Expanded View Figures

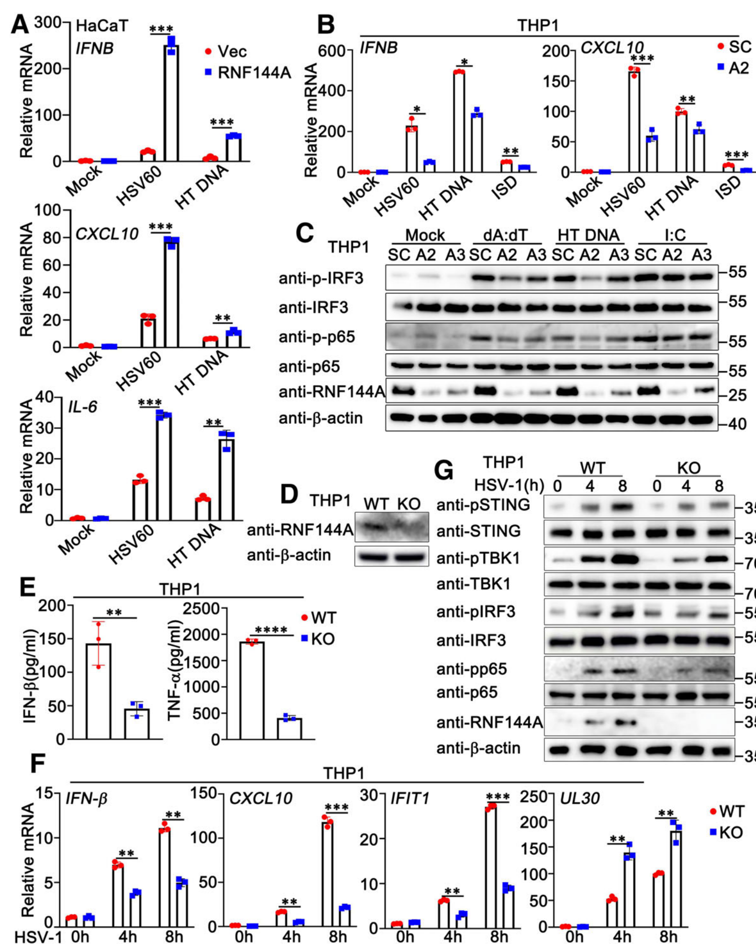

Figure EV1. RNF144A promotes DNA virus- or exogenous cytosolic DNA-triggered innate immune responses.

- A HaCaT keratinocytes were transfected with the empty vector (Vec) or the RNF144A plasmid for 24 h and then treated with HSV60 (1 μg/ml), HT-DNA (1 μg/ml), or left untreated for 8 h. The cells were lysed for real-time PCR assays.
- B PMA-THP1 cells were transfected with control siRNA (SC) or RNF144A-specific siRNA (A2). At 24 h after transfection, the cells were transfected with HSV60 (1 μg/ml), HT-DNA (1 μg/ml), or ISD (1 μg/ml) for 8 h. The cells were lysed for real-time PCR assays.
- C PMA-THP1 cells were transfected with control siRNA (SC) or RNF144A-specific siRNA (A2, A3). At 24 h after transfection, the cells were transfected with poly(dA:dT) (1 μg/ml), HT-DNA (1 μg/ml), or poly(I: C) (2.5 μg/ml) for 8 h. The cells were lysed for immunoblot assays.
- D Wild-type (WT) and *Rnf144a*-deficient (KO) PMA-THP1 cells were infected with HSV-1 (MOI = 1) for 8 h. The cells were lysed for immunoblot assays.
- E Wild-type (WT) and *Rnf144a*-deficient (KO) PMA-THP1 cells were infected with HSV-1 (MOI = 1) for 24 h. The supernatants were collected and subjected to ELISA assays.
- F Wild-type (WT) and *Rnf144a*-deficient (KO) PMA-THP1 cells were infected with HSV-1 (MOI = 1) for the indicated periods. The cells were lysed for real-time PCR assays.
- G Wild-type (WT) and *Rnf144a*-deficient (KO) PMA-THP1 cells were infected with HSV-1 (MOI = 1) for the indicated periods. The immunoblot assays were then performed.

