Expanded View Figures

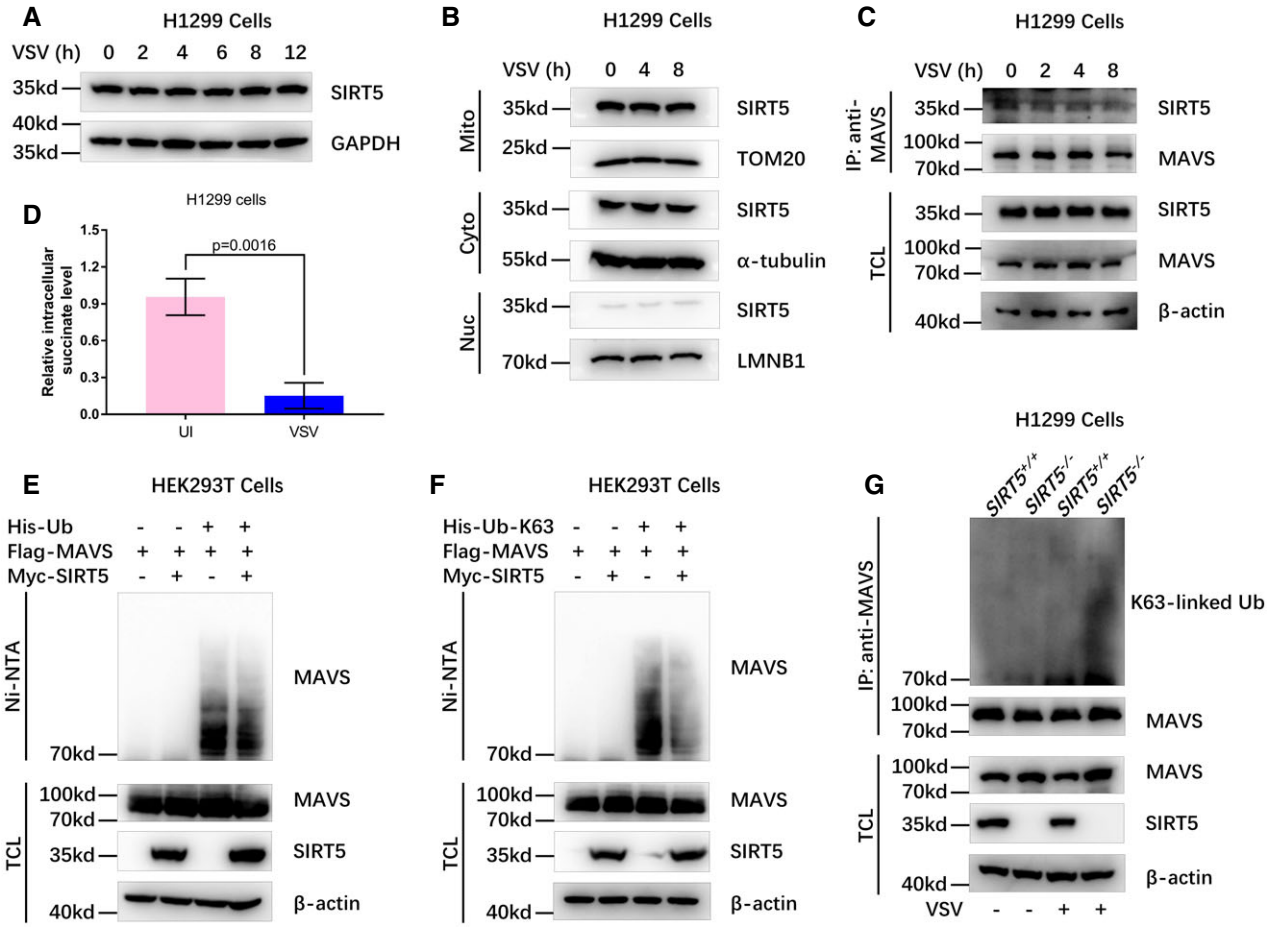

Figure EV1. VSV infection does not influence protein level, translocation of SIRT5, but attenuates SIRT5 binding to MAVS; VSV infection diminishes K63-linked polyubiquitination of MAVS.

