**Virologica Sinica**

**Supplementary Data**

**STING strengthens host anti-hantaviral immunity through an interferon-independent pathway**

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**Table S1.** Antibodies and reagents were used in this study

|  |  |  |
| --- | --- | --- |
| **Antibody Or Reagent** | **Source** | **Identifier** |
| **Antibodies** |  |  |
| Rabbit polyclonal anti-STING | Proteintech | Cat #19851-1-AP |
| Mouse monoclonal anti-STING | Proteintech | Cat #66680-1-Ig |
| Mouse monoclonal anti-GAPPDH | Sangon Biotech | Cat #D190090-0200 |
| Rabbit polyclonal anti-ERGIC-53 | Proteintech | Cat #13364-1-AP |
| Rabbit polyclonal anti-Rab5A | Proteintech | Cat #11947-1-AP |
| Rabbit polyclonal anti-Rab7A | Abcam | Cat #ab137029 |
| Rabbit polyclonal anti-RIG-I | Abcam | Cat #ab45428 |
| Rabbit polyclonal anti-cGAS | Abcam | Cat #ab176177 |
| Rabbit polyclonal anti-p-TBK1 | Abcam | Cat #ab109272 |
| Rabbit polyclonal anti-p-STAT1 | CST | Cat #9177 |
| Rabbit polyclonal anti-MAP1LC3B | Proteintech | Cat #14600-1AP |
| Rabbit polyclonal anti-SQSTM1/p62 | Proteintech | Cat #18420-1-AP |
| Rabbit polyclonal anti-Beclin1 | Proteintech | Cat #11306-1-AP |
| Rabbit polyclonal anti-HA | Abcam | Cat #ab9110 |
| Mouse polyclonal anti-Myc | Abcam | Cat #A02060-1 |
| Mouse polyclonal anti-Flag | Sino Biological | Cat #100233-MM01 |
| Mouse monoclonal anti-HTNV NP | Prepared in our Lab | N/A |
| Alexa Fluor 488-conjugated goat anti-mouse IgG | Sangon Biotech | Cat #D110090-0001 |
| Cy3-conjugated Donkey anti-rabbit IgG | Sangon Biotech | Cat #D110052-0100 |
| Cy3-conjugated Donkey anti-mouse IgG | Sangon Biotech | Cat #D110082-0100 |
| Cy5-conjugated Donkey anti-rabbit IgG | Sangon Biotech | Cat #D111133-0100 |
| Goat Anti-Rabbit 800 CW | LI-COR | Cat #D11103-05 |
| Goat Anti-Mouse 680 RD | LI-COR | Cat #C70427-05 |
| **Reagents** |  |  |
| 3-methyladenine (3-MA) | Selleck | Cat# S2767 |
| Chloroquine (CQ) | Selleck | Cat# S7544 |
| Bafilomycin A1 (BAFA1) | Selleck | Cat# S1413 |
| cGAMP | InvivoGen | Cat# tlrlnacga23-1 |
| MG132 | Sigma | Cat# M8699 |
| 4μ8c | TargetMol | Cat# 14003-96-4 |
| Brefeldin A (BFA) | BioLegend | Cat# 423303 |
| H-151 | AbMole | Cat# 941987-60-6 |
| 5,6-dimethylxanthenone-4-acetic acid (DMXAA) | AbMole | Cat# 117570-53-3 |
| ISD: 5′-TACAGATCTACTAGTGATCTATG-3′ | Prepared in our Lab | N/A |
| Lyso-Tracker™ Red DND-99 | InvivoGen | Cat# L7528 |
| Anti-Flag magnetic beads | Bimake | Cat #B26102 |
| Anti-Myc magnetic beads | Bimake | Cat #B26302 |
| Anti-HA magnetic beads | Bimake | Cat #B26202 |

