

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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17 **ABSTRACT**

18 **Background:** Infection with *Turkey Hemorrhagic Enteritis Virus* (THEV) can cause hemorrhagic enteritis,
19 which affects turkey pouls. This disease is characterized by immunosuppression (IMS) and bloody drop-
20 pings. The clinical disease usually lasts only a few days but secondary opportunistic infections due to THEV-
21 induced IMS extend the duration of illness and mortality, which exacerbates economic losses. Although an
22 avirulent THEV strain with only subclinical disease is used as a vaccine, some immunosuppressive prop-
23 erties remain in this prophylactic strain. To elucidate the mechanisms mediating THEV-induced IMS, we
24 performed the first transcriptomic analysis of a THEV infection using bulk RNA-sequencing.

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

31 **Results:** A total of 2,343 and 3,295 differentially expressed genes (DEGs) were identified at 12-hpi and
32 24-hpi, respectively. At 12-hpi, 1,079 genes were upregulated and 1,264 genes downregulated, whereas
33 1,512 genes were upregulated and 1,783 genes downregulated at 24-hpi. The DEGs contributed to multi-
34 ple biological processes including apoptosis, ER unfolded protein response, and cell maintenance. Multiple
35 pro-apoptotic genes, including *APAF1*, *BNIP3L*, *BMF*, *BAK1*, *RIPK1*, and *FAS* were upregulated. Genes
36 that play a role in ER stress-induced unfolded protein response including *VCP*, *UFD1*, *EDEM1*, *EDEM3*,
37 and *ATF4* were also upregulated and may contribute to apoptosis.

38 **Conclusions:** Our data suggest that several biological processes and pathways including apoptosis, im-
39 mune response, ER response to stress, ubiquitin-dependent protein catabolic process, and repression of
40 essential cellular maintenance are important aspects of the host cell response to THEV infection. It is
41 possible that interplay between multiple processes may mediate apoptosis of infected B-cells, leading to
42 IMS.

43 **KEY WORDS**

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

45 INTRODUCTION

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

58 It is well-established that THEV primarily infects and replicates in turkey B-cells of the bursa and spleen
59 and to a lesser extent in macrophages, inducing apoptosis and necrosis. Consequently, a significant drop
60 in the number of B-cells (specifically, IgM+ B-cells) and macrophages ensues along with increased T-cell
61 counts with abnormal ratios of T-cell subpopulations (CD4+ and CD8+). The cell death seen in the infected
62 B-cells and macrophages is generally proposed as the major cause of THEV-induced IMS as both humoral
63 and cell-mediated immunity are impaired (5, 6, 8, 11). Immunopathogenesis via cytokines from T-cells and
64 macrophages has also been suggested as a mechanism of apoptosis leading to IMS. It is thought that virus
65 replication in the spleen attracts T-cells and peripheral blood macrophages, which results in T-cell activation
66 by cytokines from activated macrophages and vice versa. The activated T-cells undergo clonal expansion
67 and secrete type I (IFN- α and IFN- β) and type II (IFN- γ) interferons as well as tumor necrosis factor (TNF),
68 while activated macrophages secrete interleukin 6 (IL-6), TNF, and nitric oxide (NO), an antiviral agent
69 with immunosuppressive properties. These cytokines may further contribute to apoptosis and necrosis in
70 bystander splenocytes, culminating in IMS (8, 11) (**Figure 1**). However, the precise molecular mechanisms
71 of THEV-induced IMS or the relevant intracellular signaling pathways are poorly understood (6). Elucidating
72 the specific mechanisms and pathways of THEV-induced IMS is a crucial step in THEV research as it will
73 present a means of mitigating IMS.

74 Next generation sequencing (NGS) is a groundbreaking technology that has significantly enhanced our
75 understanding of DNA and RNA structure and function and facilitated exceptional advancements in all do-
76 mains of biological sciences (12). Bulk mRNA sequencing (RNA-seq), an NGS approach to transcriptomic

77 studies, is a versatile, high throughput, and cost-effective technology that allows a broad scan of the en-
78 tire transcriptome of a cell population, thereby uncovering the active genes and molecular pathways and
79 processes. This technology has been leveraged in an ever-increasing number of studies to elucidate ac-
80 tive cellular processes under a wide range of treatment conditions, including viral infections (12–16). In
81 RNA-seq studies, differentially expressed genes (DEGs) identified by contrasting pairs of different experi-
82 mental conditions are key to unlocking the interesting biology or mechanism. Identified DEGs are typically
83 used for functional enrichment analyses in large curated knowledgebases such as gene ontology (GO) and
84 Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways which connect genes to specific biological
85 processes, functions, and pathways, shedding light on the biological question under study (17, 18).

86 To the best of our knowledge, no study has leveraged the wealth of information offered by RNA-seq to
87 elucidate the molecular mechanisms and pathways leading to THEV-induced IMS. To effectively counteract
88 the immunosuppressive effect of the vaccine, it is essential to unravel the host cell processes/pathways
89 influenced by the virus to bring about IMS. In this study, we present the first transcriptomic profile of THEV-
90 infected cells using paired-end bulk RNA-seq in a turkey B-cell line (MDTC-RP19), highlighting key host
91 genes, cellular/molecular processes and pathways affected during a THEV time course infection. We specif-
92 ically focus on cellular processes related to cell survivability that can elucidate THEV-induced IMS.

93 **RESULTS**

94 **Sequencing Results**

95 To identify the host transcriptome profile during THEV infection, we infected MDTC-RP19 cells with THEV
96 or no virus (mock) in triplicates or duplicates, respectively, and harvested at total RNA at 4-, 12-, 24-, and
97 72-hours post infection (hpi). mRNAs extracted from mock- or THEV-infected cells were sequenced on the
98 Illumina platform, yielding a total of **776.1** million raw reads (149-bp in length) across all samples (see **Table**
99 **1** for sequencing statistics). After trimming low-quality reads, the remaining **742.8** million total paired-end
100 trimmed reads (approximately, **34.7-47.9** million reads per sample) were mapped to the reference genome
101 of *M. galloparvo* obtained from the National Center for Biotechnology Information (NCBI). The percentage
102 of reads that mapped to the host genome across all samples ranged from **32.4** to **89.2%**. We observed
103 that the fraction of reads that mapped to the host genome decreased while those mapping to the virus
104 genome increased over the course of the infection as the viral infectious cycle progressed. Despite excellent
105 quality scores at all time points (**Table 1**), DEGs identified at 4- and 72-hpi did not yield any results in
106 the downstream functional enrichment analyses (GO term and KEGG pathway analysis) and they were
107 excluded from all subsequent analyses. In the remaining 12- and 24-hpi samples, a high consistency was
108 observed between biological replicates (**Figure 2A** and **B**).

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

110 We quantified gene expression levels with the StringTie software (19) in Fragments per kilobase of transcript
111 per million (FPKM) units. The differential expression analysis of DEGs was performed with the DESeq2 R
112 package (20) which employs a negative binomial distribution model for determining statistical significance.
113 Using a false discovery rate (FDR)-adjusted P-value cutoff ≤ 0.05 as the inclusion criteria, **2,343** and **3,295**
114 genes from THEV-infected samples were identified as differentially expressed relative to their time-matched
115 mock-infected samples at 12-hpi and 24-hpi, respectively. The results from the DEG analyses at 12- and
116 24-hpi have been deposited in NCBI Gene Expression Omnibus (<http://www.ncbi.nlm.nih.gov/geo>) under
117 accession number GSE286211 with files named total_12hrsDEGs.csv.gz and total_24hrsDEGs.csv.gz, re-
118 spectively. We compared THEV-infected samples relative to their time-matched mock-infected samples in
119 identifying the significant DEGs and in the functional enrichment analyses. At 12-hpi (THEV-infected ver-
120 sus mock-infected), we found **1,079** upregulated genes and **1,264** downregulated genes, whereas **1,512**
121 genes were upregulated and **1,783** genes downregulated at 24-hpi (THEV-infected versus mock-infected)
122 (**Figure 2C**, and **Figure 3A-C**). The log₂fold-change (FC) values at 12-hpi ranged between **-1.4** and **+1.7**
123 for **TMEM156** (Transmembrane Protein 156) and **LIPG** (Lipase G), respectively. At 24-hpi, the log₂FC val-
124 ues 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 the DEGs determined at the 12- and 24-hpi
128 timepoints with the DAVID (Database for Annotation, Visualization and Integrated Discovery; version 2021)
129 online resource (21) and the gprofiler2 R package – version **0.2.3** (22), which outputs results according
130 to the three branches of the GO directed acyclic graph – cellular components (CP), biological processes
131 (BP), and molecular functions (MF). We compared THEV-infected samples relative to their time-matched
132 mock-infected samples for each timepoint. Results with $P_{adjusted}$ -value ≤ 0.05 were considered functionally
133 enriched. The GO enrichment analyses results at 12-hpi and 24-hpi showed significant overlaps among
134 all three GO categories. At both time points, cellular breakdown processes were upregulated while cellular
135 maintenance processes and structures were downregulated in all three GO categories (**Table 2A-B** and
136 **Table 3A-B**).

