

1      Turkey B-cell Transcriptome Profile During Turkey

2      Hemorrhagic Enteritis Virus (THEV) Infection Highlights

3      Upregulated Apoptosis and Breakdown Pathways That May

4      Mediate Immunosuppression

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16

17 **ABSTRACT**

18 **Background:** Hemorrhagic enteritis, caused by *Turkey Hemorrhagic Enteritis Virus (THEV)*, is a disease  
19 affecting turkey pouls characterized by immunosuppression (IMS) and bloody droppings. The clinical dis-  
20 ease usually lasts only a few days but secondary opportunistic infections due to THEV-induced IMS extend  
21 the duration of illness and mortality, exacerbating the economic losses. Albeit an avirulent THEV strain with  
22 only subclinical disease is available as a vaccine, it still retains immunosuppressive properties, thus, still  
23 causing substantial economic. To elucidate the mechanisms mediating THEV-induced IMS, we performed  
24 the first transcriptomic analysis of a THEV infection using RNA-seq, uncovering key insights into host cell  
25 response.

26 **Methods:** After infecting a turkey B-cell line with the vaccine strain, samples in triplicates were collected at  
27 4, 12, 24, and 72 hours post-infection (hpi). Total RNA was extracted, and poly-A-tailed mRNA sequenced.  
28 Reads were mapped to the turkey genome after trimming and gene expression was counted with StringTie.  
29 Differential gene expression was performed with DESeq2 followed by functional enrichment analysis with  
30 gprofiler2 and DAVID from NCBI. We performed RT-qPCR of select genes to validate the RNA-seq data.

31 **Results:** We obtained usable data from only 12 and 24 hpi samples. In total 2,343 and 3,295 Differentially  
32 expressed genes (DEGs) were identified at 12 hpi and 24 hpi, respectively. At 12 hpi, 1,079 genes were  
33 upregulated and 1,264 genes downregulated, whereas 1,512 genes were upregulated and 1,783 genes  
34 downregulated at 24 hpi. The DEGs were related to multiple biological processes all potentially playing a  
35 role in THEV infection but the most relevant to our study are apoptosis, ER unfolded protein response, and  
36 suppressed cell maintenance processes. Multiple pro-apoptotic genes, including *APAF1*, *BNIP3L*, *BMF*,  
37 *BAK1*, *RIPK1*, and *FAS* were upregulated, indicating that, unlike most adenoviruses, THEV is not adept at  
38 thwarting the host apoptotic program. However, some anti-apoptotic genes were also stimulated. Genes  
39 such as *VCP*, *UFD1*, *EDEM1*, *EDEM3*, and *ATF4* were also upregulated, strongly suggesting an activate  
40 ER stress-induced unfolded protein response, which may also contribute to apoptosis.

41 **Conclusions:** Apoptosis has long been suggested as the mechanism for THEV-induced IMS because  
42 THEV primarily infects B-cells. Our results are in agreement with but we did not see strong support for  
43 cytokine-mediated apoptosis. Our data suggest that a non-cytokine-dependent process or a synergistic  
44 cross-talk of multiple processes is more likely to mediate direct cell death of infected cells.

45 **KEY WORDS**

46 Turkey hemorrhagic enteritis virus (THEV), Adenovirus, RNA sequencing, Apoptosis, Immunosuppression

47 **INTRODUCTION**

48 Turkey hemorrhagic enteritis virus (THEV), belonging to the family *Adenoviridae*, genus *Siadenovirus*, in-  
49 fects turkeys, chickens, and pheasants (1, 2). THEV is transmitted via the fecal-oral route and causes  
50 hemorrhagic enteritis (HE) in turkeys, a debilitating disease affecting predominantly 6-12-week-old turkey  
51 pouls characterized by immunosuppression (IMS), depression, splenomegaly, intestinal lesions leading to  
52 bloody droppings, and up to 80% mortality (3–6). The clinical disease usually persists in affected flocks for  
53 about 7-10 days. However, secondary bacterial infections may extend the duration of illness and mortality  
54 for an additional 2-3 weeks due to the immunosuppressive nature of the virus, exacerbating the economic  
55 losses (5, 7). Naturally-occurring low pathogenic (avirulent) strains of THEV have been isolated, which  
56 show subclinical infections but retain the immunosuppressive effects. Since its isolation from a pheasant  
57 spleen, the Virginia Avirulent Strain (VAS) has been used effectively as a live vaccine despite the immuno-  
58 suppressive side-effects, but the vaccinated birds are rendered more susceptible to opportunistic infections  
59 and death than unvaccinated cohorts leading to significant economic losses (4, 5, 8–10).

60 It is well-established that THEV primarily infects and replicates in turkey B-cells of the bursa and spleen and  
61 somewhat in macrophages, inducing apoptosis and necrosis. Consequently, a significant drop in number of  
62 B-cells (specifically, IgM+ B-cells) and macrophages ensue along with increased T-cell counts with abnormal  
63 T-cell subpopulation (CD4+ and CD8+) ratios. The cell death seen in the infected B-cells and macrophages  
64 is generally proposed as the major cause of THEV-induced IMS as both humoral and cell-mediated immu-  
65 nity are impaired (5, 6, 8, 11). Immunopathogenesis via cytokines from T-cells and macrophages has also  
66 been suggested as a mechanism of apoptosis leading to IMS. It is thought that the virus replication in the  
67 spleen attracts T-cells and peripheral blood macrophages to the spleen where the T-cells are activated by  
68 cytokines from activated macrophages and vice versa. The activated T-cells undergo clonal expansion and  
69 secrete interferons: type I (IFN- $\alpha$  and IFN- $\beta$ ) and type II (IFN- $\gamma$ ) as well as tumor necrosis factor (TNF)  
70 while activated macrophages secrete interleukin 6 (IL-6), TNF, and nitric oxide (NO), an antiviral agent with  
71 immunosuppressive properties. These cytokines released by T-cells and macrophages (e.g., TNF) may  
72 contribute to apoptosis and necrosis in bystander splenocytes, exacerbating the already numerous apop-  
73 totic and necrotic splenocytes, culminating in IMS (8, 11) (see **Figure 1**). However, the precise molecular  
74 mechanisms of THEV-induced IMS or pathways involved are poorly understood (6). Elucidating the spe-  
75 cific mechanisms and pathways of THEV-induced IMS is the most crucial step in THEV research as it will  
76 present a means of mitigating IMS.

77 Next generation sequencing (NGS) is a groundbreaking technology that has significantly enhanced our un-  
78 derstanding of DNA and RNA structure and function and facilitated exceptional advancements in all domains

79 of biology and the Life Sciences (12). mRNA sequencing (RNA-seq), an NGS approach to transcriptomic  
80 studies, is a versatile, high throughput, and cost-effective technology that allows a broad scan of the entire  
81 transcriptome, thereby uncovering the active genes and molecular pathways and processes. This tech-  
82 nology has been leveraged in an ever-increasing number of studies to elucidate active cellular processes  
83 under a wide range of treatment conditions, including the transcriptomics of viral infections (12–16). In  
84 RNA-seq studies, differentially expressed genes (DEGs) identified under different experimental conditions  
85 are key to unlocking the interesting biology or mechanism under study. Identified DEGs are typically used  
86 for functional enrichment analyses in large curated knowledgebases such as gene ontology (GO) and Ky-  
87 oto Encyclopedia of Genes and Genomes (KEGG) pathways which connect genes to specific biological  
88 processes, functions, and pathways, shedding light on the biological question under study (17, 18).

89 To the best of our knowledge, no study has leveraged the wealth of information offered by RNA-seq to  
90 elucidate the molecular mechanisms and pathways leading to THEV-induced IMS. To effectively counteract  
91 the immunosuppressive effect of the vaccine, it is essential to unravel the host cell processes/pathways  
92 influenced by the virus to bring about IMS. In this study, we present the first transcriptomic profile of THEV-  
93 infected cells using paired-end RNA-seq in a turkey B-cell line (MDTC-RP19), highlighting key host genes,  
94 cellular/molecular processes and pathways affected during a THEV infection. We specifically focus on  
95 cellular processes related to cell survivability that would help in elucidating THEV-induced IMS. Our RNA-  
96 seq yielded 149 bp long high quality (mean PHRED Score of 36) sequences from each end of cDNA  
97 fragments, which were mapped to the genome of domestic turkey (*Meleagris gallopavo*).

98 **RESULTS**

99 **Sequencing Results**

100 To identify the host transcriptome profile during THEV infection, MDTC-RP19 cells were THEV-infected or  
101 mock-infected in triplicates or duplicates, respectively, and harvested at 4-, 12-, 24-, and 72-hours post in-  
102 fection (hpi). mRNAs extracted from mock- or THEV-infected cells were sequenced on the Illumina platform,  
103 yielding a total of **776.1** million raw reads (149 bp in length) across all samples (see **Table 1** for sequenc-  
104 ing statistics). After trimming low-quality reads, the remaining **742.8** million total paired-end trimmed reads  
105 (approximately, **34.7-47.9** million reads per sample) were mapped to the genome of *Meleagris gallopavo*  
106 obtained from the National Center for Biotechnology Information (NCBI). The percentage of reads mapping  
107 to the host genome across all samples ranged from **32.4** to **89.2%**. The fraction of reads mapping to the  
108 host genome decreased while those mapping to the virus genome increased over course of the infection  
109 as the virus infectious cycle progressed. Despite excellent quality scores at all time points (**Table 1**), DEGs  
110 identified at 4 and 72 hpi did not yield any results in the downstream functional enrichment analyses (GO  
111 term and KEGG pathway analysis) and they were excluded from all subsequent analyses. In the remaining  
112 12 and 24 hpi samples, a high correlation was observed between biological replicates (**Figure 2A** and **B**).

113 **DEGs of THEV-infected Versus Mock-infected Cells**

114 Gene expression levels were estimated with the StringTie software (19) in Fragments per kilobase of tran-  
115 script per million (FPKM) units. The analysis of DEGs was performed with the DESeq2 R package (20)  
116 which employs negative binomial distribution model for read count comparisons. Using a  $P_{\text{adjusted}}$ -value  
117 cutoff  $\leq 0.05$  as the inclusion criteria, **2,343** and **3,295** genes were identified as differentially expressed  
118 at 12-hpi and 24-hpi, respectively. The DEG analyses results at 12 and 24-hpi have been deposited in  
119 NCBI Gene Expression Omnibus (<http://www.ncbi.nlm.nih.gov/geo>) under accession number ### with files  
120 named ~file\_name12hpi and file\_name24hpi~, respectively. At 12-hpi, **1,079** genes were upregulated and  
121 **1,264** genes downregulated, whereas **1,512** genes were upregulated and **1,783** genes downregulated at  
122 24-hpi (**Figure 2C**, and **Figure 3A-C**). The log<sub>2</sub>fold-change(FC) values at 12-hpi ranged between **-1.4** and  
123 **+1.7** for **TMEM156** (Transmembrane Protein 156) and **LIPG** (Lipase G), respectively. At 24-hpi, the log<sub>2</sub>FC  
124 values ranged between **-2.0** and **+2.6** for **C1QTNF12** (C1q And TNF Related 12) and **KCNG1** (Potassium  
125 Voltage-Gated Channel Modifier Subfamily G Member 1), respectively.

126 **Functional Enrichment Analyses (GO and KEGG pathway Analyses)**

127 Gene ontology (GO) enrichment analysis was performed for 12- and 24-hpi DEGs with the DAVID (Database  
128 for Annotation, Visualization and Integrated Discovery; version 2021) online resource (21) and the gprofiler2

129 R package – version **0.2.3** (22), which output results in three GO categories – cellular components (CP), bi-  
130 ological processes (BP), and molecular functions (MF). Results with  $P_{adjusted}$ -value  $\leq 0.05$  were considered  
131 functionally enriched. The GO enrichment analyses results at 12-hpi and 24-hpi showed significant over-  
132 laps among all three GO categories. At both time points, cellular breakdown processes were upregulated  
133 while cellular maintenance processes and structures were downregulated in all three GO categories (**Table**  
134 **2A-B** and **Table 3A-B**).

135 For upregulated DEGs at 12-hpi, GO terms annotated under the BP category broadly cluster into: apop-  
136 tosis and autophagy, cellular metabolism (catabolic processes), sterol biosynthesis, response to stimuli,  
137 and protein processing (**Figure 4A** and **Table 2A**). In the CC category, the GO terms relate primarily with  
138 cytoplasmic vacuolation, while in the MF category, they broadly fit under protein binding and kinase activity  
139 (**Table 2A**). For downregulated DEGs at 12 hpi, GO terms in BP category generally fell under transla-  
140 tion, protein biosynthesis and folding, ribosome biogenesis, nitrogen compound metabolism, nucleic acid  
141 synthesis, repair, metabolism, processing, and replication, and energy metabolism. Also, immunoglobu-  
142 lin production and isotype switching were downregulated (**Figure 4C** and **Table 2B**). In the CC category  
143 GO terms broadly grouped into ribosome, mitochondria, respirosome, nucleus, and spliceosome, while in  
144 the MF category, they generally belong to translation regulator activity, protein folding chaperone, catalytic  
145 activity (acting on a nucleic acids), and ATP hydrolysis activity (**Table 2B**).

146 At 24-hpi, the GO terms in the BP category for upregulated DEGs were connected with apoptosis and  
147 autophagy, lipid and sterol biosynthesis, catabolic process, protein ubiquitination and proteolysis, cell sig-  
148 nalling, and cell metabolism. Additionally, host defense response and genes that negatively regulate cy-  
149 tokine production were upregulated (**Figure 4B** and **Table 3A**). In the CC category, GO terms were related  
150 to cytoplasmic vacuolation and the lysosome, similar to those identified at 12-hpi. In the MF category GO  
151 terms group into protein ubiquitination activity, kinase and acyltransferase activity, and macromolecule bind-  
152 ing activity (**Table 3A**). GO terms for the downregulated DEGs were markedly similar to those at 12-hpi in  
153 all three GO categories. In the BP category, GO terms broadly group into translation, peptide biosynthesis  
154 and folding, ribosome biogenesis, aerobic respiration and ATP synthesis, and cell cycle process and nu-  
155 cleic acid replication and processing (**Figure 4D** and **Table 3B**). The GO terms in the CC category group  
156 under ribosome, mitochondrion, nucleus and chromosomes, while the MF category GO terms group into  
157 structural constituent of ribosome and translation regulator activity, catalytic activity acting on a nucleic acid  
158 and nucleic acid binding, aminoacyl-tRNA ligase activity, and NAD binding (**Table 3B**).

159 KEGG pathway analysis on the DEGs was also performed using both the gprofiler2 R package (22) and the  
160 DAVID online resource. Both resources gave similar results, but the results from DAVID (**Table 4A**) included

more information than the gprofiler2 results (**Table S2**). KEGG pathway analysis was congruent with the GO results, revealing that generally, cell maintenance and upkeep pathways were downregulated while cell death and breakdown pathways were upregulated. Cell maintenance pathways such as DNA replication and repair, ribosome biogenesis, spliceosome, and oxidative phosphorylation were downregulated at both 12- and 24-hpi. Pathways such as: autophagy, response to virus (Influenza A), and steroid biosynthesis were upregulated at 12-hpi similar to 24-hpi, where pathways such as: autophagy, ubiquitin-mediated proteolysis, lysosome, protein processing in endoplasmic reticulum, and steroid biosynthesis were upregulated.

It is well-established that THEV induces cell death (apoptosis and necrosis) in infected B-cells, which is linked to THEV-induced IMS (8, 11, 23). Hence, we are particularly interested in cellular processes and pathways associated with cell death and pathways that may affect the survivability of the host B-cells, thereby accounting for THEV-induced IMS. We highlight the upregulated cell death (apoptosis and autophagy), ubiquitin-dependent endoplasmic reticulum [ER]-mediated protein degradation, and suppressed cell maintenance pathways as well as cytokine deregulation identified by our GO and KEGG analyses as the likely key aspects of THEV-host cell interaction relevant to THEV-induced IMS.

### **Cell Death and Breakdown Pathways Upregulated by THEV**

Many virus families, including adenoviruses, herpesviruses, poxviruses, baculoviruses, parvoviruses, retroviruses, rhabdoviruses, paramyxoviruses, orthomyxoviruses, togaviruses, and picornaviruses are known to trigger apoptosis in infected host cells either through direct viral protein action or the host antiviral response (24–26). The Mastadenovirus family possess the protein, E1B-19K, used to inhibit host cell apoptosis long enough to complete their replication cycle (24, 26, 27). However, no such protein is known in THEV. A recent paper showed several novel transcripts and open reading frames (ORFs) in the genome of THEV which may offer similar anti-apoptotic functions but the functions of these novel ORFs are yet to be studied (28). Our data show that apoptotic and autophagic pathways are upregulated during THEV infection, supporting previous findings of apoptosis and necrosis of THEV-infected cells (8, 11, 23). For example, several proapoptotic members of the BCL2 (B-cell lymphoma 2) protein family such as BCL2 antagonist/killer 1 (*BAK1*), BCL2 interacting protein 3 like (*BNIP3L*), BCL2 interacting protein 3 (*BNIP3*), and Bcl2 modifying factor (*BMF*) were upregulated. Additionally, Fas cell surface death receptor (*FAS*), Fas associated via death domain (*FADD*), MAP kinase-activating death domain (*MADD*), programmed cell death 4 (*PDCD4*), RB1 inducible coiled-coil 1 (*RB1CC1*), activating transcription factor 4 (*ATF4*), receptor interacting serine/threonine kinase 1 (*RIPK1*), tumor necrosis factor receptor superfamily member 1B (*TNFRSF1B*), pro-apoptotic WT1 regulator (*PAWR*), and apoptotic peptidase activating factor 1 (*APAF1*), which are potent proapoptotic factors were upregulated. Interestingly, both the intrinsic (*BAK1*, *BNIP3L*, *BNIP3*, *BMF*, *RB1CC1*, *ATF4*, *PDCD4*, and

193 *APAF1*) and extrinsic (*FAS*, *FADD*, *TNFRSF1B*, *MADD*, and *RIPK1*) apoptotic pathways were represented.  
194 Conversely, several anti-apoptotic proteins such as BCL2 apoptosis regulator (*BCL2*), BCL2 interacting pro-  
195 tein 2 (*BNIP2*; interacts directly with adenovirus E1B-19K protein), BCL2 related protein A1 (*BCL2A1*), and  
196 apoptosis inhibitor 5 (*API5*) were also upregulated. Thus, apoptosis and its regulation pathways are clearly  
197 upregulated; this highlights the host-virus tug-of-war also typical in Mastadenovirus infections. Moreover,  
198 several genes associated with autophagy such as: TNF receptor associated factor 6 (*TRAF6*), autophagy  
199 related 9A (*ATG9A*), unc-51 like autophagy activating kinase 2 (*ULK2*), and autophagy related 4B cysteine  
200 peptidase (*ATG4B*) were upregulated.

## 201 **Downregulation of Cell Maintenance Pathways**

202 Forcibly transitioning host cell cycle to the S phase during the early phase of infection is a prerequisite for a  
203 productive adenovirus infection (29). Interaction of the viral E1A early proteins with the host pRb (retinoblas-  
204 toma) protein releases the host transcription factor E2F, which activates genes required for S phase cell  
205 cycle induction. Viral E1A also binds the host transcriptional co-activator p300/CBP (29, 30). Our GO and  
206 KEGG pathway results showed that at 12 hpi, several key genes involved with cell cycle transition were  
207 upregulated. Notably, E1A binding protein p300 (*EP300*), cyclin genes (*CCND3*, *CCNG1*, *CCNG2*, *CDK6*),  
208 anaphase promoting complex subunit 1 (*ANAPC1*), and cell division cycle 27 (*CDC27*) were upregulated.  
209 However, unlike observed in Mastadenoviruses, the cell cycle regulation at 12 hpi seems complicated as  
210 some key cell cycle related genes as well as DNA and RNA synthesis, repair, metabolism, processing, and  
211 replication were concurrently downregulated. At 24 hpi, our KEGG pathway and GO analysis show that cell  
212 cycle was unanimously downregulated.

213 We found that several essential cell maintenance processes whose suppression can trigger apoptosis,  
214 were downregulated. Severe DNA damage is a known mechanism of apoptosis induction, called DNA  
215 damage-dependent apoptosis (31). Repression of RNA and protein synthesis is also strongly associated  
216 with apoptosis (32). Several processes related to DNA and RNA synthesis, maintenance, and repair such  
217 as nucleotide biosynthesis and metabolism, double strand break repair, DNA excision repair, RNA biosyn-  
218 thesis, RNA processing, DNA replication, mitotic cell cycle process, protein-RNA complex organization, and  
219 DNA damage response were downregulated. Notable genes identified include DNA ligase 1 (*LIG1*), X-ray  
220 repair cross complementing 1 (*XRCC1*), cyclin dependent kinase 1 and 2 (*CDK1*, *CDK2*), checkpoint kinase  
221 1 (*CHEK1*), 8-oxoguanine DNA glycosylase (*OGG1*), BLM RecQ-like-helicase (*BLM*), BRCA1 DNA repair  
222 associated (*BRCA1*), and several RAD family proteins (*RAD21*, *RAD51*, *RAD51B*, *RAD51C*, *RAD54B*). Ad-  
223 ditionally, protein synthesis-related processes, including ribosome biogenesis, rRNA processing, ribosome  
224 assembly, protein folding, translational initiation, protein maturation, ribosome and ribonucleoprotein com-

plex formation, translation pre-initiation complex formation, and cytoplasmic translation were significantly downregulated. Notable genes identified include eukaryotic translation initiation factors (*EIF1*, *EIF1AX*, *EIF3E* and *EIF3F*, *EIF3H*, *EIF3I*, *EIF3L* and *EIF3M*), biogenesis of ribosomes BRX1 (*BRIX1*), MCTS1 re-initiation and release factor (*MCTS1*), and ribosomal protein subunits (*RPL8*, *RPL10a*, *RPL11*, *RP12*, *RP13*, *RP14*, *RP15*, *RP18a*, *RP19*). We speculate that these may all contribute to cell death via apoptosis; hence, THEV-induce IMS.

### Endoplasmic Reticulum (ER) Stress Response during THEV infection

The KEGG pathway analysis (**Table 4A**) show that protein processing in the ER, and ubiquitin-mediated proteolysis are significantly upregulated (**Figure 5**). The GO results (**Table 3A**) shows that specifically, ER stress and the ER-associated protein degradation (ERAD) pathway, a branch of the unfolded protein response (UPR) were upregulated during THEV infection. The ER is the major site for protein synthesis, folding and quality control, and sorting (33). Upon ER stress or continued accumulation of unfolded proteins in the ER lumen, the UPR pathways are activated to restore ER homeostasis. The ERAD pathway, a ubiquitin-proteasome-dependent pathway, is a protein quality control system activated for degradation of misfolded and unassembled proteins (33). In our results, the THEV-infected samples showed significant increase in ERAD pathway effector proteins, such as valosin containing protein (*VCP*), ubiquitin recognition factor in ER associated degradation 1 (*UFD1*), ER degradation enhancing alpha-mannosidase like proteins 1 and 3 (*EDEM1*, *EDEM3*), cullin 1 (*CUL1*), and ubiquilin 1 (*UBQLN1*). Other genes related to other UPR pathways such as *HSPA5* and *ATF4* were also upregulated. Our KEGG pathway (**Table S2**) and GO (**Figure 4B**) results indicated a significant upregulation of ubiquitin mediated proteolysis with other ubiquitination pathway proteins such as ubiquitin conjugating enzymes (*UBE2J2*, *UBE2E3*, *UBE2Z*), ubiquitin protein ligases (*UBE3A*, *UBE3B*), NPL4 homolog ubiquitin recognition factor (*NPLOC4*), and ubiquitin like modifier activating enzyme 6 (*UBA6*) showing significant upregulation. Additionally, the heat shock family of chaperone proteins such as DnaJ heat shock protein family (*HSP40*) members (*DNAJB11*, *DNAJB12*, *DNAJB2*, *DNAJC10*), heat shock protein family A (*HSP70*) members (*HSPA4L*, *HSPA5*, *HSPA8*), and heat shock protein 90 alpha family class A member 1 (*HSP90AA1*) were upregulated. Moreover, the KEGG pathway analysis (**Table 4A**) shows a significant upregulation in lysosome formation, lumen acidification, and lysosomal degradation, likely an indication of ER-to-lysosome-associated degradation. Taken together, these results suggest that THEV infection triggers significant ER-associated protein degradation, which may contribute to cell death and IMS.

### Differential Expression of Cytokine and Cytokine Receptor-encoding Genes

Our KEGG pathway results showed that a pathway similar to immune response to influenza A infection was

257 upregulated at 12 hpi. Our GO analysis also identified terms such as regulation of lymphocyte activation  
258 and regulation of cytokine production as upregulated at both 12 and 24 hpi. Genes involved include *IL18*,  
259 *IL2RB*, *IL4R*, *IL5RA*, TNF receptor associated factors (*TRAF2*, *TRAF3*, *TRAF6*, *TRAF7*, *TRAFD1*), TNF re-  
260 ceptor superfamily members (*TNFRSF1B*, *TNFRSF8*, *TNFSF4*), interferon-induced with helicase C domain  
261 1 (*IFIH1*), interferon-induced double-stranded RNA-activated protein kinase (*PKR*), and *CD80*. In contrast,  
262 cytokine inhibitors such as suppressor of cytokine signaling (*SOCS3* and *SOCS5*) were also upregulated  
263 at both 12 and 24 hpi and immunoglobulin production and isotype switching GO terms were downregu-  
264 lated at 12 hpi. This inconsistency is likely and indicator of the struggle between the virus and its host.  
265 While several cytokines were regulated by THEV as in the proposed model of THEV immunopathogene-  
266 sis (**Figure 1**), the cytokines in the model (IFN- $\alpha$ , IFN- $\beta$ , IFN- $\gamma$  TNF, IL-6, and NO) were not significantly  
267 differentially expressed in our data. However, some of the identified differentially expressed cytokines and  
268 cytokine receptors (*TNFRSF8*, *TRAF7*) are positive regulators of apoptosis; therefore, they may play a role  
269 in THEV-induced IMS.

270 **Validation of DEGs by Reverse Transcriptase Quantitative PCR (RT-qPCR)**

271 To validate the RNA-seq results, 12 DEGs (8 upregulated and 4 downregulated) were selected for RT-  
272 qPCR. The DEGs were representative of apoptosis (*APAF1*, *BMF*, *FADD*, *MADD*, and *PDCD4*), ERAD and  
273 ubiquitination (*VCP*, *UFD1*, *EDEM1*), and ribosome biosynthetic (*EIF3D*, *EIF3M*, *RPL8*, *RPL10A*) pathways.  
274 As shown in **Figure 6**, the RT-qPCR results corroborate the RNA-seq results, further reinforcing the validity  
275 of the RNA-seq transcriptomic profile results. Our RT-qPCR primers showed excellent target specificity; only  
276 one amplicon of the expected size was amplified in the melt curves (data not shown) and gel electrophoresis  
277 (**Figure S1**). According to our Student's T-test and Mann-Whitney U test, the difference in gene expression  
278 levels in all the selected genes were statistically significant.

