

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

5

6 Abraham Quaye^{†,a}, Brett E. Pickett^a, Joel S. Griffitts^a, Bradford K. Berges^a, Brian D. Poole^{a,*}

7 ^aDepartment of Microbiology and Molecular Biology, Brigham Young University

8 [†]Primary Author

9 ^{*}Corresponding Author

10 **Corresponding Author Information**

11 brian_poole@byu.edu

12 Department of Microbiology and Molecular Biology,

13 4007 Life Sciences Building (LSB),

14 Brigham Young University,

15 Provo, Utah

16

17 **ABSTRACT**

18 **Background:** Hemorrhagic enteritis, caused by *Turkey Hemorrhagic Enteritis Virus (THEV)*, is a disease
19 affecting turkey pouls characterized by immunosuppression (IMS) and bloody droppings. The clinical dis-
20 ease usually lasts only a few days but secondary opportunistic infections due to THEV-induced IMS extend
21 the duration of illness and mortality, exacerbating the economic losses. Albeit an avirulent THEV strain with
22 only subclinical disease is available as a vaccine, it still retains immunosuppressive properties, thus, still
23 causing substantial economic. To elucidate the mechanisms mediating THEV-induced IMS, we performed
24 the first transcriptomic analysis of a THEV infection using RNA-seq, uncovering key insights into host cell
25 response. **Methods:** After infecting a turkey B-cell line with the vaccine strain, samples in triplicates were
26 collected at 4, 12, 24, and 72 hours post-infection (hpi). Total RNA was extracted, and poly-A-tailed mRNA
27 sequenced. Reads were mapped to the turkey genome after trimming and gene expression was counted
28 with StringTie. Differential gene expression was performed with DESeq2 followed by functional enrichment
29 analysis with gprofiler2 and DAVID from NCBI. We performed RT-qPCR of select genes to validate the
30 RNA-seq data. **Results:** We obtained usable data from only 12 and 24 hpi samples. In total 2,343 and
31 3,295 Differentially expressed genes (DEGs) were identified at 12 hpi and 24 hpi, respectively. At 12 hpi,
32 1,079 genes were upregulated and 1,264 genes downregulated, whereas 1,512 genes were upregulated
33 and 1,783 genes downregulated at 24 hpi. The DEGs were related to multiple biological processes all
34 potentially playing a role in THEV infection but the most relevant to our study are apoptosis, ER unfolded
35 protein response, and suppressed cell maintenance processes. Multiple pro-apoptotic genes, including
36 *APAF1*, *BNIP3L*, *BMF*, *BAK1*, *RIPK1*, and *FAS* were upregulated, indicating that, unlike most adenoviruses,
37 THEV is not adept at thwarting the host apoptotic program. However, some anti-apoptotic genes were
38 also stimulated. Genes such as *VCP*, *UFD1*, *EDEM1*, *EDEM3*, and *ATF4* were also upregulated, strongly
39 suggesting an activate ER stress-induced unfolded protein response, which may also contribute to apopto-
40 sis. **Conclusions:** Apoptosis has long been suggested as the mechanism for THEV-induced IMS because
41 THEV primarily infects B-cells. Our results are in agreement with but we did not see strong support for
42 cytokine-mediated apoptosis. Our data suggest that a non-cytokine-dependent process or a synergistic
43 cross-talk of multiple processes is more likely to mediate direct cell death of infected cells.

44 **KEY WORDS**

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

46 **INTRODUCTION**

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

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

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

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

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

97 **RESULTS**

98 **Sequencing Results**

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

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

113 Gene expression levels were estimated with the StringTie software (19) in Fragments per kilobase of tran-
114 script per million (FPKM) units. The analysis of DEGs was performed with the DESeq2 R package (20)
115 which employs negative binomial distribution model for read count comparisons. Using a P_{adjusted} -value
116 cutoff ≤ 0.05 as the inclusion criteria, **2,343** and **3,295** genes were identified as differentially expressed
117 at 12-hpi and 24-hpi, respectively. The DEG analyses results at 12 and 24-hpi have been deposited in
118 NCBI Gene Expression Omnibus (<http://www.ncbi.nlm.nih.gov/geo>) under accession number ### with files
119 named ~file_name12hpi and file_name24hpi~, respectively. At 12-hpi, **1,079** genes were upregulated and
120 **1,264** genes downregulated, whereas **1,512** genes were upregulated and **1,783** genes downregulated at
121 24-hpi (**Figure 2C**, and **Figure 3A-C**). The log₂fold-change(FC) values at 12-hpi ranged between **-1.4** and
122 **+1.7** for **TMEM156** (Transmembrane Protein 156) and **LIPG** (Lipase G), respectively. At 24-hpi, the log₂FC
123 values ranged between **-2.0** and **+2.6** for **C1QTNF12** (C1q And TNF Related 12) and **KCNG1** (Potassium
124 Voltage-Gated Channel Modifier Subfamily G Member 1), respectively.

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

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

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

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

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

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

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

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

Cell Death and Breakdown Pathways Upregulated by THEV

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

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

200 **Cell Cycle and Cell Maintenance Pathway Regulation Impacting Apoptosis**

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

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

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

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

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

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

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

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

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

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

278 **DISCUSSION**

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

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

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

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

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

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

373 Regulation of genes involved in the cell cycle during THEV infection seemed complicated at 12 hpi, since

³⁷⁴ both positive and negative regulators were both up- or down-regulated.