A Western blot analysis of SIRT5 in H1299 cells infected with VSV at different time points (0, 2, 4, 6, 8, 12 h).
B Western blot analysis of SIRT5 in mitochondria, cytoplasm, and nuclei of H1299 cell infected with VSV at different time points (0, 4, 8 h).
C Western blot analysis for SIRT5 binding to MAVS in H1299 cells infected with VSV at different time points (0, 2, 4, 8 h).
D VSV infection caused a reduction of intracellular succinate level.
E Overexpression of SIRT5 attenuated polyubiquitination of MAVS in HEK293T cells.
F Overexpression of *SIRT5* attenuated K63-linked polyubiquitination of MAVS in HEK293T cells.
G Knockout of *SIRT5* in H1299 cells enhanced K63-linked polyubiquitination of endogenous MAVS in response to VSV infection.

Source data are available online for this figure.

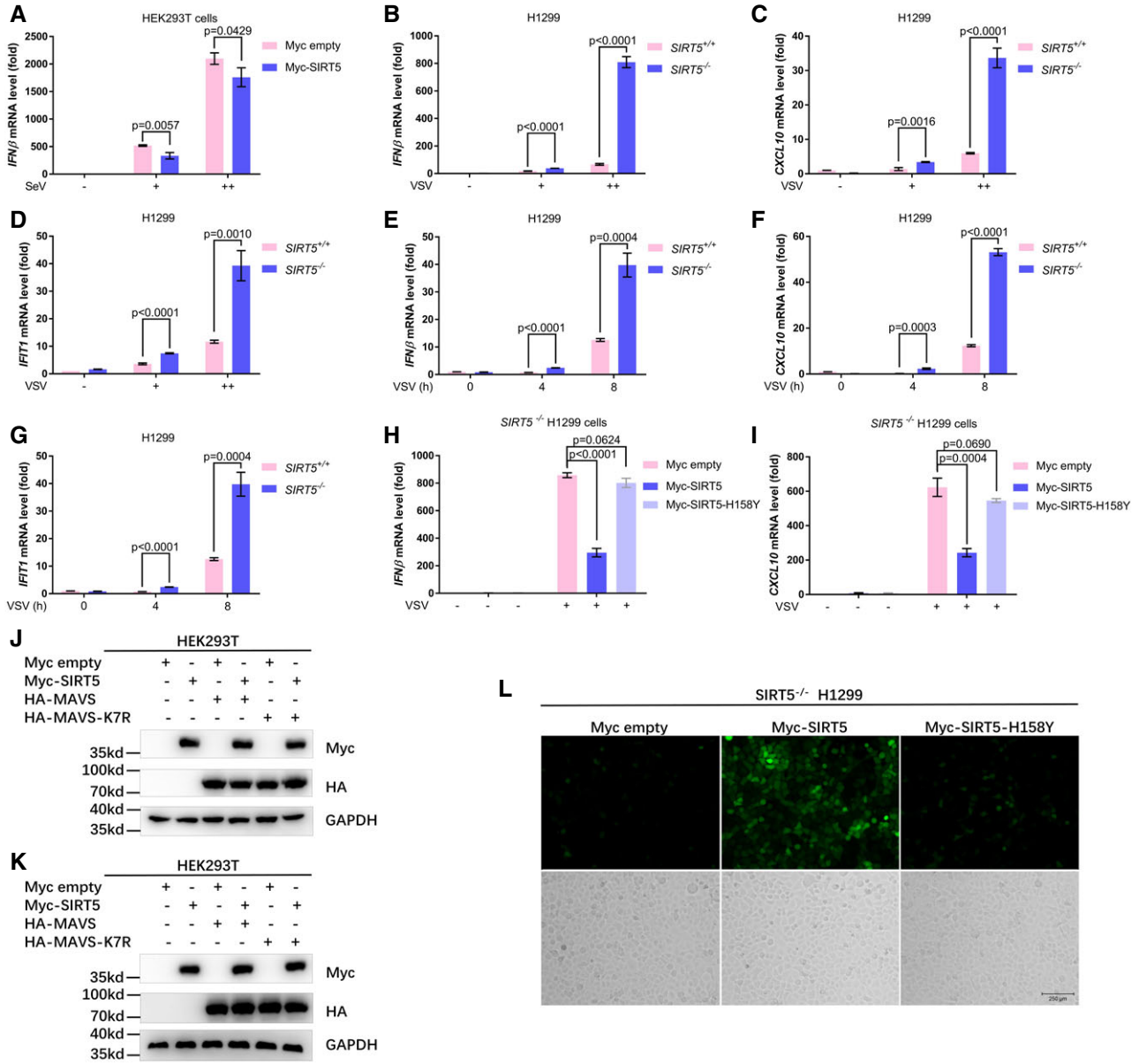

Figure EV2. *SIRT5* negatively regulates cellular antiviral response.

