Supplementary Information for
Tankyrases inhibit innate antiviral response by PARylating VISA/MAVS and
priming it for RNF146-mediated ubiquitination and degradation

Yan-Ran Xu\textsuperscript{a,b,c}, Meng-Ling Shi\textsuperscript{a,b,c#}, Yu Zhang\textsuperscript{d#}, Na Kong\textsuperscript{a,b,c}, Cong Wang\textsuperscript{a,b,c},
Yi-Feng Xiao\textsuperscript{a,b}, Shi-Shen Du\textsuperscript{b}, Qi-Yun Zhu\textsuperscript{c}, Cao-Qi Lei\textsuperscript{a,b,c*}

*Corresponding author. Dr. Cao-Qi Lei
Email: caoqilei@whu.edu.cn

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Ethics Statement and mice

The animal care and use were adhered to the Chinese National Guidelines for Ethical Review of Animal Welfare. The protocols and procedures for mice experiments in this study were approved by the College of Life Sciences Animal Care and Use Committee of Wuhan University.

*Tnks1* gene-knockout mice were kindly provided by Dr. Richard J. Hodes (NIH, USA) and Dr. Yeguang Chen (Tsinghua University, China) (1, 2). Genotyping was performed by PCR using the following pairs of primers suggested by Dr. Richard J. Hodes:

TNK1E2F-GAAGAGACAAACCAAGCGG
TNK1E2R-ACACACACAAACGACAATGAG
TNK1SR-GTCTCTCTCTGCCCCCTTATC
LoxP-TGGCTGGACGTAAACTCCTCTTCAGACCTAATAAC

Amplification of the WT allele with primer pair TNK1E2F/2R resulted in a 300-bp fragment, whereas amplification of the disrupted allele with primer pair TNK1S2R/LoxP resulted in a 200-bp fragment.

*Tnks2*^fl/+^ mice were also provided by Dr. Richard J. Hodes (NIH, USA) and Dr. Yeguang Chen (Tsinghua University, China) (1, 2). Cre-ER mice (B6.129-Gt (ROSA)26Sortm1(cre/ERT2)Tyj) were originally from the Jackson Laboratory and were kindly provided by Dr. Bo Zhong (Wuhan University, China). *Tnks2*^fl/+^ mice were crossed with Cre-ER mice to obtain *Tnks2*^fl/fl:Cre-ER^ mice.
Genotyping was performed by PCR using the following pairs of primers suggested by Dr. Richard J. Hodes:

TNK2F1-TCTCCTAACCCCTTTCTCCC
TNK2R1-GCATACACATCAAAGTTTTCCG
LoxF-GACGTAAACTCCTCTTCAGACCTAATAAC

Amplification with primer pair TNK2F1/R1/LoxF, resulted in a 300-bp fragment corresponds to the wild-type allele, and a 250-bp fragment corresponds to the conditional knockout alleles.

To achieve TNKS2-deficient mice, 8 week-old Tnks2\(^{fl/fl}\) and Tnks2\(^{fl/fl}:Cre-ER\) mice were injected intraperitoneally with tamoxifen (80 μg/g body weight, dissolved in corn oil) for a total of five consecutive days. After 7 days without treatment, mice were either subjected to euthanasia to test the knockout efficiency or infected with EMCV. To knockout TNKS2 in cultured cells, Tnks2\(^{fl/fl}\) and Tnks2\(^{fl/fl}:Cre-ER\) cells were treated with 4-OHT (1 μM) for three days. Cells were then re-seeded into culture dishes or plated in 4-OHT free medium and rested for 24 h followed by infection with SeV or EMCV.

**Preparation of BMDMs and BMDCs**

Bone marrow cells were isolated from tibiae and femur of mice, and were then cultured in RPMI 1640 medium supplemented with 10% FBS and recombinant murine M-CSF or GM-CSF-containing conditional medium for 5 or 9 days for generation of BMDMs or BMDCs, respectively.

