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REVIEW

Decoding Enterovirus 71: Molecular Functions of Structural and Nonstructural Proteins in Viral Replication and Host Manipulation

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ABSTRACT: Enterovirus 71 (EV71), a member of the family *Picornaviridae*, genus *Enterovirus*, is an agent of hand, foot, and mouth disease (HFMD) and remains a persistent global health concern, particularly among children under five years of age. Although most infections are self-limiting, a significant proportion can progress to severe neurological manifestations such as aseptic meningitis, encephalitis, and fatal pulmonary oedema. Despite substantial advances in research, no universally effective antiviral therapy or broadly protective vaccine has yet been developed. Drawing upon both foundational and recent studies, we evaluate the strength of existing evidence and delineate how these viral proteins cooperatively regulate viral entry, genome replication, immune evasion, autophagy, and host cell death. Special emphasis is placed on virus–host protein interactions, post-translational modifications, and the signaling pathways targeted by EV71 to subvert innate immune responses protein–protein interactions with host factors, post-translational modifications, and the molecular mechanisms by which EV71 subverts innate immune signaling pathways. By integrating structural, biochemical, and systems-level insights, this review pro-poses a unifying conceptual model of EV71 protein function, highlighting key molecular interfaces that underline viral replication and pathogenesis. The analysis also identifies critical knowledge gaps and emerging therapeutic targets, providing a forward-looking perspective on strategies for antiviral drug discovery, vaccine design, and host-directed interventions aimed at durable control of EV71 infection. This review was designed to critically synthesize current structural, molecular, and systems-level evidence to elucidate how EV71 structural and nonstructural proteins coordinate viral replication, immune evasion, and pathogenesis, and to identify priority targets for antiviral and vaccine development.

KEYWORDS: Enterovirus 71; *Picornaviridae*; structural protein; nonstructural protein; viral pathogenesis

1 Introduction

Enterovirus 71 (EV71), belonging to the family *Picornaviridae* and genus *Enterovirus* (species *Enterovirus A*), is a small, non-enveloped, positive-sense RNA virus and an emerging neurotropic pathogen that continues to drive seasonal outbreaks of hand, foot, and mouth disease (HFMD) with escalating severity and expanding geographic distribution [1–3]. Alongside Coxsackie virus A16 (CV-A16), EV71 remains a primary etiological agent of HFMD, yet it is uniquely associated with severe neurological and cardiopulmonary complications in a subset of pediatric patients [4,5].

HFMD predominantly affects infants and young children, typically presenting with low-grade fever, vesicular eruptions on the hands, feet, and buttocks, and oral ulcers as hallmark clinical manifestations of the disease [6]. Although most cases are self-limiting, EV71 infection can progress to serious neurological

manifestations such as aseptic meningitis, brainstem encephalitis, poliomyelitis-like flaccid paralysis, and neurogenic pulmonary edema, occasionally leading to fatal outcomes [7]. EV71 infection is initiated primarily through fecal–oral and respiratory transmission, followed by viral replication in the gastrointestinal or respiratory epithelium and subsequent systemic dissemination via viremia. The virus may invade the central nervous system through several proposed mechanisms, including retrograde axonal transport from peripheral neuromuscular junctions, disruption of the blood–brain barrier that permits paracellular or transcellular viral passage across endothelial cells, and the so-called "Trojan horse" mechanism in which infected immune cells facilitate viral entry into neural tissues [8]. Since its first detection in New Zealand in 1956, EV71 has spread globally, with major epidemics reported across the Asia–Pacific region, including Australia, Cambodia, China, India, Japan, Malaysia, Singapore, Taiwan, Thailand, and Vietnam [9,10]. In China, HFMD was classified as a noticeable Class C infectious disease in 2008 [11]. The temporary decline in HFMD incidence during the COVID-19 pandemic, attributed to non-pharmaceutical interventions (NPIs), was followed by a rapid resurgence of EV71 cases after restrictions were lifted, accompanied by shifts in genotype prevalence and an increase in neurological complications among younger age groups [7,12,13].

Genotypically, EV71 is classified into three main genogroups (A, B, and C) and multiple subgenotypes (e.g., B1–B5, C1–C5), with C4 having predominated in China over the past two decades [14,15]. Recent surveillance has revealed the emergence of novel recombinant lineages and co-circulation with other enteroviruses such as CV-A6 and CV-A16, raising concerns about limited vaccine cross-protection and the potential for immune-escape variants [16].

Although over 280 human *Enterovirus* (EV) serotypes have been documented, only four currently have approved vaccines worldwide, and no antiviral agents are available for the treatment of EV infections [17,18]. In addition, global monitoring of EV circulation remains limited and lacks coordinated standardization, making it difficult to rapidly detect and respond to new variants. Because *Enterovirus* A71 (EV-A71) has emerged as a major public health threat in China, three alum-adjuvanted and inactivated EV-A71 vaccines were launched between 2015 and 2016. However, these vaccines predominantly exhibit genotype-restricted protection and do not provide broad immunity against diverse circulating strains [17,19]. Recent studies have identified that EV71 structural (e.g., VP1) and nonstructural proteins (2A, 3C) play pivotal roles in modulating viral replication, host immune evasion, and neuropathogenesis. For instance, the host E3 ligase SPOP was found to degrade the viral 2A protease to restrict replication, while VP1 induces endoplasmic reticulum stress via PMP22 accumulation in neuronal cells, contributing to virus-induced neuropathology [20]. Similarly, the ubiquitination of VP1 by ZYG11B (Zyg-11 family member B), an E3 ubiquitin ligase adaptor protein, suppresses viral propagation, identifying VP1 as both a virulence factor and a potential drug target [21].

Despite these advances, the molecular understanding of how EV71 proteins collectively coordinate viral replication, immune evasion, and host responses remains fragmented. Previous reviews have largely focused on individual proteins or limited mechanistic aspects, lacking a holistic synthesis of structural and nonstructural protein functions. The present review aims to fill this gap by providing a comprehensive, mechanistic, and integrative analysis of all EV71-encoded proteins, summarizing their structural characteristics, host interactions, and immunomodulatory roles. By critically evaluating recent findings and highlighting unresolved questions, this review seeks to provide a unified conceptual framework that may guide future antiviral and vaccine development strategies.

2 Genome Structure and Replication Process of EV71

EV71 is a non-enveloped, positive-sense single-stranded RNA (+ssRNA) virus belonging to the family *Picornaviridae*, genus *Enterovirus*. The genome is approximately 7.4 kb in length and contains a 5′ untranslated

region (5′UTR), a single open reading frame (ORF), and a 3′UTR followed by a poly(A) tail (Fig. 1) [22]. The ORF is divided into three regions, P1, P2, and P3, where the P1 region encodes the four structural capsid proteins (VP1–VP4), while P2 and P3 encode seven nonstructural proteins (2A–3D) that regulate viral replication, host interaction, and immune evasion [3].

Recent structural analyzes have refined the topology of the EV71 genome, revealing conserved cis-acting replication elements within both the 5′ and 3′ UTRs, including a cloverleaf-like structure essential for polymerase recruitment and genome circularization [23]. Together, the 11 encoded proteins form a coordinated molecular network that drives viral replication, immune evasion, and host inflammatory responses [7]. A concise overview of VP1–VP4, including their structural features, functions, host interactions, and therapeutic potential, is provided in Table 1 to enable systematic comparison and facilitate translational interpretation.

In particular, VP1 not only contributes to receptor recognition but also triggers host stress responses and is implicated in endoplasmic reticulum dysfunction and neurotoxicity [20]. Similarly, nonstructural proteins such as 2A protease and 3C protease are multifunctional enzymes involved in viral polyprotein cleavage, host shutoff, and immune modulation. New findings indicate that 2A is tightly regulated by host ubiquitination machinery, with E3 ligase SPOP promoting its degradation and thereby limiting viral replication [19].

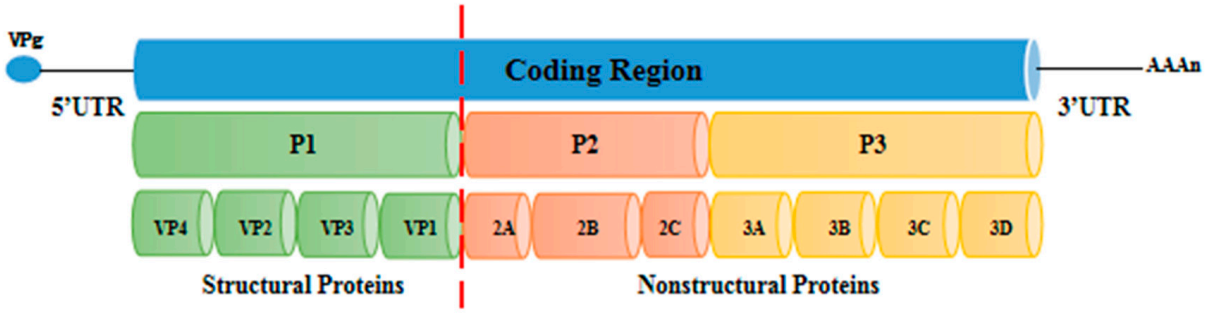

Figure 1: Structure of the enterovirus 71 (EV71) genome.

EV71 entry begins with the receptor-mediated attachment, followed by capsid rearrangement and VP4 release, which drive endocytosis and genome uncoating [24,25]. The interaction between EV-A71 capsid proteins and host receptors, followed by conformational changes leading to VP4 release, is depicted in Fig. 1, offering a visual framework that complements the mechanistic description provided in this section.

Within the host cell, the viral capsid is removed, and genomic RNA is released into the cytoplasm. The viral proteases 2A and 3C rapidly process the translated polymer proteins into structural capsid proteins (VP1–VP4) and nonstructural proteins (2A–2C, 3A–3D) of daughter viruses by directly using the parent viral RNA as mRNA. Notably, 3C has been shown to target and degrade host RNA-binding proteins and nuclear pore components, contributing to immune evasion and facilitating efficient replication [23,26].

Genome replication occurs within membrane-associated replication organelles, where the viral RNA-dependent RNA polymerase (3D) synthesizes a negative-strand RNA template, subsequently used to generate multiple positive-strand RNAs for progeny virion assembly [27]. Viral replication organelle formation depends on VPS34 (class III phosphatidylinositol 3-kinase) and its effector DFCP1, which remodels host lipid droplets and endoplasmic reticulum membranes. Inhibition of VPS34 has therefore emerged as a potential antiviral strategy [23].

Table 1: Summary of the four EV-71 structural proteins (VP1–VP4), highlighting their structural features, molecular functions, host interactions, and translational implications [28].

