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Foot-and-mouth disease virus 3D polymerase antagonizes the interferon signaling pathway by blocking STAT2 nuclear translocation

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ABSTRACT

Foot-and-mouth disease virus (FMDV) is the etiological agent of foot-and-mouth disease (FMD), which is highly contagious and extremely destructive in cloven-hoofed animals. Previous studies have shown that FMDV strongly suppress the innate immune response, and the research mainly focused on FMDV 3C and L proteinase. However, the role of FMDV 3D polymerase, an RNA-dependent RNA polymerase (RdRp), in inhibiting the IFN signaling pathway remains unclear. In this study, for the first time, we demonstrate that the highly conserved 3D polymerase of FMDV inhibits the activation of the JAK-STAT signaling pathway by targeting STAT2. Mechanistically, FMDV 3D significantly inhibits the activity of the interferon-stimulated response element promoter and down-regulates the transcription of interferon-stimulated genes. Further research revealed that 3D interacts with STAT2, hinders its phosphorylation, and inhibits its nuclear translocation, thereby blocking the activation of the JAK-STAT signaling pathway. Collectively, these findings elucidate a novel mechanism by which FMDV 3D polymerase, acting as an inhibitor, targets STAT2 to suppress IFN signaling and antagonize the host antiviral immune response. This will provide insights for the development of future anti-FMDV strategies.

1. Introduction

Foot-and-mouth disease (FMD) is an acute and highly contagious disease caused by foot-and-mouth disease virus (FMDV). The host range of FMDV is broad, encompassing over 70 cloven-hoofed animals, such as pigs, cattle, sheep, goats and African buffaloes. FMD significantly impacts the livestock industry and poses a threat to international trade in animals and animal products (Qiu et al., 2018). FMDV belongs to the *Aphthovirus* genus of the *Picornaviridae* family. The viral genome is approximately 8.3 kb in length, including the 5′-untranslated region (5′UTR), the single open reading frame (ORF), and the 3′UTR. The ORF encodes four structural proteins constitute the icosahedral capsid, and eight non-structural proteins regulate RNA replication, protein folding and viral assembly (Belsham 2005). The 3D protein is the RNA-dependent RNA polymerase (RdRP) of the virus and plays a crucial role in the process of viral replication, which is highly conserved (Nie et al. 2024).

The type I interferon (IFN) family is a crucial component of the innate immune response, serving as the first line of defense against viral infection. Upon viral infection, pattern recognition receptors (PRRs) detect viral elements, initiating a signaling cascade that results in the production of interferons (IFNs) and pro-inflammatory cytokines. This cascade activates the JAK-STAT signaling pathway, which induces the expression of interferon-stimulated genes (ISGs) to disrupt viral protein synthesis or inhibit viral replication (Li et al. 2021; Xie et al. 2025; Ma et al. 2023b). STAT1 and STAT2 are important in JAK-STAT signaling pathway. Upon the stimulation of IFN-α/β, the IFNARs receptor induces the phosphorylation and activation of JAK1 and TYK2, which in turn induces the phosphorylation and activation of STAT1/2. Tyrosine phosphorylated STAT1 and STAT2 form a heterodimer, which then combines with IRF9 to form the transcription factor complex ISGF3. With ISGF3 transported into nucleus, it binds to the promoter of interferon-stimulated response elements (ISRE), inducing the production of antiviral ISGs (Li et al. 1998).

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FMDV have developed mechanisms to counteract host innate immune system, and various viral proteins have been identified as antagonists. The Lb protease cleaves intracellular transcription factors STAT1 and STAT2 to inhibit IFN-β-induced signaling (Ma et al. 2023a). The 3C protease inhibits the type I IFN signaling pathway by preventing the nuclear translocation of STAT1 and STAT2 (Du et al. 2014; Wu et al. 2024). FMDV VP3 degrades YTHDF2 through the autophagy pathway, regulates IRF3 activity, and promotes viral replication (Liu et al. 2024). FMDV 3C and L protease cleavage MDA5 to evade the innate immune response (Kim et al. 2021; Pulido et al. 2020). FMDV 2B inhibits RLR-mediated signaling pathway by targeting RIG-I and MDA5 (Zhu et al. 2016; Li et al. 2018). The 3B protein of FMDV interacts with the viral RNA sensor RIG-I, block the activation of RIG-I to suppress IFN-related antiviral response (Zhang et al. 2020). FMDV VP1 target MAVS to inhibit type-I interferon signaling (Ekanayaka et al. 2020). However, as the conserved polymerase which is highly expressed at the early infection stage, the role of FMDV 3D polymerase in inhibiting host innate immune response remain unclear.

In this study, we found that FMDV 3D polymerase substantially inhibits IFN-β-induced activation of interferon-stimulated response elements (ISRE) promoter and reduces the transcriptional of ISGs. This led us to hypothesize that it targets a key component of the JAK-STAT pathway. Subsequently, we identified that FMDV 3D interacts with porcine STAT2 in the context of viral infection. 3D-STAT2 interaction hinders the phosphorylation of STAT2, and inhibits the activation of the JAK-STAT signaling pathway. Additionally, we observed that the presence of 3D blocks the nuclear translocation of STAT2. These results suggest that FMDV 3D protein not only facilitates viral replication by functioning as RNA-dependent RNA polymerases (RdRp) but also serves as an antagonist of innate immunity to inhibit the activation of IFN signaling pathway. FMDV is highly variable, while FMDV 3D is highly conserved. Therefore, selection of the 3D protein as an antiviral target for FMDV is highly promising in terms of application potential.