375 **CONCLUSIONS**

376 CCHFV is a high-virulent BSL-4 pathogen posing a severe threat to worldwide public health. In the current
377 study, we establish the cellular transcriptome profile of CCHFV infection in human kidney HEK293 cells by
378 RNA-seq and identify 496 DEGs, including 361 up-regulated and 135 down-regulated genes. The data sug-
379 gest that multiple biological processes including defense response to virus, response to stress, regulation
380 of viral process, immune response, metabolism, stimulus, apoptosis and protein catabolic process are likely
381 implicated in the cellular responses upon CCHFV infection. Among them, type III IFN pathways as well as
382 ER stress responses are particularly regulated and highlighted by qPCR analyses, indicating their poten-
383 tial roles in CCHFV-cell interplays. Furthermore, antiviral assays demonstrate that not only type I but also
384 type III IFNs exhibit notable anti-CCHFV activities. These findings provide new insights into CCHFV-host
385 interactions and may help advance the understanding of viral infection and pathogenesis, which perhaps
386 will eventually inform the developm

387 **MATERIALS AND METHODS**

388 **Cell culture and THEV Infection**

389 The Turkey B-cell line (MDTC-RP19, ATCC CRL-8135) was grown as a suspension culture in 1:1 complete
390 Leibovitz's L-15/McCoy's 5A medium with 10% fetal bovine serum (FBS), 20% chicken serum (ChS), 5%
391 tryptose phosphate broth (TPB), and 1% antibiotic solution (100 U/mL Penicillin and 100 μ g/mL Strepto-
392 mycin), at 41°C in a humidified atmosphere with 5% CO₂. Infected cells were maintained in 1:1 serum-
393 reduced Leibovitz's L15/McCoy's 5A media (SRLM) with 2.5% FBS, 5% ChS, 1.2% TPB, and 1% antibiotic
394 solution. A commercially available THEV vaccine was purchased from Hygieia Biological Labs (VAS strain).
395 The stock virus was titrated using an in-house qPCR assay with titer expressed as genome copy number
396 (GCN)/mL, similar to Mahshoub *et al* (38). Cells were THEV-infected or mock-infected in triplicates or du-
397 plicates, respectively at a multiplicity of infection (MOI) of 100 GCN/cell, incubated at 41°C for 1 hour, and
398 washed three times with phosphate buffered saline (PBS) to get rid of free virus particles. At each time point
399 (4-, 12-, 24-, and 72-hpi), triplicate (THEV-infected) and duplicate (mock-infected) samples were harvested
400 for total RNA extraction.

401 **RNA extraction and Sequencing**

402 Total RNA was extracted from infected cells using the Thermo Fisher RNaseous™-4PCR Total RNA Iso-
403 lation Kit (which includes a DNase I digestion step) per manufacturer's instructions. An agarose gel elec-
404 trophoresis was performed to check RNA integrity. The RNA quantity and purity was initially assessed using
405 nanodrop, and RNA was used only if the A260/A280 ratio was 2.0 ± 0.05 and the A260/A230 ratio was >2
406 and <2.2. Extracted total RNA samples were sent to LC Sciences, Houston TX for poly-A-tailed mRNA
407 sequencing. RNA integrity was checked with Agilent Technologies 2100 Bioanalyzer High Sensitivity DNA
408 Chip and poly(A) RNA-seq library was prepared following Illumina's TruSeq-stranded-mRNA sample prepa-
409 ration protocol. Paired-end sequencing, generating 150 bp reads was performed on the Illumina NovaSeq
410 6000 sequencing system. The paired-end 150bp sequences obtained during this study and all expression
411 data have been submitted to the Gene Expression Omnibus database, under accession no #####

412 **Quality Control and Mapping Process**

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

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

428 **DEG Analysis and Functional Enrichment Analysis**

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

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

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

446 SYBR™ Green master mix from Applied Biosystems with primers designed manually in the SnapGene
447 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**
448
449 **Table S1.** Relative mRNA levels were calculated by $2^{-\Delta\Delta CT}$ method (44).

450 **Statistical Analysis**

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

454 **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

455 **DATA AVAILABILITY**

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

459 **CODE AVAILABILITY**

460 All the code/scripts in the entire analysis pipeline are available on github (<https://github.com/Abraham->
 461 Quaye/host_rna_seq)

462 **ACKNOWLEDGMENTS**

463 We thank the Office of Research Computing at Brigham Young University for granting us access to the
 464 high-performance computing systems to perform the memory-intensive steps in the analysis pipeline of this
 465 work.

466 **REFERENCES**

- 467 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.
- 468 2. Davison A, Benko M, Harrach B. 2003. Genetic content and evolution of adenoviruses. The Journal
of general virology 84:2895–908.
- 469 3. Gross WB, Moore WE. 1967. Hemorrhagic enteritis of turkeys. Avian Dis 11:296–307.
- 470 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.
- 471 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.
- 472 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.
- 473 7. Pierson F, Fitzgerald S. 2008. Hemorrhagic enteritis and related infections. Diseases of Poultry
276–286.
- 474 8. Rautenschlein S, Sharma JM. 2000. Immunopathogenesis of haemorrhagic enteritis virus (HEV) in
turkeys. Dev Comp Immunol 24:237–46.
- 475 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.