**Table S2.** Primers were used for PCR in this study

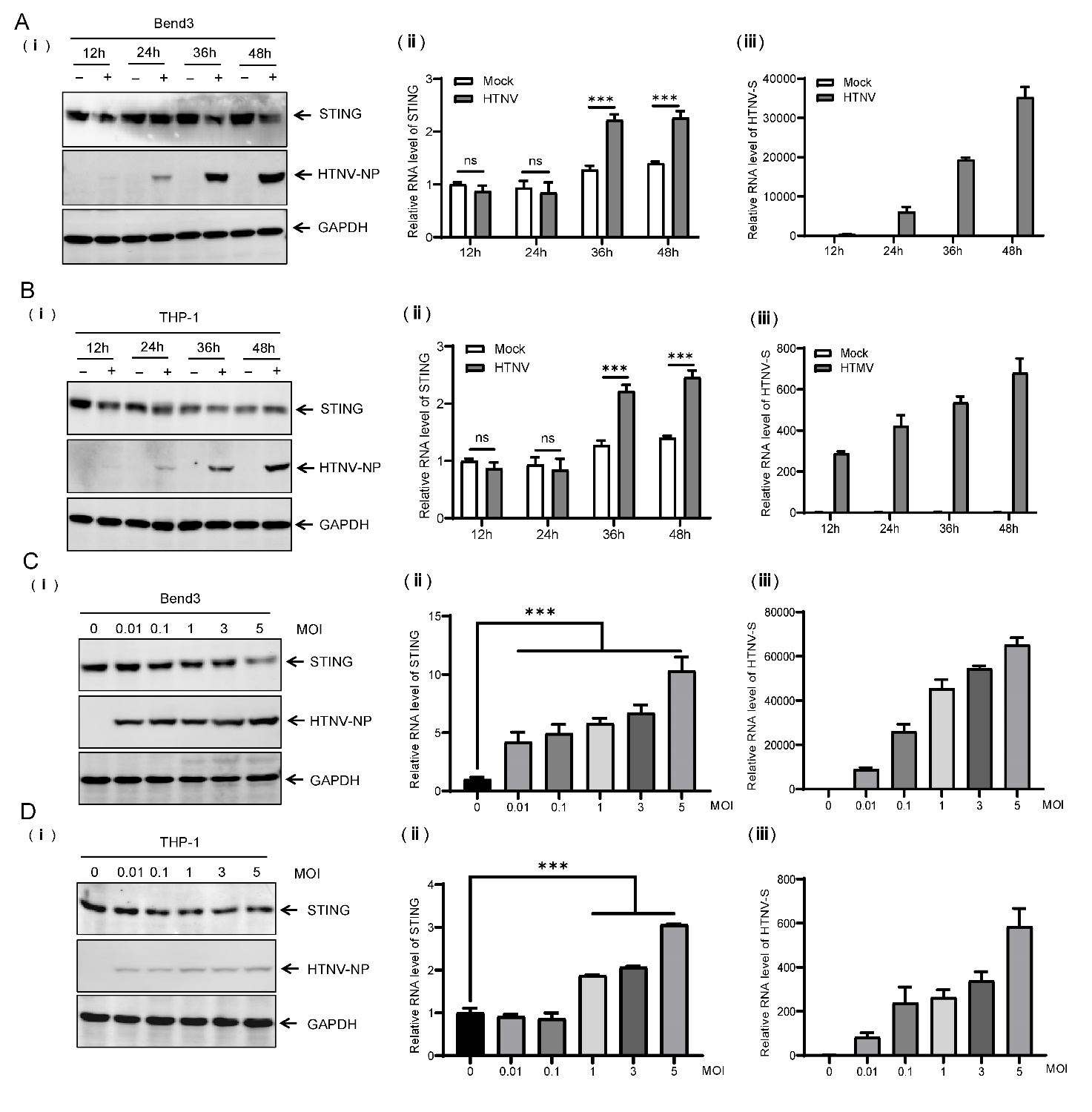
|  |  |
| --- | --- |
| Primer name | Primer sequences (5′–3′) |
| pCDNA3.1-STING-F | ATAGGATCCATGCCCC ACTCCAGCCT |
| pCDNA3.1-STING-R | GTCGAATTCAGAGAAATCCGTGCGGAG |
| pLVX-STING-F | CTAGAGGATCTATTTCCGGTGAATTCATGCCCCACTCCAGC |
| pLVX-STING-F | GGAGGGAGAGGGGCGGGATCCAAGAGAAATCCGTGC |
| pCDNA6.2-STING-F | ATAGGATCCATGCCCC ACTCCAGCCT |
| pCDNA6.2-STING-F | GTCGAATTCAGAGAAATCCGTGCGGAG |
| pCDNA6.2-△CTT-F | ATAGGATCCATGCCCC ACTCCAGCCT |
| pCDNA6.2-△CTT-F | GTCCTCGAGCTCTTCCTTTTCCTCCTGC |
| STING-TM-F | ATAGGATCCATGCCCC ACTCCAGCCT |
| STING-TM-R | GTCTCGAGGGCCAGGCCCTTGAGG |
| STING-CBD-F | GTCCTCGAGAAGAGAAATCCGTGCGGAG |
| STING-CBD-R | GTCCTCGAGCTCTT CCTTTTCCTCCTGC |
| STING-△CTT-F | ATAGGATCCATGCCCCACTCCAGCCT |
| STING-△CTT-R | GTCCTCGAGCTCTTCCTTTTCCTCCTGC |
| STING-△TM-F | GTCCTCGAGAAG AGAAATCCGTGCGGAG |
| STING-△TM-R | GTCGAATTCAGAGAAATCCGTGCGGAG |
| STING R238A-F | GATGCTGTTGCTGTAAACGGCATCCTTGATGCCAGCATG |
| STING R238A-R | CATGCTGGCATCAAGGATGCCGTTTACAGCAACAGCATC |
| STING S366A-F | GAGGGGCTTTTCCATTCCAGCGATGAGGAGCTCAGGCTCT |
| STING S366A-R | AGAGCCTGAGCTCCTCATCGCTGGAATGGAAAAGCCCCTC |

