



## Research Article

# FoxJ1 inhibits African swine fever virus replication and viral S273R protein decreases the expression of FoxJ1 to impair its antiviral effect

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## ABSTRACT

African swine fever (ASF) is a highly pathogenic swine infectious disease that affects domestic pigs and wild boar, which is caused by the African swine fever virus (ASFV). ASF has caused huge economic losses to the pig industry and seriously threatens global food security and livestock health. To date, there is no safe and effective commercial vaccine against ASF. Unveiling the underlying mechanisms of ASFV-host interplay is critical for developing effective vaccines and drugs against ASFV. In the present study, RNA-sequencing, RT-qPCR and Western blotting analysis revealed that the transcriptional and protein levels of the host factor FoxJ1 were significantly down-regulated in primary porcine alveolar macrophages (PAMs) infected by ASFV. RT-qPCR analysis showed that overexpression of FoxJ1 upregulated the transcription of type I interferon and interferon stimulating genes (ISGs) induced by poly(dA:dT). FoxJ1 revealed a function to positively regulate innate immune response, therefore, suppressing the replication of ASFV. In addition, Western blotting analysis indicated that FoxJ1 degraded ASFV MGF505-2R and E165R proteins through autophagy pathway. Meanwhile, RT-qPCR and Western blotting analysis showed that ASFV S273R inhibited the expression of FoxJ1. Altogether, we determined that FoxJ1 plays an antiviral role against ASFV replication, and ASFV protein impairs FoxJ1-mediated antiviral effect by degradation of FoxJ1. Our findings provide new insights into the antiviral function of FoxJ1, which might help design antiviral drugs or vaccines against ASFV infection.

## 1. Introduction

African swine fever (ASF) is a highly contagious and deadly viral disease that affects domestic pigs and wild boar, which is characterized by various clinical symptoms, including anorexia, moribundity, bloody diarrhea, vomiting, high fever, and hemorrhagic symptoms (Sanchez-Cordon et al., 2018). ASF first appeared in Kenya in Africa in 1921, and then reported and spread rapidly in many countries and regions around the world (Costard et al., 2013; Chenais et al., 2019; Mulumba-Mfumum et al., 2019). So far, there are no commercial vaccines and effective antiviral drugs available to prevent and control ASFV infections. Therefore, the spread of ASF has caused great economic losses to the global pig industry and seriously threatened global food security and livestock health (Tao et al., 2020).

ASFV is the only member of the *Asfarviridae* family and the only known DNA arbovirus. The double-stranded DNA (dsDNA) genome of

ASFV is around 170–193 kb and contains more than 150 open reading frames (ORFs), encoding 150 to 200 proteins (Jia et al., 2017). Previous studies have suggested that many of ASFV-encoded proteins are involved in not only the viral life cycle, including viral entry, DNA replication and repair, viral assembly, and egress, but also utilizes multiple mechanisms to inhibit and evade host immune response (such as type I interferon production and inflammatory responses) (Alonso et al., 2013). For example, ASFV E120R protein inhibits the production of IFN- $\beta$  by blocking IRF3 activation (Liu et al., 2021). ASFV multigene families (MGFs) 360 and MGF505 play important roles in determining host range and inhibiting host innate immunity (Zsak et al., 2001; Afonso et al., 2004). ASFV MGF360-9L interacts with STAT1 and STAT2 to inhibit IFN- $\beta$  signaling via degradation of STAT1 through apoptosis and STAT2 through the proteasome pathway (Zhang et al., 2022), and ASFV-MGF505-7R interacts with IKK $\alpha$  to inhibit the activation of NF- $\kappa$ B and binds to NLRP3 to suppress the formation of inflammatory bodies,

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which reduces the production of IL-1 $\beta$  (Li et al., 2021b). An iGLuc-based inflammation reconstruction system reporter and an IFN- $\beta$  reporter screening analysis showed that ASFV MGF505-2R significantly inhibited the maturation of IL-1 $\beta$  and IFN- $\beta$  expression (Li et al., 2021b). E165R, a deoxyuridine 5'-triphosphate nucleotidohydrolase encoded by ASFV genome, degrades dUTP in the cytoplasm and thus minimizes misincorporation of uracil into viral DNA, which plays an essential role in maintaining the fidelity of genome replication (Li et al., 2019; Zhang et al., 2021). ASFV S273R is a specific SUMO-1 cysteine protease, which catalyzes the maturation of the pp220 and pp62 polyprotein precursors. The overall structure of S273R protease consists of the “core domain” which shares high degree of structural similarity with chlamydial deubiquitinating enzyme, sentrin-specific protease, and adenovirus protease and the “arm domain” which maintains the activity of S273R (Li et al., 2020). However, up to now, the host proteins that affect ASFV replication remain poorly mapped.

Further exploration of host proteins interacting with ASFV and elucidating their specific mechanisms are of great significance for studying the pathogenesis of ASFV. Porcine alveolar macrophages (PAMs) are the main targets for ASFV infection and play a central role in host antiviral immune response (van Furth et al., 1972; Gordon et al., 1995; Sanchez et al., 2017). PAMs have been widely used to investigate the antiviral mechanisms of ASFV infection (Li et al., 2021b; Liu et al., 2021). MA-104 cells are non-host cells, which have also been used in ASFV study. Although the susceptibility of MA-104 cells to ASFV is lower than that of PAMs, MA-104 cells are often used in evaluation of host antiviral mechanism during ASFV infection. The reason is that the transfection efficiency in MA-104 cells is higher than that in PAMs (Rai et al., 2020; Yang et al., 2021a). In addition, several studies also used host-derived immortalized pulmonary alveolar macrophages (iPAM) to explore the mechanism of host-virus interaction (Yang et al., 2021b).

