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ZNF219, a novel transcriptional repressor, inhibits transcription of the prototype foamy virus by interacting with the viral LTR promoter

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ABSTRACT

Prototype foamy virus (PFV) is an ancient retrovirus that infects humans with persistent latent infections and non-pathogenic consequences. Lifelong latent PFV infections can be caused by restrictive factors in the host. However, the molecular mechanisms underlying host cell regulation during PFV infection are not fully understood. The aim of the study was to investigate whether a zinc finger protein (ZFP), ZNF219, as a transcription factor, can regulate the transcriptional activity of the viral promoter. Here, using transcriptome sequencing, we found that ZNF219, is downregulated in PFV infected cells and that ZNF219 suppresses viral replication by targeting the viral 5'LTR promoter region to repress its transcription. We also found that PFV infection induced abnormal expression of miRNAs targeting the ZNF219-3'UTR to downregulate ZNF219 expression. These findings indicated that ZNF219 may be a potent antiviral factor for suppressing PFV infection, and may shed light on the mechanism of virus-host interactions.

1. Introduction

Foamy viruses (FVs), also known as spumaviruses, are the only members of the Spumaretrovirinae subfamily Retroviridae (Jaguva et al., 2021). Unlike orthoretroviruses members of the Retroviridae family, such as human immunodeficiency virus (HIV) or human T-lymphotropic virus (HTLV), FVs infect most mammals with no symptoms in the host. The prototype foamy virus (PFV), previously known as human foamy virus (HFV), is the best-studied member of all FVs and is characterised by unique features in its replication strategy. (Achong et al., 1971). Unlike most complex retroviruses, the PFV genome contains two promoters. The expression of viral structural genes, including *gag*, *pol*, and *env*, are promoted by the PFV 5'long terminal repeat (LTR) promoter. The unique internal promoter (IP) of the foamy virus is located at the 3' end of the *env* gene and initiates the expression of viral accessory proteins Tas and Bet (Löchelt et al., 1993). After the viral genome integrates into the host genome during PFV infection, Tas, a regulatory protein required for viral replication, is instantaneously expressed by the IP. Subsequently, Tas regulates viral gene expression by binding to transactivation-responsive elements (TREs) on the LTR and IPs (He et al., 1996; Delelis et al., 2004).

Several host cell factors inhibit viral replication, which may be one of the reasons why FVs cause lifelong persistent infections with no symptoms. Moreover, host restriction factors can regulate the different stages of viral infection in cells. Entry of PFV into host cells is inhibited by interferon-induced transmembrane protein 3 (IFITM3). IFITM3 promotes envelope protein, a key infectious protein of retroviruses, and its degradation through the lysosomal pathway (Wang et al., 2022). For the regulation of viral genome transcription, the antiviral activity of apolipoprotein B mRNA editing enzyme catalytic subunit 3G (APOBEC3G) suppresses viral transcription associated with cytidine editing of the viral genome (Löchelt et al., 2005). Several antiviral factors that inhibit viral gene transcription are relevant to its unique transactivator, Tas. The promyelocytic leukaemia (PML) protein represses PFV transcription

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by complexing with Tas, preventing the direct binding of Tas to viral DNA (Regad et al., 2001). The N-Myc interactor (Nmi), an interferon-induced protein, interacts with the regulatory protein Tas of the prototype FV and sequesters it in the cytoplasm (Hu et al., 2014). A p53-induced protein with a RING-H2 domain (PIRH2) suppresses PFV replication by negatively impacting Tas and the transcription of two viral promoters (Dong et al., 2015). PHD finger protein 11 (PHF11) prevents Tas expression and the subsequent activation of the viral LTR promoter (Kane et al., 2020). Moreover, the viral structural proteins are affected by antiviral factors. Encoding serum/glucocorticoid-regulated kinase 1 (SGK1) inhibits the replication of PFV by affecting the function of the transcription activator Tas, and reduces the stability of the structural protein Gag (Zhang et al., 2022). Interferon-induced protein 35 (IFP35) interacts with a homologous regulatory protein of PFV and arrests viral transcription and replication (Tan et al., 2008). Schlafen family member 11 (SLFN11) impairs PFV viral protein synthesis by exploiting the distinct codon usage between the virus and host (Guo et al., 2021). During the viral release phase, human BST2 (hBST2; also called tetherin) is a host restriction factor that blocks the release of various enveloped viruses (Xu et al., 2011). TBC1 domain family member 16 (TBC1D16) reduces the transcription and translation of Tas and Gag by suppressing the transcriptional activity of PFV dual promoters. Moreover, Rab5C, a member of the Rab GTPases, is targeted by TBC1D16 to suppress PFV replication (Yan et al., 2021). Nonetheless, studies on transcription factors regulating the 5'LTR promoter of PFV are limited.

Recently, we identified a host factor TRIM28 (tripartite motif-containing protein 28, also called transcription intermediary factor 1-beta, TIF1β, or KRAB-associated protein 1, KAP1) that restricts PFV replication by enriching the PFV 5'LTR promoter region to modulate H3K9me3 marks and destabilise the viral transactivator Tas (Yuan et al., 2021). TRIM28 is recruited by the Krüppel-associated box (KRAB) type zinc finger protein (ZFP) to the LTR promoter of other retroviruses (Wolf et al., 2009; Nishitsuji et al., 2012; Nishitsuji et al., 2015). The TRIM28/KRAB-ZFP interaction is also critical for the recruitment of TRIM28 to transposable elements for epigenetic silencing, thereby preventing aberrant gene expression (Lupo et al., 2013; Ecco et al., 2017). However, the interaction between ZFPs and PFV 5'LTR promoter affecting viral replication has not been investigated. To identify new cellular factors that inhibit PFV replication, we determined the differential mRNA expression profiles of PFV-infected cells. We further analysed ZFPs with altered expression and verified that zinc finger protein 219 (ZNF219, also known as ZFP219) was downregulated in PFV-infected cells, suggesting that ZNF219 plays an essential role in PFV replication.

ZNF219 is a member of the Krüppel-like zinc finger gene family and is a 77-kDa protein containing nine sets of C_2H_2 zinc finger structures (Sakai et al., 2000). ZNF219 was initially found to be a transcriptional repressor for the high mobility group nucleosome binding domain 1 (HMGN1) promoter (Sakai et al., 2003). Subsequently, it was reported that ZNF219 is both an activator and a repressor for the 5'-promoter of α-synuclein (SNCA). These opposing regulatory functions may depend on the cell type and its interactions with other binding proteins and response elements (Clough et al., 2009). As a transcriptional activator, ZNF219 markedly enhances the transcriptional activity of SRY-box transcription factor 9 (SOX9) in the collagen type II alpha 1 chain (COL2A1) gene promoter (Takigawa et al., 2010). However, in the present study, we investigated whether ZNF219, as a transcription factor, can regulate the transcriptional activity of the viral promoter.

2. Materials and methods

2.1. Cell Culture, Reagents and Antibodies

Human embryo kidney 293T (HEK293T) cells and human fibrosarcoma (HT1080) cells were respectively cultured in Dulbecco's Modified Eagle Medium (DMEM) or Minimum Essential Medium (MEM) supplemented with fetal bovine serum (FBS; BI), and with 100 U/mL penicillin and 100 μg/mL streptomycin (1%Pen/Strep; Gibco). All cell culture medium and supplements were purchased from Hyclone (Hyclone Laboratories). Anti-Flag (#14793S) antibody was purchased from Cell Signaling Technology (CST); anti-ZNF219 antibody (#NBP1-76550) was purchased from Novu; anti-β-actin (#ab3280) antibody was obtained from Abcam. Antibody against PFV Gag was kindly provided by Professor Li Zhi (Shaanxi Normal University). Antibody against Tas protein of PFV was stored in our laboratory. Anti-Tas was produced by immunizing mouse with prokaryotic expressed Tas and purified according to standard procedures (Qiu et al., 2012). HRP-conjugated goat anti-rabbit or goat anti-mouse secondary antibodies were purchased from Proteintech (Wuhan, China).

2.2. Plasmids, siRNA, and Transfection

The ZNF219 gene was amplified from cDNA of HEK293T cells. The amplified gene fragment was inserted into the pCMV-Flag plasmid to construct a pCMV-Flag-ZNF219 plasmid. Gene silencing was performed by transfecting HEK293T or HT1080 cells with siRNA oligonucleotides (GenePharma, Shanghai, China). The siRNA sequences were shown in Table 1. For transfections in 12-well plates, 1.0×10^5 cells were seeded per well and incubated overnight, then transfected with 80 nM siZNF219 or negative negative control (siNC) using siRNA-mate (GenePharma, Shanghai, China) according to the manufacturer's protocol. At 12 h after transfection, the cells were infected with PFV for the following experiments. ZNF219-3'UTR gene was synthesized by Stargene Sci-Tech (Wuhan, China). The synthesized gene fragment was inserted into the pmirGLO vector to construct pmirGLO-ZNF219-3'UTR-luc plasmid. pGL3-PFV-LTR-luc and TK-Tas were constructed based on an infectious

Table 1
Primers for PCR amplification and Quantitative real-time PCR primers.