**Table S3.** Sequences were used for siRNA

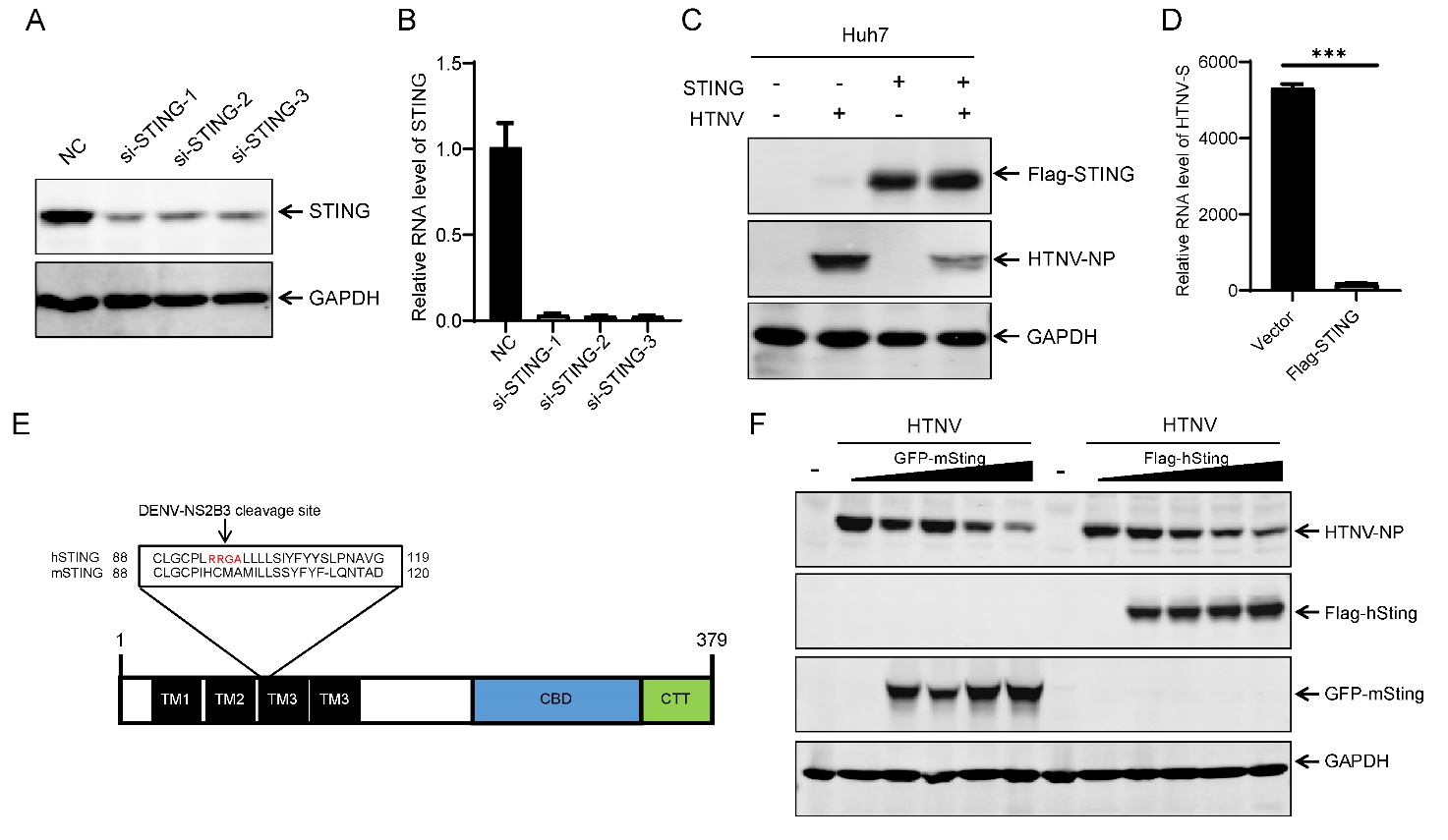
|  |  |  |
| --- | --- | --- |
| Genes | Sense (5′–3′) | Antisense (5′–3′) |
| NC | UUCUCCGAACGUGUCACGUTT | ACGUGACACGUUCGGAGAATT |
| STING-1 | GCCCGGAUUCGAACUUACAAU | AUUGUAAGUUCGAAUCCGGGC |
| STING-2 | GUCCAGGACUUGACAUCUUAA | UUAAGAUGUCAAGUCCUGGAC |
| STING-3 | CCTCATCAGTGGAATGGAATT | UUCCATTCCACTGATGAGGTT |
| cGAS | GGAAGGAAAUGGUUUCCAATT | UUGGAAACCAUUUCCUUCCTT |
| RIG-I | GAGGUGCAGUAUAUUCAGGTT | CCUGAAUAUACUGCACCUCTT |
| TBK1 | CACAAAUUUGAUAAGCAAATT | UUUGCUUAUCAAAUUUGUGTT |
| IRF3 | GGAGUGAUGAGCUACGUGA | UCACGUAGCUCAUCACUCCTT |
| ERGIC-53 | GGACAGAAUCGUAUUCAUCTT | AUGAAUACGAUUCUGUCCTT |
| SEC24C-1 | ACUUAUGUUAUCGAGUCAAUG | CAUUGACUCGAUAACAUAAGU |
| SEC24C-2 | UUGAUGUAAAGCGACUAAUAU | AUAUUAGUCGCUUUACAUCAA |
| TLR3 | GGUAACGAUUCCUUUGCUUTT | AAGCAAAGGAAUCGUUACCTT |
| TLR4 | GGAUUUAUCCAGGUGUGAATT | UUCACACCUGGAUAAAUCCTT |
| ATF4 | CCACGUUGGAUGACACUUGTT | CAAGUGUCAUCCAACGUGGTT |
| ATF6 | GCACCCAAGACUCAAACAATT | UUGUUUGAGUCUUGGGUGCTT |