A qRT–PCR analysis of *IFNβ* mRNA in HEK293T cell transfected with Myc empty vector or Myc-SIRT5 for 24 h, followed by infected with SeV in an increasing titration (+, ++) for 8 h. –, without infection.

B–D qRT–PCR analysis of *IFNβ* (B), *CXCL10* (C), and *IFIT1* (D) mRNA in *SIRT5*-deficient ($SIRT5^{-/-}$) or WT ($SIRT5^{+/+}$) H1299 cells infected with SeV in an increasing titration (+, ++) for 8 h. –, without infection.

E–G qPCR analysis of *IFNβ* (E), *CXCL10* (F), and *IFIT1* (G) mRNA in *SIRT5*-deficient ($SIRT5^{-/-}$) or WT ($SIRT5^{+/+}$) H1299 cells infected with SeV for different time points (0, 4, 8 h).

H, I qPCR analysis of *IFNβ* (H) and *CXCL10* (I) mRNA in *SIRT5*-deficient H1299 cells ($SIRT5^{-/-}$) transfected with the control plasmid (Myc empty), the plasmid expressing Myc-SIRT5 (Myc-SIRT5), or the plasmid expressing the enzyme-deficient mutant H158Y (Myc-SIRT5-H158Y) for 24 h, followed by infection with (+) or without (–) VSV for 8 h.

J Western blot analysis for Fig 4M.

K Western blot analysis for Fig 4N.

L Fluorescence microscopy images of VSV-GFP virus replication in *SIRT5*-deficient H1299 cells ($SIRT5^{-/-}$) transfected with the control plasmid (Myc empty), the plasmid expressing Myc-SIRT5 (Myc-SIRT5), or the plasmid expressing the enzyme-deficient mutant H158Y (Myc-SIRT5-H158Y) for 24 h, followed by infection with VSV-GFP (MOI = 0.1) for 12 h.

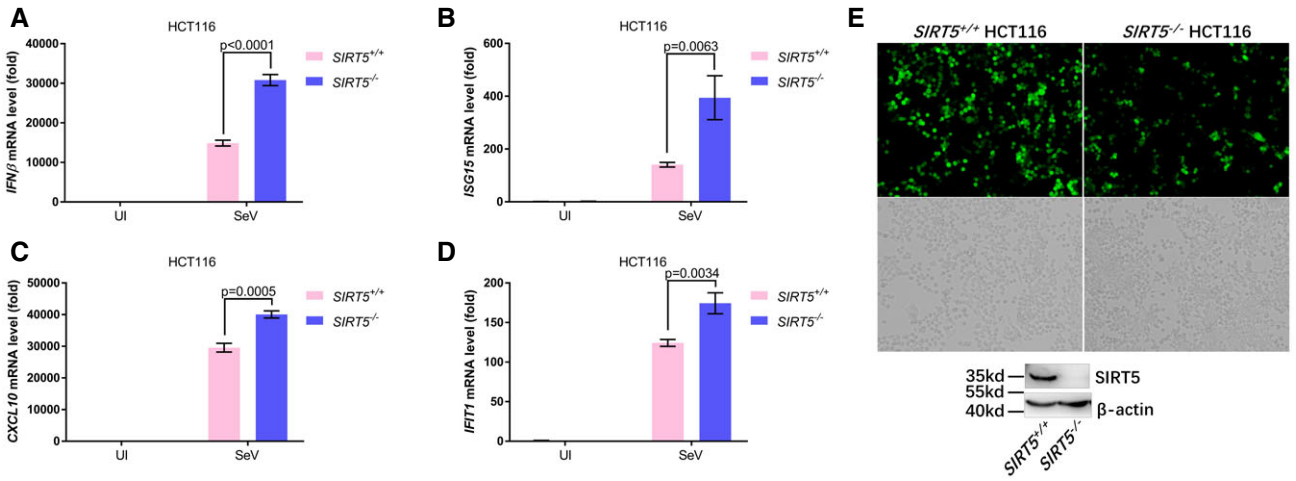

Figure EV3. *SIRT5* deficiency enhances cellular antiviral response in HCT116 cells.