137 For upregulated DEGs at 12-hpi, we observed that the GO terms annotated under the BP category broadly
138 cluster into: apoptosis and autophagy, cellular metabolism (catabolic processes), sterol biosynthesis, re-
139 sponse to stimuli, and protein processing (**Figure 4A** and **Table 2A**). In the CC category, the GO terms
140 relate primarily with cytoplasmic vacuolation, while in the MF category, they broadly fit under protein
141 binding and kinase activity (**Table 2A**). For downregulated DEGs at 12 hpi, the GO terms in BP cate-
142 gory generally fell under translation, protein biosynthesis and folding, ribosome biogenesis, nitrogen com-
143 pound metabolism, nucleic acid synthesis, repair, metabolism, processing, and replication, and energy
144 metabolism. Also, immunoglobulin production and isotype switching were downregulated (**Figure 4C** and
145 **Table 2B**). In the CC category GO terms broadly grouped into ribosome, mitochondria, respirosome, nu-
146 cleus, and spliceosome, while in the MF category, they generally belong to translation regulator activity,
147 protein folding chaperone, catalytic activity (acting on a nucleic acids), and ATP hydrolysis activity (**Table**
148 **2B**).

149 At 24-hpi, we found that the GO terms in the BP category for upregulated DEGs were associated with apop-
150 tosis and autophagy, lipid and sterol biosynthesis, catabolic process, protein ubiquitination and proteolysis,
151 cell signaling, and cell metabolism. Additionally, host defense response and genes that negatively regu-
152 late cytokine production were upregulated (**Figure 4B** and **Table 3A**). In the CC category, the GO terms
153 were related to cytoplasmic vacuolation and the lysosome, similar to those identified at 12-hpi. In the MF
154 category, the GO terms grouped into protein ubiquitination activity, kinase and acyltransferase activity, and
155 macromolecule binding activity (**Table 3A**). GO terms for the downregulated DEGs were markedly similar
156 to those at 12-hpi in all three GO categories. In the BP category, the GO terms broadly group into trans-

157 lation, peptide biosynthesis and folding, ribosome biogenesis, aerobic respiration and ATP synthesis, and
158 cell cycle process and nucleic acid replication and processing (**Figure 4D** and **Table 3B**). The GO terms in
159 the CC category group under ribosome, mitochondrion, nucleus and chromosomes, while the MF category,
160 the GO terms grouped into structural components of ribosome and translation regulator activity, catalytic
161 activity acting on a nucleic acid and nucleic acid binding, aminoacyl-tRNA ligase activity, and NAD binding
162 (**Table 3B**).

163 KEGG pathway analysis on the DEGs was also performed using both the gprofiler2 R package (22) and
164 the DAVID online resource. Both resources gave similar results, but the results from DAVID (**Table 4A**)
165 included more information than the gprofiler2 results (**Table S2**). The results from the KEGG pathway anal-
166 ysis were consistent with the GO results, revealing that generally, cell maintenance and upkeep pathways
167 were downregulated while cell death and breakdown pathways were upregulated. We observed that cell
168 maintenance pathways such as DNA replication and repair, ribosome biogenesis, spliceosome, and oxida-
169 tive phosphorylation were downregulated at both 12- and 24-hpi. Pathways such as: autophagy, response
170 to virus (Influenza A), and steroid biosynthesis were upregulated at 12-hpi, which is similar to 24-hpi, where
171 pathways such as: autophagy, ubiquitin-mediated proteolysis, lysosome, protein processing in endoplasmic
172 reticulum, and steroid biosynthesis were upregulated.

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

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

181 Many virus families, including adenoviruses, herpesviruses, poxviruses, baculoviruses, parvoviruses, retro-
182 viruses, rhabdoviruses, paramyxoviruses, orthomyxoviruses, togaviruses, and picornaviruses are known
183 to trigger apoptosis in infected host cells either through direct viral protein action or the host antiviral re-
184 sponse (24–26). Our data show that apoptotic and autophagic pathways are upregulated during THEV
185 infection, supporting previous findings of apoptosis and necrosis of THEV-infected cells (8, 11, 23). For
186 example, several proapoptotic members of the BCL2 (B-cell lymphoma 2) protein family such as BCL2
187 antagonist/killer 1 (*BAK1*), BCL2 interacting protein 3 like (*BNIP3L*), BCL2 interacting protein 3 (*BNIP3*),
188 and Bcl2 modifying factor (*BMF*) were upregulated. Additionally, Fas cell surface death receptor (*FAS*),

189 Fas associated via death domain (*FADD*), MAP kinase-activating death domain (*MADD*), programmed cell
190 death 4 (*PDCD4*), RB1 inducible coiled-coil 1 (*RB1CC1*), activating transcription factor 4 (*ATF4*), recep-
191 tor interacting serine/threonine kinase 1 (*RIPK1*), tumor necrosis factor receptor superfamily member 1B
192 (*TNFRSF1B*), pro-apoptotic WT1 regulator (*PAWR*), and apoptotic peptidase activating factor 1 (*APAF1*),
193 which are potent proapoptotic factors were upregulated at both timepoints. Interestingly, both the intrinsic
194 (*BAK1*, *BNIP3L*, *BNIP3*, *BMF*, *RB1CC1*, *ATF4*, *PDCD4*, and *APAF1*) and extrinsic (*FAS*, *FADD*, *TNFRSF1B*,
195 *MADD*, and *RIPK1*) apoptotic pathways were represented. Conversely, several anti-apoptotic proteins such
196 as BCL2 apoptosis regulator (*BCL2*), BCL2 interacting protein 2 (*BNIP2*; interacts directly with adenovirus
197 E1B-19K protein), BCL2 related protein A1 (*BCL2A1*), and apoptosis inhibitor 5 (*API5*) were also upreg-
198 ulated. Thus, apoptosis and its regulation pathways are clearly upregulated; this highlights the host-virus
199 tug-of-war also typical in Mastadenovirus infections. Moreover, several genes associated with autophagy
200 such as: TNF receptor associated factor 6 (*TRAF6*), autophagy related 9A (*ATG9A*), unc-51 like autophagy
201 activating kinase 2 (*ULK2*), and autophagy related 4B cysteine peptidase (*ATG4B*) were upregulated.

202 Downregulation of Cell Maintenance Pathways

203 Forcibly transitioning the host cell cycle to the S phase during the early phase of infection is a prerequisite
204 for a productive adenovirus infection (27). Interaction of the viral E1A early proteins with the host pRb
205 (retinoblastoma) protein releases the host transcription factor E2F, which activates genes required for S
206 phase cell cycle induction. Viral E1A also binds the host transcriptional co-activator p300/CBP (27, 28).
207 Our GO and KEGG pathway results showed that at 12-hpi, several key genes involved with cell cycle
208 transition were upregulated. Notably, E1A binding protein p300 (*EP300*), cyclin genes (*CCND3*, *CCNG1*,
209 *CCNG2*, *CDK6*), anaphase promoting complex subunit 1 (*ANAPC1*), and cell division cycle 27 (*CDC27*)
210 were upregulated. However, unlike the observation in Mastadenoviruses, the cell cycle regulation at 12-
211 hpi seems complicated as some key cell cycle-related genes as well as DNA and RNA synthesis, repair,
212 metabolism, processing, and replication were concurrently downregulated. At 24-hpi, our KEGG pathway
213 and GO analysis show that components of the cell cycle were downregulated.

214 We found that several essential cell maintenance processes whose suppression can trigger apoptosis were
215 downregulated. Severe DNA damage is a known mechanism of apoptosis induction, called DNA damage-
216 dependent apoptosis (29). Repression of host RNA and protein synthesis is also strongly associated with
217 apoptosis (30). Several processes related to DNA and RNA synthesis, maintenance, and repair such as nu-
218 cleotide biosynthesis and metabolism, double strand break repair, DNA excision repair, RNA biosynthesis,
219 RNA processing, DNA replication, mitotic cell cycle process, protein-RNA complex organization, and DNA
220 damage response were downregulated at both timepoints. Notable genes identified include DNA ligase 1

221 (*LIG1*), X-ray repair cross complementing 1 (*XRCC1*), cyclin dependent kinase 1 and 2 (*CDK1*, *CDK2*),
222 checkpoint kinase 1 (*CHEK1*), 8-oxoguanine DNA glycosylase (*OGG1*), BLM RecQ-like-helicase (*BLM*),
223 BRCA1 DNA repair associated (*BRCA1*), and several RAD family proteins (*RAD21*, *RAD51*, *RAD51B*,
224 *RAD51C*, *RAD54B*).

225 Protein synthesis-related processes, including ribosome biogenesis, rRNA processing, ribosome assembly,
226 protein folding, translational initiation, protein maturation, ribosome and ribonucleoprotein complex forma-
227 tion, translation pre-initiation complex formation, and cytoplasmic translation were significantly downregu-
228 lated at both 12- and 24-hpi. Notable genes identified include eukaryotic translation initiation factors (*EIF1*,
229 *EIF1AX*, *EIF3E* and *EIF3F*, *EIF3H*, *EIF3I*, *EIF3L* and *EIF3M*), biogenesis of ribosomes BRX1 (*BRIX1*),
230 MCTS1 re-initiation and release factor (*MCTS1*), and ribosomal protein subunits (*RPL8*, *RPL10a*, *RPL11*,
231 *RP12*, *RP13*, *RP14*, *RP15*, *RP18a*, *RP19*).