Protein	Amino Acids	Core Molecular Function	Validated Host Targets/ Genetic Determinants	Mechanistic Insight	Translational Significance
VP1	297	Receptor binding, uncoating trigger, neurotropism	SCARB2, PSGL-1; VP1-145E/Q/K switch; Nter (1–71) binds viral RNA	Receptor engagement expels the pocket factor and induces VP4 release, initiating viral genome delivery	Principal vaccine antigen; basis for genotyping; monoclonal antibodies target GH loop (e.g., 2G8, 22A12)
VP2	254	Capsid stabilization; RNA release	$VP2_{136-150}$ conserved epitope; VP2-149M synergizes with VP1-145E	Modulates capsid conformational dynamics during entry and enhances viral infectivity when mutated	Biomarker for vaccine potency; candidate for cross-protective epitopes
VP3	245	Structural scaffold; virion assembly	"Knob" region; VP3 position 74 recognized by therapeutic mAb 5H7	Conserved epitope allows differential diagnostic targeting between EV-A71 and CVA16	Target for diagnostic antibodies (mAb 10D3, 5H7)
VP4	69	RNA anchoring; early uncoating	N-terminal 1–20 aa form neutralizing epitope	Expelled first during uncoating and inserts into membrane to form pore for RNA passage	VLP-based vaccine candidate; tool for molecular epidemiology

Abb: aa, amino acids; CVA16, Coxsackievirus A16; EV-A71, Enterovirus A71; mAb, monoclonal antibody; PSGL-1, P-selectin glycoprotein ligand-1; RNA, ribonucleic acid; SCARB2, scavenger receptor class B member 2; VLP, virus-like particle; VP, viral protein.

3 Function of EV71's Structural Proteins

The EV71 capsid is formed by four structural proteins (VP1-VP4) that assemble into an icosahedral shell encapsulating the viral genome [28]. While VP1–VP3 are surface-exposed and involved in host cell receptor engagement and immune recognition, VP4 is located internally and contributes to capsid stabilization and uncoating [24]. Together, these proteins play mechanistic roles beyond structural support, participating in viral entry, host modulation, and immune evasion. A comparative summary of VP1–VP4, including their molecular functions, host interactions, and translational relevance, is provided in Table 1.

3.1 VP1 Protein

3.1.1 Antigenicity and Receptor Recognition

VP1 is the most exposed capsid protein (297 aa) and serves as the principal determinant for receptor usage, serotype, and immune recognition [3]. Its canyon and pocket-factor domains are critical for receptor-mediated viral entry, consistent with high-resolution structural analyses (Fig. 2). SCARB2 is the primary entry receptor, interacting with VP1 GH and VP2 EF loops in a pH-dependent manner to facilitate uncoating [29]. Mutations such as K98E, E145A, and L169F enhance VP1–SCARB2 binding and increase infectivity, particularly in murine models [30].

P-selectin glycoprotein ligand-1 (PSGL-1) binds explicitly to VP1 variants containing G or Q at residue 145, whereas the 145E variant abolishes PSGL1 binding [31,32]. VP1-145 acts as a molecular switch that controls both PSGL-1 usage and neutralisation sensitivity, indicating strong immune selection pressure [23]. When heparan sulfate (HS) is used as an attachment receptor, residues VP1-98 and VP1-145 regulate viral affinity and tropism, and VP1-E98K variants show reduced pathogenicity in mice [33,34].

Additional host factors, including annexin II, vimentin, fibronectin, and Galectin-1, interact with distinct VP1 regions to enhance viral entry and thermal stability, supporting EV71 survival *in vivo* [24,35]. Thermostable VP1 mutants (e.g., K215A) have shown promise for recombinant vaccine design, especially in high-temperature regions [27,36].

VP1 contains multiple conformational B-cell epitopes located in surface loops such as BC, GH, and HI, which are targets for potent monoclonal antibodies, including mAb 2G8, mAb 51, and mAb 22A12 [5,37]. Recombinant VP1 vaccines have demonstrated cross-genotype neutralisation responses, supporting its use as a universal vaccine antigen [37]. Fig. 2 illustrates the key domains and receptor-binding motifs of VP1.

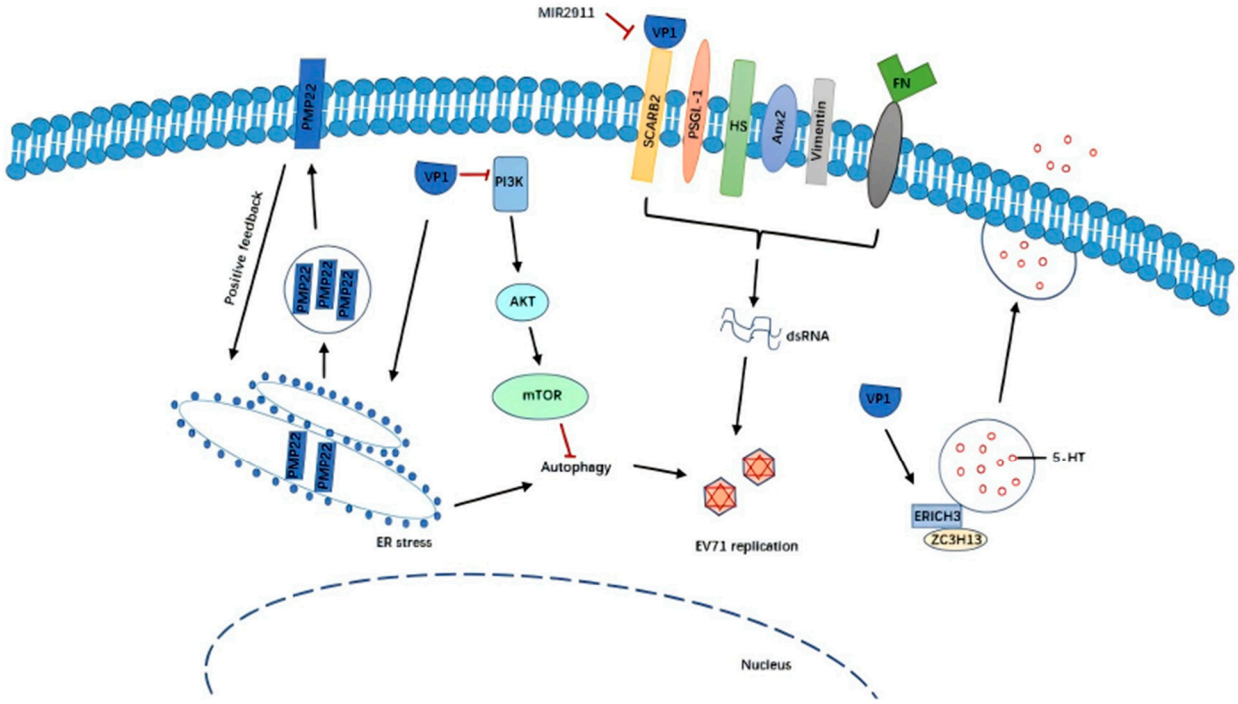

Figure 2: Molecular functions and host interactions of viral protein 1 (VP1) during EV71 infection. VP1 engages multiple host receptors—including SCARB2, PSGL-1, heparan sulfate (HS), annexin A2 (Anx2), vimentin, and fibronectin (FN)—to mediate viral attachment, entry, and uncoating. Binding of cell-surface receptors triggers the expulsion of VP4 and the exposure of the VP1 N-terminus, facilitating membrane penetration and genome release. VP1 also induces endoplasmic reticulum (ER) stress by upregulating peripheral myelin protein 22 (PMP22), thereby activating PI3K/Akt/mTOR-dependent autophagy and promoting viral replication. Additionally, VP1 modulates serotonergic signaling by regulating ERICH3–ZC3H13 interactions, contributing to neurovirulence. MIR2911, a plant-derived miRNA, directly targets VP1 and inhibits viral replication. Abbreviation: VP1, viral protein 1; SCARB2, scavenger receptor class B member 2; PSGL-1, P-selectin glycoprotein ligand-1; HS, heparan sulfate; Anx2, annexin A2; FN, fibronectin; PM P22, peripheral myelin protein 22; PI3K, phosphoinositide 3-kinase; AKT, protein kinase B; mTOR, mechanistic target of rapamycin; ER, endoplasmic reticulum; dsRNA, double-stranded RNA; 5-HT, 5-hydroxytryptamine (serotonin); ERICH3, glutamate-rich protein 3; ZC3H13, zinc finger CCCH-type containing 13; MIR2911, microRNA-2911.

3.1.2 Neurovirulence

VP1 is a major determinant of neurotropism, acting through endoplasmic reticulum (ER) stress, autophagy regulation, and mitochondrial dysfunction (Fig. 2). The VP1/ER stress/PMP22 axis modulates autophagy in Schwann cells, promoting viral replication and neuronal damage [38]. Downregulation of mTOR signaling further supports autophagic flux, facilitating viral spread and increased disease severity [39].

Mammalian target protein (mTOR) is an important signalling molecule in the phosphatidylinositol 3-kinase (PI3K)/serine/threonine kinase (Akt)/mTOR autophagy pathway, and the inhibition of mTOR activity can lead to the initiation of autophagy and the formation of autophagosomes [40]. The EV71 VP1 protein regulates viral replication in nerve cells via the mTOR autophagy signalling pathway. Experiments

have shown that autophagy promotes the release and spread of the virus, thereby increasing disease severity and the viral titer [39].

Recent works reveal that VP1 induces mitochondrial fragmentation and reactive oxygen species (ROS) accumulation, impairing axonal integrity [41]. In infected neurons, VP1 sustains autophagic flux through the PI3K/Akt/mTOR pathway, thereby suppressing apoptosis and enabling prolonged viral persistence [42]. VP1 also upregulates serotonin-related regulators ERICH3 and ZC3H13, disrupting neurotransmission via the VP1–ERICH3–ZC3H13 axis, which has been linked to brainstem encephalitis in mouse models [43,44]. Collectively, these findings implicate VP1 as a multifunctional virulence factor with roles beyond its structural role.

3.1.3 Virus Neutralisation

Due to its surface exposure, VP1 is the primary target of neutralising antibodies, especially within the GH loop. Mutations at VP1-145 modulate both receptor usage and antibody sensitivity, enabling immune escape [29,45,46]. VP2 and VP3 also contribute to neutralising epitopes, owing to shared β-sandwich "jelly-roll" folds [47]. Conserved VP2 regions (141–150 amino acids) remain stable after formalin inactivation—making them highly suitable for vaccine potency assays [48]. Synergistic mutations at VP1-145 and VP2-149 increase cell apoptosis and enhance viral fitness, suggesting coordinated modulation of receptor binding and replication [49].

