

***SIRT5* impairs aggregation and activation of the signaling adaptor MAVS through catalyzing lysine desuccinylation**

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APPENDIX

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Appendix Figure S1. Western blot analysis for protein expression in Figure 1.

- A Western blot analysis for Figure 1A.
- B Western blot analysis for Figure 1B.
- C Western blot analysis for Figure 1C.
- D Western blot analysis for Figure 1D.
- E Western blot analysis for Figure 1E.
- F Western blot analysis for Figure 1F.
- G Western blot analysis for Figure 1G.
- H Western blot analysis for Figure 1H.
- I Western blot analysis for Figure 1I.
- J Western blot analysis for Figure 1J.
- K Western blot analysis for Figure 1K.
- L Western blot analysis for Figure 1L.
- M Western blot analysis for Figure 1M.
- N Western blot analysis for Figure 1N.
- O Western blot analysis for Figure 1O.

Appendix Figure S2. Dose-titrations of different stimuli.

- A Dose-dependent activation of IFN- β promoter activity by SeV infection was suppressed by transfection of Myc-SIRT5 (200 ng) in HEK293T cells.
- B Dose-dependent activation of ISRE reporter activity by transfection of increasing amount of Myc-MDA5 (100ng, 200 ng) was suppressed by co-transfection of Myc-SIRT5 (200 ng) in HEK293T cells.
- C Dose-dependent activation of ISRE reporter activity by transfection of Myc-IRF3 (100ng, 200 ng) was not suppressed by co-transfection of Myc-SIRT5 (200 ng) in HEK293T cells.
- D Western blot analysis for A.
- E Western blot analysis for B.
- F Western blot analysis for C.
- G Activation of ISRE reporter activity by transfection of HA-cGAS (100 ng) together with Flag-STING (100ng) was not suppressed by co-transfection of Myc-SIRT5 (200 ng) in HEK293T cells.

H Activation of ISRE reporter activity by transfection of HA-MAVS (200 ng) was not suppressed by co-transfection of Myc-SIRT2 (100ng or 200 ng) in HEK293T cells.

I Activation of IFN- β promoter activity by transfection of HA-MAVS (200 ng) was not suppressed by co-transfection of Myc-SIRT2 (100ng or 200 ng) in HEK293T cells.

J Western blot analysis for G.

K Western blot analysis for H.

L Western blot analysis for I.

Appendix Figure S3. Confocal microscopy images of endogenous SIRT5 and MAVS localization in H1299 cells and HeLa cells. Mito, Mitotracker. Scale bar = 8 μ m.

Appendix Figure S4 Confocal microscopy images of endogenous SIRT5 and MAVS localization in H1299 cells, Hela cells, *SIRT5*-deficient (*SIRT5*^{-/-}) or WT (*SIRT5*^{+/+}) H1299 cells, *MAVS*-deficient (*MAVS*^{-/-}) or WT (*MAVS*^{+/+}) H1299 cells . Scale bar = 8 μ m.

A Co-localization of SIRT5 and MAVS in H1299 cells.

B Co-localization of SIRT5 and MAVS in Hela cells.

C Localization of *SIRT5* in *SIRT5*-deficient (*SIRT5*^{-/-}) or WT (*SIRT5*^{+/+}) H1299 cells.

D Localization of *MAVS* in *MAVS*-deficient (*MAVS*^{-/-}) or WT (*MAVS*^{+/+}) H1299 cells.

Appendix Figure S5. *SIRT5* has no obvious effect on MAVS protein level in H1299 cells; the enzyme-deficient mutant of SIRT5 (SIRT5-H158Y) interacts with MAVS, and the K7 succinylation site mutant of MAVS (MAVS-K7R) interacts with SIRT5.

A Immunoblotting of endogenous MAVS expression in HEK293T cells transfected with an increasing amount of Myc-SIRT5 expression plasmid.

B Immunoblotting of endogenous MAVS expression in *SIRT5*-deficient or WT H1299 cells (*SIRT5*^{-/-} or *SIRT5*^{+/+}).

C Co-immunoprecipitation analysis (Co-IP) analysis of Flag-SIRT5 and HA-MAVS or HA-MAVS-K7R mutant. HEK293T cells were co-transfected with the indicated plasmids. Anti-HA antibody conjugated agarose beads were used for immunoprecipitation and interaction was detected by immunoblotting with the indicated

antibodies.

D Co-IP analysis of HA-MAVS and Flag-SIRT5 or Flag-SIRT5-H158Y mutant. HEK293T cells were co-transfected with indicated plasmids. Anti-Flag antibody conjugated agarose beads were used for immunoprecipitation and interaction was detected by immunoblotting with the indicated antibodies.

Appendix Figure S6. *Sirt5* deficiency potentiates the host antiviral immunity in response to poly (I:C) stimulation, but does not potentiate the host antiviral immunity in response to HSV-1 infection.

A-F qPCR analysis of *Ifnβ* (A), *Ifna1* (B), *Ifna4* (C), *Ifit1* (D), *Cxcl10* (E) and *Cxcl11* (F) mRNA in WT (*Sirt5*^{+/+}) or *Sirt5*-deficient (*Sirt5*^{-/-}) MEF cells transfected with or without poly(I:C) (poly I:C or UT) for 8 h. UT, un-transfected.