FoxJ1 is a member of the Forkhead Box (Fox) family, an important regulator of immune cell development and effector function. More studies have indicated that the Fox family members play important roles in lymphatic system development and immune function regulation (Jonsson and Peng, 2005). FoxJ1 plays an important role in CD<sup>4+</sup> T cell tolerance, FoxP1 is involved in macrophage differentiation, and Foxq1 is associated with the function of natural killer cells (Coffer and Burgering, 2004; Jonsson and Peng, 2005). FoxJ1 expression can be regulated by some virus infections. For example, mouse cytomegalovirus infects airway epithelium, resulting in reduced expression of FoxJ1 in cilia, and affecting ciliary epithelial cells development (Wu et al., 2008). Respiratory paramyxovirus infection leads to decreased FoxJ1 expression in airway epithelial cells (Look et al., 2001).

Meanwhile, FoxJ1 inhibits spontaneous autoimmunity by antagonizing NF- $\kappa$ B activation in T cells. It also has a similar effect in B cells (Lin et al., 2005; Srivatsan and Peng, 2005). Therefore, FoxJ1 plays an important role in regulating immune function, but the underlying mechanism remains largely unknown. In this study, we evaluated the antiviral role of FoxJ1 and preliminarily explored the mechanism by which FoxJ1 inhibits ASFV replication. By an RNA-sequencing analysis, we found that FoxJ1 was significantly down-regulated in porcine alveolar macrophages infected with ASFV, and it was observed that FoxJ1 could degrade ASFV-MGF505-2R and ASFV-E165R through autophagy pathway and play a role in inhibiting ASFV replication. In addition, ASFV S273R could inhibit FoxJ1 expression. Our study provided new data to elucidate the interaction between ASFV and the host.

## 2. Materials and methods

### 2.1. Cells and viruses

MA-104 monkey kidney epithelial cells were cultured in Dulbecco's modified Eagles medium (Gibco, Carlsbad, CA, USA) containing 10% inactivated fetal bovine serum (Gibco, Carlsbad, CA, USA) and were cultured at 37 °C and 5% CO<sub>2</sub>. Porcine alveolar macrophages (PAMs) and

iPAM cells were cultured in RPMI 1640 medium (Gibco, Carlsbad, CA, USA) containing 10% inactivated fetal bovine serum were cultured at 37 °C and 5% CO<sub>2</sub>. The ASFV strain, a genotype II ASFV named ASFV CN/GS/2018, is provided by Lanzhou Veterinary Research Institute, CAAS (Liu et al., 2021).

### 2.2. Antibodies and reagents

The anti-FLAG (F7425), anti-Myc (M4439),  $\beta$ -Actin (A5441), anti-GSDMD (SAB4200798) was purchased from Sigma-Aldrich Co., Ltd (Shanghai, China). The anti-FoxJ1 Polyclonal Antibody was purchased from Abbkine Scientific Co., Ltd (Wuhan, China). Horseradish peroxidase HRP-Goat anti-Rabbit IgG (H+L) (SA00001-2), HRP-Goat anti-Mouse IgG (H+L) (SA00001-1) was purchased from Lanzhou Lihe Biotechnology (Lanzhou, China).

MG132, chloroquine (CQ), 3-MA, NH<sub>4</sub>Cl, and Z-VAD-FMK were purchased from MedChemExpress (New Jersey, USA). Lipofectamine2000 transfection reagent (11668019) was purchased from Thermo Fisher Scientific (Massachusetts, USA). jetPRIME and jetPEI-Macrophage were purchased from Polyplus Transfection (Strasbourg, France).

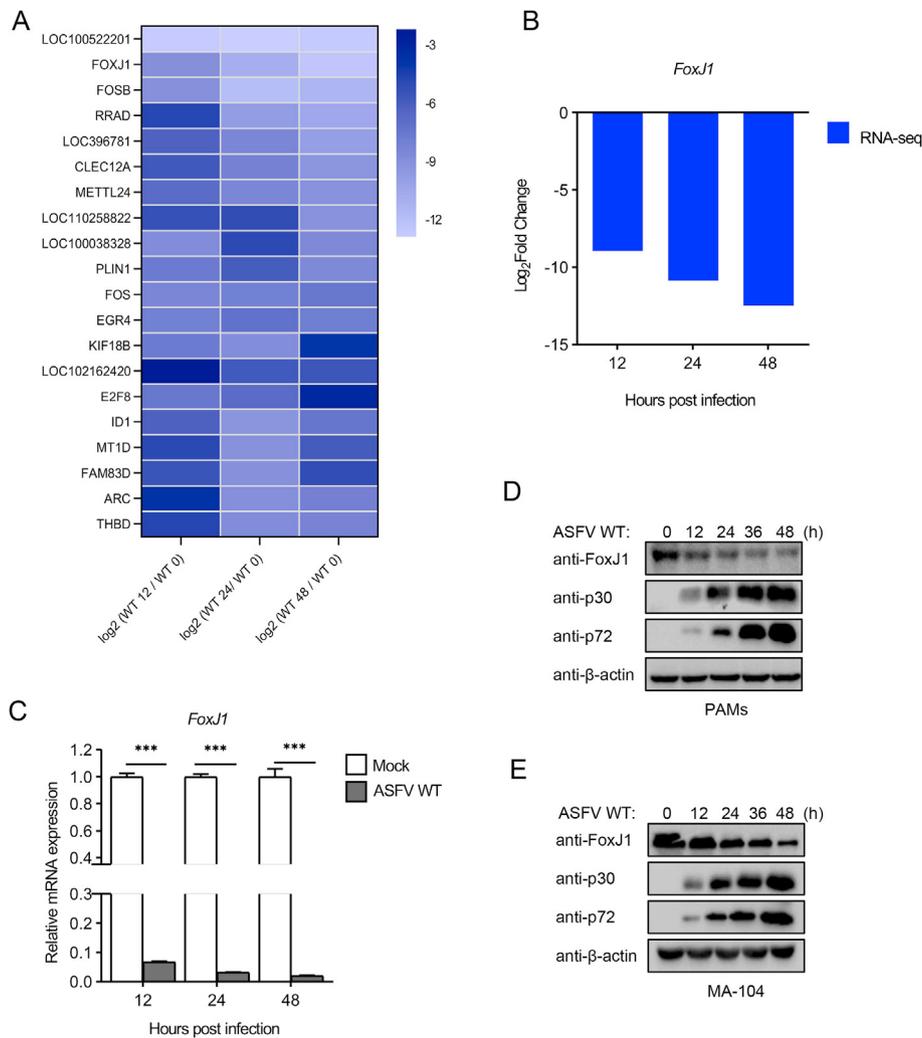
### 2.3. Plasmid construction and cell transfection

