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Severe fever with thrombocytopenia syndrome phlebovirus nonstructural protein activates TPL2 signalling pathway for viral immunopathogenesis

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Severe fever with thrombocytopenia syndrome phlebovirus (SFTSV) listed in the World Health Organization Prioritized Pathogens is an emerging Phlebovirus with a high fatality\textsuperscript{1-4}. Due to the lack of therapies and vaccines\textsuperscript{5,6}, there is a pressing need to understand SFTSV pathogenesis. SFSTV nonstructural protein (NSs) has been shown to block type-I interferon (IFN) induction\textsuperscript{7-11} and facilitate disease progression\textsuperscript{12,13}. Here, we report that SFTSV NSs targets the TPL2-ABIN2-p105 complex to induce the expression of IL-10 for viral pathogenesis. Combination of reverse genetics, TPL2 kinase inhibitor and $Tpl2^{-/-}$ mice showed that NSs interacted with ABIN2 and promoted TPL2 complex formation and signalling activity, resulting in the marked upregulation of $\textit{Il-10}$ expression. While SFTSV infection of wild-type mice led to rapid weight loss and death, $Tpl2^{-/-}$ mice or $\textit{Il-10^{-/-}}$ mice survived an infection. Furthermore, SFTSV-NSs P\textsubscript{102}A and SFTSV-NSs K\textsubscript{211}R that lost the ability to induce TPL2 signalling and IL-10 production showed drastically reduced pathogenesis. Remarkably, the exogenous administration of recombinant IL-10 effectively rescued the attenuated pathogenic activity of SFTSV-NSs P\textsubscript{102}A, resulting in a lethal infection. Our study demonstrates that SFTSV NSs targets the TPL2 signalling pathway to induce immune suppressive IL-10 cytokine production as means to dampen the host defense and promote viral pathogenesis.
SFTSV contains three segments of negative or ambisense RNA genome designated as large (L), medium (M), and small (S). The S segment encodes a nucleoprotein (N) and a nonstructural protein (NSs) via an ambisense coding strategy. As SFTSV infection has been demonstrated to lead to the suppression of host antiviral immunity, work has shown that NSs plays an important role in immune evasion by interacting with type I interferon (IFN) signalling effectors RIG-I, MAVS, TRIM25, TBK1, and STAT1, leading to their sequestration to virus-induced inclusion bodies (IB), resulting in the inhibition of IFN and NF-κB responses. Similar to SFTSV NSs, the NSs protein of other phleboviruses including Heartland-, Bhanja-, Granada-, and Lone Star viruses except for an apathogenic Uukuniemi virus (UUKV) were able to effectively suppress the activation of the IFN-β (Ifnb1) promoter induced by Sendai virus (SeV) infection (Supplementary Fig. 1a). Surprisingly, when aligned with the NSs of other pathogenic phleboviruses, SFTSV NSs exhibited limited homology, except for two conserved sequences (S97xLRWPxG104 and D282WP284). SFTSV NSs also carries two potential protein-interacting motifs (P66xxP69 for SH3 interaction and W133xxL136 for LC3 interaction) (Supplementary Fig. 1b). When NSs mutants carrying changes at these four sequences were examined for IB formation and IFN signalling suppression, three mutants (NSs-WxxL, NSs-PxxP, and NSs-DWP) formed cytoplasmic IBs similar to NSs wild-type (WT), whereas a NSs-8A mutant carrying alanine substitutions of the S97xLRWPxG104 8 amino acids could no longer form IBs (Supplementary Fig. 2a). NSs-WT and three mutants that retained the ability to form IBs potently suppressed SeV-induced IFN-β promoter activation, whereas NSs-8A that didn’t form IBs did not (Supplementary Fig. 2b). Furthermore, a NSs-P102A (PA) mutant carrying an alanine replacement within the SxLRWPxG sequence neither formed IBs, nor suppressed the expression and secretion of IFN-β upon SeV infection (Fig. 1ab and Supplementary Fig. 2c, respectively). These results indicate the direct correlation between the IB formation and the IFN signaling-inhibition ability of SFTSV NSs.

We next investigated the effects of NSs on host immune gene expression either in Raw264.7 cells expressing NSs or upon WT or mutant SFTSV infection of Ifnar^-/- mice. Using a reverse genetic system, we rescued wild-type virus (SFTSV-WT) and NSs-PA mutant virus (SFTSV-PA) (Supplementary Fig. 2d). While SFTSV-WT produced larger plaques than SFTSV-PA, both viruses had similar replication kinetics in IFN-negative Vero E6 cells (Supplementary Fig. 2ef). Total RNA isolated from the spleens of mock-, SFTSV-WT- or SFTSV-PA-infected Ifnar^-/- mice or from Raw264.7-vector or Raw264.7-NSs-WT cells with or without lipopolysaccharide (LPS) stimulation were subjected to NanoString analysis (Supplementary Fig. 3a and Supplementary Data). SFTSV-WT infection of Ifnar^-/- mice not only broadly induced
immune response genes, but also markedly suppressed IFN-responsible genes compared to
SFTSV-PA infection (Fig. 1cd and Supplementary Fig. 3bc). Remarkably, the majority of
downregulated host genes in SFTSV-WT-infected mouse spleens were B cell-specific genes
(Fig. 1c and Supplementary Fig. 3d), which appeared to be consistent with recent reports15,16.
Interestingly, nine genes (Il-10, PD-L1, Phlpp1, Sele, Socs3, Nos2, Cxcl1, Cxcl13, and Hif1α),
which were comprehensively induced by SFTSV-WT infection in Ifnar1−/− mice or by NSs-WT
expression in Raw264.7 cells, but not by SFTSV-PA infection, function in immune suppressive
pathways (Fig. 1e and Supplementary Table. 1).

In particular, IL-10 is an anti-inflammatory cytokine that limits host immune responses to
pathogens to prevent its own damage17 and is induced by many viruses to dampen the host
defense18-20. Real-time quantitative RT-PCR showed that NSs-WT markedly increased Il-10
expression in Raw264.7 cells by ~50 fold under no stimulation, and by ~200 and ~1300 fold
upon SeV infection or LPS stimulation, respectively, whereas NSs-PA induced little or no Il-10
expression under any conditions (Fig. 1f-i). Furthermore, SFTSV-WT infection, but not SFTSV-
PA infection, considerably induced Il-10 expression in Raw264.7 cells, mouse bone marrow
derived macrophages (BMDMs), and human whole blood in vitro (Fig. 1j-l). In addition, SFTSV-
WT infection led to the increase of Il-10 expression in spleens and livers of Ifnar1−/− mice by ~70
and ~200 fold, respectively, whereas SFTSV-PA infection showed little or no increase of Il-10
expression (Fig. 1mn). Finally, we also found highly-elevated levels of IL-10 in blood of SFTSV-
infected patients along with IL-6 and TNFα (Fig. 1o and Supplementary Fig. 3e). These findings
collectively indicate that the SFTSV NSs robustly induces Il-10 expression.

