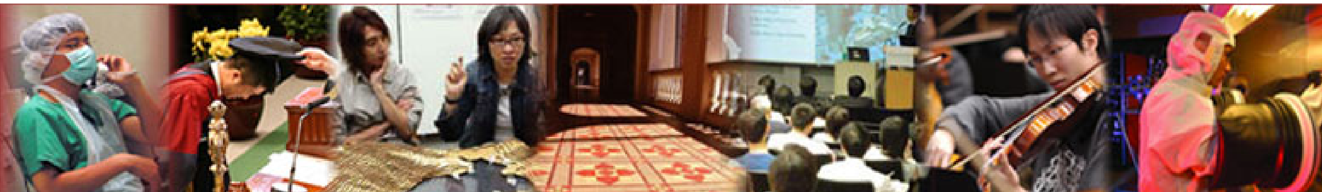
The HKU Scholars Hub The University of Hong Kong 香港大學學術庫



Title	Suppression of type I and type III interferon signalling by NSs protein of severe fever-with-thrombocytopenia syndrome virus through inhibition of STAT1 phosphorylation and activation
Author(s)	Chaudhary, V; Zhang, S; Yuen, KS; Li, C; Lui, PY; Fung, SY; Wang, PH; Chan, CP; Li, D; Kok, KH; Liang, M; Jin, D
Citation	Journal of General Virology, 2015, v. 96 n. 11, p. 3204-3211
Issued Date	2015
URL	http://hdl.handle.net/10722/216602
Rights	Journal of General Virology. Copyright © Society for General Microbiology.; This is an author manuscript that has been accepted for publication in Journal of General Virology, copyright Society for General Microbiology, but has not been copy-edited, formatted or proofed. Cite this article as appearing in Journal of General Virology. This version of the manuscript may not be duplicated or reproduced, other than for personal use or within the rule of 'Fair Use of Copyrighted Materials' (section 17, Title 17, US Code), without permission from the copyright owner, Society for General Microbiology. The Society for General Microbiology disclaims any responsibility or liability for errors or omissions in this version of the manuscript or in any version derived from it by any other parties. The final copy-edited, published article, which is the version of record, can be found at http://vir.sgmjournals.org, and is freely available without a subscription.; This work is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License.

Word count: 149 (Summary) and 2414 (Text)
Number of figures: 3
Category: Animal – RNA Viruses

Suppression of type I and type III interferon signalling by NSs protein of severe fever-with-thrombocytopenia syndrome virus through inhibition of STAT1 phosphorylation and activation

Vidyanath Chaudhary,[1]† Shuo Zhang,[2]† Kit-San Yuen,[1] Chuan Li,[2] Pak-Yin Lui,[1] Sin-Yee Fung,[1] Pei-Hui Wang,[1] Chi-Ping Chan,[1] Dexin Li,[2] Kin-Hang Kok,[3] Mifang Liang[2] and Dong-Yan Jin[1]

[1]School of Biomedical Sciences, The University of Hong Kong, Pokfulam, Hong Kong

[2]Key Laboratory for Medical Virology and National Institute for Viral Disease Control and Prevention, Chinese Centre for Disease Control and Prevention, Beijing 102206, China

[3]Department of Microbiology, The University of Hong Kong, Pokfulam, Hong Kong

Running title: Suppression of interferon signalling by SFTSV NSs protein

Correspondence

Mifang Liang
mifangl@vip.sina.com

Dong-Yan Jin
dyjin@hku.hk

†These authors contributed equally to this work.

Summary

Severe fever-with-thrombocytopenia syndrome virus (SFTSV) is an emerging tick-borne pathogen causing significant morbidity and mortality in Asia. NSs protein of SFTSV is known to perturb type I interferon (IFN) induction and signalling, but the mechanism remains to be fully understood. Here, we showed the suppression of both type I and type III IFN signalling by SFTSV NSs protein mediated through inhibition of STAT1 phosphorylation and activation. Infection with live SFTSV or expression of NSs potently suppressed IFN-stimulated genes but not NF-κB activation. NSs was capable of counteracting the activity of IFN-α1, IFN-β, IFN-λ1 and IFN-λ1. Mechanistically, NSs associated with STAT1 and STAT2, mitigated IFN-β-induced phosphorylation of STAT1 at serine 727, and reduced the expression and activity of STAT1 protein in IFN-β-treated cells, resulting in the inhibition of STAT1 and STAT2 recruitment to IFN-stimulated promoters. Taken together, SFTSV NSs protein is an IFN antagonist that suppresses phosphorylation and activation of STAT1.

Keywords: severe fever-with-thrombocytopenia syndrome virus (SFTSV); phlebovirus; NSs protein; STAT1; STAT2; interferon signalling

1 Severe fever-with-thrombocytopenia syndrome virus (SFTSV) is a novel phlebovirus
2 first isolated in 2009 in China, where it causes a tick-borne zoonosis in humans and
3 domestic animals (Yu et al., 2011; Niu et al., 2013). Human patients are presented with
4 acute fever, thrombocytopenia, leukocytopenia as well as gastrointestinal and joint
5 symptoms. In a small subset of severe cases the disease progresses rapidly to multiorgan
6 failure, hemorrhage and death, with the case fatality rate ranging from 2% to 15% (Liu
7 et al., 2014; Li, 2015). Whereas the majority of patients are farmers who live in wooded
8 upland areas and work in the fields, a few clusters of cases and human-to-human
9 transmission through direct contact with blood or respiratory secretions have also been
10 reported (Bao et al., 2011; Gai et al., 2012). Human infection of SFTSV has been
11 identified retrospectively in Korea and Japan (Kim et al., 2013; Takahashi et al., 2014).
12 SFTSV and related viruses have also been isolated in ticks collected in Korea and
13 Australia (Yun et al., 2014; Wang et al., 2014). Furthermore, Heartland virus, another
14 tick-borne phlebovirus sharing 60-73% amino acid sequence homology with SFTSV,
15 has been shown to be etiologically associated with an SFTS-like severe disease in US
16 (McMullan et al., 2012; Muehlenbachs et al., 2014). Thus, SFTSV and closely related
17 human pathogens are distributed widely.

18

19 SFTSV belongs to the *Phlebovirus* genus of the *Bunyaviridae* family. Similar to other
20 bunyaviruses, SFTSV contains a tripartite single-stranded RNA genome of negative
21 sense (Walter & Barr, 2011). Whereas the L segment encodes viral polymerase, the M
22 segment codes for envelope glycoproteins Gc and Gn. Nucleocapsid and nonstructural
23 protein NSs are expressed in opposite directions from the ambisense S segment.
24 Characterization of viral virulence factors might shed light on the mechanism of severe
25 diseases caused by SFTSV. As a major virulence factor, NSs proteins from many

1 bunyaviruses are capable of antagonizing interferon (IFN) response. Among them NSs
2 protein from Rift Valley fever virus (RVFV) is one of the most extensively studied
3 (Ikegami & Makino, 2011). However, the mechanism of NSs-mediated immune
4 evasion varies from one to another bunyavirus (Walter & Barr, 2011).

5

6 Innate immune response is triggered by the sensing of pathogen-associated molecular
7 patterns by host pattern recognition receptors (Habjan & Pichlmair, 2015). Subsequent
8 activation of the signalling cascades leads to the induction of type I and type III IFNs
9 by transcription factors IRF3 and IRF7. The binding of type I and type III IFNs to their
10 receptors results in auto-phosphorylation and activation of the receptor-associated
11 kinases TYK2 and JAK1, which regulate the activation of STAT1 and STAT2.
12 Together with IRF9, homodimers or heterodimers of phosphorylated STAT1 and
13 STAT2 form the ISGF3 complex, which translocates into the nucleus, binds to specific
14 IFN-stimulated response elements (ISREs) present in the promoters of IFN-stimulated
15 genes (ISGs), and activates their transcription (Schneider et al., 2014). Viruses have
16 evolved various IFN antagonists to counteract IFN induction and signalling at all steps
17 (Randall & Goodbourn, 2008; Hoffmann et al., 2015).