**Reagents**
RNAiso Plus and Taq DNA polymerase (9109, Takara Bio); Lipofectamine 2000 (11668019, InvivoGen); HiScript® II Q RT SuperMix for qPCR (R222-01, Vazyme); SYBR qPCR Master Mix (MQ10101,Monad); Dual-specific luciferase assay kit (E1980, Promega); Polybrene (TR-1003-G, Millipore); Poly(I:C) (tlrl-pic-5, Invivogen); Z-link Psoralen-PEG3-Biotin (29986, Thermo); GammaBind G Plus-Sepharose (17-0618-05, GE Healthcare); β-Nicotinamide adenine dinucleotide (NAD+)(B9007S, New England Biolabs); Tamoxifen (#T5648, Sigma); 4-hydroxytamoxifen (H6278, Sigma); ELISA kits for murine IFN-β (CE0116, 4A Biotech.), MCP1 (CME0046, 4A Biotech.) and IL6 (CME0006, 4A Biotech.); Recombinant human IFN-γ (300-02-100, Peprotech); Recombinant human IFN-β (300-02BC-50, Peprotech); Mouse monoclonal antibodies for Flag (M2) (F3165, Sigma; RRID: AB_259529); β-actin (A2228, Sigma; RRID: AB_476697); Hemagglutinin (HA) (CB051, Origene; RRID: AB_2622290); p-IRF3 (4947, Cell Signaling Technology; RRID: AB_823547); IRF3 (sc-33641, Santa Cruz Biotechnology; RRID: AB_627826); p-TBK1 (ab109272, Abcam; RRID: AB_10862438); TBK1 (ab40676, Abcam; RRID: 776632); Myc (AE010, ABclonal Tech.; RRID:AB_2770408); K48-linkage specific polyubiquitin (ab140601, Abcam; RRID:AB_2783797); K63-linkage specific polyubiquitin (ab197434, Abcam); Rabbit anti-TNKS antisera were kindly provided by Junjie Chen (MD Anderson Cancer Center, USA); Anti-PAR polymer monoclonal antibody (4335-MC-100, Trevigen; RRID:AB_2572318); Anti-RNF146 (ab201212, Abcam; or PA5-55544, Invitrogen; RRID:AB_2646631); Anti-VISA (sc-166583 or sc-365333, Santa Cruz Biotechnology; RRID:AB_2012300 or AB_10844335); Anti-AIF (sc-13116, Santa Cruz Biotechnology; RRID:AB_626654); Anti-TOM20 (sc-11415, Santa Cruz Biotechnology); Anti-α-tubulin (T6199, Sigma; RRID:AB_2207533); Alexa Fluor 488-conjugated goat anti-mouse IgG (A-10680, Invitrogen; RRID:AB_2534062) and Alexa Fluor 594-conjugated goat anti-
rabbit IgG (A-11012, Invitrogen; RRID:AB_141359); HEK293 and MLF cells (China Center for Type Culture Collection, CCTCC) were purchased from the indicated companies. Influenza virus A/Puerto Rico/8/1934(H1N1) (PR8) was provided by Dr. Qiyun Zhu (Lanzhou Veterinary Research Institute); SeV (Cantell strain), EMCV (BJC3 Strain) and VSV-GFP (Indiana Strain) were previously described (3, 4).

**Constructs**

SFB-tagged TNKS1, TNKS2, RNF146 were obtained from Dr. Junjie Chen (MD Anderson Cancer Center, USA); HA-, Flag- or Myc-tagged TNKS1, TNKS2, RNF146 and their mutants were constructed in the pRK5 vector by standard molecular biology techniques. pRK vector-based mammalian expression plasmids for Flag- or HA-tagged VISA and its mutants, HA-tagged RIG-I, MDA5, cGAS, MITA and TBK1, and the IFN-β promoter reporter plasmid were previously described (5, 6).

**RNAi experiments**

Double-strand oligonucleotides corresponding to the target sequences were cloned into the pSuper-Retro RNAi plasmid (Oligoengine Inc.). In this study, the following sequences were targeted for the indicated mRNA.

Human TNKS1 mRNA:

#1-GCGGCAAACGTAAATGCAA
#2-GCATGAAGCTGCTATTAAA
#3-GCGAAAGTCGACTCCTTTA

Human TNKS2 mRNA:
CRISPR-Cas9-mediated gene knockout

Genome engineering was performed using the CRISPR-Cas9 system (7, 8). Double-stranded oligonucleotides corresponding to the target sequences were cloned into the lenti-CRISPR-V2 vector and co-transfected with psPAX2 and pMD2.G into HEK293 cells. Two days after transfection, the viruses were harvested and used to infect HEK293 cells or MLFs. The infected cells were selected with puromycin (1 μg/mL) for at least 7 days to obtain TNKS1-, TNKS2- or RNF146-KO cell pools.