232 **Endoplasmic Reticulum (ER) Stress Response during THEV infection**

233 Our KEGG pathway analysis (**Table 4A**) showed that protein processing in the ER, and ubiquitin-mediated
234 proteolysis are significantly upregulated (**Figure 5**). The GO results (**Table 3A**) showed that specifically,
235 ER stress and the ER-associated protein degradation (ERAD) pathway, a branch of the unfolded protein
236 response (UPR) were upregulated during THEV infection, especially at 24-hpi. The ER is the major site for
237 protein synthesis, folding and quality control, and sorting (31). Upon ER stress or continued accumulation
238 of unfolded proteins in the ER lumen, the UPR pathways are activated to restore ER homeostasis. The
239 ERAD pathway, a ubiquitin-proteasome-dependent pathway, is a protein quality control system activated for
240 degradation of misfolded and unassembled proteins (31). In our results, the THEV-infected samples showed
241 significant increase in ERAD pathway effector proteins, such as valosin containing protein (*VCP*), ubiquitin
242 recognition factor in ER associated degradation 1 (*UFD1*), ER degradation enhancing alpha-mannosidase
243 like proteins 1 and 3 (*EDEM1*, *EDEM3*), cullin 1 (*CUL1*), and ubiquilin 1 (*UBQLN1*). Other genes related to
244 other UPR pathways such as *HSPA5* and *ATF4* were also upregulated. Our KEGG pathway (**Table S2**) and
245 GO (**Figure 4B**) results indicated a significant upregulation of ubiquitin mediated proteolysis with other ubiq-
246 uitination pathway proteins such as ubiquitin conjugating enzymes (*UBE2J2*, *UBE2E3*, *UBE2Z*), ubiquitin
247 protein ligases (*UBE3A*, *UBE3B*), NPL4 homolog ubiquitin recognition factor (*NPLOC4*), and ubiquitin like
248 modifier activating enzyme 6 (*UBA6*) showing significant upregulation. Additionally, the heat shock family of
249 chaperone proteins such as the DnaJ heat shock protein family (*HSP40*) members (*DNAJB11*, *DNAJB12*,
250 *DNAJB2*, *DNAJC10*), heat shock protein family A (*HSP70*) members (*HSPA4L*, *HSPA5*, *HSPA8*), and heat
251 shock protein 90 alpha family class A member 1 (*HSP90AA1*) were upregulated at 24-hpi. Moreover, the
252 KEGG pathway analysis (**Table 4A**) shows a significant upregulation in lysosome formation, lumen acidifi-

253 cation, and lysosomal degradation, likely an indication of ER-to-lysosome-associated degradation. Taken
254 together, these results suggest that THEV infection triggers significant ER-associated protein degradation,
255 which may contribute to cell death and IMS.

256 **Differential Expression of Cytokine and Cytokine Receptor-encoding Genes**

257 Our KEGG pathway results showed that a pathway similar to immune response to influenza A infection was
258 upregulated at 12-hpi. Our GO analysis also identified terms such as regulation of lymphocyte activation
259 and regulation of cytokine production as upregulated at both 12- and 24-hpi. Genes involved include *IL18*,
260 *IL2RB*, *IL4R*, *IL5RA*, TNF receptor associated factors (*TRAF2*, *TRAF3*, *TRAF6*, *TRAF7*, *TRAFD1*), TNF re-
261 ceptor superfamily members (*TNFRSF1B*, *TNFRSF8*, *TNFSF4*), interferon-induced with helicase C domain
262 1 (*IFIH1*), interferon-induced double-stranded RNA-activated protein kinase (*PKR*), and *CD80*. In contrast,
263 cytokine inhibitors such as suppressor of cytokine signaling (*SOCS3* and *SOCS5*) were also upregulated
264 at both 12 and 24-hpi and immunoglobulin production and isotype switching GO terms were downregulated
265 at 12-hpi. This inconsistency is likely an indicator of the struggle between the virus and its host. While sev-
266 eral cytokines were regulated by THEV as in the proposed model of THEV immunopathogenesis (**Figure**
267 **1**), the cytokines in the model (IFN- α , IFN- β , IFN- γ TNF, IL-6, and NO) were not significantly differentially
268 expressed in our data. However, some of the differentially expressed cytokines and cytokine receptors (*TN-*
269 *FRSF8*, *TRAF7*) identified in this study are positive regulators of apoptosis; therefore, they may play a role
270 in THEV-induced IMS.

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

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

280 **DISCUSSION**

281 THEV has a worldwide distribution, wreaking economic havoc on affected poultry farms, particularly due
282 to its immunosuppressive trait allowing secondary opportunistic infections to devastate turkey populations
283 (4, 6). HE in turkeys causes more economic losses than any disease caused in other birds like chicken
284 and pheasants (4). While the current vaccine strain (VAS) has proven effective at preventing clinical HE in
285 turkey poult, the retention of its immunosuppressive properties leaves some of the issues associated with
286 economic losses unresolved. Elucidating the virus-host interactions leading to IMS is most pressing for not
287 only the understanding of the viral infection and pathogenesis but also future antiviral therapy targets. Since
288 both virulent and avirulent THEV cause IMS but the avirulent are used as vaccine, we believe that studying
289 VAS would be more expedient for understanding THEV vaccine-induced IMS.

290 Only one cell line (MDTC-RP19 or RP19) has ever been found to be capable of supporting THEV infection
291 and replication (32). Thus, in this work, we establish the first transcriptome profile of THEV infection in
292 RP19 cells using paired-end RNA-seq. We attempted a multi-time point experimental design but this being
293 the first transcriptomic study of THEV infection, we faced some difficulties, including selecting our sampling
294 time points based on the only study of THEV gene expression kinetics (33), leading to only 12- and 24-hpi
295 providing useful data. In total **2,343** and **3,295** DEGs were identified at 12-hpi and 24-hpi, respectively. At
296 12-hpi, **1,079** genes were upregulated and **1,264** genes downregulated, whereas **1,512** genes were upreg-
297 ulated and **1,783** genes downregulated at 24-hpi. Being a non-model organism, a significant proportion
298 of the host (*M. gallopavo*) genes are not annotated and not recognized by the databases used for func-
299 tional enrichment analysis. Thus, the obtained results are likely sub-optimal in amount of detail relative to
300 results from well annotated and curated genomes of model organisms. The DEGs were related to multi-
301 ple biological processes all potentially playing a role in THEV infection but the most relevant to our study
302 are apoptosis, ER stress-induced unfolded protein response, suppressed cell maintenance processes, and
303 cytokine deregulation. Furthermore, the RT-qPCR results validated the RNA-seq results. Collectively, this
304 study may shed light on some significant aspects of THEV-host interactions, which may benefit further
305 mechanistic delineation of the viral infection and induction of IMS and inform future development of anti-
306 THEV strategies. The biological processes most relevant to THEV-induced IMS highlighted by this study
307 are further discussed below.

308 Apoptosis is a key defense mechanism activated by cells in response to irreversible injury and virus infection
309 to abrogate virus propagation. It is a formidable cellular defense network, non-specific to any virus family
310 and therefore an important problem for any infecting virus to tackle (24–26). The adenovirus E1A pro-
311 teins are strong inducers of apoptosis. They bind host pRb and p300/CBP protein, inducing p53-mediated

312 apoptosis, and can also sensitize infected cells to TNF α and TRAIL-induced apoptosis (27, 28). However,
313 adenoviruses have developed multiple distinct anti-apoptotic mechanisms to counter almost all cellular pro-
314 apoptotic programs. For example, E1A blocks its own induction of p53-dependent apoptosis and E1B
315 proteins (E1B-19K and E1B-55K) counteract several types of apoptosis including TNF-induced apopto-
316 sis (27, 28). Despite the rich arsenal of countermeasures, transcriptomic studies of human adenovirus
317 infections suggest a complex set of virus-host interactions where both pro- and anti-apoptotic genes are
318 turned on contemporaneously. For example, in human adenovirus 2 infection, both pro- and anti-apoptotic
319 BCL2 family genes were stimulated (27). Siadenoviruses including THEV are the smallest adenoviruses
320 and therefore encode the fewest genes (10, 34). THEV encodes a mere 34 ORFs with no anti-apoptotic
321 genes characterized (34). In agreement with these findings, in our results a strong signal indicative of
322 apoptosis was observed. However, like mastadenovirus infections, a complex relationship between pro and
323 anti-apoptotic genes were observed. Pro-apoptotic genes such as *APAF1*, *BNIP3L*, *BMF*, *BAK1*, *RIPK1*,
324 *FAS*, *FADD* and *ATF* were upregulated in concert with the anti-apoptotic genes: *BCL2*, *BNIP2*, *BCL2A1*
325 and *API5*. We speculate that this complex regulation is predictive of THEV possessing some anti-apoptotic
326 genes but not sufficiently potent to thwart the cellular apoptotic response. Interestingly, both intrinsic and
327 extrinsic pathway pro-apoptotic genes were upregulated, possibly due to a concurrent stimulation of multi-
328 ple apoptotic pathways or a positive feedback mechanism of one system activating the other. The specific
329 mechanism of apoptosis induction remains elusive. Further studies designed to elucidate these fine details
330 are warranted and would benefit future THEV therapeutics tremendously.

