## 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- $\beta$  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 cotransfection of Myc-SIRT2 (100ng or 200 ng) in HEK293T cells.

I Activation of IFN- $\beta$  promoter activity by transfection of HA-MAVS (200 ng) was not suppressed by cotransfection 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 =  $8 \mu m$ .

Appendix Figure S4 Confocal microscopy images of endogenous SIRT5 and MAVS localization in H1299 cells, Hela cells, *SIRT5*-deficient (*SIRT5*<sup>-/-</sup>) or WT (*SIRT5*<sup>+/+</sup>) H1299 cells, *MAVS*-deficient (*MAVS*<sup>-/-</sup>) or WT (*MAVS*<sup>+/+</sup>) H1299 cells . Scale bar = 8  $\mu$ 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*<sup>-/-</sup>) or WT (*SIRT5*<sup>+/+</sup>) 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 enzymedeficient 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 cotransfected 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  $Ifn\beta$  (A),  $Ifn\alpha I$  (B),  $Ifn\alpha 4$  (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 *Ifn* $\beta$  (G), and *Ifit1* (H) mRNA in WT (*Sirt5*<sup>+/+</sup>) or *Sirt5*-deficient (*Sirt5*<sup>-/-</sup>) 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 *Ifn* $\beta$  (A), *Ifn* $\alpha$ 1 (B), *Ifn* $\alpha$ 4 (C), *Cxcl10* (D) and *Ccl5* (E) mRNA in WT (*Sirt5*<sup>+/+</sup>) or *Sirt5*-deficient BMDM cells infected with or without SeV (Sev or UI) for 8 h.

F-J qPCR of  $Ifn\beta$  (F),  $Ifn\alpha I$  (G),  $Ifn\alpha 4$  (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 S7** 



Appendix Table S1. The reagents and resources

Antibodies     Mouse monoclonal anti-MAVS   Santa Cruz   Cat #sc-365333     Mouse monoclonal anti-Myc   Santa Cruz   Cat #sc-40     Mouse monoclonal anti-Myc   Santa Cruz   Cat #sc-47724     Mouse monoclonal anti-GAPDH   Santa Cruz   Cat #sc-47724     Mouse monoclonal anti-Flag   Sigma-Aldrich   Cat #sc-47724     Mouse monoclonal anti-Flag   Sigma-Aldrich   Cat #HPA022002     Rabbit polyclonal anti-SIRT5   Sigma-Aldrich   Cat #HPA022002     Rabbit polyclonal anti-SIRT5   Sigma-Aldrich   Cat #HPA022002     Rabbit polyclonal anti-SIRT5   Sigma-Aldrich   Cat #HPA021798     Rabbit polyclonal anti-succi-K7-MAVS   Abclonal   This paper     Rabbit polyclonal anti-succi-K7-MAVS   Abclonal   This paper     Rabbit polyclonal anti-nouse IgG   ThermoFisher   Cat #A11005     Scientific   Scientific   Cat #A21245     Alexa Fluor 594 goat anti-rabbit IgG   ThermoFisher   Cat #A21245     Rabbit monoclonal anti-IRF3   Cell   Signaling   Cat #A21245     Scientific   Signaling   Cat #A302S   Technology     Rabbit monoclonal anti-RG-I   Cell   <	REAGENT or RESOURCE	SOURCE IDENTIFIER		
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HSV-1   Chunfu Zheng Lab     Chemicals, Peptides, and Recombinant Proteins	VSV-GFP	Mingzhou Chen Lab		
Chemicals, Peptides, and Recombinant Proteins   Poly I:C Invivogen Cat #tlrl-piclv   Recombinant Human GM-CSF PeproTech Cat #315-03	HSV-1	Chunfu Zheng Lab		
Chemicals, Peptides, and Recombinant ProteinsPoly I:CInvivogenCat #tlrl-piclvRecombinant Human GM-CSFPeproTechCat #315-03				
Poly I:CInvivogenCat #tlrl-piclvRecombinant Human GM-CSFPeproTechCat #315-03	Chemicals, Peptides, and Recombinant Proteins			
Recombinant Human GM-CSFPeproTechCat #315-03	Poly I:C	Invivogen	Cat #tlrl-piclv	
	Recombinant Human GM-CSF	PeproTech	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	Cat #17-0618-01
	Company	
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	N/A
	Chinese Academy of	
	Sciences	
HCT116	Cell Bank of the N/A	
	Chinese Academy of	
	Sciences	
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-Sirt5 <sup>tm1Fwa</sup>	The Jackson	JAX stock 012757
	Laboratory	
Oligonucleotides		
siRNA targeting sequence: SIRT5#1:	Genepharma	N/A
5'-GCCCTTGAACATTTCCCAATG -3'		
siRNA targeting sequence: SIRT5#2:	Genepharma	N/A
5'-GCATTAGAACTACAGACAAC-3'		
Recombinant DNA		
Plasmid: pCMV-Myc	Clonteck	Cat #K6003-1
Plasmid: pCMV-HA	Clonteck	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-∆C1	This paper	N/A
Plasmid: pCMV-Flag-MAVS-∆C2	This paper	N/A
Plasmid: pCMV-Flag-MAVS-∆N1	This paper	N/A
Plasmid: pCMV-Flag-MAVS-∆N2	This paper	N/A
Plasmid: pCMV-Flag-MAVS-∆CARD	This paper	N/A
Plasmid: pCMV-Flag-MAVS-∆PR	This paper	N/A
Plasmid: pCMV-Flag-MAVS-∆TM	This paper	N/A
Plasmid: pGEX-2T	GE Life Sciences	Cat #28-9546-53
Plasmid: pET-32a	Novagen	Cat #69015-3
Plasmid: pGEX-2T-MAVS	This paper	N/A
Plasmid: pET-32α-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/