Yeast two-hybrid (Y2H) screen found that NSs interacted with A20-binding protein 2
(ABIN2). Co-immunoprecipitation (Co-IP) showed the specific interaction of NSs with
exogenous ABIN2 in HEK293T cells and endogenous ABIN2 in Raw264.7 cells (Fig. 2ab) as
well as the interaction of virally-produced NSs with ABIN2 in SFTSV-infected Raw264.7 cells
(Fig. 2c). Furthermore, NSs specifically bound to ABIN2 but not to ABIN1 and ABIN3 despite
their high homology21 (Fig. 2d). By using truncation mutagenesis, the middle region (148-220) of
NSs was found to be responsible for ABIN2-binding (Supplementary Fig. 4a). Y2H mapping and
Glutathione S-transferase (GST) pull-down assays revealed the ABIN homology domains
(AHDs) of ABIN2 for efficient NSs interaction (Supplementary Fig. 4bc). The single-molecule
pull-down (SiMPull) assay22 also revealed that SFTSV NSs effectively bound to ABIN2
(Supplementary Fig. 4d). Finally, the NSs protein of other phleboviruses showed interaction with
ABIN2 as strongly as the SFTSV NSs (Supplementary Fig. 4e). However, the UUKV NSs
neither bound ABIN2 nor induced Il-10 expression (Supplementary Fig. 4fg). In parallel, UUKV
infection failed to induce \textit{Il-10} expression compared to SFTSV and Heartland virus infection (Supplementary Fig. 4h). These suggest the strong correlation between NSs-induced \textit{Il-10} expression and phlebovirus pathogenesis.

ABIN2 preferentially forms a ternary complex with TPL2 kinase (tumor progression locus 2) and p105/NF-κB1 that is required to maintain TPL2 stability\textsuperscript{23}. Differential translational initiation of the \textit{Tpl2} mRNA gives rise to two isoforms (p58 and p52)\textsuperscript{24}. Upon LPS stimulation, p58 TPL2 is preferentially released from p105 relative to p52 TPL2 and released p58 TPL2 initiates downstream signalling and subsequently undergoes rapid degradation\textsuperscript{24}. NSs-WT-expressing Raw264.7 cells showed increased amounts of TPL2 (p58/p52) and the rapid degradation of p58 TPL2 compared to vector- or NSs-PA-expressing Raw264.7 cells (Fig. 2e).

SiMPull assay also revealed that NSs-WT, but not NSs-PA, increased the TPL2-ABIN2-p105 ternary complex formation (Fig. 2f). As this ternary complex formation governs TPL2 stability and its signalling activity, expression of the NSs-WT but not the NSs-PA increased TPL2 amounts, ultimately resulting in the enhanced activation of downstream MEK1/2 and ERK1/2 upon LPS stimulation as evidenced by increased reactivity of their activation-dependent phospho-specific antibodies (Fig. 2e). Thus, SFTSV NSs stabilizes and activates the TPL2 signalling complex through its specific interaction with ABIN2 (Supplementary Fig. 4i). Indeed, depletion of \textit{ABIN2} expression not only abrogated the interaction between NSs-WT and TPL2/p105 (Supplementary Fig. 5ab), but also led to the reduction of NSs-mediated induction of \textit{Il-10} expression (Supplementary Fig. 5c).

ABIN2 alone formed cytoplasmic punctate structures similar to NSs (Supplementary Fig. 6a). When NSs and ABIN2 were co-expressed, they showed partial co-localization. Surprisingly, NSs-PA showed much higher ABIN2 binding and co-localization compared to NSs-WT (Fig. 2g and Supplementary Fig. 6ab). Increasing expression of NSs-PA suppressed NSs-WT activity to induce TPL2-mediated \textit{Il-10} expression, despite its increased ABIN2 binding (Supplementary Fig. 7ab). Additional mutation analyses identified that the NSs-K\textsubscript{211R} (KR) mutant, carrying replacement of the lysine 211 with arginine (Supplementary Fig. 1b), neither bound ABIN2 (Fig. 2g) nor induced \textit{Il-10} expression upon LPS stimulation or SeV infection (Fig. 2h and Supplementary Fig. 7c). While the NSs-WT and NSs-PA interacted with TPL2 and p105 through ABIN2 binding, the NSs-KR showed no interaction with TPL2 and p105 due to its lack of ABIN2 binding (Supplementary Fig. 7de). Moreover, the P\textsubscript{102A} and K\textsubscript{211R} double-mutant (NSs-PK) also showed the loss of ABIN2 interaction (Supplementary Fig. 7f). Functionally, NSs-KR did not activate downstream MEK1/2 and ERK1/2 upon LPS stimulation (Supplementary Fig. 7g). Moreover, SFTSV-KR infection of Raw264.7 cells and mice led to a little or no increase of \textit{Il-10} expression.
expression (Fig. 2i and Supplementary Fig. 7h). On the other hand, NSs-KR interacted with TBK1 and suppressed SeV-induced IFN-β promoter activation as strongly as NSs-WT, whereas NSs-PA could not do both (Supplementary Fig. 7ij), suggesting that NSs-mediated regulation of TPL2 signalling is genetically separable from its regulation of IFN signalling.

As the TPL2 signalling pathway is important for Il-10 expression in macrophage25, treatment with a TPL2 inhibitor (CAS1186649-59-1) not only suppressed the basal level of Il-10 expression but also abrogated the NSs-mediated upregulation of Il-10 expression (Fig. 3a). Consistently, MEK inhibitor, but not c-Raf kinase inhibitor, suppressed the NSs-mediated Il-10 upregulation (Fig. 3b). Moreover, NSs-WT markedly induced Il-10 expression in WT BMDMs, but not in Tpl2<sup>−/−</sup> BMDMs. In contrast, NSs-PA did not induce Il-10 expression in either WT or Tpl2<sup>−/−</sup> BMDMs (Fig. 3c). Correspondingly, SFTSV-WT infection upregulated Il-10 expression in WT or Ifnar<sup>−/−</sup> BMDMs but not in Tpl2<sup>−/−</sup> BMDMs, whereas SFTSV-PA infection did not upregulate Il-10 expression under any conditions (Fig. 3d). Treatment with the TPL2 inhibitor also abrogated Il-10 expression in SFTSV-WT-infected Ifnar<sup>−/−</sup> BMDMs (Fig. 3e). Finally, Il-10 expression was considerably upregulated in spleens of SFTSV-WT-infected mice compared to those of SFTSV-WT-infected Tpl2<sup>−/−</sup> mice where both mice were pretreated with anti-IFNAR1 blocking antibody prior to infection (Fig. 3f). As an activated p58 TPL2 undergoes rapid degradation<sup>24</sup>, SFTSV-WT-infected BMDMs showed the reduced p58 TPL2 along with MEK1/2 and ERK1/2 activation compared to mock- or SFTSV-PA-infected BMDMs, resulting in the marked increase of Il-10 expression (Fig. 3gh). Intriguingly, the TPL2 inhibitor did not affect the degradation of p58 TPL2, while it detectably reduced MEK1/2 phosphorylation (Fig. 3i), suggesting that NSs specifically targets the TPL2-ABIN2-p105 complex formation, not the p58 TPL2 degradation. Of note, the TPL2 signalling pathway was not required for SeV- or LPS-induced IFN-β expression, nor for NSs-mediated IFN-β suppression (Supplementary Fig. 8ab). Moreover, NSs did not affect Tpl2 expression upon several stimulation conditions (Supplementary Fig. 8cd). These results indicate that SFTSV NSs specifically enhances TPL2 complex formation and its signalling activity, resulting in the upregulation of Il-10 expression.