- 476 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.
- 477 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.
- 478 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.
- 479 13. Pandey D, Onkara Perumal P. 2023. A scoping review on deep learning for next-generation RNA-seq.
Data analysis. *Functional & Integrative Genomics* 23.
- 480 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.
- 481 15. Choi SC. 2016. On the study of microbial transcriptomes using second- and third-generation se-
quencing technologies. *Journal of Microbiology* 54:527–536.
- 482 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.
- 483 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.
- 484 18. Kanehisa M. 2000. KEGG: Kyoto encyclopedia of genes and genomes. *Nucleic Acids Research*
28:27–30.

- 485 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.
- 486 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.
- 487 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.
- 488 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).
- 489 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.
- 490 24. Barber GN. 2001. Host defense, viruses and apoptosis. *Cell Death & Differentiation* 8:113–126.
- 491 25. Hardwick JM. 1997. Virus-induced apoptosis, p. 295–336. *In Apoptosis - pharmacological implications and therapeutic opportunities*. Elsevier.
- 492 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.
- 493 27. Ezoe H, Fatt RB, Mak S. 1981. Degradation of intracellular DNA in KB cells infected with cyt mutants of human adenovirus type 12. *Journal of Virology* 40:20–27.
- 494 28. Quaye A, Pickett BE, Griffitts JS, Berges BK, Poole BD. 2024. Characterizing the splice map of turkey hemorrhagic enteritis virus. *Virology Journal* 21.

- 495 29. Zhao H, Dahlö M, Isaksson A, Syvänen A-C, Pettersson U. 2012. The transcriptome of the adenovirus infected cell. *Virology* 424:115–128.
- 496 30. Guimet D, Hearing P. 2016. Adenovirus replication, p. 59–84. *In* Adenoviral vectors for gene therapy. Elsevier.
- 497 31. Roos WP, Kaina B. 2006. DNA damage-induced cell death by apoptosis. *Trends in Molecular Medicine* 12:440–450.
- 498 32. Martin SJ. 1993. Protein or RNA synthesis inhibition induces apoptosis of mature human CD4+ t cell blasts. *Immunology Letters* 35:125–134.
- 499 33. Christianson JC, Carvalho P. 2022. Order through destruction: How ER-associated protein degradation contributes to organelle homeostasis. *The EMBO Journal* 41.
- 500 34. Hurk JV van den. 1990. Propagation of group II avian adenoviruses in turkey and chicken leukocytes. *Avian Diseases* 34:12.
- 501 35. Aboezz 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.
- 502 36. Fribley A, Zhang K, Kaufman RJ. 2009. Regulation of apoptosis by the unfolded protein response, p. 191–204. *In* Apoptosis. Humana Press.
- 503 37. Read A, Schröder M. 2021. The unfolded protein response: An overview. *Biology* 10:384.
- 504 38. 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.

- 505 39. 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.
- 506 40. Martin M. 2011. Cutadapt removes adapter sequences from high-throughput sequencing reads.
EMBnetjournal 17:10.
- 507 41. Wickham H. 2016. *ggplot2: Elegant graphics for data analysis*. Springer-Verlag New York. <https://ggplot2.tidyverse.org>.
- 508 42. Kolde R. 2019. *Pheatmap: Pretty heatmaps*. <https://CRAN.R-project.org/package=pheatmap>.
- 509 43. Yan L. 2023. *Ggvenn: Draw venn diagram by 'ggplot2'*. <https://CRAN.R-project.org/package=ggvenn>.
- 510 44. Livak KJ, Schmittgen TD. 2001.. *Methods* 25:402–408.

