Proteomics analysis reveals heat shock proteins involved in caprine parainfluenza virus type 3 infection

Chunyan Zhong, Jizong Li*, Li Mao, Maojun Liu, Xing Zhu, Wenliang Li, Min Sun, Xinqin Ji, Fang Xiao, Leilei Yang, Wenwen Zhang and Zheng Liao

Abstract

Background: Caprine parainfluenza virus type 3 (CPIV3) is major pathogen of goat herds causing serious respiratory tract disease and economic losses to the goat industry in China. We analyzed the differential proteomics of CPIV3-infected Madin-Darby bovine kidney (MDBK) cells using quantitative iTRAQ coupled LC-MS/MS. In addition, four DEPs were validated by qRT-PCR and western blot analysis.

Results: Quantitative proteomics analysis revealed 163 differentially expressed proteins (DEPs) between CPIV3-infected and mock-infected groups (p-value < 0.05 and fold change > 1.2), among which 91 were down-regulated and 72 were up-regulated. Gene ontology (GO) analysis showed that these DEPs were involved in molecular functions, cellular components and biological processes. Biological functions in which the DEPs were involved included diseases, genetic information processing, metabolism, environmental information processing, cellular processes, and organismal systems. STRING analysis revealed that four heat shock proteins (HSPs) included HSPA5, HSPA1B, HSP90B1 and HSPA6 may be associated with proliferation of CPIV3 in MDBK cells. qRT-PCR and western blot analysis showed that the selected HSPs were identical to the quantitative proteomics data.

Conclusion: To our knowledge, this is the first report of the proteomic changes in MDBK cells after CPIV3 infection.

Keywords: Caprine parainfluenza virus type 3, Madin-Darby bovine kidney cells, Proteomic analysis, iTRAQ, LC-MS/MS

Background

In August 2013, an outbreak of severe goat respiratory disease occurred throughout the major goat herd regions of eastern China. The causative agent was identified as a novel strain of parainfluenza virus type 3 (PIV3) and was designated as caprine parainfluenza virus type 3 (CPIV3) strain JS2013 [1]. The infected goats exhibited high fever, coughing, nasal discharge and dyspnea. Necropsy of the infected goats showed mild to moderate gross lesions in the lungs, and increased amounts of secretion in the tracheas and bronchia were also observed. Genome sequence alignment and phylogenetic analysis revealed that the genome of CPIV3 strain JS2013 showed only 73.3–75.5% identity with BPIV3 and HPIV3 strains [2]. Based on phylogenetic analysis, this pathogen was designated as CPIV3, a member of the PIV3 group belonging to the Respirovirus genus within the Paramyxiviridae family. Moreover, we further demonstrated that CPIV3 strain JS2013 can be transferred horizontally between adjacent pens [3]. Recently, a seroprevalence study using 2919 serum samples in China reported a CPIV3 prevalence of 39.9% in goats [4]. Another study reported that 35% of nasal swabs and serum samples from clinically diseased goats were positive for CPIV3 by quantitative RT-PCR (qRT-PCR) [5]. It is noteworthy that the spread of CPIV3 has caused heavy economic losses in China [6].

To understand the pathogenesis of viral infection, research on virus-host interaction is critical. Virus infection can dramatically affect host cell morphology, transcription and translation patterns, the cytoskeleton, the cell cycle and innate immune responses of the host, the apoptosis

* Correspondence: lijizong22@sina.com
1Institute of Veterinary Medicine, Jiangsu Academy of Agricultural Sciences, Key Laboratory of Veterinary Biological Engineering and Technology, Ministry of Agriculture, Nanjing 210014, China
2School of Pharmacy, Linyi University, Linyi 276000, China
Full list of author information is available at the end of the article

© The Author(s). 2019 Open Access This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The Creative Commons Public Domain Dedication waiver (http://creativecommons.org/publicdomain/zero/1.0/) applies to the data made available in this article, unless otherwise stated.
pathway, and may also cause inflammation and alter stress responses [7]. Many functional and morphological changes in host cells are associated with significant changes in the patterns of expression of host cells. Therefore, information on proteome changes in the host following CPIV3 infection may be crucial to understand the host response to viral pathogenesis. In recent years, comparative proteomic analysis has emerged as a valuable tool for the establishment of the global host protein profiles in response to virus infection [8]. This technique has been widely used to investigate proteome changes in cow, yak, buffalo, goat and camel milk [9], and peste des petits ruminants virus (PPRV)-infected Vero cells [10], based on the isobaric tags for relative and absolute quantification (iTRAQ) method. In addition, this technique has also been widely employed to examine the mechanisms of viral infection through comparative investigation of the proteome changes, for example, in the case of Crimean-Congo hemorrhagic fever virus (CCHFV) [11] and bovine respiratory syncytial viruses (BSRV) [12].

However, to the best of our knowledge, no previous study has analyzed the proteomic changes in CPIV3-infected MDBK cells. Proteomic techniques are effective tools to characterize protein expression profiles, and have been widely used to investigate disease-associated proteins [13, 14]. Among current proteomics methods, quantitative high-throughput proteomics approaches are useful for the analysis of infection-associated proteins [15, 16]. In our current study, we used a quantitative proteomics approach based on an iTRAQ tandem mass spectrometry (MS/MS) technique to identify differentially expressed proteins (DEPs) between CPIV3-infected and mock-infected MDBK cells. The functions of the DEPs were analyzed to determine whether they might be associated with CPIV3 infection [17]. Our findings provide valuable insight into the changes in cellular processes that occur during CPIV3 infection.

**Results**

**CPIV3 propagation in MDBK cells**

The kinetics of CPIV3 propagation in MDBK cells were observed by monitoring the CPE at 24, 48 and 72 h post infection (hpi) (Fig. 1a), a minimal CPE was visible at 24 hpi, whereas an obvious CPE was observed at 48 hpi, and at 72hpi, almost all cells were disrupted. The TCID<sub>50</sub> showed that the viral titer reached 10<sup>3.5</sup> TCID<sub>50</sub>/ml at 24 hpi, peaked at 10<sup>7.0</sup> TCID<sub>50</sub>/ml at 72 hpi and then declined (Fig. 1b). To ensure a higher proportion of infected cells and to avoid an excessive CPE, we selected 24 hpi as the time point under our infection conditions for further proteomic analysis.

**Identification and annotation of proteins**

We detected 8153 proteins and quantified 4109 proteins, including 28,815 peptides (Additional file 1: Figure S1). Detected proteins were annotated according to the GO database in the following categories: cellular components (CC), biological processes (BP), and molecular functions (MF) (Additional file 2: Figure S2). The top 20 pathways containing the largest number of proteins among the 8153 proteins were annotated according to KEGG (Additional file 3: Figure S3). Based on the KOG, 830 of the proteins were annotated as being involved in information storage and processing, 1545 were annotated as cellular processes and signaling, 581 were annotated as metabolism, and 699 were annotated as poorly characterized (Additional file 4: Figure S4 and Data Sheet 5). Furthermore, the cutoff criteria considered for the DEPs were set with an adjusted p-value of < 0.05 and a ratio of > 1.2-fold difference. Among the DEPs, 163 proteins from the two sets of biological replicates overlapped and
were subsequently adjusted for multiple testing according to the stringent method of Benjamini and Hochberg [18]. Of these, 72 proteins were up-regulated and 91 proteins were down-regulated based on our criteria for the identification of DEPs in the MDBK-infected and mock-infected groups using the iTRAQ-MS/MS approach. Protein ratios were presented as CPIV3-infected/mock-infected. An average V/C ratio > 1 represented up-regulated proteins and an average V/C ratio < 1 represented down-regulated proteins. A list of DEPs information is shown in Table 1. DEPs between the two groups are shown as heat map and scatterplot (Additional file 5: Figure S6 and S7). Finally, the DEPs displaying the greatest increase and decrease in expression in the CPIV3-infected MDBK cells were FAM81B protein (1:0.118) and the DEP displaying the greatest decrease in expression in the CPIV3-infected MDBK cells was carboxypeptidase (1:1.206).