While SFTSV-WT and SFTSV-PA replicated in IFN-negative Vero E6 cells at similar kinetics (Supplementary Fig. 2f), the viral copy numbers of SFTSV-WT were approximately 1.5 logs higher in vitro than those of SFTSV-PA in WT, Tpl2<sup>−/−</sup>, and Ifnar<sup>−/−</sup> BMDMs as well as in human whole blood (Supplementary Fig. 9ab). SFTSV-WT also replicated slightly higher than SFTSV-PA in the spleens and livers of Ifnar<sup>−/−</sup> mice (Supplementary Fig. 9c). On the other hand, treatment with TPL2 inhibitor did not significantly affect SFTSV-WT replication in Ifnar<sup>−/−</sup> BMDMs (Supplementary Fig. 9d), suggesting that TPL2 pathway does not directly influence SFTSV in
vitro replication. To test the role of NSs in SFTSV pathogenesis, we used two lethal challenge mouse models: *Ifnar*\(^{-/-}\) mice and anti-IFNAR1-blocking antibody-treated WT mice or *Tpl2*\(^{-/-}\) mice that were passively administered 2 mg of an anti-IFNAR1-blocking antibody or isotype control IgG antibody 1 day prior to infection. Upon SFTSV-WT infection, *Ifnar*\(^{-/-}\) mice or anti-IFNAR1 antibody-treated WT mice rapidly lost weight, showed severe symptoms, and died over a period of 4-5 days or 5-6 days p.i, respectively (Fig. 4a and 4g). Remarkably, 100% of anti-IFNAR1 antibody-treated *Tpl2*\(^{-/-}\) mice recovered from initial weight loss and survived SFTSV-WT infection (Fig. 4b). Control IgG-treated WT or *Tpl2*\(^{-/-}\) mice did not show any symptom and weight loss, and survived SFTSV infection (Fig. 4ab). Furthermore, SFTSV-WT copy numbers were approximately 5 logs lower in spleens of anti-IFNAR1 antibody-treated *Tpl2*\(^{-/-}\) mice at 4 days p.i than in those of anti-IFNAR1 antibody-treated WT mice (Supplementary Fig. 9e). Remarkably, the administration of TPL2 inhibitor (CAS871307-18-5) substantially improved the survival of anti-IFNAR1 antibody-treated wild-type mice infected with SFTSV: 80% of TPL2 inhibitor-treated mice (9/11) survived SFTSV infection (Fig. 4cd). These data suggest that the NSs-mediated activation of the TPL2 signalling pathway is required for the SFTSV-induced lethal phenotype, and that the inhibition of TPL2 signalling pathway is a potent therapeutic approach to treat SFTSV pathogenesis.

To further investigate role of the NSs-mediated IL-10 production in viral pathogenesis, anti-IFNAR1 antibody-treated *Il-10*\(^{-/-}\) mice were infected with SFTSV-WT. Compared to WT mice that succumbed to infection, 70% of *Il-10*\(^{-/-}\) mice (5/7) recovered from weight loss and survived SFTSV-WT infection (Fig. 4ef). Moreover, *Ifnar*\(^{-/-}\) mice were infected with SFTSV-WT or SFTSV-PA. By striking contrast to SFTSV-WT-infected *Ifnar*\(^{-/-}\) mice that died over a period of 4-5 days p.i, 70% of SFTSV-PA-infected *Ifnar*\(^{-/-}\) mice showed minor symptoms, recovered from weight loss and survived, whereas 30% of them showed moderate symptoms and died over a period of 8-15 days p.i (Fig. 4gh). Remarkably, the administration of recombinant mouse IL-10 (rmIL-10) to SFTSV-PA-infected *Ifnar*\(^{-/-}\) mice promptly led to severe symptoms, rapid weight loss, and death at 5-8 days p.i (Fig. 4gh, yellow triangle). The rmIL-10 treatment of mock-infected *Ifnar*\(^{-/-}\) mice did not lead to any symptoms or death. Finally, compared to SFTSV-WT-infected mice, SFTSV-KR-infected mice showed no symptom and weight loss, and ultimately survived (Fig. 4ij). In summary, NSs-WT binds ABIN2 and induces TPL2-mediated IL-10 production; NSs-PA strongly binds ABIN2 but cannot induce TPL2-mediated IL-10 production; and NSs-KR neither binds ABIN2 nor induces TPL2-mediated IL-10 production (Supplementary Table 2). Correlated with TPL2 activation and IL-10 production, SFTSV-WT induces pathogenesis but SFTSV-KR and SFTSV-PA do not induce pathogenesis. Thus, NSs-WT binds ABIN2 to enhance TPL2-
ABIN2-p105 signalling activity to induce IL-10 production for viral pathogenesis.

As seen in SFTSV-infected patients (Fig. 1o), IL-10 levels were increased in the acute phase of SFTS patients and further induced to higher levels in fatal cases\textsuperscript{12,26}. This suggests that upon SFTSV infection, NSs targets TPL2 signalling pathway to produce IL-10 cytokine that creates a local immune suppressive environment to allow high viral replication, ultimately leading to viral pathogenesis. Thus, the SFTSV NSs and the host’s TPL2 signalling pathway are potential therapeutic targets to treat SFTSV-infected patients.
Methods

Bacterial strains, mammalian cell lines, and culturing conditions

_E. coli_ Top10 and DH10B were grown in LB (Lenox, Sigma) medium for genetic manipulations with appropriate antibiotics (Ampicillin 50 µg/ml, Kanamycin 50 µg/ml). HEK293T, HeLa, and mouse macrophage Raw264.7 cells were cultured in Dulbecco’s modified Eagle’s medium (DMEM, Gibco) supplemented with 10% fetal bovine serum (FBS, Seradigm) and 100 U/ml penicillin, and 100 µg/ml streptomycin (1% Pen/Strep, Gibco). All cells were maintained at 37°C with 5% CO₂. SFTSV NSs-WT, NSs-PA and NSs-KR stably expressing Raw264.7 cells were generated by transfection with pIRES-NSs-3xFlag-puro, followed by selection with puromycin (0.5 µg/ml, Gibco). For viral infection study, Heartland virus, MO-4 (NR-49771) was obtained through BEI Resources, NIAID, NIH, as part of the WRCEVA program and Uukuniemi virus was kindly provided from a collaborator.