Data information: Two-tailed unpaired Student's *t*-test, **P* < 0.05, ***P* < 0.01 and ****P* < 0.001. Data shown are representative of at least three independent biological replicates. In (A, B, F), each data point represents a technical replicate. In (E), each data point represents an independent biological replicate. Error bars are presented as mean ± SD.
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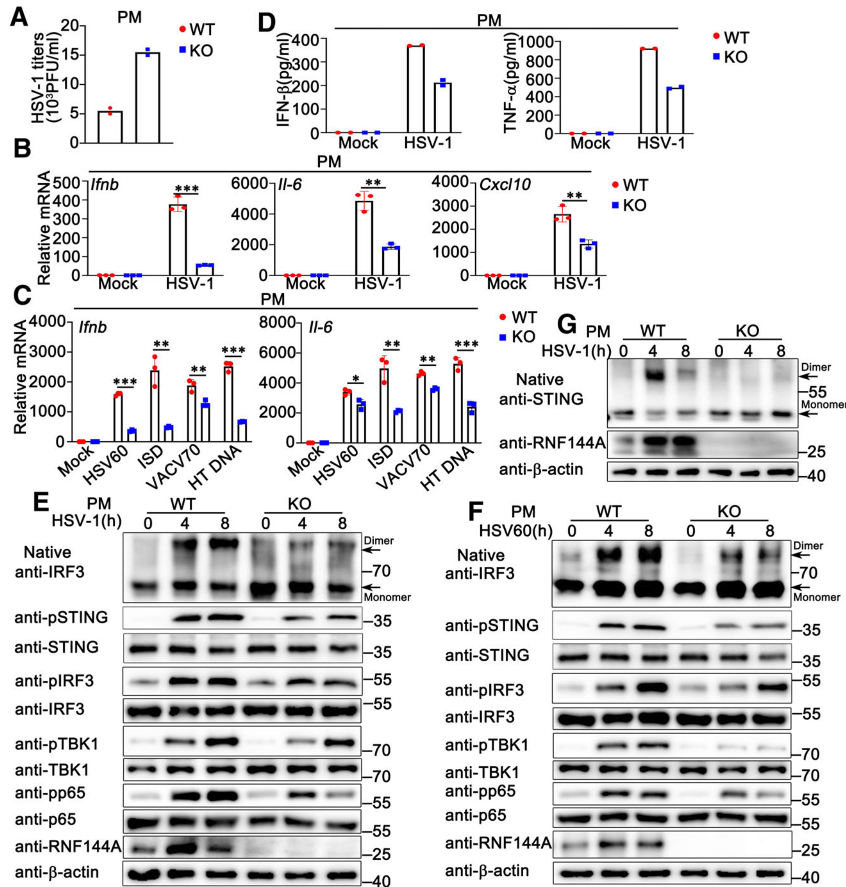

Figure EV2. RNF144A deficiency impairs DNA virus or exogenous cytosolic DNA-triggered innate immune responses in PMs.

A Wild-type (WT) and *Rnf144a*-deficient (KO) PMs were infected with HSV-1 (MOI = 1) for 24 h. The titers of HSV-1 were determined by standard plaque assay.

B Wild-type (WT) and *Rnf144a*-deficient (KO) PMs were infected with HSV-1 (MOI = 1) for 8 h. The cells were lysed for real-time PCR assays.

C Wild-type (WT) and *Rnf144a*-deficient (KO) PMs were transfected with HSV60 (1 μg/ml), ISD (1 μg/ml), VACV70 (1 μg/ml), or HT-DNA (1 μg/ml) for 8 h. The cells were lysed for real-time PCR assays.

D Wild-type (WT) and *Rnf144a*-deficient (KO) PMs were infected with HSV-1 (MOI = 1) for 24 h. The supernatants were collected and subjected to ELISA assays.

E, F Wild-type (WT) and *Rnf144a*-deficient (KO) PMs were infected with HSV-1 (MOI = 1) (E) or transfected with HSV60 (1 μg/ml) (F) for the indicated periods. Native-PAGE and SDS–PAGE assays were then performed.

G Wild-type (WT) and *Rnf144a*-deficient (KO) PMs were infected with HSV-1 (MOI = 1) for the indicated periods. Native-PAGE and SDS–PAGE assays were then performed.