G, H qPCR analysis of *Ifnβ* (G), and *Ifit1* (H) mRNA in WT (*Sirt5*^{+/+}) or *Sirt5*-deficient (*Sirt5*^{-/-}) MEF cells infected with or without HSV-1 viruses (HSV-1 or UI) for 8h. UI, un-infected.

UI, uninfected; the graphs represent fold-induction relative to the uninfected cells. All data are presented as the mean values based on three independent experiments, and error bars indicate S.E.M.

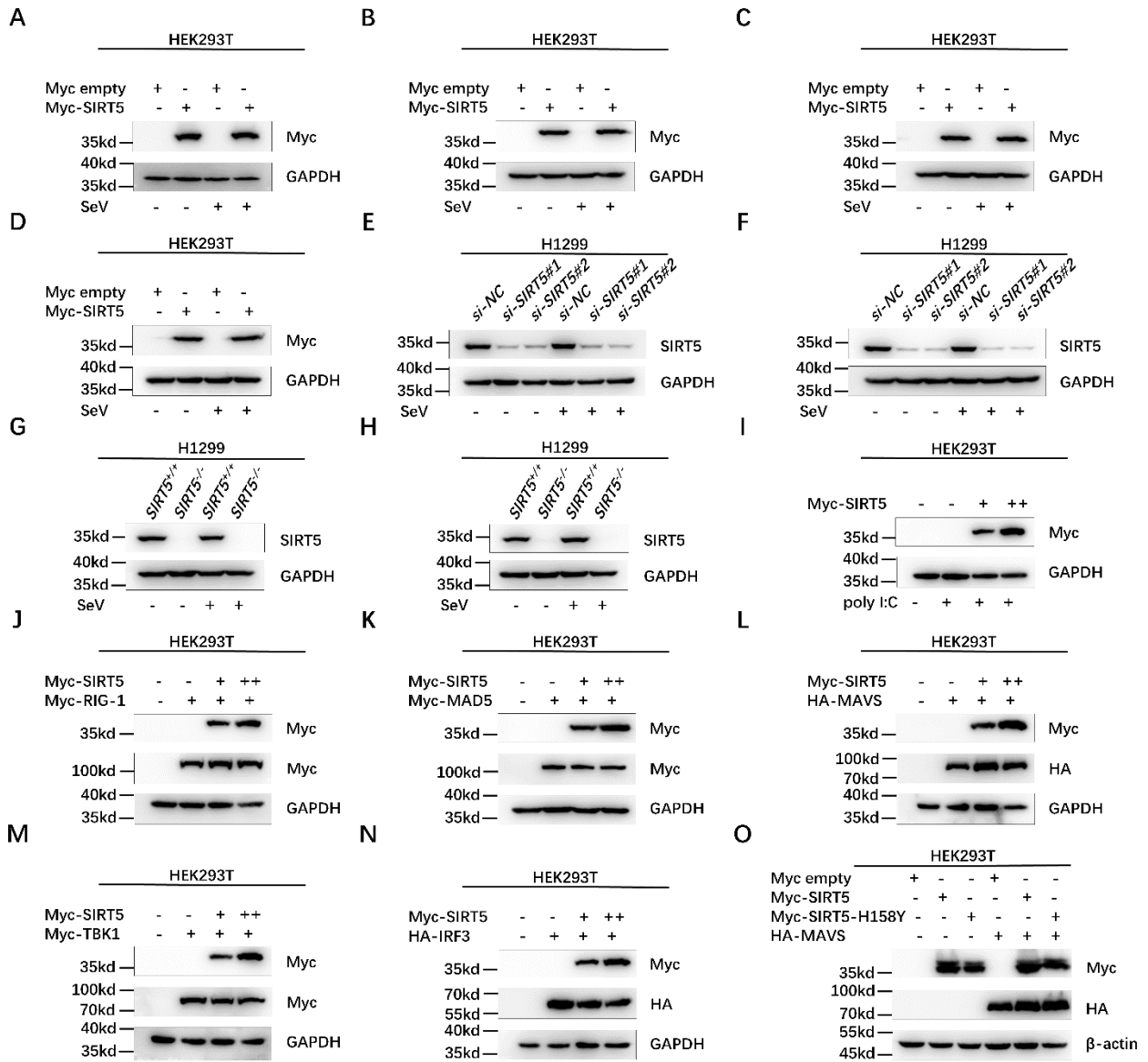
Appendix Figure S7. *Sirt5* deficiency potentiates antiviral immune response in bone marrow derived macrophage (BMDM) cells.

A-E qPCR analysis of *Ifnβ* (A), *Ifna1* (B), *Ifna4* (C), *Cxcl10* (D) and *Ccl5* (E) mRNA in WT (*Sirt5*^{+/+}) or *Sirt5*-deficient BMDM cells infected with or without SeV (Sev or UI) for 8 h.