Description	Primers
ChIP-GAPDH-pro-F	5'-GAAGGTGAAGGTCGGAGTCA-3'
ChIP-GAPDH-pro-R	5'-CCCATACGACTGCAAAGACC-3'
ChIP-LTR-U3-1-F (52-187)	5'-GGGAAGGAAGTGAAGAAC-3'
ChIP-LTR-U3-1-R (52-187)	5'-TTGGATGTCAGAGGGAGT-3'
ChIP-LTR-U3-2-F(151-166)	5'-ACTCCCTCTGACATCC-3'
ChIP-LTR-U3-2-R(353-368)	5'-TTTTCGGTGTCTGTCA-3'
ChIP-LTR-U3-3-F(355-370)	5'-AAGCCACAGACAGTAA-3'
ChIP-LTR-U3-3-R(574-589)	5'-TGCATCCCACTGTTCT-3'
ChIP-LTR-U3-4-F (573-588)	5'-CACGTAGGGTGACAAG-3'
ChIP-LTR-U3-4-R (584-800)	5'-GAGAAGTGATGAGCGAC-3'
ChIP-LTR-R-F (831-955)	5'-GCTCTTCACTACTCGCTG-3'
ChIP-LTR-R-R (831-955)	5'-GCAATCACCCTTACAATC-3'
ChIP-LTR-U5-F (1072-1167)	5'-CTTAAATGATGTAACTCCT-3'
ChIP-LTR-U5-R (1072-1167)	5'-TACAAATAAACCCGACTT-3'
ChIP-IP-1-F (9019-9108)	5'-CTGGACTTTAAAAGGCCACT-3'
ChIP-IP-1-R (9019-9108)	5'-AACCAAATGTGGTAATCT-3'
ChIP-IP-2-F (9013-9196)	5'-TTTGGTTGGAATTATTGC-3'
ChIP-IP-2-F (9013-9196)	5'-AGCTTTTGCTCTTTCAAT-3'
ZNF219-qPCR-F	5'-CATGGAGGGCTCACGTCC-3'
ZNF219-qPCR-R	5'-CTGCACGACTCTCAGACCAG-3'
β-actin-qPCR-F	5'-CACGATGGAGGGGCCGGACTCATC-3'
β-actin-qPCR-R	5'-TAAAGACCTCTATGCCAACACAGT-3'
ZNF266 -qPCR-F	5'-GCCGTTTCGGGATCTGTCAA-3'
ZNF266-qPCR-R	5'-TTCTTAGCATTGCCGCGACG-3'
ZNF580-qPCR-F	5'-AAAAAGATCTIGCCCGGAGTGCGCCCGTG-3'
ZNF580-qPCR-R	5'-GTCGTGCACGCGGAGGTGTTCGAAAAA-3'
ZNF395-qPCR-F	5'-GAAGTCGGACGCAGTGGAAA-3'
ZNF395-qPCR-R	5'-CACGGGAAGCAGAGAAGTTG-3'
ZNF217-qPCR-F	5'-GACTGTGTGTATCCGTCGCA-3'
ZNF217-qPCR-R	5'-TGACACAGGCCTTTTTCCTTCTA-3'
ZNF219 Hind III-F	5'-CCCAAGCTTATGGAGGGCTCACGTC-3'
ZNF219 Kpn I-R	5'-GGGGTACCCTACCGTTCTTG-3'
siZNF219-sense	5'-GCCACAUGCGUAAGCACAATT-3'
siZNF219-aitisense	5'-UUGUGCUUACGCAUGUGGCTT-3'
siNC-sense	5'-UUCUCCGAACGUGUCACGUTT-3'
SiNC-aitisense	5'-ACGUGACACGUUCGGAGAATT-3'

DNA clone of the full-length human spumaretrovirus genome pHSRV13 (Keller et al., 1991), which was a gift from Professor Rolf M. Flügel (German Cancer Research Center). The truncated-LTR-luc plasmids were amplified from pHSRV13 and inserted into pGL3-Basic plasmid (Promega), and were stored in our laboratory (Yuan et al., 2021). All primers used for plasmids construction were listed in Table 1. Plasmid transfections were performed by using lipofectamine 3000 reagent (Life Technologies) according to the manufacturer's instructions. microRNA (miRNA) mimics and miRNA inhibitors were synthesized and purchased from RiboBio (Guangzhou RiboBio).

2.3. Virus Preparation and Infection

The proviral plasmids pHSRV13 were transfected into HEK293T cells through PEI transfection reagent (Müllers et al., 2011). After 48 h transfection, the cells and culture supernatants were freeze-thawed three times. To prepare the first generation of virus stock, above-mentioned freeze liquid was centrifuged at 4000 × g for 10 min and filtered through a 0.22 μm-pore-size filter membrane. Then, HT1080 cells were infected with those virus stock at least for 48 h to get more viral particle. Cells and culture supernatants were freeze-thawed three times once again to release viruses. Finally, the virus stock was centrifuged at 4000 × g for 10 min and filtered through a 0.22 μm-pore-size filter membrane and stored at −80°C. The experimental steps of TCID50 are roughly as follows. HEK293T cells (1×10^4) were cultured in 96-well plates. Dilute the PFV stock solution with serum-free MEM in a 10 fold gradient for 10 consecutive gradients. Discard the culture medium from the 96-well plates and add the diluted virus solution to 96-well plates 50 μL per well. Besides, set 8 duplicate wells for each concentration, and set a group of uninfected cells as a control (mock group). Incubate the culture plate at 37°C in a cell incubator for 1.5 h, discard the virus supernatant, and add 100 μL cell culture medium (with 2% FBS) to each well. Incubate continuously for 7 days, observe and record the number of cells with pathological changes every day. Calculate the $TCID_{50}$ of the virus solution using the Spearman-Karber 50% tissue culture infectious dose method. The mock group is treated with cell media lacking the virus and then added with new media after discarding cell media. Mock supernatant-treated cells were similarly produced by transient transfection of empty vector plasmid pcDNA3.0 using the PEI transfection reagent to generate negative controls.

PFV virus titer was determined by infecting PFV indicator cells and the multiplicity of infection (MOI) was calculated according to the method of Tai et al (Tai et al., 2001). The cells were infected with PFV (third generation) at a MOI of 0.1 when cell density was almost 80% in cell culture plates. After 2 h infection, cells culture supernatants were replaced with growth medium and maintained at 37°C for the indicated time.

2.4. Real-time quantitative PCR

Total RNA from cells were harvested by TRIzol (Invitrogen, #15596018). First-strand cDNA was synthesized through Revert AidTM First Strand cDNA Synthesis Kit (Thermo Scientific, #K1622) according to the manufacturer's protocol. Gene expression was examined by a SYBR green Real-Time PCR master mix kit (Toyobo, #QPK-201T). Values for the relative quantification were calculated by the $2^{-\triangle\triangle Ct}$ method. All primers are listed in Table 1. Melting curve analysis was performed to verify the specificity of the products. Quantification of β-actin gene transcripts was used to normalize RNA amounts. All data are representative of three independent experiments with triplicate samples. Statistical significance was analyzed with a Student's *t*-test.

2.5. Western blotting

After completion of cell processing, in order to obtain whole-cell lysates, the cells were washed twice with ice-cold phosphate-buffered saline (PBS) and lysed on ice with immunoprecipitation assay buffer (Beyotime Biotechnology) containing a protease inhibitor cocktail. Then, these cellular lysates were centrifuged at 13,000 rpm for 10 min at 4°C. Whole-cell lysates samples were boiled at 100°C for 10 min with loading buffer (5% SDS, 10% glycerol, 60 mM Tris pH 6.8, 5% β-mercaptoethanol, and 0.01% bromophenol blue). Protein samples were resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene fluoride (PVDF) membranes. The membranes were blocked in 5% non-fat milk-TBST for 3 h at room temperature and incubated with primary antibodies overnight followed at 4°C. Bound antibodies were visualized by HRP-conjugated secondary antibodies for 1.5 h at room temperature and enhanced chemiluminescence (ECL) system (Advansta) with a Kodak imager. All data are representative of three independent experiments with triplicate samples. The quantitative analysis of the relative intensities of proteins (normalized to β-actin) was performed with Quantity One Software (Bio-Rad) and GraphPad Prism 5.

2.6. Luciferase reporter assay

HEK293T cells (4×10^4) were cultured in 24-well plates and transfected with the pGL3-PFV-LTR-luc or pGL3-PFV-IP-luc reporter plasmid and a Renilla luciferase reporter plasmid (pRL-TK, Promega) as an internal control. The empty vector was used to equalize the total amount of DNA. 48 h after transfection, cells were harvested by passive lysis buffer, and the firefly and Renilla luciferase activities were performed using the Dual-Luciferase Reporter Assay System following the manufacturer's protocol (Promega). All experiments were performed in triplicate. The firefly luciferase activity was normalized on the Renilla luciferase activity and expressed as the fold change relative to the activity in the vector-transfected cells. Data represent the average of three independent experiments, and error bars represent standard deviations.

2.7. Foamy virus activated luciferase (FAL) assay

PFV indicator cell line (BHK21-derived indicator cells encoding a luciferase gene driven by the PFV LTR promoter (Tai et al., 2001)) was donated by Professor Li Zhi (Shaanxi Normal University). HEK293T cells were transfected with pCMV-Flag-ZNF219 for 24 h (pCMV-Flag as an negative control), and the cells were infected with PFV for another 24 h, then those infected HEK293T were incubated with PFV indicator cell line (PIC) for 48 h to detecting PFV viral load, and RL-TK plasmid expressing Renilla (RLu) luciferase was transfected into PFV indicator cell line as an internal control 12 h before incubation. Thereafter, the firefly and Renilla luciferase activities were determined using the Dual-Glo luciferase assay (Promega) according to the manufacturer's instructions.

