Multifunctional roles of leader protein of foot-and-mouth disease viruses in suppressing host antiviral responses

Yingqi Liu, Zixiang Zhu, Miaotao Zhang, Haixue Zheng

To cite this version:

Yingqi Liu, Zixiang Zhu, Miaotao Zhang, Haixue Zheng. Multifunctional roles of leader protein of foot-and-mouth disease viruses in suppressing host antiviral responses. Veterinary Research, BioMed Central, 2015, 46 (1), pp.127. 10.1186/s13567-015-0273-1 hal-01341436
Multifunctional roles of leader protein of foot-and-mouth disease viruses in suppressing host antiviral responses

Yingqi Liu1,2†, Zixiang Zhu1†, Miaotao Zhang2 and Haixue Zheng1*

Abstract

Foot-and-mouth disease virus (FMDV) leader protein (Lpro) is a papain-like proteinase, which plays an important role in FMDV pathogenesis. Lpro exists as two forms, Lab and Lb, due to translation being initiated from two different start codons separated by 84 nucleotides. Lpro self-cleaves from the nascent viral polyprotein precursor as the first mature viral protein. In addition to its role as a viral proteinase, Lpro also has the ability to antagonize host antiviral effects. To promote FMDV replication, Lpro can suppress host antiviral responses by three different mechanisms: (1) cleavage of eukaryotic translation initiation factor 4γ (eIF4G) to shut off host protein synthesis; (2) inhibition of host innate immune responses through restriction of interferon-α/β production; and (3) Lpro can also act as a deubiquitinase and catalyze deubiquitination of innate immune signaling molecules. In the light of recent functional and biochemical findings regarding Lpro, this review introduces the basic properties of Lpro and the mechanisms by which it antagonizes host antiviral responses.

Table of Contents

1 Introduction
2 Different forms of FMDV Lpro
3 Cleavage activity of Lpro
4 Cleavage of host proteins induced by Lpro
5 Suppression of IFN production mediated by Lpro
6 Deubiquitination activity of Lpro
7 Lpro counteracts innate immune responses through its DUB activity
8 A putative SAP domain identified in Lpro
9 The SAP domain is important for Lpro activity
10 Conclusions

© 2015 Liu et al. This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The Creative Commons Public Domain Dedication waiver (http://creativecommons.org/publicdomain/zero/1.0/) applies to the data made available in this article, unless otherwise stated.
ability to inhibit the functions of a variety of host proteins, suppressing cellular immune responses [5–8]. For instance, 3C<sub>pro</sub> and L<sub>pro</sub> can induce the cleavage of host eukaryotic translation initiation factor 4 γ (eIF4G), limiting the synthesis of various host proteins [7, 9]. This could possibly include type 1 interferons (IFNs), indirectly promoting viral replication [10]. 3C<sub>pro</sub> can also cleave the nuclear factor kappa B (NF-κB) essential modulator (NEMO) and karyopherin α1 (KPNA1) to abate innate immune signaling [5, 6]. Moreover, L<sub>pro</sub> can directly cleave various other host proteins to suppress antiviral responses [11].

L<sub>pro</sub>, as a viral proteinase, self-cleaves from the nascent viral polyprotein precursor during FMDV infection and plays an important role in viral pathogenesis. L<sub>pro</sub> has two different forms (termed Lab and Lb) due to the initiation of translation at two functional AUGs that are separated by 84 nucleotides [12]. However, the Lb AUG is more efficiently used than the Lab site despite translation initiating from the Lab site [13, 14]. Hence, Lb is more abundant than Lab. The complete loss of Lab-coding region of FMDV is reported to be lethal for the virus [15], whereas the viruses with precisely deleted Lb coding regions (leaderless viruses) were viable and could replicate both in cattle and swine. However, these viruses could not induce any pathological changes and their replicative ability was attenuated [16, 17]. Furthermore, the supernatants of primary cell cultures infected with leaderless viruses possess stronger antiviral activity than the supernatants from wild-type FMDV-infected cells [18]. Recent evidence shows that the nature and extent of the residual leader protein sequences of FMDV precisely lacking the Lb-coding sequence determine different growth characteristics in different host-cell systems [19]. Based on these studies, L<sub>pro</sub> is thought to have multifunctional roles in viral pathogenicity and is considered an important virulence factor of FMDV.

L<sub>pro</sub> is known to contribute to virus propagation by suppressing host antiviral activity [20]. L<sub>pro</sub> has an antagonistic effect on host antiviral responses via at least three mechanisms. The most well-characterized mechanism is the cleavage of eIF4G by L<sub>pro</sub>, which shuts off host cap-dependent mRNA translation, and IFN translation may be included [7, 21]. Additionally, L<sub>pro</sub> also directly
involved in antiviral pathways through its deubiquitination enzyme (DUB) activity [22]. In this review, we discuss the current knowledge of these antagonistic mechanisms of Lpro against host antiviral responses.

2 Different forms of FMDV Lpro

FMDV Lpro shows similarities to the members of the cysteine proteinase family in structure and function [24]. It recognizes the junction sites between Lpro and VP4 and then cleaves itself from the polypeptide [4]. This automatic self-processing makes Lpro the first mature viral protein during FMDV infection. The two forms of Lpro (Lab and Lb) generated have been confirmed in vitro and in vivo [4, 25, 26]. Both these forms of Lpro exhibit the same enzymatic properties [27]. Each of them releases itself from the polypeptide via intermolecular or intramolecular self-cleavage [4, 25]. It is deemed that intramolecular self-processing is more efficient than intermolecular self-processing [28]. Nevertheless, the detailed mechanisms for the production of the two forms of Lpro have not been clearly elucidated. The mechanisms for selection of Lab start site (AUG1) or Lb start site (AUG2) for protein synthesis are complex. Through constructing synthetic fusion genes of AUG1 and AUG2, Belsham determined that before initiation of protein synthesis at AUG2, the ribosomes need to scan past AUG1–AUG2. The two initiation sites can both be used efficiently, whereas internal ribosome entry sites (IRESs) contribute to a slight biased utilization of the Lb site [29]. In a translation system mimicking the translation initiation pattern of the FMDV RNA observed during viral infection, the spacer region between two start codons plays a role in start codon recognition and biases the start codon selection towards the second one to initiate protein synthesis. The utilization of the first start codon depends on its sequence context [30]. Another study showed that the selection of AUG2 does not depend on the assembly of 48S complex formation on the 5' side of AUG1 [31]. A recent study based on previous work presented by Belsham [29] revealed a mechanism involving bias-usage of translation initiation sites of Lpro, suggesting that the poor nucleotide context of the Lab-initiation site restricts its translational efficiency. The ribosomes access the Lb site through linear scanning, starting from the upstream IRES proximal to the first initiation codon and this is not an independent entry process [14]. An early study by Poyry et al. suggested an alternative mechanism by which a few ribosomes reach the second initiation site [32].