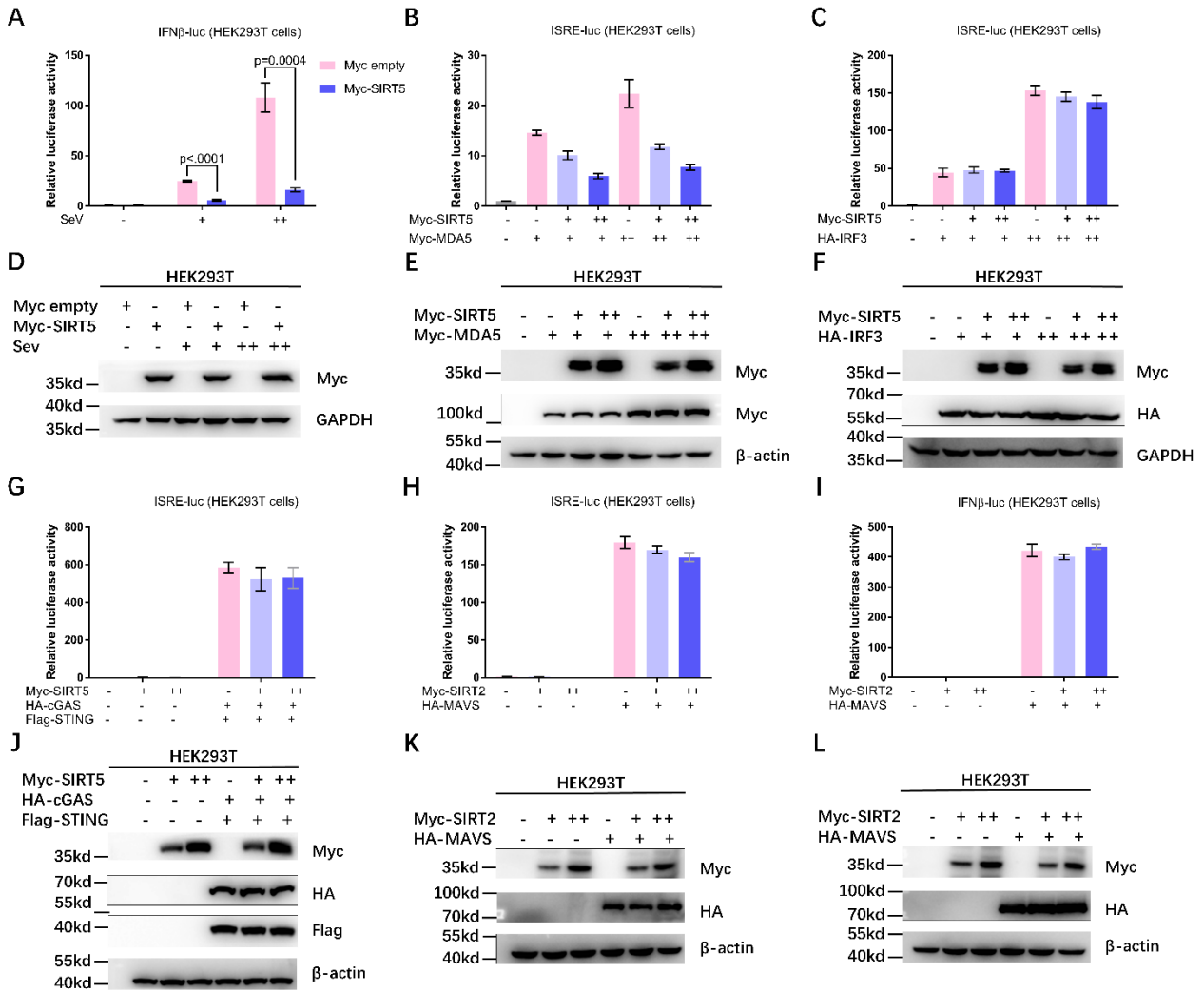
F-J qPCR of *Ifnβ* (F), *Ifna1* (G), *Ifna4* (H), *Cxcl10* (I), and *Cxcl11* (J) mRNA in WT (*Sirt5*^{+/+}) or *Sirt5*-deficient (*Sirt5*^{-/-}) BMDM cells infected with or without VSV for 8 h (VSV or UI).

UI, uninfected. The graphs represent the fold induction relative to the uninfected WT BMDM cells. All data are presented as the mean values based on three independent experiments, and error bars indicate S.E.M.