GO analysis of the DEPs
The molecular functional classes and subcellular locations of the 163 DEPs were analyzed using UniProt and the GO database. The 163 DEPs were annotated into the categories: cellular component, biological process, or molecular function, and the distribution of up-regulated and down-regulated proteins among these GO annotations are shown in Additional file 6: Figure S8. GO enrichment annotation comparisons were performed to elucidate the characteristics of the altered proteins in MDBK cells induced by CPIV3 infection, to determine any associations with virulence and pathogenicity. In terms of biological process annotation, DEPs were mainly involved in cell aggregation, cellular processes, cellular component organization or biogenesis, locomotion, metabolic processes, multicellular organismal processes and reproductive processes; in terms cellular component annotation, DEPs were mainly involved in the cell part, extracellular region part, membrane part, organelle part, protein-containing complex and supramolecular complex; in terms of molecular function annotation, DEPs were mainly involved in binding, catalytic activity, molecular carrier activity and transporter activity (Fig. 2).

KEGG (Kyoto encyclopedia of genes and genomes) pathway analysis of the DEPs
The KEGG pathway is a collection of pathway maps that represent molecular interactions and reaction networks in cell line. The 93 DEPs identified were annotated, and mapped to a total of six KEGG pathway categories, which included metabolism, disease, genetic information processing, cellular processes, environmental information processing, and organismal systems pathway categories (Additional file 7 Data Sheet 9). The enrichment annotation protein pathway information is shown in Fig. 3. The results showed that most of the abundant KEGG terms were involved in biological processes such as the p53 signaling pathway, microRNAs in cancer, alanine, aspartate and glutamate metabolism, nitrogen metabolism, the estrogen signaling pathway, mineral absorption and thyroid hormone synthesis. Functional classification by KEGG showed that the upregulated and downregulated proteins could be divided among six distinct functional sets: environmental information processing, cellular processes, metabolism, genetic information processing, organismal systems and human diseases (Fig. 4).

STRING analysis of the relationships between DEPs
With the goal of exploring the potential protein network connections for the differentially regulated proteins in detail, the STRING tool was used. The differentially regulated proteins were mainly mapped to four functional networks (Fig. 5). A specific network had at least four “focus” proteins (HSPA5, HSPA1B, HSP90B1 and HSPA6). The networks of interest corresponded to: cell-to-cell signaling, hereditary disorder, cell death and survival, cardiovascular disease, cellular developmental, RNA post-transcriptional modification, cellular growth and proliferation.

Confirmation of proteomic data by qRT-PCR
Alterations in the expression of a protein may be owing to a change in its mRNA levels. To confirm the results of the proteomic analysis by mRNA expression, transcriptional alterations in four selected proteins were measured by qRT-PCR. The qRT-PCR analysis showed that no difference in the ratio of these mRNAs between the CPIV3 infected group and the mock infected group were consistent with those obtained using quantitative proteomics analysis (Fig. 6). The mRNA expression of HSPA5, HSP90B1, HSPA1B and HSPA6 were increased in CPIV3-infected MDBK cells. Therefore, the trends in the mRNA expression were consistent with those in their corresponding proteins.

Western blot analysis of HSPA1B
We analyzed the expression levels of HSPA1B (up-regulated) in CPIV3-infected MDBK cells (Fig. 7) by western blot at 24 h and 48 h. Figure 7 shows that HSPA1B was up-regulated in CPIV3-infected MDBK cells at 24 h and 48 h. The results were consistent with those obtained using the iTRAQ labeled LC-MS/MS system.

Discussion
Proteomic techniques have become significant methodologies for determining cellular protein interactions and host cellular pathophysiological processes following virus infection [19, 20]. As a general rule, no important host cell membrane rearrangement or cytoskeleton collapse is observed following virus infection but the point at which a high virus yield is obtained is considered as the best
| Accession | Protein name | CPIV3-infected | Mock-infected | FC (CPIV3-infected vs Mock-infected) | regulate |
|-----------|--------------|----------------|---------------|--------------------------------------|----------|
| Q0VCX2   | Endoplasmic reticulum chaperone BIP (HSPA5) | 1 | 0.488 | 2.049180328 | up |
| Q95M18   | Endoplasm (HSP90B1) | 1 | 0.744 | 1.344086022 | up |
| F1MEN8   | Protein disulfide-isomerase A4 (PDIA4) | 1 | 0.816 | 1.225490106 | up |
| E1B748   | Hypoxia up-regulated protein 1 precursor (HYOU1) | 1 | 0.704 | 1.420454545 | up |
| Q27965   | Heat shock 70 kDa protein 1B (HSPA1B) | 1 | 0.793 | 1.261034048 | up |
| A6QR28   | Phosphoserine aminotransferase (PSAT1) | 1 | 0.814 | 1.228501229 | up |
| F1MWU9   | Uncharacterized protein (HSPA6) | 1 | 0.726 | 1.377410468 | up |
| Q32CA7   | G protein subunit alpha i3 (GNAI3) | 1 | 0.739 | 1.353179973 | up |
| Q1LZ3A   | Asparagine synthetase [glutamine-hydrolyzing] (ASN5) | 1 | 0.721 | 1.386925522 | up |
| P80513   | Mesencephalic astrocyte-derived neurotrophic factor (MANF) | 1 | 0.794 | 1.259445844 | up |
| Q2KHU0   | Phosphoserine phosphatase (PSPH) | 1 | 0.81 | 1.234567901 | up |
| Q3TOL2   | Endoplasmic reticulum resident protein 44 (ERP44) | 1 | 0.765 | 1.307189542 | up |
| Q08DLO   | SLC3A2 protein (SLC3A2) | 1 | 0.78 | 1.282051282 | up |
| ASPK96   | ACP1 protein (ACP1) | 1 | 0.78 | 1.282051282 | up |
| P13909   | Plasminogen activator inhibitor 1 (SERPINE1) | 1 | 0.807 | 1.239157373 | up |
| P68301   | Metallothionein-2 (MT2) | 1 | 0.491 | 2.036659878 | up |
| Q27955   | Voltage-gated potassium channel subunit beta-2 (KCNAB2) | 1 | 0.797 | 1.254705144 | up |
| A5D7C1   | Probable ATP-dependent RNA helicase DDX52 (DDX52) | 1 | 0.814 | 1.228501229 | up |
| A6H797   | MLEC protein (MLEC) | 1 | 0.807 | 1.239157373 | up |
| F1N1R3   | Mitochondrial ribosomal protein L40 (MRPL40) | 1 | 0.824 | 1.213522323 | up |
| E1BPL3   | ATP binding cassette subfamily B member 7 (ABC7) | 1 | 0.721 | 1.386925522 | up |
| ASPIN8   | Splicing factor 3A subunit 2 (SF3A2) | 1 | 0.551 | 1.814882033 | up |
| Q2XIN6   | Protein Mpv17 (MPV17) | 1 | 0.801 | 1.248439451 | up |
| Q3SZZ0   | Ribosome biogenesis protein BRX1 homolog (BRX1) | 1 | 0.799 | 1.251564456 | up |
| A6QLR4   | Flotillin-2 (FLOT2) | 1 | 0.476 | 2.100840336 | up |
| Q17Q2    | RNA polymerase II subunit A C-terminal domain phosphatase SSU72 (SSU72) | 1 | 0.796 | 1.256281407 | up |
| Q0VCX9   | Ankyrin repeat and MYND domain-containing protein 2 (ANKMY2) | 1 | 0.812 | 1.231527094 | up |
| A2VE10   | Protein CASC4 (CASC4) | 1 | 0.829 | 1.206276218 | up |
| A7MB19   | NLRX1 protein (NLRX1) | 1 | 0.804 | 1.243781095 | up |
| Q6EV2    | elf4G1 protein (elf4G1) | 1 | 0.825 | 1.212121212 | up |
| Q3SZ99   | Peptidylprolyl isomerase (AIP) | 1 | 0.774 | 1.291989664 | up |
| E1BD11   | Chromosome 11 open reading frame 84 (SPINDOC) | 1 | 0.825 | 1.212121212 | up |
| A4FUCO   | 395 ribosomal protein L37, mitochondrial (MRPL37) | 1 | 0.815 | 1.226993865 | up |
| Q2TA30   | Ninjurin 1 (NINJ1) | 1 | 0.594 | 1.683501684 | up |
| E1BNN60  | Solute carrier family 30 member 1 (SLC30A1) | 1 | 0.77 | 1.298701299 | up |
| Q3T093   | Adaptin ear-binding coat-associated protein 1 (NECAP1) | 1 | 0.83 | 1.204819277 | up |
| G3N3D6   | Phosphoinositide phospholipase C(PLCH1) | 1 | 0.823 | 1.215066829 | up |
| Q2YDF6   | 285 ribosomal protein S35, mitochondrial(MRPS35) | 1 | 0.809 | 1.236093943 | up |
| Q0BDH9   | CCCTC-binding factor(TCTF) | 1 | 0.802 | 1.246882793 | up |
| Q0BDK7   | Mitochondrial basic amino acids transporter(SLC25A29) | 1 | 0.798 | 1.253132832 | up |
| F1MBD5   | Surfeit 2(SURF2) | 1 | 0.833 | 1.200480192 | up |
| G3XN83   | Serotransferrin (TF) | 1 | 0.76 | 1.315789474 | up |
### Table 1: Statistically significant DEPs identified by iTRAQ analysis of MDBK cells infected with CPIV3 (Continued)

