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# Screening and characterization of a novel linear B-cell epitope on orf virus F1L protein

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**Background:** Orf, also known as contagious ecthyma (CE), is an acute, contagious zoonotic disease caused by the orf virus (ORFV). The F1L protein is a major immunodominant protein on the surface of ORFV and can induce the production of neutralizing antibodies.

**Methods:** The prokaryotic expression system was used to produce the recombinant F1L protein of ORFV, which was subsequently purified and used to immunize mice. Positive hybridoma clones were screened using an indirect enzyme-linked immunosorbent assay (ELISA). The reactivity and specificity of the monoclonal antibody (mAb) were verified through Western blot and indirect immunofluorescence (IFA). The linear antigenic epitope specific to the mAb was identified through Western blot, using truncated F1L proteins expressed in eukaryotic cells. A multiple sequence alignment of the ORFV reference strains was performed to evaluate the degree of conservation of the identified epitope.

**Results:** After three rounds of subcloning, a mAb named Ba-F1L was produced. Ba-F1L was found to react with both the exogenously expressed F1L protein and the native F1L protein from ORFV-infected cells, as confirmed by Western blot and IFA. The mAb recognized the core epitope <sup>103</sup>CKSTCPKEM<sup>111</sup>, which is highly conserved among various ORFV strains, as shown by homologous sequence alignment.

**Conclusion:** The mAb produced in the present study can be used as a diagnostic reagent for detecting ORFV and as a basic tool for exploring the mechanisms of orf pathogenesis. In addition, the identified linear epitope may be valuable for the development of epitope-based vaccines.

## KEYWORDS

orf virus, F1L protein, monoclonal antibody, linear B-cell epitope, homology analysis

## Introduction

Orf, also called sore mouth, contagious pustular dermatitis, or contagious ecthyma, is an acute, highly contagious, and epitheliotropic zoonotic disease caused by orf virus (ORFV) (Santiago et al., 2019). ORFV primarily infects domestic ruminants, such as goats and sheep, as well as wild ruminants like deer and camels. It can also infect mammals such as cats and dogs, and in rare cases, humans (Tack and Reynolds, 2011; Spyrou and Valiakos, 2015; Yu et al., 2023). The typical symptoms of orf in animals are ulcerating lesions on the skin and mucous membranes of the mouth, lips, nose, and udders (Du et al., 2023). The morbidity rate of orf is high, particularly in young animals. Fortunately, the fatality rate is usually low unless there are secondary infections (Demiraslan et al., 2017). However, orf could be fatal in lambs and kids due to oral lesions that may impede suckling, and lead to secondary bacterial, viral, fungal, or parasitic infections (Hosamani et al., 2009).

Orf not only has a considerable economic impact on the farming sector but also poses a threat to public health. It can be transmitted from animals to humans through direct or indirect contact with infected animals or contaminated animal products (Andreani et al., 2019; Alajlan and Alsubeeh, 2020). Cases of human-to-human transmission of orf are rare but do occur (Turk et al., 2014; Rajkomar et al., 2016; Bouscarat and Descamps, 2017). Clinical manifestation of human orf usually begins with small papules, which then progress into severe pustular dermatitis lesions, most commonly on the hands and arms (Khan et al., 2021; Qiu and Yanes, 2021). Although orf in humans is a benign and self-limiting disease, it can become complicated in patients with compromised immune systems (Lederman et al., 2007).

Orf virus, the causative agent of orf, is the prototype member of the genus *Parapoxvirus* of the family Poxviridae (Chen et al., 2017). The genome of ORFV is a double-strand linear DNA of about 138 kb with a high G + C content that up to 64% (Spyrou and Valiakos, 2015). Its genome contains 132 putative open reading frames, which are further classified into the central core area (ORFs009-111) and the terminal areas (ORFs001-008 and ORFs112-132) (Li et al., 2023). The genes in the core region are typically associated with virus replication, packaging, morphogenesis, and release, and are relatively conserved (Zhou et al., 2022). On the other hand, the terminal regions, which consist of two inverted repeats are highly variable and are mainly related to virulence, host range, and immune regulation (Bergqvist et al., 2017).

The ORF059 gene is a highly conserved gene located in the core region of the ORFV genome. It is commonly used as a molecular target for phylogenetic analysis of ORFV (Li et al., 2023). The F1L protein, encoded by ORF059, is a significant immunogenic protein that not only triggers the production of neutralizing antibodies (Scagliarini et al., 2002; Gallina et al., 2004), but also may contribute to the induction of cellular immunity (Wang et al., 2022; Zheng et al., 2024). The F1L protein has been shown to have a significant role in the adsorption and entry of the virus into host cells, owing to its ability to bind with heparin (Scagliarini et al., 2004). Furthermore, F1L is a suitable candidate for designing diagnostic methods and subunit vaccines for orf (Yogisharadhy et al., 2018). However, information on the structure and functions of F1L and its

antigenic epitopes needs to be further explored. Currently, research on antigenic epitopes of ORFV F1L protein is limited, and there is a wide potential application for the diagnosis of clinical cases using F1L linear B-cell epitope. In order to bridge the gaps between the two, this study aimed to develop and characterize an anti-F1L monoclonal antibody (mAb) and identify the specific antigenic epitope on the F1L protein. These findings have the potential to aid in the diagnosis of orf infection and the development of subunit vaccines against ORFV.

## Materials and methods

### Cell lines, viruses, and plasmids

The SP2/0 myeloma cell line, HEK293T cell line, and immortalized goat endometrial epithelial cells (iGEEC) were maintained in our laboratory and cultured at 37°C in a humidified incubator with 5% CO<sub>2</sub>. The basal medium for the SP2/0 myeloma cell line is RPMI-1640 (Share-bio, China). The basal medium for HEK293T and iGEEC is Dulbecco's modified Eagle medium (Invitrogen, USA). The cells in this study were cultured in basal medium supplemented with 10% FBS (Gibco, USA), 100 IU/ml penicillin, and 100 µg/ml streptomycin. The ORFV HCE isolate is an attenuated vaccine strain purchased from Shandong Huahong Biotechnology Company Limited. The prokaryotic expression vector pET-28a, the eukaryotic expression vector pEGFP-C1, and pCMV-HA were kept in our laboratory.