Mutations in the initiation site of Lb disables the production of progeny viruses in transfected baby hamster kidney (BHK) cells, while mutations in the Lab initiation site do not affect the production of progeny viruses [33]. The precise deletion of the Lb from the A12 strain of FMDV (serotype A) produced viable viruses in BHK cells, while the mutant virus showed a reduced growth rate and produced smaller plaques [15]. A recent report shows that FMDVs (serotype O) lacking complete Lb coding sequences can be obtained in BHK cells by modifying Lab start codons, while the precise deletion of the Lb coding region alone prevents FMDV replication in primary bovine thyroid cells [19]. In addition, the deletion of the “spacer” region between two initiation codons is not lethal for the virus. These findings imply that the Lpro sequence is physiologically associated with FMDV propagation.

Apart from Lab and Lb, another form of Lpro has been observed, which is termed sLbpro or Lb' [34, 35]. sLbpro is generated by the removal of six or seven residues from the C-terminal extension (CTE) of Lpro during FMDV infection [36]. The trimming of the CTE of Lpro results in different characteristics of sLbpro. sLbpro cannot form homodimers like Lb via interactions of the CTE of one monomer with the substrate-binding site of the neighboring one, and vice versa [34, 35]. The Lb homodimers have been observed by X-ray crystallography and nuclear magnetic resonance (NMR) [34, 35], providing weak evidence for intermolecular reactions during self-cleavage. The X-ray structures of the L protease were obtained with the two forms of the protein, Lb (not Lab) and Lb, which additionally were modified (C51A). However, both the kinetic evidence of cleavage efficiencies and the structural evidence provided by NMR study on the monomeric variant of Lb, have strongly indicated an intramolecular mechanism of self-processing. Moreover, the obvious formation of a homodimer suggests that it may have a potential function in the modulation of enzyme activity; the dimer may be a physiologically active form responsible for the cleavage activities after the self-processing [35, 37]. The loss of the last six or seven residues in the CTE does not affect the cleavage efficiencies of sLbpro on the eIF4G site. This is because both Lab and sLbpro use residue C133 and two conserved amino acid residues (D184 and E186) of CTE, mediating binding and cleavage of eIF4GI. However, the cleavage efficiencies of Lb and sLbpro are different during the intramolecular incision of the polypeptide substrate due to the lack of an intact CTE in sLbpro, as the presence of at least one intact CTE is more favorable for intermolecular cleavage [38]. Although, the exact role of sLbpro remains unknown, it is thought to have a function during FMDV infection [38]. A putative SAP domain identified in Lpro is also
involved in the biological activities and functions of \( \text{L}^\text{pro} \). The mutation in some sites of the SAP domain lead to the production of different forms of \( \text{L}^\text{pro} \), all with varying functions [39].

### 3 Cleavage activity of \( \text{L}^\text{pro} \)

\( \text{L}^\text{pro} \), the first matured protein of FMDV, self-cleaves from the viral genome ORF-encoding polyprotein. The self-release of \( \text{L}^\text{pro} \) is thought to result from both intramolecular [28] and intermolecular [4] cleavage. The sequences of KVQRKLK*GAGQSS at the junction between \( \text{L}^\text{pro} \) and viral structural protein precursor (P1-2A) are thought to be the cleavage sites [4] (Figure 2A). In addition to the self-cleavage activity of \( \text{L}^\text{pro} \), it can cleave the homologues of host eIF4G in vitro (Figure 2B). The amino acid sequence recognized as the cleavage site of eIF4GI is PSFANLG*RTTLST [40], and VPLLNVG*SRRSQP for eIF4GII [21]. However, there remain some controversies about the precise cleavage sites within eIF4GI and eIF4GII generated by the \( \text{L}^\text{pro} \), because the cleavage sites of eIF4GI or eIF4GII in the virus-infected cells have not been identified.

\( \text{L}^\text{pro} \) is a papain-like cysteine proteinase. Although sequencing shows that \( \text{L}^\text{pro} \) shares low nucleotide identity with papain family members [24], the typically conserved catalytic cysteine and histidine residues belonging to papain-like proteinase have been identified in \( \text{L}^\text{pro} \) [41]. The catalytic cysteine site is located at the top of the central α-helix, and the catalytic histidine site lies opposite to it on a turn between two β-sheets in the right-hand domain [42]. The most conserved region between papain-like proteases and \( \text{L}^\text{pro} \) structures surrounds the active center, particularly the secondary components, α1 and β5–β6 [42].

The crystal structure of \( \text{L}^\text{pro} \) (indicating the \( \text{L}^\text{pro} \) superfamily) includes a globular domain similar to other members of the papain superfamily cysteine proteinase, and a flexible CTE. \( \text{L}^\text{pro} \) also possesses the same overall folding, which resembles the cellular prototype of papain. However, the pro-peptide binding loop and many other loops found in papain are not observed in \( \text{L}^\text{pro} \). Members of the papain proteinase superfamily have a corresponding activity unit, which comprises the catalytic triad of Cys/His/Asn [43]. This catalytic unit of Cys/His/Asp is also present in \( \text{L}^\text{pro} \). According to a detailed comparison of the two active sites, certain hydrogen bonds and water molecules localized at the catalytic site are remarkably conserved. Hydrogen bonds stabilize the side-chain amide group contributing to the oxyanion hole in both enzymes. One of the carboxylate oxygen atoms of Asp164 and amide nitrogens of Asn46 form a hydrogen bond in \( \text{L}^\text{pro} \). In papain, the hydrogen bond comprises a P-Ser176 hydroxyl group and P-Gln19 amide oxygen atom. The multiple discrepancies between the structures of \( \text{L}^\text{pro} \) and cysteine protease give rise to physicochemical differences between the two enzymes. For example, in the soluble state, when the concentration of cations increases, cysteine protease displays excellent tolerance and keeps its original state, whereas the activity of \( \text{L}^\text{pro} \) changes markedly. The fluctuation of pH can significantly

![Figure 2](image-url)
influence the activity of $L^{pro}$ because its cleavage activity varies greatly in different pH ranges [34].