Data information: Two-tailed unpaired Student's *t*-test, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. Data shown are from two (A, D) or at least three independent biological replicates (B, C, E–G). In (B, C), each data point represents a technical replicate. In (A, D), each data point represents an independent biological replicate. Error bars are presented as mean ± SD.
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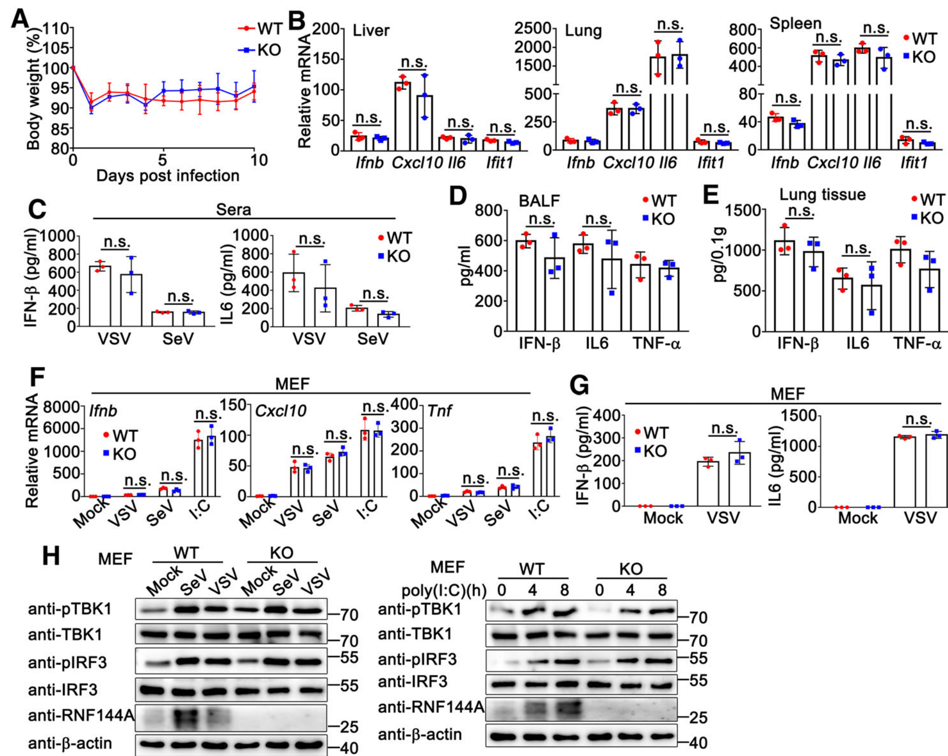

Figure EV3. RNF144A does not affect RNA virus- or cytosolic RNA-triggered innate immune responses.

A Sex and age-matched wild-type (WT) and *Rnf144a*-deficient (KO) mice ($n = 7$, 8-week-old) were intravenously infected with VSV (5×10^7PFU). The body weight loss was monitored for 10 days.

B Wild-type (WT) ($n = 3$) and *Rnf144a*-deficient (KO) ($n = 3$) mice were intravenously infected with VSV (5×10^7 PFU) for 24 h and then the lungs, livers, and spleens of the mice were subjected to real-time PCR assays.

C Serum IFN-β and IL-6 were determined by ELISA in wild-type (WT) ($n = 3$) and *Rnf144a*-deficient (KO) ($n = 3$) mice 6 h after intravenous infection with VSV or SeV.

D Wild-type (WT) ($n = 3$) and *Rnf144a*-deficient (KO) mice ($n = 3$) were intranasally infected with VSV (5×10^7 PFU) for 24 h. Bronchoalveolar lavage fluid (BALF) was collected and ELISA assays were performed.

E Following homogenization, the lung suspensions in (D) were centrifuged, and the supernatants were subjected to ELISA assays.

F Wild-type (WT) and *Rnf144a*-deficient (KO) MEFs were infected with VSV (MOI = 1), SeV (5 HA units/ml), or transfected with poly(I: C) (1 μg/ml) for 8 h. The cells were then lysed for real-time PCR assays.

G Wild-type (WT) and *Rnf144a*-deficient (KO) MEFs were infected with VSV (MOI = 1) for 24 h. The supernatants were collected and subjected to ELISA assays.

H Wild-type (WT) and *Rnf144a*-deficient (KO) MEFs were infected with VSV (MOI = 1), SeV (5 HA units/ml), or transfected with poly(I: C) (1 μg/ml) for the indicated periods and then the cells were lysed for immunoblot assays.