The following sequences were targeted for the indicated DNA:

human TNKS1:
#1- CTCCGCCAAGCTCGATCCAG
#2- CGCGGCAAACGTAAATGCAA

human TNKS2:
#1- CACTTCGCCGCAGGTAACCG
#2- CGTGGAACGAGTCAAGAGGC

murine TNKS1:
#1- CTTAGCGGAGAGTCCCGAGG
#2- CAGGGGAGAAGACTTTCGGC

murine TNKS2:
#1- GGTGAACAGCCGCGACACGG
#2- AGCCTCGAACAGCTCGCGGG

human RNF146:
#1-GTCAGATCTAGAAGGCCTGA
#2-CCCGATCGATCAGATCGATC
#3-GGCTTGTCAAGGAAATCCTC

**Tandem affinity purification of SFB-tagged protein complexes**

We firstly made a SFB-tagged (S protein tag, Flag tag and streptavidin-binding tag) VISA construct. VISA cDNA was generated by PCR and subcloned into pDONOR201 vector using Gateway Technology (Invitrogen, Carlsbad, CA) as the entry clone. Then the entry clone was subsequently recombined into lentiviral-gateway-compatible destination vector for the expression of C-terminal triple SFB-tagged fusion VISA. HEK293 cells were transfected with SFB-VISA construct. Twenty-four hours later, the cells were selected by culturing in medium containing puromycin (2 µg/mL), and the protein expression was confirmed by immunostaining and Western blotting. For affinity purification, HEK293 cells stabling expressing SFB-VISA were subjected to lysis in NETN lysis buffer (100 mM NaCl, 20 mM Tris-HCl, 0.5 mM EDTA, 0.5 % Nonidet P-40) with protease and phosphatase inhibitors at 4 °C for 20 min. The cell lysate were subjected to centrifugation at 4 °C and 14,000 rpm for 15 min. Supernatants were incubated with streptavidin-conjugated beads for 1 h at 4 °C. The beads were washed three times with NETN buffer, and bounded proteins were eluted with NETN buffer containing 2 mg/mL biotin nearly 90 min at 4 °C. The eluates were incubated with S protein beads for 1 h. The beads were then washed three times with NETN buffer and subjected to SDS-PAGE. Protein bands were excised and subjected to mass spectrometry analysis.
**Confocal microscopy**

HEK293 cells were transfected with the indicated plasmids for 24 h, and then fixed with 4% paraformaldehyde for 10 min, and permeabilized with 0.1% Triton X-100 in PBS for 15 min. The cells were blocked with 1% BSA in PBS for 1 h. Cells were then incubated with primary antibodies overnight at 4 °C. Alexa Fluor 488- and 546-conjugated secondary antibodies were incubated for 1 h. The nuclei were stained with DAPI for 2 min before images were acquired using a ZEISS confocal microscope with a 60× objective.

**Coimmunoprecipitation and immunoblot analysis**

Cells were lysed in 1 mL NP-40 lysis buffer (20 mM Tris-HCl, pH 7.4-7.5, 150 mM NaCl, 1 mM EDTA, 1% NP-40, 10 µg/mL aprotinin, 10 µg/mL leupeptin and 1 mM phenylmethylsulfonyl fluoride). The lysate were centrifuged at 12,000 rpm for 10 min at 4 °C. For each immunoprecipitation, the supernatant was incubated with 0.5 µg of the indicated antibody and 35 µl of 50% slurry of GammaBind G Plus-Sepharose at 4 °C for 2 h. The beads were then washed for three times with 1 mL lysis buffer containing 500 mM NaCl. The bound proteins were separated by SDS-PAGE and the associated proteins were analyzed by immunoblot analysis.

**Detection of ubiquitin-modified proteins**

The experiments were performed as previously described (3). Briefly, the cells were lysed in lysis buffer containing 1% SDS and denatured by heating at 95°C for 10 min. After centrifugation, the supernatants were diluted with NP-40 lysis buffer until the concentration of
SDS was decreased to 0.1%, followed by co-immunoprecipitation with the indicated antibodies. Ubiquitin-modified proteins were detected by immunoblots with the indicated antibodies.