### Determination of the antigenicity, hydrophilicity, and transmembrane regions

The characteristics of the ORFV F1L protein were analyzed using Lasergene v7.0 software (Lasergene, USA). The molecular weight (MW) and amino acid (aa) sequence of the cloned ORF059 (F1L) gene were analyzed by the EditSeq program of the Lasergene software. The secondary structure of the F1L protein was analyzed using the Protean program of the Lasergene software. The analysis focused on two aspects: antigenicity and hydrophilicity, which are important for the exogenous expression of the target proteins in *Escherichia coli*. The potential transmembrane domains of the F1L protein were predicted using the online program TMHMM.<sup>1</sup>

### Preparation and purification of recombinant F1L protein expressed in prokaryotic cells

The prokaryotic expression system was used to express a truncated form of the F1L protein, designated as tF1L, covering amino acids 1–281. The F1L gene was amplified from a sample of orf collected in the field. The F1L gene has been deposited

<sup>1</sup> <https://dtu.biolib.com/DeepTMHMM>

in GenBank with the accession number OQ686990. The tF1L gene was amplified using a pair of primers (tF1L-F and tF1L-R) (Supplementary Table 1) carrying the restriction enzyme sites *Hind*III or *Xho*I. The amplified gene was then digested with the appropriate restriction enzymes and ligated to the plasmid pET-28a between the *Hind*III and *Xho*I cleavage sites. The recombinant plasmid pET-28a-tF1L was confirmed by sequencing and subsequently transformed into competent BL21(DE3) cells (Tolobio, China). The single positive colony of pET-28a-tF1L transformed BL21(DE3) was induced to express the recombinant protein His-tF1L. The expression of His-tF1L was confirmed by Coomassie Brilliant Blue staining. The SDS-PAGE coupled gel-cutting method was used to purify His-tF1L from the inclusion bodies of the induced *E. coli* lysates, following the procedure described by Han et al. (2019). Briefly, after separation by SDS-PAGE, the protein samples were stained with 0.25 M KCl for approximately 5 min. The proteins with different MWs appeared as white bands in the polyacrylamide gels. The protein bands corresponding to the position of His-tF1L were excised, and homogenized, and an appropriate amount of PBS was added to the gels. After several cycles of freezing and thawing, a portion of the target proteins migrated from the gels into the PBS. The PBS was then separated by centrifugation and aspirated. The purified His-tF1L was identified by staining with Coomassie Brilliant Blue and Western blot analysis using an anti-His mAb (Biodragon, China).

## Indirect enzyme-linked immunosorbent assay

Enzyme-linked immunosorbent assay (ELISA) plates were coated with the purified His-tF1L protein (1 µg/ml, 100 µl/well) and incubated at 4°C overnight. The plates were then blocked with 5% skim milk in PBS at 37°C for 1 h, washed three times with PBS containing 0.05% Tween-20 (PBST), and incubated with hybridoma culture supernatant at 37°C for 1 h. After three washes with PBST, HRP-labeled goat anti-mouse IgG (Beyotime, China) was added to each well at a dilution of 1:3,000 and incubated at 37°C for 1 h. After washing with PBST, 100 µl/well of TMB substrate (Beyotime, China) was added and the color was allowed to develop for 5 min at 37°C. To stop the reaction, 50 µl of 2 M H<sub>2</sub>SO<sub>4</sub> was added to each well. Absorbance was measured at 450 nm using Multiskan FC (Thermo, USA).

## Production and characterization of murine mAb against F1L protein

Monoclonal antibodies against the recombinant F1L protein of ORFV were generated according to a previously described procedure (Zhang, 2012). Three female BALB/c mice, aged 7 weeks, were immunized three times with 100 µg of purified His-tF1L via subcutaneous injection at 21-day intervals. The antigen was emulsified with complete Freund's adjuvant for the first dose and incomplete Freund's adjuvant for the subsequent booster immunizations (Sigma, USA). The antibody levels of the immunized mice were assessed through indirect ELISA after three immunizations, using purified His-tF1L as the coat antigen.

A booster dose of 100 µg His-tF1L protein without adjuvant was administered intraperitoneally to the mouse with the highest antibody level 3 days before cell fusion. The mouse selected for the experiment was euthanized and its splenocytes were fused with SP2/0 myeloma cells according to standard procedures (Galfre and Milstein, 1981). The resulting hybridoma cell supernatants were tested for His-tF1L antibodies by indirect ELISA. The hybridoma cells were subcloned three times by limiting dilution and verified as His-tF1L specific antibody positive by ELISA. The purified hybridoma cells were then injected into the peritoneal cavity of BALB/c mice to generate ascites fluid against the F1L protein. The isotype of the F1L mAb was determined using a Mouse Monoclonal Antibody Ig Class/subclass Identification ELISA Kit (Biodragon, China) and an IsoQuick Strips and Kits for Mouse Monoclonal Isotyping (Sigma-Aldrich, MO, USA) according to the manufacturer's protocol. The reactivity of the F1L mAb with ORFV were evaluated using Western blot analysis and immunofluorescence assay (IFA).

## Expression of full or a series of truncated F1L proteins in eukaryotic cells

To determine the minimal epitope recognized by the F1L mAb, the amino acids at both ends of the tF1L protein were gradually deleted until the core epitope was identified. A series of truncated F1L proteins that were used in the study are shown in Figures 4A, 5A. The genes encoding the truncated forms of the F1L protein were inserted into the pEGFP-C1 plasmid via *Hind*III and *Kpn*I restriction sites. The HEK293T cells were transfected with the recombinant plasmids pEGFP-C1-tF1L or its derivatives to express the F1L protein and its various truncations. Simply, when the cell culture reached 70%–80% confluence, 2.5 µg of each recombinant plasmid was transfected into HEK293T cells in a six-well plate. Cells were harvested at 48 h post-transfection, and the expression of each GFP-fused protein was confirmed through Western blot analysis using both GFP mAb and F1L mAb.