VP3 harbors a conserved "knob" region, targeted by diagnostic mAbs such as 10D3 and 5H7 [50]. MicroRNAs (e.g., miR-18a and miR-452) further regulate EV71 replication by targeting VP3 transcripts, reinforcing VP3's role beyond capsid support [51,52].

VP4 (69 aa) is internal and is expelled during uncoating, inserting into host membranes to form a transient pore for genome release [53]. Its N-terminal VP4N20 peptide fused to HBcAg successfully produced virus-like particles (VLPs) that neutralized viruses across genotypes, making VP4 a promising next-generation vaccine platform [54].

VP1–VP4 function as a coordinated molecular system that drives viral attachment, entry, genome delivery, immunogenicity, and capsid dynamics. VP1 is the best-characterized component; however, mechanistic insights into VP2–VP4 remain comparatively limited. The cooperative roles of these proteins during uncoating, endosomal escape, and capsid destabilization remain incompletely resolved, particularly in physiologically relevant *in vivo* models.

Future studies should prioritize high-resolution structural imaging, protein–protein interaction mapping, and host interaction profiling to define the temporal sequence of events during early infection. Such work will be essential for rational vaccine engineering and for identifying structural determinants amenable to capsid-targeting antiviral strategies. Importantly, most available evidence regarding VP1–VP4 function derives from *in vitro* systems, with limited validation in neuronal models or animal studies. Establishing the physiological relevance of these mechanisms will require *in vivo* evaluation using organoids, transgenic mouse models, or humanized infection systems.

4 Functions of EV71's Nonstructural Proteins

EV71 nonstructural proteins (2A–3D) are translated as part of a single polyprotein and subsequently processed into enzymatic and regulatory components that coordinate viral RNA replication, polyprotein processing, intracellular membrane remodeling, and evasion of host innate and adaptive immunity [28,55]. In addition to their canonical roles in replication, accumulating evidence indicates that these proteins extensively rewire host signaling pathways, stress responses, and cell death programs, thereby shaping tissue tropism

and disease severity. To synthesize the multifaceted roles of EV71 nonstructural proteins, we provide a structured overview (Table 2) highlighting their functions, host interactions, system-level relevance, and current therapeutic opportunities. The integration of replication machinery and innate immune evasion suggests an emergent model of coordinated viral control over cellular decision-making pathways.

Table 2: Summary of EV71 nonstructural proteins (2A–3D): key mechanisms, host interactions, and therapeutic relevance [28].

Protein	Core Function	Host Interaction/Immune Modulation	Systems-Level Role	Therapeutic Potential
2A	Protease & polyprotein cleavage	Cleaves MAVS, MDA5, NLRP3, IFNAR1; blocks IFN signaling	Coordinates viral replication & innate immune suppression	Targeted by CW-33 + IFN; high-value drug target
2B	Ion channel formation	Binds Bax & VDAC3 → apoptosis & ROS	Remodels mitochondria for viral replication	DIDS inhibits 2B-mediated ion flux
2C	ATPase/helicase	Recruits RTN3 & COPI; inhibits NF-κB via RelA & IKKβ	Assembles replication organelles	Conserved drug target across enteroviruses
3A	Membrane remodeling	ACBD3–PI4KB complex; modulates ATP1B3/IFN-I	Builds replication sites/vesicle trafficking	Itraconazole inhibits 3A
3B	RNA replication primer	Anchors to 3D for VPg uridylation	Initiates RNA synthesis	Disrupting VPg uridylation blocks infection
3C	Protease & RNA binding	Cleaves TRIF, TAB2, GSDMD, PinX1	Central regulator of cell death & IFN evasion	Inhibitors: DC07090, luteoloside
3D	RNA synthesis	Activates NLRP3; inhibits IFN-γ/STAT1	Controls replication & pathogenic inflammation	Antivirals: baicalin, aurintricarboxylic acid

Abb: ATPase, adenosine triphosphatase; COPI, coat protein complex I; IFN, interferon; IFN-I, type I interferon; MAVS, mitochondrial antiviral-signaling protein; MDA5, melanoma differentiation–associated protein 5; NF-κB, nuclear factor kappa B; NLRP3, NOD-, LRR-, and pyrin domain–containing protein 3; ROS, reactive oxygen species; RNA, ribonucleic acid; STAT1, signal transducer and activator of transcription 1; VPg, viral protein genome-linked.

4.1 Protein 2A

Protein 2A is a cysteine protease of approximately 150 amino acids composed of an N-terminal four-stranded β-sheet domain and a C-terminal six-stranded β-barrel domain that coordinates a tightly bound zinc ion [56]. According to the crystal structure, 2A adopts a chymotrypsin-like fold, and the catalytic triad (H21, D39, C110) is positioned within the cleft between the N- and C-terminal domains. The cI–eI2 loop at the N terminus forms a highly conserved, surface-exposed conformation that increases hydrophilicity, whereas two C-terminal motifs, 144DEE146 and LLWL, are critical for EV71 replication [57]. Structural analysis indicates that 144DEE146 forms an acidic surface patch, with E145 and E146 more solvent-exposed than D144. Functional studies showed that a D144A substitution does not impair viral replication, whereas E145A markedly reduces replication, and the D144A/E145A double mutant is lethal despite preserving correct folding and protease activity [57,58]. Deletion of residues 146–149 (EAME) similarly abrogates viral transcriptional activity without disrupting protease function, and removal of the adjacent LLWL motif abolishes polyprotein processing and replication, indicating that this hydrophobic motif is essential for positioning the acidic motif and maintaining an active protease conformation [57]. Notably, 2A also exhibits transcriptional activity in yeast, contributing to replication and virulence independently of its protease function, as demonstrated in 2A-substituted chimeric strains [57,59].

As a protease, 2A catalyses cleavage of the capsid precursor and 3CD polyprotein, thereby generating 3C and 3D and initiating the maturation of viral proteins. Beyond viral substrates, 2A cleaves host far upstream element-binding protein 1 (FBP1) at Gly-371 to produce FBP1_1–371, which synergizes with full-length FBP1 to enhance internal ribosome entry site (IRES)–driven translation from the 5′UTR of the viral genome [60–62]. ERK1/2-dependent signaling positively regulates IRES activity, and 2A mediates both cis-cleavage of viral polyproteins and trans-cleavage of the translation initiation factor eIF4G, thereby shutting off cap-dependent host translation while favoring cap-independent viral mRNA translation [63,64]. By cleaving eIF4GI, 2A disrupts the formation of canonical stress granules and promotes atypical stress particle assembly, further biasing translation toward viral RNAs and contributing to virus-induced apoptosis [63–65]. 2A also interferes with RNA processing bodies (P-bodies) by preventing their assembly and recruiting P-body components to viral RNA, generating a microenvironment that supports efficient viral RNA synthesis [66].

2A is a central antagonist of innate antiviral pathways. It inhibits IRF3-dependent signaling by cleaving MAVS and the RIG-I-like receptor MDA5, thereby blocking type I IFN induction (Fig. 3) [67–69]. In addition, 2A reduces surface IFNAR1 expression and dampens JAK1, Tyk2, and STAT phosphorylation, attenuating IFN-stimulated gene induction and enabling escape from IFN-α/β responses [67–69]. 2A also targets the DEAD-box helicase DDX6, reducing the DDX6–viral RNA complex and impairing its positive regulatory role in RIG-I–mediated IFN-I signaling, thereby further favouring replication [70].

At the level of cellular immunity, 2A downregulates NKG2D ligands and upregulates PD-L1 on intestinal epithelial cells, reducing recognition and killing by innate lymphoid cells and $CD3^+$ intraepithelial lymphocytes [71]. Furthermore, 2A suppresses NLRP3 inflammasome activity by cleaving NLRP3 at the Q225–G226 and G493–L494 junctions, thereby preventing IL-1β maturation and permitting enhanced viral replication [72,73]. Taken together, these findings position 2A as a multifunctional enzyme that links polyprotein processing with broad suppression of interferon, inflammasome, and RNA quality-control pathways.

4.2 Protein 2B

Protein 2B is a small, ~100-residue ion channel–like protein that integrates into intracellular membranes and perturbs ion homeostasis. It contains two predicted transmembrane domains (TM1 and TM2), and 2B-mediated chloride-dependent currents elevate cytosolic Ca^{2+} by disturbing anion balance in the Golgi complex, thereby promoting EV71 replication; these currents can be inhibited by the anion exchange blocker DIDS [28]. The C-terminal segment (amino acids 63–80) mediates mitochondrial targeting during infection. Within this compartment, protein 2B interacts with the mitochondrial voltage-dependent anion channel 3 (VDAC3), thereby elevating mitochondrial ROS production and perturbing cellular redox balance—conditions conducive to efficient viral replication [74]. The N-terminal hydrophilic segment (the first 14 residues) interacts with the proapoptotic protein Bax, inducing its conformational activation and redistribution, and triggering mitochondrial apoptosis (Fig. 4).