18

19 During the course of SFTSV infection in humans, IFNs are almost undetectable in the
20 blood (Qu et al., 2012), indicating the suppression of IFN production. Consistent with
21 this, SFTSV NSs protein has been shown to suppress type I IFN production through the
22 interaction with RIG-I, TRIM25 as well as IRF3 kinases TBK1 and IKKε, leading to
23 their sequestration in virus-induced cytoplasmic subdomains separated from
24 mitochondria (Ning et al., 2014; Qu et al., 2012; Santiago et al., 2014; Wu et al., 2014).

1 In addition, SFTSV NSs has recently been found to perturb type I IFN signalling by
2 interacting with STAT2 and thus retaining STAT1 and STAT2 in the cytoplasm (Ning
3 et al., 2015). However, mechanistic details of NSs-induced suppression of IFN
4 production and signalling remain controversial and thus merit further investigations.

5

6 Although SFTSV NSs has been shown to inhibit both NF-κB and IRF3 transcription
7 factors (Qu et al., 2012), we noted the upregulated expression of many NF-κB-
8 regulated cytokines such as interleukin 6 (IL6), IL8 and tumour necrosis factor α (TNF-
9 α) in humans and primates infected with SFTSV (Sun et al., 2012; Deng et al., 2012;
10 Jin et al., 2015). To resolve this discrepancy, we compared the impact of NSs
11 expression on Sendai virus-induced activation of IFN-β promoter and canonical κB
12 elements. Dual luciferase assays were performed (Chan et al., 2010; Kok et al., 2011),
13 with reporter constructs driven respectively by IFN-β promoter (IFNβ-Luc) and by five
14 tandem copies of canonical κB element (κB-Luc). It is known that the activation of
15 IFN-β promoter by Sendai virus is mediated primarily through IRF3 (Lin et al., 1998).
16 Notably, VP35 from Ebola virus and NSs from RVFV, which are well characterized
17 suppressors of type I IFN induction (Billecocq et al., 2004; Cardenas et al., 2006;
18 Ikegami et al., 2009; Kalveram et al., 2013), were capable of impeding Sendai virus-
19 induced activation of IFN-β promoter in our assay (Fig. 1a, bars 4 and 5 versus 2). As
20 expected, VP35 and IκBα super-repressor also ablated NF-κB activation (Fig. 1b, bars
21 4 and 5 versus 2). In contrast, SFTSV NSs suppressed the activation of IFN-β promoter
22 by Sendai virus (Fig. 1a, bar 3 versus 2), but had no influence on its activation of NF-
23 κB (Fig. 1b, bar 3 versus 2). A similar pattern was also observed when we used MAVS
24 to induce the activation of IFN-β promoter and NF-κB (Fig. 1b, d). In addition, SFTSV

1 NSs had no inhibitory effect on Sendai virus- or TNF-α-induced activation of NF-κB
2 in HeLa or HepG2 cells (Fig. 1e, f). Thus, SFTSV NSs preferentially suppressed IRF3
3 but not NF-κB activity in our setting. These results were at odds with a previous report
4 (Qu et al., 2012) but might be more compatible with the overproduction of NF-κB-
5 induced cytokines in SFTSV-infected cells (Jin et al., 2015). Further studies are
6 required to determine whether NF-κB is activated by SFTSV. On the other hand, the
7 inability of SFTSV NSs to suppress NF-κB activation also implicated that it unlikely
8 suppressed general transcription or translation. Thus, its suppression of type I IFN
9 production is specific.

10

11 We next examined the effect of SFTSV NSs protein on type I and type III IFN
12 signalling using a luciferase reporter driven by ISREs (ISRE-Luc). ISRE-dependent
13 reporter expression was potently induced in HEK293 cells treated with IFN-β, IFN-λ1
14 or IFN-λ2. This activity was largely ablated when SFTSV NSs was expressed (Fig. 1g,
15 h and Fig. S1a; bar 3 versus 2). The suppressive activity of SFTSV NSs was more
16 pronounced than that of RVFV NSs (Fig. 1g, h and Fig. S1a; bar 3 versus 4). In light
17 of this, we went on to verify the suppression of IFN signalling in SFTSV-infected cells.
18 We chose THP-1 cells, which were further induced to differentiate into macrophages,
19 for the infection experiment, because macrophages are highly responsive to IFN
20 treatment and they are also thought to play an important role in SFTSV pathogenesis
21 (Jin et al., 2012). mRNA expression of five selected ISGs, namely MX1, OAS1, ISG15,
22 ISG56 and STAT1, was assessed by RT-qPCR as described (Tang et al., 2013; Yuen et
23 al., 2015). Primer sequences are presented in the supplementary material. All five ISGs
24 were strongly induced by IFN-α1 and SFTSV individually (Fig. 1i-l and Fig. S1b, bars

2 and 3 versus 1). However, the steady-state mRNA levels of the ISGs did not increase further but decreased in IFN-α1-treated and SFTSV-infected cells (Fig. 1i-l and Fig. S1b, bar 4 versus 2). Similar results were also obtained from IFN-α1-treated SFTSV-infected HEK293T cells (Fig. 1m, n and Fig. S1c), indicating that the effect was not cell type-specific. Although SFTSV replication was inhibited by IFN-α1, reasonably high copy numbers of viral RNA were still detected in IFN-α1-treated THP-1 cells (Fig. S2). The inability of the remaining SFTSV to augment or at least maintain the ISG-inducing activity of IFN-α1 suggested that SFTSV might antagonize IFN-α1. In contrast to previous findings (Ning et al., 2014), in our setting SFTSV was capable of inducing ISG expression more substantially in THP-1 and HEK293T cells. It remains to be determined whether the use of different SFTSV strains in the two studies might account for the different results.

In addition to representative ISGs, we also examined the expression of two proinflammatory cytokines IL8 and CCL5 in infected cells. Both IL8 and CCL5 are well-characterized NF-κB target genes (Kunsch & Rosen, 1993; Wickremasinghe et al., 2004). Their mRNA expression levels remained unchanged in SFTSV-infected THP-1 cells stimulated with lipopolysaccharide (LPS), a strong activator of NF-κB (Fig. 1o, p). Furthermore, expression of SFTSV NSs did not affect phorbol ester-induced nuclear translocation of p65 subunit of NF-κB in HeLa cells (Fig. 2a, panel 2; NSs-expressing versus NSs-non-expressing cells). Thus, our results from luciferase assays (Fig. 1b, d, e, f), RT-qPCR (Fig. 1o, p) and confocal staining (Fig. 2a) consistently indicated no suppression of NF-κB activation by SFTSV NSs.

The suppression of type I and III IFN signalling by SFTSV NSs protein prompted us to investigate further whether it might affect the stability and function of STAT1 and STAT2. Although SFTSV NSs has recently been shown to interact with STAT2 but not STAT1 (Ning et al., 2015), we would like to re-examine this issue in our experimental setting. We noted that STAT1, STAT2 and an active form of STAT1 phosphorylated at S727 appeared in the nucleus of IFN-β-treated HeLa cells. However, only weak and cytoplasmic staining of STAT1 and STAT2 was observed in NSs-expressing cells (Fig. 2b, c, panel 2, NSs-expressing versus NSs-non-expressing cells). In addition, nuclear staining of STAT1 phosphorylated at S727 was not seen in the presence of NSs (Fig. 2d). This suggested that NSs might exert an inhibitory effect on STAT1 and STAT2 activation. To shed further light on this, co-immunoprecipitation was performed as described (Ng et al., 2011; Tang et al., 2014). STAT1 and STAT2 were detected in the NSs-containing immunoprecipitate (Fig. 3a, lane 2 versus 1). Although NSs might indirectly interact with STAT2 through STAT1, our results were also consistent with the model that NSs could interact with both STAT1 and STAT2.