| Accession | Protein name                                                                 | CPIV3-infected | Mock-infected | FC (CPIV3-infected vs Mock-infected) | regulate |
|-----------|------------------------------------------------------------------------------|----------------|---------------|--------------------------------------|----------|
| F1MG47    | Peroxisomal N(1)-acetyl-spermine/spermidine oxidase (PAOX)                   | 1              | 0.706         | 1.416430595                         | up       |
| E1BH45    | RB1 inducible coiled-coil 1 (RB1CC1)                                        | 1              | 0.682         | 1.46627566                           | up       |
| E1BF44    | Kinase D interacting substate 220 (KIDINS220)                               | 1              | 0.684         | 1.233045623                         | up       |
| E1BI11    | ELM2 and Myb/SANT domain containing 1 (ELMSAN1)                             | 1              | 0.736         | 1.358695652                         | up       |
| Q5E9T1    | GDP-D-glucose phosphatase 1 (GDPGP1)                                         | 1              | 0.823         | 1.215066829                         | up       |
| A7Z023    | CCDC132 protein (CCDC132)                                                   | 1              | 0.819         | 1.221001221                         | up       |
| A6QR26    | UBAP1 protein (UBAP1)                                                        | 1              | 0.702         | 1.424501425                         | up       |
| E1B4J7    | Histone deacetylase (HDAC6)                                                  | 1              | 0.832         | 1.201923077                         | up       |
| Q148F0    | Ubiquitin-related modifier 1 (URM1)                                         | 1              | 0.402         | 2.487562189                         | up       |
| F1MR86    | Lemur tyrosine kinase 2 (LMTK2)                                             | 1              | 0.42          | 2.380952381                         | up       |
| QV882     | Bax inhibitor 1 (TMBMI6)                                                     | 1              | 0.766         | 1.305483029                         | up       |
| G3X6Y2    | Chromosome X open reading frame 38 (CXHxorf38)                              | 1              | 0.81          | 1.234567901                         | up       |
| G3MYB9    | UNC homeobox (UNCX)                                                         | 1              | 0.793         | 1.261034048                         | up       |
| G3NO5     | Uncharacterized protein                                                      | 1              | 0.698         | 1.432664756                         | up       |
| Q5Z2N3    | Metalloendopeptidase OMA1, mitochondrial (OMA1)                             | 1              | 0.304         | 3.289473684                         | up       |
| A7YWV9    | PHLD21 protein (PHLD1A)                                                     | 1              | 0.654         | 1.529051988                         | up       |
| A5U7Q0    | Allograft inflammatory factor 1-like (AIF1L)                                 | 1              | 0.808         | 1.237623762                         | up       |
| Q2YDD1    | FGFR1 oncogene partner (FGFR1OP)                                            | 1              | 0.664         | 1.506024096                         | up       |
| F1M3N9    | Interferon related developmental regulator 1 (IFRD1)                        | 1              | 0.521         | 1.919385797                         | up       |
| Q0I90     | Protein FAM81B (FAM81B)                                                     | 1              | 0.118         | 8.474576271                         | up       |
| QV5V95    | Calcitonin receptor-stimulating peptide 1 (CRSP1)                           | 1              | 0.818         | 1.222493888                         | up       |
| F1RMS9    | Discs large MAGUK scaffold protein 5 (DLGS)                                 | 1              | 0.795         | 1.257861635                         | up       |
| E1BFR6    | Transmembrane protease, serine 13 (TMPRSS13)                                | 1              | 0.827         | 1.209189843                         | up       |
| E1BC4     | Miasinis (MDN1)                                                            | 1              | 0.579         | 1.727115717                         | up       |
| Q5BDG0    | Nuclear receptor binding factor 2 (NRFB2)                                   | 1              | 0.434         | 2.304174706                         | up       |
| Q2K8B     | Lish domain-containing protein ARMC9 (ARMC9)                                | 1              | 0.411         | 2.4330024                           | up       |
| F1MNN5    | Sortilin related VPS10 domain containing receptor 1 (SORCS1)               | 1              | 0.759         | 1.317523057                         | up       |
| A0A1Y0T882| Uncharacterized protein CLBA1 (CLBA1)                                       | 1              | 0.635         | 1.57480315                          | up       |
| F1M7307   | Transmembrane protein 131 (TMEM131)                                        | 1              | 0.793         | 1.261034048                         | up       |
| Q28037    | Vitamin D3 receptor (VDR)                                                   | 1              | 0.826         | 1.210633753                         | up       |
| F1N2K8    | Periplakin (PPL)                                                            | 1              | 1.423         | 0.702746899                         | down     |
| F6RJG0    | 3-hydroxy-3-methylglutaryl coenzyme A synthase (HMGC51)                     | 1              | 1.371         | 0.72939602                          | down     |
| Q5KR49    | Tropomyosin alpha-1 chain (TPM1)                                            | 1              | 1.227         | 0.814999325                         | down     |
| G3MVW5    | Histone cluster 1 H1 family member e (HIST1H1E)                            | 1              | 1.264         | 0.79119241                          | down     |
| A7MAZ5    | Histone H1.3 (HIST1H1D)                                                     | 1              | 1.258         | 0.79491256                          | down     |
| Q5SYV6    | Importin subunit alpha (KPN1A2)                                             | 1              | 1.22          | 0.819672131                         | down     |
| Q3SYY8    | Thrombospondin-1 (THBS1)                                                    | 1              | 1.406         | 0.711237553                         | down     |
| A4FV84    | KRT6A protein (KRT6A)                                                       | 1              | 1.212         | 0.825082508                         | down     |
| A6QBP5    | PGM1 protein (PGM1)                                                        | 1              | 1.272         | 0.78613522                          | down     |
| G3NOV2    | Keratin 1 (KRT1)                                                           | 1              | 1.499         | 0.667111408                         | down     |
| Accession | Protein name                                           | CPIV3-infected | Mock-infected | FC (CPIV3-infected vs Mock-infected) | regulate |
|-----------|--------------------------------------------------------|----------------|---------------|--------------------------------------|----------|
| E1BNE7    | Caveolae associated protein 1 (CAVIN1)                 | 1              | 1.213         | 0.824402308                         | down     |
| Q3YJF3    | MHC class I antigen (Fragment) (BoLA)                  | 1              | 1.277         | 0.783085356                         | down     |
| Q2HJU0    | Kinesin light chain 4 (KLC4)                           | 1              | 1.209         | 0.827129859                         | down     |
| F1MX88    | Solute carrier family 25 member 13 (SLC25A13)          | 1              | 1.212         | 0.825082508                         | down     |
| F1N688    | V-type proton ATPase subunit B, kidney isoform (ATP6V1B1) | 1              | 1.352         | 0.73964497                         | down     |
| Q0VCZ8    | Acyl-CoA synthetase long-chain family member 1 (ACSL1) | 1              | 1.333         | 0.750187547                         | down     |
| A6QNZ7    | Keratin 10 (Epidermolytic hyperkeratosis; keratosis palmaris et plantaris) (KRT10) | 1              | 1.387         | 0.720980534                         | down     |
| F1N4K3    | Uncharacterized protein                                | 1              | 1.474         | 0.678462052                         | down     |
| F1MTJ9    | Terpene cyclase/mutase family member (LSS)             | 1              | 1.243         | 0.804505229                         | down     |
| Q867D1    | Stearoyl-CoA desaturase (Scd)                          | 1              | 1.427         | 0.700770848                         | down     |
| F1MH31    | Nucleoporin 214 (NUP214)                               | 1              | 1.241         | 0.805801773                         | down     |
| G3N1R5    | Uncharacterized protein                                | 1              | 1.454         | 0.687757909                         | down     |
| Q32PA5    | Ubiquitin-conjugating enzyme E2 C (UBE2C)              | 1              | 1.589         | 0.629326621                         | down     |
| Q0PSJ6    | Keratin, type I cytoskeletal 27 (KRT27)                | 1              | 1.375         | 0.772272727                         | down     |
| A7MB38    | SFRS4 protein (SRSF4)                                  | 1              | 1.22          | 0.819672131                         | down     |
| A7YW33    | DNA polymerase delta interacting protein 3 (POLDIP3)   | 1              | 1.267         | 0.789265983                         | down     |
| Q3ZC01    | Coiled-coil-helix-coiled-helix domain containing 2 (CHCHD9) | 1              | 1.298         | 0.770416025                         | down     |
| E1BJC9    | Uncharacterized protein (C18H19orf33)                  | 1              | 1.24          | 0.806451613                         | down     |