For pCMV-HA-F1L construction, the intact ORF059 gene was amplified using the primers HA-F1L-F and HA-F1L-R (Supplementary Table 1), containing *Eco*RI and *Xho*I restriction sites, respectively. The resultant amplicon was inserted into the eukaryotic expression plasmid pCMV-HA via *Eco*RI/*Xho*I restriction sites to generate the recombinant plasmid pCMV-HA-F1L. For *in vitro* expression of HA-F1L, HEK293T cells were transfected with recombinant plasmid pCMV-HA-F1L as described above.

## Indirect immunofluorescence assay

The expression of HA-F1L protein in HEK293T cells was assessed through IFA 36 h after transfection. The native F1L protein was obtained by infecting iGEEC with ORFV HCE strain at an MOI of 0.5. Briefly, iGEEC seeded in glass-bottomed culture dishes at approximately 90% confluence were inoculated with ORFV. When there were obvious cytopathic effects after infection, cells were fixed with 4% paraformaldehyde (Biosharp, China) in PBS for 30 min at room temperature, and rinsed three times with PBS for

5 min each time. The fixed cells were then permeabilized with 0.1% Triton X-100 (Macklin, China) in PBS/2% BSA (Saiguo, China) for 10 min. The liquid was aspirated and the cells were blocked with PBS/2% BSA for 30 min at 37°C. After washing with PBS, the cells were incubated with mAb Ba-F1L (1:500 dilution) for 1 h at 37°C. After washing, cells were incubated with donkey anti-mouse IgG antibody (1:1,000) conjugated to FITC (Share-bio, China) for 1 h at 37°C. Cells were washed thrice with PBS and stained with DAPI (Sigma, USA) for 10 min at room temperature before final washing with PBS. Images were captured by fluorescence microscopy (Nikon, Japan).

## Western blot analysis

Western blot was used to analyze the reactivity of mAb Ba-F1L with viral native F1L and GFP-fused F1L protein for the fine determination of the core epitope. Culture lysates containing native F1L or GFP-fused F1L polypeptides were subjected to 10% SDS-PAGE followed by transfer to polyvinylidene fluoride (PVDF) membrane. After blocking with 5% skim milk in PBS at 37°C for 1 h, PVDF membranes were incubated with mAb Ba-F1L (1:500 dilution) or GFP (1:1,000 dilution, Share-bio, China) at 4°C overnight. After three washes with PBS containing 0.05% Tween-20

(PBST), the membranes were incubated with HRP-conjugated goat anti-mouse IgG (1:3,000 dilution, Share-bio, China) at room temperature for 1 h. The membranes were washed with PBST and developed using an ECL imaging system (Amersham Imager 600, General Electric, USA).

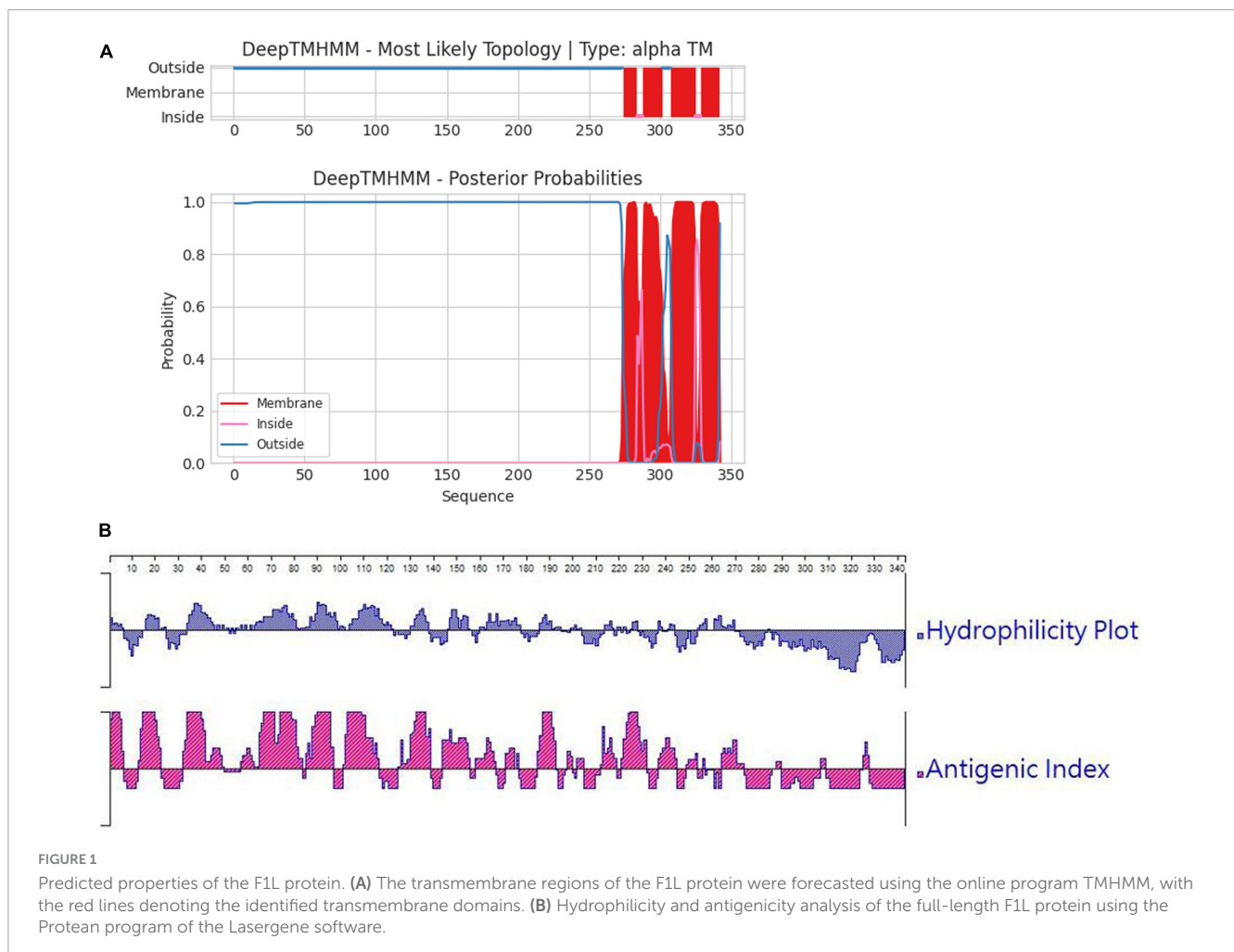
## Sequence analysis

To analyze whether the epitope identified in the present study is conservative among different ORFV strains, the epitope sequences and their flanking sequences of the F1L proteins were aligned among 17 ORFV reference strains using BioEdit software V7.2.5.