Since other viral IFN-antagonizing proteins such as simian virus 5 V protein are known to induce ubiquitination and degradation of STAT1 (Precious et al., 2005), we asked whether SFTSV NSs might also affect the steady-state levels of STAT1. Western blot analysis of whole cell extracts was carried out as described (Chin et al., 2005; Chun et al., 2013) and the results indicated that NSs had no influence on STAT1 protein stability ambiently in HEK293 cells (Fig. 3b, lane 2 versus 1). However, when STAT1 was activated by IFN-β, the steady-state amounts of STAT1 in NSs-expressing cells detected over a time course of 24 hours were diminished consistently (Fig. 3b, lane 4 versus 3, lane 6 versus 5, and lane 8 versus 7). The inhibitory effect of NSs was not

seen when cells were treated with actinomycin D, an inhibitor of RNA polymerase II (Fig. 3c, lane 5 versus 4). These results suggested that NSs-induced inhibition likely occurs at the level of STAT1 transcription. As previously shown by others (Wong et al., 2002) and in Fig. 1l, n, STAT1 is an ISG. The abrogation of the inhibitory effect of NSs on STAT1 expression in IFN-β-treated cells by actinomycin D suggested that NSs might suppress type I IFN-induced activation of STAT1 transcription.

Exactly how SFTSV NSs modulates phosphorylation and activation of STAT1 remains elusive. IFNs induce STAT1 phosphorylation at two major sites Y701 and S727. Both modifications are required for full activation of STAT1 (Wen et al., 1995; Takaoka et al., 1999). To determine the impact of NSs expression on STAT1 phosphorylation, Western blotting was performed with phospho-specific antibodies. Whereas NSs had no influence on Y701 phosphorylation of STAT1, it exerted a suppressive effect on S727 phosphorylation (Fig. 3d, lane 3 versus 2). To shed light on where this inhibition by NSs might occur, we collected and analyzed the cytosolic and nuclear fractions. NSs and STAT1 were detected in both fractions. In addition, STAT1 with phospho-S727 was also found in reduced levels in both the cytosol and the nucleus (Fig. 3d, lane 6 versus 5, and lane 9 versus 8). Although a primarily cytoplasmic staining of NSs was observed in HeLa cells (Fig. 2), we cannot rule out that a subset of NSs might enter the nucleus. Biochemical fractionation is a more sensitive method. On the other hand, the nuclear localization of NSs might not be observed by confocal microscopy when cytoplasmic NSs is more prominent. Considered together with the absence of STAT1 with phospho-S727 in NSs-expressing HeLa cells (Fig. 2d), our results were generally compatible with the notion that NSs might suppress STAT1 phosphorylation at S727 in both the cytoplasm and the nucleus. These data do not support the model in which

NSs functions solely to sequester STAT1 and STAT2 in the cytoplasm (Ning et al., 2015).

Whether SFTSV NSs affects STAT1 and STAT2 recruitment to the ISREs in ISG promoters has not yet been characterized. To address this, we performed chromatin immunoprecipitation (ChIP) assay as described (Tang et al., 2014). Primers for qPCR analysis of ISREs in IFI6 and ADAR1 promoters are presented in the supplementary material. IFI6 and ADAR1 are two representative ISGs (Samuel, 2011; Schneider et al., 2014). They were chosen in the ChIP-qPCR assay only for technical reasons. We observed that IFN-β-induced recruitment of STAT1 and STAT2 to the ISREs in both IFI6 and ADAR1 promoters was impeded in NSs-expressing HEK293 cells (Fig. S3a, b, bar 5 versus 3, and bar 6 versus 4). Consistent with this, mRNA levels of IFI6 and ADAR1 were dampened in the presence of NSs (Fig. S3c, d, bar 3 versus 2). Hence, SFTSV NSs inhibits IFI6 and ADAR1 expression by preventing the recruitment of STAT1 and STAT2 proteins to their promoters.

Several salient points concerning SFTSV NSs-dependent perturbation of IFN production and signalling emerged in our study. First, we provided evidence for differential modulation of IRF3 and NF-κB by NSs (Fig. 1a-f, i-p and Fig. 2). Second, we characterized the suppression of type III IFN signalling by NSs (Fig. 1h). Third, we demonstrated the interaction of NSs with STAT1 (Fig. 3a), the inhibition of IFN-β-induced STAT1 expression and phosphorylation at S727 but not Y701 by NSs (Fig. 2d and Fig. 3b-d). Finally, we documented the reduced recruitment of STAT1 and STAT2 to the IRSEs in ISG promoters in NSs-expressing cells (Fig. S3). Because NSs-deficient

1 viruses might be developed as attenuated SFTSV vaccines and IFNs could be tested as
2 antivirals against SFTSV infection, our work also has implications in the design and
3 development of SFTSV vaccines and antivirals.
4

Acknowledgements

We thank Genhong Cheng, Heinz Feldmann, Takashi Fujita and Shinji Makino for gifts of plasmids, and Roy Wong and Hinson Cheung for critical reading of the manuscript. This work was supported by Hong Kong Research Grants Council-Natural Science Foundation of China Joint Research Scheme (N-HKU 714/12 and 81261160504), Hong Kong Research Grants Council Collaborative Research Fund (HKU1/CRF/11G), Hong Kong Medical Research Fund (HKM-15-M01) and 973 National Basic Research Program (2011CB504704).

References

Bao, C. J., Guo, X. L., Qi, X., Hu, J. L., Zhou, M. H., Varma, J. K., Cui, L. B., Yang, H. T., Jiao, Y. J. & other authors (2011). A family cluster of infections by a newly recognized bunyavirus in eastern China, 2007: further evidence of person-to-person transmission. *Clin Infect Dis* **53**, 1208-1214.

Billecocq, A., Spiegel, M., Vialat, P., Kohl, A., Weber, F., Bouloy, M. & Haller, O. (2004). NSs protein of Rift Valley fever virus blocks interferon production by inhibiting host gene transcription. *J Virol* **78**, 9798-9806.

Cardenas, W. B., Loo, Y. M., Gale, M., Jr., Hartman, A. L., Kimberlin, C. R., Martinez-Sobrido, L., Saphire, E. O. & Basler, C. F. (2006). Ebola virus VP35 protein binds double-stranded RNA and inhibits α/β interferon production induced by RIG-I signaling. *J Virol, **80***, 5168-5178.

Chan, C. P., Mak, T. Y., Chin, K. T., Ng, I. O. L. & Jin, D. Y. (2010). N-linked glycosylation is required for optimal proteolytic activation of membrane-bound transcription factor CREB-H. *J Cell Sci* **123**, 1438-1448.

Chin, K. T., Zhou, H. J., Wong, C. M., Lee, J. M., Chan, C. P., Qiang, B. Q., Yuan, J. G., Ng, I. O. L. & Jin, D. Y. (2005). The liver-enriched transcription factor CREB-H is a growth suppressor protein underexpressed in hepatocellular carcinoma. *Nucleic Acids Res* **33**, 1859-1873.

Chun, A. C. S., Kok, K. H. & Jin, D. Y. (2013). REV7 is required for anaphase-promoting complex-dependent ubiquitination and degradation of translesion DNA polymerase REV1. *Cell Cycle* **12**, 365-378.

Deng, B., Zhang, S., Geng, Y., Zhang, Y., Wang, Y., Yao, W., Wen, Y., Cui, W., Zhou, Y. & other authors (2012). Cytokine and chemokine levels in patients with severe fever with thrombocytopenia syndrome virus. *PLoS One* **7**, e41365.

Gai, Z. T., Zhang, Y., Liang, M. F., Jin, C., Zhang, S., Zhu, C. B., Li, C., Li, X. Y., Zhang, Q. F. & other authors (2012). Clinical progress and risk factors for death in severe fever with thrombocytopenia syndrome patients. *J Infect Dis* **206**, 1095-1102.

