# Porcine reproductive and respiratory syndrome virus inhibits STAT2 signaling via nsp11-mediated downregulation

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## ABSTRACT

Interferons (IFNs) play a crucial role in host antiviral response via activating JAK/STAT (Janus kinase/signal transducer and activator of transcription) signaling pathway to induce expression of numerous genes. Porcine reproductive and respiratory syndrome virus (PRRSV) antagonizes the antiviral response by inhibiting IFN synthesis and JAK/STAT signaling. STAT2 is a key player in the IFN-activated JAK/STAT signaling. The objective of this study was to investigate the PRRSV effect on STAT2 signaling. Here, we discovered that PRRSV downregulated STAT2 to inhibit the IFN-activated signaling. PRRSV strains of both *PRRSV-1* and *PRRSV-2* species decreased STAT2 protein level, whereas its transcript had minimal change. PRRSV reduced STAT2 level in a dose-dependent manner and shortened STAT2 half-life significantly from about 30 to 10 hours. PRRSV-induced STAT2 degradation could be restored by treatment with the proteasome inhibitor MG132. In addition, PRRSV non-structural protein 11 (nsp11) was identified to reduce STAT2. The N-terminal domain (NTD) of nsp11 was responsible for STAT2 degradation and interacted with STAT2. Site-directed mutagenesis showed that three amino acids (57-59, IHK) located in the nsp11 NTD were critical for nsp11 interacting with and reducing STAT2. Together, these results demonstrate that PRRSV antagonizes STAT2 signaling via nsp11-mediated downregulation. This study provides further insight of PRRSV interference with IFN signaling and the consequent host immune response.

## INTRODUCTION

- Interferons (IFN) are indispensable components in the innate immunity.
- Both type I and type III IFNs can activate the same canonical JAK/STAT pathway, in which JAKs phosphorylate STAT1 and STAT2, followed by interaction with interferon regulatory factor 9 (IRF9) to form a heterotrimer termed IFN-stimulated gene factor 3 (ISGF3) .
- A STAT1-independent pathway, named non-canonical JAK/STAT signaling.
- STAT2 is indispensable in both canonical and non-canonical IFN-activated signaling pathways. Therefore, many viruses target STAT2 to antagonize IFN signaling.
- PRRS virus, the causative agent, is a member of the genus *Porartevirus*, the family *Arteriviridae*, the order *Nidovirales*.
- PRRSV is a small enveloped virus containing a positive-sense, single-stranded RNA genome of approximately 15 kb in length.
- PRRSV infection of pigs induces poor innate and adaptive immune responses.
- It has been shown that PRRSV has several evasion strategies to interfere with the host innate immune response.