**Subcellular fractionation**

Cell fractionation experiments were performed as previously described (6). In brief, cells were left un-infected or infected with SeV for the indicated times, the cells were then washed with PBS and lysed by douncing 40 times in 1 mL of homogenization buffer (10 mmol/L Tris-HCl, pH 7.4, 2 mmol/L MgCl₂, 10 mmol/L KCl, 250 mmol/L sucrose). The homogenized samples were centrifuged at 500 × g for 10 min. The supernatants were centrifuged at 5000 × g for 10 min to precipitate crude mitochondria (P5K) and cytosol fractions (S5K).

**Transfection and reporter assays**

The cells were transfected by standard calcium phosphate precipitation. To normalize for transfection efficiencies, pRL-TK (Renilla luciferase) reporter plasmid (0.01 μg) was added to each transfection. Empty control plasmid was added to ensure that each transfection received the same amount of total plasmid DNA. Twenty hours after transfection, cells were treated or left un-treated with the indicated stimuli before luciferase assays were performed using a dual-specific luciferase assay kit. Firefly luciferase activities were normalized based on Renilla luciferase activities.

**RT-qPCR analysis**

Total RNA was isolated for RT-qPCR analysis to measure mRNA levels of the indicated genes according to the manufacturer’s protocol of RNAiso Plus (TaKaRa). Data shown are the relative
abundance of the indicated mRNA derived from human or mouse cells normalized to that of 
*GAPDH* or *Gapdh*. The sequences of qPCR primers were as following:

**Human *GAPDH***: Forward-GAGTCAACGGATTTGGTCGT  
Reverse-GACAAGCTTCCCGTTCTCAG  
**Human *IFNB1***: Forward-CATTACCTGAAGGCAAGGA  
Reverse-CAATTGTCCAGTCCAGAGGG  
**Human *ISG56***: Forward-TCATCAGGCAAGGATAGTC  
Reverse-CCACACTGTATTTGGGTCTAGGG  
**Human *IL6***: Forward-TTCTCCCAACGGCCCTTCGGTC  
Reverse-TCTGTGTGGGGCGGCTACATCT  
**Human *IRF1***: Forward-GAGGAGGGTGAAGACACGAGCA  
Reverse-TAGCATCTCGGCTGGACTTCA  
**Human *GBP2***: Forward-GTTCCCTACATCCCTGACCATTCC  
Reverse-CCACTGCTGATGGCATTGACGT  
**Human *STAT1***: Forward-ATGGCAGTCTGGCGGCTGAATT  
Reverse-CCAAAACCAGGGCGGCTGAATT  
**Human *TNKS1***: Forward-AGAGTACCTGCTTGGCGGCTGAATT  
Reverse-AGTCCGCCACATTGACAGCAGG  
**Human *TNKS2***: Forward-GTGATTGCCAAGACACAGAGG  
Reverse-GGTGTGAAAGCCCATTTGTCC  
**Human *RNF146***: Forward-ACGGCCCGCATCTTCTTTGCA  
**Murine *Gapdh***: Forward-ACGGCCCGCATCTTCTTTGCA
Reverse-ACGGCCAAATCCGTTCACACC
Murine *Isg56*: Forward-ACAGCAACCATGGGAGAGAATGCTG
Reverse-ACGTAGGCCAGGGTTGTGCAT
Murine *Il6*: Forward-TCTGCAAGAGACTTCCATCCAGTTGC
Reverse-AGCCTCCGACTTGTGAAGTGGT
Murine *Ifb1*: Forward-CCCTATGGAGATGACGGAGA
Reverse-CTGTCTGCTGGTGAGGTTCA
Murine *Irf1*: Forward-TCCAAGTCCAGCCGAGACACTA
Reverse-ACTGCTGTGGTCATCAGGTAGG
Murine *Gbp2*: Forward-AGATGCCCACAGAAACCCTCCA
Reverse-AAGGCATCTCGCTTGGCCTACCA
Murine *Stat1*: Forward-GCCTCTCATTGTCACCGAAGAAC
Reverse-TGGCTGACGTTGGAGATCACCA
Murine *Tnks1*: Forward-GTCTACTCCGTTACACCTGGC
Reverse-TGAAGAGGTACAAGTCCACCTTT
Murine *Tnks2*: Forward-CGCCCAAGAGGTGAACAG
Reverse-TTTGCAACGTTCATCCAAG
EMCV: Forward-TCTTGGCCGCTTTGTCTAGA
Reverse-TGGCTTGGTCTCGACTAGTG