1 **Habjan, M. & Pichlmair, A. (2015).** Cytoplasmic sensing of viral nucleic acids. *Curr*
2 *Opin Virol* **11**, 31-37.

3 **Hoffmann, H. H., Schneider, W. M. & Rice, C. M. (2015).** Interferons and viruses:
4 an evolutionary arms race of molecular interactions. *Trends Immunol* **36**, 124-138.

5 **Ikegami, T. & Makino, S. (2011).** The pathogenesis of Rift Valley fever. *Viruses* **3**,
6 493-519.

7 **Ikegami, T., Narayanan, K., Won, S., Kamitani, W., Peters, C. J. & Makino, S.**
8 **(2009).** Rift Valley fever virus NSs protein promotes post-transcriptional
9 downregulation of protein kinase PKR and inhibits eIF2α phosphorylation. *PLoS*
10 *Pathog* **5**, e1000287.

11 **Jin, C., Jiang, H., Liang, M., Han, Y., Gu, W., Zhang, F., Zhu, H., Wu, W., Chen,**
12 **T. & other authors (2015).** SFTS virus infection in nonhuman primates. *J Infect Dis*
13 **211**, 915-925.

14 **Jin, C., Liang, M., Ning, J., Gu, W., Jiang, H., Wu, W., Zhang, F., Li, C., Zhang,**
15 **Q. & other authors (2012).** Pathogenesis of emerging severe fever with
16 thrombocytopenia syndrome virus in C57/BL6 mouse model. *Proc Natl Acad Sci USA*
17 **109**, 10053-10058.

18 **Kalveram, B., Lihoradova, O., Indran, S. V., Lokugamage, N., Head, J. A. &**
19 **Ikegami, T. (2013).** Rift Valley fever virus NSs inhibits host transcription
20 independently of the degradation of dsRNA-dependent protein kinase PKR. *Virology*
21 **435**, 415-424.

22 **Kim, K. H., Yi, J., Kim, G., Choi, S. J., Jun, K. I., Kim, N. H., Choe, P. G., Kim,**
23 **N. J., Lee, J. K. & Oh, M. D. (2012).** Severe fever with thrombocytopenia syndrome,
24 South Korea, 2012. *Emerg Infect Dis* **19**, 1892-1894.

25 **Kok, K. H., Lui, P. Y., Ng, M. H. J., Siu, K. L., Au, S. W. N. & Jin, D. Y. (2011).**
26 The double-stranded RNA-binding protein PACT functions as a cellular activator of
27 RIG-I to facilitate innate antiviral response. *Cell Host Microbe* **9**, 299-309.

Kunsch, C. & Rosen, C. A. (1993). NF-κB subunit-specific regulation of the interleukin-8 promoter. *Mol Cell Biol* **13**, 6137-6146.

Li, D. (2015). Severe fever with thrombocytopenia syndrome: A newly discovered emerging infectious disease. *Clin Microbiol Infect* **21**, 614-620.

Lin, R., Heylbroeck, C., Pitha, P. M. & Hiscott, J. (1998). Virus-dependent phosphorylation of the IRF-3 transcription factor regulates nuclear translocation, transactivation potential, and proteasome-mediated degradation. *Mol Cell Biol* **18**, 2986-2996.

Liu, Q., He, B., Huang, S. Y., Wei, F. & Zhu, X. Q. (2014). Severe fever with thrombocytopenia syndrome, an emerging tick-borne zoonosis. *Lancet Infect Dis* **14**, 763-772.

McMullan, L. K., Folk, S. M., Kelly, A. J., MacNeil, A., Goldsmith, C. S., Metcalfe, M. G., Batten, B. C., Albarino, C. G., Zaki, S. R. & other authors (2012). A new phlebovirus associated with severe febrile illness in Missouri. *N Engl J Med* **367**, 834-841.

Muehlenbachs, A., Fata, C. R., Lambert, A. J., Paddock, C. D., Velez, J. O., Blau, D. M., Staples, J. E., Karlekar, M. B., Bhatnagar, J. & other authors (2014). Heartland virus-associated death in tennessee. *Clin Infect Dis* **59**, 845-850.

Ng, M. H. J., Ho, T. H., Kok, K. H., Siu, K. L., Li, J. & Jin, D. Y. (2011). MIP-T3 is a negative regulator of innate type I IFN response. *J Immunol* **187**, 6473-6482.

Ning, Y. J., Feng, K., Min, Y. Q., Cao, W. C., Wang, M., Deng, F., Hu, Z. & Wang, H. (2015). Disruption of type I interferon signaling by the nonstructural protein of severe fever with thrombocytopenia syndrome virus via the hijacking of STAT2 and STAT1 into inclusion bodies. *J Virol* **89**, 4227-4236.

Ning, Y. J., Wang, M., Deng, M., Shen, S., Liu, W., Cao, W. C., Deng, F., Wang, Y. Y., Hu, Z. & Wang, H. (2014). Viral suppression of innate immunity via spatial isolation of TBK1/IKKε from mitochondrial antiviral platform. *J Mol Cell Biol* **6**, 324-337.

1 **Niu, G., Li, J., Liang, M., Jiang, X., Jiang, M., Yin, H., Wang, Z., Li, C., Zhang,**
2 **Q. & other authors (2013).** Severe fever with thrombocytopenia syndrome virus
3 among domesticated animals, China. *Emerg Infect Dis* **19**, 756-763.

4 **Precious, B., Childs, K., Fitzpatrick-Swallow, V., Goodbourn, S., & Randall, R. E.**
5 **(2005).** Simian virus 5 V protein acts as an adaptor, linking DDB1 to STAT2, to
6 facilitate the ubiquitination of STAT1. *J Virol* **79**, 13434-13441.

7 **Qu, B., Qi, X., Wu, X., Liang, M., Li, C., Cardona, C. J., Xu, W., Tang, F., Li, Z.**
8 **& other authors (2012).** Suppression of the interferon and NF-κB responses by severe
9 fever with thrombocytopenia syndrome virus. *J Virol* **86**, 8388-8401.

10 **Randall, R. E. & Goodbourn, S. (2008).** Interferons and viruses: an interplay between
11 induction, signalling, antiviral responses and virus countermeasures. *J Gen Virol* **89**, 1-
12 47.

13 **Samuel, C. E. (2011).** Adenosine deaminases acting on RNA (ADARs) are both
14 antiviral and proviral. *Virology* **411**, 180-193.

15 **Santiago, F. W., Covaleda, L. M., Sanchez-Aparicio, M. T., Silvas, J. A., Diaz-**
16 **Vizarreta, A. C., Patel, J. R., Popov, V., Yu, X. J., García-Sastre, A. & Aguilar, P.**
17 **V. (2014).** Hijacking of RIG-I signaling proteins into virus-induced cytoplasmic
18 structures correlates with the inhibition of type I interferon responses. J Virol **88**, 4572-
19 4585.

20 **Schneider, W. M., Chevillotte, M. D. & Rice, C. M. (2014).** Interferon-stimulated
21 genes: a complex web of host defenses. *Annu Rev Immunol* **32**, 513-545.

22 **Schreiber, E., Matthias, P., Muller, M. M. & Schaffner, W. (1989).** Rapid detection
23 of octamer binding proteins with 'mini-extracts', prepared from a small number of cells.
24 *Nucleic Acids Res* **17**, 6419.

25 **Sun, Y., Jin, C., Zhan, F., Wang, X., Liang, M., Zhang, Q., Ding, S., Guan, X.,**
26 **Huo, X. & other authors (2012).** Host cytokine storm is associated with disease
27 severity of severe fever with thrombocytopenia syndrome. *J Infect Dis* **206**, 1085-1094.

Takahashi, T., Maeda, K., Suzuki, T., Ishido, A., Shigeoka, T., Tominaga, T., Kamei, T., Honda, M., Ninomiya, D. & other authors (2014). The first identification and retrospective study of Severe fever with thrombocytopenia syndrome in Japan. *J Infect Dis* **209**, 816-827.

