Inhibition of orf virus replication in goat skin fibroblast cells by the HSPA1B protein, as demonstrated by iTRAQ-based quantitative proteome analysis

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Abstract
Orf virus (ORFV) infects sheep and goat tissues, resulting in severe proliferative lesions. To analyze cellular protein expression in ORFV-infected goat skin fibroblast (GSF) cells, we used two-dimensional liquid chromatography-tandem mass spectrometry coupled with isobaric tags for relative and absolute quantification (iTRAQ). The proteomics approach was used along with quantitative reverse transcription polymerase chain reaction (RT-qPCR) to detect differentially expressed proteins in ORFV-infected GSF cells and mock-infected GSF cells. A total of 282 differentially expressed proteins were identified. It was found that 222 host proteins were upregulated and 60 were downregulated following viral infection. We confirmed that these proteins were differentially expressed and found that heat shock 70-kDa protein 1B (HSPA1B) was differentially expressed and localized in the cytoplasm. It was also noted that HSPA1B caused inhibition of viral proliferation, in the middle and late stages of viral infection. The differentially expressed proteins were associated with the biological processes of viral binding, cell structure, signal transduction, cell adhesion, and cell proliferation.

Introduction
Orf, which is caused by orf virus (ORFV), is one of the most widespread viral diseases worldwide. Although ORFV mainly infects sheep and goats, it can also infect other ruminants and other mammals [18]. Genes involved in virulence and pathogenesis are distributed in the ITR regions of the ORFV genome. The virus encodes involved in immunomodulation, including viral-interleukin 10 (vIL-10, ORFV127), vascular endothelial growth factor (VEGF, ORFV132), orf virus interferon resistance protein (OVIFNR, ORFV020), chemokine binding protein (CBP, ORFV112), granulocyte–macrophage colony-stimulating factor/interleukin-2 (GM-CSF/IL-2) inhibitory protein (GIF, ORFV117), nuclear factor kappa B (NF-κB) inhibitory protein (ORFV125), and deoxyuridine 5′-triphosphate pyrophosphatase (dUTPase, ORFV007) [38].

Like other poxviruses, ORFV encodes a range of molecules that play vital roles in immune evasion by the production of anti-inflammatory proteins [13]. These proteins, which are mainly involved in the interaction with host defense mechanisms, include OVIFNR, GIF, vIL-10, and VEGF. A number of these proteins are orthologues of known mammalian proteins, whereas others do not appear to have mammalian counterparts [7, 11–13]. ORFV encodes GIF, which is a novel secreted inhibitor of the cytokines GM-CSF and IL-2 [7]. GIF co-localizes with ORFV in infected epidermal cells, as detected by immunohistochemistry (IHC) [13]. Both ORFV and ovine IL-10 (vIL-10 and ovIL-10) inhibit production of tumor necrosis factor-α (TNF-α) and IL-8 from macrophages and keratinocytes as well as production of interferon gamma (IFN-γ) from activated lymphocytes [10]. The viral VEGF is important for virulence [25, 34, 40]. It is mitogenic to endothelial cells and promotes angiogenesis in the underlying dermis as well as proliferation of epidermal cells and activation of VEGF receptors. Aside from an array of immunomodulatory factors produced by ORFV, poxviruses also produced a class
of proteins, namely ankyrin (ANK) repeat proteins [28], which can also promote viral survival. A proteomic analysis of host cellular responses to viral infection may provide new insights into the cellular mechanisms involved in viral pathogenesis.

The isobaric tag for relative and absolute quantitation (iTRAQ) technique allows comprehensive, comparative, and quantitative determination of protein expression [33]. This technique has been extensively applied to proteome analysis [3, 14, 15, 33, 39]. iTRAQ simultaneously identifies and quantifies peptides by measuring peak intensities of reporter ions using tandem mass spectroscopy (MS/MS) and has been developed to identify biomarkers for various viral diseases [2, 41]. This method has been widely utilized to study the mechanisms of viral infection through the comparative analysis of cellular protein profiles. In the present study, a comparative iTRAQ-based proteomic analysis was conducted to analyze the changes in cellular proteins of goat skin fibroblast (GSF) cells exposed to ORFV in vitro at specific time points. The experiments were performed to investigate functional changes occurring in GSF cells infected by ORFV. This is the first study on the interactions of ORFV with its host using a proteomics approach. The purpose of the present study was to investigate the adaptability and proliferation of ORFV in goat skin fibroblasts. Hence, the iTRAQ-2D-LC–MS/MS technology was used to analyze proteomic changes in ORFV-infected GSF cells. Quantitative reverse transcription polymerase chain reaction (RT-qPCR) and other methods were applied to identify differentially expressed proteins and to further analyze and confirm the function of the differentially expressed proteins and heat shock 70-kDa protein 1B (HSPA1B) in ORFV-infected host cells. These data provide a foundation for mapping of gene regulatory networks that are affected by ORFV infection.

Materials and methods

Cell culture and virus infection

GSF cells were purchased from the Kunming Institute of Zoology, Chinese Academy of Sciences (Kunming, China) and cultured in complete Dulbecco’s modified Eagle’s medium (DMEM; HyClone Laboratories Inc., Logan, UT, USA), containing 10% fetal bovine serum (FBS; Gibco, New York, NY, USA) in an incubator with 5% CO₂. The GSF cell line was tested for mycoplasma contamination, which showed an appropriate microscopic morphology. ORFV was isolated and stored in our laboratory for further experiments. ORFV was passaged continuously for 15 generations on GSFs, virus samples were selected after the 1st, 5th, 10th, and 15th generations, and the viral genome was extracted for B2L gene detection. A 1137-bp fragment of the genome corresponding to the ORFV B2L gene was sequenced, and found to be identical to an ORFV B2L gene sequence published in the Gene Bank database (GU320351).

Growth curve of ORFV in GSF cells

To analyze the growth of ORFV in GSF cells, the cells were infected at an MOI of 0.1 in a 24-well plate, and uninfected cells served as a control. Samples were collected at 2, 12, 18, 24, 36, 48, and 60 h postinfection. The ORFV copy number was estimated using RT-qPCR.

Sample preparation

The cultured GSF cells were divided into an experimental group and a control group. When the cells reached about 80% confluency, 500 μL of ORFV suspension (MOI, 0.1) was added to the cells in the experimental group, and DMEM was added to the cells in the control group. The cells were incubated for 1 h and washed three times with phosphate-buffered saline (PBS), and DMEM medium containing 2% FBS was added. The cells were collected and protein samples were prepared after 35 h. The culture medium was discarded, and the cells were washed three times with cold PBS, scraped with a cell scraper, and collected in a centrifuge tube. The cells were pelleted by centrifugation at 2000 rpm for 3 min, resuspended and washed two times, and the final cell pellet was stored at -80 °C for further experiments.

Protein extraction

Five hundred μL of protein lysate was added to the sample and lysed on ice. Phenylmethylsulfonyl fluoride (PMSF) and dithiothreitol (DTT) with final concentrations of 1 and 10 mM, respectively, were added. After sonication in an ice bath for 15 min, the sample was centrifuged, and the supernatant was treated at 56 °C for 1 h to reduce disulfide bonds. Next, iodoacetamide (IAM) was added to a final concentration of 55 mM, and the sample was kept in the dark for 45 min to block cysteine alkylation. An appropriate amount of cold acetone was added, and the sample was stored at -20 °C for 2 h. The sample centrifuged at 14,000 rpm for 20 min, the supernatant was discarded, and 200 μl of 0.5 mM tetraethylammonium borohydride (TEAB) was added to the pellet, which was sonicated for 15 min, and centrifuged at 14,000 rpm for 20 min.

Protein concentration measurement

Protein quantification was done by the Bradford method, using prepare a standard curve made from series of dilutions.
of bovine serum albumin (BSA) and measuring absorbance at 595 nm.

**Protein digestion and iTRAQ labeling**

One hundred μg of protein was trypsinized for 12 h. After enzymatic digestion, the peptide was dried using a vacuum centrifugal pump and reconstituted with 0.5 M TEAB. iTRAQ labeling was done according to the manufacturer’s instructions. Labeling reagent 116 was used to label the experimental group, and labeling reagent 119 was used to label the control group. After incubation at room temperature for 2 h, the labeled peptides of each group were mixed and separated by liquid-phase chromatography using a strong cation-exchange (SCX) column.

**SCX chromatography**

An LC-20AB high-performance liquid chromatography (HPLC) pump system (Shimadzu, Tokyo, Japan) and a 4.6×250-mm separation column were used for liquid-phase separation of the samples. The mixed peptides were labeled reconstituted and 4 ml of buffer A. Gradient elution was performed at a rate of 1 ml/min. The first elution was performed with buffer A for 10 min, which was gradually mixed with 5–35% buffer B for 11 min, and finally mixed with 35–80% buffer B and eluted for 1 min. The entire elution process was monitored by measuring the absorbance at 214 nm, and 20 components were eventually selected. Each component was desalted separately using a Strata-X C18 column (Phenomenex, Torrance, CA, USA) and then freeze-dried.

**Capillary HPLC**

The concentration of each component that was adjusted to about 0.5 μg/μl with buffer C and centrifuged at 20,000 rpm for 10 min to remove insoluble matter. Eight microliters of each component (about 4 μg of protein) was separated using a Shimadzu LC-20AD HPLC system (Shimadzu, Tokyo, Japan). The column included a trap column and an analytical column. The separation parameters were as follows: injection for 4 min at a flow rate of 8 nL/min, washing at a flow rate of 300 nL/min for 40 min, washing with gradient buffer D from 2–35% and then from 35–80%, and washing with 80% buffer D for 4 min and buffer C for 1 min.

**Electrospray ionization mass spectrometry (ESI-MS)**

The peptides were separated using a Q-Exactive mass spectrometer. The primary MS resolution was set to 70,000 full width at half maximum (FWHM). The peptides were screened using a high-energy collision mode with a collision energy of 27 (± 12). Secondary fragments were detected in Orbitrap with a resolution of 17,500 FWHM. In addition, 15 secondary spectra were plotted for primary precursor ions with peak intensities in excess of 20,000, and primary and secondary scanning was performed. Scanned mass-to-charge ratios ranged from 350 to 2000 Da.

**MS data analysis**

The original mass spectrum file was converted to MGF format. The quantitative data were analyzed by iTRAQ Result Multiple File Distiller, and the MGF file was used as an original file with Mascot2.3.02 protein identification software. A selected sheep genome annotation database (22134 sequences) and ORFV virus from the NCBI database (150 sequences) were used for searching and combined with the quantitative results.

**Bioinformatics analysis**

Functional annotation, subcellular localization, and metabolic pathway analysis of the identified differentially expressed proteins were carried out. The hypergeometric test was used to find Gene Ontology (GO) entries that were enriched compared with other proteins. These differentially expressed proteins were compared with the Clusters of Orthologous Groups of proteins (COG) database to predict the possible functions of these proteins and perform statistical analysis on their functional classification.

**RT-qPCR**

Specific primers (Table 1) were designed to amplify various target genes simultaneously according to the corresponding gene sequences of MS/MS-identified proteins, and the available genetic information was deposited in the GenBank database. The analysis was conducted using LaserGene sequence analysis software (DNASTAR, Inc., Madison, WI, USA). GSF monolayers were inoculated with ORFV for 35 h and washed three times with ice-cold PBS. They were then harvested by centrifugation at 1,000 rpm for 10 min. Total cellular RNA was extracted using an RNeasy Mini Kit (QIAGEN GmbH, Hilden, Germany) according to the manufacturer’s protocol. The RNA concentration was measured using a spectrophotometer (260/280 nm). RT-qPCR was performed using an Mx3005P Real-Time PCR System (Agilent Technologies, Inc., Santa Clara, CA, USA). A reverse transcription step was first performed at 42 °C for 5 min to make cDNA from total RNA. The PCR procedure consisted of a denaturation step at 95 °C for 10 s, followed by 40 cycles of amplification, each consisting of an extra denaturation step at 95 °C for 5 s and a primer annealing step at 60 °C for 34 s. Melting curves were plotted, and
quantitative analysis of the data was conducted using the 7500 Fast System SDS 1.3.1 software (Applied Biosystems, Foster City, CA, USA). Mock-infected GSF cells were used as a control. The RT-PCR products were analyzed by electrophoresis in a 2% agarose gel.