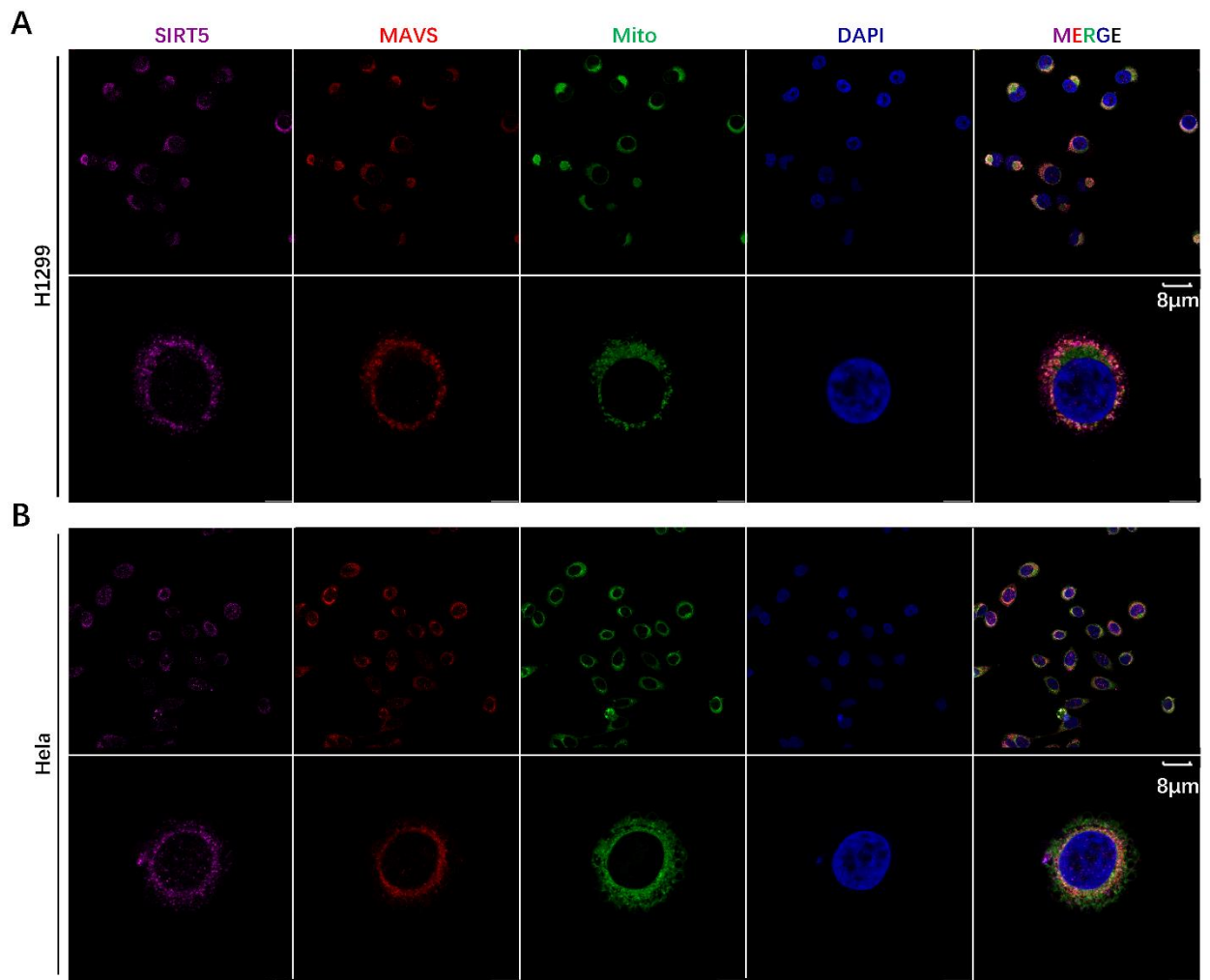
Appendix Figure S1



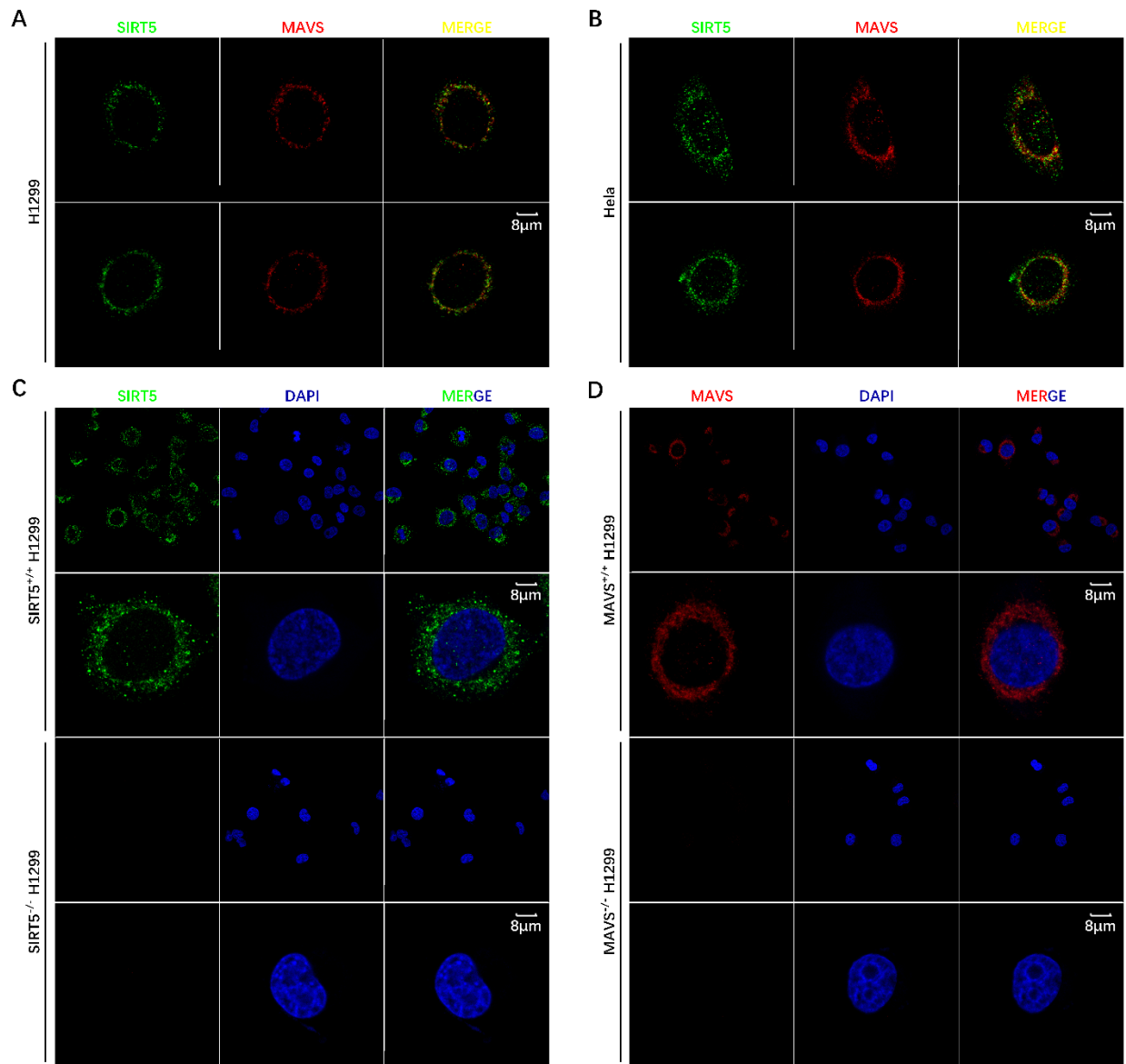
Appendix Figure S2



Appendix Figure S3

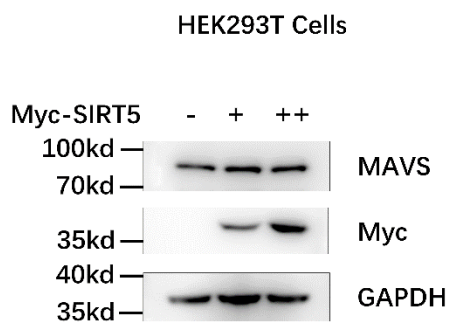


Appendix Figure S4