331 The ER serves many specialized functions including biosynthesis and assembly of membrane and secre-
332 tory proteins, calcium storage, and biosynthesis of lipids and sterols. It is also the site of protein folding and
333 post-translational modifications and maintains stringent quality control systems, ensuring correctly folded
334 exported proteins and degradation of unfolded or misfolded proteins (16, 31, 35). Disruption of ER home-
335 ostatics or ER stress leads to accumulation of incorrect proteins in the ER lumen, triggering the UPR. The
336 UPR restores ER normality by transiently attenuating general protein synthesis, increasing the luminal
337 folding capacity, and the degradation of misfolded proteins through the ERAD pathway or autophagy (16,
338 31, 35, 36). However, if incorrect luminal protein overload persists, the prolonged UPR will induce apop-
339 tosis known as ER stress-associated programmed cell death (35, 36). Many viruses, including DNA and
340 RNA viruses are reported to induce ER stress and UPR pathways during infection (16). In our results,
341 *ATF4* and PKR-like ER protein kinase (*PERK*), key proteins in the *PERK* branch of the UPR pathway were
342 upregulated. A myriad ERAD pathway proteins (e.g., *VCP*, *UFD1*, *EDEM1*, *EDEM3*, *CUL1*, *UBQLN1*),
343 ubiquitination system proteins (e.g., *UBE2J2*, *UBE2E3*, *UBE2Z*, *UBE3A*, *UBE3B*), and heat shock family of

344 chaperone proteins (e.g., *HSPA5*, *HSP4L*, *HSPA8*, *HSP90AA1*) all showed increased expression according
345 to our RNA-seq data with some validated with RT-qPCR. These data strongly suggest that THEV infection
346 triggers the ER UPR pathways leading to a massive decrease of protein synthesis and deregulation of
347 sterol biosynthesis, and ubiquitin-mediated proteolysis, all seen in our results. As noted above, a prolonged
348 UPR activation leads to ER stress-associated programmed cell death via genes such *ATF4* (35, 36). Thus,
349 we suggest that ER stress response likely plays a crucial role in the THEV-induced IMS. Nonetheless, the
350 mechanisms underlying the regulation of the UPR pathways by THEV remain to be clearly unraveled. Also,
351 whether and how ER stress response affects THEV infection and pathogenicity are also merited to be de-
352 termined in a future study. Unsurprisingly, protein degradation was more pronounced at the 24-hpi than at
353 12-hpi, reflecting the suggested two phases of UPR – phase one allows the unfolded proteins time to refold
354 without degradation and phase two degrades any proteins which have failed to fold (36).

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

376 By convention, the Mastadenovirus replication cycle is divided into two phases, an early and a late phase,
377 based on the onset of viral DNA replication (27, 28). Based on DNA microarray analysis, adenovirus type 2
378 (Ad2) infection has been divided into four stages. The first period is from 0 to 12-hpi during which, changes
379 in cellular gene expression are likely to be triggered by the virus entry process. Most of the deregulated
380 genes have functions linked to inhibition of cell growth. Therefore, growth suppression is most likely the first
381 response of the host cell to the incoming virus (27). The second period covers the time from 12 to 24-hpi
382 and follows activation of the immediate early E1A gene, which forcibly transition cell cycle to S phase (27).
383 While the temporal changes of host gene expression for a THEV infection have not been characterized in
384 prior studies, our data showed that during the first 24-hpi, cell growth was suppressed. Cell maintenance
385 processes involving nucleic acid and proteins were downregulated according to our data. Protein synthesis-
386 related processes including ribosome biogenesis, rRNA processing, ribosome assembly, protein folding,
387 translational initiation, protein maturation, and others were heavily affected. Additionally, DNA and RNA
388 synthesis, maintenance, and repair such as nucleotide biosynthesis and metabolism, double strand break
389 repair, and DNA excision repair were also repressed. As severe DNA damage leads to DNA damage-
390 dependent apoptosis (29) and repression of RNA and protein synthesis is also strongly associated with
391 apoptosis (30), these inhibitions may also play a role in THEV-induced IMS. Moreover, we speculate that
392 the ER UPR may contribute partly to the severe repression of protein synthesis as discussed above. An
393 in-depth study of temporal changes of host gene expression during THEV infection would be invaluable in
394 establishing if THEV follows the same pattern as Ad2.

395 **CONCLUSIONS**

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

408 **MATERIALS AND METHODS**

409 **Cell culture and THEV Infection**

410 The Turkey B-cell line (MDTC-RP19, ATCC CRL-8135) was grown as a suspension culture in 1:1 complete
411 Leibovitz's L-15/McCoy's 5A medium with 10% fetal bovine serum (FBS), 20% chicken serum (ChS), 5%
412 tryptose phosphate broth (TPB), and 1% antibiotic solution (100 U/mL Penicillin and 100 μ g/mL Strepto-
413 mycin), at 41°C in a humidified atmosphere with 5% CO₂. Infected cells were maintained in 1:1 serum-
414 reduced Leibovitz's L15/McCoy's 5A media (SRLM) with 2.5% FBS, 5% ChS, 1.2% TPB, and 1% antibiotic
415 solution. A commercially available THEV vaccine was purchased from Hygieia Biological Labs (VAS strain).
416 The stock virus was titrated using an in-house qPCR assay with titer expressed as genome copy number
417 (GCN)/mL, similar to Mahshoub *et al* (37). Cells were THEV-infected or mock-infected in triplicates or du-
418 plicates, respectively at a multiplicity of infection (MOI) of 100 GCN/cell, incubated at 41°C for 1 hour, and
419 washed three times with phosphate buffered saline (PBS) to get rid of free virus particles. At each time point
420 (4-, 12-, 24-, and 72-hpi), triplicate (THEV-infected) and duplicate (mock-infected) samples were harvested
421 for total RNA extraction.

422 **RNA extraction and Sequencing**

423 Total RNA was extracted from infected cells using the Thermo Fisher RNaseous™-4PCR Total RNA Iso-
424 lation Kit (which includes a DNase I digestion step) per manufacturer's instructions. An agarose gel elec-
425 trophoresis was performed to check RNA integrity. The RNA quantity and purity was initially assessed using
426 nanodrop, and RNA was used only if the A260/A280 ratio was 2.0 ± 0.05 and the A260/A230 ratio was >2
427 and <2.2. Extracted total RNA samples were sent to LC Sciences, Houston TX for poly-A-tailed mRNA
428 sequencing. RNA integrity was checked with Agilent Technologies 2100 Bioanalyzer High Sensitivity DNA
429 Chip and poly(A) RNA-seq library was prepared following Illumina's TruSeq-stranded-mRNA sample prepa-
430 ration protocol. Paired-end sequencing, generating 150-bp reads was performed on the Illumina NovaSeq
431 6000 sequencing system. The paired-end 150bp sequences obtained during this study and all expression
432 data have been submitted to the Gene Expression Omnibus database, under accession no GSE286211

433 **Quality Control and Mapping Process**

434 Sequencing reads were processed following a well-established protocol described by Pertea *et al*
435 (19), using Snakemake - version 7.32.4 (38), a popular workflow management system to drive the
436 pipeline. Briefly, raw sequencing reads were trimmed with Cutadapt - version 1.10 (39) and the quality

437 of trimmed reads evaluated using the FastQC software, version 0.12.1 (Bioinformatics Group at the
438 Babraham Institute, Cambridge, United Kingdom; www.bioinformatics.babraham.ac.uk), achieving an
439 overall Mean Sequence Quality (PHRED Score) of 36. Trimmed reads were mapped the reference *Me-*
440 *leagris gallopavo* genome file GCF_000146605.3_Turkey_5.1_genomic.fna.gz from NCBI (genome build:
441 melGal5) (https://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/000/146/605/GCF_000146605.3_Turkey_5.1/)
442 with Hisat2 - version 2.2.1 (19) using the accompanying gene transfer format (GTF) annotation file
443 (GCF_000146605.3_Turkey_5.1_genomic.gtf.gz) to build a genomic index. Samtools - version 1.21 was
444 used to convert the output Sequence Alignment Map (SAM) file to the Binary Alignment Map (BAM)
445 format. The StringTie (v2.2.1) software (19), set to expression estimation mode was used to generate
446 normalized gene expression estimates from the BAM files for genes in the reference GTF file after which
447 the prepDE.py3 script was used to extract read count information from the StringTie gene expression files,
448 providing an expression-count matrix for downstream DEG analysis.

449 **DEG Analysis and Functional Enrichment Analysis**

450 DEG analysis between mock- and THEV-infected samples was performed using the very popular DESeq2
451 (20), which employs a Negative Binomial distribution model for determining statistical significance when
452 comparing read counts from multiple replicates. Genes with (FDR)-adjusted P-value ≤ 0.05 were consid-
453 ered as differentially expressed. The sequencing data (FASTQ files), expression-count matrix, and DEG
454 analysis results from DESeq2 are deposited at NCBI Gene Expression Omnibus under accession num-
455 ber GSE286211. The functional profiling of DEGs (GO and KEGG analyses) were performed based on
456 GO databases and KEGG databases using DAVID and the R package gprofiler2 (22) with *M. gallopavo*
457 as the reference organism. Results with P_{adjusted}-value ≤ 0.05 were included as functionally enriched. All
458 visualization plots were made using ggplot2, pheatmap, and ggvenn R packages (40–42).

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

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

467 remained as the template for the RT-qPCR quantification. The RT-qPCR was performed with the PowerUp™
468 SYBR™ Green master mix from Applied Biosystems with primers designed manually in the SnapGene
469 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**
470 **Table S1.** Relative mRNA levels were calculated by $2^{-\Delta\Delta CT}$ method (43).

472 **Statistical Analysis**

473 Statistical analyses of the RT-qPCR results were performed using R (Version 4.3.3) with Student's t-test and
474 Mann-Whitney U test for the comparison between two groups. A p-value ≤ 0.05 was considered statistically
475 significant.

476 **LIST OF ABBREVIATIONS**

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

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

477 DATA AVAILABILITY

478 The raw sequencing read data (FastQ), gene expression counts, and total DEGs identified at 12- and 24-

479 hpi have been deposited at the NCBI Gene Expression Omnibus (<http://www.ncbi.nlm.nih.gov/geo>) under

480 accession number GSE286211.

481 CODE AVAILABILITY

482 All the code/scripts in the entire analysis pipeline are available on GitHub (<https://github.com/Abraham->

483 Quaye/host_rna_seq)

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486 high-performance computing systems to perform the memory-intensive steps in the analysis pipeline of this

487 work.