4 Cleavage of host proteins induced by $L^{pro}$

Eukaryotic cellular translation initiation factor 4F (eIF4F) is a protein complex that recruits ribosomes to bind to host mRNA, initiating cap-dependent translation. This recruitment process is a rate-limiting step and therefore regulates translation [44]. The eIF4F complex comprises eIF4E small cap-binding protein, eIF4G scaffolding protein, and eIF4A ATP-dependent RNA helicase with capped-mRNA. The cap binding factor eIF4E, can bind to a segment of eIF4G to facilitate the formation of the eIF4E/cap-mRNA complex. As a core apparatus of eIF4F complex, eIF4G is a scaffolding protein that provides the binding regions for eIF4E, eIF4A, and RNA elements to form the eIF4F complex. The eIF4G protein also provides binding sites that recruit the small ribosomal subunit interacting protein eIF3 (recruiting the 40S ribosomal subunits to the 5′-end of the mRNA in eIF4F complex), poly(A)-binding protein (PABP), and eIF4E kinases Mnk1 (mitogen-activated protein kinase signal-integrating kinase1) and Mnk2, regulating host mRNA translation [45].

eIF4G proteins possess two homologous proteins in yeast, eIF4G1 (TIF4631) and eIF4GII (TIF4632), sharing a similar function. Both of them contain the conserved binding sites for eIF4E, PABP, eIF3 and RNA. For eIF4G1, it is reported that its N-terminal portion provides the binding sites for eIF4E and PABP, whereas eIF4A and eIF3 bind to the C-terminal portion of eIF4G1 [46, 47]. Some picornaviruses including poliovirus, human rhinovirus 2, and FMDV can effectively cleave the eIF4G1, yielding N- and C-terminal fragments [40, 47]. FMDV Lb protease can also cleave eIF4GII, generating a C-terminal fragment [48]. The loss of integrity of eIF4G1 and eIF4GII blocks the formation of the eIF4F complexes, which directly influences the cellular cap-dependent translation. However, the C-terminal fragment of both eIF4G proteins containing the binding sites for eIF4A and eIF3 can still bind to the FMDV IRES as efficiently as the non-processing eIF4G1 and eIF4GII respectively [47, 48]. Studies over the last two decades have shown that regulation of host and viral mRNAs by eIF4G is achieved by different mechanisms. Viral protein synthesis initiated at two distinct sites from artificial fusion genes is independent of the cap-binding eIF4F complex in the presence of IRES [29]. Furthermore, the cleavage products of eIF4G1 (C-terminal portion) stimulate the translation of uncapped RNAs and those carrying IRESs [49]. The interaction of the two eIF4G proteins with IRES is an essential event for promoting IRES activity. Therefore, viral RNA translation is unaffected [48, 50].

eIF4GI is a major form of eIF4G, which correlates with inhibition of cellular cap-dependent protein synthesis within FMDV-infected cells [4, 40]. However, cellular protein synthesis can still be maintained at a reduced level, with the complete loss of intact eIF4GI when virus replication is inhibited [51]. The discovery of human eIF4GII, which appears functionally analogous to eIF4GI, has resolved this puzzle [52]. The shut-off of host cell protein synthesis significantly decreases the expression of various cytokines and the major histocompatibility complex (MHc), resulting in delayed host antiviral effects. However, viral uncapped RNA can be translated through an IRES that is independent of intact eIF4E [53]. Therefore, the virus quickly takes over the host machinery to propagate vast numbers of progeny. FMDV lacking $L^{pro}$ is unable to escape the antiviral response and is not disseminated in the infected animals [16].

Apart from the cleavage of eIF4G, $L^{pro}$ can cleave a series of cellular proteins, such as eIF3a, polypyrimidine tract-binding protein (PTB), PABP and Gemin5, which are involved in the control of translation, and death domain associated protein (Daxx), a key factor that crosslinks the apoptosis, innate immune responses and transcription control, to interfere with various cellular pathways during viral infection [54]. The events associated with the extent of cytopathic effects in FMDV-infected cells are proteolysis of PTB, which is involved in mRNA stability and RNA localization, interaction of PABP with the entire FMDV 3′-UTR, and the binding of two subunits of eIF3 (eIF3a and b) with the IRES [11]. Recently, Piñeiro et al. [54] reported that the RNA-binding protein Gemin5 is also a target of $L^{pro}$. Gemin5 is the RNA-binding factor of a large macromolecule of the survival of motor neuron (SMN) complex, which acts as a down-regulator of cellular mRNA translation and IRES-driven translation initiation [55]. $L^{pro}$ recognizes the sequence RKAR of Gemin5 and induce its proteolysis, yielding two stable products of molecular weight 85 and 57 kDa within FMDV-infected cells [54]. Daxx has also been identified as a substrate of $L^{pro}$, and the RRLR motif is the recognition site. Daxx is a ligand of Fas, acting as a multifunctional adapter protein in the process of apoptosis, innate immune responses, and in transcriptional regulation [56]. The cleavage recognition site for $L^{pro}$ in PABP1 has not been identified experimentally. The sequence similarity with other $L^{pro}$ substrates and the molecular weight of the proteolysis product imply this characteristic [11], and it is deduced that a novel motif containing sequence (R)(R/K)(L/A)(R) is a putative target sequence of $L^{pro}$. Hence, neuroguidin, an eIF4E and cytoplasmic polyadenylation element binding protein (CPEB) that plays an important role in neuronal development [57], is hypothesized to be a potential target of $L^{pro}$.
with the target sequence as AKRRALS [54]. Furthermore, elf3a and b are essential to the assembly of the translation initiation complex, and are associated with PABP and RNA-binding protein PTB. This is involved in mRNA stability and RNA localization and can be proteolysed by FMDV Lpro, whereas PABP can be partial cleaved by L pro [11]. All these studies suggest that L pro can cleave various host proteins and has potential multifunctional roles.

Other than these identified substrates of L pro, various IRES-binding factors that are targets of other picornavirus proteases may contribute to understanding the link between these proteins and L pro. These factors include poly(rC)-binding protein 2, Gemin3 (RNA helicase that is a component of the SMN complex), RIG-I (retinoic acid-inducible gene 1; a cytoplasmic RNA helicase that senses viral infection), MAVS (mitochondrial antiviral-signaling protein), TRIF (Toll/interleukin (IL)-1 receptor domain-containing adapter inducing IFN-β or innate immune adaptor molecules), and the stress granules protein G3BP [58–62].