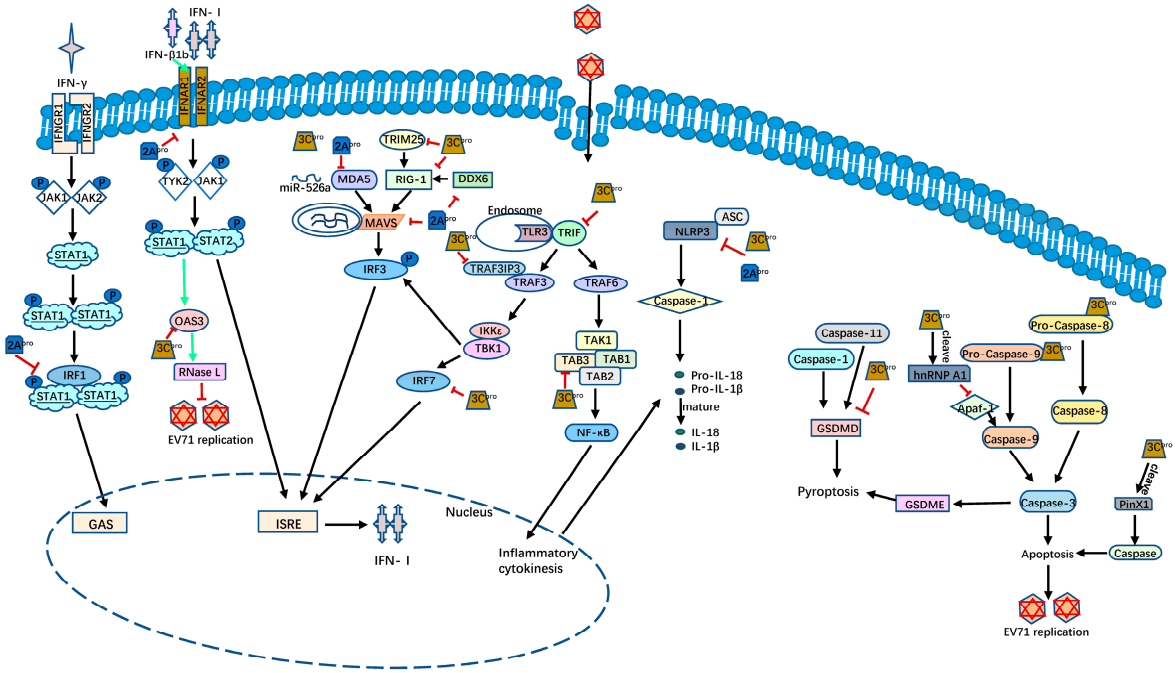

Figure 3: Mechanisms of EV71 nonstructural proteins in host immune suppression and cell death regulation. EV71 nonstructural proteins 2A and 3C employ multiple mechanisms to evade host immunity, modulate inflammatory signalling, and regulate apoptosis and pyroptosis. Both proteases inhibit type I and type II interferon responses by suppressing JAK/STAT signalling, cleaving MAVS, MDA5, TRIF, and IRF7, and disrupting the formation of antiviral transcriptional complexes. They further inhibit NF-κB activation by blocking IKK phosphorylation and cleaving key scaffold proteins (TAK1, TAB1, TAB2, TAB3). Moreover, 2A and 3C regulate programmed cell death via targeted cleavage of caspase-8, caspase-9, and gasdermin-D, thereby modulating apoptosis and pyroptosis to enhance viral replication and dissemination. Collectively, these interactions establish EV71 proteases as central determinants of virulence and immune evasion. Abbreviation: IFN-γ, interferon-gamma; IFN-I/IFN-β, type I interferon/interferon-beta; IFNAR1, interferon-alpha/beta receptor subunit 1; JAK1/JAK2, Janus kinase 1/2; TYK2, tyrosine kinase 2; STAT1/STAT2/STAT3, signal transducer and activator of transcription 1/2/3; IRF1/IRF3/IRF7, interferon regulatory factors 1/3/7; OAS3, 2′–5′ oligoadenylate synthetase 3; RNase L, ribonuclease L; GAS, gamma-activated sequence; ISRE, interferon-stimulated response element; RIG-I, retinoic acid-inducible gene I; MDA5, melanoma differentiation–associated protein 5; MAVS, mitochondrial antiviral signaling protein; TLR3, Toll-like receptor 3; TRIF, TIR-domain-containing adaptor inducing interferon-β; TRAF3/TRAF6, TNF receptor-associated factors 3/6; TRIM25, tripartite motif-containing protein 25; DDX6, DEAD-box helicase 6; TAK1, transforming growth factor-beta–activated kinase 1; TAB1/TAB2/TAB3, TAK1-binding proteins; IKKα/IKKβ, IκB kinases α/β; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; TBK1, TANK-binding kinase 1; ASC, apoptosis-associated speck-like protein containing a CARD; NLRP3, NOD-like receptor family pyrin domain containing 3; GSDMD/GSDME, gasdermin D/E; hnRNP A1, heterogeneous nuclear ribonucleoprotein A1; Apaf-1, apoptotic peptidase activating factor 1; PinX1, PIN2/TERF1-interacting telomeric protein 1; Caspase-3/-8/-9/-11, cysteine-aspartic proteases involved in apoptosis and pyroptosis.

2B also modulates host antiviral factors and transcriptional regulators. It binds interleukin enhancer-binding factor 2 (ILF2), impairs its nuclear translocation, and reduces ILF2 mRNA and protein levels, thereby antagonizing ILF2-mediated antiviral effects [75]. The ER-resident acetyltransferase NAT8 interacts with 2B, as well as 3AB and 3C, stabilizing these viral proteins in an acetyltransferase-dependent manner and

enhancing replication [76]. Collectively, 2B integrates ion dysregulation, mitochondrial stress, apoptosis, and antagonism of antiviral host factors to create a cellular environment conducive to EV71 replication.

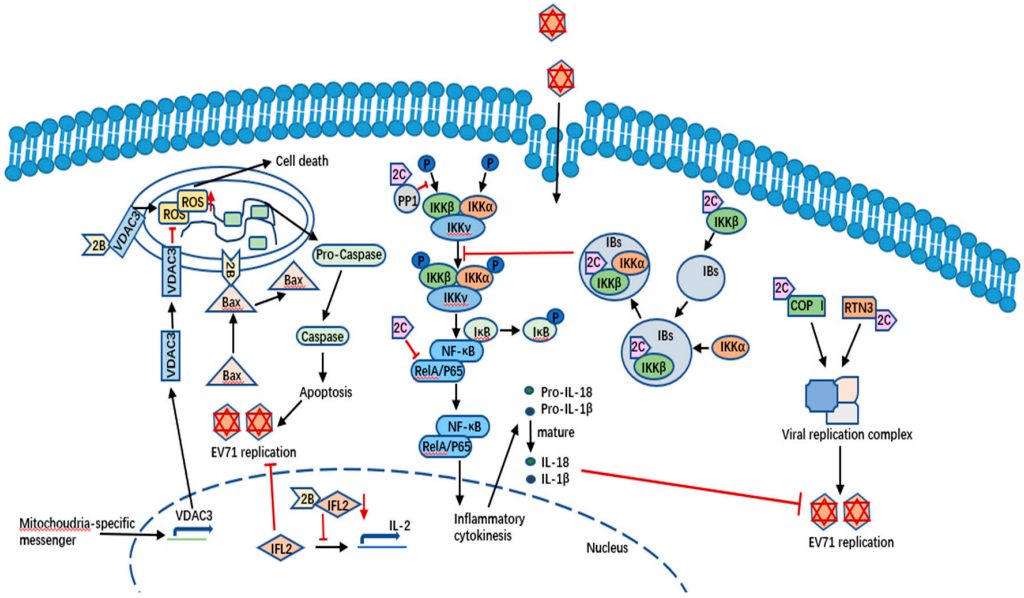

Figure 4: Functional roles of EV71 nonstructural proteins 2b and 2c in apoptosis, nf-κb suppression, and viral replication. EV71 proteins 2B and 2C orchestrate multiple host-modulating mechanisms to promote viral replication and disrupt immune defence. Protein 2B localises to mitochondria and directly interacts with VDAC3 to elevate reactive oxygen species (ROS), triggering Bax-mediated mitochondrial apoptosis and enhancing virus propagation. Additionally, 2B inhibits the transcription factor ILF2, thereby downregulating IL-2 expression and facilitating immune evasion. Protein 2C interferes with NF-κB signalling by binding to RelA/p65 and recruiting protein phosphatase 1 (PP1), which inhibits phosphorylation of IKKα/IKKβ and leads to cytosolic sequestration within inclusion bodies (IBs). Furthermore, 2C interacts with COPI and RTN3 to assemble the viral replication complex on intracellular membranes, enabling efficient genome replication and viral egress. Collectively, these mechanisms highlight 2B and 2C as central regulators of host cell death, inflammatory signalling, and replication complex formation during EV71 infection. Abbreviation: ROS, reactive oxygen species; VDAC3, voltage-dependent anion channel 3; Bax, Bcl-2-associated X protein; Caspase, cysteine-aspartic protease; ILF2, interleukin enhancer-binding factor 2; IL-2, interleukin-2; PP1, protein phosphatase 1; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; RelA/p65, NF-κB subunit p65; IKKα/IKKβ, IκB kinase α/β; IBs, inclusion bodies; COPI, coat protein complex I; RTN3, reticulon-3.

4.3 Protein 2C

2C, comprising 329 amino acid residues, is one of the most conserved nonstructural proteins of Protein 2C (329 aa) is one of the most conserved EV71 nonstructural proteins and is essential for RNA replication. It comprises an NTPase/RNA helicase domain, a zinc-binding motif, and a C-terminal helical region that supports self-oligomerisation [77,78]. 2C functions as an ATP-dependent RNA helicase and ATP-independent RNA chaperone, mediating RNA unwinding and remodeling of viral RNA structures required for replication; these two RNA-remodeling activities are mechanistically distinct yet functionally interdependent within the same viral RNA substrate [77,78]. The C-terminal oligomerisation domain is critical for both ATPase activity and formation of the replication complex.

2C is a key organizer of replication organelles. It binds the ER-associated protein reticulon-3 (RTN3) and coatomer protein complex I (COPI), recruiting these host factors to intracellular membranes to assemble viral

replication complexes (Fig. 4) [79,80]. Interference with COPI function markedly reduces EV71 replication, underscoring the importance of 2C-dependent membrane remodeling [80].

In innate immunity, 2C suppresses NF-κB signaling by targeting components of the IKK complex. 2C interacts with the IPT domain of RelA(p65), recruits protein phosphatase 1 (PP1) together with IKKβ, and inhibits IKKβ phosphorylation, thereby preventing TNF-α–induced NF-κB activation [81–83]. 2C also forms inclusion bodies that sequester IKKβ and IKKα, further limiting their phosphorylation and NF-κB–dependent transcription [81–83]. Moreover, 2C promotes degradation of the cytidine deaminase APOBEC3G (A3G) via the autophagy–lysosome pathway, counteracting A3G-mediated suppression of 5′UTR activity and enabling sustained viral replication [84]. These data identify 2C as a multifunctional hub linking replication complex formation to antagonism of NF-κB signaling and intrinsic antiviral restriction factors.

4.4 Protein 3A

Protein 3A (86 aa) is a membrane-associated protein that coordinates intracellular trafficking and the formation of replication organelles. EV71 infection increases the expression of Na^+/K^+-transporting ATPase subunit β3 (ATP1β3), which exerts antiviral activity by promoting type I IFN production; 3A interacts with ATP1β3, potentially modulating this antiviral response, although the mechanistic details remain to be clarified [84]. 3A also antagonizes innate immune sensors by targeting Ras-GTPase-activating protein SH3 domain-binding protein 1 (G3BP1). Through upregulation of the autophagy-related protein LRRC25, 3A promotes G3BP1 degradation and suppresses RIG-I-like helicase (RLH) signaling, thereby dampening IFN-β induction and enhancing EV71 replication (Fig. 5) [85].