## Results

### Amino acid characteristics prediction results

In order to ascertain the antigenicity, hydrophilicity, MW, and transmembrane region of the F1L protein, we initially conducted a targeted analysis using bioinformatics software. The results are depicted in Figures 1A, B. It was revealed that the net MW of



tF1L is approximately 31 kDa. In comparison to the full-length F1L protein, the N-terminal 281 amino acids (tF1L) exhibit higher immunogenicity and hydrophilicity. The F1L protein has multiple transmembrane regions at its C-terminus. Consequently, we chose to express the N-terminal 281 amino acids (tF1L) of the F1L protein instead of the full form F1L.

## Expression and purification of recombinant His<sub>6</sub>-tF1L

The prokaryotic expression vector pET-28a was used as the vehicle to express the F1L protein. In the present study, tF1L was expressed as a fusion protein in the form of hexahistidine-tF1L-hexahistidine. The MW of the recombinant protein His-tF1L, as estimated by the software lasergene, is approximately 37 kDa. As shown in Figure 2A, the His-tF1L protein was successfully expressed and almost all the target proteins were present in an insoluble fraction. However, the actual MW of the prokaryotic form of His-tF1L is a slightly larger than the deduced MW. We purified the insoluble His-tF1L by a method called “SDS-PAGE and gel-cutting” as described previously (Han et al., 2019). The purity of purified His-tF1L was relatively high as shown by SDS-PAGE (Figure 2B). Western blot analysis showed that the purified His-tF1L could be recognized by the anti-His mAb (Figure 2C).

## Production and characterization of F1L protein-specific mAb

The purified His-tF1L protein was used both as an immunogen and as a screening antigen for the development of hybridomas. Positive clones were screened from hybridoma supernatants using purified His-tF1L protein as the coating antigen in an indirect ELISA. After three rounds of screening and subcloning, a hybridoma cell line that secretes F1L protein-specific antibodies was selected and designated Ba-F1L. Ascites fluid was produced by infecting mice with the Ba-F1L hybridoma cell line. The isotype of the mAb Ba-F1L light chain was determined using an IsoQuick Strips and Kits for Mouse Monoclonal Isotyping. The right strip in Figure 3A has been extracted from the instructions accompanying the Strips and Kits, which provide guidance on how to judge the results. As shown in Figure 3A, the left strip developed a test line

and a kappa line, which indicated that the light chain of mAb Ba-F1L was  $\kappa$ . The heavy chain subclasses were identified by another commercial kit. The results clearly indicated that the mAb Ba-F1L heavy chain was IgG1, as demonstrated by testing at a dilution of 1:100,000 of the ascites (Figure 3B).

To assess the reactivity of the mAb Ba-F1L, we used Western blot and IFA to test its ability to recognize the F1L protein. As shown in Figure 3C, a specific band at the position of the expected MW of F1L (approximately 39 kDa) was present in ORFV-infected iGEEC but not in mock-infected cells. Green fluorescence signals were observed in both ORFV-infected iGEEC and HEK293T cells transfected with the eukaryotic plasmid pCMV-HA-F1L when using the mAb Ba-F1L as the primary antibody in IFA. However, no fluorescence signals were present in mock-infected iGEEC (Figure 3D). The fluorescence signals of HEK293T cells transfected with recombinant plasmids are stronger than those of iGEEC infected with ORFV. This may be due to the higher expression level of exogenous F1L in HEK293T compared to the viral native F1L protein in iGEEC.

## Preliminary determination of the antigenic epitope domain

The basic strategy for identifying the epitope-containing domain in F1L involves progressively splitting and truncating the positive proteins/polypeptides that react with mAb Ba-F1L. In this section, a series of four successive rounds of overlapping GFP-tagged proteins or polypeptides were constructed and expressed in HEK293T cells through transfection of the corresponding recombinant plasmids (Figure 4A). The tF1L protein is 281 aa in length. For the first round, the tF1L protein was divided into two parts, fragment A (150 aa) and fragment B (151 aa), with an overlap portion of 20 aa. The anti-GFP mAb recognized the tF1L protein, fragments A, and B, all of which bear a GFP tag (Figure 4B). However, only the tF1L protein and fragment A exhibited reactivity with the mAb, thereby signifying that the specific epitope recognized by mAb Ba-F1L was situated within fragment A (Figure 4B). Subsequently, over the course of the three rounds of epitope mapping, fragments A2, A3, and A7 were successively identified as the epitope-containing domains (Figures 4C–E). Consequently, it can be concluded that the epitope-containing region of the F1L protein is located between amino acids 96 and 115.