Subcellular localization of HSPA1B

Cells were seeded into 6-well plates and cultured overnight in DMEM in the presence of 10% FBS. The transfection mixture, which contained 2.0 μg of plasmid DNA and 6 μL of transfection reagent (Invitrogen, Carlsbad, CA, USA) in 100 μL of serum-free DMEM, was mixed for 20 min at room temperature and subsequently added to each well with complete medium for 24 h. The cells were evaluated for protein expression by fluorescence microscopy at 24–48 h post-transfection and then were fixed by conventional methods and stained with 4’,6-diamidino-2-phenylindole (DAPI). The cellular distribution of HSPA1B was analyzed by confocal laser scanning microscopy (CLSM).

Measurement of the effect of HSPA1B on ORFV replication

In order to examine the influence of HSPA1B on the proliferation of ORFV, GSF cells were transiently transfected with pEFGP-HSPA1B plasmids as described previously. Subsequently, the cells were infected with ORFV at an MOI of 0.1. After incubation for 1 h, the cells were washed three times with PBS and DMEM containing 2% FBS was added. The cultured cells were collected at 15, 24, and 36 h postinfection and the viral DNA was quantitated using RT-qPCR.

RNA interference

Small interfering RNA (siRNA) was chemically synthesized by Gene Pharma (Shanghai, China). The knockdown of endogenous HSPA1B was carried out by transfection of GSF cells with HSPA1B siRNA (517) (5’- UUU GUA GCU CAC CUG CAC CTT-3’), HSPA1B siRNA (1258) (5’- GUU GAA GAA GUC CUG CAG CTT-3’), and HSPA1B siRNA (1486) (5’- GUAGUGGUAGAAGUCUGTT-3’) using Lipofectamine 2000. Nontargeting siRNA (NC siRNA) was used as a negative control.

Western blot

For Western blotting, target proteins were resolved by SDS-PAGE and transferred to an Immobilon-P membrane (Millipore). The membrane was blocked and incubated with appropriate primary antibodies and secondary antibodies. Antibody-antigen complexes were visualized using enhanced chemiluminescence detection reagents (Thermo) [42]. Mouse anti-HSPA1B anti-β-actin antibodies were purchased from Abbkine. Mouse anti-B2L antibody was obtained from the Lanzhou Veterinary Research Institute.

Results

Growth of ORFV in GSF cells

In order to assess the growth of ORFV in GSF cells, a one-step growth curve was carried out using RT-qPCR. ORFV was adapted to GSF infection by passaging for 15
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Generations. At different time points, the supernatants and the cells were collected, and the copy number of viral DNA was determined by RT-qPCR to prepare a one-step growth curve. The results indicated that the intracellular viral DNA content increased from 2 to 36 h postinfection and reached the highest level at 36 h. The extracellular viral DNA content gradually increased (Fig. 1A), while the intracellular viral DNA content decreased during this time period, and the extracellular viral DNA content increased to form an S-shaped curve. The eclipse phase was from 0 to 12 h postinfection, during which the viral content remained at a low level. The logarithmic phase was from 12 to 48 h, and the viral DNA content increased, reaching a peak at 48 h, slowing from 48 to 60 h postinfection, and gradually reaching a plateau (Fig. 1B).

Kinetics of ORFV-induced cytopathology in cultured GSF cells

One of the key parameters for determining virus-induced alterations is the length of time until a cytopathic effect (CPE) is observed in the model system. GSF cells were infected with ORFV at an MOI of approximately 0.1, and monitored for cell viability and CPE. As shown in Fig. 2, the cells infected with ORFV that were cultured for less than 24 h demonstrated no detectable CPE (Fig. 2A-B). At 24 h postinfection, a small number of cells began to swell and had a round shape (Fig. 2C-D). At 60 h, the cells detached from the plate (Fig. 2E) and were completely destroyed after 72 h postinfection (Fig. 2F-G).

Fig. 1 One-step growth curve of ORFV infection in GSF cells. GSF cells were infected with ORFV at an MOI of 0.1 and cell supernatants and infected cells were collected at 2, 12, 18, 24, 36, 48, and 60 h postinfection. RT-qPCR was used to make a one-step growth curve to measure the copy number of the intracellular virus (Fig. 1A) and extracellular virus (Fig. 1B)

Fig. 2 Kinetics of the ORFV-induced cytopathic effect (CPE) in GSF cells. Cells at about 80% confluency were infected with ORFV at an MOI of 0.1 or mock infected, and DMEM was added to the cells in the control group, and after 1 h, the cells were washed three times with PBS, and DMEM 2% FBS was added. Morphological changes were then examined at different time points.
Differentially expressed proteins analyzed by iTRAQ-coupled 2D LC/MS-MS

The cellular proteins in ORFV-infected and mock-infected GSF cells were extracted for iTRAQ analysis. In total, 10,630 peptides and 2,776 proteins were detected. Of these, 282 proteins were found to be differentially expressed in ORFV-infected GSF cells compared with the mock-infected GSF cells, including 222 significantly upregulated proteins and 60 significantly downregulated proteins. The upregulated proteins are listed in Table 2, and the downregulated proteins are listed in Table 3. Three proteins that are involved in cell killing showed altered expression levels. The levels of CXCL6, IFNG (interferon gamma [IFNγ]), and ALBU increased after infection. In addition, 28 proteins involved in cell proliferation exhibited differential expression. NFIP1, IFNG, TKT, CD9, BAK, HS71B, CSK2B, NPM, HDGF, PA2G4, CDV3, RL23A, THIO, MCM7, CXC6L1, YBOX1, RS4X, CYR61, HMOX1, APOD, TANA, RS6, and PAI1 were upregulated, while CAV1, GBG2, ZPR1, MK01, and LAMB2 were downregulated. CXCL6 and IFNG are associated with pathways involved in apoptosis [5, 22] and proliferation [23, 29]. Changes in the levels of biological adhesion proteins were also detected, including PAI1, APOD, CTGF, ADAM9, EZRI, CYR61, CD9, and TKT, which were upregulated, and TBCD, VINC, CO3A1, 2AAA, LAMB2, and PARVA were downregulated. The abundance of the 70-kDa heat shock proteins (HSPs) HSPA6 and HSPA1B increased after infection. Moreover, several ribosomal proteins, including RPS6, RPS17, RPS3, RPS21, RPS18, RPS3A, Rps16, Rps8, RPS4X, Rps23, RPS2, RPS19, RPS12, Rps13, and RPS20 were upregulated (Table 2). However, no ribosomal proteins were downregulated after infection (Table 3).

Functional classification of differentially expressed proteins

To understand the implications of the cellular responses to ORFV infection, these proteins were categorized into three main types using the UniProt Knowledgebase (Swiss-Prot/TrEMBL) and the GO databases: cellular components, metabolic functions, and biological processes. Cell component ontology refers to subcellular structures, locations, and macromolecular complexes, such as nucleoli, telomeres, and complexes that recognize initiation. Cellular-component-based enrichment analysis identified differentially expressed proteins that are well distributed in cell components (Fig. 3A) and are mainly involved in morphogenesis, protein synthesis, metabolism, the stress response, the ubiquitin–proteasome pathway, cellular processes, metabolic processes, biological regulation, and response to stimuli. Molecular function ontology refers to the function of an individual gene product, such as carbohydrate binding or ATP hydrolase activity. Molecular-function-based enrichment analysis demonstrated that binding, catalytic activity, structural molecule activity, and enzymatic regulation were influenced by viral infection (Fig. 3B). Biological processes ontology refers to the ordered combination of molecular functions to achieve a wider range of biological functions, such as mitosis or purine metabolism. Enrichment analysis using biological processes indicated that viral infection mainly affected cellular and metabolic processes (Fig. 3C). A protein may have multiple GO annotations. To determine which biological functions were associated with differentially expressed proteins, a significant enrichment analysis of GO functions was carried out on these differentially expressed proteins. The results revealed that these differentially expressed proteins were mainly localized in the cytoplasm, ribosomes and the nucleus (Fig. 3D). Molecular enrichment analysis showed that these differentially expressed proteins included proteins with nucleic acid binding activity, threonine peptidase activity, and growth factor binding activity (Fig. 3E). Gene set enrichment analysis revealed that these differentially expressed proteins were mainly involved in the initiation and termination of transcription, which are related to virus replication and signal transduction processes (Fig. 3F). The identified differentially expressed proteins were compared with the COG database, and possible functions of these proteins were predicted. COG annotation classification indicated that the differentially expressed proteins were mainly involved in functions such as post-translational modification, protein folding, molecular chaperones, translation, ribosome structure and biosynthesis, energy generation and conversion, and signal transduction (Fig. 3G).

Confirmation of proteomic data by RT-qPCR

Changes in transcription of 12 genes selected from the differentially expressed proteins were analyzed by quantifying their mRNA transcripts. The “β-actin” (ACTB) gene was used as a control. The pattern of differences in mRNA abundance for these genes between infected and control GSF cells was similar to the pattern observed for the corresponding proteins based on LC–MS/MS data. As shown in Fig. 4, the abundance of BCLF1, MAP4, 6PGD, G3P, ZN207, and CDV3 mRNA increased. The SC24D, FIS1, COR1B, and PSME2 genes were downregulated, whereas the PARP1 gene was upregulated. An inconsistency between the RT-qPCR data and the LC–MS/MS data was observed for VMA5A, which was downregulated in the RT-qPCR analysis and upregulated in the LC–MS/MS analysis. This inconsistency might have been due to post-translational modifications, such as methylation, phosphorylation, or acetylation, or to differences in protein degradation rates for unknown reasons. These data provide transcriptional
Table 2  Upregulated proteins identified by iTRAQ analysis of ORFV-infected GSF cells. These proteins had expression ratios > 1.2 relative to the control group at the same time postinfection.