## RESULTS

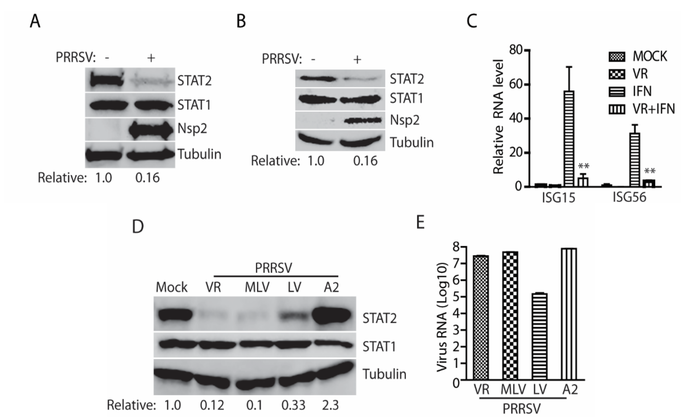


Figure 1. PRRSV infection reduces STAT2 in MARC-145 and PAM cells. A. PRRSV reduces STAT2 protein level but has minimal effect on STAT1. MARC-145 cells were infected with VR-2385 at an MOI of 1 and harvested 36 hours post-infection (hpi) for Western blotting (WB) with antibodies against STAT2, STAT1, PRRSV nsp2, and tubulin. Relative levels of STAT2 are shown below the images after normalization with tubulin in densitometry analysis. B. PRRSV reduces STAT2 in PAM cells but has minimal effect on STAT1. The cells were infected with VR-2385 at an MOI of 1 and harvested for WB at 16 hpi. C. PRRSV inhibits IFNα-activated expression of ISG15 and ISG56 detected by RT-qPCR. VR: VR-2385. D. Reduction of STAT2 by different PRRSV strains with the exception of A2 (A2MC2) in MARC-145 cells. The cells were infected with VR-2385, MLV (Ingelvac PRRS MLV), LV (Lelystad virus), and A2 at the MOI of 1. The relative levels of STAT2 are shown below the images after normalization with tubulin. E. PRRSV RNA levels of the PRRSV strains detected by RT-qPCR. Viral-infected MARC-145 cells were harvested for RNA isolation and RT-qPCR at 24 hpi. Error bars represent the standard errors of the results of three repeated experiments.

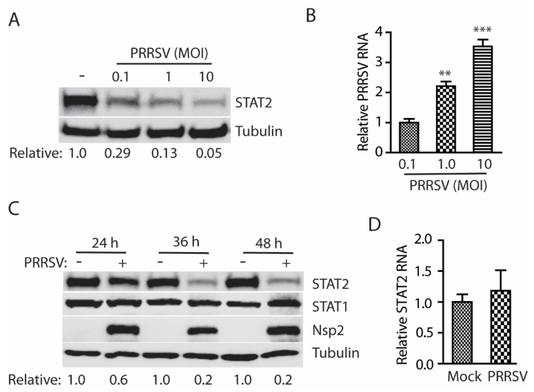


Figure 2. PRRSV reduces STAT2 in a dose and time-dependent manner. A. Dose-dependent reduction of STAT2 by PRRSV. MARC-145 cells were inoculated with incremental MOI of VR-2385 and harvested for WB at 36 hpi. Relative levels of STAT2 are shown below the images after normalization with tubulin. B. PRRSV RNA levels detected by RT-qPCR. Error bars represent the standard errors of the means of three repeated experiments. Significant differences in RNA level from an MOI of 0.1 are denoted by asterisks (**, P < 0.01; ***, P < 0.001). C. Temporal kinetics of STAT2 levels in PRRSV-infected cells. MARC-145 cells were infected with VR-2385 at an MOI of 1. At different time points, the cells were harvested for WB. Mock-infected cells at corresponding time points were included as controls. Relative levels of STAT2 are shown below the images after normalization with tubulin at corresponding time point. D. PRRSV infection has minimal effect on STAT2 mRNA level. The cells were harvested at 24 hpi for RNA isolation and RT-qPCR. The relative STAT2 mRNA levels are shown in comparison with the mock-infected cells.

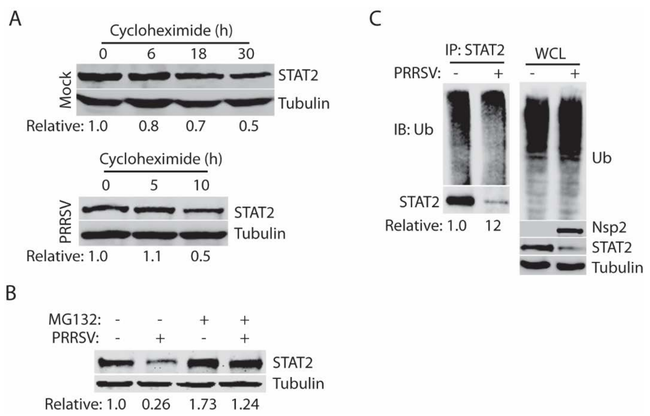


Figure 3. PRRSV infection shortens STAT2 half-life and mediates STAT2 reduction via ubiquitin-proteasome degradation pathway. A. PRRSV extends STAT2 half-life. MARC-145 cells were infected with VR-2385 at an MOI of 1. The cells were treated with cycloheximide at 24 hpi and harvested at the indicated time (h) for WB. The relative levels of STAT2 are shown below the images. B. MG132 treatment restores STAT2 level in PRRSV-infected cells. MARC-145 cells were infected with VR-2385 at an MOI of 1. At 24 hpi, the cells were treated with MG132 for 6 h and then harvested for WB. Non-treated and mock-infected cells were included as controls. C. PRRSV induces elevation of STAT2 polyubiquitination. MARC-145 cells were infected with PRRSV VR-2385 at an MOI of 1 and harvested for IP with STAT2 antibody at 36 hpi, followed by WB with the ubiquitin (Ub) antibody. WB of whole cell lysate (WCL) was conducted. The relative levels of Ub after normalization with STAT2 are shown below the images.

