Supplementary Information

MATERIALS AND METHODS

Ethics statement

BALB/c mice were purchased from VITAL RIVER (Beijing, China) and housed in individual ventilated cages in a bio-safety level-2 (BSL-2) laboratory. The animal trials were performed according to the Chinese Regulations of Laboratory Animals and the Guidelines for the Care of Laboratory Animals (Ministry of Science and Technology of People's Republic of China). The license number associated with the research protocol was SY20142023, which was approved by the Laboratory Animal Ethical Committee of Harbin Veterinary Research Institute (LAECHVRI). The 9 to 11-day-old chicken embryos used to amplify SeV were purchased from the center of Harbin Experimental Animal.

Mice and inoculation

Five-week-old female BALB/c mice were infected with EMCV-HB10 strain to determine its pathogenicity. Briefly, the mice were distributed into two groups, and inoculated intraperitoneally with 200 μl of $10^3$ TCID$_{50}$ of EMCV-HB10 and DMEM, respectively. Mice were observed every 24 h for clinical signs of disease until 21$^{th}$ day post infection (dpi).

Histology

The brains and hearts isolated from the mice mock-infected or infected with EMCV-HB10 were immediately fixed in 10% neutral buffered formalin, and then embedded in paraffin, which was cut into 4 μm coronal or sagittal sections. For routine histologic examination, the sections were stained with hematoxylin and eosin (HE) and the images of the stained sections were captured under a Nikon 90i microscope (Nikon, Japan).
Figure S1. Characterization of the biological characteristics of EMCV-HB10 strain.

(A and B) Evaluation of the pathogenicity of EMCV-HB10. 4-week-old BALB/c mice were either mock-infected or infected intraperitoneally with a high dose of EMCV-HB10 ($10^3$ TCID$_{50}$) and monitored for their clinical signs for 12 days (A). The histopathology of the brain and heart of the BALB/c female mice infected with EMCV-HB10 were analyzed (B). The inflammatory cells were pointed out with the arrows. Bar, 50 µm. (C) HeLa cells were transfected with 100 ng IFNβ reporter and 5 ng pRL-TK plasmid and then the cells were either mock-infected or infected with different doses of EMCV-HB10 (MOI: 0.01, 0.1, or 1.0) or SeV. The cells were collected and luciferase assay was performed. The results represent three independent experiments. *** represents $p < 0.001$. (D and E) HEK293T cells were infected with different doses of EMCV-HB10 and then stimulated with polyI:C stimulation (D) or infected with SeV (E). The cell lysates were analyzed by Western blotting.
Figure S2. EMCV proteins inhibit SeV-mediated type I IFN signaling.

HEK293T cells were transfected with 100 ng IFNβ reporter and 5 ng pRL-TK plasmid, along with a plasmid expressing individual EMCV protein as indicated. The cells were either mock-infected or infected with SeV. At 12 hpi, the cells were collected and the luciferase assay was performed. The cell lysates were also analyzed by Western blotting.
Figure S3. EMCV 3C inhibits SeV-mediated type I IFN signaling.

(A) HEK293T cells were transfected with 100 ng IFNβ reporter and 5 ng pRL-TK plasmids, along with different amount (0, 200, 400, or 800 ng) of a plasmid encoding the EMCV 3C. The cells were either mock-infected or infected with SeV. At 12 hpi, the cells were collected and the luciferase assay was performed. The results were representative three independent experiments. *** represents p < 0.001. (B and C) HEK293T cells were transfected with different amount (0, 200, 400, or 800 ng) of a plasmid encoding the EMCV 3C. The cells were then either mock-infected or infected with SeV. At 12 hpi, the cells were collected and the mRNA levels of the IFNβ were evaluated by qRT-PCR (B). The IFNβ production was detected by ELISA assay (C). (D and E) HEK293T cells were transfected with increasing amount of a plasmid encoding HA-tagged EMCV 3C and then infected with SeV as indicated. The cell lysates were analyzed by Western blotting. (F) The cell supernatants from panel D or E were inactivated and added to the MDBK cells for another 24 h and then infected with VSV-GFP (MOI: 1.0) for 24 h. Bright field images (top) indicated growing cells. Green fluorescence (bottom) indicated viral replication. The cells stimulated with IFNα were used as a positive control.
Figure S4. EMCV 3C activity is required for inhibition of TBK1- and IKKε-mediated IRF3 phosphorylation

(A-D) HEK293T cells were transfected with a plasmid expressing TBK1 (A and C) or IKKε (B and D), along with different amount (0, 200, 400, or 800 ng) of a plasmid encoding the EMCV 3C (A and B) or EMCV 3C-DM (C and D). The cells were collected and qRT-PCR assay was performed to detect mRNA levels of IFNβ. The results represented three independent experiments. (E-H) HEK293T cells were transfected with a plasmid expressing EMCV 3C or EMCV 3C-DM alone or in combination with a plasmid expressing TBK1 (E, G) or IKKε (F, H). The cell lysates were analyzed by Western blotting.
Figure S5. EMCV 3C inhibits IRF3-5D-mediated IFNβ promoter activation.

(A) HEK293T cells were transfected with 100 ng of IFNβ reporter and 5 ng of pRL-TK plasmid, along with different amount (0, 200, 400, or 800 ng) of a plasmid encoding EMCV 3C and 250 ng of a plasmid encoding IRF3-5D. (B) HEK293T cells were transfected with 100 ng of IFNβ reporter, along with a plasmid encoding EMCV-3C or its double mutant (EMCV 3C-DM) and a plasmid encoding IRF3-5D. The luciferase assay was performed to evaluate the activities of the IFNβ promoter activation. The results represented three independent experiments. * represents $0.01 < p < 0.05$. *** represents $p < 0.001$. The expression levels of proteins were analyzed by Western blotting with the indicated antibodies.
Figure S6. EMV 3C inhibits IRF3 phosphorylation, but not TBK1 or IKKe phosphorylation.

HEK293T cells were transfected with different mount of a plasmid expressing EMV 3C. At 24 hpt, the cells were transfected with polyI:C for another 12 h, and then cell lysates were analyzed by Western blotting.

Figure S7.
Figure S7. Cleavage of TANK disrupts the interaction between TANK and IRF3.

(A-C) HEK293T cells were transfected with 2 µg of a plasmid expressing TBK1 (A), IKKε (B), or IRF3 (C), in combination with 2 µg of a plasmid expressing full-length TANK or its deletion mutants. Co-IPs were performed and the immunoprecipitants were analyzed by Western blotting.