Data information: Two-tailed unpaired Student's *t*-test, n.s., not significant ($P > 0.05$). Data are representative of at least three independent biological replicates. In (F), each data point represents a technical replicate. In (B–E, G), each data point represents an independent biological replicate. Error bars are presented as mean ± SD. Source data are available online for this figure.

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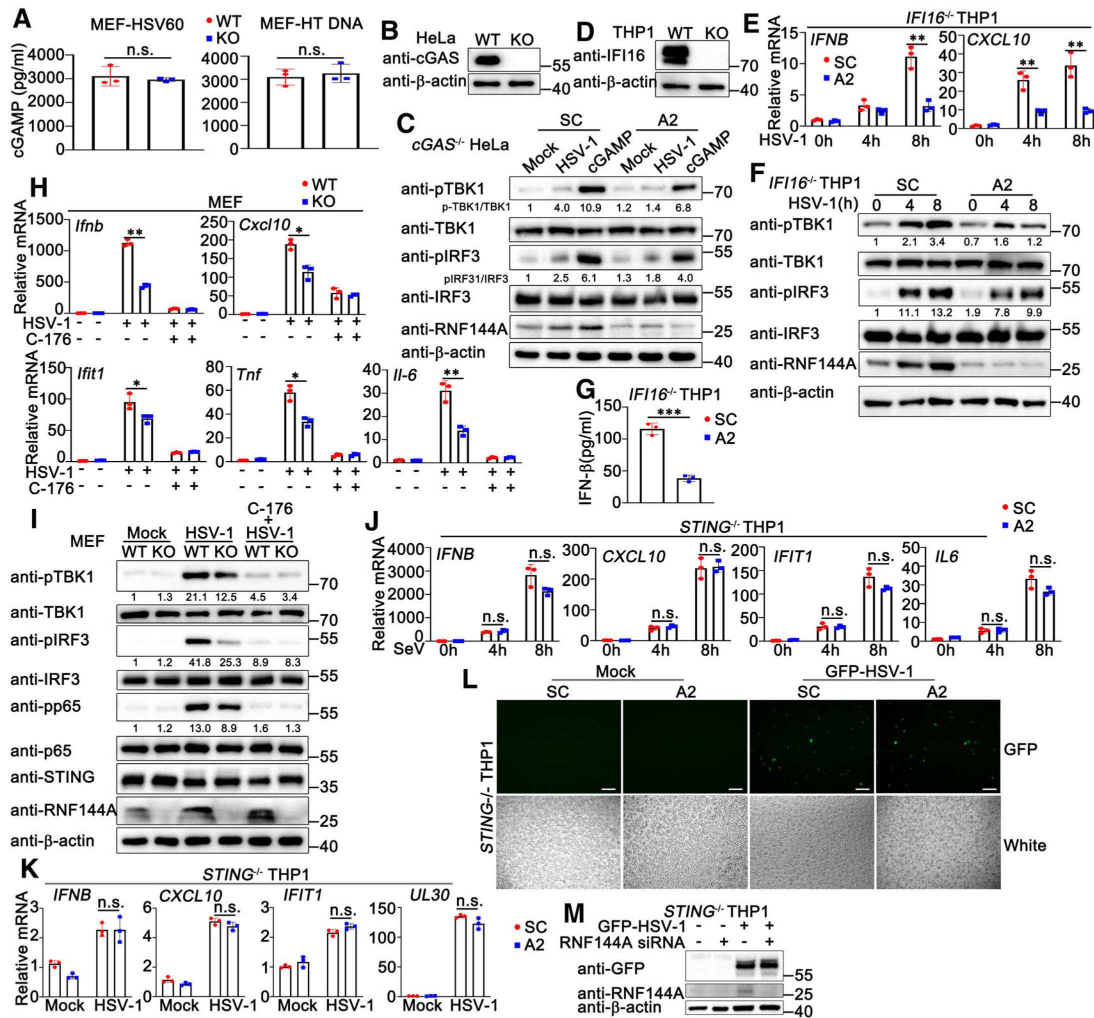

Figure EV4. RNF144A regulates STING-mediated signaling pathways.

A Wild-type (WT) and *Rnf144a*-deficient (KO) MEFs were transfected with HSV60 (1 μg/ml) or HT DNA (1 μg/ml) for 24 h. The supernatants were collected and subjected to ELISA assays.

B Wild-type (WT) and *cGAS*-deficient (KO) HeLa cells were lysed for immunoblot assays.