279 **DISCUSSION**

280 THEV has a worldwide distribution, wreaking economic havoc on affected farms, particularly due to its  
281 immunosuppressive trait allowing secondary opportunistic infections to devastate turkey populations (4,  
282 6). HE in turkeys causes more economic losses than any disease caused in other birds like chicken and  
283 pheasants (4). While the current vaccine strain (VAS) has proven effective at preventing clinical HE in turkey  
284 pouls, the retention of its immunosuppressive properties leaves the economic losses problem unresolved.  
285 Elucidating the virus-host interactions leading to IMS is most pressing for not only the understanding of the  
286 viral infection and pathogenesis but also future antiviral therapy targets. Since both virulent and avirulent  
287 THEV cause IMS but the avirulent are used as vaccine, we believe that studying VAS would be more  
288 expedient for understanding THEV vaccine-induced IMS. Only one cell line (MDTC-RP19 or RP19) has ever  
289 been developed capable of supporting THEV infection and replication (34). Thus, in this work, we establish  
290 the first transcriptome profile of THEV infection in RP19 cells using paired-end RNA-seq. We attempted  
291 a multi-time point experimental design but this being the first transcriptomic study of THEV infection, we  
292 faced some difficulties, including selecting our sampling time points based on the only study of THEV gene  
293 expression kinetics (35), leading to only 12 and 24 hpi providing useful data. In total **2,343** and **3,295** DEGs  
294 were identified at 12-hpi and 24-hpi, respectively. At 12-hpi, **1,079** genes were upregulated and **1,264** genes  
295 downregulated, whereas **1,512** genes were upregulated and **1,783** genes downregulated at 24-hpi. Being  
296 a non-model organism, a significant proportion of the host (*Meleagris gallopavo*) genes are not annotated  
297 and not recognized by the databases used for functional enrichment analysis. Thus, the obtained results  
298 are likely sub-optimal in amount of detail relative to results from well annotated and curated genomes of  
299 model organisms. The DEGs were related to multiple biological processes all potentially playing a role  
300 in THEV infection but the most relevant to our study are apoptosis, ER stress-induced unfolded protein  
301 response, suppressed cell maintenance processes, and cytokine deregulation. Furthermore, the RT-qPCR  
302 results validated the RNA-seq results. Collectively, this study may shed light on some significant aspects of  
303 THEV-host interactions, which may benefit further mechanistic delineation of the viral infection and induction  
304 of IMS and inform future development of anti-THEV strategies. The biological processes most relevant to  
305 THEV-induced IMS highlighted by this study are further discussed below.

306 Apoptosis is key defense mechanism activated by cells in response to irreversible injury and virus infection  
307 to abrogate virus propagation. It is a formidable cellular defense network, non-specific to any virus family  
308 and therefore, an important problem for any infecting virus to tackle (24–26). The adenovirus E1A pro-  
309 teins are strong inducers of apoptosis. They bind host pRb and p300/CBP protein, inducing p53-mediate  
310 apoptosis and can also sensitize infected cells to TNF $\alpha$  and TRAIL-induced apoptosis (29, 30). However,

adenoviruses have developed multiple distinct anti-apoptotic mechanisms to counter almost all cellular pro-apoptotic programs. For example E1A blocks its own induction of p53-dependent apoptosis and E1B proteins (E1B-19K and E1B-55K) counteract several types of apoptosis including TNF-induced apoptosis (29, 30). Despite, the rich arsenal of countermeasures, transcriptomic studies of human adenovirus infections suggest a complex set of virus-host interactions where both pro- and anti-apoptotic genes are turned on contemporaneously. For example in human adenovirus 2 infection, both pro- and anti-apoptotic BCL2 family genes were stimulated (29). Siadenoviruses including THEV are the smallest adenoviruses and therefore encode the fewest genes (10, 28). THEV encodes a mere 34 ORFs with no anti-apoptotic genes characterized (28). Thus, in our results a strong signal indicative of apoptosis was observed. However, like mastadenovirus infections, a complex relationship between pro and anti-apoptotic genes were observed. Pro-apoptotic genes such as *APAF1*, *BNIP3L*, *BMF*, *BAK1*, *RIPK1*, *FAS*, *FADD* and *ATF* were upregulated in concert with the anti-apoptotic genes: *BCL2*, *BNIP2*, *BCL2A1* and *API5*. We speculate that this complex regulation is predictive of THEV possessing some anti-apoptotic genes but not sufficiently potent to thwart the cellular apoptotic response. Interestingly, both intrinsic and extrinsic pathway pro-apoptotic genes were upregulated, possibly due to a concurrent stimulation of multiple apoptotic pathways or a positive feedback mechanism of one system activating the other. The specific mechanism of apoptosis induction remains elusive. Further studies designed to elucidate these fine details are warranted and would benefit future THEV therapeutics tremendously.

The ER serves many specialized functions including biosynthesis and assembly of membrane and secretory proteins, calcium storage, and biosynthesis of lipids and sterols. It is also the site of protein folding and post-translational modifications and maintains stringent quality control systems, ensuring correctly folded exported proteins and degradation of unfolded or misfolded proteins (16, 33, 36). Disruption of ER homeostasis or ER stress leads to accumulation of incorrect proteins in the ER lumen, triggering the UPR. The UPR restores ER normality by transiently attenuating general protein synthesis, increasing the luminal folding capacity, and the degradation of misfolded proteins through the ERAD pathway or autophagy (16, 33, 36, 37). However, if incorrect luminal protein overload persists, the prolonged UPR will induce apoptosis known as ER stress-associated programmed cell death (36, 37). Many viruses, including DNA and RNA viruses are reported to induce ER stress and UPR pathways during infection (16). In our results, *ATF4* and PKR-like ER protein kinase (*PERK*), key proteins in the *PERK* branch of the UPR pathway were upregulated. A myriad ERAD pathway proteins (e.g., *VCP*, *UFD1*, *EDEM1*, *EDEM3*, *CUL1*, *UBQLN1*), ubiquitination system proteins (e.g., *UBE2J2*, *UBE2E3*, *UBE2Z*, *UBE3A*, *UBE3B*), and heat shock family of chaperone proteins (e.g., *HSPA5*, *HSP4L*, *HSPA8*, *HSP90AA1*) all showed increased expression according

343 to our RNA-seq data with some validated with RT-qPCR. These data strongly suggest that THEV infection  
344 triggers the ER UPR pathways leading to a massive decrease of protein synthesis and deregulation of  
345 sterol biosynthesis, and ubiquitin-mediated proteolysis, all seen in our results. As noted above, a prolonged  
346 UPR activation leads to ER stress-associated programmed cell death via genes such *ATF4* (36, 37). Thus,  
347 we suggest that ER stress response likely plays a crucial role in the THEV-induced IMS. Nonetheless, the  
348 mechanisms underlying the regulation of the UPR pathways by THEV remain to be clearly unraveled. Also,  
349 whether and how ER stress response affects THEV infection and pathogenicity are also merited to be de-  
350 termined in a future study. Unsurprisingly, protein degradation was more pronounced at the 24 hpi than at  
351 12 hpi, reflecting the suggested two phases of UPR – phase one allows the unfolded proteins time to refold  
352 without degradation and phase two degrades any proteins which have failed to fold (37).

353 In the proposed model of THEV immunopathogenesis by Rautenschlein *et al* (**Figure 1**), while THEV di-  
354 rectly induced cell death in infected cells, cytokines are responsible for extending cell death to bystander  
355 splenocytes (8). However, the primary cytokines (IFN- $\alpha$ , IFN- $\beta$ , IFN- $\gamma$  TNF, IL-6, and NO) highlighted in  
356 the model were not significantly differentially expressed in our data. This may be explained by the fact that  
357 the model was proposed based on data from splenocytes of THEV-infected turkeys, which have the full  
358 complement of immune cells (T-cells, B-cells, macrophages) shown in the model and not from B-cell culture  
359 data as in this study. From the model, T-cells and macrophages are the principal producers of the effector  
360 cytokines; thus, there is agreement with our data that B-cells alone would poorly simulate the cytokine com-  
361 munication network. This may also explain the very few immune-associated biological processes in our data  
362 as the B-cells may require cytokines from other immune cells such as macrophages and T-cells for optimal  
363 activation. Further transcriptomic studies with splenocytes would offer a wealth of insights regarding these  
364 ideas. It also likely that cytokines may only play a dominant role in some aspects of THEV-infection such as  
365 the clinical hemorrhage of the intestines but not the associated IMS since a study using the TNF-blocking  
366 drug (thalidomide) only prevented intestinal disease, not IMS (8). While some of the upregulated cytokines  
367 and receptors in our results are positive apoptosis regulators (*TNFRSF8*, *TRAF7*), most of the cytokines  
368 are either anti-apoptotic (*TNFRSF1B*, *TRAF2*), boost host antiviral defense (*IL18*, *TNFSF4*, *PKR*, *TRAFD1*,  
369 *IFIH1*), or suppress cytokine signalling (*SOCS3*, *SOCS5*). Therefore, we speculate that a non-cytokine-  
370 mediated apoptotic process such as ER stress-associated programmed cell death is more likely to mediate  
371 direct killing of infected cells. However, whether bystander cell death occurs and if it is cytokine-mediated as  
372 suggested by Rautenschlein *et al* are important questions that can be addressed with future transcriptomic  
373 studies in splenocytes.

374 By convention, the Mastadenovirus replication cycle is divided into two phases, an early and a late phase,

375 based on the onset of viral DNA replication (29, 30). Based on DNA microarray analysis, adenovirus type 2  
376 (Ad2) infection has been divided into four stages. The first period is from 0 to 12 hpi during which, changes  
377 in cellular gene expression are likely to be triggered by the virus entry process. Most of the deregulated  
378 genes have functions linked to inhibition of cell growth. Therefore, growth suppression is most likely the first  
379 response of the host cell to the incoming virus (29). The second period covers the time from 12 to 24 hpi and  
380 follows activation of the immediate early E1A gene, which forcibly transition cell cycle to S phase (29). While  
381 the temporal changes of host gene expression for a THEV a infection has no prior study, our data showed  
382 that during the first 24 hpi, cell growth was suppressed. Cell maintenance processes involving nucleic  
383 acid and proteins were downregulated according our data. Protein synthesis-related processes including  
384 ribosome biogenesis, rRNA processing, ribosome assembly, protein folding, translational initiation, protein  
385 maturation, etc were heavily affected. Additionally, DNA and RNA synthesis, maintenance, and repair such  
386 as nucleotide biosynthesis and metabolism, double strand break repair, and DNA excision repair were also  
387 repressed. As severe DNA damage leads to DNA damage-dependent apoptosis (31) and repression of  
388 RNA and protein synthesis is also strongly associated with apoptosis (32), these inhibitions may also play  
389 a role in THEV-induce IMS. Moreover, we speculate that the ER UPR may contribute partly to the severe  
390 repression of protein synthesis as discussed above. An in-depth study of temporal changes of host gene  
391 expression during THEV infection would be invaluable in establishing if THEV follows the same pattern as  
392 Ad2.

393 **CONCLUSIONS**

394 THEV-induced IMS is a pressing concern for turkey farmers worldwide, causing substantial economic losses  
395 annually. In this study, we establish the cellular transcriptomic profile of THEV infection in turkey RP19 B-  
396 cells using paired-end RNA-seq, identifying **1,079** upregulated genes and **1,264** downregulated genes at  
397 12 hpi and **1,512** upregulated genes and **1,783** downregulated genes at 24 hpi. Our data suggest that  
398 several biological processes and pathways including apoptosis, immune response, ER response to stress,  
399 ubiquitin-dependent protein catabolic process, and repression essential cellular maintenance are significant  
400 aspects of host cell response to THEV infection. All these processes are established apoptosis inducing  
401 mechanisms, therefore, we believe that either one or synergistic interplay between multiple ones may me-  
402 diate cell death of infected B-cells, leading to IMS. These findings provide the first insights into THEV-host  
403 interactions and may help advance the understanding of non-human adenoviral infection and pathogene-  
404 sis, which may eventually inform the development of medical countermeasures for disease prevention and  
405 treatment.

406 **MATERIALS AND METHODS**

407 **Cell culture and THEV Infection**

408 The Turkey B-cell line (MDTC-RP19, ATCC CRL-8135) was grown as a suspension culture in 1:1 complete  
409 Leibovitz's L-15/McCoy's 5A medium with 10% fetal bovine serum (FBS), 20% chicken serum (ChS), 5%  
410 tryptose phosphate broth (TPB), and 1% antibiotic solution (100 U/mL Penicillin and 100 $\mu$ g/mL Strepto-  
411 mycin), at 41°C in a humidified atmosphere with 5% CO<sub>2</sub>. Infected cells were maintained in 1:1 serum-  
412 reduced Leibovitz's L15/McCoy's 5A media (SRLM) with 2.5% FBS, 5% ChS, 1.2% TPB, and 1% antibiotic  
413 solution. A commercially available THEV vaccine was purchased from Hygieia Biological Labs (VAS strain).  
414 The stock virus was titrated using an in-house qPCR assay with titer expressed as genome copy number  
415 (GCN)/mL, similar to Mahshoub *et al* (38). Cells were THEV-infected or mock-infected in triplicates or du-  
416 plicates, respectively at a multiplicity of infection (MOI) of 100 GCN/cell, incubated at 41°C for 1 hour, and  
417 washed three times with phosphate buffered saline (PBS) to get rid of free virus particles. At each time point  
418 (4-, 12-, 24-, and 72-hpi), triplicate (THEV-infected) and duplicate (mock-infected) samples were harvested  
419 for total RNA extraction.

420 **RNA extraction and Sequencing**

421 Total RNA was extracted from infected cells using the ThermoFisher RNaseous™-4PCR Total RNA Iso-  
422 lation Kit (which includes a DNase I digestion step) per manufacturer's instructions. An agarose gel elec-  
423 trophoresis was performed to check RNA integrity. The RNA quantity and purity was initially assessed using  
424 nanodrop, and RNA was used only if the A260/A280 ratio was 2.0 ± 0.05 and the A260/A230 ratio was >2  
425 and <2.2. Extracted total RNA samples were sent to LC Sciences, Houston TX for poly-A-tailed mRNA  
426 sequencing. RNA integrity was checked with Agilent Technologies 2100 Bioanalyzer High Sensitivity DNA  
427 Chip and poly(A) RNA-seq library was prepared following Illumina's TruSeq-stranded-mRNA sample prepa-  
428 ration protocol. Paired-end sequencing, generating 150 bp reads was performed on the Illumina NovaSeq  
429 6000 sequencing system. The paired-end 150bp sequences obtained during this study and all expression  
430 data have been submitted to the Gene Expression Omnibus database, under accession no #####

431 **Quality Control and Mapping Process**

432 Sequencing reads were processed following a well-established protocol described by Pertea *et al* (19),  
433 using Snakemake - version 7.32.4 (39), a popular workflow management system to drive the pipeline.  
434 Briefly, raw sequencing reads were trimmed with Cutadapt - version 1.10 (40) and the quality of trimmed

435 reads evaluated using the FastQC software, version 0.12.1 (Bioinformatics Group at the Babraham Institute,  
436 Cambridge, United Kingdom; [www.bioinformatics.babraham.ac.uk](http://www.bioinformatics.babraham.ac.uk)), achieving an overall Mean Sequence  
437 Quality (PHRED Score) of 36. Trimmed reads were mapped the reference *Meleagris gallopavo* genome file  
438 GCF\_000146605.3\_Turkey\_5.1\_genomic.fna.gz from NCBI ([https://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/000/146/605/GCF\\_000146605.3\\_Turkey\\_5.1/](https://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/000/146/605/GCF_000146605.3_Turkey_5.1/)) with Hisat2 - version 2.2.1 (19) using the accompanying  
439 gene transfer format (GTF) annotation file (GCF\_000146605.3\_Turkey\_5.1\_genomic.gtf.gz) to build a ge-  
440 nomic index. Samtools - version 1.19.2 was used to convert the output Sequence Alignment Map (SAM)  
441 file to the more manageable Binary Alignment Map (BAM) format. The StringTie (v2.2.1) software (19), set  
442 to expression estimation mode was used to generate normalized gene expression estimates from the BAM  
443 files for genes in the reference GTF file after which the prepDE.py3 script was used to extract read count  
444 information from the StringTie gene expression files, providing an expression-count matrix for downstream  
445 DEG analysis.

#### 447 **DEG Analysis and Functional Enrichment Analysis**

448 DEG analysis between mock- and THEV-infected samples was performed using the very popular DE-  
449 Seq2 (20), which employs a Negative Binomial distribution model for read count comparisons. Genes  
450 with  $P_{\text{adjusted-value}} \leq 0.05$  were considered as differentially expressed. The sequencing data (FASTQ files)  
451 and read count data are deposited at NCBI Gene Expression Omnibus under accession number ###. The  
452 functional profiling of DEGs (GO and KEGG analyses) were performed based on GO databases and KEGG  
453 databases using DAVID and the R package gprofiler2 (22) with *Meleagris gallopavo* as the reference or-  
454 ganism. Results with  $P_{\text{adjusted-value}} \leq 0.05$  were included as functionally enriched. All visualization plots  
455 were made using ggplot2, pheatmap, and ggvenn R packages (41–43).

#### 456 **Validation of DEGs by Reverse Transcriptase Quantitative PCR (RT-qPCR)**

457 The gene expression levels of representative DEGs (*APAF1*, *BMF*, *FADD*, *PDCD4*, *MADD*, *VCP*, *UFD1*,  
458 *EDEM1*, *EIF3D*, *EIF3M*, *RPL8*, *RPL10A*) were validated by quantification of relative mRNA levels with  
459 turkey *GAPDH* mRNA levels as the control gene. Briefly, the samples were infected and RNA extracted as  
460 described for the RNA sequencing samples with three biological replicates at 12 and 24 hpi each for both  
461 THEV-infected or mock-infected samples. First-strand cDNA synthesis of total RNA was performed with an  
462 oligo-dT primer to amplify poly-A-tailed mRNA using SuperScript™ IV First-Strand Synthesis System. The  
463 parent RNA were digested using RNase H after cDNA synthesis was complete to ensure that only cDNA  
464 remain as the template for the RT-qPCR quantification. The RT-qPCR was performed with the PowerUp™

465 SYBR™ Green master mix from Applied Biosystems with primers designed manually in the SnapGene  
466 software. The primers were checked for specificity using NCBI Nucleotide BLAST (<https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastn>) before use. All primers used in this study are listed in **Supplementary**  
467  
468 **Table S1.** Relative mRNA levels were calculated by  $2^{-\Delta\Delta CT}$  method (44).

469 **Statistical Analysis**

470 Statistical analyses of the RT-qPCR results were performed using R (Version 4.3.3) with Student's t-test  
471 and Mann-Whitney U test for the comparison between two groups. A difference with P-value  $\leq 0.05$  was  
472 considered statistically significant.

473 **LIST OF ABBREVIATIONS**

| <b>Abbreviation</b> | <b>Definition</b>   |
|---------------------|---|
| DAVID               | Database for Annotation,<br>Visualization and Integrated<br>Discovery |
| DEG                 | Differentially Expressed Gene   |
| ER                  | Endoplasmic Reticulum   |
| ERAD                | Endoplasmic<br>Reticulum-associated<br>Degradation                    |
| FPKM                | Fragments Per Kilobase of<br>transcript per Million mapped<br>reads   |
| GCN                 | Genome Copy Number  |
| GO                  | Gene Ontology   |
| HE                  | Hemorrhagic Enteritis   |
| IMS                 | Immunosuppression   |
| KEGG                | Kyoto Encyclopedia of Genes<br>and Genomes                            |
| NGS                 | Next Generation Sequencing  |
| ORF                 | Open Reading Frame  |
| RNA-seq             | RNA sequencing  |
| RT-qPCR             | Reverse Transcriptase<br>Quantitative Polymerase Chain<br>Reaction    |

| <b>Abbreviation</b> | <b>Definition</b>                  |
|---------------------|------------------------------------|
| THEV                | Turkey Hemorrhagic Enteritis Virus |
| UPR                 | Unfolded Protein Response          |
| VAS                 | Virginia Avirulent Strain          |
| hpi                 | Hours Post-infection               |

## **474 DATA AVAILABILITY**

475 The raw sequencing read data (FastQ), transcript expression counts, and total DEGs identified at 12 and 24  
 476 hpi have been deposited at the NCBI Gene Expression Omnibus (<http://www.ncbi.nlm.nih.gov/geo>) under  
 477 accession number #####.

## **478 CODE AVAILABILITY**

479 All the code/scripts in the entire analysis pipeline are available on github (<https://github.com/Abraham->  
 480 Quaye/host\_rna\_seq)

## **481 ACKNOWLEDGMENTS**

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 483 high-performance computing systems to perform the memory-intensive steps in the analysis pipeline of this  
 484 work.

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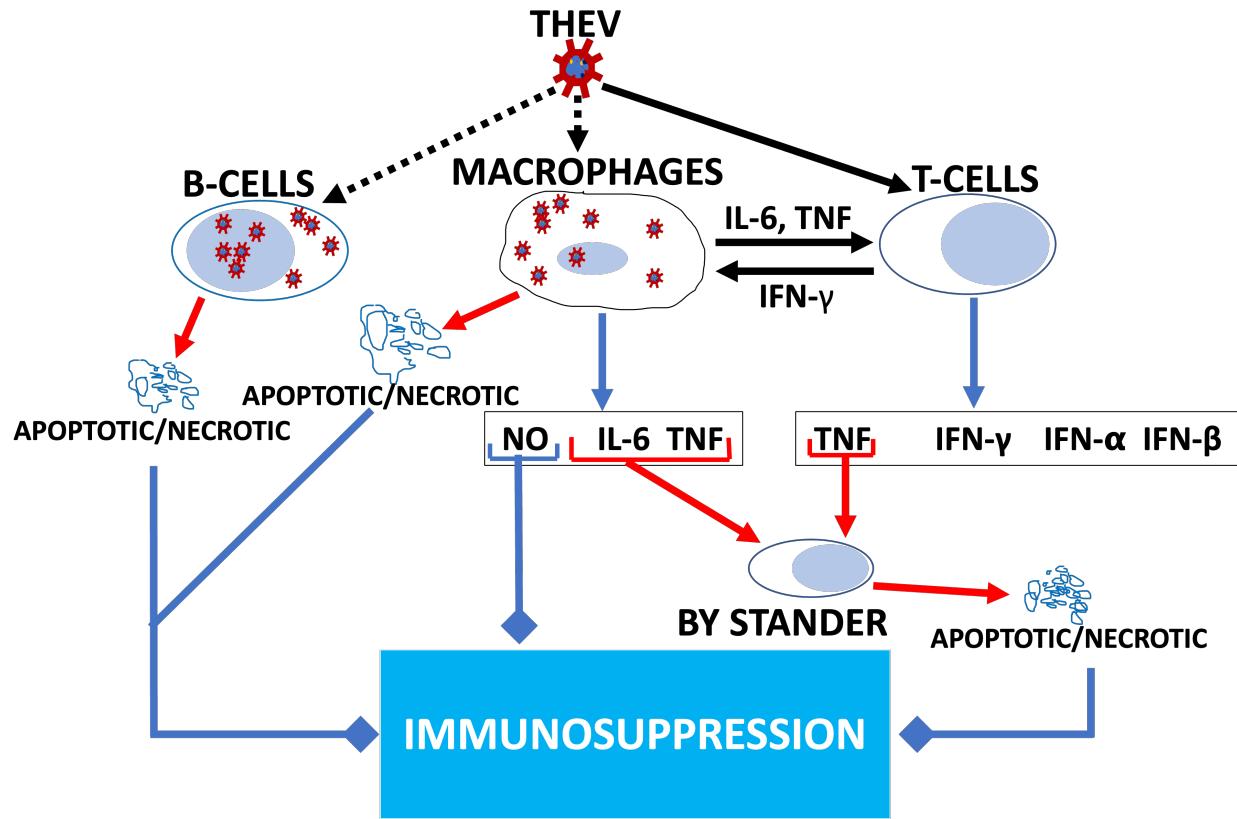
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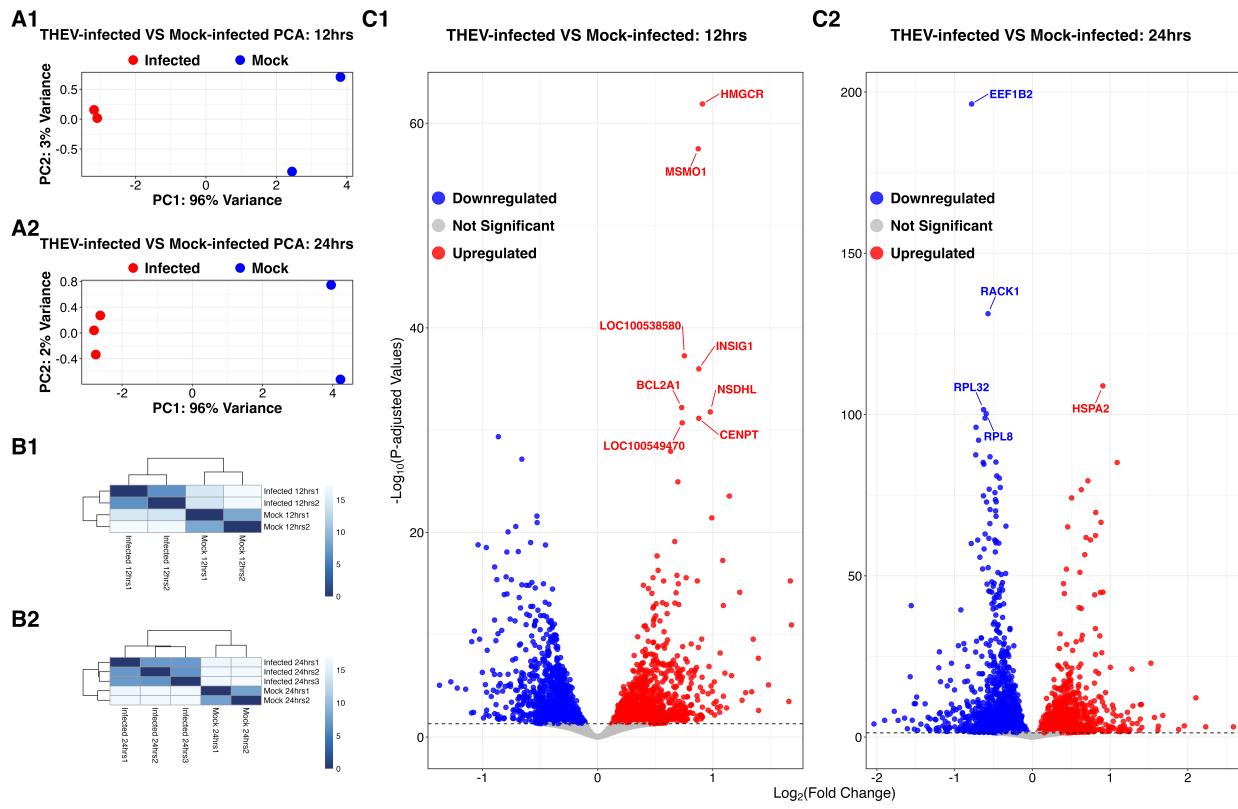
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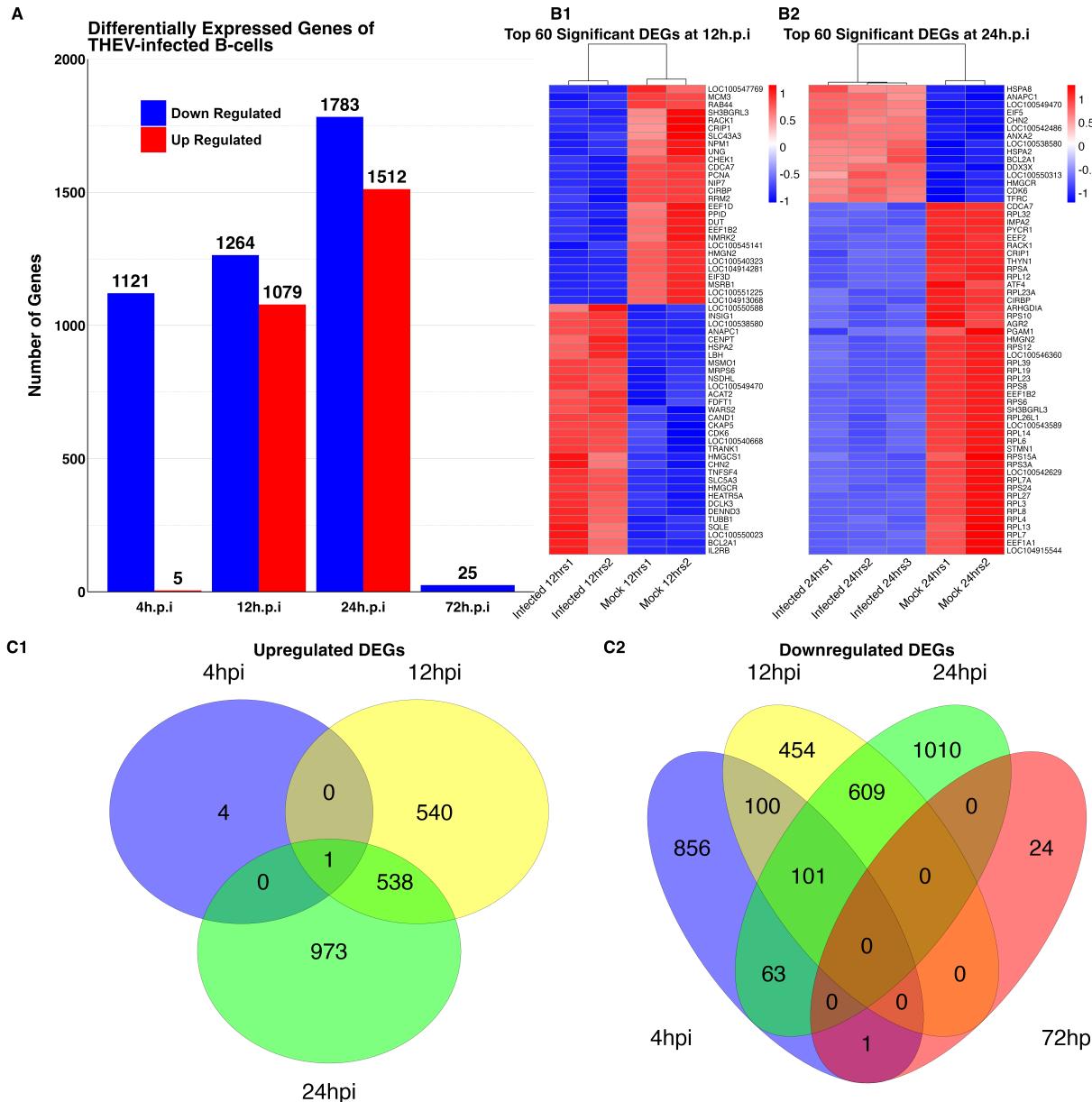
530 TABLES AND FIGURES