2. Materials and methods

2.1. Cells, viruses, and infection

HEK-293T cells (ATCC, CRL-11,268) and PK-15 cells (ATCC, CCL-33) were cultured in Dulbecco modified Eagle medium (DMEM, VivaCell) supplemented with 10 % heat-inactivated fetal bovine serum (FBS, Excell) and maintained at 37 °C (5 % CO_2). FMDV type O strain O/BY/CHA/2010 (GenBank number: JN998085) were cryopreserved at −80 °C, utilized for viral infection. The viral infection experiments were conducted in accordance with the procedures described in previous studies (Lei et al. 2010).

2.2. Plasmids and antibodies

The indicated cDNA of FMDV 3D was cloned into the pCAGGS vector to generate plasmids expressing Flag-tagged 3D protein. Mammalian expression plasmids for Myc-tagged JAK1, TYK2, STAT1, STAT2 and IRF9, and the ISRE promoter luciferase reporter plasmids, were kindly provided by Professor Hongbing Shu (Wuhan University, China).

The commercial antibodies used in this study include: anti-Flag mouse Ab (Sigma, F1804), anti-Myc mouse Ab (Sigma, M5546), anti-HA mouse Ab (Sigma, H9658), and anti-GAPDH mouse Ab (Abclonal, AC002), anti-JAK1 rabbit Ab (Cell Signaling Technology, 3332), anti-TYK2 rabbit Ab (Cell Signaling Technology, 9312), anti-STAT1 rabbit Ab (Cell Signaling Technology, 9172), anti-STAT2 rabbit Ab (Cell Signaling Technology, 4594), anti-p-STAT2 rabbit Ab (Cell Signaling Technology, 4441), anti-IRF9 rabbit Ab (Cell Signaling Technology, 76,684). Anti-3D rabbit polyclonal Ab were prepared by our laboratory previously (unpublished data). Goat anti-mouse or rabbit IgG (H + L) secondary antibodies were purchased from Biodragon company.

2.3. Transfection and reporter assays

HEK-293T cells, cultured in 48-well plates, were transfected with a combination of 50 ng of *ISRE* reporter plasmid, 5 ng of pRL-TK (Promega, Madison, WI, USA) serving as an internal control, and an increasing amount of the Flag-3D plasmid (0, 0, 10, 20, 50 ng). To standardize the transfection process, empty vector plasmids were included to ensure that equal total plasmid amounts were introduced into each well. After 24 h post transfection (hpt), the cells were stimulated with IFN-β (1000 U/mL) for another 12 h. Subsequently, the cell extracts were prepared to assess dual-luciferase activities.

2.4. RNA extraction and real-time PCR

Total RNA was extracted from the cell cultures using TRIzol Reagent (Vazyme, R401–01), and then reverse-transcribed into cDNA with ABScript Neo RT Master Mix for qPCR (Abclonal, RK20433) according to the manufacturer's instructions. The relative amounts of cDNAs were quantified and using BrightCycle Universal SYBR Green qPCR (Abclonal, RK21219) following the manufacturer's protocol to analyze the target genes. The glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene was used as an internal control, and the relative mRNA levels were calculated using $2^{-\Delta\Delta CT}$ method. The qPCR primers used in this study were shown in Table 2.

2.5. Coimmunoprecipitation and western blotting analysis

HEK-293T or PK-15 cells were cultured in 10 cm dishes, and the monolayer cells were co-transfected with various plasmids or infected with FMDV. The collected cells were then lysed by lysis buffer and immunoprecipitated with BeyoMag Protein A + G Beads (Beyotime, P2108) binding with indicated primary antibodies. For western blotting analysis, target proteins were resolved by 10 % SDS-PAGE and transferred onto the nitrocellulose membranes (Cytiva, 10,600,001). The membrane was blocked by 5 % nonfat dried milk diluted in TBST and incubated with appropriate primary antibodies and secondary antibodies, and the antibody-antigen complexes were subsequently visualized using ECL detection reagents (Thermo Fisher Scientific, K-12,045-D50).

2.6. Confocal immunofluorescence assay

PK-15 Cells were seeded into glass bottom cell culture dishes and transfected with various plasmids using jetPRIME DNA transfection reagent according to the manufacturer's instruction. At 24 hpt, the cells were infected with FMDV for 10 h or stimulated with IFN-β (1000 U/mL) for 30 min. The cells were then fixed, permeabilized and blocked as previously described (Zhu et al. 2016). The fixed cells were incubated with primary antibodies overnight, then incubated with secondary antibodies conjugated to Alexa Fluor™ 488 (Thermo Fisher Scientific, A-11,001) or Alexa Fluor™ 594 (Thermo Fisher Scientific, A-11,012) at room temperature for 2 h, cell nuclei were stained with 4′, 6′-diamidino-2-phenylindole (DAPI) for 10 min. The cells were visualized using a Leica SP2 confocal microscopy system (Leica Microsystems, Wizla, Germany).

2.7. Statistical analysis

All Statistical analysis was performed using GraphPad Prism software version 8.0. All the data are represented as the mean with SD from three independent experiments. A two-tailed Student's *t*-test were employed to assess the significance of the data. The statistical significance was indicated in the figures ($^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, ns indicated not significant).

3. Results

3.1. FMDV 3D protein inhibits IFN-mediated signaling and decreases ISGs expression

The preliminary research results showed that FMDV 3D protein inhibits IFN-mediated signaling. To elucidate the mechanisms underlying the effects of FMDV 3D on the activation of the IFN pathway, we transfected HEK-293T cells with 0, 0, 10, 20, or 50 ng of FMDV 3D expressing plasmid and evaluated the effects of FMDV 3D on the activation of IFN-β-induced *ISRE* promoter. The results revealed that FMDV 3D protein notably inhibited the activation of *ISRE* promoter in a dose-dependent manner (Fig. 1A). The influences of 3D protein on the expression of ISGs induced by IFN-β were further investigated. We observed that the mRNA expression of *ISG54, ISG56* and *OAS1*, which are stimulated by IFN-β, were significantly reduced in 3D overexpressing HEK-293T cells (Fig. 1B). Similarly, the upregulation of mRNA levels of *ISG15, ISG54* and *OAS1* induced by porcine IFN-β in PK-15 cells was significantly repressed by FMDV 3D (Fig. 1C). These results indicate that FMDV 3D protein plays a crucial role in inhibiting IFN-mediated signaling and interfering ISGs expression.