A–D qPCR analysis of *IFNβ* (A), *ISG15* (B), *CXCL10* (C), and *IFIT1* (D) mRNA in *SIRT5*-deficient ($SIRT5^{-/-}$) or WT ($SIRT5^{+/+}$) HCT116 cells infected with or without SeV (Sev or UI) for 8 h. UI, uninfected; the graphs represent the fold induction relative to the untreated control cells. All data are presented as the mean values based on three independent experiments, and error bars indicate s.e.m.

E Fluorescence microscopy images of VSV-GFP virus replication in *SIRT5*-deficient ($SIRT5^{-/-}$) or WT ($SIRT5^{+/+}$) HCT116 cells after infected with VSV-GFP viruses (MOI = 0.1) for 12 h (fluorescence, upper; bright-field, bottom). The expression of *SIRT5* in *SIRT5*-deficient ($SIRT5^{-/-}$) or WT ($SIRT5^{+/+}$) HCT116 cells was examined by Western blot analysis.

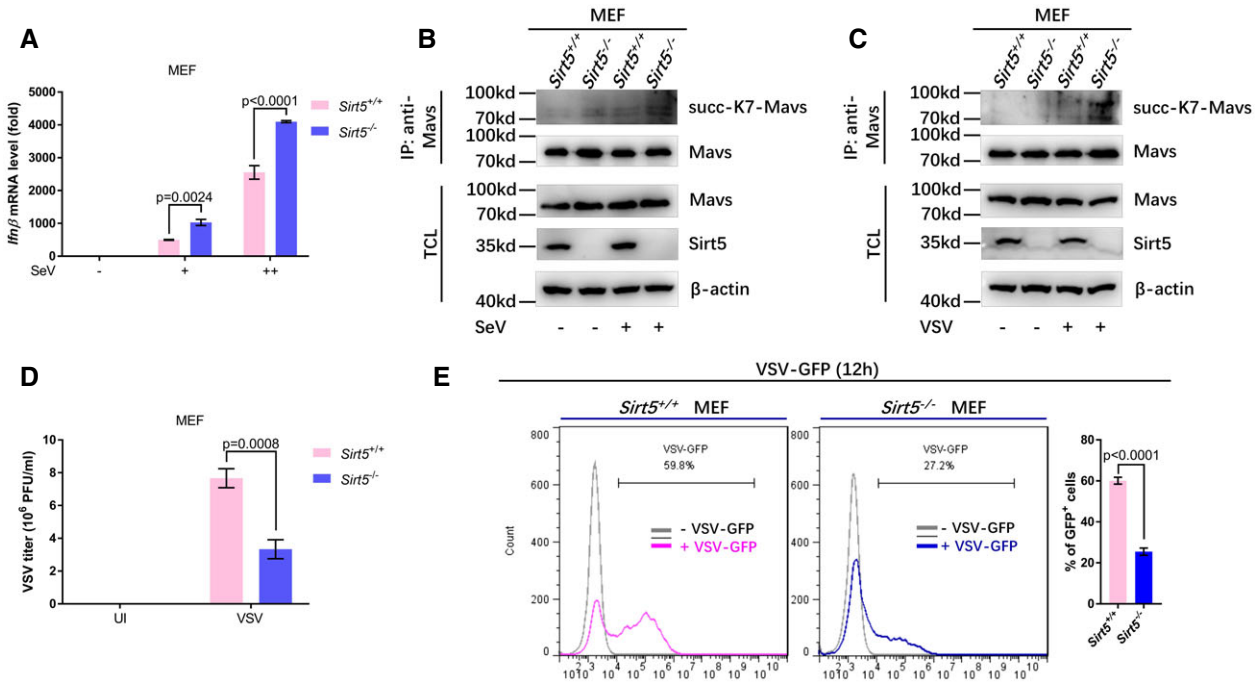

Figure EV4. *Sirt5* deficiency potentiates the host innate antiviral immune response.

A qPCR analysis of *Ifnβ* mRNA in WT (*Sirt5*$^{+/+}$) or Sirt5-deficient (*Sirt5*$^{-/-}$) MEF cells infected with an increasing titration of SeV (+, ++) for 8 h. −, without infection. The graphs represent fold induction relative to the uninfected cells.