| A5D7N6    | Kinesin-like protein (KIF23)                           | 1              | 1.373         | 0.728332119                         | down     |
| F2ZH42    | Non-histone chromosomal protein HMG-17 (HMGN2)         | 1              | 1.242         | 0.805152979                         | down     |
| A3KL9     | Superoxide dismutase (SOD3)                            | 1              | 1.36          | 0.735294118                         | down     |
| G8J KY5   | Thymosin beta-4 (TMS4X)                                | 1              | 1.547         | 0.646412411                         | down     |
| Q08DJ5    | Ras-related protein Rap-2c (RAP2C)                     | 1              | 1.207         | 0.828500414                         | down     |
| A4IF70    | GPR56 protein (GPR56)                                  | 1              | 1.233         | 0.811030008                         | down     |
| P15103    | Glutamine synthetase (GLUL)                            | 1              | 1.265         | 0.790513834                         | down     |
| E1BKT0    | Leucine zipper protein 1 (LUZP1)                       | 1              | 1.353         | 0.7390983                           | down     |
| F1MFW9    | Keratin 24 (KRT24)                                     | 1              | 2.313         | 0.43238954                          | down     |
| Q0VC74    | Trimethyllysine dioxygenase, mitochondrial (TMLHE)      | 1              | 1.217         | 0.821692687                         | down     |
| F1MLZ1    | Cytochrome b reductase 1 (CYBRD1)                      | 1              | 1.252         | 0.798722045                         | down     |
| F1MP14    | Forkhead box K1 (FOXK1)                                | 1              | 1.208         | 0.82781457                          | down     |
| F1MYS2    | FCH domain only 2 (FCHD2)                              | 1              | 1.253         | 0.798084597                         | down     |
| Q3T02J    | Guanine nucleotide-binding protein-like 3-like protein (GNL3L) | 1              | 1.336         | 0.748502994                         | down     |
| Q2NZK9    | Carboxypeptidase (SCPEP1)                              | 1              | 1.206         | 0.829187396                         | down     |
| F1N6L1    | Valyl-tRNA synthetase 2, mitochondrial (VARS2)         | 1              | 1.272         | 0.786163522                         | down     |
| G6E5Q8    | SET binding factor 1 (SBF1)                            | 1              | 1.286         | 0.777604977                         | down     |
| Q2KH47    | Regulator of G-protein signaling 10 (RGS10)            | 1              | 1.219         | 0.820344545                         | down     |
| F1N4R2    | Uncharacterized protein (MORF4L1)                      | 1              | 1.222         | 0.818330606                         | down     |
| Q5E9Q1    | Protein O-glucosyltransferase 1 (POGLUT1)              | 1              | 1.234         | 0.810372771                         | down     |
| Q2R9Z9    | WD repeat-containing protein 92 (WDR92)                | 1              | 1.26          | 0.793650794                         | down     |
| F1NSR4    | Conserved oligomeric Golgi complex subunit 8 (COG8)    | 1              | 1.271         | 0.786782061                         | down     |
| F1ML71    | Neddyd family interacting protein 2 (NDFIP2)           | 1              | 1.254         | 0.797448166                         | down     |
| Accession | Protein name                                      | CPIV3-infected | Mock-infected | FC (CPIV3-infected vs Mock-infected) | regulate |
|-----------|--------------------------------------------------|----------------|---------------|--------------------------------------|----------|
| G3N266    | G protein signaling modulator 1 (GPSM1)          | 1              | 1.235         | 0.809716599                         | down     |
| F1N0K0    | Collagen alpha-1(XI) chain (COL11A1)             | 1              | 1.212         | 0.825082508                         | down     |
| F1MFG2    | Chromodomain helicase DNA binding protein 1 (CHD1) | 1              | 1.254         | 0.797448166                         | down     |
| A6QQK2    | MAP3K7P1 protein (MAP3K7P1)                      | 1              | 1.339         | 0.74682599                          | down     |
| E1BD81    | Ras and Rab interactor 1 (RIN1)                  | 1              | 1.29          | 0.775193798                         | down     |
| E1BBR7    | HPSS, biogenesis of lysosomal organelles complex 2 subunit 2 (HPSS) | 1              | 1.252         | 0.798722045                         | down     |
| A8E646    | CARD11 protein (CARD11)                          | 1              | 1.222         | 0.818330606                         | down     |
| Q32Kl9    | B-cell receptor-associated protein 29 (BCAP29)    | 1              | 1.435         | 0.696864111                         | down     |
| E1BG66    | Regulatory factor X5 (RFX5)                      | 1              | 1.233         | 0.811030008                         | down     |
| Q3TON3    | Calcium load-activated calcium channel (TMCO1)   | 1              | 1.295         | 0.772200772                         | down     |
| E1BCR9    | Oxysterol-binding protein (OSBPL5)               | 1              | 1.241         | 0.805801773                         | down     |
| F1MQ45    | Solute carrier organic anion transporter family member (SLCO2A1) | 1              | 1.234         | 0.810372771                         | down     |
| Q32P76    | Small EDRK-rich factor 1 (SERF1)                 | 1              | 1.447         | 0.691085003                         | down     |
| A6QQ55    | WHSC2 protein (WHSC2)                            | 1              | 1.289         | 0.77579519                         | down     |
| F1MNT2    | Protein RTF2 homolog (RTF2)                      | 1              | 1.278         | 0.782472613                         | down     |
| F1MVE2    | Enoyl-[acyl-carrier-protein] reductase, mitochondrial (MECR) | 1              | 1.387         | 0.720980534                         | down     |
| A6QNX2    | DPP7 protein (DPP7)                              | 1              | 1.287         | 0.777007777                         | down     |
| E1BE80    | Transmembrane protein 236 (TMEM236)              | 1              | 1.247         | 0.801924619                         | down     |
| A4IFD1    | PDCD4 protein (PDCD4)                            | 1              | 1.209         | 0.827129859                         | down     |
| A1A4R8    | Cell division cycle protein 23 homolog (CDC23)   | 1              | 1.267         | 0.789265983                         | down     |
| E1BG49    | Centromere protein E (CENPE)                     | 1              | 1.324         | 0.755287009                         | down     |
| P07926    | ATP synthase F(0) complex subunit C2, mitochondrial (ATP5MC2) | 1              | 1.497         | 0.668002672                         | down     |
| Q402A0    | Aggrus (DPDN)                                    | 1              | 1.293         | 0.773395205                         | down     |
| Q17Q1     | Trafficking protein particle complex subunit 1 (TRAPPC1) | 1              | 1.265         | 0.790513834                         | down     |
| E1BKA4    | Uncharacterized protein (HAUS4)                  | 1              | 1.3           | 0.769230769                         | down     |
| Q2KHT6    | F-box only protein 32 (FBXO32)                    | 1              | 1.227         | 0.814999525                         | down     |
| F1M544    | Doublecortin domain containing 2 (DCDC2)         | 1              | 1.277         | 0.783085356                         | down     |
| E1BIR2    | Dipeptidase (DPEP2)                              | 1              | 1.211         | 0.825763832                         | down     |
| A5PKA5    | Sorting nexin-27 (SNX27)                         | 1              | 1.31          | 0.763358779                         | down     |
| A6H7C1    | MORF4L2 protein (MORF4L2)                        | 1              | 1.213         | 0.824402308                         | down     |
| A6QLZ5    | Protein FAM177A1 (FAM177A1)                      | 1              | 1.23          | 0.81300813                         | down     |
| P13384    | Insulin-like growth factor-binding protein 2 (IGFBP2) | 1              | 1.748         | 0.57208238                         | down     |
| A5D974    | Acyl-Coenzyme A dehydrogenase family, member 9 (ACAD9) | 1              | 1.217         | 0.821692687                         | down     |
| F1N2N9    | Coiled-coil domain containing 114 (CCDC114)      | 1              | 1.224         | 0.816993464                         | down     |
| E1BBH4    | Protein unc-93 homolog 81 (UNC93B1)              | 1              | 1.666         | 0.600240096                         | down     |
| A5PXS0    | F-box protein 22 (FBXO22)                        | 1              | 1.272         | 0.786163522                         | down     |
| E1BEG4    | Zinc finger FYVE-type containing 16 (ZFYVE16)     | 1              | 1.225         | 0.816326531                         | down     |
| E1BE6     | ATM serine/threonine kinase (ATM)                | 1              | 1.507         | 0.663570007                         | down     |
| P0C914    | Overexpressed in colon carcinoma 1 protein homolog | 1              | 1.213         | 0.824402308                         | down     |
time for proteomic analysis [21, 22]. Taking this substantial evidence into consideration, cell samples at 24 hpi were chosen for further proteomic analysis. Based on our study, the expression levels of 163 DEPs were found to be significantly altered in CPIV3-infected cells. The results of GO, KEGG pathway and STRING analysis predicted that these DEPs pertaining to different types of functional categories and signal pathways. Western blot and qRT-PCR were also applied to validate some differential proteins at the mRNA and protein levels. To date, no analysis has been reported of the differential proteomes of MDBK cells infected with CPIV3. Our data may provide an overview of the proteins altered in expression during the host response to CPIV3 infection and may provide insight in the process of CPIV3 pathogenesis.