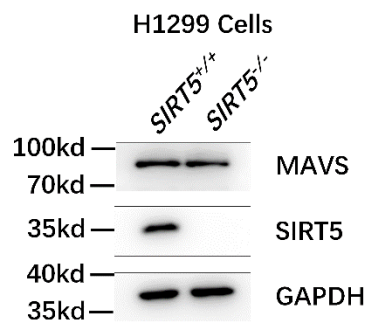


Appendix Figure S5

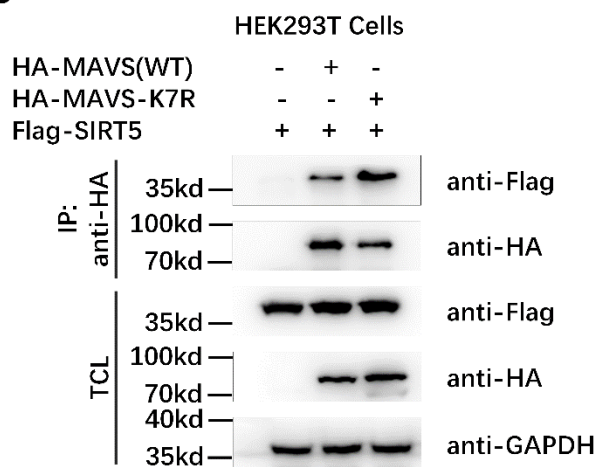
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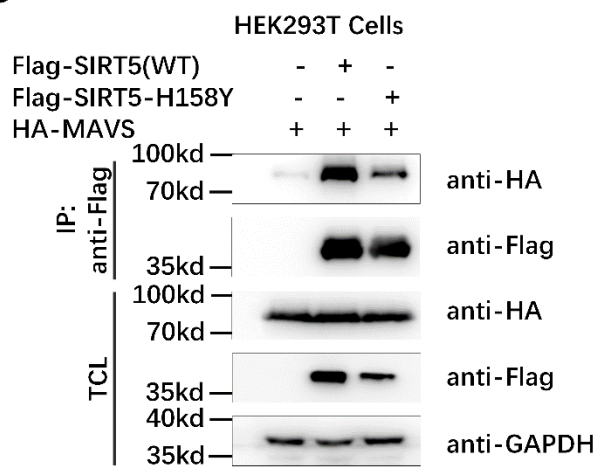