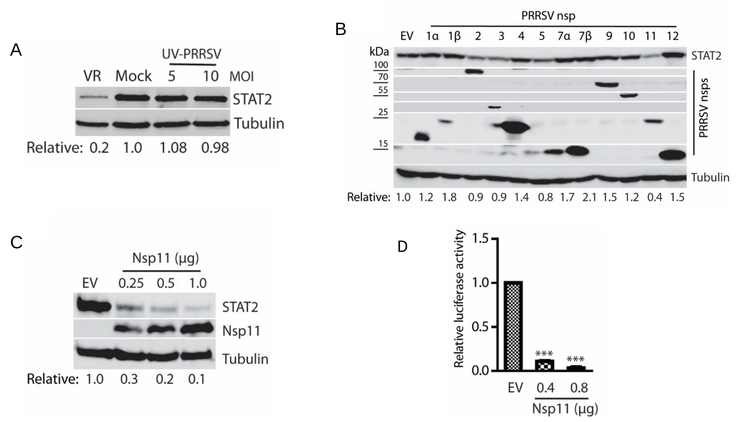


Figure 4. PRRSV nsp11 induces reduction of STAT2. A. UV-inactivated PRRSV has minimal effect on STAT2 level. The cells were inoculated with PRRSV VR-2385 at an MOI of 1 or UV-inactivated VR-2385 at the MOI of 5 and 10, followed by WB at 36 hpi. B. Nsp11 reduces STAT2 in HEK293 cells, whereas other PRRSV nsps have minimal effect. The cells were transfected with individual HA-nsp plasmids and STAT2 plasmid. An empty vector (EV) was included as a control. The relative levels of STAT2 protein are shown below the images. Molecular mass markers are added on left side of the nsp images. C. Nsp11 reduces STAT2 in HeLa cells in a dose-dependent manner. HeLa cells were transfected with nsp11 plasmid in incremental amounts. D. Nsp11 inhibits IFN-α activated expression of ISRE reporter in HEK293 cells. The cells were co-transfected with ISRE luciferase reporter, Renilla luciferase plasmid, and HA-nsp11. The relative levels of firefly luciferase activity are shown as fold compared to the EV control after normalization with the Renilla activity.