3.2. FMDV 3D protein interacts with STAT2

Type I IFN induces ISGs expression through activation of JAK-STAT pathway. To investigate whether 3D blocks the activation of JAK-STAT pathway by interacting with its components. We assessed the interaction between FMDV 3D and the key components of JAK-STAT pathway including JAK1, TYK2, STAT1, STAT2, and IRF9 using Co-Immunoprecipitation (Co-IP) assays. The results revealed that 3D specifically interacted with JAK1 and STAT2, while STAT2 showed a stronger interaction with 3D (Fig. 2A). A previous study using a NanoLuc two-hybrid assay identified the interaction between FMDV 3Dpol and IFN-pathway proteins but did not specifically identify the 3D-STAT2 interaction in porcine cells and lacked analysis in the context of viral infection (Sarry et al. 2023). To confirm the interaction between 3D and STAT2, HEK-293T cells were co-transfected with Flag-3D and Myc-STAT2 expressing plasmids, the cell lysates were used for Co-IP experiments using both anti-Myc antibodies and IgG control, which validated that Flag-3D specifically interacted with Myc-STAT2 (Fig. 2B). Similarly, subsequent reverse Co-IP experiments using anti-Flag antibodies demonstrated the consistent interaction (Fig. 2C). We also examined the interaction between FMDV 3D and cellular endogenous STAT2, and found that overexpression of Flag-3D pulled down endogenous STAT2 in PK-15 cells, which further confirmed the interaction between FMDV 3D and STAT2 (Fig. 2D). To verify whether FMDV 3D interacted with STAT2 in the context of virus infection, PK-15 cells were mock-infected or infected with FMDV for 12 h, and the cells lysates were immunoprecipitated with anti-3D antibody and subjected to western blotting analysis. The results showed that 3D interacted with STAT2 during FMDV infection (Fig. 2E). Additionally, we investigated the subcellular localization of FMDV 3D and STAT2. PK-15 cells were transfected with porcine HA-STAT2 expressing plasmids, and the cells were subjected to mock infection or infection with FMDV for 10 h. A colocalization of STAT2 and 3D was clearly observed in the cytoplasm upon FMDV infection (Fig. 2F). Taken together, these results confirmed that FMDV 3D interacts with host STAT2 protein.

3.3. FMDV 3D protein does not alter the protein and mRNA level of STAT2

To assess the impact of 3D on the JAK-STAT signaling pathway, we overexpressed the Flag-3D in PK-15 cells and examined the expression levels of JAK1, TYK2, STAT1, STAT2, and IRF9. The results showed that overexpression of 3D did not affect their protein expression (Fig. 3A). To further investigate the influence of FMDV 3D on the expression of STAT2, we overexpressed Flag-3D in PK-15 cells. Despite increasing 3D expression levels, we observed no downregulation of STAT2 protein. Therefore, 3D did not affect the expression of STAT2 (Fig. 3B). Additionally, to assess the influence of 3D on STAT2 transcription, we transfected PK-15 cells with a Flag-3D expression plasmid and measured STAT2 mRNA levels. The results indicated that the presence of 3D did not significantly affect STAT2 mRNA expression (Fig. 3C). In addition, we examined the expression of STAT2 and STAT2 phosphorylation with