**Table S4.** The sequences for qRT-PCR primers

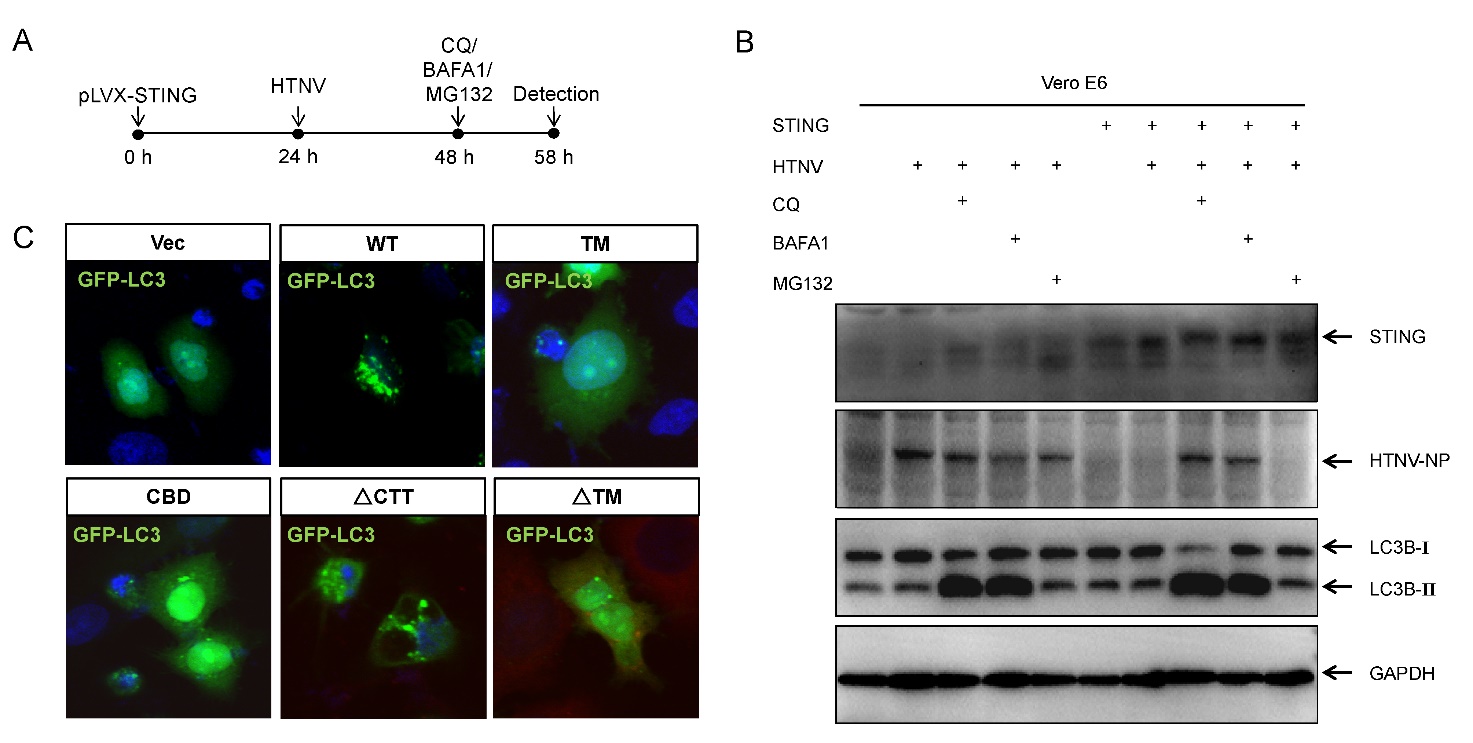
|  |  |  |
| --- | --- | --- |
| Genes | Forward (5′–3′) | Reverse (5′–3′) |
| *Homo*-*cGAS* | AGGAAGCAACTACGACTAAAGCC | CGATGTGAGAGAAGGATAGCCG |
| *Homo*-*STING* | CCTGAGTCTCAGAACAACTGCC | GGTCTTCAAGCTGCCCACAGTA |
| *Mus-Sting* | GGTCACCGCTCCAAATATGTAG | CAGTAGTCCAAGTTCGTGCGA |
| *Homo*-*GAPDH* | GTCTCCTCTGACTTCAACAGCG | ACCACCCTGTT GCTGTAGCCAA |
| *Mus--Gapdh* | CATCACTGCCACCCAGAAGACTG | ATGCCAGTGAGCTTCCCGTTCAG |
| *Homo*-*IFNβ* | CATTACCTGAAGGCCAAGGA | CAATTGTCCAGTCCCAGAGG |
| *Mus-Ifnβ* | GCCTTTGCCATCCAAGAGATGC | ACACTGTCTGCTGGTGGAGTTC |
| *Homo*-*Mx1* | GGCTGTTTACCAGACTCCGACA | CACAAAGCCTGGCAGCTCTCTA |
| *Mus-Il-6* | TACCACTTCACAAGTCGGAGGC | CTGCAAGTGCATCATCGTTGTTC |
| *Mus-Il-1β* | CCTTCCAGGATGAGGACATGA | TGAGTCACAGAGGATGGGCTC |
| *Mus-Tnf-α* | GCCTCTTCTCATTCCTGCTT | CTCCTCCACTTGGTGGTTTG |
| *Homo*-*ISG56* | GCCTTGCTGAAGTGTGGAGGAA | ATCCAGGCGATAGGCAGAGATC |
| HTNV S | TCTAGTTGTATCCCCATCGACTG | ACATGCGGAATACAAATTATGGC |
| *Homo-IRF3* | TCTGCCCTCAACCGCAAAGAAG | TACT GCCTCCACCATTGGTGTC |
| *Homo-TBK1* | CAACCTGGAAGCGGCAGAGTTA | ACCTGGAGATAATCTGCTGTCGA |
| *Homo-TLR3* | GCGCTAAAAAGTGAAGAACTGGA | GCTGGACATTGTTCAGAAAGAG |
| *Homo-TLR4* | CCCTGAGGCATTTAGGCAGCTA | AGGTAGAGAGGTGGCTTAGGCT |
| *Homo-RIG-I* | CACCTCAGTTGCTGATGAAGGC | GTCAGAAGGAAGCACTTGCTACC |
| *Homo-ATF4* | TTCTCCAGCGACAAGGCTAAGG | CTCCAACATCCAATCTGTCCCG |
| *Homo-ATF6* | CAGACAGTACCAACGCTTATGCC | GCAGAACTCCAGGTGCTTGAAG |

