluted with 100, 200, 300, and 500 mM imidazole.

Original immunoblot for Fig.6

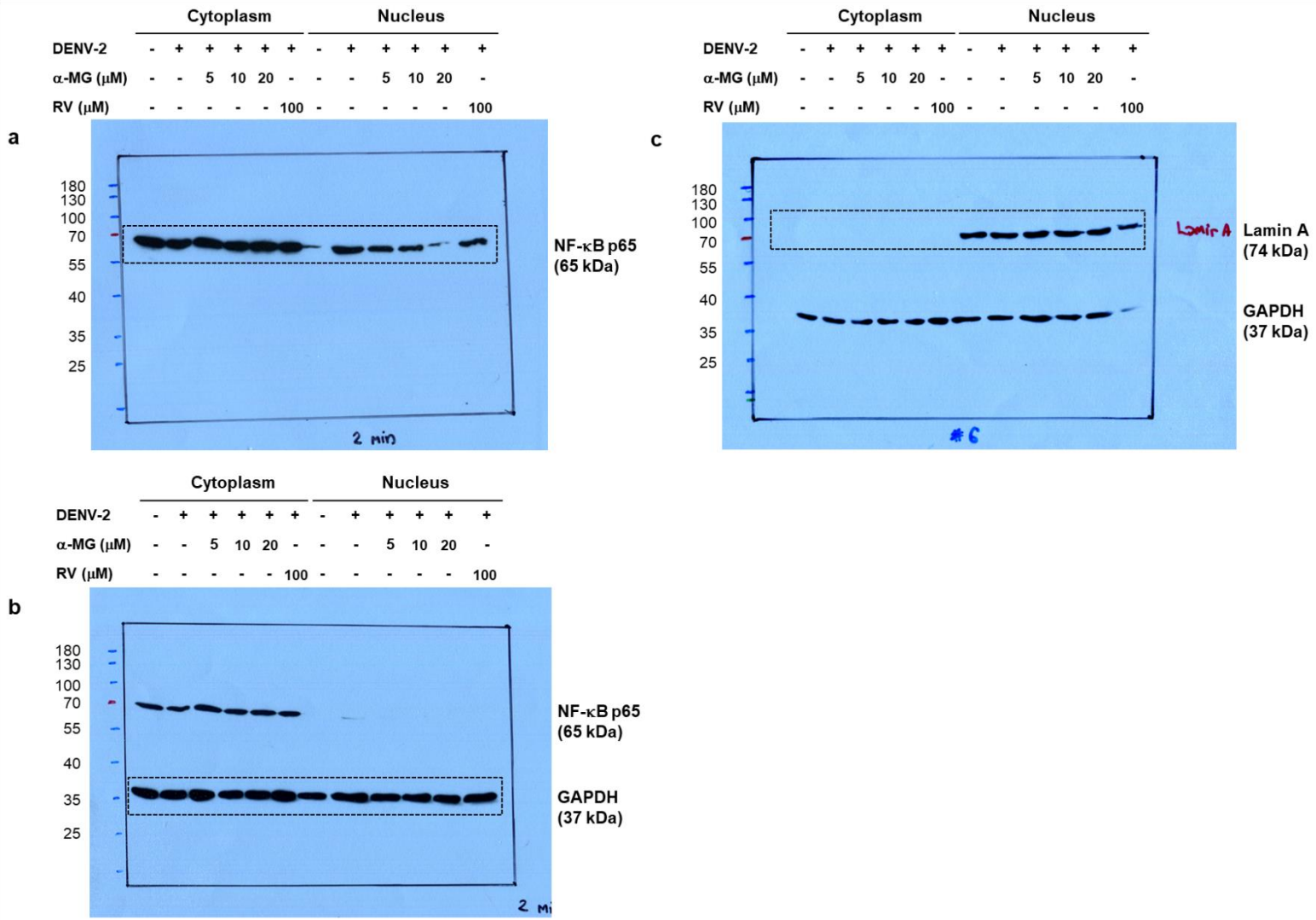

Fig. S3 Immunoblot images with cropped areas indicated by dashed lines. The results are the representatives from three independent experiments. Quantitative comparisons between samples were performed on the same blot. (a) The membrane was incubated with anti-NF-κB p65 antibody. (b) The same membrane was incubated with anti-GAPDH antibody. (c) The samples were obtained from the same experiment, the blot was processed in parallel, and the membrane was incubated with anti-lamin A antibody. Then, the membrane was incubated with anti-GAPDH antibody.