Studies have shown that HSPs may play an important role in virus host cell interactions during in vivo and in vitro infection [23, 24]. Inhibitors of HSP90 can inhibit herpes simplex virus type 1 (HSV-1) infection [25]. Bovine viral diarrhea virus (BVDV) structural proteins comprise the C nucleocapsid protein and three envelope glycoproteins, Erns, E1 and E2 [26]. A previous study found that HSP110 enhanced the presentation of E2 to CD4+ T cells in vitro to improve the immunogenicity of an E2 vaccine in cattle [27]. Previous work demonstrated that HSP70 is actively released into the extracellular milieu and acts as a cytokine and peptide adjuvant, thereby promoting both the innate and adaptive immune responses [28]. In our analysis, four proteins (HSPA5, HSPA1B, HSP90B1 and HSPA6) were identified

**Fig. 2** GO enriched histogram of DEPs. Each column in the figure is a GO terms, the abscissa text indicates the name and classification of GO, and the height of the column indicates the enrichment rate. The color indicates the significance of the enrichment (p-value). The darker the color, the more significant the enrichment of the GO term (*P < 0.05; **P < 0.01; ***P < 0.001)

**Fig. 3** KEGG enrichment annotation of the DEPs. Each column in the figure is a pathway. The abscissa text indicates the name and classification of the pathway, and the height of the column indicates the enrichment rate. The color indicates the significance of the enrichment (p-value). The darker the color, the more significant the enrichment of the pathway (*P < 0.05; **P < 0.01; ***P < 0.001)**
following CPIV3 infection. HSP90B1 is proposed to be associated with poor survival from hepatocellular carcinoma (HCC), whereas high levels of HSPA5 and HSPA6 may be associated with earlier recurrence of HCC [29]. HSPA1B, also known as heat shock protein 72, is a member of the HSP70 family. HSP70 expression levels rapidly increased in response to cellular stresses such as heat shock, or in response to certain viral infections [30–33].

In the current study, HSP70 was rarely detected in the mock-infected group, whereas it was notably present in the CPIV3 group. CPIV3 infection resulted in the up-regulated secretion of exosomes and packaging of the viral proteins into exosomes, and these results suggested that CPIV3 infection may enhance HSP70-mediated exosome release (unpublished data). In addition, HSP70 is actively released into the extracellular milieu, thereby promoting innate and adaptive immune responses [34]. In this study, HSPA5, HSPA1B, HSP90B1 and HSPA6 were up-regulated at 24 hpi to various degrees following CPIV3 -infection of MDBK cells. Different expression levels of HSPA1B were detected by western blot analysis at 24 hpi and 48 hpi after CPIV3 -infection of MDBK cells. This may indicate that HSPA1B affects the proliferation of CPIV3 in MDBK cells. HSPA1B is an endogenous ligand for toll-like receptor

| Cellular Processes         | Metabolism            |
|----------------------------|-----------------------|
| Phagosome                  | Other types of O-glucan biosynthesis |
| Peroxisome                 | Metabolic pathways    |
| Endocytosis                | Carbon metabolism     |
| Tight junction             | Biogenesis of amino acids |
| Fetal alcohol              | Oxidative phosphorylation |
| p53 signaling pathway      | Nitrogen metabolism   |
| Oxytocin                   | Glycine and diacylthiole metabolism |
| Neuropeptide               | Lysine degradation    |
| Cellular senescence        | Glycine, serine and threonine metabolism |
| Cell cycle                 | Arginine biosynthesis |
|                           | Alanine, aspartate and glutamate metabolism |

| Organismal Systems         |
|----------------------------|
| Glutamatergic synapse      |
| GABAergic synapse          |
| IL-17 signaling pathway    |
| Complement and coagulation cascades |
| Antigen processing and presentation |
| Endocrine and other factor-regulated calcium resorption |
| Thermogenesis              |
| Thyroid hormone synthesis  |
| Progesterone-mediated oocyte maturation |
| Parathyroid hormone synthesis, secretion and action |
| Insulin signaling pathway  |
| Estrogen signaling pathway |
| Mineral absorption         |
| Cardiac muscle contraction |
| Adrenergic signaling in cardiomyocytes |
| Longevity regulating pathway - multiple species |