5 Suppression of IFN production mediated by L pro
FMDV infection triggers the activation of various pattern recognition receptors (PRRs) and induces a series of antiviral responses; with the transcription factor NF-κB acting as a sensor in response to the general alteration of the cellular environment. After the PRRs recognize the pathogens, the coordinated activation of various transcription factors including NF-κB, IFN regulatory factor (IRF)3 and IRF7, are initiated to induce early expression of type I IFNs and activate host antiviral responses [63].

PRR-induced signal transduction can activate NF-κB to translocate into the nucleus through degradation of NF-κB inhibitor. Nuclear translocation of NF-κB is followed by its binding to the promoter sequences of many genes to initiate their transcription. The expression of various cytokine genes such as the proinflammatory factors, chemokines, and adherence factors is greatly enhanced to induce antiviral responses [64, 65]. NF-κB also promotes secretion of IFN-α/β and their binding to corresponding receptors. This activates the JAK/STAT signaling pathway, which subsequently induces the expression of hundreds of IFN-α/β-stimulated genes (ISGs). ISGs are a class of antiviral genes that directly encode antiviral proteins that suppress virus propagation at different stages of the viral replication cycle [66]. It was recently reported that the enhanced expression of ISGs increases antiviral effects on FMDV [67]. IRFs are transcription factors that are pivotal for inducing activation of IFN-α/β during virus infection; IRF3 and IRF7 are crucial for virus-triggered IFN-α/β secretion [68]. IFN-α/β belong to the family of type I IFNs and serve as the first line of host defenses, displaying critical antiviral activity [69]. In addition, IFN-λ, a type III IFN, possesses IFN-like activity and is suggested to be a potent antiviral factor that is effective against many viruses [70, 71].

FMDV L pro acts as an antagonist of innate immune responses mainly eliciting the IFN-α/β specific antiviral activity at both protein and mRNA levels. The down-regulation of IFN expression at least in part corresponds to the cleavage of elf4G by L pro [64]. Both genetically engineered FMDV lacking L pro (A12-LLV2) and wild-type FMDV (A12-IC) were observed to induce the production of IFN-α/β mRNAs in secondary cells from susceptible animals. However, the A12-LLV2 mutant induces greater antiviral activity than the wild type as a consequence of failing to shut off the expression of host cell protein, including IFN-α/β [18]. L pro, blocks IFN protein synthesis, as well as synthesis of IFN-β mRNA and at least three ISGs mRNAs [10], including double-stranded RNA-dependent protein kinase (PKR) which plays an important role in inhibition of FDMV replication, 2′, 5′ oligoadenylate synthetase 1 (OAS1) and myxovirus resistance protein 1 (Mx1). Using microarray technology, a transcriptional profile associated with the antiviral responses against FMDV was systematically analyzed. The results suggested that L pro significantly inhibits NF-κB-dependent gene expression including expression of IFN-β and ISGs during FMDV infection [72]. Furthermore, it was found that during the acute infection phase, levels of type 1 IFN in the serum from infected animals significantly increased [73]. These studies indicate that type 1 IFN production is associated with antiviral effects against FMDV infection and is important in antiviral immune regulation. L pro as a critical virulence factor of FMDV is capable of using multiple strategies to suppress the production of IFNs.

Many picornviruses have evolutionarily developed subtle strategies that target host factors to subvert IFNs signaling pathways, and survive and replicate in host cells. For example, enterovirus 2A pro counteracts IFNs responses in infected cells by cleaving melanoma differentiation-associated protein 5 (MDA5) and MAVs [74], while the mengovirus utilizes L pro to prevent the production of IFN-α/β by inactivating iron/ferritin-mediated activation of NF-κB [75]. Cardioirus L pro induces cellular nuclear transport inhibition by binding to a key trafficking regulator RanGTPase [76].

Accumulating evidence shows that L pro of FMDV inhibits IFN production through interfering with the IFN signaling pathways. De Los Santos et al. determined that L pro can restrict the induction of IFN-β mRNA [10]. The restriction is partially built on the control of transcription factors and their upstream signaling factors by L pro. L pro was shown to be associated with the downregulation of nuclear p65/RelA during FMDV infection [8]. P65/RelA is the core component of NF-κB, and a decrease
in the integrity of p65/RelA may lead to the reduction of NF-kB. This ultimately results in downregulation of IFN-β expression and attenuation of host innate immune responses [8]. The mechanism involved in the down-regulation of p65/RelA induced by Lpro remains unclear. Whether the disappearance of p65/RelA is mediated by the cleavage activity of Lpro has not been confirmed, since no cleavage products of p65/RelA have been determined and no cleavage sites have been mapped until now. Wang et al. observed that Lpro decreases IRF-3-induced IFN-α/β expression by reducing ubiquitination of several transcription factors, thereby inactivating IFN transcription. Lpro can also use its deubiquitination activity to prevent IFN-α/β production by reducing ubiquitination of several of these signaling events [66, 78]. How-ever, there are also deubiquitinating enzymes (DUBs) [79] that can inactivate this complex by cleaving ubiquitin from its substrate proteins [80]. DUBs belong to the proteinase superfamily, of which 100 members have been identified in humans. DUBs can be classified into two main categories, metallocysteine deubiquitinating enzymes, and cysteine proteases [79]. The DUBs such as, A20, cylindromatosis (CYLD) protein, and deubiquitinating enzyme A (DUBA) negatively regulate the ubiquitination process, and hence, are key regulators in antiviral responses. For example, A20 is involved in downregulation of NF-κB activation, negatively regulating host antiviral responses. A20 is a DUB that can remove K63-linked ubiquitin from the ubiquitinated receptor-interacting protein (RIP) [80]. RIP is a serine/threonine kinase that contains a death domain which can interact with the death receptors Fas and tumor necrosis factor (TNF) receptor 1 to mediate activation of NF-κB [81]. Deubiquitination of RIP directly abates activation of the NF-κB signaling pathway [80]. Yokota et al. recently reported that measles virus P protein upregulates A20 to repress Toll-like receptors, inhibiting activation of NF-κB [82].

Bioinformatics analysis suggests that Lb has a potential DUB structure and conserved DUB catalytic residues (Cys51 and His148). The observed catalytic residues are highly conserved in the Lb of all seven serotypes of FMDV. Structural analysis indicates that Lb possesses a topology similar to DUB ubiquitin-specific 1A and resembles papain-like protease (PLpro) of severe acute respiratory syndrome coronavirus (SARS-CoV) [22, 83]. It has been observed that mutation of the SAP box (I83A/L86A) or the catalytically active site (C51A or D163 N/D164 N) of Lb results in the inactivation of DUB activity of Lpro [22].