A central function of 3A is to remodel Golgi-derived membranes for viral RNA synthesis. 3A interacts with the Golgi resident protein acyl-CoA–binding domain containing 3 (ACBD3), which is required to recruit phosphatidylinositol 4-kinase IIIβ (PI4KB) to sites of genomic replication [86,87]. The 3A–ACBD3–PI4KB axis generates PI4P-enriched membranes that serve as scaffolds for the replication complex; mutations at residues I44 or H54 in 3A disrupt these interactions and strongly inhibit RNA replication [86,87]. More recently, 3A has been shown to bind vacuolar protein sorting 25 (VPS25) on exosomes, enhancing exosome biogenesis and promoting the release of EV71-containing vesicles (Fig. 5) [88]. This exosome-mediated dissemination pathway may facilitate immune evasion and long-range viral spread.

4.5 Protein 3B

Protein 3B, also known as viral protein genome-linked (VPg), is a 22-residue nucleic acid chaperone covalently linked to the 5′ end of the viral genome. VPg binds 3Dl and anchors into a site near residue 311 at the base of the polymerase, adopting a V-shaped extended conformation that positions its hydroxylated Tyr3 in the active site [28,89,90]. Uridylation of VPg by 3D generates VPg-pUpU, which primes RNA synthesis; mutations that impair VPg uridylation severely compromise viral replication [90]. A bimolecular model proposes that one 3D position VPg, while a second 3D catalyses uridylation, underscoring the complexity of this priming reaction [91]. Although 3B is small, its essential role in replication initiation and its high conservation make it an attractive candidate for antiviral targeting.

4.6 Protein 3C

Protein 3C (3C, 183 aa) is a chymotrypsin-like cysteine protease that is indispensable for polyprotein processing and also functions as an RNA-binding protein and potent immune antagonist. Structural studies revealed that 3C comprises two β-barrel domains forming a catalytic cleft, and a flexible β-loop containing

hinge residues Gly123 and His133 that regulate conformational dynamics and hydrolytic activity [92]. 3C cleaves multiple sites within the viral precursor polyprotein, generating both structural and nonstructural proteins required for virion maturation.

3C harbors two principal RNA-binding motifs, KFRDI (residues 82–86) and VGK (154–156), that mediate interaction with viral RNA; mutations in these motifs impair both RNA binding and protease activity, whereas catalytic-site mutations do not abolish RNA binding [93]. A single amino acid substitution at position 69 significantly reduces replication and virulence in recombinant viruses, highlighting this residue as a virulence determinant [94,95].

Beyond its proteolytic role, 3C extensively remodels innate immune pathways. It cleaves the cytosolic sensor RIG-I and reduces expression of the E3 ligase TRIM25, thereby blocking RIG-I ubiquitination and activation and suppressing type I IFN induction. 3C also cleaves IRF7 and modulates miR-526a, further dampening IFN-I signaling. By targeting the TAK1–TAB1/TAB2/TAB3 complex, 3C prevents NF-κB activation, and by cleaving TRIF, it disrupts TLR3-mediated antiviral signaling (Fig. 3) [96]. 3C additionally cleaves the antiviral effector OAS3 at Q982–G983 and the NLRP3 inflammasome component at Q225–G226, weakening OAS3-dependent RNA degradation and inhibiting inflammasome activation [97,98]. TRAF3-interacting protein 3 (TRAF3IP3) and promyelocytic leukemia protein IV (PMLIV), both antiviral factors, are similarly targeted and cleaved by 3C, leading to disruption of PML nuclear bodies and diminished antiviral autophagy [99–101]. NAT8-mediated stabilization of 3C further amplifies these immune evasion effects [76].

3C also orchestrates multiple forms of programmed cell death. It cleaves telomere-binding protein PinX1 at Q51–G52, promotes caspase-8 and caspase-9 activation, and indirectly induces caspase-3–dependent apoptosis (Fig. 3) [102–104]. By cleaving hnRNP A1, 3C relieves repression of apaf-1 IRES translation, further enhancing caspase-3 activation and apoptotic cell death [103,104]. In pyroptosis, 3C directly cleaves gasdermin D (GSDMD) at Q193–G194, inactivating its pore-forming N-terminal fragment and suppressing pyroptotic cell death, while 3C-driven caspase-3 activation contributes indirectly to gasdermin E (GSDME) cleavage [71,105]. These combined actions ensure efficient viral release while limiting inflammatory cell death pathways that could restrict viral spread.

4.7 Protein 3D

3D polymerase (3D, 462 aa) is the viral RNA-dependent RNA polymerase responsible for synthesizing negative- and positive-strand RNA during replication and for catalyzing VPg uridylation. Post-translational modification by SUMO-1 stabilizes 3D, and SUMO-1–dependent ubiquitination further enhances polymerase abundance and activity, leading to increased EV71 replication [106]. 3D also interacts with the methyltransferase METTL3, promoting N^6-methyladenosine (m6A) modification of EV71 RNA and facilitating viral RNA translation and stability [106]. Similarly, N4-acetylcytidine (ac4C) modification of viral RNA augments translation and RNA–3D binding, with ac4C-deficient mutants showing reduced pathogenicity in mice [107,108].

3D can manipulate cell cycle progression and innate immunity. It induces S-phase arrest, creating a cellular state favorable to viral replication [109,110]. 3D directly binds NLRP3 to promote assembly of the 3D–NLRP3–ASC inflammasome complex and IL-1β release (Fig. 5), yet it concurrently suppresses antiviral signaling by inhibiting IFN-γ–STAT1 signaling and interacting with the CARD domain of MDA5 to block IFN-β promoter activation [68,111]. 3D also engages the autophagy machinery via Beclin1, whose ECD and CCD domains bind 3D to support the formation of autophagic structures that are exploited as replication sites [112]. Furthermore, 3D interacts with acyl-CoA oxidase 1 (ACOX1), reduces peroxisome biogenesis,

and enhances ROS production by inhibiting the DJ-1/Nrf2/HO-1 antioxidant pathway, thereby inducing autophagy and apoptosis in neural cells and contributing to neuropathogenesis [112,113].

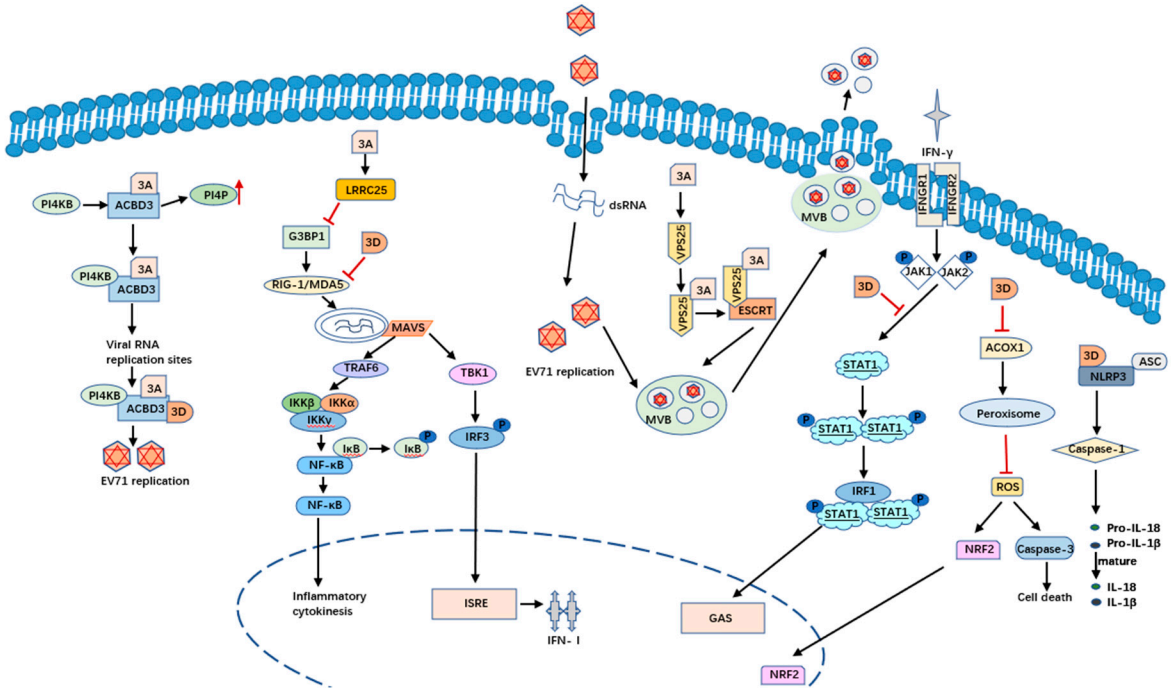

Figure 5: Multifunctional roles of EV71 proteins 3A and 3D in viral replication, innate immune evasion, and host cell fate regulation. EV71 nonstructural proteins 3A and 3D coordinate distinct yet complementary mechanisms to enhance viral replication and suppress host antiviral responses. Protein 3A disrupts innate immune signalling by interacting with ATP1B3 to suppress IFN-I production, and by promoting degradation of G3BP1 via LRRC25 to block RIG-I/MDA5-mediated RLH signalling. It further recruits ACBD3 to engage PI4KB at viral replication sites, facilitating membrane remodeling and genome synthesis. Additionally, 3A binds VPS25 on exosomes, enabling exosome-mediated viral dissemination. Protein 3D functions as an RNA-dependent RNA polymerase and modulates host cell fate by promoting NLRP3 inflammasome activation, inducing S-phase arrest, driving autophagy via Beclin1, and impairing peroxisomal function through ACOX1-mediated oxidative stress. Furthermore, 3D suppresses IFN-γ signaling and MDA5-mediated IFN-β production, serving as a dual regulator of viral immune evasion and host cell survival. Together, 3A and 3D represent key molecular determinants of EV71 pathogenesis and therapeutic intervention targets. **Abbreviation:** IFN-I, type I interferon; IFN-γ, interferon-gamma; ATP1B3, sodium/potassium-transporting ATPase subunit beta-3; G3BP1, Ras-GTPase–activating protein SH3 domain–binding protein 1; LRRC25, leucine-rich repeat-containing protein 25; RIG-I, retinoic acid-inducible gene I; MDA5, melanoma differentiation-associated protein 5; RLH, RIG-I-like helicase pathway; ACBD3, acyl-CoA-binding domain–containing protein 3; PI4KB, phosphatidylinositol-4-kinase IIIβ; VPS25, vacuolar protein sorting–associated protein 25; 3Dpol, EV71 RNA-dependent RNA polymerase; NLRP3, NOD-like receptor family pyrin domain containing 3; ASC, apoptosis-associated speck-like protein containing a CARD; Beclin1, autophagy regulator; ACOX1, acyl-CoA oxidase 1.

