Figure S1

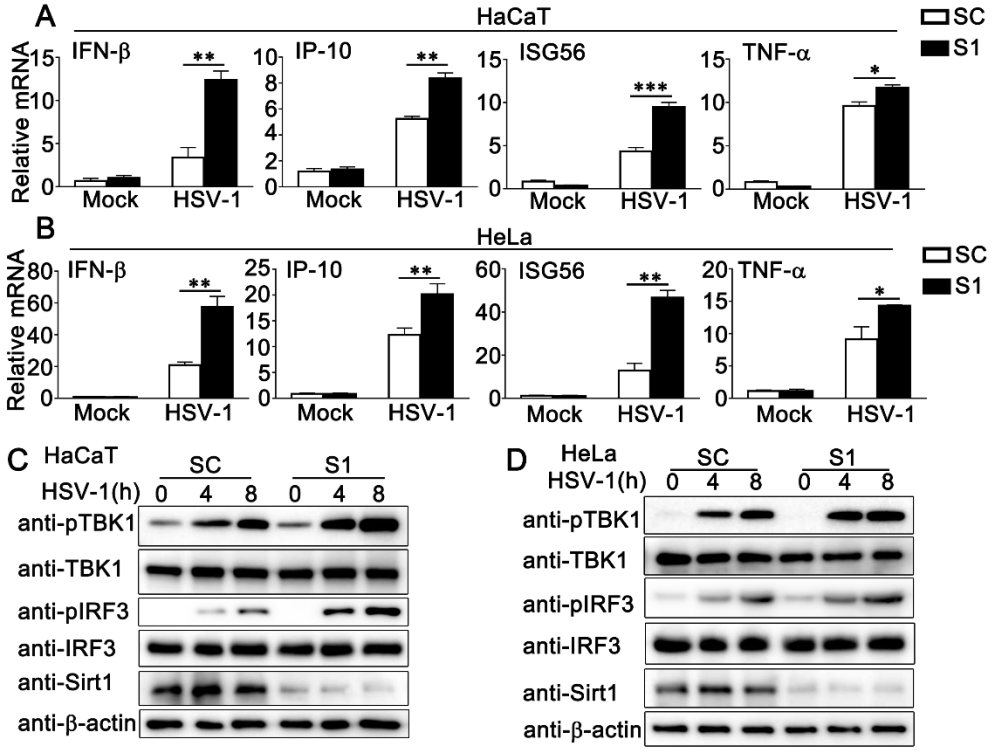

Figure S1 Sirt1 knockdown promotes HSV-1-induced innate immune responses in HaCaT and HeLa cells.

A HaCaT cells were transfected with control siRNA (SC) or Sirt1-specific siRNA (S1) for 24 h, and then infected with HSV-1 (MOI=1) or left uninfected for 4 h. The cells were lysed for real-time PCR analyses.

B HeLa cells were transfected with control siRNA (SC) or Sirt1-specific siRNA (S1) for 24 h, and then infected with HSV-1 (MOI=1) or left uninfected for 4 h. The cells were lysed for real-time PCR analyses.

C HaCaT cells were transfected with control siRNA (SC) or Sirt1-specific siRNA (S1) for 24 h, and then infected with HSV-1 (MOI=1) or left uninfected for 4 h. The cell lysates were subjected to immunoblot analysis.

D HeLa cells were transfected with control siRNA (SC) or Sirt1-specific siRNA (S1)

for 24 h, and then infected with HSV-1 (MOI=1) or left uninfected for 4 h. The cell lysates were subjected to immunoblot analysis.

β-actin served as a loading control in all the immunoblot assays. The data are representative of three independent experiments and are presented as mean ± SD. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