| Environmental Information Processing | Genetic Information Processing |
|--------------------------------------|--------------------------------|
| TGF-beta signaling pathway           | Ribosome biogenesis in eukaryotes |
| Rap1 signaling pathway               | mRNA surveillance pathway      |
| PI3K-Akt signaling pathway           | Spliceosome                    |
| MAPK signaling pathway               | Ubiquitin mediated proteolysis |
| Hippo signaling pathway              | Sulfur relay system            |
| HIP-1 signaling pathway              | Protein processing in endoplasmic reticulum |
| FoxO signaling pathway               | Protein export                 |

| Human Diseases                  |
|---------------------------------|
| Parkinson's disease             |
| Alzheimer's disease             |
| Influenza A                     |
| HTLV-1 Infection                |
| Toxoplasmosis                   |
| Chagas disease (American trypanosomiasis) |
| Salmonella infection            |
| AGE-RAGE signaling pathway in diabetic complications |
| Fluid shear stress and atherosclerosis |
| Prostate cancer                 |
| Proteoglycans in cancer         |
| MicroRNAs in cancer             |

Fig. 4 Functional characterization of DEPs. a Cellular processes, metabolism and organismal systems. b Environmental information processing, genetic information processing and human diseases. More information is available in Additional file 5: Figure S7.
TLR4, thereby stimulating innate immunity [35], and HSPA1B regulates the NF-κB pathway via TLR2 and TLR4 in fibroblasts. However, fibroblasts and macrophages interact with each other to mediate the immune response. Activation of the NF-κB pathway then results in enhanced secretion of pro-inflammatory cytokines (TNF-α, IL-6 and IL-1β) and neutrophil chemoattractant MIP-2 and Cxcl1 from macrophages [36]. This evidence indicates that HSPA1B may be associated with the proliferation of CPIV3 in MDBK cells through an ability to interact with key components of the NF-κB pathway, moreover, those involved in innate immunity, but the detailed mechanism remains
unknown. However, the detailed functions of these pathways and proteins changes in CPIV3 infection therefore requires further verification.

**Conclusions**
The proteomic changes in CPIV3-infected MDBK cells were analyzed using iTRAQ combined with LC-MS/MS. To the best of our knowledge, this is the first time proteomics has been used to explore the virus–host protein interaction network in CPIV3-infected MDBK cells. The results revealed 163 DEPs, among which 72 were up-regulated and 91 were down-regulated. In addition, four DEPs were validated by qRT-PCR and HSPA1B was validated by western blot analysis. These results were consistent with those of label-free LC-MS analysis. Our analyses of the DEPs were descriptive, and further functional investigations are required to elucidate the pathogenic mechanisms and cellular responses to CPIV3 infection.

**Methods**
**Cell culture and virus infection**
CPIV3 strain JS2013 isolated in Jiangsu Province was used for virus infection. MDBK cells were cultured in Dulbecco’s modified Eagle’s medium (DMEM; Sigma, CA, USA) supplemented with 10% fetal bovine serum (FBS; HyClone, UT, USA), at 37 °C in an atmosphere of 5% CO₂. When the cells grow to 70–80% confluence, they were inoculated with CPIV3 at a multiplicity of infection (MOI) of 1. After 1 h of adsorption, infected cells were maintained in fresh medium containing 2% FBS. Uninfected cells were used as a control. The CPIV3- or mock-infected cells were collected at 24 hpi. Viral propagation was confirmed by the observation of a cytopathic effect (CPE).

**Protein sample preparation and labeling with iTRAQ reagent**
The CPIV3- and mock-infected cell samples were washed three times with cold phosphate-buffered saline (PBS) and then treated with lysis buffer containing 8 M urea, 4% CHAPS, 2 M thiourea, and 30 mM Tris-HCl on ice for 30 min until the cell line were completely lysed. The supernatant was collected by centrifugation at 12000×g for 30 min at 4 °C after ultrasonication treatment for 2 min. The protein concentration in the supernatants was quantified using the Bradford protein assay. After reduction and cysteine-blocking as described in the iTRAQ protocol (AB Sciex, Concord, ON, USA), solutions containing 100 μg protein were digested overnight at 37 °C with sequence grade modified trypsin (Promega, Madison, WI, USA) and then labeled with...
different iTRAQ tags. The labeled samples were then mixed and dried with a rotary vacuum concentrator.

**LC-MS analysis**
Ten microliters (μl) of each fraction were analyzed by Q Exactive (Thermo, USA) mass spectrometer coupled to a Proxeon Biosystem Easy-nLC 1200 (Thermo Fisher Scientific, Waltham, MA, USA) in the LC-MS experiments. The peptide mixture (5 g) was loaded onto a C18 column (75 μm × 25 cm, Thermo, USA) packed with RP-C18 (5 m) resin in buffer A (2% ACN with 0.1% formic acid), and eluted with a linear gradient of buffer B (80% ACN with 0.1% formic acid) at a flow rate of 300 nl/min for 120 min using IntelliFlow technology. The equate underwent electrospray ionization for LC-MS analysis. The MS/MS instrument was run in the peptide recognition mode, and the spectra were acquired using a data-dependent top-20 method based on the selection of the most abundant precursor ions from the survey scan (350–1300 m/z) for HCD fragmentation. Determination of the target value was based on the predictive automatic gain control, and the dynamic exclusion duration was 18 s. Survey scans were acquired at a resolution of 70,000 at m/z 200, and the resolution for the HCD spectra was set to 17,500 at m/z 200. The normalized collision energy was 30 eV, and the underfill ratio, which specifies the minimum percentage of the target value likely to be reached at maximum fill time, was defined as 0.1%. Thermo Xcalibur 4.0 (Thermo, USA) was used to collect MS analysis data via DDA mode.

**Data analysis**
The MS data were analyzed using Proteome Discoverer™ software 2.1. When the library was searched, the raw file was submitted to the Proteome Discoverer server searched against the Uniprot Bos taurus database (197,939 total sequences, downloaded April 26, 2018). The following parameters were used for protein identification: a precursor mass tolerance of 20 ppm; a fragment mass tolerance of 0.05 Da; trypsin digestion; max. Missed cleavage sites of 2; the variable dynamic modifications included oxidation (M), iTRAQ8plex (Y) and acetyl (protein N-terminus), and the fixed static modifications included carbamidomethyl (C), iTRAQ8plex (K) and iTRAQ8plex (N-term). The cutoff for the global false discovery rate (FDR) for peptide and protein identification was set to 0.01. The value of the quantitative ratio for each protein relative to the internal reference was calculated, and averaged to obtain the quantitative ratio (V/C) of the proteins identified in the treatment groups [37]. Proteins with a fold change > 1.2 and a p-value < 0.05 were considered to show significantly different expression. Auto bias-correction was executed to decrease the artificial error. Statistical analysis was performed using Excel 2007 software. The DEPs were annotated using gene ontology (GO) and KEGG database. The Cluster of Orthologous Groups of proteins (COG or KOG) were retrieved, and mapped to pathways in the KEGG database [38]. In addition, DEPs were analyzed using STRING for predicting functional association networks of proteins.

**CPIV3 yield quantification**
MDBK cells were seeded in 96-well plates and incubated for 24 h. Then, CPIV3 samples were 10-fold serially diluted and added to each well in quadruplicate. MDBK cells exhibit CPE were scored positive for viral growth and the TCID$_{50}$ was calculated by the Reed–Muench method [39].