Takaoka, A., Tanaka, N., Mitani, Y., Miyazaki, T., Fujii, H., Sato, M., Kovarik, P., Decker, T., Schlessinger, J. & Taniguchi, T. (1999). Protein tyrosine kinase Pyk2 mediates the Jak-dependent activation of MAPK and Stat1 in IFN-γ, but not IFN-α, signaling. *EMBO J* **18**, 2480-2488.

Tang, H. M. V., Gao, W. W., Chan, C. P., Cheng, Y., Chaudhary, V., Deng, J. J., Yuen, K. S., Wong, C. M., Ng, I. O. L. & other authors (2014). Requirement of CRTC1 coactivator for hepatitis B virus transcription. *Nucleic Acids Res* **42**, 12455-12468.

Tang, H. M. V., Gao, W. W., Chan, C. P., Siu, Y. T., Wong, C. M., Kok, K. H., Ching, Y. P., Takemori, H. & Jin, D. Y. (2013). LKB1 tumor suppressor and salt-inducible kinases negatively regulate human T-cell leukemia virus type 1 transcription. *Retrovirology* **10**, 40.

Walter, C. T. & Barr, J. N. (2011). Recent advances in the molecular and cellular biology of bunyaviruses. *J Gen Virol* **92**, 2467-2484.

Wang, J., Selleck, P., Yu, M., Ha, W., Rootes, C., Gales, R., Wise, T., Crameri, S., Chen, H. & other authors (2014). Novel phlebovirus with zoonotic potential isolated from ticks, Australia. *Emerg Infect Dis* **20**, 1040-1043.

Wen, Z., Zhong, Z. & Darnell, J. E., Jr. (1995). Maximal activation of transcription by Stat1 and Stat3 requires both tyrosine and serine phosphorylation. *Cell* **82**, 241-250.

Wickremasinghe, M. I., Thomas, L. H., O'Kane, C. M., Uddin, J. & Friedland, J. S. (2004). Transcriptional mechanisms regulating alveolar epithelial cell-specific CCL5 secretion in pulmonary tuberculosis. *J Biol Chem* **279**, 27199-27210.

Wong, L. H., Sim, H., Chatterjee-Kishore, M., Hatzinisiriou, I., Devenish, R. J., Stark, G. & Ralph, S. J. (2002). Isolation and characterization of a human STAT1

1 gene regulatory element. Inducibility by interferon (IFN) types I and II and role of IFN
2 regulatory factor-1. *J Biol Chem* **277**, 19408-19417.

3 **Wu, X., Qi, X., Qu, B., Zhang, Z., Liang, M., Li, C., Cardona, C. J., Li, D. & Xing,**
4 **Z. (2014).** Evasion of antiviral immunity through sequestering of TBK1/IKKε/IRF3
5 into viral inclusion bodies. *J Virol* **88**, 3067-3076.

6 **Yu, X. J., Liang, M. F., Zhang, S. Y., Liu, Y., Li, J. D., Sun, Y. L., Zhang, L.,**
7 **Zhang, Q. F., Popov, V. L. & other authors (2011).** Fever with thrombocytopenia
8 associated with a novel bunyavirus in China. *N Engl J Med* **364**, 1523-1532.

9 **Yuen, K. S., Chan, C. P., Wong, N. H., Ho, C. H., Ho, T. H., Lei, T., Deng, W.,**
10 **Tsao, S. W., Chen, H. & other authors (2015).** CRISPR/Cas9-mediated genome
11 editing of Epstein-Barr virus in human cells. *J Gen Virol* **96**, 626-636.

12 **Yun, S. M., Lee, W. G., Ryou, J., Yang, S. C., Park, S. W., Roh, J. Y., Lee, Y. J.,**
13 **Park, Cchaudhary. & Han, M. G. (2014).** Severe fever with thrombocytopenia
14 syndrome virus in ticks collected from humans, South Korea, 2013. *Emerg Infect Dis*
15 **20**, 1358-1361.

16

17

1 **Figure Legends**

2 **Fig 1.** SFTSV NSs suppresses IFN induction and signalling. (a-h) HEK293 cells
3 expressing the indicated proteins and reporter were infected with 100 hemagglutinating
4 units/ml of Sendai virus (SENV; a, b, e), transfected with 200 ng/ml of MAVS
5 expression vector (c, d) or treated with TNF-α (10 ng/ml, f), IFN-β (1000 U/ml
6 purchased from PBL; g) or IFN-λ1 (100 ng/ml from Peprotech; h) for 24 hours. NSs
7 from Hb29 strain of SFTSV was cloned into pVR1012 expression vector and 200 ng/ml
8 of this plasmid was used to transfect cells. Dual luciferase assay was performed. Results
9 represent means ± SD (n = 3). S-NSs: SFTSV NSs. VP35: Ebola virus VP35. R-NSs:
10 RVFV NSs. IκB-sr: IκBα super-repressor with S→A mutations at positions 32 and 36
11 (EMD Millipore). The difference between groups 2 and 3 in b was statistically not
12 significant (n.s.) by Student's t test ($p = 0.22$). (i-p) SFTSV suppression of IFN-α1
13 signalling. THP-1 cells were treated with 100 nM of phorbol 12-myristate 12-acetate
14 for 72 hours to induce the differentiation into macrophages. HEK293T cells and
15 induced THP-1 cells were then either mock-infected or infected with Hb29 strain of
16 SFTSV at 1000 $TCID_{50}$/ml. At 24 hours post infection, cells were either mock-treated
17 or treated with 10 ng/ml of IFN-α1 or 100 ng/ml of LPS for an additional 16 hours.
18 mRNA levels of the indicated ISGs were measured by RT-qPCR. Results represent
19 means ± SD (n = 3). Differences between selected groups were highlighted with
20 asterisks and statistically assessed by Student's t test. The p values are 0.018 (i), 0.0009
21 (j), 0.040 (k), 0.008 (l), 0.025 (m), 0.003 (n), 0.49 (o) and 0.39 (p).

22

23 **Fig. 2.** Influence of SFTSV NSs on NF-κB and STAT1 activation. (a) HeLa cells were
24 transfected with an expression vector for HA-tagged NSs protein for 48 hours. Cells

1 were stimulated with 40 nM of phorbol 12-myristate 12-acetate for 30 minutes and then
2 stained for HA and p65. The NSs- (green) and p65-specific (red) fluorescent signals
3 were merged in panel 3. (b-d) HA-tagged NSs was expressed in HeLa cells for 48 hours.
4 Cells were treated with IFN-β (1000 U/ml) for 30 minutes and then stained for HA
5 (green) and STAT1, STAT2 or phospho-S727 STAT1 (red). Nuclear morphology (blue)
6 was visualized with 4′,6-diamidine-2′-phenylindole dihydrochloride (DAPI). Different
7 fluorescent signals were merged in panel 4. Arrows point to NSs-expressing cells,
8 whereas NSs-non-expressing cells in the same field are highlighted by arrowheads. Bar,
9 20 μm.

10

11 **Fig. 3.** Interaction of SFTSV NSs with STAT1 and STAT2. (a) Immunoprecipitation
12 (IP). HEK293 cells were either mock-transfected or transfected with an expression
13 vector for HA-tagged NSs. Cell lysates were collected and subjected to precipitation
14 with mouse anti-HA antibody. Precipitates and cell lysates (input) were analyzed by
15 Western blotting with antibodies against the indicated proteins. All blots were exposed
16 for 3 minutes. Rabbit polyclonal anti-STAT1 and anti-STAT2 antibodies were
17 purchased from Santa-Cruz. Similar results were also obtained for V5-tagged NSs. (b,
18 c) Steady-state levels of STAT1 protein in NSs-expressing HEK293 cells treated with
19 IFN-β (1000 U/ml). Relative levels of STAT1 protein normalized to β-actin (STAT1/β-
20 actin) are determined by densitometry and indicated below the panel. Some cells were
21 treated with of 5 μg/ml of actinomycin D (ActD) for 6 hours before harvest. (d) NSs
22 inhibits STAT1 phosphorylation at S727. HEK293 cells were treated with IFN-β (1000
23 U/ml) for 24 hours. Whole cell extracts (WCE) as well as cytosolic and nuclear
24 fractions were prepared and probed for the indicated proteins. Rabbit polyclonal

1 phospho-specific antibodies recognizing phospho-Y701 (Y701p) and phospho-S727
2 (S727p) of STAT1 were purchased from Cell Signalling. Cell fractionation was
3 performed as described (Schreiber et al., 1989). Relative levels of STAT1 S727p
4 protein normalized to total STAT1 and either glyceraldehyde-3-phosphate
5 dehydrogenase (GAPDH) or lamin C (rel. S727p) are indicated below the panels.