488 **REFERENCES**

- 489 1. Harrach B. 2008. Adenoviruses: General features, p. 1–9. In Mahy, BWJ, Van Regenmortel, MHV
(eds.), Encyclopedia of virology (third edition). Book Section. Academic Press, Oxford.
- 490 2. Davison A, Benko M, Harrach B. 2003. Genetic content and evolution of adenoviruses. The Journal
of general virology 84:2895–908.
- 491 3. Gross WB, Moore WE. 1967. Hemorrhagic enteritis of turkeys. Avian Dis 11:296–307.
- 492 4. Beach NM. 2006. Characterization of avirulent turkey hemorrhagic enteritis virus: A study of the
molecular basis for variation in virulence and the occurrence of persistent infection. Thesis.
- 493 5. Dhamma K, Gowthaman V, Karthik K, Tiwari R, Sachan S, Kumar MA, Palanivelu M, Malik YS, Singh
RK, Munir M. 2017. Haemorrhagic enteritis of turkeys – current knowledge. Veterinary Quarterly
37:31–42.
- 494 6. Tykałowski B, Śmiałek M, Koncicki A, Ognik K, Zduńczyk Z, Jankowski J. 2019. The immune re-
sponse of young turkeys to haemorrhagic enteritis virus infection at different levels and sources of
methionine in the diet. BMC Veterinary Research 15.
- 495 7. Pierson F, Fitzgerald S. 2008. Hemorrhagic enteritis and related infections. Diseases of Poultry
276–286.
- 496 8. Rautenschlein S, Sharma JM. 2000. Immunopathogenesis of haemorrhagic enteritis virus (HEV) in
turkeys. Dev Comp Immunol 24:237–46.
- 497 9. Larsen CT, Domermuth CH, Sponenberg DP, Gross WB. 1985. Colibacillosis of turkeys exacerbated
by hemorrhagic enteritis virus. Laboratory studies. Avian Dis 29:729–32.

- 498 10. Beach NM, Duncan RB, Larsen CT, Meng XJ, Sriranganathan N, Pierson FW. 2009. Persistent
infection of turkeys with an avirulent strain of turkey hemorrhagic enteritis virus. *Avian Diseases*
53:370–375.
- 499 11. Rautenschlein S, Suresh M, Sharma JM. 2000. Pathogenic avian adenovirus type II induces apop-
tosis in turkey spleen cells. *Archives of Virology* 145:1671–1683.
- 500 12. Satam H, Joshi K, Mangrolia U, Waghoo S, Zaidi G, Rawool S, Thakare RP, Banday S, Mishra AK,
Das G, Malonia SK. 2023. Next-generation sequencing technology: Current trends and advance-
ments. *Biology* 12:997.
- 501 13. Pandey D, Onkara Perumal P. 2023. A scoping review on deep learning for next-generation RNA-seq.
Data analysis. *Functional & Integrative Genomics* 23.
- 502 14. Wang B, Kumar V, Olson A, Ware D. 2019. Reviving the transcriptome studies: An insight into the
emergence of single-molecule transcriptome sequencing. *Frontiers in Genetics* 10.
- 503 15. Choi SC. 2016. On the study of microbial transcriptomes using second- and third-generation se-
quencing technologies. *Journal of Microbiology* 54:527–536.
- 504 16. Mo Q, Feng K, Dai S, Wu Q, Zhang Z, Ali A, Deng F, Wang H, Ning Y-J. 2023. Transcriptome
profiling highlights regulated biological processes and type III interferon antiviral responses upon
crimean-congo hemorrhagic fever virus infection. *Virologica Sinica* 38:34–46.
- 505 17. Ashburner M, Ball CA, Blake JA, Botstein D, Butler H, Cherry JM, Davis AP, Dolinski K, Dwight
SS, Eppig JT, Harris MA, Hill DP, Issel-Tarver L, Kasarskis A, Lewis S, Matese JC, Richardson JE,
Ringwald M, Rubin GM, Sherlock G. 2000. Gene ontology: Tool for the unification of biology. *Nature
Genetics* 25:25–29.
- 506 18. Kanehisa M. 2000. KEGG: Kyoto encyclopedia of genes and genomes. *Nucleic Acids Research*
28:27–30.

- 507 19. Pertea M, Kim D, Pertea GM, Leek JT, Salzberg SL. 2016. Transcript-level expression analysis of RNA-seq experiments with HISAT, StringTie and ballgown. *Nature Protocols* 11:1650–1667.
- 508 20. Love MI, Huber W, Anders S. 2014. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biology* 15:550.
- 509 21. Sherman BT, Hao M, Qiu J, Jiao X, Baseler MW, Lane HC, Imamichi T, Chang W. 2022. DAVID: A web server for functional enrichment analysis and functional annotation of gene lists (2021 update). *Nucleic Acids Research* 50:W216–W221.
- 510 22. Kolberg L, Raudvere U, Kuzmin I, Vilo J, Peterson H. 2020. gprofiler2— an r package for gene list functional enrichment analysis and namespace conversion toolset g:profiler. *F1000Research* 9 (ELIXIR).
- 511 23. Saunders GK, Pierson FW, Hurk JV van den. 1993. Haemorrhagic enteritis virus infection in turkeys: A comparison of virulent and avirulent virus infections, and a proposed pathogenesis. *Avian Pathology* 22:47–58.
- 512 24. Barber GN. 2001. Host defense, viruses and apoptosis. *Cell Death & Differentiation* 8:113–126.
- 513 25. Hardwick JM. 1997. Virus-induced apoptosis, p. 295–336. *In Apoptosis - pharmacological implications and therapeutic opportunities*. Elsevier.
- 514 26. Verburg SG, Lelievre RM, Westerveld MJ, Inkol JM, Sun YL, Workenhe ST. 2022. Viral-mediated activation and inhibition of programmed cell death. *PLOS Pathogens* 18:e1010718.
- 515 27. Zhao H, Dahlö M, Isaksson A, Syvänen A-C, Pettersson U. 2012. The transcriptome of the adenovirus infected cell. *Virology* 424:115–128.
- 516 28. Guimet D, Hearing P. 2016. Adenovirus replication, p. 59–84. *In Adenoviral vectors for gene therapy*. Elsevier.

- 517 29. Roos WP, Kaina B. 2006. DNA damage-induced cell death by apoptosis. *Trends in Molecular Medicine* 12:440–450.
- 518 30. Martin SJ. 1993. Protein or RNA synthesis inhibition induces apoptosis of mature human CD4+ t cell blasts. *Immunology Letters* 35:125–134.
- 519 31. Christianson JC, Carvalho P. 2022. Order through destruction: How ER-associated protein degradation contributes to organelle homeostasis. *The EMBO Journal* 41.
- 520 32. Hurk JV van den. 1990. Propagation of group II avian adenoviruses in turkey and chicken leukocytes. *Avian Diseases* 34:12.
- 521 33. Aboeazz ZR, Mahsoub HM, El-Bagoury G, Pierson FW. 2019. In vitro growth kinetics and gene expression analysis of the turkey adenovirus 3, a siadenovirus. *Virus Research* 263:47–54.
- 522 34. Quaye A, Pickett BE, Griffitts JS, Berges BK, Poole BD. 2024. Characterizing the splice map of turkey hemorrhagic enteritis virus. *Virology Journal* 21.
- 523 35. Fribley A, Zhang K, Kaufman RJ. 2009. Regulation of apoptosis by the unfolded protein response, p. 191–204. *In Apoptosis*. Humana Press.
- 524 36. Read A, Schröder M. 2021. The unfolded protein response: An overview. *Biology* 10:384.
- 525 37. Mahsoub HM, Evans NP, Beach NM, Yuan L, Zimmerman K, Pierson FW. 2017. Real-time PCR-based infectivity assay for the titration of turkey hemorrhagic enteritis virus, an adenovirus, in live vaccines. *Journal of Virological Methods* 239:42–49.
- 526 38. Mölder F, Jablonski KP, Letcher B, Hall MB, Tomkins-Tinch CH, Sochat V, Forster J, Lee S, Twardziok SO, Kanitz A, Wilm A, Holtgrewe M, Rahmann S, Nahnsen S, Köster J. 2021. Sustainable data analysis with snakemake. *F1000Research* 10:33.

- 527 39. Martin M. 2011. Cutadapt removes adapter sequences from high-throughput sequencing reads.
EMBnetjournal 17:10.
- 528 40. Wickham H. 2016. ggplot2: Elegant graphics for data analysis. Springer-Verlag New York. <https://ggplot2.tidyverse.org>.
- 529 41. Kolde R. 2019. Pheatmap: Pretty heatmaps. <https://CRAN.R-project.org/package=pheatmap>.
- 530 42. Yan L. 2023. Ggvenn: Draw venn diagram by 'ggplot2'. <https://CRAN.R-project.org/package=ggvenn>.
- 531 43. Livak KJ, Schmittgen TD. 2001.. Methods 25:402–408.