**mRNA quantitation by qRT-PCR**
Total cellular RNA was extracted from the CPIV3-infected and mock-infected MDBK cells using Transzol UP reagent (Transgen Co. Ltd., Beijing, China) according to the manufacturer’s protocol. Specific primers for amplifying various genes were as follows: for GAPDH mRNA analysis, 5'–GATTGTGACGAAATGCCTCCT–3' (forward) and 5'–GTCATAAGTCCTCCACAGA–3' (reverse) were used; for HSPA5 mRNA analysis, 5'–GTGCCCAACCAAGAAGTCTCA–3' (forward) and 5'–TCTTTGCTACCGGATTCCGTC–3' (reverse) were used; for HSPA6 mRNA analysis, 5'–TCAAGGGTGTGTTGGACTCG–3' (forward) and 5'–GC TGAAGTGCTTCACGGGAA–3' (reverse) were used; for HSP90B1 mRNA analysis, 5'–TGCCCAAAATCCACACCC–3' (forward) and 5'–GC TGAAGTGCTTCACGGGAA–3' (reverse) were used; for HSPA1B mRNA analysis, 5'–GTGCTGTCACAGGGTGCTGC–3' (forward) and 5'–GCAAGTCGCTCCCTC–3' (reverse) were used; for HSPA2B mRNA analysis, 5'–GGATGGCGGGCTTCAAGGG–3' (forward) and 5'–GGATGGCGGGCTTCAAGGG–3' (reverse) were used. GAPDH was employed as an internal reference gene. The first-strand cDNA was synthesized via PrimeScript™ RT Master Mix (TaKaRa, Dalian, China). Then qRT-PCR was performed using the SYBR Premix Ex Taq™ II Kit (TaKaRa) on an ABI Step One thermocycler (Applied Biosystems, CA, USA). The relative expression level of each mRNA was calculated by the 2$^{-ΔΔct}$ method. Three independent biological replicates were performed for each gene.

**Western blot analysis**
To further verify the variation in the DEPs identified by the proteomic approaches, HSPA1B was selected for western blot analysis. The CPIV3- and mock-infected cells were collected at 24 and 48 hpi. Equivalent amounts of cell lysate from each sample were collected. After measuring the protein concentrations, equivalent amounts of cellular proteins were separated by SDS-PAGE and transferred onto nitrocellulose PVDF membranes (Millipore, USA). The membranes were incubated overnight at 4°C with primary rabbit polyclonal antibodies of anti-HSPA1B (Biyotime,
Shanghai, China). Then the membranes were further incubated for 1 h with horseradish peroxidase-conjugated goat anti-rabbit secondary antibody (BIOSS, Beijing, China). The protein bands were detected using the ECL Detection Kit (Vazyme, Nanjing, China). β-actin protein was used as an internal control.

Additional files

Additional file 1: Figure S1. Information on the detected proteins in CPIV3-infected MDBK cells (JPG 357 kb)
Additional file 2: Figure S2. Detected proteins were annotated in the GO database (JPG 1406 kb)
Additional file 3: Figure S3. The top 20 pathways annotated by KEGG (JPG 738 kb)
Additional file 4: Figure S4 and Data Sheet 5. Proteins were annotated based on the KOG (ZIP 669 kb)
Additional file 5: Figure S6 and S7. Heat map and scatterplot (ZIP 1116 kb)
Additional file 6: Figure S8. GO annotations for the up-regulated and down-regulated proteins (JPG 2482 kb)
Additional file 7: Data Sheet 9. The 93 DEPs were annotated into six KEGG pathway categories (XLS 32 kb)

Abbreviations
COG: Cluster of orthologous groups of proteins; CPE: Cytopathic effect; CPIV3: Caprine parainfluenza virus type 3; DEPs: Differentially expressed proteins; DMEM: Dulbecco’s modified Eagle’s medium; FDR: False discovery rate; GO: Gene ontology; HSPs: Heat shock proteins; iTRAQ: Isobaric tags for relative and absolute quantification; KEGG: Kyoto encyclopedia of genes and genomes; MDBK: Madin-Darby bovine kidney; MOI: Multiplicity of infection

Acknowledgments
We thank Kate Fox, DPhil, from Liwen Bianji, Edanz Group China (https://www.liwenbianji.cn/), for editing the English text of a draft of this manuscript.

Funding
This work was supported by the National Natural Science Foundation of China (31702272, 31802196), Natural Science Foundation of Jiangsu Province, China (BK20170595), Natural Science Foundation of Shandong Province, China (ZR2016CP08), and the National Key R&D Program of China (2017YFD0500908, 2018YFD0502100). The funders had no role in study design, in the collection, analysis and interpretation of data, in the writing of the manuscript, or in the decision to submit the article for publication.

Availability of data and materials
The datasets contained in this study are available from the corresponding author upon request.

Authors’ contributions
CZ and JL took part in all the experiments and wrote the manuscript. LM, ML, XZ and WL helped to designed the whole project and draft the manuscript. MS, FX, LY, WZ and ZL conducted cell culture and sample processing for sequencing. XJ conducted data analysis. All authors read and approved the final manuscript.

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Publisher’s Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Author details
1Institute of Veterinary Medicine, Jiangsu Academy of Agricultural Sciences, Key Laboratory of Veterinary Biological Engineering and Technology, Ministry of Agriculture, Nanjing 210014, China. 2School of Pharmacy, Linyi University, Linyi 276000, China. 3College of Animal Science, Guizhou University, Guiyang 550025, China. 4Key Lab of Food Quality and Safety of Jiangsu Province-State Key Laboratory Breeding Base, Nanjing 210014, China.