C *cGAS*-deficient (KO) HeLa cells were transfected with control siRNA (SC) or RNF144A-specific siRNA (A2) for 24 h and then infected with HSV-1 or transfected with cGAMP (1 μg/ml) for 8 h. The cells were lysed for immunoblot assays.

D Wild-type (WT) and *IFI16*-deficient (KO) PMA-THP1 cells were lysed for immunoblot assays.

E, F *IFI16*-deficient (KO) PMA-THP1 cells were transfected with control siRNA (SC) or RNF144A-specific siRNA (A2) for 24 h and then infected with HSV-1 (MOI = 1) for the indicated periods. Afterward, the cells were lysed for real-time PCR (E) or immunoblot assays (F).

G *IFI16*-deficient (KO) PMA-THP1 cells were transfected with control siRNA (SC) or RNF144A-specific siRNA (A2) for 24 h and then infected with HSV-1 (MOI = 1) for 24 h. The supernatants were collected and subjected to ELISA analysis.

H, I Wild-type (WT) and *Rnf144a*-deficient (KO) MEFs were treated with C-176 (1 μM) or left untreated for 24 h and then infected with HSV-1 (MOI = 1) for 8 h. Afterward, the cells were lysed for real-time PCR (H) or immunoblot assays (I).

J *STING*-deficient (KO) PMA-THP1 cells were transfected with control siRNA (SC) or RNF144A-specific siRNA (A2) for 24 h and then infected with SeV for the indicated periods. Afterward, the cells were lysed for real-time PCR assays.

K *STING*-deficient (KO) PMA-THP1 cells were transfected with control siRNA (SC) or RNF144A-specific siRNA (A2) for 24 h and then infected with HSV-1 for 8 h. Afterward, the cells were lysed for real-time PCR assays.

L, M *STING*-deficient (KO) PMA-THP1 cells were transfected with control siRNA (SC) or RNF144A-specific siRNA (A2) for 24 h and then infected with GFP-HSV-1 for 24 h. Afterward, the cells were subjected to image acquisition (L) or immunoblot assays (M). Scale bar: 200 μm (L). White: bright field microscopy (L).

Data information: Two-tailed unpaired Student's *t*-test, $^{*}P < 0.05$, $^{**}P < 0.01$, n.s., not significant ($P > 0.05$). Data shown are representative of at least three independent biological replicates. In (E, H, J, K), each data point represents a technical replicate. In (A, G), each data point represents an independent biological replicate. Error bars are presented as mean ± SD.
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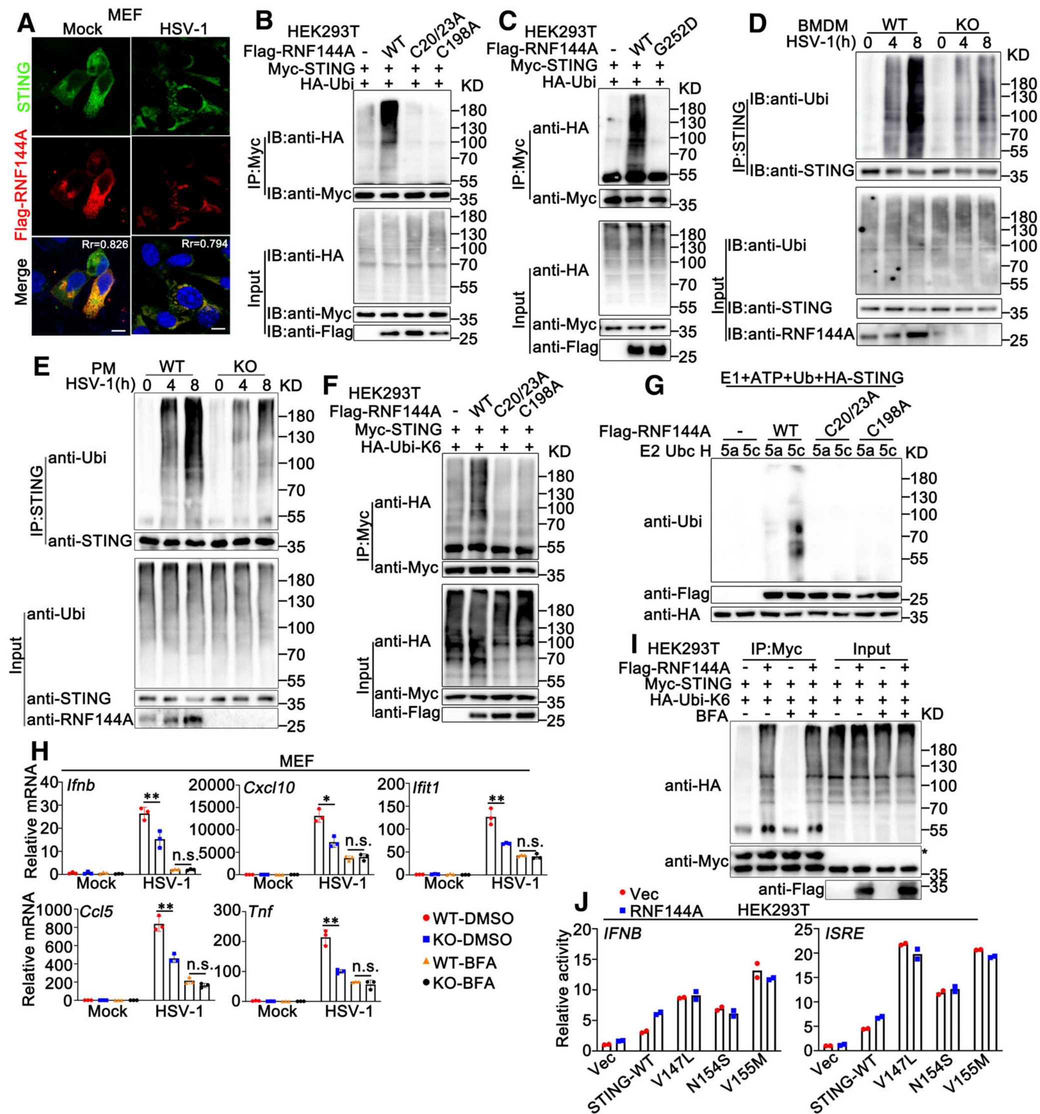