The Flag-ASFV-D1133L, Flag-ASFV-B962L, Flag-ASFV-NP868R, Flag-ASFV-Q706L, Flag-ASFV-MGF505-2R, Flag-ASFV-MGF505-7R, Flag-ASFV-MGF505-6R, Flag-ASFV-MGF505-9R, Flag-ASFV-C475L, Flag-ASFV-NP419L, Flag-ASFV-MG360-3L, Flag-ASFV-MG360-12L, Flag-ASFV-I329L, Flag-ASFV-E296R, Flag-ASFV-S273R, Flag-ASFV-MGF360-15R, Flag-ASFV-A238L, Flag-ASFV-A224L, Flag-ASFV-E165R, Flag-ASFV-DP96R, Flag-SVA-2B, HA-JAK2, HA-MAVS, expression plasmids were stored by our laboratory previously. To construct a porcine FoxJ1 expressing plasmid, the full-length CDS fragment of FoxJ1 was amplified with the primers (FoxJ1-F: 5'-CGCCTCGAGATGGCGGAGAGCTG GCTACGC-3'; FoxJ1-R: 5'-CGCGGTACCTTACAAGAAGGCCACCCACGCT-3') from the mRNA obtained from iPAM cells and inserted into the pcDNA<sup>TM</sup>3.1/myc-His(-) A vector by using *Xho*I (Cat. no: 1094A, Takara, Otsu, Japan) and *Kpn*I (Cat. no: 1068A, Takara, Otsu, Japan) restriction enzyme sites. All of the constructed plasmids were analyzed and verified by DNA sequencing. According to the manufacturer's protocol, the plasmids were transfected into MA-104 cells or iPAM cells using jetPRIME transfection reagent (Polyplus).

### 2.4. Real-time qPCR

PAMs were seeded in a 12-well plate and were collected after being infected with ASFV for the indicated times. MA-104 cells were seeded in a 12-well plate, and the cells were transfected with Myc-FoxJ1 expressing plasmids prior to ASFV infection at the indicated times. Total RNA was extracted from PAMs or MA-104 cells using TRIzol reagent and reverse transcribed with PrimeScript RT kit according to the manufacturer's direction (Takara, Otsu, Japan). qPCR was performed using the Power Up SYBR Green Master Mix on the ABI StepOnePlus system. All data were analyzed using the StepOnePlus software, and the relative mRNA level of these genes was normalized to porcine or monkey GAPDH mRNA level. In addition, the relative expression of mRNA was calculated based on the comparative cycle threshold ( $2^{-\Delta\Delta CT}$ ) method (Schmittgen and Livak, 2008). The primer sequences were listed in Supplementary Table S1.

The supernatant of PAMs or MA-104 cells was collected at the indicated time after ASFV infection. Real-time quantitative PCR analysis was performed using ASFV p72 as a target gene to detect the copy number of ASFV genomic DNA. The DNA was extracted using QIAamp® DNA Mini Kits as the manufacturer's instruction (Qiagen, Germany), and then qPCR was carried out on a Bio-Rad system using the p72 primers: ASFV-p72-F: 5'-GAT ACC ACA AGA TCA GCC GT-3'; ASFV-p72-R: 5'-CTG CTC ATG



**Fig. 1.** ASFV infection decreases FoxJ1 expression. **A** The heat map of the top 20 significantly down-regulated genes in PAMs in response to ASFV infection. **B** RNA-seq analysis of the *FoxJ1* expression changes in PAMs infected with ASFV (MOI = 1) for 12, 24, and 48 h, respectively. Y-axis coordinates for the ratio of ASFV infection group and mock group. Genes with  $P < 0.05$  and  $|\log_2FC| > 1$  were defined as differentially expressed genes. **C**, **D** PAMs were infected with ASFV (MOI = 1) for 12, 24, 36, or 48 h, the transcriptional and protein levels of FoxJ1 were detected by RT-qPCR (**C**) and Western blotting (**D**). GAPDH and  $\beta$ -actin were used as the internal control for RT-qPCR and Western blotting, respectively. **E** MA-104 cells were infected with ASFV (MOI = 1) for 12, 24, 36, and 48 h, the protein levels of FoxJ1 were detected by Western blotting. \*\*\*,  $P < 0.001$ .

GTA TCA ATC TTA TCG A-3'; Taqman: 5'-CCA CGG GAG GAA TAC CAA CCC AGT G-3'.