B WT (*Sirt5*$^{+/+}$) or *Sirt5*-deficient (*Sirt5*$^{-/-}$) MEF cells were infected with (+) or without (−) SeV for 8 h, and the cell lysates were immunoprecipitated with anti-MAVS antibody, followed by immunoblotting with anti-succ-K7-MAVS antibody.

C WT (*Sirt5*$^{+/+}$) or *Sirt5*-deficient (*Sirt5*$^{-/-}$) MEF cells were infected with (+) or without (−) VSV for 8 h, and the cell lysates were immunoprecipitated with anti-MAVS antibody, followed by immunoblotting with anti-succ-K7-MAVS antibody.

D VSV titer was determined in WT (*Sirt5*$^{+/+}$) or *Sirt5*-deficient (*Sirt5*$^{-/-}$) MEF cells after infected with or without VSV for 12 h. UI, uninfected.

E WT (*Sirt5*$^{+/+}$) or *Sirt5*-deficient (*Sirt5*$^{-/-}$) MEF cells were infected with (+) or without (−) VSV-GFP viruses (relative lower titration) for 12 h, and viral infectivity was detected by flow cytometry analysis (n = 3).

Data information: All data are presented as the mean values based on three independent experiments, and error bars indicate s.e.m. (unpaired two-tailed Student's t-test).

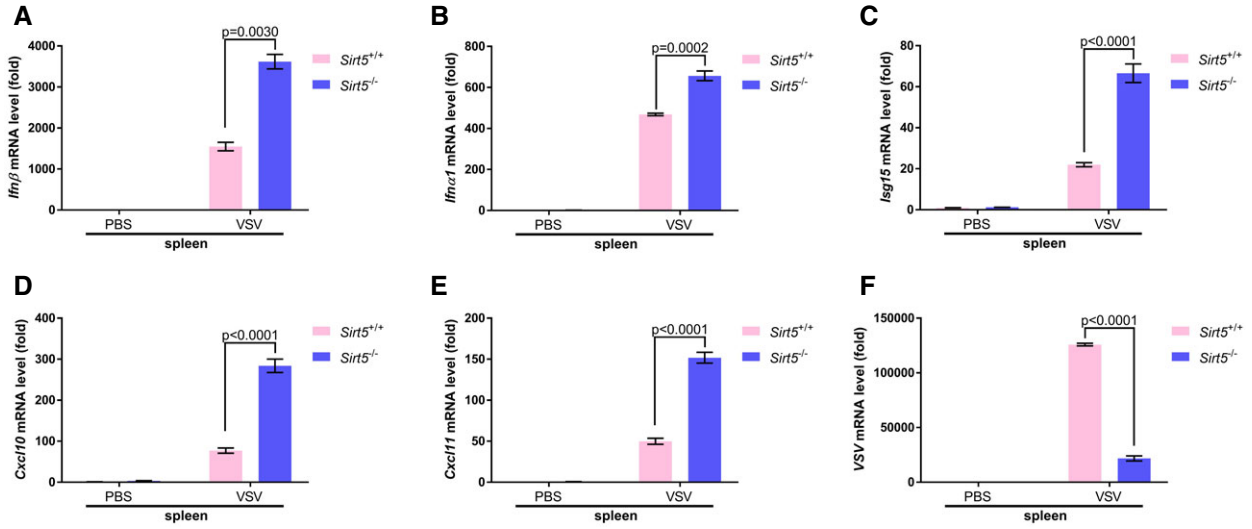

Figure EV5. *Sirt5* deficiency potentiates antiviral immune response in spleen.

A–E qPCR analysis of *Ifnβ* (A), *Ifnα1* (B), *Isg15* (C), *Cxcl10* (D), and *Cxcl11* (E) mRNA in the spleens of WT ($Sirt5^{+/+}$) or *Sirt5*-deficient ($Sirt5^{-/-}$) mice injected intraperitoneally with VSV (1×10^7 plaque-forming units (PFU) per mouse) or PBS control for 24 h.

F qPCR of VSV mRNA in the spleens of WT ($Sirt5^{+/+}$) or *Sirt5*-deficient ($Sirt5^{-/-}$) mice injected intraperitoneally with VSV (1×10^7 plaque-forming units (PFU) per mouse) or PBS control for 24 h.

Data information: PBS, phosphate-buffered saline. The graphs represent the fold induction relative to the untreated WT mice. All data are presented as the mean values based on three independent experiments, and error bars indicate s.e.m. (unpaired two-tailed Student's *t*-test).