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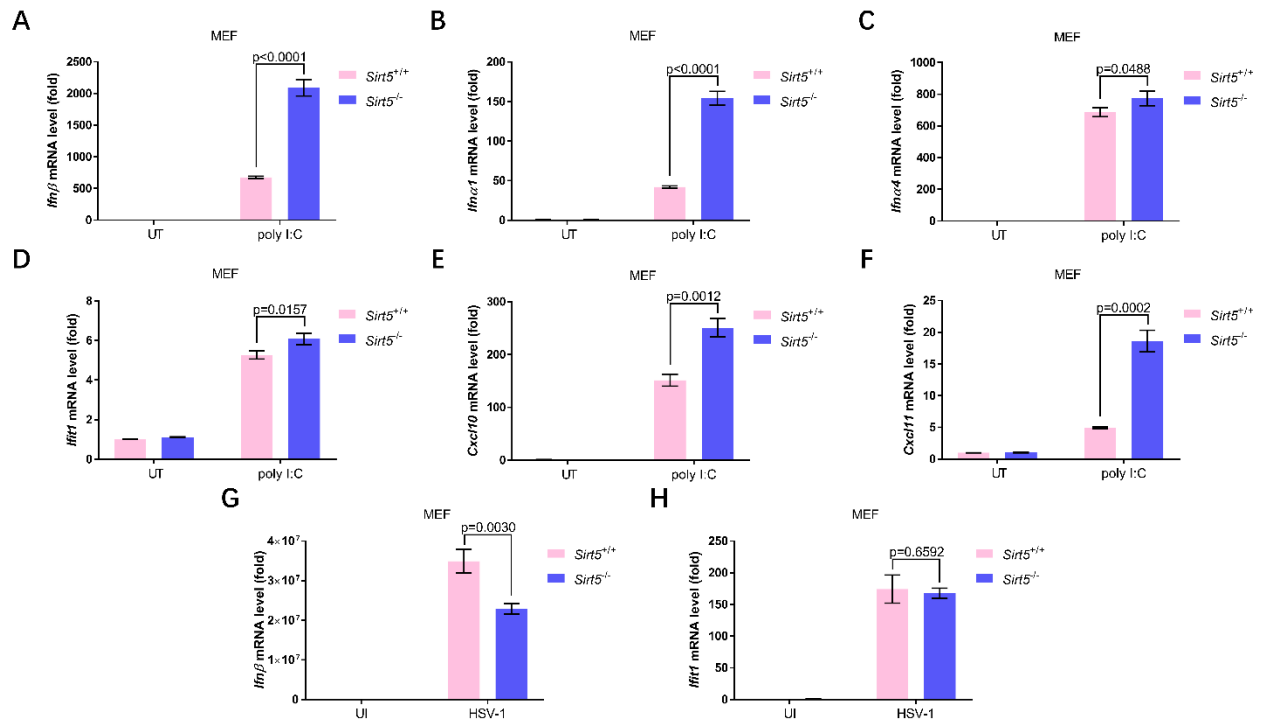
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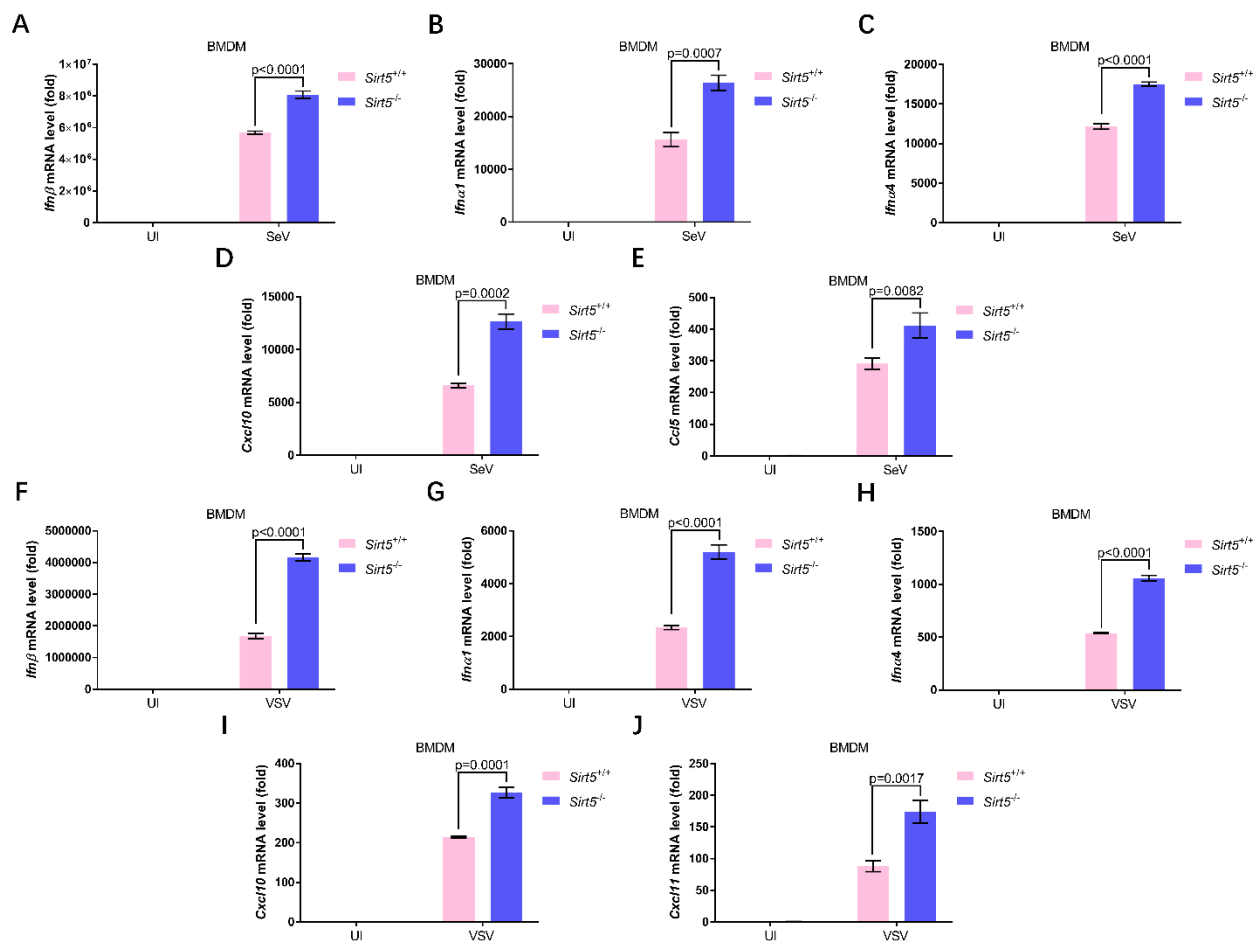
D