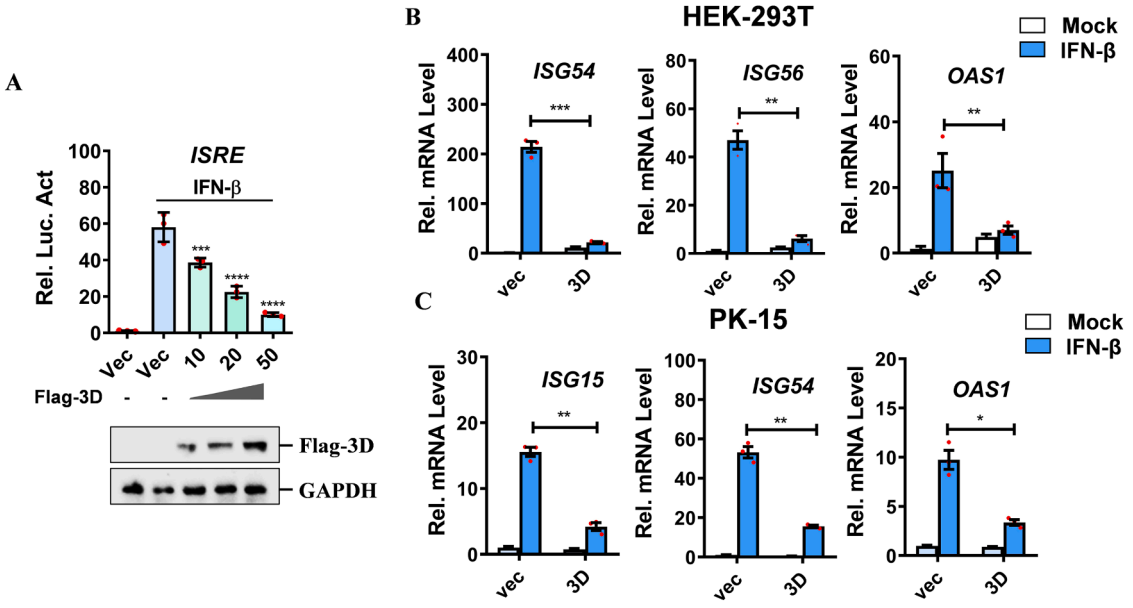

Fig. 1. FMDV 3D protein inhibits IFN signaling pathway. **(A)** HEK-293T cells were co-transfected with *ISRE* (50 ng) luciferase reporter plasmids and an internal control plasmid PRL-TK (5 ng), along with increasing amount of the Flag-3D plasmids (0, 10, 20, or 50 ng) for 24 h. The cells were then treated with IFN-β to activate the promoter *ISRE* promoter for an additional 12 h. Luciferase activity was measured using a dual-luciferase assay. (**B**) HEK-293T cells were transfected with either an empty vector (Vec) or the Flag-3D plasmid and treated with IFN-β, the mRNA levels of *ISG54, ISG56*, and *OAS1* were measured by qPCR. **(C)** PK-15 cells were transfected with either Vec or Flag-3D plasmids and treated with porcine IFN-β for another 12 h, the mRNA levels of *ISG15, ISG54*, and *OAS1* were detected by qPCR. All experiments were repeated three times, with similar results. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, ns, not significant.

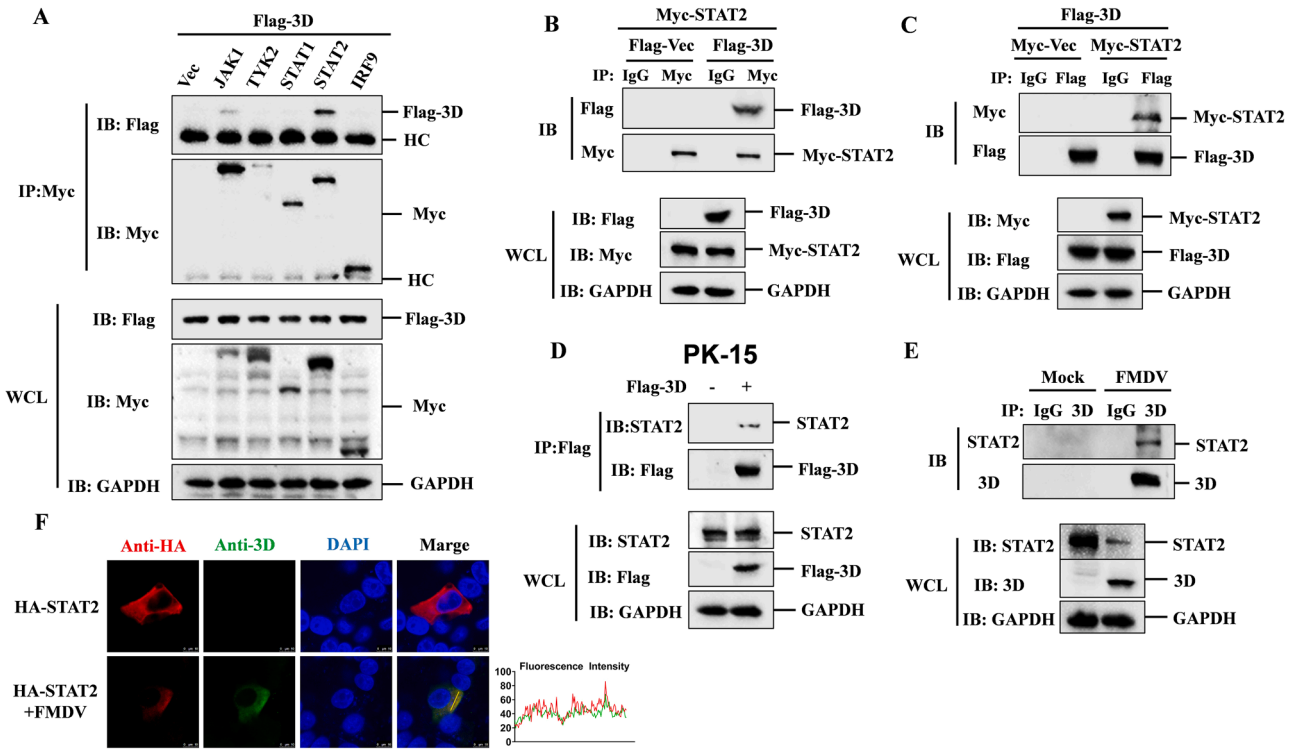

Fig. 2. FMDV 3D protein interacts with STAT2. **(A)** HEK-293T cells were co-transfected with Flag-3D and various Myc-tagged innate immune molecule-expressing plasmids (JAK1, TYK2, STAT1, STAT2, or IRF9). At 36 hpt, the cell lysates were subjected to Co-IP assay analysis. Immunoprecipitated proteins and whole-cell lysates (WCL) were analyzed by western blotting using specified antibodies. **(B)** HEK-293T cells were co-transfected with Myc-STAT2 and either an empty vector or Flag-3D expressing plasmids for 36 h. Cell lysates were immunoprecipitated with anti-Myc or control IgG antibodies and analyzed by western blotting. **(C)** HEK-293T cells were co-transfected with Flag-3D along with Vec or Myc-STAT2. At 36 hpt, cell lysates were subjected to Co-IP assay. Immunoprecipitated proteins and WCL were analyzed by western blotting. **(D)** PK-15 cells were transfected with Flag-3D or empty vector plasmids. At 36 hpt, cell lysates were immunoprecipitated with anti-Flag antibodies and analyzed by western blotting. **(E)** PK-15 cells were mock-infected or infected with FMDV for 12 h, cell lysates were immunoprecipitated with anti-3D antibodies and analyzed by western blotting with the indicated antibodies. **(F)** PK-15 cells were transfected with porcine HA-STAT2 expressing plasmids for 24 h, then mock-infected or infected with FMDV (MOI = 0.1) for 10 h. Colocalization of HA-STAT2 (red) and FMDV 3D (green) was assessed by immunofluorescence assay (IFA). Nuclei were counterstained with DAPI (blue).