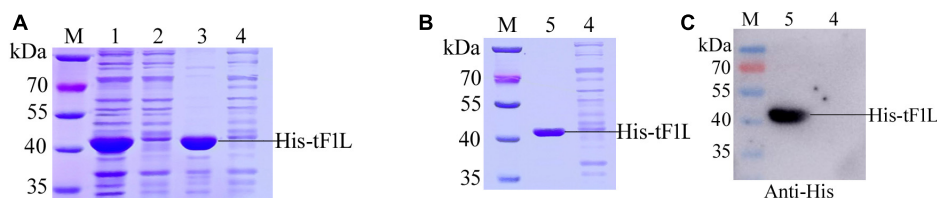
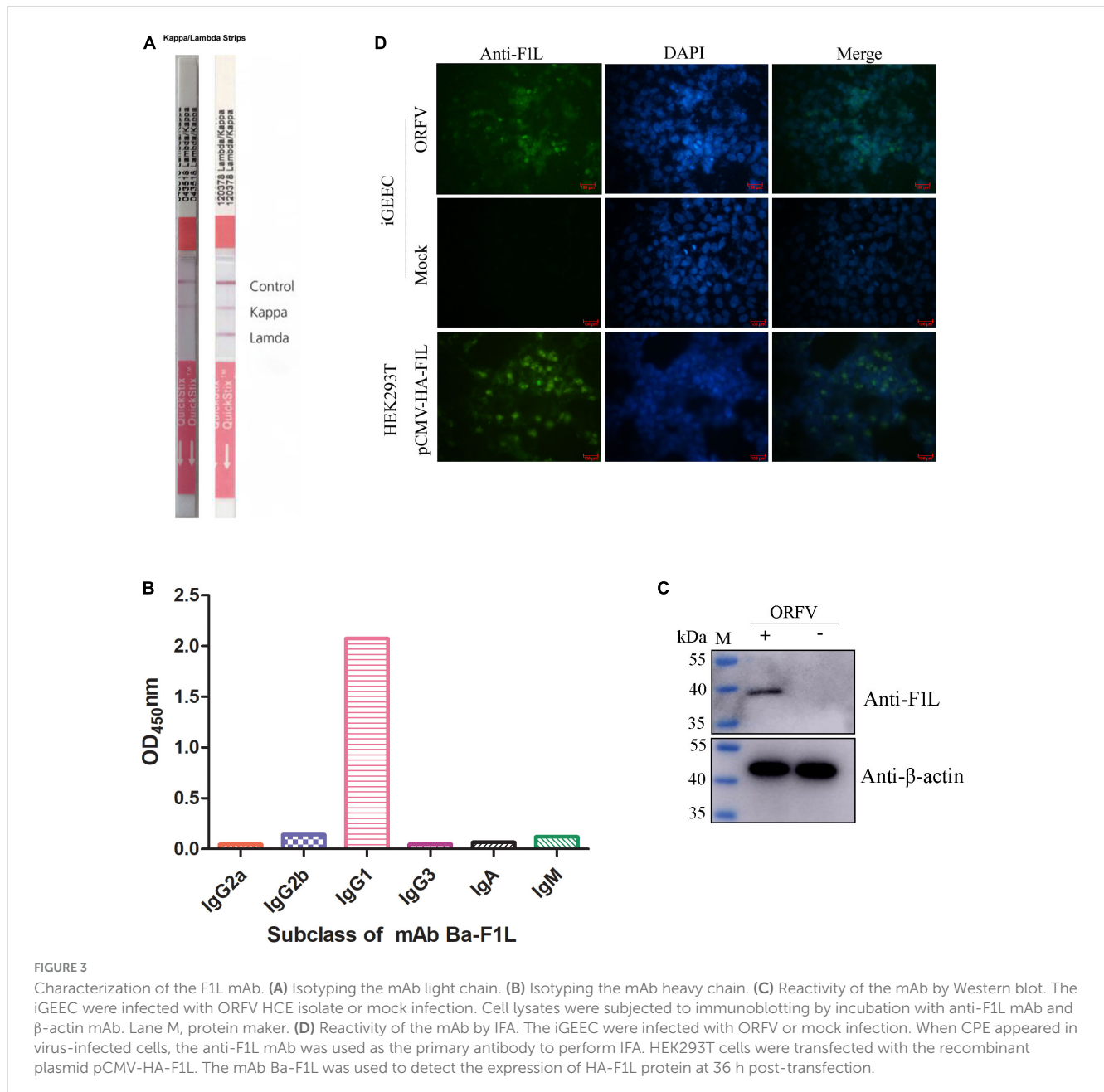


FIGURE 2

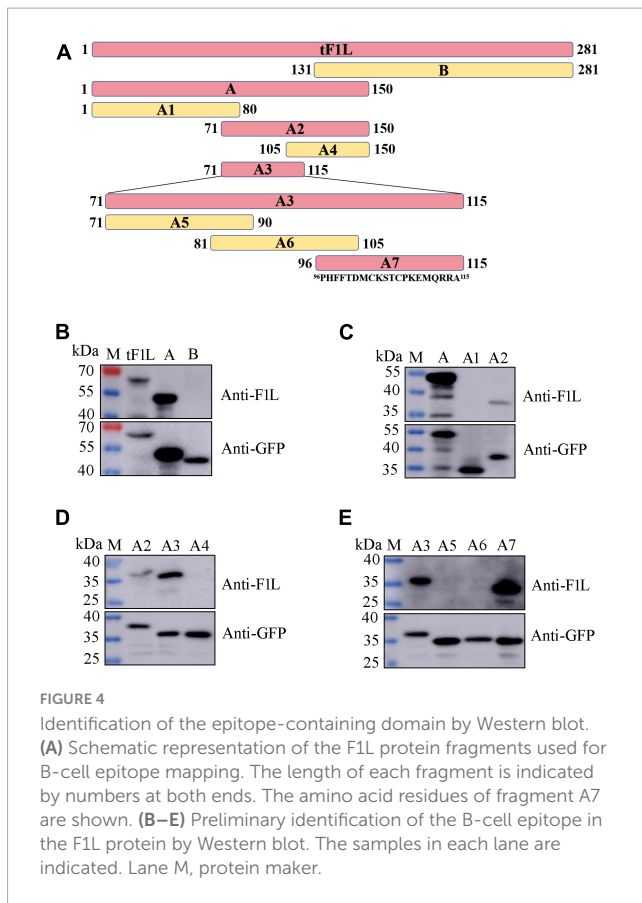
Expression, purification, and identification of the recombinant His-tF1L protein. Bacterial lysates from *E. coli* transformed with recombinant plasmid pET-28a-tF1L or empty plasmid pET-28a were subjected to SDS-PAGE analysis (A). Analysis of the purified His-tF1L protein by Coomassie blue staining (B) and Western blot (C). Lane M, protein molecular weight maker; lanes 1–3, the whole cell lysates, the supernatant and the precipitation of *E. coli* cells transformed with the recombinant plasmid pET-28a-tF1L; lane 4, the whole cell lysates of *E. coli* cells transformed with the empty plasmid pET-28a; lane 5, the purified His-tF1L protein.