| Group ID | Accession no          | Score | %Cov (95%) | Peptide (95%) | Unique peptide | vorfM_116:CK_119 | Protein abbreviation | Protein description                                      | COG function description               |
|----------|-----------------------|-------|------------|---------------|----------------|------------------|---------------------|----------------------------------------------------------|-----------------------------------------|
| 1        | 1::GOAT_ENSP00000369757 | 185   | 21.1       | 4             | 3              | 1.263            | RPS6                | 40S ribosomal protein S6                                    | Ribosomal protein S6E (S10)                 |
| 2        | 1::GOAT_ENSBTAP0000024092 | 82    | 14.2       | 5             | 5              | 1.234            | AHCY                | Adenosylhomocysteinase                                     | S-adenosylhomocysteine hydrolase              |
| 3        | 1::GOAT_ENSBTAP0000016634 | 130   | 4.2        | 1             | 1              | 1.472            | IFNG                | Interferon gamma                                          | -                                        |
| 4        | 1::GOAT_ENSBTAP0000029157 | 45    | 2.8        | 1             | 1              | 1.319            | FAM98B              | Protein FAM98B                                            | -                                        |
| 5        | 1::GOAT_ENSBTAP0000008184 | 45    | 2.5        | 2             | 2              | 1.219            | RPS17               | 40S ribosomal protein S17                                   | Ribosomal protein S17E                      |
| 6        | 1::GOAT_ENSBTAP0000019643 | 61    | 2.5        | 4             | 4              | 1.392            | CD9                 | CD9 antigen                                               | -                                        |
| 7        | 1::GOAT_ENSP00000410059 | 306   | 12.3       | 4             | 4              | 1.347            | EEF1D               | Elongation factor 1-delta                                  | Translation elongation factor EF-1beta      |
| 8        | 1::GOAT_ENSBTAP0000015277 | 190   | 9.3        | 7             | 7              | 1.247            | PYGL                | Glycogen phosphorylase, liver form                        | Glucan phosphorylase                        |
| 9        | 1::GOAT_ENSBTAP00000278572 | 244   | 39.9       | 8             | 8              | 1.337            | RPS3                | 40S ribosomal protein S3                                   | Ribosomal protein S3                        |
| 10       | 1::GOAT_ENSBTAP00000325376 | 361   | 12.7       | 9             | 9              | 1.343            | HNRNPM              | Heterogeneous nuclear ribonucleoprotein M                  | RNA-binding proteins (RRM domain)            |
| 11       | 1::GOAT_ENSBTAP000002587 | 48    | 16.1       | 3             | 3              | 1.231            | SNRNP70             | U1 small nuclear ribonucleoprotein 70 kDa                 | RNA-binding proteins (RRM domain)            |
| 12       | 1::GOAT_ENSBTAP00000391481 | 131   | 14.7       | 5             | 5              | 1.267            | TKT                 | Transketolase                                             | Transketolase                              |
| 13       | 1::GOAT_ENSBTAP00000408263 | 379   | 41.7       | 13            | 13             | 1.226            | SELENBP1            | Selenium-binding protein 1                                 | -                                        |
| 14       | 1::GOAT_ENSBTAP00000313007 | 266   | 12.8       | 7             | 4              | 1.398            | PABPC1              | Polyadenylate-binding protein 1                            | RNA-binding proteins (RRM domain)            |
| 15       | 1::GOAT_ENSBTAP0000027991 | 166   | 16.3       | 9             | 8              | 1.261            | Khsrp               | Far upstream element-binding protein 2                     | -                                        |
| 16       | 1::GOAT_ENSBTAP00000264703 | 94    | 18.4       | 5             | 5              | 1.291            | ELAVL1              | ELAV-like protein 1                                        | RNA-binding proteins (RRM domain)            |
| 17       | 1::GOAT_ENSBTAP0000041860 | 241   | 34         | 3             | 3              | 1.246            | TXN                 | Thioredoxin                                                | Thiol-disulfide isomerase and thioredoxins  |
| 18       | 1::GOAT_ENSBTAP000002045 | 71    | 18.1       | 5             | 5              | 1.317            | MESDC2              | LDLR chaperone MESD                                        | -                                        |
| 19       | 1::GOAT_ENSBTAP0000022576 | 208   | 20.5       | 3             | 1              | 1.352            | h2afv               | Histone H2A.V                                              | Histone H2A                                |
| 20       | 1::GOAT_ENSBTAP00000367550 | 1243  | 19.1       | 8             | 2              | 1.335            | TPM2                | Tropomyosin beta chain                                     | -                                        |
| 21       | 1::GOAT_ENSBTAP0000027713 | 50    | 12.3       | 1             | 1              | 2.11             | RPS21               | 40S ribosomal protein S21                                   | Cold shock proteins                         |
| 22       | 1::GOAT_ENSBTAP0000023094 | 167   | 20.6       | 3             | 2              | 1.974            | YBX1                | Nuclelease-sensitive element-binding protein 1             | -                                        |
| 23       | 1::GOAT_ENSBTAP0000021345 | 19    | 0.3        | 1             | 1              | 1.418            | RGPD5               | RANBP2-like and GRIP domain-containing protein 5/6         | -                                        |
| 24       | 1::GOAT_ENSBTAP00000225972 | 188   | 19         | 5             | 5              | 1.332            | LRRC59              | Leucine-rich repeat-containing protein 59                  | Leucine-rich repeat (LRR) protein           |
| 25       | 1::GOAT_ENSBTAP0000000630 | 63    | 16.6       | 4             | 4              | 1.32             | AKR1A1              | Alcohol dehydrogenase [NADP(+)]                           | Aldo/keto reductases, related to diketogulonate reductase |
| Group ID | Accession no       | Score | %Cov  | Peptide | Unique peptide | vortM_116: | Protein description                                                                 |
|----------|--------------------|-------|-------|---------|---------------|-----------|-------------------------------------------------------------------------------------|
| 26       | 1::GOAT_ENSP00000279230 | 75    | 0.7   | 1       | 1             | 1.578     | PLCB3 1-phosphatidylinositol 4,5-bis-phosphate phosphodiesterase beta-3               |
| 27       | 1::GOAT_ENSBTAP0000040666 | 80    | 5.8   | 2       | 2             | 1.441     | TOE1 Target of EGR1 protein 1                                                       |
| 28       | 1::GOAT_ENSBTAP0000004408 | 113   | 4.9   | 4       | 4             | 1.24      | SMARCA5 SWI/SNF-related matrix-associated actin-dependent regulator of chromatin    |
|          |                     |       |       |         |               |           | subfamily A member 5                                                                |
| 29       | 1::GOAT_ENSP00000325905 | 102   | 10.9  | 2       | 1             | 1.527     | Srsf7 Serine/arginine-rich splicing factor 7                                         |
| 30       | 1::GOAT_ENSBTAP0000021643 | 262   | 10.9  | 2       | 2             | 1.376     | Snrpf Small nuclear ribonucleoprotein protein F                                      |
| 31       | 1::GOAT_ENSP00000246789  | 38    | 10    | 3       | 3             | 1.221     | PRMT1 Protein arginine N-methyltransferase 1                                         |
| 32       | 1::GOAT_ENSBTAP0000029007 | 87    | 11.2  | 3       | 3             | 1.288     | ZC3H15 Zinc finger CCCH domain-containing protein 15 SV =1                           |
| 33       | 1::GOAT_ENSP00000416110  | 111   | 13.8  | 3       | 3             | 1.474     | RPS18 40S ribosomal protein S18                                                     |
| 34       | 1::GOAT_ENSP00000253024  | 169   | 6.6   | 3       | 3             | 1.274     | TRIM28 Transcription intermediary factor 1-beta                                    |
| 35       | 1::GOAT_ENSP00000327539  | 485   | 25.2  | 8       | 2             | 1.44      | HNRNPH1 Heterogeneous nuclear ribonucleoprotein H                                  |
| 36       | 1::GOAT_ENSBTAP0000020081 | 26    | 5.3   | 1       | 1             | 1.488     | SGTA Small glutamine-rich tetra-tripeptide repeat-containing protein alpha          |
| 37       | 1::GOAT_ENSP00000356420  | 64    | 7.8   | 3       | 3             | 1.266     | UCHL5 Ubiquitin carboxyl-terminal hydrolase isozyme L5                             |
| 38       | 1::GOAT_ENSP00000253332  | 194   | 6.3   | 6       | 6             | 1.236     | AKAP12 A-kinase anchor protein 12                                                   |
| 39       | 1::GOAT_ENSP00000296755  | 297   | 5.5   | 9       | 8             | 1.206     | MAP1B Microtubule-associated protein 1B                                             |
| 40       | 1::GOAT_ENSBTAP0000008386 | 137   | 12.2  | 5       | 5             | 1.771     | GPI Glucose-6-phosphate isomerase                                                   |
| 41       | 1::GOAT_ENSBTAP0000012977 | 141   | 7.6   | 2       | 2             | 1.266     | CYR61 Protein CYR61                                                                |
| 42       | 1::GOAT_ENSBTAP00000081111 | 103   | 14.7  | 6       | 6             | 1.424     | RBM45 RNA-binding protein 45                                                       |
| 43       | 1::GOAT_ENSP00000346120  | 179   | 11    | 6       | 6             | 1.294     | DDX21 Nucleolar RNA helicase 2                                                      |
| 44       | 1::GOAT_ENSP00000262193  | 101   | 27.4  | 5       | 5             | 1.381     | PSMB1 Proteasome subunit beta type-1                                               |
| 45       | 1::GOAT_ENSBTAP0000013079 | 234   | 22.7  | 5       | 5             | 1.425     | RPS3A 40S ribosomal protein S3a                                                     |