## 2.5. Western blotting

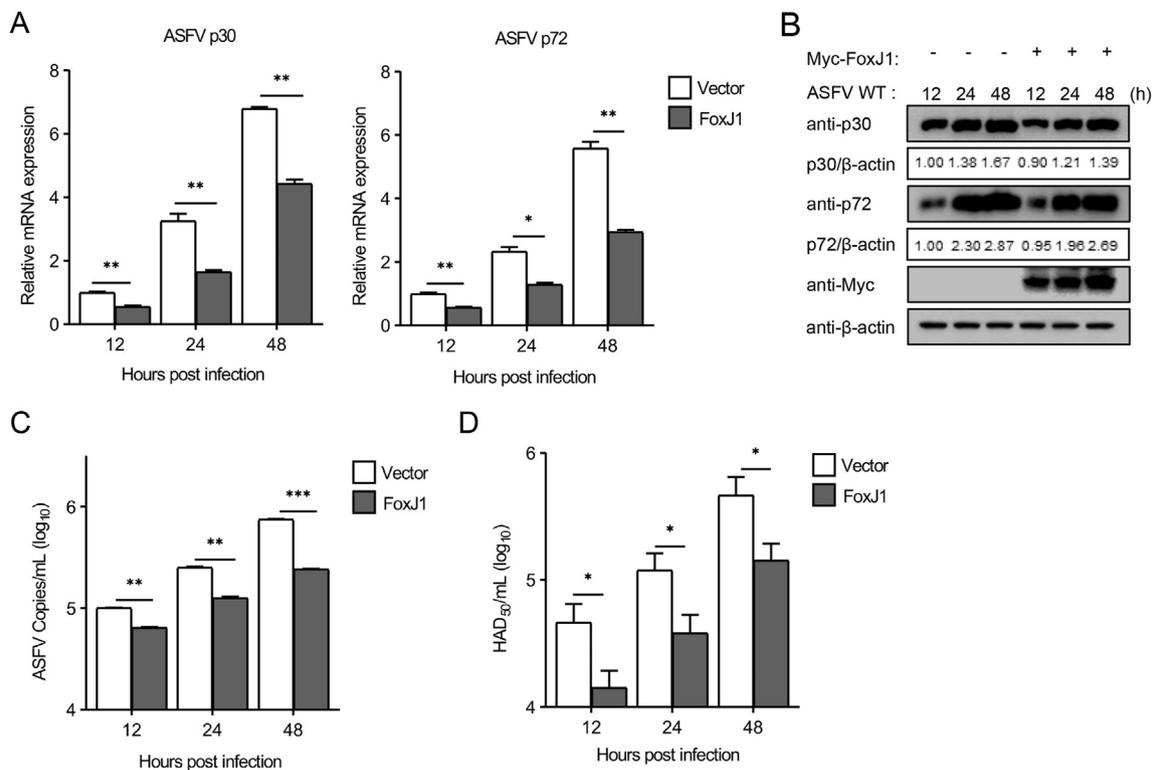
Cells were collected and lysed for Western blotting analysis as the indicated experiments, the target proteins were analyzed by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto Immobilon-P membrane (EMD Millipore, Billerica, MA, USA), which was blocked with 5% skim milk in Tris-buffered saline with Tween 20 (TBST) at room temperature for 2 h and then incubated with appropriate primary antibodies at 4 °C overnight. Membranes were washed three times with TBST and exposed for 1 h to the designated secondary antibodies. Chemiluminescence detection was performed by ECL prime (Milli-poreSigma). Antibody-antigen complexes were visualized with chemiluminescence detection reagents (Thermo Scientific). The relative expression levels of the target proteins were determined as previously described (Gassmann et al., 2009). The relative expression of the target protein in the control cells was arbitrarily set at 1. Densitometric analysis of immunoreactive bands was performed using Image J Software (NIH).

## 2.6. Virus titration

Wild-type ASFV CN/GS/2018 virus was quantified using the hemadsorption assay described previously (Malmquist and Hay, 1960) with minor modifications. PAMs were seeded in 96-well plates, and the viruses were added to the plates and titrated in triplicate using 10-fold serial dilutions. Hemadsorption dose (HAD) was determined on day 7 post-inoculation, and 50% HAD doses (HAD<sub>50</sub>) were calculated using the method of Reed and Muench (Reed, 1938).

## 2.7. RNA interference experiments

The specific small interfering RNA (siRNA) designed for targeting FoxJ1 was synthesized by Genepharma company (Shanghai, China). MA-104 cells or PAMs were transfected with negative control (NC) or FoxJ1-siRNAs. At 6 h post-transfection (hpt), the cells were mock-infected or infected with ASFV at a multiplicity of infection (MOI) of 1 for the indicated time. The mRNA expression levels of the target gene and viral indicators (ASFV *p30* and *p72* genes) were detected by qPCR and Western blotting. The siRNA sequence information is presented in Supplementary Table S1.



**Fig. 2.** Overexpression of FoxJ1 inhibits ASFV replication. **A, B** MA-104 cells were transfected with an empty vector (2  $\mu$ g/well) or Myc-FoxJ1 (2  $\mu$ g/well) expressing plasmids for 24 h, and the cells were then infected with 1 MOI dose of ASFV for 12, 24, or 48 h. The transcriptional and protein levels of ASFV p30 (CP204L) and p72 (B646L) were detected by RT-qPCR (**A**) and Western blotting (**B**), respectively. The relative fold-change of viral mRNA expression was measured by the  $2^{-\Delta\Delta Ct}$  method. **C, D** The samples were prepared as described in Fig. 2A. The viral genome copies of ASFV (**C**) and viral titers (**D**) were determined by qPCR and HAD<sub>50</sub> methods at 12, 24 and 48 hpi, respectively. Data are presented as mean  $\pm$  SD of three independent experiments. Statistical significance between groups was determined using a *t*-test with GraphPad Prism software. \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ; \*\*\*,  $P < 0.001$ .

## 2.8. RNA sequencing

Total RNAs were extracted from mock-infected or ASFV WT-infected PAMs using TRIzol reagents for transcriptomic analysis. All experiments were carried out in triplicate. The quality and integrity of the extracted RNAs were determined using 1% (wt/vol) agarose gel and the RNA Nano 6000 assay kit for the Bioanalyzer 2100 system (Agilent Technologies, Santa Clara, USA), respectively. All extracted RNA samples were submitted to Lc-Bio Technologies Co. Ltd. (Hangzhou, China) for transcriptome sequencing (RNA-seq). The top 10 genes that were most significantly down-regulated at each time point after ASFV infection are presented in Supplementary Table S2.

## 2.9. Statistical analysis

All data are presented as mean values  $\pm$  standard errors of at least three independent experiments. The significance of the experimental results was analyzed using GraphPad Prism v.8 (San Diego, CA, USA). \*,  $P < 0.05$  was considered statistically significant. \*\*,  $P < 0.01$  and \*\*\*,  $P < 0.001$  was considered highly statistically significant, "ns" indicated not significant.