Appendix Figure S6



Appendix Figure S7



Appendix Table S1. The reagents and resources

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Mouse monoclonal anti-MAVS	Santa Cruz	Cat #sc-365333
Mouse monoclonal anti-MAVS	Santa Cruz	Cat #sc-166583
Mouse monoclonal anti-Myc	Santa Cruz	Cat #sc-40
Mouse monoclonal anti-GAPDH	Santa Cruz	Cat #sc-47724
Mouse monoclonal anti-HA	Covance	Cat #901515
Mouse monoclonal anti-Flag	Sigma-Aldrich	Cat #F1804
Rabbit polyclonal anti-SIRT5	Sigma-Aldrich	Cat #HPA022002
Rabbit polyclonal anti-SIRT5	Sigma-Aldrich	Cat #HPA021798
Rabbit polyclonal anti-SIRT5	Frdbio Bioscience & Technology	This paper
Rabbit polyclonal anti-succi-K7-MAVS	Abclonal	This paper
Rabbit polyclonal anti-pan-succinyl-K	PTM Company	Cat #PTM-401
Rabbit monoclonal anti-histone H3	Cell Signaling Technology	Cat #4499S
Alexa Fluor 594 goat anti-mouse IgG	ThermoFisher Scientific	Cat #A11005
Alexa Fluor 647 goat anti-rabbit IgG	ThermoFisher Scientific	Cat #A21245
Rabbit monoclonal anti-IRF3	Cell Signaling Technology	Cat #4302S
Rabbit monoclonal anti-Phospho-IRF3 (Ser396)	Cell Signaling Technology	Cat #29047S
Rabbit monoclonal anti-RIG-I	Cell Signaling Technology	Cat #3743S
Rabbit monoclonal anti-K63-linkage specific polyubiquitin	Cell Signaling Technology	Cat#5621S
Mouse monoclonal anti-GFP	Abclonal	Cat#AE012;
Rabbit monoclonal anti-TOM20	Abclonal	Cat# A19403
Rabbit polyconal anti-LMNB1	Abclonal	Cat# A16909
Bacterial and Virus Strains		
<i>DH5α</i>	Transgen Biotech	Cat #CD201
<i>Rosetta</i>	Transgen Biotech	Cat #CB108
<i>SeV</i>	Bo Zhong Lab	
<i>VSV-GFP</i>	Mingzhou Chen Lab	
<i>HSV-1</i>	Chunfu Zheng Lab	
Chemicals, Peptides, and Recombinant Proteins		
Poly I:C	Invivogen	Cat #tlrl-piclv
Recombinant Human GM-CSF	PeptoTech	Cat #315-03

Recombinant Human M-CSF	PeproTech	Cat #315-02
succinyl-CoA	Sigma-Aldrich	Cat #S1129
Critical Commercial Assays		
Anti-HA Ab-conjugated agarose beads	Sigma-Aldrich	Cat #A2095
Anti-Myc Ab-conjugated agarose beads	Sigma-Aldrich	Cat #A7470
Anti-Flag Ab-conjugated agarose beads	Sigma-Aldrich	Cat #A2220
Protein G Sepharose	GE HealthCare Company	Cat #17-0618-01
Dual-luciferase reporter assay system	Promega	Cat #E194A
Mouse Ifn- β ELISA kit	LEGEND MAX	Cat #439408
Succinate Assay kit	Abcam	Cat# ab204718
Experimental Models: Cell Lines		
HEK293T	ATCC	Cat #CRL-11268
H1299	Cell Bank of the Chinese Academy of Sciences	N/A
HCT116	Cell Bank of the Chinese Academy of Sciences	N/A
SIRT5-knockout H1299	This paper	N/A
SIRT5-knockout HCT116	This paper	N/A
MAVS-knockout H1299	This paper	N/A
Experimental Models: Organisms/Strains		
B6;129- <i>Sirt5^{tm1Fwa}</i>	The Jackson Laboratory	JAX stock 012757
Oligonucleotides		
siRNA targeting sequence: SIRT5#1: 5'-GCCCTTGAACATTTCCCAATG-3'	Genepharma	N/A
siRNA targeting sequence: SIRT5#2: 5'-GCATTAGAACTACAGACAAC-3'	Genepharma	N/A
Recombinant DNA		
Plasmid: pCMV-Myc	Clontek	Cat #K6003-1
Plasmid: pCMV-HA	Clontek	Cat #631604
Plasmid: pCMV-Flag	This paper	N/A
Plasmid: pCMV-Myc-SIRT5	This paper	N/A
Plasmid: pCMV-Myc-SIRT5-H158Y	This paper	N/A
Plasmid: pCMV-HA-SIRT5	This paper	N/A