A3G, discussed above, can associate with 3D and viral RNA and be packaged into progeny virions, thereby restricting infectivity; however, EV71 counteracts this through 2C- and 3D-mediated modulation of A3G stability and function [114,115]. Overall, 3D integrates RNA synthesis with extensive post-transcriptional RNA modification and modulation of cell stress pathways, positioning it as a prime target for broad-spectrum antiviral intervention.

Nonstructural proteins 2A–3D serve as the functional core of the EV71 replication machinery and orchestrate immune evasion, intracellular remodeling, and regulated cell death. However, most mechanistic insights originate from *in vitro* overexpression systems or limited animal models, and the temporal hierarchy of these proteins during natural human infection remains unresolved. Deciphering how these proteins interact as a coordinated network—rather than as isolated effectors—will be crucial to understanding EV71 pathogenesis.

Future research should prioritize *in vivo* validation of protein–host interactions, temporal mapping of immune evasion events, and evaluation of 2A, 3C, and 3D as conserved enzymatic targets for small-molecule inhibition. The integration of stem cell–derived neural organoids, CRISPR-based functional screening, and multi-omics profiling may provide a physiologically relevant platform to dissect viral strategies and identify actionable host dependencies. Ultimately, systematic dissection of the nonstructural protein network—through structure-guided drug design and advanced infection models—will be essential to convert current mechanistic insights into clinically viable antiviral strategies.

5 Conclusions and Future Perspectives

EV71 remains a persistent threat to child health due to its neurotropic potential, genetic diversity, and absence of broadly protective antiviral or vaccine strategies. The coordinated actions of its structural proteins (VP1–VP4) and nonstructural proteins (2A–3D) extend beyond viral assembly and replication, orchestrating receptor binding, immune evasion, intracellular remodeling, and host cell death. This expanding mechanistic landscape suggests that EV71 operates as a systems-level pathogen rather than through isolated linear pathways.

Key host factors—including ACBD3, RTN3, VPS25, NAT8, METTL3, and SPOP—have emerged as central nodes in virus–host interplay, indicating that targeting viral–host interfaces may offer broader therapeutic potential than virus-directed approaches alone. However, much of the current evidence stems from *in vitro* studies, and mechanistic validation in physiologically relevant models—such as human organoids, hSCARB2 knock-in mice, and primary neuronal cultures—remains limited.

To accelerate translational progress, several priority questions warrant systematic investigation. A central objective is to determine whether cross-genotype vaccines can be rationally engineered by exploring conserved structural motifs or epitope-scaffolding strategies to achieve broad and durable protection. Equally important is the identification of viral proteins and post-translational modifications that constitute the most tractable druggable nodes within the EV71 interactome, thereby enabling the development of targeted antiviral interventions. Advances in computational biology further invite exploration into whether artificial intelligence–assisted protein design platforms, including RFdiffusion and AlphaFold2-multimer, can generate synthetic antibodies or receptor mimetics with broad-spectrum neutralizing capacity. In parallel, the therapeutic feasibility of host-directed strategies—such as inhibition of METTL3 or VPS34—should be rigorously evaluated to determine whether viral replication can be suppressed without perturbing essential cellular homeostasis. Finally, a deeper mechanistic understanding is needed to clarify how EV71 remodels neuroimmune crosstalk during the earliest stages of infection, particularly within brainstem neurons, microglia, and gut-resident immune compartments.

A unified model of EV71 pathogenesis will require combining structural virology with systems immunology and translational biology. By bridging these disciplines, the field can advance toward broad-spectrum antivirals and next-generation vaccine platforms capable of mitigating the global burden of EV71 and related enteroviruses.

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References

1. Nayak G, Bhuyan SK, Bhuyan R, Sahu A, Kar D, Kuanar A. Global emergence of Enterovirus 71: a systematic review. Beni Suef Univ J Basic Appl Sci. 2022;11(1):78. [CrossRef].
2. Ge A, Cui W, Qu S, Wang N, Zhang W, Wei L, et al. A meta-analysis of influencing factors for reinfection of hand, foot and mouth disease in China, based on adjusted effect estimates. Pathogens. 2026;15(1):50. [CrossRef].
3. Chen J, Zhao T, Ji M, Xia B. Antiviral candidates for enterovirus 71: targeting viral proteome and stage-specific lifecycle interventions. Virol J. 2025;22(1):355. [CrossRef].
4. Huang CY, Su SB, Chen KT. A review of enterovirus-associated hand-foot and mouth disease: preventive strategies and the need for a global enterovirus surveillance network. Pathog Glob Health. 2024;118(7–8):538–48. [CrossRef].
5. Zhang X, Sun R, Hou J, Jia W, Li P, Shi S, et al. Clinical characteristics of fatal cases of hand, foot and mouth disease in children. Front Pediatr. 2025;13:1522164. [CrossRef].
6. Fahim IH, Haque MA, Aslam S, Al Mazid Bhuiyan MR. Atypical hand, foot and mouth disease with secondary infection in a toddler: a case study. Med Rep. 2024;3:100029. [CrossRef].
7. Wei Y, Liu H, Hu D, He Q, Yao C, Li H, et al. Recent advances in enterovirus A71 infection and antiviral agents. Lab Investig. 2024;104(2):100298. [CrossRef].
8. Lai J, Li Z, Pan L, Huang Y, Zhou Z, Ma C, et al. Research progress on pathogenic and therapeutic mechanisms of Enterovirus A71. Arch Virol. 2023;168(10):260. [CrossRef].
9. Yi Z, Pei S, Suo W, Wang X, Huang Z, Yi A, et al. Epidemiological characteristics, routine laboratory diagnosis, clinical signs and risk factors for hand, -foot-and-mouth disease: a systematic review and meta-analysis. PLoS One. 2022;17(4):e0267716. [CrossRef].
10. Zhang W, Rui J, Cheng X, Deng B, Zhang H, Huang L, et al. Transmissibility of hand, foot, and mouth disease in 97 counties of China. Sci Rep. 2022;12(1):4103. [CrossRef].
11. Fan C, Yang Y, Zhan S, Sun X, Fu C. The effectiveness and impact of the enterovirus 71 vaccine on the onset of hand, foot, and mouth disease in children aged ≤5 years: a 7-year study. J Infect. 2024;89(4):106244. [CrossRef].
12. Liu G, Zhu D, Feng K, Peng H, Yang S, Huang L, et al. The neurological damage caused by enterovirus 71 infection is associated with hsa_circ_0069335/miR-29b/PMP22 pathway. J Virol. 2025;99(1):e00844–24. [CrossRef].
13. Zhao Z, Zheng C, Qi H, Chen Y, Ward MP, Liu F, et al. Impact of the coronavirus disease 2019 interventions on the incidence of hand, foot, and mouth disease in mainland China. Lancet Reg Health West Pac. 2022;20:100362. [CrossRef].
14. Huang YP, Lin TL, Kuo CY, Lin MW, Yao CY, Liao HW, et al. The circulation of subgenogroups B5 and C5 of enterovirus 71 in Taiwan from 2006 to 2007. Virus Res. 2008;137(2):206–12. [CrossRef].

15. Tee KK, Lam TT, Chan YF, Bible JM, Kamarulzaman A, William Tong CY, et al. Evolutionary genetics of human enterovirus 71: origin, population dynamics, natural selection, and seasonal periodicity of the VP1 gene. J Virol. 2010;84(7):3339–50. [CrossRef].
16. Liu Y, Maisimu M, Ge Z, Xiao S, Wang H. The pathogenesis and virulence of the major enterovirus pathogens associated with severe clinical manifestations: a comprehensive review. Cells. 2025;14(20):1617. [CrossRef].
17. Jartti M, Flodström-Tullberg M, Hankaniemi MM. Enteroviruses: epidemic potential, challenges and opportunities with vaccines. J Biomed Sci. 2024;31(1):73. [CrossRef].
18. Ma ZH, Nawal Bahoussi A, Tariq Shah P, Guo YY, Dong L, Wu C, et al. Phylogeographic dynamics and molecular characteristics of Enterovirus 71 in China. Front Microbiol. 2023;14:1182382. [CrossRef].
19. Zang L, Yang X, Chen Y, Huang F, Yuan Y, Chen X, et al. Ubiquitin E3 ligase SPOP is a host negative regulator of enterovirus 71-encoded 2A protease. J Virol. 2023;97(10):e00786–23. [CrossRef].
20. Zhu D, Liu G, Feng K, Li S, Hu D, Yang S, et al. Gp78 regulates PMP22 and causes ER stress and autophagy in EV71-VP1-overexpressing mouse Schwann cells. Biocell. 2024;48(4):653–64. [CrossRef].
21. Tian L, Mi Z, Yang W, Chen J, Wei X, Zhang W, et al. ZYG11B suppresses multiple enteroviruses by triggering viral VP1 degradation. J Virol. 2025;99(4):e00030–25. [CrossRef].
22. Wang Q, Wu X, Liu M, Li L, Wang Y, He Q, et al. Hijacking the host cell for replication: pro-viral host factors involved in EVA71 infection. Int J Mol Sci. 2025;26(16):7992. [CrossRef].
23. Wu J, Lu J, Pan M, Gu X, Wang Y, Dai L. Inhibition of neutral sphingomyelinase-2 restrains enterovirus 71 infection by autophagy. Microb Pathog. 2025;200:107326. [CrossRef].
24. Hu K, Onintsoa Diarimalala R, Yao C, Li H, Wei Y. EV-A71 mechanism of entry: receptors/co-receptors, related pathways and inhibitors. Viruses. 2023;15(3):785. [CrossRef].
25. Yuan M, Yan J, Xun J, Chen C, Zhang Y, Wang M, et al. Enhanced human enterovirus 71 infection by endocytosis inhibitors reveals multiple entry pathways by enterovirus causing hand-foot-and-mouth diseases. Virol J. 2018;15(1):1. [CrossRef].
26. Swain SK, Panda S, Sahu BP, Sarangi R. Activation of host cellular signaling and mechanism of enterovirus 71 viral proteins associated with hand, foot and mouth disease. Viruses. 2022;14(10):2190. [CrossRef].
27. Ang PY, Chong CWH, Alonso S. Viral determinants that drive Enterovirus-A71 fitness and virulence. Emerg Microbes Infect. 2021;10(1):713–24. [CrossRef].
28. Yuan J, Shen L, Wu J, Zou X, Gu J, Chen J, et al. Enterovirus A71 proteins: structure and function. Front Microbiol. 2018;9:286. [CrossRef].
29. Bifani AM, Tee HK, Antanasijevic A, Clément S, Tapparel C. Enterovirus A71 priorities, challenges, and future opportunities in humoral immunity and vaccine development. npj Vaccines. 2025;10(1):194. [CrossRef].
30. Zhou D, Zhao Y, Kotecha A, Fry EE, Kelly JT, Wang X, et al. Unexpected mode of engagement between enterovirus 71 and its receptor SCARB2. Nat Microbiol. 2019;4(3):414–9. [CrossRef].
31. Zaongo SD, Liu Y, Harypursat V, Song F, Xia H, Ma P, et al. P-selectin glycoprotein ligand 1: a potential HIV-1 therapeutic target. Front Immunol. 2021;12:710121. [CrossRef].
32. You L, Chen J, Cheng Y, Li Y, Chen YQ, Ying T, et al. Antibody signatures in hospitalized hand, foot and mouth disease patients with acute enterovirus A71 infection. PLoS Pathog. 2023;19(6):e1011420. [CrossRef].
33. Ke X, Zhang Y, Liu Y, Miao Y, Zheng C, Luo D, et al. A single mutation in the VP1 gene of enterovirus 71 enhances viral binding to heparan sulfate and impairs viral pathogenicity in mice. Viruses. 2020;12(8):883. [CrossRef].
34. Meng T, Wong SM, Chua KB. Sulfonated azo dyes enhance the genome release of enterovirus A71 VP1-98K variants by preventing the virions from being trapped by sulfated glycosaminoglycans at acidic pH. Virology. 2021;555:19–34. [CrossRef].
35. Tonello F, Massimino ML, Peggion C. Nucleolin: a cell portal for viruses, bacteria, and toxins. Cell Mol Life Sci. 2022;79(5):271. [CrossRef].
36. Yuan S, Li G, Wang Y, Gao Q, Wang Y, Cui R, et al. Identification of positively charged residues in enterovirus 71 capsid protein VP1 essential for production of infectious particles. J Virol. 2015;90(2):741–52. [CrossRef].
37. Tang J, Zhang X, Zhang J, Zhao Z, Ding Z. Environmental surveillance reveals co-circulation of distinctive lineages of enteroviruses in southwest China's border cities, 2020-2022. J Appl Microbiol. 2024;135(3):lxae060. [CrossRef].