532 TABLES AND FIGURES

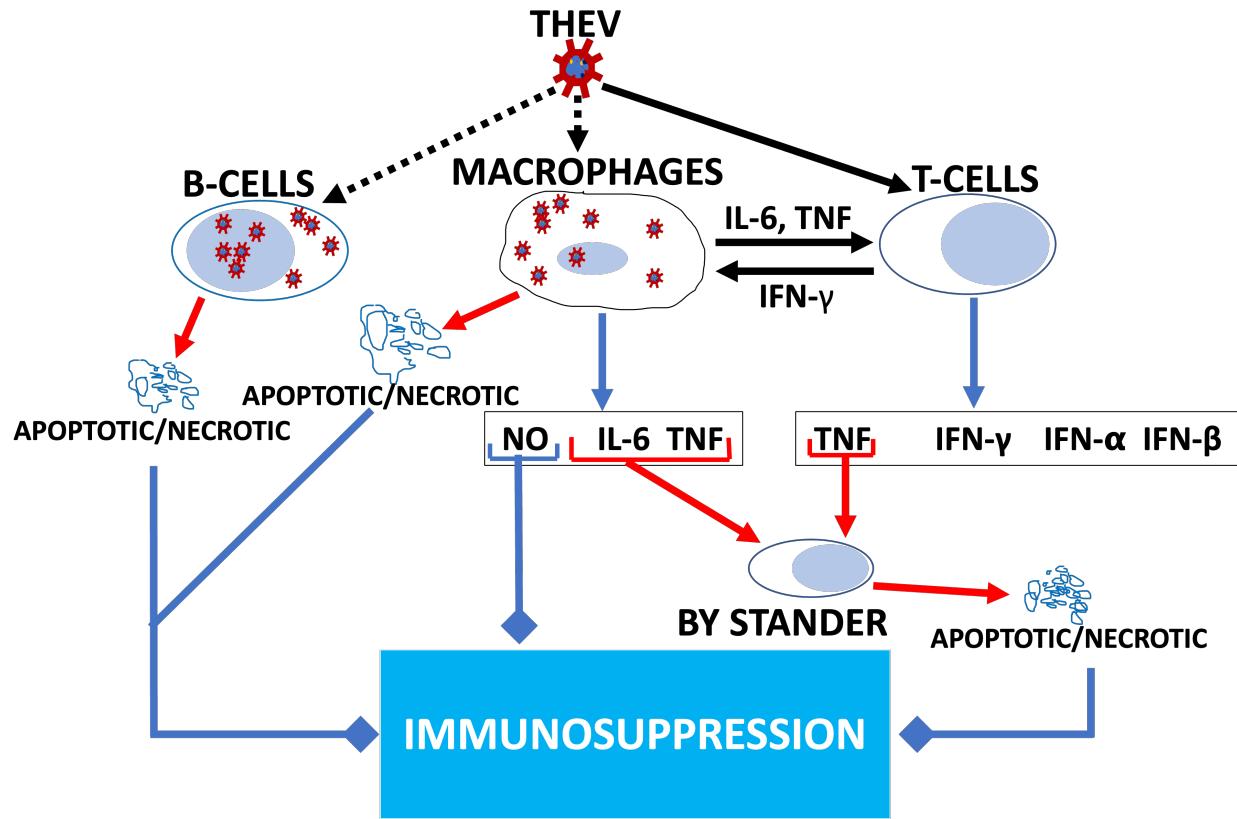


Figure 1: Model of THEV-induced immunosuppression in turkeys. THEV infection of target cells is indicated with black dotted arrows. Black unbroken arrows indicate cell activation. Red arrows indicate signals leading to apoptosis. Blue arrows indicate all cytokines released by the cell. Blue arrows with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

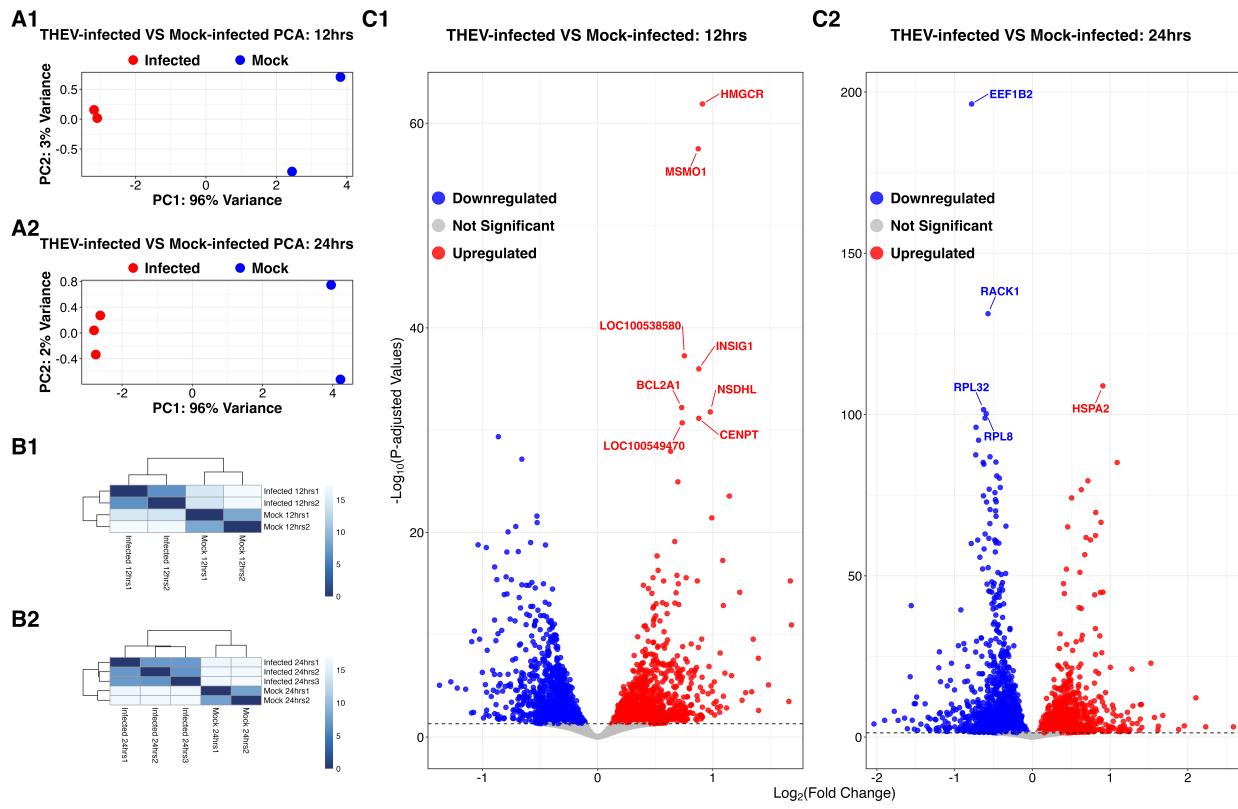


Figure 2. (A) Principal component analysis (PCA) of turkey B-cells during THEV infection. At 12-hpi (**A1**), the results indicate that the first (PC1) and second (PC2) principal components account for 96% and 3% of the variation in the samples, respectively. Whereas PC1 and PC2 account for 96% and 2% 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**).