Preparation of bone marrow-derived macrophages (BMDMs)

Bone marrow cells (BMs) were removed from femur and tibia, and red blood cells were lysed. For differentiation into BMDMs, BMs were suspended in DMEM supplemented with 20% MCSF containing L929 supernatants, 10% FBS (Seradigm) and 1% Pen/Strep (Gibco) and plated in a 10 cm non-treated plate (1.5x10⁷ cells/plate). On Day 3 or 4, the fresh media containing 10% MCSF was added. After 7 days, BMDMs were used for experiment (3x10⁵ cells/well of a 24-well culture plate).

Plasmids and reagents

DNA encoding NSs from SFTS, Heartland, Bhanja, Granada or Lone Star phleboviruses were synthesized by Integrated DNA Technologies (IDT). cDNAs for human ABIN1, 2, 3, TPL2, and NFκB1/p105 were obtained from Addgene and Origene. All constructs for transient and stable expression in mammalian cells include pEF-MCS-IRES-puro, pEBG-GST-MCS, pCDH-CMV-MCS-EF1-puro (System Biosciences, Dual promoter) vectors. All NSs-expression plasmids contain a C-terminal 3xFlag epitope tag, ABIN1/2/3-expression plasmids have a C-terminal V5 epitope tag, TPL2-expression plasmids have N-terminal Myc epitope tag or C-terminal GFP tag for SiMPull assay, and the p105-expression plasmid contains a C-terminal mCherry tag for SiMPull assay. Substitution and deletion mutants were constructed by a standard PCR cloning strategy. For the generation of recombinant viruses, five plasmids were transfected to BHK21-T7 cells. pTVT7-ppL, pTVT7-ppM, and pTVT7-S encode three viral antigenomic segments
under T7 promoter and pTM1-ppL and pTM1-N produce RdRp and N protein, respectively, to support viral replication. For MAPKs inhibition, TPL2 kinase inhibitors (616404, Calbiochem and sc-204351, Santa Cruz), MEK kinase inhibitor (U0126, Calbiochem), and c-Raf1 kinase inhibitor (475958, Calbiochem) were used along with DMSO as a control.

**Mouse infection**
The USC Institutional Animal Care and Use Committee (IACUC) approved all animal studies. Tpl2-/- mice (C57BL/6 background) and PD-L1-/- mice (BALB/c background) were bred as littermate controls. C57BL/6 wild-type, BALB/c wild-type, Ifnar-/- mice (A129, C57BL/6 background), Il-10-/- mice (C57BL/6 background) were purchased from the Jackson Laboratory. All mice were maintained in pathogen-free barrier at the USC animal facilities and were transferred into the BSL3 facility immediately prior to, and for the duration of the infection study. Mice were 8-12-weeks old during the course of the experiments and were age- and sex-matched in each experiment. Sample size was based on empirical data from pilot experiments and publications. No additional randomization or blinding was used to allocate experimental groups. Immediately prior to infection, frozen SFTSV stock was thawed and centrifuged. Mice were intramuscularly infected with 10^5 PFU or 10^2 PFU of SFTSV per mouse in 150 μl total volume of inoculum. For disease reconstitution, a recombinant mouse IL-10 (BioLegend at 1 μg/mouse) was injected intraperitoneally three times daily. To block the IFN signalling, anti-mouse IFNAR1 IgG (MAR1-5A3, BioXcell at 2 μg/mouse) and its isotype IgG (MOPC-21, BioXcell at 2 μg/mouse) were injected intraperitoneally 1 day prior to virus infection. TPL2 inhibitor CAS871307-18-5 (naphthyridine ATP-competitive inhibitor) was dissolved in DMSO and sesame oil (S3547, Sigma) was used as a vehicle to inject mice. To neutralize the PD-L1 signalling, anti-mouse PD-L1 IgG (B7-H1, 10F.9G2, BioXcell) and its isotype IgG (LTF-2, BioXcell) were injected intraperitoneally 1 day after to virus infection. For the survival experiment, mice were weighed daily after infection. Spleen and liver tissues were collected and used for quantitative PCR.

**Co-immunoprecipitation (Co-IP) and GST pull-down (GST-PD)**
HEK293T or Raw264.7 cells were transfected with indicated DNA plasmids using standard polyethylenimine (PEI) or Viromer® RED, respectively. For SFTSV-NSs-GFP infection, NSs-GFP-FUSE virus was kindly provided by a collaborator. Raw264.7 cells were infected by SFTSV-NSs-GFP for 24 h. Cells were collected at 48 h post-transfection followed by PBS washing and cell pellets were re-suspended in 1% NP-40 lysis buffer containing 50mM Tris-HCl.
(pH 8.0), 150mM NaCl, 1% Nonidet P-40 (Sigma) supplemented with complete protease inhibitor EDTA-free cocktail (Roche). After sonication or freeze/thaw three times, whole-cell extracts (WCEs) were pre-cleared with Sepharose beads rotating at 4°C for 2 h, followed by filtering through a 0.45 μm polyethersulfone (PES) filter (Thermo Fisher). For Co-IP, pre-cleared WCEs were incubated with indicated antibodies at 4°C for 3-12 h, followed by further incubation with protein A/G agarose beads (Thermo Fisher) at 4°C for 3 h. For GST-PD, pre-cleared WCEs were incubated with glutathione S transferase (GSH)-conjugated Sepharose beads (GE) at 4°C for 2 h. Immobilized immune complexes or GST complexes containing beads were extensively washed five times using lysis buffer containing 1% NP40 buffer with various concentrations of NaCl (150-500 mM). Beads were eluted in 2X Laemmli dye and heated for 5 min at 95°C and then subjected immunoblotting analysis.