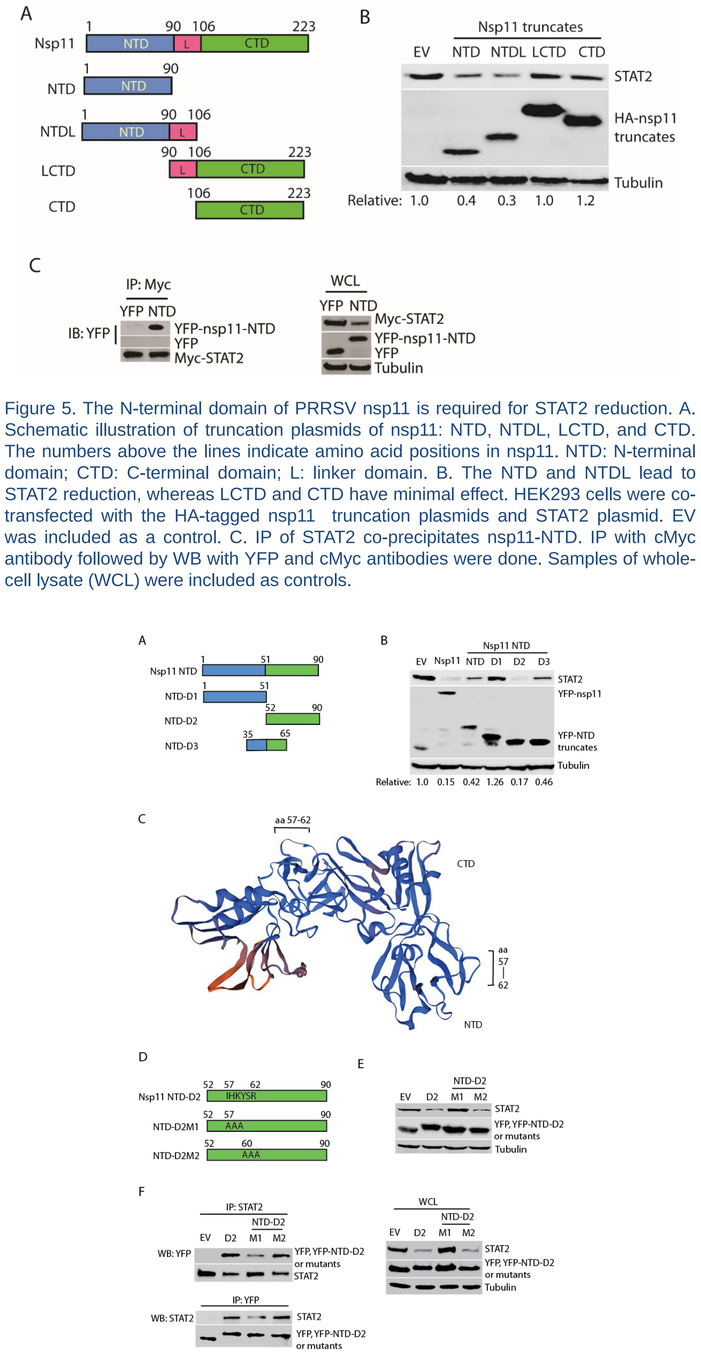


Figure 5. The N-terminal domain of PRRSV nsp11 is required for STAT2 reduction. A. Schematic illustration of truncation plasmids of nsp11: NTD, NTDL, LCTD, and CTD. The numbers above the lines indicate amino acid positions in nsp11. NTD: N-terminal domain; CTD: C-terminal domain; L: linker domain. B. The NTD and NTDL lead to STAT2 reduction, whereas LCTD and CTD have minimal effect. HEK293 cells were co-transfected with the HA-tagged nsp11 truncation plasmids and STAT2 plasmid. EV was included as a control. C. IP of STAT2 co-precipitates nsp11-NTD. IP with cMyc antibody followed by WB with YFP and cMyc antibodies were done. Samples of whole-cell lysate (WCL) were included as controls.




Figure 6. Mapping motifs in Nsp11-NTD that are required for STAT2 reduction. A. Schematic illustration of truncation plasmids nsp11-NTD: D1, D2 and D3. B. The NTD-D2 and NTD-D3 lead to STAT2 reduction, whereas NTD-D1 has minimal effect. C. A model of nsp11 protein. The loop of aa 57-62 is shown on upper and right side of the nsp11 dimer. D. Schematic illustration of mutant plasmids of nsp11-NTD-D2: D2M1 (I57A, H58A, and K59A) and D2M2 (Y60A, S61A, and R62A). E. The NTD-D2M1 fails to reduce STAT2, whereas NTD-D2M2 induces STAT2 decrease similarly to wild type NTD-D2. F. IP of STAT2 co-precipitates nsp11 NTD-D2 and NTD-D2M2 but much less NTD-D2M1. IP of YFP-tagged nsp11 NTD-D2 and NTD-D2M2 co-precipitates STAT2, whereas much less STAT2 in NTD-D2M1 pulldown. WB of WCL was done.

## Summary

- PRRSV infection reduces STAT2 protein level.
- PRRSV reduces STAT2 protein level in a dose and time-dependent manner.
- PRRSV infection shortens STAT2 half-life.
- PRRSV mediates STAT2 reduction via the ubiquitin-proteasome degradation pathway.
- PRRSV nsp11 reduces STAT2 protein level.
- The N-terminal domain of nsp11 appears to be responsible for the reduction of STAT2 via interacting with STAT2.
- Amino acids 57-59 of nsp11 are crucial for the interaction with STAT2 and STAT2 reduction.