2.8. Chromatin immunoprecipitation (ChIP) assay

ChIP assays were performed as previously Wu's lab described (Wu et al., 2008). Briefly, cells were fixed with 1% formaldehyde for 10 min and quenched by 0.125 M glycine for 5 min at room temperature. After cross-linking, the cells were washed three times with precooled PBS and harvested by precooled PBS. Harvest cross-linked cells were centrifuged at 3000 rpm for 5 min, then removd the supernatant. Precipitated cells were resuspended by ChIP lysis buffer (50 mM Tris-HCl pH 8.0, 0.5% SDS, 5 mM EDTA). Resuspended cells were followed by ultrasound lysis to produce 400-600bp DNA fragments. After testing the size of DNA fragments using gel electrophoresis, cell lysate was centrifuged at 12000 rpm, 4°C for 10 min. Transfer the supernatant to a new EP tube and ChIP dilution buffer (20 mM Tris-HCl, pH 8.0, 150 mM NaCl, 2 mM EDTA, 1% Triton X-100) was added to the supernatant (4:1 volume). The resulting lysate was then incubated with pretreated protein G beads and antibodies at 4°C overnight. The beads were washed five times (a. Centrifuge at 3000 rpm, 4°C for 1 min,collection of sediment and disposal of

supernatant. b. Beads were washed by wash buffer I (20 mM Tris-HCl PH = 8 (0.242 g/100mL), 150 mM NaCl, 2 mM EDTA, 1% Triton X-100, 0.1% SDS (0.1 g/100mL), centrifuge at 3000 rpm, 4°C for 1 min, collection of beads and disposal of supernatant. c. Beads were washed by wash buffer II (20 mM Tris-HCl PH=8, 500 mM NaCl (5.844 g/100mL), 2 mM EDTA, 1% Triton X-100, 0.1% SDS (0.1 g/100mL), centrifuge at 3000 rpm, 4°C for 1 min, collection of beads and disposal of supernatant. d. Beads were washed by wash buffer III (10 mM Tris-HCl PH = 8 (0.242 g/100mL), 0.25 M LiCl, 1 mM EDTA, 1% deoxycholate 1g/100mL, 1% NP-40 1mL/100mL), centrifuge at 3000 rpm, 4°C for 1 min, collection of beads and disposal of supernatant. e. Beads were washed by TE buffer twice, centrifuge at 3000 rpm, 4°C for 1 min, collection of beads and disposal of supernatant. Finally DNA was eluted by ChIP elution buffer (0.1 M $NaHCO_3$, 1% SDS and 30 μg/mL proteinase K). The elution was incubated at 65°C overnight and DNA was extracted with a DNA purification kit (Tiangen). The purified DNA was assayed by quantitative PCR with a SYBR green Real-Time PCR master mix kit (Toyobo). The primer information is listed in Table 1. Data shown are mean ± standard deviations (SD) of representative experiments. At least three biological replicates were analyzed in each experiment. A *t*-test was used for statistical analysis.

2.9. Statistical analysis

Data were expressed as the means ± SD. Statistical analyses were performed using GraphPad Prism to evaluate the differences between experimental groups. Statistical significance was determined using Student's *t*-test and expressed as *p*-values. *$*p < 0.05$* was considered to be statistically significant. (*$^{ns}p > 0.05$, $*p < 0.05$, $**p < 0.01$, $***p < 0.001$*)

3. Result

3.1. ZNF219 is significantly downregulated in PFV-infected cells

To investigate host factors that may inhibit PFV replication, transcriptome sequencing (mRNA-seq) of PFV-infected HEK293T cells was performed 24 h post-infection (hpi) (Xu et al., 2017). The top 10 upregulated and downregulated ZFP genes were selected for heatmap analysis. A heatmap was used to visualise the changing trends of the ZFP genes among the groups and was drawn using Heml 2.0 (Ning et al., 2022). (Fig. 1A and Supplementary Table S1). To obtain an overall corroboration of the mRNA-seq results, 5 downregulated genes were selected for quantitative real-time reverse transcription-PCR (qRT-PCR). The altered expression of these five genes, identified by mRNA-seq, was consistent with the qRT-PCR results (Fig. 1B). Among these candidates, the downregulated ZFP, ZNF219, attracted our attention. Our results showed that the mRNA expression level of ZNF219 was markedly downregulated in these PFV-infected HEK293T cells compared to that in the mock group. In PFV-infected HT1080 cells, a PFV-permissive cell line, ZNF219 mRNA expression was downregulated (Fig. 1C). Western blotting also revealed that the expression of ZNF219 was significantly downregulated in PFV infected HEK293T or HT1080 cells (Fig. 1D).

3.2. ZNF219 overexpression inhibits PFV transcription and replication

To investigate the effect of ZNF219 on PFV replication, we first used a PFV indicator cell line (PIC), in which a luciferase gene driven by the PFV 5'LTR promoter was stably transfected into baby hamster kidney-21 (BHK-21) cells. In this foamy virus-activated luciferase (FAL) assay, pCMV-Flag-ZNF219 was transfected into HEK293T cells for 24 h (pCMV-Flag was used as a control), and the cells were infected with PFV (MOI = 0.1) for an additional 48 h. The infected HEK293T cells were subsequently incubated with the PIC for 48 h, and the RL-TK plasmid expressing *Renilla* luciferase was transfected into the PIC as an internal

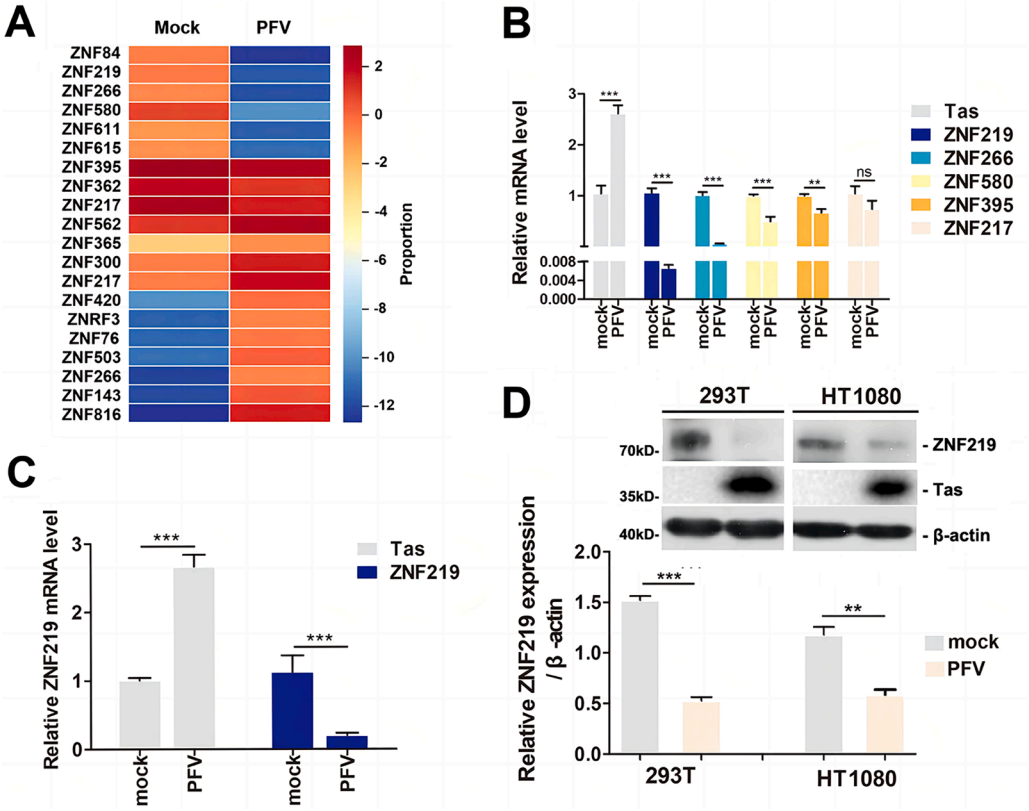

Fig. 1. The expression of ZNF219 is downregulated in PFV-infected HEK293T or HT1080 cells. A. Heatmap representation of the mean fold change in zinc finger proteins expression, as determined by RNA-seq analysis of PFV-infected HEK293T cells. And a scale from 2.83 (red) to -13.42 (blue) represents fold of differential expression (log2 flod change). B. Validating differential expression of 5 selected ZFPs by qRT-PCR in PFV-infected HEK293T cells. C. qRT-PCR detection of ZNF219 mRNA expression in PFV-infected HT1080 cells. β-actin gene transcripts serve as a loading control in qRT-PCR assay. D. The expression of ZNF219 in HEK293T and HT1080 cells after PFV infection. Quantitation analysis intensity from the western blot used quantity one software (Bio-Rad). Unless noted otherwise, all results in this and other figures were representative of at least three independent experiments. The plot shows densitometry from three separate experiments shown as mean values ± SD; *$^{ns}p > 0.05$, $p < 0.05$, $**p < 0.01$, $***p < 0.001$*.

control 12 h before incubation. The FAL assay results showed that PFV was able to activate the PIC more prominently than the control group, and the Luc/Rlu ratio was significantly suppressed in Flag-ZNF219-transfected virus-infected cells compared to that in the control group (Fig. 2A), suggesting that overexpression of ZNF219 significantly suppressed PFV particle production. To further investigate the effect of ZNF219 on PFV viral protein production, HEK293T or HT1080 cells were transfected with pCMV-Flag-ZNF219 for 24 h and subsequently infected with PFV (MOI = 0.1). The levels of the PFV viral proteins Gag (1.7-fold change) and Tas (2.2-fold change) were analysed using quantitative western blotting and were found to be downregulated ZNF219 overexpression (Fig. 2B). To further explore whether ZNF219 suppresses viral transcription, the relative mRNA levels of all viral RNA, primers located in the Tas region (*tas*), or of the LTR-derived genomic RNA (*gag*) were analysed using qRT-PCR in infected cells, which overexpressed ZNF219. The number of all viral transcripts containing the *tas* region (3.8-fold change in 293T cells and 4.1-fold change in HT1080 cells) and genomic RNA *gag* (1.7-fold change in 293T cells and 3.8-fold change in HT1080 cells) were markedly decreased in cells overexpressing ZNF219 compared to those in the empty control group (Fig. 2C and 2D). Thus, ZNF219 overexpression inhibited viral gene transcription and viral protein expression in PFV-infected cells, and thereby it may inhibit viral replication as PFV is a retrovirus.