**Immunoblotting analysis**

Whole-cell lysates (WCLs) were lysed in 1% NP40 buffer and measured for protein concentration by Bradford protein assay (Thermo Fisher) to equalize protein loading. Proteins were resolved on SDS-PAGE gels and transferred to PVDF membrane by semi-dry transfer at 25 V for 30 min (Trans-Blot Turbo, Bio-Rad). All membranes were blocked in 5% milk in Tris buffered saline with Tween20 (TBST, pH 8.0, Sigma) and probed with indicated antibodies in 5% milk or 5% BSA in TBST. Primary antibodies included: ABIN2 (H-300; Santa Cruz), TPL2 (Cot, M-20; Santa Cruz), NF-κB1 (p105/p50, D4P4D; Cell Signaling), NF-κB (p65, D14E12; Cell Signaling), ERK1/2 (p44/42; Cell Signaling), Phospho-ERK1/2 (Phospho-p44/42, E10; Cell Signaling), MEK1/2 (9122, Cell Signaling), Phospho-MEK1/2 (Ser217/221; Cell Signaling), JNK (SAPK, Cell Signaling), Phospho-JNK (Phospho-SAPK, Thr183/Tyr185; Cell Signaling), p38 (Cell Signaling), Phospho-p38 (Thr180/Tyr182; Cell signaling), IKKβ (L570; Cell Signaling), Phospho-IKKα/β (Ser176/180, 16A6; Cell Signaling), GFP (B-2; Santa Cruz), GST (B-14; Santa Cruz), β-actin (C4; Santa Cruz), Flag (rabbit-Flag; F7425; Sigma, mouse-Flag; F1804, Sigma), HA (rabbit-HA; PRB-101P; Covance, mouse-HA; 16B12; BioLegend), V5 (rabbit-V5; A190; Bethyl, mouse-V5; Invitrogen), Myc (rabbit-Myc; Poly9063; BioLegend, mouse-Myc; 9E10; BioLegend). For detection of viral proteins NSs and Gn, rabbit anti-SFTSV-NSs Ab 14 and mouse anti-SFSTV-Gn Ab 28 were kindly provided from collaborators. Appropriate horseradish peroxidase (HRP)-conjugated secondary antibodies were incubated on membranes in 5% milk in TBST at room temperature for 1 h and bands were developed with enhanced chemiluminescence (ECL) reagent (Thermo Fisher) and imaged on an LAS-4000 imager (Fuji).
or a ChemiDoc Touch (Bio-Rad). All source gels with uncropped blots are available in Supplementary information.

Yeast two-hybrid assay

Yeast two-hybrid assay was performed using the Matchmaker GAL4 Two-Hybrid System 3 (Clontech Laboratories, Inc.) per manufacturer's instructions. SFTSV NSs (2-293 amino acids, aa) was subcloned into the vector pGBK7 as bait. Saccharomyces cerevisiae AH109 cells expressing NSs were transformed with a human leukocyte matchmaker complementary DNA library. To test the interactions between proteins, the transformants were grown onto low-stringency (lacking leucine and tryptophan) and high-stringency (lacking adenine, histidine, leucine and tryptophan) selection plates.

Luciferase reporter assay

HEK293T cells were seeded in 12-well plates at 4x10^5 cells per well in the growth medium and cultured overnight. To measure IFN-β, HEK293T cells were co-transfected with 100 ng of IFN-β luciferase reporter plasmid, 20 ng of TK-renilla luciferase plasmid and 100 ng of plasmid encoding NSs proteins. 48 h after transfection, WCLs were prepared and subjected to the Dual-Glo luciferase assay per manufacturer's instructions (Promega). Results are presented with renilla luciferase levels normalized by the firefly luciferase levels. Luciferase report assay was performed in triplicate with at least three independently transfected cell populations.

Immunofluorescence confocal microscopy

HeLa cells and Raw264.7 stable cells were seeded onto glass coverslips. After 24 h, cells were transfected using Lipofectamine 2000 (Invitrogen). Cells were washed 18 h after transfection, fixed in 4% paraformaldehyde, permeabilized with 0.5% Triton X-100, and blocked with 5% BSA for 1 h. Cells were stained with the designated primary antibodies, followed by labeling with anti-mouse Alexa Fluor 568 or 488 and anti-rabbit Alexa Fluor 568 or 488 (Molecular Probes). After antibody labeling, cells were counterstained with Hoechst (33342, Molecular Probes). Cells were imaged with a confocal microscope (Nikon) and were processed with NIS-elements confocal (Nikon).

Quantitative RT-PCR (qRT-PCR)

Total RNA was extracted using TRI reagent (Sigma) and digested with DNaseI (Sigma). 1 μg of total RNA was reverse-transcribed into cDNA using iScript cDNA synthesis kit (Bio-Rad).
Diluted cDNAs (1:5 or 1:10) were quantified using the iQ SYBR Green Supermix kit (Bio-Rad) per manufacturer’s instructions. DNase and RNase free water (Sigma W4502) and filter tips were used. CFX96 PCR machine (Bio-Rad) was used for qRT-PCR analysis with the following thermocycler conditions: 95°C for 3 min, (95°C for 10 sec, 59°C for 20 sec, 72°C for 20 sec) x 40 cycles, 95°C for 10 sec, melt curve analysis with 65°C to 95°C, increment 0.5°C for 5 sec, plate read added. Threshold cycle (Ct) of each gene was normalized to internal reference gene (either β-actin or GAPDH); \( \Delta C_t = C_{t, \text{target gene}} - C_{t, \text{reference gene}} \). Gene expression results are presented as relative mRNA level or fold change. Relative mRNA levels are calculated using the 2^{-\Delta Ct} method. Fold changes are calculated using the 2^{-\Delta\Delta Ct} method; where the target gene relative to the control gene is determined by; \( \Delta\Delta C_t = \Delta C_{t, \text{target sample}} - \Delta C_{t, \text{control sample}} \). Gene-specific probes (5’ to 3’) for qRT-PCR are mouse Il-10 (NM_010548, Forward-CCCTGGGTGAGAAGCTGAAG and Reverse-CACTGCCTTGCTCTTTATTTTACCA), mouse Ifnb1 (NM_010510, Forward-GCACTGGGTGGAATGAGACT and Reverse-AGTGAGAGGACTTGAGGACA), mouse Actin (NM_007393, Forward-TGAGAGGAAATCGTGCCTGAC and Reverse-AAGAAGGAAGGCTGGAAAGAG), human Il-10 (NM_000572, Forward-GTGGCCAAGCCTTGCTGA and Reverse-AGGGAGTTCACATGCCGTCT), and human Gapdh (NM_002046, Forward-GAAGGTGAAGGTCGGAGTC and Reverse-GAAGATGATGATGGGATTTC).