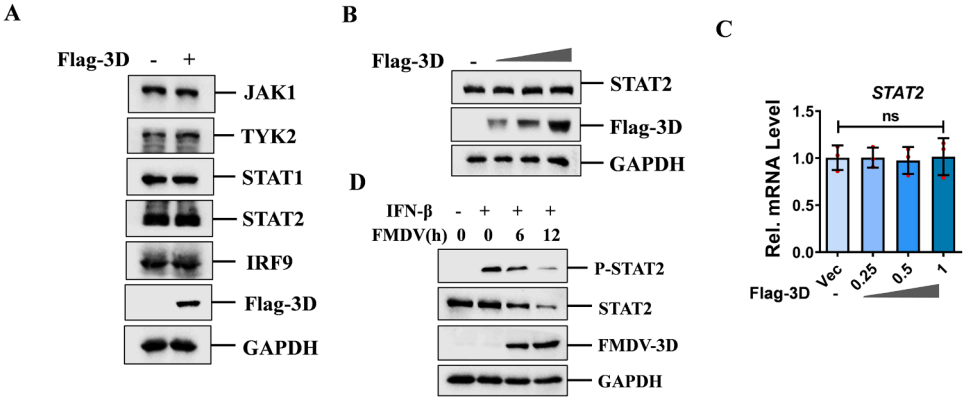

Fig. 3. FMDV 3D protein does not alter the protein level of STAT2. **(A)** PK-15 cells were transfected with empty vector or Flag-3D expressing plasmids. At 24 hpt, cell lysates were analyzed by western blotting with specified antibodies. **(B)** PK-15 cells were transfected with 0, 0.25, 0.5 or 1 μg of Flag-3D expressing plasmids for 24 h. Endogenous STAT2 protein expression levels were detected by western blotting. **(C)** PK-15 cells were transfected with an increasing amount of Flag-3D expressing plasmids (0, 0.25, 0.5 or 1 μg) for 24 h. Total RNA was extracted, and STAT2 mRNA levels were quantified by qPCR. **(D)** PK-15 cells mock-infected or infected with FMDV (MOI=0.1) for 6 and 12 h, followed by IFN-β (1000 U/mL) or mock treatment for 30 min. The expression and phosphorylation levels of STAT2 were detected by western blotting.

FMDV infection. The results showed that FMDV infection could down-regulate the expression and phosphorylation levels of STAT2, which might be caused by FMDV Lb protease. Previous reports showed that FMDV Lb protease cleaves intracellular transcription factors STAT1 and STAT2 to antagonize IFN-β-induced signaling (Ma et al. 2023a). Therefore, these data indicate that FMDV 3D inhibits JAK-STAT signaling pathway not through alter the translation or transcription of STAT2.

3.4. FMDV 3D suppresses the phosphorylation and nuclear translocation of STAT2

Upon binding of IFN-β to its receptor (IFNAR), JAK1 and TYK2 are activated. Subsequently, STAT1 and STAT2 undergo phosphorylation, bind to IRF9 to form interferon-stimulated gene factor 3 (ISGF3), and translocate to the nucleus. This process activates the ISRE promoter, leading to the production of ISGs, which exert antiviral functions. Given that 3D did not affect the protein and mRNA expression of STAT2, we investigated whether 3D interferes with STAT2 phosphorylation. Our results revealed that IFN-β-induced STAT2 phosphorylation was remarkably inhibited by FMDV 3D (Fig. 4A). Moreover, overexpression of FMDV 3D reduced STAT2 phosphorylation in a dose-dependent manner (Fig. 4B). Since 3D affected STAT2 phosphorylation, we examined whether 3D influences the formation of the STAT1-STAT2 complex in response to IFN-β. We found that the presence of 3D reduced the amount of STAT1 precipitated by STAT2 (Fig. 4C). To further confirm the suppressive effect of 3D on STAT2 nuclear translocation, PK-15 cells were mock-infected or infected with FMDV, followed by IFN-β treatment, and then the subcellular localization of STAT2 was evaluated. Our observations revealed that STAT2 nuclear translocation was initiated by IFN-β treatment, but this process was inhibited by FMDV infection (Fig. 4D). To determine whether the effect of 3D on STAT2 nuclear transport depends on nuclear localization signal receptor, we examined the influence of 3D on the expression of KPNA1, KPNA3 and KPNA4. The results showed that 3D did not affect the expression of KPNA1, KPNA3 and KPNA4 proteins (Fig. 4E). In addition, we detected the effect of FMDV 3D protein on the replication of FMDV, and the results showed that as the dose of 3D increased, FMDV replication was enhanced (Fig. 4F). Collectively, these data indicate that 3D suppresses STAT2 phosphorylation, hinders the dimerization of STAT1-STAT2, and blocks STAT2 nuclear translocation, thereby inhibiting the IFN signaling pathway, promoting the replication of FMDV.

3.5. Predicting the key interaction residues between STAT2 and 3D

To identify the interaction interfaces and residues involved in the STAT2–3D interaction, we used the SWISS-MODEL online services for homology modeling to create the models of STAT2 and 3D proteins. Subsequently, we employed the ZDOCK online service (https://zdock.wenglab.org/) to predict the interactions between 3D and STAT2. The predictions suggested that STAT2 has a strong interaction with 3D, forming five salt bridges, four attractive charge interactions, and twenty-one hydrogen bonds with 3D Specifically, residues D850, D399, E788, D794, and R687 of STAT2 form salt bridges with R168, R234, K400, R456, and E33 of 3D, respectively. Additionally, residues E686, D850, E724, and R807 of JAK1 engage in attractive charge interactions with R174, K369, R440, and D469 of 3D Residues P845, S849, N683, P848, C809, M459, T800, S835, R836, S838, Y841, S849, S849, E717, T800, K335, H693, P789, P817, H839, and P848 of STAT2 form hydrogen bonds with I167, R168, R174, K387, R461, Y394, G468, D222, G216, D109, D220, I167, P169, P445, A470, Y394, G28, S434, E451, Y336, and I167 of 3D, respectively (Table 1). Furthermore, the phosphorylation site Y690 of STAT2 forms a Pi-Alkyl interaction with the P23 residue of 3D Additionally, residues G28, E33, R174, and R174 of 3D interact with H693, R687, E683, and E686 of STAT2, respectively, forming a pocket that masks the Y690 residue of STAT2. This interaction explains the inhibition of STAT2 phosphorylation by 3D (Fig. 5).