## Precise mapping of mAb Ba-F1L-specific epitope

For fine mapping of the core epitope, a stepwise deletion of amino acids was carried out from either the N-terminal or C-terminal of fragment A7 (96–115 aa), resulting in a total of 14 polypeptides derived from A7 (Figure 5A). These polypeptides were expressed as GFP-fused proteins through the transient transfection of the corresponding plasmids into HEK293T cells. Subsequently, Western blot analysis was conducted on lysates from cells transfected with each recombinant plasmid to assess their reactivity with either the GFP mAb, F1L mAb, or both. As depicted in Figures 5B, C, all polypeptides exhibited reactivity with the GFP mAb, indicating the successful expression of each recombinant protein. Notably, the polypeptide A7-7 failed to be recognized

by the F1L mAb, implying the essential nature of the cysteine at position 103 in F1L for the mAb-specific epitope (Figure 5B). Polypeptides A7-8 to A7-14 share the same amino acid <sup>103</sup>C in their N-terminus, differing solely in their C-terminus due to the progressive deletion of one amino acid at a time (Figure 5A). In Figure 5C, it is evident that polypeptides A7-8 and A7-9 exhibited a strong reaction with the F1L mAb, whereas A7-10 only yielded a weak signal. The remaining polypeptides, A7-11 to A7-14, showed no reactivity with the F1L mAb (Figure 5C). The polypeptide <sup>103</sup>CKSTCPKE<sup>110</sup> displayed weak reactivity with the mAb Ba-F1L. However, the addition of the amino acid M<sup>111</sup> to the polypeptide <sup>103</sup>CKSTCPKE<sup>110</sup> resulted in a strong reactivity with the F1L mAb, as observed in sample A7-9 in Figure 5C. These findings indicate that the core epitope recognized by the mAb Ba-F1L is <sup>103</sup>CKSTCPKEM<sup>111</sup> within the F1L protein.



## Homology analysis of the F1L epitope in ORFV strains

Sequence alignment was performed to determine the conservation level of the epitope recognized by mAb Ba-F1L among 17 representative ORFV strains from China and other countries (Table 1). The results showed that the epitope  $^{103}\text{CKSTCPKEM}^{111}$  exhibits complete conservation across all ORFV reference strains, both domestic and abroad (Figure 6). The polypeptide  $^{103}\text{CKSTCPKEM}^{111}$  represents a highly conserved epitope on the F1L protein of ORFV, indicating the mAb Ba-F1L has the potential to react with any ORFV strain globally.

## Discussion

China has the largest population of domestic small ruminants in the world, with an estimated population of over 300 million sheep and goats (Mao et al., 2022). With the rapid development of the sheep and goat-raising industry in recent years, small ruminant-associated diseases have emerged and reemerged in China. These include peste des petits ruminants, caprine parainfluenza type 3, border disease, enzootic nasal tumor, caprine herpesvirus 1 infection, enterovirus infection, and orf (Zhao et al., 2010; Mao et al., 2022). Orf is a zoonotic disease that is distributed globally and has caused significant financial losses in the breeding industry (Li et al., 2023). Additionally, it may have an impact on human health, especially for individuals who come into contact with

ORFV-infected animals or their products, such as veterinarians and farmers (Du et al., 2023).

The expeditious and accurate diagnosis of orf is imperative for effective disease control. Initial diagnosis relies heavily on clinical manifestations and epidemiological history (Pang and Long, 2023). However, the accuracy of the initial diagnosis is predominantly contingent upon individual professional expertise, which may not consistently yield reliable results. The differentiation of orf from similar diseases such as foot and mouth disease, peste des petits ruminants, bluetongue disease, and goat pox or sheep pox can be challenging due to clinical similarities and the potential for co-infections (Gou et al., 2022; Pan et al., 2023). Molecular methods such as standard PCR, real-time PCR, virus isolation, ELISA, and electron microscopy are commonly used in the laboratory to confirm the preliminary diagnosis (Pang and Long, 2023). Nevertheless, the implementation of these molecular methods is often hindered on sheep or goat farms in China due to the shortage of skilled personnel to conduct the tests. Consequently, there is an urgent need for a straightforward and convenient diagnostic approach, such as an immunocolloidal gold test strip. However, the immunocolloidal gold test strips for the detection of ORFV are not commercially available. The development of immunocolloidal gold test strips necessitates monoclonal antibodies with high specificity and affinity, which are also valuable for various molecular immunology techniques utilized in fundamental research. Hence, we embarked on the development of the mAb against ORFV.

F1L protein is the immunodominant envelope antigen of ORFV and participates in viral adsorption and entry of target cells (Czerny et al., 1997; Scagliarini et al., 2004). Several research groups have demonstrated the potential utility of the F1L protein as a candidate for subunit vaccines against various ORFV strains (Zhao et al., 2011; Wang et al., 2022, 2023). Considering the important roles played by F1L, it is essential to develop F1L mAbs and identify its antigenic epitopes. Czerny et al. (1997) generated several mAbs against ORFV, and one of the mAbs (named 4D9) exhibited high affinity for the 39 kDa protein in purified ORFV particles. The mAb 4D9 had neutralizing capacity and was able to recognize the F1L protein in Western blots (Scagliarini et al., 2002). Another group developed a panel of 27 mouse mAbs against ORFV, of which 8 mAbs could react with native F1L protein (Housawi et al., 1998). The above-mentioned F1L mAbs were produced using the purified ORFV particles as immunogen. In another report, 10 mAbs were generated by immunizing mice with bacterially expressed F1L protein. Two mAbs (A3 and G3) with the greatest reactivity among these 10 mAbs were shown to react with viral native F1L protein (Li et al., 2012). The mAb developed in this study was also able to recognize naturally and artificially expressed F1L protein, both in IFA and Western blot analysis.