7 Lpro counteracts innate immune responses through its DUB activity

Over the course of long-term evolutionary processes, many viruses have developed sophisticated strategies to antagonize host antiviral responses. Redirecting the cellular ubiquitination system to suppress innate antiviral immune signaling pathways is one of the strategies. For example, rotavirus NSP1 blocks NF-κB- and IRF- dependent transcription of type I IFN by inducing proteosome-mediated degradation of IRF3/5/7 or inhibiting IκB-α (inhibitor of NF-κB) degradation to prevent NF-κB activation [84, 85]. The accessory proteins, Viral Protein R and Virion Infectivity Factor of HIV can independently hijack the cellular ubiquitination system to decrease IRF-3 expression through proteasomal degradation and promote virus replication [86]. As a result, the production of host antiviral ISGs and proinflammatory factors is reduced and the antiviral innate responses are attenuated. Moreover, many viruses can hijack host ubiquitination systems to facilitate viral evasion, genomic replication, and exocytosis [87].

In addition to hijacking the host ubiquitination system for virus replication, many viruses have also developed the ability to disrupt cellular ubiquitination machinery to terminate or block several signaling transduction pathways responsible for the induction of antiviral responses [88]. So far, the PLpro of several coronavirus such as, porcine epidemic diarrhea virus, SARS-CoV, and Middle East respiratory syndrome (MERS-CoV) have been shown to possess deubiquitination activity that antagonizes IFN production, indicating that PLpro is a multifunctional protein [89, 90]. Similarly, FMDV Lpro is a papain-like protease that acts as an antagonist of IFN by negatively regulating IFN transcription and IFN mRNA translation [8, 18, 42, 77].

A recent study from Wang et al. has identified a DUB-like activity of Lb of FMDV [22]. It was observed that Lb significantly inhibited ubiquitination of several adapter signaling molecules of type I IFN pathway, including RIG-I, TBK1, TRAF3, and TRAF6 (Figure 3). The results of sequence alignment and structural bioinformatics analyses indicate that Lpro and ubiquitin-specific
protease (USP14) share similar topology [91]. The DUB activity of Lb was further confirmed through observation of the inhibitory effects of Lb on ubiquitination of RIG-I, TRAF3, TRAF6, and TBK1, which eventually prevents activation of the type I IFN pathway. This DUB activity can be abrogated through mutation of the conserved catalytic sites of Lb. The deubiquitinating processes mediated by Lb are similar to those mediated by DUBA and CYLD. Future studies should focus on whether the DUB activity of Lb is involved in the signaling pathways regulated by A20.

8 A putative SAP domain identified in \( L^{pro} \)

De Los Santos et al. discovered that FMDV \( L^{pro} \) contains a putative SAP domain (scaffold-attachment factor (SAF) A and SAFB, apoptotic chromatin-condensation inducer in the nucleus (ACINUS), and protein inhibitor of activated STAT (PIAS) domain) [39]. SAP is a conserved domain which usually exists in the eukaryotic proteins and involved in nucleic acid binding, DNA metabolism, DNA repair, chromosomal organization, apoptosis, transcriptional regulation, and immune regulation [92].

SMART software analysis of FMDV \( L^{pro} \) predicted an SAP domain between amino acids 47 and 83 of Lb. This putative SAP domain in \( L^{pro} \) shows >80% amino acid homology with other SAP domains of eukaryotic proteins. Three-dimensional analysis indicates that \( L^{pro} \) and the eukaryotic cellular SAP domains share almost the same α-helix-turn-α-helix structure, in which only two amino acid insertions found in the two α-helices of \( L^{pro} \) differed from other cellular SAP domains [39]. Furthermore, a motif of IQKL sequence in \( L^{pro} \) resembles the LXXLL signature motif that is mostly found in the SAP domain of PIAS. All these observations demonstrate the presence of a putative SAP domain in \( L^{pro} \).

The eukaryotic SAP domain is usually implicated in PIAS-associated functions. The SAP motif in PIAS has been conserved in evolution, from yeast to humans, and this functional motif can recognize and bind to the AT-rich sequence of scaffold/matrix attachment regions (S/MARs) of eukaryotic chromosomes. S/MAR is usually located close to the enhancer sequence so that it provides a special microenvironment for transcription [93]. PIAS is a negative regulator in host antiviral immunity.

Figure 3 DUB activity of \( L^{pro} \) in innate immune signaling pathways. \( L^{pro} \) can deubiquitinate several adaptor proteins including RIG-I, TRAF3, TRAF6, and TBK1. Deubiquitination of these proteins contributes to the attenuation of host innate immune responses.
For instance, pias gene knockout mice show more resistance to bacterial infection and improved antiviral responses to vesicular stomatitis virus. It is proposed that PIAS affects the expression of >60 genes, most of which are cytokine-induced and pathogen-activated genes involved in NF-κB and STAT signaling pathways. PIAS1 and PIASy are key proteins of the PIAS family and act as inhibitors to negatively regulate NF-κB- and STAT-dependent gene expression [94]. Furthermore, PIASy adopts distinctive mechanisms to inhibit virus-induced and IFN-stimulated transcription [95]. Intriguingly, some viral proteins are localized in the S/MAR regions, suggesting an interaction between viral proteins and that S/MAR may block host antiviral activities [96]. In addition, there is evidence showing that the VP35 protein of Ebola virus utilizes PIAS to promote sumoylation of IRF7, thus contributing to inhibition of IFN production in immune cells [97]. Until now, whether Lpro can adopt an analogous way of using PIAS in inhibiting cellular antiviral activities remains unclear. However, the N-terminal portion of PIAS3 containing the SAP domain was verified to block the NF-κB activation through binding to the p65/RelA subunit of NF-κB [98], whether Lpro can use this manner to interrupt activation of NF-κB remains unclear.