4. Discussion

The innate immune system serves as the first line of defense against

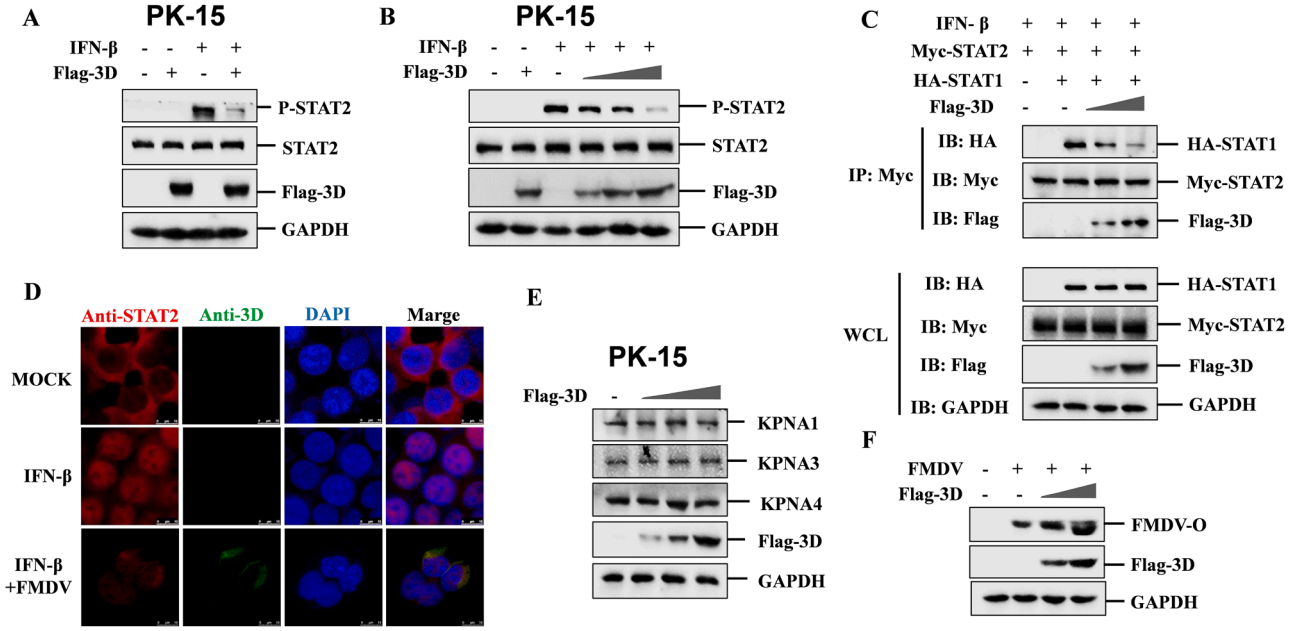

Fig. 4. FMDV 3D protein suppresses phosphorylation and nuclear translocation of STAT2. **(A)** PK-15 cells were transfected with either an empty vector or Flag-3D expressing plasmids. At 24 hpt, cells were treated with IFN-β (1000 U/mL) or solvent control for 30 min. Cell lysates were analyzed by western blotting with specified antibodies. **(B)** PK-15 cells were transfected with increasing amounts of Flag-3D expressing plasmids (0, 0.25, 0.5, or 1 μg). At 24 hpt, cells were treated with IFN-β (1000 U/mL) for 30 min. Cell lysates were analyzed by western blotting with indicated antibodies. **(C)** HEK-293T cells were co-transfected with Myc-STAT2 and HA-STAT1 or HA-vector, along with increasing amounts Flag-3D expressing plasmids. At 36 hpt, the cells were treated with IFN-β (1000 U/mL) for 30 min. The cell lysates were immunoprecipitated with anti-Myc antibodies. The immunoprecipitated proteins and WCL were analyzed by western blotting using the specified antibodies. **(D)** PK-15 cells mock-infected or infected with FMDV (MOI=0.1) for 10 h, followed by IFN-β (1000 U/mL) or mock treatment for 30 min. The subcellular localization of STAT2 and FMDV 3D was assessed by IFA. Nuclei were stained with DAPI (blue), and fluorescence was visualized for 3D (green) and STAT2 (red). **(E)** PK-15 cells were transfected with an increasing amounts Flag-3D expressing plasmids for 24 h, the cell lysates were analyzed by western blotting using the specified antibodies. **(F)** PK-15 cells were transfected with Flag-3D expressing plasmids for 24 h, then mock-infected or infected with FMDV (MOI=0.1) for 12 h. Cell lysates were analyzed by western blotting with indicated antibodies.

Table 1
The crucial residues involved in STAT2 and 3D interaction.