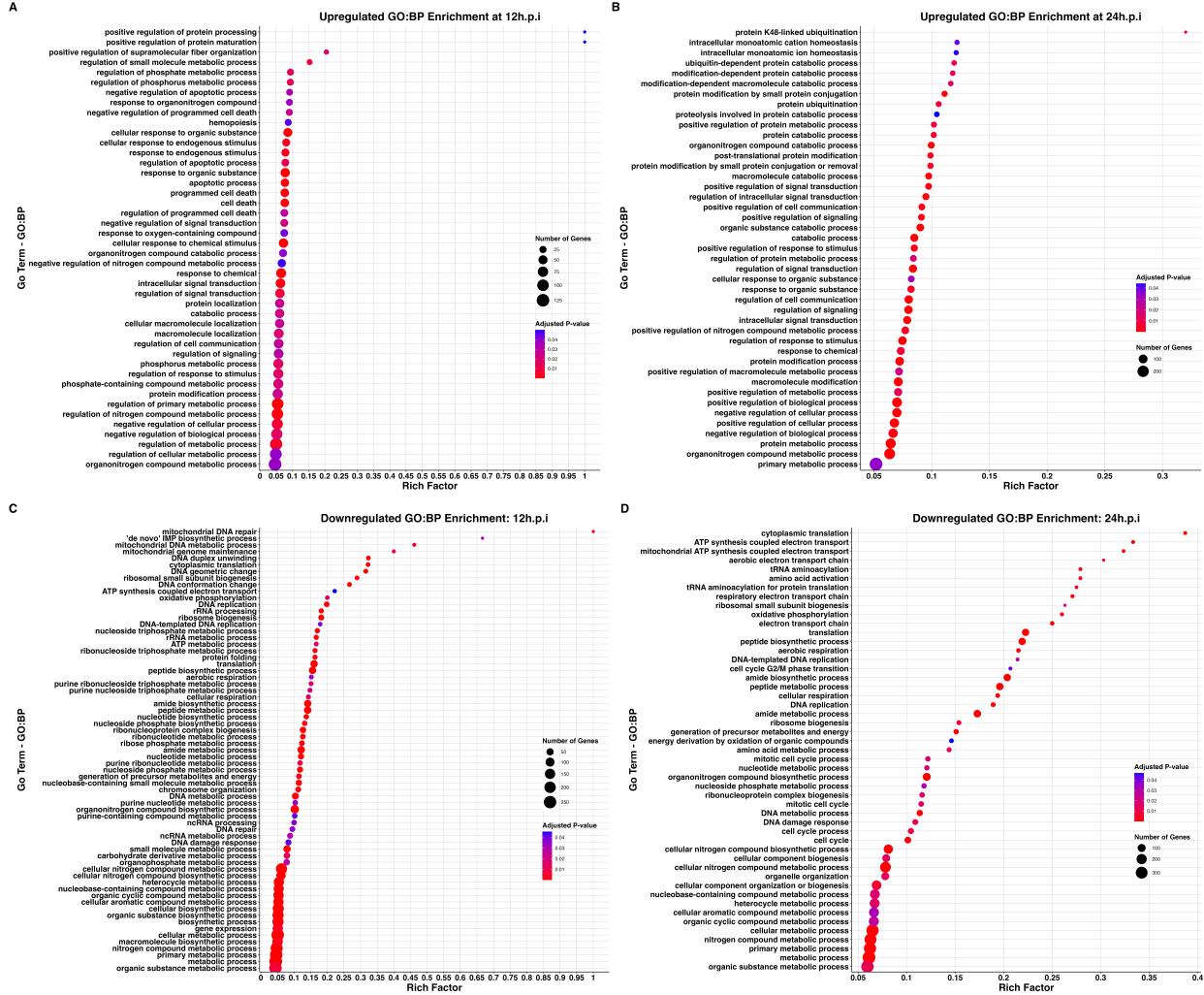
**Figure 1: Model of THEV-induced immunosuppression in turkeys.** THEV infection of target cells is indicated with black dotted arrows. Black unbroken arrows indicate cell activation. Red arrows indicate signals leading to apoptosis. Blue arrows indicate all cytokines released by the cell. Blue arrows with square heads indicate an event leading to IMS. Adapted from Rautenschlein *et al.* (8).



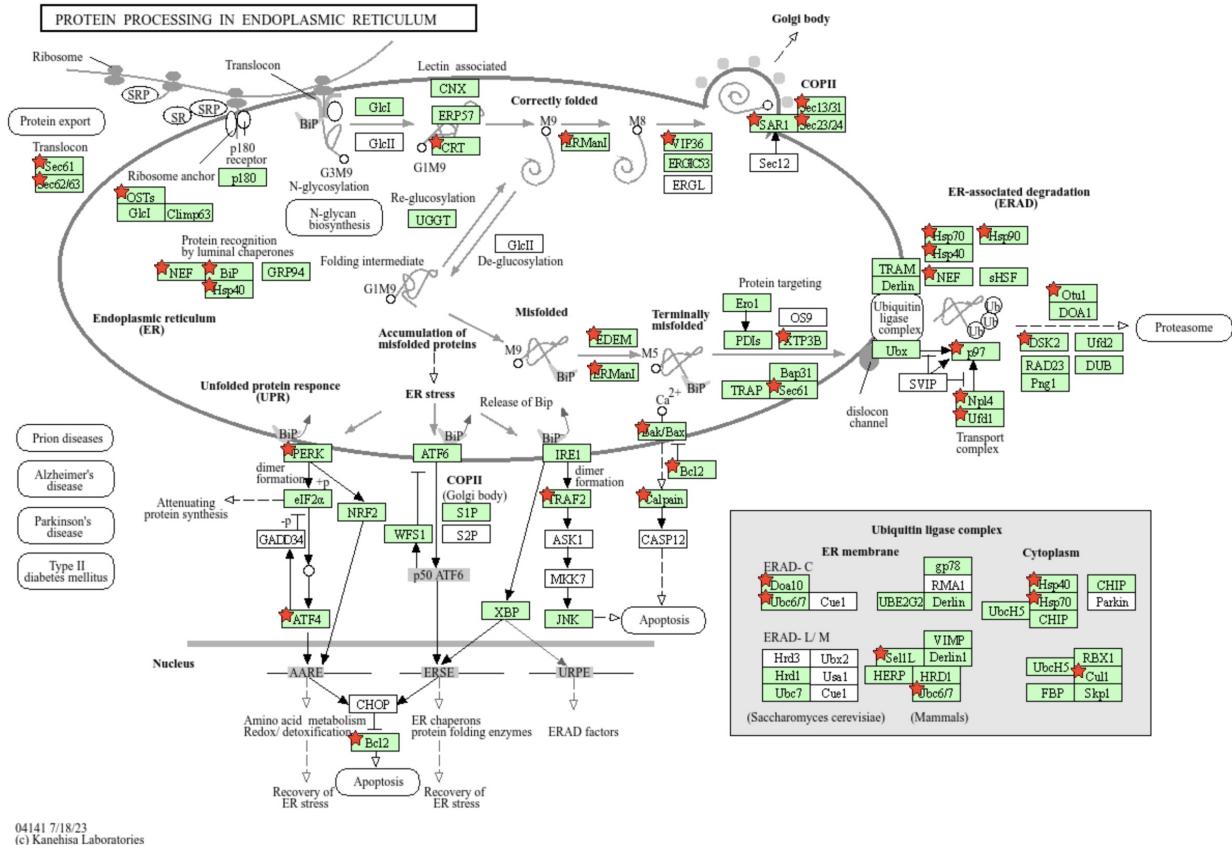
**Figure 2. (A) Principal component analysis (PCA) of turkey B-cells during THEV infection.** At 12-hpi (**A1**), the results indicate that the first (PC1) and second (PC2) principal components account for 96% and 3% of the variation in the samples, respectively. Whereas PC1 and PC2 account for 96% and 2% of the variation, respectively at 24-hpi (**A2**). **(B) Poisson distance matrices illustrating the RNA-seq library integrity within treatment (infected versus mock) groups.** The color scale represents the distances between biological replicates for both 12-hpi samples (**B1**) and 24-hpi samples (**B2**). Dark colors represent high correlation (similarity) between the samples involved. **(C) Volcano plots of DEGs between THEV-infected versus mock-infected cells at 12- and 24-hpi.** Red, blue, and grey dots represent upregulated, downregulated, and non-significant genes, respectively for both 12-hpi samples (**C1**) and 24-hpi samples (**C2**).



**Figure 3: DEGs of THEV-infected versus mock-infected samples at different time points. (A) Bar plot of number DEGs identified.** Red represents upregulated genes and blue represents downregulated genes. **(B) Heatmaps of scaled expression data (Z-scores) of DEGs.** DEGs identified at 12-hpi are shown in (B1) and DEGs at 24-hpi in (B2). **(C) Venn diagrams showing the number of DEGs identified at different time points.** For the upregulated genes (C1), the red circle represents genes at 4-hpi, the blue circle, 12-hpi, and the grey circle, 24-hpi. For the downregulated genes (C2), the green circle represents genes at 72-hpi, while all the other time points retain the colors from (C1).



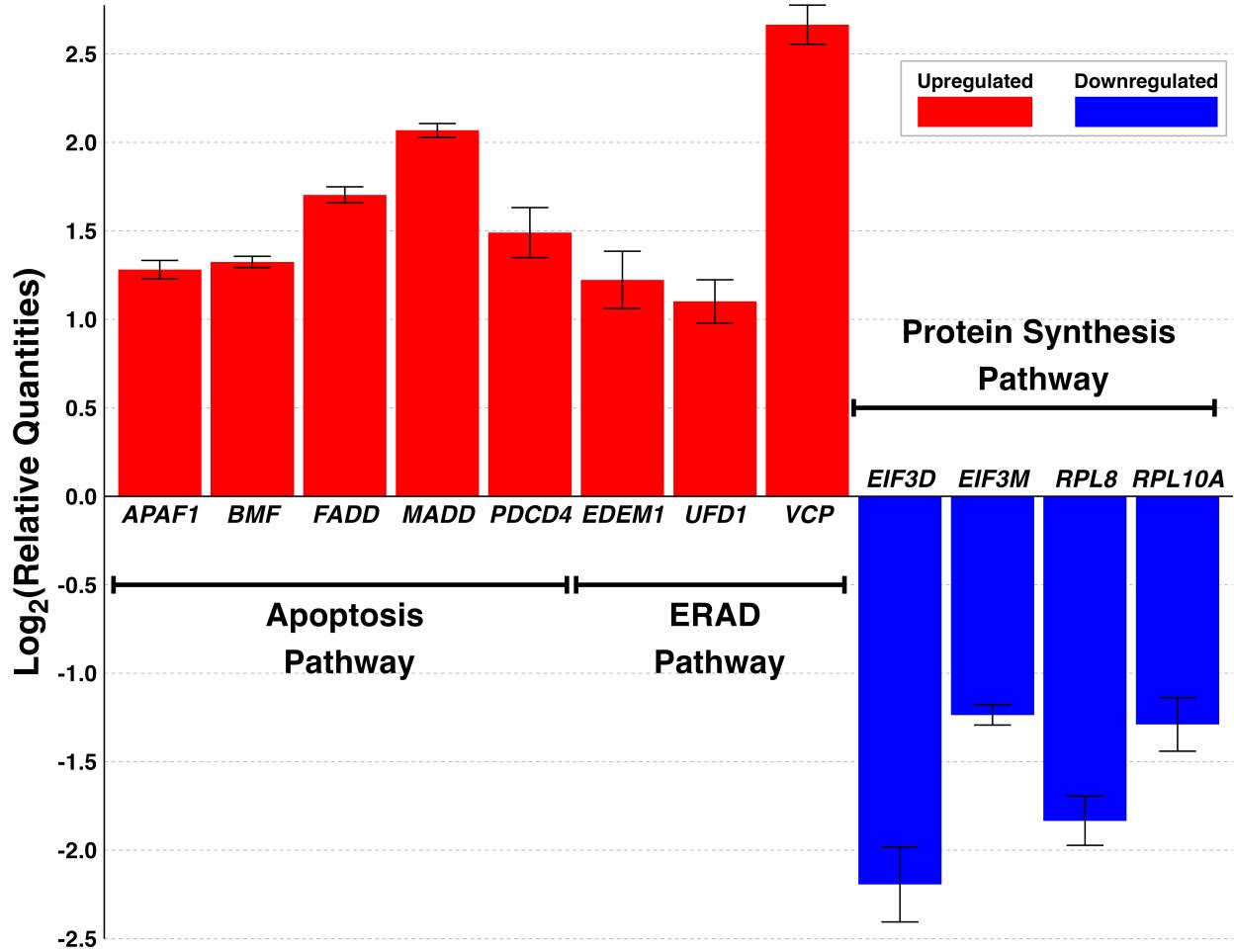
**Figure 4: Dotplot of Enriched Gene Ontology Biological Processes (BP).** Significant BP GO terms identified for upregulated DEGs at 12-hpi and 24-hpi are shown in (A) and (B), respectively. Significant BP GO terms for downregulated DEGs at 12-hpi and 24-hpi are shown in (C) and (D), respectively. The y-axis indicates GO terms and the x-axis represents the rich factor, which indicates the ratio of the number of DEGs annotated to the term to the total number of genes annotated to the term. The diameter indicates the number of genes overlapping the gene ontology term and the color indicates the enrichment P-value.



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(c) Kanehisa Laboratories

**Figure 5: Upregulation of ER Unfolded Protein Response (UPR).** KEGG Pathway analysis shows multiple key genes involved in the ER UPR were upregulated. All genes from our DEG list are annotated with the red star. Notably, *ATF4*, *PERK*, *VCP (p97)*, *TRAF2*, *UFD1* and several *BCL2* and heat shock proteins are upregulated. We see that the PERK branch of the UPR pathway linked to apoptosis is upregulated. Another pathway linked to apoptosis via *BAX* is shown as well as the ERAD protein degradation pathway. Note that due limited annotation of the host genome, a significant proportion of the DEGs were not recognized by the database; hence not shown here.

## RT-qPCR Validation of Select DEGs



**Figure 6: Validation of representative DEGs involved in Apoptosis, Protein synthesis, and ER-stress responses by RT-qPCR.** MDTC-RP19 cells infected with THEV or mock infected were subjected to RT-qPCR analysis for the relative expression of the indicated DEGs at 24hpi. GAPDH was used as the internal control. Data are expressed as the mean  $\pm$  SD. All genes are statistically differentially expressed based on Student's t-test and Mann-Whitney U test.

Table 1: Summary of sequencing, quality control, and mapping processes

| Sample                   | Raw Reads <sup>M</sup> | Trimmed Reads <sup>M</sup> | Mapped Reads <sup>M</sup> | Uniquely Mapped Reads <sup>M</sup> | Non-uniquely Mapped Reads <sup>M</sup> | Q20%  | Q30%  | GC Content (%) |
|--------------------------|------------------------|----------------------------|---------------------------|------------------------------------|--|-------|-------|----------------|
| I_12hrsS1 <sup>Inf</sup> | 40.6                   | 39.0                       | 34.7 (88.92%)             | 33.1 (84.78%)                      | 1.6 (4.14%)                            | 99.95 | 97.23 | 47.5           |
| I_12hrsS3 <sup>Inf</sup> | 38.8                   | 37.3                       | 33.1 (88.78%)             | 31.7 (84.95%)                      | 1.4 (3.83%)                            | 99.95 | 97.53 | 47.5           |
| I_24hrsS1 <sup>Inf</sup> | 42.7                   | 41.0                       | 36.2 (88.13%)             | 34.5 (84.2%)                       | 1.6 (3.93%)                            | 99.95 | 96.95 | 46.5           |
| I_24hrsS2 <sup>Inf</sup> | 42.0                   | 40.4                       | 35.6 (88.1%)              | 33.9 (83.83%)                      | 1.7 (4.27%)                            | 99.94 | 97.05 | 46.5           |
| I_24hrsS3 <sup>Inf</sup> | 40.5                   | 38.9                       | 34.2 (88.01%)             | 32.7 (84.12%)                      | 1.5 (3.89%)                            | 99.95 | 97.08 | 47.0           |
| I_4hrsS1 <sup>Inf</sup>  | 39.1                   | 37.4                       | 33 (88.16%)               | 31.2 (83.43%)                      | 1.8 (4.73%)                            | 99.93 | 97.04 | 48.5           |
| I_4hrsS2 <sup>Inf</sup>  | 41.3                   | 39.6                       | 35.3 (89.24%)             | 33.6 (84.92%)                      | 1.7 (4.33%)                            | 99.95 | 97.15 | 47.0           |
| I_4hrsS3 <sup>Inf</sup>  | 41.5                   | 39.8                       | 35.5 (89.2%)              | 33.2 (83.29%)                      | 2.4 (5.91%)                            | 99.95 | 97.11 | 47.5           |
| I_72hrsS1 <sup>Inf</sup> | 41.2                   | 39.8                       | 28.3 (71.09%)             | 26.9 (67.7%)                       | 1.3 (3.38%)                            | 99.96 | 97.23 | 44.5           |
| I_72hrsS2 <sup>Inf</sup> | 39.3                   | 38.0                       | 27 (71.11%)               | 25.8 (67.86%)                      | 1.2 (3.25%)                            | 99.96 | 97.34 | 44.5           |
| I_72hrsS3 <sup>Inf</sup> | 39.9                   | 37.1                       | 28.3 (76.36%)             | 26.1 (70.3%)                       | 2.2 (6.05%)                            | 99.87 | 96.14 | 52.5           |
| U_12hrsN1 <sup>Mk</sup>  | 42.1                   | 40.4                       | 35.9 (88.72%)             | 34.1 (84.39%)                      | 1.7 (4.33%)                            | 99.95 | 97.04 | 47.5           |
| U_12hrsN2 <sup>Mk</sup>  | 41.0                   | 39.3                       | 34.7 (88.4%)              | 33.2 (84.53%)                      | 1.5 (3.86%)                            | 99.94 | 97.08 | 47.5           |
| U_24hrsN1 <sup>Mk</sup>  | 38.4                   | 37.0                       | 32.7 (88.46%)             | 31.4 (84.74%)                      | 1.4 (3.72%)                            | 99.96 | 97.48 | 47.5           |
| U_24hrsN2 <sup>Mk</sup>  | 39.9                   | 38.4                       | 34 (88.58%)               | 32.6 (84.96%)                      | 1.4 (3.61%)                            | 99.95 | 96.95 | 47.0           |
| U_4hrsN1 <sup>Mk</sup>   | 39.4                   | 37.9                       | 33.7 (88.9%)              | 32 (84.41%)                        | 1.7 (4.49%)                            | 99.96 | 97.36 | 47.0           |
| U_4hrsN2 <sup>Mk</sup>   | 37.6                   | 34.7                       | 22 (63.43%)               | 18.5 (53.18%)                      | 3.6 (10.25%)                           | 99.80 | 94.96 | 61.0           |
| U_72hrsN1 <sup>Mk</sup>  | 50.3                   | 47.9                       | 15.5 (32.4%)              | 11.7 (24.5%)                       | 3.8 (7.9%)                             | 99.88 | 96.54 | 56.0           |
| U_72hrsN2 <sup>Mk</sup>  | 40.5                   | 38.9                       | 34.5 (88.82%)             | 32.7 (84.14%)                      | 1.8 (4.68%)                            | 99.95 | 97.04 | 46.5           |

| Sample | Raw Reads <sup>M</sup> | Trimmed Reads <sup>M</sup> | Mapped Reads <sup>M</sup> | Uniquely Mapped<br>Reads <sup>M</sup> | Non-uniquely<br>Mapped Reads <sup>M</sup> | Q20% | Q30% | GC<br>Content (%) |
|--------|------------------------|----------------------------|---------------------------|---------------------------------------|---|------|------|-------------------|
|--------|------------------------|----------------------------|---------------------------|---------------------------------------|---|------|------|-------------------|

<sup>M</sup>All values for number of reads are in millions; <sup>Inf</sup>These are infected samples indicated by the letter 'I' and 'S' in sample names; <sup>Mk</sup>These are mock-infected samples indicated by the letters 'U' and

'N' in sample names;

Table 2A: Gene ontology analysis of Significantly Upregulated DEGs identified at 12-hpi

| GO Category               | GO:Term   | Fold Enrichment | Number of DEGs | P-value<br>(Adjusted) |
|---------------------------|---|-----------------|----------------|-----------------------|
| <b>Biological Process</b> |   |                 |                |                       |
| GO:BP                     | DNA-templated transcription   | 2.17            | 26             | 1.53e-02              |
| GO:BP                     | alcohol biosynthetic process  | 3.77            | 19             | 3.45e-04              |
| GO:BP                     | androgen receptor signaling pathway                                       | 10.03           | 5              | 3.18e-02              |
| GO:BP                     | apoptotic process   | 2.75            | 47             | 6.09e-07              |
| GO:BP                     | apoptotic signaling pathway   | 3.32            | 20             | 8.19e-04              |
| GO:BP                     | appendage development   | 4.20            | 9              | 3.40e-02              |
| GO:BP                     | appendage morphogenesis   | 4.59            | 8              | 4.22e-02              |
| GO:BP                     | autophagy   | 2.59            | 23             | 4.43e-03              |
| GO:BP                     | biological process involved in interspecies interaction between organisms | 1.80            | 40             | 1.74e-02              |
| GO:BP                     | biological regulation   | 1.14            | 517            | 8.20e-04              |
| GO:BP                     | catabolic process   | 1.51            | 108            | 1.03e-03              |
| GO:BP                     | cell cycle  | 1.68            | 72             | 1.34e-03              |
| GO:BP                     | cell cycle phase transition   | 3.29            | 11             | 4.63e-02              |
| GO:BP                     | cell cycle process  | 1.70            | 59             | 4.85e-03              |
| GO:BP                     | cell death  | 2.85            | 51             | 4.99e-08              |
| GO:BP                     | cell division   | 2.20            | 26             | 1.31e-02              |
| GO:BP                     | cellular catabolic process  | 1.64            | 44             | 4.22e-02              |
| GO:BP                     | cellular component disassembly  | 2.46            | 21             | 1.31e-02              |

| GO Category | GO:Term   | Fold Enrichment | Number of DEGs | P-value<br>(Adjusted) |
|-------------|---|-----------------|----------------|-----------------------|
| GO:BP       | cellular lipid biosynthetic process                             | 9.36            | 7              | 3.16e-03              |
| GO:BP       | cellular lipid metabolic process                                | 1.67            | 67             | 3.03e-03              |
| GO:BP       | cellular localization   | 1.45            | 145            | 4.34e-04              |
| GO:BP       | cellular macromolecule localization                             | 1.58            | 104            | 4.21e-04              |
| GO:BP       | cellular metabolic process                                      | 1.23            | 307            | 8.52e-04              |
| GO:BP       | cellular response to biotic stimulus                            | 3.21            | 12             | 3.47e-02              |
| GO:BP       | cellular response to chemical stimulus                          | 1.56            | 60             | 2.49e-02              |
| GO:BP       | cellular response to decreased oxygen levels                    | 4.86            | 8              | 3.18e-02              |
| GO:BP       | cellular response to hypoxia                                    | 5.02            | 8              | 2.79e-02              |
| GO:BP       | cellular response to lipid                                      | 2.66            | 20             | 8.44e-03              |
| GO:BP       | cellular response to lipopolysaccharide                         | 3.56            | 11             | 2.92e-02              |
| GO:BP       | cellular response to molecule of bacterial origin               | 3.34            | 11             | 4.22e-02              |
| GO:BP       | cellular response to oxygen levels                              | 5.02            | 9              | 1.27e-02              |
| GO:BP       | cellular response to oxygen-containing compound                 | 1.92            | 33             | 1.98e-02              |
| GO:BP       | cellular response to stress                                     | 1.77            | 81             | 1.11e-04              |
| GO:BP       | cholesterol biosynthetic process                                | 6.92            | 10             | 7.48e-04              |
| GO:BP       | cholesterol metabolic process                                   | 3.76            | 12             | 1.20e-02              |
| GO:BP       | deadenylation-independent decapping of nuclear-transcribed mRNA | 14.33           | 5              | 8.47e-03              |
| GO:BP       | developmental growth  | 2.58            | 19             | 1.53e-02              |
| GO:BP       | embryo development  | 1.93            | 29             | 3.43e-02              |

| GO Category | GO:Term  | Fold Enrichment | Number of DEGs | P-value<br>(Adjusted) |
|-------------|--|-----------------|----------------|-----------------------|
| GO:BP       | embryonic morphogenesis                            | 2.36            | 24             | 9.41e-03              |
| GO:BP       | endoderm development                               | 6.42            | 8              | 7.66e-03              |
| GO:BP       | ergosterol biosynthetic process                    | 12.77           | 7              | 5.94e-04              |
| GO:BP       | ergosterol metabolic process                       | 12.77           | 7              | 5.94e-04              |
| GO:BP       | establishment of localization                      | 1.24            | 195            | 2.46e-02              |
| GO:BP       | establishment of localization in cell              | 1.56            | 104            | 5.94e-04              |
| GO:BP       | establishment of protein localization              | 1.61            | 73             | 3.79e-03              |
| GO:BP       | establishment of protein localization to organelle | 2.04            | 34             | 7.41e-03              |
| GO:BP       | establishment or maintenance of cell polarity      | 2.51            | 20             | 1.48e-02              |
| GO:BP       | extrinsic apoptotic signaling pathway              | 4.18            | 10             | 1.89e-02              |
| GO:BP       | gland development                                  | 3.06            | 16             | 9.41e-03              |
| GO:BP       | growth   | 2.58            | 19             | 1.53e-02              |
| GO:BP       | hemopoiesis  | 2.16            | 26             | 1.69e-02              |
| GO:BP       | homeostasis of number of cells                     | 3.27            | 14             | 1.27e-02              |
| GO:BP       | intracellular lipid transport                      | 5.02            | 8              | 2.79e-02              |
| GO:BP       | intracellular protein transport                    | 2.00            | 50             | 5.94e-04              |
| GO:BP       | intracellular signal transduction                  | 1.54            | 97             | 1.48e-03              |
| GO:BP       | intracellular transport                            | 1.51            | 79             | 1.02e-02              |
| GO:BP       | intrinsic apoptotic signaling pathway              | 3.70            | 12             | 1.31e-02              |
| GO:BP       | limb development                                   | 4.20            | 9              | 3.40e-02              |