Table 2 (continued)
| Group ID | Accession no | Score | %Cov (95%) | Peptide (95%) | Unique peptide | vorfM_116: CK_119 | Protein abbreviation | Protein description | COG function description |
|----------|--------------|-------|------------|---------------|----------------|-------------------|---------------------|---------------------|------------------------|
| 46       | 1::GOAT_ENSP00000378669 | 382   | 40.9       | 11            | 11             | 1.216             | ALDOA               | Fructose-bisphosphate aldolase A | Fructose-1,6-bisphosphate aldolase |
| 47       | 1::GOAT_ENSP00000307288 | 65    | 11         | 6             | 6              | 1.245             | MCM7                | DNA replication licensing factor MCM7 | Predicted ATPase involved in replication control, Cdc46/Mcm family |
| 48       | 1::GOAT_ENSP00000357876 | 69    | 10.3       | 3             | 3              | 1.404             | PSMD4               | 26S proteasome non-ATPase regulatory subunit 4 | 26S proteasome regulatory complex, subunit RPN10/PSMD4 |
| 49       | 1::GOAT_ENSBTAP0000001351 | 90    | 6.7        | 2             | 1              | 1.413             | GDF10               | Bone morphogenetic protein 3B | - |
| 50       | 1::GOAT_ENSP00000262584 | 162   | 14         | 2             | 2              | 1.511             | RPL8                | 60S ribosomal protein L8 | Ribosomal protein L2 |
| 51       | 1::GOAT_ENSP00000359910 | 134   | 31.5       | 5             | 5              | 1.265             | PSMA7               | Proteasome subunit alpha type-7 | 20S proteasome, alpha and beta subunits |
| 52       | 1::GOAT_ENSBTAP0000019980 | 23    | 4.6        | 1             | 1              | 1.675             | CG059               | UPF0539 protein C7orf59 homolog | - |
| 53       | 1::goat_GLEAN_10016260 | 255   | 25.7       | 3             | 1              | 1.255             | FIL                 | Ferritin light chain (Fragment) | Ferritin-like protein |
| 54       | 1::GOAT_ENSP00000324111-D9 | 45    | 2.3        | 1             | 1              | 1.46              | OR10AG1             | Olfactory receptor 10AG1 | - |
| 55       | 1::GOAT_ENSP00000377385 | 61    | 1.8        | 1             | 1              | 1.34              | Ppun                | Suppressor of SWI4 1 homolog | - |
| 56       | 1::GOAT_ENSBTAP0000001566 | 1208  | 17.9       | 8             | 8              | 1.666             | CALD1               | Caldesmon | - |
| 57       | 1::GOAT_ENSBTAP000000240 | 107   | 16.9       | 2             | 2              | 1.205             | ATP6V1G1            | V-type proton ATPase subunit G1 | - |
| 58       | 1::GOAT_ENSP00000376290 | 232   | 24.7       | 5             | 5              | 1.695             | TRA2B               | Transformer-2 protein homolog beta | RNA-binding proteins (RRM domain) |
| 59       | 1::GOAT_ENSP00000407602 | 300   | 8.4        | 13            | 13             | 1.511             | MAP4                | Microtubule-associated protein 4 | - |
| 60       | 1::GOAT_ENSBTAP0000053740 | 364   | 9.7        | 8             | 8              | 1.379             | RRP1                | Ribosome-binding protein 1 | PPE-repeat proteins |
| 61       | 1::GOAT_ENSP00000328773 | 46    | 6.6        | 2             | 2              | 1.415             | HEXIM1              | Protein HEXIM1 | - |
| 62       | 1::GOAT_ENSBTAP0000043789 | 80    | 8.9        | 2             | 2              | 1.454             | APOD                | Apolipoprotein D | Bacterial lipocalin |
| 63       | 1::goat_GLEAN_10013438 | 162   | 13.2       | 6             | 6              | 1.325             | HNRNPU              | Heterogeneous nuclear ribonucleoprotein U | - |
| 64       | 1::GOAT_ENSBTAP00000015619 | 41    | 10.3       | 2             | 2              | 1.289             | MRPL14              | 39S ribosomal protein L14, mitochondrial | Ribosomal protein L14 |
| 65       | 1::GOAT_ENSP00000415615 | 49    | 8.1        | 2             | 2              | 1.332             | Csnk2b              | Casein kinase II subunit beta | Casein kinase II, beta subunit |
| 66       | 1::GOAT_ENSP000000315309 | 71    | 20.5       | 4             | 4              | 1.571             | PSMA1               | Proteasome subunit alpha type-1 | 20S proteasome, alpha and beta subunits |
| 67       | 1::GOAT_ENSBTAP00000022763 | 293   | 13.3       | 7             | 7              | 1.359             | ALB                 | Serum albumin | - |
| 68       | 1::GOAT_ENSP00000281537 | 47    | 2.7        | 4             | 4              | 1.357             | TJP1                | Tight junction protein ZO-1 | - |
| Group ID | Accession no             | Score | %Cov  | Peptide | Unique peptide | vormM_116: CK_119 | Protein abbreviation | Protein description                                      | COG function description                                      |
|----------|--------------------------|-------|-------|---------|----------------|------------------|--------------------|----------------------|----------------------------------------------------------|
| 69       | 1::GOAT_ENSBTAP00000013796 | 125   | 14.4  | 5       | 5              | 1.42             | PA2G4              | Proliferation-associated protein 2G4                     | Methionine aminopeptidase                                     |
| 70       | 1::GOAT_ENSBTAP00000013522 | 56    | 8.6   | 2       | 2              | 1.234            | DHRSL1             | Dehydrogenase/reductase SDR family member 1              | Dehydrogenases with different specificities (related to short-chain alcohol dehydrogenases) |
| 71       | 1::GOAT_ENSBTAP00000011762 | 75    | 20.8  | 2       | 2              | 1.228            | PFDN5              | Prefoldin subunit 5                                       | Predicted prefoldin, molecular chaperone implicated in de novo protein folding |
| 72       | 1::goat_GLEAN_10013034    | 1401  | 24.9  | 5       | 3              | 1.45             | NPMI               | Nucleosomin                                                | -                                                          |
| 73       | 1::GOAT_ENSP00000422319   | 229   | 13.8  | 11      | 11             | 1.235            | MATR3              | Matrin-3                                                   | -                                                          |
| 74       | 1::GOAT_ENSP00000380362   | 83    | 4     | 2       | 2              | 1.455            | EIF3D              | Eukaryotic translation initiation factor 3 subunit D     | -                                                          |
| 75       | 1::GOAT_ENSP00000367806   | 102   | 7.5   | 1       | 1              | 1.283            | Rps16              | 40S ribosomal protein S16                                 | Ribosomal protein S9                                         |
| 76       | 1::GOAT_ENSP00000357206   | 1438  | 27.6  | 25      | 25             | 1.307            | TANA               | Tanabin                                                    | -                                                          |
| 77       | 1::GOAT_ENSP0000031785    | 217   | 12.7  | 6       | 6              | 1.207            | HNRN-PUL2          | Heterogeneous nuclear ribonucleoprotein U-like protein 2 | -                                                          |
| 78       | 1::GOAT_ENSBTAP00000016560| 192   | 12    | 8       | 8              | 1.261            | CDCP1              | CUB domain-containing protein 1                           | -                                                          |
| 79       | 1::GOAT_ENSBTAP00000026323| 44    | 14.9  | 2       | 2              | 1.488            | PRKCDBP            | Protein kinase C delta-binding protein                     | -                                                          |
| 80       | 1::GOAT_ENSP00000379888   | 285   | 42.5  | 7       | 7              | 1.564            | Rps8               | 40S ribosomal protein S8                                   | Ribosomal protein S8E                                       |
| 81       | 1::GOAT_ENSP00000381785   | 147   | 20.1  | 7       | 7              | 1.239            | HSDL2              | Hydroxysteroid dehydrogenase-like protein 2              | Dehydrogenases with different specificities (related to short-chain alcohol dehydrogenases) |
| 82       | 1::GOAT_ENSBTAP00000028994| 37    | 9.3   | 3       | 3              | 1.303            | DNAJB4             | DnaJ homolog subfamily B member 4                         | DnaJ-class molecular chaperone                               |
| 83       | 1::GOAT_ENSBTAP00000021229| 51    | 4.1   | 1       | 1              | 1.225            | SCN2B              | Sodium channel subunit beta-2                             | -                                                          |
| 84       | 1::GOAT_ENSBTAP0000017837 | 111   | 5.3   | 4       | 4              | 1.363            | RBM12B             | RNA-binding protein 1B                                     | -                                                          |
| 85       | 1::GOAT_ENSBTAP0000008609 | 46    | 12.4  | 2       | 2              | 1.591            | HDGF               | Hepatoma-derived growth factor                            | -                                                          |
| 86       | 1::GOAT_ENSBTAP0000004635 | 67    | 6.3   | 3       | 3              | 1.455            | HPX                | Hemopexin                                                  | -                                                          |
| 87       | 1::GOAT_ENSP00000350990   | 104   | 2.9   | 3       | 3              | 1.274            | TNKS1BP1           | 182 kDa tankyrase-1-binding protein                       | -                                                          |
| 88       | 1::GOAT_ENSBTAP0000007584 | 27    | 0.5   | 1       | 1              | 2.199            | NPHP3              | Nephrocystin-3                                            | FOG: TPR repeat                                             |
| 89       | 1::GOAT_ENSP00000309415   | 69    | 12    | 2       | 2              | 1.414            | CLTB               | Clathrin light chain B                                     | -                                                          |
| Group ID | Accession no       | Score | %Cov (95%) | Peptide (95%) | Unique peptide | vortM_116: CK_119 | Protein abbreviation | Protein description                  | COG function description             |
|---------|--------------------|-------|------------|--------------|----------------|------------------|----------------------|----------------------|-------------------------------------|
| 90      | 1::GOAT_ENSP00000361777 | 136   | 16.8       | 4            | 4              | 1.376            | SET                  | Protein SET              | -                                   |
| 91      | 1::GOAT_ENSP00000401336 | 515   | 30.6       | 9            | 9              | 1.348            | Srsfl                | Serine/arginine-rich splicing factor 1 | RNA-binding proteins (RRM domain)    |
| 92      | 1::GOAT_ENSP00000370739 | 682   | 22.3       | 6            | 1              | 1.632            | ENO3                 | Enolase                | -                                   |
| 93      | 1::GOAT_ENSP00000353878 | 31    | 11.5       | 2            | 2              | 1.216            | BAK1                 | Bel-2 homologous antagonist/killer  | -                                   |
| 94      | 1::GOAT_ENSBTAP0000012939 | 109   | 50         | 3            | 3              | 1.322            | CXCL6                | C-X-C motif chemokine 6 | -                                   |
| 95      | 1::GOAT_ENSBTAP0000019232 | 208   | 18.2       | 5            | 5              | 1.397            | SERPINE1             | Serine protease inhibitor 1       | -                                   |
| 96      | 1::GOAT_ENSBTAP0000041265 | 206   | 16.6       | 5            | 5              | 1.211            | NDUFV3               | NADH dehydrogenase (ubiquinone) flavoprotein 3, mitochondrial | -                                   |
| 97      | 1::GOAT_ENSBTAP0000029400 | 52    | 3.2        | 3            | 3              | 1.258            | SPECC1L              | Cytospin-A             | Ca2+ -binding actin-bundling protein limbrin/plastin (EF-Hand superfamily) |
| 98      | 1::GOAT_ENSP00000362744 | 353   | 28.6       | 6            | 6              | 1.229            | RPS4X                | 40S ribosomal protein S4, X isoform | Ribosomal protein S4E             |
| 99      | 1::GOAT_ENSP00000355011 | 210   | 21.1       | 5            | 5              | 1.272            | ILF2                 | Interleukin enhancer-binding factor 2 | -                                   |
| 100     | 1::GOAT_ENSP00000296674 | 67    | 15.5       | 2            | 2              | 1.38             | Rps23                | 40S ribosomal protein S23         | Ribosomal protein S12             |
| 101     | 1::GOAT_ENSBTAP0000053088 | 71    | 6.2        | 1            | 1              | 1.293            | H1FX                 | Histone H1.x OS = Homo sapiens | -                                   |
| 102     | 1::GOAT_ENSBTAP0000007571 | 96    | 6.4        | 3            | 3              | 1.36             | FUS                  | RNA-binding protein FUS          | -                                   |
| 103     | 1::goat_GLEAN_1000207 | 721   | 48.1       | 5            | 3              | 1.342            | Tpm3                 | Tropomyosin alpha-3 chain       | -                                   |
| 104     | 1::GOAT_ENSBTAP0000019184 | 94    | 29.3       | 3            | 3              | 1.232            | RPL22                | 60S ribosomal protein L22        | -                                   |
| 105     | 1::GOAT_ENSBTAP0000051668 | 327   | 9.6        | 4            | 2              | 1.497            | HSPA6                | Heat shock 70 kDa protein 6      | Molecular chaperone                 |
| 106     | 1::GOAT_ENSBTAP0000053339 | 133   | 9.2        | 9            | 9              | 1.325            | ERC1                 | ELKS/Rab6-interacting/CAST family member 1 | -                                   |
| 107     | 1::GOAT_ENSBTAP0000051256-D3 | 151   | 14.6       | 3            | 3              | 1.6              | HIST1H1C             | Histone H1.2                 | -                                   |
| 108     | 1::GOAT_ENSBTAP0000012735 | 182   | 19.6       | 3            | 2              | 1.46             | YBX1                 | Nuclease-sensitive element-binding protein 1 | Cold shock proteins            |
| 109     | 1::GOAT_ENSBTAP0000012544 | 283   | 37.4       | 9            | 9              | 1.519            | RPS2                 | 40S ribosomal protein S2         | Ribosomal protein S5              |
| 110     | 1::GOAT_ENSBP00000349428 | 212   | 11         | 4            | 3              | 1.222            | PTBP1                | Polypyrimidine tract-binding protein 1 | -                                   |
| Group ID | Accession no | Score | %Cov (95%) | Peptide (95%) | Unique peptide | vortM_116:CK_119 | Protein abbreviation | Protein description | COG function description |
|----------|--------------|-------|------------|--------------|---------------|------------------|--------------------|---------------------|----------------------|
| 111      | 1::GOAT_ENSBTAP00000011484 | 300   | 19.5       | 6            | 6             | 1.485            | SSB                | Lupus La protein homolog | La protein, small RNA-binding pol III transcript stabilizing protein and related La-motif-containing proteins involved in translation |
| 112      | 1::GOAT_ENSBTAP00000053296 | 129   | 4.3        | 3            | 3             | 1.418            | PALM2              | Paralemmin-2          | -                    |
| 113      | 1::GOAT_ENSBTAP00000020452 | 286   | 19.7       | 3            | 3             | 1.273            | RPL18              | 60S ribosomal protein L18 | Ribosomal protein L18E |
| 114      | 1::GOAT_ENSBTAP00000049167 | 47    | 1.9        | 1            | 1             | 1.352            | SUCNR1             | Succinate receptor 1    | -                    |
| 115      | 1::GOAT_ENSBTAP00000049804 | 100   | 8.4        | 2            | 2             | 1.616            | NUCKS1             | Nuclear ubiquitous casein and cyclin-dependent kinase substrate 1 | - |
| 116      | 1::goat_GLEAN_10006421     | 106   | 7          | 2            | 2             | 1.525            | DYNC1I2            | Cytoplasmic dynein 1 intermediate chain 2 | - |
| 117      | 1::GOAT_ENSP0000015769     | 186   | 6.1        | 5            | 5             | 1.368            | ITIH3              | Inter-alpha-trypsin inhibitor heavy chain H3 | Uncharacterized protein containing a von Willebrand factor type A (vWA) domain |
| 118      | 1::GOAT_ENSP00000359506     | 154   | 8.8        | 4            | 3             | 1.509            | FMR1               | Fragile X mental retardation protein 1 | - |
| 119      | 1::GOAT_ENSBTAP0000042575 | 28    | 0.7        | 1            | 1             | 1.349            | PPP4R4             | Serine/threonine-protein phosphatase 4 regulatory subunit 4 | - |
| 120      | 1::GOAT_ENSP00000359345     | 133   | 25         | 7            | 7             | 1.535            | RPL5               | 60S ribosomal protein L5 | Ribosomal protein L18 |
| 121      | 1::GOAT_ENSP00000340278     | 249   | 38.6       | 5            | 5             | 1.292            | PARK7              | Protein DJ-1           | Putative intracellular protease/amidase |
| 122      | 1::GOAT_ENSBTAP0000019203 | 55    | 6.9        | 2            | 2             | 1.334            | PSMA4              | Proteasome subunit alpha type-4 | 20S proteasome, alpha and beta subunits |
| 123      | 1::GOAT_ENSP00000353224     | 776   | 27.8       | 18           | 18            | 1.262            | TFRC               | Transferrin receptor protein 1 | Permeases of the major facilitator superfamly |
| 124      | 1::GOAT_ENSBTAP0000040563 | 32    | 3.8        | 2            | 2             | 1.218            | SLC2A1             | Solute carrier family 2, facilitated glucose transporter member 1 | - |
| 125      | 1::GOAT_ENSP00000376159     | 89    | 4.5        | 3            | 2             | 2.17             | BCLAF1             | Bel-2-associated transcription factor 1 | - |
| 126      | 1::GOAT_ENSP00000379733     | 155   | 38.8       | 9            | 6             | 1.512            | RPL7               | 60S ribosomal protein L7 | Ribosomal protein L30/L7E |
| 127      | 1::GOAT_ENSP00000362110     | 174   | 15.6       | 6            | 6             | 1.27             | SF3A3              | Splicing factor 3A subunit 3 | Splicing factor 3a, subunit 3 |
| 128      | 1::GOAT_ENSBTAP0000002349 | 137   | 28.4       | 2            | 2             | 1.235            | RPL36              | 60S ribosomal protein L36 | Ribosomal protein L36E |
| 129      | 1::goat_GLEAN_10017787      | 518   | 16.4       | 10           | 6             | 1.206            | EZR                | Ezrin                | - |
| 130      | 1::GOAT_ENSP00000378720     | 262   | 7.5        | 8            | 8             | 1.239            | KTN1               | Kinectin              | - |
| 131      | 1::GOAT_ENSP00000365950     | 111   | 8.1        | 1            | 1             | 1.656            | RBM3               | Putative RNA-binding protein 3 | RNA-binding proteins (RRM domain) |
Inhibition of orf virus replication by HSPA1B