3.3. Silencing of ZNF219 expression accelerates PFV transcription and viral protein production

Due to the significant downregulation of ZNF219 expression by PFV infection, co-incubation of PFV-infected cells transfected with siZNF219 with PIC cells will result in the downregulation of endogenous ZNF219 expression in PIC cells. It is improper to detect the influence of silencing ZNF219 on PFV particle release using siZNF219-transfected PFV-infected cells in the FAL assay. HEK293T or HT1080 cells were transfected with small interfering RNA (siRNA) targeting ZNF219 or NC siRNA for 24 h and were subsequently infected with PFV at an MOI of 0.1. At 24 hpi, cells were collected to determine ZNF219 mRNA and protein levels using qRT-PCR and western blotting, respectively, and to determine PFV transcription and viral protein production. The results showed that the protein expression of endogenous ZNF219 was significantly reduced by transfection with siZNF219 in PFV-infected HEK293T or HT1080 cells compared to that of the negative control siNC group (Fig. 3A). Silencing of ZNF219 enhanced the expression of the viral proteins Gag (1.6-fold change in 293T and HT1080 cells) and Tas (2.3-fold change in 293T cells and 1.6-fold change in HT1080 cells) (Fig. 3A). Furthermore, the relative mRNA levels of viral RNA and primers located in the Tas region (*tas*) (2.9-fold change in 293T cells and 2.6-fold change in HT1080 cells) or of the LTR derived genomic RNA (*gag*) (2.4-fold change in 293T cells and 2.0-fold change in HT1080 cells) were restored by transfection with

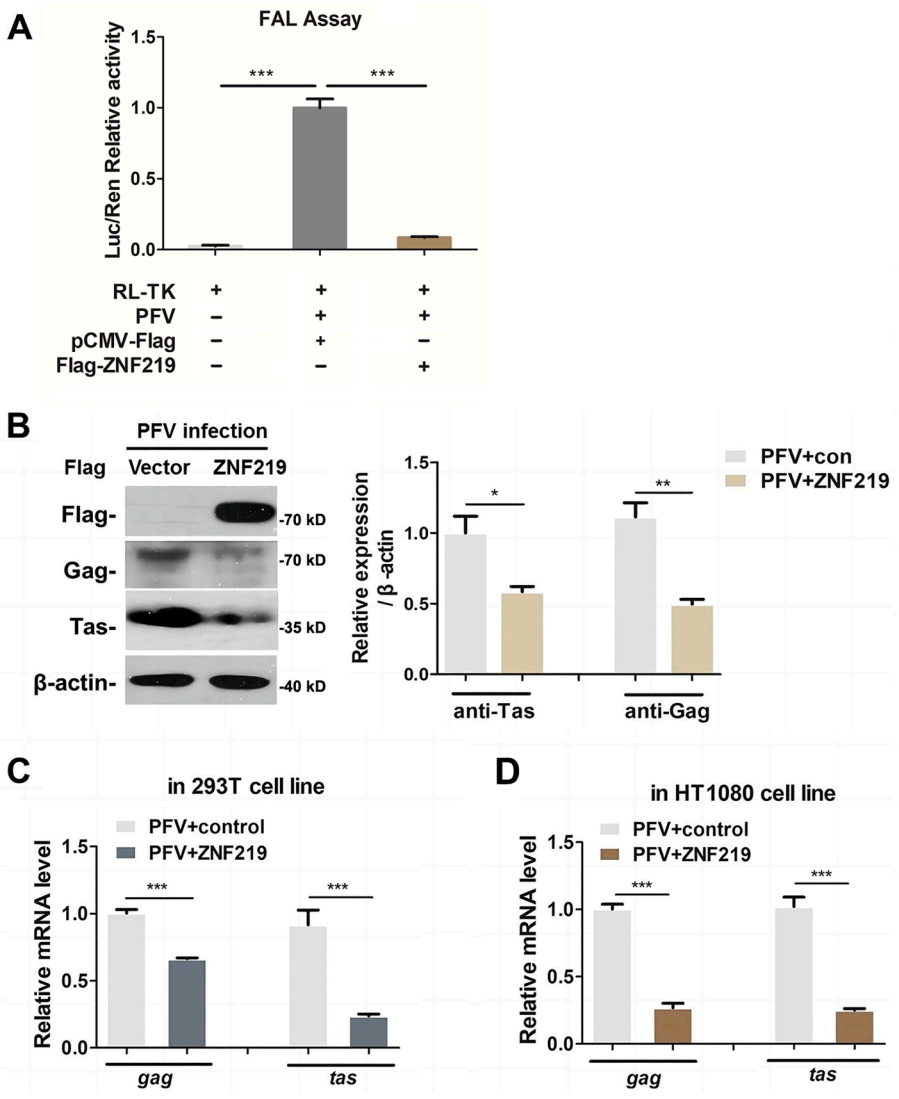

Fig. 2. ZNF219 overexpression inhibits PFV transcription and replication. A. ZNF219 inhibits the replication of PFV. The relative viral titers in the presence or absence of overexpressed with ZNF219 expression was analyzed using the FAL assay with a PFV indicator cell line. RL-TK (5 μg) was transfected as an internal control. B. HEK293T cells were respectively transfected with pCMV-Flag-ZNF219 (ZNF219) or pCMV-Flag (vector). After 24 h transfection, cells were infected with PFV for another 24 h. Western blotting of PFV viral proteins Gag and Tas in HEK293T cells which were overexpressed with Flag-ZNF219 during PFV infection. β-actin serves as a loading control. Quantitation analysis intensity from the western blot used Quantity one software (Bio-Rad). C. qRT-PCR detection of viral gene *gag* and *tas* in HEK293T and HT1080 cells which were overexpressed with Flag-ZNF219 during PFV infection. β-actin gene transcripts serve as a loading control in qRT-PCR assay. Unless noted otherwise, all results in this and other figures were representative of at least three independent experiments. The plot shows densitometry from three separate experiments shown as mean values ± SD; $*p<0.05$, $**p<0.01$, $***p<0.001$.

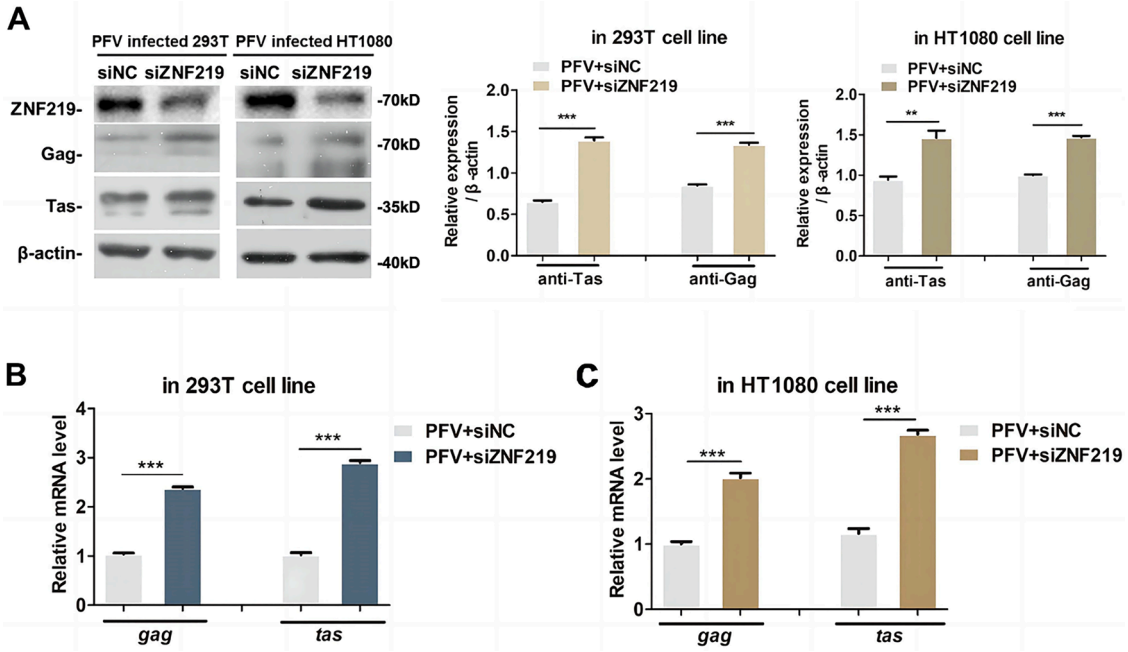

Fig. 3. Silencing of ZNF219 expression promotes PFV transcription and viral protein production. A. HEK293T and HT1080 cells were transfected with siRNAs targeting ZNF219 (siZNF219, siNC serves as negative control). Western blotting analysis of and PFV viral proteins Gag and Tas in HEK293T cells which were silenced of ZNF219 during PFV infection. ZNF219 expression was detected to confirm the silencing efficiency, β-actin serves as a loading control. Quantitation analysis intensity from the Western blot used Quantity one software (Bio-Rad). B. C. qRT-PCR detection of viral gene *gag* and *tas* in HEK293T and HT1080 cells which were downregulated with siZNF219 during PFV infection. The plot shows densitometry from three separate experiments shown as mean values ± SD; $*p<0.05$, $**p<0.01$, $***p<0.001$.

siZNF219 in PFV infected HEK293T or HT1080 cells compared to those of the negative control siNC group (Fig. 3B and 3C). Silencing ZNF219 accelerated viral gene transcription and protein expression in PFV infected cells. These results indicated that ZNF219 is a part of the host defence mechanism against PFV infection.