511 TABLES AND FIGURES

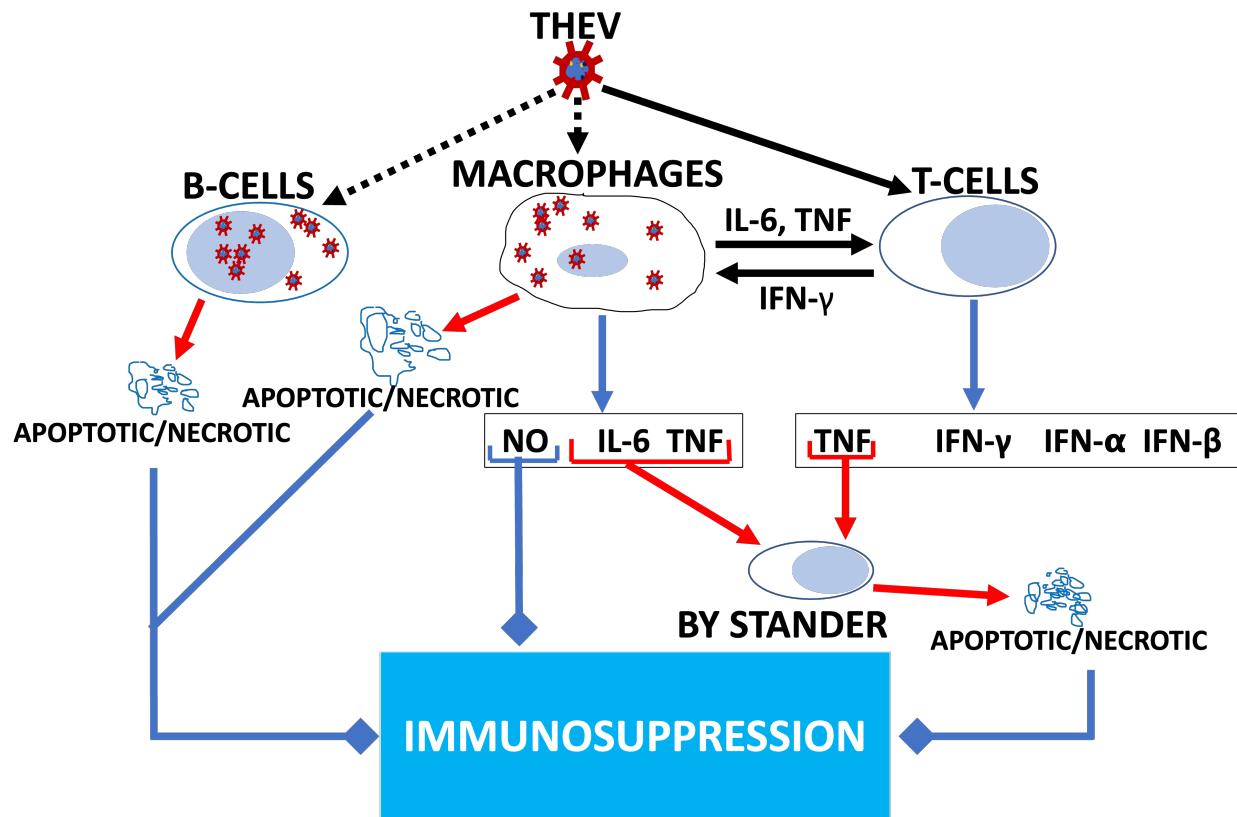


Figure 1: Model of THEV-induced immunosuppression in turkeys. THEV infection of target cells is indicated with black dotted arrows. Black unbroken arrows indicate cell activation. Red arrows indicate signals leading to apoptosis. Blue arrows indicate all cytokines released by the cell. Blue arrows with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

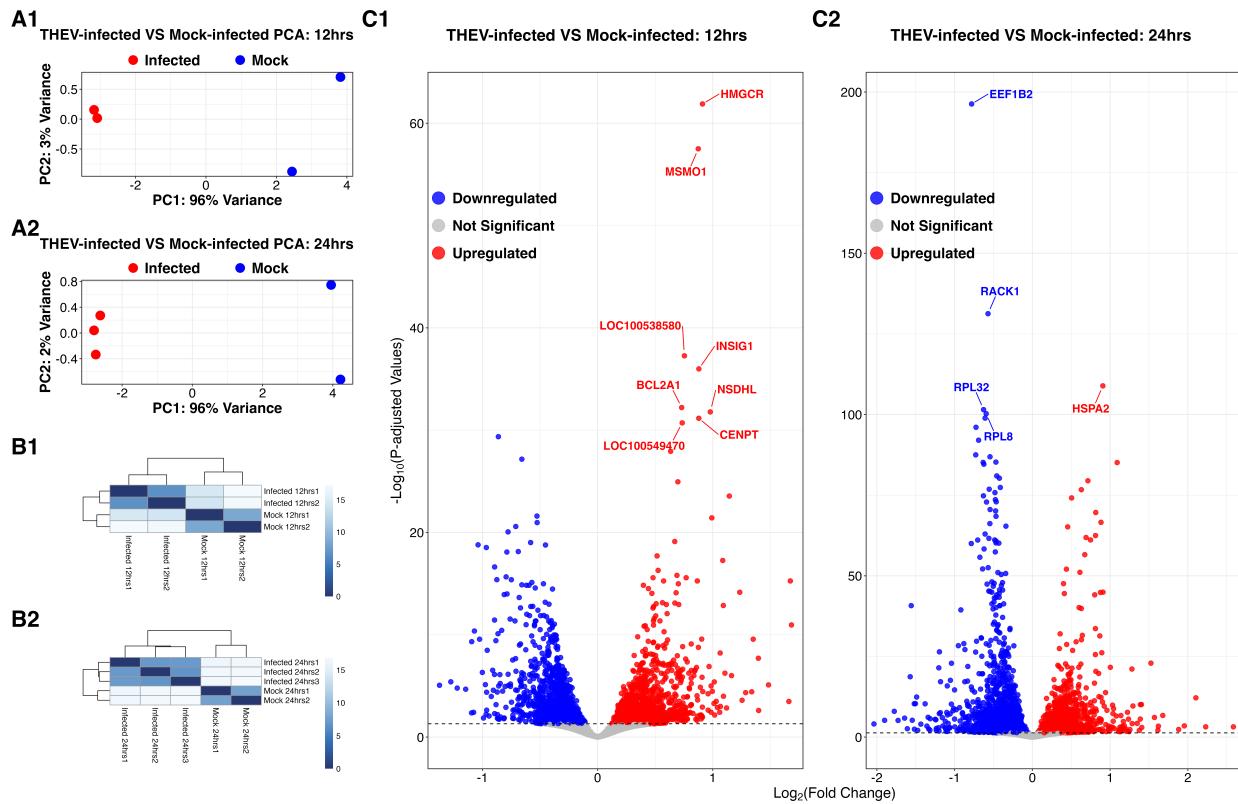


Figure 2. (A) Principal component analysis (PCA) of turkey B-cells during THEV infection. At 12-hpi (**A1**), the results indicate that the first (PC1) and second (PC2) principal components account for 96% and 3% of the variation in the samples, respectively. Whereas PC1 and PC2 account for 96% and 2%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