| Group ID   | Accession no                  | Score | %Cov (95%) | Peptide (95%) | Unique peptide | vortM_116:CK_119 | Protein abbreviation | Protein description | COG function description                  |
|------------|-------------------------------|-------|------------|---------------|----------------|------------------|----------------------|-----------------------|--------------------------------------------|
| 132        | 1::GOAT_ENSP00000354314       | 31    | 1.7        | 1             | 1              | 1.38             | HAO2                 | Hydroxyacid oxidase 2 | L-lactate dehydrogenase (FMN-dependent) and related alpha-hydroxy acid dehydrogenases |
| 133        | 1::GOAT_ENSP00000306099       | 42    | 4.5        | 2             | 2              | 1.534            | FGB                  | Fibrinogen beta chain | -                                         |
| 134        | 1::GOAT_ENSBTAP0000003636     | 55    | 8.5        | 2             | 2              | 1.325            | PSMA3                | Proteasome subunit alpha type-3 | 20S proteasome, alpha and beta subunits |
| 135        | 1::GOAT_ENSP00000229270       | 278   | 47.2       | 6             | 5              | 1.897            | TPI1                 | Triosephosphate isomerase   | Triosephosphate isomerase |
| 136        | 1::GOAT_ENSBTAP0000019039     | 186   | 17.3       | 4             | 4              | 1.234            | HNRNPH3              | Heterogeneous nuclear ribonucleoprotein H3 | -                                         |
| 137        | 1::GOAT_ENSBTAP0000036278     | 37    | 5.7        | 3             | 3              | 1.27             | CNP                  | 2',3'-cyclic-nucleotide 3'-phosphodiesterase | -                                         |
| 138        | 1::GOAT_ENSBTAP0000025094     | 163   | 9.4        | 3             | 3              | 1.206            | HARS                 | Histidine-tRNA ligase, cytoplasmic | Histidyl-tRNA synthetase |
| 139        | 1::GOAT_ENSP00000316042       | 136   | 14.4       | 2             | 2              | 1.23             | HNRNPA0              | Heterogeneous nuclear ribonucleoprotein A0 | RNA-binding proteins (RRM domain) |
| 140        | 1::GOAT_ENSP00000393738-D2    | 49    | 1.2        | 1             | 1              | 1.564            | GALNT13              | Polypeptide N-acetylgalactosaminyltransferase 13 | -                                         |
| 141        | 1::GOAT_ENSP00000340176       | 68    | 17.7       | 3             | 3              | 1.208            | RBPMS                | RNA-binding protein with multiple splicing | -                                         |
| 142        | 1::goat_GLEAN_10004749        | 243   | 17.5       | 6             | 4              | 1.328            | KRT10                | Keratin, type I cytoskeletal 10 | -                                         |
| 143        | 1::GOAT_ENSP00000346634       | 159   | 6.2        | 5             | 4              | 1.551            | THRAP3               | Thyroid hormone receptor-associated protein 3 | -                                         |
| 144        | 1::GOAT_ENSBTAP0000023664     | 90    | 32.7       | 3             | 3              | 1.307            | ERH                  | Enhancer of rudimentary homolog (Fragment) | -                                         |
| 145        | 1::GOAT_ENSP00000349140       | 148   | 14.4       | 1             | 1              | 1.337            | MTPN                 | Myotrophin               | FOG: Ankyrin repeat |
| 146        | 1::GOAT_ENSP00000405965       | 82    | 13.5       | 1             | 1              | 2.004            | SUMO2                | Small ubiquitin-related modifier 2   | Ubiquitin-like protein (sentrin) |
| 147        | 1::GOAT_ENSP00000409666       | 82    | 10.1       | 1             | 1              |               |                     |                       |                                           |
| 148        | 1::GOAT_ENSBTAP0000001518     | 78    | 8.4        | 2             | 2              | 1.49             | CLTA                 | Clathrin light chain A | -                                         |
| 149        | 1::GOAT_ENSBTAP00000050222    | 91    | 11.2       | 8             | 8              | 1.344            | KIAA1967             | DBIRD complex subunit KIAA1967 | -                                         |
| 149        | 1::goat_GLEAN_10016995        | 380   | 23.7       | 4             | 4              | 1.562            | RPL17                | 60S ribosomal protein L17 | Ribosomal protein L22 |
| 150        | 1::GOAT_ENSBTAP0000041518     | 56    | 1          | 1             | 1              | 1.589            | Ky                   | Kyphoscoliosis peptidase           | Uncharacterized protein involved in cytokinesis, contains TGc (transglutaminase/protease-like) domain |

**Table 2 (continued)**
| Group ID | Accession no | Score | %Cov (95%) | Peptide (95%) | Unique peptide | vortM_116: CK_119 | Protein abbreviation | Protein description | COG function description |
|----------|--------------|-------|------------|--------------|----------------|----------------|---------------------|---------------------|------------------------|
| 151      | 1::GOAT_ENSBTAP0000016153 | 61    | 47.9       | 5            | 5              | 1.275 | SNRPD2 | Small nuclear ribonucleoprotein Sm D2 | Small nuclear ribonucleoprotein (snRNP) homolog |
| 152      | 1::GOAT_ENSP00000420195   | 102   | 16.7       | 4            | 4              | 1.259 | Srsf10 | Serine/arginine-rich splicing factor 10 | - |
| 153      | 1::GOAT_ENSBTAP0000004122 | 48    | 12.6       | 2            | 2              | 1.369 | NDUFAF4 | NADH dehydrogenase [ubiquinone] 1 alpha subcomplex assembly factor 4 | - |
| 154      | 1::GOAT_ENSP00000318195   | 675   | 22.8       | 15           | 13             | 1.499 | NCL    | Nucleolin | RNA-binding proteins (RRM domain) |
| 155      | 1::goat_GLEAN_1000538     | 34    | 9.8        | 2            | 2              | 1.407 | HVM63  | Ig heavy chain Mem5 (Fragment) | - |
| 156      | 1::GOAT_ENSBTAP0000047729-D2 | 217  | 20.5       | 7            | 6              | 1.382 | LDHA   | L-lactate dehydrogenase A chain | Malate/lactate dehydrogenases |
| 157      | 1::GOAT_ENSP00000253814   | 50    | 7.5        | 2            | 2              | 1.578 | NDFIP1 | NEDD4 family-interacting protein 1 | - |
| 158      | 1::GOAT_ENSBTAP0000002326 | 699   | 85.2       | 7            | 7              | 1.386 | RPLP2  | 60S acidic ribosomal protein P2 (Fragment) | Ribosomal protein L12E/L44/L45/RPP1/RPP2 |
| 159      | 1::GOAT_ENSBTAP0000008357 | 43    | 7.5        | 2            | 2              | 1.435 | CTGF   | Connective tissue growth factor | - |
| 160      | 1::GOAT_ENSP00000364119   | 90    | 13.5       | 3            | 3              | 1.263 | EIF2S2 | Eukaryotic translation initiation factor 2 subunit 2 | Translation initiation factor 2, beta subunit (eIF-2beta)/eIF-5 N-terminal domain |
| 160      | 1::goat_GLEAN_10014323    | 90    | 13.5       | 3            | 3              | 1.477 | PSME4  | Proteasome activator complex subunit 4 | - |
| 162      | 1::GOAT_ENSBTAP0000027001 | 32    | 0.4        | 1            | 1              | 1.414 | PSMB6  | Proteasome subunit beta type-6 | 20S proteasome, alpha and beta subunits |
| 163      | 1::GOAT_ENSP00000338727   | 93    | 1.9        | 1            | 1              | 1.279 | Lrrfip2 | Leucine-rich repeat flightless-interacting protein 2 | - |
| 164      | 1::GOAT_ENSBTAP0000011029 | 93    | 7.7        | 4            | 4              | 1.553 | CAST   | Calpastatin | - |
| 165      | 1::GOAT_ENSBTAP0000017988 | 158   | 10.4       | 5            | 5              | 1.531 | PGD    | 6-phosphogluconate dehydrogenase, decarboxylating | 6-phosphogluconate dehydrogenase |
| 166      | 1::GOAT_ENSP00000376055   | 195   | 38.7       | 5            | 5              | 1.226 | EEF1B  | Elongation factor 1-beta | Translation elongation factor EF-1beta |
| 167      | 1::GOAT_ENSBTAP0000029284 | 117   | 30.7       | 4            | 4              | 1.723 | SUB1   | Activated RNA polymerase II transcriptional coactivator p15 | - |
| 168      | 1::GOAT_ENSP00000377469   | 158   | 26.1       | 4            | 4              | 1.247 | NACA   | Nascent polypeptide-associated complex subunit alpha | Transcription factor homologous to NACalpha-BTF3 |
Table 2 (continued)