| GO Category | GO:Term  | Fold Enrichment | Number of DEGs | P-value<br>(Adjusted) |
|-------------|--|-----------------|----------------|-----------------------|
| GO:BP       | limb morphogenesis                                   | 4.59            | 8              | 4.22e-02              |
| GO:BP       | lipid biosynthetic process                           | 1.94            | 46             | 1.94e-03              |
| GO:BP       | lipid metabolic process                              | 1.53            | 79             | 7.41e-03              |
| GO:BP       | localization   | 1.24            | 219            | 1.25e-02              |
| GO:BP       | mRNA transcription                                   | 7.80            | 7              | 7.79e-03              |
| GO:BP       | macroautophagy                                       | 2.98            | 18             | 5.47e-03              |
| GO:BP       | macromolecule catabolic process                      | 1.76            | 60             | 1.77e-03              |
| GO:BP       | macromolecule localization                           | 1.58            | 128            | 3.56e-05              |
| GO:BP       | macromolecule metabolic process                      | 1.21            | 286            | 6.45e-03              |
| GO:BP       | macromolecule modification                           | 1.43            | 138            | 9.09e-04              |
| GO:BP       | metabolic process                                    | 1.19            | 426            | 3.93e-04              |
| GO:BP       | mitotic cell cycle                                   | 1.94            | 47             | 1.70e-03              |
| GO:BP       | mitotic cell cycle phase transition                  | 3.34            | 11             | 4.22e-02              |
| GO:BP       | mitotic cell cycle process                           | 2.14            | 41             | 8.20e-04              |
| GO:BP       | motor neuron apoptotic process                       | 10.03           | 5              | 3.18e-02              |
| GO:BP       | multicellular organismal-level homeostasis           | 2.48            | 22             | 9.41e-03              |
| GO:BP       | negative regulation of apoptotic process             | 2.37            | 36             | 4.54e-04              |
| GO:BP       | negative regulation of biological process            | 1.56            | 187            | 1.90e-07              |
| GO:BP       | negative regulation of biosynthetic process          | 1.73            | 68             | 1.03e-03              |
| GO:BP       | negative regulation of cellular biosynthetic process | 1.74            | 68             | 8.52e-04              |

| GO Category | GO:Term   | Fold Enrichment | Number of DEGs | P-value<br>(Adjusted) |
|-------------|---|-----------------|----------------|-----------------------|
| GO:BP       | negative regulation of cellular metabolic process                           | 1.79            | 80             | 9.46e-05              |
| GO:BP       | negative regulation of cellular process                                     | 1.59            | 174            | 1.90e-07              |
| GO:BP       | negative regulation of gene expression                                      | 2.16            | 40             | 8.52e-04              |
| GO:BP       | negative regulation of intracellular signal transduction                    | 2.07            | 28             | 1.78e-02              |
| GO:BP       | negative regulation of macromolecule biosynthetic process                   | 1.70            | 65             | 2.25e-03              |
| GO:BP       | negative regulation of macromolecule metabolic process                      | 1.66            | 82             | 7.20e-04              |
| GO:BP       | negative regulation of metabolic process                                    | 1.70            | 91             | 1.10e-04              |
| GO:BP       | negative regulation of programmed cell death                                | 2.35            | 37             | 4.29e-04              |
| GO:BP       | nitrogen compound transport   | 1.57            | 79             | 4.15e-03              |
| GO:BP       | nuclear transport   | 2.24            | 22             | 2.79e-02              |
| GO:BP       | nuclear-transcribed mRNA catabolic process, deadenylation-independent decay | 14.33           | 5              | 8.47e-03              |
| GO:BP       | nucleobase-containing compound catabolic process                            | 2.05            | 24             | 4.40e-02              |
| GO:BP       | nucleocytoplasmic transport   | 2.24            | 22             | 2.79e-02              |
| GO:BP       | nucleoside bisphosphate metabolic process                                   | 3.03            | 13             | 3.39e-02              |
| GO:BP       | organic hydroxy compound biosynthetic process                               | 2.93            | 20             | 3.11e-03              |
| GO:BP       | organonitrogen compound metabolic process                                   | 1.29            | 260            | 3.93e-04              |
| GO:BP       | organophosphate metabolic process   | 1.59            | 65             | 1.09e-02              |
| GO:BP       | phosphate-containing compound metabolic process                             | 1.63            | 145            | 1.11e-06              |
| GO:BP       | phosphorus metabolic process  | 1.63            | 146            | 1.11e-06              |
| GO:BP       | phosphorylation   | 1.80            | 74             | 1.96e-04              |

| GO Category | GO:Term  | Fold Enrichment | Number of DEGs | P-value<br>(Adjusted) |
|-------------|--|-----------------|----------------|-----------------------|
| GO:BP       | phytosteroid biosynthetic process                      | 12.04           | 9              | 3.75e-05              |
| GO:BP       | phytosteroid metabolic process                         | 12.04           | 9              | 3.75e-05              |
| GO:BP       | positive regulation of apoptotic process               | 2.85            | 24             | 9.34e-04              |
| GO:BP       | positive regulation of biological process              | 1.33            | 193            | 1.29e-03              |
| GO:BP       | positive regulation of catabolic process               | 2.30            | 27             | 6.45e-03              |
| GO:BP       | positive regulation of cell communication              | 1.55            | 55             | 4.22e-02              |
| GO:BP       | positive regulation of cellular biosynthetic process   | 1.44            | 72             | 4.95e-02              |
| GO:BP       | positive regulation of cellular metabolic process      | 1.56            | 98             | 8.52e-04              |
| GO:BP       | positive regulation of cellular process                | 1.34            | 173            | 1.83e-03              |
| GO:BP       | positive regulation of macromolecule metabolic process | 1.46            | 100            | 5.97e-03              |
| GO:BP       | positive regulation of metabolic process               | 1.53            | 116            | 4.34e-04              |
| GO:BP       | positive regulation of programmed cell death           | 2.74            | 24             | 1.58e-03              |
| GO:BP       | positive regulation of signal transduction             | 1.62            | 51             | 2.85e-02              |
| GO:BP       | positive regulation of signaling                       | 1.55            | 55             | 4.22e-02              |
| GO:BP       | primary metabolic process                              | 1.22            | 380            | 1.08e-04              |
| GO:BP       | process utilizing autophagic mechanism                 | 2.59            | 23             | 4.43e-03              |
| GO:BP       | programmed cell death                                  | 2.85            | 51             | 4.99e-08              |
| GO:BP       | protein catabolic process                              | 1.66            | 42             | 4.40e-02              |
| GO:BP       | protein localization                                   | 1.58            | 104            | 4.21e-04              |
| GO:BP       | protein localization to organelle                      | 1.90            | 52             | 1.15e-03              |

| GO Category | GO:Term  | Fold Enrichment | Number of DEGs | P-value<br>(Adjusted) |
|-------------|--|-----------------|----------------|-----------------------|
| GO:BP       | protein metabolic process                        | 1.27            | 198            | 8.44e-03              |
| GO:BP       | protein modification process                     | 1.55            | 138            | 3.56e-05              |
| GO:BP       | protein phosphorylation                          | 2.33            | 61             | 9.07e-07              |
| GO:BP       | protein transport                                | 1.58            | 62             | 1.45e-02              |
| GO:BP       | purine nucleoside bisphosphate metabolic process | 3.03            | 13             | 3.39e-02              |
| GO:BP       | regulation of DNA-templated transcription        | 1.35            | 142            | 7.41e-03              |
| GO:BP       | regulation of RNA biosynthetic process           | 1.35            | 142            | 7.59e-03              |
| GO:BP       | regulation of RNA metabolic process              | 1.34            | 154            | 5.97e-03              |
| GO:BP       | regulation of anatomical structure morphogenesis | 1.81            | 32             | 4.74e-02              |
| GO:BP       | regulation of apoptotic process                  | 2.15            | 57             | 2.79e-05              |
| GO:BP       | regulation of autophagy                          | 2.61            | 20             | 1.02e-02              |
| GO:BP       | regulation of biological process                 | 1.15            | 497            | 5.83e-04              |
| GO:BP       | regulation of biosynthetic process               | 1.40            | 210            | 3.56e-05              |
| GO:BP       | regulation of catabolic process                  | 2.01            | 47             | 8.20e-04              |
| GO:BP       | regulation of cell communication                 | 1.36            | 120            | 1.31e-02              |
| GO:BP       | regulation of cell cycle                         | 1.67            | 43             | 3.66e-02              |
| GO:BP       | regulation of cell cycle process                 | 1.79            | 33             | 4.65e-02              |
| GO:BP       | regulation of cellular biosynthetic process      | 1.41            | 210            | 2.24e-05              |
| GO:BP       | regulation of cellular catabolic process         | 2.35            | 21             | 2.18e-02              |
| GO:BP       | regulation of cellular metabolic process         | 1.48            | 252            | 2.56e-08              |

| GO Category | GO:Term  | Fold Enrichment | Number of DEGs | P-value<br>(Adjusted) |
|-------------|--|-----------------|----------------|-----------------------|
| GO:BP       | regulation of cellular process                                 | 1.16            | 473            | 4.21e-04              |
| GO:BP       | regulation of cytokine production                              | 2.21            | 23             | 2.61e-02              |
| GO:BP       | regulation of developmental process                            | 1.59            | 73             | 4.88e-03              |
| GO:BP       | regulation of epithelial cell apoptotic process                | 5.02            | 8              | 2.79e-02              |
| GO:BP       | regulation of gene expression                                  | 1.38            | 201            | 1.06e-04              |
| GO:BP       | regulation of intracellular signal transduction                | 1.60            | 73             | 4.75e-03              |
| GO:BP       | regulation of leukocyte differentiation                        | 3.00            | 13             | 3.64e-02              |
| GO:BP       | regulation of macromolecule biosynthetic process               | 1.39            | 205            | 6.25e-05              |
| GO:BP       | regulation of macromolecule metabolic process                  | 1.42            | 248            | 9.05e-07              |
| GO:BP       | regulation of metabolic process                                | 1.47            | 279            | 6.08e-09              |
| GO:BP       | regulation of mitotic cell cycle phase transition              | 2.34            | 18             | 4.74e-02              |
| GO:BP       | regulation of nucleobase-containing compound metabolic process | 1.36            | 167            | 1.29e-03              |
| GO:BP       | regulation of phosphate metabolic process                      | 1.79            | 37             | 2.89e-02              |
| GO:BP       | regulation of phosphorus metabolic process                     | 1.79            | 37             | 2.89e-02              |
| GO:BP       | regulation of primary metabolic process                        | 1.40            | 226            | 1.02e-05              |
| GO:BP       | regulation of programmed cell death                            | 2.07            | 57             | 6.77e-05              |
| GO:BP       | regulation of protein metabolic process                        | 1.50            | 59             | 4.97e-02              |
| GO:BP       | regulation of response to stimulus                             | 1.39            | 137            | 3.49e-03              |
| GO:BP       | regulation of response to stress                               | 1.91            | 46             | 2.54e-03              |
| GO:BP       | regulation of signal transduction                              | 1.44            | 110            | 4.85e-03              |

| GO Category | GO:Term  | Fold Enrichment | Number of DEGs | P-value<br>(Adjusted) |
|-------------|--|-----------------|----------------|-----------------------|
| GO:BP       | regulation of signaling                          | 1.37            | 121            | 1.25e-02              |
| GO:BP       | regulation of transcription by RNA polymerase II | 1.41            | 111            | 7.98e-03              |
| GO:BP       | response to chemical                             | 1.56            | 95             | 1.24e-03              |
| GO:BP       | response to lipid                                | 2.41            | 25             | 5.91e-03              |
| GO:BP       | response to nitrogen compound                    | 1.97            | 30             | 2.46e-02              |
| GO:BP       | response to organonitrogen compound              | 2.19            | 29             | 7.59e-03              |
| GO:BP       | response to oxygen-containing compound           | 1.90            | 44             | 4.16e-03              |
| GO:BP       | response to stress                               | 1.44            | 112            | 3.79e-03              |
| GO:BP       | ribonucleoside bisphosphate metabolic process    | 3.03            | 13             | 3.39e-02              |
| GO:BP       | secondary alcohol biosynthetic process           | 7.67            | 13             | 1.58e-05              |
| GO:BP       | secondary alcohol metabolic process              | 3.70            | 14             | 4.85e-03              |
| GO:BP       | small molecule biosynthetic process              | 2.38            | 45             | 3.75e-05              |
| GO:BP       | small molecule metabolic process                 | 1.44            | 93             | 1.24e-02              |
| GO:BP       | steroid biosynthetic process                     | 3.96            | 16             | 8.52e-04              |
| GO:BP       | steroid metabolic process                        | 2.54            | 18             | 2.46e-02              |
| GO:BP       | sterol biosynthetic process                      | 7.24            | 13             | 2.79e-05              |
| GO:BP       | sterol metabolic process                         | 3.86            | 15             | 1.83e-03              |
| GO:BP       | tissue development                               | 1.58            | 51             | 4.22e-02              |
| GO:BP       | transport  | 1.24            | 183            | 3.18e-02              |
| GO:BP       | vesicle-mediated transport                       | 1.51            | 80             | 9.41e-03              |

| GO Category               | GO:Term                                  | Fold Enrichment | Number of DEGs | P-value<br>(Adjusted) |
|---------------------------|--|-----------------|----------------|-----------------------|
| <b>Cellular Component</b> |  |                 |                |                       |
| GO:CC                     | Golgi apparatus                          | 1.52            | 69             | 1.40e-02              |
| GO:CC                     | bounding membrane of organelle           | 1.70            | 92             | 3.49e-05              |
| GO:CC                     | chromatin                                | 1.84            | 40             | 9.58e-03              |
| GO:CC                     | chromosome                               | 1.58            | 63             | 1.10e-02              |
| GO:CC                     | cytoplasm                                | 1.28            | 590            | 7.06e-17              |
| GO:CC                     | cytoplasmic vesicle                      | 1.57            | 88             | 1.12e-03              |
| GO:CC                     | cytoplasmic vesicle membrane             | 1.69            | 41             | 2.85e-02              |
| GO:CC                     | cytosol                                  | 1.69            | 166            | 6.96e-10              |
| GO:CC                     | early endosome                           | 2.11            | 22             | 3.70e-02              |
| GO:CC                     | endomembrane system                      | 1.48            | 200            | 3.53e-07              |
| GO:CC                     | endoplasmic reticulum                    | 1.54            | 86             | 2.07e-03              |
| GO:CC                     | endosome                                 | 1.69            | 48             | 1.37e-02              |
| GO:CC                     | endosome membrane                        | 2.02            | 25             | 3.21e-02              |
| GO:CC                     | intracellular anatomical structure       | 1.19            | 774            | 8.23e-20              |
| GO:CC                     | intracellular membrane-bounded organelle | 1.29            | 578            | 1.08e-16              |
| GO:CC                     | intracellular organelle                  | 1.23            | 655            | 7.52e-16              |
| GO:CC                     | intracellular organelle lumen            | 1.51            | 135            | 4.91e-05              |
| GO:CC                     | intracellular vesicle                    | 1.55            | 88             | 1.62e-03              |
| GO:CC                     | membrane-bounded organelle               | 1.26            | 595            | 7.52e-16              |

| GO Category               | GO:Term                                  | Fold Enrichment | Number of DEGs | P-value<br>(Adjusted) |
|---------------------------|--|-----------------|----------------|-----------------------|
| GO:CC                     | membrane-enclosed lumen                  | 1.51            | 135            | 4.91e-05              |
| GO:CC                     | nuclear lumen                            | 1.55            | 119            | 6.06e-05              |
| GO:CC                     | nucleoplasm                              | 1.75            | 104            | 1.57e-06              |
| GO:CC                     | nucleus                                  | 1.42            | 371            | 1.33e-13              |
| GO:CC                     | organelle                                | 1.21            | 666            | 2.49e-14              |
| GO:CC                     | organelle lumen                          | 1.51            | 135            | 4.91e-05              |
| GO:CC                     | organelle membrane                       | 1.59            | 154            | 3.53e-07              |
| GO:CC                     | organelle subcompartment                 | 1.56            | 65             | 1.21e-02              |
| GO:CC                     | perinuclear region of cytoplasm          | 2.51            | 25             | 2.11e-03              |
| GO:CC                     | phagophore assembly site                 | 4.73            | 8              | 2.83e-02              |
| GO:CC                     | protein-DNA complex                      | 1.76            | 42             | 1.40e-02              |
| GO:CC                     | spindle                                  | 2.04            | 25             | 2.85e-02              |
| GO:CC                     | transcription regulator complex          | 1.99            | 30             | 1.40e-02              |
| GO:CC                     | vacuole                                  | 1.82            | 34             | 2.73e-02              |
| GO:CC                     | vesicle                                  | 1.51            | 95             | 1.94e-03              |
| GO:CC                     | vesicle membrane                         | 1.74            | 43             | 1.40e-02              |
| <b>Molecular Function</b> |  |                 |                |                       |
| GO:MF                     | ATP binding                              | 1.33            | 128            | 2.71e-02              |
| GO:MF                     | DNA-binding transcription factor binding | 2.73            | 22             | 5.83e-03              |
| GO:MF                     | R-SMAD binding                           | 10.31           | 5              | 4.03e-02              |

| GO Category | GO:Term   | Fold Enrichment | Number of DEGs | P-value<br>(Adjusted) |
|-------------|---|-----------------|----------------|-----------------------|
| GO:MF       | RNA polymerase II-specific DNA-binding transcription factor binding | 2.94            | 18             | 1.05e-02              |
| GO:MF       | adenyl nucleotide binding   | 1.30            | 133            | 4.38e-02              |
| GO:MF       | adenyl ribonucleotide binding                                       | 1.33            | 130            | 2.71e-02              |
| GO:MF       | binding   | 1.09            | 714            | 4.02e-04              |
| GO:MF       | enzyme binding  | 2.20            | 102            | 8.60e-11              |
| GO:MF       | enzyme regulator activity   | 1.50            | 82             | 1.38e-02              |
| GO:MF       | identical protein binding   | 2.07            | 54             | 1.96e-04              |
| GO:MF       | ion binding   | 1.19            | 325            | 1.05e-02              |
| GO:MF       | kinase activity   | 1.58            | 86             | 4.95e-03              |
| GO:MF       | kinase binding  | 2.14            | 35             | 5.83e-03              |
| GO:MF       | manganese ion binding   | 5.62            | 9              | 1.05e-02              |
| GO:MF       | molecular adaptor activity  | 1.90            | 73             | 5.57e-05              |
| GO:MF       | myosin phosphatase activity   | 4.64            | 9              | 2.71e-02              |
| GO:MF       | nuclear androgen receptor binding                                   | 12.88           | 5              | 1.83e-02              |
| GO:MF       | phosphotransferase activity, alcohol group as acceptor              | 1.59            | 80             | 5.83e-03              |
| GO:MF       | protein binding   | 1.27            | 427            | 8.52e-08              |
| GO:MF       | protein domain specific binding                                     | 2.51            | 25             | 5.83e-03              |
| GO:MF       | protein homodimerization activity                                   | 2.29            | 23             | 2.51e-02              |
| GO:MF       | protein kinase activity   | 1.59            | 67             | 1.20e-02              |
| GO:MF       | protein kinase binding  | 2.10            | 31             | 1.18e-02              |

| GO Category | GO:Term   | Fold Enrichment | Number of DEGs | P-value<br>(Adjusted) |
|-------------|---|-----------------|----------------|-----------------------|
| GO:MF       | protein serine/threonine kinase activity                        | 1.68            | 44             | 4.27e-02              |
| GO:MF       | protein-macromolecule adaptor activity                          | 1.98            | 67             | 5.57e-05              |
| GO:MF       | purine ribonucleoside triphosphate binding                      | 1.28            | 148            | 4.38e-02              |
| GO:MF       | signaling adaptor activity                                      | 3.44            | 12             | 3.01e-02              |
| GO:MF       | small molecule binding  | 1.19            | 338            | 5.83e-03              |
| GO:MF       | transcription coregulator activity                              | 1.93            | 38             | 1.18e-02              |
| GO:MF       | transcription factor binding                                    | 2.38            | 27             | 6.59e-03              |
| GO:MF       | transferase activity  | 1.30            | 176            | 1.05e-02              |
| GO:MF       | transferase activity, transferring phosphorus-containing groups | 1.51            | 96             | 5.83e-03              |

Table 2B: Gene ontology analysis of Significantly Downregulated DEGs identified at 12-hpi

| GO Category               | GO:Term                                      | Fold Enrichment | Number of DEGs | P-value<br>(Adjusted) |
|---------------------------|--|-----------------|----------------|-----------------------|
| <b>Biological Process</b> |  |                 |                |                       |
| GO:BP                     | 'de novo' AMP biosynthetic process           | 10.16           | 5              | 2.26e-02              |
| GO:BP                     | 'de novo' IMP biosynthetic process           | 11.43           | 5              | 1.51e-02              |
| GO:BP                     | 'de novo' XMP biosynthetic process           | 18.29           | 4              | 1.71e-02              |
| GO:BP                     | 'de novo' post-translational protein folding | 8.31            | 10             | 6.05e-05              |
| GO:BP                     | 'de novo' protein folding                    | 7.95            | 10             | 8.96e-05              |
| GO:BP                     | AMP biosynthetic process                     | 7.84            | 6              | 1.71e-02              |
| GO:BP                     | ATP biosynthetic process                     | 6.58            | 9              | 1.26e-03              |
| GO:BP                     | ATP metabolic process                        | 4.29            | 19             | 1.86e-05              |
| GO:BP                     | ATP synthesis coupled electron transport     | 6.10            | 18             | 1.95e-07              |
| GO:BP                     | DNA damage response                          | 2.35            | 69             | 7.26e-09              |
| GO:BP                     | DNA geometric change                         | 4.33            | 9              | 2.22e-02              |
| GO:BP                     | DNA integrity checkpoint signaling           | 3.75            | 16             | 6.72e-04              |
| GO:BP                     | DNA metabolic process                        | 2.74            | 82             | 3.18e-14              |
| GO:BP                     | DNA recombination                            | 2.07            | 23             | 3.64e-02              |
| GO:BP                     | DNA repair                                   | 2.39            | 51             | 1.44e-06              |
| GO:BP                     | DNA replication                              | 4.95            | 39             | 2.28e-14              |
| GO:BP                     | DNA replication checkpoint signaling         | 6.36            | 8              | 5.00e-03              |
| GO:BP                     | DNA replication initiation                   | 5.23            | 8              | 1.71e-02              |

| GO Category | GO:Term  | Fold Enrichment | Number of DEGs | P-value<br>(Adjusted) |
|-------------|--|-----------------|----------------|-----------------------|
| GO:BP       | DNA strand elongation  | 6.97            | 8              | 2.67e-03              |
| GO:BP       | DNA strand elongation involved in DNA replication                                    | 7.32            | 8              | 1.93e-03              |
| GO:BP       | DNA-templated DNA replication  | 5.29            | 33             | 8.34e-13              |
| GO:BP       | DNA-templated DNA replication maintenance of fidelity                                | 4.43            | 8              | 3.69e-02              |
| GO:BP       | GMP biosynthetic process   | 9.15            | 6              | 8.32e-03              |
| GO:BP       | GMP metabolic process  | 7.84            | 6              | 1.71e-02              |
| GO:BP       | NADH dehydrogenase complex assembly  | 4.57            | 8              | 3.13e-02              |
| GO:BP       | RNA biosynthetic process   | 2.55            | 135            | 4.64e-22              |
| GO:BP       | RNA export from nucleus  | 3.77            | 13             | 4.16e-03              |
| GO:BP       | RNA localization   | 3.69            | 22             | 2.39e-05              |
| GO:BP       | RNA metabolic process  | 2.49            | 152            | 6.25e-24              |
| GO:BP       | RNA modification   | 2.30            | 19             | 3.26e-02              |
| GO:BP       | RNA processing   | 3.04            | 120            | 6.65e-26              |
| GO:BP       | RNA splicing   | 2.51            | 33             | 1.35e-04              |
| GO:BP       | RNA splicing, via transesterification reactions                                      | 2.70            | 28             | 2.22e-04              |
| GO:BP       | RNA splicing, via transesterification reactions with bulged adenosine as nucleophile | 2.70            | 28             | 2.22e-04              |
| GO:BP       | RNA transport  | 3.70            | 18             | 2.32e-04              |
| GO:BP       | XMP biosynthetic process   | 18.29           | 4              | 1.71e-02              |
| GO:BP       | XMP metabolic process  | 18.29           | 4              | 1.71e-02              |
| GO:BP       | aerobic electron transport chain   | 5.97            | 16             | 2.15e-06              |

| GO Category | GO:Term  | Fold Enrichment | Number of DEGs | P-value<br>(Adjusted) |
|-------------|--|-----------------|----------------|-----------------------|
| GO:BP       | aerobic respiration                            | 5.10            | 29             | 1.17e-10              |
| GO:BP       | amino acid activation                          | 3.25            | 11             | 3.83e-02              |
| GO:BP       | biosynthetic process                           | 2.25            | 377            | 3.04e-57              |
| GO:BP       | carbohydrate derivative biosynthetic process   | 1.64            | 49             | 2.14e-02              |
| GO:BP       | carbohydrate derivative metabolic process      | 1.49            | 69             | 2.28e-02              |
| GO:BP       | cell cycle                                     | 1.47            | 69             | 3.10e-02              |
| GO:BP       | cell cycle DNA replication                     | 6.33            | 9              | 1.67e-03              |
| GO:BP       | cell cycle checkpoint signaling                | 3.59            | 20             | 1.16e-04              |
| GO:BP       | cell cycle process                             | 1.50            | 57             | 4.88e-02              |
| GO:BP       | cellular biosynthetic process                  | 2.29            | 329            | 4.05e-50              |
| GO:BP       | cellular component assembly                    | 1.34            | 117            | 2.26e-02              |
| GO:BP       | cellular component biogenesis                  | 1.83            | 182            | 5.57e-14              |
| GO:BP       | cellular component organization or biogenesis  | 1.26            | 310            | 9.53e-05              |
| GO:BP       | cellular metabolic process                     | 1.77            | 482            | 3.42e-45              |
| GO:BP       | cellular process                               | 1.10            | 827            | 3.50e-07              |
| GO:BP       | cellular respiration                           | 4.50            | 29             | 2.81e-09              |
| GO:BP       | cellular response to stress                    | 1.79            | 90             | 5.95e-06              |
| GO:BP       | chaperone cofactor-dependent protein refolding | 7.84            | 9              | 3.37e-04              |
| GO:BP       | chaperone-mediated protein folding             | 5.03            | 11             | 1.45e-03              |
| GO:BP       | chromosome organization                        | 2.15            | 38             | 6.51e-04              |