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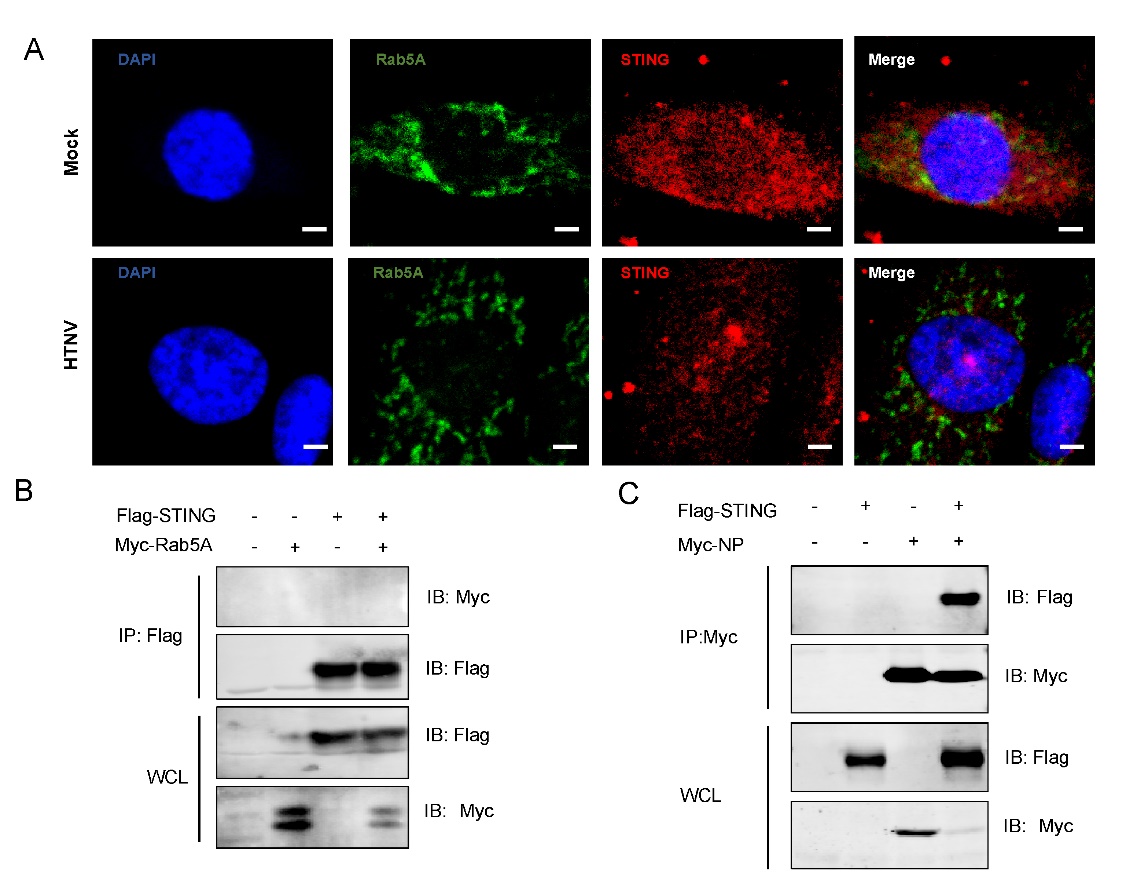
**Supplementary Fig. S1.** The reaction of STING during HTNV infection. **A, B** Bend3 (**A**) or THP-1 cells (**B**) were infected with HTNV at different time points (MOI = 1), and the lysates were harvested for immunoblot analysis of STING (**A-ⅰ and B-ⅰ**). The mRNA levels of *STING* (**A-ⅱ and B-ⅱ**)or *HTNV S* (**A-ⅲ and B-ⅲ**)were detected through qRT-PCR with *GAPDH* as the internal control. **C, D** Bend3 (**C**) or THP-1 cells (**D**) were infected with HTNV under different MOI for 36 h, and the lysates were harvested for immunoblot analysis of STING (**C-ⅰ and D-ⅰ**). The mRNA levels of *STING* (**C-ⅱ and D-ⅱ**)or *HTNV S* (**C-ⅲ and D-ⅲ**)were detected through qRT-PCR with *GAPDH* as the internal control. Dates are expressed as mean ± SEM (n = 3). Statistical significance was calculated using Student’s *t*-test or one-way ANOVA; \* *P* < 0.05; \*\* *P* < 0.01; \*\*\* *P* < 0.001; ns, not significant.