| Group ID | Accession no | Score | %Cov (95%) | Peptide (95%) | Unique peptide | vortM_116: CK_119 | Protein abbreviation | Protein description | COG function description |
|----------|--------------|-------|------------|--------------|----------------|-------------------|---------------------|----------------------|-------------------------|
| 169      | 1::GOAT_ENSBTAP00000015875-D2 | 108   | 24.2       | 4            | 4              | 1.596             | RPS19               | 40S ribosomal protein S19 | Ribosomal protein S19E (S16A) |
| 170      | 1::GOAT_ENSP000000403265    | 1786  | 41.3       | 21           | 21             | 1.606             | PKM2                | Pyruvate kinase isozymes M1/M2 | Pyruvate kinase |
| 171      | 1::GOAT_ENSBTAP00000028486  | 68    | 24.3       | 5            | 3              | 1.755             | ANP32B              | Acidic leucine-rich nuclear phosphoprotein 32 family member B | - |
| 172      | 1::GOAT_ENSBTAP00000003340  | 86    | 23.5       | 5            | 4              | 1.221             | Fbl                 | rRNA 2′-O-methyltransferase fibrillin | Fibrillarin-like rRNA methylase |
| 173      | 1::GOAT_ENSBTAP0000001791-D2 | 96    | 18.1       | 2            | 2              | 1.252             | RPS12               | 40S ribosomal protein S12 | Ribosomal protein HS6-type (S12/L30/L7a) |
| 173      | 1::goat_GLEAN_10019219     | 96    | 18.7       | 2            | 2              |                   |                     |                      |                         |
| 174      | 1::GOAT_ENSBTAP00000009803  | 137   | 23.5       | 3            | 3              | 1.211             | RPL10               | 60S ribosomal protein L10 | Ribosomal protein L16/L10E |
| 175      | 1::goat_GLEAN_10019253     | 401   | 32.8       | 5            | 2              | 1.541             | PPIA                | Peptidyl-prolyl cis–trans isomerase A | Peptidyl-prolyl cis–trans isomerase (rotamase)—cyclophilin family |
| 176      | 1::GOAT_ENSBTAP00000031070 | 73    | 11         | 4            | 4              | 1.211             | CNDP2               | Cytoplasmic non-specific dipeptidase | Acetylornithine deacetylase/Succinyl-diaminopimelate desuccinylase and related deacetylases |
| 177      | 1::GOAT_ENSBTAP00000013650  | 92    | 12.1       | 2            | 2              | 1.866             | TALDO1              | Transaldolase (Fragment) | Transaldolase |
| 178      | 1::GOAT_ENSBTAP00000025691  | 144   | 6.9        | 2            | 2              | 1.247             | MDH1                | Malate dehydrogenase, cytoplasmic | Malate/lactate dehydrogenases |
| 179      | 1::GOAT_ENSP00000408907-D2  | 427   | 26.2       | 9            | 3              | 1.231             | HSPA1B              | Heat shock 70 kDa protein 1B | Molecular chaperone |
| 180      | 1::GOAT_ENSBTAP00000014585  | 71    | 8.9        | 1            | 1              | 1.518             | TTR                 | Transhydrogenin | Transhydrogenin-like protein |
| 181      | 1::GOAT_ENSBTAP0000006383   | 468   | 52.2       | 11           | 11             | 1.414             | PRDX6               | Peroxiredoxin-6 (Fragments) | Peroxiredoxin |
| 182      | 1::GOAT_ENSP00000381916     | 557   | 10.1       | 6            | 6              | 1.307             | EIF3C               | Eukaryotic translation initiation factor 3 subunit C | - |
| 183      | 1::GOAT_ENSBTAP0000001309   | 129   | 19.7       | 3            | 3              | 1.291             | PSMA2               | Proteasome subunit alpha type-2 | 20S proteasome, alpha and beta subunits |
| 183      | 1::goat_GLEAN_10020553      | 129   | 20.1       | 3            | 3              |                   |                     |                      |                         |
| 184      | 1::GOAT_ENSBTAP00000037502  | 104   | 1.6        | 3            | 3              | 1.345             | IGF2R               | Cation-independent mannose-6-phosphate receptor | - |
| 185      | 1::GOAT_ENSP00000369421     | 21    | 1.1        | 1            | 1              | 2.041             | FUT10               | Alpha-(1,3)-fucosyltransferase 10 | - |
| 186      | 1::GOAT_ENSBTAP00000053565  | 339   | 11.1       | 8            | 8              | 1.298             | ILF3                | Interleukin enhancer-binding factor 3 | - |
| 187      | 1::GOAT_ENSBTAP0000002642   | 232   | 13.7       | 3            | 3              | 1.273             | RPL14               | 60S ribosomal protein L14 | Ribosomal protein L14E/L6E/L27E |
| Group ID | Accession no | Score | %Cov (95%) | Peptide (95%) | Unique peptide | vortM_116:CK_119 | Protein abbreviation | Protein description | COG function description |
|----------|--------------|-------|------------|--------------|----------------|----------------|-------------------|---------------------|-----------------------|
| 188      | 1::GOAT_ENSP0000389536 | 102   | 11.4       | 7            | 5              | 1.328           | FUBP1             | Far upstream element-binding protein 1 | -                     |
| 189      | 1::GOAT_ENSBTAP0000020701 | 148   | 24.3       | 5            | 5              | 1.271           | HMOX1             | Heme oxygenase 1 | Heme oxygenase         |
| 190      | 1::GOAT_ENSP0000354884  | 35    | 1.9        | 1            | 1              | 1.539           | RASSF9            | Ras association domain-containing protein 9 | -                     |
| 191      | 1::GOAT_ENSP0000317786  | 138   | 4          | 4            | 4              | 1.298           | MPRIP             | Myosin phosphatase Rho-interacting protein | -                     |
| 192      | 1::GOAT_ENSBTAP0000019001 | 87    | 3.8        | 2            | 2              | 1.311           | TOMM70A           | Mitochondrial import receptor subunit TOM70 | FOG: TPR repeat       |
| 193      | 1::GOAT_ENSBTAP0000022382 | 53    | 15         | 2            | 2              | 1.437           | PFDN6             | Prefoldin subunit 6 | Prefoldin, chaperonin cofactor |
| 194      | 1::GOAT_ENSBTAP0000023197 | 46    | 7.6        | 4            | 4              | 1.348           | ADAM9             | Disintegrin and metalloproteinase domain-containing protein 9 | -                     |
| 195      | 1::GOAT_ENSP0000362352  | 64    | 11.3       | 3            | 2              | 1.204           | H2AFY2            | Core histone macro-H2A.2 | Histone H2A          |
| 196      | 1::GOAT_ENSBTAP0000021658 | 115   | 6          | 3            | 3              | 1.431           | PREP              | Prolyl endopeptidase | Serine proteases of the peptidase family S9A |
| 197      | 1::GOAT_ENSP00000215909  | 750   | 62.3       | 6            | 6              | 1.507           | LGALS1            | Galectin-1                  | -                     |
| 198      | 1::GOAT_ENSP00000378172  | 149   | 5.2        | 2            | 2              | 1.422           | ZNF207            | Zinc finger protein 207 | -                     |
| 199      | 1::GOAT_ENSBTAP0000025659 | 40    | 6.2        | 3            | 3              | 1.29            | NARS              | Asparagine-tRNA ligase, cytoplasmic | Aspartyl/asparaginyl-tRNA synthetases |
| 200      | 1::GOAT_ENSP00000346022  | 160   | 18.8       | 3            | 3              | 1.441           | RPL9              | 60S ribosomal protein L9 | Ribosomal protein L6P/L9E |
| 201      | 1::GOAT_ENSP00000321016  | 121   | 14         | 6            | 6              | 1.297           | PUF60             | Poly(U)-binding-splicing factor PUF60 | RNA-binding proteins (RRM domain) |
| 202      | 1::GOAT_ENSP0000003532  | 92    | 2.1        | 1            | 1              | 1.49            | RNGT              | mRNA-capping enzyme | mRNA capping enzyme, guanylyltransferase (alpha) subunit |
| 203      | 1::GOAT_ENSP00000363676  | 218   | 16.9       | 3            | 3              | 1.379           | RPL11             | 60S ribosomal protein L11 | Ribosomal protein L5 |
| 204      | 1::GOAT_ENSP00000369317-D4 | 302   | 11.3       | 5            | 3              | 1.663           | KRT1              | Keratin, type II cytoskeletal 1 | -                     |
| 205      | 1::GOAT_ENSBTAP0000008363 | 21    | 10.7       | 1            | 1              | 1.844           | NHP2              | H/ACA ribonucleoprotein complex subunit 2 | Ribosomal protein HS6-type (S12/L30/L7a) |
| 206      | 1::GOAT_ENSBTAP000001113  | 239   | 9.1        | 7            | 7              | 1.202           | PARP1             | Poly [ADP-ribose] polymerase 1 | -                     |
| 207      | 1::GOAT_ENSP00000265753  | 149   | 18.1       | 3            | 3              | 1.249           | EIF4H             | Eukaryotic translation initiation factor 4H | RNA-binding proteins (RRM domain) |
| 208      | 1::GOAT_ENSP00000338095  | 513   | 16.6       | 5            | 5              | 1.211           | HNRNPC            | Heterogeneous nuclear ribonucleoprotein C | -                     |
| 209      | 1::GOAT_ENSP00000368927  | 65    | 18         | 2            | 2              | 1.369           | Eif1ax            | Eukaryotic translation initiation factor 1A, X-chromosomal | Translation initiation factor 1 (IF-1) |
### Table 2 (continued)