**Knockdown of gene expression**

Small interfering RNA (siRNA) targeting human ABIN2 (isoform accession numbers: NM_024309, NM_001161527, NM_001292016, purchased from Santa Cruz, sc-44638) was transfected into HEK293T cells using Lipofectamine 2000 (Invitrogen). After 24 h incubation, Co-IP experiment was performed as described earlier. For control, siRNA-A (sc-37007, Santa Cruz) was included as a non-targeting 20-25 nt siRNA. Short hairpin RNA (shRNA) targeting mouse ABIN2 gene (5’-GCAGGAAGTTGAAAGACTTAC-3’) was introduced into Raw264.7 cells by lentivirus-mediated transduction. shRNA was cloned into pLKO.1 vector and lentivirus was produced by transfection of HEK293T cells with shRNA plasmid, packaging plasmid and envelope plasmid. Raw264.7 cells were spin-infected by lentivirus with polybrene (8 μg/ml) and were subjected to gene expression assay.

**Enzyme-linked immunosorbent assay**

Secreted cytokines in supernatants were quantified by VeriKine mouse IFN-β ELISA kit (PBL Biomedical Laboratories), OptEIA mouse IL-10 ELISA kit (BD Biosciences) per manufacturer’s
protocols. Raw264.7 cells were treated with LPS priming (20 ng/ml, 0111:B4 Sigma) or Sendai virus infection and the cell culture supernatants were collected at indicated time points. Control healthy participant sera and SFTSV-infected patient sera collected from Chungbuk National University College of Medicine and Korea National Research Institute of Health were subjected to Lumina Bio-Plex Multiplex Immunoassay to measure cytokines.

Single-molecule pull-down (SiMPull) assay

SiMPull technique combines the principles of a conventional pull-down assay with single-molecule fluorescence microscopy and enables direct visualization of individual protein-protein interactions. Protein-protein interaction and TPL2 complex formations were assessed using SiMPull assay. Briefly, HEK293T cells were transfected with the indicated DNA plasmids (NSs-Flag, ABIN2-V5, TPL2-GFP and p105-mCherry) and lysates were applied to slides coated with biotinylated anti-V5 antibody (GTX77436, GeneTex) for ABIN2-V5 pull down. For binding, anti-Flag-Cy3 antibody (Sigma) was used for detecting NSs-Flag. For complex formations, GFP and mCherry fluorescence were imaged for TPL2-GFP and p105-mCherry, respectively. Proteins immobilized on the slides were visualized by a TIRF microscope equipped with excitation laser 488 nm (GFP) and 561 nm (mCherry or RFP). 20 different regions of the imaging surface were imaged and molecular numbers were quantified by IDL and MATLAB programs.

Reverse genetics

Recombinant viruses were generated as previously described. Briefly, BHK21-T7 cells (1.5x10^5/ml) were transfected with 0.1 μg of pTM1-HB29ppL, 0.5 μg of pTM1-HB29N, and 1 μg of each pTVT7-based plasmid expressing cDNA copies of the viral antigenomic segments, using 3 μl of TransIT-LT1 (Mirus Bio LLC) per μg of DNA as transfection reagent. After 7 days, the virus-containing supernatants were collected, clarified by low-speed centrifugation, and stored at -80°C. Stocks of recombinant viruses were grown in Vero E6 cells (1.5x10^5/ml) at 37°C by infecting at MOI=0.01 and harvesting culture medium at 7 days p.i. The genome segments of recovered viruses were amplified by RT-PCR (M-MLV Reverse Transcriptase, Promega), and their nucleotide sequences were determined to confirm the lack of mutations. pTVT7-S-NSs-PA and pTVT7-S-NSs-KR plasmids were constructed by using site-direct mutagenesis according to the manufacturer’s protocol and subsequently used to make SFTSV-PA or -KR viruses (GENEART Site-Directed Mutagenesis System, Invitrogen).

Virus titration by plaque assay
Vero E6 cells were infected with serial dilutions of virus and incubated under an overlay consisting of DMEM supplemented with 2.5% FBS, 0.5% minimum essential amino acids, 0.5% sodium pyruvate, 0.5% Glutamax, 1% Pen/Strep and 1.2% Avicel (FMC BioPolymer) at 37°C for 14 days. Cell monolayers were fixed with 10% formaldehyde in PBS. Following fixation, cell monolayers were stained with 1% Crystal Violet in 20% ethanol to visualize plaques.

**Viral copy number**

Viral loads of SFTSV-infected cells or tissues of infected mice were determined by qPCR. For M segment, forward primer was SFTS-M-F: 5’-AAGAAGTGGCTGTTCATCATTATTG-3’, reverse primer was SFTS-M-R: 5’-GCCTTAAGGACATTGGTGAGTA-3’ and the probe was SFTS-M-Probe: 5’-6FAM-TCATCCTCTTGGATATGCAGGCCTCA-TAM-3’ (synthesized by Sigma). qPCR cycling was performed using 10 ng of total RNA with SsoAdvanced™ Universal Probes Supermix (BioRad) per manufacturer's instructions. The copy numbers were calculated as a ratio of the copy numbers to the standard control.

**Whole blood infection**

Peripheral blood specimens were obtained from healthy donors and stored temporarily at 37 °C in lithium heparin-containing tube (BD Biosciences) prior to infection. Blood specimens used in Fig. 1i were obtained from male and female subjects of equal distribution. SFTSV infections of whole blood were performed using MOI=1. Each infection mix consisted of 1 ml blood and 1 ml virus suspension prepared in serum-free DMEM. Samples were incubated at 37°C for 24 h with intermittent shaking. Mock infections using the cell-free fractions from Vero-E6 cultures were performed in parallel. All procedures performed in studies using human whole blood were in accordance with the ethical standards of the USC code of ethics.

**NanoString analysis**

Total RNA was isolated from Raw264.7 cells expressing NSs with or without LPS treatment and spleens of Ifnar<sup>-/-</sup> mice infected by SFTSV, and 100 ng was used to determine the absolute levels of gene expression through nCounter mouse immunology panel (~550 immune-related genes). Hybridization and nCounter were performed by the Molecular Genomics Core of USC, according to the manufacturer's protocol (NanoString Technologies). In brief, reactions were hybridized for 20 h at 65°C, after which the products were used to run on the nCounter preparation station for removal of excess probes. Data were collected with the nCounter digital analyzer by counting individual barcodes. Data generated from the nCounter digital analyzer
were examined with the nSolver Analysis software 3.0 (NanoString Technologies). Data were normalized to the geometric means of spiked-in positive controls for assay efficiency and spiked-in negative controls to normalize for background. The data were further normalized to the housekeeping genes like Gapdh, Hprt and Tubb5, and reported as normalized RNA counts (means ± SEM). For further analysis, differential expression (DE) of genes in response to SFTSV-WT/SFTSV-PA infection versus Mock infection sample was performed and visualized as a volcano plot by using the Advanced Analysis module. In volcano plot, the \(-\log_{10}\) \(P\) value of each gene is plotted (on the vertical axis) against its log2 fold-change (on the horizontal axis). For each gene, a single linear regression was fit using all selected covariates to predict expression. Output is shown with nonadjusted \(P\) value as well as Benjamini-Hochberg FDR. The genes of interest were both high in the graph (corresponding to a very small \(p\)-value) and at either the right or left side (corresponding to increased or decreased expression).