A	ppendix	Table S2.	The	primer	sequences
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Primers	Sequence (5' to 3')
Human-GAPDH-RT-F	ATGACATCAAGAAGGTGGTG
Human-GAPDH-RT-R	CATACCAGGAAATGAGCTTG
Human- <i>IFNβ</i> -RT-F	ACGCCGCATTGACCATCTATG
Human- <i>IFNβ</i> -RT-R	CGGAGGTAACCTGTAAGTCTGT
Human-CXCL10-RT-F	GCTCTACTGAGGTGCTATGTTC
Human-CXCL10-RT-R	GGAGGATGGCAGTGGAAGTC
Human-IFIT1-RT-F	CCTCCTTGGGTTCGTCTACA
Human-IFIT1-RT-R	GGCTGATATCTGGGTGCCTA
Human-ISG15-RT-F	GAGAGGCAGCGAACTCATCTT
Human-ISG15-RT-R	CCAGCATCTTCACCGTCAGG
Mouse- <i>β-actin</i> -RT-F	TGGAATCCTGTGGCATCCATGAAAC
Mouse-β-actin-RT-R	TAAAACGCAGCTCAGTAACAGTCCG
Mouse- <i>Ifnβ</i> -RT-F	TACAACAGATACGCCTGGAT
Mouse- <i>Ifnβ</i> -RT-R	AGTCCGCCTCTGATGCTTAA
Mouse-Ifit1-RT-F	CCAAGTGTTCCAATGCTCCT
Mouse-Ifit1-RT-R	GGATGGAATTGCCTGCTAGA
Mouse-Cxcl10-RT-F	CCAAGTGCTGCCGTCATTTT
Mouse-Cxcl10-RT-R	GATAGGCTCGCAGGGATGAT
Mouse-Cxcl11-RT-F	GTCATAGCCACACTCAAGAA
Mouse-Cxcl11-RT-R	AGACAGGTGCCATCAGAG
Mouse-Ccl5-RT-F	CCTCACCATATGGCTCGGAC
Mouse-Ccl5-RT-R	TCTTCTCTGGGTTGGCACAC
Mouse-Ifna-RT-F	CCTTCCACAGGATCACTGTGTACCT
Mouse-Ifna-RT-R	TTCTGCTCTGACCACCTCCC
Mouse- <i>Ifnα4</i> -RT-F	AGGATCACTGTGTACCTGAGA
Mouse-Ifna4-RT-R	TCTCCACACTTTGTCTCAGGA
Mouse-Isg15-RT-F	CCTCTGAGCATCCTGGTGAG
Mouse-Isg15-RT-R	ACTGGTCTTCGTGGACTTGTT
Mouse-Rig-I-RT-F	ATTGTCGGCGTCCACAAAG
Mouse- <i>Rig-I</i> -RT-R	GTGCATCGTTGTATTTCCGCA
Mouse-Irf7-RT-F	GAGACTGGCTATTGGGGGGAG
Mouse-Irf7-RT-R	GACCGAAATGCTTCCAGGG