| GO Category | GO:Term   | Fold Enrichment | Number of DEGs | P-value<br>(Adjusted) |
|-------------|---|-----------------|----------------|-----------------------|
| GO:BP       | cytoplasmic translation   | 9.15            | 27             | 7.21e-17              |
| GO:BP       | cytoplasmic translational initiation  | 7.48            | 9              | 4.85e-04              |
| GO:BP       | double-strand break repair via break-induced replication                      | 7.32            | 6              | 2.28e-02              |
| GO:BP       | electron transport chain  | 5.08            | 20             | 5.49e-07              |
| GO:BP       | energy derivation by oxidation of organic compounds                           | 3.00            | 30             | 1.49e-05              |
| GO:BP       | establishment of RNA localization   | 3.70            | 18             | 2.32e-04              |
| GO:BP       | establishment of protein localization to mitochondrion                        | 3.09            | 12             | 3.30e-02              |
| GO:BP       | establishment of protein localization to organelle                            | 1.91            | 35             | 1.13e-02              |
| GO:BP       | formation of cytoplasmic translation initiation complex                       | 11.64           | 7              | 3.80e-04              |
| GO:BP       | gene expression   | 3.07            | 289            | 4.61e-70              |
| GO:BP       | generation of precursor metabolites and energy                                | 2.77            | 40             | 1.13e-06              |
| GO:BP       | immunoglobulin production involved in immunoglobulin-mediated immune response | 5.82            | 7              | 2.28e-02              |
| GO:BP       | import into nucleus   | 2.69            | 15             | 3.01e-02              |
| GO:BP       | import into the mitochondrion   | 3.92            | 12             | 5.82e-03              |
| GO:BP       | isotype switching   | 9.15            | 5              | 3.13e-02              |
| GO:BP       | mRNA metabolic process  | 2.21            | 47             | 4.07e-05              |
| GO:BP       | mRNA processing   | 2.50            | 39             | 2.09e-05              |
| GO:BP       | mRNA splicing, via spliceosome  | 2.70            | 28             | 2.22e-04              |
| GO:BP       | macromolecule biosynthetic process  | 2.69            | 313            | 8.24e-63              |
| GO:BP       | macromolecule metabolic process   | 1.79            | 466            | 6.20e-45              |

| GO Category | GO:Term  | Fold Enrichment | Number of DEGs | P-value<br>(Adjusted) |
|-------------|--|-----------------|----------------|-----------------------|
| GO:BP       | macromolecule methylation  | 2.81            | 14             | 3.04e-02              |
| GO:BP       | maturation of 5.8S rRNA  | 6.79            | 13             | 1.21e-05              |
| GO:BP       | maturation of LSU-rRNA   | 8.13            | 16             | 1.88e-08              |
| GO:BP       | maturation of LSU-rRNA from tricistronic rRNA transcript (SSU-rRNA, 5.8S rRNA, LSU-rRNA) | 5.57            | 7              | 2.86e-02              |
| GO:BP       | maturation of SSU-rRNA   | 6.22            | 17             | 4.15e-07              |
| GO:BP       | maturation of SSU-rRNA from tricistronic rRNA transcript (SSU-rRNA, 5.8S rRNA, LSU-rRNA) | 6.27            | 12             | 8.09e-05              |
| GO:BP       | metabolic process  | 1.57            | 618            | 9.00e-47              |
| GO:BP       | mitochondrial ATP synthesis coupled electron transport                                   | 6.10            | 17             | 5.49e-07              |
| GO:BP       | mitochondrial DNA metabolic process  | 9.85            | 7              | 1.15e-03              |
| GO:BP       | mitochondrial DNA replication  | 9.15            | 5              | 3.13e-02              |
| GO:BP       | mitochondrial electron transport, NADH to ubiquinone                                     | 6.58            | 9              | 1.26e-03              |
| GO:BP       | mitochondrial gene expression  | 2.93            | 12             | 4.86e-02              |
| GO:BP       | mitochondrial genome maintenance   | 6.10            | 7              | 1.85e-02              |
| GO:BP       | mitochondrial respiratory chain complex I assembly                                       | 4.57            | 8              | 3.13e-02              |
| GO:BP       | mitochondrial transmembrane transport  | 3.70            | 18             | 2.32e-04              |
| GO:BP       | mitochondrial transport  | 3.47            | 22             | 6.14e-05              |
| GO:BP       | mitochondrion organization   | 2.51            | 39             | 1.97e-05              |
| GO:BP       | mitotic cell cycle   | 1.69            | 45             | 2.01e-02              |
| GO:BP       | mitotic cell cycle checkpoint signaling  | 3.13            | 13             | 2.10e-02              |
| GO:BP       | mitotic cell cycle process   | 1.71            | 36             | 4.39e-02              |

| GO Category | GO:Term  | Fold Enrichment | Number of DEGs | P-value<br>(Adjusted) |
|-------------|--|-----------------|----------------|-----------------------|
| GO:BP       | negative regulation of DNA metabolic process           | 3.43            | 12             | 1.71e-02              |
| GO:BP       | negative regulation of cell cycle                      | 2.19            | 24             | 1.71e-02              |
| GO:BP       | negative regulation of cell cycle phase transition     | 2.49            | 20             | 1.22e-02              |
| GO:BP       | negative regulation of cell cycle process              | 2.26            | 21             | 2.46e-02              |
| GO:BP       | non-membrane-bounded organelle assembly                | 1.99            | 30             | 1.66e-02              |
| GO:BP       | nuclear DNA replication                                | 6.33            | 9              | 1.67e-03              |
| GO:BP       | nuclear export   | 3.33            | 20             | 3.06e-04              |
| GO:BP       | nuclear transport                                      | 2.79            | 30             | 5.99e-05              |
| GO:BP       | nucleic acid biosynthetic process                      | 2.57            | 142            | 1.38e-23              |
| GO:BP       | nucleic acid metabolic process                         | 2.56            | 226            | 1.57e-39              |
| GO:BP       | nucleic acid transport                                 | 3.70            | 18             | 2.32e-04              |
| GO:BP       | nucleobase-containing compound biosynthetic process    | 2.57            | 178            | 2.25e-30              |
| GO:BP       | nucleobase-containing compound metabolic process       | 2.41            | 276            | 9.74e-45              |
| GO:BP       | nucleobase-containing compound transport               | 3.22            | 22             | 1.83e-04              |
| GO:BP       | nucleobase-containing small molecule metabolic process | 2.04            | 53             | 7.60e-05              |
| GO:BP       | nucleocytoplasmic transport                            | 2.79            | 30             | 5.99e-05              |
| GO:BP       | nucleoside monophosphate biosynthetic process          | 5.12            | 14             | 9.79e-05              |
| GO:BP       | nucleoside monophosphate metabolic process             | 4.57            | 14             | 3.08e-04              |
| GO:BP       | nucleoside phosphate biosynthetic process              | 2.50            | 31             | 2.80e-04              |
| GO:BP       | nucleoside phosphate metabolic process                 | 2.00            | 46             | 4.85e-04              |

| GO Category | GO:Term  | Fold Enrichment | Number of DEGs | P-value<br>(Adjusted) |
|-------------|--|-----------------|----------------|-----------------------|
| GO:BP       | nucleoside triphosphate biosynthetic process                     | 5.23            | 16             | 1.41e-05              |
| GO:BP       | nucleoside triphosphate metabolic process                        | 3.95            | 27             | 2.73e-07              |
| GO:BP       | nucleotide biosynthetic process                                  | 2.53            | 31             | 2.28e-04              |
| GO:BP       | nucleotide metabolic process                                     | 2.10            | 46             | 1.68e-04              |
| GO:BP       | organelle organization   | 1.23            | 174            | 4.94e-02              |
| GO:BP       | organonitrogen compound biosynthetic process                     | 2.74            | 170            | 3.69e-32              |
| GO:BP       | organonitrogen compound metabolic process                        | 1.50            | 331            | 2.81e-14              |
| GO:BP       | oxidative phosphorylation  | 6.29            | 22             | 1.17e-09              |
| GO:BP       | positive regulation of gene expression                           | 1.74            | 37             | 3.13e-02              |
| GO:BP       | positive regulation of signal transduction by p53 class mediator | 13.06           | 5              | 8.24e-03              |
| GO:BP       | positive regulation of translation                               | 3.59            | 11             | 2.11e-02              |
| GO:BP       | primary metabolic process  | 1.63            | 556            | 1.30e-44              |
| GO:BP       | protein folding  | 4.00            | 35             | 6.76e-10              |
| GO:BP       | protein import into nucleus                                      | 2.80            | 15             | 2.20e-02              |
| GO:BP       | protein localization to mitochondrion                            | 3.01            | 12             | 4.05e-02              |
| GO:BP       | protein localization to nucleus                                  | 2.49            | 17             | 3.01e-02              |
| GO:BP       | protein maturation   | 2.16            | 42             | 2.32e-04              |
| GO:BP       | protein metabolic process  | 1.52            | 261            | 2.39e-11              |
| GO:BP       | protein stabilization  | 3.85            | 16             | 4.94e-04              |
| GO:BP       | protein targeting  | 2.11            | 22             | 3.80e-02              |

| GO Category | GO:Term  | Fold Enrichment | Number of DEGs | P-value<br>(Adjusted) |
|-------------|--|-----------------|----------------|-----------------------|
| GO:BP       | protein targeting to mitochondrion                       | 3.66            | 12             | 1.06e-02              |
| GO:BP       | protein-RNA complex assembly                             | 5.76            | 40             | 3.24e-17              |
| GO:BP       | protein-RNA complex organization                         | 5.54            | 40             | 1.35e-16              |
| GO:BP       | protein-containing complex assembly                      | 2.19            | 83             | 2.81e-09              |
| GO:BP       | protein-containing complex organization                  | 1.95            | 112            | 1.17e-09              |
| GO:BP       | proton motive force-driven ATP synthesis                 | 6.86            | 9              | 9.28e-04              |
| GO:BP       | purine nucleoside monophosphate biosynthetic process     | 4.57            | 8              | 3.13e-02              |
| GO:BP       | purine nucleoside triphosphate biosynthetic process      | 4.91            | 11             | 1.78e-03              |
| GO:BP       | purine nucleoside triphosphate metabolic process         | 3.69            | 21             | 4.37e-05              |
| GO:BP       | purine nucleotide metabolic process                      | 1.76            | 33             | 4.40e-02              |
| GO:BP       | purine ribonucleoside monophosphate biosynthetic process | 4.57            | 8              | 3.13e-02              |
| GO:BP       | purine ribonucleoside triphosphate biosynthetic process  | 5.03            | 11             | 1.45e-03              |
| GO:BP       | purine ribonucleoside triphosphate metabolic process     | 3.88            | 21             | 2.09e-05              |
| GO:BP       | purine ribonucleotide metabolic process                  | 1.94            | 29             | 2.52e-02              |
| GO:BP       | rRNA metabolic process                                   | 5.57            | 63             | 4.00e-27              |
| GO:BP       | rRNA modification  | 5.14            | 9              | 7.66e-03              |
| GO:BP       | rRNA processing  | 5.97            | 62             | 1.61e-28              |
| GO:BP       | regulation of DNA metabolic process                      | 2.92            | 30             | 2.42e-05              |
| GO:BP       | regulation of DNA replication                            | 6.23            | 16             | 1.18e-06              |
| GO:BP       | regulation of DNA strand elongation                      | 8.31            | 5              | 4.39e-02              |

| GO Category | GO:Term   | Fold Enrichment | Number of DEGs | P-value<br>(Adjusted) |
|-------------|---|-----------------|----------------|-----------------------|
| GO:BP       | regulation of DNA-templated DNA replication             | 7.84            | 6              | 1.71e-02              |
| GO:BP       | regulation of G2/M transition of mitotic cell cycle     | 3.41            | 11             | 2.94e-02              |
| GO:BP       | regulation of apoptotic process                         | 1.58            | 46             | 4.77e-02              |
| GO:BP       | regulation of apoptotic signaling pathway               | 2.29            | 20             | 2.86e-02              |
| GO:BP       | regulation of cell cycle                                | 1.95            | 55             | 1.68e-04              |
| GO:BP       | regulation of cell cycle phase transition               | 2.42            | 30             | 6.83e-04              |
| GO:BP       | regulation of cell cycle process                        | 2.18            | 44             | 1.16e-04              |
| GO:BP       | regulation of protein stability                         | 3.41            | 19             | 3.76e-04              |
| GO:BP       | regulation of signal transduction by p53 class mediator | 6.10            | 7              | 1.85e-02              |
| GO:BP       | regulation of translation                               | 2.26            | 20             | 3.13e-02              |
| GO:BP       | respiratory electron transport chain                    | 4.99            | 18             | 4.43e-06              |
| GO:BP       | response to stress                                      | 1.45            | 123            | 9.23e-04              |
| GO:BP       | ribonucleoprotein complex biogenesis                    | 5.52            | 108            | 1.71e-47              |
| GO:BP       | ribonucleoside monophosphate biosynthetic process       | 4.88            | 12             | 8.23e-04              |
| GO:BP       | ribonucleoside monophosphate metabolic process          | 4.39            | 12             | 2.07e-03              |
| GO:BP       | ribonucleoside triphosphate biosynthetic process        | 5.45            | 14             | 5.12e-05              |
| GO:BP       | ribonucleoside triphosphate metabolic process           | 4.14            | 24             | 8.49e-07              |
| GO:BP       | ribonucleotide biosynthetic process                     | 2.42            | 22             | 8.55e-03              |
| GO:BP       | ribonucleotide metabolic process                        | 2.12            | 34             | 2.14e-03              |
| GO:BP       | ribose phosphate biosynthetic process                   | 2.55            | 24             | 2.07e-03              |

| GO Category | GO:Term   | Fold Enrichment | Number of DEGs | P-value<br>(Adjusted) |
|-------------|---|-----------------|----------------|-----------------------|
| GO:BP       | ribose phosphate metabolic process  | 2.19            | 36             | 7.72e-04              |
| GO:BP       | ribosomal large subunit assembly  | 10.16           | 10             | 9.32e-06              |
| GO:BP       | ribosomal large subunit biogenesis  | 8.18            | 34             | 8.01e-20              |
| GO:BP       | ribosomal small subunit assembly  | 8.54            | 7              | 2.70e-03              |
| GO:BP       | ribosomal small subunit biogenesis  | 7.36            | 31             | 2.14e-16              |
| GO:BP       | ribosome assembly   | 8.13            | 20             | 6.51e-11              |
| GO:BP       | ribosome biogenesis   | 5.80            | 86             | 2.32e-39              |
| GO:BP       | small molecule metabolic process  | 1.50            | 106            | 8.46e-04              |
| GO:BP       | somatic diversification of immunoglobulins involved in immune response    | 9.15            | 5              | 3.13e-02              |
| GO:BP       | somatic recombination of immunoglobulin genes involved in immune response | 9.15            | 5              | 3.13e-02              |
| GO:BP       | tRNA aminoacylation   | 3.41            | 11             | 2.94e-02              |
| GO:BP       | tRNA metabolic process  | 2.81            | 31             | 3.49e-05              |
| GO:BP       | tRNA transport  | 14.63           | 4              | 3.24e-02              |
| GO:BP       | telomere maintenance  | 3.35            | 11             | 3.13e-02              |
| GO:BP       | telomere organization   | 3.19            | 11             | 4.28e-02              |
| GO:BP       | translation   | 6.31            | 110            | 7.08e-55              |
| GO:BP       | translational elongation  | 4.30            | 8              | 4.31e-02              |
| GO:BP       | translational initiation  | 5.78            | 12             | 1.79e-04              |
| GO:BP       | viral gene expression   | 18.29           | 5              | 1.45e-03              |
| GO:BP       | viral translation   | 18.29           | 4              | 1.71e-02              |

| GO Category               | GO:Term                               | Fold Enrichment | Number of DEGs | P-value<br>(Adjusted) |
|---------------------------|---------------------------------------|-----------------|----------------|-----------------------|
| <b>Cellular Component</b> |                                       |                 |                |                       |
| GO:CC                     | 90S preribosome                       | 8.05            | 20             | 1.61e-11              |
| GO:CC                     | Arp2/3 protein complex                | 7.89            | 5              | 2.33e-02              |
| GO:CC                     | Ctf18 RFC-like complex                | 15.77           | 5              | 1.30e-03              |
| GO:CC                     | DNA replication preinitiation complex | 9.46            | 5              | 1.18e-02              |
| GO:CC                     | INO80-type complex                    | 5.68            | 6              | 2.68e-02              |
| GO:CC                     | Ino80 complex                         | 7.89            | 5              | 2.33e-02              |
| GO:CC                     | MCM complex                           | 7.28            | 5              | 3.08e-02              |
| GO:CC                     | Sm-like protein family complex        | 3.57            | 13             | 2.69e-03              |
| GO:CC                     | U2-type prespliceosome                | 6.31            | 6              | 1.77e-02              |
| GO:CC                     | U2-type spliceosomal complex          | 4.32            | 13             | 4.48e-04              |
| GO:CC                     | catalytic complex                     | 1.63            | 136            | 2.38e-07              |
| GO:CC                     | catalytic step 2 spliceosome          | 3.40            | 14             | 2.49e-03              |
| GO:CC                     | chaperonin-containing T-complex       | 7.36            | 7              | 2.67e-03              |
| GO:CC                     | chromatin                             | 1.64            | 41             | 2.10e-02              |
| GO:CC                     | chromosome                            | 1.98            | 91             | 1.20e-08              |
| GO:CC                     | cytochrome complex                    | 5.10            | 7              | 1.77e-02              |
| GO:CC                     | cytoplasm                             | 1.29            | 685            | 9.10e-22              |
| GO:CC                     | cytosol                               | 2.09            | 237            | 4.82e-28              |
| GO:CC                     | cytosolic large ribosomal subunit     | 10.51           | 40             | 1.85e-29              |

| GO Category | GO:Term   | Fold Enrichment | Number of DEGs | P-value<br>(Adjusted) |
|-------------|---|-----------------|----------------|-----------------------|
| GO:CC       | cytosolic ribosome  | 10.70           | 69             | 3.41e-52              |
| GO:CC       | cytosolic small ribosomal subunit                         | 12.20           | 29             | 1.62e-23              |
| GO:CC       | endopeptidase complex                                     | 3.11            | 13             | 8.59e-03              |
| GO:CC       | eukaryotic 43S preinitiation complex                      | 12.62           | 8              | 1.11e-05              |
| GO:CC       | eukaryotic 48S preinitiation complex                      | 15.14           | 8              | 1.98e-06              |
| GO:CC       | eukaryotic translation initiation factor 3 complex        | 12.17           | 9              | 2.20e-06              |
| GO:CC       | eukaryotic translation initiation factor 3 complex, eIF3m | 15.14           | 4              | 1.26e-02              |
| GO:CC       | exosome (RNase complex)                                   | 5.16            | 6              | 3.92e-02              |
| GO:CC       | fibrillar center  | 3.44            | 10             | 1.94e-02              |
| GO:CC       | inner mitochondrial membrane protein complex              | 4.50            | 29             | 6.05e-10              |
| GO:CC       | intracellular anatomical structure                        | 1.26            | 951            | 6.67e-51              |
| GO:CC       | intracellular membrane-bounded organelle                  | 1.39            | 719            | 2.65e-36              |
| GO:CC       | intracellular non-membrane-bounded organelle              | 1.85            | 361            | 2.45e-34              |
| GO:CC       | intracellular organelle                                   | 1.33            | 823            | 1.15e-41              |
| GO:CC       | intracellular organelle lumen                             | 2.62            | 271            | 6.67e-51              |
| GO:CC       | large ribosomal subunit                                   | 8.79            | 52             | 1.03e-33              |
| GO:CC       | membrane-bounded organelle                                | 1.34            | 729            | 1.48e-31              |
| GO:CC       | membrane-enclosed lumen                                   | 2.62            | 271            | 6.67e-51              |
| GO:CC       | mitochondrial envelope                                    | 2.71            | 68             | 3.51e-12              |
| GO:CC       | mitochondrial inner membrane                              | 3.34            | 50             | 3.51e-12              |

| GO Category | GO:Term  | Fold Enrichment | Number of DEGs | P-value<br>(Adjusted) |
|-------------|--|-----------------|----------------|-----------------------|
| GO:CC       | mitochondrial intermembrane space  | 4.73            | 9              | 5.04e-03              |
| GO:CC       | mitochondrial large ribosomal subunit  | 4.73            | 13             | 1.79e-04              |
| GO:CC       | mitochondrial matrix   | 3.99            | 42             | 9.69e-13              |
| GO:CC       | mitochondrial membrane   | 2.58            | 60             | 8.01e-10              |
| GO:CC       | mitochondrial protein-containing complex                                     | 4.57            | 55             | 9.42e-20              |
| GO:CC       | mitochondrial proton-transporting ATP synthase complex                       | 6.08            | 9              | 8.94e-04              |
| GO:CC       | mitochondrial proton-transporting ATP synthase complex, coupling factor F(o) | 6.76            | 5              | 3.92e-02              |
| GO:CC       | mitochondrial respirasome  | 5.16            | 6              | 3.92e-02              |
| GO:CC       | mitochondrial ribosome   | 5.03            | 21             | 5.82e-08              |
| GO:CC       | mitochondrial small ribosomal subunit  | 6.06            | 8              | 2.67e-03              |
| GO:CC       | mitochondrion  | 2.46            | 177            | 2.97e-28              |
| GO:CC       | non-membrane-bounded organelle   | 1.85            | 362            | 1.14e-34              |
| GO:CC       | nuclear chromosome   | 3.03            | 25             | 3.48e-05              |
| GO:CC       | nuclear envelope   | 2.30            | 34             | 1.90e-04              |
| GO:CC       | nuclear lumen  | 2.49            | 221            | 4.84e-37              |
| GO:CC       | nuclear membrane   | 2.38            | 16             | 2.43e-02              |
| GO:CC       | nuclear pore   | 3.51            | 13             | 3.05e-03              |
| GO:CC       | nuclear protein-containing complex   | 2.21            | 137            | 2.38e-17              |
| GO:CC       | nucleolus  | 4.68            | 111            | 8.60e-42              |
| GO:CC       | nucleoplasm  | 1.94            | 133            | 3.47e-12              |

| GO Category | GO:Term                                  | Fold Enrichment | Number of DEGs | P-value<br>(Adjusted) |
|-------------|--|-----------------|----------------|-----------------------|
| GO:CC       | nucleus                                  | 1.53            | 462            | 4.75e-26              |
| GO:CC       | organellar large ribosomal subunit       | 4.73            | 13             | 1.79e-04              |
| GO:CC       | organellar ribosome                      | 5.03            | 21             | 5.82e-08              |
| GO:CC       | organellar small ribosomal subunit       | 6.06            | 8              | 2.67e-03              |
| GO:CC       | organelle                                | 1.30            | 829            | 4.21e-37              |
| GO:CC       | organelle envelope                       | 2.56            | 101            | 8.81e-17              |
| GO:CC       | organelle envelope lumen                 | 4.37            | 9              | 8.53e-03              |
| GO:CC       | organelle inner membrane                 | 3.14            | 54             | 4.23e-12              |
| GO:CC       | organelle lumen                          | 2.62            | 271            | 6.67e-51              |
| GO:CC       | organelle membrane                       | 1.25            | 140            | 3.13e-02              |
| GO:CC       | oxidoreductase complex                   | 4.27            | 14             | 2.31e-04              |
| GO:CC       | peptidase complex                        | 2.70            | 15             | 1.18e-02              |
| GO:CC       | preribosome                              | 8.06            | 43             | 6.98e-26              |
| GO:CC       | preribosome, large subunit precursor     | 8.60            | 10             | 1.27e-05              |
| GO:CC       | preribosome, small subunit precursor     | 7.10            | 6              | 1.05e-02              |
| GO:CC       | prespliceosome                           | 6.31            | 6              | 1.77e-02              |
| GO:CC       | protein folding chaperone complex        | 7.33            | 12             | 4.35e-06              |
| GO:CC       | protein-DNA complex                      | 1.89            | 52             | 2.33e-04              |
| GO:CC       | protein-containing complex               | 1.73            | 467            | 5.00e-40              |
| GO:CC       | proton-transporting ATP synthase complex | 5.87            | 9              | 1.16e-03              |

| GO Category               | GO:Term  | Fold Enrichment | Number of DEGs | P-value<br>(Adjusted) |
|---------------------------|--|-----------------|----------------|-----------------------|
| GO:CC                     | proton-transporting ATP synthase complex, coupling factor F(o) | 7.10            | 6              | 1.05e-02              |
| GO:CC                     | proton-transporting two-sector ATPase complex                  | 3.26            | 10             | 2.66e-02              |
| GO:CC                     | replication fork   | 4.82            | 13             | 1.51e-04              |
| GO:CC                     | respirasome  | 5.22            | 8              | 6.59e-03              |
| GO:CC                     | respiratory chain complex                                      | 5.82            | 8              | 3.33e-03              |
| GO:CC                     | ribonucleoprotein complex                                      | 5.28            | 181            | 7.13e-79              |
| GO:CC                     | ribosomal subunit  | 9.31            | 90             | 8.18e-62              |
| GO:CC                     | ribosome   | 8.45            | 100            | 9.07e-64              |
| GO:CC                     | rough endoplasmic reticulum                                    | 3.88            | 8              | 3.13e-02              |
| GO:CC                     | small nuclear ribonucleoprotein complex                        | 3.65            | 11             | 7.47e-03              |
| GO:CC                     | small ribosomal subunit  | 10.00           | 37             | 3.59e-26              |
| GO:CC                     | small-subunit processome                                       | 8.20            | 26             | 1.78e-15              |
| GO:CC                     | sno(s)RNA-containing ribonucleoprotein complex                 | 7.57            | 8              | 6.08e-04              |
| GO:CC                     | spliceosomal complex   | 2.63            | 29             | 8.28e-05              |
| GO:CC                     | spliceosomal snRNP complex                                     | 3.86            | 11             | 4.98e-03              |
| GO:CC                     | spliceosomal tri-snRNP complex                                 | 4.88            | 8              | 9.58e-03              |
| GO:CC                     | translation preinitiation complex                              | 13.10           | 9              | 1.03e-06              |
| <b>Molecular Function</b> |  |                 |                |                       |
| GO:MF                     | ATP hydrolysis activity  | 1.81            | 38             | 1.92e-02              |
| GO:MF                     | ATP-dependent activity, acting on DNA                          | 2.75            | 20             | 4.94e-03              |

| GO Category | GO:Term  | Fold Enrichment | Number of DEGs | P-value<br>(Adjusted) |
|-------------|--|-----------------|----------------|-----------------------|
| GO:MF       | ATP-dependent protein folding chaperone  | 4.92            | 15             | 1.11e-04              |
| GO:MF       | DNA helicase activity  | 4.66            | 13             | 1.02e-03              |
| GO:MF       | NADH dehydrogenase (ubiquinone) activity   | 9.68            | 6              | 7.91e-03              |
| GO:MF       | RNA binding  | 3.20            | 181            | 1.08e-43              |
| GO:MF       | catalytic activity, acting on DNA  | 2.37            | 32             | 1.02e-03              |
| GO:MF       | catalytic activity, acting on RNA  | 2.07            | 41             | 1.27e-03              |
| GO:MF       | catalytic activity, acting on a nucleic acid                                       | 2.13            | 71             | 3.41e-07              |
| GO:MF       | catalytic activity, acting on a tRNA   | 2.83            | 19             | 4.94e-03              |
| GO:MF       | electron transfer activity   | 5.16            | 8              | 2.07e-02              |
| GO:MF       | heat shock protein binding   | 3.45            | 13             | 1.17e-02              |
| GO:MF       | helicase activity  | 2.66            | 21             | 4.94e-03              |
| GO:MF       | heterocyclic compound binding  | 1.23            | 180            | 4.54e-02              |
| GO:MF       | hydrolase activity, acting on acid anhydrides                                      | 1.66            | 71             | 1.87e-03              |
| GO:MF       | hydrolase activity, acting on acid anhydrides, in phosphorus-containing anhydrides | 1.64            | 70             | 2.65e-03              |
| GO:MF       | identical protein binding  | 2.09            | 58             | 2.05e-05              |
| GO:MF       | isomerase activity   | 2.25            | 21             | 3.26e-02              |
| GO:MF       | mRNA binding   | 2.26            | 35             | 1.02e-03              |
| GO:MF       | nucleic acid binding   | 1.74            | 292            | 1.36e-21              |
| GO:MF       | nucleoside phosphate binding   | 1.24            | 173            | 3.69e-02              |
| GO:MF       | nucleotide binding   | 1.24            | 173            | 3.69e-02              |

| GO Category | GO:Term   | Fold Enrichment | Number of DEGs | P-value<br>(Adjusted) |
|-------------|---|-----------------|----------------|-----------------------|
| GO:MF       | organic cyclic compound binding   | 1.43            | 438            | 2.05e-17              |
| GO:MF       | oxidoreductase activity   | 1.55            | 68             | 1.17e-02              |
| GO:MF       | oxidoreductase activity, acting on NAD(P)H  | 4.63            | 11             | 4.18e-03              |
| GO:MF       | oxidoreductase activity, acting on NAD(P)H, quinone or similar compound as acceptor | 7.74            | 8              | 2.16e-03              |
| GO:MF       | oxidoreduction-driven active transmembrane transporter activity                     | 6.77            | 7              | 1.26e-02              |
| GO:MF       | poly(U) RNA binding   | 9.68            | 5              | 3.33e-02              |
| GO:MF       | protein folding chaperone   | 4.01            | 17             | 2.99e-04              |
| GO:MF       | protein-folding chaperone binding   | 3.45            | 13             | 1.17e-02              |
| GO:MF       | proton transmembrane transporter activity   | 2.79            | 17             | 1.23e-02              |
| GO:MF       | pyrophosphatase activity  | 1.65            | 70             | 2.23e-03              |
| GO:MF       | rRNA binding  | 7.82            | 21             | 8.21e-11              |
| GO:MF       | ribonucleoprotein complex binding   | 3.39            | 17             | 1.91e-03              |
| GO:MF       | ribonucleoside triphosphate phosphatase activity                                    | 1.57            | 61             | 1.65e-02              |
| GO:MF       | ribosome binding  | 3.99            | 13             | 3.66e-03              |
| GO:MF       | single-stranded DNA binding   | 3.64            | 16             | 1.61e-03              |
| GO:MF       | single-stranded DNA helicase activity   | 7.04            | 8              | 3.73e-03              |
| GO:MF       | snoRNA binding  | 10.84           | 14             | 7.89e-09              |
| GO:MF       | structural constituent of nuclear pore  | 5.42            | 7              | 3.82e-02              |
| GO:MF       | structural constituent of ribosome  | 9.21            | 88             | 5.28e-59              |
| GO:MF       | structural molecule activity  | 2.69            | 109            | 8.75e-19              |

| GO Category | GO:Term  | Fold Enrichment | Number of DEGs | P-value<br>(Adjusted) |
|-------------|--|-----------------|----------------|-----------------------|
| GO:MF       | translation elongation factor activity               | 7.53            | 7              | 7.78e-03              |
| GO:MF       | translation factor activity, RNA binding             | 5.36            | 23             | 1.83e-08              |
| GO:MF       | translation initiation factor activity               | 5.58            | 15             | 2.21e-05              |
| GO:MF       | translation regulator activity                       | 4.84            | 29             | 8.60e-10              |
| GO:MF       | translation regulator activity, nucleic acid binding | 4.94            | 24             | 3.61e-08              |
| GO:MF       | unfolded protein binding                             | 4.65            | 25             | 4.96e-08              |