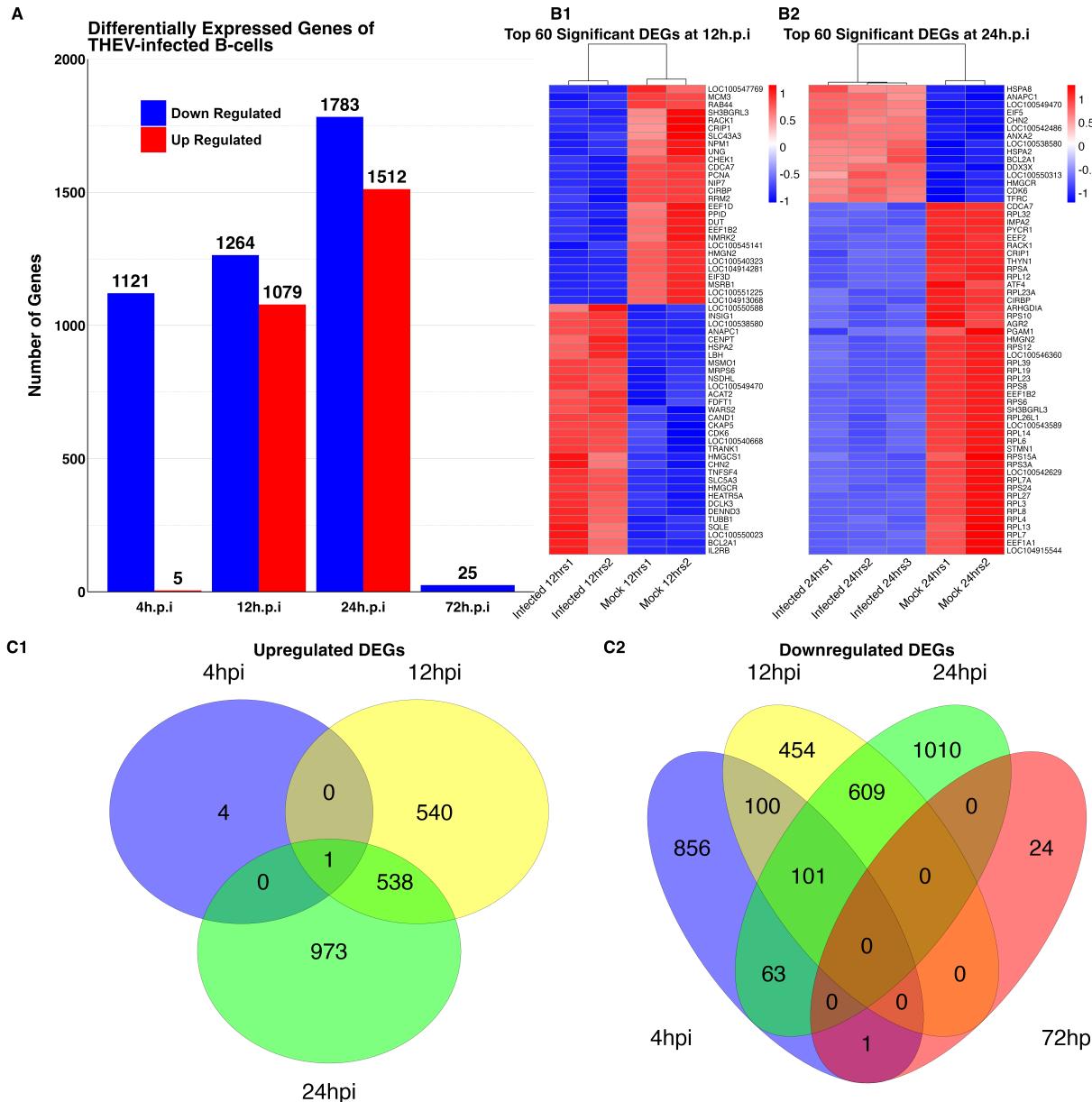


Figure 3: DEGs of THEV-infected versus mock-infected samples at different time points. (A) Bar plot of number DEGs identified. Red represents upregulated genes and blue represents downregulated genes. **(B) Heatmaps of scaled expression data (Z-scores) of DEGs.** DEGs identified at 12-hpi are shown in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