STAT2	3D	Interaction type	STAT2	3D	Interaction type
D850	R168	Salt Bridge	T800	G468	Hydrogen Bond
D399	RR234	Salt Bridge	S835	D222	Hydrogen Bond
E788	K400	Salt Bridge	R836	G216	Hydrogen Bond
D794	R456	Salt Bridge	S838	D109	Hydrogen Bond
R687	E33	Salt Bridge	Y841	D220	Hydrogen Bond
E686	R174	Attractive Charge	S849	I167	Hydrogen Bond
D850	K369	Attractive Charge	S849	P169	Hydrogen Bond
E724	R440	Attractive Charge	E717	P445	Hydrogen Bond
R807	D469	Attractive Charge	T800	A470	Hydrogen Bond
P845	I167	Hydrogen Bond	K335	Y394	Hydrogen Bond
S849	R168	Hydrogen Bond	H693	G28	Hydrogen Bond
N683	R174	Hydrogen Bond	P789	S434	Hydrogen Bond
P848	K387	Hydrogen Bond	P817	E451	Hydrogen Bond
C809	R461	Hydrogen Bond	H839	Y336	Hydrogen Bond
M459	Y394	Hydrogen Bond	P848	I167	Hydrogen Bond

Table 2
The primers sequences used in this study.

Primers	Sequences (5′–3′)
Human ISG54	Forward: 5′- ACGGTATGCTTGGAACGATTG –3′ Reverse: 5′- AACCCAGAGTGTGGCTGATG –3′
Human ISG56	Forward: 5′- TCACAGGTCAAGGATAGTC –3′ Reverse: 5′- CCACACTGTATTTGGTGTCTAGG –3′
HumanOAS1	Forward: 5′- TCCACAGCCTCACTTCATTCC –3′ Reverse: 5′- ACATTAGACATTACCCTCCCATCAG –3′
Human GAPDH	Forward: 5′- CGGGAAGCTTGTGATCAATGG –3′ Reverse: 5′- GGCAGTGATGGCATGGACTG –3′
Porcine ISG15	Forward: 5′- GATCGGTGTGCCTGCCTTC –3′ Reverse: 5′- CGTTGCTGCGACCCTTGT –3′
Porcine ISG54	Forward: 5′- CTGGCAAAGAGCCCTAAGGA –3′ Reverse: 5′- CTCAGAGGGTCAATGGAATTCC –3′
Porcine OAS1	Forward: 5′- AAGCATCAGAAGCTTTGCATCTT –3′ Reverse: 5′- CAGGCCTGGGTTTCTTGAGTT –3′
Porcine GAPDH	Forward: 5′- ACATGGCCTCCAAGGAGTAAGA –3′ Reverse: 5′- GATCGAGTTGGGGCTGTGACT –3′
Porcine STAT2	Forward: 5′- GCAGGAAAGGGCAACAATAA –3′ Reverse: 5′- GAGGGTGTCCGTTGTCAGTT –3′

viral invasions, initiating host antiviral responses. Upon viral infection, pattern recognition receptors (PRRs) identify viral elements, prompting the production of IFNs. These IFNs subsequently stimulate the expression of ISGs, which have direct antiviral effects and inhibit viral replication (Liu et al. 2017). STAT2 is a crucial molecule in the innate immune system, and various viruses have evolved strategies to target STAT2, thereby evading host innate immunity and promoting viral replication. For instance, porcine reproductive and respiratory syndrome virus (PRRSV) nsp11 antagonizes IFN signaling by mediating STAT2 degradation (Yang et al. 2019). Porcine deltacoronavirus (PDCoV) NS7a interacts with STAT2 and IRF9, inhibits STAT1 phosphorylation, and blocks the formation and nuclear accumulation of ISGF3 (Mou et al. 2024). Heartland virus disrupts type I and III interferon antiviral signaling by blocking the phosphorylation and nuclear translocation of STAT1 and STAT2 (Feng et al. 2019). The alpha-coronavirus E protein disrupts the JAK-STAT signaling pathway by inducing STAT2 degradation (Huang et al. 2025). Zika virus NS2A protein disrupts interferon signaling through the degradation of STAT1 and STAT2 (Fanunza et al. 2021). African swine fever virus (ASFV) cysteine protease pS273R inhibits type I IFN signaling by targeting STAT2 for degradation (Li et al. 2023). Alongshan virus (ALSV) nonstructural protein NSP1 interacts with STAT2, leading to its degradation (Zhao et al. 2025). Mpox virus (MPXV) PoxS interacts with STAT2 and sequesters it in the cytoplasm to antagonize the type I IFN antiviral response (Chan et al. 2025). ASFV pB475L evades host antiviral innate immunity by targeting STAT2, inhibiting IFN-I signaling (Huang et al. 2024). In this study, we found that STAT2 is also a target of FMDV for inhibiting the host antiviral response, which broadens the understanding of how viruses target STAT2 to exert antagonistic effects.

The 3D protein of picornaviruses not only functions as an RNA-dependent RNA polymerase (RdRP) to promote viral replication but also counteracts host immune responses. For instance, enterovirus 71 (EV71) and coxsackievirus B3 3D proteins inhibit the activation of the IFN-β promoter and promote viral replication by interacting with MDA5 (Kuo et al. 2019). The 3D protein of EV71 degrades STAT1 to suppress IFN-γ signaling (Wang et al. 2015). Additionally, Senecavirus A (SVA) 3D protein interacts with IKKα and IKKβ, leading to the activation of NF-κB and promoting the transcription of IL-1β (Choudhury et al. 2022). These findings demonstrate the diverse strategies by which picornavirus 3D proteins suppress host immune responses; however, no reports have previously described the suppression of innate immunity mediated by FMDV 3D in the context of viral infection. As a conserved RdRP, it can be used as a promising target for antiviral strategies development.

In this study, we observed that FMDV 3D significantly inhibits the activation of the IFN-β-induced *ISRE* promoter and reduces the expression of ISGs induced by IFN-β. Further investigation revealed that 3D interacts with STAT2 to inhibit STAT2 phosphorylation, blocking its nuclear transport, and suppressing the activation of the *ISRE* promoter, ultimately inhibiting the JAK-STAT signaling pathway. For the first time, we discovered FMDV 3D polymerase targets STAT2, inhibit the phosphorylation of STAT2, thereby hindering the activation of the JAK-STAT pathway and promoting the replication of FMDV. In summary, we have uncovered a novel mechanism by which the FMDV 3D protein targets STAT2 to antagonize the JAK-STAT signaling pathway (Fig. 6). These findings highlight the potential of the 3D protein as a promising antiviral target for FMDV, given its highly conserved genetic stability.