Figure S2

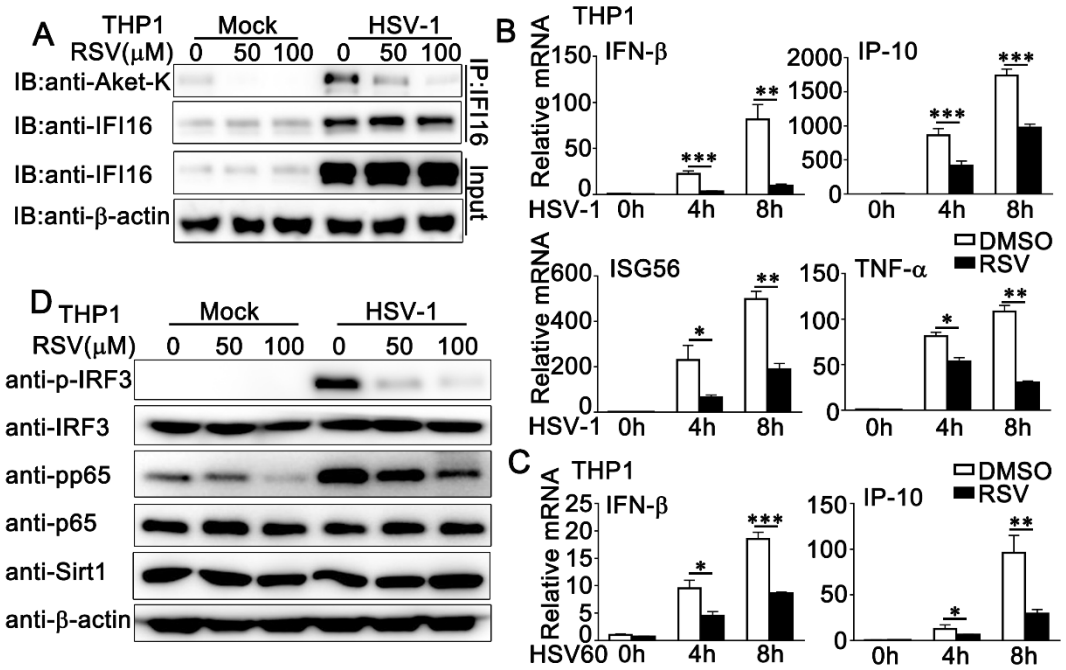

Figure S2 Sirt1 activator RSV inhibits HSV-1- or HSV60-induced innate immune responses

A PMA-THP1 cells were treated with increasing amounts of Resveratrol (RSV) (0, 50, and 100 μM) for 12 h, and then infected with HSV-1(MOI=1) for another 12 h. The cells were subjected to immunoprecipitation (IP) and immunoblot (IB) analysis.

B PMA-THP1 cells were treated with Resveratrol (RSV) (100 μM) for 12 h and then infected with HSV-1(MOI=1) for indicated periods. Afterward, the cells were lysed for real-time PCR analysis.

C PMA-THP1 cells were treated with Resveratrol (RSV) (100 μM) for 12 h and then transfected with HSV60 (1μg/ml) for indicated periods. Afterward, real-time PCR analysis was performed.

D PMA-THP1 cells were treated with increasing amounts of Resveratrol (RSV) (0, 50, and 100 μM) for 12 h, and then infected with HSV-1(MOI=1) for another 8 h. The cells

were subjected to immunoblot analysis.

β-actin served as a loading control in all the immunoblot assays. The data are representative of three independent experiments and are presented as mean ± SD. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