3.4. ZNF219 is enriched in the PFV 5'LTR promoter to inhibit promoter activity

ZNF219 functions as a transcription factor that regulates the promoters of various genes (Sakai et al., 2003; Clough et al., 2009). Based on these results, ZNF219 overexpression inhibited viral gene mRNA expression (Fig. 2C), indicating that ZNF219 may function during early viral gene transcription. Therefore, we speculated that ZNF219 may regulate the transcription of the viral promoter transcription as a transcription factor. To explore whether ZNF219 could affect PFV 5'LTR promoter activity, we co-transfected a PFV 5'LTR-mediated luciferase reporter plasmid with a plasmid expressing ZNF219. As shown in Fig. 4B, overexpression of ZNF219 significantly inhibits the transcription activity of the PFV 5'LTR promoter. Compared to the control group, the inhibitory effect of ZNF219 on the LTR promoter was approximately 100 times greater. PFV 5'LTR promoter transcriptional activity increased in cells transfected with siZNF219 compared to that in the siNC group (Fig. 4B). Thus, we investigated the functions of ZNF219 for PFV 5'LTR transcription repression. We designed a series of primer sets at the PFV 5'LTR promoter (Fig. 4A). ChIP analysis showed that the binding of Flag-ZNF219 majorly in the U3 and R regions rather than the U5 regions of the PFV 5'LTR promoter occurs during overexpression of the PFV 5'LTR-mediated luciferase reporter plasmid (Fig. 4C). To confirm these results, we constructed a series of truncated LTR reporter plasmids to functionally define the ZNF219 responsive region. As shown in Fig. 4D, overexpression of ZNF219 inhibited Tas-dependent PFV LTR promoter activity in constructs encompassing the U3 regions (Fig. 4D). The U3 region of the 5'LTR promoter was strictly Tas-dependent and is virtually silent in its absence. To explore whether the inhibition of ZNF219 on the U3 promoter is dependent on Tas expression, we transfected a considerable amount of the pGL3-U3-luc plasmid, which only contains the U3 region of the LTR, to transcribe the U3 promoter. As shown in Fig. 4E, in the absence of Tas, the overexpression of ZNF219 significantly inhibited the transcriptional activity of the LTR-U3 promoter.

In addition, we designed two ChIP-qPCR primers targeting the IP region (Fig. 4A). ChIP-qPCR analysis revealed that Flag-ZNF219 showed almost no enrichment in the IP region (Fig. 4F). To explore the effect of ZNF219 on the transcriptional activity of the IP, an IP-mediated luciferase reporter plasmid (IP-Luc) was co-transfected with Flag-ZNF219 or siZNF219 (pCMV-Flag and siNC as negative controls). As shown in Fig. 4G, overexpression of ZNF219 promoted the transcriptional activity of the IP, however, the increase was only 2-fold. Downregulation of ZNF219 expression by transfecting siZNF219 with cells did not affect IP activation compared to that of the siNC group (Fig. 4G). The IP can be transiently transcribed when Tas is absent, i.e., when there is no viral protein expression in the early stages of viral infection (Delelis et al., 2004). We further assessed whether the overexpression of ZNF219 affected the basal transcriptional activity of the IP in the absence of Tas. As shown in Fig. 4E, overexpression of ZNF219 does not affect the transcriptional activity of the IP promoter when Tas is absent. These results indicated that ZNF219 is enriched in the 5'LTR promoter region of the PFV genome and not in the IP region. ZNF219 significantly inhibited the transcriptional activity of the PFV 5'LTR promoter in a Tas-inessential manner.

3.5. PFV infection induces abnormal expression of microRNAs (miRNAs) targeting ZNF219-3'UTR

It is well established that miRNAs exert their biological functions through binding to the 3'-untranslated region (3'-UTR) of mRNA targets in a sequence-specific manner, thus facilitating translation inhibition (Gu et al., 2009). To investigate whether the mechanism of the mRNA expression of ZNF219 was downregulated during PFV infection, we used