## 3. Results

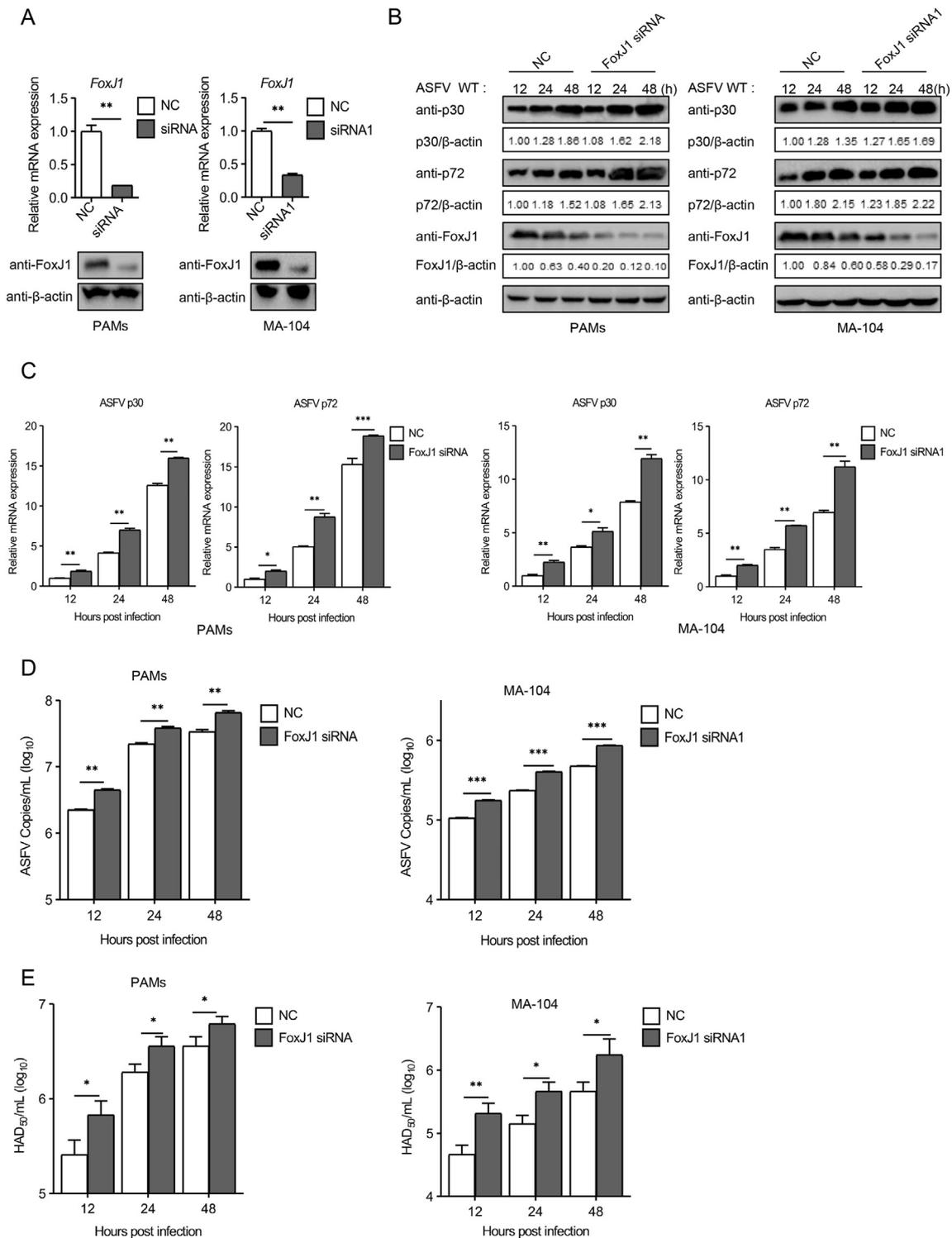
### 3.1. ASFV infection decreases FoxJ1 expression

RNA sequencing method was used to identify the host genes in PAMs regulated by ASFV infection. PAMs were mock-infected or infected with ASFV (MOI = 1) for 12, 24 or 48 h, and the changes of gene expression profile were analyzed by RNA-sequencing. The top 20 most significantly down-regulated genes during ASFV infection were

