Demalonylation of DDX3 by Sirtuin 5 promotes antiviral innate

immune responses

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Supplementary Figure 1. (A) Q-PCR analysis of knockdown efficiency in BMDMs transfected with siRNA specific for Sirt1-7 for 48h. The results are normalized to *Gapdh* expression. (B) Immunoblot analysis of BMDMs transfected with indicated Si-RNA for 48 h and then infected with VSV for 8h with indicated antibodies. Data are presented as mean \pm standard deviation (SD) of three independent experiments. (* *P*<0.05)

sFig. 2



Supplementary Figure 2. ELISA of TNF α and IL-6 production in supernatants from BMDMs transfected with siRNA specific for Sirt5 for 48h and then infected with VSV for indicate time. Data are presented as mean \pm standard deviation (SD) of three independent experiments. (* *P*<0.05)

sFig. 3



Supplementary Figure 3. (A), ELISA of IFN- β production in supernatants from *Sirt5*^{+/-} or *Sir5*^{-/-} BMDMs infected with VSV, SeV and HSV with indicated time. (B) Cytotoxicity was measured by subtracting LDH content in supernatants from *Sirt5*^{+/-} or *Sir5*^{-/-} BMDMs infected with indicated viruses compared to total LDH in untreated controls. (C) Q-PCR of IFN- α and IFN- γ mRNA expression in *Sirt5*^{+/-} or *Sir5*^{-/-} BMDMs infected with VSV, SeV and HSV for 8 hours. Data are presented as mean ± standard deviation (SD) of three independent experiments. (* *P*<0.05)



Supplementary Figure 4. ELISA of TNF α , IL-6 and IFN- β production in supernatants from *Sirt5*^{+/-} or *Sir5*^{-/-} peritoneal macrophages, bone marrow stroma cells and DCs stimulated with ssRNA40/Lyo VecTM (1 µg/ml) for 12 hours. Data

are presented as mean \pm standard deviation (SD) of three independent experiments.



Supplementary Figure 5. Immunoblot analysis of $Sirt5^{+/-}$ or $Sir5^{-/-}$ BMDMs infected with HSV for indicated time and with indicated antibodies. Data are representative of three independent experiments.



Supplementary Figure 6. Mass-Spectro analysis of Sirt5 interacting proteins. Raw264.7 cells overexpressed with Flag-tagged Sirt5 or empty vector were infected with VSV for 6h. The Flag-tagged SIRT5 was immunoprecipitated and with anti-Flag antibody and loaded on SDS-page. After sliver staining, the indicated bands were analyzed by Mass-Spectro and the peptides gained from band 3 were marked in red color in the sequence of DDX3.

supplementaryTable1.MASS-SPECTROdata

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prot_hit_nıprot_acc	prot_desc	prot_score	prot_m ass	prot_m atcl	prot <u>m</u> atcl
9 Q 62167	ATP-depe	775	73455	38	20
9 Q 62167	ATP-depe	775	73455	38	20
9 Q 62167	ATP-depe	775	73455	38	20
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