6

7

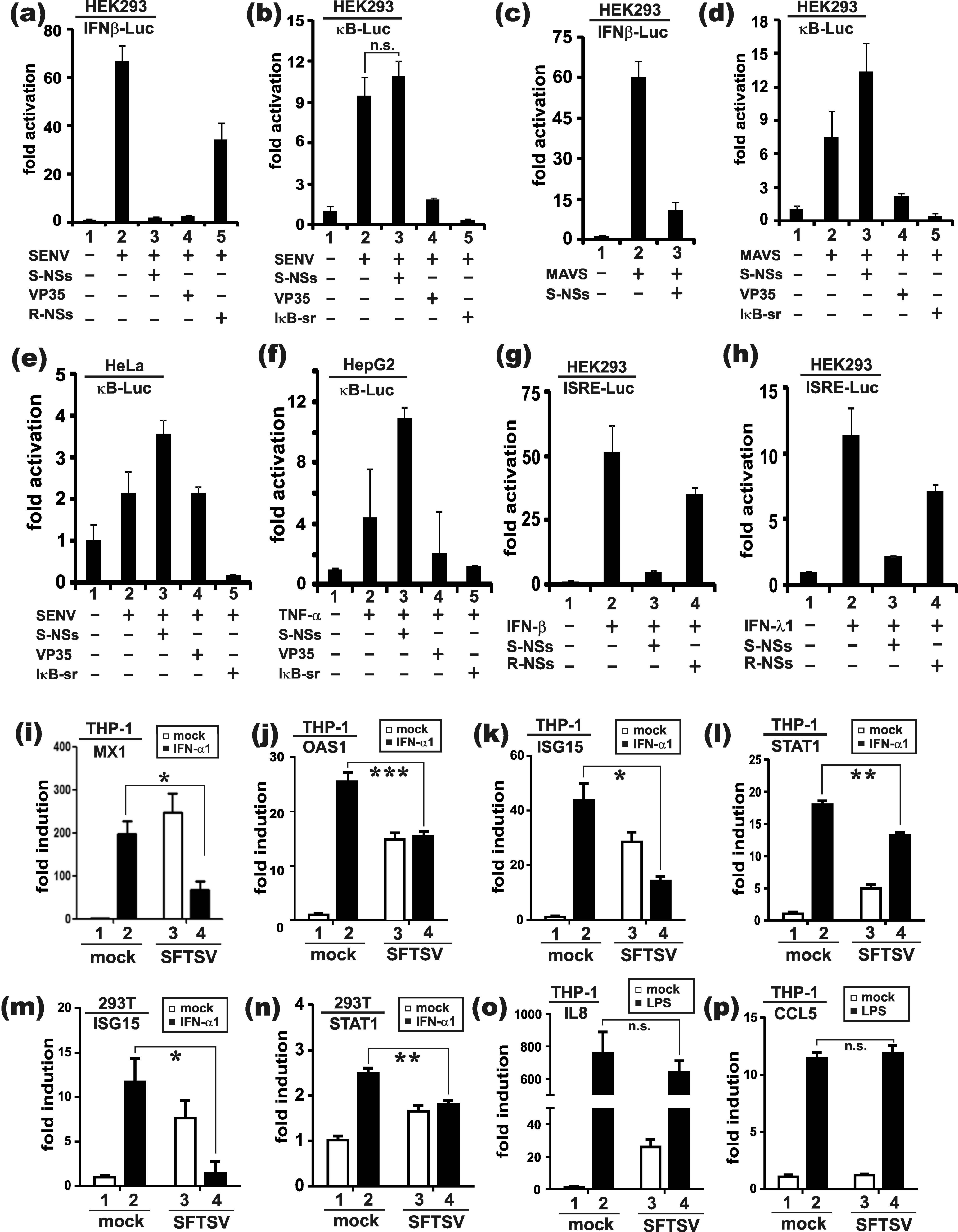


Figure 1

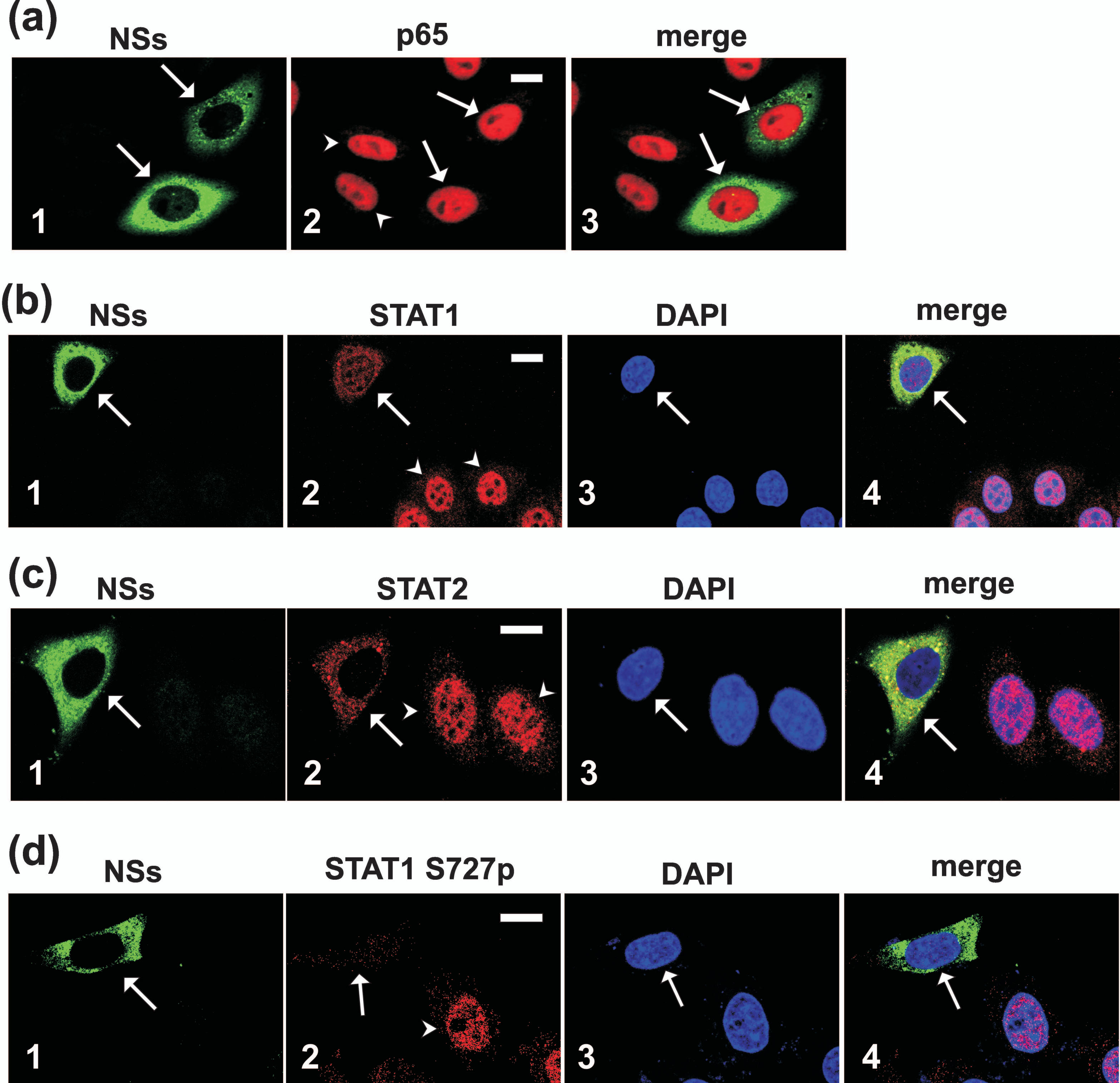


Figure 2

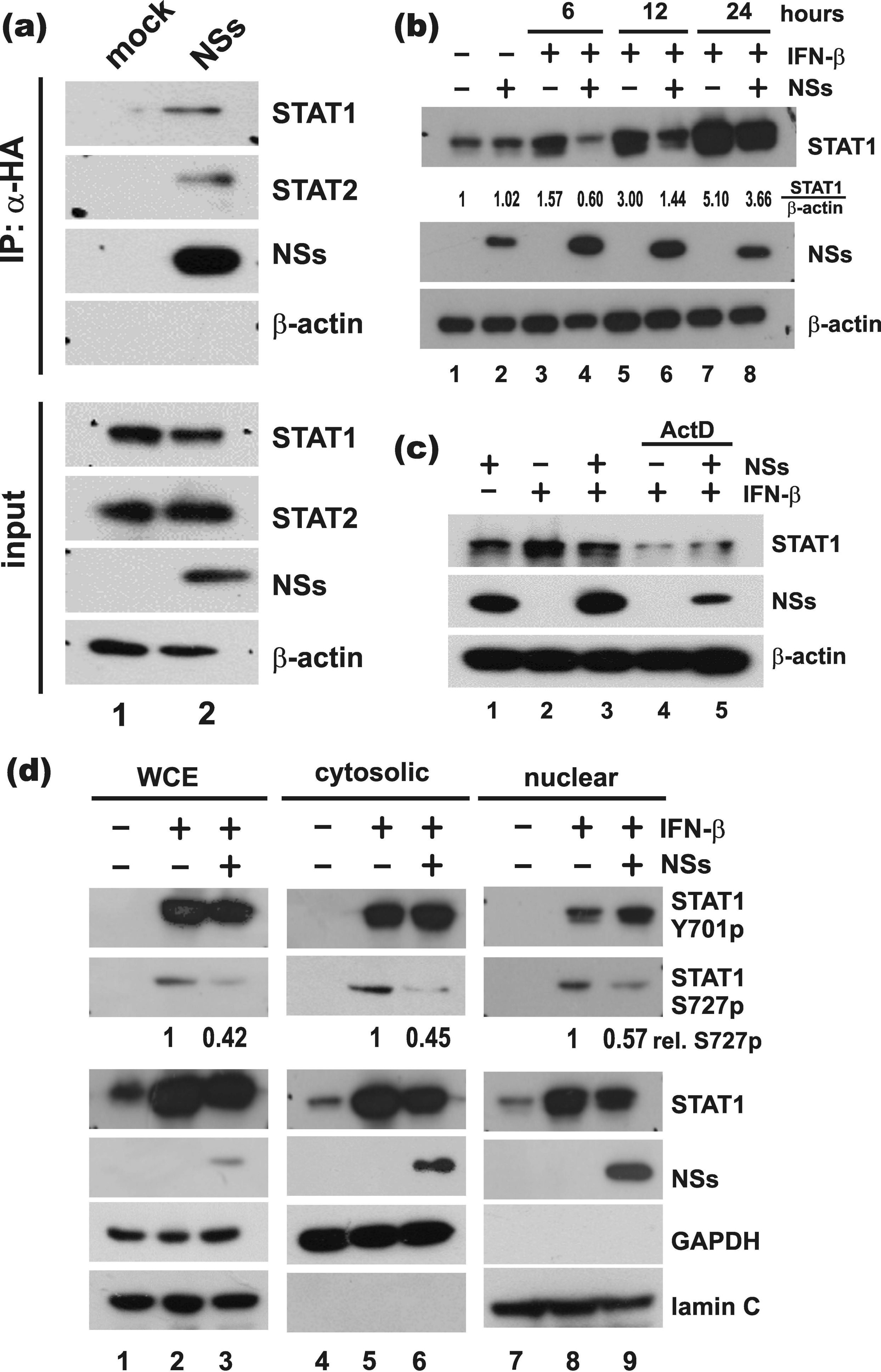


Figure 3

Table S1. Primers used in this study

Primer	Sequence
ISG56-F	5′-GCAGCCAAGTTTTACCGAAG-3′
ISG56-R	5′-GCCTTTCTCCGAAGTTTCCT-3′
ISG15-F	5′-GACCTGACGGTGAAGATGCT-3′
ISG15-R	5′-GAAGGTCAGCCAGAACAGGT-3′
MX1-F	5′-CTACACACCGTGACGGATATG-3′
MX1-R	5′-CGAGCTGGATTGGAAAGCCC-3′
STAT1-F	5′-CATCTTCTCTGGCGACAG-3′
STA1-R	5′-CAGTAAGATGCATGATGCCC-3′
OAS1-F	5′-CATCCGCCTAGTCAAGCACTG-3′
OAS1-R	5′-CACCACCCAAGTTTCCTGTAG-3′