The bacterium *E. coli* is the most widely used vehicle for the overexpression of exogenous proteins. The ORF059 gene cloned in this study (GenBank No. OQ686990) is 1,029 bp long and the deduced amino acids of the F1L protein are 342. At the beginning of the study, we cloned the entire ORF059 gene into a bacterial expression vector pET-28a. However, the expression level of the full-length F1L protein was very low, even when different expression conditions were tested, such as incubation temperatures, induction times, and IPTG concentrations. This may

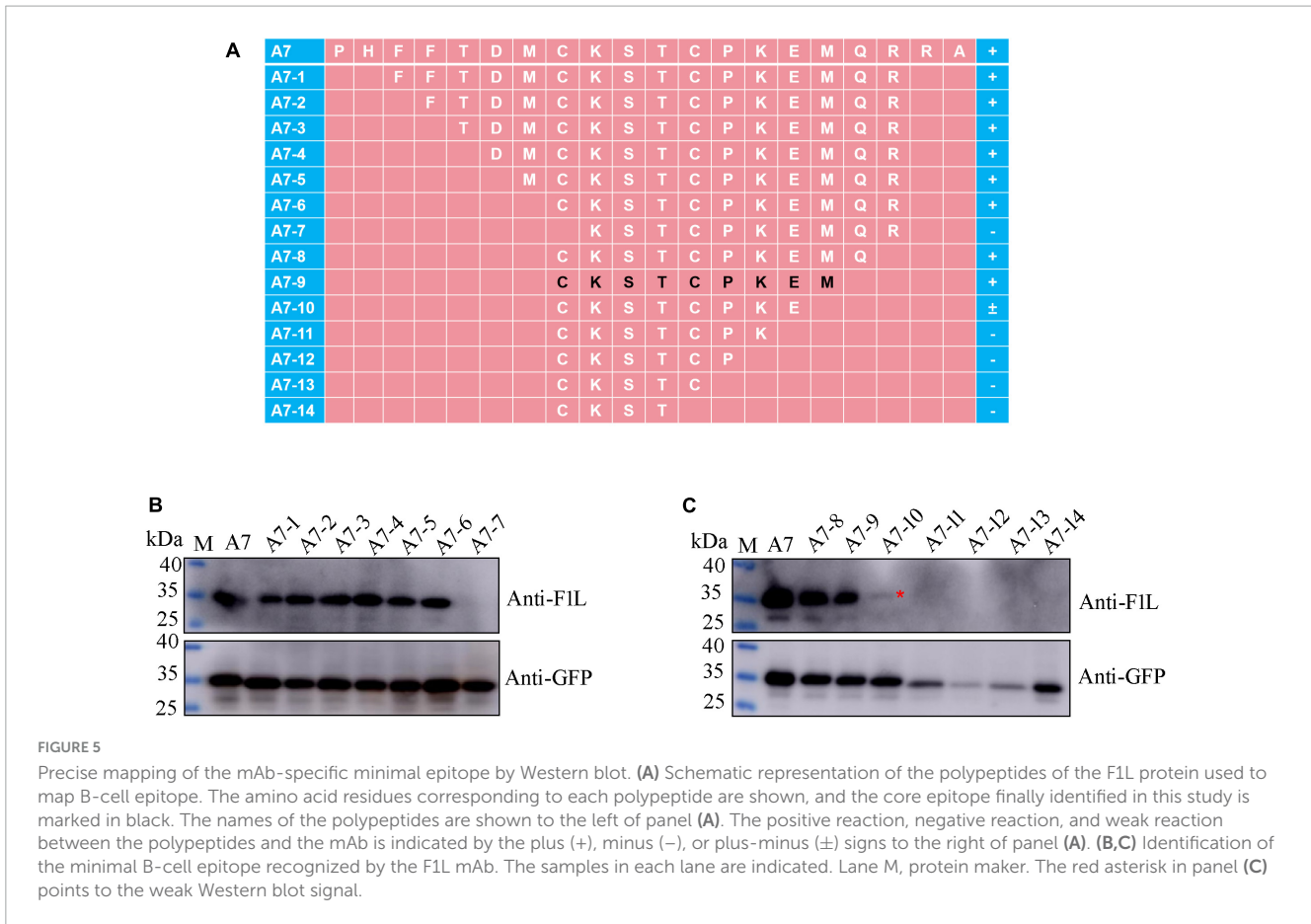


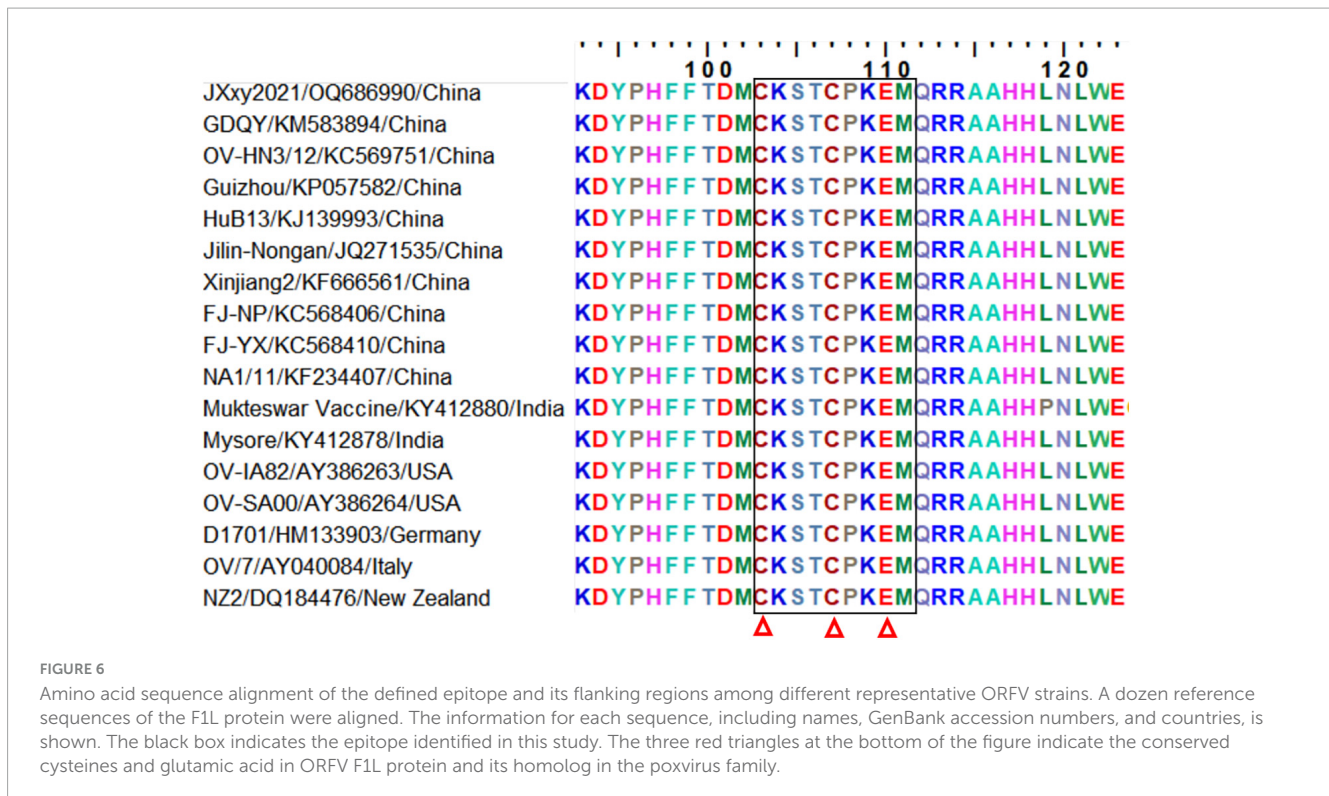
TABLE 1 The F1L genes of ORFV reference strains used in this study.