38. Li P, Yang S, Hu D, Wei D, Lu J, Zheng H, et al. Enterovirus 71 VP1 promotes mouse Schwann cell autophagy via ER stress-mediated PMP22 upregulation. Int J Mol Med. 2019;44(2):759–67. [CrossRef].
39. Liu ZW, Zhuang ZC, Chen R, Wang XR, Zhang HL, Li SH, et al. Enterovirus 71 VP1 protein regulates viral replication in SH-SY5Y cells via the mTOR autophagy signaling pathway. Viruses. 2019;12(1):11. [CrossRef].
40. He Y, Sun MM, Zhang GG, Yang J, Chen KS, Xu WW, et al. Targeting PI3K/Akt signal transduction for cancer therapy. Signal Transduct Target Ther. 2021;6(1):425. [CrossRef].
41. Zhang W, Yang H, Liu Z, Wang S, Chen T, Song H, et al. Enterovirus 71 leads to abnormal mitochondrial dynamics in human neuroblastoma SK-N-SH cells. Virus Res. 2024;339:199267. [CrossRef].
42. Chen L, Yang L, Li Y, Liu T, Yang B, Liu L, et al. Autophagy and inflammation: regulatory roles in viral infections. Biomolecules. 2023;13(10):1454. [CrossRef].
43. Zhu D, Liu G, Song Y, Li S, Yang S, Hu D, et al. Enterovirus 71 VP1 promotes 5-HT release by upregulating the expression of $ERICH_3$ and methyltransferase ZC3H13. Virus Res. 2022;318:198843. [CrossRef].
44. Wu Q, He Q, Zhang X, Chen S, Xue X. Systemic modulators: potential mechanism for the 5-HT system to mediate exercise amelioration in Alzheimer's disease. Aging Dis. 2024;16(5):2770–802. [CrossRef].
45. Ku Z, Ye X, Shi J, Wang X, Liu Q, Huang Z. Single neutralizing monoclonal antibodies targeting the VP1 GH loop of enterovirus 71 inhibit both virus attachment and internalization during viral entry. J Virol. 2015;89(23):12084–95. [CrossRef].
46. Fujii K, Sudaka Y, Takashino A, Kobayashi K, Kataoka C, Suzuki T, et al. VP1 amino acid residue 145 of enterovirus 71 is a key residue for its receptor attachment and resistance to neutralizing antibody during Cynomolgus Monkey infection. J Virol. 2018;92(15):e00682–18. [CrossRef].
47. Wang L, Zhu M, Fang Y, Rong H, Gao L, Liao Q, et al. Bioinformatics-based prediction of conformational epitopes for enterovirus A71 and coxsackievirus A16. Sci Rep. 2021;11(1):5701. [CrossRef].
48. Liu CC, Chou AH, Lien SP, Lin HY, Liu SJ, Chang JY, et al. Identification and characterization of a cross-neutralization epitope of Enterovirus 71. Vaccine. 2011;29(26):4362–72. [CrossRef].
49. Huang SW, Wang YF, Yu CK, Su IJ, Wang JR. Mutations in VP2 and VP1 capsid proteins increase infectivity and mouse lethality of enterovirus 71 by virus binding and RNA accumulation enhancement. Virology. 2012;422(1):132–43. [CrossRef].
50. Anasir MI, Poh CL. Advances in antigenic peptide-based vaccine and neutralizing antibodies against viruses causing hand, foot, and mouth disease. Int J Mol Sci. 2019;20(6):1256. [CrossRef].
51. Yang Z, Zhuo Q, Qin W, Wang J, Wang L, Po T. microRNAs miR-18a and miR-452 regulate the replication of enterovirus 71 by targeting the gene encoding VP3. Virus Genes. 2021;57(4):318–26. [CrossRef].
52. Pei Z, Wang H, Zhao Z, Chen X, Huan C, Zhang W. Chemokine PF4 inhibits EV71 and CA16 infections at the entry stage. J Virol. 2022;96(11):e00435–22. [CrossRef].
53. Tan YW, Hong WJ, Chu JJH. Inhibition of enterovirus VP4 myristoylation is a potential antiviral strategy for hand, foot and mouth disease. Antivir Res. 2016;133:191–5. [CrossRef].
54. Phanthong S, Densumite J, Seesuay W, Thanongsaksrikul J, Teimoori S, Sookrung N, et al. Human antibodies to VP4 inhibit replication of enteroviruses across subgenotypes and serotypes, and enhance host innate immunity. Front Microbiol. 2020;11:562768. [CrossRef].
55. Andronik A, Lewandowski D, Sulik A, Toczylowski K. The host immune response to enterovirus A71 (EV-A71): from viral immune evasion to immunopathology and prognostic biomarkers of severe disease—a narrative review. Viruses. 2025;17(12):1540. [CrossRef].
56. Chen T, Grauffel C, Yang WZ, Chen YP, Yuan HS, Lim C. Efficient strategy to design protease inhibitors: application to enterovirus 71 2A protease. ACS Bio Med Chem Au. 2022;2(4):437–49. [CrossRef].
57. Cai Q, Yameen M, Liu W, Gao Z, Li Y, Peng X, et al. Conformational plasticity of the 2A proteinase from enterovirus 71. J Virol. 2013;87(13):7348–56. [CrossRef].
58. Liu Y, Lv P, Wang W, Zhang J, Zhou X, Qiu Y, et al. Structural insight into EV-A71 3A protein and its interaction with a peptide inhibitor. Virol Sin. 2023;38(6):975–9. [CrossRef].
59. Li C, Qiao Q, Hao SB, Dong Z, Zhao L, Ji J, et al. Nonstructural protein 2A modulates replication and virulence of enterovirus 71. Virus Res. 2018;244:262–9. [CrossRef].
60. Lai MC, Chen HH, Xu P, Wang RYL. Translation control of Enterovirus A71 gene expression. J Biomed Sci. 2020;27(1):22. [CrossRef].