IL8-F	5′-TCTGCAGCTCTGTGTGAAGGTGCAGTT-3′
IL8-R	5′-AACCCTCTGCACCCAGTTTTCCT-3′
CCL5-F	5′-GCATCTGCCT CCCCATATT-3′
CCL5-R	5′-AGCACTTGCC ACTGGTGTAG-3′
IFI6-F	5′-TAAGAAAAAGTGCTCGGAGAGCTC-3′
IFI6-R	5′-CCGACGGCCATGAAGGT-3′
ADAR1-F	5′-GCTCTCCGTGTCTTGATTGG-3′
ADAR1-R	5′-CTGCCAGTGAGAGGGAGTGT-3′
GAPDH-F1	5′-GGAGCGAGATCCCTCCAAAAT-3′
GAPDH-R1	5′-GGCTGTTGTCATACTTCTCATGG-3′
GAPDH-F2	5′-ACCACAGTCCATGCCATCAC-3′
GAPDH-R2	5′-TCCACCACCCTGTTGCTGTA-3′
qChIP-IFI6-F	5′-GCAGGCAGCACACAAATG-3′
qChIP-IFI6-R	5′-CAATCCCTGTCGGAGTTTCT-3′
qChIP-ADAR1-F	5′-AAGCGTGGCGCAAGATTT-3′
qChIP-ADAR1-R	5′-GATGGCTCCGGTTCAATTT-3′