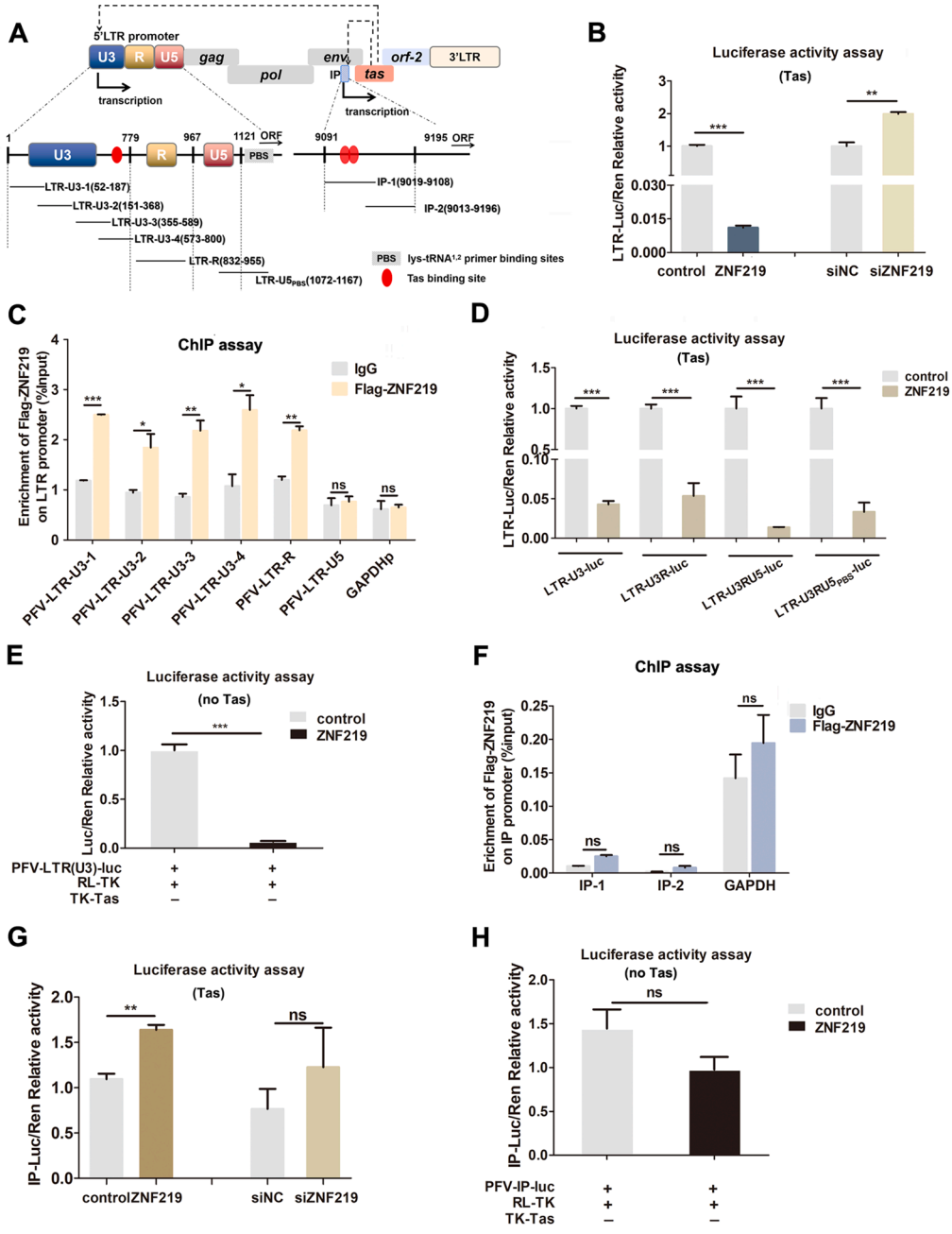

Fig. 4. ZNF219 is enriched in PFV 5'LTR to negatively regulate the activity of LTR promoter. A. Illustration of the primer sets at the PFV 5'LTR and IPs used in the ChIP assays. The numbers are relative to the transcription start site nucleotide +1. B. ZNF219 negatively regulates Tas-dependent transactivation of the PFV 5'LTR promoter activity. PFV 5'LTR firefly luciferase reporter activity were monitored in HEK293T cell lines. The luciferase activity was measured by Promega DLU-luc system. Firefly luciferase values were normalized to Renilla luciferase values from a simultaneously transfected Renilla control vector. The transfection amount of each pore particle in the 24-well plate was as follows: pGL3-LTR-luc (40 ng), pCMV-Flag-ZNF219 (400 ng, or pCMV-Flag 400 ng as control), TK-Tas (50 ng) and RL-TK (5 ng). C. ZNF219 was enriched in the PFV 5'LTR promoter regions. ChIP assays of anti-Flag in the presence of Flag-ZNF219 and pGL3-PFV-LTR-luc co-expression. ChIP-qPCR data was normalized by the fold enrichment method (ChIP signals were divided by the IgG signals) with GAPDH promoter region as the negative control. The data presented are means the standard errors of the means of three independent experiments. D. The effect of ZNF219 in regulating the activity of Tas-mediated transactivation of the PFV 5'LTR and truncated-LTR promoter activity. E. The effect of ZNF219 in regulating the activity of Tas-independent transactivation of the PFV 5'LTR-U3 promoter activity. The transfection amount of each pore particle in the 24-well plate was as follows: pGL3-PFV-LTR-luc (200 ng), pCMV-Flag-ZNF219 (200 ng, or pCMV-Flag 200ng as control) and RL-TK (5 ng) (with no Tas). F. ZNF219 was not enriched in the PFV IP regions. ChIP assays of Flag-ZNF219 in the presence of Flag-ZNF219 and pGL3-IP-luc coexpression. ChIP-qPCR data was normalized by the fold enrichment method (ChIP signals were divided by the IgG signals) with GAPDH promoter region as the negative control. The data presented are means the standard errors of the means of three independent experiments. G. The effect of ZNF219 in regulating the activity of Tas-mediated transactivation of the PFV IP activity. PFV IP-firefly luciferase reporter activity were monitored in HEK293T cell lines. The luciferase activity was measured by Promega DLU-luc system. Firefly luciferase values were normalized to Renilla luciferase values from a simultaneously transfected Renilla control vector. The transfection amount of each pore particle in the 24-well plate was as follows: pGL3-PFV-IP-luc (20 ng), pCMV-Flag-ZNF219 (400 ng, or pCMV-Flag 400 ng as control), TK-Tas (10 ng) and RL-TK (5 ng).
H. The effect of ZNF219 in regulating the activity of Tas-independent transactivation of the PFV IP promoter activity. The transfection amount of each pore particle in the 24-well plate was as follows: pGL3-PFV-IP-luc (40 ng), pCMV-Flag-ZNF219 (400 ng, or pCMV-Flag 400 ng as control)and RL-TK (5 ng) (with no Tas).

TargetScanHuman to predict miRNAs targeting ZNF219-3'UTR (Agarwal et al., 2015). We explored the relative expression of ZNF219 mRNA in the 10 selected miRNAs (Fig. 5A). miRNA mimics were transfected into HEK293T cells for 24 h. Our results showed that ZNF219 mRNA expression was downregulated by eight miRNAs among these (Fig. 5B). We selected four of these miRNAs, miR-124-3p, miR-126-3p, miR-506-3p, and miR-5000-3p, which notably downregulated ZNF219-3'UTR changes. Western blot results also corroborated that the protein level of ZNF219 was decreased by miR-124-3p, miR-126-3p, miR-506-3p, and miR-5000-3p in HEK293T cells (Fig. 5C). Luciferase reporter assays were performed to validate whether ZNF219 is a target gene of miR-124-3p, miR-126-3p, miR-506-3p, or miR-5000-3p. Our

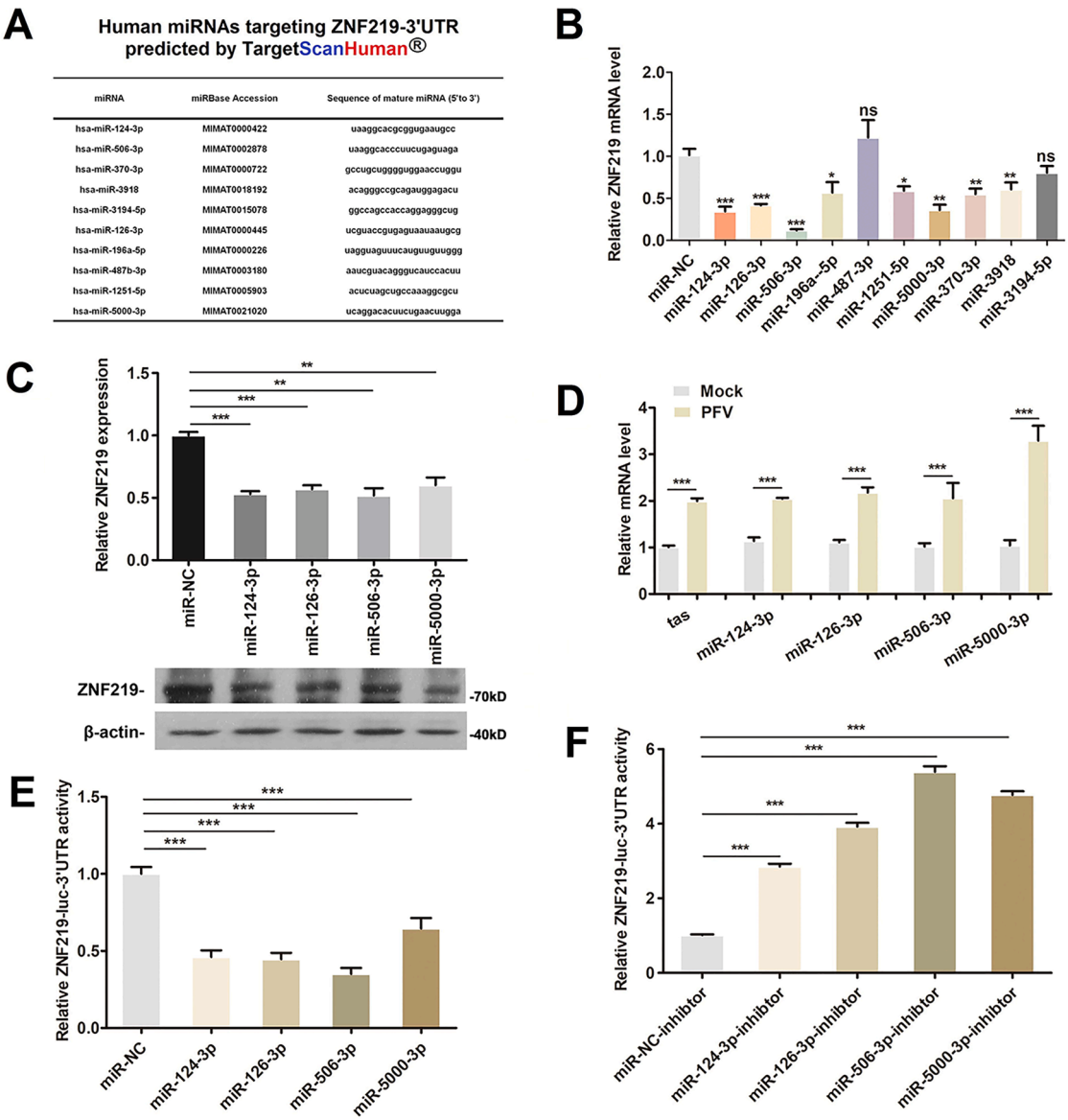

Fig. 5. PFV infection induces abnormal expression of miRNAs targeting ZNF219-3'UTR. A. TargetScanHuman predicted 10 miRNAs targeting ZNF219-3'UTR. B. qRT-PCR analysis of ZNF219 expression when HEK293T cells were transfected with miRNAs mimics or control. C. Western blot analysis of ZNF219 expression in HEK293T cells transfected with miRNAs mimics targeting ZNF219-3'UTR. D. qRT-PCR analysis of miRNAs expression during PFV infection in HEK293T. E. HEK293T cells were co-transfected with the reporter plasmid pmirGLO-ZNF219-3'UTR-luc and miRNA mimics. The luciferase activity was measured by Promega DLU-luc system. Firefly luciferase values were normalized to Renilla luciferase values from a simultaneously transfected Renilla control vector. F. HEK293T cells were co-transfected with the reporter plasmid pmirGLO-ZNF219-3'UTR-luc and miRNA inhibitors. The relative fluorescence value was detected by luciferase reporter gene assays. The plot shows densitometry from three separate experiments shown as mean values ± SD; $^{ns}p>0.05$, $p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$.

results showed that the luciferase activity of reporter plasmids carrying the ZNF219 3'-UTR was significantly decreased in miRNA mimics (miR-124-3p, miR-126-3p, miR-506-3p, and miR-5000-3p) that were separately transfected into HEK293T cells (Fig. 5D), and increased in these miRNA inhibitor transfected groups, compared with that of the control group (Fig. 5E). Coincidently, through qRT-PCR and analyses, we found that level of miR-124-3p, miR-126-3p, miR-506-3p, and miR-5000-3p are all increased in PFV-infected cells compared to that of the mock group (Fig. 5D), indicating that PFV infection induces abnormal expression of miRNAs targeting ZNF219-3'UTR.

4. Discussion

As a non-pathogenic retrovirus, PFV can be expected to be used as a vector for gene therapy. However, the interaction between PFV and the host immune response is complicated and is not fully understood. In this study, we screened several candidate ZFPs that were downregulated in PFV-infected cells using transcriptome sequencing. We showed that a novel ZFP ZNF219 acts as a restriction factor of PFV transcription and viral protein production.