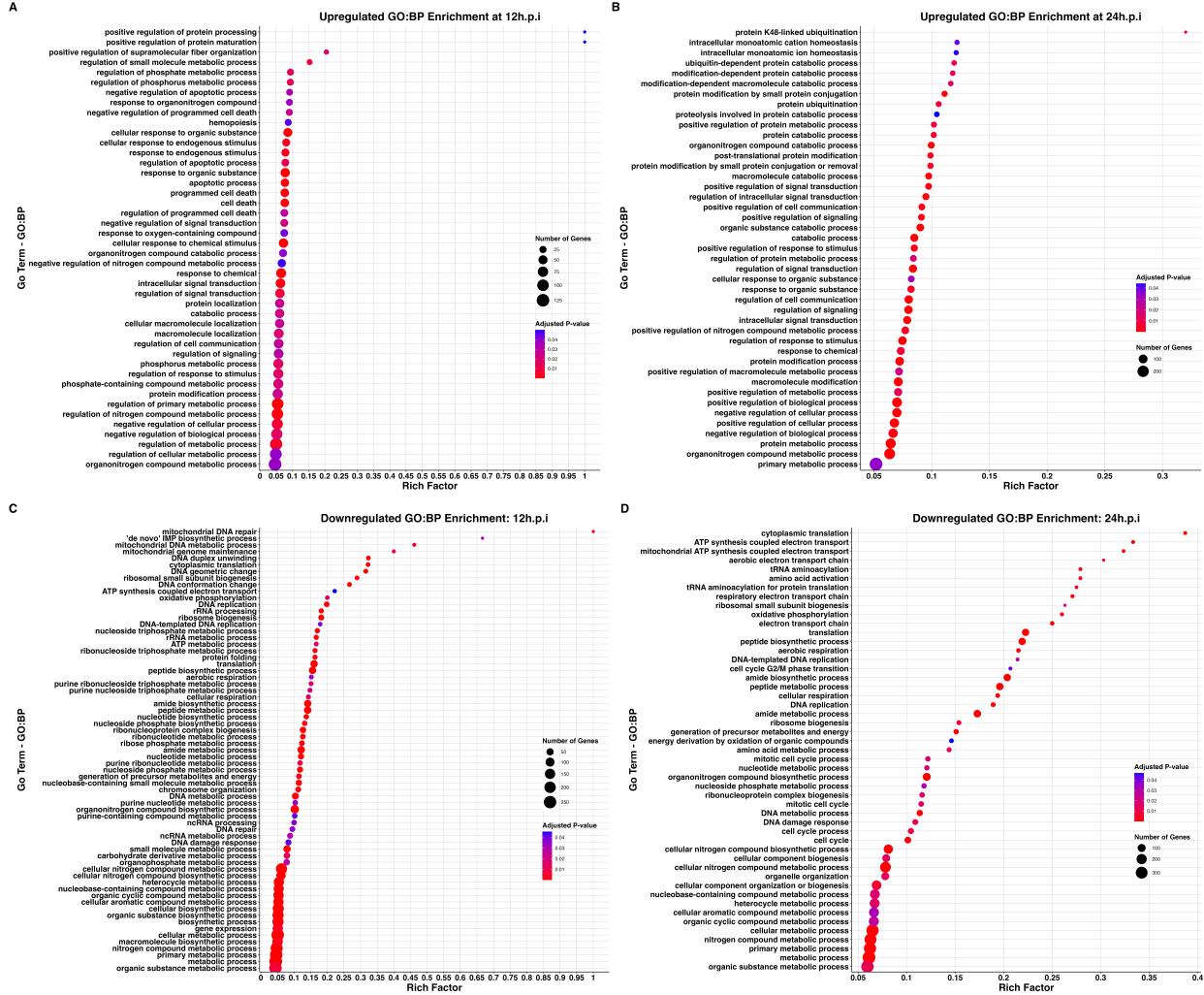


Figure 4: Dotplot of Enriched Gene Ontology Biological Processes (BP). Significant BP GO terms identified for upregulated DEGs at 12-hpi and 24-hpi are shown in (A) and (B), respectively. Significant BP GO terms for downregulated DEGs at 12-hpi and 24-hpi are shown in (C) and (D), respectively. The y-axis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