Strains	GenBank no.	Host	Country	Year
Jxxy2021	OQ686990	Goat	China	2021
GDQY	KM583894	Goat	China	2014
OV-HN3/12	KC569751	Sheep	China	2012
Guizhou	KP057582	Goat	China	2010
HuB13	KJ139993	Goat	China	2013
Jilin-Nongan	JQ271535	Sheep	China	2011
Xinjiang2	KF666561	Goat	China	2013
FJ-NP	KC568406	Goat	China	2011
FJ-YX	KC568410	Goat	China	2012
NA1/11	KF234407	Sheep	China	2011
Mukteswar vaccine	KY412880	Goat	India	2005
Mysore	KY412878	Sheep	India	2010
OV-IA82	AY386263	Sheep	USA	1982
OV-SA00	AY386264	Goat	USA	2003
D1701	HM133903	Sheep	Germany	2010
OV/7	AY040084	Sheep	Italy	2001
NZ2	DQ184476	Sheep	New Zealand	2005

be due to the properties of the F1L protein. The F1L protein has multiple transmembrane domains at its C-terminal as predicted by the online program (TMHMM) for transmembrane prediction.

It is widely recognized that exogenous proteins containing transmembrane domains are typically expressed at low levels or not at all in *E. coli* (Wagner et al., 2007). We decided to express





the N-terminal 281 amino acids of the F1L protein (tF1L), from which almost all the transmembrane regions of the F1L protein have been eliminated. At the same time, the tF1L protein retained good antigenicity and hydrophilicity, making it suitable for use as an immunogen. The tF1L protein was successfully expressed and its expression level was relatively high. For the same reason, part of the F1L protein was expressed in *E. coli* and an indirect ELISA was established based on the rF1L protein, which lacks the transmembrane regions (289–334 aa) (Yogisharadhy et al., 2018). In contrast, the intact F1L protein was highly expressed in another report using the bacterial expression plasmid pET28a-ORF059 (Li et al., 2012). The inconsistency observed in the expression patterns of the full-length F1L protein in *E. coli* across various research groups remains ambiguous and necessitates thorough investigation.

The ORFV F1L protein is a homolog of the vaccinia HL3 protein, the Capripoxvirus P32 protein, and the lumpy skin disease virus P32 protein because of their structural similarities (Housawi et al., 1998; Heine et al., 1999). A pair of cysteine residues at 103 and 107 and a glutamic acid at 110 of the identified epitope <sup>103</sup>CKSTCPKEM<sup>111</sup> are highly conserved and identical in the ORFV F1L protein as well as in the F1L homologs of animal poxviruses (Lin et al., 2000). In other words, other viruses in the Poxviridae family have at least five or six different amino acids in their counterparts of the ORFV F1L protein epitope <sup>103</sup>CKSTCPKEM<sup>111</sup>. As the epitope sequence identified in this study varies widely among different poxviruses, it is plausible that the mAb Ba-F1L may not interact with sheep poxvirus, goat poxvirus, and other viruses in the Poxviridae family. Consequently, the monoclonal antibody Ba-F1L holds potential utility in devising a methodology to differentiate ORFV from other poxviruses.

## Conclusion

A novel mAb directed against ORFV was successfully generated, with the mAb-specific linear epitope identified at residues <sup>103</sup>CKSTCPKEM<sup>111</sup> within the F1L protein. This report presents the first identification of a linear antigenic epitope on the ORFV F1L protein. Owing to the high conservation of the identified epitope among ORFV isolates, the mAb exhibits the potential to serve as a universal diagnostic tool for all ORF viruses. Furthermore, it holds promise for contributing to elucidating the structural and functional aspects of the F1L protein through future investigations. Moreover, the epitope's characteristics could contribute to the development of epitope-based vaccines.

## Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary material.

## Ethics statement

The animal study was approved by the Animal Care and Ethics Committee of Yichun University. The study was conducted in accordance with the local legislation and institutional requirements.

## Author contributions

ZZ: Investigation, Writing – original draft, Conceptualization, Funding acquisition. XZ: Investigation, Funding acquisition, Writing – review & editing. KF: Investigation, Writing – review & editing. SB: Investigation, Writing – review & editing. TY: Formal analysis, Funding acquisition, Software, Writing – review & editing. JG: Validation, Writing – review & editing. ZY: Data curation, Writing – review & editing. HZ: Data curation, Resources, Writing – review & editing. ZS: Project administration, Supervision, Writing – review & editing. PL: Conceptualization, Funding acquisition, Project administration, Writing – original draft.

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## Conflict of interest

HZ was employed by Xinyu Mengling Animal Husbandry Development Co., Ltd.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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## Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fmicb.2024.1373687/full#supplementary-material>

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