**Biosafety**

All work with infectious agents for SFTSV was done in the Wright Foundation and Hasting Foundation Laboratories Animal Biosafety Level 3 (BSL3/ABSL3) Facility at the Keck School of Medicine of USC.

**Statistical analysis**

All results are presented as mean ± SD. All experiments were repeated at least twice with a representative gel or plot being shown. Statistical analysis was performed using Prism 6.0 (Graph Pad Software). The n values in the figure legend indicate the number of independent experiments and each experiment was conducted with two to three technical replicates. Where appropriate, column analyses were performed using an unpaired, two-tailed \(t\)-test with Welch's correction or one-way ANOVA with Bonferroni’s or Dunnett’s test was used for multi-component comparisons. For grouped analyses two-way ANOVA with Bonferroni’s or Dunnett’s test was performed. \(P\) values less than 0.05 (95% confidence interval) were considered significant. Comparison of mouse survival was estimated using Kaplan–Meier method and analyzed by log-rank analysis.

**Data availability**

The data that support the findings of this study are available from the corresponding author upon request.
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Author Contributions
Y.C. performed and analyzed all experiments, prepared the figures and wrote the first draft of the manuscript. S.J.P., Y.S., J.S.Y., S.P., S.S.F., W.J.S., S.B.C., P.N.T., W.J.L., J.S.L., W.L., B.B., and Y.K.C. collaborated in the experimental design and interpretation. S.J.P. tested human patient samples. Y.S. and W.L. provided SFTSV-Gn antibody. J.S.Y, S.P., and W.J.S. worked in BSL3 for viral infection studies. S.B.C. performed SiMPull assay. S.S.F. designed whole blood infection study. P.N.T. provided mouse strain. W.J.L. provide human patient samples. B.B., provided materials for reverse genetics and viral strains. Y.C. and J.U.J. jointly conceived the experimental design, interpreted the results and wrote subsequent drafts of the manuscript.

Competing financial interests
None
References


**Figure 1. SFTSV NSs-mediated alteration of host gene expression.**

**a,** Inclusion body (IB) formation of NSs in Raw264.7 cells expressing Vector, NSs-WT, or NSs-PA was shown by confocal microscopy. NSs was detected by Alexa Fluor 568 (red) and nuclear DNA was stained with Hoechst (blue). Scale bars, 10 μm. This represents three independent experiments. **b,** Expressions of IFN-β mRNA and protein in Raw264.7 cells expressing Vector, NSs-WT, or NSs-PA (n=3 each) were measured by quantitative RT-PCR (qRT-PCR) and Enzyme-linked immunosorbent assay (ELISA) upon Sendai virus (SeV) stimulation for 12 h. **c,** Immune profiling in spleens. RNA from splenocytes of Ifnar<sup>−/−</sup> mice infected by SFTSV-WT (n=2) or SFTSV-PA (n=2) at 10<sup>5</sup> PFU/mice for 4 days was subjected to NanoString analysis. Differential gene expression in SFTSV-WT (c) and SFTSV-PA (d) infection compared to Mock (n=2) infection was presented as a volcano plot. **e,** Comparison of gene expression between macrophages expressing NSs-WT and spleens infected with SFTSV-WT in Venn diagram and Heat map. **f,** IL-10 mRNA in Raw264.7 cells (n=3) was measured by qRT-PCR. **g,** IL-10 ELISA in Raw264.7 cells at various time points (n=2 each) after LPS (20 ng/ml) treatment. **h,** IL-10 mRNA in Raw264.7 cells (n=2) was measured by qRT-PCR after SeV infection for 9 h. **i,** IL-10 ELISA in Raw264.7 cells (n=2) after SeV infection for 12 h. **j-1,** IL-10 mRNA levels were measured by qRT-PCR in Raw264.7 cells (n=2) (j), mouse bone marrow derived macrophages (BMDMs) (n=2) (k), and human whole blood (n=3) (l) after SFTSV-WT or SFTSV-PA infection (MOI 0.1) for 48 h. **m,** IL-10 mRNA expression was assessed by qRT-PCR in spleens (n=3) (m) and livers (n=3) (n) at 4 days after infection of 10<sup>5</sup> PFU of SFTSV-WT or SFTSV-PA. **o,** IL-10 expression in healthy participant sera (n=5) and SFTSV-infected patient sera (n=32) was measured by Bio-Plex human cytokine assay. Data are represented as mean ± SD. *P* values for multiple *t*-test (two-tailed) in (g), one-way or two-way ANOVA using Bonferroni’s multiple comparisons test in (b, f, h-n), and unpaired *t*-test (two-sided) with Welch’s correction in (o). Statistical information is described in Supplementary Table 3.

**Figure 2. NSs activates TPL2 ternary complex formation through the interaction with ABIN2.**

**a,** HEK293T cells were transfected by NSs-3xFlag and ABIN2-V5 and whole cell extracts (WCEs) were immunoprecipitated by anti-Flag antibody (Ab) or anti-V5 Ab, followed by immunoblotting with indicated Ab. **b,** Lysates of Raw264.7 cells expressing NSs-WT were used for immunoprecipitation with anti-Flag Ab, followed by immunoblotting with anti-ABIN2 Ab. **c,** Raw264.7 cells were infected with SFTSV-NSs-GFP for 24 h and immunoprecipitated by anti-GFP Ab, followed by immunoblotting with anti-ABIN2 Ab. **d,** HEK293T cells were transfected by NSs-3xFlag, ABIN1-V5, ABIN2-V5 and ABIN3-V5 and immunoprecipitated by anti-V5 Ab,
followed by immunoblotting with anti-Flag Ab. e, Raw264.7 cells expressing Vector, NSs-WT, or NSs-PA were treated with LPS (1 μg/ml) for 0, 15, 30, and 60 min and cell lysates were immunoblotted for indicated proteins. f, HEK293T cells were transfected by p105-mCherry (P), TPL2-GFP (T), ABIN2-V5 (A), NSs-WT-3xFlag (NSs), or NSs-PA-3xFlag (PA) and cell lysates were applied to Single Molecular Pull-down (SiMPull) analysis. Six representative images were shown in upper panel and molecular numbers were counted in lower panel. Bar graph with average number of fluorophores per image. Error bars represent SD of the mean across >20 images. This represents three independent experiments. Scale bars, 5 μm. g, HEK293T cells were transfected by NSs-3xFlag (WT, PA, and KR) and ABIN2-V5 and immunoprecipitated by anti-Flag Ab, followed by immunoblotting with anti-V5 Ab. h, IL-10 mRNA in Raw264.7 cells expressing NSs (WT, PA and KR, n=4) was measured by qRT-PCR. i, IL-10 mRNA expression was measured by qRT-PCR in spleens and livers (n=2 each) at 4 days after infection with 10^5 PFU of SFTSV-WT and SFTSV-KR. Data are represented as mean ± SD. P values for two-way ANOVA using Bonferroni’s multiple comparisons test in (h and i). Statistical information is described in Supplementary Table 3. Western blots in (a-e, g) are representative of three independent experiments.