9 The SAP domain is important for Lpro activity
Zhu et al. found that expression of various IFN-inducible genes, chemokines or transcription factors, especially NF-κB-dependent gene expression in Lpro SAP domain mutant FMDV-infected bovine cells was significantly enhanced compared with the wild-type FMDV-infected cells [72]. De los Santos and his co-workers revealed that SAP domain is a determinant for Lpro nuclear subcellular localization. In FMDV-infected cells, Lpro progressively translocates to the nucleus, whereas mutation of two residues at positions 55 and 58 of Lpro (SAP mutant) significantly prevents nuclear translocation of Lpro without affecting the cleavage of eIF4G. This suggests that the SAP domain affects retention of Lpro in the nucleus within the FMDV-infected cells. The proper subcellular localization of Lpro in the nucleus is deemed to mediate the Lpro-dependent degradation of p65/RelA. Observations concerning SAP-related cellular antiviral responses suggest that in SAP-mutant FMDV-infected cells, the mRNA expression levels of several NF-κB-dependent cytokines, chemokines, and ISGs are higher than in wild-type FMDV-infected cells [39]. Collectively, the aforementioned results demonstrate that subcellular localization of Lpro in the nucleus is an important factor in the suppression of innate immune responses, and that the SAP domain is involved in this process. Besides, a recent study demonstrated that the catalytic activity and SAP domain of Lpro were required for suppressing poly(I:C)-induced IFN-λ1 production [23].

Diaz-San Segundo et al. found that inoculation of pigs with SAP-mutant FMDV (I55A and L58A mutations were introduced in Lpro) can induce early protection against FMD [99]. No clinical signs of FMD, viremia, or virus shedding were observed, even when the pigs were inoculated at 100-fold higher doses than those required to cause clinical signs with wild-type FMDV. The SAP-mutant FMDV elicited strong adaptive immune responses that provided complete protection against wild-type FMDV infection. Impressively, the neutralizing antibody response was induced as early as 2 days post-inoculation and lasted for at least 21 days after inoculation. In the blood of pigs inoculated with SAP mutant virus, expression of IFN-α, TNF-α, IL-1, and IL-6 was higher than in pigs inoculated with the wild-type virus. Zhu et al. reported that FMDV manipulates ubiquitin-activating enzyme one to promote viral replication, and the SAP domain of Lpro was involved in this process, which indicates that SAP maybe has a novel role [100]. All these studies suggest that FMDV Lpro plays an important role in virus replication process, and the SAP domain may be a critical region for the maintenance of the biological activities of Lpro.

10 Conclusions
FMDV has evolved numerous strategies to evade host antiviral responses. In order to survive and replicate in host cells, the virus has developed various ways to impair or suppress the induction and activation of antiviral responses, utilizing viral nonstructural proteins. Lpro and 3Cpro are the main viral factors that antagonize host immune responses, with Lpro being one of the most well-characterized proteins. Lpro can cleave numerous host proteins, inhibit cellular protein expression, and deubiquitinate some crucial molecules that are essential for the activation of antiviral pathways and signal transduction. Intensive study of FMDV Lpro has uncovered several mechanisms by which FMDV replicates in host cells and suppresses host antiviral responses utilizing Lpro (Table 1). However, these observations represent only the “tip of the iceberg” and several questions regarding the different forms of Lpro and the pathways involved in Lpro-mediated antagonistic effects need to be answered. Further studies are necessary to elucidate these unanswered questions and the multifunctional role of Lpro in FMDV infection.
Table 1  The target proteins and the multifunctional role of Lpro.

| Biological functions of Lpro | Auto cleavage | Cleavage activities | Deubiquitination activity | Unknown activity |
|-----------------------------|--------------|---------------------|---------------------------|-----------------|
| Target proteins             | Viral polyprotein [4]     | eIF4GI [40]; eIF4GII [21] | Gemin5 [54] eIF3a and β; PTB;PABP1 [11] | RIG-I,TRAF3 and TRAF6 [22] |
| Recognition site/region     | KVQRKLX201*GAGQSS | eIF4GIPSFANLGC72*RTTLST; eIF4GII/VPLLNVG700*SRRSQP | DaxxVLARRLR180*ENRSLAL (RRR motif) | Unknown |
| The effects induced by Lpro | Directing the release of Lpro from the nascent polyprotein | Shutting off cellular translation | Regulation of apoptosis and innate immune antiviral responses | Restricting antiviral activities of IFN-α/β and IFN-λ1 |

Unknown: unknown
Abbreviations
FMD: foot-and-mouth disease; FMDV: foot-and-mouth disease virus; Lpro, leader protein; 5′-UTR, 5′ untranslated region; 3′-UTR, 3′ untranslated region; ORF: the open reading frame; eIF4G: eukaryotic translation initiation factor 4 gamma; NF-κB: nuclear factor-kappa B; NEMO: NF-κB essential modulator; KPNA1: karyopherin α1; IFN: interferon; DUB: deubiquitinase; NMR: nuclear magnetic resonance; elfIF4F: eukaryotic cellular translation initiation factor 4F; PABP: poly(A)-binding protein; Mnk: mitogen-activated protein kinase signal-integrating kinase 1; IRES: internal ribosome entry site; MHC: major histocompatibility complex; PTBP: poly(rC)-mRNA tract-binding protein; Daxx: death-domain associated protein; CPEB: cytoplasmic polyadenylation element binding protein; PCBP: poly(rC)-binding protein; RIG-I: retinoic acid-inducible gene 1; MAVS: mitochondrial antiviral-signaling protein; TRIF: Toll/interleukin (IL)-1 receptor domain-containing adaptor inducing interferon-β or innate immune adaptor molecules; PRRs: pattern recognition receptors; IFR: IFN regulatory factor; OAS1: 2′, 5′ oligoadenylate synthetase 1; RIG: receptor interacting protein; TRAF: TRIF receptor-associated factor; TBK1: TANK binding kinase 1; PL: papain-like protease; PEDV: porcine epidemic diarrhea virus; SARS-CoV: severe acute respiratory syndrome coronavirus; MERS-CoV: middle East respiratory syndrome coronavirus; IF-κBα, 5′-UTR: 5′ untranslated leader protein; 5′ leader proteinase; 5′ leader proteinase cleavage site in vitro. J Virol 78:3271–3278