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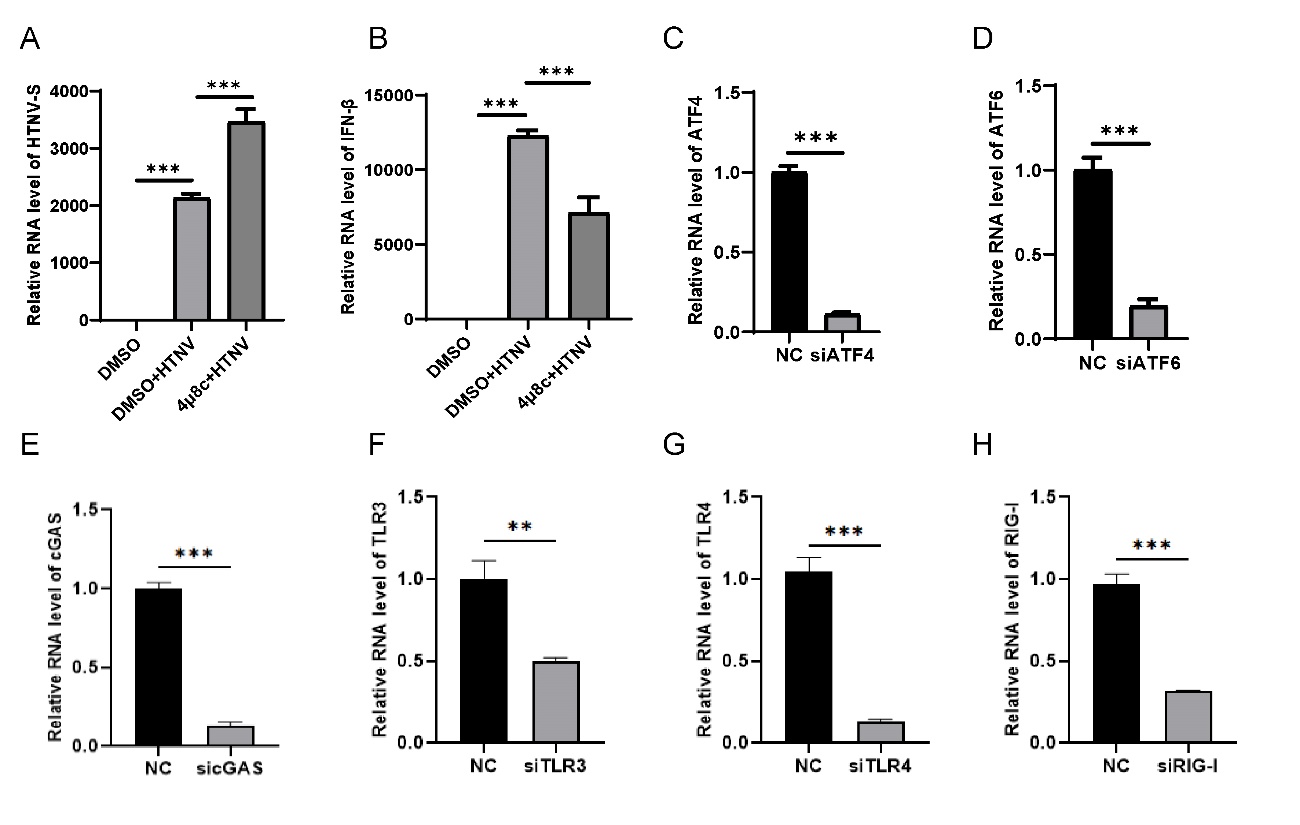
**Supplementary Fig. S2.** STING inhibits HTNV replication. **A** HUVECs were transfected with STING-specific siRNA or a negative control sequence (NC) for 24 h. The ablation efficiency of silencing STING was confirmed by immunoblotting. **B** HUVECs were transfected with STING-specific siRNA or a negative control sequence (NC) for 24 h. The ablation efficiency of silencing STING was confirmed by qRT-PCR. **C** Huh7 cells were transfected with Flag-STING for 24 h and then infected with HTNV (MOI=1) for 36 h. HTNV NP and Flag-STING were detected by immunoblotting. **D** Huh7 cells were transfected with Flag-STING for 24 h and then infected with HTNV (MOI = 1) for 36 h. The mRNA levels of IFN-β were detected through qRT-PCR with *GAPDH* as the internal control. **E** Schematic illustration of the domain of human STING as indicated. Highlighted in red amin-acid sequence alignment of human STING are the cleavage site of DENV NS2B3. **F** Huh7 cells were transfected with Flag-hSting or GFP-mting at indicated concentration for 24 h and then infected with HTNV (MOI = 1) for 36 h. HTNV NP and STING were detected by immunoblotting. Dates are expressed as mean ± SEM (n = 3). Statistical significance was calculated using Student’s *t*-test or one-way ANOVA; \**P* < 0.05; \*\**P* < 0.01; \*\*\**P* < 0.001; ns, not significant.