Table 3A: Gene ontology analysis of Significantly Upregulated DEGs identified at 24-hpi

| GO Category               | GO:Term  | Fold Enrichment | Number of DEGs | P-value<br>(Adjusted) |
|---------------------------|--|-----------------|----------------|-----------------------|
| <b>Biological Process</b> |  |                 |                |                       |
| GO:BP                     | ERAD pathway   | 6.28            | 14             | 1.77e-05              |
| GO:BP                     | alcohol biosynthetic process                         | 3.22            | 22             | 3.23e-04              |
| GO:BP                     | autophagy  | 2.33            | 28             | 3.55e-03              |
| GO:BP                     | biosynthetic process                                 | 1.21            | 252            | 1.72e-02              |
| GO:BP                     | carbohydrate derivative metabolic process            | 1.47            | 84             | 1.58e-02              |
| GO:BP                     | catabolic process                                    | 1.71            | 165            | 3.58e-09              |
| GO:BP                     | cell death   | 1.73            | 42             | 2.50e-02              |
| GO:BP                     | cellular biosynthetic process                        | 1.24            | 220            | 1.58e-02              |
| GO:BP                     | cellular catabolic process                           | 1.76            | 64             | 1.02e-03              |
| GO:BP                     | cellular homeostasis                                 | 1.64            | 45             | 3.99e-02              |
| GO:BP                     | cellular lipid biosynthetic process                  | 7.89            | 8              | 1.81e-03              |
| GO:BP                     | cellular lipid metabolic process                     | 1.60            | 87             | 1.20e-03              |
| GO:BP                     | cellular localization                                | 1.44            | 196            | 1.35e-05              |
| GO:BP                     | cellular macromolecule localization                  | 1.51            | 135            | 1.34e-04              |
| GO:BP                     | cellular metabolic process                           | 1.25            | 422            | 7.24e-06              |
| GO:BP                     | cellular response to stress                          | 1.69            | 105            | 1.87e-05              |
| GO:BP                     | cellular response to topologically incorrect protein | 2.87            | 13             | 4.82e-02              |
| GO:BP                     | chaperone-mediated protein folding                   | 3.70            | 10             | 3.73e-02              |

| GO Category | GO:Term  | Fold Enrichment | Number of DEGs | P-value<br>(Adjusted) |
|-------------|--|-----------------|----------------|-----------------------|
| GO:BP       | chemical homeostasis                               | 1.61            | 54             | 2.28e-02              |
| GO:BP       | cholesterol biosynthetic process                   | 7.14            | 14             | 3.72e-06              |
| GO:BP       | cholesterol metabolic process                      | 3.70            | 16             | 1.34e-03              |
| GO:BP       | cytosolic transport                                | 2.78            | 16             | 2.09e-02              |
| GO:BP       | embryonic epithelial tube formation                | 3.89            | 10             | 2.70e-02              |
| GO:BP       | embryonic morphogenesis                            | 2.03            | 28             | 2.34e-02              |
| GO:BP       | endocytosis  | 1.83            | 41             | 1.20e-02              |
| GO:BP       | epithelial tube formation                          | 3.79            | 10             | 3.22e-02              |
| GO:BP       | ergosterol biosynthetic process                    | 10.76           | 8              | 1.76e-04              |
| GO:BP       | ergosterol metabolic process                       | 10.76           | 8              | 1.76e-04              |
| GO:BP       | establishment of localization                      | 1.40            | 298            | 9.39e-08              |
| GO:BP       | establishment of localization in cell              | 1.60            | 145            | 2.78e-06              |
| GO:BP       | establishment of protein localization              | 1.63            | 100            | 1.68e-04              |
| GO:BP       | establishment of protein localization to organelle | 1.85            | 42             | 7.96e-03              |
| GO:BP       | glycoprotein metabolic process                     | 1.88            | 40             | 8.79e-03              |
| GO:BP       | heparan sulfate proteoglycan biosynthetic process  | 7.75            | 11             | 5.17e-05              |
| GO:BP       | homeostatic process                                | 1.60            | 73             | 3.78e-03              |
| GO:BP       | intracellular monoatomic cation homeostasis        | 1.82            | 36             | 2.50e-02              |
| GO:BP       | intracellular monoatomic ion homeostasis           | 1.82            | 36             | 2.63e-02              |
| GO:BP       | intracellular pH reduction                         | 6.12            | 12             | 1.68e-04              |

| GO Category | GO:Term  | Fold Enrichment | Number of DEGs | P-value<br>(Adjusted) |
|-------------|--|-----------------|----------------|-----------------------|
| GO:BP       | intracellular protein transport                        | 2.03            | 69             | 6.74e-06              |
| GO:BP       | intracellular signal transduction                      | 1.39            | 119            | 8.90e-03              |
| GO:BP       | intracellular transport                                | 1.66            | 118            | 8.14e-06              |
| GO:BP       | lipid biosynthetic process                             | 1.77            | 57             | 2.31e-03              |
| GO:BP       | lipid metabolic process                                | 1.50            | 105            | 1.77e-03              |
| GO:BP       | localization   | 1.34            | 322            | 9.94e-07              |
| GO:BP       | lysosomal lumen acidification                          | 10.57           | 5              | 2.34e-02              |
| GO:BP       | macroautophagy   | 2.32            | 19             | 4.03e-02              |
| GO:BP       | macromolecule catabolic process                        | 1.95            | 90             | 5.00e-07              |
| GO:BP       | macromolecule localization                             | 1.48            | 162            | 4.06e-05              |
| GO:BP       | macromolecule metabolic process                        | 1.35            | 435            | 1.22e-10              |
| GO:BP       | macromolecule modification                             | 1.62            | 212            | 2.68e-10              |
| GO:BP       | metabolic process                                      | 1.25            | 609            | 1.89e-10              |
| GO:BP       | modification-dependent macromolecule catabolic process | 1.84            | 49             | 3.12e-03              |
| GO:BP       | modification-dependent protein catabolic process       | 1.84            | 49             | 2.99e-03              |
| GO:BP       | monoatomic cation homeostasis                          | 1.75            | 39             | 2.96e-02              |
| GO:BP       | monoatomic ion homeostasis                             | 1.75            | 40             | 2.65e-02              |
| GO:BP       | morphogenesis of embryonic epithelium                  | 3.70            | 11             | 2.25e-02              |
| GO:BP       | negative regulation of biological process              | 1.33            | 216            | 5.68e-04              |
| GO:BP       | negative regulation of biosynthetic process            | 1.56            | 83             | 3.32e-03              |

| GO Category | GO:Term   | Fold Enrichment | Number of DEGs | P-value<br>(Adjusted) |
|-------------|---|-----------------|----------------|-----------------------|
| GO:BP       | negative regulation of cellular biosynthetic process      | 1.57            | 83             | 2.79e-03              |
| GO:BP       | negative regulation of cellular metabolic process         | 1.64            | 99             | 1.56e-04              |
| GO:BP       | negative regulation of cellular process                   | 1.37            | 203            | 1.70e-04              |
| GO:BP       | negative regulation of cytokine production                | 3.15            | 13             | 2.50e-02              |
| GO:BP       | negative regulation of gene expression                    | 2.15            | 54             | 3.27e-05              |
| GO:BP       | negative regulation of intracellular signal transduction  | 2.24            | 41             | 2.58e-04              |
| GO:BP       | negative regulation of macromolecule biosynthetic process | 1.53            | 79             | 8.47e-03              |
| GO:BP       | negative regulation of macromolecule metabolic process    | 1.45            | 97             | 9.56e-03              |
| GO:BP       | negative regulation of metabolic process                  | 1.55            | 112            | 3.27e-04              |
| GO:BP       | neural tube formation                                     | 4.03            | 9              | 4.08e-02              |
| GO:BP       | nitrogen compound transport                               | 1.73            | 118            | 1.35e-06              |
| GO:BP       | organic hydroxy compound biosynthetic process             | 2.48            | 23             | 6.55e-03              |
| GO:BP       | organonitrogen compound catabolic process                 | 1.84            | 100            | 1.01e-06              |
| GO:BP       | organonitrogen compound metabolic process                 | 1.39            | 381            | 1.64e-10              |
| GO:BP       | peptidyl-amino acid modification                          | 2.40            | 42             | 4.49e-05              |
| GO:BP       | peptidyl-serine modification                              | 2.87            | 13             | 4.82e-02              |
| GO:BP       | peptidyl-threonine modification                           | 5.28            | 10             | 3.48e-03              |
| GO:BP       | phosphate-containing compound metabolic process           | 1.31            | 158            | 1.20e-02              |
| GO:BP       | phospholipid biosynthetic process                         | 2.03            | 24             | 4.91e-02              |
| GO:BP       | phospholipid metabolic process                            | 1.71            | 39             | 4.17e-02              |

| GO Category | GO:Term  | Fold Enrichment | Number of DEGs | P-value<br>(Adjusted) |
|-------------|--|-----------------|----------------|-----------------------|
| GO:BP       | phosphorus metabolic process                             | 1.31            | 160            | 9.82e-03              |
| GO:BP       | phosphorylation  | 1.42            | 79             | 4.67e-02              |
| GO:BP       | phytosteroid biosynthetic process                        | 9.86            | 10             | 1.77e-05              |
| GO:BP       | phytosteroid metabolic process                           | 9.86            | 10             | 1.77e-05              |
| GO:BP       | positive regulation of apoptotic process                 | 2.19            | 25             | 1.78e-02              |
| GO:BP       | positive regulation of biological process                | 1.26            | 249            | 2.53e-03              |
| GO:BP       | positive regulation of catabolic process                 | 2.38            | 38             | 1.68e-04              |
| GO:BP       | positive regulation of cell communication                | 1.53            | 74             | 1.13e-02              |
| GO:BP       | positive regulation of cellular process                  | 1.24            | 217            | 1.54e-02              |
| GO:BP       | positive regulation of intracellular signal transduction | 1.61            | 49             | 3.55e-02              |
| GO:BP       | positive regulation of macromolecule metabolic process   | 1.32            | 123            | 3.22e-02              |
| GO:BP       | positive regulation of metabolic process                 | 1.38            | 142            | 3.34e-03              |
| GO:BP       | positive regulation of programmed cell death             | 2.10            | 25             | 2.71e-02              |
| GO:BP       | positive regulation of protein catabolic process         | 2.64            | 18             | 1.71e-02              |
| GO:BP       | positive regulation of protein metabolic process         | 1.67            | 50             | 1.78e-02              |
| GO:BP       | positive regulation of response to stimulus              | 1.43            | 90             | 2.09e-02              |
| GO:BP       | positive regulation of signal transduction               | 1.64            | 70             | 3.22e-03              |
| GO:BP       | positive regulation of signaling                         | 1.53            | 74             | 1.13e-02              |
| GO:BP       | post-translational protein modification                  | 1.73            | 75             | 4.24e-04              |
| GO:BP       | primary metabolic process                                | 1.32            | 558            | 9.52e-14              |

| GO Category | GO:Term   | Fold Enrichment | Number of DEGs | P-value<br>(Adjusted) |
|-------------|---|-----------------|----------------|-----------------------|
| GO:BP       | process utilizing autophagic mechanism                            | 2.33            | 28             | 3.55e-03              |
| GO:BP       | programmed cell death   | 1.73            | 42             | 2.50e-02              |
| GO:BP       | proteasomal protein catabolic process                             | 2.36            | 42             | 6.24e-05              |
| GO:BP       | proteasome-mediated ubiquitin-dependent protein catabolic process | 1.90            | 28             | 4.97e-02              |
| GO:BP       | protein catabolic process   | 2.07            | 71             | 2.36e-06              |
| GO:BP       | protein export from nucleus                                       | 4.55            | 8              | 4.17e-02              |
| GO:BP       | protein folding   | 2.40            | 26             | 3.87e-03              |
| GO:BP       | protein localization  | 1.51            | 135            | 1.30e-04              |
| GO:BP       | protein localization to organelle                                 | 1.53            | 57             | 4.32e-02              |
| GO:BP       | protein localization to vacuole                                   | 2.76            | 14             | 4.39e-02              |
| GO:BP       | protein maturation  | 1.70            | 41             | 3.55e-02              |
| GO:BP       | protein metabolic process   | 1.48            | 313            | 1.04e-10              |
| GO:BP       | protein modification by small protein conjugation                 | 1.59            | 56             | 2.50e-02              |
| GO:BP       | protein modification by small protein conjugation or removal      | 1.73            | 73             | 5.68e-04              |
| GO:BP       | protein modification by small protein removal                     | 2.51            | 18             | 2.60e-02              |
| GO:BP       | protein modification process                                      | 1.68            | 202            | 1.04e-10              |
| GO:BP       | protein phosphorylation   | 1.80            | 64             | 5.84e-04              |
| GO:BP       | protein transport   | 1.62            | 86             | 8.20e-04              |
| GO:BP       | protein ubiquitination  | 1.60            | 51             | 3.47e-02              |
| GO:BP       | proteoglycan biosynthetic process                                 | 3.85            | 13             | 4.99e-03              |

| GO Category | GO:Term  | Fold Enrichment | Number of DEGs | P-value<br>(Adjusted) |
|-------------|--|-----------------|----------------|-----------------------|
| GO:BP       | proteoglycan metabolic process   | 3.70            | 15             | 2.31e-03              |
| GO:BP       | proteolysis  | 1.45            | 101            | 6.55e-03              |
| GO:BP       | proteolysis involved in protein catabolic process                        | 1.97            | 63             | 5.57e-05              |
| GO:BP       | regulation of apoptotic process  | 1.62            | 58             | 1.54e-02              |
| GO:BP       | regulation of autophagy  | 2.31            | 24             | 1.20e-02              |
| GO:BP       | regulation of catabolic process  | 2.09            | 66             | 5.48e-06              |
| GO:BP       | regulation of cell communication   | 1.33            | 158            | 7.93e-03              |
| GO:BP       | regulation of cellular catabolic process                                 | 2.07            | 25             | 3.36e-02              |
| GO:BP       | regulation of cellular metabolic process                                 | 1.26            | 290            | 8.57e-04              |
| GO:BP       | regulation of cellular pH  | 3.19            | 19             | 1.47e-03              |
| GO:BP       | regulation of cytokine production  | 2.27            | 32             | 2.03e-03              |
| GO:BP       | regulation of cytoplasmic pattern recognition receptor signaling pathway | 3.89            | 10             | 2.70e-02              |
| GO:BP       | regulation of defense response   | 1.88            | 34             | 2.33e-02              |
| GO:BP       | regulation of intracellular pH   | 3.13            | 18             | 2.85e-03              |
| GO:BP       | regulation of intracellular signal transduction                          | 1.60            | 99             | 3.58e-04              |
| GO:BP       | regulation of lysosomal lumen pH   | 8.07            | 6              | 1.86e-02              |
| GO:BP       | regulation of macromolecule metabolic process                            | 1.22            | 291            | 3.83e-03              |
| GO:BP       | regulation of metabolic process  | 1.27            | 329            | 6.35e-05              |
| GO:BP       | regulation of pH   | 2.99            | 19             | 3.08e-03              |
| GO:BP       | regulation of primary metabolic process                                  | 1.20            | 262            | 2.50e-02              |

| GO Category | GO:Term   | Fold Enrichment | Number of DEGs | P-value<br>(Adjusted) |
|-------------|---|-----------------|----------------|-----------------------|
| GO:BP       | regulation of programmed cell death                             | 1.58            | 59             | 2.12e-02              |
| GO:BP       | regulation of proteasomal protein catabolic process             | 2.60            | 16             | 3.55e-02              |
| GO:BP       | regulation of protein catabolic process                         | 2.47            | 27             | 2.19e-03              |
| GO:BP       | regulation of protein metabolic process                         | 1.60            | 85             | 1.41e-03              |
| GO:BP       | regulation of proteolysis involved in protein catabolic process | 2.78            | 19             | 6.74e-03              |
| GO:BP       | regulation of response to stimulus                              | 1.34            | 179            | 2.19e-03              |
| GO:BP       | regulation of response to stress                                | 1.78            | 58             | 1.81e-03              |
| GO:BP       | regulation of signal transduction                               | 1.41            | 146            | 1.27e-03              |
| GO:BP       | regulation of signaling   | 1.32            | 158            | 1.06e-02              |
| GO:BP       | response to chemical  | 1.49            | 123            | 6.61e-04              |
| GO:BP       | response to endoplasmic reticulum stress                        | 3.24            | 23             | 1.76e-04              |
| GO:BP       | response to nitrogen compound                                   | 1.93            | 40             | 5.15e-03              |
| GO:BP       | response to organonitrogen compound                             | 2.06            | 37             | 3.12e-03              |
| GO:BP       | response to stress  | 1.42            | 149            | 8.20e-04              |
| GO:BP       | response to topologically incorrect protein                     | 3.00            | 15             | 1.61e-02              |
| GO:BP       | secondary alcohol biosynthetic process                          | 7.40            | 17             | 6.60e-08              |
| GO:BP       | secondary alcohol metabolic process                             | 3.50            | 18             | 8.20e-04              |
| GO:BP       | small molecule biosynthetic process                             | 1.72            | 44             | 2.28e-02              |
| GO:BP       | steroid biosynthetic process                                    | 3.65            | 20             | 1.68e-04              |
| GO:BP       | steroid metabolic process                                       | 2.50            | 24             | 4.19e-03              |

| GO Category               | GO:Term  | Fold Enrichment | Number of DEGs | P-value<br>(Adjusted) |
|---------------------------|--|-----------------|----------------|-----------------------|
| GO:BP                     | sterol biosynthetic process                      | 6.99            | 17             | 1.44e-07              |
| GO:BP                     | sterol metabolic process                         | 3.60            | 19             | 3.27e-04              |
| GO:BP                     | sulfur compound biosynthetic process             | 2.75            | 18             | 1.13e-02              |
| GO:BP                     | sulfur compound metabolic process                | 1.95            | 30             | 2.66e-02              |
| GO:BP                     | tissue morphogenesis                             | 2.10            | 24             | 3.43e-02              |
| GO:BP                     | transport  | 1.41            | 283            | 9.39e-08              |
| GO:BP                     | ubiquitin-dependent protein catabolic process    | 1.89            | 49             | 1.89e-03              |
| GO:BP                     | vacuolar acidification                           | 6.16            | 10             | 1.18e-03              |
| GO:BP                     | vacuolar transport                               | 2.47            | 23             | 7.10e-03              |
| GO:BP                     | vacuole organization                             | 2.24            | 20             | 4.33e-02              |
| GO:BP                     | vesicle organization                             | 1.89            | 29             | 4.52e-02              |
| GO:BP                     | vesicle-mediated transport                       | 1.66            | 119            | 8.14e-06              |
| <b>Cellular Component</b> |  |                 |                |                       |
| GO:CC                     | ATPase complex                                   | 2.46            | 19             | 1.18e-02              |
| GO:CC                     | ATPase dependent transmembrane transport complex | 3.96            | 11             | 6.39e-03              |
| GO:CC                     | Golgi apparatus                                  | 1.68            | 105            | 4.83e-06              |
| GO:CC                     | Golgi apparatus subcompartment                   | 1.83            | 28             | 4.19e-02              |
| GO:CC                     | Golgi cisterna                                   | 3.11            | 12             | 2.23e-02              |
| GO:CC                     | Golgi membrane                                   | 2.28            | 45             | 1.13e-05              |
| GO:CC                     | bounding membrane of organelle                   | 1.89            | 141            | 1.37e-11              |

| GO Category | GO:Term                                  | Fold Enrichment | Number of DEGs | P-value<br>(Adjusted) |
|-------------|--|-----------------|----------------|-----------------------|
| GO:CC       | catalytic complex                        | 1.30            | 130            | 2.23e-02              |
| GO:CC       | cation-transporting ATPase complex       | 4.14            | 11             | 4.36e-03              |
| GO:CC       | clathrin-coated vesicle                  | 2.72            | 16             | 1.23e-02              |
| GO:CC       | coated vesicle                           | 2.38            | 26             | 1.90e-03              |
| GO:CC       | cytoplasm                                | 1.30            | 827            | 6.20e-27              |
| GO:CC       | cytoplasmic vesicle                      | 1.65            | 128            | 5.29e-07              |
| GO:CC       | cytoplasmic vesicle membrane             | 1.85            | 62             | 9.07e-05              |
| GO:CC       | cytosol                                  | 1.58            | 214            | 4.32e-10              |
| GO:CC       | early endosome                           | 1.94            | 28             | 2.07e-02              |
| GO:CC       | endocytic vesicle                        | 2.26            | 18             | 3.61e-02              |
| GO:CC       | endomembrane system                      | 1.68            | 315            | 1.99e-20              |
| GO:CC       | endoplasmic reticulum                    | 1.88            | 145            | 8.54e-12              |
| GO:CC       | endoplasmic reticulum membrane           | 1.98            | 84             | 1.17e-07              |
| GO:CC       | endoplasmic reticulum subcompartment     | 1.98            | 85             | 9.98e-08              |
| GO:CC       | endosome                                 | 1.81            | 71             | 3.79e-05              |
| GO:CC       | endosome membrane                        | 2.16            | 37             | 4.17e-04              |
| GO:CC       | intracellular anatomical structure       | 1.16            | 1,043          | 5.44e-20              |
| GO:CC       | intracellular membrane-bounded organelle | 1.30            | 805            | 3.83e-25              |
| GO:CC       | intracellular organelle                  | 1.19            | 881            | 2.20e-16              |
| GO:CC       | intracellular organelle lumen            | 1.53            | 190            | 5.31e-08              |

| GO Category | GO:Term   | Fold Enrichment | Number of DEGs | P-value<br>(Adjusted) |
|-------------|---|-----------------|----------------|-----------------------|
| GO:CC       | intracellular protein-containing complex                      | 1.47            | 79             | 1.13e-02              |
| GO:CC       | intracellular vesicle   | 1.63            | 128            | 1.01e-06              |
| GO:CC       | lysosomal membrane  | 3.03            | 32             | 1.47e-06              |
| GO:CC       | lysosome  | 2.62            | 52             | 2.58e-08              |
| GO:CC       | lytic vacuole   | 2.59            | 52             | 3.79e-08              |
| GO:CC       | lytic vacuole membrane  | 3.03            | 32             | 1.47e-06              |
| GO:CC       | membrane  | 1.12            | 612            | 2.43e-03              |
| GO:CC       | membrane microdomain  | 2.66            | 16             | 1.48e-02              |
| GO:CC       | membrane raft   | 2.69            | 16             | 1.35e-02              |
| GO:CC       | membrane-bounded organelle                                    | 1.26            | 822            | 4.43e-22              |
| GO:CC       | membrane-enclosed lumen                                       | 1.53            | 190            | 5.31e-08              |
| GO:CC       | nuclear body  | 1.81            | 33             | 2.23e-02              |
| GO:CC       | nuclear lumen   | 1.55            | 164            | 4.35e-07              |
| GO:CC       | nuclear outer membrane-endoplasmic reticulum membrane network | 1.94            | 84             | 2.90e-07              |
| GO:CC       | nucleolus   | 1.62            | 46             | 2.25e-02              |
| GO:CC       | nucleoplasm   | 1.67            | 137            | 9.98e-08              |
| GO:CC       | nucleus   | 1.24            | 447            | 1.08e-06              |
| GO:CC       | organelle   | 1.17            | 893            | 9.33e-14              |
| GO:CC       | organelle lumen   | 1.53            | 190            | 5.31e-08              |
| GO:CC       | organelle membrane  | 1.82            | 244            | 1.57e-19              |

| GO Category               | GO:Term  | Fold Enrichment | Number of DEGs | P-value<br>(Adjusted) |
|---------------------------|--|-----------------|----------------|-----------------------|
| GO:CC                     | organelle subcompartment                           | 1.93            | 111            | 1.94e-09              |
| GO:CC                     | perinuclear region of cytoplasm                    | 2.47            | 34             | 6.02e-05              |
| GO:CC                     | protein-containing complex                         | 1.13            | 366            | 4.13e-02              |
| GO:CC                     | proton-transporting V-type ATPase complex          | 4.52            | 8              | 2.23e-02              |
| GO:CC                     | vacuolar membrane                                  | 2.73            | 39             | 9.21e-07              |
| GO:CC                     | vacuolar proton-transporting V-type ATPase complex | 6.33            | 8              | 2.95e-03              |
| GO:CC                     | vacuole  | 2.48            | 64             | 2.52e-09              |
| GO:CC                     | vesicle  | 1.56            | 136            | 3.62e-06              |
| GO:CC                     | vesicle membrane                                   | 1.94            | 66             | 9.55e-06              |
| <b>Molecular Function</b> |  |                 |                |                       |
| GO:MF                     | acyltransferase activity                           | 1.63            | 72             | 7.45e-03              |
| GO:MF                     | binding  | 1.07            | 924            | 1.02e-02              |
| GO:MF                     | catalytic activity                                 | 1.16            | 522            | 1.47e-03              |
| GO:MF                     | catalytic activity, acting on a protein            | 1.28            | 230            | 4.88e-03              |
| GO:MF                     | enzyme binding                                     | 1.95            | 120            | 2.10e-09              |
| GO:MF                     | identical protein binding                          | 2.17            | 75             | 2.02e-07              |
| GO:MF                     | kinase binding                                     | 2.08            | 45             | 1.47e-03              |
| GO:MF                     | lipid binding                                      | 1.57            | 62             | 4.54e-02              |
| GO:MF                     | manganese ion binding                              | 4.71            | 10             | 2.12e-02              |
| GO:MF                     | misfolded protein binding                          | 7.40            | 10             | 9.76e-04              |

| GO Category | GO:Term                               | Fold Enrichment | Number of DEGs | P-value<br>(Adjusted) |
|-------------|---------------------------------------|-----------------|----------------|-----------------------|
| GO:MF       | protein binding                       | 1.24            | 551            | 4.61e-08              |
| GO:MF       | protein domain specific binding       | 2.12            | 28             | 3.51e-02              |
| GO:MF       | protein kinase binding                | 2.04            | 40             | 4.88e-03              |
| GO:MF       | steroid binding                       | 3.20            | 14             | 3.61e-02              |
| GO:MF       | transferase activity                  | 1.30            | 233            | 2.67e-03              |
| GO:MF       | ubiquitin-like protein ligase binding | 2.50            | 19             | 4.88e-02              |