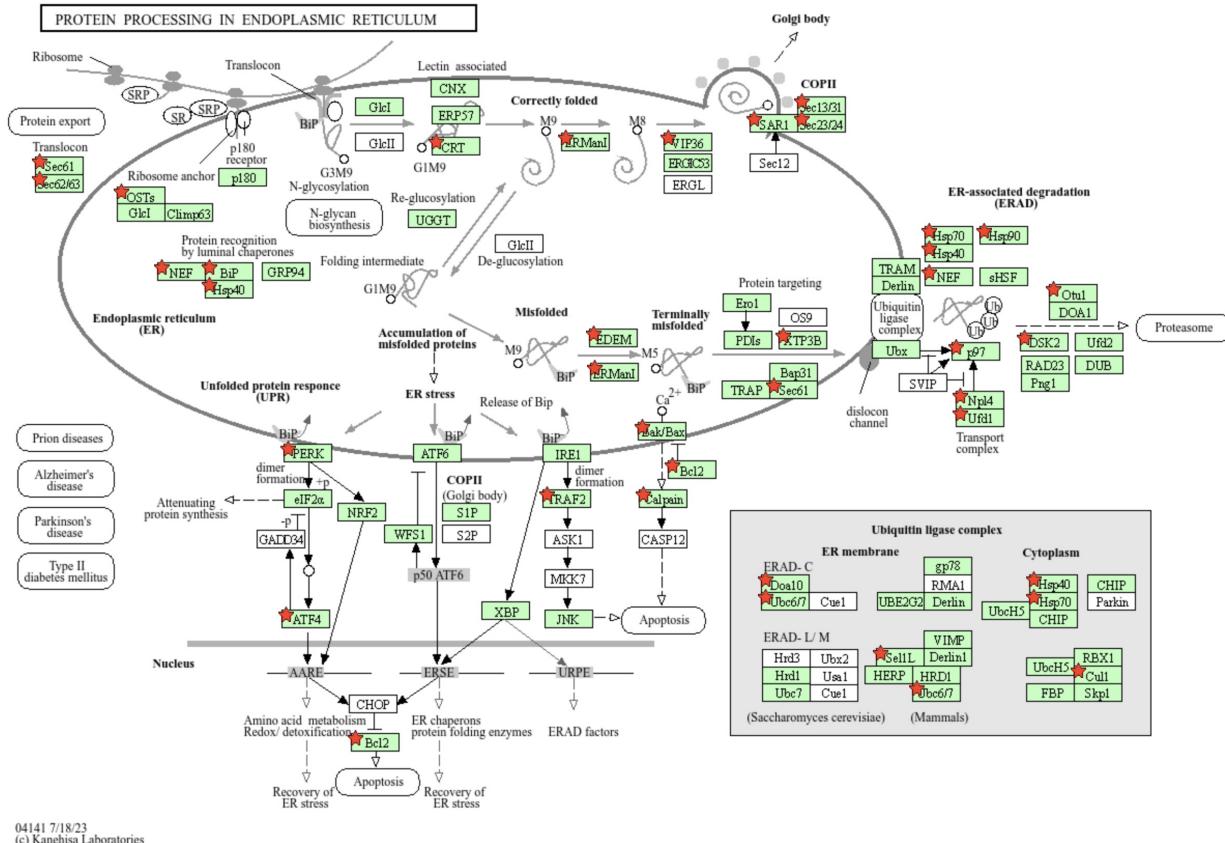


Figure 5: Upregulation of ER Unfolded Protein Response (UPR). KEGG Pathway analysis shows multiple key genes involved in the ER UPR were upregulated. All genes from our DEG list are annotated with the red star. Notably, *ATF4*, *PERK*, *VCP (p97)*, *TRAF2*, *UFD1* and several *BCL2* and heat shock proteins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

RT-qPCR Validation of Select DEGs

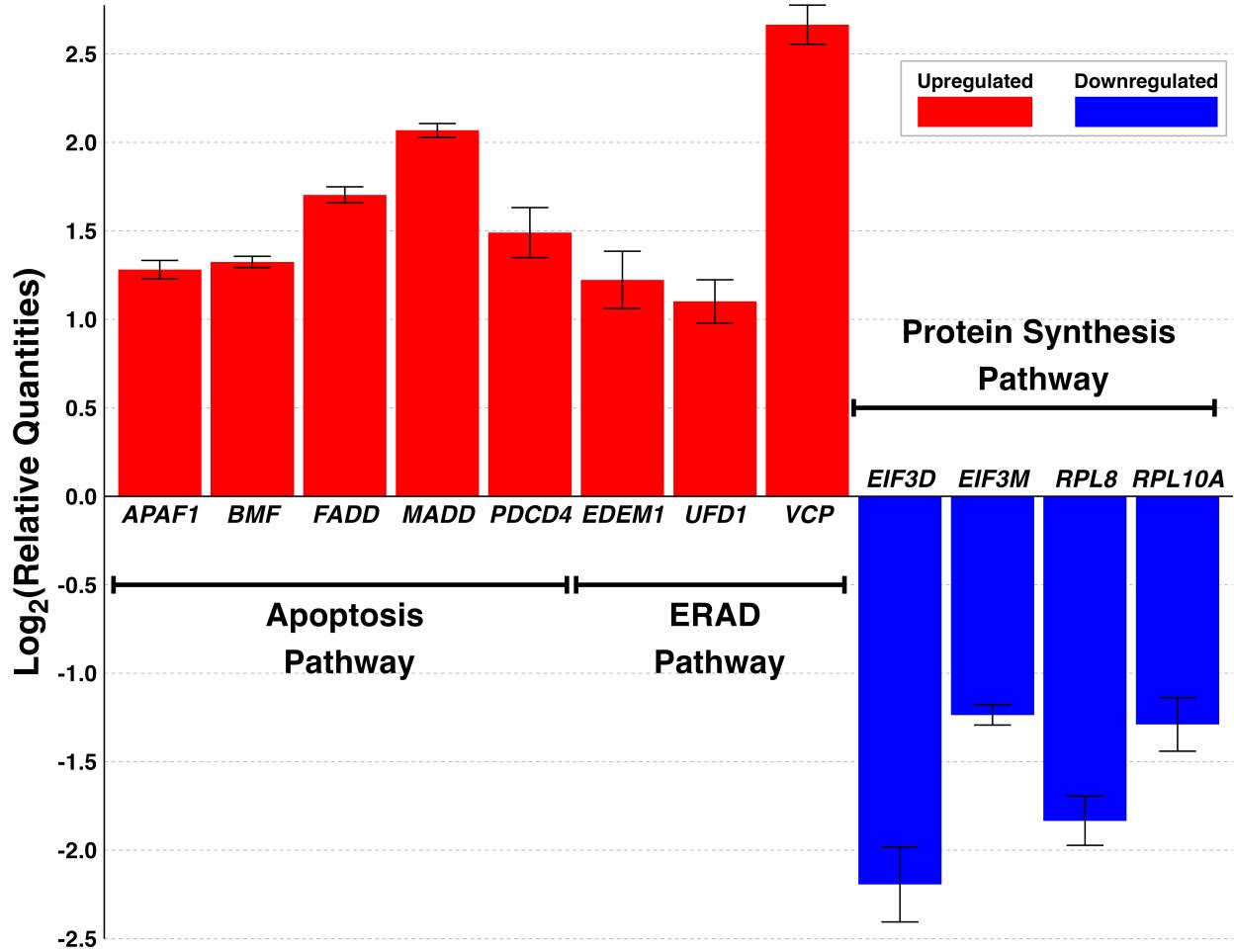


Figure 6: Validation of representative DEGs involved in Apoptosis, Protein synthesis, and ER-stress responses by RT-qPCR. MDTC-RP19 cells infected with THEV or mock infected were subjected to RT-qPCR analysis for the relative expression of the indicated DEGs at 24hpi. GAPDH was used as the internal control.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