Figure S3

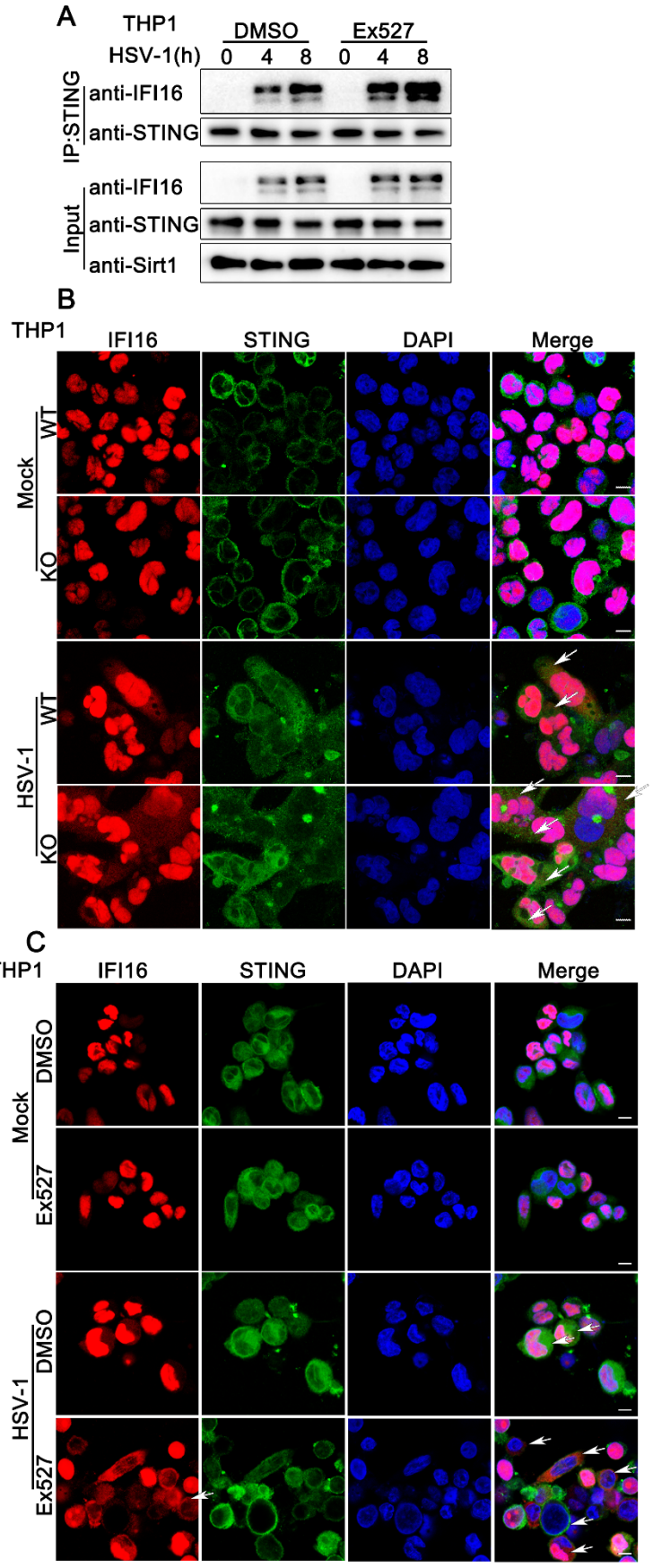
A
THP1
DMSO
Ex527
HSV-1(h)
0 4 8 0 4 8
IP:STING
anti-IFI16
anti-STING
Input
anti-IFI16
anti-STING
anti-Sirt1
B
THP1
IFI16
STING
DAPI
Merge
Mock
WT
KO
HSV-1
WT
KO
C
THP1
IFI16
STING
DAPI
Merge
Mock
DMSO
Ex527
HSV-1
DMSO
Ex527

Figure S3 Sirt1 regulates IFI16-STING interaction.

A PMA-THP1 cells were treated with control DMSO or Ex527 (5μM) for 12 h and then infected with HSV-1 (MOI=1) for indicated periods. Afterward, immunoprecipitation (IP) and immunoblot (IB) analysis were performed.

B Wild-type (WT) and Sirt1-deficient (KO) PMA-THP1 cells were infected with HSV-1 (MOI=1) or left uninfected for 8 h. Immunofluorescence was performed using anti-IFI16 (red) and anti-STING (green). Nuclei were stained with DAPI. Scale bars in the merge are 10μm. White arrows point at occurrences of cytoplasmic IFI16.

C PMA-THP1 cells were treated with control DMSO or Ex527 (5μM) for 12 h and then infected with HSV-1 (MOI=1) for 8 h. Immunofluorescence was performed using anti-IFI16 (red) and anti-STING (green). Nuclei were stained with DAPI. Scale bars in the merge are 10μm. White arrows point at occurrences of cytoplasmic IFI16.

The data are representative of three independent experiments.