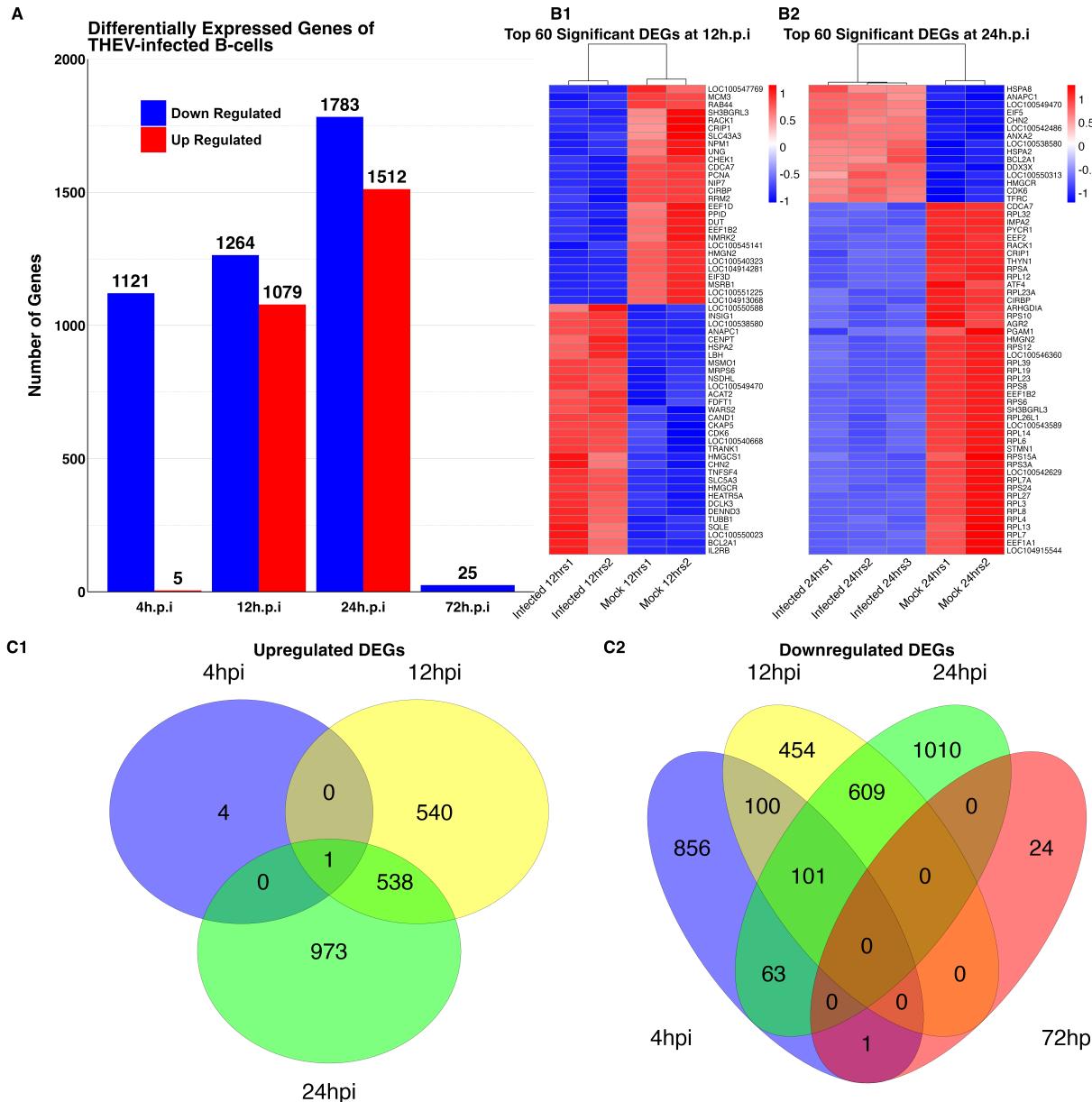
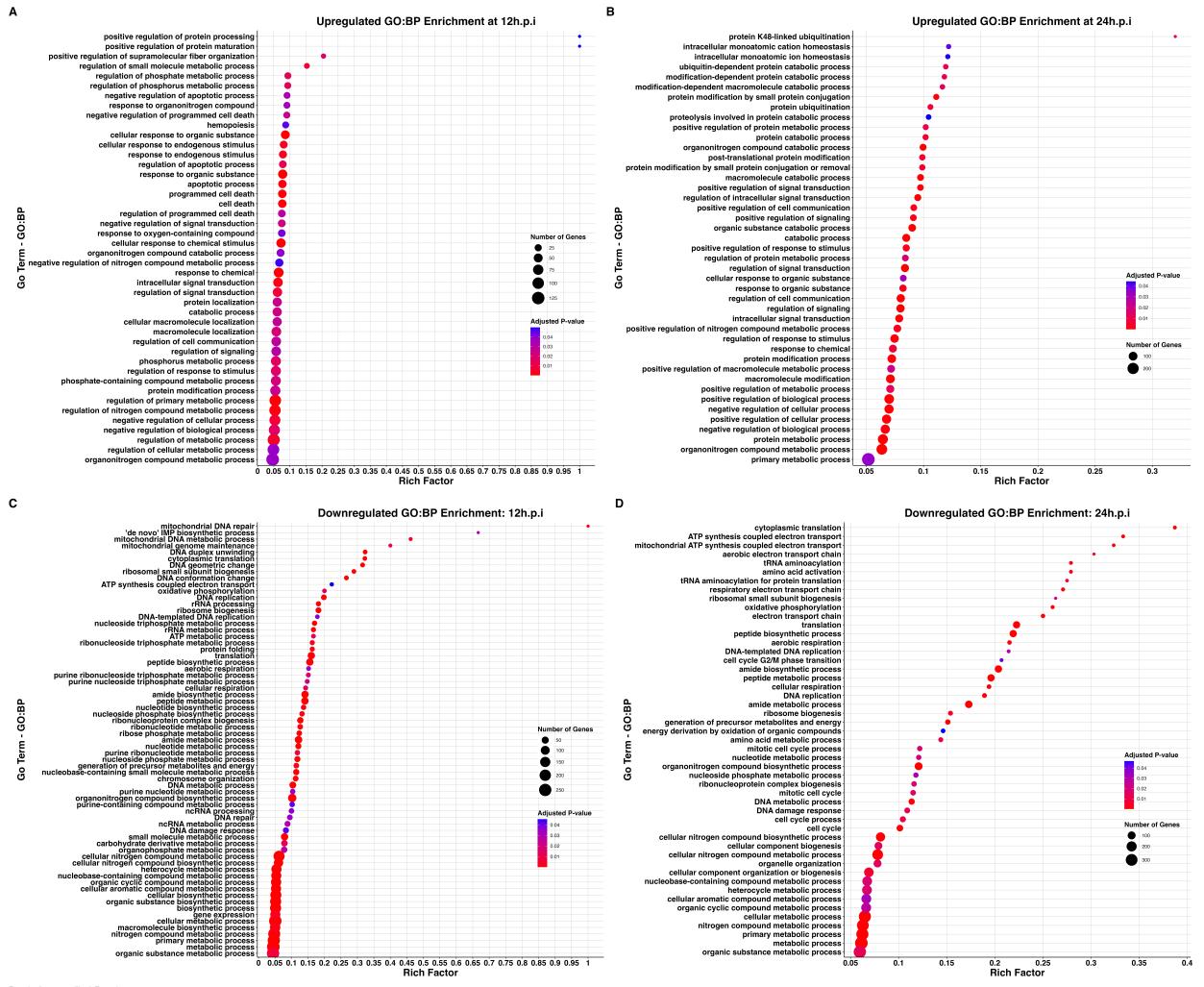


Figure 3: DEGs of THEV-infected versus mock-infected samples at different time points. (A) Bar plot of number DEGs identified. Red represents upregulated genes and blue represents downregulated genes. **(B) Heatmaps of scaled expression data (Z-scores) of DEGs.** DEGs identified at 12-hpi are shown in (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).



Results from gprofiler2 R package

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.

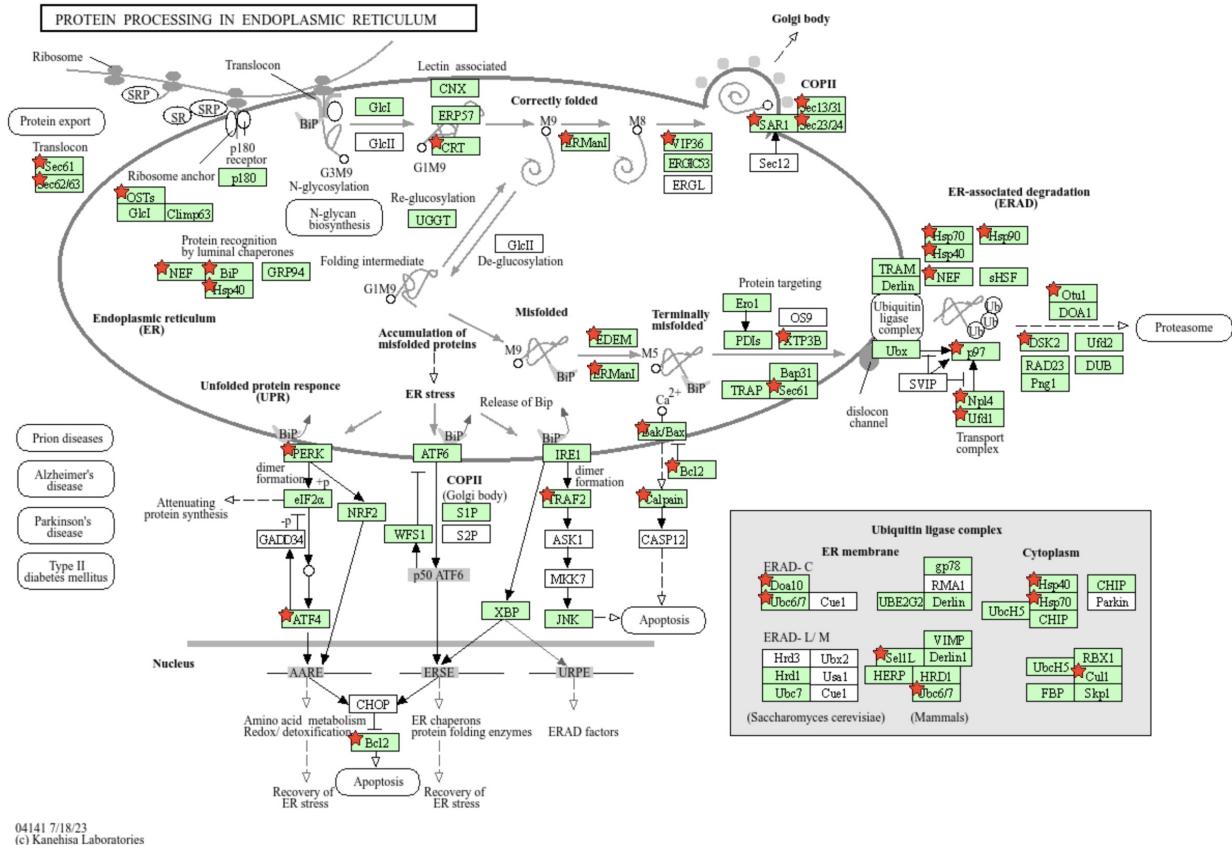


Figure 5: Upregulation of ER Unfolded Protein Response (UPR). KEGG Pathway analysis shows multiple key genes involved in the ER UPR were upregulated. All genes from our DEG list are annotated with the red star. Notably, *ATF4*, *PERK*, *VCP (p97)*, *TRAF2*, *UFD1* and several *BCL2* and heat shock proteins 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