*In vitro* PARylation assay

Samples containing TNKS1/2 or their mutants (purified from HEK293 cells) and 1 μg recombinant *Escherichia coli*-derived His-VISA (provided by Dr. Qiyun Zhu, Lanzhou
Veterinary Research Institute) were incubated in 50 μL PARP reaction buffer (50 mM Tris-HCl, pH 8.0, 4 mM MgCl₂, 0.2 mM dithiothreitol) with 25 μM NAD⁺ at 25 °C for 30 min. Reactions were terminated by the addition of 2× SDS loading buffer and the samples were further subjected to immunoblot analysis (9).

**EMCV or PR8 infection**

Age- and sex-matched TNKS1- or TNKS2-deficient mice and wild-type mice were infected with EMCV through intraperitoneal route or PR8 through intranasal inhalation. The survival of the infected mice was monitored every 12 h. The blood was collected for ELISA measurement of cytokine levels after EMCV or PR8 infection for the indicated times. The indicated organs were collected for RT-qPCR or histological analysis at 3 days after infection.

**Immunohistochemistry analysis**

The experiments were performed as previously described (10). Briefly, tissues were dehydrated and embedded in paraffin. Paraffin-embedded tissue blocks were sectioned with 4 μm thickness. The sections were processed by hematoxylin and eosin (H&E) staining and pictures were acquired using Leica Aperio versa Slide Scanning Microscope equipped with a HistoFAXS system.

**Statistical analysis**

Unpaired two-tailed Student’s t test was used for statistical analysis with Microsoft Excel and GraphPad Prism Software. p < 0.05 was considered significant. For animal survival analysis, the
Kaplan-Meier method was adopted to generate graphs, and the survival curves were analyzed with log-rank analysis.
References:

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Fig. S1: TNKS1 and TNKS2 are associated with VISA through their ANK domain. (A, B) TNKS1 (A) or TNKS2 (B) specifically interacts with VISA. HEK293 cells were transfected with the indicated plasmids for 20 h before co-IP and immunoblot analysis. (C, D) Domain mapping of the interaction between VISA and TNKS1 (C) or TNKS2 (D). HEK293 cells were transfected with the indicated plasmids for 20 h before co-IP and immunoblot analysis. (E) Immunostaining of Flag-TNKS1 or TNKS2 (green) and TOM20 (red) in HEK293 cells. WT, wild type; IP, immunoprecipitation; HA, hemagglutinin; IgG, immunoglobulin G.
Fig. S2: TNKS1 and TNKS2 negatively regulate virus-triggered signaling