Figure S4

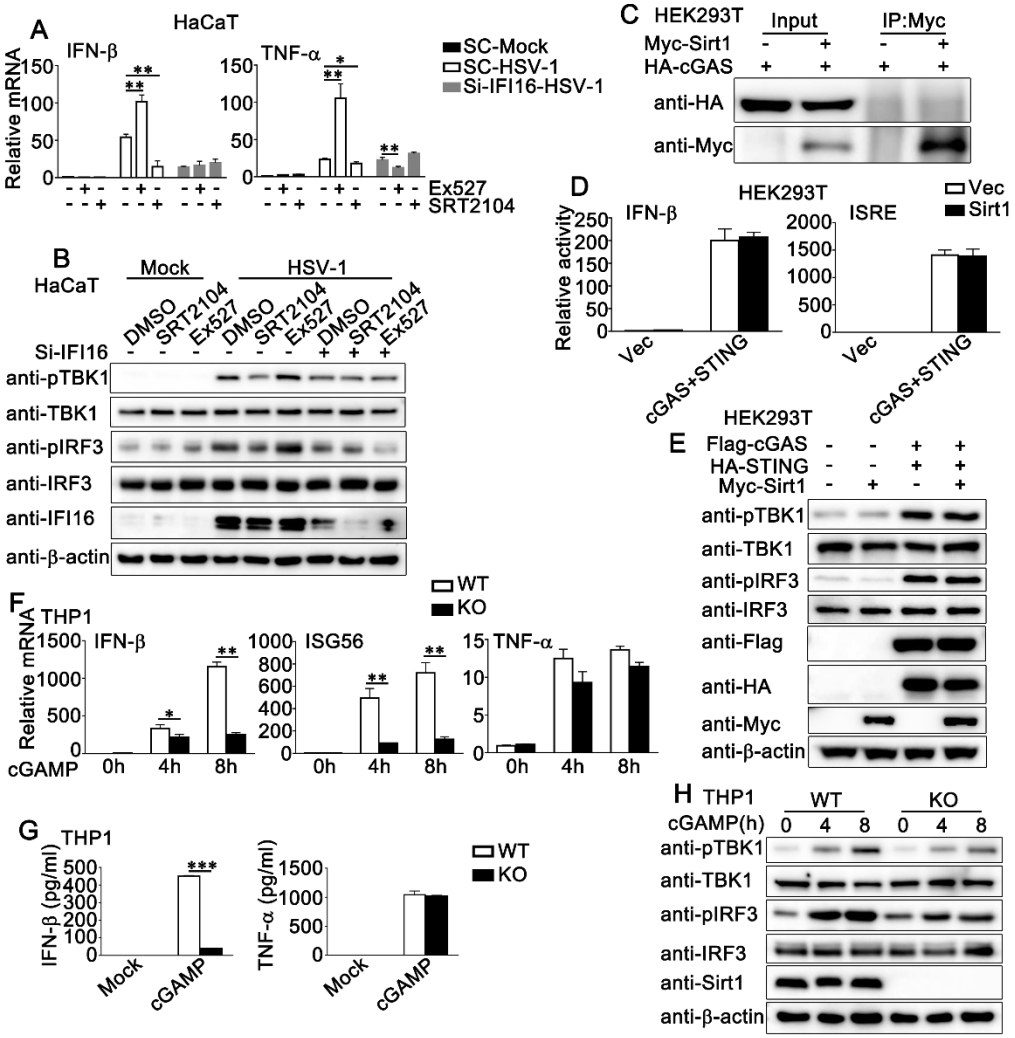

Figure S4 Sirt1 inhibits DNA virus- or viral DNA-triggered innate immune responses in an IFI16-dependent pattern.

A HaCaT cells were treated with control DMSO, Ex527 (5μM), or SRT2104 (5μM) for 12 h, and then transfected with control siRNA (SC) or IFI16-specific siRNA for 24 h. The cells were infected with HSV-1 (MOI=1) or left uninfected for another 8 h and then lysed for real-time PCR analyses.

B HaCaT cells were treated with control DMSO, Ex527 (5μM), or SRT2104 (5μM) for 12 h, and then transfected with control siRNA (SC) or IFI16-specific siRNA for 24 h.

The cells were infected with HSV-1 (MOI=1) or left uninfected for another 8 h and then subjected to immunoblot analysis.

C HEK293T cells were transfected with indicated plasmids. 24 h later, immunoprecipitation (IP) and immunoblot (IB) analysis were performed.

D HEK293T cells were transfected with Vector (Vec) or Sirt1, and IFN-β or ISRE luciferase reporter, with or without the cGAS and STING expressing plasmids. At 24 h after transfection, the cells were lysed for luciferase assay.

E HEK293T cells were transfected with Vector (Vec) or Sirt1, together with or without the cGAS and STING-expressing plasmids. At 24 h after transfection, the cell lysates were subjected to immunoblot analysis.

F Wild-type (WT) and Sirt1-deficient (KO) PMA-THP1 cells were stimulated with cGAMP (1μg/ml) for indicated periods. Then the cells were lysed for real-time PCR analysis.

G Wild-type (WT) and Sirt1-deficient (KO) PMA-THP1 cells were stimulated with cGAMP (1μg/ml) for 24 h. The supernatants were collected and subjected to ELISA analysis.

H Wild-type (WT) and Sirt1-deficient (KO) PMA-THP1 cells were stimulated with cGAMP (1μg/ml) for indicated periods. Then the cell lysates were subjected to immunoblot analysis.

β-actin served as a loading control in all the immunoblot assays. The data are representative of three independent experiments and are presented as mean ± SD. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.