Table 3B: Gene ontology analysis of Significantly Downregulated DEGs identified at 24-hpi

| GO Category               | GO:Term   | Fold Enrichment | Number of DEGs | P-value<br>(Adjusted) |
|---------------------------|---|-----------------|----------------|-----------------------|
| <b>Biological Process</b> |   |                 |                |                       |
| GO:BP                     | 'de novo' IMP biosynthetic process                    | 8.46            | 5              | 3.63e-02              |
| GO:BP                     | 'de novo' XMP biosynthetic process                    | 13.53           | 4              | 3.42e-02              |
| GO:BP                     | 2'-deoxyribonucleotide biosynthetic process           | 7.89            | 7              | 3.48e-03              |
| GO:BP                     | 2'-deoxyribonucleotide metabolic process              | 5.26            | 7              | 3.23e-02              |
| GO:BP                     | ADP catabolic process                                 | 3.54            | 11             | 2.13e-02              |
| GO:BP                     | ADP metabolic process                                 | 3.46            | 11             | 2.38e-02              |
| GO:BP                     | ATP biosynthetic process                              | 5.95            | 11             | 2.49e-04              |
| GO:BP                     | ATP metabolic process                                 | 3.84            | 23             | 4.27e-06              |
| GO:BP                     | ATP synthesis coupled electron transport              | 6.26            | 25             | 1.40e-11              |
| GO:BP                     | DNA damage response                                   | 2.04            | 81             | 1.12e-07              |
| GO:BP                     | DNA integrity checkpoint signaling                    | 2.95            | 17             | 5.00e-03              |
| GO:BP                     | DNA metabolic process                                 | 2.27            | 92             | 2.37e-11              |
| GO:BP                     | DNA recombination                                     | 2.13            | 32             | 3.19e-03              |
| GO:BP                     | DNA repair  | 2.28            | 66             | 5.27e-08              |
| GO:BP                     | DNA replication                                       | 3.29            | 35             | 9.38e-08              |
| GO:BP                     | DNA-templated DNA replication                         | 3.44            | 29             | 8.52e-07              |
| GO:BP                     | DNA-templated DNA replication maintenance of fidelity | 3.69            | 9              | 4.84e-02              |
| GO:BP                     | GMP biosynthetic process                              | 6.76            | 6              | 2.73e-02              |

| GO Category | GO:Term  | Fold Enrichment | Number of DEGs | P-value<br>(Adjusted) |
|-------------|--|-----------------|----------------|-----------------------|
| GO:BP       | L-amino acid biosynthetic process  | 3.09            | 13             | 2.14e-02              |
| GO:BP       | L-amino acid metabolic process   | 2.46            | 28             | 9.12e-04              |
| GO:BP       | RNA biosynthetic process   | 1.71            | 122            | 4.31e-07              |
| GO:BP       | RNA metabolic process  | 1.89            | 156            | 1.03e-12              |
| GO:BP       | RNA processing   | 1.85            | 99             | 2.56e-07              |
| GO:BP       | RNA splicing   | 2.25            | 40             | 1.31e-04              |
| GO:BP       | RNA splicing, via transesterification reactions                                      | 2.42            | 34             | 1.68e-04              |
| GO:BP       | RNA splicing, via transesterification reactions with bulged adenosine as nucleophile | 2.42            | 34             | 1.68e-04              |
| GO:BP       | XMP biosynthetic process   | 13.53           | 4              | 3.42e-02              |
| GO:BP       | XMP metabolic process  | 13.53           | 4              | 3.42e-02              |
| GO:BP       | aerobic electron transport chain   | 6.35            | 23             | 9.80e-11              |
| GO:BP       | aerobic respiration  | 5.33            | 41             | 7.30e-17              |
| GO:BP       | alpha-amino acid biosynthetic process  | 3.05            | 14             | 1.48e-02              |
| GO:BP       | alpha-amino acid metabolic process   | 2.16            | 29             | 5.33e-03              |
| GO:BP       | amino acid activation  | 4.80            | 22             | 1.38e-07              |
| GO:BP       | amino acid metabolic process   | 2.61            | 52             | 4.48e-08              |
| GO:BP       | biosynthetic process   | 1.78            | 404            | 6.23e-33              |
| GO:BP       | carbohydrate derivative metabolic process  | 1.42            | 89             | 1.91e-02              |
| GO:BP       | carboxylic acid metabolic process  | 1.76            | 78             | 6.39e-05              |
| GO:BP       | catabolic process  | 1.30            | 138            | 2.31e-02              |

| GO Category | GO:Term  | Fold Enrichment | Number of DEGs | P-value<br>(Adjusted) |
|-------------|--|-----------------|----------------|-----------------------|
| GO:BP       | cell cycle                                     | 2.28            | 145            | 5.63e-19              |
| GO:BP       | cell cycle DNA replication                     | 4.68            | 9              | 1.22e-02              |
| GO:BP       | cell cycle checkpoint signaling                | 3.05            | 23             | 1.96e-04              |
| GO:BP       | cell cycle phase transition                    | 2.83            | 14             | 2.68e-02              |
| GO:BP       | cell cycle process                             | 2.35            | 121            | 1.51e-16              |
| GO:BP       | cell division                                  | 2.28            | 40             | 9.83e-05              |
| GO:BP       | cellular biosynthetic process                  | 1.83            | 356            | 7.55e-31              |
| GO:BP       | cellular component assembly                    | 1.51            | 179            | 8.36e-07              |
| GO:BP       | cellular component biogenesis                  | 1.61            | 217            | 5.62e-11              |
| GO:BP       | cellular component disassembly                 | 2.06            | 26             | 2.14e-02              |
| GO:BP       | cellular component organization                | 1.20            | 381            | 8.36e-04              |
| GO:BP       | cellular component organization or biogenesis  | 1.25            | 417            | 3.54e-06              |
| GO:BP       | cellular metabolic process                     | 1.55            | 571            | 2.12e-32              |
| GO:BP       | cellular modified amino acid metabolic process | 2.19            | 21             | 3.21e-02              |
| GO:BP       | cellular process                               | 1.10            | 1,121          | 1.42e-10              |
| GO:BP       | cellular respiration                           | 4.93            | 43             | 2.46e-16              |
| GO:BP       | cellular response to stress                    | 1.74            | 118            | 2.81e-07              |
| GO:BP       | centromere complex assembly                    | 4.92            | 8              | 2.09e-02              |
| GO:BP       | chromatin organization                         | 2.10            | 51             | 4.41e-05              |
| GO:BP       | chromatin remodeling                           | 2.07            | 37             | 1.70e-03              |

| GO Category | GO:Term   | Fold Enrichment | Number of DEGs | P-value<br>(Adjusted) |
|-------------|---|-----------------|----------------|-----------------------|
| GO:BP       | chromosome organization                                 | 2.47            | 59             | 2.36e-08              |
| GO:BP       | chromosome segregation                                  | 2.49            | 47             | 1.02e-06              |
| GO:BP       | cytoplasmic translation                                 | 8.27            | 33             | 2.36e-20              |
| GO:BP       | cytoplasmic translational initiation                    | 7.38            | 12             | 7.93e-06              |
| GO:BP       | deoxyribonucleotide biosynthetic process                | 6.37            | 8              | 3.90e-03              |
| GO:BP       | deoxyribonucleotide metabolic process                   | 4.51            | 8              | 3.17e-02              |
| GO:BP       | deoxyribose phosphate biosynthetic process              | 7.89            | 7              | 3.48e-03              |
| GO:BP       | deoxyribose phosphate metabolic process                 | 4.98            | 7              | 4.10e-02              |
| GO:BP       | double-strand break repair                              | 2.15            | 31             | 3.48e-03              |
| GO:BP       | double-strand break repair via homologous recombination | 2.29            | 20             | 2.53e-02              |
| GO:BP       | electron transport chain                                | 5.64            | 30             | 1.03e-12              |
| GO:BP       | energy derivation by oxidation of organic compounds     | 3.40            | 46             | 5.23e-11              |
| GO:BP       | fatty acid beta-oxidation                               | 3.57            | 14             | 3.37e-03              |
| GO:BP       | fatty acid oxidation                                    | 3.27            | 14             | 7.85e-03              |
| GO:BP       | formation of cytoplasmic translation initiation complex | 9.84            | 8              | 1.51e-04              |
| GO:BP       | formation of translation preinitiation complex          | 9.66            | 5              | 2.30e-02              |
| GO:BP       | gene expression   | 2.26            | 288            | 1.04e-39              |
| GO:BP       | generation of precursor metabolites and energy          | 3.07            | 60             | 1.49e-12              |
| GO:BP       | glycolytic process                                      | 3.63            | 11             | 1.81e-02              |
| GO:BP       | import into the mitochondrion                           | 3.14            | 13             | 1.89e-02              |

| GO Category | GO:Term   | Fold Enrichment | Number of DEGs | P-value<br>(Adjusted) |
|-------------|---|-----------------|----------------|-----------------------|
| GO:BP       | lipid oxidation   | 3.21            | 14             | 9.33e-03              |
| GO:BP       | mRNA metabolic process                                    | 1.95            | 56             | 1.28e-04              |
| GO:BP       | mRNA processing   | 2.14            | 45             | 1.28e-04              |
| GO:BP       | mRNA splicing, via spliceosome                            | 2.42            | 34             | 1.68e-04              |
| GO:BP       | macromolecule biosynthetic process                        | 2.03            | 319            | 5.47e-35              |
| GO:BP       | macromolecule catabolic process                           | 1.43            | 72             | 4.46e-02              |
| GO:BP       | macromolecule metabolic process                           | 1.54            | 540            | 3.53e-29              |
| GO:BP       | maturation of LSU-rRNA                                    | 3.76            | 10             | 2.42e-02              |
| GO:BP       | meiosis I cell cycle process                              | 2.43            | 16             | 4.50e-02              |
| GO:BP       | meiotic cell cycle  | 2.42            | 29             | 8.58e-04              |
| GO:BP       | meiotic cell cycle process                                | 2.71            | 28             | 1.68e-04              |
| GO:BP       | meiotic nuclear division                                  | 2.71            | 24             | 8.57e-04              |
| GO:BP       | metabolic process   | 1.43            | 761            | 3.14e-36              |
| GO:BP       | microtubule cytoskeleton organization involved in mitosis | 2.67            | 28             | 2.13e-04              |
| GO:BP       | microtubule-based process                                 | 1.44            | 85             | 1.63e-02              |
| GO:BP       | mitochondrial ATP synthesis coupled electron transport    | 6.37            | 24             | 2.94e-11              |
| GO:BP       | mitochondrial electron transport, NADH to ubiquinone      | 5.41            | 10             | 1.70e-03              |
| GO:BP       | mitochondrial electron transport, succinate to ubiquinone | 13.53           | 4              | 3.42e-02              |
| GO:BP       | mitochondrial gene expression                             | 3.07            | 17             | 3.37e-03              |
| GO:BP       | mitochondrial translation                                 | 3.64            | 14             | 2.78e-03              |

| GO Category | GO:Term  | Fold Enrichment | Number of DEGs | P-value<br>(Adjusted) |
|-------------|--|-----------------|----------------|-----------------------|
| GO:BP       | mitochondrial transmembrane transport              | 2.89            | 19             | 2.75e-03              |
| GO:BP       | mitochondrial transport                            | 2.68            | 23             | 1.42e-03              |
| GO:BP       | mitochondrion organization                         | 1.81            | 38             | 1.58e-02              |
| GO:BP       | mitotic DNA integrity checkpoint signaling         | 3.06            | 12             | 3.38e-02              |
| GO:BP       | mitotic cell cycle                                 | 2.64            | 95             | 5.25e-16              |
| GO:BP       | mitotic cell cycle checkpoint signaling            | 3.03            | 17             | 3.78e-03              |
| GO:BP       | mitotic cell cycle phase transition                | 2.87            | 14             | 2.38e-02              |
| GO:BP       | mitotic cell cycle process                         | 2.68            | 76             | 9.49e-13              |
| GO:BP       | mitotic nuclear division                           | 2.82            | 20             | 2.41e-03              |
| GO:BP       | mitotic sister chromatid segregation               | 2.92            | 19             | 2.39e-03              |
| GO:BP       | mitotic spindle organization                       | 2.76            | 22             | 1.43e-03              |
| GO:BP       | negative regulation of cell cycle                  | 2.03            | 30             | 1.10e-02              |
| GO:BP       | negative regulation of cell cycle phase transition | 2.30            | 25             | 6.05e-03              |
| GO:BP       | negative regulation of cell cycle process          | 2.15            | 27             | 9.53e-03              |
| GO:BP       | non-membrane-bounded organelle assembly            | 2.40            | 49             | 1.65e-06              |
| GO:BP       | nuclear DNA replication                            | 4.68            | 9              | 1.22e-02              |
| GO:BP       | nuclear chromosome segregation                     | 2.47            | 32             | 2.06e-04              |
| GO:BP       | nuclear division                                   | 2.71            | 42             | 6.47e-07              |
| GO:BP       | nucleic acid biosynthetic process                  | 1.75            | 131            | 2.42e-08              |
| GO:BP       | nucleic acid metabolic process                     | 2.02            | 241            | 3.92e-25              |

| GO Category | GO:Term  | Fold Enrichment | Number of DEGs | P-value<br>(Adjusted) |
|-------------|--|-----------------|----------------|-----------------------|
| GO:BP       | nucleobase-containing compound biosynthetic process    | 1.80            | 168            | 6.72e-12              |
| GO:BP       | nucleobase-containing compound catabolic process       | 1.84            | 32             | 2.83e-02              |
| GO:BP       | nucleobase-containing compound metabolic process       | 1.99            | 308            | 3.62e-32              |
| GO:BP       | nucleobase-containing small molecule metabolic process | 1.91            | 67             | 2.86e-05              |
| GO:BP       | nucleoside diphosphate metabolic process               | 2.91            | 14             | 2.17e-02              |
| GO:BP       | nucleoside monophosphate biosynthetic process          | 3.25            | 12             | 2.30e-02              |
| GO:BP       | nucleoside monophosphate metabolic process             | 2.90            | 12             | 4.90e-02              |
| GO:BP       | nucleoside phosphate biosynthetic process              | 2.09            | 35             | 2.32e-03              |
| GO:BP       | nucleoside phosphate metabolic process                 | 1.96            | 61             | 3.63e-05              |
| GO:BP       | nucleoside triphosphate biosynthetic process           | 4.35            | 18             | 2.11e-05              |
| GO:BP       | nucleoside triphosphate metabolic process              | 3.36            | 31             | 5.03e-07              |
| GO:BP       | nucleotide biosynthetic process                        | 2.05            | 34             | 3.69e-03              |
| GO:BP       | nucleotide metabolic process                           | 2.00            | 59             | 3.40e-05              |
| GO:BP       | organelle assembly                                     | 1.63            | 79             | 8.36e-04              |
| GO:BP       | organelle fission                                      | 2.44            | 42             | 1.11e-05              |
| GO:BP       | organelle organization                                 | 1.38            | 265            | 8.36e-07              |
| GO:BP       | organic acid metabolic process                         | 1.70            | 80             | 1.79e-04              |
| GO:BP       | organonitrogen compound biosynthetic process           | 2.54            | 213            | 1.45e-35              |
| GO:BP       | organonitrogen compound metabolic process              | 1.46            | 436            | 4.46e-17              |
| GO:BP       | oxidative phosphorylation                              | 6.34            | 30             | 2.57e-14              |

| GO Category | GO:Term  | Fold Enrichment | Number of DEGs | P-value<br>(Adjusted) |
|-------------|--|-----------------|----------------|-----------------------|
| GO:BP       | oxoacid metabolic process  | 1.75            | 79             | 6.92e-05              |
| GO:BP       | peptidyl-amino acid modification                                 | 1.78            | 34             | 3.42e-02              |
| GO:BP       | peptidyl-proline modification                                    | 4.51            | 9              | 1.58e-02              |
| GO:BP       | positive regulation of apoptotic process                         | 2.00            | 25             | 3.42e-02              |
| GO:BP       | positive regulation of signal transduction by p53 class mediator | 9.66            | 5              | 2.30e-02              |
| GO:BP       | primary metabolic process  | 1.45            | 670            | 5.01e-32              |
| GO:BP       | protein metabolic process  | 1.41            | 327            | 6.60e-10              |
| GO:BP       | protein peptidyl-prolyl isomerization                            | 6.37            | 8              | 3.90e-03              |
| GO:BP       | protein-DNA complex assembly                                     | 3.28            | 23             | 6.53e-05              |
| GO:BP       | protein-DNA complex organization                                 | 2.23            | 62             | 3.95e-07              |
| GO:BP       | protein-RNA complex assembly                                     | 4.26            | 40             | 1.03e-12              |
| GO:BP       | protein-RNA complex organization                                 | 4.20            | 41             | 8.95e-13              |
| GO:BP       | protein-containing complex assembly                              | 2.07            | 106            | 1.19e-10              |
| GO:BP       | protein-containing complex organization                          | 1.97            | 153            | 5.98e-14              |
| GO:BP       | proteinogenic amino acid biosynthetic process                    | 3.09            | 13             | 2.14e-02              |
| GO:BP       | proteinogenic amino acid metabolic process                       | 2.46            | 26             | 1.73e-03              |
| GO:BP       | proton motive force-driven ATP synthesis                         | 6.20            | 11             | 1.68e-04              |
| GO:BP       | purine nucleoside diphosphate catabolic process                  | 3.46            | 11             | 2.38e-02              |
| GO:BP       | purine nucleoside diphosphate metabolic process                  | 3.61            | 12             | 1.02e-02              |
| GO:BP       | purine nucleoside triphosphate biosynthetic process              | 4.29            | 13             | 1.08e-03              |

| GO Category | GO:Term   | Fold Enrichment | Number of DEGs | P-value<br>(Adjusted) |
|-------------|---|-----------------|----------------|-----------------------|
| GO:BP       | purine nucleoside triphosphate metabolic process        | 3.25            | 25             | 2.63e-05              |
| GO:BP       | purine nucleotide biosynthetic process                  | 1.95            | 26             | 3.69e-02              |
| GO:BP       | purine nucleotide metabolic process                     | 1.90            | 48             | 1.11e-03              |
| GO:BP       | purine ribonucleoside diphosphate catabolic process     | 3.46            | 11             | 2.38e-02              |
| GO:BP       | purine ribonucleoside diphosphate metabolic process     | 3.38            | 11             | 2.75e-02              |
| GO:BP       | purine ribonucleoside triphosphate biosynthetic process | 4.06            | 12             | 3.66e-03              |
| GO:BP       | purine ribonucleoside triphosphate metabolic process    | 3.28            | 24             | 3.71e-05              |
| GO:BP       | purine ribonucleotide biosynthetic process              | 2.09            | 23             | 3.25e-02              |
| GO:BP       | purine ribonucleotide metabolic process                 | 2.03            | 41             | 1.00e-03              |
| GO:BP       | purine-containing compound biosynthetic process         | 1.93            | 27             | 3.50e-02              |
| GO:BP       | purine-containing compound metabolic process            | 1.86            | 50             | 1.20e-03              |
| GO:BP       | pyridine nucleotide catabolic process                   | 3.46            | 11             | 2.38e-02              |
| GO:BP       | pyridine-containing compound catabolic process          | 3.38            | 11             | 2.75e-02              |
| GO:BP       | pyruvate metabolic process                              | 2.87            | 14             | 2.38e-02              |
| GO:BP       | rRNA metabolic process                                  | 2.48            | 38             | 2.68e-05              |
| GO:BP       | rRNA processing   | 2.56            | 36             | 2.63e-05              |
| GO:BP       | recombinational repair                                  | 2.24            | 20             | 3.24e-02              |
| GO:BP       | regulation of DNA metabolic process                     | 2.01            | 28             | 1.89e-02              |
| GO:BP       | regulation of DNA replication                           | 3.74            | 13             | 3.86e-03              |
| GO:BP       | regulation of G2/M transition of mitotic cell cycle     | 3.44            | 15             | 2.78e-03              |

| GO Category | GO:Term   | Fold Enrichment | Number of DEGs | P-value<br>(Adjusted) |
|-------------|---|-----------------|----------------|-----------------------|
| GO:BP       | regulation of apoptotic process                           | 1.53            | 60             | 2.68e-02              |
| GO:BP       | regulation of apoptotic signaling pathway                 | 2.11            | 25             | 1.93e-02              |
| GO:BP       | regulation of cell cycle                                  | 2.05            | 78             | 2.06e-07              |
| GO:BP       | regulation of cell cycle G2/M phase transition            | 3.18            | 16             | 3.60e-03              |
| GO:BP       | regulation of cell cycle phase transition                 | 2.32            | 39             | 8.76e-05              |
| GO:BP       | regulation of cell cycle process                          | 2.13            | 58             | 5.99e-06              |
| GO:BP       | regulation of cellular response to stress                 | 2.09            | 30             | 6.75e-03              |
| GO:BP       | regulation of chromosome organization                     | 2.35            | 17             | 4.54e-02              |
| GO:BP       | regulation of chromosome segregation                      | 3.10            | 14             | 1.27e-02              |
| GO:BP       | regulation of double-strand break repair                  | 2.90            | 12             | 4.90e-02              |
| GO:BP       | regulation of metaphase/anaphase transition of cell cycle | 3.76            | 10             | 2.42e-02              |
| GO:BP       | regulation of mitotic cell cycle                          | 2.05            | 37             | 1.99e-03              |
| GO:BP       | regulation of mitotic cell cycle phase transition         | 2.28            | 26             | 5.00e-03              |
| GO:BP       | regulation of mitotic metaphase/anaphase transition       | 3.87            | 10             | 2.13e-02              |
| GO:BP       | regulation of mitotic sister chromatid separation         | 3.80            | 9              | 4.04e-02              |
| GO:BP       | regulation of translation                                 | 2.00            | 24             | 4.09e-02              |
| GO:BP       | respiratory electron transport chain                      | 5.74            | 28             | 5.17e-12              |
| GO:BP       | response to stress  | 1.40            | 161            | 3.57e-04              |
| GO:BP       | ribonucleoprotein complex biogenesis                      | 3.17            | 84             | 5.63e-19              |
| GO:BP       | ribonucleoside diphosphate catabolic process              | 3.38            | 11             | 2.75e-02              |

| GO Category | GO:Term  | Fold Enrichment | Number of DEGs | P-value<br>(Adjusted) |
|-------------|--|-----------------|----------------|-----------------------|
| GO:BP       | ribonucleoside diphosphate metabolic process     | 3.25            | 12             | 2.30e-02              |
| GO:BP       | ribonucleoside triphosphate biosynthetic process | 4.03            | 14             | 1.00e-03              |
| GO:BP       | ribonucleoside triphosphate metabolic process    | 3.32            | 26             | 1.07e-05              |
| GO:BP       | ribonucleotide biosynthetic process              | 2.04            | 25             | 2.81e-02              |
| GO:BP       | ribonucleotide metabolic process                 | 2.03            | 44             | 5.34e-04              |
| GO:BP       | ribose phosphate biosynthetic process            | 2.20            | 28             | 5.00e-03              |
| GO:BP       | ribose phosphate metabolic process               | 2.16            | 48             | 4.64e-05              |
| GO:BP       | ribosomal large subunit biogenesis               | 3.74            | 21             | 2.61e-05              |
| GO:BP       | ribosomal small subunit assembly                 | 6.31            | 7              | 1.33e-02              |
| GO:BP       | ribosomal small subunit biogenesis               | 3.69            | 21             | 3.07e-05              |
| GO:BP       | ribosome assembly                                | 5.71            | 19             | 9.60e-08              |
| GO:BP       | ribosome biogenesis                              | 2.90            | 58             | 5.23e-11              |
| GO:BP       | sexual reproduction                              | 1.74            | 51             | 4.56e-03              |
| GO:BP       | signal transduction in response to DNA damage    | 2.54            | 15             | 4.38e-02              |
| GO:BP       | sister chromatid segregation                     | 2.91            | 20             | 1.62e-03              |
| GO:BP       | small molecule metabolic process                 | 1.54            | 147            | 7.02e-06              |
| GO:BP       | spindle organization                             | 2.62            | 32             | 6.68e-05              |
| GO:BP       | tRNA aminoacylation                              | 5.04            | 22             | 5.27e-08              |
| GO:BP       | tRNA aminoacylation for protein translation      | 4.92            | 20             | 5.03e-07              |
| GO:BP       | tRNA metabolic process                           | 2.28            | 34             | 5.83e-04              |

| GO Category               | GO:Term                                       | Fold Enrichment | Number of DEGs | P-value<br>(Adjusted) |
|---------------------------|---|-----------------|----------------|-----------------------|
| GO:BP                     | tetrahydrofolate metabolic process            | 6.24            | 6              | 3.75e-02              |
| GO:BP                     | translation                                   | 5.77            | 136            | 3.59e-65              |
| GO:BP                     | translational elongation                      | 4.77            | 12             | 8.57e-04              |
| GO:BP                     | translational initiation                      | 5.34            | 15             | 1.59e-05              |
| GO:BP                     | tricarboxylic acid cycle                      | 5.34            | 15             | 1.59e-05              |
| <b>Cellular Component</b> |   |                 |                |                       |
| GO:CC                     | Arp2/3 protein complex                        | 6.92            | 6              | 1.02e-02              |
| GO:CC                     | Sm-like protein family complex                | 3.21            | 16             | 1.16e-03              |
| GO:CC                     | U1 snRNP                                      | 5.10            | 7              | 1.67e-02              |
| GO:CC                     | U12-type spliceosomal complex                 | 4.62            | 7              | 2.88e-02              |
| GO:CC                     | U2 snRNP                                      | 5.54            | 8              | 3.72e-03              |
| GO:CC                     | U2-type spliceosomal complex                  | 3.64            | 15             | 5.33e-04              |
| GO:CC                     | U4 snRNP                                      | 10.39           | 6              | 1.12e-03              |
| GO:CC                     | U5 snRNP                                      | 7.55            | 6              | 6.68e-03              |
| GO:CC                     | aminoacyl-tRNA synthetase multienzyme complex | 8.08            | 7              | 1.12e-03              |
| GO:CC                     | catalytic complex                             | 1.64            | 188            | 1.31e-10              |
| GO:CC                     | catalytic step 2 spliceosome                  | 3.02            | 17             | 1.37e-03              |
| GO:CC                     | centrosome                                    | 1.78            | 55             | 5.33e-04              |
| GO:CC                     | chromatin                                     | 1.69            | 58             | 1.18e-03              |
| GO:CC                     | chromosomal region                            | 2.85            | 41             | 6.00e-08              |