(A) Effects of TNKS1 and TNKS2 on SeV-triggered activation of IFN-β promoter. IFN-β reporter assays for HEK293 cells transfected with the indicated plasmids and were then infected with SeV for 10 h. (B) Effects of TNKS1 and TNKS2 on the IFN-γ-triggered activation of IRF1 promoter. IRF1 reporter assays for HEK293 cells transfected with the indicated plasmids and were then stimulated with IFN-γ for 10 h. (C) Effects of TNKS1 and TNKS2 RNAi plasmids on expression of TNKS1 and TNKS2. The HEK293 cells were transfected with Flag-TNKS1 or TNKS2, HA-β-actin and the indicated RNAi plasmids for 24 h before immunoblotting analysis. (D) Effects of TNKS1- and TNKS2-RNAi on SeV-triggered activation of the IFN-β promoter. HEK293 cells were transfected with IFN-β promoter along with the indicated RNAi plasmids for 36 h, and the cells were then infected with SeV for 10 h before luciferase assays. (E) Effects of double knockdown of TNKS1 and TNKS2 on SeV- or poly(I:C)-triggered activation of the IFN-β promoter. HEK293 cells were transfected with IFN-β promoter along with the indicated RNAi plasmids for 36 h, and the cells were then infected with SeV or transfected with poly(I:C) for 10 h before luciferase assays and immunoblotting analysis. (F) Effects of TNKS1- and TNKS2-RNAi on the SeV-triggered phosphorylation of TBK1 and IRF3. HEK293 cells were transfected with IFN-β promoter along with the indicated RNAi plasmids. Twelve hours after transfection, puromycin (1 μg/mL) was added into the culture medium. The cells were selected for 24 h and then untreated or infected with SeV for the indicated time points before immunoblotting analysis. (G) Effects of TNKS1- and TNKS2-RNAi on IFN-γ-triggered activation of IRF1 promoter. HEK293 cells were transfected with IRF1 promoter along with the indicated RNAi plasmids for 36 h, and the cells were then stimulated with IFN-γ for 10 h before luciferase assays. (H-I) Effects of TNKS1 or TNKS2 deficiency on SeV-triggered transcription of downstream genes in MEFs or HEK293 cells. RT-qPCR analysis of mRNA abundance of the indicated genes in WT and TNKS1- or TNKS2-KO MEFs or HEK293 cells infected with SeV for 10 h. (J) Effects of TNKS1 or TNKS2 deficiency on IFN-γ-triggered transcription of downstream genes in HEK293 cells and MLFs. RT-qPCR analysis of mRNA abundance of the indicated genes in WT and TNKS1- or TNKS2-KO HEK293 cells (upper panel) or MLFs (lower panel) stimulated with IFN-γ for 4 h. *p < 0.05, **p < 0.01, and ***p < 0.001 (Student’s t test). Vec., vector; Con., control; mRNA, messenger RNA.
Fig. S3: TNKS1 or TNKS2 deficiency potentiates virus-triggered signaling in BMDMs
(A) Effects of TNKS2 deficiency on SeV- or EMCV-induced transcription of downstream genes in BMDMs. *Tnks2^fl/fl^ and *Tnks2^fl/fl::Cre-ER^+^ BMDMs were treated with 4-OHT (1 μM) for 3 days and followed by infection with SeV or EMCV for the indicated times before RT-qPCR analysis. *p < 0.05, **p < 0.01, and ***p < 0.001 (Student’s t test). mRNA, messenger RNA.

(B) Effects of TNKS1 deficiency on SeV- or EMCV-induced transcription of downstream genes in BMDMs. *Tnks1^+/-^ and *Tnks1^-/-^ BMDMs were left untreated or infected with SeV or EMCV for the indicated times before RT-qPCR analysis. *p < 0.05, **p < 0.01, and ***p < 0.001 (Student’s t test). mRNA, messenger RNA.
Fig. S4: TNKS1-deficient mice exhibit reduced liver size and more severe liver damages after virus infection

(A) Sex- and age-matched Tnks2fl/fl and Tnks2fl/fl:Cre-ER+ mice were injected intraperitoneally with tamoxifen (80 μg/g dissolved in corn oil) for five consecutive days, and seven days later the mice were intraperitoneally injected with EMCV (1x10^8 PFU per mouse) for 3 days, the indicated organs or tissues were retrieved for RT-qPCR analysis. (B) RT-qPCR analysis of the mRNA abundance of Tnks1 and Tnks2 in the indicated tissues of mice. (C, D) Sex- and age-matched Tnks1+/+ and Tnks1−/− mice were intraperitoneally injected with EMCV (1x10^8 PFU per mouse) for 3 days, the indicated organs were further weighted and normalized to the body weight (C) or the livers of the mice were analyzed by H&E staining (D). (E) Effects of TNKS1 deficiency on serum levels of IFN-β, IL6 and MCP1 induced by PR8 infection. Sex- and age-matched Tnks1+/+ and Tnks1−/− mice (n = 5) were intranasally infected with PR8 (10^6 EID50) for the indicated time points before measurement of the indicated serum cytokines by ELISA. Each symbol represents an individual mouse. (F) Sex- and age-matched Tnks1+/+ and Tnks1−/− mice were intranasally infected with PR8 (10^6 EID50) for 48 h, the livers of the mice were analyzed by H&E staining. (G) Effects of TNKS1 deficiency on PR8-induced death. Sex- and age-matched Tnks1+/+ and Tnks1−/− mice were intranasally infected with PR8 (10^6 EID50, n = 8), and mouse survival was observed and recorded every 6 h. (H, I) Sex- and age-matched Tnks2fl/fl and Tnks2fl/fl:Cre-ER+ mice were injected intraperitoneally with tamoxifen (80 μg/g dissolved in corn oil) for five consecutive days, and seven days later the mice were intraperitoneally injected with EMCV (1x10^8 PFU per mouse) for 3 days, the indicated organs were further weighted and normalized to the body weight (H) or the livers of the mice were analyzed by H&E staining (I). *p <0.05, and **p < 0.01 (Student’s t test). mRNA, messenger RNA.
Fig. S5: TNKS1 and TNKS2 inhibit virus-triggered signaling by destabilizing VISA