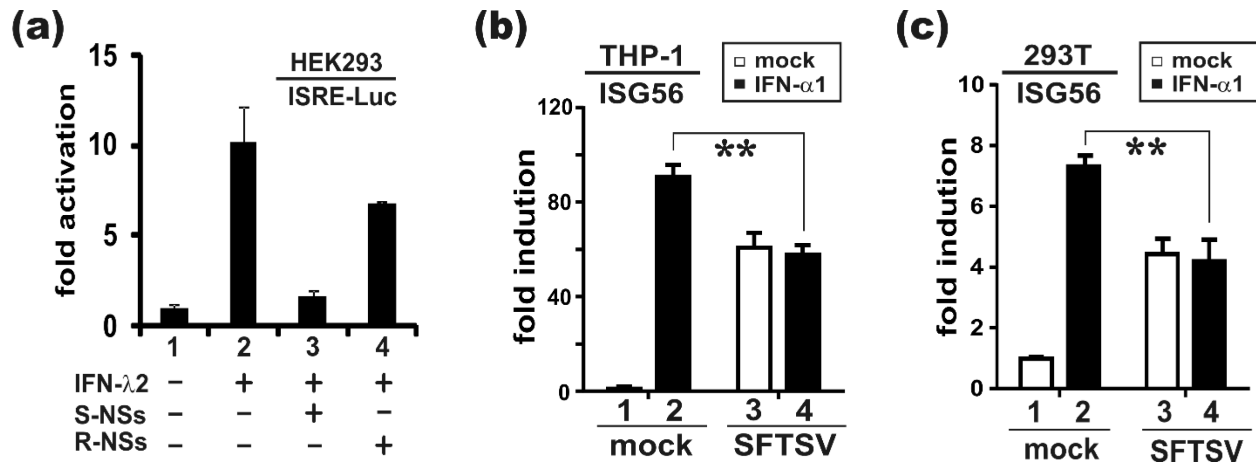


Fig S1. SFTSV NSs suppresses IFN-λ2 signalling and ISG56 induction. (a) Suppression of IFN-λ2 signalling by SFTSV-NSs. HEK293 cells transfected with ISRE-Luc reporter were treated with IFN-λ2 (100 ng/ml from PBL) for 24 hours. Dual luciferase assay was performed. Results represent means ± SD (n = 3). S-NSs: SFTSV NSs. R-NSs: RVFV NSs. (b, c) Phorbol 12-myristate 12-acetate-induced THP-1 cells and HEK293T cells were mock-infected or infected with SFTSV. Cells were then mock-treated or treated with 10 ng/ml of IFN-α1 at 24 hours post infection. Levels of ISG56 mRNA were analysed by RT-qPCR. Results represent means ± SD (n = 3). Differences between the selected groups were highlighted with asterisks and statistically assessed by Student's t test. The p values are 0.005 (b) and 0.007 (c).

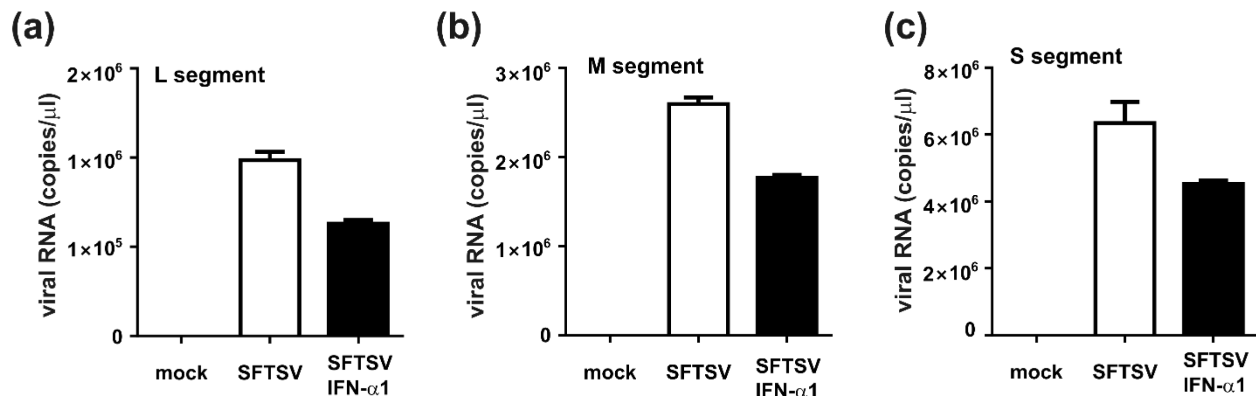


Fig. S2. RT-qPCR analysis of SFTSV RNA segments. Copy numbers of L, M and S segments in infected and IFN-α1-treated THP-1 cells were calculated as described (Jin et al., 2012).

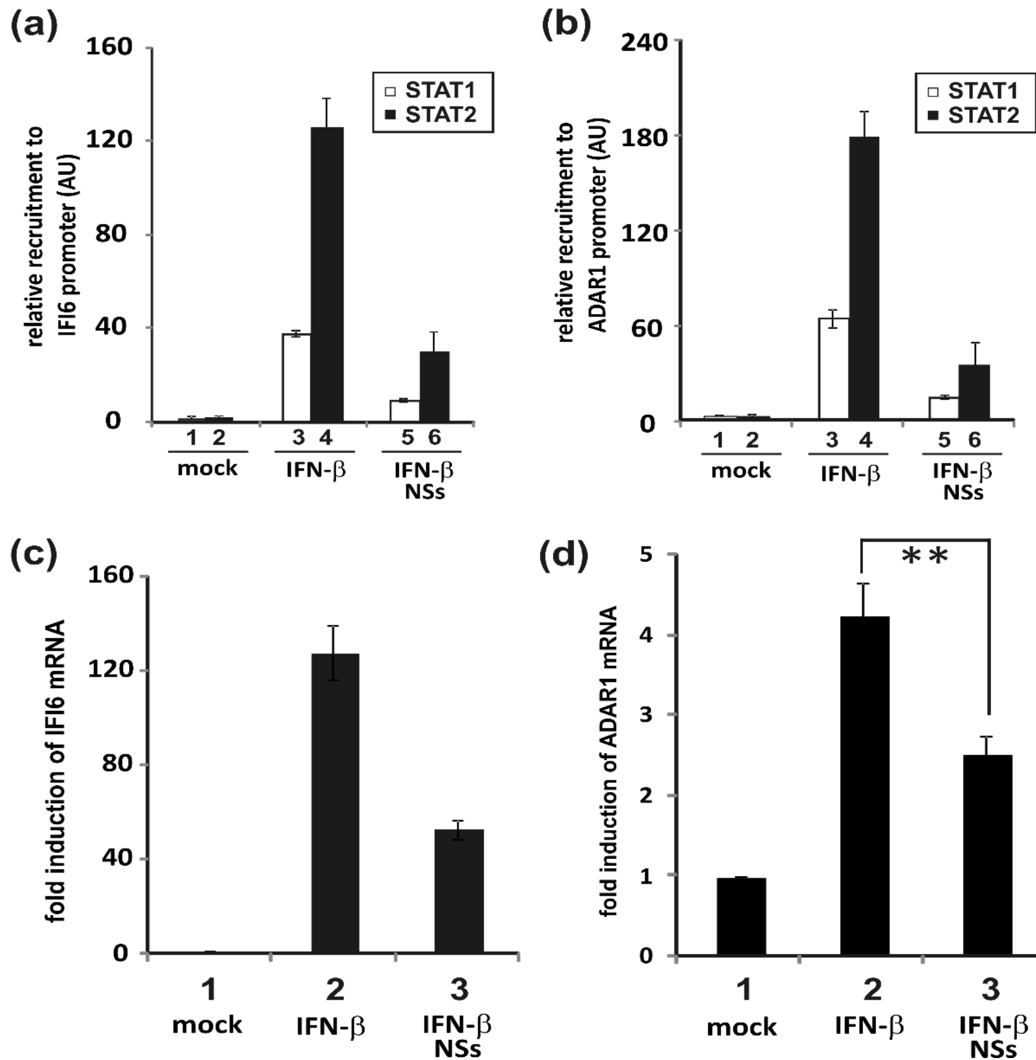


Fig. S3. SFTSV NSs inhibits ISG expression by impeding STAT1 and STAT2 recruitment. (a, b) STAT1 and STAT2 recruitment to the ISREs in IFI6 and ADAR1 promoters in IFN-β-treated HEK293 cells was blunted when NSs was overexpressed. ChIP was performed with anti-STAT1 and anti-STAT2. The STAT-bound ISREs were analyzed by qPCR. Expression of SFTSV N protein had no influence on STAT recruitment (data not shown). AU: arbitrary unit. (c, d) Verification of mRNA expression. Levels of IFI6 and ADAR1 mRNA in NSs-expressing and IFN-β-treated HEK293 cells were analyzed by RT-qPCR. The difference between bars 2 and 3 in d ($p = 0.0027$; highlighted with **) was statistically significant by Student's t test.