61. Hung CT, Kung YA, Li ML, Brewer G, Lee KM, Liu ST, et al. Additive promotion of viral internal ribosome entry site-mediated translation by far upstream element-binding protein 1 and an enterovirus 71-induced cleavage product. PLoS Pathog. 2016;12(10):e1005959. [CrossRef].
62. Liu Q, Long JE. Insight into the life cycle of enterovirus-A71. Viruses. 2025;17(2):181. [CrossRef].
63. Duan H, Zhu M, Xiong Q, Wang Y, Xu C, Sun J, et al. Regulation of enterovirus 2A protease-associated viral IRES activities by the cell's ERK signaling cascade: implicating ERK as an efficiently antiviral target. Antiviral Res. 2017;143:13–21. [CrossRef].
64. Yang X, Hu Z, Fan S, Zhang Q, Zhong Y, Guo D, et al. Picornavirus 2A protease regulates stress granule formation to facilitate viral translation. PLoS Pathog. 2018;14(2):e1006901. [CrossRef].
65. Bai J, Chen X, Liu Q, Zhou X, Long JE. Characteristics of enterovirus 71-induced cell death and genome scanning to identify viral genes involved in virus-induced cell apoptosis. Virus Res. 2019;265:104–14. [CrossRef].
66. Fan S, Xu Z, Liu P, Qin Y, Chen M. Enterovirus 71 2A protease inhibits P-body formation to promote viral RNA synthesis. J Virol. 2021;95(19):e00922–21. [CrossRef].
67. Jian Z, Ma R, Zhu L, Deng H, Li F, Zhao J, et al. Evasion of interferon-mediated immune response by arteriviruses. Front Immunol. 2022;13:963923. [CrossRef].
68. Kuo RL, Chen CJ, Wang RYL, Huang HI, Lin YH, Tam EH, et al. Role of enteroviral RNA-dependent RNA polymerase in regulation of MDA5-mediated beta interferon activation. J Virol. 2019;93(10):e00132–19. [CrossRef].
69. Lu J, Yi L, Zhao J, Yu J, Chen Y, Lin MC, et al. Enterovirus 71 disrupts interferon signaling by reducing the level of interferon receptor 1. J Virol. 2012;86(7):3767–76. [CrossRef].
70. Taschuk F, Cherry S. DEAD-box helicases: sensors, regulators, and effectors for antiviral defense. Viruses. 2020;12(2):181. [CrossRef].
71. Dong S, Shi Y, Dong X, Xiao X, Qi J, Ren L, et al. Gasdermin E is required for induction of pyroptosis and severe disease during enterovirus 71 infection. J Biol Chem. 2022;298(5):101850. [CrossRef].
72. Wei J, Lv L, Wang T, Gu W, Luo Y, Feng H. Recent progress in innate immune responses to enterovirus A71 and viral evasion strategies. Int J Mol Sci. 2024;25(11):5688. [CrossRef].
73. Wang H, Lei X, Xiao X, Yang C, Lu W, Huang Z, et al. Reciprocal regulation between enterovirus 71 and the NLRP3 inflammasome. Cell Rep. 2015;12(1):42–8. [CrossRef].
74. Cheng ML, Wu CH, Chien KY, Lai CH, Li GJ, Liu YY, et al. Enteroviral 2B interacts with VDAC3 to regulate reactive oxygen species generation that is essential to viral replication. Viruses. 2022;14(8):1717. [CrossRef].
75. Jin J, Wang W, Ai S, Liu W, Song Y, Luo Z, et al. Enterovirus 71 represses interleukin enhancer-binding factor 2 production and nucleus translocation to antagonize ILF2 antiviral effects. Viruses. 2019;12(1):22. [CrossRef].
76. Zhao X, Yuan H, Yang H, Liu Y, Xun M, Li X, et al. N-acetyltransferase 8 promotes viral replication by increasing the stability of enterovirus 71 nonstructural proteins. J Virol. 2022;96(6):e00119–22. [CrossRef].
77. Xia H, Wang P, Wang GC, Yang J, Sun X, Wu W, et al. Human enterovirus nonstructural protein 2CATPase functions as both an RNA helicase and ATP-independent RNA chaperone. PLoS Pathog. 2015;11(7):e1005067. [CrossRef].
78. Guan H, Tian J, Qin B, Wojdyla JA, Wang B, Zhao Z, et al. Crystal structure of 2C helicase from enterovirus 71. Sci Adv. 2017;3(4):e1602573. [CrossRef].
79. Yin C, Zhao H, Xia X, Pan Z, Li D, Zhang L. Picornavirus 2C proteins: structure-function relationships and interactions with host factors. Front Cell Infect Microbiol. 2024;14:1347615. [CrossRef].
80. Wang J, Wu Z, Jin Q. COPI is required for enterovirus 71 replication. PLoS One. 2012;7(5):e38035. [CrossRef].
81. Ji L, Yang E, He S, Jin Y, Chen D. Enterovirus 2C protein suppresses IKKα phosphorylation by recruiting IKKβ and IKKα into viral inclusion bodies. Viral Immunol. 2021;34(4):218–26. [CrossRef].
82. Li Q, Zheng Z, Liu Y, Zhang Z, Liu Q, Meng J, et al. 2C proteins of enteroviruses suppress IKKβ phosphorylation by recruiting protein phosphatase 1. J Virol. 2016;90(10):5141–51. [CrossRef].
83. Du N, Cong H, Tian H, Zhang H, Zhang W, Song L, et al. Cell surface vimentin is an attachment receptor for enterovirus 71. J Virol. 2014;88(10):5816–33. [CrossRef].
84. Lu Y, Hou H, Wang F, Qiao L, Wang X, Yu J, et al. ATP1B3: a virus-induced host factor against EV71 replication by up-regulating the production of type-I interferons. Virology. 2016;496:28–34. [CrossRef].

85. Jayabalan AK, Griffin DE, Leung AKL. Pro-viral and anti-viral roles of the RNA-binding protein G3BP1. Viruses. 2023;15(2):449. [CrossRef].
86. Lei X, Xiao X, Zhang Z, Ma Y, Qi J, Wu C, et al. The Golgi protein ACBD3 facilitates Enterovirus 71 replication by interacting with 3A. Sci Rep. 2017;7:44592. [CrossRef].
87. Xiao X, Lei X, Zhang Z, Ma Y, Qi J, Wu C, et al. Enterovirus 3A facilitates viral replication by promoting phosphatidylinositol 4-kinase IIIβ-ACBD3 interaction. J Virol. 2017;91(19):e00791–17. [CrossRef].
88. Ruan Z, Liang Y, Chen Z, Yin J, Li C, Pan P, et al. Enterovirus 71 non-structural protein 3A hijacks vacuolar protein sorting 25 to boost exosome biogenesis to facilitate viral replication. Front Microbiol. 2022;13:1024899. [CrossRef].
89. Jackson T, Belsham GJ. Picornaviruses: a view from 3A. Viruses. 2021;13(3):456. [CrossRef].
90. Chen C, Wang Y, Shan C, Sun Y, Xu P, Zhou H, et al. Crystal structure of enterovirus 71 RNA-dependent RNA polymerase complexed with its protein primer VPg: implication for a trans mechanism of VPg uridylylation. J Virol. 2013;87(10):5755–68. [CrossRef].
91. Sun Y, Wang Y, Shan C, Chen C, Xu P, Song M, et al. Enterovirus 71 VPg uridylation uses a two-molecular mechanism of 3D polymerase. J Virol. 2012;86(24):13662–71. [CrossRef].
92. Cui S, Wang J, Fan T, Qin B, Guo L, Lei X, et al. Crystal structure of human enterovirus 71 3C protease. J Mol Biol. 2011;408(3):449–61. [CrossRef].
93. Shih SR, Chiang C, Chen TC, Wu CN, Hsu JT, Lee JC, et al. Mutations at KFRDI and VGK domains of enterovirus 71 3C protease affect its RNA binding and proteolytic activities. J Biomed Sci. 2004;11(2):239–48. [CrossRef].
94. Li B, Yue Y, Zhang Y, Yuan Z, Li P, Song N, et al. A novel enterovirus 71 (EV71) virulence determinant: the 69th residue of 3C protease modulates pathogenicity. Front Cell Infect Microbiol. 2017;7:26. [CrossRef].
95. Ma HY, Lu CY, Tsao KC, Shih HM, Cheng AL, Huang LM, et al. Association of EV71 3C polymorphisms with clinical severity. J Microbiol Immunol Infect. 2018;51(5):608–13. [CrossRef].
96. Xiao H, Li J, Yang X, Li Z, Wang Y, Rui Y, et al. Ectopic expression of TRIM25 restores RIG-I expression and IFN production reduced by multiple enteroviruses 3C(pro). Virol Sin. 2021;36(6):1363–74. [CrossRef].
97. Zheng B, Zhou X, Tian L, Wang J, Zhang W. IFN-β1b induces OAS3 to inhibit EV71 via IFN-β1b/JAK/STAT1 pathway. Virol Sin. 2022;37(5):676–84. [CrossRef].
98. Zhou X, Tian L, Wang J, Zheng B, Zhang W. EV71 3C protease cleaves host anti-viral factor OAS3 and enhances virus replication. Virol Sin. 2022;37(3):418–26. [CrossRef].
99. Li H, Yao Y, Chen Y, Zhang S, Deng Z, Qiao W, et al. $TRAF_3IP_3$ is cleaved by EV71 3C protease and exhibits antiviral activity. Front Microbiol. 2022;13:914971. [CrossRef].
100. Li Z, Wu YN, Li H, Li W, Tan J, Qiao W. 3C protease of enterovirus 71 cleaves promyelocytic leukemia protein and impairs PML-NBs production. Virol J. 2021;18(1):255. [CrossRef].
101. Chen D, Feng C, Tian X, Zheng N, Wu Z. Promyelocytic leukemia restricts enterovirus 71 replication by inhibiting autophagy. Front Immunol. 2018;9:1268. [CrossRef].
102. Li J, Yao Y, Chen Y, Xu X, Lin Y, Yang Z, et al. Enterovirus 71 3C promotes apoptosis through cleavage of PinX1, a telomere binding protein. J Virol. 2017;91(2):e02016–16. [CrossRef].
103. Song F, Yu X, Zhong T, Wang Z, Meng X, Li Z, et al. Caspase-3 inhibition attenuates the cytopathic effects of EV71 infection. Front Microbiol. 2018;9:817. [CrossRef].
104. Li ML, Lin JY, Chen BS, Weng KF, Shih SR, Calderon JD, et al. EV71 3C protease induces apoptosis by cleavage of hnRNP A1 to promote apaf-1 translation. PLoS One. 2019;14(9):e0221048. [CrossRef].
105. Lei X, Zhang Z, Xiao X, Qi J, He B, Wang J. Enterovirus 71 inhibits pyroptosis through cleavage of gasdermin D. J Virol. 2017;91(18):e01069–17. [CrossRef].
106. Liu Y, Zheng Z, Shu B, Meng J, Zhang Y, Zheng C, et al. SUMO modification stabilizes enterovirus 71 polymerase 3D to facilitate viral replication. J Virol. 2016;90(23):10472–85. [CrossRef].
107. Hao H, Hao S, Chen H, Chen Z, Zhang Y, Wang J, et al. N6-methyladenosine modification and METTL3 modulate enterovirus 71 replication. Nucleic Acids Res. 2019;47(1):362–74. [CrossRef].
108. Hao H, Liu W, Miao Y, Ma L, Yu B, Liu L, et al. N4-acetylcytidine regulates the replication and pathogenicity of enterovirus 71. Nucleic Acids Res. 2022;50(16):9339–54. [CrossRef].

109. Yu J, Zhang L, Ren P, Zhong T, Li Z, Wang Z, et al. Enterovirus 71 mediates cell cycle arrest in S phase through non-structural protein 3D. Cell Cycle. 2015;14(3):425–36. [CrossRef].
110. Xu C, Wang M, Cheng A, Yang Q, Huang J, Ou X, et al. Multiple functions of the nonstructural protein 3D in picornavirus infection. Front Immunol. 2024;15:1365521. [CrossRef].
111. Wang LC, Chen SO, Chang SP, Lee YP, Yu CK, Chen CL, et al. Enterovirus 71 proteins 2A and 3D antagonize the antiviral activity of gamma interferon via signaling attenuation. J Virol. 2015;89(14):7028–37. [CrossRef].
112. Xiang Q, Wan P, Yang G, Huang S, Qin M, Yang H, et al. Beclin1 binds to enterovirus 71 3D protein to promote the virus replication. Viruses. 2020;12(7):756. [CrossRef].
113. You L, Chen J, Liu W, Xiang Q, Luo Z, Wang W, et al. Enterovirus 71 induces neural cell apoptosis and autophagy through promoting ACOX1 downregulation and ROS generation. Virulence. 2020;11(1):537–53. [CrossRef].
114. Wang H, Zhong M, Li Y, Li K, Wu S, Guo T, et al. APOBEC3G is a restriction factor of EV71 and mediator of IMB-Z antiviral activity. Antiviral Res. 2019;165:23–33. [CrossRef].
115. Hu J, Wang H, Yang L, Wu S, Li Y, Li Y, et al. Compound IMB-Z inhibits hepatitis B virus replication through increasing APOBEC3G expression and incorporation into viral nucleocapsids. J Glob Antimicrob Resist. 2022;31:371–8. [CrossRef].