5. Conclusion

This study revealed FMDV 3D protein targets STAT2, inhibiting

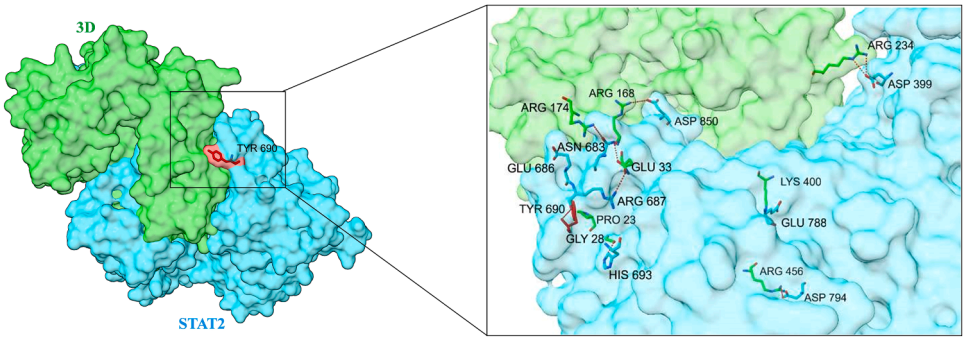

Fig. 5. Prediction the key interaction residues between STAT2 and FMDV-3D The interaction model between STAT2 and FMDV-3D was constructed using the ZDOCK online service. Predicted salt bridge interactions, Pi-Alkyl interaction of STAT2 phosphorylation residue Y690 with 3D P23, and key residues STAT2 R687, E686, N683, H693 and 3D E33, R174, R174, G28 were highlighted using PyMOL software.

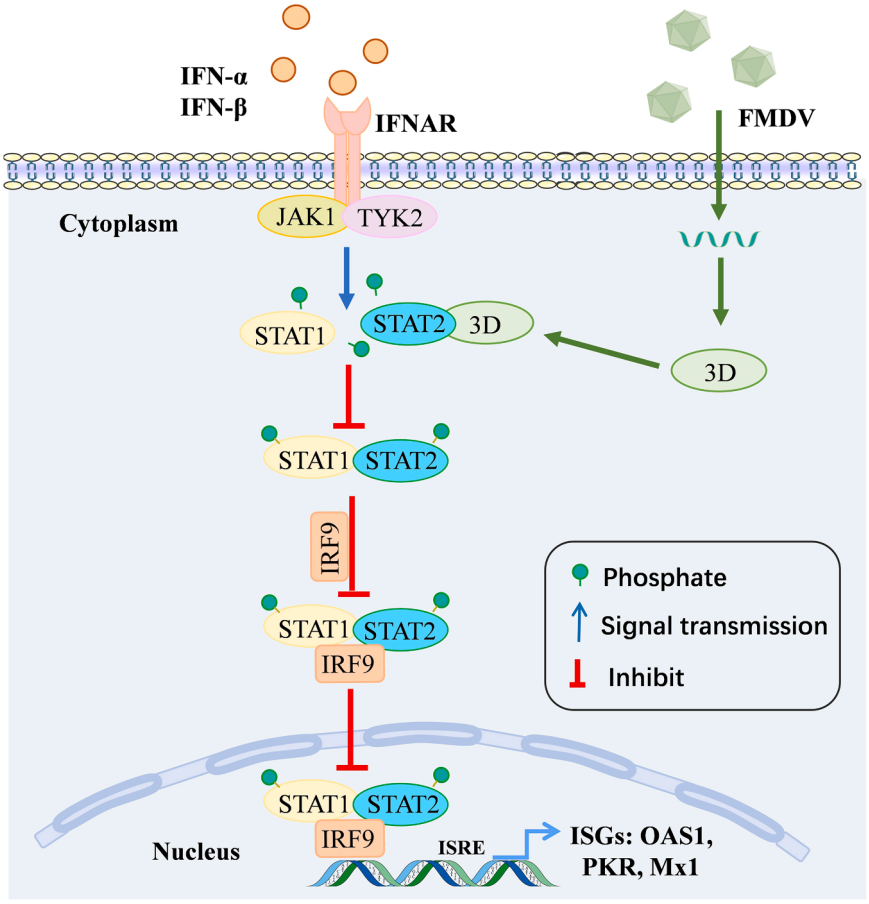

Fig. 6. Mechanisms by which FMDV 3D protein targets STAT2 to impede the activation of the JAK-STAT signaling pathway. Upon FMDV infection of host cells, the viral 3D protein interacts with STAT2, hinders the phosphorylation and inhibits the nuclear translocation of STAT2, thereby blocks the activation of the JAK-STAT signaling pathway and suppresses host antiviral response.

STAT2 phosphorylation, then blocking its nuclear transport, antagonize the JAK-STAT signaling pathway. Our results demonstrated that FMDV 3D protein acts as an antagonist, targeting STAT2 to inhibit the JAK-STAT signaling pathway.

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CRediT authorship contribution statement

Kangli Li: Writing – original draft, Formal analysis, Data curation. **Boning Zhu:** Methodology, Data curation. **Shuo Wang:** Methodology, Formal analysis. **Xiangle Zhang:** Supervision, Resources, Project administration. **Xiaodan Wen:** Methodology, Investigation. **Weijun Cao:** Supervision, Resources. **Guoliang Zhu:** Validation. **Haixue Zheng:** Supervision, Resources, Project administration. **Fan Yang:** Supervision, Resources, Project administration. **Zixiang Zhu:** Writing – review & editing, Resources, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no conflicts of interest.

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Data availability

Data will be made available on request.

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