| Group ID | Accession no          | Score | %Cov (95%) | Peptide (95%) | Unique peptide | vortM_116: CK_119 | Protein abbreviation | Protein description                                                                 | COG function description |
|----------|-----------------------|-------|------------|---------------|----------------|-------------------|----------------------|-------------------------------------------------------------------------------------|--------------------------|
| 210      | 1::GOAT_ENSP00000358939 | 119   | 13.4       | 6             | 6              | 1.266             | SARS                 | Serine–tRNA ligase, cytoplasmic                                                     | Seryl-tRNA synthetase    |
| 211      | 1::GOAT_ENSP00000404545 | 76    | 6.6        | 4             | 4              | 1.502             | SaFB                 | Scaffold attachment factor B1                                                      | -                        |
| 212      | 1::GOAT_ENSBTAP0000041837 | 130   | 10.4       | 3             | 3              | 1.243             | Raly                 | RNA-binding protein Raly                                                        | -                        |
| 213      | 1::GOAT_ENSBTAP0000027348 | 126   | 16.9       | 6             | 5              | 1.296             | IDH1                 | Isocitrate dehydrogenase [NADP] cytoplasmic                                       | Isocitrate dehydrogenases |
| 214      | 1::GOAT_ENSBTAP0000018888 | 30    | 6.9        | 1             | 1              | 1.917             | RPL35A               | 60S ribosomal protein L35a                                                       | -                        |
| 215      | 1::GOAT_ENSBTAP0000009564 | 334   | 13.6       | 10            | 10             | 1.368             | TF                   | Serotransferrin                                                           | -                        |
| 216      | 1::GOAT_ENSBTAP0000020564 | 77    | 5.3        | 2             | 2              | 1.312             | SRSF11               | Serine/arginine-rich splicing factor 11                                           | -                        |
| 217      | 1::GOAT_ENSBTAP00000052105 | 213   | 31.5       | 4             | 4              | 1.437             | Rps13                | 40S ribosomal protein S13                                                       | Ribosomal protein S15P/S13E |
| 218      | 1::GOAT_ENSBTAP00000031700 | 45    | 13.3       | 2             | 2              | 1.546             | RPL28                | 60S ribosomal protein L28                                                        | -                        |
| 219      | 1::GOAT_ENSBTAP00000040484 | 1295  | 33.1       | 9             | 9              | 1.677             | GAPDH                | Glyceraldehyde-3-phosphate dehydrogenase (Fragment)                               | Glyceraldehyde-3-phosphate dehydrogenase/erythrose-4-phosphate dehydrogenase |
| 220      | 1::GOAT_ENSBTAP00000037041 | 99    | 19.9       | 3             | 3              | 1.405             | RpL23a               | 60S ribosomal protein L23a                                                      | Ribosomal protein L23    |
| 221      | 1::GOAT_ENSBTAP00000025484 | 74    | 18.3       | 2             | 2              | 1.34              | RPS20                | 40S ribosomal protein S20                                                      | Ribosomal protein S10    |
| 222      | 1::GOAT_ENSBTAP00000023484 | 111   | 14.9       | 2             | 2              | 2.097             | CDV3                 | Protein CDV3 homolog                                               | -                        |
| Group ID | Accession no | Score (95%) | Cov (95%) | Unique peptide | Protein abbreviation | Protein description | COG function description |
|---------|--------------|-------------|-----------|----------------|---------------------|---------------------|------------------------|
| 1       | 1::GOAT_ENSP00000385942 | 173 | 7.2 | 6 | 6 | 0.784 | Xpo1 | Exportin-1 |
| 2       | 1::GOAT_ENSP00000362335 | 203 | 30.8 | 5 | 3 | 0.824 | SAR1A | GTP-binding protein SAR1a |
| 3       | 1::GOAT_ENSP00000304408 | 833 | 14.4 | 13 | 12 | 0.566 | COL3A1 | Collagen alpha-1(III) chain |
| 4       | 1::GOAT_ENSBTAP00000007515 | 451 | 51.1 | 6 | 6 | 0.732 | CRABP2 | Cellular retinoic acid-binding protein 2 |
| 5       | 1::GOAT_ENSP00000414942 | 72 | 5.1 | 1 | 1 | 0.688 | IST1 | IST1 homolog |
| 6       | 1::GOAT_ENSBTAP0000014801 | 125 | 14.6 | 1 | 1 | 0.775 | NDUFA4 | NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 4 |
| 7       | 1::GOAT_ENSP00000357980 | 386 | 39 | 9 | 9 | 0.745 | HTRA1 | Serine protease HTRA1 |
| 8       | 1::GOAT_ENSP00000216479 | 96 | 17 | 4 | 4 | 0.805 | AHSA1 | Activator of 90 kDa heat shock protein ATPase homolog 1 |
| 9       | 1::GOAT_ENSP00000360939 | 66 | 6.2 | 1 | 1 | 0.808 | CMPK1 | UMP-CMP kinase |
| 10      | 1::GOAT_ENSP00000262288 | 199 | 18.8 | 4 | 4 | 0.8 | SCPEP1 | Retinoid-inducible serine carboxypeptidase |
| 11      | 1::GOAT_ENSBTAP00000031937 | 111 | 17.3 | 4 | 4 | 0.812 | Ech1 | Delta(3,5)-Delta(2,4)-dienoyl-CoA isomerase, mitochondrial |
| 12      | 1::GOAT_ENSBTAP0000017298 | 167 | 2.6 | 2 | 2 | 0.739 | PLAA | Phospholipase A-2-activating protein |
| 13      | goat_GLEAN_1009864 | 87 | 8.5 | 2 | 2 | 0.704 | TWF2 | Twinfilin-2 |
| 14      | 1::GOAT_ENSP00000381607 | 87 | 11.9 | 1 | 1 | 0.72 | GSTP1 | Glutathione S-transferase P |
| 15      | 1::GOAT_ENSP00000361508 | 84 | 13.6 | 4 | 4 | 0.746 | PLTP | Phospholipid transfer protein |
| 16      | 1::GOAT_ENSBTAP00000045426 | 183 | 3.3 | 2 | 2 | 0.832 | NAA15 | N-alpha-acetyltransferase 15, NatA auxiliary subunit |
| 17      | 1::GOAT_ENSP00000269321 | 446 | 41.7 | 6 | 6 | 0.818 | ARHGDIA | Rho GDP-dissociation inhibitor 1 |
| 18      | 1::GOAT_ENSP00000205061 | 106 | 7.5 | 6 | 6 | 0.754 | Glg1 | Golgi apparatus protein 1 |
| 19      | 1::GOAT_ENSP00000348775 | 91 | 6 | 3 | 3 | 0.816 | ACOX3 | Peroxisomal acyl-coenzyme A oxidase 3 |
| 20      | 1::GOAT_ENSBTAP0000003959 | 122 | 22.5 | 1 | 1 | 0.815 | GNG2 | Guanine nucleotide-binding protein G(1)G(S)/G(O) subunit gamma-2 |
| 21      | 1::GOAT_ENSBTAP00000043782 | 102 | 15.1 | 2 | 2 | 0.813 | CAV1 | Caveolin-1 |
| 22      | 1::GOAT_ENSBTAP0000005837 | 56 | 3.7 | 2 | 2 | 0.778 | Ip69 | Importin-9 |
| 23      | 1::GOAT_ENSBTAP0000010389 | 80 | 15.1 | 2 | 2 | 0.722 | FIS1 | Mitochondrial fission 1 protein |
| 24      | 1::GOAT_ENSP000000416650 | 102 | 14.6 | 3 | 3 | 0.772 | PSME2 | Proteasome activator complex subunit 2 |
| 25      | 1::GOAT_ENSP00000360860 | 73 | 12.9 | 5 | 5 | 0.751 | IFT5 | Interferon-induced protein with tetratricopeptide repeats 5 |
| Group ID | Accession no | Score (95%) | Cov (95%) | Peptide Unique peptide | vorM_116:CK_119 | Protein abbreviation | Protein description | COG function description |
|---------|--------------|-------------|-----------|------------------------|-----------------|---------------------|---------------------|------------------------|
| 26      | 1::GOAT_ENSP00000407726 | 237 | 14.5 | 9 | 9 | 0.676 | VWA5A | von Willebrand factor A domain-containing protein 5A | Uncharacterized protein containing a von Willebrand factor type A (vWA) domain |
| 27      | 1::goat_GLEAN_10015090  | 107 | 22.2 | 2 | 2 | 0.812 | ATP5J | ATP synthase-coupling factor 6, mitochondrial | - |
| 28      | 1::GOAT_ENSP00000381803  | 116 | 18.8 | 5 | 4 | 0.816 | MAPK1 | Mitogen-activated protein kinase 1 | Serine/threonine protein kinase |
| 29      | 1::GOAT_ENSBTAP0000007028  | 133 | 16.9 | 3 | 3 | 0.71 | ARPC3 | Actin-related protein 2/3 complex subunit 3 | - |
| 30      | 1::GOAT_ENSP00000264933  | 106 | 23.2 | 3 | 3 | 0.789 | Pdcd6 | Programmed cell death protein 6 | - |
| 31      | 1::GOAT_ENSBTAP0000020484  | 209 | 8.1 | 8 | 8 | 0.8 | TBBCD | Tubulin-specific chaperone D | Beta-tubulin folding cofactor D |
| 32      | 1::GOAT_ENSBTAP0000030311  | 135 | 20 | 3 | 3 | 0.706 | SAA1 | Serum amyloid A protein | - |
| 33      | 1::GOAT_ENSP00000409204  | 211 | 13.3 | 7 | 7 | 0.805 | SUN2 | SUN domain-containing protein 2 | - |
| 34      | 1::GOAT_ENSBTAP0000036255  | 182 | 24.6 | 5 | 5 | 0.796 | LEPREL4 | Synaptosomal complex protein SC65 | - |
| 35      | 1::GOAT_ENSBTAP0000252951-D2 | 180 | 14.8 | 2 | 2 | 0.777 | HBA | Hemoglobin subunit alpha-1/2 | - |
| 36      | 1::GOAT_ENSBTAP0000005713  | 645 | 22.2 | 7 | 7 | 0.803 | SERPINC1 | Antithrombin-III | Serine protease inhibitor |
| 37      | 1::GOAT_ENSP00000394338  | 73 | 7.1 | 2 | 2 | 0.779 | PPP2R4 | Serine/threonine-protein phosphatase 2A activator | Phosphotyrosyl phosphatase activator |
| 38      | 1::GOAT_ENSP00000382533  | 151 | 14.8 | 4 | 3 | 0.761 | NAP1L4 | Nucleosome assembly protein 1-like 4 | - |
| 39      | 1::GOAT_ENSBTAP00000053644  | 253 | 43.4 | 39 | 39 | 0.827 | Vcl | Vinculin | - |
| 40      | 1::GOAT_ENSBTAP0000024445-D2 | 338 | 34.5 | 11 | 11 | 0.744 | SERPINB1 | Leukocyte elastase inhibitor | Serine protease inhibitor |
| 41      | 1::GOAT_ENSBTAP00000051810  | 72 | 3.1 | 1 | 1 | 0.823 | UBP1 | Upstream-binding protein 1 | - |
| 42      | 1::GOAT_ENSBTAP0000032864  | 412 | 45.3 | 7 | 2 | 0.755 | PGAM1 | Phosphoglycerate mutase 1 | Phosphoglycerate mutase 1 |
| 43      | 1::GOAT_ENSBTAP00000242277  | 148 | 14.8 | 5 | 5 | 0.795 | DAK | Bifunctional ATP-dependent dihydroxyacetone kinase/FAD-AMP lyase (cyclizing) | Dihydroxyacetone kinase |
| 44      | 1::GOAT_ENSBTAP0000026449  | 449 | 15.9 | 7 | 7 | 0.825 | PPP2R1A | Serine/threonine-protein phosphatase 2A 65 kDa regulatory subunit A alpha isoform | - |
| 45      | 1::GOAT_ENSP00000407487  | 153 | 9.2 | 7 | 7 | 0.813 | UNC45A | Protein unc-45 homolog A | FOG: TPR repeat |
| 46      | 1::GOAT_ENSP00000423006  | 98 | 6.4 | 3 | 3 | 0.825 | ACSL1 | Long-chain-fatty-acid-CoA ligase 1 | Long-chain acyl-CoA synthetases (AMP-forming) |
| 47      | 1::GOAT_ENSP00000227322  | 65 | 10.5 | 4 | 4 | 0.826 | ZNF259 | Zinc finger protein ZPR1 | C4-type Zn-finger protein |
| 48      | 1::GOAT_ENSP00000334008  | 218 | 15.9 | 4 | 4 | 0.761 | PARVA | Alpha-parvin | - |
| 49      | 1::GOAT_ENSP00000351740  | 94 | 7.1 | 3 | 3 | 0.795 | FAM14A1 | Protein NOXP20 | - |
| 50      | 1::GOAT_ENSBTAP00000359840  | 104 | 5.8 | 4 | 4 | 0.806 | DNM2 | Dynamin-2 | Predicted GTPases (dynamins related) |
information complementary to the protein expression data obtained by proteomics analysis.

### The subcellular localization of HSPA1B

GSF cells were transfected with the recombinant plasmid pEGFP-HSPA1B for transient expression. The pEGFP-HSPA1B protein was used to observe the subcellular localization of HSPA1B using CLSM (Fig. 5A). Following DAPI staining, the nuclear excitation of the blue fluorescence was monitored (Fig. 5B) while the subcellular localization of pEGFP-N1 was monitored (Fig. 5D and E). Compared with pEGFP-N1 (Fig. 5F), the overlap of fluorescence in Fig. 5A indicated that the HSPA1B protein was mainly distributed in the cytoplasm of GSF cells (Fig. 5C).

