Supporting Information

The Nonstructural Protein of Guertu Virus Disrupts Host Defenses by Blocking Antiviral Interferon Induction and Action

Yuan-Qin Min[1, 2], Chen Shi[1, 3], Ting Yao[1, 3], Kuan Feng[1, 3], Qiong Mo[1, 3], Fei Deng[1, 2, *], Hualin Wang[1, 2, *], Yun-Jia Ning[1, 2, *]

[1]State Key Laboratory of Virology, Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan 430071/430207, China; [2]Center for Biosafety Mega-Science, Chinese Academy of Sciences, Wuhan 430071/430207, China; [3]the University of Chinese Academy of Sciences, Beijing 101408, China

[*]**Correspondence:**

Hualin Wang, Dr., Prof. Mailing address: Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan, China; Tel: 86-27-87199353; E-mail: h. wang@wh.iov.cn

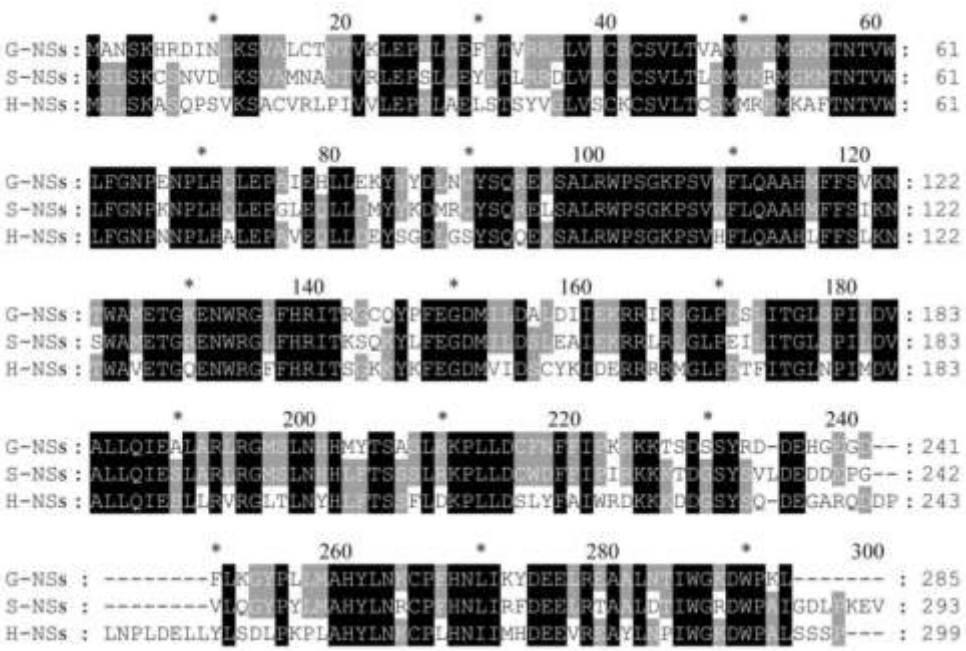
Fei Deng, Dr., Prof. Mailing address: Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan, China; Tel: 86-027-87198465; E-mail: df@wh.iov.cn

Yun-Jia Ning, Dr. Mailing address: Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan, China; Tel: 86-027-87197200; E-mail: nyj@wh.iov.cn

Pages: S1-S5

Figures: 4

A



B

Pairwise sequence identity values of NSs

	G-NSs	S-NSs	H-NSs
G-NSs	-	61	51
S-NSs	73	-	52
H-NSs	59	59	-

Figure S1. Homology analysis of G-NSs, S-NSs, and H-NSs. (A) Amino acid sequence alignment of G-NSs (YP_009666939.1), S-NSs (YP_009666135.1), and H-NSs (YP_009047243.1) were performed by Clustal X Version 2.1 and viewed by Gene Doc Version 2.7. (B) Summary of the pairwise sequence identity values of NSs. Nucleotide sequence identities in pairwise comparison are shown on upper right, and the amino acid sequence identities are shown on lower left.