Original immunoblot for Fig.7

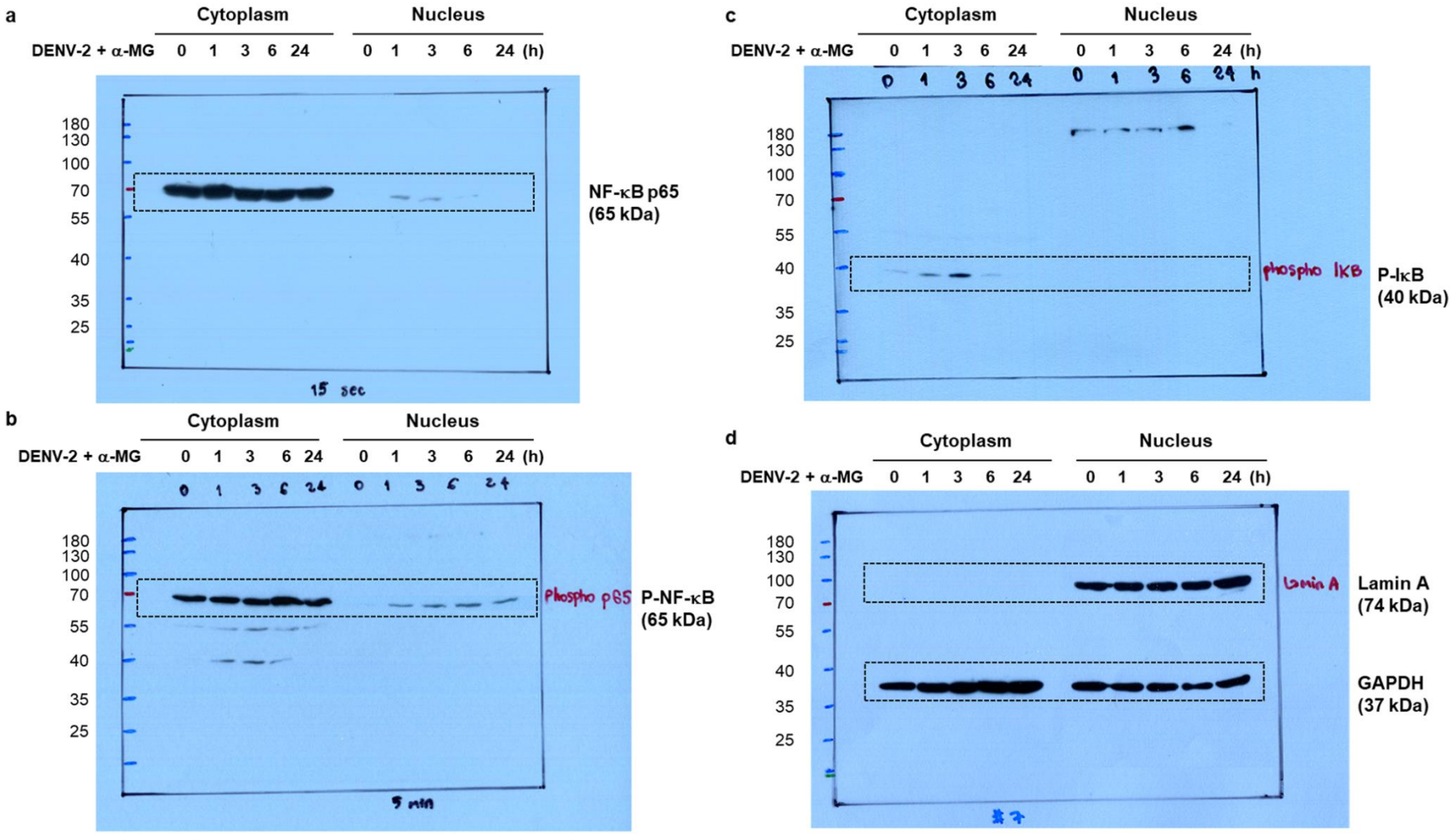

Fig. S4 Immunoblot images with cropped areas indicated by dashed lines. The results are the representatives from three independent experiments. The samples were obtained from the same experiment and the blots were processed in parallel. Each membrane was separately incubated with specific antibody to detect either (a) NF-κB p65, (b) P-NF-κB p65, or (c) P-IκB. (d) The membrane was incubated with anti-lamin A and anti-GAPDH antibodies to detect lamin A and GAPDH.

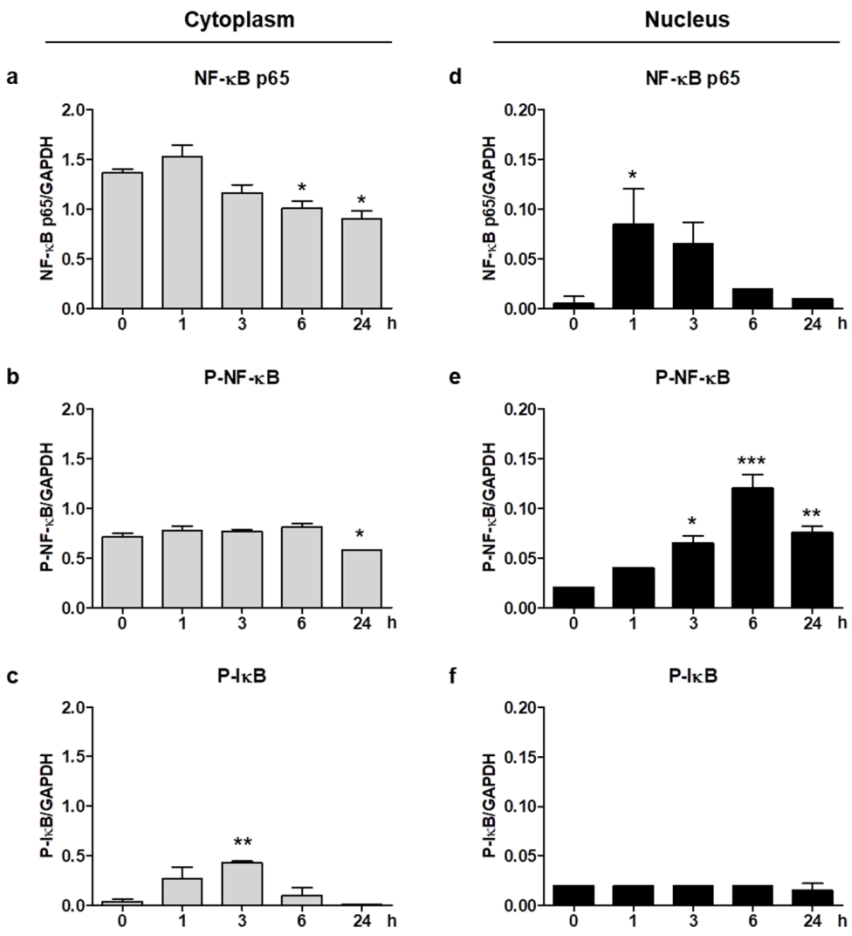

Fig. S5 α-MG suppresses NF-κB activation. HepG2 were infected with DENV-2 at a MOI of 5 and treated with 20 μM of α-MG. The cells were harvested at 0, 1, 3, 6, and 24 h after treatment. Cell lysates were separated into cytoplasmic and nuclear fractions. The expressions of NF-κB p65, phosphorylated NF-κB (P-NF-κB), and phosphorylated IκB (P-IκB) were determined by immunoblot assay using anti-NF-κB p65, anti-P-NF-κB, and anti-P-IκB antibodies, respectively (Fig. 7a). The optical densities normalized to GAPDH are shown. One-way ANOVA followed by Tukey's HSD test were used to evaluate for differences in protein levels as compared to 0 h post- treatment of α-MG (*p<0.05, **p<0.01, ***p<0.001).