ZNF219 is a member of the Krüppel-like zinc finger gene family and is a 77-kDa protein containing nine sets of C_2H_2 zinc finger structures (Sakai et al., 2000). ZNF219 initially functions as a transcriptional repressor of HMGN1, and the core element of ZNF219 required for DNA binding is CCCCC(A) (Sakai et al., 2003). Some studies have also found ZNF219 is both an activator and a repressor for the 5'-promoter of α-synuclein. They predicted that this opposing regulatory function may depend on cell type and interactions with other binding proteins and response elements (Clough et al., 2009). Here, we found that the PFV 5'LTR promoter does not contain the CCCC(A) sequence. However, in

our study, ZNF219 still inhibited the transcriptional activity of the 5'LTR promoter. We predicted a mechanism by which the repressor ZNF219 directly or indirectly recruits a chromatin-modulating factor, such as the nucleosome remodelling and deacetylation (NuRD) complex and represses promoter activity. ZNF219 interacts with NuRD complex components in several cell lines *in vitro*, and this interaction involves the N-terminal zinc-fingers of ZNF219 and the C-terminal end of Chd4 (Tabar et al., 2019). In mammals, the NuRD complex is approximately 1 MDa and comprises at least six to seven proteins, which generally exist as two or more paralogs: CHD4 (and its paralogues CHD3 and CHD5), MTA1 (plus MTA2 and MTA3), HDAC1 (and HDAC2), GATAD2A (and GATAD2B), MBD3 (and MBD2), RBBP4 (and RBBP7), and CDK2AP1 (Low et al., 2016). Physical interaction between CHD4 and ZNF219 has been reported in cardiac tissues (El Abdellaoui-Soussi et al., 2022). Recently, we found that the host protein TRIM28 is enriched in the PFV 5'LTR promoter region and positively regulates H3K9me3 marks (Yuan et al., 2021). TRIM28, also known as transcription intermediary factor 1-beta (TIFIβ) or KRAB-associated protein 1 (KAP1), acts as a bridge molecule by recruiting the NuRD complex co-repressor for KRAB domain-containing ZFPs (KRAB-ZFPs) to enrich on the retroviral LTR promoter (Friedman et al., 1996). TRIM28 mediates gene silencing by recruiting CHD3, a subunit of the nucleosome remodelling and deacetylation (NuRD) complex, and SETDB1 (which specifically methylates histone H3 at 'Lys-9' (H3K9me)) to the promoter regions of KRAB target genes (Schultz et al., 2001; Schultz et al., 2002). TRIM28 was enriched in the 5'LTR instead of the IP regions of the PFV genome, and significantly enriched in R, U5, and downstream in the PBS regions (Yuan et al., 2021). In this study, we found that ZNF219 was enriched in the U3 and R regions of the PFV 5'LTR promoter. There is an overlap in the enrichment regions of ZNF219 and TRIM28 in the PFV 5'LTR promoter region. This suggested that there may be an interaction between ZNF219 and TRIM28 in this region, which regulates the transcriptional activity of LTR.

The FV contains two promoters: the canonical LTR promoter and the IP. The transcription of the viral Tas protein is initiated by the IP during the early period of viral infection. Tas can use feedback to activate IP and increase its synthesis (Campbell et al., 1994). FV gene expression is believed to be initiated by the IP. TPA or activator protein-1 (AP1) increases basal transcription of IP; however, it does not affect basal transcription of the promoter in the LTR (Wu et al., 2010). AP-1 and BCL2-associated athanogene 3, 2 types of vital proteins associated with the NF-κB and PKC pathways, could activate the basal activity of 5'LTR and IP; however, they inhibit the Tas-regulated activity of both promoters. This suggested that the same transcription factor exhibits different regulatory effects on dual viral promoters. The regulatory effects of transcription factors on viral promoter transcription may be related to Tas expression (Wei et al., 2022). Our study found that Flag-ZNF219 was not enriched in the transfected IP-containing promoter sequences. However, ZNF219 was enriched in the 5'LTR promoter region and had a high degree of inhibition on LTR promoter activity with or without Tas expression. In contrast to the effect of ZNF219 on the LTR promoter, the overexpression of ZNF219 marginally promoted the transcriptional activity of the IP, suggesting that the activation effect of ZNF219 on the IP was limited. In the absence of Tas, its overexpression did not affect the IP. miRNAs are small non-coding RNAs that bind to the 3' untranslated region (3'UTR) of target mRNA, inducing mRNA degradation or transcriptional inhibition (Fabian et al., 2010). To date, the miRNAs that regulate ZNF219 mRNA expression have not been identified. A possible mechanism underlying ZNF219 downregulation in PFV-infected cells was determined. We found that the expression of ZNF219 was downregulated in HEK293T cells after transfection with mimics of miR-124-3p, miR-126-3p, miR-506-3p, and miR-5000-3p compared to the control group. Coincidentally, these miRNAs were abnormally highly expressed in PFV-infected cells. PFV infection could upregulated the expression of miR-124-3p, miR-126-3p, miR-506-3p, and miR-5000-3p, resulting in the downregulation of ZNF219. These miRNAs negatively regulate the mRNA expression of ZNF219 by targeting the 3'UTR of ZNF219. The influenza A virus infection upregulated microRNA-203. Ectopic expression of miR-203 inhibits H5N1 virus replication by downregulating of transcription 1 (DR1) (Zhang et al., 2018). In this study, we found that PFV infection induced the abnormal expression of miRNAs targeting the ZNF219-3'UTR. PFV infection upregulated the levels of miRNAs that regulate ZNF219 expression, which may be a mechanism by which the host interacts with the virus to control viral replication. The regulation of abnormal miR-124-3p, miR-126-3p, miR-506-3p, and miR-5000-3p expression warrants further exploration. The abnormal expression of miRNAs regulating ZNF219-3'UTR is one of the mechanisms that explain the downregulation of ZNF219 expression by PFV infection.

5. Conclusions

In summary, our study demonstrated that the expression of ZNF219 is significantly downregulated in PFV-infected cells. ZNF219 negatively regulated the transcription and replication of PFV through enrichment of the PFV 5'LTR region. Furthermore, we identified miRNAs that regulate the abnormal expression of ZNF219-3'UTR that may be due to PFV infection. This study expands our knowledge of the molecular mechanisms by which ZNF219 acts as a transcriptional repressor of PFV.

CRediT authorship contribution statement

Peipei Yuan: Investigation, Methodology, Data curation, Writing – original draft, Visualization, Funding acquisition, Writing – review & editing. **Shuang Wang:** Data curation, Formal analysis, Visualization. **Tongtong Du:** Methodology. **Luo Liu:** Methodology. **Xiong Chen:** Methodology, Funding acquisition. **Jun Yan:** Investigation, Data curation. **Song Han:** Supervision, Methodology. **Biwen Peng:** Supervision, Writing – review & editing. **Xiaohua He:** Supervision, Project administration. **Wanhong Liu:** Conceptualization, Formal analysis, Visualization, Supervision, Project administration, Writing – original draft, Writing – review & editing, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.virusres.2023.199161.

References

Achong, B.G., Mansell, P.W., Epstein, M.A., Clifford, P., 1971. An unusual virus in cultures from a human nasopharyngeal carcinoma. Journal of the National Cancer Institute 46 (2), 299–307.

Agarwal, V., Bell, G.W., Nam, J.W., Bartel, D.P., 2015. Predicting effective microRNA target sites in mammalian mRNAs. eLife 4, e05005.

Campbell, M., Renshaw-Gegg, L., Renne, R., Luciw, P.A., 1994. Characterization of the internal promoter of simian foamy viruses. Journal of virology 68 (8), 4811–4820.

Clough, R.L., Dermentzaki, G., Stefanis, L., 2009. Functional dissection of the alpha-synuclein promoter: transcriptional regulation by ZSCAN21 and ZNF219. Journal of neurochemistry 110 (5), 1479–1490.

Delelis, O., Lehmann-Che, J., Saïb, A., 2004. Foamy viruses–a world apart. Current opinion in microbiology 7 (4), 400–406.

Dong, L., Cheng, Q., Wang, Z., Yuan, P., Li, Z., Sun, Y., Han, S., Yin, J., Peng, B., He, X., Liu, W., 2015. Human Pirh2 is a novel inhibitor of prototype foamy virus replication. Viruses 7 (4), 1668–1684.

Ecco, G., Imbeault, M., Trono, D., 2017. KRAB zinc finger proteins. Development (Cambridge, England) 144 (15), 2719–2729.

El Abdellaoui-Soussi, F., Yunes-Leites, P.S., López-Maderuelo, D., García-Marqués, F., Vázquez, J., Redondo, J.M., Gómez-Del Arco, P., 2022. Interplay between the Chd4/NuRD Complex and the Transcription Factor Znf219 Controls Cardiac Cell Identity. International journal of molecular sciences 23 (17), 9565.

Fabian, M.R., Sonenberg, N., Filipowicz, W., 2010. Regulation of mRNA translation and stability by microRNAs. Annual review of biochemistry 79, 351–379.

Friedman, J.R., Fredericks, W.J., Jensen, D.E., Speicher, D.W., Huang, X.P., Neilson, E.G., Rauscher 3rd, F.J., 1996. KAP-1, a novel corepressor for the highly conserved KRAB repression domain. Genes & development 10 (16), 2067–2078.

Gu, S., Jin, L., Zhang, F., Sarnow, P., Kay, M.A., 2009. Biological basis for restriction of microRNA targets to the 3' untranslated region in mammalian mRNAs. Nature structural & molecular biology 16 (2), 144–150.

Guo, G., Wang, Y., Hu, X.M., Li, Z.R., Tan, J., Qiao, W.T., 2021. Human Schlafen 11 exploits codon preference discrimination to attenuate viral protein synthesis of prototype foamy virus (PFV). Virology 555, 78–88.