Figure EV5. RNF144A promotes the ubiquitination of STING and regulates its translocation.

A MEFs were transfected with expressing plasmids for Flag-RNF144A. At 24 h after transfection, MEFs were infected with HSV-1 (MOI = 1) or left uninfected for 8 h. Immunofluorescence assays were performed using anti-STING (green) and anti-Flag (red). Nuclei were stained with DAPI. Scale bar: 10 μm. Pearson's correlation coefficient was calculated using ImageJ software. Rr, Pearson's correlation coefficient.

B, C HEK293T cells were transfected with the indicated plasmids. At 24 h after transfection, the cells were lysed and subjected to immunoprecipitation (IP) and immunoblot (IB) assays.

D, E Wild-type (WT) and *Rnf144a*-deficient (KO) BMDMs (D) or PMs (E) were infected with HSV-1 (MOI = 1) for the indicated periods. Afterward, the cells were lysed and subjected to immunoprecipitation (IP) and immunoblot (IB) assays.

F HEK293T cells were transfected with the indicated plasmids. At 24 h after transfection, the cells were lysed and subjected to immunoprecipitation (IP) and immunoblot (IB) assays.

G Immunoblot analysis of STING ubiquitination *in vitro*. STING, wild-type RNF144A, and its mutants were quickly translated *in vitro*. Then, the biotin-ubiquitin E1 and indicated E2s were added for the *in vitro* ubiquitination assays.

H Wild-type (WT) and *Rnf144a*-deficient (KO) MEFs were treated with BFA (5 μg/ml) or left untreated for 3 h and then infected with HSV-1 (MOI = 1) for 8 h. Afterward, the cells were lysed for real-time PCR assays.

I HEK293T cells were transfected with the indicated plasmids. At 24 h after transfection, the cells were treated with BFA (5 μg/ml) or left untreated for 3 h. Afterward, the cells were lysed and subjected to immunoprecipitation (IP) and immunoblot (IB) assays. *Heavy chain of the antibody.

J Luciferase activity in HEK293 cells transfected with an *IFNB* or *ISRE* luciferase reporter, together with plasmids as indicated.

Data information: Two-tailed unpaired Student's *t*-test, $*P < 0.05$, $**P < 0.01$, n.s., not significant ($P > 0.05$). Data shown are from two (J) or at least three independent biological replicates (A–I). In (H), each data point represents a technical replicate. In (J), each data point represents an independent biological replicate. Error bars are presented as mean ± SD.

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