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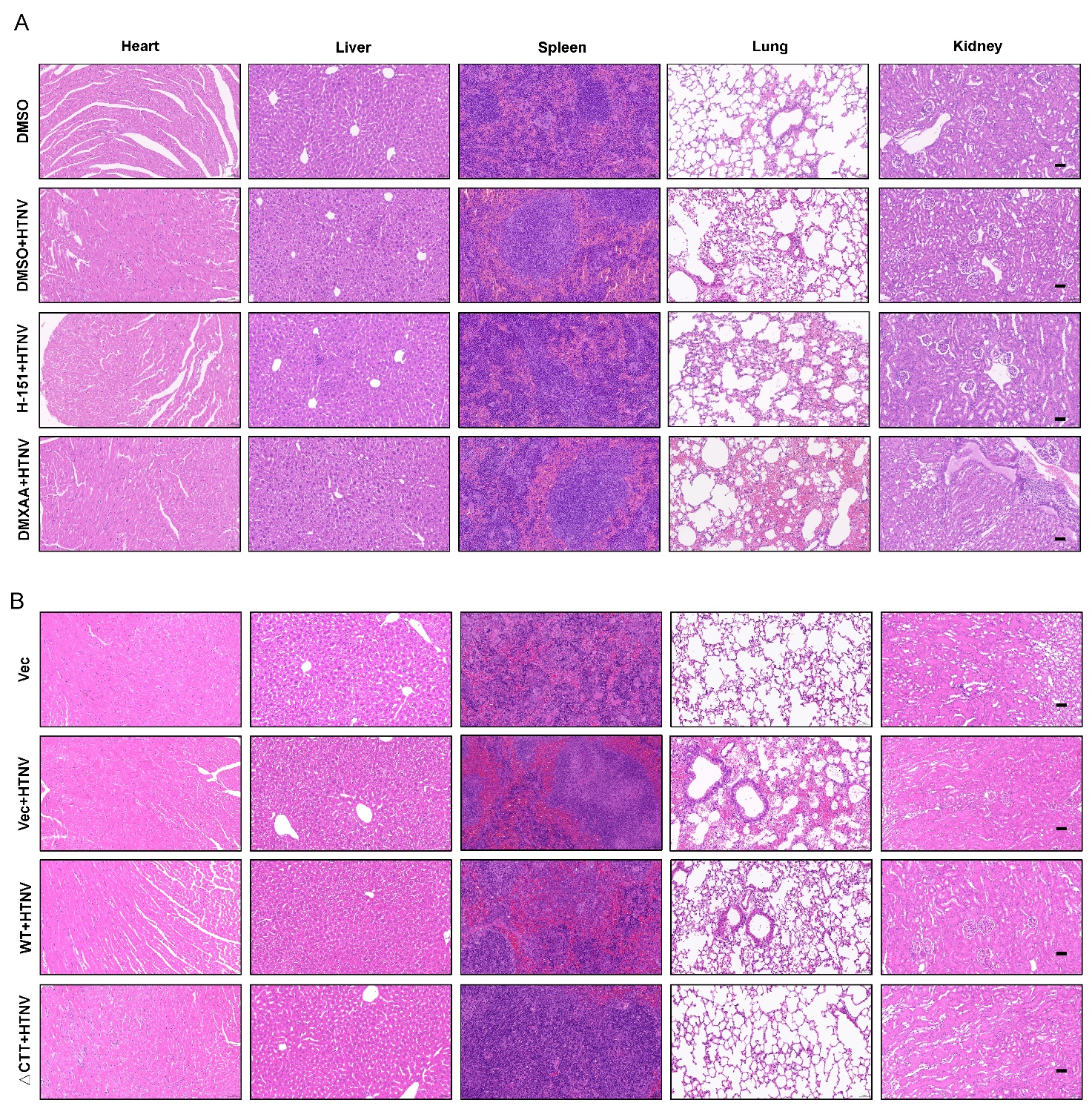
**Supplementary Fig. S3.** STING-mediated autophagy restricts HTNV replication. **A** Diagram of the assay for (**B**). **B** Vero E6 cells with overexpression STING by lentivirus infection and then infected with HTNV (MOI = 1) for 36 h, and subsequently treated with BAFA1 or MG132 for 4 h or 8 h. HTNV NP and LC3B were detected by immunoblotting. **C** HeLa cells were co-transfected with GFP-LC3 and indicated plasmids (Flag-Vec, Flag-WT, Flag-TM, Flag-CBD, Flag-△CTT, Flag-△TM) for 24 hrs. The cells were fixed and then observed using confocal microscopy. Scale bars, 10 µm.