He, F., Blair, W.S., Fukushima, J., Cullen, B.R., 1996. The human foamy virus Bel-1 transcription factor is a sequence-specific DNA binding protein. Journal of virology 70 (6), 3902–3908.

Hu, X., Yang, W., Liu, R., Geng, Y., Qiao, W., Tan, J., 2014. N-Myc interactor inhibits prototype foamy virus by sequestering viral Tas protein in the cytoplasm. Journal of virology 88 (12), 7036–7044.

Jaguva Vasudevan, A.A., Becker, D., Luedde, T., Gohlke, H., Münk, C, 2021. Foamy Viruses, Bet, and APOBEC3 Restriction. Viruses, 13 (3), 504.

Kane, M., Mele, V., Liberatore, R.A., Bieniasz, P.D., 2020. Inhibition of spumavirus gene expression by PHF11. PLoS pathogens 16 (7), e1008644.

Keller, A., Partin, K.M., Löchelt, M., Bannert, H., Flügel, R.M., Cullen, B.R., 1991. Characterization of the transcriptional trans activator of human foamy retrovirus. Journal of virology 65 (5), 2589–2594.

Löchelt, M., Muranyi, W., Flügel, R.M., 1993. Human foamy virus genome possesses an internal, Bel-1-dependent and functional promoter. Proceedings of the National Academy of Sciences of the United States of America 90 (15), 7317–7321.

Löchelt, M., Romen, F., Bastone, P., Muckenfuss, H., Kirchner, N., Kim, Y.B., Truyen, U., Rösler, U., Battenberg, M., Saib, A., Flory, E., Cichutek, K., Münk, C., 2005. The antiretroviral activity of APOBEC3 is inhibited by the foamy virus accessory Bet protein. In: Proceedings of the National Academy of Sciences of the United States of America, 102, pp. 7982–7987.

Low, J.K., Webb, S.R., Silva, A.P., Saathoff, H., Ryan, D.P., Torrado, M., Brofelth, M., Parker, B.L., Shepherd, N.E., Mackay, J.P., 2016. CHD4 Is a Peripheral Component of the Nucleosome Remodeling and Deacetylase Complex. The Journal of biological chemistry 291 (30), 15853–15866.

Lupo, A., Cesaro, E., Montano, G., Zurlo, D., Izzo, P., Costanzo, P., 2013. KRAB-Zinc Finger Proteins: A Repressor Family Displaying Multiple Biological Functions. Current genomics 14 (4), 268–278.

Müllers, E., Stirnnagel, K., Kaulfuss, S., Lindemann, D., 2011. Prototype foamy virus gag nuclear localization: a novel pathway among retroviruses. Journal of virology 85 (18), 9276–9285.

Ning, W., Wei, Y., Gao, L., Han, C., Gou, Y., Fu, S., Liu, D., Zhang, C., Huang, X., Wu, S., Peng, D., Wang, C., Xue, Y., 2022. HemI 2.0: an online service for heatmap illustration. Nucleic acids research 50 (W1), W405–W411.

Nishitsuji, H., Abe, M., Sawada, R., Takaku, H., 2012. ZBRK1 represses HIV-1 LTR-mediated transcription. FEBS letters 586 (20), 3562–3568.

Nishitsuji, H., Sawada, L., Sugiyama, R., Takaku, H., 2015. ZNF10 inhibits HIV-1 LTR activity through interaction with NF-κB and Sp1 binding motifs. FEBS letters 589 (15), 2019–2025.

Oiu, Y., Zhu, G., Dong, L., Wang, Z., Zhang, Y., Li, Z., Sum, Y., Liu, W., L, He, X, 2012. Prokaryotic expression and polyclonal antibody production of transactivator Tas for potential application in detection of human foamy virus infection. African Journal of Microbiology Research 6, 1497–1503.

Regad, T., Saib, A., Lallemand-Breitenbach, V., Pandolfi, P.P., de Thé, H., Chelbi-Alix, M. K., 2001. PML mediates the interferon-induced antiviral state against a complex retrovirus via its association with the viral transactivator. The EMBO journal 20 (13), 3495–3505.

Sakai, T., Toyoda, A., Hashimoto, K., Maeda, H., 2000. Isolation and characterization of a novel zinc finger gene, ZNF219, and mapping to the human chromosome 14q11 region. DNA research: an international journal for rapid publication of reports on genes and genomes 7 (2), 137–141.

Sakai, T., Hino, K., Wada, S., Maeda, H., 2003. Identification of the DNA binding specificity of the human ZNF219 protein and its function as a transcriptional repressor. DNA research: an international journal for rapid publication of reports on genes and genomes 10 (4), 155–165.

Schultz, D.C., Friedman, J.R., Rauscher 3rd, F.J., 2001. Targeting histone deacetylase complexes via KRAB-zinc finger proteins: the PHD and bromodomains of KAP-1 form a cooperative unit that recruits a novel isoform of the Mi-2alpha subunit of NuRD. Genes & development 15 (4), 428–443.

Schultz, D.C., Ayyanathan, K., Negorev, D., Maul, G.G., Rauscher 3rd, F.J., 2002. SETDB1: a novel KAP-1-associated histone H3, lysine 9-specific methyltransferase that contributes to HP1-mediated silencing of euchromatic genes by KRAB zinc-finger proteins. Genes & development 16 (8), 919–932.

Sharifi Tabar, M., Mackay, J.P., Low, J., 2019. The stoichiometry and interactome of the Nucleosome Remodeling and Deacetylase (NuRD) complex are conserved across multiple cell lines. The FEBS journal 286 (11), 2043–2061.

Tai, H.Y., Sun, K.H., Kung, S.H., Liu, W.T., 2001. A quantitative assay for measuring human foamy virus using an established indicator cell line. Journal of virological methods 94 (1-2), 155–162.

Takigawa, Y., Hata, K., Muramatsu, S., Amano, K., Ono, K., Wakabayashi, M., Matsuda, A., Takada, K., Nishimura, R., Yoneda, T., 2010. The transcription factor Znf219 regulates chondrocyte differentiation by assembling a transcription factory with Sox9. Journal of cell science 123 (Pt 21), 3780–3788.

Tan, J., Qiao, W., Wang, J., Xu, F., Li, Y., Zhou, J., Chen, Q., Geng, Y., 2008. IFP35 is involved in the antiviral function of interferon by association with the viral tas transactivator of bovine foamy virus. Journal of virology 82 (9), 4275–4283.

Wang, Z., Tuo, X., Zhang, J., Chai, K., Tan, J., Qiao, W., 2022. Antiviral role of IFITM3 in prototype foamy virus infection. Virology journal 19 (1), 195.

Wei, J., Sun, Y., Wang, T.T., Zhu, G., Liu, W.H., He, X.H., Li, Z., 2022. The Regulation of Prototype Foamy Virus 5'Long Terminal Repeats and Internal Promoter by Endogenous Transcription Factors. Intervirology 65 (1), 17–28.

Wolf, D., Goff, S.P., 2009. Embryonic stem cells use ZFP809 to silence retroviral DNAs. Nature 458 (7242), 1201–1204.

Wu, M., Wang, P.F., Lee, J.S., Martin-Brown, S., Florens, L., Washburn, M., Shilatifard, A., 2008. Molecular regulation of H3K4 trimethylation by Wdr82, a component of human Set1/COMPASS. Molecular and cellular biology 28 (24), 7337–7344.

Wu, Y., Tan, J., Su, Y., Qiao, W., Geng, Y., Chen, Q., 2010. Transcription factor AP1 modulates the internal promoter activity of bovine foamy virus. Virus research 147 (1), 139–144.

Xu, F., Tan, J., Liu, R., Xu, D., Li, Y., Geng, Y., Liang, C., Qiao, W., 2011. Tetherin inhibits prototypic foamy virus release. Virology journal 8, 198.

Xu, S., Dong, L., Shi, Y., Chen, L., Yuan, P., Wang, S., Li, Z., Sun, Y., Han, S., Yin, J., Peng, B., He, X., Liu, W., 2017. The Novel Landscape of Long Non-Coding RNAs in Response to Human Foamy Virus Infection Characterized by RNA-Seq. AIDS research and human retroviruses 33 (5), 452–464.

Yan, J., Zheng, Y., Yuan, P., Wang, S., Han, S., Yin, J., Peng, B., Li, Z., Sun, Y., He, X., Liu, W., 2021. Novel Host Protein TBC1D16, a GTPase Activating Protein of Rab5C, Inhibits Prototype Foamy Virus Replication. Frontiers in immunology 12, 658660.

Yuan, P., Yan, J., Wang, S., Guo, Y., Xi, X., Han, S., Yin, J., Peng, B., He, X., Bodem, J., Liu, W., 2021. Trim28 acts as restriction factor of prototype foamy virus replication by modulating H3K9me3 marks and destabilizing the viral transactivator Tas. Retrovirology 18 (1), 38.

Zhang, S., Li, J., Li, J., Yang, Y., Kang, X., Li, Y., Wu, X., Zhu, Q., Zhou, Y., Hu, Y., 2018. Up-regulation of microRNA-203 in influenza A virus infection inhibits viral replication by targeting DR1. Scientific reports 8 (1), 6797.

Zhang, J., Han, C., Xiong, Z., Qiu, M., Tuo, X., Wang, C., Qiao, W., Tan, J., 2022. SGK1, a Serine/Threonine Kinase, Inhibits Prototype Foamy Virus Replication. Microbiology spectrum 10 (3), e0199521.