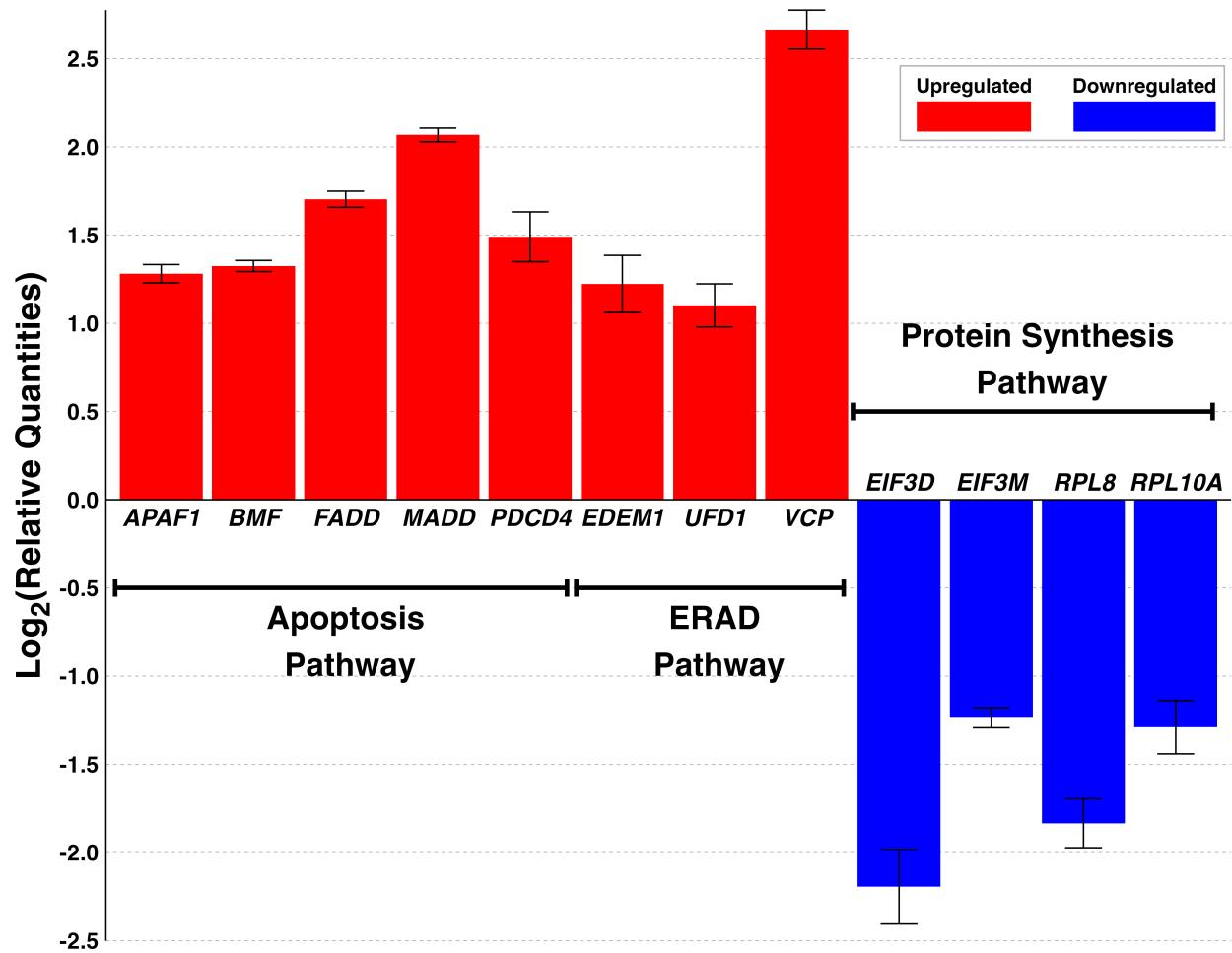


Figure 6: Validation of representative DEGs involved in Apoptosis, Protein synthesis, and ER-stress responses by RT-qPCR. MDTC-RP19 cells infected with THEV or mock infected were subjected to RT-qPCR analysis for the relative expression of the indicated DEGs at 24-hpi. GAPDH was used as the internal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

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

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

533 SUPPLEMENTARY MATERIALS

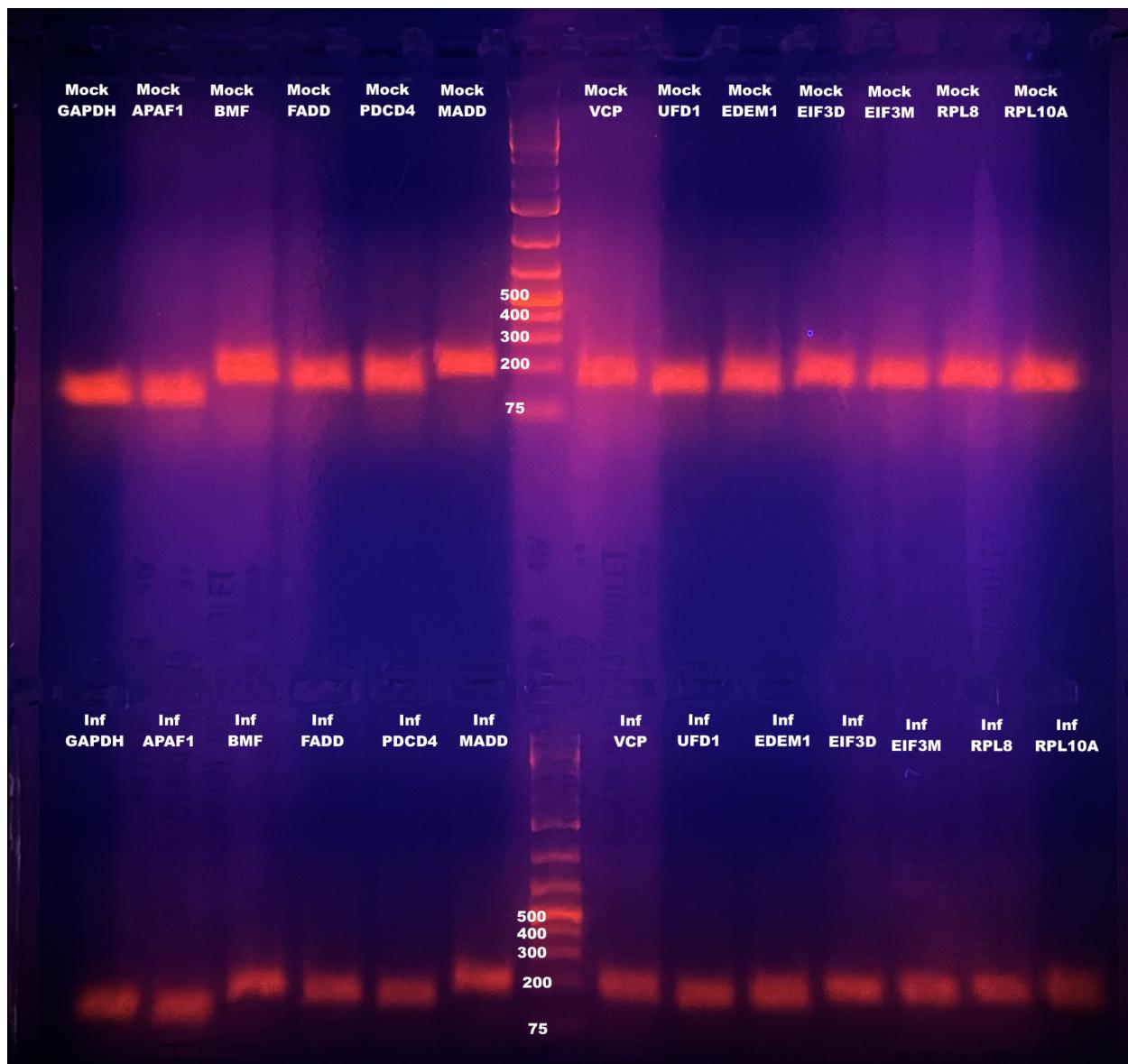


Figure S1: Gel Electrophoresis of RT-qPCR validation reactions. We run a gel electrophoresis of the RT-qPCR reactions in 2% agarose gel to confirm primer specificity. The 13 primer pairs all show excellent target specificity, each amplifying one amplicon of the expected size (see Table S1 for expected 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 ^{ExJ}	GCCAGACACAGCATCTGTCACAC ^{ExJ}	133 bp
100550591	<i>BMF</i>	CGGAGACTCTCTATGGGAATGCTGG ^{ExJ}	CTGCTGATGCCGCTGTATGTGG ^{ExJ}	189 bp
100543065	<i>EDEM1</i>	CTGGACTACAGGTGTTGATAGGAGACG ^{ExJ}	CCACTAACTCTGGCCTCAGTGG	159 bp
100545922	<i>EIF3D</i>	GCACAGAGGAACCTCGGAGAG ^{ExJ}	GTCACGAGGCTCTGCTGTGAC ^{ExJ}	180 bp
100545633	<i>EIF3M</i>	CTCTCAGACTGCAGCTACTGAGC ^{ExJ}	GTCTGTGCTGAGGTTCCAGTCAG	179 bp
100540536	<i>FADD</i>	GGAGCTCTGCAACTTCCTCATGG	CCTTCATGTCAGGCCACTCATCAG	167 bp
100303685	<i>GAPDH^{HK}</i>	CACTATCTTCAGGAGCGTGACC ^{ExJ}	CTGAGATGATAACACGCTTAGCACAC	146 bp
100551463	<i>MADD</i>	GAGCTGACGAGGTTGAACTTGCTG ^{ExJ}	CTGGCTCCAATGATAACAAGGTAGTCG	200 bp
100547583	<i>PDCD4</i>	GCACAGTAGAAGTGGAGAACATGAGTG ^{ExJ}	CTTCCTCAACCGCCTCTTGC	161 bp
100544053	<i>RPL10A</i>	GGCACCGTCAGGCTGAAGTC ^{ExJ}	GGCATCGTACTTCTTAGCCAGCTTC ^{ExJ}	177 bp
100544011	<i>RPL8</i>	GCCGAGAGACATGGCTACATCAAGG	CAGCTGAGCTTCTGCCACAG ^{ExJ}	186 bp
104913522	<i>UFD1</i>	GTGGTCTGCTTCAACATCTGGTC ^{ExJ}	GATCTATGAGCTTCGGTAATGGAGAC ^{ExJ}	154 bp
100548376	<i>VCP</i>	CAAGGCCATAGGAGTGAAGCCTC ^{ExJ}	CTCAGGTTGCTCTCAGACTCACC	171 bp

^{HK} Control (house-keeping) gene^{ExJ} Primer spans exon-exon junction;

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

⁵⁴⁰ 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