Figure 3. SFTSV NSs induces IL-10 expression by activating TPL2 signalling pathway. a, IL-10 ELISA of Raw264.7 cells expressing Vector, NSs-WT, or NSs-PA with DMSO (NT), TPL2 inhibitor (INH, 5 μM), LPS (20 ng/ml), or LPS+TPL2 inhibitor (n=2 each) for 12 h. b, IL-10 ELISA of LPS (20 ng/ml) treated Raw264.7 cells (n=2) as described in (a) for 12 h with DMSO, TPL2 inhibitor (5 μM), c-Raf inhibitor (10 μM), MEK inhibitor (U0126, 10 μM). c, IL-10 mRNA was measured by qRT-PCR in wild-type (WT) and Tpl2^-/- BMDMs (n=4) infected with Vector, NSs-WT, or NSs-PA lentivirus, followed by LPS (1 μg/ml) treatment for 6 h. d, IL-10 mRNA was measured by qRT-PCR in wild-type (WT), Tpl2^-/- and Ifnar^-/- BMDMs (n=2) upon SFTSV-WT or SFTSV-PA infection for 12, 24, and 48 h. e, Ifnar^-/- BMDMs (n=2) were infected by mock and SFTSV-WT for 24 and 48 h with or without TPL2 inhibitor (INH, 10 μM), and IL-10 mRNA was then measured by qRT-PCR. f, IL-10 mRNA was measured by qRT-PCR in spleens of wild-type C57BL/6 and Tpl2^-/- mice (n=4) pre-treated with anti-IFNAR1 or isotype IgG at 4 days p.i with mock, SFTSV-WT. g, h, Ifnar^-/- BMDMs were infected by mock, SFTSV-WT and SFTSV-PA (n=2 each) for 24 and 48 h, and IL-10 mRNA levels were then assessed by qRT-PCR (g) and cell lysates were analyzed by immunoblotting for indicated proteins (h). i, Ifnar^-/- BMDMs were infected by mock, SFTSV-WT and SFTSV-PA for 48 h with or without TPL2 inhibitor (INH, 10
μM), and cell lysates were immunoblotted for indicated proteins. Data are represented as mean ± SD. *P* values for one-way or two-way ANOVA using Dunnett's multiple comparisons test in (a–e, h) and unpaired *t*-test (two-sided) in (a, c, f). Statistical information is described in Supplementary Table 3. Western blots in (g, i) are representative of two independent experiments.

**Figure 4. NSs-mediated activation of TPL2 signalling pathway is required for SFTSV-induced lethal phenotype.** a, b, Wild-type C57BL/6 and *Tpl2*−/− mice were treated with anti-IFNAR1 (n=7 for WT, n=10 for *Tpl2*−/−) or isotype IgG (n=3 for each) intraperitoneally 1 day prior to intramuscular infection with 10⁵ PFU of SFTSV-WT. Mice were then monitored (a) and weighed (b) for 15 days. c, d, Wild-type C57BL/6 mice were treated with anti-IFNAR1 IgG or isotype IgG intraperitoneally 1 day prior to intramuscular infection with 10⁵ PFU of SFTSV-WT. Subsequently, infected mice were treated with TPL2 inhibitor (INH, 10 mg/kg at day 1, 2, 3, p.i, n=3 for Isotype, n=11 for anti-IFNAR1) or DMSO (n=3 for each), and then monitored (c) and weighed (d) for 15 days. e, f, Wild-type C57BL/6 and *Il-10*−/− mice were treated with anti-IFNAR1 IgG or isotype IgG intraperitoneally 1 day prior to intramuscular infection with 10⁵ PFU of SFTSV-WT (n=7 for each). For infection control, three mice (n=3) were only treated with anti-IFNAR1 IgG. Mice were then monitored (e) and weighed (f) for 15 days. g, h, *Ifnar*−/− mice were intramuscularly infected with 10² PFU of SFTSV-WT (n=10), SFTSV-PA (n=7) or SFTSV-PA together with recombinant mouse IL-10 (rmIL-10, 1 μg/mouse at day 1, 2, 3 p.i, n=7) intraperitoneally, and mice were monitored (g) and weighed (h) for 15 days. i, j, Wild-type C57BL/6 mice were treated with anti-IFNAR1 IgG or isotype IgG intraperitoneally 1 day prior to intramuscular infection with 10⁵ PFU of SFTSV-WT (n=8) or SFTSV-KR (n=7) and then monitored (i) and weighed (j) for 15 days. The survival curves were estimated using Kaplan–Meier method and analyzed by log-rank analysis with *P* values (two-sided). Statistical information is described in Supplementary Table 3.
Day post infection
Relative weight (%)

**a**
- WT Isotype (n=3)
- WT anti-IFNAR1 (n=7)
- Tpl2-/- Isotype (n=3)
- Tpl2-/- anti-IFNAR1 (n=10)

**b**
- WT Isotype/DMSO (n=3)
- WT Isotype/TPL2 INH (n=3)
- WT anti-IFNAR1/DMSO (n=9)
- WT anti-IFNAR1/TPL2 INH (n=11)

**c**
- WT Isotype (n=3)
- WT anti-IFNAR1 (n=7)
- Tpl2-/- anti-IFNAR1 no infection (n=3)

**d**
- WT anti-IFNAR1 SFTSV-WT (n=8)
- WT anti-IFNAR1 SFTSV-KR (n=7)

**e**
- Tpl2-/- SFTSV-PA (n=7)
- Tpl2-/- SFTSV-WT (n=10)
- Tpl2-/- SFTSV-PA/rmIL-10 (n=7)

**f**
- Ifnar-/- SFTSV-WT (n=10)
- Ifnar-/- SFTSV-PA (n=7)
- Ifnar-/- SFTSV-PA/rmIL-10 (n=7)

**g**
- Ifnar-/- SFTSV-WT (n=7)
- Ifnar-/- SFTSV-KR (n=7)
- Ifnar-/- anti-IFNAR1 no infection (n=3)

**h**
- Ifnar-/- anti-IFNAR1 SFTSV-WT (n=8)
- Ifnar-/- anti-IFNAR1 SFTSV-KR (n=7)

**i**
- P < 0.0001
- P = 0.0002
- P = 0.0021
- P = 0.0009
- P < 0.0001

**P** values provided for statistical significance.