References
1. Ding YZ, Chen HT, Zhang J, Zhou H, Ma LN, Zhang L, Gu Y, Liu YS (2013) An overview of control strategy and diagnostic technology for foot-and-mouth disease in China. Virol J 10:78
2. Kwon JH, Bryan MJ, Baat B (2004) Foot-and-mouth disease. Clin Microbiol Rev 17:465–493
3. Conda-Sheridan M, Lee SS, Preslar AT, Stupp SI (2014) Esterase-activated release of naproxen from supramolecular nanofibres. Chem Commun (Camb) 50:13757–13760
4. Strebel K, Beck E (1986) A second protease of foot-and-mouth disease virus. J Virol 58:893–899
5. Du Y, Bi J, Liu J, Liu X, Wu X, Jiang P, Yoo D, Zhang Y, Wu J, Wan R, Zhao X, Guo L, Sun W, Cong X, Chen L, Wang J (2014) Identification of foot-and-mouth disease virus antagonizes the interferon signaling pathway by blocking STAT1/STAT2 nuclear translocation. J Virol 88:4908–4920
6. Wang D, Fang L, Li K, Zhong H, Fan J, Ouyang C, Zhang H, Duan E, Luo R, Zhang Z, Liu X, Chen H, Xiao S (2012) Foot-and-mouth disease virus 3C protease cleaves NEMO to impair innate immune signaling. J Virol 86:9311–9322
7. Devaney MA, Vakharia VN, Lloyd RE, Ehrenfeld E, Grubman MJ (1988) Leader proteinase of foot-and-mouth disease virus is required for cleavage of the capsid protein complex. J Virol 62:4407–4419
8. de Los Santos T, Diaz-San Segundo F, Grubman MJ (2007) Derepression of nuclear factor kappa B during foot-and-mouth disease virus infection. J Virol 81:12803–12815
9. Belsham GJ, McInerney GA, Ross-Smith N (2000) Foot-and-mouth disease virus 3C protease induces cleavage of translation initiation factors elf4A and elf4G within infected cells. J Virol 74:272–280
10. de Los Santos T, de Avila Botton S, Weblen R, Grubman MJ (2006) The leader protease of foot-and-mouth disease virus inhibits the induction of beta interferon mRNA and blocks the host innate immune response. J Virol 80:1906–1914
11. Rodriguez Pulido M, Serrano P, Saiz M, Martinez-Salas E (2007) Foot-and-mouth disease virus infection induces proteolytic cleavage of PTB, elf3a, b, and PABP RNA-binding proteins. Virology 364:466–474
12. Esteban-Torres M, Landete JM, Reveron I, Santamaria L, de las Rivas B, Munoz R (2015) A Lactobacillus plantarum esterase active on a broad range of phenolic esters. Appl Environ Microbiol 81:3235–3242
13. Gu X, Kumar S, Kim E, Kim Y (2015) A whole genome screening and RNA interference identify a juvenile hormone esterase-like gene of the diamondback moth, Plutella xylostella. J Insect Physiol 80:81–87
14. Poyry TA, Jackson RJ (2011) Mechanisms governing the selection of translation initiation sites on foot-and-mouth disease virus RNA. J Virol 85:10178–10188
15. Piccone ME, Rieder E, Mason PW, Grubman MJ (1995) The foot-and-mouth disease virus leader protease gene is not required for viral replication. J Virol 69:5376–5382
16. Brown CC, Piccone ME, Mason PW, Mckenna TS, Grubman MJ (1996) Pathogenesis of wild-type and leaderless foot-and-mouth disease virus in cattle. J Virol 70:5638–5641
17. Chinsangaram J, Mason PW, Grubman MJ (1998) Protection of swine by live and inactivated vaccines prepared from a leader proteinase-deficient serotype A12 foot-and-mouth disease virus. Vaccine 16:1516–1522
18. Chinsangaram J, Piccone ME, Grubman MJ (1999) Ability of foot-and-mouth disease virus to form plaques in cell culture is associated with suppression of alpha/beta interferon. J Virol 73:9891–9896
19. Belsham GJ (2013) Influence of the Leader protein coding region of foot-and-mouth disease virus on virus replication. Gen Virol 94:1486–1495
20. Steinberger J, Skern T (2014) The leader protease of foot-and-mouth disease virus: structure-function relationships in a proteolytic virulence factor. Biol Chem 395:1179–1185
21. Gradi A, Foeger N, Strong R, Svikitin YV, Sonenberg N, Skern T, Belsham GJ (2004) Cleavage of eukaryotic translation initiation factor 4GII within foot-and-mouth disease virus-infected cells: identification of the L-protease cleavage site in vitro. J Virol 78:3271–3278
22. Wang D, Fang L, Li P, Sun L, Fan J, Zhang Q, Luo R, Liu X, Li K, Chen H, Chen Z, Xiao S (2011) The leader protease of foot-and-mouth disease virus negatively regulates the type I interferon pathway by acting as a viral deubiquitina. J Virol 85:3758–3766
23. Wang D, Fang L, Liu L, Zhong H, Chen Q, Luo R, Liu X, Zhang Z, Chen H, Xiao S (2011) Foot-and-mouth disease virus (FMDV) leader protease negatively regulates the porcine interferon-lambdα1 pathway. Mol Immunol 49:407–412
24. Gorbalkaeya AE, Kooren EV, Lai MM (1991) Putative papain-related thiol proteases of positive-strand RNA viruses. Identification of rubi- and aphthovirus proteases and delineation of a novel conserved domain.
Andersen J, VanScy C, Cheng TF, Gomez D, Reich NC (2008) IRF-3-dependent and augmented target genes during viral infection. Genes Immun 9:168–175

Taniguchi T, Takaoka A (2002) The interferon-alpha/beta system in antiviral responses: a multimodal machinery of gene regulation by the IRF family of transcription factors. Curr Opin Immunol 14:111–116

Ank N, West H, Bartholdy C, Eriksson K, Thomesen AR, Paludan SR (2006) Lambda interferon (IFN-lambda), a type III IFN, is induced by viruses and IFNs and displays potent antiviral activity against select virus infections in vivo. J Virol 80:4501–4509

Kotenko SV, Gallagher G, Baunin VV, A, Shen M, Shah NK, Langer JA, Sheikh F, Dickensheets H, Donnelly RP (2003) IFN-lambdas mediate antiviral protection through a distinct class II cytokine receptor complex. Nat Immunol 4:69–77

Zhu J, Weiss M, Grubman MJ, de los Santos T (2010) Differential gene expression in bovine cells infected with wild type and leaderless foot-and-mouth disease virus. Virology 404:32–40

Stenfeldt C, Heegaard PM, Stockmarr A, Tjornehoj K, Belsheim GJ (2011) Analysis of the acute phase responses of serum amyloid A, haptoglobin and type I interferon in cattle experimentally infected with foot-and-mouth disease virus serotype O. Vet Res 42:66