**Supplementary Fig. S4.** STING trafficking from ER to late endosome participate in viral protein degradation. **A** HUVECs were mock-infected or HTNV-infected (MOI = 1) for 24 h, and incubated with mouse anti-STING and rabbit anti-Rab5A antibodies, following stained with Cy3-conjugated anti-mouse and FITC-conjugated anti-rabbit secondary antibodies. Nuclei were stained with DAPI. Scale bar, 20 μm. **B** HEK293T cells were single or co-transfected with Flag-STING or Myc-Rab5A for 36 h. The cell lysates were immunoprecipitated with anti-Flag antibody,and detected by immunoblotting withanti-Flag or anti-Myc antibody. **C** HEK293T cells were single or co-transfected with Flag-STING or Myc-NP for 36 h. The cell lysates were immunoprecipitated with anti-Myc antibody,and detected by immunoblotting withanti-Flag or anti-Myc antibody.



**Supplementary Fig. S5.** IRE1-XBP1-RIG-I signalling initiates STING-mediated autophagy post HTNV infection. **A** HUVECs were treated with 4μ8c or DMSO for 24 h and then infected with HTNV (MOI=1) for 36 h. The mRNA levels of HTNV S were detected through qRT-PCR with *GAPDH* as the internal control. **B** HUVECs were treated with 4μ8c or DMSO for 24 h and then infected with HTNV (MOI = 1) for 36 h. The mRNA levels of IFN-β were detected through qRT-PCR with *GAPDH* as the internal control. **C**–**D** HUVECs were transfected with siRNA targeting ATF4, ATF6 or NC for 24 h. The ablation efficiency of silencing ATF4 (**C**) or ATF6 (**D**) was confirmed by qRT-PCR. **E** HUVECs were transfected with siRNA targeting cGAS or NC for 24 h. The ablation efficiency of silencing cGAS was confirmed by qRT-PCR. **F**–**H** HUVECs were transfected with siRNA targeting TLR3, TLR4, RIG-I or NC for 24 h. the ablation efficiency of silencing TLR3 (**F**), TLR4 (**G**) or RIG-I (**H**)was confirmed by qRT-PCR. Dates are expressed as mean ± SEM (n = 3). Statistical significance was calculated using Student’s *t-*test or one-way ANOVA; \* *P* < 0.05; \*\* *P* < 0.01; \*\*\* *P* < 0.001; ns, not significant.



**Supplementary Fig. S6.** STING enhances anti-hantaviral immunity responses *in vivo.* **A** Nude mice (6–8 weeks) were intraperitoneally inoculated with HTNV (4×104 TCID50/g per mouse) and intraperitoneal administration of vehicle, H-151, or DMXAA (starting at 2 day after infection). Pathology assessment by H&E images of different nude mice tissues at 6 dpi. Scale bar, 50 μm. **B** Nude mice (6-8 weeks) were intraperitoneally inoculated with HTNV (4×104 TCID50/g per mouse) and intraperitoneal administration of vector, WT, or △CTT (starting at 2 day after infection). Pathology assessment by H&E staining of different nude mice tissues at 6 dpi. Scale bar, 50 μm.