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

Sample	Raw Reads ^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 (%)
--------	------------------------	----------------------------	---------------------------	---------------------------------------	---	------	------	-------------------

^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

512 SUPPLEMENTARY INFORMATION/MATERIALS

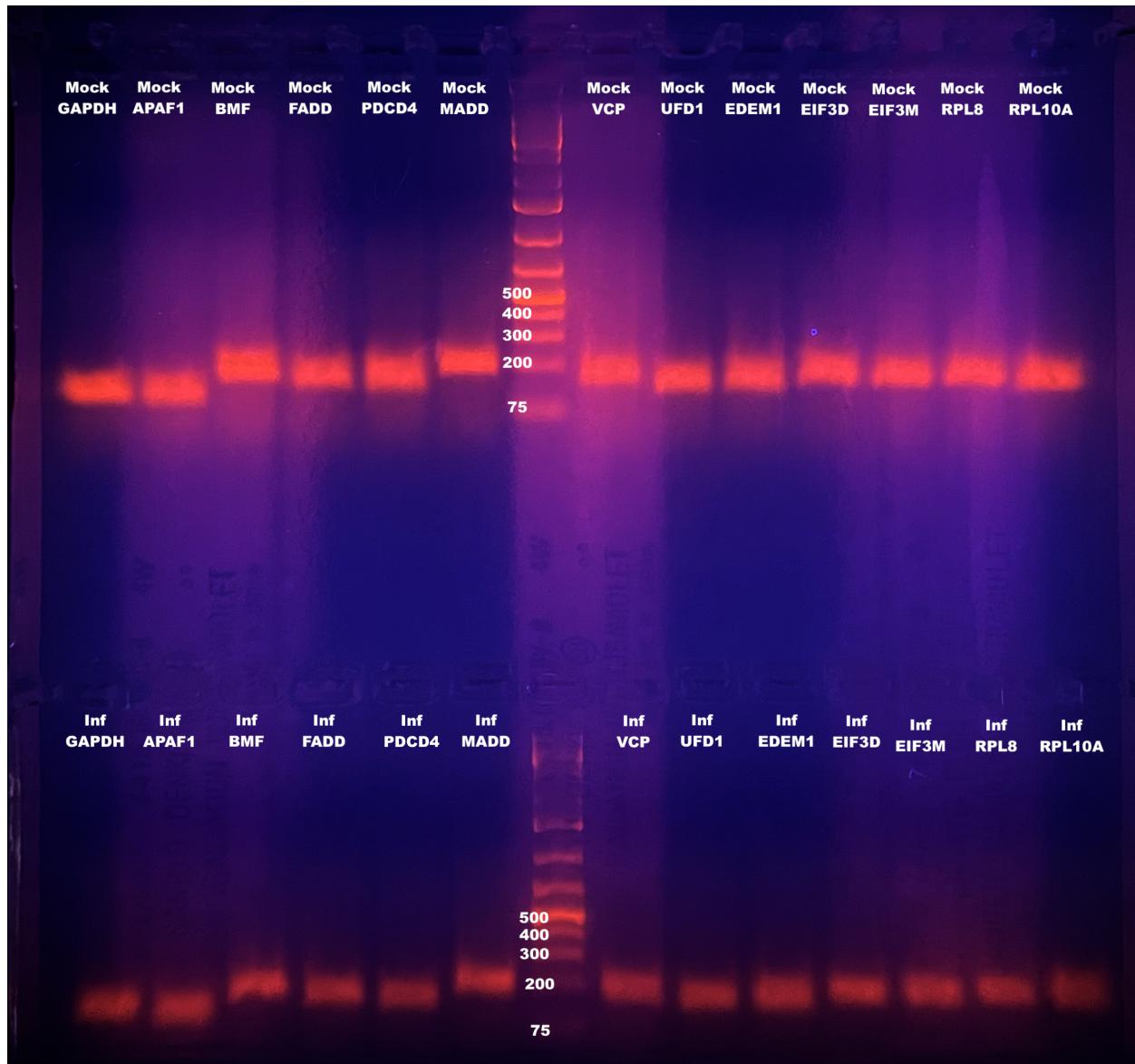


Figure S1: Gel Electrophoresis of RT-qPCR validation reactions. We run a gel electrophoresis of the RT-qPCR reactions in 2% agarose gel to confirm primer specificity. The 13 primer pairs all show excellent target specificity, each amplifying one amplicon of the expected size (see Table S1 for expected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

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>	CGGAGACTCTTCTATGGGAATGCTGG ^{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>	CACTATCTTCCAGGAGCGTGACC ^{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>	GTGGTCTGCTTCAACATCTGTGGTC ^{ExJ}	GATCTATGAGCTTCGGGTAATGGAGAC ^{ExJ}	154 bp
100548376	<i>VCP</i>	CAAGGCCATAGGAGTGAAGCCTC ^{ExJ}	CTCAGGTTGCTCTCAGACTCACC	171 bp

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

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