| GO Category | GO:Term   | Fold Enrichment | Number of DEGs | P-value<br>(Adjusted) |
|-------------|---|-----------------|----------------|-----------------------|
| GO:CC       | chromosome  | 2.09            | 131            | 2.56e-14              |
| GO:CC       | chromosome, centromeric region                            | 2.94            | 34             | 7.34e-07              |
| GO:CC       | cleavage furrow   | 4.82            | 8              | 9.12e-03              |
| GO:CC       | condensed chromosome                                      | 2.77            | 36             | 1.29e-06              |
| GO:CC       | condensed chromosome, centromeric region                  | 2.70            | 24             | 3.03e-04              |
| GO:CC       | cytochrome complex  | 5.86            | 11             | 1.01e-04              |
| GO:CC       | cytoplasm   | 1.28            | 929            | 7.97e-28              |
| GO:CC       | cytosol   | 1.95            | 302            | 3.27e-30              |
| GO:CC       | cytosolic large ribosomal subunit                         | 8.46            | 44             | 4.02e-29              |
| GO:CC       | cytosolic ribosome  | 8.63            | 76             | 4.32e-52              |
| GO:CC       | cytosolic small ribosomal subunit                         | 9.54            | 31             | 1.85e-22              |
| GO:CC       | eukaryotic 43S preinitiation complex                      | 9.23            | 8              | 9.12e-05              |
| GO:CC       | eukaryotic 48S preinitiation complex                      | 11.08           | 8              | 1.63e-05              |
| GO:CC       | eukaryotic translation initiation factor 3 complex        | 8.90            | 9              | 2.36e-05              |
| GO:CC       | eukaryotic translation initiation factor 3 complex, eIF3m | 11.08           | 4              | 3.24e-02              |
| GO:CC       | inner mitochondrial membrane protein complex              | 3.86            | 34             | 4.75e-10              |
| GO:CC       | intracellular anatomical structure                        | 1.23            | 1,265          | 8.08e-52              |
| GO:CC       | intracellular membrane-bounded organelle                  | 1.28            | 905            | 4.52e-26              |
| GO:CC       | intracellular non-membrane-bounded organelle              | 1.72            | 459            | 4.71e-35              |
| GO:CC       | intracellular organelle                                   | 1.29            | 1,087          | 1.05e-42              |

| GO Category | GO:Term  | Fold Enrichment | Number of DEGs | P-value<br>(Adjusted) |
|-------------|--|-----------------|----------------|-----------------------|
| GO:CC       | intracellular organelle lumen  | 1.94            | 275            | 3.97e-27              |
| GO:CC       | intracellular protein-containing complex                                     | 1.44            | 89             | 4.68e-03              |
| GO:CC       | kinetochore  | 2.89            | 24             | 1.01e-04              |
| GO:CC       | large ribosomal subunit  | 6.31            | 51             | 3.76e-26              |
| GO:CC       | membrane-bounded organelle   | 1.26            | 933            | 9.54e-25              |
| GO:CC       | membrane-enclosed lumen  | 1.94            | 275            | 3.97e-27              |
| GO:CC       | microtubule cytoskeleton   | 1.48            | 111            | 4.41e-04              |
| GO:CC       | microtubule organizing center  | 1.54            | 60             | 1.02e-02              |
| GO:CC       | mitochondrial envelope   | 2.13            | 73             | 3.18e-08              |
| GO:CC       | mitochondrial inner membrane   | 2.94            | 60             | 2.55e-12              |
| GO:CC       | mitochondrial matrix   | 3.48            | 50             | 4.42e-13              |
| GO:CC       | mitochondrial membrane   | 2.17            | 69             | 4.32e-08              |
| GO:CC       | mitochondrial protein-containing complex                                     | 3.58            | 59             | 2.73e-16              |
| GO:CC       | mitochondrial proton-transporting ATP synthase complex                       | 6.43            | 13             | 4.00e-06              |
| GO:CC       | mitochondrial proton-transporting ATP synthase complex, coupling factor F(o) | 5.93            | 6              | 2.21e-02              |
| GO:CC       | mitochondrial respirasome  | 5.66            | 9              | 1.12e-03              |
| GO:CC       | mitochondrial ribosome   | 3.68            | 21             | 9.69e-06              |
| GO:CC       | mitochondrial small ribosomal subunit  | 6.65            | 12             | 8.40e-06              |
| GO:CC       | mitochondrion  | 2.39            | 235            | 6.18e-36              |
| GO:CC       | mitotic spindle  | 2.16            | 17             | 4.58e-02              |

| GO Category | GO:Term                                  | Fold Enrichment | Number of DEGs | P-value<br>(Adjusted) |
|-------------|--|-----------------|----------------|-----------------------|
| GO:CC       | non-membrane-bounded organelle           | 1.72            | 460            | 2.54e-35              |
| GO:CC       | nuclear chromosome                       | 1.95            | 22             | 3.89e-02              |
| GO:CC       | nuclear lumen                            | 1.85            | 224            | 6.28e-19              |
| GO:CC       | nuclear protein-containing complex       | 1.60            | 136            | 7.62e-07              |
| GO:CC       | nucleolus                                | 2.34            | 76             | 1.62e-10              |
| GO:CC       | nucleoplasm                              | 1.80            | 169            | 1.02e-12              |
| GO:CC       | nucleus                                  | 1.36            | 561            | 2.14e-17              |
| GO:CC       | organellar ribosome                      | 3.68            | 21             | 9.69e-06              |
| GO:CC       | organellar small ribosomal subunit       | 6.65            | 12             | 8.40e-06              |
| GO:CC       | organelle                                | 1.26            | 1,100          | 9.05e-39              |
| GO:CC       | organelle envelope                       | 1.93            | 104            | 2.99e-09              |
| GO:CC       | organelle inner membrane                 | 2.73            | 64             | 1.16e-11              |
| GO:CC       | organelle lumen                          | 1.94            | 275            | 3.97e-27              |
| GO:CC       | oxidoreductase complex                   | 4.24            | 19             | 4.39e-06              |
| GO:CC       | pICln-Sm protein complex                 | 9.89            | 5              | 8.41e-03              |
| GO:CC       | preribosome                              | 2.74            | 20             | 1.16e-03              |
| GO:CC       | proteasome core complex                  | 6.23            | 9              | 5.43e-04              |
| GO:CC       | protein-DNA complex                      | 1.72            | 65             | 3.03e-04              |
| GO:CC       | protein-containing complex               | 1.54            | 570            | 5.76e-32              |
| GO:CC       | proton-transporting ATP synthase complex | 6.21            | 13             | 5.94e-06              |

| GO Category               | GO:Term  | Fold Enrichment | Number of DEGs | P-value<br>(Adjusted) |
|---------------------------|--|-----------------|----------------|-----------------------|
| GO:CC                     | proton-transporting ATP synthase complex, coupling factor F(o) | 5.19            | 6              | 3.99e-02              |
| GO:CC                     | proton-transporting two-sector ATPase complex                  | 3.58            | 15             | 6.27e-04              |
| GO:CC                     | replication fork   | 3.53            | 13             | 2.44e-03              |
| GO:CC                     | respirasome  | 5.25            | 11             | 3.03e-04              |
| GO:CC                     | respiratory chain complex                                      | 5.86            | 11             | 1.01e-04              |
| GO:CC                     | respiratory chain complex IV                                   | 5.70            | 7              | 9.06e-03              |
| GO:CC                     | ribonucleoprotein complex                                      | 3.78            | 177            | 5.05e-54              |
| GO:CC                     | ribosomal subunit  | 7.04            | 93             | 1.92e-53              |
| GO:CC                     | ribosome   | 6.68            | 108            | 4.42e-59              |
| GO:CC                     | small nuclear ribonucleoprotein complex                        | 3.64            | 15             | 5.33e-04              |
| GO:CC                     | small ribosomal subunit  | 8.11            | 41             | 3.74e-26              |
| GO:CC                     | small-subunit processome                                       | 2.77            | 12             | 3.26e-02              |
| GO:CC                     | spindle  | 2.33            | 45             | 4.67e-06              |
| GO:CC                     | spliceosomal complex   | 2.45            | 37             | 1.52e-05              |
| GO:CC                     | spliceosomal snRNP complex                                     | 3.59            | 14             | 1.12e-03              |
| GO:CC                     | spliceosomal tri-snRNP complex                                 | 4.47            | 10             | 2.72e-03              |
| GO:CC                     | translation preinitiation complex                              | 9.59            | 9              | 1.15e-05              |
| GO:CC                     | tricarboxylic acid cycle heteromeric enzyme complex            | 6.92            | 5              | 3.76e-02              |
| <b>Molecular Function</b> |  |                 |                |                       |
| GO:MF                     | ATP-dependent activity, acting on DNA                          | 2.32            | 23             | 1.60e-02              |

| GO Category | GO:Term                                      | Fold Enrichment | Number of DEGs | P-value<br>(Adjusted) |
|-------------|--|-----------------|----------------|-----------------------|
| GO:MF       | NAD binding                                  | 3.78            | 17             | 5.51e-04              |
| GO:MF       | NAD+ binding                                 | 6.22            | 7              | 2.33e-02              |
| GO:MF       | RNA binding                                  | 2.37            | 182            | 6.82e-26              |
| GO:MF       | aminoacyl-tRNA ligase activity               | 5.08            | 20             | 5.57e-07              |
| GO:MF       | binding                                      | 1.05            | 999            | 2.45e-02              |
| GO:MF       | catalytic activity                           | 1.13            | 553            | 9.04e-03              |
| GO:MF       | catalytic activity, acting on DNA            | 2.07            | 38             | 2.37e-03              |
| GO:MF       | catalytic activity, acting on RNA            | 1.89            | 51             | 1.40e-03              |
| GO:MF       | catalytic activity, acting on a nucleic acid | 1.91            | 87             | 7.53e-07              |
| GO:MF       | catalytic activity, acting on a tRNA         | 3.17            | 29             | 8.40e-06              |
| GO:MF       | electron transfer activity                   | 5.21            | 11             | 1.73e-03              |
| GO:MF       | heterocyclic compound binding                | 1.24            | 247            | 8.80e-03              |
| GO:MF       | identical protein binding                    | 1.77            | 67             | 5.59e-04              |
| GO:MF       | isomerase activity                           | 2.20            | 28             | 9.04e-03              |
| GO:MF       | ligase activity                              | 2.63            | 45             | 6.96e-07              |
| GO:MF       | ligase activity, forming carbon-oxygen bonds | 5.08            | 20             | 5.57e-07              |
| GO:MF       | mRNA binding                                 | 1.99            | 42             | 2.32e-03              |
| GO:MF       | nucleic acid binding                         | 1.49            | 340            | 2.94e-13              |
| GO:MF       | nucleoside phosphate binding                 | 1.25            | 236            | 9.04e-03              |
| GO:MF       | nucleotide binding                           | 1.25            | 236            | 9.04e-03              |

| GO Category | GO:Term   | Fold Enrichment | Number of DEGs | P-value<br>(Adjusted) |
|-------------|---|-----------------|----------------|-----------------------|
| GO:MF       | organic cyclic compound binding   | 1.30            | 544            | 9.27e-12              |
| GO:MF       | oxidoreductase activity   | 1.61            | 96             | 3.56e-04              |
| GO:MF       | oxidoreductase activity, acting on NAD(P)H  | 3.71            | 12             | 1.38e-02              |
| GO:MF       | oxidoreductase activity, acting on NAD(P)H, quinone or similar compound as acceptor   | 5.69            | 8              | 1.42e-02              |
| GO:MF       | oxidoreductase activity, acting on the CH-NH group of donors, NAD or NADP as acceptor | 6.63            | 7              | 1.60e-02              |
| GO:MF       | proton transmembrane transporter activity   | 2.41            | 20             | 2.45e-02              |
| GO:MF       | rRNA binding  | 6.56            | 24             | 3.02e-11              |
| GO:MF       | single-stranded DNA binding   | 2.84            | 17             | 1.33e-02              |
| GO:MF       | structural constituent of ribosome  | 7.30            | 95             | 1.45e-55              |
| GO:MF       | structural molecule activity  | 2.26            | 125            | 1.73e-15              |
| GO:MF       | translation elongation factor activity  | 5.53            | 7              | 4.38e-02              |
| GO:MF       | translation factor activity, RNA binding  | 4.28            | 25             | 2.61e-07              |
| GO:MF       | translation initiation factor activity  | 4.37            | 16             | 1.74e-04              |
| GO:MF       | translation regulator activity  | 3.92            | 32             | 1.15e-08              |
| GO:MF       | translation regulator activity, nucleic acid binding                                  | 3.93            | 26             | 5.88e-07              |

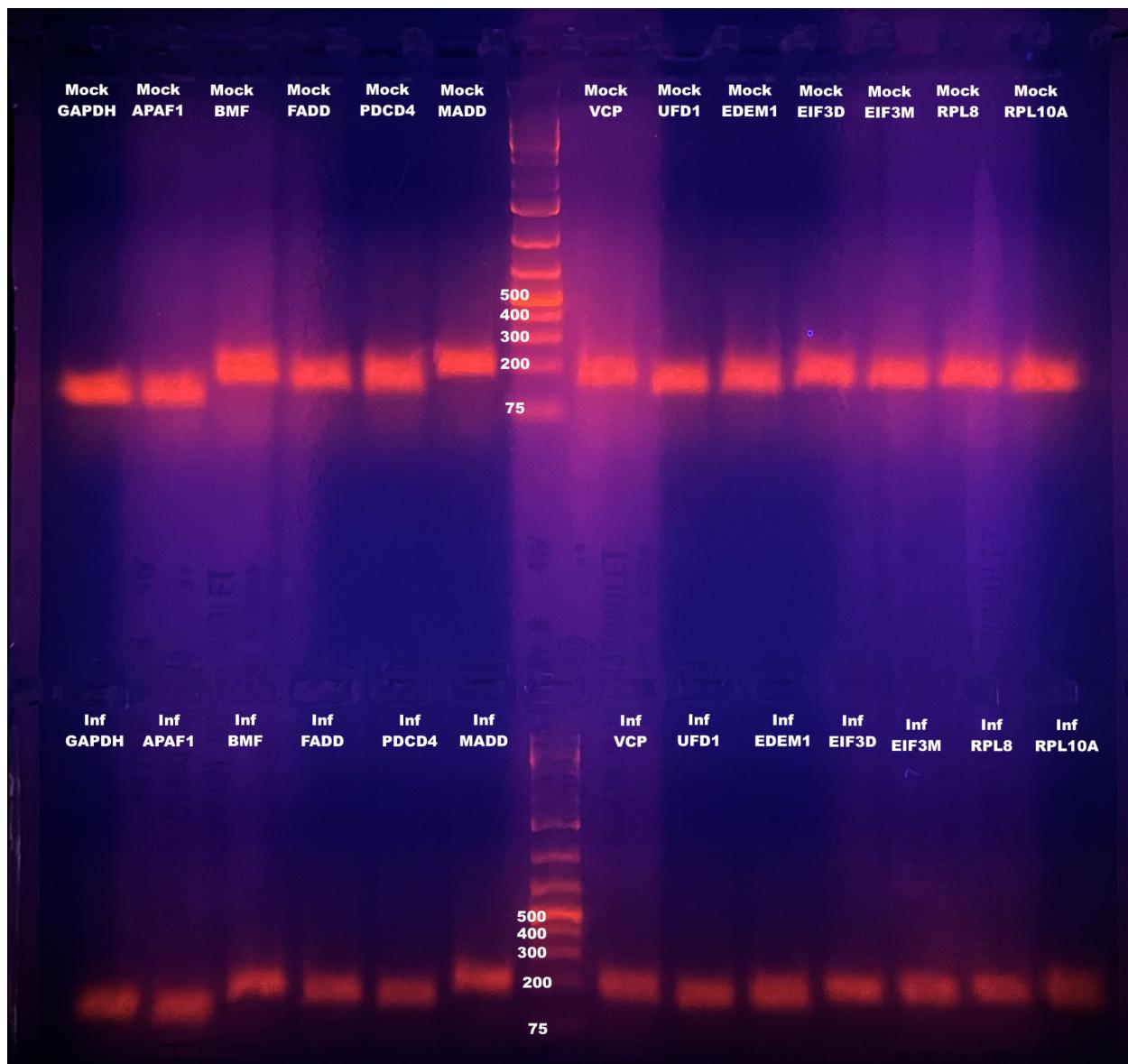
Table 4A: Significantly Enriched KEGG Pathways from DEGs identified at 12 and 24-hpi (Results from the DAVID online resource)

| Time Point | Regulation | KEGG Term                         | DEG Count | Fold Enrichment | P-value<br>(Adjusted) |
|------------|------------|-----------------------------------|-----------|-----------------|-----------------------|
| 12-hpi     | down       | Ribosome                          | 80        | 6.68            | 3.16e-49              |
| 12-hpi     | down       | Oxidative phosphorylation         | 37        | 3.22            | 1.08e-08              |
| 12-hpi     | down       | DNA replication                   | 18        | 6.01            | 1.09e-08              |
| 12-hpi     | down       | Ribosome biogenesis in eukaryotes | 27        | 4.03            | 1.09e-08              |
| 12-hpi     | down       | Spliceosome                       | 30        | 2.50            | 1.25e-04              |
| 12-hpi     | down       | Nucleocytoplasmic transport       | 22        | 2.29            | 1.00e-02              |
| 12-hpi     | down       | Base excision repair              | 13        | 3.10            | 1.13e-02              |
| 12-hpi     | down       | Mismatch repair                   | 9         | 4.29            | 1.13e-02              |
| 12-hpi     | down       | Nucleotide excision repair        | 14        | 2.86            | 1.29e-02              |
| 12-hpi     | up         | Steroid biosynthesis              | 10        | 6.14            | 1.65e-03              |
| 12-hpi     | up         | Autophagy - animal                | 29        | 2.34            | 2.12e-03              |
| 12-hpi     | up         | Cell cycle                        | 27        | 2.30            | 3.90e-03              |
| 12-hpi     | up         | Influenza A                       | 22        | 2.13            | 4.74e-02              |
| 24-hpi     | down       | Ribosome                          | 88        | 5.54            | 2.81e-49              |
| 24-hpi     | down       | Oxidative phosphorylation         | 50        | 3.28            | 2.71e-13              |
| 24-hpi     | down       | Carbon metabolism                 | 39        | 2.98            | 1.08e-08              |
| 24-hpi     | down       | Aminoacyl-tRNA biosynthesis       | 22        | 3.78            | 1.10e-06              |
| 24-hpi     | down       | Biosynthesis of amino acids       | 24        | 3.02            | 2.50e-05              |

Table 4A: Significantly Enriched KEGG Pathways from DEGs identified at 12 and 24-hpi (Results from the DAVID online resource)

| Time Point | Regulation | KEGG Term  | DEG Count | Fold Enrichment | P-value<br>(Adjusted) |
|------------|------------|--|-----------|-----------------|-----------------------|
| 24-hpi     | down       | Citrate cycle (TCA cycle)                                  | 15        | 4.36            | 2.50e-05              |
| 24-hpi     | down       | DNA replication  | 15        | 3.78            | 1.93e-04              |
| 24-hpi     | down       | Spliceosome  | 33        | 2.08            | 1.09e-03              |
| 24-hpi     | down       | Metabolic pathways   | 225       | 1.22            | 3.04e-03              |
| 24-hpi     | down       | Cell cycle   | 36        | 1.89            | 3.04e-03              |
| 24-hpi     | down       | Propanoate metabolism                                      | 12        | 3.24            | 7.53e-03              |
| 24-hpi     | down       | Fatty acid degradation                                     | 14        | 2.86            | 7.77e-03              |
| 24-hpi     | down       | Glycolysis / Gluconeogenesis                               | 17        | 2.42            | 1.19e-02              |
| 24-hpi     | down       | One carbon pool by folate                                  | 9         | 3.78            | 1.35e-02              |
| 24-hpi     | down       | Nucleotide excision repair                                 | 15        | 2.31            | 3.73e-02              |
| 24-hpi     | down       | Pyruvate metabolism  | 12        | 2.59            | 4.20e-02              |
| 24-hpi     | up         | Steroid biosynthesis                                       | 11        | 5.15            | 1.92e-03              |
| 24-hpi     | up         | Lysosome   | 29        | 2.24            | 3.94e-03              |
| 24-hpi     | up         | Terpenoid backbone biosynthesis                            | 9         | 4.43            | 1.73e-02              |
| 24-hpi     | up         | Glycosaminoglycan biosynthesis - heparan sulfate / heparin | 10        | 3.90            | 1.73e-02              |
| 24-hpi     | up         | Protein processing in endoplasmic reticulum                | 30        | 1.94            | 1.73e-02              |
| 24-hpi     | up         | Autophagy - animal   | 30        | 1.85            | 3.19e-02              |

## 531 SUPPLEMENTARY MATERIALS



**Figure S1: Gel Electrophoresis of RT-qPCR validation reactions.** We run a gel electrophoresis of the RT-qPCR reactions in 2% agarose gel to confirm primer specificity. The 13 primer pairs all show excellent target specificity, each amplifying one amplicon of the expected size (see Table S1 for expected amplicon sizes of each primer pair). This was also confirmed in the RT-qPCR melt curves (not shown). Thermo Scientific™ generuler 1 kb plus DNA ladder was used. Mock-infected samples are shown in the top row prefixed with “mock” and infected samples are shown in the bottom row prefixed with “inf”

Table S1: Primers for RT-qPCR Validation of RNA-seq data

| Entrez ID | Target Gene               | Forward Primer                             | Reverse Primer                            | Amplicon Size |
|-----------|---------------------------|--|---|---------------|
| 100549497 | <i>APAF1</i>              | GCTGCGCAAATACCCGAGGTC <sup>ExJ</sup>       | GCCAGACACAGCATCTGTCACAC <sup>ExJ</sup>    | 133 bp        |
| 100550591 | <i>BMF</i>                | CGGAGACTCTCTATGGGAATGCTGG <sup>ExJ</sup>   | CTGCTGATGCCGCTGTATGTGG <sup>ExJ</sup>     | 189 bp        |
| 100543065 | <i>EDEM1</i>              | CTGGACTACAGGTGTTGATAGGAGACG <sup>ExJ</sup> | CCACTAACTCTGGCCTCAGTGG                    | 159 bp        |
| 100545922 | <i>EIF3D</i>              | GCACAGAGGAACCTCGGAGAG <sup>ExJ</sup>       | GTCACGAGGCTCTGCTGTGAC <sup>ExJ</sup>      | 180 bp        |
| 100545633 | <i>EIF3M</i>              | CTCTCAGACTGCAGCTACTGAGC <sup>ExJ</sup>     | GTCTGTGCTGAGGTTCCAGTCAG                   | 179 bp        |
| 100540536 | <i>FADD</i>               | GGAGCTCTGCAACTTCCTCATGG                    | CCTTCATGTCAGGCCACTCATCAG                  | 167 bp        |
| 100303685 | <i>GAPDH<sup>HK</sup></i> | CACTATCTTCAGGAGCGTGACC <sup>ExJ</sup>      | CTGAGATGATAACACGCTTAGCACAC                | 146 bp        |
| 100551463 | <i>MADD</i>               | GAGCTGACGAGGTTGAACTTGCTG <sup>ExJ</sup>    | CTGGCTCCAATGATAACAAGGTAGTCG               | 200 bp        |
| 100547583 | <i>PDCD4</i>              | GCACAGTAGAAGTGGAGAACATGAGTG <sup>ExJ</sup> | CTTCCTCAACCGCCTCTTGC                      | 161 bp        |
| 100544053 | <i>RPL10A</i>             | GGCACCGTCAGGCTGAAGTC <sup>ExJ</sup>        | GGCATCGTACTTCTTAGCCAGCTTC <sup>ExJ</sup>  | 177 bp        |
| 100544011 | <i>RPL8</i>               | GCCGAGAGACATGGCTACATCAAGG                  | CAGCTGAGCTTCTGCCACAG <sup>ExJ</sup>       | 186 bp        |
| 104913522 | <i>UFD1</i>               | GTGGTCTGCTTCAACATCTGGTC <sup>ExJ</sup>     | GATCTATGAGCTTCGGTAATGGAGAC <sup>ExJ</sup> | 154 bp        |
| 100548376 | <i>VCP</i>                | CAAGGCCATAGGAGTGAAGCCTC <sup>ExJ</sup>     | CTCAGGTTGCTCTCAGACTCACC                   | 171 bp        |

<sup>HK</sup> Control (house-keeping) gene<sup>ExJ</sup> Primer spans exon-exon junction;

532 Gene symbols: glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*); apoptotic peptidase activating fac-  
 533 tor 1 (*APAF1*); Bcl2 modifying factor (*BMF*); FAS-associated protein with death domain (*FADD*); pro-  
 534 grammed cell death 4 (*PDCD4*); MAP kinase activating death domain (*MADD*); valosin containing protein  
 535 (*VCP/p97*); Ubiquitin Recognition Factor in ER Associated Degradation 1 (*UFD1*); ER degradation enhanc-  
 536 ing alpha-mannosidase like protein 1 (*EDEM1*); eukaryotic translation initiation factor 3 subunit D (*EIF3D*);  
 537 eukaryotic translation initiation factor 3 subunit M (*EIF3M*); ribosomal protein L8 (*RPL8*); ribosomal protein

<sub>538</sub> L10a (*RPL10A*)

Table S2: Significantly Enriched KEGG Pathways from DEGs identified at 12 and 24-hpi (Results from the gprofiler2 R package)

| Time Point | Regulation | KEGG Term                         | DEG Count | P-value (Adjusted) |
|------------|------------|-----------------------------------|-----------|--------------------|
| 12-hpi     | down       | Ribosome                          | 35        | 7.70e-24           |
| 12-hpi     | down       | DNA replication                   | 11        | 5.07e-07           |
| 12-hpi     | down       | Oxidative phosphorylation         | 19        | 3.10e-04           |
| 12-hpi     | down       | Base excision repair              | 9         | 1.15e-03           |
| 12-hpi     | down       | One carbon pool by folate         | 6         | 1.27e-03           |
| 12-hpi     | down       | Mismatch repair                   | 6         | 3.49e-03           |
| 12-hpi     | down       | Ribosome biogenesis in eukaryotes | 9         | 1.77e-02           |
| 12-hpi     | down       | Nucleotide excision repair        | 8         | 3.36e-02           |
| 12-hpi     | up         | Autophagy - animal                | 13        | 2.09e-02           |
| 24-hpi     | down       | Ribosome                          | 41        | 4.71e-28           |
| 24-hpi     | down       | Aminoacyl-tRNA biosynthesis       | 12        | 3.04e-04           |
| 24-hpi     | down       | Oxidative phosphorylation         | 22        | 4.35e-04           |
| 24-hpi     | down       | Base excision repair              | 9         | 1.15e-02           |
| 24-hpi     | down       | Carbon metabolism                 | 14        | 3.14e-02           |
| 24-hpi     | down       | Propanoate metabolism             | 6         | 3.99e-02           |
| 24-hpi     | up         | Ubiquitin mediated proteolysis    | 17        | 7.26e-03           |
| 24-hpi     | up         | Steroid biosynthesis              | 5         | 2.63e-02           |