### Inhibition of ORFV proliferation by HSPA1B

GSF cells were transfected with pEGFP-HSPA1B, cultured for 18 h, and infected with ORFV. Cell suspension samples were collected at different time points after infection to measure virus proliferation by RT-qPCR. The results indicated that the difference in viral proliferation at 6 and 15 h postinfection between HSPA1B overexpressing cells and the control cells was not statistically significant. However, at 24 and 36 h postinfection, the viral genome copy number was considerably lower in cells overexpressing HSPA1B, indicating that this protein inhibits proliferation of ORFV in GSF cells (Fig. 6A). Next, we examined ORFV replication in HSPA1B-downregulated cells. HSPA1B was knocked down in GSF cells using RNAi. Three HSPA1B small interfering RNAs (siRNAs) were designed and synthesized, and their silencing efficiency was evaluated using a Western blot assay. SiRNA-517 was found to be the most efficient for decreasing HSPA1B expression (Fig. 6B). GSF cells were transfected with negative-control (NC) siRNA or siRNA-517 and then infected with equal amounts of ORFV. The siRNA knockdown efficiency was confirmed by Western blotting, and the levels of viral RNA and viral proteins in the siRNA-517 cells were compared to NC siRNA cells at different time points after virus infection. The levels of ORFV replication were higher in HSPA1B siRNA cells than

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### Table 3 (continued)

| Group ID | Accession no | Score | Cov (95%) | Unique peptide | Peptide | Protein abbreviation | Protein description |
|----------|--------------|-------|-----------|----------------|---------|----------------------|---------------------|
| 51       | 1::GOAT_ENSP00000369059 | 57    | 3         | 3              | 0.785   | SEC24D | Protein transport protein Sec24D Vesicle coat complex COPII, subunit SEC24/subunit SFB2/subunit SFB3 |
| 52       | 1::GOAT_ENSBTAP0000001138 | 188   | 27.1      | 8              | 0.784   | SULT1A1 | Sulfotransferase 1A1 |
| 53       | 1::GOAT_ENSP00000340211 | 122   | 10.6      | 3              | 0.709   | CORO1B | Corin-1B |
| 54       | 1::GOAT_ENSP00000395277 | 41    | 13        | 2              | 0.825   | K0913 | Zinc finger SWIM domain-containing protein KIAA0913 |
| 55       | 1::GOAT_ENSBTAP00000051130 | 116   | 26.5      | 3              | 0.721   | LAMB2 | Laminin subunit beta-2 |
| 56       | 1::GOAT_ENSP00000432911 | 122   | 8         | 3              | 0.743   | Up50 | Ubiquitin carboxyl-terminal hydrolase 5 |
| 57       | 1::GOAT_ENSBTAP00000017069 | 162   | 26.2      | 8              | 0.812   | SI120N2 | Plasminogen activator inhibitor 2 |
| 58       | 1::GOAT_ENSP00000337711 | 1409  | 27.9      | 25             | 0.702   | Collagen-alpha-2(I) chain |
| 59       | 1::GOAT_ENSBTAP0000014960 | 45    | 9.8       | 3              | 0.819   | ASAH1 | Acid ceramidase |
| 60       | 1::GOAT_ENSBTAP0000039059 | 188   | 27.1      | 8              | 0.784   | SULT1A1 | Sulfotransferase 1A1 |

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![Fig. 3](https://www.genego.com/) Classification of the identified proteins based on their functional annotations using Gene Ontology enrichment analysis. (A) Cellular components of the identified proteins. (B) Molecular functions of the identified proteins. (C) Biological processes of the identified proteins. (D) Gene Ontology enrichment analysis of cellular components of differentially expressed proteins. (E) Gene Ontology enrichment analysis of molecular functions of differentially expressed proteins. (F) Gene Ontology enrichment analysis of biological processes of differentially expressed proteins. (G) COG classification of differentially expressed proteins. P-values were calculated using MetaCore in the GeneGO package (https://www.genego.com/).
Inhibition of orf virus replication by HSPA1B
in control cells (Fig. 6C-E), suggesting that ORFV replication was significantly enhanced in the HSPA1B knockdown cells. These data suggest that HSPA1B has an inhibitory effect on ORFV replication in GSF cells.

**Discussion**

ORFV is an epitheliotropic virus that infects damaged or scarified skin and replicates in regenerating epidermal
Inhibition of orf virus replication by HSPA1B

In a previous study, healthy OFTu cells were inoculated with a filtered viral suspension, and CPE was observed after 7–9 days. After five blind passages of ORFV, approximately 80% of the cells exhibited rounding, detachment, and clustering, creating a net-like form [9]. In the present study, the proliferation of GSF cells infected with ORFV was studied for the first time, using virus titration, microscopic observations, and RT-qPCR. The data indicated that GSF cells are susceptible to ORFV infection. The proliferation rate was initially slow but increased from 12 to 72 h postinfection. A one-step growth curve of ORFV in GSF cells showed that the intracellular viral level was the highest at 36 h postinfection. By 60 h, the cells had detached from the cell plate and the viral titer decreased, possibly due to the lysis of the infected cells. Since GSF cells are derived from the primary host of ORF, they provide a good model system for understanding the changes that occur in these cells following infection with ORFV. In the present...
study, iTRAQ LC − MS/MS was applied for the first time to identify differentially expressed proteins in ORFV-infected GSF cells. The data demonstrated that 282 proteins were differentially expressed, 222 of which were upregulated and 60 of which were downregulated. Changes in mRNA levels measured by RT-qPCR were in agreement with the changes observed for the corresponding proteins by iTRAQ. These findings may be helpful for elucidating the molecular mechanisms by which target cells interact with the virus.

The host cytoskeletal network participates in the transport of viral components, particularly during the stages of entry and exit of the virus [30]. Viral components either hijack the cytoplasmic membrane traffic or interact directly with the cytoskeletal transport machinery [8]. In the present study, the expression levels of two specific proteins involved in cytoskeleton networks and cell communication were altered following ORFV infection. TBCD expression was downregulated, and SPECC1L expression was upregulated.

CD9 promotes adeno-associated virus type 2 infection of mammary carcinoma cells with low cell surface expression of heparan sulfate proteoglycans [19]. Parseval et al. [6] showed that the monoclonal antibody MAb vpg15, which targets a determinant of the feline cell surface marker CD9, which may serve as a receptor or co-receptor for feline immunodeficiency virus (FIV), markedly delayed infection with that virus. In the present study, CD9 was found to be upregulated in infected cells.

We also observed differential expression of components of several ubiquitin-mediated protein degradation pathways in ORFV-infected GSF cells. UB5 is able to hydrolyze conjugates of the ubiquitin-like protein ISG15, as demonstrated in previous experiments in which these conjugates were shown to bind to the suicide probe ISG15-VS, which in turn inhibited protein degradation [36]. In the present study, UB5 expression was downregulated, and PSMA7 expression was upregulated in ORFV-infected GSF cells. PSMA7 is a component of the 26S proteasome, participating in protein degradation and cell apoptosis. However, PMSA6 and PSME3, which belong to the same protein family, exhibited downregulated expression in infectious bursal disease virus (IBDV)-infected chicken embryo fibroblast (CEF) cells, suggesting that different viruses may use different pathways to regulate protein degradation and cell apoptosis [43].

It is noteworthy that ATP synthase-coupling factor 6 (ATP5J), which is involved in ATP synthesis-coupled proton transport [37], exhibited decreased expression in ORFV-infected cells. A total of six proteins with receptor activity were identified in ORFV-infected GSF cells. TR150, K2C1,
Inhibition of orf virus replication by HSPA1B

SUCR1, MPRI, O10AG, and TKT were upregulated. Transketolase (TK) catalyzes several reactions in the non-oxidative branch of the pentose phosphate pathway (PPP) and serves as a bridge between the oxidative part of the PPP and the oxidative decarboxylation of glucose [17]. Recently, it has been reported that TK and its cofactor thiamine have a very high growth control coefficient. TR150 has been shown to be involved in pre-mRNA splicing and was previously believed to participate in transcriptional co-activation via its association with the TRAP complex. However, studies have not shown TR150 to be a subunit of a stable mediator complex [16, 20]. K2C1 may regulate the activity of kinases, such as PKC and SRC via binding to integrin beta-1 (ITB1) and the receptor of activated protein kinase C (RACK1/GNB2L1). Additionally, it can form a complex with C1QB, which is a high-affinity receptor for kininogen-1/HMWK. The functions of these proteins in infected host cells are not well understood.

HSPA1B is a member of the HSP70 family. The expression level of HSP70 rapidly increases in response to cellular stresses (e.g., heat shock) or in response to certain viral infections [4, 21, 24, 26]. Genetic variations in HSP70 have

Fig. 6 HSPA1B inhibits ORFV replication. (A) Overexpression of HSPA1B suppresses ORFV replication. GSF cells were transfected with pEGFP-HSPA1B and pEGFP-N1 and infected 18 h later with ORFV at an MOI of 0.1. Cultured cells were collected at 6, 15, 24, and 36 h postinfection, and the viral DNA content was measured by RT-qPCR. (B) Evaluation of the efficiency of NC or HSPA1B siRNA in silencing HSPA1B expression. GSF cells were transfected with 150 nM HSPA1B siRNA or NC, and the expression of HSPA1B mRNA or protein was detected by Western blotting. (C-E) Downregulation of HSPA1B promotes ORFV replication. GSF cells were transfected with NC siRNA or HSPA1B siRNA and subsequently infected with equal amounts of ORFV at an MOI of 0.1. The expression of HSPA1B and viral mRNA or protein was detected by RT-qPCR or Western blotting.
been found to be associated with individual susceptibility to several diseases by alterations in protein expression and/or function. Studies have shown that HSPs may play a significant role in virus-host cell interactions during viral infection in vivo and in vitro [1, 31]. HSP70 stabilizes proteins against aggregation and mediates the folding of newly translated polypeptides in the cytosol, as well as within organelles. It can bind with nucleotides via an ATP-dependent process and is involved in the response to stress as well as in cell apoptosis. HSP70 is associated with membrane microdomains (lipid rafts) in response to dengue virus infection and acts as a receptor complex in human cell lines and in monocytes/macrophages that are susceptible to dengue virus (DENV) infection [32]. Therefore, we hypothesize that the increased expression level of HSP70 protein may play a substantial role in the replication of ORFV. In the present study, the recombinant plasmid construct pEGFP-HSPA1B transiently expressed in GSF cells and was found to be localized in the cytoplasm. Interestingly, it has been reported that HSP70 and HSP90 are clustered around CD14, preventing them from interacting with DENV, when monocytes are incubated and HSP90 are clustered around CD14, preventing them from interacting with DENV, when monocytes are incubated and HSP90 are clustered around CD14, preventing them from interacting with DENV, when monocytes are incubated and HSP90 are clustered around CD14, preventing them from interacting with DENV, when monocytes are incubated and HSP90 are clustered around CD14, preventing them from interacting with DENV, when monocytes are incubated.

The results suggested that HSPA1B inhibits proliferation of ORFV in GSF cells, and this appears to occur in the middle of the viral replication cycle. Furthermore, ORFV replication was significantly enhanced in HSPA1B knockdown cells, again suggesting that HSPA1B plays an important role in ORFV-infected GSF cells. Importantly, animals are not protected against ORFV reinfections, which may be in part due to short-lived ORFV-specific adaptive immunity. Poxviruses encode a considerable number of gene products that allow them to evade the host immune response [35]. These evasive strategies may play a major role in supporting ORFV replication and allowing ORFV reinfections to occur.

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