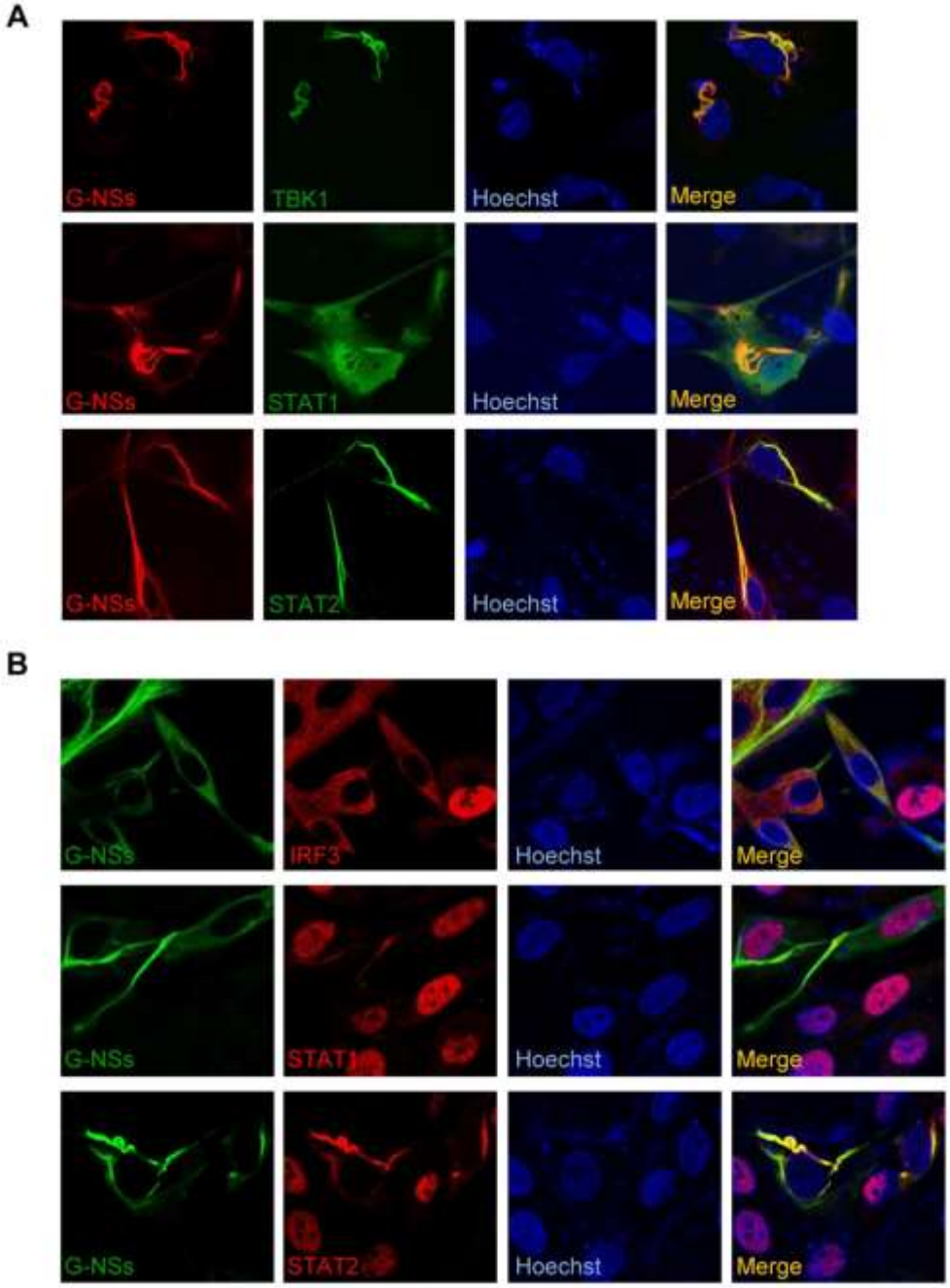


Figure S2. GTV NSs relocates TBK1 and STAT2 into the induced FSs, blocking nuclear translocation of IRF3 and STAT2. (A) Highly efficient relocation of TBK1 and STAT2 by GTV NSs into FSs. HUVEC cells were transfected with the G-NSs expression plasmid or the control vector, together with the TBK1, STAT2, or STAT1 expression plasmids. Twenty-four hours later, cells were fixed for IFA and confocal microscopy, as performed in Figure 5. (B) Blockade of IRF3 and STAT2 nuclear accumulation by GTV NSs. HUVEC cells were transfected with the G-NSs expression plasmid. Twenty-four hours post transfection, cells were infected with SeV for 8 hours or treated with IFN-α for 30 min to respectively induce IRF3 or STAT nuclear translocation. Cells were then delivered to IFA and confocal microscopy for visualization of protein expression and localization. Nuclei stained with Hoechst were shown in blue.

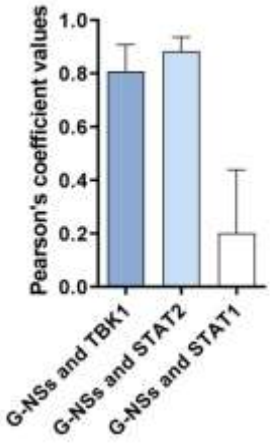


Figure S3. Colocalization efficiency of G-NSs with host proteins. Cells cotransfected with the G-NSs plasmid and the plasmids encoding the host proteins (as described in Figure 5) were used for quantitative analysis of protein colocalization. Pearson's co-localization coefficients of the G-NSs immunofluorescence signal with those of TBK1, STAT2, or STAT1 were quantified using ImageJ sofware in more than 60 cells coexpressing G-NSs and the indicated host proteins from at least 15 fields per group. Data are shown as means ± SD.

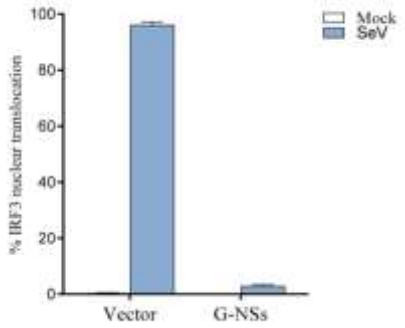


Figure S4. Nuclear translocation efficiency of IRF3. Cells were treated as in Figure 6B and then scored for SeV-induced nuclear translocation of IRF3. For the groups transfected with the G-NSs expression plasmid, cells expressing G-NSs were counted. At least 200 cells were included for each sample in the counting. Data are shown as means ± SD.