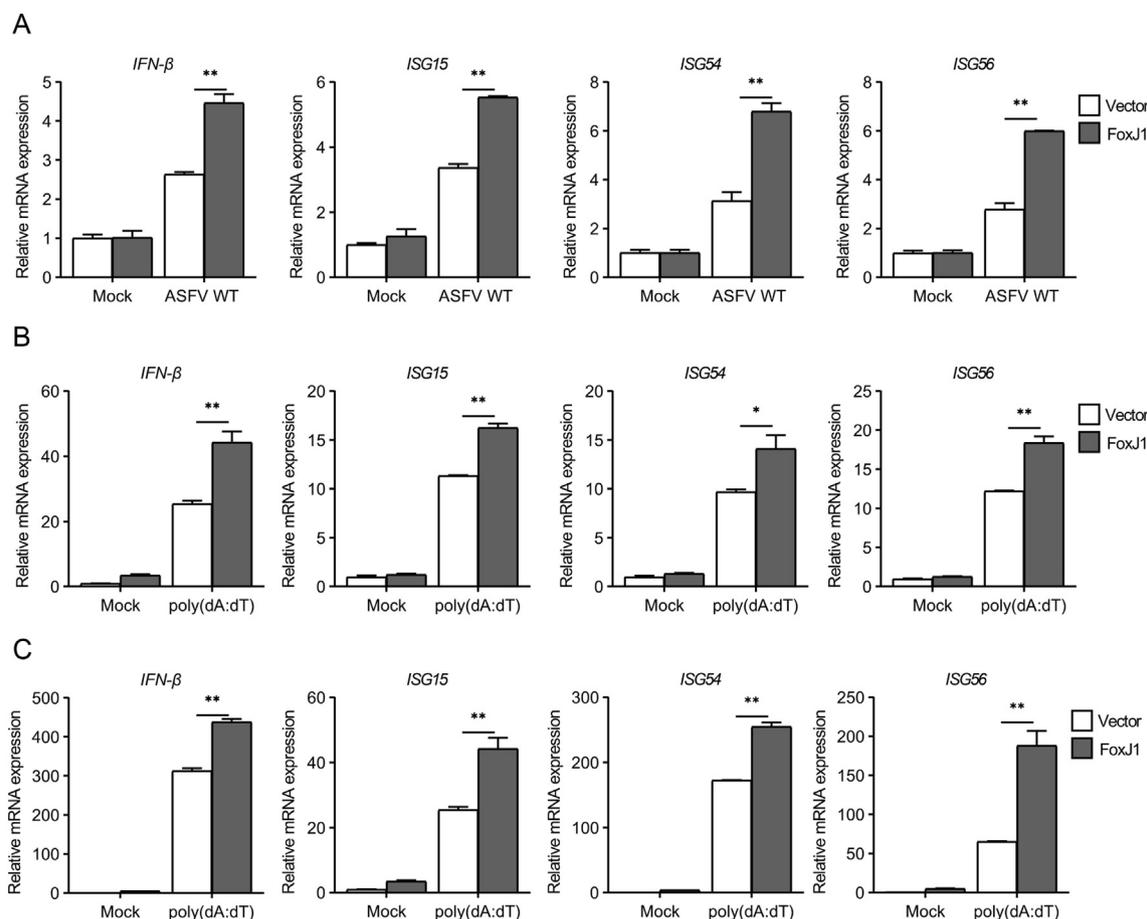
listed and analyzed (Fig. 1A). The results showed that the mRNA expression of *FoxJ1* was significantly down-regulated in ASFV-infected PAMs compared with that in the mock-infected PAMs, and the expression level of *FoxJ1* gradually decreased as ASFV infection progressed (Fig. 1B). The changes of *FoxJ1* transcripts during ASFV infection were further verified by qPCR analysis. At 12, 24, 36, or 48 h post-infection (hpi) with ASFV, PAMs were collected and analyzed by RT-qPCR and Western blotting. The results showed that both the mRNA and protein expression levels of FoxJ1 were significantly down-regulated in ASFV-infected PAMs (Fig. 1C and D). In addition, the decreased expression of FoxJ1 was also detected in ASFV-infected MA-104 cells (Fig. 1E).

### 3.2. Overexpression of FoxJ1 inhibits ASFV replication

ASFV infection leads to down-regulation of FoxJ1 expression (Fig. 1). The viral replication of ASFV in FoxJ1 overexpressing cells was evaluated to investigate the role of FoxJ1 in ASFV replication. The MA-104 cells were transfected with an increasing amount of Myc-FoxJ1 expressing plasmids followed by ASFV infection, and the cells were collected at 12, 24, or 48 hpi with ASFV (MOI = 1). The transcriptional and protein expression levels of ASFV p30 (CP204L) and p72 (B646L) were detected by RT-qPCR and Western blotting. The results showed that the transcriptional and protein levels of ASFV p30 and p72 in FoxJ1 overexpressing cells were significantly downregulated at 12, 24, and 48 hpi (Fig. 2A and B). Similarly, the viral DNA copies in the supernatant evaluated by the TaqMan qPCR confirmed that overexpression of FoxJ1 inhibited ASFV replication (Fig. 2C). Consistently, the viral titers were significantly decreased in FoxJ1 overexpressing cells (Fig. 2D). These results suggested that FoxJ1 has an antiviral function against ASFV replication.



**Fig. 3.** Knockdown of FoxJ1 promotes ASFV replication. **A** Evaluation of the knockdown efficiency of FoxJ1-siRNA. PAMs or MA-104 cells were transfected with 150 nmol/L NC or FoxJ1 siRNA, and the expression of FoxJ1 transcription and protein was detected by RT-qPCR and Western blotting respectively. **B** The PAMs or MA-104 cells were transfected with 150 nmol/L NC or si-FoxJ1 prior to ASFV infection (MOI = 1). Effects of FoxJ1 siRNA on ASFV replication were determined. The protein levels of ASFV p30 and p72 in PAMs and MA-104 cells were detected by Western blotting respectively. The relative expression levels of p30, p72 and FoxJ1 were determined by densitometric analysis using Image J Software (NIH). **C** The mRNA levels of ASFV p30 and p72 were detected by RT-qPCR at 12, 24, or 48 hpi. **D, E** The PAMs or MA-104 cells were transfected with 150 nmol/L NC or FoxJ1 siRNA before ASFV infection (MOI = 1). The viral genome copies of ASFV and viral titer were determined by qPCR and HAD<sub>50</sub> methods at 12, 24, and 48 hpi, respectively. Data are presented as mean  $\pm$  SD of three independent experiments. Statistical significance between groups was determined using a *t*-test with GraphPad Prism software. \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ; \*\*\*,  $P < 0.001$ .



**Fig. 4.** Overexpression of FoxJ1 leads to enhancement of type I IFN response. **A** MA-104 cells were transfected with empty vector or Myc-FoxJ1 expressing plasmids for 24 h and then infected with ASFV (MOI = 1) for 24 h. The mRNA expression levels of *IFN-β*, *ISG15*, *ISG54*, and *ISG56* were then detected by RT-qPCR. **B, C** MA-104 and iPAM cells were transfected with empty vector (2 μg/well) or Myc-FoxJ1 (2 μg/well) expressing plasmids for 24 h, and then mock-transfected or transfected with poly(dA:dT) (1 mg/mL) for 12 h. The transcriptional levels of *IFN-β*, *ISG15*, *ISG54*, and *ISG56* were detected by RT-qPCR. \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ .

### 3.3. Knockdown of FoxJ1 promotes ASFV replication

Next, we tried to investigate the endogenous function of FoxJ1 against ASFV replication. Prior to ASFV infection, FoxJ1 was knocked down by siRNAs in PAMs and MA-104 cells, respectively. As shown in Fig. 3A, the knockdown efficiency of FoxJ1 was measured in PAMs, and MA-104 cells, respectively, and the results indicated that transfection of the indicated siRNA targeting FoxJ1 could efficiently downregulate the expression of FoxJ1 in the two cell lines (Fig. 3A). The replication of ASFV in FoxJ1 knockdown cells was then evaluated. Western blotting analysis confirmed that knockdown of FoxJ1 expression increased the expression of ASFV p30 and p72 proteins in PAMs and MA-104 cells as well (Fig. 3B). As shown in Fig. 3C, the transcriptional levels of ASFV p30 and p72 in FoxJ1 knockdown PAMs and MA-104 cells were significantly increased at 12, 24, and 48 h after ASFV infection (MOI = 1), compared to that in the negative control siRNA (NC) transfected cells. In addition, we detected the ASFV genomic copies and titers in ASFV-infected FoxJ1 knockdown PAMs and MA-104 cells respectively, which showed that ASFV genomic copies and titers in PAMs and MA-104 cells were significantly increased in FoxJ1 knockdown cells compared to that in the control cells (Fig. 3D and E). The results suggested that down-regulation of FoxJ1 expression was beneficial for ASFV replication.

### 3.4. Overexpression of FoxJ1 leads to enhancement of type I IFN response

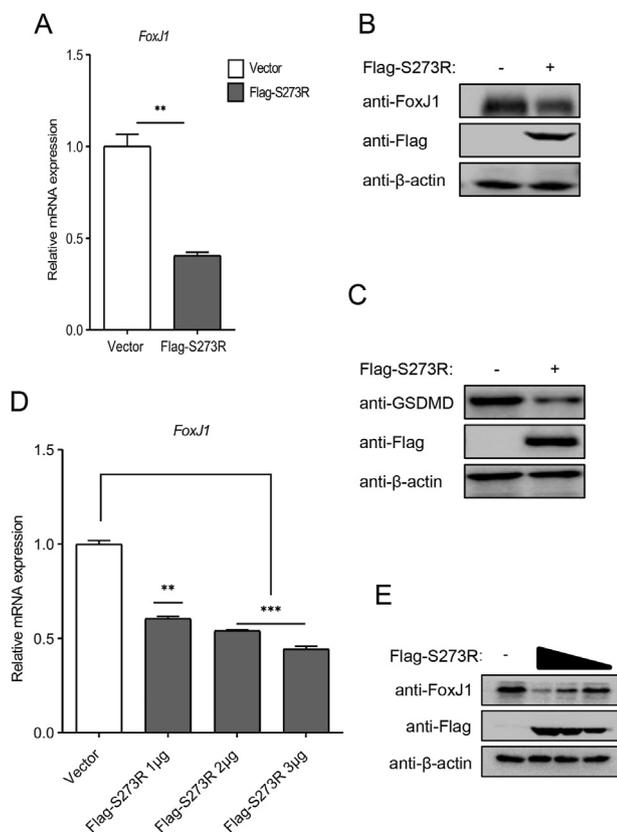
FoxJ1 showed an antiviral function against ASFV. A Previous study reported that FoxJ1 plays an important role in regulating immune

function. FoxJ1 modulates inflammatory reactions and prevents autoimmunity by antagonizing proinflammatory transcriptional activities (Lin et al., 2004). However, the post-transcriptional modification and regulation of FoxJ1 in many immune processes remain unknown. In the present study, we evaluated the expression of *IFN-β* and ISGs in ASFV-infected FoxJ1 overexpressing cells. We found that the expression levels of *IFN-β*, *ISG15*, *ISG54* and *ISG56* were all upregulated in FoxJ1 overexpressing cells compared with that in the empty vector-transfected cells during ASFV infection (Fig. 4A). We evaluated whether FoxJ1 affects the host's innate immune response. Poly(dA:dT) is a synthetic mimetic of B form double-stranded (ds) DNA, which has been widely used to activate the IFN signaling pathway-mediated antiviral immunity through initiation of cGAS-STING pathway activation (Wu et al., 2013). MA-104 and iPAM cells were transfected with Myc-FoxJ1 expressing plasmids and then transfected with poly(dA:dT) (1 mg/mL) for 12 h. The transcriptional levels of *IFN-β* and ISGs were detected. The results showed that overexpression of FoxJ1 enhanced the upregulation of *IFN-β*, *ISG15*, *ISG54*, and *ISG56* expression induced by poly(dA:dT) (Fig. 4B and C). These results suggest that FoxJ1 positively regulates type I IFN and ISGs expression to inhibit ASFV replication.

### 3.5. FoxJ1 degrades ASFV MGF505-2R and E165R proteins through the autophagy pathway

Many host proteins inhibit viral replication by suppressing viral protein expression. Here, we investigated whether FoxJ1 could inhibit the expression of ASFV proteins. iPAM cells were co-transfected with





**Fig. 6.** ASFV S273R inhibits FoxJ1 expression. **A, B** iPAM cells were transfected with an empty vector (2 µg/well) or Flag-ASFV-S273R (2 µg/well) for 24 h. The mRNA expression of *FoxJ1* was detected by RT-qPCR (A). The protein levels of FoxJ1 were detected by Western blotting (B). **C** iPAM cells were transfected with an empty vector (2 µg/well) or Flag-ASFV-S273R (2 µg/well) for 24 h. The protein levels of GSDMD were detected by Western blotting. **D, E** iPAM cells were transfected with an increasing amount of Flag-S273R (1 µg, 2 µg and 3 µg) expressing plasmids for 24 h, and the mRNA and protein expression of FoxJ1 was detected by RT-qPCR and Western blotting respectively. \*\*,  $P < 0.01$ ; \*\*\*,  $P < 0.001$ .

by upregulating the expression of type I IFN. For example, TRIM35 inhibits influenza virus replication by ubiquitin modification of TRAF3, a key molecule of retinoic acid-induced gene I signal pathway, promoting the formation of VISA-TBK1 complex and the production of type I IFN (Sun et al., 2020). Host Esterase D proteins enhances type I IFN signal transduction to suppress foot-and-mouth disease virus replication (Li et al., 2016).