Plasmid: pCMV-Flag-SIRT5	This paper	N/A
Plasmid: pCMV-Flag-SIRT5-H158Y	This paper	N/A
Plasmid: pCMV-HA-MAVS	This paper	N/A
Plasmid: pCMV-HA-MAVS-K7R	This paper	N/A
Plasmid: pCMV-Flag-MAVS	This paper	N/A
Plasmid: pCMV-Flag-MAVS- Δ C1	This paper	N/A
Plasmid: pCMV-Flag-MAVS- Δ C2	This paper	N/A
Plasmid: pCMV-Flag-MAVS- Δ N1	This paper	N/A
Plasmid: pCMV-Flag-MAVS- Δ N2	This paper	N/A
Plasmid: pCMV-Flag-MAVS- Δ CARD	This paper	N/A
Plasmid: pCMV-Flag-MAVS- Δ PR	This paper	N/A
Plasmid: pCMV-Flag-MAVS- Δ TM	This paper	N/A
Plasmid: pGEX-2T	GE Life Sciences	Cat #28-9546-53
Plasmid: pET-32 α	Novagen	Cat #69015-3
Plasmid: pGEX-2T-MAVS	This paper	N/A
Plasmid: pET-32 α -SIRT5	This paper	N/A
Plasmid: pCMV-Myc-MDA5	This paper	N/A
Plasmid: pCMV-Myc-RIG-I	This paper	N/A
Plasmid: pCMV-Myc-TBK1	This paper	N/A
Plasmid: pCMV-HA-IRF3	This paper	N/A
Plasmid: pCMV-HA-c-GAS	This paper	N/A
Plasmid: pCMV-Flag-STING-	This paper	N/A
Plasmid: pCMV-Myc-SIRT2	This paper	N/A
Plasmid: pCI-His-Ub	This paper	N/A
Plasmid: pCI-His-Ub-K63	This paper	N/A
Software and Algorithms		
ImageJ	Schneider et al., 2012	https://imagej.nih.gov/ij/
GraphPad Prism		https://www.graphpad.com/scientific-software/prism/
FlowJo		https://www.flowjo.com/

Appendix Table S2. The primer sequences

Primers	Sequence (5' to 3')
Human- <i>GAPDH</i> -RT-F	ATGACATCAAGAAGGTGGTG
Human- <i>GAPDH</i> -RT-R	CATACCAGGAAATGAGCTTG
Human- <i>IFN</i> β -RT-F	ACGCCGCATTGACCATCTATG
Human- <i>IFN</i> β -RT-R	CGGAGGTAACCTGTAAGTCTGT
Human- <i>CXCL10</i> -RT-F	GCTCTACTGAGGTGCTATGTTC
Human- <i>CXCL10</i> -RT-R	GGAGGATGGCAGTGGAAAGTC
Human- <i>IFIT1</i> -RT-F	CCTCCTTGGGTTCGTCTACA
Human- <i>IFIT1</i> -RT-R	GGCTGATATCTGGGTGCCTA
Human- <i>ISG15</i> -RT-F	GAGAGGCAGCGAACTCATCTT
Human- <i>ISG15</i> -RT-R	CCAGCATCTTCACCGTCAGG
Mouse- β - <i>actin</i> -RT-F	TGGAATCCTGTGGCATCCATGAAAC
Mouse- β - <i>actin</i> -RT-R	TAAAACGCAGCTCAGTAACAGTCCG
Mouse- <i>Ifn</i> β -RT-F	TACAACAGATACGCCTGGAT
Mouse- <i>Ifn</i> β -RT-R	AGTCCGCCTCTGATGCTTAA
Mouse- <i>Ifit1</i> -RT-F	CCAAGTGTTCCAATGCTCCT
Mouse- <i>Ifit1</i> -RT-R	GGATGGAATTGCCTGCTAGA
Mouse- <i>Cxcl10</i> -RT-F	CCAAGTGCTGCCGTCATTTT
Mouse- <i>Cxcl10</i> -RT-R	GATAGGCTCGCAGGGATGAT
Mouse- <i>Cxcl11</i> -RT-F	GTCATAGCCACACTCAAGAA
Mouse- <i>Cxcl11</i> -RT-R	AGACAGGTGCCATCAGAG
Mouse- <i>Ccl5</i> -RT-F	CCTCACCATATGGCTCGGAC
Mouse- <i>Ccl5</i> -RT-R	TCTTCTCTGGGTTGGCACAC
Mouse- <i>Ifna</i> -RT-F	CCTTCCACAGGATCACTGTGTACCT
Mouse- <i>Ifna</i> -RT-R	TTCTGCTCTGACCACCTCCC
Mouse- <i>Ifna4</i> -RT-F	AGGATCACTGTGTACCTGAGA
Mouse- <i>Ifna4</i> -RT-R	TCTCCACACTTTGTCTCAGGA
Mouse- <i>Isg15</i> -RT-F	CCTCTGAGCATCCTGGTGAG
Mouse- <i>Isg15</i> -RT-R	ACTGGTCTTCGTGGACTTGTT
Mouse- <i>Rig-I</i> -RT-F	ATTGTCGGCGTCCACAAAG
Mouse- <i>Rig-I</i> -RT-R	GTGCATCGTTGTATTTCGCA
Mouse- <i>Irf7</i> -RT-F	GAGACTGGCTATTGGGGGAG
Mouse- <i>Irf7</i> -RT-R	GACCGAAATGCTTCCAGGG