Feng Q, Langerreais ME, Lork M, Nguyen M, Hato SV, Lanke K, Emadl M, Bhoopathi P, Fisher PB, Lloyd RE, van Kuppeveld FJ (2014) Enterovirus 2Apro targets MDAS and MAVS in infected cells. J Virol 88:3369–3378

Zoll J, Melders-WJ, Galanna JM, van Kuppeveld FJ (2002) The mungo virus leader protein suppresses alpha/beta interferon production by inhibition of the iron/ferritin-mediated activation of NF-kappa B. J Virol 76:9664–9672

Bacot-Davis VR, Palmenberg AC (2013) Encephalomyocarditis virus leader protein hinge domain is responsible for interactions with Ran GTPase. Virology 443:177–185

Wang D, Fang L, Luo R, Ye R, Fang Y, Xie L, Chen H, Xiao S (2010) Foot-and-mouth disease virus leader protein inhibits dsiRNA-induced type I interferon transcription by decreasing interferon regulatory factor 3/7 in protein levels. Biochem Biophys Res Commun 399:72–78

Ribéreau-Poirier A, Servant MJ (2008) Roles of ubiquitination in pattern-recognition receptors and type I interferon receptor signaling. Cytokine 43:359–367

Nijman SM, Luna-Vargas MP, Velds A, Brummelkamp TR, Dirac AM, Sixma TK, Bernards R (2005) A genomic and functional inventory of deubiquitinating enzymes. Cell 123:773–786

Wertz I, O’Rourke KM, Zhu H, Eby M, Aravind L, Seshagiri S, Wu P, Weismann C, Baker R, Boone DL, Ma A, Koonin EV, Dixt VM (2004) De-ubiquitination and ubiquitin ligase domains of A20 down-regulate NF-kappaB signalling. Nature 430:694–699

Kellibrer MA, Grimm S, Ishida Y, Kuo F, Stanger BZ, Leder P (1998) The death domain kinase RIP mediates the TNF-induced NF-kappaB signal. Immunity 8:297–303

Yokota S, Okabayashi T, Yokosawa N, Fujii N (2008) Measles virus p protein suppresses Toll-like receptor signal through up-regulation of ubiquitin-modifying enzyme A20. FASEB J 22:74–83

Ratia K, Saikatendu KS, Santaniello BD, Barretto N, Baker SC, Stevens RC, Mexecar AD (2006) Severe acute respiratory syndrome coronavirus papain-like protease: structure of a viral deubiquitinating enzyme. Proc Natl Acad Sci U S A 103:5717–5722

Kim S, Kim H, Choi Y, Kim Y (2015) A new strategy for fluorogenic esterase probes displaying low levels of non-specific hydrolysis. Chemistry 21:9645–9649

Dev L, Pawar RM, Makala H, Goel S (2015) Conserved expression of ubiquitin carboxyl-terminal esterase L1 (UCHL1) in mammalian testes. Indian J Exp Biol 53:305–312

Okumura A, A, Lubybova E, Ezhile H, Strebek P, Pritha PM (2008) HIV-1 accessory proteins VPR and Vif modulate antiviral response by targeting IRF-3 for degradation. Virology 373:85–97

Gale M Jr, Sen GC (2009) Viral evasion of the interferon system. J Interferon Cytokine Res 29:475–476

Viswanathan K, , DeFilippis V (2010) Viral hijacking of the host ubiquitin system to evade interferon responses. Curr Opin Microbiol 13:517–523

Yang X, Chen X, Biau G, Li J, Xing Y, Wang Y, Chen Z (2014) Proteolytic processing, deubiquitination and interferon antagonist activities of Middle East respiratory syndrome coronavirus papain-like protease. J Gen Virol 95:614–626

Gazzard L, Williams K, Chen H, Asford L, Blackwood E, Burton R, Chapman K, Crackett P, Drobnick J, Elwood C, Epler J, Flaggella M, Garcia E, Gill M, Goodacre S, Halladay J, Hewitt J, Hunt H, Kintz S, Lyssikatos J, Macleod C, Major S, Meard G, Nanukulla R, Ramiscal J, Schmidt S, Seward E, Wiesmann C, Wu P, Yee S, Yen L, Malek S (2015) Mitigation of acetycholine esterase activity in the 1,7-diazacarbazole series of inhibitors of checkpoint kinase 1. J Med Chem 58:5053–5074

Hu M, Li P, Song L, Jeffrey PD, Chenova TA, Wilkinson KD, Cohen R, Shi Y (2005) Structure and mechanisms of the proteosome-associated deubiquitinating enzyme USP14. EMBO J 24:3747–3756

Aravind L, Koonin EV (2000) SAP—a putative DNA-binding motif involved in chromosomal organization. Trends Biochem Sci 25:112–114

Kipp M, Gohring F, Ostendorp T, van Drunen CM, van Diei R, Przybylsky M, Fackelmayer FO (2000) SAF-Box, a conserved protein domain that specifically recognizes scaffold attachment region DNA. Mol Cell Biol 20:7480–7489

Shuai K (2006) Regulation of cytokine signaling pathways by PIAS proteins. Cell Res 16:196–202

Kubota T, Matsuoka M, Xu S, Otsuki N, Kato A, Ozato K (2011) PIASy inhibits virus-induced and interferon-stimulated transcription through distinct mechanisms. J Biol Chem 286:8165–8175

Everett RD, Chelbi-Alix MK (2007) PML and PML nuclear bodies: implications in antiviral defence. Biochemie 89:819–830

Chang TH, Kubota T, Matsuoka M, Jones S, Bradfute SB, Bray M, Ozato K (2008) Ebola Zaire virus blocks type I interferon production by exploiting the host SUMO modification machinery. PLoS Pathog 5:e1000493

Jang HD, Yoon K, Shin YJ, Lee J, Lee SY (2004) PIAS3 suppresses NF-kappaB-mediated transcription by interacting with the p65/RelA subunit. J Biol Chem 279:24873–24880

Diaz-San Segundo F, Weiss M, Perez-Martin E, Dias CC, Grubman MJ, de los Santos T (2012) Inoculation of swine with foot-and-mouth disease virus induces early protection against disease. J Virol 86:1316–1327

Zhu Z, Yang F, Zhang K, Cao W, Jin Y, Wang G, Mao R, Li D, Guo J, Liu X, Zheng H (2015) Comparative proteomic analysis of wild-type and SAP domain mutant foot-and-mouth disease virus-infected porcine cells identifies the ubiquitin-activating enzyme UBE1 required for virus replication. J Proteom Res 14:4194–4206