Received: 14 February 2019 Accepted: 1 May 2019
Published online: 17 May 2019

References
1. Li W, Mao L, Cheng S, Wang Q, Huang J, Deng J, Wang Z, Zhang W, Yang L, Hao F, et al. A novel parainfluenza virus type 3 (PIV3) identified from goat herds with respiratory diseases in eastern China. Vet Microbiol. 2014;174(1–2):100–6.
2. Yang L, Li W, Mao L, Hao F, Wang Z, Zhang W, Deng J, Jiang J. Analysis on the complete genome of a novel caprine parainfluenza virus 3. Infect Genet Evol. 2016;38:29–34.
3. Li W, Hao F, Mao L, Wang Z, Zhou T, Deng J, Li J, Zhang W, Yang L, Ly V, et al. Pathogenicity and horizontal transmission studies of caprine parainfluenza virus type 3 J52013 strain in goats. Virus Res. 2016;223:80–7.
4. Mao L, Li W, Zhou T, Yang L, Hao F, Li J, Zhang W, Luo X, Jiang J. Development of a blocking ELISA for caprine parainfluenza virus type 3. J Virol Methods. 2017;250:59–65.
5. Li J, Li W, Mao L, Hao F, Yang L, Zhang W, Jiang J. Rapid detection of novel caprine parainfluenza virus type 3 (PIV3) using a TaqMan-based RT-qPCR. J Virol Methods. 2016;236:126–31.
6. Mao L, Yang L, Li W, Liang P, Zhang S, Li J, Sun M, Zhang W, Wang L, Zhong C, et al. Epidemiological investigation and phylogenetetic analysis of caprine parainfluenza virus type 3 in sheep of China. Transbound Emerg Dis. 2019. https://doi.org/10.1111/tbed.13149.
7. Zheng J, Sugrue RJ, Tang K. Mass spectrometry based proteomic studies on viruses and hosts—a review. Anal Chim Acta. 2011;702(2):149–59.
8. Han K, Zhao D, Liu Y, Liu Q, Huang X, Yang J, An F, Li Y. Quantitative proteomic analysis of duck ovarian follicles infected with duck Tumbes virus by label-free LC-MS. Front Microbiol. 2016;7:463.
9. Yang Y, Bu D, Zhao X, Sun P, Wang J, Zhou L. Proteomic analysis of cow, yak, buffalo, goat and camel milk whey proteins: quantitative differential expression patterns. J Proteome Res. 2013;12(4):1660–7.
10. Pandey A, Sahu AR, Wani SA, Saxena S, Kanchan S, Sahu VH, Rajak KK, Khanduri A, Sahoo AP, Tiwari AK, et al. Modulation of host mRNAs transcription in lung and spleen of Peste des Petits ruminants virus infected sheep and goats. Front Microbiol. 2017;8:1146.
11. Fernandez de Mera IG, Chaligiannis I, Hernandez-Jarguin A, Villar M, Mateos-Hernandez L, Papa A, Sotriaki S, Ruiz-Fons F, Cabezás-Cruz A, Gotorca C, et al. Combination of RT-PCR and proteomics for the identification of Crimean-Congo hemorrhagic fever virus in ticks. Heliyon. 2017;3(7):e00353.
12. Hagglund S, Blodom K, Naslund K, Vargmar K, Lind SB, Mi J, Araning M, Riffault S, Taylor G, Pringle J, et al. Proteome analysis of bronchoalveolar lavage from calves infected with bovine respiratory syncytial virus-insights in pathogenesis and perspectives for new treatments. PLoS One. 2017;12(10):e0186594.
13. Sun D, Zhang H, Guo D, Sun A, Wang H. Shotgun proteomic analysis of plasma from dairy cattle suffering from footot: characterization of potential disease-associated factors. PLoS One. 2013;8(2):e59573.
14. He Y, Li W, Liao G, Xie J. Mycobacterium tuberculosis-specific phagosome proteome and underlying signaling pathways. J Proteome Res. 2012;11(5):2635–43.
15. Zeng S, Zhang H, Ding Z, Luo R, An K, Liu L, Bi J, Chen H, Xiao S, Fang L. Proteome analysis of porcine epidemic diarrhea virus (PEDV)-infected Vero cells. Proteomics. 2015;15(11):1819–28.
16. Linde ME, Colquhoun DR, Ubara Mohien C, Kole T, Aquino V, Cotter R, Edwards N, Hildreth JE, Graham DR. The conserved set of host proteins incorporated into HIV-1 virions suggests a common egress pathway in multiple cell types. J Proteome Res. 2013;12(5):2045–54.
17. Sun D, Shi H, Guo D, Chen J, Shi D, Zhu Q, Zhang X, Feng L. Analysis of protein expression changes of the Vero E6 cells infected with classic PEDV

Note
Publisher's Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.
18. Cho YE, Singh TS, Lee HC, Moon PG, Lee JE, Lee MH, Choi EC, Chen YJ, Kim SH, Baek MC. In-depth identification of pathways related to cisplatin-induced hepatotoxicity through an integrative method based on an informatics-assisted label-free protein quantitation and microarray gene expression approach. Mol Cell Proteomics. 2012;11(1):M111 018884.

19. Zhang X, Zhou J, Wu Y, Zheng X, Ma G, Wang Z, Jin Y, He J, Yan Y. Differential proteome analysis of host cells infected with porcine circovirus type 2. J Proteome Res. 2009;8(11):5111–9.

20. Maxwell KL, Frappier L. Viral proteomics. Microbiol Mol Biol Rev. 2007;71(2):398–411.

21. An K, Fang L, Luo R, Wang D, Xie L, Yang J, Chen H, Xiao S. Quantitative proteome analysis reveals that transmissible gastroenteritis virus activates the JAK-STAT1 signaling pathway. J Proteome Res. 2014;13(12):5376–80.

22. Zhang LK, Chai F, Li HY, Xiao G, Guo L. Identification of host proteins involved in Japanese encephalitis virus infection by quantitative proteomics analysis. J Proteome Res. 2013;12(6):2666–78.

23. Biaga ACS, Camelo BM, Batista MN, Alinaga MM, Bittar C, Rahal P. Heat shock proteins HSPB8 and DNAJC5B have HCV antiviral activity. PLoS One. 2017;12(11):e0188467.

24. Rathore AP, Haystead T, Das PK, Merits A, Ng ML, Vasudevan SG. Chikungunya virus nsP3 & nsP4 interacts with HSP-90 to promote virus replication: HSP-90 inhibitors reduce CHIKV infection and inflammation in vivo. Antivir Res. 2014;103(7–16).

25. Zhong M, Zheng K, Chen M, Xiang Y, Jin F, Ma K, Qiu X, Wang Q, Peng T, Kitaoka K, et al. Heat-shock protein 90 promotes nuclear transport of herpes simplex virus 1 capsid protein by interacting with acetylated tubulin. PLoS One. 2014;9(6):e99425.

26. Patton JT, Chizhikov V, Taraporewala Z, Chen D. Virus replication. Methods Mol Med. 2000;34:33–66.

27. McLaughlin K, Carr VB, Kjøb K, LeFevre EA, Robinson L, Prentice HC, Charleston B. Hsp110-mediated enhancement of CD4+ T cell responses to the envelope glycoprotein of members of the family Flaviviridae in vitro does not occur in vivo. Clin Vaccine Immunol. 2011;18(2):311–7.

28. Hunter-Lavin C, Davies EL, Baccar MM, Marshall MJ, Andrew SM, Williams JH. Hsp70 release from peripheral blood mononuclear cells. Biochem Biophys Res Commun. 2004;324(2):511–7.

29. Yang Z, Zhuang L, Szatmary P, Wen L, Sun H, Liu Y, Xu Q, Chen X. Upregulation of heat shock proteins (HSPA12A, HSP90B1, HSPA4, HSPA5 and HSPA6) in tumour tissues is associated with poor outcomes from HBV-related early-stage hepatocellular carcinoma. Int J Med Sci. 2015;12(3):563–63.

30. Cheung RK, Dosch HM. The growth transformation of human B cells involves superinduction of hsp70 and hsp90. Virology. 1993;193(2):700–8.

31. Lefeuvre A, Contamin H, Decelle T, Fournier C, Lang J, Deubel V, Marianneau P. Host-cell interaction of attenuated and wild-type strains of yellow fever virus can be differentiated at early stages of hepatocyte infection. Microbes Infect. 2006;8:1530–8.

32. Liao WJ, Fan PS, Fu M, Fan XL, Liu YF. Increased expression of 70 kD heat shock protein in cultured primary human keratinocytes induced by human papillomavirus 16 E6/E7 gene. Chin Med J. 2005;118(24):2058–62.

33. Mayer MP. Recruitment of Hsp70 chaperones: a crucial part of viral survival strategies. Rev Physiol Biochem Pharmacol. 2005;153:1–46.

34. Mansilla MJ, Costa C, Eixarch H, Tepavcevic V, Castillo M, Martin R, Lubetzki C, Aigrot MS, Montalban X, Espejo C. Hsp70 regulates immune response in experimental autoimmune encephalomyelitis. PLoS One. 2014;9(8):e105737.

35. Rusai K, Banki NF, Prokai A, Podracka L, Szebeni B, Tulassay T, Reusz GS, Sallay P, Komendy R, Szabo AJ, et al. Heat shock protein polymorphism predisposes to urinary tract malformations and renal transplantation in children. Transplant Proc. 2010;42(8):2309–11.

36. Zulaziz N, Azhim A, Himeno N, Tanaka M, Satoh Y, Kinoshita M, Miyazaki H, Saitoh D, Shinomiya N, Morimoto Y. Photodynamic therapy mediates innate immune responses via fibroblast-macrophage interactions. Hum Cell. 2015;28(4):159–66.

37. Unwin RD, Griffiths JR, Whetton AD. Simultaneous analysis of relative protein expression levels across multiple samples using iTRAQ isobaric tags with 20 nano LC-MS/MS. Nat Protoc. 2010;5(5):1574–82.

38. Reed LJ, Muench H. A simple method of estimation of fifty percent endpoint. Am J Epidemiol. 1938;27:493–7.