(A, B) Knockdown of TNKS1 or TNKS2 potentiates VISA-mediated IFN-β activation. HEK293 cells were transfected with control or TNKS-RNAi plasmids. Thirty-six hours later, cells were further transfected with the indicated plasmids along with IFN-β promoter for 20 h before luciferase assays. (C) Effects of TNKS1- or TNKS2-deficient on the transcription of Visa. The wild-type and TNKS1- or TNKS2-deficient BMDCs were uninfected or infected with SeV for 8 h before RT-qPCR analysis. (D) TNKS1 and TNKS2 destabilize wild-type but not VISA-AA mutant. HEK293 cells (2×10^5) were transfected with the indicated plasmids for 24 h, and the cells were then analyzed by immunoblotting analysis. *p < 0.05, and **p < 0.01 (Student’s t test). Vec., vector; T1, TNKS1; T2, TNKS2.
Fig. S6: TNKS1 and TNKS2 catalyze the PARylation of VISA at Glu 137

(A) Effects of TNKS2 on IFN-β activation mediated by VISA and its mutants. HEK293 cells were transfected with the indicated plasmids for 20 h before luciferase assays. (B) Effects of TNKS2 on expression levels of VISA and its mutants. HEK293 cells were transfected with the indicated plasmids for 20 h before immunoblotting analysis. (C) Effects of TNKS1 on IFN-β activation mediated by VISA and E137A mutant. HEK293 cells were transfected with the indicated plasmids for 20 h before luciferase assays. (D) Effects of TNKS1 on expression levels of VISA and E137A mutant. HEK293 cells were transfected with the indicated plasmids for 20 h before immunoblotting analysis. WT, wild-type; E137A, VISA-E137A mutant.
**Fig. S7: RNF146 mediated polyubiquitination of VISA at Lys 7**

(A) HEK293 cells were transfected with the indicated plasmids for 24 h, and the cell lysates were then analyzed by immunoblot analysis. (B) HEK293 cells were transfected with the indicated plasmids for 24 h, the cell lysates were denatured and reimmunoprecipitated with anti-Flag and then analyzed by immunoblot with anti-HA (upper panel). The expression levels of the proteins were analyzed by immunoblot analysis with the indicated antibodies (lower panel). WT, wild type; K7R, VISA-K7R mutant.
**Fig. S8: RNF146 inhibited SeV-triggered activation of IFN-β promoter**

(A) IFN-β reporter assays for HEK293 cells transfected with the increased amounts of RNF146 expression plasmids and were then infected with SeV for 10 h. (B, C) IRF1 reporter assays for HEK293 cells transfected with the increased amounts of RNF146 expression plasmids (B) or the indicated RNF146-RNAi plasmids (C) and were then stimulated with IFN-γ for 10 h. (D) The effects of RNF146-gRNAi #3 on SeV-triggered transcription of downstream genes in HEK293 cells. RNF146 deficient HEK293 cells were generated by CRISPR-Cas9 system using RNF146-gRNA #3. Then the RNF146-KO and control HEK293 cells were untreated or infected with SeV for 10 h before RT-qPCR analysis. *p < 0.05, **p < 0.01, and ***p < 0.001 (Student’s t test). Vec., vector.
Fig. S9: A schematic presentation for the roles of TNKS-RNF146 axis in regulating VISA-mediated innate antiviral response

Following virus infection, the viral RNAs are recognized by cytosolic sensors including RIG-I and MDA5, which undergoes conformational changes to expose N-terminal CARD domain and induces the oligomerization of the mitochondrial adaptor protein VISA. VISA further activates transcription factors IRF3 and NF-κB through TBK1 and IKKs, thus inducing the expression of downstream antiviral genes such as IFNs, and leading to the innate antiviral response. Constant infection also upregulates the expression of TNKS1 and TNKS2, which are further recruited to the mitochondria. TNKS1 and TNKS2 interact with and PARylate VISA, which is then recognized by E3 ligase RNF146, thereby priming VISA for K48-linked polyubiquitination and proteasomal degradation, thus attenuating the innate antiviral response.