In the study of virus infection, the effect of cytoplasmic DNA on innate immune response is particularly important. The synthetic dsDNA mimetic poly(dA:dT) can trigger intracellular DNA sensors (such as DDX41, DNA-dependent RNA Polymerase III, cGAS and IFI16, etc.), thus activating STING and downstream transcription factor IRF3 to produce type I IFN (Ablasser et al., 2009; Zhang et al., 2011; Xia et al., 2016). Interestingly, in this study, we found that overexpression of FoxJ1 promoted ASFV-infected and poly(dA:dT)-induced transcription of *IFN-β* and *ISGs*. Therefore, we speculate that the antiviral effect of FoxJ1 may be indirectly regulated by the pathway. Knockdown of FoxJ1 enhanced ASFV replication. Immunological attention of FoxJ1 was drawn during microarray studies of novel transcription factors in autoimmunity (Lin et al., 2004). Previous studies have reported that FoxJ1 is required for modulation of NF-κB activity and maintaining T cell tolerance. Abnormality in FoxJ1 expression, function, and/or metabolism may cause autoimmunity and/or other inflammatory conditions (Jonsson and Peng, 2005). FoxJ1 is the main regulator of motor cilia, which are components of the innate immune system (Kuek and

Lee, 2020). Cilia are distributed on the surface of most mammalian cells. Paramyxovirus and murine cytomegalovirus significantly down-regulate the expression of FoxJ1 and the occurrence of cilia and change the cell phenotype, which may be related to the cytokines released after virus interference, such as *IFN-α/β*, *IFN-γ* (Look et al., 2001; Wu et al., 2008), implying a potential relationship between FoxJ1 and innate immune pathway.

Commonly, host restriction factors play an antiviral role by degrading viral key proteins. It is reported that TRIM25 inhibits infectious bursal disease virus replication by targeting VP3 for ubiquitination and degradation (Wang et al., 2021). Twenty ASFV proteins that have been confirmed or predicted to regulate ASFV replication and type I IFN production were selected. After coexpression these viral proteins with FoxJ1, we found that FoxJ1 could degrade ASFV MGF505-2R and E165R proteins. The ASFV MGF505 family has been shown to inhibit the secretion of type I IFN (Golding et al., 2016). E165R is a highly specific dUTP nucleotide hydrolase (dUTPase) encoded by the ASFV genome, which is necessary for efficient replication of ASFV in PAMs (Li et al., 2019). We speculated that FoxJ1 induced the degradation of MGF505-2R and E165R, which rescued the host's innate immune response and suppressed ASFV replication. 3-MA, a purine analog, blocks autophagosome elongation by inhibiting the class III PI3K signaling cascade (Seglen and Gordon, 1982; Petiot et al., 2000). CQ mainly inhibits autophagy by impairing autophagosome fusion with lysosomes (Mauthe et al., 2018). We found that 3-MA and CQ completely inhibited FoxJ1-induced degradation of MGF505-2R and E165R. It is confirmed that FoxJ1 degraded MGF505-2R and E165R through the autophagy pathway. In some cases, autophagy is a powerful tool for host cells to resist viral infection. It has been reported that many host proteins inhibit virus replication through autophagy (Sumpter and Levine, 2011). For example, interferon-induced protein SCOTIN degrades hepatitis C virus (HCV) nonstructural protein 5A through autophagy, thus limiting HCV replication (Kim et al., 2016). Although we have confirmed that FoxJ1 can degrade ASFV MGF505-2R and E165R by autophagy, the underlying mechanism of the degradation of viral proteins by FoxJ1 through autophagy remains unknown and needs to be further explored.

Besides, in the present study, we found that ASFV S273R protein could reduce the expression of FoxJ1 showing a dose-dependent manner, which may be a new antagonistic strategy for ASFV to evade host antiviral response. ASFV pS273R, a specific SUMO-1 cysteine protease, catalyzes the maturation of ASFV pp220 and pp62 polyprotein precursors into core-shell proteins (Li et al., 2020). ASFV pS273R protease has a catalytic triad of C232-H168-N187 that resembles that of other cysteine protease catalytic triads (Cys-His-Asp/-Glu) (Mossessova and Lima, 2000; Pruneda et al., 2016). This active site usually catalyzes the ubiquitin or a ubiquitin-like protein (such as SUMO, smt3, NEDD8, and ISG15), which is involved in the proteolysis of a polyprotein that leads to the maturation of structural proteins in some viruses, including ASFV, poliovirus, flavivirus, and coronavirus (Mukhopadhyay et al., 2005; de Wit et al., 2016). In addition, ASFV pS273R has been shown to induce the cleavage porcine GSDMD, a novel mechanism to disrupt the pyroptosis mediated by GSDMD (Zhao et al., 2022). Therefore, ASFV pS273R protease may antagonize host antiviral response by modulating multiple host proteins.

## 5. Conclusions

In summary, we found that the host protein FoxJ1 inhibit ASFV replication and confirmed that overexpression of FoxJ1 can enhance the transcription of *IFN-β* and *ISGs*. In addition, FoxJ1 degrades ASFV MGF505-2R and E165R through autophagy. However, ASFV S273R protein can reduce the expression of FoxJ1. This study provides a reference for revealing the interaction mechanism between host protein and ASFV and provides novel insights for developing vaccines or antivirals against ASFV.

## Data availability

All the data generated during the current study are included in the manuscript. The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

## Ethics statement

This article does not contain any studies with human or animal subjects performed by any authors.

## Author contributions

Caina Ma: conceptualization, formal analysis, investigation, writing-original draft, writing-review and editing. Shasha Li: data curation, methodology, writing-original draft. Fan Yang: methodology, supervision. Weijun Cao: methodology. Huisheng Liu: writing-review and editing. Tao Feng: methodology. Keshan Zhang: project administration. Zixiang Zhu: writing-review and editing, supervision. Xiangtao Liu: project administration. Yonghao Hu: conceptualization, funding acquisition, resources, supervision, writing-review and editing. Haixue Zheng: funding acquisition, resources, supervision, project administration. All authors have read and agreed to the published version of the manuscript.

## Conflict of interest

The authors declare that they have no conflict of interest.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.virs.2022.04.008>.

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